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The Most Reactive Amide As a Transition-State Mimic For *cis*–*trans* Interconversion

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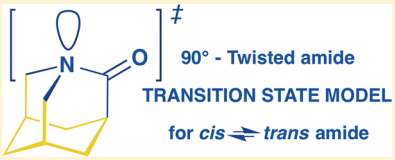
Supporting Information

ABSTRACT: 1-Azatricyclo[3.3.1.1^{3,7}]decan-2-one (**3**), the parent compound of a rare class of 90°-twisted amides, has finally been synthesized, using an unprecedented transformation. These compounds are of special interest as transition-state mimics for the enzyme-catalyzed *cis*–*trans* rotamer interconversion of amides involved in peptide and protein folding and function. The stabilization of the amide group in its high energy, perpendicular conformation common to both systems is shown for the rigid tricyclic system to depend, as predicted by calculation, on its methyl group substitution pattern, making **3** by some way the most reactive known "amide".



INTRODUCTION

There is a compelling body of evidence that the *cis*–*trans* rotamer interconversion of amide bonds in peptides and proteins plays a fundamental role in countless biochemical processes. In addition to its involvement as the rate-limiting step in protein folding,[1−3] recent studies have also identified key roles for the *cis*–*trans* interconversion in protein function. For example, signal transduction in immune cells involves peptidyl–prolyl bond *cis*–*trans* isomerization (Figure 1) in secondary messengers, catalyzed by rotamase enzymes.[4]

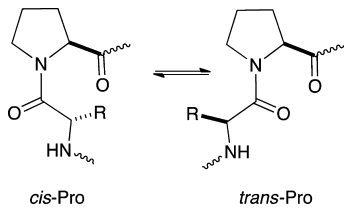


Figure 1. Peptidyl–prolyl bond *cis*–*trans* interconversion.

Similar deformations of the amide bonds in peptide substrates could in principle contribute to the catalytic efficiency of other classes of enzymes, including proteases[5−8] and oligosaccharyltransferase,[9] as well as to self-activation of proteins during splicing.[10,11] And *cis*–*trans* isomerization in the peptide backbone of anion channel, 5-hydroxytryptamine type 3 (5-HT$_3$) receptor, is reported to trigger the opening of the channel upon neurotransmitter binding.[12] We report the synthesis and a theoretical and experimental study of a molecule (**3**) which represents the closest transition-state (TS) model for the *cis*–*trans* interconversion of simple amides.

Our current understanding of the mechanisms of biochemical transformations of this sort relies in large part upon detailed knowledge of the structure and properties of reaction intermediates and TSs in simpler systems. TSs correspond to lowest saddle points on reaction potential energy surfaces, and their structures typically involve unusual bond angles and partial bonds which cannot be mimicked using direct connectivity with first or second row elements.[13,14] However, it is possible to design a stable molecule as a meaningful TS mimic if any conformational motion of the molecular fragments bearing the characteristic features of the TS would lead to a sharp overall potential energy increase. This is possible in rigid systems, where conformational motions are severely restricted. Of course a stable TS mimic can only offer a crude approximation of the corresponding short-lived TS,[15] but the degree of the approximation can be assessed by high-level computational techniques.

In 1998 we reported the synthesis of the "highly twisted amide" **1**,[16] an extreme case of a bridged lactam,[17] with the N—C═O fragment embedded in the rigid 1-azaadamantane skeleton, as a potential TS mimic for the *cis*–*trans* rotamer interconversion of the amide bond. Subsequent computational studies by Morgan et al.[18] came to the conclusion that "the use of amide **1** as a model of the TS to amide C–N bond rotation carries some risk". Comparing the calculated enthalpies for the hydrolysis of **1** and its di-, mono-, and nonmethyl-substituted derivatives revealed significant buttressing effects of the methyl substituents on the reactivity of **1**, leading to "artificial" stabilization of this TS mimic. More recently Tani and Stoltz[19]

