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Stereoselective Synthesis of the γ-Lactam Hydrolysate of the Thiopeptide Cyclothiazomycin

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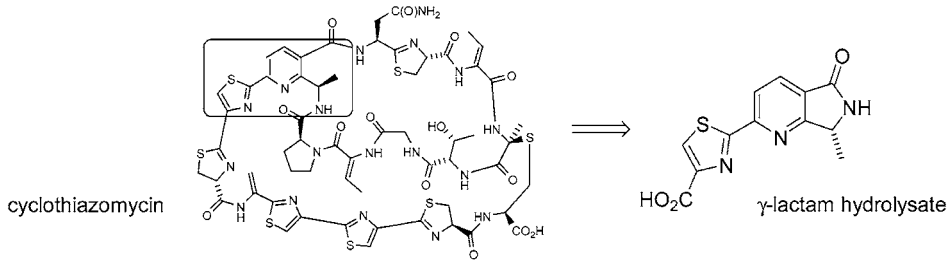
ABSTRACT



Bohlmann–Rahtz pyridine synthesis of a chiral nonracemic enamine and thiazolylpropynone gives a terminal-protected pyridine-containing γ-amino acid in high optical purity in a sequential one-pot multicomponent reaction that proceeds with total control of regiochemistry and with minimal racemization. Further elaboration has established the synthesis of the γ-lactam acidic hydrolysate of the macrocyclic thiopeptide antibiotic cyclothiazomycin, a selective renin inhibitor, in only four steps and 30% overall yield and has confirmed its structure.

The thiopeptide or thiazolylpeptide antibiotics, according to Bérdy's structural classification,[1] comprise at least 29 different families of sulfur-containing macrocyclic peptide secondary metabolites produced by actinomycetes, Gram-positive mycelial sporulating bacteria. They possess a wide range of biological properties, most inhibiting bacterial protein synthesis by impeding conformational changes in the bacterial ribosome, binding to the complex of 23S rRNA and protein L11,[2] or by preventing the formation of the aminoacyl−tRNA·Ef−Tu·GTP ternary complex.[3] Cyclothiazomycin (**1**), one of 76 structurally distinct actinomycete thiopeptide natural products, was isolated from the fermentation broth of *Streptomyces* sp. NR0516 obtained from a soil sample collected at Kanagawa, Japan, and is a selective inhibitor of human plasma renin at an IC_{50} of 1.7 μM.[4] Its unique structure, consisting of a (1-amino-1-ethyl)pyridine heterocyclic domain embedded in two macrocyclic peptide loops,[5] and absolute stereochemistry were determined using a combination of spectroscopic and analytical methods, supported by chemical degradation studies that isolated a heterocyclic amino acid as γ-lactam **2** and saramycetic acid I (**3**) from the acid hydrolysate of the natural product (Scheme 1).[6] The preparation of some of the unusual structural motifs present in cyclothiazomycin has attracted synthetic interest,[7,8] but the stereospecific synthesis of its γ-amino acid central heterocyclic domain has not been addressed. Heterocyclic amino acids are of particular interest as modified components of peptides, proteins, peptide nucleic acids (PNAs), and peptidoglycans and can function as mediators of neuronal signal transduction.[9] Although the stereospecific synthesis of pyrimidyl, isoxazolyl, pyrazolyl, and pyridyl α-amino acids[9] and conformationally constrained

