Supplemental Information

New Charge-Bearing Amino Acid Residues that Promote β-Sheet Secondary Structure

Stacy J. Maynard, Aaron M. Almeida, Yasuharu Yoshimi, and Samuel H. Gellman

Table of Contents

Additional Chemical Shift Deviation Data (ΔδCαH) and Peptide Sequence Summary……Supplementary Section 1, S2
Thermodynamic Calculations………………………………Supplementary Section 2, S10
Cross Strand NOE Summaries…………………………Supplementary Section 3, S11
Chemical Shifts of Peptides from 2D NMR…………Supplementary Section 4, S21
NMR Sample Preparation and Data Acquisition……Supplementary Section 5, S43
NMR Structure Calculations…………………………Supplementary Section 6, S43
Circular Dichroism (CD)…………………………Supplementary Section 7, S47
Distribution Coefficients…………………………Supplementary Section 8, S53
Peptide Synthesis………………………………Supplementary Section 9, S54
Amino Acid Synthesis…………………………Supplementary Section 10, S55
Dilution Studies………………………………Supplementary Section 11, S66
Peptide Purity Checks…………………………Supplementary Section 12, S77
NMR Spectra………………………………Supplementary Section 13, S83
MALDI-MS Data…………………………Supplementary Section 14, S119
NMR Structure Calculation Restraint Files………Supplementary Section 15, S139
Supplementary Section 1
Additional Chemical Shift Deviation Data ($\Delta \delta C_{\alpha}H$) and Peptide Sequence Summary

**General.** All chemical shift deviations ($\Delta \delta C_{\alpha}H$) are calculated by subtracting the chemical shift of the $\alpha$ proton for each residue of the peptide of interest from the chemical shift of the $\alpha$ proton for the corresponding residue in an unfolded control peptide. Unless otherwise noted, the unfolded control peptide is the diastereomer of the parent peptide with LPro instead of DPro (Figure 3 in the main text). It has previously been shown that the LPro-Gly segment cannot adopt the type of two-residue turn necessary to form the $\beta$-hairpin conformation. The parent peptide has the same sequence as the peptide of interest except at the position where a natural residue has been substituted for TO$^+$, TS$^+$, or TS$^-$. The $\Delta \delta C_{\alpha}H$ at or adjacent to the substitution site cannot be directly compared between parent and derivative peptides due to changes in dynamic range for $\Delta \delta C_{\alpha}H$ that result from the replacement. The dynamic range is determined by comparing the unfolded control peptide (defined above) with a fully folded control peptide (macrocycle formed by connecting the termini of the peptide in question with a second DPro-Gly segment, as illustrated in main text Figure 3. For example, if the dynamic range is 1 ppm, then a $\Delta \delta C_{\alpha}H$ of 0.5 ppm means a 50% folded population. However, if the dynamic range is 0.5 ppm, a $\Delta \delta C_{\alpha}H$ of 0.5 ppm means the peptide is fully folded. We found that the dynamic range of $\Delta \delta C_{\alpha}H$ more than one residue away from the substitution site is approximately constant; therefore, $\Delta \delta C_{\alpha}H$ at these positions can be directly compared between peptides containing different residues at the substitution site. Note that with the proper control peptides available, the calculated folded populations may be directly compared near substitutions (see SI Section 2). All unfolded control peptides were characterized by 2D NMR, and no non-sequential NOEs were observed, which is consistent with the hypothesis that they are unfolded. Unless otherwise noted, all chemical shifts were determined for the peptide in sodium acetate buffer (100 mM, pH=3.8) in 9:1 H$_2$O:D$_2$O at 4°C. The reporter positions in each peptide are the residues at which $\Delta \delta C_{\alpha}H$ is assumed to reflect most accurately the population of the $\beta$-hairpin conformation. We define reporter positions as the hydrogen bonding positions in the core of the peptide not adjacent to a substitution, based on previous studies.
Figure S1. Summary of peptides synthesized and characterized. (A) Peptides derived from the parent sequence described by Syud et al.\(^2\) (B) Peptides derived from the parent sequence described by Stanger et al.\(^3\). The sequence is an extended version of the sequence described by Syud et al.\(^2\) with two extra Thr residues at each terminus. (C) Peptides derived from the parent sequence described by Espinosa et al.\(^4\) (D) Peptides derived from the parent sequence from Haque et al.\(^1b\).
Figure S2. Comparison of the chemical shift deviations ($\Delta \delta_{\text{C,H}}$) of V (Lys), VI (TO$^+$), and VII (TS$^+$), peptides derived from a sequence from Haque et al.$^{1b}$ (MQIFVKSpGKTITLK-V-NH$_2$), at reporter positions. The $\Delta \delta_{\text{C,H}}$ at or adjacent to the substitution (position 15) cannot be directly compared due to differences in the dynamic range. The reporter positions in each peptide are the residues for which $\Delta \delta_{\text{C,H}}$ is believed to reflect most accurately the population of the $\beta$-hairpin conformation. We define reporter positions as the hydrogen bonding positions in the core of the peptide not adjacent to the substitution, as these sites have been shown to most accurately reflect the population of a $\beta$-hairpin conformation.$^2$ The unfolded control peptide is the diastereomer of the parent peptide with an LPro instead of a DPro. It has previously been shown that the LPro-Gly segment cannot adopt the type of two-residue turn necessary to form the $\beta$-hairpin conformation.$^1$
**Figure S3.** Comparison of the chemical shift deviations (ΔδCαH) of V (Ile), VIII (Glu), and IX (TS), peptides derived from a sequence from Haque et al.,

(MQIFVKSpGKTTLKV-NH2), at (A) all positions and (B) reporter positions. The ΔδCαH of positions at or adjacent to the substitution (position 3) cannot be directly compared due to changes in the dynamic range. The reporter positions in each peptide are the residues for which ΔδCαH is believed to reflect most accurately the population of the β-hairpin conformation. We define reporter positions as the hydrogen bonding positions in the core of the peptide not adjacent to the substitution, as these sites have been shown to most accurately reflect the population of a β-hairpin conformation. The unfolded control peptide is the diastereomer of the parent peptide with an LPro instead of a DPro. It has previously been shown that the LPro-Gly segment cannot adopt the type of two-residue turn necessary to form the β-hairpin conformation.

(A)

(B)
**Figure S4.** Comparison of the chemical shift deviations ($\Delta \delta C_{\alpha} H$) of V (Ile), X (TO$^+$), and XI (TS$^+$), peptides derived from a sequence from Haque et. al.$^{1b}$ (MQIFVKSpGKTTLK-V-NH$_2$), at (A) all positions and (B) reporter postions. The $\Delta \delta C_{\alpha} H$ of positions at or adjacent to the substitution cannot be directly compared due to changes in the dynamic range. The reporter positions in each peptide are the residues for which $\Delta \delta C_{\alpha} H$ is believed to reflect most accurately the population of the $\beta$-hairpin conformation. We define reporter positions as the hydrogen bonding positions in the core of the peptide not adjacent to the substitution, as these sites have been shown to most accurately reflect the population of a $\beta$-hairpin conformation.$^2$ The unfolded control peptide is the diastereomer of the parent peptide with an LPro instead of a dPro. It has previously been shown that the LPro-Gly segment cannot adopt the type of two-residue turn necessary to form the $\beta$-hairpin conformation.$^1$

(A)
Figure S5. Comparison of the chemical shift deviations (ΔδCαH) of XII (Thr), XIII (TO*), and XIV (TS*) of an extended (16mer) version of peptide I derived from a sequence from Stanger et al.1 (TTRYVEVpGOKILQTT-NH₂) at (A) core positions and (B) reporter positions. All peptides were referenced relative to an unfolded control of the 12mer parent peptide I. Positions at the end of the sequence are not shown as there is not an appropriate unfolded chemical shift for reference. Although the β-sheet propensity of TS* appears to be close to Thr in this peptide, the substitution was made near the end of the peptide and may be part of the ‘frayed end’ rather than strictly within the hairpin. The reporter positions in each peptide are the residues for which ΔδCαH is believed to reflect most accurately the population of the β-hairpin conformation. We define reporter positions as the hydrogen bonding positions in the core of the peptide not adjacent to the substitution, as these sites have been shown to most accurately reflect the population of a β-hairpin conformation.2 The unfolded control peptide is the diastereomer of the parent peptide with an LPro instead of a DPro. It has previously been shown that the LPro-Gly segment cannot adopt the type of two-residue turn necessary to form the β-hairpin conformation.1

(A)

(B)
Figure S6. Comparison of the chemical shift deviations (ΔδCαH) of XV (Lys8) and XVI (TO'), peptides derived from a sequence in Espinosa et al.⁴ (RWQYpGKFTVQ-NH₂), at (A) all positions and (B) reporter positions at 4°C. All peptides were referenced relative to an unfolded control that was taken at 3°C. The ΔδCαH of positions at or adjacent to the substitution cannot be directly compared due to differences in the dynamic range. The reporter positions in each peptide are the residues for which ΔδCαH is believed to reflect most accurately the population of the β-hairpin conformation. We define reporter positions as the hydrogen bonding positions in the core of the peptide not adjacent to the substitution, as these sites have been shown to most accurately reflect the population of a β-hairpin conformation.¹ The unfolded control peptide is the diastereomer of the parent peptide with an LPro instead of a DPro. It has previously been shown that the LPro-Gly segment cannot adopt the type of two-residue turn necessary to form the β-hairpin conformation.¹
**Figure S7.** Comparison of the chemical shift deviations (ΔδCαH) of XV (Thr10) and XVII (TO'), peptides derived from Espinosa et al.4 (RWQYVpGKFTVQ-NH2), at (A) all positions and (B) reporter positions. All peptides were referenced relative to an unfolded control of the parent peptide that was taken at 3°C. The ΔδCαH of positions at or adjacent to the substitution cannot be directly compared due to differences in the dynamic range. Note that the substitution of Thr10 for a TO' puts an extra positive charge in the peptide that should be close in space to positively charged Lys8 in the folded peptide. Therefore, charge-charge repulsion may contribute to the destabilization of the TO' peptide relative to the parent peptide. The reporter positions in each peptide are the residues for which ΔδCαH is believed to reflect most accurately the population of the β-hairpin conformation. We define reporter positions as the hydrogen bonding positions in the core of the peptide not adjacent to the substitution, as these sites have been shown to most accurately reflect the population of a β-hairpin conformation.\(^2\) The unfolded control peptide is the diastereomer of the parent peptide with an LPro instead of a DPro. It has previously been shown that the LPro-Gly segment cannot adopt the type of two-residue turn necessary to form the β-hairpin conformation.\(^1\)
Supplementary Section 2
Thermodynamic Calculations

The β-sheet populations and ΔG\text{Fold} values were calculated for the peptides derived from the Syud et. al\textsuperscript{2} (Peptides I-IV). For each peptide, the population is measured using the chemical shifts of the α protons (δC\textsubscript{α}H) of the hydrogen bonded positions in the core of the peptide (Val 3, Val 5, Orn 8, Ile 10).\textsuperscript{2,5} These reporter positions are thought to most accurately reflect the β-sheet population of the hairpin.\textsuperscript{2} The β-sheet population was calculated with equation 1

\[
\text{Beta Sheet Population (\%) = } \frac{\delta_{\text{Obs}} - \delta_{U}}{\delta_{F} - \delta_{U}} \quad \text{Equation 1}
\]

where δ\textsubscript{Obs} is the δC\textsubscript{α}H of the peptide of interest, δ\textsubscript{F} is the δC\textsubscript{α}H of the cyclic peptide of matching sequence, and δ\textsubscript{U} is the δC\textsubscript{α}H of a diastereomer of the peptide of the same sequence with an LPro instead of a DPro at the turn. (See Figure 3 in the main text for an illustration.)

The equilibrium constant was calculated with equation 2.

\[
K_F = \frac{\text{Beta Sheet Population}}{1 - \text{Beta Sheet Population}} \quad \text{Equation 2}
\]

The ΔΔG\text{Fold} was calculated with equation 3.

\[
\Delta G_{\text{Fold}} = -RT\ln(K_F) \quad \text{Equation 3}
\]

The ΔΔG\text{Fold} for each substitution was calculated at each reporter position, and the four resulting values were averaged. The error in the averaged value was calculated using the student’s T test at the 95% confidence interval.

Note that ΔδC\textsubscript{α}H at or adjacent to a substitution cannot be directly compared because the dynamic range of the measurement may have changed. However, with folded and unfolded controls for each substitution, the population folded may be directly compared as changes in dynamic range are accounted for through the use of folded and unfolded control peptides containing the appropriate substitution.
Supplementary Section 3
Summary of Cross-Strand NOEs

Figure S8. Cross-strand NOEs observed in peptide II (Lys9 → TO+) at 4°C. Medium NOEs are blue and weak NOEs are green.

Figure S9. Cross-strand NOEs observed in cyclic analogue (fully folded control) of peptide II (cyclic Lys9 → TO+) at 4°C. Strong NOEs are red, medium NOEs are blue, and weak NOEs are green.
Figure S10. Cross-strand NOEs observed in peptide III (Lys9 → TS’) at (A) 4°C and (B) 10°C. Tyr2 side-chain aromatic position 2,6 has the same chemical shift as Gln12 NH2 side-chain. As a result, none of those NOEs are definitively assigned or shown. Medium NOEs are blue and weak NOEs are green.
**Figure S11.** Cross-strand NOEs observed in cyclic analogue (fully folded control) of peptide III (cyclic Lys9 → TS') at 4°C. Strong NOEs are red, medium NOEs are blue, and weak NOEs are green.

**Figure S12.** Cross-strand NOEs observed in peptide IV (Glu4 → TS) at 4°C. Strong NOEs are red, medium NOEs are blue, and weak NOEs are green.
**Figure S13.** Cross-strand NOEs observed in cyclic analogue (fully folded control) of peptide IV (cyclic Glu⁴→TS) at 4°C. Strong NOEs are red, medium NOEs are blue, and weak NOEs are green.

**Figure S14.** Cross-strand NOEs observed in parent peptide I at 4°C from Syud et al.². Strong and medium NOEs are black and weak NOEs are green.
**Figure S15.** Cross-strand NOEs observed in peptide XIII (Thr2 → TO⁺) at 4°C. Strong NOEs are red, medium NOEs are blue, and weak NOEs are green.

**Figure S16.** Cross-strand NOEs observed in peptide XIV (Thr2 → TS⁺) at 4°C. Medium NOEs are blue and weak NOEs are green. Chemical shifts of Val 5 and 7-NH overlap, so cross-strand backbone Val5-NH to Ile12-NH and Val7-NH to Orn10-NH cannot be definitively assigned, but are detected.
Figure S17. Cross-strand NOEs observed in parent hairpin V derived from Haque et al.\textsuperscript{1b} at 4°C. Medium NOEs are blue. There is an additional strong or medium NOE(s) from Lys10 to Ser7 and/or Gly9, which is consistent with the backbone hydrogen bonding pattern of the expected hairpin structure. In general, fewer NOEs are observed for this parent peptide and its derivatives than the other parent sequences. It is probable that this peptide sequence is not as well folded as the sequence derived from Syud et al.\textsuperscript{2} However, the observed NOEs and \(\Delta \delta C_{\alpha}H\) values support that the sequence does form a hairpin (Figure S2, S3).

Figure S18. Cross-strand NOEs observed in peptide VI (Lys15 $\rightarrow$ TO*) at 4°C. Strong NOEs are red and medium NOEs are blue.
**Figure S19.** Cross-strand NOEs observed in peptide VII (Lys15 → TS*) at 4°C. Strong NOEs are red and medium NOEs are blue.

![Peptide VII](image)

**Figure S20.** Cross-strand NOEs observed in peptide X (Ile3 → TO*) at 4°C. Strong NOEs are red and weak NOEs are green.

![Peptide X](image)
Figure S21. Cross-strand NOEs observed in peptide XI (Ile3 $\rightarrow$ TS') at 4°C. Strong NOEs are red and medium NOEs are blue.

Figure S22. Cross-strand NOEs observed in peptide IX (Ile3 $\rightarrow$ TS') at 4°C. Strong NOEs are red and medium NOEs are blue.
**Figure S23.** Cross-strand NOEs observed in peptide **VIII** (Ile3 → TS) at 4°C. Strong NOEs are red. Additional strong or medium NOE(s) are observed from Lys10 to Ser7 and/or Gly9 which is consistent with the expected hydrogen bonding pattern.

![Figure S23](image)

**Figure S24.** Cross-strand NOEs observed in peptide **XVII** (Thr10 → TO) at 4°C. Strong NOEs are red, medium NOEs are blue, and weak NOEs are green.

![Figure S24](image)
**Figure S25.** Cross-strand NOEs observed in peptide XVI (Lys8 $\rightarrow$ TO$^+$) at 4°C. Strong NOEs are red, medium NOEs are blue, and weak NOEs are green. **(A)** Backbone-backbone and backbone-side chain NOEs. **(B)** Sidechain-sidechain NOEs.
Supplementary Section 4
Chemical Shifts of Peptides from 2D NMR

General. Details for the sample preparation and acquisition of NMR spectra can be found in supplementary section 5. All unfolded controls are the peptide sequence of interest with LPro in place of DPro. See supplementary section 1 for further explanation. All cyclic peptides (fully folded controls) are the sequence of interest cyclized with a two-residue DPro-Gly turn (see main text Figure 3 for an example).

