

Peptidomimetics

Sulfono- γ -AApeptides as a New Class of Nonnatural Helical FoldamerHaifan Wu,^[a] Qiao Qiao,^[a] Yaogang Hu,^[a] Peng Teng,^[a] Wenyang Gao,^[a] Xiaobing Zuo,^[b] Lukasz Wojtas,^[a] Randy W. Larsen,^[a] Shengqian Ma,^[a] and Jianfeng Cai^{*[a]}

Abstract: Foldamers offer an attractive opportunity for the design of novel molecules that mimic the structures and functions of proteins and enzymes including biocatalysis and biomolecular recognition. Herein we report a new class of nonnatural helical sulfono- γ -AApeptide foldamers of varying lengths. The crystal structure of the sulfono- γ -AApeptide monomer **S6** illustrates the intrinsic folding propensity of sulfono- γ -AApeptides, which likely originates from the bulkiness of tertiary sulfonamide moiety. The two-dimensional so-

lution NMR spectroscopy data for the longest sequence **S1** demonstrates a 10/16 right-handed helical structure. Optical analysis using circular dichroism further supports well-defined helical conformation of sulfono- γ -AApeptides in solution containing as few as five building blocks. Future development of sulfono- γ -AApeptides may lead to new foldamers with discrete functions, enabling expanded application in chemical biology and biomedical sciences.

Introduction

Natural biopolymers including proteins and nucleic acids adopt well-defined and compact three-dimensional folding conformations providing a structural basis for their complex biological functions.^[1] This paradigm suggests that nonnatural foldamers with new molecular frameworks and folding propensities may also exhibit unique biomimetic properties that can be exploited in pharmaceutical development and advanced biotechnology applications.^[2] Specifically, nonnatural foldamers offer opportunities to better understand biomolecular structure-function relationships, facilitate the design of novel nanostructures, and develop targeted diagnostic agents and potential drug candidates.^[3] Because nonnatural monomers have an enormous diversity in size, shape, and backbone structure, nonnatural foldamers can theoretically be developed to display a wide variety of three-dimensional conformations and biomimetic function.^[4] In addition, many nonnatural foldamers are resistant to proteolytic degradation, augmenting their potential application in biological systems. The potential importance of nonnatural foldamers has led to the development of numerous foldamer systems including β -peptides,^[5]

α -aminoxy-peptides,^[6] peptoids,^[7] and oligoureas.^[8] However, nonnatural foldamers have just recently begun to find biological applications and thus the continued development of new building blocks, molecular frameworks, and backbones are of key interest.

We recently developed a new class of peptidomimetics- γ -AApeptides, oligomers of *N*-acylated-*N*-aminoethyl amino acids (Figure 1).^[9] As half of the side chains are introduced through

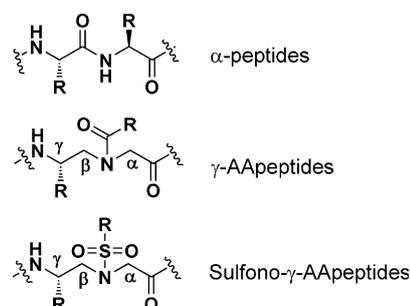


Figure 1. The general chemical structures of α -peptides, γ -AApeptides, and sulfono- γ -AApeptides.

acylation, γ -AApeptides have virtually limitless potential in functional group diversity. Although γ -AApeptides are based upon a chiral PNA backbone,^[10] they are designed to capture the function of bioactive peptides rather than nucleic acids.^[11] For instance, certain γ -AApeptides display both antimicrobial activity^[12] and anti-inflammatory activity^[13] by mimicking host-defense peptides, whereas others mimic the Tat peptide by binding to HIV-1 RNA with high affinity^[11b] and permeating cell membranes with excellent efficiency.^[11a] In addition, γ -AApeptides have been developed to mimic the RGD peptide^[14] and to

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form one-bead-one-compound libraries for the discovery of bioactive protein/peptide based ligands.^[15] Furthermore, γ -AApeptides can also form novel nanostructures akin to peptide-based biomaterials.^[16] The emerging importance of γ -AApeptides has heightened the interest in the folding propensity regarding biomolecular structure.

