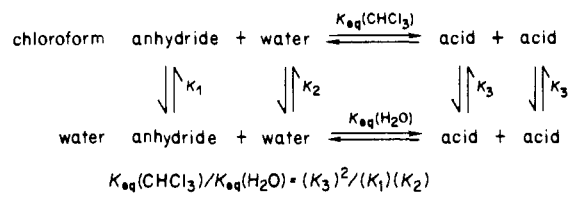
Scheme I



$$K_{eq}(CHCl_3)/K_{eq}(H_2O) = (K_3)^2/(K_1)(K_2)$$

Table I. Estimated Free Energies of Hydrolysis of Acetic Anhydride, Pyrophosphoric Acid, and ATP in Wet Chloroform

	Ac_2O	PP	ATP
ΔG(app) for reaction in dilute aqueous solution at pH 7[a]	−21.8[e]	−8.0[f]	−7.6[g]
ΔG(app) for reaction of nonionized compounds[b]	−15.7	−8.9	−6.6[g]
ΔG(app) incorporating actual concentration of water[c]	−13.3	−6.5	−4.4
ΔG for reaction in wet chloroform at 25 °C[d]	−11.6	−1.8	+0.3

[a] Free energies (in kcal) based on a standard state of 1 M total stoichiometric concentrations of reactants and products (except hydrogen ion) in neutral aqueous solution, with water activity taken as unity (convention III, ref 3). [b] Free energies based on a standard state of 1 M uncharged reactants and products and activity of pure water taken as unity (convention I, ref 3). K_a values of reactants and products were obtained from ref 3 for Ac_2O, from ref 16 for PP, and from ref 17 for ATP. [c] Obtained by dividing apparent equilibria of hydrolysis of nonionized compounds (convention I) by 55.5 M. [d] Obtained by correcting for the difference in distribution coefficients between reactants and products (see text). [e] 25 °C, ref 10. [f] 25 °C, ref 16. [g] 30 °C, ref 17, for cleavage to ADP and inorganic phosphate.

At 20 °C and ionic strength 0.3, distribution coefficients favored the chloroform phase by a factor of 19 for acetic anhydride and 40 for tetraethyl pyrophosphate. Self-association of these anhydrides had not been expected to occur to a significant extent in either chloroform or water.[8] These distribution coefficients showed no systematic or significant variation (less than 20%) as the total amount of anhydride present was caused to vary over a 50-fold range, from 0.02 to 1.00 M introduced into the chloroform phase at the outset. Variation would have been expected if either of these anhydrides tended to self-associate in either of the two phases.

Distribution coefficients between water and wet $CHCl_3$ have been measured previously for water ($K_2 = 5.6 \times 10^{-4}$), acetic acid ($K_3 = 0.025$),[9] and diethyl phosphate ($K_3 = 2.7 \times 10^{-3}$).[8] By comparing distribution coefficients of reactants and products (Scheme I), one is led to infer that the equilibrium constant for hydrolysis of acetic anhydride is less favorable in wet chloroform than in water by a factor of 17, i.e., 3.7×10^8, as compared with a value of 6.3×10^9 measured in water.[10] This effect is more pronounced in the case of tetraethyl pyrophosphate, so that the equilibrium constant for its hydrolysis is 3070-fold less favorable in wet chloroform than in water. If these factors are applied to equilibrium constants that have been determined for aqueous reactions, it becomes evident (Table I) that hydrolysis of acetic anhydride to unionized products remains highly exergonic in wet chloroform, whereas equilibrium constants for hydrolysis of pyrophosphoric acid derivatives approach unity.

Other work indicates that myosin binds ATP in such a way that its free energy of hydrolysis becomes much less negative than it was in free solution.[11] The present results support suggestions by George et al.[5] and by Hayes et al.[4] that this could be achieved by abstracting reacting portions of the substrates from solution into relatively waterless surroundings. The considerable energetic cost of stripping water from ATP could presumably be paid in part by binding of the relatively hydrophobic[12] adenosine moiety, and there is evidence in adenylate kinase for a dominant role of distant binding sites for both the phosphoryl donor and the acceptor in stabilizing intermediates in the transfer of phosphoryl groups.[13] Actin binding appears to promote dissociation of ATP from myosin, perhaps by inducing a conformation change.[11] This effect could presumably arise if the binding sites of ATP were transformed, in the course of actin binding, from a relatively buried to a relatively exposed configuration. The present results are also of interest in relation to the suggestion that evolutionary pressure may have tended to favor enzyme mechanisms in which equilibria between reactants and products approach unity on the catalytic surface more closely than they do in free solution, a tendency that has been observed in numerous kinases.[14] This objective might tend to be accomplished if binding sites for ATP and phosphoryl group acceptors had evolved in such a way as to remove the reacting portions of substrates and products, at least in part, from water.

