Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry


**Supplementary Information**

# Positional effects of click cyclization on β-hairpin structure, stability, and function

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**Table S1**

Proton Assignments (ppm) for Term-U

| | α | β | γ | δ | ε |
|---|---|---|---|---|---|
| AzK | 4.32 | 1.78 | 1.43 | 1.60 | 3.30 |
| R | 4.64 | 1.79 | 1.58 | 3.19 | |
| W | 5.35 | 3.28, 3.00 | | | |
| V | 4.73 | 2.00 | 0.91 | | |
| K | 4.36 | 1.06, 1.16 | -0.11 | 0.46 | 2.31 |
| V | 4.14 | 1.85 | 0.87 | | |
| N | 4.37 | 3.08, 2.75 | | | |
| G* | 0.74 | | | | |
| O | 4.70 | 1.93 | 1.76 | 3.07 | |
| W | 5.05 | 3.20, 3.08 | | | |
| I | 4.79 | 1.98 | 1.25,1.46 | 0.96 ($\gamma$,$CH_3$) | 0.83 ($\delta$,$CH_3$) |
| K | 4.03 | 1.26 | 0.39, 0.47 | 0.67 | 2.65 |
| Q | 4.49 | 2.06, 1.87 | 2.25 | | |
| Pra | 4.70 | 2.51 | | | |

*Glycine splitting value

**Table S2**

Proton Assignments (ppm) for Term-C

| | **α** | **β** | **γ** | **δ** | **ε** |
|---|---|---|---|---|---|
| **K-$N_3$** | 4.07 | 1.87 | 1.11 | 1.47, 1.57 | 4.36 |
| **R** | 4.67 | 1.79 | 1.60 | 3.19 | |
| **W** | 5.30 | 3.29, 3.04 | | | |
| **V** | 4.69 | 2.00 | 0.90 | | |
| **K** | 4.35 | 1.13, 1.06 | -0.10 | 0.43 | 2.29 |
| **V** | 4.13 | 1.84 | 0.86 | | |
| **N** | 4.36 | 3.08, 2.75 | | | |
| **G*** | 0.74 | | | | |
| **O** | 4.70 | 1.92 | 1.75 | 3.06 | |
| **W** | 5.04 | 3.18, 3.07 | | | |
| **I** | 4.76 | 1.96 | 1.44, 1.24 | 0.93 ($\gamma$,$CH_3$) | 0.84 ($\delta$,$CH_3$) |
| **K** | 4.18 | 1.18 | 0.41 | 0.70 | 2.47 |
| **Q** | 4.46 | 2.00, 1.84 | 2.23 | | |
| **Pra** | 4.80 | 2.92, 2.76 | | | |

*Glycine splitting value

**Table S3**

Proton Assignments (ppm) for Term-rev-U

| | α | β | γ | δ | ε |
|---|---|---|---|---|---|
| **K-$N_3$** | 4.38 | 1.62 | 1.34 | 1.47 | 3.24 |
| **R** | 4.58 | 1.85, 1.77 | 1.58 | 3.19 | |
| **W** | 5.28 | 3.29, 3.00 | | | |
| **V** | 4.67 | 2.00 | 0.89 | | |
| **K** | 4.32 | 1.06, 1.16 | -0.10 | 0.46 | 2.32 |
| **V** | 4.12 | 1.84 | 0.86 | | |
| **N** | 4.36 | 3.06, 2.73 | | | |
| **G*** | 0.72 | | | | |
| **O** | 4.67 | 1.91 | 1.74 | 3.06 | |
| **W** | 5.01 | 3.18, 3.05 | | | |
| **I** | 4.78 | 1.97 | 1.44, 1.23 | 0.95 (γ,$CH_3$) | 0.82 (δ,$CH_3$) |
| **K** | 4.21 | 1.22 | 0.46 | 0.86 | 2.56 |
| **Q** | 4.50 | 2.05, 1.87 | 2.26 | | |
| **Pra** | 4.46 | 2.71, 2.45 | | | |

*Glycine splitting value

**Table S4**

Proton Assignments (ppm) for Term-rev-C

| | α | β | γ | δ | ε |
|---|---|---|---|---|---|
| **K-$N_3$** | 4.09 | 1.65 | 0.43 | 1.42 | 4.35 |
| **R** | 4.64 | 1.72 | 1.56 | 3.16 | |
| **W** | 5.27 | 3.19,2.95 | | | |
| **V** | 4.67 | 1.94 | 0.83 | | |
| **K** | 4.31 | 1.00, 1.10 | -0.19 | 0.41 | 2.24 |
| **V** | 4.09 | 1.79 | 0.83 | | |
| **N** | 4.32 | 3.04, 2.71 | | | |
| **G*** | 0.74 | | | | |
| **O** | 4.66 | 1.94 | 1.74 | 3.03 | |
| **W** | 4.98 | 3.14, 3.03 | | | |
| **I** | 4.78 | 1.97 | 1.41, 1.24 | 0.91 (γ,$CH_3$) | 0.82 (δ,$CH_3$) |
| **K** | 4.07 | 1.39 | 0.60 | 0.99 | 2.71 |
| **Q** | 4.46 | 1.89, 1.72 | 2.14 | | |
| **Pra** | 4.70 | 3.28, 2.93 | | | |

*Glycine splitting value

**Table S5**

Proton Assignments (ppm) for NHB-U

| | α | β | γ | δ | ε |
|---|---|---|---|---|---|
| **R** | 4.61 | 1.85 | 1.69 | 3.01 | |
| **K-N3** | 4.37 | 1.78 | 1.63 | 1.94 | |
| **V** | 4.57 | 1.99 | 0.88 | | |
| **K** | 4.30 | 1.08, 1.18 | 0.09, 0.14 | 0.52 | 2.32 |
| **V** | 4.10 | 1.83 | 0.85 | | |
| **N** | 4.38 | 3.00, 2.72 | | | |
| **G*** | 0.63 | | | | |
| **O** | 4.99 | 1.70, 1.81 | 1.39 | 3.05 | |
| **W** | 4.87 | 3.10 | | | |
| **I** | 4.58 | 1.94 | 1.39, 1.18 | 0.89 ($\gamma$,$CH_3$) | 0.82 ($\delta$,$CH_3$) |
| **Pra** | 4.76 | 2.71 | | | |
| **Q** | 4.38 | 2.11, 1.94 | 2.31 | | |

*Glycine splitting value

**Table S6**

Proton Assignments (ppm) for NHB-C

| | α | β | γ | δ | ε |
|---|---|---|---|---|---|
| **R** | 4.30 | 1.78 | 1.51 | 3.21 | |
| **K-N3** | 4.29 | 1.64 | 0.71 | 1.06 | 4.56 |
| **V** | 4.65 | 1.97 | 0.85 | | |
| **K** | 4.37 | 1.12 | -0.11, 0.16 | 0.40, 0.91 | 2.04 |
| **V** | 4.09 | 1.79 | 0.84 | | |
| **N** | 4.33 | 3.04, 2.71 | | | |
| **G*** | 0.72 | | | | |
| **O** | 4.62 | 1.86 | 1.69 | 3.00 | |
| **W** | 4.84 | 3.12 | | | |
| **I** | 4.59 | 1.98 | 1.35, 1.28 | 0.89 ($\gamma$,$CH_3$) | 0.78 ($\delta$,$CH_3$) |
| **Pra** | 4.09 | 2.42, 2.31 | | | |
| **Q** | 4.41 | 2.11, 2.01 | 2.33 | | |

*Glycine splitting value

**Table S7**

Proton Assignments (ppm) for NHB-rev-U

| | α | β | γ | δ | ε |
|---|---|---|---|---|---|
| **R** | 4.40 | 1.84, 1.79 | 1.65 | 3.20 | |
| **K-N3** | 4.54 | 1.77 | 1.43 | 1.60 | |
| **V** | 4.49 | 2 | 0.87 | | |
| **K** | 4.31 | | 0.63 | 1.27, 1.17 | 2.42 |
| **V** | 4.10 | 1.87 | 0.85 | | |
| **N** | 4.42 | 3.00, 2.73 | | | |
| **G*** | 0.55 | | | | |
| **O** | 4.57 | 1.83 | 1.69 | 3.01 | |
| **W** | 5.08 | 2.73, 2.67 | | | |
| **I** | 4.45 | 1.91 | 1.40, 1.19 | 0.88 ($\gamma$,$CH_3$) | 0.77 ($\delta$,$CH_3$) |
| **Pra** | 4.80 | 3.11 | | | |
| **Q** | 4.37 | 2.10, 1.97 | 2.33 | | |

*Glycine splitting value

**Table S8**

Proton Assignments (ppm) for NHB-rev-C

| | α | β | γ | δ | ε |
|---|---|---|---|---|---|
| **R** | 4.32 | 1.80 | 1.63 | 3.21 | |
| **K-N3** | 4.21 | 1.78, 1.98 | 0.94, 1.13 | 1.56 | 4.43, 4.37 |
| **V** | 4.65 | 1.98 | 0.86 | | |
| **K** | 4.29 | 1.07, 1.16 | 0.00 | 0.44 | 2.29 |
| **V** | 4.10 | 1.81 | 0.84 | | |
| **N** | 4.36 | 3.04, 2.71 | | | |
| **G*** | 0.71 | | | | |
| **O** | 4.65 | 1.87 | 1.71 | 3.03 | |
| **W** | 5.34 | 3.17, 3.06 | | | |
| **I** | 4.57 | 1.92 | 1.36, 1.21 | 0.88 ($\gamma$,$CH_3$) | 0.81 ($\delta$,$CH_3$) |
| **Pra** | 4.87 | 3.14 | | | |
| **Q** | 4.31 | 2.05, 1.96 | 2.29 | | |

*Glycine splitting value

**Table S9**

Proton Assignments (ppm) for HB-U

| | α | β | γ | δ | ε |
|---|---|---|---|---|---|
| **R** | 4.32 | 1.69 | 1.54 | 3.14 | |
| **W** | 4.99 | 3.27, 3.10 | | | |
| **K-N3** | 4.63 | 1.74, 1.67 | 1.26 | 1.52 | 3.25 |
| **K** | **4.00** | 1.39 | 0.68 | 0.86 | 2.73 |
| **V** | 4.11 | 2.01 | 0.89 | | |
| **N** | 4.45 | 2.98, 2.73 | | | |
| **G*** | 0.48 | | | | |
| **O** | 4.58 | 1.88 | 1.70 | 3.03 | |
| **W** | 4.90 | 3.27, 3.12 | | | |
| **Pra** | 4.78 | 2.67, 2.41 | | | |
| **K** | **4.31** | 1.35, 1.19 | 0.48 | 0.79, 0.64 | 2.43 |
| **Q** | 4.27 | 2.06, 1.92 | 2.30 | | |

*Glycine splitting value

**Table S10**

Proton Assignments (ppm) for HB-C

| | α | β | γ | δ | ε |
|---|---|---|---|---|---|
| **R** | 4.49 | 1.75 | 1.63 | 3.22 | |
| **W** | 5.30 | 3.32, 3.13 | | | |
| **K-N3** | 4.82 | 1.93, 1.67 | 0.86 | 1.16 | 4.30, 4.26 |
| **K** | 3.90 | 1.28 | 0.33, 0.57 | 1.17 | 2.68 |
| **V** | 4.21 | 1.97 | 0.92 | | |
| **N** | 4.41 | 3.05, 2.80 | | | |
| **G*** | 0.66 | | | | |
| **O** | 4.80 | 1.75 | 1.63 | 3.12 | |
| **W** | 5.07 | 3.31, 3.02 | | | |
| **Pra** | 5.13 | 3.35 | | | |
| **K** | 4.54 | | 0.44 | 1.06, 0.93 | |
| **Q** | 4.32 | 2.08, 1.94 | 2.33 | | |

*Glycine splitting value

**Table S11**

Proton Assignments (ppm) for HB-rev-U

| | α | β | γ | δ | ε |
|---|---|---|---|---|---|
| **R** | 4.41 | 1.68 | 1.58 | 3.17 | |
| **W** | 5.17 | 3.29, 3.06 | | | |
| **K-N3** | 4.71 | 1.82, 1.75 | 1.40 | 1.59 | 3.32 |
| **K** | 4.34 | 1.28 | 0.53 | 1.15 | 2.39 |
| **V** | 4.11 | 1.90 | 0.90 | | |
| **N** | 4.41 | 3.02, 2.76 | | | |
| **G*** | 0.59 | | | | |
| **O** | 4.64 | 1.91 | 1.75 | 3.06 | |
| **W** | 4.92 | 3.25, 3.12 | | | |
| **Pra** | 4.87 | 2.67 | | | |
| **K** | 3.92 | 1.37 | 0.63 | 0.82 | 2.72 |
| **Q** | 4.28 | 2.04, 1.91 | 2.29 | | |

*Glycine splitting value

**Table S12**

Proton Assignments (ppm) for HB-rev-C

| | α | β | γ | δ | ε |
|---|---|---|---|---|---|
| **R** | 4.48 | 1.77 | 1.61 | 3.18 | |
| **W** | 5.32 | 3.35, 3.07 | | | |
| **K-N3** | 4.79 | 1.96 | 1.26 | 1.75 | 4.32 |
| **K** | 4.34 | 1.00 | 0.03, 0.18 | 0.27 | 2.30 |
| **V** | 4.15 | 1.88 | 0.82 | | |
| **N** | 4.39 | 3.03, 2.77 | | | |
| **G*** | 0.69 | | | | |
| **O** | 4.71 | 1.93 | 1.75 | 3.08 | |
| **W** | 4.92 | 3.26, 3.07 | | | |
| **Pra** | 5.20 | 3.35 | | | |
| **K** | 4.05 | 1.32, 1.20 | 0.43 | 0.7 | 2.71 |
| **Q** | 4.27 | 2.01, 1.86 | 2.20 | | |

*Glycine splitting value

**Table S13**

Proton Assignments (ppm) for Term-pG-U

| | α | β | γ | δ | ε |
|---|---|---|---|---|---|
| **Pra** | 4.68 | 2.48 | | | |
| **R** | 4.62 | 1.78 | 1.57 | 3.18 | |
| **W** | 5.31 | 3.24, 3.01 | | | |
| **V** | 4.68 | 1.99 | 0.89 | | |
| **K** | 4.30 | 1.19, 0.97 | -0.07 | 0.5 | |
| **V** | 4.50 | 1.90 | 0.88 | | |
| **dP** | 4.33 | 2.32 | 2.08, 1.93 | 3.80, 3.73 | |
| **G*** | | | | | |
| **O** | 4.72 | 1.90 | 1.75 | 3.04 | |
| **W** | 5.09 | 3.17, 3.04 | | | |
| **I** | 4.76 | 1.95 | 1.42, 1.22 | 0.92 ($\gamma$,$CH_3$) | 0.80 ($\delta$,$CH_3$) |
| **K** | 3.99 | 1.24 | 0.46. 0.31 | 0.60 | 2.61 |
| **Q** | 4.46 | 2.02, 1.85 | 2.23 | | |
| **K-$N_3$** | 4.30 | 2.75 | 1.41 | 1.58 | 3.29 |

**Table S14**

Proton Assignments (ppm) for Term-pG-C

| | α | β | γ | δ | ε |
|---|---|---|---|---|---|
| **Pra** | 4.82 | 2.94, 2.77 | | | |
| **R** | 4.69 | 1.82 | 1.62 | 3.20 | |
| **W** | 5.27 | 3.29, 3.07 | | | |
| **V** | 4.67 | 2.02 | 0.92 | | |
| **K** | 4.31 | 1.20, 0.99 | 0.04 | 0.51 | 2.38 |
| **V** | 4.50 | 1.92 | 0.88 | | |
| **dP** | 4.34 | 2.33 | 1.95, 2.11 | 3.81, 3.74 | |
| **G*** | | | | | |
| **O** | 4.74 | 1.93 | 1.77 | 3.07 | |
| **W** | 5.10 | 3.18, 3.03 | | | |
| **I** | 4.76 | 1.97 | 1.45, 1.24 | 0.95 ($\gamma$,$CH_3$) | 0.84 ($\delta$,$CH_3$) |
| **K** | 4.15 | 1.17 | 0.45, 0.23 | 0.65 | 2.47 |
| **Q** | 4.47 | 2.02, 1.84 | 2.24 | | |
| **K-$N_3$** | 4.06 | 1.89 | 1.12 | 1.58, 1.47 | 4.38, 4.36 |

**Table S15**

Proton Assignments (ppm) for NHB-pG-U

| | α | β | γ | δ | ε |
|---|---|---|---|---|---|
| **R** | 4.36 | 1.75 | 1.61 | 3.19 | |
| **K-$N_3$** | 5.03 | | 1.36 | 1.80, 1.71 | 3.03 |
| **V** | 4.58 | 1.97 | 0.85 | | |
| **K** | 4.22 | 1.16, 0.95 | 0.01 | 0.49 | 2.30 |
| **V** | 4.46 | 1.94 | 0.87 | | |
| **dP** | 4.31 | 2.29 | 1.90, 2.04 | 3.76, 3.69 | |
| **G*** | | | | | |
| **O** | 4.66 | 1.85 | 1.70 | 2.99 | |
| **W** | 4.94 | 3.07, 2.96 | | | |
| **I** | 4.62 | 1.94 | 1.38, 1.17 | 0.89 (γ,$CH_3$) | 0.78 (δ,$CH_3$) |
| **Pra** | 4.80 | 2.70 | | | |
| **Q** | 4.37 | 2.09, 1.94 | 2.30 | | |

