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Cis–Trans Conformational Analysis of δ-Azaproline in Peptides

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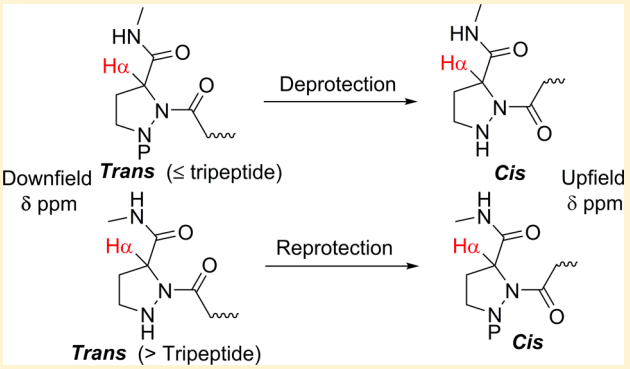
Supporting Information

ABSTRACT: The cis–trans isomerization and conformer specificity of δ-azaproline and its carbamate-protected form in linear and cyclic peptides were investigated using NMR and α-chymotrypsin assay. Comparisons of the chemical shift value of the α-hydrogen in each case of δ-azaproline-containing peptides with conformer-specific locked diketopiperazines reveal the fact that an upfield chemical shift value corresponds to cis conformer and a downfield value corresponds to a trans conformer. δ-Azaproline adopts cis-conformation in simple amides, dipeptides, and tripeptides whereas its carbamate-protected form adopts trans-conformation. In the case of longer, linear or cyclic peptides, vice versa results are obtained. Interestingly, in all these peptides exclusively one conformer, either cis or trans, is stabilized. This cis–trans isomerization is independent of both temperature and solvents; only the δ-nitrogen protecting group plays key role in the isomerization. δ-Azaproline is conformer-specific in either of its protected or deprotected forms, which is a unique property of this proline. Unlike other covalently modified proline surrogates, this isomerization of δ-azaproline can be tuned easily by a protecting group. The mechanism of cis–trans isomerization of δ-azaproline during deprotection and reprotection is supported by theoretical calculations.



INTRODUCTION

Cyclic α-hydrazino acids (**1**) (Figure 1) have emerged as one of the most promising nonproteinogenic amino acid candidates,

Figure 1. General structure of cyclic α-hydrazino acids.

and they may find use as proline surrogates to improve peptide pharmacokinetics[1] and/or bioavailability. However, before being considered as a proline substitute, their effect on the structure of peptides needs to be thoroughly investigated, because the function of peptides and proteins largely depend on their structure.

In protein structures, due to significant delocalization of the lone pair of electrons on the nitrogen atom the peptide bond attains a partial double bond character. The partial double bond renders the amide group planar, allowing it to adopt either a cis or a trans conformation. These bonds are free to adopt either of the conformations in the unfolded state but are only allowed to adopt the energetically more favorable trans conformation in the folded state.[2]

Proline is unique among other naturally occurring amino acids, in that the imino acid proline residue restricts the conformational space of the peptide chain. However, Xaa-Pro peptide groups tend to have a roughly 3:1 cis–trans ratio, apparently because the symmetry between the C^{α} and C^{δ} atoms of proline makes both conformers nearly equal in energy. Consequently, any change in α and δ positions will be reflected in the cis–trans ratio, as the symmetry will be disrupted. Because of this unique conformational property, proline plays an important role in the structural and biological properties of peptides and proteins.[3a−f] Loss of symmetry owing to any substitution at the α[3g−i] or δ position[3j−o] of proline has effects on the structure of peptides as have been investigated by several groups. In most cases, conformational studies of peptides or protein folding is hampered by the heterogeneity of the Xaa-Pro peptide group due to the presence of cis/trans mixtures. To eliminate complications arising from the cis/trans isomerization of proline peptide groups, the scientific community has constantly been on the lookout for either cis- or trans-stabilizing proline analogues[3,4] or surrogates (diminishing or enlarging the pyrrolidine ring size).[4k] In this context, the conformational behavior of pyrazolidines as proline equivalents in peptides has been extensively explored by Marshall and co-workers.[5] To some extent, pyrazolidines resembled proline and could stabilize the cis conformer in organic solvents and in the solid phase; however, it loses its conformer specificity in aqueous solutions. Taking a cue from these studies, we contemplated the use of δ-azaproline **1** as a proline surrogate to achieve conformer specificity.

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δ-Azaproline **1**, a five-membered cyclic α-hydrazino acid, closely resembles proline in structure and ring size, the only difference being a nitrogen atom at the δ position instead of carbon. Herein we report the effect of δ-azaproline and a carbamate protecting group at the δ-nitrogen on the cis−trans conformation of δ-azaproline-containing linear and cyclic peptides.

RESULTS AND DISCUSSION

Model Study. The cis−trans conformation of δ-azaproline containing peptide is shown in Figure 2, which is defined by the spatial arrangement of peptide chains, either lying syn or anti to each other is termed as the cis or trans conformer, respectively.

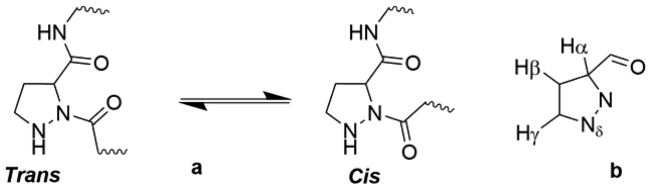


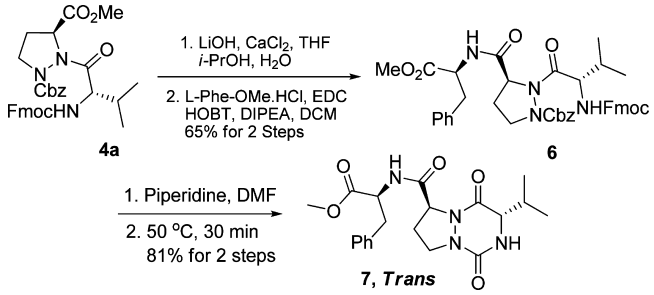
Figure 2. (a) Cis−trans isomerization in δ-azaproline. (b) Relative positions in δ-azaproline.

Chemical shift values of the α-hydrogen in ^{1}H-NMR was our preferred tool for the identification of cis and trans conformers of δ-azaproline and its peptides. Initially we investigated the chemical shift of the α-H corresponding to the cis-locked and trans-locked diketopiperazines of δ-azaproline, and accordingly the compounds were synthesized. Compound **2** was treated with 20% TFA in DCM followed by selective benzyloxy carbonyl protection of δ-nitrogen to yield **3**.[6] Compound **3** was heated with Fmoc-Val-Cl and AgCN in benzene[7] to yield **4**, which on Fmoc deprotection and heating in the same pot yielded the diketopiperazine **5**. Compound **5** is locked in cis conformation (Scheme 1).

Similarly, we attempted to synthesize trans-locked diketopiperazine from **4** but isolated **5** as the sole product. Accordingly, Scheme 2 was followed to synthesize the desired product. Compound **4a** was hydrolyzed by LiOH in $CaCl_2$ medium[8a] followed by peptide coupling with L-phenylalanine methyl ester to yield **6**.[8b] Fmoc deprotection and in situ heating yielded the diketopiperazine **7**. The diketopiperazine **7** is locked in trans conformation (Scheme 2).

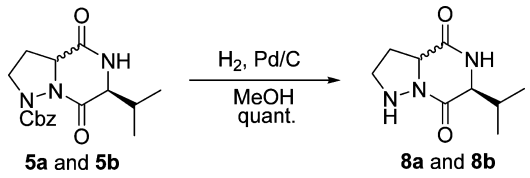
To establish the electronic effect of the carbonyl group adjoining the δ-nitrogen on the α-H ppm value, compound **5**

Scheme 2. Synthesis of Trans-Locked Diketopiperazine 7



was hydrogenated to remove the Cbz protection to yield **8** (Scheme 3).

Scheme 3. Synthesis of Deprotected Cis-Locked Diketopiperazine 8



NMR (^{1}H, HMQC, and COSY in Supporting Information) studies on compounds **5**, **7**, and **8** were performed to establish the signals corresponding to the α-hydrogen, and the observations were as follows.

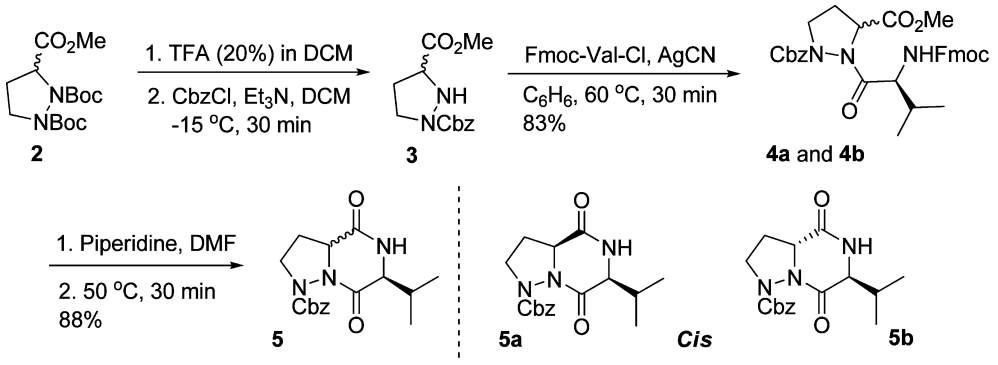
Compound 5. The α-hydrogen signal of diketopiperazine **5** was identified at δ 4.25. As **5** is locked in cis conformation, this value (ppm) was taken as the standard region for cis conformers of δ-azaproline-containing peptides (Figure 3).

Compound 7. The α-hydrogen signal of diketopiperazine **7a** was identified at δ 4.86. As **7a** is locked in trans conformation, this value (ppm) was taken as the standard region for trans conformers of δ-azaproline-containing peptides (Figure 4).

Compound 8. The α-hydrogen signal of the deprotected diketopiperazine **8a** was identified at 4.27. As **8a** is locked in cis conformation, this value (ppm) was taken as the standard region for deprotected cis conformers of δ-azaproline-containing peptides (Figure 5).

In conclusion, the cis conformer generally can be identified by an upfield α-hydrogen signal, and the trans conformer by a downfield α-hydrogen signal. Also the protecting group at the

Scheme 1. Synthesis of Cis-Locked Diketopiperazine 5



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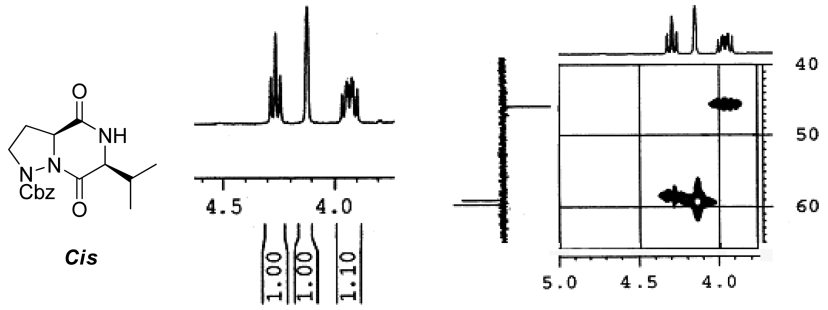


Figure 3. [1]H NMR and HMQC spectra of compound **5a**.

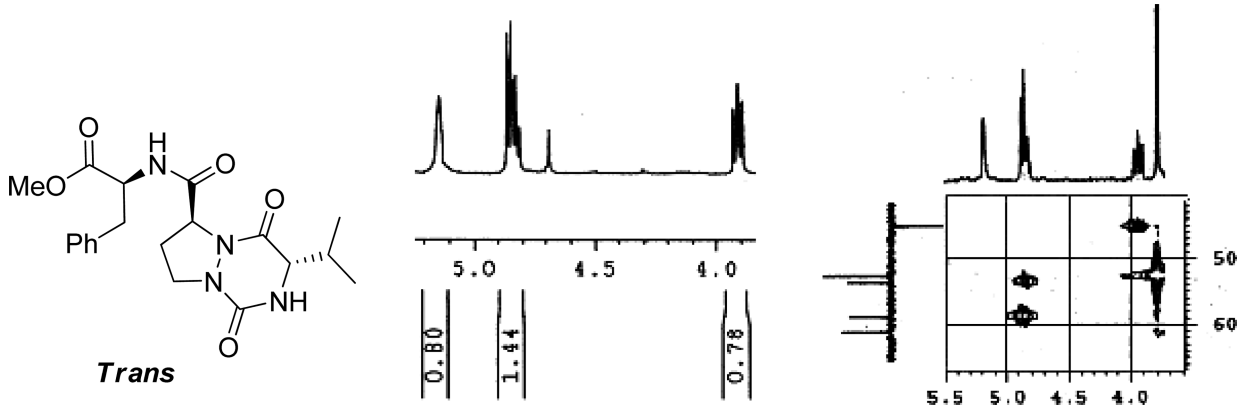


Figure 4. [1]H NMR and HMQC spectra of compound **7**.

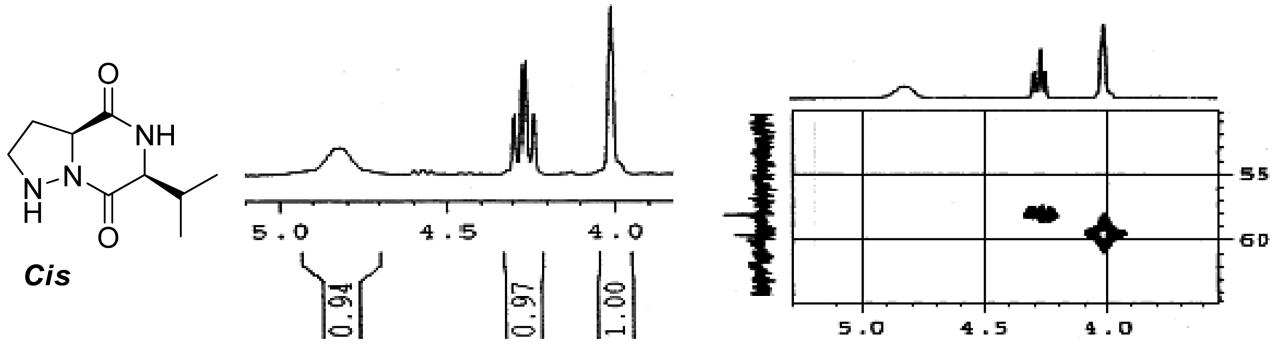


Figure 5. [1]H NMR and HMQC spectra of compound **8a** (for detailed NMR spectra, see Supporting Information).

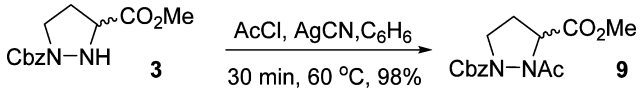
δ-nitrogen has no notable electronic effect on the chemical shift of α-hydrogen.

Obtaining the required information from the above experiments, we proceeded to determine the actual inclination of protected and unprotected δ-azaproline toward the cis or trans conformation. To establish the cis−trans conformation in δ-azaproline, we considered a model study based on previous literature reports on *N*-acetylproline methyl ester.[9] The [1]H NMR spectrum of *N*-acetylproline methyl ester showed the presence of two isomers in roughly 3:1 ratio (78:22), i.e., 78% trans and 22% cis, characterized by the α-H peaks at δ 4.33 (dd, J = 8.7, 3.6 Hz, 0.78H) corresponding to trans and at δ 4.27 (dd, J = 8.6, 2.8 Hz, 0.22H) for cis conformers, respectively (see Supporting Information).

Analogous azaproline-based substrate **9** was prepared by acetylating **3** with acetyl chloride and AgCN in benzene (Scheme 4).

[1]H NMR and HMQC studies on **9** indicated the presence of only one conformer (Figure 6). Comparing those with the chemical shift values from the previous section, we can say that Cbz-protected δ-azaproline exists exclusively in the trans conformation.

Scheme 4. Synthesis of Model Substrate 9



We have synthesized another azaproline derivative **10** with a photolabile protecting group. Such protecting groups are known to be cleaved in the presence of light, and this strategy could be useful in biological research. Compound **2** was converted to **11** by selectively protecting with veratroyloxy chloroformate followed by acetylation (Scheme 5).

1H and 2D NMR studies of **11** again illustrated the presence of trans conformer exclusively, similar to that of compound **9** (Figure 7). The role of electronic parameters (presence of

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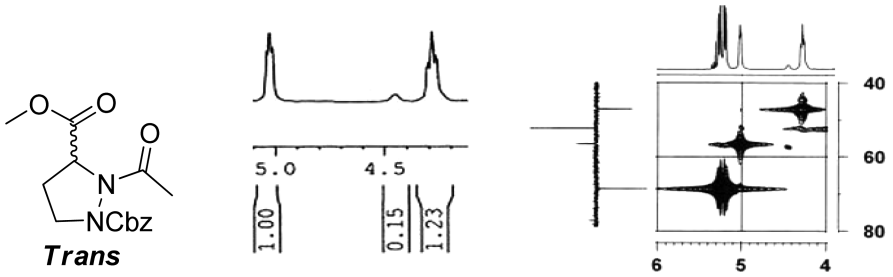
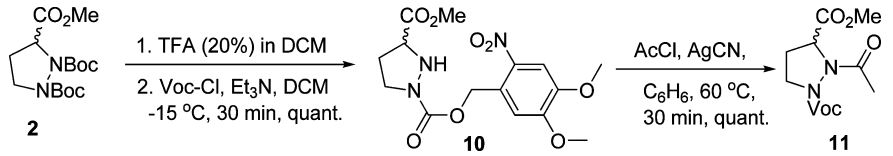


Figure 6. [1]H NMR and HMQC spectra of **9**.

