Supporting Information

Discovery of C13-Aminobenzoyl Cycloheximide Derivatives that Potently Inhibit Translation Elongation

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Supplemental Figures

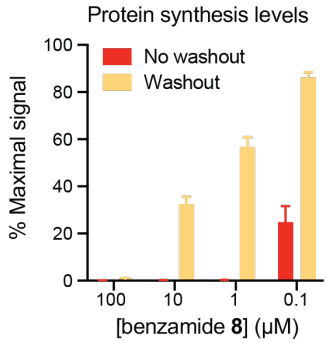


Figure S1: Washout assay for benzamide **8**. Error bars represent SE for *n*=3.

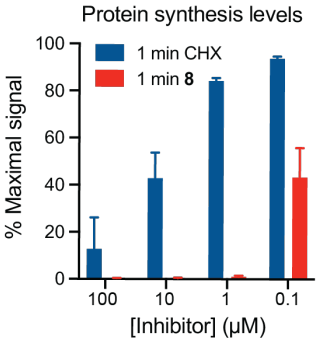


Figure S2: Protein synthesis levels after 1 minute treatment with either CHX (**1**) or benzamide **8**. All of the assay conditions except for the length of compound treatment were the same as the representative procedure. Error bars represent SE for *n*=3.

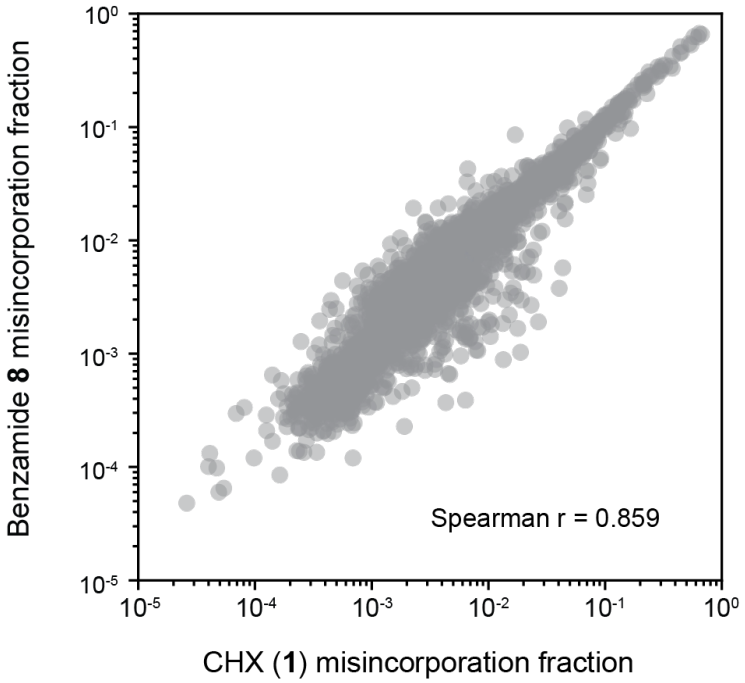


Figure S3: A scatter plot showing mutation rates of 28S and 18S rRNA due to DMS modification comparing *in vivo* pretreatment with **8** (y-axis) and with CHX (**1**; x-axis).

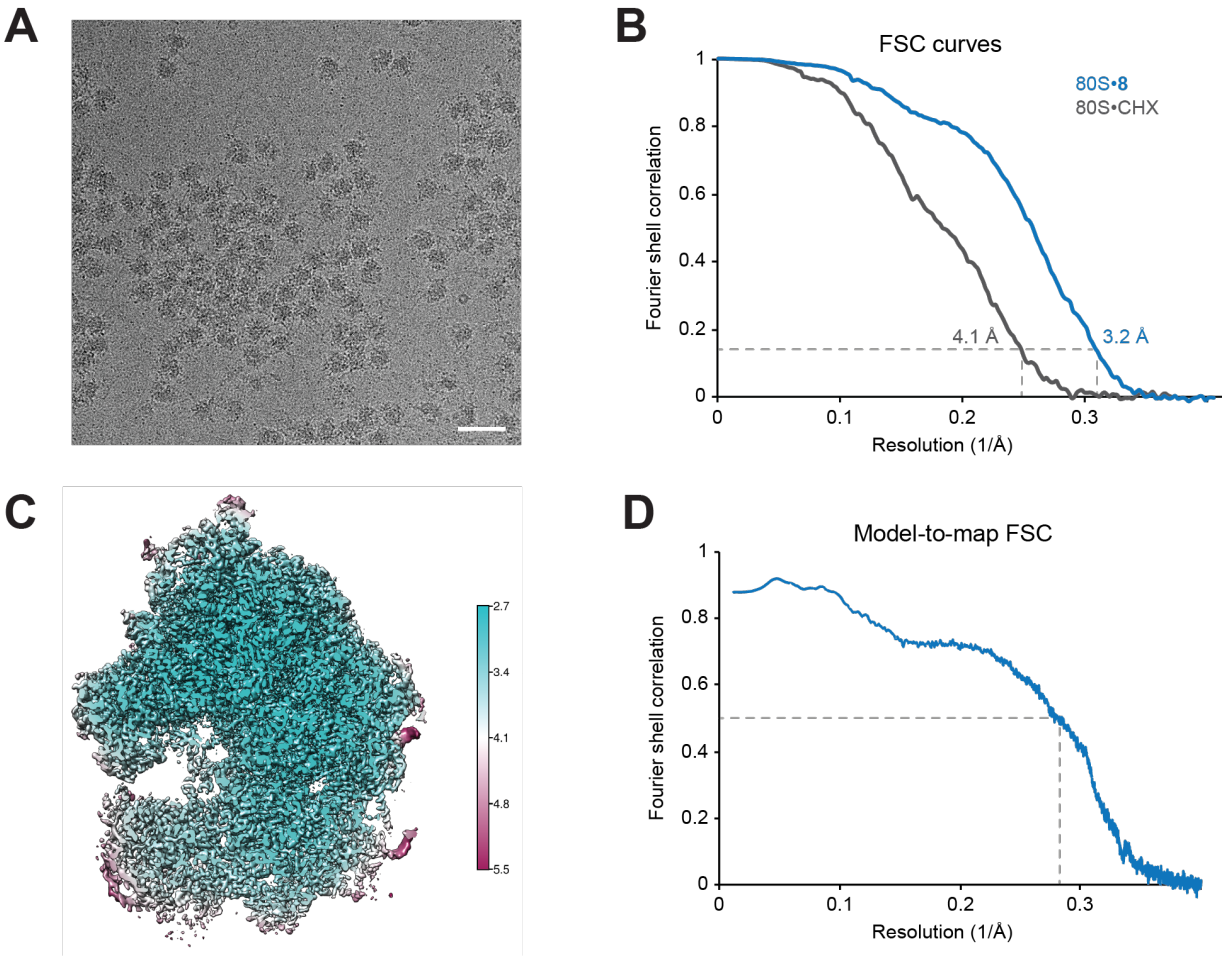


Figure S4: Assessment of model quality. **A**) A representative micrograph. The white scalebar is 500 Angstroms. **B**) Fourier shell correlation (FSC) curves of 80S•**8**•P-site tRNA (blue) and 80S•CHX•P-site tRNA (gray) cryo-EM maps. Resolutions of 3.2 Å and 4.1 Å were estimated at FSC=0.143. **C**) A cross-section of the 80S•**8**•P-site tRNA map colored by local resolution. **D**) FSC curve of the 80S•**8**•P-site tRNA model versus the EM map.

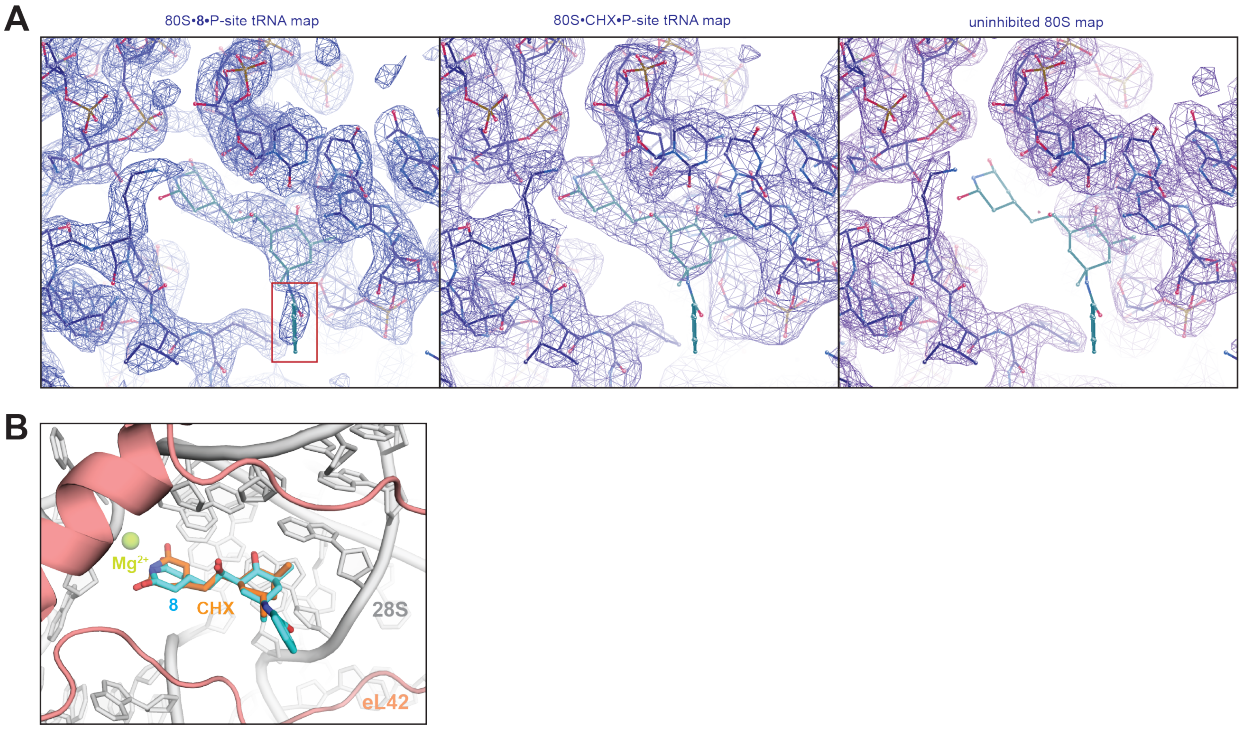


Figure S5: A) Side-by-side comparison of Coulomb potential maps around the canonical CHX binding pocket. The 80S•**8**•P-tRNA model (PDB 7MDZ) was overlayed with the 80S•**8**•P-tRNA (EMD-23785, left), 80S•CHX•P-tRNA (EMD-23787, center) or uninhibited 80S map (EMD-10181, right). The red box highlights the extra density corresponding to the benzamide group of **8**. **B**) Overlay of the 80S•**8**•P-tRNA model with the *S. cerevisiae* 80S•CHX structure (PDB 4U3U).

Table S1. Data collection, processing, and model statistics.

	80S•**8**• P-site tRNA EMDB 23785 PDB 7MDZ	80S•CHX• P-site tRNA EMDB 23787
Data collection and processing		
Voltage (kV)	300	300
Pixel size (Å)	1.23	1.36
Defocus range (μm)	-0.5 to -3	-1.5 to -3
Electron exposure ($e^-/Å^2$)	47	35
No. micrographs used	2,827	960
No. initial particles	222,917	102,474
No. final particles	34,783	18,241
Map resolution (Å) FSC threshold	3.2 0.143	4.1 0.143
Model composition		
Protein residues	11,315	
RNA bases	5,594	
Ligands (Zn^{2+}/Mg^{2+}/BNZ)	8/277/1	
Refinement		
Initial model (PDB code)	6SGC	
Model resolution (Å) FSC threshold	3.6 0.5	
Map sharpening B-factor ($Å^2$)	-44.2	
Model-to-map correlation		
CC (mask) CC (volume)	0.78 0.76	
Rms deviations		
Bond angles (Å)	0.004	
Angles (º)	0.74	
Ramachandran plot		
Favored (%)	94.4	
Allowed (%)	5.6	
Validation		
Molprobity score	1.83	
Clash score	8.11	
Rotamer outliers (%)	0.05	
EMRinger score	2.7	

Table S2. List of oversampled genes and undersampled genes in **8**-treated samples compared to CHX (**1**)-treated samples in ribosome profiling (cutoff: $|\log_2(\text{fold change})| > 1$, adjusted p-value < 0.05).

Ensembl Gene ID	Gene Gene ID	Log_2(fold change)	Adjusted p-value
ENSG00000100568.11	VTI1B	2.878	7.43E-08
ENSG00000105619.13	TFPT	4.113	1.03E-14
ENSG00000117984.14	CTSD	3.830	5.79E-03
ENSG00000125845.7	BMP2	4.824	1.09E-03
ENSG00000130520.10	LSM4	4.436	4.79E-68
ENSG00000137857.17	DUOX1	4.265	2.91E-29
ENSG00000145016.16	RUBCN	3.630	2.47E-03
ENSG00000172350.10	ABCG4	5.555	2.42E-06
ENSG00000252521.1	RNU5D-2P	5.122	2.88E-09
ENSG00000256618.2	MTRNR2L1	2.242	3.90E-03
ENSG00000269028.3	MTRNR2L12	3.689	2.70E-13
ENSG00000277399.4	GPR179	2.887	2.09E-02
ENSG00000109705.7	NKX3-2	-3.861	1.40E-02
ENSG00000139722.7	VPS37B	-2.693	2.19E-02
ENSG00000181143.15	MUC16	-5.651	6.81E-04
ENSG00000189433.6	GJB4	-2.914	4.95E-02
ENSG00000198786.2	MT-ND5	-2.483	2.54E-04
ENSG00000199377.1	RNU5F-1	-2.686	2.58E-10
ENSG00000200156.1	RNU5B-1	-2.276	3.57E-16
ENSG00000200972.1	RNU5A-8P	-2.577	7.64E-04
ENSG00000202538.1	RNU4-2	-3.400	1.34E-02
ENSG00000237973.1	MTCO1P12	-2.810	3.82E-02
ENSG00000251741.1	RNU4ATAC13P	-4.429	1.73E-31

Materials and Methods

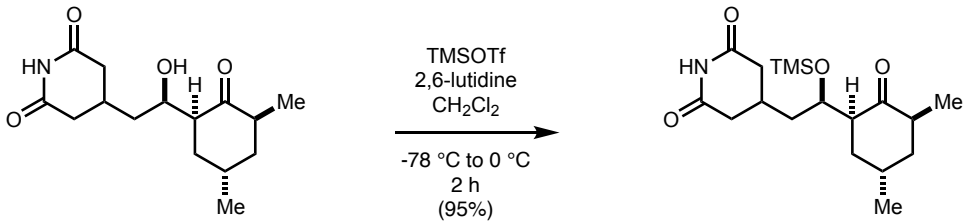
Organic Chemistry. All reactions were performed in oven-dried or flame-dried glassware under a positive pressure of nitrogen unless otherwise noted. Column chromatography was performed with a Biotage Isolera system employing silica gel 60 (230-400 mesh, Silicycle). Celite filtration was performed using Celite® 545 (EMD Millipore). Analytical thin-layer chromatography (TLC) was performed using 0.25 mm silica gel 60 F254 (EMD Millipore). TLC plates were visualized by fluorescence quenching under ultraviolet light (UV) and exposure to a solution of ceric ammonium molybdate, *p*-anisaldehyde, or potassium permanganate stain followed by heating on a hot plate. Reversed-phase chromatography was performed on an Agilent 1200 series HPLC using water and acetonitrile as a mobile phase and Agilent Pursuit C18 as a stationary phase.

Commercial reagents and solvents were used as received with the following exceptions: tetrahydrofuran (THF), diethyl ether (Et_2O), dichloromethane (CH_2Cl_2), toluene (PhMe), acetonitrile (MeCN), and *N*,*N*-dimethylformamide (DMF) were degassed with argon and passed through a solvent purification system (designed by Pure Process Technology) utilizing alumina columns.

NMR spectra were recorded with a Varian INOVA-500 spectrometer, are reported in parts per million (δ), and are calibrated using residual undeuterated solvent as an internal reference ($CDCl_3$: δ 7.26 for 1H NMR and δ 77.00 for 13C NMR; CD_3CN: δ 1.94 for 1H NMR and δ 118.26; CD_3OD: δ 3.31 for 1H NMR and δ 49.00 for 13C NMR). Data for 1H NMR spectra are reported as follows: chemical shift (δ ppm) (multiplicity, coupling constant (Hz), integration). Multiplicities are reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, or combinations thereof. High-resolution mass spectra (HRMS) were recorded using electrospray ionization (ESI) mass spectroscopy experiments on an Agilent 6210 TOF LC/MS.

