Instability of Amide Bond with Trifluoroacetic Acid (20%): Synthesis, Conformational Analysis, and Mechanistic Insights into Cleavable Amide Bond Comprising β-Troponylhydrazino Acid

Nihar Ranjan Dalabehera,* Sagarika Meher, Bibhuti Bhusana Palai,* and Nagendra K. Sharma*

ABSTRACT: The instability of an amide bond with dilute trifluoroacetic acid (TFA) is a rare chemical event. The native amide bonds are stable even in the neat TFA, which is one of the reagents that releases the peptides from the solid support in the solid-supported peptide synthesis method. In the repertoire of unnatural peptidomics, α-/β-hydrazidroxy acids and their peptides are explored for the synthesis of N-amino peptide derivatives, and their amide bonds are stable in TFA (~100%) as natural amide bonds. This report describes the synthesis of a β-hydrazidroxy analogue as β-troponylhydrazino acid, containing a nonbenzenoid natural troponyl scaffold. The structural and conformational studies of their hybrid di-/tripeptides with the natural amino acid show that the 2-aminotroponyl residue is involved in hydrogen bonding. Surprisingly, the amide bond of β-troponylhydrazino peptides is cleavable with TFA (~20%) through the formation of a new heterocyclic molecule N-troponylpyrazolidinone or troponylpyrazolidinone. Tropolone and related compounds are excellent biocompatible chromophores. Hence, β-troponylhydrazino acid could be employed for tuning the peptide structure and considered a promising chromophoric acid-sensitive protecting group of a free amine of amino acids/peptides. It could be applied for the estimation of the free amine group functionality by a UV–vis spectrophotometer.

INTRODUCTION

Natural amide bonds are pretty stable, with an estimated half-life of around ~350–600 years for spontaneous hydrolysis at neutral pH and room temperature (RT). The natural amide bonds are resonance-stabilized. The carbonyl group of the natural amide is inert toward the nucleophilic addition reaction. The cleavage/hydrolysis of amide bonds could be achieved under extreme conditions as the heating under strong acidic or basic conditions. However, the cyclic amides (lactams) are more cleavable as compared to the linear amides because of ring-strained amides. A large number of ring-strained lactams are synthetized and their poor stability is reported even under mild conditions because of the resonance decoupling through N=C=O torsion, which induces the strong electrophilicity at the C=O group as ketonic carbonyl. The cleavage of an amide bond without metal ions becomes a center point of discussion. Brown and co-workers have shown that the resonance decoupling enhances the hydrolysis rate in the strained amide bond because of the direct nucleophilic attack. For instance, the twisted amide of 1-aza-2-admantanone derivatives is a highly strained lactam ring and readily cleavable under mild conditions. This twisted amide also shows the dual reactivities such as (i) nucleophilic character of amine and (ii) electrophilicity of the carbonyl. The hydrolysis of linear amide bonds is also possible by decoupling the N=C=O resonance stability within the structurally modified amide bonds. However, the sequence-specific amide is cleaved/hydrolyzed with enzymes such as proteases. The zinc metal-dependent peptidase cleaves the specific amide bond through zinc ion mediation. These results encourage the synthetic chemists for the development of artificial peptidases. Mashima and co-workers have explored the role of zinc ions in the cleavage of amides bearing β-hydroxyethyl using Lewis acid Zn(OTf)2. Recently, the activation of specific amide bonds has been explored using a metal catalyst. For example, Garg, Houk, and co-workers have shown the conversion of an amide functional group into an ester group by cleaving the C=N bond of amide with the Ni-catalyst. The cleavage of the amide bond under near-physiological conditions is still challenging. Booker-Milburn and co-workers have reported the solvolysis of acyclic synthetic amide bonds at RT under neutral conditions via the formation of ketene intermediates. They have shown that an electron-withdrawing group, at the α-position, of amide carbonyl enhances the protonation of sterically hindered amide amine and facilitates the formation of ketene by cleaving the C=N bond of amide. In a recent report, the cleavage of the terminal...
amide bond occurs with ammonium salt/aqueous hydrazine under heating conditions via hydrazinolysis. The cleavage of modified N-terminal amide bonds, such as aminopyrazolonyloxy-containing acetamides, occurs under mild acidic conditions. Another reactivity of amide bond as transamidation also reported, such as the transamination reaction of the amide bond using Zr/Hf-catalyst. For the development of peptide-based materials, various aromatic amino acids/peptides are synthesized and explored for novel peptidomimetics. In addition to benzenoid aromatic peptides, recently, non-benzenoid aromatic amino acids/peptides have also been synthesized from the tropolone molecule and unnatural amino acid backbone for evaluating the role of tropolonyl carbonyl in the structural and functional changes of peptides (Figure 1a). The α-troponylalkyl amino acid and its peptides exhibit rare characteristic chemical properties as the cleavage of amide bond under mild acidic conditions [5% trifluoroacetic acid (TFA)] along with the reversible amidation and transamidation activities under basic conditions. However, their β-analouges as β-troponylalkyl amino acid derivatives are stable like other natural amide bonds, even with neat TFA (~100%) (Figure 1b). In repertoire of unnatural peptidomimetics, α-hydrazino acids and their peptides as N-amino peptide (NAP) derivatives are explored, and it was found that those peptides exhibit improved biostability and bioactivity as compared to control (Figure 1c). Thus, we designed a β-troponylhydrazino acid analogue to explore the role of the troponyl group for novel peptidomimetics (Figure 1d). This report describes the synthesis of β-troponylhydrazino acid and its hybrid peptides with the amino group of natural α-amino acid/peptide ester derivative (Figure 1b). For conformational studies, the DMSO-d₆ titration experiment and X-ray studies are performed with representative peptides. For practical utilities, the stability of such peptides is also investigated under near-physiological pH conditions (mild acidic/alkaline conditions) by nuclear magnetic resonance (NMR) and electrospray ionization mass spectrometry (ESI-MS) techniques, which reveal the cleavage of their amide bonds with dilute TFA (20%).

