

Supporting Information

**Unveiling the active isomer of cycloalanopine, a cyclic opine from *Lactobacillus rhamnosus* LS8, through synthesis and analog production**

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## General synthetic procedures

Reactions involving air or moisture sensitive reagents were conducted under a positive pressure of argon in flame-dried glassware. Commercially available solvents and chemicals were supplied from Sigma-Aldrich and used as received unless otherwise stated. Anhydrous solvents required were dried according to the procedures outlined in Perrin and Armarego.<sup>1</sup> Removal of solvent was performed under reduced pressure below 40 °C using a Büchi rotary evaporator. All reactions and fractions from column chromatography were monitored by thin layer chromatography (TLC). Analytical TLC was done on glass plates (5 × 1.5 cm) pre-coated (0.25 mm) with silica gel (normal SiO<sub>2</sub>, Merck 60 F254). Compounds were visualized by exposure to UV light and/or by exposing the plates to permanganate (KMnO<sub>4</sub>: K<sub>2</sub>CO<sub>3</sub>: NaOH: H<sub>2</sub>O, 1.5 g: 10 g: 0.12 g: 200 mL) solution, followed by heating. Flash chromatography was performed on silica gel (EM Science, 60 Å pore size, 230-400 mesh).

## *Compound Characterization*

Optical rotations were measured on a Perkin Elmer 241 polarimeter with a microcell (10 cm, 1 mL) at 25 °C. Infrared spectra (IR) were recorded on a Nicolet Magna 750. Cast film refers to the evaporation of a solution on a NaCl plate. Nuclear magnetic resonance (NMR) spectra were obtained on Varian Inova 500 or 700 MHz spectrometer. <sup>1</sup>H NMR chemical shifts are reported in parts per million (ppm) using the residual proton resonance of solvents as reference: CDCl<sub>3</sub> δ 7.26, DMSO-d<sub>6</sub> δ 2.50, or CD<sub>2</sub>Cl<sub>2</sub> δ 5.32. <sup>13</sup>C NMR chemical shifts are reported relative to CDCl<sub>3</sub> δ 77.1, DMSO-d<sub>6</sub> δ 39.5, or CD<sub>2</sub>Cl<sub>2</sub> δ 53.8. Mass spectra were recorded on a Kratos AEIMS-50 (high resolution, electron impact ionization (EI)) or an Agilent Technologies 6220

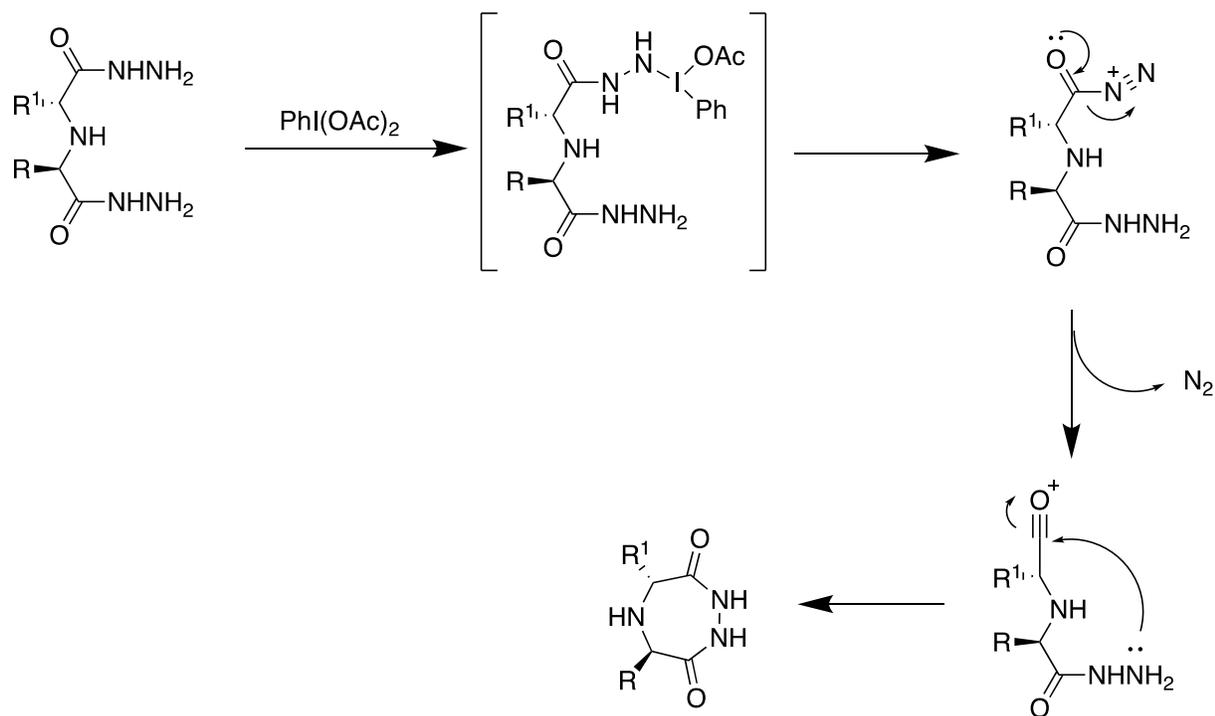
oaTOF instrument equipped with +ve and –ve ion ESI ionization source, and full-scan MS (high resolution analysis) with two-point lock mass correction operating mode. The instrument inlet was an Agilent Technologies 1200 SL HPLC system.

### **Biological assays**

Spot-on-lawn assays were performed to determine the antibacterial activity of the various opine analogs. *Salmonella typhimurium*, *Escherichia coli*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* were grown in Luria broth medium at 37 °C. *Enterococcus faecalis* and *Listeria monocytogenes* were grown in All Purpose Tween medium at 25 °C, whereas *Staphylococcus aureus* was grown in Tryptic Soy Broth medium at 25 °C. Overnight cultures were used to inoculate 5 mL of soft agar (0.75% agar) containing the appropriate medium and poured onto hard agar media (1.5% agar) plates.

Compounds to be tested for inhibitory activity were dissolved in MQ-H<sub>2</sub>O or DMSO and various concentrations were made by series of two-fold dilution of the opine stock solutions. An aliquot of 10 µL from each concentration was spotted onto the plates and, after drying, plates were incubated overnight at the appropriate temperature. Minimum inhibitory concentration was determined from triplicates of this assay based on inhibited growth observed at the location of opine solution spotting.

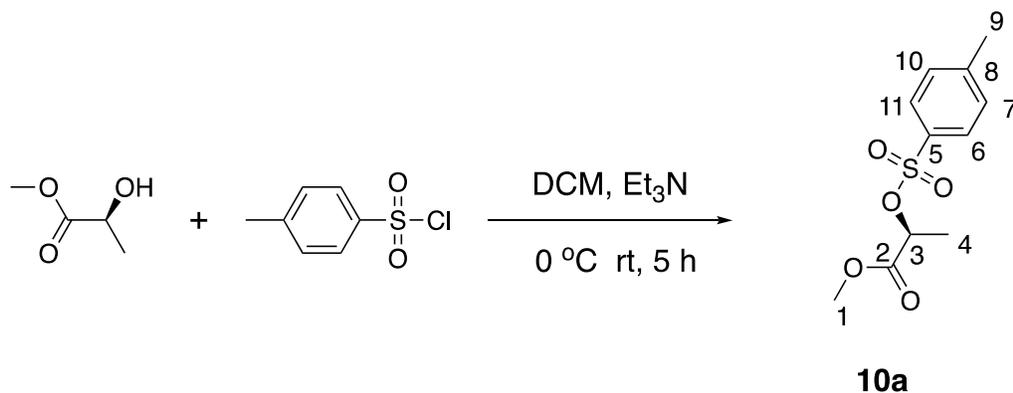
### Plausible mechanism for the cyclization



Scheme S1: Plausible mechanism for the cyclization of bishydrazides.

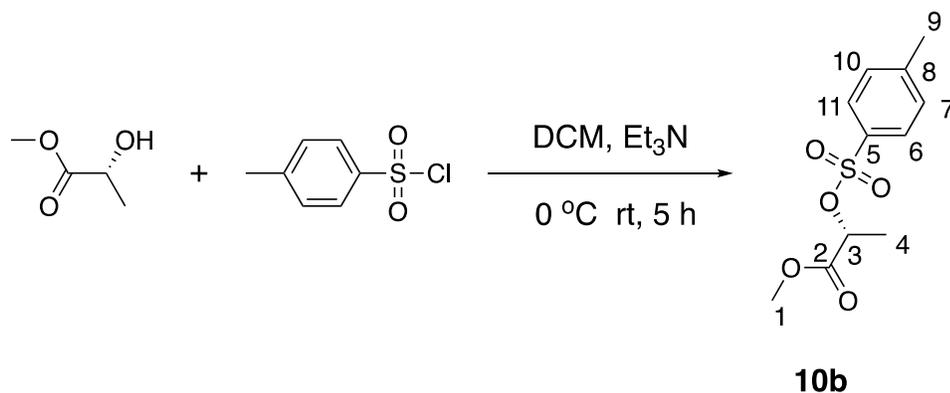
## Synthetic Procedures

### Methyl (*S*)-2-(tosyloxy)propanoate (**10a**)



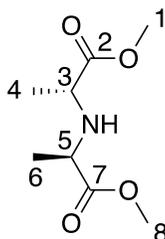
This known compound was synthesized following a literature protocol<sup>2</sup> to give the final product as a pale yellow oil (30.5 g, 96%).  $R_f = 0.34$  in 10% EtOAc in hexanes;  $[\alpha]_D^{25} = -29.59$  ( $c$  1.00, CHCl<sub>3</sub>); IR (CHCl<sub>3</sub>, cast) 2996, 2956, 1761, 1595, 1190, 1176, 1082, 664 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz)  $\delta$  7.79 (d,  $J = 7.93$  Hz, 2H, H6, H11), 7.36 (d,  $J = 7.93$  Hz, 2H, H7, H10), 4.96 (q,  $J = 7.2$  Hz, 1H, H3), 3.67 (s, 3H, H1), 2.49 (s, 3H, H9), 1.51 (d,  $J = 6.98$  Hz, 3H, H4); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz)  $\delta$  169.5 (C2) (C5), 145.1 (C5), 133.4 (C8), 130.3 (C7, C10), 127.9 (C6, C11), 74.1 (C3), 52.6 (C1), 21.7 (C9), 18.4 (C4); HRMS (ESI) Calcd for C<sub>6</sub>H<sub>9</sub>NNaOS<sub>2</sub> [M+Na]<sup>+</sup> 281.0454, found 281.0453.

### Methyl (*R*)-2-(tosyloxy)propanoate (**10b**)



Compound **10b** was prepared following the same method as described for **10a**<sup>2</sup> to give the final product as a pale yellow oil (30.5 g, 96%).  $R_f = 0.38$  in 10% EtOAc in hexanes;  $[\alpha]_D^{25} = 27.87$  ( $c$  1.00, CHCl<sub>3</sub>); IR (CHCl<sub>3</sub>, cast) 2930, 2850, 1759, 1465, 1368, 1180, 1085, 820, 555 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz)  $\delta$  7.79 (d,  $J = 7.93$  Hz, 2H, H6/ H11), 7.33 (d,  $J = 7.93$  Hz, 2H, H7, H10), 4.93 (q,  $J = 7.01$  Hz, 1H, H3), 3.64 (s, 3H, H1), 2.43 (s, 3H, H9), 1.48 (d,  $J = 7.01$  Hz, 3H, H4); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz)  $\delta$  169.5 (C2), 145.2 (C5), 133.3 (C8), 129.8 (C7, C10), 127.9 (C6, C11), 74.1 (C3), 52.6 (C1) 21.6 (C9), 18.3 (C4); HRMS (ESI) Calcd for C<sub>11</sub>H<sub>14</sub>NaO<sub>5</sub>S<sub>2</sub> [M+Na]<sup>+</sup> 281.0454, found 281.0453.

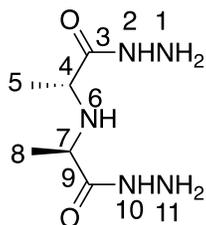
### Dimethyl 2,2'-azanediyl(2*R*,2'*R*)-dipropionate (**2a**)



**2a**

This known compound<sup>3</sup> was synthesized *via* an alternative route. To a solution of D-alanine methyl ester hydrochloride (5.0 g, 35.8 mmol) in MeCN (30 mL) was added NaHCO<sub>3</sub> (6.0 g, 71.6 mmol) and stirred for 1 h at room temperature. Compound **10a** (9.2 g, 35.8 mmol) in MeCN (10 mL) was added and reaction stirred under reflux for 12 h. TLC analysis of reaction mixture (30% EtOAc in hexanes, R<sub>f</sub> = 0.33) showed completion of reaction. The reaction mixture was allowed to cool to room temperature, filtered and the filtrate was concentrated *in vacuo* to give a pale yellow oil. The crude compound was purified by column chromatography (SiO<sub>2</sub>, 40% EtOAc in hexanes), yielding the product as a pale yellow oil (6.2 g, 91%).  $[\alpha]_D^{25} = 48.91$  (*c* 1.00, CHCl<sub>3</sub>); IR (CHCl<sub>3</sub>, cast) 3454, 3341, 2981, 2954, 2876, 1737, 1453, 1374, 1202, 1095, 983, 743, cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 700 MHz)  $\delta$  3.71 (s, 6H, H1, H8), 3.41 (q, *J* = 7.03 Hz, 2H, H3, H5) 1.31 (d, *J* = 7.03 Hz, 6H, H4, H6); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz)  $\delta$  175.5 (C2, C7), 55.1 (C3, C5), 51.9 (C1, C8), 19.3 (C4, C6); HRMS (ESI) Calcd for C<sub>8</sub>H<sub>16</sub>NO<sub>4</sub> [M + H]<sup>+</sup> 190.1074, found 190.1072.

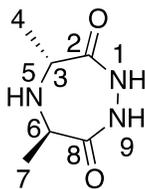
**(2*R*,2'*R*)-2,2'-azanediyldi(propanehydrazide) (6a)**



**6a**

This new compound was synthesized following literature procedure.<sup>4</sup> To a solution of **2a** (3.0 g, 15.9 mmol) in MeOH (10 mL) was added hydrazine monohydrate (1.5 mL, 31.8 mmol) and stirred for 12 h. A white precipitate formed and was filtered, washed with Et<sub>2</sub>O (5 × 20 mL), and dried to give a white solid which was recrystallized from Et<sub>2</sub>O to give the product as white powder (2.8 g, 93%).  $[\alpha]_D^{25} = 37.25$  (*c* 1.00, MeOH); IR (MeOH, cast) 3301, 3208, 3041, 2991, 2979, 1653, 1534, 1322, 971, 689, cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 500 MHz) δ 8.92 (s, 2H, H2, H10), 4.15 (s, 4H, H1, H11), 3.31 (s, 1H, H6), 2.95 (q, *J* = 7.03 Hz, 2H, H4, H7) 1.28 (d, *J* = 7.03 Hz, 6H, H5, H8); <sup>13</sup>C NMR (DMSO-d<sub>6</sub>, 125 MHz) δ 173.3 (C3, C9), 54.7 (C4, C7), 19.6 (C5, C8); HRMS (ESI) Calcd for C<sub>6</sub>H<sub>16</sub>N<sub>5</sub>O<sub>2</sub> [M + H]<sup>+</sup> 190.1299, found 190.1275.

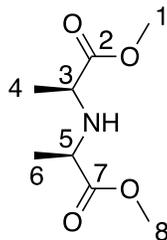
**(4*R*,6*R*)-4,6-dimethyl-1,2,5-triazepane-3,7-dione (1a)**



**1a**

This new compound was synthesized following literature procedure.<sup>5</sup> To a solution of **6a** (1.2 g, 6.3 mmol) in dry MeCN (5 mL) was added  $\text{PhI}(\text{OAc})_2$  (2.1 g, 6.3 mmol) in four portions. The reaction became exothermic with the evolution of gas. After stirring for 10 min the reaction became homogeneous and stirring was continued for additional 20 min at room temperature. TLC monitoring of the reaction (10% MeOH in EtOAc,  $R_f = 0.41$ ) showed completion. The solvent was reduced *in vacuo* to a volume of about 1 mL and the crude was purified by column chromatography ( $\text{SiO}_2$ , 10% MeOH in EtOAc), yielding the product as a yellow solid (0.89 g, 90%).  $[\alpha]_D^{25} = 43.23$  (*c* 0.89, MeOH); IR (MeOH, cast) 3354, 2991, 2944, 2843, 1714, 1423, 1109, 1056, 755,  $\text{cm}^{-1}$ ;  $^1\text{H NMR}$  ( $\text{D}_2\text{O}$ , 500 MHz)  $\delta$  4.06 (q,  $J = 7.02$  Hz, 2H, H3, H6) 1.51 (d,  $J = 7.02$  Hz, H4, H7);  $^{13}\text{C NMR}$  ( $\text{D}_2\text{O}$ , 125 MHz)  $\delta$  174.9 (C2, C8), 51.2 (C3, C6), 16.1 (C4, C7); HRMS (ESI) Calcd for  $\text{C}_6\text{H}_{12}\text{N}_3\text{O}_2$   $[\text{M} + \text{H}]^+$  158.0924, found 158.0926.

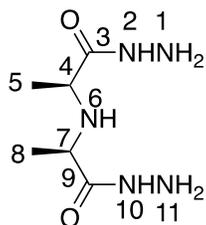
**Methyl ((*R*)-1-methoxy-1-oxopropan-2-yl)-L-alaninate (2b)**



**2b**

This known compound<sup>3</sup> was synthesized using the same method as described for **2a**. After purification by column chromatography (SiO<sub>2</sub>, 40% EtOAc in hexanes), the product was obtained as a yellow oil (6.8 g, 92%). R<sub>f</sub> = 0.41 in 30% EtOAc in hexanes). [α]<sub>D</sub><sup>25</sup> = 0.00 (*c* 1.00, CHCl<sub>3</sub>); IR (CHCl<sub>3</sub>, cast) 3461, 3335, 2981, 2954, 2873, 1740, 1453, 1376, 1209, 1056, 982, 755, cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) δ 3.70 (s, 6H, H1, H8), 3.41 (q, *J* = 7.02 Hz, 2H, H3, H5) 1.31 (d, *J* = 7.02 Hz, 6H, H4, H6); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) δ 175.2 (C2, C7), 54.4 (C3), 51.8 (C5), 18.7 (C4, C6); HRMS (ESI) Calcd for C<sub>8</sub>H<sub>16</sub>NO<sub>4</sub> [M + H]<sup>+</sup> 190.1074, found 190.1081.

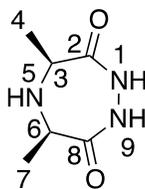
**(S)-2-(((R)-1-hydrazinyl-1-oxopropan-2-yl)amino)propanehydrazide (6b)**



**6b**

This new compound was synthesized following the same method as described for **6a**.<sup>4</sup> Recrystallization from Et<sub>2</sub>O afforded the product as a white powder (2.8 g, 93%).  $[\alpha]_D^{25} = 0.03$  (*c* 1.00, MeOH); IR (MeOH, cast) 3301, 3208, 3041, 2991, 2979, 1653, 1534, 1322, 971, 689, cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 500 MHz)  $\delta$  8.93 (s, 2H, H2, H10), 4.21 (s, 4H, H1, H11), 3.32 (s, 1H, H6), 3.01 (q, *J* = 7.02 Hz, 2H, H4, H9), 1.31 (d, *J* = 7.02 Hz, 6H, H5, H8); <sup>13</sup>C NMR (DMSO-d<sub>6</sub>, 125 MHz)  $\delta$  174.2 (C3, C9), 54.9 (C4, C7), 19.7 (C5, C8); HRMS (ESI) Calcd for C<sub>6</sub>H<sub>16</sub>N<sub>5</sub>O<sub>2</sub> [M + H]<sup>+</sup> 190.1299, found 190.1300.

**(4S,6R)-4,6-dimethyl-1,2,5-triazepane-3,7-dione (1b)**

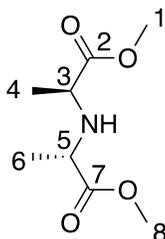


**1b**

This new compound was synthesized following the same method as described for the synthesis of **1a**.<sup>5</sup> After purification by column chromatography (SiO<sub>2</sub>, 10% MeOH in EtOAc), the product

was obtained as a yellow solid (0.87 g, 88%).  $R_f = 0.48$  in 10% MeOH in EtOAc.  $[\alpha]_D^{25} = 0.01$  ( $c$  0.89, MeOH); IR (MeOH, cast) 3349, 2998, 2954, 2863, 1721, 1424, 1121, 1054, 746,  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ , 500 MHz)  $\delta$  3.14 (q,  $J = 7.02$  Hz, 2H, H3, H6) 1.21 (d,  $J = 7.02$  Hz, 6H, H4, H7);  $^{13}\text{C}$  NMR ( $\text{D}_2\text{O}$ , 125 MHz)  $\delta$  176.4 (C2, C8), 55.4 (C3, C6), 18.8 (C4, C7); HRMS (ESI) Calcd for  $\text{C}_6\text{H}_{12}\text{N}_3\text{O}_2$   $[\text{M} + \text{H}]^+$  158.0924, found 158.0921.

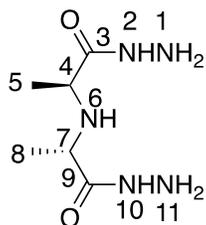
### Dimethyl 2,2'-azanediyl(2*S*,2'*S*)-dipropionate (**2c**)



**2c**

This known compound<sup>3</sup> was prepared using the same method as described for the synthesis of **2a**. After purification by column chromatography ( $\text{SiO}_2$ , 40% EtOAc in hexanes), the product was obtained as a yellow oil (6.3 g, 94%).  $R_f = 0.34$  in 30% EtOAc in hexanes.  $[\alpha]_D^{25} = -47.87$  ( $c$  0.86,  $\text{CHCl}_3$ ); IR ( $\text{CHCl}_3$ , cast) 3453, 3321, 2986, 2962, 2886, 1745, 1452, 1369, 1202, 1098, 986, 746,  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 700 MHz)  $\delta$  3.72 (s, 6H, H1, H8), 3.42 (q,  $J = 7.02$  Hz, 2H, H3, H5) 1.32 (d,  $J = 7.02$  Hz, 6H, H4, H6);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  175.5 (C2, C7), 55.0 (C3), 51.9 (C5), 19.2 (C4, C6); HRMS (ESI) Calcd for  $\text{C}_8\text{H}_{16}\text{NO}_4$   $[\text{M} + \text{H}]^+$  190.1074, found 190.1082.

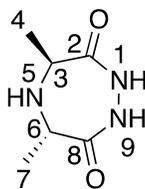
**(2*S*,2'*S*)-2,2'-Azanediyl-di(propanehydrazide) (6c)**



**6c**

This new compound was synthesized following the same method as described for the synthesis of **6a**.<sup>4</sup> Recrystallization from Et<sub>2</sub>O afforded the product as a white powder (2.8 g, 94%). [ $\alpha$ ]<sub>D</sub><sup>25</sup> = -36.98 (*c* 0.94, MeOH); IR (MeOH, cast) 3312, 3207, 3032, 2988, 2974, 1662, 1553, 1352, 963, 679, cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 500 MHz)  $\delta$  9.11 (s, 2H, H2, H10), 3.32 (s, 4H, H1, H11), 3.13 (q, *J* = 7.04 Hz, 2H, H4, H7) 1.27 (d, *J* = 7.04 Hz, 6H, H5, H8); <sup>13</sup>C NMR (DMSO-d<sub>6</sub>, 125 MHz)  $\delta$  173.1 (C3, C9), 54.5 (C4, C7), 19.3 (C5, C8); HRMS (EI) Calcd for C<sub>6</sub>H<sub>16</sub>N<sub>5</sub>O<sub>2</sub> [M + H]<sup>+</sup> 190.1299, found 190.1301.

**(4*S*,6*S*)-4,6-dimethyl-1,2,5-triazepane-3,7-dione (1c)**

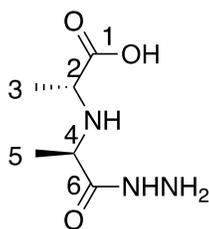


**1c**

This new compound was synthesized following the method as described for the synthesis of **1a**.<sup>5</sup> After purification by column chromatography (SiO<sub>2</sub>, 10% MeOH in EtOAc), the product was

obtained as a yellow solid (0.80 g, 86%).  $R_f = 0.42$  in 10% MeOH in EtOAc.  $[\alpha]_D^{25} = -43.21$  ( $c$  0.92, MeOH); IR (MeOH, cast) 3364, 2988, 2943, 2844, 1716, 1427, 1118, 1054, 753,  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ , 500 MHz)  $\delta$  4.06 (q,  $J = 7.04$  Hz, 2H, H3, H6) 1.52 (d,  $J = 7.02$  Hz, 6H, H4, H7);  $^{13}\text{C}$  NMR ( $\text{D}_2\text{O}$ , 125 MHz)  $\delta$  174.9 (C2, C8), 51.2 (C3, C6), 16.2 (C4, C7); HRMS (ESI) Calcd for  $\text{C}_6\text{H}_{12}\text{N}_3\text{O}_2$   $[\text{M} + \text{H}]^+$  158.0924, found 158.0927.

