P/00/008b 12/11/91
Section 29 (1)
Regulation 3.1 (2)

AUSTRALIA

Patents Act 1990

NOTICE OF ENTITLEMENT

(To be filed before acceptance)

We, HOECHST AKTIENGESELLSCHAFT
of Postfach 80 03 20, 6230 Frankfurt/Main 80, Germany,
being the applicant in respect of Application No. 83402/91 state the following:-

☐ The Person nominated for the grant of the patent has entitlement from the actual inventors by assignment.

☐ The person nominated for the grant of the patent is the applicant of the basic application listed on the patent request form.

☐ The basic application listed on the request form is the first application made in a Convention country in respect of the invention.

By our Patent Attorneys,
WATERMARK PATENT & TRADEMARK ATTORNEYS

.....
Darryl B. Mischlewski
Registered Patent Attorney

19 October 1993
.....
(Date)

P/00/001 28/5/91
Section 29

AUSTRALIA

Patents Act 1990

646488

PATENT REQUEST: STANDARD PATENT/PATENT OF ADDITION

I/We, being the person(s) identified below as the Applicant, request the grant of a patent to the person identified below as the Nominated Person, for an invention described in the accompanying standard complete specification.

Full application details follow.

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[54] Invention Title: A PROCESS FOR THE PREPARATION OF PEPTIDES BY SOLID PHASE SYNTHESIS

[72] Name(s) of actual Inventor(s): Gerhard BREIPOHL, Jochen KNOLLE, Wolfgang KÖNIG

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BASIC CONVENTION APPLICATION(S) DETAILS

[31] Application Number	[33] Country	Country Code	[32] Date of Application
P40 27 394 . 6	Germany	DE	30th August 1990

Basic Applicant(s): Hoechst AG

Drawing number recommended to accompany the abstract .....

By our Patent Attorneys,
WATERMARK PATENT & TRADEMARK ATTORNEYS

.....
Darryl B. Mischlewski

3rd December 1993
.....
(Date)

Registered Patent Attorney

AUSTRALIAN PATENT OFFICE

AU9183402

(12) PATENT ABRIDGMENT (11) Document No. AU-B-83402/91
(19) AUSTRALIAN PATENT OFFICE (10) Acceptance No. 646488

(54) Title
A PROCESS FOR THE PREPARATION OF PEPTIDES BY SOLID-PHASE SYNTHESIS

(51)[5] International Patent Classification(s)
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(57) The invention relates to a process for the preparation of peptides with C-terminal aza-amino amide by solid-phase synthesis.

The object of the invention is to develop a low-racemization process for the preparation of peptides with C-terminal aza-amino amides by solid-phase synthesis.

Claim

1. A process for the preparation of peptides of the formula I

$$(X)_n - A - NH_2 \qquad (I)$$

in which

X is a natural or unnatural amino acid, aza-amino acid or imino acid,

n is an integer from 1 to 50, preferably 1 to 30, and

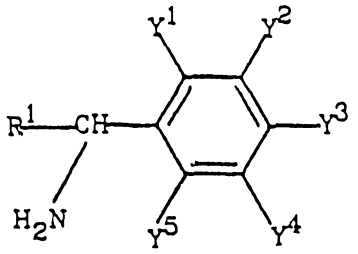
A is an aza-amino acid,

and the physiologically tolerated salts thereof, which comprises converting a spacer into a form capable of acylation, reacting the latter with a suitable formic acid derivative and subsequently with an appropriate

.../2

amino hydrazide, where appropriate converting the protective group into a protective group which is base-labile or labile to weak acids, coupling the spacer obtained in this way to a resin, synthesizing the required peptide stepwise from the C-terminal end, subsequently cleaving the peptide off the resin and, where appropriate, converting it into physiologically tolerated salts thereof.

