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Publication Date

DOI

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Abstract

Although β-hairpins are widespread in proteins, there is still no universal tool to coax any small peptide to adopt a β-hairpin conformation, regardless of sequence. Here, we report that δ-linked γ(*R*)-methyl-ornithine (δMeOrn) provides an improved β-turn template for inducing a β-hairpin conformation in peptides. We have developed a synthesis of protected δMeOrn as a building block suitable for use in Fmoc-based solid-phase peptide synthesis. The synthesis begins with L-leucine and affords gram quantities of the N^α-Boc-N^δ-Fmoc-γ(*R*)-methyl-ornithine building block. X-ray crystallography confirms that the δMeOrn turn unit adopts a folded structure in a macrocyclic β-hairpin peptide. CD and NMR spectroscopic experiments allow comparison of the δMeOrn turn template to the δ-linked ornithine (δOrn) turn template that our laboratory has previously introduced and also to the popular D-Pro-Gly turn template. Collectively, these studies demonstrate that the folding of the δMeOrn turn template is substantially better than that of δOrn and is comparable to D-Pro-Gly.

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δ-Linked γ(*R*)-methyl-ornithine (δMeOrn) provides an improved β-turn template for inducing a β-hairpin conformation in peptides.
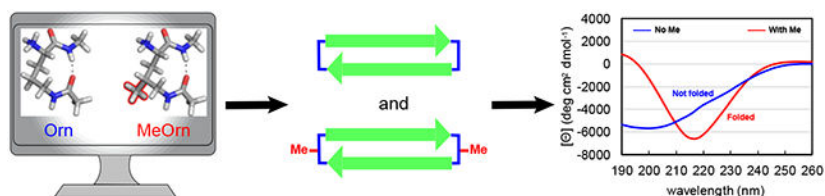
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Supporting information for this article is given via a link at the end of the document

Keywords

β-hairpin; β-turn; amino acid; peptidomimetics; peptides

Introduction

Although almost three decades have elapsed since initial reports of small peptides that fold to form β-hairpins in aqueous solution, there is still no universal tool to coax any small peptide to adopt a β-hairpin conformation, regardless of sequence. β-Hairpins occur widely in folded peptides and proteins and are defined by two hydrogen-bonded antiparallel β-strands connected by a short loop. Even though β-hairpins are nearly ubiquitous within proteins, most β-hairpins will not retain a folded structure in aqueous solution when excised from the protein. Instead, most peptides that fold into β-hairpins in aqueous solution require relatively specific sequences for the β-strands and loop. One approach to stabilizing β-hairpin formation in peptides involves replacing the loop region with a turn template.[1–8] The template mimics the loop and enforces proximity, hydrogen bonding, and an antiparallel alignment between the β-strands.

Early efforts to stabilize β-hairpins involved turn templates composed of either α-amino acids or other amino acid building blocks. A D-proline residue at the *i+1* position proved particularly effective for driving the formation of a β-turn. Balaram and co-workers first observed the D-Pro-L-Pro motif acting as a turn in the X-ray crystal structure of pivaloyl-D-Pro-L-Pro-L-Ala-*N*-methylamide, and later reported the crystal structure of a designed octapeptide containing a D-Pro-Gly sequence that folds as a β-hairpin.[9,10] Gellman and co-workers subsequently demonstrated that D-Pro-Gly is favorable for inducing a β-hairpin conformation in aqueous solution in several model peptide sequences.[11–13] Not all turn templates are composed of α-amino acids. Kelly and co-workers demonstrated that a dibenzofuran amino acid can induce–a β-sheet structure in analogues of gramicidin S.[14] A number of other cleverly designed templates based on aromatic and alkene scaffolds have also been described.[15–18]

Amongst the turn templates reported to date, the D-Pro-L-Pro and D-Pro-Gly turn templates have emerged as favorites for stabilizing useful β-hairpin peptides. Schneider and co-workers have developed a series of biomaterials based on MAX1, a β-hairpin peptide containing the D-Pro-L-Pro turn motif that self-assembles into a hydrogel.[19] Further investigations of MAX1 have revealed antibacterial properties for non-sterile injections and lead to other analogues with useful attributes.[20,21] Robinson and co-workers have applied the D-Pro-L-Pro turn to construct cyclic β-hairpin peptides inspired by natural antibiotics. These peptidomimetic antibiotics exhibit enhanced activity and have revealed lipopolysaccharide transport proteins as cellular targets in the outer membrane of Gram-negative bacteria.[22–24] Wetzel and co-workers have used the D-Pro-Gly turn motif to create polyglutamine-containing β-hairpin peptides as chemical models for the aggregation of polyglutamine-containing proteins in Huntington's disease.[25–26]

In 2003, our laboratory introduced δ-linked ornithine ($^{\delta}$Orn) as a new turn-forming unit to induce a β-hairpin conformation in peptides.[27] We have subsequently used the $^{\delta}$Orn

turn unit to stabilize a growing repertoire of macrocyclic β-hairpin peptides — containing sequences from Aβ,[28–30] α-synuclein,[31] IAPP,[32] and β2-microglobulin[33] — to provide insight into the structures of amyloid oligomers.[34] In the current study, we set out to improve the δOrn turn. Here, we report that introduction of a single methyl group at the γ-position with R stereochemistry substantially improves folding of the δOrn turn unit.

Results and Discussion

Design of an improved δ-linked ornithine turn.

We hypothesized that the introduction of a methyl group into the side chain of ornithine, at the right position and with the right stereochemistry, might limit the number of unfolded conformers and thus favor a folded β-turn conformation. We used Monte Carlo Stochastic Dynamics (MC/SD) calculations to guide the design of a methylated δOrn turn with improved folding properties.[35,36] We examined the equilibrium between the unfolded and folded states of the methylated ornithine derivatives shown in Figure 1 by MC/SD simulation in MacroModel using the MMFFs force field with GB/SA water solvation at 300 K. We explored the effect of methylation at the β-, γ-, and δ-positions and found that methylation at the γ-position with R stereochemistry shifts the equilibrium from 0.8% folded (for the unmethylated ornithine) to 20.3% folded — an enhancement of 2.08 kcal/mol (Figure S1). Molecular modeling studies suggest that other than enhanced folding, there should be little difference between the γ(R)-methyl-ornithine turn and the unmethylated ornithine turn, as the minimum-energy turn conformations (local minima) of the ornithine derivatives are virtually identical (Figure 2). These simulations provided us with the confidence to pursue the synthesis and study of turn formation by γ(R)-methyl-ornithine.

Synthesis of orthogonally protected γ(R)-methyl-ornithine.

We have developed a synthesis of orthogonally protected γ(R)-methyl-ornithine as a building block suitable for use in standard Fmoc-based solid-phase peptide synthesis. The synthesis begins with L-leucine and relies on the recently reported finding by Renata and co-workers that L-leucine can be chemoenzymatically oxidized to 4-hydroxyleucine **2** with high stereoselectivity and that the resulting product can be isolated as the Boc-protected lactone **3**.[37]

Ring opening of the lactone was achieved by treatment with *N,O*-dimethylhydroxylamine hydrochloride and trimethylaluminum to afford the Weinreb amide (Scheme 1). The liberated alcohol group was then converted to the corresponding mesylate using methanesulfonyl chloride, resulting in an overall two-step transformation with 71% yield of the mesylated intermediate **4**. This procedure was previously reported by the Renata group en route to (*2S,4R*)-N^α-Boc-methylproline, but the intermediate **4** was not isolated.[38]

Displacement of the mesylate group with sodium azide afforded the Weinreb protected azidoleucine **5** in 85% yield. The reaction was run at 70 °C to increase solubility of sodium azide in dimethylformamide and facilitate S_N2 displacement. Subsequent hydrolysis of the Weinreb amide with lithium hydroxide at 55 °C in a 1:1 mixture of water and THF furnished the Boc-azidoleucine intermediate **6** in a 93% yield. Reduction of the azide group to the

corresponding amine was then carried out by catalytic hydrogenation with H_2 and Pd/C, followed by Fmoc protection with Fmoc-OSu in a 1:1 mixture of water and THF to give N^α-Boc-N^δ-Fmoc-$\gamma(R)$-methyl-ornithine (**1**, Boc-MeOrn(Fmoc)-OH) in gram quantities and a yield of 57% over the two steps.

