ChemComm

COMMUNICATION

View Article Online
View Journal | View Issue

Cite this: *Chem. Commun.*, 2016, 52, 10389

Received 29th May 2016,
Accepted 26th July 2016

DOI: 10.1039/c6cc04508a

www.rsc.org/chemcomm

An in-tether sulfilimine chiral center induces helicity in short peptides†

Huacan Lin, Yixiang Jiang, Qingzhou Zhang, Kuan Hu and Zigang Li*

A precisely positioned sulfilimine chiral center in the tether of a stabilized peptide would determine the peptide's secondary structure. Peptide sulfilimines could be prepared by a facile chloramine T oxidation and the two resulting peptide diastereomers showed significant differences in their secondary structures, which were supported by circular dichroism spectroscopy and NMR.

Protein–protein interactions (PPIs) mediate various kinds of biological processes including transcription regulation and signal transduction.[1] Short helices in the length of 4–15 amino acids count for about 30% interaction subdomains of all known PPIs.[2] Thus, chemically induced helical conformation of short peptides is a focused research area of peptide sciences. Side chain–side chain ligation is the most important stabilization strategy, including disulfide,[3] lactam bridges,[4] all hydrocarbon linkers,[5] oxime,[6] perfluorobenzyl thioether[7] and click reactions.[8] Recently, Moore *et al.* reported that an in-tether chiral center would influence the secondary structures of stapled peptides.[9] Meanwhile, we reported that an in-tether chiral center will dominate the secondary structure of peptides with single-bonded tethers.[10] In this report, we further demonstrate that the chirality-induced helicity concept could be translated into a sulfur-based chiral center of sulfilimine (Fig. 1). Structural elucidation of a helical peptide containing an in-tether carbon chiral center unambiguously showed that the absolute configuration of the chiral center should be R as shown in Fig. 1,[10] and we propose that the sulfilimine center will also have an R configuration.

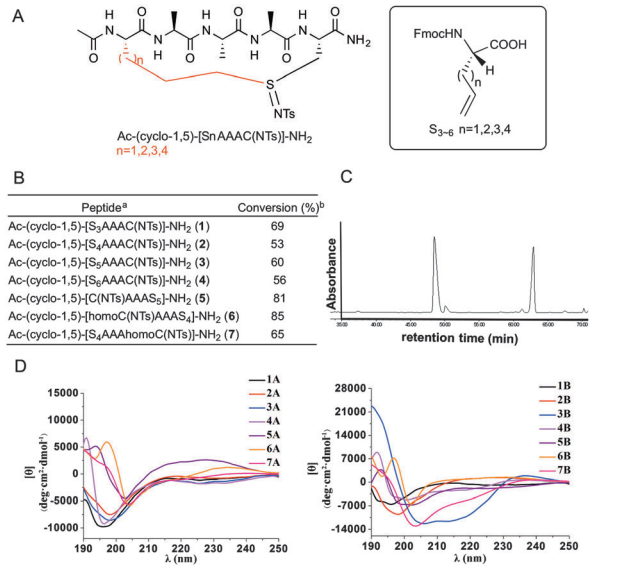
Fig. 1 An in-tether chiral center will influence the backbone peptides' secondary structure. Abbreviation: Ts, *p*-tolylsulfonyl group.

Pentapeptides were prepared to mimic a single-turn helix, to avoid any sequence perturbation. Different methods for sulfilimine preparation were tested and chloramine T stood out as the best nitrenoid transfer reagent (see Tables S1–S3 for detailed conditions screened, ESI†).[11] Compared with other reported reactions such as RCM and azide–alkyne click reactions to construct a tether, the chloramine T oxidation method was free of metal catalysts and compatible with aqueous solution. Then the resulting peptide diastereomers were separated by reverse phase HPLC and their helicity contents were examined by circular dichroism (CD) spectroscopy and are summarized in Fig. 2. Peptides **1**–**7** with different tether lengths (peptides **1**–**4**) or different linking patterns (peptides **5**–**7**) were prepared and converted into the corresponding sulfilimine easily by chloramine-T oxidation as shown in Fig. 2A. Sulfilimines were obtained in satisfying yields varying from 53% to 85% as summarized in Fig. 2. The peptide diastereomers were separated by HPLC and the separation spectrum of peptide **3** is presented in Fig. 2C. The peptide diastereomers were called peptides **A** and **B** based on their HPLC retention time respectively. The CD spectra of peptides **1A** to **7A** and peptides **1B** to **7B** are summarized in Fig. 2D. The results clearly indicated that only peptide **3B** showed characteristic features of a helical structure in water. When trifluoroethanol (TFE) was added to aqueous **3B** solution, the CD spectrum of **3B** showed some 3_{10} helix features (Fig. S6, ESI†), indicated by the increased intensity at 204 nm and reduced intensity at 190 nm. Notably, for a seven-atom tether, when the S = NTs chiral center was switched to positions other than peptide **3**, the helicity greatly diminished in peptides **5**, **6** and **7** (Fig. 2D). Thus, 20-membered ring size with the S = NTs chiral center positioned at the second site from the C terminus was determined as the optimal linking strategy, similar to the carbon chiral center case.[10]