Cell Culture. For cell culture, all media were supplemented with 100 U mL-1 penicillin and 100 μg mL-1 streptomycin (Life Technologies) and fetal bovine serum (FBS, Peak Serum). K562 cells were maintained in RPMI-1640 (Life Technologies), 10% FBS. 293T cells were maintained in Dulbecco's Modified Eagle Medium (DMEM, Life Technologies) supplemented with 10% FBS. All cell lines were authenticated by STR profiling and routinely tested for mycoplasma (Sigma-Aldrich).

Experimental Procedures



cycloheximide (**1**) **5**

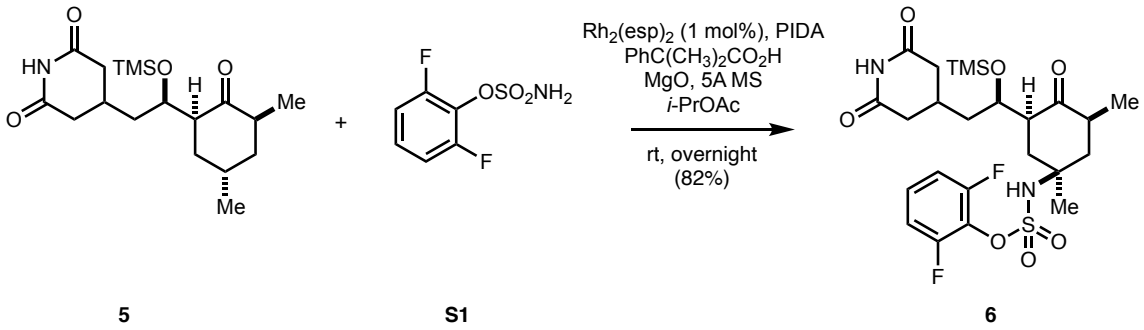
Silyl ether 5

To a cooled (–78 °C) solution of cycloheximide (**1**) (2.00 g, 7.11 mmol) in CH_2Cl_2 (35 mL, 0.2 M) was added 2,6-lutidine (1.74 mL, 14.9 mmol, 2.1 equiv) and TMSOTf (2.74 mL, 14.9 mmol, 2.1 equiv). The reaction mixture was warmed to 0 °C and stirred for 2 hours. The reaction was quenched by the addition of saturated aqueous $NaHCO_3$ solution, and then diluted with EtOAc and water. The layers were separated, and the organic layer was washed with 0.1 M aqueous HCl solution. The layers were separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (hexane/EtOAc, 4:1 to 2:1) to afford **5** as a white solid (2.40 g, 6.79 mmol, 95%).

1H NMR (500 MHz, $CDCl_3$) δ 7.73 (s, 1H), 4.24 (ddd, J = 9.1, 6.0, 3.0 Hz, 1H), 2.86–2.78 (m, 1H), 2.72–2.62 (m, 1H), 2.55 (dp, J = 13.1, 7.0, 6.5 Hz, 1H), 2.43 (dt, J = 12.3, 5.6 Hz, 1H), 2.40–2.15 (m, 4H), 2.05–1.93 (m, 1H), 1.92–1.81 (m, 1H), 1.71 (td, J = 13.1, 4.9 Hz, 1H), 1.66–1.55 (m, 2H), 1.49–1.35 (m, 1H), 1.24 (d, J = 7.2 Hz, 3H), 0.99 (d, J = 6.5 Hz, 3H), 0.11 (s, 9H).

13C NMR (126 MHz, $CDCl_3$) δ 213.4, 172.8, 172.6, 67.6, 50.8, 42.5, 41.4, 40.5, 38.9, 37.4, 35.6, 27.0, 26.9, 18.3, 14.4, 0.7.

HRMS (ESI) (m/z) calc'd for $C_{18}H_{32}NO_4Si$ $[M+H]^+$: 354.2095, found 354.2086.



5 **S1** **6**

Sulfamate 6[1]

Flame dried MgO (456 mg, 11.3 mmol, 4 equiv), flame dried 5Å MS (1.6 mm pellets, 566 mg, 200 mg per 1 mmol of **5**), **S1** (769 mg, 3.68 mmol, 1.3 equiv), 2-phenylisobutyric acid (232 mg, 1.41 mmol, 0.5 equiv), $Rh_2(esp)_2$ (21 mg, 0.0283 mmol, 1 mol%), and **5** (1.00 g, 2.83 mmol, 1 equiv) was suspended in *i*-PrOAc (5.7 mL, 0.5 M) at room temperature. After 5 minutes, PIDA (1.82 g, 5.66 mmol, 2 equiv) was added. The reaction was stirred overnight, filtered through a pad of celite, and purified by column chromatography (hexane/EtOAc, 4:1 to 1:4) to afford **6** as a white solid (1.30 g, 2.32 mmol, 82%).

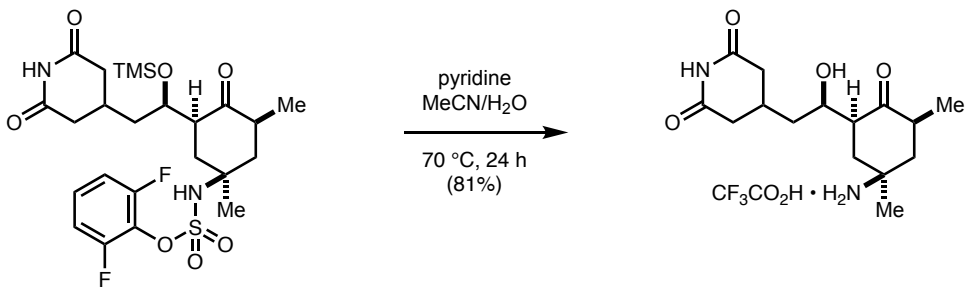
1H NMR (500 MHz, $CDCl_3$) δ 7.98 (s, 1H), 7.25–7.16 (m, 1H), 7.06–6.97 (m, 2H), 5.20 (s, 1H), 4.45–4.31 (m, 1H), 2.79 (ddd, *J* = 16.8, 4.0, 1.8 Hz, 1H), 2.68 (ddd, *J* = 16.6, 3.8, 1.7 Hz, 1H), 2.50 (dp, *J* = 12.4, 6.3 Hz, 1H), 2.44–2.17 (m, 6H), 2.09 (t, *J* = 12.3 Hz, 1H), 1.89 (t, *J* = 13.2 Hz, 1H), 1.81 (s, 3H), 1.66–1.54 (m, 1H), 1.41 (ddd, *J* = 13.8, 8.7, 3.5 Hz, 1H), 1.06 (d, *J* = 6.5 Hz, 3H), 0.10 (s, 9H).

13C NMR (126 MHz, $CDCl_3$) δ 210.1, 172.3, 172.1, 156.0 (dd, *J* = 253.4, 3.6 Hz), 127.40 (t, *J* = 9.1 Hz), 126.85 (t, *J* = 15.6 Hz), 112.53 (dd, *J* = 18.5, 4.1 Hz), 66.8, 57.4, 51.1, 45.6, 41.0, 38.9, 38.6, 37.2, 27.0, 23.3, 14.2, 0.5.

19F NMR (471 MHz, $CDCl_3$) δ –124.78. ($PhCF_3$ as an internal standard; referenced to –63.72)

HRMS (ESI) (m/z) calc'd for $C_{24}H_{35}F_2N_2O_7SSi$ $[M+H]^+$: 561.1897, found 561.1882.

[1] Reference for C−H amination: Roizen, J. L., Zalatan, D. N., Du Bois, J. Selective Intermolecular Amination of C−H Bonds at Tertiary Carbon Centers. *Angew. Chem. Int. Ed.* **2013**, *52*, 11343–11346.



6 **7**

Amine 7[2]

To a stirred solution of **6** (1.30 g, 2.32 mmol, 1 equiv) in MeCN/H_2O (2/1, v/v, 24 mL, 0.1 M) was added pyridine (3.74 mL, 46.4 mmol, 20 equiv). The mixture was stirred at 70 °C for 24 hours and concentrated under reduced pressure. The residue was purified by reversed-phase column chromatography (5% to 50% MeCN in water, 0.1% TFA) with concomitant desilylation to afford **7** as a white solid (770 mg, 1.88 mmol, 81%).

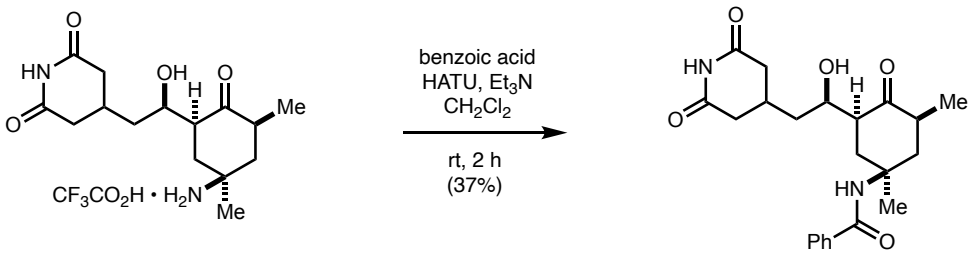
1H NMR (500 MHz, D_2O) δ 4.37–4.29 (m, 1H), 3.02–2.74 (m, 4H), 2.68–2.49 (m, 2H), 2.40 (ddd, *J* = 12.8, 5.6, 3.6 Hz, 1H), 2.34 (ddd, *J* = 12.7, 5.7, 3.7 Hz, 1H), 2.21 (d, *J* = 6.7 Hz, 1H), 2.07 (t, *J* = 13.2 Hz, 1H), 1.90 (t, *J* = 13.3 Hz, 1H), 1.82 (s, 3H), 1.68 (ddd, *J* = 14.3, 10.1, 4.4 Hz, 1H), 1.62–1.50 (m, 1H), 1.13 (d, *J* = 6.5 Hz, 3H).

13C NMR (126 MHz, D_2O/CD_3CN, 2/1) δ 212.7, 175.5, 175.4, 64.4, 52.6, 49.2, 42.6, 39.6, 37.8, 36.7, 35.4, 35.3, 26.2, 20.7, 12.5.

HRMS (ESI) (m/z) calc'd for $C_{15}H_{25}N_2O_4$ $[M+H]^+$: 297.1809, found 297.1802.

[2] Reference for deprotection: Chiappini, N. D., Mack, J. B. C., Du Bois, J. Intermolecular C(sp^3)−H Amination of Complex Molecules. *Angew. Chem. Int. Ed.* **2018**, *130*, 5050–5053.

Representative Procedure for Amide Bond Formation:



7 **8**

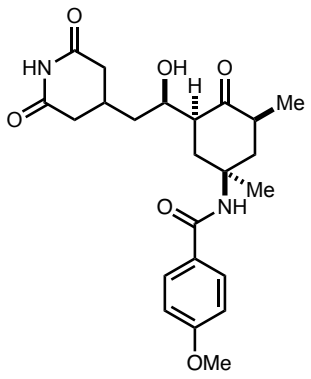
Amide 8

To a stirred solution of **7** (25 mg, 0.0609 mmol, 1 equiv) in CH_2Cl_2 was added benzoic acid (8 mg, 0.0670 mmol, 1.1 equiv), HATU (26 mg, 0.0670 mmol, 1.1 equiv), and Et_3N (43 µL, 0.305 mmol, 5 equiv). The mixture was stirred for 2 hours, concentrated under reduced pressure, purified by column chromatography (hexane/EtOAc, 1:1 to EtOAc only), and purified by reversed-phase HPLC (5% to 60% MeCN in water) to afford **8** as a colorless oil (9 mg, 0.0225 mmol, 37%).

1H NMR (500 MHz, $CDCl_3$) δ 7.94 (s, 1H), 7.69 (d, *J* = 7.4 Hz, 2H), 7.49 (t, *J* = 7.3 Hz, 1H), 7.42 (t, *J* = 7.5 Hz, 2H), 6.03 (s, 1H), 4.30–4.23 (m, 1H), 2.84–2.73 (m, 3H), 2.63 (dq, *J* = 12.6, 6.3 Hz, 1H), 2.57–2.43 (m, 5H), 2.34 (ddd, *J* = 19.6, 16.8, 10.2 Hz, 2H), 2.25–2.14 (m, 1H), 1.92 (t, *J* = 13.1 Hz, 1H), 1.83 (s, 3H), 1.68 (ddd, *J* = 15.9, 10.9, 5.2 Hz, 1H), 1.35–1.18 (m, 1H), 1.06 (d, *J* = 6.3 Hz, 3H).

13C NMR (126 MHz, $CDCl_3$) δ 214.0, 172.0, 171.8, 167.0, 135.2, 131.5, 128.6, 126.7, 66.3, 53.1, 50.7, 45.7, 41.0, 38.4, 37.9, 37.2, 37.0, 27.7, 23.0, 14.0.

HRMS (ESI) (m/z) calc'd for $C_{22}H_{29}N_2O_5$ $[M+H]^+$: 401.2071, found 401.2061.



9

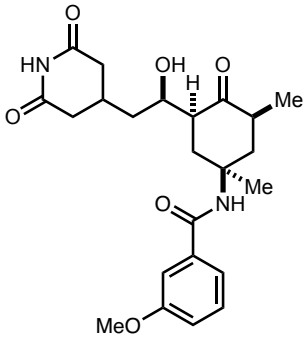
Amide 9

Following the representative procedure above, Amide **9** was obtained as a colorless oil (7 mg, 0.0163 mmol, 67%).

1H NMR (500 MHz, $CDCl_3$) δ 7.78 (s, 1H), 7.66 (d, *J* = 8.8 Hz, 2H), 6.91 (d, *J* = 8.8 Hz, 2H), 5.92 (s, 1H), 4.30–4.24 (m, 1H), 3.85 (s, 3H), 2.86–2.73 (m, 2H), 2.74–2.57 (m, 2H), 2.56–2.41 (m, 4H), 2.41–2.24 (m, 2H), 2.23–2.12 (m, 1H), 1.90 (t, *J* = 13.0 Hz, 1H), 1.82 (s, 3H), 1.69 (ddd, *J* = 14.3, 11.2, 5.3 Hz, 1H), 1.24 (dd, *J* = 14.0, 8.4 Hz, 1H), 1.06 (d, *J* = 6.5 Hz, 3H).

13C NMR (126 MHz, $CDCl_3$) δ 214.1, 171.9, 171.7, 166.5, 162.2, 128.5, 127.4, 113.8, 66.4, 55.4, 53.0, 50.7, 45.8, 41.0, 38.5, 37.8, 37.3, 37.0, 27.7, 23.1, 14.0.

HRMS (ESI) (m/z) calc'd for $C_{23}H_{31}N_2O_6$ $[M+H]^+$: 431.2177, found 431.2165.



10

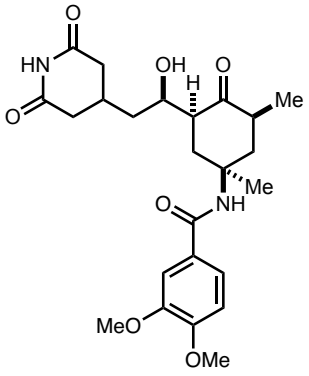
Amide 10

Following the representative procedure above, Amide **10** was obtained as a colorless oil (8 mg, 0.0186 mmol, 76%).

1H NMR (500 MHz, $CDCl_3$) δ 7.87 (s, 1H), 7.37–7.29 (m, 2H), 7.25–7.17 (m, 1H), 7.12–6.99 (m, 1H), 6.03 (s, 1H), 4.29 (dd, *J* = 9.2, 2.1 Hz, 1H), 3.86 (s, 3H), 2.89–2.76 (m, 2H), 2.73 (dd, *J* = 4.2, 1.6 Hz, 1H), 2.65 (dq, *J* = 12.8, 6.6 Hz, 1H), 2.58–2.44 (m, 4H), 2.36 (ddd, *J* = 19.4, 17.0, 10.2 Hz, 2H), 2.26–2.17 (m, 1H), 1.93 (t, *J* = 13.1 Hz, 1H), 1.84 (s, 3H), 1.70 (ddd, *J* = 14.0, 11.0, 5.2 Hz, 1H), 1.26 (dd, *J* = 14.6, 7.9 Hz, 1H), 1.08 (d, *J* = 6.3 Hz, 3H).

13C NMR (126 MHz, $CDCl_3$) δ 214.0, 171.9, 171.8, 166.8, 159.9, 136.6, 129.6, 118.4, 117.7, 112.2, 66.3, 55.4, 53.1, 50.7, 45.7, 41.0, 38.5, 37.8, 37.3, 36.9, 27.7, 23.0, 14.0.

HRMS (ESI) (m/z) calc'd for $C_{23}H_{31}N_2O_6$ $[M+H]^+$: 431.2177, found 431.2167.