X-ray crystallographic structure of a macrocyclic β-hairpin peptide containing the $^\delta$MeOrn turn.

To confirm the stereochemistry and conformation of the $^\delta$MeOrn turn unit within a β-hairpin motif, we turned to X-ray crystallography. Our laboratory has previously reported the X-ray crystallographic structure of a β-hairpin peptide derived from β2-microglobulin (peptide **1a**, PDB 4P4Z).[33] Peptide **1a** is a macrocyclic β-hairpin peptide containing two heptapeptide strands linked by two $^\delta$Orn turn units. In the X-ray crystallographic structure of peptide **1a**, only one of the two $^\delta$Orn turn units (right side) adopts the characteristic hydrogen-bonded turn conformation shown in Figure 2A. The other $^\delta$Orn turn unit (left side) does not adopt this hydrogen-bonded conformation. To visualize the $^\delta$MeOrn turn unit and determine whether it adopts the hydrogen-bonded conformation hypothesized in Figure 2B, we prepared homologue **1b** and studied its structure by X-ray crystallography (PDB 7LIB).

We crystallized peptide **1b** using similar conditions to those used for peptide **1a** and determined the X-ray crystallographic structure at 1.1 Å. In the X-ray crystallographic structure, peptide **1b** adopts a well-defined macrocyclic β-hairpin conformation (Figure 3A). The $^\delta$MeOrn turn unit adopts the hypothesized hydrogen-bonded conformation, with the $\gamma(R)$-methyl group clearly visible in the electron density map (Figure 3B). The conformation of the $^\delta$MeOrn turn unit in peptide **1b** is particularly noteworthy, since the corresponding $^\delta$Orn turn unit in peptide **1a** does not adopt this well-defined hydrogen-bonded conformation (Figure S2). The improved folding of this turn in peptide **1b** corroborates the MC/SD prediction that the $^\delta$MeOrn turn unit is superior to the $^\delta$Orn turn unit.

Circular dichroism (CD) spectroscopic studies of a macrocyclic β-hairpin peptide containing the $^\delta$MeOrn turn.

To investigate the propensity of the $^\delta$MeOrn turn unit to induce β-hairpin formation in the solution state, we compared a macrocyclic β-hairpin peptide containing two $^\delta$MeOrn turn units to a homologue containing two $^\delta$Orn turn units by CD spectroscopy. Our laboratory has previously described the X-ray crystallographic structure of a β-hairpin peptide derived from the β-amyloid peptide Aβ (peptide **2a**).[28] Peptide **2a** contains a heptapeptide strand derived from Aβ$_{17–23}$ and a heptapeptide strand derived from Aβ$_{30–36}$, which are linked by two $^\delta$Orn turns to form a macrocycle. The CD spectrum of peptide **2a** exhibits an extended region of negative ellipticity below 250 nm, with a minimum at 202 nm and an additional small minimum at 192 nm (Figure 4). These features reflect a predominance of random coil structure in aqueous solution, even though the peptide adopts a β-hairpin conformation in the crystal state.

Replacement of the two $^\delta$Orn turn units with $^\delta$MeOrn turn units affords peptide **2b**. The CD spectrum of peptide **2b** shows a well-defined minimum centered at 216 nm, with a maximum positive ellipticity at ca. 194 nm (Figure 4). These features suggest a predominance of β-sheet character. The striking difference between the CD spectra of peptides **2a** and **2b** provides additional evidence that the $^\delta$MeOrn turn is more effective at inducing a β-hairpin conformation than the $^\delta$Orn turn.

NMR and CD spectroscopic studies of β-hairpin peptides containing d-Pro-Gly, $^\delta$Orn, and $^\delta$MeOrn turns.

To further evaluate the propensity of the $^\delta$MeOrn turn unit to induce β-hairpin formation in solution, we used NMR spectroscopy to compare a set of β-hairpin peptides containing D-Pro-Gly, $^\delta$Orn, and $^\delta$MeOrn turns. In 1998, Gellman and co-workers reported that a turn comprising D-Pro-Gly induces a β-hairpin conformation in peptide **3**, a 12-residue linear peptide.[12] We prepared peptides **4a** and **4b** as homologues of peptide **3** in which the D-Pro-Gly turn is replaced by $^\delta$Orn and $^\delta$MeOrn turn units. We used TOCSY and NOESY experiments in D_2O and in 90:10 H_2O:D_2O to assign key NOE crosspeaks associated with β-hairpin folding for each of the three peptides (Figure 5).

The NOEs represented in Figure 5 for peptides **3**, **4a**, and **4b** suggest that all three peptides adopt a folded conformation in aqueous solution. Peptides **3** and **4b** exhibit an extensive network of cross-strand NOEs associated with hydrogen-bonded β-hairpin formation. An expected NOE between the α-protons of Lys_9 and Glu_4 could not be observed in peptide **4b**, because the α-proton resonances occur at very similar chemical shifts (δ = 0.03 ppm). Unlike peptides **3** and **4b**, peptide **4a** exhibits only three key cross-strand NOEs, which suggests that it is less well-folded than peptides **3** and **4b**.

In addition to the cross-strand NOEs, each of the peptides also exhibits key NOEs associated with turn formation (Figure 5). Peptide **3** exhibits an NOE between the NH protons of Orn_8 and Gly_7. Peptide **4b** exhibits NOEs between the NH proton of Orn_8 and the δ-protons of $^\delta$MeOrn. Peptide **4b** also displays NOEs between the α-proton and δ-protons of $^\delta$MeOrn. Peptide **4a** only exhibits NOEs between the α-proton and δ-protons of $^\delta$Orn. The diastereotopic δ-proton resonances of the $^\delta$MeOrn turn in peptide **4b** are separated by 0.72 ppm, reflecting the formation of a well-defined turn conformation, in which the *pro-S* δ-proton is proximal to the Val_5 carbonyl group.[39] In contrast, the diastereotopic δ-proton resonances of the $^\delta$Orn turn in peptide **4a** are only separated by 0.14 ppm, reflecting the formation of a significantly less well-defined turn structure.

To further compare the folding of peptides **3**, **4a**, and **4b**, we examined the chemical shifts of the α-protons, which are widely used to probe the secondary structure of peptides and proteins.[40–44] The chemical shifts of α-protons in a β-sheet are typically more downfield than those of a random coil or an α-helix. The differences between the α-proton chemical shifts of peptides **3**, **4a**, and **4b** and those of a random coil are plotted in Figure 6. In this figure, residues 2–4 and 8–11 are grouped together, because they represent regions of the β-hairpin that can be compared in a meaningful fashion across peptides **3**, **4a**, and **4b**.

Residues 1, 5, and 12 are grouped separately, because they either terminate the β-hairpin are attached to the differing turn units.[45,46]

The difference in α-proton chemical shifts relative to random coil values for residues 2–3 and 8–11 of peptide **4b** are similar to those of peptide **3**, with all but residue 11 shifted substantially downfield. This downfield shifting reflects β-hairpin formation and suggests that both peptides exhibit comparable degrees of β-hairpin folding. The α-protons of Leu_{11} in peptides **3** and **4b** are shifted upfield by ca. 0.2 ppm, which may result from the proximity of the aromatic ring of Tyr_2. The α-proton of Glu_4 in peptide **3** is substantially downfield to that of peptide **4b** (δ = 0.30 ppm), suggesting subtle differences in the folded β-hairpin conformations of the two peptides.

The chemical shifts of the α-protons of peptide **4a** are similar to those observed for a random coil (Figure 6), suggesting that peptide **4a** does not form a well-folded β-hairpin. This result seems to contradict the NOEs observed for peptide **4a** (Figure 5) and may reflect that peptide **4a** adopts an ensemble of poorly folded β-hairpin conformations, with significant cross-strand proximity between Leu_{11} and Tyr_2 and between Lys_9 and Glu_4. Taken together, the NOE and chemical shift data suggest that peptide **4a** exhibits substantially less β-hairpin formation than peptides **3** and **4b**.