Table S1. $^1$H chemical shifts of peptide II (Lys9 → TO$^+$) at 4°C in 9:1 H$_2$O:D$_2$O, pH 3.8, 100 mM sodium acetate buffer (pH not corrected for isotope effects)

|      | H-N  | H $\alpha$ | H $\beta$ | H $\gamma$ | H $\delta$ | H $\varepsilon$ | Sidechain |
|------|------|------------|-----------|------------|------------|-----------------|-----------|
| Arg1 | 4.021| 1.917      | 1.56      | 3.199      |            |                 | 7.256     |
| Tyr2 | 9.003| 5.027      | 2.864     |            | 2.916      |                 | 6.832     |
| Val3 | 8.775| 4.285      | 1.994     | 0.843      |            |                 | 7.035     |
| Glu4 | 8.596| 4.787      | 1.914     | 2.28       |            |                 |           |
| Val5 | 8.768| 4.565      | 1.988     | 0.934      |            |                 |           |
| dPro6| 4.396| 1.993      | 2.044     | 3.853      | 2.374      | 2.124           |           |
| Gly7 | 8.678| 3.815      |           | 3.997      |            |                 |           |
| Orn8 | 8.028| 4.643      | 1.865     | 1.713      | 3.022      |                 | 7.684     |
| TO$^+$| 8.725| 4.742      | 3.758     | 1.094      | 3.365      | 3.047           | 3.628     |
| Ile10| 8.933| 4.417      | 1.849     | 0.886      | 0.8138     |                 | 1.160     |
| Leu11| 8.667| 4.276      | 1.607     | 1.491      | 0.7218     |                 |           |
| Gln12| 8.555| 4.301      | 2.317     | 1.887      |            | 2.049           | 7.283     |
| Cterm| 7.247|            |           |            |            | 7.835           |           |
Table S2. $^1$H chemical shifts of cyclic analogue of peptide II (cyclic Lys9 → TO*) at 4°C in 9:1 H$_2$O:D$_2$O, pH 3.8, 100 mM sodium acetate buffer (pH not corrected for isotope effects)

|      | H-N  | H α   | H β   | H γ   | H δ   | H ε   | Side Chain |
|------|------|-------|-------|-------|-------|-------|------------|
| Arg1 | 7.789| 4.627 | 1.813 | 1.566 | 3.182 |       | 7.184      |
| Tyr2 | 8.744| 5.033 | 2.656 | 2.811 |       | 0.847 | 6.781      |
|      |      |       |       |       | 0.885 |       | 6.957      |
| Val3 | 9.321| 4.483 | 2.025 | 0.847 |       | 0.914 |           |
| Glu4 | 8.689| 4.985 | 1.915 | 2.281 |       |       |            |
| Val5 | 9.009| 4.614 | 1.943 | 0.889 |       |       |            |
|      |      |       |       |       | 0.914 |       |            |
| dPro6| 4.385| 1.983 | 2.063 | 3.827 |       |       |            |
|      |      | 2.386 | 2.157 | 3.897 |       |       |            |
| Gly7 | 8.812| 3.819 |       |       |       |       | 7.697      |
| Orn8 | 7.934| 4.752 | 1.822 | 1.702 | 3.015 |       |            |
|      |      |       |       | 1.876 |       |       |            |
| TO*9 | 8.84 | 5.074 | 3.714 | 1.102 | 3.368 | 3.657 |            |
|      |      |       |       | 3.034 |       |       |            |
| Ile10| 9.365| 4.645 | 1.849 | 0.887 | 1.105 | 0.817 |            |
|      |      |       |       |       | 1.105 |       |            |
|      |      |       |       |       | 1.348 |       |            |
| Leu11| 8.579| 4.329 | 1.182 | 1.115 | 0.512 |       |            |
|      |      |       |       | 1.578 |       | 0.178 |            |
| Gln12| 9.232| 4.868 | 1.813 | 2.194 |       | 7.464 |            |
|      |      |       |       | 2.003 |       |       |            |
|      |      |       |       | 2.241 |       |       |            |
| dPro13| 4.305| 1.941 | 2.009 | 3.715 |       |       |            |
|      |      |       |       | 2.325 |       | 3.786 |            |
| Gly14| 8.555| 3.8135|       |       |       |       |            |
Table S3. \(^1\)H chemical shifts of LPro diasteromer (unfolded control) of peptide II (unfolded Lys9 \(\rightarrow\) TO') at 4°C in 9:1 H\(_2\)O:D\(_2\)O, pH 3.8, 100 mM sodium acetate buffer (pH not corrected for isotope effects)

|        | H-N   | H \(\alpha\) | H \(\beta\) | H \(\gamma\) | H \(\delta\) | H \(\varepsilon\) | Side Chain |
|--------|-------|--------------|-------------|--------------|--------------|------------------|------------|
| Arg1   | 4.024 | 1.891        | 1.582       | 3.195        |              |                  | 7.258      |
| Tyr2   | 8.893 | 4.622        | 2.913       |              | 3.029        |                  | 6.803      |
| Val3   | 8.149 | 3.959        | 3.959       | 0.843        |              |                  | 7.103      |
| Glu4   | 8.443 | 4.209        | 1.984       | 2.367        |              |                  |            |
| Val5   | 8.491 | 4.403        | 2.062       | 0.953        |              |                  |            |
| LPro 6 | 8.409 | 4.409        | 1.933       | 1.991        | 3.696        | 2.319            | 2.053      |
| Gly7   | 8.618 | 3.954        |              |              |              |                  |            |
| Orn8   | 8.398 | 4.461        | 1.796       | 1.718        | 3.027        | 1.887            | 1.782      |
| TO*9   | 8.576 | 4.483        | 3.97        | 1.187        | 3.631        | 3.813            | 3.179      |
| Ile10  | 8.45  | 4.176        | 1.821       | 1.167        | 0.886        | 1.485            | 0.930      |
| Leu11  | 8.589 | 4.383        | 1.634       | 1.581        | 0.867        | 0.930            |            |
| Gln12  | 8.553 | 4.282        | 1.976       | 2.371        |              | 2.094            |            |
| C Term.| 7.232 |              |             |              |              |                  | 7.745      |
Table S4. $^1$H chemical shifts of peptide III (Lys9 $\rightarrow$ TS*) at 4°C in 9:1 H$_2$O:D$_2$O, pH 3.8, 100 mM sodium acetate buffer (pH not corrected for isotope effects)

|     | H-N   | H$\alpha$ | H$\beta$ | H$\gamma$ | H$\delta$ | H$\varepsilon$ | Sidechain |
|-----|-------|-----------|----------|-----------|-----------|----------------|-----------|
| Arg1| 4.049 | 1.899     | 1.94     | 3.204     | 7.246     |                |           |
| Tyr2| 9.031 | 5.134     | 2.845    |           |           | 6.867          |           |
| Val3| 9.019 | 4.405     | 2.006    | 0.85      |           | 6.928          |           |
| Glu4| 8.661 | 5.052     | 2.291    | 1.913     |           |                |           |
| Val5| 8.882 | 4.615     | 1.973    | 0.9302    |           |                |           |
| dPro6| 4.394 | 2.389     | 2.059    | 3.88      |           |                |           |
| Gly7| 8.743 | 3.9195    |          |           |           | 7.7            |           |
| Orn8| 8.032 | 4.65      | 1.849    | 1.680     | 3.02      | 1.768          |           |
| TS*9| 8.97  | 4.85      | 3.11     | 1.192     | 2.793     | 3.091          |           |
| Ile10| 9.155 | 4.557     | 1.892    | 0.876     | 0.806     | 1.118          |           |
| Leu11| 8.649 | 4.23      | 1.614    | 1.425     | 0.599     | 0.722          |           |
| Gln12| 8.57  | 4.303     | 2.027    | 2.283     | 7.007     | 7.553          |           |
| Cterm| 7.25  |           |          |           |           | 7.878          |           |
**Table S5.** $^1$H chemical shifts of peptide III (Lys9 → TS') at 10°C in 9:1 H$_2$O:D$_2$O, pH 3.8, 100 mM sodium acetate buffer (pH not corrected for isotope effects)

|      | H-N  | H α  | H β  | H γ  | H δ  | H ε  | Side Chain |
|------|------|------|------|------|------|------|------------|
| Arg1 | 4.046| 1.903| 1.557| 3.195|      |      | 7.224      |
| Tyr2 | 8.993| 5.12 | 2.837|      |      |      | 6.815      |
| Val3 | 8.986| 4.393| 1.999| 0.8568|
| Glu4 | 8.619| 5.034| 1.914| 2.27  |
|      |      |      |      | 2.005|
| Val5 | 8.846| 4.612| 1.969| 0.933|
| dPro6| 4.38 | 1.99 | 2.053| 3.864|
|      |      | 2.384| 2.151|
| Gly7 | 8.71 | 3.82,|      |      |
|      |      | 4.019|      |      |
| Orn8 | 8.021| 4.642| 1.841| 1.667| 3.009|
|      |      |      |      | 1.728|
| TS'9 | 8.921| 4.84 | 3.106| 1.19 | 2.788| 3.095|
| Ile10| 9.12 | 4.536| 1.889| 0.972| 0.8166| 1.125| 1.359|
|      |      |      |      |      |      |      | 6.955      |
| Leu11| 8.611| 4.251| 1.442| 1.394| 0.609|      | 7.516      |
| Gln12| 8.533| 4.321| 1.836| 2.269| 2.285|
|      |      |      | 2.022|      |      |      | 6.955      |
| Cterm| 7.205|      |      |      |      |      | 7.825      |
Table S6. $^1$H chemical shifts of cyclic analogue of peptide III (cyclic Lys9 → TS$^*$) at 4°C in 9:1 H$_2$O:D$_2$O, pH 3.8, 100 mM sodium acetate buffer (pH not corrected for isotope effects)

|       | H-N     | Hα     | Hβ     | Hγ     | Hδ     | Hε     | Side Chain |
|-------|---------|--------|--------|--------|--------|--------|------------|
| Arg1  | 7.791   | 4.622  | 1.751  | 1.565  | 3.178  |        | 7.181      |
| Tyr2  | 8.719   | 5.005  | 2.648  |        |        |        | 6.775      |
|       |         |        |        |        |        |        | 6.957      |
| Val3  | 9.351   | 4.486  | 2      | 0.846  |        | 0.882  |            |
| Glu4  | 8.711   | 5.067  | 1.925  | 2.269  |        |        |            |
|       |         |        |        |        |        |        | 2.050      |
| Val5  | 9.055   | 4.633  | 1.946  | 0.9194 |        |        |            |
| DPro6 | 4.376   | 1.991  | 2.053  | 3.878  |        |        |            |
|       |         |        |        |        |        |        | 2.398      |
|       |         |        |        |        |        |        | 2.154      |
| Gly7  | 8.809   | 3.850  |        |        |        |        |            |
|       |         |        |        |        |        |        | 4.053      |
| Orn8  | 7.971   | 4.724  | 1.835  | 1.694  | 3.009  |        | 7.694      |
| TS$^*$9 | 9.036  | 5.061  | 3.117  | 1.238  | 2.827  | 3.113  |            |
| Ile10 | 9.302   | 4.717  | 1.878  | 0.865  | 0.8085 |        |            |
|       |         |        |        |        |        |        | 1.053      |
|       |         |        |        |        |        |        | 1.248      |
| Leu11 | 8.516   | 4.357  | 1.173  | 1.101  | 0.150  |        |            |
|       |         |        |        |        |        |        | 1.550      |
|       |         |        |        |        |        |        | 1.152      |
| Gln12 | 9.238   | 4.875  | 1.802  | 2.205  |        |        | 6.932      |
|       |         |        |        |        |        |        | 1.998      |
|       |         |        |        |        |        |        | 7.499      |
| Pro13 | 4.303   | 1.932  | 2.013  | 3.718  |        |        |            |
|       |         |        |        |        |        |        | 2.324      |
|       |         |        |        |        |        |        | 2.139      |
| Gly14 | 8.541   | 3.645  |        |        |        |        | 3.938      |


Table S7. $^1$H chemical shifts of LPro diastereomer (unfolded control) peptide III (unfolded Lys9 → TS$^+$) at 4°C in 9:1 H$_2$O:D$_2$O, pH 3.8, 100 mM sodium acetate buffer (pH not corrected for isotope effects)

|        | H-N  | H $\alpha$ | H $\beta$ | H $\gamma$ | H $\delta$ | H $\varepsilon$ | Side Chain |
|--------|------|------------|-----------|------------|------------|-----------------|------------|
| Arg1   | 4.018| 2.324      | 1.682     | 3.198      |            |                 | 7.263      |
| Tyr2   | 8.893| 4.619      | 2.908     | 3.029      | 0.824      |                 | 6.807      |
| Val3   | 8.133| 3.951      | 1.864     | 0.824      | 0.866      |                 | 7.095      |
| Glu4   | 8.454| 4.192      | 1.957     | 2.371      |            |                 |            |
| Val5   | 8.505| 4.4        | 2.066     | 0.958      |            |                 |            |
| LPro6  | 4.419| 1.932      | 1.982     | 3.694      | 2.219      | 2.059           | 3.879      |
| Gly7   | 8.636| 3.966      | 1.77      |            |            |                 |            |
| Orn8   | 8.414| 4.415      | 1.784     | 1.707      | 1.876      |                 |            |
| TS$^+$ | 8.646| 4.492      | 3.215     | 1.299      | 2.897      | 3.222           | 7.819      |
| Ile10  | 8.633| 4.178      | 1.834     | 0.888      | 0.8437     | 1.212           |            |
|        |      |            |           |            |            | 1.482           |            |
| Leu11  | 8.606| 4.382      | 1.645     | 1.571      | 0.867      | 0.927           |            |
| Gln12  | 8.551| 4.285      | 1.975     | 2.377      | 2.092      |                 | 6.985      |
| C Term | 7.226|            |           |            |            |                 | 7.665      |
|        | 7.740|            |           |            |            |                 |            |
Table S8. $^1$H chemical shifts of peptide IV (Glu4 → TS) at 4°C in 9:1 H$_2$O:D$_2$O, pH 3.8, 100 mM sodium acetate buffer (pH not corrected for isotope effects)

|       | H-N   | H-α    | H-β    | H-γ    | H-δ    | H-ε    | Side Chain |
|-------|-------|--------|--------|--------|--------|--------|------------|
| Arg1  | 4.056 | 1.891  | 1.548  | 3.201  |        |        | 7.27       |
| Tyr2  | 9.015 | 5.343  | 2.802  |        |        |        | 6.819      |
| Val3  | 9.006 | 4.496  | 2.049  | 0.843  | 0.892  |        | 6.962      |
| TS4   | 8.934 | 5.19   | 3.105  | 1.093  |        |        | 2.587      |
| Val5  | 8.938 | 4.659  | 1.972  | 0.942  |        |        | 2.833      |
| dPro6 | 8.938 | 4.359  | 1.972  | 2.060  | 3.876  |        | 2.833      |
| Gly7  | 8.483 | 3.673  | 2.399  | 2.172  | 3.927  |        |            |
| Orn8  | 7.988 | 4.622  | 1.821  | 1.650  | 3.006  | 1.723  | 7.695      |
| Lys9  | 8.663 | 4.721  | 1.548  | 1.118  | 1.226  | 2.357  | 7.376      |
| Ile10 | 9.4   | 4.538  | 1.926  | 0.876  | 0.795  | 1.276  | 2.462      |
| Leu11 | 8.79  | 4.176  | 1.457  | 1.432  | 0.600  | 1.178  |            |
| Gln12 | 8.543 | 4.33   | 1.816  | 2.273  |        | 0.737  |            |
| C Term.| 7.256 | 7.898  |        |        |        |        |            |
Table S9. $^1$H chemical shifts of cyclic analogue of peptide IV (cyclic Glu4 → TS') at 4°C in 9:1 H$_2$O:D$_2$O, pH 3.8, 100 mM sodium acetate buffer (pH not corrected for isotope effects)

|     | H-N  | H $\alpha$ | H $\beta$ | H $\gamma$ | H $\delta$ | H $\varepsilon$ | Side Chain |
|-----|------|------------|-----------|------------|------------|-----------------|------------|
| Arg1| 7.779| 4.662      | 1.798     | 1.524      | 3.183      |                 |            |
|     |      |            | 1.832     | 1.611      |            |                 |            |
| Tyr2| 8.688| 5.164      | 2.649     | 2.809      |            | 6.782          | 6.944      |
|     |      |            |           |            |            |                 |            |
| Val3| 9.325| 4.525      | 2.048     | 0.843      | 0.894      | 2.523          | 2.593      |
|     |      |            |           |            |            |                 | 2.807      |
| TS'4| 8.904| 5.167      | 3.103     | 1.099      |            |                 |            |
| Val5| 8.994| 4.665      | 1.96      | 0.900      | 0.9435     | 2.523          | 2.593      |
| dPro6| 4.347| 1.967      | 2.057     | 3.869      |            |                 |            |
|     |      |            | 2.397     | 2.172      | 3.929      |                 |            |
| Gly7| 8.487| 3.67       | 3.99      |            |            |                 |            |
| Orn8| 7.946| 4.672      | 1.822     | 1.639      | 3.01       | 7.688          |            |
|     |      |            |           |            |            | 1.698          |            |
| Lys9| 8.668| 4.898      | 1.614     | 1.183      | 1.36       | 2.399          | 7.364      |
|     |      |            | 1.677     | 1.297      |            |                 |            |
| Ile10| 9.46 | 4.655     | 1.88      | 0.8876     | 0.8065     | 1.123          | 1.366      |
|     |      |            |           |            |            |                 |            |
| Leu11| 8.59 | 4.241    | 1.209     | 1.131      | 0.214      |                 |            |
|     |      |            | 1.556     | 0.214      |            |                 |            |
| Gln12| 9.184| 4.86      | 1.811     | 2.213      | 2.003      | 6.915          | 7.476      |
|     |      |            | 2.003     | 2.005      | 3.686      |                 |            |
| dPro13| 4.314| 1.936    | 2.005     | 2.005      | 3.686      |                 |            |
|     |      |            | 2.305     | 2.138      | 3.769      |                 |            |
| Gly14| 8.576| 3.74      | 3.74      | 3.915      |            |                 |            |
Table S10. $^1$H chemical shifts of lPro diastereomer (unfolded control) of peptide IV (Glu4 → TS') at 4°C in 9:1 H$_2$O:D$_2$O, pH 3.8, 100 mM sodium acetate buffer (pH not corrected for isotope effects)

