and, after stabilization at -20 °C, their ^{31}P spectra.

Methods. The $^{31}P\{^1H\}$ NMR spectra were recorded at 121.5 MHz on a Bruker WM 300 spectrometer with appropriate temperature control for spectra recorded below ambient temperature. Splitting patterns were simulated by using software (NIC-SIM) provided with the Nicolet NT-300 spectrometer. The latter instrument was also used for routine ^1H spectra. UV-vis spectra and the kinetics measurements in the slower reactions were measured on a Cary Model 219 spectrophotometer. Kinetic data were fit to suitable equations by standard least-squares programs. More rapid reactions were monitored at suitable wavelengths on a Canterbury Model SF-3A stopped-flow spectrophotometer interfaced with an OLIS 3820 Data System for data collection, storage, and analysis.

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Mimics of Transaminase Enzymes

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Abstract: Pyridoxamine has been attached to the primary side and to the secondary side of β-cyclodextrin; the resulting compounds convert α-keto acids to amino acids with substrate selectivity and some stereoselectivity. Pyridoxamine has also been attached to a synthetic macrocycle; the attached binding group showed substrate selectivity. Chains carrying catalytic basic groups have been attached to pyridoxamine; appropriate systems catalyze the prototropic rearrangement characteristic of transamination. A catalyzed HCl elimination involving chloropyruvic acid was also observed. A tetrahydroquinoline system related to pyridoxamine was synthesized to permit the stereochemically defined placement of a basic catalytic group. This converted keto acids to amino acids with good stereoselectivity for the formation of optically active products.

Pyridoxal phosphate and pyridoxamine phosphate are the two central coenzymes of amino acid metabolism.[6] The synthesis of almost all amino acids is achieved by the biochemical reaction of pyridoxamine phosphate with α-keto acids, in one of the steps of an overall process referred to as transamination (Figure 1). The pyridoxamine phosphate is converted to pyridoxal phosphate, the other coenzyme form; this can react with a second amino acid to regenerate pyridoxamine phosphate and convert the amino acid to its corresponding keto acid. As a result, a keto acid and an unrelated amino acid interchange functionality. Of the order of 30 different transaminase enzymes have been identified, which are reasonably specific for the particular pair of amino acid/keto acid reactants whose functionality is catalytically interchanged.

Pyridoxal phosphate has a number of other functions as well. Indeed it is the coenzyme for most of the interesting metabolic transformations of amino acids, catalyzing such processes as α-decarboxylation, β-decarboxylation, α,β-elimination reactions, β-substitution reactions (as in the reaction of serine with indole to form tryptophan), etc. In all of these cases the first step is the formation of a Schiff base between the pyridoxal phosphate aldehyde group and the amino group of the amino acid. The subsequent detailed chemistry is determined by the specific conformation of this adduct and of enzymatic catalytic groups within the enzyme-substrate-coenzyme complex.

The pioneering work of Snell[7] and of Braunstein[8] helped to clarify the mechanisms of all these reactions. It was shown that in simple model systems pyridoxal (with or without its phosphate group) and pyridoxamine could perform essentially all of the reactions in which they are involved metabolically. The rates in these nonenzymatic reactions were very slow, and the characteristic selectivities of the enzymatic processes were not present.

Considerable improvement in rate could be achieved in these model systems when polyvalent metal cations were included,[9] although no evidence has been found for the requirement of any such cations in enzymatic reactions. Metal complexing in the model system helps to fix the geometry of the coenzyme-substrate Schiff base, a task performed by the enzyme in the biochemical reaction. Martell showed[10] that there are situations in which nonaqueous solvents (that may better mimic the interior of the enzyme) can have an advantage in pyridoxal model reactions.

Some years ago we started a series of studies on model systems for these transaminase enzymes, with several goals. First of all, we wanted to incorporate more of the features of the enzymatic system itself. In particular, we wanted to incorporate a binding group to increase the extent of capture of the substrate and add some selectivity to the processes. In transaminase enzymes the protein itself helps to bind particular substrates, in cooperation with the covalent binding to the coenzyme. Furthermore, the enzyme acts to catalyze the steps of the transamination process.

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(4) American Cancer Society postdoctoral fellow, 1981-1983. Current address: Department of Chemistry, University of Chicago, Chicago, IL 60637.
(5) Taken in part from: Zimmerman, S. Ph.D. Thesis Columbia University, 1983. Current address: Department of Chemistry, the University of Illinois, Urbana, IL 61801.
(6) For reviews, see: 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. Note that in enzymatic transaminations the lysine Schiff base of pyridoxal phosphate is the catalytically important structure, rather than the free aldehyde.
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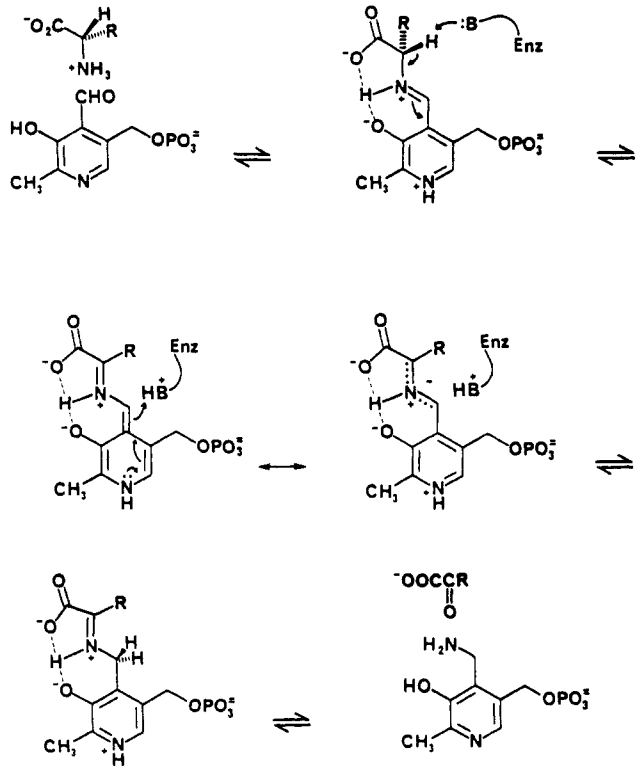


Figure 1. Enzymatic transamination. The pyridoxal phosphate shown actually is present as an imine with an enzymatic amino group. For reversible catalysis, the entire process is run backward with a different keto acid to form pyridoxal phosphate and a new amino acid.

The enzyme accelerates the formation and cleavage of the various Schiff bases, so the substrates can be bound rapidly and products rapidly extruded. The enzyme also furnishes catalysis to assist the proton transfers involved in the isomerization of ketimines to aldimines in the transamination process of Figure 1. Under most circumstances this isomerization is the rate-limiting step in the enzymatic processes. We hoped that by the incorporation of catalytic groups to accelerate these proton transfers we could produce better and more selective catalysts.

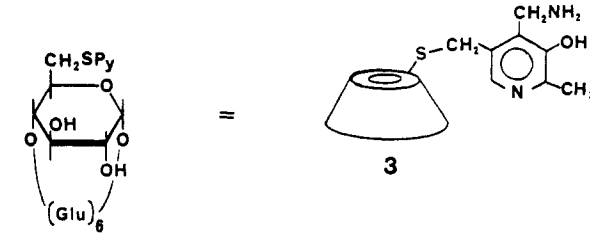
Enzymatic reactions typically show four types of selectivity,[11] three of which are of significant interest in the current work. First of all, enzymes are selective with respect to their *substrates*, using shape recognition and specific binding interactions to select a particular substrate from the reaction medium. This is the type of selectivity that we hoped to mimic by incorporating binding groups. Second, enzymes are selective with respect to the chemical *reaction* performed. This is particularly striking in the biochemistry of amino acids in which pyridoxal phosphate acts as coenzyme. For instance, the amino acid serine can undergo racemization, decarboxylation, loss of formaldehyde, loss of water and hydrolysis, or substitution of the hydroxyl group by various nucleophiles including indole. The specific conformation of the enzyme–substrate–coenzyme complex determines which of these is the preferred process. We would hope that in an artificial enzyme system in which the geometry of binding and of catalytic group positioning is controlled one might also achieve such chemical selectivity.

Enzymes also show *regioselectivity*, performing chemical transformations in a particular geometric region of the substrate. This is not a general problem with amino acid metabolism involving pyridoxal phosphate, but it is an attractive kind of selectivity to achieve. Geometrically controlled steroid functionalization is an example.[11,12] Finally, enzymes show *stereospecificity*, both in their selection of the original substrate and in the products that are produced. In amino acid metabolism, the most important example of this is the conversion of keto acids to optically active amino acids, in which the stereospecificity of the reaction is determined by the enzyme geometry. We hoped to imitate this by producing a mimic of the enzyme in which the proton that is added to the incipient optically active center during transamination (Figure 1) is delivered with good geometric control by a catalytic group in a defined orientation.

In this paper we will describe the attachment of pyridoxamine to the primary C-6 carbon of β-cyclodextrin and also to the secondary C-3 carbon of β-cyclodextrin. We will also describe attachment to a synthetic cavity. We have published preliminary accounts of these studies,[13-15] and a full description of a related molecule in which pyridoxamine is attached to the C-6 carbon of a β-cyclodextrin on which all the other primary hydroxyls had been removed.[16] We will also describe the attachment of catalytic chains to pyridoxamine, including a system in which the highly defined orientation leads to excellent stereoselectivity in transamination. Preliminary accounts of some of this work have appeared.[17,18] We have also examined some derivatives of pyridoxal as selective amino acid racemases[19] and a system in which pyridoxal attached to β-cyclodextrin acts as a mimic of tryptophan synthetase.[20] These studies will not be discussed here.

Results and Discussion

1. Syntheses of Pyridoxamines Carrying Binding Groups. Pyridoxamine dihydrochloride, on treatment with 48% HBr, was converted into the known dihydrobromide of the bromomethyl derivative (**1**).[21] On treatment with potassium thioacetate and



then acetic anhydride, this was converted to the *O,S,N*-triacetyl derivative of thiopyridoxamine (**2**), isolated in analytical purity and high yield. When this was heated with 48% HBr the acetyl groups were removed, affording the dihydrobromide of thiopyridoxamine, an air-sensitive compound. This was dissolved in water with ammonium bicarbonate and the solution was heated with β-cyclodextrin 6-tosylate. The resulting compound **3** carrying a cyclodextrin group on the C-5 methylene of pyridoxamine was isolated by chromatography, in analytical purity. The structure of this compound was confirmed by ^{1}H NMR spectroscopy.