Scheme 5. Synthesis of *N*-Voc-Protected Model Substrate 11



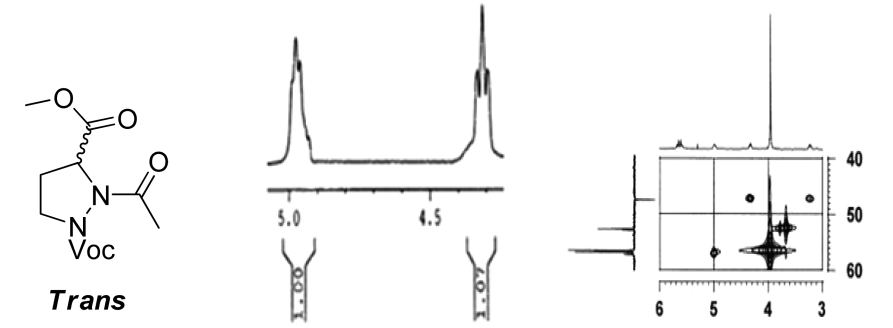
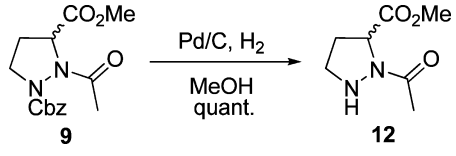


Figure 7. [1]H NMR and HMQC spectra of **11**.

NO_2) on the chemical shift of α-hydrogen was ruled out by our previous study of compound **8** (Scheme 3, Figure 5).

To spot the effect of deprotection, compound **9** was subjected to hydrogenation conditions to yield **12** (Scheme 6). NMR analysis of **12** showed the presence of only cis conformer, indicating a crossover from trans conformation during the deprotection (Figure 8).

Scheme 6. Synthesis of Deprotected Model Substrate 12



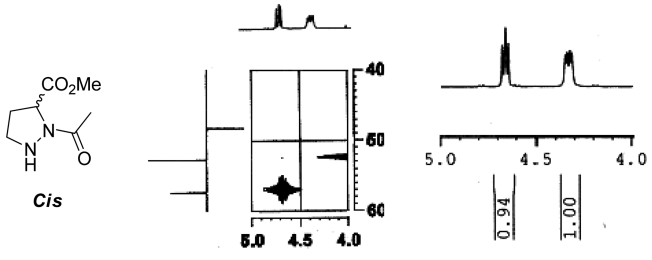


Figure 8. [1]H NMR and HMQC spectra of **12**.

The effect of electronic factor on the chemical shift value was again ruled out by comparing with compound **8**.

The possible preference for cis conformation of the deprotected form (**12**) was initially thought to be due to the formation of an intramolecular H-bonding between the hydrogen attached to the δ-nitrogen and the adjacent oxygen of *N*-acetyl (highlighted in red), but rapid deuterium exchange with D_2O suggested otherwise (Figure 9). A proton which is part of an intramolecular hydrogen bond is expected to exchange slowly if at all.[10]

After this investigation, we tried to establish the temperature dependence of cis−trans isomerization between the two conformers. Accordingly, high temperature NMR studies on both compounds **9** and **12** were performed. The compounds were dissolved in DMSO-d_6, and proton NMR spectra were recorded at an interval of 10 °C (25 °C to 105 °C).

Both the Cbz-protected model substrate **9** and the deprotected substrate **12** did not undergo any cis−trans isomerization in the temperature range between 25 °C to 105 °C (Figure 10).

Apart from the expected shift, the change of solvent ($CDCl_3$ to DMSO-d_6) also had no effect on the isomerization as evident from the chemical shift values of the α-H of **9** and **12** under investigation.

These investigations also beg the question about the fate of **12** on reinstallation of a protecting group; consequently, it was subjected to standard Cbz protection conditions (DMAP, Et_3N, and CbzCl) only to recover the starting material. Harsher conditions (AgCN, CbzCl, at 60 °C) yielded protected δ-azaproline which was identical to previously synthesized **9** in all aspects (Scheme 7).

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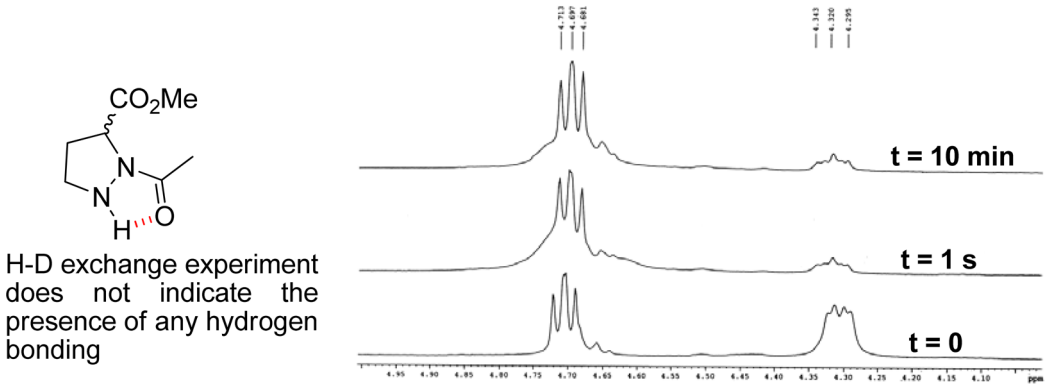


Figure 9. H−D exchange experiment on **12**.

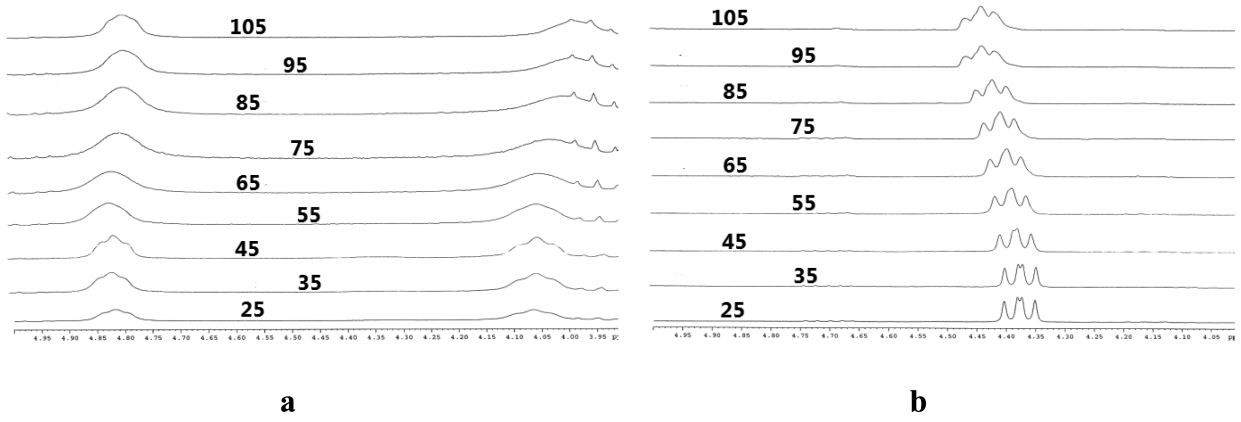
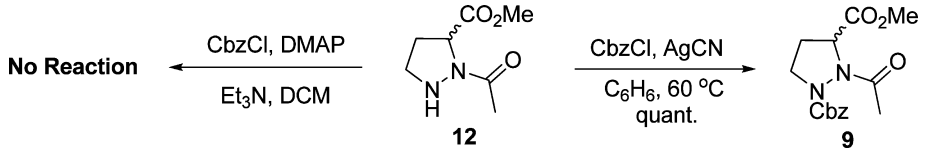


Figure 10. (a) Variable temperature NMR spectra of **9**. (b) Variable temperature NMR spectra of **12** (values in the spectra denote the temperature).

Scheme 7. Reprotection of Model Substrate 12



From the above model studies, it was concluded that the δ-azaproline in simple amides exclusively adopts a trans conformation in its protected form and cis conformation in its native form. These conformations resist interconversion even at elevated temperatures; only protection or deprotection seems to bring about the conformational changes. At this point, to gain insight into the mechanism of isomerization during deprotection or reprotection, we turned toward theoretical calculations.

1. Computational Details. We have optimized each intermediate and transition state using ωB97X-D[11] functional with Pople's 6-31++g(d,p) basis set on each atom except Ag. For the Ag atom, we have used LANL2 pseudopotential for core electrons and LANL2DZ basis set for valence electrons. All optimizations were performed in the presence of continuum with the help of SMD solvent model[12] and the appropriate dielectric constant of solvent. For deprotection of **9t** (**t** stands for trans) using Pd/H_2, we have used DMSO as solvent, and for protection of **12c** (**c** stands for cis) using AgCN and CbzCl, we have used benzene as solvent. Determination of entropy of a species in solution phase is difficult to compute, so an approximation proposed by Wertz[13] to compute solution-phase entropies from gas-phase entropies based on experimental results was used. This approximation is frequently employed for evaluating free energies by static quantum chemical calculations.[14] We have calculated and incorporated the decrease of entropy in the solution phase by reducing gas-phase entropy to 0.5 times its actual value. All theoretical calculations are performed using the standard Gaussian09 quantum chemical package.[15]

2. Mechanism of Deprotection of Cbz-Protected Amide Using Pd/H_2. The optimized structure of **9t** (shown in Figure 11) shows a strong CH−π interaction between the methyl CH of the amide bond present nearest to the chiral center and the benzene ring present in the benzyl group which is used for protection of the amine. In the optimized structure of **9t**, the amide C−N bond distance is 1.37 Å. It is well-known that during deprotection of the Cbz-protected amine by Pd/H_2, a carbamic acid intermediate is generated which undergoes decarboxylation to produce the deprotected amine.[16] We have optimized the in situ-generated carbamic acid **9t_1** (shown in Figure 11) from **9t**. The optimized structure of **9t_1** shows hydrogen bonding between the proton of the carbamic acid and the amide nitrogen and between the proton of the carbamic acid and the carbonyl group of the substituent attached to the chiral carbon. The proton of the carboxylic acid in **9t_1** can be

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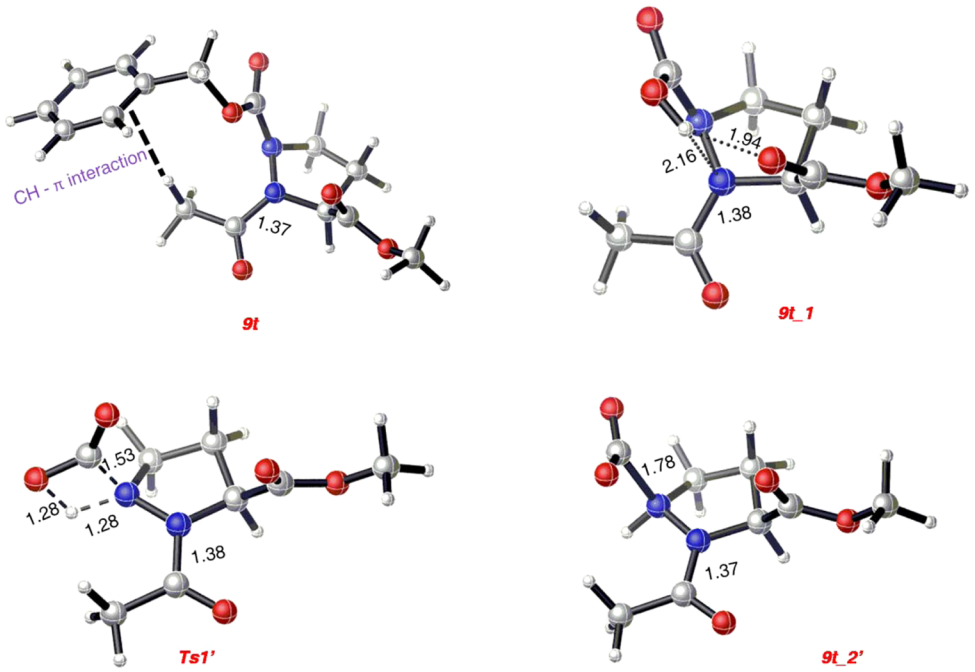

Figure 11. Optimized structures of **9t**, **9t_1**, **Ts1**′, and **9t_2**′. Bond distances are shown in angstroms. C, N, and O are shown in gray, blue, and red. H atoms are shown in gray also but they have smaller van der wall radii compared to C atoms. This color coding is maintained throughout this section.

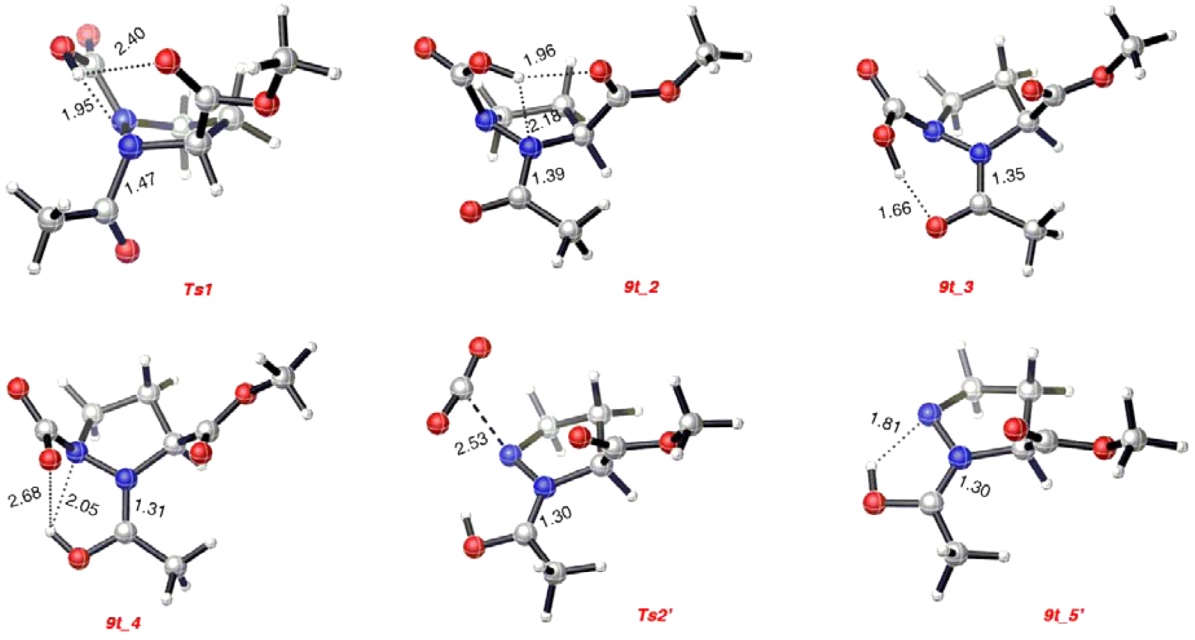

Figure 12. Optimized structure of **Ts1**, **9t_2**, **9t_3**, **9t_4**, **Ts2**′, and **9t_5**′. Bond distances are shown in angstroms.

transferred through a four-membered transition state **Ts1**′ (shown in Figure 11) to the nitrogen atom to produce **9t_2**′ (shown in Figure 11) with a free energy activation barrier of 37.3 kcal/mol. This barrier height is insurmountable under the reaction conditions. Also if this decarboxylation happens, we would get a trans isomer of **12**. But our experimental results show that the cis isomer of **12** is obtained selectively. The optimized structure of **9t_1** (shown in Figure 11) shows that C−N amide bond length is 1.38 Å, which is longer compared to **9t** (shown in Figure 11). So we looked into the possibility of rotation of the amide bond to produce the cis isomer of the carbamic acid, **9t_2** (shown in Figure 12). Our theoretical computation predicts a very low free energy activation barrier ($\Delta G^{\ddagger}$ = 15.3 kcal/mol) for the amide bond rotation via **Ts1**, and the reason behind this low activation barrier is the stabilization provided by the proton of the carboxylic acid to the lone pair of electrons on nitrogen during rotation. As a result of amide bond rotation, **9t_3** is obtained and it consists of a strong hydrogen bonding interaction between the carbonyl oxygen of the amide group and the proton of the carboxylic acid. Starting with **9t_3**, an intramolecular proton transfer from

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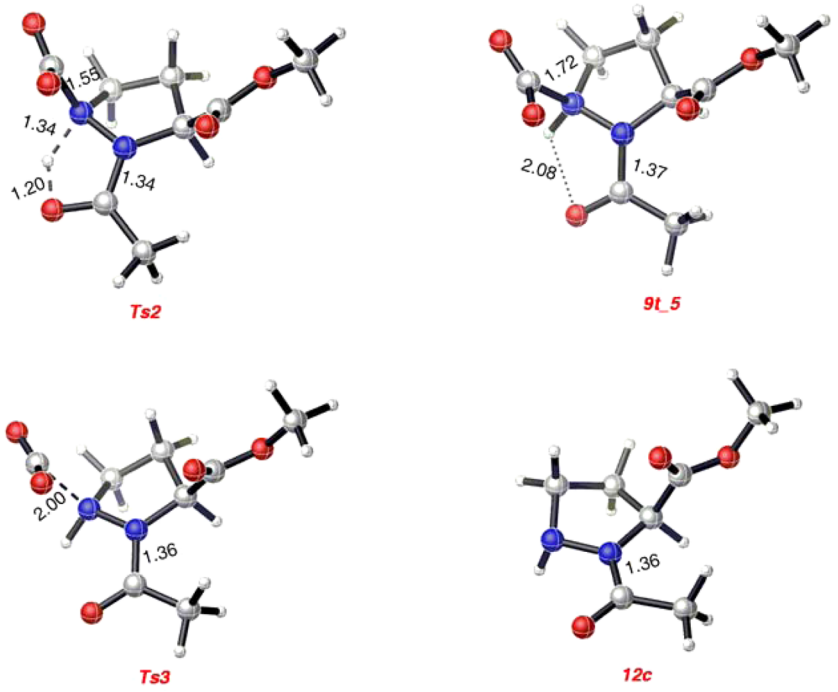

Figure 13. Optimized structure of **Ts2**, **9t_5**, **Ts3** and **12c**. Bond distances are shown in angstroms.

