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Synthetic Catalysis of Amide Isomerization

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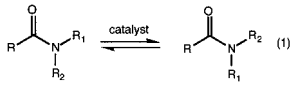
ABSTRACT

Rotation about the C−N bond in amides can be catalyzed by Brønsted and Lewis acids, as well as nucleophiles and bases. Catalysis of amide isomerization occurs in biological systems via "rotamase" enzymes; however, the mechanisms by which these proteins operate are not completely understood. We outline investigations that provide experimental support for mechanisms believed to be feasible for the catalysis of amide isomerization and present practical applications that have resulted from this work.

Introduction

The observation of slow cis-to-trans isomerization (rotation) about the C−N bond in amides (eq 1) and its implications for structure and reactivity have fascinated chemists for many years. As with many other "slow"



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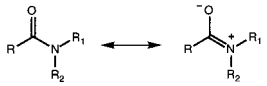
processes, chemists have sought ways to "speed it up", i.e., catalyze it by a variety of mechanisms, involving Brønsted and Lewis acids, as well as nucleophilic and basic catalysis. Nature has also devised ingenious ways to catalyze amide rotation by means of "rotamase" enzymes, otherwise known as the peptidyl prolyl isomerases (PPIases). Much attention has recently been paid to these novel enzymes due to their importance as biological receptors for the immunosuppressive drugs cyclosporin A and FK-506. Additionally, they may play other roles in vivo, including the catalysis of protein folding, functioning as auxiliary enzymes in HIV-1 protease-mediated reactions, modulation of calcium release, and in mitotic regulation. The mechanisms by which these enzymes, including the FK506 binding proteins (FKBPs), cyclophilins, and the newly discovered parvulin class, isomerize amides are not completely understood. In various guises, distortion, desolvation, Brønsted acid/base catalysis, and nucleophilic catalysis have been proposed to play pivotal roles in the enzymatic mechanisms of action.[1] It has proven challenging in these biological systems to deconvolute each contributing factor to discern fundamental mechanistic characteristics of the enzymes. Recently, model systems have been devised in which the viability of several of these mechanistic candidates could be evaluated, free from other interfering effects. In this Account, we document biologically relevant intramolecular catalysis and nucleophilic catalysis of amide isomerization; base-catalyzed amide isomerization and Lewis acid-catalyzed amide isomerization are also discussed in turn. Although the biological relevance of these last two mechanisms remains to be established, Lewis acid catalysis would seem to be a possible way to catalyze protein folding in vitro, and experiments along these lines on collagen model systems are discussed. Finally, we also reveal how the catalysis of amide isomerization may relate to the reaction chemistry of *N*-acylaziridines.

The Amide Group: A Brief Overview

The amide is one of the most significant functional groups in all of chemistry, forming the basic building block of biologically important polymers such as peptides and proteins, as well as commercially important ones such as nylon. The resonance theory Pauling advanced many years ago explains many properties of amides, such as short C−N bond lengths,[2] carbonyl stretching frequencies in the IR spectrum,[3] kinetic stability toward nucleophilic attack,[4] and the barrier to rotation about the C−N bond.[5] As explained by resonance theory, amides are essentially planar due to delocalization of the lone pair of electrons on nitrogen into the π-orbital of the carbonyl group, resulting in substantial double-bond character in the C−N bond (form **1b**).[6,7]



The observation of hindered rotation about the C−N bond in amides was realized in the earliest days of NMR spectroscopy and represents the first application of dynamic NMR to mechanistic organic chemistry.[8] Although the barrier to C−N rotation is readily surmountable at room (or physiological) temperature, the reaction is slow on the NMR and biological time scales; for instance, the barrier to rotation ($\Delta G^{\ddagger}$) of neat dimethylacetamide at 25 °C is about 18 kcal/mol,[9] which leads to a rate constant of 0.4 s^{-1}. In more heavily substituted amides, $\Delta G^{\ddagger}$ can approach 22 kcal/mol (5×10^{-4} s^{-1}), and it is easy to imagine that any reaction dependent upon cis−trans interconversion could be rate limited by such a process. In fact, it is now well known that the cis−trans isomerization of proline residues is the slow step in the folding of a number of peptides and proteins.[1b]

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Tom Lectka is a native of Michigan who graduated from Oberlin College in 1985. He attended graduate school at Cornell University in John McMurry's laboratory. After a Humboldt Fellowship to study at Heidelberg in 1991, he studied in Dave Evans's laboratory at Harvard. In 1994 he moved to Johns Hopkins University, where he was promoted to Associate Professor in 1999. His research interests include catalytic, enantioselective reactions of imines and amides, "switchable" mechanisms in synthesis, and synthetic rotamase catalysts.