2. The process as claimed in claim 1, wherein a compound of the formula II



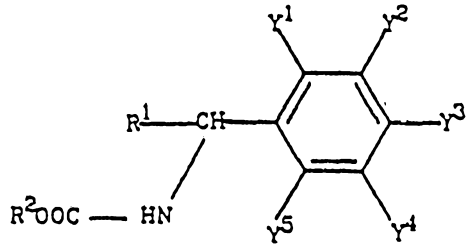
(II)

in which

Y^1, Y^2, Y^3, Y^4 and Y^5 are hydrogen, C_1-C_4-alkyl, C_1-C_4-alkoxy or $-O-(CH_2)_n-COOH$, $-(CH_2)_n-COOH$ or $-NH-CO-(CH_2)_n-COOH$, where the radicals can be identical or different, but at least one radical is $-O-(CH_2)_n-COOH$, $-(CH_2)_n-COOH$ or $-NH-CO-(CH_2)_n-COOH$, n is an integer from 1 to 6, preferably 1 to 3, and

R^1 is hydrogen or C_1-C_6-alkoxy-C_6-C_{12}-aryl,

is reacted with a silylating reagent in a solvent suitable for this purpose, and subsequently the silylated compound is converted with a chloroformic acid derivative into compounds of the formula III



(III)

in which

R^1, Y^1, Y^2, Y^3, Y^4 and Y^5 are as defined above, and

R^2 is a C_6-C_{12}-aryl radical which is substituted by electron-attracting substituents, preferably nitro and halogen,

the compounds of the formula III obtained in this way are reacted with an amino hydrazide of the formula IV

$$R^3 \cdot X \cdot CO \cdot NH \cdot NH \cdot R^4 \qquad (IV)$$

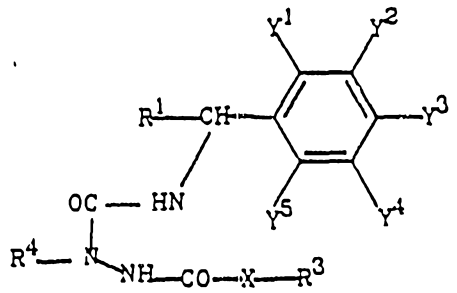
in which

X is a natural or unnatural amino acid or imino acid and is as defined above,

R^3 is a protective group which is base-labile or labile to weak acids or hydrogenation, and

R^4 is C_1-C_8-alkyl, C_3-C_9-cycloalkyl, C_6-C_{12}-aryl, C_6-C_{12}-aryl-C_1-C_8-alkyl, heteroaryl or heteroaryl-C_1-C_8-alkyl or hydrogen,

in a suitable solvent to give the compounds of the formula V



(V)

in which R^1, R^3, R^4 and Y^1, Y^2, Y^3, Y^4 and Y^5 have the abovementioned meanings, if R^3 is a protective group labile to hydrogenation this protective group is removed by hydrogenation on a Pd catalyst and, before the subsequent reaction, converted into a urethane protective group which is base-labile or labile to weak acids, subsequently the compound of the formula V in which R^1, R^4 Y^1, Y^2, Y^3, Y^4 and Y^5 have the abovementioned meanings, and R^3 is a urethane protective group which is labile to bases and weak acids, is coupled with the coupling reagents

customary in peptide chemistry via the $-O-(CH_2)_n-COOH$, $-(CH_2)_n-COOH$ or $-NH-CO-(CH_2)_n-COOH$ group to a resin, the protective group R^3 is eliminated, natural or unnatural amino, imino or aza-amino acids which have been temporarily protected by amino-protective groups which are base-labile or labile to weak acids and which are optionally in the form of their activated derivatives are coupled on stepwise, and, after the synthesis is complete, the peptides of the formula I are liberated from the resin by treatment with a moderately strong acid, with elimination again, simultaneously or by suitable measures subsequent thereto, of temporarily introduced side-chain protective groups.

P/00/011 28/5/91
Regulation 3.2(2)

AUST

Patents

646488

ORIGINAL
COMPLETE SPECIFICATION
STANDARD PATENT

Application Number:

Lodged:

Invention Title: A PROCESS FOR THE PREPARATION OF PEPTIDES BY SOLID-PHASE SYNTHESIS

The following statement is a full description of this invention, including the best method of performing it known to :- US

Abstract of the disclosure:

A process for the preparation of peptides by solid-phase synthesis

A process for the preparation of peptides of the formula $(X)_n$-A-NH_2 is described, in which X is a natural or unnatural amino acid, aza-amino acid or imino acid, n is an integer from 1 to 50, preferably 1 to 30, and A is an aza-amino acid, and the physiologically tolerated salts thereof, which comprises converting a spacer into a form which is capable of acylation, reacting the latter with a suitable formic acid derivative and subsequently with an appropriate amino hydrazide, where appropriate converting the protective group into a protective group which is base-labile or labile to weak acids, coupling the spacer obtained in this way to a resin, synthesizing the required peptide stepwise from the C-terminal end, subsequently cleaving the peptide off the resin and, where appropriate, converting it into physiologically tolerated salts thereof.

