duration of the animals, though to a lesser degree (Table 6). As the results of the study show, the most pronounced antibacterial activity was shown by the pimelic acid derivatives (see Tables 5 and 6). These compounds can probably be regarded as pimelic acid antimetabolites, which are known to participate in the matter exchange processes of various bacteria, fungi, and molds [1, 9].

The healthy animals tolerated a single injection of the test compounds in 2000 and 3000 mg/kg doses without any apparent change in their condition or behavior. No animals died over the ten-day observation period. Tests were not carried out for higher dosages.

In summary the tested compounds were found to be slightly toxic and the N,N'-pimeloyldiglycine derivatives proved the most active. It was also shown that the activity of polymethylenedicarbonyldiglycine derivatives increases when they are transformed into the corresponding esters.

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SYNTHESIS OF CHEMOTAXIC TRIPEPTIDE ANALOGS CONTAINING L- AND D-(S-TRIFLUOROMETHYL)-HOMOCYSTEINE AND HAVING HYPOTENSIVE ACTIVITY

V. V. Ryakhovskii, S. V. Agafonov,
O. B. Vinogradova, Yu. M. Kosyrev,
M. V. Kiselevskii, and V. S. Dobryanskii

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Chemotaxis is one of the main processes for the delivery of polymorphonuclear leucocytes from the blood to an inflamed site, where the inflammation is caused by an infectant or allergen [4]. One of the most powerful known agents inducing chemotaxis is the FMLP tripeptide (I) [8].*

For-Met-Leu-Phe
|

As known, introduction of this compound in vivo is accompanied by a change in several physiological parameters of the organism and, in particular, by a sharp decrease in the blood pressure. It is clear that analogs of formyltripeptide having a milder hypotensive action, may be considered as potential medicinal preparations.

*Abbreviations by IUPAC – IUB [7]. Boc_2O – di-tert-butylpyrocarbonate; AP – arterial pressure; DMFA – dimethylformamide; DOR – dispersion of optical rotation; THF – tetrahydrofuran; TFA – trifluoroacetic acid; EA – ethyl acetate.

State Scientific-Research Institute of Organic Chemistry and Technology, Moscow.

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In order to search for compounds having the above property, within the series of analogs of a chemotaxis tripeptide, we synthesized derivatives of I containing a fluorinated residue of methionine (II) and (III):

For-HomoCys(S—CF_3)-Leu-Phe
II

For-D-HomoCys(S—CF_3)-Leu-Phe
III

Trifluoromethionine – S-trifluoromethylhomocysteine – inhibits the growth of several microorganisms [9]. Introduction of this amino acid into the composition of the peptide may broaden the spectrum of activity of the latter and impart new useful properties to it.

The synthesis of the desired compounds II and III can be tentatively divided into two stages. The first is the synthesis of the suitable N-substituted S-trifluoromethylhomocysteine derivative, and the second – the direct synthesis of peptides.

Only two investigations devoted to the synthesis of S-trifluoromethylhomocysteine are described in the literature [5, 6]. In [5] the preparation of the racemic amino acid is reported being based on the addition of trifluoromethylmercaptan to acrolein, followed by the Strecker synthesis. We did not use this method because of the multiplicity of stages and difficulty in obtaining trifluoromethylmercaptan. The preparation of an optically active amino acid from a racemic N-acetylhomocysteine by alkylation of the latter by trifluoroiodomethane with UV irradiation and subsequent enzymatic splitting of the acetyl group is described in [6]. The low yield (29%) of S-trifluoromethylhomocysteine, the lack of details for the synthesis and also the necessity of the subsequent introduction of the protective function to the amino group were the reasons for our developing a new scheme of preparation of N-tert-butoxycarbonyl-S-trifluoromethylhomocysteine (VII).

D, L-$[SCH_2CH_2CH(NH_2)COOH]_2$
IV
↓ Boc_2O
D, L-$[SCH_2CH_2CH(NHBoc)COOH]_2$
V
↓ $CH_2SHCHOHCHOHCH_2SH$
D, L-$HSCH_2CH_2CH(NHBoc)COOH$
VI
↓ CF_3I
D, L-$F_3CSCH_2CH_2CH(NHBoc)COOH$
VII

We used D,L-homocystine (IV) as the starting compound into which the Boc-protection was introduced by means of Boc_2O. The method of preparation of Boc-amino acids by means of Boc_2O is generally known [3]. However, difficulties arose in the preparation of N,N'-di-tert-butoxycarbonylhomocystine (V) due to the extraordinarily low solubility in water and in organic solvents of both compound IV and the Na salt of IV, usually used for introducing the Boc-protection into amino acids. Thus, on using the Na salt of IV, a double excess of Boc_2O in a DMFA-water medium at 50°C and at 24 h duration of the reaction, the yield of V does not exceed 20%. It was shown that under the same conditions but using the K-salt, the yield increases to 50%, while the use of a Cs-salt gives a yield of 80%.

