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Design and Synthesis of Dipeptide Mimetics of the Brain-Derived Neurotrophic Factor

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Abstract—Low-molecular-weight mimetics of loops 1 and 4 of the brain-derived neurotrophic factor (BDNF) have been designed and synthesized. The compounds represent monomeric and dimeric amides of *N*-acyldipeptides. Their dipeptide fragments coincide in sequence with the central regions of beta-turns of the corresponding neurotrophin loops, and acyl groups are the bioesosteres of preceding amino acid residues. Hexa- or heptamethylenediamines were used as spacers to link the C-terminal regions of dipeptides in dimeric mimetics of BDNF. These compounds were synthesized by classical methods of peptide synthesis in solution and received the laboratory codes GSB-104 (HO–Suc-Ser-Lys–NH_2), GSB-106 {[HO–Suc-Ser-Lys–NH–$(CH_2)_3$–]$_2$}, GSB-207 (HO–Suc-Met-Ser–NH_2), and GSB-214 ([HO–Suc-Met-Ser–NH–$(CH_2)_{7/2}$–]$_2$). It was shown using immortalized hippocampal cells of the HT22 line under conditions of oxidative stress that the dimeric mimetics of both loops at concentrations of 10^{-5}–10^{-8} M possess a neuroprotective activity. The monomeric loop 1 mimetic GSB-207 in the same concentration range is inactive, and the monomeric loop 4 mimetic GSB-104 at a concentration of 10^{-7} impairs the survival of neurons. The finding that only dimeric mimetics possess the neuroprotective activity is consistent with the data indicating that BDNF is active in the homodimeric form. As opposed to the dimeric loop 1 mimetic GSB-214, the dimeric loop 4 mimetic GSB-106 exhibits the antidepressant activity typical for BDNF in the Porsolt test on rats at doses of 0.1 and 1 mg/kg injected intraperitoneally. This suggests that the antidepressant activity of BDNF is related to its 4th loop. We believe that the compounds obtained will be useful in studies of the mechanism of action of BDNF and may form the basis for the design of a novel group of drugs with antidepressant and neuroprotective activities.

Keywords: BDNF, mimetics, dipeptides, antidepressant activity, neuroprotective activity

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INTRODUCTION

The brain-derived neurotrophic factor belongs to the family of neurotrophins, which involves the nerve growth factor, neurotrophin-3, and neurotrophin-4/5. Owing to the ability to increase the survival of neurons, neurotrophins are considered as promising antineurodegenerative drugs. BDNF is particularly attractive in this respect since it improves the survival and prevents the degeneration of neuronal populations involved in diseases such as amyotrophic lateral sclerosis (motoneurons), sensory neuropathies (sensory neurons), Alzheimer's disease (basal cholinergic neurons of the forebrain), and Parkinson's disease (dophaminergic neurons of the black substance). In addition, BDNF was shown to play an important role in the etiology of Huntington's disease and depression [1, 2].

The biological activity of BDNF and other neurotrophins is effected through two types of transmembrane receptors. The binding of neurotrophins to a specific member of the Trk family of tyrosine kinase receptors (BDNF interacts with TrkB and does not interact with TrkA and TrkC) results in the homodimerization of the receptor followed by self-phosphorylation and the triggering of a great many pathways of transduction of the biological response, including those that lead to neuronal survival. Conversely, glycoprotein p75 acts as a common low-affinity receptor for all neurotrophins. It is assumed to be involved in the regulation of apoptosis and the modulation of the signaling cascade associated with the Trk receptor [2].

Despite the promising data of preclinical studies, clinical trials of BDNF in the treatment of amyo-

Abbreviations: BDNF, brain-derived neurotrophic factor; BuONO, butyl nitrite; DCU, 1,3-dicyclohexylurea; DIEA, *N*,*N*-diisopropylethylamine; DMAPA, 3-(dimethylamino)propylamine; HOSu, *N*-hydroxysuccinimide; HONp, 4-nitrophenol; IBCF, isobutyl chloroformate; NGF, nerve growth factor; NMM, *N*-methylmorpholine; TEA, triethylamine; TFA, trifluoroacetic acid; Z(Cl), 2-chlorbenzyloxycarbonyl.

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trophic lateral sclerosis have not been successful owing to pharmacokinetic limitations, such as a short lifetime in the blood flow (half-life of neurotrophin in rats is less than 1 min) and a low capacity to permeate through the hematoencephalic barrier [3]. In this connection, several laboratories are concerned with the construction of low-molecular-weight functional BDNF mimetics. Thus, a team of Australian researchers has designed a number of mimetics based on BDNF loops: monomeric monocyclic peptides with the antagonistic activity on the basis of loops 1, 2, and 4 [4, 5]; dimeric bicyclic and tricyclic peptides with the agonistic activity based on loop 2 [6]; a cyclopentapeptide based on the loop 4 tripeptide fragment -Lys-Lys-Arg-, which was created as a ligand for the p75 receptor and which enhances the survival of sensory neurons in vitro [7], and its lipophilic derivatives [8].

A team of American investigators implanted BDNF fragments in the structure of the NGF and determined the region of loop 2 involved in the specific interaction of BDNF with TrkB, -Ser-Lys-Gly-Gln-Leu- [9]. Based on this structure, a pharmacophoric hypothesis was advanced, 35 million described compounds were virtually screened using this hypothesis, and 1785 candidate compounds were identified. With regard to the criteria of molecular weight and the accessibility of the pharmacophoric moiety for the interaction with the receptor, the number of the candidates was reduced to 14. Seven of these 14 compounds were commercially available. After in vitro tests on cell models, one compound based on 1,3,5-benzoltricarboxylic acid (LM22A-4) was chosen. It was shown in rats that, on intranasal administration, this compound is capable of restoring the spatial memory impaired by a brain trauma.

Here we describe monomeric and dimeric linear *N*-acyldipeptide mimetics of BDNF constructed on the basis of its 1st and 4th loops. These dipeptides were chosen since they have a low molecular weight and better permeate across biological barriers, including the hematoencephalic barrier. In the gastrointestinal tract, there is a special system for the transport of dipeptides, and they can freely penetrate into the blood. Therefore, it can be expected that, on systemic administration, including the peroral administration, they can exhibit activity which is of importance for their potential therapeutic application.

RESULTS AND DISCUSSION

When constructing BDNF mimetics, we relied on the literature data on the crystalline structure of the heterodimer BDNF/NT-3 (pdb ID 1b8m) [10]. Like other members of the neurotrophin family, BDNF is a symmetrical homodimer whose monomeric units contain seven β-strands linked by three hairpin loops exposed to solvent (loops 1, 2, 4). A visual analysis of this structure shows that loops 4 and 1 of BDNF are most exposed to solvent. In addition, the central fragments of the β-turns of these loops occupy the position geometrically most advantageous for the interaction with the receptor. Therefore, the sequences of β-turns -Asp^{93}-Ser^{94}-Lys^{95}-Lys^{96}- and -Asp^{30}-Met^{31}-Ser^{32}-Gly^{33}- were chosen as the basis for modeling.

To reproduce the active conformation, mimetics of neurotrophin loops are usually obtained by covalent cyclization of their fragments. We used another approach, which we have earlier approved to synthesize dipeptide mimetics of the NGF [11]. This approach involves the synthesis of the amides of *N*-acyldipeptides, which often reproduce the β-turn conformation stabilized by the intramolecular hydrogen bond [12]. The dipeptide coincided in sequence with the central fragment of the β-turn of the loop, and the preceding amino acid residue was substituted for by its bioisostere. In the present study, the aspartic acid residue Asp^{93} or Asp^{30} was substituted for by a succinic acid residue. The residue following the central fragment was represented by the amide group.

As a result, we constructed the mimetic of the BNDF loop 4 β-turn HO–Suc-Ser-Lys–NH_2 (GSB-104) and the mimetic of loop 1 β-turn HO–Suc-Met-Ser–NH_2 (GSB-207).

