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Novel and General Method for the Preparation of Peptidyl Argininals

Susan Y. Tamura,* J. Edward Semple, Robert J. Ardecky, Pamela Leon, Steven H. Carpenter, Yu Ge, Brian M. Shamblin, Michael I. Weinhouse, William C. Ripka, Ruth F. Nutt

Corvas International, Inc. , 3030 Science Park Rd., San Diego, CA 92121

Abstract: A general method for the synthesis of peptidyl argininals was developed which utilizes the novel building block N^{g}-nitro-L-argininal ethyl aminal•HCl. The final aldehyde structure is generated by hydrolysis of the peptidic aminal moiety and can be applied to highly functionalized peptidic structures. The method is amenable to the preparation of large quantities of enantiomerically pure peptidyl argininals and was applied to the synthesis of two potent thrombin inhibitors. Copyright © 1996 Elsevier Science Ltd

Peptidyl argininals which inhibit Factor VIIa, Factor Xa and thrombin, trypsin-like serine proteases involved in the coagulation cascade, are of current high interest as therapeutic targets for the treatment of thrombotic vascular disease.[1] A currently favored method for the synthesis of peptidyl argininals utilizes the late stage hydride reduction of a N^{g}-Cbz-arginine lactam intermediate.[2] However, this strategy is limited to substrates which are stable to lithium aluminum hydride and thus could not be applied to our targets which contain ester, carboxylic acid, and other sensitive functionalities. Argininal syntheses via semicarbazone intermediates have been reported using both solution and solid phase methods;[3] however, these protocols were unacceptable for multigram scale preparations of these reactive molecules. The development of novel, efficient and general synthetic routes to several peptidyl argininal targets has become of paramount importance as studies of these inhibitors have advanced from the laboratory to preclinical and clinical stages.[4]

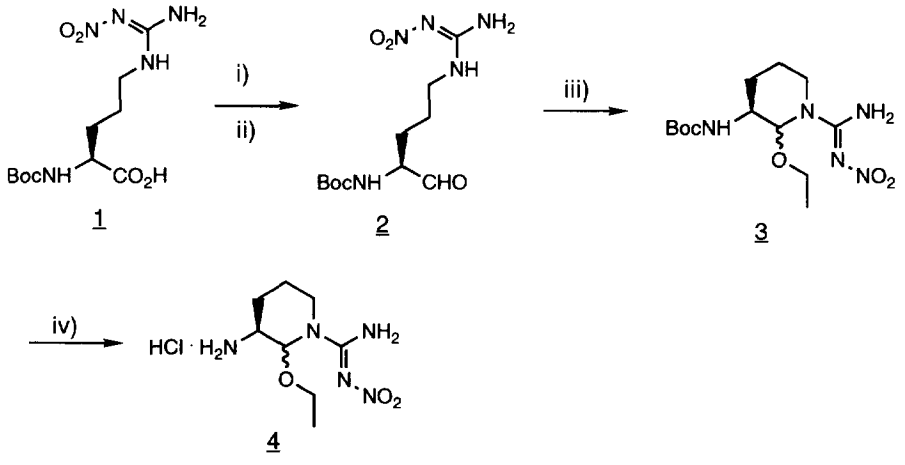
Thus, our criteria for the preparation of peptidyl argininals were the following: 1) a novel protected argininal synthon is formed early in the synthesis, 2) racemization is supressed during all steps of the synthesis, 3) the intermediates are easily handled, 4) the final step to release the aldehyde moiety involves a simple hydrolysis which can be easily monitored and rapidly quenched with base, 5) the final hydrolysis to unmask the aldehyde is highly selective in the presence of other sensitive moieties, and 6) this methodology is amenable to the preparation of hundred gram quantities of peptidyl argininals.

Herein are described the syntheses for two related peptidyl aldehydes: 2-propylpentanoylAsp(OMe)-ProArg-H (prpnD(OMe)PR-H) **10**, CVS 1123, and 2-propylpentanoylAspProArg-H (prpnDPR-H) **11**, CVS 738. Both are potent, orally active thrombin inhibitors, and each presented significant synthetic challenges for development.[5] Significantly, the final hydrolysis which provided CVS 1123 was carried out successfully in the presence of a methyl ester when performed with a non-nucleophilic acid.