|      | H-N  | H $\alpha$ | H $\beta$ | H $\gamma$ | H $\delta$ | H $\varepsilon$ | Side Chain |
|------|------|------------|-----------|------------|-------------|-----------------|------------|
| Arg1 | 4.004 | 1.888      | 1.577     | 3.195      | 7.264       |                 |            |
| Tyr2 | 8.827 | 4.688      | 2.987     |            |             | 6.820          |            |
|      |       |            |           |            |             | 7.122          |            |
| Val3 | 8.296 | 4.12       | 1.945     | 0.8813     |             | 2.551          |            |
|      |       |            |           |            |             | 2.793          |            |
| TS'  | 8.362 | 4.41       | 3.169     | 1.275      |             | 2.551          |            |
|      |       |            |           |            |             | 2.793          |            |
| Val5 | 8.614 | 4.414      | 2.066     | 0.9653     |             |                 |            |
| L-Pro6 | 4.392 | 1.926      | 1.973     | 3.698      | 3.899       |                 |            |
| Gly7 | 8.616 | 3.957      |           |            |             |                 |            |
| Orn8 | 8.359 | 4.355      | 1.858     | 1.737      | 3.012       | 7.693          |            |
| Lys9 | 8.556 | 4.311      | 1.763     | 1.376      | 1.679       | 2.989          | 7.617      |
|      |       |            |           |            |             |                 |            |
| Ile10 | 8.477 | 4.122      | 1.833     | 0.888      | 0.8497      |                 |            |
|      |       |            |           |            |             |                 |            |
| Leu11 | 8.558 | 4.381      | 1.646     | 1.586      | 0.866       | 0.938          |            |
| Gln12 | 8.519 | 4.283      | 1.977     | 2.376      | 2.107       | 6.989          | 7.660      |
| C Term. | 7.234 | 7.737      |           |            |             |                 |            |
Table S11. $^1$H chemical shifts of peptide XIII (Thr2 $\rightarrow$ TO$^+$) at 4°C in 9:1 H$_2$O:D$_2$O, pH 3.8, 100 mM sodium acetate buffer (pH not corrected for isotope effects)

|        | H-N  | H α   | H β   | H γ   | H δ   | H ε   | Side Chain          |
|--------|------|-------|-------|-------|-------|-------|--------------------|
| Thr 1  | 3.991| 4.148 |       | 1.123 |       |       |                    |
| TO$^+$2| 8.959| 4.708 | 3.809 | 1.123 | 3.159 |       |                    |
| Arg 3  | 8.671| 4.475 | 1.726 | 1.484 | 3.139 | 3.159 | 3.751 3.466       |
| Tyr 4  | 8.571| 5.124 | 2.795 |       |       |       |                    |
| Val 5  | 8.905| 4.380 | 1.991 | 0.852 |       |       |                    |
| Glu 6  | 8.655| 4.907 | 1.977 | 2.267 |       |       |                    |
| Val 7  | 8.942| 4.595 | 1.968 | 0.943 |       |       |                    |
| dPro 8 | 4.378| 1.990 | 2.066 | 3.881 |       |       |                    |
| Gly 9  | 8.712| 3.790 |       |       |       |       |                    |
| Orn 10 | 7.977| 4.589 | 1.831 | 1.676 | 3.010 | 7.697 | 7.464 (NH$_3$)     |
| Lys 11 | 8.604| 4.573 | 1.570 | 1.146 | 1.368 | 2.635 |                    |
| Ile 12 | 9.105| 4.468 | 1.920 | 1.231 | 0.802 |       |                    |
| Leu 13 | 8.669| 4.229 | 1.520 | 1.432 | 0.701 |       |                    |
| Gln 14 | 8.817| 4.509 | 4.509 | 2.295 |       |       | 7.021, 7.491 (NH$_2$) |
| Thr 15 | 8.587| 4.576 | 4.214 | 1.19  |       |       |                    |
| Thr 16 | 8.29 | 4.36  | 4.241 | 1.184 |       |       |                    |
| C-Term | 7.284| 7.781 |       |       |       |       |                    |
Table S12. $^1$H chemical shifts of peptide XIV (Thr2 $\rightarrow$ TS$^+$) at 4°C in 9:1 H$_2$O:D$_2$O, pH 3.8, 100 mM sodium acetate buffer (pH not corrected for isotope effects)

|      | H-N   | H $\alpha$ | H $\beta$ | H $\gamma$ | H $\delta$ | H $\varepsilon$ | Side Chain |
|------|-------|------------|-----------|------------|------------|-----------------|------------|
| Thr1 | 3.926 | 4.11       | 1.273     |            |            |                 |            |
| TS$^+$2 | 9.046 | 4.581      | 3.138     | 1.163      | 2.876      | 3.203           |            |
| Arg3 | 8.726 | 4.498      | 1.700     | 1.504      | 3.142      |                 | 7.179      |
|       |       |            | 1.786     |            |            |                 |            |
| Tyr4  | 8.599 | 5.109      | 2.781     |            |            |                 | 6.769      |
|       |       |            |           |            |            |                 | 6.944      |
| Val5  | 8.918 | 4.365      | 2.005     | 0.852      |            |                 |            |
|       |       |            |           | 0.872      |            |                 |            |
| Glu6  | 8.643 | 4.928      | 1.878     | 2.241      |            |                 |            |
|       |       |            | 1.971     |            |            |                 |            |
| Val7  | 8.921 | 4.609      | 1.983     | 0.9342     |            |                 |            |
| dPro8 | 4.382 | 1.990      | 2.061     | 3.875      |            |                 |            |
|       |       |            | 2.384     | 2.126      |            |                 |            |
| Gly9  | 8.711 | 4.015      | 3.779     |            |            |                 |            |
| Orn10 | 7.97  | 4.598      | 1.827     | 1.703      | 3.016      |                 | 7.687      |
| Lys11 | 8.593 | 4.582      | 1.562     | 1.145      | 1.358      | 2.626           | 7.449      |
|       |       |            | 1.645     |            |            |                 |            |
| Ile12 | 9.095 | 4.48       | 1.923     | 0.893      | 0.8036     |                 |            |
|       |       |            |           | 1.220      | 1.402      |                 |            |
| Leu13 | 8.678 | 4.189      | 1.518     | 1.426      | 0.7101     |                 |            |
|       |       |            |           | 1.565      |            |                 |            |
| Gln14 | 8.768 | 4.491      | 1.846     | 2.282      |            |                 | 7.023      |
|       |       |            |           | 2.045      |            |                 | 7.473      |
| Thr15 | 8.562 | 4.576      | 4.256     | 1.193      |            |                 |            |
| Thr16 | 8.215 | 4.364      | 4.281     | 1.191      |            |                 |            |
| C Term| 7.287 |           |           |            |            | 7.770           |            |
Table S13. \(^1\)H chemical shifts of parent peptide V at 4°C in 9:1 H\(_2\)O:D\(_2\)O, pH 3.8, 100 mM sodium acetate buffer (pH not corrected for isotope effects)

| Sequence | NH     | Ha  | Hb     | Hg     | Hd     | He     | Side Chain |
|----------|--------|-----|--------|--------|--------|--------|------------|
| Met1     | 4.15   | 2.153 | 2.558  |        |        |        |            |
| Gln2     | 8.840  | 4.512 | 1.806  | 2.110  |        |        | 7.011      |
|          |        |      | 1.857  | 2.179  |        |        | 7.570      |
| Ile3     | 8.516  | 4.189 | 1.755  | 0.822  | 0.789  |        |            |
|          |        |      |        | 1.081  |        |        |            |
|          |        |      |        | 1.381  |        |        |            |
| Phe4     | 8.562  | 5.064 | 2.883  |        | 2.965  |        | 7.187      |
|          |        |      |        |        |        |        | 7.296      |
|          |        |      |        |        |        |        | 7.236      |
| Val5     | 8.757  | 4.243 | 1.998  | 0.877  |        |        |            |
| Lys6     | 8.621  | 4.661 | 1.751  | 1.402  | 1.646  | 2.965  | 7.566      |
| dPro7    | 4.868  | 1.981 | 2.030  | 3.791  |        |        |            |
|          |        |      |        | 2.334  |        |        |            |
|          |        |      |        | 2.083  |        |        |            |
| Ser7     | 8.725  | 4.422 | 3.725  |        |        |        |            |
| Gly9     | 8.717  | 3.926 |        |        |        |        |            |
| Lys10    | 8.131  | 4.565 | 1.828  | 1.421  | 1.683  | 2.983  | 7.618      |
| Thr11    | 8.587  | 4.56  | 4.007  | 1.116  |        |        |            |
| Ile12    | 8.933  | 4.436 | 1.892  | 0.881  | 0.824  |        |            |
|          |        |      |        | 1.408  |        |        |            |
| Thr13    | 8.503  | 4.534 | 4.022  | 1.080  |        |        |            |
| Leu14    | 8.634  | 4.414 | 1.528  | 1.524  | 0.827  | 0.885  |            |
| Lys15    | 8.524  | 4.409 | 1.731  | 1.344  | 1.658  | 2.976  | 7.617      |
|          |        |      |        | 1.788  |        |        |            |
| Val16    | 8.346  | 4.095 | 1.998  | 0.926  |        |        |            |
| C Term   | 7.229  |      |        |        |        |        | 7.883      |


Table S14. $^1$H chemical shifts of LPro diasteromer (unfolded control) of V at 4°C in 9:1 H$_2$O:D$_2$O, pH 3.8, 100 mM sodium acetate buffer (pH not corrected for isotope effects)

|       | H-N  | H α   | H β   | H γ   | H δ   | H ε   | Side Chain |
|-------|------|-------|-------|-------|-------|-------|------------|
| Met1  | 4.142| 2.158 |       | 2.582 |       |       |            |
| Gln2  | 8.865| 4.361 | 1.911 | 2.182 |       |       | 7.008      |
|       |      |       |       |       |       |       | 7.631      |
| Ile3  | 8.45 | 4.123 | 1.763 | 0.837 | 0.8404| 1.757 |            |
|       |      |       |       |       |       |       |            |
| Phe4  | 8.643| 4.669 | 2.979 |       | 3.049 |       | 7.217      |
|       |      |       |       |       |       |       | 7.310      |
|       |      |       |       |       |       |       | 7.264      |
| Val5  | 8.276| 4.012 | 1.934 | 0.8833|       |       |            |
| Lys6  | 8.534| 4.234 | 1.781 | 1.442 | 1.705 | 2.997 |            |
| Ser7  | 8.597| 4.727 | 3.853 |       |       |       |            |
| LPro8 | 4.438| 1.976 | 2.035 | 3.745 | 2.295 | 3.814 |            |
| Gly9  | 8.511| 3.898 | 3.986 |       |       |       |            |
| Lys10 | 8.367| 4.403 | 1.828 | 1.434 | 1.742 | 2.994 | 7.613      |
| Thr11 | 8.431| 4.313 | 4.108 | 1.184 |       |       |            |
| Ile12 | 8.527| 4.248 | 1.858 | 0.889 | 0.8525| 1.189 | 1.488      |
|       |      |       |       |       |       |       |            |
| Thr13 | 8.486| 4.313 | 4.103 | 1.185 |       |       |            |
| Leu14 | 8.502| 4.361 | 1.627 | 1.559 | 0.858 | 0.919 |            |
| Lys15 | 8.512| 4.338 | 1.801 | 1.406 | 1.672 | 2.991 | 7.611      |
| Val16 | 8.359| 4.067 | 2.027 | 0.9537|       |       |            |
| Cterm | 7.24 |       |       |       |       |       | 7.871      |
Table S15. $^1$H chemical shifts of peptide VI (Lys15 → TO') at 4°C in 9:1 H$_2$O:D$_2$O, pH 3.8, 100 mM sodium acetate buffer (pH not corrected for isotope effects)

|       | NH  | Ha  | Hb  | Hg  | Hd  | He  | Side Chain |
|-------|-----|-----|-----|-----|-----|-----|------------|
| Met1  | 4.161 | 2.151 | 2.567 |
| Gln2  | 8.839 | 4.501 | 1.805 | 2.116 |
|       |      |      | 1.850 | 2.189 |
| Ile3  | 8.5  | 4.176 | 1.754 | 0.827 | 0.7956 | 7.018 | 7.571 |
|       |      |      |      | 1.076 | 1.372 |
| Phe4  | 8.564 | 5.073 | 2.908 | 2.984 |
| Val5  | 8.765 | 4.24 | 2.004 | 0.8885 |
| Lys6  | 8.624 | 4.671 | 1.747 | 1.419 | 1.642 | 2.94 | 7.569 |
| Ser7  | 8.73  | 4.874 | 3.733 |
| DPro8 | 4.424 | 1.993 | 2.035 | 3.801 |
|       |      |      | 2.342 | 2.081 |
| Gly9  | 8.726 | 3.897 | 3.941 |
| Lys10 | 8.135 | 4.578 | 1.801 | 1.429 | 1.688 | 2.993 | 7.621 |
|       |      |      | 1.859 |
| Thr11 | 8.6  | 4.588 | 4.015 | 1.125 |
| Ile12 | 8.954 | 4.435 | 1.902 | 0.890 | 0.8329 | 7.712 |
|       |      |      | 1.143 |
| Thr13 | 8.519 | 4.558 | 4.021 | 1.079 |
| Leu14 | 8.716 | 4.55 | 1.573 | 1.568 | 0.8538 | 0.902 |
| TO'15 | 8.545 | 4.625 | 4.018 | 1.182 | 3.605 | 3.155 | 3.822 |
| Val16 | 8.311 | 4.122 | 2.008 | 0.942 |
| C Term. | 7.260 | 7.918 |
Table S16. $^1$H chemical shifts of peptide VII Lys15 $\rightarrow$ TS* at 4°C in 9:1 H$_2$O:D$_2$O, pH 3.8, 100 mM sodium acetate buffer (pH not corrected for isotope effects)

| Side Chain | H-N  | H α   | H β   | H γ   | H δ   | H ε   |
|------------|------|-------|-------|-------|-------|-------|
| Met1       | 4.16 | 2.155 | 2.53  |       |       |       |
| Gln2       | 8.881| 4.791 | 1.748 | 2.148 |       |       |
|            |      | 1.850 | 2.211 |       |       |       |
| Ile3       | 8.652| 4.241 | 1.76  | 0.826 | 0.7843|       |
|            |      |       |       | 1.029 |       | 1.336 |
| Phe4       | 8.573| 5.223 | 2.917 |       |       |       |
| Val5       | 8.936| 4.331 | 2.015 | 0.8829|       |       |
| Lys6       | 8.688| 4.788 | 1.761 | 1.418 | 1.63  | 2.936 |
| Ser7       | 8.766| 4.911 | 3.709 |       |       |       |
| Pro8       | 4.421| 1.985 | 2.042 | 3.776 |       |       |
|            |      | 2.356 | 2.100 | 3.844 |       |       |
| Gly9       | 8.807| 3.926 |       |       |       |       |
| Lys10      | 8.085| 4.62  | 1.827 | 1.428 | 1.69  | 3.001 |
|            |      | 1.865 |       |       |       |       |
| Thr11      | 8.636| 4.692 | 3.987 | 1.123 |       |       |
| Ile12      | 9.138| 4.485 | 1.912 | 0.903 | 0.8395|       |
|            |      | 1.150 | 1.410 |       |       |       |
| Thr13      | 8.561| 4.674 | 4.007 | 1.033 |       |       |
| Leu14      | 8.894| 4.563 | 1.514 | 1.506 | 0.828 |       |
|            |      |       |       |       | 0.864 |       |
| TS*15      | 8.743| 4.734 | 3.212 | 1.272 | 2.897 | 3.194 |
| Val16      | 8.556| 4.199 | 2.016 | 0.877 | 0.923 |       |
| C Term     | 7.290|       |       |       |       |       |
|            | 7.967|       |       |       |       |       |
Table S17. $^1$H chemical shifts of peptide X (Ile3 → TO∗) at 4°C in 9:1 H$_2$O:D$_2$O, pH 3.8, 100 mM sodium acetate buffer (pH not corrected for isotope effects)

|       | H-N  | H α  | H β  | H γ  | H δ  | H ε  | Side Chain |
|-------|------|------|------|------|------|------|------------|
| Met1  | 4.161| 2.164| 2.598|      |      |      | 7.027      |
| Gln2  | 8.937| 4.479| 1.951| 2.269|      |      | 7.633      |
| TO∗3  | 8.62 | 4.441| 3.927| 1.14 |      |      | 3.128      |
|       |      |      |      |      |      |      | 3.445      |
|       |      |      |      |      |      |      | 3.766      |
| Phe4  | 8.45 | 5.306| 2.953|      |      |      | 7.202      |
|       |      |      |      |      |      |      | 7.314      |
|       |      |      |      |      |      |      | 7.264      |
| Val5  | 8.582| 4.17 | 1.99 |      | 0.8923|      |            |
| Lys6  | 8.579| 4.539| 1.791| 1.444| 1.671| 2.981| 7.608      |
| Ser7  | 8.695| 4.835| 3.771|      |      |      |            |
| dPro8 | 4.435| 1.983| 2.051| 3.794|      |      |            |
| Gly9  | 8.639| 3.925|      |      |      |      |            |
| Lys10 | 8.199| 4.511| 1.819| 1.438| 1.675| 2.978|            |
| Thr11 | 8.562| 4.46 | 4.039| 1.125|      |      |            |
| Ile12 | 8.757| 4.349| 1.876| 0.895| 0.848|      |            |
|       |      |      |      | 1.155|      |      |            |
|       |      |      |      | 1.4476|     |      |            |
| Thr13 | 8.52 | 4.418| 4.076| 1.152|      |      |            |
| Leu14 | 6.207| 4.376| 4.376| 1.562| 0.852| 0.905|            |
| Lys15 | 8.538| 4.356| 1.791| 1.437| 1.673| 2.978|            |
| Val16 | 8.34 | 4.065| 2.025| 0.948|      |      |            |
| Cterm | 7.234| 7.873|      |      |      |      |            |
Table S18. $^1$H chemical shifts of peptide XI (Ile3 $\rightarrow$ TS$^\dagger$) at 4°C in 9:1 H$_2$O:D$_2$O, pH 3.8, 100 mM sodium acetate buffer (pH not corrected for isotope effects)

