ORGANIC LETTERS

2004
Vol. 6, No. 15
2627−2630

Solid-Phase Synthesis and Stereochemical Assignments of Tenuecyclamides A–D Employing Heterocyclic Amino Acids Derived from Commercially Available Fmoc α-Amino Acids

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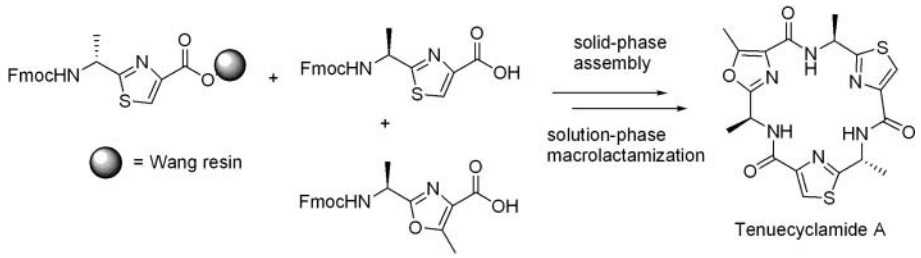
Received May 27, 2004

ABSTRACT



The solid-phase assembly of heterocyclic amino acids enabled the total synthesis of numerous diastereoisomers of tenuecyclamides A–D, establishing or correcting the stereochemistry of each natural product. This strategy provides a very efficient route to synthesize thiazole- and oxazole-containing macrolactams from heterocyclic amino acids that are readily prepared from Fmoc-α-amino acids. This methodology appears to be broadly applicable to the synthesis of natural product libraries incorporating unnatural heterocyclic amino acid residues for the purpose of drug discovery.

Heterocyclic amino acids, including those composed of thiazoles (**1**), oxazoles (**2**), thiazolines (**3**), and oxazolines (**4**) (Figure 1), are substructures comprising numerous macrolactam natural products having interesting biological activities.[1] These include cytotoxicity, P-glycoprotein pump inhibition, and metal binding properties that have motivated chemists to develop methodology to prepare these compounds.[2] Included in this family of macrolactams are the tenuecyclamides A−D, isolated from the cyanobacterium *Nostoc spongiaeforme* var. *tenue*, which inhibit the division of sea urchin embryos with an ED_{100} of 9.0−19.1 μM (Figure 2A).[3] The stereochemistry of tenuecyclamides A and B could not be fully assigned due to racemization of chiral centers neighboring the thiazole rings during the degradation procedure used to assign their structures.

The activity of the tenuecyclamides warrants the development of efficient chemistry to synthesize libraries of these and related natural products for the purpose of structural verification and biological evaluation. While solid-phase synthesis[4] has become an important tool in lead discovery, very few examples of the solid-phase synthesis of heterocycle-containing macrolactams have been reported.[5]

The solid-phase synthesis of these macrocycles requires heterocyclic amino acid building blocks. The efficient synthesis of these building blocks is now practical. We recently reported the synthesis of thiazoline amino acids from

10.1021/ol049020m CCC: $27.50
Published on Web 06/24/2004

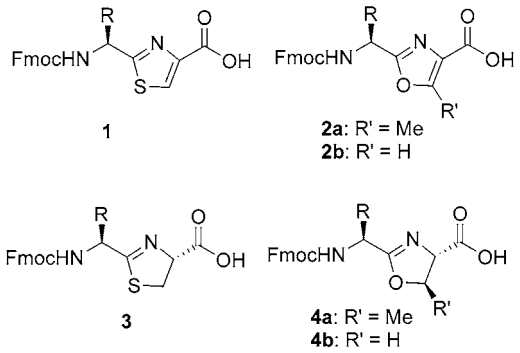


Figure 1. Heterocycle-containing amino acids often found in macrolactam natural products.

