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The synthesis of head-to-tail cyclic sulfono-γ-AApeptides†

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We report an efficient method for the preparation of unprecedented head-to-tail cyclic sulfono-γ-AApeptides. Following this method, a number of cyclic sequences bearing two to five subunits were efficiently synthesized. In addition, the X-ray crystal structure study of a three-membered cyclic sulfono-γ-AApeptide revealed a type II β-turn-like character.

Cyclic peptides play an important role in the area of drug discovery.[1] The conformational rigidity conferred by macrocyclization is often associated with increased activities compared with linear peptides, especially in the modulation of protein–protein interactions (PPIs).[2–5] Over the years, extensive efforts have been focused on the synthesis and structural modification of cyclic peptides.[6–8] In the meantime, a number of classes of peptidomimetics were developed to mimic the structure of peptides.[9,10] These compounds were shown to display similar, even enhanced functions compared to peptides and possess much better stability towards proteolysis.[9] Similar to peptides, conformational constraints, such as cyclization, have been introduced to peptidomimetics such as peptoids, further enhancing their structural rigidity and therefore potential biological activity.[11–14]

To expand the structural diversity of peptidomimetics, we have recently developed a new class of peptidomimetics termed as "γ-AApeptides".[15] γ-AApeptides contain *N*-acylated *N*-aminoethyl amino acid units derived from γ-PNAs (Fig. 1). They can be efficiently synthesized by solid phase synthesis methods.[15–17] Previous studies of γ-AApeptides have revealed that γ-AApeptides are highly resistant to proteolysis and are highly amendable to chemical diversification.[18] In addition, many γ-AApeptides were reported to bear promising biological functions.[18–26] We thus believe that further development of γ-AApeptides will broaden the scope of their applications in the future.

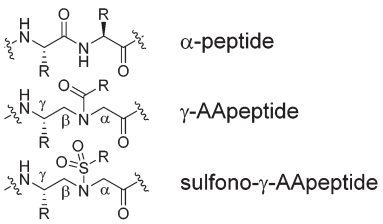

Fig. 1 Structural presentation of a γ-AApeptide as compared with an α-peptide.

Inspired by cyclic peptides and cyclic peptidomimetics, we also seek to extend the structural and functional diversity of γ-AApeptides by macrocyclization. In an initial study, an on-resin head-to-side chain cyclization method was successfully developed[23] and resulted in the efficient preparation of cyclic γ-AApeptides that exhibited broad-spectrum antimicrobial activities superior to those of linear γ-AApeptides.[23] However, since the cyclization was on the side chains, the resulting cyclic γ-AApeptides exhibited asymmetrical structures, and therefore structural studies and rational design of those cyclic sequences are difficult. As such, we have directed interest toward the development of the head-to-tail cyclic γ-AApeptides, especially cyclic sulfono-γ-AApeptides which may present a more rigid structure by avoiding *cis-trans* isomerization of tertiary amide bonds in a γ-AApeptide. Herein, we report for the first time an efficient method for the synthesis of cyclic sulfono-γ-AApeptides in a head-to-tail fashion. In order to assess the potential of cyclic sulfono-γ-AApeptides to mimic functions of peptides, structural analysis of a three-membered cyclic sulfono-γ-AApeptide **γ5** was subsequently conducted.

Among successful methods for peptide macrocyclization, in-solution head-to-tail cyclization of linear peptide precursors in the presence of powerful coupling reagents has found the greatest number of applications. We initially attempted to synthesize linear sulfono-γ-AApeptide precursors by following the similar method. As such, a regular Fmoc γ-AApeptide building

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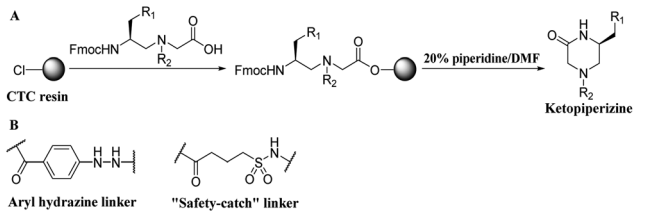