It seems fair to conclude that the large negative free energies of reactions of phosphoric anhydrides, like those of intermediates in protein biosynthesis,[15] can be understood to a large extent in terms of the differing strengths of solvation of reactants and products, leaving relatively little to be explained in terms of intrinsic chemical properties of reactants and products as they would be observed in the absence of solvent water.

Acknowledgment. We are grateful to the National Science Foundation (Grant No. PCM-7823016) for support of this work.

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Asymmetric Oxygenation of Chiral Imide Enolates. A General Approach to the Synthesis of Enantiomerically Pure α-Hydroxy Carboxylic Acid Synthons

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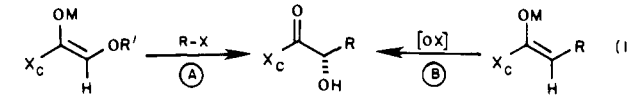
Homochiral α-hydroxy acids, and simple derivatives thereof, have proven to be a versatile class of molecules which have been extensively exploited in asymmetric synthesis.[1] For example, malic, mandelic, and tartaric acids are routinely employed as chiral synthons as well as precursers to both chiral ligands and auxiliaries.[2,3] By inspection it is evident that the generation of these target structures might be accomplished via the application of chiral enolate technology (eq 1). Recently, two independent



(1)

reports have described the development of camphor-based chiral glycolate enolate synthons and their respective diastereoselective alkylation studies (eq 1A).[4,5] In addition, Tamm and co-workers have demonstrated that MoOPH oxygenates camphor-derived ester enolates with moderate levels of diastereoselection (eq 1B).[6]

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Scheme I

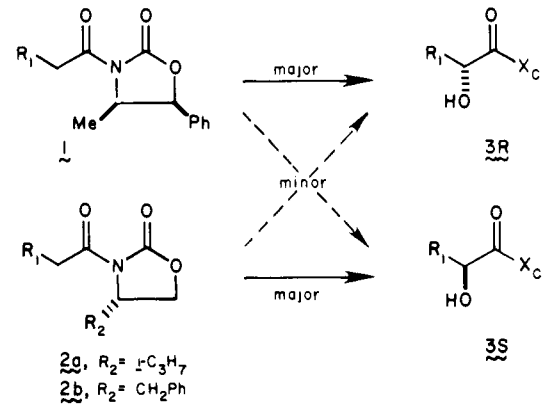


Table I. Diastereoselective Hydroxylation of Chiral Carboximide Enolates (Scheme I)[13,14]

entry	imide	ratio[a] 3R:3S	yield, %[b]
A	**1** (R_1 = CH_2Ph)	94:6	86 (**3R**)
B	**2a** (R_1 = CH_2Ph)	5:95	85 (**3S**)
C	**2b** (R_1 = CH_2Ph)	5:95	83 (**3S**)
D	**1** (R_1 = Ph)	90:10	77 (**3R**)
E	**1** (R_1 = Et)	94:6	86 (**3R**)
F	**1** (R_1 = $CH_2CH{=}CH_2$)	95:5	91 (**3R**)
G	**1** (R_1 = t-C_4H_9)	99:1	94 (**3R**)
H	**2a** (R_1 = i-C_3H_7)	1:99	86 (**3S**)