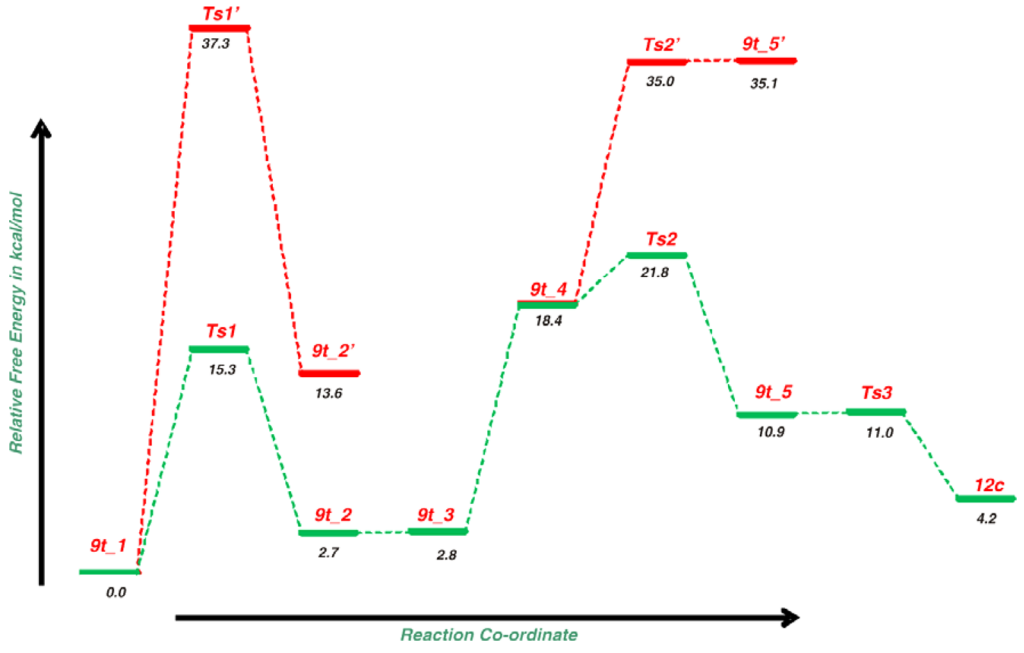

Figure 14. Relative free energy profile for the decarboxylation process of the carbamic acid generated from **9t** during deprotection using Pd/H_2.

the carboxylic acid to the carbonyl oxygen atom of the adjacent amide group yielded **9t_4** (shown in Figure 12).

The optimized structure of **9t_4** shows that the amide C−N bond length is shortened and the adjacent carbonyl C−O bond length has increased appreciably compared with that of **9t_3**, suggesting the lone pair donation to the π^* orbital of the protonated carbonyl group. We did not get any transition state for the **9t_3** to **9t_4** transformation. We have performed a relaxed scan to locate the transition state for the above-mentioned process, but no transition state for this proton transfer process could be identified. Hydrogen bonding interaction between the proton of the imidic acid form of the protonated amide and the lone pair of the adjacent nitrogen atom in **9t_4** (shown in Figure 12) clearly suggests another possible intramolecular proton transfer. This proton transfer happens via **Ts2** (structure shown in Figure 13) with a free energy activation barrier of 21.8 kcal/mol.

As a result of this proton transfer, **9t_5** is formed (an exoergic process), followed by decarboxylation of **9t_5** via **Ts3** with an activation barrier of 11.0 kcal/mol, leading to the formation of **12c**. Though formation of **12c** is endoergic in nature, the deprotection process is favored, as the release of CO_2 from solution phase to gas phase creates a non-equilibrium situation.

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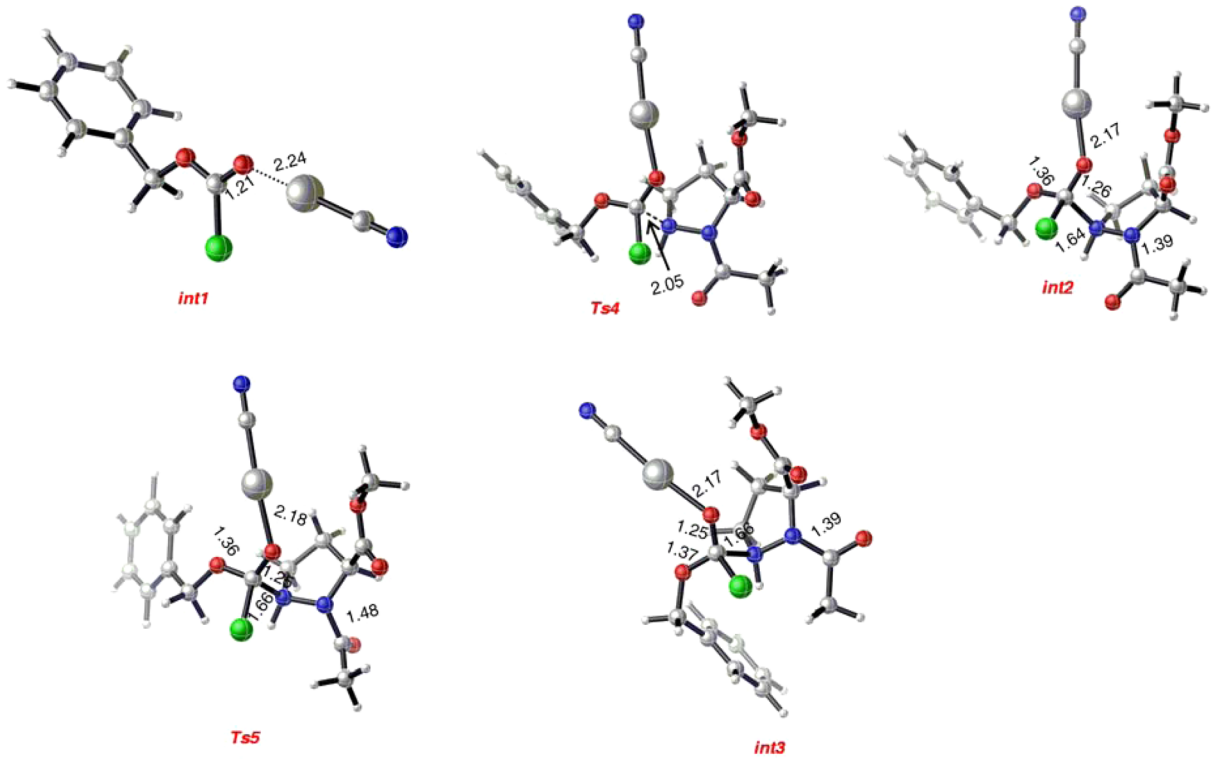
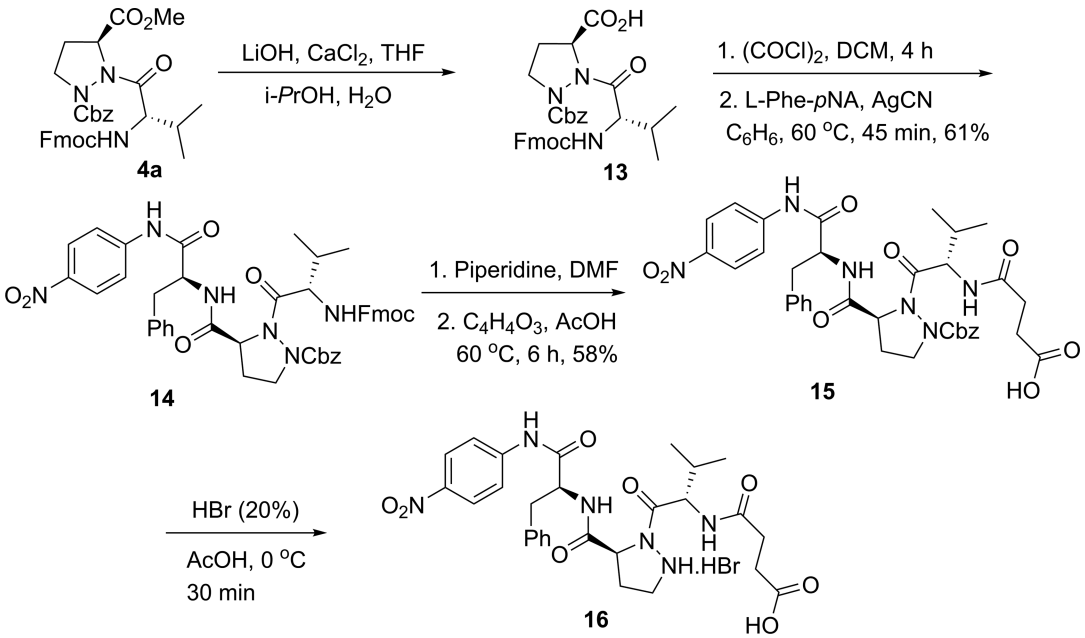


Figure 15. Optimized structures of important intermediates and transition states related to the conformational change of the amide bond of **12c** during protection using AgCN and Cbz. Bond distances are shown in angstroms.

Scheme 8. Synthesis of Chymotrypsin Substrates 15 and 16



Decarboxylation can also happen before intramolecular proton transfer, i.e., from **9t_4** via **Ts2′** (Figures 12 and 14). Because this route requires an activation energy of 35.0 kcal/mol, it is very unlikely for the reaction to follow this path.

Our detailed theoretical investigation shows that the minimum energy pathway for deprotection of **9t** involves rotation of the amide bond, which plays a crucial role in the deprotonation of the carbamic acid and subsequently facilitates the decarboxylation process. So our theoretical calculations offer an explanation for the crossover from trans to cis isomer during deprotection of the amide (**9t**).

3. Mechanism for Protection of Amine Using Cbz and AgCN. In this section we have investigated the mechanistic details of conformational change in the amide group during protection of **12c** with AgCN and Cbz. We found that the carbonyl oxygen of the Cbz can coordinate with AgCN to produce **int1** (see Figure 15). The optimized structure of **int1** shows an increase in C−O (present in Cbz) bond length (the

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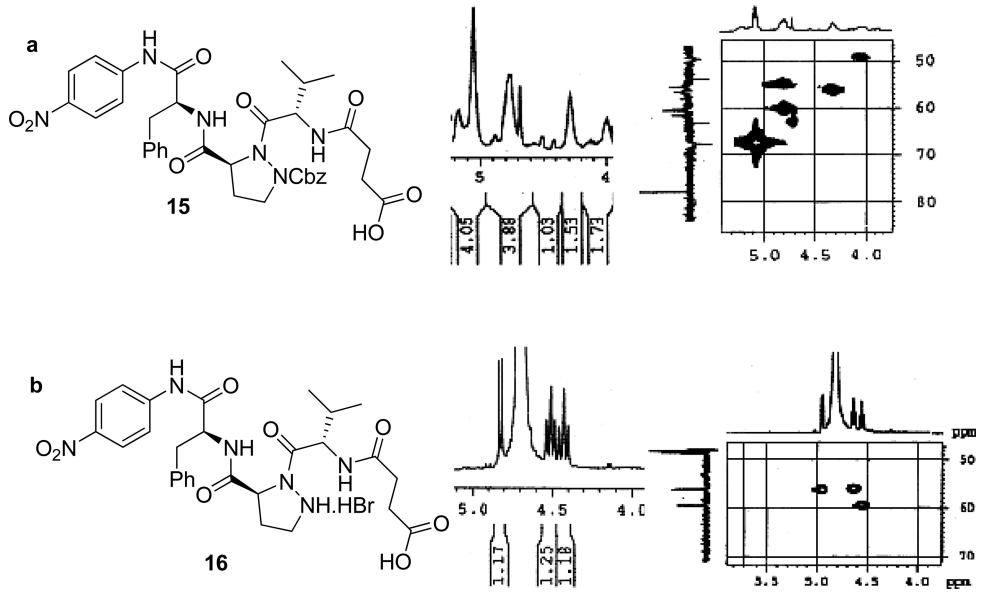

Figure 16. (a) ^{1}H NMR and HMQC spectra of **15**. (b) ^{1}H NMR and HMQC spectra of **16**.

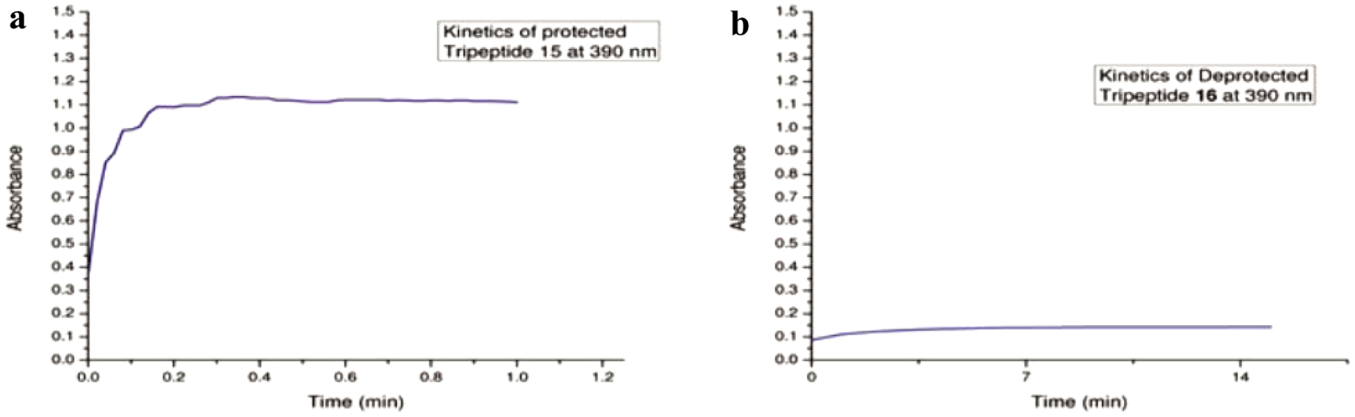

Figure 17. (a) Chymotrypsin assay of **15**. (b) Chymotrypsin assay of **16**.

bond length of C═O group of **int1** is 1.21 Å whereas the C═O bond length of Cbz is 1.19 Å) (see Figure 15). So AgCN acts as a Lewis acid to polarize the carbonyl group, facilitating subsequent nucleophilic addition by amine **12c**. Formation of **int1** is exothermic by 7.2 kcal/mol in terms of free energy. Nucleophilic addition of **12c** to the carbonyl group of **int1** produces **int2** via **Ts4** with a free energy activation barrier of 3.7 kcal/mol (see Figure 15). Formation of **int2** is endothermic compared to the separated reactants (**int1** and **12c**) by 2.34 kcal/mol. The optimized structure of **int2** shows that the amide C−N bond length is 1.39 Å (Figure 15), which is longer compared to **9t_1**. Starting with **int2**, the amide bond can rotate through **Ts5** (with a free energy activation barrier of 17.6 kcal) to attain the trans form of the nucleophilic addition product, **int3** (Figure 15). So this low free energy activation barrier allows the conversion of cis to trans form of the amide during protection of **12c** by AgCN and Cbz, which is observed experimentally.

To experimentally establish and generalize this observation in tripeptides, the chymotrypsin coupled assay developed by Fischer et al.[17a] was used. Short model peptides of type Suc-Val-ΨPro-Phe-*p*NA are hydrolyzed by chymotrypsin between Phe and *p*NA only if the Val-ΨPro peptide bond is in the trans conformation (the degree of tolerance of enzymes toward proline surrogates was demonstrated by M. Mutter et al.).[18,3a] A high rate of Phe-pNA hydrolysis by the enzyme is expected of the protected δ-azaproline-containing model peptide (known as burst phase kinetics); on the other hand, the deprotected δ-azaproline-containing model peptide is not expected to undergo hydrolysis.

The azaproline-containing model peptides for chymotrypsin studies were prepared from **4a**. The free acid **13** on treatment with oxalyl chloride followed by AgCN-mediated coupling with L-Phe-*p*NA provided the tripeptide **14**. Compound **14** on Fmoc deprotection and subsequent N-succinylation[19] provided the protected model peptide **15**. Tripeptide **15** was then treated with 20% HBr in acetic acid[20] to yield the deprotected tripeptide **16** (Scheme 8).

NMR analysis of the tripeptides **15** (protected) and **16** (deprotected) showed the anticipated results. In other words, **15** adopted a trans conformation (Figure 16a) and compound **16** adopted a cis conformation (Figure 16b).

Chymotrypsin Assay. The compound **15** (protected) has a trans δ-azaproline and hence is expected to undergo hydrolysis, releasing *p*-nitroaniline. This was confirmed by the

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assay, as the reaction (release of *p* -nitroaniline) was completed within 12 s (Figure 17a).