**((R)-1-hydrazineyl-1-oxopropane-2-yl)-D-alanine (3a)**

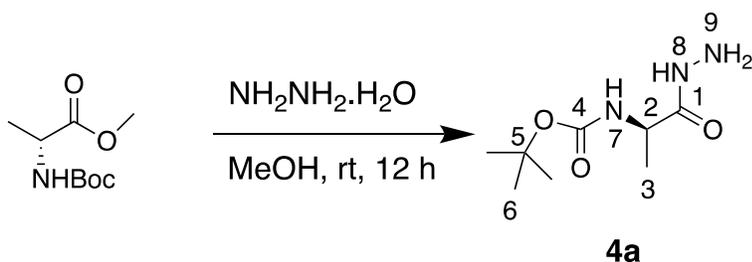


**3a**

This new compound was synthesized following literature procedure.<sup>7</sup> A solution of LiOH (0.24 g, 0.010 mol) in MeOH (10 mL) was added to **2a** (2.0 g, 0.010 mmol) and the mixture was stirred for 1 h at 0 °C. The solvent was then removed, and Et<sub>2</sub>O (20 mL) and H<sub>2</sub>O (30 mL) were added. The aqueous layer was acidified with concentrated HCl to pH 3 and extracted with Et<sub>2</sub>O (3 × 20 mL). The combined organic layers were washed with brine and dried over MgSO<sub>4</sub>. The solvent was removed *in vacuo* to give the half acid as a light yellow oil (1.5 g) which was used in the next step without further purification. The half acid (1.2 g, 0.0068 mol) was dissolved in MeOH (10 mL) and hydrazine monohydrate (0.33 mL, 0.0068 mol) was added and the reaction was stirred at room temperature for 6 h. After this time, a white precipitated formed which was filtered and washed with Et<sub>2</sub>O (5 × 20 mL). This solid was allowed to dry and was recrystallized

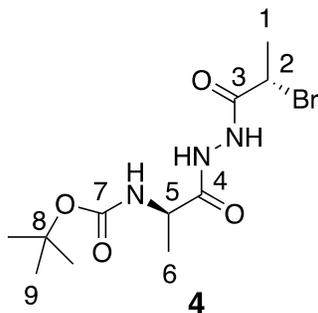
from Et<sub>2</sub>O to give the product as white powder (0.92 g, 76%).  $[\alpha]_D^{25} = 41.10$  (*c* 1.00, MeOH); IR (MeOH, cast) 3320, 2977, 1714, 1552, 1101 cm<sup>-1</sup>; <sup>1</sup>H NMR (D<sub>2</sub>O, 500 MHz)  $\delta$  2.95 (q, *J* = 7.03 Hz, 2H, H<sub>2</sub>, H<sub>4</sub>) 1.31 (d, *J* = 7.03 Hz, 3H, H<sub>3</sub>), 1.28 (d, *J* = 7.03 Hz, 3H, H<sub>5</sub>); <sup>13</sup>C NMR (D<sub>2</sub>O, 125 MHz)  $\delta$  175.3 (C<sub>1</sub>), 170.3 (C<sub>6</sub>), 51.1 (C<sub>2</sub>), 49.1 (C<sub>4</sub>), 18.86 (C<sub>3</sub>), 17.6 (C<sub>5</sub>); HRMS (ESI) Calcd for C<sub>6</sub>H<sub>12</sub>N<sub>3</sub>O<sub>3</sub> [M - H]<sup>-</sup> 174.0884 found 174.0887.

**Tert-butyl (*R*)-(1-hydrazineyl-1-oxopropan-2-yl)carbamate (4a)**



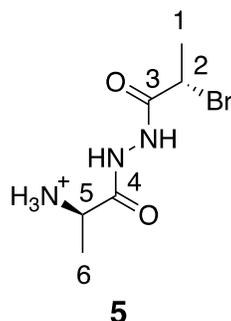
This known compound<sup>10</sup> was synthesized following the same procedure as described for the synthesis of **6a** to give the final product as a white powder (5.2 g, 95%).  $[\alpha]_D^{25} = 36.55$  (*c* 0.94, CHCl<sub>3</sub>); IR (CHCl<sub>3</sub>, cast) 3316, 2979, 1701, 1671, 1525, 1169, 1046, 757, cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 500 MHz)  $\delta$  8.93 (s, 1H, H<sub>7</sub>), 4.09 (s, 2H, H<sub>9</sub>), 3.91 (q, *J* = 7.14 Hz, 1H, H<sub>2</sub>), 1.35 (s, 9H, H<sub>6</sub>), 1.12 (d, *J* = 7.14 Hz, 3H, H<sub>3</sub>); <sup>13</sup>C NMR (DMSO-d<sub>6</sub>, 125 MHz)  $\delta$  172.1 (C<sub>1</sub>), 154.6 (C<sub>4</sub>), 77.8 (C<sub>5</sub>), 48.3 (C<sub>2</sub>), 28.1 (C<sub>6</sub>), 18.5 (C<sub>3</sub>); HRMS (ESI) Calcd for C<sub>8</sub>H<sub>17</sub>N<sub>3</sub>NaO<sub>3</sub> [M + Na]<sup>+</sup> 226.1162, found 226.1165.

**Tert-butyl ((*R*)-1-(2-(*S*)-2-bromopropanoyl)hydrazineyl)-1-oxopropan-2-yl)carbamate (**4**)**

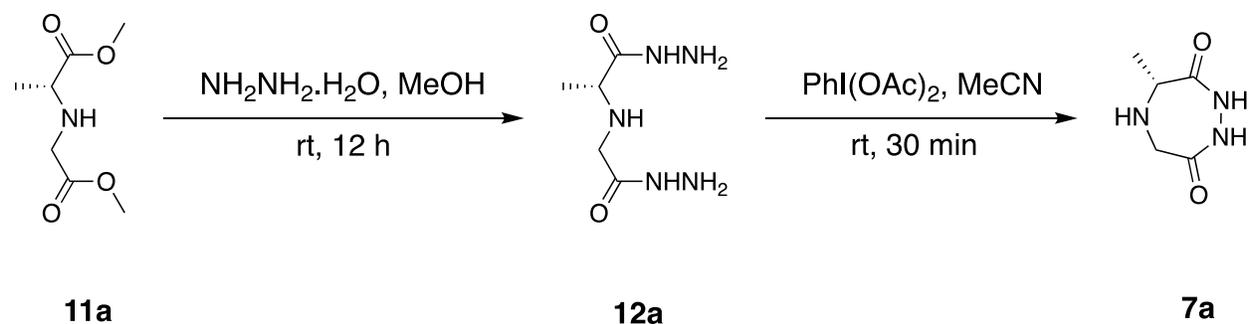


This new compound was synthesized following the same procedure as described for the synthesis of **2a** to give the final product as a white powder (4.2 g, 77%).  $R_f = 0.41$  in 30% EtOAc in Hexanes.  $[\alpha]_D^{25} = 25.25$  ( $c$  0.94, MeOH); IR (MeOH, cast) 3313, 2959, 1721, 1681, 1555, 1159, 1041, 755,  $\text{cm}^{-1}$ ;  $^1\text{H NMR}$  ( $\text{CD}_3\text{OD}$ , 500 MHz)  $\delta$  4.52 (q,  $J = 7.15$  Hz, 1H, H5), 4.14 (q,  $J = 6.80$  Hz, 1H, H2), 1.68 (d,  $J = 6.80$  Hz, 3H, H1), 1.21 (d,  $J = 7.14$  Hz, 3H, H6);  $^{13}\text{C NMR}$  ( $\text{CD}_3\text{OD}$ , 175 MHz)  $\delta$  171.1 (C3), 167.4 (C4), 154.9 (C7), 80.5 (C8), 49.3 (C5), 41.3 (C2), 28.3 (C9), 21.6 (C1), 17.5 (C6); HRMS (EI) Calcd for  $\text{C}_{11}\text{H}_{20}\text{BrN}_3\text{NaO}_4$   $[\text{M} + \text{Na}]^+$  360.0528, found 360.0529.

**(R)-1-(2-((S)-2-bromopropanoyl)hydrazineyl)-1-oxopropan-2-aminium (5)**

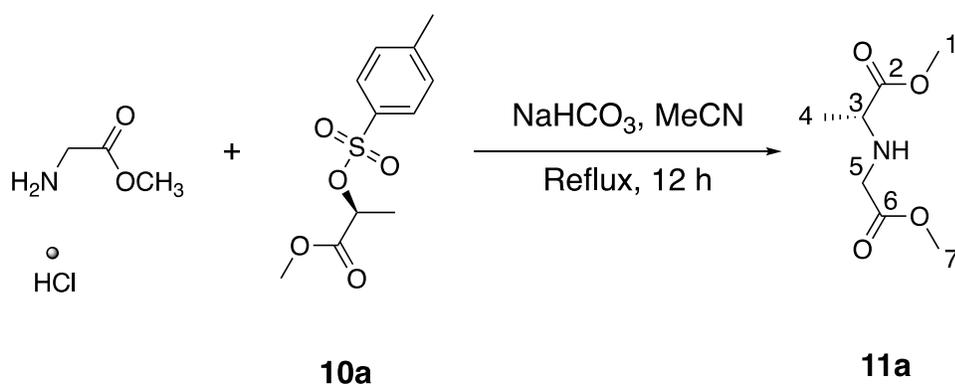


This new compound was synthesized following literature protocol.<sup>6</sup> To solution of compound **4** (4.2 g, 0.012 mol) in DCM (20 mL) at 0 °C was added TFA (20 mL) and the reaction mixture was stirred for 4 h. The solvent was removed and coevaporated with toluene (3× 50 mL) to give compound **5** as a white solid (2.7 g, 93%) which was used without further purification.  $[\alpha]_D^{25} = 29.45$  (*c* 0.94, MeOH); IR (MeOH, cast) 3316, 2979, 1701, 1671, 1525, 1169, 1046, 757,  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (DMSO, 500 MHz)  $\delta$  4.59 (q, *J* = 7.15 Hz, 1H, H5), 3.89 (q, *J* = 6.80 Hz, 1H, H2), 1.68 (d, *J* = 6.80 Hz, 3H, H1), 1.21 (d, *J* = 7.14 Hz, 3H, H6);  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 125 MHz)  $\delta$  171.1 (C3), 167.4 (C4), 154.9 (C7), 49.3 (C5), 41.3 (C2), 21.6 (C1), 17.5 (C6); HRMS (EI) Calcd for  $\text{C}_6\text{H}_{13}\text{BrN}_3\text{O}_2$   $[\text{M} + \text{H}]^+$  238.0850, found 238.0852.



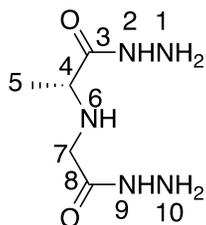
**Scheme S2:** Synthetic scheme for the preparation of (R)-4-methyl-1,2,5-triazepane-3,7-dione

**Methyl (2-methoxy-2-oxoethyl)-D-alaninate (11a)**



Compound **11a** was prepared using the same method as described for the synthesis of **2a**. After purification by column chromatography (SiO<sub>2</sub>, 40% EtOAc in hexanes), the product was obtained as a yellow oil (12.3 g, 88%). R<sub>f</sub> = 0.21 in 50% EtOAc in hexanes. [α]<sub>D</sub><sup>25</sup> = 27.31 (*c* 1.00, CHCl<sub>3</sub>); IR (CHCl<sub>3</sub>, cast) 3623, 3342, 2955, 1739, 1437, 1205, 1157, 978, 754, cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 3.70 (s, 6H, H1, H7), 3.47 – 3.33 (m, 3H, H3, H5) 1.32 (d, *J* = 7.02 Hz, 3H, H4); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 175.1 (C2), 172.2 (C6), 55.8 (C3), 51.9 (C1), 51.8 (C7), 48.8 (C5), 18.7 (C4); HRMS (ESI) Calcd for C<sub>7</sub>H<sub>14</sub>NO<sub>4</sub> [M + H]<sup>+</sup> 176.0917, found 176.0920.

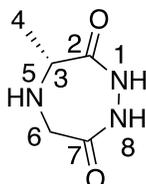
**(R)-2-((2-hydrazineyl-2-oxoethyl)amino)propanehydrazide (12a)**



**12a**

This new compound was synthesized following the method same method as described for the synthesis of **6a**.<sup>4</sup> Recrystallization from Et<sub>2</sub>O afforded the product as a white powder, (10.9 g, 89%).  $[\alpha]_D^{25} = 26.81$  (*c* 0.90, MeOH); IR (MeOH, cast) 3424, 3332, 3206, 3031, 2988, 2971, 1714, 1550, 1358, 958, 674, cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 500 MHz)  $\delta$  3.01 – 2.98 (m, 3H, H4, H7), 1.09 (d, *J* = 7.02 Hz, 3 H, H5); <sup>13</sup>C NMR (DMSO-d<sub>6</sub>, 125 MHz)  $\delta$  173.2 (C3), 170.1 (C8), 55.8 (C4), 49.2 (C7), 18.8 (C5); HRMS (ESI) Calcd for C<sub>5</sub>H<sub>14</sub>N<sub>5</sub>O<sub>2</sub> [M + H]<sup>+</sup> 176.1142, found 176.1141.

**(R)-4-methyl-1,2,5-triazepane-3,7-dione (7a)**

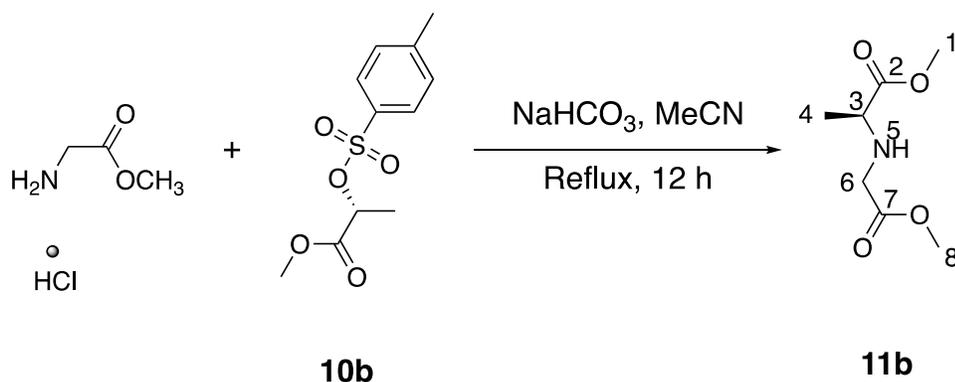


**7a**

This new compound was synthesized following the same method as described for the synthesis of **1a**.<sup>5</sup> After purification by column chromatography (SiO<sub>2</sub>, 10% MeOH in EtOAc), the product

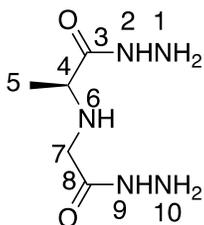
was obtained as a white solid (1.79 g, 67%).  $R_f = 0.4$  in 20% MeOH in EtOAc.  $[\alpha]_D^{25} = 25.5$  ( $c$  1.00, MeOH); IR (MeOH, cast) 3354, 2978, 2953, 2832, 1721, 1422, 1128, 1051, 763,  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ , 500 MHz)  $\delta$  3.28 – 3.24 (m, 3H, H3, H6) 1.24 (d,  $J = 7.02$  Hz, 3H, H4);  $^{13}\text{C}$  NMR ( $\text{D}_2\text{O}$ , 125 MHz)  $\delta$  173.2 (C2), 170.1 (C7), 55.8 (C3), 49.2 (C6), 18.8 (C4); HRMS (ESI) Calcd for  $\text{C}_5\text{H}_{10}\text{N}_3\text{O}_2$   $[\text{M} + \text{H}]^+$  144.0697, found 144.0694.

### Methyl (2-methoxy-2-oxoethyl)-*L*-alaninate (**11b**)



Compound **11b** was prepared using the same method as described for the synthesis of **2a**. After purification by column chromatography ( $\text{SiO}_2$ , 40% EtOAc in hexanes), the product was obtained as a light-yellow oil (12.8 g, 90%).  $R_f = 0.28$  in 50% EtOAc in hexanes.  $[\alpha]_D^{25} = 27.31$  ( $c$  1.00,  $\text{CHCl}_3$ ); IR ( $\text{CHCl}_3$ , cast) 3623, 3342, 2955, 1739, 1437, 1205, 1157, 978, 754,  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  3.63 (s, 6H, H1, H7), 3.38 – 3.28 (m, 3H, H3, H6) 1.25 (d,  $J = 7.03$ , 3H, H4);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  175.0 (C2), 172.1 (C7), 55.8 (C3), 51.8 (C1), 51.7 (C8), 48.8 (C7), 18.6 (C4); HRMS (ESI) Calcd for  $\text{C}_7\text{H}_{14}\text{NO}_4$   $[\text{M} + \text{H}]^+$  176.0917, found 176.0921.

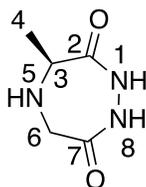
**(S)-2-((2-hydrazineyl-2-oxoethyl)amino)propanehydrazide (12b)**



**12b**

This new compound was synthesized following the same method as described for the synthesis of **6a**.<sup>4</sup> Recrystallization from Et<sub>2</sub>O afforded the product as a white powder (8.9 g, 89%).  $[\alpha]_D^{25} = 26.81$  (*c* 0.90, MeOH); IR (MeOH, cast) 3434, 3312, 3207, 2998, 2974, 1721, 1554, 1348, 953, 676, cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 500 MHz)  $\delta$  3.04 – 2.98 (m, 3H, H4, H7) 1.07 (d, *J* = 7.02, 3H, H5); <sup>13</sup>C NMR (DMSO-d<sub>6</sub>, 124 MHz)  $\delta$  174.2 (C3), 171.1 (C8), 55.7 (C4), 49.5 (C7), 18.6 (C5); HRMS (ESI) Calcd for C<sub>5</sub>H<sub>14</sub>N<sub>5</sub>O<sub>2</sub> [M + H]<sup>+</sup> 176.1142, found 176.1140.

**(S)-4-methyl-1,2,5-triazepane-3,7-dione (7b)**

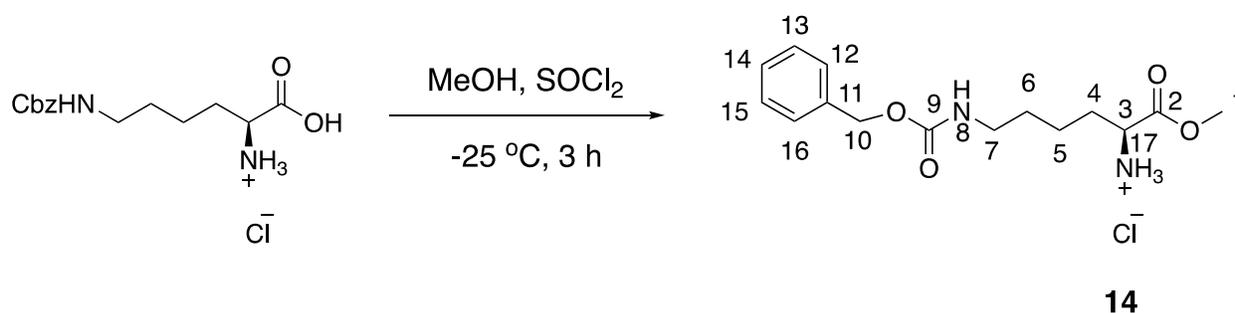


**7b**

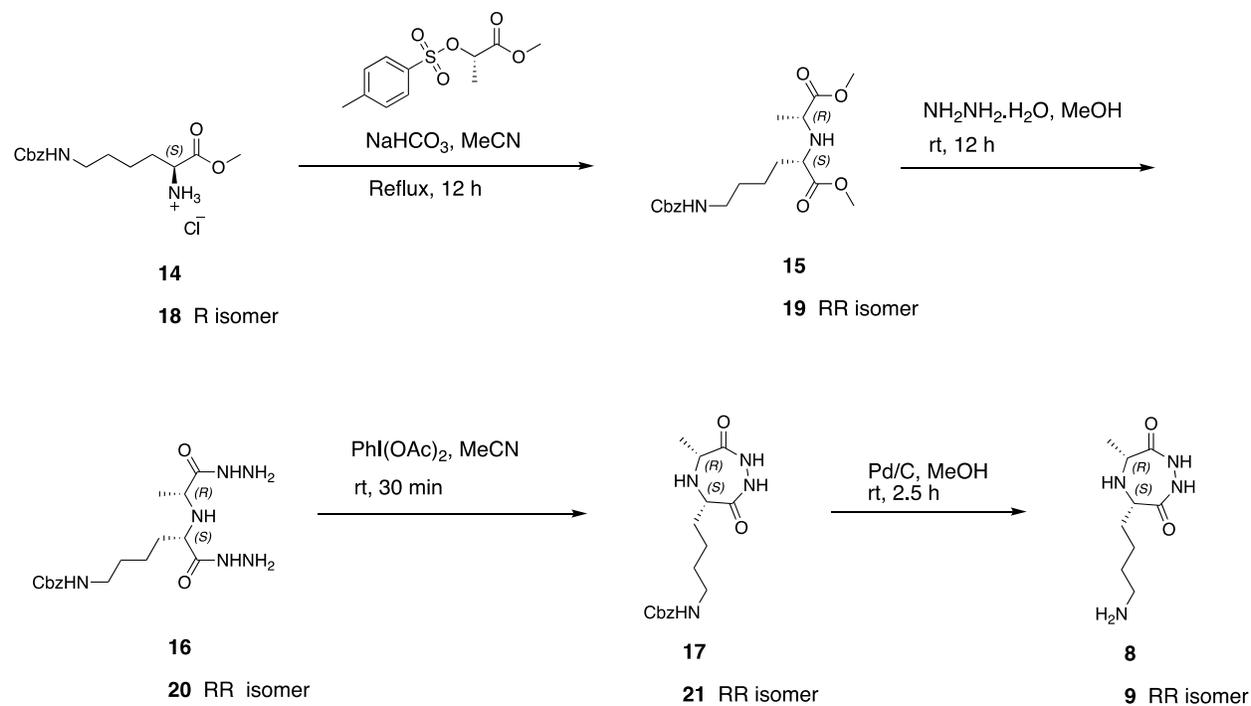
This new compound was synthesized following the method as described for **1a**.<sup>5</sup> After purification by column chromatography (SiO<sub>2</sub>, 10% MeOH in EtOAc), the product was obtained as a white solid (1.3 g, 72%). *R<sub>f</sub>* = 0.51 in 20% MeOH in EtOAc.  $[\alpha]_D^{25} = 25.5$  (*c* 1.00, MeOH);

IR (MeOH, cast) 3352, 2988, 2962, 2835, 1719, 1411, 1127, 1054, 765,  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ , 500 MHz)  $\delta$  3.26 – 3.22 (m, 3H, H3, H6) 1.23 (d,  $J = 7.02$ , 3H, H4);  $^{13}\text{C}$  NMR ( $\text{D}_2\text{O}$ , 125 MHz)  $\delta$  173.4 (C2), 170.5 (C7), 55.9 (C3), 49.4 (C6), 18.7 (C4); HRMS (EI) Calcd for  $\text{C}_5\text{H}_{10}\text{N}_3\text{O}_2$  [ $\text{M} + \text{H}$ ] $^+$  144.0697, found 144.0695.

**N<sup>ε</sup>-benzyloxycarbonyl-L-lysine methyl ester hydrochloride (14)**

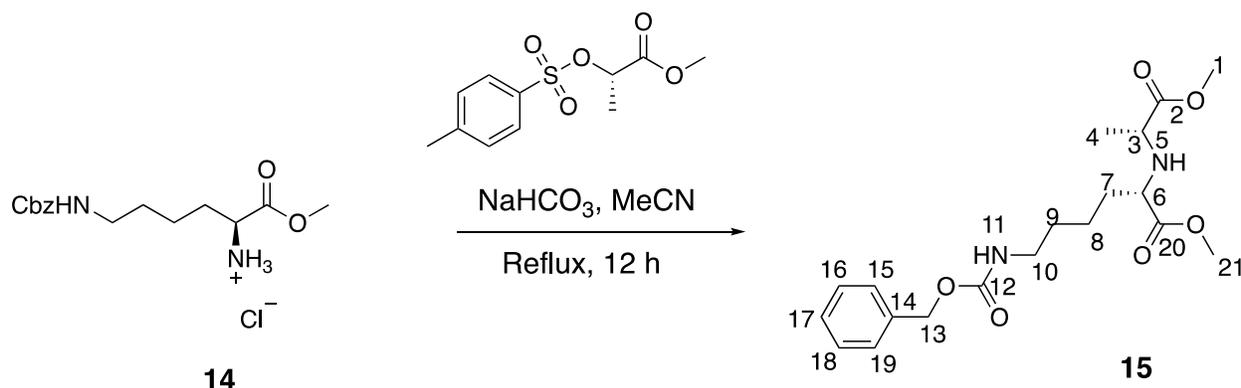


This known compound was synthesized following literature protocol.<sup>8</sup> The compound was lyophilized to afford **14** as a white solid (quant.).  $[\alpha]_{\text{D}}^{25} = 15.62$  ( $c$  1.00, MeOH); IR (MeOH, cast) 3309, 3032, 2951, 1749, 1697, 1527, 1253, 1135, 776  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR, (DMSO- $d_6$ , 500 MHz)  $\delta$  8.65 (s, 1H, H8), 7.31 – 7.27 (m, 5H, H12, H13, H14, H15, H16), 5.01 (s, 2H, H10), 4.02 (t,  $J = 6.25$ , 1H, H3), 3.8 (s, 3H, H1), 3.33 (s, 3H, H17), 2.98 – 2.96 (m, 2H, H7), 1.81 – 1.78 (m, 2H, H4), 1.41 – 1.38 (m, 4H, H5, H6), 1.28 – 1.26 (m, 1H, H3);  $^{13}\text{C}$  NMR (DMSO- $d_6$ , 125 MHz)  $\delta$  169.9 (C2), 156.1 (C9), 137.2 (C11), 128.3 (C13, C15), 127.7 (C12, C16), 127.6 (C14), 65.1 (C10), 52.6 (C1), 51.7 (C7), 29.6 (C4), 28.7 (C7), 21.4 (C5); HRMS (ESI) Calcd for  $\text{C}_{15}\text{H}_{23}\text{N}_2\text{O}_4$  [ $\text{M} + \text{H}$ ] $^+$  295.1580 found 295.1581.



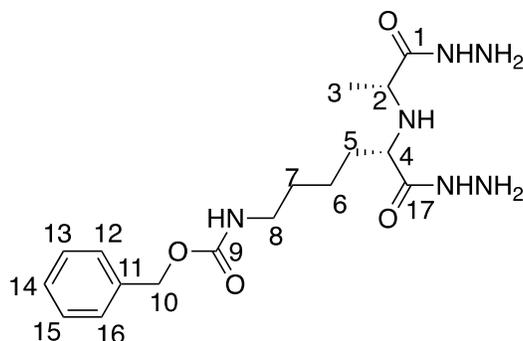
**Scheme S3:** Synthetic scheme for the preparation of (4*S*,6*R*)-4-(4-aminobutyl)-6-methyl-1,2,5-triazepane-3,7-dione.

