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Stability of Medium-Bridged Twisted Amides in Aqueous Solutions

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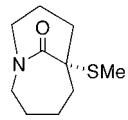

This twisted amide is **soluble** and **stable** in water

"Twisted" amides containing nonstandard dihedral angles are typically hypersensitive to hydrolysis, a feature that has stringently limited their utility in water. We have synthesized a series of bridged lactams that contain a twisted amide linkage but exhibit enhanced stability in aqueous environments. Many of these compounds were extracted unchanged from aqueous mixtures ranging from the strongly basic to the strongly acidic. NMR experiments showed that tricyclic lactams undergo reversible hydrolysis at extreme pH ranges but that a number of compounds in this structure class are indefinitely stable under physiologically relevant pH conditions; one bicyclic example was additionally water-soluble. We examined the effect of structure on the reversibility of amide bond hydrolysis, which we attributed to the transannular nature of the amino acid analogs. These data suggest that medium-bridged lactams of these types should provide useful platforms for studying the behavior of twisted amides in aqueous systems.

Introduction

The amide bond is one of the most important linkages in nature because of its presence in peptides and protein structures; its suitability for this central role derives from its resistance to hydrolysis.[1] The stability of planar amide bonds has been classically explained by resonance delocalization of the nitrogen lone pair into the carbonyl group (e.g., in the most common trans isomer, where $\omega = 180°$, Figure 1a). Although the resonance model has been the subject of vigorous reexamination,[2−6] the importance of C−N bond rotation on the properties of the amide bond has been solidly established. Thus, the nonplanar or "twisted" amides that approach ω angles of 90° are hypersensitive to hydrolysis and more basic than standard amides, contain pyramidal rather than planar nitrogen atoms, and react with protons or electrophiles at nitrogen rather than oxygen (Figure 1b).[7,8] Though much rarer than standard amides, such species are important because they are encountered during cis−trans isomerization processes essential to protein folding,[9−11] have been proposed as intermediates in amide bond cleavage,[12−14] and are required to experimentally examine the effect of bond rotation on any type of amide reactivity such as protonation[15−17] or other reactions.[8,18−20]

The most effective way of capturing an amide bond in a twisted conformation is to constrain it into a bicyclic ring system in which the amide nitrogen is present at a bridgehead

(1) *Amide Linkage: Selected Structural Aspects in Chemistry, Biochemistry, and Materials Science*; Greenberg, A., Breneman, C. M., Liebman, J. F., Eds.; Wiley-Interscience: New York, 2000.
(2) Lauvergnat, D.; Hiberty, P. C. *J. Am. Chem. Soc.* **1997**, *119*, 9478–9482.
(3) Basch, H.; Hoz, S. *Chem. Phys. Lett.* **1998**, *294*, 117–125.
(4) Wiberg, K. In *The Amide Linkage: Structural Significance in Chemistry, Biochemistry, and Materials Science*; Greenberg, A., Breneman, C. M., Liebman, J. F., Eds.; Wiley-Interscience: New York, 2000; pp 33−45.
(5) Quinonero, D.; Frontera, A.; Capo, M.; Ballester, P.; Suner, G. A.; Garau, C.; Deya, P. M. *New J. Chem.* **2001**, *25*, 259–261.
(6) Ilieva, S.; Hadjieva, B.; Galabov, B. *J. Org. Chem.* **2002**, *67*, 6210–6215.
(7) Greenberg, A. In *Structure and Reactivity*; Liebman, J. F., Greenberg, A., Eds.; VCH: New York, 1988; pp 139−178.
(8) Greenberg, A. In *The Amide Linkage: Structural Significance in Chemistry, Biochemistry, and Materials Science*; Greenberg, A., Breneman, C. M., Liebman, J. F., Eds.; Wiley-Interscience: New York, 2000; pp 47−83.
(9) Shao, H.; Jiang, X.; Gantzel, P.; Goodman, M. *Chem. Biol.* **1994**, *1*, 231–4.
(10) Fischer, G.; Schmid, F. X. In *Molecular Chaperones and Folding Catalysts*; Harwood Academic Publishers: Amsterdam, 1999; pp 461−489.
(11) Schmid, F. X. *Adv. Protein Chem.* **2002**, *59*, 243–282.
(12) Somayaji, V.; Brown, R. S. *J. Org. Chem.* **1986**, *51*, 2676–2686.
(13) Brown, R. S. In *The Amide Linkage: Structural Significance in Chemistry, Biochemistry, and Materials Science*; Greenberg, A., Breneman, C. M., Liebman, J. F., Eds.; Wiley-Interscience: New York, 2000; pp 85−114.
(14) Mujika, J. I.; Mercero, J. M.; Lopez, X. *J. Am. Chem. Soc.* **2003**, *127*, 4445–4453.
(15) Werstiuk, N. H.; Brown, R. S.; Wang, Q. *Can. J. Chem.* **1996**, *74*, 524–532.
(16) Mujika, J. I.; Mercero, J. M.; Lopez, X. *J. Phys. Chem. A* **2003**, *107*, 6099–6107.
(17) Ly, T.; Krout, M.; Pham, D. K.; Tani, K.; Stoltz, B. M.; Julian, R. R. *J. Am. Chem. Soc.* **2007**, *129*, 1864–1865.
(18) Kirby, A. J.; Komarov, I. V.; Kowski, K.; Rademacher, P. *J. Chem. Soc., Perkin Trans. 2* **1999**, 1313–1316.

