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The Total Synthesis of Pantocin B

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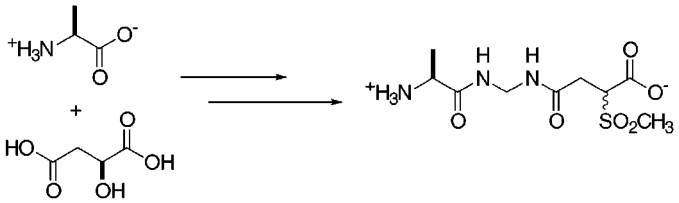
ABSTRACT



Pantocin B, an unusual antibiotic produced by *Erwinia herbicola*, effectively controls *E. amylovora,* the pathogen causing the plant disease fire blight. A total synthesis of pantocin B from L-alanine, glycine, and L-malic acid is reported.

Fire blight, a devastating disease of apples, pears, and other rosaceous plants, is caused by the pathogenic bacterium *Erwinia amylovora.* A closely related bacterium, *E. herbicola* (syn. *Pantoea agglomerans*), produces antibiotics that effectively control *E. amylovora* in the laboratory and the field. The large number of antibiotics produced by *E. herbicola* complicated the isolation of the antibiotics from wild-type strains, and a genomic library and heterologous expression approach was used to simplify the problem.[1] A cosmid library from *E. herbicola* strain 318 (Eh 318) contained two antibiotic producing clones, and the antibiotics they produced were trivially named pantocin A and B.[1] A recent publication from this laboratory dealt with the structure and biological activity of pantocin B (**1**, Figure 1). The total synthesis of

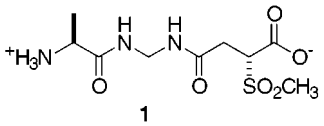


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Figure 1. Structure of crystalline pantocin B (see text).

pantocin B (**1**) was undertaken both to provide material to investigate the antibiotic's biological activity and to explore the feasibility of a chemical approach to constructing the multiple antibiotics produced by *E. herbicola.*

Pantocin B (**1**) and related molecules can be constructed from the coupling of two roughly equal fragments, each containing one of the stereocenters (Figure 2). One fragment can be derived from L-alanine and glycine, while the other can be derived from L-malic acid.

This approach also should prove adaptable for the synthesis of analogues.

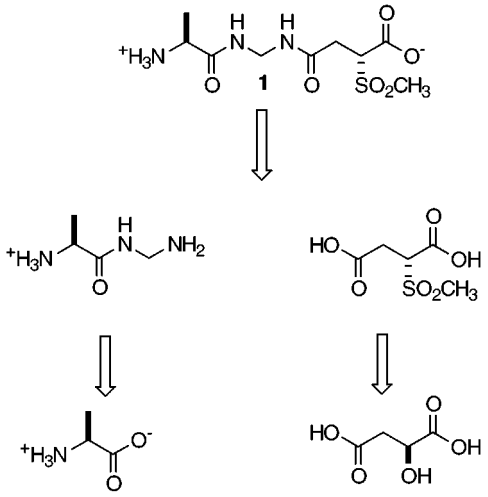

Figure 2. Retrosynthetic analysis of pantocin B.

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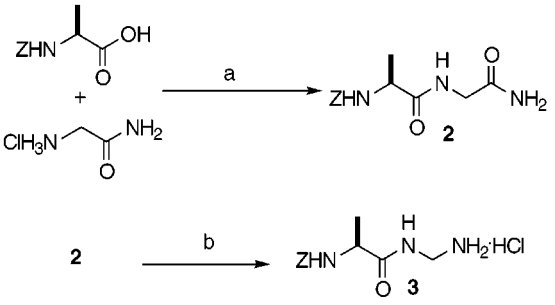
The least well-precedented part of the synthesis involves the methylenediamine moiety in the L-alanyl fragment. Most work concerning the synthesis of such *N*-(1-aminoalkyl)-amides focuses on their role as intermediates in carboxyl-terminal peptide sequencing.[2] Only a few examples exist where such geminal amino amides were directly synthesized.[3] The one precedent for synthesizing such an *N*-(1-amino-methyl)amide in a peptidic molecule had an overall yield of only 14%.[4] Following Loudon's procedure employing [*I*,*I*′-bis(trifluoroacetoxy)iodo]benzene (PIFA) to affect an acidic Hofmann rearrangement, we were able to synthesize the desired amine (**3**) from the CBZ-L-alanylglycinamide derivative (**2**) in 48% yield (Scheme 1). The *N*-(1-aminomethyl)-

