Scheme I

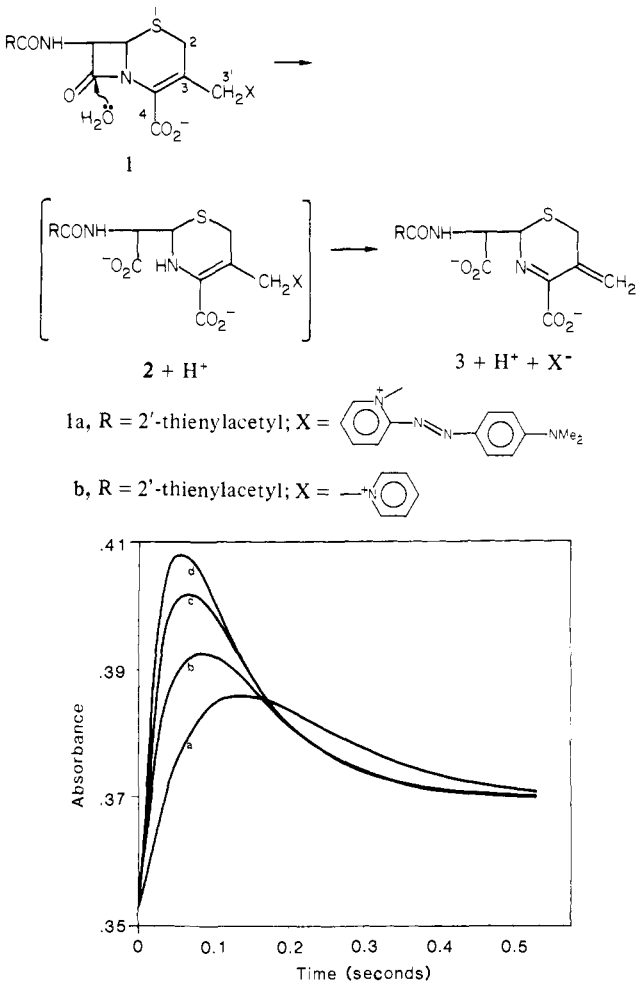

Figure 1. Absorption changes at 498 nm on mixing 13 μM PADAC with (a) 3.0, (b) 6.0, (c) 9.0, and (d) 12.0 μM TEM-2 β-lactamase.

I. It is well-known that two protons are released on hydrolysis of cephaloridine.[5]

The above evidence shows that elimination of pyridinium leaving groups from the 3'-position of these cephalosporins is not concerted with β-lactam ring opening when the latter is catalyzed by the TEM-2 β-lactamase. This may also be true with other leaving groups and when the β-lactam ring is opened by nonenzymic nucleophiles.[21] Other enzymes, either β-lactamases or bacterial cell wall transpeptidases, may influence the situation differently. For example, we have found that the reaction of PADAC (12 μM) with the β-lactamase II of *Bacillus cereus*[22] (60 μM) occurs in a single enzyme-catalyzed step yielding Y and at a rate much faster than the rate of breakdown of C'-P to C' and Y under the same conditions. In this case the enzyme must be catalyzing departure of the leaving group in what could be either a concerted or a nonconcerted reaction on the enzyme surface. The application of these findings to further studies of the active sites of the β-lactam-specific enzymes is being pursued.

Acknowledgment. We are grateful to Hoechst-Roussel Pharmaceuticals Inc. and to Eli Lilly and Co. for generous gifts of PADAC and cephaloridine, respectively. This work was supported by Wesleyan University and the National Institute of Health.

(21) Only one phase of reaction was observed in reactions of PADAC or cephaloridine with hydroxide ion (up to 0.5 M). Either the elimination step is concerted with ring opening or ring opening is rate determining in a two-step mechanism.

(22) Obtained as a mixture with β-lactamase I from the PHLS Centre for Applied Microbiology, Porton Down, England, and purified by the method of Davies et al. (Davies, R. B.; Abraham, E. P.; Melling, J. *Biochem. J.* **1974**, *143*, 115-127).

Asymmetric Synthesis of Amino Acids by Pyridoxamine Enzyme Analogues Utilizing General Base-Acid Catalysis

