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Facile Chemoselective Modification of Thio-Ethers Generates Chiral Center-Induced Helical Peptides

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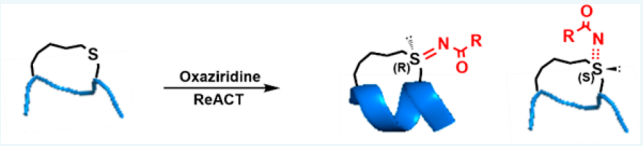
Supporting Information

ABSTRACT: A precisely positioned sulfimide chiral center on-tether of a thio-ether tethered peptide determines the peptide secondary structure by chemoselective oxaziridine modification. This method provides a facile way to tune peptides' secondary structures and biophysical properties.

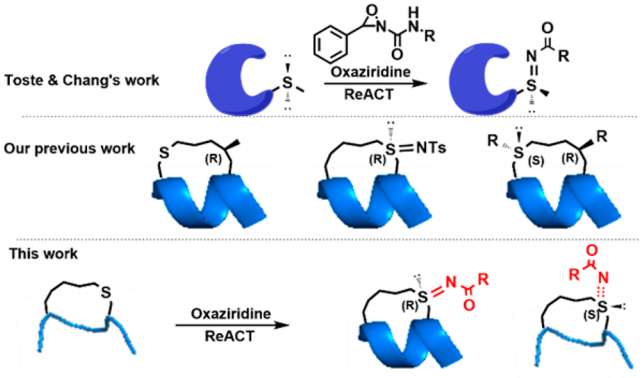


A majority of intra- and intercellular biological processes are modulated by protein−protein interactions (PPIs). Many important PPIs are potential therapeutic targets and peptides with suitable modifications which are viewed as preferable modulators for various PPIs.[1] Stabilizing peptides with artificial tethers could potentially improve the peptides' target selectivity, binding affinity, and cellular uptake.[2−9] A variety of chemical methods have been reported to construct the artificial tethers, including disulfide-bond or lactam-bridge formation,[10−15] click reaction,[16−19] ring-closing alkene and alkyne metathesis,[20−23] cysteine alkylation,[24,25] incorporation of perfluoroarenes,[26−28] and so on.[29−31] These methods substantially enrich the chemical and functional diversity of stabilized peptides.[32]

In addition to tethering methodology, expanding the chemical space and increasing the chemical diversity of peptide molecules is crucial for peptide drug design.[33−37] Chang et al., Phillips et al., and Baek et al. reported that the peptide tethers may have additional interactions with the target proteins, despite it being originally designed on the solvent exposure face.[38−40] One important approach is to expand the on-tether peptide modification methods.[41] Recently, Greenbaum et al. and Spring et al. reported several tether modification strategies, in which the tailored modifications were capable of modulating the peptides' binding affinities and cellular uptakes.[42,43] However, most of the tether modifications were performed without knowing the precise influence of modification on the backbone peptide's secondary structure.

Recently, our group reported a chirality-induced helicity (CIH) strategy for peptides stabilization, of which a carbon chiral center precisely placed in a single-bonded tether dominates the backbone peptide's helicity.[44−46] Moreover, we demonstrated that a further on-tether sulfonium modification of helical CIH peptides could maintain the helical secondary structures at a precisely chosen position.[47,48] However, this sulfonium method requires multiple chemical synthesis steps, and a facile secondary structure-fixed on-tether modification method is still to be developed. Recently, Chang and Toste et al. reported a highly chemoselective protein methionine bioconjugation method through oxaziridine oxidation (Scheme 1).[49,50] The ease of synthesis and high specificity of this reaction inspired us to explore oxaziridine modification on a thio-ether tethered peptide, which could allow further modifications.

Scheme 1. Schematic Presentation of Toste and Chang's Work on Methionine Modification on Proteins (ReACT: Redoxactivated Chemical Tagging) and Our On-Tether Chiral Center Induced Helical Peptide Strategies[a]



[a]Left: carbon chiral center, middle: sulfimide chiral center, right: dual chiral center. The bottom is a schematic presentation of peptide thioether functionalization.

