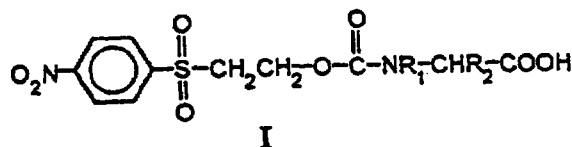


N_ε-2-(4-Nitrophenylsulfonyl)ethoxycarbonyl-amino acids**Background of the Invention**

The field of the invention concerns protected amino acid derivatives for

solid phase peptide synthesis, namely,

5 N_ε-2-(4-nitorphenylsulfonyl)ethoxycarbonyl-amino acids having the general formula I:



10 wherein R₁ represents hydrogen atom, and R₂ may represent hydrogen, methyl, isopropyl, 1-methylpropyl, 2-methylpropyl, tert-butoxymethyl, 1-tert-butoxyethyl, 2-methylthioethyl, benzyl, carboxamidomethyl, 2-carboxamidoethyl, tert-butoxycarbonylmethyl, 2-(tert-butoxycarbonyl)ethyl, 4-(tert-butoxycarbamido)butyl, 4-tert-butoxybenzyl, indolyl-3-methyl,

15 S-(triphenylmethyl)thiomethyl, 1-(triphenylmethyl)imidazolyl-4methyl, 3-(N^G-mesitylenesulfonyl)guanidinopropyl, N-xanthylcarboxamidomethyl, 2-(N-xanthylcarboxamido)ethyl or S-(acetamidomethyl)thiomethyl.

or R₁ and R₂ together represent propylene radical,

employed as N_α-protected amino acid derivatives for solid phase peptide synthesis.

Solid phase peptide synthesis is widely employed for the preparation of biologically active peptides which are used in medical and biological research and also as active substances in pharmacy, veterinary and diagnostics.

The essence of solid phase peptide synthesis can be outlined as a stepwise elongation of a peptide chain by means of repeated cycles of chemical reactions, beginning from the first C-terminal amino acid attached to an insoluble carrier. During the course of the synthesis target products of all reactions remain bound to the carrier, whereas excessive reactants and side-products are removed by filtration and washing of the carrier.

In order to perform the solid phase synthesis of a peptide, the first amino acid(C-terminal of the target amino acid sequence) with the protected α-amino group is linked covalently to an insoluble polymeric carrier through the free α-carboxyl group by ester or amide bond formation. Then N_α-protective group is selectively cleaved from thus obtained N_α-protected aminoacyl-polymer, and the aminoacyl-polymer with the free α-amino group

is formed. This polymer is further acylated with the next N_α -protected amino acid, thus giving N_α -protected dipeptidyl-polymer. Such synthetic cycles, which consist of N_α -protection cleavage and of subsequent acylation of free amino group with following N_α -protected amino acid, are repeated
5 until the assembly of target amino acid sequence is completed.

In practical solid phase synthesis large molar excesses(2 to 10-fold) of acylating reagents are usually employed to assure complete conversion, therefore, all reactive groups in side chains of the amino acids, such as amino, carboxyl, hydroxyl, thiol, guanidino groups, should be blocked with
10 appropriate protective groups. The protective groups for this purpose must be selected carefully to provide reliable and permanent protection of the side chains under conditions of peptidyl-polymer acylations and during the cleavage of temporary N_α -protection. On the other hand, these side-chain protective groups must provide the opportunity to deprotect the synthesized
15 peptide in one or two stages quantitatively and without damage of its structure. In most cases the peptidyl-polymer linkage also should be cleaved simultaneously. It is evident that the structure and the chemical properties of permanent protective groups for side-chains of amino acids

are determined not only by the nature of reactive function to be protected but in a great extent by the structure and the chemical properties of the employed temporary N_α-protective group. Therefore, temporary N_α-protection is the key element of the whole strategy of solid phase peptide synthesis.

Well known and widely used in solid phase peptide synthesis are N_α-tert-butoxycarbonyl amino acids(Boc-amino acids) described for this purpose by R.B. Merrifield in *Biochemistry*, 1964, V. 3, p.1385. tert-Butoxycarbonyl(Boc) group can be cleaved by the action of acidic reagents of medium strength, such as, for example, trifluoroacetic acid and its solutions in chlorinated hydrocarbons, solutions of hydrogen chloride in organic solvents, boron trifluoride/diethyl ether complex and some other acids, with the formation of isobutylene and carbon dioxide.

Together with temporary N_α-Boc-protection for the permanent blocking of side chains protective groups are employed, which are stable during N_α-Boc cleavage but can be cleaved by more strong acidic reagents with the simultaneous fission of peptidyl-polymer bond. Known reagents used for this purpose are liquid hydrogen fluoride, trifluoromethanesulfonic acid

and their mixtures with anisole, thioanisole, dimethylsulfide. The main drawback of the synthetic strategy with the use of temporary N_α-Boc-protection is the application of acidolysis for the cleavage of both temporay and permanent protective groups, that cannot provide complete stability of the permanent protection. As the length of synthesized peptide grows, permanent protective groups undergo cumulative action of acidic reagents during Boc cleavage steps, that can result in partial loss of these groups and accumulation of side-products. Apart of this, the final treatment of assembled peptidyl-polymer with superacidic reagents can cause partial destruction of the target peptide. It also should be mentioned that extremely hazardous propeties of superacids require special equipment and appropriate safety measures during handing.