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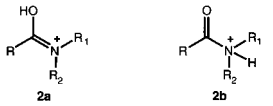
Solvent effects are well known to play a large role in the barrier to C−N rotation. For instance, $\Delta G^{\ddagger}$ can be increased by up to 3 kcal/mol (>100-fold rate decrease) simply by changing the environment from a nonpolar, non-hydrogen-bonding solvent to water.[10] This effect has been explained by selective stabilization of the more polar ground state in water, versus the transition state of isomerization, wherein amide resonance is disrupted and charge separation diminished.[9] The reverse process, transfer of an amide from water to a hydrophobic environment, termed "desolvation", has been proposed to be important biologically as a mechanism for the catalysis of amide isomerization.[11]

The Brønsted acid-catalyzed isomerization of amides has also been well studied.[1a] Even though the carbonyl oxygen is universally believed to be the thermodynamically preferred site of protonation in amides (**2a**),[12] the catalysis of amide isomerization by strong Brønsted acids is a well-known process that is most easily rationalized as occurring through a small but kinetically significant quantity of N-protonated intermediate **2b**.[13] For example,



the rate of amide isomerization of dimethylacetamide increases 130-fold when the pH of the solution is changed from 7.0 to 1.8.[14] Other investigations into the isomerization of amides have focused on isotope[15] and substituent effects.[16] Collectively, these mechanistic observations suggest that the resonance model is a useful guide for understanding the reactivity of the amide group, and they also indicate that interactions which disrupt resonance should, in theory, catalyze amide isomerization.

Intramolecular Catalysis of Amide Isomerization

In a notable theoretical study,[17] Karplus proposed that intramolecular catalysis of amide isomerization, by donation of a weak hydrogen bond from the backbone NH of a proline residue to the amide nitrogen (N_a), plays a role in the mechanism of FKBP-catalyzed peptide folding (Figure 1A). This stabilizing interaction was postulated to contribute 1.4 kcal/mol of the 6.2 kcal/mol decrease in $\Delta G^{\ddagger}$ for FKBP-catalyzed proline isomerization. On the other hand, cyclophilin is believed to bind substrates in a so-called type VIb proline turn, in which the adjacent amide proton is not properly aligned to induce intramolecular catalysis. However, there is an Arg residue close in the tertiary structure within the active site of cyclophilin (but not in FKBP) that may act as the hydrogen bond donor during catalysis (Figure 1B).[17] In an earlier study of the folding of dihydrofolate reductase (DHFR), the authors proposed that an analogous intramolecular interaction between an Arg residue and a key Pro catalyzes folding.[18] In fact, intramolecular hydrogen bonding between a prolyl nitrogen and nearby H bond donors is

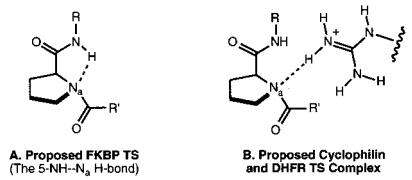
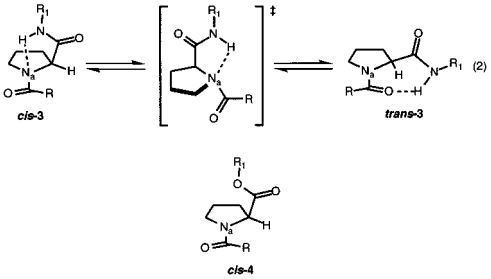


FIGURE 1. Intramolecular catalysis in biological systems.

commonplace in structural protein chemistry,[19,20] yet its role in the folding and stabilization of proteins is yet to be defined.

In an effort to provide experimental support for this mechanistic hypothesis, we postulated that small peptides containing the correct structural features should show intramolecular catalysis in an organic medium that mimics the hydrophobic environment of the FKBP active site. For example, it seemed reasonable that if the activation barriers for two sterically similar prolines were compared—one proline containing the catalytic NH general acid in the side chain, the other not—in both organic and in aqueous solution, the difference in the barriers would be a reflection of intramolecular catalysis. Amides **3** and esters **4** fulfill these requirements. In nonpolar solution, it is expected that the cis form of amides **3** contains an H bond between the side chain and the prolyl N_a (a 5-NH- - N_a bond); this interaction should be strengthened in the transition state for cis-to-trans amide isomerization as N_a becomes more basic (eq 2). It was expected that, in



aqueous solution, intramolecular catalysis would be eliminated by competition from the strongly H-bond-accepting solvent molecules. Intramolecular catalysis (IC) was thus defined as $\Delta(\Delta G^{\ddagger})$ in the change from aqueous solution to an organic solvent for model amides, with the analogous $\Delta(\Delta G^{\ddagger})$ for model esters subtracted (eq 3). However, for isosteric substrates, there is no reason to believe that the simpler eq 4 would not serve just as well and can expand the range of model systems amenable to investigation due to the often unfavorable separation of NMR resonances in aqueous solution.[21]

Intramolecular Catalysis in Terms of $\Delta G^{\ddagger}$ (with Aqueous Correction):

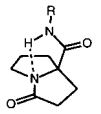
$$IC = [\Delta G^{\ddagger}_{amide(aqueous)} - \Delta G^{\ddagger}_{amide(organic)}] - [\Delta G^{\ddagger}_{ester(aqueous)} - \Delta G^{\ddagger}_{ester(organic)}] \quad (3)$$

Intramolecular Catalysis in Terms of $\Delta G^{\ddagger}$:

$$IC = [\Delta G^{\ddagger}_{ester(organic)} - \Delta G^{\ddagger}_{amide(organic)}] \quad (4)$$