Description

A process for the preparation of peptides by solid-phase synthesis

The invention relates to a process for the preparation of peptides with C-terminal aza-amino amide by solid-phase synthesis.

The object of the invention is to develop a low-racemization process for the preparation of peptides with C-terminal aza-amino amides by solid-phase synthesis.

This object is achieved according to the invention by the process for the preparation of peptides of the formula I

$$(X)_n \cdot A \cdot NH_2 \qquad (I)$$

in which

X is a natural or unnatural amino acid, aza-amino acid or imino acid,

n is an integer from 1 to 50, preferably 1 to 30, and

A is an aza-amino acid,

and the physiologically tolerated salts thereof, which comprises converting a spacer into a form capable of acylation, reacting the latter with a suitable formic acid derivative and subsequently with an appropriate amino hydrazide, where appropriate converting the protective group into a protective group which is base-labile or labile to weak acids, coupling the spacer obtained in this way to a resin, synthesizing the required peptide stepwise from the C-terminal end, subsequently cleaving the peptide off the resin and, where appropriate, converting it into physiologically tolerated salts thereof.

Natural or unnatural amino acids can, if chiral, be in the D or L form. α-Amino acids are preferred.

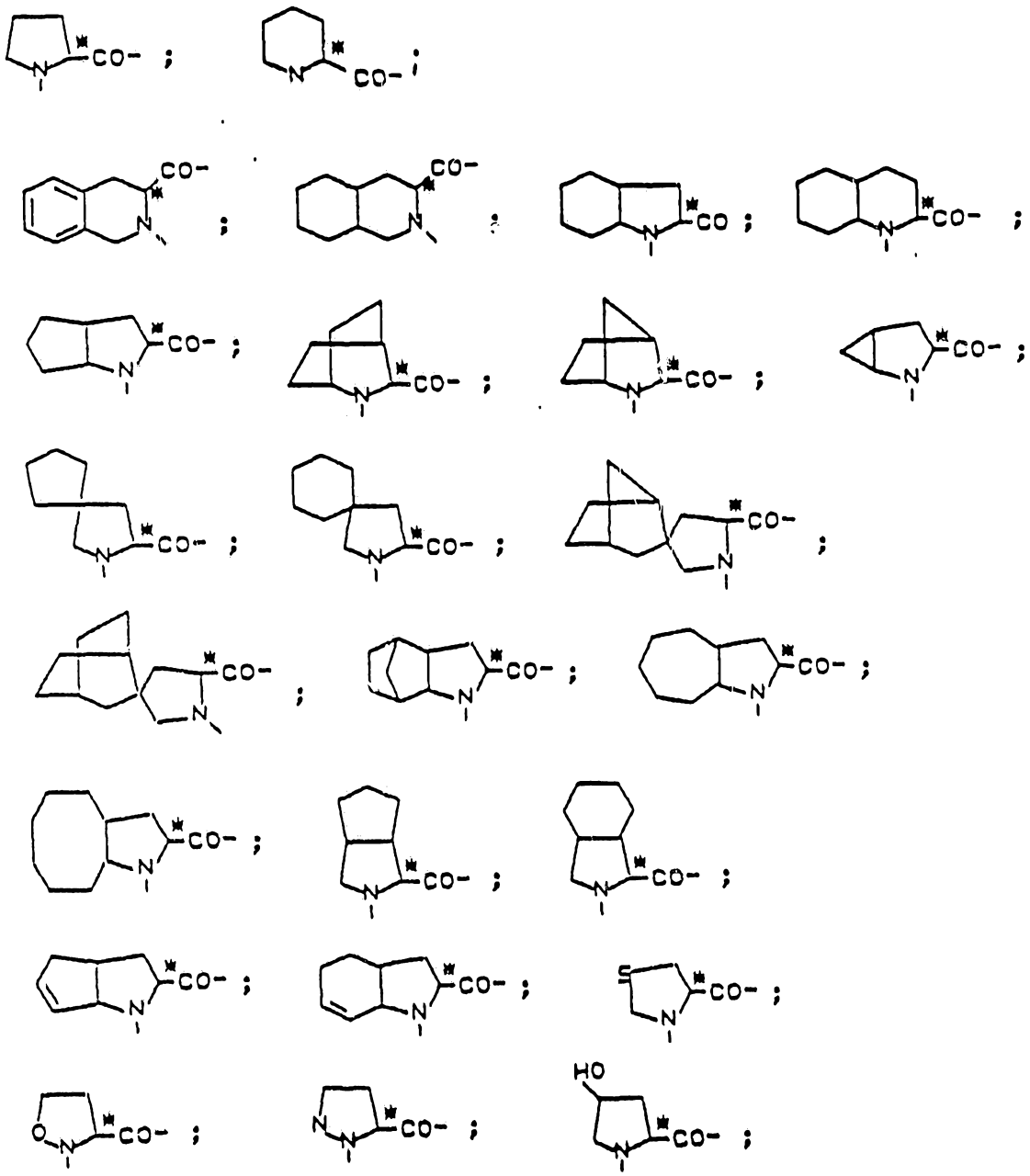
Examples which may be mentioned are: Aad, Abu, γAbu, ABz, 2ABz, εAca, Ach, Acp, Adpd, Ahb, Aib, βAib, Ala, βAla, ΔAla, Alg, All, Ama, Amt, Ape, Apm, Apr, Arg, Asn, Asp, Asu, Aze, Azi, Bai, Bph, Can, Cit, Cys, $(Cys)_2$, Cyta, Daad, Dab, Dadd, Dap, Dapm, Dasu, Djen, Dpa, Dtc, Fel, Gln, Glu, Gly, Guv, hAla, hArg, hCys, hGln, hGlu, His, hIle, hLeu, hLys, hMet, hPhe, hPro, hSer, hThr, hTrp, hTyr, Hyl, Hyp, 3Hyp, Ile, Ise, Iva, Kyn, Lant, Lcn, Leu, Lsg, Lys, βLys, ΔLys, Met, Mim, Min, nArg, Nle, Nva, Oly, Orn, Pan, Pec, Pen, Phe, Phg, Pic, Pro, ΔPro, Pse, Pya, Pyr, Pza, Qin, Ros, Sar, Sec, Sem, Ser, Thi, βThi, Thr, Thy, Thx, Tia, Tle, Tly, Trp, Trta, Tyr, Val, Nal, Tbg, Npg, Cha, Chg, Thia (cf., for example, Houben-Weyl, Methoden der organischen Chemie (Methods of organic chemistry), Volume XV/1 and 2, Stuttgart, 1974).