To aviod the use of superacidic reagents for the final peptide deprotection, several highly acid-sensitive groups were proposed more recently as a temporary N_α-protection, which are considered to be compatible to permanent side-chain protection of so-called tert-butyl type cleavable by acidic reagents of medium strength. An example of such N_α-protective group is

1-(3,5-di-tert-butylphenyl)-1-methyl-ethoxycarbonyl(t-Bumeoc)group, which is described in *Collect. Czech. Chem. Commun.*, 1992, V.57, p.1707.

N_α-t-Bumeoc-group is cleaved by 1% trifluoroacetic acid in dichloromethane and can be used together with permanent protective groups of t-butyl type

5 cleavable by neat trifluoroacetic acid or its concentrated solutions. In this case the employment of superacids is excluded but general principle of differential acidolysis still remains unchanged.

A different approach to the strategy of solid phase peptide synthesis is outlined by R.B. Merrifield in *Science*, 1986, V.232, p.341. This approach, 10 called "orthogonality principle", is based on the assumption that temporary and permanent protective groups should be removable by totally distinct reagents according to totally distinct chemical mechanisms, so that temporary N_α-protection could be cleaved with absolute selectivity providing full preservation of permanent protection, and *vice versa*. At present time 15 the "orthogonality principle" is commonly accepted as a guideline for the development of efficient strategies of solid phase peptide synthesis.

As an example of implementation of the "orthogonality principle" the employment of N_α-dithiasuccinylamino acids(Dts-amino acids) in solid

phase synthesis is described in *Int. J. Peptide and Protein Res.*, 1987, V.30.

p.740. N_α -Dithiasuccinyl(Dts) protective group is quite resistant to acidic reagents of medium strength and is cleaved smoothly by thiol reagents in neutral media with the liberation of amino group and formation of carbon
5 thiooxide. Application of Dts-amino acids in practical synthesis is still limited due to the lack of effective methods for their preparation.

The most known and widely employed strategy of solid phase synthesis, which corresponds to the "orthogonality principle", is based on the use of N_α -9-fluorenylmethoxycarbonylamino acids(Fmoc-amino acids), as
10 described by C.D. Chang and J. Meienhofer in *Int. J. Peptide and Protein Res.*, 1975, V.11, p.246. N_α -9-Fluorenylmethoxycarbonyl(Fmoc) group is resistant to to acidic reagents and is cleaved according to the β -elimination mechanism by organic bases in aprotic solvents, for example, by morpholine diethylamine, piperazine, or piperidine in
15 dimethylformamide(DMF) or dichloromethane, amino group being liberated and dibenzofulvene together with CO_2 being formed. In solid phase synthesis the cleavage of Fmoc group is preferably performed by the treatment of N_α -protected peptidyl-polymer with 20 to 50% piperidine in

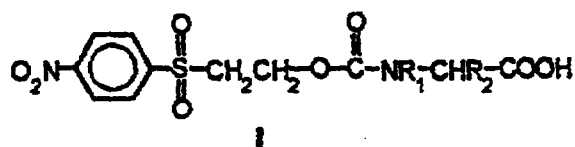
DMF during 10 to 30 min. Said conditions allow to use permanent acid sensitive protection of t-butyl type together with temporary N_ε-Fmoc-protection, thus providing the "orthogonality" of the synthetic strategy.

- 5 N_ε-Fmoc-amino acids are widely used in manual solid phase peptide synthesis, as well as in automatic and semi-automatic synthesizer of all types. However, it should be noted that extreme base sensitivity of N_ε-Fmoc-protection and some its unstability in neutral aprotic solvents require to control carefully acylation conditions and also the purity of
- 10 employed solvents. Special care should be taken when N_ε-Fmoc-amino acids are used for the synthesis of peptides exceeding 30 residues in length. Besides, relatively high cost of production prevents the use of Fmoc-derivatives in large scale peptide preparations.

Summary of the Invention

- 15 It is, therefore, desirable to develop new N_ε-protected amino acid derivatives which may be useful for the development of efficient strategies in solid phase peptide synthesis. An object of the present invention is to provide new amino acids derivatives, more particularly,

N_ε-2-(4-nitrophenylsulfonyl)ethoxycarbony-amino acids(N_ε-Nsc-amino acids)having the general formula I:



5

wherein R₁ represents hydrogen atom, and R₂ may represent hydrogen, methyl, isopropyl, 1-methylpropyl, 2-methylpropyl, tert-butoxymethyl, 1-tert-butoxyethyl, 2-methylthioethyl, benzyl, carboxamidomethyl, 2-carboxamidoethyl, tert-butoxycarbonylmethyl, 2-(tert-butoxycarbonyl)ethyl, 10 4-(tert-butoxycarbamido)butyl, 4-tert-butoxybenzyl, indolyl-3-methyl, S-(triphenylmethyl)thiomethyl, 1-(triphenylmethyl)imidazolyl-4-methyl, 3-(N^G-mesitylenesulfonyl)guanidinopropyl, N-xanthylcarboxamidomethyl, 2-(N-xanthylcarboxamido)ethyl or S-(acetamidomethyl)thiomethyl;

or R₁ and R₂ together represent propylene radical,

15 which can be employed as N_ε-protected amino acid derivatives in solid phase peptide synthesis.

