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

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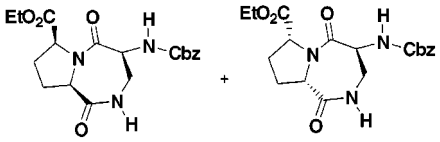
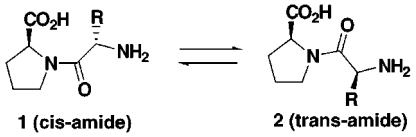
## ABSTRACT



**A short synthesis that generates two isomeric bicyclic dipeptides having constrained, *trans*-proline amide bonds has been developed. One of these bicyclic dipeptides corresponds to an Xxx-L-Pro dipeptide (4), while the other isomer corresponds to an Xxx-D-Pro dipeptide (5). The two isomers are readily distinguished by their $^1$H NMR spectra.**

Owing to the stability obtained from $\pi$-bonding between the N, C, and O atoms, amides only assume two conformations that differ in orientation by 180°. The limited conformational freedom of amides is an important part of protein structure. Under normal circumstances most amide bonds in proteins adopt only one of the two possible orientations, the *trans*-amide conformation. The one exception to this are proline amide bonds, which are known to assume either the *cis*- (**1**) or *trans-amide* (**2**) conformation.[1] Since proline amides



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

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 signaling,[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 biochemical events involving proline would be peptides that constrain proline to either the *cis*- or *trans*-amide conformation. In previous work we have detailed a short synthesis that generates constrained *cis*-proline dipeptides.[6] Here, a short synthesis that generates the corresponding *trans*-proline dipeptides is presented.

(1) Grathwohl, C.; Wuthrich, K. *Biopolymers* **1981**, *20*, 2623.

(2) (a) Brandts, J. F.; Halvorson, H. R.; Brennan, M. *Biochemistry* **1975**, *14*, 953. For recent references, see: (b) Borden, K. L.; Richards, F. M. *Biochemistry* **1990**, *29*, 3071. (c) Texter, F. L.; Spencer, D. B.; Rosenstein, R.; Matthews, C. R. *Biochemistry* **1992**, *31*, 5687. (d) Shalongo, W.; Jagannadham, M. V.; Heid, P.; Stellwegen, E. *Biochemistry* **1992**, *31*, 11390. (e) Lang, K.; Schmid, F. X. *J. Mol. Biol.* **1990**, *212*, 185. (f) Kiefhaber, T.; Kohler, H. H.; Schmid, F. X. *J. Mol. Biol.* **1992**, *224*, 217. (g) Kiefhaber, T.; Kohler, H. H.; Schmid, F. X. *J. Mol. Biol.* **1992**, *224*, 231.

(3) (a) Brandl, C. J.; Deber, C. M. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 917. (b) Williams, K. A.; Deber, C. M. *Biochemistry* **1991**, *30*, 8919. (c) Vogel, H.; Nilsson, L.; Rigler, R.; Meder, S.; Boheim, G.; Beck, W.; Kurth, H. H.; Jung, G. *Eur. J. Biochem.* **1993**, *212*, 305. (d) Wess, J.; Nanavati, S.; Vogel, Z.; Maggio, R. *EMBO J.* **1993**, *12*, 331. (e) Suchyna, T. M.; Xu, L. X.; Gao, F.; Fourtner, C. R.; Nicholson, B. J. *Nature* **1993**, *365*, 847.

(4) Richards, N. G. J.; Hinds, M. G.; Brennand, D. M.; Glennie, M. J.; Welsh, J. M.; Robinson, J. A. *Biochem. Pharm.* **1990**,*40*, 119.

(5) (a) Yaron, A.; Naider, F. *Crit. Rev. Biochem. Mol. Biol.* **1993**, *28*, 31. (b) London, R. E.; Stewart, J. M.; Cann, J. R. *Biochem. Pharm.* **1990**, *40*, 41. (b) Yaron, A. *Biopolymers* **1987**, *26*, S215.

(6) Curran, T. P.; McEnaney, P. M. *Tetrahedron Lett.* **1995**, *36*, 191.

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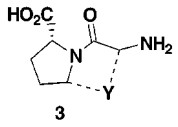
The obvious approach for constraining an Xxx-Pro dipeptide to the *trans*-amide conformation is to tether the δ-carbon of the pyrrolidine ring of proline to the α-carbon on the preceding amino acid using a linker Y, as shown in general structure **3**. A number of species that correspond to general



**3**

structure **3** have been prepared and described in recent years, and they are shown in Figure 1.[7] The target molecules in the present work are **4** and **5**, which employ a lactam methylene (CO-NH-$CH_2$) linker. Two attractive features of this linker are that the lactam is easy to assemble and that it limits the conformational mobility of the bicyclic ring system. The two isomers **4** and **5** significantly differ in their configuration at the carbon bearing the carboxylic acid. In **4** the carboxylic acid has the configuration identical to that of L-proline, while in **5** the carboxylic acid has the configuration identical to that of D-proline. Thus, **4** is a constrained, *trans*-Xxx-L-Pro dipeptide, while **5** is a constrained, *trans*-Xxx-D-Pro dipeptide. Another attractive feature of the synthesis detailed below is that it generates an easily separable mixture of derivatives of **4** and **5**.