At the stage of the introduction of the trifluoromethyl group into the N-protected homocysteine (VI), the work was based on the procedure used for the alkylation of thiols by trifluoroiodomethane [2]. Before carrying out the alkylation, the S–S bond in V was preliminarily reduced by adding a 1.1 equivalent of dithioethythritol to its methanolic solution. According to the TLC data, the reduction was completed in 1 h at 20°C to the extent of 100%. Compound VI formed was treated without isolation in a methanolic solution with metallic Na and was alkylated with trifluoroiodomethane in a dry nitrogen atmosphere, at 0°C, in a quartz reactor with UV irradiation. After the chromatographic purification on silica gel, the yield of the desired product VII was 51%. The acetone and acetonitrile solvents, which, according to [2], ensure the maximal yields during the trifluoromethylation of thiols, were found to be unsuitable in the present case because of the low solubility of VI in them. An attempt to use THF led to a strong resinification of the reaction mixture, which reduced the yield of VII to 30%.

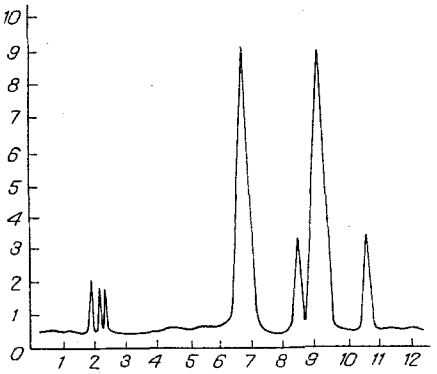


Fig. 1

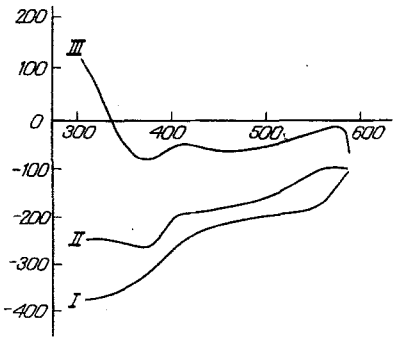
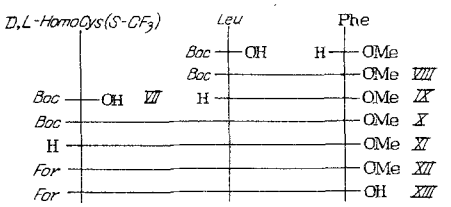


Fig. 2

Fig. 1. Chromatogram of the reaction mixture after the saponification of the ester in XII. A C8 (0.46 × 15 cm) Zorbax column, an isocratic mixture of 60% methanol in 0.1% TFA, 1.5 ml/min, detection at 230 nm. On abscissa) retention time, min; on ordinate) optical density (in rel. units).

Fig. 2. Dispersion of optical rotation (DOR) curves of compounds I, II, and III; concentration c 1.25 (methanol). On abscissa) wave length; on ordinate) molar rotation angle, degrees.

The synthesis of tripeptides II and III was carried out by classical method in a solution by chain propagation from its C-end. Isobutyl chloroformate was used as condensing agent and also for introducing the formic acid residue. The Boc-protection was removed by the action of 3 N solution of hydrogen chloride in dioxane. The saponification of phenylalanine methyl ester at the last stage was carried out by using two equivalents of a methanolic solution of potassium hydroxide for 3 h at 20°C.



After the introduction of the racemic VII residue into peptide (X), the latter was isolated already as a mixture of two epimers, which, however, could not be separated by means of TLC on a stationary silica gel layer on both Silufol plates or on Merck plates. A mixture of epimers of tripeptides XI and XII also could not be separated. However, after the saponification of the methyl ester XII, the mixture of epimers of tripeptide XIII gave two well separated spots during the TLC on Merck plates, but not on the Silufol plates. The high performance liquid chromatography (HPLC) on a C8 reversed phase gave two main peaks in a ratio of 1:1 (Fig. 1). Further, the two epimers were isolated in a pure state by means of liquid chromatography on a preparative column with a reversed phase. The mass spectra of the two epimers showed the presence of a molecular ion MH^+ 492, which corresponds to their calculated molecular weight.

