$(RNH_2)Fe^{II}TPP$ and $:CCl_2$ moieties by t-$BuNH_2$, aniline, and pyridine in the dissociative mechanism are thermodynamically favored reactions with small activation energies. Therefore, it is not surprising that there is little sensitivity of k_2 to amine pK_a.

Cytochrome P-450 destruction by haloalkanes may occur through formation of Fe(II)-bonded carbenes which, through a dissociative pathway, generates $:CCl_2$ (a reactive species) that migrates to porphyrin or protein functionalities. Callot and Schaeffer[16] have established electrophilic alkyl group migration from porphyrincobalt(III) alkyl to yield (alkylporphyrinato)cobalt(III).

Acknowledgment. This work was supported by grants from the National Institutes of Health and the American Cancer Society.

(16) Callot, H. J.; Schaeffer, E. *Tetrahedron* Lett. **1980**, *21*, 1335.

A Novel P-450 Model System for the *N*-Dealkylation Reaction

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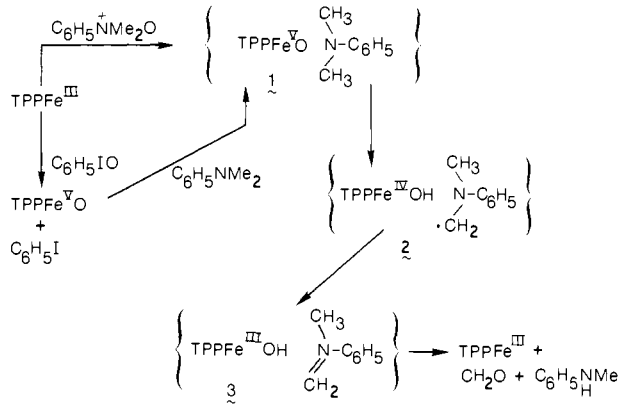
The enzymatic oxidations of secondary and tertiary xenobiotic and naturally occurring amines are initiated by flavin and cytochrome P-450 monooxygenases.[1] Whereas flavin-dependent monooxygenases are capable of N-oxidation, the heme-protein dependent monooxygenases carry out both N-oxidation and N-dealkylation of amines and, along with other oxygen insertion reactions of P-450 monooxygenases, constitute an area of wide concern and growing scientific interest. Both the flavoenzyme reaction mechanism[2] and, that of its biomimetic counterpart, the noncatalytic 4a-hydroperoxyflavin N-oxidation reaction[3] have been investigated; the essential features of both processes appear to be comparable. In concert with our investigation of the flavin dependent reaction, we have pursued the modeling of the P-450 N-dealkylation of amines.

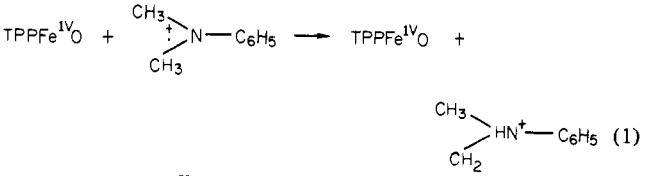
The P-450 enzyme juxtaposes the penultimate oxidizing species and substrate by catalyzing, in a sequence of steps, two one-electron reductions of enzyme–substrate–$^3O_2$ complex. The formation of product occurs following the rate-determining addition of the second electron; the oxidative event is too rapid to allow any characterization of the iron–oxygen species. Therefore, the actual oxidative event is rendered kinetically invisible, and the oxidizing reagent has eluded absolute identification. In a number of studies porphyrin bound Fe^VO^{+3} has been suggested to be responsible for substrate oxidation. Generation of cytochrome P-450 oxidizing species has been accomplished by the use of a wide variety of "oxene" transfer reagents such as iodosobenzene, peroxy acids, and numerous other peroxy compounds including hydrogen peroxide.[4] We report here the use of *N*,*N*-dimethylaniline *N*-oxide (DMANO) as a novel means of entrance into the catalytic cycle in a biomimetic system. In reactions of chlorotetraphenylporphyrinatoiron(III) [$TPPFe^{III}$] with DMANO, the arylamine *N*-oxide acts as both the oxene donor and, thereafter, the juxtaposed substrate (**1** in Scheme I) as in the enzymatic reaction.