The CD spectrum of peptide **3** exhibits a well-defined negative ellipticity centered at 215 nm and a maximum positive ellipticity centered at 201 nm, reflecting a β-sheet-like conformation (Figure 7). The CD spectrum of peptide **4b** has a broader minimum at ca. 205–220 nm and a second minimum centered at 196 nm, indicating a mixture of β-sheet and random coil conformations. The CD spectrum of peptide **4a** displays a strong minimum at 197 nm, with a small inflection at ca. 217 nm, suggesting a predominance of random coil conformation. These observations corroborate the substantial improvement of the δMeOrn turn over the δOrn turn in inducing β-hairpin formation in peptides, while also suggesting that the D-Pro-Gly turn is superior to the δMeOrn turn.

NMR and CD spectroscopic studies of a second set of β-hairpin peptides.

We further investigated the effect of the δMeOrn turn with a second β-hairpin peptide reported by the Gellman group, peptide **5**.[13] We prepared peptides **6a** and **6b** as homologues of peptide **5** in which the D-Pro-Gly turn is replaced by δOrn and δMeOrn turn units. The NOEs represented in Figure 8 for peptides **5**, **6a**, and **6b** suggest that all three peptides adopt a folded conformation in aqueous solution. All three peptides exhibit an extensive network of cross-strand NOEs associated with hydrogen-bonded β-hairpin formation. An expected NOE between the NH protons of Thr_{10} and Gln_3 could not be observed in peptides **6a** and **6b**, because the NH proton resonances occur at very similar chemical shifts (δ = 0.03 ppm).

Peptides **5**, **6a**, and **6b** each show NOE patterns associated with turn formation. Peptide **5** exhibits NOEs between the NH proton of Lys_8 and both the NH proton of Gly_7 and α-proton of D-Pro_6 (Figure 8). Peptide **6a** displays NOEs between the NH proton of Lys_8 and the δ-protons of δOrn. Peptide **6a** also displays NOEs between the α-proton and δ-protons of δOrn. Peptide **6b** has the same pattern of NOEs associated with the δMeOrn turn. The

diastereotopic δ-proton resonances of the $^\delta$MeOrn turn in peptide **6b** are separated by 0.89 ppm, indicating that the turn is well-folded, while the diastereotopic δ-proton resonances of the $^\delta$Orn turn in peptide **6a** are separated by 0.35 ppm, reflecting only moderate folding.[39]

The α-proton chemical shifts of peptides **5**, **6a**, and **6b** suggest that peptides **6a** and **6b** fold in a similar fashion to each other but a different fashion from peptide **5** (Figure 9). In both peptides **6a** and **6b**, the α-protons of Trp_2, Gln_3, Lys_8, Phe_9, and Thr_{10} are shifted downfield, while the α-protons of Tyr_4 and Val_{11} are shifted upfield. The downfield shifting for Trp_2, Gln_3, Lys_8, Phe_9, and Thr_{10} is greater in peptide **6b** than in **6a**, reflecting a greater degree of folding for peptide **6b**. Peptide **5** exhibits a somewhat different pattern of chemical shifts, with the α-proton of Gln_3 showing only slight downfield shifting, the α-proton of Phe_9 showing slight upfield shifting, and the α-proton of Tyr_4 showing substantial downfield shifting. These differences may result from subtle differences in the β-hairpin conformations of the peptides, with the magnetic anisotropies from the aromatic rings of Trp_2, Tyr_4, and Phe_9 affecting the chemical shifts of many of the α-protons (Figure S3).

The CD spectrum of peptide **5** has a maximum at 230 nm, a broad minimum at ca. 210–220 nm and a second more intense minimum at 200 nm, reflecting a mixture of β-sheet and random coil conformations (Figure 10). The CD spectrum of peptide **6b** is similar to peptide **5**, but with a more intense minimum at ca. 204–208 nm instead of ca. 210–220 nm. The combination of minima at 200 nm and at ca. 204–208 nm in peptide **6b** suggest a mixture of β-sheet and random coil conformations, with the shifted minimum at ca. 204–208 nm reflecting significant contributions from the packing of the aromatic residues. The CD spectrum of peptide **6a** exhibits a pair of small minima at 196 nm and 200 nm, reflecting a predominance of random coil conformations. These observations stand in agreement with our findings that the $^\delta$MeOrn turn is more effective than the $^\delta$Orn turn in inducing β-hairpin formation in peptides.

Conclusion

Stereospecific incorporation of a single methyl group substantially enhances the propensity of δ-linked ornithine to induce β-hairpin formation in peptides. A recently reported chemoenzymatic hydroxylation of L-leucine selectively sets the *R* stereochemistry of the methyl group at the γ-position and enables the subsequent gram-scale synthesis of the protected γ(*R*)-methyl-ornithine derivative, Boc-MeOrn(Fmoc)-OH. This derivative bears orthogonal protecting groups compatible with solid-phase peptide synthesis and can be readily incorporated into different peptide sequences.

The X-ray crystallographic structure of peptide **1b** unambiguously confirms the stereochemistry of the methyl group and the predicted hydrogen-bonded conformation of the $^\delta$MeOrn turn. CD spectroscopic studies of peptides **2a** and **2b** reveal a distinct improvement in β-hairpin folding when both the $^\delta$Orn turns are replaced with $^\delta$MeOrn turns. NMR and CD spectroscopic studies of β-hairpin peptides **3–6** also indicate a substantial improvement in β-hairpin folding induced by $^\delta$MeOrn. NMR and CD spectroscopic studies further indicate comparable β-hairpin folding induced by $^\delta$MeOrn and D-Pro-Gly, with the latter perhaps being somewhat superior.