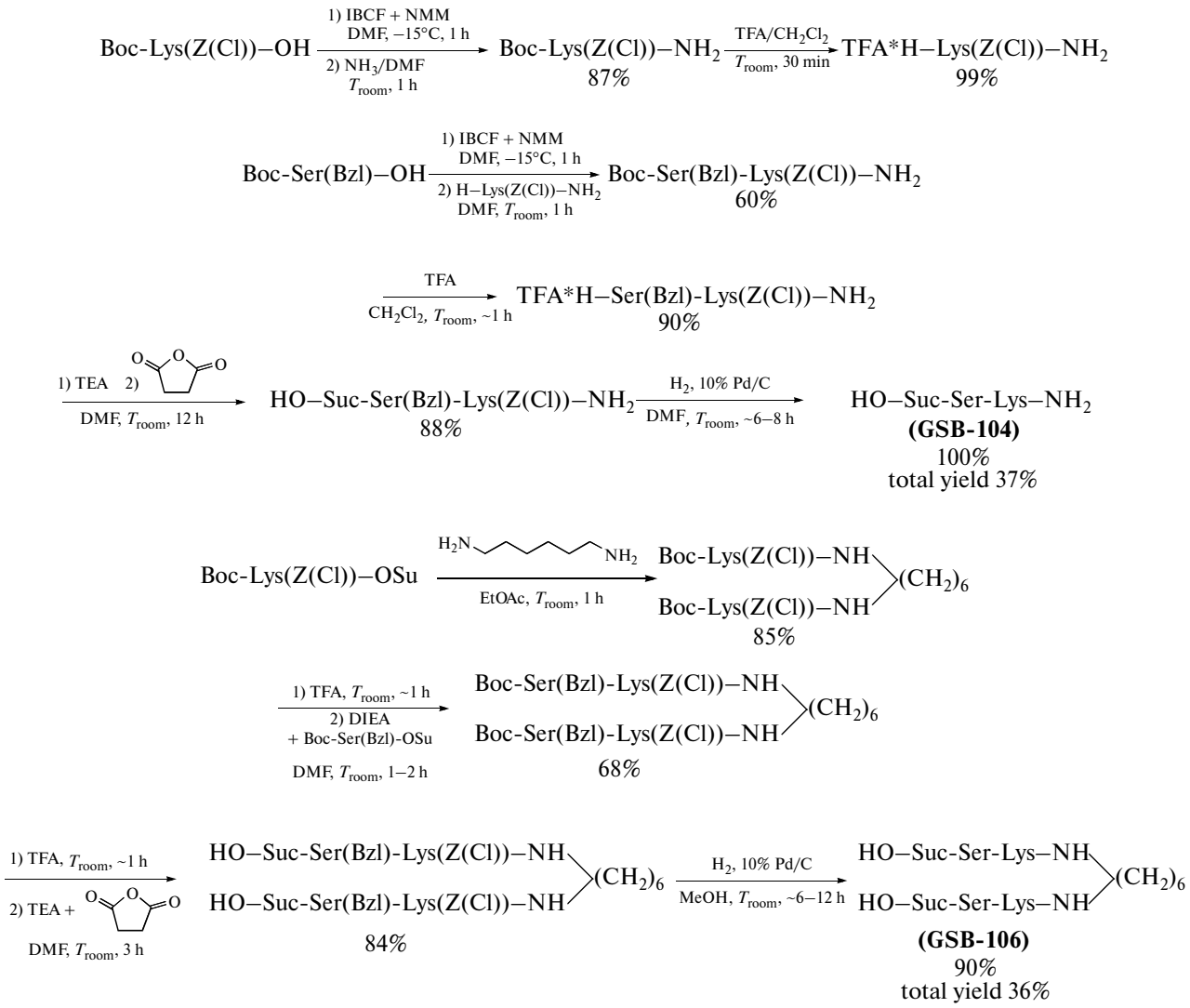
Because BDNF interacts with the TrkB receptor in the dimeric form, the agonistic activity was achieved by dimerizing the mimetics of the β-turn by hexa- or heptamethylenediamine. It has been earlier shown for similar NGF mimetics that these spacers are optimal since the reduction in the length to pentamethylenediamine led to a decrease and then the reverse of activity, and the elongation did not enhance the biological activity [13].

Thus, the BDNF loop 4 mimetic bis(*N*-monosuccinyl-seryllysine) hexamethylenediamide (GSB-106) and the BDNF loop 1 mimetic bis(*N*-monosuccinyl-methionylserine) heptamethylenediamide (GSB-214) were constructed.

The mimetics designed were synthesized by the methods of classical peptide synthesis in solution, including the methods of activated esters, mixed anhydrides, and the azide method with the use of the strategy of Boc/Z protecting groups.

The monomeric dipeptide mimetic of loop 4 GSB-104 was obtained by the condensation of Boc-Ser(Bzl)–OH with H–Lys(Z(Cl))–NH_2 by the method of mixed anhydrides under Andersen's conditions [14] followed by the removal of the Boc-group, the acylation of the product by succinic anhydride, and Z(Bzl) deblocking (Scheme 1).

The dimeric dipeptide mimetic of loop 4 GSB-106 was obtained by linking the hexamethylenediamine spacer to Boc-Lys(Z(Cl))–OSu with subsequent elongation of the peptide chain with the residue of the protected serine by the method of oxysuccinimide esters. The Boc protecting group was then removed, and the bisdipeptide was acylated by succinic anhydride. The removal of side protecting groups by hydrogenolysis gave the dipeptide GSB-106 with a total yield of 36% (Scheme 1).



IBCF, isobutyl chloroformate.

Scheme 1. Synthesis of BDNF loop 4 mimetics.

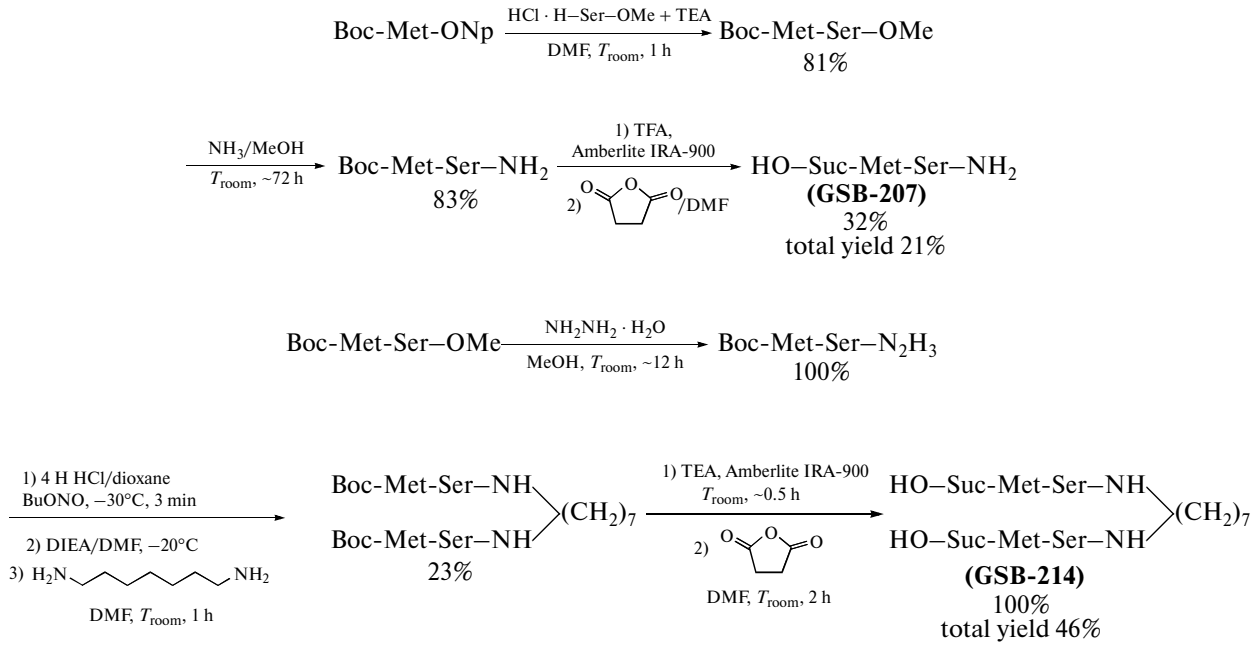
The monomeric dipeptide GSB-207 was synthesized from serine methyl ester and Boc-methionine *p*-nitrophenyl ester with subsequent ammonolysis, the removal of the protecting group by trifluoroacetic acid, and acylation by succinic anhydride with a total yield of 21% (Scheme 2).