To minimize the sequence influences on peptide secondary structure, a single turn pentapeptide was chosen as a model shown in Figure 1A.[51] The reaction of peptide Ac-(cyclo-1,5)-S_5AAAC-NH_2 (**1**) with **Ox1** completed within approximately 5 min in 1:1 methanol/water (v/v), and two sulfimide peptide epimers **1a**/**b** were generated (Figure 1A). Epimer **1a** and **1b**

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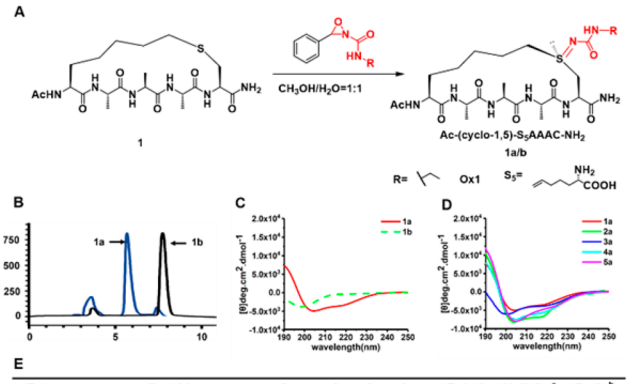

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Entry	Peptide	Conversion of a epimer	Relative Helicity[a]	Ratio[b]
1a	Ac-(cyclo-1,5)-S5AAAC-NH2	0.41	0.53	1:1.2
2a	Ac-(cyclo-1,5)-S5AGAC-NH2	0.37	1	1:1.4
3a	Ac-(cyclo-1,5)-S5AQAC-NH2	0.43	0.57	1.1:1
4a	Ac-(cyclo-1,5)-S5AAIC-NH2	0.62	0.86	2:1
5a	Ac-(cyclo-1,5)-S5AEAC-NH2	0.46	0.76	1:1

Figure 1. (A) Cyclic sulfimide pentapeptide synthesis. (B) HPLC separation of peptide epimer **1a** and **1b**. (C) CD spectra of pentapeptides **1a** and **1b** in H_2O at 25 °C. (D) CD spectra of helical peptides **1a**−**5a** in H_2O at 25 °C. Concentrations of samples are about 0.5 mM. (E) Peptide sequences and relative helicity percentage of **1a**−**5a** in H_2O at 25 °C. [a]The final relative helical content presented were calculated based on the helicity of peptide **2a**, which was set as 100% helical.[51] [b]The ratios were calculated based on the integration of the peak areas of peptide epimers **a**:epimers **b** in HPLC.

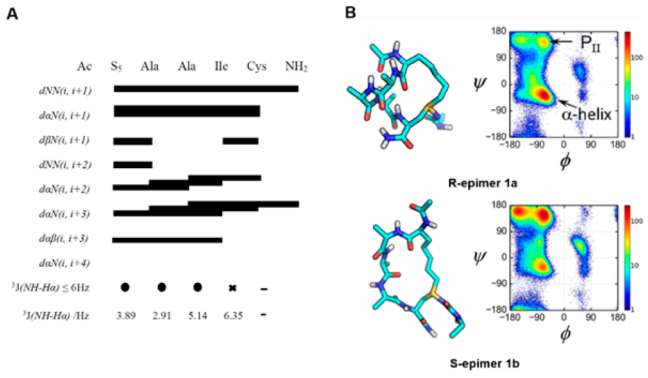

Figure 2. (A) NOE diagram of peptide **4a** (measured in 10% D_2O in H_2O, 25 °C). Bar thickness reveals the intensity of NOE signals. (B) Representative structures and Ramachandran plots of **1a** and **1b** (R/S epimers) from room temperature (300 K) replicas of REMD simulations.

