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Graphical Abstract

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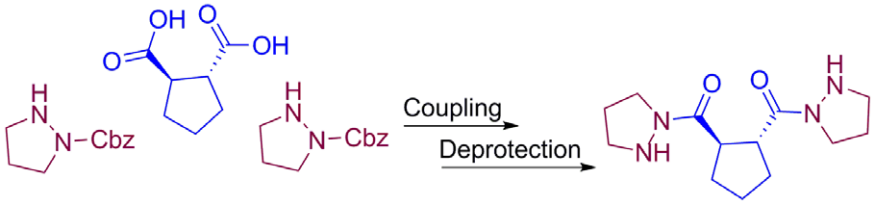

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Mimicking a Proline Tripeptide with Pyrazolidines and a Cyclopentane Linker

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ABSTRACT

In this work the five-step assembly of a peptidomimetic structurally resembling Prolyl-Prolyl-Proline tripeptide is reported. Proline units are mimicked by two pyrazolidine rings connected to *trans*-1,2-cyclopentanedicarboxylic acid. The Pro-Pro-Pro mimetic resembles a tri-L-proline unit but with increased lipophilicity and structural constraints imposed by the linker.

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Introduction

Peptides play an increasingly important role in the treatment of diabetes, cancer, metabolic, cardiovascular, and infectious diseases.[1] Based on the fact that the FDA approved six peptides in 2012 (lucinactant, peginesatide, pasireotide, carfilzomib, linaclotide, and teduglutide), peptide and peptidomimetic therapeutic compositions have spurred additional efforts in drug discovery.[2]

L-Proline is often found at the end of an α helix or in protein turns or loops and is known to change amino acid triplets to the *cis-trans-cis* configuration.[3] The Met[97]TyrProProProTyr[102] motif of T-lymphocyte-associated protein 4 (CTLA-4) and CD28 sequences is critical in mediating interaction with B7 membrane protein. Mutagenesis experiments indicate that the polyproline motif is essential for the binding of CG152, CD28, and ICOS receptors to their respective ligands.[4,5] The *cis-trans-cis* configuration of the three consecutive proline residues in CTLA-4 and their relatively low B factors, suggests that these proline residues play crucial roles in binding B7.[4] Constrained unnatural amino acids **1** and **2** (Figure 1), showing structural features similar to proline, have been used to explore the capabilities of β-turn scaffolds.[6] In a library of proline mimetics **2**, the R^2 substituents were diversified and the five-membered ring was constrained as the 1,2,3-triazole moiety.[7] Cyclohexane-1,2-dicarboxylic acid and 2-aminocyclohexanecarboxylic acid are proline isosteres in thrombin inhibitors.[8] Proline mimetics containing cyclopentane-1,2-dicarboxylic acid **3** and cyclopentene-1,5 dicarboxylic acid **4** have been prepared and crystallized with thrombin to study the interaction with the binding site of these thrombin inhibitors.[9]

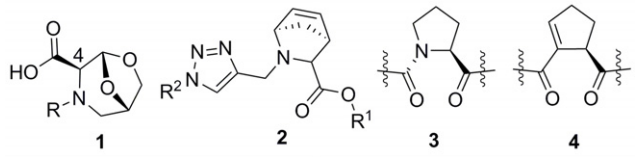


Figure 1. Reported mimetics of L-proline.

Peptidomimetics with aza-moieties are known to be more resistant to protease cleavage.[10,11] Aza-Pro **5** has been successfully incorporated into solid-phase organic synthesis providing stable aza-Pro-containing peptidomimetics.[12] Aza-Pro **6**, was included in SAR studies of small molecules which mimic the FKBR binding protein.[13]

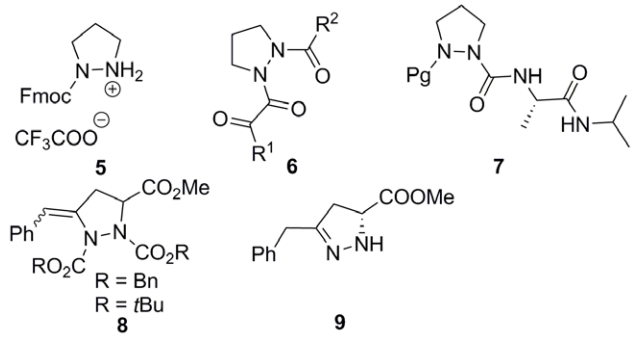
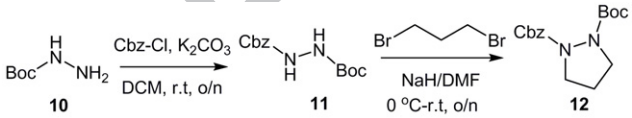


Figure 2. Reported aza-mimetics **5-9** of L-proline unit.