11

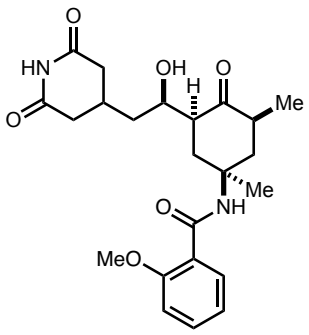
Amide 11

Following the representative procedure above, Amide **11** was obtained as a colorless oil (10 mg, 0.0217 mmol, 89%).

1H NMR (500 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.37 (d, *J* = 2.1 Hz, 1H), 7.17 (dd, *J* = 8.4, 2.1 Hz, 1H), 6.84 (d, *J* = 8.3 Hz, 1H), 5.96 (s, 1H), 4.31–4.25 (m, 1H), 3.93 (s, 3H), 3.92 (s, 3H), 2.87–2.74 (m, 2H), 2.73–2.69 (m, 1H), 2.64 (dp, *J* = 12.3, 6.0, 5.6 Hz, 1H), 2.57–2.42 (m, 4H), 2.41–2.28 (m, 2H), 2.19 (t, *J* = 12.1 Hz, 1H), 1.91 (t, *J* = 13.1 Hz, 1H), 1.83 (s, 3H), 1.69 (ddd, *J* = 14.0, 11.0, 5.1 Hz, 1H), 1.30–1.18 (m, 1H), 1.06 (d, *J* = 6.3 Hz, 3H).

13C NMR (126 MHz, $CDCl_3$) δ 214.1, 171.9, 171.7, 166.6, 151.8, 149.1, 127.7, 118.9, 110.5, 110.2, 66.3, 56.0, 53.0, 50.7, 45.8, 41.0, 38.5, 37.8, 37.3, 37.0, 27.7, 23.1, 14.1.

HRMS (ESI) (m/z) calc'd for $C_{24}H_{33}N_2O_7$ $[M+H]^+$: 461.2282, found 461.2270.



12

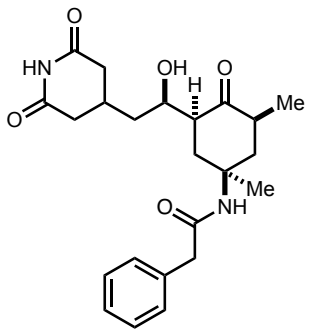
Amide 12

Following the representative procedure above, Amide **12** was obtained as a colorless oil (4 mg, 0.00929 mmol, 38%).

1H NMR (500 MHz, CD_3OD) δ 7.81 (d, *J* = 7.8 Hz, 1H), 7.51–7.44 (m, 1H), 7.12 (d, *J* = 8.3 Hz, 1H), 7.04 (t, *J* = 7.5 Hz, 1H), 4.18–4.06 (m, 1H), 3.96 (s, 3H), 2.82–2.53 (m, 5H), 2.51–2.31 (m, 4H), 2.09 (t, *J* = 14.4 Hz, 1H), 1.93 (t, *J* = 13.4 Hz, 1H), 1.82 (s, 3H), 1.59–1.43 (m, 2H), 1.02 (d, *J* = 6.3 Hz, 3H).

13C NMR (126 MHz, CD_3OD) δ 214.0, 175.6, 175.5, 167.4, 158.8, 133.8, 131.6, 124.3, 122.0, 112.9, 67.1, 56.6, 54.5, 52.3, 46.8, 42.0, 41.0, 39.5, 39.4, 37.9, 29.0, 23.2, 14.6.

HRMS (ESI) (m/z) calc'd for $C_{23}H_{31}N_2O_6$ $[M+H]^+$: 431.2177, found 431.2165.



13

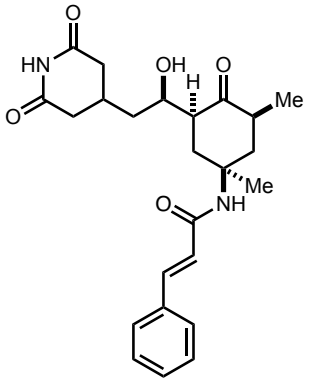
Amide 13

Following the representative procedure above, Amide **13** was obtained as a colorless oil (4.5 mg, 0.0109 mmol, 45%).

1H NMR (500 MHz, CD3OD) δ 7.39–7.16 (m, 5H), 4.08 (dt, *J* = 8.4, 3.9 Hz, 1H), 3.44 (s, 2H), 2.81–2.69 (m, 1H), 2.69–2.57 (m, 2H), 2.57–2.42 (m, 2H), 2.42–2.24 (m, 4H), 1.95 (t, *J* = 12.8 Hz, 1H), 1.81 (t, *J* = 13.2 Hz, 1H), 1.68 (s, 3H), 1.56–1.36 (m, 2H), 0.97 (d, *J* = 6.5 Hz, 3H).

13C NMR (126 MHz, CD3OD) δ 214.0, 175.6, 175.5, 173.4, 137.3, 130.0, 129.5, 127.8, 127.3, 67.1, 54.2, 52.3, 46.7, 44.6, 41.9, 41.0, 39.3, 39.1, 37.9, 29.0, 23.0, 14.6.

HRMS (ESI) (m/z) calc'd for $C_{23}H_{31}N_2O_5$ $[M+H]^+$: 415.2227, found 415.2218.



14

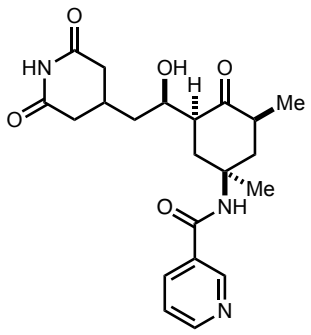
Amide 14

Following the representative procedure above, Amide **14** was obtained as a colorless oil (4.4 mg, 0.0103 mmol, 42%).

1H NMR (500 MHz, CD_3OD) δ 7.60–7.30 (m, 6H), 6.62 (d, J = 15.7 Hz, 1H), 4.15–4.08 (m, 1H), 2.84–2.62 (m, 3H), 2.61–2.48 (m, 2H), 2.44–2.31 (m, 4H), 2.13–2.00 (m, 1H), 1.90 (t, J = 13.7 Hz, 1H), 1.77 (s, 3H), 1.61–1.39 (m, 2H), 1.01 (d, J = 6.5 Hz, 3H).

13C NMR (126 MHz, CD_3OD) δ 214.1, 175.6, 175.5, 168.0, 141.2, 136.4, 130.7, 129.9, 128.8, 123.0, 67.1, 54.3, 52.3, 46.8, 42.0, 41.0, 39.4, 39.3, 38.0, 29.0, 23.1, 14.7.

HRMS (ESI) (m/z) calc'd for $C_{24}H_{31}N_2O_5$ $[M+H]^+$: 427.2227, found 427.2217.



15

Amide 15

Following the representative procedure above, Amide **15** was obtained as a colorless oil (3.5 mg, 0.00872 mmol, 36%).

1H NMR (500 MHz, CD_3OD) δ 8.89 (s, 1H), 8.65 (d, *J* = 5.0 Hz, 1H), 8.16 (d, *J* = 7.9 Hz, 1H), 7.51 (dd, *J* = 8.2, 4.9 Hz, 1H), 4.20–4.12 (m, 1H), 2.83–2.55 (m, 5H), 2.47 (dq, *J* = 13.3, 3.2 Hz, 1H), 2.43–2.31 (m, 3H), 2.20–1.91 (m, 2H), 1.82 (s, 3H), 1.61–1.41 (m, 2H), 1.02 (d, *J* = 6.3 Hz, 3H).

13C NMR (126 MHz, CD_3OD) δ 214.0, 175.6, 175.5, 167.9, 152.3, 149.1, 137.1, 133.4, 125.0, 67.0, 55.0, 52.2, 46.4, 41.9, 41.0, 39.4, 39.0, 38.0, 29.0, 23.0, 14.8.

HRMS (ESI) (m/z) calc'd for $C_{21}H_{28}N_3O_5$ $[M+H]^+$: 402.2023, found 402.2012.

O-Propargyl Puromycin (OPP) Flow Cytometry Translation Inhibition Assays[3]

Representative procedure:

K562 cells were plated into 96-well plates (10^5 cells in 100 μL media per well) in R10 media (RPMI with glutamine, 10% FBS and 1% penicillin-streptomycin) in triplicate per condition. Stock solutions of inhibitors CHX and benzamide **8** were prepared in DMSO (100 mM). Stock solutions of *O*-propargyl puromycin (OPP, 120 mM) were prepared in DMSO.

Well #	1	2	3	4	5	6	7	8	9	10
CHX (μM)	0	0	100	32	10	3.2	1	0.32	0.1	0.032
OPP (μM)	0	20	20	20	20	20	20	20	20	20
DMSO (%)	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12

Table S2. Concentrations of each reagent for dose-curve analysis.

Inhibitor (100 μM to 31.6 nM final concentration in R10) or DMSO (0.1% final concentration in R10) was added to cells in triplicate, and the cells were incubated at 37 °C for 30 minutes (**Table S2**). The cells were then treated with *O*-propargyl puromycin (20 μM final concentration in R10) or DMSO (0.12% final concentration in R10), and the cells were incubated at 37 °C for 60 minutes. The media was removed, and the cells were washed with PBS twice. The cells were then fixed in 1% formaldehyde in PBS at 0 °C for 15 min. The formaldehyde solution was removed, and the cells were washed with PBS once. The cells were permeabilized by re-suspension in 20 μL of PBS containing 0.1% saponin and 2% BSA at room temperature. After 10 minutes, the Click-iT Cell Reaction Kit (Life Technologies) was used to tag the alkyne-labeled peptides with MB 488 picolyl azide (Click Chemistry Tools, 10 μM final concentration). After 30 minutes at room temperature, the reaction solution was removed, and the cells were washed twice in PBS containing 0.1% saponin and 2% BSA, resuspended in the same buffer, and analyzed by flow cytometry (>10^4 events/well).

Gating was performed as previously described.[4] Briefly, a polygon gate was drawn to exclude dead cells using forward and side scatter. Then, a bi-range gate was set up to exclude approximately 99% of DMSO-treated cells to define a FITC-positive population. A maximum signal was obtained when cells were only treated with OPP, and a minimum signal was obtained when cells were only treated with DMSO. % Maximal signal was defined as ($FITC_{drug,OPP}$ – $FITC_{DMSO,DMSO}$) / ($FITC_{DMSO,OPP}$ – $FITC_{DMSO,DMSO}$). From these values, a dose-response curve was generated using GraphPad Prism.

[3] Liu, J.; Xu, Y.; Stoleru, D.; Salic, A. Imaging Protein Synthesis in Cells and Tissues with an Alkyne Analog of Puromycin. *Proc. Natl. Acad. Sci.* **2012**, *109* (2), 413 LP – 418.

[4] Park, Y.; Koga, Y.; Su, C.; Waterbury, A. L.; Johnny, C. L.; Liau, B. B. Versatile Synthetic Route to Cycloheximide and Analogues That Potently Inhibit Translation Elongation. *Angew. Chemie - Int. Ed.* **2019**, *58* (16), 5387–5391.

Compound Washout Experiments

K562 cells were plated into 96-well plates (10^5 cells in 100 µL media per well) in R10 media in triplicate per condition. After being treated with drugs (see above for details) or DMSO (0.1% v/v) in R10, the cells were incubated at 37 °C for 30 min and were then washed twice with R10 and resuspended in 0.1% DMSO in R10. The washes were not performed for non-washout samples. The cells were treated with *O*-propargyl puromycin (20 µM final concentration in R10) or DMSO (0.11% v/v) at 37 °C for 60 min. Fixation, permeabilization, cycloaddition, and flow cytometry analysis were performed as described above.

Polysome Profiling

293T cells were grown on 10 cm^2 tissue culture plates to approximately 90% confluency. All stock solutions of drugs including CHX were made in DMSO. The cells were washed with 5 mL PBS twice before drug treatment. Benzamide **8** (100 µM final concentration in PBS) or DMSO (0.1% final concentration in PBS) was added to cells, and the cells were incubated at 37 °C for 30 minutes, washed twice with either PBS containing 100 µM CHX (for CHX control) or in PBS (for **8**-treated cells), scraped, and collected by brief centrifugation. The cell pellet was resuspended in 300 µL polysome lysis buffer (5 mM Tris-HCl pH 7.4, 2.5 mM $MgCl_2$, 1.5 mM KCl) with 70 U RNase inhibitor added, and 3.5 µL of either 10 mM CHX dissolved in 10% DMSO in PBS (for CHX control) or 10% DMSO in PBS (for **8**-treated cells) was added immediately. Subsequently, 7 µL 0.1 mM DTT was added, vortexed, 17.5 µL 10% Triton-X 100 and 17.5 µL 10% sodium deoxycholate were added, vortexed again, and the mixture was centrifuged for 2 min at 14,000 × g. The supernatant was frozen immediately in isopropanol/dry ice and stored at –80 °C until the sucrose gradient was ready. The thawed supernatant (amount normalized based on OD260 measurement) was loaded onto 10%–50% sucrose gradients prepared in 10 mM HEPES pH 7.4, 100 mM KCl, 5 mM $MgCl_2$ and centrifuged in an SW41 ultracentrifuge at 40,000 × g for 2 hours at 4 °C. Gradients were analyzed by BioComp Gradient Profiler connected to Bio-Rad Model EM-1 Econo™ UV Monitor.

Ribosome Profiling and Data Processing

Ribosome profiling using CHX and benzamide **8** was carried out according to the previously described protocol[5] with minor modifications described below. 293T cells were grown on 10 cm^2 tissue culture plates to approximately 90% confluency and were treated for 1 min with 350 µM CHX or 350 µM benzamide **8** at 37 °C. Cells were lysed and the lysates were clarified by centrifugation for 10 min at 20,000g, 4 °C. For each condition, lysate containing 30 µg total RNA (determined by Qubit RNA BR Assay Kit, Thermo Fisher Scientific) was used for nuclease footprinting. The digestion was transferred to 8 × 34 mm thick wall polycarbonate ultracentrifuge tube (Beckman 343776) on top of 0.25 mL sucrose cushion. Ribosome recovery was carried out by centrifugation in a TLA 100.1 rotor at 100,000 rpm, 4 °C for 3 h 40 min. The RNA from the pelleted ribosomes was purified first using the Direct-zol RNA Miniprep kit (Zymo Research) and

[5] McGlincy, N. J.; Ingolia, N. T. Transcriptome-Wide Measurement of Translation by Ribosome Profiling. *Methods* **2017**, *126*, 112–129.

then by precipitating with isopropanol. Size selection by running a 15% polyacrylamide TBE-Urea gel for 65 min at 200 V in 1X TBE, staining the gel for 3 min with 1X SYBR Gold in TBE, and excising the 17 nt – 34 nt region. The size-selected RNA was extracted in the gel extraction buffer and purified by isopropanol precipitation. The resulting RNA was resuspended in 10 µL 10 mM Tris pH 8, and end repaired by T4 polynucleotide kinase (New England Biolabs, hereafter NEB). Linker ligation was performed using T4 RNA ligase 2 truncated K227Q (NEB) and linkers pre-adenylated using 5' DNA adenylatioin kit (NEB). NI-812 linker (barcode CGTAA) was used for footprints from CHX-treated cells and NI-810 (barcode ATCGT) was used for footprints from **8**-treated cells. Depletion of the unligated linker from the ligation reaction was carried out by adding 1 µL yeast 5'-deadenylase 10 U/µL and 1 µL RecJ exonuclease 10 U/µL (epicenter definition) directly to the ligation reaction and incubating for 45 min at 30 °C. After pooling and purification of ligations, ribosomal RNA was depleted using RiboMinus™ Eukaryote System v2 (Thermo Fisher Scientific) according to the manufacturer's instruction. The resulting RNA was reverse transcribed using SuperScript III RT (Invitrogen) and the reverse transcription products were separated from the unextended primers and no-insert background products by running a 15% polyacrylamide TBE-Urea gel for 40 min at 180 V. The reverse transcription products were extracted from the excised bands in the DNA gel extraction buffer and were purified by isopropanol precipitation. The resulting cDNA was circularized by CircLigase II (EpiCentre) and was quantified by qPCR. Library construction was carried out by 9 cycles of PCR using Q5 Hot Start High-Fidelity DNA Polymerase (NEB). After purification of the DNA library, library length distribution was analyzed by TapeStation 2200 (Agilent) using High Sensitivity D1000 ScreenTape. The first technical replicate was sequenced for 51 cycles (paired end) on an Illumina MiSeq, and the second replicate was sequenced for 150 cycles (single read) on an Illumina NextSeq.

