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New Class of Heterogeneous Helical Peptidomimetics

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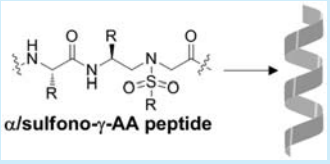
Supporting Information

ABSTRACT: A new class of unnatural heterogeneous foldamers is reported to contain alternative α-amino acid and sulfono-γ-AA amino acid residues in a 1:1 repeat pattern. Two-dimensional NMR data show that two 1:1 α/sulfono-γ-AA peptides with diverse side chains form analogous right-handed helical structures in solution. The effects of sequence length, side chain, N-capping, and temperature on folding propensity were further investigated using circular dichroism and small-angle X-ray scattering.



Unnatural oligomers that fold into well-defined three-dimensional structures, so-called "foldamers", have attracted considerable interest in the past decade.[1] Examples include β-peptides,[2] oligoureas,[3,4] peptoids,[5] β-sulfonamido peptides,[6,7] α-aminoxy peptides,[8] $\alpha/\beta/\gamma$ peptides,[9,10] urea/amide and urea/carbamate,[11] β-sheet mimetics,[12] etc. These foldamers are designed based on either homogeneous or heterogeneous backbones. They are designed to not only retain the structural and functional message of peptides or proteins but also display novel functions due to the presence of unnatural backbones and their discrete folding propensities.[2a,b,11] Compared to native peptides, unnatural foldamers can be advantageous in biological applications due to their enhanced resistance against proteolytic degradation and increased functional diversity.[10] The creation of these frameworks has led to synthetic oligomers, which exhibit a range of interesting structures and useful functions.

Based on the chiral PNA backbone that comes from the reduced dipeptides,[13] we have recently developed a new class of peptidomimetics termed "γ-AA peptides" (Figure 1),[14] which are

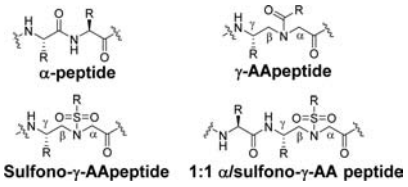


Figure 1. General structures of α-peptides, γ-AA peptides, sulfono-γ-AA peptides, and 1:1 α/sulfono-γ-AA heterogeneous peptides.

oligomers of N-acylated-N-aminoethyl amino acids. Akin to other peptidomimetics, γ-AA peptides are resistant to enzymatic degradation and are mendable for extensive diversification.[14,15] Meanwhile, γ-AA peptides have shown great promise in biomedical and material sciences.[16]

One of the most attractive features of γ-AA peptides is that the potential to introduce chemically diverse functional groups is enormous, as one-half of the side chains are introduced by any acylating agents, which are not limited to carboxylic acids. For instance, a myriad of functional side chains can be introduced by reacting sulfonyl chlorides with the secondary nitrogen on the backbone, which leads to the creation of sulfono-γ-AA peptides (Figure 1).[17] We have recently shown that homogeneous sulfono-γ-AA peptides can adopt the α-helix-like conformation in solution.[18] However, peptidomimetics based on heterogeneous backbones have recently attracted considerable interest, as they significantly increase the availability of molecular frameworks, three-dimensional structures, and functions.[9,11,19] We believe it is also important to understand the folding propensity of the α/sulfono-γ-AA heterogeneous peptides to develop a new class of foldamers with novel functions. As each sulfono-γ-AA peptide building block is comparable to a dipeptide residue, an α/sulfono-γ-AA heterogeneous peptide theoretically projects the same number of side chains as the α-peptide of the same length (Figure 1), suggesting its potential for α-peptide mimicry. Sulfonamide groups in α/sulfono-γ-AA peptides are bulky and may induce a curvature conformation of the backbone.[18] Furthermore, the presence of α-amino acid residues in the heterogeneous backbone contributes more amide hydrogens to the current sulfono-γ-AA peptide backbone, which can potentially stabilize the folding conformation through intramolecular hydrogen bonding. In addition, polar sulfonyl groups may also participate in hydrogen bond formation to enhance the folding propensity. Thus, we hypothesize that α/sulfono-γ-AA heterogeneous peptides may possess certain folding conformations.