Scheme I



Reaction of excess DMANO with $TPPFe^{III}$ (10^{-5} M) in anhydrous (Mg dried), nitrogen outgassed ethanol (30 °C) results in an initial shift in the porphyrin absorption bands (from λ_{max} 415, 575 nm to λ_{max} 403, 567, 607 nm) with a concomitant and continuing increase in absorbance at 290–295 nm accompanying product formation. Analysis by HPLC and GC/MS revealed that *N*,*N*-dimethylaniline (DMA) and the N-demethylated product, *N*-methylaniline (NMA), as well as formaldehyde and varying, lesser amounts of aniline are produced catalytically in the reaction. Under the condition [DMANO] ≫ [$TPPFe^{III}$], product formation is linear with time, and the zero-order velocities are proportional to [$TPPFe^{III}$] and initial [DMANO] (Figure 1A). The yields of the products depend only on the total amount of initial DMANO present. No other products were detected even after greater than 500 turnovers of the catalyst. Product formation was terminated in certain reactions by the crystallization of an ethanol insoluble TPPFe complex. Upon dissolution of this complex in $CHCl_3$, CH_2Cl_2, etc. under anaerobic conditions, the absorbance spectrum smoothly and rapidly (30 °C) converts to that of authentic $TPPFe^{III}$. Further characterization of this crystalline precipitate is in progress.

The mechanism of the reaction may be envisioned as in Scheme I. The entity **1** (the $TPPFe^VO$ moiety is written as such for electron bookkeeping purposes; the exact structure, as in the case of P-450, is unknown) must possess a lifetime of sufficient duration to allow escape of DMA, since the formation of aniline can only be accounted for by the exchange of NMA product for DMA in complex **1**. Formation of **2** is consistent with either (i) transfer of an electron from the amine nitrogen to Fe^VO to yield an anilinium cation radical and subsequent H-atom transfer from C to N (eq 1) or (ii) transfer of an H atom directly from an *N*-methyl



substituent to Fe^VO. Though, in both chemical and electrochemical oxidations of amines, the transfer of an electron from N to yield an aminium cation radical is often the rate-determining step,[5] this may not be so as a result of $TPPFe^VO$ involvement in

(1) Gorrod, J. W. "Biological Oxidation of Nitrogen", Elsevier/North Holland Biomedical Press: New York, 1978.

(2) (a) Poulsen, L. L.; Kadlubar, F. F.; Ziegler, D. M. *Arch. Biochem. Biophys.* **1974**, *164*, 774. (b) Ziegler, D. M.; Mitchell, C. H. *Ibid.* **1972**, *150*, 116. (c) Poulsen, L. L.; Ziegler, D. M. *J. Biol. Chem.* **1979**, *254*, 6449. (d) Beaty, N. B.; Ballou, D. P. *J. Biol. Chem.* **1980**, *255*, 3817.

(3) (a) Ball, S.; Bruice, T. C. *J. Am. Chem. Soc.* **1979**, *101*, 4017. (b) *Ibid.* **1980**, *102*, 6498.

(4) (a) Nordblom, G. D.; White, R. E.; Coon, M. J. *Arch. Biochem. Biophys.* **1976**, *175*, 524. (b) Hrycay, E. G.; Gustafsson, J.; Ingelman-Sundberg, M.; Ernster, L. *Biochem. Biophys. Res. Commun.* **1975**, *66*, 209. (c) Lichtenberger, F.; Ullrich, V. "Microsomes and Drug Oxidations"; Ullrich, V., Ed.; Pergamon Press: New York, 1976; p 218. (d) Groves, J. T.; Nemo, T. E.; Myers, R. S. *J. Am. Chem. Soc.* **1979**, *101*, 1032. (e) Chang, C. K.; Kuo, M.-S. *Ibid.* **1979**, *101*, 3413. (f) Akhrem, A. A.; Bokut, S. B.; Metelitza, D. I. *Biochem. Biophys. Res. Commun.* **1977**, *77*, 20. (g) Rahimtula, A. D.; O'Brien, P. J. *Ibid.* **1974**, *60*, 440. (h) Gustafsson, J.; Hrycay, E. G.; Ernster, I. *Arch. Biochem. Biophys.* **1976**, *174*, 440. (i) Lichtenberger, F.; Nastainczyk, W.; Ullrich, V. *Biochem. Biophys. Res. Commun.* **1976**, *70*, 939. (j) Tabushi, I.; Koga, N. *J. Am. Chem. Soc.* **1979**, *101*, 6456.

