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# N-Trifluoroacyl Lysine Derivatives in the Synthesis of L-Lysyl-L-glutamic Acid

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**Abstract**—Conditions were developed for simultaneous preparation of $N^{a}$-trifluoroacetyl-L-lysine and $N^{\alpha}$,$N^{a}$-bis(trifluoroacetyl)-L-lysine at overall conversion of initial lysine monohydrochloride up to 82%. By reaction of dimethyl L-glutamate with $N^{\alpha}$,$N^{a}$-bis(trifluoroacetyl)-L-lysyl chloride in the presence of triethylamine or with $N^{\alpha}$-carboxyanhydride of $N^{a}$-trifluoroacetyl-L-lysine with subsequent removing protecting groups in the formed dipeptides by treating with water-ethanol solution of sodium hydroxide we obtained L-lysyl-L-glutamic acid. Physicochemical characteristics of samples obtained coincided with characteristics of L-lysyl-L-glutamic acid described in the literature thus suggesting that no racemization occurred either at the stage of peptide bond formation or at deprotection.

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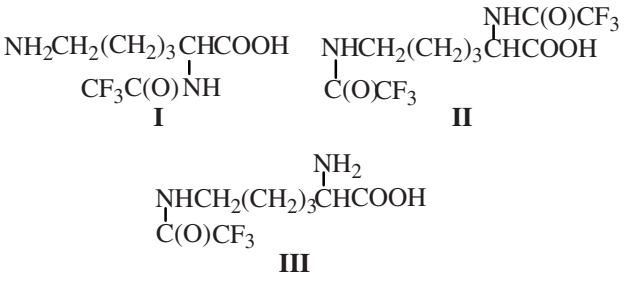
Development of efficient drugs with no side action is an urgent target of modern synthetic and pharmaceutical chemistry. One of the ways to solution of this problem is application amino acids and their derivatives as drugs substance. Recently the attention of researchers was directed to amino acids derivatives like di- and polypeptides. The interest to these compounds and considerable efforts applied to their synthesis and investigation originates from the high pharmaceutical activity of quite a number of this class substances. Based on peptides are prepared and widely used in medical practice cardiovascular and antinuclear prepartions, analgesics, immunomodulators, cardioprotectors etc. Notwithstanding the large number of peptide preparations used in medicine the development of new convenient procedures for the synthesis of applied peptides, producing new representatives of this class compounds, the study of their physiological and physicochemical characteristics is very pressing.

One among the important problems of the peptide synthesis is the choice of an optimum combination of protective groups. Nowadays the amino groups of amino acids are commonly protected by uretane type moieties (Boc, Cbz). The application of trifluoroacetyl structures for protection of amino groups is only just mentioned in the most treatises on peptide synthesis. Nonetheless, recently the trifluoroacyl protective groups attract attention of researchers more frequently. For instance, $N^{\varepsilon}$-trifluoroacetyl-L-lysine was used in the synthesis of lysinopril a substance of an antihypertension drug [1]. The use in the peptide synthesis was suggested of $N^{\alpha}$,$N^{\varepsilon}$-bis-(trifluoroacetyl)-L-lysyl chloride [2]. The low price and easy removal of the trifluoroacyl groups predict that they are promising for application to the peptide synthesis.

This study is dedicated to investigation of the possible use of trifluoroacyl protective groups in the synthesis of L-lysyl-L-glutamic acid, a dipeptide endowed with a strong immunostimulating action that improves the indices of cell and humoral immunity at a dose 10–100 times less that the currently applied immunomodulating drug thymogen [3].