To test our hypothesis, we synthesized a few heterogeneous peptides of variable lengths containing alternative α and sulfono-γ-AA amino acid residues (Figure 2). **h1** and **h2** are two of the longest sequences with different side groups and were chosen to study their solution structures by 2D NMR. To ensure the solution structures could be determined by 2D NMR unambiguously based on NOEs, a few different hydrophilic

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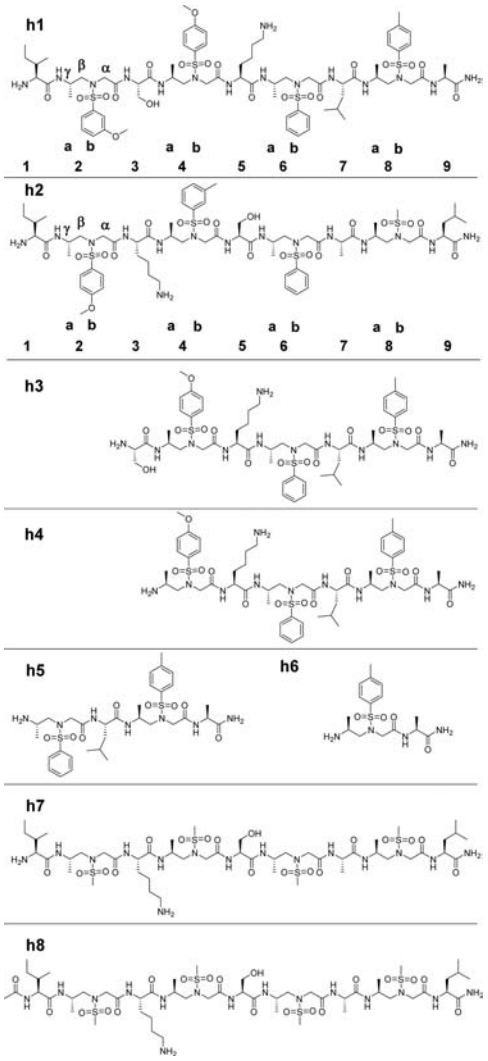

Figure 2. Structures of the heterogeneous peptides **h1**−**h8** that contain alternative α and sulfono-γ-AA amino acid residues. In **h1** and **h2**, odd-numbered residues are α-amino acid residues; even-numbered residues are sulfono-γ-AA amino acid residues. In each sulfono-γ-AA amino acid residue, **a** denotes the chiral side chain and **b** represents the sulfonyl side chain. α, β, and γ represent three different carbons in a sulfono-γ-AA amino acid residue.

and hydrophobic side chains were chosen in both sequences. Sequences **h3**−**h8** were also synthesized to understand factors that may affect the folding propensity of this class of peptidomimetics, such as lengths, side chains, and capping groups. All α/sulfono-γ-AA heterogeneous peptides were synthesized on the solid phase following a procedure adapted from our previous protocol (Scheme S1).

The structure of **h1** was analyzed by 2D NMR (2 mM, CD_3OH, 10 °C). The NMR peaks were assigned based on NOESY, DQF-COSY, and zTOCSY spectra (Figure S1). Although the heterogeneous backbone of **h1** is different from that of canonical peptides, the assignment of protons could be achieved. Briefly, determination of protons in α-amino acid residues and the chiral side chains of γ-AA amino acid residues was identical to the assignment strategies of protons in α-peptides. The aromatic rings of side chains 2b, 4b, 6b, and 8b were different from each other and could be identified unambiguously. Protons on 2β, 4β, 6β, and 8β were extrapolated based on the cross-peaks between aromatic protons and/or amide protons of the following α-amino acid residues in the NOESY spectrum (Figure S1). Protons on 2α, 4α, 6α, and 8α were also assigned under the assistance of the TOCSY and DQF-COSY spectra (Table S1). Thus, plenty of NOEs, including both sequential and nonsequential, were determined by NOESY (Figures S2 and Figure 3).