Scheme 1



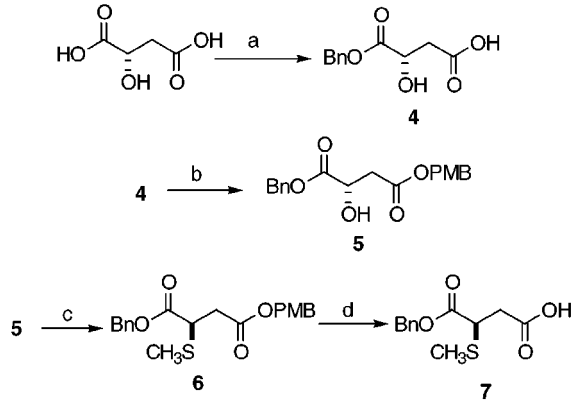
a) CDI, NEt_3, THF, 73% (b) i. PIFA, aq MeCN ii. HCl, 48%

amides are remarkably stable to hydrolysis, surviving under a large range of acidic (pH ≥ 1) as well as moderately basic conditions (pH ≤ 11).[5]

The malic acid derived portion of pantocin B proved to be more synthetically challenging due to the acidity of the methylene protons. The choice of protecting groups had to allow for selective deprotection under conditions mild enough to prevent unwanted side reactions. After some experimentation, the benzyl and *p*-methoxybenzyl esters were selected. Malic acid was selectively converted to 2-hydroxysuccinic acid 1-benzyl ester (**4**) following Miller's procedure (Scheme 2).[6]

The use of triflate as a leaving group in the substitution reaction is essential to avoid a competing elimination−addition reaction, observed with both the mesylate and the tosylate under a variety of conditions. The unwanted addi-

Scheme 2

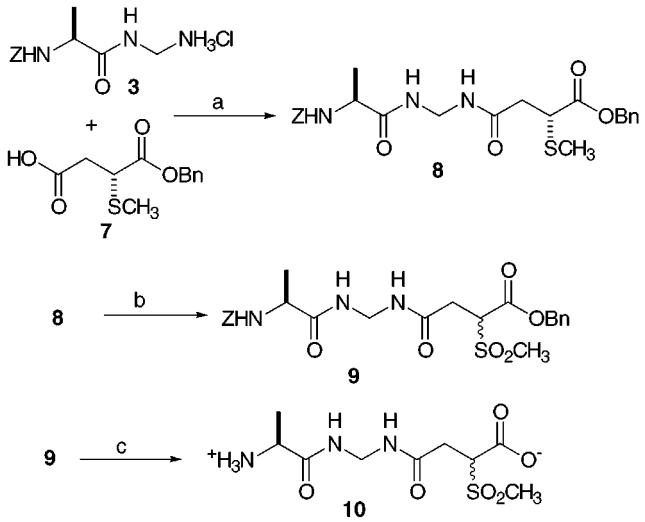


a) i. TFAA, 0 °C ii. BnOH, 80% (b) *p*-methoxybenzyl chloride, K_2CO_3, DMF, 74% (c) i. Tf_2O, 2,6 lutidine, DCM, -78 °C ii. CH_3SNa, 15-crown-5, DMF, -78 °C to RT, 78% d)10% TFA in DCM, 0 °C, 100%

tion−elimination reaction leads to a loss of both stereochemistry and regiochemistry. The triflate gives the desired substitution reaction with predominant inversion. A 5:1 diastereoselective ratio as was observed by the anisotropic shifts in the ^{1}H NMR of the (*S*)-mandelate derivative of **7**.[7] A single-crystal X-ray structure of **6**, using anomalous diffraction to fix the absolute configuration, confirmed the stereochemical assignments. Finally, the selective cleavage of the *p*-methoxybenzyl ester gives **7** in essentially quantitative yield.

