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Instability of Amide Bond Comprising the 2-Aminotropone Moiety: Cleavable under Mild Acidic Conditions

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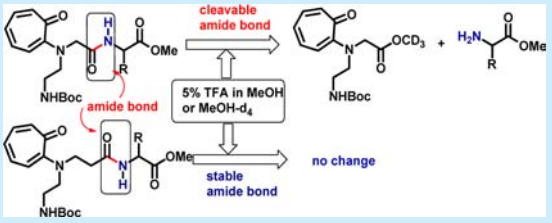
Supporting Information

ABSTRACT: An unusual hydrolysis/solvolysis of the classical acyclic amide bond, derived from *N*-troponylaminoethylglycine (*Traeg*) and α-amino acids, is described under mild acidic conditions. The reactivity of this amide bond is possibly owed to the protonation of the troponyl carbonyl functional group. The results suggest that the *Traeg* amino acid is a potential candidate for protecting and caging of the amine functional group of bioactive molecules via a cleavable amide bond.



The amide functional group has been known to be inert at neutral pH and room temperature for over 100 years.[1] However, enzymes (peptidases) hydrolyze the amide bond specifically and efficiently under physiological conditions. Hydrolysis of the amide bond requires drastic conditions such as elevated temperatures and extreme pH. At high pH, direct nucleophilic addition at the amide carbonyl followed by elimination of amine occurs, while at low pH, protonation of amide carbonyl is followed by nucleophilic addition with pronounced elimination of amine. In both cases, the reaction proceeds through a tetrahedral intermediate at the C atom of amide carbonyl.

Structurally, the classical acyclic amide bond (−NHCO−) is planar in nature, which is favorable for delocalization of nonbonding electrons of nitrogen toward the π-orbital of carbonyl.[2] As a result, the partial double bond character $^{+}$N═C-O^{-} arises in the amide bond owing to resonance, which decreases the electrophilicity of the amide carbonyl group. In the case of ring-strained cyclic amides, the constituent atoms of the amide bond deviate from planarity, which results in poor delocalization of electrons and significantly decreases the partial double bond character of the amide bond. Subsequently, the reactivity of the amide carbonyl group increases toward nucleophilic addition reaction.

In the late 1990s, Kirby and co-workers reported the hydrolysis of the amide bond of 1-aza-2-adamantanone in the presence of water within 1 min, where the amide *N*-atom is at the bridgehead of the bicycle, which prevents amide resonance and diminishes the stability of the amide bond.[3] The hydrolysis/solvolysis of bridged bicyclic lactams and acyclic distorted amides is influenced by the electronic and steric effects.

For two decades, there have been notable reports on metal-free[4] and metal-mediated[5] cleavage of acyclic amide bonds. The most fascinating among these is the Lloyd-Jones and Booker-Milburn activated acyclic amide (Figure 1A), which undergoes hydrolysis/solvolysis at room temperature in water/protic solvents, respectively.[1a,6] Importantly, the solvolysis of that acyclic amide reportedly relies on electron-withdrawing substituents at the α-C atom of amide carbonyl and steric crowding at the *N*-atom of amide amine. The hydrolyzable amide bond near physiological conditions could be applicable in protection/deprotection of carboxyl and amine functional groups in different environments. In the repertoire of unnatural aromatic amino acids/peptides, we explored the hydrogen bonding ability of troponyl carbonyl in unnatural peptides.[8] In expansion of our previous studies, we have found an unusual instability of the amide bond containing 2-aminotroponyl residue at α-C position of the amide carbonyl (Figure 1B).