Results and Discussion

One of the most attractive features of γ -AApeptides is that half of the side chains do not have to be derived from carboxylic acids. As shown in Figure 1, replacement of carboxylic acids with sulfonyl chlorides leads to the generation of sulfonyl- γ -AApeptides.^[17] As a subclass of γ -AApeptides, sulfonyl- γ -AApeptides possess essentially unlimited functional diversity, as a wide variety of functionalized sulfonyl chlorides are either available commercially or can be readily synthesized.^[18] Moreover, Sulfonyl- γ -AApeptides contain the same number of side chains as regular peptides of the same length, affording the potential of sulfonyl- γ -AApeptides to mimic bioactive peptides. The presence of protons in the second amide moieties in sulfonyl- γ -AApeptides indicate these polymers may exhibit folding propensities through intramolecular hydrogen bonding akin to α -peptides. In addition, the tertiary sulfonamido moieties are sufficiently bulky to induce intrinsic curvature into the sulfonyl- γ -AApeptide backbone. Furthermore, half of the side chains of sulfonyl- γ -AApeptides are chiral, which may also impose conformational bias to further promote the formation of specific secondary conformation.

To test the hypothesis that sulfonyl- γ -AApeptide foldamers can form discrete secondary structures, we have synthesized a series of sulfonyl- γ -AApeptides of differing lengths. The longest sulfonyl- γ -AApeptide (**S1**) contains eight building blocks, comparable in length to a 16-mer peptide. The shortest sequences **S5** and **S6** are sulfonyl- γ -AApeptide monomers, which are equivalent to dipeptides (Figure 2).

The sulfonyl- γ -AApeptides **S1–S5** were obtained through solid-phase synthesis following our previously published protocol (Scheme 1).^[15a,17] In brief, the desired N-alloc γ -AApeptide residues^[15a,17] were attached sequentially on the solid support. After each N-alloc γ -AApeptide residue was added, the alloc protecting group was removed with Pd(PPh₃)₄ (10 mol% equiv) and Me₂NH·BH₃ (6 equiv) in DCM.^[19] Subsequently, the sulfonyl side group was introduced by reacting a sulfonyl chloride with the secondary nitrogen on the γ -AApeptide backbone. The synthetic cycle was repeated until the desired sequence was

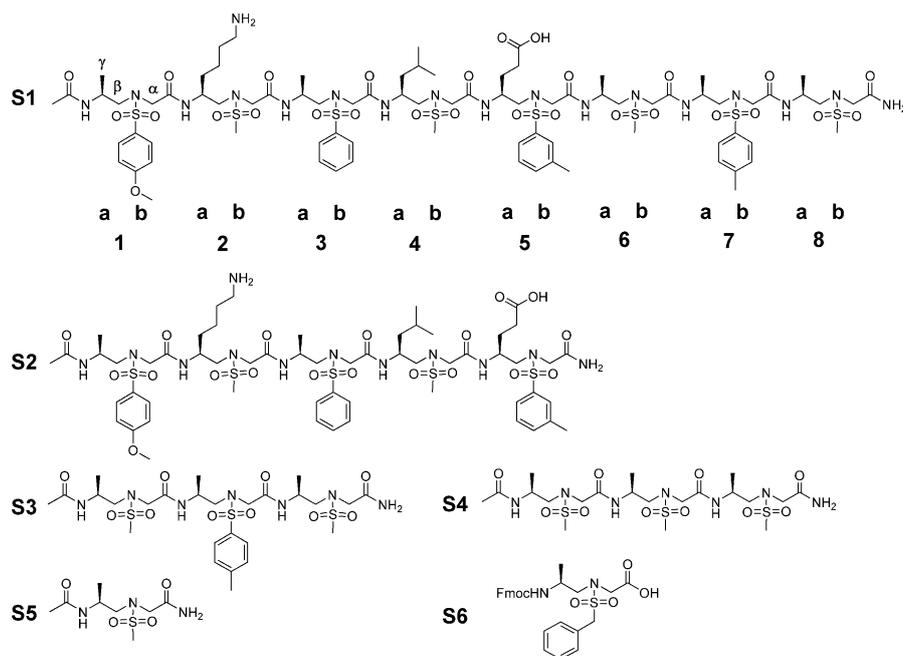
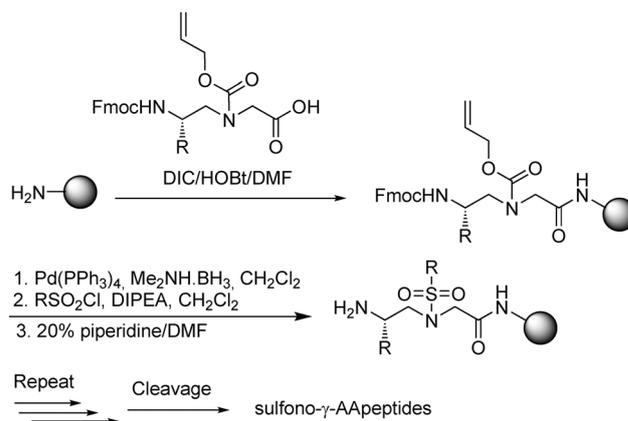


