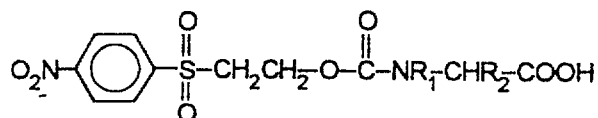


N_α-2-(4-Nitrophenylsulfonyl)ethoxycarbonyl-amino acidsBackground of the invention

The field of the invention concerns new protected amino acid derivatives useful for peptide synthesis, namely, N_α-2-(4-nitrophenylsulfonyl)ethoxycarbonyl-amino acids having the general formula I:

**I**

10

wherein R₁ represents hydrogen atom, and R₂ may represent hydroxymethyl, 1-hydroxyethyl, 4-hydroxybenzyl, imidazolyl-2-methyl, benzyloxymethyl, 1-benzyloxyethyl, 4-benzyloxybenzyl, benzyloxycarbonylmethyl, 2-(benzyloxycarbonyl)ethyl, S-
 15 benzylthiomethyl, S-(diphenylmethyl)thiomethyl, 4-(benzyloxycarbamido)butyl, 3-guanidinopropyl, 3-(N^G-toluenesulfonyl)guanidinopropyl, 3-(N^G-nitro)guanidinopropyl, 3-[N^G-(4-methoxy-2,3,6-trimethylbenzenesulfonyl)]-guanidinopropyl, N-(4,4'-dimethoxybenzhydryl)carboxamidomethyl,

2-[N-(4,4'-dimethoxybenzhydryl)carboxamido]ethyl, S-tert-butyl
dithiomethyl, 4-[2-(4-nitrophenylsulfonyl)ethoxycarbamido]
-butyl, propyl, butyl, ethyl, 3-(benzyloxycarbamido)propyl, or
3-(tert-butoxycarbamido)propyl, and methods for the preparation
5 thereof.

N_α -2-(4-Nitrophenylsulfonyl)ethoxycarbonyl-amino acids
(Nsc-amino acids) I represent a class of protected amino acid
10 derivatives which are used in the chemical synthesis of
peptides. In these derivatives, N_α -2-(4-nitrophenylsulfonyl)
-ethoxycarbonyl (Nsc) group serves as a temporary N_α -protection
which can be selectively removed after each step of the peptide
chain elongation. Nsc-Group is fairly resistant to acidic
15 reagents and is cleaved according to the β -elimination
mechanism by organic bases in aprotic solvents. Mild
conditions of the cleavage allow to use the temporary N_α -Nsc-
protection in the peptide synthesis together with the acid-
sensitive side chain protection of widely used t-butyl or

benzyl type, thus providing the so-called "orthogonality" of the synthetic strategy.

Recently N_α -Nsc-amino acids, methods for their preparation, and their employment for the solid phase peptide synthesis have been disclosed in International Publication No. WO96/25394. However, only minimal set of N_α -Nsc-derivatives of proteogenic amino acids has been described, which is intended for the solid phase peptide synthesis with full side-chain protection of acid-labile tert-butyl or compatible type.

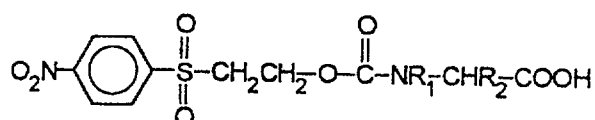
10 Summary of the Invention

It is, therefore, desirable to develop new N_α -Nsc-amino acids to extend the list of derivatives available for solid phase synthesis. On the other hand, it is useful to provide N_α -Nsc-amino acid derivatives with another type of side
15 protection(e.g., benzyl or compatible) or without side protection which may give new opportunities for liquid phase peptide synthesis.