The dimeric dipeptide GSB-214 was synthesized by the azide method from Boc-methionyl-serine methyl ester by its conversion into hydrazide and then into azide followed by the condensation of the azide with heptamethylenediamine. After the removal of the Boc protection, bis(methionylserine) heptamethylenediamide was acylated by succinic anhydride. GSB-214 was obtained with a total yield of 46% (Scheme 2).

All target compounds were purified by recrystallization. The products were homogeneous, as indicated by the TLC and HPLC data. The structure was confirmed by ^{1}H NMR spectroscopy. The diastereomeric purity was no less than 98%, as indicated by ^{1}H NMR data.

The neuroprotective activity of the dipeptides was revealed using a model of oxidative stress with hydrogen peroxide on a culture of immortalized rat hippocampal cell line HT22 [15]. The activity was calculated in percent of the maximum possible effect, which enabled one to quantitatively estimate both the neuroprotective and neurodegenerative (negative neuroprotective) activities. The peptides were examined in a concentration range of 10^{-5}–10^{-8} M. Both dimeric mimetics increased the viability of neurons (see table). The 4 loop mimetic GSB-106 and the 1 loop mimetic GSB-214 exhibited the neuroprotective activity in a concentration range of 10^{-5}–10^{-8} M. The monomeric loop 4 mimetic GSB-104 at a concentration of 10^{-7} M decreased the neuron viability twofold, i. e., exhibited

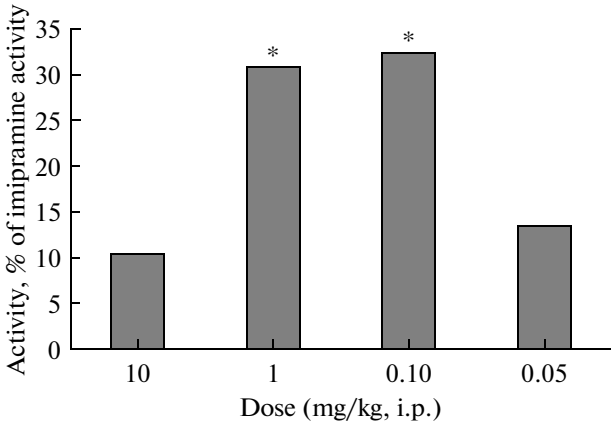
the negative neuroprotective activity. This may be due to its antagonism toward endogenous BDNF. The monomeric loop 1 mimetic was inactive. These data are consistent with the fact that BDNF exhibits the activity when interacting with the TrkB receptor in the dimeric form.



Scheme 2. Synthesis of BDNF loop 1 mimetics.

Because BDNF is involved in the pathogenesis of depression and possesses the antidepressant activity [2, 16], we studied the antidepressant properties of the BDNF dimeric mimetics on a classical "forced swimming" model in the original Porsolt test [17] by comparison with the classical antidepressant imipramine.

Only the loop 4 mimetic GSB-106 exhibited the activity. It produced a statistically significant antidepressant effect at doses of 1 and 0.1 mg/kg i.p. (see figure). The magnitude of the effect amounted to 30% of the effect of the most optimal dose of the standard antidepressant imipramine on this model (25 mg/kg i.p.) [18].



Antidepressant activity of the BDNF dipeptide mimetic GSB-106 in the Porsolt forced swimming test as compared with the classical antidepressant imipramine (25 mg/kg, i.p.). *$p < 0.05$ relative to the control by the Mann—Whitney U-test.

Presumably, its fourth loop is responsible for the activity of BDNF. Note that the selectivity associated with loop structures was also found in the case of dipeptide mimetics of NGF: loop 4 was responsible for the neuroprotective activity of this neurotrophin, and loop 1, for its differentiation activity [11].

Thus, we designed and synthesized for the first time in vitro and in vivo active BDNF dipeptide mimetics, which can be used to study the mechanism of action of neurotrophin and serve as the basis for the development of novel neuroprotective and antidepressant drugs.

EXPERIMENTAL

Commercially available amino acids and their derivatives (Fluka, Sigma) were used. Solvents were purified and dried by standard methods. ^{1}H NMR spectra (δ, ppm) were recorded on a Bruker AC-250 spectrometer (Germany) in DMSO-d_6 solutions with tetramethyl silane as an internal standard. The melting point was determined in open capillaries and was not corrected. Specific optical rotation was measured on a Perkin-Elmer-241 polarimeter (England). TLC was per-

Effect of dipeptide mimetics of BDNF on neuron viability under the conditions of oxidative stress

Compound	Concentration, M	Optical absorption		Activity, %
		with H_2O_2	without H_2O_2	
GSB-104	10^{-5}	0.114 ± 0.036		26
	10^{-6}	0.100 ± 0.020		–19
	10^{-7}	0.089 ± 0.010*		–55
	10^{-8}	0.107 ± 0.025		3
	0 (control)	0.106 ± 0.023#	0.137 ± 0.026	
GSB-106	10^{-5}	0.097 ± 0.010*		31
	10^{-6}	0.095 ± 0.008*		27
	10^{-7}	0.095 ± 0.010*		26
	10^{-8}	0.089 ± 0.009		10
	0 (control)	0.085 ± 0.006#	0.124 ± 0.010	
GSB-207	10^{-5}	0.081 ± 0.010		–7
	10^{-6}	0.087 ± 0.010		13
	10^{-7}	0.082 ± 0.005		–3
	10^{-8}	0.084 ± 0.010		3
	0 (control)	0.083 ± 0.005#	0.113 ± 0.007	
GSB-214	10^{-5}	0.096 ± 0.008*		34
	10^{-6}	0.098 ± 0.011*		40
	10^{-7}	0.095 ± 0.006*		31
	10^{-8}	0.088 ± 0.004*		11
	0 (control)	0.084 ± 0.004#	0.119 ± 0.006	
BDNF	50 ng/mL (~10^{-9})	0.090 ± 0.003		78
	0 (control)	0.076 ± 0.003#	0.094 ± 0.004	

Notes: Experiments were carried out on hippocampal neurons of mice, line HT22. Cell viability was determined by the MTT test.

* $p < 0.05$ by the Student's t-test relative to the active control (with H_2O_2).

# $p < 0.05$ by the Student's t-test relative to the passive control (without H_2O_2).

formed on Kieselgel 60 G/F_{254} plates (Merck, Germany) in solvent systems: chloroform–methanol 9 : 1 (A), dioxane–water 9 : 1 (B), chloroform–methanol–water–acetic acid 15 : 10 : 2 : 3 (C), pyridine–water–acetic acid–ethyl acetate 20 : 11 : 6 : 120 (D), hexane–ethyl acetate 4 : 1 (E), and *n*-butanol–acetic acid–water 4 : 1 : 1 (F). Amino-containing compounds were detected by ninhydrin; compounds containing amide groups, in iodine vapors; compounds with an open carboxyl group, by bromocresol green; and compounds containing aromatic groups, in UV rays. The elemental analysis was carried out on a device for carbon and hydrogen determination with four electric ovens (600–900°C, model MA-G/6P; LETO plant, Russia) in a flow of oxygen and on a device for nitrogen determination with three similar electric ovens in a flow of carbon dioxide. The elemental analysis data on the percent content of C, H, and N of compounds deviate from the theoretical data by no more than 0.4%.

The analytical HPLC of the dipeptide GSB-106 was performed using a Wellchrom 2001 chromatographic system (KNAUER, Germany) on a Diasorb-130-C16T column (4.6 × 150 0 mm, 7 μm) in a gradient of concentrations of isopropyl alcohol in 0.5% TFA (0–25%, 25 min). The flow rate was 1 mL/min, and the detection was at 214 nm.

