Pergamon

Tetrahedron Letters, Vol. 37, No. 12, pp. 1933-1936, 1996

0040-4039/96 $15.00 + 0.00

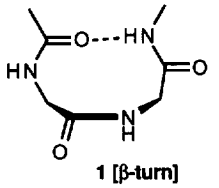
S0040-4039(96)00307-3

N-α-Benzoyl-*Cis*-4-Amino-L-Proline: A γ-Turn Mimetic

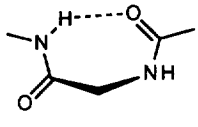
Timothy P. Curran*, Nicole M. Chandler, Robert J. Kennedy and Meghan T. Keaney
Department of Chemistry, College of the Holy Cross, Worcester, MA 01610-2395 USA

Abstract: Peptides containing N-α-benzoyl-*cis*-4-amino-L-proline are shown to adopt γ-turn conformations in chloroform solutions.

The β- (**1**) and γ- (**2**) turn secondary structures are thought to play important roles in biochemical molecular recognition. Studies have suggested that many peptide hormones,



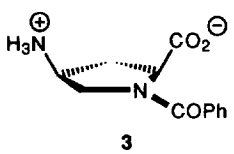
1 [β-turn]



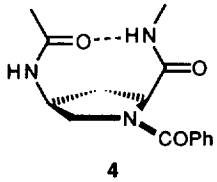
2 [γ-turn]

when bound to their protein receptors, adopt turn conformations.[1] Also, turns have been identified as sites for posttranslational modification of proteins; this is particularly true with respect to phosphorylation and glycosylation.[1] Additionally, formation of β- or γ-turns may initiate formation of β-sheets in protein folding.[2] Because of their biochemical importance a number of peptide and non-peptides that confer a β- or γ-turn conformations, called β-turn mimetics, have been described.[3,4]

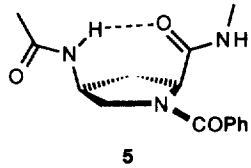
As part of another research project aimed at preparing structural variants of an α-helix template[5], N-α-benzoyl-*cis*-4-aminoproline, **3**, was prepared (see below). An obvious feature of **3** is the close proximity of the amine and carboxylic acid enforced by the pyrrolidine ring. In examining models of **3** and its derivatives, it appeared likely that were the acid converted to a secondary amide and the amine acylated then the resulting species might adopt either a β- (**4**) or γ-turn (**5**) type of conformation, depending on the location of the intramolecular hydrogen bond. To determine whether **3** directs formation of a β- or γ-turn, peptides incorporating this compound were prepared and examined.