Attachment of pyridoxamine through this thioether linkage to the secondary side of cyclodextrin was more challenging. It had been reported[22] that cyclodextrin could be selectively tosylated

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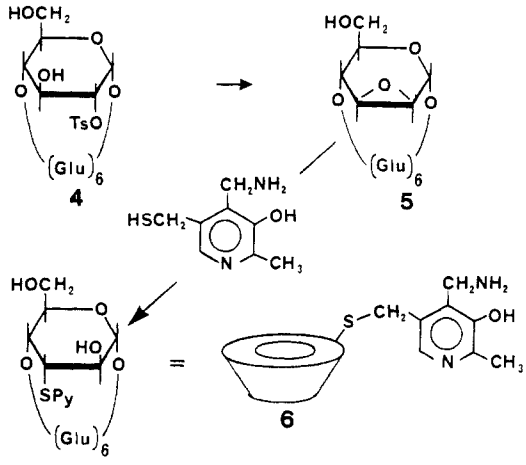
on the secondary side under special conditions but, as we have reported elsewhere,[23] we were not able to confirm this earlier report. However, we did find that[23] it was possible to prepare the C-2 tosylate of β-cyclodextrin (**4**) by tosyl transfer from bound



m-nitrophenyl tosylate. When this authentic C-2 tosylate derivative was treated with base, it formed the 2,3-epoxide on one of the glucose units. The resulting manno epoxide **5** showed the expected ^{1}H NMR signal at 3.3 ppm with a 3.7-Hz coupling to H-3, for the C-2 proton of the glucose epoxide residue, and an unsplit one-proton signal at 4.5 ppm for the C-1 proton of that residue, in addition to the other normal signals. In glucose manno epoxides $J_{1,2}$ is normally close to 0 Hz, while in allo epoxides it is 2.5–4.5 Hz. Thus this spectrum confirms the assignment of structure **5** as the manno epoxide.

The epoxide was allowed to react with thiopyridoxamine, prepared as described above. The opening of sugar epoxides normally occurs by axial attack with the formation of a diaxial product,[24] in which the leaving oxygen is converted to an axial hydroxyl group. However, in structure **5** this would require axial attack by a nucleophile located inside the cyclodextrin cavity. Thus one could have imagined a violation of the usual diaxial opening rule, but the violation was not observed. We found that the pyridoxamine unit was attached at C-3 in structure **6**, as expected from diaxial opening. The 300-MHz NMR spectrum of **6** showed the expected signals from pyridoxamine and cyclodextrin protons and a one-proton signal at 3.05 ppm, the expected position for a cyclodextrin CH unit carrying the sulfur atom. This signal appeared as a doublet of doublets, with J = 11.2 and 3.4 Hz. Decoupling of the 3.4-Hz splitting occurred by irradiation at 3.95 ppm and of the 11.2-Hz signal at 3.8 ppm. Thus the CH–S unit is at C-3, with coupling to protons at C-2 and C-4. If it had been attached at C-2 instead it would have been coupled to H-1 of the cyclodextrin unit, a hydrogen that is expected to be found in the region of 4.9 ppm. The anomeric hydrogens at C-1 of cyclodextrin are of course downfield-shifted by the two oxygen substituents, and since they are located on the outside of the cyclodextrin cavity their expected positions could not be significantly shifted by anisotropic effects from the bound pyridoxamine unit. Thus the conclusion that the attachment is at C-3 seems to be completely unambiguous.

This point is of particular importance, since the details of the NMR spectrum indicate that the pyridoxamine unit is not in fact attached axially to the glucose in the final isolated product. A coupling constant as large as 11.2 Hz for the C-3 proton indicates that that proton must itself be axial, not equatorial.[25] Thus after ring opening of the epoxide to form a cyclodextrin–pyridoxamine conjugate with axial pyridoxamine and hydroxyl units on one glucose, there must be a conformational change of that glucose unit. The easiest interpretation would be a chair flip to the $^1C_4$ conformation,[26] so that now the hydroxyl group and pyridoxamine unit would be equatorial. The 11.2-Hz coupling would then be $J_{2,3}$ between axial protons, while $J_{3,4}$ of 3.4 Hz involves axial–equatorial coupling. This ring flip pulls the pyridoxamine unit out of the cyclodextrin cavity, although the inverted chair produces an indentation into the cavity. Molecular models suggest that with this geometry it would still be possible for aromatic sidechains of keto acids to bind into this indented cavity while undergoing transamination with the pyridoxamine unit, and the results below confirm that indeed this occurs. Of course our data do not yet exclude the possibility that this glucose residue, instead of undergoing a complete chair flip, simply undergoes some sort of flexure to move the pyridoxamine unit into a pseudoequatorial position.

Many laboratories throughout the world are involved in the synthesis of artificial binding cavities. Those constructed of hydrocarbon segments but carrying groups to solubilize them in water have the potential to bind substrates by the same sort of hydrophobic interactions characteristic of cyclodextrin binding and of the binding by many enzymes. It seemed to us desirable to generalize our studies on pyridoxamine–cyclodextrin compounds to include an example of such a synthetic cavity. First of all, the synthetic cavities clearly have the advantage of geometric versatility, while with the cyclodextrins one is restricted to substrates that can use the cyclodextrin geometry or various simple modifications of it. Second, it seemed to us desirable to make such a comparison so that we could evaluate the question of whether the experience with cyclodextrins can be easily extrapolated to some of these novel synthetic cavities. For this reason we have attached pyridoxamine to a water-soluble macrocycle with a good hydrophobic binding cavity.

In choosing such a system we were attracted to the cavities described by Koga,[27] since X-ray and NMR studies on complexes with his compounds had demonstrated[28] that substrates are indeed bound into the cavity. In some other systems it has turned out that proposed cavities actually collapse, and substrates are simply occluded on the face of the cavity system.

Koga originally reported only the tetraamine systems, which required strong acid in order to dissolve in water. For our purposes this was not suitable, so we made the quaternary derivatives of the Koga compounds and evaluated their binding constants. As we have reported elsewhere,[15] both the binding constants and the NMR shifts associated with binding indicate clearly that substrates bind into the cavity of the quaternary tetracation in just the same fashion in which they bind into the protonated Koga systems and with comparable binding constants. These macrocycles are most conveniently made by attaching both aliphatic chains to a diphenylmethane-4,4′-disulfonamide dianion in a tetraalkylation reaction, but this procedure is not suitable for the preparation of a monosubstituted derivative. Accordingly, we synthesized compound **7** by reaction of the monoanion of *N*-acetyl-*N*′-tosylbis(4-aminophenyl)methane (**8**) with 1,5-dibromopentane, to introduce the unsubstituted chain. After deacetylation of the nitrogens and tosylation of each of them, the second chain was introduced by alkylation with 3-[*tert*-butyldimethylsilyl)oxy]-1,5-dibromopentane. The resulting macrocycle was detosylated and desilylated with sodium metal in 1-butanol, and the tetraamino alcohol was N-methylated with formaldehyde and $NaBH_3CN$ to afford compound **9**. This was converted to the thioacetate by Mitsunobu reaction with thioacetic acid, and the product was then quaternized on all four nitrogens with methyl iodide. The acetyl group was removed with alkali in the presence of sodium borohydride (to protect the thiol anion from oxidation) and reacted directly with bromomethyl pyridoxamine dihydrobromide **1**. The product **10** was isolated, by Sephadex chromatography with an aqueous ammonium bicarbonate eluent, as a pentabicarbonate

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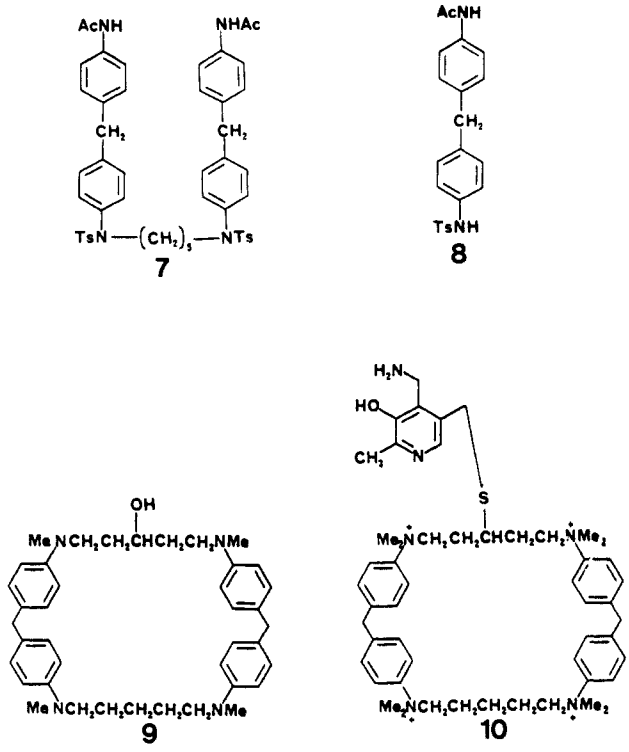


salt. It was characterized by analysis and NMR spectroscopy.

2. Transaminations by the Binding Group–Pyridoxamine Molecules. We expected that these pyridoxamine derivatives **3**, **6**, and **10**, carrying hydrophobic binding groups, should be able to accelerate the reductive amination (transamination) of keto acids carrying hydrophobic substituents. In particular, molecular models suggested that indolepyruvic acid and phenylpyruvic acid can form the ketimine derivative with the attached pyridoxamines and hold the geometry so that the ketimine can be converted to aldimine while the hydrophobic substituent is bound into the cavity of cyclodextrin or of the synthetic macrocycle. Of course the flexibility of these systems is such that cooperative binding is permitted but not required. To detect cooperation, we have compared transamination rates for indolepyruvic acid and phenylpyruvic acid with those for simple pyruvic acid, which cannot utilize the binding site, and for α-ketovaleric acid, which can use it less effectively than the aromatic derivatives.

Our earliest studies[13] involved rudimentary competition experiments. For instance, when simple pyridoxamine was allowed to react with 1 equiv each of pyruvic acid and of phenylpyruvic acid, and the products were then identified by dinitrophenylation and quantitative HPLC, it was found that approximately equal amounts of alanine and phenylalanine were formed. This indication that, as expected, simple pyridoxamine shows no significant selectivity among these keto acids was also observed in one-to-one competition between pyruvic acid and indolepyruvic acid, in which again approximately equal yields of alanine and of tryptophan were formed. The situation was quite different with the pyridoxamine derivative **3** attached at C-6 of β-cyclodextrin. Here a one-to-one competition between simple pyruvic acid and phenylpyruvic acid or indolepyruvic acid led to the formation of at least a 50/1 ratio of the aromatic amino acid products relative to alanine. Thus judged on this simple basis the rate for the aromatic keto acids is being increased at least 50-fold by the subsidiary hydrophobic binding into the cyclodextrin cavity.