Aza-amino acids are derived from natural or unnatural amino acids with the central -CHR- or $-CH_2-$ unit being replaced by -NR- or -NH- respectively. Examples which may be mentioned are azaglycine, azavaline, azaleucine, azaisoleucine and azaphenylalanine.

By an imino acid are meant in general natural or unnatural amino acids whose amino group is monosubstituted. Particular mention may be made in this connection of compounds which are substituted by C_1-C_8-alkyl, which in turn is optionally mono- or diunsaturated and can be substituted by up to 3 identical or different radicals from the series comprising mercapto; hydroxyl; C_1-C_7-alkoxy; carbamoyl; C_1-C_8-alkanoyloxy; carboxyl; C_1-C_7-alkoxycarbonyl; F; Cl; Br; I; amino; amidino which can optionally be substituted by one, two or three C_1-C_8-alkyl radicals; guanidino which can optionally be substituted by one or two benzyloxycarbonyl radicals or by one, two, three or four C_1-C_8-alkyl radicals; C_1-C_7-alkylamino; di-C_1-C_7-alkylamino; C_1-C_6-alkoxycarbonylamino; C_7-C_{15}-aralkoxycarbonyl; C_7-C_{15}-aralkoxycarbonylamino; phenyl-C_1-C_4-alkoxy; 9-fluorenylmethoxycarbonylamino; C_1-C_6-alkylsulfonyl; C_1-C_6-alkylsulfinyl; C_1-C_6-alkylthio; hydroxyamino;

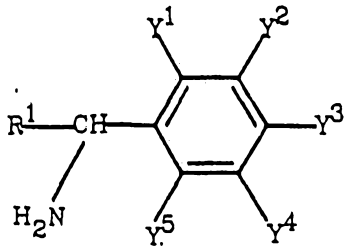
hydroxyimino; sulfamoyl.