Figure 2. The chemical structures of the sulfonyl- γ -AApeptides **S1–S6**. In each sulfonyl- γ -AApeptide, the residues from the N-terminus are numbered as 1, 2, etc. In each sulfonyl- γ -AApeptide residue, **a** denotes the chiral side chain derived from the cognate α -amino acid, and **b** represents the sulfonyl side group coming from sulfonyl chlorides.



Scheme 1. Synthesis of the sulfonyl- γ -AApeptides **S1–S5**.

assembled, followed by cleavage and purification by HPLC (Figure S1). The monomer **S6** was prepared as previously reported.^[15a,17]

The crystal structure of the monomer **S6** was successfully obtained and shown in Figure 3a. The crystal structure indicates that the **S6** adopts a right-handed turn conformation. Consistent with our hypothesis, the bulky tertiary sulfonamido group appears to force the formation of the backbone curvature. In addition, there is a hydrogen bond formation between NH of N-termini and CO of C-termini. An overlay of **S6** with a canonical α -helical scaffold reveals that this turn curvature matches that of the α -helical sense (Figure 3b). The demonstration that such a short sulfonyl- γ -AApeptide has a defined pre-organized structure due to the intrinsic folding propensity

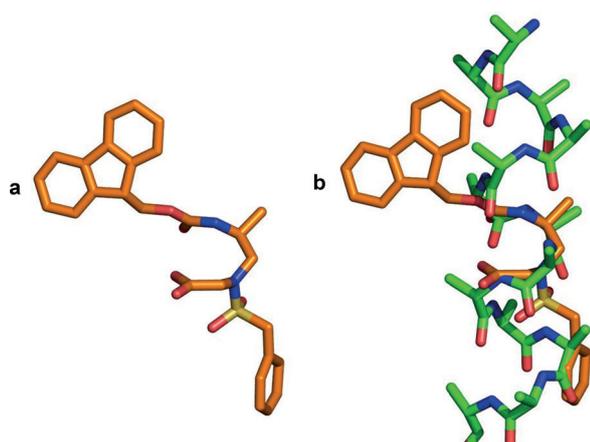


Figure 3. a) Crystal structure of the sulfono- γ -AApeptide monomer **56**; b) overlay of **56** on an α -helical polyalanine scaffold.

leads to the possibility that longer sulfono- γ -AApeptides can be formed with more defined and stable secondary structures.

We then carried out NMR studies of the longest sequence, **S1**. In order to solve the structure unambiguously, different hydrophobic and hydrophilic groups are included in the **S1** sequence. The NMR spectra were collected on an Agilent dd600 with a triple resonance cold probe. The 1D ^1H NMR spectra were first obtained with differing concentrations (0.05–1 mM) and the chemical shifts of the backbone protons were compared. There were no obvious changes in the chemical shifts, suggesting that **S1** does not aggregate under the experimental conditions (Figure S4). Next, 2D NMR spectroscopy was employed to investigate the solution structure of **S1** in methanol (2 mM in CD_3OH , 10°C). Two-dimensional spectra (zTOCSY, NOESY) were collected using standard pulse sequences with the number of acquisitions typically set to 200 for the NOESY and 6 for the zTOCSY spectra. The Water suppression through Enhanced T1 Effects (WET) method was used to suppress the proton peak in the CD_3OH solvent. In general, a 2 s delay was applied before each scan. Experiments were collected with 2 K complex data points in F2 for each of 300 t_1 increments with a sweep width of 6009 Hz in each dimension. Residue-specific assignments were made based on a combination of DQFCOSY, zTOCSY, ROESY, and NOESY spectra. The presence of different side chains eliminates potential overlaps between proton signals and is helpful for the unambiguous assignment of

different building blocks. The $\text{C}\alpha\text{H}$ s were successfully assigned based on the short-range and/or sequential NOEs with neighbor side chains or amide proton (Figure 4).