**RESULTS AND DISCUSSION**

We used commercially available N-Boc-hydrazine (1) for N-alkylation with 3-bromopropionate ester under basic conditions that produced alkylated hydrazine derivative (2). This derivative was treated with O-tosylate tropolone (3) under reflux conditions for 4 days for N-troponylation of amine that is converted into a new unnatural amino acid as a 2-aminotroponyl hydrazine derivative (4). However, the O-tosylate tropolone (3) was derived from the commercially available tropolone molecule. For the synthesis of an amide bond, the ester group of derivative (4) was hydrolyzed into the carboxylate derivative under alkaline conditions, followed by

---

**Scheme 1. Synthesis of N-Troponylated-β-Hydrazino Acid/Peptides**

- **(a)** N-Troponylated-β-Hydrazino Acid/Cleavable in TFA
- **(b)** N-Troponylated-β-Hydrazino Acid/Stable in TFA

**Figure 1.** (i) Previously reported tropolonyl/hydrazine-containing amides and (ii) rationally designed β-troponylhydrazinyl peptides and their instability under acidic conditions.
Coupling with various natural α-L-amino acid esters (Gly, Ala, Leu, Val, Ile, Phe, and Pro)/peptide ester (Leu−Phe) derivatives using peptide coupling reagents. Subsequently, the hybrid di-/tripeptide derivatives (5a−5g/5h) were isolated (Scheme 1). These peptides were well characterized by NMR and ESI high-resolution MS (ESI-HRMS) (see the Supporting Information). Pleasantly, we obtained the single crystal of one dipeptide (5a) from the organic solvent mixture (EtOAc/hexane), which was analyzed by an X-ray diffractometer, which confirmed the structures of peptide 5a as Boc-β-troponylhydrazino-glycine ester (Supporting Information, Table S4 and Figure S69). Its crystal data were deposited to the Cambridge Crystallographic Data Centre (CCDC) with number CCDC 2003629 (5a).

For comparative studies, we synthesized three types of control β-hydrazino acid derivatives without containing a troponyl scaffold from the same β-hydrazino acid ester (2) and their hybrid peptides with natural α-amino acids (Scheme 2). The β-hydrazino acid ester (2) was treated with benzyl bromide under the basic condition, which produced N-benzyl-β-hydrazino ester (2-Bn). Similarly, N-hexyl-β-hydrazino ester (2-hexyl) was prepared by ester (2) and hexyl bromide under the basic condition. However, the N-amide derivative of β-hydrazino acid ester (2) was developed with picolinic acid

Figure 2. (a) 1H NMR DMSO-d_6 titration plot for BocNH (A) and amide NH (B) and (b) proposed conformation of β-troponylhydrazino peptides/control peptide (C).
under peptide coupling reaction conditions, which produced β-picolinylhydrazino ester (2-picolamidate). These non-troponyl β-hydrazino ester derivatives (2-Bn/2-hexyl/2-picolamidate) were hydrolyzed into the respective carboxylate with LiOH and then directly coupled with the amine group of α-amino acid ester under peptide coupling conditions. As resultsants, the control di-/tripeptides β-benzylhydrazino peptides (5a/6b), β-picolinylhydrazino peptide (7), and β-hexylhydrazino peptide (8) were synthesized for further studies. The characterization data are described in the Experimental Section, while their NMR and mass spectra are provided in the Supporting Information (Figures S1–S34). We also attempted to synthesize a more resemble analogue as N-phenyl-β-hydrazino ester for control studies but could not achieve it.

Herein, we also attempted the involvement of amine protons (BocNH/amide NH) in intramolecular hydrogen bonding with carboxyl oxygen (troponyl/Boc/Amide) in solution state by DMSO-δ6-titration 1H NMR experiments. In this experiment, the chemical shift of intramolecular hydrogen-bonded proton remains constant or exhibits a small downfield shift (N-H), while the chemical shift of intermolecular hydrogen-bonded proton exhibits a significant downfield shift with increasing concentration of DMSO-δ6 (a strong hydrogen bond acceptor solvent).19 The strength of the intramolecular hydrogen bond is inversely proportional to the downfield shift of N-H by dimethyl sulfoxide (DMSO) addition. We assigned proton resonance signals of BocNH (NH1) and amide N-H (NH2) in troponylated dipeptides (5a/5e) and a control peptide (7). Pleasantly, we performed the DMSO titration experiment by recording the consecutive 1H NMR spectra of the respective peptides (5a–5e/7) in CDCl3 with successive addition of DMSO-δ6 in a small amount. Their 1H NMR titration spectra are provided in the Supporting Information (Figures S35–40). We extracted the chemical shift value of BocNH and amide N-H with respect to the volume of DMSO-δ6 addition and then generated a plot as a chemical shift (ppm) versus the DMSO-δ6 volume (μL). These plots are depicted in Figure 2A,B, which exhibit a marginal downfield shift in the chemical shift of BocN−H/amide N-H in troponylated peptides (5a–5e) as compared to the BocN−H of control peptide (7). Importantly, we noticed that the extent of downfield shift in BocNH and amide N-H is almost equal in troponoyl peptide (5a/5c). However, the extent of downfield shift in BocNH of the troponoyl peptide (5b/5d/5e) is lower than its respective amide N-H, which is almost equal to that of control peptide (7). Hence, intramolecular hydrogen bond in troponyl peptides (5a–5e) due to BocNH is equal to or stronger than the respective amide N-H. Our 1H NMR titration results reveal two intramolecular hydrogen bonds in β-troponoylhydrazino peptides while one control peptide. Herein, we propose the preferable intramolecular hydrogen bonding in β-troponoylhydrazino peptides (5a–5e)/control peptide as shown in Figure 2C. In troponyl peptides (5a–5e), the intramolecular hydrogen bond between BocN−H−O=C (troponoyl carbonyl), six-membered ring, could be slightly stronger than another intramolecular hydrogen bond between amide N-H−O=C (Boc carbonyl), nine-membered ring in solution state. In the literature, a nine-membered ring α-N-O turn is reported in peptide containing α-amino acid.20