In the earliest work[13] a very rough kinetic estimate was obtained by the observation that in a noncompetitive reaction indolepyruvic acid was converted to a few percent tryptophan in 10 min, while with the same concentration conditions pyruvic acid required 30 h to form an equivalent amount of alanine. This ratio of 180-fold in the amount of time required to produce these two different amino acids can be deceptive, however. The transamination reactions are quite slow, even with the binding group attached,

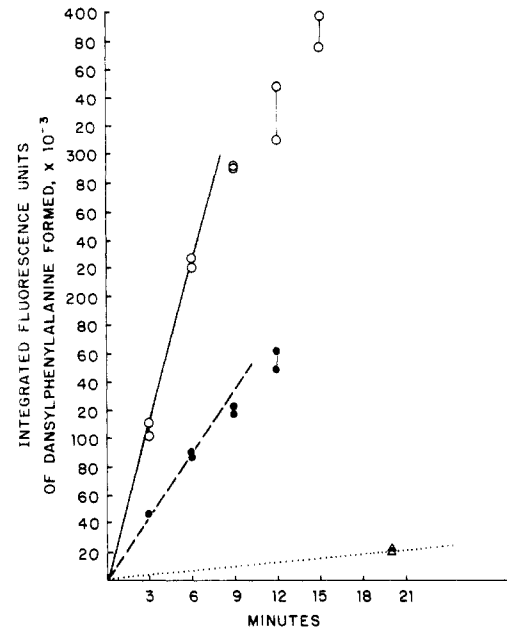


Figure 2. Typical kinetic runs for the formation of phenylalanine from phenylpyruvic acid by pyridoxamine (Δ···Δ), by the cyclodextrin derivative **3** (●--●) and by the macrocyclic derivatives *10* (○—○). As can be seen, the rates fall off with time.

and many side reactions occur in the course of the process. Thus over 30 h much of the original pyruvic acid is lost to side reactions; this makes the slow reaction appear even slower and thus overemphasizes the kinetic advantage associated with hydrophobic binding. In later work we examined initial rates, for which such problems are minimized.

Direct studies were done on solutions 0.5 mM in the keto acids and in the pyridoxamine derivative with a rather concentrated 2.7 M phosphate buffer of pH 9.3. Samples were diluted and treated with dansyl chloride. HPLC analysis with fluorescence detection, in a system that had been quantitatively calibrated with authentic dansyl amino acid solutions, was used to follow the production of amino acids. Data were taken over only the first few percent of reaction, to avoid the loss of starting material through side reactions observed if the reactions were allowed to proceed further. Typical runs are plotted in Figure 2. In these studies the pyridoxamine–β-cyclodextrin derivative **3** accelerated the conversion of phenylpyruvic acid to phenylalanine by a factor of 15 ± 2 compared with the rate observed for this same amination process by simple pyridoxamine. This comparison is probably the most reliable. It differs from our estimate of a 50-fold rate effect in the direct competition experiments presumably in part because the reaction conditions were somewhat different and possibly also because the direct competition reactions were run over a longer period of time in which side reactions that change the concentrations of starting materials have begun to be appreciable. By this kinetic assay, **3** accelerated the formation of tryptophan from indolepyruvic acid by a factor of 12.

The evaluation of compound **6**, with the pyridoxamine attached to the secondary face of cyclodextrin, was done by the same kinetic method, but over longer times. We found that compound **6** produced tryptophan from indolepyruvic acid at about 20 times the rate at which pyridoxamine acted on indolepyruvic acid and with phenylpyruvic acid **6** showed a similar rate advantage. Thus attachment of pyridoxamine at C-3 of cyclodextrin, with an apparent ring inversion of the glucose residue to which the attachment is made, still leads to a catalyst that can cooperatively utilize hydrophobic binding and imine formation, with effects of the same general magnitude as those observed when the pyridoxamine is attached to the primary carbon.

The pyridoxamine derivative **10** carrying a synthetic macrocycle was if anything slightly better in this cooperative interaction. The kinetic data show that **10** accelerates the conversion of phenylpyruvic acid to phenylalanine by a factor of 31 ± 3 compared with

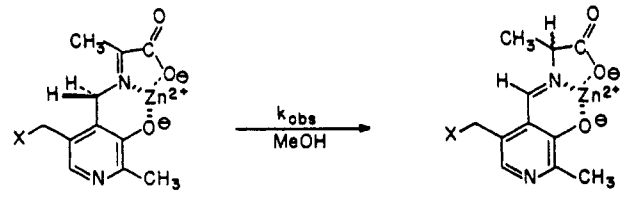


Figure 3. Rapid formation of a ketimine is followed by rate-determining isomerization to an aldimine. This isomerization is the process catalyzed by appropriate side-arm groups in compounds **11–20** and **36**.

the rate for simple pyridoxamine. It accelerates the conversion of indolepyruvic acid to tryptophan by a factor of 12 ± 1.5. The conversion of α-ketovaleric acid was only 6 ± 1 times as fast as it was with pyridoxamine, as expected because of the smaller hydrophobic tail. Taking all these data together, it can be seen that the attachment of a hydrophobic binding group to pyridoxamine, with the flexible link used, results in general in a preferential acceleration of reaction with aromatic keto acids carrying good hydrophobic units by more than 1 order of magnitude, but less than 2 orders of magnitude. We have recently observed a similar selective acceleration, for substrates that can utilize the hydrophobic binding cavity, in a molecule with thiamine and various analogues attached by a flexible linkage to C-6 of β-cyclodextrin.[29]

3. Stereoselective Product Formation. The association of optically active cyclodextrin units with pyridoxamine in reagents **3** and **6** suggested that the product amino acids might be formed with some stereoselectivity. We determined this by using the chiral chromatographic method of Karmen,[30] in which dansyl amino acids are analyzed by HPLC with a copper–proline eluent. The phenylalanine formed by transamination with the pyridoxamine–C-6-cyclodextrin derivative **3** had a 5:1 preference for the L enantiomer. With indolepyruvic acid the product tryptophan was formed with a 2:1 preference for the L enantiomer. By contrast, the pyridoxamine–C-3-cyclodextrin **6** showed no detectable enantiomeric selectivity in the formation of phenylalanine and a 1.8:1 preference for the formation of D-tryptophan. While it is interesting that the cyclodextrin chirality is expressed in these reactions, the expression is clearly not reliable or predictable. For this reason we set out to prepare pyridoxamine derivatives in which chiral induction in the products is produced by the active direction of an asymmetrically placed catalytic group, not by the accident of geometry in cyclodextrin binding.

4. Pyridoxamines Carrying Attached Proton-Transfer Catalysts. A basic group of the enzyme catalyzes the proton transfers characteristic of transaminations. Bruice found that imidazole buffer can catalyze transamination reactions in model systems,[31] presumably imitating the basic catalytic group of the enzyme that is involved in proton removals in the course of transamination (Figure 1). However, no previous study has been done on the incorporation of such a base into the pyridoxal or pyridoxamine molecule itself. It seemed to us that synthetic approaches to such compounds should be straightforward and that these catalysts could be better mimics of the transaminase enzymes than are the simple coenzymes without such catalytic groups. Furthermore, if a catalytic group could be demonstrated to be playing a complete role in proton transfer, removing the proton from the 4′-CH_2 group of the ketimine intermediate and attaching it to the α-carbon of the new amino acid in the aldimine intermediate, one would expect that appropriate geometric control of this catalytic group could be used to direct asymmetric synthesis of the amino acid.

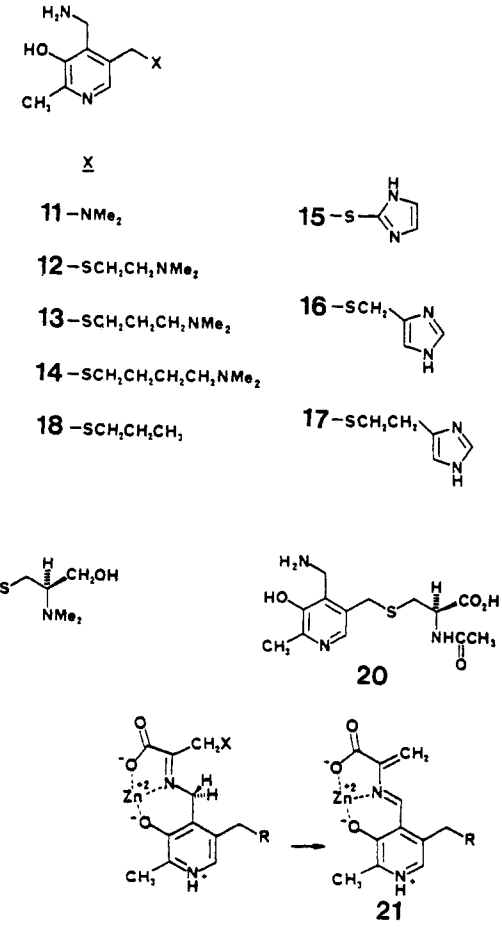
Our first approach to this question involved the synthesis of a series of simple pyridoxamine derivatives carrying basic groups at the end of flexible chains of various lengths.[17] The 5-(bromomethyl) dihydrobromide derivative of pyridoxamine **1** was allowed to react with a variety of nucleophiles, and the resulting products were isolated as the hydrobromides. In methanol at pH 4.00 they reacted rapidly with pyruvic acid to form the ketimine, and we followed the rate of ketimine to aldimine isomerization (Figure 3). The rate data are listed in Table I.

Table I. Rates of Conversions of Ketimines to Aldimines and of Elimination of HCl from the Ketimine of α-Chloropyruvic Acid at pH 4.00[a] in Methanol (30.0 °C)

reagent	side chain	k_{trans}[b]	k_{rel}	k_{elim}[c]	k_{rel}
18	SPr	0.009	1.0	0.77	1.0
pyridoxamine	OH	0.012	1.4		
11	NMe_2	0.13	14	3.3	4.3
12	$S(CH_2)_2NMe_2$	0.23	26	9.2	12
13	$S(CH_2)_3NMe_2$	0.33	37	2.6	3.4
14	$S(CH_2)_4NMe_2$	0.11	13		
15	*S*-Im	0.05	5.6	6.4	8.3
16	*S*-CH_2Im	0.11	12	5.3	6.9
17	*S*-$(CH_2)_2$Im	0.68	76	2.8	3.6
19	*N,N*-dimethylcysteinol	0.23	26[d]		
20	*N*-acetylcysteine	0.096	11		

[a] "pH" as read with a glass electrode. The pH's were unchanged at the end of the reaction. [b] Pseudo-first-order rate constant for conversion of the ketimine to the aldimine in transamination with pyruvic acid, $\times 10^3$ s^{-1}. [c] Pseudo-first-order rate constant for development of the chromophor at 414 nm from α-chloropyruvic acid, $\times 10^3$ s^{-1}. [d] With 2-ketovaleric acid as substrate, which is ca. 20% slower than pyruvic acid.

