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Synthesis of (3*R*)-Carboxy Pyrrolidine (a β-Proline Analogue) and its Oligomer

Yong Jip Kim,[a] Donald A. Kaiser,[b] Thomas D. Pollard[b] and Yoshitaka Ichikawa[a,*]

[a]*Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA*
[b]*Structural Biology Laboratory, Salk Institute for Biological Studies, La Jolla, CA 92037, USA*

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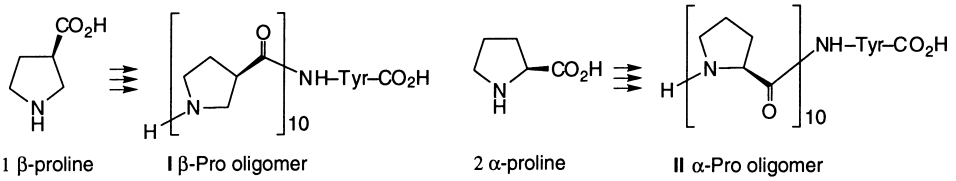
Abstract—A decamer of a β-amino acid analogue of L-proline, (3*R*)-carboxy pyrrolidine (β-proline), was synthesized from a readily available (*R*)-glycidol. It was found to possess a rigid secondary structure, as evidenced by its CD spectrum. The β-proline decamer, however, failed to bind to profilin, whereas the corresponding α-L-proline decamer bound tightly to this protein. © 2000 Published by Elsevier Science Ltd.

Since naturally occurring peptides are prone to being hydrolyzed by cellular proteases, their usefulness as therapeutic agents is very limited. Therefore, a new class of peptide analogues has been actively studied with the expectation that they would be resistant to proteolytic degradation and yet retain biological activity similar to that of their natural counterparts.[1] β-Amino acids possess an additional methylene unit between the amino and carboxyl groups, and their oligomers have been found to form an α-helical-like conformation, the L + 2 helix, as evidenced by NMR and X-ray crystallographic studies.[2–4] However, only a few of these designed oligomers have yet been shown to have biological activities similar to those of the naturally occurring peptides composed of α-amino acids.[5–7]

In the course of our ongoing research program of developing a new class of peptide analogues, we decided to simply replace the α-amino acid of a peptide with the corresponding β-amino acid and to examine its conformation and biological activity, and we chose a proline oligomer as our target peptide.

Yuki and co-workers have previously prepared polymers of β-proline analogue (3-carboxy pyrrolidine) and indicated that these polymers exist in random conformations.[8] Seebach et al. have prepared oligomers of β^2- and β^3-homoprolines (2-carboxymethyl- and 2-carboxyethyl-pyrrolidine, respectively) and suggested that they form rigid secondary structures.[9] Recently, Gellman et al. have reported that oligomers of a β-proline analogue, (3*S*)-carboxy pyrrolidine, form a rigid secondary structure, as indicated by their CD spectra.[10] The report from Gellman's group has prompted us to report our preliminary work on the synthesis and biological evaluation of an oligomer of a different β-proline analogue, (3*R*)-carboxy pyrrolidine **1**, which is an enantiomer of Gellman's compound. In this communication, we will describe a facile synthesis of **1** and the biological evaluation of its decamer I as a potential ligand of profilin.

Profilin interacts with a variety of biological molecules, including actin monomers and poly-proline rich sequences of proteins, and it plays a key role in regulating the



*Corresponding author. Tel.: +1-410-614-2892; fax: +1-410-955-3023; e-mail: ichikawa@jhmi.edu

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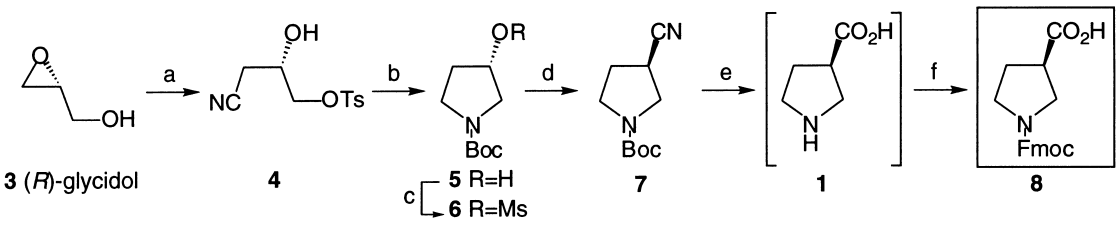