(5) Of numerous examples, see: (a) Smith, P. A. S.; Loeppky, R. N. *J. Am. Chem. Soc.* **1967**, *89*, 1147. (b) Deno, N. C.; Fruit, R. E. *Ibid.* **1968**, *90*, 3502. (c) Lindsay Smith, J. R.; Mead, L. A. V. *J. Chem. Soc., Perkins Trans. 2* **1973**, 206. (d) Smith, P. J.; Mann, C. K. *J. Org. Chem.* **1969**, *34*, 1821. (e) Portis, L. C.; Bhat, V. V.; Mann, C. K. *Ibid.* **1970**, *35*, 2175. (f) Lindsay Smith, J. R.; Matsheder, D. J. *J. Chem. Soc., Perkins Trans 2* **1976**, 47. (g) Cohen, S. G.; Parola, A.; Parsons, G. H. *Chem. Rev.* **1973**, *73*, 141.

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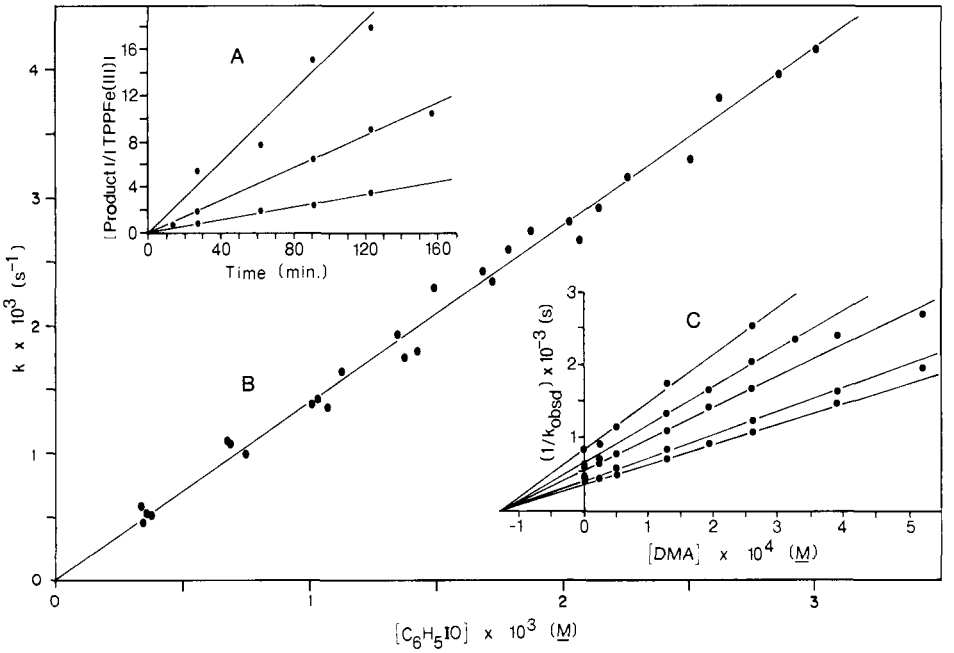
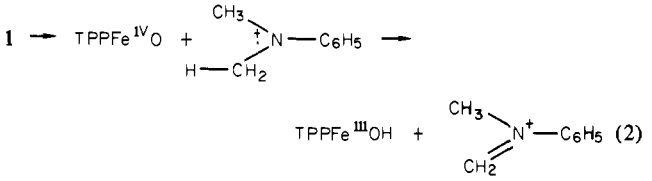