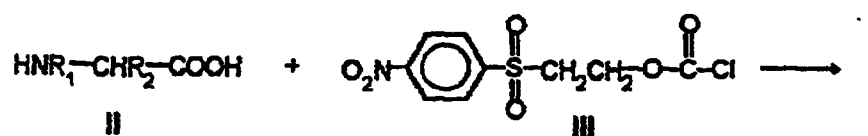
Another object of the present invention is to provide methods for the preparation of said N_ε-Nsc-amino acids. Still another object of the invention

is to provide a process for solid phase peptide synthesis using the N_α -Nsc-amino acids. These and other object of the present invention will be apparent from the following description.

Detailed Description of the Invention

- 5 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-(nitrophenylsulfonyl)chloroformate III in mixed aqueous/organic solvent in the presence of base and at the temperature from 0 to 40°C, preferably
- 10 from 0 to 20°C (Scheme 1).

Scheme 1

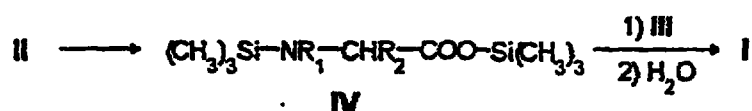


- 15 Chloroformate III is introduced into the reaction in amounts from 0.5 to 1.5 molar equivalents, preferably from 0.7 to 0.9, as related to amino acid. As an organic component of the solvent any aprotic organic solvent may be used which is capable to dissolve the acylating reagent and is mixible with

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, triethylamine, N-methylmorpholine.

According to another method of the present invention, amino acids of the general formula II are firstly converted into N,O-bis-trimethylsilyl derivatives IV using methods known in the art and then treated with chloroformate III in anhydrous organic solvent, for example, dichloromethane. After aqueous hydrolysis of intermediate trimethylsilyl derivatives desirable N_α-N_ω-amino acids I are obtained in a free form (Scheme 2).

Scheme 2



Derivatives of the formula I, wherein R₁ is hydrogen, and R₂ represents N-xanthylcarboxamidomethyl or 2-(N-xanthylcarboxamido)ethyl, may be prepared by the reaction of the derivatives of the formula I, wherein R₁ is hydrogen, and R₂ represents carboxamidomethyl or 2-(carboxamido)ethyl, with xanthidrol in aprotic organic solvent in the

presence of acid. As a solvent DMF may be used, and preferable acid is organic acid, for example, trifluoroacetic, methanesulfonic or p-toluenesulfonic acid.

It is seen from molecular formula that compounds I have an asymmetric α -carbon atom(except for compound where $R_1 = R_2 = H$). 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 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 starting compound II.

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 naturally occurring amino acids containing or not containing protective groups known in the art, mostly the groups of tert-butyl type or similar to them in relation to the cleavage conditions(Table 1).

Table 1
Meanings of R₁ and R₂ substituents in compounds I

No	R ₁	R ₂	Amino acid	Abbreviation
5	I-1	H	Glycine	Nsc-Gly-OH
	I-2	H	Alanine	Nsc-Ala-OH
	I-3	H	Valine	Nsc-Val-OH
	I-4	H	1-Methylpropyl	Nsc-Ile-OH
	I-5	H	2-Methylpropyl	Nsc-Leu-OH
10	I-6	H	tert-Butoxymethyl	Nsc-Ser(tBu)-OH
	I-7	H	1-tert-Butoxymethyl	Nsc-Thr(tBu)-OH
	I-8	H	2-Methylthioethyl	Nsc-Met-OH
	I-9	H	Benzyl	Nsc-Phe-OH
	I-10	H	Carboxamidomethyl	Nsc-Asn-OH
15	I-11	H	2-Carboxamidoethyl	Nsc-Gln-OH
	I-12	H	tert-Butoxycarbonylmethyl	Nsc-Asp(OtBu)-OH
	I-13	H	2-(tert-Butoxycarbonyl)ethyl	Nsc-Glu(OtBu)-OH
	I-14	H	4-(tert-Butoxycarbamido)butyl	Nsc-Lys(Boc)-OH
	I-15	H	4-tert-Butoxybenzyl	Nsc-Tyr(tBu)-OH
15	I-16	H	Indolyl-3-methyl	Nsc-Trp-OH
	I-17	H	S-(Triphenylmethyl)thiomethyl	Nsc-Cys(Trt)-OH
	I-18	H	1-(Triphenylmethyl)imidazolyl-4-methyl	Nsc-His(Trt)-OH
	I-19	H	3-(N ^G -Mesitylenesulfonyl)-guanidinopropyl	Nsc-Arg(Mts)-OH
	I-20	H	N-Xanthylicarboxamido-methyl	Nsc-Asn(Xan)-OH
	I-21	H	2-(N-Xanthylicarboxamido)ethyl	Nsc-Gln(Xan)-OH
	I-22	H	S-(Acetamidomethyl)thiomethyl	Nsc-Cys(Acm)-OH
	I-23	R ₁ +R ₂ =Propylene	Proline	Nsc-Pro-OH

Apparently the compounds of the formula I shown in Table I represent a full set of protected proteogenic amino acid derivatives required for the synthesis of a peptide of any amino acid composition. It is also apparent that N_α-Nsc-derivatives of amino acids carrying another types of backbone protection as well as N_α-Nsc-derivatives of non-preteogenic or unusual amino acids can be synthesized by the provided method.