Although the compound **11** with a simple dimethylamino group



attached to the C-5 methylene could in principle act as an internal base to deprotonate the ketimine intermediate, the resulting six-membered ring is not stereoelectronically ideal. One would expect a somewhat longer basic chain to be better so the basic nitrogen can reach the proton to be removed when the CH bond is perpendicular to the pyridine ring. Hine has found that for a related internal deprotonation process (not involving pyridoxal), an eight- or nine-membered ring is better, since it meets these stereoelectronic requirements.[32]

(29) Hilvert, D.; Breslow, R. *Bioorg. Chem.* **1984**, *12*, 206–220.
(30) Lam, S.; Chow, F.; Karmen, A. *J. Chromatogr.* **1980**, *199*, 295–305.
(31) (a) Bruice, T. C.; Topping, R. M. *J. Am. Chem. Soc.* **1962**, *84*, 2448–2450. (b) Bruice, T. C.; Topping, R. M. *J. Am. Chem. Soc.* **1963**, *85*, 1480–1488. (c) Bruice, T. C.; Topping, R. M. *J. Am. Chem. Soc.* **1963**, *85*, 1488–1493.

The compound (**12**) with a sulfur and two methylene groups carries the dimethylamino function so far out that it would form a nine-membered ring on removing the proton from the methylene group of the ketimine. From the work of Hine,[32] this is more than enough length to permit such a process, but interestingly this was not the optimum chain. The compound with one additional methylene (**13**) showed an improved rate. This makes no sense if the dimethylamino group is functioning only as a base, but this increased chain length would be needed if after removing the proton from the methylene group the dimethylamino catalytic group then transferred the proton to the α-carbon of the developing amino acid, in the isomerization to the aldimine. Thus this preference for increased chain length seems to argue for such a dual catalytic role of the amine. As expected from this, the compound (**14**) with yet an additional methylene group, which is no longer needed to permit the reach to the α-carbon, is now less active. In general of course the incorporation of extra flexibility in such chains will decrease rates unless the flexibility and length are needed for the catalytic process.

Related but even more striking effects are seen with the imidazole catalysts. The compound in which an imidazole is linked through its C-2 carbon to a sulfur and then to the pyridoxamine (**15**) is a moderate catalyst, although simple proton removal would involve a nine-membered ring. Some improvement is found when an extra methylene group is incorporated in compound **16**, but the best catalyst by far is compound **17** with a three-atom chain linking the imidazole to the pyridoxamine methylene group. The distance of the imidazole nitrogen from the pyridoxamine ring is identical with that in compound **13**, the previous optimal structure. Again, our conclusion is that the extra chain length required would make no sense if this catalytic group were functioning only as a base to deprotonate the ketimine, but it is as expected if the chain must then extend out to the amino acid α-carbon and add the proton in the conversion of ketimine to aldimine.

It is interesting that the imidazole compounds are significantly better catalysts than are the dimethylamino derivatives. This might simply reflect the rigidity in an imidazole ring, with a resulting entropy advantage for these systems. However, imidazole is a considerably weaker base than is an alkyl amine, and weaker basicity should be an *advantage* in this catalytic process.

The important catalytic species in the first step of the isomerization reaction is the pyridoxamine derivative carrying one extra proton. For the reaction to occur the proton should be attached to the pyridine ring nitrogen, but such a species will of course be in equilibrium with an isomer in which the proton is instead attached to the basic catalytic group. As the basicity of that catalytic group is decreased, the concentration of the catalytically useful isomer with the proton on the pyridine ring will increase, while the rate constant for deprotonation by the basic catalytic group will decrease. The Brönsted coefficient for base-catalyzed deprotonation reactions is normally considerably less than one.[33] Thus the increased concentration of the correct isomer will more than make up for the decreased rate constant of that isomer as the basicity of the catalytic group is lowered. Because of these considerations the ideal catalytic group would have a basicity close to that of the pyridine ring itself, rather than the much increased basicity of a saturated amine. The observed catalytic rate advantage for the imidazole compounds is therefore expected from their decreased basicity.

As predicted from all this, the rate advantage of the compounds carrying cataytic groups is observed to be greater at low pH than at higher pH. In the comparison of compound **13** with the reference compound **18** carrying a simple propyl group, the largest rate ratio is found at pH 4.00. As the pH is raised to 8.00 the observed rate for the simple propyl compound **18** increases dramatically while that for the compound **13** carrying a basic side chain increases much less. The result is that at pH 8.00 the rate ratio is only 4-fold, not the 38-fold of Table I.

An additional piece of evidence for the overall mechanism, in which the catalyst first removes the proton and then adds it to the other carbon, can be seen with the catalyst **19** carrying an *optically active* basic unit. This compound produced a 39% enantiomeric excess of D-norvaline, indicating that the chirality is influencing the direction of protonation of the intermediate to form the aldimine. In compound **20**, carrying a related chiral unit that is not able to reach the α-carbon and protonate it, no detectable enantiomeric preference was observed.

Further evidence for this two-step mechanism, with the catalyst serving first as base and then as acid, was obtained by looking at a related process in which the second step does not occur. We find that the reaction of chloropyruvic acid with pyridoxamine and its derivatives in methanol with zinc acetate leads to rapid ketimine formation, with a spectroscopic maximum at 328 nm; on standing this undergoes a subsequent reaction to form a new chromophore with spectroscopic maximum at 414 nm. This then more slowly reacts further to produce a new chromophore at 380 nm. It seems certain that the intermediate with λ_{max} 414 nm is the dehydroalanine derivative **21**. A λ_{max} of 412 nm has been reported[34] for a closely related compound, and this position is also that expected from the general rule[35] that addition of a double bond to a linear conjugated system will extend λ_{max} by approximately 30 nm. The intermediate **21** can be thought of as an aldimine with such an additional double bond.

As expected from this, the basic catalytic groups in the side chains of compounds **11**–**17** were able to accelerate the HCl elimination process to form intermediate **21**, but the structural dependence of this elimination rate was very different from that for the isomerization described above. We list the pseudo-first-order rate constants for these elimination reactions also in Table I, where they can be compared with the relative rate constants for isomerization of ketimines to aldimines using simple pyruvic acid as substrate. The elimination reactions are much faster than the related isomerization processes, but more important the relative rates among pyridoxamines carrying different catalyst side arms show striking reversals.

For the elimination reactions, the extension of a simple dimethylamino group in compound **11** to a dimethylamino group at the end of a three-atom chain in compound **12** leads to an improvement of rate, as expected from our earlier discussion on the stereoelectronic requirements for deprotonation. However, when the chain is made one atom longer in **13** the elimination rate drops significantly, while this change had led to an improvement in the isomerization rate. Similarly, in the imidazole series the extension of the chain from compound **15**–**16** to **17** leads to a drop in rate for HCl elimination, while it had lead to a significant increase in rate in the isomerization reactions.

These reversals strikingly confirm our previous conclusions. When the catalytic group is required to act only as a base, the shorter chains are better. In the isomerization reactions the preference for the longer chains must mean that the catalyst group indeed has to reach out far enough to deliver the proton to the α-carbon of the developing amino acid.

The much higher rates for HCl elimination than for isomerization are also striking. We believe that the elimination process involves deprotonation to the same kind of intermediate involved in transamination, with loss of chloride ion in a second step. The evidence for this is that in the few cases examined bromopyruvic acid had essentially the same rate as did chloropyruvic acid, while if HX elimination had been a concerted process one might have expected a much faster rate for the HBr case. If this conclusion is correct, it seems that in our systems the transamination intermediate is reprotonated to generate the ketimine approximately 100 times as often as it protonates to isomerize to the aldimine. Thus one of the problems in elaborating better catalysts will be

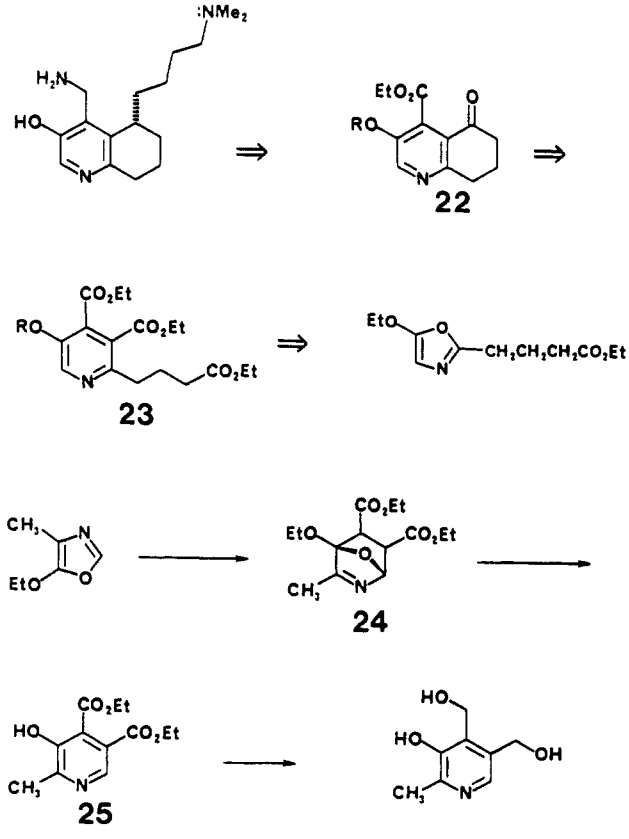
(32) Hine, J. *Acc. Chem. Res.* **1978**, *11*, 1–7.
(33) Jencks, W. P. "Catalysis in Chemistry and Enzymology"; McGraw-Hill: New York, 1969; p 198.
(34) Matsushima, Y.; Karube, Y.; Kono, A. *Chem. Pharm. Bull.* **1979**, *27*, 703–709.
(35) Fieser, L.; Fieser, M. "Steroids"; Van Nostrand Reinhold: New York, 1959; p 15.

to produce catalytic systems, such as bifunctional systems, that can more easily protonate the intermediate at the remote carbon atom.

