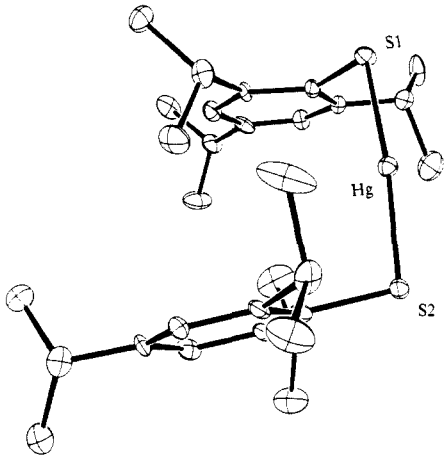

Figure 3. ORTEP diagram of $[Hg(S\text{-}2,4,6\text{-}i\text{-}Pr_3C_6H_2)_2]$ (3). Selected bond distances (angstroms) and angles (degrees): Hg1-S1, 2.322 (6); Hg1-S2, 2.322 (5); S2-Hg1-S1, 174.2 (2); Hg1-S1-C11, 97.8 (6); Hg1-S2-C21, 101.1 (7).

The reaction of 2 equiv of $LiS\text{-}2,4,6\text{-}i\text{-}Pr_3C_6H_2$ with $HgCl_2$ gives the two-coordinate complex $[Hg(S\text{-}2,4,6\text{-}i\text{-}Pr_3C_6H_2)_2]$ (3) (Figure 3).[21] The Hg-S bond distance (2.322 (6) Å) is similar to those of other $[Hg(SR)_2]$ complexes.[22] Linear $[M(SR)_2]$ complexes of Cd and Zn remain a synthetic objective.

The average M-S distances in **1** and **2** are nearly equal (Table I). The close similarity of Hg-S and Cd-S distances is also found in $[M(SR)_4]^{2-}$ compounds.[23] Our studies suggest that cadmium should be an excellent spectroscopic probe for monomeric Hg-cysteine centers particularly in cases where there is a protein-imposed coordination geometry. EXAFS studies have indicated a Hg-S distance of 2.42 Å for the MerR protein.[3] This distance is in close agreement with the trigonal-planar $[Hg(SR)_3]^{1-}$. Very recent biochemical studies have provided strong evidence for $[Hg(S\text{-}cys)_3]$ coordination.[24] If this is indeed the case, a Cd derivative of the MerR protein might be expected to also have $[M(S\text{-}cys)_3]$ coordination.[25]

Continued study of the similarity and differences in the coordination chemistry of the group 2B metals with thiolate ligands should provide insight in the metal-binding specificity of group 2B metal-cysteine proteins.

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Registry No. **1**·Ph_4P, 124511-86-0; **1**·Ph_4P·DMF·*i*-PrOH, 124511-87-1; **2**-*n*-Pr_4N·CH_3OH, 124511-90-6; **3**, 124511-91-7.

Supplementary Material Available: Tables of crystallographic parameters, atomic coordinates, thermal parameters, and bond distances and angles for **1-3** (30 pages); tables of observed and calculated structure factors for **1-3** (69 pages). Ordering information is given on any current masthead page.

(21) $[Hg(S\text{-}2,4,6\text{-}i\text{-}Pr_3C_6H_2)_2]$ crystallizes in the monoclinic space group $C2/c$ with a = 37.56 (1) Å, b = 8.645 (4) Å, c = 21.05 (1) Å, β = 113.87 (5)°, V = 6251 Å^3, Z = 8. Final least-squares refinement using 2356 unique reflections with $I > 3\sigma(I)$ gave $R(R_w)$ = 0.066 (0.081).

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(25) **Note Added in Proof:** The ability of the MerR protein to bind Cd^{2+} has been demonstrated: (a) Ralston, D. M.; Frantz, B.; Shin, M.; Wright, J. G.; O'Halloran, T. V. *UCLA Symp. Mol. Cell. Biol., New Ser.* **1989**, *98*, 407. (b) Helmann, J. D.; Walsh, C. T., private communication.

Synthesis of [1-^{15}N]Purine Ribonucleoside by a Novel Rearrangement

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We report the facile conversion of the 1-methylpurinium ribonucleoside cation (I, Scheme I) in aqueous ammonia to purine ribonucleoside (II, Scheme I). This reaction, which appears to involve addition of ammonia at C-6, followed by a rearrangement with elimination of methylamine, offers a method for specific incorporation of ^{15}N into heterocyclic compounds.