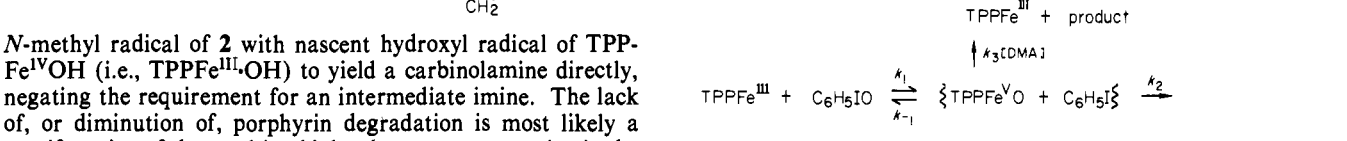
Figure 1. (A) [Product]/[TPPFe^{III}] vs. time in the reaction of DMANO (0.03 M) with TPPFe^{III}. In order of decreasing slope, the products are DMA, CH_2O, and NMA (solvent C_2H_5OH, 30 °C, anerobic). (B) Dependence of the pseudo-first-order rate constants (k) for the disappearance of TPPFe^{III} upon the concentration of iodosobenzene (TPPFe^{III} employed at $\sim 1 \times 10^{-5}$ M, 30 °C, in absolute and O_2-free C_2H_5OH). (C) Dependence of the reciprocal of the pseudo-first-order rate constants for the disappearance of TPPFe^{III} on reaction with iodosobenzene as a function of [DMA]. Initial [C_6H_5IO] employed in order of decreasing slope: 8.13×10^{-4}, 1.17×10^{-3}, 1.33×10^{-3}, 1.73×10^{-3}, and 2.01×10^{-3} M. Reaction conditions as in (B).

the reaction. Thus, although formation of the anilinium radical in the thermolysis of *O*-acetoxy-*N*,*N*-dimethylaniline gives rise to high yields of ortho-radical coupling products,[6] no such products were detected in this investigation. This finding may be explained by condition ii above or a very favorable H-atom transfer from the aminium cation radical to TPPFe^{IV}O without formation of **2** (eq 2). An alternate possibility is the recombination of the



N-methyl radical of **2** with nascent hydroxyl radical of TPP-Fe^{IV}OH (i.e., TPPFe^{III}·OH) to yield a carbinolamine directly, negating the requirement for an intermediate imine. The lack of, or diminution of, porphyrin degradation is most likely a manifestation of the resulting high substrate concentration in the porphyrin microenvironment during the ensuing reaction.

In order to provide an alternate entrance (Scheme I) to the reaction cycle, we explored the reaction of TPPFe^{III} with iodosobenzene and DMA. No kinetic analysis for the reaction of TPPFe^{III}–C_6H_5IO couple have been reported, although Groves[4d,7] and Chang[4e] and co-workers have investigated the TPPFe^{III}–C_6H_5IO couple in oxidation reactions. The reaction of TPPFe^{III} with C_6H_5IO was carried out under the pseudo-first-order conditions of [C_6H_5IO] $\gg$ [TPPFe^{III}] in anhydrous ethanol at 30 °C under inert atmosphere.[8] The kinetics for porphyrin decomposition were monitored at the Soret maximum (415 nm) and were found to be first order (up to 7–$t_{1/2}$) in both iodosobenzene and porphyrin ($k = 1.36 \pm 0.03$ M^{-1} s^{-1}, Figure 1B). We made no attempt to determine the structure of the porphyrin degradation product(s) resulting from apparent self-hydroxylation.[4e] Inclusion of the substrate, DMA, in the reaction mixture results in a significant inhibition of the loss of the porphyrin, without alteration in the spectral characteristics of the porphyrin catalyst (350–800 nm). The decreased rate of TPPFe^{III} decomposition in the presence of DMA was observed to obey first-order kinetics as above and result in the production of *N*-methylaniline and formaldehyde as major products. Both aniline and *N*,*N*-dimethylaniline *N*-oxide were detected in variable, small yields (<10% relative to [NMA]).[9] These products were not detected in incubations of solutions of iodosobenzene and DMA. The simplest kinetic scheme for the partitioning of a reactive intermediate between decomposition and trapping by DMA ia summarized in eq. 3.