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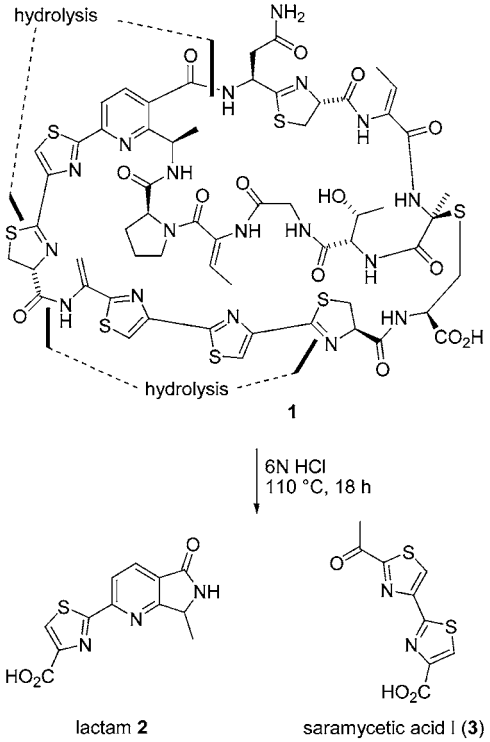
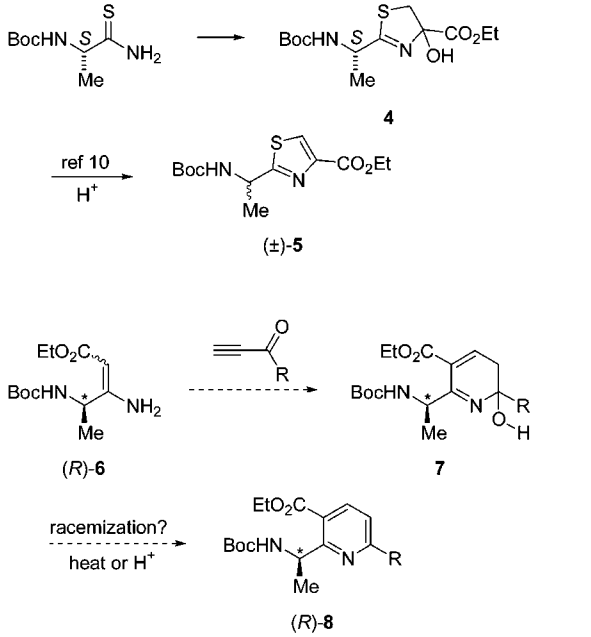
Scheme 1. Cyclothiazomycin (**1**) and Its Hydrolysates



Scheme 2. Racemization in Heteroannulation Reactions[a]



[a] Top: Racemization in the Hantzsch synthesis of thiazole-containing δ-amino acid **5**. Bottom: Proposed Bohlmann−Rahtz synthesis of pyridine-containing (*R*)-γ-amino acid **8**.

azole-containing δ-amino acids has received considerable attention,[10] the preparation of the corresponding γ-amino acids has been somewhat neglected. This communication describes the stereoselective synthesis of conformationally constrained pyridine-containing γ-amino acids and prepares both the cyclothiazomycin domain and its lactam hydrolysate **2** in order to verify natural product structure and facilitate its future synthesis.

Our approach utilizes a Bohlmann−Rahtz reaction[11] to assemble the pyridine domain from acyclic precursors starting from the corresponding amino acid, a highly successful heteroannulation method used in the synthesis of promothiocin A,[12] dimethyl sulfomycinamate,[13] and the tris(thiazolyl)pyridine domain of the amythiamicins.[14] However, the effectiveness of a Bohlmann−Rahtz strategy, in this case hitherto unreported, relies upon the ready availability of chiral enamine **6**, which must proceed through the heteroannulation without racemization (Scheme 2). The synthesis of a related heterocyclic δ-amino acid, alanine-derived thiazole **5**, by Hantzsch cyclocondensation of the corresponding thioamide and ethyl bromopyruvate, results in considerable racemization due to the acid instability of hydroxythiazoline **4** (Scheme 2).[10a] The significant challenge in a stereospecific approach to the cyclothiazomycin lactam **2** concerns how to prevent the loss of optical purity in an analogous process by epimerization of 6-hydroxy-5,6-dihydropyridine **7** when the double-bond isomerization/cyclodehydration Bohlmann−Rahtz sequence inherent in the reaction requires high temperatures or an acid catalyst in order to facilitate pyridine **8** heteroannulation.[15]

To this end, reacting the known (*R*)-ketoester **9**[16] with ammonium acetate gave enamine **6**; however, during its formation or purification, this chiral intermediate racemized on exposure to heat (ethanol at reflux), Brønsted acids (5:1 toluene−acetic acid), or silica gel and could only be isolated in 70% yield and 92% ee by carrying out the reaction at room temperature in ethanol and using the crude material without purification (Scheme 3).

The Bohlmann−Rahtz reaction of enamine **6** and readily available propynone **10**[14] under traditional heteroannulation conditions, Michael addition at 50 °C for 10 min followed by cyclodehydration at 135 °C (entry 1), gave pyridine **8a** as a single regioisomer in 73% yield, albeit only in 14% ee. The microwave-assisted reaction[17] resulted in appreciable loss

(9) For lead references and recent examples of the synthesis of heterocyclic α-amino acids, see: (a) Dondoni, A.; Massi, A.; Minghini, E.; Sabbatini, S.; Bertolasi, V. *J. Org. Chem.* **2003**, *68*, 6172. (b) Adlington, R. M.; Baldwin, J. E.; Catterick, D.; Pritchard, G. J.; Tang, L. T. *J. Chem. Soc., Perkin Trans. 1* **2000**, 303. (c) Adlington, R. M.; Baldwin, J. E.; Catterick, D.; Pritchard, G. J.; Tang, L. T. *J. Chem. Soc., Perkin Trans. 1* **2000**, 2311.

