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# Improved Synthesis of Arginine Peptide Aldehydes

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**Abstract**: An improved method for the synthesis of peptide argininals by the use of a new aldehyde protecting group (diphenylmethyl semicarbazide) is reported.

There have been reports of various methods for the solution synthesis of peptide aldehydes[1-6]. Recently one of these procedures[1] has gained acceptance for the synthesis of peptide argininal inhibitors of thrombin.[7,8] This procedure relies on the reduction of an arginine lactam derivative with $LiAlH_4$. This procedure is limited to the synthesis of derivatives that do not contain groups that would be reduced by $LiAlH_4$. Procedures using this powerful reducing agent are not suited for the synthesis of derivatives containing, for example, protected aspartic acid or glutamic acid residues. An alternate approach uses the unsubstituted semicarbazide group as an aldehyde protecting reagent for the solution synthesis of these peptide transition-state analogs.[2,6] The latter method is potentially applicable to the solution synthesis of derivatives that are beyond the scope of the lactam procedure. In practice, however, the solution synthesis of these derivatives, using unsubstituted semicarbazones, is of restricted utility. The synthesis of derivatives that contain aspartic or glutamic esters has not been reported using this approach. In part this is due to the limited solubility of the protected argininal semicarbazone derivatives. The synthesis of a variety of peptide aldehydes that contain reduction sensitive groups has recently been reported. This method uses a substituted heterobifunctional semicarbazone linker group and represents a general automated solid-phase synthesis of these challenging synthetic targets.[9]

We report here a procedure which is based on this solid-phase method. The present method is useful for the preparation of larger amounts of peptide aldehydes than would normally be obtained by the use of the solid-phase method.[9] This procedure uses the diphenylmethyl semicarbazone group, which gives synthetic intermediates that have desirable solubility characteristics, in standard organic solvents. The diphenylmethyl semicarbazone group protection also allows for the switching of solubility characteristics, when desired, via removal of the diphenylmethyl group by anhydrous HF/anisole, which is concomitant with the removal of the amino acid side-chain protecting groups. Alternately, the one-pot complete deprotection of the protected peptide aldehyde is achieved, by hydrogenolysis of protecting groups and the accompanying simultaneous hydrolysis of the semicarbazone, via hydrogenation under acidic aqueous conditions.

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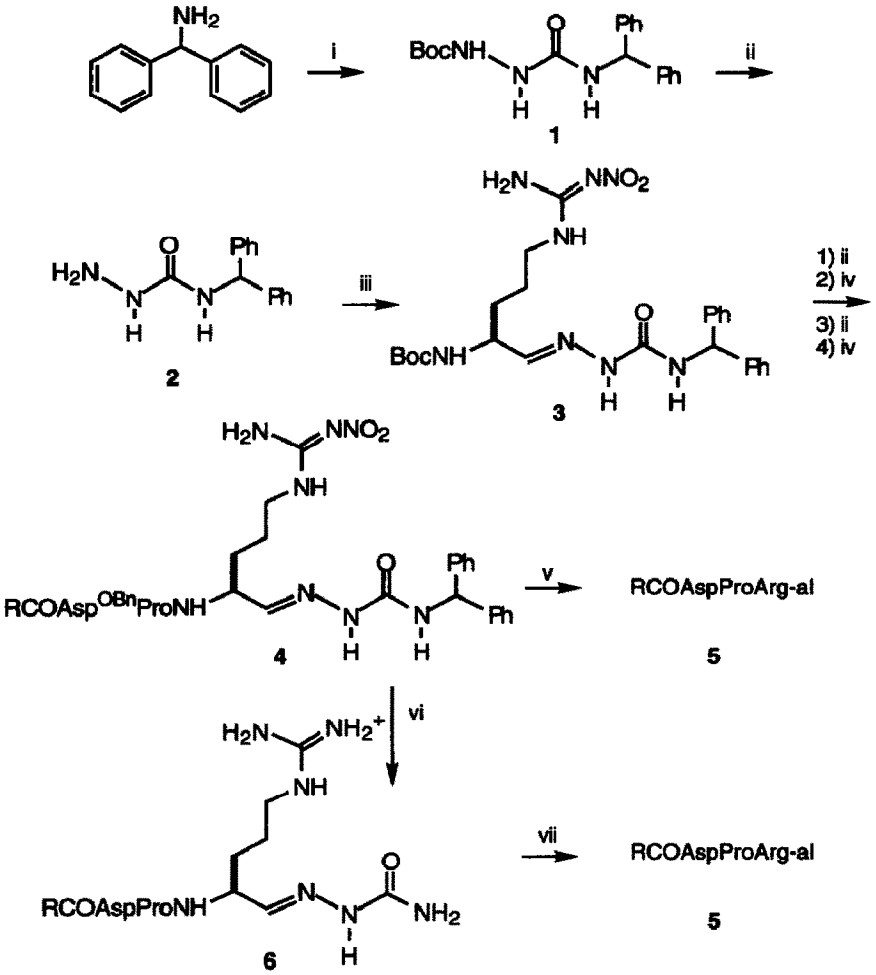
**Scheme 1.**