**Methyl *N*<sup>6</sup>-((benzyloxy)carbonyl)-*N*<sup>2</sup>-((*R*)-1-methoxy-1-oxopropan-2-yl)-L-lysinate (15)**



This new compound was synthesized following the same method as described for synthesis of **2a**. After purification by column chromatography (SiO<sub>2</sub>, 40% EtOAc in hexanes), the product was obtained as a light-yellow oil (10.3 g, 90%).  $R_f = 0.60$  in 50% EtOAc in hexanes.  $[\alpha]_D^{25} = 3.91$  ( $c$  1.50, CHCl<sub>3</sub>); IR (CHCl<sub>3</sub>, cast) 3336, 2950, 1731, 1527, 1239, 1057, 697 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 700 MHz)  $\delta$  7.33 – 7.24 (m, 5H, H15, H16, H17, H18, H19), 5.02 (s, 2H, H13), 3.64 (s, 6H, H1, H21), 3.23 (q,  $J = 7.02$  Hz, 1H, H3), 3.19 (t,  $J = 7.63$  Hz, 1H, H3), 3.17 – 3.11 (m, 2H, H9), 1.67 (td,  $J = 7.79$  Hz, 7.30 Hz, 2H, H7), 1.51 – 1.45 (m, 2H, H8), 1.40 – 1.34 (m, 2H, H10), 1.27 (d,  $J = 7.02$  Hz, 3H, H4); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz)  $\delta$  175.1 (C2), 175.0 (C20), 156.4 (C12), 136.7 (C14), 129.6 (C16), 128.5 (C18), 128.0 (C17), 127.2 (C15, C19), 66.5 (C13), 59.2 (C6), 55.0 (C5), 51.9 (C1), 51.8 (C21), 40.8 (C10), 33.0 (C7), 29.6 (C9), 22.7 (C8), 18.6 (C4); HRMS (ESI) Calcd for C<sub>19</sub>H<sub>29</sub>N<sub>2</sub>O<sub>6</sub> [M + H]<sup>+</sup> 381.2020, found 381.2016.

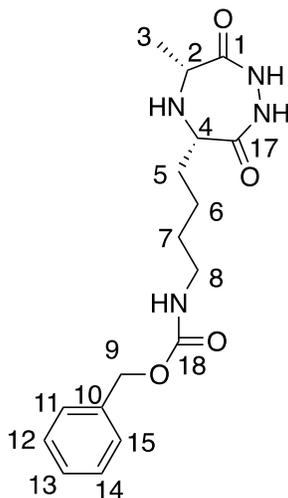
**Benzyl((S)-6-hydrazineyl-5-(((R)-1-hydrazineyl-1-oxopropan-2-yl)amino)-6-oxohexyl)carbamate (16)**



**16**

This new compound was synthesized following the same method as described for the synthesis of **6a**.<sup>4</sup> Recrystallization from Et<sub>2</sub>O afforded the product as a white powder (4.47 g, 89%).  $[\alpha]_D^{25} = 3.95$  (*c* 1.40, MeOH); IR (MeOH, cast) 3445, 3339, 2961, 1741, 1537, 1236, 1057, 695 cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 700 MHz)  $\delta$  7.36 – 7.29 (m, 5H, H12, H13, H14, H15, H16), 4.99 (s, 2H, H10), 3.01 – 2.98 (m, 2H, H2, H4), 1.67 (td, *J* = 7.79 Hz, 7.30 Hz, 2H, H5), 1.51 – 1.45 (m, 6H, H6, H7, H8), 1.27 (d, *J* = 7.02 Hz, 3H, H3); <sup>13</sup>C NMR (DMSO-d<sub>6</sub>, 125 MHz)  $\delta$  173.2 (C10), 172.8 (C12), 156.1 (C9), 137.2 (C11), 128.3 (C13, C15), 127.7 (C12, C16), 127.6 (C14), 65.0 (C10), 58.6 (C4), 54.2 (C2), 40.1 (C8), 32.8 (C5), 29.3 (C7), 22.5 (C6), 18.5 (C3); HRMS (ESI) Calcd for C<sub>17</sub>H<sub>29</sub>N<sub>6</sub>O<sub>4</sub> [M + H]<sup>+</sup> 381.2245, found 381.2243.

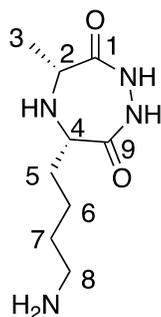
**Benzyl (4-((4*S*,6*R*)-6-Methyl-3,7-dioxo-1,2,5-triazepan-4-yl)butyl)carbamate (17)**



**17**

This new compound was prepared using the same method described for the synthesis of **1a**.<sup>5</sup> After purification by column chromatography (SiO<sub>2</sub>, 10% MeOH in EtOAc), the product was obtained as a yellow solid (0.8 g, 73%). R<sub>f</sub> = 0.6 in 20% MeOH in EtOAc. [α]<sub>D</sub><sup>25</sup> = 3.92 (c 1.30, MeOH); IR (MeOH, cast) 3339, 2971, 1724, 1545, 1238, 1061, 698 cm<sup>-1</sup>; <sup>1</sup>H NMR (D<sub>2</sub>O, 700 MHz) δ 7.36 – 7.29 (m, 5H, H10, H11, H12, H13, H14, H15), 4.99 (s, 2H, H9), 3.23 (q, *J* = 7.02 Hz, 1H, H2), 3.03 – 3.29 (m, 3H, H4, H8), 1.47 (td, *J* = 7.79 Hz, 7.30 Hz, 2H, H5), 1.38 – 1.36 (m, 2H, H6), 1.24 – 1.22 (m, 2H, H7), 1.10 (d, *J* = 7.02 Hz, 3H, H3); <sup>13</sup>C NMR (D<sub>2</sub>O, 125 MHz) δ 173.3 (C1), 172.7 (C17), 156.4 (C18), 137.6 (C10), 128.4 (C12, C14), 127.8 (C13), 127.6 (C11, C15), 65.2 (C9), 58.8 (C4), 54.3 (C2), 40.4 (C8), 32.6 (C5), 29.4 (C7), 22.7 (C6), 18.6 (C3); HRMS (ESI) Calcd for C<sub>17</sub>H<sub>24</sub>N<sub>4</sub>NaO<sub>4</sub> [M + Na]<sup>+</sup> 371.1690, found 371.1689.

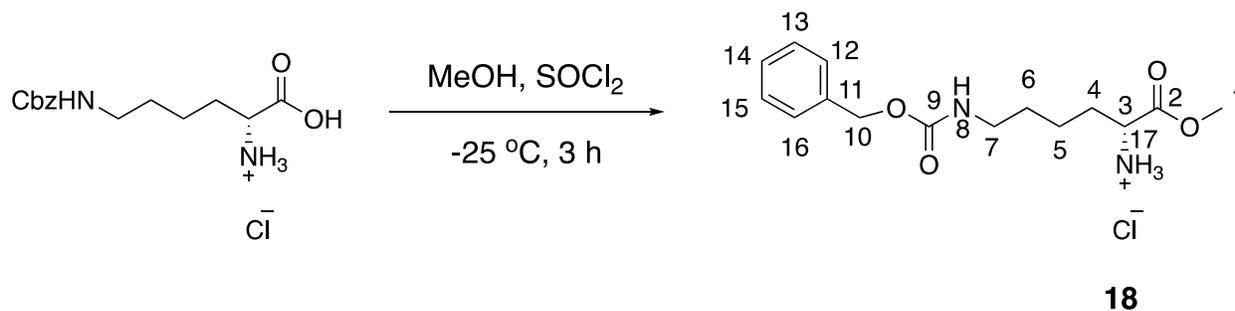
**(4*S*,6*R*)-4-(4-aminobutyl)-6-methyl-1,2,5-triazepane-3,7-dione (8)**



**8**

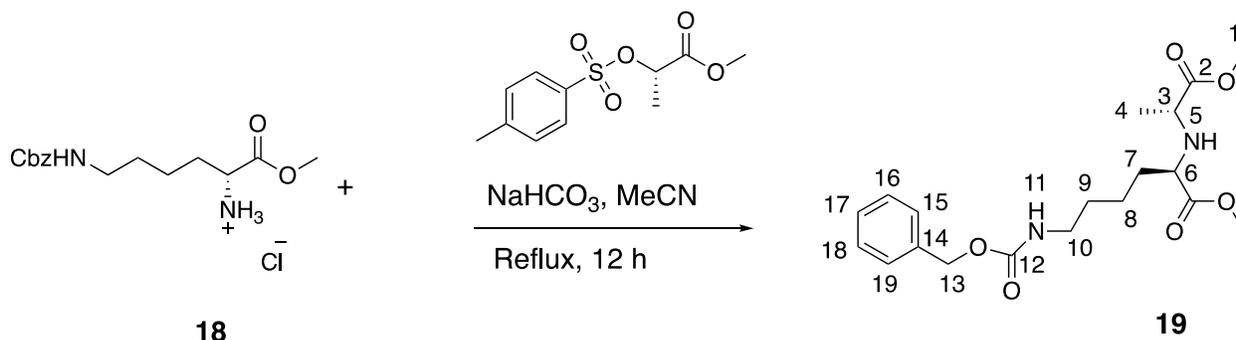
This new compound was synthesized following literature procedure.<sup>9</sup> A solution of **17** (0.5 g, 1.4 mmol) in dry MeOH (10 mL) was degassed by flushing with argon for 5 min and palladium on carbon 10% w/w (640 mg) was added. The reaction was stirred under hydrogen atmosphere at room temperature for 2.5 h. The reaction was filtered through a pad of Celite and washed with MeOH (10 mL) and the solvent was removed *in vacuo* and the crude was purified by column chromatography (SiO<sub>2</sub>, 10% MeOH in EtOAc), yielding the product as a yellow solid (0.36 g, 84%).  $R_f = 0.4$  in 10% MeOH in EtOAc.  $[\alpha]_D^{25} = 4.10$  ( $c$  1.60, MeOH); IR (MeOH, cast) 3339, 2971, 1724, 1545, 1238, 1061, 698  $\text{cm}^{-1}$ ; <sup>1</sup>H NMR (D<sub>2</sub>O, 700 MHz)  $\delta$  3.96 (t,  $J = 7.04$  Hz, 1H, H4), 3.66 (q,  $J = 7.02$  Hz, 1H), 2.64 (t,  $J = 7.30$  Hz, 2H, H8), 1.47 (td,  $J = 7.79$  Hz, 7.30 Hz, 2H, H5), 1.38 – 1.36 (m, 2H, H6), 1.24 – 1.22 (m, 2H, H7), 1.10 (d,  $J = 7.02$  Hz, 3H, H3); <sup>13</sup>C NMR (D<sub>2</sub>O, 125 MHz)  $\delta$  164.7 (C1), 164.7 (C9), 60.3 (C4), 55.7 (C2), 41.1 (C8), 29.3 (C5), 27.7 (C7), 22.1 (C6), 17.5 (C3); HRMS (ESI) Calcd for C<sub>9</sub>H<sub>19</sub>N<sub>4</sub>O<sub>2</sub> [M + H]<sup>+</sup> 215.1430 found 215.1434.

**N<sup>ε</sup>-benzyloxycarbonyl-D-lysine methyl ester hydrochloride (18)**



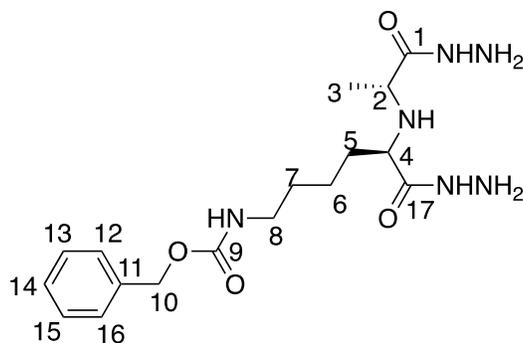
This known compound was synthesized using the same method as described for the synthesis of **14** to give the final product as white powder (quant.).  $[\alpha]_D^{25} = -14.32$  ( $c$  1.00, MeOH); IR (MeOH, cast) 3307, 3034, 2949, 1749, 1697, 1528, 1252, 1135, 736  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR, (DMSO- $d_6$ , 700 MHz)  $\delta$  8.65 (s, 1H, H8), 7.37 – 7.26 (m, 5H, H12, H13, H14, H15, H16), 4.99 (s, 2H, H10), 3.95 (t,  $J = 6.25$ , 1H, H3), 3.72 (s, 3H, H1), 3.35 (s, 3H, H17), 2.98 – 2.96 (m, 2H, H7), 1.81 – 1.78 (m, 2H, H4), 1.42 – 1.38 (m, 4H, H5, H6), 1.28 – 1.26 (m, 1H, H3);  $^{13}\text{C}$  NMR (DMSO- $d_6$ , 125 MHz)  $\delta$  169.9 (C2), 156.1 (C9), 137.2 (C11), 128.3 (C13, C15), 127.7 (C12, C16), 127.6 (C14), 65.1 (C10), 52.6 (C1), 51.7 (C7), 29.6 (C4), 28.7 (C7), 21.4 (C5); HRMS (ESI) Calcd for  $\text{C}_{15}\text{H}_{23}\text{N}_2\text{O}_4$   $[\text{M} + \text{H}]^+$  295.1580 found 295.1579.

**MethylN<sup>6</sup>-((benzyloxy)carbonyl)-N<sup>2</sup>-((*R*)-1-methoxy-1-oxopropan-2-yl)-*D*-lysinate (19)**



This new compound was synthesized following the same method as described for the synthesis of **2a** to give the final product as a light-yellow oil (10.6 g, 92%).  $R_f = 0.56$  in 50% EtOAc in hexanes.  $[\alpha]_D^{25} = 18.58$  ( $c$  1.50, CHCl<sub>3</sub>); IR (CHCl<sub>3</sub>, cast) 3346, 2950, 1730, 1528, 1244, 1202, 1053, 776 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 700 MHz)  $\delta$  7.21 – 7.16 (m, 5H, H15, H16, H17, H18, H19), 5.19 (s, 2H, H13), 3.59 (s, 6H, H1, H21), 3.25 (q,  $J = 7.02$  Hz, 1H, H3), 3.19 (t,  $J = 7.63$  Hz, 1H, H3), 3.17 – 3.11 (m, 2H, H9), 1.67 (td,  $J = 7.79$  Hz, 7.30 Hz, 2H, H7), 1.57 – 1.55 (m, 2H, H8), 1.30 – 1.27 (m, 2H, H10), 1.19 (d,  $J = 7.02$  Hz, 3H, H4); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz)  $\delta$  175.1 (C2), 175.1 (C20), 156.4 (C12), 136.7 (C14), 129.6 (C16), 128.5 (C18), 128.0 (C17), 127.2 (C15, C19), 66.5 (C13), 59.2 (C6), 55.0 (C5), 51.9 (C1), 51.8 (C21), 40.8 (C10), 32.9 (C7), 29.6 (C9), 22.7 (C8), 19.0 (C4); HRMS (EI) Calcd for C<sub>19</sub>H<sub>29</sub>N<sub>2</sub>O<sub>6</sub> [M + H]<sup>+</sup> 381.2020, found 381.2017.

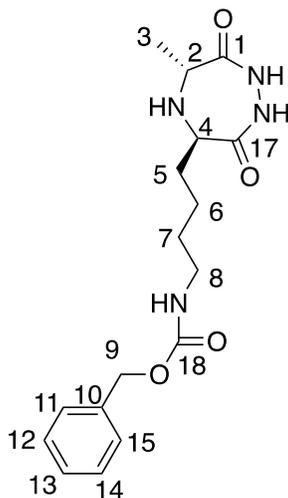
**Benzyl((*R*)-6-hydrazineyl-5-(((*R*)-1-hydrazineyl-1-oxopropan-2-yl)amino)-6-oxohexyl)carbamate (20)**



**20**

This new compound was synthesized following the method as described for the synthesis of **6a**<sup>4</sup> to give the final product as a white powder (4.51 g, 92%).  $[\alpha]_{\text{D}}^{25} = 18.32$  ( $c$  1.40, MeOH); IR (MeOH, cast) 3443, 3337, 2959, 1739, 1567, 1236, 1057, 695  $\text{cm}^{-1}$ ; <sup>1</sup>H NMR (DMSO- $d_6$ , 700 MHz)  $\delta$  7.38 – 7.29 (m, 5H, H12, H13, H14, H15, H16), 5.00 (s, 2H, H10), 3.02 – 2.98 (m, 2H, H2, H4), 1.67 (td,  $J = 7.79$  Hz, 7.30 Hz, 2H, H5), 1.51 – 1.45 (m, 6H, H6, H7, H8), 1.27 (d,  $J = 7.02$  Hz, 3H, H3); <sup>13</sup>C NMR (DMSO- $d_6$ , 125 MHz)  $\delta$  173.2 (C10), 172.8 (C12), 156.1 (C9), 137.2 (C11), 128.3 (C13, C15), 127.7 (C12, C16), 127.6 (C14), 65.0 (C10), 58.6 (C4), 54.2 (C2), 40.1 (C8), 32.8 (C5), 29.3 (C7), 22.5 (C6), 18.5 (C3); HRMS (ESI) Calcd for  $\text{C}_{17}\text{H}_{28}\text{N}_6\text{NaO}_4$  [ $\text{M} + \text{Na}$ ]<sup>+</sup> 403.2064, found 403.2059.

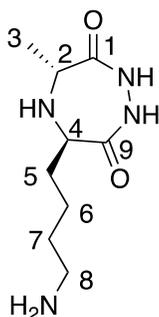
**Benzyl (4-((4*R*,6*R*)-6-methyl-3,7-dioxo-1,2,5-triazepan-4-yl)butyl)carbamate (21)**



**21**

This new compound was synthesized following the same method as described for the synthesis of **1a**<sup>5</sup> to give the final product as a white solid (0.82 g, 75%).  $R_f = 0.5$  in 20% MeOH in EtOAc.  $[\alpha]_D^{25} = 18.21$  ( $c$  1.30, MeOH); IR (MeOH, cast) 3339, 2971, 1724, 1545, 1238, 1061, 698  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ , 700 MHz)  $\delta$  7.36 – 7.29 (m, 5H, H10, H11, H12, H13, H14, H15), 4.99 (s, 2H, H9), 3.21 (q,  $J = 7.02$  Hz, 1H, H2), 3.04 – 3.01 (m, 3H, H4, H8), 1.44 (td,  $J = 7.79$  Hz, 7.30 Hz, 2H, H5), 1.38 – 1.36 (m, 2H, H6), 1.24 – 1.22 (m, 2H, H7), 1.10 (d,  $J = 7.02$  Hz, 3H, H3);  $^{13}\text{C}$  NMR ( $\text{D}_2\text{O}$ , 125 MHz)  $\delta$  173.3 (C1), 172.7 (C17), 156.4 (C18), 137.6 (C10), 128.4 (C12, C14), 127.8 (C13), 127.6 (C11, C15), 65.2 (C9), 58.8 (C4), 54.3 (C2), 40.4 (C8), 32.6 (C5), 29.4 (C7), 22.7 (C6), 18.6 (C3); HRMS (ESI) Calcd for  $\text{C}_{17}\text{H}_{24}\text{N}_4\text{NaO}_4$   $[\text{M} + \text{Na}]^+$  371.1690, found 371.1688.

**(4*R*,6*R*)-4-(4-aminobutyl)-6-methyl-1,2,5-triazepane-3,7-dione (9)**



**9**

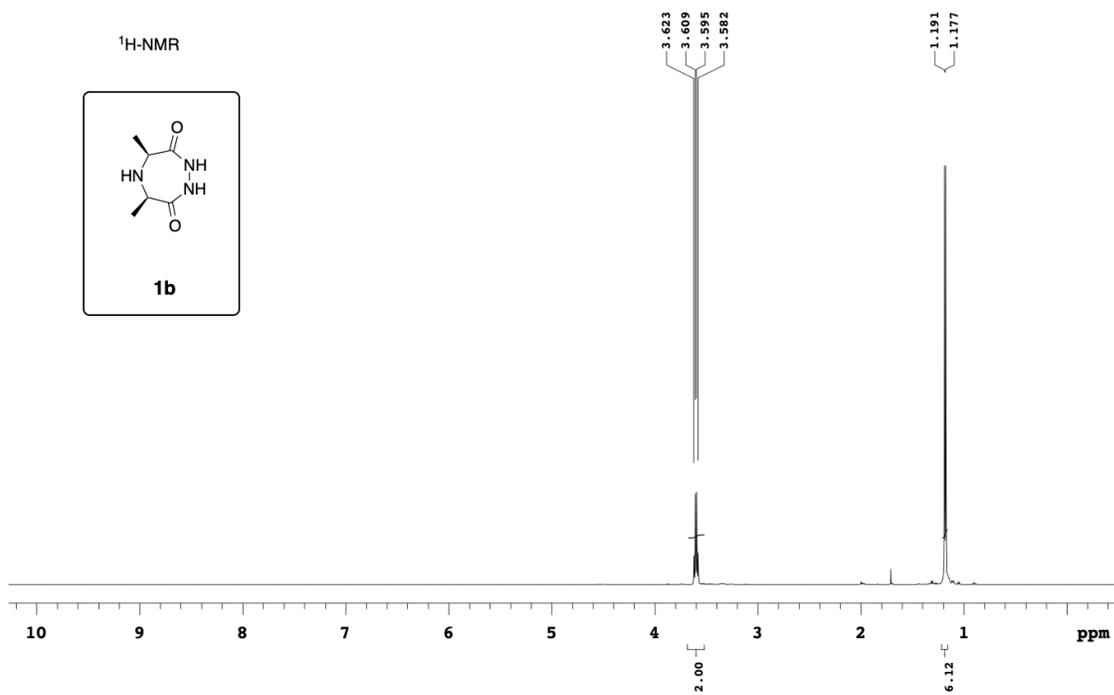
This new compound was synthesized following the same method as described for the synthesis of **8**<sup>9</sup> to give the final product as a white solid (0.32 g, 79%).  $R_f = 0.43$  in 10% MeOH in EtOAc.  $[\alpha]_D^{25} = 20.10$  ( $c$  1.60, MeOH); IR (MeOH, cast) 3339, 2971, 1724, 1545, 1238, 1061, 698  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (DMSO- $d_6$ , 700 MHz)  $\delta$  3.96 (t,  $J = 7.04$  Hz, 1H, H4), 3.66 (q,  $J = 7.02$  Hz, 1H), 2.64 (t,  $J = 7.30$  Hz, 2H, H8), 1.47 (td,  $J = 7.79$  Hz, 7.30 Hz, 2H, H5), 1.38 – 1.36 (m, 2H, H6), 1.24 – 1.22 (m, 2H, H7), 1.10 (d,  $J = 7.02$  Hz, 3H, H3);  $^{13}\text{C}$  NMR (DMSO- $d_6$ , 125 MHz)  $\delta$  164.7 (C1), 164.7 (C9), 60.3 (C4), 55.7 (C2), 41.1 (C8), 29.3 (C5), 27.7 (C7), 22.1 (C6), 17.5 (C3); HRMS (ESI) Calcd for  $\text{C}_9\text{H}_{18}\text{N}_4\text{O}_2$   $[\text{M} + \text{Na}]^+$  214.1430 found 214.1438.