To investigate the role of the troponoyl carbonyl group in β-troponoylhydrazino acid containing hybrid peptides, we attempted to crystallize hybrid peptides under various solvent systems. Pleasantly, we obtained the single crystal of one dipeptide (5a) and one tripeptide (5h). Their crystal data are submitted to CCDC with number CCDC 2003629 for peptide 5a and CCDC 2003628 for peptide 5h. We extracted their packing diagram in unit cell and supramolecular self-assembled structure using software Diamond 3.2. The structural analyses of dipeptide (5a) in solid state are depicted in Figure 3, which includes Oak Ridge thermal ellipsoid plot (ORTEP) diagram (Figure 3a), unit cell (Figure 3b), packing arrangement (Figure 3c), and supramolecular helical structure (Figure 3d). However, other crystal data are provided in the Supporting Information. Importantly, peptide 5a forms an intramolecular hydrogen bonding between troponoyl carbonyl with amide N−H (C==O−H−N) and hydrazine NH with amide carbonyl (N−H···C==O), which leads to a novel supramolecular helical structure with a pitch of 6.02 (Å) (Figure 3d). Thus, troponoyl carbonyl has a significant role in the conformational changes of peptides for interesting supramolecular self-assembled structures in solid state.

Similarly, the structural analysis data of tripeptide (5h) are depicted in Figure 4, which describe the ORTEP diagram (Figure 4a), unit cell (Figure 4b), packing diagram (Figure 4c), and exceptional supramolecular helical structure (Figure 4d). Other crystal data are provided in the Supporting Information. This peptide forms inter- and intramolecular hydrogen bonding and generates a new supramolecular self-assembled helical structure. Most importantly, we noticed two intramolecular hydrogen bonding—(a) troponoyl carbonyl with adjacent amide NH (Leu) (C==O−H−N, 2.04 Å) as i + 9 helical structure and (b) hydrazine Boc carbonyl with N−H of the third residue (Phe) (C==O−H−N, 2.1 Å). Other carbonyl and NH of amide form intermolecular hydrogen bonding and assemble into a supramolecular helical supramolecular structure.

We also obtained the single crystal of control non-troponoyl hybrid peptide (6a), β-benzylhydrazino acid containing peptide, and analyzed its structural conformation by X-ray studies (Figure 5). Other crystal data are provided in the Supporting Information. Their crystal data are submitted to CCDC with number CCDC 2003626 for peptide 6a. The
ORTEP/unit cell packing diagram of 6a is shown in Figure 5a,b, respectively. It has circular packing rearrangement by self-assembly through hydrogen bonding, as shown in Figure 5c. There are two types of intermolecular hydrogen bonding: BocN−H···O=Boc (2.1 Å) and amide N−H···O=C amide (2.0 Å) (Figure 5c). These intermolecular hydrogen bonds of peptide 6a form a unique ladder type of supramolecular helical structure, as shown in Figure 5e. Thus, nonpropyl β-hydrazino acid containing peptide is also a building block of a new peptidomimetics.

For peptide coupling at the N-terminal of hybrid peptides, we attempted to remove the Boc group of di-/tripeptides (5a−5h) with versatile reagent 20−30% TFA in dichloromethane (DCM) (Scheme 3). Unexpectedly, we isolated a new pyrazolidinone derivative as tropolone pyrazolidinone (9) from all respective hybrid peptides (5a−5h) under similar conditions by unusual cleavage of the amide bond. The structure of tropolonepyrazolidinone (9) is confirmed by NMR and HRMS. Their spectra are provided in the Supporting Information. We also obtained the single crystal of tropolonepyrazolidinone (9), which was analyzed by an X-ray diffractometer. Crystal details and unit cells are provided in the Supporting Information (Figure S72). The X-ray analysis result confirms the structure of tropolonepyrazolidinone (9). The X-ray data are also submitted to CCDC with number 2003627. The ORTEP diagram of one dipeptide (5a) and its cleaved product tropolonepyrazolidinone (9) is depicted in Figure 6. We also noticed two types of intramolecular hydrogen bonds: (i) N−H of pyrrolidinone with tropolone carbonyl (1.8 Å) and (ii) N−H of pyrazolidinone with its carbonyl (2.5 Å). We also examined the instability of such amide bonds under different acids such as HCl (4.0 N), HClO4 (4.0 N), PTSA (10 equiv), and AcOH (4 N) by the ESI-MS technique (see the Supporting Information, Figure 5c).
S68). For peptide 5a, our mass analysis results reveal the cleavage of the amide bond of 5a with acids (HCl, HClO4, and PTSA) and the formation of the same cyclic derivative troponylpyrazolidinone (9). We could not notice the cleavage of the Boc group and amide bond cleavage with AcOH, which is a relatively weak organic acid. To examine the role of tropolone residue for the cleavage of such amide bonds, we performed control studies with similar types of non-troponyl-β-alkylhydrazino acid containing hybrid peptides (6a, 6b, 7, and 8) and TFA (~20%), which were analyzed by ESI-MS and NMR techniques (Figures S49–S52). Except Boc group deprotection, we could not find the cleavage of amide bond in control peptides. However, the control peptide 8 also forms a trifluoroacetylated salt derivative by acylation at the pyridine ring of picolamide residue. Hence, tropolyn residue has a critical role for the cleavage of amide bonds containing β-troponylhydrazino acid (peptides 5a–5h).