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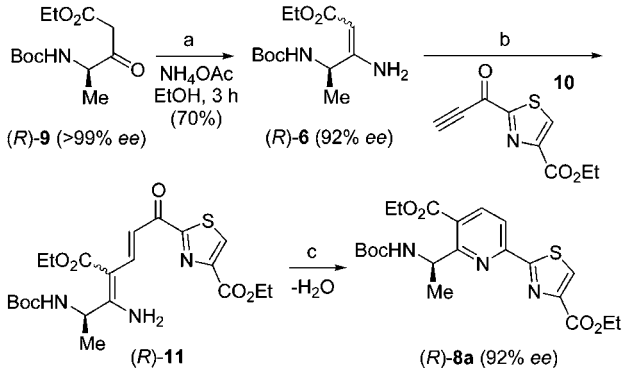
Scheme 3. Synthesis of the Cyclothiazomycin Domain



entry	reagents & conditions (yield)	*ee*%
1	b) EtOH, 50 °C, 10 min; c) neat, 135 °C, 4 h (73%)	14
2	b & c) μwave, 170 °C, 20 min (10%)	33
3	b & c) PhMe, AcOH, 60 °C, 90 min (73%)	47
4	b) EtOH, 50 °C, 10 min; c) PhMe-AcOH, 60 °C, 90 min (66%)	81
5	b) EtOH, 50 °C, 10 min; c) NIS, 0 °C, 15 min (71%)	92
6	a-c) NH_4OAc, EtOH, 4 h; **10**, 1 h; NIS, 0 °C, 15 min (55%)	96

of material but did improve the optical purity somewhat (entry 2). As predicted, the one-pot acid-catalyzed heteroannulation process[18] was much more efficient but did little to prevent racemization throughout the process (entry 3), presumably as a consequence of the acid instability of hydroxydihydropyridine **7**. However, in combination with a Michael addition under traditional Bohlmann−Rahtz conditions, the acid-catalyzed cyclodehydration of the diaminodienone intermediate **11** at 60 °C caused a significant increase in the optical activity of pyridine **8a** (entry 4).

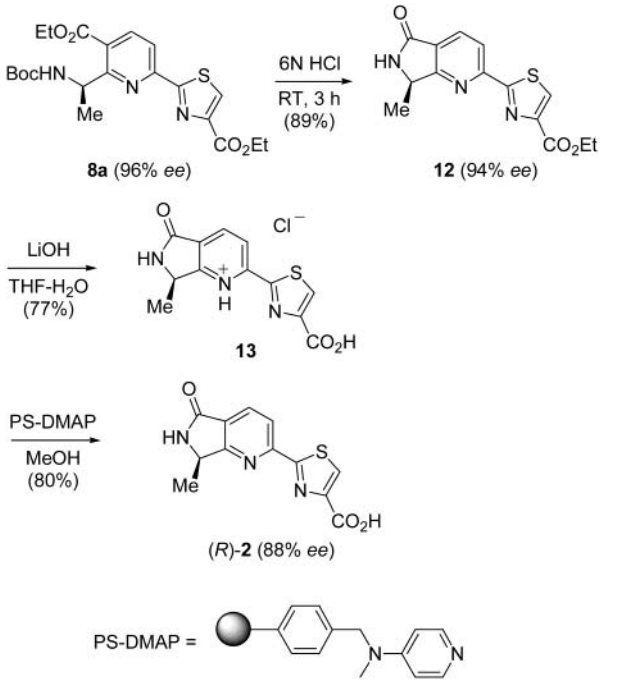
Changing the cyclodehydrating agent from a Brønsted acid to *N*-iodosuccinimide (NIS), a reagent effective in this transformation at 0 °C,[19] further improved the stereoselectivity of the process (entry 5), giving pyridine **8a** in 92% ee. Although an excellent result, the optical purity of the product was still limited by the stereochemical instability of enamine **6** following isolation of this Bohlmann−Rahtz precursor.