3

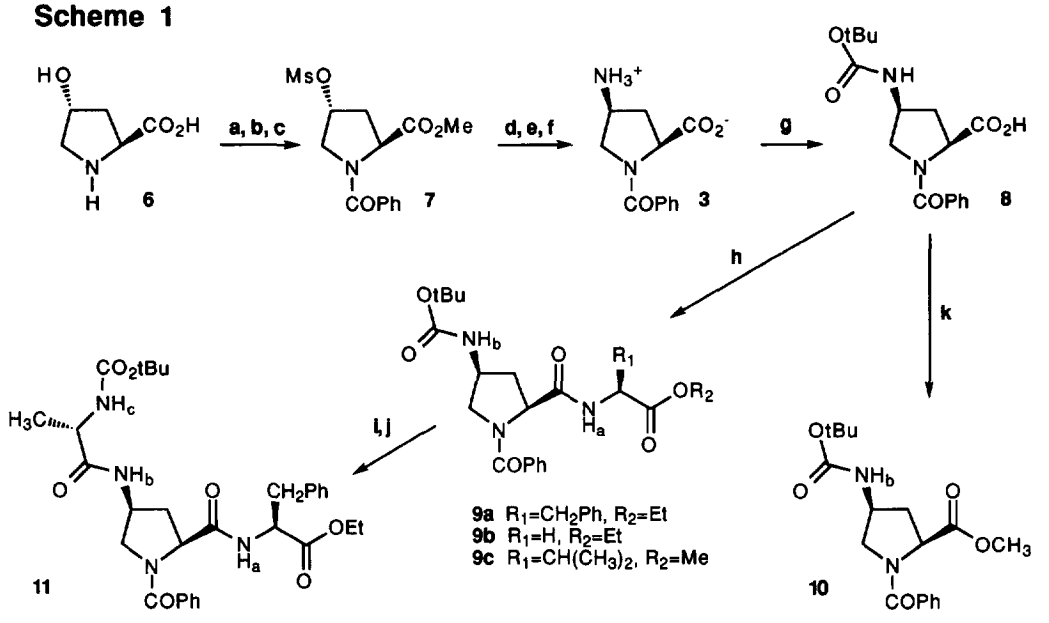


4



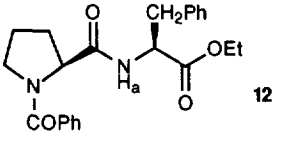
5

Scheme 1



Legend: a. $SOCl_2$/MeOH. b. PhCOCl, $NaHCO_3$, dioxane-H_2O (a & b, 82%). c. MsCl, pyridine (77%). d. NaN_3, DMF, 60°C (96%). e. LiOH, THF-H_2O (87%). f. H_2, Pd-C, MeOH (100%). g. Boc_2O, $NaHCO_3$, THF-H_2O (76%). h. H-Phe-OEt · HCl or H-Gly-OEt · HCl or H-Val-OMe · HCl, EDC, CH_2Cl_2 (50-70%). i. CF_3CO_2H, CH_2Cl_2. j. Boc-Ala-ONp, Et_3N, CH_2Cl_2 (i & j, 27%). k. CH_2N_2 (91%).

The synthesis of **3** and its derivatives (Scheme 1) follow established routes to *cis*-4-aminoprolines.[6] Starting with *trans*-4-hydroxy-L-proline (**6**), functionalization of the carboxyl, amine and alcohol groups yields mesylate **7** as a crystalline solid in an overall yield of 63%. Displacement of the mesylate with azide ion, followed by hydrolysis of the methyl ester and reduction of the azide to the amine produces **3** in high yield. Protection of the primary amine of **3** with the Boc group provides **8**, which can readily be obtained in 10-50 g amounts. Subsequent acylation of **8** with amino acid ester hydrochlorides produces dipeptides **9a-c**, while treatment of **8** with diazomethane produces **10**. Tripeptide **11** was readily obtained in two steps from **9a** by



12

removal of the Boc group and acylation of the resulting amine. Finally, dipeptide **12** (a comparison molecule) was prepared using standard peptide synthesis methods.

FT-IR and NMR spectroscopies were employed to determine whether peptide derivatives of

Table I. Spectroscopic data for NH bonds in **9a-c**, **10**, **11** and **12** in $CHCl_3$ (FT-IR) or $CDCl_3$ (NMR).

		^{1}H NMR					
	FT-IR	NH_a		NH_b		NH_c	
Compound	ν^a	δ^b	$-\Delta\delta/\Delta T^c$	δ^b	$-\Delta\delta/\Delta T^c$	δ^b	$-\Delta\delta/\Delta T^c$
9a	3421, 3371	7.64	3.4	6.07	5.6	-----	----
9b	3445, 3356	7.73	4.0	6.20	6.4	-----	----
9c	3440, 3356	7.67	3.0	6.27	5.8	-----	----
10	3440, 3387	-----	----	5.42	5.6	-----	----
11	3430, 3326	7.51	1.9	8.10	6.7	5.11	2.9
12	3421	-----	----	-----[d]	----	-----	----

[a]Peaks in the NH region of the IR. Values given for the peaks are in the units cm^{-1}.
[b]Chemical shift values given were obtained at 295K and are expressed in the units ppm.
[c]Values for the change in chemical shift with respect to temperature given in the units ppb/K.
[d] In the ^{1}H NMR spectrum of **12**, the NH_b resonance is masked by the resonances of the phenyl hydrogens.