The cleavage of the amide bond in peptides (5a–5h), under acidic pH, was dependent only on the concentration of peptides and thus considered as a first-order kinetic reaction. Thus, we performed kinetic studies of dipeptide (5a/5b) cleavage with the time-dependent 1H NMR experiment under acidic conditions (20% TFA in CDCl3). Their NMR spectra are provided in the Supporting Information (Figures S41–S48). After addition of TFA, 1H NMR spectra arrays of dipeptide 5a exhibit the significant downfield shift of α-/β-hydrogen resonance signals such as the resonance signal δ2.7 (α-H) shifted to δ3.0 and δ3.8 (β-H) shifted to δ4.2. Simultaneously, the new signals appeared at δ3.2 and δ4.4, which presumably belong to new cyclic derivative troponylpyrazolidinone (9). The NMR spectral arrays also show an exponential decrease in the intensity α-H signal (δ3.0) while an exponential increase in the intensity of new signals (δ3.2) with respect to time. After completion of NMR experiments, we recorded their mass spectra, which are provided in the Supporting Information (Figures S49–S52). Their mass spectra confirm the removal of the Boc group, followed by the formation of cyclic compound 9. Thus, the amide bond of a dipeptide (5a) was cleaved with TFA (20%). We repeated the similar NMR kinetic experiments with another representative peptide (5b) and obtained almost similar results. To determine the kinetic parameters (equilibrium constant and half-life of amide bond cleavage), we extracted mole fractions of reactant (5a) and its product (9) at different intervals of time from their respective NMR (see the Supporting Information, Tables S1 and S2). Then, we generated a kinetic plot (mole fraction vs time) for the cleavage amide bond (5a) and formation of a new cyclic product (9) (Figure 7A). We also obtained similar results with peptide (5b), and its kinetic plot is provided in the Supporting Information (Figure S56A). Our kinetic results clearly indicate that the cleavage of amide bond (5a/5b) and the formation of cyclic derivative are the first-order kinetic reactions. By following our previous report,16 we extracted the equilibrium constant (k) as 0.016 and 0.009 min⁻¹ for peptides 5a and 5b, respectively (Figures 7B and S56B). We also compared our experimental kinetic results with

Scheme 3. Reaction of β-Troponylhydrazino Peptides/Non-troponyl-β-hydrazino Peptides with TFA

Figure 6. Conformational analyses of troponylpyrazolidinone crystal (9): (a) ORTEP diagram, (b) unit cell packing, and (C) intramolecular hydrogen bonding.
the simulated kinetic model (COPASI) using reported software (see Supporting Information, Figures S57–S58). Then, we calculated the half-life of amide bond cleavage (5a/5b) from their respective logarithmic plots (mole fraction vs time) by following the previous reports (see the Supporting Information). We obtained the half-life 41.0 and 71.0 min for the cleavage of respective amide bonds 5a and 5b.

We noticed similar results with dipeptide 5b from the time-dependent UV–vis studies and mass analyses under the same acidic conditions (Supporting Information, Figures S65/S66). Hence, tropolonylhydrazino hybrid peptides produced the same cyclic intermediate tropolonepyrazolidinone (9) after the removal of N-Boc, followed by the cleavage of the amide bond under acidic conditions.

Finally, we propose the plausible mechanism for the cleavage of β-tropolonoylhydrazino-containing amide bond (Figure 9). First, TFA (20%) removed the N-Boc group of the peptide (5) and produced the protonated hydrazinyl derivative (5-Boc)* under the acidic condition that facilitated the cleavage of an adjacent amide bond by the formation of a new cyclic molecule tropolonepyrazolidinone (9). We assumed that the protonated hydrazinyl derivative (5-Boc)* activated its amide bond when the tropolone residue was present at the N-atom. Herein, the delocalization of cationic hydrazinyl proton possibly occurs through the tropolone ring as a tautomeric intermediate (T1 and T2), possessing a hydrazine amine (−NH₂) nucleophile. This nucleophile is attacked at the protonated amide carbonyl group via the nucleophilic addition reaction and then a reactive cyclic-1,1-aminol intermediate (T3) is generated. Then, the protonation of the aminol amine group followed by elimination leads to the stable molecule N-tropolonopyrazolidinone (9) and amine residue. In the case of control peptides, the delocalization of cationic hydrazinyl proton is not possible, and we could not notice the cleavage of amide bond or formation of tropolonepyrazolidinone (9) under similar acidic conditions. Thus, tropolone played a crucial role in the cleavage of the amide bond in tropolonated-β-hydrazinopeptide (5) under acidic conditions, possibly through the proposed mechanism.

### CONCLUSIONS

β-Tropolonylhydrazino acid analogues and their hybrid peptides are synthesized from natural amino acid derivatives. Conformational analyses of β-hydrazinoy acid-containing peptides are demonstrated by extracting hydrogen bonding in representative peptides in solid state. The intramolecular hydrogen bonding of amide N–H has been shown in β-tropolonoylhydrazino peptide by DMSO titration methods in solution state. X-ray studies reveal the role of the tropolone group in the self-assembled supramolecular structures in solid state. Most importantly, the troponyl-β-hydrazinoy acid-containing hybrid peptides show a unique feature as the cleavage of the amide bond through the formation of a new cyclic molecule.
troponylpyrazolidinone under mild acidic conditions. Time-dependent NMR studies determine the equilibrium constant and half-life of amide cleavage. The cleavage of the amide bond and formation of troponylpyrazolidinone are explained with the plausible mechanism. Hence, β-troponylhydrazidroxy acid could be a promising chromophoric acid-sensitive protecting group of free amines. It could also be useful to estimate the free amine group by a UV–vis spectrophotometer.

■ EXPERIMENTAL SECTION

Materials. All required materials were obtained from commercial suppliers and used without any further purification. Dimethylformamide (DMF) was distilled over calcium hydride. Reactions were monitored by thin-layer chromatography (TLC) and visualized by UV and ninhydrin. Column chromatography was performed in 230–400 mesh silica. Mass spectra and HRMS were obtained using a Bruker microOTOF-Q II spectrometer. 1H NMR and 13C NMR spectra were recorded on Bruker AV-400 or 700 MHz at 298 K. 1H and 13C over Na2SO4, and concentrated under low pressure to a pH was adjusted to 6 evaporated under vacuum to half of their volume and the esters were completed within 3.0 h. The solvents are hydrolysis reactions. The hydrolyses of compounds having ester group (s, singlet; d, doublet; dd, doublet of doublet; t, triplet; q, quartet; dq, doublet of quartet; and m, multiplet.