Compound 16 (deprotected). Because compound **16** has a cis δ-azaproline, consequently it is not expected to undergo hydrolysis in the presence of chymotrypsin, which was also corroborated with the experiment (Figure 17b).

A steep rise in absorbance in the case of **15** (Figure 17a) is a characteristic of burst phase kinetics, denoting the exclusive presence of trans conformer in solution. On the other hand, a flat line (Figure 17b) in the case of **16** indicates no reaction at all due to the exclusive presence of cis conformer.

After a thorough investigation of the cis−trans profile of δ-azaproline **1** in amide and tripeptides, we decided to investigate the same for a longer linear peptide and a cyclic peptide. Accordingly, a proline containing naturally occurring cyclic pentapeptide **17**, isolated from endolichenic *Xylaria* sp.,[21] was chosen as a target, the linear precursor will serve the purpose of a linear model, and compound **18** will serve as the cyclic model. The D-amino acids were replaced by their corresponding L isomers, and the D isomer of δ-azaproline instead of proline was used for this study (Figure 18).

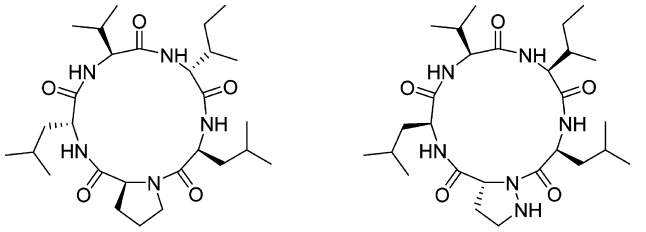


Figure 18. Naturally occurring cyclic peptide **17** and our target **18**.

The retrosynthetic analysis of our target **18** identified Fmoc-L-Leu, Boc-L-Ile, L-Leu-OMe, L-Val-OMe, and δ-*N*-Cbz-D-azaproline-OMe **3** as the required starting materials (Scheme 9).

The forward synthesis commenced from the synthesis of δ-azaproline-containing dipeptide **21** using the previously employed silver cyanide method. The diastereomers hence obtained were separated, and the dipeptide δ-*N*-Cbz-D-azaproline-OMe-Fmoc-L-Leu **21b** was used for further reactions (Scheme 10).

Methyl ester deprotection followed by coupling with L-Leu-OMe yielded the tripeptide **22**. Compound **22** on Fmoc deprotection in piperidine followed by another coupling with Boc-L-Ile yielded the *N*-Cbz deprotected tetrapeptide **23**.

At this point we envisaged that due to steric factors the secondary δ-nitrogen would be unable to take part in further peptide coupling and hence proceeded with **23**. The tetrapeptide **23** on ester hydrolysis followed by coupling with L-Val-OMe yielded the linear pentapeptide **24**. Ester hydrolysis and N-Boc deprotection of **24** yielded the linear precursor which was cyclized using $NaHCO_3$ and BOP in DMF[22] to yield the cyclic peptide **18*T*** (***T*** stands for the trans conformer, Scheme 11).

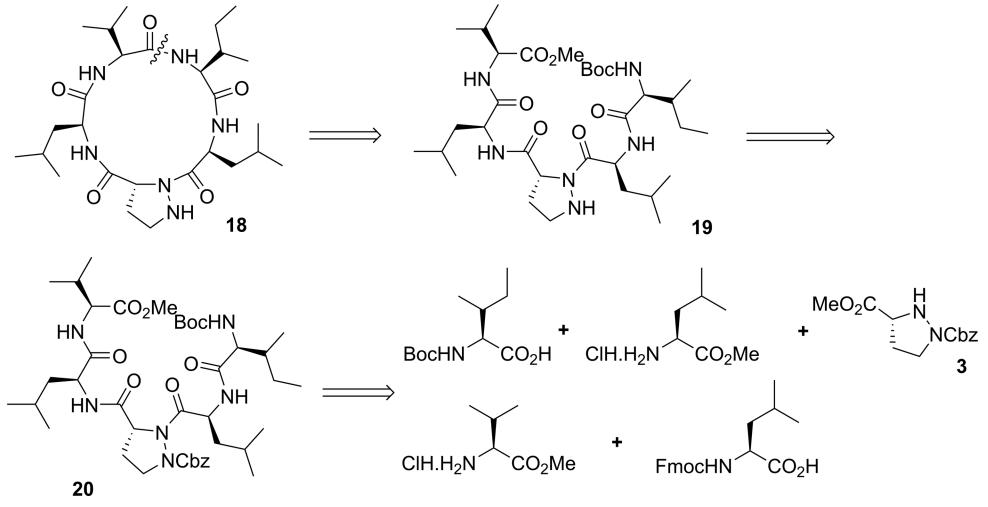
NMR studies of the compound **18*T*** showed epimerization at the valine center that is indicative of slower rate of cyclization which in turn designates the trans conformation at the δ-azaproline amide bond. It was confirmed by X-ray studies of **18*T*** (Figure 19).

This might appear as a contradiction to our claim and theoretical studies in the previous section, but an analysis of the Cbz deprotection (Scheme 12) provided the answer, i.e., for base-mediated deprotection of an otherwise acid-labile protecting group; the trans conformation is essential, and the conversion of trans to cis becomes restricted once the deprotection takes place. This trans conformation is carried forward until the final step and is evident from the NMR and X-ray studies (Figure 19).

Product **25** and byproduct **26** were identified using HRMS (Supporting Information), substantiating the above proposed mechanism. The H-bonding between the NH and the carbonyl of the Cbz increases the electrophilicity at the carbonyl carbon, allowing nucleophilic piperidine to carry out the deprotection.

From the above analysis it was hypothesized that the in situ Cbz deprotection could be arrested by solvating the liable NH_2 group with a polar aprotic solvent such as DMF (**27**). Accordingly, Fmoc deprotection was performed in DMF using piperidine which yielded the desired Fmoc deprotected product **28**. Product **28** under EDC coupling conditions in DCM yielded the Cbz-deprotected tetrapeptide **23** with a trans conformation (Scheme 13).

Scheme 9. Retrosynthetic Analysis of Target Molecule 18



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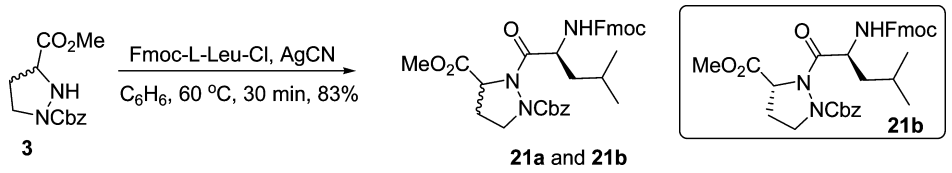
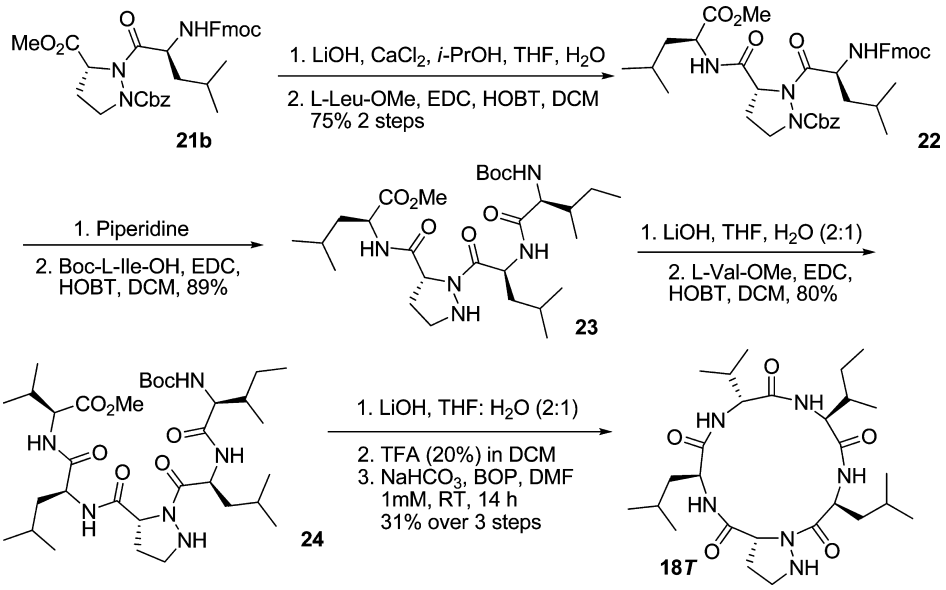
Scheme 10. Synthesis of Dipeptide 21



Scheme 11. Synthesis of Cyclic Peptide 18*T*



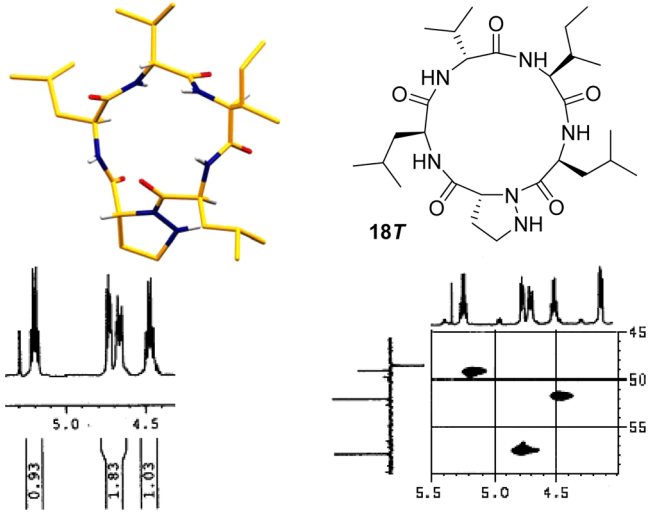


Figure 19. X-ray structure and ^{1}H NMR and HMQC spectra of epimerized cyclic peptide **18*T***.

The effect of reprotection at the δ-N is noteworthy at this point (because in the previous section and theoretical calculations we observed that reprotection induces isomerization); as a result the deprotected linear tetrapeptide **23** was treated with Cbz-Cl in the presence of AgCN. The reaction proceeded with a change in the conformation to yield **29** (Scheme 14). The isolated product was in cis conformation (confirmed from 2D NMR studies, Supporting Information).

The compound **29** on hydrolysis followed by EDC-mediated peptide coupling with L-Val-OMe·HCl proceeded with Cbz deprotection and conformation change to yield the pentapeptide **24**, which was indistinguishable from the previously synthesized **24** (Scheme 11) in all aspects.

The pentapeptide **24** on reprotection exhibits a trend similar to that of tetrapeptide **23** on Cbz protection of **24** to yield **30** (Scheme 14).

To investigate the effect of reprotection of the δ-nitrogen on cyclic peptide **18*T*** with a trans conformation, it was subjected to Cbz protection to yield **31** (Scheme 15).

NMR analysis shows an upfield shift of the α-hydrogen to 4.02 ppm from 4.72 ppm, indicating a crossover to cis conformation (Figure 20).

Compound **31** had a cis conformation; interestingly, removal of the Cbz group from the δ-N under hydrogenolysis conditions yielded the deprotected cyclic peptide **18*T*** with a trans conformation (Scheme 15). NMR analysis of **18*T*** and tallying it with that of previously synthesized **18*T*** indicated that both compounds are indeed identical.

CONCLUSION

δ-Azaproline, exclusively adopts a trans conformation in simple amides to tripeptides when its δ-N is protected with a carbamate group and changes over to cis conformation during deprotection which was confirmed by NMR spectroscopy and chymotrypsin-mediated digestion studies. In longer linear and cyclic peptides due to an in situ deprotection the behavior reverses, but the reluctance to isomerize holds true in all the cases. This behavior of δ-azaproline will be beneficial in cases of solid-phase peptide synthesis where its deprotected form in longer peptides will stabilize the trans conformation, thereby minimizing aggregate formation. Theoretical studies give clear insight into the mechanism of isomerization during protection or deprotection. The behavior of unprotected δ-azaproline, in

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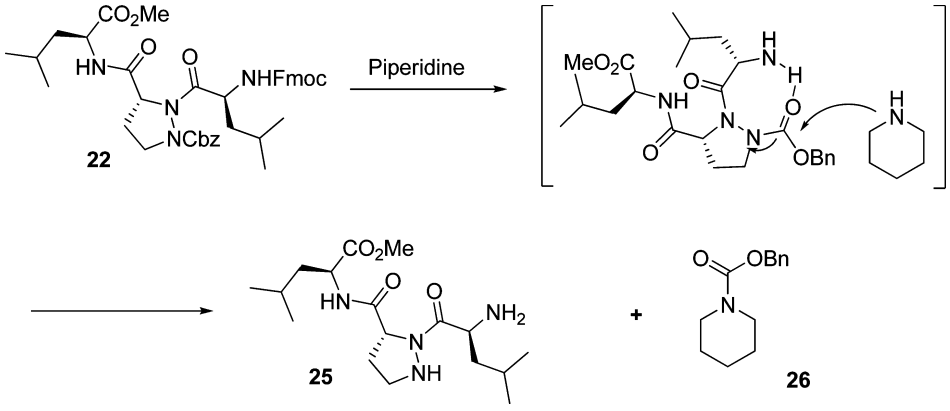
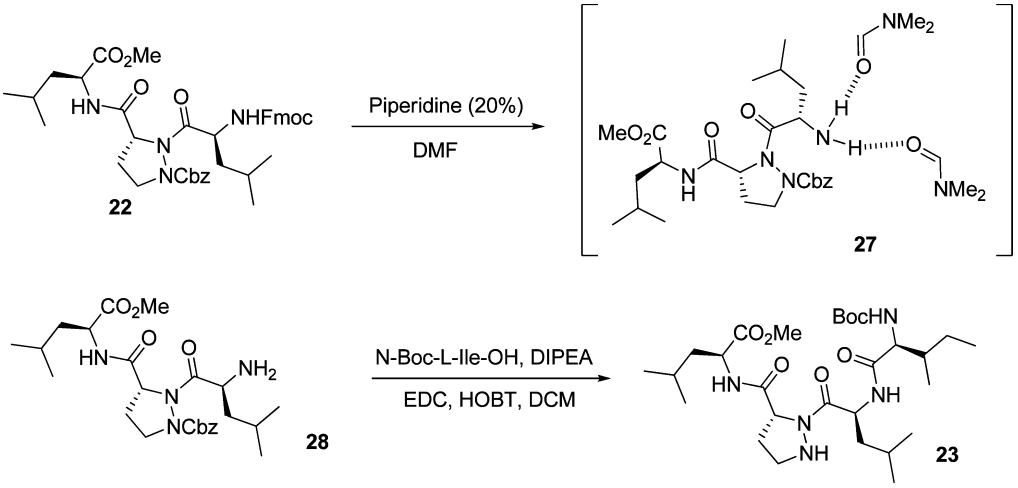
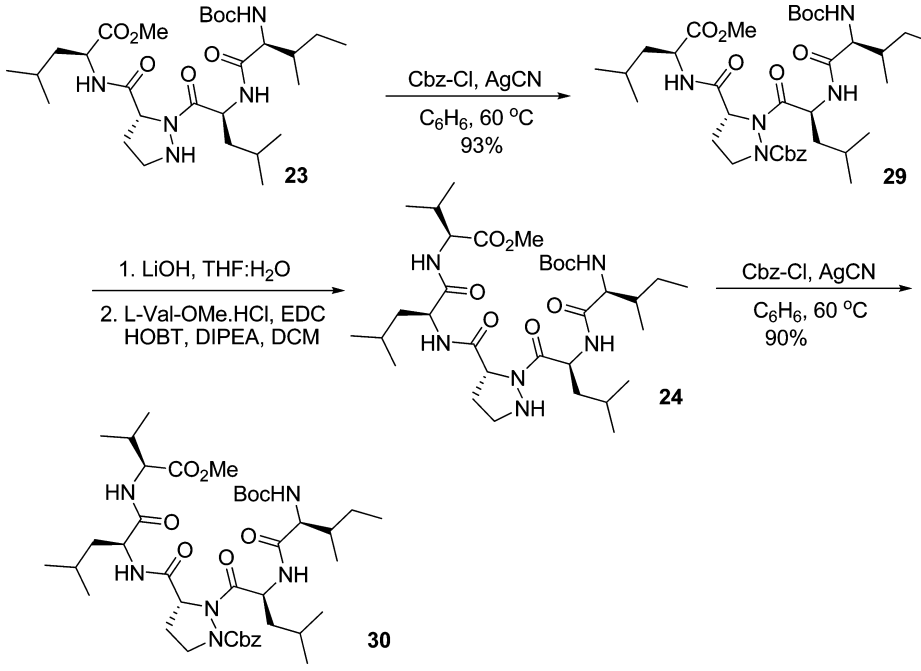
Scheme 12. Mechanism of Cbz Deprotection of 22



Scheme 13. Addressing in Situ Cbz Deprotection



Scheme 14. Effect of Isomerization after Cbz Reprotection of Linear Peptides



the case of tripeptides, remains the same even if the δ-N is protonated, which was evident from the NMR studies of **16** which, despite being an hydrobromide salt, adopted a cis conformation. An interesting observation is that the chemical

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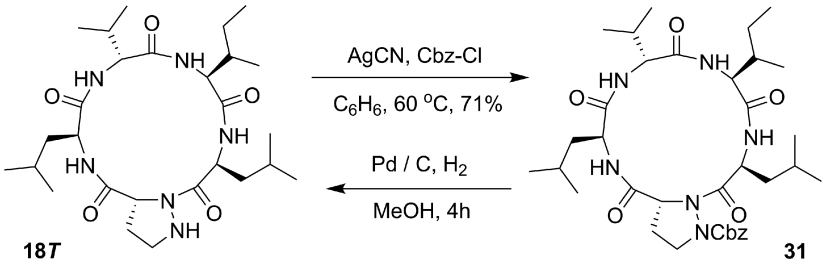
Scheme 15. Cbz Reprotection of 18*T* and Deprotection of 31



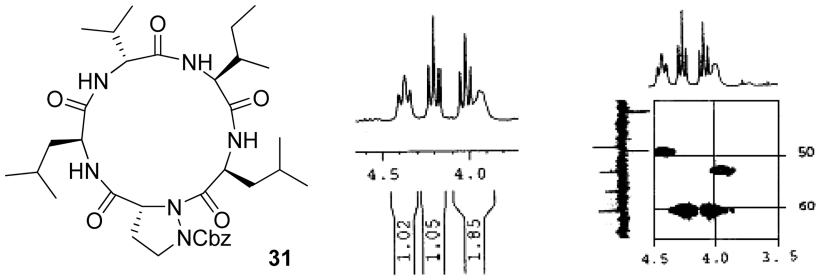


Figure 20. ^{1}H NMR and HMQC spectra of Cbz-protected cis-cyclic peptide **31**.

shift values of the α-H of δ-azaproline, which are more upfield for the cis-conformer than for the trans-conformer, a similar trend is also exhibited by the cis−trans conformers of proline.[9] During the course of our work, a related work was published by Jamart-Grégoire et al. where they tried to prove similar preferences of δ-azaproline in simple tripeptides with the help of IR and NMR studies.[23] For the first time, δ-azaproline has also been used for the synthesis of medicinally important diketopiperazines, which are employing both of the "N"s. Among several substituted prolines, δ-azaproline is unique because of its ability to stabilize one conformer exclusively, and the δ-N provides a much required site for the spatiotemporal control of bioactive peptides, e.g., prolyloligopeptidase inhibitors with a suitable protecting group or a photolabile group. Such applications of δ-azaproline with a photolabile protecting group in biological systems are ongoing and will be reported shortly. Effects of non-carbamate protecting groups on the cis−trans profile of δ-azaproline are also being investigated.

