Supporting Information

Structure-Based Design and Synthesis of Macrocyclic Inhibitors of Norovirus Protease: Structural, Biochemical, Spectroscopic, and Antiviral Studies.

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1. **Representative assignments of 2D NMR resonances of macrocyclic precursor ester *28g***

The spectral details, 2D and variable temperature (VT) NMR spectra were taken using a Varian XL-400 MHz NMR spectrometer. The sample was prepared by dissolving 30 mg of compound in one mL of DMSO-d_6. Assignments of NH and CHα resonances were done using 1D 1H NMR, COSY, ROESY and HMBC spectral data recorded at 273 K. Temperature dependence of amide NH chemical shifts (Δδ/T) were examined within the temperature range of 298-343 K in 5 degree increments.

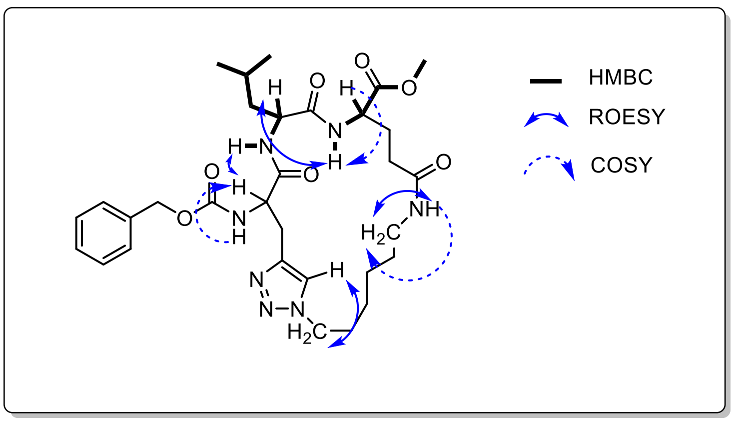


FIGURE S1. Selected HMBC, ROESY and COSY correlations of macrocyclic precursor ester ***28g***