N_α-Nsc-amino acids I are crystalline compounds unsoluble or slightly soluble in water and soluble in polar organic solvents, stable at long-term storage at -10° to 25°C.

According to the present invention a process is provided for solid phase peptide synthesis using the N_α-Nsc-amino acids of the formula I.

By this process the first N_α-Nsc-amino (C-terminal of the target amino sequence) is linked covalently to an unsoluble polymeric carrier through the free α-carboxyl group by ester or amide bond formation, N_α-Nsc-aminoacyl-polymer being obtained. A variety of polymers may be used as a polymeric carrier, such as cross-linked or macroporous polystyrene, cross-linked poly-N,N-dimethylacrylamide in granular form or as a composite with kieselguhr, cross-linked dextrans, celluloses, papers

and other polymers known in the art and employed for this purpose.

For the attachment of the first N_α -Nsc-amino acid the polymeric carrier should contain appropriate anchor groups. In most instances anchor groups are preferable which provide the cleavage of synthesized peptide
5 from polymeric carrier with the liberation of C-terminal carboxyl or carboxamide group during the treatment of peptidyl-polymer with acidic reagents, such as trifluoroacetic acid and its solutions or hydrogen chloride solutions in organic solvent. Such anchor groups for the ester type attachment may be 4-hydroxymethylphenoxyalkyl, 4-chloro- or
10 4-bromomethylphenoxyalkyl, α -hydroxydiphenylmethyl and other groups known in the art; for the carboxamide type attachment there may be known di- and trialkoxybenzhydrylamine groups, 4-aminomethyl-3,5-dimethoxyphenoxyalkyl group and also other known groups employed for this purpose.

15 Attachment of the C-terminal N_α -Nsc-amino acid to anchor groups of polymeric carrier may be performed by the methods known in the art.

In order to cleave N_α -protective group from the obtained N_α -Nsc-aminoacyl-polymer, said protected aminoacyl-polymer is treated

with basic reagent. Preferable basic reagents for this purpose are nitrogen bases, e.g. ammonia, morpholine, piperidine, piperazine, diethylamine, 1,8-diazabicyclo[5,4,0]undec-7-ene, 1,1,3,3-tetramethylguanidine and their solutions in aprotic organic solvents. More preferable basic reagent is 20
5 to 50% solution of piperidine in DMF. In this instance Nsc-group is cleaved with the formation of N-[2-(4-nitrophenylsulfonyl)ethyl]piperidine and carbon dioxide, α -amino group being liberated.

Further the aminoacyl-polymer with the free α -amino group is acylated with the next N_α-Nsc-amino acid, thus giving N_α-Nsc-dipeptidyl-polymer.
10 For this purpose methods are employed known in the art and usually used for this purpose. As acylating agents may be used, for example, 4-nitrophenyl, pentachlorophenyl, pentafluorophenyl, 1-hydroxybenzotriazolyl esters of N_α-Nsc-amino acids and other known types of active esters used in solid phase peptide synthesis; symmetric anhydrides of N_α-Nsc-amino
15 acids. Acylation may also be performed with N_α-Nsc-amino acids in the presence of known coupling reagents, e.g. dicyclohexylcarbodiimide, diisopropylcarbodiimide, benzotriazolyl-1-oxy-(tris-dimethylamino)phosphonium hexafluorophosphate.

Synthetic cycles, which consist of N_α-Nsc-group cleavage and of subsequent acylation of free amino group with following N_α-Nsc-amino acid, are repeated until the assembly of target amino acid sequence is completed.

- 5 After the assembly of desired N_α-Nsc-peptidyl-polymer N_α-terminal protective group is cleaved using methods described above, then in most instances the target peptide is detached from the anchor group of the carrier with concurrent cleavage of permanent protection from side chains of amino acids. For this purpose acidic reagents may be employed known
- 10 in the art for the cleavage of tert-butyl type protective groups, e. g., trifluoroacetic acid, solutions of methanesulfonic or p-toluenesulfonic acid, containing or not containing known additives for trapping of evolved carbonium ions, for instance, water, anisole, thioanisole, dimethylsulfide, ethanedithiol-1,2,-trisopropylsilane.
- 15 Optionally, target peptide may be deblocked without cleavage from polymeric carrier. In such instances known anchor groups should be employed which can provide an acid-resistant peptidyl-polymer linkage.

In comparison to Fmoc, Nsc-group is more resistant to basic reagents,

and its cleavage rates are markedly slower under similar conditions, but the time, which is usually allocated for the cleavage of N_ε-protection according to protocols of solid phase synthesis(15-20 min), is sufficient for quantitative cleavage of N_ε-Nsc-group from protected peptidyl-polymer by the such basic reagent as 20 to 50% solution of piperidine in DMF. On the other part, increased resistance of Nsc-group to basic reagents provides its more pronounced stability in neutral and weakly basic media, which are preferably employed for performing of acylation steps.