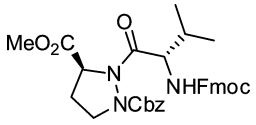
EXPERIMENTAL SECTION

General Methods. All the reactions were performed in argon atmosphere with dry solvents and reagents unless mentioned otherwise. Reagents purchased from commercial sources were used directly except DCM, DMF (dried from CaH_2), THF, benzene, and toluene (dried over sodium). Column chromatography was performed on 230−400 mesh silica gel. Thin-layer chromatography (TLC) was carried out on aluminum sheets, silica gel 60 F254 (layer thickness 0.25 mm). Visualization of the developed chromatogram was performed by ceric ammonium molybdate (CAM) or $KMnO_4$ staining. ^{1}H and 2-D spectra were recorded at 300, 400, or 500 MHz and ^{13}C NMR at 75, 100, or 125 MHz, using $CDCl_3$, CD_3OD, or D_2O as solvent. Chemical shifts (δ) are given in parts per million. The following abbreviations are used for multiplicity of NMR signals: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad. High resolution mass spectra (HRMS) were measured in a QTOF I (quadrupole-hexapole-TOF) mass spectrometer. Kinetics was measured by a diode array spectrophotometer with a thermostated cuvette holder.

*1-Benzyl 3-Methyl 2-(2-(((9H-Fluoren-9-yl)methoxy)-carbonylamino)-3-methylbutanoyl)pyrazolidine-1,3-dicarboxylate (**4**).* Fmoc-protected L-valine (0.689 g, 2.03 mmol) was stirred with oxalyl chloride (0.72 mL, 8.4 mmol) in DCM at rt for 4 h. Then DCM and excess oxalyl chloride were removed in vacuo from the deep yellow (to red) solution.

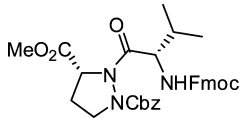
The acid chloride generated above was redissolved in dry benzene and added to a benzene solution of **3** (0.447 g, 1.7 mmol). To the above mixture, AgCN (0.453 g, 3.38 mmol) was added and heated at 60 °C for 30 min. On completion of the reaction (TLC), benzene was removed, and the mixture was extracted with EtOAc and filtered through a sintered funnel. The organic layer was then washed with satd $NaHCO_3$ followed by water and brine. Removal of the organic layer yielded the crude diasteriomeric mixture which was separated by a flash column chromatography (25% ethyl acetate in petroleum ether). Overall yield 83% (0.826 g, 1.41 mmol, mixture 0.099 g).

(S)-1-Benzyl 3-Methyl 2-((S)-2-(((9H-Fluoren-9-yl)methoxy)-carbonylamino)-3-methylbutanoyl)pyrazolidine-1,3-dicarboxylate



(4a). Yield, 35%, 0.35 g, 0.6 mmol. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.73 (d, 2H, J = 7.5 Hz), 7.57 (d, 2H, J = 7.5 Hz), 7.24−7.39 (m, 9H), 5.59 (d, 0.23H, J = 9 Hz), 5.05−5.30 (m, 4H), 4.62−4.73 (m, 0.77H), 4.17−4.41 (m, 3H), 3.74 (s, 0.86H), 3.61 (s, 2.13H), 3.35−3.41 (m, 0.35H), 3.04−3.16 (m, 0.65H), 2.12−2.38 (m, 4H), 0.82−1.02 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.3, 170.8, 170.5, 158.7, 158.1, 156.3, 156.0, 143.8, 141.3, 135.2, 135.1, 128.6, 128.5, 127.9, 127.7, 127.3, 127.0, 125.1, 120.0, 69.5, 68.9, 66.9, 57.6, 57.4, 57.2, 56.8, 55.5, 52.5, 48.5, 48.1, 47.1, 31.4, 31.1, 29.7, 29.0, 19.9, 19.5, 16.7, 16.3. HRMS (ESI) $(M + Na)^+$ calculated for $C_{33}H_{35}N_3O_7Na^+$ = 608.2373, found 608.2371.

(R)-1-Benzyl 3-Methyl 2-((S)-2-(((9H-Fluoren-9-yl)methoxy)-carbonylamino)-3-methylbutanoyl)pyrazolidine-1,3-dicarboxylate



(4b). Yield, 37%, 0.38 g, 0.64 mmol. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74 (d, 2H, J = 7.2 Hz), 7.54 (d, 2H, J = 7.5 Hz), 7.14−7.40 (m, 9H), 5.42 (br d, 1H, J = 8.1 Hz), 5.29 (d, 1H, J = 12 Hz), 5.09 (d, 1H, J = 12.3 Hz), 4.25−4.31 (m, 2H), 4.76−4.86 (m, 2H), 3.51 (s, 3H), 3.08−3.16 (br m, 1H), 2.26−2.41 (m, 2H), 1.84−1.95 (m, 1H), 0.87 (dd,

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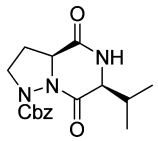
6H, J = 13.8, 6.9 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.7, 170.3, 157.9, 155.8, 143.9, 143.8, 141.2, 141.1, 135.3, 128.3, 128.0, 127.6, 127.0, 125.1, 125.0, 119.9, 68.9, 66.8, 57.2, 56.4, 52.4, 47.4, 47.0, 31.5, 29.7, 19.2, 17.3. HRMS (ESI) (M + Na)$^+$ calculated for $C_{33}H_{35}N_3O_7Na^+$ = 608.2373, found 608.2371.

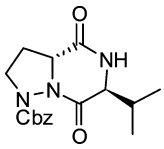
*6-Benzyl 6-Isopropyl-4,7-dioxohexahydropyrazolo[1,5-a]-pyrazine-1(2H)-carboxylate (**5**).* To a solution of **4** (mixture of diasterioisomer, 0.099 g, 0.17 mmol) in DMF (2.4 mL) was added piperidine (0.6 mL), and the mixture was stirred overnight. The reaction mixture was then heated at 50 °C for 30 min following which the DMF was removed in vacuo. The crude reaction mixture was then purified by flash column chromatography (50% ethyl acetate in petroleum ether) to yield two diasterioisomers. Overall yield 88% (0.049 g, 0.15 mmol).

*(3aS,6S)-Benzyl 6-Isopropyl-4,7-dioxohexahydropyrazolo[1,5-a]-pyrazine-1(2H)-carboxylate (**5a**).* Yield, 53%, 0.03 g, 0.09 mmol, ^{1}H



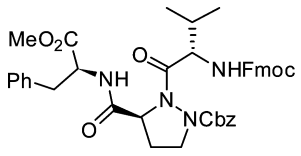
NMR (400 MHz, $CDCl_3$) δ 7.32−7.37 (m, 5H), 5.95 (br s, 1H), 5.23 (d, 2H, J = 12.4 Hz), 4.26 (td, 1H, J = 8.4, 0.8 Hz), 4.12 (t, 1H, J = 1.2 Hz), 3.93 (qt, 1H, J = 8.4, 2.4 Hz), 3.44−3.51 (m, 1H), 2.60−2.67 (m, 2H), 2.26−2.34 (m, 1H), 1.07 (d, 3H, J = 7.2 Hz), 0.91 (d, 3H, J = 6.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.9, 162.9, 156.1, 135.7, 128.8, 128.5, 128.2, 68.8, 59.5, 58.9, 45.8, 30.9, 29.4, 19.1, 16.2. HRMS (ESI) (M + Na)$^+$ calculated for $C_{17}H_{21}N_3O_4Na^+$ = 354.1430, found 354.1433.

*(3aR,6S)-Benzyl 6-Isopropyl-4,7-dioxohexahydropyrazolo[1,5-a]-pyrazine-1(2H)-carboxylate (**5b**).* Yield, 33%, 0.02 g, 0.057 mmol, ^{1}H



NMR (400 MHz, $CDCl_3$) δ 7.31−7.36 (m, 5H), 6.54 (d, 1H, J = 2.4 Hz), 5.21 (d, 1H, J = 12 Hz), 5.16 (d, 1H, J = 12 Hz), 4.23 (t, 1H, J = 8.8 Hz), 3.94 (qt, 1H, J = 8.8, 2 Hz), 3.79 (t, 1H, J = 4.8 Hz), 3.47 (td, 1H, J = 10, 5.2 Hz), 2.61−2.70 (m, 1H), 2.29−2.38 (m, 1H), 2.15−2.19 (m, 1H), 0.98 (d, 3H, J = 7.2 Hz), 0.94 (d, 3H, J = 6.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.0, 162.5, 156.2, 135.4, 128.7, 128.6, 128.5, 69.0, 62.55, 58.3, 45.2, 34.0, 31.0, 18.6, 17.1. HRMS (ESI) (M + Na)$^+$ calculated for $C_{17}H_{21}N_3O_4Na^+$ = 354.1430, found 354.1428.

(S)-Benzyl 2-((S)-2-(((9H-Fluoren-9-yl)methoxy)carbonylamino)-3-methylbutanoyl)-3-((S)-1-methoxy-1-oxo-3-phenylpropan-2-



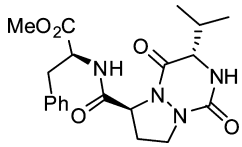
*ylcarbamoyl)pyrazolidine-1-carboxylate (**6**).* The methyl ester **4a** (0.152 g, 0.26 mmol) was dissolved in 2-propanol (4.5 mL) and tetrahydrofuran (2 mL). $CaCl_2$ was added (0.461 g, 4.15 mmol). Separately, $LiOH·H_2O$ (0.042 g, 1.03 mmol) was dissolved in H_2O (2 mL). The aqueous solution was then added to the reaction mixture, and the cloudy white solution was stirred for 45 min. The organic solvents were removed under reduced pressure, and the resulting residue was taken up in 10% potassium carbonate (K_2CO_3) (15 mL) as a cloudy white suspension. The aqueous layer was partitioned in diethyl ether (Et_2O) (4 × 3 mL) to remove the Fmoc deprotection side products (if any), after which it was acidified to pH 2 with concentrated HCl. It was then extracted with EtOAc (30 mL). The organic layers were dried over Na_2SO_4 and concentrated to a white foamy solid **13**.

Diisopropylethylamine (45.28 μL, 0.26 mmol) was added dropwise to a stirred suspension of L-phenylalanine methyl ester hydrochloride (0.084 g, 0.39 mmol) in dichloromethane (20 mL) at room temperature under an atmosphere of nitrogen. On dissolution, the solution was cooled to 0 °C and then **13** (crude product from above reaction) and 1-hydroxybenzotriazole (0.53 g, 0.39 mmol) were added successively, each in one portion. The suspension was stirred at 0 °C for further 15 min and then EDC·HCl (0.075 g, 0.39 mmol) was added in one portion. The mixture was allowed to warm to room temperature over the course of 12 h, and the mixture was evaporated in vacuo.

The residue was taken up in ethyl acetate (40 mL) and washed with saturated aqueous sodium bicarbonate solution and brine. The combined organic extracts were dried and evaporated in vacuo to leave the crude product which was purified by chromatography (50% EtOAc in petroleum ether) to give the tripeptide **6** in 65% yield (0.127 g, 0.17 mmol) as a light yellow solid.

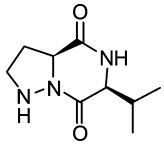
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45 (d, 2H, J = 7.5 Hz), 7.56−7.60 (m, 3H), 7.08−7.40 (m, 13H), 7.06 (d, 2H, J = 10 Hz), 5.20 (d, 1H, J = 9 Hz), 5.13 (d, 1H, J = 12 Hz), 4.98 (br s, 1H), 4.78−4.81 (m, 2H), 4.59 (dd, 1H, J = 9, 4 Hz), 4.37 (dd, 1H, J = 10.5, 7 Hz), 4.29−4.32 (m, 1H), 4.15−4.20 (m, 2H), 3.17 (s, 3H), 3.07−3.17 (m, 2H), 2.93 (dd, 1H, J = 14.5, 7 Hz), 2.38−2.44 (m, 1H), 2.15−2.17 (m, 1H), 1.88−1.96 (m, 1H), 0.84−0.89 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.3, 171.5, 169.4, 158.8, 156.4, 143.9, 141.5, 136.3, 135.0, 129.4, 129.0, 128.9, 128.7, 128.5, 127.9, 127.2, 127.1, 125.2, 120.1, 69.3, 67.1, 60.6, 55.9, 53.3, 52.4, 49.7, 47.3, 38.1, 30.2, 30.0, 19.9, 16.5. HRMS (ESI) (M + Na)$^+$ calculated for $C_{42}H_{44}N_4O_8Na^+$ = 755.3057 found 755.3054.

*(S)-Methyl 2-((3S,6S)-3-Isopropyl-1,4-dioxohexahydro-1H-pyrazolo[1,2-a][1,2,4]triazine-6-carboxamido)-3-phenylpropanoate (**7**).* Compound **6** (0.019 g, 0.026 mmol) was dissolved in 1.6 mL of DMF and 0.4 mL of piperidine. The mixture was stirred overnight followed by heating at 50 °C for 30 min. After completion of the reaction (TLC, 5% MeOH in DCM), excess DMF and piperidine were removed in vacuo. The compound was purified by column chromatography to yield **7** in 81% yield (8.4 mg, 0.021 mmol).



^{1}H NMR (500 MHz, $CDCl_3$) δ 7.50 (d, 1H, J = 7.5 Hz), 7.20−7.30 (m, 2H), 7.08 (d, 2H, J = 7 Hz), 5.14 (br s, 1H), 4.81−4.86 (m, 2H), 3.91 (t, 1H, J = 8.5 Hz), 3.76 (s, 3H), 3.61 (dd, 1H, J = 5, 2.5 Hz), 3.38−3.44 (m, 1H), 3.23 (dd, 1H, J = 14, 5 Hz), 2.97 (dd, 1H, J = 14, 7.5 Hz), 2.71 (dd, 1H, J = 13, 5.5 Hz), 2.22−2.27 (m, 1H), 2.09−2.17 (m, 1H), 1.21 (t, 1H, J = 7 Hz), 1.03 (d, 3H, J = 7 Hz), 0.97 (d, 3H, J = 11.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.6, 167.4, 161.9, 154.5, 136.0, 129.3, 128.7, 127.2, 61.4, 58.9, 53.7, 52.6, 45.2, 38.0, 30.5, 25.4, 19.0, 17.6. HRMS (ESI) (M + Na)$^+$ calculated for $C_{20}H_{26}N_4O_5Na^+$ = 425.1801, found 425.1803.

*(3aS,6S)-6-Isopropylhexahydropyrazolo[1,5-a]pyrazine-4,7-dione (**8a**).* To a solution of **5a** (5 mg, 0.015 mmol) in MeOH (1 mL) was



added 10% Pd/C (3 mg), and the mixture was stirred for 4 h in hydrogen atmosphere. The reaction mixture was filtered through Celite followed by MeOH removal in vacuo. The crude reaction mixture was then purified by flash column chromatography (5% MeOH in DCM) to yield **8a** in quantitative yield (3 mg, 0.015 mmol).