**Table S16**

Proton Assignments (ppm) for NHB-pG-C

| | α | β | γ | δ | ε |
|---|---|---|---|---|---|
| **R** | 4.30 | 1.80 | 1.59 | 3.22 | |
| **K-$N_3$** | 4.30 | 1.66, 1.54 | 0.70 | 1.05 | 4.55, 4.54 |
| **V** | 4.58 | 1.96 | 0.88 | | |
| **K** | 4.30 | 1.21, 0.88 | -0.06 | 0.28, 0.46 | |
| **V** | 4.46 | 1.86 | 0.86 | | |
| **dP** | 4.30 | 2.3 | 2.07, 1.90 | 3.80, 3.69 | |
| **G*** | | | | | |
| **O** | 4.63 | 1.86 | 1.71 | 3.01 | |
| **W** | 4.91 | 3.06, 2.85 | | | |
| **I** | 4.58 | 1.96 | 1.27 | 0.88 (γ,$CH_3$) | 0.80 (δ,$CH_3$) |
| **Pra** | 4.92 | 3.23 | | | |
| **Q** | 4.41 | 2.14,2.01 | 2.34 | | |

**Table S17**

Proton Assignments (ppm) for HB-pG-U

| | α | β | γ | δ | ε |
|---|---|---|---|---|---|
| **R** | 4.43 | 1.71 | 1.62 | 3.19 | |
| **W** | 5.12 | 3.28, 3.08 | | | |
| **K-$N_3$** | 4.83 | 1.55 | | 1.33 | 1.77 |
| **K** | 4.27 | 1.32 | 0.30, 0.57 | 1.04 | 2.40 |
| **V** | 4.48 | 2.04 | 0.90 | | |
| **dP** | 4.36 | 2.31 | 2.07, 1.94 | 3.72 | |
| **G*** | | | | | |
| **O** | 4.71 | 1.91 | 1.77 | 3.07 | |
| **W** | 5.09 | 3.27, 3.07 | | | |
| **Pra** | 4.99 | 2.73, 2.66 | | | |
| **K** | 3.96 | 1.30 | 0.46, 0.58 | 0.76 | 2.67 |
| **Q** | 4.29 | 2.06, 1.91 | 2.31 | | |

**Table S18**

Proton Assignments (ppm) for NHB-pG-C

| | α | β | γ | δ | ε |
|---|---|---|---|---|---|
| **R** | 4.47 | 1.77 | 1.59, 1.69 | 3.19 | |
| **W** | 5.35 | 3.28, 3.06 | | | |
| **K-$N_3$** | 4.29 | 1.70 | 0.92 | 1.15 | |
| **K** | 3.88 | 1.18 | 0.32 | 0.53 | 2.64 |
| **V** | 4.53 | 2.01 | 0.92 | | |
| **dP** | 4.37 | 2.32 | 2.07, 1.94 | 3.75, 3.67 | |
| **G*** | | | | | |
| **O** | 4.80 | 1.93, 1.84 | 1.67 | 3.07 | |
| **W** | 5.06 | 3.27, 3.01 | | | |
| **Pra** | 5.14 | 3.32 | | | |
| **K** | 4.53 | | 0.05, 0.42 | 1.-2 | 2.50 |
| **Q** | 4.30 | 2.05, 1.89 | 2.31 | | |

**Table S19**

Proton Assignments for Random Coil strands

| | **Hα** | | **Hα** |
|---|---|---|---|
| **R** | 4.25 | **G** | 3.98 |
| **W** | 4.74 | **O** | 4.16 |
| **Azk/V** | 4.36/4.00 | **W** | 4.70 |
| **K** | 4.13 | **Pra/I** | 4.30/4.06 |
| **V** | 4.08 | **K** | 4.29 |
| **N** | 4.67 | **Q** | 4.29 |

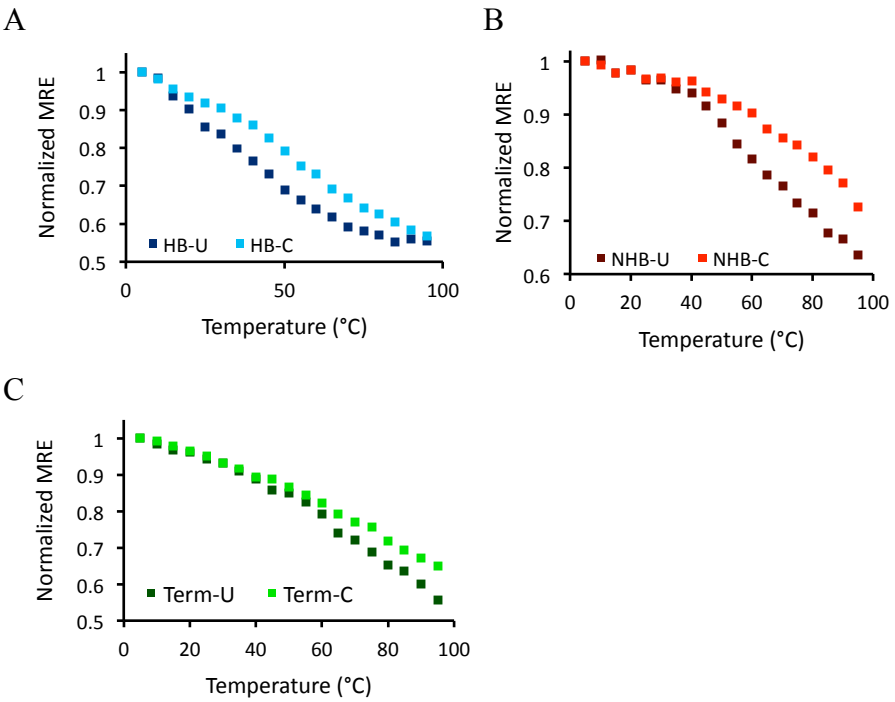



**Figure S1.** Thermal stability assessed by circular dichroism of (A) HB at 213 nm, (B) NHB at 215 nm, and (C) Term at 212 nm from 5 °C to 95 °C in 10 mM phosphate buffer, pH 7.5.

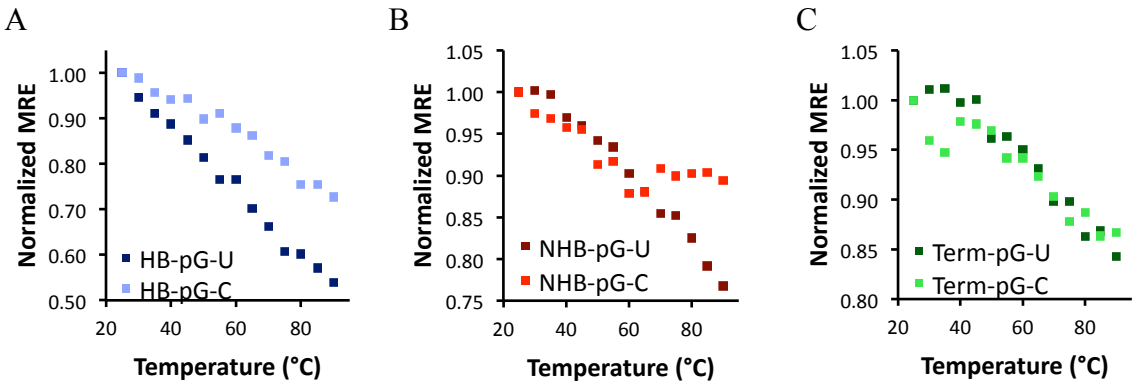



**Figure S2.** Thermal stability assessed by circular dichroism of (A) **HB-pG**, (B) **NHB-pG**, and (C) **Term-pG** peptides from 20 °C to 90 °C in 10 mM phosphate buffer, pH 7.5.

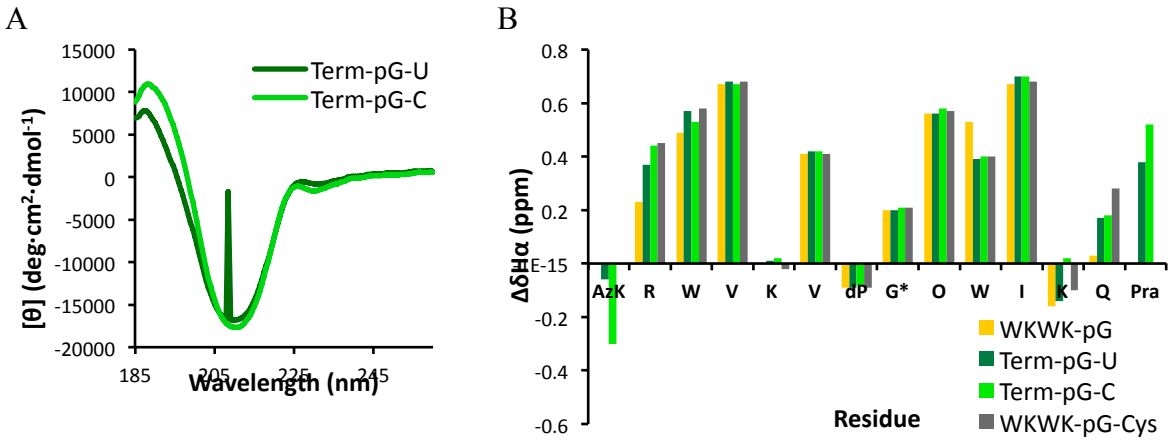



**Figure S3.** (A) Circular dichrosim spectra of **Term-pG-U** (dark green) compared to **Term -pG-C** (light green) at 298 K in 10mM sodium phosphate pH 7.0 buffer. (B) Hα chemical shift differences for **Term -pG-U** (dark green), **Term -pG-C** (light green), **WKWK-pG** (orange), and **WKWK-pG-Cys** (grey) from random coil peptides. Values calculated from data obtained at 293 K in 50 mM potassium phosphate-*d2*, pD 7.0 (uncorrected). *The Gly bars reflect the glycine splitting.

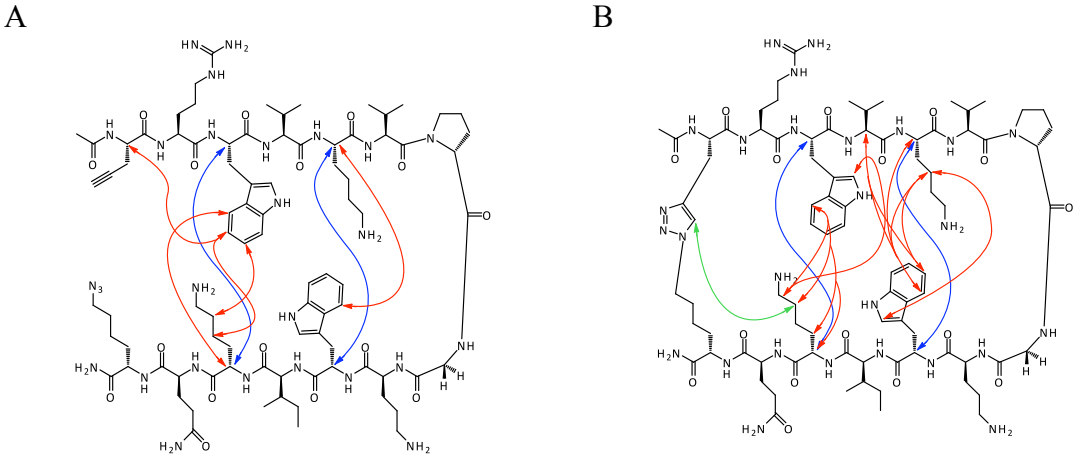



**Figure S4.** NOEs observed between cross-strand Hα protons (blue), side-chain □ side-chain (red), and the triazole proton (green) in (A) **Term-pG-U** and (B) **Term-pG-C**.

**Table S20** Sequences of Term-pG-AzX peptides

| Peptide | Sequence |
|---|---|
| **Term-pG-AzK** | Ac-(AzK)R**W**V**K**VNGO**W**I**K**Q(Pra)-$NH_2$ |
| **Term-pG-AzO** | Ac-(AzO)R**W**V**K**VNGO**W**I**K**Q(Pra)-$NH_2$ |
| **Term-pG-AzB** | Ac-(AzB)R**W**V**K**VNGO**W**I**K**Q(Pra)-$NH_2$ |
| **Term-pG-AzP** | Ac-(AzP)R**W**V**K**VNGO**W**I**K**Q(Pra)-$NH_2$ |

Prefixes of "U" and "C" denote linear unclicked and cyclic clicked peptides. p = dPro, AzO = Orn($N_3$) (azido-ornithine), AzB = Dab($N_3$) (azido-homoalanine), AzP = Dap($N_3$) (azido-alanine)

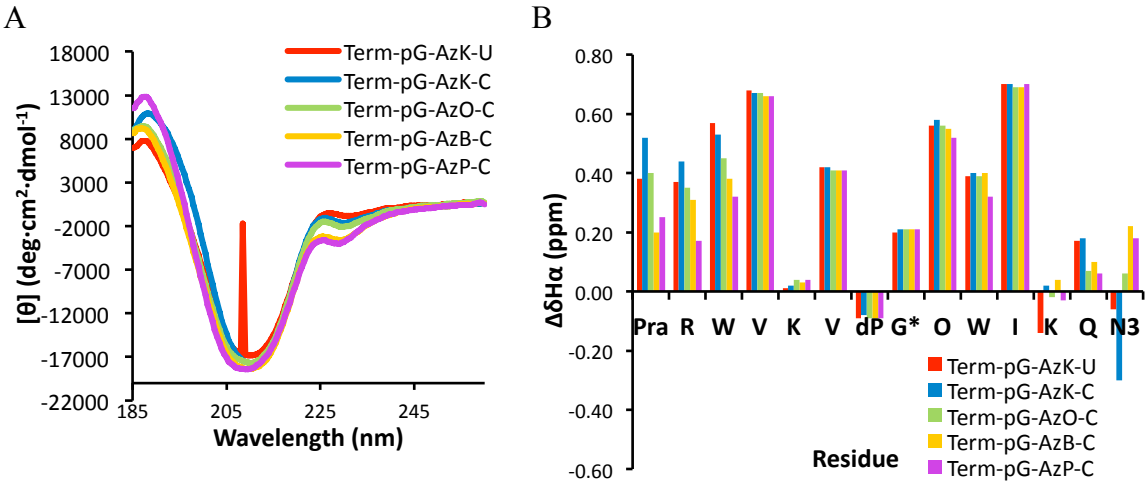



**Figure S5.** (A) Circular dichrosim spectra of **Term-pG-AzK-U** (red), **Term-pG-AzK-C** (blue), **Term-pG-AzO-C** (green), **Term-pG-AzB-C** (orange), and **Term-pG-AzP-C** (purple) at 298 K in 10mM sodium phosphate pH 7.0 buffer. (B) Hα chemical shift differences for **Term-pG-AzK-U** (red), **Term-pG-AzK-C** (blue), **Term-pG-AzO-C** (green), **Term-pG-AzB-C** (orange), and **Term-pG-AzP-C** (purple) from random coil peptides. Values calculated from data obtained at 293 K in 50 mM potassium phosphate-*d2*, pD 7.0 (uncorrected). Glycine shifts were unclear due to overlap with the Hδ protons of dPro. The residue N3 represents the azide that was incorporated.

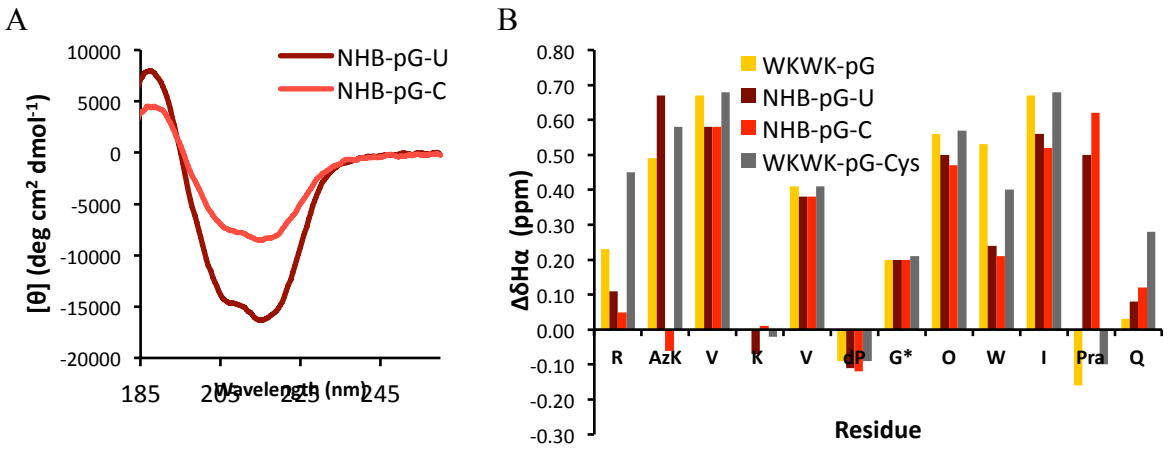



**Figure S6.** (A) Circular dichrosim spectra of **NHB-pG-U** (dark red) compared to **NHB-pG-C** (light red) at 298 K in 10mM sodium phosphate pH 7.0 buffer. (B) Hα chemical shift differences for **NHB-pG-U** (dark red), **NHB-pG-C** (light red), **WKWK-pG** (orange), and **WKWK-pG-Cys** (grey) from random coil peptides. Values calculated from data obtained at 293 K in 50 mM potassium phosphate-*d2*, pD 7.0 (uncorrected). *The Gly bars reflect the glycine splitting.

