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CHEMOENZYMATIC SYNTHESIS AND INCORPORATION OF L-2-QUINOXALYLALANINE INTO A TANDEM β-TURN PEPTIDE MOTIF

Xiaofen Huang and Eric C. Long*

Department of Chemistry, Indiana University Purdue University-Indianapolis (IUPUI)
402 North Blackford Street, Indianapolis, Indiana 46202

Abstract: L-2-Quinoxalylalanine was synthesized chemoenzymatically and incorporated through solid-phase peptide synthesis at both termini of one repeat unit of the carboxy-terminal domain (CTD) of RNA polymerase II. The resulting structure closely resembles members of the quinoxaline class of bis-intercalating antitumor antibiotics and may eventually assist in probing the DNA binding mechanism of the CTD.

The carboxy-terminal domain (CTD) of RNA polymerase II contains a unique, repeating heptad motif based on -(Ser-Pro-Thr-Ser-Pro-Ser-Tyr)$_n$- that occurs up to 52 times in mammals.[1] While the specific function of this domain is unclear, several proposed functions[2] include its involvement in transcriptional initiation, elongation, post-transcriptional RNA processing, nuclear localization, and DNA binding.[3] Among these functions, the act of DNA association has been suggested as a means to alter the conformation of the CTD to create a more efficient substrate for the kinases involved in the phosphorylation of this motif *in vivo*.[4]

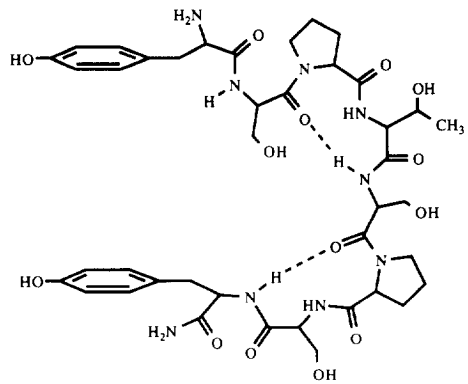


Figure 1. The proposed structure of Tyr-Ser-Pro-Thr-Ser-Pro-Ser-Tyr (**1**)

The ability of the CTD and its individual repeat units to bind DNA is possibly related to its unique structure; previous NMR studies[5] of one repeat of the CTD, containing an additional amino-terminal tyrosine (Tyr-Ser-Pro-Thr-Ser-Pro-Ser-Tyr, **1**), determined that the peptide contains two overlapping β-turns within each of the two Ser-Pro-Xaa-Xaa sequences found within one repeat unit (Figure 1). This structure thus permits a parallel

alignment of the flanking tyrosine residues of contiguous repeat units with a gap distance of ~ 10.2 Å, a structure seemingly poised for the simultaneous insertion of each aromatic side chain between the stacked Watson-Crick base pairs of the DNA helix in a bis-intercalative fashion. Indeed, it has been noted[3] that the presence of tandem β-turns allows an individual motif to quite closely resemble known DNA bis-intercalators of the quinoxaline family[6] of antitumor nautral products (e.g., echinomycin and triostin A). Further, in support of this form of peptide-DNA association, biophysical and hydrodynamic investigations of DNA binding by these individual motifs have indicated that both aromatic residues partially stack between the DNA base pairs.[3a,7]

Given the structural similarities that exist between the individual repeat units of the CTD and members of the quinoxaline family of antitumor agents, our laboratory has sought to determine if a "hybrid" of the CTD repeat unit could be created that contains elements inherent to both the native peptide motif and the quinoxaline antibiotics. Such a conjugate may eventually facilitate investigations of the interaction of the CTD with its DNA (or enzyme) substrate along with potentially creating a new class of DNA bisintercalators with unique site selectivities. Accordingly, we have chosen to replace the tyrosine residues of the native sequence with L-2-quinoxalylalanine. Described herein is the chemoenzymatic synthesis of this amino acid and its incorporation, using solid phase peptide synthesis, into the tandem β-turn system found in the CTD of RNA polymerase II.