5. Asymmetric Induction in Amino Acid Synthesis by a Bicyclic Pyridoxamine Derivative Carrying an Oriented Catalytic Side Arm. With the clear evidence that a basic side arm attached to pyridoxamine could act not only to remove a proton but also subsequently to add it to the remote carbon, eventually the α-carbon of the product amino acid, it seemed attractive to learn how to do this reaction with high stereoselectivity. By the use of a metal complex of the ketimine intermediate it is possible to hold the system in a well-defined flat geometry. Thus for stereoselective synthesis of a product amino acid it is necessary simply that the catalytic side arm operate on one face or the other of this planar system. We decided that this could be achieved by fusing a ring to the pyridoxamine unit, so that a side arm coming off this ring would necessarily be aimed either forward or backward.

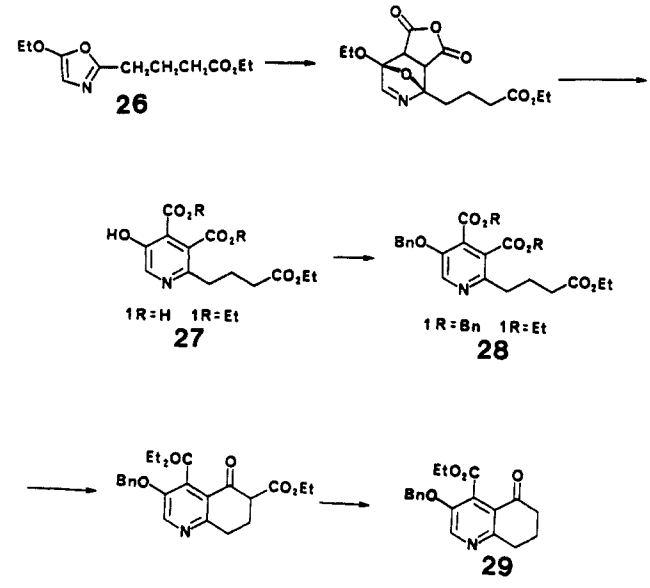
Our target was a pyridoxamine with a saturated six-membered ring fused to it in positions 5 and 6. We then intended to have the side arm come off the carbon attached to C-5, putting it in the same position as in the simple systems described above. Compounds of this general type were not previously known, but it seemed to us that a synthesis proceding through the general framework **22** should let us prepare the desired structures. We omitted the C-2 methyl group characteristic of pyridoxal and pyridoxamine, since it has no obvious function. In the synthesis as developed it would have been possible to incorporate it if this seemed desirable.

None of the classical syntheses of vitamin B_6 appeared easily modified to include the added functionality needed to prepare **22**, which looked most accessible by a Dieckmann condensation–decarboxylation sequence from **23**. However, Firestone reported a novel synthesis of vitamin B_6 in 1962, utilizing a heterodiene Diels–Alder reaction.[36] In his synthesis diethyl maleate reacted with 5-ethoxy-4-methyloxazole to afford adduct **24**, which on treatment with acidic ethanol was converted to **25**. In order to



modify this synthesis for our purposes, it was necessary simply to prepare an oxazole carrying the appropriate chain at C-2.

Ethyl glutaryl chloride was condensed with glycine ethyl ester, and the resulting amide was cyclized with phosphorous pentoxide, affording the desired substituted oxazole derivative **26**. We found that this was unreactive with diethyl maleate, but would react with maleic anhydride. The resulting Diels–Alder adduct was isomerized and ethanolized with ethanolic HCl, leading to a mixture of ester acids **27**. Rather than separate these isomers, we treated the mixture with *O*-benzyl-*N*,*N*-diisopropylurea. The reagent benzylated not only the phenolic oxygen but also the free carboxyl group; 2 equiv of the reagent were used to prepare a benzyl ether mixed triester **28** in a 32% overall yield from the oxazole **26**. This crude triester was submitted to Dieckmann cyclization with sodium ethoxide, and the product was hydrolyzed and decarboxylated by refluxing it with 2.4 N hydrochloric acid. These conditions were sufficiently selective that the aromatic ester group remained unhydrolyzed, and product **29** was isolated. With more vigorous



acid conditions some debenzylation of the phenol also occurred, interestingly before appreciable hydrolysis of the aromatic ester group of **29**.

For attachment of a catalytic side arm, we initially considered a system in which the chain contained only carbons. Attempted Grignard additions of the appropriate chain to compound **29** were relatively unsuccessful, and Wittig reactions with this ketone were also unrewarding. Thus we decided to attach a chain through a sulfur atom, as had been carried out in the studies described above.

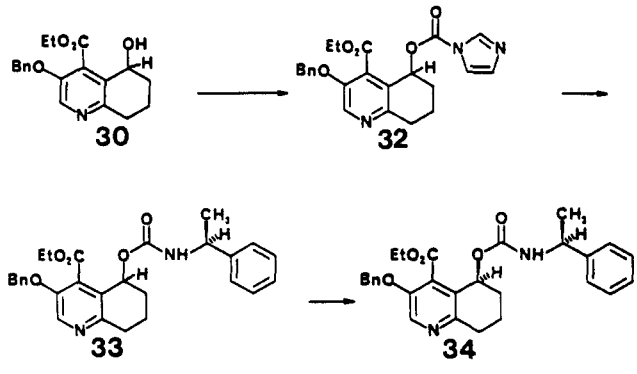
Reduction of the keto ester **29** with DIBAL afforded the corresponding hydroxyaldehyde, but various attempts to protect the aldehyde group while functionalizing the hydroxyl further were poorly successful. We decided to leave the ester group unreduced and treated the keto ester **29** with sodium borohydride. Remarkably, under normal conditions this completely reduced both the ketone and the ester, but by carrying out the reaction for a short time at 0 °C we were able to do a selective ketone reduction and prepare the desired hydroxy ester (**30**) in a very high yield. The hydroxyl group was mesylated, and the resulting mesylate was used to attach various side chains by thiol nucleophilic reaction.

After preliminary studies with the racemic compound, which will not be detailed here, we set out to prepare the optically resolved target compound **31** (or its mirror image, in separated form) by performing an optical resolution of intermediate **30**. The alcohol was allowed to react with carbonyl diimidazole, and the resulting imidazole carbamate **32** was heated at reflux with α-(*S*)-(−)-phenethylamine and boron trifluoride etherate.[37,38] This afforded

(36) Firestone, R. A.; Harris, E. E.; Reuter, W. *Tetrahedron* **1967**, *23*, 943–945.

(37) Cf.: Pirkle, W. H.; Hauske, J. R. *J. Org. Chem.* **1977**, *42*, 1839–1844.
(38) Pirkle, W. H.; Hauske, J. R. *J. Org. Chem.* **1977**, *42*, 2781–2782.

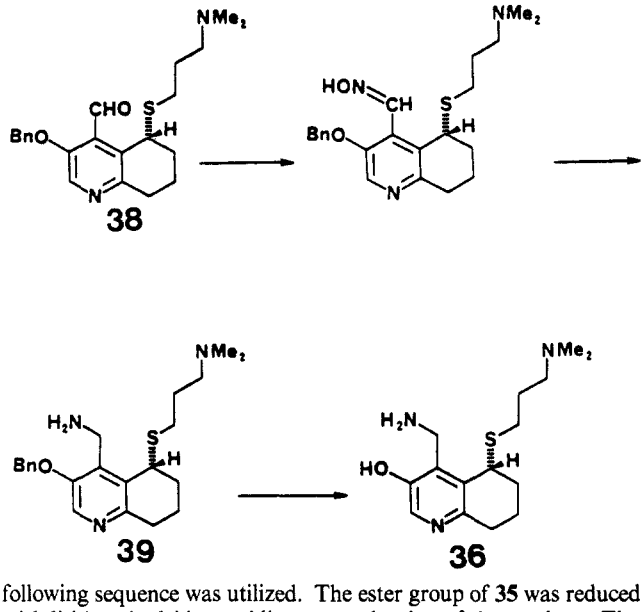
a mixture of the two diastereomers of the carbamate **33**, which were separated by medium-pressure liquid chromatography on a silica column. After two or three passes through the column, the earlier eluting carbamate isomer **34** was purified from its



isomer by a factor of at least 200 to 1, as judged by HPLC analysis. This would correspond to the optical purity of intermediate **34**, assuming that the original phenethylamine is optically pure, but the carbamate would also contain the epimer to the extent that the phenethylamine were itself contaminated with its enantiomer. The assignment of the configuration in intermediate **34** was made on the basis of the further studies to be described below but has not yet been independently confirmed by a direct method.

The alcohol was regenerated by treating the carbamate with triethylamine and trichlorosilane,[38] and the resulting optically active alcohol **31** was mesylated. By this point the results of the study described above were available, so it was decided to attach a (dimethylamino)propanethiol side arm. The desired thioether **35** was obtained in high yield.

After a number of studies on an appropriate sequence to generate the aminomethyl group of the target compound **36**, the



following sequence was utilized. The ester group of **35** was reduced with lithium hydride, avoiding overreduction of the product. The resulting alcohol **37** was oxidized to the aldehyde **38** with Collins' reagent, after various other oxidants proved unsatisfactory. The conversion of a pyridoxal to a pyridoxamine is in principle no problem, since it should be possible to do it by transamination. However, the side reactions that occur during transamination can make this an unattractive synthetic procedure. Reductive aminations of such aldehydes with sodium cyanoborohydride and ammonium chloride[39] or with ammonium hydroxide and sodium dithionite[40] gave very poor yields of the desired amine. Kuzuhara[41]

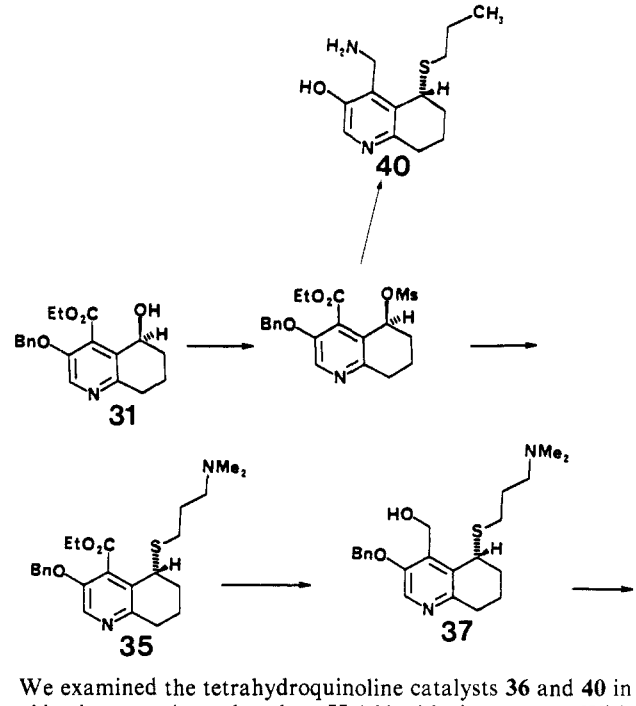
Table II. Rates of Conversion of Ketimine to Aldimine in Methanol at "pH 4.00" (30.0 °C) and Optical Inductions in Product Amino Acids[a]

compd	amino acid[b]	k_{obsd}, s^{-1} [c]	rel rate[d]	% conversion[e]	ratio[f] D:L
13	alanine	3.3×10^{-4}	38		
18	alanine	8.7×10^{-6}	1		
36	alanine	1.5×10^{-3}	172	83	93:7
36	alanine			68	91:9
36	norvaline	9.5×10^{-4}	109	68	96:4
36	norvaline			35	95:5
36	tryptophan	1.0×10^{-4}	115	89	94:6
40	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 keto acid. Reactions were performed as in ref 17, 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 **18** with pyruvic acid. [e] At the time of product isolation, relative to final equilibrium absorbance (UV). [f] Determined by chiral HPLC, as described in the Experimental Section.

had performed such a transformation by converting the aldehyde to its oxime, then reducing it with Lalancette's reagent.[42] In our hands this procedure led to a mixture of products, but we found that the oxime of **38** could be smoothly reduced with zinc and acetic acid. The resulting amine **39** was debenzylated with 6 N HCl, leading to the desired product **36**.