Adapter sequences were trimmed by the sequencing center at Bauer Core Facility at Harvard University. The paired reads were merged by NGmerge[6] (first replicate only). After barcode splitting by FASTX-Toolkit,[7] barcodes were trimmed and reads shorter than 8 bp were filtered out by Cutadapt.[8] UMI was processed using custom code. Low quality reads (below 15) were discarded by Cutadapt. After removing reads mapping to ribosomal RNA, transfer RNA and noncoding RNA using Bowtie2, the reads were aligned to the hg38 genome using STAR.[9] Out of 28.7-44.7 million unprocessed reads, 0.8-1.3 million remained after removal of non-coding RNA and reads mapped multiple times. Two-component Gaussian mixture models were fit to the footprint length histograms using mixtools[10] to produce point estimates of the probability that each footprint was "long," P_{long}. From there, each footprint was classified to be "long" if $P_{long} > 0.99$, "intermediate" if $0.01 \leq P_{long} \leq 0.99$, or "short if $P_{long} < 0.01$. The scatter plot comparing the two samples was generated using Matplotlib.[11] The metagene profile and codon usage analyses were

[6] Gaspar, J. M. NGmerge: Merging Paired-End Reads via Novel Empirically-Derived Models of Sequencing Errors. *BMC Bioinformatics* **2018**, *19* (1), 536.
[7] http://hannonlab.cshl.edu/fastx_toolkit/
[8] Martin, M. Cutadapt Removes Adapter Sequences from High-Throughput Sequencing Reads. *EMBnet.journal; Vol 17, No 1 Next Gener. Seq. Data Anal.* **2011**.
[9] Dobin, A.; Davis, C. A.; Schlesinger, F.; Drenkow, J.; Zaleski, C.; Jha, S.; Batut, P.; Chaisson, M.; Gingeras, T. R. STAR: Ultrafast Universal RNA-Seq Aligner. *Bioinformatics* **2013**, *29* (1), 15–21.
[10] Benaglia, T.; Chauveau, D.; Hunter, D. R.; Young, D. S. Mixtools: An R Package for Analyzing Finite Mixture Models. *J. Stat. Softw.* **2009**, *32* (6), 1–29.
[11] Hunter, J. D. Matplotlib: A 2D Graphics Environment. *Comput. Sci. Eng.* **2007**, *9* (3), 90–95.

done using riboWaltz package.[12] Codon usage is defined as the frequency of in-frame P-site codon within the coding sequence. The metagene profile plot was generated using GraphPad Prism, and the codon usage scatter plot was generated using Matplotlib.

DMS-MaPSeq and data processing

Whole cell DMS-MaPSeq was carried out with CHX and benzamide **8** as previously described.[13] 293T cells were grown in 15 cm^2 tissue culture plates to roughly 80% confluency. Cells were treated with 350 μM of CHX, 350 μM benzamide **8**, or vehicle for 10 min at 37 ºC before adding 600 μL of dimethylsulfate (DMS) to each plate, gently swirling to mix. DMS-treated cells and no DMS-treated cells were included as additional controls. The DMS modification was quenched by the addition of 10 mL of a solution of 30% v/v 2-mercaptoethanol in PBS to each plate. Cells were transferred to a conical tube, pelleted, washed with PBS, and then resuspended in 1 mL TRIzol, after which RNA isolation was performed according to manufacturer protocol. RNA samples were resuspended in 50 μL of RNase-free water and quantified by NanoDrop.

10 μg of RNA was used for library preparation as outlined in McClary *et al.*[13] RNA was fragmented with 10 mM $ZnCl_2$ and incubated for 5 minutes at 95 ºC before quenching with 20 mM EDTA on ice. Samples were ethanol precipitated and size selected using a 10% TBE-Urea PAGE gel, staining with SYBR Gold in TBE, and cutting out a slice between 60 and 70 bp. Size-selected fragments were gel extracted and purified before performing 3' end healing with T4 polynucleotide kinase (NEB) followed by linker ligation to AppCACTCGGGCACCAAGGA/3ddC/ using T4 RNA ligase 2 truncated K227Q (NEB). Residual linker was degraded by the addition of yeast 5'-deadenylase and RecJ exonuclease. 10 μM L reverse transcription reactions were carried out using half of the ligated RNA and adding 1X first strand buffer, 0.5 mM dNTPs, 0.5 μL SUPERase-In, 100 nM of RT primer (5Phos/AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGC/iSP18/CACTCA/iSp18/TTCAGACGTGTGCTCTTCCGATCTGTCCTTGGTGCCCGAGT), 5 mM dithiothreitol, and 0.5 μM TGIRTIII (InGex) for 1 hour at 60 ºC. Full-length RT products were purified using a 10% TBE-Urea PAGE gel, followed by gel extraction and purification. cDNA was circularized using CircLigase II (EpiCentre) and prepared for Illumina sequencing using forward primer AATGATACGGCGACCACCGAGATCTACAC and barcoded reverse primer CAAGCAGAAGACGGCATACGAGATNNNNNNNNGTGACTGGAGTTCAGACGTGTGCTCTTCC. 15 cycles of PCR were performed using Phusion High-Fidelity DNA polymerase (NEB). Full-length libraries were gel purified from a 10% TBE-PAGE gel, quantified by bioanalyzer, pooled, and sequenced. The libraries were sequenced for 150 cycles, single end on an Illumina NextSeq with roughly 20 million reads allocated per condition.

Raw sequencing reads were trimmed and aligned to rRNA reference indices using DMS-MaPseq data analysis software provided at https://github.com/borisz264/mod_seq/. Briefly, the 3' linker sequence was trimmed from all reads and the 5'-most 3 nucleotides were removed. Pre-

[12] Lauria, F.; Tebaldi, T.; Bernabò, P.; Groen, E. J. N.; Gillingwater, T. H.; Viero, G. RiboWaltz: Optimization of Ribosome P-Site Positioning in Ribosome Profiling Data. *PLOS Comput. Biol.* **2018**, *14* (8), e1006169.

[13] McClary, B.; Zinshteyn, B.; Meyer, M.; Jouanneau, M.; Pellegrino, S.; Yusupova, G.; Schuller, A.; Reyes, J. C. P.; Lu, J.; Guo, Z.; et al. Inhibition of Eukaryotic Translation by the Antitumor Natural Product Agelastatin A. *Cell Chem. Biol.* **2017**, *24* (5), 605-613.e5.

processed reads were fed into ShapeMapper 2.0 to count mismatches relative to human ribosomal 28S and 18S sequences (NCBI accession NR_003287.2 for 28S, and X03205.1 for 18S).

Cryo-EM Sample Preparation and Data Processing

Inhibitor-stalled mammalian ribosomes were isolated as described previously.[14] Briefly, *in vitro* translation reactions of substrates containing an N-terminal 3XFLAG in a rabbit reticulocyte system[15] were initiated by incubation at 32°C, supplemented with 50 μg/mL CHX or benzamide **8** after 7 min, and incubated at 32°C for an additional 13 min. 2-4 mL of translation reaction was directly incubated with 100 μL packed volume of anti-Flag M2 affinity resin (Sigma A2220) for 1 hr at 4°C. The resin was washed extensively and eluted with 0.1-0.2 mg/mL 3X Flag peptide (Sigma F4799) in 50 mM Hepes-KOH pH 7.4, 100 mM KOAc, 5 mM $Mg(OAC)_2$, 1 mM DTT at room temperature for 20-45 min. Elutions were directly frozen to grids, or ribosomes were pelleted by centrifugation at 100,000 rpm at 4°C for 40 min in a TLA-120.2 rotor (Beckman Coulter), resuspended and adjusted to 100-120 nM. 3 μL of purified ribosomes were applied to glow-discharged Cu300 R2/2 grids (Quantifoil) covered with a thin layer (~50 Å) of continuous carbon for 30 s, blotted for 3 s, and plunge frozen in liquid ethane using an FEI Vitrobot Mark II or Mark III operated at 4°C and 100% humidity.

Semi-automated data collection of benzamide-CHX-stalled was performed with SerialEM[16] using a Tecnai Polara F30 (ThermoScientific) operated at 300 kV and a K2 Summit direct electron detector (Gatan) in super-resolution mode at a nominal magnification of 31,000 x (calibrated magnification of 40,607 x) yielding a physical pixel size of 1.23 Å. The total exposure time was 7 s fractionated into 35 frames with a total exposure of ~47 electrons/$Å^2$. Semi-automated data collection of CHX-stalled ribosomes was performed with EPU (FEI) using a Tecnai G2 Polara microscope (ThermoScientific) operated at an acceleration voltage of 300 kV and a Falcon II direct electron detector (FEI) at a calibrated magnification of 102,942 x yielding a pixel size of 1.36 Å. The total exposure time was 1.5 s fractionated into 30 frames with a total exposure of ~35 electrons/$Å^2$.

Data processing was performed using RELION-3.0.[17] Global and local (5x5 patches) beam-induced motion was corrected using MotionCor2.[18] GCTF[19] was used to estimate parameters of the contrast transfer function (CTF). 2D class averages from particles manually picked from 50-100 micrographs were used as templates for automated particle picking in RELION-3.0. Subsequent classification steps were performed with particles extracted with a box size of 360 or downscaled to a box size of 180. Iterative 2D classifications were used to remove poor quality particles, followed by 3D refinement and 3D classification using EMDB-4129[14] as an initial model. After 3D classification, approximately 2/3 of both datasets were of rotated ribosomes containing hybrid A/P and P/E tRNAs but with no detectable inhibitor density, while the remaining

[14] Shao, S.; Murray, J.; Brown, A.; Taunton, J.; Ramakrishnan, V.; Hegde, R. S. Decoding Mammalian Ribosome-MRNA States by Translational GTPase Complexes. *Cell* **2016**, *167* (5), 1229-1240.e15.
[15] Feng, Q.; Shao, S. In Vitro Reconstitution of Translational Arrest Pathways. *Methods* **2018**, *137*, 20–36.
[16] Schorb, M.; Haberbosch, I.; Hagen, W. J. H.; Schwab, Y.; Mastronarde, D. N. Software Tools for Automated Transmission Electron Microscopy. *Nat. Methods* **2019**, *16* (6), 471–477.
[17] Zivanov, J.; Nakane, T.; Forsberg, B. O.; Kimanius, D.; Hagen, W. J. H.; Lindahl, E.; Scheres, S. H. W. New Tools for Automated High-Resolution Cryo-EM Structure Determination in RELION-3. *Elife* **2018**, *7*, e42166.
[18] Zheng, S. Q.; Palovcak, E.; Armache, J.-P.; Verba, K. A.; Cheng, Y.; Agard, D. A. MotionCor2: Anisotropic Correction of Beam-Induced Motion for Improved Cryo-Electron Microscopy. *Nat. Methods* **2017**, *14* (4), 331–332.
[19] Zhang, K. Gctf: Real-Time CTF Determination and Correction. *J. Struct. Biol.* **2016**, *193* (1), 1–12.

1/3 were of unrotated ribosomes containing a P-site tRNA and density corresponding to the inhibitors in the E-site. The P-site tRNA classes in each dataset were combined for further refinement. One round of focused classification with signal subtraction centered on the P-site of the **8**-stalled ribosome was used to increase P-site tRNA occupancy. After refinement, CTF refinement, Bayesian polishing, and post-processing was performed (**Table S1**).

The unrotated rabbit 80S ribosome (PDB 6SGC)[20] was used as a starting model. We removed the nascent protein chain and replaced the P-site lysyl-tRNA and poly(A) stall-inducing mRNA sequences with that of valyl-tRNA and a complementary mRNA sequence,[14] although our reconstruction is likely an average of ribosomes stalled on different P-site codons. We also removed peripheral ribosomal features that were poorly resolved, such as the L1 stalk. After rigid body fitting in Chimera,[21] local differences of protein and rRNA around the CHX binding site were modeled in Coot.[22] The structure and restraint files of **8** were created using Phenix eLBOW from the SMILES string, and docked manually in Coot. The complete model was refined using multiple rounds of Phenix real space refine[23] with manual model correction in Coot in between rounds of refinement. The final model was validated using MolProbity[24] and EMRinger[25] (**Table S1**).