An object of the present invention is to provide new N_α -Nsc-amino acids derivatives, more particularly, N_α -2-

4

(nitrophenylsulfonyl)ethoxycarbonyl-amino acids having the general formula I:



I

5

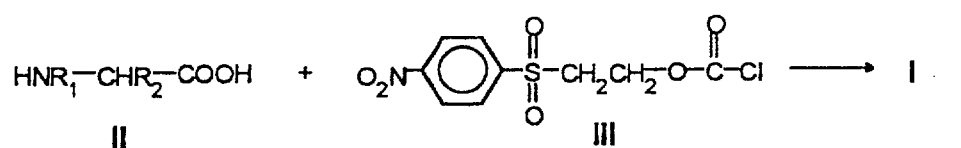
wherein R_1 represents hydrogen atom, and R_2 may represent hydroxymethyl, 1-hydroxyethyl, 4-hydroxybenzyl, imidazolyl-2-methyl, benzyloxymethyl, 1-benzyloxyethyl, 4-benzyloxybenzyl, benzyloxycarbonylmethyl, 2-(benzyloxycarbonyl)ethyl, S-10 benzylthiomethyl, S-(diphenylmethyl)thiomethyl, 4-(benzyloxycarbamido)butyl, 3-guanidinopropyl, 3-(N^G -toluenesulfonyl)guanidinopropyl, 3-(N^G -nitro)guanidinopropyl, 3-[N^G -(4-methoxy-2,3,6-trimethylbenzenesulfonyl)]-guanidinopropyl, N-(4,4'-dimethoxybenzhydryl)carboxamidomethyl, 15 2-[N-(4,4'-dimethoxybenzhydryl)carboxamido]ethyl, S-tert-butylldithiomethyl, 4-[2-(4-nitrophenylsulfonyl)ethoxycarbamido]-butyl, propyl, butyl, ethyl, 3-(benzyloxycarbamido)propyl, or 3-(tert-butoxycarbamido)propyl, which can be employed as N_α -protected amino acid derivatives in peptide synthesis.

Another object of the present invention is to provide methods for the preparation of said N_α -Nsc-amino acids. These and other objects of the present invention will be apparent from the following description

5 Detailed Description of the Invention

N_α -Nsc-amino acids of the present invention(I) can be prepared by the treatment of amino acids of the general formula II, wherein R_1 and R_2 represent meanings given for formula I, with 2-(4-nitrophenylsulfonyl)ethylchloroformate III in mixed
10 aqueous/organic solvent in the presence of base and at the temperature from 0 to 40°C, preferably from 0 to 20°C(Scheme 1).

Scheme 1



15

Chloroformate III is introduced into the reaction in amounts from 0.5 to 1.5 molar equivalents, preferably from 0.7 to 0.9, as related to amino acid. As an organic component of the solvent any aprotic organic solvent may be used which is

capable to dissolve the acylating reagent and is mixible with water, for example, acetonitrile, DMF, tetrahydrofuran or dioxane. A base may be organic or inorganic base, for example, sodium or potassium carbonate, magnesium or calcium oxide, 5 triethylamine, N-methylmorpholine.

According to another method of the present invention, amino acids of the general formula II are firstly converted into N,O-trimethylsilyl derivatives using methods known in the art and then treated with equivalent amount of chloroformate 10 III in anhydrous organic solvent, for example, dichloromethane in the presence of base, for example, tertiary amine. After aqueous hydrolysis of intermediate acylated trimethylsilyl derivatives desirable N_α-Nsc-amino acids I are obtained in a free form.

15 Derivatives of the formula I, wherein R₁ is hydrogen, and R₂ represents N-(4,4'-dimethoxybenzhydryl)carboxamidomethyl or 2-[N-(4, 4'-dimethoxybenzhydryl)carboxamido]ethyl, may be prepared by the reaction of the known derivatives I, wherein R₁ is hydrogen, and R₂ represents carboxamidomethyl or 2-

(carboxamido)ethyl, with 4,4'-dimethoxybenzhydrol in organic solvent in the presence of strong acid. As a solvent acetic acid may be used, and as a strong acid-sulfuric or methanesulfonic acid.

5 Derivative of the formula I, wherein R_1 is hydrogen, and R_2 represents 3-guanidinopropyl (N_α -Nsc-Arg-OH), may be prepared by the reaction of arginine with 2-(4-nitrophenylthio)-ethylchloroformate in water in the presence of sodium bicarbonate, resulting N_α -2-(4-nitrophenylthio)ethoxycarbonyl-
10 arginine is then oxidized into desired N_α -Nsc-arginine with hydrogen peroxide in organic solvent, for example, ethanol or acetic acid.