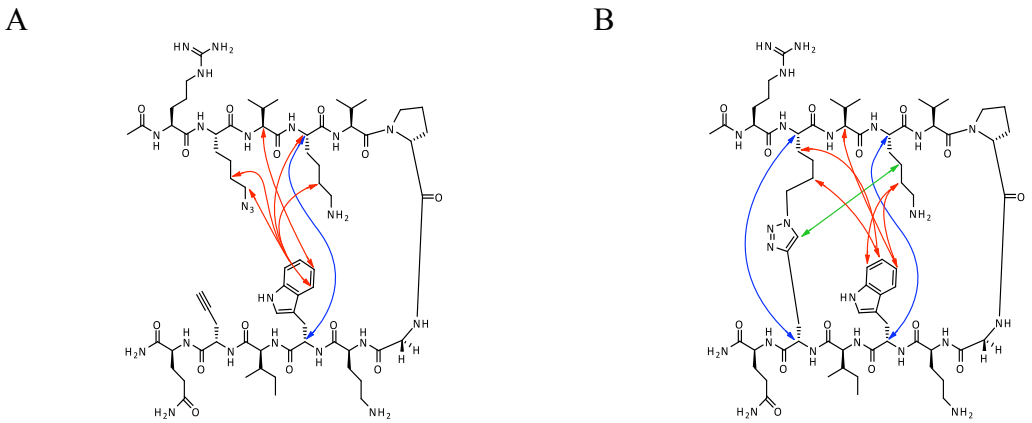



**Figure S7.** NOEs observed between cross-strand Hα protons (blue), side-chain □ side-chain (red), and the triazole proton (green) in (A) **NHB-pG-U** and (B) **NHB-pG-C**.

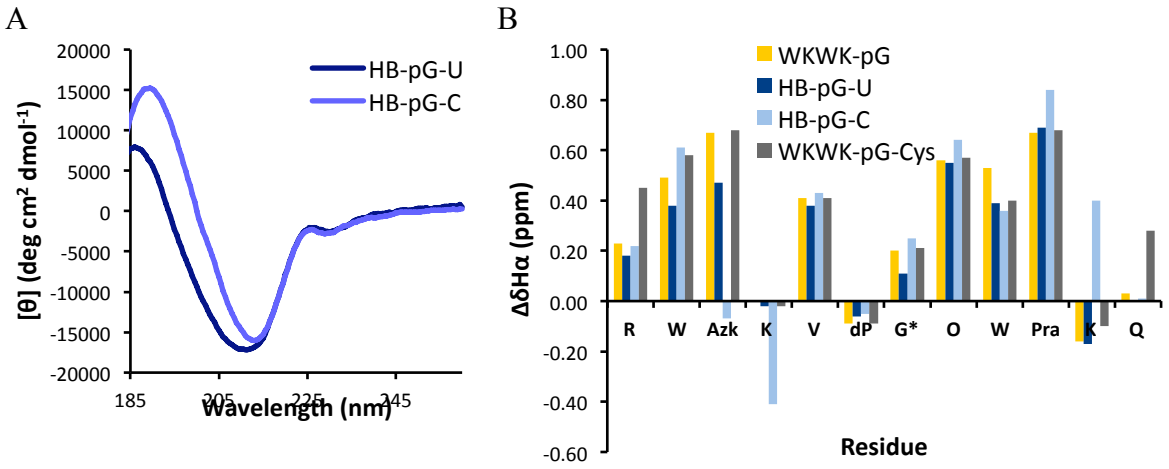



**Figure S8.** (A) Circular dichrosim spectra of **HB-pG-U** (dark blue) compared to **HB-pG-C** (light blue) at 298 K in 10mM sodium phosphate pH 7.0 buffer. (B) Hα chemical shift differences for **HB-pG-U** (dark blue), **HB-pG-C** (light blue), **WKWK-pG** (orange), and **WKWK-pG-Cys** (grey) from random coil peptides. Values calculated from data obtained at 293 K in 50 mM potassium phosphate-*d2*, pD 7.0 (uncorrected)

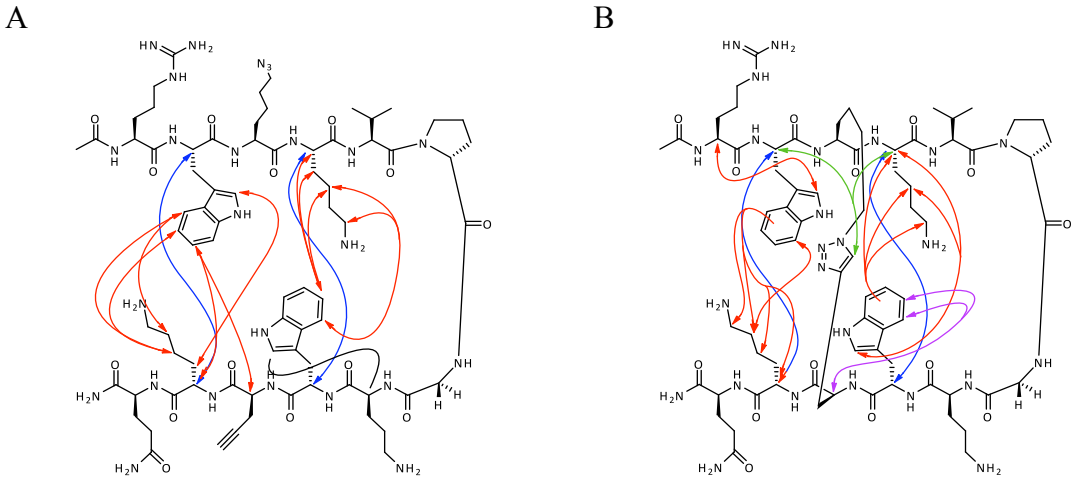



**Figure S9.** NOEs observed between cross-strand Hα protons (blue), side-chain □ side-chain (red), and the triazole proton (green) in (A) **HB-pG-U** and (B) **HB-pG-C**.