The epimer having the higher mobility in TLC and a shorter retention time in HPLC was identified by us as a peptide containing L-trifluoromethionine. It was crystallized from aqueous methanol in the form of characteristic needle-like crystals similarly to tripeptide I from the firm Sigma, while the second epimer did not give any ordered crystals. The DOR curves were plotted for the two epimers and for the tripeptides 1 (Fig. 2). The similarity of the DOR curves of standard I and of the epimer with shorter retention time during the HPLC confirms the L-configuration of the trifluoromethionine residue in this epimer.

EXPERIMENTAL (CHEMICAL)

The course of the reaction and the purity of the compounds were monitored by TLC on Silufol silica gel plates from the firm Kavalier and Kieselguhr-60 plates from the firm Merck in systems: chloroform–methanol 9:1 (A), 85:15 (B); chloroform–ethyl acetate–acetic

TABLE 1. Influence of Compounds II and III on AP in Hypertensive Rats

Compound	Dose, mg/kg	Initial AP, mm Hg	AP (mm Hg) up to the corresponding moment of time after application					
			1 min	5 min	10 min	20 min	30 min	1 h
II	0,1	159±3	125±5*	150±2	155±3	157±4	159±3	158±2
	1,0	162±3	95±8*	125±7*	130±2*	135±8*	135±6*	145±7
	2,5	166±5	70±9*	105±8*	122±6*	128±3*	130±5*	142±7*
III	0,1	167±4	160±7	156±8	162±6	166±3	165±5	166±4
	1,0	165±3	140±5*	150±4*	155±7	157±5	166±4	166±5
	2,5	165±4	105±6*	125±5*	130±7*	128±4*	137±5*	157±8

*The differences significant at $p < 0.05$.

acid 8:2:0.5 (C); chloroform–methanol–water 9:1:0.1 (D); chloroform–methanol–acetic acid 9:0.5:1 (E). The Silufol plates were developed by calcination and with ninhydrin. The Merck plates were developed after the chromatography with benzidine. The HPLC was carried out on a Gilson system. The molecular weight was determined on a MX1321A apparatus with a bombardment with accelerated argon atoms. The energy of the ions was 175-180 eV. Glycerin was used as the matrix. The melting points were determined in an open capillary without correction on a Mettler FP-5 apparatus. The specific rotation angles and the DOR curves were obtained on a Perkin–Elmer 141 spectropolarimeter. A bactericidal therapeutic lamp was used as the UV source. In the investigation protected amino acids from the firm "Reanal", Boc_2O and trifluoromethane from the firm Aldrich were used. The results of the elemental analyses corresponded to the calculated values.

N,N'-Ditert-butoxycarbonyl-D,L-homocystine (V). A solution of 5.1 g (15.6 mmoles) of Cs_2CO_3 in a mixture of 30 ml of water and 30 ml of DMFA was added to 3.5 g (13 mmoles) of homocystine. A 13 g portion (59.6 mmoles) of Boc_2O was added with stirring to the above suspension in the course of 5 h, while a temperature of about 50°C was maintained. The mixture was then stirred for another 1 h and then filtered. The precipitate was washed with 30 ml of water and the filtrate was evaporated. The residue was dissolved in 70 ml of water and washed with ether (2 × 15 ml). The aqueous phase was acidified with citric acid, saturated with sodium chloride and extracted with 200 ml of EA. The extract was washed with a saturated solution of NaCl (2 × 40 ml), dried over $MgSO_4$, and evaporated. Yield, 4.9 g (80%), mp 150.2°C, R_f 0.52 (Silufol, B).

N-Tert-butoxycarbonyl-S-trifluoromethyl-D,L-homocysteine (VII). A 0.3 g portion (0.64 mmole) of V was dissolved in 4.5 ml of methanol in a quartz test tube. A 0.118 g portion (0.76 mmole) of dithioerythritol was added and the mixture was allowed to stand for 1 h at 20°C in a nitrogen atmosphere. Then 0.092 g (4 mmoles) of metallic sodium was added, and after its dissolution, the reaction mixture was cooled to −73°C, and 3 g (15.3 mmoles) of trifluoroiodomethane was condensed in it from a cylinder. The temperature was then raised to 0°C, the reactor was illuminated with UV light for 1 h while passing a slow current of nitrogen over the reaction mixture, which was then evaporated. The residue was dissolved in 20 ml of water. The aqueous solution was washed with EA (2 × 4 ml), saturated with NaCl, acidified with citric acid, and extracted with 30 ml of EA. The extract was washed with a saturated solution of NaCl (10 ml) and evaporated. The residue was chromatographed on a column with silica gel, with elution of the compound with system (C). Yield 0.2 g (51%), mp 102.7°C, R_f 0.47 (Silufol C).