We planned to use in the preparation of the target dipeptide both mono- and bis(trifluoroacetyl)-L-lysine. Versatile procedures are described for the synthesis of mono- and bistrifluoroacyl lysisne derivatives, and therewith depending on the character of acylating agent, reagents ratio, and the solvent used one or the other reaction product can be obtained in a good yield. The acylation of D,L-lysine with trifluoroacetic anhydride in trifluoroacetic acid solution depending on the reagents ratio led to the formation either of $N^{\alpha}$-trifluoroacetyl-D,L-lysine (**I**), or $N^{\alpha}$,$N^{\varepsilon}$-bis(trifluoroacetyl)-D,L-lysine (**II**) [4]. In acylation of lysine with S-ethyl trifluorothio-

acetate 5] or with ethyl trifluoroacetate [6] in water medium formed $N^{\varepsilon}$-trifluoroacetyllysine (**III**), and the course of the reaction involving ethyl trifluoroacetate strongly depended on the pH of the reaction mixture [6]. The same reaction carried out in dioxane an a large excess of ethyl trifluoroacetate resulted in bis(trifluoroacetyl)-lysine [7]. $N^{\alpha}$,$N^{\varepsilon}$-Bis(trifluoroacetyl)-L-lysine was obtained in a high yield at treating lysine dispersion in methyl trifluoroacetate with excess 1,1,3,3-tetramethylguanidine [8].

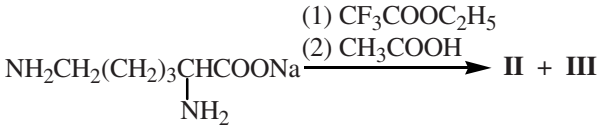


**I** **II**



**III**

In the present study we developed conditions for simultaneous synthesis of mono- and di(trifluoroacyl) lysine derivatives with an overall conversion over 80%. The method consisted in the reaction of L-lysine sodium salt with ethyl trifluoroacetate in anhydrous ethanol.



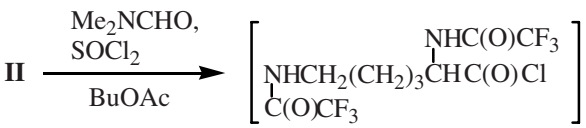
The use of ethanol as solvent provided a possibility to synthesize the lysine sodium salt from the available lysine monohydrochloride (the arising sodium chloride insoluble in ethanol was easily removed by filtration), and also to isolate from the reaction mixture on acidifying the formed sparingly soluble in ethanol $N^{\varepsilon}$-trifluoroacetyl-L-lysine by simple filtration.

Already at the molar ratio lysine–ethyl trifluoroacetate 1:1.25 in the reaction mixture alongside the monotrifluoroacetyllysine a significant amount is present of bis(trifluoroacetyl)lysine. The larger amount of ethyl trifluoroacetate introduced into the reaction resulted in greater yield of bis(trifluoroacetyl)-L-lysine, and lysine conversion attained 80%. At the reagents ratio 1:2.25 the bis(trifluoroacetyl)-L-lysine is the main reaction product. Only trace amounts of the monotrifluoroacetyllysine were isolated from the reaction mixture: The quantity of precipitate obtained by filtration of the reaction mixture after acidifying did not exceed 0.02% calculated on the initial lysine hydrochloride (the IR spectrum of the precipitate was identical to that of authentic $N^{\varepsilon}$-trifluoroacetyl-L-lysine).

Variation of the reaction temperature in the range from 0 to 15°did not virtually affect the yield of the reaction products.

The $^1$H NMR spectrum of monotrifluoroacyl derivative contains single resonances from the CH group (δ 3.72 ppm, t, 1H), and from $CH_2N$ group (δ 3.36 ppm, t, 2H) indicating that acylation occurred exclusively at one definite nitrogen atom. The melting point of samples obtained (251–255°C) is considerably higher than the melting point of $N^{\alpha}$-trifluoroacetyllysine (233°C [4]) suggesting that our experiments yielded $N^{\varepsilon}$-trifluoroacetyllysine. The same conclusion of formation of $N^{\varepsilon}$-trifluoroacetyllysine follows from the identity of the IR spectra of our samples and of $N^{\varepsilon}$-trifluoroacetyl-L-lysine prepared by method [9] [stretching vibrations $\nu(C{=}O_{amide})$ give rise to a band at 1698 $cm^{-1}$, bending vibrations $\delta(NH_{amide})$, at 1581 $cm^{-1}$]. The slightly lower melting point and specific rotation of the samples obtained as compared to pure $N^{\varepsilon}$-trifluoroacetyl-L-lysine may be due to partial racemization (mp of $N^{\varepsilon}$-trifluoroacetyl-D,L-lysine is 226–231°C [5]).