Derivative of the formula I, wherein R_1 is hydrogen, and R_2 represents imidazolyl-2-methyl (N_α -Nsc-His-OH), may be
15 prepared from N_α -Nsc- N_{im} -protected histidine derivative by selective deprotection of imidazole ring, for example, by selective acidic detritylation of known N_α -Nsc- N_{im} -triphenylmethyl-histidine.

It is seen from molecular formula that compounds I have

an asymmetric α -carbon atom. Because α -carbon atom does not participate in reactions employed for the preparation of compounds I, so the configuration of this chiral center existing in starting amino acids II is retained in resulting
5 N_α -Nsc-derivatives I. Therefore it is obvious that the methods of the present invention can be used for the preparation of N_α -Nsc-amino acids I in any chiral form(L or D), as well as racemic compounds, depending on the configuration of the compound II.

10 Meanings of R_1 and R_2 substituents in derivatives of the formula I according to the present invention correspond to structures of side chains of known α -amino acids containing or not containing protective groups known in the art(Table 1).

Table 1

Meanings of R₁ and R₂ substituents in compounds I

No	R ₁	R ₂	Amino acid	Abbreviation
I-1	H	Hydroxymethyl	Serine	Nsc-Ser-OH
5	I-2	H 1-Hydroxyethyl	Threonine	Nsc-Thr-OH
	I-3	H 4-Hydroxybenzyl	Tyrosine	Nsc-Tyr-OH
	I-4	H Imidazolyl-2-methyl	Histidine	Nsc-His-OH
	I-5	H Benzyloxymethyl	O-Benzyl-serine	Nsc-Ser(Bzl)-OH
	I-6	H 1-Benzyloxyethyl	O-Benzyl-threonine	Nsc-Thr(Bzl)-OH
10	I-7	H 4-Benzyloxybenzyl	O-Benzyl-tyrosine	Nsc-Tyr(Bzl)-OH
	I-8	H Benzyloxycarbonylmethyl	Aspartic acid β -benzyl -ester	Nsc-Asp(OBzl)-OH
	I-9	H 2-(Benzyloxycarbonyl)ethyl	Glutamic acid γ -benzyl -ester	Nsc-Glu(OBzl)-OH
15	I-10	H S-Benzylthiomethyl	S-Benzyl-cysteine	Nsc-Cys(Bzl)-OH
	I-11	H S-(Diphenylmethyl)thio	S-Diphenylmethyl-cysteine	Nsc-Cys(Dpm)-OH -methyl

No	R1	R2	Amino acid	Abbreviation	
I-12	H	4-(Benzyloxycarbamido) -butyl	N ϵ -Benzyloxycarbonyl -lysine	Nsc-Lys(Z)-OH	
5	I-13	H	3-Guanidinopropyl	Arginine	Nsc-Arg-OH
I-14	H	3-(N ^G -Toluenesulfonyl) guanidinopropyl	N ^G -Toluenesulfonyl -arginine	Nsc-Arg(Tos)-OH	
I-15	H	3-(N ^G -Nitro)guanidino -propyl	N ^G -Nitro-arginine	Nsc-Arg(NO ₂)-OH	
10	I-16	H	3-[N ^G -(4-Methoxy-2,3,6-trimethyl-benzenesulfonyl)] guanidinopropyl	N ^G -(4-Methoxy-2,3,6-trimethylbenzenesulfonyl) -arginine	Nsc-Arg(Mtr)-OH
I-17	H	N-(4,4'-Dimethoxybenzhydryl)carboxamidomethyl	N β -(4,4'-Dimethoxybenzhydryl)-asparagine	Nsc-Asn(Mbh)-OH	
15	I-18	H	2-[n-(4,4'-Dimethoxybenzhydryl)carboxamido]ethyl	N γ -(4,4'-Dimethoxybenzhydryl)-glutamine	Nsc-Gln(Mbh)-OH
I-19	H	S-tert-Butyldithiomethyl	S-tert-Butylthio-cysteine	Nsc-Cys(StBu)-OH	

