Synthetic Communications

ISSN: 0039-7911 (Print) 1532-2432 (Online) Journal homepage: http://www.tandfonline.com/loi/lsyc20

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To cite this article: Michael S. Bernatowicz & Gary R. Matsueda (1993) An Improved Synthesis of N^G-Allyl-(L)-Arginine, Synthetic Communications, 23:5, 657-661, DOI: 10.1080/00397919308009824

To link to this article: http://dx.doi.org/10.1080/00397919308009824

SYNTHETIC COMMUNICATIONS, 23(5), 657-661 (1993)

AN IMPROVED SYNTHESIS OF N^G-ALLYL-(L)-ARGININE

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ABSTRACT: A novel, more efficient and practical synthesis of the title compound, recently reported as a potent inhibitor of nitric oxide synthase is described. Reaction of (L)-ornithine with 1H-pyrazole-N-allyl-1-carboxamidine allowed facile isolation of the title product in 70% yield.

Recently this laboratory reported on the utility of 1H-pyrazole-1-carboxamidine hydrochloride (1-guanylpyrazole hydrochloride) **1** as a reagent for the guanylation of amines including its use in solid phase peptide synthesis strategies employing the conversion of ornithine to arginine residues.[1] That study suggested that substituted derivatives of **1** could be useful in the preparation of more highly substituted guanidines such as interesting arginine derivatives. To test this hypothesis, a potentially important and pharmacologically relevant target, N^G-allyl-(L)-arginine,[2] was chosen for synthesis.

N^G-allyl-(L)-arginine **2** has been recently reported to behave as a potent (K_I = 3.4 μM) irreversible inhibitor of the inducible murine macrophage nitric oxide synthase.[2] Nitric oxide synthase, a recently discovered mammalian enzyme which catalyzes the conversion of L-arginine to citrulline and nitric oxide, is currently the subject of intensive research since its regulation has the potential for therapeutic applications[3] related to

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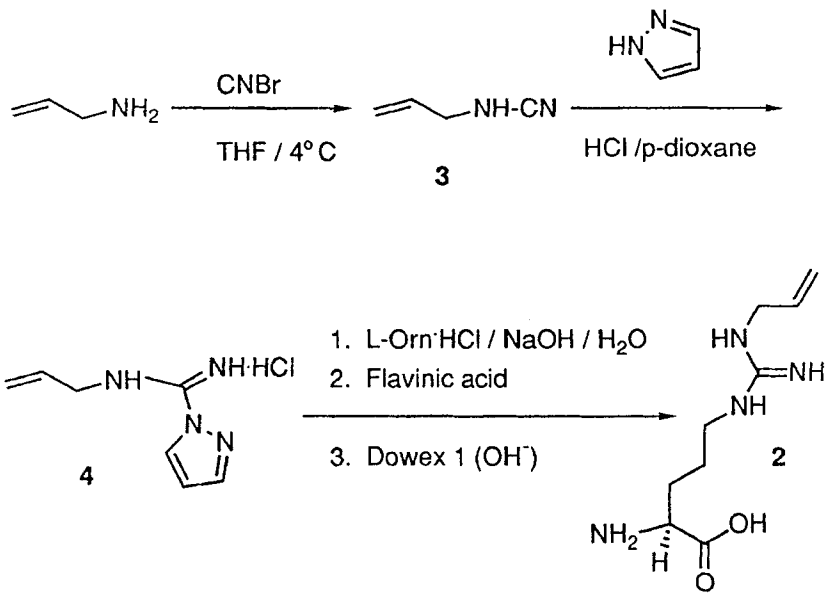

Scheme 1

homeostatic regulation of blood pressure and antiaggregatory effects on platelets.[4] In addition, nitric oxide liberated by this enzyme in macrophages has been shown to be needed for the cytotoxic and cytostatic functions of these cells.[5] Recent studies have also indicated that nitric oxide plays a role in the brain and may be important for memory formation.[6]

The preparation of **2** from L-ornithine hydrochloride is outlined in Scheme 1. Reaction of cyanogen bromide with allylamine produced crude allylcyanamide **3** which, without purification was promptly condensed with pyrazole in the presence of dry HCl in p-dioxane to give a quantitative isolated yield (based on allylamine limiting reagent) of crystalline 1H-pyrazole-N-allyl-1-carboxamidine hydrochloride **4** . Pyrazole **4** was used directly to essentially completely guanylate L-ornithine at Nδ to allow direct isolation of the pure monoflavinate salt of **2** from the reaction mixture in 70% yield. The free base **2** was obtained quantitatively from its monoflavinate after neutralization with Dowex 1 ion exchange resin in the

hydroxide form. The melting point of the monoflavinate salt of **2** thus obtained was in perfect agreement with that reported[2] and the ^1H NMR and mass spectra, as well as TLC (ninhydrin detection) and amino acid analysis by HPLC[7] of free base **2** were consistent with the literature describing a single ornithine-free product obtained by specific guanylation of ornithine at N^{δ}.[2]

Several properties of guanylating reagent **4** are noteworthy. Solution studies indicated that **4** was substantially less reactive with amines when compared to the previously reported unsubstituted guanylating reagent, 1-guanylpyrazole hydrochloride.[1] Nevertheless, the utility of **4** as a reagent for the preparation of **2** is obvious and dramatic. It is also interesting that **4**, like the commonly used pseudothiouronium salt guanylating reagents previously used to prepare N^G-substituted arginines,[2, 8, 9] was found to specifically produce the ornithine N^{δ} guanylation product. Thus Cu(II) complexation of ornithine in order to protect N^{α} [10, 11] is also not necessary using **4**.