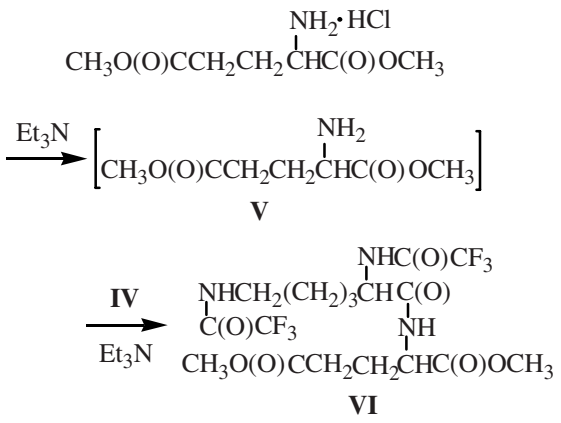
As already mentioned, till recently the use of trifluoroacyl protective groups in the peptide synthesis was limited due to racemization occurring both in the stage of preparation of activated derivatives of trifluoroacetylamino acids and in the stage of peptide bond formation. However in 2003 conditions were found [2] practically excluding the racemization and permitting the preparation of acyl chlorides from *N*-trifluoroacetylamino acids and their appliction in the peptide synthesis. We used this procedure in the present study for preparation of L-lysyl-L-glutamic acid. Acyl chloride **IV** was obtained from $N^{\alpha}$,$N^{\varepsilon}$-bis(trifluoroacetyl)-L-lysine (**II**) and on distilling off excess thionyl chloride in a vacuum the substance was without isolation used in the synthesis of the peptide.



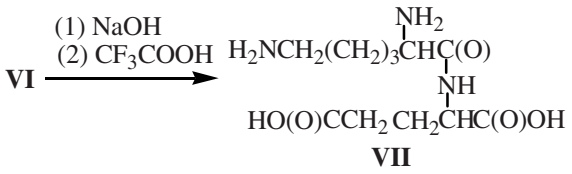
**IV**

The reaction of the solution of compound **IV** with a mixture obtained by treating dimethyl L-glutamate hydrochloride (**V**) with triethylamine led to the formation

in up to 55% yield of dimethyl $N^{\alpha}$,$N^{\varepsilon}$-bis(trifluoroacetyl)-L-lysyl-L-glutamate (**VI**).

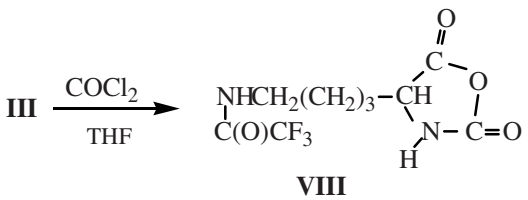


The treatment of the ethanol solution of compound **VI** with water-ethanol sodium hydroxide solution followed by acidifying of the reaction mixture with the trifluoroacetic acid provided L-lysyl-L-glutamic acid (**VII**) in up to 80% yield.