Fig. 2 (A) Ketopiperazine formation, which prevents the sequence elongation. (B) Aryl hydrazine linker and 4-sulfamylbutyryl "safety-catch" linker used to eliminate ketopiperazine formation.

block[15] was first attached on the 2-chlorotrityl chloride (CTC) resin (Fig. 2A). The Fmoc protecting groups were subsequently removed by 20% piperidine in DMF. However, ninhydrin test of the resulting resin showed negative, indicative of the failure of the first attempt. The LCMS analysis of Fmoc deprotection elution revealed the ketopiperazine formation during the Fmoc-deprotection process (Fig. 2A). This is not surprising, as a similar phenomenon was observed on the attempted synthesis of cyclic PNAs.[27] Since γ-AApeptides have the same backbone as chiral PNA, it is reasonable that synthesis of γ-AApeptides on CTC resin was unsuccessful.

To overcome the observed difficulty, we tested the feasibility of two different linkers, an aryl hydrazine linker and a 4-sulfamylbutyryl "safety-catch" linker, for the preparation of head-to-tail cyclized sulfono-γ-AApeptides (Fig. 2B). The linkers were introduced to eliminate the potential six-membered ring formation of ketopiperazine in the first step. Both hydrazine and 4-sulfamylbutyryl linkers contain unique functionality, which can be selectively activated by oxidation and alkylation, respectively.[28,29] Upon activation, sequences can be cleaved from solid support by nucleophiles, such as amines, alcohols, and hydroxide, forming the corresponding products.[30,31] In the case of intramolecular nucleophilic attack by N-termini amines, cyclic sequences in a head-to-tail fashion can be prepared.[32,33] These methods have successfully provided a four-membered cyclic sulfono-γ-AApeptide **γ1**. However, the yields were very low (less than 20% for both methods), thus limiting their potential applications.

It is known that the formation of ketopiperazines that lead to self-cleavage off the solid support is significant under basic condition.[27] Thus, we hypothesized that if the reaction is carried out under neutral condition, the potential formation of ketopiperazine would be minimized. As such, we introduced the Fmoc-*N*-alloc γ-AApeptide building block (Fig. 3A), with the assumption that neutral alloc deprotection condition could bypass the auto-cleavage of ketopiperazines. We then tested the feasibility of this method by synthesizing a four-membered cyclic sulfono-γ-AApeptide **γ1**. (Fig. 3A) In a general method, an Fmoc-*N*-alloc γ-AApeptide building block (**γ-BB-1**) was first attached on the CTC resin. Fmoc protecting groups were removed by 20% piperidine/DMF solution, followed by the modification of the secondary amine by methanesulfonyl chloride. Next, the alloc protecting groups were removed in the presence of $Pd(PPh_3)_4$ and $Me_2NH{\cdot}BH_3$ in dichloromethane,[34] which indeed significantly prevented ketopiperazine formation. The rest of residues in the sequence were assembled with the regular γ-AApeptide synthesis method.[17] Linear protected sulfono-γ-AApeptides were cleaved from the solid support with the regular CTC resin cleavage cocktail (acetic acid–trifluoroethanol–dichloromethane = 1 : 1 : 8). Finally, seven conditions were investigated for the efficiency of head-to-tail cyclization in solution (Table 1). We first employed PyBOP and HBTU (entry 1 and 2), which are common activating agents for peptide lactamization.[35,36] Both of them gave modest results with 70% and 55% yields for PyBOP and HBTU, respectively. Surprisingly, an alternative method with the use of EDC[37] (entry 3 and 4) showed even poorer yield (<5%). The most efficient cyclization was performed in dichloromethane with TBTU, HOBt, and DMAP as coupling reagents (entry 6).[38] Under this condition, the four-membered cyclic sulfono-γ-AApeptide **γ1** was prepared with high yield (>95%) based on the analytical HPLC trace of the crude compound (Fig. 4). No oligomerization was detected.