General Procedure for the Hydrolysis of Ester into Acid. Compounds having ester group (4), (2-Bn), (2-Hexyl), and (2-Picolamide) were dissolved in tetrahydrofuran containing 2 equiv of LiOH at 0 °C and then brought to RT with stirring. TLC monitored the completion of those ester hydrolysis reactions. The hydrolyses of N-alkylated-β-hydrazidroxy esters were completed within 3.0 h. The solvents are evaporated under vacuum to half of their volume and the pH was adjusted to 6–7 with 1 M HCl and then extracted thrice with EtOAc. The organic layers were combined, dried over Na2SO4, and concentrated under low pressure to afford the acid derivative products. Without any further characterization, we proceed for the next step (amidine coupling). These N-alkylated-β-hydrazidroxy acids were coupled with natural α-amino acids in the presence of amide coupling reagents as HOAT (1.3 equiv), N-methyl morpholine (3 equiv) was EDC-HCl (1.3 equiv), and was dissolved in dry DMF (1.5 M). After stirring for 10 min, cooled it to 0 °C and added.

General Procedure for Peptide Synthesis. After hydrolysis of the ester group into the acid group, it is directly used for the peptide coupling reaction without any further purifications, where the corresponding amines (1.2 equiv) and HOAT (1.3 equiv) were dissolved in dry DMF (1.5 M). After stirring for 10 min, N-methyl morpholine (3 equiv) was added dropwise and cooled to 0 °C, and EDC-HCl was added (1.3 equiv). After 20 min, it was allowed to cool to RT, followed by heating at 55 °C for 14 h. The crude reaction mixture was evaporated under reduced pressure. The resultant crude was purified by column chromatography with MeOH in CH2Cl2 (1–3%). The obtained product was characterized using 1H/13C by NMR and HRMS by ESI-MS techniques. The characterization data of all synthesized hybrid peptides are provided in the following sections.

General Procedure for Boc Deprotection. TFA (30%) in CH2Cl2 (5 mL) was added to compound (5a) at RT and stirred for 3 h. The solvents were removed under vacuum, resulting in a red color residue (only in the case of troponyl derivative). The residual viscous oil was purified via chromatography with 3% MeOH in CH2Cl2. The obtained product was characterized using 1H/13C by NMR and HRMS by ESI-MS techniques. tert-Butyl-2-(3-ethoxy-3-oxopropyl)hydrazinyl peptide propenamide cleavage under mild acidic conditions.

Figure 9. Proposed mechanism of β-troponylhydrazinyl peptide propenamide cleavage under mild acidic conditions.
tert-Butyl-2-(3-((2S,3R)-1-methoxy-3-methyl-1-oxopentan-2-yl)amino)-3-oxopropyl)-2-(7-oxocyclohepta-1,3,5-trien-1-yl)hydrazine-1-carboxylate (5a). The dipeptide was synthesized by following the general procedure. The pure product (155 mg, 63% yield) was obtained as a yellow color solid. 1H NMR (700 MHz, deuterated solvent CDCl3): δ 7.92 (s, 1H), 7.18−6.99 (m, 4H), 6.75 (t, J = 9.0 Hz, 1H), 4.53−4.45 (m, 1H), 4.03 (s, 2H), 3.75 (s, 3H), 3.69−3.63 (m, 1H), 3.59−3.51 (m, 1H), 2.98−2.84 (m, 1H), 2.77−2.65 (m, 1H), 2.25−2.13 (m, 1H), 1.97−1.95 (m, 3H), 1.45 (s, 9H). 13C NMR (101 MHz, CDCl3): δ 182.62, 174.98, 174.77, 171.96, 171.15, 171.10, 139.46, 133.09, 129.90, 129.26, 128.49, 127.30, 126.18, 118.76, 118.67, 118.41, 78.41, 76.39, 52.76, 34.52, 33.84, 17.53. HRMS (ESI-TOF) m/z: [M + Na]+ calcd for C21H29N3O6, 442.1949; found, 442.1984.

tert-Butyl-(S)-2-(3-((1-methoxy-1-oxo-3-phenylpropan-2-yl)amino)-4-methyl-1-oxopentan-2-yl)amino)-3-oxopropyl)-2-(7-oxocyclohepta-1,3,5-trien-1-yl)hydrazine-1-carboxylate (5b). The dipeptide was synthesized by following the general procedure. The pure product (320 mg, 42% yield) was obtained as a yellow viscous liquid. 1H NMR (400 MHz, deuterated solvent CDCl3): δ 7.92 (s, 1H), 7.18−6.99 (m, 4H), 6.75 (t, J = 9.0 Hz, 1H), 4.53−4.45 (m, 1H), 4.03 (s, 2H), 3.75 (s, 3H), 3.69−3.63 (m, 1H), 3.59−3.51 (m, 1H), 2.98−2.84 (m, 1H), 2.77−2.65 (m, 1H), 2.25−2.13 (m, 1H), 1.97−1.95 (m, 3H), 1.45 (s, 9H). 13C NMR (101 MHz, CDCl3): δ 182.62, 174.98, 174.77, 171.96, 171.15, 171.10, 139.46, 133.09, 129.90, 129.26, 128.49, 127.30, 126.18, 118.76, 118.67, 118.41, 78.41, 76.39, 52.76, 34.52, 33.84, 17.53. HRMS (ESI-TOF) m/z: [M + Na]+ calcd for C21H29N3O6, 442.1949; found, 442.1984.

tert-Butyl-2-(3-((2S,3R)-1-methoxy-3-methyl-1-oxopentan-2-yl)amino)-3-oxopropyl)-2-(7-oxocyclohepta-1,3,5-trien-1-yl)hydrazine-1-carboxylate (5c). The dipeptide was synthesized by following the general procedure. The pure product (18 mg, 94%) was obtained as a red color solid and characterized by NMR (1H/13C) and MS. 1H NMR (400 MHz, deuterated solvent CDCl3): δ 7.57 (s, 1H), 7.24 (s, 1H), 7.20−6.99 (m, 3H), 6.83 (t, J = 9.1 Hz, 1H), 4.47 (t, J = 6.4 Hz, 1H), 4.00−3.86 (m, 1H), 3.70 (s, 4H), 2.77−2.47 (m, 2H), 1.86 (s, 1H), 1.43 (s, 9H), 1.30−1.13 (m, 2H), 0.87 (t, J = 7.8 Hz, 6H). 13C NMR (101 MHz, CDCl3): δ 182.21, 172.80, 171.81, 157.29, 155.67, 137.00, 133.95, 129.02, 81.39, 75.11, 75.14, 73.22, 34.90, 28.20, 25.29, 15.48, 11.54. HRMS (ESI-TOF) m/z: [M + Na]+ calcd for C21H23N3O6, 458.2262; found, 458.2295.