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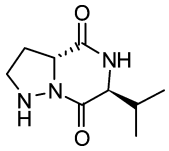
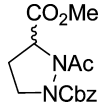
^{1}H NMR (300 MHz, $CDCl_3$) δ 5.81 (br s, 1H), 4.83 (br s, 1H), 4.27 (td, 1H, J = 8.4, 1.2 Hz), 4.01 (t, 1H, J = 2.1 Hz), 3.25−3.33 (m, 1H), 3.10−3.18 (m, 1H), 2.61−2.73 (m, 1H), 2.48−2.58 (m, 2H), 1.09 (d, 3H, J = 7.2 Hz), 0.94 (d, 3H, J = 6.9 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.4, 161.2, 150.1, 129.9, 59.8, 58.1, 45.3, 30.4, 28.6, 19.4, 16.1. HRMS (ESI) $(M + Na)^+$ calculated for $C_9H_{15}N_3O_2Na^+$ = 220.1062, found 220.1059.

*(3aR,6S)-6-Isopropylhexahydropyrazolo[1,5-a]pyrazine-4,7-dione (**8b**).* Yield, 83%, 5 mg, 0.025 mmol from 9 mg, 0.03 mmol of



5b, ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.42−6.44 (m, 1H), 5.30 (br s, 1H), 4.28 (t, 1H, J = 8.4 Hz), 3.77 (t, 1H, J = 4.8), 3.21−3.30 (m, 1H), 3.07−3.16 (m, 1H), 2.54−2.63 (m, 1H), 2.38−2.53 (m, 1H), 2.18−2.27 (m, 1H), 1.08 (d, 3H, J = 6 Hz), 1.03 (d, 3H, 5.7). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.1, 161.4, 64.5, 63.0, 62.9, 57.6, 44.7, 33.2, 31.1, 29.7, 19.2, 19.1, 17.9, 17.6. HRMS (ESI) $(M + Na)^+$ calculated for $C_9H_{15}N_3O_2Na^+$ = 220.1062, found 220.1060.

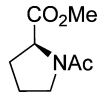
*1-Benzyl 3-Methyl 2-Acetylpyrazolidine-1,3-dicarboxylate (**9**).* To a solution of **3** (0.057 g, 0.21 mmol) in benzene (3 mL) were added



acetyl chloride (33 μL, 0.46 mmol) and AgCN (43 mg, 0.34 mmol). The mixture was then heated at 60 °C for 30 min followed by removal of benzene in vacuo. The residue was taken up in EtOAc (10 mL) and filtered through Celite (to remove AgCl ppt). The filtrate was then washed with satd $NaHCO_3$ and brine. Organic extract was dried and evaporated in vacuo to leave the crude product which was purified by chromatography (30% EtOAc in petroleum ether) to give **9** as colorless liquid in quantitative yield (0.064 g, 0.21 mmol) which slowly formed a white solid on cooling.

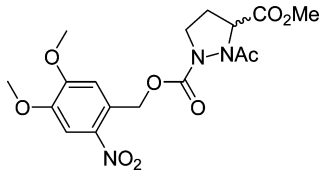
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32−7.38 (m, 5H), 5.21 (dd, 2H, J = 21.6, 12 Hz), 5.00 (dd, 1H, J = 8.7, 5.7 Hz), 4.22−4.30 (m, 1H), 3.61 (s, 3H), 3.12−3.24 (m, 1H), 2.22−2.44 (m, 2H), 2.12 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.3, 171.0, 157.9, 135.4, 128.6, 128.4, 128.1, 68.7, 56.7, 52.4, 47.2, 29.8, 20.7. HRMS (ESI) $(M + Na)^+$ calculated for $C_{15}H_{18}N_2O_5Na^+$ = 329.1113, found 329.1114.

N-Acetyl-L-proline Methyl Ester.[9] ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.33 (dd, J = 8.7, 3.6 Hz, 0.78H), 4.27 (dd, J = 8.6, 2.8 Hz, 0.22H),



3.63 (s, 0.6H), 3.58 (s, 2.4H), 3.48−3.56 (m, 1H), 3.33−3.42 (m, 1H), 1.78−2.23 (m, 7H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.7, 172.4, 169.4, 169.2, 59.9, 58.3, 52.4, 51.9, 47.6, 46.1, 31.2, 29.2, 24.6, 22.6, 22.0.

*1-(4,5-Dimethoxy-2-nitrobenzyl) 3-Methyl 2-Acetylpyrazolidine-1,3-dicarboxylate (**11**).* A solution of **2** (0.035 g, 0.1 mmol) in DCM



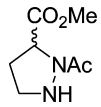
(1.6 mL) was cooled to 0 °C, and to it was added TFA (0.4 mL). The reaction mixture was stirred at the same temperature for 4 h. Removal of the organic solvent yielded the *N*-Boc-deprotected compound.

DCM (1 mL) solution of 4,5-dimethoxy-2-nitrobenzyl alcohol (0.022 g, 0.1 mmol) was added to a solution of triphosgene (16 mg, 0.053 mmol) and aliquot 336 (catalytic) in DCM at 0 °C and stirred for 12 h at rt. Removal of the organic solvent yielded the crude chloroformate. The residue was taken up in DCM (5 mL), and a solution of *N*-Boc-deprotected compound was added in DCM (2 mL). The reaction mixture was then cooled to −20 °C (ice salt mixture) followed by addition of Et_3N (60 μL, 0.4 mmol). It was then stirred at the same temperature for 30 min. Upon completion (TLC, 15% EtOAc in DCM), the reaction was quenched with satd NH_4Cl solution (0.1 mL). DCM was removed in vacuo from the reaction mixture followed by extraction with EtOAc (10 mL). The EtOAc layer was washed with satd $NaHCO_3$ solution and brine. Organic extract was dried and evaporated in vacuo to leave the crude product **10** (0.046 g).

The crude compound **10** was dissolved in dry benzene (5 mL), and to it were added acetyl chloride (15 μL, 0.21 mmol) and AgCN (0.021 g, 0.16 mmol). The mixture was heated at 60 °C for 30 min followed by the removal of benzene. The residue was taken up in EtOAc (10 mL) and filtered through Celite (to remove AgCl ppt). The filtrate was then washed with satd $NaHCO_3$ and brine. Organic extract was dried and evaporated in vacuo to leave the crude product which was purified by chromatography (50% EtOAc in petroleum ether) to give **11** as a yellow liquid in quantitative yield (0.043 g, 0.1 mmol).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.69 (s, 1H), 7.25 (s, 1H), 5.54−5.65 (m, 2H), 4.95 (t, 1H, J = 7 Hz), 4.28 (t, 1H, J = 9 Hz), 3.92 (s, 6H), 3.62 (s, 3H), 3.19 (q, 1H, J = 11 Hz), 2.44−2.46 (m, 1H), 2.21−2.26 (m, 1H), 2.15 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.1, 171.0, 157.5, 154.1, 148.2, 139.3, 127.0, 109.9, 108.1, 65.9, 57.1, 56.7, 56.4, 52.5, 47.3, 30.2, 20.7, 12.2. HRMS (ESI) $(M + Na)^+$ calculated for $C_{17}H_{21}N_3O_9Na^+$ = 434.1175, found 434.1176.

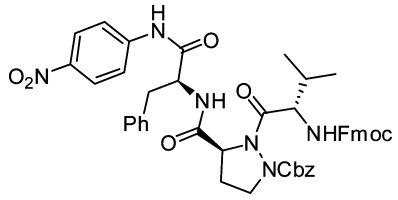
*Methyl 2-Acetylpyrazolidine-3-carboxylate (**12**).* To a solution of **9** (0.052 g, 0.17 mmol) in MeOH (3 mL) was added 10% Pd/C (6



mg). The reaction mixture was stirred in hydrogen atmosphere at rt for 4 h. Upon completion (TLC, 50% EtOAc in petroleum ether), the mixture was filtered through Celite. Organic extract was dried and evaporated in vacuo to yield the pure product **12** as a colorless liquid in quantitative yield (0.029 g, 0.17 mmol).

^{1}H NMR (500 MHz, $CDCl_3$) δ 4.66 (t, 1H, J = 8.5 Hz), 4.33 (dd, 1H, J = 12, 5.5 Hz), 3.71 (s, 3H), 3.20−3.25 (m, 1H), 2.80−2.88 (m, 1H), 2.47−2.51 (m, 1H), 2.17 (s, 3H), 1.98−2.06 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.9, 171.3, 57.1, 52.5, 48.0, 33.5, 21.4. HRMS (ESI) $(M + Na)^+$ calculated for $C_7H_{12}N_2O_3Na^+$ = 195.0746, found 195.0740.

(S)-Benzyl 2-((S)-2-(((9H-Fluoren-9-yl)methoxy)carbonylamino)-3-methylbutanoyl)-3-((S)-1-(4-nitrophenylamino)-1-oxo-3-phenyl-



*propan-2-ylcarbamoyl)pyrazolidine-1-carboxylate (**14**).* A solution of **13** (0.11 g, 0.19 mmol) and oxalyl chloride (83 μL, 0.97 mmol) in DCM (3 mL) was stirred at rt for 4 h during which it turned deep yellow (indicating completion of reaction). The excess DCM and oxalyl chloride were removed, and the residue was taken up in dry benzene. To it were added AgCN (0.04 g, 0.29 mmol) and L-phenylalanine 4-nitroanilide (0.061 g, 0.21 mmol). The mixture was heated at 60 °C for 30 min. Upon completion (TLC, 50% EtOAc in petroleum ether), the excess benzene was removed. The residue was taken up in EtOAc (15 mL) and filtered through a Celite bed. The filtrate was washed with satd $NaHCO_3$ followed by 0.2 N HCl solution

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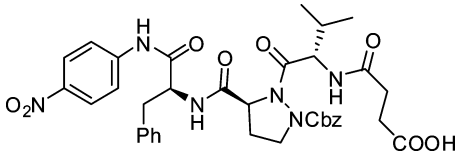
and brine. Organic extract was dried and evaporated in vacuo. Column chromatography yielded the pure product **14** in 61% yield (0.097 g, 0.116 mmol) as a yellow solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.54−8.56 (m, 2H), 8.10−8.15 (m, 1H), 7.96 (d, 1H, J = 10 Hz), 7.75 (d, 2H, J = 9.5 Hz), 7.64 (d, 1H, J = 10 Hz), 7.58 (d, 2H, J = 9.5 Hz), 7.48 (t, 1H, J = 10 Hz), 7.39 (s, 6H), 7.22−7.32 (m, 6H), 5.16−5.29 (m, 3H), 4.91 (q, 1H, J = 6 Hz), 4.64−4.69 (m, 2H), 4.54 (t, 1H, J = 10 Hz), 4.31−4.41 (m, 2H), 4.12−4.21 (m, 2H), 3.44 (dd, 1H, J = 18, 5 Hz), 3.15−3.30 (m, 1H), 2.93−2.99 (m, 1H), 2.45−2.55 (m, 1H), 2.08−2.13 (m, 2H), 1.60 (br s, 1H), 0.82−0.90 (m, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.2, 170.0, 169.4, 160.3, 156.5, 148.7, 143.9, 143.8, 141.5, 139.1, 136.7, 134.6, 129.9, 129.8, 129.3, 129.0, 128.6, 128.5, 127.9, 127.2, 127.1, 125.7, 125.3, 125.2, 120.2, 119.0, 115.1, 70.1, 67.3, 62.9, 61.7, 56.3, 54.7, 50.8, 47.3, 37.1, 32.1, 30.5, 20.0, 16.9. HRMS (ESI) (M + Na)$^+$ calculated for $C_{47}H_{46}N_6O_9$ Na^+ = 861.3218, found 861.3220.

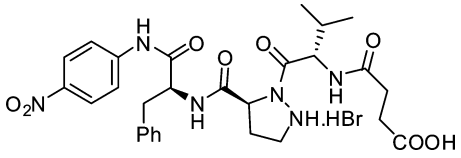
4-((S)-1-((S)-2-(Benzyloxycarbonyl)-5-((S)-1-(4-nitrophenylamino)-1-oxo-3-phenylpropan-2-ylcarbamoyl)pyrazolidin-1-yl)-3-



*methyl-1-oxobutan-2-ylamino)-4-oxobutanoic Acid (**15**).* To a solution of **14** (0.058 g, 0.069 mmol) in DMF (1.6 mL) was added piperidine (0.4 mL). The mixture was stirred for 12 h at rt. Upon completion (TLC, 5% MeOH in DCM), the excess DMF was removed in vacuo. The residue was taken up in AcOH (0.7 mL), and succinic anhydride (0.014 g, 0.14 mmol) was added. The mixture was heated at 60 °C for 6 h. Excess AcOH was removed, and the residue was washed with 10% EtOAc in petroleum ether (4 × 3 mL, to remove the Fmoc deprotection byproduct). Column chromatography purification yielded the pure product **15** in 58% (0.028 g, 0.04 mmol) yield as a foamy yellow solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.67 (br s, 1H), 8.43−8.45 (m, 2H), 7.84−8.10 (m, 5H), 7.16−7.45 (m, 7H), 5.05−5.17 (m, 2H), 4.68−4.89 (m, 2H), 4.29 (br s, 1H), 3.99−4.19 (m, 1H), 3.30−3.35 (m, 2H), 2.88−2.95 (m, 2H), 2.31−2.73 (m, 3H), 1.96−2.23 (m, 2H), 1.86 (br s, 1H), 0.68−1.04 (m, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.3, 170.6, 169.7, 168.7, 163.0, 156.5, 148.6, 139.0, 136.5, 129.9, 129.7, 129.3, 129.2, 129.1, 128.9, 128.7, 128.6, 128.3, 127.1, 125.8, 119.0, 115.0, 67.4, 60.2, 56.5, 55.3, 49.4, 37.1, 30.6, 30.6, 29.7, 29.0, 28.2, 20.0, 17.2. HRMS (ESI) (M + Na)$^+$ calculated for $C_{36}H_{40}N_6O_{10}Na^+$ = 739.2704, found 739.2747.

4-((S)-3-Methyl-1-((S)-5-((S)-1-(4-nitrophenylamino)-1-oxo-3-phenylpropan-2-ylcarbamoyl)pyrazolidin-1-yl)-1-oxobutan-2-yla-



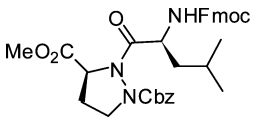
*mino)-4-oxobutanoic Acid Hydrobromide Salt (**16**).* To a cooled solution (0 °C) of **15** (0.017 g, 0.024 mmol) in AcOH (0.4 mL) was added 33% HBr in AcOH (0.6 mL). The mixture was stirred at the same temperature for 1 h. Upon completion of the reaction (TLC, 10% MeOH in DCM), excess AcOH and HBr were removed in vacuo. The residue was washed with hexane (5 × 1 mL) to remove the benzyl bromide (byproduct of Cbz deprotection). Drying the mixture yielded the pure product in 71% (0.011 g, 0.017 mmol) yield as a foamy white solid.

^{1}H NMR (500 MHz, CD_3OD) δ 8.18 (t, 1H, J = 2.8 Hz), 7.90 (ddd, 1H, J = 11, 3.2, 1.2 Hz), 7.52−7.56 (m, 1H), 7.44 (t, 1H, J = 10.8 Hz), 7.11−7.23 (m, 5H), 4.82 (d, 1H, J = 9.2 Hz), 4.51 (t, 1H, J = 10.8 Hz), 4.43 (t, 1H, J = 10.8 Hz), 2.97−3.14 (m, 3H), 2.74−2.83 (m, 1H), 2.39−2.52 (m, 3H), 1.82−2.02 (m, 2H), 1.14−1.20 (m, 2H), 0.81 (t, 6H, J = 8.8 Hz). ^{13}C NMR (75 MHz, CD_3OD) δ 175.5, 173.8, 173.7, 172.3, 149.1, 139.1, 137.0, 131.0, 129.6, 128.2, 128.0, 120.6, 116.4, 60.4, 57.3, 57.1, 38.3, 33.4, 31.4, 31.2, 30.9, 19.4, 19.2, 18.3 (one peak merged with solvent). HRMS (ESI) (M + Na)$^+$ calculated for $C_{28}H_{34}N_6O_8Na^+$ = 605.2330, found 605.2321.

Chymotrypsin Coupled Assay. A solution of **15** in DMSO (∼10 mg/mL) was prepared, of which 3 μL was pipetted to a solution of 50 μL of α-chymotrypsin (∼25 mg/mL, 1 mM HCl) and 1150 μL of buffer (HEPES 0.035 M, pH 7.8) at room 25 °C. Kinetics was measured by a diode array spectrophotometer with a thermostated cuvette holder. Total measuring time was kept at 10 min with each cycle time 10 s. The absorption of the released p-nitroaniline (ε = 11814 M^{-1} cm^{-1}) was monitored at 390 nm. The same procedure was used for **16** as a substrate.

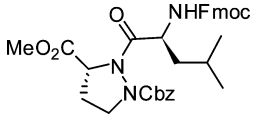
*1-Benzyl 3-Methyl 2-(2-(((9H-Fluoren-9-yl)methoxy)-carbonylamino)-4-methylpentanoyl)-pyrazolidine-1,3-dicarboxylate (**21**).* Compound **3** (0.496 g, 1.88 mmol) was dissolved in benzene (15 mL), and AgCN (0.3 g, 2.26 mmol) was added followed by the addition of Fmoc-L-Leu-Cl (0.77 g, 2.07 mmol) in benzene (10 mL). The mixture was then heated at 60 °C for about 1 h. The completion of the reaction was adjudged by TLC. Solvent was removed in vacuo. The residue was taken up in ethyl acetate and filtered, and the filtrate was then washed with saturated aqueous sodium bicarbonate solution, water, and brine. The combined organic extracts were dried and evaporated in vacuo to leave the crude product which was purified by flash column chromatography using 7:3 petroleum ether−EtOAc as eluent to give the overall dipeptide as a yellow solid in 83% yield (0.94 g, 1.56 mmol).