Analogous cyclic pentapeptides **8**–**14** with different substituted amino acid residues were prepared and tested for their secondary structure in H_2O as shown in Fig. 3. Yields comparable to

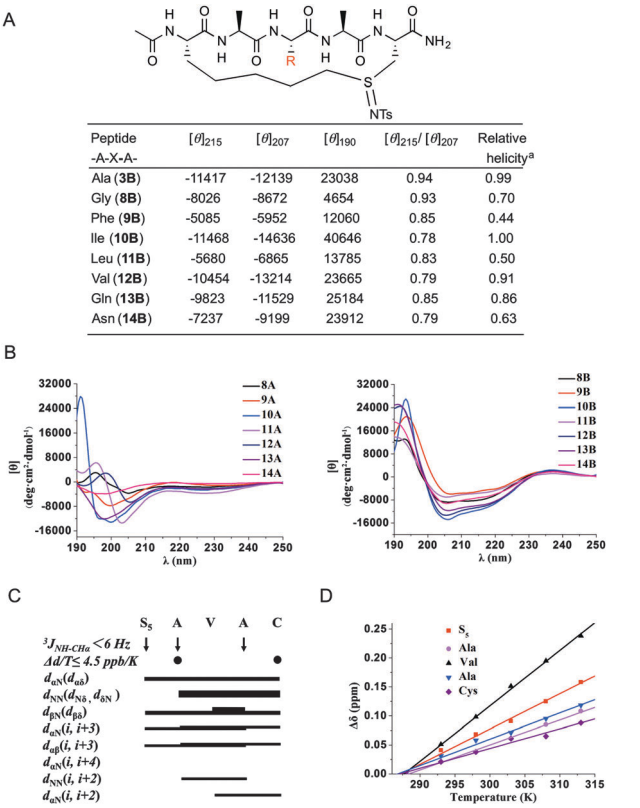
School of Chemical Biology and Biotechnology, Peking University Shenzhen Graduate School, Shenzhen, 518055, China. E-mail: lizg@pkusz.edu.cn

† Electronic supplementary information (ESI) available: Experimental procedures, supporting tables and figures. See DOI: 10.1039/c6cc04508a



Peptide[a]	Conversion (%)[b]
Ac-(cyclo-1,5)-[S_3AAAC(NTs)]-NH_2 (**1**)	69
Ac-(cyclo-1,5)-[S_4AAAC(NTs)]-NH_2 (**2**)	53
Ac-(cyclo-1,5)-[S_5AAAC(NTs)]-NH_2 (**3**)	60
Ac-(cyclo-1,5)-[S_6AAAC(NTs)]-NH_2 (**4**)	56
Ac-(cyclo-1,5)-[C(NTs)AAAS_5]-NH_2 (**5**)	81
Ac-(cyclo-1,5)-[homoC(NTs)AAAS_4]-NH_2 (**6**)	85
Ac-(cyclo-1,5)-[S_4AAAhomoC(NTs)]-NH_2 (**7**)	65

Fig. 2 (A) Structures of cyclic sulfilimine pentapeptides **1–4**. (B) Conversion of thioether peptides into sulfilimine peptides **1–7**. Abbreviation: homoC, homocysteine. (C) Representative HPLC separation of peptide diastereomers. (D) CD spectroscopy of peptides **1A–7A** and **1B–7B** in H_2O at 25 °C. [a] Reaction conditions: thioether peptide (1.0 equiv.), chloramine-T (1.2 equiv.) in CH_3CN at r.t. for 24 h. [b] Yield after HPLC purification and calculated using 1,3,5-tribromobenzene as the internal standard.