The advantages of the synthesis of **2** as described in this report are clear and significant: 1.) Guanylating reagent **4** can be easily prepared in 2 steps in quantitative yield from readily available starting materials in contrast to N-allyl-S-methylpsedothiouronium iodide which was prepared in 4 steps with a 22% overall yield,[2] 2.) The isolated yield of **2** was improved from 5.4% to 70% for the final guanylation step, 3.) Although relatively slow, reaction of **4** with ornithine was essentially complete and specific using a modest 30% excess of reagent which allowed for direct isolation with simultaneous purification of the product as the crystalline monoflavinate salt thereby avoiding the need for ion exchange chromatography to separate unreacted ornithine from product.

The procedure reported here with appropriate minor modification is likely to provide efficient access to a variety of N^G-substituted arginine analogs of potential mechanistic and pharmacological interest starting with variously substituted analogs of **4**. Studies in this laboratory are in progress to provide a better understanding of the influence of substitution on reactivity and further define the synthetic scope and limitations of other N-substituted 1-guanylpyrazole analogs for the preparation of di and trisubstituted guanidines in general.

EXPERIMENTAL

Materials: Allylamine, cyanogen bromide, pyrazole, L-ornithine hydrochloride, and flavinic acid hydrate were obtained from Aldrich Chemical Co. (Milwaukee, WI) and were used as purchased. Dowex 1 (2% crosslinked, Cl^- form, 50-100 mesh) was purchased from Sigma (St. Louis, MO) and was converted to the hydroxide form by treatment with aqueous 2 N NaOH followed by washing with water to remove excess hydroxide. Tetrahydrofuran (THF) was freshly distilled from sodium benzophenone ketyl. HCl (4 N) in p-dioxane was obtained from Pierce Chemical Co. (Rockford, IL). Ether refers to anhydrous diethyl ether. All other solvents were of the highest quality available.

1H-Pyrazole-N-allyl-1-carboxamidine hydrochloride (4). To allylamine (11.3 ml, 151mmol) in 20 ml of dry THF at 4 °C was added cyanogen bromide (10.0 g, 94.4 mmol) in 10 ml of THF dropwise with stirring under a nitrogen atmosphere. The reaction was stirred at 4 °C for 3 h after which 70 ml of ether were added. The resulting mixture was cooled to 4 °C and the solid allylamine hydrobromide byproduct was removed by filtration. Evaporation and partial drying *in vacuo* of the filtrate furnished 7.50 g of crude allylcyanamide **3** as a slightly yellow liquid which was used immediately as obtained for the next reaction. Mass spectral analysis of a small sample of **3** produced a peak at $(M + H)^+$ =83, calcd 82 (M). To the entire sample of **3** thus obtained was added 30 ml of p-dioxane, pyrazole (5.17g, 76.0 mmol) and 20 ml of 4 N HCl in p-dioxane and the mixture was stirred for 18 h at room temperature after which 30 ml of ether were added and the mixture cooled to 4 °C. The crystalline product was collected by filtration, washed with ether, partially dried and stirred with a mixture of 50 ml of ether and 15 ml of acetone. The insoluble product was collected by filtraton, washed with ether and dried *in vacuo* to constant weight to yield 14.2 g (100% based on starting allylamine) of the title compound **4** as a nearly pure crystalline solid which was used for the next step without further purification. mp 120 °C (softens) 131-34 °C. ^1H NMR (DMSO-d_6) δ 4.20-4.35 (br m, 2), 5.15-5.50 (m, 2), 5.85-6.05 (m, 1), 6.83 (d, 1), 8.16 (s, 1), 9.22 (d, 1), 9.92 (br s, 1), 10.32 (br s, 1), 10.62 (br t, 1). MS 151 $(M + H)^+$, calcd (as free base) 150 (M).

N^G-allyl-(L)-arginine (2). A mixture of L-ornithine hydrochloride (1.68 g, 10 mmol), **4** (2.42 g, 13 mmol), 2.3 ml of 10 M aqueous NaOH, amd 0.5 ml of water was stirred for 5 days at room temperature. To the mixture was added flavinic acid

hydrate (3.15 g, 10 mmol) in 12 ml of water in portions with mixing. The mixture was cooled to 4 °C and the yellow crystalline monoflavinate salt of **2** was collected by filtration, washed with small portions of ice cold water followed by ethanol and dried *in vacuo* to yield 3.72 g (70%). mp 238-40 °C (lit.[2] mp 238-40 °C). To 3.40 g (6.44 mmol) of the monoflavinate salt was added 45 ml water and 35 ml Dowex 1 (see **Materials**) and the mixture was stirred overnight to produce an essentially colorless supernatant. The resin was removed by filtration and washed with water (3 X 10 ml). The combined filtrate and washes were lyophilized to yield 1.54 g of a sticky solid. This residue was dissolved in methanol and rotary evaporated. The amorphous solid thus obtained was suspended in acetone and the acetone was evaporated at reduced pressure to produce a foamed amorphous solid which was dried to constant weight *in vacuo* to yield 1.48 g (107%) of **2** which could be pulverized and easily handled as a dry powder. The mass and ^1H NMR spectra (D_2O) of **2** were in agreement with those reported[2] except an additional small singlet at 2.18 ppm δ was observed indicating the presence of 7% by weight of nonvolatile acetone in the product.

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(Received in USA 15 September, 1992)