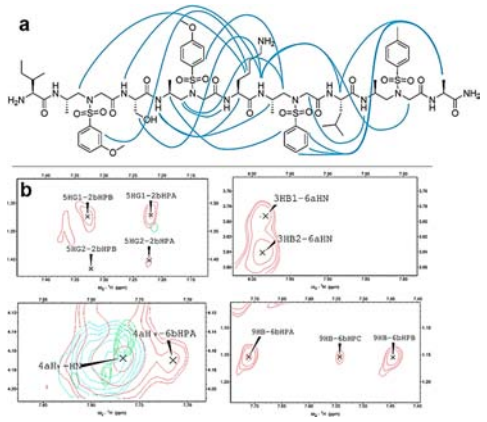

Figure 3. (a) Structure of α/sulfono-γ-AA peptide **h1** with NOEs observed in CD_3OH between nonadjacent residues indicated by curved lines. (b) Examples of NOEs showing interaction between *i* and *i*+2/*i*+3 residues.

As shown in Figure 3a,b, a number of interactions between *i* and *i*+2 or *i*+3 are observed, implying there is a regular pattern in the conformation of the solution structure of **h1**. This suggests that **h1** may have a well-defined secondary folding structure. As such, Maestro Macromodel[20] was used to carry out NOE-restrained molecular dynamics calculations (Tables S2 and S3). The 10 best structures were generated (Figure 4a), and they

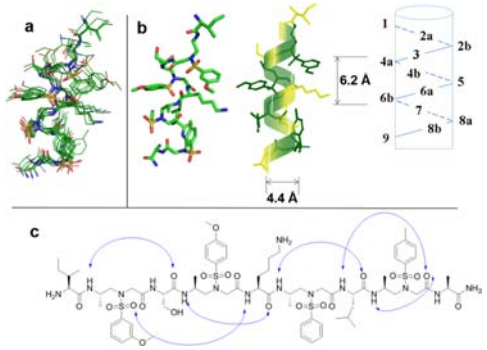

Figure 4. (a) Superimposition of the 10 best structures of **h1** generated by NOE-restrained molecular dynamics. (b) Left: average of the 10 structures. Center: helical scaffold overlaid on the average structure to guide the view. Right: approximate positions of residues on a helical cylinder (position of residue 1 is hypothesized as it is unstructured at the termini). (c) Possible hydrogen bonds based on the average structure.

display a good overlap on their backbones (rmsd = 0.89 ± 0.15 Å, Table S4). The average structure of these 10 helical structures for **h1** is shown in Figure 4b. Interestingly, it suggests that **h1** adopts a right-handed helical conformation in methanol, with a helical radius (2.2 Å) nearly the same as that of the canonical α-helix (2.3 Å). However, the helical pitch (6.2 Å) closely resembles the polyproline I helix (6.3 Å). The NMR structure suggests that each turn contains approximately four side chains (Figure 4b), which is similar to that of the α-helix (3.6 residues/turn).

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Potential hydrogen bonds are identified and shown in Figure 4c. As expected, both backbone amides and sulfonyl groups have contributed to the formation of hydrogen bonds, which stabilize the helical structure.

The existence of hydrogen bonds in **h1** was also assessed by H/D exchange studies (Figure S3). Some NH resonances disappeared in 1 h, but most of the backbone NH resonances were still discernible, and some could even be detected after 24 h. The result suggests that the helical structure is stabilized by hydrogen bonds that do not participate in H/D exchange quickly.

To compare the solution structure of **h1** with another α/sulfono-γ-AA peptide bearing different side chains, to assess the impact of functional groups on the α/sulfono-γ-AA heterogeneous scaffold, the structure of **h2** in methanol was then also analyzed by 2D NMR (2 mM, CD_3OH, 10 °C). Again, plenty of NOEs were detected by NOESY (Figure S4). Similar to **h1**, there is a defined pattern of NOEs observed between i and i+2 residues (Figures S5 and S6), strongly suggesting the existence of the secondary folding structure.

