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Diastereoselective Synthesis of 5-Heteroaryl-Substituted Prolines Useful for Controlling Peptide-Bond Geometry

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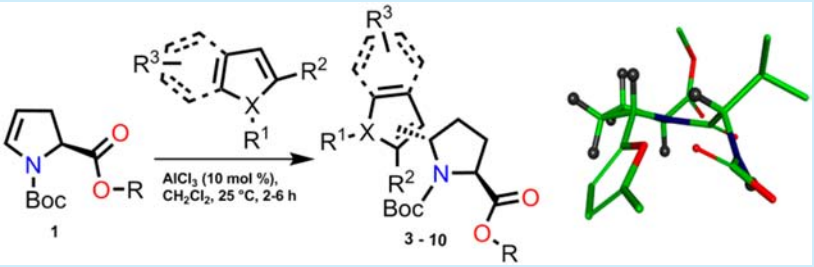
Supporting Information



ABSTRACT: A versatile diastereoselective Friedel−Crafts alkylation reaction of heteroaryl systems with a cyclic enecarbamate for the preparation of 5-heteroaryl-substituted proline analogues in good yields has been developed. These heterocyclic tethered cyclic amino acid building blocks constitute important structural motifs in many biologically active molecules. The impact of the substitution on proline *cis*/*trans* isomerization was explored by carrying out solution conformational studies by NMR on 5-furanyl-substituted proline-containing peptides. Conformational analysis revealed that the peptide bond is constrained in an exclusively *trans* conformation.

Proline, being a conformationally constrained cyclic amino acid, induces strong secondary structural motifs such as β-turns and helical kinks in proteins and peptides. Because of this inherent nature, it performs a significant role in numerous biological processes, i.e., collagen biosynthesis, protein folding, as well as in recognition events. Conformationally rigid peptides often are preferred over small molecule based therapeutics as they alleviate the drawbacks caused by the latter. For designing peptide-based therapeutics, proline was often a preferred choice, as it brings conformationally rigidity.[1] Furthermore, proline and its derivatives have been extensively used as organocatalysts in stereoselective reactions.[2] These unique properties of proline, both as key components of biomolecules and as popular organocatalysts, attracted the attention of bio-organic chemists for the development of new proline analogues useful for controlling the secondary structures of bioactive peptides. Numerous examples of proline derivatives substituted at the 2, 3, and 4 positions are reported in the literature,[3−5] whereas the 5-substituted prolines are less explored. Interestingly, 5-substituted prolines[4c,6] are reported as a key structural motif in bioactive molecules including natural products like prolinalin A[7a] and radicamine B[7b,c] as well as in medicinally important synthetic molecules like cholecystokinin (CCK) antagonist (RP 66803)[7d] and angiotensin-converting enzyme (ACE) inhibitor I[7e,f] (Figure 1). In addition, 5-substituted prolines comprise important structural components in several other biologically active molecules such as serine protease prolyl oligopeptidase (POP) inhibitors[6a] and sphingosine-1-phosphate-1 (S1P1) receptor agonists.[6e] Despite

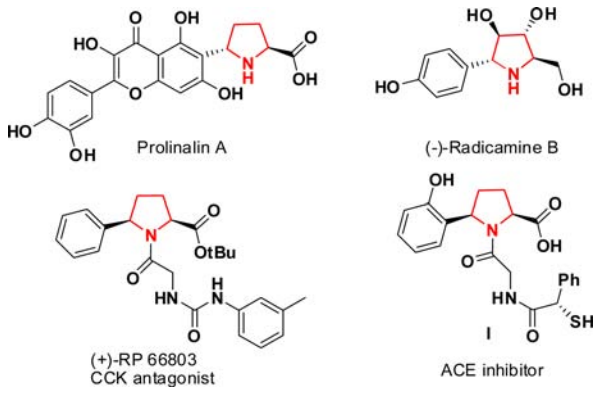


Figure 1. Bioactive molecules containing 5-substituted proline.

their importance as key structural elements, 5-substituted proline derivatives are relatively underexplored due to the limited availability of synthetic methodologies.[4c,6] Therefore, exploring synthetic methodologies to prepare new 5-substituted proline derivatives is highly desirable. Heterocyclic substituted amino acids are emerging as an important building blocks for the synthesis of novel peptidomimetics.[8] Several examples of 5-aryl-substituted prolines were reported, but to the best of our knowledge, 5-substituted heteroaryl prolines are yet to be

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reported. These attractive building blocks will be highly useful for conformationally rigid peptide-based therapeutics.