As described above, Nsc-group may be cleaved quantitatively by basic reagents in the presence of tert-butyl type protective groups, which are resistant towards organic bases. On the other hand, Nsc-group is perfectly resistant to the action of acidic reagents usually used for the cleavage of protective groups of tert-butyl type. Thus the employment of N_ε-Nsc-amino acids of general formula I, which contain in side chains protective group preferably of tert-butyl type or similar to them in relation to cleavage conditions, allows to develop new strategies of solid phase peptide synthesis in concordance to the "orthogonality principle".

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

5

N.-Nsc-Asparagine (I-10)

3.95g of asparagine and 7.7g of potassium carbonate were dissolved in 100ml of water-dioxane mixture(3 : 1, v/v) and cooled in ice bath, then solution of 7.5g of 2-(4-nitrophenylsulfonyl)ethyl chloroformate III in 70ml 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. 100ml under reduced pressure and transferred into separating funnel. 100ml of water was added, and the resulting solution was extracted with 2 × 50ml of ethyl acetate. Aqueous layer was separated, acidified to pH 2 with 40% sulfuric acid and cooled in ice bath. After 30min the formed precipitate was filtered off, washed extensively with ice-cold water and air-dried yielding the desired compound I-10 as a white crystalline powder(71%), For characterization see Table 2(Example 5).

15

Example 2

N_α-Nsc-Leucine(I -5)

4.92g of leucine and 90ml of anhydrous dichloromethane were placed into 250ml round-bottom flask equipped with reflux condenser and dropping funnel. To the suspension 9.5ml 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 9.1ml of triethylamine and 9.0g of chloroformate III were added with stirring. The mixture was stirred for 20min in ice bath, then for additional 1.5hr at room temperature. The solvent was evaporated at reduced pressure, and the residue was distributed between 200ml of ethyl acetate and 250ml of 2.5% aqueous sodium bicarbonate. Aqueous layer was separated, washed with 50ml of ether, acidified to pH 2 with 1 N hydrochloric acid, then extracted with 3×70ml of ethyl acetate. Combined extracts were dried with anhydrous sodium sulfate and evaporated at reduced pressure. Recrystallization of the residue from hexane-ethyl acetate gave the desired product I-5 in a form of white crystalline powder(80%). For characterization see Table 2(Example 5).

Example 3

N.-Nac-Aspartic Acid β -tert-Butyl Ester(I -12).

7.09g of aspartic acid β -tert-butyl ester and 90ml of anhydrous dichloromethane were placed into 250ml round-bottom flask equipped with reflux condenser and dropping funnel. To the mixture 12.7ml of
5 diisopropylethylamine and then 9.5ml of chlorotrimethylsilane were added with vigorous stirring, and the mixture was heated to boiling for 1.5hr. The reaction mixture was then cooled in ice bath, 9.0g of chloroformate III was added at once, and stirring was continued for 1.5hr at room temperature. The solvent was evaporated at reduced pressure, and the residue was
10 distributed between 200ml of ethyl acetate and 250ml of 2.5% aqueous sodium bicarbonate. Aqueous layer was separated, washed with 50ml of ether, acidified to pH 2 with 1 N hydrochloric acid, then extracted with 3 $\times$ 70ml of ethyl acetate. Combined extracts were dried with anhydrous sodium sulfate and evaporated at reduced pressure. Recrystallization of the
15 residue from hexane-ethyl acetate gave the desired product I -12 in a form of which crystalline powder(86%). For characterization see Table 2(Example 5).

Example 4

N_ε-Nsc-N-Xanthyl-Asparagine(I -20).

3.89g of N_ε-Nsc-asparagine(I -10)and 2.6g of xanthidrol were dissolved in 20ml of dry DMF. To the solution 0.4ml of methanesulfonic acid was added, and the mixture was allowed to stand for 2 days at room temperature. The resulting mixture was then poured into 100ml of ice-cold water with mixing, the formed precipitate was filtered off, washed with water and then with ethyl acetate and ether. The crude product was dissolved in 10ml 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 -20 as a crystalline powder(74%). For characterization see Table 2(Example 5).

Example 5**Properties of N_ε-Nsc-amino acids I .**

Shown in Table 2 are the compounds of formula I which were prepared utilizing provided methods described in detail in example 1-4. Figures in the column "Method" correspond to numbers of examples where particular methods are described. Specific optical rotations $[\alpha]_D^{25}$ were measured on DIP-320 polarimeter(JASCO, Japan) in 10 cm cuvettes.

Melting points were determined in open capillaries and were not corrected.

Chromatographic mobility values R_f were shown for thin-layer chromatography sheets Alufolien Kieselgel 60 F₂₅₄ (Merck, Darmstadt, Germany); chloroform/methanol/acetic acid, 95:5:3, (A) and

5 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 Cr^{252} radiation-promoted desorption (Electron SPA, Sumy, Ukraine).