Medium/long range NOEs revealed clear $i-i+3$ correlations between related side chains, that is, 1bHPA–3aHB, 2aHB–3bHPA, 3bHPA–5aHB, 4aHG–5bHPA, 5bHPE–7aHB, 6aH γ –7bHPA (Figure 5). The $i-i+3$ correlation pattern implies that there is a defined folding pattern in **S1**, which displays proximity between every first and third building block.

The NMR solution structure was solved and is displayed in Figure 6. Schrödinger Macromodel^[20] was used to perform molecular dynamics calculations based upon the NOE constraints, which resulted in the ten best structures. As shown in Figure 6b, the structures display very good overlap among backbone atoms ($\text{rmsd} = (0.72 \pm 0.29) \text{ \AA}$, Figure 6b, and Table S3 in the Supporting Information). The average of the ten helical structures for **S1** is also displayed in Figure 6c and d. The data demonstrate that **S1** adopts a well-defined right-handed helical conformation in methanol, with the side chains pointing away from the helical scaffold. Further analysis of the structure of **S1** reveals a helical radius (2.3 \AA) that is the equivalent to the canonical α -helix. In addition, the average of helical pitches is 5.7 \AA , which is also very close to that of the peptide-based α -helix (5.4 \AA). Furthermore, the structure indicates that each turn contains four side chains (Figure 6e), relative to 3.6 residues per turn found in α -helical peptides. This assignment is also consistent with the observation of $i-i+3$ NOE patterns. These features suggest that the sulfono- γ -AApeptide could be developed to mimic the structure and function of α -helices.

The NMR structure further suggests that **S1** has a 10/16 helix hydrogen-bonding pattern (Figure 7). It is known that the

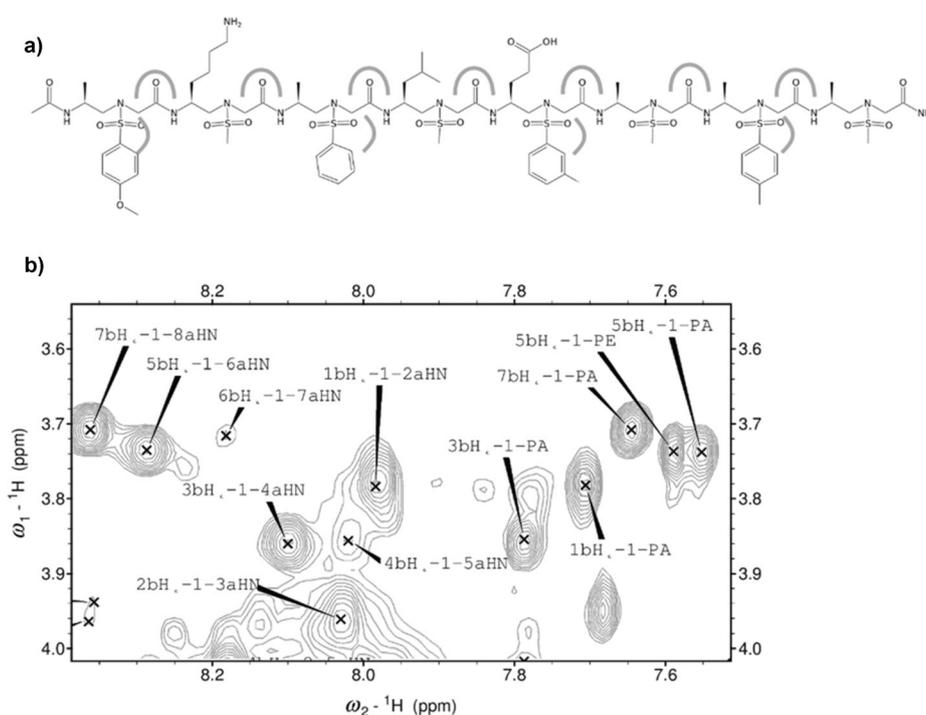


Figure 4. a) Typical sequential NOEs observed for **S1**. Residue numbers are labeled; b) corresponding peaks in NOE spectrum.