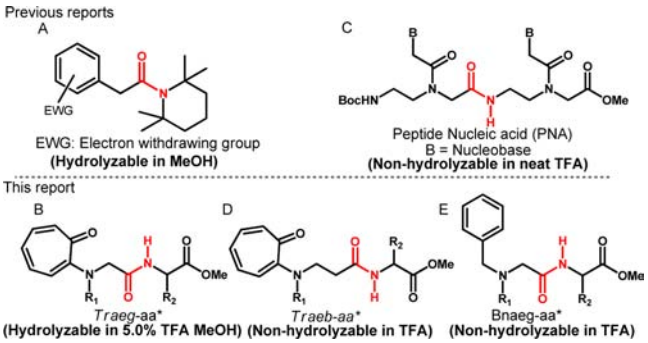


Figure 1. (A) Hydrolyzable amide bond (reported); (B) PNA with *aeg*-backbone (reported); (C, D) *Traeg* and *Traeb* peptides; (E) *Bnaeg* peptide.

Herein, we report the selective cleavage of the acyclic amide bond in peptide (*Traeg*-aa) under mild acidic conditions, which derived from *Traeg* and natural α-amino acid derivatives (Figure 1B). However, the amide bonds of structurally related peptides such as peptide nucleic acid (PNA) are stable like other acyclic amide bonds even in neat TFA (Figure 1C).[7] For

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comparative studies, the rationally designed peptides *Traeb*-aa (Figure 1D) and *Bnaeg*-aa (Figure 1E) are considered as control peptides.

We began this study with the syntheses of unnatural aromatic amino acid derivatives **1**−**4** (Figure 2). The synthesis of *Traeg*

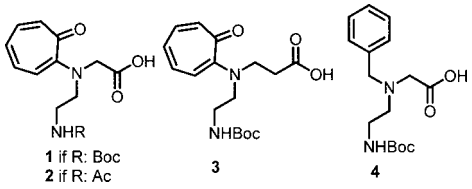


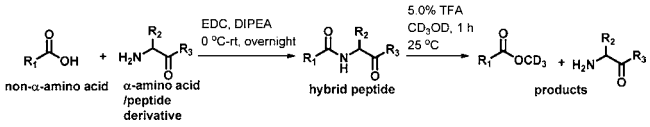
Figure 2. Synthesized unnatural amino acids.

amino acid derivative, BocNH-*Traeg*-OH (**1**), was accomplished by following the reported procedure.[8,9] We performed the syntheses of other derivatives such as AcNH-*Traeg*-OH (**2**), BocNH-*Traeb*-OH (**3**), and BocNH-*Bnaeg*-OH (**4**) as described in the Supporting Information. These amino acids were employed for the synthesis of hybrid peptides **6**−**9** with natural α-amino acid ester derivatives (**5a**−**h**) (Table 1, entries 1−11). The *Traeg*-derived hybrid peptides **6a**−**h** were synthesized from BocNH-*Traeg*-OH (**1**) and the respective α-amino acid ester derivatives **5a**−**h** (Table 1, entries 1−8). The *N*-acylated hybrid peptide **7** was accomplished from AcNH-*Traeg*-OH (**2**) and α-amino acid ester **5d** (Table 1, entry 9). For control studies, the dipeptide **8** was synthesized from BocNH-*Traeb*-OH (**3**) and α-amino acid ester **5c** (Table 1, entry 10), while the other dipeptide **9** was synthesized from BocNH-*Bnaeg*-OH (**4**) and α-amino acid ester **5d** (Table 1, entry 11). The characterization data of the newly synthesized compounds **2**−**4**, **6a**−**h**, and **7**−**9** are provided in the Supporting Information.

For the syntheses of unnatural aromatic peptide mimics comprising an aminotroponyl moiety, we attempted to remove the *N*-Boc group of peptides **6** with 20% TFA in DCM. Unfortunately, we failed to prepare the amino-fuctionalized peptides from **6** under these acidic conditions. From ESI-MS (electrospray ionization mass spectrometry) analyses, surprisingly, we found the cleavage of the amide bond derived from *Traeg*. For example, when the peptide **6a** was treated with 20% TFA in DCM, the formation of *Traeg* amino acid (NH_2-*Traeg*-OH) and methyl ester of glycine (*Gly*-OMe, **5a**) was observed along with Boc deprotection. Next, we attempted to optimize the concentration of TFA for cleavage of the *Traeg*-derived amide bond. With successive trial experiments, we found that only 5.0% TFA in DCM (~6.0 equiv) is sufficient to cleave the *Traeg*-derived amide bond selectively. Importantly, we also found that the Boc protecting group (carbamate bond) was quite stable under the above-optimized conditions (5.0% TFA). However, TFA (5.0%) in MeOH cleaved the dipeptide **6a** into the methyl ester as BocNH-*Traeg*-*OMe* (**1-OMe**) and α-amino acid ester instead of the carboxylic acid as BocNH-*Traeg*-*OH* (Figure S13). Curiously, we treated peptide **6a** with neat acetic acid and found that the amide bond was stable in acetic acid.