We began our study by obtaining kinetic data for the cis−trans isomerization of prolinamide **3a** (R = 2-fluorophenyl; R_1 = hexyl) and control ester **4a** (R = 2-fluorophenyl; R_1 = hexyl).[22] In 60% MeOD/D_2O,[23] the barriers to amide isomerization of amide **3a** and isosteric ester **4a** were found to be identical, as expected. The equilibrium constants (K = [trans]/[cis]) were also roughly equivalent. In $CDCl_3$ however, $\Delta G^{\ddagger}$ in amide **3a** dropped by 2.4 kcal/mol for trans-to-cis isomerization, and by 3.6 kcal/mol for cis-to-trans, whereas in ester **4a** the respective barrier lowerings were both 1.0 kcal/mol (in line with a simple solvent effect).[10a] Employing eq 3 thus provides differences of 1.4 kcal/mol (trans-to-cis) and 2.6 kcal/mol (cis-to-trans) that are attributed to intramolecular catalysis from the 5-NH- -N_a H bond.

Prolinamides and controls with anilide side chains of different acidities were also analyzed kinetically. Amide **3b** (R = methyl; R_1 = phenyl) affords intramolecular catalysis of 2.8 kcal/mol (cis−trans) at 25 °C in CD_2Cl_2. Electron-donating substituents (**3c**, *p*-OMe; **3d**, *p*-NMe_2) remotely placed on the aryl group show less catalysis (2.5 and 2.2 kcal/mol, cis−trans), whereas a remote electron-withdrawing substituent (**3e**, *p*-COOMe) exhibits the greatest degree of catalysis (3.3 kcal/mol, cis−trans). This latter result represents a 260-fold rate enhancement of amide isomerization over the corresponding control ester. A Hammett plot of the data indicates that the relative rate of catalysis is directly proportional to the acidity of the side chain NH, supporting the mechanistic hypothesis. The proposed hydrogen-bonding interaction was also examined by IR spectroscopy, wherein a stretch at 3430 cm^{-1} was assigned to the 5-NH- -N_a H bond. Additional evidence for a 5-NH- -N_a H bond was obtained by X-ray crystallography of amide **5** (R = *p*-bromophenyl), which is "locked" in the cis form. The structure revealed a



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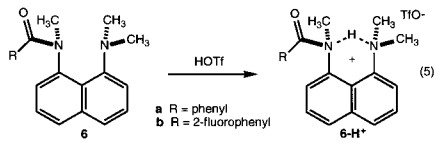
distance from the backbone NH hydrogen to the ring N_a of 2.35 Å, an N−N distance of 2.79 Å, and a NH- -N_a bond angle of 120 ± 4°. These bond distances and corresponding angles classify the observed 5-NH- -N_a interaction as a weak H bond.[24] Further spectroscopic and kinetic investigations on prolyl carbamates provided additional support for the proposed mechanism of catalysis.[22]

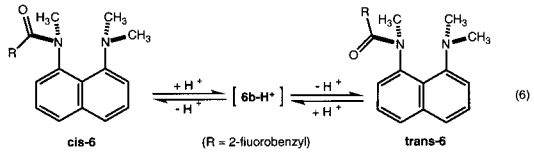
Charged Donors for Intramolecular Catalysis of Amide Isomerization

Although N-protonated amides are unknown species, we felt it would still be worthwhile and feasible to observe strong H bonding to the amide nitrogen, given a appropriate, spatially proximate *charged* donor.[25] To realize this goal we synthesized amide **6**, based on the proton sponge scaffold, with the hope that the amino group, when protonated, would act as a donor suitably positioned to engage in a strong intramolecular H bond with the amide nitrogen rather than with the carbonyl oxygen (eq 5). Spectroscopic and crystallographic investigations of



6-H$^+$ were consistent with such a species. For example, upon protonation of **6a** in acetonitrile-d_3, the amide C=O stretch shifts +47 cm^{-1} from 1637 to 1684 cm^{-1}, consistent with a more ketone-like carbonyl. Additionally, the X-ray structure of **6b-H**$^+$ reveals a bridging hydrogen placed between the amino and the amide nitrogens. The H bond distance of 2.17 Å and angle of 136° in **6b-H**$^+$ classify it as a "moderately strong" H bond.[24] Further evidence in support of a strong interaction was obtained by examining the pyramidalization of the amide nitrogen in **6b-H**$^+$ and comparing it to the X-ray structure of the free base **6b**.[25]

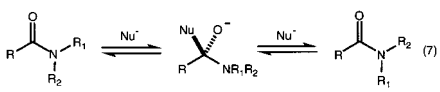
As expected, this H bond leads to a large increase in the rate of rotation about the C−N bond (eq 6).[26] Upon



the addition of 0.5 equiv of chloroacetic acid, the rate of amide isomerization of **6** increased greatly, with $\Delta G^{\ddagger}$ lowered from 20.9 to 15.9 kcal/mol at room temperature.[27] This corresponds to a 2500-fold rate acceleration at room temperature, the largest degree of intramolecular catalysis we have accurately observed. Stronger acids catalyzed the process so efficiently that we could not perform kinetic analyses. In summary, these observations of intramolecular catalysis with both neutral and charged donors provide experimental support for the action of analogous mechanisms in enzymatic systems.