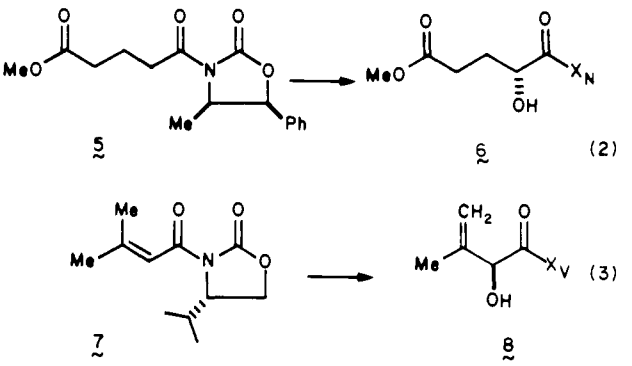
[a] Determined by capillary GLC analysis after acylation (Ac_2O–DMAP). Analyses of entries E, G, and H were carried out in a similar fashion on the (+)-MPTA esters of **3R** and **3S**.[19] [b] Isolated yields of diastereomerically pure (greater than 99%) material as analyzed by capillary GLC.

As a complement to these studies, we wish to report that the diastereoselective hydroxylation of chiral imide enolates with oxaziridine oxidants shows exceptional promise as a convenient approach to the asymmetric synthesis of α-hydroxy acid synthons (eq 1B).[7]

As an extension of our earlier studies associated with the development of chiral enolates derived from oxazolidinone carboximides,[8] we have found that the illustrated α-hydroxylation reactions of these systems (Scheme I) proceed with exceptional facility with the oxidant 2-(phenylsulfonyl)-3-phenyloxaziridine (**4**).[9,10] In direct analogy with prior reports,[8b] the illustrated carboximides **1**, **2a**, and **2b** were transformed into their respective *Z* sodium enolates (1.2 equiv of $NaN(Me_3Si)_2$, THF, −78 °C) and treated with a slight excess of oxaziridine **4** in THF (1.5 equiv. 1.0 M in THF, −78 °C).[11] After an immediate reaction, the solution was quenched with ca. 5 equiv of the soluble proton source camphor sulfonic acid (0.5 M in THF)[12] to give the α-hydroxy carboximides **3R** and **3S** illustrated in Scheme I.[13]

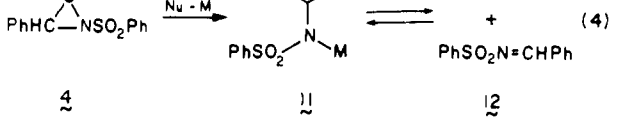
From the data summarized in Table I it is evident that the sense of asymmetric induction in these enolate hydroxylation experiments parallels the observations made in our original alkylation study.[8b] The first three entries in Table I (A–C) illustrate that the enolates derived from all three carboximides **1**, **2a**, and **2b** (R = Ch_2Ph) exhibit comparable levels of reaction diastereoselection.[14] Accordingly it appears that the selection of a given chiral oxazolidone auxialiary need *not* be predicated upon the criterion of reaction stereoselectivity. During the course of this study we have also uncovered another substrate-dependent trend in reaction diastereoselection (entries E–H). From these cases it appears that *increased* steric requirements in the R_1 moiety vicinal to the prochiral enolate center appear to amplify the diastereofacial bias for a given chiral auxiliary.

In addition to the cases summarized in Table I we have also examined the less conventional substrates **5** and **7** illustrated below (eq 2, 3). Selective enolization of the half-ester imide of glutaric



acid **5**[15] with 1.0 equiv of $NaN(TMS)_2$ (−78 °C, 5 min) and subsequent hydroxylation with oxaziridine **4** afforded a 96:4 ratio of α-hydroxy imides from which the *R* isomer **6** was isolated after flash chromatography in 68% yield (eq 2).[14,15] We thus project that selective enolization proximal to the imidic carbonyl in the presence of esters is possible. The regioselective hydroxylation of dienolates also appears to be feasible (eq 3). Enolization and subsequent hydroxylation of **7** afforded a 96:4 ratio of C_2 diastereomers from which the *S* isomer **8** was isolated in 75% yield (**8S**:**8R** > 99:1) after flash chromatography.[14,16] In all of the hydroxylation studies carried out to date, the diastereomeric α-hydroxy imides (**3R** and **3S**) have been found to be readily separable by flash chromatographic technique.[16] It is also significant to note that, in all cases encountered, the major reaction diastereomer exhibits the higher elution rate on silica gel. Hence, it appears that one might begin to rely upon this stereoregular elution order.