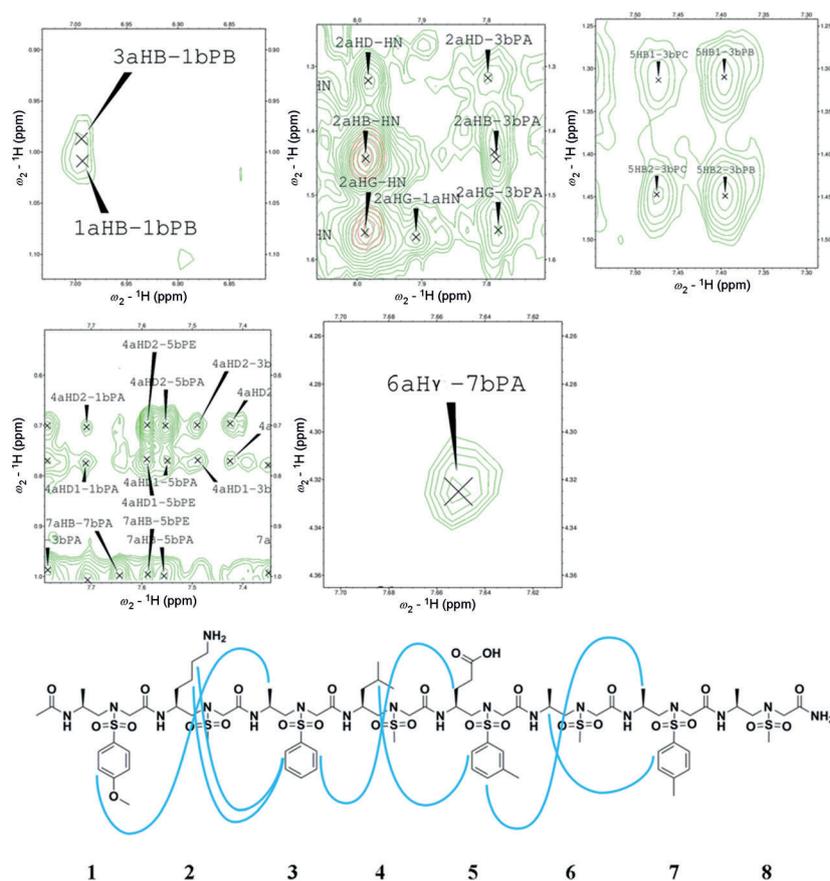


Figure 5. Typical medium or long range NOEs showing $i-i+3$ correlations in **S1**. Residue numbers are labeled. i stands for side chains.

