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LETTERS TO THE EDITOR

Synthesis of *N*-[*N*^{1}-(2,4,6-Trimethylphenylsulfonyl)-carbamimidoyl]-L-proline

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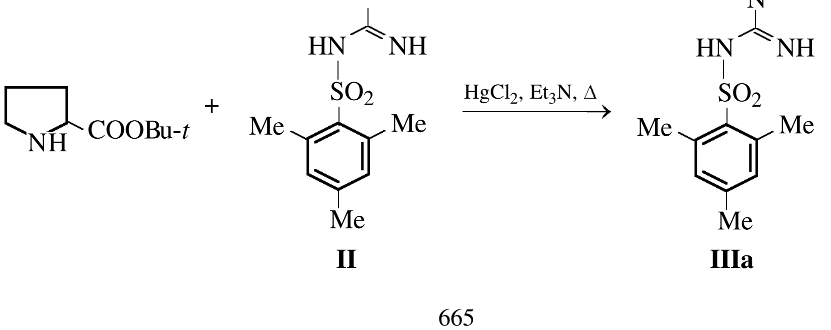
Unnatural amino acids are widely used in the synthesis of drugs on the basis of peptides and peptidomimetics [1, 2]. Introduction of nonproteinogenous amino acids possessing a guanidine moiety into a peptide chain increases the stability of peptides and their analogs to enzymatic cleavage and in some cases favors stabilization of their “biologically active” conformation. As a version of such modification we selected *N*-carbamimidoyl-L-proline (**I**) [3]. The use of this amino acid in peptide synthesis implies preparation of protected derivatives which should be soluble in organic solvents and should prevent the guanidine moiety from participating in side reactions. As protecting group we selected 2,4,6-trimethylsulfonyl (mesitylsulfonyl, Mts) which is used to block the guanidine function in arginine [4].

Kent et al. [5] described the synthesis of related compounds from 2-methyl-2-thiopseudourea (**II**) protected with various groups. Following an analogous scheme, derivative **IIIa** was obtained in 36% yield (Scheme 1). Analysis of the optical purity of the product via separation of diastereoisomeric pairs of protected dipeptides **IVa** and **IVb** by reversed-phase high-performance liquid chromatography (HPLC) indicated partial racemization during the synthesis of **IIIa** (the fraction of the *D* isomer was greater than 3%).

The limited accessibility of the initial reactants in combination with partial loss of optical activity prompted us to search for an alternative synthetic scheme. We believed that the most promising is direct protection of the guanidine moiety in compound **I** which can be prepared in a fairly good yield (53%) according to Scheme 2. The subsequent treatment of **I** with mesitylenesulfonyl chloride (**V**) gave compound **IIIb** with a high optical purity. *N*-carbamimidoyl-L-proline **IIIb** is readily soluble in organic solvents, and it can be used in the synthesis of peptides using both classical and solid-phase techniques.

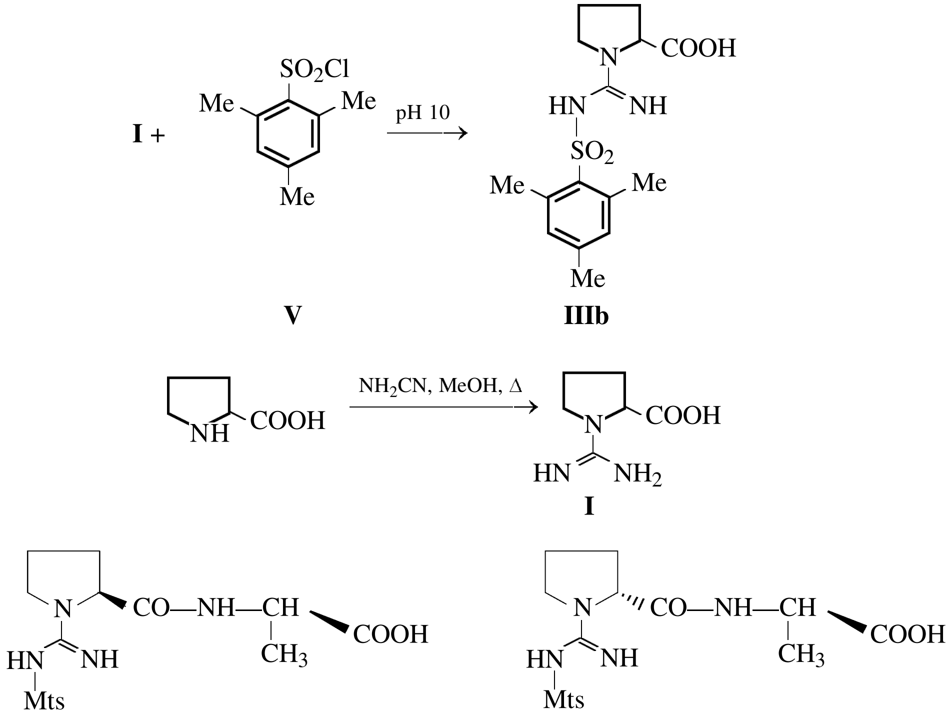
2-Methyl-1-(2,4,6-trimethylphenylsulfonyl)-2-thiopseudourea (II). A solution of 314 mg of mesitylenesulfonyl chloride (**V**) in 1 ml of THF was added dropwise under stirring over a period of 5 min to a solution of 50 mg of 2-methyl-2-thiopseudourea

