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Enantiotracin

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Abstract—The enantiomer of the antibiotic bacitracin A was prepared by solid-phase total synthesis. *ent*-Bacitracin A was found to be equally potent to the natural enantiomer in in vitro susceptibility assays. This supports the notion that bacitracin exerts its antibacterial effects through interaction with bactoprenylpyrophosphate, an achiral ligand.

We report the synthesis and in vitro analysis of the enantiomer of the antibacterial agent bacitracin A (*ent*-bacitracin A, Fig. 1). This work was undertaken in order to provide a stereochemical probe of the mechanism of action of bacitracin.

Treatment of bacterial cells with bacitracin leads to accumulation of undecaprenylpyrophosphate (bactoprenylpyrophosphate, BPP), the lipid carrier of precursors to cell wall peptidoglycan.[1] Strominger, Stone, and Storm demonstrated that bacitracin binds in a metal-dependent fashion to BPP.[2–4] This led them to propose that bacitracin's direct sequestration of BPP prevents it from being recycled for use in the cell wall biosynthesis cycle. As an achiral molecule, BPP is non-enantiodifferentiating with respect to potential receptors; that is BPP will bind to bacitracin A and *ent*-bacitracin A with equal affinity. Thus, if the biological activity of bacitracin A derives from its association with BPP, then *ent*-bacitracin A should exhibit activity equal to that of bacitracin A so long as the bioassay employed is not influenced by differences in, for example transport and metabolism of the enantiomeric forms.

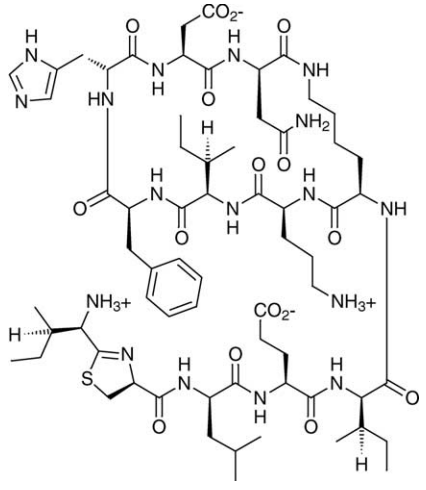

Figure 1. *ent*-Bacitracin A.

Our method for the solid-phase total synthesis of natural bacitracin A made it possible to prepare *ent*-bacitracin A by substituting enantiomeric Fmoc-protected amino acids.[5] Aloc and allyl ester protection of the L-Orn-7 side chain amino group and D-Asn-12 α-carboxyl groups, respectively, were employed to allow for concurrent, orthogonal deprotection and on-resin macrocyclization. 2-[1′-(*R*)-*t*-Butyloxycarbonylamino-2′-(*S*)-methylbutyl]-4-(*S*)-carboxy-Δ^2-thiazoline was used for installation of the N-terminal moiety. After synthesis, the product was deprotected and cleaved from the resin using trifluoroacetic acid/phenol/triisopropyl-silane (50:2.7:1), isolated by precipitation from 2:1 ether/pentane, fractionated by reverse-phase HPLC, and recovered by lyopholization. The purified product, obtained in 6.2% overall yield, co-chromatographed with both natural and synthetic bacitracin A. The ion-spray mass spectrum (m/z 1423.0 corresponding to MH^+) and ^1H NMR spectrum of the product were also indistinguishable from those of natural bacitracin A.

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Table 1. In vitro susceptibility testing of bacitracin A enantiomers

Compd (source)	MIC (μg/mL)[a]		
	M. luteus[b]	*S. aureus*[c]	*S. epidermidis*[d]
Bacitracin A (natural)	<0.013	4	32
ent-Bacitracin *A* (synthetic)	<0.013	4	32

[a]Minimal concentrations of agents required to inhibit bacterial cell growth in a broth microdilution assay. Assays were carried out in cation-adjusted Mueller–Hinton broth. Values reported are averages from replicate experiment.
[b]*Micrococcus luteus* strain 147.
[c]*Staphylococcus aureus* strain 33591, methicillin-resistant.
[d]*Staphylococcus epidermidis* strain MED-392, methicillin-resistant.

The biological activity of *ent*-bacitracin A was compared to that of natural bacitracin A, which had been purified by reverse-phase HPLC. Standard in vitro susceptibility assays were performed with a series of Gram-positive pathogens including *Micrococcus luteus*, *Staphylococcus aureus* and *Staphylococcus epidermidis* (Table 1). *ent*-Bacitracin A displays minimum inhibitory concentrations (MIC values) against these organisms that are indistinguishable from those of the naturally occurring enantiomer. Thus, we conclude that the activity of bacitracin A derives from its ability to interact with one or more non-enantiodifferentiating targets. Accordingly, these data support the BPP-complexation model for bacitracin action derived previously from findings that (1) bacitracin A complexes with BPP at concentrations similar to those at which it inhibits bacterial cell growth, and (2) structural modifications of bacitracin A that abolish affinity for BPP also abolish biological activity.[2–4] However, our data are not sufficient to conclude that bacitracin/BPP complexation represents the *sole* basis for activity. Bacitracin also alters and permeabilizes membranes at concentrations similar to those required for BPP binding and for inhibition of cell wall biosynthesis,[6–8] and studies have shown that the bacterial cell membrane is not highly stereodiscriminatory when it comes to the action of membrane-disrupting peptides (e.g., cecropins and magainins).[9,10]

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