Synthesis of $HOOC(CH_2)_2CO$–Ser-Lys–NH_2 (GSB-104)

Boc-Lys(Z(Cl))–NH_2. NMM (0.34 mL, 3.62 mmol) and IBCF (0.52 mL, 3.62 mmol) were simultaneously added from two dropping funnels to a solution of Boc-Lys(Z(Cl))–OH (1.5 g, 3.62 mmol) in DMF (10 mL) at –10°C; the temperature was maintained at values no higher than –5°C. After mixing for 2–3 min, DMF (10 mL), saturated by cooling (0°C) with ammonia (pH 8), was added. The reaction mixture was stirred for 1 h at –10°C and then for 1 h at room temperature. The solvent was removed on a rotor evaporator, and the oily residue was dissolved in chloroform (30 mL) and successively washed with water, 5% $NaHCO_3$, 1 N HCl, and again with water (30 mL each). Then the solution was evaporated, the oily residue was triturated with diethyl ether, and the precipitate was filtered and dried in a desiccator over P_2O_5 (15 mmHg). A chromatographically homogenous white powder was obtained. Yield: 1.3 g (87%); R_f 0.49 (A); mp 105–

106°C, $[\alpha]_D^{20}$ −8.5° (*c* 0.4, chloroform); ^{1}H NMR: 1.36 (9 H, s, $OC(CH_3)_3$), 1.17–1.65 (6 H, m, $C^{\beta}\underline{H}_2C^{\gamma}\underline{H}_2C^{\delta}\underline{H}_2$ Lys), 2.97 (2 H, m, $C^{\varepsilon}H_2$ Lys), 3.80 (1 H, m, $C^{\alpha}H$ Lys), 5.07 (2 H, s, $-OC\underline{H}_2C_6H_4Cl$), 6.73 (1 H, d, *J* 7.7 Hz, NH Lys), 6.96 and 7.25 (2 H, two s, NH_2 amide), 7.37 (1 H, t, *J* 5.6 Hz, $N^{\varepsilon}H$ Lys), 7.30–7.50 (4 H, m, $OCH_2C_6\underline{H}_4Cl$).

$CF_3COOH \cdot$ H–Lys(Z(Cl))–NH_2. A solution of Boc-Lys(Z(Cl))–NH_2 (400 mg, 1.00 mmol) in TFA (3 mL) and dichloromethane (3 mL) was stirred for 30 min at room temperature. The solvent was removed in vacuo, the residue was evaporated twice with diethyl ether (10 mL) and dried over P_2O_5 in vacuo (15 mmHg). The product was obtained as a trifluoroacetate salt. Yield: 410 mg (99%); R_f 0.05 (F); mp 109–111°C; ^{1}H NMR: 1.31 (2 H, m, $C^{\gamma}H_2$ Lys), 1.40 (2 H, m, $C^{\delta}H_2$ Lys), 1.70 (2 H, m, $C^{\beta}H_2$ Lys), 2.99 (2 H, m, $C^{\varepsilon}H_2$ Lys), 3.68 (1 H, m, $C^{\alpha}H$ Lys), 5.08 (2 H, s, $OC\underline{H}_2C_6H_4Cl$), 7.31–7.52 (4 H, m, $OCH_2C_6\underline{H}_4Cl$), 7.57 and 7.88 (2 H, two s, NH_2 amide), 8.09 (3 H, s, $N\underline{H}_3^+$ Lys).

Boc-Ser(Bzl) Lys(Z(Cl))–NH_2. NMM (0.09 mL, 0.82 mmol) was added to a solution of Boc-Ser(Bzl)–OH (240 mg, 0.82 mmol) in DMF (10 mL) at −15°C after which IBCF (0.13 mL, 0.82 mmol) was added. After 2–3 min, a solution of trifluoroacetic salt Lys(Z(Cl))–NH_2 (350 mg, 0.82 mmol) and NMM (0.09 mL, 0.82 mmol) in DMF (10 mL) was added, which was prepared on cooling (0°C) beforehand. The reaction mixture was stirred for 1 h at −5…−10°C and for 1 h at room temperature. The resulting reaction mixture was diluted with ethyl acetate (200 mL), successively washed with water (50 mL), 2% H_2SO_4 (30 mL), 3% K_2CO_3 (30 mL), and water (2 × 50 mL), and dried over Na_2SO_4. The solvent was removed in vacuo, the residue was triturated with diethyl ether, and the precipitate was filtered. A white powder was obtained (510 mg) in which two components were identified by TLC, with R_f 0.55 (A) and R_f 0.60 (A). The composition of the compound with R_f 0.55 was not determined. After separation on a column of silica gel (Kieselgel 130–270, Aldrich), the elution with the system chloroform—ethanol 9 : 1 was carried out. The final product was obtained as a white powder. Yield: 370 mg (60%); R_f 0.60 (A); mp 127–129°C, $[\alpha]_D^{20}$ −2.5° (*c* 0.4, DMF); ^{1}H NMR: 1.15–1.85 (6 H, m, $-C^{\beta}\underline{H}_2C^{\gamma}\underline{H}_2C^{\delta}\underline{H}_2$-Lys), 1.37 (9 H, s, $OC(CH_3)_3$), 2.93 (H, s, $-C\underline{H}_2C_6H_5$), 5.06 (1 H, s, $OC\underline{H}_2C_6H_4Cl$), 7.05 (1 H, d, *J* 6.8 Hz, NH Ser), 7.09 and 7.25 (2 H, two s, NH_2 amide), 7.2–7.5 (9 H, m, $-CH_2C_6\underline{H}_5$, $OCH_2C_6\underline{H}_4Cl$), 7.45 (1 H, t, *J* 5.6 Hz, $N^{\varepsilon}H$ Lys).

$CF_3COOH \cdot$ H-Ser(Bzl)-Lys(Z(Cl))–NH_2. A solution of Boc-Ser(Bzl)-Lys(Z(Cl))–NH_2 (287 mg, 0.5 mmol) in TFA (2 mL) was stirred for 30 min at 20°C and evaporated. The crystallization of the residue under diethyl ether yielded 265 mg (90%) of the product as white crystals; mp 153–155°C, $[\alpha]_D^{20}$ +6.25° (*c* 0.4, DMF); ^{1}H NMR: 1.27 (2 H, m, $C^{\gamma}H_2$ Lys), 1.39 (2 H, m, $C^{\delta}H_2$ Lys), 1.54 and 1.65 (2 H, two m, $C^{\beta}H_2$ Lys), 2.97 (2 H, m, $C^{\varepsilon}H_2$ Lys), 3.67 (2 H, m, $C^{\beta}H_2$ Ser), 4.11 (1 H, m, $C^{\alpha}H$ Ser), 4.24 (1 H, m, $C^{\alpha}H$ Lys), 4.54 (2 H, s, $-C\underline{H}_2C_6H_5$), 5.07 (2 H, s, $-OC\underline{H}_2C_6H_4Cl$), 7.14 and 7.45 (9 H, two m, $-CH_2C_6\underline{H}_5$, $-OCH_2C_6\underline{H}_4Cl$), 7.35 (1 H, t, *J* 5.7 Hz, $N^{\varepsilon}H$ Lys), 8.25 (3 H, s, br, N^+H_3 Ser), 8.57 (1 H, d, *J* 7.7 Hz, NH Lys).

$HOOC(CH_2)_2CO$–Ser(Bzl)-Lys(Z(Cl))–NH_2. TEA (0.06 mL, 0.44 mmol) was added to a solution of $CF_3COOH \cdot$ H–Ser(Bzl)-Lys(Z(Cl))–NH_2 (240 mg, 0.40 mmol) in DMF (4 mL), the mixture was stirred for 15 min, and succinic anhydride (60 mg, 0.6 mmol) was added. The reaction mixture was stirred for 12 h, and after the termination of the reaction (TLC control) water (25 mL) was added. The white precipitate was filtered, washed with water, and dried in vacuo (15 mmHg) over P_2O_5. The yield of the product was 207 mg (88%); R_f 0.28 (A); mp 158–166°C; ^{1}H NMR: 1.25 (2 H, m, $C^{\gamma}H_2$ Lys), 1.35 (2 H, m, $C^{\delta}H_2$ Lys), 1.52 and 1.68 (2 H, two m, $C^{\beta}H_2$ Lys), 2.41 (4 H, m, $HOOCC\underline{H}_2C\underline{H}_2$), 2.94 (2 H, m, $C^{\varepsilon}H_2$ Lys), 3.60 (2 H, m, $C^{\beta}H_2$ Ser), 4.13 (1 H, m, $C^{\alpha}H$ Lys), 4.47 (1 H, m, $C^{\alpha}H$ Ser), 4.48 (2 H, s, $-C\underline{H}_2C_6H_5$), 5.07 (2 H, s, $-OC\underline{H}_2C_6H_4Cl$), 7.06 and 7.14 (2 H, two s, NH_2 amide), 7.20–7.50 (9 H, m, $-CH_2C_6\underline{H}_5$, $-OCH_2C_6\underline{H}_4Cl$), 7.34 (1 H, t, *J* 5.7 Hz, $N^{\varepsilon}H$ Lys), 7.92 (1 H, d, *J* 7.7 Hz, NH Lys), 8.25 (1 H, d, *J* 8.0 Hz, NH Ser).