Nucleophilic Catalysis of Amide Isomerization

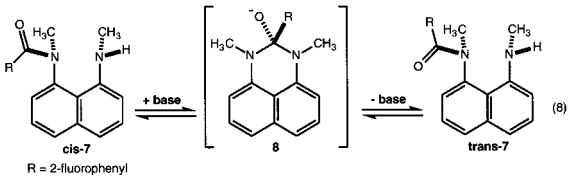
Nucleophilic catalysis, in which the formation of a tetrahedral intermediate disrupts amide resonance and thus facilitates rotation about the C−N bond (eq 7), has had a tortuous history in the biochemical literature. Fischer et



al. originally proposed that nucleophilic catalysis, involving attack of a cysteine-based sulfur on the amide carbonyl to form a hemithioorthoamide intermediate, plays a key role in the mechanism of cyclophilin-catalyzed prolyl isomerization.[28] Subsequent studies involving site-directed mutagenesis (SDM) on the native enzymes,[29] as well as kinetic isotope effects on small peptidic substrates,[30] have suggested that this hypothesis was incorrect for the cyclophilins and the FKBPs. However, the recent discovery

of the parvulin rotamases[31] has regenerated interest in enzyme-mediated nucleophilic catalysis. For instance, the human PPIase Pin1 and the closely related Ess1 in yeast are essential in the regulation of mitosis.[32] A nucleophilic component to the catalytic mechanism was proposed on the basis of the X-ray structure of a Pin1-AlaPro dipeptide complex, and on site-directed mutagenesis experiments.[1c] The mutation $Cys^{113} \rightarrow Ala^{113}$ diminishes k_{rel} by a factor of 120, and led the authors to propose that the active site His^{59} deprotonates Cys^{113}, which then attacks the amide carbonyl to catalyze cis−trans isomerization; however, no direct evidence for such a pathway was provided.

To synthesize a model system for the documentation of nucleophilic catalysis, we once again exploited the favorable juxtaposition of the *peri*-substituents in substituted naphthalenes. It was anticipated that amide **7**, following deprotonation of the amino proton, would produce tetrahedral intermediate **8**. If formation and breakdown of **8** are faster than the rate of uncatalyzed amide isomerization, interconversion of *cis*- and *trans*-**7** will be catalyzed (eq 8). The ^1H, ^{19}F, and ^{13}C NMR as well



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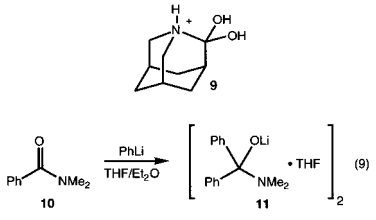
as IR spectra of **7** in CD_3CN with substoichiometric amounts of potassium bis(trimethylsilyl)amide indicated the presence of stable tetrahedral intermediate **8**, whose formation and breakdown were slow on the NMR time scale.[33]

X-ray analysis of the potassium salt of **8** revealed an anionic tetrahedral intermediate derived from nucleophilic attack on an amide carbonyl, a species that is widely accepted as an intermediate in the action of serine and cysteine proteases. Two notable examples of amide tetrahedral intermediates precede ours. In the first, Kirby et al. reported the synthesis of a remarkable hydrated amide tetrahedral intermediate **9** based on the adamantylamide framework.[34] Additionally, an X-ray structure of anionic tetrahedral intermediate **11** was reported by Adler et al.[35] What is especially remarkable about this structure is that it resulted solely from an *inter*molecular reaction of phenyllithium with *N*,*N*-dimethylbenzamide.



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The slow breakdown of intermediate **8** led us to investigate the more biologically relevant system **12**, in which the attacking nucleophile is sulfur. In this system, breakdown of tetrahedral intermediate **13** is, in fact, fast on the NMR time scale. Note that the C−N bond of

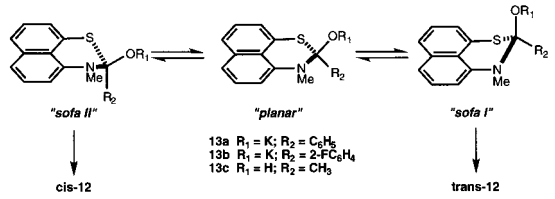
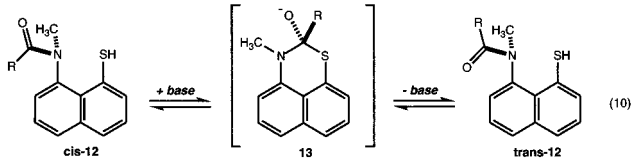


FIGURE 2. Proposed pathway of amide isomerization in **12**.

intermediate **13** cannot undergo uninhibited rotation (as in the simple analogue in eq 7) because it is constrained within a ring. However, interconversion of the two sofa conformers of **13**, sofa I and sofa II, followed by their respective breakdown, also interconverts the cis and trans rotamers (Figure 2); theoretical calculations indicate that interconversion of sofa I and sofa II should be very fast.