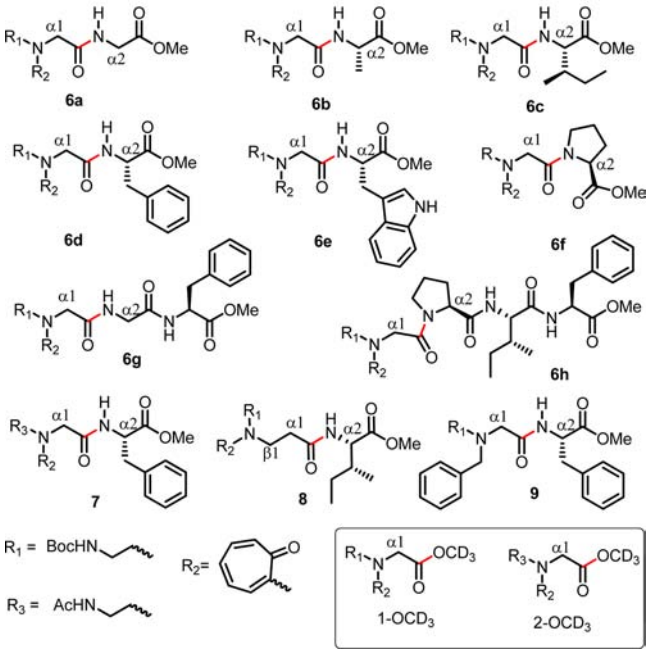
Further, we performed a time-dependent ^{1}H NMR experiment with *Traeg*-derived peptides and control peptides under the above-optimized conditions to explore the mechanistic aspects of the hydrolysis/solvolysis of the *Traeg*-derived amide bonds. Thus, a series of ^{1}H NMR spectra of the peptides **6a**−**h** and **7**, including control peptides **8** and **9**, were recorded with 7.0 min time intervals for 1.0 h after the addition of TFA. We

Table 1. Synthesis of Hybrid Peptides from *Traeg*/*Traeb*/*Bnaeg* Unnatural Amino Acids and α-Amino Acid/Peptide



entry	non-α-amino acid*	α-amino acid/peptide derivative*	hybrid peptide	products
1	**1**	**5a** (Gly-OMe)	**6a**	**1**-OCD_3 and**5a**
2	**1**	**5b** (Ala-OMe)	**6b**	**1**-OCD_3 and **5b**
3	**1**	**5c** (Ile-OMe)	**6c**	**1**-OCD_3 and **5c**
4	**1**	**5d** (Phe-OMe)	**6d**	**1**-OCD_3 and **5d**
5	**1**	**5e** (Trp-OMe)	**6e**	**1**-OCD_3 and **5e**
6	**1**	**5f** (Pro-OMe)	**6f**	**1**-OCD_3 and **5f**
7	**1**	**5g** (Gly-Phe-OMe)	**6g**	**1**-OCD_3 and **5g**
8	**1**	**5h**(Pro-Ile-Phe-OMe)	**6h**	**1**-OCD_3 and **5h**
9	**2**	**5d** (Phe-OMe)	**7**	**2**-OCD_3 and **5d**
10	**3**	**5c** (Ile-OMe)	**8**	no change
11	**4**	**5d** (Phe-OMe)	**9**	no change