| Side Chain | H-N  | H $\alpha$ | H $\beta$ | H $\gamma$ | H $\delta$ | H $\varepsilon$ |
|------------|------|------------|-----------|------------|------------|----------------|
| Met1       | 4.164 | 2.155      | 2.586     |            |            |                |
| Glu2       | 8.916 | 4.479      | 1.87      | 2.216      |            |                |
| TS$^\dagger$ | 8.663 | 4.458      | 3.165     | 1.261      | 2.837      | 3.17           |
| Phe4       | 8.66  | 4.959      | 2.923     | 3.015      |            |                |
| Val5       | 8.669 | 4.187      | 1.995     | 0.8931     |            |                |
| Lys6       | 8.601 | 4.58       | 1.752     | 1.417      | 1.655      | 2.972          |
| Ser7       | 8.705 | 4.849      | 3.76      |            |            |                |
| Pro8       | 4.423 | 1.988      | 2.062     | 3.795      |            |                |
| Gly9       | 8.661 | 3.927      |            |            |            |                |
| Lys10      | 8.185 | 4.525      | 1.82      | 1.432      | 1.679      | 2.977          |
| Thr11      | 8.571 | 4.495      | 4.034     | 1.125      |            |                |
| Ile12      | 8.808 | 4.378      | 1.888     | 0.887      | 0.8414     | 1.159          |
| Thr13      | 8.527 | 4.441      | 4.06      | 1.131      |            | 1.424          |
| Leu14      | 8.549 | 4.39       | 1.554     | 1.562      | 0.843      | 0.894          |
| Lys15      | 8.546 | 4.369      | 1.793     | 1.414      | 1.673      | 2.982          |
| Val16      | 8.332 | 4.076      | 2.019     | 0.9429     |            |                |
| C Term     | 7.233 |            |           |            |            | 7.878          |
**Table S19.** $^1$H chemical shifts of peptide IX (Ile3 → TS) at 4°C in 9:1 H$_2$O:D$_2$O, pH 3.8, 100 mM sodium acetate buffer (pH not corrected for isotope effects)

| Side Chain | H-N   | H-α   | H-β   | H-γ   | H-δ   | H-ε   | Side Chain |
|------------|-------|-------|-------|-------|-------|-------|------------|
| Met1       | 4.153 | 2.159 | 2.569 |       |       |       |            |
| Gln2       | 8.847 | 4.545 | 1.871 | 2.2   |       |       | 7.021      |
| TS3        | 8.568 | 4.466 | 3.153 | 1.24  |       |       | 2.536      |
| Phe4       | 8.596 | 5.011 | 2.912 |       |       |       | 2.734      |
|            |       |       |       |       |       |       |            |
| Val5       | 8.688 | 4.215 | 2.004 | 0.8951|       |       | 7.604      |
| Lys6       | 8.617 | 4.642 | 1.756 | 1.416 | 1.651 | 2.976 |            |
| Ser7       | 8.706 | 4.847 | 3.748 |       |       |       |            |
| dPro8      | 4.425 | 1.991 | 2.032 | 3.794 |       |       |            |
|            |       |       |       |       | 2.335 | 2.080 |            |
| Gly9       | 8.679 | 3.923 |       |       |       |       |            |
| Lys10      | 8.157 | 4.546 | 1.837 | 1.427 | 1.686 | 2.994 | 7.615      |
| Thr11      | 8.577 | 4.541 | 4.023 | 1.117 |       |       |            |
| Ile12      | 8.874 | 4.408 | 1.895 | 0.894 | 0.8353|       |            |
|            |       |       |       |       | 1.146 | 1.426 |            |
| Thr13      | 8.516 | 4.486 | 4.061 | 1.12  |       |       |            |
| Leu14      | 8.579 | 4.408 | 1.554 | 1.559 | 0.838 | 0.881 |            |
|            |       |       |       |       | 0.838 | 0.881 |            |
| Lys15      | 8.522 | 4.398 | 1.753 | 1.411 | 1.668 | 2.979 | 7.599      |
| Val16      | 8.333 | 4.084 | 2.015 | 0.9386|       |       |            |
| C Term     | 7.235 | 7.886 |       |       |       |       |            |
Table S20. $^1$H chemical shifts of peptide VIII (Ile3 $\rightarrow$ Glu) at 4°C in 9:1 H$_2$O:D$_2$O, pH 3.8, 100 mM sodium acetate buffer (pH not corrected for isotope effects)

|                | H-N | H $\alpha$ | H $\beta$ | H $\gamma$ | H $\delta$ | H $\epsilon$ | Side Chain |
|----------------|-----|------------|-----------|------------|------------|-------------|------------|
| Met1           |     | 4.148      | 2.162     | 2.584      |            |             |            |
| Gln2           | 8.868 | 4.395   | 1.915     | 2.224      |            | 7.009       |            |
| Glu3           | 8.653 | 4.358   | 1.863     | 2.240      | 1.956      | 2.329       | 7.669      |
| Phe4           | 8.509 | 4.946   | 2.920     | 3.047      |            |             | 7.193      |
| Val5           | 8.622 | 4.2     | 1.998     | 0.8947     |            |             | 7.314      |
| Lys6           | 8.606 | 4.627   | 1.756     | 1.414      | 1.659      | 2.944       | 7.248      |
| Ser7           | 8.696 | 4.854   | 3.75      |            |            |             |            |
| Pro8           | 8.425 | 4.425   | 1.992     | 2.032      | 2.330      | 2.071       |            |
| Gly9           | 8.665 | 3.923   |           |            |            |             |            |
| Lys10          | 8.167 | 4.533   | 1.826     | 1.462      | 1.686      | 2.99        | 7.616      |
| Thr11          | 8.564 | 4.514   | 4.025     | 1.119      |            |             |            |
| Ile12          | 8.837 | 4.383   | 1.891     | 0.885      | 0.8375     | 1.152       | 1.428      |
| Thr13          | 8.523 | 4.436   | 4.058     | 1.137      |            |             |            |
| Leu14          | 8.536 | 4.38    | 1.551     | 1.546      | 0.8623     |             |            |
| Lys15          | 8.524 | 4.365   | 1.742     | 1.366      | 1.667      | 2.97        | 7.596      |
| Val16          | 8.341 | 4.075   | 2.015     | 0.9385     |            |             |            |
| C Term         | 7.233 |         |           |            |            |             | 7.877      |
Table S21. $^1$H chemical shifts of peptide XVI (Lys8 $\rightarrow$ TO*) at 4°C in 9:1 H$_2$O:D$_2$O, pH 3.8, 100 mM sodium acetate buffer (pH not corrected for isotope effects)

|      | H-N | H $\alpha$ | H $\beta$ | H $\gamma$ | H $\delta$ | H $\varepsilon$ | Side Chain |
|------|-----|------------|-----------|------------|------------|----------------|------------|
| Arg1 | 4.06| 1.908      | 1.598     | 3.16       |            | 7.225         |            |
| Trp2 | 8.866| 4.9        | 2.999     | 3.061      |            | 10.190, 7.164 | 7.320, 7.049 | 7.217, 7.453 |
| Gln3 | 8.761| 4.47       | 1.877     | 2.115      |            | 6.973, 7.469  |            |
| Tyr4 | 8.65 | 4.79       | 2.732     | 2.870      |            | 6.800 6.707   |            |
| Val5 | 8.62 | 4.54       | 1.995     | 0.898      | 0.926      |                |            |
| dPro6| 4.39 | 1.966      | 2.05      | 3.783      |            |                |            |
| Gly7 | 8.291| 4.114      | 3.733     |            |            |                |            |
| Thr8 | 7.95 | 4.71       | 3.951     | 1.176      | 3.598      | 7.266         | 7.763      |
| Phe9 | 8.893| 4.71       | 2.813     | 2.901      |            | 6.898         | 7.075      | 7.029      |
| TO*10| 8.605| 4.51       | 4.028     | 1.147      |            |                |            |
| Val11| 8.359| 3.9        | 1.779     | 0.732      | 0.785      |                |            |
| Gln12| 8.444| 4.28       | 1.850     | 2.282      |            |                |            |
| Cterm| 6.952| 7.530      |           |            |            |                |            |
Table S22. $^1$H chemical shifts of peptide XVII (Thr10 $\rightarrow$ TO$^+$) at 4°C in 9:1 H$_2$O:D$_2$O, pH 3.8, 100 mM sodium acetate buffer (pH not corrected for isotope effects)

|               | H-N  | H $\alpha$ | H $\beta$ | H $\gamma$ | H $\delta$ | H $\varepsilon$ | Side Chain |
|---------------|------|------------|-----------|------------|------------|-----------------|------------|
| Arg1          |      |            |           |            |            | 7.221           |            |
| Trp2          | 8.902| 4.781      | 1.895     | 1.587      | 3.166      |                 | 10.16      |
|               |      |            |           |            |            |                 | 7.124      |
|               |      |            |           |            |            |                 | 7.382      |
|               |      |            |           |            |            |                 | 7.061      |
|               |      |            |           |            |            |                 | 7.208      |
|               |      |            |           |            |            |                 | 7.461      |
| Gln3          | 8.641| 4.388      | 1.884     | 2.084      |            |                 | 6.935      |
|               |      |            |           |            |            |                 | 7.463      |
| Tyr4          | 8.421| 4.733      | 2.722     |            |            |                 | 6.883      |
|               |      |            |           |            |            |                 | 6.712      |
| Val5          | 8.68 | 4.469      | 1.995     | 0.9015     |            |                 |            |
| dPro6         |      | 4.381      | 1.942     | 2.009      | 3.709      |                 |            |
| Gly7          | 8.333| 3.782      | 3.892     |            |            |                 |            |
|               |      |            |           |            |            |                 |            |
| Lys8          | 7.93 | 4.438      | 1.735     | 1.347      | 1.638      | 2.94            | 7.611      |
| Phe9          | 8.561| 4.714      | 2.943     | 2.837      |            |                 | 6.988      |
|               |      |            |           |            |            |                 | 7.182      |
|               |      |            |           |            |            |                 | 7.063      |
| Thr10         | 8.507| 4.577      | 3.791     | 1.067      | 3.582      | 3.119           | 7.709      |
|               |      |            |           |            |            |                 |            |
| Val11         | 8.136| 3.983      | 1.852     | 0.806      | 0.726      |                 |            |
|               |      |            |           |            |            |                 |            |
| Gln12         | 8.512| 4.241      | 1.860     | 2.282      |            |                 | 6.937      |
|               |      |            |           |            |            |                 | 7.556      |
| C Term        | 7.250| 7.797      |           |            |            |                 |            |
Supplementary Section 5
NMR Sample Preparation and Data Acquisition

Lyophilized peptide was dissolved in 9:1 H₂O:D₂O sodium acetate buffer (100 mM, pH=3.8) with a 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) standard. Sample concentrations were determined by UV if a Tyr was present in the sequence or by mass. For each peptide, COSY, TOCSY, ROESY, and 1D data were taken on a 600 mHz INOVA Varian NMR with a 3 mm probe (¹H/¹³C/¹⁵N with 3 axis PFG) with standard Varian pulse sequences gcosy, wgtocsy, wgroesy, and presat, respectively. The 1D and COSY experiments used solvent suppression with a 0.6-1.5 second solvent presaturation. The TOCSY and ROESY experiments used watergate solvent suppression. The COSY was taken in absolute value mode with gradient echo coherence selection. The TOCSY used a mixing time of 80 ms, and the ROESY had a mixing time of 250 ms. The TOCSY and ROESY were taken in the sensitive mode with hypercomplex phase cycling (States-Haberkorn method). In the f2 direction, 2048 points were collected, and in the f1 direction, 220-512 point were collected. Varian VNMR 6.1 software was used to process the data, and sine bell window functions were used before Fourier transformation. Resonance assignments were made with the Sparky program.

Supplementary Section 6
NMR Structure Calculations

NMR Structure Ensemble Generation

Distance restraints were derived from the integration of NOEs in the 2D NMR data. Distances restraints where calculated using the approximation of I=cr⁶ where I is NOE intensity, c is a constant derived from an NOE of known distance, and r is the distance between protons. Distance restraints were classified as strong (≤3.0), medium (≤3.5), or weak (≤4.5). Simulated annealing was done in CNS version 1.1 with the default settings. Restraints as entered into CNS are shown below in Section 12. Only distance restraints were used; dihedral, chemical shift, and hydrogen bonding restraints were disabled. For each calculation, 1000 structures were generated, and 10-20 of the lowest energy structures were selected.
**Figure S26.** The backbone atoms of the 18 lowest energy structures of the cyclic analogue of hairpin III (cyclic Lys9 → TS*).

![Cyclic analogue of hairpin III](image)

**Table S23.** NMR Statistics from CNS calculations for the cyclic analogue of hairpin III (cyclic Lys9 → TS*).

| NMR Distance and Dihedral Constraints | Protein |
|--------------------------------------|---------|
| **Distance Constraints**              |         |
| Total NOEs                           | 33      |
| Intra-residue                        | 0       |
| Inter-residue                        | 33      |
| Sequential (|i-j|=1)                     | 16      |
| Medium-range (|i-j|<4)                      | 6       |
| Long-range (|i-j|>5)                      | 11      |
| Intermolecular                       | 0       |
| Hydrogen bonds                       | 0       |
| **Total dihedral angle restraints**  |         |
| ϕ                                    | 0       |
| ψ                                    | 0       |
| **Structure statistics**             |         |
| Violations (mean and s.d.)           | 0       |
| Distance constraints violations (Å)  | 0       |
| Dihedral angle constraints (°)       | 0       |
| Max. dihedral angle violation (°)    | 0       |
| Max. distance constraint violation (Å)| 0   |
| **Deviations from idealized geometry** |     |
| Bond lengths (Å)                     | ±0.0018 |
| Bond angles (°)                      | ±0.302  |
| Impropers (°)                        | ±0.474  |
| **Average pairwise r.m.s. deviation (Å) (18 structures)** | 1.6 ± 0.3 |
| Heavy                                | 0.5 ± 0.1 |
Figure S27. The backbone atoms of the 16 lowest energy structures of the cyclic analogue of hairpin II (cyclic Lys9 → TO*).

Table S24. NMR statistics from CNS calculations for the cyclic analogue of hairpin II (cyclic Lys9 → TO*)

| NMR Distance and Dihedral Constraints                  | Protein |
|------------------------------------------------------|---------|
| **Distance Constraints**                              |         |
| Total NOEs                                           | 34      |
| Intra-residue                                        | 0       |
| Inter-residue                                        | 34      |
| Sequential (|i-j|=1)                                       | 17      |
| Medium-range (|i-j|<4)                                   | 4       |
| Long-range (|i-j|>5)                                   | 13      |
| Intermolecular                                       | 0       |
| Hydrogen bonds                                       | 0       |
| **Total dihedral angle restraints**                   |         |
| φ                                                     | 0       |
| ϕ                                                     | 0       |
| **Structure statistics**                              |         |
| Violations (mean and s.d.)                           | 0       |
| Distance constraints (Å)                             | 0       |
| Dihedral angle constraints (°)                        | 0       |
| Max. dihedral angle violation (°)                     | 0       |
| Max. distance constraint violation (Å)                | 0       |
| **Deviations from idealized geometry**                |         |
| Bond lengths (Å)                                     | ±0.0016 |
| Bond angles (°)                                      | ±0.314  |
| Impropers (°)                                        | ±0.49   |
| **Average pairwise r.m.s. deviation (Å) (16 structures)** |         |
| Heavy                                                | 3.3 ± 0.7 |
| Backbone                                             | 1.3 ± 0.4 |
**Figure S28.** The backbone atoms of the 11 lowest energy structures of the cyclic analogue of hairpin IV (cyclic Lys9 \(\rightarrow\) TS).

**Table S25.** NMR statistics from CNS calculations for the cyclic analogue of hairpin IV (cyclic Lys9 \(\rightarrow\) TS)

| NMR Distance and Dihedral Constraints | Protein |
|---------------------------------------|---------|
| **Distance Constraints**               |         |
| Total NOEs                             | 50      |
| Intra-residue                          | 0       |
| Inter-residue                          | 50      |
| Sequential (\(i-j\)=1)                | 29      |
| Medium-range (\(i-j\)<4)              | 7       |
| Long-range (\(i-j\)>5)                | 14      |
| Intermolecular                         | 0       |
| Hydrogen bonds                         | 0       |
| **Total dihedral angle restraints**    |         |
| \(\phi\)                              | 0       |
| \(\psi\)                              | 0       |
| **Structure statistics**               |         |
| Violations (mean and s.d.)             | 0       |
| Distance constraints (A)               | 0       |
| Dihedral angle constraints (\(^o\))    | 0       |
| Max. dihedral angle violation (\(^o\)) | 0       |
| Max. distance constraint violation (A) | 0       |
| **Deviations from idealized geometry** |         |
| Bond lengths (A)                       | \(\pm 0.0024\) |
| Bond angles (\(^o\))                   | \(\pm 0.362\) |
| Impropers (\(^o\))                     | \(\pm 0.794\) |
| **Average pairwise r.m.s. deviation (A) (11 structures)** |         |
| Heavy                                 | 1.9 \(\pm 0.4\) |
| Backbone                              | 0.7 \(\pm 0.2\) |
Supplementary Section 7
Circular Dichroism (CD)