11

No	R1	R2	Amino acid	Abbreviation
I-20	H	4-[2-(4-Nitrophenylsulfonyl)ethoxycarbamido]butyl	Nε-[2-(4-Nitrophenylsulfonyl)ethoxy carbonyl]-lysine	Nsc-Lys(Nsc)-OH
I-21	H	Propyl	Norvaline	Nsc-Nva-OH
5	I-22	H Butyl	Norleucine	Nsc-Nle-OH
I-23	H	Ethyl	α-Aminobutyric acid	Nsc-Abu-OH
I-24	H	3-(benzyloxycarbamido)-propyl	Nδ-Benzyloxycarbonyl-omitine	Nsc-Om(Z)-OH
I-25	H	3-(tert-Butoxycarbamido)-propyl	Nδ-tert-Butoxycarbonyl-omitine	Nsc-Om(Boc)-OH
10				

Together with N_α -Nsc-amino acids disclosed in International Publication No. WO96/25394, the compounds of the formula I shown in Table 1 may be used to implement a wide variety of tactical approaches to the peptide synthesis, including not only solid phase but also solution syntheses. Using N_α -Nsc-protection it is possible now to develop synthetic schemes of peptide assembly in solution on the basis of tert-butyl type or benzyl type or mixed type of side-chain protection, and also to apply minimal side-chain protection tactics.

The present invention, therefore, is regarded as an addition to, and a continuation of, aforementioned International Publication No. WO96/25394.

The invention will now be described by way of examples which are provided as illustration and are not intended as being limiting. All of the amino acids in the following description have L-configuration unless otherwise indicated.

Example 1

N_α -Nsc-O-Benzyl-Tyrosine(I-7)

8.15 g of O-benzyl-tyrosine and 7.7 g of potassium carbonate were dissolved in 150 ml of water-dioxane mixture(3:1, v/v) and cooled in ice bath, then solution of 7.5 g of 2-(4-nitrophenylsulfonyl)ethyl chloroformate III in 70 ml of dioxane was added dropwise within 15 min with stirring. Cooling bath was removed and mixture was stirred for additional 20 min, then evaporated to ca. 100 ml under reduced pressure and transferred into separating funnel. 100 ml of water was added, and the resulting solution was extracted with 2 x 50 ml of ethyl acetate. Aqueous layer was separated, acidified to pH 2 with 40% sulfuric acid with cooling in ice bath, then extracted with 3 x 80 ml of ethyl acetate. Combined extracts were dried over anhydrous sodium sulfate and evaporated at reduced pressure. Recrystallization of the residue from ethyl acetate-hexane gave the desired product I-7 in a form of white crystalline powder(70%). For characterization see Table 2(Example 7).

Example 2

N_α-Nsc-Threonine(I-2).

3.58 g of threonine and 100 ml anhydrous dichloromethane were placed into 250 ml round-bottom flask equipped with reflux condenser and dropping funnel. To the suspension 9.7 ml of chlorotrimethylsilane was added with vigorous stirring, and the mixture was heated to boiling for 1 hr. The resulting solution was cooled in ice bath, then 9.0 ml of triethylamine and 9.0 g of chloroformate III were added with stirring. The mixture was stirred for 20 min in ice bath, then for additional 1.5 hr at room temperature. The solvent was evaporated at reduced pressure, and the residue was distributed between 200 ml of ethyl acetate and 250 ml of 2.5% aqueous sodium bicarbonate. Aqueous layer was separated, washed with 50 ml of ether, acidified to pH with 1 N hydrochloric acid, then extracted with 3 x 70 ml of ethyl acetate. Combined extracts were dried with anhydrous sodium sulfate and evaporated at reduced pressure. Trituration of the residue with petroleum ether gave the desired product I-2 in a form of white powder (75%). For characterization see Table 2 (Example 7).

Example 3

N_α-Nsc-N_ε-Benzyloxycarbonyl-Ornithine(I-24).