Several peptidomimetics of type **7** with the aza-Pro at different positions have been reported and suggest that in contrast to the natural proteins, the presence of aza-Pro in the chain prevents the formation of a β-turn for the following residue.[14] Recently reported aza-prolines **8** and **9** synthesized by 5-*exo-dig* cyclization (favored over 6-*endo-dig*) expanded the range to optically active aza-prolines.[15]

In this work a new approach towards the *cis-trans-cis* Pro-Pro-Pro triplet has been developed by combining two pyrazolidine rings with *trans*-DL-1,2-cyclopentanedicarboxylic acid. In this way, L-proline is mimicked by the aza-proline units as well as the cyclopentane ring.

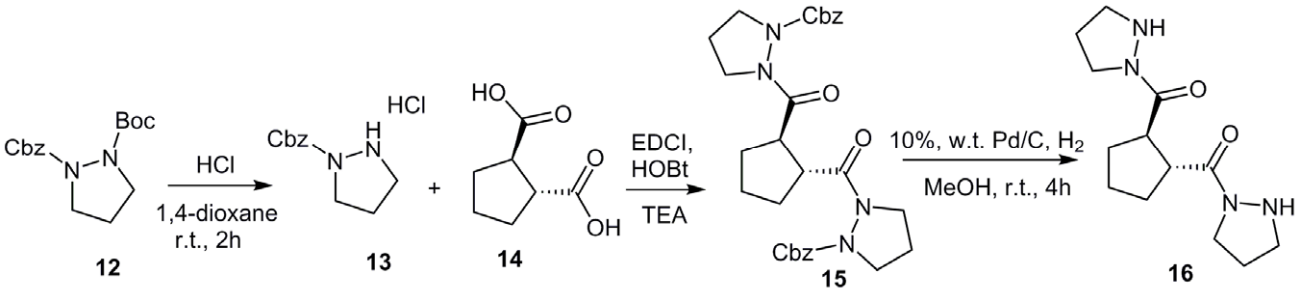
Synthesis of the N-Boc-N-Cbz di-protected hydrazine **10** proceeded first by mono-protection of hydrazine hydrate with Boc-anhydride, followed by protection of the remaining amino group with Cbz. N,N'-Diprotected hydrazine **11** was then cyclized to form the pyrazolidine ring by reaction with1,3-dibromopropane and sodium hydride in DMF to give **12** in 78% yield (Scheme 1).



Scheme 1. Assembly of N,N'-diprotected pyrazolidine ring **12**.

The Boc group of N,N'-diprotected intermediate **12** was deprotected by 2N HCl/MeOH solution to give hydrochloride **13** in quantitative yield. N-Cbz-protected pyrazolidine **13** was coupled with *trans*-DL-1,2-cyclopentanedicarboxylic acid **14** using standard EDCI/HOBt-mediated coupling to give Cbz-protected mimetic tri-proline residue **15**. Intermediate **15** was deprotected *in situ* to give the final dipyrazolidine system **16** in 89% yield. Thus, a tri-proline mimic was synthesized in five steps, namely, ring assembly, coupling, protection and deprotection.

The peptidomimetic **16** was analyzed computationally to compare it with the native triprolyl scaffold. For computational purposes, the C-terminus was removed from Pro-Pro-Pro to make it comparable with **16**. Molecular structures were drawn with ChemBioDraw Ultra 12 and optimized with PC Model using the MMX force field. Physical properties listed in Table 1 were derived from the same force field. Although rather similar to triprolyl in terms of the steric energy and heat of formation, compound **16** shows higher strain due to the conformationally restricted N-N bonds of the pyrazolidine moieties. As indicated by the dipole moments, peptidomometic **16** is significantly less polar than the triprolyl scaffold, which translates into higher lipophilicity. This enhances pharmacokinetic properties such as absorption and distribution.



Scheme 2. Synthesis of trans-DL-cyclopentane-1,2-diylbis(pyrazolidin-1-ylmethanone) **16**.