**General.** All peptides were dissolved in phosphate buffer (100 mM, pH=7.0), and an aliquot was used to determine the concentration by UV in 6 M guanidinium hydrochloride. CD samples were prepared by serial dilutions of the stock sample. All CD spectra were taken on an Aviv Model 420 Circular Dichroism Spectrometer with 1 mm pathlength cells. The parent peptide is shown in black or gray, the TS' peptide is shown in blue, and TS' peptide is shown in green.
**Figure S29.** CD spectra of parent peptide I (Arg-Tyr-Val-Val-dPro-Gly-Orn-Lys-Ile-Leu-Gln-NH$_2$) in phosphate buffer (pH=7.0, 100 mM) at variable concentrations at (A) 4°C, (B) 20°C, and (C) 37°C. All spectra have a minimum near 215 nm, which is characteristic of β-sheet secondary structure. The CD spectra at different concentrations are approximately the same, as expected if there is no self-association.
Figure S30. CD spectra of peptide III (Arg-Tyr-Val-Val-dPro-Gly-Orn-TS*-Ile-Leu-Gln-NH$_2$, Lys9→TS*) in phosphate buffer (pH=7.0, 100mM) at variable concentrations at (A) 4°C, (B) 20°C, and (C) 37°C. All spectra have a minimum near 215 nm, which is characteristic of β-sheet secondary structure. The CD spectra at different concentrations are the approximately the same, as expected if there is no self-association.
Figure S31. CD spectra of peptide IV (Arg-Tyr-Val-TS-Val-dPro-Gly-Orn-Lys-Ile-Leu-Gln-NH$_2$, Lys9→TS) in phosphate buffer (pH=7.0, 100mM) at variable concentrations at (A) 4°C, (B) 20°C, and (C) 37°C. All spectra have a minimum near 215 nm, which is characteristic of β-sheet secondary structure. The CD spectra at different concentrations are approximately the same, as expected if there is no self-association. (Note that the CD signature at 125 µM is less accurate due to the poor S/N.)
**Figure S32.** CD spectra scan of peptides I, III, and IV (parent sequence Arg-Tyr-Val-Glu-Val-DPro-Gly-Orn-Lys-Ile-Leu-Gln-NH₂, 250 µM) in phosphate buffer (pH=7.0, 100mM) (A) 4°C, (B) 20°C, and (C) 37°C. All spectra have a minimum near 215 nm, which is characteristic of β-sheet secondary structure. The relative intensity of the minima at 215 nm is consistent with the relative stability of the peptides observed by NMR in acetate buffer at pH 3.8.
Figure S33. CD spectra of peptides I, III, and IV (parent sequence Arg-Tyr-Val-Glu-Val-dPro-Gly-Orn-Lys-Ile-Leu-Gln-NH$_2$, 250 µM) in acetate buffer (pH=3.8, 100mM, 4°C). All spectra have a minimum near 215 nm, which is characteristic of β-sheet secondary structure. The relative intensity of the minima at 215 nm is similar for all three peptides, though slightly less intense for the parent peptide. Although the data does not show a clear difference between the stability of the peptides, the data is consistent with the relative stabilities determined by NMR because the NMR values are more accurate and precise. (Additionally, note that β-sheet CD signatures are less intense than α-helix signatures and therefore less useful in determining the relative stability of peptides.)
Supplementary Section 8
Distribution Coefficient Studies

In order to compare the hydrophobicities of our new amino acids to those of natural amino acids, we measured distribution coefficients of 4-nitrobenzoyl amino acid derivatives with previously described methods.\textsuperscript{15,16} The 4-nitrobenzoyl carboxamide amino acid derivatives of Ile, Thr, Gly, Glu, Lys, TS\textsuperscript{+}, and TS\textsuperscript{-} were synthesized as described in supplementary section 10. The distribution coefficient between octanol and phosphate buffer (pH 7.0, 100 mM) was measured as follows. The amino acid derivatives were dissolved in an equal amount of octanol and water and rocked on a rocker for at least 24 hours. The phases were separated and the relative concentration of amino acid was determined by analytical HPLC monitored at 275 nm. For each residue, at least three replicates were measured. The normalized distribution coefficient, $\Pi$, was calculated from equation 4:

$$\Pi = \log(D_{\text{amino acid}}) - \log(D_{\text{glycine}}) \quad \text{(Equation 4)}$$
Supplementary Section 9  
Peptide Synthesis

**Materials.** Peptides were synthesized on NovaPEG rink amide resin or Chemmatrix rink amide resin. Protected amino acids $\text{TS}^+$, $\text{TS}^-$, and $\text{TO}^+$ were synthesized as described in Section 7. All other amino acids were purchased from Chemimpex International or Novabiochem. All other reagents were purchased from Sigma-Aldrich.

**Synthesis.** Peptides were synthesized by standard Fmoc solid phase peptide synthesis (SPPS) on a CEM Mars microwave instrument. Synthesis was done on a 100 $\mu$mol scale with 4 equivalents of amino acid, 4 equivalents coupling reagent HCTU (2-(6-chloro-1H-benzotriazole-1-yl)-1,1,3,3-tetramethylammonium hexafluorophosphate) or PyBOP ((benzotriazol-1-yloxy)trispyrrolidinophosphoniumhexafluorophosphate), 4 equivalents Cl-HOBt (1-hydroxy-6-chloro-benzotriazole), and 8 equivalents diisopropylethylamine (DIEA) in 4 ml DMF with a 2 minute ramp to 70°C and a 4 minute hold at 70°C. In some cases, only two equivalents of the protected unnatural amino acids $\text{TS}^+$, $\text{TS}^-$, and $\text{TO}^+$ were used for the coupling. Deprotections were done with 4 ml of 20% piperidine in DMF with a 2 minute ramp to 80°C and a 2 minute hold at 80°C.

Peptides were cleaved by stirring resin in a solution of ethane dithiol (200 $\mu$L), water (200 $\mu$L), triisopropylsilane (80 $\mu$L), and trifluoroacetic acid (8 mL) for 2-6 hours. The solution was drained from the resin, and the peptide was precipitated with cold ether. The ether solution was centrifuged, and the solvent was decanted.

**Purification.** All peptides were purified on a Shimadzu semi-preparative HPLC with a C18 or C5 column (25x21.2 cm) and eluted with a gradient of acetonitrile and water with 0.01% trifluoroacetic acid. Purity was checked on a Shimadzu analytical HPLC with a 10-60% acetonitrile over 50 minutes gradient. All peptides were greater than 95% pure. Analytical HPLC traces can be found in supplementary section 10. MALDI-TOF MS data were taken on a Bruker REFLEX® II. MALDI-TOF MS data can be found in supplementary section 12.
Supplementary Section 10
Amino Acid Synthesis

General. Unless otherwise noted, all chemicals were purchased from Sigma-Aldrich, Chemimpex, or Fluka Analytical. All NMR spectra were taken on a Bruker AC+300a (300 mHz), Bruker Avance-400 (400 mHz), Bruker Avance-500 with a DCH cryoprobe (500 mHz), Varian INOVA 600 (600 mHz), or Varian MercuryPlus 300 (300 mHz). All optical rotations were taken on a Rudolph Autopol III automatic polarimeter with a 1 mL cell and a 5 cm path length. NMR spectra can be found in supplementary section 11.

Scheme 1. Synthesis of the protected TO\(^+\) building block
2-Amino-\(N\)-(9-Fluorenylmethoxycarbonyl)ethanol (8)

\[
\text{NH\text{Moc}}
\]

2-Aminoethanol (2.0 g, 32.7 mmol) was dissolved in 2:1 acetone: sat. aq. sodium bicarbonate (300 ml). \(N\)-(9-Fluorenylmethoxycarbonyloxy)succinimide (11.04 g, 32.7 mmol) was added. The reaction mixture was stirred at room temperature for 4 hours. The acetone was removed under reduced pressure. \(\text{H}_2\text{O}\) (200 ml) and ethyl acetate (200 ml) were added, and the mixture was acidified with 1 M HCl. The layers were separated, and the organic layer was washed with 1 M HCl (3x200 ml), saturated aqueous \(\text{NaHCO}_3\) (1x200 ml), and brine (1x200 ml). The organic layer was dried and filtered. The solvent was removed under reduced pressure to yield a white solid in a quantitative yield.

ESI-MS: Calc. \([\text{C}_{17}\text{H}_{18}\text{NO}_3]^+ = 284.1\), Obsd. \([\text{C}_{17}\text{H}_{18}\text{NO}_3]^+ = 284.1\); Melting Point: 144.5-144.9°C; \(\text{H NMR (CDCl}_3\text{, 500 MHz): } \delta 7.77 (d, J = 7.5 Hz, 2H), 7.59 (d, J = 7.5 Hz, 2H), 7.41 (t, J = 7.4 Hz, 2H), 7.32 (t, J = 7.4 Hz, 2H), 5.15 (s, 1H), 4.43 (d, J = 6.7 Hz, 2H), 4.22 (t, J = 6.5 Hz, 1H), 3.77–3.67 (m, 2H), 3.40–3.30 (m, 2H), 2.06 (t, J = 4.8 Hz, 1H). \(\text{C NMR (CDCl}_3\text{, 125 MHz): } \delta 157.14, 143.86, 141.33, 127.70, 127.05, 125.00, 119.98, 66.76, 62.36, 47.24, 43.45\).

\((2R, 3R)\)-Allyl-3-methyl-1-tritylaziridine-2-carboxylate (9)

\[
\begin{array}{c}
\text{TrtN} \\
OAllyl
\end{array}
\]

Synthesized as previously described by Vederas et al (Scheme 1).\(^{10}\)

\((2S, 3S)\)-Allyl-3-methyl-1-benzylcarbonylaziridine-2-carboxylate (1)

Aziridine 9 was dissolved in methanol (32 ml) and dichloromethane (32 ml). Trifluoroacetic acid (32 ml) was added dropwise over 10 minutes. The reaction mixture was stirred for 10 minutes, and the solvent was removed under reduced pressure. The reaction was dissolved in ether (150 ml) and \(\text{H}_2\text{O}\) (150 ml). The layers were separated, and the ether layer was extracted with \(\text{H}_2\text{O}\) (3 x 30 ml). The combined aqueous layers were cooled to 0°C and made alkaline with solid sodium bicarbonate. Benzyl chloroformate (3.1 g) and ethyl acetate (300 ml) were added, and the reaction mixture was stirred vigorously for 24 hours. The layers were separated. The organic layer was dried with \(\text{MgSO}_4\), and the solvent was removed under reduced pressure. The crude product was purified by chromatography on silica gel (EtOAc:Hexanes 1:4). The product was a clear liquid (3.2 g, 11.6 mmol, 31% yield). ESI-MS: Calc. \([\text{C}_{15}\text{H}_{18}\text{NO}_4]^+ = 276.1231\), Obsd. \([\text{C}_{15}\text{H}_{18}\text{NO}_4]^+ = 276.1225\); \([\alpha]_{D}^{25} = +73 \text{ (c 1.15, CHCl}_3\text{)}\); \(\text{H NMR (CDCl}_3\text{, 500 MHz): } \delta 7.40-7.32 \text{ (m, 5H), 5.93 (ddt, J = 16.4, 10.5, 5.9 Hz, 1H), 5.35 (dd, J=17.2, 1.2 Hz, 1H), 5.27 (dd, J=10.4, 1.0 Hz) 5.13 (AB, J=13.8 Hz, 2H), 4.68 (d, J = 5.8 Hz, 2H), 3.20 (d, J = 4.8 Hz, 2H), 1.91-1.82 (m, 2H), 1.77-1.71 (m, 2H), 1.66-1.61 (m, 2H), 1.54-1.48 (m, 2H), 1.49-1.43 (m, 2H), 1.38-1.32 (m, 2H), 1.26-1.21 (m, 2H), 1.15-1.10 (m, 2H), 1.08-1.03 (m, 2H), 0.98-0.93 (m, 2H), 0.92-0.87 (m, 2H), 0.87-0.82 (m, 2H), 0.82-0.77 (m, 2H), 0.77-0.72 (m, 2H), 0.72-0.67 (m, 2H), 0.67-0.62 (m, 2H), 0.62-0.57 (m, 2H), 0.57-0.52 (m, 2H), 0.52-0.47 (m, 2H), 0.47-0.42 (m, 2H), 0.42-0.37 (m, 2H), 0.37-0.32 (m, 2H), 0.32-0.27 (m, 2H), 0.27-0.22 (m, 2H), 0.22-0.17 (m, 2H), 0.17-0.12 (m, 2H), 0.12-0.07 (m, 2H), 0.07-0.02 (m, 2H), 0.02-0.00 (m, 2H)\).
= 6.7 Hz, 1H), 2.83 (dq, J = 6.5, 5.7 Hz, 1H), 1.36 (d, J = 5.6 Hz, 3H); $^{13}$C NMR (CDCl$_3$, 125 mHz): δ 166.87, 161.61, 135.30, 131.47, 128.57, 128.49, 128.38, 119.15, 68.59, 66.13, 39.86, 38.99, 12.86.

(2R, 3R) Allyl-2-(benzylcarbonylamino)-3-(9-Flurenylmethoxycarbonyl aminoethyloxy)butane carboxylate (2):

Aziridine 1 (5.53 g, 20.1 mmol) and alcohol 8 (38.5 g, 136 mmol) were suspended in 1000 ml of toluene. Boron trifluoride diethyl etherate (1.24 ml, 10.0 mmol) was added dropwise. The reaction mixture was refluxed for three hours. The solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (20% EtOAc in hexanes). The product was a clear oil (2.2 g, 4.0 mmol, 20% yield). ESI-MS: Calculated [C$_{32}$H$_{35}$N$_2$O$_7$]$^+$= 581.2259, Measured [C$_{32}$H$_{35}$N$_2$O$_7$]$^+$= 581.2254; $^1$H NMR (400 MHz, CDCl$_3$): δ 7.75 (d, J = 7.5 Hz, 2H), 7.60 (d, J = 7.1 Hz, 2H), 7.41-7.28 (m, 9H), 5.86 (ddt, J = 16.8, 10.6, 5.7 Hz, 1H), 5.50 (d, J = 9.3 Hz, 1H), 5.29 (d, J = 9.3 Hz, 1H), 5.14-5.10 (m, 3H), 4.67-4.58 (m, 2H), 4.43-4.32 (m, 3H), 4.20 (t, J = 6.9 Hz, 1H), 4.11-3.99 (m, 1H), 3.63-3.60 (m, 1H), 3.33-3.28 (m, 3H), 1.22 (d, J = 6.1 Hz, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$): δ 170.64, 156.65, 143.94, 141.30, 136.17, 131.42, 128.57, 128.25, 128.12, 127.69, 127.06, 125.12, 119.97, 119.15, 75.47, 68.26, 67.23, 66.76, 66.13, 58.60, 47.23, 49.97, 16.11.

(2R, 3R) Allyl-2-(benzylcarbonylamino)-3-(tert-butylmethoxycarbonyl aminoethyloxy)butane carboxylate (10):

Compound 2 (1.6 g, 2.9 mmol) was dissolved in 20% piperidine in DMF (100 ml), and the solution was stirred for 15 minutes. Solvent was removed under reduced pressure by azeotroping with toluene. The residue was dissolved in dichloromethane (100 ml). Triethylamine (0.4 ml, 2.9 mmol) and di-tert-butyl dicarbonate (4.1 g, 18.9 mmol) were added. The reaction mixture was stirred for 3 hours. The solvent was removed under reduced pressure, and the residue was dissolved in ethyl acetate (100 ml). This solution was washed with 1 M HCl (3x100 ml), saturated aqueous sodium bicarbonate (1x100 ml), and brine (1x100 ml). The organic layer was dried with magnesium sulfate and filtered. The solvent was removed under reduced pressure. The crude product was purified by silica column chromatography (20% EtOAc in hexanes) to yield a clear oil (1.3 g, 2.9 mmol, quantitative yield). ESI-MS: Calc. [C$_{22}$H$_{33}$N$_2$O$_7$]$^+$= 437.2283, Obsd. [C$_{22}$H$_{33}$N$_2$O$_7$]$^+$ = 437.2294; $^1$H NMR (400 MHz, CDCl$_3$) δ 7.38-7.32 (m, 5H), 5.91 (ddt, J
= 17.0, 10.6, 5.7 Hz, 2H), 5.47 (d, J = 9.6 Hz, 1H), 5.35 (d, J=18.3 Hz, 1H), 5.27 (d, J=10.4 Hz, 1H), 5.14 (s, 2H), 4.77 (s, 2H), 4.67 (d, J = 5.8 Hz, 4H), 4.39 (dd, J = 11.9, 2.2 Hz, 1H), 4.05 (qd, J = 6.2, 2.2 Hz, 1H), 3.65-3.55 (m, 1H), 3.35 – 3.14 (m, 3H), 1.43 (s, 9H), 1.21 (d, J = 6.3 Hz, 6H). 13C NMR (125 MHz, CDCl3): δ170.56, 156.65, 155.85, 136.18, 131.50, 128.55, 128.21, 128.10, 119.22, 79.35, 75.42, 68.44, 67.18, 66.15, 58.65, 40.44, 28.39, 16.08.