We measured the rate of isomerization of **12** in CD_3CN by ^1H saturation transfer NMR, and the cis−trans interconversion was found to occur with $\Delta G^{\ddagger} = 19.0$ kcal/mol at 25 °C, $\Delta H^{\ddagger} = 18.0$ kcal/mol, and $\Delta S^{\ddagger} = -3 \pm 3$ cal mol^{-1} K^{-1}. Upon addition of 1 equiv of potassium imidazolate (K-Im), the ^1H NMR remained essentially unaltered with the exception of a modest change in the cis:trans ratio. The IR stretch of the carbonyl moved -20 cm^{-1} to 1636 cm^{-1}, consistent with increased electron density of the naphthyl system due to deprotonation of the thiol. Attempts to observe the putative tetrahedral intermediate **13** by ^{13}C NMR were unsuccessful, presumably due to its extremely short lifetime and/or small population. However, kinetic analysis of the cis−trans isomerization was straightforward: $\Delta G^{\ddagger} = 16.2 \pm 0.3$ kcal/mol at 25 °C, $\Delta H^{\ddagger} = 5.8 \pm 0.3$ kcal/mol, $\Delta S^{\ddagger} = -35 \pm 4$ cal mol^{-1} K^{-1}, indicating a 2.8 kcal/mol lowering of $\Delta G^{\ddagger}$ due to nucleophilic catalysis. Additionally, if we analyze the results at −25 °C, a sizable 4.3 kcal/mol reduction in $\Delta G^{\ddagger}$ is observed.



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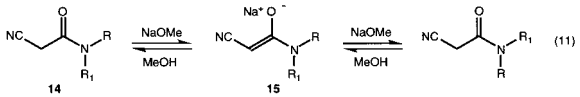
We found that the degree of catalysis observed was proportional to the quantity of base added, as 1 equiv of K-Im produced an approximately 3-fold greater rate increase than 0.25 equiv of K-Im. Numerous control reactions were performed to rule out intermolecular interactions, as well as undesired through-bond electronic effects. For example, kinetic investigations indicate that the rate of isomerization of **12** with 1 equiv of K-Im is first order in substrate concentration between 5 and 20 mg/mL, and remote thiolates do not have a barrier-lowering effect on the rate of amide isomerization. The 2.8 kcal/mol lowering of $\Delta G^{\ddagger}$ relates to a 110-fold increase in the rate of cis−trans isomerization at room temperature and represents a well-documented experimental observation of nucleophilic catalysis of amide isomerization in a model system.

Future Directions: Base Catalysis of Amide Isomerization

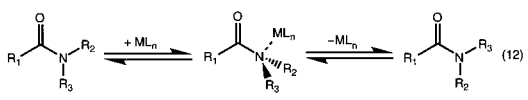
Enolization at the α-position of amides is also expected to diminish amide resonance, thus substantially lowering the barrier to rotation about the C−N bond. Although a simple enough concept, it has not been well demonstrated to date. Streitwieser et al. recently reported an effort to measure the C−N rotational barrier in an amide enolate; however, the attempt was unsuccessful due to the fact that C−N rotation in this case was presumed to be too fast.[36] In principle, only a very small amount of enolate need be present in solution to dramatically lower the observed barrier, if there exists fast proton exchange between the two components—not a trivial assumption, considering the well-known tendency of carbon acids to exhibit kinetically slow proton exchange.[37] The barriers to C−N bond rotation in amide enolates can also provide useful information on the extent to which "amide character" is retained, depending on the precise nature of the substituents and counterions. We have obtained unpublished preliminary data that amide **14**, containing a highly acidic α-proton, undergoes a barrier lowering of 4.1 kcal/mol upon treatment with 10 mol % sodium methoxide in methanol (eq 11). Most notably, 1.1 equiv of proton



sponge produces a 2.2 kcal/mol lowering in this system. Although the isomerization may proceed through the putative intermediate **15**, thorough follow-up studies are underway to fully document the phenomenon.

Metal-Catalyzed Amide Isomerization

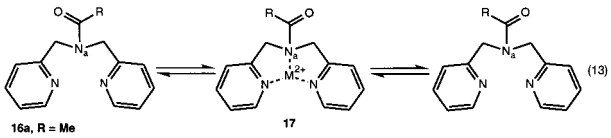
Historically, investigations into the effect of metal ions on the cis−trans isomerization of amides indicate that metal-based Lewis acids, in general, raise the barrier to rotation.[6,38] This finding is easily rationalized by assuming that metal coordination occurs on the more basic oxygen atom, reinforcing the double bond character of the C−N bond. On the other hand, coordination of the metal to N_a should disrupt amide resonance and catalyze amide isomerization (eq 12). In fact, an early computational study predicts



a lowering of $\Delta G^{\ddagger}$ upon coordination of Li^+ ions,[39] and experimental investigations have indicated that very high concentrations of Ag^+ ions in solution can reduce the $\Delta G^{\ddagger}$ for the isomerization of *N*,*N*-dimethylacetamide.[40]

Whether metal ion catalysis of amide isomerization has any biological relevance remains to be determined. To date, only one PPIase, SlyD (a member of the FKBP class), is known to be regulated by metal binding;[41] however, the activity of SlyD is shut off upon binding nickel ions, suggesting a purely structural role for the metal. Still, the possibility remains that an unidentified class of enzymes exists that utilizes catalytically active Lewis acids. It is recognized that slow protein folding reactions in vitro can experience problems due to misfolding of intermediate structures and subsequent aggregation. A proposed origin of these complications is the slow cis−trans isomerization of critical proline residues in proteins.[42] The development of small, synthetic catalysts that do not suffer from the known inability of the PPIases to catalyze the isomerization of partially buried prolines[43] could be applied to the refolding of denatured proteins in vitro. We felt metal ions could potentially aid in the synthetic catalysis of protein folding, although many potential pitfalls can be imagined. This section describes some initial efforts toward such a goal.