Methyl-(3-((tert-butoxycarbonyl)-1-(7-oxocyclohepta-1,3,5-trien-1-yl)hydrazin-1-yl)propanoyl)-L-prolinate (5g). The dipeptide was synthesized by following the general procedure. The pure product (96 mg, 43% yield) was obtained as a yellow viscous liquid. 1H NMR (400 MHz, deuterated solvent CDCl3): δ 7.73 (s, 1H), 7.60 (s, 1H), 7.27−7.13 (m, 7H), 7.11−7.03 (m, 2H), 6.86 (t, 1H), 4.84 (q, J = 7.8 Hz, 1H), 3.78 (s, 2H), 3.71 (s, 3H), 3.16 (dd, J = 14.0, 5.5 Hz, 1H), 3.04 (dd, J = 14.0, 8.1 Hz, 1H), 2.66−2.47 (m, 2H), 1.44 (s, 9H). 13C NMR (101 MHz, CDCl3): δ 182.24, 172.48, 171.55, 157.15, 155.41, 137.14, 136.52, 136.18, 133.95, 129.16, 128.44, 126.87, 81.41, 53.69, 52.27, 50.42, 37.48, 34.53, 28.23. HRMS (ESI-TOF) m/z: [M + H]+ calcd for C25H31N3O6, 470.2286; found, 470.2291.

-7-Oxocyclohepta-1,3,5-trien-1-yl)pyrazolidin-3-one (9). Troponylpyrazolidinone was synthesized by following the general procedure. The product was purified by column chromatography with MeOH in CH2Cl2 (2%). The pure product (18 mg, 94%) was obtained as a red color solid and characterized by NMR (1H/13C) and MS. 1H NMR (400 MHz, deuterated solvent CDCl3): δ 12.74 (s, 1H), 7.21−7.06.
(m, 2H), 6.94 (d, J = 11.6 Hz, 1H), 6.64 (t, J = 9.3 Hz, 1H), 6.36 (d, J = 10.8 Hz, 1H), 3.99 (t, J = 8.6 Hz, 2H), 2.80 (t, J = 8.6 Hz, 2H). 13C NMR (101 MHz, CDCl3): δ 178.12, 169.05, 150.01, 137.76, 135.62, 132.02, 124.59, 113.50, 47.68, 29.16. HRMS (ESI-TOF) m/z: [M + H]+ calcld for C_{16}H_{23}N_{3}O_{5}, 456.2493; found, 456.2501.

tert-Butyl-2-benzyl-2-(3-ethoxy-3-oxopropyl)hydrazine-1-carboxylate (2-Bn). The Boc-protected β-hydrazino acid derivative 2 (4.0 g, 17.24 mmol) was dissolved in ACN and K$_2$CO$_3$ (4.75 g, 34.48 mmol) was added to it and stirred at RT, followed by addition of benzyl bromide (3.07 mL, 25.86 mmol). This reaction mixture was allowed to stir at RT for 24 h until the disappearance of the starting material (2). The reaction was monitored by TLC with 40% ethyl acetate in hexane. After completion of the reaction, the reaction mixture was filtrated and concentrated under reduced pressure to obtain the crude product. The concentrated crude product was purified by silica gel column chromatography (230–400 mesh) in 10–20% ethyl acetate in hexane as the mobile phase. The desired product (2.8 g, 50%) was obtained as a white color solid and characterized by NMR (1H/13C) and MS methods.

1H NMR (400 MHz, deuterated solvent CDCl3): δ 7.31 (d, J = 4.3 Hz, 4H), 7.27 (d, J = 3.8 Hz, 1H), 5.57 (s, 1H), 4.13 (q, J = 7.1 Hz, 2H), 4.01 (s, 2H), 3.13 (s, 2H), 2.54 (t, J = 6.9 Hz, 2H), 1.39 (s, 9H), 1.24 (t, J = 7.1 Hz, 3H). 13C NMR (101 MHz, CDCl3): δ 172.40, 154.97, 136.81, 129.35, 128.23, 127.41, 79.95, 60.36, 51.56, 33.14, 28.22, 14.17. HRMS (ESI-TOF) m/z: [M + Na]$^+$ calcld for C$_{24}$H$_{25}$N$_{3}$O$_{5}$, 439.1779; found, 439.1749.

tert-Butyl-(S)-2-benzyl-2-(3-((1-methoxy-1-oxo-3-phenylpropan-2-yl)amino)-3-oxopropyl)hydrazine-1-carboxylate (6a). The dipeptide was synthesized by following the general procedure. The pure product (400 mg, 55% yield) was obtained as a white viscous liquid. 1H NMR (400 MHz, deuterated solvent CDCl3): δ 8.51 (s, 1H), 4.10 (q, J = 7.1 Hz, 2H), 2.98 (s, 2H), 2.67 (s, 2H), 2.50 (t, J = 6.5 Hz, 2H), 1.41 (s, 11H), 1.32–1.18 (m, 9H), 0.85 (t, J = 6.5 Hz, 3H). 13C NMR (101 MHz, CDCl3): δ 172.56, 155.06, 79.68, 60.37, 58.20, 32.74, 31.69, 28.26, 27.38, 22.54, 14.15, 14.00. HRMS (ESI-TOF) m/z: [M + Na]$^+$ calcld for C$_{38}$H$_{45}$N$_{4}$O$_{5}$, 539.2524; found, 539.2423.

