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Solid-Phase Guanidinylation as a Diversification Strategy of Poly-L-proline Type II Peptide Mimic Scaffolds

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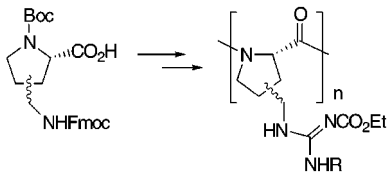
ABSTRACT



Solid-phase guanidinylation of proline-templated amino acids is studied as a diversification strategy of poly-L-proline type II scaffolds.

The poly-L-proline type II (PPII) secondary structure is uniquely characterized by an extended left-handed helical fold with $\phi \approx -75°$ and $\psi \approx 145°$. In the past decade, PPII helices have been found to mediate a number of cellular signaling pathways. For example, SH2 domains, SH3 domains, MHC class II proteins and other proteins bind peptide ligands in the PPII conformation or in a similar geometry.[1,2] Although PPII helices derive their name from the conformation of polyproline, short spans of PPII helices in globular proteins are often composed of amino acids other than proline. Because it is often these nonprolyl amino acids that are critical for recognition of the PPII helix by a receptor, we are involved in developing a program that both mimics the PPII three-dimensional structure and incorporates the critical nonprolyl functionality. Our strategy for mimicking this secondary structure involves first the synthesis of what we call proline-templated amino acids (PTAAs) and then the synthesis of peptides from PTAAs (Figure 1).[3] Oligo-PTAAs are designed to adopt the PPII conformation in solution since (1) $\phi \approx -75°$ as a result of the constraints of the PTAA pyrrolidine ring, (2) ψ is constrained to ~145° by pseudo-A(1,3) strain between adjacent PTAAs, and (3) the trans amide bond conformation is favored. Our design strategy is validated by solution studies showing that a unit as small as a PTAA dimer is conformationally constrained to the ϕ/ψ angles of a PPII helix.[3] OligoPTAAs therefore provide a conformationally defined scaffold that can vary both the orientation of the nonprolyl side chains as well as the nature of these side chains.

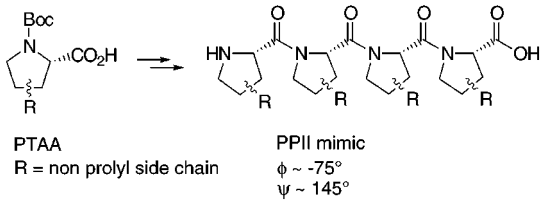


Figure 1. PPII mimic design strategy.

Because PTAAs are in essence amino acids, it is conceivable that PPII mimic libraries could be generated from

(1) For a review see: Siligardi, G.; Drake, A. F. *Pept. Sci.* **1995**, *37*, 281.

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(3) (a) Zhang, R.; Brownewell, F.; Madalengoitia, J. S. *J. Am. Chem. Soc.* **1998**, *120*, 3894. (b) Zhang, R.; Madalengoitia, J. S. *J. Org. Chem.* **1999**, *64*, 330.

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appropriately protected PTAAs as potential inhibitors of signaling pathways that involve PPII recognition and binding. One potential drawback to this goal, however, is the labor required for the synthesis of a large diversity of these PTAAs. For example, 10 synthetic steps are required for the synthesis of the leucine PTAA **1**, and 13 steps are required for the synthesis of the arginine PTAA **2** (Figure 2).[4] Highly

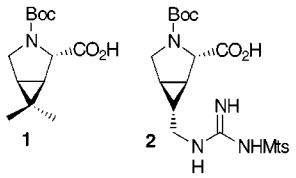
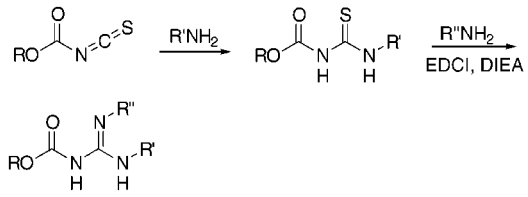


Figure 2. Representative PTAAs.

functionalized PTAAs that would be more desirable would of course require more steps to make. In this paper we begin to address how a large diversity of PPII mimics may be generated without the need for a large diversity of PTAAs. We propose to use the concept of a "diversifiable" PTAA, i.e., a PTAA that can be deprotected on a solid support and allowed to react with a number of reagents as a means of diversification without the need for the solution synthesis of all PTAAs that would eventually be generated. This strategy could, of course, take many forms depending on the diversification reaction chosen. Since several receptor-bound PPII helices have been shown to incorporate arginine residues,[1] we begin by exploring the solid-phase guanidinylation of two proline-templated ornithines (**6** and **9**) as the means of diversification. The two ornithine analogues are chosen to highlight that the diversity of the library can be increased through the use of PTAAs with different side chain orientations.