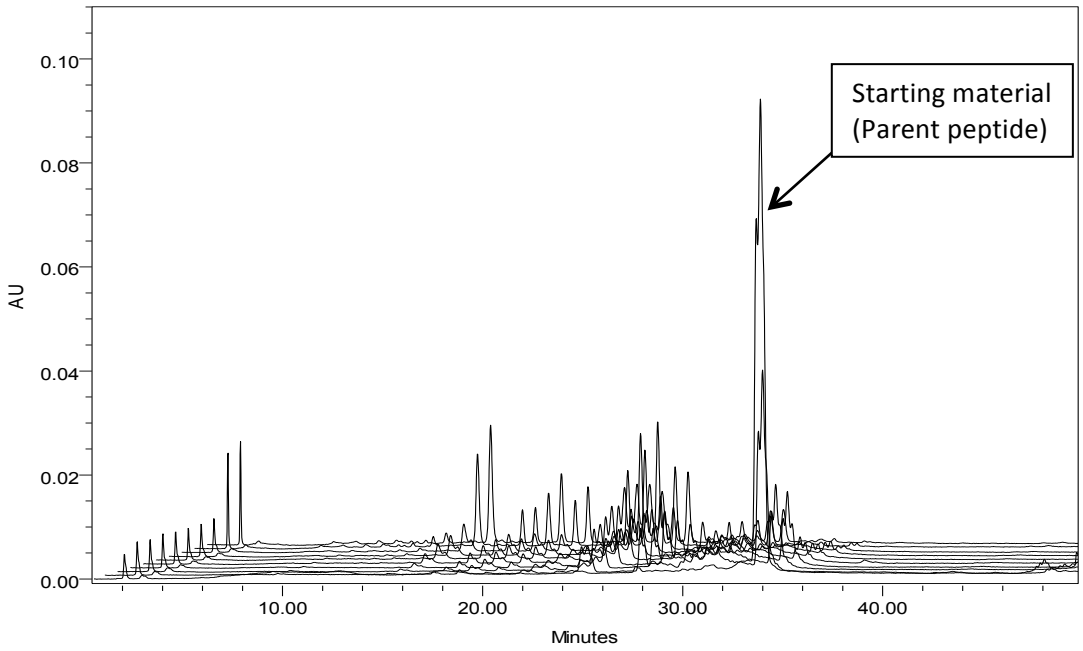



**Figure S10.** Overlay of HPLC traces monitoring Pronase E degradation of **Scramble** at 280 nm over 24 hours.

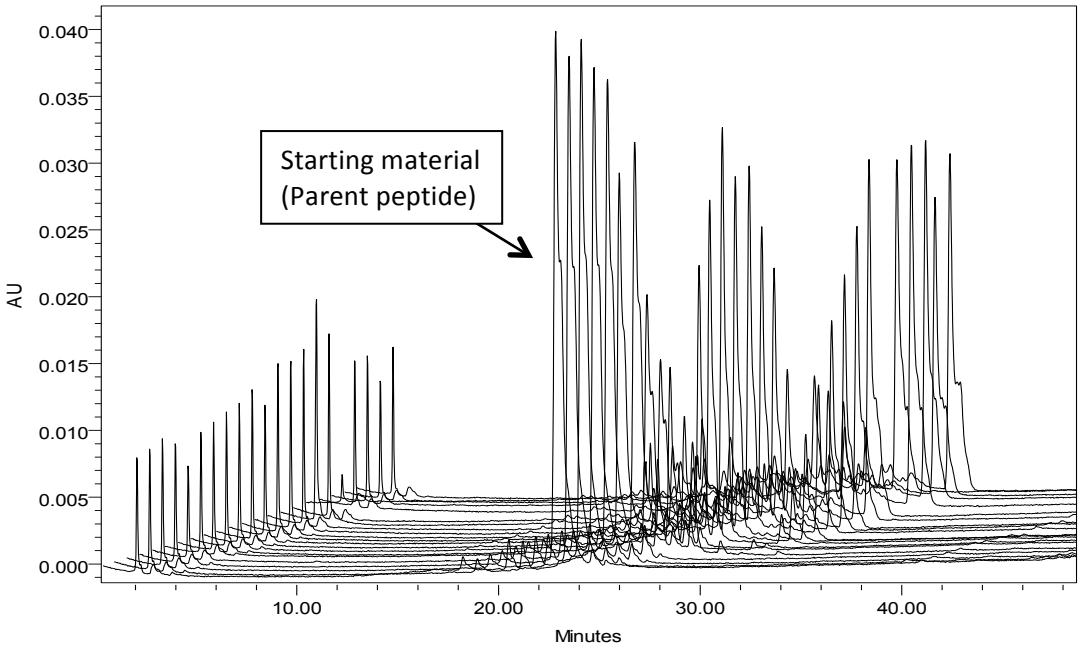



**Figure S11.** Overlay of HPLC traces monitoring Pronase E degradation of **HB-pG-U** at 280 nm over 24 hours.

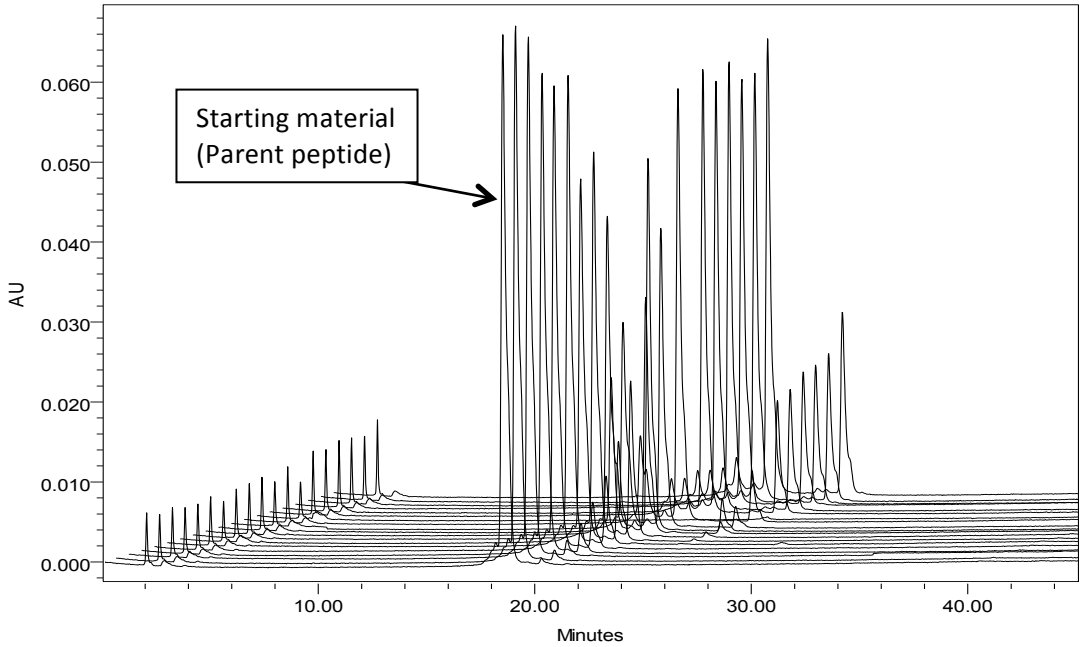



**Figure S12.** Overlay of HPLC traces monitoring Pronase E degradation of **HB-pG-C** at 280 nm over 24 hours.

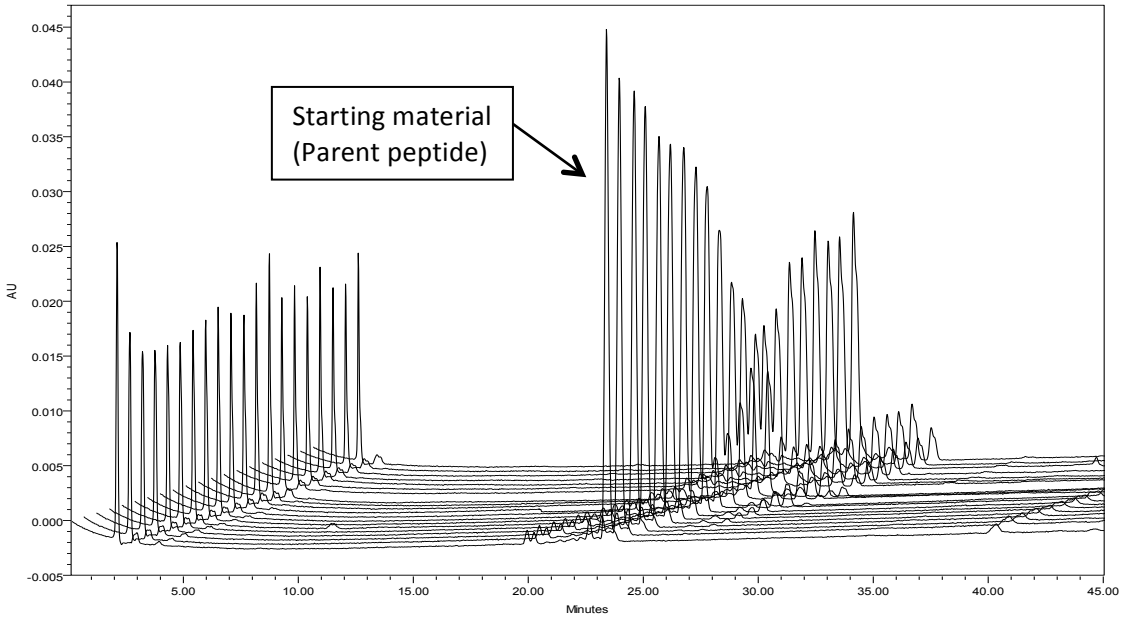



**Figure S13.** Overlay of HPLC traces monitoring Pronase E degradation of **NHB-pG-U** at 280 nm over 24 hours.

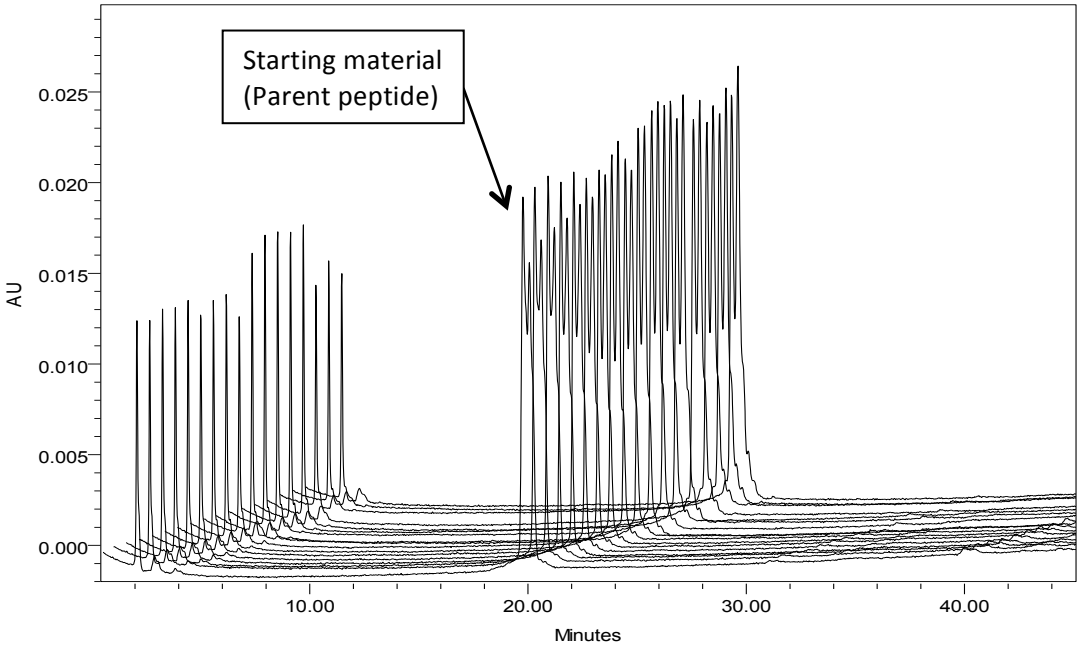



**Figure S14.** Overlay of HPLC traces monitoring Pronase E degradation of **NHB-pG-C** at 280 nm over 24 hours.

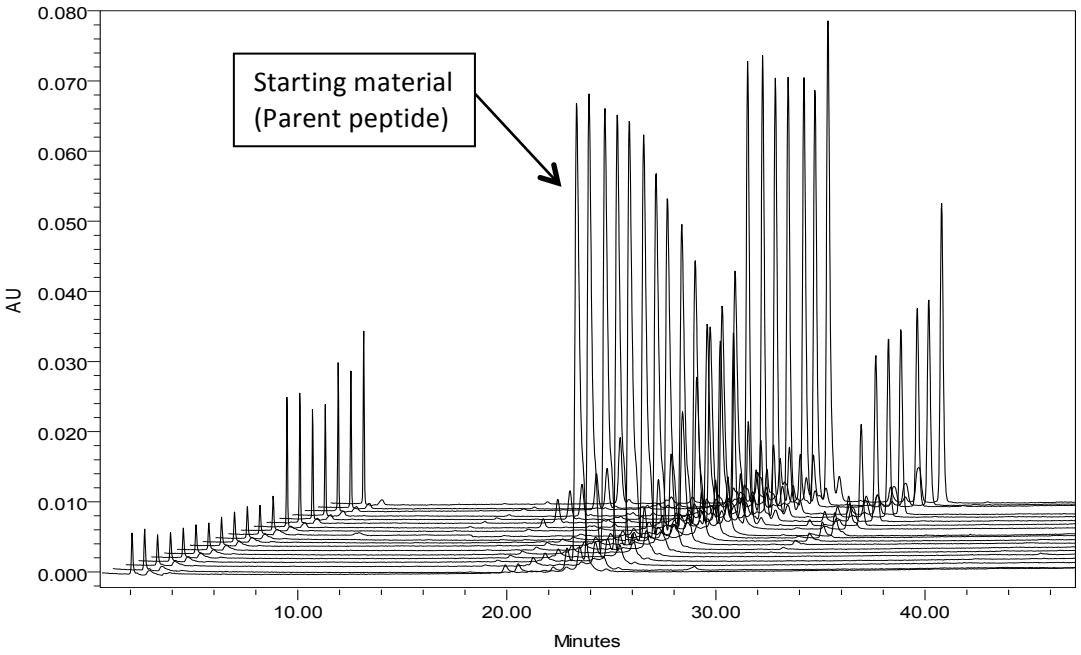



**Figure S15.** Overlay of HPLC traces monitoring Pronase E degradation of **Term-pG-U** at 280 nm over 24 hours.

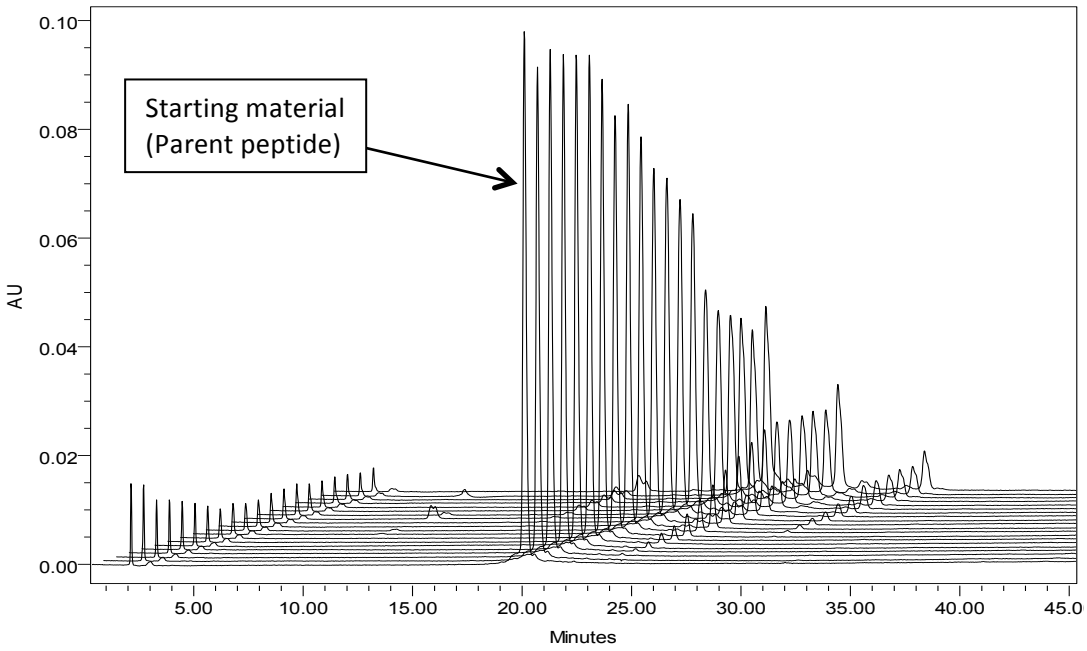



**Figure S16.** Overlay of HPLC traces monitoring Pronase E degradation of **Term-pG-C** at 280 nm over 24 hours.

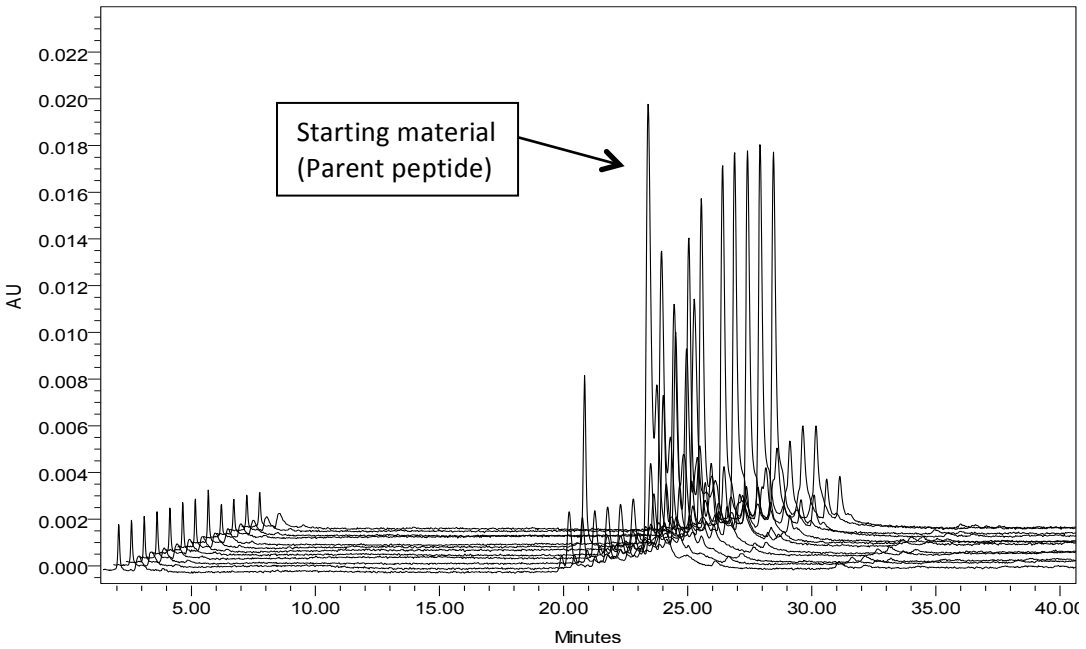



**Figure S17.** Overlay of HPLC traces monitoring Trypsin degradation of **NHB-pG-U** at 280 nm over 24 hours.

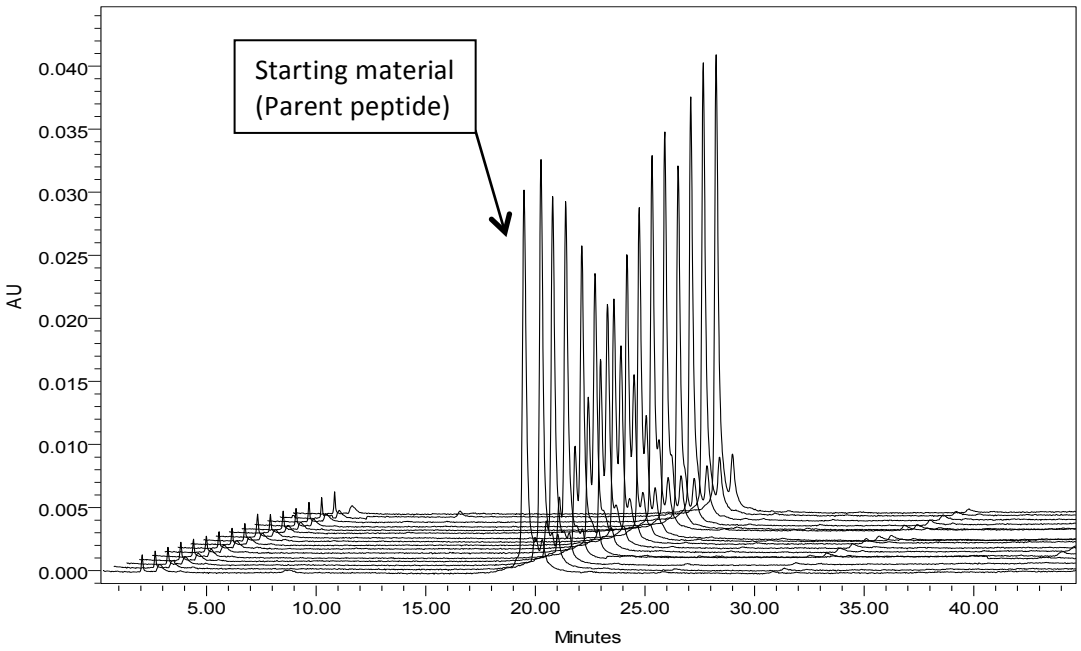
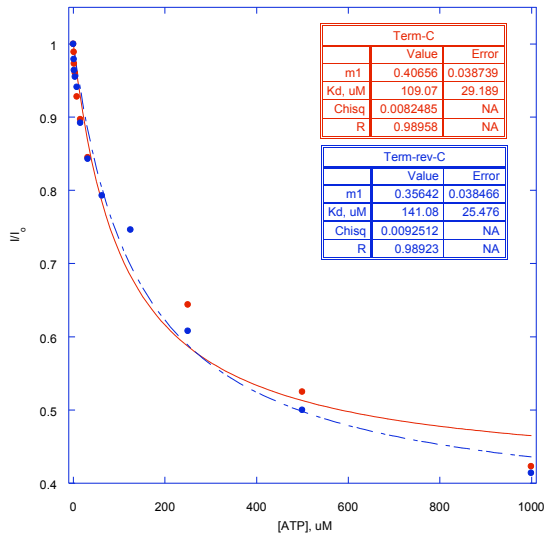



**Figure S18.** Overlay of HPLC traces monitoring Trypsin degradation of **NHB-pG-C** at 280 nm over 24 hours.

A




B

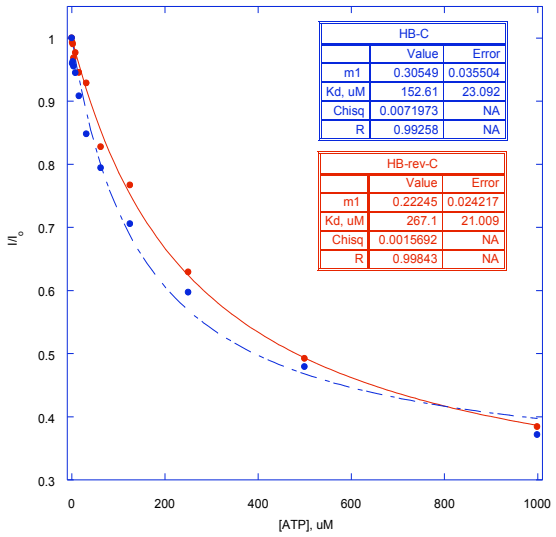



**Figure S19.** Fluorescence quenching titrations of (A) **Term-C** (red) and **Term-rev-C** (blue) and (B) **HB-C** (blue) and **HB-rev-C** (red) with ATP at 5 μM peptide in 10 mM acetate buffer, pH 5.0 at 25 °C.

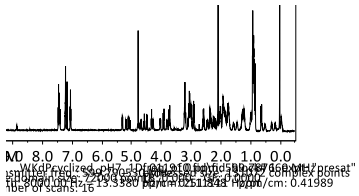


**Figure S1** $^{1}$H NMR spectrum for **WKWK-pG-Cys**: Ac-Cys-Arg-Trp-Val-Lys-Val-dPro-Gly-Orn-Trp-Ile-Lys-Gln-Cys-$NH_2$

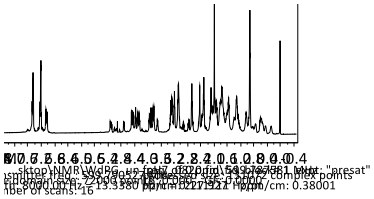


**Figure S2** $^{1}$H NMR spectrum for **HB-pG-U**: Ac-Arg-Trp-AzK-Lys-Val-dPro-Gly-Orn-Trp-Pra-Lys-Gln-$NH_2$

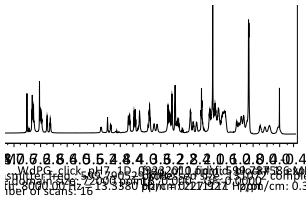


**Figure S3** $^{1}$H NMR spectrum for **HB-pG-C**: Ac-Arg-Trp-AzK-Lys-Val-dPro-Gly-Orn-Trp-Pra-Lys-Gln-$NH_2$

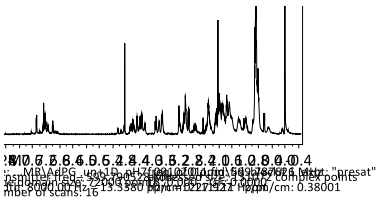


**Figure S4** $^{1}$H NMR spectrum for **NHB-pG-U**: Ac-Arg-AzK-Val-Lys-Val-dPro-Gly-Orn-Trp-Ile-Pra-Gln-$NH_2$

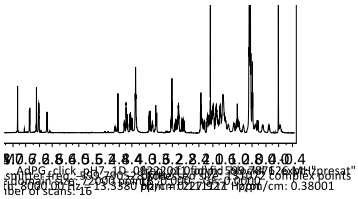


**Figure S5** $^{1}$H NMR spectrum for **NHB-pG-C**: Ac-Arg-AzK-Val-Lys-Val-dPro-Gly-Orn-Trp-Ile-Pra-Gln-$NH_2$

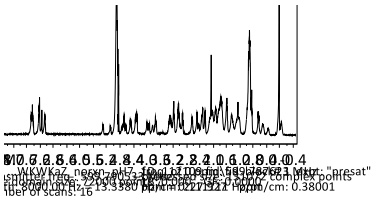


**Figure S6** $^{1}$H NMR spectrum for **Term-pG-U**: Ac-Pra-Arg-Trp-Val-Lys-Val-dPro-Gly-Orn-Trp-Ile-Lys-Gln-AzK-$NH_2$

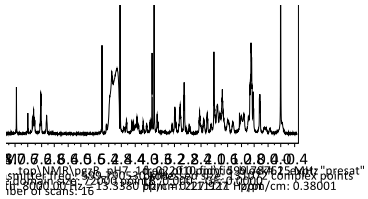


**Figure S7** $^{1}$H NMR spectrum for **Term-pG-C**: Ac-Pra-Arg-Trp-Val-Lys-Val-dPro-Gly-Orn-Trp-Ile-Lys-Gln-AzK-$NH_2$

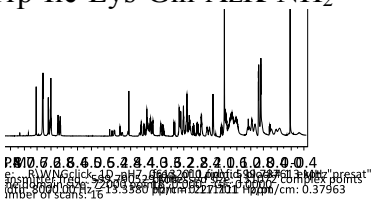


**Figure S8** $^{1}$H NMR spectrum for **HB-U**: Ac-Arg-Trp-AzK-Lys-Val-Asn-Gly-Orn-Trp-Pra-Lys-Gln-$NH_2$

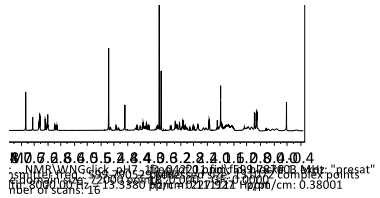


**Figure S9** $^{1}$H NMR spectrum for **HB-C**: Ac-Arg-Trp-AzK-Lys-Val-Asn-Gly-Orn-Trp-Pra-Lys-Gln-$NH_2$

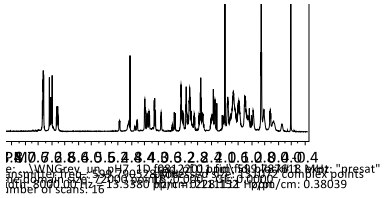


**Figure S10** $^{1}$H NMR spectrum for **HB-rev-U**: Ac-Arg-Trp-Pra-Lys-Val-Asn-Gly-Orn-Trp-AzK-Lys-Gln-$NH_2$

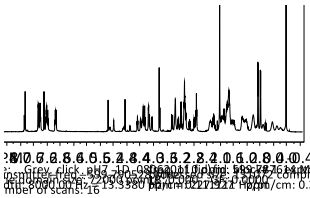


**Figure S11** $^{1}$H NMR spectrum for **HB-rev-C**: Ac-Arg-Trp-Pra-Lys-Val-Asn-Gly-Orn-Trp-AzK-Lys-Gln-$NH_2$

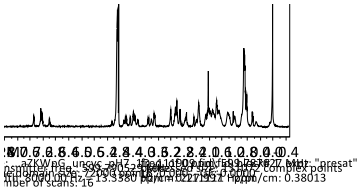


**Figure S12** $^{1}$H NMR spectrum for **NHB-U**: Ac-Arg-AzK-Val-Lys-Val-Asn-Gly-Orn-Trp-Ile-Pra-Gln-$NH_2$

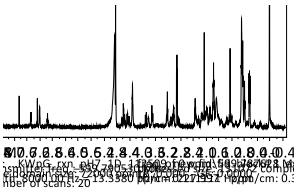


**Figure S13** $^{1}$H NMR spectrum for **NHB-C**: Ac-Arg-AzK-Val-Lys-Val-Asn-Gly-Orn-Trp-Ile-Pra-Gln-$NH_2$

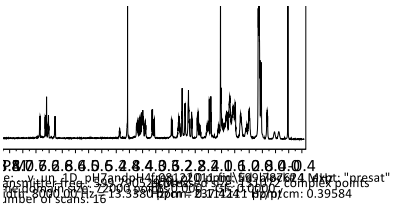


**Figure S14** $^{1}$H NMR spectrum for **NHB-rev-U**: Ac-Arg-Pra-Val-Lys-Val-Asn-Gly-Orn-Trp-Ile-Lys-AzK-Gln-$NH_2$

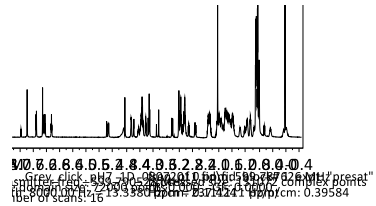


**Figure S15** $^{1}$H NMR spectrum for **NHB-rev-C**: Ac-Arg-Pra-Val-Lys-Val-Asn-Gly-Orn-Trp-Ile-Lys-AzK-Gln-$NH_2$

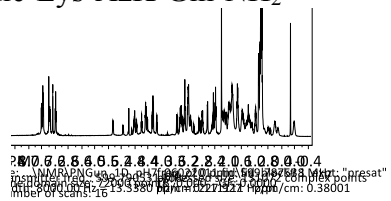


**Figure S16** $^{1}$H NMR spectrum for **Term-U**: Ac-Pra-Arg-Trp-Val-Lys-Val-Asn-Gly-Orn-Trp-Ile-Lys-Gln-AzK-$NH_2$

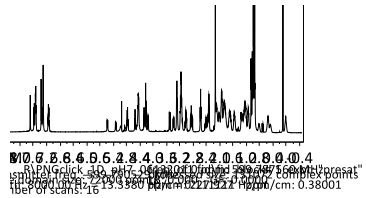


**Figure S17** $^{1}$H NMR spectrum for **Term-C**: Ac-Pra-Arg-Trp-Val-Lys-Val-Asn-Gly-Orn-Trp-Ile-Lys-Gln-AzK-$NH_2$

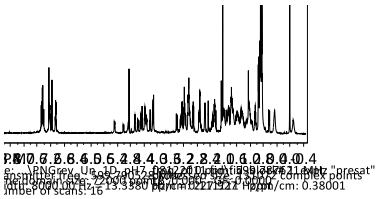


**Figure S18** $^{1}$H NMR spectrum for **Term-rev-U**: Ac-AzK-Arg-Trp-Val-Lys-Val-Asn-Gly-Orn-Trp-Ile-Lys-Gln-Pra-$NH_2$