The ability of proline to form *cis*-peptide bonds and undergo *cis-trans* isomerization has been well-studied.[6] The conformations of poly-proline peptides have been calculated[16, 17] and it was suggested that the *trans-cis* conversion of poly-proline chain may occur not only at the terminus but also in the middle of the chain.[18, 19] X-ray analysis of several aza-proline containing peptides revealed that because of steric hindrance, both nitrogen atoms are not co-planar. Reduced electronic conjugation in the two aza-Pro adjacent amide groups explains the longer amide bond distances and the weak proton-accepting character of the two pyrazolidine nitrogens. The absolute configuration of both aza-Pro-nitrogens depends on the chemical nature of the sequence but in all cases the aza-Pro residue assumes the same three-dimensional structure and causes folding opposite to that induced by proline.[20]

Table 1. Comparison of calculated physical properties

Property	*Trans* isomer **16**	Triprolyl
Steric energy, kcal/mol	36.1	33.2
Strain energy, kcal/mol	23.8	17.7
Heat of formation, kcal/mol	87.6	88.7
Dipole moment, D	3.97	5.46
Log P (calc.)	4.43	1.37

In conclusion, a novel mimetic of L-Pro-L-Pro-L-Pro **16** consisting of two pyrazolidine moieties linked by amide bonds to a cyclopentane ring in the *trans* configuration has been synthesisized in five steps from N,N'-diprotected hydrazine. Mono N-terminus deprotection of the product followed by coupling with *trans*-DL-1,2-cyclopentanedicarboxylic acid and deprotection of the remaining secondary amino group, resulted in a di-aza mimic of the tri-proline peptide. Molecular structures of the original Pro-Pro-Pro tripeptide and the di-aza mimic were studied using molecular mechanics, and it was concluded that the non-polar cyclopentane ring of **16** increases the lipophilicity of the peptidomimetic, while the pyrazolidine moieties instil conformational constraints that induce turn.

Acknowledgments

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*Preparation of tert-butyl hydrazinecarboxylate **10***: Hydrazine hydrate (113 mg, 2.26 mmol, 2.26 eq.) was dissolved in IPA (5 mL) and cooled down to 0 °C. Di-*tert*-butyl carbonate (174 mg, 1 mmol, 1 eq.) in IPA (1 mL) was added drop-wise and the mixture was stirred for 2 hours. The solvent was then evaporated and the residue was dissolved in DCM, dried over $MgSO_4$, and filtered. Evaporation of the solvent gave *tert*-butyl hydrazinecarboxylate **10** as a white semi-solid (128 mg, 97 %). ^{1}H NMR

(300 MHz, $CDCl_3$) δ 6.73 (s, 1H), 3.83 (s, 2H), 1.46 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.2, 80.1, 28.2.[21]

*Preparation of 1-benzyl 2-(tert-butyl) hydrazine-1,2-dicarboxylate **11***: *Tert*-butyl hydrazinecarboxylate **10** (132 mg, 1 mmol) was dissolved in dry DCM and cooled down to -78 °C. Benzyl chloroformate (20.5 mg 1.2 mmol) was added drop-wise followed by Na_2CO_3 (106 mg, 1 mmol). The reaction mixture was allowed to r. t. and stirred overnight. Filtration, evaporation of solvents, and recrystallization from hexanes/EtOAc 50:1 gave 1-benzyl 2-(*tert*-butyl) hydrazine-1,2-dicarboxylate **11** as a white solid (226 mg, 85%), mp 75.0–77.0 °C, lit. 72–74 °C. ^1H NMR (300 MHz, $CDCl_3$) δ 7.34 (s, 5H), 6.80 (s, 1H), 6.49 (s, 1H), 5.16 (s, 2H), 1.46 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.9, 155.9, 135.8, 128.7, 128.5, 128.4, 81.9, 67.8, 28.3.[22]