Chemical structure of hybrid peptides



also recorded the NMR of these peptides in CD_3OD before the addition of TFA. After the completion of NMR experiments, the same samples were analyzed by ESI-MS. The spectral data and time-dependent ^{1}H NMR and ESI-MS data are provided in the Supporting Information. Moreover, the extended region of the time dependent ^{1}H NMR spectra of dipeptide **6b** and mass spectra of **6b**,**h** are depicted in Figure 3. Before analyzing the time-dependent NMR spectra of peptides, we assigned all the protons signals of peptides **6a**,**b**,**e**,**h** and **7**−**9**, by comparing with the NMR data of our previously reported monomer **1** and peptides **6c**,**d**,**f**,**g**.[8] After the addition of TFA, significant changes in the chemical shifts of α_1/α_2-CH_2 proton signals

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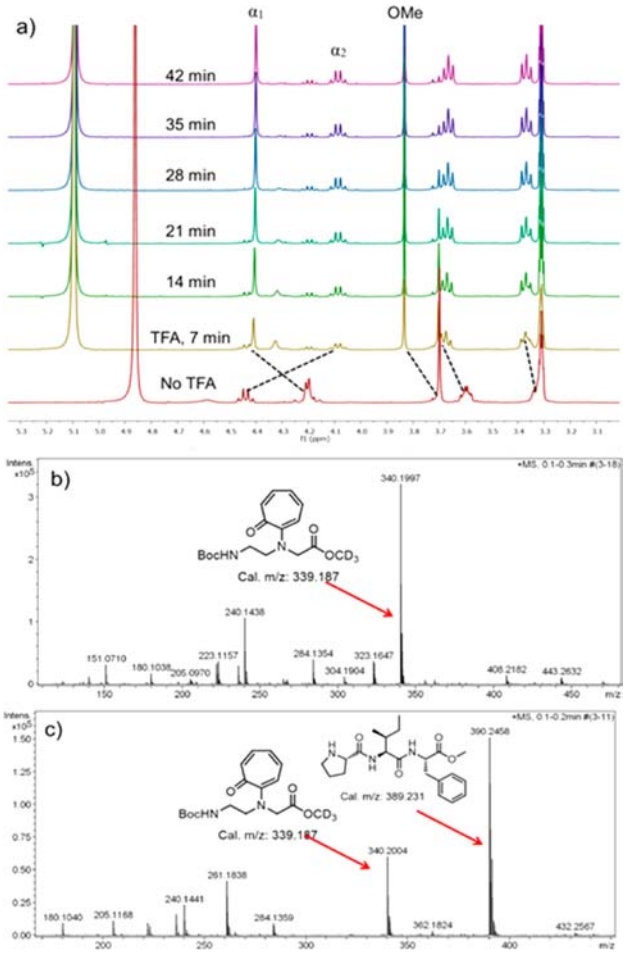

Figure 3. (a) Time-dependent ^{1}H NMR spectra of dipeptide **6b**; (b) ESI-MS spectrum of dipeptide **6b** after time-dependent NMR; (c) ESI-MS spectrum of tetrapeptide **6h** after time-dependent NMR.

and their intensities were observed in the ^{1}H NMR spectra of dipeptide **6b** with respect to time (Figure 3a).