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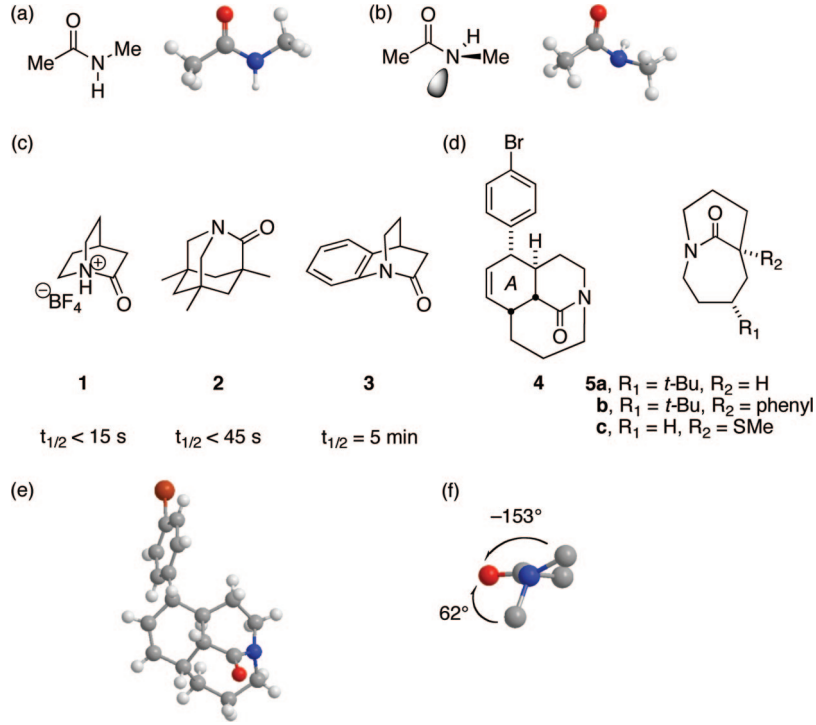

FIGURE 1. Generalized depictions of (a) a trans amide linkage and (b) a twisted, N-pyramidalized amide. (c) Literature examples of twisted amides reported by the groups of Stoltz (**1**),[21] Kirby (**2**),[22−24] and Brown (**3**).[13] Approximate half-lives for hydrolysis of these amides in water solution are indicated. (d) Bridged bicyclic amides examined in the present study. (e) Ball and stick depiction of the X-ray structure of compound **4** and (f) the isolated amide region of this structure along with the two ω values.

position;[7,19,25−31] iconic examples include the parent quinuclidone **1**[21] and the adamantane analog **2** (Figure 1c).[22−24] Such compounds are challenging to prepare, in part as a result of the extreme lability of their amide bond relative to ordinary amides. In particular, the predicted sensitivity of these compounds to hydrolysis has been amply demonstrated; half-lives of hydrolysis in water of less than 1 min are commonly observed for known bridgehead lactams. This lability has limited the study of twisted amide reactivity in water.[23,24] In addition, although orthogonal amides in principle have promise as inhibitors of protease or isomerase enzymes, this instability has ruled out their study for such purposes altogether.

There is no reason why the study of amides ought be limited to fully planar or fully twisted examples. To wit, we have presented a new class of bridged amides in which the amide linkage spans a medium size, 9-membered ring.[20,32] Prepared by the intramolecular ring-expansion reaction of a precursor azidoalkyl ketone, these compounds were shown to exhibit spectroscopic properties typically associated with other types of twisted amides and also displayed novel chemistry previously unobserved in any other standard or twisted amides. *We now report that such medium bridged lactams are also remarkably stable in aqueous solutions, both kinetically and thermodynamically, and present one example that is both soluble and indefinitely persistent in pure water.*

Results and Discussion

Two kinds of bridged lactams were used in this study: the tricyclic derivative **4** and several bicyclic examples **5** (Figure 1d). Lactam **4** contains a heavily pyramidalized nitrogen atom with C−C(O)−N−C dihedral angles of −153.4° and 62.0° [33] and a Dunitz−Winkler twist angle[34] of $\tau = 50.7°$ (the τ value for a planar amide is 0° and a fully orthogonal one is 90°). A previously reported analog of **5** (where R_1 = C(O)-pyrrolidinyl

(19) Bashore, C. G.; Samardjiev, I. J.; Bordner, J.; Coe, J. W. *J. Am. Chem. Soc.* **2003**, *125*, 3268–3272.
(20) Lei, Y.; Wrobleski, A. D.; Golden, J. E.; Powell, D. R.; Aubé, J. *J. Am. Chem. Soc.* **2005**, *127*, 4552–4553.
(21) Tani, K.; Stoltz, B. M. *Nature* **2006**, *441*, 731–734.
(22) Kirby, A. J.; Komarov, I. V.; Wothers, P. D.; Feeder, N. *Angew. Chem., Int. Ed.* **1998**, *37*, 785–786.
(23) Kirby, A. J.; Komarov, I. V.; Feeder, N. *J. Chem. Soc., Perkin Trans. 2* **2001**, 522–529.
(24) Kirby, A. J.; Komarov, I. V.; Feeder, N. *J. Am. Chem. Soc.* **1998**, *120*, 7101–7102.
(25) Greenberg, A.; Moore, D. T.; DuBois, T. D. *J. Am. Chem. Soc.* **1996**, *118*, 8658–8668.
(26) Goodman, M.; Shao, H.; Jiang, X.; Gantzel, P. *Peptides: Chemistry, Structure and Biology−Proceedings of the 14th American Peptide Symposium*; Intercept Ltd.: U.K., 1996; pp 477−478.
(27) Yamada, S. In *The Amide Linkage: Structural Significance in Chemistry, Biochemistry, and Materials Science*; Greenberg, A., Breneman, C. M., Liebman, J. F., Eds.; Wiley-Interscience: New York, 2000; pp 215−246.
(28) Sparks, S. M.; Vargas, J. D.; Shea, K. J. *Org. Lett.* **2000**, *2*, 1473–1475.
(29) Chow, C. P.; Shea, K. J.; Sparks, S. M. *Org. Lett.* **2002**, *4*, 2637–2640.
(30) Sparks, S., M.; Chow, C., P.; Zhu, L.; Shea, K. J. *J. Org. Chem.* **2004**, *69*, 3025–3035.
(31) Ribelin, T. P.; Judd, A. S.; Akritopoulou-Zanze, I.; Henry, R. F.; Cross, J. L.; Whittern, D. N.; Djuric, S. W. *Org. Lett.* **2007**, *9*, 5119–5122.
(32) Golden, J. E.; Aubé, J. *Angew. Chem., Int. Ed.* **2002**, *41*, 4316–4318.
(33) Compound **4** was previously published,[20] but a new crystal structure was determined for this work. The crystal of lactam **4** ($C_{18}H_{20}NOBr$) used for the X-ray structure determination was a pseudo-merohedral twin having two domains with nearly equal (56%/44%) volumes. These domains were related by a 180° rotation about the [−1 0 1] direction of the monoclinic unit cell chosen for the final refinement. This monoclinic unit cell had $a = c$ and metrically mimicked a C-centered orthorhombic unit cell for which the intensity data gave $R_{sym} = 0.079$ when averaged according to orthorhombic Laue symmetry. See cif file (Supporting Information) for details.
(34) Winkler, F. K.; Dunitz, J. D. *J. Mol. Biol.* **1971**, *59*, 169–82.