Maestro Macromodel[20] was then used to carry out NOE-restrained molecular dynamics calculations (Tables S7 and S8). The 10 best structures were generated (Figure S8a) that display a good overlap on their backbones (rmsd = 1.08 ± 0.34 Å, Table S9). The average of these 10 helical structures for **h2** is shown in Figure S8b.

NMR results suggest that **h2** adopts a right-handed helical structure very similar to that in **h1**. Consistent with **h1**, H/D exchange study of **h2** (Figure S7) suggests that the helical structure is stabilized by hydrogen bonds, which are not replaced by deuterium from the solvent as fast as the free protons. In addition, although the number of hypothesized hydrogen bonds are slightly different (Figure S8c), the helical scaffolds of **h1** and **h2** overlap very well (Figures S8d and S9). The positions of their side chains are similar on the scaffold, except for the N-terminus, which is less structured in solution. As the side chains of **h1** and **h2** are all random, the results suggest that this class of α/sulfono-γ-AA heterogeneous peptides has a general folding propensity to adopt right-handed helical structures. Note that hydrogen bonding patterns in both sequences are rather different. We believe this is because these solution structures are calculated based on the average of ensemble dynamic structures. Meanwhile, we intentionally chose unconstrained sequences in the studies to assess the general folding propensity of this class of peptidomimetics. As the backbone is flexible, it is difficult to propose a well-defined folding pattern at this point. However, crystallization of some constrained sequences may help to gain insight into this issue, which is currently under investigation in our lab.

Compared to classic γ-AA peptides, α/sulfono-γ-AA peptides have much more defined folding propensity. This is because classic γ-AA peptides contain tertiary amido moieties, which could adopt cis/trans conformations, making solution structural analysis impossible. However, replacement of these moieties with sulfonamido groups eliminates the issue. More importantly, as sulfonamido groups are bulky, they force the formation of curvatures in the sequences, which induces the helical propensity. This has been observed in our recently reported homogeneous sulfono-γ-AA peptides. It is possible that oxygens in sulfonamido groups participate in hydrogen bonding in the sequences, as seen in Figure 4; however, we believe that this effect is minor in the formation of helical structures compared to their bulkiness. As α/sulfono-γ-AA peptides contain α-amino acid residues, we also compared the conformation of these residues to α-amino acid residues in an α-helix. Again, they are quite different from each other (Tables S5 and S10), which may due to their unconstrained backbone in the solution and rather different folding conformations existing in α/sulfono-γ-AA peptides and α-helices.

To correlate the helical structure to the circular dichroism (CD) spectrum, to quickly assess the folding propensity of α/sulfono-γ-AA peptides in the future, we then first carried out the CD studies (Figure 5) for both **h1** and **h2**. They display very

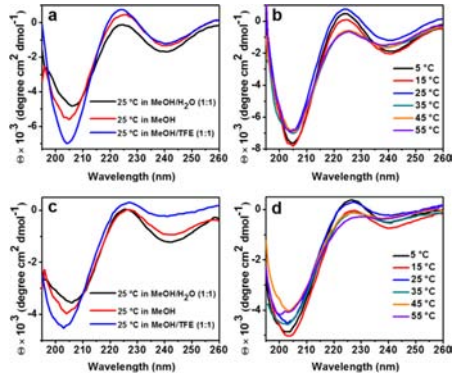

Figure 5. CD data for α/sulfono-γ-AA peptides **h1** and **h2**. (a) **h1** (100 μM) in CH_3OH, 1:1 (v/v) CH_3OH/H_2O, and 1:1 (v/v) CH_3OH/TFE. (b) **h1** (100 μM) in CH_3OH at various temperatures. (c) **h2** (100 μM) in CH_3OH, 1:1 (v/v) CH_3OH/H_2O, and 1:1 (v/v) CH_3OH/TFE. (d) **h2** (100 μM) in CH_3OH at various temperatures.