The ^{1}H NMR signal of α_1-CH_2 (δ 4.2, d) was shifted to δ 4.4 (s), and that of α_2-CH_2 (δ 4.46−4.41, q) was shifted to δ 4.06 (q). Importantly, the intensity of proton signal at δ 4.4 (s) increases with time. After 1 h, the α_1-CH_2 protons are completely deshielded (δ 4.4, s), while the α_2-CH_2 protons are shielded (δ 4.06, q). These ^{1}H NMR changes clearly indicate the formation of new derivatives. The mass spectrum of the same NMR sample of **6b** exhibits the new mass peak at m/z 340.0 instead of dipeptide **6b** mass at m/z 394.0 $(M + H)^+$. However, the mass peak at m/z 340.0 is equivalent to the calculated mass of the *BocNH-Traeg-OCD*$_3$ (**1-OCD**$_3$). This is only possible due to the methanolysis of the *Traeg*-derived amide bond of dipeptide **6b** in CD_3OD in the presence of TFA (5.0%). Almost similar spectral changes are observed in the ^{1}H NMR spectra of other *Traeg*-derived peptides **6a,c−h** with respect to time after the addition of TFA. Similarly, the mass peak at (m/z) 340.0 (M + H) is also constantly observed in the mass spectrum of the other *Traeg*-derived peptides after time-dependent ^{1}H NMR experiments. Overall, the time-dependent ^{1}H NMR experiments and ESI-MS studies strongly support the selective methanolysis of *Traeg*-derived amide bond.

However, the ESI-MS spectrum of tetrapeptide **6h** exhibits two prominent mass peaks at m/z 340.0 and 390.0 after ^{1}H NMR experiment. These two mass peaks represent the formation of **1-OCD**$_3$ (M + H) and tripeptide **5h**, respectively (Figure 3c). These mass results reveal that the other amide bonds of tripeptide **5h** are stable under the same acidic conditions. Similarly, the other non-*Traeg* amide bonds of tripeptide **6g** and *N*-acylated dipeptide **7** are also found stable. Pleasingly, we calculated the rate constant (k = 0.21423 min^{-1}) and half-life ($t_{1/2}$ = 3.23 min) of methanolysis of dipeptide **6b** by considering it a pseudo-first-order reaction. The kinetic studies, plots, and calculations are provided in the Supporting Information.

In contrast, the time-dependent ^{1}H NMR spectra of control dipeptides **8** and **9** show only the downfield shift in their proton signals after the addition of TFA (Figures S59 and S73). The mass spectra of control dipeptides **8** and **9** remained unchanged after TFA treatment (Figures S60 and S74), but the deprotection of the Boc group was noticed from the mass spectra of both control peptides **8** and **9** after the same NMR samples were left for a longer time under the same acidic conditions (Figure S75). These comparative studies reveal that only *Traeg*-derived amide bonds are cleavable under the above-optimized conditions.

The selective cleavage of the *Traeg*-derived amide bond in the presence of other *non-Traeg* amides encouraged us to think about the mechanism of solvolysis. From the above studies, it is clear that the aminotroponyl moiety has an important role in the cleavage of the amide bond. Since tropolone derivatives are UV active. Therefore, we recorded UV-absorption spectra of *Traeg* peptides **6b,e,h** and control peptide **8** in non-nucleophilic solvent, anhydrous CH_3CN, before and after addition of TFA, to find the role of aminotroponyl group. To prevent the hydrolysis/methanolysis, these samples were analyzed with ESI-MS by using anhydrous CH_3CN:3−5% TFA solvent system, instead of CH_3CN/H_2O (0.1% HCOOH) or $MeOH/H_2O$ (0.1% HCOOH). The UV and mass spectral data are provided in the Supporting Information (Figures S77−S85).

From above UV/mass studies, we found the formation of charged troponyl lactone intermediate in acidic CH_3CN after the cleavage of the amide bond of peptide **6b** and the conversion into methyl ester (**1-OCH**$_3$) after the addition of MeOH. The formation of such a lactone intermediate is from the O atom of troponyl carbonyl and the C atom of amide carbonyl of peptide **6** after cleavage of the *Traeg*-derived amide bond in non-nucleophilic solvent CH_3CN. However, tropylium cation salts are reportedly stable under anhydrous conditions.[11] The absorption spectrum of peptide **6b** in CH_3CN after the addition of TFA has shown only one peak at λ = 362 nm. The similar absorption changes were also observed with other *Traeg*-derived peptides such as **6e**/**h**. In contrast, the control peptide **8** and monomer has not shown similar changes like *Traeg* peptides.