Peptide -A-X-A-	$[\theta]_{215}$	$[\theta]_{207}$	$[\theta]_{190}$	$[\theta]_{215}/[\theta]_{207}$	Relative helicity[a]
Ala (**3B**)	-11417	-12139	23038	0.94	0.99
Gly (**8B**)	-8026	-8672	4654	0.93	0.70
Phe (**9B**)	-5085	-5952	12060	0.85	0.44
Ile (**10B**)	-11468	-14636	40646	0.78	1.00
Leu (**11B**)	-5680	-6865	13785	0.83	0.50
Val (**12B**)	-10454	-13214	23665	0.79	0.91
Gln (**13B**)	-9823	-11529	25184	0.85	0.86
Asn (**14B**)	-7237	-9199	23912	0.79	0.63

Fig. 3 (A) Molar ellipticities $[\theta]_{215}$, $[\theta]_{207}$, $[\theta]_{190}$, $[\theta]_{215}/[\theta]_{207}$ and relative helicity of **3B** and **8B–14B**. (B) CD measurements of **8A–14A** and **8B–14B** in H_2O at 25 °C. (C) Two dimensional NMR summary results of **12B** performed in 90% H_2O : 10% D_2O at 25 °C. Distance restraints in ROEs and their intensities were reflected in the bar thickness. (D) The dependence of amide NH chemical shifts on temperature for each residue at 288 K, 293 K, 298 K, 303 K, 308 K and 313 K. [a]Relative helicity refers to $[\theta]_{215}$(**X**)/$[\theta]_{215}$(**10B**).

peptides **1–7** were achieved, indicating that the preparation of sulfilimines using chloramine-T was compatible with different functional groups (Table S4, ESI†). CD spectra of peptides **8A–14A** showed a single deep minimum at 200–205 nm, supporting the adoption of a random coil structure in H_2O, while their **B** diastereomers **8B–14B** displayed good characteristic features of an α-helix, indicated by a deep minimum at 215/207 nm and a high maximum at 190 nm (Fig. 3B). The significant difference between A and B diastereomers of peptides **8–14** in CD spectra confirmed the decisive stabilization effect of the S = NTs chiral center. Details of molar ellipticities at λ = 215, 207 and 190 nm are summarized in Fig. 3. The high $[\theta]_{215}/[\theta]_{207}$ ratio of peptides **3B** and **8B–14B** demonstrated a well-defined α-helix in H_2O. The highest ratio 0.94 was found in peptide **3B**, while peptide **10B** with isoleucine had the lowest value of 0.78. Even in the case of substitution with an α-helix breaking residue glycine, peptide **8B** still showed satisfactory α-helicity with $[\theta]_{215}/[\theta]_{207}$ of 0.93. These results illustrated our strategy's good residue tolerance. As for the absolute configuration of the in-tether chiral center, although we didn't have direct evidence, it was very reasonable to propose an R absolute configuration for the sulfilimine center because the current sulfilimine system was translated from our previous report of an in-tether chiral carbon center.[10]

2D ^{1}H NMR spectroscopy in $H_2O:D_2O$ (9 : 1) at different temperatures was also conducted with peptide **12B**. The low amide coupling constants $^3J_{NH\text{-}CH\alpha} < 6$ Hz of S_5 and two alanine residues suggested their involvement in the helical structure (Fig. 3C). In addition, the temperature coefficient $\Delta\delta/T < 4.5$ ppb K^{-1} of the residues was also consistent with the helical structure. The low temperature coefficient $\Delta\delta/T$ of alanine and cysteine was indicative of their involvement in the α-helix. The temperature coefficient $\Delta\delta/T$ of each residue could be determined by line slopes intuitively (Fig. 3D). Among the five residues, the first alanine and fifth cysteine had the minimum line slopes. The observation of strong signals at $d_{\alpha N}(i,i+3)$ and $d_{\alpha\beta}(i,i+3)$ of **12B** suggested the adoption of α-helical conformation in H_2O.

To verify whether our α-helix stabilizing strategy was applied to induce left-handed α-helicity in D-pentapeptides, we prepared **15B** composed of all D amino acid residues, termed D-Ac-(cyclo-1,5)-[S_5AIAC(NTs)]-NH_2, which was the mirror image of **10B** composed of all L-residues. The CD spectrum of **15B** showed opposite absorption characteristics at 215 nm, 207 nm and 190 nm, suggesting a left-handed α-helix in **15B** (Fig. 4A). The neutralization titration by adding **15B** to **10B** solution showed that the right-handed α-helicity characteristic in the CD spectrum exhibited by **10B** was gradually offset by the addition of **15B**. The absorption curve returned to the baseline when the quantity ratio of **15B**/**10B** was 1 : 1. Then excess addition of **15B** to the L/D mixture exhibited left-handed α-helicity characteristic in the CD spectrum, which certified the feasibility of left-handed α-helicity induced by the sulfilimine chiral center with all D-residues (Fig. 4B).