Scheme 1.



II **IIIa**

Scheme 2.



V IIIb

I

IVa IVb

in 1.5 ml of THF and 2.5 ml of a saturated solution of sodium carbonate. The mixture was stirred for 12 h at room temperature, THF was removed under reduced pressure, and the remaining aqueous solution was extracted with ethyl acetate. The organic phase was washed with water and a saturated solution of sodium chloride and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the residue was crystallized from petroleum ether. Yield 77.4 mg (79%), mp 127–129°C. ^1H NMR spectrum ($CDCl_3$), δ, ppm: 2.28 d (6H), 2.67 s (6H), 6.92 s (2H).

***N*-[*N*^{1}-(2,4,6-Trimethylphenylsulfonyl)carbamimidoyl]-L-proline *tert*-butyl ester (IIIa).** Triethylamine, 0.33 ml, and mercury(II) chloride, 0.35 g, were added to a solution of 0.2 g of L-proline *tert*-butyl ester and 0.381 g of compound **II** in 2 ml of anhydrous THF. The mixture was heated for 14 h under reflux, and additional portion, 0.191 g, of compound **II** was added, and the mixture was heated for 4 h more under reflux. The mixture was evaporated, the residue was dissolved in ethyl acetate, the solution was filtered through a filter paper, and the filtrate was washed in succession with water, a saturated solution of $NaHCO_3$, 0.1 M hydrochloric acid, and a saturated solution of NaCl and dried over anhydrous Na_2SO_4. The organic phase was evaporated under reduced pressure, and the oily residue was subjected to chromatography on silica gel using ethyl acetate–petroleum ether as eluent. Yield 166.7 mg (36%), mp 107–109°C. ^1H NMR spectrum ($CDCl_3$), δ, ppm: 1.27 s (9H), 1.76 m (1H), 2.00 m (3H), 2.23 s (3H), 2.66 s (6H), 3.27–3.47 m (2H), 4.3 m (1H), 6.25 s (2H), 6.84 s (2H).

***N*-[*N*^{1}-(2,4,6-Trimethylphenylsulfonyl)carbamimidoyl]-*L*-proline (IIIb).** Compound **I**, 0.1 g, was dissolved in a mixture of 1.7 ml of a 1 M solution of NaOH and 0.5 ml of acetone, and a solution of 0.25 g of mesitylenesulfonyl chloride (**V**) in 1 ml of acetone was added dropwise over a period of 10 min at room temperature, maintaining pH 10–11. After 24 h, the solvent was removed under reduced pressure, the residue was dissolved in water, and the solution was washed with ethyl acetate. The aqueous phase was acidified to pH 2 with 1 M sulfuric acid, and the product was extracted into ethyl acetate. The extract was washed in succession with 1 M H_2SO_4, water, and a saturated solution of NaCl and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure, and the residue was crystallized from diethyl ether. Yield 35.3 mg (18%), mp 161–164°C. Mass spectrum, *m/z*: 339.09 $[M]^+$. Calculated: *M* 339.13. ^1H NMR spectrum (DMSO-d_6), δ, ppm: 1.91–1.98 m (3H), 2.12 m (1H), 2.23 s (3H), 2.56 s (6H), 3.30–3.38 m (2H), 4.26 m (1H), 6.88 s (2H), 6.99 s (2H).

The ^1H NMR spectra were obtained on a Bruker

AC-200 spectrometer at 200 MHz using TMS as reference. Reversed-phase HPLC was performed on a Beckman System-Gold chromatograph. The mass spectra were recorded on an MKh-5303 instrument (electrospray ionization, time-of-flight analyzer).

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