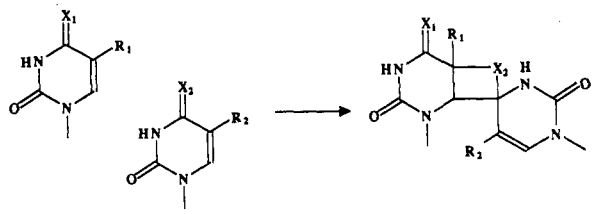

X_1 = O, NH X_2 = O, NH, S

R_1 = CH_3, H R_2 = CH_3, H

Figure 1. Presumed precursors of (6-4) bipyrimidines.

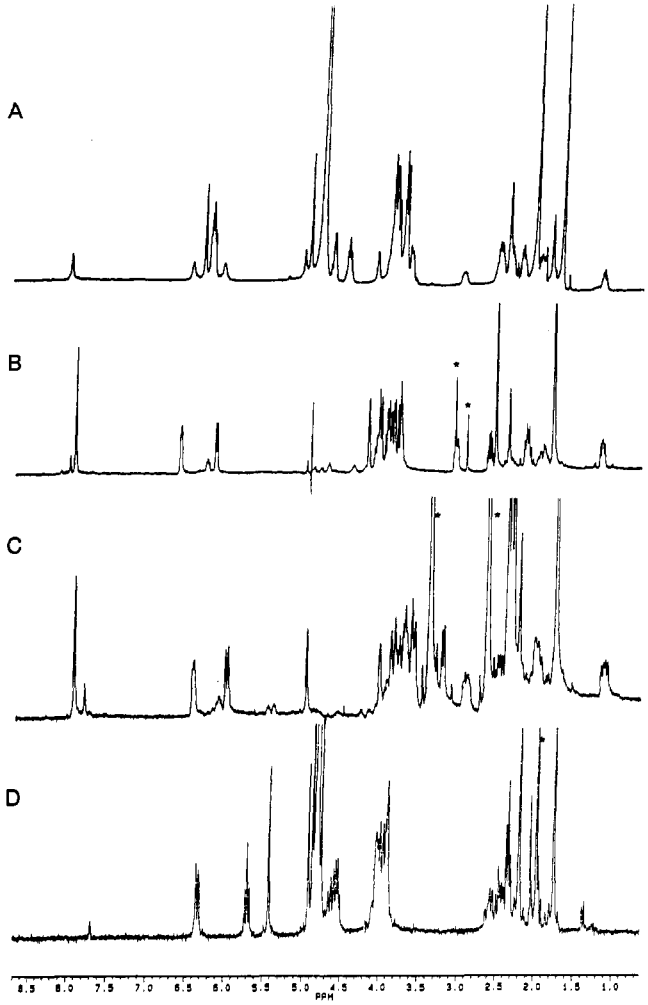

Figure 2. ^1H NMR spectra (D_2O) of photoproducts and derivatives: spectrum A, **2** + **3**; spectrum B, **3** (* denotes impurities); spectrum C, **8** (* denotes methyl methanethiosulfonate signals); spectrum D, **4** (* denotes ammonium acetate).

that the (2 + 2) cycloaddition proceeds with the two bases in an anti conformation, despite the proximity between the two bulky methyl substituents, and that the anti conformation of the pT part is retained during and after ring opening as it is also the case for the Dewar isomer **4**.

Our results demonstrate that **1** undergoes a very efficient photochemical conversion into a bipyrimidine of the (6-4) type. It is likely that this will prove useful for the study of the chemical behavior of related adducts in model systems and more particularly in double-stranded oligodeoxynucleotides. In the latter case we are currently interested in raising antibodies that could detect the (6-4) lesions of cellular DNA.[11] Finally, this work also supports the mechanism of (5-4) bipyrimidine formation in tRNAs containing 4-thiouridine.[12]

Supplementary Material Available: Experimental conditions, ^1H NMR spectra of **1** and **5**, and ^{13}C NMR chemical shifts of **1-7** (4 pages). Ordering information is given on any current masthead page.

(11) Mitchell, D. L.; Allison, J. P.; Nairn, S. N. *Radiat. Res.* **1990**, *123*, 299-303.