Previous results with protic acids suggest that metal-based Lewis acids, with help from other properly oriented binding groups, could possibly be induced to coordinate preferentially with the amide nitrogen rather than oxygen. This tendency should be enhanced by more azaphilic metals, such as low-valent, late transition metals, rather than harder, more oxophilic metals. The first amides that were tested for Lewis acid catalysis were highly "rigged" for N coordination on both steric and entropic grounds. For example, the bis-pyridyl amide system **16** should provide an ideal environment for N coordination—treatment of **16** with an azaphilic metal should produce tight tridentate complex **17** containing two five-membered rings involving N_a (eq 13). If the metal were to coordinate

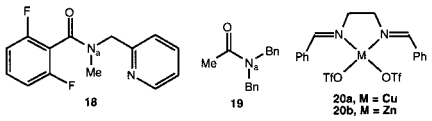


to the oxygen, it would have to do so through a less favored seven-membered chelate. In fact, ligand **16d** has been reported to undergo an unusual hydrolysis reaction in the presence of Cu(II) ions, presumably through a mechanism involving Cu−N_a coordination.[44]

A number of more "azaphilic" transition metals were initially screened for their ability to catalyze the isomerization of **16**.[45] Of the metals screened [Cu(I), Cu(II), Ni(II), Zn(II), Ag(I), and Pd(II)], Cu(II) was found to be the most effective catalyst for the reaction; however, paramagnetic broadening in the ^1H NMR limited the useful range of Cu(II) to 2−10 mol %. Additionally, highly dissociable triflate counterions were advantageous, as the tighter binding chloride ions produced significantly less catalysis. The rotational barriers for tridentate amides **16a**−**c** were measured under various conditions in the presence of Cu(II) ions, and we observed as much as a 6 kcal/mol reduction in $\Delta G^{\ddagger}$ (in the case of **16c**) with only 5 mol % $Cu(OTf)_2$, representing a 25 000-fold rate enhancement. In general, we found that the potential for barrier lowering is greatest in amides with the highest rotational barriers. The other metals screened, especially Cu(I), Zn(II), and

Ag(I), produced catalytic effects comparable to that observed with Cu(II), but required a substantially higher loading of metal.

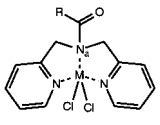
Bidentate amide **18**, in which we expect less efficient catalysis due to reduced binding ability to N_a, undergoes a reduction in $\Delta G^{\ddagger}$ of only 1.1 kcal/mol with 10 mol % $Cu(OTf)_2$. In this case, we used ^{19}F saturation transfer NMR to measure the barrier in the presence of a relatively large amount of paramagnetic metal, demonstrating the usefulness of the ^{19}F nucleus for this purpose. Substoichiometric



amounts of metal confirm that Cu(II) undergoes fast exchange and is a true catalyst, while the lack of any catalysis in the simple amide **19** emphasizes the importance of additional binding sites. We also found that preformed complexes can catalyze amide isomerization, a fact that has importance for the flexible design of soluble synthetic catalysts. Unfortunately, measuring catalyzed amide isomerization when amides **16** are treated with the $Cu(OTf)_2$−bis(imine) complex **20a** is impossible due to paramagnetic broadening in the ^1H NMR spectrum; however, the Zn(II) complex **20b** (25 mol %) lowers the barrier of amide **16a** by 2.5 kcal in $CDCl_3$.

We also gathered evidence that a Cu−N_a interaction was present in solution. To this end, we first studied the change in the carbonyl stretching frequency of amides **16** in CH_2Cl_2 upon the addition of 1 equiv of $Cu(OTf)_2$. For instance, the carbonyl stretch of **16b** shifts from 1635 cm^{-1} in the free ligand to 1730 cm^{-1} upon the addition of metal, a shift of 95 cm^{-1} that is indicative of a more ketone-like carbonyl. Similar shifts of 50−100 cm^{-1} were observed by Maslak in his Cu(II)− and Ni(II)−N_{urea} complexes, whereas the O-bound Zn(II) produced a shift of −50 cm^{-1}.[46] Evidence for Cu−N_a coordination was also obtained from EPR spectroscopy, as pictured in Figure 3. A notable difference in superhyperfine splitting in the spectra of **16b**·$Cu(OTf)_2$ at −110 °C in CH_2Cl_2 was observed when $^{15}N_a$ was substituted for $^{14}N_a$, a result consistent with direct Cu−N_a bonding.[47]

The best evidence for catalysis by N coordination is the X-ray structure of a crystalline **16b**·$CuCl_2$ complex (**21**) that reveals clear N_a coordination by Cu(II). In the crystal,



