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Report

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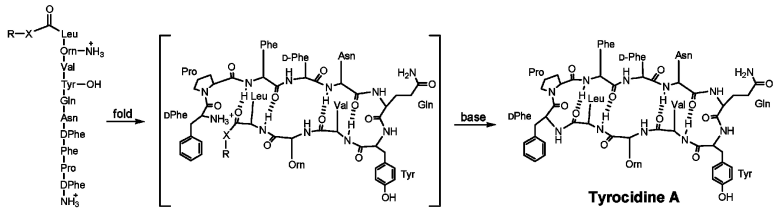

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A Chemical Approach to Generate Molecular Diversity Based on the Scaffold of Cyclic Decapeptide Antibiotic Tyrocidine A

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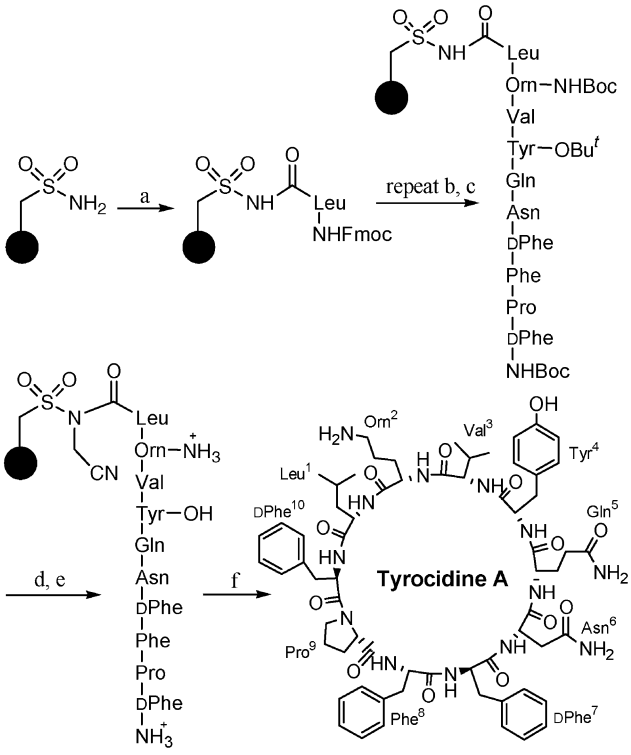
Macrocyclic peptide natural products are a class of structurally diverse complex molecules exhibiting a broad spectrum of biological activities, ranging from insecticidal, antimicrobial, antiviral, and tumor-promoting, to antiinflammatory and immunosuppressing activities.[1] It is of high interest to generate molecular diversity based on these natural structures to search for new biological functions or to optimize their original activities. Currently, intense effort is being invested in utilizing the modular biosynthesis of the peptide natural products to achieve this goal.[2] For example, thioesterases of the nonribosomal peptide synthases (NRPS) have been thoroughly investigated and used to generate analogues for enhancement of the therapeutic index of the natural products.[3] In contrast, available chemical methods for cyclic peptide synthesis[4] have been perceived not to be suitable for this engineering purpose, largely because of the poor cyclizing tendency of the precursors and the tedious side chain protection−deprotection necessitated in the ring closure. However, it is well known that chemical synthesis of these natural peptide products, particularly in the ring closure, can be greatly facilitated by the conformational preference of their linear precursors,[5] rendering the hope that a convenient strategy can be developed to generate molecular diversity on the basis of these natural structures. Recently, we found that the linear biosynthetic precursor of the decapeptide antibiotic tyrocidine A possesses such a conformational preference to self-cyclize.[6] Here, we report utilization of this conformational propensity to develop a simple and efficient method adaptable to high-throughput synthesis of cyclic peptide molecules based on the scaffold of tyrocidine A.

