Enantio- and Regioselective Synthesis of a (*Z*)-Alkene *cis*-Proline Mimic

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Proline is the unique dialkylated member of the 20 common natural amino acids, resulting in cis/trans amide bond isomerization. Isomerization of proline has profound effects on peptide and protein structure[1] and results in structural issues for every peptide containing a proline. Several bioactive peptides contain *cis*-Pro,[2−5] a type-VI β-turn,[6] though it is not always known whether the bioactive conformation is *trans*- or *cis*-Pro.[7−12] We have synthesized an isosteric mimic of a *cis*-Pro dipeptide that will be useful in rational drug design and protein folding studies, Figure 1. Our mimic is suitable for stabilizing the bioactive conformation of peptides and proteins. The *cis*-Pro mimic will be incorporated into substrate analogues for the peptidyl-prolyl isomerase enzymes, cyclophilin and FK506 binding protein (FKBP), that preferentially bind *cis*-Pro substrates.[13]

The peptide Ac-Ala-*cis*-Pro-NHMe and the corresponding mimic, **1a** (Figure 1), were subjected to a MonteCarlo conformational search and minimized using the Amber force field with water solvation in MacroModel v. 3.5a. The lowest energy structure of the mimic overlayed with the peptide on the bonds marked with vectors with root-mean-square deviation of 0.17 Å, Figure 1. The vectors[14] in this case are part of the peptide backbone, giving direction to attached peptide chains.

The conformationally rigid (*Z*)-alkene isostere of Ala-*cis*-Pro was synthesized in a form suitably protected for incorporation into peptidomimetics. The (*Z*)-alkene mimic is a very good isostere for the *cis*-amide bond and does not introduce additional functionality that could interfere with peptide structure. Surprisingly, this simple dipeptide mimic has not yet been synthesized. Other *cis*-proline mimics have been reviewed.[5,15] Nonproline *cis*-amide alkene isosteres have been made,[16] some as *E*/*Z* mixtures.[17,18] The fluoro-

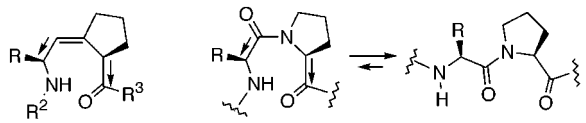
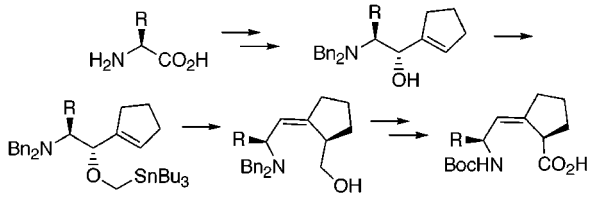
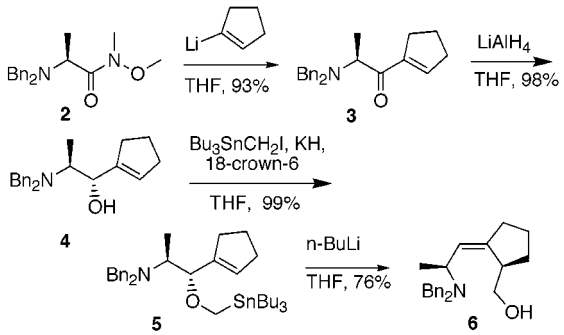


Figure 1. *cis*-Pro mimics and *cis*-/*trans*-Pro equilibrium.

Scheme 1



Scheme 2



alkene *trans* isosteres have been made.[19] Stereoselective routes to *trans*-alkene dipeptide isosteres[16,20,21] and *trans*-Pro mimics are known.[22]

The synthetic challenges associated with the *cis*-Pro mimic were as follows: (1) versatility for introducing a variety of amino acid side chains to mimic the residue N-terminal to Pro, (2) enantiospecific synthesis of the key *S*,*R* enantiomer of the mimic, and (3) formation of the (*Z*)-alkene exocyclic to a cyclopentyl ring to mimic the *cis*-amide. The α-amino acid starting material permits introduction of a wide variety of amino acid side chains into the mimic and is the origin of stereoselectivity for the synthesis, Scheme 1. A Still−Wittig [2,3]-sigmatropic rearrangement introduced the (*Z*)-alkene and transferred chirality to the cyclopentyl ring.[23]