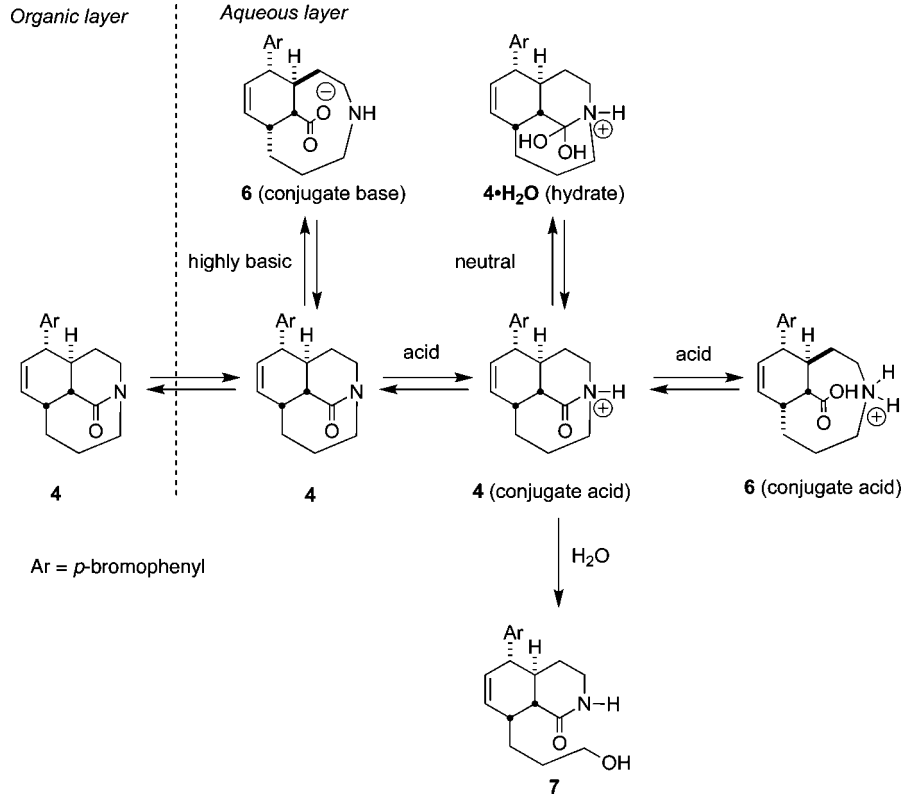

FIGURE 2. Proposed species for compound **4** in organic (ethyl acetate) and aqueous layers of different pH.

and the seven-membered ring containing a double bond[20] has $\tau = 50.3$. Lactams were prepared via the intramolecular ring insertion of an alkyl azide into a carbonyl group as previously reported,[20,32] generally as the minor component of a mixture also containing the regioisomeric fused lactam; the additional six-membered ring (labeled *A* in Figure 1d) or *tert*-butyl group were incorporated to enhance the proportion of bridged lactam obtained in this synthesis.

TABLE 1. Extraction Studies of Lactam 4

entry	conditions[a]	time, temp	result
1	1:4 H_2O/CH_3CN	20 h, rt	>85% recovery of **4**
2	1:8 aq NaOH/CH_3CN (pH ca. 14)	20 h, rt	>85% recovery of **4**
3	1:24 aq NaOH/CH_3CN (pH ca. 14)	22 h, 80 °C	>80% recovery of **4**
4	1:8 aq HCl/CH_3CN (pH ca. 1)	20 h, rt	>80% recovery of **4**
5	1:8 aq HCl/CH_3CN (pH ca. 1)	8 days, rt	>85% recovery of **4**
6	1:24 aq HCl/CH_3CN (pH ca. 1)	23 h, 80 °C	conversion to **7** (95% yield)

[a] The pH values noted refer to the aqueous layers.