Thus, the optimized coupling condition was selected to synthesize more cyclic sequences so as to demonstrate its generality. It is known that the ring size is another factor that affects the efficiency of synthesis. For instance, cyclization of ring sizes less than seven amino acids in peptides are sometimes problematic due to the backbone steric strain.[39] To test the efficiency of cyclization, we investigated the effects of ring size on the cyclization of sulfono-γ-AApeptides by the preparation of a two-, a three-, and a five-membered cyclic sulfono-γ-AApeptide, **γ2**, **γ3**, and **γ4** respectively. Surprisingly, all of them showed high yields (Table S1†). Even the shortest one **γ2**, which bears same backbone size as a cyclotetrapeptide, displayed remarkable high yields. Short cyclic peptides, such as cyclotetrapeptides have attracted a lot of attention for their potent biological activities.[40] However, the synthesis still remain a challenge.[7] Providing the highly efficient synthetic method, short cyclic sulfono-γ-AApeptides may serve as a novel scaffold to mimic the biological functions of short cyclic peptides. In addition, such method can be employed to prepare amphiphilic cyclic sequences (**γ6** and **γ7**) with more than 50% yield (Table S1†).

The functions of peptides/peptidomimetics are tightly associated to their structures. Therefore, it is very intriguing to probe the structural conformation of head-to-tail cyclic sulfono-γ-AApeptides, so as to rationally design new molecules with predictable functions. To this end, we have successfully obtained a monocrystal of the three-membered cyclic sulfono-γ-AApeptide **γ5** by diffusing pentane vapor into a chloroform solution of **γ5**. The structure was then elucidated by X-ray crystallographic study (Fig. 5).

The crystal unit cell contains two **γ5** molecules, with each molecule displays segregated side chains on two faces. The top face is comprised of two 4-chlorophenyl sulfonyl groups and a methyl group whereas the bottom face contains two methyl groups and a 4-chlorophenyl sulfonyl group. All three tertiary sulfonamide groups adopt anti conformations. The unusual asymmetry of **γ5** contradicts what is predicted based on its