3 (**9a-c**, **10**, **11**) assume turn conformations. First, FT-IR was used to establish whether these compounds exhibit intramolecular hydrogen bonding. Previous work has shown that amide NH protons involved in an intramolecular hydrogen bond appear as broad absorptions between 3300-3400 cm^{-1}, while amide NH protons not internally hydrogen bonded appear as sharp absorptions between 3400-3500 cm^{-1}.[4a,7] FT-IR spectra of $CHCl_3$ solutions of **9a-c**, **10**, and **11** in the region between 3200-3500 cm^{-1} were recorded, and the results are given in Table I. Compounds **9a-c** exhibit two peaks of roughly equal intensity: a broad absorbance between 3350-3380 cm^{-1} and another between 3450-3420 cm^{-1}. This suggests that one of the NH protons in **9a-c** is involved in an intramolecular hydrogen bond and the other is not. In order to locate the intramolecular hydrogen bond, both **10** and **12** were examined. With **12**, which lacks NH_b and the urethane group at the 4 position on the proline ring as compared to **9a-c**, the FT-IR spectra shows only a single peak at 3421 cm^{-1}, showing that NH_a in this compound is not involved in an intramolecular hydrogen bond. This indicates that in **9a-c** the intramolecular hydrogen bond is not between NH_a and the benzoyl carbonyl. With ester **10**, which lacks proton NH_a as compared to **9a-c**, the FT-IR spectra shows two peaks at 3440 and 3387 cm^{-1}, indicating that NH_b in this molecule is partially involved in an intramolecular hydrogen bond with the urethane carbonyl. This suggests that in **9a-c** the intramolecular hydrogen bond is between NH_b and the urethane carbonyl; formation of this hydrogen bond confers a γ-turn type conformation to these compounds as depicted in structure **5**. The FT-IR data for tripeptide **11** also is consistent with the γ-turn type conformation.

To confirm that the NH_b protons are involved in an intramolecular hydrogen bond and that the NH_a protons are not, values for the temperature dependence of the chemical shifts ($-\Delta\delta/\Delta T$) of the NH protons in **9a-c**, **10** and **11** were measured (Table 1). It is well established that amide

and urethane NH protons involved in intramolecular hydrogen bonds change their chemical shift with respect to temperature at different rates than similar protons not involved in an intramolecular hydrogen bond.[8] Although there have been a few examples to the contrary,[9] recent work from a two laboratories has shown that in $CDCl_3$ and CD_2Cl_2 $-\Delta\delta/\Delta T$ values around 3 ppb/K are indicative of NH protons not involved in intramolecular hydrogen bonds, while $-\Delta\delta/\Delta T$ values significantly higher than 3 ppb/K are indicative of NH protons involved in intramolecular hydrogen bonds.[4a,7] As the data in Table 1 shows, for **9a-c** $-\Delta\delta/\Delta T$ values for NH_b indicate that this proton is involved in an intramolecular hydrogen bond, while $-\Delta\delta/\Delta T$ values for NH_a indicate that this proton is not involved in an intramolecular hydrogen bond. Likewise, the data for **10** indicates that NH_b in this compound is also involved in an intramolecular hydrogen bond. For tripeptide **11** the $-\Delta\delta/\Delta T$ data indicate that NH_b in this compound is involved in an intramolecular hydrogen bond, while NH_a and NH_c are not. That NH_b in **11** is involved in an intramolecular hydrogen bond is also indicated by the deshielding of this proton as exhibited by its chemical shift (8.10 ppm). Overall, the $-\Delta\delta/\Delta T$ data for **9a-c**, **10** and **11** are consistent with the conclusion that these compounds adopt the γ-turn type conformation shown in structure **5**.

Taken together, the FT-IR and NMR data in $CHCl_3/CDCl_3$ indicate that **3** serves as a template for formation of a γ-turn in the compounds examined. Further studies to explore the conformational behavior of **3** and to modify its structure in order to gain a β-turn mimetic are in progress.