The first indication that this class of compounds had unusual properties came from a simple set of extraction experiments. Thus, samples of tricyclic lactams **4** were dissolved in aqueous acetonitrile solutions, vigorously stirred for ca. 20 h, and then extracted with an organic solvent (ethyl acetate). A twisted amide with a short half-life toward hydrolysis would be expected to afford amino acid under such conditions. In sharp contrast to this expectation, it proved possible to recover unchanged compound **4** in high (generally >85%) yield after this treatment (it is likely that the recoveries were <100% due to modest solubility in the acetonitrile-containing aqueous layer). As shown in Table 1, similar results were obtained when the lactams were challenged by extremely acidic or basic conditions, longer dissolution times in acid, and higher temperatures in base. In fact, the only irreversible chemical reaction that was observed in these experiments took place when **4** was placed in aqueous HCl/acetonitrile at 80 °C for 23 h, which led to the regioselective cleavage of one of the C−N bonds adjacent to the amide bond, affording **7** (Figure 2). This unconventional mode of amide hydrolysis is emblematic of the unusual reactivity imparted by the twisted amide linkage.[20]

The recovery of high quantities of amide from this range of aqueous solutions is consistent with two possible scenarios. The first would be that the amide linkage is thermodynamically stabilized in these molecules. Thus, dissolution of **4** in an aqueous environment could result in reversible amide hydrolysis,[24] with reclosure of the amide occurring in the presence of a large excess of water, permitting re-extraction of the original compound. Some of the species that would be expected in water under acid or basic conditions are depicted in Figure 2. The second possibility is that the amide bond is kinetically stabilized toward hydrolysis.

We addressed this issue by dissolving the twisted amides in D_2O/THF-d_8 solutions at neutral, acidic, or basic pH and directly observing these solutions via ^{13}C NMR spectroscopy (Figure 3 and Table 2). The chemical shift of the signal arising from the carbonyl carbon provided evidence for the kinetic stability under neutral conditions, with other species being observed under strongly basic or acidic condition. Thus, the ^{13}C NMR signal for the carbonyl carbon in $CDCl_3$ is at 187 ppm (Figure 3a); upon dissolution in 1:1 D_2O/THF-d_8, this signal moves slightly downfield to 189 (Figure 3b). When **4** is dissolved in other organic solvents or in 1:1 D_2O/THF-d_8, the carbonyl signal always appears between 185 and 190 ppm. At pH ca. 14, this

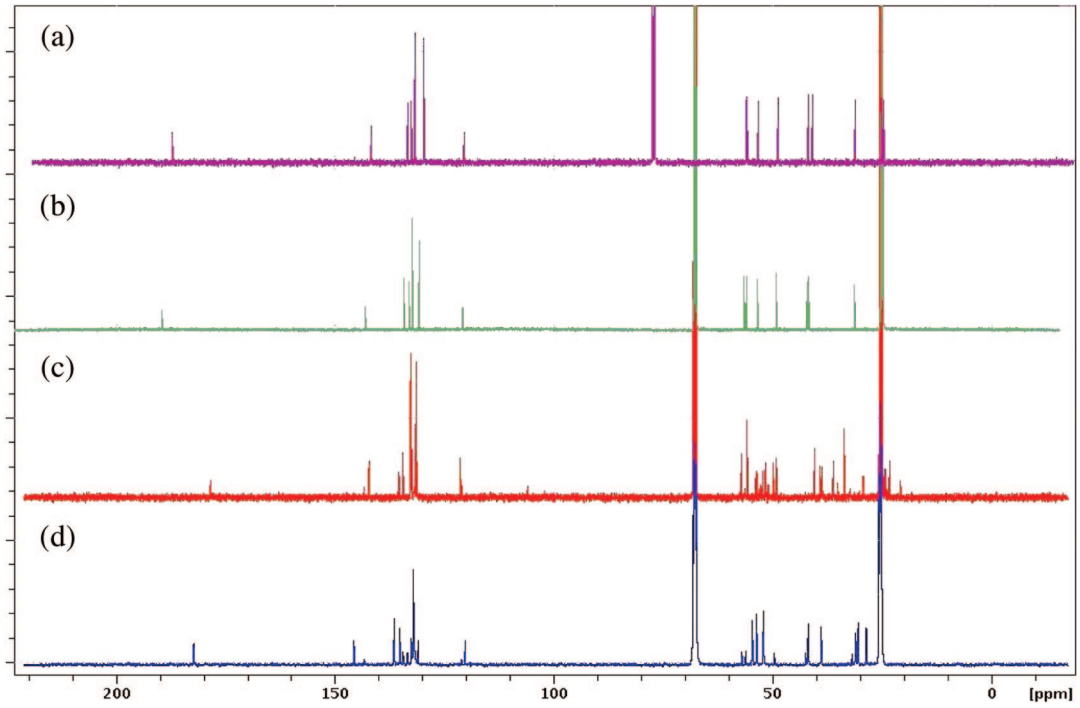

FIGURE 3. ^{13}C NMR spectra of compound **4** dissolved in (a) $CDCl_3$, (b) 1:1 D_2O/THF-d_8, (c) 1:6 DCl (1 N in D_2O)/THF-d_8, and (d) 1:6 NaOD (1 N in D_2O)/THF-d_8.

TABLE 2. ^{13}C NMR Carbonyl Shifts of Lactam 4 and Its Derivatives

entry	conditions	assignment	shift (ppm)
1	$CDCl_3$	**4**	187.1
2	THF-d_8	**4**	185.0
3	DMSO-d_6	**4**	186.2
4	1:1 D_2O/THF-d_8	**4**	189.6
5	1:6 DCl (1 N in D_2O)/THF-d_8	**6** (conjugate acid)	178.5
		4·H_2O (hydrate)	106.1
6	1:6 DCl (1 N in D_2O)/DMSO-d_6	**6** (conjugate acid)	178.5
		4 (conjugate acid)	176.9
		4·H_2O (hydrate)	104.9
7	1:6 NaOD (1 N in D_2O)/THF-d_8	**6** (conjugate base)	182.3
8	1:6 NaOD (1 N in D_2O)/DMSO-d_6	**6** (conjugate base)	179.0
9	$CDCl_3$	**4** (conjugate acid)	176.9