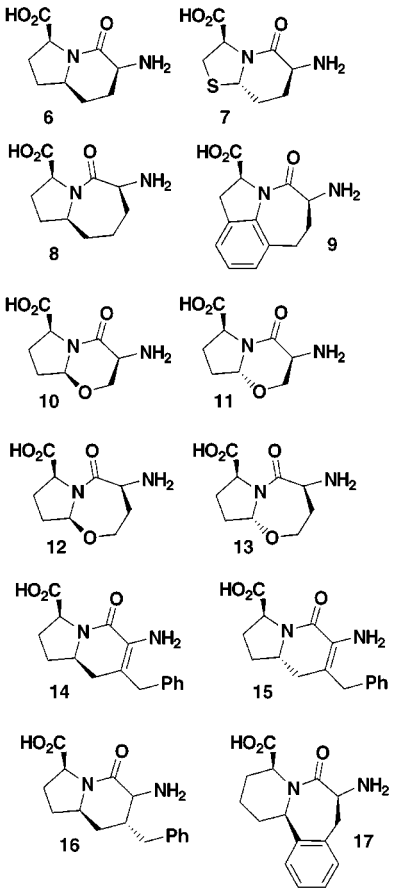
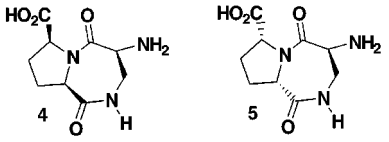
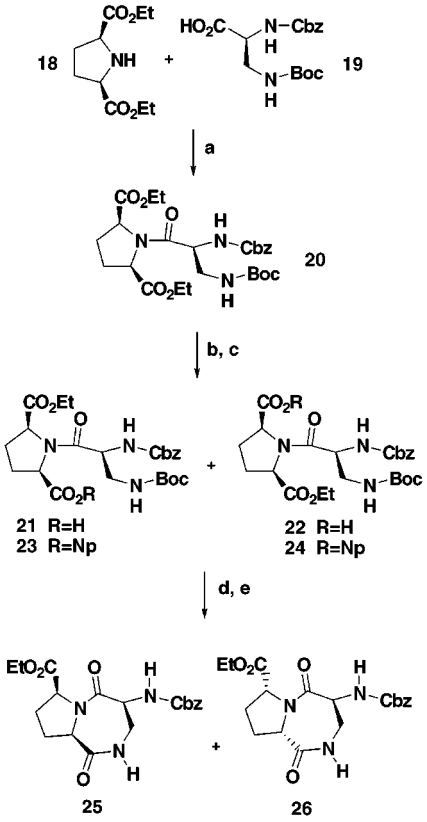


**Figure 1.** Bicyclic dipeptides conforming to general structure **3**.



The preparation of **4** and **5** is outlined in Scheme 1. Coupling of *cis*-2,5-dicarbethoxypyrrolidine (**18**, obtained in two steps from diethyl *meso*-1,4-dibromoadipate[8]) with *N*-α-Cbz-*N*-β-Boc-L-aminoalanine (**19**, obtained either by addition

**Scheme 1**[a]



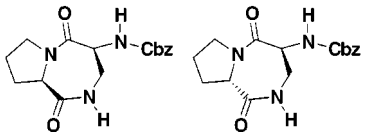
[a] (a) EDC, $CH_2Cl_2$ (95%); (b) LiOH, $H_2O$, EtOH (78%); (c) 4-nitrophenol, DCC, $CH_2Cl_2$ (54%); (d) $CF_3CO_2H$, $CH_2Cl_2$; (e) pyridine (d and e: 44% **25**, 8% **26**).

of the Boc group to commercially available *N*-α-Cbz-L-aminoalanine[9] or by a facile two-step synthesis starting from Cbz-Asn-OH[10]) yields dipeptide **20**. Treatment of **20** with 1 equiv of LiOH leads to hydrolysis of one of the two esters to generate a mixture of half-esters **21** and **22**. Esterification of this mixture with DCC and 4-nitrophenol afforded an inseparable mixture of diesters **23** and **24**. Last, treatment of the mixture of **23** and **24** 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 5.5:1 mixture of the isomeric, bicyclic dipeptides **25** and **26**. The abundance of **25** relative to **26** must arise during the treatment of **20** with LiOH. Either one of the esters reacts more quickly than the other because of assistance from the neighboring aminoalanine residue or reaction at one of the esters is hindered

by the neighboring aminoalanine. In either case, the chiral aminoalanine must exert an influence on the course of the reaction. The two bicyclic dipeptides **25** and **26** were easily separated and purified by flash chromatography.[11]