i) CDI/ t-Boc hydrazide followed by diphenylmethylamine . ii) TFA/ DCM. iii) Boc-N$^{g}$-nitroargininal/ NaOAc. iv) Protected amino acid / BOP/ NMM/ DMF. v) $H_2$/ Pd, $H_3O^+$. vi) HF/anisole. vii) $CH_2O$/ $H_3O^+$.

The synthesis of peptide argininals using this method begins with the synthesis of the protected diphenylmethyl semicarbazide derivative **2** (see Scheme 1). This type of procedure has been previously used to prepare semicarbazones of similar general structure.[9,10,11,12] The semicarbazide **2** is allowed to react with alpha-N-Boc-N$^{g}$-nitroargininal to give the protected argininal derivative **3**, using conditions that are identical to those published for the semicarbazones of similar structure.[10,13] The Boc derivative **3** is converted to the free amine with trifluoroacetic acid (TFA) in dichloromethane (DCM),[14] and protected amino acid derivative are added (either sequentially, or as blocks) using conditions that are standard for peptide couplings, for

example Bop in NMM/DMF. Fully protected peptide aldehydes such as **4** are obtained in this way. Compounds protected in this way can be purified by flash silica chromatography (0-10% MeOH/DCM). Products such as **4** can then be deprotected in one step, by hydrogenation in acidic aqueous methanol, to give **5** in modest yield (20%). The semicarbazones spontaneously hydrolyze under these conditions.[15] Alternately, treatment of **4** with anhydrous HF/anisole[16] gives the water soluble derivative **6**, in which the side chain protecting groups have been removed, along with the diphenylmethyl group. This derivative can then be directly converted to **5** by treatment with formaldehyde in aqueous acid, in 40-60% overall yield from **4**.[17] The final products can be purified by HPLC, as previously reported.[9] The overall yields are significantly better using this approach than those reported for the synthesis of the less functionalized derivatives, using the $LiAlH_4$ procedure.[7,8]

Using this procedure we have synthesized derivatives **6** where R= t-ButylO-, $PhCH_2CH_2$-, 2-propylpentanoyl-, and t-Butyl- in 40-60% yield, from the corresponding protected peptide aldehyde **4**. This method facilitates the synthesis of these formidable synthetic targets on a 0.1 to one gram scale. The side-chain protecting groups can be removed with hydrogen/Pd on carbon, but this is accompanied by partial reduction of the semicarbazone group, which lowers the yield of the desired product. The use of HF/anisole gives better yields and allows for the use of side-chain protecting groups that are standard in the synthesis of peptides.

**References and Notes;**

1) Bajusz, S.; Sznell, E.; Bagdy, D.; Barabas, E.; Horvath, G.; Dioszegi, M.; Fittler, Z.; Szabo, G.; Juhasz, A.; Tomori, E.; Szilagyi, G. *J. Med. Chem.* **1990**, *33:* 1729-1735 .

2) McConnell, R. M.; Barnes, G. E.; Hoyng, C. F.; Gunn, J. M. *J. Med. Chem.* **1990**, *33*:86-93 . McConnell, R. M.; York, J. L.; Frizzel, D.; Ezell, C. *J. Med. Chem.* **1993**, *36*: 1084-1089.

3) Kawamura, K.; Kondo, S.; Maeda, K.; Umezawa, H. *Chem. Pharm. Bull.* **1969**, *17*, 1902.

4) Someno, T. and Ishii, S., *Chem. Pharm. Bull.*, **1986**, *34*, 1748-1754 .

5) Westerik and Wolfenden, *J. Biol. Chem.* **1972**, *247*, 8195.

6) Ito, A.; Takahashi, R.; Baba, Y. *Chem. Pharm. Bull.* **1975**, *23*, 3081-3087 .

7) Balasubramanian, N.; St. Laurent, D. R.; Federici, M. E.; Meanwell, N. A.; Wright, J. J.; Schumacher, W. A.; Seiler, S. M. *J. Med. Chem.* **1993**,*36*, 300-303.

8) Shuman, R. T.; Rothenberger, R. B.; Campbell C. S.; Smith, G. S.; Gifford-Moore, D. S.; Gesellchen, P. D. *J. Med. Chem.* **1993**, *36*: 314-319.