N-tert-butoxycarbonylleucylphenylalanine methyl ester (VIII). A 2 g portion (8.7 mmoles) of Boc-Leu was dissolved in 20 ml of THF, 0.88 g (8.7 mmoles) of N-methylmorpholine was added and the solution was cooled to −20°C. A 1.2 g portion (8.7 mmoles) of isobutyl chloroformate was added with stirring and the mixture was allowed to stand for 5 min at −15°C. Then a solution of 1.9 g (8.7 mmoles) of HCl·H-Phe-OMe in 20 ml of DMFA containing 0.88 g (8.7 mmoles) of N-methylmorpholine was added. The reaction mixture was allowed to stand for 1 h at −15°C and for 1 h at 20°C. The solvent was evaporated, and the residue was dissolved in 50 ml of EA and the solution was washed with a 10% solution of $KHSO_4$ (15 ml), water (15 ml), a saturated solution of $KHCO_3$ (2 × 15 ml), and water (10 ml). Ethyl acetate was evaporated and the residue was dried in vacuo. Yield, 3.1 g (91.2%), mp 88°C, R_f 0.68 (Silufol, A). $[\alpha]_D^{20}$ −19.5° (c 1.0; DMFA).

Leucylphenylalanine methyl ester hydrochloride (IX). A 3 g portion (7.6 mmoles) of VIII was dissolved in 20 ml of a 3 N solution of HCl in dioxane, the mixture was allowed

to stand for 1 h and was evaporated. The residue was crystallized by grinding with 70 ml of a heptane-ether (1:1) mixture. Yield 2.4 g (95.6%), mp 190.5°C. R_f 0.44 (Merck, D), $[\alpha]_D^{20}$ +14° (c 1.25; methanol).

N-Tert-butoxycarbonyl-S-trifluoromethyl-D,L-homocysteinylleucylphenylalanine methyl ester (X) was obtained from 0.25 g (0.82 mmole) of VII and 0.3 g (0.9 mmole) of IX, as described for VIII. Yield, 0.46 g (96.6%), mp 119.8°C, R_f 0.55 (Silufol, A), $[\alpha]_D^{20}$ −17° (c 1.25 methanol).

S-Trifluoromethyl-D,L-homocysteinylleucylphenylalanine methyl ester hydrochloride (XI) was obtained from 0.46 g (0.8 mmole) of X as described for IX. Yield, 0.39 g (95.1%), mp 205.2°C, R_f 0.58 (Merck, D), $[\alpha]_D^{20}$ −7.5° (c 1.25 methanol).

N-formyl-S-trifluoromethyl-D,L-homocysteinylleucylphenylalanine methyl ester (XII) was obtained from 0.051 g (1.1 mmole) of COH and 0.37 g (0.73 mmole) of XI, as described for VIII. Yield, 0.34 g (92.6%), mp 45.7°C, R_f 0.52 (Merck, D), $[\alpha]_D^{20}$ −20.2° (c 1.25; methanol).

N-Formyl-S-trifluoromethylhomocysteinylleucylphenylalanine (II) and N-formyl-S-trifluoromethyl-D-homocysteinyleucylphenylalanine (III). A 0.34 g portion (0.67 mmole) of XII was dissolved in 4 ml of methanol, 0.1 ml of water and 0.07 g (1.25 mmole) of KOH were added, and the mixture was allowed to stand for 3 h at 20°C. Methanol was evaporated, the residue was dissolved in 20 ml of water, washed with an ether-heptane (1:1) mixture (10 ml), acidified with citric acid, and the precipitate that separated out was filtered off and washed on the filter with 10 ml of water. The product was precipitated twice from ethanol with water and chromatographed on a Lihroprep RP8 preparative column (2.5 × 1 cm) from the firm Merck in a methanol gradient in 0.1% TFA from 60 to 80%. Yield, 0.065 g (19.7%) for II, 0.068 g (20.6%) for III, mp 223.6°C (II), mp 205.1°C (III), R_f 0.44 (II), R_f 0.36 (E) (III), R_t 6.61 min (II), R_t 9.05 min (III) (Zorbax C8, 0.46 × 15 cm, 60% methanol in 0.1% TFA, 1.5 ml/min). The content of the main compound in both epimers was not less than 99% (detection at 230 nm), $[\alpha]_D^{20}$ −21° (II), $[\alpha]_D^{20}$ −13° (III) (c 1.25; methanol).