signal is replaced by an upfield signal at 182 ppm. In strong base (Figure 3d), the most reasonable assignment for this signal is for the carboxylate anion arising from clean conversion to the conjugate base of the amino acid **6** (Figure 2). This assignment is consistent with normal values for simple carboxylic acids. For example, the carbonyl of acetic acid in $CDCl_3$ appears at 178.1 ppm and the corresponding signal for the conjugate base is at 181.5 in aqueous solution.[35] In mixtures of DCl (pH ca. 1) and either THF-d_8 (Figure 3c) or DMSO-d_6, several species can be observed. In the former, the predominant peak occurs at 178 ppm (entry 5), whereas two peaks in the same range appear when DMSO is used as the cosolvent (176.9 and 178.5, entry 6). We assign the more downfield of these peaks to the N-protonated form of **6** shown in Figure 2 and the 176.9 peak in DMSO to the protonated form of lactam **4**. This tentative assignment is based on the fact that the corresponding signal in fully characterized salts of **4** and closely related compounds appear between 176−177.5 ppm in $CDCl_3$ (entry 9).[36] However, solvent effects could modulate the location of these peaks, leaving open the possibility that we are simply observing N-protonation of **4** in acid/THF as opposed to ring opening (obviously, in acid/DMSO *both* occurrences provide the only reasonable explanation for the data). This would still be remarkable, however, given the extreme tendence of most twisted lactams to quickly hydrolyze under these conditions. In addition, we assigned the small signal observed at 106 ppm to the hydrate **4**·H_2O, also protonated on nitrogen. Finally, the unconventional conversion of lactam **4** to alcohol **7** entails nonreversible attack of water on a carbon adjacent to the amine in the N-protonated **4**; the group that specifically undergoes this attack is that revealed by X-ray to have the most severe deviation from planarity ($\omega = -62°$; Figure 1f).

The presence of the various species noted in Figure 2 was also supported by mass spectrometry (Table 3). Thus, MS measurements were taken from samples prepared as described above using neutral, strongly basic, or strongly acidic conditions. Aliquots from each experiment were diluted by the solvents noted in the table to prepare them for ionization. These results supported the qualitative data as discerned from the NMR work described above. Thus, only starting lactam **4** was observed in MS measurements from samples of **4** dissolved in D_2O/THF, regardless of whether samples were diluted by THF or water prior to injection. Under basic conditions (entries 3−6) the solvent used for ionization played an important role in determining whether parent lactam **4** (MW 346) or the corresponding amino acid (MW 364) was observed. Thus, when the samples prepared from strongly basic NaOD and THF were dissolved in nonpolar solvents (THF or CH_3CN), only **4** could be detected by MS (entries 3 and 4). In contrast, however, when the same

(35) Silverstein, R. M.; Bassler, G. C.; Morrill, T. C. *Spectroscopic Identification of Organic Compounds*; John Wiley and Sons: New York, 1991.

(36) N-Protonation is a common and distinctive feature of twisted amides (regular amides protonate predominantly on nitrogen). The conjugate acid of compound **3** can be made by simply adding toluenesulfonic acid to the amide in ether; the resulting salt has been purified by recrystallization and characterized by X-ray crystallography. Lei, Y. Ph.D. Dissertation, University of Kansas, 2006.

TABLE 3. ESI MS Experiments with Lactam 4[a]

entry	conditions	solvent used for ionization	exact mass (es) observed	assignments
1	1:1 D_2O/THF	THF	346.0818	**4**
2	1:1 D_2O/THF	H_2O	346.0797	**4**
3	1:6 NaOD (1 N in D_2O)/THF	THF	346.0856	**4**
4	1:6 NaOD (1 N in D_2O)/THF	CH_3CN	346.0792	**4**
5	1:6 NaOD (1 N in D_2O)/THF	H_2O	364.0918	**6**
6	1:6 NaOD (1 N in D_2O)/THF	DMSO	364.0920	**6**
7	1:6 DCl (1 N in D_2O)/THF	THF	346.0752; 364.0903 (ratio ca. 1:1)	**4** and **6**
8	1:6 DCl (1 N in D_2O)/THF	H_2O	346.0722; 364.0913	**4** and **6**
9	1:6 DCl (1 N in D_2O)/THF	MeOH/H_2O/HCOOH	346.0761; 378.1022 (ratio ca. 3:1)	**4** and the methyl ester of **6**
10	1:6 DCl (1 N in D_2O)/THF	CH_3CN	346.0776; 364.0916 (ratio ca. 1:1)	**4** and **6**

[a] Relevant HRMS calculations: HRMS calcd for $C_{18}H_{21}BrNO$ ($M^+ + H$) 346.0806 (compound **4**); HRMS calcd for $C_{18}H_{23}BrNO_2$ ($M^+ + H$) 364.0912 (compound **6**); and HRMS calcd for $C_{19}H_{25}BrNO_2$ ($M^+ + H$) 378.1069 (methyl ester of compound **6**).

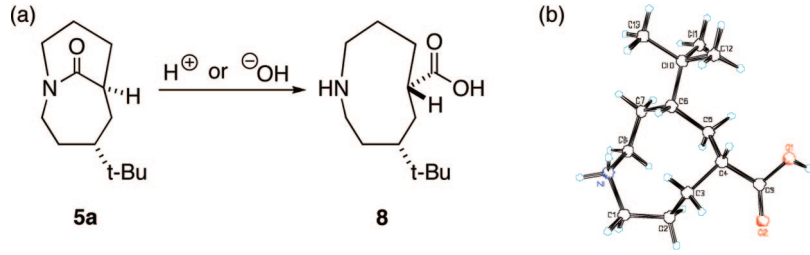

FIGURE 4. (a) Results of treating **5a** with strong acid or base. (b) X-ray structure of amino acid **8**.