In the present study, we have explored the synthesis of 5-heteroarylprolines using a Friedel–Crafts alkylation reaction on *N*-Boc-4,5-dehydroproline benzyl ester as cyclic enecarbamate **1** and further investigated their conformational priorities by incorporating these novel proline residues into peptides. The indole and indole pyrrolidine play key roles as structural motifs in numerous biologically active molecules.[9] Therefore, we have selected the indole moiety for alkylation on enecarbamate **1**. Several examples of the Friedel–Crafts alkylation reaction of enamides and enecarbamate with indole moiety have been reported in the literature.[8c,10] Considering these interesting reactions, we have prepared 5-indolylprolines by the reaction of indole with *N*-Boc-4,5-dehydroproline benzyl ester as cyclic enecarbamate using $AlCl_3$ as the catalyst (Table 1, entry 2).

Table 1. Optimization of Reaction Conditions

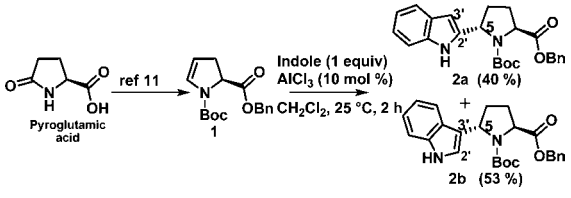
entry[a]	catalyst (mol %)	time (h)	solvent	yield of **2a**[b] (%)	yield of **2b**[b] (%)
1		24	CH_2Cl_2	NR[c]	NR[c]
2	$AlCl_3$ (10)	2	CH_2Cl_2	40	53
3	$Al(OTf)_3$ (10)	2	CH_2Cl_2	25	30
4	$Cu(OTf)_2$ (10)	1	CH_2Cl_2	35	40
5	AgOTf (10)	12	CH_2Cl_2	27	31
6	$AuCl_3$ (10)	1	CH_2Cl_2	38	50
7	TfOH (10)[d]	2	CH_2Cl_2		
8	$FeCl_3$ (10)[d]	2	CH_2Cl_2		
9	$BF_3.Et_2O$ (10)[d]	2	CH_2Cl_2		
10	$AlCl_3$ (10)	6	DCE	39	50
11	$AlCl_3$ (10)	2	CH_3CN	34	54
12	$AlCl_3$ (10)	4	THF	26	38
13	$AlCl_3$ (10)	2	ether	25	34
14	$AlCl_3$ (10)	2	toluene	NR[c]	NR[c]

[a]Reaction was carried out using an equimolar amount of **1** and indole. [b]Isolated yield. [c]No reaction. [d]Starting material consumed but product not identified.

The cyclic enecarbamate, *N*-Boc-4,5-dehydroproline benzyl ester **1**, was prepared from easily accessible pyroglutamic acid in three steps according to the procedure reported in the literature.[11] The reaction resulted in the formation of two products, namely **2a** (alkylation at C2′ of indole) and **2b** (alkylation at C3′ of the indole) with a slight excess of **2b** (Scheme 1).

Generally, the Friedel–Crafts alkylation reaction of indole will not take place at the second position, but surprisingly, we obtained alkylated products at both the second (**2a**) and third positions (**2b**) of the indole ring with a slight excess of alkylated

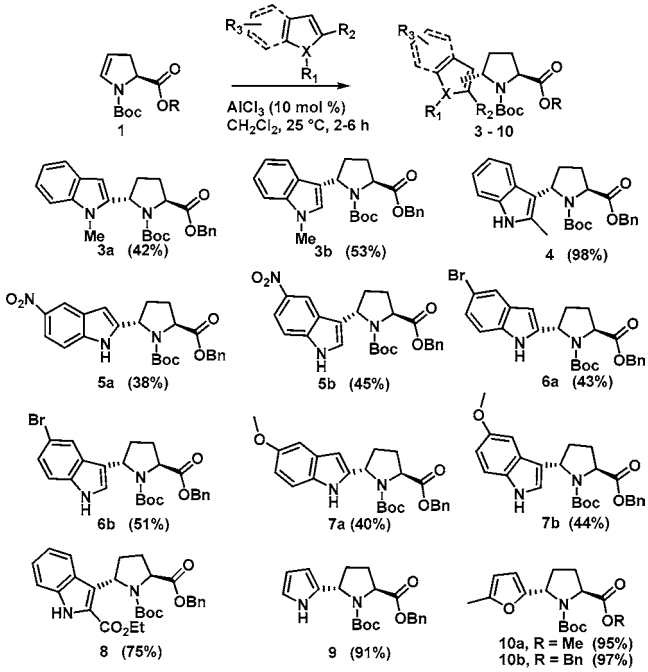
Scheme 1. Friedel–Crafts Alkylation Reaction of Indole with *N*-Boc-4, 5-dehydroproline Benzyl Ester 1