Assuming steady-state levels of TPPFe^{V}O during reaction and that $k_2 \gg k_{-1}$, equ 4 may be obtained for the observed rate of

$$1/k_{obsd} = 1/k_1[C_6H_5IO] + k_3[DMA]/k_1k_2[C_6H_5IO] \quad (4)$$

TPPFe^{III} decomposition. A plot of $1/k_{obsd}$ as a function of [DMA] for given values of [C_6H_5IO] produces a family of lines with a common intercept, $-k_2/k_3$, at the abscissa (see Figure 1C). The partitioning ratio between product formation and porphyrin degradation is $k_3[DMA]/k_2$ (i.e., at a value of [DMA] = 1.0 M the rate of DMA oxidation exceeds that for catalyst destruction by 8000).

Whereas the P-450 catalyzed N-demethylation of arylamines, particularly that of DMA has been under careful scrutiny, a number of questions which include the necessity of an intermediate *N*-oxide or imine remain.[11,12] The present study suggests that

(6) Oae, S.; Kitao, T.; Kitaoka, Y. *J. Am. Chem. Soc.* **1962**, *84*, 3366.
(7) Groves, J. T.; Kruper, W. T. *J. Am. Chem. Soc.* **1979**, *101*, 7613.
(8) In order to eliminate possible spurious reactions due to decomposed chlorocarbon solvent, rigorously degassed, or nitrogen outgassed, dry ethanol was employed as a solvent. Iodosobenzene is soluble in ethanol in excess of 0.01 M. Concentrated stock solutions of TPPFe^{III} were in dry benzene. The resultant small concentration of benzene present during catalysis has negligible effect on the reactions.
(9) Aryl product analysis was carried out by HPLC separation, GC/MS of HPLC isolated products, and GC/MS of unadultered reactions mixtures. Formaldehyde was determined colormetrically[10] following bulb-to-bulb distillation of reaction mixtures. DMANO was determined by the method of Ziegler.[11]
(10) Nash, T. *Biochem. J.* **1953**, *55*, 416.

the two are not mutually exclusive. Though model chemistry resulting in the metal porphyrin mediated ring hydroxylation of aniline has been reported,[13] no prior biomimetic work has been carried out in which catalytic N-demethylation has been demonstrated. We are currently exploring the reaction of DMANO with P-450 enzymes.

Acknowledgment. P.S. gratefully thanks the National Institutes of Health for the support as a postdoctoral fellow. This study was supported by grants from the National Institutes of Health and the American Cancer Society.

(11) Ziegler, D. M.; Pettit, F. H. *Biochem. Biophys. Res. Commun.* **1964**, *15*, 188.
(12) (a) Miwa, G. T.; Garland, W. A.; Hodshon, B. J.; Lu, A. Y. H.; Northrop, D. B. *J. Biol. Chem.* **1980**, *255*, 6049. (b) Hlavica, P.; Aichinger, G. *Biochim. Biophys. Acta* **1978**, *544*, 185. (c) Hlavica, P.; Kohl, M. *Biochem. J.* **1977**, *164*, 487. (d) Willi, P.; Bickel, M. H. *Arch. Biochem. Biophys.* **1973**, *156*, 772. (e) Hlavica, P. *Biochim. Biophys. Acta* **1972**, *273*, 318.
(13) (a) Sakurai, H.; Shimomura, S.; Fukuzawa, K.; Ishizu, K. *Chem. Pharm. Bull.* **1978**, *26*, 1348 and references cited. (b) Adams, P. A.; Berman, M. C.; Baldwin, D. A. *J. Chem. Soc., Chem. Commun.* **1979**, 856.