EXPERIMENTAL (BIOLOGICAL)

The experiments were carried out on non-narcotized hypertensive rats (SHR) of both sexes, each weighing 180-220 g. The AP level in the femoral artery was measured by a direct electromanometric method. The AP was recorded on an RM-86 polygraph from the Nichon Kohden firm (Japan). The tested solutions were introduced into the tail vein of the animals. The ED_{50}, the dose causing a decrease in the AP of the animals of 50 mm Hg, was determined by the Litchfield–Wilcoxon method [1].

The data on the effect of the compounds studied on the AP level in hypertensive rats are given in Table 1.

The data in Table 1 show that compound II has a more pronounced and prolonged antihypertensive action than compound III. Compound II caused a reliable decrease in the AP of rats in a dose of 0.1 mg/kg, such that the duration of this effect did not exceed 2-3 min. In a dose of 1.0 mg/kg, this compound caused a pronounced decrease in the AP for 20-30 min. The maximum decrease in the AP level of 60-70 mm Hg was recorded in 1-2 min. An intravenous administration of compound II in a dose of 2.5 mg/kg caused a more prolonged antihypertensive action. A reliable decrease for 1 h in the AP was noted. In this dose, the AP decreased by 80-100 mm Hg 1/2 min after the administration. Introduction of compound III in a dose of 1.0 mg/kg led to a reliable decrease in the AP by 15-20 mm Hg, and this effect lasted for 10 min. Introduction of compound III in a dose of 2.5 mg/kg caused a reliable antihypertensive action for 30 min. The maximal decrease in the AP in this dose was at 1 min after the administration reached 60 mm Hg. For compounds II and III the ED_{50} are equal to 0.25 (0.08-1.3) mg/kg and 2.4 (0.7-3.2) mg/kg, respectively.

Thus, a method has been developed for the preparation of N-tert-butoxycarbonyltrifluoromethionine which is suitable for use in a peptide synthesis. Two analogs of a chemotaxic FMLP tripeptide having hypotensive activity were obtained from this compound, containing D- and L-trifluoromethionine.

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SYNTHESIS OF BUTOXYBUTENYNE BASES AND THEIR QUATERNARY SALTS AND INVESTIGATION OF THEIR PHARMACOLOGICAL PROPERTIES

G. A. Losev, V. N. Voshchula,
A. S. Sokolova, N. T. Novikova,
R. Ya. Mushii, and V. I. Dulenko

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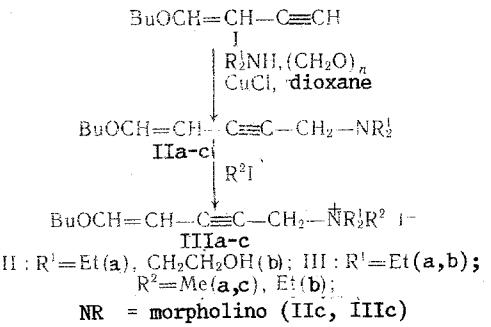
Acetylenic Mannich bases have a wide spectrum of pharmacological activity (see [1]), but only a few of them have been tested for their antitumorigenic activity, in particular, the Mannich bases obtained from propargyl ethers.

To study the antitumorigenic activity, we synthesized Mannich bases (IIa-c) obtained from 1-butoxy-but-1-en-3-yne [1], which is the main component of the flotation agent MIG-4. Methoxybutenyne Mannich bases are described in the literature, but their pharmacological properties have not been investigated [5].

Acetylenic amines II were synthesized under the conditions of a catalytic Mannich reaction, similar to those described in [5]. The quaternary salts III were obtained by quaternization of amines II with methyl or ethyl iodide in an acetonitrile medium. The structure of compounds II and III was confirmed by IR and PMR spectra.



EXPERIMENTAL (CHEMICAL)

The IR spectra were run on "UR-20" and "Specord" spectrophotometers, the PMR spectra — on a "Tesla BS-467" spectrometer (60 MHz), of amines (II) in CCl_4, of salts III in deuteroacetone, using TMS as internal standard. The characteristics of the synthesized compounds are given in Table 1. The results of the elemental analyses correspond to the calculated values.

Central Chemical Laboratory of Synthesis, S. Ordzhonikidze All-Union Scientific-Research Chemical Pharmaceutical Institute, Moscow. Institute of Physical Organic Chemistry and Carbon Chemistry, Ukrainian Academy of Sciences, Donetsk. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 26, No. 2, pp. 49-50, February, 1992. Original article submitted April 24, 1991.

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