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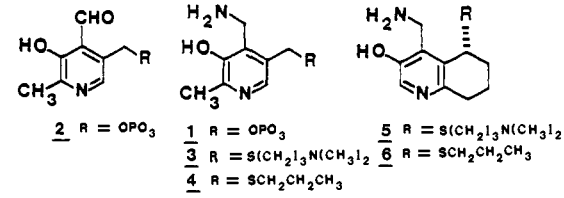
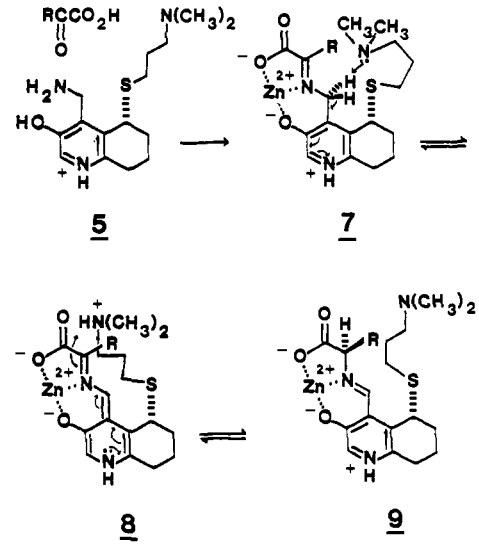
Pyridoxamine (**1**) and pyridoxal (**2**) are the coenzymes for numerous transformations in amino acid metabolism[1] including transaminations that interconvert α-keto acids and amino acids. In transaminations a single enzyme catalytic group, possibly a lysine amino group,[2] acts as a general base to remove the *pro-S* hydrogen from the 4'-methylene of a ketimine intermediate (analogous to **7**). It then moves up the same face of the intermediate and, acting as an acid, reprotonates it in the α-position on the *si* face, yielding the chiral aldimine (analogous to **9**).[1,3] The chiral amino acid is then released. We recently demonstrated[4]



that this base-acid sequence could be duplicated in a transaminase analogue by pyridoxamine derivatives carrying basic side arms, such as **3**. However, only modest stereoselectivity was observed[4] when the side arm was chiral; some stereoselectivity has also been observed in transaminations by pyridoxamine-cyclodextrin derivatives[5,6] or other chiral pyridoxamines.[7] We not wish to describe a closely biomimetic system (**5**) in which a rigidly mounted



side arm is constrained to perform proton transfers on one face of the transamination intermediate, as in the enzyme. Extraordinary stereoselectivity results from this constraint.

As Scheme I indicates, the synthesis of **5** proceeded through

(1) (a) Bruice, T. C.; Benkovic, S. "Bioorganic Mechanisms"; W. A. Benjamin: New York, 1966; Vol. 2, Chapter 8. Walsh, C. "Enzymatic Reaction Mechanisms"; W. H. Freeman: San Francisco, CA, 1979; Chapter 24.

(2) Morino, Y.; Tanase, S. *J. Biol. Chem.* **1978**, *253*, 252-256.

(3) (a) Enzymatic transaminations occur in the absence of metal ions which are frequently used in nonenzymatic transaminations. (b) Matsushima, Y.; Martell, A. E. *J. Am. Chem. Soc.* **1967**, *89*, 1331-1335.

(4) Zimmerman, S. C.; Czarnik, A. W.; Breslow, R. *J. Am. Chem. Soc.* **1983**, *105*, 1694-1695.

(5) Breslow, R.; Hammond, M.; Lauer, M. *J. Am. Chem. Soc.* **1980**, *102*, 421-422.

(6) Breslow, R.; Czarnik, A. W. *J. Am. Chem. Soc.* **1983**, *105*, 1390-1391.

(7) Tachibana, Y.; Ando, M.; Kuzuhara, H. *Chem. Lett.* **1982**, 1765-1768.

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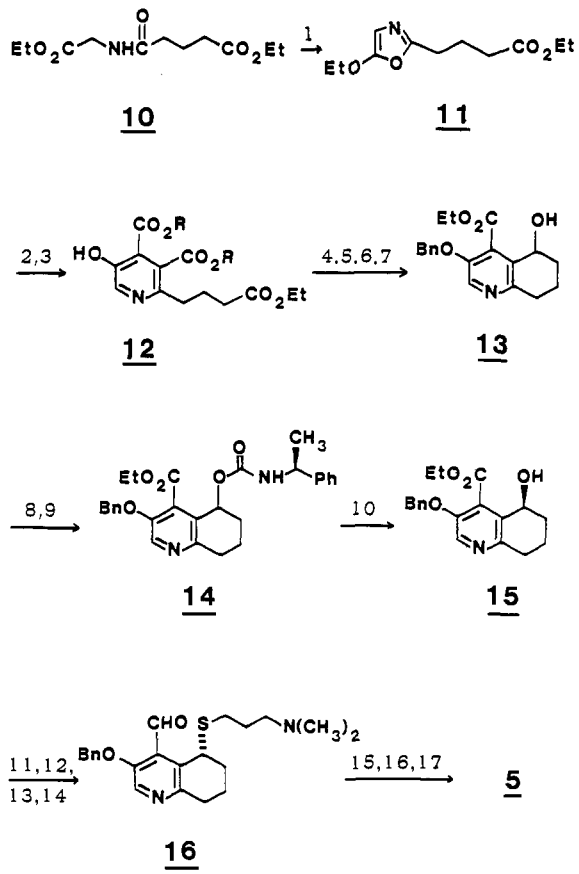
Scheme I[a]



