adopts a conformation that makes the ester inaccessible to hydroxylamine.

We next plan to investigate whether a second active site nucleophile will make the postulated attack at pH 7.5 in the absence of a nucleophilic buffer. We have found that crystals grown at pH 5.0 (acetate buffer) can be converted to pH 7.5 (HEPES buffer) and then the inhibitor diffused into the crystals.

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Supplementary Material Available: X-ray data for native and complex crystals of PPE including tables of bond lengths and angles, nonbonded contacts, atomic coordinates, and temperature factors (12 pages). Ordering information is given on any current masthead page.

Mimics of Tryptophan Synthetase and of Biochemical Dehydroalanine Formation

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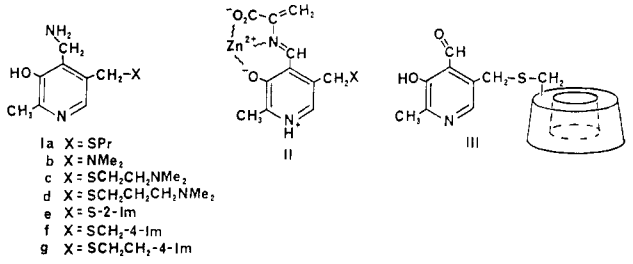
We have described compounds consisting of pyridoxamine coupled to the primary side of β-cyclodextrin,[2] the secondary side of β-cyclodextrin,[3] or a synthetic macrocyclic binding group.[4] These converted keto acids into amino acids with selectivity for substrates, such as indolepyruvic acid, that bound into the macrocyclic cavities while adopting the correct geometry for transamination. The enzyme tryptophan synthetase uses pyridoxal to dehydrate serine to a pyridoxal–dehydroalanine Schiff base (cf. structure II) while binding indole so as to promote carbon–carbon



bond formation.[5] To mimic this enzyme, we have prepared the pyridoxal–β-cyclodextrin conjugate III. Molecular models suggested that III should be able to imitate tryptophan synthetase, binding indole into the cyclodextrin cavity and holding it near a dehydroalanine group on the pyridoxal unit of III (see Scheme I). The product would be the doubly bound tryptophan that was involved in our earlier[5] transamination study.

Although III is formed in transaminations by the pyridoxamine–cyclodextrin conjugate we have described previously,[2] we

Scheme I

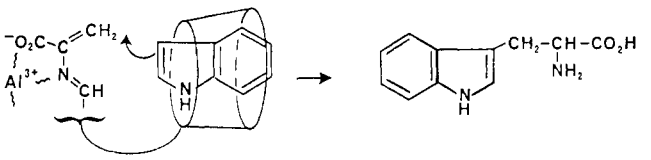


Table I. Rates of Dehydroalanine–Pyridoxal Imine Formation from Chloropyruvic Acid and of Ketimine to Aldimine Conversion with Pyruvic Acid by the Pyridoxamine Derivatives Ia–g

reagent	X	k_{elim}[a]	k_{rel}	k_{trans}[b]	k_{rel}
Ia	SPr	0.77	1.0	0.009	1.0
Ib	NMe_2	3.3	4.3	0.13	14
Ic	$S(CH_2)_2NMe_2$	9.2	12	0.23	26
Id	$S(CH_2)_3NMe_2$	2.6	3.4	0.33	37
Ie	SIm	6.4	8.3	0.05	5.6
If	SCH_2Im	5.3	6.9	0.11	12
Ig	$S(CH_2)_2Im$	2.8	3.6	0.68	76

[a] Pseudo-first-order rate constant for development of the chromophore at 414 nm, $\times 10^3$ s^{-1}, at 30.0 °C in methanol at pH 4.0. [b] Pseudo-first-order rate constant for conversion of the ketimine to the aldimine in transamination, $\times 10^3$ s^{-1}, at 30.0 °C in methanol at pH 4.0—data from ref 2.