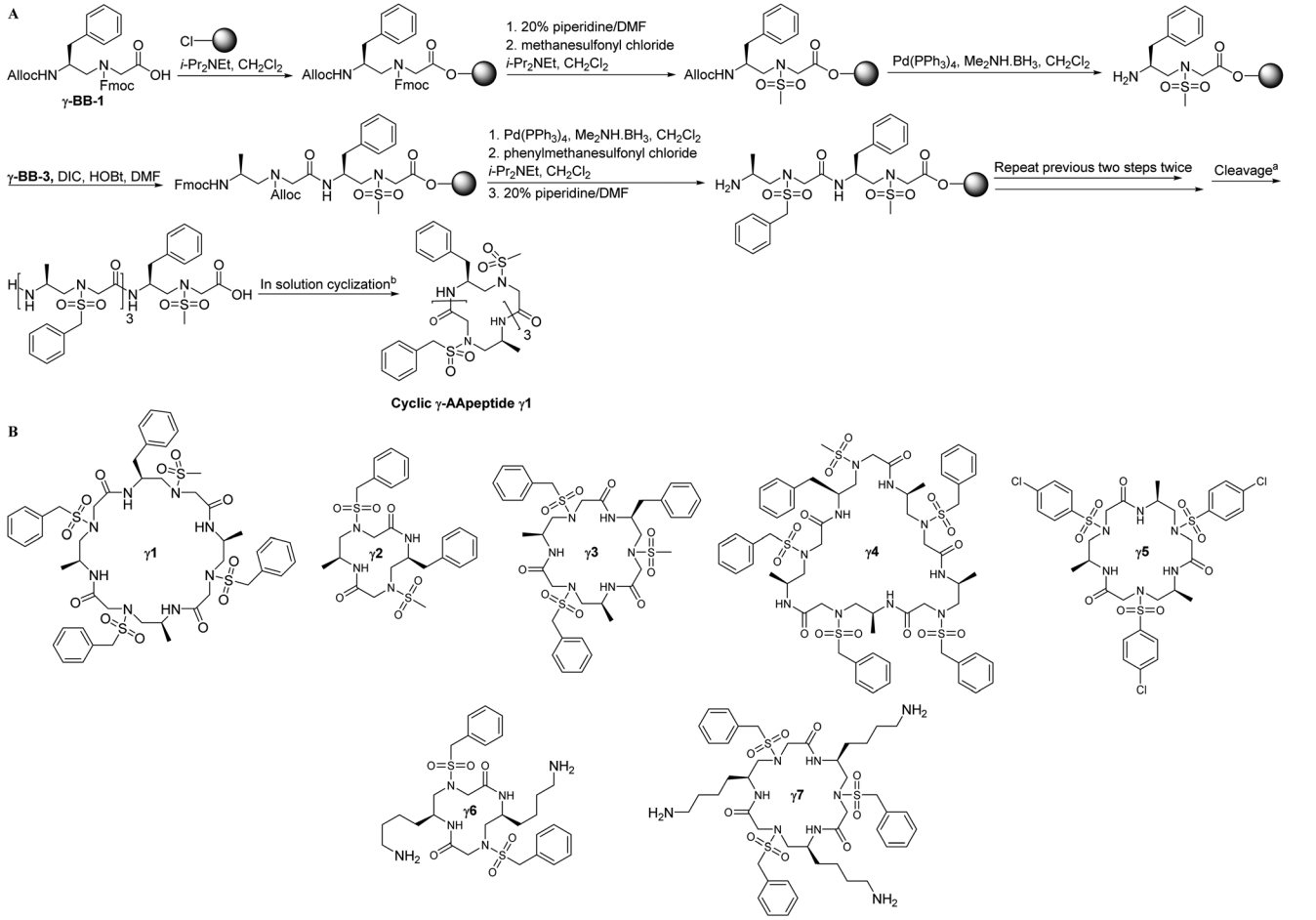

Fig. 3 (A) Scheme for the preparation of cyclic sulfono-γ-AApeptide **γ1**. (B) Structures of cyclic sulfono-γ-AApeptides in this study. [a]Acetic acid–trifluoroethanol–dichloromethane = 1 : 1 : 8 for 2 h; [b]optimized cyclization condition: 0.5 mM linear precursors in dichloromethane with *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyl-uronium tetrafluoroborate (TBTU) (3 equiv.), hydroxybenzotriazole (HOBt) (3 equiv.), and 4-dimethylaminopyridine (DMAP) (5 equiv.) at room temperature for 6 h.

Table 1 Cyclization conditions for **γ1**

Entry	Cyclization conditions[a] (equiv.)	Solvent	Yield[b] (%)
1	PyBOP/HOBt/i-Pr_2NEt (4, 4, 8)	DMF	70
2	HBTU/HOBt/i-Pr_2NEt (4, 4, 8)	DMF	55
3	EDC/HOBt/i-Pr_2NEt (4, 4, 8)	DMF	<5
4	EDC/HOBt/i-Pr_2NEt (4, 4, 8)	CH_2Cl_2	<5
5	TBTU/HOBt/i-Pr_2NEt (3, 3, 5)	DMF	50
6	TBTU/HOBt/DMAP (3, 3, 5)	CH_2Cl_2	95
7	TBTU/HOBt/DMAP (3, 3, 5)	DMF	70

[a] The cyclization reactions were conducted with 0.5 mM linear precursor at room temperature for 6 h. [b] Yields were determined by analytical HPLC traces. PyBOP: benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluoro-phosphate; HBTU: 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; EDC: 1-ethyl-3-(3-dimethylaminopropyl)carbo-diimide.

primary structure as well as observed from other macrocycles, such as cyclohexapeptoids.[11,37,41]

