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# Microwave-assisted guanidinylation in solid phase peptide synthesis: comparison of various reagents

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ABSTRACT

The guanidinylation of a peptide chain on a polymeric support under microwave conditions using derivatives of thioureas—*S*-alkylisothioureas, pyrazole-carboxamidine, and guanidine as guanidinylating reagents is described. The best results are obtained with *N*,*N*′-di-Z-*S*-methylisothiourea and *N*,*N*′-di-Z(2-Cl)-*S*-methylisothiourea. It is found that guanidinylation with reagents containing Boc groups is accompanied by side reactions.



Arginine, which possesses a guanidine group in the side chain, is the most basic of all the natural amino acids and plays an important role in many physiological processes. The guanidine group is responsible for forming both electrostatic and direct hydrogen bond interactions with polar and anionic molecules, via arginine or arginine residues in peptides and proteins in a number of biological systems.[1] It is especially important in ligand-receptor interactions, for example, in peptide A7R in interactions with neuropilin.[2] Peptides containing arginine or arginine-like moieties are used as model compounds for biological research as well as therapeutic purposes.[3] The synthesis of peptides containing an arginine or arginine-like residue is, in many cases, difficult due to the side reactions observed in the presence of arginine derivatives.[4] In order to avoid these difficulties, one of the proposed approaches is the conversion of an ornithine-containing peptide into an arginine-containing peptide by selective guanidinylation of the ornithine side chain amino group.[4a–c,5] This strategy also allows the formation of peptides with a modified arginine residue, with the side chain longer or shorter than the side chain of arginine (e.g., *homo*-arginine, 4-guanidino-2-aminobutanoic acid). Various guanidinylating reagents (i.e., compounds which convert an amino group into a guanidine group) have been proposed and used in syntheses of protected and unprotected guanidines,[6] but not all of them can be applied in solid phase peptide synthesis (SPPS). In SPPS, conversion of an amino group in the side chain of a basic amino acid residue into a guanidine group is usually carried out using thiourea, *S*-alkylisothiourea, or pyrazole-carboxamidine derivatives. The rate of guanidinylation with such reagents, in a number of cases, is low and the yield of the target products, despite the large excess of guanidinylating agent, is far from quantitative.

Recently, it was demonstrated that SPPS can be successfully accelerated with microwave irradiation,[7] therefore we envisaged that this method could be applied to improve the rate and yield of the peptide side chain guanidinylation on a solid support.

To investigate the effect of microwave irradiation on the course of guanidinylation, the side-chain amino group of model peptides on a solid support was converted into the guanidino group using a variety of reagents, with or without microwave irradiation. In this paper we present the results of these studies. The general procedure employed in our studies is described schematically in Figure 1.

*Guanidinylating reagents*. The following reagents were selected for this study: (**I**) *O*-methylisourea,[5d,8] (**II**) *S*-methylisothiourea,[9] (**III**) 3,5-dimethyl-1*H*-pyrazole-1-carboxyamidine,[5b,9a,10] (**IV**) *N*,*N*′-di-Boc-*S*-methylisothiourea,[11] (**V**) *N*,*N*′-di-Boc-*S*-benzylisothiourea,[12] (**VI**) *N*,*N*′-di–Boc-*N*″-trifluoromethanesulfonylguanidine,[13]

*Abbreviations:* Boc, *tert*-butyloxycarbonyl; Dab, 2,4-diaminobutyric acid; Dap, 2,3-diaminopropionic acid; Dap(g), 3-guanidino-2-aminopropionic acid; DIC, 1,3-diisopropylcarbodiimide; DIEA, diisopropylethylamine; *h*Arg(g), 2,6-diguanidinohexanoic acid; MW, microwave; TBTU, 2(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate; TFA, trifluoroacetic acid; TIS, triisopropylsilane; Z, benzyloxycarbonyl; Z(2-Cl), 2-chlorobenzyloxycabonyl.

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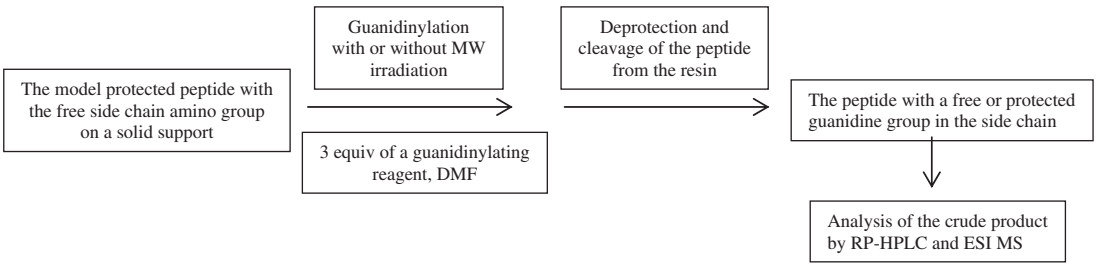


**Figure 1.** Flow diagram of the guanidinylating methodology.

(**VII**) *N*,*N*′-di-Z-*S*-methylisothiourea,[9b,11a,14] and (**VIII**) *N*,*N*′-di-Z(2-Cl)-*S*-methylisothiourea[15] (Fig. 2).