The optical activity of our samples of compound **VII** {$[\alpha]_D^{20}$ 22.4° (*c* 3.0, $H_2O$)} is practically identical to that of the L-lysyl-L-glutamic acid samples described in the literature {$[\alpha]_D^{19}$ 22.9° (*c* 2.0, $H_2O$) [10], $[\alpha]_D^{20}$ 20.0° (*c* 3.0, $H_2O$) [3]}. This fact may be considered as a proof that no racemization occurs either at the stage of peptide bond formation or at deprotection. The developed method of L-lysyl-L-glutamic acid preparation has great advantages compared with the published procedures [3, 10] because the removal of protective groups occurs in one stage without application of an expensive palladium catalyst .

In this study we used in the preparation of L-lysyl-L-glutamic acid also $N^{\varepsilon}$-trifluoroacetyl-L-lysine which was preliminary converted into *N*-carboxyanhydride of $N^{\varepsilon}$-trifluoroacetyl-L-lysine (**VIII**).



The reaction of *N*-carboxyanhydride **VIII** with dimethyl L-glutamate at the temperature below –50°C in THF followed by the treatment of the reaction mixture with with water-ethanol sodium hydroxide solution followed by acidifying with the trifluoroacetic acid resulted in dipeptide **VII**.

The data of this study demonstrated the promising character of application of N-trifluoroacyl lysine derivatives in the synthesis of lysine-containing dipeptides.

## EXPERIMENTAL

IR spectra were recorded on a IR-Fourier spectrophotometer Protege-460 from samples pelletized with KBr. $^1$H NMR spectra were registered on spectrometer Tesla BS-567A, chemical shifts of protons were measured with respect to TMS. The syntheses were carried out in anhydrous solvents.

Dimethyl L-glutamate hydrochloride was prepared by procedure [11].

***N*$^{\alpha}$,*N*$^{\varepsilon}$-Bis(trifluoroacetyl)-L-lysine (II).** To a solution of 4.623 g (201 mmol) of sodium in 200 ml of ethanol was added 18.25 g (100 mmol) of L-lysine hydrochloride. The reaction mixture was stirred for 1 h, cooled to 5°C, then slowly dropwise was added at vigorous stirring 31.95 g (225 mmol) of ethyl trifluoroacetate. After the end of addition the reaction mixture was stirred for 2 h at 5°C, then 3 h at room temperature. To the dispersion thus obtained was added 11.514 g (101 mmol) of trifluoroacetic acid, and the stirring was continued for 1 h more. The solvent was distilled off at reduced pressure, the residue was dissolved in 200 ml of water, the reaction product was extracted into ether (3×150 ml). The extract was dried with sodium sulfate. The solution obtained was filtered and concentrated by distilling off ether in a vacuum to a volume of 75 ml. The precipitate separated on addition of 300 ml of hexane was filtered off, washed with hexane, and dried in a vacuum. Then it was reprecipitated from ether with hexane. Yield 27.9 g (82.5%), colorless powder, mp 114–116°C, $[\alpha]_D^{20}$ –6.2° (*C* 2.0, ethanol) {116–118°C, $[\alpha]_D^{20}$ –6° (*C* 2.0, ethanol) [12]}. IR spectrum, ν, cm$^{-1}$: 1738 ($C{=}O_{carboxyl}$), 1704 ($C{=}O_{amide}$), 1560 ($NH_{amide}$). $^1$H NMR spectrum [$(CD_3)_2CO$], δ, ppm: 1.3–2.0 m (6H, $3CH_2$), 3.25–3.40 m (2H, $CH_2$), 4.30–4.65 m (1H, CH), 7.6 s (NH), 8.5 br.s (NH). Found, %: C 35.70; H 3.69; N 8.14. $C_{10}H_{12}F_6N_2O_4$. Calculated, %: C 35.51; H 3.58; N 8.28.