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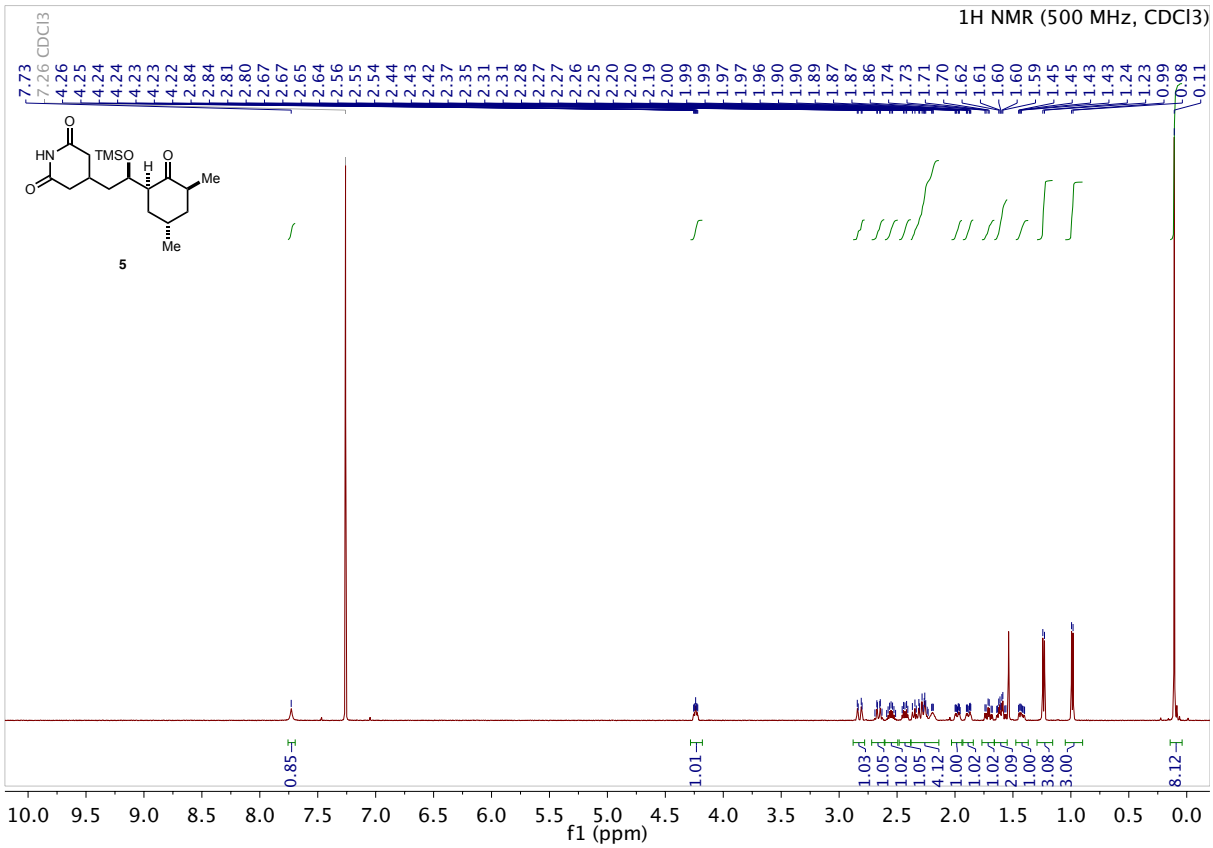
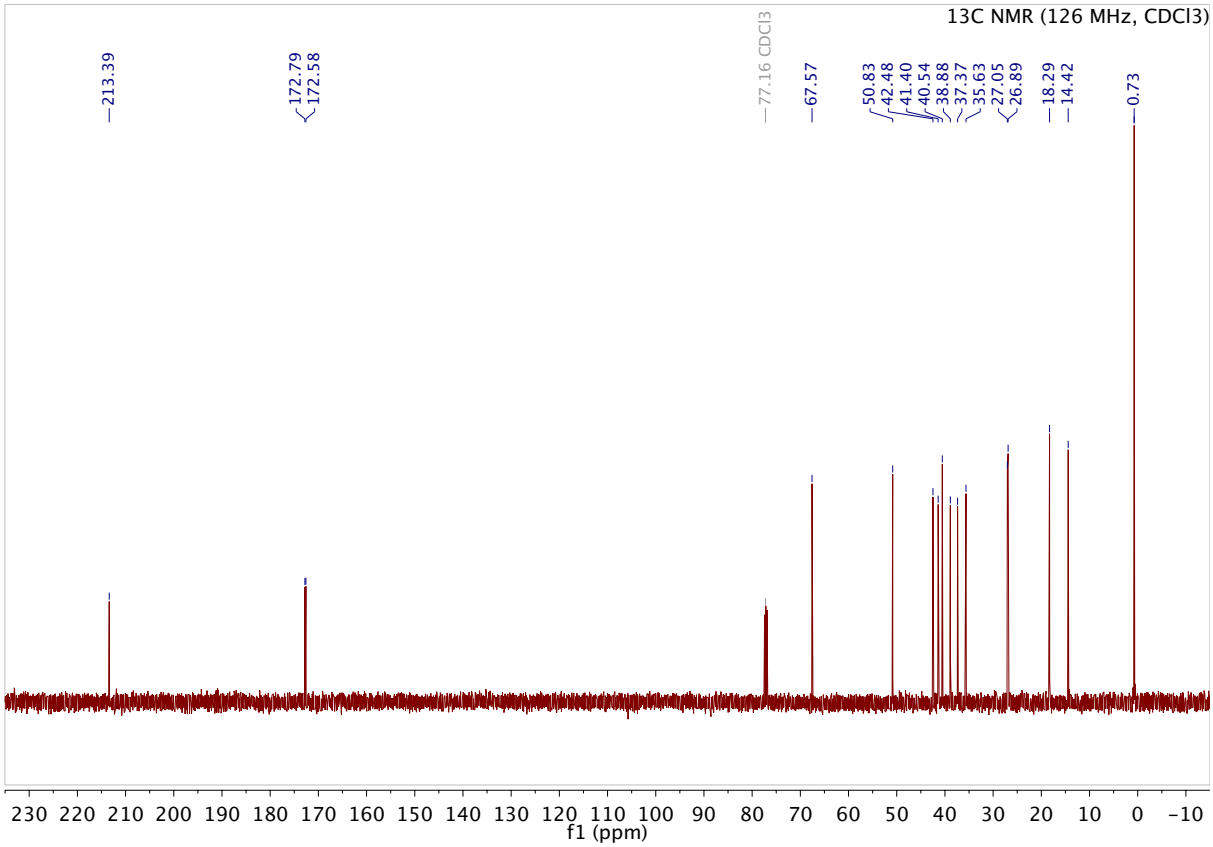
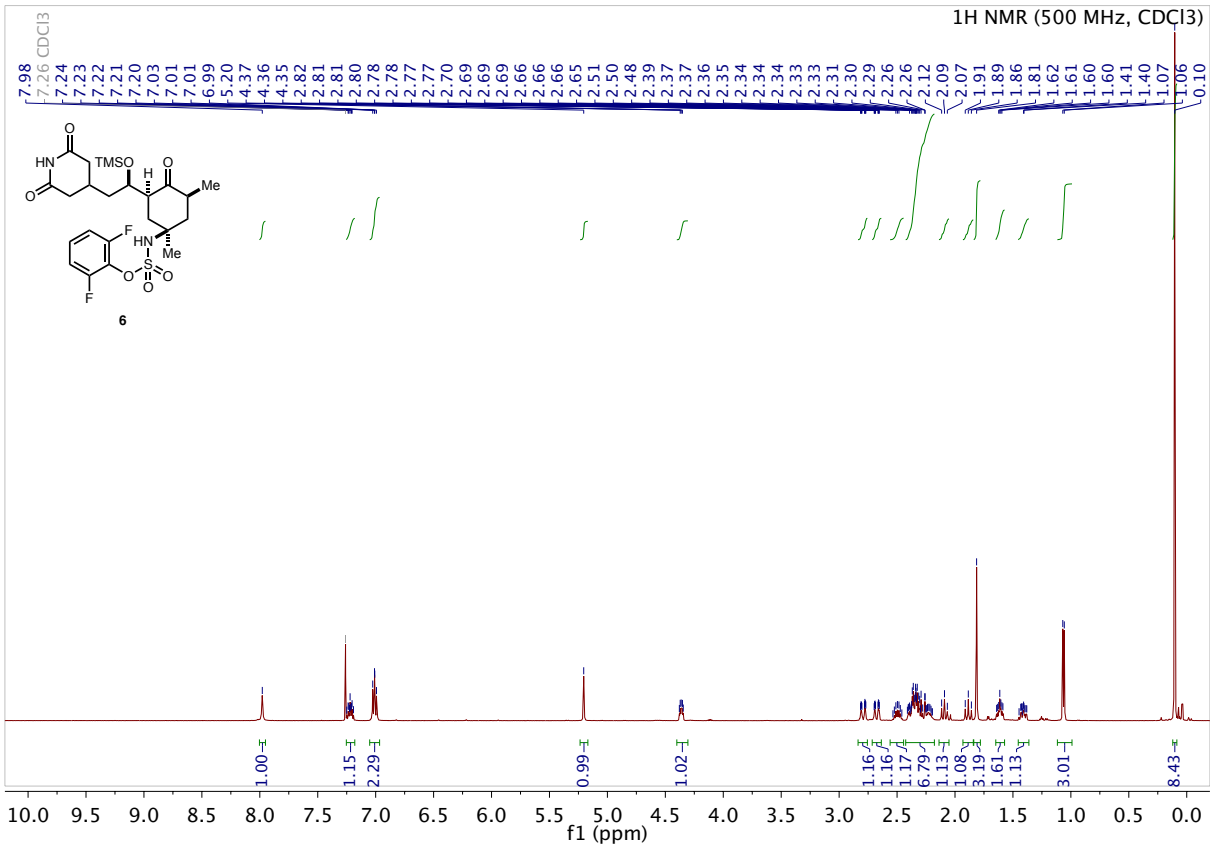
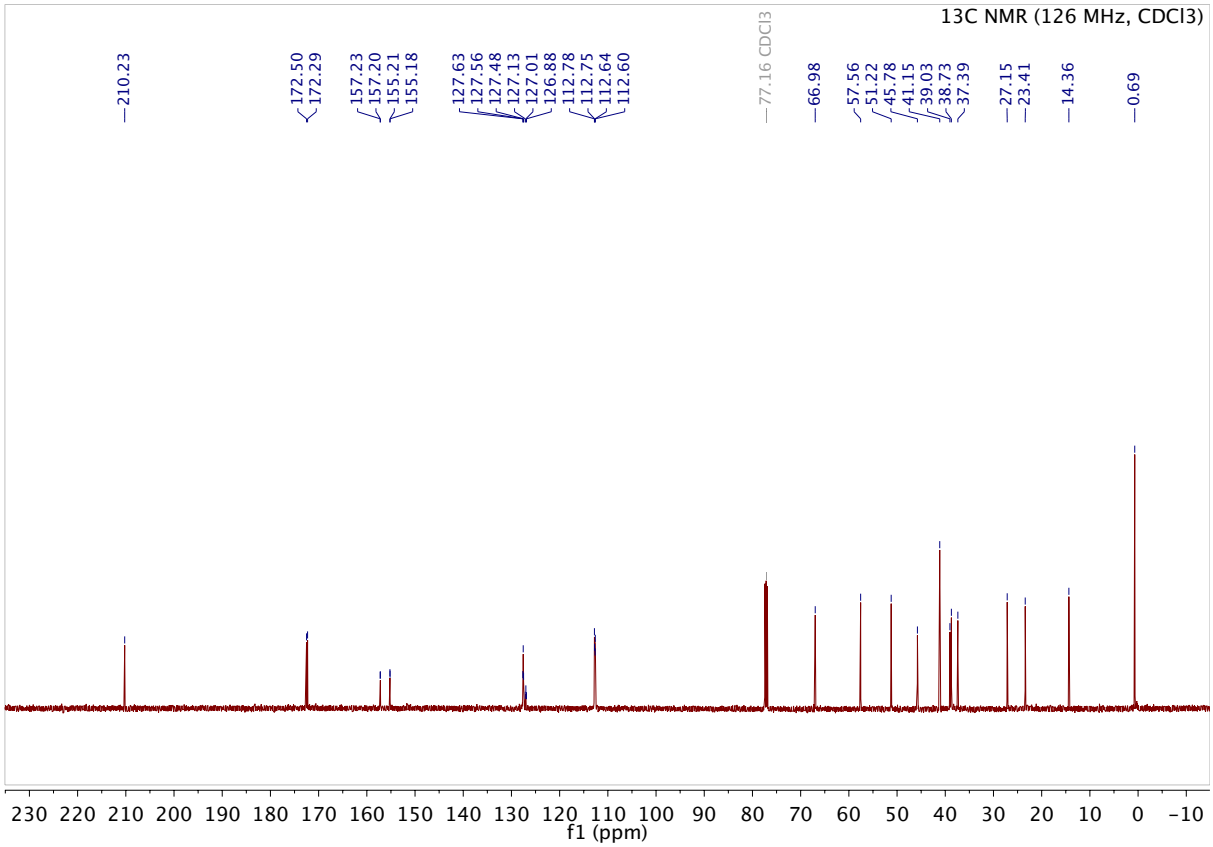
Silyl ether **5**