The improved β-hairpin formation provided by the [δ]MeOrn turn places it in the same league as the popular D-Pro-L-Pro and D-Pro-Gly turns and opens the door to useful applications. We anticipate using it in our own laboratory to improve the mimicry of amyloid oligomers composed of β-hairpins.[34] We also envision that the free α-amino group of the [δ]MeOrn turn will offer advantages over the D-Pro-L-Pro and D-Pro-Gly turns in solubility and could also serve as a handle for further functionalization.[30,38] Although there is still no universal tool to coax any small peptide — polyalanine, for example — to adopt a β-hairpin conformation, the [δ]MeOrn turn represents a worthy addition to the toolbox of turn templates. MC/SD calculations predict that $\beta(R),\gamma(R)$-dimethyl-ornithine may be even better at inducing β-hairpin formation in peptides (Figure S1). We look forward to future synthetic advances or clever application of existing methodology that enable the facile preparation of derivatives of $\beta(R),\gamma(R)$-dimethyl-ornithine suitable for use in solid-phase peptide synthesis, so that this hypothesis can be tested.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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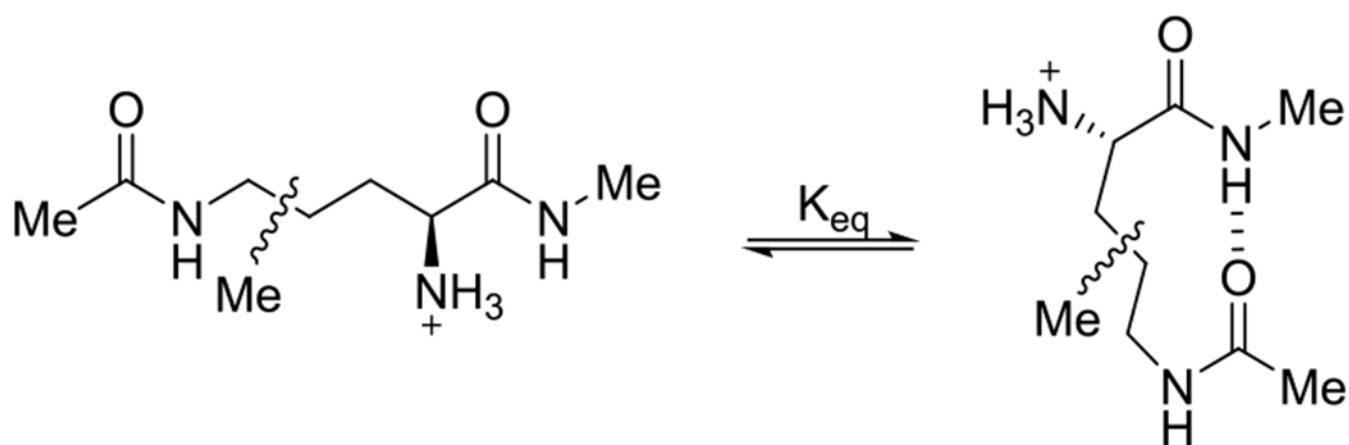
Figure 1.
Equilibrium between unfolded and folded conformations of methylated ornithine derivatives.
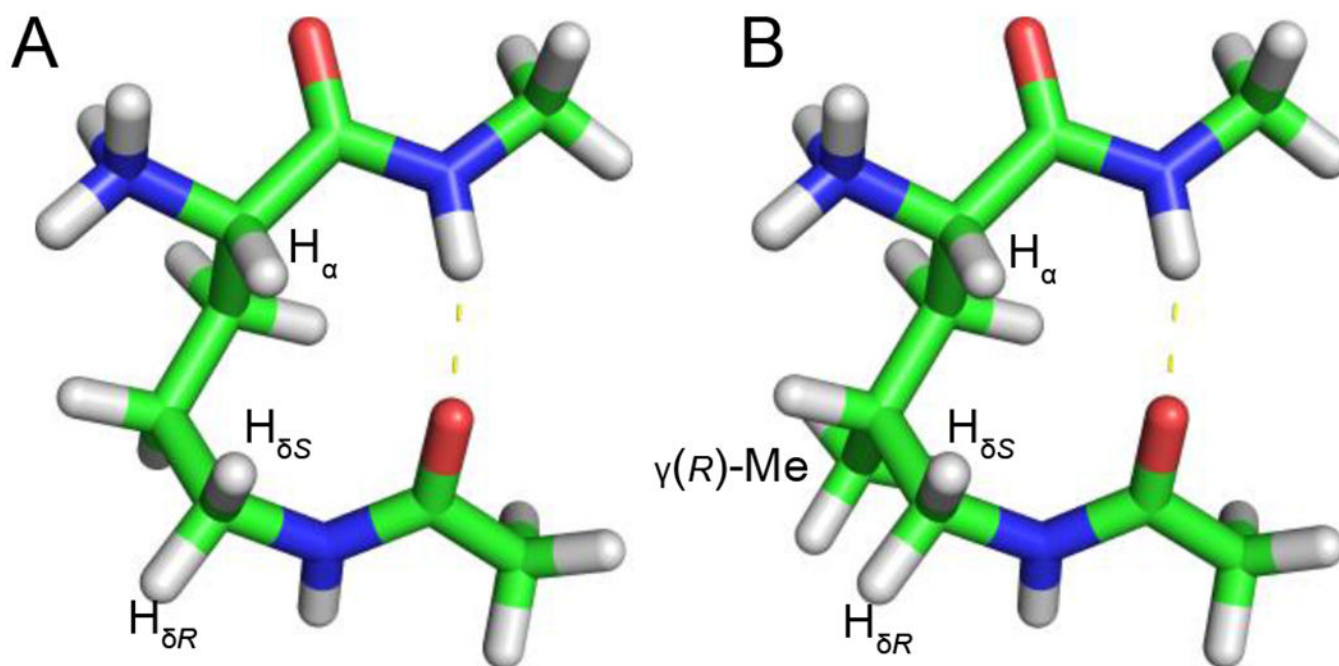
Figure 2.
Minimum-energy models (local minima) of ornithine and $\gamma(R)$-methyl-ornithine derivatives Ac-$^\delta$Orn-NHMe (A) and Ac-$^\delta$MeOrn-NHMe (B) in folded conformations.
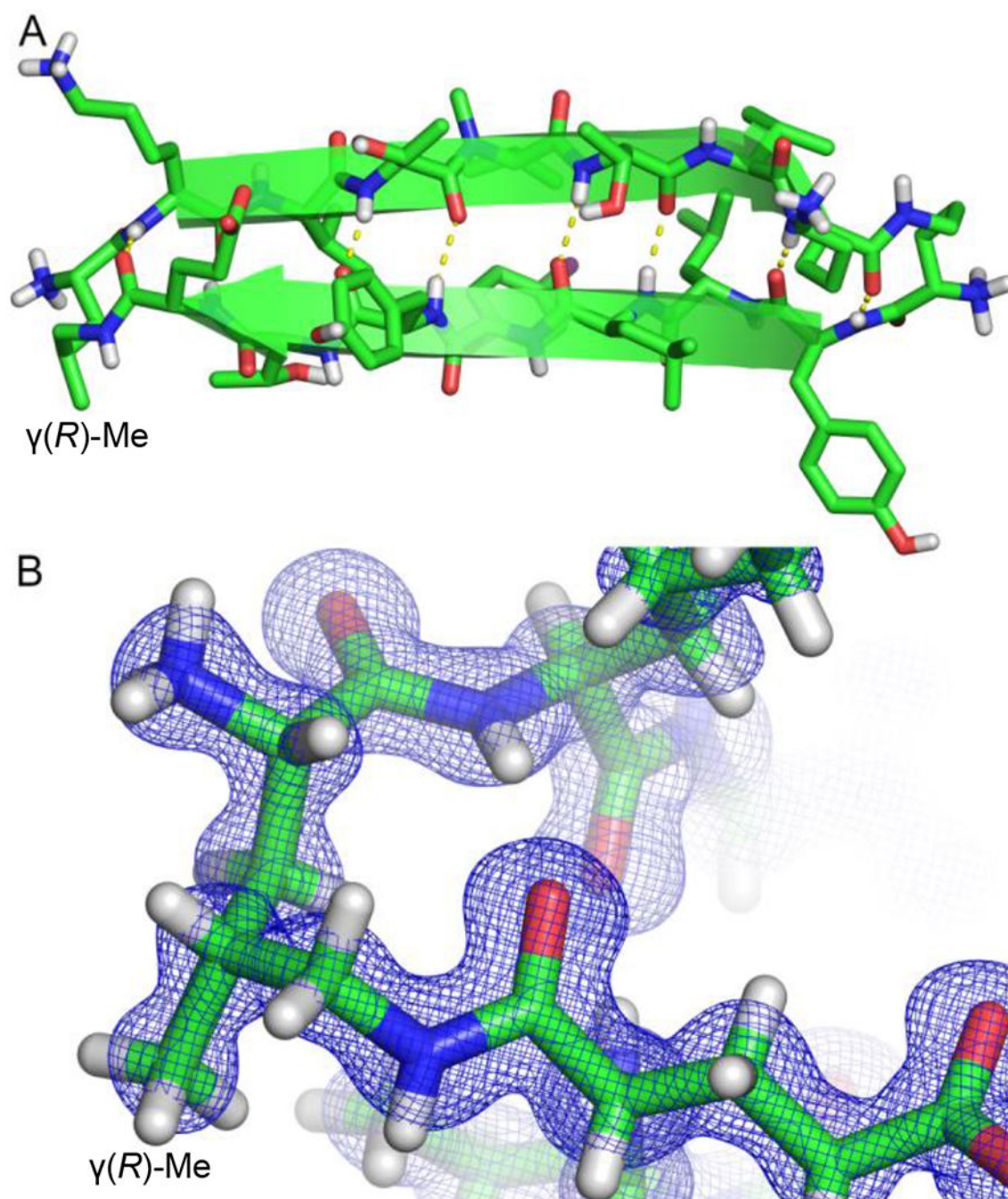
Figure 3.
(A) X-ray crystallographic structure of peptide **1b**. (B) $2F_O$-F_C electron density map contoured at the 1 sigma level, showing the conformation of the δMeOrn turn unit and the stereochemistry of the γ-carbon.
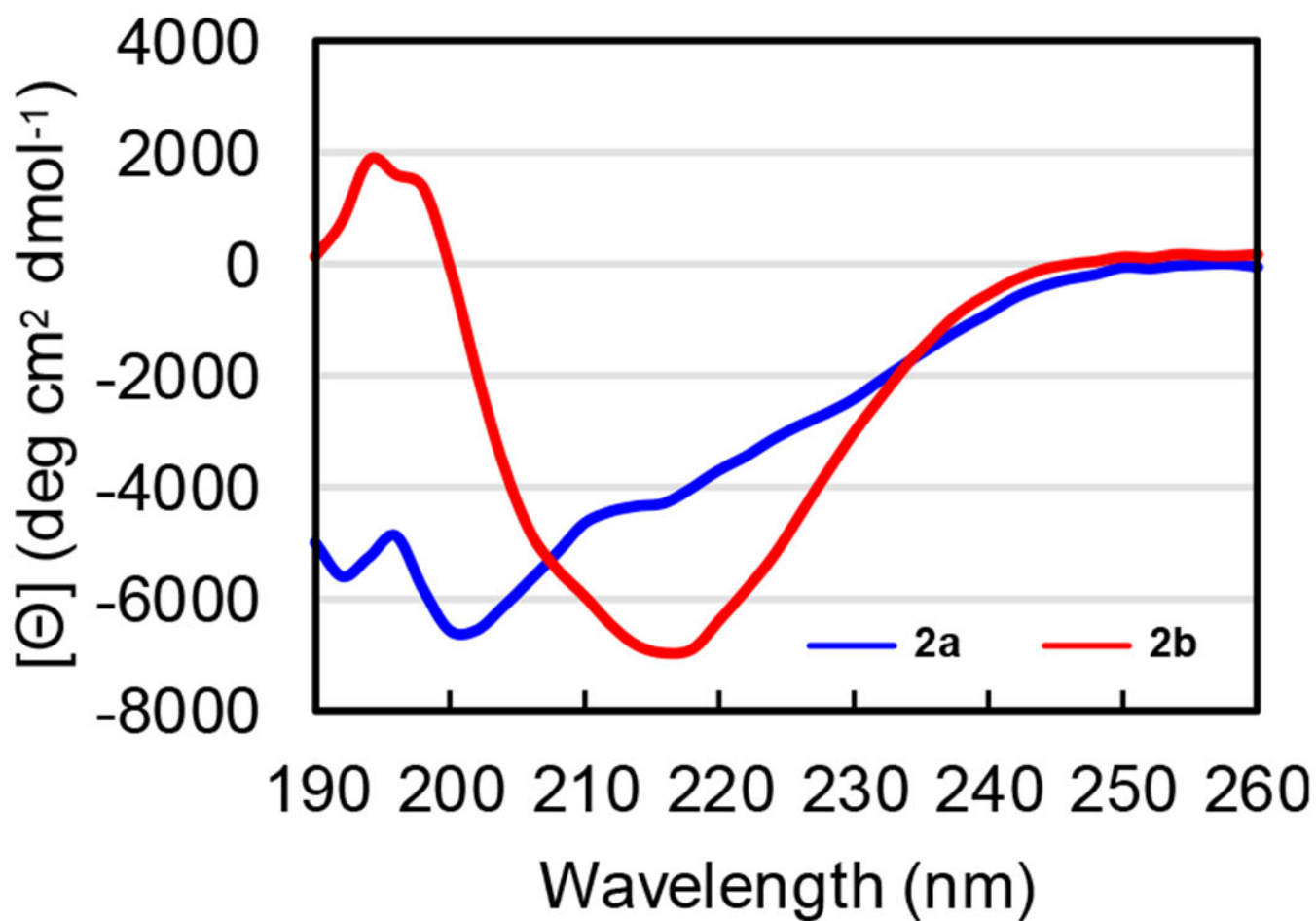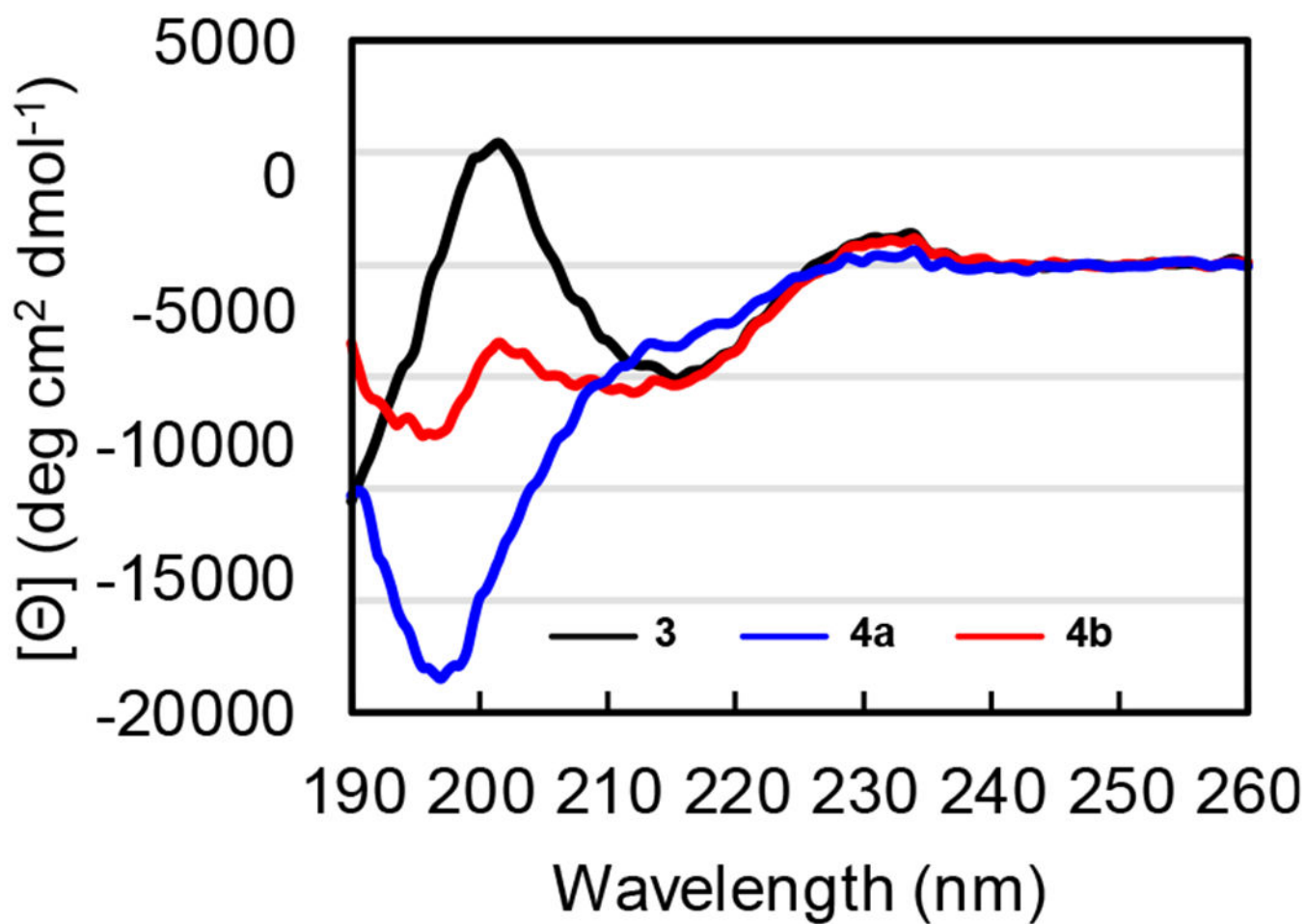