Cu(II) is approximately trigonal bipyramidal, and the Cu−N_a distance of 2.49 Å is well within bonding distance. Support for a significant Cu−N_a interaction is also revealed by the lengthening of the C−N bond distance from 1.34 Å in analogous uncoordinated amides to 1.39 Å in **21**; N_a is also significantly pyramidalized. To our knowledge, **21**

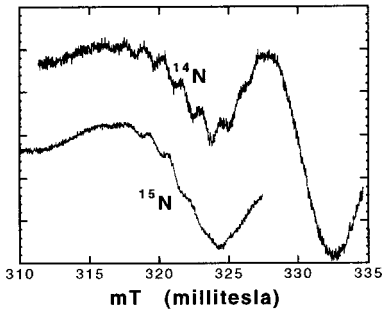
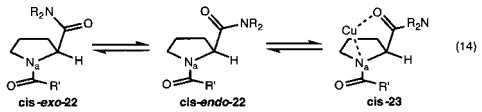


FIGURE 3. EPR spectra of a 1:1 complex of **16b**·$Cu(OTf)_2$ in CH_2Cl_2 at −110 °C. The top spectrum is for **16b** of natural abundance; the bottom spectrum is **16b** that was enriched (>98%) with ^{15}N at the amide nitrogen.

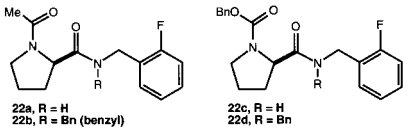
represented the first proof of metal coordination to N_a of a tertiary amide.[48]

Toward our goal of developing synthetic catalysts for peptide folding, we sought evidence that metal-based Lewis acids could catalyze the isomerization of substituted prolines, not just "rigged" tridentate amides such as **16**. Because of their cyclic structure and their conformation in solution, prolines in peptides contain what appears to be a natural binding site for metals involving N_a (eq 14). Due to $A_{1,3}$ strain, the proline unit should prefer to dispose



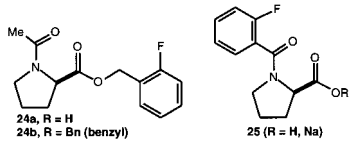
its C_α substituent pseudoaxially, and when the C_α carbonyl is *endo*, it is poised to form a five-membered metal chelate containing the ring N_a (*cis*-**23**). Even though the prolyl N_a is known to be more highly pyramidalized (and thus more basic) than "normal" tertiary amides,[49] the amide carbonyl of the side chain is not expected to bind as favorably to azaphilic metals as did the pyridyl nitrogens in ligands **16**.

Treatment of prolyl amide **22a** with 5 mol % $Cu(OTf)_2$ in THF lowered $\Delta G^{\ddagger}$ from 17.8 to 16.8 kcal/mol [$\Delta(\Delta G^{\ddagger} =$ 1.0 kcal/mol] for trans-to-cis isomerization, as monitored by ^{19}F ST NMR. Catalysis was enhanced in prolyl carbam-

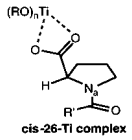


ate **22c** (1.3 kcal/mol), which contains a more electron-rich N_a. Under the same conditions, the barrier in prolyl amide **22c** dropped by 2.0 kcal/mol, and we observed the largest energy lowering (4.3 kcal/mol) in proline **22d**.[50] Ag(I) was also found to be effective for these isomerizations, but, with substrate **22a** for instance, 50 mol % Ag(I) lowered the barrier by the same amount as only 5 mol % Cu(II), confirming the superior nature of Cu(II) as a catalyst for the reaction. There was no perturbation of the cis:trans equilibrium constants in any of these systems, consistent with the metal's role as a catalyst. In both *N*-acetylpyrrolidine and *N*-Cbz-pyrrolidine, no energy

lowering occurred under standard conditions with 5 mol % $Cu(OTf)_2$. Interestingly, less catalysis was observed when an ester side chain, as in **24**, was substituted for the amide. Additionally, the barrier to rotation about the side chain amide bond, easily measured for **22b**, was not altered upon the addition of Cu(II). Taken together, these observations are consistent with the ability of the side chain amide group to bind the metal (through oxygen, structure **23**) and catalyze amide isomerization in proline-containing peptides.



Studies were also performed to determine the effect of Lewis acids [mainly Cu(II) and Ag(I)] in water on the barrier to isomerization in water-soluble prolines, such as **25**; however, the results are preliminary at this point, and no certain conclusions can be drawn as of yet. It was also found that a metal-bound phosphine was capable of catalyzing proline isomerization in organic solvents; for example, the barrier to rotation in **22b** was reduced by 1.3 kcal/mol by 50 mol % of a Pd(II)−BINAP complex in THF. We also sought evidence that Lewis acid catalysis of amide isomerization could occur by through-bond effects. For example, tight coordination of a metal to the side chain of a proline (such as in titanate ester **26**) could be expected to withdraw electron density from the amide nitrogen by a though-bond mechanism. However, no barrier lowering was observed upon complexation of oxophilic metals such as titanium to the sodium salts of N-acyl prolines.