similar CD signatures. For both **h1** and **h2**, the CD data reveal a minimum around 204 nm (Figure 5a,c), which is similar to the CD of a helical peptide containing an $\alpha/\beta/\gamma$ backbone reported recently.[9] Compared with homogeneous sequences containing only sulfono-γ-AA peptide residues, introduction of α-amino acid residues led to a blue shift in the CD spectra. Such a pattern was also revealed in the cases of α/β-peptides and α/γ-peptides.[21−24] The solvent effect on helical stability was also investigated and is shown in Figure 5a,c. It is not surprising that the sequences adopt the best helical conformations in TFE (trifluoroethanol), an excellent solvent-promoting secondary structure folding of peptides. It is noted that the sequences also retained a certain degree of helicity in water, although the population is less than that in TFE and methanol. Because canonical α-peptides only form α-helices with lengths >15, the folding propensity of α/sulfono-γ-AA peptides is fairly strong. The stability of the helical propensity of the sequences in methanol was further evaluated by temperature-dependent CD studies. The intensity of the minimum at 204 nm only slightly decreased when temperature increased from 5 to 55 °C (Figure 5b,d). Compared with sulfono-γ-AA peptides, which showed a considerable decrease in helical contents as temperature increased,[18] α/sulfono-γ-AA peptides display a much higher helix propensity.

To understand the relationship between helical folding propensity and the length of the sequence, the CD of shorter sequences **h3**−**h6** were also investigated. As shown in Figure S10a, there is a length-dependent decrease in signal intensity at 204 nm from **h1** to **h4**, akin to α-helical peptides. As expected, two short sequences, **h5** and **h6**, which are comparable to a hexa- and a tripeptide, respectively, disfavor the formation of a helical structure.

The effects of aromatic substituents were also investigated by CD. Sequence **h7** was prepared by replacing all the aromatic

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sulfonamido side chains of **h2** with alkyl side chains (methanesulfonamides). The CD spectra of **h7** show a minimum at 201 nm, with a slight blue shift compared with **h2** (Figure S10b). Such change was also revealed in the studies of peptoid helical foldamers.[25,26] However, in the region of 220−240 nm, there is a change in the shape, missing a slight maximum at 222 nm (Figure S10b). It is likely due to the substitution of alkyl side chains, as methoxylphenyl side chains mimic tyrosine residues, which are known to have positive contribution to the ellipticity at 220 nm.[27] In addition, N-Terminal capping groups were shown to stabilize helical foldamers (Figure S10b). Overall, the CD studies further support the potential of α/sulfono-γ-AA peptides for helical mimicry.

Small-angle X-ray scattering (SAXS) has become a powerful tool in the analysis of the solution structures of biomolecules and polymers. We have conducted SAXS analysis of a few sequences to provide additional information for the folding of α/γ-sulfono-AA peptides along with the CD data (Supporting information).

In summary, we have identified a new class of heterogeneous foldamer, which forms a right-handed helical structure in solution. The structural consistency of two different sequences demonstrates the general folding propensity of this foldamer class. Akin to regular α-peptides, the folding propensity of this class of 1:1 α/sulfono-γ-AA heterogeneous peptides in solution is dependent on their lengths. Since the helical structure can be further stabilized by a range of methods such as hydrocarbon stapling[28] and inclusion of constrained residues[9] and it is convenient to introduce a wide variety of functional groups into the sulfono-γ-AA peptides, we envision that this new type of foldamer will facilitate the development of novel molecules for many biological applications, such as molecular recognition, protein−protein interaction, catalysis, etc. This is also the first report of the α/sulfono-γ-AA heterogeneous peptide foldamer, and we believe the findings will greatly expand the scope of γ-AA peptides in biomedical research.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details, synthesis and characterization of **h1**−**h8**, 1D and 2D NMR spectra including NOEs and MD simulations, CD, and SAXS. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b01608.

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Author Contributions

§H.W. and Q.Q. contributed equally.

Notes

The authors declare no competing financial interest.

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