## References

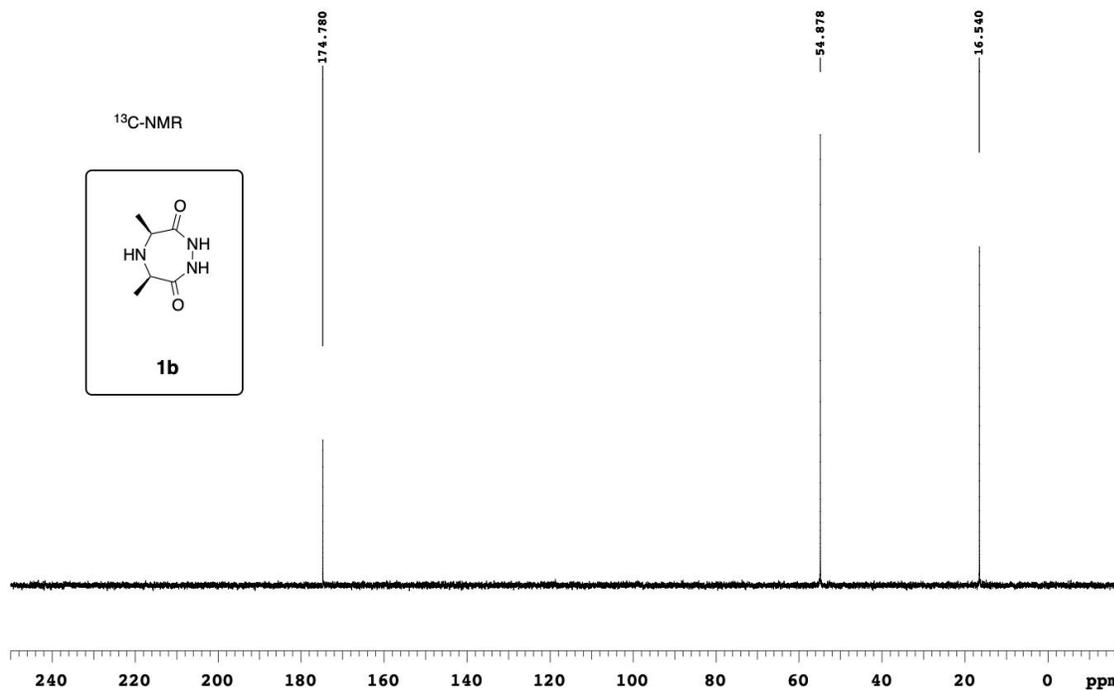
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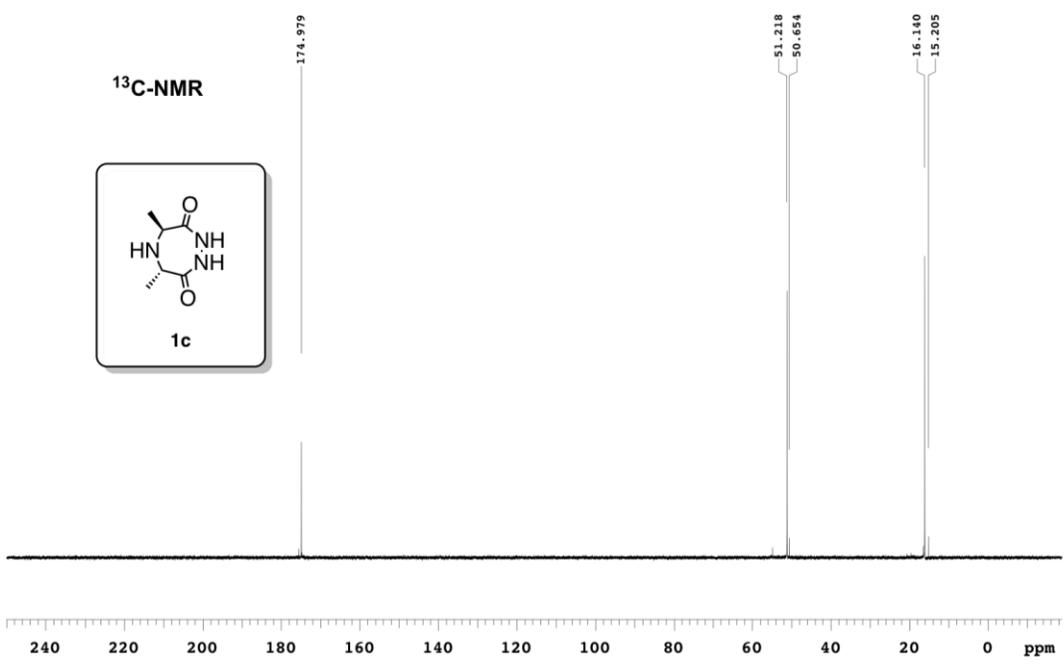
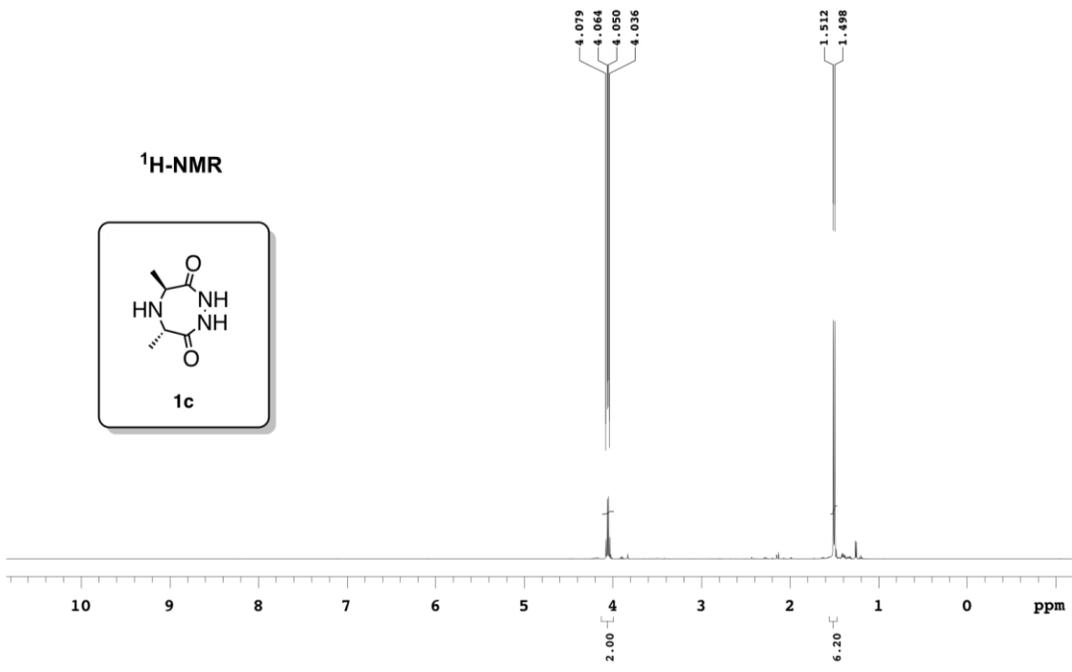


temp 27.7 C -> actual temp = 27.0 C, cold dual probe



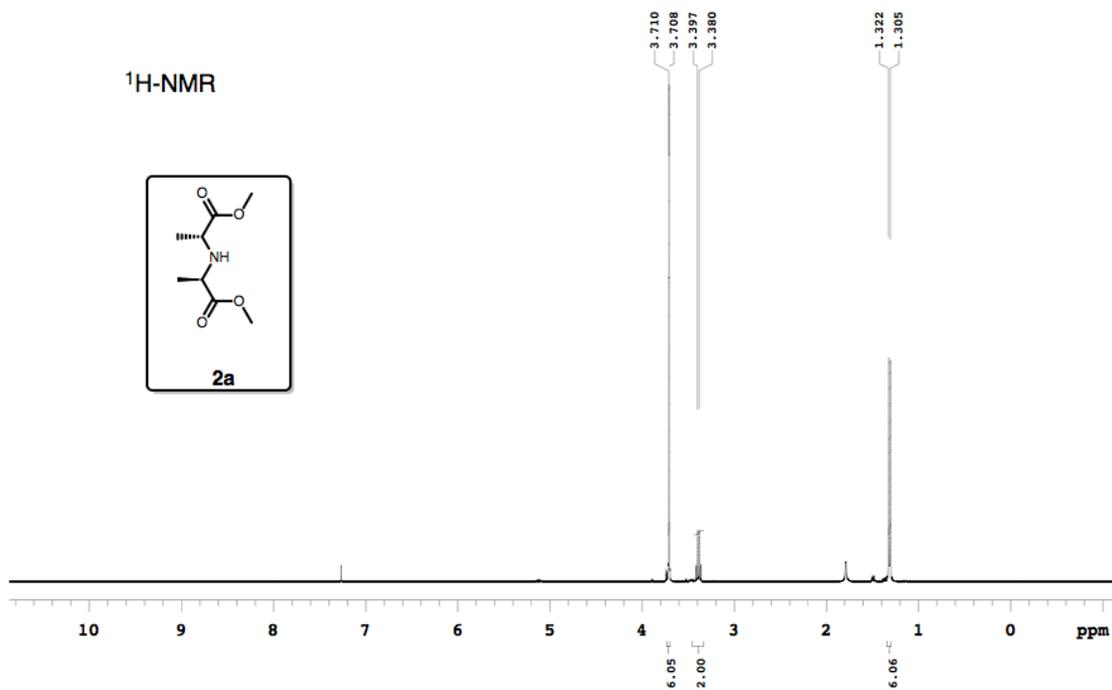
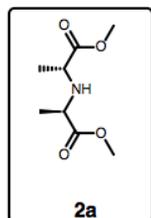
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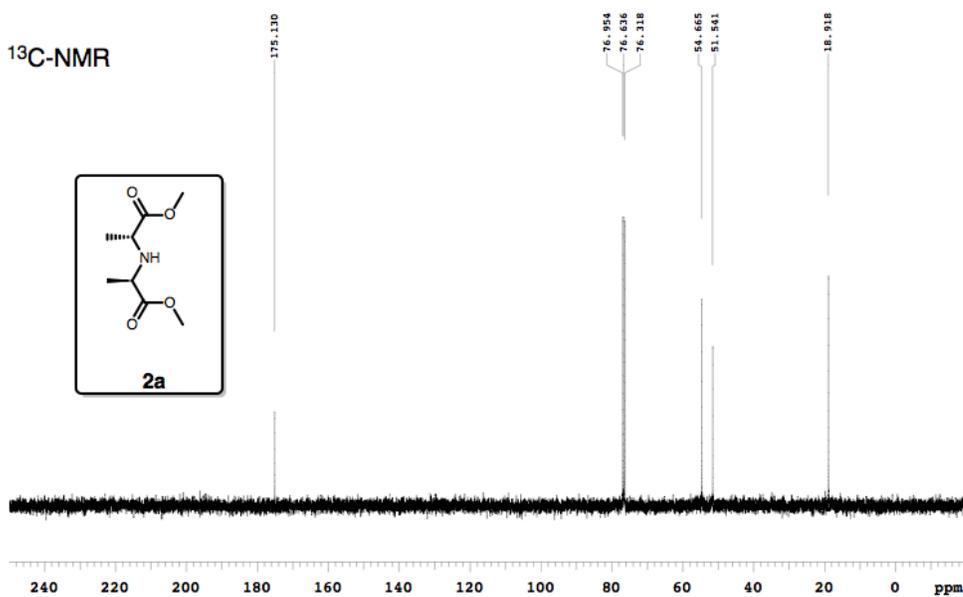
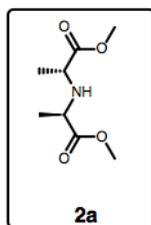
Isaac, IA-4-23-2  
399.794 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm)  
temp 26.5 C -> actual temp = 27.0 C, autoxzb probe

### <sup>1</sup>H-NMR

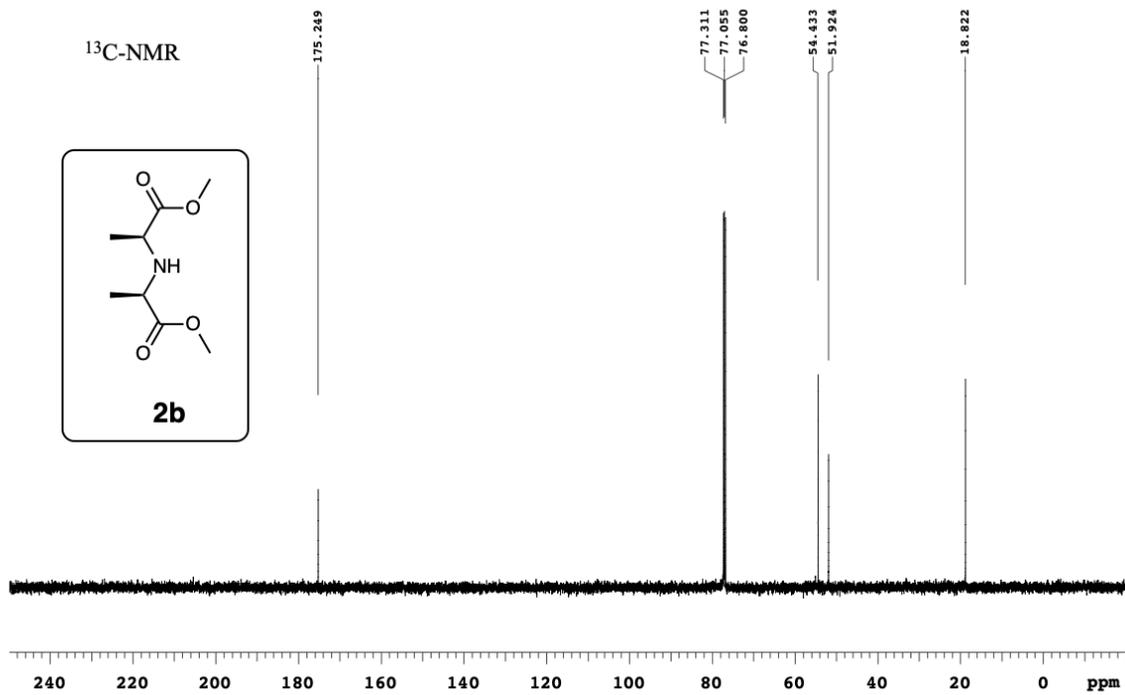


Isaac, IA-4-23-2  
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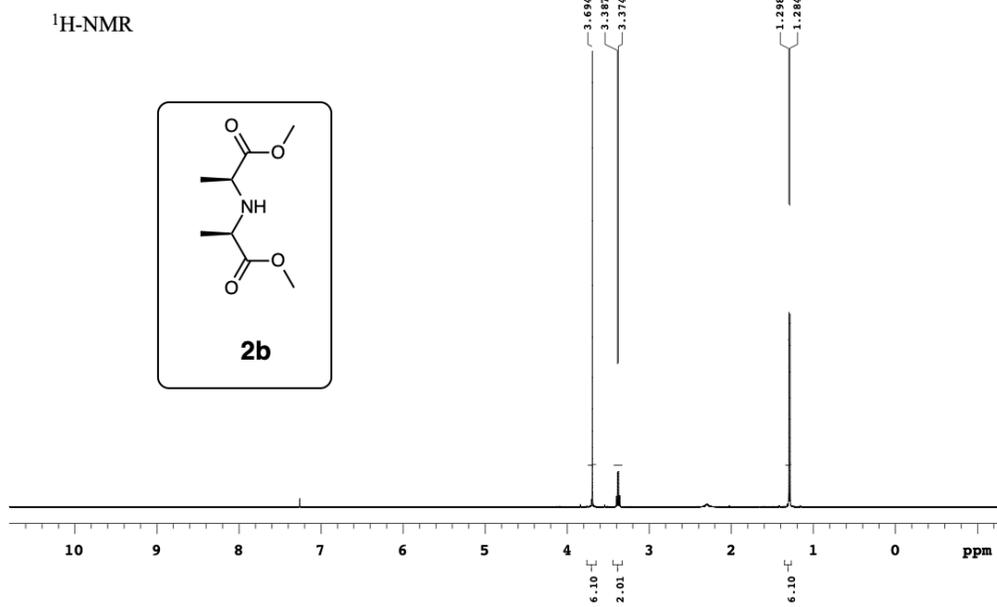
### <sup>13</sup>C-NMR



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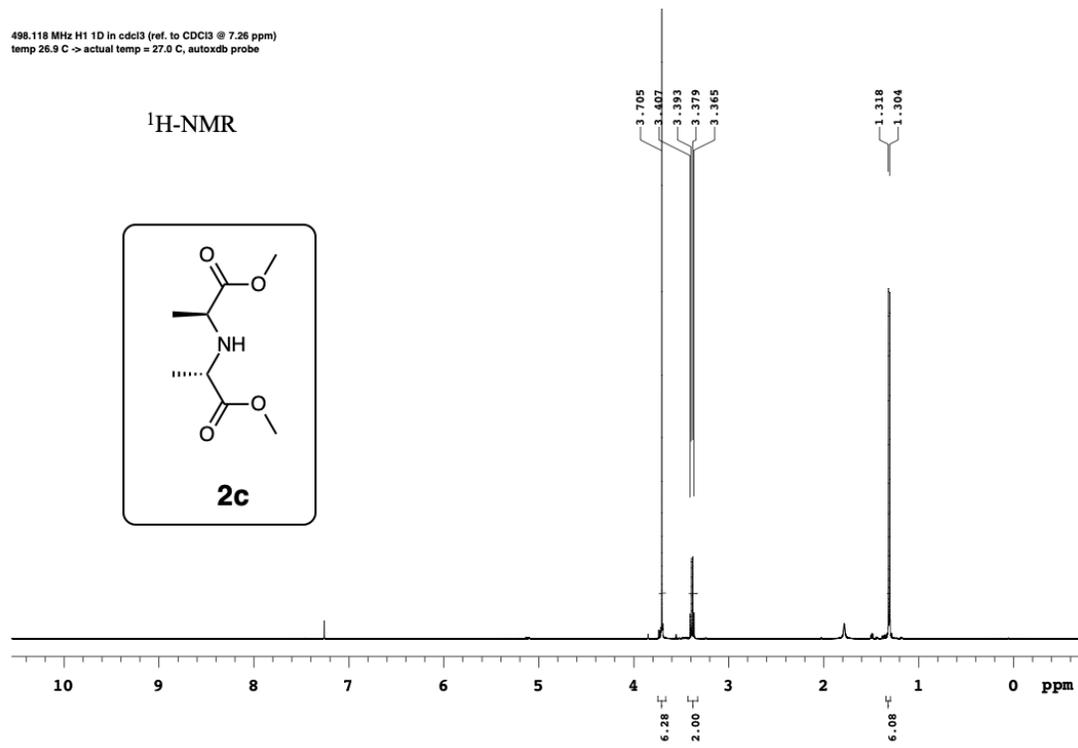
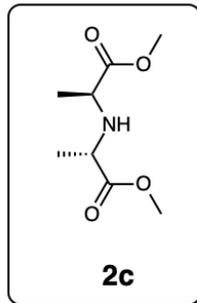


498.118 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm)  
temp 26.9 C -> actual temp = 27.0 C, autotxnb probe



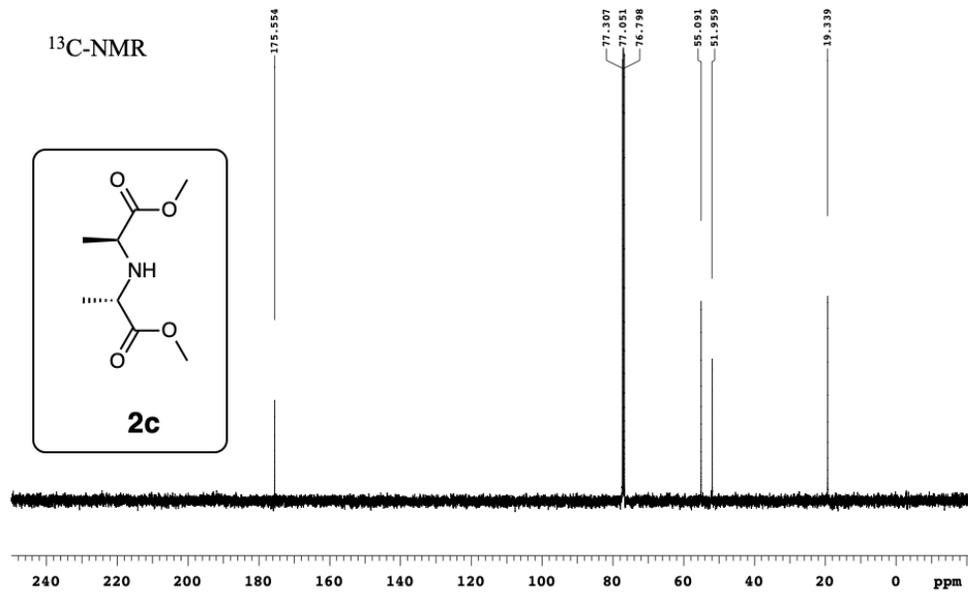
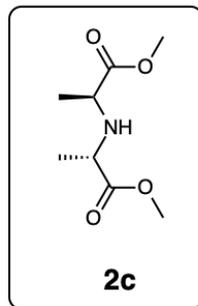
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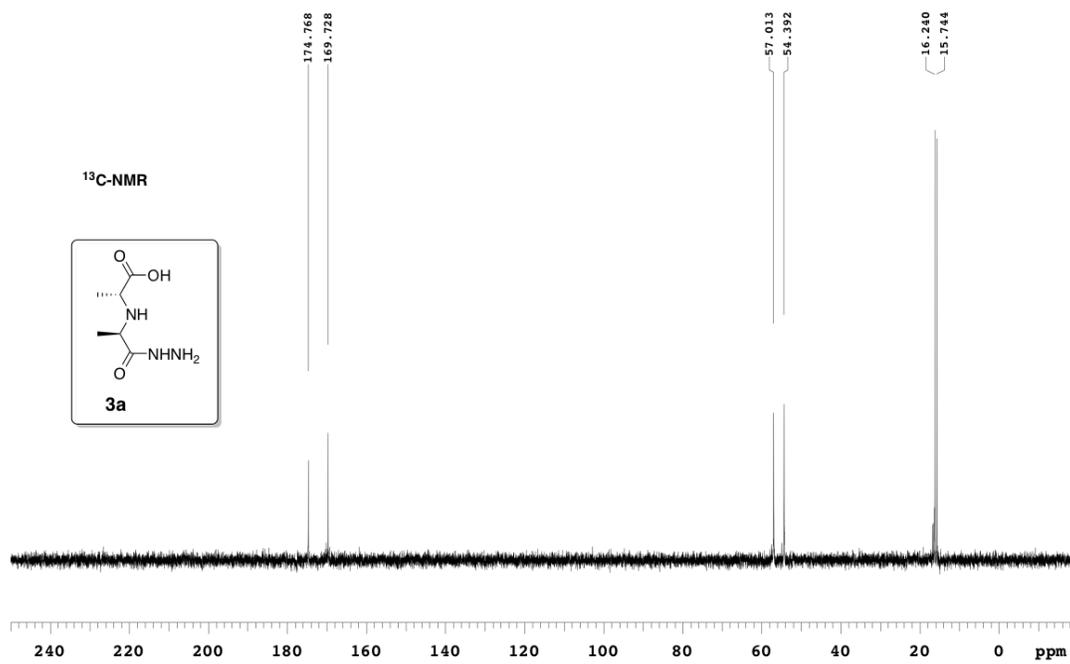
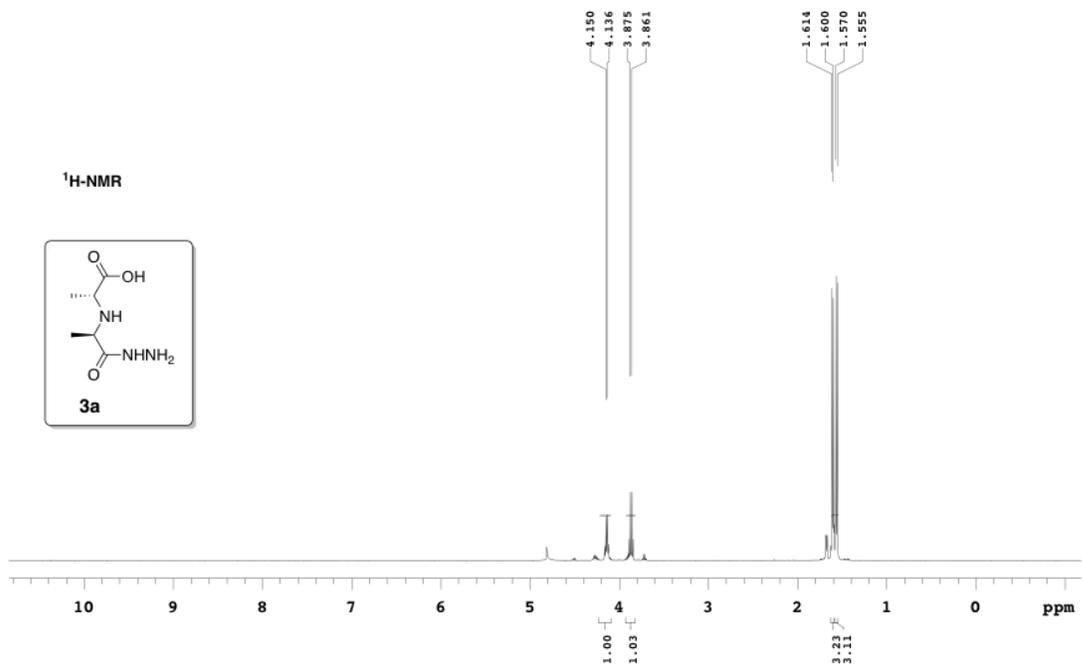
### <sup>1</sup>H-NMR

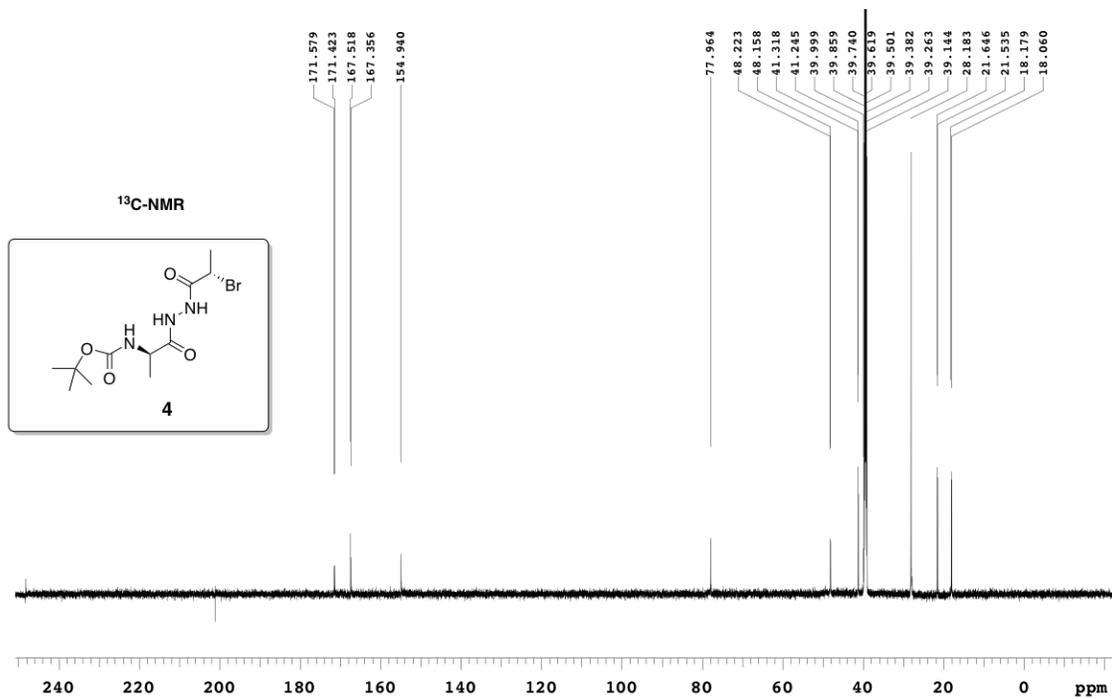
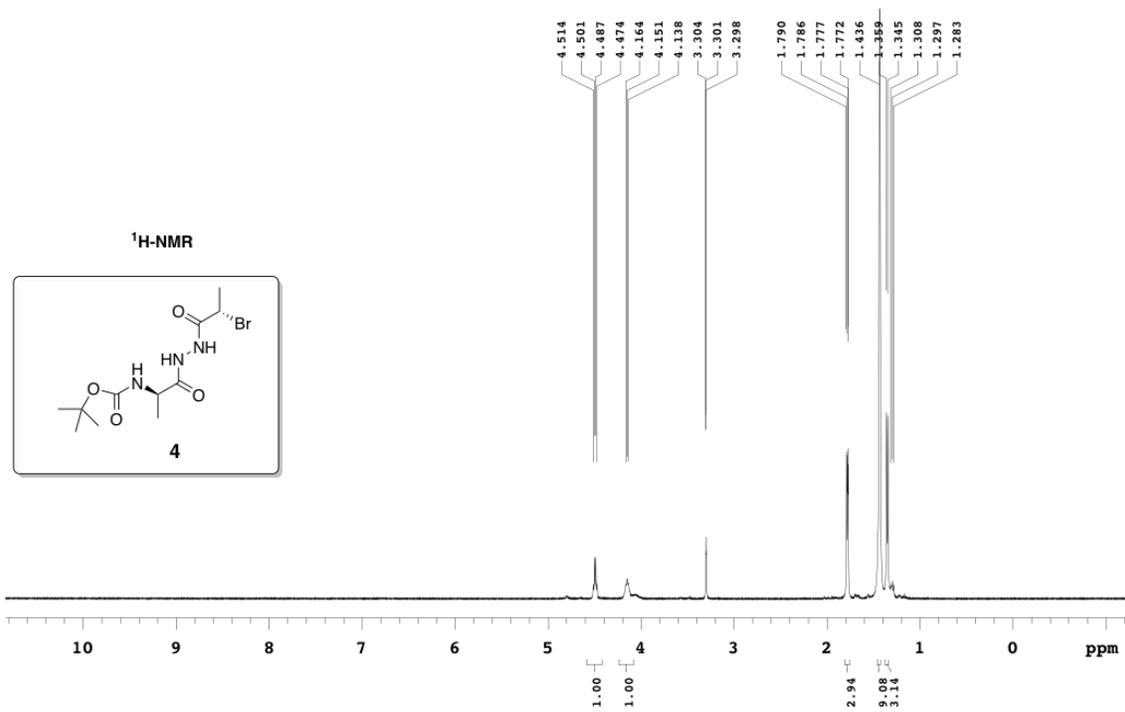