Tyrocidine A is an amphiphilic cyclic decapeptide antibiotic produced by *Bacillus brevis*;[7] it possesses a rigid antiparallel β-pleated sheet structure.[8] It has been found that the linear biosynthetic precursor of tyrocidine A adopts a backbone conformation highly favorable for ring closure.[6] Since formation of the favorable conformation involved four strong backbone hydrogen bonds,[8] it was expected that the side chains of the constituent amino acids would have little effect on the self-cyclizing tendency, making it an ideal template for display of functionalities. In addition, the scaffold molecule itself is biologically important. This microbicide is unique in its mode of action by disrupting cell membrane function[9] and, as a result, its low risk of provoking microbial resistance.[10] On the basis of this scaffold, the synthesized compounds will at least be useful in functional selection to find analogues maintaining the natural product's high antibiotic activities while eliminating its high hemolytic activity[11] for potential treatment of resistant microbial pathogens that have become a public health threat.[12]

Scheme 1[a]



[a] (a) PyBOP, DIPEA, $CHCl_3$, Fmoc−Leu−OH, - 20 °C, 8 h, repeat once. (b) 20% piperidine in DMF. (c) Fmoc−amino acid (Boc−DPhe−OH for the last residue), DIC, HOBt, 2h. (d) ICH_2CN, NMP, DIPEA, 24 h. (e) CF_3COOH/phenol/*i*-Pr_3SiH/H_2O = 88:5:5:2, 1 h. (f) 20% DIPEA/THF.

We used a safety-catch linker, shown to be suitable for cyclic peptide synthesis,[13] to assemble the high-purity linear tyrocidine A precursor that can be activated at the carboxyl terminus for the conformation-dependent cyclization. Use of a linker other than the native phosphopantetheinyl group[2b] was not expected to change the precursor's self-cyclizing propensity that is dependent on the backbone hydrogen bonds.[6] The biomimetic synthesis of the natural product is outlined in Scheme 1. After solid-phase synthesis, the linear precursor was deprotected and cyclized to give tyrocidine A in an overall yield of 25%.

A FAB-MS spectrum of the cyclization product showed only one molecular ion peak at 1270.8 ($[M + H]^+$), consistent with the calculated mass of 1269.65 for tyrocidine A, indicating an absence of hydrolytic products or other truncated peptide products. In addition, HPLC analysis of the cyclization product showed only one major product peak

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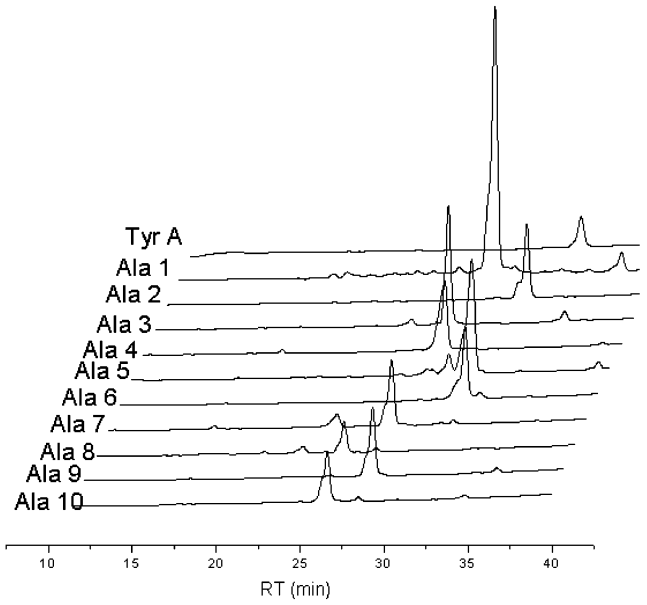
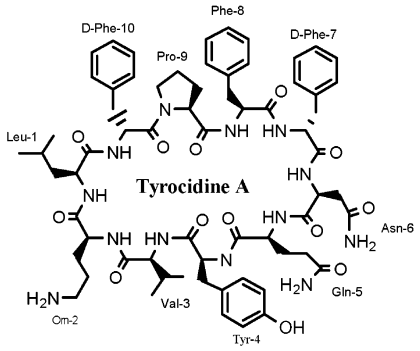