For comparisons of rates and products, we also prepared a related compound **40** carrying a simple propylthio side arm. Importantly, this material was synthesized from the same optically resolved alcohol (**31**) as was our catalyst compound **36**.



We examined the tetrahydroquinoline catalysts **36** and **40** in our kinetic system in methanol at pH 4.00 with zinc acetate. With various keto acids both compounds gave good pseudo-first-order kinetic plots for conversion of the ketimines to the aldimines, linear from 20% to 98% of the reaction (the early data is sometimes less good, because of still incomplete formation of the original ketimines). The data are listed in Table II. Even the tetrahydroquinoline derivative **40** carrying no extra catalytic side arm was 5 times faster in its reaction with α-ketovaleric acid than was the simple pyridoxamine **18** with a propylthio side arm. Since we have found that transaminations with α-ketovaleric acid are generally

(39) Cf.: Borch, R. F.; Bernstein, M. D.; Durst, H. D. *J. Am. Chem. Soc.* **1971**, *93*, 2897–2904.
(40) Tabushi, I. *Chem. Abstr.* **1985**, *78*, 13507r.
(41) Kuzuhara, H.; Komatsu, T.; Emoto, S. *Tetrahedron Lett.* **1978**, 3563–3566.
(42) Lalancette, J. M.; Brindle, J. R. *Can. J. Chem.* **1970**, *48*, 735–737.

1.2–1.5 time slower than with pyruvic acid, this means that the tetrahydroquinoline catalyst with a simple propylthio side arm has a 6–7.5-fold rate advantage over the pyridoxamine with the same side arm.

The basic catalytic group in the tetrahydroquinoline **36** was quite effective in the conversion of ketimine to aldimine. As Table II indicates, the rates for this isomerization with α-ketovaleric acid, indolepyruvic acid, and pyruvic acid were over 100 times as fast as those for the simple pyridoxamine derivative **18**. Relative to the tetrahydroquinoline catalyst **40** without a catalytic side arm, the catalyst **36** was 20–30 times as effective. We expected that this kinetic acceleration by the side arm would be reflected in asymmetric induction in the product amino acids.

With α-ketovaleric acid, compound **36** underwent transamination to afford a 95:5 ratio of D- to L-norvaline. As Table II shows, a similar preference was seen in the formation of tryptophan from indolepyruvic acid, but a somewhat lower preference was seen for the formation of D-alanine. The table also shows that the optical yield of these reactions is the same, within experimental error, if they are stopped at different times, so no racemization of the products is occurring competitive with their asymmetric synthesis.

Compound **40**, with a propylthio group in the same stereochemical position as is the basic side arm of catalyst **36**, produces a 1.5:1 excess of L-norvaline, in contrast to the large preference for D amino acids with the dimethylamino side arm in place. This finding certainly indicates that our stereochemical and mechanistic assignments are correct. The small preference for the L amino acid must indicate that pyridoxamine derivatives **36** and **40**, which have the same stereochemistry, have the *R* configuration at the asymmetric center. This would lead to a small amount of steric shielding of the *si* face of the intermediate, so protonation by solvent occurs from the *re* face with preferential formation of the L amino acid. Then the very large preferential protonation on the *si* face when the catalytic side arm is present in compound **36** can be explained only if it is actively putting the proton onto that face, as desired.

The formation of small amounts of the incorrect isomers has several possible explanations. Catalyst **36** may not be completely resolved as the *R* isomer; any mistakes here would show up in the products. Alternatively, since the rate accelerations (Table II) by the catalytic side arm are only of the order of 20–30-fold compared with the situation without that side arm, there may be a few percent of reaction performed by catalytic species in solution, with steric preference for the formation of the L amino acid. Finally, flexibility in the transamination intermediate may be a problem, either permitting the side arm to reach the other face of the intermediate or permitting rotation of the keto acid portion if metal complexing is not completely effective in holding the geometry. Further work will be needed to clarify these questions and to solve them so that even higher stereochemical preferences are observed.

Conclusions

This work demonstrates that nonenzymatic transamination reactions by pyridoxamine can be made more selective for substrate by attaching appropriate binding groups. Then it can be made faster by attaching catalytic groups to promote proton transfers and stereoselective if these catalytic groups are held in a defined geometry. Obviously these lines can be combined, by constructing pyridoxamine derivatives carrying both a binding group and an asymmetrically mounted catalytic group. Two approaches to such molecules have been pursued and will be the subject of forthcoming publications.[43,44]

Experimental Section

5-Pyridoxaminyl-6′-β-cyclodextrinyl Sulfide (3) and 5-Pyridoxaminyl-6′-α-cyclodextrinyl Sulfide. As described,[21] pyridoxamine dihydrochloride was converted to **2-methyl-3-hydroxy-4-(aminomethyl)-5-(bromomethyl)pyridine dihydrobromide (1)** in 87% yield by heating under reflux with 48% aqueous HBr for 45 min. To 8.2 g of this bromide in 150 mL of methanol a solution of 5.2 g of KOH and 6.6 mL of CH_3COSH in 100 mL of methanol was added, and the solution was heated under reflux for 1 h. Solvent was evaporated, 15 mL of acetic anhydride was added, and the mixture was allowed to stand for 15 min. Then 200 mL of CH_2Cl_2 was added, KBr was removed by filtration, and the product was crystallized by addition of 600 mL of ether and cooling to −20 °C. A second crystallization from CH_2Cl_2/ether afforded ***O*,*S*,*N*-triacetylthiopyridoxamine (2)** in 5.3-g (85%) yield, mp 168–169 °C. Anal. Calcd for $C_{14}H_{18}N_2O_4S$ (found): C, 54.12 (53.95); H, 5.80 (5.78); N, 9.02 (8.88); S, 10.31 (10.58). ^1H NMR ($CDCl_3$) δ 2.0 (s, 3 H), 2.35–2.45 (3 s, 9 H), 4.15 (s, 2 H), 4.40 (d, J = 7 Hz, 2 H), 6.0 (NH), 8.35 (s, 1 H). m/e = 310.

The acetyl groups were removed by 5-h heating of **2** under reflux in 48% aqueous HBr, and the unstable (air oxidation) crystals of **5-thiopyridoxamine dihydrobromide** were collected in quantitative yield and used directly. ^1H NMR (D_2O) δ 2.9 (s, 3 H), 4.1 (s, 2 H), 4.6 (s, 2 H), 8.3 ppm (s, 1 H).

To 350 mg of the above thiol dihydrobromide in 60 mL of H_2O under N_2, 600 mg of solid NH_4HCO_3 was added carefully. Then 1.3 g of cycloheptaamylose 6-tosylate was added and the solution was held at 60 °C for 16 h. Concentration of the colorless solution to 15 mL and chromatography on Sephadex CM 25 (with 25 g of NH_4HCO_3 in 4L H_2O as eluent) afforded 500 mg (ca. 50% yield) of pure **5-pyridoxaminyl-6′-β-cyclodextrinyl sulfide (3)**, isolated by lypohilization and vacuum removal of NH_4HCO_3. Anal. Calcd for $C_{50}H_{80}N_2O_{35}S{\cdot}6H_2O$ (found): C, 42.61 (42.42); H, 6.58 (6.69); N, 1.99 (1.70); S, 2.27 (1.81).

In a similar fashion, reaction of cyclohexaamylose 6-(2-naphthalenesulfonate) with the thiol dihydrobromide afforded **5-pyridoxaminyl-6′-α-cyclodextrinyl sulfide** in ca. 15% yield. Anal. Calcd for $C_{44}H_{70}N_2O_{30}S{\cdot}6H_2O$ (found): C, 42.38 (42.87); H, 6.62 (6.77); N, 2.25 (2.16); S, 2.57 (2.58).

Thioether 6, with Pyridoxamine-5-thiol Attached to C-3 of β-Cyclodextrin. As we have described,[23] β-cyclodextrin 2-tosylate (**4**) was prepared by reacting β-cyclodextrin with *m*-nitrophenyl tosylate in DMF. A solution of 360 mg of **4** and 700 mg of NH_4HCO_3 in 5 mL of H_2O was heated at 60 °C for 3.5 h. This formed the epoxide **5**, which could be isolated by chromatography on Sephadex G-15 resin. The epoxide showed a one-proton doublet at 3.3 ppm (J = 3.7 Hz) for C-2 and an unsplit one-proton signal at 5.1 ppm for C-1 of the glucose epoxide residue, in addition to normal cyclodextrin signals. For the pyridoxamine coupling, the reaction mixture after 3.5 h of heating was used directly.

5-Thiopyridoxamine dihydrobromide (200 mg) was added, and the solution was stirred under N_2 at 60 °C in the dark for 19 h. After solvent evaporation the product was chromatographed on Sephadex CM-25, eluting with a 0–0.125 M NH_4HCO_3 in H_2O gradient. The desired product **6** was isolated in 12% yield as a colorless solid. Anal. Calcd for $C_{50}H_{80}N_2O_{35}S{\cdot}11.5H_2O$ (found): C, 39.81 (40.06); H, 6.88 (6.70); N, 1.85 (1.70); S, 2.13 (1.93). The ^1H NMR (D_2O/dilute H_2SO_4) showed the expected pyridoxamine and cyclodextrin peaks, and a one-proton signal at 3.05 ppm that must be the cyclodextrin CH–S peak. It was a doublet of doublets, J = 11.2 and 3.4 Hz. Decoupling occurred by irradiation at 3.95 (J = 3.4 Hz) and 3.8 ppm (J = 11.2 Hz).