***N*$^{\varepsilon}$-Trifluoroacetyl-L-lysine (III).** To a solution of 4.623 g (201 mmol) of sodium in 200 ml of ethanol was

added 18.25 g (100 mmol) of L-lysine hydrochloride. The reaction mixture was stirred for 1 h, the separated precipitate was filtered off in a nitrogen flow. To the filtrate cooled to 5°C slowly dropwise was added at vigorous stirring 21.3 g (150 mmol) of ethyl trifluoroacetate. After the end of addition the reaction mixture was stirred for 2 h at 5°C, then 1 h at room temperature. The precipitate separated on adding 6.06 g (101 mmol) of acetic acid was filtered off and washed in succession with ethanol and acetone, then dried in a vacuum. Then it was reprecipitated from water with ethanol. Yield 15.01 g (62%), colorless powder, mp 252–254°C (decomp.), $[\alpha]_D^{20}$ +16.7° (*c* 1.0, 1 N HCl) {260–262°C (decomp.), $[\alpha]_D^{25}$ +18.6° (*c* 1.0, 1 ν. HCl) [9]}. IR spectrum, ν, $cm^{-1}$: 1698 ($C{=}O_{amide}$), 1581 ($NH_{amide}$). $^1H$ NMR spectrum ($D_2O$), δ, ppm: 1.3–2.0 m (6H, $3CH_2$), 3.36 t (2H, $CH_2$), 3.72 t (1H, CH). Found, %: C 39.81; H 5.63; N 11.42. $C_8H_{13}F_3N_2O_3$. Calculated, %: C 39.67; H 5.42; N 11.57.

**Simultaneous preparation of compounds II and III.** To a solution of 4.623 g (201 mmol) of sodium in 200 ml of ethanol was added 18.25 g (100 mmol) of L-lysine hydrochloride. The reaction mixture was stirred for 1 h, the separated precipitate was filtered off in a nitrogen flow. To the filtrate cooled to 5°C slowly dropwise was added at vigorous stirring 24.85 g (175 mmol) of ethyl trifluoroacetate. After the end of addition the reaction mixture was stirred for 2 h at 5°C, then 2 h at room temperature. The precipitate separated on adding 11.514 g (101 mmol) of trifluoroacetic acid was filtered off and washed in succession with ethanol and acetone, then dried in a vacuum.. Yield of compound **III** 6.05 g (25%).

From the filtrate after separation of the compound **III** precipitate the solvent was distilled off at reduced pressure, the residue was dissolved in 200 ml of water, the reaction product was extracted into ether (3×150 ml). The extract was dried with sodium sulfate. The solution obtained was filtered and concentrated by distilling off ether in a vacuum to a volume of 75 ml. The precipitate separated on addition of 300 ml of hexane was filtered off, washed with hexane, and dried in a vacuum. Yield of compound **II** 18.94 g (56%).

**Dimethyl $N^{\alpha}$,$N^{\varepsilon}$-bis(trifluoroacetyl)-L-lysyl-L-glutamate (VI).** To cooled to –15°C solution of 16.9 g (50 mmol) of compound **II** and 8.395 g (115 mmol) dimethylformamide in 150 ml of butyl acetate was slowly added dropwise 13.685 g (115 mmol) of thionyl chloride. The reaction mixture was stirred at –15°C for 4 h, and excess thionyl chloride was distilled off in a vacuum. The solution obtained was cooled to –5°C, 11.11 g (110 mmol) of triethylamine was added, and to the formed dispersion cooled to –5°C was added dropwise 10.575 g (50 mmol) of dimethyl L-glutamate hydrochloride (**V**) in 150 ml of butyl acetate. The reaction mixture was stirred at –5°C for 2 h, the precipitate was filtered off, the solution was washed with water (2×100 ml), and dried with sodium sulfate. The butyl acetate was distilled off in a vacuum, the reaction product was extracted from the residue with dichloromethane (3×100 ml). The solution obtained was filtered and concentrated to a volume of 75 ml. On adding 300 ml of ethyl ether the separated precipitate was filtered off, washed with ether, and dried in a vacuum. Then it was reprecipitated from dichloromethane with ether. Yield 13.365 g (54%), colorless powder, mp 104–106°C, $[\alpha]_D^{20}$ –26.2° (*C* 3.0, ethanol). IR spectrum, ν, $cm^{-1}$: 1742, 1733 ($C{=}O_{carboxyl}$), 1702, 1667 ($C{=}O_{amide}$), 1560, 1548 ($NH_{amide}$). $^1H$ NMR spectrum ($CDCl_3$), δ, ppm: 1.30–2.55 m (10H, 5 $CH_2$), 3.22–3.52 m (2H, $CH_2$), 3.67 s (3H, $CH_3$), 3.75 s (3H, $CH_3$), 4.39–4.73 m (2H, 2 CH), 7.15 br.s (1H, NH), 7.33 d (1H, NH), 7.60 d (1H, NH). Found, %: C 41.42; H 4.81; N 8.24. $C_{17}H_{23}F_6N_3O_7$. Calculated, %: C 41.22; H 4.68; N 8.48.