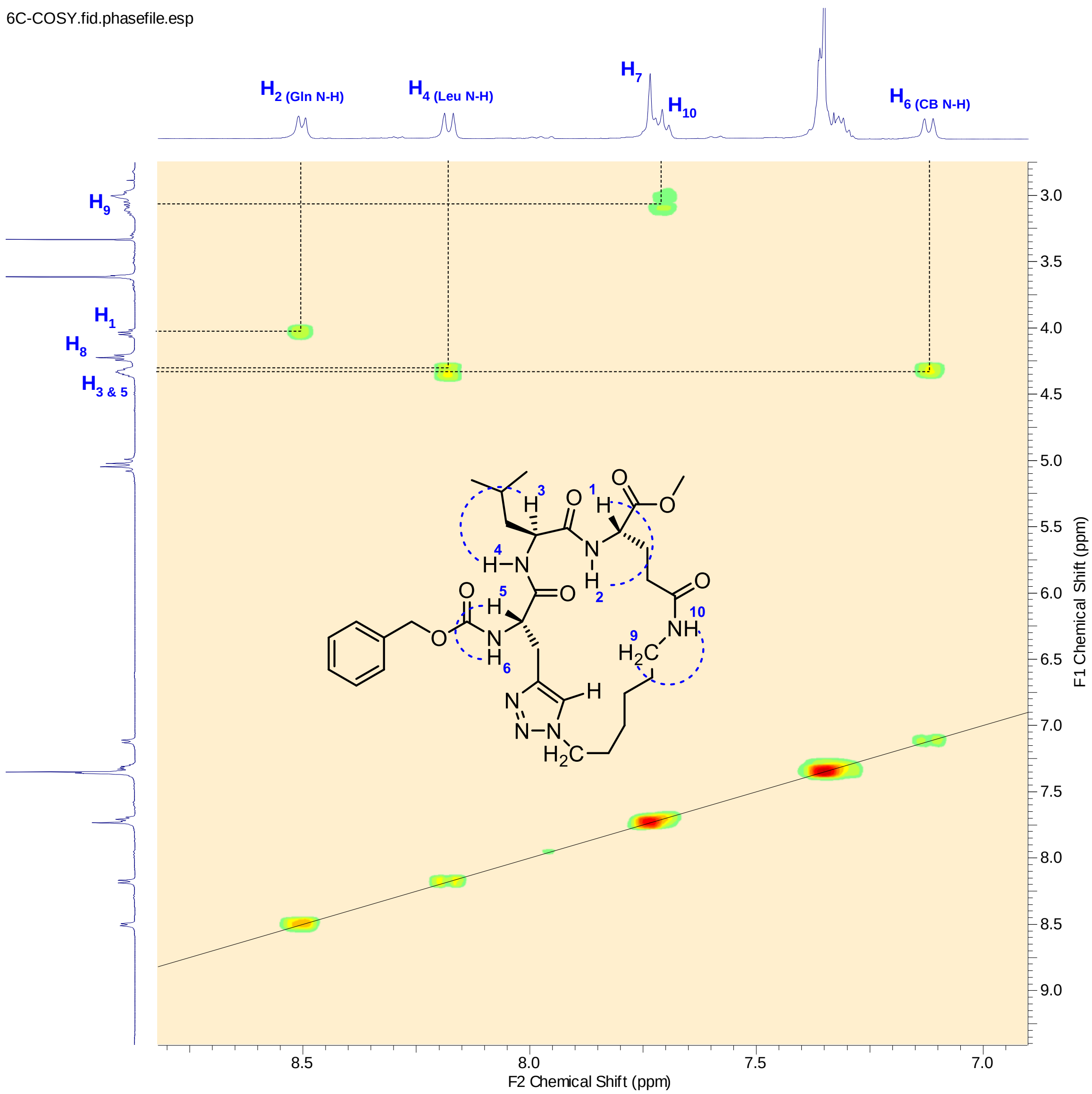


FIGURE S2. Selected COSY correlations of macrocyclic precursor ester ***28g***

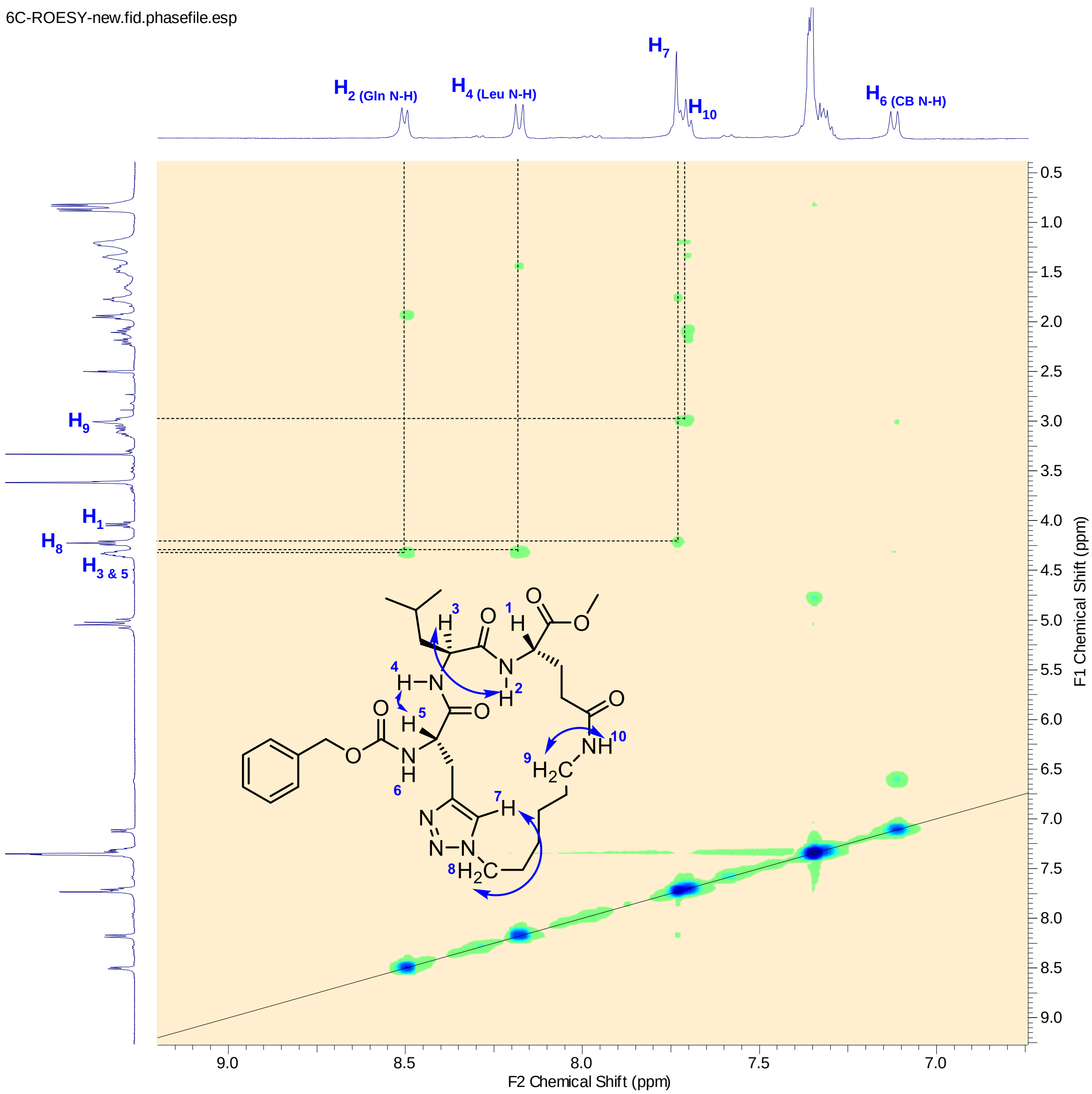


FIGURE S3. Selected ROESY correlations of macrocyclic precursor ester ***28g***

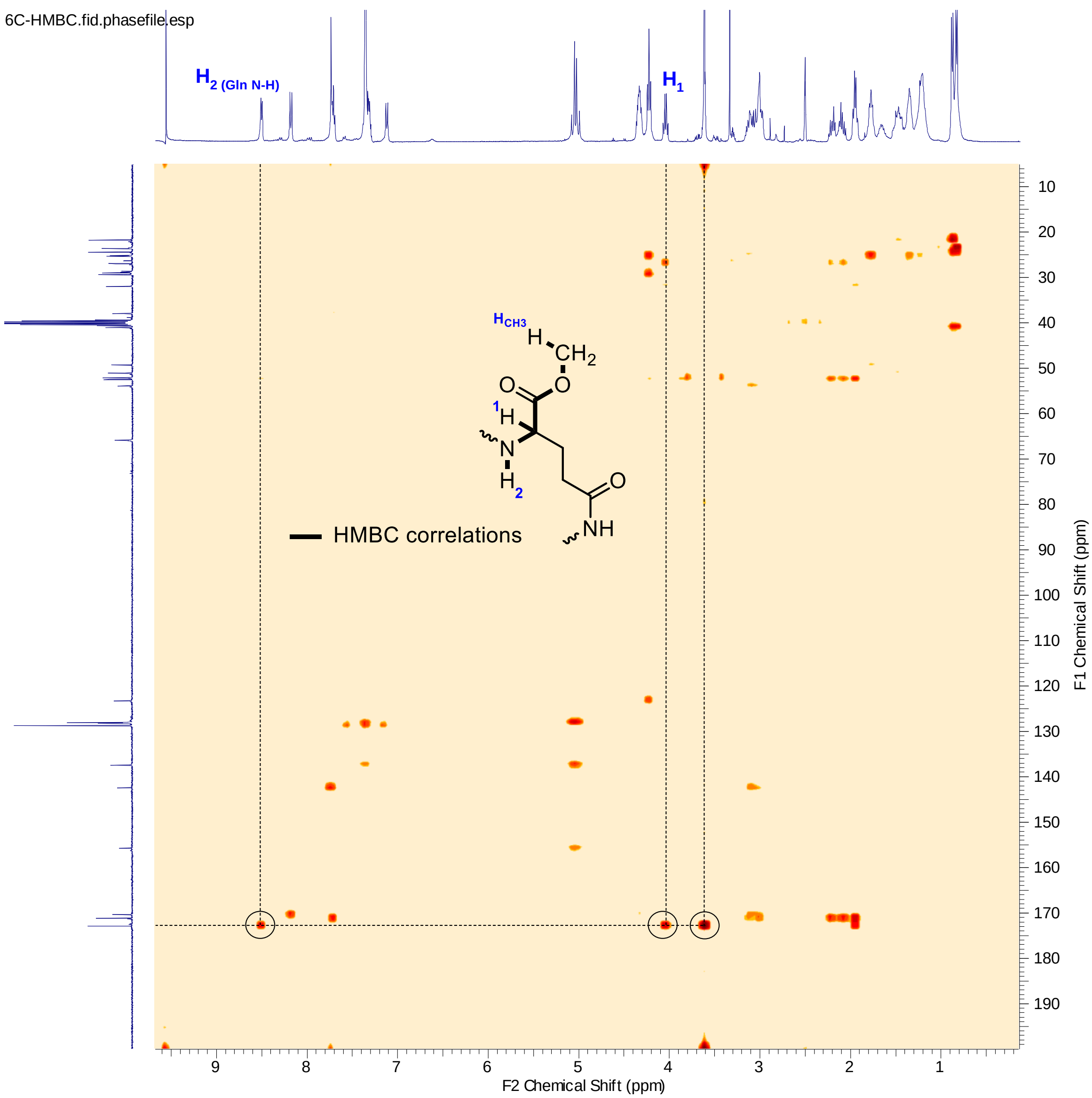


FIGURE S4. Selected HMBC correlations of macrocyclic precursor ester ***28g***

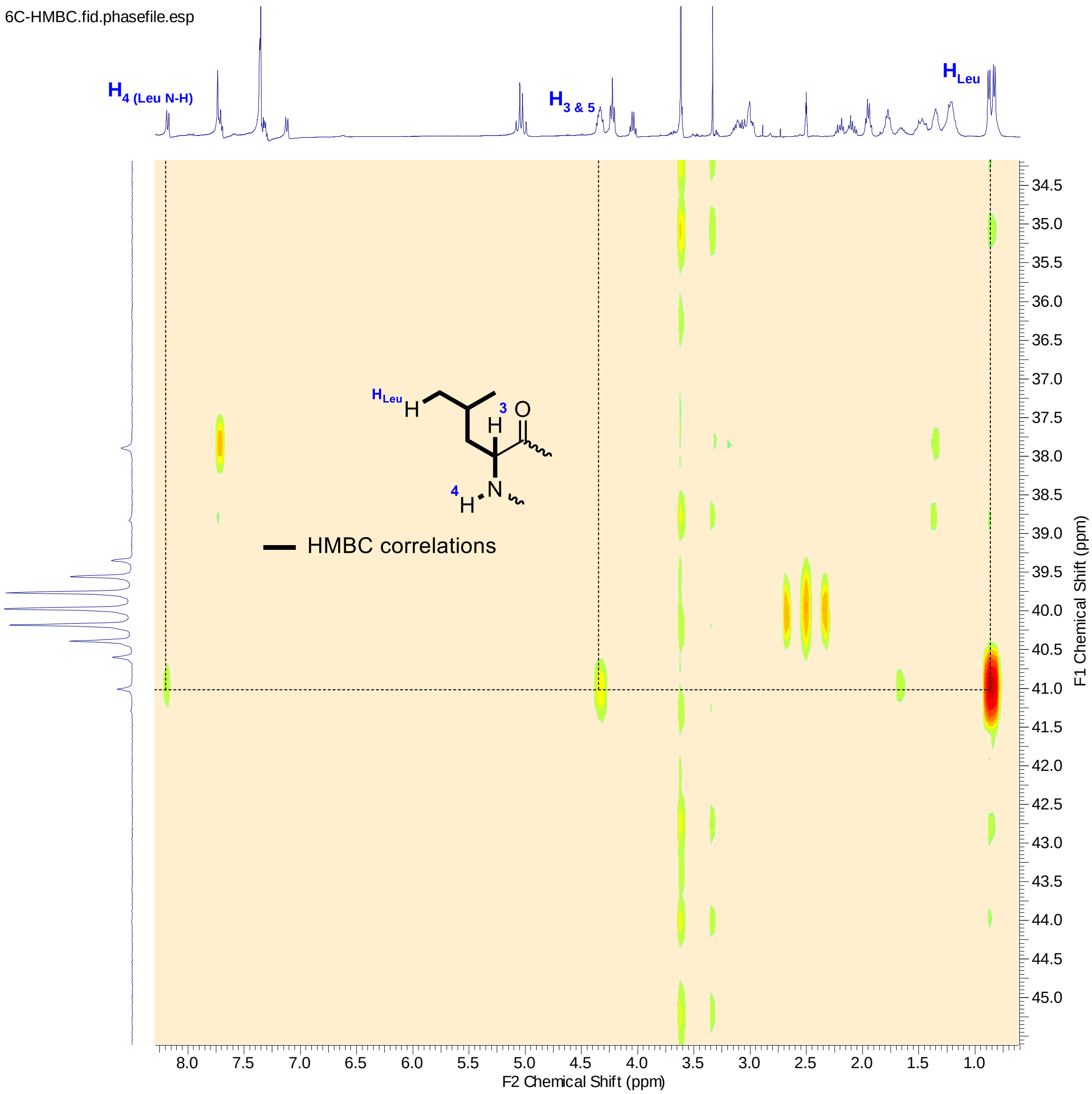


FIGURE S5. Selected HMBC correlations of macrocyclic precursor ester ***28g***

2. Variable temperature NMR studies of compound *28a*, *28e* & *28g*

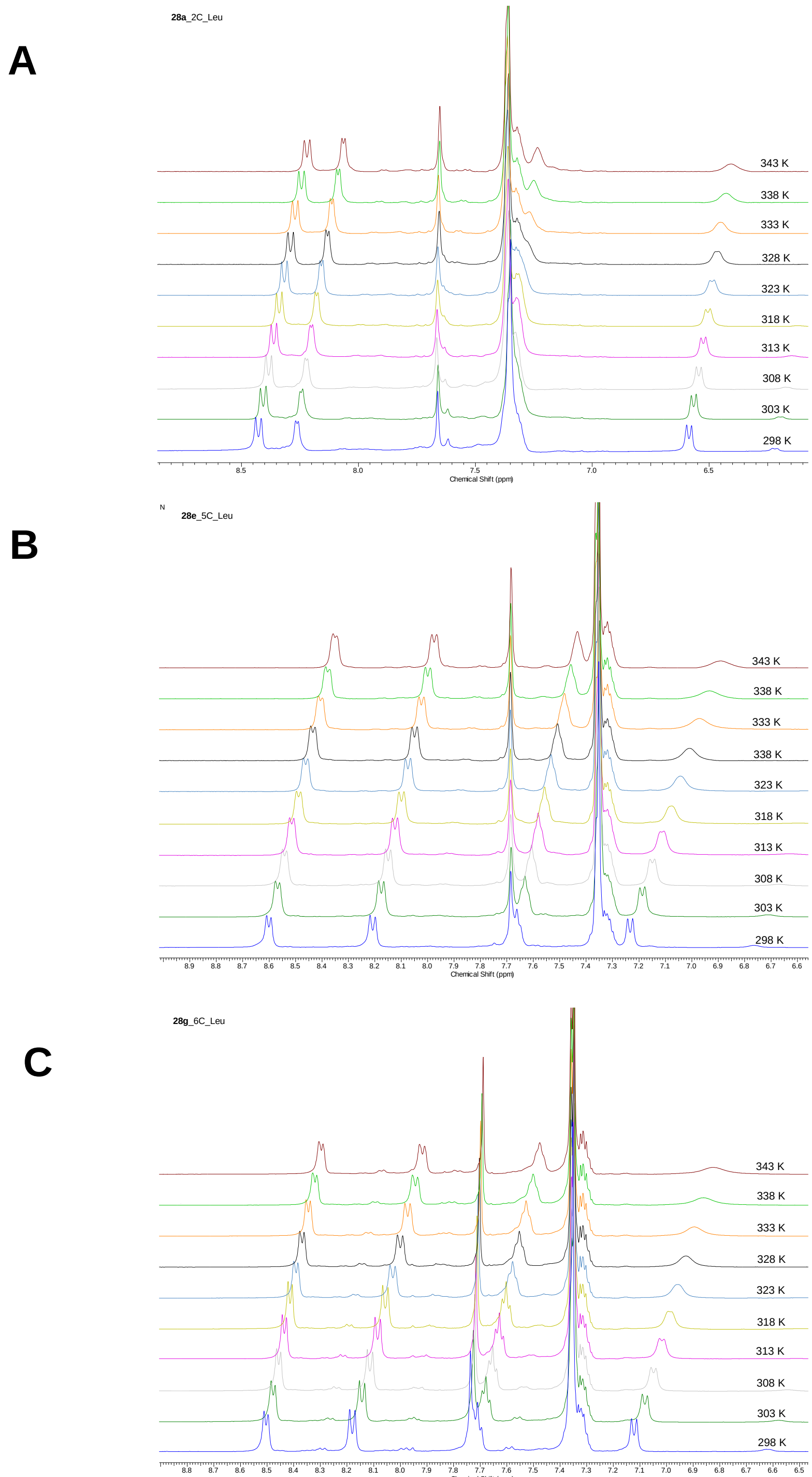


FIGURE S6. Overlap of VT-NMR experiment spectra and line broadening A) ***28a*** B) ***28e*** and C) ***28g***