TABLE 4. Extraction Studies of Bicyclic Lactams

entry	lactam	solvent	time, conditions	result
1	**5a**	1: 1 D_2O/THF-d_8	13 d, rt	ca. 50% recovery of **5a**
2	**5a**	aq HCl (1.0N)	0.25 h, rt	conversion to **8**
3	**5a**	aq NaOH (1.0N)	3 h, rt	conversion to **8**
4	**5b**	buffer (pH 4)/CH_3CN	2 h, rt	>90% recovery of **5b**
5	**5b**	buffer (pH 10)/CH_3CN	2 h, rt	>90% recovery of **5b**
6	**5c**	D_2O	6 d, rt	>90% recovery of **5c**
7	**5c**	buffer (pH 4)	2 h, rt	>90% recovery of **5c**
8	**5c**	buffer (pH 10)	2 h, rt	>90% recovery of **5c**

samples were diluted by either water or DMSO, no parent ion corresponding to **4** was observed. Instead, weak signals corresponding to **6** were present in each spectra (we ascribe the weakness of these signals to the relative difficulty of forming a positive ion in the MS from zwitterions **6**). Samples in which **4** was dissolved in 1 N aqueous DCl/THF mixtures generally

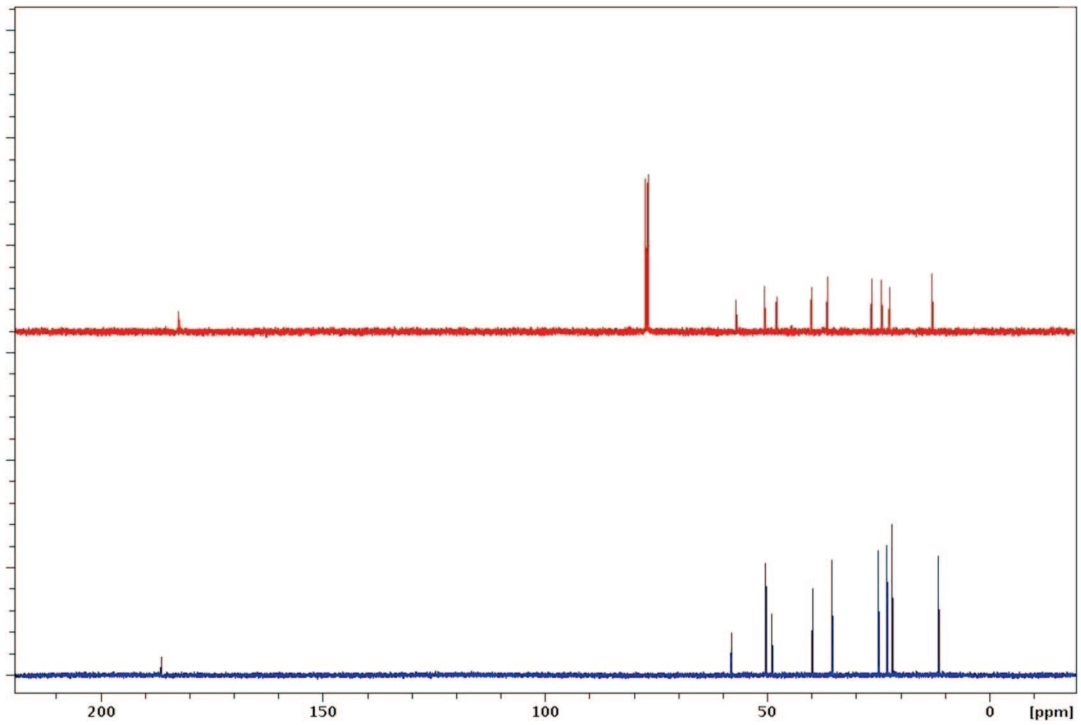

FIGURE 5. ^{13}C NMR spectra of lactam **5c** in (a) $CDCl_3$ and (b) D_2O.

led to well-behaved MS spectra in which both **4** or **6** could be observed regardless of how the samples were prepared for the MS experiments. Interestingly, the ratio of **4** over **6** was higher when the samples were prepared using water as a diluent versus either THF or CH_3CN. Additionally, the methyl ester of **6** was observed when the MS experiments were prepared by dissolution with a ca. 95:4:1 MeOH/water/aqueous formic acid mixture (entry 9). Overall, these experiments are consistent with the assignments made from the NMR experiments.

These data are consistent with both kinetic and thermodynamic stability at neutral pH. When the amides are subjected to strong acid or base, hydrolysis occurs, but the amide bond is able to reform even in a medium containing 50% water. It is possible that small amounts of unobserved amide persist in water under extreme pH conditions, but the high yields of recovered bridged amide jibe with the NMR results only if reclosure of the amino acid form is possible. Numerous attempts were made to retrieve validated samples of zwitterions **6** or derivated thereof by concentrating the aqueous samples (acid or basic solutions) and examining the residues by NMR. However, only starting lactam **4** was observed under these conditions, strongly suggesting that the removal of water from these samples by any means is sufficient to shift the equilibrium of the compound back to lactam **4**.