(S)-1-Benzyl 3-Methyl 2-((S)-2-(((9H-Fluoren-9-yl)methoxy)-carbonylamino)-4-methylpentanoyl)pyrazolidine-1,3-dicarboxylate



*(**21a**).* Yield, 40%, 0.45 g, 0.75 mmol, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (d, 2H, J = 7.5 Hz), 7.58 (d, 2H, J = 7.2 Hz), 7.41−7.18 (m, 9H), 5.50 (d, 1H, J = 8.4 Hz), 5.32−5.09 (m, 2H), 4.91 (br s, 2H), 4.12−4.39 (m, 4H), 3.53 (br s, 3H), 3.17 (d, 1H, J = 7.8 Hz), 2.31−2.40 (m, 2H), 1.66−1.61 (m, 1H), 1.41−1.44 (m, 1H), 1.26−1.30 (m, 1H), 0.88−0.90 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.9, 170.4, 157.9, 155.7, 144.1, 143.9, 141.3, 135.4, 128.6, 128.5, 128.3, 127.7, 127.1, 125.2, 125.1, 120.0, 69.2, 66.9, 60.4, 57.5, 53.5, 52.6, 50.7, 47.2, 42.7, 29.9, 24.7, 23.4, 21.7, 21.1, 14.2. HRMS (ESI) (M + Na)$^+$ calculated for $C_{34}H_{37}N_3O_7Na^+$ = 622.2529, found 622.2528.

(R)-1-Benzyl 3-Methyl 2-((S)-2-(((9H-Fluoren-9-yl)methoxy)-carbonylamino)-4-methylpentanoyl)pyrazolidine-1,3-dicarboxylate



*(**21b**).* Yield, 43%, 0.48 g, 0.80 mmol), ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (d, 2H, J = 7.5 Hz), 7.58 (d, 2H, J = 7.2 Hz), 7.19−7.48 (m, 9H), 5.47 (br d, 1H, J = 8.1 Hz), 5.14−5.32 (m, 2H), 4.91 (br s, 2H), 4.15−4.36 (m, 4H), 3.54 (s, 3H), 3.18 (br d, 1H, J = 8.7 Hz), 2.42−2.32 (m, 2H), 1.55−1.61 (m, 1H), 1.36−1.44 (m, 1H), 1.26−1.28 (m, 1H), 0.89 (d, 6H, J = 6.3 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.3, 170.5, 157.9, 155.8, 144.1, 144.0, 141.4, 135.4, 128.5, 128.3, 127.7, 127.1, 125.3, 120.0, 69.2, 67.0, 57.5, 52.6, 50.8, 46.8, 42.7, 29.9, 24.8, 23.4, 21.8. HRMS (ESI) (M + Na)$^+$ calculated for $C_{34}H_{37}N_3O_7Na^+$ = 622.2529, found 622.2528.

*(R)-Benzyl 2-((S)-2-(((9H-Fluoren-9-yl)methoxy)carbonylamino)-4-methylpentanoyl)-3-((S)-1-methoxy-4-methyl-1-oxopentan-2-ylcarbamoyl)pyrazolidine-1-carboxylate (**22**).* The methyl ester **21b** (0.48 g, 0.80 mmol) was dissolved in 2-propanol (14 mL), and tetrahydrofuran (4.5 mL). $CaCl_2$ (1.42 g, 12.81 mmol) was added. Separately, $LiOH·H_2O$ (0.135 g, 3.2 mmol) was dissolved in H_2O (6 mL). The aqueous solution was then added to the reaction mixture,

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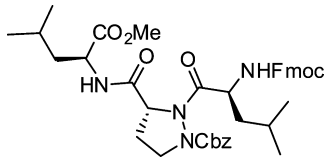
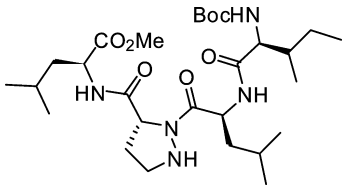


and the cloudy white solution was stirred for 45 min. The organic solvents were removed under reduced pressure, and the resulting residue was taken up in 10% potassium carbonate (K_2CO_3) (40 mL) as a cloudy white suspension. The aqueous layer was partitioned in diethyl ether (Et_2O) (2 × 10 mL) to remove the Fmoc deprotection side products (if any), after which it was acidified to pH 2 with concentrated HCl. It was then extracted with EtOAc (100 mL). The organic layers were dried over Na_2SO_4 and concentrated to a white foamy solid in 89% yield (0.42 g, 0.71 mmol).

DIPEA (0.12 mL, 0.71 mmol) was added dropwise to a stirred suspension of L-Leu-OMe·HCl (0.155 g, 0.85 mmol) in dichloromethane (10 mL) at room temperature under an atmosphere of nitrogen. On dissolution, the solution was cooled to 0 °C and then the acid obtained above (0.42 g, 0.71 mmol) and 1-hydroxybenzotriazole (0.12 g, 0.85 mmol) were added successively, each in one portion. The suspension was stirred at 0 °C for a further 15 min, and then EDC (0.16 g, 0.85 mmol) was added in one portion. The mixture was allowed to warm to room temperature over the course of 12 h and then filtered, and the filtrate was evaporated in vacuo. The residue was taken up in ethyl acetate and filtered, and the filtrate was then washed with 10% aqueous citric acid solution followed by saturated aqueous sodium bicarbonate solution. The combined organic extracts were dried and evaporated in vacuo to leave the crude product which was purified by chromatography on silica using 1:1 petroleum ether−EtOAc as eluent to give the tripeptide **22** (0.43 g, 0.60 mmol) as a yellow solid in 85% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74 (d, 2H, J = 7.5 Hz), 7.56 (d, 2H, J = 7.5 Hz), 7.26−7.41 (m, 9H), 5.27−5.29 (m, 1H), 5.14−5.18 (m, 3H), 4.87 (t, 1H, J = 7.8 Hz), 4.78 (t, 1H, J = 9.0 Hz), 4.47−4.55 (m, 1H), 4.32−4.36 (m, 2H), 4.16−4.21 (m, 2H), 3.72 (s, 3H), 3.26 (q, 1H, J = 9.6 Hz), 2.34−2.42 (m, 2H), 1.50−1.69 (m, 6H), 0.87−0.95 (m, 10H), 0.78 (d, 2H, J = 5.4 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.0, 172.8, 169.2, 158.5, 156.3, 147.1, 143.9, 141.4, 135.0, 128.9, 128.6, 128.5, 128.3, 127.8, 127.2, 125.2, 120.1, 69.3, 67.2, 59.8, 52.4, 51.1, 49.7, 49.4, 47.3, 41.2, 40.7, 29.01, 24.9, 24.8, 23.4, 23.0, 21.9, 20.9. HRMS (ESI) $(M + Na)^+$ calculated for $C_{40}H_{48}N_4O_8Na^+$ = 735.3370, found 735.3371.

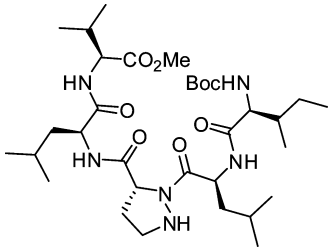
*(S)-Methyl 2-((R)-2-((S)-2-((2S,3S)-2-(tert-Butoxycarbonylamino)-3-methylpentanamido)-4-methylpentanoyl)pyrazolidine-3-carboxamido)-4-methylpentanoate (**23**).* Compound **22** (0.070 g, 0.098 mmol) was treated with piperidine (1 mL) and stirred at room temperature for about 10 min. Piperidine was then removed in vacuo, and the white solid obtained was directly used for the next step without purification.



The crude compound was dissolved in dry DCM. On dissolution, the solution was cooled to 0 °C, and then L-Ile-NHBoc (0.025 g, 0.108 mmol) and 1-hydroxybenzotriazole (0.016 g, 0.118 mmol) were added successively, each in one portion. The suspension was stirred at 0 °C for a further 15 min, and then EDC (0.023 g, 0.118 mmol) was added in one portion. The mixture was allowed to warm to room temperature over the course of 12 h and filtered, and the filtrate was evaporated in vacuo. The residue was taken up in ethyl acetate and filtered, and the filtrate was then washed with 10% aqueous citric acid solution followed by saturated aqueous sodium bicarbonate solution. The combined organic extracts were dried and evaporated in vacuo to leave the crude product which was purified by chromatography on silica using 1:1 petroleum ether−EtOAc as eluent to give the tetrapeptide **23** (0.055 g, 0.096 mmol, 89%) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 (d, 1H, J = 7.8 Hz), 6.65 (d, 1H, J = 6.9 Hz), 5.23 (dd, 1H, J = 13.8, 7.8 Hz), 5.15 (d, 1H, J = 9 Hz), 4.72 (dd, 1H, J = 8.7, 6.9 Hz), 4.47−4.55 (m, 1H), 4.42 (dd, 1H, J = 12.6, 4.5 Hz), 3.96 (t, 1H, J = 8.0 Hz), 3.69 (s, 3H), 3.27−3.34 (m, 1H), 2.70−2.85 (m, 1H), 2.33−2.57 (m, 2H), 1.79 (br s, 1H), 1.48−1.69 (m, 7H), 1.46 (s, 9H), 1.11−1.19 (m, 1H), 0.87−0.97 (m, 18H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.3, 173.1, 171.6, 170.5, 155.8, 79.7, 59.2, 58.9, 52.2, 51.1, 49.7, 48.0, 41.0, 40.8, 37.5, 30.8, 28.4, 25.0, 24.9, 23.2, 22.9, 22.0, 21.8, 15.5, 11.4. HRMS (ESI) $(M + Na)^+$ calculated for $C_{28}H_{51}N_5O_7Na^+$ = 592.3686, found 592.3685.

*(S)-Methyl 2-((S)-2-((R)-2-((S)-2-((2S,3S)-2-(tert-Butoxycarbonylamino)-3-methylpentanamido)-4-methylpentanoyl)pyrazolidine-3-carboxamido)-4-methylpentanamido)-3-methylbutanoate (**24**).* The compound **23** (76 mg, 0.13 mmol) was taken up in THF (3 mL) and MeOH (0.3 mL). A solution of LiOH·H_2O (56 mg, 1.3 mmol) in H_2O (0.9 mL) was added dropwise, ultimately attaining a molarity of 1.5 M. The reaction mixture was stirred for about 30 min. The solvent was removed, acidified to pH 4 at 0 °C, and extracted with ethyl acetate. The organic layer was washed with water and brine to yield the acid (70 mg, 0.1 mmol) in 77% yield.



Diisopropylethylamine (73 μL, 0.42 mmol) was added dropwise to a stirred suspension of L-Val-OMe·HCl (22 mg, 0.0.13 mmol) in dichloromethane (3 mL) at room temperature under an atmosphere of nitrogen. On dissolution, the solution was cooled to 0 °C and then the acid obtained above (70 mg, 0.1 mmol) and 1-hydroxybenzotriazole (18 mg, 0.13 mmol) were added successively, each in one portion. The suspension was stirred at 0 °C for a further 15 min, and then EDC (25 mg, 0.13 mmol) was added in one portion. The mixture was allowed to warm to room temperature over the course of 12 h, and the solvent was then evaporated in vacuo. The residue was taken up in ethyl acetate and washed with cold 0.1 N HCl followed by saturated aqueous sodium bicarbonate solution. The combined organic extracts were dried and evaporated in vacuo to leave the crude product which was purified by chromatography on silica using 1:1 petroleum ether−EtOAc as eluent to give the pentapeptide **24** (70 mg, 0.10 mmol, 80%) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 (d, 1H, J = 7.2 Hz), 6.96 (d, 1H, J = 9.0 Hz), 6.67 (d, 1H, J = 5.7 Hz), 5.18−5.11 (m, 1H), 5.01 (d, 1H, J = 9.3 Hz), 4.87 (dd, 1H, J = 12.6, 4.8 Hz), 4.72 (dd, 1H, J = 9.0, 6.9 Hz), 4.51 (dd, 1H, J = 9, 5.7 Hz), 4.35−4.42 (m, 1H), 3.94 (t, 1H, J = 8.4 Hz), 3.72 (s, 3H), 3.27−3.35 (m, 1H), 2.72−2.86 (m, 1H), 2.35−2.58 (m, 2H), 2.08−2.20 (m, 1H), 1.48−1.80 (m, 8H), 1.41 (s, 9H), 1.08−1.19 (m, 1H), 0.87−0.97 (m, 24H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.2, 172.9, 172.4, 172.1, 170.8, 155.7, 79.9, 59.0, 57.0, 53.3, 52.2, 50.1, 47.9, 40.4, 37.5, 31.2, 30.8, 28.4, 25.0, 23.1, 22.9, 22.2, 22.0, 19.1, 18.1, 17.9, 15.6, 11.3. HRMS (ESI) $(M + Na)^+$ calculated for $C_{33}H_{60}N_6O_8Na^+$ = 691.4370, found 691.4368.

*(3aR,6S,9R,12S,15S)-12-sec-Butyl-6,15-diisobutyl-9-isopropyldecahydro-1H-pyrazolo[1,5-a][1,4,7,10,13]pentaazacyclopentadecine-4,7,10,13,16(2H)-pentaone (**18T**).* Compound **24** (55 mg, 0.082 mmol) was taken up in THF−MeOH (2 mL:0.2 mL) mixture. A solution of LiOH·H_2O (42 mg, 0.82 mmol) in 0.5 mL of H_2O was added dropwise, ultimately attaining a molarity of 1.5 M. The reaction mixture was stirred for 30 min. The solvent was removed, acidified to pH 4 at 0 °C, and extracted with ethyl acetate. The organic layer was washed with water and brine to yield the carboxylic acid as white

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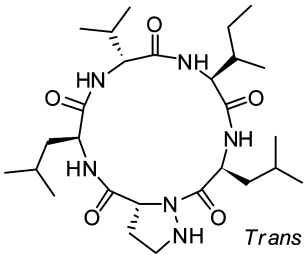
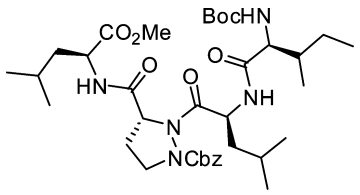


foamy solid. The crude product so obtained was dissolved in dry DCM (1.6 mL), and 0.4 mL of TFA was added at 0 °C and stirred for 2 h. Removal of DCM in vacuo yielded a brownish viscous liquid which was used in the next step without further purification.

The brownish solid obtained above (54 mg, 0.08 mmol) was dissolved in dry DMF (80 mL) to attain a concentration of 1 mM. To it was added $NaHCO_3$ (34 mg, 0.4 mmol) followed by the addition of BOP (39 mg, 0.088 mmol) at room temperature. The reaction mixture was then stirred overnight at rt. Removal of DMF followed by a flash column chromatography (5% MeOH−DCM) chromatography yielded **18*T*** (14 mg, 0.025 mmol) in 31% yield.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79 (d, 1H, J = 9.5 Hz), 7.09 (d, 1H, J = 8.0 Hz), 6.61 (d, 1H, J = 9.0 Hz), 6.44 (d, 1H, J = 7.0 Hz), 5.20 (dd, 1H, J = 16.5, 7.5 Hz), 4.73 (dd,1H, J = 9, 5.5 Hz), 4.67 (dd, 1H, J = 12, 5 Hz), 4.47 (dd, 1H, J = 17.0, 7.5 Hz), 4.09 (dd, 1H, J = 7.5, 6.0 Hz), 3.77 (t, 1H, J = 9.0 Hz), 3.32−3.33 (m, 1H), 2.74−2.83 (m, 2H), 2.09 (s, 1H), 1.94−1.99 (m, 1H), 1.43−1.70 (m, 8H), 1.10−1.16 (m, 1H), 0.86−1.00 (m, 24H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.3, 172.6, 171.9, 170.6, 169.3, 61.2, 60.1, 57.7, 51.8, 48.9, 48.4, 40.4, 39.0, 35.7, 30.0, 27.1, 25.0, 24.9, 22.9, 22.8, 22.7, 22.5, 19.4, 16.3, 11.6. HRMS (ESI) $(M + Na)^+$ calculated for $C_{27}H_{48}N_6O_5Na^+$ = 559.3584, found 559.3582.

