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Efficient Introduction of Protected Guanidines in BOC Solid Phase Peptide Synthesis

Yongda Zhang and Alan J. Kennan*

Department of Chemistry, Colorado State University, Fort Collins, Colorado 80523

kennan@lamar.colostate.edu

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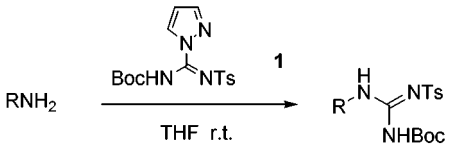
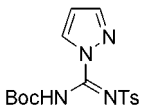
ABSTRACT



Reaction of primary amines with pyrazole 1 results in rapid and efficient guanidinylation, either in solution or on solid phase. The reaction affords sulfonamide-protected products required for BOC solid phase peptide synthesis (SPPS) in a single step under mild conditions. Incorporation of orthogonally protected side chain amines permits the synthesis of peptides containing arginine analogues, one of which could not be prepared by coupling of preformed amino acids.

The widespread occurrence of guanidine groups in natural products has inspired considerable development of synthetic methodology.[1] In particular, a number of elegant methods for on-resin functionalization have been disclosed, though most involve more than one step.[2] Here we report a new reagent (**1**), capable of guanidinylating primary amines with impressive speed and efficiency. Reaction with side chain amines during peptide synthesis allows one-step introduction



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of protected guanidines compatible with BOC methodology. The synthesis of **1** and its application in both solution and solid phase contexts is described.

In the course of peptide-based molecular recognition studies, we sought to prepare sequences containing chain-shortened or -elongated arginine analogues. Given the standard use of *N*-tosyl-protected arginine in BOC synthesis, initial efforts targeted *N*-tosyl monomers. Although the corresponding amino acids proved simple to prepare, their use in peptide synthesis was impractical.[3] Thus, an alternative strategy of on-resin functionalization was pursued. Incorporation of amino acids bearing FMOC-protected side chain amines, followed by sequential treatment with piperidine and a guanidinylating agent, was expected to afford the desired peptides.

An appropriate reagent was suggested by reports that pyrazole **2** produces bis-Boc protected guanidines from

(1) (a) Moroni, M.; Koksch, B.; Osipov, S. N.; Crucianelli, M.; Frigerio, M.; Bravo, P.; Burger, K. *J. Org. Chem.* **2001**, *66*, 130−133. (b) Brewer, M.; Rich, D. H. *Org. Lett.* **2001**, *3*, 945−948. (c) Katritzky, A. R.; Rogovoy, B. V.; Chassaing, C.; Vvedensky, V. *J. Org. Chem.* **2000**, *65*, 8080−8082. (d) Barawkar, D. A.; Kwok, Y.; Bruice, T. W.; Bruice, T. C. *J. Am. Chem. Soc.* **2000**, *122*, 5244−5250. (e) Linton, B. R.; Carr, A. J.; Orner, B. P.; Hamilton, A. D. *J. Org. Chem.* **2000**, *65*, 1566−1568. (f) Schneider, S. E.; O'Neil, S. N.; Anslyn, E. V. *J. Am. Chem. Soc.* **2000**, *122*, 542−543. (g) Baker, T. J.; Luedtke, N. W.; Tor, Y.; Goodman, M. *J. Org. Chem.* **2000**, *65*, 9054−9058. (h) Luedtke, N. W.; Baker, T. J.; Goodman, M.; Tor, Y. *J. Am. Chem. Soc.* **2000**, *122*, 12035−12036. (i) Feichtinger, K.; Sings, H. L.; Baker, T. J.; Matthews, K.; Goodman, M. *J. Org. Chem.* **1998**, *63*, 8432−8439. (j) Feichtinger, K.; Zapf, C.; Sings, H. L.; Goodman, M. *J. Org. Chem.* **1998**, *63*, 3804−3805.

(2) (a) Burgess, K.; Chen, J. In *Solid-Phase Organic Synthesis*; Burgess, K., Ed.; John Wiley & Sons: New York, 2000; pp 1−23. (b) Gomez, L.; Gellibert, F.; Wagner, A.; Mioskowski, C. *Chem.—Eur. J.* **2000**, *6*, 4016−4020. (c) Dahmen, S.; Braese, S. *Org. Lett.* **2000**, *2*, 3563−3565. (d) Zapf, C. W.; Creighton, C. J.; Tomioka, M.; Goodman, M. *Org. Lett.* **2001**, *3*, 1133−1136. (e) Ghosh, A. K.; Hol, W. G. J.; Fan, E. *J. Org. Chem.* **2001**, *66*, 2161−2164. (f) Mamai, A.; Madalengoitia, J. S. *Org. Lett.* **2001**, *3*, 561−564.