8.0 g of N_ε-benzyloxycarbonyl-ornithine and 120 ml of anhydrous dichloromethane were placed into 250 ml round-bottom flask equipped with reflux condenser and dropping funnel. To
5 the mixture 12.7 ml of triethylamine and then 7 ml of chlorotrimethylsilane were added with vigorous stirring, and the mixture was heated to boiling for 1.5 hr. The reaction mixture was then cooled in ice bath, 9.0 g of chloroformate III was added at once, and stirring was continued for 1.5 hr at
10 room temperature. The solvent was evaporated at reduced pressure, and the residue was distributed between 250 ml of ethyl acetate and 250 ml of 2.5% aqueous sodium bicarbonate. Aqueous layer was separated, washed with 50 ml of ether, acidified to pH 2 with 1 N hydrochloric acid, then extracted
15 with 2 x 100 ml of ethyl acetate. Combined extracts were dried with anhydrous sodium sulfate and evaporated at reduced pressure. Recrystallization of the residue from ethyl acetate-hexane gave the desired product I-24 in a form of white powder(84%). For characterization see Table 2(Example 7).

Example 4

N_{α} -Nsc-N(4,4'-Dimethoxybenzhydryl)-Asparagine(I-17).

3.89 g of N_{α} -Nsc-asparagine and 2.6 g of 4,4'-dimethoxybenzhydrol were dissolved in 25 ml of glacial acetic acid. To the solution 0.4 ml of methanesulfonic acid was added, and the mixture was allowed to stand overnight at room temperature. The resulting mixture was then poured into 100 ml of ice-cold water with mixing, the formed precipitate was filtered off, washed with water and then with ether. The crude product was dissolved in 10 ml of warm DMF, filtered and reprecipitated with ether. The precipitate was collected by filtration, washed with ether and dried in vacuo yielding the desired compound I-17 as a crystalline powder(80%). For characterization see Table 2(Example 7).

Example 5

N_{α} -Nsc-Arginine(I-13).

To the solution of 1.7 g of sodium bicarbonate and 4.22 g of arginine hydrochloride in 25 ml of water stirred at room temperature 5.74 g of 2-(4-nitrophenylthio)ethylchloro-formate

was added in five equal portions during 30 min, then stirring was continued for additional 2 hrs. The mixture was saponified to pH 8.5 with conc. ammonia solution and left for 3 hrs at 4°C. Precipitate formed was collected, washed with ice-cold water, dried on the filter with suction and dissolved in 30 ml of acetic acid. To the solution 4 ml of 34% hydrogen peroxide was added, and the mixture was left for 30 hrs at room temperature. Then the mixture was evaporated to dryness, and the residue was triturated with ethyl acetate, collected by filtration, washed with ether and dried on air to give the desired product I-13(88%). For characterization see Table 2(Example 7).

Example 6

N_α -Nsc-Histidine(I-4).

6.6 g of N_α -Nsc- N_{im} -triphenylmethyl-histidine was dissolved in 30 ml 90% aqueous acetic acid and boiled under reflux for 30 min. After cooling the precipitate was filtered off, and the filtrate was evaporated. The residue was triturated with ether and collected by filtration giving the desired compound I-4 in

95% yield. For characterization see Table 2 (Example 7).

Example 7

Properties of N_α-Nsc-amino acids I.

Shown in Tabel 2 are the compounds of formula I which were
5 prepared utilizing provided methods described in detail in
examples 1-6. Figures in the column "Method" correspond to
numbers of examples where particular methods are described.
Chromatographic mobility values R_f are shown for thin-layer
chromatography sheets Alufolien Keselgel 60 F₂₅₄ (Merck,
10 Darmstadt, Germany); chloroform/methanol/acetic acid, 95:5:3,
(A) and benzene/acetone/acetic acid, 100:50:3, (B), were used
as developing solvents, spots were detected by UV-absorbance
and/or by ninhydrin reaction. Molecular ion masses (M+H)⁺ were
measured using MS-BC-1 time-of-flight mass spectrometer with
15 Cf²⁵² radiation-promoted desorption (Electrom SPA, Sumy,
Ukraine).