The enantio- and regioselective synthesis of the dipeptide mimic of L-Ala-*cis*-L-Pro is shown in Schemes 2 and 3. Beginning with L-Ala, the Weinreb amide **2** was prepared according to published procedures.[24] The reaction of **2** with cyclopentenyllithium[25,26] gave the desired ketone **3** in 93%

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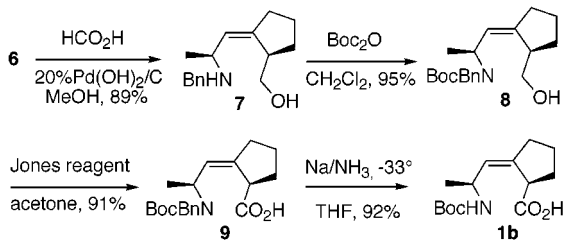
Scheme 3



yield.[27,28] Simple $LiAlH_4$ reduction of ketone **3** resulted in greater than 95% diastereoselectivity of the Felkin−Ahn product **4**.[29]

The tributyltin intermediate **5** was prepared and Still−Wittig rearrangement gave the desired (*Z*)-alkene **6**, with transfer of chirality to the cyclopentyl ring to give a single diastereomer in the NMR. (*Z*)-Alkene regiochemistry was confirmed by NOE (see the Supporting Information). Alcohol **6** crystallized after evaporation of ethyl acetate. X-ray crystallographic analysis provided definitive evidence for the desired isomer (see the Supporting Information). Similar steric features of the five-membered ring that induce cis/trans isomerization in Pro peptides appear to be responsible for the high (*Z*)-stereoselectivity obtained in this case.[17]

Protection of the amine in a form suitable for peptide synthesis and oxidation of the alcohol to the acid were intertwined synthetic steps, Scheme 3. Didebenzylation via dissolving metal reduction (Na or Li in NH_3/THF) failed. One of the two benzyl groups could be removed by catalytic transfer hydrogenolysis in formic acid on Pearlman's catalyst.[30] Protection of benzylamine **7** with *tert*-butoxycarbonyl (Boc) facilitated removal of the second benzyl via Na/NH_3 reduction; however, Jones oxidation of the resulting alcohol gave decomposition. Instead, oxidation of alcohol **8** with the doubly protected amine proceeded in high yield to give acid **9** from which the benzyl group was easily removed via Na/NH_3 reduction to give the target **1b**. Concerns about β,γ-unsaturated acid or amide isomerization to the α,β-unsaturated compound proved unfounded.[31] The (*E*)-alkene dipeptide isosteres are also stable toward isomerization.[20,21]

We have synthesized **1b**, a new (*Z*)-alkene isostere of a *cis*-Pro dipeptide. This route uses a single chiral precursor for the enantio- and regioselective synthesis. The Ala-*cis*-Pro mimic was prepared in eight steps and 49% overall yield from the Weinreb amide of dibenzyl-Ala. These results demonstrate the ease of preparing the (*Z*)-alkene mimic starting with a chiral amino acid in a quantity required for peptide synthesis. The Ala-*cis*-Pro mimic has been incorporated into a cyclophilin substrate analogue (Hart, S. A.; Etzkorn, F. A., unpublished results).

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Supporting Information Available: Experimental procedures for all compounds, ^1H NMR spectrum of an oxazolidinone derivative of compound **4**, 1D NOE spectrum of compound **6**, X-ray crystallographic data for compound **6**, and the ^1H and ^{13}C NMR spectra of compound **7** (24 pages).

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(31) The double bond was inert to isomerization both under conditions of amide coupling to the activated ester and toward heating with tertiary amine base. Acid **1b** was coupled to L-Phe-*p*-nitroanilide using standard solution-phase methods (Hart, S. A.; Etzkorn, F. A., unpublished results). No migration of the alkene was observed in the product. This product was treated with 2.5 equiv of Et_3N at 80 °C for 16 h, and no isomerization was observed by ^1H NMR.