tert-Butyl-(R)-2-hexyl-2-3-(1-methoxy-1-oxo-3-phenylpropan-2-yl)amino)-3-oxopropyl)hydrazine-1-carboxylate (7). The dipeptide was synthesized by following the general procedure. The pure product (150 mg, 45% yield) was obtained as a yellow viscous liquid. 1H NMR (400 MHz, deuterated solvent CDCl3): δ 8.45–8.30 (m, 1H), 7.34–7.16 (m, 5H), 5.35 (s, 1H), 4.81 (q, J = 7.8 Hz, 1H), 3.70 (s, 3H), 3.15 (dd, J = 2H), 2.98–2.76 (m, 2H), 2.74–2.53 (m, 2H), 2.40–2.24 (m, 2H), 1.47 (s, 9H), 1.34–1.23 (m, 0.9H), 0.90 (t, J = 6.7 Hz, 3H). 13C NMR (101 MHz, CDCl3): δ 172.35, 172.26, 155.83, 137.14, 129.30, 128.58, 128.29, 126.70, 80.28, 58.33, 54.41, 53.74, 52.04, 37.56, 33.73, 31.79, 29.69, 28.30, 26.82, 26.67, 22.58, 14.06. HRMS (ESI-TOF) m/z: [M + Na]$^+$ calcld for C$_{36}$H$_{43}$_N$_{4}$O$_{5}$, 472.2782; found, 472.2783.

tert-Butyl-2-(3-Ethoxy-3-oxopropyl)-2-picolinoylhydrazine-1-carboxylate (2-Picolamide). The picolinoyl derivative peptide ester was synthesized by following the general procedure. The pure product (1.6 g, 55% yield) was obtained as a yellow viscous liquid. 1H NMR (400 MHz, deuterated solvent CDCl3): δ 8.54 (s, 1H), 7.90–7.69 (m, 3H), 7.41–7.28 (m, 1H), 4.16 (q, J = 7.0 Hz, 2H), 4.02 (t, J = 6.4 Hz, 2H), 2.77 (t, J = 6.7 Hz, 2H), 1.31–1.17 (m, 12H). 13C NMR (101 MHz, CDCl3): δ 171.87, 169.76, 153.00, 147.74, 136.91, 124.81, 124.02, 81.43, 60.65, 45.30, 32.14, 27.84, 14.14. HRMS (ESI-TOF) m/z: [M + Na]$^+$ calcld for C$_{36}$H$_{43}$_N$_{4}$O$_{5}$, 338.1767; found, 338.1710.

tert-Butyl-(S)-2-3-(1-methoxy-1-oxo-3-phenylpropan-2-yl)amino)-3-oxopropyl)hydrazine-1-carboxylate (8). The tripeptide was synthesized by following the general procedure. The pure product (120 mg, 40% yield) was obtained as a white color solid and characterized by NMR (1H/13C) and MS methods. 1H NMR (400 MHz, deuterated solvent CDCl3): δ 5.41 (s, 1H), 4.10 (q, J = 7.1 Hz, 2H), 2.98 (s, 2H), 2.67 (s, 2H), 2.50 (t, J = 6.5 Hz, 2H), 1.41 (s, 11H), 1.32–1.18 (m, 9H), 0.85 (t, J = 6.5 Hz, 3H). 13C NMR (101 MHz, CDCl3): δ 172.56, 155.06, 79.68, 60.37, 58.20, 32.74, 31.69, 28.26, 27.38, 22.54, 14.15, 14.00. HRMS (ESI-TOF) m/z: [M + Na]$^+$ calcld for C$_{42}$H$_{50}$_N$_{5}$O$_{6}$, 591.3220; found, 591.3233.
General Procedure for the Synthesis of N-Troponyl-
pyrazolidinone (9). The Boc-protected troponyl peptides (~100 mmol), containing β-troponyl hydrazino acid, were added to 20–30% TFA in DCM (~5.0 mL) and stirred for 2–3 h at RT. The reaction was monitored by TLC before characterization by ESI-MS. The reaction mixture was purified through the silica column chromatography technique. The yield of the isolated cyclic product was ~94%.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/10.1021/acsomega.0c03729.

1. NMR (1H/13C) and ESI-HRMS spectra of new intermediates and β-hydrazine acids/peptides and time-dependent NMR in TFA

Crystallographic data for compound 5a* (tert-butyl ester) (CIF)

Crystallographic data for compound 5b (CIF)

Crystallographic data for compound 6a (CIF)

Crystallographic data for compound 9 (CIF)

AUTHOR INFORMATION

Corresponding Author
Nagendra K. Sharma — National Institute of Science Education and Research (NISER)-Bhubaneswar, Jatni, Odisha 752050, India; Homi Bhabha National Institute (HBNI), Mumbai 400 094, India; orcid.org/0000-0003-0901-0523; Email: nagendra@niser.ac.in

Authors
Nihar Ranjan Dalabehera — National Institute of Science Education and Research (NISER)-Bhubaneswar, Jatni, Odisha 752050, India; Homi Bhabha National Institute (HBNI), Mumbai 400 094, India

Sagarika Meher — National Institute of Science Education and Research (NISER)-Bhubaneswar, Jatni, Odisha 752050, India; Homi Bhabha National Institute (HBNI), Mumbai 400 094, India

Bibhuti Bhusana Palai — National Institute of Science Education and Research (NISER)-Bhubaneswar, Jatni, Odisha 752050, India; Homi Bhabha National Institute (HBNI), Mumbai 400 094, India

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.0c03729

Author Contributions
N.R.D., S.M., and B.B.P. authors contributed equally.

ACKNOWLEDGMENTS

N.R.D. thanks Dr Amarnath Bolu for analyzing the NMR kinetic data. B.B.P. thanks UGC-New Delhi for JRF/SRF Fellowship. N.K.S. thanks CSIR-HRDG-New Delhi, grant number 02(0166)/13/EMR, for financial support.

REFERENCES

(1) Barrett, A.; Woessner, J.; Rawlings, N. D. Enzymes can of course efficiently orchestrate amide cleavage under physiological conditions. *Handbook of Proteolytic Enzymes*, 2nd ed.; Academic Press: San Diego, 2004; pp 1–30.