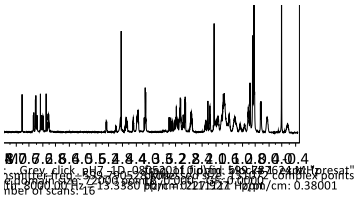


**Figure S19** $^{1}$H NMR spectrum for **Term-rev-C**: Ac-AzK-Arg-Trp-Val-Lys-Val-Asn-Gly-Orn-Trp-Ile-Lys-Gln-Pra-$NH_2$

**Analytical HPLC trace for peptides:** Lyophilized peptides were reconstituted in water for HPLC analysis. 5 µL of sample was injected on a Waters 2695 HPLC using an Atlantis C-18 column with a gradient of 0 to 50% solvent B in 25 minutes, where solvent B is 95% acetonitrile, 5% water, and 0.1% trifluoroacetic acid and solvent A is 95:5:0.1 of $H_2O$:ACN:TFA.

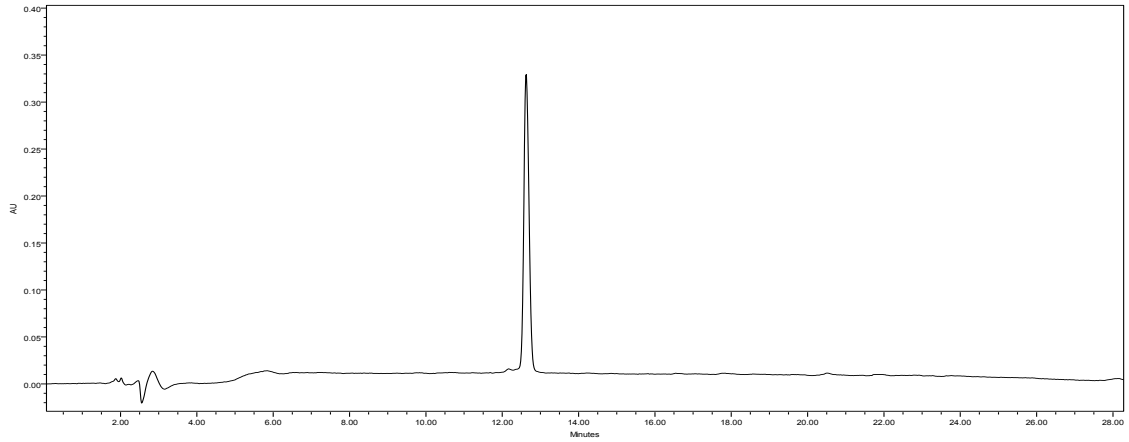



**Figure S20.** HPLC trace of Term-rev-C

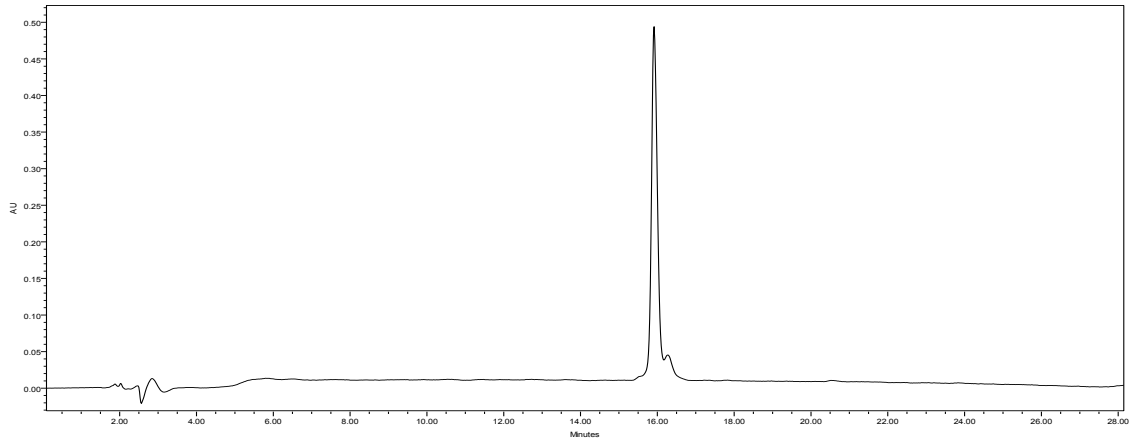



**Figure S21.** HPLC trace of Term-rev-U

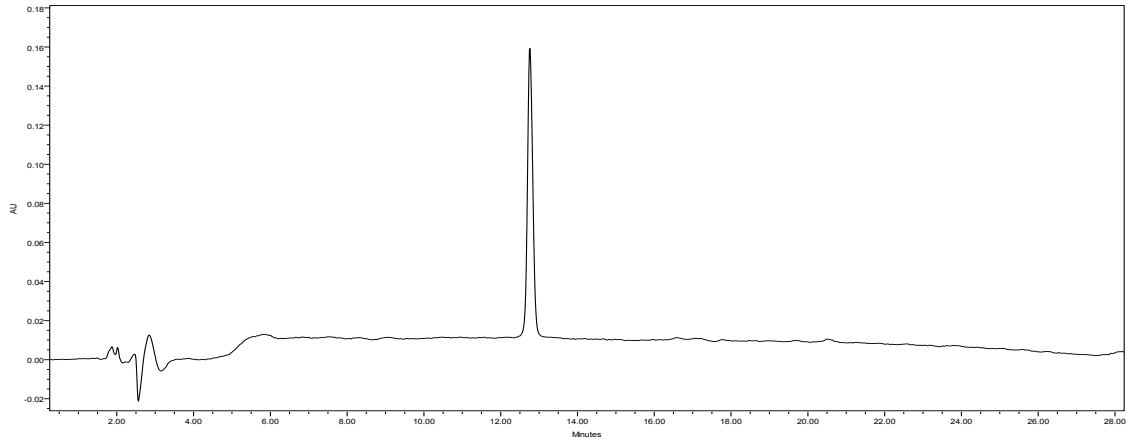



**Figure S22.** HPLC trace of Term-C

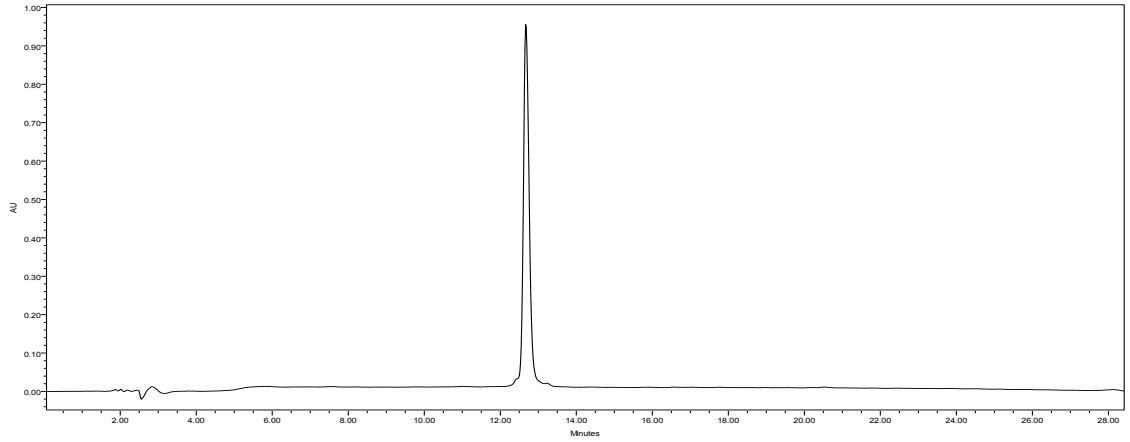



**Figure S23.** HPLC trace of WKWK

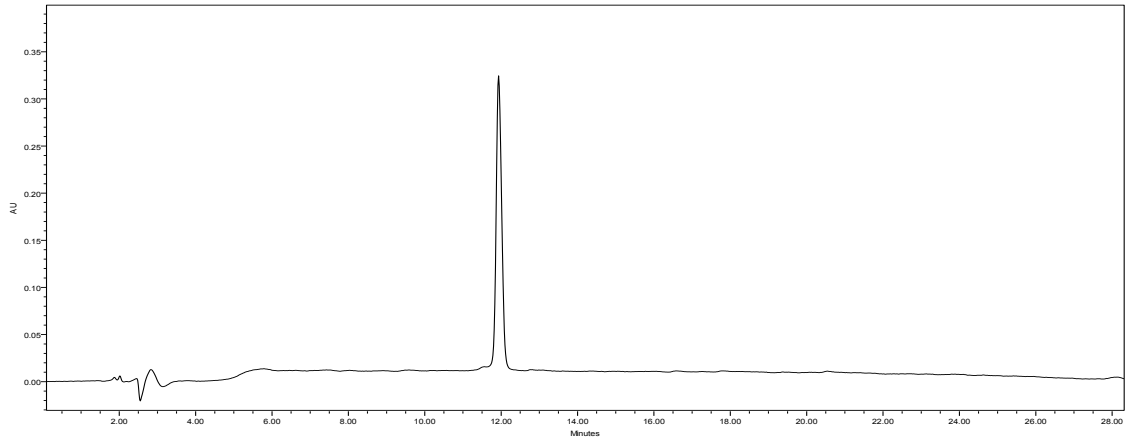