We hypothesize that the bridged amides are stabilized by a scaffolding effect of the medium ring. In addition, once the lactam bond is hydrolyzed, the resulting amino acid has the carboxylic acid and amine moieties on the opposite sides of a medium-size ring where they should be subject to strong proximity effects. While transannular reactions across medium- or large-size rings have been frequently observed, the formation of a twisted amide in water is highly unusual. The most relevant prior observations are the spontaneous formation of the quinuclidone system under mass spectrometry conditions[17] and a single example of amide bond formation in the highly constrained adamantane system in acid.[24]

The role of conformation in constraining the open nine-membered amino acid form was of interest as species **6** in Figure 2 exhibits *in*/*out* isomerism.[37] Thus, the six-membered ring holds the carboxylic acid in place following amide hydrolysis to the medium-size ring. The importance of this was underlined when exposure of lactam **5a**, lacking this ring, to strongly acidic or basic conditions did not permit reisolation of the unchanged amide, as was the case for compound **4**. Removal of all solvent did afford a quantitative yield of amino acid **8**. Here, X-ray crystallography established that this compound was able to undergo a conformational change that afforded an amino acid in which the carboxylic acid has now flipped to the outside perimeter of the nine-membered ring, where it is unable to reach the amide group (Figure 4).[38] These observations are consistent with the proposed role of transannular interactions in contributing to the reversibility of the amide bond formation.

The determination of the limits of thermodynamic stability in simple bicycles such as **5a** allowed us to examine the kinetic stability of this series of amides through extraction experiments using organic solvents (either CH_3CN or tetrahydrofuran (THF)) and buffer (Table 4). Although the unsubstituted amide **5a** was largely stable in neutral solutions (with a half-life of ca. 13 days, as determined by NMR), it underwent irreversible conversion to amino acid under even moderately acidic (pH ca. 4) or basic (pH ca. 10) conditions. However, the phenyl-substituted analog **5b** could be completely recovered following exposure to mixed solvents.

A lactam containing an α-methylthio group and lacking the *tert*-butyl substituent (**5c**) was fully water-soluble, which allowed its study in pure aqueous solutions at pH = 4, 7, and 10. Under all of these conditions, lactam **5c** was stable throughout the course of study (Table 4, entries 6−8). The kinetic stability of this compound was further demonstrated by comparing its ^{13}C NMR spectra taken in $CDCl_3$ and D_2O, which were similar (Figure 5). These studies establish that the enhanced stability of these "mid-range" twisted amides is not solely due to scaffolding/reversibility effects but may also depend on their degree of twist or steric effects arising from embedding the amide carbonyl group into the middle of the ring system.

Summary

This new class of medium bridged lactams will further the study of the effect of dihedral angle and twist value on chemical reactivity. These compounds are clearly twisted amides due to their substantial twist values of ca. 60°, characteristic spectral and chemical properties (i.e., N-protonation), and the fact that they undergo unprecedented chemical reactivity such as adjacent N−C cleavage (e.g., **4** → **7**).[20] However, they offer superior kinetic and thermodynamic water stability relative to known classes of twisted amides and are readily amenable to synthesis and structural variation. It has been suggested that twisted amides would provide an attractive platform for the study of enzymatic folding and proteolysis properties.[1] However, existing systems were sufficiently unstable in water and/or insufficiently diversifiable that nearly all aqueous studies of twisted amides to date have been limited to their hydrolytic behavior. We anticipate that medium bridged amides will enhance the range of chemical and biological studies possible with compounds containing unconventional amide linkages.

Experimental Section

6-(Methylthio)-1-azabicyclo[4.3.1]decan-10-one (5c) and 9a-(Methylthio)hexahydro-1*H*-pyrrolo[1,2-*a*]azepin-5(6*H*)-one. To a solution of 2-(3′-azidopropyl)-2-(methylthio)cyclohexanone (0.0910 g, 0.40 mmol, 1.0 equiv, Supporting Information) in CH_2Cl_2 (8.0 mL, 0.05 M) was added TfOH (0.18 mL, 2.0 mmol, 5.0 equiv) in one portion at 0 °C, and the resulting solution was stirred at 0 °C for 2.5 min. The reaction was quenched with saturated $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The organic layer was washed with brine (1 × 20 mL), dried (Na_2SO_4), and concentrated. Flash chromatography (1/2 EtOAc/hexanes, followed by EtOAc) afforded compound **5c** as a pale yellow oil (R_f = 0.57, 1/1 EtOAc/hexanes), yield 65% (0.0525 g, 0.26 mmol) and 9a-(methylthio)hexahydro-1*H*-pyrrolo[1,2-*a*]azepin-5(6*H*)-one as a colorless oil (R_f = 0.31, 1/1 EtOAc/hexanes), yield 15% (0.0120 g, 0.06 mmol). Compound **5c**: ^1H NMR (400 MHz, $CDCl_3$) δ 1.53−1.79 (complex, 4H), 1.80−1.99 (complex, 4H), 2.05−2.14 (complex, 4H), 2.22−2.29 (m, 1H), 2.80−2.86 (m, 1H), 3.18−3.24 (m, 1H), 3.43 (dt, J = 2.8, 12.0 Hz, 1H), 3.86−3.94 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.8, 22.5, 24.3, 26.5, 36.4, 40.1, 47.9, 50.6, 57.0, 182.4; ^1H NMR (500 MHz, D_2O) δ 1.37−1.46 (m, 1H), 1.49−1.64 (complex, 2H), 1.65−1.71 (complex, 2H), 1.78−1.86

(37) Alder, R. W.; East, S. P. *Chem. Rev.* **1996**, *96*, 2097–2111.

(38) The crystal of compound **8** contained 0.25 mol of cocrystallized NaCl per mole of $[C_{13}H_{26}NO_2][Cl]$. The Na ion was disordered over four general position sites and was therefore included in the structural model with an occupancy factor of 0.25. See cif file (Supporting Information) for details.