(2R, 3R) 2-(9-Fluorenlymethoxycarbonylamino)-3-(2-tert-butyloxycarbonylaminoethoxy)butane carboxylic acid (3):

Compound 10 (0.4226g, 1.0 mmol) was dissolved in methanol (100 ml). Ammonium formate (0.60 g) was added, and the solution was stirred under N2 for 20 minutes. Palladium on carbon (0.36 g, 10% loading by weight on a wet support) was added, and the mixture was stirred under N2 for 24 hours. The mixture was filtered through celite, and the solvent was removed from the filtrate under reduced pressure. The residue was dissolved in 2:1 acetone:saturated aqueous sodium bicarbonate (50 ml). N-(9-Fluorennlymethoxycarbonyloxy)succinimide was added, and the mixture was stirred for 5 hours. The acetone was removed under reduced pressure, and ethyl acetate was added (50 ml). The solution was acidified with 1 M HCl and extracted with ethyl acetate (3x50 ml). The combined organic layers were dried with magnesium sulfate and filtered. The solvent was removed under reduced pressure to give a white solid (0.5g, 1.0 mmol, quantitative yield). EMM-MS: Calc. [C26H32N2O7NH4]⁺=502.2548, Obsd. [C26H32N2O7NH4]⁺= 502.2549; Melting Point: 108.5-111.0°C; 1H NMR (500 MHz, CD3OD): δ7.82 (d, J = 7.5 Hz, 2H), 7.76 – 7.68 (m, 2H), 7.41 (t, J = 7.5 Hz, 2H), 7.34 (t, J = 7.4 Hz, 2H), 4.41 (dd, J = 7.0, 2.2 Hz, 2H), 4.32 – 4.23 (m, 2H), 4.11 (qd, J = 6.2, 2.6 Hz, 1H), 3.61-3.57 (m, 1H), 3.41 (dt, J = 9.8, 5.3 Hz, 1H), 3.35-3.15 (m, 2H), 1.46 (s, 9H), 1.21 (d, J = 6.3 Hz, 3H); 13C NMR (125 MHz, CD3OD): δ 174.16, 159.22, 158.56, 145.38, 145.15, 142.60, 128.78, 128.19, 128.17, 126.31, 126.28, 120.92, 120.91, 80.13, 76.52, 69.45, 68.13, 60.10, 41.58, 28.78, 26.28, 16.75.
Scheme 2. Synthesis of the protected TS<sup>+</sup> and TS<sup>-</sup> building blocks

2-(tert-Butylmethoxycarbonylamino)ethyl bromide (7a)
Synthesized as previously described in Sawai et al.\textsuperscript{10}

(2S, 3S)-Allyl-3-methyl-1-(9-Flurenylmethoxycarbonyl)aziridine-2-carboxylate (4)

The synthesis was based on methods modified from Vederas et al.\textsuperscript{10} Trityl protected aziridine 11 (65.5 g, 170.7 mmol) was dissolved in dichloromethane (100 ml) and methanol (100 ml). The solution was cooled to 0°C. Trifluoroacetic acid was added dropwise over 10 minutes. The solution was stirred for 10 minutes. The solvent was removed under reduced pressure. The resulting brown oil was dissolved in ether (500 ml) and water (500 ml). The organic layer was extracted with water (3 x 100 ml). The
combined water layers were cooled to 0°C. Sodium bicarbonate was added until the pH was basic. Ethyl acetate (1000 ml) and Fmoc-Cl (55.2 g) were added. The solution was stirred vigorously at room temperature for 20 hours. The organic and aqueous layers were separated and the aqueous layer was extracted with ethyl acetate (3 x 200 ml). The organic layer was washed with brine (3 x 500 ml), dried with magnesium sulfate, and filtered. The solvent was removed under reduced pressure. The resulting brown oil was purified by flash chromatography on silica gel (10% ethyl acetate in hexanes). The product was a clear oil (22.1 g, 51% yield). ESI-MS: Calc. [C_{22}H_{22}NO_{4}]^{+}=364.1544, Obsd. [C_{22}H_{22}NO_{4}]^{+}=364.1555; ^1H NMR (CDCl3, 500 mHz): δ 7.74 (d, J = 7.5 Hz, 2H), 7.57 (d, J = 7.5 Hz, 2H), 7.38 (t, J = 7.5 Hz, 2H), 7.30 (t, J = 7.5 Hz, 2H), 5.93 (ddt, J = 16.3, 10.6, 5.8 Hz, 1H), 5.36 (dq, J = 17.1, 1.3 Hz, 1H), 5.27 (dq, J = 10.4, 1.0 Hz, 1H), 4.74–4.61 (m, 2H), 4.41 (d, J = 7.0 Hz, 2H), 4.21 (t, J = 7.0 Hz, 2H), 4.21 (t, J = 7.0 Hz, 1H), 3.09 (d, J = 6.7 Hz, 1H), 2.80 (p, J = 7.5 Hz, 1H), 1.36 (d, J = 5.7 Hz, 3H); ^13C NMR (CDCl3, 125 mHz): δ 166.88, 161.62, 143.43, 141.34, 141.36, 131.55, 127.92, 127.17, 127.18, 125.13, 125.17, 120.16, 119.12, 68.47, 66.12, 46.90, 39.83, 39.07, 12.93.

(2R, 3R) Allyl-N-(9-Fluorenylmethoxycarbonyl)-3-methylcysteine carboxylate (5):

\[
\begin{align*}
\text{Fmoc} & \quad \text{SH} \\
\text{O} & \quad \text{O} \\
\end{align*}
\]

The synthesis was based on methods derived from Narayan and VanNieuwenhze. Aziridine 4 (13.8 g, 38.0 mmol, 1 eq) was dissolved in dry dichloromethane (300 ml) and cooled to 0°C. Boron trifluoride diethyl etherate (9.9 ml, 80.2 mmol, 2.1 eq) was dissolved in dichloromethane (0°C, 60 ml) under N2. Triphenylmethanethiol (37 g, 133.9 mmol, 3.5 eq) was added to the aziridine solution. The boron trifluoride solution was added dropwise to the aziridine solution over 5 minutes. The mixture was stirred at 0°C for 2.5 hours. The reaction was quenched with saturated aqueous sodium bicarbonate and stirred for an hour. The aqueous and organic layers were separated. The aqueous layer was extracted with dichloromethane. The combined organic layers were dried with magnesium sulfate and filtered. The solvent was removed under reduced pressure. The crude mixture was purified by flash chromatography on silica gel (5% ethyl acetate and 10% dichloromethane in hexanes). A crude yellow oil (11.9 g) was obtained. The oil was dissolved in triisopropylsilane (12 ml), dichloromethane (300 ml), and trifluoroacetic acid (30 ml). The reaction was stirred for 20.5 hours. The solvent was removed under reduced pressure and the reaction was purified by flash chromatography (5% dichloromethane, 5% ethyl acetate in hexanes). A white solid was obtained (11.3 g, 28.4 mmol, 74% yield). Two rotomers were observed in the ^1H NMR at room temperature. Peaks begin to coalesce at higher temperatures (45°C). A COSY was taken and the connectivity is consistent with the expected regiosomer. ESI-MS: Calc. [C_{22}H_{22}NO_{4}S]^{+}=398.1421, Obsd. [C_{22}H_{22}NO_{4}S]^{+}=398.1419; ^1H NMR (CDCl3, 500 mHz) δ 7.77 (d, J = 7.5 Hz, 2H), 7.66–7.58 (m, 2H), 7.46–7.36 (m, 4H), 5.91 (ddt, J = 16.3, 11.0, 5.7 Hz, 1H), 5.50 (d, J = 9.6 Hz, 1H), 5.40–5.12 (m, 3H), 4.70 (d, J = 5.6 Hz, 2H), 4.62–4.37 (m, 2H), 4.25 (t, J = 7.1 Hz, 1H), 1.39 (2d rotomers, J = 6.3 Hz, 3H); ^13C NMR (CDCl3, 125 mHz): δ 171.17, 169.14, 169.12, 156.77, 156.60, 143.81, 143.58, 141.53, 141.34, 141.32, 131.24, 127.78,
(2R, 3R) Allyl-2-(9-Fluorenyl methoxycarbonyl amino)-3-(2-tert-butyloxycarbonylaminoethylthio)butane carboxylate (15a):

Thiol 5 (0.55 g, 1.4 mmol, 1eq) was dissolved in ethyl acetate and saturated aqueous sodium bicarbonate (24 ml, 1:1). Tetrabutylammonium bisulfate (1.7 g) and excess solid sodium bicarbonate were added. Alkyl bromide 14a (0.6 g, 2.8 mmol, 2eq) was added. The reaction was stirred vigorously for 16 hours. The layers were separated, and the aqueous layer was extracted with ethyl acetate. The organic layers were dried with magnesium sulfate and filtered. The solvent was removed under reduced pressure. The crude product was purified by flash chromatography on silica gel (10-20% ethyl acetate in hexanes). The product was a clear oil (0.28 g, 38% yield). EMM-MS: Calc. [C\textsubscript{29}H\textsubscript{37}N\textsubscript{2}O\textsubscript{6}S]\textsuperscript{+}=541.2367, Obsd. [C\textsubscript{29}H\textsubscript{37}N\textsubscript{2}O\textsubscript{6}S]\textsuperscript{+}=541.2371; \textsuperscript{1}H NMR (CDCl\textsubscript{3}, 500 MHz): \begin{align*}
\delta & \quad 7.77 \ (d, \ J= 7.5 \ Hz, 2H), 7.62 \ (d, \ J= 7.1 \ Hz, 2H), 7.41 \ (t, \ J= 7.3 \ Hz, 2H), 7.32 \\
& \quad \ (t, \ J= 7.2 \ Hz, 2H), 5.94 \ (ddt, \ J = 16.5, 11.2, 5.9 \ Hz, 1H), 5.62 \ (d, \ J= 8.9 \ Hz, 1H), 5.38 \\
& \quad (d, \ J= 17.2 \ Hz, 1H), 5.29 \ (d, \ J= 10.4 \ Hz, 1H), 4.88 \ (s, 1H), 4.68 \ (d, \ J= 5.6 \ Hz, 2H), \\
& \quad 4.60 \ (dd, \ J= 9.2, 3.1 \ Hz, 1H), 4.46-4.33 \ (m, 2H), 4.25 \ (t, \ J= 7.1 \ Hz, 1H), 3.47-3.35 \ (m, 1H), 3.35-3.20 \ (m, 2H), 2.64 \ (ddt, \ J= 19.7, 13.1, 6.6 \ Hz, 2H), 1.45 \ (s, 9H), 1.37 \ (d, \ J= 7.0 \ Hz, 3H) \\
& \quad 13\text{C}-\text{NMR} \ (CDCl\textsubscript{3}, 125 \text{mHz}): \delta \quad 170.53, 156.62, 155.92, 144.05, 143.87, \\
& \quad 141.52, 131.49, 127.95, 127.31, 125.35, 120.22, 120.21, 119.76, 79.77, 75.52, 66.66, \\
& \quad 58.80, 47.35, 42.91, 40.12, 31.95, 28.60, 19.94.
\end{align*}

(2R, 3R) Allyl-2-(9-Fluorenyl methoxycarbonyl amino)-3-(2-tert-butyloxycarbonylpropylthio)butane carboxylate (15b):

Thiol 5 (0.55 g, 1.38 mmol) was dissolved in EtOAc: sat. aq. NaHCO\textsubscript{3} (1:1). Alkyl bromide 14b (0.46 ml, 2.76 mmol) was added. Tetrabutylammonium bisulfate (1.7 g) was added. The mixture was stirred vigourously for 16 hours. The aqueous and organic layers were separated, and the aqueous layer was extracted with ethyl acetate. Combined organic layers were dried with magnesium sulfate and filtered. Solvent was removed under reduced pressure. Crude product was purified by flash chromatography on silica gel (10% ethyl acetate in hexanes). The product was a clear oil (0.3 g, 0.571 mmol, 41% yield). ESI-MS: Calc. [C\textsubscript{29}H\textsubscript{36}NO\textsubscript{6}S]\textsuperscript{+}=526.2258, Obsd. [C\textsubscript{29}H\textsubscript{36}NO\textsubscript{6}S]\textsuperscript{+}=526.2242; \textsuperscript{1}H
NMR (500 MHz, CDCl$_3$) $\delta$ 7.77 (d, $J = 7.5$ Hz, 2H), 7.68 – 7.58 (m, 2H), 7.36 (dt, $J = 41.8$, 7.4 Hz, 4H), 5.94 (ddt, $J = 16.5$, 10.9, 5.9 Hz, 1H), 5.70 (d, $J = 9.3$ Hz, 1H), 5.38 (d, $J = 16.5$ Hz, 1H), 5.28 (d, $J = 10.3$ Hz, 1H), 4.74-4.61 (m, 1H), 4.59 (dd, $J = 9.3$, 3.1 Hz, 1H), 4.46 – 4.35 (m, 2H), 4.26 (t, $J = 7.3$ Hz, 1H), 3.47 (qd, $J = 7.0$, 3.2 Hz, 1H), 2.81-2.61 (m, 2H), 2.49 (t, $J = 7.2$ Hz, 2H), 1.46 (s, 9H), 1.37 (d, $J = 7.1$ Hz, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 141.30, 131.34, 127.71, 127.09, 119.98, 119.65, 81.04, 67.30, 66.37, 58.64, 47.14, 42.90, 35.95, 28.09, 26.49, 19.70.

(2R, 3R) 2-(9-Fluorenylethoxycarbonylamino)-3-(2-tert-butyloxy carbonylaminoethythio)butane carboxylic acid (6a):

Thioether 15a (0.28 g, 0.52 mmol) was dissolved in methanol (20 ml). Ammonium formate (2g) was added and the solution was stirred under N$_2$ for 10 minutes. Palladium on carbon (0.3g, 10% loading by weight, wet support) was added and the reaction was stirred overnight. The reaction was filtered through celite and the solvent was removed under reduced pressure. The product was a white solid (0.3 g, quantitative yield). ESI-MS: Calc. [C$_{26}$H$_{33}$N$_2$O$_6$S]$^+$ = 501.2054, Obsd. [C$_{26}$H$_{33}$N$_2$O$_6$S]$^+$ = 501.2063; Melting Point: 112.8-116.6°C; $^1$H NMR (CD$_2$OD, 500 mHz): $\delta$ 7.70 (d, $J = 7.6$ Hz, 2H), 7.64 – 7.55 (m, 2H), 7.29 (t, $J = 7.5$ Hz, 2H), 7.21 (t, $J = 7.5$ Hz, 2H), 4.32-4.24 (m, 2H), 4.16 (t, $J = 7.0$ Hz, 1H), 3.29-3.21 (m, 2H), 3.17-3.07 (m, 2H), 2.49 (t, $J = 7.2$ Hz, 2H), 1.46 (s, 9H), 1.37 (d, $J = 7.1$ Hz, 3H); $^{13}$C NMR (CD$_2$OD, 125 mHz): $\delta$ 172.37, 157.31, 143.91, 143.72, 141.17, 127.38, 126.78, 124.90, 119.50, 78.75, 66.76, 58.87, 42.25, 40.05, 30.84, 27.35, 18.73.

(2R, 3R) 2-(9-Fluorenylethoxycarbonylamino)-3-(2-tert-butyloxy carbonylamino ethylthio)butane carboxylic acid (6b):

Thioether 15b (0.61g, 1.2 mmol) was dissolved in methanol (100 ml). Ammonium formate (2g) was added and the solution was stirred under N$_2$ for 10 minutes. Palladium on carbon (0.3g , 10% loading by weight, wet support) was added, and the mixture was stirred overnight. The mixture was filtered through celite, and the solvent was removed under reduced pressure. The product was a white solid (0.36g, 0.75 mmol, 65% yield). ESI-MS: Calc. [C$_{26}$H$_{31}$NO$_6$SNH$_4$]$^+$ = 503.2211, Obsd. [C$_{26}$H$_{31}$NO$_6$SNH$_4$]$^+$ = 503.2200;
[α]D25 = +18 (c 0.5, MeOH); Melting Point: 131.5-133.1°C; 1H NMR (CD3OD, 500 mHz):
δ 7.80 (d, J = 7.5 Hz, 2H), 7.72 – 7.66 (m, 2H), 7.39 (t, J = 7.5 Hz, 2H), 7.31 (t, J = 7.5 Hz, 2H), 4.45 – 4.30 (m, 3H), 4.25 (t, J = 6.9 Hz, 1H), 3.42 – 3.33 (m, 1H), 2.85-2.72 (m, 2H), 2.51 (t, J = 7.0 Hz, 2H), 1.45 (s, 9H), 1.32 (d, J = 7.1 Hz, 3H). 13C NMR (CD3OD, 125 mHz): δ 173.74, 173.05, 158.72, 145.32, 145.14, 142.60, 128.79, 128.19, 126.33, 126.30, 120.93, 82.04, 68.14, 60.19, 43.79, 37.07, 28.34, 27.53, 20.04, 18.79.

Notes on Aziridine Ring Opening Stereochemistry

Scheme 3. Selected Aziridine Ring Opening Examples from the Literature13

Aziridine ring openings under Lewis acid conditions are known to proceed with high stereoselectivity in a predictable manner.14 The stereochemistry of the products of the ring opening reactions we conducted is assigned based on closely related ring openings in the literature for which the absolute stereochemistry of the final product has been established (Scheme 3).13
N-4-Nitrobenzoyl carboxamides:

Amino acid derivatives were synthesized by standard Fmoc solid phase peptide synthesis. For amino acid derivatives of Ile, Thr, Gly, Glu, Lys, and TS', protected amino acids were couple to Chemmatrix rink amide resin on a 100 µmol scale with 4 eq PyBOP ((benzotriazol-1-yloxy)tripyrrolidinophosphoniumhexafluorophosphate), 4 eq Cl-HOBt (1-hydroxy-6-chloro-benzotriazole), and 8 equivalents diisopropylethylamine (DIEA) in 4 ml DMF for 40 minutes. Resin was rinsed with DMF (3x10 ml) and resin was stirred in 20% piperidine in DMF (4 ml) for 15 minutes. Resin was rinsed with DMF (3x10 ml). Added 4 eq 4-nitrobenzoyl chloride and 8 eq diisopropylethylamine in DMF and stirred for 1 hour. Rinsed resin with DMF (3x10 ml) and DCM (3x10 ml). Cleaved amino acid from resin with 8 ml trifluoroacetic acid (TFA), 200 µl water, and 200 µl triisopropylsilane for 1 hour. Diluted resulting solution with water and acetonitrile and purified on a Shimadzu semiprep HPLC with a gradient acetonitrile and water. Fraction with product were combined and the solvent was removed under reduced pressure. For the TS' derivative, the synthesis was done as described above with the following modifications. The synthesis was done on a 5 µmol scale and only 1 eq of monomer, 1 eq PyBOP, 1 eq Cl-HOBt, and 2 eq DIEA in 1 ml DMF. The coupling was done on a CEM microwave for 12 minutes at 70°C. All amino acid derivatives were characterized by NMR and ESI-MS.

4-Nitrobenzoyl isoleucine carboxamide. ESI-MS: Calc. [C$_{13}$H$_7$N$_3$O$_4$Na]$^+$ = 302.1112, Obsd. [C$_{13}$H$_7$N$_3$O$_4$Na]$^+$ = 302.1102; $^1$H NMR (CD$_3$OD, 500 mHz): δ 8.24 (d, $J = 8.9$ Hz, 2H), 7.96 (d, $J = 8.9$ Hz, 2H), 4.37 (d, $J = 8.1$ Hz, 1H), 1.99 - 1.79 (m, 1H), 1.59-1.51 (multiplet, 1H), 1.26 - 1.11 (m, 1H), 0.94 (d, $J = 6.8$ Hz, 3H), 0.87 (t, $J = 7.4$ Hz, 3H); $^{13}$C NMR (CD$_3$OD, 125 mHz): 176.28, 168.50, 151.25, 141.12, 130.08, 124.79, 120.53, 74.89, 59.87, 37.98, 26.36, 16.12, 11.46.

4-Nitrobenzoyl threonine carboxamide. ESI-MS: Calc. [C$_{11}$H$_{13}$N$_3$O$_5$Na]$^-$ = 290.0748, Obsd. [C$_{11}$H$_{13}$N$_3$O$_5$Na]$^-$ = 290.0755; $^1$H NMR (500 MHz, CD$_3$OD): δ 8.35 (d, $J = 8.9$ Hz, 1H), 8.11 (d, $J = 8.9$ Hz, 1H), 4.57 (d, $J = 4.0$ Hz, 1H), 4.29 (qd, $J = 6.4$, 4.1 Hz, 1H), 1.26 (d, $J = 6.4$ Hz, 3H); $^{13}$C NMR (126 MHz, MeOD): δ 175.15, 168.47, 151.36, 141.12, 130.11, 124.79, 68.67, 60.75, 20.53.