preferred to prepare it in analytical purity[6] by conversion of pyridoxal to the dithioacetal with ethanedithiol, conversion of the CH_2OH group to CH_2SH (by Mitsunobu thioacetylation, then hydrolysis), displacement by the thiol on β-cyclodextrin-6-tosylate, and deprotection with $AgNO_3$. Pure III, from Sephadex SP-C50-120 cation exchange chromatography, showed the expected ^1H NMR and IR spectra. The UV spectrum at pH 10 had λ_{max} 398 nm; in H_2O, pyridoxal has λ_{max} 391 nm at this pH, shifting to 400 nm in 50% H_2O/THF solution. This spectroscopic indication that the pyridoxal group is partially bound in the cyclodextrin cavity of III is also supported by the strong CD of III at the pyridoxal chromophore. We have previously observed a related situtation in a thiamine–cyclodextrin conjugate.[7]

Metzler and Snell have reported[8] a ca. 1% yield of tryptophan from serine and indole with pyridoxal; we observe somewhat higher yields when *d,l*-β-chloroalanine is substituted for serine. We find that III indeed produces 3–5 times more tryptophan (still only a few percent) when it (at 21 mM) is incubated with 10 mM indole, 51 mM β-chloroalanine, and 2.5 mM $Al_2(SO_4)_3$ for 30 min at 100 °C and pH 5.2, compared with the same reaction in which III is replaced by pyridoxal. As expected, this kinetic advantage disappears at higher (40–80 mM) concentrations of indole, because of saturation of the binding group. Furthermore, the tryptophan produced with 10 mM indole has ca. 10% excess of the L enantiomer; under milder conditions conversion of indolepyruvic acid to tryptophan by the pyridoxamine derivative related to III had produced a 33% excess of the L isomer.[3] In these different syntheses the new asymmetric center is introduced by an identical step, so the difference in optical yield must reflect the more vigorous conditions in our present study.

The magnitude of the cooperative effect was undoubtedly diminished by alternative modes of binding of indole to III and by the internal binding detected in III itself. However, that such a cooperative effect is observed at all is good evidence for the dual binding of reagents to III, as a mimic of enzymes such as tryptophan synthetase that promote reaction of two bound substrates.

We have also described[9,10] mimics of transaminase enzymes in which basic groups attached to pyridoxamine by flexible chains acted to transfer protons from the CH_2 group of pyridoxamine

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to the α-carbon of the newly forming amino acid. Part of our evidence was the higher rate (Table I) of transamination by systems such as Id in which the chain was long enough to permit proton delivery compared with shorter chains (e.g., Ib, Ic).[9] Of course, this mechanism was also the basis for our successful stereoselective transamination process.[10]

As a further check on these conclusions, we have now examined the effect of chain length in compounds Ia–g on the reaction with chloropyruvic acid. As in the previous study,[9] reaction in methanol at pH 4.0 and addition of zinc acetate led to rapid ketimine formation (λ_{max} 328 nm), and we followed the rate of the subsequent process. In this case HCl elimination formed the dehydroalanine derivative II, with λ_{max} 414 nm,[11] which then reacted more slowly (presumably by adding solvent) to form an aldimine with λ_{max} 380 nm.[12] Dehydroalanine–pyridoxal structures related to II are intermediates in a number of biochemical processes, including the condensation of serine with indole by tryptophan synthetase discussed above.

In the dimethylamino series (Ib–d) the HCl elimination rates (Table I) were fastest[13] with the shorter chain of Ic, as expected for a process that requires only proton removal from the pyridoxamine 4′-CH_2 group. The contrast with the data for transamination (Table I), in which Id with the longer chain was the fastest, supports our previous contention[9,10] that in transamination the catalytic group also performs the protonation at the amino acid α-carbon. In the imidazole series (Ie–g) the shortest chain system Ie was also fastest in HCl elimination. The striking contrast to the data for transamination fully supports the proposition that in our transamination studies the catalysis is sequential, with proton transfer by the catalytic group to the remote position of the intermediate.

(11) Cf.: Matsushima, Y.; Karube, Y.; Kono, A. *Chem. Pharm Bull.* **1979**, *27*, 703–709 for a related compound with λ_{max} 412 nm.
(12) This elimination apparently resembles Elcb, not E2, since with bromopyruvate the reaction is slightly slower.
(13) The six-atom transition state with Ib is too small, for stereoelectronic reasons; cf.: Hine, *J. Acc. Chem. Res.* **1978**, *11*, 1–7.