Further, we examined the formation of charged lactone intermediate from peptide **6a** in CD_3CN in the presence of 5% TFA by ^{13}C NMR studies (Figures S86−S89). The ^{13}C NMR spectrum has shown that the resonating signal of tropone carbonyl carbon at 181.9 ppm in CD_3CN disappeared after the addition of TFA. The mass peak at m/z 305.0 (M^+) was observed from the ESI-MS analysis of the same sample, which is equivalent to the mass of lactone. After 24 h, we added CD_3OD (10%) in the same NMR sample and recorded the ^{13}C NMR and ESI-MS. We found that the lactone was converted into methyl ester. Overall, the above studies support the cleavage of *Traeg*-derived amide bond via a stable lactone

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intermediate in CH_3CN or CD_3CN (in the absence of nucleophilic solvents ROH/H_2O).

Finally, we here propose a mechanism in Figure 4 by considering the outcomes of the above observations. The

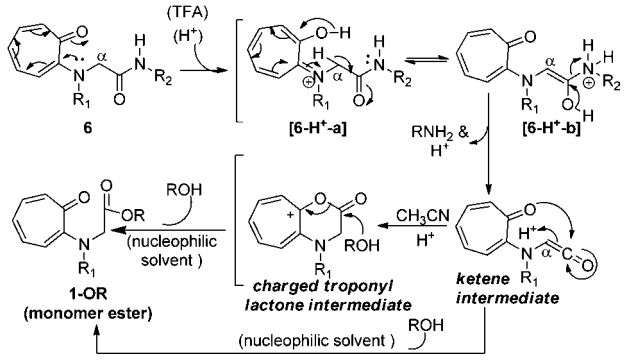

Figure 4. Possible mechanism of *Traeg* amide bond cleavage.

protonation of troponyl carbonyl in peptide **6** preferably occurs in TFA and leads to the formation of intermediate **6-H$^+$-a**, which is further stabilized by delocalization of charge via the troponyl ring. The protonation of troponyl carbonyl group is well-known in acidic medium which leads to the formation of stable tropylium cation.[11] The intermediate **6-H$^+$-a** facilitates the enolization of the amide carbonyl from its α_1-CH_2 protons (**6-H$^+$-b**), and the protonation of *N*-atom of the amide occurs in acidic medium. Consequently, the cleavage of the amide C−N bond is furnished by liberation of reactive ketene intermediate and respective amine derivative **5**. The newly generated ketene intermediate proceeds toward nucleophilic addition to convert into stable ester/acid in the presence of respective nucleophilic solvents alcohol/water. The hydrolysis of amide bond via the formation ketene intermediate is illustrated.[6,10c] Although we attempted to characterize the ketene intermediate in anhydrous CH_3CN/TFA (5.0%), the attempt failed and a lactone intermediate resulted. The isolation of reactive ketene intermediate, having an electron-withdrawing group, is extremely difficult, especially at low pH in the presence of nucleophilc solvents.[10e]

In conclusion, we have successfully demonstrated the instability of 2-aminotroponyl containing an amide bond in the presence of 5% TFA in CD_3OD. The role of the 2-aminotroponyl substituent is established in the selective cleavage of the *Traeg*-derived amide bond via acid (TFA) mediated hydrolysis and methanolysis. Most importantly, we have demonstrated the formation of a reactive charged troponyl lactone intermediate after the cleavage of the amide bond in CH_3CN with 5.0% TFA, possibly via ketene intermediate and proposed a plausible mechanism of hydrolysis/solvolysis. The outcomes of this report will provide enormous opportunities to employ chromophoric *Traeg* amino acid in caging/protection of free amine functionality of bioactive molecules. Hence, the reactivity of those molecules may be regulated by UV spectrophotometry in a temporal and spatially controlled manner.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b01535.

Experimental procedures and characterization data of new amino acids **2**−**4** and peptides **6a,b,e,h** and **7**−**9**; time-dependent NMR and mass spectra; UV-absorption spectra of peptides **6b,e,h** (PDF)

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Notes

The authors declare no competing financial interest.

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