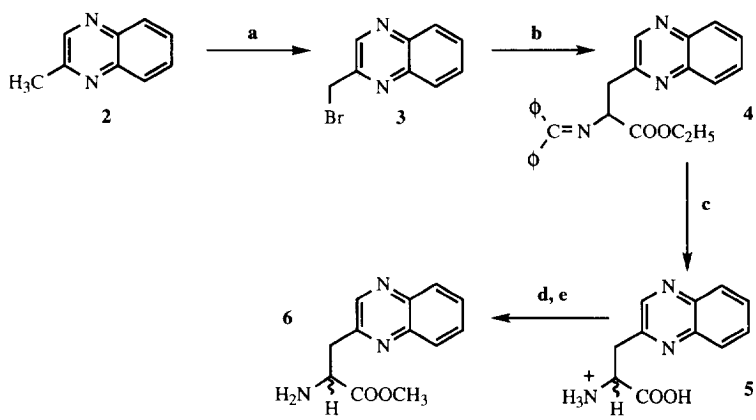


Figure 2. Synthesis of racemic L-2-quinoxalylalanine. (a) 1,3 dibromo-5,5-dimethylhydantoin, benzoyl peroxide, hv, CCl_4, reflux 3 hrs; (b) N-(diphenylmethylene)glycine ethyl ester, NaOH, tetrabutylammonium bromide, CH_2Cl_2, 8 hrs; (c) 6 N HCl, 6 hrs; (d) MeOH, HCl gas; (e) $NaHCO_3/CHCl_3$ extraction.

Initially, 2-bromomethylquinoxaline (**3**) was prepared as described[8] from 1,3-dibromo-5,5-dimethylhydantoin (6.76 g, 23.6 mmol) and 2-methylquinoxaline (**2**, 6.15 g, 43.0 mmol) in the presence of benzoyl peroxide (0.93 g, 3.8 mmol) and hv (150W spotlight) in 250 mL of CCl_4 (Figure 2). Employing 2-bromomethylquinoxaline (**3**) as an electrophile (5.78 g, 26.0 mmol), the Schiff base protected glycine equivalent N-(diphenylmethylene)glycine ethyl ester (5.77 g, 21.6 mmol) was alkylated under phase transfer conditions (43 mL of 45% NaOH, 220 mL CH_2Cl_2, 1.39 g, 4.3 mmol tetrabutylammonium bromide) to yield compound **4** (86% yield).[9] This Schiff base protected, racemic α-amino acid was purified by a combination of flash chromatography (1:3, v/v, ethyl acetate/hexanes, 200 - 400 mesh silica, Aldrich) and crystallization (1:3, v/v,

ethyl acetate/hexanes). Upon purification, **4** (7.17 g, 17.5 mmol) was hydrolyzed in 6 N HCl (250 mL) to yield free amino acid **5** which, without further purification, was esterified[10] (**6**) and crystallized (54% yield from **4**).