During the course of this study we have made several observations that are relevant to the mechanism of oxygen atom transfer from oxiaziridine **4** to the metal enolate system. Although Davis has speculated that nucleophile oxygenation *could* be stepwise (eq 4),[17] no evidence for (or against) this postulate exists. We



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(11) Oxaziridine **4** was prepared by an improved procedure developed by Davis (Davis, F. A.; Stringer, O. D. *J. Org. Chem.* **1982**, *47*, 1774) with a slight modification in product isolation. In our hands, oxaziridine **4** could be obtained in high purity and good chemical yield (90%) by filtering the concentrated reaction mixture through flash silica gel to remove the phase-transfer catalyst. Complete experimental details are given in the supplementary material.

(12) This asymmetric hydroxylation procedure is extremely sensitive to the method by which the reaction is quenched. Approximately 20% of a product arising from intramolecular attack of the alkoxide oxygen at the oxazolidinone carbonyl can be isolated when "standard" quenching conditions (saturated aqueous ammonium chloride, −78 to 25 °C) are employed (see eq 5, **3R** to **9**). However, complete suppression of this acyl-transfer process can be achieved by the use of a *soluble* proton source such as camphor sulfonic acid in THF (−78 °C).

(13) Satisfactory spectral data and elemental analyses were obtained on all compounds reported herein.

(14) For each hydroxylation experiment, an authentic sample of the minor diastereomer was prepared by one of two possible routes: (a) the alkylation of (*p*-methoxybenzyl)oxyacetimide with the appropriate electrophile followed by oxidative removal of the *p*-methoxybenzyl group with DDQ; (b) the oxidation of the trimethylsilyl enol ether derived from imides **1** and **2** with CrO_2Cl_2 (Lee, T. V.; Toczek, J. *Tetrahedron Lett.* **1982**, *23*, 2917.

(15) Half-ester–imide **5** was prepared from the corresponding half-ester–acid chloride (Aldrich) under standard conditions; see: Evans, D. A.; Bartroli, J.; Shih, T. L. *J. Am. Chem. Soc.* **1981**, *103*, 2127.

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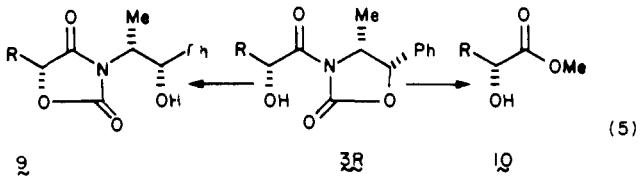
wish to propose that the counterion-dependent product distribution observed in these enolate oxidations could be associated with the intervention and collapse of the hemiaminal **11**. It is relevant that lithium, sodium, and potassium enolates all react rapidly with **4**. However, only the hydroxylation of lithium enolates is complicated by accompanying aldol addition with the product sulfonyl imine **12**.[18] For example, the lithium enolate derived from **1** (R_1 = CH_2Ph) afforded **3R** (54%) as well as 44% of the aldol adduct derived from **12**. In the analogous experiment with the derived sodium enolate, less than 2% of this aldol adduct was observed in competition with hydroxylation. A convincing demonstration that the sulfonyl imine **12** is *not* generated in stoichiometric quantities upon sodium enolate oxidation follows from the reaction of **4** with 2 equiv of the sodium and lithium enolates derived from **1** (R_1 = CH_2Ph) under standard conditions (-78 °C, 5 min). The presence of the second equivalent of enolate was shown in independent experiments to be an effective trap for sulfonyl imine **12**.[18] From these experiments the lithium enolate afforded a 1:1 ratio of hydroxylation and imide aldol adducts while the analogous ratio from the sodium enolate was 85:15. It has been concluded that the sulfonyl imine **12** is *not* stoichiometrically generated in the latter reaction. We speculate that the tetrahedral intermediate **11** (M = Li, Na) may well be involved in the reaction (eq 4) and that the subsequent **11** $\rightleftarrows$ **12** equilibrium is possibly counterion dependent ($K_{eq} > 1$, M = Li, $K_{eq} < 1$, M = Na). Additional experiments to further substantiate this point are being explored.