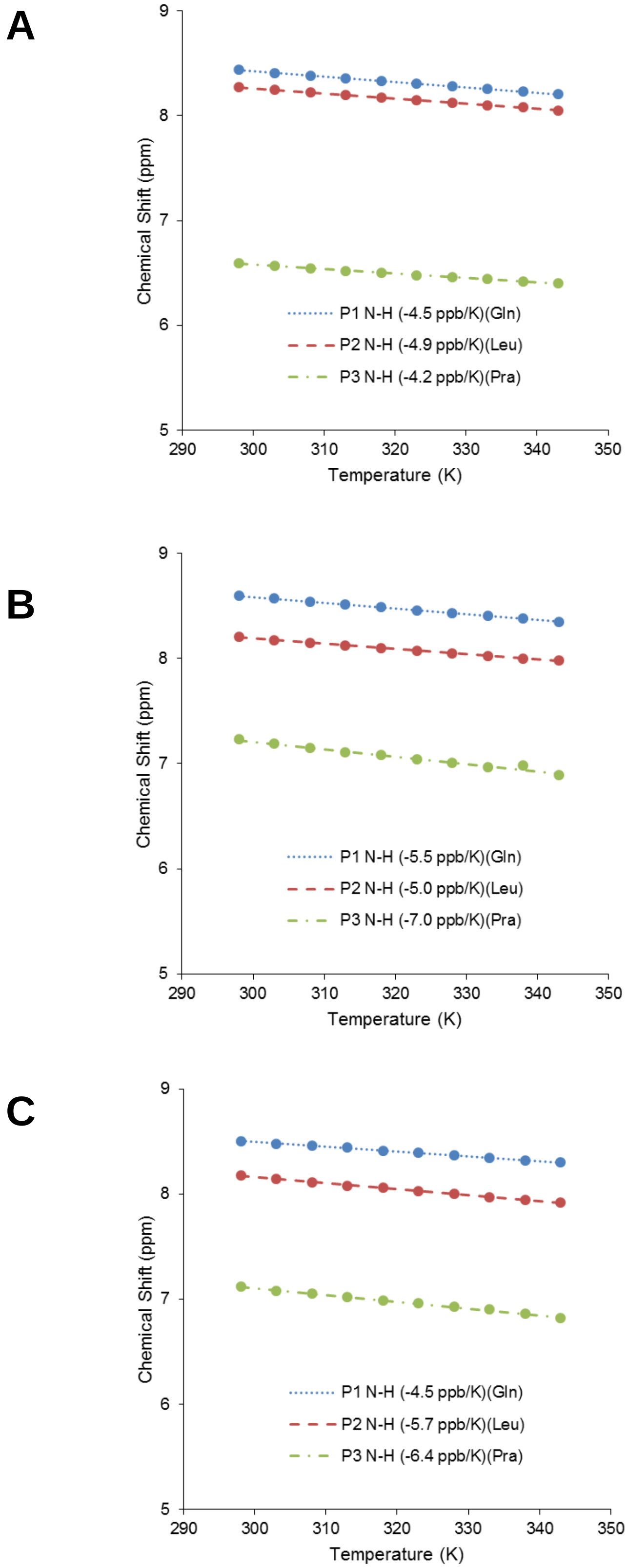
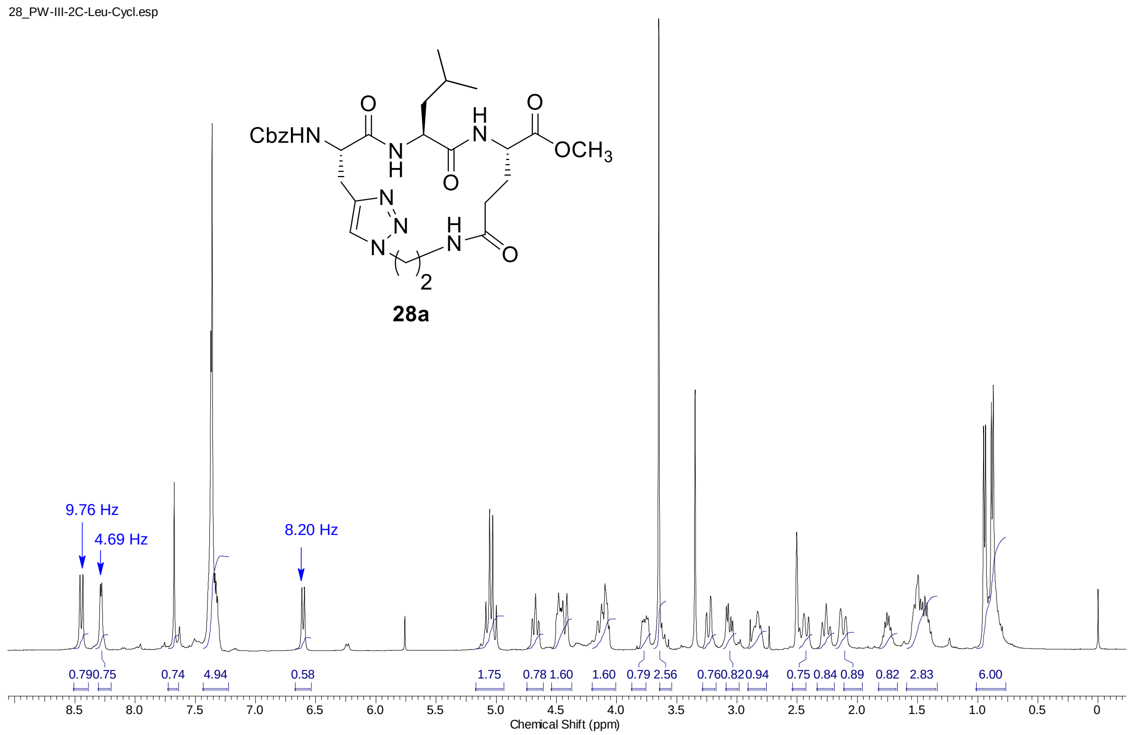


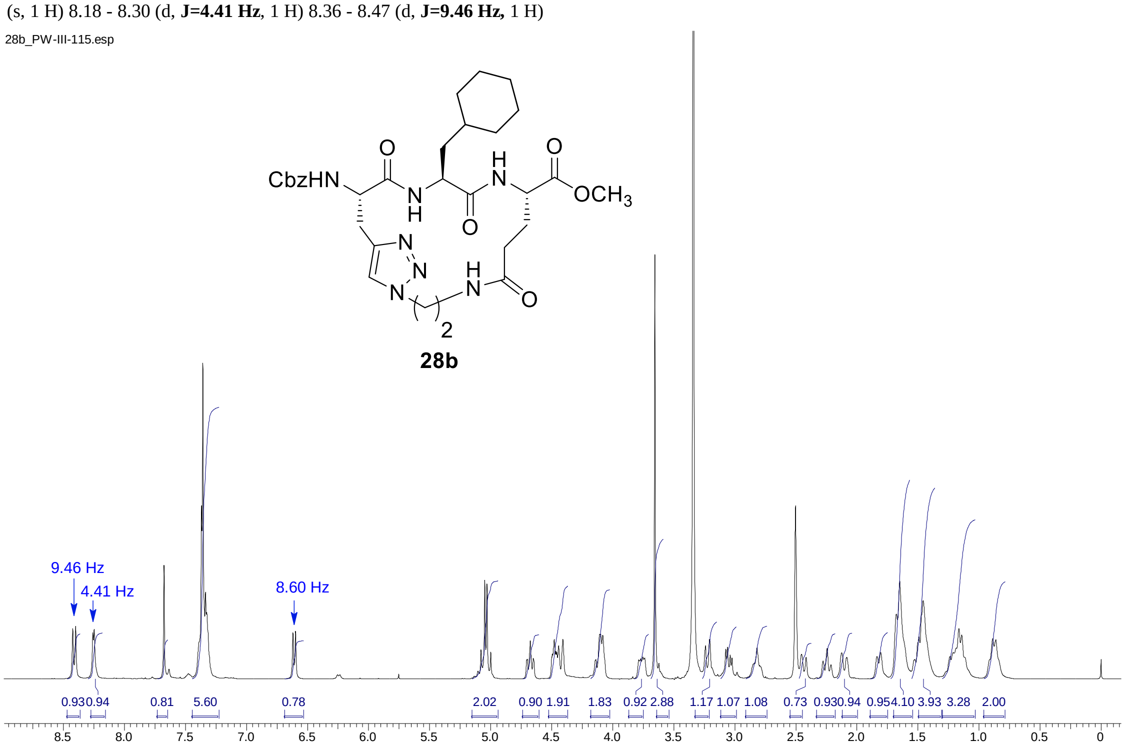
FIGURE S7. Temperature dependence of amide protons A) ***28a*** B) ***28e*** and C) ***28g***

3. 1D $^{3}J_{NH\text{-}CH\alpha}$ coupling constants of precursor macrocyclic esters

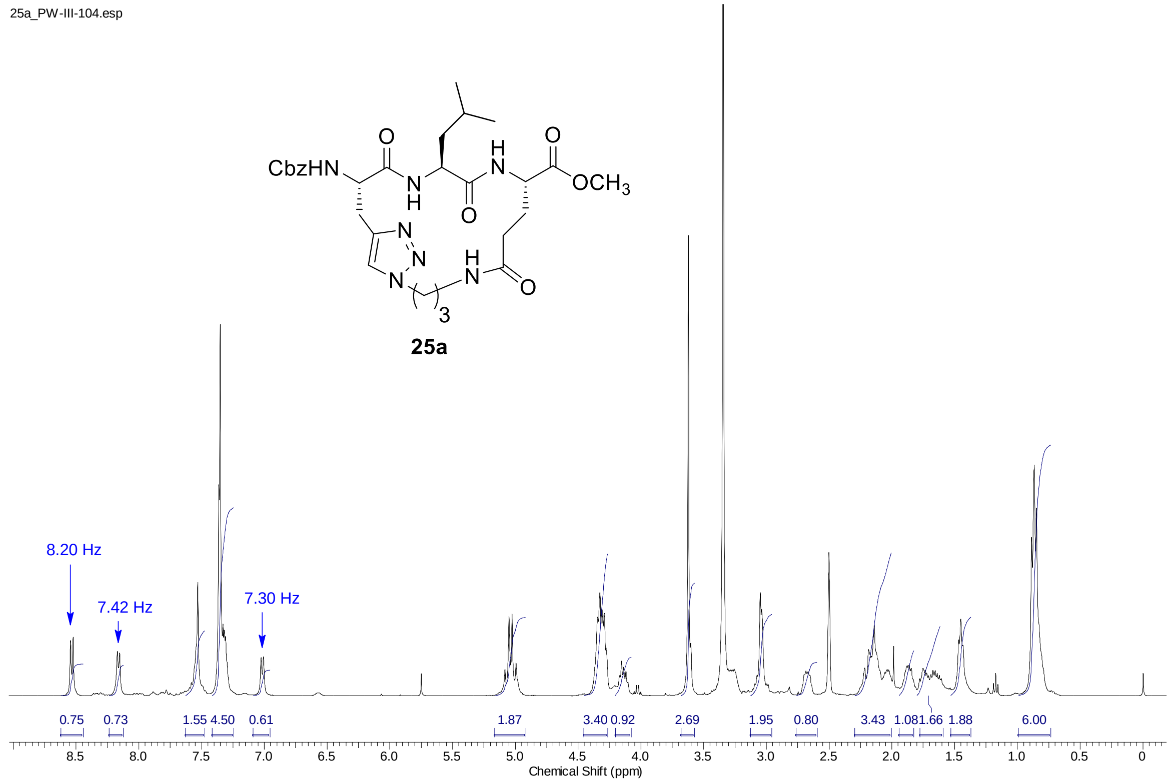
^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.77 - 1.01 (m, 6 H) 1.34 - 1.59 (m, 3 H) 1.76 (dt, *J*=13.28, 6.64 Hz, 1 H) 2.12 (d, *J*=16.80 Hz, 1 H) 2.26 (t, *J*=12.89 Hz, 1 H) 2.37 - 2.48 (m, 1 H) 2.75 - 2.91 (m, 1 H) 3.01 - 3.11 (m, 1 H) 3.24 (d, *J*=12.89 Hz, 1 H) 3.59 - 3.69 (m, 3 H) 3.71 - 3.83 (m, 1 H) 4.01 - 4.20 (m, 2 H) 4.37 - 4.54 (m, 2 H) 4.61 - 4.74 (m, 1 H) 4.94 - 5.17 (m, 2 H) 6.60 (d, ***J*=8.20 Hz**, 1 H) 7.22 - 7.43 (m, 6 H) 7.68 (s, 1 H) 8.28 (d, ***J*=4.69 Hz**, 1 H) 8.45 (d, ***J*=9.76 Hz**, 1 H)



