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Nucleophilic Catalysis of Amide Isomerization

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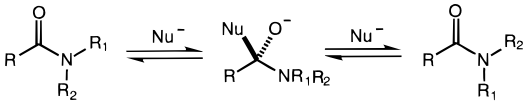
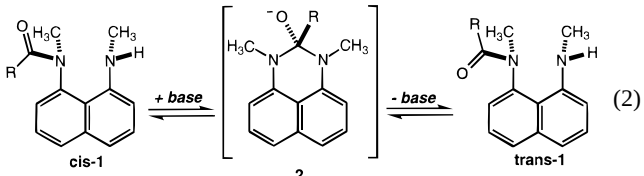
Studies on the Brønsted acid-catalyzed cis−trans isomerization of amides have served as an elegant testing ground for a number of classical NMR techniques in physical organic chemistry.[1] A potentially complementary process, the nucleophilic catalysis of amide isomerization, whereby the formation of a tetrahedral intermediate disrupts amide resonance and facilitates rotation about the C−N bond (eq 1), remains uncharacterized although a number



(1)

of intriguing proposals imply its biological significance. For example, the human PPIase Pin1, and the closely related Ess1 in yeast, are essential in the regulation of mitosis[2] and are thus potential therapeutic targets for cancer chemotherapy. Noel and coworkers have recently suggested a nucleophilic component in the mechanism of Pin1 catalysis based on the X-ray structure of a Pin1−AlaPro dipeptide complex, as well as on site-directed mutagenesis data.[3] These authors propose that the active site His[59] deprotonates Cys[113], which then attacks the amide carbonyl and catalyzes cis−trans isomerization; however, no direct evidence was provided to support this hypothesis. In this report, we unveil a model system in which nucleophilic catalysis of amide isomerization is characterized for the first time, as well as the first X-ray structure of an anionic tetrahedral intermediate resulting from nucleophilic attack on an amide carbonyl.[4]

We postulated that amide **1**, following deprotonation of the amino proton, would produce tetrahedral intermediate **2**. If formation and breakdown of **2** are faster than the rate of uncatalyzed amide isomerization, interconversion of cis and trans **1** will be catalyzed (eq 2). The ^{1}H and ^{13}C NMR spectra of **1a** in CD_3CN



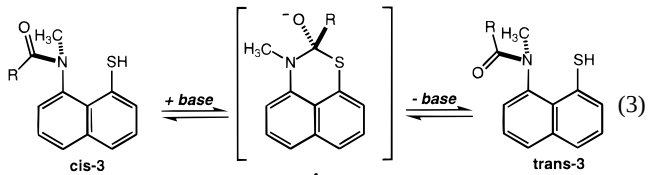
(2)

a R = C_6H_5; ^{13}C-carbonyl
b R = 2-FC_6H_4
c R = 4-BrC_6H_4

indicated a predominance of one species (cis/trans >20:1), and the ^{13}C shift of the labeled carbon at 172.5 ppm was as expected for an amide carbonyl. Additionally, the IR spectrum of **1b** in CD_3CN showed a typical amide carbonyl stretch at 1657 cm^{-1}. However, upon addition of 1 equiv of potassium hexamethyldisilazane (KHMDS), ^{13}C NMR revealed a single resonance at 102.5 ppm, and no carbonyl stretch was visible in the IR. The upfield shift of 70 ppm for the ^{13}C resonance is diagnostic for a carbon that underwent a change in hybridization[5] from sp^2 to sp^3 and, along with the IR data, indicated the formation of stable tetrahedral intermediate **2**. This conclusion was confirmed by X-ray analysis of the potassium salt of **2c**, as pictured in Figure 1.[6] To our knowledge, this is the first X-ray structure of an anionic tetrahedral intermediate derived from nucleophilic attack on an amide carbonyl, a species believed to be involved in the mechanism of peptide hydrolysis catalyzed by serine and cysteine proteases.[7]

With evidence for the ability of **1** to form the key tetrahedral intermediate, we investigated the behavior of this system with a substoichiometric quantity of base. When 0.5 equiv of KHMDS was added to **1a** in CD_3CN, ^{13}C NMR revealed resonances at 172.5 and 102.5 ppm with approximately equal intensity; thus, tetrahedral intermediate **2** appeared to be too stable, and its breakdown was expected to be slower than background amide isomerization. This conclusion was supported by the investigation of **1b** by ^{19}F saturation transfer (ST) NMR.[8] With no base present, the cis and trans conformers interconverted with $\Delta G^{\ddagger} = 16.8 \pm 0.2$ kcal mol^{-1} (cis-to-trans[9]). Upon addition of 0.5 equiv of KHMDS, a third ^{19}F resonance appeared (attributed to the formation of **2**), but the rate of isomerization remained unchanged.