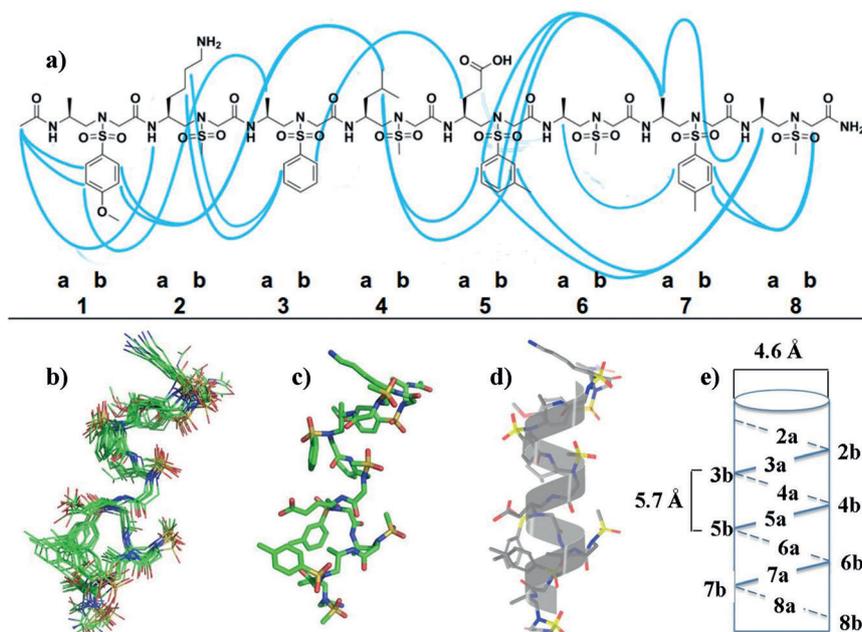


Figure 6. a) The chemical structure of the sulfono- γ -AApeptide **S1**, as well as nonsequential NOEs indicated by curved lines; b) overlay of the ten lowest energy three-dimensional structures of **S1** with lowest energies calculated based on NOE constraints (2D NMR analyses were carried out in CD_3OH at 10°C) and using MD simulations; c) the average structure based upon b); d) a helical ribbon is drawn to guide the review; e) Approximate positions of side chains on the helical scaffold. Residue 1, which is less ordered in solution, is omitted.

α -helix is the 13-helix; however, the same hydrogen pattern cannot be formed in sulfono- γ -AApeptides due to an alternative secondary amide and tertiary sulfonamide functionalities. Nonetheless, each 10/16 helix cycle in a sulfono- γ -AApeptide is equivalent to two successive 13-helices in the α -helix. This feature may partially explain why the helical pitch and the radius of **S1** are similar to those of the α -helix. Additionally, as expected, sulfonyl groups also contribute to the stability of the helical structure by directly participating in hydrogen bonding. SO_2 groups (except the first and last one) point away from the helical scaffold and do not participate in hydrogen bonding.

Circular Dichroism (CD) spectroscopy can also provide an assessment of the folding propensity of oligomers including proteins and peptides, as well as nonnatural molecules such as β -peptides and peptoids. The CD spectra of **S1** under different solvent conditions are displayed in Figure 8a. The spectra exhibit a maximum at ≈ 220 nm. Interestingly, it appears that the sequence adopts a more stable helical conformation in PBS buffer relative to TFE, suggesting the potential of sulfono- γ -AApeptide for the mimicry of the α -helix and modulation of protein interactions in biological systems. The CD data further indicate that the sequence is not aggregated under the concentration range examined here, as a ten-fold dilution in TFE has little or no effect on the spectrum. The stability of the helix was further examined by temperature-dependent CD analysis (Figure 8b). As expected, **S1** forms more defined helical structures at low temperatures. However, the secondary folding structure is still discernable even up to 55°C . To assess the general folding propensity of sulfono-

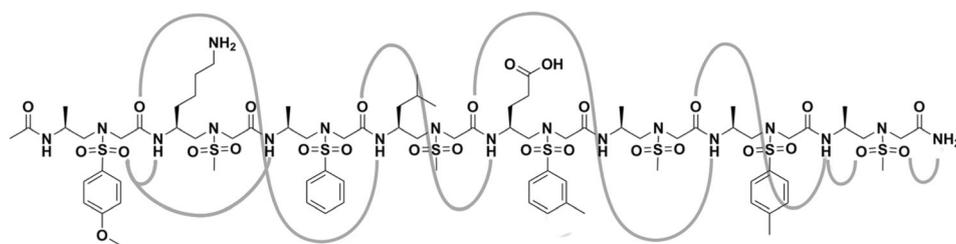


Figure 7. Possible hydrogen-bonding pattern suggested by the average of the ten best structures of **S1** generated by NOE-restrained molecular dynamics. The numbers indicate the number of atoms in the hydrogen-bonded rings, respectively.