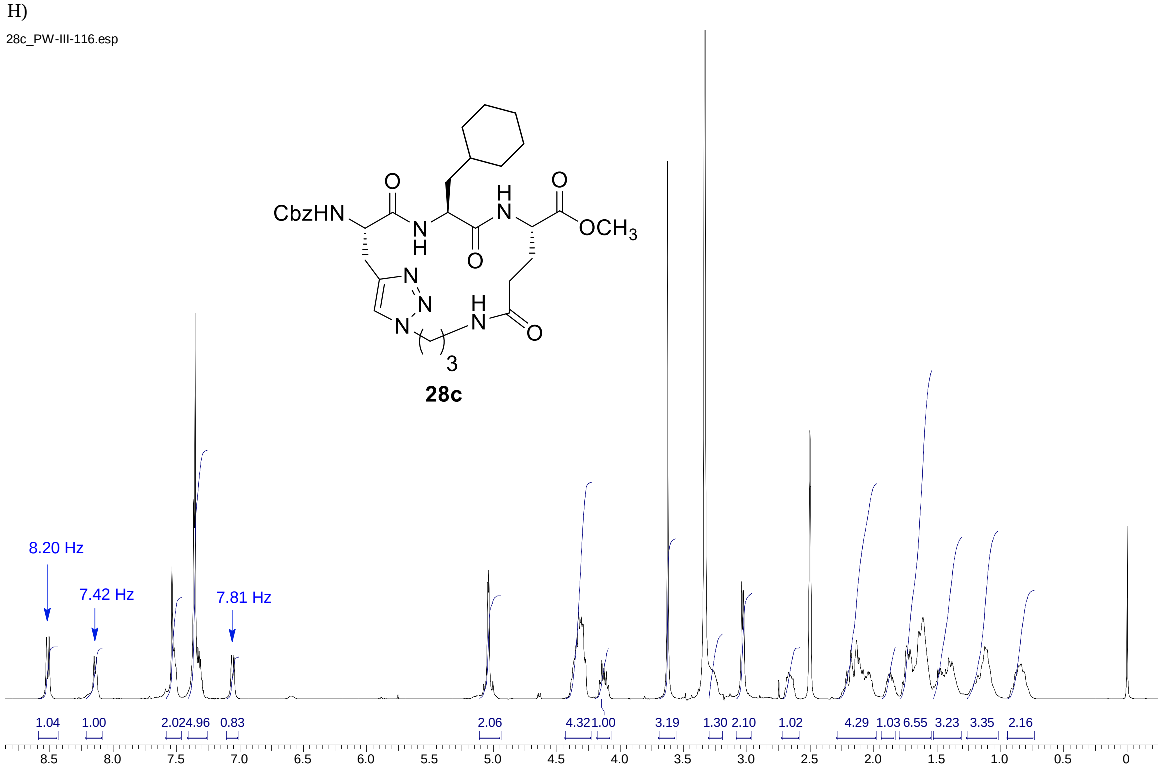
^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.79 - 0.96 (m, 2 H) 1.03 - 1.30 (m, 3 H) 1.46 (br. s., 4 H) 1.57 - 1.72 (m, 4 H) 1.75 - 1.89 (m, 1 H) 2.04 - 2.16 (m, 1 H) 2.18 - 2.33 (m, 1 H) 2.37 - 2.47 (m, 1 H) 2.74 - 2.91 (m, 1 H) 2.99 - 3.12 (m, 1 H) 3.15 - 3.26 (m, 1 H) 3.65 (s, 3 H) 3.71 - 3.82 (m, 1 H) 4.02 - 4.18 (m, 2 H) 4.37 - 4.52 (m, 2 H) 4.60 - 4.74 (m, 1 H) 5.04 (d, *J*=7.03 Hz, 2 H) 6.53 - 6.69 (d, **J=8.60 Hz**, 1 H) 7.22 - 7.44 (m, 6 H) 7.68 (s, 1 H) 8.18 - 8.30 (d, **J=4.41 Hz**, 1 H) 8.36 - 8.47 (d, **J=9.46 Hz,** 1 H)



^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.73 - 0.99 (m, 6 H) 1.45 (q, J=6.60 Hz, 3 H) 1.62 - 1.80 (m, 2 H) 1.83 - 1.94 (m, 1 H) 2.00 - 2.30 (m, 4 H) 2.60 - 2.76 (m, 1 H) 3.04 (d, J=4.30 Hz, 2 H) 3.57 - 3.68 (m, 3 H) 4.08 - 4.20 (m, 1 H) 4.26 - 4.45 (m, 4 H) 5.04 (d, J=9.76 Hz, 2 H) 7.03 (d, ***J*=7.03 Hz**, 1 H) 7.24 - 7.42 (m, 5 H) 7.53 (s, 2 H) 8.16 (d, ***J*=7.42 Hz**, 1 H) 8.53 (d, ***J*=8.20 Hz**, 1 H)



^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.73 - 0.95 (m, 2 H) 1.02 - 1.26 (m, 3 H) 1.30 - 1.53 (m, 3 H) 1.54 - 1.79 (m, 6 H) 1.83 - 1.93 (m, 1 H) 1.98 - 2.29 (m, 4 H) 2.66 (dd, J=13.28, 6.25 Hz, 1 H) 3.03 (d, J=5.86 Hz, 2 H) 3.19 - 3.30 (m, 1 H) 3.63 (s, 3 H) 4.13 (dt, J=13.67, 6.44 Hz, 1 H) 4.22 - 4.43 (m, 4 H) 4.94 - 5.11 (m, 2 H) 7.06 (d, ***J*=7.81 Hz**, 1 H) 7.25 - 7.41 (m, 5 H) 7.46 - 7.58 (m, 2 H) 8.14 (d, ***J*=7.42 Hz**, 1 H) 8.52 (d, ***J*=8.20 Hz**, 1 H)



^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.87 (dd, J=12.69, 6.44 Hz, 6 H) 1.14 - 1.32 (m, 2 H) 1.35 - 1.56 (m, 3 H) 1.57 - 1.83 (m, 4 H) 2.03 - 2.32 (m, 3 H) 2.73 (t, J=6.25 Hz, 1 H) 2.98 - 3.14 (m, 2 H) 3.54 - 3.70 (m, 3 H) 4.11 - 4.32 (m, 3 H) 4.32 - 4.44 (m, 2 H) 4.92 - 5.16 (m, 2 H) 6.91 (d, ***J*=7.81 Hz**, 1 H) 7.26 - 7.41 (m, 5 H) 7.67 - 7.83 (m, 2 H) 8.22 (d, ***J*=7.42 Hz**, 1 H) 8.36 (d, ***J*=7.81 Hz**, 1 H)

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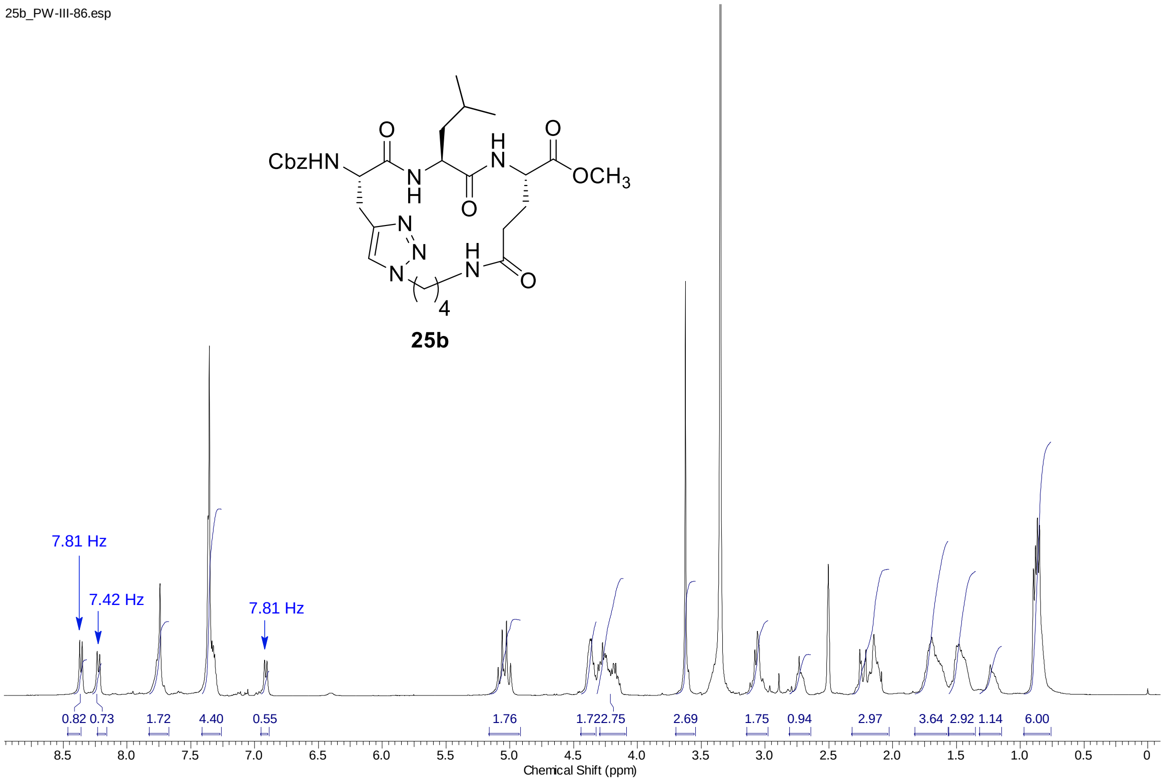

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.75 - 0.99 (m, 2 H) 1.03 - 1.29 (m, 4 H) 1.44 (d, J=9.76 Hz, 3 H) 1.50 - 1.81 (m, 10 H) 2.04 - 2.31 (m, 3 H) 2.65 - 2.79 (m, 1 H) 2.95 - 3.16 (m, 2 H) 3.62 (s, 3 H) 4.10 - 4.20 (m, 1 H) 4.22 - 4.31 (m, 2 H) 4.32 - 4.49 (m, 2 H) 4.93 - 5.14 (m, 2 H) 7.00 (d, ***J*=7.42 Hz**, 1 H) 7.25 - 7.47 (m, 5 H) 7.76 (s, 2 H) 8.22 (d, ***J*=7.42 Hz**, 1 H) 8.35 (d, ***J*=7.81 Hz**, 1 H)