Stabilities of α-helicity induced by the sulfilimine chiral center were explored by thermal denaturation and guanidine·HCl

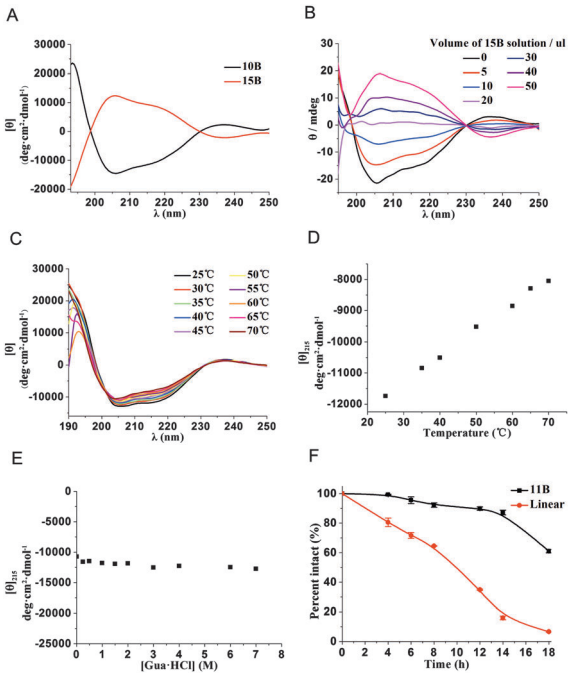


Fig. 4 (A) CD spectrum comparison between L-peptide **10B** and D-analog **15B**. (B) The absorption change trend of the CD spectrum in the neutralization titration experiment. Peptide **15B** solution was titrated into **10B** solution slowly until **15B** was excessive. (C) Full CD spectra of **3B** at different temperatures from 25 °C to 70 °C. (D) Molar ellipticity at 215 nm of **3B** at different temperatures from 25 °C to 70 °C. (E) Molar ellipticity at 215 nm of **12B** under the condition of increasing guanidine·HCl at 25 °C. (F) *In vitro* serum digestion assay of peptide **11B** and its linear analog.

denaturants (Fig. 4). CD measurements of **3B** in H_2O from 25 °C to 70 °C showed that **3B** maintained a well-defined α-helix even at 70 °C (Fig. 4C). A gradual increase in molar ellipticity was observed at 215 nm when the temperature was increased from 25 °C to 70 °C, which indicated that the α-helix content decreased in **3B** (Fig. 4D). Despite some α-helix unwinding, over 60% α-helicity was preserved even at 70 °C, which suggested that the sulfilimine chiral center-induced α-helicity was thermally stable. Pentapeptide **12B** was used for chemical denaturation under the condition of increasing guanidine·HCl from 0 to 7 M. The molar ellipticity at 215 nm of **12B** remained almost unchanged with increasing guanidine·HCl, suggesting that the α-helical conformation of **12B** was stable even in the presence of high concentration of guanidine·HCl (Fig. 4E). Besides, the *in vitro* serum stability assay showed that more than 90% of the helical peptide **11B** with the sulfilimine chiral center remained intact after 10 hours while over 50% degradation of its linear analog, termed Ac-S_5ALAC(SH)-NH_2, was observed (Fig. 4F). Thus, peptide **11B** with enhanced helicity induced by the sulfilimine chiral center showed better proteolytic resistance.

In summary, we have demonstrated a novel and effective helix-stabilizing strategy by introducing a precisely positioned S = NTs chiral center *via* chloramine-T oxidation. The tether length and S = NTs positions were screened for the best helix stabilization effects. Furthermore, we have proven that the absolute configuration of the S = NTs chiral center dominates the backbone peptides' secondary structure. Namely, the B diastereomers with longer retention time in HPLC spectra showed better helical contents in H_2O while A diastereomers appeared as random coils. CD and 2D NMR experiments provided strong evidence to support the secondary structure determination. This new stapling methodology presented good tolerance to different residues, including glycine. Notably, this novel helicity-induced method showed good thermal stability, in guanidine·HCl denaturation as well as serum digestion. This discovery enriches the toolbox of peptide stabilization and helps in further understanding the correlation between the in-tether chiral center and the backbone peptides' secondary structures, and it also provides one valuable modification site for further applications.