were readily separated by high performance liquid chromatography (HPLC). The retention time of **1b** is longer than that of **1a** which suggested a significantly different secondary conformation (Figure 1B). Circular dichroism (CD) measurements showed that **1a** has a helical conformation while **1b** is nonhelical in solution (Figure 1C). Subsequently, we tested other peptides (entries 2−5) with amino acids bearing different functional groups. The sequence and structure information were summarized in Figure 1E. Consistently, peptides **2a**−**5a** showed helical structures (Figure 1D), while peptides **2b**−**5b** were nonhelical (Figure S1).[52] The epimers **1a**−**5a** were obtained in conversions varying from 37% to 62% for the **a** epimer alone based on HPLC peak integration. Notably, the epimer ratio was determined to be roughly 1:1 based on HPLC peak integration, except peptide **4a**/**b** (Figure 1D,E).

To better illustrate the secondary structure of the sulfimide peptide conformation in aqueous solution, two-dimensional ^{1}H NMR spectroscopy study of peptide **4a** was performed in 10% D_2O in H_2O (by volume) at 25 °C. Spectral features characteristic of a well-defined helical structure were observed with the exception of cysteine residue. Low coupling constants were observed ($^3J_{NH-CH\alpha}$ < 6 Hz) for all amide resonances except cysteine and isoleucine (Figure 2A; Supporting Information Table S2).[53] In addition, the NOESY spectra showed nonsequential medium range $d_{\alpha N}$ (i, i+3) and $d_{\alpha\beta}$ (i, i +3) NOEs which further suggested the peptide's helical structure in solution.[54]

Each helical epimer (**1a**−**5a**) showed shorter HPLC retention time compared with their nonhelical counterparts (**1b**−**5b**). To confirm the chirality of the sulfimide center, we carried out replica-exchange molecular dynamics (REMD) simulations[55] on peptides **1a** and **1b**. Our recently developed residue-specific force field RSFF2[56,57] was used for all residues except for the non-natural side-chain tether, which was described by generalized Amber force field (GAFF).[58] This computational method has been used to study our previous **CIH** peptides[59,60] with reliable structure predictions. The simulation results showed that the representative structure of **R**-epimer exhibited a well-defined helical conformation while the representative structure of **S**-epimer was nonhelical. The obviously different conformational preferences of both peptide epimers were also demonstrated from their simulated Ramachandran plots (Figure 2B). The calculated helicity of **1a** and **1b** were 36.8% and 0.2%, respectively. The results were consistent with our previous studies,[59] which suggested the absolute configuration of helix-inducing tether also to be **R**.

In our previous studies,[59] helical epimers usually have longer retention time because the internal hydrogen bonds make them less polar. The shorter retention times of helical epimers observed here could be attributed to the amphipathicity introduced by the relatively hydrophilic substitution group on the tether. In our simulations, the on-tether polar group in the representative structure of helical epimer **1a** was exposed to the solvent. In contrast, the on-tether polar group in nonhelical epimer **1b** structure was found to have more contacts to both terminals of the peptide (Figure S2), which may increase the retention time. This reversal of elution mode was not found for longer peptides we studied below, suggesting that the conformation and polarity of the on-tether substitution played a more important role in shorter peptides.

Using estrogen receptor-α (ERα) peptide ligands as an example, we further explored the influence of different on-tether substituents on their biophysical properties.[39,61] Peptide epimers **6-Ac-a** and **6-Ac-b** (Figure 3C) were obtained by oxaziridine oxidation of peptide **6**. Based on the CD spectra, peptide **6-Ac-b** showed a helical structure. Then, another three oxaziridines bearing different functional groups were let to react with peptide **6** to generate peptides **7-Ac-a**/**b**, **8-Ac-a**/**b**, and **9-Ac-a**/**b** as shown in Figure 3A. Consistently, the results suggested that peptides **6-Ac-b** to **9-Ac-b** (with longer HPLC retention time) had helical structures (Figure 3C,D), while peptides **6-Ac-a** to **9-Ac-a** were nonhelical (Figure S3A). Then, the thermo- and chemostability of peptides **7-Ac-b** and **6** were tested at different temperatures (20−70 °C) (Figure S3B for CD spectra of peptides **7-Ac-b** to **9-Ac-b** at different T) and different concentrations of guanidine hydrochloride (0−8 M), as shown in Figure 3E and F. A gradual increase in molar ellipticity was observed at 215 nm when the temperature was increased, which indicated that the helix content decreased in