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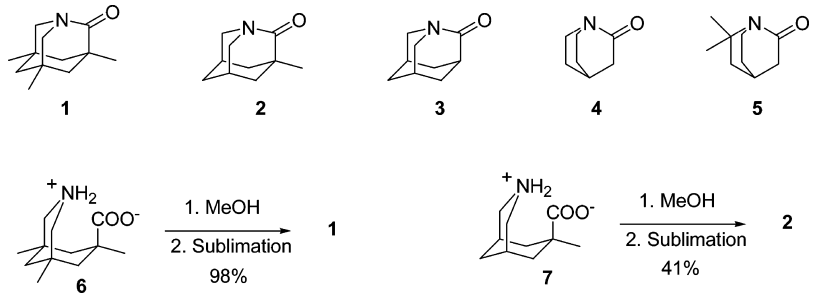

Figure 2. Highly twisted amide "family" compounds **1**−**5** and the preparation of **1** and **2**.

reported on the parent system **4** of the known Pracejus 2-quinuclidone **5**, which is similarly stabilized by methyl substitution.[20] In the absence of this evidently substantial stabilization the free base **4** could not be isolated, though the tetrafluoroborate salt was obtained, and its crystal structure reported.[19]

RESULTS AND DISCUSSION

We report the synthesis and spectroscopic characterization of **2**, the monomethyl derivative of **1**, and of the parent system **3** together with single crystal X-ray diffraction structures for **2** and for the conjugate acid **3**·HBF_4 of the parent system (Figure 2). Detailed comparisons of chemical reactivity, consistent with the calculations of the Morgan group, make **3** the most reactive known amide.

Synthesis of Twisted Amides. Twisted amide **1** was prepared from the amino acid **6** (obtained in seven steps from the Kemp triacid),[21] and **6** is converted spontaneously to some 80% of **1** in solution in methanol under mild, neutral conditions.[22] The monomethyl derivative **2** is also formed spontaneously on dissolving the corresponding monomethyl-substituted amino acid **7** in methanol, but significantly (about 10-fold) more slowly. In both cases evaporation of the methanolic solution followed by sublimation provides pure twisted amide. However, the synthesis of **3** (Figure 3) presented a major challenge.

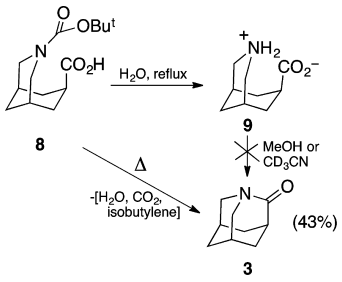

Figure 3. Preparation of **3**.

According to Morgan's calculations,[18] the methyl alpha to the carboxyl group should exert the biggest stabilizing effect. So it was no surprise to find that the amino acid **9**, lacking the α-methyl group, did not react when dissolved in methanol or in dry CD_3CN, in which both **6** and **7** cyclized faster than we could run their ^{1}H NMR solution spectra. Standard methods of amide formation also failed.

The clue to the successful synthesis of the parent compound **3** came from the observation of a strong peak at *m*/*e* 151 during the GS-MS identification of the N-Boc protected amino acid **8**[23] (molecular ion *m*/*e* 269, see the Supporting Information, SI). Boc protection is known to be thermally labile,[24] suggesting that the *m*/*e* 151 peak could correspond to the twisted amide **3**, formed by decomposition of **8** under the conditions of the gas chromatography (column temperature 210 °C).

The preparative synthesis of **3** was seriously hampered by the released water. Compounds **3** and **8** both prove to be highly volatile and difficult to separate from water in the gas phase. Thus, simple sublimation of **8** in vacuum at temperatures between 180 and 250 °C leads consistently to **3** heavily contaminated with starting material **8** and the amino acid **9**, the hydrolysis product of the evidently very reactive twisted amide. After extensive experimentation, we found that vacuum sublimation at elevated temperature of **8** preabsorbed on silica gel produces pure **3** in moderate (43%) yield. Under these conditions, the twisted amide product **3** presumably remains trapped on the silica surface, while water evaporates off finally compound **3** collects on the condenser. (The method also works well for the preparation of twisted amides **1** and **2**, from the corresponding N-Boc-protected amino acids, in yields of 63% and 51%, respectively.) Full details of the synthesis, spectroscopic evidence and crystal structure determinations confirming the structural assignments appear in the SI.