Figure 4.
CD spectra of peptides **2a** (blue) and **2b** (red). CD spectra were acquired for each peptide at 150 μM in 10 mM phosphate buffer at pH 7.4; the ellipticity was normalized for the number of residues in each peptide.
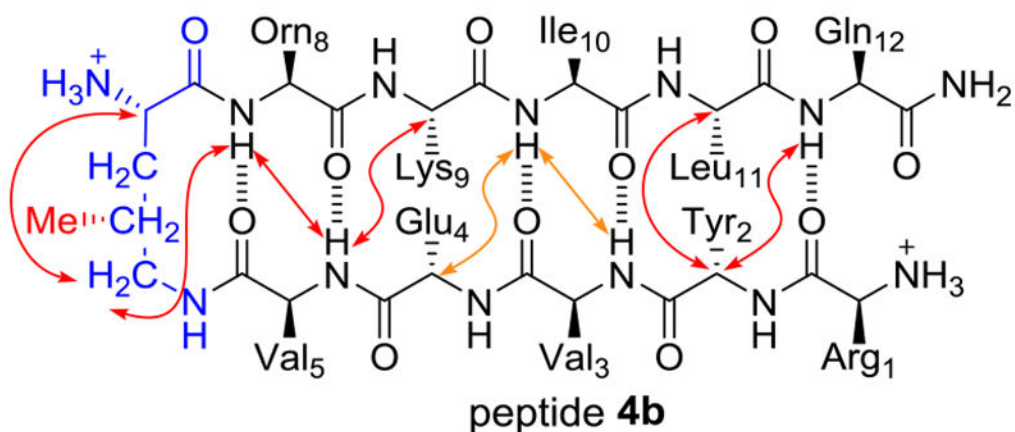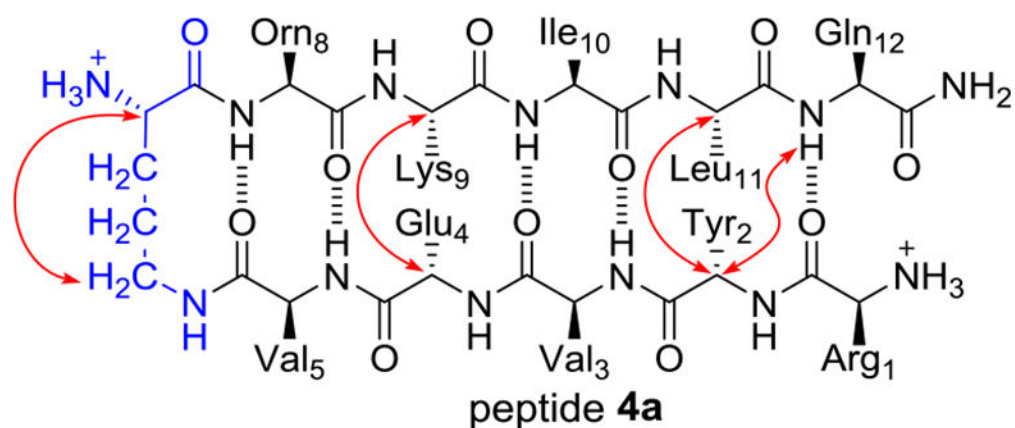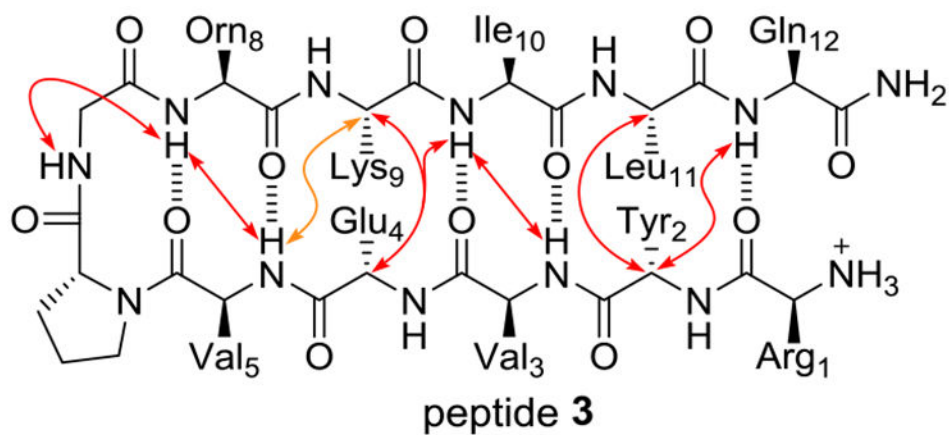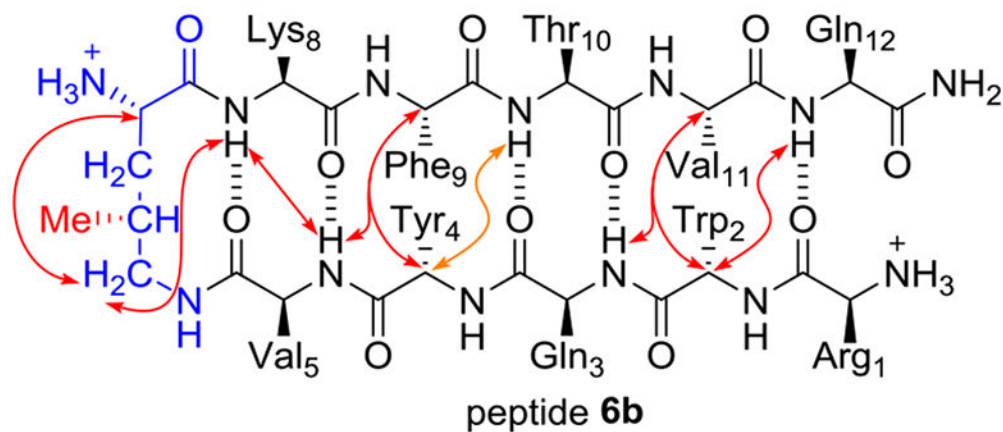
Figure 5.
Chemical structures of peptide **3** and homologues **4a** and **4b**. Key NOEs associated with solution-state folding for each peptide are represented with double-headed arrows. Red arrows represent unambiguous NOEs. An orange arrow in peptide **3** and two orange arrows in peptide **4b** represent NOEs in which overlap with other resonances preclude unambiguous assignment. The resonances of the α-protons of Lys$_9$ and Glu$_4$ are nearly coincident in peptide **4b**, preventing identification of an NOE between these protons. NMR spectra were

