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A Short Synthesis of Bicyclic Dipeptides Corresponding to Xxx-L-Pro and Xxx-D-Pro Having Constrained *Cis*-Proline Amides

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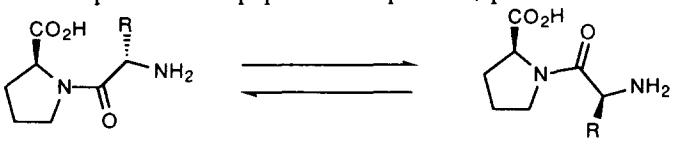
Abstract: A short synthesis that generates two isomeric bicyclic dipeptides having constrained, *cis*-proline amide bonds has been developed. One of these bicyclic dipeptides corresponds to an Xxx-L-Pro dipeptide (**17**), while the other isomer corresponds to an Xxx-D-Pro dipeptide (**18**).

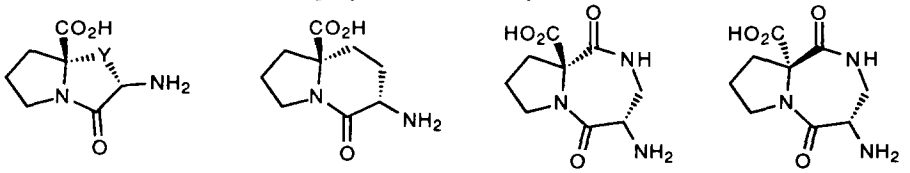
Unlike the other 19 common amino acids, which predominantly assume *trans*-amide conformations when incorporated into peptides and proteins, proline amides display an equal



1 (cis-amide) **2 (trans-amide)**

tendency to assume both the *cis*- (**1**) and *trans*-amide (**2**) conformation.[1] Because only proline amides possess this conformational flexibility, it has been speculated that *cis-trans* proline isomerization plays many important biochemical roles, including controlling the rate of protein folding,[2] triggering receptor-mediated transmembrane signalling,[3] providing a recognition element in peptide antigens,[4] and regulating both the activation and breakdown of peptide hormones.[5] Of potential utility in studying these biochemical events would be peptides that constrain proline to either the *cis*- or *trans*-amide conformation. Detailed here is a short synthesis that generates two isomeric, bicyclic dipeptides that covalently constrain the proline amide to the *cis* conformation.

The obvious approach for constraining an Xxx-Pro dipeptide is to tether the α carbons of the two amino acids using a linker Y, as shown in general structure **3**. The synthesis and characterization of **4**, in which an alkyl linker (Y=CH_2CH_2) is used to constrain the amide to the *cis* conformation, has recently been described by two groups.[6] The target molecules in the present work are **5** and **6**, which employ a lactam-methylene (CO-NH-CH_2) linker. An attractive