product at the third position. The structures of compounds **2a** and **2b** were verified by extensive NMR analysis. The HMBC cross peaks between H5↔C2′ in **2a** and H5↔C3′ in **2b** confirmed the substitutions are indeed at C2′ and C3′ of the indole, respectively.[12] In order to investigate whether these products could be prepared regioselectively, we optimized the reaction using several catalysts and solvents (Table 1). There was no reaction without catalyst even after 24 h (entry 1), whereas in the other two sets of reactions we could not obtain significant regioselectivity as presented in Table 1 (for catalyst: Table 1, entries 2–9; for solvents: Table 1 entries 10–14). In all of these reactions, we obtained only diastereoselectively *trans* product with respect to the α position of proline.

We have extended the scope of the reaction (Scheme 2) by reacting **1** with *N*-methylindole and indole substituted with

Scheme 2. Reaction of Cyclic Enecarbamate 1 with Indole, Pyrrole, and Furan Derivatives



electron-withdrawing, -neutral, and -rich groups, which also resulted into two products with substitutions at both C2′ as well as at the C3′ position (**3a**, **3b**, **5a**, **5b**, **6a**, **6b**, **7a**, and **7b**) in good yields. In the case of 2-methylindole and 2-(ethoxycarbonyl)indole, we obtained only third-substituted products **4** and **8** exclusively in excellent yields. The reaction with other heteroaromatic residues like pyrrole and furan resulted in formation of the corresponding 5-substituted prolines **9** and **10**, respectively, in excellent yield. It is noteworthy that in all the cases only one diastereomer, (2*S*,5*S*)-5-heteroarylproline, was obtained as the exclusive product. Structures of these compounds and their stereospecific assignments were carried out by extensive NMR analysis. The $^1J_{C-H}$ and $^nJ_{C-H}$ values from proton-coupled HSQC and HMBC spectra support the structure of compound **9** as a 2-substituted product.[12]

We have demonstrated a straightforward and diastereoselective synthesis of (2*S*,5*S*)-5-heteroarylprolines in excellent yields. The steric influences of 5-substituted prolines resulted in the stabilization of the *cis*/*trans* geometry of the peptide bond

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as evident from the literature reports. The (2*S*,5*R*)-5-*tert*-butylproline resulted in a predominant *cis* peptide bond, whereas the 5,5-dimethylproline (dmP) has the ability to lock the peptide bond in a *cis* conformation.[13] It is therefore pertinent to study the steric influence on pyrrolidine ring puckering and peptide bond geometry of the new 5-substituted prolines. Initially, structural studies were carried out on protected 5-furanyl-substituted proline **10b** by NMR spectroscopy. The pyrrolidine ring-puckering study in **10b** revealed at least two major rotamers were evident with two sets of peaks for each proton in the ^{1}H NMR spectrum. Both of these rotamers were characterized independently. The rotamers were observed due to the rotation between the *N*-Boc bond.[6d] This is evident from the similarity of the NOEs and vicinal coupling constants for the pyrrolidine ring protons. We have observed very weak NOE between CαH−CγH, coupled with a very small coupling constant (~1 Hz) between CδH−CγH and CαH−Cβ(*pro*-R)H as compared to characteristic NOE between CδH and CβH(*pro*-R). These observations support the pyrrolidine ring being in the Cγ-exo conformation. Similar results were obtained from the 5nS Molecular Dynamics (MD) simulations as given in the Figure 2, using distance and torsional restraints derived from the NOEs and ^3J values, respectively.[12]

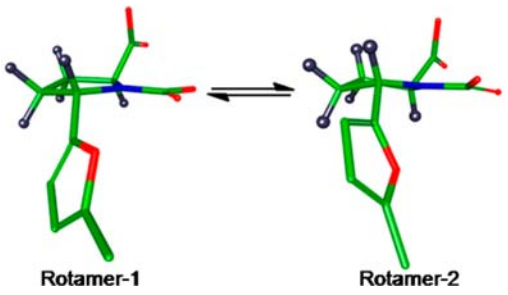


Figure 2. Average structures of rotamers **1** and **2** from 5 nS MD simulations for the compound **10b** showing that both rotamers are in the Cγ-exo form of proline; protecting group (Bn and *tert*-butyl) removed for clarity.