The preparation of the key building block, N^{g}-nitro-L-argininal ethyl aminal•HCl, **4**, is described in **Scheme 1**. Boc N^{g}-nitro-L-arginine **1** was converted into the Weinreb amide in 88% yield via the mixed

anhydride method.[6] Reduction with lithium aluminum hydride following a modified procedure of Castro et al.[6] afforded Boc Nᵍ-nitro-L-argininal **2** in 74% yield.[7] Treatment of Boc Nᵍ-nitro-L-argininal **2** with ethanol in the presence of a catalytic amount of hydrochloric acid at ambient temperature produced the aminal **3** in 81% yield as a 2:1-6:1 (SS/SR) mixture of anomers.[8,9] The Boc group is quantitatively cleaved with HCl in ethanol to afford amino-aminal **4** as a 12:1 mixture of anomers.[10] The observed change in the ratio of anomers during deprotection is most likely a consequence of their thermodynamic stabilities. In this way 80g quantities of the intermediate aminal **3** could be easily prepared in our laboratory.

Scheme 1[a]



[a]Reagents: i) i-BuOCOCl, N-methylpiperidine; CH_3ONHCH_3•HCl, N-methylpiperidine, 88%; ii) $LiAlH_4$, THF, -60°C, 74%; iii) EtOH, HCl, 81%; iv) sat'd HCl in EtOH, 100%.

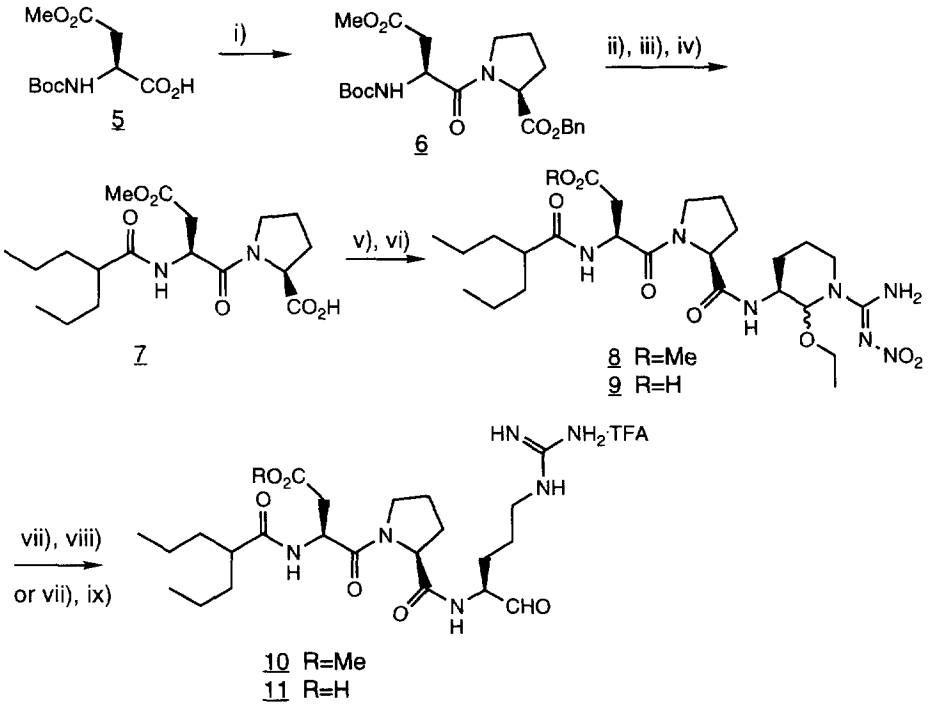
The preparation of the remaining peptide segment **7**, prpnD(OMe)P-OH, was accomplished in four steps from BocAsp(OMe)OH **5** (see **Scheme 2**). A mixed anhydride coupling was used to synthesize the dipeptide fragment **6** (91%). Removal of the Boc protecting group with HCl in ethyl acetate, coupling with the 2-propylpentanoic acid fragment, and hydrogenolysis of the benzyl ester gave the P_4-P_2 segment **7** in 52% overall yield. The coupling of fragment **7** to the aminal **4** was best accomplished with EDC/HOBt, and produced a 70% yield of crystalline intermediate **8**. This intermediate was used in the synthesis of the two peptidyl argininals.