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Lewis Acid Induced Internal Proton Return: Enantiocontrolled Protonation of an Amide Enolate

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We report a new technique for enantiocontrolled amide enolate protonation in the enolate complex with a chiral secondary amine. We have found that BF_3·Et_2O and certain other Lewis acids induce the protonation of amine-containing enolates derived from acids, esters, and amides. Under these conditions, there can be no competition from enolate quenching by external proton sources, and proton transfer is more likely to occur within a specific, highly chirotopic environment. Our experiment is an example of the internal proton return process (IPR).[1-3] This phenomenon was encountered early in the LDA era, usually as an undesired side reaction in the attempted deuterium labeling of LDA-derived lithium enolates.[1,2] Evidence for IPR has also been detected in the course of certain alkylation experiments.[2c] We are not aware of prior studies designed to maximize and exploit this process, but pioneering work by Seebach et al. reveals a close relationship between IPR and aggregate structures in amine-containing lithium enolates.[3,4] The most relevant X-ray analogy (Figure 1) has a secondary amine ligand N-H proton within H-bonding distance of the amide enolate nitrogen. As noted by Seebach et al., a relatively small geometrical change is needed for internal transfer of this proton from nitrogen to carbon if ligand N-H acidity is increased by the interaction of the enolate-amine complex with an electrophile.

Our experiments were designed to maximize the IPR process by using electrophilic quenching agents that might attack amine ligand nitrogen in the amine-enolate complex in preference to enolate carbon or oxygen. Naproxen amides **1** were chosen for the initial optimization study because the α-substituents differ sufficiently in size to allow reasonable control for one major enolate isomer. Thus, treatment of racemic **1a** with *sec*-BuLi (2 equiv, 10 min in THF, -78 °C) afforded a yellow anion **2**, and quenching with TMSCl gave 14:1 *Z*:*E* mixture of enol silanes **3Z**:**3E**.[5] We

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(2) (a) Seebach, D.; Boes, M.; Naef, R.; Schweizer, W. B. *J. Am. Chem. Soc.* **1983**, *105*, 5390. (b) High internal return from a chiral amine has been demonstrated in the enolate of 2,2,6-trimethylcyclohexanone enolate, resulting in a solvent-dependent 15-46% ee: Eleveld, M. B.; Hogeveen, H. *Tetrahedron Lett.* **1986**, *27*, 631. (c) Aebi, J. D.; Seebach, D. *Helv. Chim. Acta* **1985**, *68*, 1507. El Achqar, A.; Roumstant, M. L.; Villefont, P. *Tetrahedron Lett.* **1988**, *20*, 2441. Polt, R.; Seebach, D. *J. Am. Chem. Soc.* **1989**, *111*, 2622.

(3) For an excellent review of the role of the coordinating amine in enolate chemistry and a number of examples of chiral diamine induced asymmetric transformations, see: Seebach, D. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 1624. Further examples of chiral diamine or triamine modified enolate reactions are discussed by Hansen: Hansen, J. Ph.D. Dissertation No. 7863, ETH, Zürich, 1985.

(4) Seebach, D.; Laube, V. T.; Dunitz, J. D. *Helv. Chim. Acta* **1985**, *68*, 1373.

(5) NMR (200 MHz, $CDCl_3$): major (**3Z**) δ 2.07 (3 H, s), 1.16 (12 H, d, J = 6.0 Hz), -0.26 (9 H, s); minor (**3E**) δ 2.04 (3 H, s), 0.96 (12 H, d, J = 6.0 Hz), 0.30 (9 H, s).

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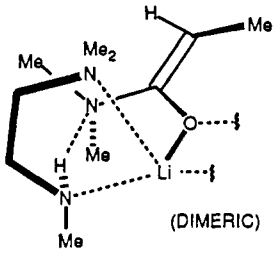

Figure 1.

assume that this ratio reflects the enolate *Z*:*E* ratio. When the solution of enolate **2** and excess *sec*-BuLi (1:1 ratio) was combined with triamine **4a**[6] (2 equiv, −78 °C), the yellow color deepened to wine red. Subsequent addition of BF_3·Et_2O (2 equiv, −78 °C) and warming to −23 °C resulted in the gradual disappearance of the color. The naproxen amide **1a** could be recovered in >90% yield after filtration chromatography over silica gel, with an enantiomeric excess of 82 ± 1%. Simple crystallization could then be used to upgrade the optical purity of this material.[7] The optimum result was obtained by quenching a solution that contained 1 equiv of excess lithium amide **4b** for each equivalent of enolate. A small excess of **4b** was less effective (a ratio of 0.2:1 of **4b**:**2** gave 34% ee), but there was no further benefit from increasing the ratio of **4b**:**2** to levels in the range of 2–4:1.