Figure 1. HPLC chromatograms of the alanine-substituted analogues of tyrocidine A. Eluted products were monitored at 220 nm. Analysis was performed with a Waters 600E system with a reversed-phase semipreparative XTerra RP_{18} column, 7 μM, 7.8 × 300 mm. Separation conditions were: 3.0 mL min^{-1} flow rate, a linear gradient of 80−20% A in 25 min, 20−0% A in another 10 min, washed with 100% B for 10 min, and then calibrated at 80% A for 15 min. Solution A was 0.1% TFA in double-deionized H_2O, and solution B was 0.1% TFA in acetonitrile.

(Figure 1, >95% pure) with a retention time identical to that of the authentic tyrocidine A, free of contamination of cyclic aminolytic product from the unprotected ornithine side chain δ-NH_2. Moreover, the ^{1}H NMR spectrum of the cyclization product was found to be completely consistent with that of the wild-type tyrocidine A.[8] Finally, the cyclic product was as active as the wild-type tyrocidine A toward a *Bacillus subtilis* strain using a standard microtiter assay.[11b,14] These results clearly showed that the simple synthetic scheme efficiently afforded the expected head-to-tail cyclic product in high purity without interference from the unprotected side chain amine group.

Next, we examined the effect of side chains on the self-cyclizing tendency of the scaffold molecule. The constituent amino acids of the linear precursor were sequentially substituted by alanine in a process called "alanine-scanning". D-Alanine was used to replace the D-phenylalanines in the parent molecule, since configuration of individual amino acid had been shown to be important to the conformation of the linear precursors.[6] The substituted linear precursors were synthesized and cyclized in parallel using IRORI's AccuTag-100 Combinatorial Chemistry System,[15] according to the method shown in Scheme 1. Starting from 30 mg of 4-sulfamylbutyryl AM resin (0.8 mmol/g) for each compound, the alanine-substituted products were obtained in good yields and characterized without purification, as summarized in Table 1. Mass spectroscopic results showed that all the obtained products resulted from cyclization of the commensurate linear peptides. Moreover, HPLC analysis found that the products were of high purity (Figure 1, mostly >90%), similar to that for the wild-type scaffold molecule. No linear hydrolytic products which should appear around

Table 1. Synthesis of Alanine-Substituted Tyrocidine A



compd	substituted residual	formula	R_t[a] (min)	calcd mass	found[b] (M + 1)	purity[c] (%)	yield[d] (%)
Ala-1	Leu-1	$C_{63}H_{81}N_{13}O_{13}$	27.2	1227.6	1228.6	95.8	15.7
Ala-2	Orn-2	$C_{64}H_{82}N_{12}O_{13}$	33.0	1226.6	1227.5	99.2	21.5
Ala-3	Val-3	$C_{64}H_{83}N_{13}O_{13}$	29.0	1241.6	1242.5	94.6	37.8
Ala-4	Tyr-4	$C_{60}H_{83}N_{13}O_{12}$	29.5	1177.6	1178.5	99.0	34.6
Ala-5	Gln-5	$C_{64}H_{84}N_{12}O_{12}$	29.0	1212.6	1213.5	93.7	35.0
Ala-6	Asn-6	$C_{65}H_{86}N_{12}O_{12}$	32.1	1226.7	1227.7	96.1	23.7
Ala-7	DPhe-7	$C_{60}H_{83}N_{13}O_{13}$	28.5	1193.6	1194.4	92.5	28.6
Ala-8	Phe-8	$C_{60}H_{83}N_{13}O_{13}$	26.3	1193.6	1194.6	81.4	30.1
Ala-9	Pro-9	$C_{64}H_{85}N_{13}O_{13}$	28.6	1243.6	1244.5	98.5	30.0
Ala-10	DPhe-10	$C_{60}H_{83}N_{13}O_{13}$	26.5	1193.6	1195.7	96.7	26.6

[a] Retention time of the product peak on the HPLC chromatograms. [b] Molecular ion from FAB-MS. [c] Calculated as percentage of the product peak area over the total peak area between 10 and 35 min. [d] Overall yield based on the loading value of the resin after first amino acid coupling.