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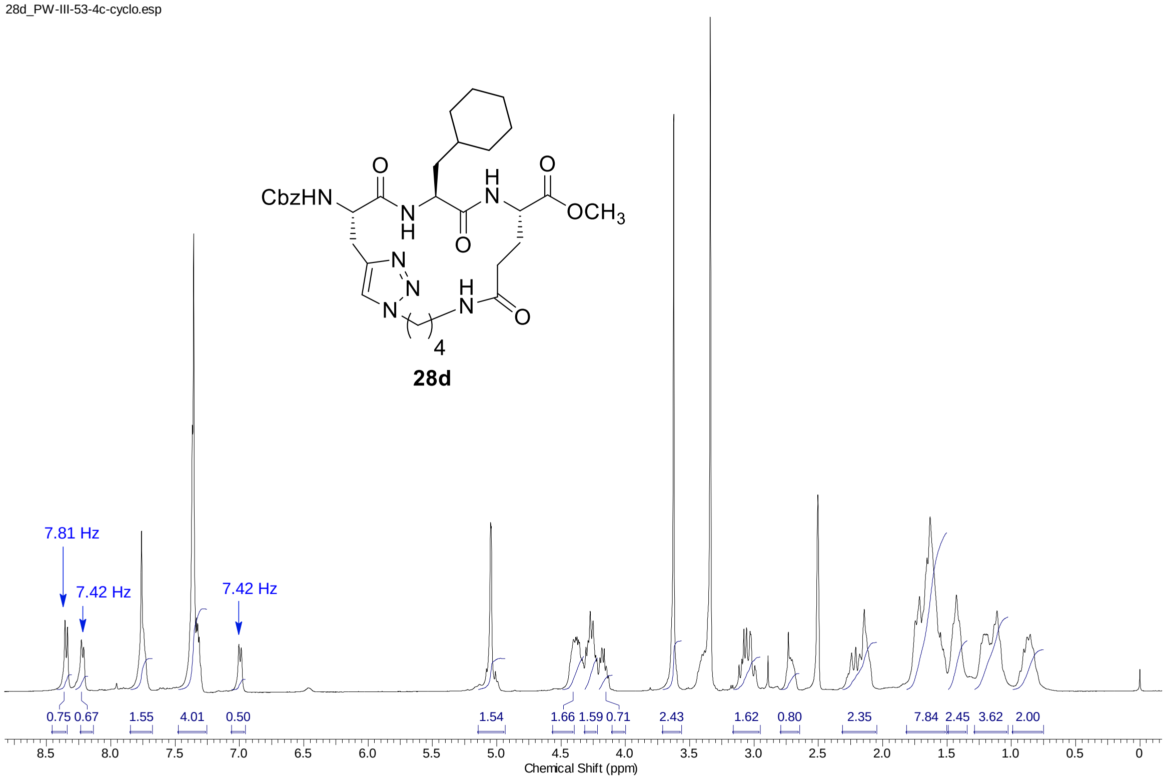
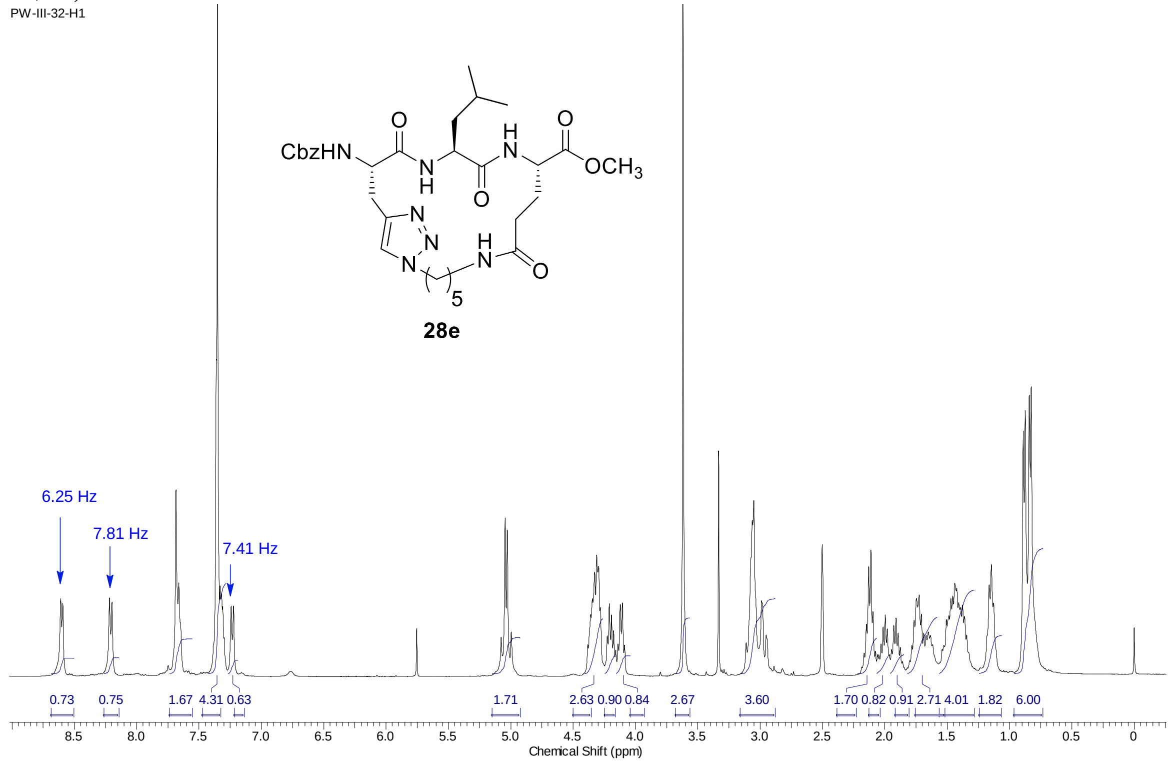


[1]H NMR (400 MHz, DMSO-d_6) δ ppm 0.86 (dd, *J*=19.53, 6.25 Hz, 6 H) 1.06 - 1.24 (m, 2 H) 1.28 - 1.56 (m, 4 H) 1.58 - 1.82 (m, 3 H) 1.91 (dt, *J*=14.06, 7.03 Hz, 1 H) 1.98 - 2.06 (m, 1 H) 2.07 - 2.22 (m, 2 H) 2.88 - 3.16 (m, 4 H) 3.62 (s, 3 H) 4.11 (q, *J*=6.77 Hz, 1 H) 4.16 - 4.25 (m, 1 H) 4.26 - 4.41 (m, 3 H) 4.92 - 5.15 (m, 2 H) 7.23 (d, ***J*=7.42 Hz**, 1 H) 7.28 - 7.43 (m, 5 H) 7.55 - 7.74 (m, 2 H) 8.21 (d, ***J*=7.81 Hz**, 1 H) 8.60 (d, ***J*=6.25 Hz**, 1 H)

PW-III-32-H1



[1]H NMR (400 MHz, DMSO-d_6) δ ppm 0.70 - 0.98 (m, 2 H) 1.03 - 1.27 (m, 5 H) 1.30 - 1.47 (m, 4 H) 1.49 - 1.85 (m, 8 H) 1.90 (dt, *J*=14.06, 7.03 Hz, 1 H) 1.96 - 2.06 (m, 1 H) 2.11 (dq, *J*=14.84, 7.42 Hz, 2 H) 2.91 - 3.18 (m, 4 H) 3.54 - 3.77 (m, 3 H) 4.07 - 4.16 (m, 1 H) 4.16 - 4.24 (m, 1 H) 4.25 - 4.48 (m, 3 H) 4.93 - 5.18 (m, 2 H) 7.24 - 7.47 (m, 5 H) 7.59 - 7.79 (m, 2 H) 8.19 (d, ***J*=7.81 Hz**, 1 H) 8.57 (d, ***J*=6.64 Hz**, 1 H)

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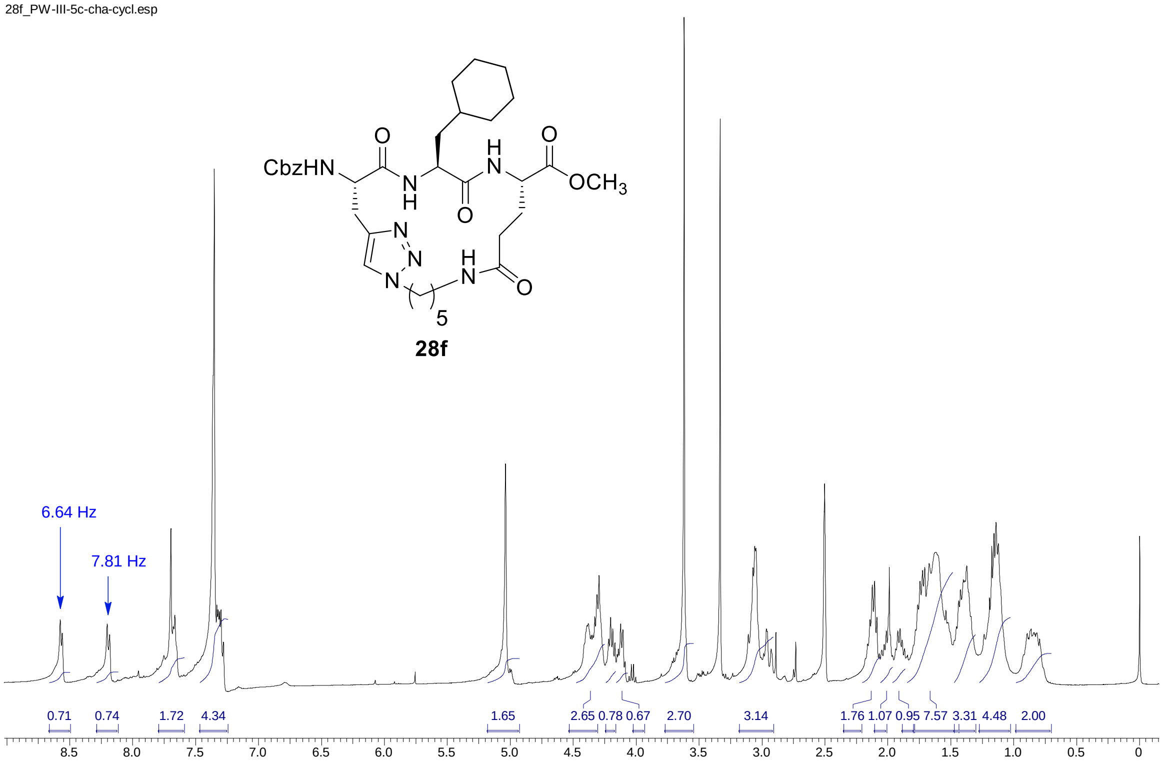

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.85 (dd, *J*=18.36, 6.25 Hz, 6 H) 1.22 (d, *J*=10.16 Hz, 4 H) 1.35 (br. s., 2 H) 1.42 - 1.55 (m, 2 H) 1.59 - 1.71 (m, 1 H) 1.72 - 1.86 (m, 2 H) 1.95 (q, *J*=6.51 Hz, 2 H) 2.04 - 2.15 (m, 1 H) 2.16 - 2.27 (m, 1 H) 2.91 - 3.21 (m, 4 H) 3.62 (s, 3 H) 4.04 (q, *J*=7.03 Hz, 1 H) 4.16 - 4.28 (m, 2 H) 4.29 - 4.41 (m, 2 H) 4.89 - 5.16 (m, 2 H) 7.12 (d, ***J*=7.81 Hz**, 1 H) 7.27 - 7.44 (m, 5 H) 7.65 - 7.78 (m, 2 H) 8.18 (d, ***J*=8.20 Hz**, 1 H) 8.50 (d, ***J*=5.86 Hz**, 1 H)

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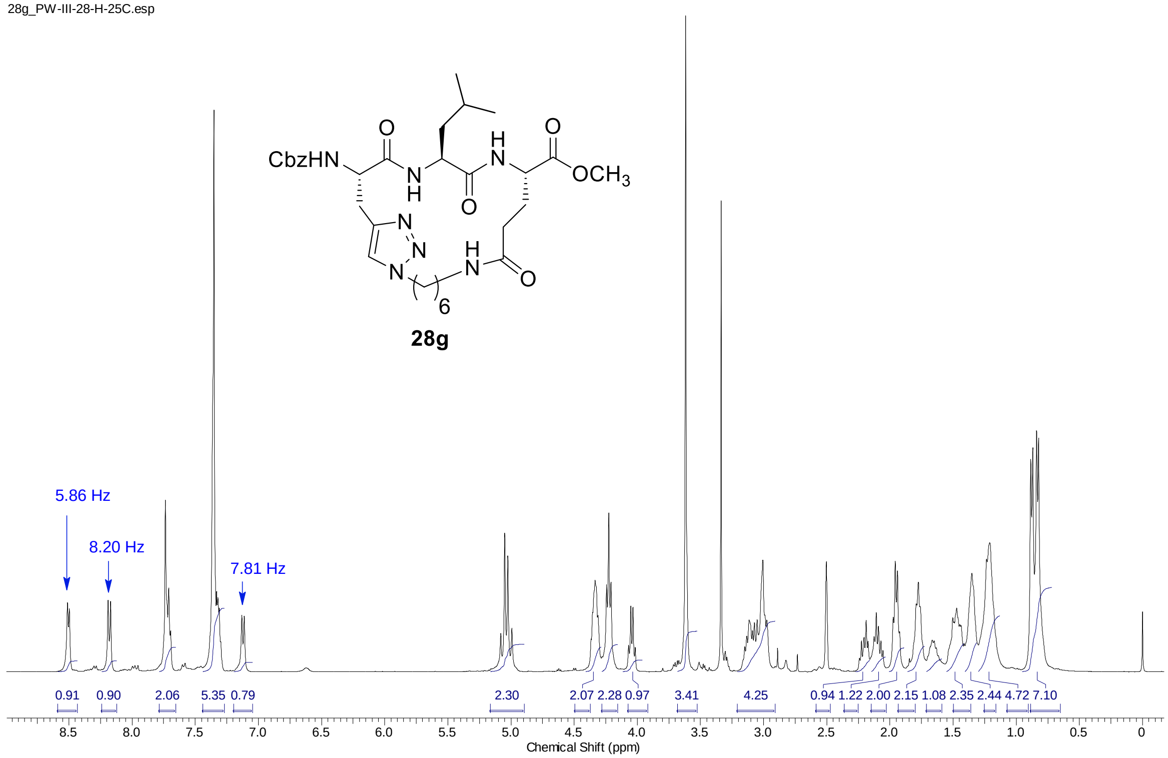