N-acylated cysteine substructures using bis(triphenyl) oxodiphosphonium trifluoromethane sulfonate.[6] Thiazole amino acids were easily obtained by oxidation of the corresponding thiazolines.[7,8] In addition, oxazole amino acids were obtained from cyclodehydration of a β-ketodipeptide using the same bisphosphonium salt in solution.[7] Herein we utilize the solid-phase assembly of oxazole and thiazole amino acids, e.g., **1** and **2**, to establish the stereochemistry of tenuecyclamides A and B and to reassign the stereochemistry of tenuecyclamides C and D.

Establishing the stereochemistry of tenuecyclamides A and B (Figure 2A) requires the synthesis of the enantiomeric thiazole amino acids, synthesized as described above using established methodologies.[6−8] The availability of the Fmoc-protected enantiomeric thiazole amino acids facilitates the preparation of the four diastereomeric macrolactams (Table 1) required to establish the stereochemistry of tenuecyclamides A and B. The retrosynthesis of one of the diastereomeric macrolactams, **7**, is outlined in Figure 3. Disconnection at the three amide bonds requires two thiazole (**5**) and one oxazole amino acid (**6**) building blocks. The proposed nomenclature for each residue, surrounded by bold paren-

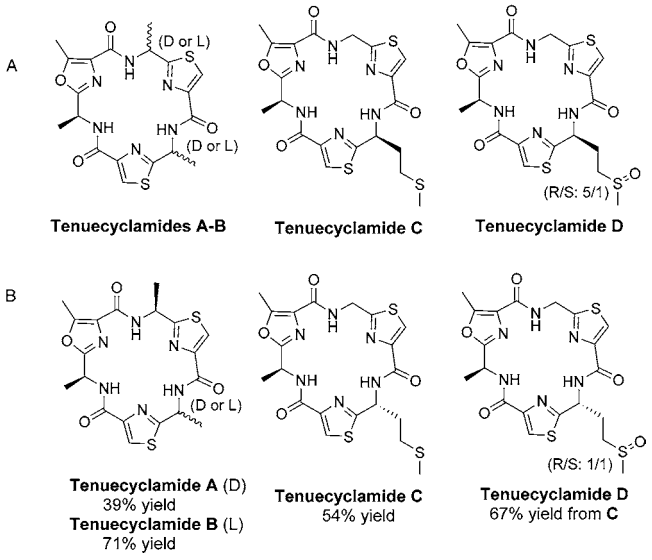


Figure 2. (A) Tenuecyclamides A−D as initially reported. (B) Tenuecyclamides A−D with their stereochemistry assigned or reassigned (isolated yields are reported).

Table 1. Syntheses of Diastereomers of Tenuecyclamides A and B

entry	compound	sequence[a]	yield (%)
1	**7**	[-((L-Ala)-Oxz(L-Thr))-((L-Ala)-Thz(L-Cys))-((L-Ala)-Thz(L-Cys))-]	71
2	**8**	[-((L-Ala)-Oxz(L-Thr))-((D-Ala)-Thz(L-Cys))-((L-Ala)-Thz(L-Cys))-]	41
3	**9**	[-((L-Ala)-Oxz(L-Thr))-((L-Ala)-Thz(L-Cys))-((D-Ala)-Thz(L-Cys))-]	39
4	**10**	[-((L-Ala)-Oxz(L-Thr))-((D-Ala)-Thz(L-Cys))-((D-Ala)-Thz(L-Cys))-]	38

[a] Sequence written N-to-C, synthesized C-to-N.

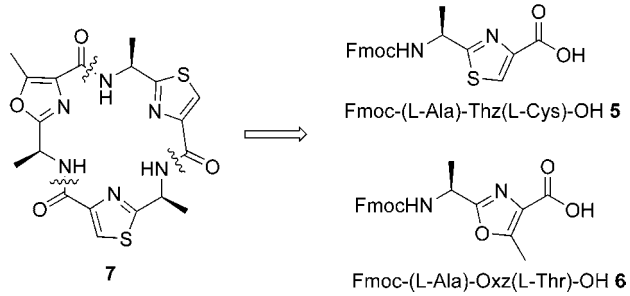


Figure 3. Retrosynthetic analysis of diastereomer **7**.