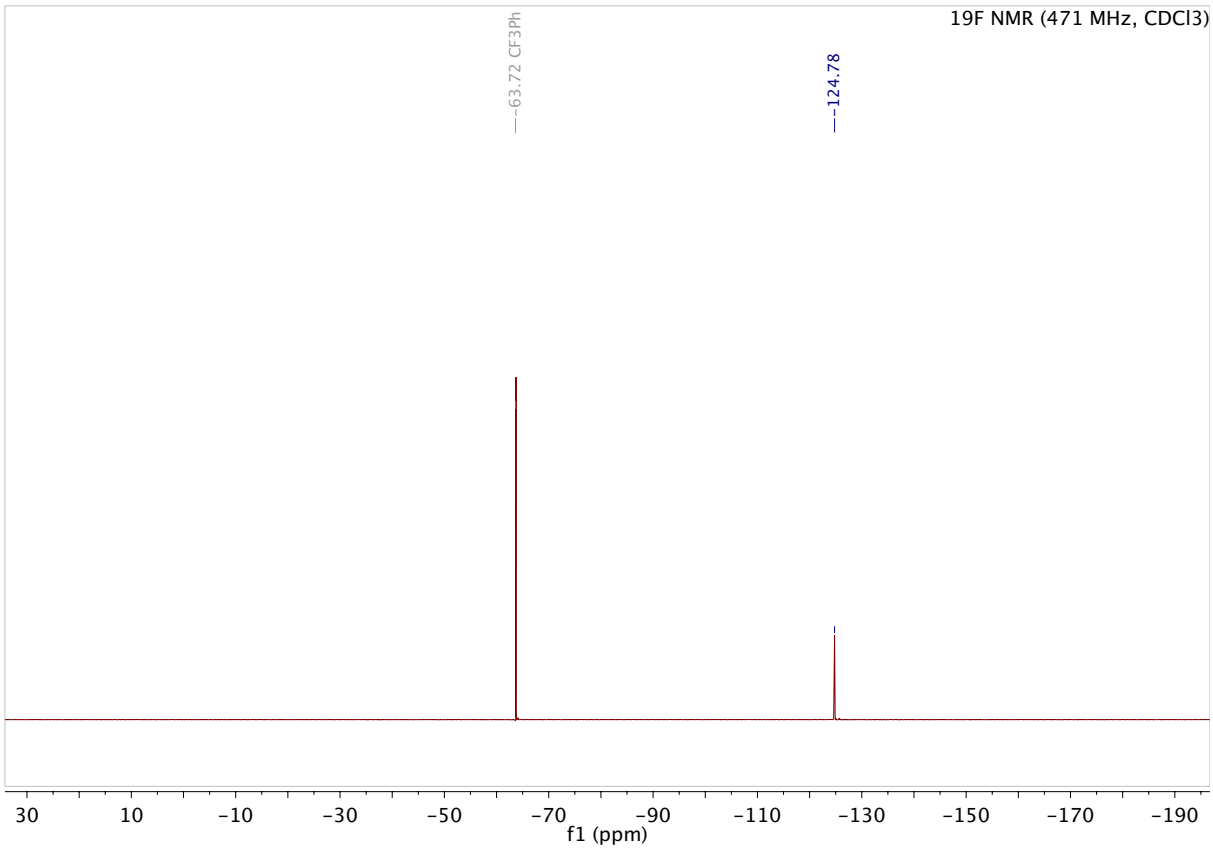




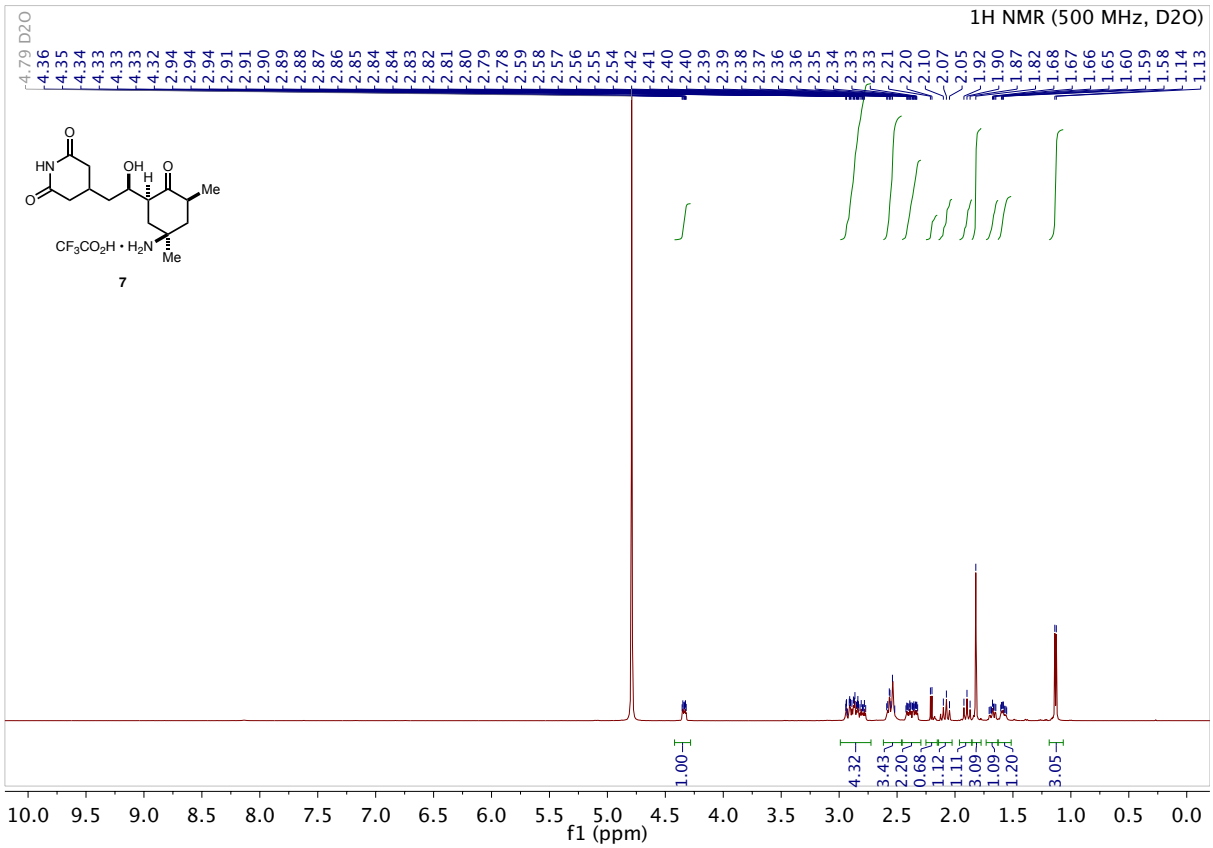
Sulfamate **6**

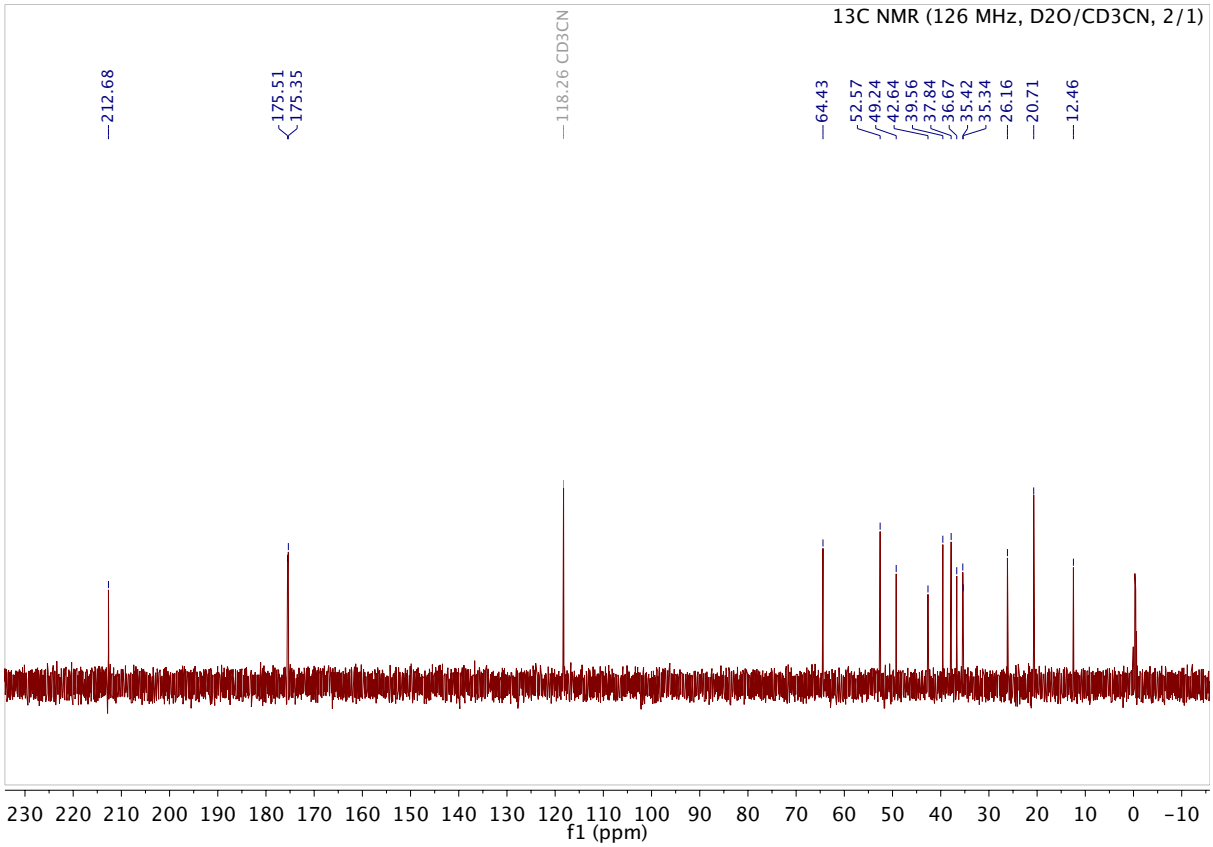




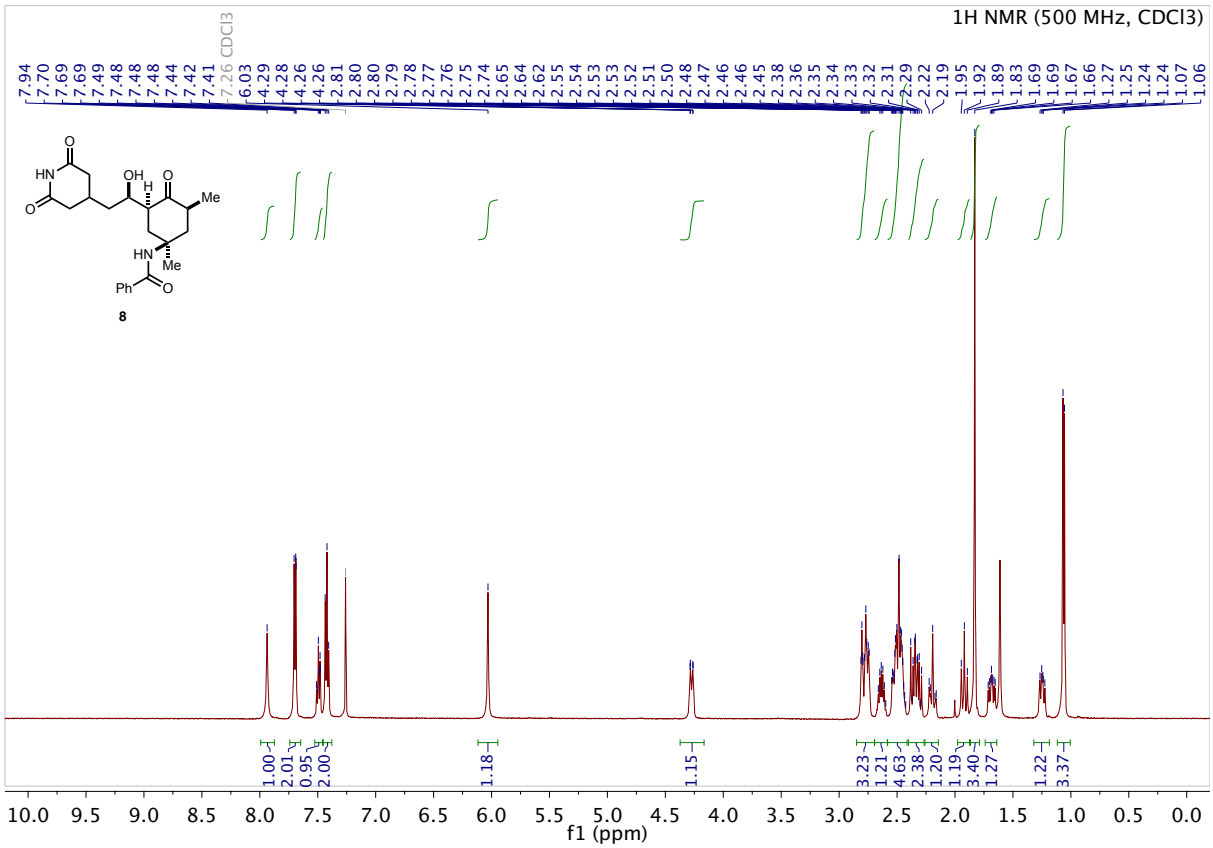

19F NMR (471 MHz, CDCl3)
-63.72 CF3Ph
-124.78
30
10
-10
-30
-50
-70
-90
-110
-130
-150
-170
-190
f1 (ppm)

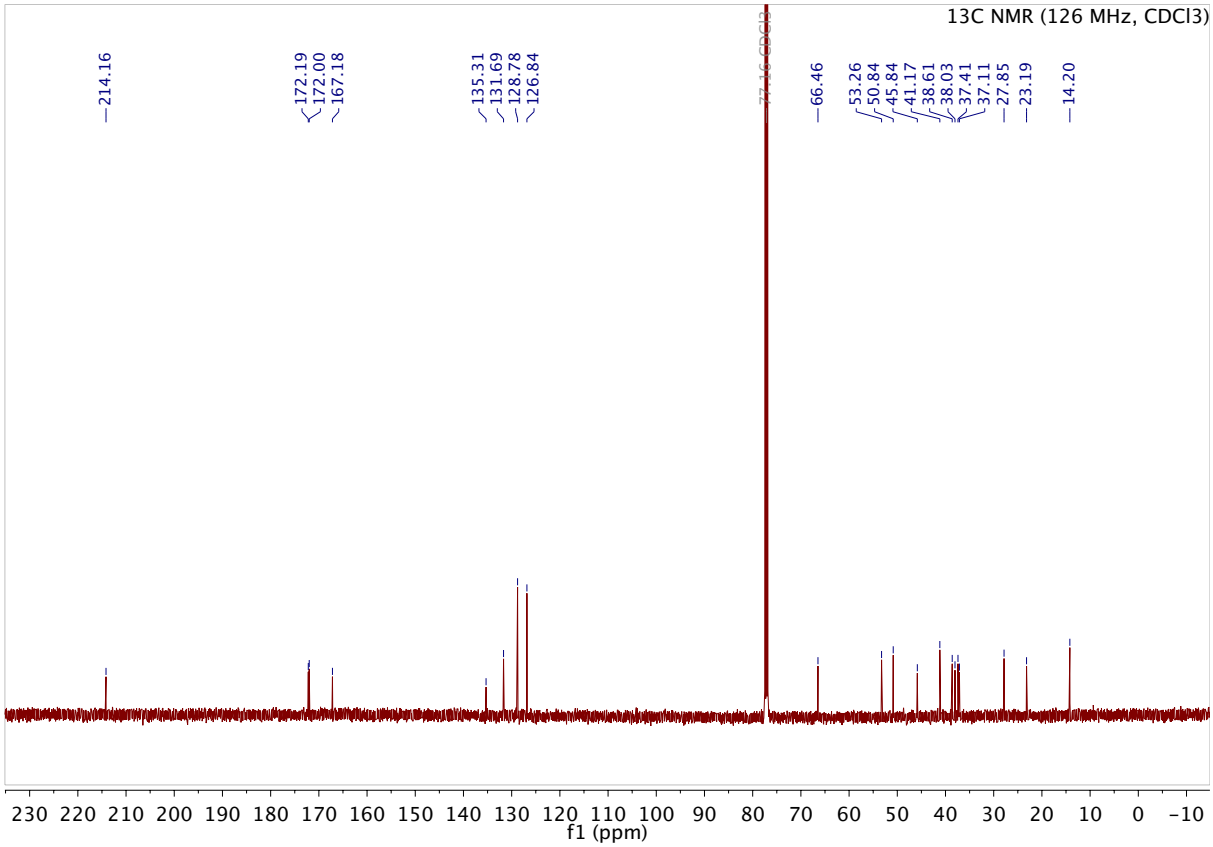
Amine **7**


1H NMR (500 MHz, D2O)
4.79 D2O
4.36 4.35 4.34 4.33 4.33 4.32 2.94 2.94 2.91 2.91 2.90 2.89 2.88 2.87 2.86 2.85 2.84 2.84 2.83 2.82 2.81 2.80 2.79 2.78 2.59 2.58 2.57 2.56 2.55 2.54 2.42 2.41 2.40 2.40 2.39 2.39 2.38 2.37 2.36 2.36 2.35 2.34 2.33 2.33 2.21 2.20 2.10 2.07 2.05 1.92 1.90 1.87 1.82 1.68 1.67 1.66 1.65 1.60 1.59 1.58 1.14 1.13
HN
O
O
OH
H
O
Me
CF3CO2H • H2N
Me
7
1.00
4.32
3.43
2.20
0.68
1.12
1.11
3.09
1.09
1.20
3.05
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

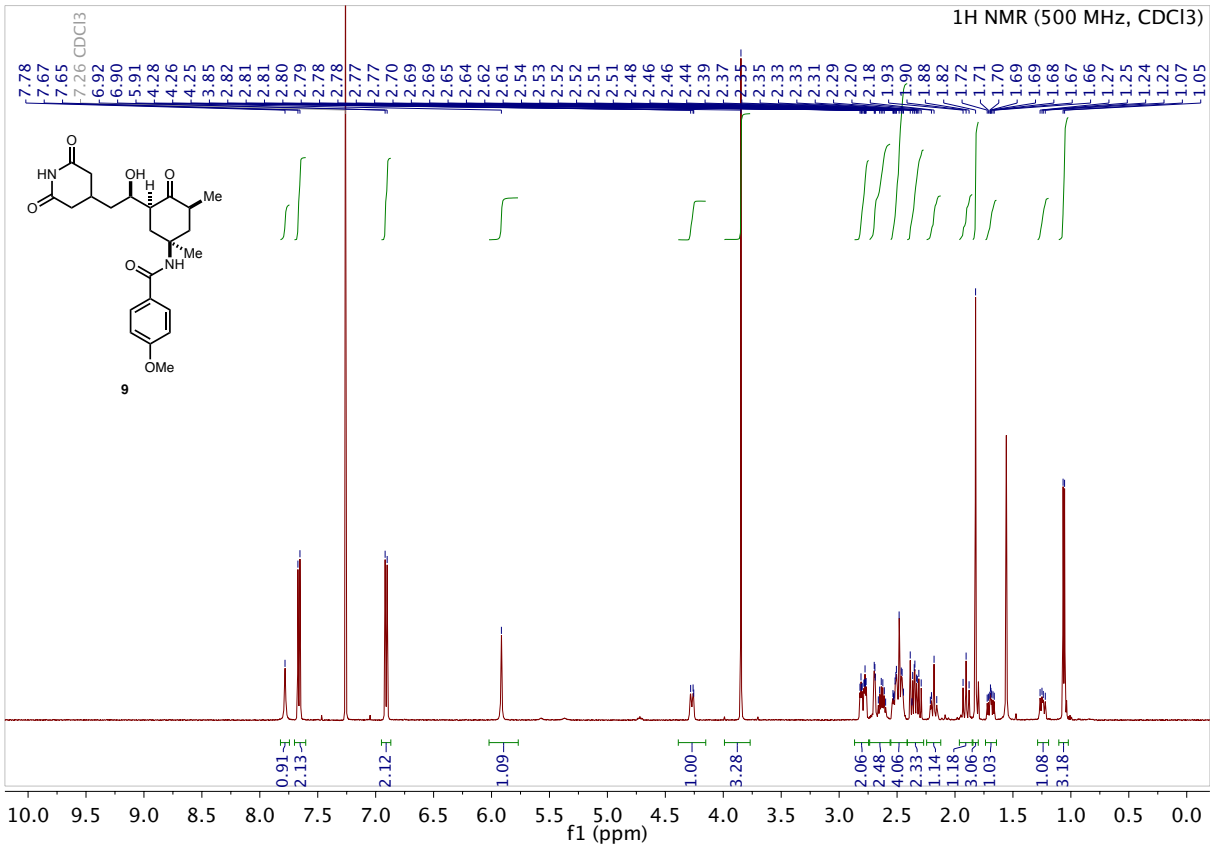

13C NMR (126 MHz, D2O/CD3CN, 2/1)
212.68
175.51
175.35
118.26 CD3CN
64.43
52.57
49.24
42.64
39.56
37.84
36.67
35.42
35.34
26.16
20.71
12.46
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