Hydrogenation, followed by selective hydrolysis with HPF_6 produced the final aldehyde, prpnD(OMe)PR-al (CVS 1123) **10**, in 41% overall yield after preparative HPLC (Vydac C-18, 0.1% TFA/10-20% aqueous CH_3CN).[11] Hydrolysis of the intermediate ester **8** with lithium hydroxide produced the acid **9** in 47% yield. Hydrogenation, followed by hydrolysis with 3.0N HCl afforded prpnDPR-al (CVS 738), **11** in 29% overall yield after preparative HPLC.

In conclusion, a variety of peptidyl argininals may be routinely synthesized in multigram quantities using the Nᵍ-nitro-L-argininal ethyl aminal•HCl **4** as a novel building block. A convenient four step sequence from commercially available Boc Nᵍ-nitro-L-arginine produces the ethyl aminal **4** in 53% overall

yield. This method of preparation of peptidyl argininals is general, and has been used to synthesize a large number of peptidyl argininal targets. Novel serine protease inhibitors prepared using this methodology will form the subject of forthcoming publications from our laboratories.

Scheme 2[a]



[a]Reagents: i) i-BuOCOCl, 4-methylmorpholine; H-Pro-OBn•HCl, 91%; ii) HCl, EtOAc; iii) 2-propylpentanoic acid, i-BuOCOCl, 4-methylmorpholine, 90%; iv) H_2, 10% Pd/C, 58%; v) EDC, HOBt, 4-methylmorpholine, 70%; vi) LiOH, MeOH, 47%; vii) H_2, 10% Pd/C, EtOH, HOAc, H_2O, 100%; viii) 60% HPF_6, CH_3CN, H_2O, 41%; ix) 3.0N HCl, 29%.

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7. Boc N^g-nitro-L-argininal **2** was prepared according to a modified procedure of Goel et al.[4a] NMR and HPLC analyses indicate that **2** exists as a mixture of the two cyclic hemiaminal forms and the free aldehyde.
8. Details on the NMR structure determination will be reported in due course.
9. The experimental procedure for the preparation of **3**: Aldehyde **2** (41.6 g, 137 mmol) was dissolved in ethanol (200 ml) and conc. HCl (1.0 ml) was added. After the reaction was complete by TLC (silica gel, 10% methanol/dichloromethane), the solvent was removed under vacuum. The crude product was purified by flash chromatography using 0-10% ethyl acetate/dichloromethane as eluent to afford 36.88 g (81% yield) of ethyl aminal **3** as a pale yellow foam. R_f: 0.62 (silica gel, 5% methanol/dichloromethane). ^{1}H NMR (400 MHz, CD_3OD) δ 1.30 (2 overlapping t, 3H, Et), 1.43-1.85 (m, Boc+Arg β, γ) + 2.11 (tt, J=13.7, 4.5Hz, total 13H, Arg β, γ), 3.12 (t, J=12Hz, Arg δ) + 3.24 (dt, J=13.2, 2.6Hz, total 1H , Arg δ), 3.55-3.59 (m, 3H, Arg δ+Et), 3.70-3.82 (m, 1H, αCH),5.71 (brs) + 5.61 (brs, total 1H, anomeric CH).
10. The experimental procedure for the preparation of **4**: To a solution of ethyl aminal **3** (120.37 g) in absolute ethanol (1.1 l) at 0°C was added slowly HCl in ethanol (500 ml of a saturated solution). After 30 min at 0°C, this mixture was allowed to warm to room temperature over a period of 2h. Some of the HCl(g) was removed with a stream of nitrogen, and the solvent from the resulting solution was reduced to approximately 250 ml. Diethyl ether (2.0 l) was added, and the precipitate was filtered. The precipitate was washed thoroughly with ether, then immediately dried *in vacuo*. The resulting pale yellow solid, N^g-nitro-L-argininal ethyl aminal•HCl **4** (101.26 g, quantitative yield) was used without further purification. R_f: 0.45 (silica gel, methanol:dichloromethane:conc. ammonium hydroxide 5:25:1). ^{1}H NMR (400 MHz, CD_3OD) δ 1.30 (t, J= 7.0Hz, 3H), 1.67-2.05 (m, 4H), 3.26 (dt, J=13.4Hz, 2.8Hz, 1H), 3.55-3.41 (m, 1H), 3,55-3.70 (m, 2H), 3.72-3.79 (m, 1H), 5.95 (d, J=3.3Hz) + 6.05 (d, J=3.3Hz, total 1H).
11. The methyl ester is hydrolyzed in minute quantities (<3%) under these conditions.

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