[a] (1) P_2O_5, $CHCl_3$, reflux/54%; (2) maleic anhydride neat; (3) EtOH-saturated HCl_g, reflux/55%; (4) 1,3-diisopropyl-2-benzylisourea, neat, 110 °C/57%; (5) NaOEt-EtOH, PhH, reflux/99%; (6) 2.4 N HCl, EtOH, reflux/41%; (7) $NaBH_4$, EtOH, 5% $NaOH_{aq}$/99%; (8) carbonyldiimidazole, CH_2Cl_2, room temperature; (9) α-1-(-)-phenethylamine, PhH, $BF_3 \cdot O_2Et$, reflux/58%; (10) MPLC; $HSiCl_3$, Et_3N, PhH, reflux/85%. (11) MsCl, Et_3N, CH_2Cl_2, 0 °C/99%; (12) $HS(CH_2)_3N(CH_3)_2$, NaH, THF, 0 °C-room temperature/82%; (13) LAH, THF, room temperature/58%; (14) $CrO_3 \cdot Pyr_2$, CH_2Cl_2, room temperature; (15) $HONH_2$, NaOAc, H_2O, EtOH; (16) Zn, HOAc; H_2S, CH_3OH; CM25 sephadex/26% (steps 14-16); (17) 6 N HCl, reflux, 30 min; CM25 sephadex/82%.

Table I. Rates of Conversion of Ketimine to Aldimine in Methanol at "pH 4.00" (30.0 °C) and Optical Inductions in Product Amino Acids[a]

compd	amino acid[b]	k_{obsd}, s^{-1} [c]	rel rate[d]	% conversion[e]	ratio[f] D:L
3	alanine	3.3×10^{-4}	38		
4	alanine	8.7×10^{-6}	1		
5	alanine	1.5×10^{-3}	172	83	93:7
5	alanine			68	91:9
5	norvaline	9.5×10^{-4}	109	68	96:4
5	norvaline			35	95:5
5	tryptophan	1.0×10^{-4}	115	89	94:6
6	norvaline	4.4×10^{-5}	5	75	42:58

[a] Methanol solutions 0.16 mM in pyridoxamine derivative and in zinc acetate and 1.6 mM in ketoacid. Reactions were performed as in ref 4, with the "pH" as read on a glass electrode calibrated against aqueous buffer. [b] Obtained from the corresponding α-keto acid and analyzed as the dansyl derivative. [c] Standard deviations for all runs were <1% with duplicate runs within 10%. [d] Relative to 4 with pyruvic acid. [e] At the time of product isolation, relative to final equilibrium absorbance (UV). [f] Determined by chiral HPLC, as described in ref 4.

a maleic anhydride/oxazole Diels-Alder reaction[8] and a Dieckmann cyclization. The racemic intermediate **13** was resolved[9] by MPLC of its carbamate **14**, and the isomer whose carbamate eluted first was used for the synthesis of **5**[10] and of the related **6**.[10] The steps are detailed in Scheme I. To establish catalysis the compounds were then evaluated (as in our previous work[4]) for the rate at which the ketimines, formed with various α-keto acids in methanol, underwent isomerization to the corresponding aldimines. The data are listed in Table I. Furthermore, the product amino acids, from hydrolysis of the aldimines, were examined for chirality by chiral HPLC[11] of their dansyl derivatives (as in our previous work[4]). These data are also in Table I.

Catalysis by the basic side arm of **5** is clearly established by the significant rate accelerations in Table I relative to the rate for **6**, whose side arm is not basic. The chiral inductions by **5** are striking. Indeed the D/L enantiomeric ratio of 95:5 for norvaline, for instance, is a minimum value. We cannot yet exclude a few percent contamination of **5** by its enantiomer from incomplete resolution or partial racemization. The data in Table I do show that catalyzed racemization of the product amino acids, at the aldimine stage, is not a problem since enantioselectivity did not fall when reactions were allowed to run to higher conversions over longer times.

The mechanism involved in the chiral selectivity and the absolute configuration of **5** are established by the results with compound **6**. Its noncatalytic side chain helps shield the *re* face of the intermediate, leading to some preference for L-norvaline. Since **5** has the same absolute configuration as **6** but shows a strong D preference, **5** must be catalyzing proton transfer along the *re* face.

Even if the few percent nonspecific product from **5** proves to be genuine, rather than the result of optical contamination of **5** itself, the stereospecificity of these biomimetic transaminations is striking. It remains to be seen whether these or related systems will prove to be practical catalysts for chiral amino acid synthesis.

Acknowledgment. This work was supported by a grant from NIH.

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(9) Cf.: Pirkle, W. H.; Hauske, J. R. *J. Org. Chem.* **1977**, *42*, 1839-1844; **1977**, *42*, 2781-2782.
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(11) Lam, S.; Chow, F.; Karmen, A. *J. Chromatogr.* **1980**, *199*, 295-305.

Nucleophilic Attack of a Phosphorus-Phosphorus Double Bond

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Recently, several compounds have been isolated that exhibit double bonding between the heavier main-group elements.[1] Current attention has turned to examining the reactivities of these novel species. In the context of group 5A, it has been found that diphosphenes (RP=PR), phosphaarsenes (RP=AsR), and diarsenes (RAs=AsR) react with electrophiles such as HX,[2] peracids,[3] elemental sulfur,[4] halogens,[5] *t*-BuX radicals,[6] and metal

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