$HOOC(CH_2)_2CO$–Ser-Lys–NH_2 (GSB-104). To a solution of $HOOC(CH_2)_2CO$–Ser(Bzl)-Lys(Z(Cl))–NH_2 (180 mg, 0.30 mmol) in DMF (10 mL), 10% Pd/C (180 mg) was added, and the reaction mixture was stirred in an atmosphere of hydrogen for 10 h until the initial compound disappeared (TLC control). Then, the catalyzer was filtered, and the filtrate was evaporated. The product was obtained as a white powder. Yield: 95 mg (100%, total yield 37%); R_f 0.11 (F); mp 136–138°C, $[\alpha]_D^{20}$ −15.75° (*c* 0.4. DMF). Found, %: C 46.80; H 7.39; N 16.68; $C_{13}H_{24}N_4O_6$. Calculated, %: C 46.99; H 7.23; N 16.87. ^{1}H NMR: 1.29 (2 H, m, $C^{\gamma}H_2$ Lys), 1.53 (2 H, m, $C^{\beta}H_2$ Lys), 1.79 (2 H, m, $C^{\delta}H_2$ Lys), 2.51 (4 H, m, $HOOCC\underline{H}_2C\underline{H}_2$), 3.91 and 4.16 (2 H, two m, $C^{\beta}H_2$ Ser), 4.40 (1 H, m, $C^{\alpha}H$ Lys), 4.55 (1 H, m, $C^{\alpha}H$ Ser), 7.21 (2 H, s, NH_2 amide), 8.00 (1 H, d, *J* 7.6 Hz, NH Lys), 8.31 (1 H, d, *J* 8.1 Hz, NH Ser).

Synthesis of (HOOC(CH$_2$)$_2$CO–Ser-Lys–NH–)$_2$(CH$_2$)$_6$ (GSB-106)

Boc-Lys(Z(Cl))–OSu. *N*-Hydroxysuccinimide (2.08 g, 18 mmol) was added to a solution of Boc-Lys(Z(Cl))–OH (7.60 g, 17 mmol) in THF (150 mL), the solution was cooled to –5°C, and a solution of DCC (3.50 g, 17 mmol) in THF (20 mL) was added dropwise for 30 min; care was taken that the temperature was no higher than 0°C. Then the reaction mixture was stirred for 30 min at –5°C and for 4 h at room temperature. The white DCU precipitate was filtered, and the filtrate was evaporated. The resulting viscous oil was dissolved in dichloromethane (30 mL). The solution was kept for 24 h at 0°C, the DCU precipitate was filtered, and the filtrate was evaporated in vacuo. A chromatographically homogeneous oily product was obtained. Yield: 6.80 g (84%); R_f 0.40 (A).

Boc-Ser(Bzl)–OSu. *N*-Hydroxysuccinimide (1.17 g, 11 mmol) was added to a solution of Boc-Ser(Bzl)–OH (2.89 g, 10 mmol), the solution was cooled to –5°C, and a solution of DCC (2.07 g, 10 mmol) in THF (20 mL) was added dropwise for 20 min. The reaction mixture was stirred for 3 h at room temperature. The white DCU precipitate was filtered, the filtrate was evaporated, and the resulting oil was crystallized from isopropyl alcohol. The crystals that precipitated were filtered and dried in vacuo (15 mmHg) over $CaCl_2$. Yield: 3.20 g (83%); R_f 0.60 (A), R_f 0.70 (E); mp 106–108°C, $[\alpha]_D^{20}$ –7.0° (*c* 1; DMF); ^{1}H NMR: 1.39 (9 H, s, –OC(CH$_3$)$_3$), 2.42 (4 H, m, HOOC–C$\underline{H}_2$C$\underline{H}_2$), 3.70 (2 H, m, C$^\beta$H$_2$ Ser), 4.39 (1 H, m, C$^\alpha$H Ser), 4.50 (2 H, s, C$\underline{H}_2$C$_6$H$_5$), 7.20–7.50 (5 H, m, –CH$_2$C$_6\underline{H}_5$), 7.72 (1 H, d, *J* 8.0 Hz, NH Ser).

(Boc-Lys(Z(Cl))–NH–)$_2$(CH$_2$)$_6$. A solution of hexamethylenediamine (380 mg, 3.30 mmol) in ethyl acetate (5 mL) was added to a solution of Boc-Lys(Z(Cl))–OSu (3.50 g, 6.80 mmol) in ethyl acetate (40 mL). The reaction mixture was stirred for 1 h and evaporated. The residue was dissolved in ethyl acetate (100 mL) and washed successively with 1 N H_2SO_4 (50 mL), 3% K_2CO_3 (50 mL), and water (2 × 50 mL). The filtrate was dried over Na_2SO_4 and evaporated. The resulting solid crystalline residue was dried in vacuo (15 mmHg) over $CaCl_2$ to yield 2.40 g (85%) of the product; R_f 0.45 (A), mp 101–103°C, $[\alpha]_D^{20}$ –7.3° (*c* 0.5, DMF); ^{1}H NMR: 1.29 (8 H, m, 2 C$^\gamma$H$_2$ Lys, –NH(CH$_2$)$_2$C$\underline{H}_2$C$\underline{H}_2$(CH$_2$)$_2$NH–), 1.42 (18 H, s, 2 –OC(CH$_3$)$_3$), 1.55 (8 H, m, 2 C$^\delta$H$_2$ Lys, –NHCH$_2$C$\underline{H}_2$(CH$_2$)$_2$C$\underline{H}_2$CH$_2$NH–), 1.79 (4 H, m, 2 C$^\beta$H$_2$ Lys), 2.96 (4 H, m, 2 C$^\varepsilon$H$_2$ Lys), 3.20 (4 H, m, –NHC$\underline{H}_2$(CH$_2$)$_4$C$\underline{H}_2$NH–), 4.53 (2 H, m, 2 C$^\alpha$H Lys), 5.34 (4 H, c, 2 C$\underline{H}_2$C$_6$H$_4$Cl), 7.10 (2 H, d, *J* 7.8 Hz, 2NH Lys), 7.39 (2 H, t, *J* 5.7 Hz, 2 N$^\varepsilon$H Lys), 8.01 (2 H, t, *J* 4.7 Hz, N$\underline{H}$(CH$_2$)$_6$N$\underline{H}$).

2CF$_3$COOH · (H–Lys(Z(Cl))–)$_2$(CH$_2$)$_6$. TFA (15 mL) was poured to (Boc-Lys(Z(Cl))–NH–)$_2$(CH$_2$)$_6$ (1.42 g, 1.71 mmol), and the solution was stirred for 2 h. After the termination of the reaction (TLC control), the solution was evaporated with diethyl ether (2 × 20 mL). The resulting foam (solidified oil) of the product was triturated with diethyl ether. Yield: 1.2 g (90%); R_f 0.10 (C), mp 87–90°C; ^{1}H NMR: 0.95–1.75 (20 H, m, 2 –C$^\beta\underline{H}_2$C$^\gamma\underline{H}_2$C$^\delta\underline{H}_2$Lys, –NHCH$_2$(C$\underline{H}_2$)$_4$CH$_2$NH–), 2.98 (4 H, m, –NHC$\underline{H}_2$(CH$_2$)$_4$C$\underline{H}_2$NH–), 3.09 (4 H, m, 2 C$^\varepsilon$H$_2$ Lys), 3.70 (2 H, m, 2 C$^\alpha$H Lys), 5.07 (4 H, s, 2 –OC$\underline{H}_2$C$_6$H$_4$Cl), 7.37 (2 H, t, *J* 4.9 Hz, –N$\underline{H}$(CH$_2$)$_6$N$\underline{H}$–), 7.45 (8 H, m, 2 –OCH$_2$C$_6\underline{H}_4$Cl), 8.23 (6 H, m, 2 N$^+$H$_3$ Lys), 8.59 (2 H, t, *J* 6.0 Hz, 2 N$^\varepsilon$H Lys).

