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Facile solid-phase synthesis of cyclic decapeptide antibiotic streptocidins A–D ☆

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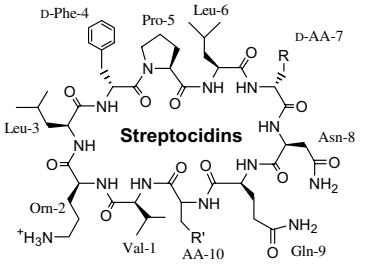
Abstract—Total synthesis of decapeptide antibiotics streptocidins A–D from *Streptomyces* sp. Tü 6071 was accomplished for the first time by solid-phase peptide synthesis followed by traceless cyclization of the activated linear precursors, without protection of nucleophilic side chain. Synthetic products were equally active as the natural products isolated from the bacterial source and found to possess similar bacterial selectivity as other members in the amphipathic antimicrobial cyclic decapeptide family.

Streptocidins are novel antibiotics recently isolated from the culture broth of *Streptomyces* sp. Tü 6071.[1] They consists of four structurally related compounds, streptocidins A–D, on a cyclic decapeptide backbone (Fig. 1). The reported structural characterization and conformational analysis of these natural products[2] showed that they can be structurally classified to a family of antimicrobial cyclic decapeptides such as tyrocidines (A–E),[3] loloatins (A–D),[4] and gramicidin S.[5]



	Streptocidin	AA_7	AA_{10}
1	A	Tyr	Trp
2	B	Trp	Trp
3	C	DTrp	Trp
4	D	Trp	Phe

Figure 1. Structure of streptocidins A–D.

Tyrocidines and gramicidin S in this antimicrobial family have been known to act on microbial cell membranes where their accumulation results in disruption of the barrier functions[1,6] and thus are unlikely to provoke microbial resistance.[7] In light of the widespread microbial resistance that has become a threat to public health,[8] this class of natural products have become attractive targets for drug development for containment of the resistance.[9] However, it has also been well known that these membrane-interacting peptides are also toxic to mammalian cells.[1,6]

On the basis of similar sequence and structure, we anticipated that the newly discovered streptocidins adopt the same unique antibiotic mechanism and may have bacterial specificity different from other members in the family of amphipathic peptide antibiotics. Thus, we embarked on their total synthesis to verify the structures, to study their biological activities, and to develop a convenient method for analogue synthesis in an attempt to improve their selectivity against bacteria.

A number of methods have been developed for synthesis of cyclic peptides.[10] Traditionally, C-terminal activation by *N*-hydroxysuccinimide ester (ONSu) and azide of the linear peptide was used in the synthesis of tyrocidines and gramicidins.[11] More recently, cyclization of peptides anchored on resin through the side-chain functionalities has been widely employed to afford the cyclic peptides.[12] Moreover, 'safety-catch' methods have been developed

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for synthesis of head-to-tail cyclic products.[13] However, these methods involve tedious side-chain protection–deprotection necessitated in the ring closure and are often hampered by the poor cyclizing tendency of the linear peptide precursors. Furthermore, thioesterases of the nonribosomal peptide synthases (NRPS) have been successfully applied in synthesis of tyrocidine A and analogues, and suggested to be a general method for generation molecular diversity for enhancement of the therapeutic index of the natural products.[14] Recently, we found that the biosynthetic precursors of tyrocidine A[15] and gramicidin S[16] adopt a pre-organized conformation, which is highly favorable for specific head-to-tail cyclization. This led to a simpler synthetic method for the tyrocidine A using acylsulfonamide safety-catch linker,[17] without the need to protect the reactive side chain functionalities in the cyclization-product release step.

Streptocidins differ from tyrocidines in three positions.[1] The Leu-6 in streptocidins is Phe or Trp in tyrocidines. Either DPhe or DTrp occupies the position of AA_7 (Fig. 1) in both tyrocidines and streptocidines. In tyrocidines, L-amino acids Tyr/Trp/Phe occupies the position of AA_{10}, whereas Trp/Phe is present at the same location in streptocidins A, B, and D, while a D-amino acid, DTrp, is present at this position in streptocidin C. Based on the structural similarities between tyrocidines and streptocidins, it is believed that the linear precursors are likely to adopt a pre-organized conformation favorable for cyclization, like that for tyrocidine A. Thus, the convenient safety-catch linker method for tyrocidine A is directly employed to synthesize the novel cyclic decapeptide antibiotics.

As shown in Scheme 1, the precursors were synthesized and cyclized similar to that of tyrocidine A,[17] without protection of the side chain $-NH_2$ group in the cyclization. Summary and characterization of the reactions are showed in Table 1. HPLC analysis of the final products without chromatographic purification showed that they are of high purity. FAB-MS results indicated that molecular ions of the products were consistent with the calculated values of the expected cyclic peptides. Relatively overall low yield (10–18%) of the products were probably due to the overestimation of the resin loading value determined after attachment of the first amino acid (Fmoc–Leu–OH). No free amine group was found after the cyclization reaction by Kaiser's test.