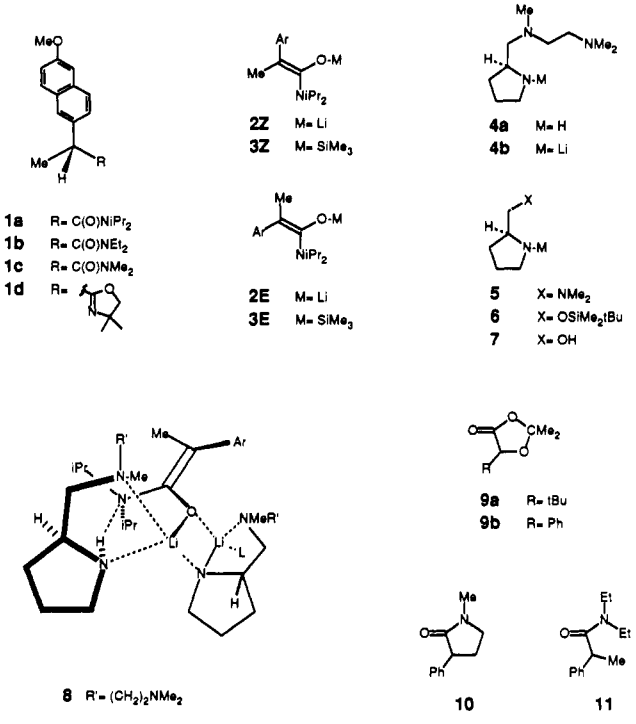

Table I summarizes observations made in the course of studies to optimize the Lewis acid. High levels of IPR are maintained by using a variety of electrophiles as judged by the yields of recovered **1**, but high enantiomeric excess is restricted to the boron fluoride Lewis acids. Aluminum or silicon electrophiles afforded

Table I. Comparison of Lewis Acids in the Conversion of **2** to **1a**

entry	Lewis acid	% ee (*R*/*S*) of **1a**	% yield of **1a**[a]
1	BF_3·Et_2O	82 (*R*)[b]	92–97
2	BF_3·SMe_2	56 (*R*)	89
3	$PhBF_2$	39 (*R*)	92
4	BCl_3	12 (*R*)	32
5	$PhBCl_2$	7 (*R*)	63
6	$B(OMe)_3$	0	54
7	Me_3SiCl	4 (*S*)	85
8	Et_2AlCl	15 (*S*)	86
9	Et_3Al	1 (*S*)	75

[a] Yield of isolated **1a** after filtration chromatography over silica gel. [b] Enantiomeric excess was confirmed by using the chiral NMR shift reagent tris[3-[(trifluoromethyl)hydroxymethylene]-(+)-camphorato]-europium(III), $Eu(tfc)_3$, in deuterioacetone, ±1% integral error; ee values were estimated by polarimetry for entries 2–9 and were confirmed to be within 3% of the Eu(tfc) values in several cases.

1a with a marginal preference for the "*S*" enantiomer. The highest selectivity for "*S*" **1a** (26% ee) resulted when the mixture of **2** + **4a** + **4b** (1:1:1) was quenched with excess triflic acid in THF at −109 °C. Other protic acids (NH_4Cl/H_2O, 1% ee, *S*; CF_3CO_2H, 6% ee, *S*) gave poor results. A control experiment with CF_3CO_2D afforded **1a** with 50% deuterium incorporation, suggesting that at least part of the reason for minimal enantiomeric excess in the protic acid quenching experiments is the competition between internal (N–H) and external (O–H) proton donors.[2b]

Most of the enolate enantioface discrimination can be achieved by using BF_3-induced IPR from the diamine **5** in place of **4a** (73% ee vs 82% ee, *R*). On the other hand, the amino ether **6** (32% ee, *R*), the amino alcohol **7** (5–9% ee, *S*), and several chiral monoamines were found to be relatively ineffective. Marginal ee values were also obtained when the optimized triamine conditions were modified by replacing THF with other solvents (ether, 48% ee, *R*; toluene, 33% ee, *R*; THF + DMPU, 2% ee, *R*). Various metal salt additives ($MgBr_2$, 8% ee, *R*; $Ti(OiPr)_4$, 36% ee, *R*; $ZnCl_2$, 6% ee, *S*) were not helpful. Lower empirical enantiomeric excesses were also noted with the diethylamide **1b** (50% ee, *R*), the dimethylamide **1c** (33% ee, *R*), and the ethyl ester of naproxen (<20% ee) under the best conditions developed for **1a**. On the other hand, the relatively bulky oxazolidine **1d** gave better results (50–60% ee). Changes in the order of mixing of the critical ingredients (enolate; amine; BF_3·Et_2O) invariably reduced ee values. For example, treatment of the enolate **2** with a premixed solution of BF_3·Et_2O and **4a** gave **1a** with only 25% ee (*R*). This experiment also resulted in nearly instantaneous discharge of anion color at −78 °C while the optimum procedure (BF_3·Et_2O added last) was characterized by slow fading of color over hours at −78 °C or minutes at −23 °C.