20−25 min in the HPLC profiles were identified. Furthermore, for each substituted product, ^{1}H NMR signals of the backbone amide protons of the unchanged amino acids were only very slightly alternated from that of the wild-type tyrocidine A,[8] indicating that the cyclic products possessed a ring structure closely resembling the antiparallel natural product and that the cyclization of the linear precursors was predominantly head-to-tail with little interference from the unprotected amine or other active side-chain functionalities. Indeed, these results showed that the side chains of the tyrocidine A scaffold have minimal effect on the strong tendency of the linear precursors to self-cyclize and that they are replaceable for generation of molecular diversity to enhance the natural product's activity or evolve new biological functions. On the basis of this conclusion, a library of 192 tyrocidine analogues, including simultaneous variations at four loci on the scaffold, have been successfully synthesized.[16]

In summary, we have demonstrated that the predisposed conformation of linear peptide precursors can be utilized to design a simple and efficient synthetic method as a means of molecular diversity generation based on the scaffold of the natural peptide antibiotic tyrocidine A. In comparison to the reported chemoenzymatic method,[3d] this chemical approach allows more flexibility in the precursor sequence and, thus, a much larger structural space to be explored. Since similar conformation preference has been observed for many other natural peptide or non-peptide macrocyclic products, this work may lead to analogous chemical strategies for efficient generation of their analogues for structural optimization or discovery of new biological functions.

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Supporting Information Available. Experimental procedure, FAB-MS spectra, and comparison of ^{1}H NMR signals for α, β, and backbone amide protons of the constituent amino acids of tyrocidine A with that of the alanine-scanning analogues. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) (a) Waki, M.; Izumiya, N. *Biochemistry of Peptide Antibiotics: Recent Advances in the Biotechnology of β-Lactams and Microbial Bioactive Peptides*; Kleinkaug, H., von Döhren, H., Eds.; Walter de Gruyter: Berlin, 1990; pp 205−244. (b) Fusetani, N.; Matsunaga, S. *Chem. Rev.* **1993**, *93*, 1793. (c) Davidson, B. S. *Chem. Rev.* **1993**, *93*, 1771.

(2) (a) Döhren, H.; Keller, U.; Vater, J.; Zocher, R. *Chem. Rev.* **1997**, *97*, 2675. (b) Marahiel, M. A.; Stachelhaus, T.; Mootz, H. D. *Chem. Rev.* **1997**, *97*, 2661. (c) Walsh, C. T. *ChemBioChem* **2002**, *3*, 124.

(3) (a) Trauger, J. W.; Kohli, R. M.; Mootz, H. D.; Marahiel, M. A.; Walsh, C. T. *Nature* **2000**, *407*, 215. (b) Trauger, J. W.; Kohli, R. M.; Walsh, C. T. *Biochemistry* **2001**, *40*, 7092. (c) Kohli, R. M.; Trauger, J. W.; Schwarzer, D.; Marahiel, M. A.; Walsh, C. T. *Biochemistry* **2001**, *40*, 7099. (d) Kohli, R. M.; Walsh, C. T.; Burkart, M. D. *Nature* **2002**, *418*, 658. (e) Kohli R. M.; Walsh, C. T. *Chem. Commun.* **2003**, *297*, 297.

(4) (a) Wipf, P. *Chem. Rev.* **1995**, *95*, 2115. (b) Humphrey, J. M.; Chamberlin, A. R. *Chem. Rev.* **1997**, *97*, 2243. (c) Lambert, J. N.; Mitchell, J. P.; Roberts, K. D. *J. Chem. Soc., Perkin Trans. 1* **2001**, 471.