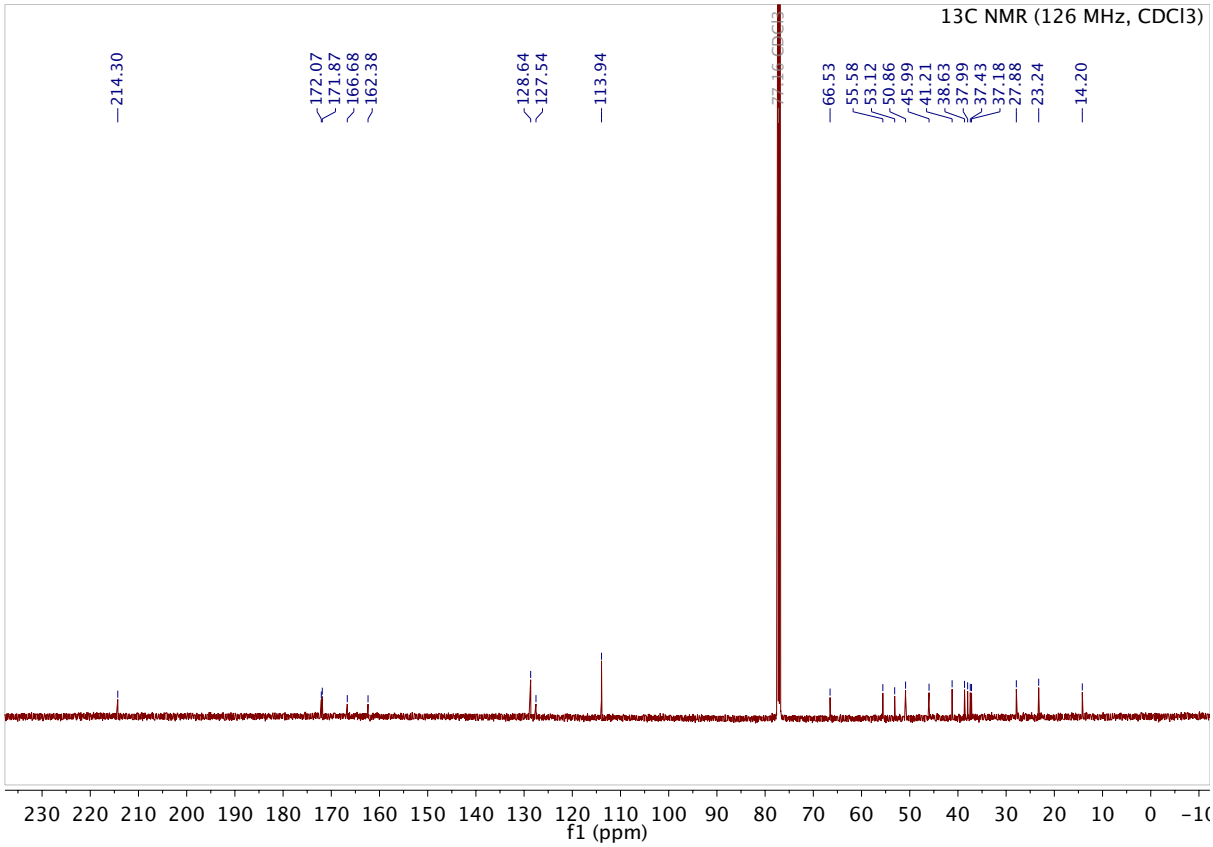
Amide **8**



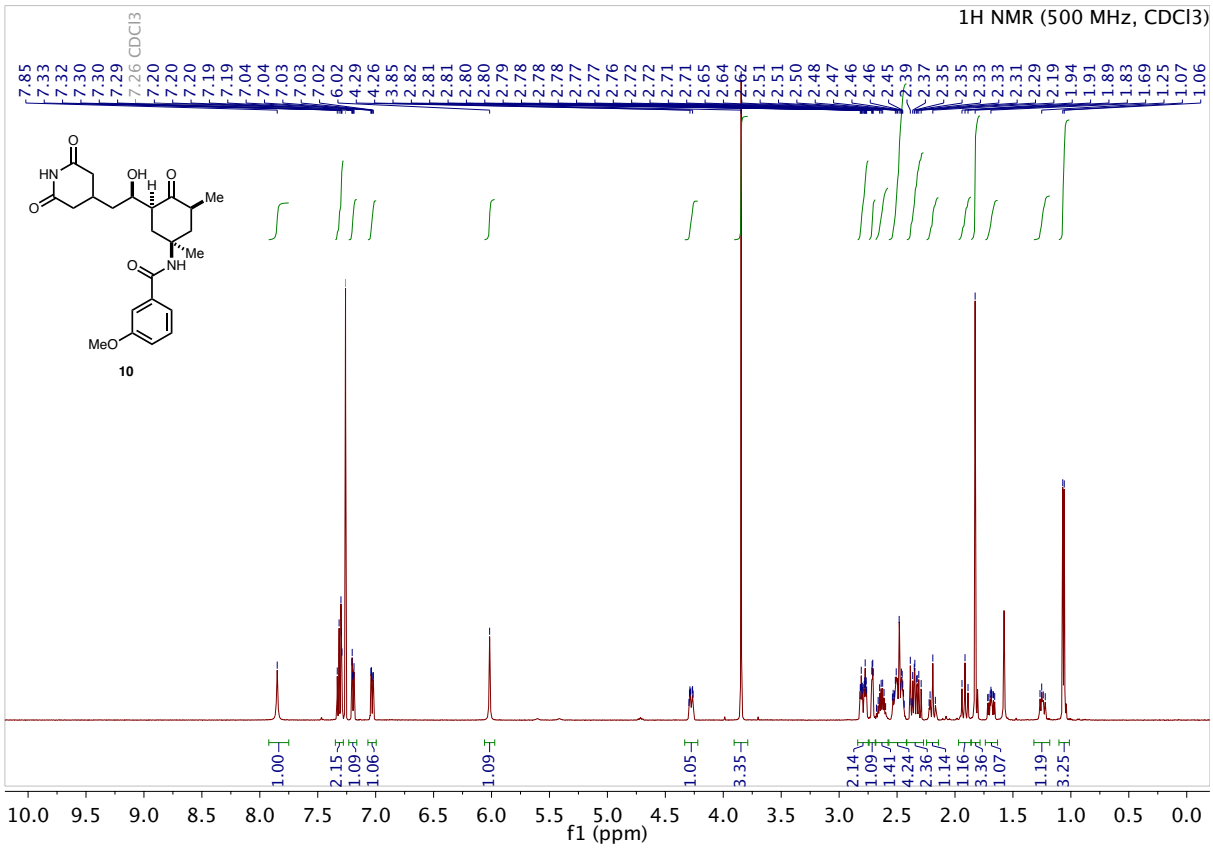


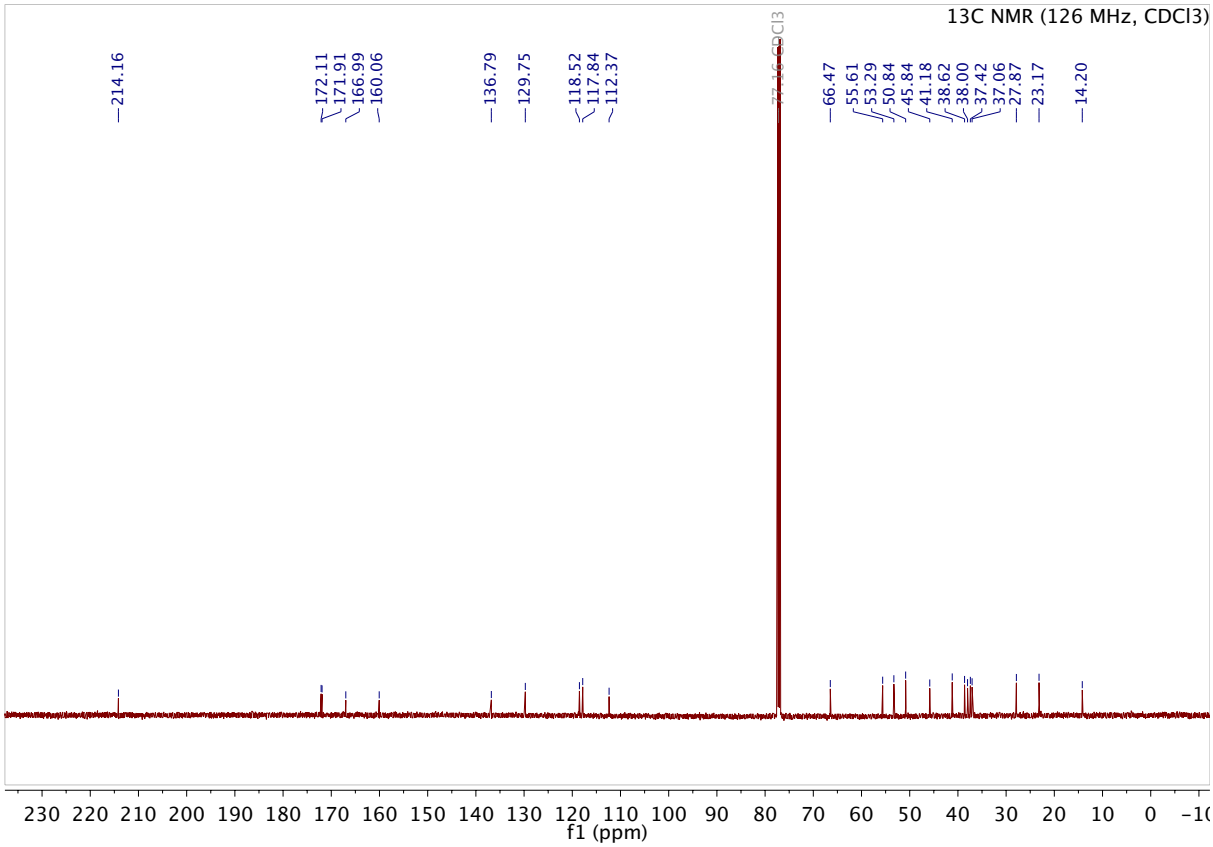
Amide **9**



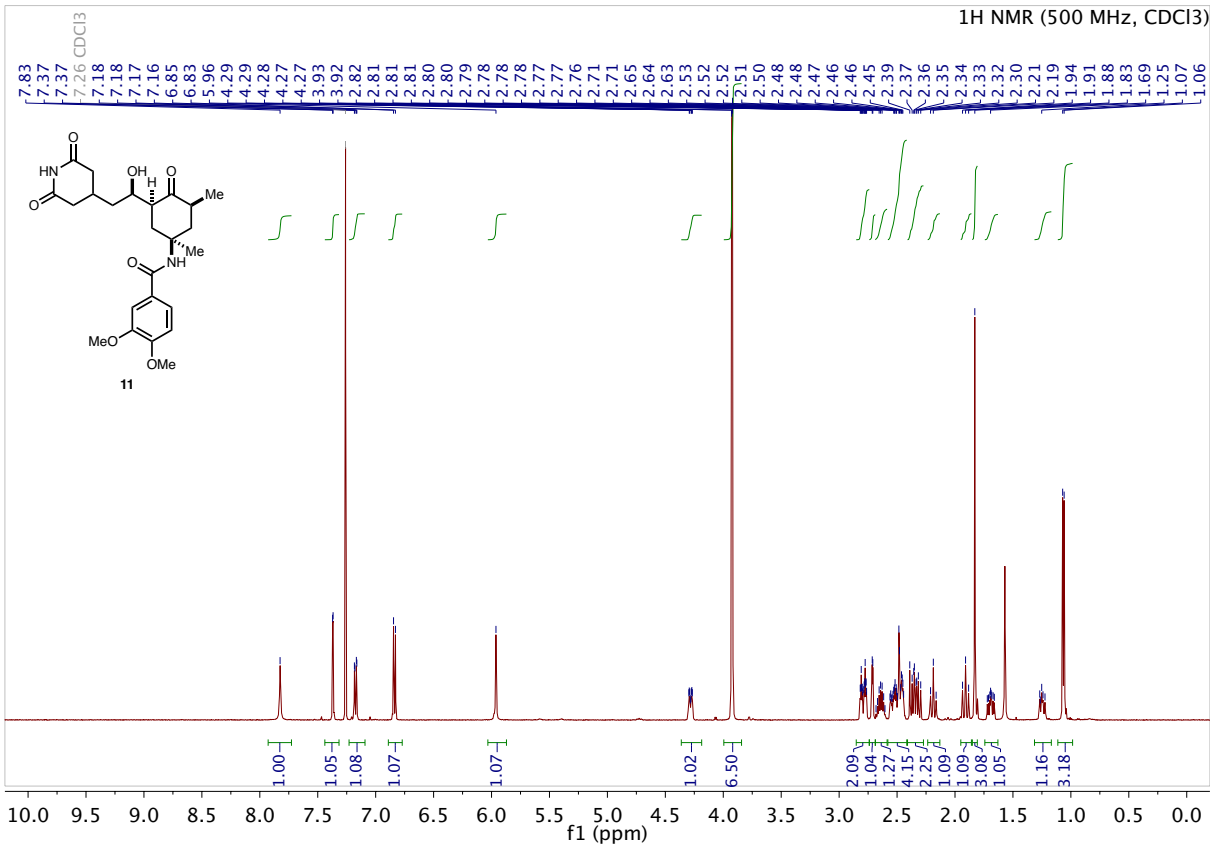


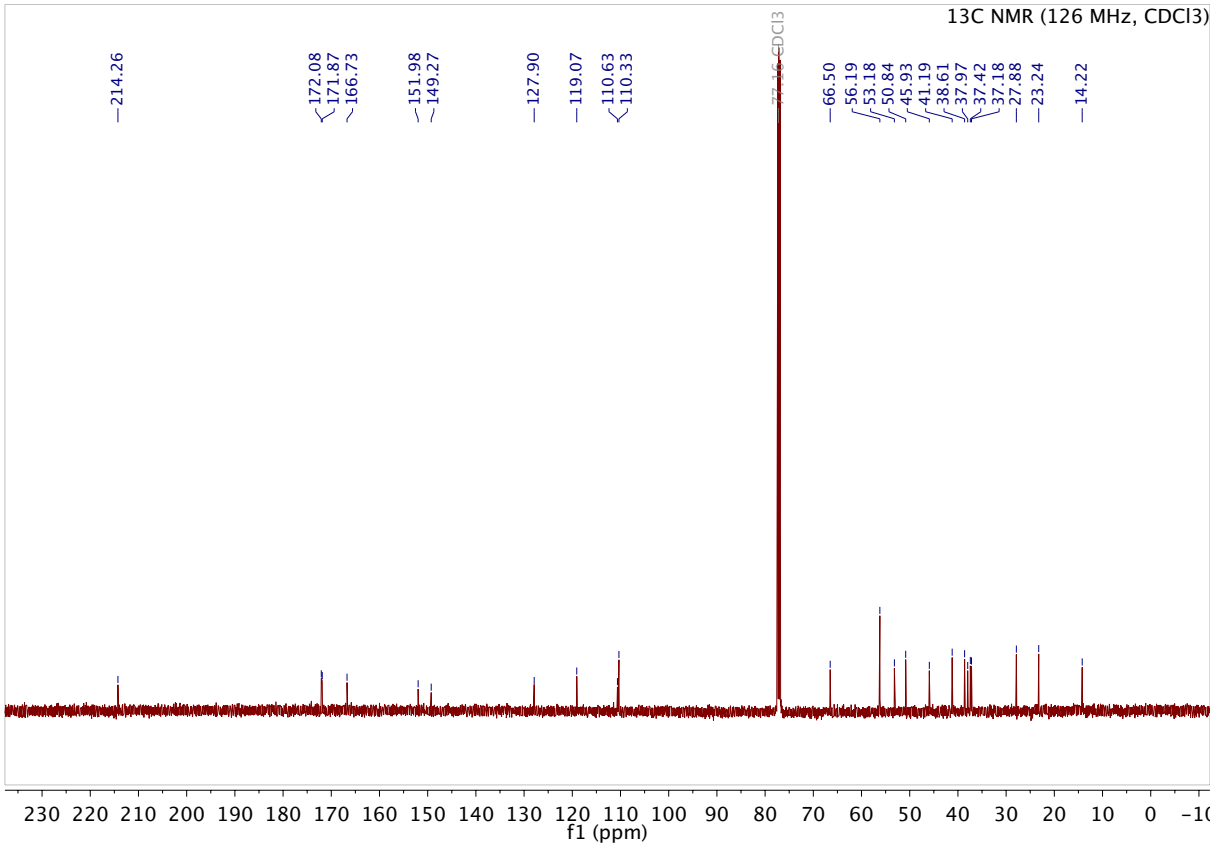
Amide **10**



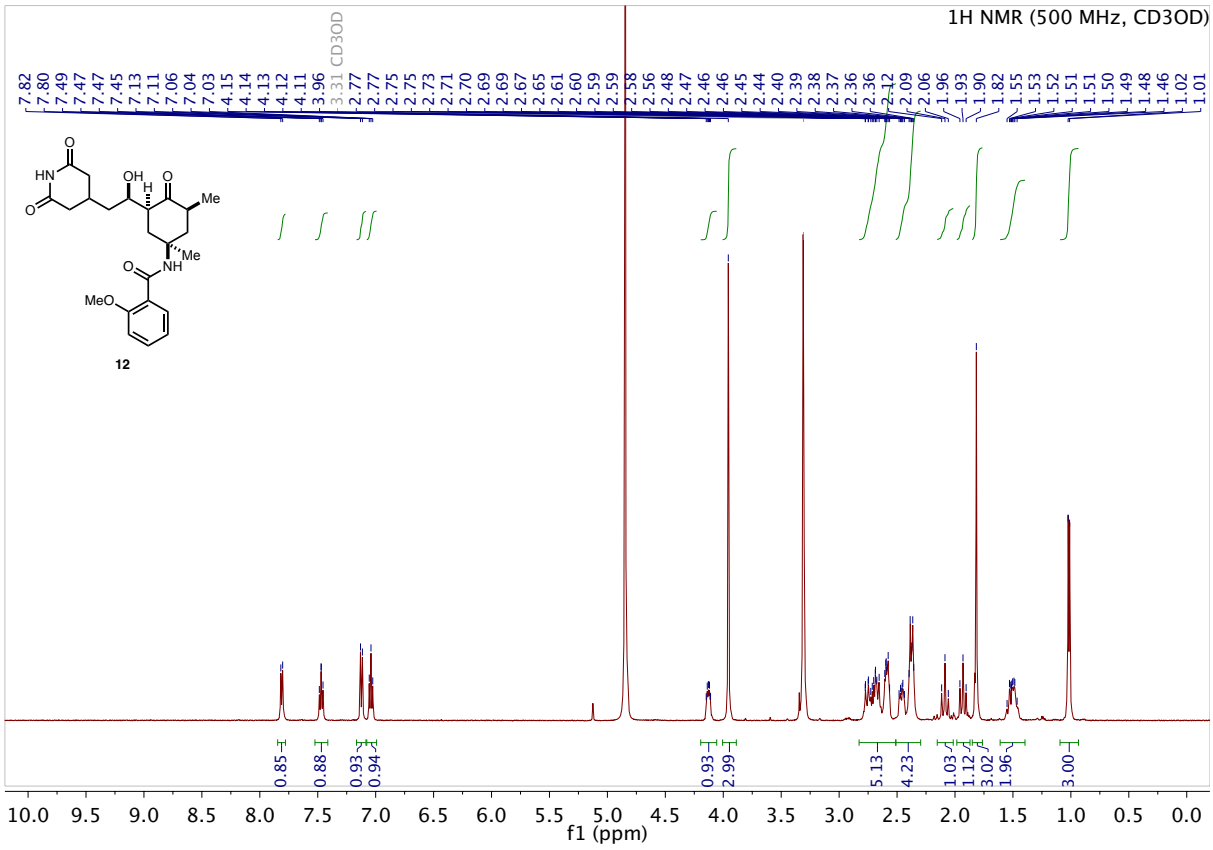


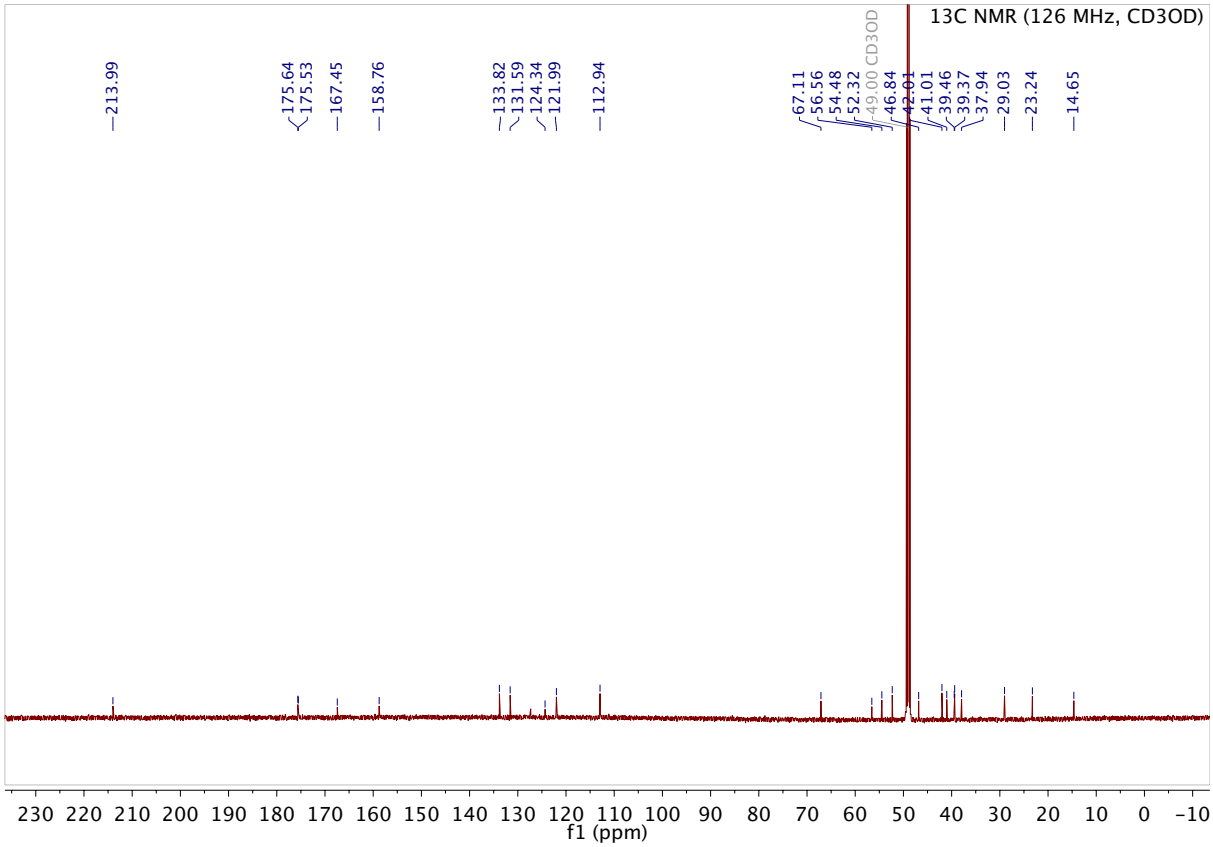