**Figure S24.** HPLC trace of HB-rev-C

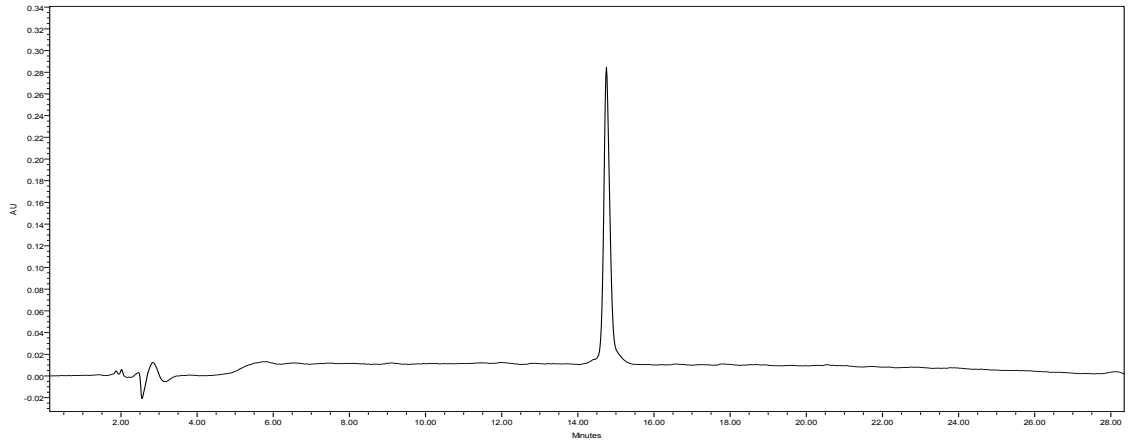



**Figure S25.** HPLC trace of HB-rev-U

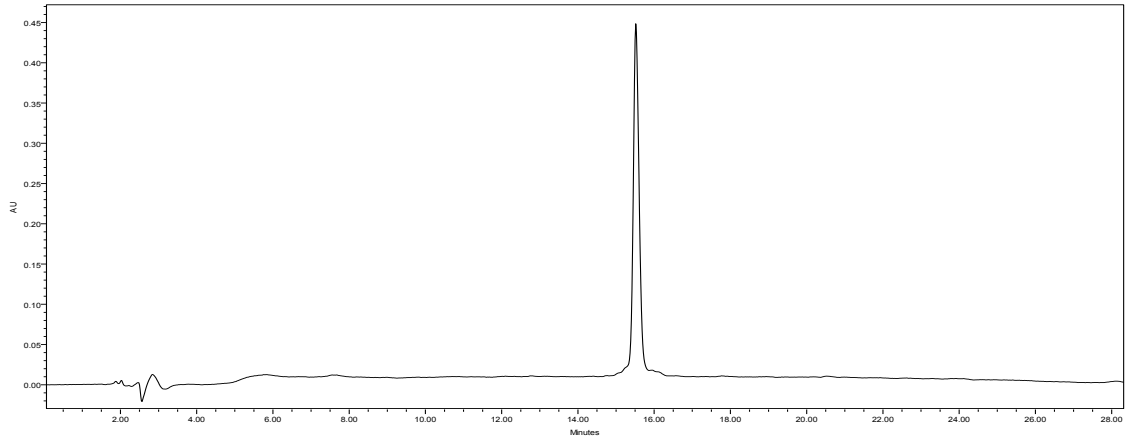



**Figure S26.** HPLC trace of Term-U

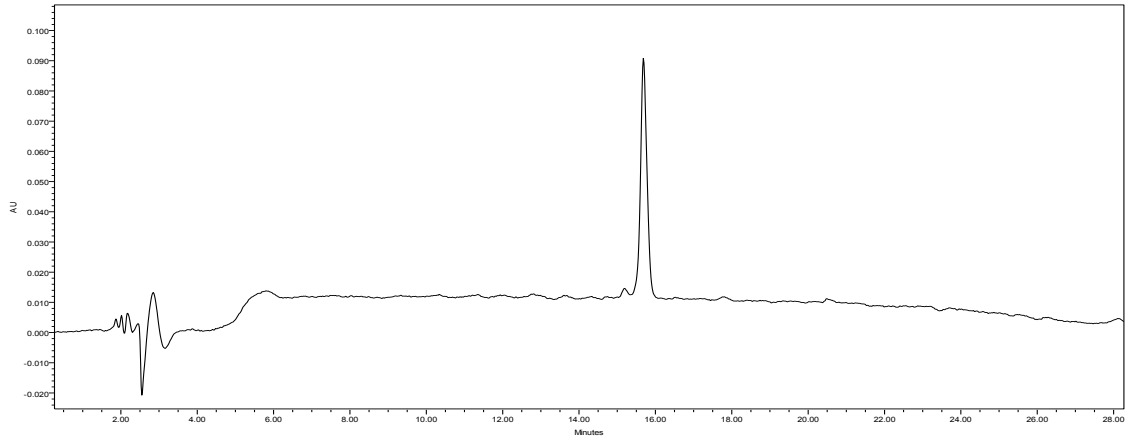



**Figure S27.** HPLC trace of NHB-rev-U

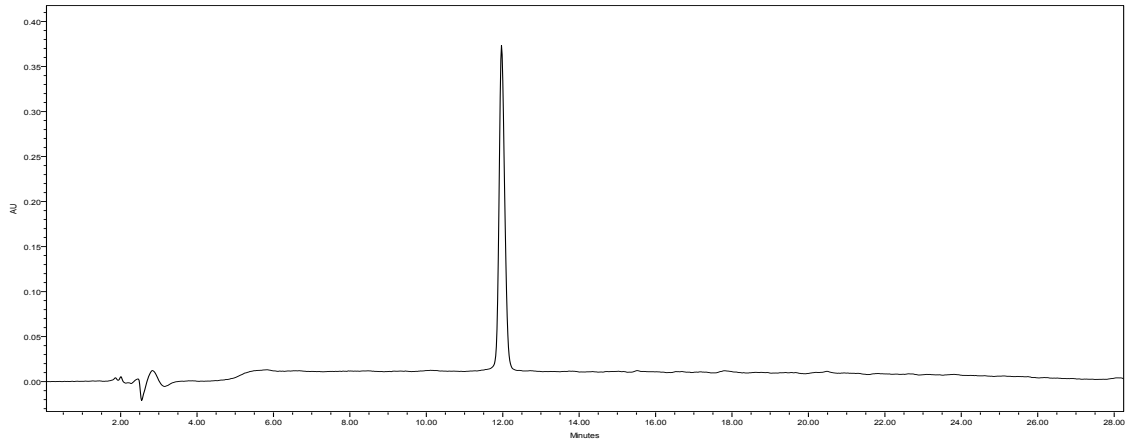



**Figure S28.** HPLC trace of HB-C

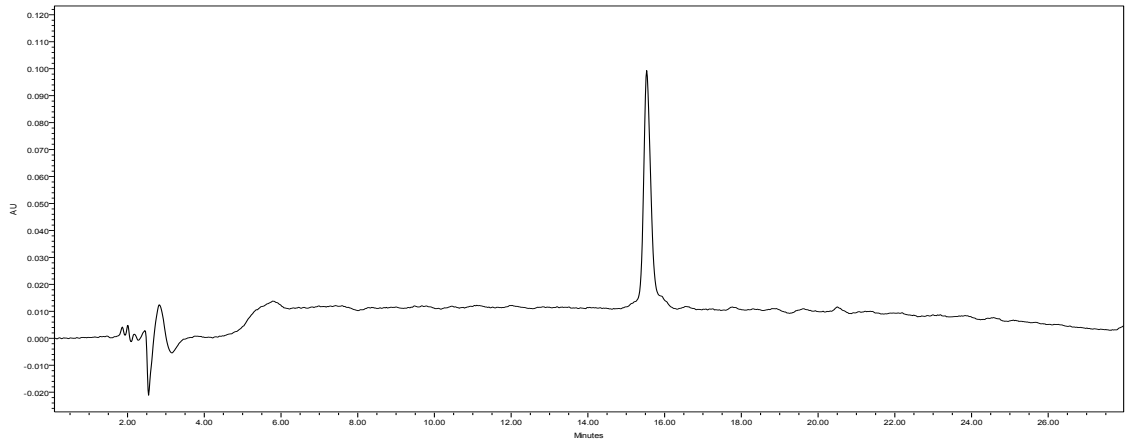



**Figure S30.** HPLC trace of HB-U

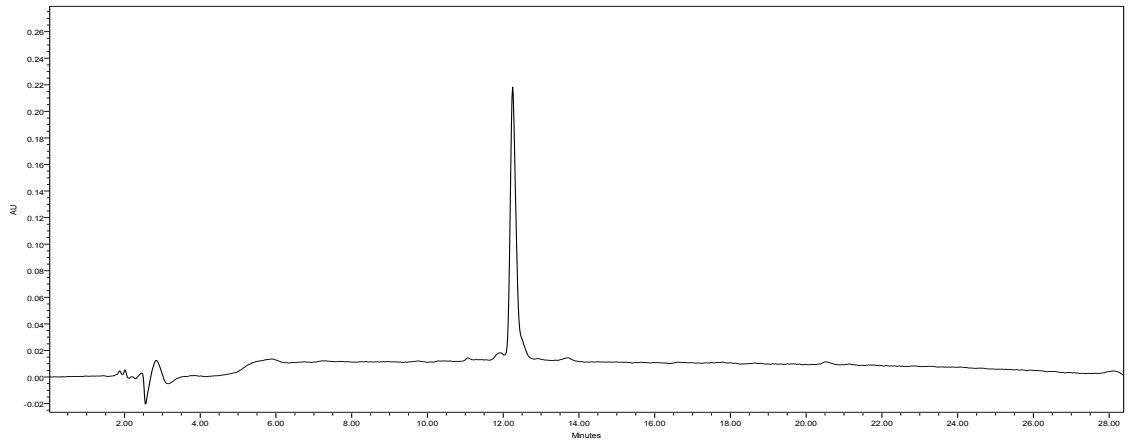



**Figure S31.** HPLC trace of NHB-rev-C

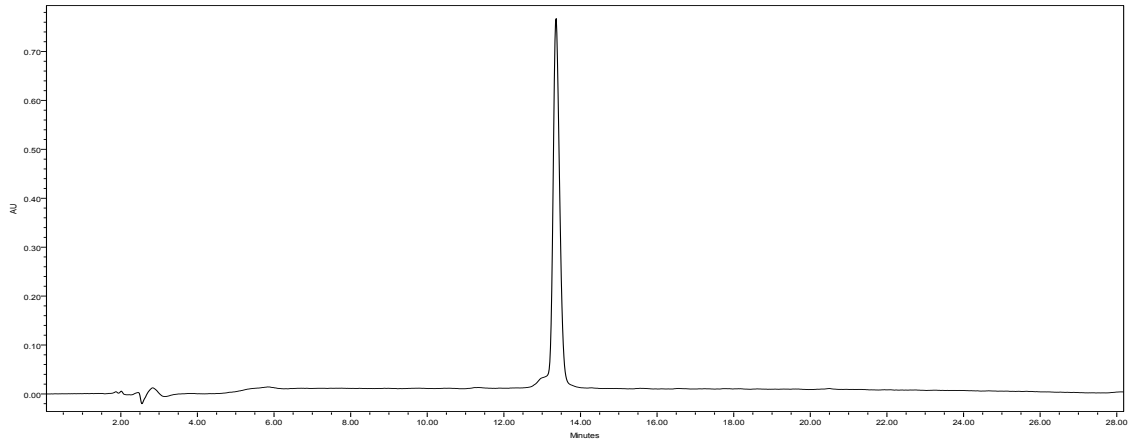



**Figure S32.** HPLC trace of Term-pG-C

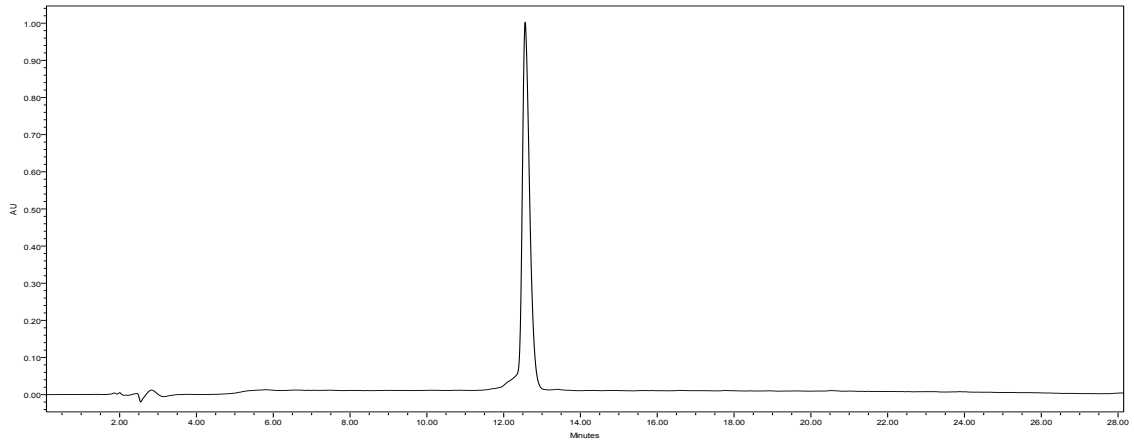



**Figure S33.** HPLC trace of HB-pG-C

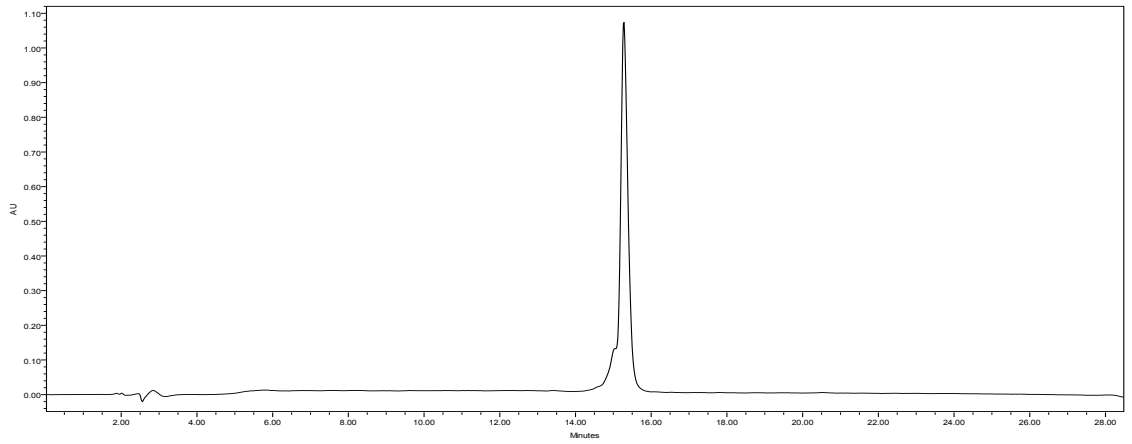



**Figure S34.** HPLC trace of Term-pG-U

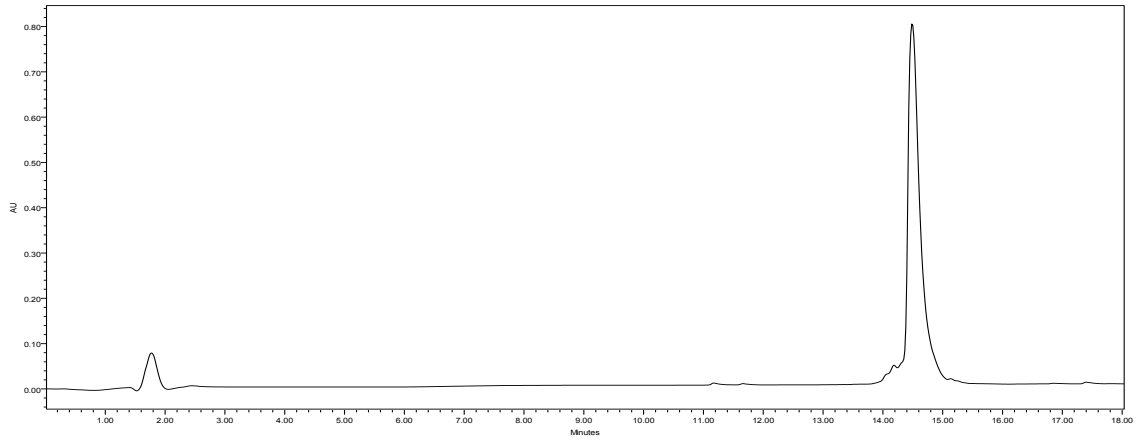



**Figure S35.** HPLC trace of NHB-U

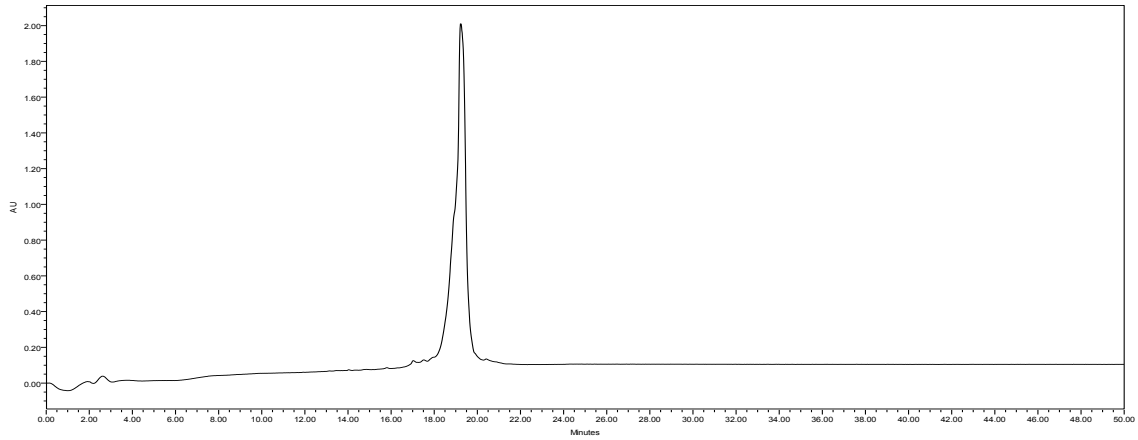



**Figure S36.** HPLC trace of Scramble

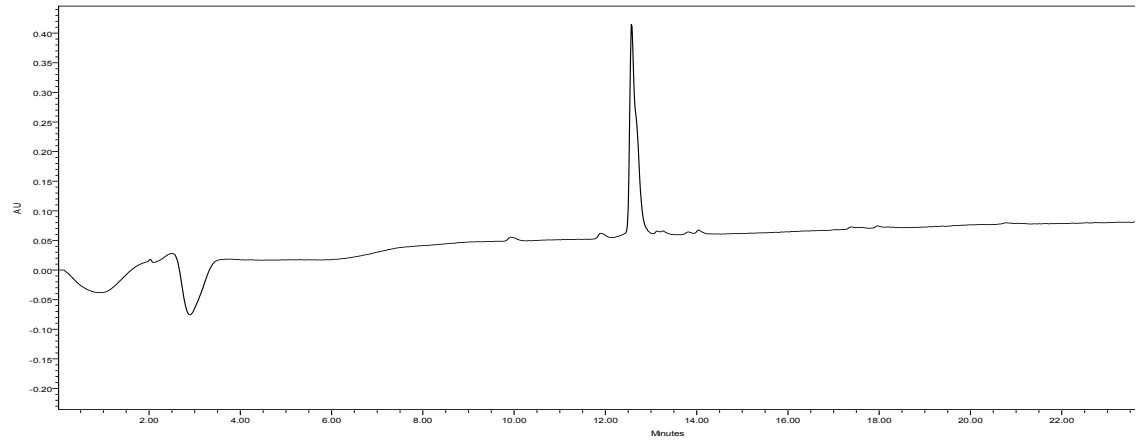