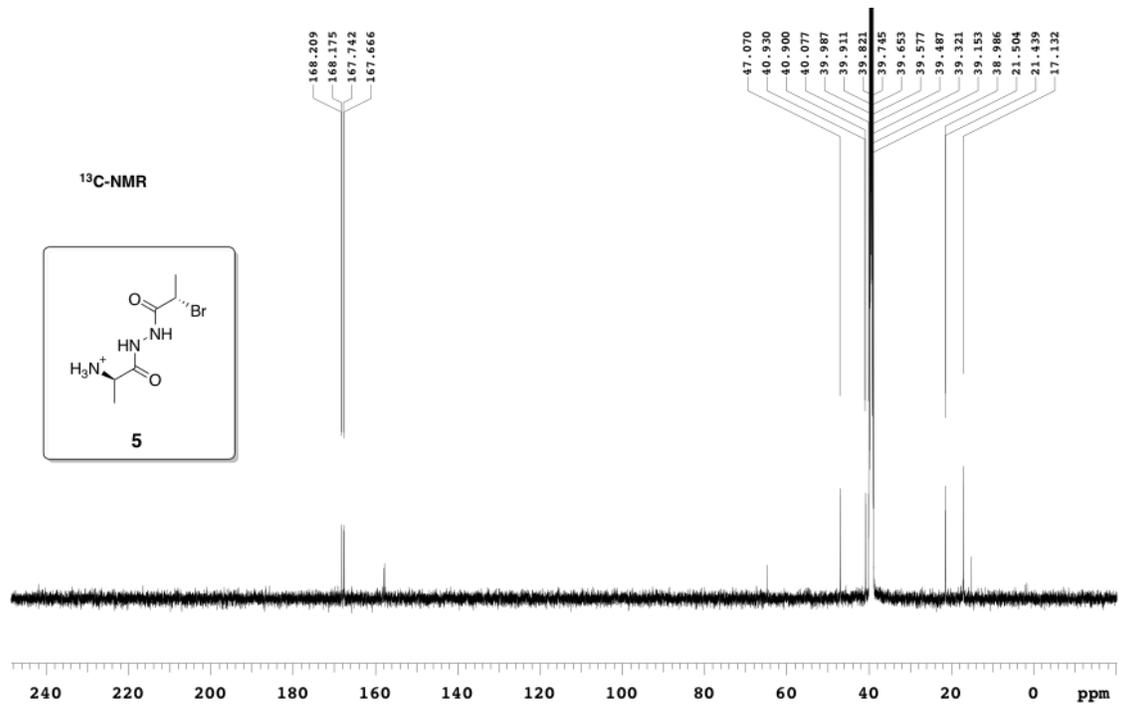
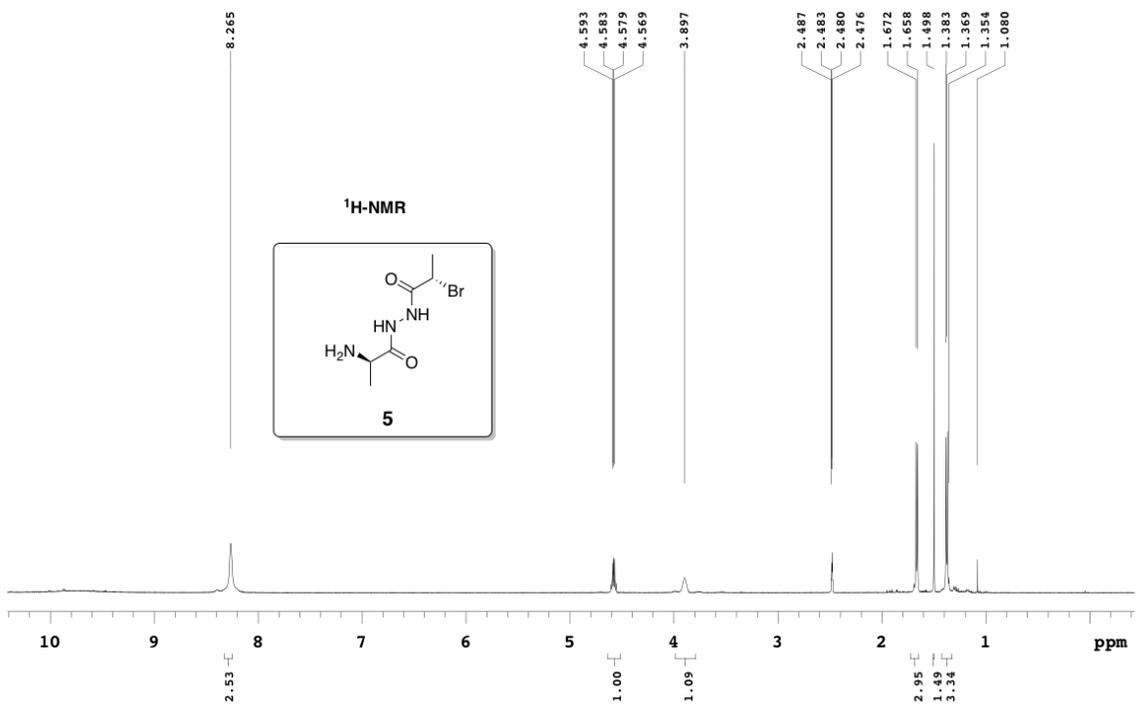
125.266 MHz C13(H1) 1D in cdcl3 (ref. to CDCl3 @ 77.06 ppm)  
temp 26.9 C -> actual temp = 27.0 C, autoxtdb probe

### <sup>13</sup>C-NMR

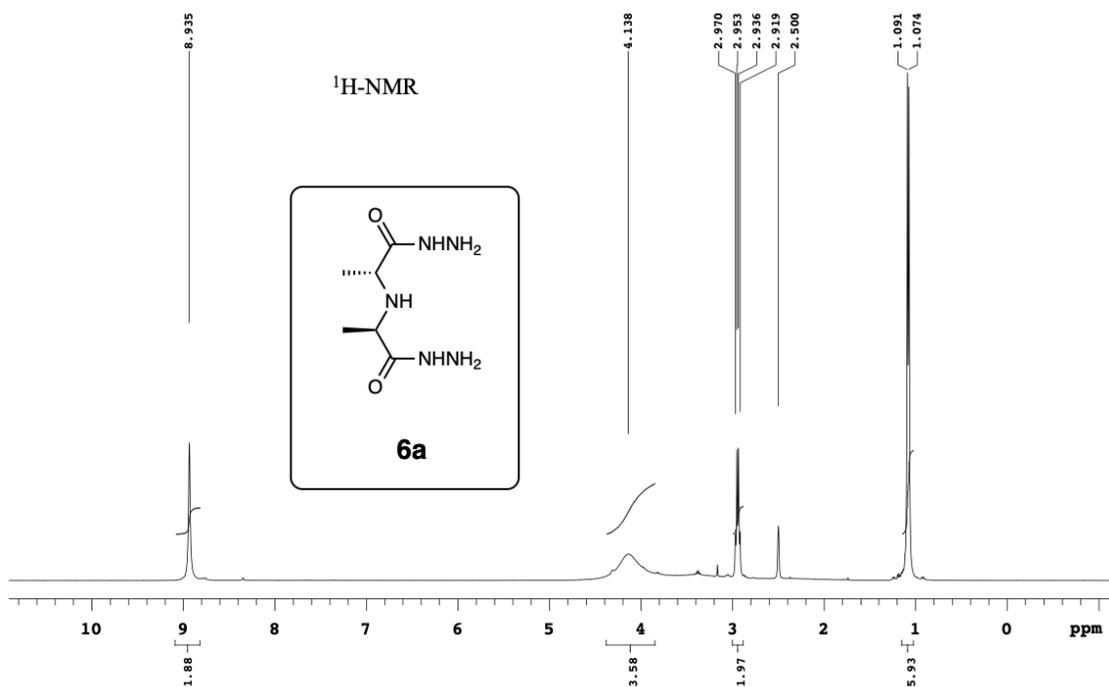




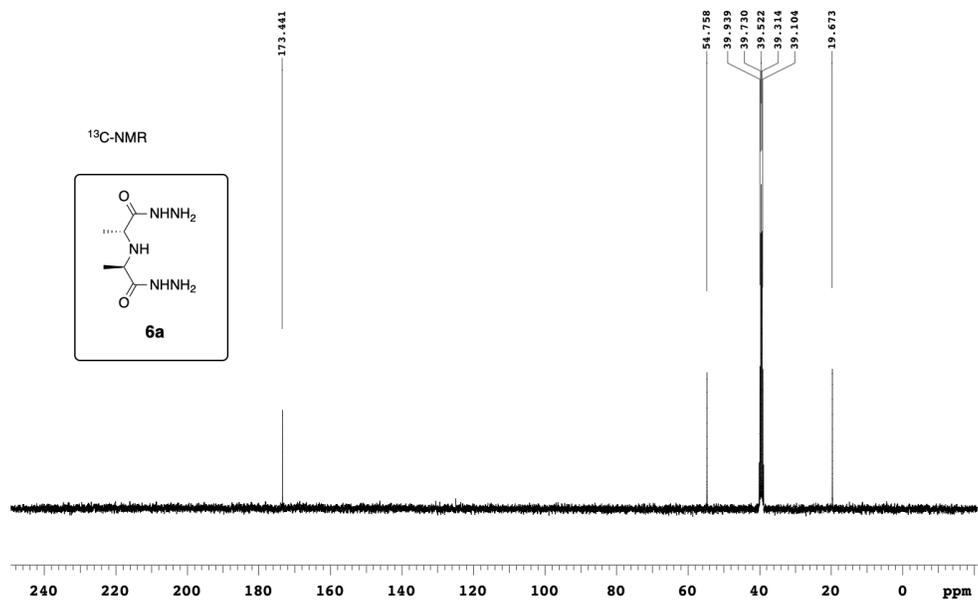




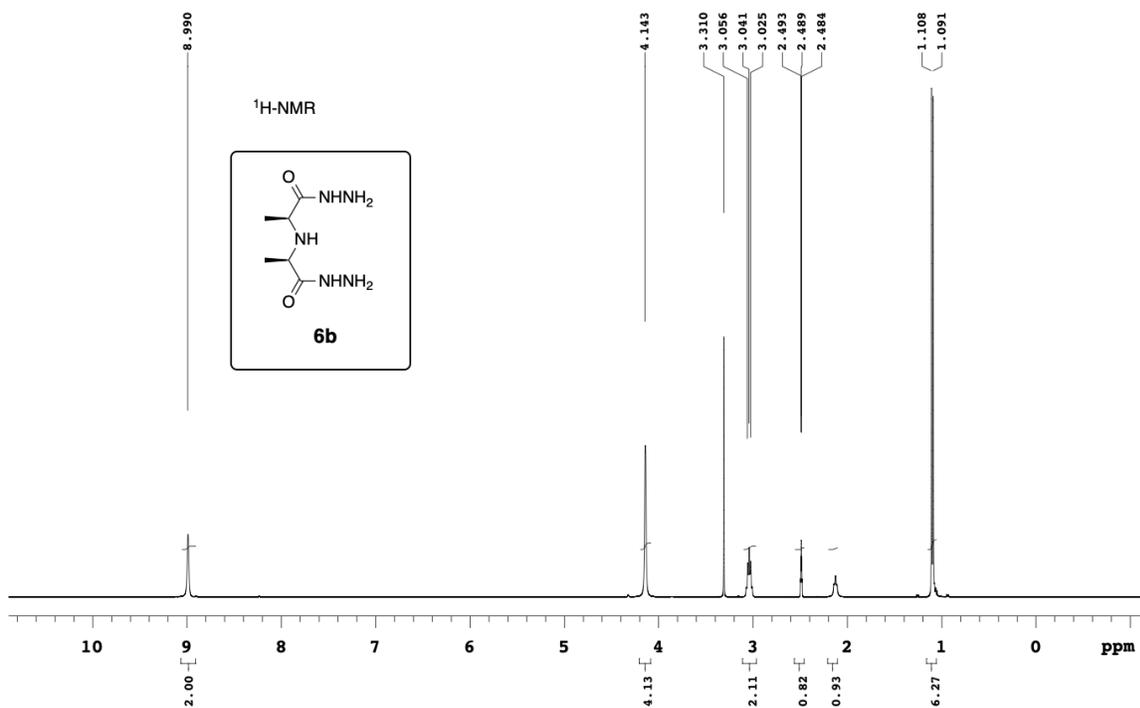
399.980 MHz H1 1D in dms0 (ref. to DMSO @ 2.49 ppm)  
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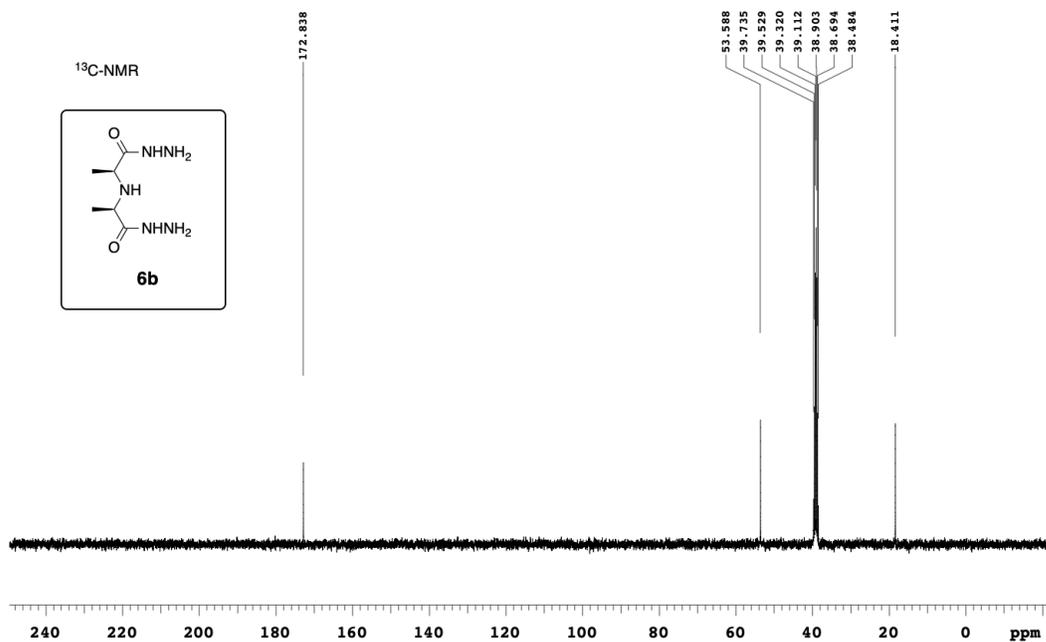
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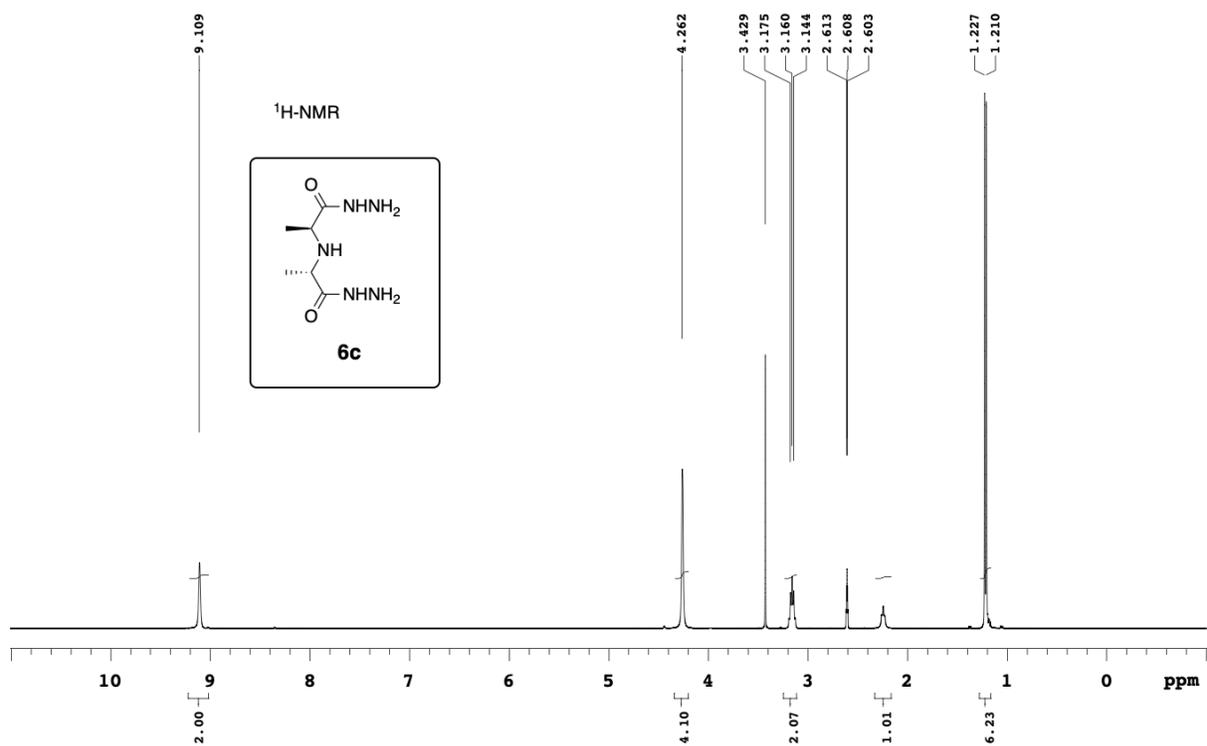
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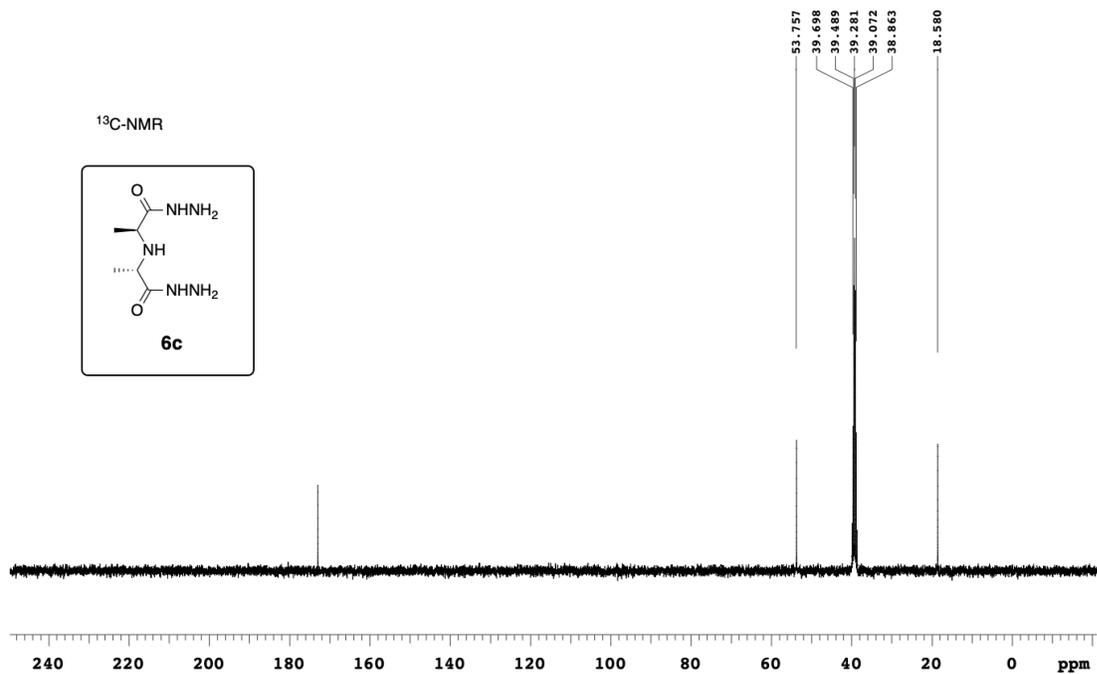
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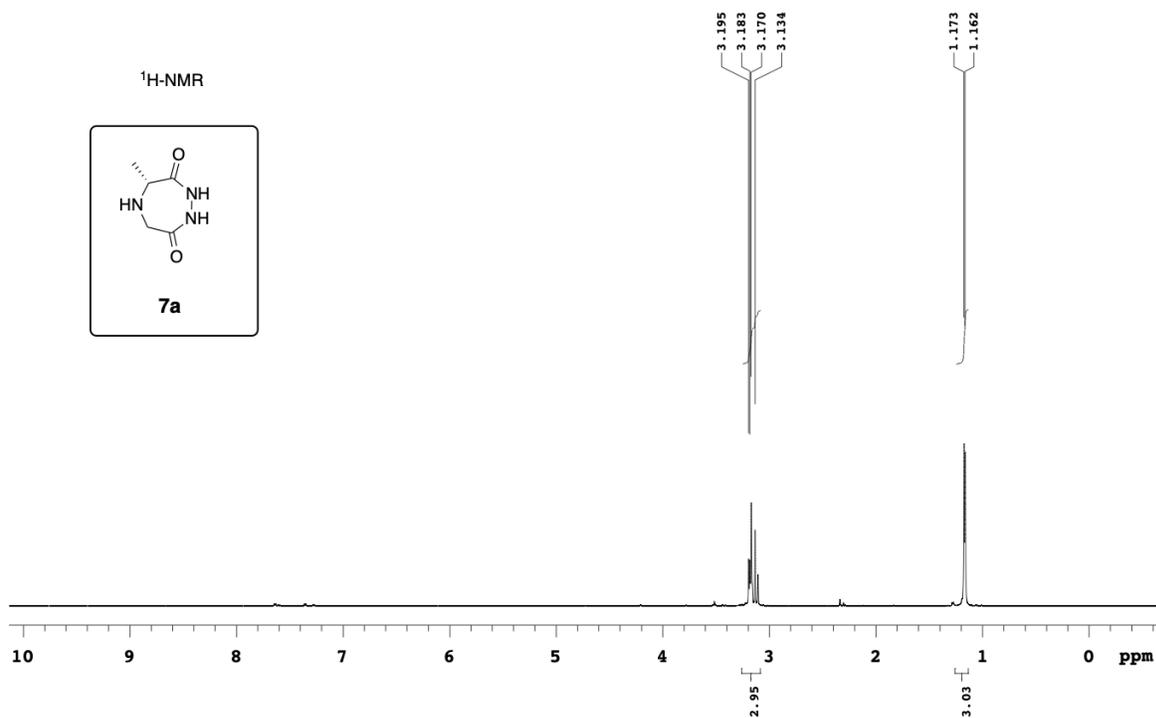
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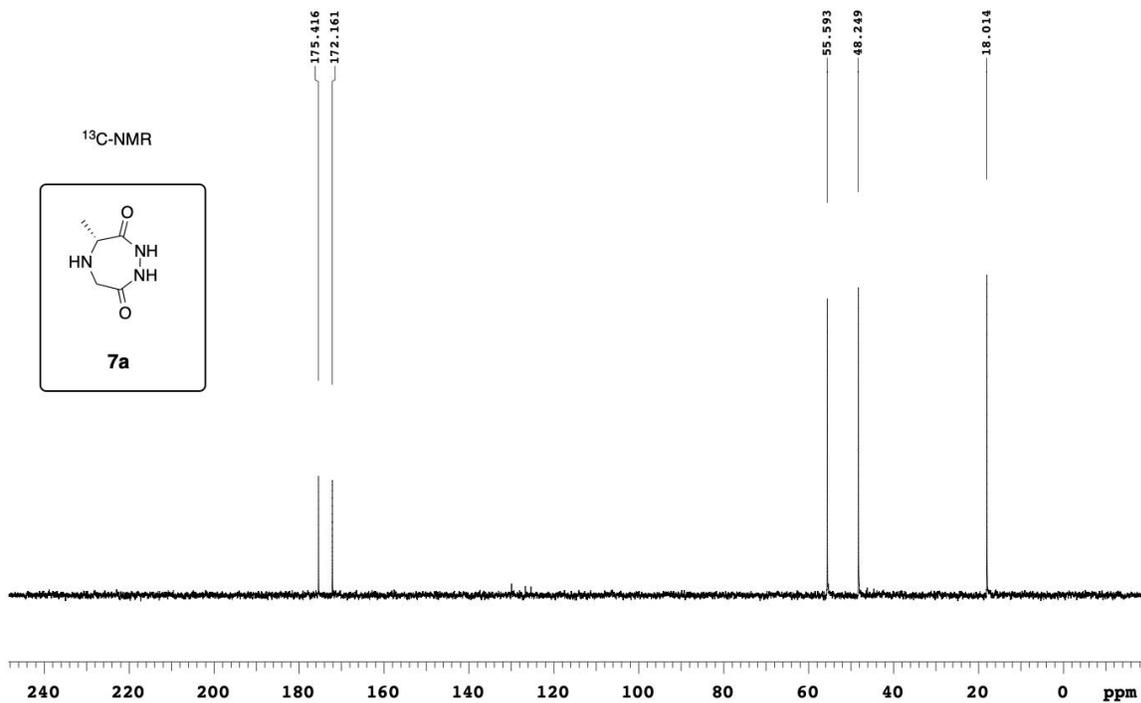
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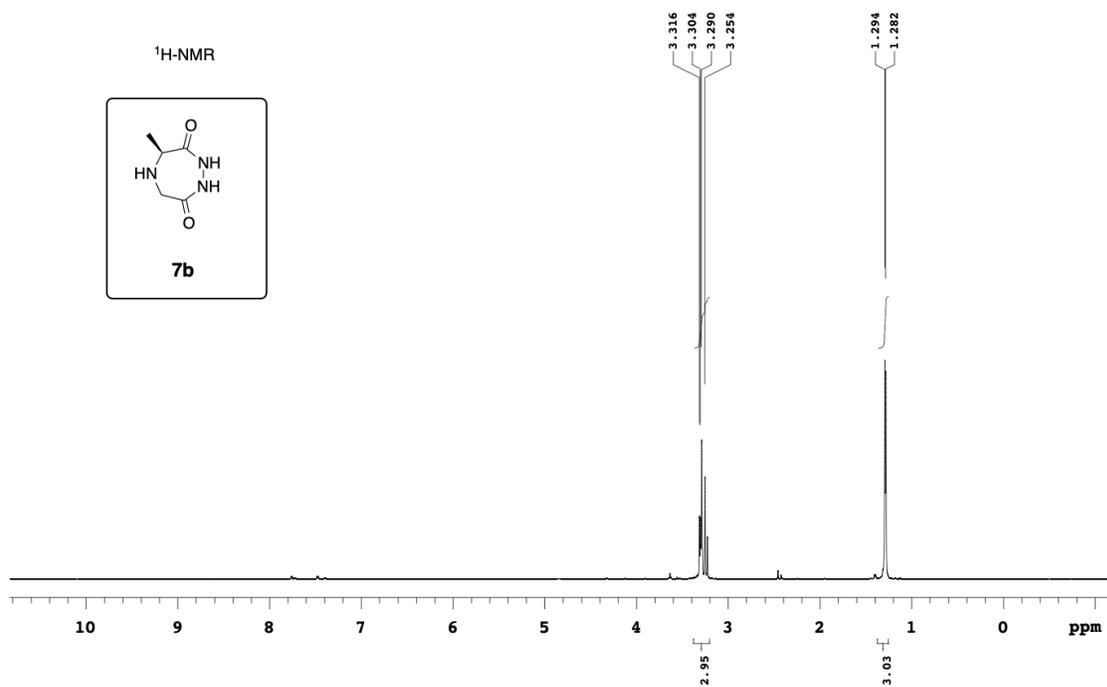
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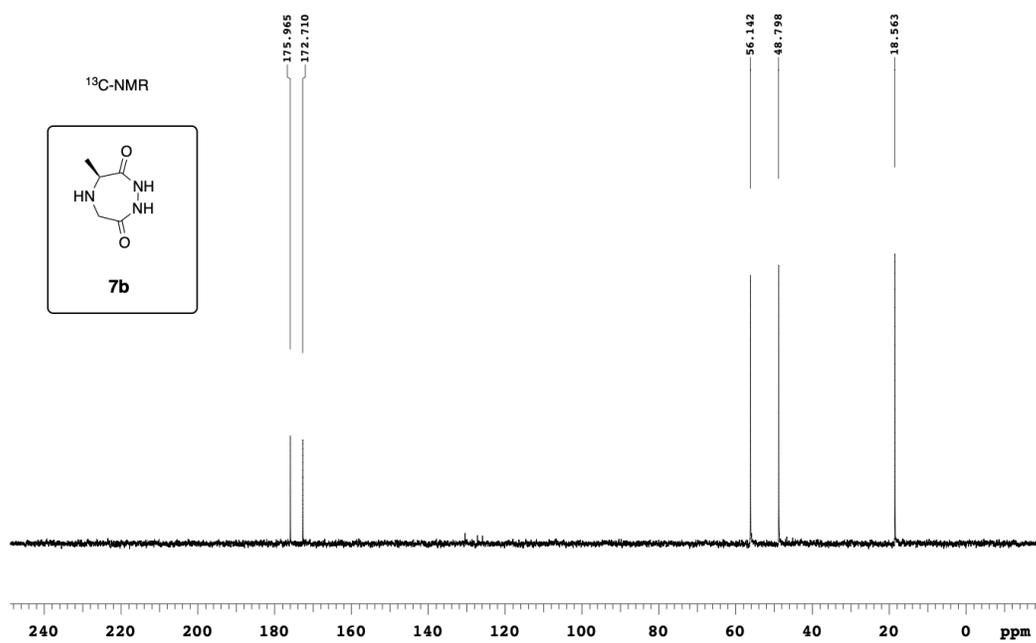
150.868 MHz  $^{13}\text{C}\{^1\text{H}\}$  1D in  $\text{d}_2\text{o}$  (ref. to external acetone @ 31.07 ppm)  
temp 26.2 C -> actual temp = 27.0 C, autoxid probe