Acknowledgments. Acknowledgment is made to the Donors of The Petroleum Research Fund, administered by the American Chemical Society, for the support of this research (ACS-PRF # 25517-GB). The NMR spectrometer used for the experiments was purchased with a grant from the National Science Foundation (NSF-ILI USE-8852774).

References and Notes.

1. Smith, J.A.; Pease, L.G. *CRC Crit. Rev. Biochem.* **1980**, *8*, 315.
2. Rose, G.D.; Gierasch, L.G.; Smith, J.A. *Adv. Prot. Chem.* **1985**, *37*, 1.
3. For reviews see: (a) Ball, J.B.; Alewood, P.F. *J. Mol. Recogn.* **1990**, *3*, 55. (b) Holzemann, G. *Kontakte (Darmstadt)* **1991**, 3. (c) Holzemann, G. *Kontakte (Darmstadt)* **1991**, 55. (d) Rizo, J.; Gierasch, L.M. *Ann. Rev. Biochem.* **1992**, *61*, 387. (e) Morgan, B.A.; Gainor, J.A. *Ann. Rep. Med. Chem.* **1989**, *24*, 243.
4. For recent work see: (a) Tsang, K.Y.; Diaz, H.; Graciani, N.; Kelly, J.W. *J. Am. Chem. Soc.* **1994**, *116*, 3988. (b) Hermkens, P.H.H.; v. Dinther, T.G.; Joukema, C.W.; Wagenaars, G.N.; Ottenheijm, H.C.J. *Tetrahedron Lett.* **1994**, *35*, 9271. (c) Gardner, B.; Nakanishi, H.; Kahn, M. *Tetrahedron* **1993**, *49*, 3433. (d) Callahan, J.F.; Newlander, K.A.; Burgess, J.L.; Eggleston, D.S.; Nichols, A.; Wong, A.; Huffman, W.F. *Tetrahedron* **1993**, *49*, Johnston, M.E. *Tetrahedron* **1993**, *49*, 3489. (e) Nagai, U.; Sato, K.; Nakamura, R.; Kato, R. *Tetrahedron* **1993**, *49*, 3577. (f) Ripka, W.C.; De Lucca, G.V.; Bach, A.C. II; Pottorf, R.S.; Blaney, J.M. *Tetrahedron* **1993**, *49*, 3593, 3609. (g) Nowick, J.S.; Powell, N.A.; Martinez, E.J.; Smith, E.M.; Noronha, G. *J. Org. Chem.* **1992**, *57*, 3763. (h) Kemp, D.S.; Li, Z.Q. *Tetrahedron Lett.* **1995**, *36*, 4175. (i) Kemp, D.S.; Li, Z.Q. *Tetrahedron Lett.* **1995**, *36*, 4179.
5. (a) Kemp, D.S.; Curran, T.P.; Davis, W.M.; Boyd, J.G.; Muendel, C. *J. Org. Chem.* **1991**, *56*, 6672. (b) Kemp, D.S.; Curran, T.P.; Boyd, J.G.; Allen, T.J. *J. Org. Chem.* **1991**, *56*, 6683. (c) Kemp, D.S.; Boyd, J.G.; Muendel, C. C. *Nature* **1991**, *352*, 451.
6. (a) Abraham, D.J.; Mokotoff, M.; Sheh, L.; Simmons, J.E. *J. Med. Chem.* **1983**, *26*, 549. (b) Webb, T.R.; Eigenbrot, C. *J. Org. Chem.* **1991**, *56*, 3009.
7. Gellman, S.H.; Dado, G.P.; Liang, G.-B.; Adams, B.R. *J. Am. Chem. Soc.* **1991**, *113*, 1164.
8. Kessler, H. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 512.
9. See reference 5b.

(*Received in USA* 13 *July* 1995; *revised* 29 *December* 1995; *accepted* 2 *January* 1996)