*Preparation of 1-benzyl 2-(tert-butyl) pyrazolidine-1,2-dicarboxylate **12***: 1-Benzyl 2-(*tert*-butyl) hydrazine-1,2-dicarboxylate **11** (266 mg, 1 mmol, 1 eq.) was dissolved in anhydrous DMF (10 mL) and cooled down to 0°C. Sodium hydride 60% dispersion in mineral oil (84 mg, 2.1 mmol, 2.1 eq.) was added and the mixture was stirred for 30 minutes under nitrogen atmosphere. 1,3-Dibromopropane (0.11 mL, 1.05 mmol, 1.05 eq.) was added to the mixture and it was stirred overnight at room temperature. The mixture was then acidified with saturated citric acid solution and extracted three times with diethyl ether. The combined organic layers were washed with citric acid solution three times, dried over $MgSO_4$, filtered and the solvent was evaporated. Purification by column chromatography (hexanes-EtOAc 3:1) gave 1-benzyl 2-(*tert*-butyl) pyrazolidine-1,2-dicarboxylate **12** as a colorless oil (238 mg, 78 %). ^1H NMR (300 MHz, $CDCl_3$) δ 7.42–7.24 (m, 5H), 5.18 (d, *J* = 31.3 Hz, 2H), 3.92 (d, *J* = 23.3 Hz, 2H), 3.39–3.07 (m, 2H), 2.09–1.95 (m, 2H), 1.42 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.7, 156.2, 136.2, 128.4, 128.0, 127.8, 81.4, 67.6, 46.7, 46.2, 28.0, 25.6.[22]

*Preparation of benzyl pyrazolidine-1-carboxylate hydrochloride **13***: 1-benzyl 2-(*tert*-butyl) pyrazolidine-1,2-dicarboxylate (310 mg, 1 mmol, 1 eq.) was dissolved in 2N HCl/MeOH and it was stirred at r. t. for 24 hours. Evaporation of the solvent followed by recrystallization from ether gave benzyl pyrazolidine-1-carboxylate hydrochloride **13** as a colorless oil (503 mg, 86 %); ^1H NMR (300 MHz, CD_3OD) δ 7.61–7.25 (m, 5H), 5.28 (d, *J* = 1.7 Hz, 3H), 3.77 (td, *J* = 6.9, 1.9 Hz, 2H), 3.63 (td, *J* = 7.0, 1.9 Hz, 2H), 2.38 (pd, *J* = 6.8, 1.8 Hz, 2H); ^{13}C NMR (75 MHz, CD_3OD) δ 136.7, 129.9, 129.8, 129.7, 70.3, 47.6, 25.7.[23]

*Preparation of trans-DL-cyclopentane-1,2-diylbis(pyrazolidin-1-ylmethanone) **16**:* Benzyl pyrazolidine-1-carboxylate hydrochloride **13** (534 mg, 2.2 mmol), *trans*-DL-1,2-cyclopentanedycarboxylic acid **14** (158 mg, 1 mmol), EDCI (341 mg, 2.2 mmol), and 1*H*-benzo[*d*][1,2,3]triazol-1-ol hydrate (368 mg, 2.4 mmol) were dissolved in dry THF (20 mL) and stirred at 0 °C. TEA (0.3 mL, 2.1 mmol) was added to the mixture, which was then stirred at 0 °C for 30 minutes. After that, it was allowed to r. t. and stirred overnight. After the solvent was evaporated, the residue was dissolved in EtOAc, washed with water and brine, dried over $MgSO_4$, and the solvent was evaporated again. Without further purification, the crude intermediate **15** was deprotected using standard hydrogenation conditions (H_2/Pd/C, r. t. in 10 mL of MeOH). After filtration and evaporation of the solvent, the crude product was purified by preparative HPLC. Product **16** is a colorless oil (236 mg, 89 %). ^1H NMR (600 MHz, D_2O) δ 3.70 – 3.57 (m, 1H), 3.55–3.46 (m, 1H), 3.39 (t, *J* = 7.5 Hz, 3H), 3.11 (q, *J* = 9.0 Hz, 1H), 3.03–2.83 (m, 4H), 2.09 (p, *J* = 6.9 Hz, 1H), 2.06–1.93 (m, 5H), 1.79–1.66 (m, 2H), 1.66–1.50 (m, 2H); ^{13}C NMR (150 MHz, D_2O) δ 174.9, 174.6, 170.8, 170.5, 47.3, 47.3, 46.8, 46.3, 46.3, 46.2, 46.1, 45.8, 45.6, 45.5, 44.9, 44.8, 29.9, 29.7, 29.5, 26.8, 26.1, 24.8, 24.7. HRMS ($C_{13}H_{22}N_4O_2$) Calcd: 266.17, Found: 267.3

Supplementary Material

^1H and ^{13}C spectra of all novel compounds listed in experimental section. This material is available free of charge electronically