9) A. M. Murphy, R. Dagnino Jr., P. L. Vallar, A. J. Trippe, S. L. Sherman, R. H. Lumpkin, S. Y. Tamura, and T. R. Webb, *J. Amer. Chem. Soc.* **1992** , *114*, 3156-3157.

10) J. Reiner, R. Dagnino, E. Goldman, and T. R. Webb, *Tetrahedron Lett.* **1993**, *34*, 5425-5428.

11) 1-t-Butoxycarbonyl-semicarbazidyl-4-diphenylmethane (1); A solution of 16.2 g (0.10 mole) of carbonyldiimidazole (CDI) in 225 mL of dimethylformamide (DMF) was prepared at room temperature and allowed to stir under nitrogen. A solution of 13.2 g (0.100 moles) t-butyl carbazate in 225 mL DMF was

then added dropwise over a 30 min. period. Next 18.3 g (0.10 moles) of diphenylmethylamine was added over a 30 min. period. The reaction was allowed to stir at room temperature under nitrogen for one hour. Water (10 mL) was added and this mixture was concentrated to about 150 mL under vacuum. This solution was poured into 500mL water and extracted with 400 mL of ethyl acetate. The ethyl acetate phase was extracted two times each with 75 mL 1N HCl, $H_2O$, $NaHCO_3$, NaCl and dried with $MgSO_4$. The mixture was filtered and the solution was concentrated to give 29.5 g (85% yield) of a white foam: mp 142-143 ° C.

12) Semicarbazidyl-4-diphenylmethane trifluoroacetate salt (**2**); A solution of 3.43 g (10 mmole) of **1** in 12.5 mL of dichloromethane was treated with 12.5 mL of trifluoroacetic acid (TFA) at 0°C and allowed to stir for 30 min at this temperature. After this time the solution was added dropwise to 75 mL of ether. A precipitate formed, and the mixture was filtered and washed with ether. Weight of crude product was 2.7 g (80% yield): mp 182-184 ° C.

13) α-N-(t-Butoxycarbonyl)-N$^g$-nitro-argininal-semicarbazonyl-4-N-diphenylmethane (**3**); A solution of 2.65 g (7.8 mmoles) of **2**, and 2.36 g (7.8 mmoles) of (alpha-N-(t-Butoxycarbonyl)-N$^g$-nitro-argininal) in 20 mL ethanol containing 6 mL of water, was treated with 1.2 g (8.8 mmoles) of NaOAc and refluxed for one hour. This solution was allowed to cool and then poured into water and extracted three times with ethyl acetate. The combined organic phase was washed with water, 0.1 N HCl, brine, dried ($MgSO_4$) and concentrated to a small volume. The white solid residue was recrystallized from acetonitrile/ether. This gave 3.2 g (78% yield): mp 78-79° C.

14) N$^g$-Nitro-argininal-semicarbazonyl-4-N-diphenylmethane trifluoroacetate salt; A solution of 0.53 g (1.0 mmole) of compound **3** in 5 mL of dichloromethane was treated with 5 mL of trifluoroacetic acid (TFA) at 0°C and allowed to stir for 30 min at this temperature. After this time the solution was added dropwise to 40 mL of ether. A precipitate formed, and the mixture was filtered and washed with ether. This gave 0.51 g of a pure white solid (97% yield): mp 159-160 ° C.

15) N-(2,2-Dimethylpropanoyl)-L-aspartyl-L-prolyl-L-argininal (**5**). A solution of 1.0 g (1.2 mmole) α-N-(2,2-dimethylpropanoyl)-L-aspartyl-β-(benzyl ester)-L-prolyl-L-N$^g$-nitro-argininal-semicarbazonyl-4-N-diphenylmethane (**4**) in 10 mL of 10% water in methanol is treated with 0.300 mL 1 N HCl and 0.200 g palladium on carbon. The solution is shaken with hydrogen at 5 psig for 45 minutes, then is passed through a fine fritted filter with Celite. The filter is washed with 10% water in methanol and the combined filtrates are concentrated to give the crude peptide aldehyde. The crude product is purified using reverse phase HPLC on a 10 micron particle size, 300 angstrom pore size C-18 column, eluting with a water-acetonitrile gradient (containing 0.1% trifluoroacetic acid) where the gradient is run from 5% to 40% acetonitrile.

16) Yamashiro, D.; Blake, J.; Li, C. H. *J. Am. Chem. Soc.* **1972**, 94, 2855-2859.

17) The general procedure is as follows; The crude unprotected semicarbazone (0.5 mmol), after treatment as in reference 16, is dissolved in a mixture of 5 mL THF, 1 mL glacial acetic acid, and 0.1 mL of 1 **M** aqueous HCl. This solution is then treated with 1 mL of 37% aqueous formaldehyde and allowed to stir for 1 h at r.t. This solution is diluted with water and purified as in note 15.

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