Synthesis of 10. To 6.46 g of *N*-tosyl-*N*′-acetylbis(4-aminophenyl)-methane[45] and 6.78 g of K_2CO_3 in 150 mL of DMF was added 2.07 g of 1,5-dibromopentane, and the solution was stirred at 130 °C for 20 h. Evaporation of the solvent and aqueous $CHCl_3$ workup afforded 7.8 g of crude **7**. This was deacetylated in 60 mL of EtOH with 60 mL of 20% HCl in H_2O at 80 °C for 5 h, and the resulting diamine was ditosylated with tosyl chloride in pyridine, producing a tetrakis(tosylamide) derivative. 3-(*tert*-Butyldimethylsiloxy)-1,5-dibromopentane was prepared by silylation of diethyl 3-hydroxyglutamate, then reduction ($LiBH_4$), ditosylation, and displacement with LiBr. Now this dibromide (3.60 g) in 350 mL of benzene and the tetrakis(tosylamide) derivative (7.73 g) in 300 mL of benzene and 50 mL of 1,2-dichloroethane were added simultaneously to a stirred boiling mixture of 1 L of benzene and 500 mL of aqueous 5% NaOH solution with 1.8 g of $Bu_4N^+I^-$ in a high dilution apparatus[46] over 8 h. After 12 h of additional heating and stirring, workup afforded a 26% yield of the macrocycle (structure **9**, but with Ts instead of CH_3 groups and a *tert*-butyldimethylsilyl on the OH), purified by flash chromatography.

The tosyl groups and the silyl group were removed by treating the macrocycle with excess sodium metal in 1-butanol.[47] Compound **9** was then prepared by treating the above product (**9**, lacking the CH_3 groups) with CH_2O and $NaBH_3CN$.[34] Then **9** was treated with thioacetic acid,

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triphenylphosphine, and diethyl azodicarboxylate.[48] The resulting crude thioacetate, ^{1}H NMR 2.25 ppm (CH_3COS–), was alkylated with CH_3I in DMF overnight in the dark, and the product (**10**, but with acetyl instead of pyridoxamine on sulfur) was crystallized from methanol. Then 50 mg of this thioacetate tetracation and 10 mg of $NaBH_4$ were dissolved in 0.4 mL of Me_2SO and 0.4 mL of degassed aqueous 0.2 N NaOH under Ar and stirred at room temp for 6 h. To the product, 60 mg of **1** was added and the solution was stirred overnight under Ar. After adjustment to pH 7, chromatography on Sephadex CM-25 with 0–1 M NH_4HCO_3 gradient elution afforded **10** as a hydrate of the pentabicarbonate salt of the pentacation (including CH_2–NH_3^+). Anal. Calcd for $C_{57}H_{80}N_6SO_{16}\cdot 7H_2O$ (found): C, 54.18 (54.46); H, 7.50 (7.42); N, 6.65 (6.87).

Rates of Aminations by 3, 6, and 10. Preliminary competition studies[13] were done by mixing 1 equiv each (0.05–5 mM) of pyridoxamine or of pyridoxamine–cyclodextrin **3** with pyruvic acid, phenylpyruvic acid, or indolepyruvic acid in 4.0 M phosphate buffer, pH 8.0. After 10 min an excess of 2,4-dinitrofluorobenzene in ethanol–water was added and the mixture was heated at 60 °C for 90 min. The mixture was cooled, acidified, and extracted with ether, and the concentrated extract was examined by HPLC on Partisil PXS 10 with 40% v/v CH_3CN–H_2O containing 2.5 mL of HOAc and 0.4 g of NaOAc/L. The dinitrophenyl derivatives of alanine, phenylalanine, and tryptophan were quantitatively determined by calibration with authentic samples. It was found that with pyridoxamine the three keto acids had similar reactivity, producing ca. 1:1 ratios of the corresponding product amino acids. With the cyclodextrin–pyridoxamine **3**, phenylpyruvic acid was ca. 50 times as reactive as pyruvic acid, while indolepyruvic acid was ca. 32 times as reactive as pyruvic acid.

In another preliminary assay,[13] separate reactions of pyruvic acid or of phenylpyruvic acid with pyridoxamine or with **3** were performed as above but with varying times before dinitrophenylation. After 1 h, 0.2–0.5% of alanine was formed from pyruvic acid with either pyridoxamine or **3**. After 10 min, the phenylalanine formed from phenylpyruvic acid and **3** was equal (ca. 1–5%, depending on pH and initial concentration) to that formed after ca. 30 h with pyridoxamine.

The isomers **3** and **6** were evaluated by a rough kinetic study.[14] Solutions were made up 0.5 mM in phenylpyruvic acid and in pyridoxamine **3** or **6** and 3.2 M in pH 9.1 phosphate buffer in H_2O at room temperature. At time intervals of 10, 30, 60, 120, 180, and 825 min, samples were taken, diluted with H_2O, and treated with excess dansyl chloride in ethanol overnight. The dansyl amino acids were analyzed by chromatography on a C-18 column with fluorescence detection. A similar set of experiments was performed by using indolepyruvic acid. Reasonable linear kinetic plots were seen, but after 8 h the product concentrations *decreased*. From these data the reaction of phenylpyruvic or indolepyruvic acid with **6** was ca. 20 times as fast as with pyridoxamine, while with **3** they were ca. 40 times as fast. Errors are estimated as at least 25%.

A careful kinetic study was later performed with **3** and **10**. Rates were studied at 0.5 mM concentrations of phenylpyruvic acid or of 2-ketovaleric acid and 0.5 mM of pyridoxamine **3** or **10** in 2.7 M phosphate buffer, pH 9.3, at 26 °C. Aliquots (100 μL) were taken every 3 min for 15 min, diluted with 200 μL of H_2O, and treated with 100 μL of 7.4 mM dansyl chloride in acetone. HPLC analysis was performed on a C-18 radial compression column, with fluorescence detection. The macrocyclic derivative **10** was 31 ± 3 times as reactive as was pyridoxamine in the phenylalanine synthesis and 6 ± 1 times as reactive in the amination of 2-ketovaleric acid. The cyclodextrin derivative **3** was 15 ± 2 times as reactive as pyridoxamine with 2-ketovaleric acid.

Stereoselective Transaminations with 3 and 6. The reactions of pyridoxamine, of **3**, and of **6** with phenylpyruvic acid were performed as in the rough kinetic study described above, and dansylation was carried out after 2 h. Chromatography with Cu^{2+} and L-proline, as described elsewhere,[30] was used to determine the ratios of D- and L-phenylalanine. With pyridoxamine we observed the expected 1:1 ratio, with **3** we observed a (5.5 ± 0.2)/1 preference for the formation of the L enantiomer, while with **6** the ratio was not distinguishable from 1:1. With indolepyruvic acid, examined in the same way, **3** showed a 2:1 preference for the formation of L-tryptophan, while **6** showed a 1.8:1 preference for the formation of D-tryptophan.

Preparation of the Side Chain Pyridoxamine Derivatives 11-20. The dimethylamine derivative **11** was prepared by treating 1.0 g of **1** with an excess (20 mL) of 40% dimethylamine in H_2O. After evaporation the residue was evaporated again with ethanol and 1 M HBr and then crystallized from ethanol. The product trihydrobromide of **11**, mp 243–244 °C dec, was obtained as a cream-colored powder (900 mg, 79% yield). CI, *m/e* 196 (MH^+ – 3HBr). Anal. Calcd for $C_{10}H_{20}Br_3N_3O$ (found): C, 27.42 (27.43); H, 4.61 (4.62); Br, 54.73 (54.68); N, 9.59 (9.56).

The thioethers **12–20** were prepared by reaction of the appropriate thiol in methanolic NaOMe with **1** added dropwise in methanol. After evaporation of solvent, the residue was taken up in 2-propanol, adjusted to ca. pH 1 with 48% HBr, filtered, and allowed to crystallize. The products generally contained some NaBr, but were used directly for the transamination studies after adding enough extra NaBr to produce constant ionic strength: **12**·3HBr, CI, *m/e* 256 (MH^+ – 3HBr); **13**·3HBr, CI, *m/e* 270 (MH^+ – 3HBr); **14**·3HBr, expected ^{1}H NMR; **15**·3HBr, CI, *m/e* 251 (MH^+ – 3HBr); **16**·3HBr, CI, *m/e* 265 (MH^+ – 3HBr); **17**, ^{1}H NMR as expected; **18**·2HBr, CI, *m/e* 227 (MH^+ – 2HBr); **19**·3HBr[49] purified by Sephadex CM 25 chromatography, CI, *m/e* 286 (MH^+ – 3HBr); **20**·2HBr, correct by ^{1}H NMR. All compounds prepared had the expected ^{1}H NMR spectra.

Transaminations by 11–20. A kinetic system related to that of Martell[10] was used. Methanol solutions 0.16 mM in pyridoxamine derivative, 0.16 mM in $Zn(OAc)_2$, and 1.6 mM in pyruvic acid or sodium pyruvate were brought to the appropriate "pH" with 10 mM NaOH in methanol at 30.1 ± 0.1 °C. The "pH" was read with a glass electrode calibrated against aqueous buffer and is not corrected for the solvent change. For calibration, **13**·3HBr was titrated in methanol with methanolic NaOH by using this electrode and showed "pK_a's" of 3.1, 7.2, 8.0, and ≥10. Titration of **18**·2HBr gave pK_a's of 2.9, 7.2, and ≥10. These are very similar to the known values in H_2O (e.g., pyridoxamine, pK_a = 3.37, 8.01, 10.13).

The kinetics were monitored on a Beckman DU-8 spectrophotometer by repetitive scans from 460 to 220 nm. At "pH" 5.00 the pyridoxamine peak at 291 nm disappeared in less than 5 min, with simultaneous appearance of a new peak at 324 nm, the zinc complex of the ketimine. In the slow step this species was quantitatively converted to the zinc complex of the aldimine, with a UV maximum at 383 nm. Clear isosbestic points were observed at 288 and 342 nm in all kinetic runs. At pH 4.00 the ketimine with λ_{max} 324 nm was in rapid equilibrium with a second species, with λ_{max} 290 nm, apparently the phenolic O-protonated ketimine. Rates of conversion of ketimine to aldimine were followed at 383 nm. Good first-order plots were linear from 10% to 98% completion, with repeated runs in agreement within 10%.