**$N^{\varepsilon}$-Trifluoroacetyl-L-lysine $N^{\alpha}$-carboxyanhydride (VIII).** Through a dispersion of 12.1 g (50 mmol) of compound **III** in 200 ml of dioxane was passed for 2 h a flow of phosgene at room temperature. The reaction mixture was heated at 45°C for 2 h, dioxane was partially evaporated at a reduced pressure (100 ml), and to the mixture obtained 250 ml of hexane was added. The precipitate was filtered off, washed with hexane, and dried in a vacuum. Then it was reprecipitated from ethyl acetate with hexane. Yield 9.38 g (70%), colorless powder, mp 92–93°C (decomp.), $[\alpha]_D^{20}$ –31.3° (*C* 3.0, dioxane) {94°C (decomp.), $[\alpha]_D^{20}$ –32.2° (*C* 3.0, dioxane) [13]}. Found, %: C 40.48; H 4.30; N 10.32. $C_9H_{11}F_3N_2O_4$. Calculated, %: C 40.31; H 4.13; N 10.45.

**L-Lysyl-L-glutamic acid (VII).** *a*. To a solution of 24.77 g (50 mmol) of compound **VI** in 150 ml of ethanol was added dropwise a solution of 8.2 g (205 mmol) of sodium hydroxide in 100 ml of 90% ethanol. The reaction mixture was stirred for 20 h at room temperature and then filtered. To the filtrate was added dropwise 11.97 g (105 mmol) of trifluoroacetic acid. The reaction mixture was stirred for 2 h, the precipitate was filtered off and washed in succession with ethanol (2×50 ml) and acetone (2×50 ml), and dried in a vacuum. Then it was

reprecipitated from water with ethanol. Yield 11.01 g (80%), colorless powder, mp 195–197°C (197°C [10], 194–196°C [3]). Found, %: C 48.21; H 7.77; N 15.04. $C_{11}H_{21}N_3O_5$. Calculated, %: C 47.99; H 7.69; N 15.26.

*b*. To a solution of 3.5 g (20 mmol) of dimethyl glutamate (**V**) in 100 ml of THF cooled to –60°C was added dropwise cooled to –50°C solution of 5.36 g (20 mmol) of compound **VIII** in 500 ml of THF. The reaction mixture was stirred at–50°C for 3 h then slowly warmed to room temperature. The solvent was distilled off in a vacuum, the residue was dissolved in 100 ml of ethanol, and then filtered. To the filtrate was added dropwise at vigorous stirring a solution of 2.4 g (60 mmol) of sodium hydroxide in 50 ml of 90% ethanol. The reaction mixture was stirred for 20 h and acidified with trifluoroacetic acid to pH 6.0. The separated precipitate was filtered off, washed with ethanol, acetone, and dried in a vacuum. Then it was reprecipitated from water with ethanol. Yield 2.47 g (45%), colorless powder, mp 194–195°C, $[\alpha]_D^{20}$ 20.3° (*c* 3.0, water).

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