The final point of interest to the current study has been the development of a reliable procedure for the nondestructive removal of the chiral auxiliary by transesterification without concurrent racemization. Methanolysis of the reported α-hydroxy carboximides may be successfully carried out with 2 equiv of a 0.08 M magnesium methoxide solution in methanol (0 °C, 15 min). The enantiomeric purity of the resultant α-hydroxy methyl esters was determined by subsequent conversion to the (+)- and (-)-MPTA esters[19] which were subjected to capillary gas chromatographic analysis. In the cases reported, this methanolysis procedure affords the derived methyl esters in yields ranging from 80 to 90% with a conservative estimate of less than 0.3% racemization. No detectable level of racemization was seen even in the methanolysis of **3R** (R_1 = Ph) which should be particularly susceptable to enolization. The derived (*R*)-methylmandelate, $[\alpha]_D$ -181.9° (*c* 0.69, C_6H_6) was isolated in 86% yield.[20] One potential problem associated with carboximide methanolysis has been found to be the competing based-catalyzed intramolecular acyl transfer illustrated in the conversion of **3R** to **9** (eq 5).[12] Under the



(5)

conditions described above, this intramolecular acyl transfer is still the prevalent reaction in several of the hindered cases (e.g.: **3R**, R = *t*-C_4H_9; **3S**, R = *i*-C_3H_7).

In conclusion, we have found the asymmetric synthesis of enantiomerically pure α-hydroxy acid synthons via enolate oxidation to be quite general. There are two significant advantages of this protocol (eq 1B) over asymmetric glycolate alkylation (eq 1A). First, enolate oxidation rates with oxaziridine **4** appear to be essentially substrate invariant. Second, α-hydroxy acids inaccessible via the glycolate alkylation route (e.g., **3**, R_1 = Ar) are readily prepared by this method. Finally, during the course of this study we have had the opportunity to compare the relative merits of oxaziridine **4** and MoOPH in stereoselective enolate oxidation. In contrast to observations made by Davis,[9] we have found MoOPH to be slightly more stereoselective and far less reactive than oxaziridine **4**.[21] We estimate that enolate oxidation rates for these two reagents differ by more than 10^{+3}.[22] Overall, the superior yields associated with the oxaziridine reagent render it the reagent of choice.

Acknowledgment. This research has been supported by the National Science Foundation (CHE-8342576) and the National Institutes of Health (GM-33327-02). We also thank the NIH BRS shared instrumentation Grant Program 1 S10 RRoa748-01A1 for work facilities.

Supplementary Material Available: General experimental procedures for hydroxylation and diastereomer analysis as well as ^1H and ^{13}C NMR data for all new compounds prepared (15 pages). Ordering information is given on any current masthead page.

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(21) This control experimenta was performed by treating the sodium enolate of **2b** (R_1 = $PhCH_2$) with MoOPH (1.7 equiv, -78 °C, 1.0 h), followed by normal quench and product isolation. The **3R**:**3S** ratio was 2:98 and **3S** (R_1 = $PhCH_2$) was isolated in 37% yield along with 30% of **2b** (R_1 = $PhCH_2$).

(22) Since completing this manuscript the diastereoselective acetoxylation of camphor-based enolates has been reported: Oppolzer, W.; Dudfield, P. *Helv. Chim. Acta* **1985**, *68*, 216.

X-ray Absorption Spectroscopic Studies of the Copper(II) Sites in Bovine Plasma Amine Oxidase

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Primary amines are ubiquitous in nature and have numerous important functions. Oxidative deamination (in which the oxidation of an amine to an aldehyde plus ammonia is coupled to the reduction of O_2 to H_2O_2) is usually the first step in primary amine catabolism.[3] Copper-containing amine oxidases are often found to be the catalysts for this reaction and are also responsible for cross-linking the connective tissue proteins elastin and collagen.[3] These enzymes are composed of two noncovalently bound subunits, containing two Cu(II) ions, with a total M_r ~180000. The copper site structure and function in amine oxidases are still not very well understood, although the available evidence strongly indicates that copper is essential to catalysis and is probably involved in the reoxidation of the substrate-reduced enzyme by O_2.[3-7] It is known that the amine oxidase Cu(II) sites are tetragonal; EPR param-

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