(2) Radzicka, A.; Wolfenden, R. Rates of uncatalyzed peptide bond hydrolysis in neutral solution and the transition state affinities of proteases. *J. Am. Chem. Soc.* 1996, 118, 6105–6109.

(3) Bennet, A. J.; Somayaji, V.; Brown, R. S.; Santarsiero, B. D. The influence of altered amidic resonance on the infrared and carbon-13 and nitrogen-15 NMR spectroscopic characteristics and barriers to rotation about the NC(O) bond in some anilides and toluamides. *J. Am. Chem. Soc.* 1991, 113, 7563–7571.

(4) Lide, D. R. *CRC Handbook of Chemistry and Physics*, 3rd ed.; CRC, Boca Raton, Florida, 2000; pp 3–84.

(5) Clayden, J. Organic chemistry: Stabilizers cause instability. *Nature* 2012, 481, 274–275.

(6) Clayden, J.; Moran, W. J. The Twisted Amide 2-Quinuclidinone: 60 Years in the Making. *Angew. Chem., Int. Ed. Engl.* 2006, 45, 7118–7120.

(7) Sztokas, M.; Aubé, J. Medium-bridged lactams: a new class of non-planar amides. *Org. Biomol. Chem.* 2011, 9, 27–35.

(8) Tani, K.; Stoltz, B. M. Synthesis and structural analysis of 2-quinuclidinum tetrafluoroborate. *Nature* 2006, 441, 731–734.

(9) Somayaji, V.; Brown, R. S. Distorted amides as models for activated peptide NC: O units produced during enzyme-catalyzed acyl transfer reactions. I. The mechanism of hydrolysis of 3, 4-dihydro-2-oxo-1, 4-ethanoquinoline and 2, 3, 4, 5-tetrahydro-2-oxo-1, 5-ethanobenzazepine. *J. Org. Chem.* 1986, 51, 2676–2686.

(10) Komarov, I. V.; Yanik, S.; Ishchenko, A. Y.; Davies, J. E.; Goodman, J. M.; Kirby, A. J. The most reactive amide as a transition-state mimic For cis-trans interconversion. *J. Am. Chem. Soc.* 2015, 137, 926–930.

(11) Balachandra, C.; Sharma, N. K. Novel fluorophores: Syntheses and photophysical studies of boron-aminotriponitrones. *Dyes Pigment.* 2017, 137, 532–538.

(12) Kita, Y.; Nishii, Y.; Onoue, A.; Mashima, K. Combined catalytic system of scandium trflate and boronic ester for amide bond cleavage. *Adv. Synth. Catal.* 2013, 355, 3391–3395.

(13) Hie, L.; Fine Nathel, N. F.; Shah, T. K.; Baker, E. L.; Hong, X.; Yang, Y.-F.; Liu, P.; Houk, K. N.; Garg, N. K. Conversion of amides to esters by the nickel-catalyzed activation of amide C–N bonds. *Nature* 2015, 524, 79.

(14) Hutchby, M.; Houlden, C. E.; Haddow, M. F.; Tyler, S. N. G.; Lloyd-Jones, G. C.; Booker-Milburn, K. I. Switching pathways: room-temperature neutral solvolysis and substitution of amides. *Angew. Chem., Int. Ed. Engl.* 2012, 51, 548–551.

(15) Shimizu, Y.; Noshita, M.; Mukai, Y.; Morimoto, H.; Ohshima, T. Cleavage of unactivated amide bonds by ammonium salt-accelerated hydrazinolysis. *Chem. Commun.* 2014, 50, 12623–12625.

(16) Bolu, A.; Sharma, N. K. Cleavable Amide Bond: Mechanistic Insight into Cleavable 4-Aminopyrazoloxy Acetamide at Low pH. *J. Org. Chem.* 2019, 84, 5596–5602.

(17) Stephenson, N. A.; Zhu, J.; Gellman, S. H.; Stahl, S. S. Catalytic transamination reactions compatible with tertiary amide methatization under ambient conditions. *J. Am. Chem. Soc.* 2009, 131, 10003–10008.

(18) Avan, I.; Hall, C. D.; Katritzky, A. R. Peptidomimetics via modifications of amino acids and peptide bonds. *Chem. Soc. Rev.* 2014, 43, 3575–3594.

(19) Balachandra, C.; Sharma, N. K. Synthesis and conformational analysis of new troponyl aromatic amida acid. *Tetrahedron* 2014, 70, 7464–7469.

(20) Balachandra, C.; Sharma, N. K. Instability of Amide Bond Comprising the 2-Aminotropone Moiety: Cleavable under Mild Acidic Conditions. *Org. Lett.* 2015, 17, 3948–3951.

(21) Balachandra, C.; Sharma, N. K. Direct/Reversible Amidation of Troponyl Alkylglycinates via Cationic Troponyl Lactones and Mechanistic Insights. *ACS Omega* 2018, 3, 997–1013.

(22) Kang, C. W.; Sarnowski, M. P.; Elbattawi, Y. M.; Del Valle, J. R. Access to enantiopure α-hydradnoic acids for N-amino peptide synthesis. *J. Org. Chem.* 2017, 82, 1833–1841.

(23) Li, X.; Yang, D. Peptides of aminxoy acids as foldamers. *Chem. Commun.* 2006, 3367–3379.
(24) Kent, E.; Hoops, S.; Mendes, P. Condor-COPASI: high-throughput computing for biochemical networks. *BMC Syst. Biol.* 2012, 6, 91.

(25) Breheret, E. F.; Martin, M. M. Electronic relaxation of troponoids: tropolone fluorescence. *J. Lumin.* 1978, 17, 49–60.

(26) Bordessa, A.; Keita, M.; Maréchal, X.; Formicola, L.; Lagarde, N.; Rodrigo, J.; Bernadat, G.; Bauvais, C.; Soulier, J.-L.; Dufau, L.; Milcentau, T.; Crousse, B.; Reboud-Ravaux, M.; Ongeri, S. α-and β-hydrazino acid-based pseudopeptides inhibit the chymotrypsin-like activity of the eukaryotic 20S proteasome. *Eur. J. Med. Chem.* 2013, 70, 505–524.