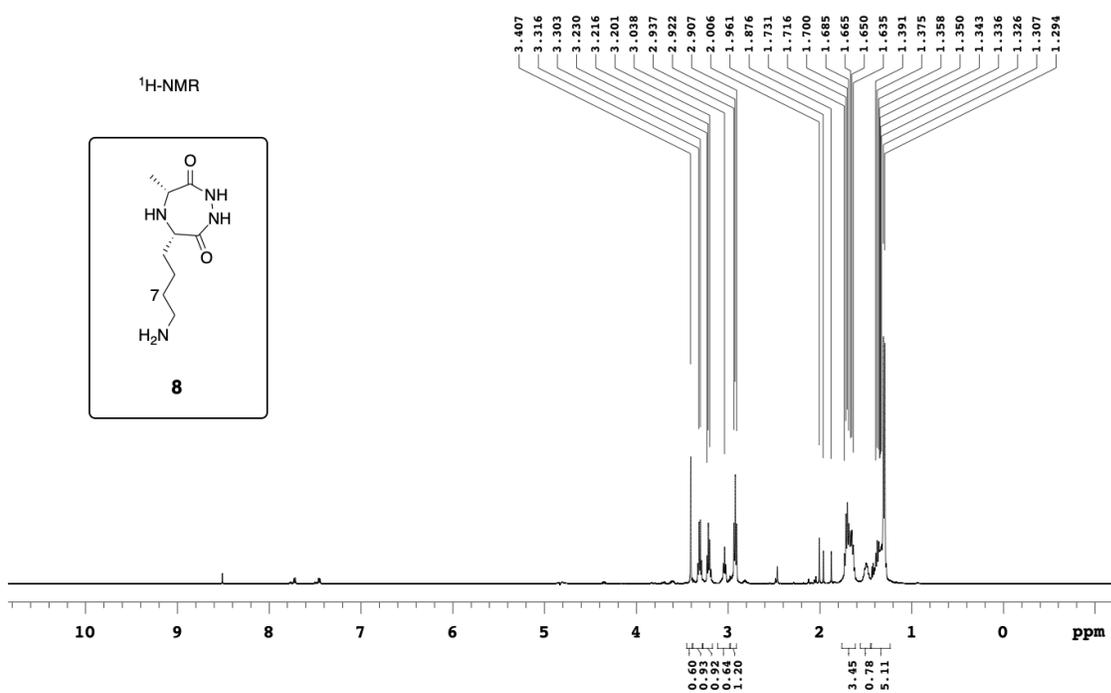
599.927 MHz <sup>1</sup>H 1D in d<sub>2</sub>O (ref. to external acetone @ 2.225 ppm)  
temp 26.2 C -> actual temp = 27.0 C, autoxid probe



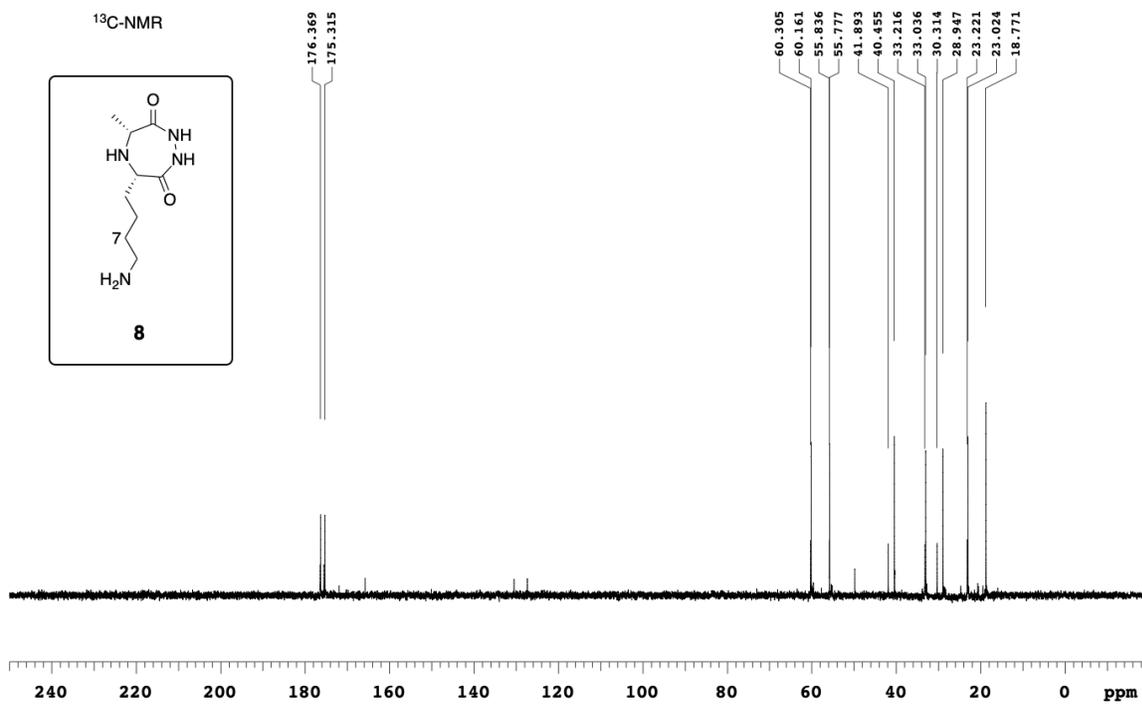
150.868 MHz <sup>13</sup>C(H) 1D in d<sub>2</sub>O (ref. to external acetone @ 31.07 ppm)  
temp 26.2 C -> actual temp = 27.0 C, autoxid probe



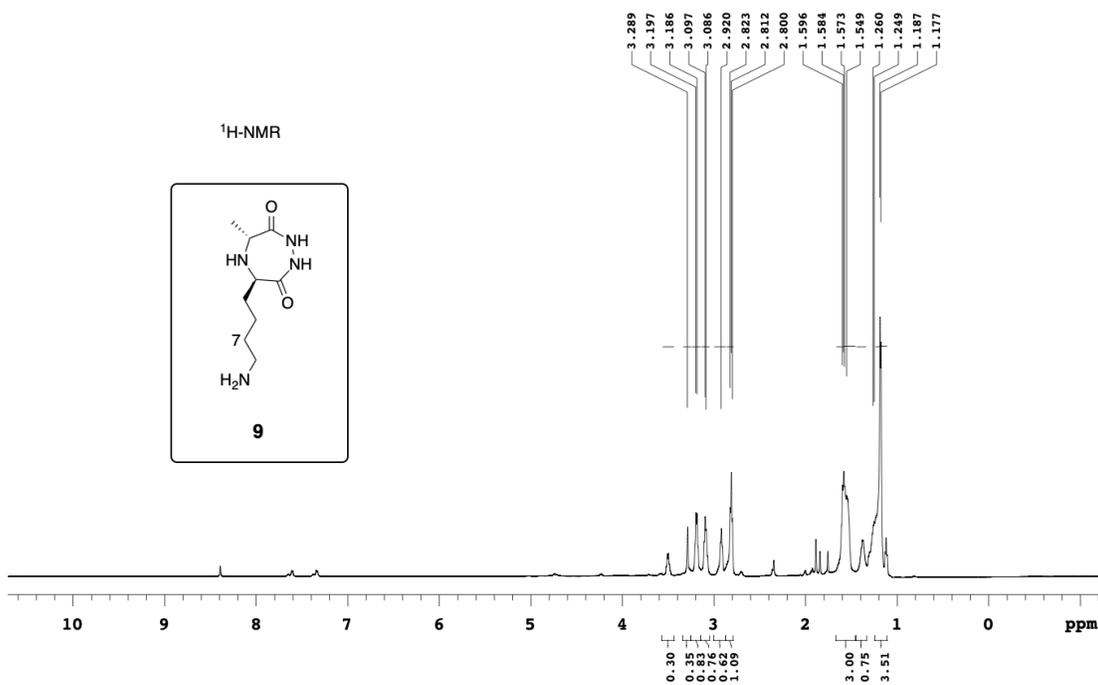
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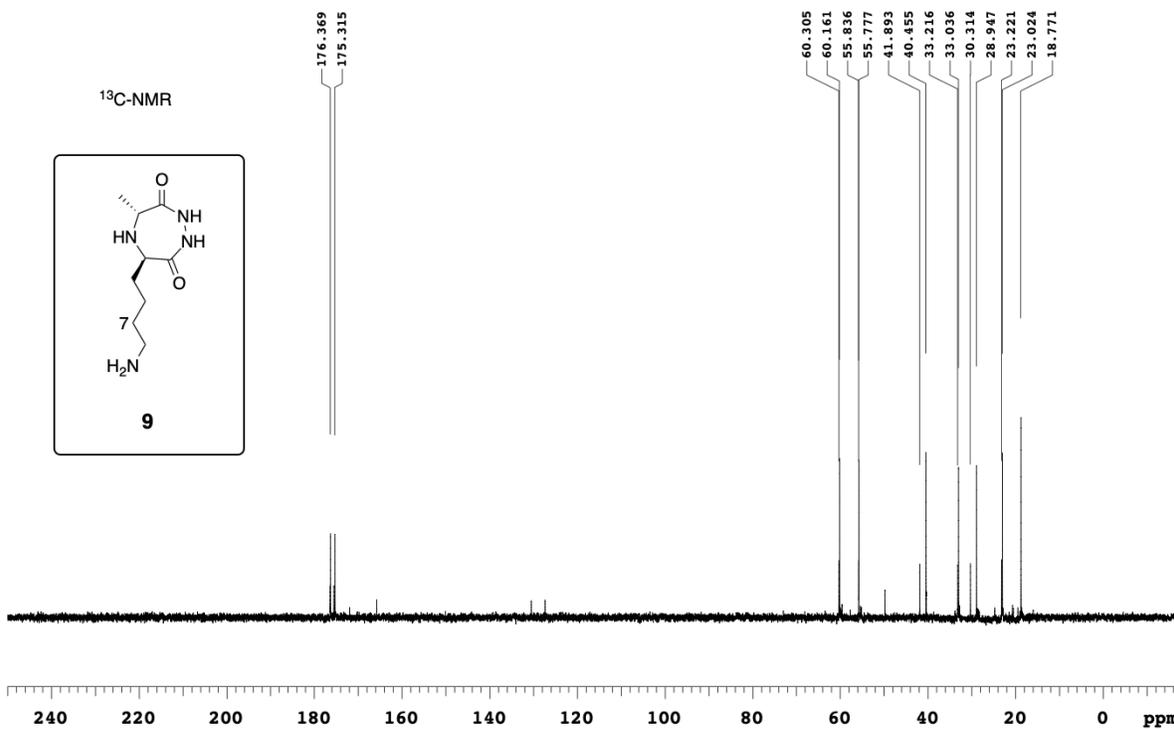
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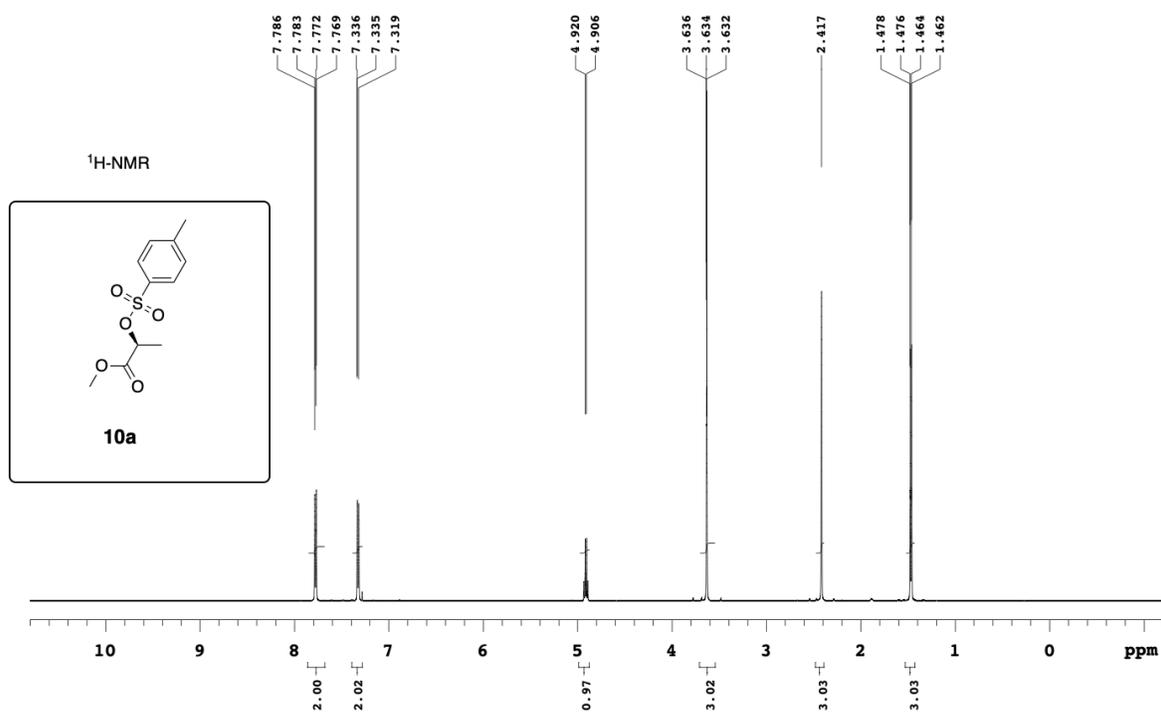
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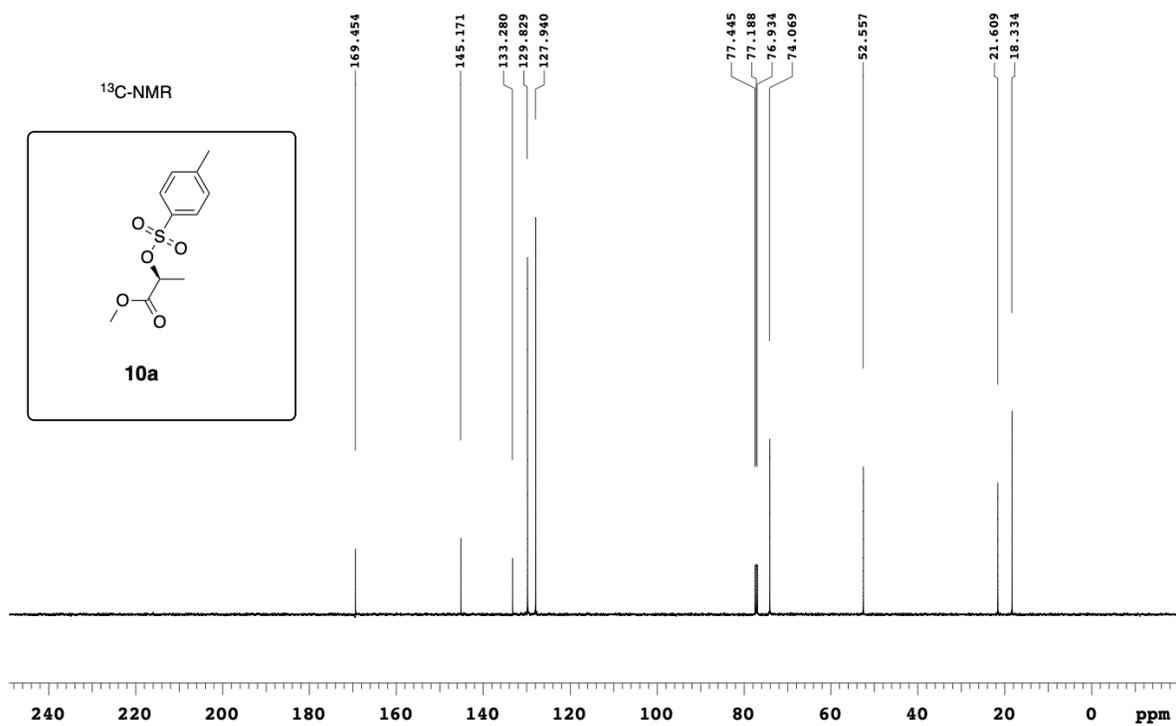
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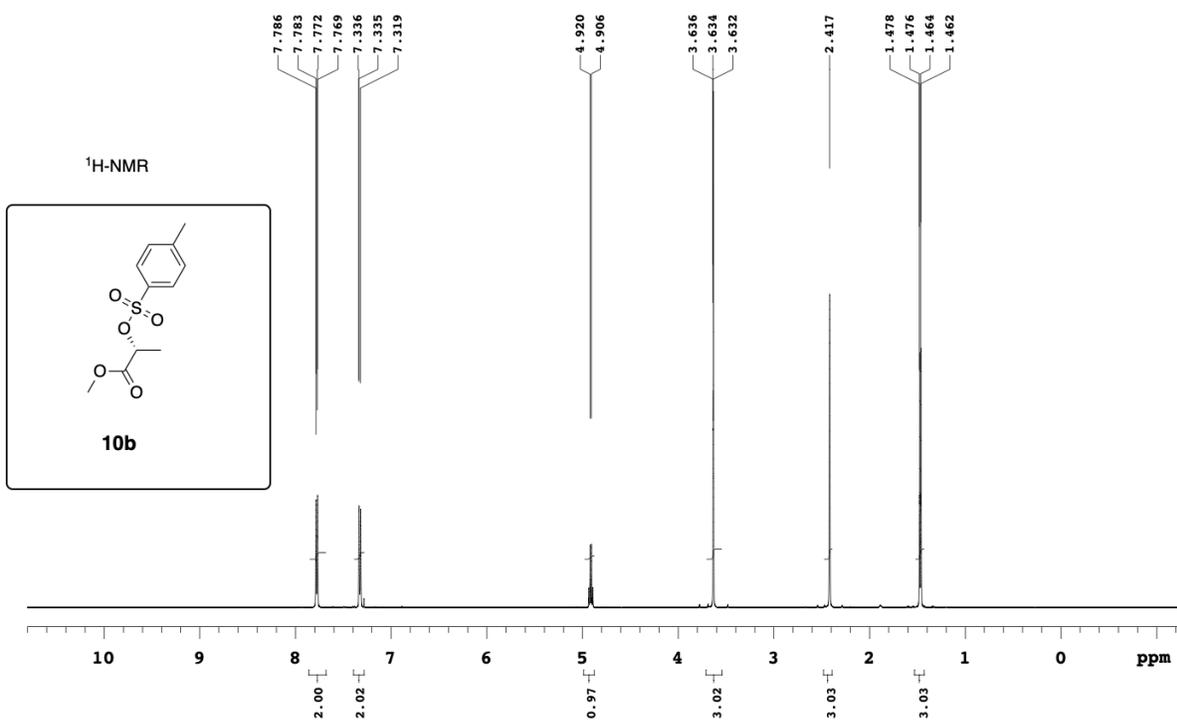
499.787 MHz <sup>1</sup>H 1D in cdcl<sub>3</sub> (ref. to CDCl<sub>3</sub> @ 7.26 ppm)  
temp 27.7 C -> actual temp = 27.0 C, coldlual probe