If 100% enolate face selectivity for IPR is assumed for each enolate isomer **2Z** and **2E**, then the 14:1 *Z*:*E* ratio could give a maximum empirical ee of 86%. Ironically, the empirical result could actually improve if the minor enolate isomer were to undergo protonation with no facial preference at all (93% ee is the theoretical limit, assuming 100% ee for the major isomer)! There is one previous report of >80% empirical ee in the protonation of a nonconstrained enolate by Galindo and Fehr.[8] Their result (84% ee; ketone substrate) is the best so far with an acyclic enolate and was also obtained by using an enolate mixture (9:1 ratio). As in our experiment, the intrinsic enolate facial selectivities are not yet known for the individual enolate isomers. Promising results (60–70% ee) have also appeared for the protonation of certain α-heteroatom-containing enolates or cyclic enolates,[9] including a preliminary report of 91% ee for the protonation of **9a** using a chiral tricyclic lactam as the proton source.[10] On the other

(6) (*S*)-*N*-(Benzyloxycarbonyl)proline was coupled with *N*,*N*,*N*′-trimethylethylenediamine by using bis(2-oxo-3-oxazolidinyl)phosphinic chloride according to Tung and Rich: Tung, R. D.; Rich, D. H.; *J. Am. Chem. Soc.* **1985**, *107*, 4342. The resulting amide was subjected to Paar hydrogenation (methanol/Pd–C) followed by $LiAlH_4$ reduction (THF) to obtain triamine **4a** (91% over three steps on 14-mmol scale). The full experimental procedure is included as supplementary material.

(7) Optical purity was determined by using the chiral shift reagent $Eu(tfc)_3$ in C_2D_6CO solution. Qualitative ee determination was also performed by polarimetry, $[\alpha]^{20}_D$ +107 (*c* = 2.7, ethanol) for purified (*S*)-**1a**. Optically enriched **1a** was upgraded to 97% ee (70% recovery) by selectively precipitating the less soluble racemate of **1a** from ether (two times), followed by recrystallization from hexane–toluene, mp 114–116 °C.

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(9) (a) Duhamel, L.; Plaquevent, J. C. *J. Am. Chem. Soc.* **1978**, *100*, 7415. Duhasmel, L.; Launay, J. C. *Tetrahedron Lett.* **1983**, *24*, 4209. Duhamel, L.; Fouquay, S.; Plaquevent, J. C. *Tetrahedron Lett.* **1986**, *27*, 4975. Duhamel, L.; Duhamel, P.; Fouquay, S.; Eddine, J. J.; Peschard, O.; Plaquevent, J. C.; Ravard, A.; Solliard, R.; Valnot, J. Y.; Vincens, H. *Tetrahedron* **1988**, *44*, 5495. (b) Hunig, S.; Gerlach, U. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 1283.

hand, the same technique affords 0% ee with **9b**. None of the methods currently known approaches the desired goal of a substrate-independent asymmetric protonation of enolates. Our IPR method is also not effective with **9b** (11% ee), but it does work with reasonable generality for α-aryl propionamide systems. Thus, both **10** (50% ee) and **11** (60% ee) gave comparable results to those obtained with naproxen amide **1b**. These experiments were carried out by using the procedure optimized for **1a**, without individually optimizing nitrogen substituents or reaction conditions.

The essential role of diamine or triamine ligands in our experiment can be interpreted as evidence for bidentate coordination of lithium, similar to that in Seebach's X-ray structure of an amide enolate (Figure 1).[3,4] Since the optimum stoichiometry for high ee involves a 1:1:1 ratio of enolate **2**:lithium amide **4b**:neutral amine **4a**, the mixed aggregate representation **8** can be used as a starting point for developing predictive models. This structure is based on the Seebach precedent[4] and on an X-ray structure for a mixed aggregate of an enolate with LDA reported by Williard et al.[11] If the BF_3-induced internal proton transfer occurs rapidly relative to structural changes within the mixed aggregate, then a geometry similar to **8** could explain the observed *R* selectivity for IPR. However, important details of the activation process and the identity of the actual proton donor remain to be evaluated. Experiments to address these issues and to explore other substrates are in progress.

Acknowledgment. This work was supported by the National Institutes of Health (GM44724).

Supplementary Material Available: Experimental details for the preparation of **4a** and the deracemization of **1a** (2 pages). Ordering information is given on any current masthead page.