The two fragments were then coupled using a water-soluble carbodiimide and *N*-hydroxybenzotriazole yielding **8** in 73% yield (Scheme 3).

Scheme 3



a) EDAC, HOBT, N-ethylmorpholine, DCM, 73%
b) mCPBA, DCM, 0 °C
c) Pd-black, 5% HCOOH in MeOH, 47% from **8**

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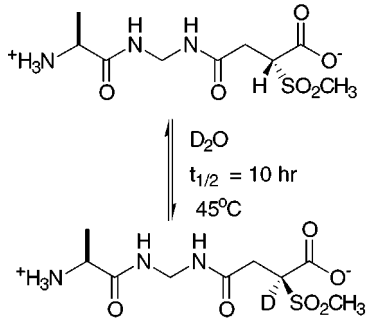
Oxidation of **8** with either Oxone or *m*-CPBA under a variety of conditions inevitably led to epimerization of the stereocenter bearing the methylsulfonyl group. The ^{1}H NMR spectra of **10** in both DMSO-d_6 and D_2O as well as the X-ray crystal structure matched those of the isolated antibiotic. The stereochemical scrambling was consistent with our experience in the isolation and structure elucidation of pantocin B from natural sources. During the structure determination of the natural product, the ^{1}H NMR spectra obtained in DMSO-d_6 indicated two species thought to be epimers. In part, the synthesis of pantocin B was undertaken to determine whether epimerization occurred during the isolation of the natural product or under typical in vivo conditions. The epimerization of **9** coupled with the observed acidity of the methine protons of the malic acid derivative support the epimerization occurring under typical biological conditions.

An NMR study to investigate epimerization or possible conformational equilibria was undertaken. The ^{1}H NMR of compound **10** does not exhibit line broadening, from 25 to 85 °C in either DMSO-d_6 or D_2O, which would be expected with a slow conformational equilibrium. A variable-temperature NMR in D_2O exhibits a decrease in the intensity of the sulfone-bearing methine, suggesting H/D exchange. The resonance at δ 4.32, that of the sulfone-bearing methine, also disappears over the course of 10 days at room temperature. Over a similar period of time the magnitude of the optical rotation decreases from 19.9° to 14.3° (c = 0.71, H_2O, 25 °C). Adjusting the pH to 3.5 accelerates the H/D exchange 3-fold. The $t_{1/2}$ of the observed H/D is 10 h at 45 °C (Scheme 4).[8] The isolated antibiotic was observed to have a similar H/D exchange at room temperature over the course of 2 weeks.[1] We believe that pantocin B naturally exists as an epimeric pair, one of which preferentially crystallizes.

Synthetic pantocin B exhibits activity against *E. amylovora* in a disk diffusion assay. Zones of inhibited growth of *E. amylovora* were measured at antibiotic loading of 125, 63, and 31 ng. The antibiotic exhibited activity at all three concentrations, having kill zones of 19, 17, and 14 mm, respectively—essentially identical to the natural antibiotic. Pantocin B is equipotent with streptomycin on a weight basis. Pantocin B resistant mutants appear in laboratory cultures of *E. amylovora* (approximately three resistant colonies per million cells). Synthetic material is similarly ineffective against these resistant colonies. The antibiotic activity of natural pantocin B can be suppressed by adding arginine to the test media. Synthetic material shows identical arginine suppression. Thus, by the criteria of antibiotic potency, resistant mutants, and arginine suppression, synthetic and natural pantocin B are indistinguishable.

Scheme 4



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Supporting Information Available: Text giving the experimental procedures and characterization for compounds **2**−**10**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL991264X

(7) Tyrrell, E.; Tsang, M. W. H.; Skinner, G. A.; Fawcett, J. *Tetrahedron* **1996**, *52*, 9841.

(8) Kinetic data were collected at 400 MHz using a sealed tube containing 10 mg of **10** in 0.4 mL of D_2O at 45 °C, pH =7 for 1.5 half-lives. The half-life and rate constant, k, were determined by linear regression.