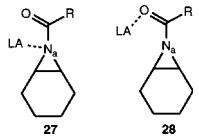


Metal-Catalyzed "Folding" in a Model System

Poly-L-proline is a remarkably structured "switch" polypeptide that reversibly interconverts between all-cis (PPI, right-handed helix) and all-trans (PPII, left-handed helix) forms, depending on the solvent environment.[51] PPII helix conformations have been found to be important structural motifs for both protein structure and biorecognition,[52] and poly-L-proline has been studied as a model for the folding of the collagen triple helix, one of the few documented cases of PPIase-catalyzed folding in vivo.[53] As a prelude to the study of more complex systems, we investigated the ability of Cu(II) ions to catalyze the interconversion of PPII to PPI in CD_2Cl_2. In the presence of $Cu(OTf)_2$ (10 wt % Cu(II) relative to poly-L-proline), we found that the rate of trans-to-cis conversion increases by a factor of 10 at 23 °C (1.4 kcal/mol of catalysis). As a logical extension of our work with poly-L-proline, we are currently interested in the catalysis of protein folding by Lewis acids in aqueous solution. Recent work has demonstrated the stability and activity of certain Lewis acids in water, including those based on lanthanide(III) ions, Cu(II) and Ag(I).[54] Collagen, or especially proline-rich synthetic model systems thereof, present interesting targets for catalysis of folding in aqueous solution.

Reaction Chemistry Involving Possible Metal−Amide N Coordination. "Orthogonal" Lewis Acids: Catalyzed Ring Opening and Rearrangement of Acylaziridines

To this point, we have discussed reversible cis−trans amide isomerization. The question arises as to whether N coordination of metals and protons, effective at catalyzing isomerization, can also impart interesting reaction chemistry. A good place to address this issue is in the case of *N*-acylaziridines, which possess highly pyramidalized amide nitrogens that may be basic enough to bind metals in competition with the corresponding carbonyl oxygen. Experimental as well as theoretical evidence indicates that acylaziridines may undergo N-protonation.[55] They rearrange to oxazolines[56] and can function as electrophiles[57] or as possible probes for Lewis-acid-catalyzed reaction pathway selectivity. We postulated that coordination of a Lewis acid to the amide nitrogen of acylaziridines (**27**) might be expected to catalyze a rearrangement to the oxazoline, whereas coordination to the carbonyl O (**28**) may be better at activating the acylaziridine toward external nucleophilic attack. These predictions have borne out in practice.[58]



We found that catalytic quantities of relatively oxophilic metals activate *N*-acylaziridines predominantly toward external nucleophilic attack, whereas more azaphilic, or "orthogonal", Lewis acids catalyze the oxazoline rearrangement (Figure 4).[59] Along these lines, the reaction of acylated cyclohexenimine derivatives **29** was studied. Compounds **29a**−**d** were converted to ring-opened products **30a**−**d** by $TMSN_3$ in the presence of 10 mol % Yb-(2,2′-biphenol)OTf. The complexes $Zr(Cp)_2(SbF_6)_2$ and $Ti(OiPr)_4$ were also found to catalyze nucleophilic attack of $TMSN_3$. Remote electron-withdrawing substituents accelerate the reaction, as indicated by a linear correlation of $\log[k/k_0]$ to Hammett σ values. More "azaphilic" Lewis

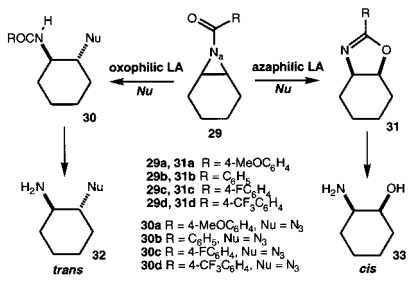


FIGURE 4. Rearrangement and ring opening of acylaziridines.

acids, such as $Zn(OTf)_2$, $Cu(OTf)_2$, and $Sn(OTf)_2$, did not catalyze the addition of nucleophiles to acylaziridines, but instead promoted the rearrangement of **29a**−**d** to 2-aryloxazolines **31a**−**d**, even in the presence of nucleophiles. Competition experiments lead to the conclusion that electron-donating substituents increase the rate of reaction, a trend opposite to that of the oxophilic Lewis-acid-catalyzed additions analyzed above. Mechanistic information derived from stereochemical and solvent polarity studies suggests that the reaction proceeds through a tight ion pair. This study represents the first instance where control of reaction pathway is governed by the identity of a Lewis acid, and the products are valuable precursors to chiral ligands and natural products.

Conclusion

In summary, we have outlined recent investigations that provide experimental support for several mechanisms by which amide isomerization can be catalyzed, accompanied by a synthetic application manifested from this work. It would be appropriate to mention here future possibilities for the catalysis of amide isomerization. For example, one study underway in our laboratories involves the catalysis of cyclic peptide formation. The formation of cyclic peptides is often impeded by rate-determining isomerization of a thermodynamically stable trans amide to a less stable cis amide. Theoretically, the rates of such cyclizations could be accelerated by the catalysis of amide isomerization. Consequently, the effect on product distributions and yields of these chemical reactions could be beneficial. Accordingly, we are attempting to couple cyclization reactions with fast trans-to-cis isomerization of peptides to afford a practical benefit to the theoretical groundwork of amide isomerization laid over the past decades.

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