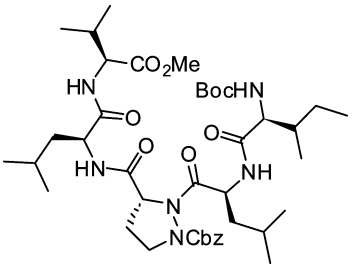
(R)-Benzyl 2-((S)-2-((2S,3S)-2-(tert-Butoxycarbonylamino)-3-methylpentanamido)-4-methylpentanoyl)-3-((S)-1-methoxy-4-



*methyl-1-oxopentan-2-ylcarbamoyl)pyrazolidine-1-carboxylate (**29**).* Cbz-Cl (0.026 mL, 0.18 mmol) was added to a benzene solution of **23** (68 mg, 0.12 mmol). To the above mixture was added AgCN (23 mg, 0.024 mmol), and the mixture was heated at 60 °C for 30 min. After completion of the reaction (as indicated by TLC), benzene was removed and the mixture was extracted with EtOAc and filtered through a sintered funnel. The organic layer was then washed with satd $NaHCO_3$ followed by water and brine. Removal of the organic layer yielded the crude mixture which was purified by a flash column chromatography (3% MeOH in DCM) to yield **29** (78 mg, 0.11 mmol) in 93% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31−7.41 (m, 6H), 6.36 (d, 1H, J = 8.4 Hz), 5.26 (s, 2H), 5.00−5.10 (m, 2H), 4.83 (br s, 1H), 4.37−4.43 (m,1H), 4.16−4.23 (m, 1H), 3.93 (t, 1H, J = 8.4 Hz), 3.65 (s, 3H), 3.08−3.25 (m, 1H), 2.39−2.55 (m, 2H), 2.00 (br s, 1H), 1.76−1.87 (m, 1H), 1.48−1.66 (m, 5H), 1.44 (s, 9H), 1.12−1.33 (m, 2H), 0.85−1.00 (m, 18H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.9, 171.7, 171.2, 169.9, 155.8, 135.0, 128.8, 79.9, 70.0, 60.1, 59.3, 57.0, 52.3, 51.5, 51.0, 50.4, 49.3, 42.0, 40.8, 40.2, 37.6, 37.4, 30.7, 28.4, 25.1, 25.0, 24.9, 23.5, 23.3, 23.0, 22.0, 21.9, 21.7, 21.5, 15.6, 14.3, 11.5. HRMS (ESI) $(M + Na)^+$ calculated for $C_{36}H_{57}N_5O_9Na^+$ = 726.4054, found 726.4051.

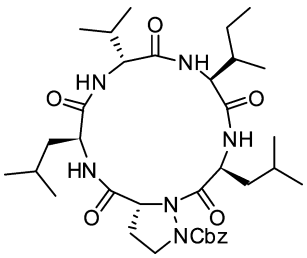
*(R)-Benzyl 2-((S)-2-((2S,3S)-2-(tert-Butoxycarbonylamino)-3-methylpentanamido)-4-methylpentanoyl)-3-((S)-1-((S)-1-methoxy-3-methyl-1-oxobutan-2-ylamino)-4-methyl-1-oxopentan-2-ylcarbamoyl)pyrazolidine-1-carboxylate (**30**).* Cbz-Cl (0.013 mL, 0.087 mmol) was added to a benzene solution of **24** (39 mg, 0.058 mmol). To the above mixture was added AgCN (15 mg, 0.12 mmol),



and the mixture was heated at 60 °C for 30 min. After completion of the reaction (as indicated by TLC), benzene was removed and the mixture was extracted with EtOAc and filtered through a sintered funnel. The organic layer was then washed with satd $NaHCO_3$ followed by water and brine. Removal of the organic layer yielded the crude diasteriomeric mixture which was separated by a flash column chromatography (3% MeOH in DCM) to yield **30** (35 mg, 0.052 mmol) in 90% yield.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33−7.40 (m, 6H), 6.54 (d, 1H, J = 8.0 Hz), 6.28 (d, 1H, J = 7.5 Hz), 5.19−5.28 (m, 2H), 5.06 (m, 1H), 4.91 (dd, 1H, J = 11.5, 4 Hz), 4.80 (br s, 1H), 4.50 (dd, 1H, J = 9, 5.5 Hz), 4.28−4.32 (m, 1H), 4.24 (br s, 1H), 3.92 (t, 1H, J = 8 Hz), 3.69 (s, 3H), 3.16−3.19 (m, 1H), 2.55−2.61 (m, 1H), 2.31 (br s, 1H), 2.11−2.17 (m, 1H), 1.81 (br s, 1H), 1.56−1.63 (m, 6H), 1.44 (s, 9H), 1.24−1.30 (m, 1H), 1.12−1.21 (m, 1H), 0.77−0.94 (m, 24H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.7, 172.2, 171.6, 171.4, 170.1, 159.1, 155.9, 148.6, 134.9, 128.8, 79.9, 70.1, 60.0, 59.3, 57.1, 52.8, 52.2, 49.6, 41.5, 40.4, 40.3, 37.3, 32.1, 31.6, 31.3, 29.5, 28.5, 25.0, 24.9, 23.3, 23.1, 23.0, 22.8, 22.0, 21.9, 21.5, 19.1, 18.1, 18.0, 15.6, 14.3, 11.5, 11.4. HRMS (ESI) $(M + Na)^+$ calculated for $C_{41}H_{66}N_6O_{10}Na^+$ = 825.4738, found 825.4741.

(3aR,6S,9R,12S,15S)-Benzyl 12-sec-Butyl-6,15-diisobutyl-9-isopropyl-4,7,10,13,16-pentaoxohexadecahydro-1H-pyrazolo[1,5-a]-



*[1,4,7,10,13]pentaazacyclopentadecine-1-carboxylate (**31**).* Cbz-Cl (5.3 μL, 0.037 mmol) was added to a benzene solution of **18*T*** (10 mg, 0.019 mmol). To the above mixture was added AgCN (5 mg, 0.037 mmol), and the mixture was heated at 60 °C for 30 min. On completion of the reaction (as indicated by TLC), benzene was removed and the mixture was extracted with EtOAc and filtered through a sintered funnel. The organic layer was then washed with satd $NaHCO_3$ followed by water and brine. Removal of the organic layer yielded the crude mixture which was purified by a flash column chromatography (5% MeOH in DCM) to yield **31** (9 mg, 0.013 mmol) in 71% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (d, 1H, J = 8.4 Hz), 7.49 (d, 2H, J = 7.2 Hz), 7.28−7.41 (m, 3H), 7.20 (br s, 1H), 6.82 (d, 1H, J = 7.8 Hz), 5.95 (brs, 1H), 5.49 (d, 1H, J = 12.6 Hz), 4.99−5.10 (m, 2H), 4.78 (dd, 1H, J = 8.4, 4.8 Hz), 4.37 (t, 1H, J = 9.7 Hz), 4.20 (t, 1H, J = 8.7 Hz), 4.02 (t, 1H, J = 9.3 Hz), 3.94 (br m, 1H), 3.10 (q, 1H, J = 9.0 Hz), 2.80−2.94 (m, 1H), 1.98−2.17 (m, 2H), 1.79 (br s, 2H), 1.35−1.63 (m, 6H), 1.02−1.19 (m, 1H), 0.84−0.98 (m, 24H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.6, 172.1, 170.8, 170.0, 167.8, 158.5, 136.3, 128.7, 128.4, 128.3, 69.6, 60.5, 60.4, 56.9, 53.6, 53.2, 49.5, 48.8, 42.3, 42.1, 36.3, 30.8, 25.6, 25.3, 25.1, 22.9, 22.7, 22.3, 19.6, 19.0, 15.7, 10.9. HRMS (ESI) $(M + Na)^+$ calculated for $C_{35}H_{54}N_6O_7Na^+$ = 693.3952, found 693.3950.

(3aR,6S,9R,12S,15S)-12-sec-Butyl-6,15-diisobutyl-9-isopropyldecahydro-1H-pyrazolo[1,5-a][1,4,7,10,13]pentaazacyclopenta-

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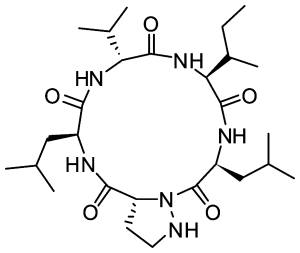

*decine-4,7,10,13,16(2H)-pentaone (**18T**).* To a solution of **31** (4 mg, 0.006 mmol) in MeOH (1 mL) was added 10% Pd/C (3 mg), and the mixture was stirred for 4 h in hydrogen atmosphere. The reaction mixture was filtered through Celite, and MeOH was removed in vacuo. The crude reaction mixture was then purified by flash column chromatography (5% MeOH in DCM) to yield **18*T*** in 72% yield (2.3 mg, 0.0043 mmol).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.5b01668.

X-ray crystallographic data for **18*T*** (CIF)
Copies of NMR (^{1}H, ^{13}C) spectra of all the new compounds along with 2-D NMR (COSY and HMQC) spectra of relevant compounds (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Lelais, G.; Seebach, D. *Helv. Chim. Acta* **2003**, *86*, 4152.

(2) (a) Stewart, D. E.; Sarkar, A.; Wampler, J. E. *J. Mol. Biol.* **1990**, *214*, 253. (b) Weiss, M. S.; Jabs, A.; Hilgenfeld, R. *Nat. Struct. Mol. Biol.* **1998**, *5*, 676.

(3) (a) Fischer, G. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1415. (b) Yaron, A.; Naider, F. *Crit. Rev. Biochem. Mol. Biol.* **1993**, *28*, 31. (c) Cohen, G. B.; Ren, R.; Baltimore, D. *Cell* **1995**, *80*, 237. (d) Lewis, P. N.; Momany, F. A.; Scheraga, H. A. *Proc. Natl. Acad. Sci. U. S. A.* **1971**, *68*, 2293. (e) Vitagliano, L.; Berisio, R.; Mastrangelo, A.; Mazzarella, L.; Zagari, A. *Protein Sci.* **2001**, *10*, 2627. (f) Garcia, J.; Dumy, P.; Rosen, O.; Anglister, J. *Biochemistry* **2006**, *45*, 4284. (g) Flores-Ortega, A.; Jiménez, A. I.; Cativiela, C.; Nussinov, R.; Alemán, C.; Casanovas, J. *J. Org. Chem.* **2008**, *73*, 3418. (h) Baures, P. W.; Ojala, W. H.; Gleason, W. B.; Johnson, R. L. *J. Pept. Res.* **1997**, *50*, 1. (i) Flippen-Anderson, J. L.; Gilardi, R.; Karle, I. L.; Frey, M. H.; Opella, S. J.; Gierasch, L. M.; Goodman, M.; Madison, V.; Delaney, N. G. *J. Am. Chem. Soc.* **1983**, *105*, 6609. (j) Halab, L.; Lubell, W. D. *J. Am. Chem. Soc.* **2002**, *124*, 2474. (k) Halab, L.; Lubell, W. D. *J. Org. Chem.* **1999**, *64*, 3312. (l) Beausoleil, E.; Lubell, W. D. *J. Am. Chem. Soc.* **1996**, *118*, 12902. (m) An, A. S. S.; Lester, C. C.; Peng, J.-L.; Li, Y.-J.; Rothwarf, D. M.; Welker, E.; Thannhauser, T. W.; Zhang, L. S.; Tam, J. P.; Scheraga, H. A. *J. Am. Chem. Soc.* **1999**, *121*, 11558. (n) Dumy, P.; Keller, M.; Ryan, D. E.; Rohwedder, B.; Wöhr, T.; Mutter, M. *J. Am. Chem. Soc.* **1997**, *119*, 918. (o) Wedemeyer, W. J.; Welker, E.; Scheraga, H. A. *Biochemistry* **2002**, *41*, 14637.

(4) (a) Keller, M.; Sager, C.; Dumy, P.; Schutkowski, M.; Fischer, G. S.; Mutter, M. *J. Am. Chem. Soc.* **1998**, *120*, 2714. (b) Montelione, G. T.; Arnold, E.; Meinwald, Y. C.; Stimson, E. R.; Denton, J. B.; Huang, S.-G.; Clardy, J.; Scheraga, H. A. *J. Am. Chem. Soc.* **1984**, *106*, 7946. (c) Montelione, G. T.; Hughes, P.; Clardy, J.; Scheraga, H. A. *J. Am. Chem. Soc.* **1986**, *108*, 6765. (d) Magaard, V. W.; Sanchez, R. M.; Bean, J. W.; Moore, M. L. *Tetrahedron Lett.* **1993**, *34*, 381. (e) Zerkout, S.; Dupont, V.; Aubry, A.; Vidal, J.; Collet, A.; Vicherat, A.; Marraud, M. *Int. J. Pept. Protein Res.* **1994**, *44*, 378. (f) Mikhailov, D.; Daragan, V. A.; Mayo, K. H. *Biophys. J.* **1995**, *68*, 1540. (g) Flores-Ortega, A.; Casanovas, J.; Assfeld, X.; Alemán, C. *J. Org. Chem.* **2009**, *74*, 3101. (h) Jhon, J. S.; Kang, Y. K. *J. Phys. Chem. B* **2007**, *111*, 3496. (i) Savrda, J. In *14th Proceedings of European Peptide Symposium*, Wepion, Belgium, April 11−17, 1976; Loffet, A., Ed.; Editions de l'Université de Bruxelles: Bruxelles, 1976; p 653. (j) Kantharaju; Raghothama, S.; Raghavender, U. S.; Aravinda, S.; Shamala, N.; Balaram, P. *Biopolymers* **2009**, *92*, 405.

(5) (a) Zhang, W. J.; Berglund, A.; Kao, J. L.-F.; Couty, J. P.; Gershengorn, M. C.; Marshall, G. R. *J. Am. Chem. Soc.* **2003**, *125*, 1221. (b) Che, Y.; Marshall, G. R. *J. Org. Chem.* **2004**, *69*, 9030. (c) Chalmers, D. K.; Marshall, G. R. *J. Am. Chem. Soc.* **1995**, *117*, 5927.

(6) Duttagupta, I.; Goswami, K.; Sinha, S. *Tetrahedron* **2012**, *68*, 8347.

(7) Hale, K. J.; Cai, J. *Chem. Commun.* **1997**, 2319.

(8) (a) Kelleman, A.; Mattern, R. H.; Pierschbacher, M. D.; Goodman, M. *Biopolymers* **2003**, *71*, 686. (b) McKeever, B.; Pattenden, G. *Tetrahedron* **2003**, *59*, 2731.

(9) (a) Jakobsche, C. E.; Choudhary, A.; Miller, S. J.; Raines, R. T. *J. Am. Chem. Soc.* **2010**, *132*, 6651. (b) Williams, K. R.; Adhyaru, B.; German, I.; Alvarez, E. *J. Chem. Educ.* **2002**, *79*, 372.

(10) Steffel, L. R.; Cashman, T. J.; Reutershan, M. H.; Linton, B. R. *J. Am. Chem. Soc.* **2007**, *129*, 12956.

(11) Chai, J. D.; Head-Gordon, M. *Phys. Chem. Chem. Phys.* **2008**, *10*, 6615.

(12) Marenich, A. V.; Cramer, C. J.; Truhlar, D. G. *J. Phys. Chem. B* **2009**, *113*, 6378.

(13) Wertz, D. H. *J. Am. Chem. Soc.* **1980**, *102*, 5316.

(14) (a) Yu, Z.-X.; Houk, K. N. *J. Am. Chem. Soc.* **2003**, *125*, 13825. (b) Li, H.; Wang, X.; Wen, M.; Wang, Z.-X. *Eur. J. Inorg. Chem.* **2012**, *2012*, 5011−5020. (c) Li, H.; Wen, M.; Wang, Z.-X. *Inorg. Chem.* **2012**, *51*, 5716. (d) Zhao, L.; Huang, F.; Lu, G.; Wang, Z.-X.; Schleyer, P. V. R. *J. Am. Chem. Soc.* **2012**, *134*, 8856. (e) Qu, S.; Dang, Y.; Song, C.; Wen, M.; Huang, K.-W.; Wang, Z.-X. *J. Am. Chem. Soc.* **2014**, *136*, 4974. (f) Ding, L.; Ishida, N.; Murakami, M.; Morokuma, K. *J. Am. Chem. Soc.* **2014**, *136*, 169.

(15) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.;Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.;Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A.; Peralta, Jr. J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, O.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. *Gaussian 09*, Revision A.02 ed.; Gaussian, Inc.: Pittsburgh, PA, 2009.

(16) Ewing, S. P.; Lockshon, D.; Jencks, W. P. *J. Am. Chem. Soc.* **1980**, *102*, 3072.

(17) (a) Fischer, G.; Bang, H.; Mech, C. *Biomed. Biochim. Acta* **1984**, *43*, 1101. (b) Berger, A.; Schechter, I. *Philos. Trans. R. Soc., B* **1970**, *B257*, 249.

DOI: 10.1021/acs.joc.5b01668
J. Org. Chem. 2015, 80, 10585−10604

(18) Keller, M.; Sager, C.; Dumy, P. L.; Schutkowski, M.; Fischer, G. S.; Mutter, M. *J. Am. Chem. Soc.* **1998**, *120*, 2714.
(19) Sakai, A.; Xiang, D. F.; Xu, C.; Song, L.; Yew, W. S.; Raushel, F. M.; Gerlt, J. A. *Biochemistry* **2006**, *45*, 4455.
(20) Paul, S.; Pattanayak, S.; Sinha, S. *Tetrahedron Lett.* **2011**, *52*, 6166.
(21) Wu, W.; Dai, H.; Bao, L.; Ren, B.; Lu, J.; Luo, Y.; Guo, L.; Zhang, L.; Liu, H. *J. Nat. Prod.* **2011**, *74*, 1303.
(22) Lécaillon, J.; Gilles, P.; Subra, G.; Martinez, J.; Amblard, M. *Tetrahedron Lett.* **2008**, *49*, 4674.
(23) Humbert-Voss, E.; Arrault, A.; Jamart-Grégoire, B. *Tetrahedron* **2014**, *70*, 363.

DOI: 10.1021/acs.joc.5b01668
J. Org. Chem. 2015, 80, 10585−10604