Asymmetric, Biogenetically Modeled Synthesis of (-)-3-Aminonocardicinic Acid

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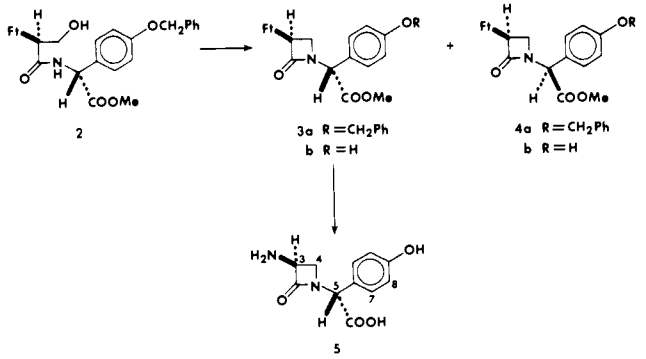
Biosynthetic results obtained in these laboratories support the view that nocardicin A (**1**) is derived from L-methionine, L-serine, and two units of L-(*p*-hydroxyphenyl)glycine (Chart I).[1] Incorporation studies with doubly labeled serine have established that β-lactam formation takes place by cyclization of the seryl residue without alternation of the oxidation state at the β carbon.[1] If generation of a peptide precursor prior to β-lactam formation is presumed, as is now known[2] to be the case in penicillin biosynthesis, a direct mechanistic rationale for β-lactam formation is nucleophilic displacement by amide nitrogen of the presumably activated seryl hydroxyl. This proposal is subject to stereochemical and chemical test. In this communication we demonstrate the latter in a biogenetically modeled cyclization of the appropriately protected, optically active dipeptide **2** (Ft = phthalimido) to a 2:1 mixture of **3** and **4**, the former being a derivative of (-)-3-aminonocardicinic acid (**5**), the structural element common to all the known nocardicins.[3]



In considering the possible modes of serine activation in vivo, phosphorylation (or the corresponding pyrophosphate) is at once mechanistically attractive and precedented in L-3-phosphoserine, the immediate precursor of L-serine from the intermediates of glycolysis. Similarly, chemical syntheses of β-lactams involving displacements of primary and secondary halides by amide anions generated typically by sodium hydride in dimethylformamide–methylene chloride had been carried out earlier by Kishi,[4] Baldwin,[5] Koppel,[6] and Wasserman.[7] However, to more closely mimic the hypothetical in vivo cyclization to the key four-membered ring, a milder reaction system was sought to generate the desired O-phosphorylated intermediate in situ and the necessary amide anion as well, e.g., **7**. The Mitsunobu reaction[8] held promise to fulfill these two requirements. Recently Miller[9] has reported the conversion of seryl *O*-alkyl hydroxamates to the corresponding N-oxidized β-lactams using the Mitsunobu reaction. While hydroxamates have biochemical relevance in other contexts, presuming the obligate intermediacy of peptide precursors in penicillin[2] and nocardicin[1] formation, their direct involvement in β-lactam biosynthesis is uncertain. In the event, we have found that whatever enhancement in acidity of amide hydrogen afforded by oxidation to the corresponding hydroxamate ($pk_a \sim 6$–10),[9] it is unnecessary for the sake of cyclization as treatment of serine-containing peptides as **2** under Mitsunobu conditions proceeds rapidly and cleanly at room temperature to yield the corresponding β-lactam to the exclusion of acrylamide or γ-lactam products derived from anion formation at C-3 or C-5, respectively.

The biogenetically modeled synthesis was initially attempted by using the racemic dipeptide **6**. *N*-Phthaloyl-DL-serine[10] was condensed with DL-(*p*-benzyloxyphenyl)glycine methyl ester in dry dimethylformamide at room temperature with 1.1 equiv of dicyclohexylcarbodiimide and 2.0 equiv of 1-hydroxybenzotriazole hydrate[11] to afford **6**, after crystallization from ethyl acetate–hexanes (83%, mp 126.5–135 °C), as a 10:1 mixture of diastereomers (favoring **2** and its enantiomer) as judged by ^{1}H NMR spectroscopy at 300 MHz and HPLC analysis. Crystalline **6** was treated under an inert atmosphere with 2.5 equiv each of triphenylphosphine and diethyl diazodicarboxylate in dry tetrahydrofuran at room temperature. After 15 min[12] excess cyclizing reagent was destroyed by addition of water and a mixture of **3a** (and enantiomer) and **4a** (and enantiomer) was isolated as a viscous oil by chromatography on silica gel (ethyl acetate–hexanes 1:1). Coeluting diethyl hydrazodicarboxylate side product **8** was removed by fractional crystallization from chloroform–hexanes. Hydrogenation at atmospheric pressure (sonication) of the oily mixture of isomers (methanol–acetic acid 1:1) with a 50% weight of 5% palladium on carbon gave a 2:1 mixture of racemic **3b** and **4b**, respectively, whose ^{1}H NMR spectra were identical with published data.[13] The change in diastereomeric composition in