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.72 - 0.97 (m, 2 H) 1.04 - 1.29 (m, 7 H) 1.30 - 1.46 (m, 4 H) 1.48 - 1.73 (m, 6 H) 1.74 - 1.83 (m, 2 H) 1.94 (q, *J*=6.71 Hz, 2 H) 2.09 (d, *J*=7.02 Hz, 1 H) 2.14 - 2.27 (m, 1 H) 2.90 - 3.20 (m, 4 H) 3.61 (s, 3 H) 4.03 (d, *J*=6.71 Hz, 1 H) 4.22 (t, *J*=7.02 Hz, 2 H) 4.28 - 4.45 (m, 2 H) 5.04 (s, 2 H) 7.18 (d, ***J*=7.63 Hz**, 1 H) 7.26 - 7.44 (m, 5 H) 7.63 - 7.80 (m, 2 H) 8.16 (d, ***J*=7.63 Hz**, 1 H) 8.46 (d, ***J*=6.10 Hz**, 1 H)

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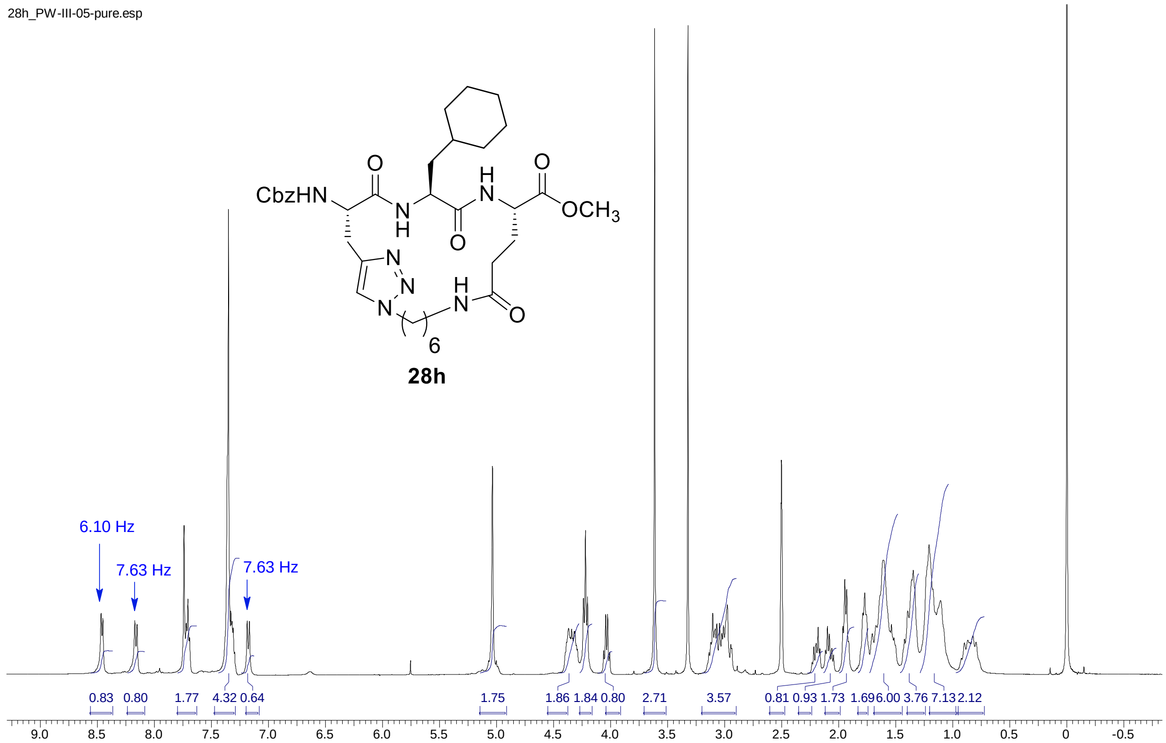
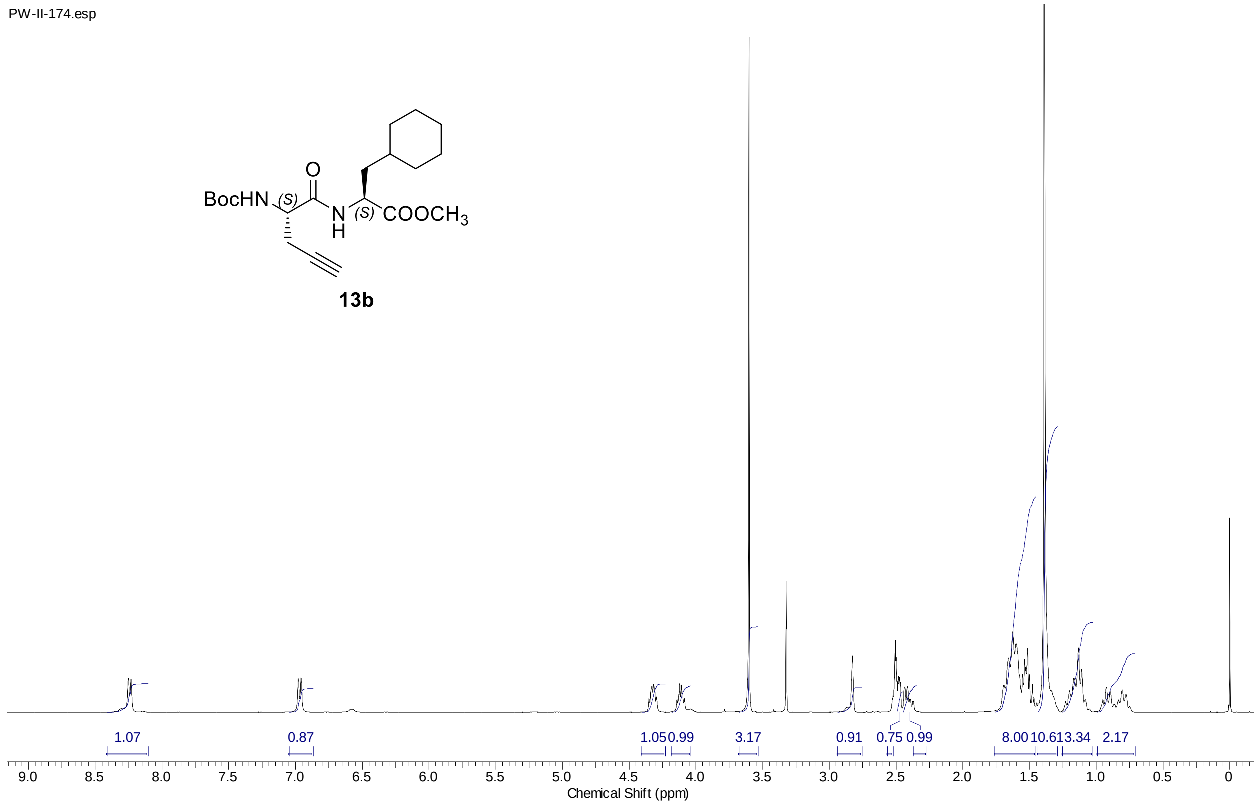
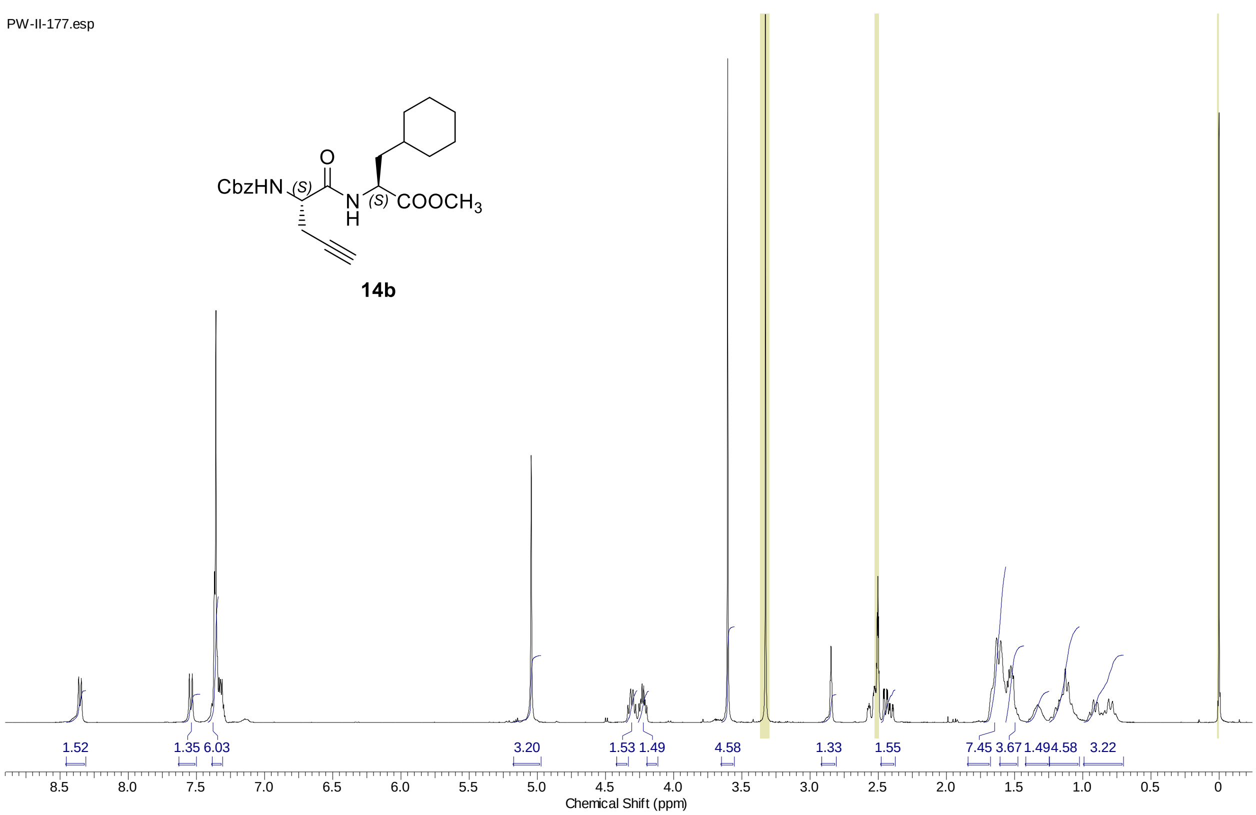
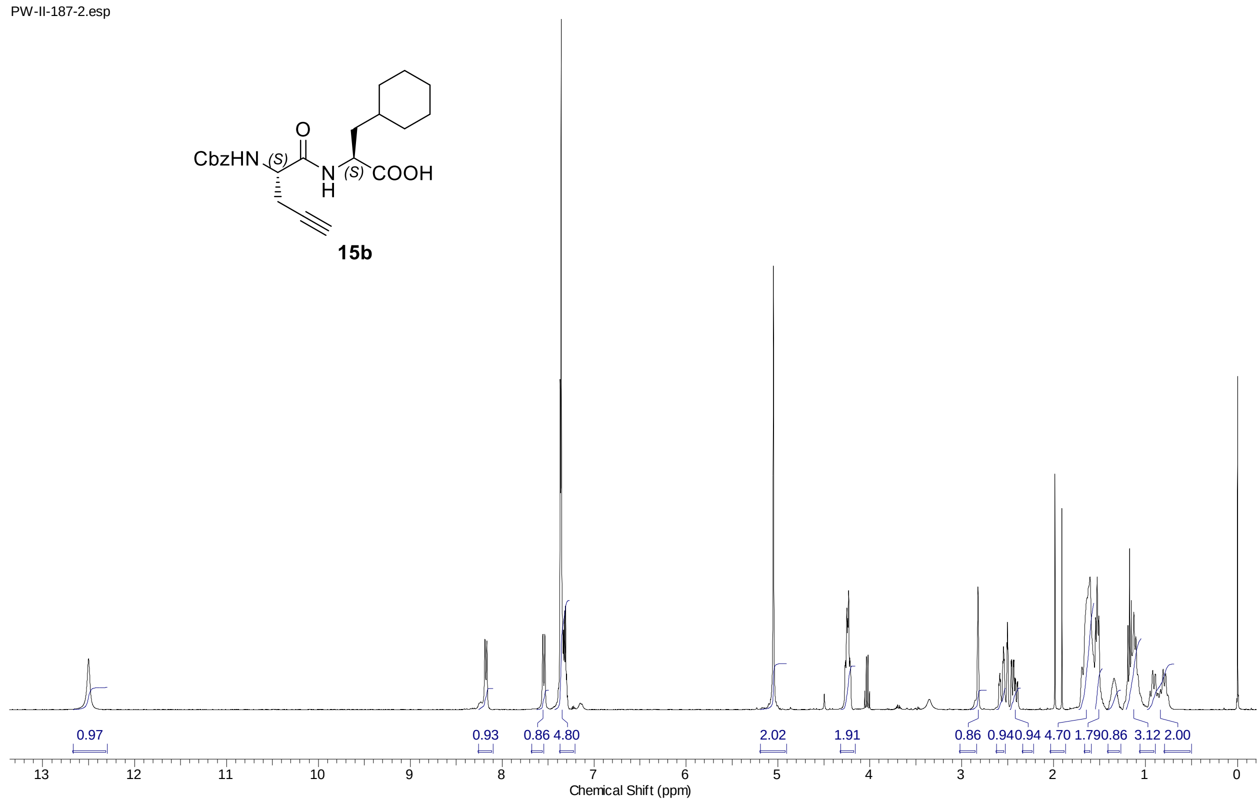