Further, we have carried out conformational studies on peptides containing **10b**, as it was evident that the substitution pattern on proline rings dictates the geometry preferences of the Xaa-Pro peptide bond.[13,14] Starting from 5-(5-methylfuran-2-yl) proline **10b**, peptides **11** and **12** were prepared with a standard solution-phase synthesis method (Scheme 3).

Generally, the peptide bonds exist predominantly in the *trans* conformation,[15a] except for the instance of Xaa-Pro peptide bond. The Xaa-Pro bond geometry exists as mixture of *cis* and *trans* forms due to less steric hindrance. This behavior of *cis*/*trans* isomerization among Xaa-Pro peptide bonds can be attributed to a small free enthalpy difference between the *cis* and *trans* Xaa-Pro bond isomers (2.0 kJ mol^{-1} compared to 10.0 kJ mol^{-1} for other Xaa-non-Pro peptide bonds).[15b]

Conformational studies were carried out in solutions of peptides **11** and **12** at 10 mM concentration in DMSO-d_6 by utilizing solution NMR techniques. A single set of resonances in the NMR spectra indicated the existence of single conformation for peptides **11** and **12** in the NMR time scale. We have observed broadness of the resonances for **12** due to the rotation of the *N*-Me group of Ala. This is a common phenomenon observed among *N*-methylated peptides.[16] However, for both **11** and **12**, the coupling constant values and NOEs for the pyrrolidine ring were similar, as observed in **10b**. These observations suggested that the pyrrolidine ring in these peptides is also in Cγ-exo conformation.[14d] Further, the NOE between Val CαH↔Pro CδH, characteristic for a *trans* peptide bond were observed for both **11** and **12**, as illustrated in Figure 3. This clearly suggests that the Val-Pro peptide

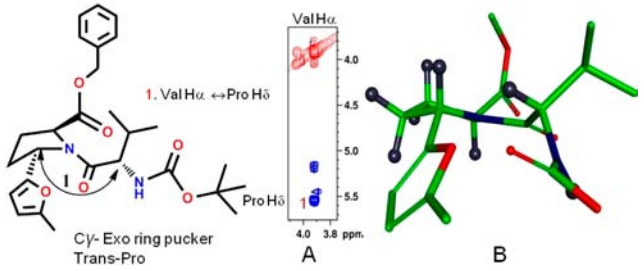
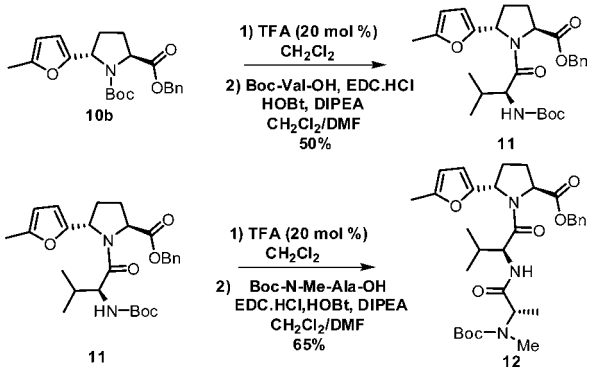


Figure 3. (A) ROESY expansion of the peptide **11** showing characteristic NOE correlation between ValHα↔ProHδ (1) in peptide **11**, confirming the *trans* Val-Pro peptide bond. (B) Average structure of **11**, from 5nS Molecular Dynamics; protecting group (Bn and *t*-butyl) removed for the clarity.

Scheme 3. Synthesis of Peptides 11 and 12



bonds are in a *trans* configuration in peptides **11** and **12**. Critical analysis of NOE correlations did not show any *i*, *i* − 1 CαH↔CαH correlations, characteristic of *cis* peptide bonds, which further supports the conclusion that the Val-Pro peptide bond is exclusively in the *trans* configuration.

In conclusion, we have developed a simple and straightforward diastereoselective method for the synthesis of a variety of novel 5-heteroaryl-substituted prolines in good yields. NMR studies revealed that the Val-Pro bond exists exclusively in the *trans* form in 5-furanyl-substituted proline-containing peptides. The ability of these novel 5-heteroaryl-substituted proline analogues to form an exclusive *trans* bond may be useful as a valuable tool in the design of conformationally constrained peptides and peptidomimetics possessing a stable conformation.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b00863.

Experimental details and spectral data (PDF)

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Notes

The authors declare no competing financial interest.

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