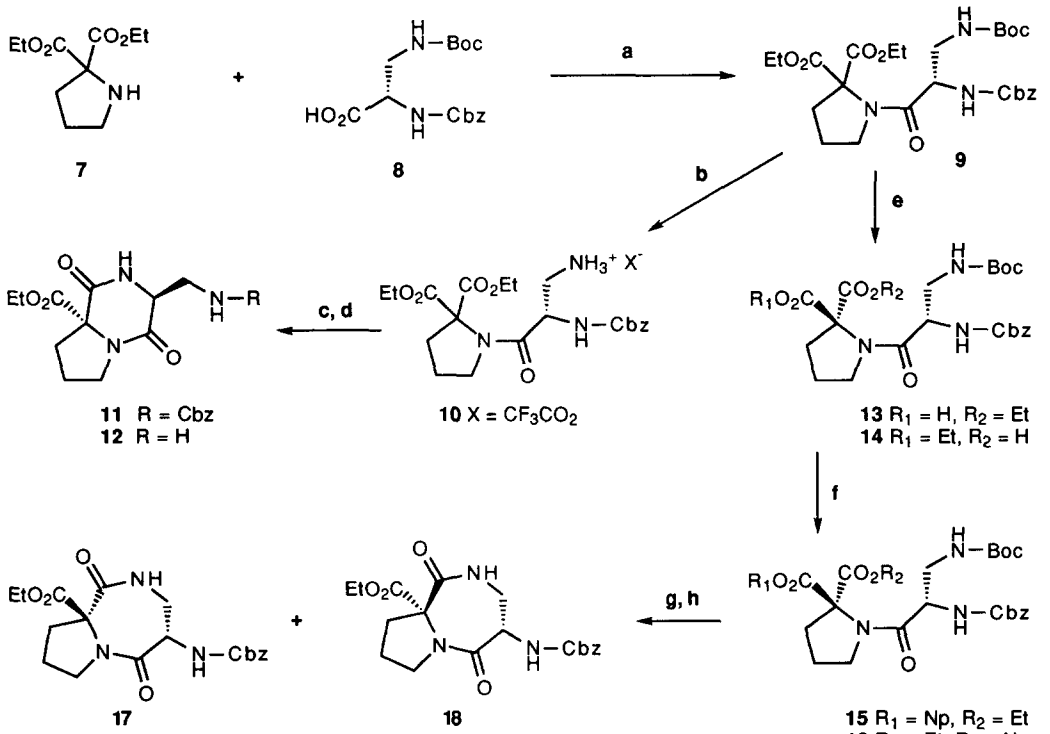


3 **4** **5** **6**

feature of this linker is that the lactam provides additional constraint to the bicyclic framework. The two isomers **5** and **6** differ in their configuration at the carbon bearing the carboxylic acid. In **5** the carboxylic acid has the configuration identical to that of L-proline, while in **6** the carboxylic acid has the configuration identical to that of D-proline. Thus, **5** is a constrained, *cis*-Xxx-L-Pro dipeptide, while **6** is a constrained, *cis*-Xxx-D-Pro dipeptide. Another attractive feature of the synthesis detailed below is that it generates a separable 1:1 mixture of derivatives of **5** and **6**.

To access these bicyclic dipeptides diethyl-2,2-pyrrolidinedicarboxylate (**7**, prepared in one step from 1,3-dibromopropane and diethylaminomalonate[7]) is first coupled to N-α-Cbz-N-β-Boc-L-aminoalanine (**8**, available commercially[8] or prepared in two steps from Cbz-Asn-OH[9])

Scheme 1



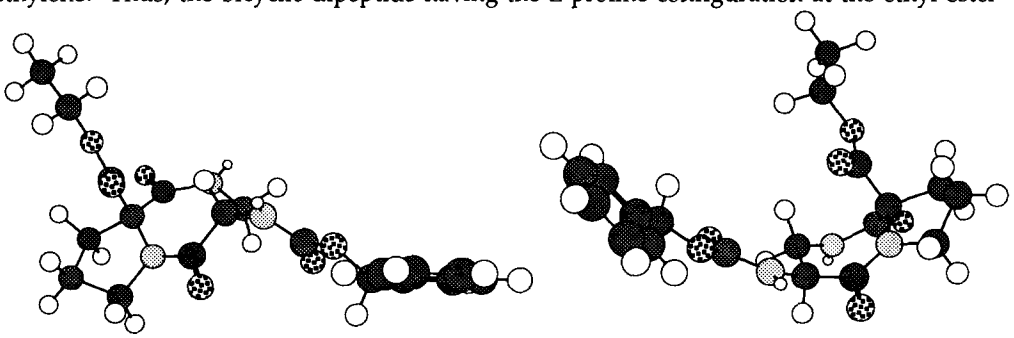
a. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, CH_2Cl_2 (54%).
b. CF_3CO_2H, CH_2Cl_2 (100%). c. Et_3N, $CHCl_3$, reflux. d. H_2, Pd-C, EtOH (c & d 88%).
e. LiOH, H_2O, MeOH (62%). f. 4-Nitrophenol, DCC, CH_2Cl_2 (70%).
g. CF_3CO_2H, CH_2Cl_2. h. Pyridine (g & h 55%).

using a water soluble carbodiimide to generate dipeptide **9** (see Scheme 1). Initial attempts to generate the 7-membered ring lactam involved treating **9** with trifluoroacetic acid in CH_2Cl_2 to remove the Boc protecting group; the resulting amine salt **10** was then dissolved in a $CHCl_3$

solution containing excess Et_3N. After stirring for 24 h at 23 °C, no loss of the **10** was detected. However, when this solution was brought to reflux, **10** was lost and a single new product, identified as diketopiperazine **11**, was formed over the course of 48 h. Identification of **11** was based on two observations. First, the Cbz NH proton in the ^1H NMR spectrum of the product appears as a triplet, indicating that the NH is adjacent to a methylene[10]; second, the *trans* orientation of the ethyl ester and methyleneamine groups was indicated by the failure of **12** (prepared from **11** by removal of the Cbz group) to cyclize in a refluxing $Et_3N/CHCl_3$ solution. Formation of **11**, which does possess a constrained *cis*-proline amide, must be initiated by transfer of the Cbz group from the α-amine to the β-amine. It is intriguing to note that the rearrangement and cyclization leading to **11** produces only one of the two possible diketopiperazine isomers.