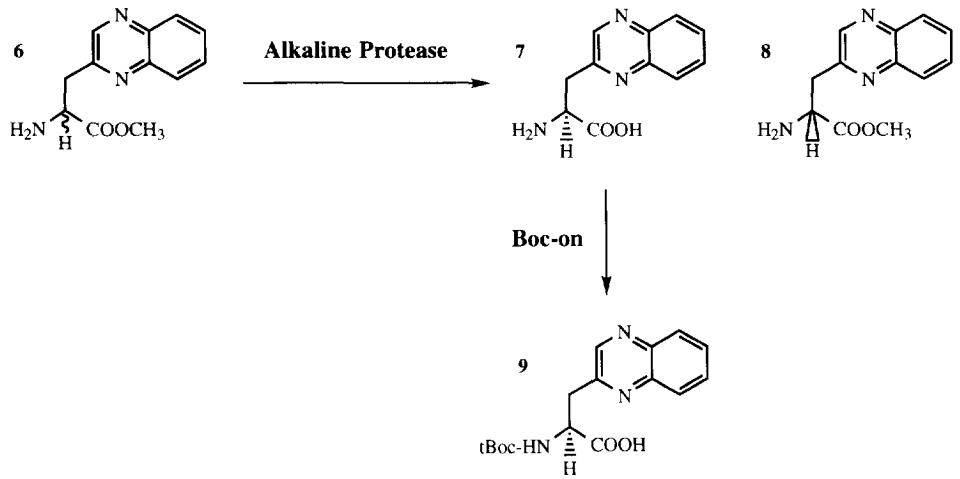
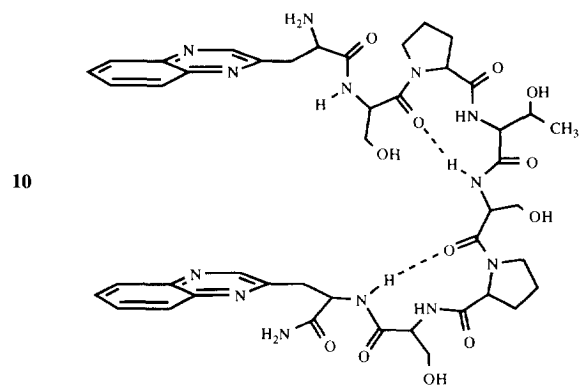


Figure 3. Kinetic resolution of racemic L-2-quinoxalylalanine using alkaline protease.

Following esterification, the racemic amino acid (2.4 g, 10.4 mmol) was kinetically resolved (Figure 3) using alkaline protease[11] (37 mg, 250 - 600 units, Sigma type VIII bacterial protease from bacillus licheniformis) for approximately 1.5 hours in 0.2 N $NaHCO_3$ (150 mL) to produce L-2-quinoxalylalanine (51% yield) and D-2-quinoxalylalanine methyl ester which were separated and purified through ion-exchange chromatography (Dowex 50WX8-100, 30% aqueous NH_4OH). The enantiomeric excess of L-2-quinoxalylalanine was determined through the treatment of the amino acid with the acyl chloride of Mosher's acid.[12] The resulting MTPA derivative was analyzed[13] by ^1H NMR revealing an enantiomeric excess of 94%.[14]



Having obtained the amino acid of choice, **7** was next protected in a fashion suitable for Boc-benzyl solid-phase peptide synthesis.[15] Accordingly, **7** (0.49 g, 2.26 mmol) was treated with 2-(t-butoxycarbonyloxyimino)-

2-phenylacetonitrile (Boc-on, 0.62 g, 2.49 mmol) in 5 mL of 50% dioxane/H_2O in the presence of triethylamine (0.53 mL) to give **9**, the corresponding N-Boc derivatized α-amino acid (63% yield). Subsequently, this amino acid was employed in the solid phase synthesis of a derivative of **1** which replaced both terminal tyrosine residues with L-2-quinoxalylalanine (**10**); peptide synthesis utilizing **9** proceeded smoothly with regards to coupling to the solid support (mBHA resin), subsequent steps of Boc-benzyl methodology (>99% coupling by the Kaiser ninhydrin test), and final deprotection and resin cleavage with 1 M TFMSA in TFA.[16]

The preceding experimentation demonstrates that L-2-quinoxalylalanine can be efficiently prepared chemoenzymatically and applied in Boc-benzyl solid phase peptide syntheses. This amino acid thus enables a peptide to be easily equipped with an identical functional group as found in the quinoxaline class of antitumor agents which, in the example presented, has permitted the synthesis of a hybrid peptide that quite closely resembles agents such as echinomycin or the triostins. Ongoing studies seek to determine the influence of this unique amino acid on the binding and recognition properties of the tandem β-turn motif found in the CTD of RNA polymerase II.