[Please change titles below.]

4-nitrobenzoyl glycine carboxamide. ESI-MS: Calc. [C$_9$H$_8$N$_3$O$_4$Na]$^-$ = 246.0486, Obsd. [C$_9$H$_8$N$_3$O$_4$Na]$^-$ = 246.0478; $^1$H NMR (400 MHz, D$_2$O) δ 8.30 (d, $J = 8.8$ Hz, 2H), 8.04 (d, $J = 9.2$ Hz, 2H), 7.87 (t, 1H), 3.70 (s, 2H).
4-nitrobenzoyl glutamic acid carboxamide. ESI-MS: Calc. [C\textsubscript{12}H\textsubscript{13}N\textsubscript{3}O\textsubscript{6}Na]\textsuperscript{+}=318.0697, Obsd. [C\textsubscript{12}H\textsubscript{13}N\textsubscript{3}O\textsubscript{6}Na]\textsuperscript{+}=318.0696; \textsuperscript{1}H NMR (500 MHz, Methanol-d4) δ 8.24 (d, J = 8.8 Hz, 2H), 8.00 (d, J = 8.9 Hz, 2H), 4.59 – 4.42 (m, 1H), 2.40 (t, J = 7.6 Hz, 2H), 2.26 – 2.07 (m, 1H), 2.05 - 1.97 (m, J = 14.3, 9.2, 7.0 Hz, 1H); \textsuperscript{13}C NMR (126 MHz, Methanol-d4) δ 176.78, 176.36, 168.41, 151.31, 141.10, 130.13, 124.72, 54.92, 31.60, 28.39.

4-nitrobenzoyl thioether anionic threonine carboxamide. ESI-MS: Calc. [C\textsubscript{14}H\textsubscript{17}N\textsubscript{3}O\textsubscript{6}SNa]\textsuperscript{+}=378.0731, Obsd. [C\textsubscript{14}H\textsubscript{17}N\textsubscript{3}O\textsubscript{6}SNa]\textsuperscript{+}=378.0734; \textsuperscript{1}H NMR (500 MHz, Methanol-d4): δ 8.26 (d, J = 8.9 Hz, 2H), 7.99 (d, J = 8.9 Hz, 2H), 4.62 (d, J = 6.9 Hz, 1H), 3.30 (quint, J = 7.0 Hz, 1H), 2.87 – 2.71 (m, 2H), 2.62 – 2.41 (m, 2H), 1.32 (d, J = 7.0 Hz, 3H); \textsuperscript{13}C NMR (126 MHz, MeOD): δ 175.71, 174.57, 168.25, 141.21, 130.14, 124.79, 59.06, 43.75, 35.71, 26.72, 19.62.

4-nitrobenzoyl lysine carboxamide: ESI-MS: Calc. [C\textsubscript{13}H\textsubscript{19}N\textsubscript{4}O\textsubscript{4}]\textsuperscript{+}=295.1401, Obsd. [C\textsubscript{13}H\textsubscript{19}N\textsubscript{4}O\textsubscript{4}]\textsuperscript{+}=295.1396; \textsuperscript{1}H NMR (500 MHz, Methanol-d4): δ 8.25 (d, J = 8.9 Hz, 2H), 8.00 (d, J = 8.9 Hz, 2H), 4.51 (dd, J = 9.0, 5.4 Hz, 1H), 2.87-2.84 (m, 2H), 1.97 – 1.84 (m, 1H), 1.77 (dt, J = 14.1, 9.4, 5.2 Hz, 1H), 1.65 (tt, J = 14.7, 6.5 Hz, 2H), 1.56 – 1.31 (m, 2H); \textsuperscript{13}C NMR (126 MHz, MeOD): δ 176.64, 168.42, 151.34, 141.07, 130.12, 124.74, 54.99, 40.68, 32.64, 28.30, 24.19.

4-nitrobenzoyl thioether cationic threonine carboxamide: ESI-MS: Calc. [C\textsubscript{13}H\textsubscript{19}N\textsubscript{4}O\textsubscript{4}S]\textsuperscript{+}=327.1122, Obsd. [C\textsubscript{13}H\textsubscript{19}N\textsubscript{4}O\textsubscript{4}S]\textsuperscript{+}=327.1121; \textsuperscript{1}H NMR (500 MHz, Deuterium Oxide) δ 8.37 (d, J = 9.2 Hz, 2H), 8.00 (d, J = 8.4 Hz, 2H), 3.24 (t, J = 6.9 Hz, 1H), 3.04 – 2.86 (m, 2H), 3.54 – 3.46 (m, 1H), 1.43 (d, J = 6.6 Hz, 3H).
General. Dilution Studies were performed for all peptides in NMR buffer (100 mM sodium acetate in 9:1 H$_2$O:D$_2$O at pH=3.8) at 4°C to check for aggregation. The most concentrated samples were the concentration used for acquisition of the 2D NMR spectra. Water suppression was performed with a presaturation pulse sequence as described in supplementary section 4. Where needed, baselines were corrected by manually defining baseline regions. In all cases, spectra of the concentrated and diluted peptide were the same, which indicates that the peptide aggregation state did not change over this concentration range. We assume that each peptide was therefore monomeric (i.e., not self-associated) throughout the range. All parent peptides were previously characterized by sedimentation equilibrium analytical ultracentrifugation experiments.\textsuperscript{1b,2-4} All unfolded analogues are diastereomers of the peptides of interest with LPro instead of DPro. See supplementary section 1 for further explanation. All cyclic peptides, used to represent the fully populated β-hairpin, have the sequence of interest cyclized with a DPro-Gly turn (see main text Figure 3 for an example). For further sequence information refer to supplementary section 1.

Figure S33. Dilution Study of Peptide II, Arg-Tyr-Val-Glu-Val-DPro-Gly-Orn-TO’-Ile-Leu-Gln-NH$_2$
**Figure S34.** Dilution study of cyclic analogue of peptide II (fully folded control), Arg-Tyr-Val-Glu-Val-dPro-Gly-Orn-TO^+^-Ile-Leu-Gln-dPro-Gly

![Dilution study of cyclic analogue of peptide II](image)

**Figure S35.** Dilution study of lPro diasteromer of II (unfolded control), Arg-Tyr-Val-Glu-Val-lPro-Gly-Orn-TO^+^-Ile-Leu-Gln-NH₂

![Dilution study of lPro diasteromer of II](image)
Figure S36. Dilution study of peptide III, Arg-Tyr-Val-Glu-Val-dPro-Gly-Orn-TS'-Ile-Leu-Leu-Gln-NH₂

Figure S37. Dilution study of cyclic analogue of peptide III (fully folded control) Arg-Tyr-Val-Glu-Val-dPro-Gly-Orn-TS'-Ile-Leu-Gln-dPro-Gly
**Figure S38.** Dilution study of l-Pro diasteromer of III (unfolded control), Arg-Tyr-Val-Glu-Val-l-Pro-Gly-Orn-TS'-Ile-Leu-Gln-NH$_2$

![Dilution study of l-Pro diasteromer of III](image)

**Figure S39.** Dilution study of peptide IV, Arg-Tyr-Val-TS'-Val-dPro-Gly-Orn-Lys-Ile-Leu-Gln-NH$_2$

![Dilution study of peptide IV](image)
**Figure S40.** Dilution study of cyclic analogue of IV (fully folded control), Arg-Tyr-Val-TS-Val-dPro-Gly-Orn-Lys-Ile-Leu-Gln-dPro-Gly

![Cyclic analogue of IV](image)

---

**Figure S41.** Dilution study of L-Pro diasteromer of IV (unfolded control), Arg-Tyr-Val-TS-Val-L-Pro-Gly-Orn-Lys-Ile-Leu-Gln-NH$_2$

![L-Pro diasteromer of IV](image)
Figure S42. Dilution study of peptide XIII, Thr-TO^-Arg-Tyr-Val-Glu-Val-L-Pro-Gly-Orn-Lys-Ile-Leu-Gln-Thr-Thr-NH₂

Figure S43. Dilution study of peptide XIV, Thr-TS^-Arg-Tyr-Val-Glu-Val-L-Pro-Gly-Orn-Lys-Ile-Leu-Gln-Thr-Thr-NH₂
Figure S44. Dilution study of parent peptide V, Met-Gln-Ile-Phe-Val-Lys-Ser-dPro-Gly-Lys-Thr-Ile-Thr-Leu-Lys-Val-NH$_2$

![Dilution study of parent peptide V](image)

Figure S45. Dilution study of LPro diasteromer of V (unfolded control), Met-Gln-Ile-Phe-Val-Lys-Ser-LPro-Gly-Lys-Thr-Ile-Thr-Leu-Lys-Val-NH$_2$

![Dilution study of LPro diasteromer of V](image)
**Figure S46.** Dilution study of peptide VI, Met-Gln-Ile-Phe-Val-Lys-Ser-dPro-Gly-Lys-Thr-Ile-Thr-Leu-TO\(^+\)-Val-NH\(_2\)

![Peptide VI Dilution Study](image)

**Figure S47.** Dilution study of peptide VII, Met-Gln-Ile-Phe-Val-Lys-Ser-dPro-Gly-Lys-Thr-Ile-Thr-Leu-TS\(^+\)-Val-NH\(_2\)

![Peptide VII Dilution Study](image)
**Figure S48.** Dilution study of peptide X, Met-Gln-TO\(^+\)-Phe-Val-Lys-Ser-dPro-Gly-Lys-Thr-Ile-Thr-Leu-TO\(^+\)-Val-NH\(_2\).

**Figure S49.** Dilution study of peptide XI, Met-Gln-TS\(^+\)-Phe-Val-Lys-Ser-dPro-Gly-Lys-Thr-Ile-Thr-Leu-TO\(^+\)-Val-NH\(_2\).
**Figure S50.** Dilution study of peptide IX, Met-Gln-TS-Phe-Val-Lys-Ser-dPro-Gly-Lys-Thr-Ile-Thr-Leu-TO\(^+-\)Val-NH\(_2\)

![Dilution study of peptide IX](image1)

---

**Figure S51.** Dilution study of peptide VIII, Met-Gln-Glu-Phe-Val-Lys-Ser-dPro-Gly-Lys-Thr-Ile-Thr-Leu-TO\(^+-\)Val-NH\(_2\)

![Dilution study of peptide VIII](image2)
Figure S52. Dilution study of peptide XVII, Arg-Trp-Gln-Tyr-Val-dPro-Gly-Lys-Phe-TO'-Val-Gln-NH₂

Figure S53. Dilution study of peptide XVI, Arg-Trp-Gln-Tyr-Val-dPro-Gly-TO'-Phe-Thr-Val-Gln-NH₂
Supplementary Section 12
Peptide Purity Check

**Figure S54.** Purity check of peptide II, Arg-Tyr-Val-Glu-Val-dPro-Gly-Orn-TO'-Ile-Leu-Gln-NH₂

**Figure S55.** Purity check of cyclic analogue of II, Arg-Tyr-Val-Glu-Val-dPro-Gly-Orn-TO'-Ile-Leu-Gln-dPro-Gly

**Figure S56.** Purity check of LPro diastereomer of II, Arg-Tyr-Val-Glu-Val-LPro-Gly-Orn-TO'-Ile-Leu-Gln-NH₂
Figure S57. Purity check of peptide III, Arg-Tyr-Val-Glu-Val-dPro-Gly-Orn-TS'-Ile-Leu-Gln-NH₂

Figure S58. Purity check of cyclic analogue of III, Arg-Tyr-Val-Glu-Val-dPro-Gly-Orn-TS'-Ile-Leu-Gln-dPro-Gly

Figure S59. Purity check of LPro diastereomer of III (unfolded control), Arg-Tyr-Val-Glu-Val-LPro-Gly-Orn-TS'-Ile-Leu-Gln-NH₂

Figure S60. Purity check of peptide IV, Arg-Tyr-Val-TS'-Val-dPro-Gly-Orn-Lys-Ile-Leu-Gln-NH₂
**Figure S61.** Purity check of cyclic analogue of peptide IV (fully folded control), Arg-Tyr-Val-TS-Val-dPro-Gly-Orn-Lys-Ile-Leu-Gln-dPro-Gly

![Graph](image1)

**Figure S62.** Purity check of lPro diastereomer IV (unfolded control), Arg-Tyr-Val-TS'-Val-lPro-Gly-Orn-Lys-Ile-Leu-Gln-NH₂

![Graph](image2)

**Figure S63.** Purity check of peptide XIII, Thr-T'O'-Arg-Tyr-Val-Glu-Val-lPro-Gly-Orn-Lys-Ile-Leu-Gln-Thr-Thr-NH₂

![Graph](image3)

**Figure S64.** Purity check of peptide XIV, Thr-T'S'-Arg-Tyr-Val-Glu-Val-lPro-Gly-Orn-Lys-Ile-Leu-Gln-Thr-Thr-NH₂

![Graph](image4)
**Figure S65.** Purity check of parent peptide V, Met-Gln-Ile-Phe-Val-Lys-Ser-dPro-Gly-Lys-Thr-Ile-Thr-Leu-Lys-Val-NH\textsubscript{2}

![Graph](image)

**Figure S66.** Purity check of L-Pro diastereomer of V (unfolded control), Met-Gln-Ile-Phe-Val-Lys-Ser-L-Pro-Gly-Lys-Thr-Ile-Thr-Leu-Lys-Val-NH\textsubscript{2}

![Graph](image)

**Figure S67.** Purity check of peptide VI, Met-Gln-Ile-Phe-Val-Lys-Ser-dPro-Gly-Lys-Thr-Ile-Thr-Leu-TO\textsuperscript{+}-Val-NH\textsubscript{2}

![Graph](image)

**Figure S68.** Purity check of peptide VII, Met-Gln-Ile-Phe-Val-Lys-Ser-dPro-Gly-Lys-Thr-Ile-Thr-Leu-TS\textsuperscript{-}-Val-NH\textsubscript{2}

![Graph](image)
**Figure S69.** Purity check of peptide X, Met-Gln-TO'-Phe-Val-Lys-Ser-dPro-Gly-Lys-Thr-Ile-Thr-Leu-TO'-Val-NH₂

![Graph of Figure S69](image)

**Figure S70.** Purity check of peptide XI, Met-Gln-TS'-Phe-Val-Lys-Ser-dPro-Gly-Lys-Thr-Ile-Thr-Leu-TO'-Val-NH₂

![Graph of Figure S70](image)

**Figure S71.** Purity check of peptide IX, Met-Gln-TS'-Phe-Val-Lys-Ser-dPro-Gly-Lys-Thr-Ile-Thr-Leu-TO'-Val-NH₂

![Graph of Figure S71](image)

**Figure S72.** Purity check of peptide VIII, Met-Gln-Glu-Phe-Val-Lys-Ser-dPro-Gly-Lys-Thr-Ile-Thr-Leu-TO'-Val-NH₂

![Graph of Figure S72](image)
**Figure S73.** Purity check of peptide XVII, Arg-Trp-Gln-Tyr-Val-dPro-Gly-Lys-Phe-TO'-Val-Gln-NH₂

![Graph](image1)

**Figure S74.** Purity check of peptide XVI, Arg-Trp-Gln-Tyr-Val-dPro-Gly-TO'-Phe-Thr-Val-Gln-NH₂

![Graph](image2)
Compound 4 in CDCl₃
500 mHz NMR, ¹³C-NMR
Referenced to CDCl₃ (77.2 ppm)
Compound 5 in CDCl₃
400 mHz NMR, $^{13}$C-NMR
Referenced to TMS (0 ppm)
Compound 5 in CDCl₃
500 mHz NMR, ¹³C-NMR
Referenced to CDCl₃ (77.2 ppm)
Compound 5 in CDCl$_3$

600 mHz NMR, COSY

Referenced to TMS (0 ppm)
Compound 5 in CDCl₃
600 mHz NMR, COSY
Referenced to TMS (0 ppm)
Compound 15a in CDCl$_3$

500 mHz NMR, $^{13}$C-NMR

Referenced to CDCl$_3$ (77.2 ppm)
Compound 15b in CDCl₃
500 MHz NMR, ¹H-NMR

\begin{align*}
\text{H} & 2.47 \\
\text{t} & 2.71 \\
\text{Bu} & 1.38 \\
\text{O} & 4.27 \\
\text{O} & 3.98 \\
\text{O} & 4.42 \\
\text{O} & 4.58 \\
\text{O} & 4.67 \\
\text{S} & 5.27 \\
\text{S} & 5.39 \\
\text{S} & 5.40 \\
\text{S} & 5.69 \\
\text{S} & 5.71 \\
\text{S} & 7.0 \text{2} \\
\text{S} & 7.39 \\
\text{S} & 7.40 \\
\text{S} & 7.42 \\
\text{S} & 7.61 \\
\text{S} & 7.63 \\
\text{S} & 7.64 \\
\text{S} & 7.76 \\
\text{S} & 7.8 \\
\end{align*}
Compound 15b in CDCl₃ 500 MHz NMR, ¹³C-NMR

- 0.00
- 1.970
- 26.49
- 38.09
- 35.95
- 42.90
- 47.14
- 58.64
- 66.37
- 67.30
- 81.04

9.36
9.98
12.518
12.70
12.71
13.134
14.130
14.370
14.389
15.47
170.35
170.89
Compound 6a in CD$_3$OD
500 mHz NMR, $^1$H-NMR
Referenced to CD$_3$OD
Compound 6a in CD$_3$OD
500 MHz NMR.$^{13}$C-NMR
Referenced to CD$_3$OD

- 20.14
- 28.76
- 32.25
- 41.46
- 43.66
- 60.28
- 68.18
- 80.16
- 120.93
- 126.32
- 128.19
- 128.80
- 142.60
- 145.14
- 145.33
- 158.73
- 173.79
Compound 6b in CD$_3$OD
500 MHz NMR, $^1$H-NMR
Referenced to CD$_3$OD

$^1$H-NMR (500 MHz, CD$_3$OD)

$^1$H-NMR spectra of compound 6b in CD$_3$OD.