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Mechanism of Adenylate Kinase. 10. Reversing Phosphorus Stereospecificity by Site-Directed Mutagenesis[1]

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The stereospecificity toward different diastereomers of nucleoside phosphorothioates is a well-established property for many enzymes[2] and has been useful in many research areas of biochemistry and molecular biology.[3] A typical example is shown in Figure 1A for adenylate kinase (AK): when AMP is substituted by AMPS, phosphorylation occurs at the *pro-R* oxygen specifically to give (S_p)-ADPαS;[4,5] the latter can be further converted to (S_p)-ATPαS since the S_p isomer is also preferred over the R_p isomer at the MgATP site.[6-8]

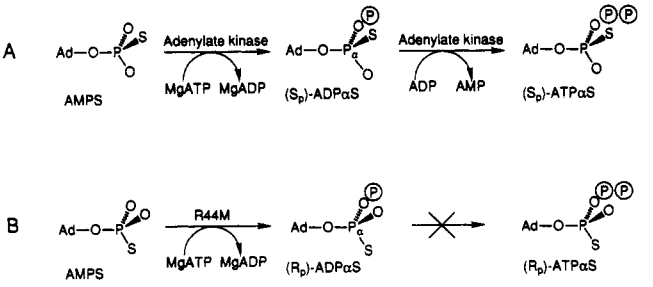

Figure 1. Schemes showing the stereospecificity of wild-type adenylate kinase (A) and R44M (B). As implied by the schemes, the orientation of the acceptor oxygen of AMPS should not change, and the reversal in stereospecificity is likely to be due to a rotation in the *bridging* O–P bond.

Such stereospecificity (not the steric course, which is a different issue) should arise from restricted orientations of the P–O and P–S bonds at the active site, so that the unnatural sulfur atom assumes the position "least painful" to the enzyme. The exact orientations depend on the combined effects of the interactions between the phosphorothioate group and the active-site residues and/or the metal ion. It has been established that changing the metal ion could perturb the stereospecificity.[9] We predict that removal of one of such interactions by site-directed mutagenesis can also change the overall interactions and potentially perturb the stereospecificity.

We tested our prediction using AK from chicken muscle overproduced in *Escherichia coli*.[10] Recently we found that substitution of Arg-44 by Met (to give the R44M AK) resulted in 36-fold and 22-fold increases in the Michaelis and the dissociation constants, respectively, of AMP but not MgATP, and only a 3-fold decrease in k_{cat}.[11] After detailed structural analysis to ensure that the conformation of the mutant AK had not been perturbed, we concluded that Arg-44 interacts specifically with AMP starting from the binary complex. Although the kinetic results do not reveal *how* Arg-44 interacts with AMP, the positively charged arginine is likely to interact with the negatively charged phosphate, as also suggested by the crystal structure of a yeast AK–inhibitor complex.[12]

Using ^{31}P NMR, we monitored the conversion of AMPS to ADPαS (at the AMP site) and its subsequent conversion to ATPαS (at the MgATP site). As shown in Figure 2A, the products from wild-type (WT) AK are mainly the S_p isomers of both ADPαS and ATPαS. Separate experiments using (R_p+S_p)-ADPαS confirmed that the S_p isomer is indeed preferred over the R_p isomer at the MgATP site. Thus chicken muscle AK behaves like other types of AK in the stereospecificity at the P_α of both AMP and MgATP.[4-8]

As shown in Figure 2B, *the stereospecificity at the AMP site is completely reversed in the reaction catalyzed by R44M AK* since the product is mainly (R_p)-ADPαS. The fact that no ATPαS formed in Figure 2B suggests that *the stereospecificity at the P_α of the MgATP site has not changed.* This was further confirmed by addition of (R_p+S_p)-ADPαS to the sample, which resulted in specific conversion of the S_p isomer to (S_p)-ATPαS (Figure 2, C and D). The stereospecificity of the reactions catalyzed by R44M is shown in Figure 1B. Although we have predicted a change in stereospecificity, it was surprising that the change was not to relax the stereospecificity, and fortuitous that it was not to enhance the existing stereospecificity.

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(1) Supported by Research Grant DMB-8904727 from the NSF. Paper 9 in the series: Yan, H.; Tsai, M.-D. *Biochemistry* **1991**, *30*, 5539-5546. Abbreviations: ADP, adenosine 5′-diphosphate; ADPαS, adenosine 5′-(1-thiodiphosphate); AK, adenylate kinase; AMP, adenosine, 5′-monophosphate; AMPS, adenosine 5′-monothiophosphate; ATP, adenosine 5′-triphosphate; ATPαS, adenosine 5′-(1-thiotriphosphate); WT, wild-type.
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