(Boc-Ser(Bzl)-Lys(Z(Cl))–NH)$_2$(CH$_2$)$_6$. To a solution of 2CF$_3$COOH · (H–Lys(Z(Cl))–NH–)$_2$(CH$_2$)$_6$ (1.13 g, 1.25 mmol) in DMF (25 mL), first DIEA (0.46 mL, 2.64 mmol) and then Boc-Ser(Bzl)–OSu (2.48 g, 2.60 mmol) were added. The reaction mixture was stirred for 1 h at room temperature, DMAPA (0.5 mL) was added, and the mixture was stirred for 15 min. The reaction mixture was diluted with ethyl acetate (150 mL), successively washed with water, 3% H_2SO_4, 2% K_2CO_3, and again with water (40 mL each), dried over Na_2SO_4, and evaporated. The recrystallization of the crystalline residue from methanol yielded 1.25 g (75%) of the product as a white powder; R_f 0.40 (A), R_f 0.60 (E); mp 143–151°C, $[\alpha]_D^{20}$ –2.5° (*c* 0.4; DMF); ^{1}H NMR: 0.93–1.83 (38 H, m, 2 –OC(CH$_3$)$_3$, 2 –C$^\beta\underline{H}_2$C$^\gamma\underline{H}_2$C$^\delta\underline{H}_2$- Lys, –NHCH$_2$(C$\underline{H}_2$)$_4$CH$_2$NH–), 2.93 (8 H, m, 2 C$^\varepsilon$H$_2$ Lys, –NHC$\underline{H}_2$(CH$_2$)$_4$C$\underline{H}_2$NH–), 3.59 (4 H, m, 2 C$^\beta$H$_2$ Ser), 4.20 (4 H, m, 2 C$^\alpha$H Lys, 2 C$^\alpha$H Ser), 4.47 (4 H, s, 2 –C$\underline{H}_2$C$_6$H$_5$), 5.07 (4 H, s, 2 –OC$\underline{H}_2$C$_6$H$_4$Cl), 7.00 (2 H, d, *J* 8.1 Hz, 2 NH Ser), 7.19–7.50 (20 H, m, 2 –CH$_2$C$_6\underline{H}_5$, 2 –OCH$_2$C$_6\underline{H}_4$Cl, –N$\underline{H}$(CH$_2$)$_6$N$\underline{H}$–), 7.77 (2 H, t, *J* 5.8 Hz, 2 N$^\varepsilon$H Lys), 7.91 (2 H, d, *J* 7.8 Hz, 2 NH Lys).

2CF$_3$COOH · (H–Ser(Bzl)-Lys(Z(Cl))–NH–)$_2$(CH$_2$)$_6$. (Boc-Ser(Bzl)-Lys(Z(Cl))–NH–)$_2$ (CH$_2$)$_6$ (1.00 g, 0.84 mmol) was dissolved in TFA (3 mL), and the solution was stirred for 40 min and evaporated. The residue was reevaporated with diethyl ether (2 × 15 mL). After the trituration of oil with diethyl ether, 0.92 g (92%) of a chromatographically homogeneous beige powder was obtained; R_f 0.33 (A), R_f 0.50 (C); mp 117–122°C, $[\alpha]_D^{20}$ +4.75° (*c* 0.4; DMF); ^{1}H NMR: 1.15–1.75 (20 H, m, 2 –C$^\beta\underline{H}_2$C$^\gamma\underline{H}_2$C $^\delta\underline{H}_2$-Lys, –NHCH$_2$(C$\underline{H}_2$)$_4$CH$_2$NH–), 3.00 (8 H, m, 2 C$^\varepsilon$H$_2$ Lys, –NHC$\underline{H}_2$(CH$_2$)$_4$C$\underline{H}_2$NH–), 3.90 (4 H, m, 2 C$^\beta$H$_2$ Ser), 4.25 (4 H, m, 2 C$^\alpha$H Lys, 2 C$^\alpha$H Ser), 4.46 (4 H, s, 2 –C$\underline{H}_2$C$_6$H$_5$), 5.10 (4 H, s, 2 –OC$\underline{H}_2$C$_6$H$_4$Cl), 7.04 (6 H, s, 2 N$^+$H$_3$ Ser), 7.20–7.50 (20 H, m, 2 –CH$_2$C$_6\underline{H}_5$, 2 –OCH$_2$C$_6\underline{H}_4$Cl, –N$\underline{H}$(CH$_2$)$_6$N$\underline{H}$–),

7.74 (2 H, t, *J* 5.6 Hz, 2 $N^{\varepsilon}H$ Lys), 7.88 (2 H, d, *J* 7.6 Hz, 2 NH Lys).

$(HOOC(CH_2)_2CO–Ser(Bzl)\text{-}Lys(Z(Cl))–NH–)_2(CH_2)_6$. TEA (0.1 mL, 1.50 mmol) was added to a suspension of 2 $CF_3COOH \cdot (H–Ser(Bzl)\text{-}Lys(Z(Cl))–NH)_2(CH_2)_6$ (0.80 g, 0.72 mmol) in DMF (5 mL), the solution was stirred for 30 min, and succinic anhydride (0.408 g, 4.08 mmol) was added. The reaction mixture was stirred for 3 h. After the disappearance of the starting compound (TLC control), the reaction mixture was poured into cold water, and the white precipitate was filtered, washed with water, and dried over $CaCl_2$ in vacuo (15 mmHg). The yield of the product was 0.72 g (91%); R_f 0.40 (D), R_f 0.95 (C), mp 128–130°C (from ethyl acetate), $[\alpha]_D^{20}$ −11.25° (*c* 0.4; DMF); ^{1}H NMR: 0.95–1.78 (20 H, m, 2 $-C^{\beta}\underline{H}_2C^{\gamma}\underline{H}_2C^{\delta}\underline{H}_2$ Lys, $-NHCH_2(C\underline{H}_2)_4CH_2NH-$), 2.42 (8 H, m, 2 $HOOCC\underline{H}_2C\underline{H}_2$), 2.97 (8 H, m, 2 $C^{\varepsilon}H_2$ Lys, $-NHC\underline{H}_2(CH_2)_4C\underline{H}_2NH-$), 3.60 (4 H, m, 2 $C^{\beta}H_2$ Ser), 4.14 (2 H, m, 2 $C^{\alpha}H$ Lys), 4.44 (2 H, m, 2 $C^{\alpha}H$ Ser), 4.45 (4 H, s, 2 $-C\underline{H}_2C_6H_5$), 5.07 (4 H, s, 2 $-C\underline{H}_2C_6H_4Cl$), 7.20–7.49 (20 H, m, 2 $-CH_2C_6\underline{H}_5$, 2 $-CH_2C_6\underline{H}_4Cl$, $-N\underline{H}(CH_2)_6N\underline{H}-$), 7.53 (2 H, t, *J* 5.6 Hz, 2 $N^{\varepsilon}H$ Lys), 7.89 (2 H, d, *J* 7.3 Hz, 2 NH Lys), 8.24 (2 H, d, *J* 8.1 Hz, 2 NH Ser).