The slow breakdown of tetrahedral intermediate **2** led us to investigate the more biologically relevant system **3**, in which the attacking nucleophile is sulfur. In this system, breakdown of tetrahedral intermediate **4** was expected to be faster, owing to the greater stability of the attacking/leaving thiolate (eq 3), but the



(3)

a R = C_6H_5; ^{13}C-carbonyl
b R = 2-FC_6H_4

possibility of transesterification to form a thioester was a potential pitfall. Consideration of intermediate **4** indicates that, unlike the hypothetically simple open chain analogue in eq 1, the C−N bond cannot undergo uninhibited rotation because it is constrained

(1) (a) Berger, A.; Loewenstein, A.; Meiboom, S. *J. Am. Chem. Soc.* **1959**, *81*, 62; (b) Steinberg, I. Z.; Harrington, W. F.; Berger, A.; Sela, M.; Katchalski, E. *J. Am. Chem. Soc.* **1960**, *82*, 5263; (c) Jackman, L. M.; Kavanagh, T. E.; Haddon, R. C. *Org. Magn. Reson.* **1969**, *1*, 109; (d) Stewart, W. E.; Siddall, T. H., III *Chem. Rev.* **1970**, *70*, 517; (e) Gerig; J. T. *Biopolymers* **1971**, *10*, 2435; (f) Perrin, C. L. *Acc. Chem. Res.* **1989**, *22*, 268.

(2) Lu, K. P.; Hanes, S. D.; Hunter, T. *Nature* **1996**, *380*, 544.

(3) Ranganathan, R.; Lu, K. P.; Hunter, T.; Noel, J. P. *Cell* **1997**, *89*, 875.

(4) Kirby has recently reported the crystal structure of the protonated hydrate of a *highly twisted* amide that corresponds to the proposed tetrahedral intermediate in acid-catalyzed hydrolysis of normal amides. See: Kirby, A. J.; Komarov, I. V.; Feeder, N. *J. Am. Chem. Soc.* **1998**, *120*, 7101.

(5) (a) Rich, D. H.; Bernatowicz, M. S.; Schmidt, P. G. *J. Am. Chem. Soc.* **1982**, *104*, 3535; (b) Conroy, J. L.; Seto, C. T. *J. Org. Chem.* **1998**, *63*, 2367.

(6) Crystals were grown in the drybox by treatment of a solution of **1c** in THF with 1.1 equiv of KH, followed by slow diffusion of pentane. Crystal data for **2c**: orthorhombic; *Pnnm*; yellow irregular block; $a = 20.135(5)$ Å; $b = 11.290(3)$ Å; $c = 12.291(3)$ Å; $V = 2794.0(13)$ Å^3; $Z = 4$; $R = 0.0374$; GOF = 0.911.

(7) Numerous studies have characterized tetrahedral intermediates of inhibitors wherein the carbonyl that was attacked was not an amide. See: (a) Poulos, T. L.; Alden, R. A.; Freer, S. T.; Birktoft, J. J.; Kraut, J. *J. Biol. Chem.* **1976**, *251*, 1097; (b) ref 5; (c) Gamcsik, M. P.; Malthouse, P. G.; Primrose, W. U.; Mackenzie, N. E.; Boyd, A. S. F.; Russell, R. A.; Scott, A. I. *J. Am. Chem. Soc.* **1983**, *105*, 6324; (d) Takahashi, L. H.; Radhakrishnan, R.; Rosenfield, R. E., Jr.; Meyer, E. F., Jr.; Trainor, D. A. *J. Am. Chem. Soc.* **1989**, *111*, 3368; (e) Ganesh, V.; Lee, A. Y.; Clardy, J.; Tulinsky, A. *Protein Sci.* **1996**, *5*, 825.

(8) For the use of ST NMR in the investigation of amide isomerization, see: (a) Perrin, C. L.; Thoburn, J. D.; Kresge, J. *J. Am. Chem. Soc.* **1992**, *114*, 8800; (b) Cox, C.; Ferraris, D.; Murthy, N. N.; Lectka, T. *J. Am. Chem. Soc.* **1996**, *118*, 5332; (c) Cox, C.; Lectka, T. *J. Am. Chem. Soc.* **1998**, *120*, 10660.