The backbone of **γ5** displays a "twisted" boat-like shape containing three *trans* amide bonds. (Fig. 5B) Two carbonyl groups point outside the ring and one inside the ring. The dihedral angles were calculated and presented in Table S3.† Six torsion angles ϕ, θ, η, ζ, ψ, and ω are defined to describe backbone dihedral angles of a γ-AApeptide (Table S3†). Noticeable variations in dihedral angles ϕ, θ, η, and ζ of the three subunits present the asymmetry of this molecule. Similar to cyclic peptoids, ψ values show almost planar geometry for all three subunits.[11] Three *trans* amide bonds are also revealed by ω values. The mean ω value is 179.2° with a standard deviation of 7.5°, which is close to what was reported for cyclic peptides.[42]

The backbone also displays a hydrogen bond between C=O (subunit 1) and N–H (subunit 3), suggesting a turn-like structure. The turn contains same number of atoms as a peptide β-turn motif. Two carbon atoms (C_1^{α} and C_3^{γ}), which mimic two C^{α} atoms in a peptide β-turn motif, show close proximity (6.103 Å). In addition, superimposition of **γ5** backbone at the turn region of a type II β turn (PDB: 1YCC) reveals high similarity (Fig. 5D). The preference of **γ5** to resemble type II β turn is shown by comparison with a type I β-turn (Fig. S4†).

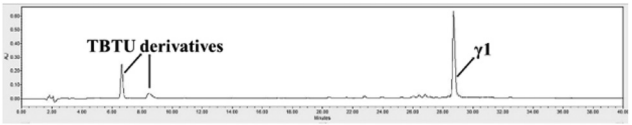

Fig. 4 HPLC trace of crude cyclic sulfono-γ-AApeptide **γ1**.

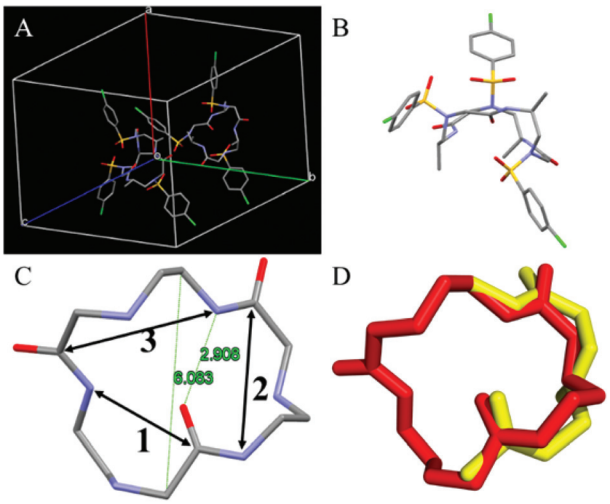

Fig. 5 (A) Crystal unit cell of cyclic sulfono-γ-AApeptide **γ5**. (B) Side view of the crystal structure showing spatial segregation of side chains. (C) Top view of the backbone with subunit 1, 2, and 3. (D) Superimposition of **γ5** backbone (red) with a turn region of a type II β turn (PDB: 1YCC) (yellow).

The structural conformation of **γ5** suggests the potential of cyclic sulfono-γ-AApeptides to mimic the protein type II β-turn structure. Such β-turn mimics may find applications in various biomedical and material research.

Conclusions

In conclusion, we report an efficient method for the preparation of unprecedented head-to-tail cyclic sulfono-γ-AApeptides. Keto-piperazine formation was greatly reduced by introducing a unique Fmoc-*N*-alloc γ-AApeptide building block for the first attachment on the CTC resin. Head-to-tail macrocyclization of the linear precursors was achieved with high efficiency by using TBTU, HOBt, and DMAP as coupling reagents. Following this method, cyclic sulfono-γ-AApeptides varying from two subunits to five subunits were readily synthesized with high yields. In order to elucidate its structural properties, we present for the first time the X-ray crystal structure of a three-membered cyclic sulfono-γ-AApeptide **γ5**. The crystal structure shows a spatial segregation of side chains in an unusual asymmetrical pattern. More interestingly, **γ5** exhibits a turn-like structure with patterns similar to a peptide type II β-turn structure. By demonstrating the robust synthetic method of cyclic sulfono-γ-AApeptides, their capability to mimic peptide β-turn structure, and the ability to introduce different side functional groups, we believe that such macrocycles will find important applications soon after.

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