The observed first-order rate constants for ketimine to aldimine conversion of compounds **11–20** at "pH" 4.00 are listed in Table I. This was the "pH" of highest catalytic rate advantage for **13** vs. **18**. The rates increased up to "pH" 8, before decreasing again, but this rate increase (apparently involving MeO^- catalysis) was greater for **18**.

Reaction of **19** and of **20** with 2-ketovaleric acid as above, then dansylation, and chiral HPLC as in the studies with **6** showed that **19** produced a 39% enantiomeric excess of D-norvaline after 2 h (65% conversion), while *20* produced a 1:1 D,L mixture.

In a similar fashion, kinetic studies were done with chloropyruvate to produce **21**. These rate constants are also listed in Table I.

Synthesis of the Tetrahydroquinolines 39 and 40. Ethyl glutaryl chloride (500 g) in 600 mL of CH_2Cl_2 with ethyl glycinate hydrochloride (391 g) was stirred while adding 781 mL of triethylamine over 2 h. After a further 2-h gentle reflux the triethylamine hydrochloride was filtered off, and normal aqueous workup afforded **ethyl *N*-(ethoxyglutaryl)-glycinate**, as a crude orange liquid (531 g, 83% yield). This could be used directly in the next step; a small sample was purified by distillation (135 °C, 0.17 mm) as a colorless liquid: CI, *m/e* 228 (MH^+); ^{1}H NMR and IR as expected.

To 100 g of this amide in 500 mL of $CHCl_3$ was added 180 g of P_2O_5 in one portion with vigorous stirring. After 6 h of heating at reflux, workup with ice/K_2CO_3 and normal workup afforded 105 g of yellow liquid. Vacuum distillation (95–100 °C, 0.12 mm) afforded **5-ethoxy-2-(3-(ethoxycarbonyl)propyl)oxazole (26)** as a colorless clear liquid (53.8 g, 54% yield). IR 2950, 1730, 1690, 1620 cm^{-1}; ^{1}H NMR δ 1.26 (t, 3 H), 1.41 (t, 3 H), 2.05 (m, 2 H), 2.40 (t, 2 H), 2.70 (t, 2 H), 4.10 (m, 4 H), 5.96 (s, 1 H).

The above oxazole (11.8 g) was chilled to 0 °C and 5.6 g of purified maleic anhydride was added with stirring. The ice bath was allowed to warm to room temperature over 3.5 h, then 125 mL of ethanol saturated with gaseous HCl was added and the solution was heated under reflux for 12 h. Workup to isolate acidic and neutral (pH >12) material afforded 9.12 g of crude **2-(3-(ethoxycarbonyl)propyl)-5-hydroxy-3,4-dicarboxypyridine** mixed monoethyl esters **27**: CI *m/e* 326 (MH^+).

This product (9.12 g) was directly heated with 13.5 g of *O*-benzyl-*N*,*N*-diisopropylurea[50] at 110 °C for 2 h, affording 8.3 g of **2-(3-eth-**

(48) Mitsunobu, O. *Synthesis* **1981**, 1.

(49) The thiol was prepared by $LiAlH_4$ reduction of *S*-benzyl-*N*,*N*-dimethylcysteine, then debenzylation.

(50) Schmidtchen, F. P.; Rapoport, H. *J. Am. Chem. Soc.* **1977**, *99*, 7014–7019.

oxycarbonyl)propyl)-3-(ethoxycarbonyl)-4-((benzyloxy)carbonyl)-5-(benzyloxy)pyridine (28) after flash chromatography as a mixture of 3,4 positional isomers: CI, *m/e* 506 (MH^+). This mixed ester (10.2 g) was heated for 50 min at reflux in benzene with NaOEt/EtOH to afford 7.9 g of the crude β-keto ester (**29**, but with a CO_2Et group α to the ketone): CI, *m/e* 398 (MH^+). This was heated at reflux in 2.4 N aqueous HCl with 10% ethanol for 2 h to afford a 41% yield of the ketone **3-(benzyloxy)-4-(ethoxycarbonyl)-5-oxo-5,6,7,8-tetrahydroquinoline (29)** as a waxy solid after flash chromatography: CI, *m/e* 326 (MH^+); ^1H NMR δ 1.38 (t, 3 H), 2.16 (m, 2 H), 2.68 (t, H), 3.09 (t, 2 H), 4.49 (q, 2 H), 5.23 (s, 2 H), 7.73 (m, 5 H), 8.44 (s, 1 H); IR 2950, 1735, 1695 cm^{-1}.

The ketone **29** in ethanol was reduced with 4 equiv of $NaBH_4$ in 5% aqueous NaOH for 10 min at 0 °C and then quenched with aqueous NH_4Cl. The product **3-(benzyloxy)-4-(ethoxycarbonyl)-5,6,7,8-tetrahydro-5-hydroxyquinoline (30)** was obtained as a waxy solid in 99% yield: CI, *m/e* 328 (MH^+). This was optically resolved to afford the *S* enantiomer **31** by reaction with carbonyl diimidazole to produce **32** and then reaction with (*S*)-(−)-(2-phenylethyl)amine (Aldrich) to produce the diastereomeric carbamates **33** which were separated by MPLC on a size B Lobar Li Chroprep Si60 column with EtOAc/petroleum. The fractions were monitored by HPLC, and all fractions with more than 90% of the first eluting peak were pooled and rechromatographed. Base-line separation was obtained, and the first peak (**34**) was typically obtained with less than 0.5% contamination, as judged by HPLC. This first peak was cleaved back to the resolved alcohol **31** by treatment with 1.7 equiv of $HSiCl_3$[38] and 1.2 equiv of Et_3N in benzene heated under reflux for 30 min.

Resolved alcohol **31** was converted to the mesylate ester, which was immediately allowed to react with 3-(dimethylamino)propanethiol and NaH in THF. Silica preparative plate chromatography afforded the pure thioether **35** in 82% yield as a tan oil, with correct IR and ^1H NMR. This was reduced with $LiAlH_4$ and the product **37** was isolated by preparative silica plate chromatography in 58% yield; it had the expected IR and ^1H NMR spectra. This was oxidized with CrO_3–pyridine complex in CH_2Cl_2, affording aldehyde **38** in 81% yield (IR 2950, 1725, 1710 cm^{-1}; ^1H NMR includes a 1 H singlet at δ 10.59). With hydroxylamine this gave the oxime in 87% yield, which was reduced in acetic acid with an excess of zinc dust for 30 min at room temperature. The product amine **39** was isolated by Sephadex CM25 chromatography (NH_4HCO_3 gradient) in 26% yield and converted to **5-((3-(dimethylamino)propyl)-thio)-4-(aminomethyl)-3-hydroxyl-5,6,7,8-tetrahydroquinoline (36)** by 30-min heating under reflux in 6 N aqueous HCl. The product, isolated by Sephadex CM25 chromatography, was obtained in 82% yield as an off-white air-sensitive solid. Field-desorption (FD) MS, *m/e* 296 (MH^+); ^1H NMR δ 1.87 (m, 4 H), 2.1–2.9 (m, 8 H), 2.29 (s, 6 H), 4.11 (s, 2 H), 4.26 (br, 1 H), 7.84 (s, 1 H).

By a similar sequence **31** was converted to **40**, FD MS, *m/e* 252 (MH^+); ^1H NMR δ 0.97 (t, 3 H), 1.2–2.9 (m, 10 H), 3.97 (center of dd, *J* = 12 Hz, 2 H), 4.26 (brs, 1 H), 7.82 (s, 1 H).

Transaminations by 36 and 40. Reactions with pyruvic acid, with 2-ketovaleric acid, and with indolepyruvic acid were performed in methanol at pH 4.00 and 30.0 °C with solutions 0.16 mM in the pyridoxamine derivative and in zinc acetate and 1.6 mM in keto acid. The kinetic studies were done as described above for compounds **11–20**; the stereochemical product analyses were done as described above for compound **6**. The data are listed in Table II.

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Acidity-Oxidation-Potential (AOP) Values as Estimates of Relative Bond Dissociation Energies and Radical Stabilities in Dimethyl Sulfoxide Solution

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Abstract: Oxidation potentials (E_{ox}) for the fluorenide ion and 21 substituted fluorenide ions have been measured in Me_2SO solution. The E_{ox} values for 2-substituted fluorenide ions were found to plot linearly with the corresponding pK_{HA} values in Me_2SO for the conjugate acids of these anions. Points for 3-methoxy-, 3-methyl-, 3-(methylthio)-, 3-(phenylthio)-, 1,2-benzo- and 2,3-benzofluorenide ions deviated from this plot. A combination of pK_{HA} and E_{ox} values gave acidity-oxidation-potential (AOP) values, which, when related to the AOP for fluorene itself, indicate that these remote substituents stabilize the fluorenyl radical by 0.4–1.5 kcal/mol. The 3-F and 3-$PhSO_2$ substituents do not stabilize the radical appreciably. A similar analysis for $GC_6H_4CHCN^-$ anions revealed radical stabilization by 4-Me_2N and 4-Ph, as well as by 4-MeO, 4-Me, and 4-PhS substituents, but not by 4-F or 4-CF_3. A similar analysis for 9-substituted fluorenes revealed much larger Δ(AOP) values, indicating radical-stabilizing effects for Me_2N, MeO, Me, MeS, and Ph donor substituents and also for the acceptor substituents, H_2NCO, MeOCO, and CN. These effects for substituents attached to a carbon atom bearing a relatively high spin density were in the range of about 2–11 kcal/mol. The effects on anion and radical stabilizations deduced from ΔpK_a and Δ(AOP) values, respectively, are compared.

Electrochemical redox potentials have been correlated with a number of experimentally derived properties including pK_a values of organic acids, Hammett σ constants, NMR chemical shifts, gas-phase ionization potentials, and kinetic data. Breyer demonstrated as early as 1938 that acidity constants of para-substituted benzenearsonic acid, *p*-$GC_6H_4AsO_3H$, could be correlated with their reduction potentials.[1] Later Zuman, in his book on polarography, collected numerous examples where E_{ox} values had been correlated with Hammett-type σ constants.[2] Federlin and his colleagues have published pK_a values in Me_2SO solution for over 100 carbon acids, mostly of the type RCOCHGG′, where G is a second acidifying function such as CN, CO_2R, $CONH_2$, COR, SO_2R, or Ar and G′ is also of this type or is hydrogen. The pK_a values for several of these families were correlated with the oxidation potentials of the corresponding anions.[3] More recently, Bank and his students have reported a correlation between pK_a values in cyclohexylamine for a series of substituted diphenyl- and triphenylmethanes vs. the oxidation potentials of their lithium salts in DME solution.[4] Also, Breslow and his students have related

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