(5) (a) Kopple, K. D. *J. Pharm. Sci.* **1972**, *61*, 1345. (b) Brady, S. F.; Varga, S. L.; Freidinger, R. M.; Schwenk, D. A.; Mendlowski, M.; Holly, F. W.; Veber, D. F. *J. Org. Chem.* **1979**, *44*, 3101. (c) Wenger, R. M. *Helv. Chim. Acta* **1984**, *67*, 502. (d) Cavelier-Frontin, F.; Pépe, G.; Verducci, J.; Siri, D.; Jacquier, R. *J. Am. Chem. Soc.* **1992**, *114*, 8885. (e) Gibbs, A. C.; Kondejewski, L. H.; Gronwald, W.; Nip, A. M.; Hodges, R. S.; Sykes, B. D.; Wishart, D. S. *Nat. Struct. Biol.* **1998**, *5*, 284.

(6) Bu, X.; Wu, X.; Xie, G.; Guo, Z. *Org. Lett.* **2002**, *4*, 2893.

(7) (a) Hotchkiss, R. D.; Craig, L. C. *J. Biol. Chem.* **1940**, *136*, 803. (b) Ruttenberg, M. A.; Mach, B. *Biochemistry* **1966**, *5*, 2864.

(8) (a) Kuo, M.-C.; Gibbons, W. A. *Biochemistry* **1979**, *18*, 5855. (b) Kuo, M.-C.; Gibbons, W. A. *J. Biol. Chem.* **1979**, *254*, 6278. (c) Kuo, M.-C.; Drakenberg, T.; Gibbons, W. A. *J. Am. Chem. Soc.* **1980**, *102*, 520. (d) Kuo, M.-C.; Gibbons, W. A. *Biophys. J.* **1980**, *32*, 807. (e) Zhou, N.; Mascagni, P.; Gibbons, W. A.; Niccolai, N.; Rossi, C.; Wyssbrod, H. *J. Chem. Soc., Perkin Trans. 2* **1985**, *4*, 581.

(9) Prenner, E. J.; Lewis, R. N. A. H.; McElhaney, R. N. *Biochim. Biophys. Acta* **1999**, *1462*, 201.

(10) Hancock, R. E. W. *Lancet* **1997**, *349*, 418.

(11) (a) Kondejewski, L. H.; Farmer, S. W.; Wishart, D. S.; Kay, C. M.; Hancock, R. E. W.; Hodges, R. S. *J. Biol. Chem.* **1996**, *271*, 25261. (b) Kondejewski, L. H.; Jelokhani-Niaraki, M.; Farmer, S. W.; Bruce, L.; Kay, C. M.; Sykes, B. D.; Hancock, R. E. W.; Hodges, R. S. *J. Biol. Chem.* **1999**, *274*, 13181. (c) McInnes, C.; Kondejewski, L. H.; Hodges, R. S.; Sykes, B. D. *J. Biol. Chem.* **2000**, *275*, 14287.

(12) (a) Neu, H. C. *Science* **1992**, *257*, 1064. (b) Cohen, M. L. *Science* **1992**, *257*, 1050.

(13) (a) Backes, B. J.; Virgilio, A. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1996**, *118*, 3055. (b) Yang, L.; Morriello, G. *Tetrahedron Lett.* **1999**, *40*, 8197.

(14) Kondejewski, L. H.; Farmer; S. W.; Wishart, D. S.; Hancock, R. E. W.; Hodges, R. S. *Int. J. Pept. Protein Res.* **1996**, *47*, 460.

(15) Nicolaou, K. C.; Xiao, X.-Y.; Parandoosh, Z.; Senyei, A.; Nova, M. P. *Angew. Chem. Int. Ed., Engl.* **1995**, *34*, 2289.

(16) The library was formed by substituting Tyr^4 with Tyr, Trp, Phe, or Ala; Gln^5 with Gln or Asn; DPhe^7 with DAla, DLeu, DPhe, or DTrp; and Phe^8 with Ala, Leu, Val, Phe, Tyr, or Trp. Characterization and screening of the library will be reported elsewhere.

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