Structure and Spectroscopic Properties. Compound **2** is sufficiently stable in the absence of (other) nucleophiles for full characterization. Crystalline **3** is thermally moderately stable but polymerizes in solution within a few hours. Nevertheless, with care we could characterize compound **3** spectroscopically. We also prepared single crystals of both **2** and **3** for X-ray study, by slow vacuum sublimation. The molecular structure of **2** is shown in Figure 4, but the crystal

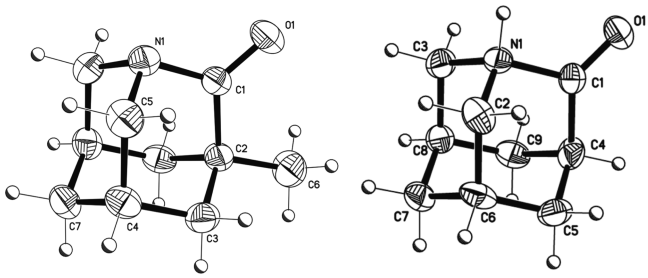

Figure 4. Molecular structures of twisted amides **2** (left) and **3**·H^+.

structure of **3**, typically for an adamantane derivative,[25] appears to be severely disordered, and no solution has so far proved possible. The crystal structure of the conjugate acid **3**·HBF_4 was solved and is included in Figure 4. Selected structural and spectroscopic parameters for 90° twisted amides **1**, **2**, and **3** and

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Table 1. Selected Structural Parameters and Spectroscopic Properties for Twisted Amides 1−3 and 4, for *N*-Methyl-2-piperidone 10,[26] and for *N,N*-Dimethylacetamide 11, Including TSs for its *cis*−*trans* Interconversion[a] (ref 18)

compound	1	2	3[b]	4[b]	4[c]	10	11	11 TS *syn*	11 TS *anti*
Selected Structural Parameters									
twist angle τ, °	90.5	90.0	90.55	105.05	90.0	2.5	0	90	90
Σ bond angles at N	325.7	325.6	326.9	324.2	327.1	358.9	360	338.6	332.7
Σ bond angles at C═O	359.9	359.9	360	359.8	360	359.9	360	360	360
C−N bond length, Å	1.475	1.448	1.450	1.443	1.433	1.375	1.38	1.45	1.46
C═O bond length, Å	1.196	1.201	1.203	1.203	1.183	1.232	1.23	1.21	1.21
Selected Spectroscopic Data[d]									
δ^{13}C of C═O[e]	199.5	199.8	198.6			165	171	–	–
IR ν_{max} of C═O, cm^{-1}	1732	1732	1733			1653	1645	–	–
EI-MS 100% peak	(M-CO)$^+$					**10**. CH_2═N^+Me		**11**. $MeCO^+$	

[a]Data from X-ray structures (**1**, **2**) or calculation: this work (**3**, **4**) and Morgan (**10**, **11**).[18] See the text. [b]Calculations using 6-31++G**/M06-2X. This work (for details see SI). [c]Calculations at the RHF/6-31G* level: Greenberg, A.; Venanzi, C. A. *J. Am. Chem. Soc.* **1993**, *116*, 6951. [d]Data from this work (**1**−**3**) and standard literature sources. [e]^{13}C NMR spectra run in CD_3CN.

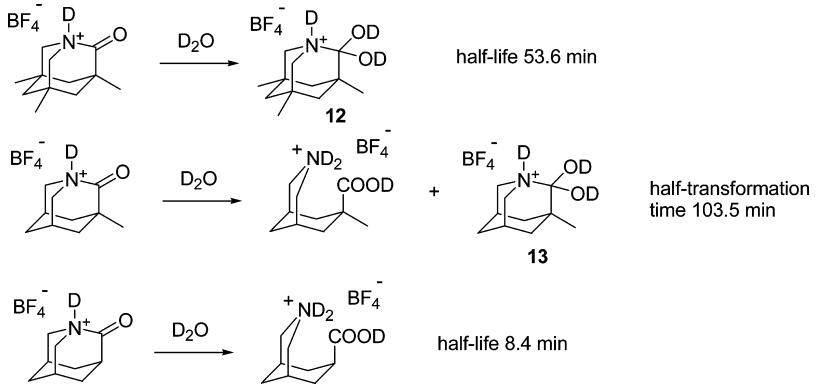

Figure 5. Hydrolysis of the HBF_4 salts of **1**−**3** (0.086 M solutions in CD_3CN, 5 equiv of D_2O, 23 °C).