4. Representative ^{1}H-NMR spectrum for synthesis of compound 10



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N
H
(S)
COOH
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0.86
4.80
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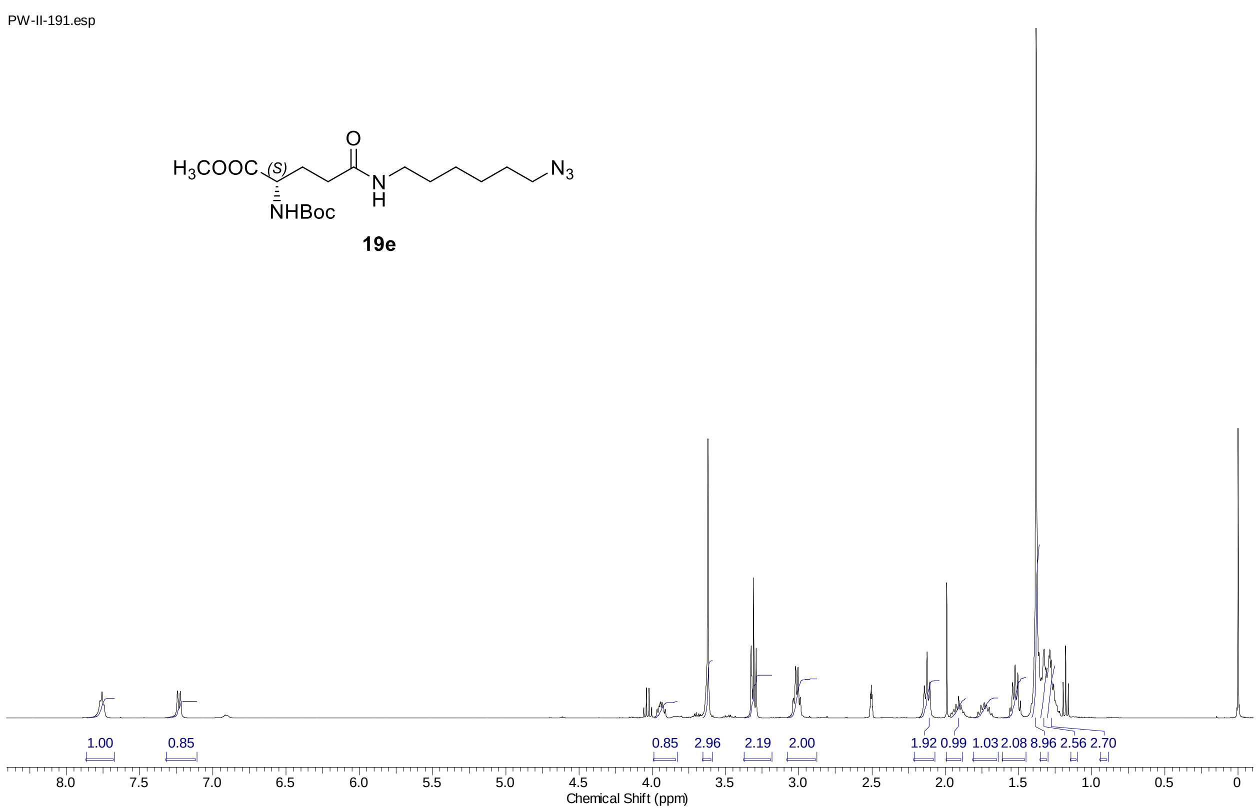
0.94
0.94
4.70
1.79
0.86
3.12
2.00
Chemical Shift (ppm)


PW-II-191.esp
H_3COOC
(S)
O
N
H
N_3
NHBoc
19e
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0.85
2.96
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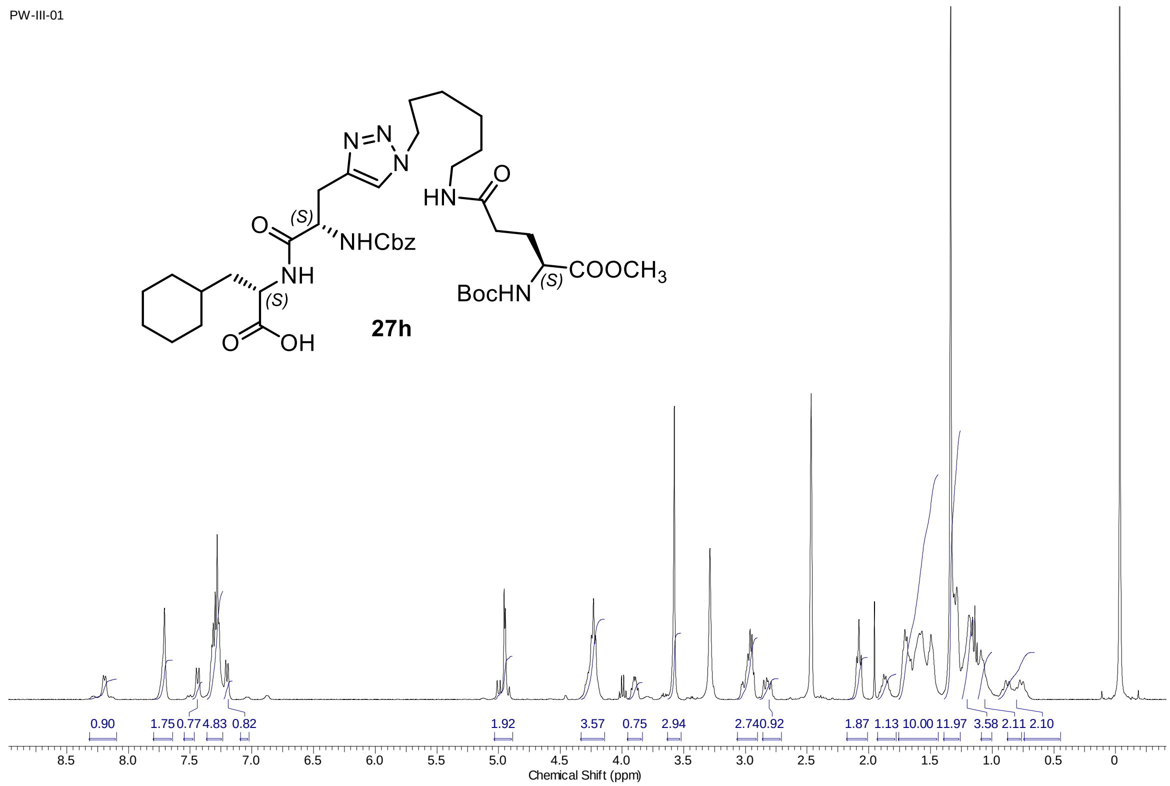
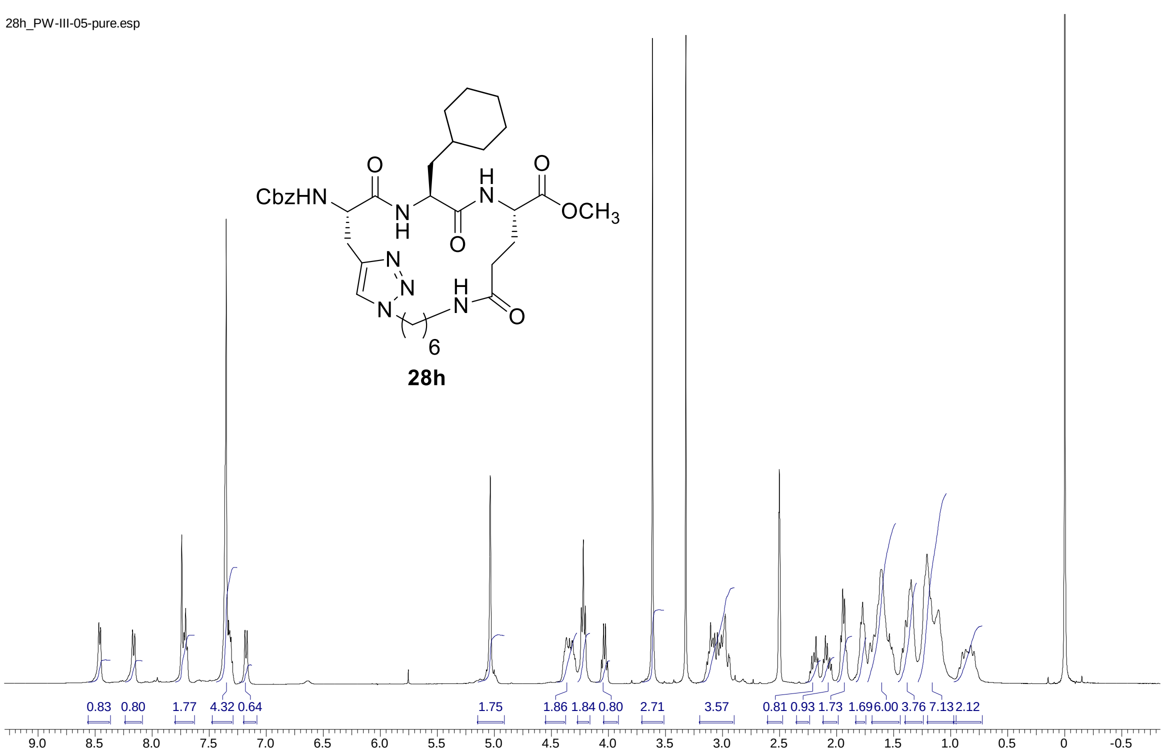
2.00
1.92
0.99
1.03
2.08
8.96
2.56
2.70
Chemical Shift (ppm)