A number of guanidinylation protocols have been described in the literature.[5] Of these, we decided to explore the procedure recently described by Hamilton because it appeared to offer an expedient and easy method for the introduction of structural diversity (Scheme 1).[5c] Hamilton's

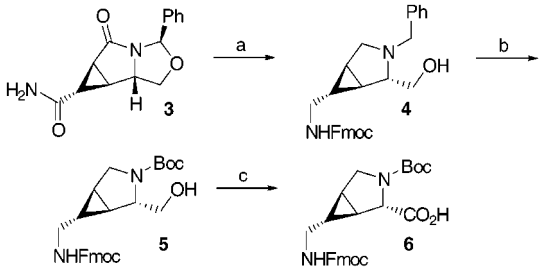
Scheme 1



method generates a number of *N*-carbamoyl-*N*′-alkyl thioureas by reaction of *N*-carbamoyl isothiocyanates with amines. The *N*-carbamoyl-*N*′-alkyl thioureas then serve as guanyl transfer reagents in the presence of EDCI and DIEA (Scheme 1).

The synthesis of the guanyl acceptor PTAAs was accomplished as shown in Schemes 2 and 3. The cyclopropyl-

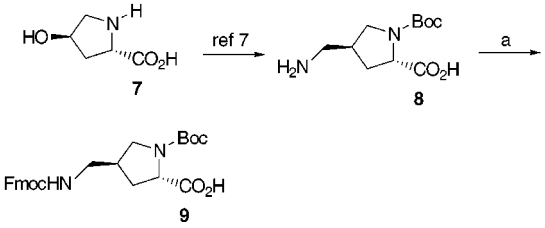
Scheme 2[a]



[a] Reagents and conditions: (a) (i) LAH, THF, reflux; (ii) FmocCl $NaHCO_3$, dioxane/H_2O, 76%; (b) H_2, Pd−C, Boc_2O, EtOAc, 95%; (c) TEMPO, $NaClO_2$, bleach CH_3CN, 90%.

substituted PTAA **6** was synthesized from tricycle **3**, which we can make in multigram quantities.[4] LAH in refluxing THF served to reduce both amides as well as the oxazolidine ring.

Scheme 3[a]



[a] Reagents and conditions: (a) FmocCl, $NaHCO_3$, dioxane/H_2O (70%).

The crude amine was then protected with FmocCl in a saturated aqueous $NaHCO_3$/dioxane mixture to afford **4** in 76% yield for both steps. N-debenzylation in the presence of Boc_2O afforded the *N*-Boc prolinol **5** in 95% yield. Oxidation of the primary alcohol to the carboxylic acid was accomplished with TEMPO/$NaClO_2$/bleach to give the PTAA **6** suitably protected for these studies.[6] The second

(4) Zhang, R.; Mamai, A.; Madalengoitia, J. S. *J. Org. Chem.* **1999**, *64*, 547.

(5) (a) Feichtinger, K.; Zapf, C.; Sings, H. L.; Goodman, M. *J. Org. Chem.* **1998**, *63*, 3804. (b) Feichtinger, K.; Sings, H. L.; Baker, T. J.; Matthews, K.; Goodman, M. *J. Org. Chem.* **1998**, *63*, 8432. (c) Linton, B. R.; Carr, A. J.; Orner, B. P.; Hamilton, A. D. *J. Org. Chem.* **2000**, *65*, 1566. (d) Kim, K. S.; Qian, L. *Tetrahedron Lett.* **1993**, *34*, 7677. (e) Bernatowicz, M. S.; Wu, Y.; Matsueda, G. R. *Tetrahedron Lett.* **1993**, *34*, 3389, (f) Poss, M. A. Iwanowicz, E.; Reid, J. A.; Lin, J.; Gu, Z. *Tetrahedron Lett.* **1992**, *33*, 5933. (g) Yong, Y. F.; Kowalski, J. A.; Lipton, M. A. *J. Org. Chem.* **1997**, *62*, 1540. (h) Robinson, S.; Roskamp, E. J. *Tetrahedron* **1997**, *53*, 6697. (i) Dodd, D. S.; Wallace, O. B. *Tetrahedron Lett.* **1998**, *39*, 5701. (j) Wilson, L. J.; Klopfenstein, S. R.; Li, M. *Tetrahedron Lett.* **1999**, *40*, 3999. (k) Dodd, D. S.; Kozikowski, A. P. *Tetrahedron Lett.* **1994**, *35*, 977.