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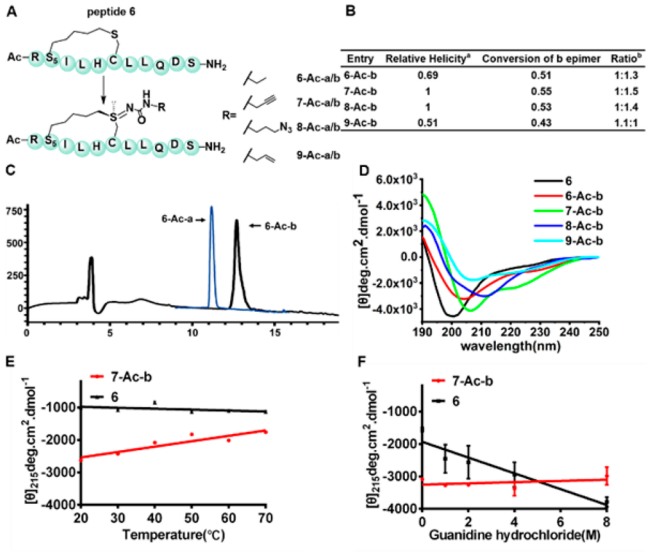

Figure 3. (A) Chemical structures of peptides. (B) Calculated relative helicity, conversion of peptide **b** epimers and **a**/**b** epimer ratios. [a]The final helical content presented relative to peptide **7-Ac-b**, where the helicity of peptide **7-Ac-b** was fixed as 100% helical.[51] [b]The ratios were calculated based on the integration of the peak areas of peptide epimer **a** versus epimer **b** in HPLC. (C) HPLC separation of peptide epimers **6-Ac-a** and **6-Ac-b**. (D) CD spectra of peptides **6**, **6-Ac-b** to **9-Ac-b**. The CD spectroscopy measurements were performed in H_2O at 25 °C. Concentrations of samples are about 1.5 mM. (E) Molar ellipticity at 215 nm of **7-Ac-b**, **6** from 20 to 70 °C with 10 °C interval. (The experiment was replicated twice.) (F) Molar ellipticity at 215 nm of **7-Ac-b**, **6** under the condition of increasing guanidine hydrochloride from 0 to 8 M at 25 °C with serial dilution. (The experiment was replicated twice.)

7-Ac-b. Despite some helix unwinding, over 65% helicity was preserved even at 70 °C. The peptide **6** was nonhelical, so its molar ellipticity at 215 nm remained almost unchanged with increasing temperature (Figure 3E). As the concentration of guanidine hydrochloride increased, the molar ellipticity at 215 nm of peptide **7-Ac-b** remained stable while the molar ellipticity at 215 nm of peptide **6** decreased (Figure 3F). These results showed that these helical sulfimide peptides were of good thermo- (Figure 3E) and chemo- (Figure 3F) stability.

Peptides' cellular permeability is crucial for their potential applications to target intracellular PPIs. To study the influence of sulfimide modifications on peptides' cellular uptake, HeLa cells were treated with peptides **6-FITC-βA-a/b** to **9-FITC-βA-a/b** at 37 °C for 2 h. The flow cytometry results showed that peptides **6-FITC-βA-b** to **9-FITC-βA-b** were of better cellular uptakes than their **a**-epimer counterparts and precursor peptide **6** (Figure 4A, Figure S4). These results clearly indicated the enhancement of peptides' helical content would increase the cellular uptake to some extent,[62] consistent with our previously reported results.[59] Then we investigated the target binding affinity of peptide epimers **6-FITC-βA-a/b** using a fluorescent polarization assay. **6-FITC-βA-b** showed increased binding affinity to ERα (K_D = 144.4 ± 21 nM), while the precursor peptide **6-FITC-βA** showed a K_D > 1000 nM. **6-FITC-βA-a** showed no binding to ERα (Figure 4B). The superior binding of peptide **6-FITC-βA-b** comparing with it is **a**-epimer and precursor peptide **6-FITC-βA clearly** indicated the importance of maintaining a peptide's secondary structure.[45]