In basic solution, cation I was earlier found to undergo reversible addition of oxygen and sulfur nucleophiles at C-6, with a change of λ_{max} from 269 nm to much longer wavelengths, 284 nm for oxygen addition and 302 nm for sulfur addition.[1] In ammonia buffers, we observed very different behavior. When cation I (0.2 M, prepared as described earlier[1]) was dissolved in aqueous $^{15}NH_4Cl$-KOH (1 M) at pH 9.2, its UV absorption spectrum underwent complete conversion in a few minutes at room temperature from that of I (λ_{max} = 269 nm) to that of II (λ_{max} = 263 nm). The elution behavior of the product, upon C-18 reverse-phase HPLC, was identical with that of an authentic sample of II, and proton-coupled ^{15}N NMR spectroscopy of the reaction solution at pH 7 revealed a doublet of doublets 248 ppm downfield from [^{15}N]ammonium ion, with a principal ^{15}N-H coupling constant of 14.3 Hz. This chemical shift is approximately 4 ppm downfield from chemical shifts reported for N-1 of purine and 7-methylpurine, each of which also shows a doublet of doublets ($J_{^{15}N\text{-}H}$ = 14.3 and 14.6 Hz, respectively).[2] Thus, I rearranges to II with incorporation of ammonia from the solvent. The other reaction product was identified as *N*-methylamine by GC-MS analysis of the waxy yellow crystals obtained by derivatizing the reaction mixture with 2,4-dinitrofluorobenzene, revealing *N*-methyl-2,4-dinitroaniline.

In ammonia buffers of varying pH, the velocity of this reaction was found to reach a sharp maximum near pH 10, with a half-time of approximately 4 min at 25 °C in aqueous NH_4Cl (5 M in total ammonia), with 100% conversion to purine ribonucleoside. In separate experiments, the rate of reaction was found to vary in direct proportion to the concentration of I ($2.5\text{-}12.5 \times 10^{-3}$ M) and of NH_4Cl (2-8 M). At pH values near 10, attack by NH_3 at C-6 appears to determine the overall rate of reaction, but at more basic pH values, instantaneous equilibrium formation of the pseudobase (accompanied by slower irreversible ring opening[1]) competes with this process. Rearrangement was found to proceed at a similar rate in dry DMSO and in water, at equivalent concentrations of ammonia, so that mechanisms involving hydrated intermediates can be excluded. The reaction probably proceeds instead by a mechanism involving addition of ammonia, followed by ring opening, ring closing, and elimination of methylamine as shown in Scheme I. In the only previous synthesis that appears to have been reported, 1-aminoadenosine after long exposure to methanolic [^{15}N]ammonia at elevated temperature yielded a mixture of products that included purine ribonucleoside in which ^{15}N was partially incorporated at the 1-position.[3]

The scope of the present reaction may be general, since rearrangement was also found to proceed smoothly to completion with the 3-methylquinazolinium ion under similar conditions. The product of the present reaction, purine ribonucleoside, is of interest as the parent compound from which major constituents of nucleic acids are derived, and because it exists in equilibrium with an extremely rare 1,6-hydrated species.[4] The active site of adenosine

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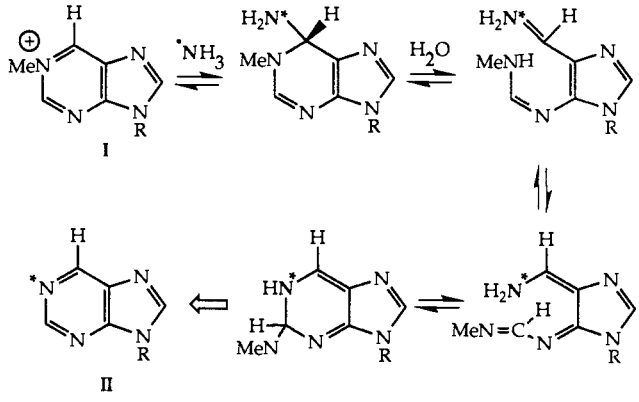
Scheme I



deaminase binds this covalent hydrate, believed to resemble an unstable intermediate in substrate hydrolysis, with an extremely high affinity approaching that expected of an ideal transition state analogue inhibitor,[1] and to show remarkable powers of discrimination between this and closely related compounds.[5] ^{15}N-labeled purine ribonucleoside may prove useful in investigating the structural details of this and other enzyme–ligand interactions.