acquired for each peptide at 4.0 mM and 277 K in D_2O or 90:10 H_2O:D_2O, with a buffer of 100 mM CD_3COOD and 100 mM CD_3COONa.
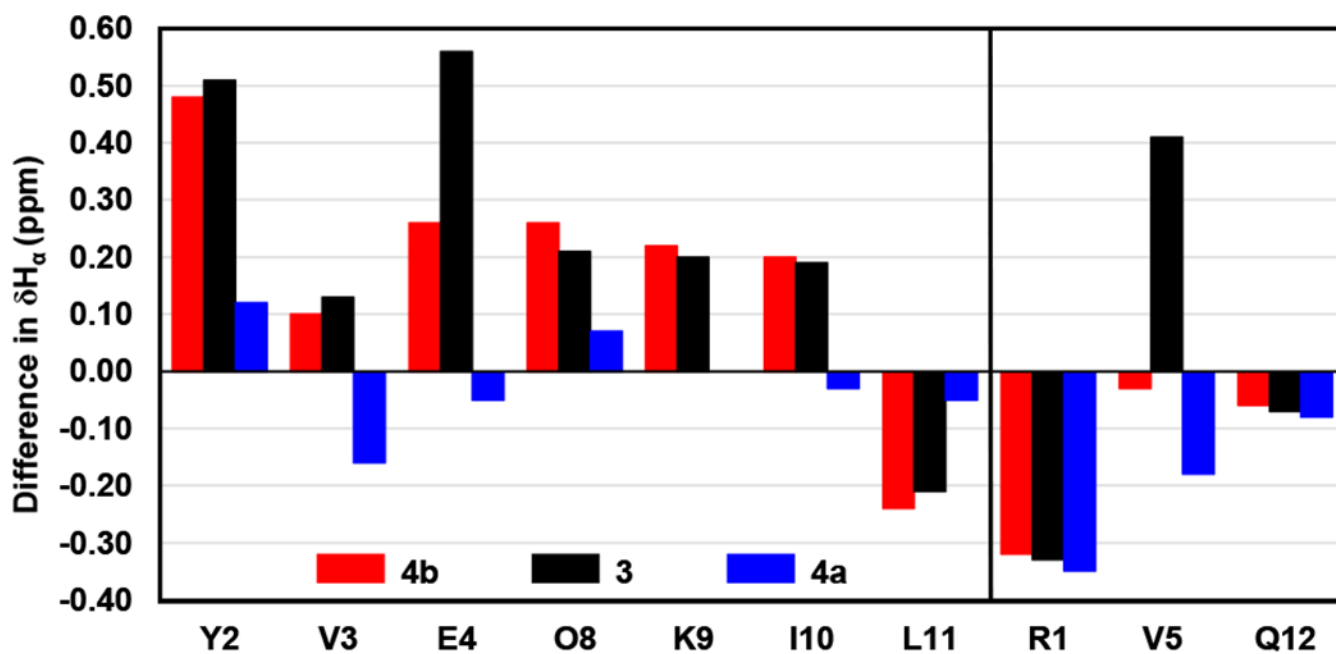
Figure 6.
Chemical shift differences between the α-protons of each residue in peptides **3** (black), **4a** (blue), and **4b** (red) and random coil values reported by Wüthrich.[44] NMR spectra were acquired for each peptide at 4.0 mM and 277 K in D_2O in a buffer of 100 mM CD_3COOD and 100 mM CD_3COONa with 0.06 mM DSA as a reference standard.[47]