Also suitable are heterocycles from the following group: pyrrolidine-2-carboxylic acid; piperidine-2-carboxylic acid; 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid; decahydroisoquinoline-3-carboxylic acid; octahydroindole-2-carboxylic acid; decahydroquinoline-2-carboxylic acid; octahydrocyclopenta[b]pyrrole-2-carboxylic acid; 2-azabicyclo[2.2.2]octane-3-carboxylic acid; 2-azabicyclo-[2.2.1]heptane-3-carboxylic acid; 2-azabicyclo[3.1.0]-hexane-3-carboxylic acid; 2-azaspiro[4.4]nonane-3-carboxylic acid; 2-azaspiro[4.5]decane-3-carboxylic acid; spiro[(bicyclo[2.2.1]heptane)-2,3-pyrrolidine-5-carboxylic acid; spiro[(bicyclo[2.2.2]octane)-2,3-pyrrolidine-5-carboxylic acid; 2-azatricyclo[4.3.0.$1^{6,9}$]decane-3-carboxylic acid; decahydrocyclohepta[b]pyrrole-2-carboxylic acid; octahydrocyclopenta[c]pyrrol-2-carboxylic acid; octahydroisoindole-1-carboxylic acid; 2,3,3a,4,6a-hexahydrocyclopenta[b]pyrrole-2-carboxylic acid; 2,3,3a,4,5,7a-hexahydroindole-2-carboxylic acid; tetrahydrothiazole-4-carboxylic acid; isoxazolidine-3-carboxylic acid; pyrazolidine-3-carboxylic acid; hydroxyproline-2-carboxylic acid; all of which can optionally be substituted:



By salts of compounds of the formula I are meant, in particular, pharmaceutically utilizable or non-toxic salts. Particularly suitable are alkali metal or alkaline earth metal salts, salts with physiologically tolerated amines and salts with inorganic or organic acids, such as, for example, HCl, HBr, H_2SO_4, H_3PO_4, maleic acid, fumaric acid, citric acid, tartaric acid and acetic acid.

The process is advantageously carried out in such a way that a compound of the formula II



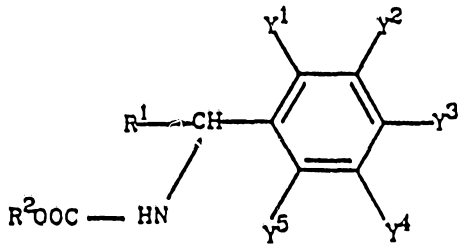
(II)

in which

Y^1, Y^2, Y^3, Y^4 and Y^5 are hydrogen, C_1-C_4-alkyl, C_1-C_4-alkoxy or $-O-(CH_2)_n-COOH$, $-(CH_2)_n-COOH$ or $-NH-CO-(CH_2)_n-COOH$, where the radicals can be identical or different, but at least one radical is $-O-(CH_2)_n-COOH$, $-(CH_2)_n-COOH$ or $-NH-CO-(CH_2)_n-COOH$, n is an integer from 1 to 6, preferably 1 to 3, and

R^1 is hydrogen or C_1-C_6-alkoxy-C_6-C_{12}-aryl, preferably 4-methoxyphenyl,

is reacted with a silylating reagent, for example tert. butyldimethylsilyl chloride, tert. butylphenylsilyl chloride, trimethylchlorosilane, especially trimethylchlorosilane, in a solvent suitable for this purpose, such as, for example, THF, acetonitrile, methylene chloride, dimethylformamide or mixtures thereof, and subsequently the silylated compound is converted with a chloroformic acid derivative, especially the substituted ester derivatives, into compounds of the formula III



(III)

in which

R^1, Y^1, Y^2, Y^3, Y^4 and Y^5 are as defined above, and

R^2 is a C_6-C_{12}-aryl radical which is substituted by electron-attracting substituents, preferably nitro and halogen, for example F or Cl,

the compounds of the formula III obtained in this way are reacted with an amino hydrazide of the formula IV

$$R^3 \cdot X \cdot CO \cdot NH \cdot NH \cdot R^4 \qquad (IV)$$

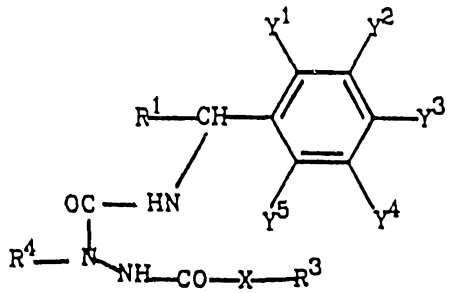
in which

X is a natural or unnatural amino acid or imino acid and is as defined above,

R^3 is a protective group which is base-labile or labile to weak acids or hydrogenation, such as, for example, a urethane protective group (see, for example, Hubbuch, Kontakte (Merck) 1979, No. 3, pages 14 to 23), and

