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Efficient Synthesis of a Peculiar Vicinal Diamine Semiochemical from *Streptomyces natalensis*

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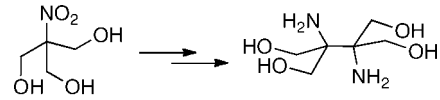
ABSTRACT

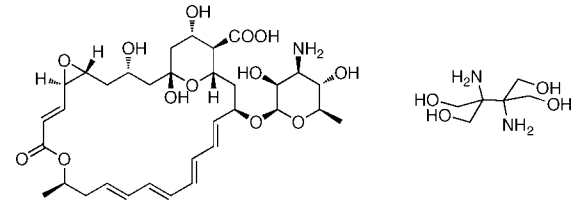


PI factor

The pimaricin-inducing (PI) factor, produced by *Streptomyces natalensis* is a proposed pheromone with a peculiar vicinal diamine structure. The first synthesis of this molecule is reported. It features oxidative dimerization of an *aci*-nitro anion derived from tris(hydroxymethyl)nitromethane and disproportionation catalyst-facilitated hydrogenation of the resulting vicinal tertiary dinitro compound. As the synthesis requires only four steps with no chromatographic separations, it provides a convenient route to prepare PI factor for biological studies and industrial applications.

Streptomyces natalensis is a soil-dwelling bacterium that produces a commercially important antifungal polyene macrolide called pimaricin (Figure 1).[1] This natural product is widely used in clinical and veterinary medicine for treatment of fungal infections and in the food and agricultural industries as a preservative. Notably, pimaricin is the only FDA-approved natural antifungal food preservative on the market. Tons of pimaricin are produced annually by fermentation of *Streptomyces* bacteria.

In 2004, a small molecule termed the "pimaricin-inducing factor" (PI factor, **1**) was isolated from wild type *S. natalensis* (Figure 1).[2] It is believed to be a critical regulator of pimaricin biosynthesis in *S. natalensis*.[3] The proposed structure of PI factor is a tetrahydroxy vicinal diamine. Interestingly, no other bacterial chemical messenger with a similar structure has been reported.[4] PI factor is reported to increase pimaricin production in the wild type *S. natalensis* by up to 40% at concentrations as low as 50 nM.[5] The mechanism of action of PI factor is entirely unknown, and its utility in industrial fermentations has yet to be established. Studies of PI factor have been limited by access to pure preparations of the semiochemical. The low quantities in which *S. natalensis* produces PI factor (nanograms/liter of culture) and the high polarity of the molecule make large-scale isolation impractical.[2] The only viable means for producing useful quantities of PI factor is by chemical synthesis.



pimaricin PI Factor

Figure 1. Pimaricin and the pimaricin-inducing factor (PI factor, **1**) from *S. natalensis*.

Although PI factor is a very low-molecular weight compound lacking stereocenters, its density of polar functional groups makes it a challenging synthetic target. In exploring synthetic routes toward PI factor, we found a lack of general and efficient methods for the preparation of vicinal diamines. The most

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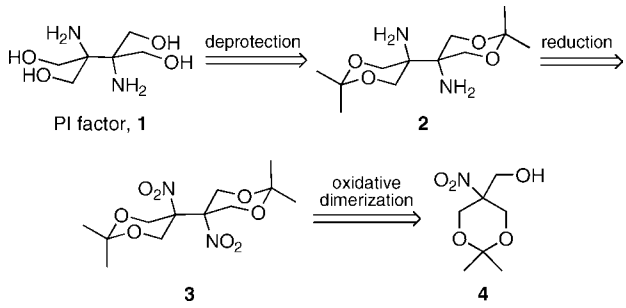
commonly reported routes to these compounds involve reductive couplings of imines, diamination of alkenes, conjugate additions of amines to α,β-unsaturated nitro compounds, and ring-opening of aziridines by nitrogen nucleophiles.[6] None of these methods are ideal for the synthesis of PI factor.