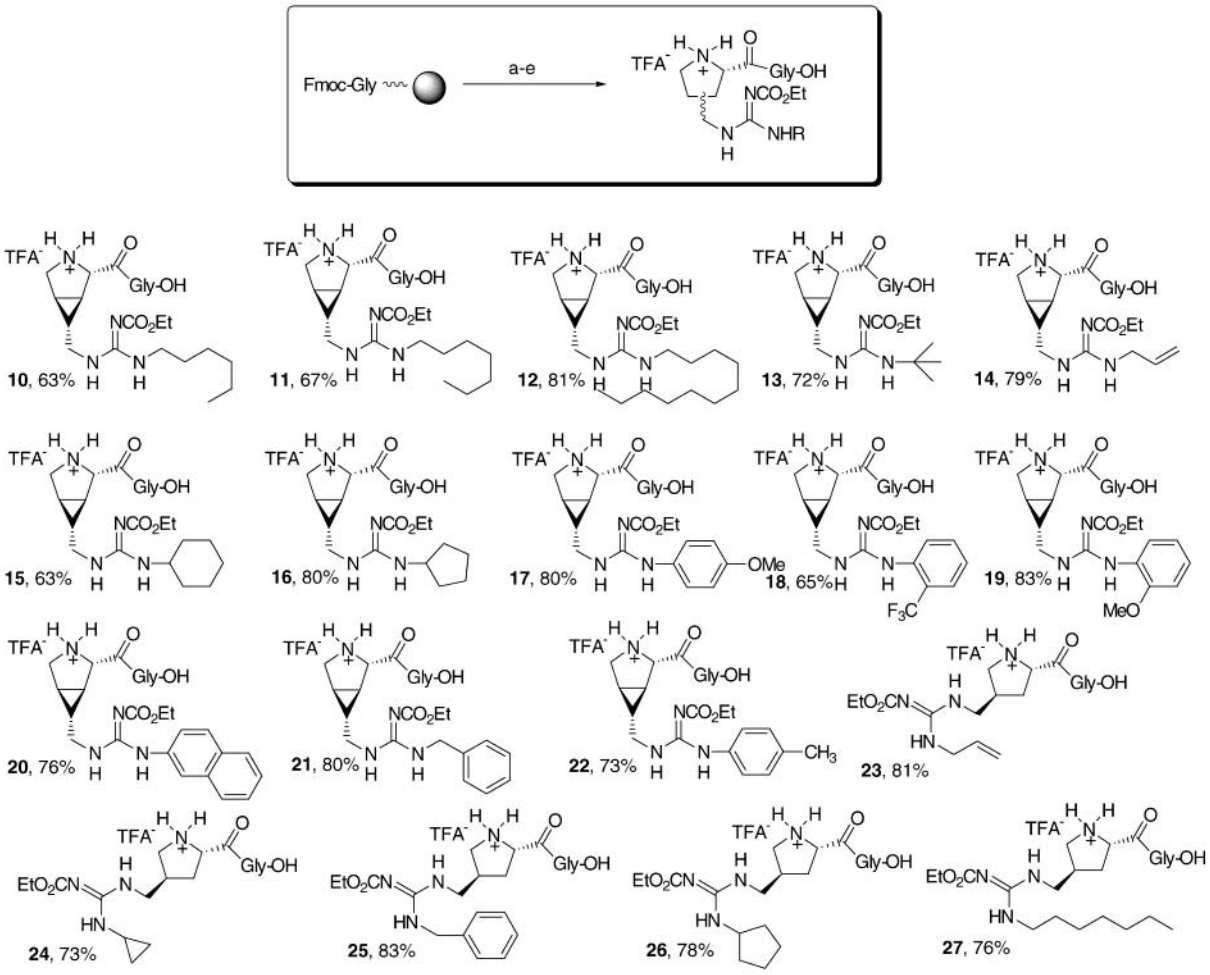


Figure 3. Reagents and conditions: (a) 20% piperidine/DMF; (b) 1.3 equiv of **6** or **9**, DIC, HOBt; (c) 20% piperidine/DMF; (d) NH-(CO_2Et)C═SNHR, EDCI, DIEA; (e) 50% TFA/CH_2Cl_2.

PTAA **9** was obtained by the simple Fmoc-protection of the known PTAA **8**.[7]

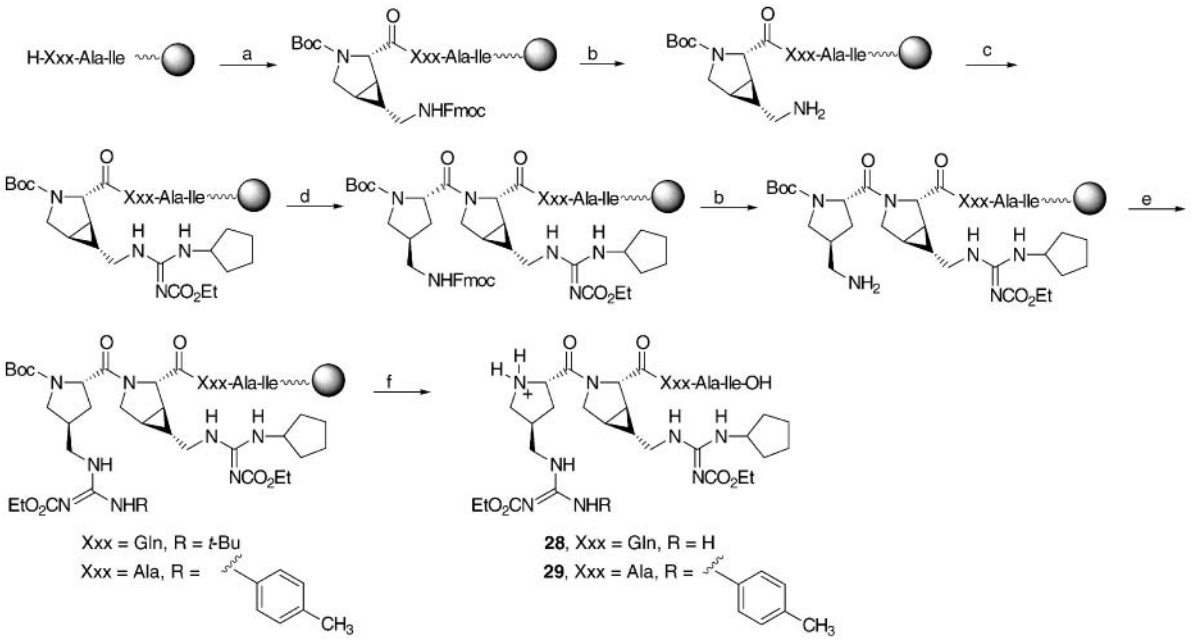
Our initial studies were designed to probe the feasibility of using the guanidinylation reaction as a means of easily and cleanly affording the guanidine-possessing products. For these studies, the Boc-PTAA(Fmoc)-OH amino acids were initially coupled to H-Gly-Wang resin. Because the synthesis of the these PTAAs still requires a number of steps, we explored the use of less than the standard 3−5 equiv of amino acid in the coupling reaction in order to reduce the amount of PTAA wasted. We are pleased to report that the reaction proceeded to completion with 1.3 equiv of Boc-PTAA-(Fmoc)-OH under standard conditions (diisopropylcarbodiimide/HOBt). Side chain deprotection was then accomplished with 20% piperidine in DMF. The guanidinylation reaction was next explored with a number of different thioureas. Figure 3 highlights the flexibility and ease with which a diversity of groups can be introduced. The guanidine substitution was chosen to reflect a number of different properties (hydrophobicity, conformational flexibility, conformational restriction, etc.) An advantage of using an *N*-carbamoyl thiourea is that since the guanyl group is transferred already protected, the progress of the guanidinylation reaction can monitored by a standard Kaiser or TNBS resin test. Typically, the reactions showed a negative Kaiser test after 24 h, but to ensure full couplings without large excesses of *N*-carbamoyl-*N*′-alkyl thioureas, the reactions were allowed to proceed for 40 h. TFA treatment of the resin resulted in Boc-deprotection and liberation of the dimer from the resin. We wanted to determine how cleanly this sequence of steps proceeded, so the purity of the peptides was examined after trituration with Et_2O.[8] Most of these compounds were obtained in >90% purity as determined by integration of the crude ^{1}H NMR spectra. The only compound not obtained in >90% purity was **20**.[9] As evidence of purity, crude spectra are included in Supporting Information.