$(HOOC(CH_2)_2CO\text{-}Ser\text{-}Lys–NH–)_2(CH_2)_6$ (GSB-106). To a solution of $(HOOC(CH_2)_2CO–Ser(Bzl)\text{-}Lys(Z(Cl))–NH)_2(CH_2)_6$ (600 mg, 0.50 mmol) in methanol (50 mL), 10% Pd/C (500 mg) was added, and the solution was stirred in an atmosphere of hydrogen for 6–12 h until the starting compound completely disappeared (TLC control). After the termination of hydration, the catalyzer was filtered, the filtrate was evaporated, and the solid residue was washed with diethyl ether and dried in vacuo (15 mmHg). If the product was isolated as oil, the residue was then triturated with diethyl ether. The yield of the product was 450 mg (90%, total yield 23%); R_f 0.10 (F), R_f 0.40 (C), $\tau = 15 \pm 1$ min; mp 143–145°C, $[\alpha]_D^{20}$ −24.7° (*c* 0.4; DMF). Found, %: C 51.54; H 8.03; N 15.18; $C_{32}H_{58}N_8O_{12}$. Calculated, %: C 51.47; H 7.77; N 15.01. ^{1}H NMR: 1.10 (4 H, m, 2 $C^{\gamma}H_2$ Lys), 1.34 (4 H, m, 2 $C^{\delta}H_2$ Lys), 1.70 (4 H, m, 2 $C^{\beta}H_2$ Lys), 1.00–1.75 (8 H, m, $-NHCH_2(C\underline{H}_2)_4CH_2NH-$), 2.42 (8 H, m, 2 $HOOCC\underline{H}_2C\underline{H}_2$), 2.74 (4 H, m, 2 $C^{\varepsilon}H_2$ Lys), 3.00 (4 H, m, $-NHC\underline{H}_2(CH_2)_4C\underline{H}_2NH-$), 3.63 (4 H, m, 2 $C^{\beta}H_2$ Ser), 4.14 (2 H, m, 2 $C^{\alpha}H$ Lys), 4.27 (2 H, m, 2 $C^{\alpha}H$ Ser), 7.68 (2 H, t, *J* 4.9 Hz, $-N\underline{H}(CH_2)_6N\underline{H}-$), 7.72 (4 H, s br, 2 $N^{\varepsilon}H_2$ Lys), 7.95 (2 H, d, *J* 7.8 Hz, 2 NH Lys), 8.10 (2 H, d, *J* 8.1 Hz, 2 NH Ser).

Synthesis of $HOOC(CH_2)_2CO–Met\text{-}Ser–NH_2$ (GSB-207)

Boc-Met-Ser–OMe. TEA (5.7 mL, 41 mmol) was added under stirring to a solution of H–Ser–OMe hydrochloride (6.38 g, 41 mmol) and Boc-Met–ONp (15.8 g, 41 mmol) in DMF (50 mL). The reaction mixture was kept for 18 h at room temperature. After the termination of the reaction (TLC control), DMAPA (2 mL) was added, and the solution was stirred for 30 min and evaporated. Ethyl acetate (150 mL) and water (100 mL) were added to the residue. The organic phase was successively washed with 3% K_2CO_3 (3 × 60 mL) and 2% HCl (100 mL), dried over Na_2SO_4, and evaporated. The residue was dissolved in diethyl ether (50 mL), and heptane (25 mL) was added to turbidity. The crystals that precipitated were filtered, washed with heptane, and dried. The yield of the product was 11.6 g (80.7%); R_f 0.80 (C), R_f 0.67 (D); mp 65–68°C, $[\alpha]_D^{20}$ −20.75° (*c* 0.4, methanol); ^{1}H NMR: 1.36 (9 H, s, $-OC(CH_3)_3$), 1.81 (2 H, m, $C^{\beta}H_2$ Met), 2.07 (3 H, s, $-SCH_3$), 2.45 (2 H, t, *J* 5.3 Hz, $C^{\gamma}H_2$ Met), 3.62 (3 H, s, $-OCH_3$), 3.70 (2 H, m, $C^{\beta}H_2$ Ser), 4.08 (1 H, m, $C^{\alpha}H$ Met), 4.33 (1 H, m, $C^{\alpha}H$ Ser), 5.08 (1 H, m, –OH Ser), 6.98 (1 H, d, *J* 9.2 Hz, NH Met), 8.07 (1 H, d, *J* 8.0 Hz, NH Ser).

Boc-Met-Ser–NH_2. A solution of Boc-Met-Ser-OMe (4 g, 11.4 mmol) in methanol (10 mL) was saturated with ammonia at 0°C and kept for 3 days at room temperature in a closed flask. Then the solution was evaporated, and the residue was crystallized with a mixture of diethyl ether (30 mL) and ethanol (5 mL). The crystals formed were filtered off and dried. The yield of the product was 3.19 g (83.4%); R_f 0.71 (C), R_f 0.57 (D); mp 120–121°C, $[\alpha]_D^{20}$ −12.25° (*c* 0.4; MeOH); ^{1}H NMR: 1.38 (9 H, s, $-OC(CH_3)_3$), 1.82 (2 H, m, $C^{\beta}H_2$ Met), 2.03 (3 H, s, $-SCH_3$), 2.46 (2 H, t, *J* 5.2 Hz, $C^{\gamma}H_2$ Met), 3.56 (2 H, m, $C^{\beta}H_2$ Ser), 4.00 (1 H, m, $C^{\alpha}H$ Met), 4.17 (1 H, m, $C^{\alpha}H$ Ser), 7.12 and 7.23 (2 H, two s, NH_2 amide), 7.14 (1 H, d, *J* 9.1 Hz, NH Met), 7.65 (1 H, d, *J* 8.1 Hz, NH Ser).

$HOOC(CH_2)_2CO–Met–Ser–NH_2$ (GSB-207). A solution of Boc-Met-Ser-NH_2 (2.99 g, 8.9 mmol) in TFA (15 mL) was stirred for 1 h and evaporated. The residue was triturated with diethyl ether, and the crystals formed were filtered and washed with diethyl ether. Then the crystals were dissolved in 50% ethanol (20 mL), treated with Amberlite IRA-900 resin (–OH form) to pH ~ 9, filtered, and evaporated. The crystals were evaporated again with the addition of DMF. The residue was dissolved in DMF (10 mL), and succinic anhydride (1 g, 10 mmol) was added. The reaction mixture was stirred for 12 h, and water (25 mL) was added. The white precipitate was filtered, washed with water, and dried. The yield of the product was 0.96 g

(32%; total yield 21.5%); R_f 0.45 (C), R_f 0.11 (D); mp 101–105°C, $[\alpha]_D^{20}$ –14.25° (*c* 0.4; MeOH). Found, %: C 42.85; H 6.50; N 12.68; $C_{12}H_{21}N_3O_6S$. Calculated, %: C 42.99; H 6.27; N 12.54. ^{1}H NMR: 1.75 and 1.90 (2 H, two m, $C^\beta H_2$ Met), 2.03 (3 H, s, $-SCH_3$), 2.41 (4 H, m, HOOCC$\underline{H}_2$C$\underline{H}_2$), 2.44(2 H, t, *J* 5.2 Hz, $C^\gamma H_2$ Met), 3.58 (2 H, m, $C^\beta H_2$ Ser), 4.31 (1 H, m, C^αH Met), 4.16 (1 H, m, C^αH Ser), 4.90 (1 H, m, –OH Ser), 7.08 and 7.17 (2 H, two s, NH_2 amide), 8.16 (1 H, d, *J* 9.1 Hz, NH Met), 7.75 (1 H, d, *J* 8.2 Hz, NH Ser).

Synthesis of (HOOC(CH$_2$)$_2$CO–Met-Ser–NH–)$_2$(CH$_2$)$_7$ (GSB-214)

Boc-Met-Ser–N_2H_3. Hydrazine hydrate (3 mL) was added to a solution of Boc-Met-Ser-OMe (6.31 g, 18 mmol) (obtained earlier during the synthesis of GSB-207) in MeOH (10 mL), and the solution was kept for 2 h. After the disappearance of the starting compound (TLC control), water (10 mL) was added to the reaction mixture, and the mixture was kept at –20°C for 18 h. The precipitate was filtered, washed with cooled aqueous methanol, and air-dried. The yield of the product was 6.31 g (100%); R_f 0.66 (C), R_f 0.47 (D); mp 160–162°C, $[\alpha]_D^{20}$ –21.75° (*c* 0.4; MeOH); ^{1}H NMR: 1.37 (9 H, s, $-OC(CH_3)_3$), 1.80 (2 H, m, $C^\beta H_2$ Met), 2.02 (3 H, s, $-SCH_3$), 2.43 (2 H, t, *J* 5.2 Hz, $C^\gamma H_2$ Met), 3.54 (2 H, m, $C^\beta H_2$ Ser), 4.00 (1 H, m, C^αH Met), 4.19 (1 H, m, C^αH Ser), 4.39 (2 H, d, *J* 4.0 Hz, –NH–N$\underline{H}_2$), 4.88 (1 H, t, *J* 4.0 Hz, –OH Ser), 7.07 (1 H, d, *J* 9.1 Hz, NH Met), 7.69 (1 H, d, *J* 8.0 Hz, NH Ser), 9.02 (1 H, t, *J* 4.0 Hz, –N$\underline{H}$–NH_2).