Amide **11**





Amide **12**





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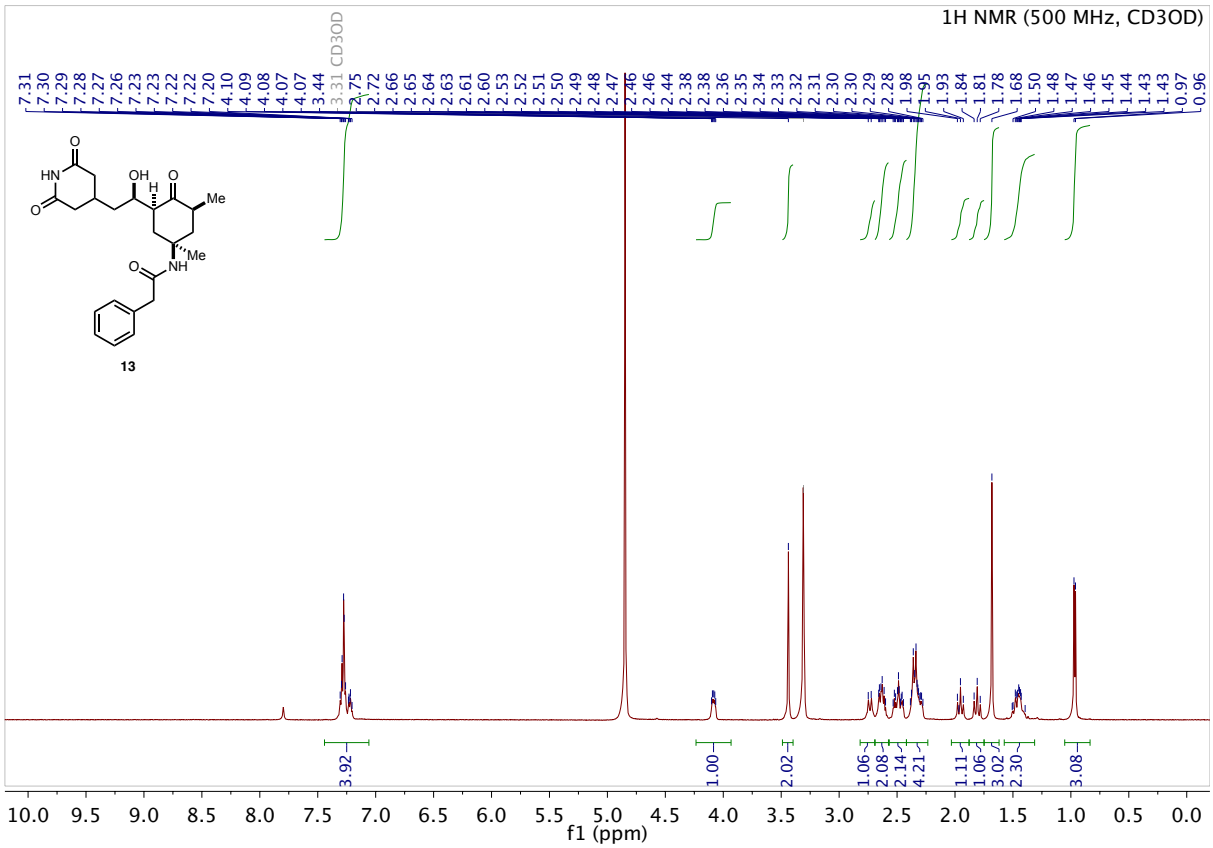

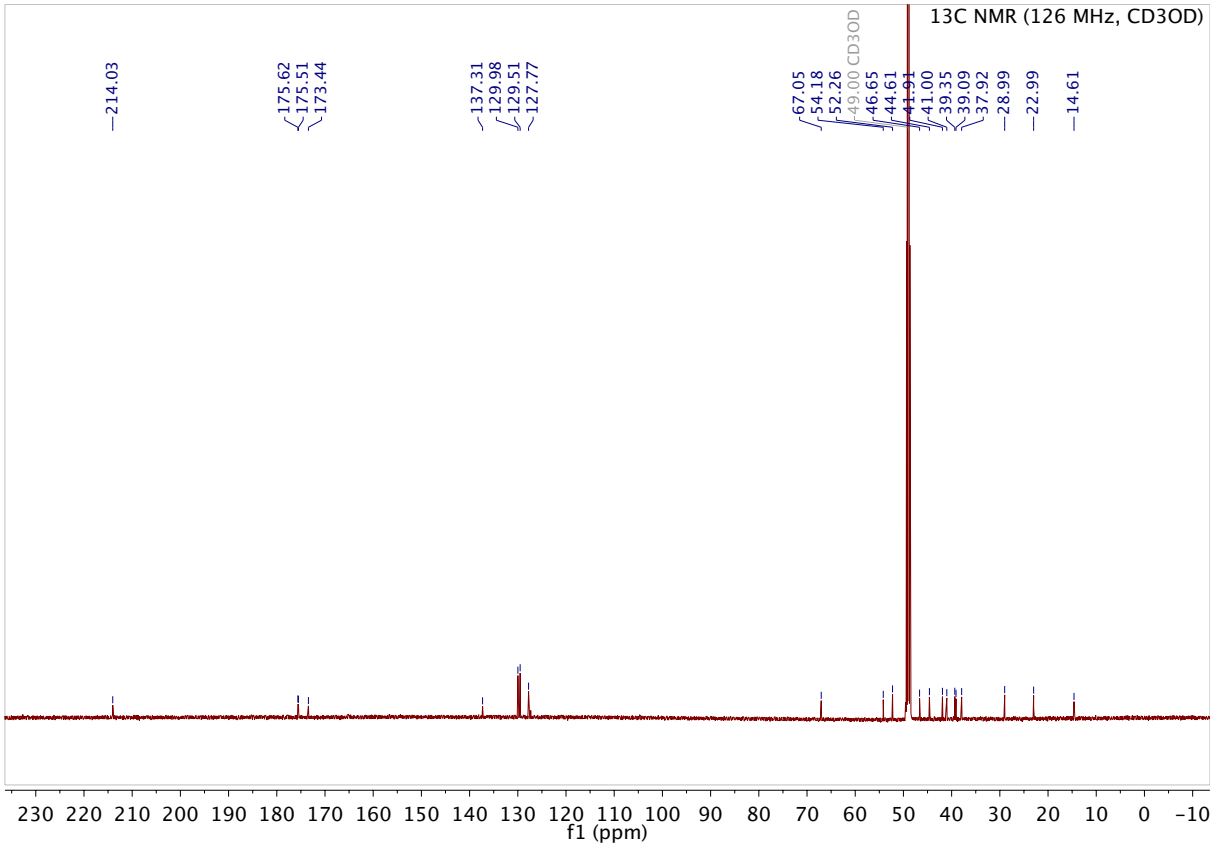

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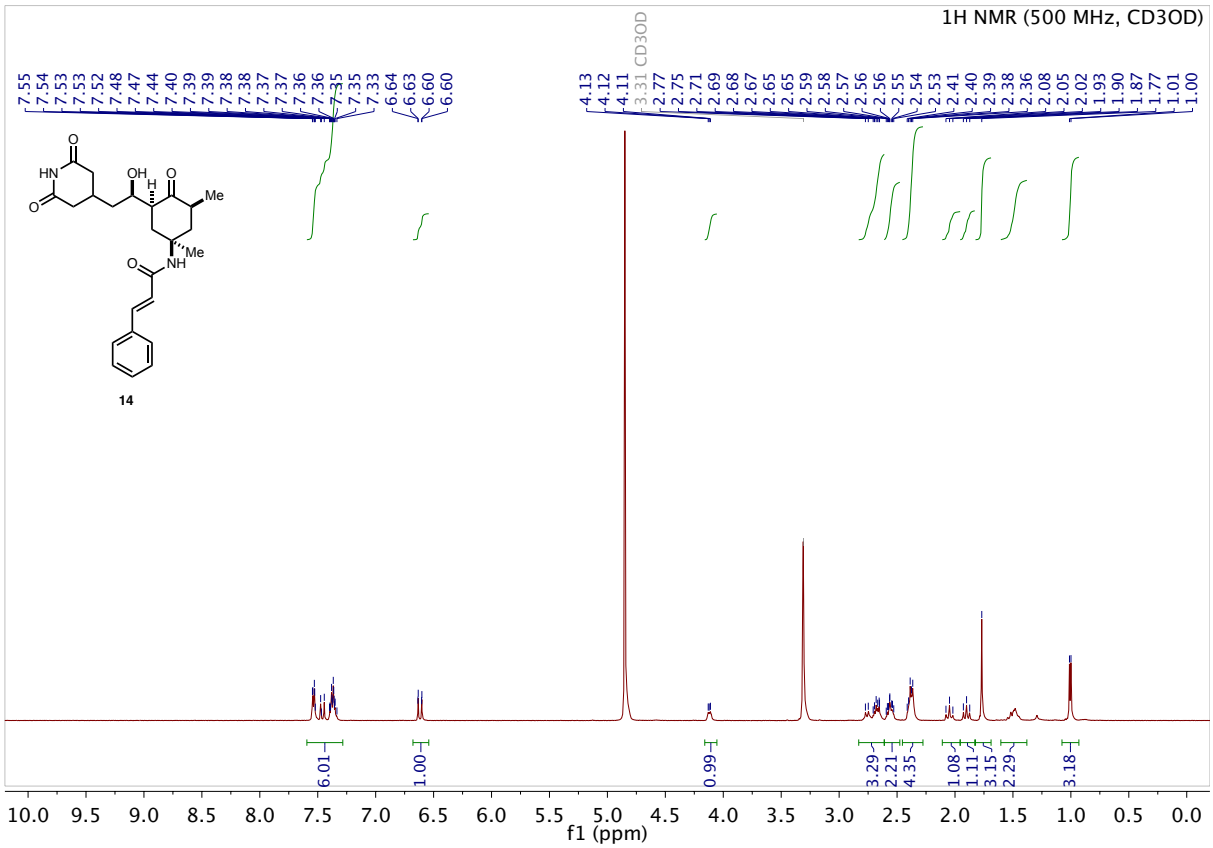

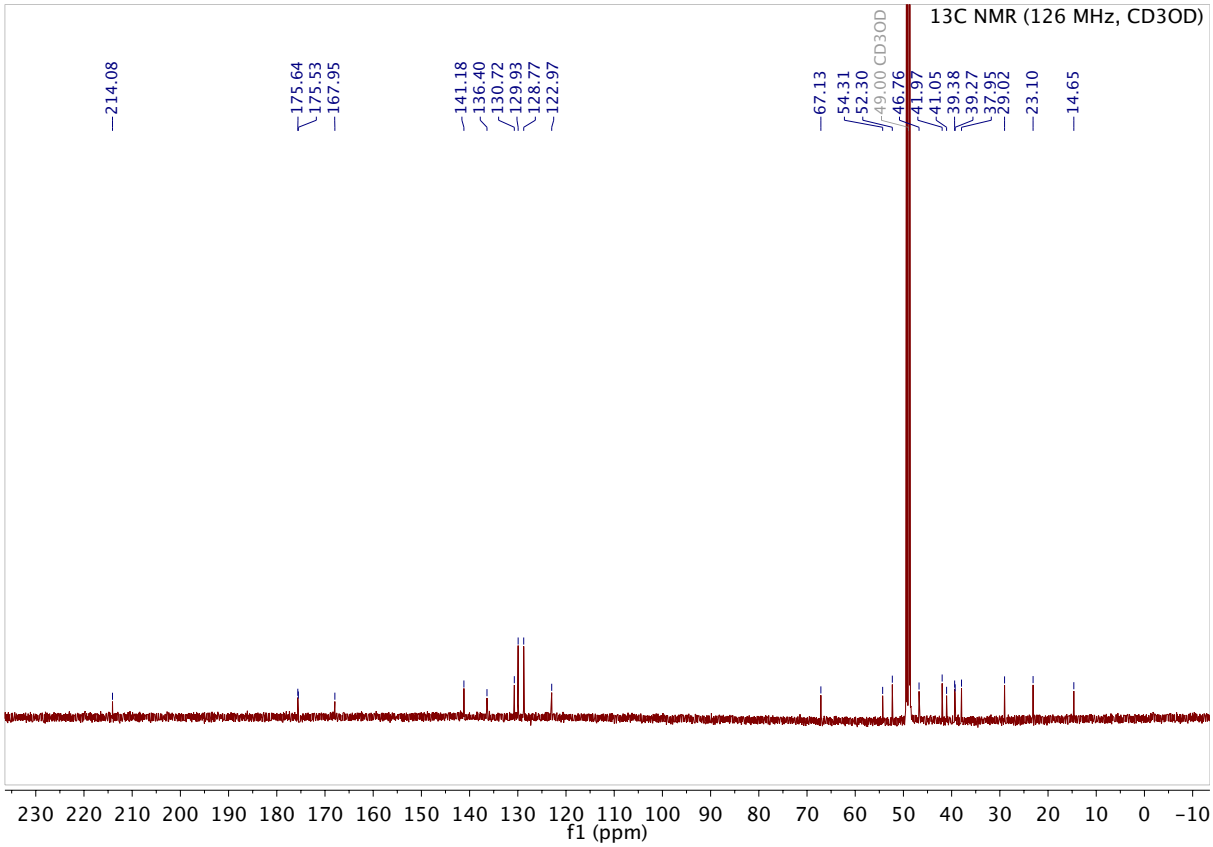

Amide **15**