10

15

Table 2
Properties of N_α-Nsc-amino acids I

Entry	Compound	Method	[α] _D ²⁵ (c1, DMF)	mp, °C	R _f (A)	R _f (B)	Molecularion, (M+H) ⁺	
							Calcd	Found
1	2	3	4	5	6	7	8	9
I-1	Nsc-Gly-OH	2	-	152-154	0.40	0.21	333.30	333.4
I-2	Nsc-Ala-OH	2	-25.6°	134-136	0.53	0.35	347.32	347.4
5 I-3	Nsc-Val-OH	2	-12.0°	72-74	0.62	0.50	375.38	375.4
I-4	Nsc-Ile-OH	2	-13.0°	120-121	0.62	0.52	389.41	389.6
I-5	Nsc-Leu-OH	2	-33.0°	160-162	0.68	0.42	389.41	389.7
I-6	Nsc-Ser(tBu)-OH	3	+3.3°	109-111	0.68	0.46	419.43	418.9
I-7	Nsc-Thr(tBu)-OH	3	-9.0°	64-66	0.68	0.50	433.46	433.2
I-8	Nsc-Met-OH	2	-28.7°	88-90	0.65	0.37	407.47	406.9
I-9	Nsc-Phe-OH	2	-23.0°	160-163	0.66	0.40	423.42	423.3
I-10	Nsc-Asn-OH	1	-1.3°	205-207	0.05	0.05	390.35	390.2
I-11	Nsc-Gln-OH	1	-9.7°	192-194	0.10	0.05	404.38	404.3
I-12	Nsc-Asp(OtBu)-OH	3	-12.7°	72-75	0.64	0.38	447.44	447.2
I-13	Nsc-Gln(OtBu)-OH	3	-17.0°	94-96	0.67	0.38	461.47	461.4
10 I-14	Nsc-Lys(Boc)-OH	3	-11.8°	110-112	0.62	0.35	504.54	505.9
I-15	Nsc-Tyr(tBu)-OH	3	-6.3°	82-84	0.68	0.44	495.53	495.2
I-16	Nsc-Trp-OH	3	-14.7°	188-190	0.53	0.33	462.46	461.7
I-17	Nsc-Cys(Trt)-OH	3	+22.3°	108-110	0.75	0.52	621.71	619.3
I-18	Nsc-His(Trt)-OH	3	+5.0°	112-115	0.42	0.1	655.71	656.8
I-19	Nsc-Arg(Mts)-OH	3	-4.0°	115-120	0.25	0.05	615.68	614.4
I-20	Nsc-Asn(Xan)-OH	4	+2.7°	198-200	0.43	0.25	570.56	568.8
I-21	Nsc-Gln(Xan)-OH	4	-13.7°	155-158	0.58	0.23	584.58	582.9
I-22	Nsc-Cys(Acm)-OH	2	-31.0°	124-126	0.17	0.05	450.47	451.2
I-23	Nsc-Pro-OH	2	-31.5°	115-117	0.53	0.37	371.35	371.3

Example 6**Solid phase synthesis of dodecapeptide****Ala-Ser-Ser-Thr-Ile-Ile-Lys-Phe-Gly-Ile-Asp-Lys.**

a) Insertion of anchor group into polymeric carrier.

5 To 250mg of aminomethylated styrene-1% divinylbenzene
copolymer(1.0meq. NH_2/g) in 3ml of DMF 0.75mmol of 2,4,5-trichlorophenyl
4-hydroxymethylphenoxypionate and 0.75mmol of 1-hydroxybenzotriazole
were added, and the suspension was shaken for 24 hrs at room
temperature. Polymer was filtered off, washed with DMF, ethanol, ether
10 and, finally, with hexane and dried in vacuo over phosphorus pentaoxide for
24hrs.

b) Attachment of Nsc-Lys(Boc)-OH to anchor group.

The obtained polymer was swollen in 4ml of
1,2-dichloroethane/N-methylpyrrolidine mixture(3:1), then 0.75 mmol of
15 Nsc-Lys(Boc)-OH (I-14), 0.1mmol of 4-dimethylaminopyridine and 0.75mmol
of dicyclohexylcarbodiimide were added. The suspension was shaken for
24 hrs at room temperature. Polymer was filtered off, thoroughly washed
with chloroform, chloroform/methanol mixture(1:1), ethanol, ether and,

finally, with hexane and dried yielding 400mg of Nsc-Lys(Boc)-polymer.

c)Peptide assembly.

Nsc-Lys(Boc)-polymer(200mg) was placed into 10ml polypropylene syringe equipped at the bottom with polypropylene frit. The polymer in the
5 syringe was washed with DMF, and further synthetic cycles were performed
according to the following operational protocol:

1. Prewash: 33% piperidine/DMF, 4ml; 0.5min.
2. Deblocking: 33% piperidine/DMF, 4ml; 15min
3. Wash: DMF, 6×(4ml; 1min)
- 10 4. Acylation: N_ε-Nsc-amino acid I, 0.5mmol;
benzotriazoly-1-oxy-
-(trisdimethylamino)phosphonium
hexafluorophosphate,
0.5mmol;1-hydroxybenzotriazole, 0.5mmol;
15 N-methyl-morpholine, 0.75mmol; DMF,
2ml; 60min(90min for NSC-Ile-OH).
5. Wash: DMF, 5×(4ml; 1min)

N_ε-Nsc-amino acids were introduced into synthetic cycles in the

following order. Nsc-Asp(OtBu)-OH, Nsc-Ile-OH, Nsc-Gly-OH, Nsc-Phe-OH,
Nsc-Lys(Boc)-OH, Nsc-Ile-OH, Nsc-Ile-OH, Nsc-Thr(tBu)-OH,
Nsc-Ser(tBu)-OH, Nsc-Ser(tBu)-OH, Nsc-Ala-OH.