Scheme 1. Reagents and conditions: (a) ref 18; (b) (1) H_2/Raney® Ni/EtOH; (2) Boc_2O/Et_3N/MeOH (70% in two steps); (c) $MsCl/Et_3N/CH_2Cl_2$ (93%); (d) LiCN/DMF (84%); (e) concd HCl; (f) $FmocCl/Na_2CO_3/H_2O$–dioxane (72% in two steps).

dynamics of actin polymerization, which is responsible for many motile process in live cells.[11] Although the physiological significance of profilin binding to poly-proline is still unknown, the binding site on profilin was found to lie in a shallow groove between the N- and C-terminal helices and the underlying β-sheet.[12] In water, poly-proline forms a left-handed helix with a *trans*-peptide bond and three residues per turn (a type II helix), whereas in organic solvents it is known to form a right-handed *cis* helix (type I).[13] A recent X-ray crystallographic study of profilin complexed with an L-α-proline decamer has shown that the binding is due to specific hydrogen bonding between the amido carbonyl group of the proline oligomer and the NH group of profilin as well as the hydrophobic interaction between the pyrrolidine framework of α-L-prolines and the tryptophane residues of profilin.[14] We therefore planned to synthesize decamers of a β-proline analogue, (3*R*)-carboxyl pyrrolidine, as a β-peptide and of α-proline as a control and to evaluate the binding affinities of these decamers for a recombinant amoeba profilin in order to examine the possibility that β-peptide can substitute for the natural peptide.

Synthesis of β-Proline: (3*R*)-Carboxy Pyrrolidine

Although several synthetic procedures have been reported for the preparation of the optically active β-proline analogue 3-carboxylic pyrrolidine: by optical resolution of the racemic compounds,[8] from *trans* (4*R*)-hydroxy-L-proline[15] as used by Gellman et al.,[9] from L-aspartic acid[16] or by microbial optical resolution of a cyclobutenone derivative,[17] we decided to develop a new and facile synthetic route because we were interested in oligomers of a β-proline analogue with (3*R*) stereochemistry (see **1** and **2**).

Our synthesis of **1** started with a commercially available (*R*)-glycidol **3** (Scheme 1). According to the Sharpless procedure,[18] **3** was converted to the 3-nitrile derivative **4**, which was then reductively cyclized[19] to form a pyrrolidine derivative **5**.[20] The 3-OH group of **5** was mesylated and subsequently displaced with a nitrile group to give **7**.[20] The nitrile group of **7** was hydrolyzed with concentrated HCl to give **1**, which was isolated as the N-Fmoc derivative **8**.[20] Oligomers of **1** and **2** were prepared by standard solid-phase Fmoc chemistry,[21] and the L-tyrosine residue was incorporated into the C-terminus of each peptide for future tracer experiments.

Binding Study of Profilin and the Decamers I and II

Because the binding of poly(α-L-proline) enhances the intrinsic tryptophan fluorescence of profilin, we used fluorescence spectroscopy to evaluate the affinity of the decamers **I** and **II** for the purified *Acanthamoeba* profilin-I.[22] As expected, the decamer of α-proline **II** showed an increased fluorescence (excitation wavelength = 295 nm; emission wavelength = 300–380 nm) that is consistent with previous observations of the L-proline decamer. However, no significant increase in fluorescence was observed when the decamer of β-proline I was mixed

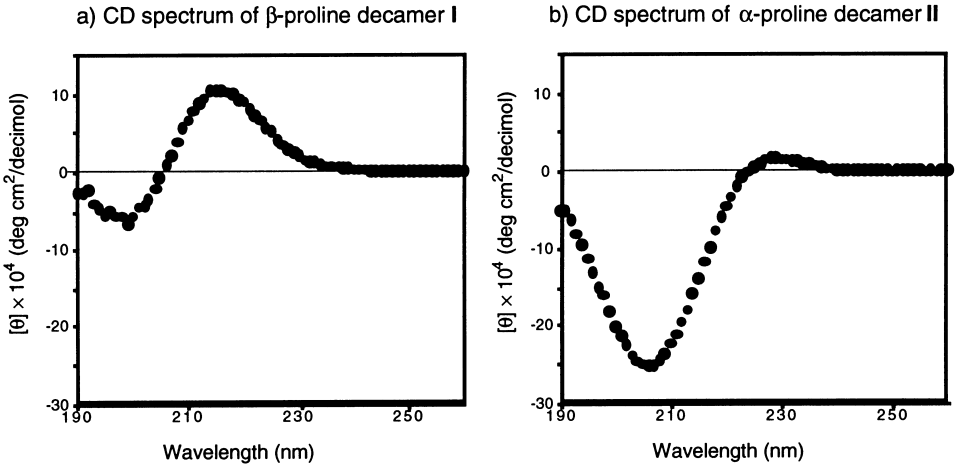

Figure 1. CD spectral data of decamers I and II (0.5 mM) in 10 mM potassium phosphate buffer (pH 7.0).

with profilin at concentrations as high as 100 mM, whereas the maximum fluorescence of profilin was achieved in the presence of α-proline decamer **II** at 318 nM.