2-quinuclidone **4** are shown in Table 1: bond lengths and angles are calculated where crystal structures are unavailable.

The key spectroscopic parameters for twisted amides **1**−**3** are almost identical and differ substantially from those for the unconstrained amides. As previously described for compound **1**, these properties indicate that twisted amides **2** and **3** lack the resonance interaction between the n_N and $\pi^*_{C=O}$ orbitals that defines a typical amide group. Consequences are the major downfield ^{13}C(O) NMR shift compared to that of the unconstrained amides, the IR C═O stretching band above 1730 cm^{-1}, and ready fragmentation of the molecular ion, with the loss of a CO molecule, in the mass spectra (EI ionization).

The crystal structures of **2** and **3**·HBF_4 (Figure 4) confirm that these compounds belong to the "most twisted amide" family, with twist angles close to 90°. The close agreement of calculated with crystal structures where both are available (in Table S.1.1) is strong evidence that our calculated geometry (Table 1) gives an accurate description of the parent compound **3**.

Structurally, all three twisted amides **1**−**3** correspond closely to the *syn*-TS structure calculated for the *cis*−*trans* rotamer interconversion of *N,N*-dimethylacetamide **11** by Morgan et al.[18] (Table 1) and by other authors.[7] Planar carbonyl groups, highly pyramidalized nitrogen atoms, and substantially elongated C−N and shortened C═O bonds are the key structural features characterizing these compounds. The addition of methyl groups has no appreciable influence on the spectroscopic and structural parameters of the twisted amide fragment. However, the chemical reactivity of the three twisted amides **1**−**3** depends significantly on the number of bridgehead methyl groups. Comparisons with the 2-quinuclidone system **4** remain of interest,[27] but we chose **3** as our ultimate synthetic target because the six-membered rings in the 2,2,2-system are restricted to the energetically less favorable boat conformation, with the majority of its substituent groups conformationally eclipsed. The calculated twist angle τ of 105.5° shown for **4** in Table 1 and the "barrel distortion" involved (Figure S1 of the SI) apply to a minimum in our new high-level gas phase calculation; more significant is the related shallow dependence of the energy of the system on conformation change, which makes the 2-quinuclidone less rigid than the azaadamantanone system.

Chemical Behavior. The chemistry of the monomethyl-substituted twisted amide **2** is closely similar to that of the trimethyl homologue **1**. It reacts with nucleophiles slightly faster than **1**, also at rates inconceivable for normal, unconstrained amides. However, the parent compound **3** is significantly more reactive toward nucleophiles than either **1** or **2**. It reacts readily with trace amounts of water in "dry" solvents, so that NMR spectra need to be run as fast as practical. For direct comparisons we use the conditions described previously by Stoltz[19] for the hydrolysis of the conjugate acid of the parent 2-quinuclidone **4**. The hydrolysis experiments for **3** and **3**·HBF_4 were run in duplicate, and the half-lives found to be reproducible within a few seconds.

The half-life of **4**·HBF_4 (0.084M) in acetonitrile-d_3 solution in the presence of 5 equiv of water was reported to be 135 min. We prepared the analogous tetrafluoroborate salts of **1**−**3** by

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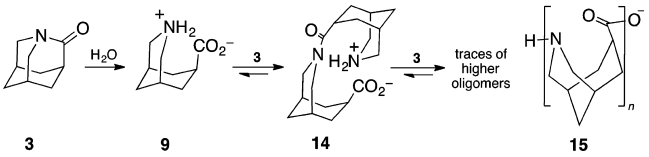
HBF_4 neutralization of the free base in dry acetonitrile followed by precipitation of the salts with diethyl ether. The salts are relatively stable in dry solvents but react with water at very different rates. Under the Stoltz conditions the half-transformation times of **1**·HBF_4, **2**·HBF_4, and **3**·HBF_4 are 53.6, 103.5, and 8.4 min, respectively. The relative rates are close to those calculated by Morgan for the hydration of the twisted amides, rather than those for hydrolysis to the amino acids.[18]

In fact only **3**·HBF_4 is hydrolyzed cleanly to the corresponding amino acid (**9**·HBF_4). Compound **1**·HBF_4 is converted to the tricyclic protonated hemiaminal **12** described previously,[22] while hydrolysis of **2**·HBF_4 produces an equilibrium mixture of three compounds in solution: the starting material **2**·HBF_4, the corresponding protonated tricyclic hemiaminal **13**, and the amino acid **7**·HBF_4 in approximate ratios of 1:3:4 (Figure 5).