(9) We believe that the cis forms of **1** and **3** are thermodynamically favored, in which case the barriers reported throughout represent the cis-to-trans isomerization of the amide bond. This conclusion is based on several pieces of data: (1) previous experimental (Itai, A.; Toriumi, Y.; Saito, S.; Kagechika, H.; Shudo, K. *J. Am. Chem. Soc.* **1992**, *114*, 10649) and theoretical (Saito, S.; Toriumi, Y.; Tomioka, N.; Itai, A. *J. Org. Chem.* **1995**, *60*, 4715) results indicate that *N*-methylanilides are more stable in the cis conformation; (2) single-crystal X-ray structures of related 1,8-disubstituted naphthyl amides indicate that cis is the favored form in the solid state (Cox, C.; Wack, H.; Lectka, T. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 798).

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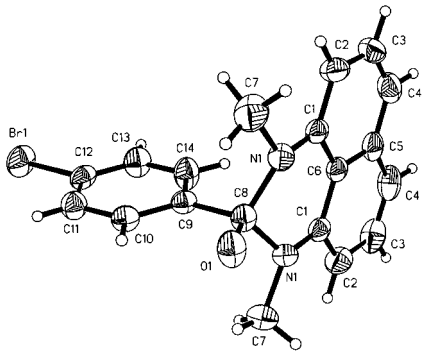


Figure 1. X-ray crystal structure of the potassium salt of tetrahedral intermediate **2c** (50% ellipsoids); the potassium cation coordinated to the oxygen has been excluded for clarity. The structure is perfectly symmetrical, with atoms C5, C6, C8, O1, C9−C14, and Br1 in a plane that bisects the naphthyl ring. Selected bond distances (Å): N1−C8, 1.517(3); O1−C8, 1.314(4). Selected angles (deg): O1−C8−N1, 110.3(2)°; N1−C8−C9, 109.3(2)°.

Scheme 1

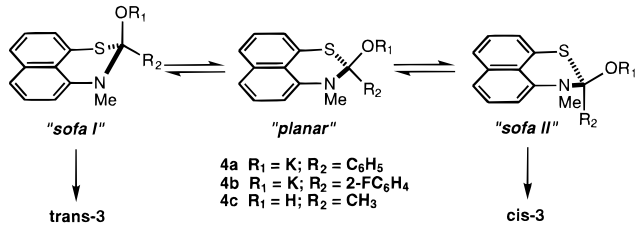


Table 1. Kinetic Parameters for the Catalyzed and Uncatalyzed Amide Isomerization of **3** and **6**[a]

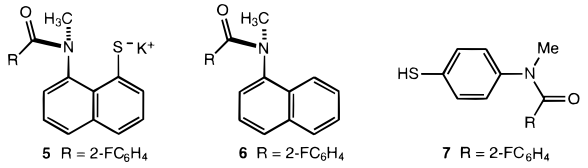
entry	substrate	T (°C)	additive	$\Delta G^{\ddagger}$[b]	cis/trans ratio
1	**3b**	25		19.0	7:1
2	**3b**	25	1 equiv of PS[c]	18.5	8:1
3	**3b**	25	1 equiv of K-Im	16.2[d]	12:1
4	**3b**	−25		18.8[d]	10:1
5	**3b**	−25	1 equiv K-Im	14.5	20:1
6	**6**	25		17.8	7:1
7	**6**	25	1 equiv of $PhS^- K^+$	17.6	7:1
8	**6**	25	1 equiv of K-Im	17.8	7:1

[a] Kinetic measurements were performed at 10 mg/mL in CD_3CN by ^1H ST NMR. [b] Cis-to-trans; ± 0.2 kcal mol^{-1}. [c] PS = Proton Sponge. [d] Calculated from the Eyring plot; ±0.3 kcal mol^{-1}.

within a ring. However, interconversion of the two sofa[10] conformers of **4** (Scheme 1), sofa I and sofa II, followed by their respective breakdown, also interconverts the cis and trans rotamers. We performed density functional (DFT) calculations to model this conformational interconversion, and at the pBP/DN* level of theory, the relative energy of the simplified planar form **4c** (R_1 = H; R_2 = CH_3) is only 5.7 kcal/mol higher than that of sofa I and 1.6 kcal/mol higher than sofa II; thus, a low barrier may separate the two forms and catalysis should be observable.