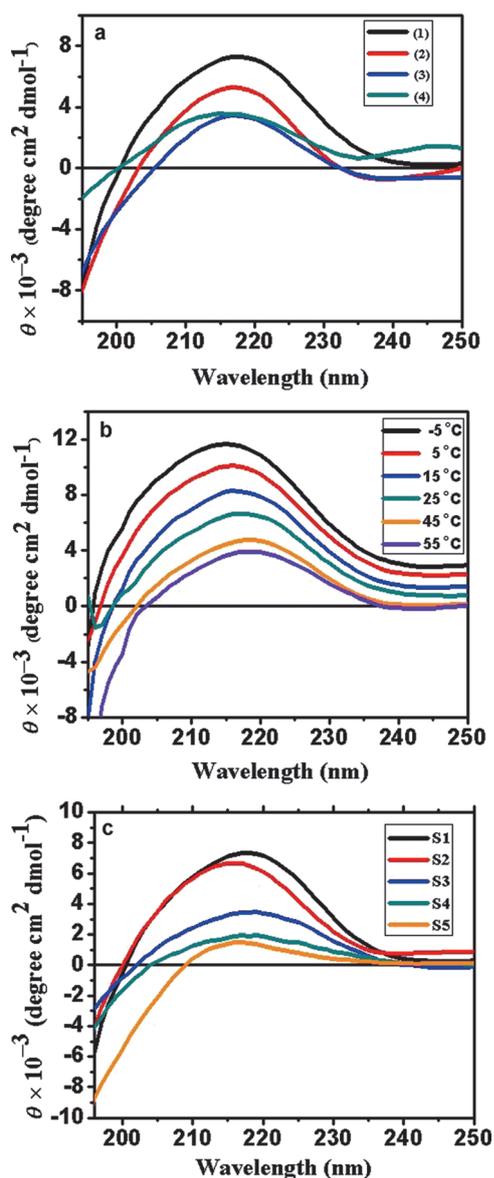


Figure 8. a), CD spectra of **S1** in different solutions at 25 °C. 1) in methanol, 200 μM ; 2) in 1:1 methanol/H₂O, 200 μM ; 3) in 1:1 methanol/TFE, 200 μM ; 4) in 1:1 methanol/TFE, 20 μM ; b), CD spectra of **S1** at various temperatures in methanol. c) CD spectra of **S1**–**S5** (200 μM) in methanol at 25 °C.

γ -AApeptides, CD analysis was also carried out for the **S2**–**S5** sulfono- γ -AApeptide sequences. As shown in Figure 8c, even

the shortest sequence **S5** displayed some degree of helicity, which is consistent with the crystal structure of **S6** foldamer. The clear trend is that longer sequences form better helical structures. The **S4** is a trimer sequence that displays increased helicity relative to **S5**. This is similar to the helical propensity of α -peptides. Additionally, the bulky group appears to stabilize the helical conformation, as observed with another trimer sequence **S3**, which contains an aromatic group and exhibits much more discernable helicity than **S4**.

Surprisingly, the pentamer sequence **S2**, which is comparable to a decamer peptide, displays almost identical helicity to the longest sequence **S1**. This indicates that the general helical propensity of sulfono- γ -AApeptides is quite high. However, as our peptidomimetics do not have canonical peptide backbone, CD data is just used as the supporting data for NMR structures, and should not be overly interpreted. For example, the dichroic bands from the arylsulfonamido chromophores may have a potentially overlapping and disrupting role. In addition, the Cotton effect near 220 nm may not be the accurate indication of molecular helicity.

Conclusion

In summary, we have identified a new class of nonnatural helical foldamer-sulfono- γ -AApeptides. The crystal structure indicates that even the shortest sulfono- γ -AApeptide (monomer) possesses a pre-organized folding structure. NMR studies further suggest that sulfono- γ -AApeptides adopt well-defined right-handed helical conformations in solution, similar to peptide-based α -helices. Similar to α -peptides, the sulfono- γ -AApeptide **S1** is also stabilized by intramolecular hydrogen bonding. CD studies suggest that the similar folding propensity is generally observed throughout the sulfono- γ -AApeptide library examined here, and longer sequences exhibit more pronounced helicity in their secondary structures. Because a virtually endless set of functional groups can be incorporated into sulfono- γ -AApeptides, the folding propensity can be further programmed by a number of chemical approaches, such as inclusion of constrained residues^[24] and hydrocarbon stapling,^[25] we envision that sulfono- γ -AApeptide foldamers can be readily developed to address a variety of challenges in chemical biology.