**Figure S37.** HPLC trace of NHB-C

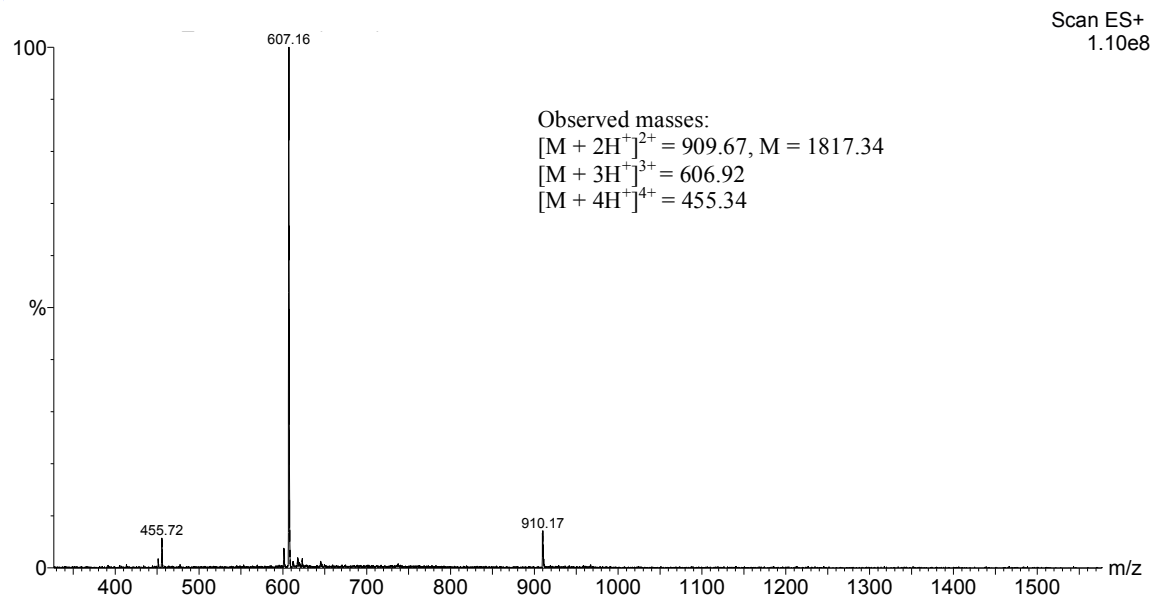



**Figure S38.** ESI-MS of Term-rev-C, calculated mass: 1817.03

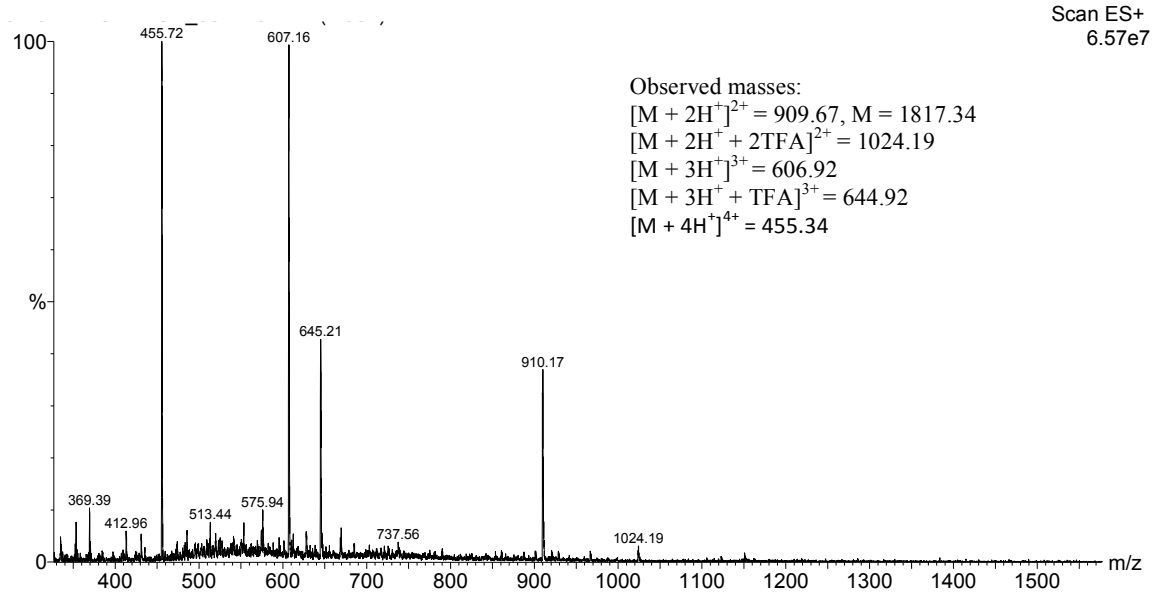



**Figure S39.** ESI-MS of Term-rev-U, calculated mass: 1817.03

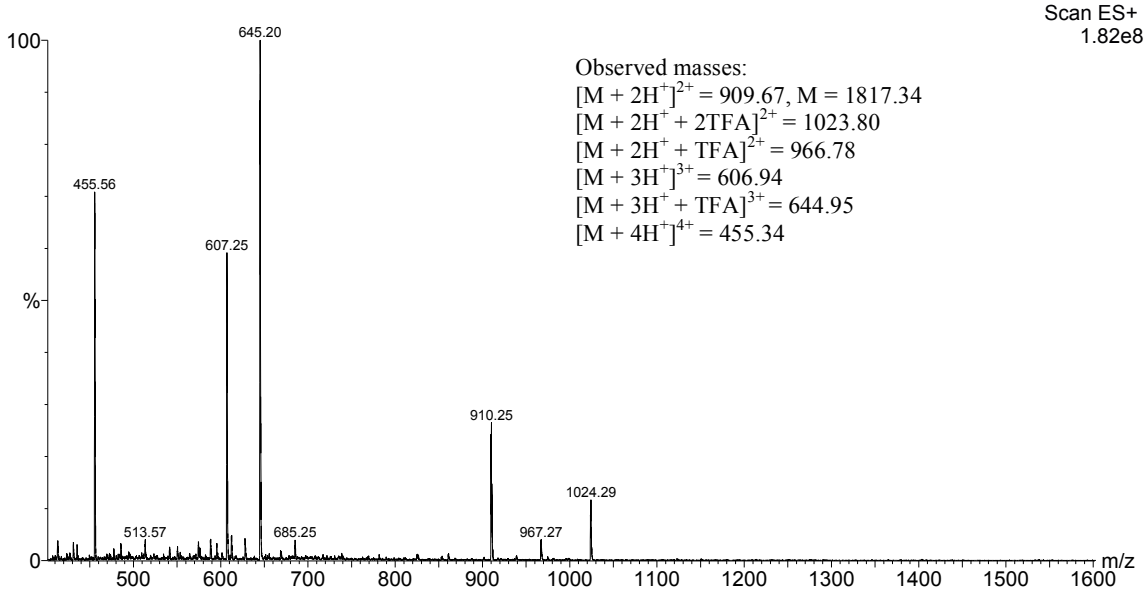



**Figure S40.** ESI-MS of Term-C, calculated mass: 1817.03

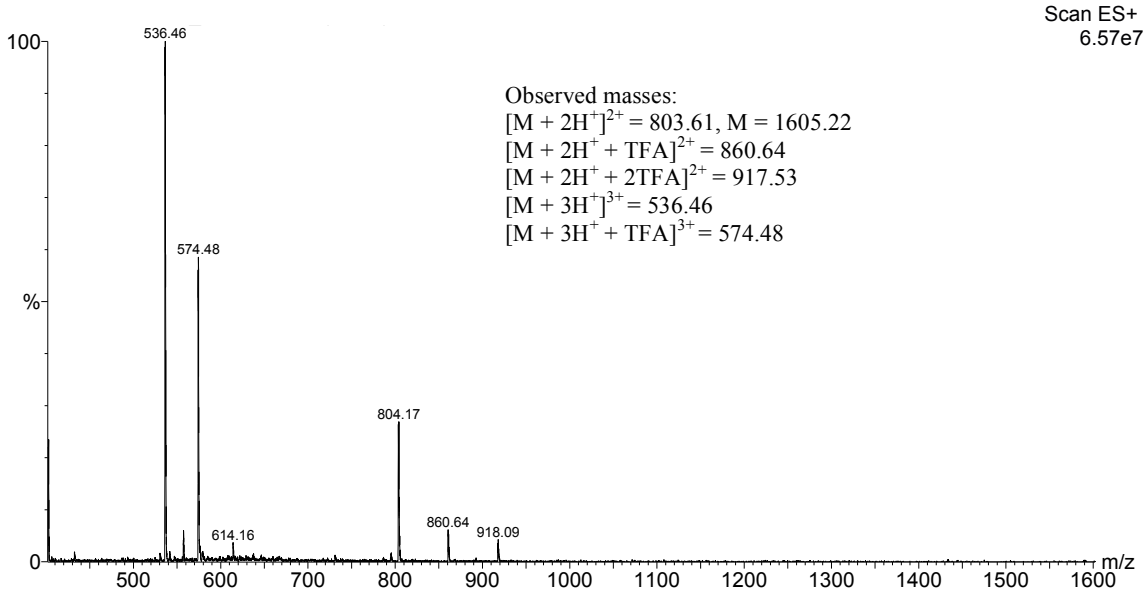



**Figure S41.** ESI-MS of HB-rev-C, calculated mass: 1604.88

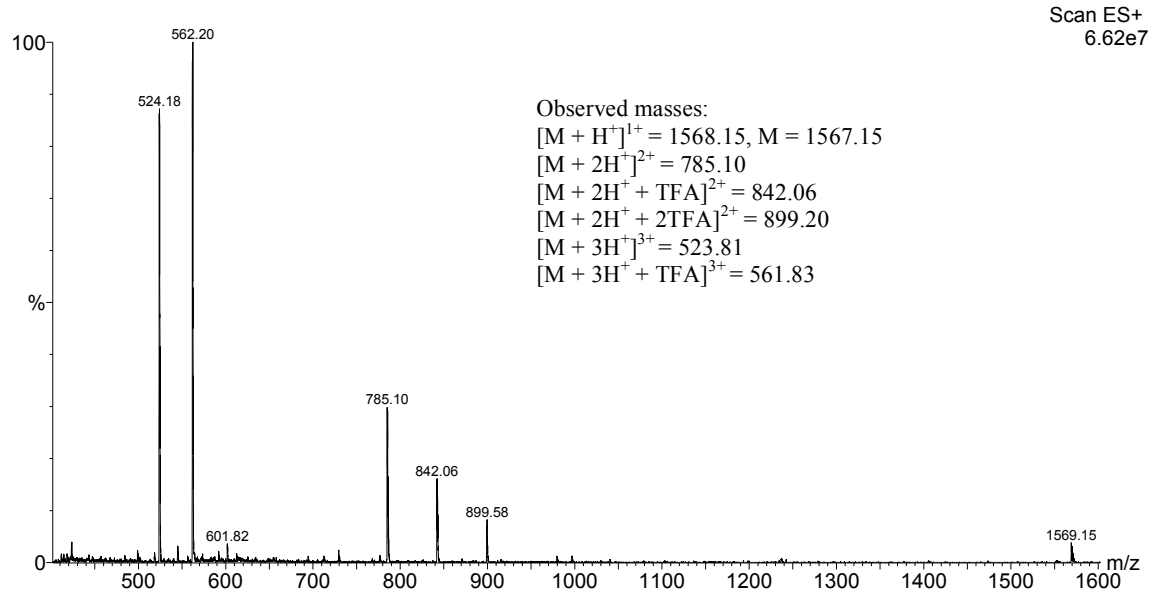



**Figure S42.** ESI-MS of WKWK, calculated mass: 1567.91

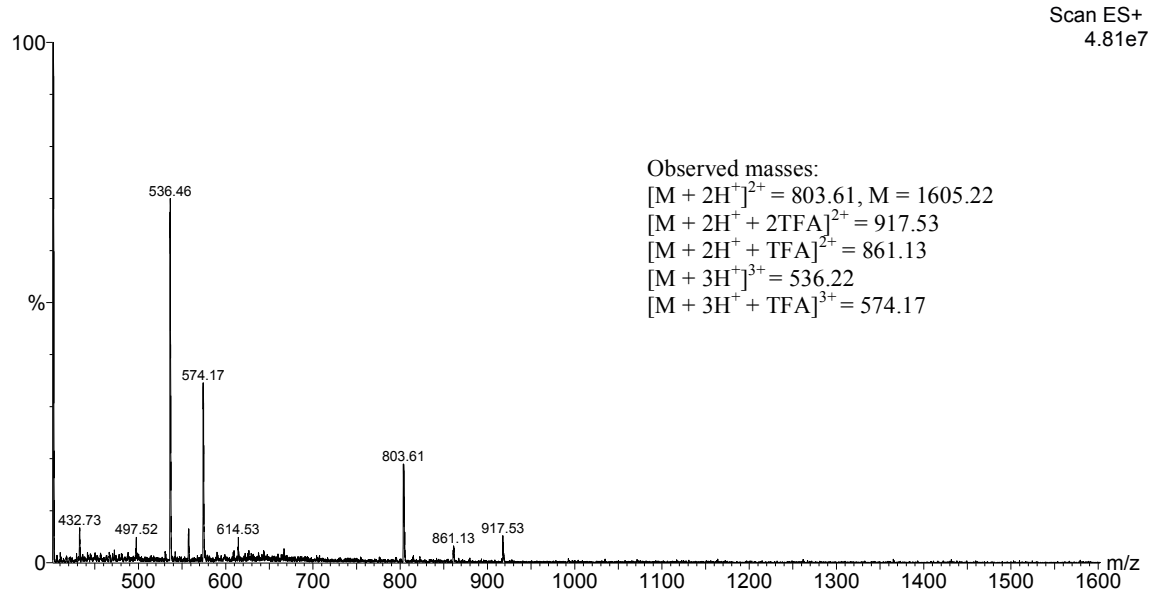



**Figure S43.** ESI-MS of HB-rev-U, calculated mass: 1604.88

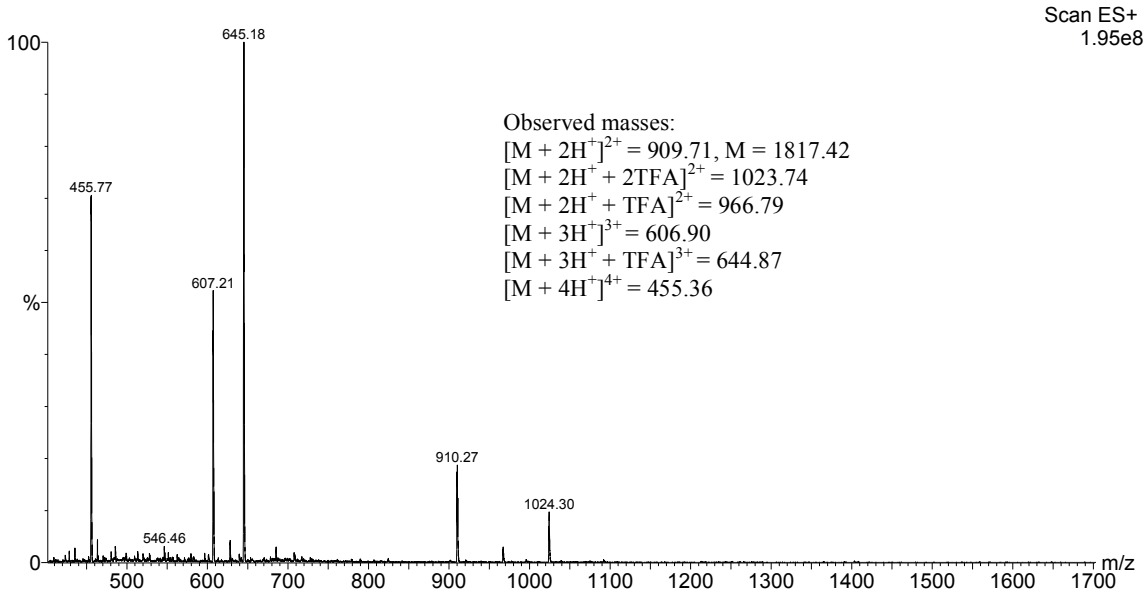



**Figure S44.** ESI-MS of Term-U, calculated mass: 1817.03

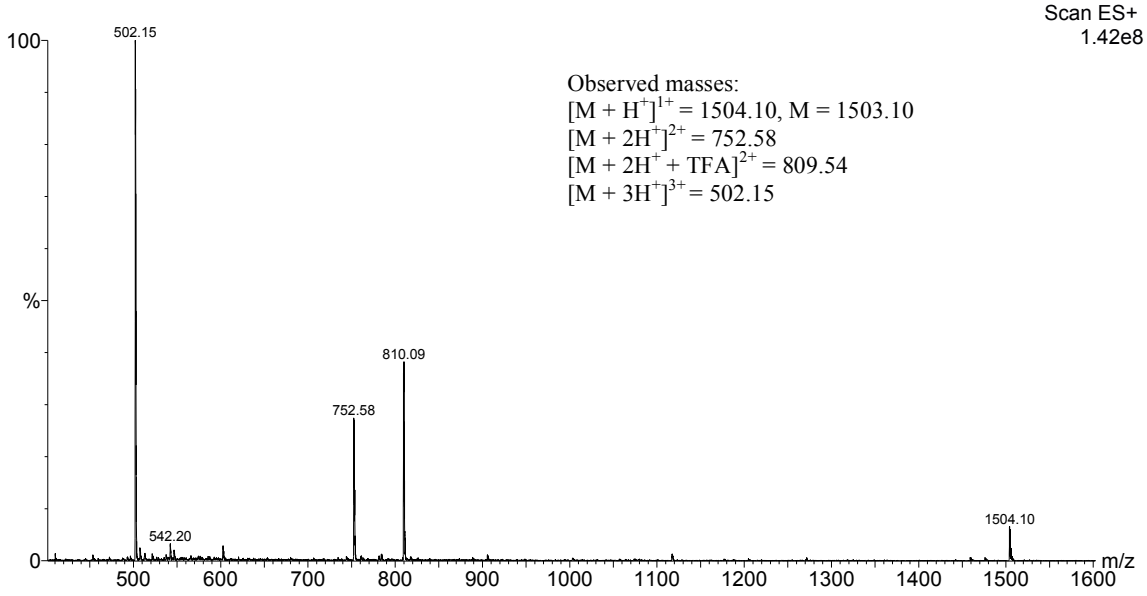



**Figure S45.** ESI-MS of NHB-rev-U, calculated mass: 1502.86

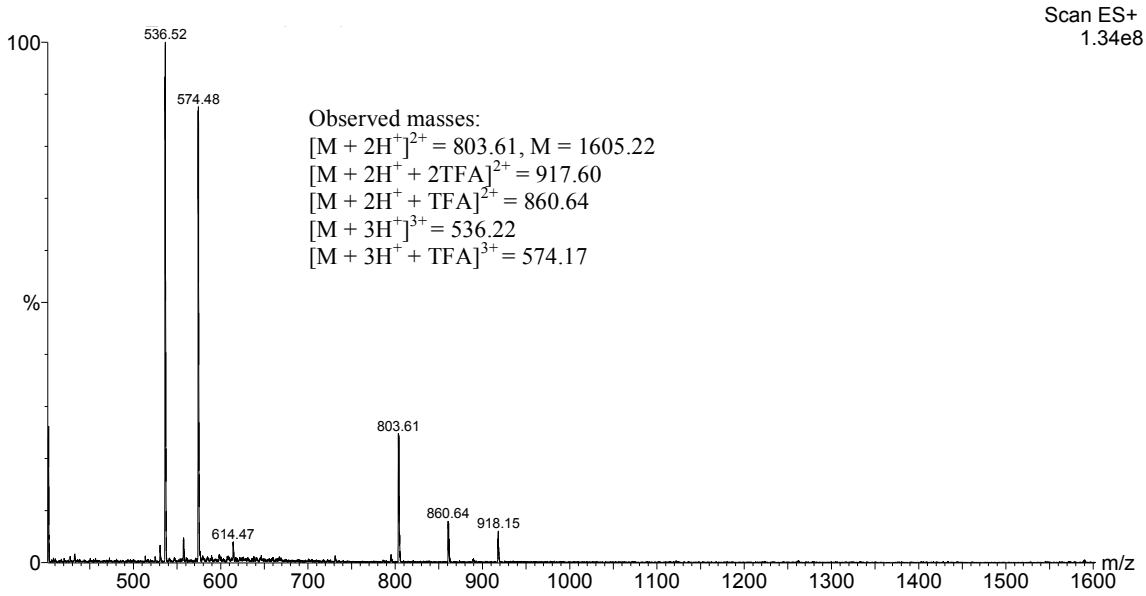