The two isomers **25** and **26** were readily distinguished by the appearance of the lactam methylene $CH_2$ group in their respective $^1$H NMR spectra. For the first isomer to elute off the chromatography column, the $^1$H NMR and COSY spectra showed the resonances of this $CH_2$ appearing as a doublet of doublets at 3.44 ppm and a multiplet at 3.63 ppm. The hydrogen appearing as the doublet of doublets was only coupled to its geminal partner and the neighboring methine; it was not coupled to the lactam NH. The absence of coupling here indicates that the dihedral angle between the lactam NH and one of the hydrogens on the $CH_2$ is approximately 90°. Conversely, for the second isomer to elute off the chromatography column, the $^1$H NMR and COSY spectra showed the resonances of the $CH_2$ appearing as two multiplets centered at 3.57 and 3.95 ppm. Both multiplets in the second isomer were coupled to the lactam NH. Inspection of Dreiding models of both **25** and **26** showed that only **25** could assume a conformation in which one of the hydrogens on the lactam $CH_2$ had a nearly 90° dihedral angle with the neighboring lactam NH. Thus, it was concluded that **25** was the first isomer to elute off the column, while **26** was the second isomer.

To confirm this conclusion, model compounds **27** and **28**, which could be prepared with established configurations at all chiral centers, were examined. **27**, which has the same




**27** **28**

bicyclic ring system as **25**, was prepared from **19** and D-proline methyl ester using the route outlined in Scheme 1. The $^1$H NMR and COSY spectra of **27** bear many similarities to the $^1$H NMR and COSY spectra of **25**. Like **25**, the two hydrogens of the lactam methylene $CH_2$ in **27** appear as a doublet of doublets at 3.3 ppm and a multiplet at 3.65 ppm, with the hydrogen appearing as the doublet of doublets not showing any coupling to the lactam NH. Similarly **28**, which has the same bicyclic ring system as **26**, was prepared from **19** and L-proline methyl ester. Its $^1$H NMR and COSY spectra also bear many similarities to its structural relative **26**. Like **26**, the two hydrogens of the lactam methylene $CH_2$ appear as multiplets centered at 3.6 and 3.9 ppm, and both resonances show coupling to the neighboring lactam methylene NH. The near identical appearance of the lactam methylene resonances in **25** and **27**, and in **26** and **28**, support the assignments of **25** and **26**. They also indicate that the bicyclic ring systems here have little conformational mobility and assume one defined conformation in solution. Finally, the spectroscopic data for **25**−**28** show that the appearance of the lactam methylene in the $^1$H NMR spectrum can be used to assign the stereochemistry in these bicyclic dipeptides.

Also supporting the assignments of **25** and **26** were molecular modeling experiments in which low-energy conformations of **25**−**28** were obtained using an MM2 energy minimization. In the low-energy conformations of **25** and **27**, the calculated dihedral angles for the lactam methylene $CH_2$ were −81° and 33°, and −85° and 30°, respectively. For **26** and **28**, the calculated dihedral angles for the lactam methylene $CH_2$ were −132° and −16°, and −127° and −11°, respectively. These calculated dihedral angles are in agree-

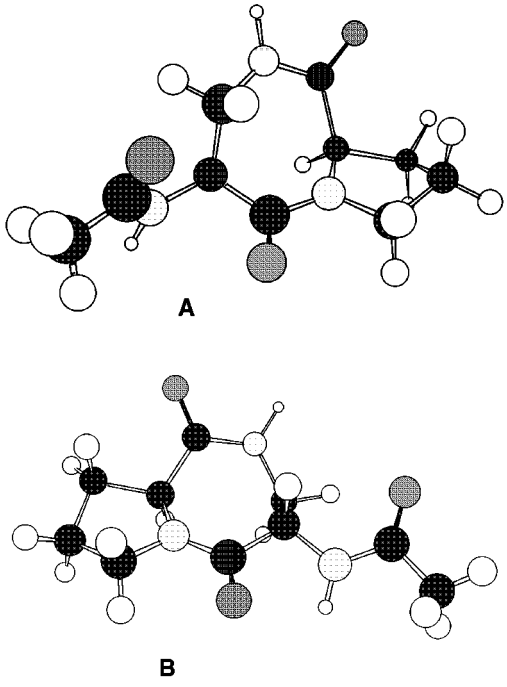



**Figure 2.** Low-energy conformations of **27** (A) and **28** (B). For clarity, the Cbz group has been truncated to an acetamide. The carbons are darkly shaded, the nitrogens are lightly spotted, the oxygens are heavily spotted, and the hydrogens are white.