The ^1H NMR spectrum of **3b** in CD_3CN indicated two isomers in equilibrium with a cis/trans ratio of 7:1, and IR analysis revealed an amide carbonyl stretch at 1656 cm^{-1}. Additionally, ^{13}C NMR of **3a** revealed amide carbonyl resonances at 171.2 and 169.9 ppm corresponding to the cis and trans rotamers. The rate of isomerization of **3b** in CD_3CN was examined by ^1H ST NMR, and the cis−trans interconversion was found to occur with $\Delta G^{\ddagger}$ = 19.0 ± 0.2 kcal mol^{-1} at 25 °C, $\Delta H^{\ddagger}$ = 18.0 ± 0.3 kcal mol^{-1}, and $\Delta S^{\ddagger}$ = −3 ± 3 cal mol^{-1} K^{-1} (Table 1, entry 1). Upon addition of 1 equiv of Proton Sponge [1,8-bis(dimethylamino)naphthalene], we observed a small but measurable decrease of 0.5 kcal mol^{-1} in $\Delta G^{\ddagger}$ (entry 2). The ^1H NMR of this mixture revealed little deprotonation of the thiol, and a stronger base was required to increase the catalytic effect. The potassium salt of imidazole (K-Im) was found to be an excellent base that did not promote transacylation under strictly anhydrous conditions.

Upon addition of 1 equiv of K-Im to **3b** in CD_3CN, the ^1H NMR remained essentially unaltered with the exception of a modest change in the cis/trans ratio.[11] The IR stretch of the carbonyl moved −20 cm^{-1} to 1636, consistent with increased electron density of the naphthyl system due to deprotonation of the thiol.[12] All attempts to observe the putative tetrahedral intermediate **4a** by ^{13}C NMR were unsuccessful, presumably due to its extremely short lifetime and/or small population. Nevertheless, kinetic analysis of cis−trans isomerization of **3b** with 1 equiv of K−Im was straightforward: $\Delta G^{\ddagger}$ = 16.2 ± 0.3 kcal mol^{-1} at 25 °C, $\Delta H^{\ddagger}$ = 5.8 ± 0.3 kcal mol^{-1}, and $\Delta S^{\ddagger}$ = −35 ± 4 cal mol^{-1} K^{-1}, indicating a 2.8 kcal mol^{-1} lowering of $\Delta G^{\ddagger}$ (110-fold rate increase) due to nucleophilic catalysis (entries 1 and 3).[13] The large negative $\Delta S^{\ddagger}$ is indicative of a highly ordered transition state and is consistent with rapid formation and breakdown of putative tetrahedral intermediate **4b** as being the catalytically competent mechanism of action. The concentration of charge from the delocalized thioaryloxide anion **5** to the oxygen



in **4**, with attendant strengthening of solvent and counterion coordination, may also be in part responsible for the magnitude of $\Delta S^{\ddagger}$.[14] The amount of catalysis 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. Additionally, if we analyze the results at −25 °C (entries 4 and 5), a sizable 4.3 kcal mol^{-1} reduction in $\Delta G^{\ddagger}$ is observed. Control compound **6** in the presence of potassium thiophenoxide or K-Im showed no lowering of $\Delta G^{\ddagger}$ (entries 6−8), suggesting that the well-defined intramolecular nature of **3** is paramount to the success of the catalytic interaction.[15]

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Supporting Information Available: Experimental procedures including the synthesis and characterization of compounds reported herein, X-ray data for **2c**, plus details of saturation transfer experiments and Eyring analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) The term "sofa" has been used for a six-membered ring conformation in which five of the ring atoms lie approximately in a plane. For an interesting discussion of the genesis of the "sofa" moniker, see: Nickon, A.; Silversmith, E. F. *Organic Chemistry: The Name Game. Modern Coined Terms and their Origins*; Pergamon Press: New York, 1987; Chapter 7.

(11) This change is most likely due to an electronic effect of the deprotonated sulfur atom. The original cis/trans ratio is re-established immediately upon the addition of HOAc to the NMR tube.

(12) IR analysis indicated that 1 equiv of K-Im deprotonates only 75% of **3b** under these conditions. As a control, we synthesized thiol **7** and noted a similar shift of −12 cm^{-1} (from 1651 to 1639 cm^{-1}) upon treatment with K-Im.

(13) We investigated control amide **7** in CD_3CN by ^{19}F ST NMR and found no change in $\Delta G^{\ddagger}$ upon addition of 1 equiv of K-Im, and an increase of 1.0 kcal mol^{-1} upon addition of 2 equiv of K-Im.

(14) (a) Fernandez, L. P.; Hepler, L. *J. Am. Chem. Soc.* **1959**, *81*, 1783; (b) Liotta, C. L.; Hopkins, H. P., Jr.; Kasudia, P. T. *J. Am. Chem. Soc.* **1974**, *96*, 7153.

(15) We also found the rate of isomerization in **3b** with 1 equiv of K-Im to be first-order in substrate concentration between 5 and 20 mg/mL, further suggesting an intramolecular interaction.