Experimental Section

Synthesis of sulfono- γ -AApeptides

Solid-phase synthesis of **S1** was carried out on 100 mg Rink-amide resin (0.7 mmol g⁻¹) at room temperature. The resin was swelled in DMF for 1 h before use. The Fmoc protecting group was removed by treating the resin with piperidine/DMF solution (3 mL 20%) for

15 min (x2). The resin was washed with dichloromethane (3 × 3 mL) and DMF (3 × 3 mL). A premixed solution of N-alloc γ -AApeptide building block^[15a] (3 equiv), HOBT (6 equiv, HOBT = 1-hydroxybenzotriazole), and DIC (6 equiv) in DMF (2 mL) was added to the resin. The mixture was shaken for 4 h. After being washed with DCM and DMF, the resin was treated with Pd(PPh₃)₄ (8 mg, 0.007 mmol) and Me₂NH-BH₃ (25 mg, 0.42 mmol) in DCM (3 mL) for 10 min (x2), then reacted with the desired sulfonyl chloride (4 equiv) and N,N-diisopropylethylamine (DIPEA) (6 equiv) in DCM (3 mL) for 30 min (x2). The reaction cycles were repeated until the desired sequence was assembled on the solid phase. After that, the resin was washed with DCM and dried in vacuo. The sulfono- γ -AApeptide cleavage was achieved in a 4 mL vial by treating the resin with TFA/H₂O/TIS (95/2.5/2.5; TFA = trifluoroacetic acid, TIS = triisopropylsilane) for 2 h. The solvent was evaporated and the crude was analyzed and purified on analytical (1 mL min⁻¹) and preparative (16 mL min⁻¹) Waters HPLC systems, respectively. 5% to 100% linear gradient of solvent B (0.1% TFA in acetonitrile) in A (0.1% TFA in water) over 40 min was used. The HPLC trace was detected at 215 nm. The desired fraction was collected and lyophilized, and confirmed on an Applied Biosystems 4700 Proteomics Analyzer. Then, the desired fraction was lyophilized. Other sequences **S2–S5** were synthesized using the same synthetic protocol as **S1**.

2D NMR spectroscopic analysis of sulfono- γ -AApeptide **S1**

The sulfono- γ -AApeptide **S1** was dissolved in approximately CD₃OH (0.5 mL) in a 5 mm NMR tube. The ¹H shift assignment was achieved by sequential assignment procedures based on zTOCSY and NOESY measurements. TOCSY and NOESY spectra were acquired with the wet solvent suppression. All these experiments were performed by collecting 6009 points in f2 and 300 points in f1. A DIPSI2 spin-lock sequence with a spin-lock field of 6 kHz and a mixing time of 80 ms were used in zTOCSY. NOESY experiments used a mixing time of 200 ms. Vnmrj was used to process the data and 2D NMR spectra were analyzed by using the SPARKY program.^[26]

X-ray crystallography

The crystal of **S6** was obtained by slow solvent evaporation of 1:1 CH₂Cl₂/isopropanol containing **S6** (5 mg mL⁻¹). The X-ray diffraction data for **S6** were collected on a Bruker D8 Venture PHOTON 100 CMOS system equipped with a Cu_{K α} INCOATEC Imsu microfocus source ($\lambda = 1.54 \text{ \AA}$). Indexing was performed using APEX2 (difference vectors method).^[27] Data integration and reduction were performed using SaintPlus 6.01.^[28] Absorption correction was performed using a multiscan method implemented in SADABS^[29] Space groups were determined using XPREP implemented in APEX2. The structure was solved using SHELXS-97 (direct methods) and refined using SHELXL-2013 (full-matrix least-squares on F₂) contained in APEX2, WinGX v1.70.01^[30], and OLEX2^[31]. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms of –CH, –CH₂, –CH₃, –OH and –NH groups were placed in geometrically calculated positions and included in the refinement process using a riding model with isotropic thermal parameters: $U_{iso}(H) = 1.2U_{eq}(-CH, -CH_2, -NH)$ and $U_{iso}(H) = 1.5U_{eq}(-CH_3, -OH)$. Disordered benzyl group has been refined using constraints (AFIX66 for phenyl group) and restraints RIGU and SADI. The atomic displacement parameter (ADP) values for disordered C21A and C21B atoms have been set to be equal (EADP). CCDC–1018941 (**S6**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cam-

bridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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Keywords: 2D NMR spectroscopy · helical foldamer · peptidomimetics · sulfono- γ -AApeptides · X-ray crystal structure

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