(7) (a) **6**: Lombart, H.-G.; Lubell, W. D. *J. Org. Chem.* **1996**, *61*, 9437. (b) **6**: Mueller, R.; Revesz, L. *Tetrahedron Lett.* **1994**, *35*, 4091. (c) **7**: Nagai, U.; Sato, K.; Nakamura, R.; Kato, R. *Tetrahedron* **1993**, *49*, 3577. (d) **8**: Colombo, L.; Di Giacomo, M.; Papeo, G.; Carugo, O.; Scolastico, C.; Manzoni, L. *Tetrahedron Lett.* **1994**, *35*, 4031. (e) **9**: De Lombaert, S.; Blanchard, L.; Stamford, L. B.; Sperbeck, D. M.; Grim, M. D.; Jenson, T. M.; Rodriguez, H. R. *Tetrahedron Lett.* **1994**, *35*, 7513. (f) **10**−**11**: Claridge, T. D. W.; Hulme, C.; Kelly, R. J.; Lee, V.; Nash, I. A.; Schofield, C. J. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 485. (g) **10**−**11**: Slomczynska, U.; Chalmers, D. K.; Cornille, F.; Smythe, M. L.; Beusen, D. D.; Moeller, K. D.; Marshall, G. R. *J. Org. Chem.* **1996**, *61*, 1198. (h) **12**−**13**: Cornille, F.; Slomczynska, U.; Smythe, M. L.; Beusen, D. D.; Moeller, K. D.; Marshall, G. R. *J. Am. Chem. Soc.* **1995**, *117*, 909. (i) **14**−**15**: Li, W.; Hanau, C. E.; d'Avignon, A.; Moeller, K. D. *J. Org. Chem.* **1995**, *60*, 8155. (j) **16**: Hanessian, S.; Ronan, B.; Laoui, A. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1397. (k) **17**: Flynn, G. A.; Giroux, E. L.; Dage, R. C. *J. Am. Chem. Soc.* **1987**, *109*, 7914.

(8) (a) Cignarella, G.; Nathansohn, G. *J. Org. Chem.* **1961**, *26*, 1500. (b) Lowe, G.; Ridley, D. D. *J. Chem. Soc., Perkin Trans. 1* **1973**, 2024. (c) Kemp, D. S.; Curran, T. P. *J. Org. Chem.* **1988**, *53*, 5729.

(9) Available from Bachem Bioscience, Inc., Catalog no. C-3315.

(10) Waki, M.; Kitajima, Y.; Izumiya, N. *Synthesis* **1981**, 266.

ment with the $^1$H NMR coupling constant data for **25**−**28**. Figure 2 shows the low-energy conformations for **27** and **28**.

In summary, a short synthesis that generates two isomeric, constrained *trans*-proline amide dipeptides has been developed. The two isomeric peptides are easily separated by chromatography and are readily distinguished by $^1$H NMR. The spectroscopic data also indicate that these two bicyclic structures adopt well-defined conformations. The use of these constrained prolines as probes of the enzymatic activity of the protease CD26 are currently being pursued.

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(11) **25** and **26** are separable by flash chromatography (EtOAc). **25**: $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.35 (5H, s), 6.07 (1H, d, $J$ = 5.1 Hz), 6.05 (1H, d, $J$ = 5.9 Hz), 5.12 (2H, s), 4.94 (1H, m), 4.67 (2H, m), 4.18 (2H, q, $J$ = 7.1 Hz), 3.63 (1H, m), 3.44 (1H, dd, $J$ = 11.6, 11.6 Hz), 2.65 (1H, m), 2.30 (1H, m), 2.17 (1H, m), 2.06 (1H, m), 1.30 (3H, t, $J$ = 7.1 Hz). TLC (EtOAc) $R_f$ = 0.47. Anal. Calcd for $C_{19}H_{23}N_3O_6$: C, 58.60; H, 5.95; N, 10.79. Found: C, 58.26; H, 6.17; N, 10.62. **26**: $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.35 (5H, s), 6.39 (1H, s), 5.82 (1H, d, $J$ = 4.3 Hz), 5.08 (2H, dd, $J$ = 9.3, 12.1 Hz), 4.73 (2H, m), 4.15 (2H, m), 3.95 (2H, m), 3.57 (1H, m), 2.67 (1H, m), 2.13 (3H, m), 1.24 (3H, t, $J$ = 7.1 Hz). TLC (EtOAc) $R_f$ = 0.24. Anal. Calcd for $C_{19}H_{23}N_3O_6$: C, 58.60; H, 5.95; N, 10.79. Found: C, 58.48; H, 6.10; N, 10.59.