(6) Zhao, M.; Li, J.; Mano, E.; Song, Z.; Tsachen, D. M.; Grabowski, E. J. J.; Reider, P. J. *J. Org. Chem.* **1999**, *64*, 2564.

(7) Webb, T. R.; Eigenbrot, C. *J. Org. Chem.* **1991**, *56*, 3009.

(8) The yield of peptide **12** is without trituration because it was found to be ether-soluble.

Scheme 4[a]



[a] Reagents and conditions: (a) 1.3 eq **6**, DIC, HOAt; (b) 20% piperidine/DMF; (c) NH(CO_2Et)C═SNH(cyclopentyl), EDCI, DIEA; (d) (i) 50% TFA/CH_2Cl_2; (ii) 30% DIEA/DMF; (iii) 1.3 eq **9**, DIC, HOAt; (e) NH(CO_2Et)C═SNHR (R = *t*-Bu, or R = p-C_6H_5Me), EDCI, DIEA; (f) TMSOTf, TFA, thioanisole, 0 °C.

As proof of principle that the chemistry could be used in iterative cycles to diversify PPII scaffolds, we synthesized two model peptides: PTAA-PTAA-Gln-Ala-Ile (**28**) and PTAA-PTAA-Ala-Ala-Ile (**29**) on PAM resin. These model pentapeptides are based on the sequence Arg-Arg-Asn-Ala-Ile from the *c*AMP-dependent protein kinase (PKA) inhibitor PKI that has been shown to bind to the PKA active site in the PPII conformation. The synthesis strategy is shown in Scheme 4. The second and third amino acids were incorporated using standard conditions (DIC/HOBt). PTAA **6** was then introduced, and the side chain was deprotected (20% piperidine/DMF). Guanidinylation of the side chain was accomplished with *N*-ethoxycarbonyl-*N*′-cyclopentyl thiourea/EDCI. The N-terminus was then deprotected (TFA), and PTAA **9** was coupled. Side chain deprotection (20% piperidine/DMF) and guanidinylation of the resultant amine with either *N*-ethoxycarbonyl-*N*′-*tert*-butyl thiourea or *N*-ethoxycarbonyl-*N*′-*p*-toluidine thiourea completed the solid-phase synthesis. Cleavage and deprotection was accomplished with TMSOTf/TFA/thioanisole.[10] Peptides were then precipitated with ether and analyzed by ^{1}H NMR and MS. Inspection of the ^{1}H NMR spectra revealed that peptide **28** had lost the *tert*-butyl group during cleavage and deprotection but was otherwise remarkably clean. As a result of the inherent "greasy" character of the peptides,[11] the major impurity detected in the crude ^{1}H NMR spectra was thioanisole and thioanisole byproducts. Crude spectra are included in Supporting Information.

In conclusion, we have shown a novel strategy for the synthesis of PPII mimics that should be easily applicable to the synthesis of PPII mimic libraries.

Acknowledgment. Financial Support for this work was provided by CHE 9870003 from the NSF.

Supporting Information Available: Experimental procedures and characterization data for **4**, **5**, **6**, and **9** and copies of crude ^{1}H NMR spectra of **10**−**28**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) The synthesis of **20** was repeated, but **20** was still obtained in lower purity than the other compounds.

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(11) One of the referees asked for an estimate of the aqueous solubility of the pentapeptides (solubility > 30 mg/mL H_2O).