The ready formation of **11** indicated that to obtain the desired 5,7 ring system, the cyclization reaction would have to be performed under milder conditions. To accomplish this, **9** was treated with one equivalent of LiOH to effect a monohydrolysis and generate a 1:1 mixture of half esters **13** and **14**. Esterification of this mixture with DCC and 4-nitrophenol then afforded a 1:1 mixture of diesters **15** and **16**. Treatment of the **15** and **16** mixture with CF_3CO_2H in CH_2Cl_2, followed by addition of the resulting amine salts to anhydrous pyridine at 23 °C led to the formation of a 1:1 mixture of the isomeric bicyclic dipeptides **17** and **18**, which could be separated by flash chromatography.[11]

The two isomers **17** and **18** display distinctly different ^1H NMR spectra[11] which facilitated their assignment. For one of the isomers the lactam NH proton appears as a doublet, indicating that the dihedral angle between the NH and one of the adjacent methylene protons is close to 90°. For the other isomer the lactam NH appears as a doublet of doublets, indicating that neither of the dihedral angles between the NH and the adjacent methylene protons is near 90°. Inspection of Dreiding models of both **17** and **18** shows that, of the two isomers, only **17** can assume a conformation having a nearly 90° dihedral angle between the NH and the adjacent methylene. Thus, the bicyclic dipeptide having the L-proline configuration at the ethyl ester



17 **18**

Figure 1. Low energy conformations of **17** and **18**. The carbons are darkly shaded, the nitrogens are lightly shaded, the oxygens are spotted and the hydrogens are white.

(**17**) possesses the amide NH that appears as a doublet, while the bicyclic dipeptide having the D-proline configuration at the ethyl ester (**18**) possesses the amide NH that appears as a triplet.

These assignments were confirmed by molecular modeling experiments in which low energy conformations of **17** and **18** (see Figure 1) were obtained using an MM2 energy minimization.[12] In the low energy conformation obtained for **17**, the calculated dihedral angles between the lactam NH and the adjacent methylene protons are -86° and +28°. For the low energy conformation obtained for **18**, the calculated dihedral angles are -116° and -3°. Of the two isomers, only **17** shows a dihedral angle (-86°) close to 90°, in agreement with both the NMR and Dreiding model data.

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10. **11**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34 (5H, s), 6.90 (1H, s, amide NH), 5.50 (1H, t, J=6.2 Hz), 5.10 (2H, 2d, J=12 Hz), 4.27 (2H, m), 4.17 (1H, t, J=3.6 Hz), 3.81 (1H, m), 3.69 (1H, m), 3.64 (2H, m), 2.61 (1H, m), 2.44 (1H, m), 1.94 (2H, m), 1.30 (3H, t, J=7.0 Hz). MS (CI, CH_4) 430 $(M+C_3H_5)^+$, 418 $(M+C_2H_5)^+$, 390 $(M+H)^+$, 346 $(M-NHCO)^+$. TLC (2:1 EtOAc/hexane) R_f=0.26.
11. **17** and **18** are separable by flash chromatography (3:1 EtOAc/hexane). **17**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (5H, s), 6.20 (1H, d, J=4.4 Hz), 6.11 (1H, d, J=4.9 Hz), 5.10 (2H, 2d, J=13 Hz), 4.57 (1H, m), 4.34 (2H, m), 3.85 (1H, m), 3.70 (1H, m), 3.58 (1H, m), 3.28 (1H, m), 3.01 (1H, m), 2.41 (1H, m), 1.87 (2H, m), 1.32 (3H, t, J=7.3 Hz). MS (CI, CH_4) 418 $(M+C_2H_5)^+$, 390 $(M+H)^+$, 346 $(M-NHCO)^+$. TLC (3:1 EtOAc/hexane) R_f=0.30.
18: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (5H, s), 6.37 (1H, dd, J=6.4, 5.9 Hz), 5.66 (1H, d, J=3.4 Hz), 5.11 (2H, 2d, J=12 Hz), 4.30 (2H, m), 4.23 (1H, m), 3.88 (1H, m), 3.72 (2H, m), 3.16 (1H, m), 3.00 (1H, m), 2.24 (1H, m), 1.84 (2H, m), 1.30 (3H, t, J=7.3 Hz). MS (CI, CH_4) 418 $(M+C_2H_5)^+$, 390 $(M+H)^+$, 346 $(M-NHCO)^+$. TLC (3:1 EtOAc/hexane) R_f=0.40.
12. Energy minimizations were performed using Chem3D™ 3.1.2 on a Macintosh LC II computer equipped with FPU simulation software.

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