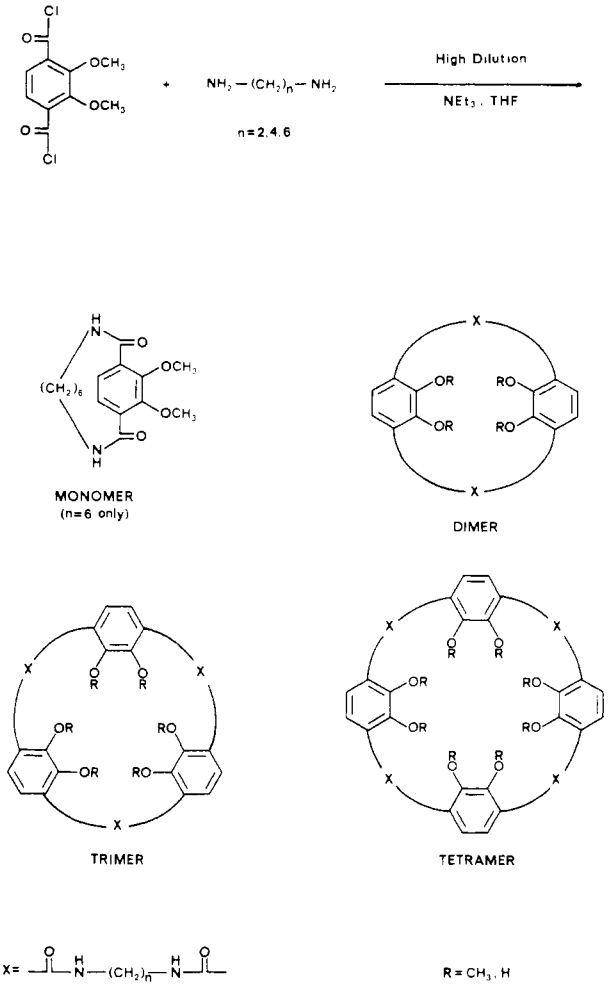

Figure 1. High-dilution synthesis of polycatecholate macrocycles.

High-Dilution Synthesis of Macrocyclic Polycatecholates

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There exists a clear need for new iron chelating agents in medicine,[1] particularly for the treatment of the iron overload syndromes. The siderophores[2] are a class of microbially produced iron chelators that are powerful sequestering agents for Fe(III) and other trivalent (or tetravalent) metal ions of similar charge to ionic radius ratios. As such, the structures of siderophores serve as important models for the biomimetic design of new chelating agents. Synthetic polycatecholate ligands modeled on the siderophores enterobactin and agrobactin have been found to form strong Fe(III) complexes,[3] to remove iron rapidly from the mammalian iron transport protein, transferrin,[4] and to be effective Pu(IV) removal agents in vivo.[5]

Among the structures of the hydroxamate siderophores are examples of acyclic, endocyclic, and exocyclic ligands.[2] The beneficial entropic aspects of incorporating the chelating moieties within a macrocyclic ring can be seen in endocyclic hydroxamate siderophores (such as ferrioxamine E), which possess the highest Fe(III) formation constants[6] of all the hydroxamate siderophores. A general theoretical discussion of the advantages of such complexes has been described, along with a number of proposed ligand structures, on the basis of these considerations.[7] All catecholate siderophores isolated to date are either acyclic or exocyclic. The recent introduction[8,9] of the symmetric 2,3-dihydroxyterephthalate moiety as a synthon for Fe(III) chelators makes possible the synthesis of a host of endocyclic polycatecholate chelators that have yet to be explored[10] and that potentially could have even greater iron-binding properties than previously known ligands. We report here the synthesis of a new class of endocyclic polycatecholate ligands.

Under high-dilution conditions[11] a series of alkane diamines (n = 2, 4, 6) react with 2,3-dimethoxyterephthaloyl chloride[12] in

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