Tabel 2

Properties of N_α-Nsc-amino acids I

Entry	Compound	Method	R _f (A)	R _f (B)	Molecular formula	Molecular ion, (M+H) ⁺		
						Calcd	Found	
5	1	2	3	4	5	6	7	8
	I-1	Nsc-Ser-OH	2	0.40	0.30	C ₁₂ H ₁₄ N ₂ O ₉ S	363.32	363.6
	I-2	Nsc-Thr-OH	2	0.42	0.35	C ₁₃ H ₁₆ N ₂ O ₉ S	377.35	377.6
	I-3	Nsc-Tyr-OH	2	0.40	0.37	C ₁₈ H ₁₈ N ₂ O ₉ S	439.44	439.9
	I-4	Nsc-His-OH	6	0.15	0.10	C ₁₅ H ₁₆ N ₄ O ₈ S	413.40	413.1
10	I-5	Nsc-Ser(Bzl)-OH	1,3	0.70	0.55	C ₁₉ H ₂₀ N ₂ O ₉ S	453.46	453.8
	I-6	Nsc-Thr(Bzl)-OH	3	0.70	0.55	C ₂₀ H ₂₂ N ₂ O ₉ S	467.49	467.9
	I-7	Nsc-Tyr(Bzl)-OH	1,3	0.75	0.55	C ₂₅ H ₂₄ N ₂ O ₉ S	529.57	530.1
	I-8	Nsc-Asp(OBzl)-OH	3	0.60	0.45	C ₂₀ H ₂₀ N ₂ O ₁₀ S	481.47	480.9
	I-9	Nsc-Asp(OBzl)-OH	3	0.62	0.43	C ₂₁ H ₂₂ N ₂ O ₁₀ S	459.50	495.1
15	I-10	Nsc-Cys(Bzl)-OH	3	0.75	0.60	C ₁₉ H ₂₀ N ₂ O ₈ S ₂	469.53	469.3
	I-11	Nsc-Cys(Dpm)-OH	1,3	0.75	0.70	C ₂₅ H ₂₄ N ₂ O ₈ S ₂	545.63	545.2
	I-12	Nsc-Lys(Z)-OH	3	0.65	0.50	C ₂₃ H ₂₇ N ₃ O ₁₀ S	538.58	538.3

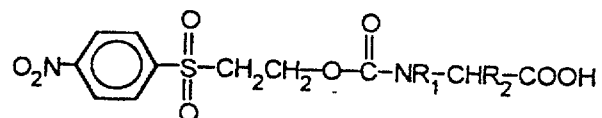
Entry	Compound	Method	R _f (A)	R _f (B)	Molecular formula	Molecular ion, (M+H) ⁺	
						Calcd	Found
1	2	3	4	5	6	7	8
I-13	Nsc-Arg-OH	5	0.10	0.05	C ₁₅ H ₂₁ N ₅ O ₈ S	432.45	432.8
5 I-14	Nsc-Arg(Tos)-OH	1,2	0.35	0.30	C ₂₂ H ₂₇ N ₅ O ₁₀ S ₂	586.64	586.9
I-15	Nsc-Arg(NO ₂)-OH	2	0.25	0.15	C ₁₅ H ₂₀ N ₆ O ₁₀ S	477.45	477.1
I-16	Nsc-Arg(Mtr)-OH	3	0.43	0.35	C ₂₅ H ₃₃ N ₅ O ₁₀ S ₂	628.73	629.4
I-17	Nsc-Asn(Mbh)-OH	4	0.55	0.38	C ₂₈ H ₂₉ N ₃ O ₁₁ S	616.65	616.1
I-18	Nsc-Gln(Mbh)-OH	4	0.58	0.33	C ₂₉ H ₃₁ N ₃ O ₁₁ S	630.68	630.2
10 I-19	Nsc-Cys(StBu)-OH	3	0.60	0.45	C ₁₆ H ₂₂ N ₂ O ₈ S ₃	467.57	567.2
I-20	Nsc-Lys(Nsc)-OH	3	0.55	0.40	C ₂₄ H ₂₈ N ₄ O ₁₄ S ₂	661.66	662.1
I-21	Nsc-Nva-OH	2	0.65	0.50	C ₁₄ H ₁₈ N ₂ O ₈ S	375.39	375.6
I-22	Nsc-Nle-OH	2	0.65	0.50	C ₁₅ H ₂₀ N ₂ O ₈ S	389.42	389.6
I-23	Nsc-Abu-OH	2	0.60	0.47	C ₁₃ H ₁₆ N ₂ O ₈ S	375.40	375.8
15 I-24	Nsc-Om(Z)-OH	3	0.62	0.47	C ₂₂ H ₂₅ N ₃ O ₁₀ S	533.55	534.0
I-25	Nsc-Om(Boc)-OH	3	0.65	0.43	C ₁₉ H ₂₇ N ₃ O ₁₀ S	490.53	491.0