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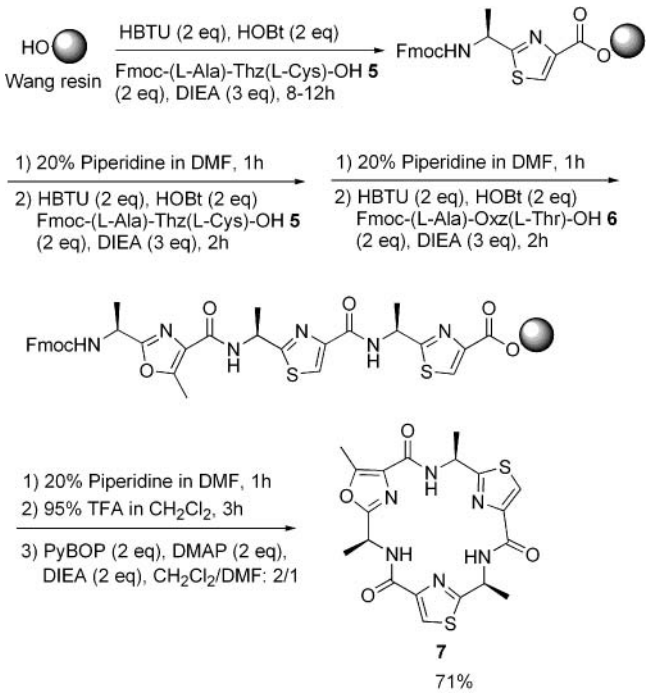
theses (Table 1), specifies the configuration and constitution of the α-amino acids used to make the heterocyclic amino acids by including their three-letter code in parentheses. Thz indicates a thiazole heterocycle, and Oxz indicates an oxazole heterocycle.

An outline of the representative solid-phase synthesis of **7** on Wang resin is shown in Scheme 1.[9] The first coupling

Scheme 1. Solid-Phase Synthesis of Diastereomer **7**[a]



[a] Abbreviations: DIEA = *N*,*N*-diisopropylethylamine, DMF = *N*,*N*-dimethylformamide, HOBt = *N*-hydroxybenzotriazole, HBTU = 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate.

between the resin and Fmoc-(L-Ala)-Thz(L-Cys)-OH **5** was performed for 8−12 h to ensure completion of ester bond formation. Removal of the Fmoc group was accomplished with 20% piperidine in DMF (1 h). Subsequent amide bond formation between the resin-bound amine and the next heterocyclic amino acid component (**5**) of the growing chain was enabled using HBTU/HOBt activation (2 h). After coupling the third heterocyclic amino acid (**6**) as described above, the terminal Fmoc group was removed and the heterocycle-containing diamide was cleaved from the Wang resin using 95% TFA in CH_2Cl_2. Removal of the solvent yielded the amino acid macrocyclization precursor that was transformed into the macrocycle using a combination of PyBOP and DMAP in solution.[7,8]

The analogous solid-phase approach yielded the three remaining diastereomers required to assign the stereochemistry of tenuecyclamides A and B (Table 1). We found that diastereomer **9** [-((L-Ala)-Oxz(L-Thr))-((L-Ala)-Thz(L-Cys))-((D-Ala)-Thz(L-Cys))-] had ^{1}H and ^{13}C NMR spectra identical with those reported for tenuecyclamide A.[3] Compound 7 [-((L-Ala)-Oxz(L-Thr))-((L-Ala)-Thz(L-Cys))-((L-Ala)-Thz(L-Cys))-] had ^{1}H and ^{13}C NMR spectra that corresponded to those reported for tenuecyclamide B. Their optical rotations were also comparable to those disclosed in the literature.[3]