(m, 1H), 1.88−1.94 (m, 1H), 1.95−1.98 (m, 1H), 2.00 (s, 3H), 2.07−2.14 (m, 1H), 2.20−2.25 (m, 1H), 2.84−2.89 (m, 1H), 3.15−3.19 (m, 1H), 3.40 (dt, J = 2.9, 12.2 Hz, 1H), 3.61−3.66 (m, 1H); ^{13}C NMR (125 MHz, D_2O) δ 11.5, 22.0, 23.1, 25.0, 35.5, 39.8, 49.0, 50.3, 58.0, 186.2; IR (neat) 2927, 2860, 1686, 1445, 1173 cm^{-1}; HRMS calcd for $C_{10}H_{18}NOS$ (M^+ + H) 200.1109, found 200.1107.

9a-(Methylthio)hexahydro-1*H*-pyrrolo[1,2-*a*]azepin-5(6*H*)-one: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45−1.57 (m, 1H), 1.62−1.90 (complex, 4H), 1.97−2.22 (complex, 6H), 2.41−2.47 (m, 1H), 2.50−2.56 (m, 1H), 3.20 (dt, J = 2.2, 13.8 Hz, 1H), 3.48−3.57 (m, 1H), 3.68−3.75 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.2, 21.1, 23.7, 24.9, 37.2, 39.3, 43.1, 49.6, 174.7; IR (neat) 2926, 1632, 1429, 1406 cm^{-1}; HRMS calcd for $C_{10}H_{18}NOS$ (M^+ + H) 200.1109, found 200.1105.

General Procedure for Extraction Studies of Lactam 4. Lactam **4** was dissolved in a specified amount of CH_3CN at room temperature. After addition of water, aqueous HCl, or aqueous NaOH the reaction mixture was vigorously stirred under conditions specified in Table 1. Reactions were cooled to room temperature (if necessary) and extracted with EtOAc (4 × 10 mL). Combined organic layers were washed with water (1 × 10 mL) and brine (1 × 10 mL), dried (Na_2SO_4), and concentrated to afford the title compound. For entries 4−6 (Table 1) the reactions were neutralized with saturated $NaHCO_3$ before extraction with EtOAc.

Recovery of Lactam 4 from H_2O/CH_3CN Mixture. According to the general procedure, lactam **4** (40.0 mg, 0.116 mmol) was dissolved in 6.0 mL of CH_3CN, and 1.5 mL of H_2O was added. After stirring at room temperature for 20 h the reaction mixture was extracted with EtOAc (4 × 10 mL), and combined organic layers were washed with water (1 × 10 mL) and brine (1 × 10 mL), dried (Na_2SO_4), and concentrated to afford 34.9 mg (0.101 mmol) of the title compound. Yield 87%.

Conversion of Lactam 4 to Compound 7. According to the general procedure, lactam **4** (19.2 mg, 0.056 mmol) was dissolved in 6.0 mL of CH_3CN, and 0.25 mL of 1.0 N HCl was added. After stirring at room temperature for 30 min, the reaction was refluxed for 23 h. The reaction was cooled to room temperature, basified with saturated $NaHCO_3$, and extracted with EtOAc (4 × 10 mL), and the combined organic layers were washed with water (1 × 10 mL) and brine (1 × 10 mL), dried (Na_2SO_4), and concentrated. Flash chromatography (1/4 EtOAc/hexanes) afforded compound **7** as a colorless film, yield 95% (19.1 mg, 0.53 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.41−1.53 (m, 2H), 1.65−1.77 (m, 2H), 1.81−2.05 (m, 3H), 2.21−2.36 (br, 1H), 2.52 (dd, J = 4.8, 11.9 Hz, 1H), 2.79−2.84 (m, 1H), 3.06 (dd, J = 1.4, 10.0 Hz, 1H), 3.16 (dt, J = 3.6, 12.1 Hz, 1H), 3.24−3.29 (m, 1H), 3.51−3.58 (m, 1H), 3.60−3.67 (m, 1H), 5.56−5.59 (m, 1H), 6.04−6.09 (m, 1H), 6.26−6.34 (br, 1H), 7.01 (d, J = 8.3 Hz, 2H), 7.46 (d, J = 8.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.3, 29.1, 29.8, 33.7, 36.5, 40.4, 44.3, 46.1, 48.8, 119.6, 128.7, 128.9, 130.5, 130.6, 141.4, 171.3; IR (neat) 3223, 3135, 2995, 2890, 1630, 1455, 795, 705 cm^{-1}; HRMS calcd for $C_{18}H_{23}BrNO2$ (M^+ + H) 364.0912, found 364.0910.

Synthesis of Amino Acid 8 under Basic Conditions. A 10-mL round-bottom flask was charged with lactam **5a** (20.0 mg, 0.096 mmol, 1.0 equiv) and NaOH (1.0 N in H_2O) (0.10 mL, 0.096 mmol, 1.0 equiv). The flask was stirred for 3 h. The solvent was evaporated, and the flask was left under vacuum overnight to give the title compound as a white solid. Yield: 100% (24.0 mg, 0.096 mmol). Mp > 300 °C; ^{1}H NMR (400 MHz, D_2O) 0.87 (s, 9H), 1.18−1.42 (m, 3H), 1.46−1.57 (m, 1H), 1.64−1.92 (m, 5H), 2.22−2.35 (m, 1H), 2.66−2.79 (m, 2H), 2.81−2.96 (m, 2H); ^{13}C NMR (100 MHz, D_2O) 20.2, 24.9, 26.6, 27.7, 28.7, 33.5, 41.2, 42.0, 46.6, 48.2, 186.3; IR (KBr) 3370, 2880, 1525, 1375 cm^{-1}; HRMS calcd for $C_{13}H_{26}NO_2$ (M^+ + H) 228.1963, found 228.1958.

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Supporting Information Available: Experimental details, characterization data for new compounds, and cif files for compounds **4** and **8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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