(3) For example, use of *N*-Boc-α-amino-γ-*N*-tosylguanidinobutyric acid gave little or no desired product even using extended coupling times and excess reagents.

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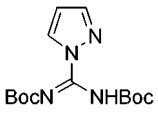
primary amines.[4] Also encouraging was an analogue's ability



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to functionalize an ornithine side chain on solid phase, although the guanidiniylation demanded somewhat elevated temperatures and extended reaction times.[5] More problematically, either analogue would require that guanidinylation immediately precede cleavage from the resin, since removal of the α-amino Boc group would also deprotect any newly formed guanidine. In the context of longer sequences, this would require orthogonal side chain protecting groups that could withstand repeated coupling iterations. A simpler approach involving side chain deprotection and functionalization before further synthesis would permit the use of protecting groups that need not be removed until final cleavage from the resin. Thus, an ideal reagent would efficiently generate BOC-compatible protected guanidines from side chain amines. In keeping with the sulfonamide strategy, we examined *N*-tosyl-protected **1** and discovered that it was an extremely efficient means of accomplishing this objective (Figure 1).

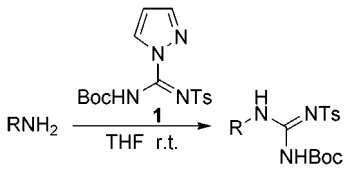
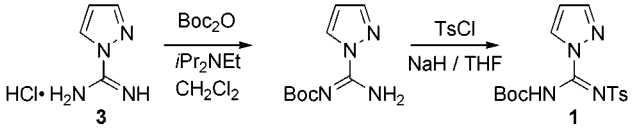


Figure 1. General guanidinylation reaction.

The required reagent can be prepared on a gram scale from commercially available pyrazole **3** via a two-step sequence (Scheme 1).[6] The mono Boc derivative of **3** (Boc_2O, iPr_2-

Scheme 1. Reagent Preparation



NEt/CH_2Cl_2, 90%) produced **1** upon reaction with TsCl (NaH/THF 46%).

To probe the reactivity of **1** toward amines, solution couplings with a handful of simple substrates were attempted (Figure 2). Reactions with simple primary amines were rapid

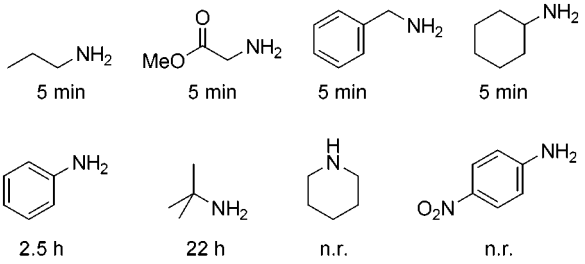


Figure 2. Solution phase reactions. Reaction times for guanidinylation of each substrate with 1.1 equiv of **1** in THF at room temperature. Reaction yields were quantitative, except for substrates marked n.r., which gave no product after 1 day.

and quantitative upon mixing with 1.1 equiv of **1** in THF at room temperature.[7] Aniline and *tert*-butylamine were also reasonable substrates, though considerably more sluggish. Further reduction in nucleophilicity (e.g., *p*-nitroaniline) suppressed the reaction entirely. Piperidine was similarly unreactive, confirming that residual base from side chain deprotections would not compete for **1**.

With the efficacy of **1** in solution demonstrated, attention turned to its use in peptide synthesis. Target sequences were selected to address a variety of issues (Figure 3). Guanidi-

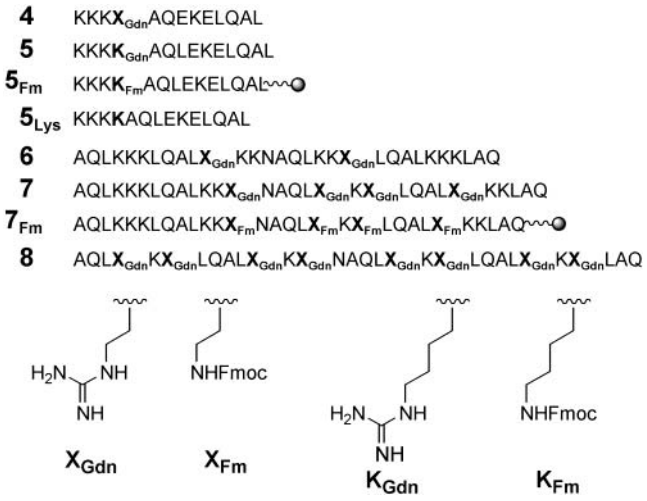


Figure 3. Test sequences for on-resin guanidinylation, presented using one-letter codes.[11] Unusual side chains are depicted explicitly. Peptides $\mathbf{5_{Fm}}$ and $\mathbf{7_{Fm}}$ were prepared only in resin-bound form.