In light of the limitations of methods for vicinal diamine synthesis, we reasoned that the best way to construct the skeleton of PI factor, **1**, would be through oxidative dimerization of an *aci*-nitro anion followed by hydrogenation of the resulting dinitro product, **3** (Scheme 1). We envisioned

Scheme 1. Retrosynthetic Analysis of the PI Factor

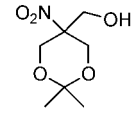
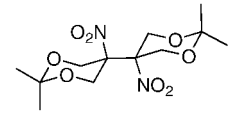
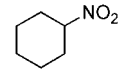
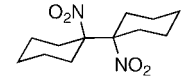
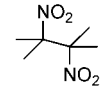
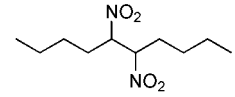
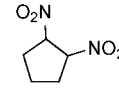


that the requisite *aci*-nitro anion could be generated from tris(hydroxymethyl)nitromethane via a retro-Henry reaction. In fact, oxidative dimerization of the *aci*-nitro anion derived from ketal-protected tris(hydroxymethyl)nitromethane,[7] **4**, using hydrogen peroxide as an oxidant was reported in a patent.[8] Although this reaction provided the desired product, its yield was only 11%. We set out to develop reaction conditions for the oxidative dimerization of the requisite dinitro compound that were higher yielding.

After extensive screening of bases and solvents, it was found that the retro Henry reaction yielding the *aci*-nitro anion from **4** proceeded best with NaH in THF. To effect the single electron oxidation of the anion, our oxidant of choice was ceric ammonium nitrate (CAN). This reagent has been shown to be effective in the oxidation of *aci*-nitro anions.[9] CAN-promoted oxidative dimerization yielded the desired product **3** in a 3-fold higher yield than same reaction using a peroxide oxidant. We reasoned that the yield of the CAN-promoted oxidation of the *aci*-nitro anion was negatively effected by the presence of the sodium counterion. We found that inclusion of the sodium chelator 15-crown-5 increased the yield of the CAN-promoted oxidation from 38% to 68%.

In the course of our work, Chavez and co-workers reported oxidative dimerization of **4** using aqueous sodium persulfate as the oxidant.[10] The yield of the reaction under our conditions and that of the reaction effected by aqueous sodium persulfate are virtually identical. Given the limited solubility of nitroalkanes in water, we suspected that our conditions for dimerization would be compatible with a wider range of substrates. Thus, we carried out a series of CAN-mediated oxidative dimerizations with both primary and secondary nitroalkanes. We were gratified to find that inter- and intramolecular oxidative couplings of aliphatic nitro substrates were achieved in moderate yields (Table 1).

Table 1. Substrate Scope of Oxidative Nitro Dimerization

starting material		product		% yield
4[a]		3		68
5[b]		9		66
6[b]		10		52
7[b]	$CH_3(CH_2)_4NO_2$	11		46
8[c]	$NO_2(CH_2)_5NO_2$	12		48

[a] 2.4 equiv of NaH in THF, 8 h at rt; 2.2 equiv of CAN in MeOH, 15 h. [b] 1.2 equiv of NaH in THF, 1.5−4 h at rt; 2.2 equiv of CAN in MeOH, 15 h. [c] Reaction performed as in *b* but at 0.015 M to promote intramolecular reaction.

Having prepared the desired dinitro compound **3**, the next transformation in our synthetic scheme was hydrogenation to yield the vicinal diamine **2**. Although this transformation seems trivial, it is fraught with difficulties. Accumulation of dangerous hydroxylamine intermediates that are difficult to reduce is a major problem in nitro reductions.[11] Additionally, tertiary vicinal dinitro compounds can undergo sequential elimination of both nitro groups after single electron transfer to generate an undesired alkene side product.[12,13] There is a single precedent for the reduction of hindered vinical dinitro compounds, but it requires harsh conditions (Sn metal with 12 N hydrochloric acid) that would remove the ketal protecting groups and complicate recovery of the desired product.[14] The efficacy of hydrazinium monoformate in reductions of both aromatic and aliphatic nitro compounds

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(8) Senkus, M. US Patent 2,543,472, 1951.
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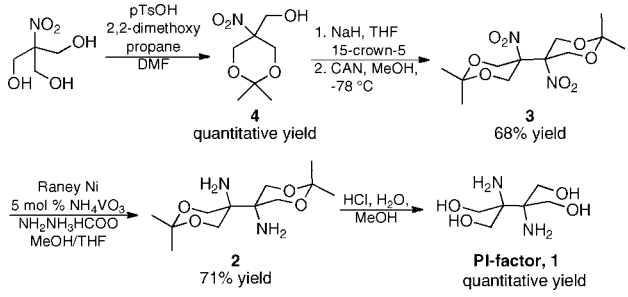
(12) Bowyer, W. J.; Evans, D. H. *J. Org. Chem.* **1988**, *53*, 5234–5239.
(13) Indeed using SmI_2/MeOH, $NaBH_4/NiCl_2$, $NaBH_4/CoCl_2$, Zn, Fe, Mg, $LiAlH_4$, and Pd/C led to the formation of alkene 2,2,2′,2′-tetramethyl-4,4′,6,6′-tetrahydro-5,5′-bi(1,3-dioxinylidene) as the major product.
(14) Asaro, M. F.; Nakayama, I.; Wilson, R. B., Jr. *J. Org. Chem.* **1992**, *57*, 778–782.

