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Kinetic Control of Proline Amide Rotamers: Total Synthesis of *trans,trans*- and *cis,cis*-Ceratospongamide

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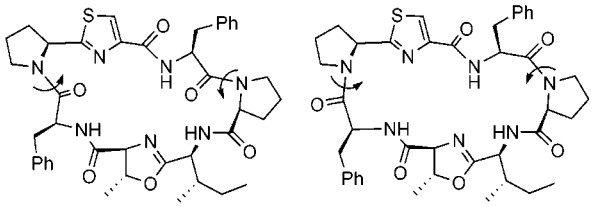
Cyclic peptides constitute a structurally diverse class of natural products that can exert profound effects on mammalian cells. Their biosynthesis is thought to occur primarily through the action of nonribosomal peptide synthetases.[1] Proline and *N*-methyl amino acids are common structural elements in bioactive cyclic peptides and dramatically affect their conformational preferences.[2] *N*-alkylation increases the number of conformational states by decreasing the free energy difference between *cis*- and *trans*-amide rotamers. At the same time, the *N*-alkyl substituent limits the number of low energy conformers by constraining adjacent torsion angles through allylic-type 1,3-interactions.



ceratospongamide

The ceratospongamides (**1** and **2**) are modified cyclic heptapeptides that were recently isolated from a marine alga/sponge symbiont.[3] Both ceratospongamides contain two proline residues. The peptide backbone is further constrained by a threonine-derived oxazoline and a cysteine-derived thiazole.[4] Remarkably, **1** and **2** are stable conformational isomers and are easily resolved by HPLC. They were characterized by extensive NMR experiments as having two *trans* and two *cis* proline amide bonds, respectively. Even when heated to 90 °C, **1** and **2** do not interconvert. The isolation of two noninterconverting proline amide rotamers from a natural source is, to our knowledge, unprecendented.

The three-dimensional structures and hence, the biological activities of **1** and **2** are predicted to be completely different from one another. Indeed, *trans,trans*-ceratospongamide **1**, but not **2**, was found to be a potent inhibitor (IC_{50} 32 nM) of transcriptional activation induced by the inflammatory cytokine, interleukin-1β (IL-1β).[3] The molecular basis for this cellular effect is not known. We therefore initiated studies toward a total synthesis of the ceratospongamideswith the ultimate goal of identifying the molecular target of **1**. We were also intrigued by the exceptional kinetic stability of **1** and **2**. The Shioiri group recently disclosed a synthesis of *cis,cis*-ceratospongamide (**2**).[5] However, they were unable to prepare the biologically active *trans,trans*-conformer. Here we report the first total synthesis of *trans,trans*-ceratospongamide (**1**).

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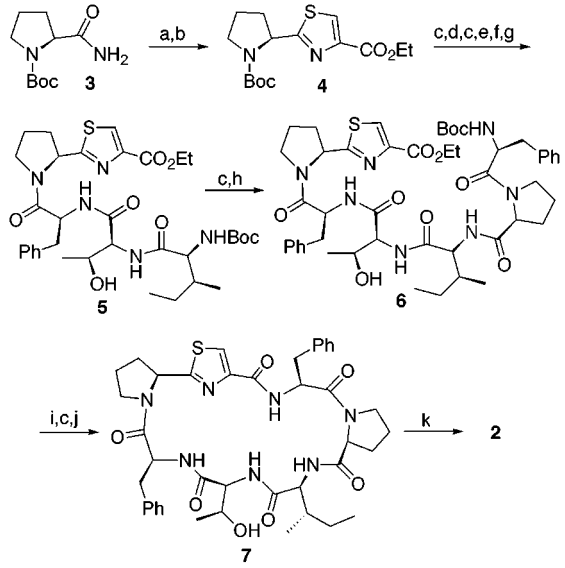
Scheme 1 [a]



[a] Reagents and conditions: (a) Lawesson's reagent, THF, rt (99%) (b) $KHCO_3$, ethyl bromopyruvate, DME, then $(CF_3CO)_2O$ (76%) (c) HCl, dioxane, CH_2Cl_2 (d) Boc-Phe-OH, EDC, HOBT, NMM, CH_2Cl_2 (77% for two steps) (e) Fmoc-*allo*-Thr-OH, EDC, HOBT, NMM, CH_2Cl_2 (98%) (f) morpholine, CH_2Cl_2 (100%) (g) Boc-Ile-OH, EDC, HOBT, NMM, CH_2Cl_2 (83%) (h) Boc-Phe-Pro-OH, EDC, HOBT, NMM, CH_2Cl_2 (98%) (i) LiOH, MeOH, H_2O (j) BOP, DMAP, DMF (95% for three steps) (k) Deoxo-fluor, CH_2Cl_2, −78 °C rt (88%).

Our results underscore the importance of the oxazoline ring in determining the kinetic distribution of conformational isomers.