CD Spectra of Decamers I and II

The decamer of α-L-proline **II** showed a CD spectrum typical of a type II poly (L-proline) helix, with a small positive band at 228 nm and a large negative band at 208 nm, in 10 mM potassium phosphate buffer (pH 7.0); these values are in good agreement with the reported[22] figures (Fig. 1b). In contrast, the other decamer of β-proline **I** had a large positive band at 215 nm and a negative band at 198 nm in the same buffer solution (Fig. 1a), yielding a curve opposite to that reported by Gellman et al. for the enantiomer of **1**,[10] indicating that the β-proline decamer **I** may have a rigid, ordered conformation.[23]

Summary

We have developed an efficient synthetic route for a β-proline analogue, (3*R*)-carboxy pyrrolidine, from an optically active (*R*)-glycidol employing a CN as a facile source of aminomethyl and carboxylate groups. The resulting decameric peptide indicated a rigid secondary structure based on its CD spectrum; however, it failed to bind to profilin, which shows a tight hydrogen bonding to the amido backbone of the α-proline decamer **II**.

Acknowledgements

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20. Compound **5**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.31 (s, 9H), 1.80 (br, 2H), 3.15–3.38 (m, 4H), 4.21 (br d, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.2, 33.1, 33.6, 43.3, 53.7, 53.9, 69.4, 70.2, 79.1, 154.6; HRMS FAB calcd for $C_9H_{17}NO_3$ $(M+H)^+$ 188.1287, found 188.1288. Coumpound **6**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.34 (s, 9H), 2.05 (br, 2H), 2.94 (s, 3H), 3.32–3.54 (m, 4H), 5.13 (br, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.3, 31.5, 32.4, 38.4, 43.1, 43. 5, 51.6, 52.0, 79.5, 80.1, 154.5; HRMS FAB calcd for $C_{10}H_{20}NO_5S$ $(M+H)^+$ 266.1062, found 266.1060. Compound **7**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 (s, 9H), 2.06–2.26 (m, 2H), 3.06 (dt, $J=6.6$, 13.5 Hz), 3.32–3.64 (m, 4H), 5.13 (br, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.4, 28.2, 29.1, 29.9, 44.2, 48.6, 79.9, 119.8, 153.6; HRMS FAB calcd for $C_{10}H_{17}N_2O_2$ $(M+H)^+$ 197.1290, found 197.1289. Compound **8**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.23 (m, 2H), 3.15 (dt, $J=7.2$, 14.1 Hz), 3.48–3.58 (m, 2H), 3.72 (d, 2H, $J=6.9$ Hz), 4.23–4.27 (m, 1H), 4.33–4.43 (m, 2H), 7.31 (dd, 2H, $J=7.2$, 9.0 Hz), 7.40 (dd, 2H, $J=7.5$ Hz), 7.60 (d, 2H, $J=7.5$ Hz), 7.76 (d, 2H, $J=7.5$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 47.4, 61.3, 65.95, 65.99, 67.5, 99.0, 119.9, 125.0, 127.0, 127.6, 141.3, 144.0, 154.9, 177.3; HRMS FAB calcd for $C_{20}H_{20}NO_4$ $(M+H)^+$ 338.1392, found 338.1392.
21. Both peptides **I** and **II** were prepared by the Core Facility at Johns Hopkins University School of Medicine using a standard Fmoc chemistry on a solid phase and were HPLC-purified (>98%).
22. The buffer solution for intrinsic fluorescence assay contained: 5 μM profilin-1, 75 mM KCl, 10 mM Tris, pH 7.5, 1 mM NaN_3; for the detailed experimental procedure, see Petrella, E. C.; Machesky, L. M.; Kaiser, D. A.; Pollard, T. D. *Biochemistry* **1996**, *35*, 16535.
23. Further study of the conformation by NMR is in progress in order to determine the conformational similarity or difference between the oligomers of β-proline **I** and the corresponding α-proline counterpart **II**.