(1) Townsend, C. A.; Brown, A. M. *J. Am. Chem. Soc.* **1981**, *103*, 2873-4.
(2) Fawcett, P. A.; Usher, J. J.; Huddleston, J. A.; Bleaney, R. C.; Nisbet, J. J.; Abraham, E. P. *Biochem. J.* **1976**, *157*, 651-660. O'Sullivan, J.; Bleaney, R. C.; Huddleston, J. A.; Abraham, E. P. *Ibid.* **1979**, *184*, 421-426. Konomi, T.; Herchen, S.; Baldwin, J. E.; Yoshida, M.; Hunt, N. A.; Demain, A. L. *Ibid.* **1979**, *184*, 427-430. Sawada, Y.; Baldwin, J. E.; Singh, P. D.; Solomon, N. A.; Demain, A. L. *Antimicrob. Agents Chemother.* **1980**, *18*, 465-470. Adriaens, P.; Vanderhaeghe, H. *FEMS Microbiol. Lett.* **1978**, *4*, 19-21. Meesschaert, B.; Adriaens, P.; Eyssen, H. *J. Antibiot.* **1980**, *33*, 722-730.
(3) Hashimoto, M.; Komori, T.; Kamiya, T. *J. Am. Chem. Soc.* **1976**, *98*, 3023-3025. *J. Antibiot.* **1976**, *29*, 890-901. Hosoda, J.; Konomi, T.; Tani, N.; Aoki, H.; Imanaka, H. *Agric. Biol. Chem.* **1977**, *41*, 2013-2020.
(4) Nakatsuka, S.; Tanino, H.; Kishi, Y. *J. Am. Chem. Soc.* **1975**, *97*, 5008-5010, 5010-5012.
(5) Baldwin, J. E.; Au, A.; Christie, M.; Haber, S. B.; Hesson, D. *J. Am. Chem. Soc.* **1975**, *97*, 5957-5958. Baldwin, J. E.; Christie, M. A. *Ibid.* **1978**, *100*, 4597-4599.
(6) Koppel, G. A.; McShane, L.; Jose, F.; Cooper, R. D. G. *Ibid.* **1978**, *100*, 3933-3935.
(7) Wasserman, H. H.; Hlasta, D. J. *J. Am. Chem. Soc.* **1978**, *100*, 6780-6781. Wasserman, H. H.; Hlasta, D. J.; Tremper, A. W.; Wu, J. S. *Tetrahedron Lett.* **1979**, 549-552. See also: Takahata, H.; Ohnishi, Y.; Yamazaki, T. *Heterocycles* **1980**, *14*, 467-469.
(8) Mitsunobu, O.; Wada, M.; Sano, T. *J. Am. Chem. Soc.* **1972**, *94*, 679-680. Wada, M.; Mitsunobu, O. *Tetrahedron Lett.* **1972**, 1279-1282. Kurihara, T.; Nakajima, Y.; Mitsunobu, O. *Ibid.* **1976**, 2455-2458.
(9) Mattingly, P. G.; Kerwin, Jr., J. F.; Miller, M. J. *J. Am. Chem. Soc.* **1979**, *101*, 3983-3985. Miller, M. J.; Mattingly, P. G.; Morrison, M. A.; Kerwin, Jr., J. F. *Ibid.* **1980**, *102*, 7026-7032.
(10) Nefkins, G. H. L.; Tesser, G. I.; Nivard, P. J. F. *Rec. Trav. Chim. Pays-Bas* **1960**, *79*, 688-698.
(11) König, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 2034-2040.
(12) Monitoring the reaction by thin-layer chromatography (silica gel, ethyl acetate–hexanes 2:1) showed clean conversion of **6** to a single less polar spot.

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