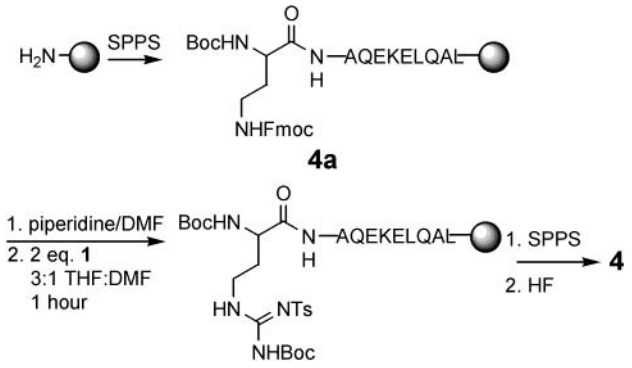
nylation conditions were tested by preparation of a short monoguanidine peptide (**4**), which could not be prepared by standard methods. Thus, fragment **4a**, terminating in an α-BOC-γ-FMOC-protected diaminobutyric acid, was syn-

(4) (a) Drake, B.; Patek, M.; Lebl, M. *Synthesis* **1994**, 579−582. (b) Bernatowicz, M. S.; Wu, Y.; Matsueda, G. R. *Tetrahedron Lett.* **1993**, *34*, 3389−3392.

(5) Bernatowicz, M. S.; Wu, Y.; Matsueda, G. R. *J. Org. Chem.* **1992**, *57*, 2497−2502.

(6) See Supporting Information for experimental details.

Scheme 2. Example of Peptide Guanidinylation



thesized by SPPS (Scheme 2).[8] Following FMOC removal, quantitative guanidinylation of the side chain amine was best achieved using 2 equiv of **1** in 3:1 THF:DMF at room temperature for 1 h.[9] Completion of the synthesis and cleavage from the resin with anhydrous HF afforded the desired peptide **4**. An analogous homoarginine peptide (**5**) was prepared using the same methods, confirming the ability to functionalize side chains of different length.[10]

Although crude products were judged reasonably clean by reverse-phase HPLC, verification was desired that observed side products were not due to inefficient guanidinylation. Thus, peptide $\mathbf{5_{Fm}}$ was prepared, in which the functionalization site of **5** remains FMOC-protected. The full-length peptide was then deprotected, and half the resulting resin was reacted with **1** under standard conditions. Individual cleavage of both resin samples afforded samples of **5** and $\mathbf{5_{Lys}}$, whose side chain is not guanidinylated. Since the two peptides differ only in whether they were guanidinylated, a comparison of crude HPLC traces should reflect the reaction efficiency. An overlay of the two traces demonstrates that indeed all significant byproducts are present in both samples and are thus attributable to errors in peptide synthesis rather than guanidinylation (Figure 4).

Once the basic application of **1** in peptide synthesis had been demonstrated, multiple substitutions were investigated. Since α-amino-γ-guanidinobutyric acid residues could not be installed by coupling functionalized monomers, they were selected as a challenging test case for the new methodology.

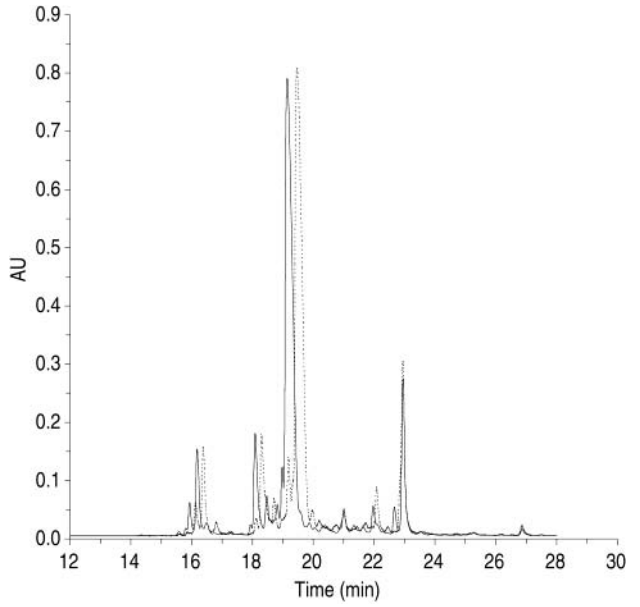


Figure 4. Reaction efficiency. Crude HPLC chromatograms of peptides **5** and $\mathbf{5_{Lys}}$. Both were prepared from a common synthesis and differ only in whether the deprotected amine side chain was guanidinylated (**5**, dotted line) or not ($\mathbf{5_{Lys}}$, solid line) before cleavage from the resin.