After the assembly of the target amino acid sequence the
5 peptidyl-polymer was treated with 33% piperidine/DMF(4ml)for 20min, then
washed with DMF, dichloromethane, ethanol, ether and finally, with hexane.

d)Deblocking and purification

The peptidyl-polymer was shaken with 5ml of 50% trifluoroacetic acid in
1,2-dichloroethane for 60min at room temperature. Polymer was filtered off,
10 washed with 5ml of 50% trifluoroacetic acid in 1,2-dichloroethane, and
combined washings were diluted with 100ml of ice-cold anhydrous ether.
Precipitate was filtered off, washed with ether and dried in vacuo, giving
170mg of crude dodecapeptide(purity 70% by analytical reversed phase high
performance liquid chromatography).

15 Crude dodecapeptide was dissolved in 3ml of 1M aqueous acetic acid
and was chromatographed on the 1.5 × 70cm column packed with TSK
HW-40F(Merck, Darmstadt, Germany), equilibrated and eluted with the same
buffer. Fractions contained pure peptide were pooled and lyophilized. Final

yield off the target dodecapeptide was 104mg(41%), purity more than 95% as estimated by analytical reversed phase high performance liquid chromatography. Amino acid composition(after 6 N HCl hydrolysis, 110°C, 24 and 48hrs): Asp 1.02(1); Ser 1.84(2); Thr 0.93(1); Glu 0.94(1); Gly 1.03(1); Ala 1.00(1); Ile 2.78(3); Lys 2.04(2).

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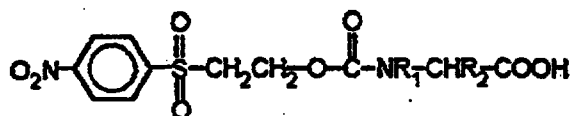
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What is claimed is;

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1. N_c-2-(4-nitrophenylsulfonyl)ethoxycarbonyl-amino acids having the general formula:

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wherein R₁ represents hydrogen atom, and R₂ represents hydrogen, methyl, isopropyl, 1-methylpropyl, 2-methylpropyl, tert-butoxymethyl, 1-tert-butoxyethyl, 2-methylthioethyl, benzyl, carboxamido-methyl, 2-carboxamidoethyl, tert-butoxycarbonylmethyl, 2-(tert-butoxycarbonyl)ethyl, 4-(tert-butoxycarbonyl)butyl, 4-tert-butoxybenzyl, indolyl-3-methyl, S-(triphenylmethyl)thiomethyl, 1-(triphenylmethyl)imidazolyl-4-methyl, 3-(N^o-mesitylenesulfonyl)guanidinopropyl, N-xanthylcarboxamidomethyl, 2-(N-xanthylcarboxamido)ethyl or S-(acetamidomethyl)thiomethyl;

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or R₁ and R₂ together represent propylene radical.

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



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wherein R_1 and R_2 represent radicals according to the claim 1, 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
5 comprising:

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;

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

4. A method for preparing of compounds according to the claim 1,
wherein R_1 represents hydrogen atom, and R_2 represents
15 N-xanthylcarboxamidomethyl or 2-(N-xanthylcarboxamido)ethyl, comprising
reacting of compounds according to the claim 1, wherein R_1 represents
hydrogen atom, and R_2 represents carboxamidomethyl or
2-(carboxamido)ethyl, with xathydrol in organic solvent in the presence of



acid.

5 5. A process for preparing peptides by means of sequential addition of protected amino acid monomers to a growing peptide chain attached to an insoluble polymeric support, wherein said protected amino acid monomers are compounds according to the claim 1, said process comprising:

a) coupling of C-terminal protected monomer to an amino or hydroxyl function of anchor group bound to said polymeric support through free carboxyl group of said protected monomer to provide protected aminoacyl-polymer,

10 b) deblocking of said protected aminoacyl-polymer by treatment with basic reagent to provide aminoacyl-polymer with free α -amino group;

c) coupling of following protected amino acid monomer to free α -amino group of said aminoacyl-polymer to provide protected peptidyl-polymer,

15 d) repeating steps b) and c) until the ultimate protected amino acid monomer is coupled, then performing step b).

6. A process according to the claim 5, wherein said basic reagent is nitrogen base selected from the group consisting of ammonia, morpholine, piperidine, piperazine, diethylamine, 1,8-diazabicyclo[5,4,0]undec-7-ene,

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1,1,3,3-tetramethylguanidine and their solutions in aprotic organic solvents.

7. A process according to the claim 6, wherein said nitrogen base is piperidine, and the organic solvent is dimethylformamide.