Key signals:
- 1.33 ppm (multiplet, $f_1$)
- 2.18 ppm (singlet, $f_2$)
- 3.45 ppm (singlet, $f_3$)
- 4.4 ppm (singlet, $f_4$)

Additional signals at:
- 0.95 ppm
- 2.31 ppm
- 4.35 ppm
- 7.8 ppm

Structural formula of Fmoc-OBu-S-CHO

Note: The image contains detailed spectral data and structural information, which are essential for understanding the compound's properties.
Compound 6b in CD$_3$OD
500 mHz NMR, $^{13}$C-NMR
Referenced to CD$_3$OD
Compound B in CDCl$_3$
500 mHz NMR, $^1$H-NMR
Referenced to TMS
Compound 8 in CDCl₃
500 mHz NMR, ¹³C-NMR
Referenced to CDCl₃
Compound 1 in CDCl₃
500 MHz NMR, ¹H-NMR
Referenced to TMS at 0 ppm
Compound 1 in CDCl₃
500 mHz NMR, $^{13}$C-NMR
Referenced to TMS at 0 ppm
Compound 2 in CDCl₃, 400 MHz NMR, ¹H NMR, Referenced to TMS at 0 ppm.
Compound 2 in CDCl₃
500 MHz NMR, ¹³C-NMR
Compound 10 in CDCl$_3$
400 mHz NMR, $^1$H-NMR
Referenced to TMS at 0 ppm
Compound 10 in CDCl₃
500 MHz NMR, ¹³C-NMR

N
H
O
O
O
NHBoc

CDCl₃
Compound 3 in CD$_3$OD
500 mHz NMR, $^1$H-NMR
Referenced to Methanol
Compound 3 in CDCl₃
500 mHz NMR, ¹³C-NMR
4-benzoyl isoleucine carboxamide in CD$_3$OD

125 mHz NMR, $^{13}$C-NMR

Referenced to methanol
4-benzoyl threonine amide in CD$_3$OD
500 mHz NMR, $^1$H-NMR
Referenced to methanol
4 benzoyl threonine carboxamide in CD$_3$OD
125 mHz NMR, $^{13}$C-NMR
Referenced to methanol

- 20.53
- 60.75
- 68.67
- 124.79
- 130.11
- 141.12
- 151.36
- 168.47
- 175.15
4-benzoyl glycine amide in D2O
400 mHz NMR, $^1$H-NMR
Referenced to ethanol
4-benzoyl glutamic acid carboxamide in CD$_3$OD
500 mHz NMR, $^1$H-NMR
Referenced to methanol
4-benzoyl glutamic acid amide in CD$_3$OD
125 mHz NMR, $^{13}$C-NMR
Referenced to methanol
4-benzoyl thioether anionic threonine carboxyamide in CD$_3$OD
500 mHz NMR, $^1$H-NMR
Referenced to methanol
4-benzoyl thioether anionic threonine carboxamide in CD$_3$OD
125 mHz NMR, $^{13}$C-NMR
Referenced to methanol
4-benzoyl lysine carboxamide in CD$_3$OD

125 MHz NMR, $^{13}$C-NMR

Referenced to methanol

- 24.19
- 28.30
- 32.64
- 40.68
- 54.99
- 124.74
- 130.12
- 141.07
- 151.34
- 168.42
- 176.64
4-benzoyl thioether cationic threonine carboxamide in D2O
400 MHz NMR, 1H-NMR
Referenced to ethanol

\[ \text{Acetone} \]

\[ \text{Acetonitrile} \]

\[ \text{EtOH} \]
Supplementary Section 14
MALDI-MS

MALDI of peptide II in CHCA
Expected: 1430.8 m/z (M+1)
MALDI of cyclic peptide II in CHCA
Expected: 1567.9 m/z (M+1), 1589.9 m/z (M+Na)
MALDI of unfolded peptide II in CHCA
Expected: 1430.8 m/z (M+1)
MALDI of cyclic peptide III in CHCA
Expected: 1583.8 m/z (M+1), 1605.8 m/z (M+Na)
MALDI of unfolded peptide III in CHCA
Expected: 1446.8 m/z (M+1), 1468.8 m/z (M+Na)
MALDI of peptide IV in CHCA
Expected: 1474.8 m/z (M+1), 1496.8 m/z (M+Na)
MALDI of cyclic peptide IV in CHCA  
Expected: 1610.9 m/z (M+1)
MALDI of unfolded peptide IV in CHCA
Expected: 1474.8 m/z (M+1), 1496.8 m/z (M+Na)
MALDI of peptide XI in CHCA
Expected: 1862.1 m/z (M+1), 1884.1 m/z (M+Na)
MALDI of peptide XII in CHCA
Expected: 1878.1 m/z (M+1)
MALDI of peptide V in CHCA
Expected: 1789.1 m/z (M+1), 1811.1 m/z (M+Na)
MALDI of unfolded peptide V in CHCA
Expected: 1789.1 m/z (M+1), 1811.1 m/z (M+Na), 1827.1 (M+K)
MALDI of peptide VI in CHCA
Expected: 1805.1 (M+1), 1827.1 m/z (M+Na)
MALDI of peptide VII in CHCA
Expected: 1821.0 (M+1), 1843.0 m/z (M+Na)
MALDI of peptide X in CHCA
Expected: 1821.0 (M+1), 1843.0 m/z (M+Na)
MALDI of peptide XI in CHCA
Expected: 1836.1 (M+1), 1858.1 m/z (M+Na)
MALDI of peptide IX in CHCA
Expected: 1865.0 (M+1), 1887.0 m/z (M+Na)
MALDI of Peptide XIII in CHCA
Expected: 1805.0 (M+H), 1827.0 m/z (M+Na)
MALDI of peptide XVII in CHCA
Expected: 1550.9 m/z (M+1)
MALDI of peptide XVI in CHCA
Expected: 1523.8 (M+1)
Supplementary Section 15
NMR Structure Calculation Restraint Files

Restraints as entered in CNS for cyclic peptide III (Lys9 → TS+):

!!Sequential and Short Range NOEs

assign (residue 1 and name HA) (residue 2 and name HN) 2.2 0.4 0.9
assign (residue 1 and name HN) (residue 14 and name HN) 2.5 0.4 0.9
assign (residue 2 and name HA) (residue 3 and name HN) 1.2 0.4 0.9
assign (residue 3 and name HA) (residue 4 and name HN) 1.6 0.4 0.9
assign (residue 5 and name HA) (residue 6 and name HG#) 2.6 0.4 0.9
assign (residue 5 and name HA) (residue 6 and name HD#) 2.0 0.4 0.9
assign (residue 5 and name HG##) (residue 6 and name HG#) 2.4 0.4 0.9
assign (residue 6 and name HA) (residue 7 and name HN) 2.6 0.4 0.9
assign (residue 7 and name HN) (residue 8 and name HN) 2.5 0.4 0.9
assign (residue 8 and name HA) (residue 9 and name HN) 2.1 0.4 0.9
assign (residue 8 and name HB#) (residue 9 and name HG#) 2.4 0.4 0.9
assign (residue 9 and name HA) (residue 10 and name HN) 2.5 0.4 0.9
assign (residue 10 and name HA) (residue 11 and name HN) 2.2 0.4 0.9
assign (residue 11 and name HA) (residue 12 and name HN) 2.2 0.4 0.9
assign (residue 12 and name HA) (residue 13 and name HD2) 2.3 1.0 0.4
assign (residue 12 and name HA) (residue 13 and name HD1) 2.3 1.0 0.4

!!! Medium and Long Range NOEs

assign (residue 1 and name HN) (residue 12 and name HN) 2.6 0.4 0.9
assign (residue 1 and name HN) (residue 12 and name HE2#) 2.5 0.4 0.9
assign (residue 2 and name HB#) (residue 4 and name HG#) 2.6 0.4 0.9
assign (residue 2 and name HB#) (residue 9 and name HG2#) 2.4 0.4 0.9
assign (residue 2 and name HD#) (residue 9 and name HG2#) 2.4 0.4 0.9
assign (residue 2 and name HE#) (residue 9 and name HG2#) 2.9 0.4 0.9
assign (residue 2 and name HD#) (residue 10 and name HN) 2.7 0.4 0.9
assign (residue 2 and name HA) (residue 11 and name HA) 2.1 0.4 0.9
assign (residue 2 and name HE#) (residue 11 and name HG) 2.4 0.4 0.9
assign (residue 2 and name HE#) (residue 11 and name HD##) 4.1 0.4 0.9
assign (residue 2 and name HE#) (residue 11 and name HD##) 3.1 0.4 0.9
assign (residue 3 and name HN) (residue 10 and name HN) 2.1 0.4 0.9
assign (residue 4 and name HG#) (residue 9 and name HD1#) 2.2 0.4 0.9
assign (residue 4 and name HB#) (residue 9 and name HD1#) 2.5 0.4 0.9
assign (residue 5 and name HA) (residue 8 and name HN) 2.6 0.4 0.9
assign (residue 5 and name HG##) (residue 8 and name HG#) 2.6 0.4 0.9
assign (residue 11 and name HD##) (residue 14 and name HA#) 2.7 0.4 0.9

Restraints as entered in CNS for cyclic peptide III (Lys9 → TO+):
!!! Sequential and Short Range NOEs

assign (residue 1 and name HA) (residue 2 and name HN) 2.2 1.0 0.9
assign (residue 1 and name HB#) (residue 2 and name HN) 2.6 1.0 0.9
assign (residue 1 and name HN) (residue 14 and name HN) 2.6 1.0 0.9
assign (residue 1 and name HN) (residue 14 and name HA#) 2.8 1.0 0.9
assign (residue 2 and name HA) (residue 3 and name HN) 2.4 1.0 0.9
assign (residue 3 and name HA) (residue 4 and name HN) 2.2 1.0 0.9
assign (residue 4 and name HA) (residue 5 and name HN) 3.0 1.0 0.9
assign (residue 6 and name HA) (residue 7 and name HN) 2.6 1.0 0.9
assign (residue 7 and name HN) (residue 8 and name HN) 2.6 1.0 0.9
assign (residue 8 and name HN) (residue 14 and name HN) 2.6 1.0 0.9
assign (residue 9 and name HA) (residue 10 and name HN) 2.1 1.0 0.9
assign (residue 9 and name HB) (residue 10 and name HN) 2.9 1.0 0.9
assign (residue 10 and name HA) (residue 11 and name HN) 2.2 1.0 0.9
assign (residue 10 and name HB) (residue 11 and name HN) 1.8 1.0 0.9
assign (residue 11 and name HA) (residue 12 and name HN) 2.2 1.0 0.9
assign (residue 11 and name HD##) (residue 12 and name HN) 3.5 1.0 0.9

!!! Medium and Long Range NOEs

assign (residue 1 and name HN) (residue 11 and name HD##) 3.0 1.0 0.9
assign (residue 1 and name HN) (residue 12 and name HN) 2.7 1.0 0.9
assign (residue 2 and name HB1) (residue 9 and name HB) 2.9 1.0 0.9
assign (residue 2 and name HD#) (residue 10 and name HN) 2.8 1.0 0.9
assign (residue 2 and name HD#) (residue 11 and name HN) 3.1 1.0 0.9
assign (residue 2 and name HD#) (residue 11 and name HA) 2.9 1.0 0.9
assign (residue 2 and name HD#) (residue 11 and name HD##) 3.1 1.0 0.9
assign (residue 2 and name HE#) (residue 11 and name HB) 2.5 1.0 0.9
assign (residue 2 and name HE#) (residue 11 and name HD##) 2.9 1.0 0.9
assign (residue 5 and name HN) (residue 8 and name HN) 2.7 1.0 0.9
assign (residue 9 and name HB) (residue 11 and name HD##) 2.7 1.0 0.9
assign (residue 9 and name HD#) (residue 11 and name HD##) 2.7 1.0 0.9

!! Second Round

assign (residue 2 and name HD#) (residue 9 and name HB) 2.8 1.0 0.9
assign (residue 2 and name HD#) (residue 9 and name HG2#) 2.5 1.0 0.9
assign (residue 2 and name HE#) (residue 11 and name HB) 2.5 1.0 0.9

Restraints as entered in CNS for cyclic peptide IV (Glu4 \(\rightarrow\) TS):
!!Sequential and Short Range NOEs
assign (residue 1 and name HN) (residue 14 and name HN) 2.4 0.4 0.9
assign (residue 1 and name NH) (residue 14 and name HA1) 3.1 0.4 0.9
assign (residue 2 and name HN) (residue 2 and name HD#) 2.5 0.4 0.9
assign (residue 2 and name HB#) (residue 2 and name HD#) 2.4 0.4 0.9
assign (residue 2 and name HB#) (residue 2 and name HD#) 2.3 0.4 0.9
assign (residue 2 and name HB#) (residue 2 and name HE#) 3.2 0.4 0.9
assign (residue 2 and name HA) (residue 3 and name HN) 2.1 0.4 0.9
assign (residue 2 and name HA) (residue 3 and name HG##) 2.8 0.4 0.9
assign (residue 2 and name HB#) (residue 3 and name HD#) 2.7 0.4 0.9
assign (residue 2 and name HB#) (residue 3 and name HG##) 2.7 0.4 0.9
assign (residue 2 and name HB#) (residue 3 and name HD#) 2.6 0.4 0.9
assign (residue 2 and name HB#) (residue 3 and name HD#) 2.3 0.4 0.9
assign (residue 2 and name HB#) (residue 3 and name HA) 2.2 0.4 0.9

!!Medium and Long Range NOEs
assign (residue 1 and name HD#) (residue 3 and name HG##) 2.5 0.4 0.9
assign (residue 1 and name HN) (residue 11 and name HD##) 3.0 0.4 0.9
assign (residue 1 and name HN) (residue 12 and name HN) 2.6 0.4 0.9
assign (residue 2 and name HB#) (residue 9 and name HD#) 2.4 0.4 0.9
assign (residue 2 and name HB#) (residue 9 and name HE#) 3.1 0.4 0.9
assign (residue 2 and name HD#) (residue 9 and name HE#) 2.4 0.4 0.9
assign (residue 2 and name HE#) (residue 9 and name HE#) 3.0 0.4 0.9
assign (residue 2 and name HD#) (residue 10 and name HN) 2.8 0.4 0.9
assign (residue 2 and name HD#) (residue 11 and name HN) 2.7 0.4 0.9
assign (residue 2 and name HD#) (residue 11 and name HA) 2.3 0.4 0.9
assign (residue 2 and name HD#) (residue 11 and name HD##) 3.0 0.4 0.9
assign (residue 2 and name HD#) (residue 11 and name HD##) 3.0 0.4 0.9
assign (residue 2 and name HE#) (residue 11 and name HN) 2.8 0.4 0.9
assign (residue 2 and name HE#) (residue 11 and name HD##) 2.8 0.4 0.9
assign (residue 2 and name HE#) (residue 11 and name HD##) 2.9 0.4 0.9
assign (residue 2 and name HE#) (residue 11 and name HD##) 3.5 3.0 0
assign (residue 2 and name HE#) (residue 11 and name HD##) 2.6 0.4 0.9
assign (residue 2 and name HE#) (residue 11 and name HD##) 2.7 0.4 0.9
assign (residue 2 and name HD##) (residue 14 and name HA#) 2.7 0.4 0.9
assign (residue 2 and name HD##) (residue 14 and name HA#) 2.8 0.4 0.9

Supplementary Information References

(1) (a) Stanger, H. E.; Gellman, S. H. J. Am. Chem. Soc. 1998, 120, 4236. (b) Haque, T. S.; Gellman, S. H. J. Am. Chem. Soc. 1997, 119, 2303.

(2) Syud, F. A., Espinosa, J. F., Gellman, S. H. J. Am. Chem. Soc. 1999, 121, 11577.

(3) Stanger, H. E.; Syud, F. A.; Espinosa, J. F.; Giriat, I.; Muir, T.; Gellman, S. H. Proc. Natl. Acad. Sci. U. S. A. 2001, 98, 12019.

(4) Espinosa, J. F.; Gellman, S. H. Angew. Chem. Int. Ed. 2000, 39, 2330.

(5) Wishart, D. S., Sykes, B. D., Richards, F. M. Biochemistry 1992, 31, 1647.

(6) (a) Bothner-By A. A.; Stephens R. L.; Lee, J.; Warren, C. D.; Jeanloz, R. W.; J. Am. Chem. Soc. 1984; 106, 811. (b) Bax, A.; Davies, D. G.; J. Mag. Res, 1985; 65, 355.

(7) Goddard, T. D.; Kneller, D. G. Sparky 3; University of California: San Francisco; 1986.

(8) Wuthrich, K.; NMR of Proteins and Nucleic Acids; Wiley-Interscience: New York, 1986.

(9) Brunger, A. T.; Adams, P.D.; Clore, G. M.; Gros, P.; Grosse-Kunstleve, R.W.; Jiang, J. S.; Kuszewski, J.; Nilges, N.; Pannu, N.S.; Read, R. J.; Rice, L. M.; Simonson, T.; Warren, G. L. Acta Cryst. 1998, 54, 905.

(10) Liu, H.; Pattabiraman, V. R.; Vederas, J. C. Org. Lett. 2007, 9, 4211.

(11) Shoji, A.; Kuwahara, M.; Ozaki, H.; Sawai, H. J. Am. Chem. Soc. 2007, 129, 1456.

(12) Narayan, R. S.; VanNieuwenhze, M. S. Org. Lett. 2005, 7, 2655.

(13) (a) Liu, W.; Chan, A. S. H.; Liu, H.; Cochrane, S. A.; Vederas, J. C. J. Am. Chem. Soc. 2011, 133, 14216. (b) Tarrade, A.; Dauban, P.; Dodd, R. H. J. Org. Chem. 2003, 68, 9521.
(14) Tanner, D. *Angew. Chem. Int. Ed.* **1994**, *33*, 599.

(15) Lee, K.; Lee, H.; Slutsky, M. M.; Anderson, J. T.; Marsh, E. N. G. *Biochemistry* **2004**, *43*, 16277.

(16) Woll, M. G.; Hadley, E. B.; Mecozzi, S.; Gellman, S. H. *J. Am. Chem. Soc.* **2006**, *128*, 15932.