Thus, longer sequences containing two (**6**), four (**7**), and eight (**8**) such groups were examined (Figure 3). It was anticipated

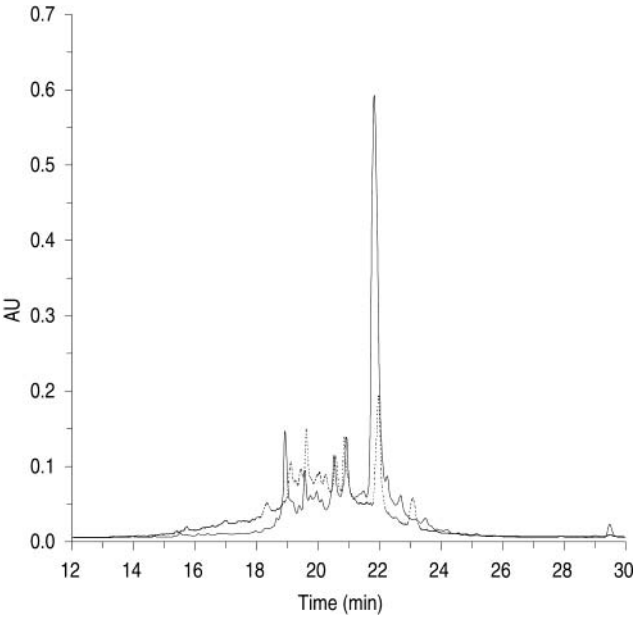


Figure 5. Sequential vs global guanidinylation. Crude HPLC chromatograms of peptide **7** synthesized by either: FMOC removal and guanidinylation immediately after incorporation of α-aminobutyric acid (solid line) or simultaneous deprotection and guanidinylation of all four amines in the fully synthesized peptide (dotted line).

(7) **Sample procedure:** A mixture of **1** (21.8 mg, 0.06 mmol) and amine (0.055 mmol) in 0.5 mL of THF was stirred at room temperature for 5 min. Water and ethyl acetate were added, and the organic layer was washed with 0.5 M citric acid, water, and brine and dried over Na_2SO_4. After removal of solvent in vacuo, the residue was chromatographed (SiO_2; 3:1 ethyl acetate:hexane) to afford pure product.

(8) Peptides were synthesized according to standard Boc protocols, employing the in situ neutralization procedure developed by Kent: Schnoelzer, M.; Alewood, P.; Jones, A.; Alewood, D.; Kent, S. B. H. *Int. J. Pept. Protein Res.* **1992**, *40*, 180−193.

(9) Functionalization of all resin-bound amines was verified by the Kaiser test. Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595−598.

(10) The identity of each peptide was confirmed by electrospray mass spectrometry of a sample purified by reverse-phase HPLC. See Supporting Information.

(11) One-letter abbreviations: A = alanine, E = glutamic acid, K = lysine, L = leucine, N = aspartic acid, Q = glutamine, X = α-aminobutyric acid.

that the ability to introduce robustly protected guanidines should be particularly useful in these contexts. Indeed, all three peptides were easily prepared using **1**. Each guanidine was introduced in the same manner as above, by side chain deprotection and functionalization prior to chain elongation. Crude HPLC analysis confirmed synthetic efficiency.

To further demonstrate the benefits of sequential guanidinylation, peptide **7** was also prepared according to a global functionalization strategy. Thus, $\mathbf{7_{Fm}}$, which retains all four FMOC groups, was synthesized. The completed peptide was then exposed to piperidine in DMF to liberate the amine side chains. Simultaneous functionalization of all four positions proceeded without incident, and after cleavage from the resin the crude material was compared to that prepared above via the sequential approach.

As expected, the globally functionalized material suffered a considerable degradation in purity. Presumably this is due to undesired FMOC removal during repeated exposure to coupling and deprotection conditions. An overlay of crude HPLC traces (Figure 5) reveals an increase in side products (from 18 to 22 min) and a dramatic reduction of the desired product (22 min).

The investigations reported above clearly demonstrate the value of **1** as an efficient reagent for the preparation of guanidine-containing peptides. The reagent can be prepared on large scale from inexpensive commercial materials. It permits one-step installation of *N*-tosyl guanidines under mild conditions and affords products compatible with subsequent exposure to repeated coupling cycles. In particular, the method has been validated in the context of several sequences that could not be prepared by simple coupling of prefunctionalized monomers. This chemistry should prove extremely useful in the construction of peptides containing a wide variety of guanidinylated side chains.

Acknowledgment. We thank Colorado State University and the Petroleum Research Fund, administered by the American Chemical Society, for financial support.

Supporting Information Available: Experimental details for the preparation of **1**, along with characterization data for solution and peptide products. This material is available free of charge via the Internet at http://www.acs.org.

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