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REFERENCES AND NOTES

1. (a) Corden, J. L.; Cadena, D. L.; Ahearn, J. M. Jr.; Dahmus, M. E. *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 7934; (b) Young, R. A. *Annu. Rev. Biochem.* **1991**, *60*, 689.
2. (a) Woychik, N. A.; Young, R. A. *Trends Biochem. Sci.* **1990**, *15*, 347; (b) Corden, J. L. *Trends Biochem. Sci.* **1990**, *15*, 383; (c) Kelly, W. G.; Dahmus, M. E.; Hart, G. W. *J. Biol. Chem.* **1993**, *268*, 10416; (d) Kim, W. K.; Dahmus, M. E. *J. Biol. Chem.* **1986**, *261*, 14219; (e) Cadena, D. L.; Dahmus, M. E. *J. Biol. Chem.* **1987**, *262*, 12468; (f) Zhang, J.; Corden, J. L. *J. Biol. Chem.* **1991**, *266*, 2290; (g) Zhang, J.; Corden, J. L. *J. Biol. Chem.* **1991**, *266*, 2297.
3. (a) Suzuki, M. *Nature* **1990**, *344*, 562; (b) Peterson, C. L.; Kruger, W.; Hershkowitz, I. *Cell* **1991**, *64*, 1135.
4. Serizawa, H.; Conaway, R. C.; Conaway, J. W. *J. Biol. Chem.* **1993**, *268*, 17300.
5. Harding, M. M. *J. Med. Chem.* **1992**, *35*, 4658.
6. (a) Waring, M. J.; Wakelin, L. P. G. *Nature* **1974**, *252*, 653; (b) Quigley, G. J.; Ughetto, G.; van der Marel, G. A.; van Boom, J. H.; Wang, A. H.-J.; Rich, A. *Science* **1986**, *232*, 1255.
7. (a) Huang, X.; Shullenberger, D. F.; Long, E. C. *Biochem. Biophys. Res. Commun.* **1994**, *198*, 712; (b) Shullenberger, D. F.; Long, E. C. *BioMed. Chem. Lett.* **1993**, *3*, 333.
8. Butera, J. A.; Spinelli, W.; Anantharaman, V.; Marcopulos, N.; Parsons, R. W.; Moubarak, I. F.; Callinan, C.; Bagli, J. F. *J. Med. Chem.* **1991**, *34*, 3212.
9. O'Donnell, M. C.; Bennett, W. D.; Wu, S. *J. Am. Chem. Soc.* **1989**, *111*, 2353.
10. Rachele, J. R. *J. Org. Chem.* **1963**, *28*, 2898.
11. Chen, S.-T.; Wang, K.-T.; Wong, C.-H. *J. Chem. Soc., Chem. Commun.* **1986**, 1514; (b) Imperiali, B.; Prins, T. J.; Fisher, S. L. *J. Org. Chem.* **1993**, *58*, 1613.
12. Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543.
13. Kabuto, K.; Yasuhara, F.; Yamaguchi, S. *Tetrahedron Lett.* **1980**, *21*, 307.
14. All new compounds yielded satisfactory characterization data by elemental analyses, MS, and ^1H NMR; ^1H NMR for **7**: 300 MHz, D_2O, δ 8.80 (s, 1H), δ 8.06 (m, 2H), δ 7.88 (m, 2H), δ 4.14 (dd, 1H), δ 3.50 (m, 2H); $[\alpha]_D^{25}$ +12 (0.2 N HCl); for an alternative chemical synthesis of heteroaryl alanines see: Krippner, G. Y.; Harding, M. M. *Tetrahedron: Asymmetry* **1994**, *5*, 1793.
15. Stewart, J. M.; Young, J. D. *Solid-Phase Peptide Synthesis*; Pierce Chemical Co.: Rockford, IL, 1984.
16. FAB-MS of **10** revealed an m/z value of 973.6 (M + H).

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