The reagents **I**–**III** were purchased from Sigma and reagents **IV**–**VIII** were synthesised according to the appropriate procedures described in the literature.[5h,12c,13a,14,15a] *O*-Methylisourea sulfate (**I**) and *S*-methylisothiourea sulfate (**II**) are commonly used for guanidinylation in an aqueous environment, therefore preliminary attempts were performed to verify if they were effective as guanidinylating agents in an aqueous-organic environment. The use of water was necessary due to the limited solubilities of **I** and **II** in organic solvents. Both reagents were used in the guanidinylation of the ε-amino group of $N^{\alpha}$-protected lysine (Boc-Lys-OH) to yield $N^{\alpha}$-protected *homo*-arginine (Boc-*h*Arg-OH). Reactions were carried out with a 1.5-fold excess of the reagent **I** or **II** under MW conditions (40 W, 50 °C), using DMF/water or 1,4-dioxane/water mixtures as solvents, at pH 10. Each reaction was conducted for 6 h and was monitored by TLC. The crude products were analyzed by ESI-MS. It was found that in the case of the reagent **II** the guanidinylation was complete and the crude product contained only Boc-*h*Arg-OH. In contrast to reagent **I**, both Boc-*h*Arg-OH and Boc-Lys-OH were present in the crude product. Reagent **I** proved to be not reactive enough in an aqueous-organic environment and therefore was not used in further studies.

*Model peptides*. The peptides used in these studies contained amino acid residues with an amino group in the side chain: lysine or L-2,3-diaminopropionic acid (Dap). The sequences of these peptides are shown in Table 1. The peptides were synthesised on Wang or Pam resin using standard Fmoc or Boc solid-phase peptide synthesis, respectively. TBTU or DIC were used as the coupling reagents. In all the peptides the side-chain amino function of the Lys and Dap residues was protected with an Fmoc group. To confirm the structure of each model peptide on the solid support, a small portion of peptide-resin was collected, then the peptide was cleaved and the crude product was analyzed by RP-HPLC and mass spectrometry. It was found that the expected peptide content in the crude product for peptides **1a** and **2a** was >90%, and for peptides **3a** and **4a** was >80%.

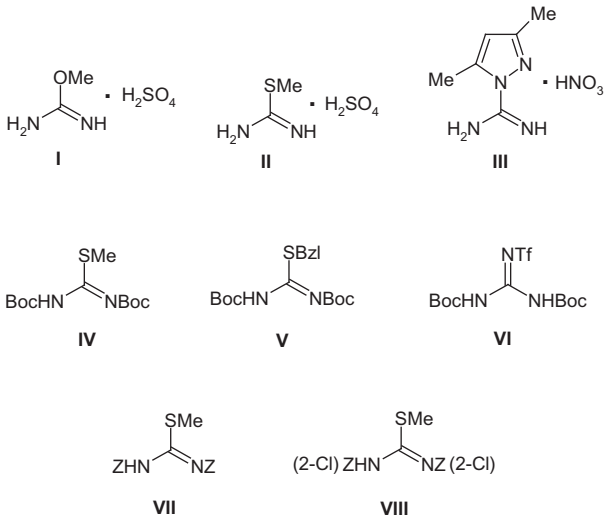


**Figure 2.** Guanidinylating reagents used in this study.

*Guanidinylation*. The Fmoc protection on the side-chain amino function of the lysine and 2,3-diaminopropionic acid residues were removed using a 30% piperidine solution in DMF before guanidinylation. Guanidinylation was carried out with a 3-fold excess of the reagents **II**–**VIII** in the presence of DIEA (pH ~9), both with and without (at room temperature) microwave irradiation. DMF was used as the solvent for the reactants **III**–**VIII** and a mixture of DMF/water (2:3 v/v) for reagent **II**. Microwave conditions: a temperature of 50 or 60 °C and a power of 20 or 40 W were typically applied to accelerate the reactions in solid phase peptide synthesis.[7b,7c] The progress of the guanidinylation was checked by qualitative determination of the free amino group (Kaiser test) every 24 h for reactions carried out at room temperature, and every hour for the reactions conducted under microwave conditions. The reaction was considered complete when a negative Kaiser test was obtained, or if no color change of the subsequent Kaiser test was observed. It was assumed that the reaction would not be carried out for longer than 10 days without microwave irradiation, and for 6 h in a microwave reactor, regardless of the Kaiser test result. Finally, it was found that the reaction time was 1–10 days in the case of the reaction at room temperature and 1–6 h under microwave conditions. Guanidinylation of **3** with **II** and **1** with **III** was only carried out under microwave conditions, since our previous (unpublished) experiments have shown that the time for guanidinylation of the peptide on the solid support with these reagents was longer than 10 days. After completion of the guanidinylation, the peptides were cleaved from the resin using HF (peptides **1** and **2**) or a TFA/TIS/$H_2O$ (95:2.5:2.5) (peptides **3** and **4**). The crude products were analyzed by RP-HPLC and ESI-MS. Details of the experimental process are provided in the Supplementary data section. The results are presented in Table 2.

The data presented show that better results (a higher content of the desired peptide and less by-products) were obtained by conducting the guanidinylation at room temperature without MW irradiation as compared to the same reaction with MW irradiation. It turned out that the use of microwave irradiation caused an increase in the content of side products. In particular, this relates to reagents **IV** and **VI**, the contribution of by-products in the crude product was 59–95% (entries 6, 8, 12, and 14). Only in two cases did the use of the microwave irradiation result in the expected effect, that is, during the guanidinylation of **3** with reagents **VII** and **VIII** (entries 16 and 20). In these cases, in a much shorter time (6 and 3 h, respectively) the desired product was obtained in a similar yield as the guanidinylation without microwave irradiation which required four days (entries 15 and 19).

In most cases, the nonguanidinylated peptide was found in the crude product. In some cases, this resulted from the fact that the