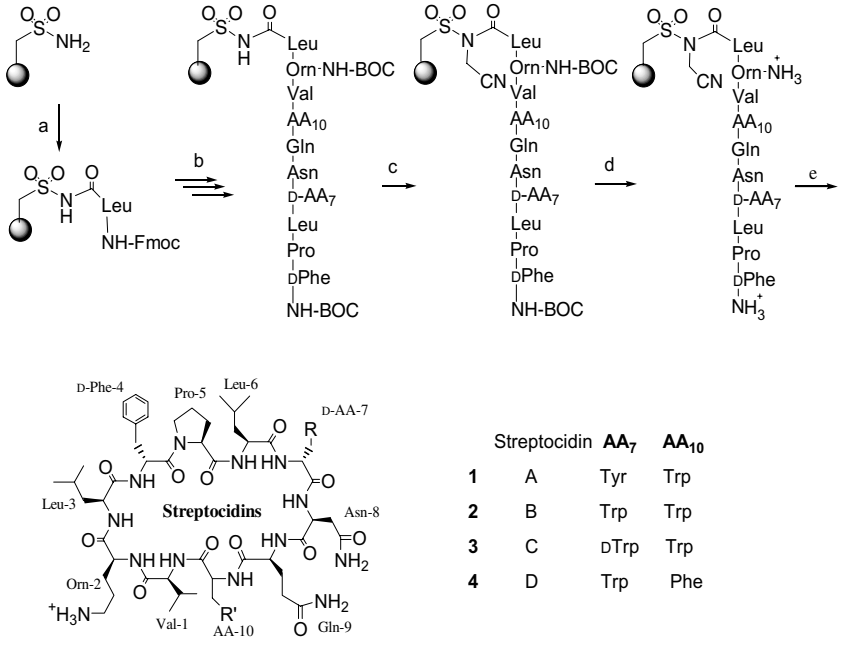
After HPLC purification of the final products, they were subject to structural determination by ^1H NMR. The spectroscopic data are summarized in the Supplementary Materials. Data for streptocidins C and D are in complete consistence with that of the corresponding

Table 1. Synthetic results of streptocidins A–D (**1**–**4**)

Entry	AA_7	AA_{10}	Calculated M	$[M+H]^+$	Purity[a] (%)	Yield[b] (%)
1	Tyr	Trp	1275.7	1276.7	94	16.0
2	Trp	Trp	1298.7	1299.7	91	14.4
3	DTrp	Trp	1298.7	1299.7	87	10.8
4	Trp	Phe	1259.7	1260.7	95	17.6

[a] Purity was determined from HPLC analysis of the unpurified cyclization products.

[b] Overall yields calculated from the loading value of the resin after first amino acid attachment.



	Streptocidin	AA_7	AA_{10}
1	A	Tyr	Trp
2	B	Trp	Trp
3	C	DTrp	Trp
4	D	Trp	Phe

Scheme 1. Synthesis of streptocidins A–D. Reactions and conditions: (a) Fmoc–Leu–OH, PyBOP, DIPEA, $CHCl_3$, −20 °C, 8 h, repeat once; (b) 20% piperidine/DMF; then Fmoc–AA–OH (Boc–DPhe–OH for the last residue), DIC/HOBt, 2 h; (c) ICH_2CN, NMP, DIPEA, 24 h; (d) CF_3COOH/phenol/*i*-$Pr_3SiH/H_2O = 88:5:5:2$, 1 h; (f) 20% DIPEA/THF.

Table 2. Antibiotic and hemolytic activities of tyrocidine A (**Ta**) and streptocidins A–D (**1**–**4**)

Cells	MIC or MHC (μg/mL)				
	Ta	**1**	**2**	**3**	**4**
Bacillus subtilis	8.3	5	2.5	4	3.5
Escherichia coli K12	>50	>50	>50	>65	>60
Pseudomonas aeruginosa	>50	>50	>50	>65	>60
Pichia pastoris GS115	33	2.5	2.5	10	15
Candida albicans	15	12	8	15	13
Human erythrocyte (for MHC)	8.5	9.0	2.0	6.0	2.5

natural products isolated from the bacterial source.[2] In comparison to the spectroscopic data of streptocidins C and D, the data of the synthetic streptocidins A and B, which differ from streptocidins C and D, respectively at only one residual (Fig. 1), indicate that they are the correct head-to-tail cyclization products. These results show that the synthetic scheme indeed affords the correct head-to-tail cyclic products without interference from the reactive side chain $-NH_2$ and –OH groups in the cyclization step, as expected.

Next, we examined the antibiotic activity of the synthetic products using a modified broth dilution method.[9a,18] As shown in Table 2, minimum inhibition concentrations (MIC) of these products showed they are indeed potent antibiotics against gram-positive bacterium *Bacillus substilis*, with comparable potency in comparison with the isolated natural products.[1] This result provided further evidence that the synthetic products are identical in structure to the natural products isolated from the bacterial source.

Further analysis of the antimicrobial activities of the streptocidins found that they are modestly potent toward yeasts and fungi such as *Pichia pastoris* GS115 or *Candida albicans*, but are inactive toward gram-negative bacteria such as *Escherichia coli* K12 or *Psudomonas aeruginosa*. This bacterial specificity of streptocidins is similar to that of tyrocidine A, but differs from the specificity of gramicidin S, which also exhibits high activity towards the gram-negative bacteria.[18]

However, determination of the minimum hemolysis concentrations (MHC)[9b,19] showed that streptocidins do not have a higher selectivity discriminating microbes against mammalian cells. Their therapeutic indices (MHC/MIC) are essentially equal to that of tyrocidine A. This result showed that structural variations in streptocidins do not bring about the anticipated differentiation of antibacterial and hemolytic activities. Nevertheless, the simple synthetic strategy adopted in this study provides a convenient access to analogues of these novel peptide antibiotics for improvement of their bacterial specificity.

In summary, the total synthesis of streptocidins A–D has been successfully accomplished for the first time through a simple method based on acylsulfonamide safety-catch linker, without protection of nucleophilic side chains. The biological activity study showed that the newly discovered streptocidins do not have different bacterial selectivity from other members in the family of amphipathic cyclic decapeptide antibiotics. The success of the synthetic strategy employed in this study should enable access to the analogues for functional optimization of these natural products.

Supplementary material

HPLC chromatograms, ^1H NMR data and spectra are available for streptocidins A–D (**1**–**4**). Details are available from the author on request.

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