To overcome the limiting racemization of enamine **6**, a new sequential one-pot process was investigated for the stereoselective synthesis of the cyclothiazomycin domain. Ammonium acetate was added to a solution of β-ketoester **9** (>99% ee) in ethanol. After 4 h at room temperature, thiazolylpropynone **10** was added and the mixture was stirred to complete the Michael addition. The mixture was then cooled to 0 °C, and *N*-iodosuccinimide was added (Scheme 3, entry 6). Pleasingly, after column chromatography, C- and N-terminal-protected amino acid **8a** was isolated directly in 55% yield and 96% ee from this one-pot reaction, demonstrating a facile stereoselective route to pyridyl γ-amino acids from the corresponding β-ketoester that avoids isolation of chiral enamine intermediates.

From chiral nonracemic pyridine **8a** it was anticipated that the total synthesis of the cyclothiazomycin lactam **2** would be realized simply by hydrolysis in accordance with the degradation studies on the natural product (Scheme 1).[6]

To our surprise, when pyridine **8a** was heated to 110 °C in 6 N hydrochloric acid, a complex mixture of products was obtained. Under milder conditions, when the reaction was repeated at room temperature, the N- and C-terminal protecting groups were cleaved but the ethyl thiazole-4-carboxylate group remained untouched to give γ-lactam **12** in 94% ee (Scheme 4). Base-catalyzed hydrolysis at room

Scheme 4. Synthesis of the Cyclothiazomycin Hydrolysate, γ-Lactam **2**



temperature, with acidic workup, was expected to complete the synthesis of the cyclothiazomycin hydrolysate **2**, but the UV and ^{1}H and ^{13}C NMR spectroscopic data of the material isolated from the natural product,[6] which was identified as hydrochloride **13**, failed to match that of the colorless precipitate obtained from the reaction. Even more surprising was the observation that elution on a solid-phase extraction column, a procedure used to isolate hydrolysate **2** from the natural product,[20,21] returned only the pyridinium salt.[22] However, when hydrochloride **13** was stirred with 1 equiv of polymer-supported 4-(dimethylamino)pyridine (PS-DMAP)

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in dry methanol for 18 h, filtered, and evaporated, chiral nonracemic 2-(2-pyridyl)thiazole-4-carboxylic acid **2** was obtained in 80% yield and 88% ee, the spectroscopic characteristics and chromatographic behavior of which were in agreement with reported literature data.[6,20,23] The entire synthetic sequence gives the C- and N-terminal-protected heterocyclic domain of cyclothiazomycin in 96% ee and 55% yield in only one step from the known (*R*)-β-ketoester **9** and generates the γ-lactam hydrolysate of the natural product in 88% ee and 30% overall yield in only four steps.

In summary, we have described the stereoselective synthesis of a chiral diaminoalkenoate, diaminodienone, and the γ-lactam form of a heterocyclic amino acid, corresponding to Bohlmann−Rahtz enamine, dienamine intermediate, and pyridine product, respectively, in high optical purity, good yield, and very few synthetic steps from a chiral pool precursor with total regiocontrol. The utility of our facile approach, centered on a sequential one-pot, three-component process, has been demonstrated in the first successful synthesis of the cyclothiazomycin γ-lactam hydrolysate, which verifies its structure, with minimum racemization and now is expected to be used in the total synthesis of the actinomycete thiopeptide secondary metabolite.

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Supporting Information Available: Experimental procedures and characterization data for all compounds, including methods used for the determination of optical purity. This material is available free of charge via the Internet at http://pubs.acs.org.

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(21) Hydrolysis of **1** (refs 6 and 20) in aq HCl (6 N) at 110 °C under nitrogen gave a residue that was suspended in water and transferred to a solid-phase extraction column (Sep-pak C18), gradient eluting with water and methanol. Purification by column chromatography on Sephadex LH20, eluting with methanol, gave hydrolysate **2** as a film, R_f 0.64 on silica, eluting with *n*BuOH−AcOH−water (3:1:1).

(22) Pyridinium hydrochloride **13** was obtained as a colorless solid, R_f 0.58 on silica, eluting with *n*BuOH−AcOH−water (3:1:1), mp 287 °C dec (from aqueous methanol): [α] +30.8 (*c* 0.26, MeOH); UV λ_{max} (MeOH) 234 nm (ϵ 12 400), 314 nm (ϵ 17 000). See Supporting Information for more experimental details and spectroscopic data.

(23) Dihydropyrrolo[3,4-*b*]pyridine **2** was obtained as a colorless solid, mp 185 °C (dec): [α] +22.7 (*c* 0.12, MeOH); UV λ_{max} (MeOH) 230 nm (ϵ 12 200), 321 nm (ϵ 14 400). See Supporting Information for more experimental details and spectroscopic data.