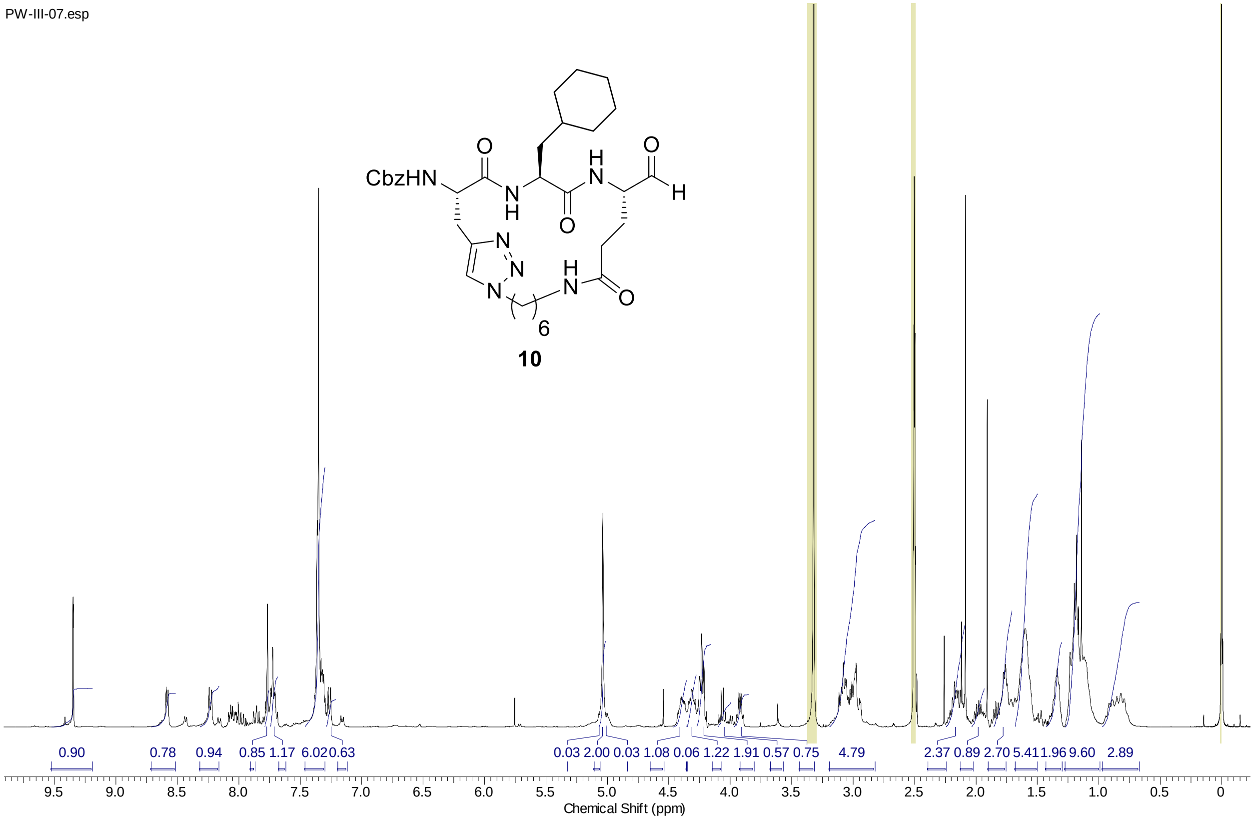
PW-III-01



28h_PW-III-05-pure.esp



PW-III-07.esp


CbzHN
H
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10
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0.03
2.00
0.03
1.08
0.06
1.22
1.91
0.57
0.75
4.79
2.37
0.89
2.70
5.41
1.96
9.60
2.89
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
Chemical Shift (ppm)

5. X-ray crystallography-derived information

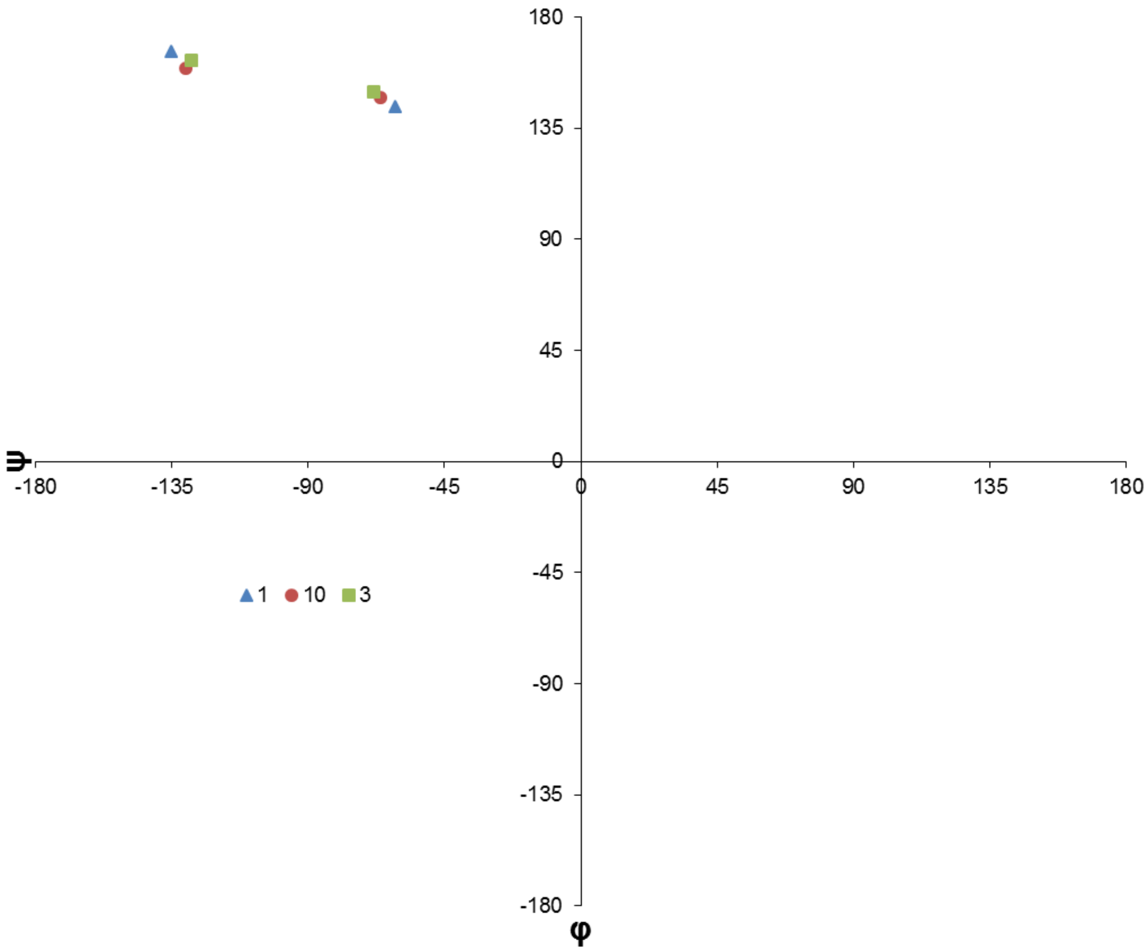

FIGURE S8. The coordinates of the Ramachandran plot of six ϕ and ψ angle pairs of the corresponding P_2 and P_3 residues, extracted from the X-ray crystal structures.

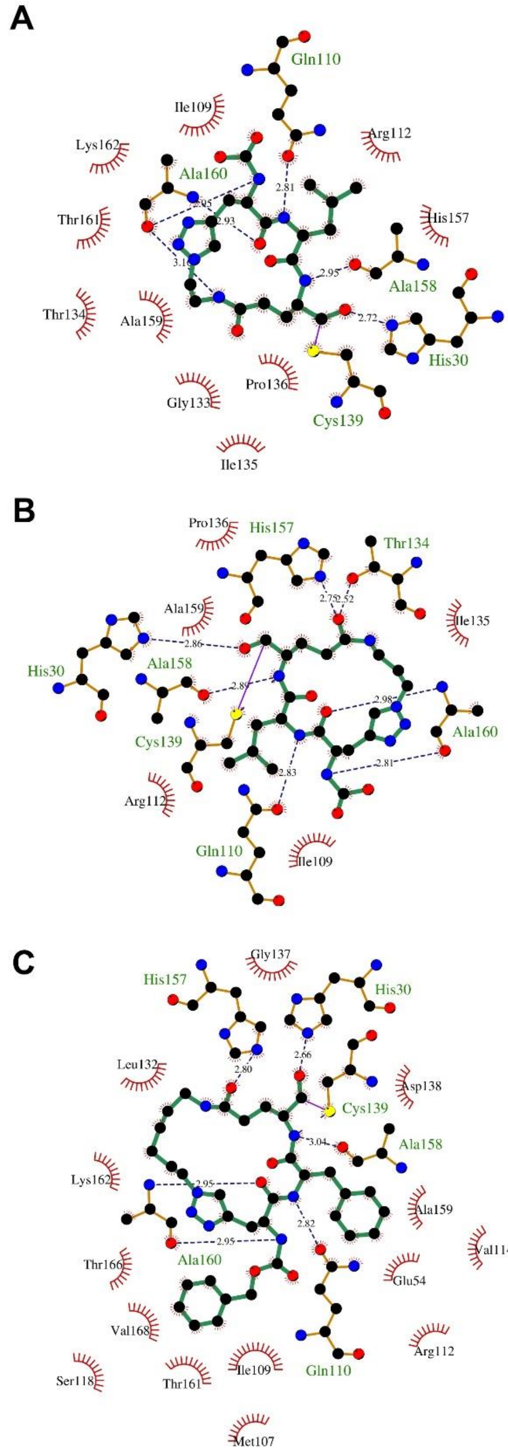

FIGURE S9. LigPlot diagrams of the X-ray crystal structures. A) NV 3CLPro:***1*** complex. B) NV 3CLPro:***3*** complex. C) NV 3CLPro:***10*** complex

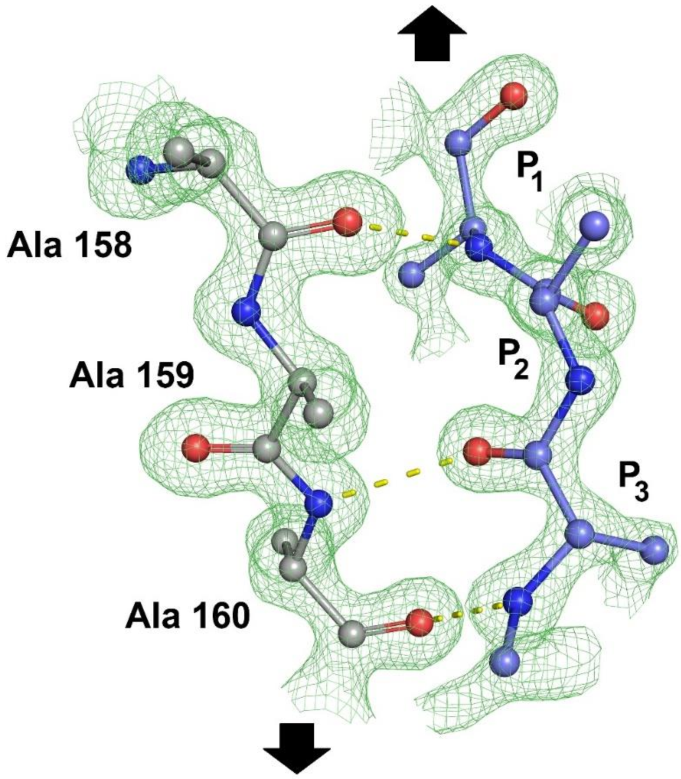

FIGURE S10. 2-Stranded antiparallel β-sheet arrangement between NV 3CLPro and backbone of inhibitor ***11*** from the X-ray crystal structure. Hydrogen bonds are shown in yellow color dashes and direction of the two strands are shown in black color arrows. Oxygen atoms are in red color, Nitrogen atoms are in blue color and hydrogen atoms are omitted for convenience. NV 3CLPro carbons atoms are in ash color and inhibitor carbon atoms are in light blue color. Sidechains are shown only up to the first sidechain carbon atom. 2Fo-Fc electron density map is shown in green color at 1.5σ.