Commercially available Boc-L-proline carboxamide (**3**) was transformed into the thiazole ethyl ester **4** in two steps and 65% yield according to a modified Hantzsch protocol (Scheme 1).[6] After Boc deprotection (HCl, dioxane, CH_2Cl_2), **4** was converted to the tetrapeptide-thiazole **5** using standard solution-phase peptide synthesis methods. The overall yield for this six-step sequence was 62%. The Boc protecting group was removed from **5**, and the free amino terminus was coupled to the dipeptide Boc-phenylalanine-proline to provide the hexapeptide-thiazole **6** in 98% yield. After removal of the ethyl ester and Boc groups, the linear peptide was cyclized by slow addition to a solution of the BOP reagent[7] (12.5 mM) and DMAP (25 mM) in DMF. This three-step procedure afforded the cyclic peptide **7** in 95% yield. Deoxo-Fluor efficiently promoted cyclodehydration[8] of the *allo*-threonine side chain to afford *cis,cis*-ceratospongamide (**2**) in 88% yield. A small amount (ca. 10%) of a related cyclic peptide, possibly the *cis,trans*- or the *trans,cis*-conformer, was also produced in this reaction and could not be separated from **2**.[9] However, the desired *trans,trans*-ceratospongamide (**1**) was not detected by HPLC or NMR analysis

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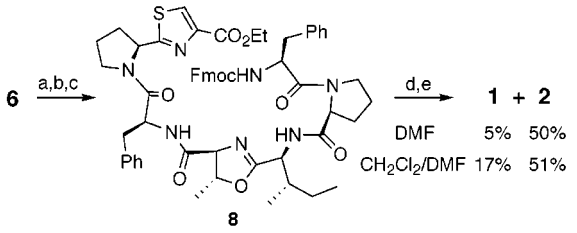
Scheme 2 [a]



[a] Reagents and conditions: (a) HCl, dioxane, CH_2Cl_2 (b) Fmoc-OSuc, DIPEA, THF (c) Deoxo-Fluor, CH_2Cl_2, −78 °C rt (100% for three steps) (d) LiOH, MeOH, H_2O (d) BOP, DMAP, syringe pump addition (depicted yields of **1** and **2** are of HPLC-purified material).

of the crude reaction mixture. These results are in accord with those obtained by the Shioiri group, who prepared **2** by a similar route.[10]

If macrocyclization to form **7**, rather than oxazoline formation, were the conformer-determining step, then closing the macrocycle at a different amino acid position might provide the *trans*,*trans*-conformer. In d_6-DMSO, **7** gives a single set of sharp peaks, indicating a single conformer or rapid interconversion between multiple conformers. In contrast, there are at least three conformers in $CDCl_3$ (see Supporting Information). These observations demonstrate that with cyclic peptide **7**, conformer interconversion occurs at room temperature. Thus, cyclization of any linear peptide to give intermediate **7** would provide the same, thermodynamic ratio of conformers, regardless of the reaction conditions or the position of ring closure.

We therefore investigated an alternative strategy of closing the macrocycle *after* forming the oxazoline, reasoning that the transition state of the macrocyclization step would be significantly different from the transition state of the oxazoline-forming reaction that provided **2**. Because the oxazoline moiety is unstable to acid, the Boc group in **6** was switched to an Fmoc group before treatment with Deoxo-Fluor (Scheme 2). The Fmoc-protected oxazoline **8** was thus obtained in quantitative yield. Simultaneous deprotection of both termini with LiOH, followed by macrocyclization with BOP/DMAP/DMF afforded a 1:10 ratio of *trans*,*trans*- and *cis*,*cis*-ceratospongamide in 55% overall yield. *Trans*,*trans*-ceratospongamide was easily purified by HPLC and was identical in all respects to naturally derived **1** (^{1}H and ^{13}C NMR, HPLC co-injection). To improve the yield of **1**, we investigated alternative cyclization conditions. Changing the solvent from DMF to a mixture of DMF and CH_2Cl_2 increased the *trans*,*trans*:*cis*,*cis* ratio to 1:3 (17% HPLC-purified yield of **1**), which is similar to the ratio of conformers that were isolated from the marine sponge.[3] Closing the macrocycle at the isoleucine−proline junction in either DMF or DMF/CH_2Cl_2 provided a 1:10 conformer ratio.

It is remarkable that macrocyclization of the oxazoline-containing peptide provided a kinetic distribution of conformers that was virtually identical to the biosynthetic ratio, whereas formation of the oxazoline ring after macrocyclization failed to give a trace of the *trans*,*trans*-conformer. Ceratospongamide biosynthesis is most likely mediated by a nonribosomal peptide synthetase (NRPS). The fact that ceratospongamide biosynthesis produces a mixture of conformers is consistent with the idea, first established by Walsh and colleagues, that NRPS cyclization domains are somewhat promiscuous and do not make specific contacts with the entire peptide chain.[11]

Assuming that the biosynthesis of *trans*,*trans*- and *cis*,*cis*-ceratospongamide is under kinetic control, it was of interest to determine their relative thermodynamic stabilities. Gerwick and colleagues reported that *cis*,*cis*-ceratospongamide could be converted into the *trans*,*trans*-conformer by heating in DMSO at 175 °C. However, their HPLC data showed at least eight additional peaks, with the *trans*,*trans*-conformer being a minor component.[3] In striking contrast, we found that *cis*,*cis*-ceratospongamide **2** was smoothly transformed (175 °C, degassed d_6-DMSO) into a 5:1 equilibrium mixture favoring *trans*,*trans*-ceratospongamide **1** (see Supporting Information). By subjecting pure **1** to the same conditions, we again obtained a 5:1 ratio of conformers. This experiment suggests that the thermodynamic ratio is the inverse of the kinetic and biosynthetic ratios. In addition, it provides a high-yielding route for the synthesis of **1**.

Our synthetic route is amenable to the preparation of suitable quantities of both ceratospongamide conformers for further biological testing. Future studies will be directed toward elucidating the mechanism by which *trans*,*trans*-ceratospongamide blocks IL-1β-mediated gene expression as well as defining the structural elements responsible for ceratospongamide's unique conformational properties. Adapting our route to the solid phase will facilitate the preparation of combinatorial libraries of cell-permeable cyclic peptides with novel biological activities.

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Supporting Information Available: Experimental procedures and spectral data for all new compounds (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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