Under these same conditions (5 equiv of water in CD_3CN) the half-life of compound **3**, as the free base, is 16.9 min, making **3** the most reactive twisted amide. The trimethyl compound **1** does not react at all, while the monomethyl derivative **2** is converted, after >3 h, to an equilibrium mixture with the zwitterionic amino acid (**2**:**7** ≈ 1.3:1, half-transformation time 17 min). The reaction of **3** with water is different again. It involves the formation of an intermediate, to which we assign the dimeric structure **14** (based on ^{1}H- and ^{13}C NMR data; see the SI). The formation of **14** (together with traces of the corresponding trimer **15**, n = 3) was confirmed by quenching the mixture obtained from reaction in THF-d_8 with $LiAlH_4$ (experimental details appear in the SI). LC-MS analysis of the quenching products confirmed the formation of trimeric amino alcohols formed from **15**, n = 3 (with the terminal CO_2^- reduced to CH_2OH but amide bonds intact) or (depending on the conditions of the quenching) **15**, n = 3 fully reduced to triamine alcohol, together with the dimeric equivalents based on **14** as the main products. Dimer **14** is presumably formed via acylation by unreacted **3** of its primary hydrolysis product, the zwitterionic amino acid **9**. (Higher oligomers **15**, n > 2 would be formed similarly by acylation of the dimer, etc.).



Remarkably, these oligomeric intermediates **14** and **15** are themselves hydrolyzed under the conditions, eventually, after ca. 6 h, giving **9** almost exclusively. Intriguingly, the compounds with the carboxyl groups reduced withstand the aqueous workup of the quenching products, suggesting a specific role for the carboxyl group in the rapid hydrolysis of **14** and the higher oligomers. Efficient intramolecular catalysis of the hydrolysis of nonactivated amides by properly positioned carboxyl groups is described in the literature.[28,29]

CONCLUSIONS

Structural parameters of the twisted amide groups in the three 1-azaadamantanones **1**–**3** closely match those for the TS for a model amide *cis*–*trans* interconversion, as calculated at high levels of theory. Both theory and experiment identify the parent molecule **3** as a valid TS model. The 90°-twisted conformation of the amide group in the rigid azaadamantanone systems **1**–**3** is stabilized further by bridgehead methyl substituents. Just as the TS for the 90°-twisted conformation of a peptide amide group in the pre-organized binding site of a rotamase enzyme must be stabilized by contributions from remote amino acid side chains.[30]

METHODS

Standard methods were used for the preparation, isolation, and analysis of all new compounds (for details and characterization see the SI).

Preparation of 3 by Pyrolysis/Sublimation of 8. The pyrolysis/sublimation was carried out in a 50 mL round-bottom flask attached to a condenser equipped with a central coldfinger. The N-Boc amino acid **8** (0.5–1.5 mmol: for its preparation see the SI) was dissolved in dry methanol (6 mL) in the 50 mL round-bottom flask. Silica gel (Kieselgel Merck 60, 5× the weight of the N-Boc amino acid) was added to the solution, the methanol evaporated on a rotary evaporator (water vacuum pump, bath temperature 40 °C), and the residue dried in vacuum (water vacuum pump, bath temperature 40 °C) for ∼30 min. Then the flask was connected to the condenser and a vacuum oil pump (∼0.5 mmHg). Connection to the vacuum has to be done with great care, because the mixture may produce volatile dust. The flask was immersed completely (up to the upper rim) in an oil bath, and the bath heated. Sublimation of product started at 115 °C (bath temperature). The temperature of the bath was increased to 150 °C over 1 h, and the product **3** was collected in the sublimer over 4 h, while maintaining a bath temperature of 150 ± 5 °C.

ASSOCIATED CONTENT

Supporting Information

Crystallographic information files, calculated geometries, details of syntheses and kinetic measurements, and spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org

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Notes

The authors declare no competing financial interest.
§Deceased.

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