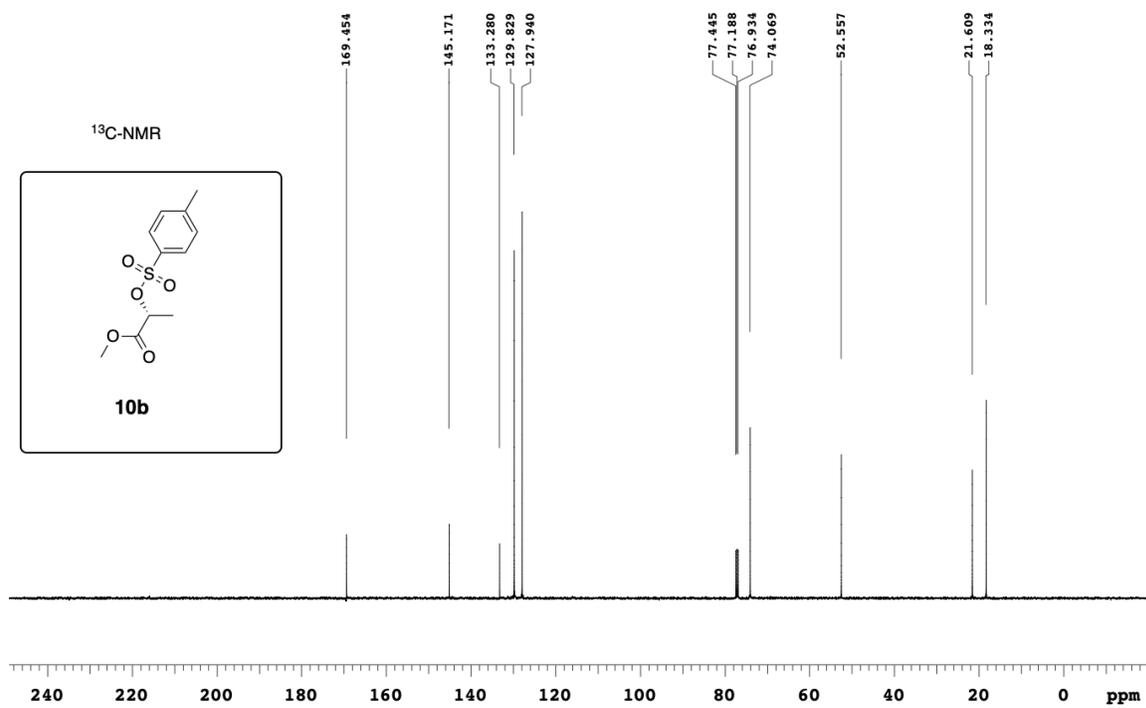
125.685 MHz <sup>13</sup>C{<sup>1</sup>H} 1D in cdcl<sub>3</sub> (ref. to CDCl<sub>3</sub> @ 77.06 ppm)  
temp 27.7 C -> actual temp = 27.0 C, coldlual probe



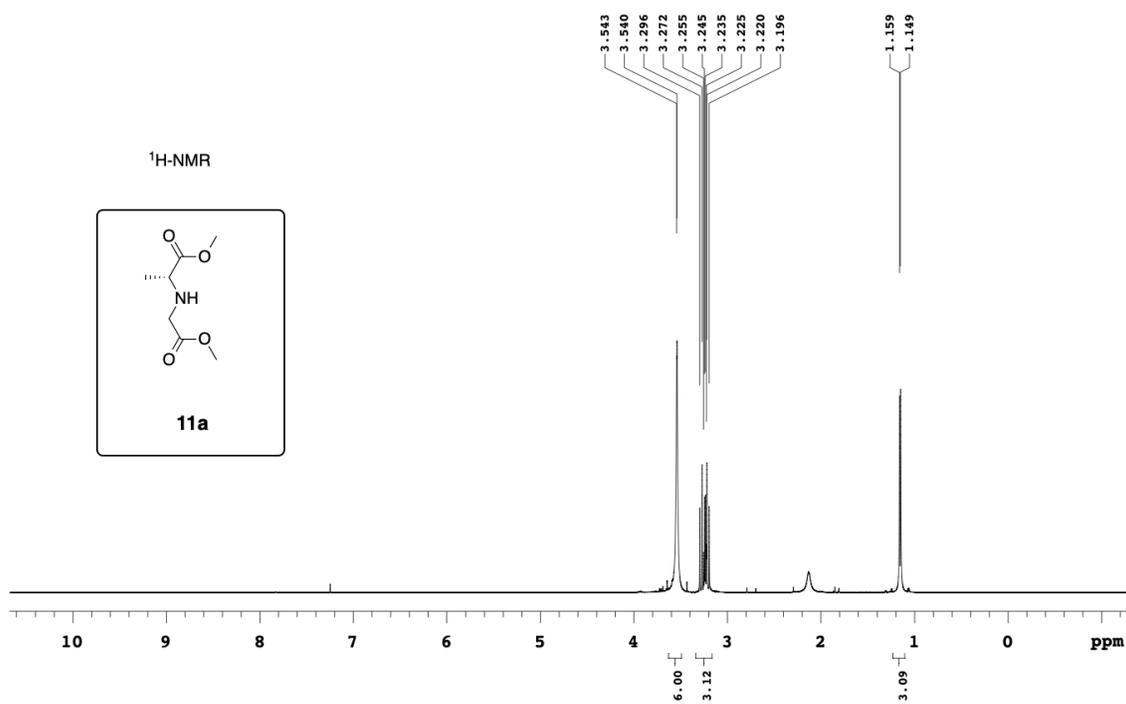
499.187 MHz <sup>1</sup>H in CDCl<sub>3</sub> (ref. to TMS) @ 7.26 ppm  
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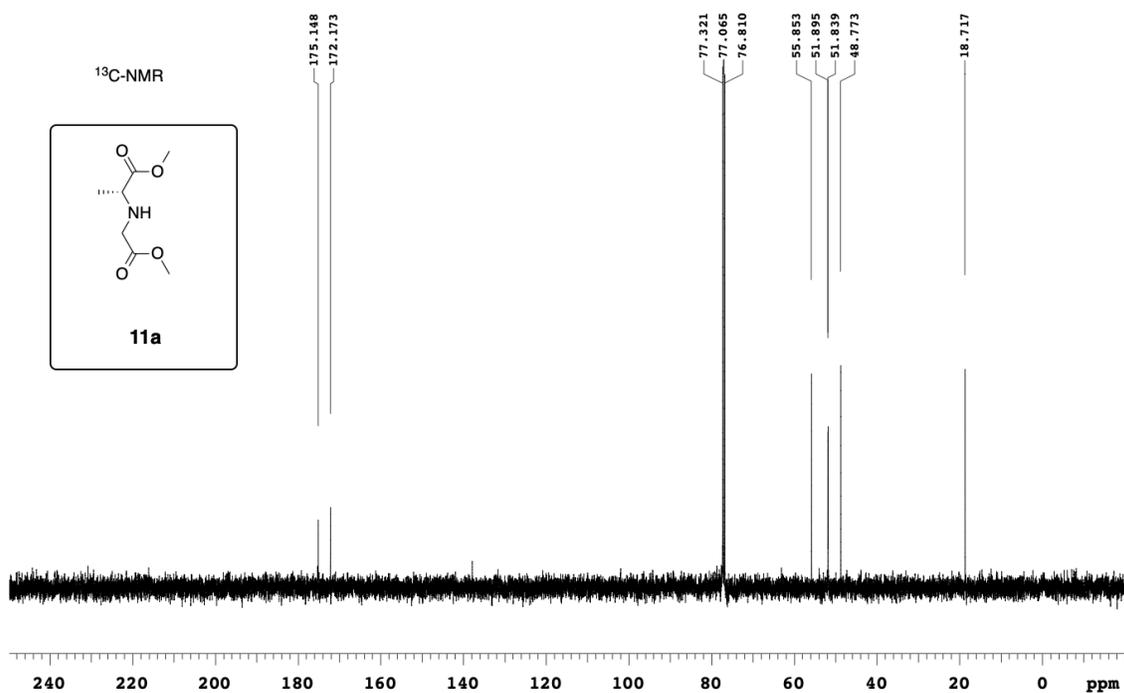
163.888 MHz <sup>13</sup>C in CDCl<sub>3</sub> (ref. to TMS) @ 77.00 ppm  
temp 27.7 C -> actual temp = 27.0 C, coldludal probe



19860\_19-0-173  
699.762 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm)  
temp 27.5 C -> actual temp = 27.0 C, coldid probe

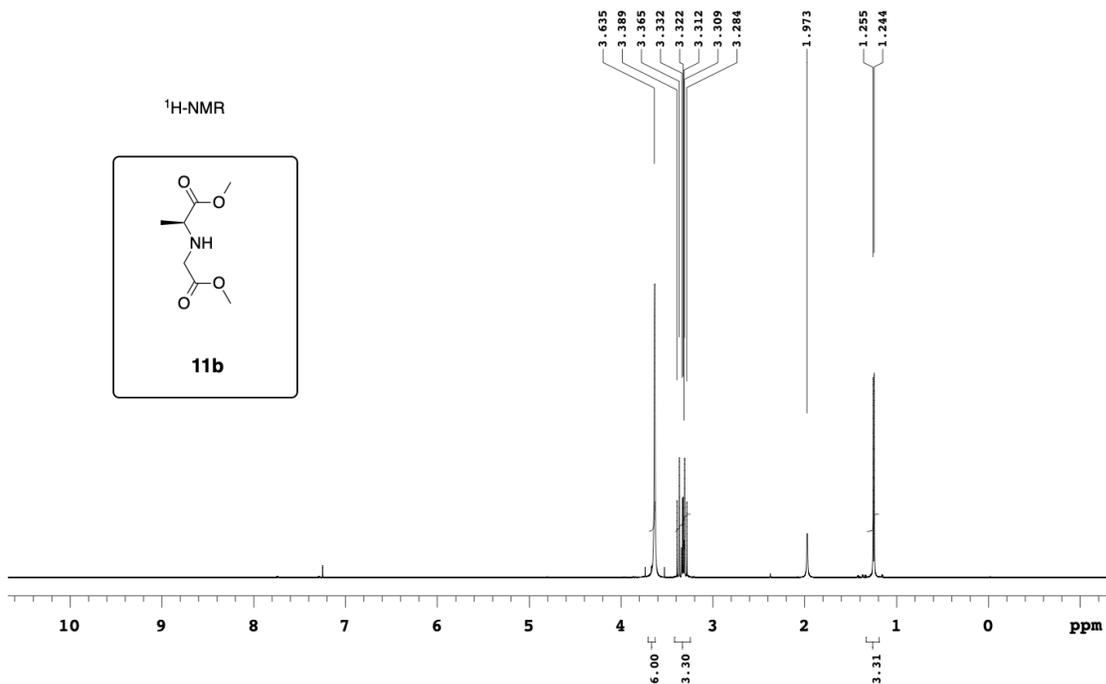
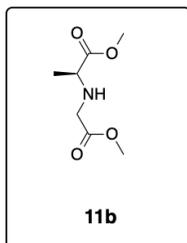


125.266 MHz C13(H1) 1D in cdcl3 (ref. to CDCl3 @ 77.06 ppm)  
temp 26.9 C -> actual temp = 27.0 C, autotdx probe



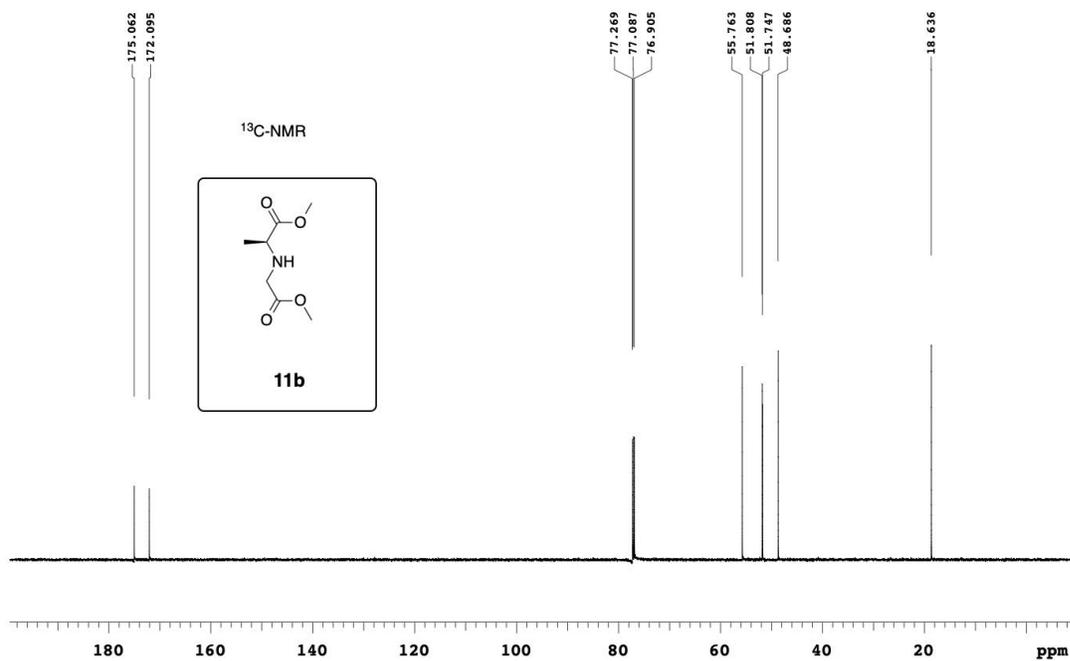
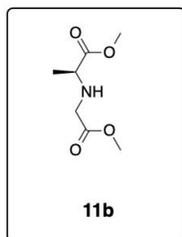
699.762 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm)  
temp 27.5 C -> actual temp = 27.0 C, coldid probe

<sup>1</sup>H-NMR

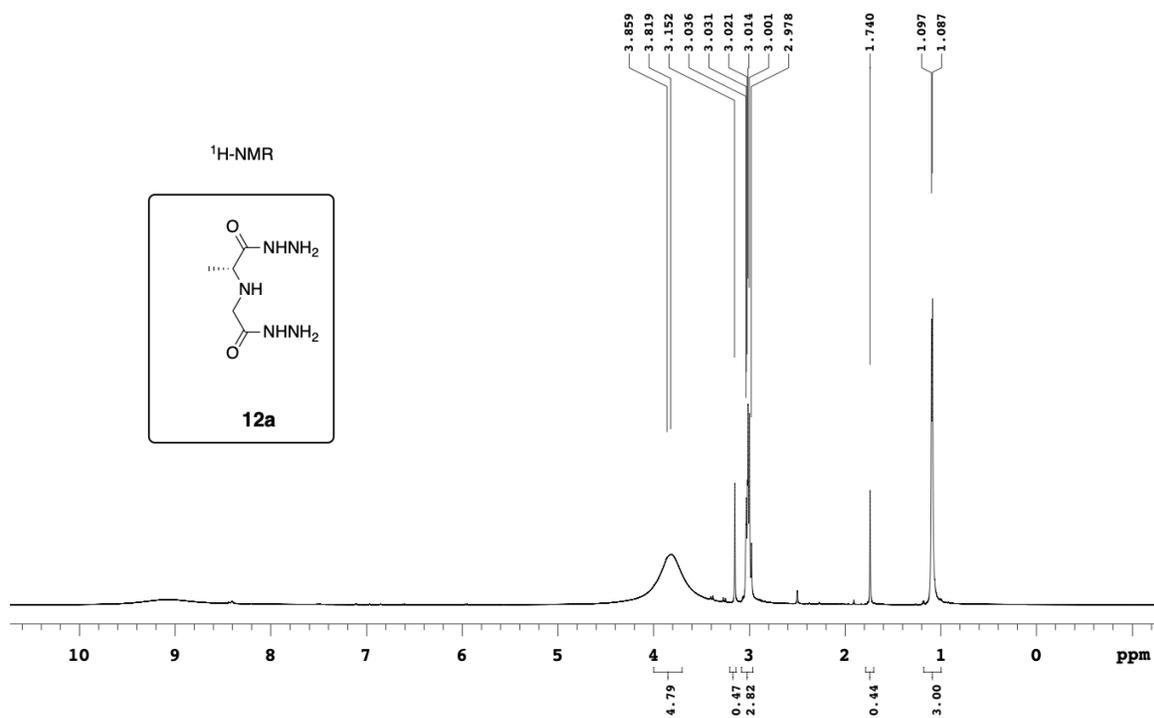


175.971 MHz C13(H1) 1D in cdcl3 (ref. to CDCl3 @ 77.06 ppm)  
temp 27.5 C -> actual temp = 27.0 C, coldid probe

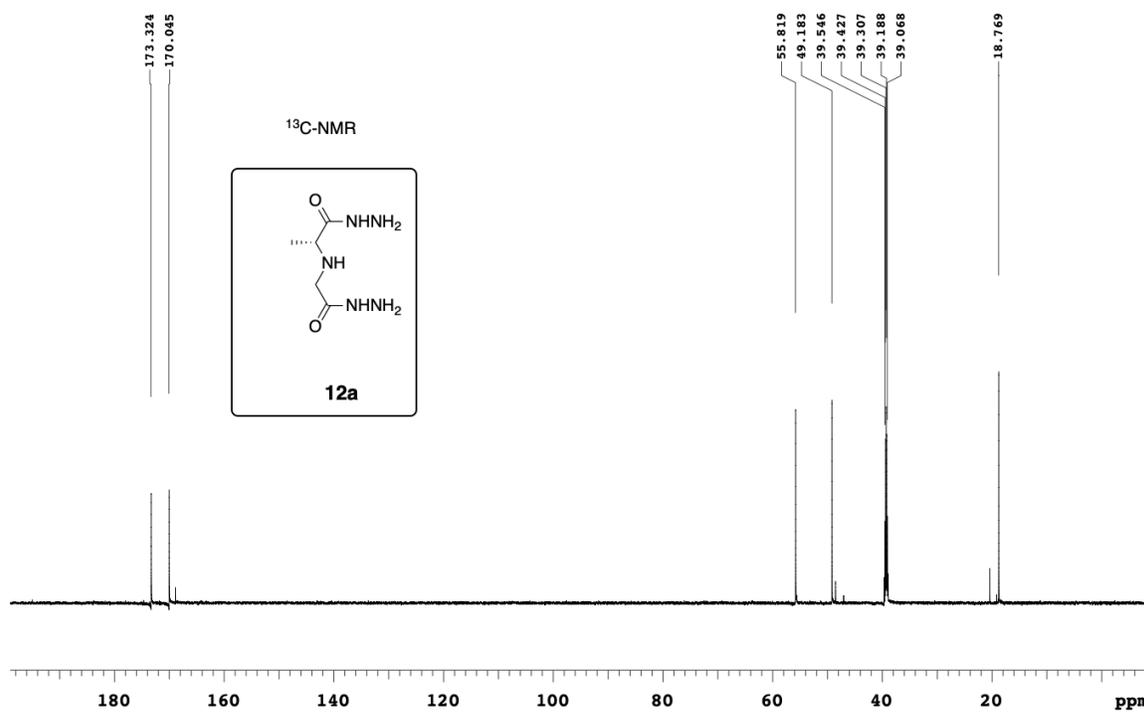
<sup>13</sup>C-NMR



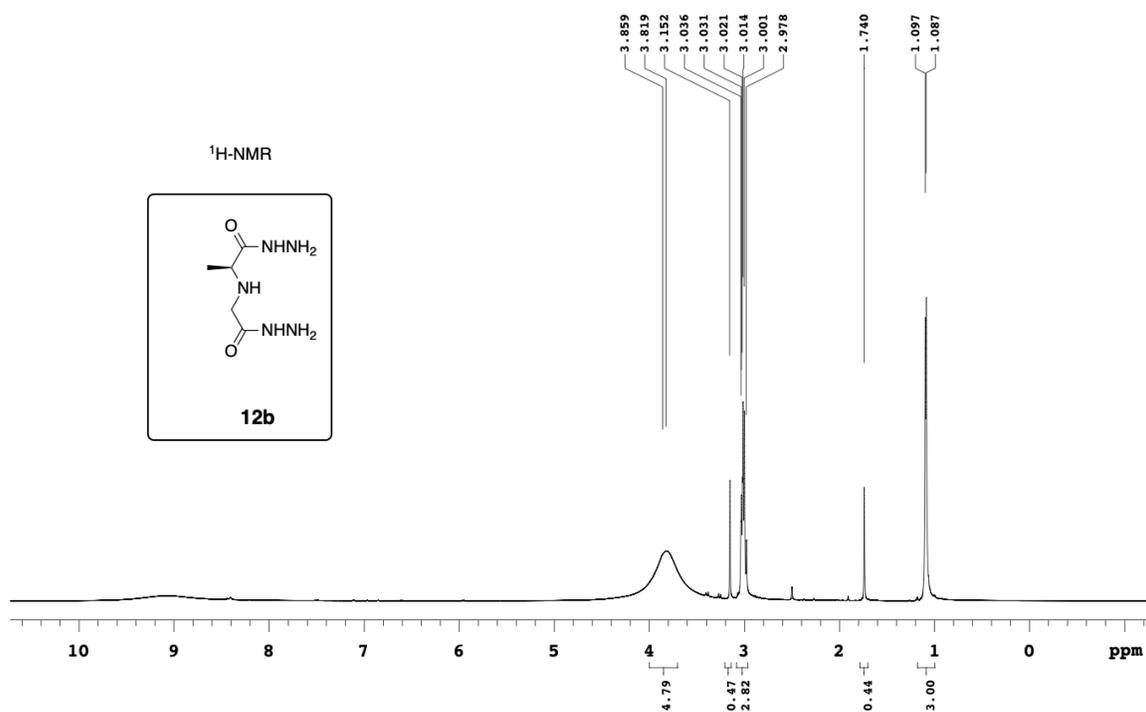
699.765 MHz H1 1D in dms0 (ref. to DMSO @ 2.49 ppm)  
temp 27.5 C -> actual temp = 27.0 C, coldid probe



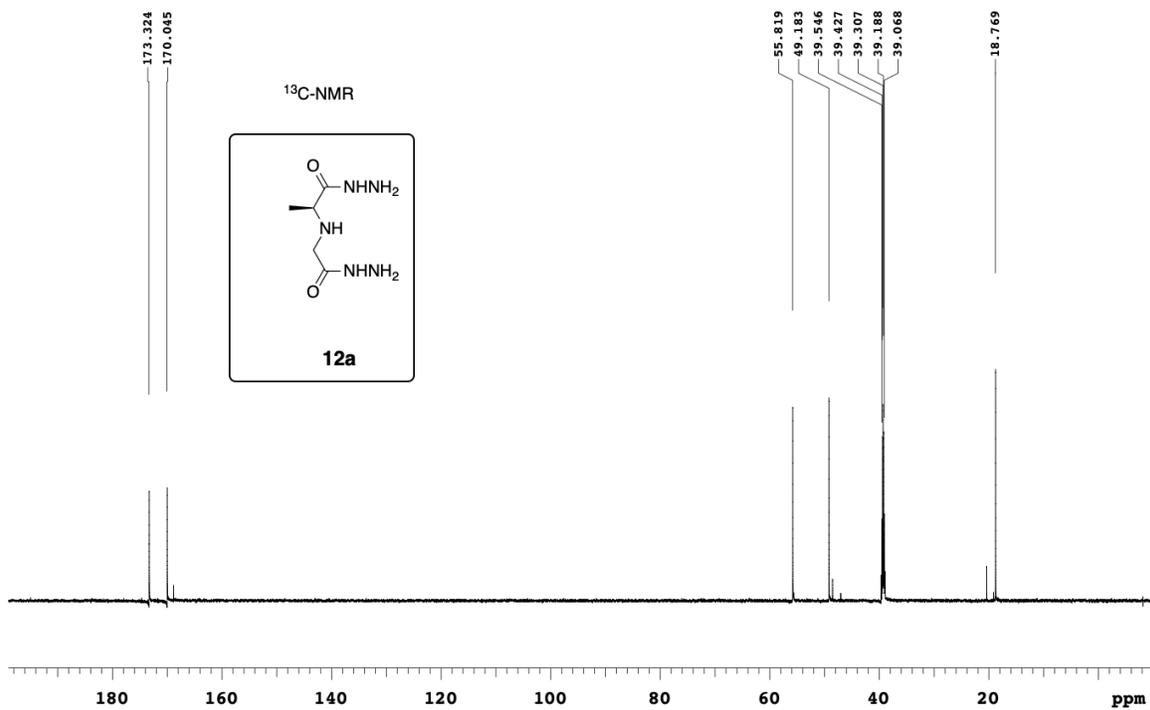
temp 27.5 C -> actual temp = 27.0 C, coldid probe