with a variety of catalysts[15] prompted us to attempt the desired transformation using this hydrogen source and Raney nickel as the catalyst. Under these reaction conditions only a minor amount of the desired vicinal diamine was formed, but the major products were partially hydrogenated (i.e., dihydroxylamine and aminohydroxylamine). We hypothesized that this problem could be remedied by using ammonium metavanadate as a disproportionation catalyst.[11] This reagent catalyzes the conversion of two molecules of hydroxylamine to the corresponding amine and the more easily reduced nitroso compound.[11] Indeed, inclusion of 5 mol % of ammonium metavanadate in the hydrogenation afforded the diamine **2** in 71% yield with no detectable traces of partially hydrogenated products.

Scheme 2. Total Synthesis of PI Factor



To complete the PI factor synthesis, the ketal protecting groups were removed in quantitative yield using 1:1 6 M HCl in methanol (Scheme 2). The recrystallized product was analyzed by NMR, mass spectrometry, and X-ray crystallography (Figure 2) confirming that our synthetic material was identical to the structure proposed by Martin and coworkers.[2] Thus, PI factor was synthesized from tris(hydroxymethyl)nitromethane in 48% yield over four steps.

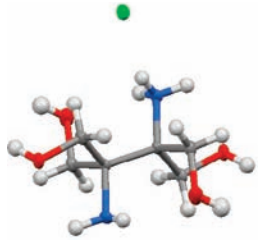


Figure 2. Crystal structure of the mono-HCl salt of PI factor, **1**.

A curious feature of the reported ^{1}H NMR spectrum of PI factor was that the methylene protons appeared as a singlet.[2] One would anticipate that these diastereotopic, geminal protons would have nonequivalent chemical shifts and appear as a doublet of doublets, especially due to the potential for intramolecular hydrogen-bonding. We suspected that the chemical shifts and coupling of these protons would be affected by pH. Using an internally referenced D_2O buffer at various pHs, the pH dependence of the ^{1}H NMR signals was investigated (Figure 3).[16] We found that the signal from these methylene protons

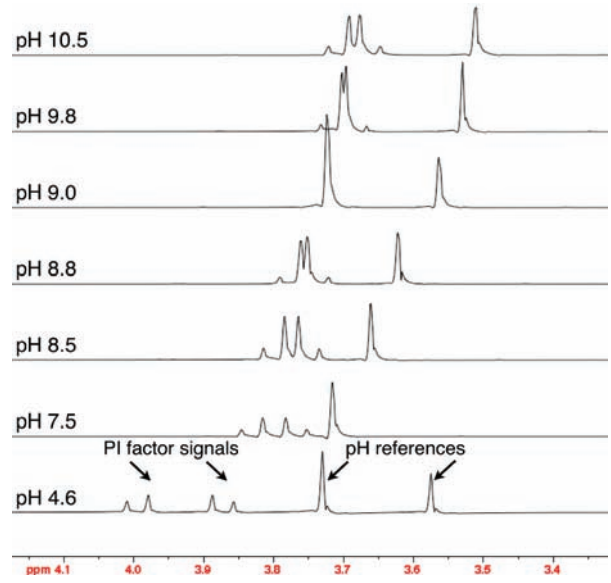


Figure 3. pH dependence of PI factor: ^{1}H NMR spectra.

appears as a singlet in a pH range near 9 and as a doublet of doublets with coupling constants of 12 Hz (typical of geminal protons) at other pHs. The structural basis of this NMR behavior is not entirely clear, but these observations suggest that the ionization state influences molecular conformation.

We report the first chemical synthesis of the proposed structure of the pimaricin-inducing factor from *S. natalensis*. Given the polarity of the synthetic intermediates and the final product, a strength of our route is that it requires no chromatographic steps. Most importantly, it provides a means to prepare sufficient quantities of the compound for biological testing and for an assessment of its utility as an additive to pimaricin fermentations.[5]

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Supporting Information Available: Procedures and spectroscopic data for all compounds. Crystallographic data for diamine **2** and PI factor, **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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