Figure 7.
CD spectra of peptides **3** (black), **4a** (blue), and **4b** (red). CD spectra were acquired for each peptide at 100 μM in 10 mM phosphate buffer at pH 7.4; the ellipticity was normalized for the number of residues in each peptide.
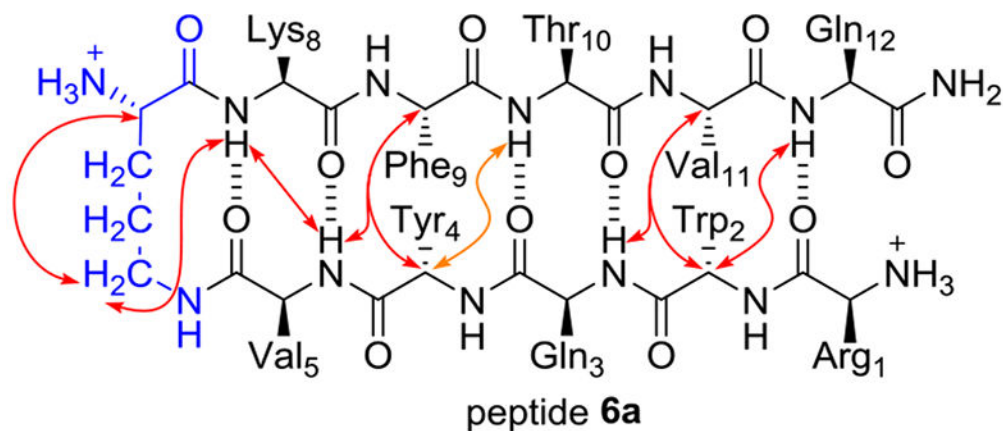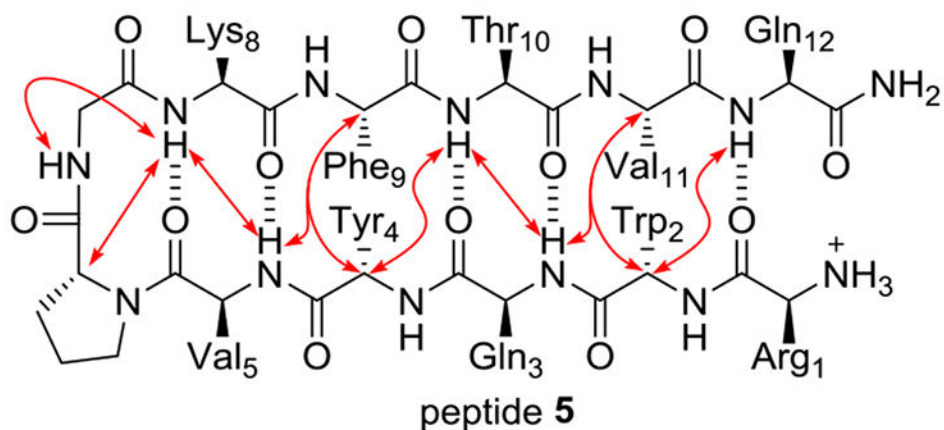
Figure 8.
Chemical structures of peptide **5** and homologues **6a** and **6b**. Key NOEs associated with solution-state folding for each peptide are shown with double-headed arrows. An orange arrow in peptides **6a** and **6b** represent NOEs in which overlap with other resonances preclude unambiguous assignment. The resonances of the NH protons of Thr_{10} and Gln_3 are nearly coincident in peptides **6a** and **6b**, preventing identification of an NOE between these protons. NMR spectra were acquired for each peptide at 4.0 mM and 277 K in D_2O or 90:10 H_2O:D_2O, with a buffer of 100 mM CD_3COOD and 100 mM CD_3COONa.
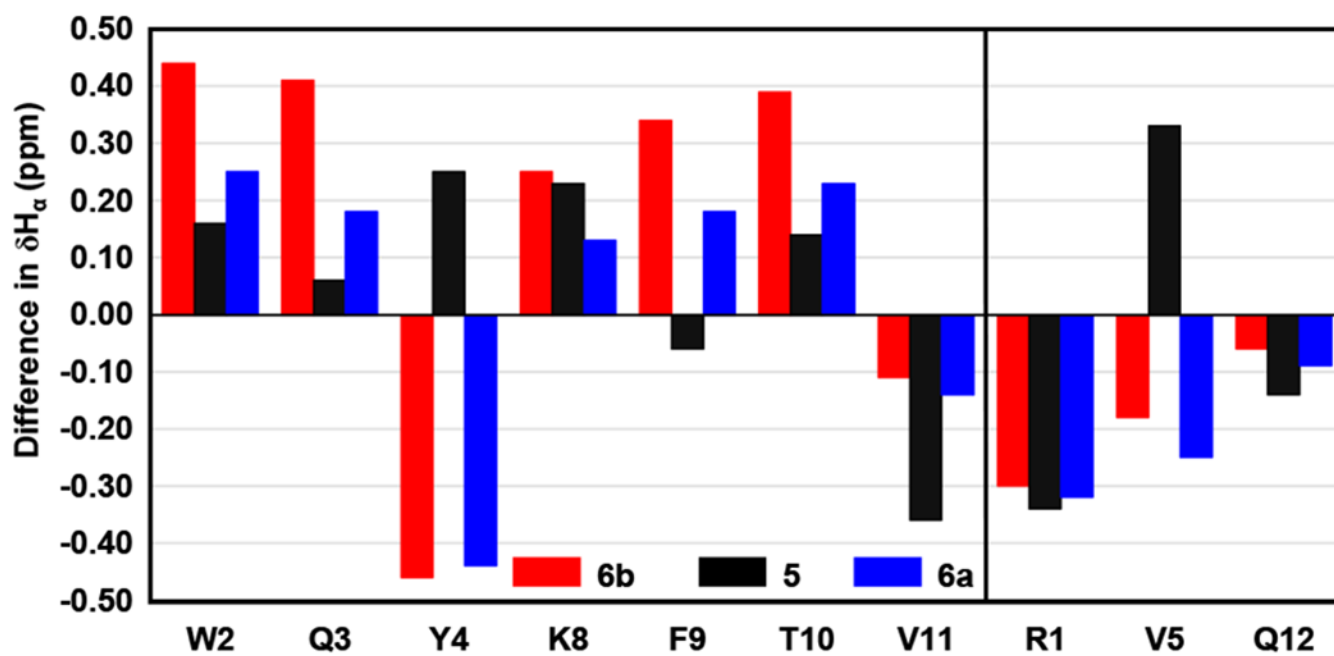
Figure 9.
Chemical shift differences between the α-protons of each residue in peptides **5** (black), **6a** (blue), and **6b** (red) and random coil values reported by Wüthrich.[44] NMR spectra were acquired for each peptide at 4.0 mM and 277 K in D_2O in a buffer of 100 mM CD_3COOD and 100 mM CD_3COONa with 0.06 mM DSA as a reference standard.[47]
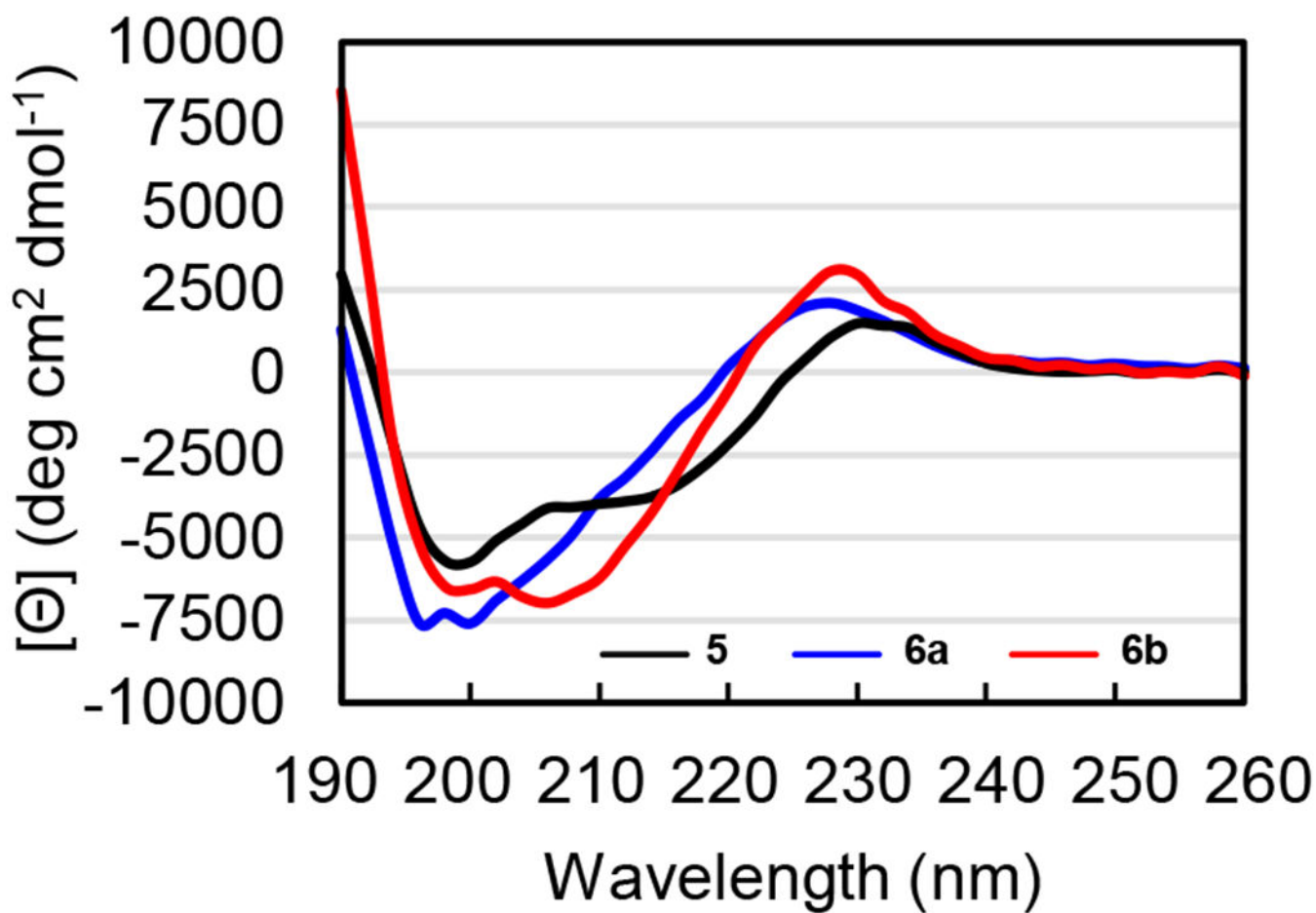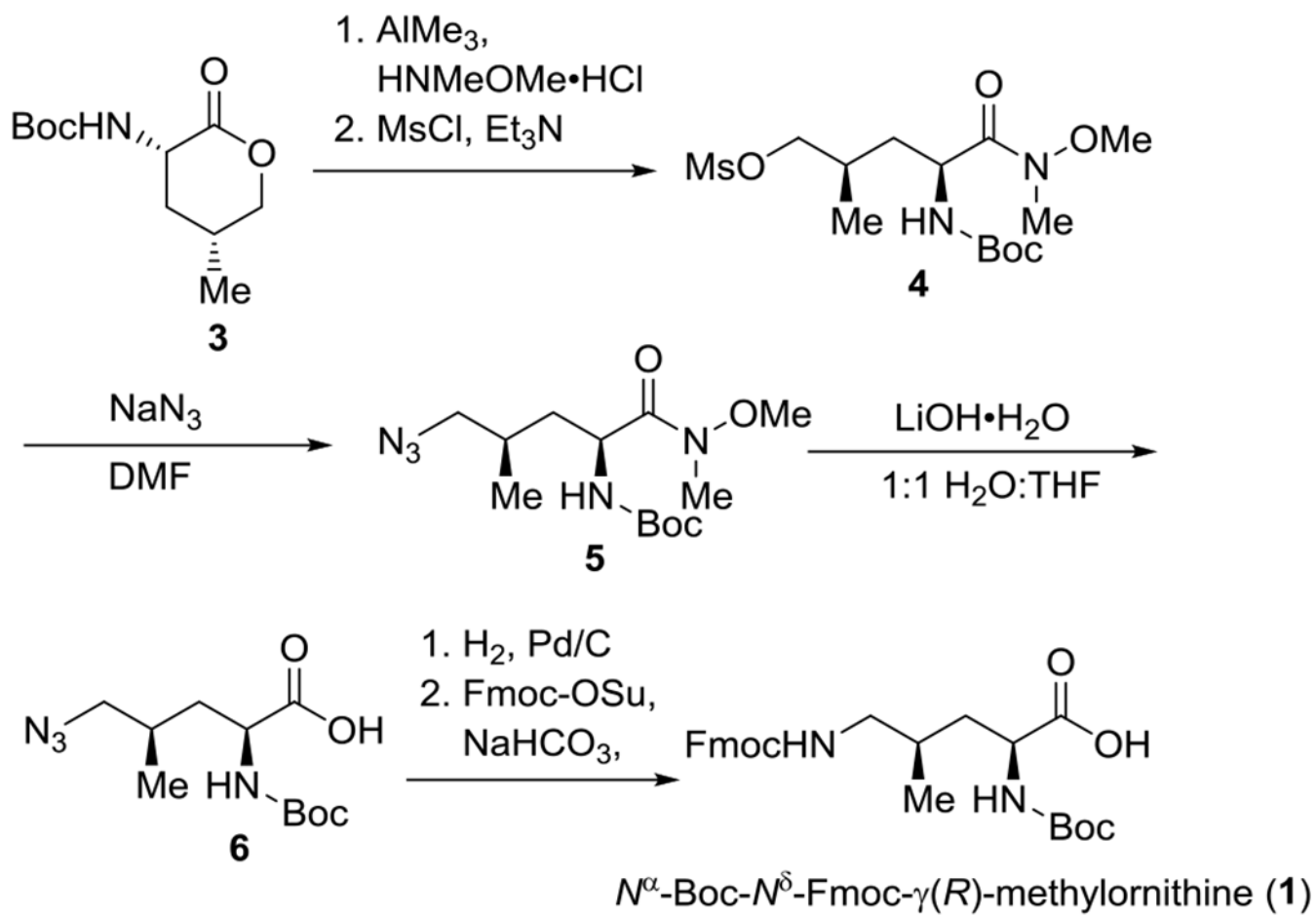
Figure 10.
CD spectra of peptides **5** (black), **6a** (blue), and **6b** (red). CD spectra were acquired for each peptide at 100 μM in 10 mM phosphate buffer at pH 7.4; the ellipticity was normalized for the number of residues in each peptide.

Scheme 1.
Synthesis of $N\alpha$-Boc-$N\delta$-Fmoc-$\gamma(R)$-methyl-ornithine (**1**) from Boc-protected lactone **3**.