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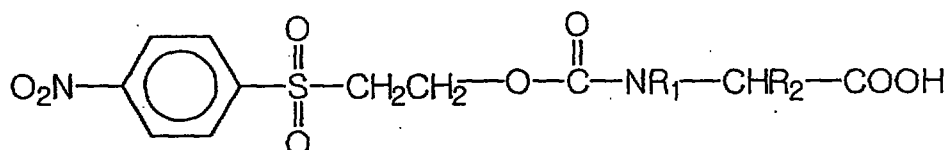


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CLAIMS

1. $\text{Na-2-(4-nitrophenylsulfonyl)ethoxycarbonyl-amino}$ acids having the general formula:



wherein, R_1 represents hydrogen atom, and R_2 represents isopropyl, 2-methylpropyl, 2-methylthioethyl, benzyl, carboxamido-methyl, 2-carboxamidoethyl, 4-ter-butoxybenzyl, indolyl-3-methyl, S-(triphenylmethyl)thiomethyl, 1-(triphenylmethyl)imidazolyl-4-methyl, 3-(N^G -mesitylenesulfonyl)guanidinopropyl, N-zanthylcarboxamidomethyl, 2-(N-xanthylcarboxamido)ethyl or S-(acetamidomethyl)thiomethyl; or R_1 and R_2 together represent propylene radical.

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 claim 1, with 2-(4-nitrophenylsulfonyl)ethoxycarbonyl chloroformate in mixed aqueous-organic solvent in the presence of base.



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

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 2-(4-nitrophenylsulfonyl)ethoxycarbonyl chloroformate in aprotic solvent in the presence of base with subsequent hydrolysis.

4. A method for preparing of compounds according to the claim 1, wherein R_1 represent hydrogen atom, and R_2 represents N-zanthylcarboxamidomethyl or 2-(N-xanthylcarboxamido)ethyl, comprising reacting of compounds according to the claim 1, wherein R_1 represents hydrogen atom and R_2 represents carboxamidomethyl or 2-(carboxamido)ethyl, with xanthidrol in organic solvent in the presence of acid.

5. A process for preparing peptides by means of sequential addition of protected amino acid monomers to a growing peptide chain attached to an insoluble polymeric support, wherein said protected



amino acid monomers are compounds according to the claim 1, said process comprising:

- a) coupling of C-terminal protected monomer to an amino or hydroxyl function of anchor group bound to said polymeric support through free carboxyl group of said protected monomer to provide protected aminoacyl-polymer,
- b) deblocking of said protected aminoacyl-polymer by treatment with basic reagent to provide aminoacyl-polymer with free α -amino group,
- c) coupling of following protected amino acid monomer to free α -amino group of said aminoacyl-polymer to provide protected peptidyl-polymer,
- d) repeating steps b) and c) until the ultimate protected amino acid monomer is coupled, then performing step b).

6. A process according to the claim 5, wherein said basic reagent is nitrogen base selected from the group consisting of ammonia, morpholine, piperidine, piperazine, diethylamine, 1,8-diazabicyclo[5,4,0]undec-7-ene, 1,1,3,3-tetramethylguanidine and their solutions in aprotic organic solvents.

7. A process according to the claim 6, wherein said nitrogen base is piperidine, and the organic solvent is dimethylformamide.

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(21) International Application Number: PCT/KR96/00012 (22) International Filing Date: 27 January 1996 (27.01.96) (30) Priority Data: 95102102 / 15 February 1995 (15.02.95) / RU (71) Applicant: HYUNDAI PHARM. IND. CO., LTD. [KR/KR]; 1110-2, Hwagok-dong, Gangseu-gu, Seoul 157-019 (KR). (72) Inventors: SAMUKOV, Vladimir Vasilyevich; Koltsovo, 23- 49, Novosibirsk Reg., 633159 (RU). SABIROV, Ayda Nadzhatovich; Koltsovo, 9-70, Novosibirsk Reg., 633159 (RU). POZDNYARKOV, Pavel Ivanovich; Koltsovo, 1-47, Novosibirsk Reg., 633159 (RU). (74) Agents: KIM, Yong, In et al.; Kims International, Dae Heung Building, 13th floor, 648-23, Yeoksam-dong, Kangnam-gu, Seoul 135-080 (KR).	(81) Designated States: AU, CA, CN, CZ, FL, HU, JP, KR, NO, PL, (SG) European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i>	
(54) Title: Na-2-(4-NITROPHENULSULFONYL)ETHOXYCARBONYL-AMINO ACIDS (57) Abstract Protected amino acid derivatives of general formula (I) wherein R₁ represents hydrogen atom, and R₂ represents hydrogen, methyl, isopropyl, 1-methylpropyl, 2-methylpropyl, tert-butoxymethyl, 1-tert-butoxyethyl, 2-methylthioethyl, benzyl, carboxamidomethyl, 2-carboxamidoethyl, tert-butoxycarbonylmethyl, 2-(tert-butoxycarbonyl)ethyl, 4-(tert-butoxycarbamido)butyl, 4-tert-butoxybenzyl, indolyl-3-methyl, S(triphenylmethyl)thiomethyl, 1-(triphenylmethyl)imidazolyl-4-methyl, 3-(N^G-mesitylenesulfonyl)guanidinopropyl, N-xanthylcarboxamidomethyl, 2-(N-xanthylcarboxamido)ethyl or S-(acetamidomethyl)thiomethyl; or R₁ and R₂ together represent propylene radical. Methods for the preparation of said derivatives are provided, and a process for solid phase peptide synthesis using said derivatives is described. $\text{O}_2\text{N}-\text{C}_6\text{H}_4-\text{SO}_2-\text{CH}_2\text{CH}_2-\text{O}-\text{C}(=\text{O})-\text{NR}_1-\text{CH}(\text{R}_2)-\text{COOH} \quad (\text{I})$		