We acknowledge financial support from the National Natural Science Foundation of China (Grant 21102007, 31300600 and 21372023), MOST 2015DFA31590, MOST 2013CB911500, the Shenzhen Science and Technology Innovation Committee (KQCX20130627103353535, SGLH20120928095602764, ZDSY2013-0331145112855 and JSGG20140519105550503) and the Shenzhen Peacock Program (KQTD201103). We thank the Beijing NMR Center at Peking University for their help.

Notes and references

1 D. P. Ryan and J. M. Matthews, *Curr. Opin. Struct. Biol.*, 2005, **15**, 441.
2 (*a*) D. J. Barlow and J. M. Thornton, *J. Mol. Biol.*, 1988, **201**, 601; (*b*) D. P. Fairlie, M. L. West and A. K. Wong, *Curr. Med. Chem.*, 1998, **5**, 29.
3 (*a*) M. Pellegrini, M. Royo, M. Chorev and D. F. Mierke, *J. Pept. Res.*, 1997, **49**, 404; (*b*) D. Y. Jackson, D. S. King, S. Singh and P. G. Schultz, *J. Am. Chem. Soc.*, 1991, **113**, 9391; (*c*) J. H. B. Pease, R. W. Storrs and D. E. Wemmer, *Proc. Natl. Acad. Sci. U. S. A.*, 1990, **87**, 5643.
4 (*a*) J. W. Taylor, *Biopolymers*, 2002, **66**, 49; (*b*) N. E. Shepherd, H. N. Hoang, G. Abbenante and D. P. Fairlie, *J. Am. Chem. Soc.*, 2005, **127**, 2974; (*c*) G. Osapay and J. W. Taylor, *J. Am. Chem. Soc.*, 1992, **114**, 6966.
5 (*a*) H. E. Blackwell and R. H. Grubbs, *Angew. Chem., Int. Ed.*, 1998, **37**, 3281; (*b*) C. E. Schafmeister, J. Po and G. L. Verdine, *J. Am. Chem. Soc.*, 2000, **122**, 5891; (*c*) H. E. Blackwell, J. D. Sadowsky, R. J. Howard, J. N. Sampson, J. A. Chao, W. E. Steinmetz and R. H. Grubbs, *J. Org. Chem.*, 2001, **66**, 5291; (*d*) F. Bernal, M. Wade, M. Godes, T. N. Davis, D. G. Whitehead, A. L. Kung and L. D. Walensky, *Cancer Cell*, 2010, **18**, 411; (*e*) F. Bernal, A. F. Tyler, S. J. Korsmeyer, L. D. Walensky and G. L. Verdine, *J. Am. Chem. Soc.*, 2007, **129**, 2457.
6 C. M. Haney, M. T. Loch and W. S. Horne, *Chem. Commun.*, 2011, **47**, 10915.
7 A. M. Spokoyny, Y. K. Zou, J. J. Ling, H. T. Yu, Y. S. Lin and B. L. Pentelute, *J. Am. Chem. Soc.*, 2013, **135**, 5946.
8 Y. H. Lau, P. Andrade, N. Skold, G. J. Mckenzie, C. Verma, D. P. Lane and D. R. Spring, *Org. Biomol. Chem.*, 2014, **12**, 4074.
9 T. E. Speltz, S. W. Fanning, C. G. Mayne, E. Tajkhorshid, G. L. Greene and T. W. Moore, *Angew. Chem., Int. Ed.*, 2016, **55**, 4252.
10 K. Hu, H. Geng, Q. Z. Zhang, Q. S. Liu, M. S. Xie, H. C. Lin, F. Jiang, T. Wang, Y. D. Wu and Z. G. Li, *Angew. Chem., Int. Ed.*, 2016, **55**, 8013.
11 For details of synthesis of sulfilimines, see (*a*) F. Collet, R. H. Dodd and P. Dauban, *Org. Lett.*, 2008, **10**, 5473; (*b*) O. G. Mancheno and C. Bolm, *Org. Lett.*, 2006, **8**, 2349; (*c*) O. G. Mancheno, J. Dallimore, A. Plant and C. Bolm, *Org. Lett.*, 2009, **11**, 2429; (*d*) G. Y. Cho and C. Bolm, *Org. Lett.*, 2005, **7**, 4983; (*e*) A. L. Marzinzik and K. B. Sharpless, *J. Org. Chem.*, 2001, **66**, 594.