**Figure S46.** ESI-MS of HB-C, calculated mass: 1604.88

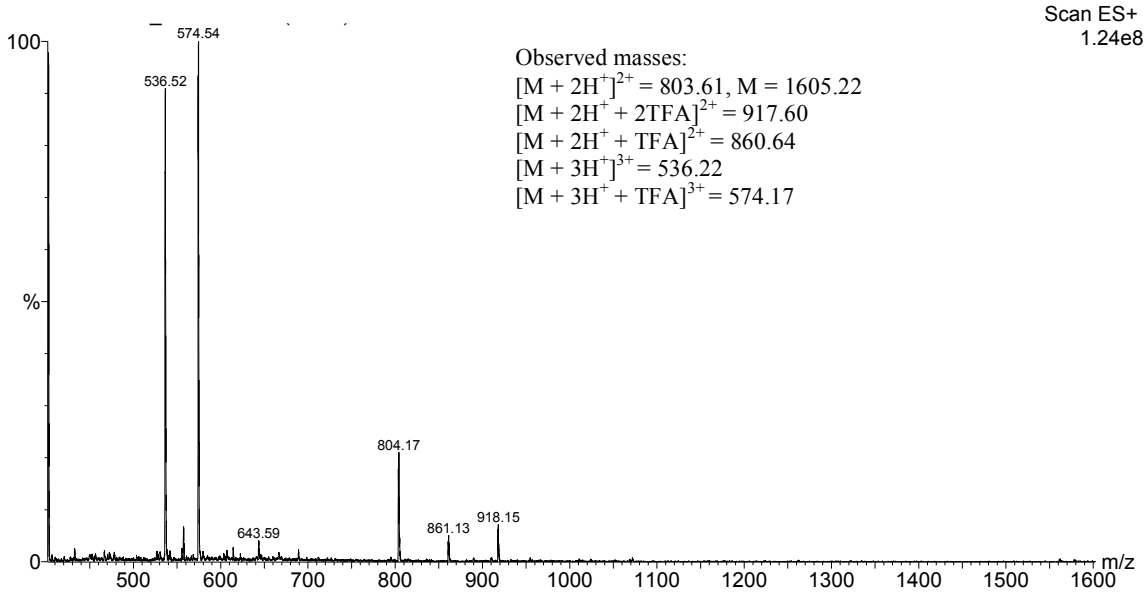



**Figure S47.** ESI-MS of HB-U, calculated mass: 1604.88

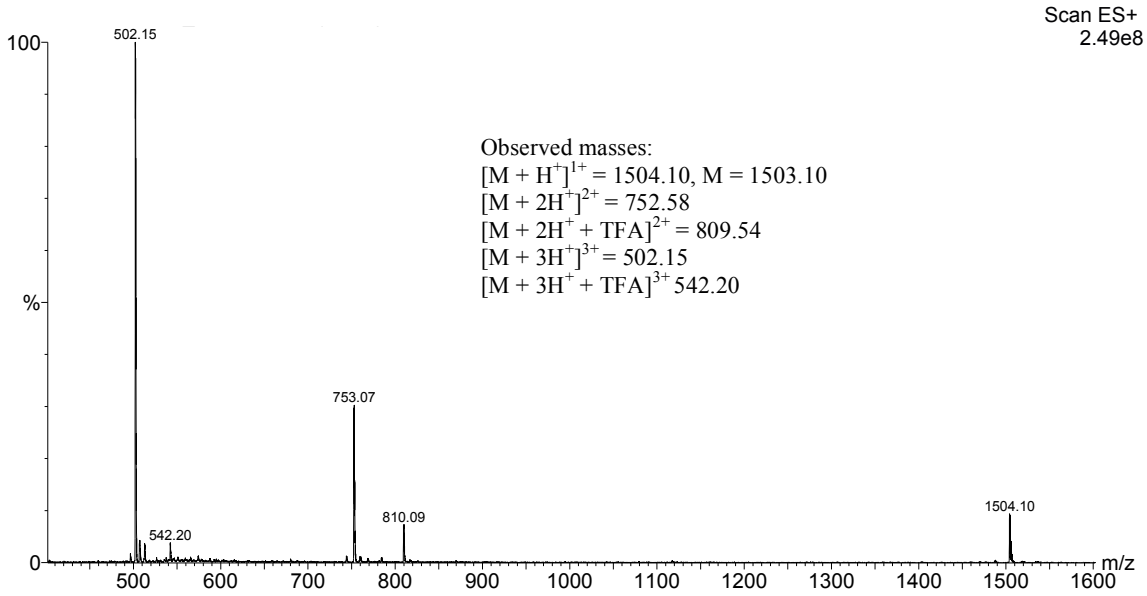



**Figure S48.** ESI-MS of NHB-rev-C, calculated mass: 1502.86

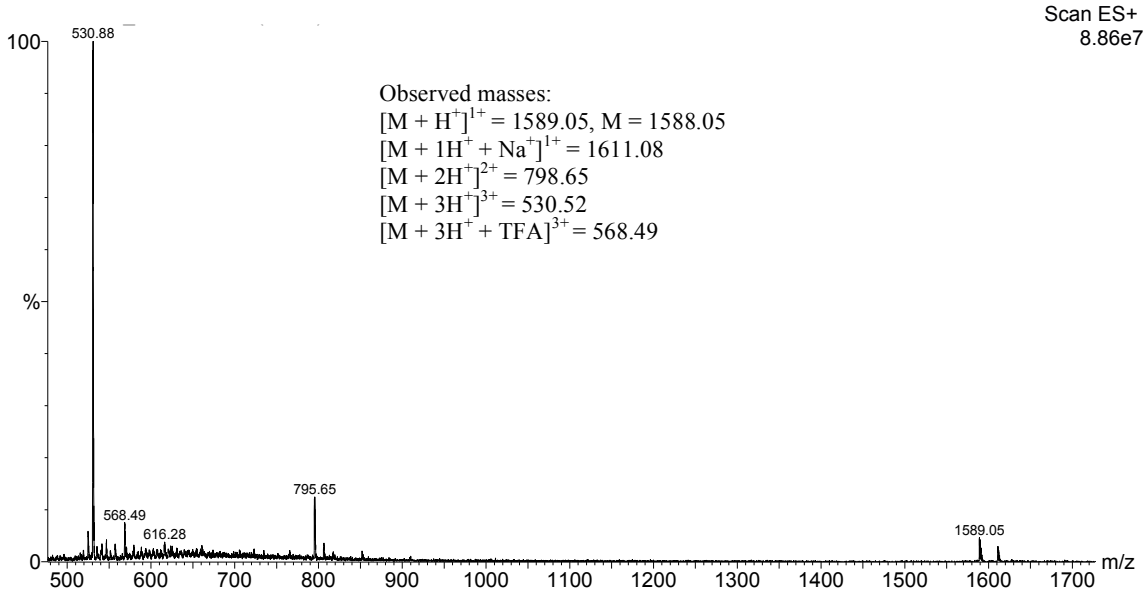



**Figure S49.** ESI-MS of HB-pG-U, calculated mass: 1587.89

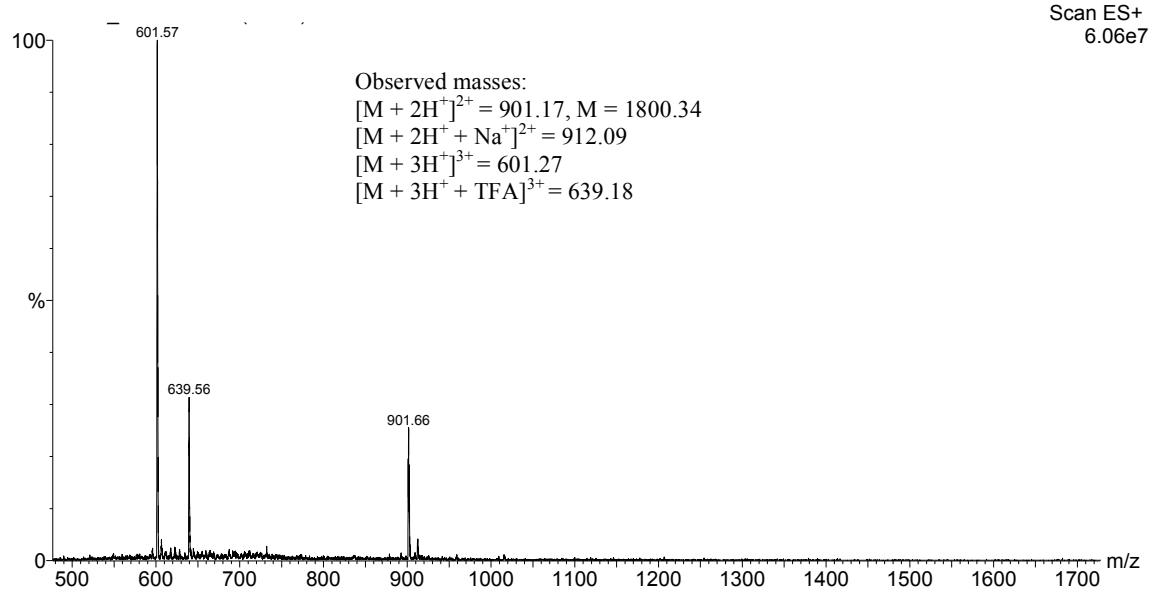



**Figure S50.** ESI-MS of Term-pG-C, calculated mass: 1800.04

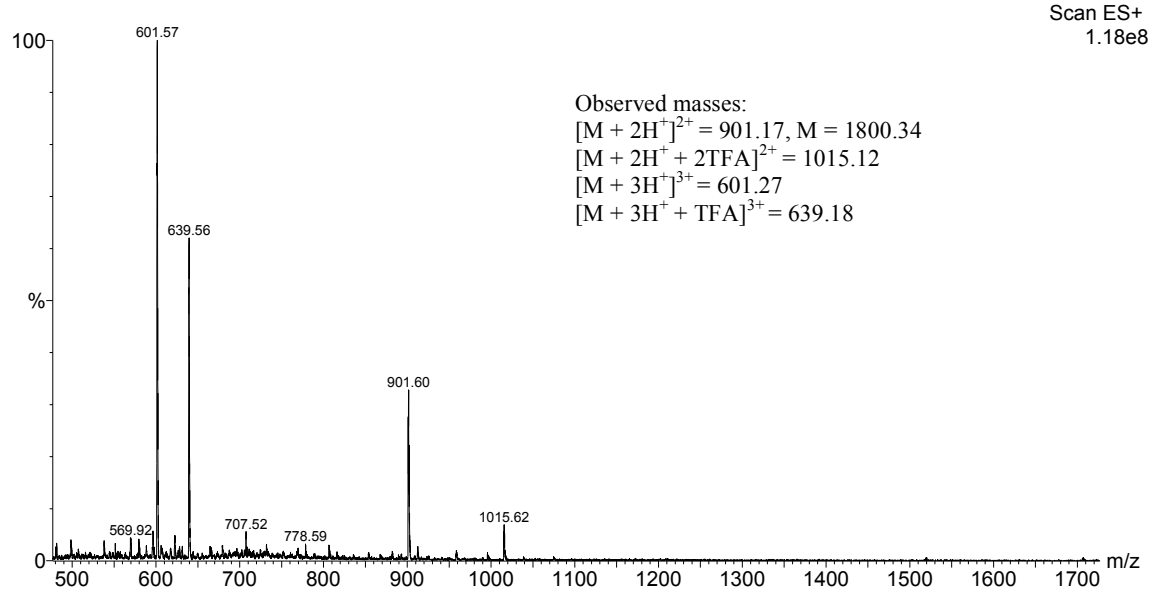



**Figure S51.** ESI-MS of Term-pG-U, calculated mass: 1800.04

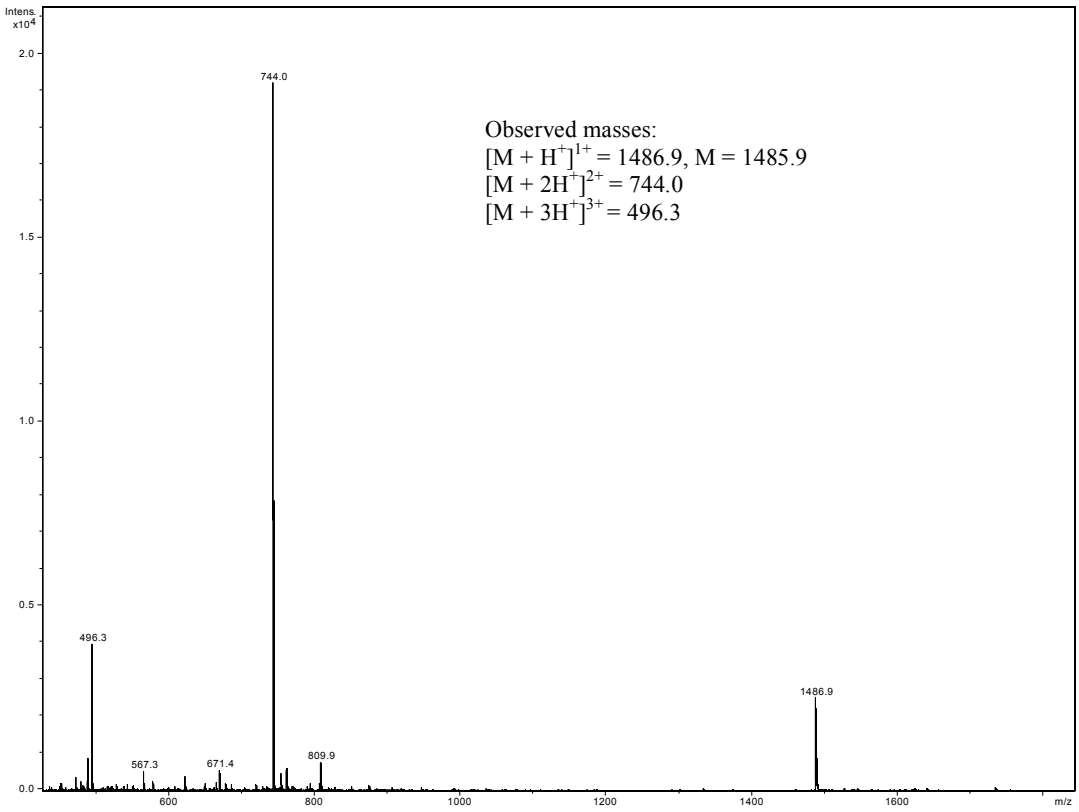



**Figure S52.** ESI-MS of NHB-pG-U, calculated mass: 1485.87

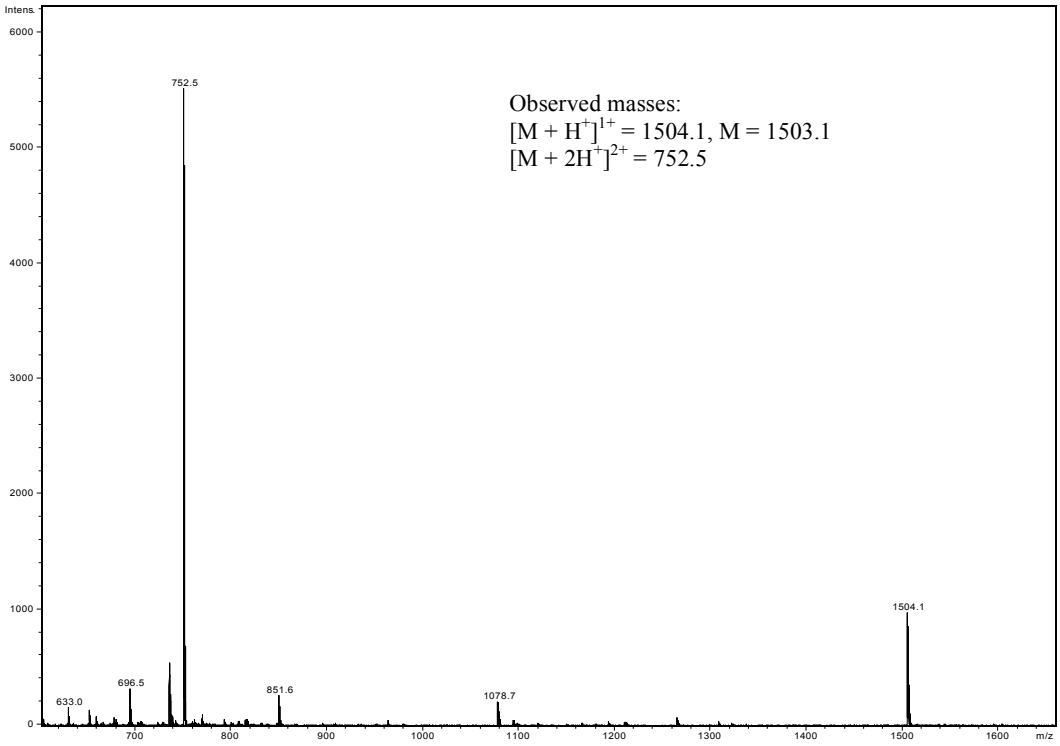



**Figure S53.** ESI-MS of NHB-U, calculated mass: 1502.86

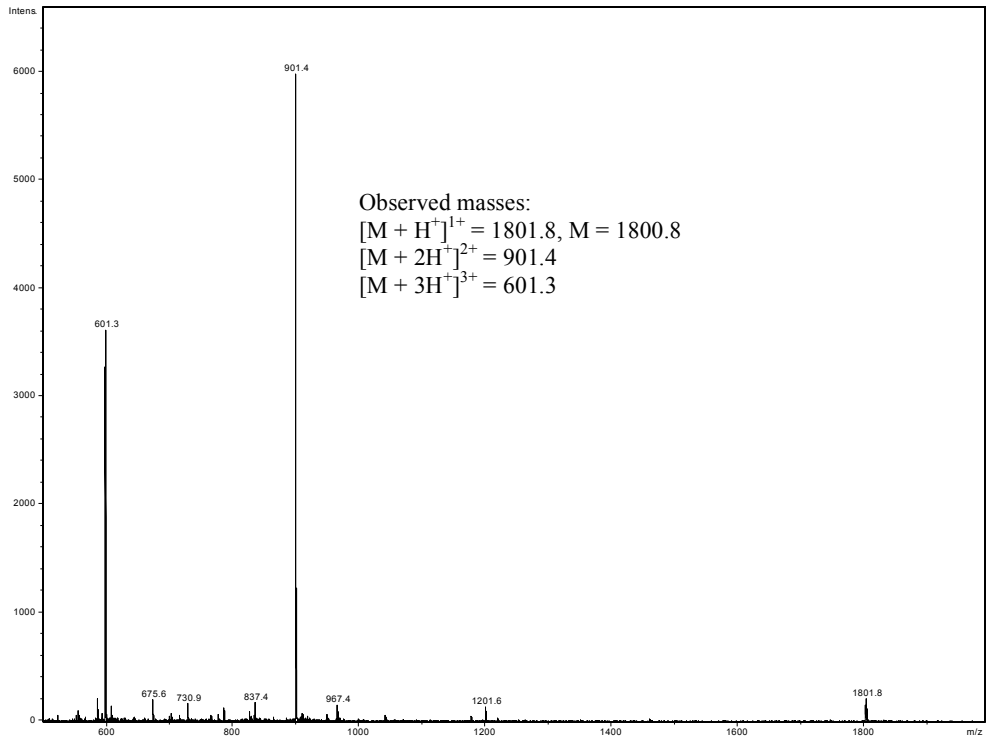



**Figure S54.** ESI-MS of Scramble, calculated mass: 1800.04