Tenuecyclamide C was also synthesized by this solid-phase strategy (Table 2, entry 1). Compound **11** [-**(**(L-Ala)-Oxz(L-Thr)**)-(**(Gly)-Thz(L-Cys)**)-(**(L-Met)-Thz(L-Cys)**)-**] has the structure reported for tenuecyclamide C, but its ^{1}H and ^{13}C NMR spectra are distinct from those reported in the literature. To determine the actual structure of tenuecyclamide C, we first synthesized compound **12** [-**(**(L-Ala)-Oxz(L-Thr)**)-(**(L-Met)-Thz(L-Cys)**)-(**(Gly)-Thz(L-Cys)**)-**], in which the positions of (L-Met) and (Gly) were swapped, and found that it also had NMR spectra different from those reported in the literature. Then, we synthesized compound **13** [-**(**(D-Ala)-Oxz(L-Thr)**)-(**(Gly)-Thz(L-Cys)**)-(**(L-Met)-Thz(L-Cys)**)-**], which had identical NMR spectra but an opposite optical rotation. Finally, we synthesized compound **14** [-**(**(D-Ala)-Oxz(L-Thr)**)-(**(Gly)-Thz(L-Cys)**)-(**(D-Met)-Thz(L-Cys)**)-**], the enantiomer of compound **13**, and found that all of its properties matched those reported in the literature. Thus, we have established that tenuecyclamide C is composed of a (D-Met), instead of (L-Met), as previously reported.[3]

Table 2. Syntheses of Enantiomer and Diastereomers of Tenuecyclamide C

entry	compound	sequence[a]	yield (%)
1	**11**	[-**(**(L-Ala)-Oxz(L-Thr)**)-(**(Gly)-Thz(L-Cys)**)-(**(L-Met)-Thz(L-Cys)**)-**]	40
2	**12**	[-**(**(L-Ala)-Oxz(L-Thr)**)-(**(L-Met)-Thz(L-Cys)**)-(**(Gly)-Thz(L-Cys)**)-**]	33
3	**13**	[-**(**(D-Ala)-Oxz(L-Thr)**)-(**(Gly)-Thz(L-Cys)**)-(**(L-Met)-Thz(L-Cys)**)-**]	39
4	**14**	[-**(**(L-Ala)-Oxz(L-Thr)**)-(**(Gly)-Thz(L-Cys)**)-(**(D-Met)-Thz(L-Cys)**)-**]	54

[a] Sequence written N-to-C, synthesized C-to-N.

Oxidation (D-Met) of tenuecyclamide C (**14**) using 1.2 equiv of *m*-CPBA led to tenuecyclamide D (**15**) in 67% yield. The synthetic tenuecyclamide D is a 1:1 diastereomeric mixture of R and S sulfoxides, while the natural product appears to be a 5:1 diastereomeric mixture of R and S sulfoxides, respectively. In summary, the total synthesis and

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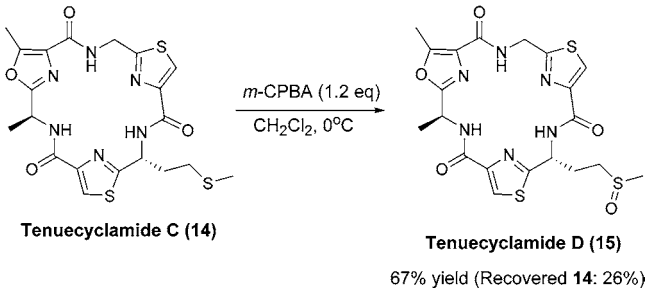
Scheme 2. Synthesis of Tenuecyclamide D (**15**)



Tenuecyclamide C (**14**)

Tenuecyclamide D (**15**)

67% yield (Recovered **14**: 26%)

stereochemical assignments of tenuecyclamides A−D have been accomplished by the solid-phase assembly of heterocyclic amino acids of defined stereochemistry. This method provides a very efficient means to synthesize thiazole- and oxazole-containing natural and unnatural macrolactam products. We believe that this methodology will be of broad interest for the synthesis of natural product libraries for the purpose of drug discovery.

Acknowledgment. We gratefully acknowledge NIH Grant GM63212 and the Skaggs Institute for Chemical Biology for generous financial support. We thank Professor Evan T. Powers for helpful discussions.

Supporting Information Available: Experimental details and NMR spectra for compounds **7**−**15**. This material is available free of charge via the Internet at http://pubs.acs.org.

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