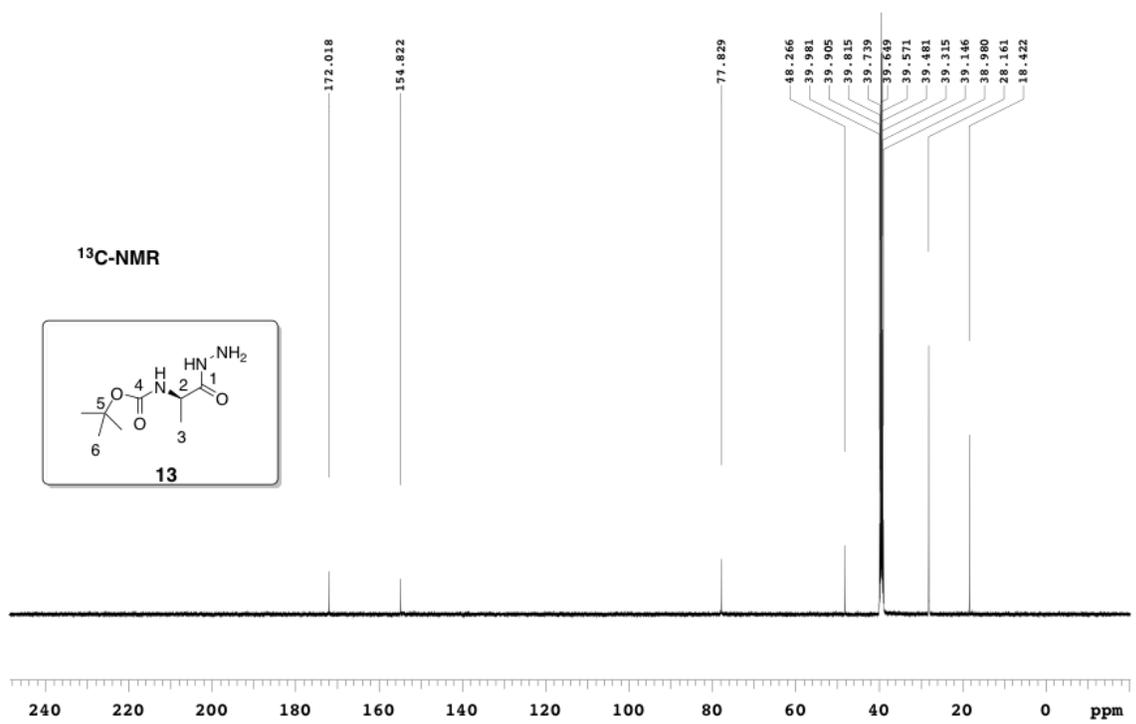
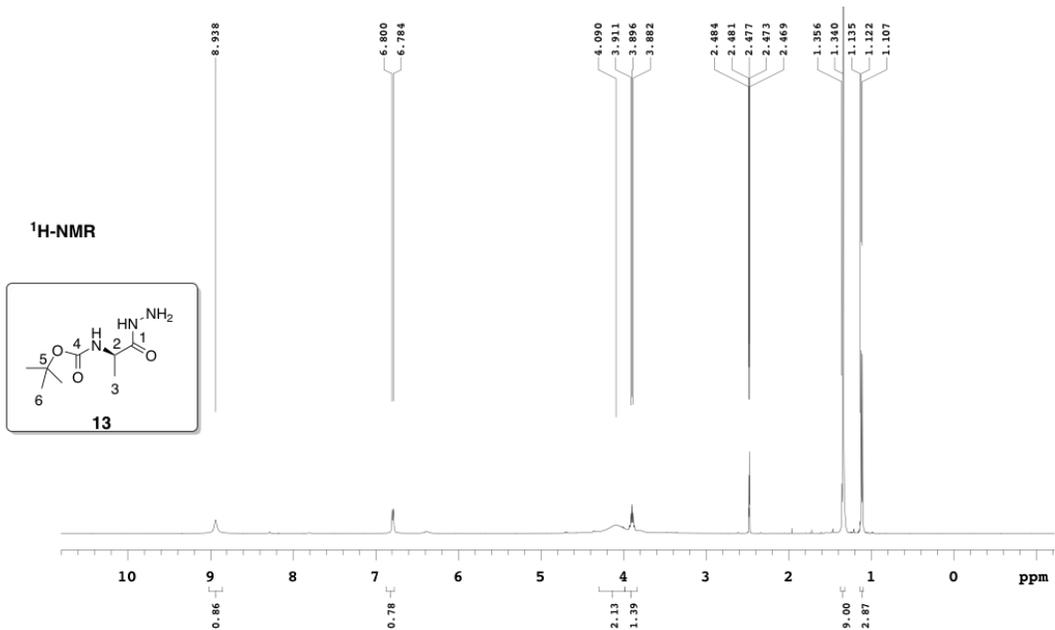


temp 27.5 C -> actual temp = 27.0 C, coldid probe

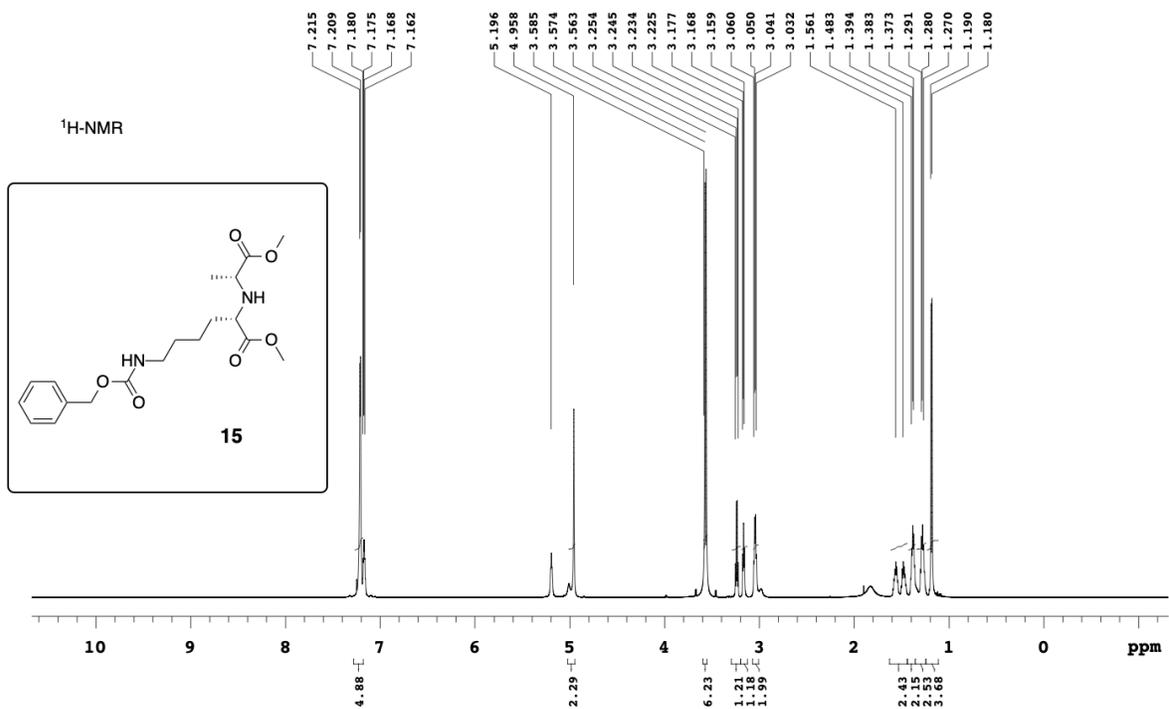


temp 27.5 C -> actual temp = 27.0 C, coldid probe

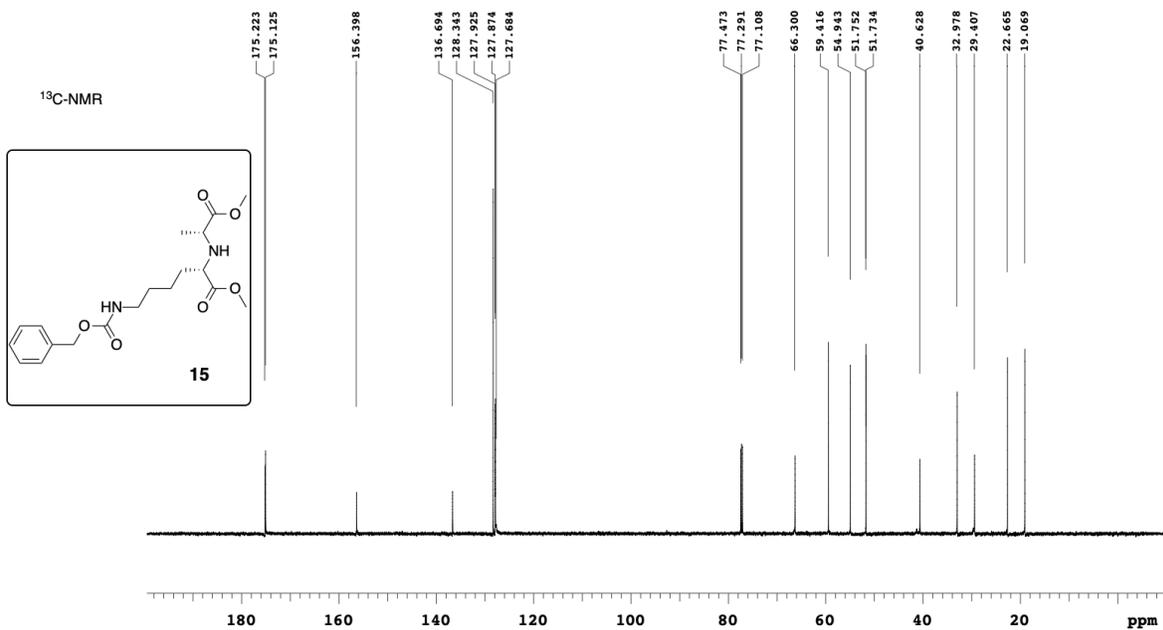


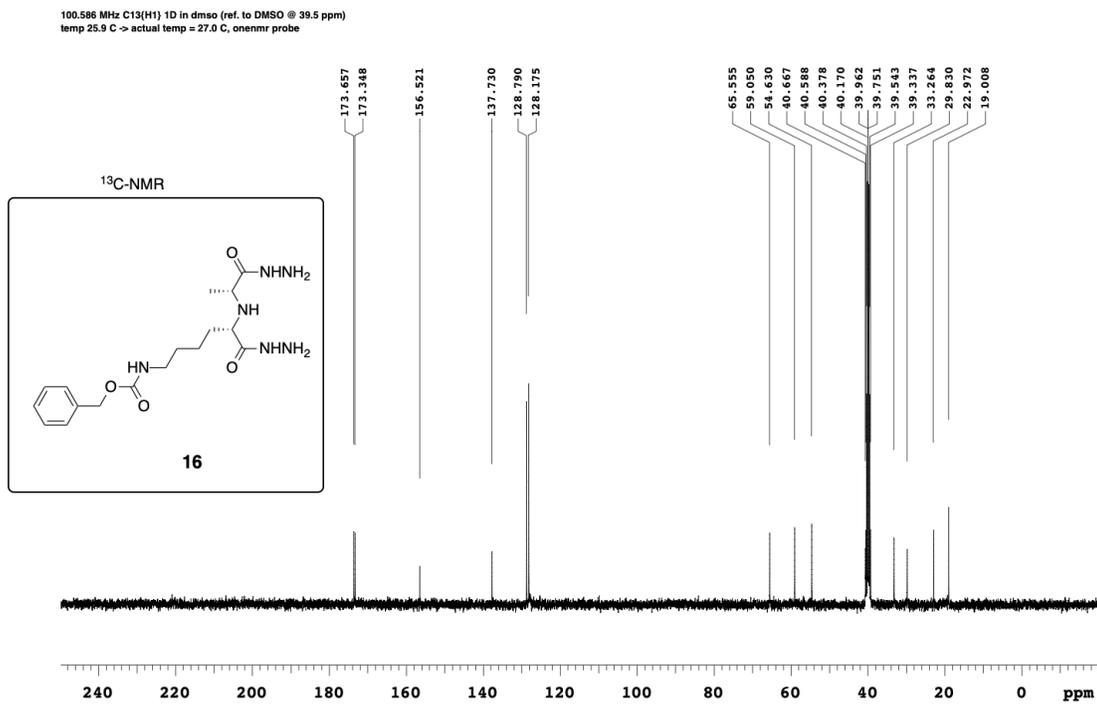
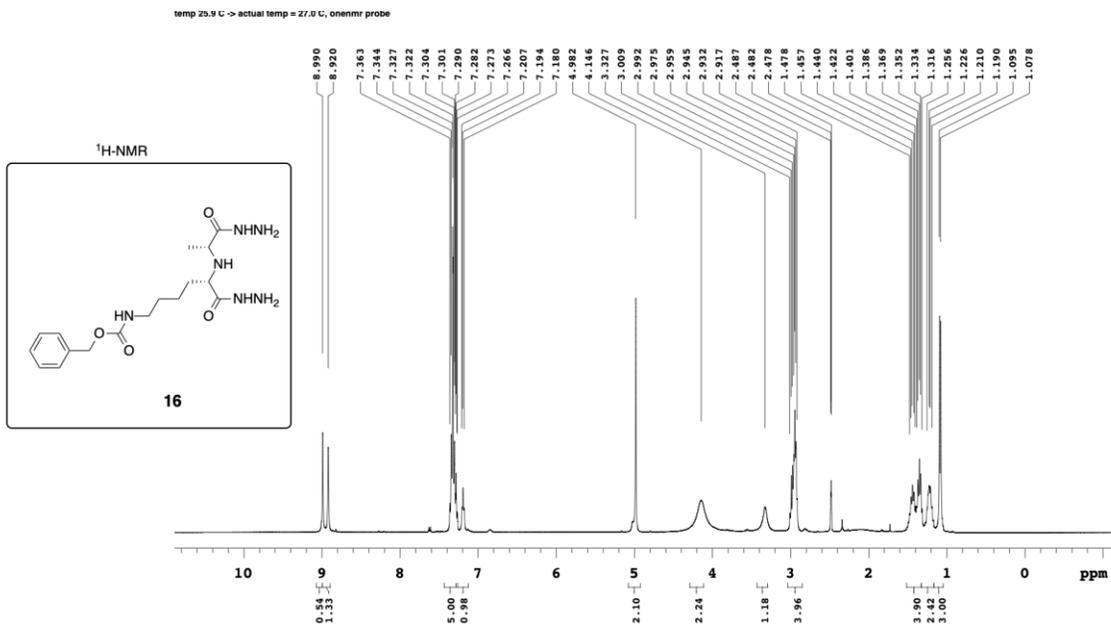


temp 27.5 C -> actual temp = 27.0 C, coldid probe

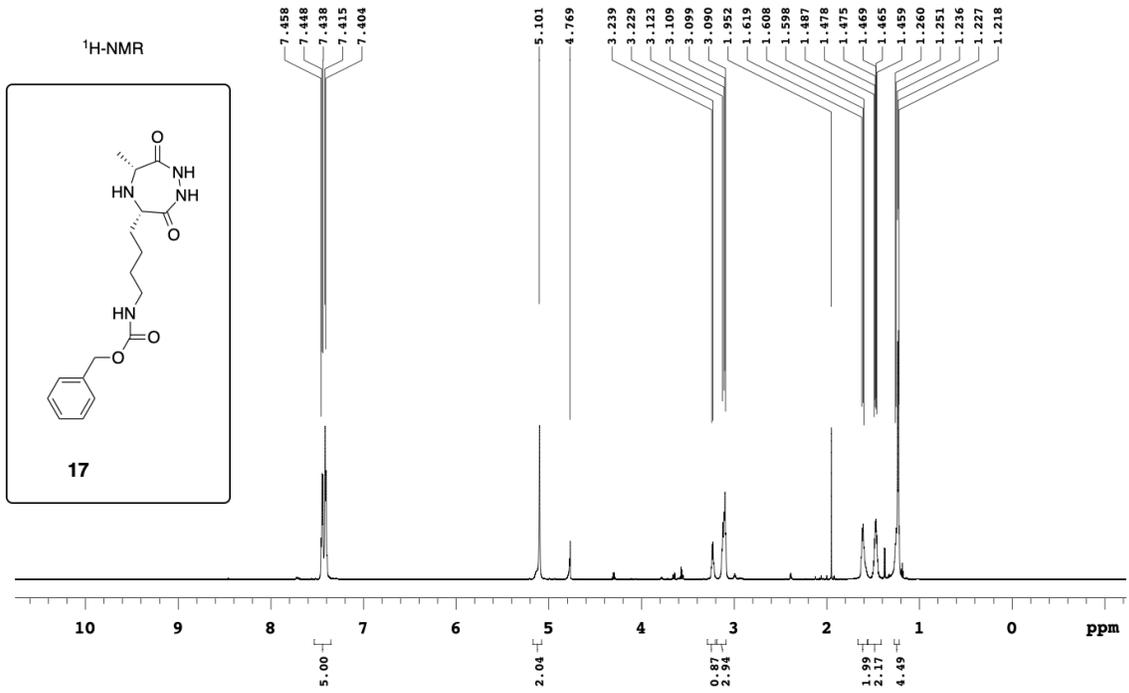


175.971 MHz C13(H1) 1D in cdcl3 (ref. to CDCl3 @ 77.06 ppm)  
temp 27.5 C -> actual temp = 27.0 C, coldid probe

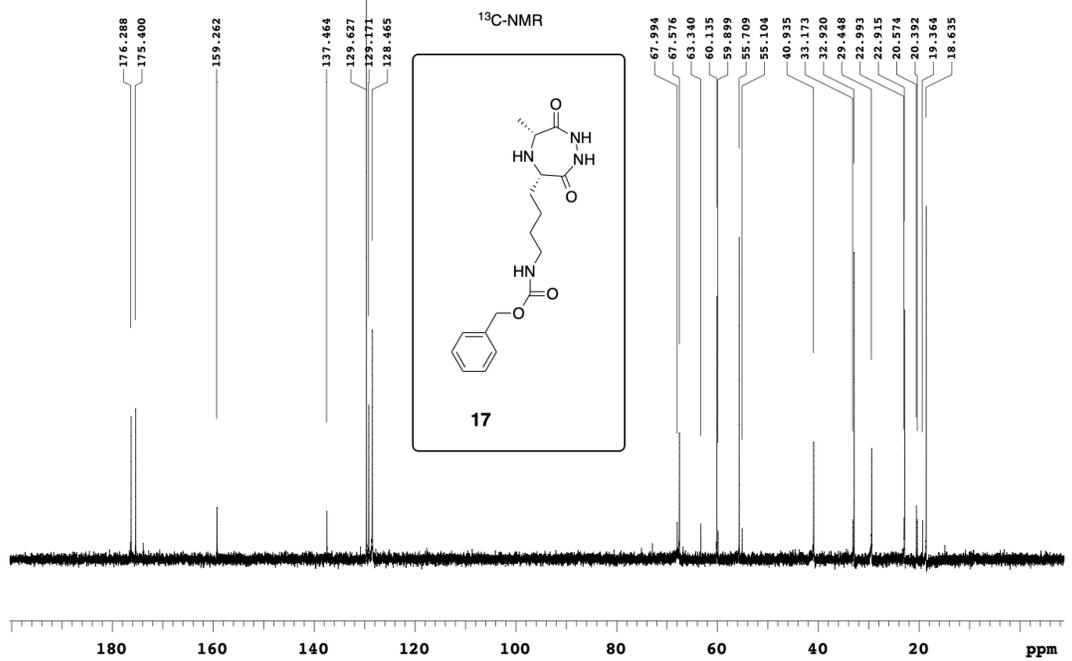




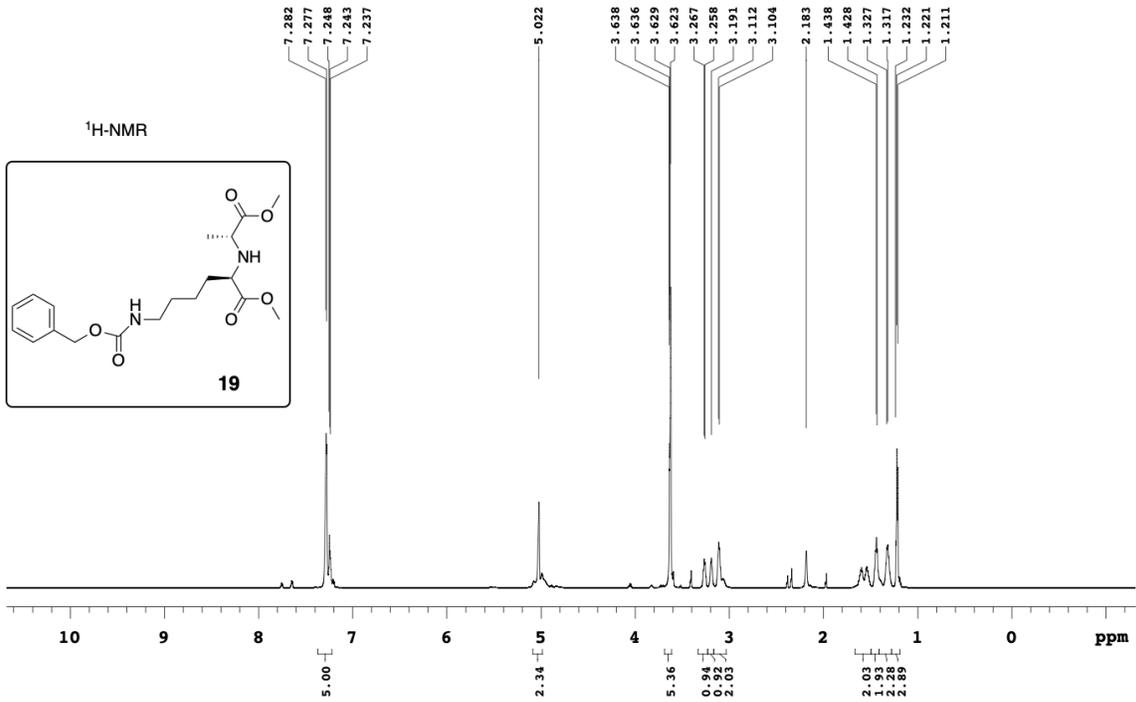
699.64 MHz H1 1U in d2o (ref. to external acetone @ 2.225 ppm)  
temp 27.5 C -> actual temp = 27.0 C, coldid probe



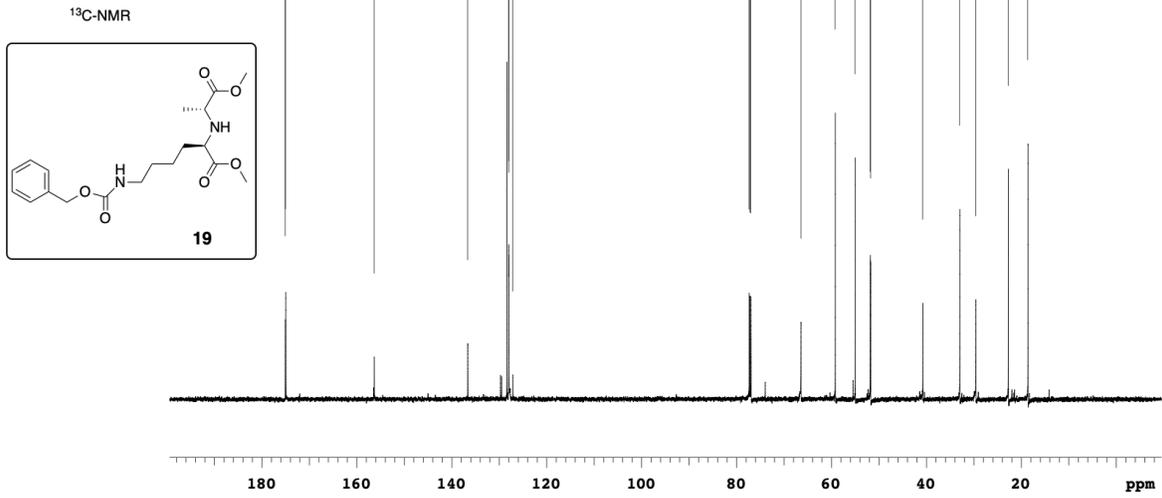
temp 27.5 C -> actual temp = 27.0 C, coldid probe

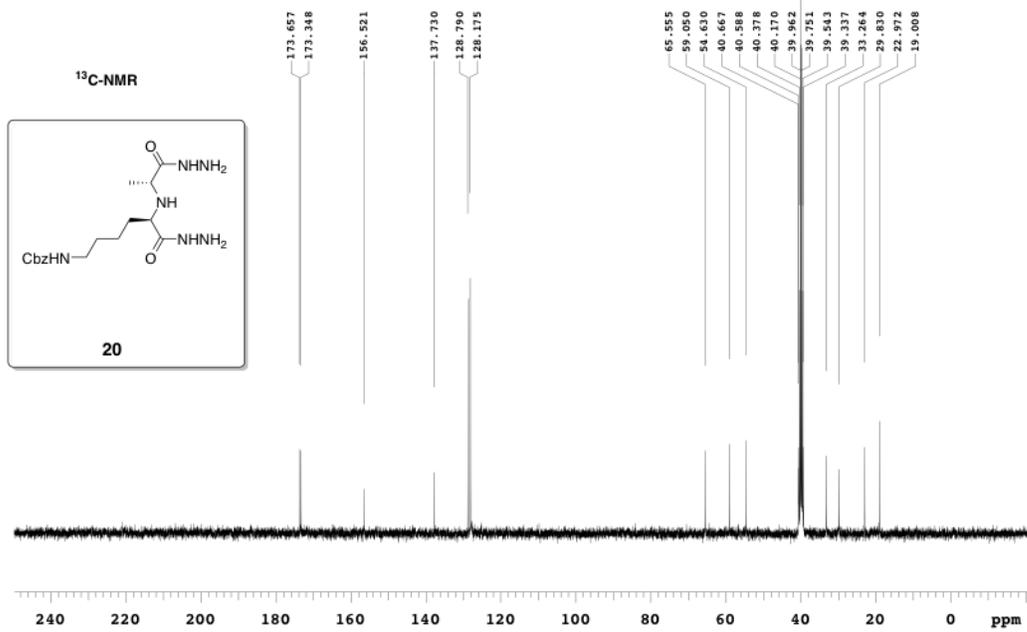
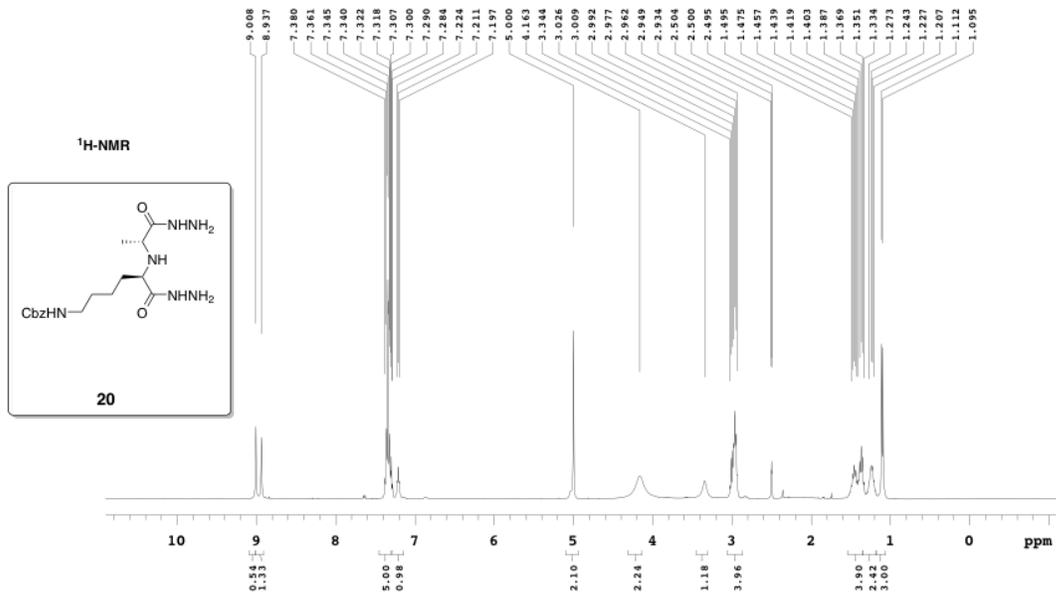


temp 27.5 C -> actual temp = 27.0 C, coldid probe



Isaac, IR-3-173  
175.971 MHz C13{H1} 1D in cdcl3 (ref. to CDCl3 @ 77.06 ppm)  
temp 27.5 C -> actual temp = 27.0 C, coldid probe





temp 27.5 C -> actual temp = 27.0 C, coldid probe