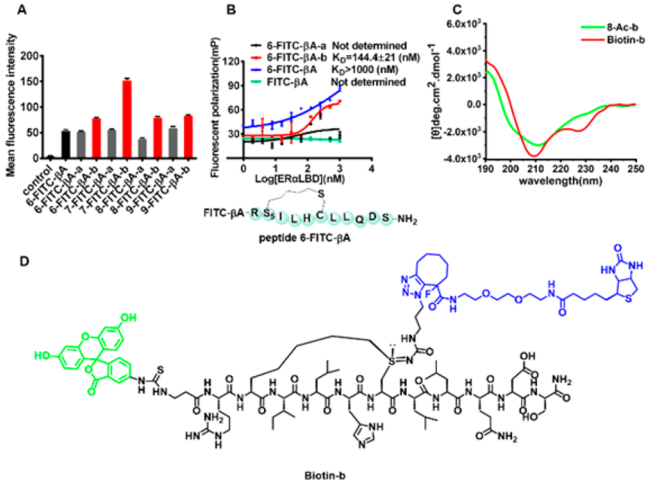

Figure 4. (A) Flow cytometry analysis of HeLa cells with 5 μM of fluoresceinated peptides **6-FITC-βA-9-FITC-βA-a/b** at 37 °C for 2 h. (The experiment was repeated three times.) (B) Binding of **6-FITC-βA-a/b**, **6-FITC-βA**, and **FITC-βA** with ERα ligand binding domain. The binding affinities were measured using fluorescence polarization at 20 °C. (The experiment was replicated twice.) **FITC**, Fluorescein isothiocyanate. **βA**, β-Alanine. (C) CD spectra of peptides **8-Ac-b** and **Biotin-b**. The CD spectroscopy measurements were performed in H_2O at 25 °C. Concentrations of samples are about 1.5 mM. (D) The chemical structure of **Biotin-b**. The compound was synthesized as described in Supporting Information.

To further test the feasibility of secondary modification on the sulfimide peptides, we tried to conjugate a biotin motif on the chiral center. By using a Cu-free click reaction between biotin-cyclooctyne derivative and **8-FITC-βA-b**, peptide **Biotin-b** was obtained with 90% conversion.[63] Its CD spectrum clearly showed the maintenance of helical features. This result indicated that this method was applicable with complex molecules like biotin and the modification with complex molecules does not damage the secondary structure of the peptide. Based on this approach, this method could be used for making molecules for other purposes, such as biotinylated probes.

In summary, we first performed oxaziridine modification of thioether tether on short model peptides, showing good sequence tolerance, specificity, and reaction efficiency. Based on our previous study of (R) hydrocarbon chiral center and sulfoxide chiral center could induce peptide helicity,[48,59] the sulfimide center would also follow the same pattern. The helical structure was speculated by CD, NMR evidence, and the absolute configuration preferences was supported by MD simulations. Finally, the secondary structure conformation, cell permeability, thermo-/chemostability, and binding affinity of longer peptides were also studied. This work is an important supplement to the existing **CIH** peptides methodologies and provides a facile way for on-tether modifications, expanding the chemical space of on-tether peptides and providing an elegant way to tune the peptide's biophysical properties in late-stage synthesis. Our work can substantially enrich the chemical toolbox for designing more effective peptide ligands.

■ ASSOCIATED CONTENT

Supporting Information

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Additional details on materials and methods. Figures showing auxiliary ligands, chemical structures, synthetic processes, CD spectra, experimental results, a comparison between peptides and epimers, temperature dependence of the mean residue ellipticity of peptides, HPLC analysis, NOE summaries, and cell penetration assays and mean fluorescence intensity. Tables showing chemical shifts and MS values. Selected NMR, HPLC, and LC−MS data. (PDF)

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Notes

The authors declare no competing financial interest.

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