What is claimed is:

1. N_x-2-(4-nitrophenylsulfonyl)ethoxycarbonyl-amino acids

having the general formula:



5

wherein R₁ represents hydrogen atom, and R₂ may represent hydroxymethyl, 1-hydroxyethyl, 4-hydroxybenzyl, imidazolyl-2-methyl, benzyloxymethyl, 1-benzyloxyethyl, 4-benzyloxybenzyl, benzyloxycarbonlmethyl, 2-(benzyloxycarbonyl)ethyl, S-
 10 benzylthiomethyl, S-(diphenylmethyl)thiomethyl, 4-(benzyloxycarbamido)butyl, 3-guanidinopropyl, 3-(N^G-toluenesulfonyl)guanidinopropyl, 3-(N^G-nitro)guanidinopropyl, 3-[N^G-(4-methoxy-2,3,6-trimethylbenzenesulfonyl)]-guanidinopropyl, N-(4,4'-dimethoxybenzhydryl)carboxamidomethyl,
 15 2-[N-(4,4'-dimethoxybenzhydryl)carboxamido]ethyl, S-tert-butylldithiomethyl, 4-[2-(4-nitrophenylsulfonyl)ethoxycarbamido]-butyl, propyl, butyl, ethyl, 3-(benzyloxycarbamido)propyl, or 3-(tert-butoxycarbamido)propyl, or 3-(tert-butoxycarbamido)-propyl.



2. A method for preparing of compounds according to the claim 1 comprising reacting amino acid of the general formula

wherein R_1 and R_2 represent radicals according to the
5 claim, with 2-(4-nitrophenylsulfonyl)ethoxycarbonyl
chloroformate in mixed aqueous-organic solvent in the presence
of base.

3. A method for preparing of compounds according to the claim 1 comprising:

10 a) converting of amino acid of the general formula

wherein R_1 and R_2 represent radicals according to the claim 1, into O,N-trimethylsilylated derivatives;

b) reacting of said O,N-trimethylsilylated derivatives with
15 2-(4-nitrophenylsulfonyl)ethoxycarbonyl chloroformate in
aprotic solvent in the presence of base with subsequent
hydrolysis.

4. A method for preparing of compound according to the claim 1, wherein R_1 represents hydrogen atom, and R_2 represents



N-(4,4'-dimethoxybenzhydryl)carboxamidomethyl or 2-[N-(4,4'-dimethoxybenzhydryl)carboxamido]ethyl, comprising reacting of N_{α} -2-(4-nitrophenylsulfonyl)-ethoxycarbonyl-asparagine or N_{α} -2-(4-nitrophenylsulfonyl)ethoxycarbonyl-glutamine, with 4-4'-dimethoxybenzhydrol in organic solvent in the presence of acid.

5 5. A method for preparing of compound according to the claim 1, wherein R_1 represents hydrogen atom, and R_2 represents 3-guanidinopropyl, comprising reacting of arginine with 2-(4-nitro-phenylthio)ethylchloroformate in water in the presence of sodium bicarbonate and oxidizing thus formed N_{α} -2-(4-nitrophenylthio)ethoxycarbonyl-arginine with hydrogen peroxide in organic solvent.

6. A method for preparing of compound according to the claim 1, wherein R_1 represents hydrogen atom, and R_2 represents imidazolyl-2-methyl, comprising reacting of N_{α} -2-(4-nitrophenylsulfonyl)ethoxycarbonyl- N_{im} -triphenylmethyl - histidine with acid.