(Boc-Met-Ser–NH–)$_2$(CH$_2$)$_7$. To Boc-Met-Ser–N_2H_3 (5.23 g, 14.9 mmol) in DMF (25 mL) cooled to –20°C, first HCl in dioxane (~4 M, 50 mL) and then *n*-butyl nitrite (1.75 mL, 15 mmol) were added; the temperature was maintained at –20°C…–30°C. The reaction mixture was kept for 3 min after which first DIEA (7.7 mL, 45 mmol) and then a solution of heptamethylenediamine (0.91 g, 7 mmol) in DMF (3 mL) were added. The reaction mixture was kept for 12 h at room temperature after which ethyl acetate (250 mL) and water (200 mL) were added. The organic phase was washed with 2% HCl (2 × 100 mL) and 3% $NaHCO_3$ (100 mL) and evaporated to a volume of ~60 mL. A gradual settling of a precipitate was observed, and the amount of the precipitate increased as the solution was cooled to –20°C. The precipitate was filtered at room temperature, washed with ethyl acetate, and dried. The yield of the product was 3.3 g (57%); R_f 0.77 (C), R_f 0.59 (D); mp 160–162°C, $[\alpha]_D^{20}$ –28.75° (*c* 0.4, methanol); ^{1}H NMR: 1.18–1.40 (10 H, m, –NHCH$_2$(C$\underline{H}_2$)$_5$CH$_2$NH–), 1.37 (18 H, s, 2 –OC(CH$_3$)$_3$), 1.80 (4 H, m, 2 $C^\beta H_2$ Met), 2.02 (6 H, s, 2 $-SCH_3$), 2.44 (4 H, t, *J* 5.5 Hz, 2 $C^\gamma H_2$ Met), 3.02 (4 H, m, –NHC$\underline{H}_2$(CH$_2$)$_5$C$\underline{H}_2$NH–), 3.53 (4 H, m, 2 $C^\beta H_2$ Ser), 3.99 (2 H, m, 2 C^αH Met), 4.17 (2 H, m, 2 C^αH Ser), 7.12 (2 H, d, *J* 9.1 Hz, 2 NH Met), 7.68 (2 H, d, *J* 8.0 Hz, 2 NH Ser), 7.71 (2 H, t, *J* 5.5 Hz, –N$\underline{H}$(CH$_2$)$_7$N$\underline{H}$–).

(HOOC(CH$_2$)$_2$CO–Met-Ser–NH–)$_2$(CH$_2$)$_7$ (GSB-214). (Boc-Met-Ser–NH)$_2$(CH$_2$)$_7$ (3.14 g, 4.1 mmol) was dissolved in TFA (20 mL), and the solution was stirred for 30 min and evaporated. The residue was triturated with diethyl ether, decanted, dissolved in 50% ethanol (50 mL), and treated with Amberlite IRA-900 resin (OH-form) to pH ~ 10. The solution was evaporated and evaporated again with DMF (40 mL). The residue was dissolved in DMF (10 mL), and succinic anhydride (1 g, 10 mmol) was added. The reaction mixture was stirred for 2 h and evaporated. The residue was triturated with acetone and filtered. Ethanol (30 mL) was added to the solid residue, and the mixture was boiled for 30 min (the residue does not dissolve completely), kept for 30 min at –20°C, filtered off, and successively washed with cold ethanol, diethyl ether, and hexane. After drying, 1.75 g of the product (100%, total yield 46%) was obtained; R_f 0.57 (C), R_f 0.0 (D); mp 162–163°C, $[\alpha]_D^{20}$ –9.00° (*c* 0.4; DMF). Found, %: C 48.72; H 7.30; N 10.88; $C_{31}H_{54}N_6O_{12}S_2$. Calculated, %: C 48.56; H 7.05; N 10.97. ^{1}H NMR: 1.20–1.40 (10 H, m, –NHCH$_2$(C$\underline{H}_2$)$_5$CH$_2$NH–), 1.76 and 1.89 (4 H, two m, 2 $C^\beta H_2$ Met), 2.02 (6 H, s, 2 $-SCH_3$), 2.40 (8 H, m, 2 HOOCC$\underline{H}_2$C$\underline{H}_2$), 2.42 (4 H, m, 2 $C^\gamma H_2$ Met), 3.01 (4 H, m, –NHC$\underline{H}_2$(CH$_2$)$_5$C$\underline{H}_2$NH–), 3.55 (4 H, m, 2 $C^\beta H_2$ Ser), 4.17 (2 H, m, 2 C^αH Ser), 4.30 (2 H, m, 2 C^αH Met), 7.61 (2 H, m, –N$\underline{H}$(CH$_2$)$_7$N$\underline{H}$–), 7.76 (2 H, d, *J* 8.1 Hz, 2 NH Ser), 8.16 (2 H, d, *J* 9.2 Hz, 2 NH Met).

Study of the biological activity in vitro. Immortalized murine hippocampal cells of line HT22 were seeded in 96-well plates with a density of 3.5 thousand cells per well in medium DMEM (HyClon) containing 5% calf fetal serum (Invitrogen) and 2 mM *L*-glutamine (ICN) and incubated at 37°C in 5% CO_2 until a monolayer formed. Oxidative stress was modeled using H_2O_2 at a final concentration of 1.5 mM. Cells were incubated with H_2O_2 in an atmosphere of 5% CO_2 at 37°C for 30 min. Then the medium was replaced by normal medium, and after 4 h cell viability was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) (Sigma). Optical absorption was measured on a Multiscan EX spectrophotometer (Thermo) at 600 nm. Peptides were introduced 24 h prior to the addition of hydrogen peroxide. BDNF (Sigma) at a final concentration of 50 ng/mL was used as a positive control.

Each experiment was carried out in 16 replicas (16 wells). The neuroprotective activity was determined using the average values. The activity in experiments on the counteraction to oxidative stress was calculated by the formula:

$$A(\%) = (D_{exp} - D_{act})/(D_{contr} - D_{act}) \times 100,$$

where D_{exp} is the optical absorption of solution in experiment, D_{act} is the optical absorption of a solution of active control (with H_2O_2), and D_{contr} is the optical absorption of a solution of passive control (without H_2O_2). The statistical significance of the difference between the experiment and control was determined by the Student's *t*-test.

Study of the biological activity in vivo. The activity was examined in the "forced swimming" test [17]. Balb/c male mice weighing 20–22 g from a nursery of laboratory animals in Pushchino (10 animals in each experimental group) were used. Animals were housed for 7 days before the test in a vivarium of the Zakusov Research Institute of Pharmacology, Russian Academy of Medical Sciences, under standard conditions with free access to water and briquetted feed under a natural light/dark regime. All experiments were carried out in the autumn–winter period, from 10 to 13 a. m. On the first day of the experiment, animals were housed for 10 min in a cylinder (height 30 cm, diameter 10 cm) filled with water (22°C) to 65%. After 60 min, peptides were injected to the animals. After 24 h, the animals were placed again in the same conditions, and the time during which the animal retained a typical form of immobility (refusal from active defensive and exploratory behavior) was registered for 5 min. The antidepressant imipramine at a dose of 25 mg/kg served as a positive control; it was injected intraperitoneally 60 min prior to the test. The peptides were injected intraperitoneally in the form of aqueous solutions (GSB-104 and GSB-106) or a water–Tween suspension (GSB-207 and GSB-214), 5 mL per 1 kg of body weight of the mouse. Control animals were injected with either distilled water or 0.05% aqueous Tween-80 (Merck).

The data on the duration of immobility were processed using nonparametric statistics. The data were presented, not as arithmetic means, but as median values of samples representing a value that is in the middle of the series of immobility durations for all animals in the group in the order of increasing value. To access the scatter of sampling, instead of root-mean-square deviations, the lower and the upper quartiles (the values of a parameter being measured that cut off, respectively, one fourth of its values from below and one fourth from the top in this series) were used. The activity of the compounds was calculated by the formula:

$$A(\%) = (T_{contr} - T_{exp})/(T_{contr} - T_{im\text{-}in}) \times 100,$$

where T_{contr} is the duration of immobility (s) in the control group, T_{exp} is the duration of immobility (s) in the experimental group, and $T_{im\text{-}in}$ is the duration of immobility (s) in the group Imipramine. For the calculation, the median values of samples were used. The differences between control and experimental groups were determined using the nonparametric Mann–Whitney *U*-test.

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