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Phosphoenolpyruvate as a Natural Bisubstrate Analogue Inhibitor of Pig Kidney Prolidase

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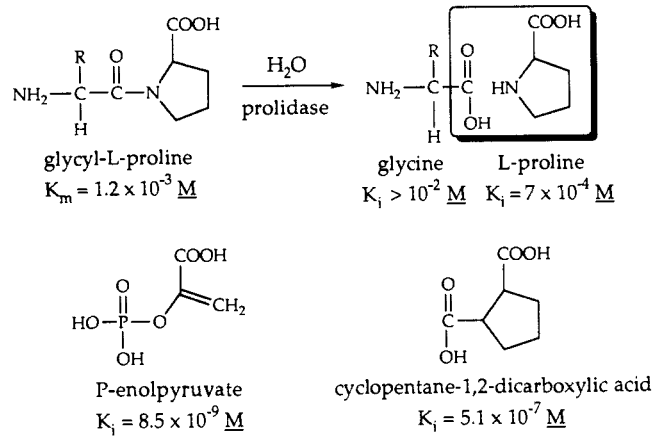
Among the few enzymes that are known to catalyze hydrolysis of peptide bonds involving the nitrogen atom of proline are prolidase (EC 3.4.13.9), a manganese-dependent dipeptidase that cleaves substrates of the type X-Pro,[1] and several virally encoded endoproteases that are responsible for processing the gene products *gag* and *pol* in HIV-1 and other retroviruses.[2] Prolidase is present in microorganisms and most mammalian tissues, where it permits terminal degradation of exogenous or endogenous polypeptides, and humans deficient in this enzyme suffer from skin lesions and limb abnormalities.[3] It has been suggested that prolidase may be involved in the intracellular metabolism of procollagen[4] and that, since the enzyme is cytosolic, a potent in vivo inhibitor should be capable of penetrating cell membranes.[5]

With a view to developing strong competitive inhibitors of pig kidney prolidase, we sought to combine structural features of the

Scheme I



two substrates for reversal of dipeptide hydrolysis within a single molecule, expecting that the resulting multisubstrate analogue inhibitor might exhibit a substantially higher binding affinity than the combined affinities of the two substrates.[6] At their moment of inception, the combined products of hydrolysis of a typical dipeptide substrate, Gly-Pro, seem likely to be arranged as shown in Scheme I. A similar arrangement would then be present at the active site when these compounds acted as substrates for the reverse, peptide bond forming, reaction. Compounds such as 1,2-cyclopentanedicarboxylic acid, bearing some structural resemblance to this arrangement (Scheme I), might then serve as effective inhibitors of prolidase in vitro.

For convenience in examining potential inhibitors,[7] we developed a continuous spectrophotometric assay, following the disappearance of the substrate Gly-Pro at 222 nm ($\Delta\epsilon_m = -904$) in K^+-MES buffer (0.01 M pH 6.0) at 20 °C. Double reciprocal plots of initial reaction velocities against substrate concentration, determined in the presence and absence of inhibitors, were linear, intersecting at the ordinate as expected for competitive inhibition. *trans*-1,2-Cyclopentanedicarboxylic acid exhibited a K_i value (5.1×10^{-7} M) much lower than the K_m value observed for the substrate Gly-Pro (1.7×10^{-3} M) and considerably lower than K_i values reported for the strongest inhibitors described previously, Cbz-Pro (9×10^{-5} M) and pyrrolidine-2-phosphonic acid (9.5×10^{-4} M).[5,8,9]

When other potential inhibitors were examined, we were surprised to find that the activity of 1,2-cyclopentanedicarboxylic acid was surpassed by that of the well-known glycolytic intermediate P-enolpyruvate, a competitive inhibitor with a K_i value of 8.5×10^{-9} M. Scheme I shows that P-enolpyruvate exhibits several structural features expected of a multisubstrate analogue, if its phosphoryl group is considered to replace one of the carboxyl groups of the two substrates for peptide-bond formation,[10] and its vinyl group projects into a hydrophobic cavity that might normally be occupied by the pyrrolidine ring of proline.

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(9) Captopril has been reported to give slightly stronger inhibition ($K_i = 3 \times 10^{-5}$ M) of rat kidney and liver prolidases (Ganapathy, V.; Pashley, S. J.; Roesel, R. A.; Pashley, D. H.; Leibach, F. H. *Biochem. Pharmacol.* **1985**, *34*, 1287), but this was not observed for the pig kidney enzyme by King et al.[5] We have confirmed the findings of the latter investigators.
(10) The phosphoryl group of P-enolpyruvate could be superimposed on either of the original carboxyl groups of 1,2-cyclopentanedicarboxylic acid, which are related by symmetry. This phosphoryl group might interact with a Mn^{2+} ion at the active site, even more effectively than would a carboxyl group. In a possibly similar case, replacement of the carboxyl group of L-leucine by a phosphonyl group has been found to improve its binding affinity for pig kidney microsomal leucine aminopeptidase by a factor of 55 [compare results reported by Andersson et al. (Andersson, L.; Isley, T. C.; Wolfenden, R. *Biochemistry* **1982**, *21*, 4711) with those reported by Lejczak et al. (Lejczak, B.; Kafarski, P.; Zygmunt, J. *Biochemistry* **1989**, *28*, 3549)].

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