R^4 is C_1-C_8-alkyl, C_3-C_9-cycloalkyl, C_6-C_{12}-aryl, C_6-C_{12}-aryl-C_1-C_8-alkyl, heteroaryl or heteroaryl-C_1-C_8-alkyl or hydrogen,

in a solvent in which the compounds of the formula III and IV are soluble, such as, for example, DMF, to give the compounds of the formula V



(V)

in which R^1, R^3, R^4 and Y^1, Y^2, Y^3, Y^4 and Y^5 have the abovementioned meanings, if R^3 is a protective group which is labile to hydrogenation, preferably benzyloxycarbonyl, this protective group is removed by hydrogenation on a Pd catalyst and, before the subsequent reaction, converted into a base-labile urethane protective group, preferably Fmoc, or a urethane protective group which is labile to

weak acids, preferably Bpoc,
subsequently the compound of the formula V in which R^1, R^4, Y^1, Y^2, Y^3, Y^4 and Y^5 have the abovementioned meanings, and R^3 is a urethane protective group which is labile to bases or weak acids, is coupled with the coupling reagents customary in peptide chemistry via the $-O-(CH_2)_n-COOH$, $-(CH_2)_n-COOH$ or $-NH-CO-(CH_2)_n-COOH$ group to a resin, the protective group R^3 is eliminated, natural or unnatural amino, imino or aza-amino acids which have been temporarily protected by amino-protective groups which are base-labile or labile to weak acids and which are optionally in the form of their activated derivatives are coupled on stepwise, and, after synthesis is complete, the peptides of the formula I are liberated from the resin by treatment with a moderately strong acid, with elimination again, simultaneously or by suitable measures subsequent thereto, of temporarily introduced side-chain protective groups.

The compounds of the formula IV are prepared by reacting the natural or unnatural amino acids or imino acid with the appropriate hydrazines by coupling methods customary in peptide chemistry.

Alkyl can be straight-chain or branched. A corresponding statement applies to radicals derived therefrom, such as, for example, alkoxy, alkylthio, alkylamino, dialkylamino and alkanoyl. Alkyl is, in particular, C_1-C_4-alkyl.

Cycloalkyl also means alkyl-substituted radicals such as, for example, 4-methylcyclohexyl or 2,3-dimethylcyclopentyl.

Halogen is fluorine, chlorine, bromine or iodine, especially fluorine or chlorine.

C_6-C_{12}-aryl is, for example, phenyl or naphthyl, preferably phenyl. Heteroaryl is the radical of a 5- to 7-membered monocyclic or 8- to 10-membered bicyclic

aromatic ring system which can be benzo-fused and which can contain as hetero elements one, two, three or four different radicals from the group comprising N, O, S, NO, SO and SO_2 and which is substituted by 1 to 6 hydroxyl or by one, two or three identical or different radicals from the series comprising F, Cl, Br, I; hydroxyl, mono-, di- or trihydroxy-C_1-C_4-alkyl; trifluoromethyl; formyl; carboxamido; mono- or di-C_1-C_4-alkylaminocarbonyl; nitro; C_1-C_7-alkoxy; C_1-C_7-alkyl; C_1-C_7-alkoxycarbonyl; amino; C_1-C_7-alkylamino; di-C_1-C_7-alkylamino; carboxyl; carboxymethoxy; amino-C_1-C_7-alkyl; C_1-C_7-alkylamino-C_1-C_7-alkyl; di-C_1-C_7-alkylamino-C_1-C_7-alkyl; C_1-C_7-alkoxycarbonylmethoxy; carbamoyl; sulfamoyl; C_1-C_7-alkoxysulfonyl; C_1-C_8-alkylsulfonyl; sulfo-C_1-C_8-alkyl; guanidino-C_1-C_8-alkyl and C_1-C_6-alkoxycarbonyl and/or mono-, di- or trisubstituted by oxo. Particular mention may be made of: furyl, thienyl, imidazolyl, pyrazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, tetrazolyl, pyridyl, pyrazinyl, pyrimidinyl, indolyl, isoindolyl, indazolyl, phthalazinyl, quinolyl, isoquinolyl, quinoxalinyl, quinazolinyl, cinnolinyl, β-carbolinyl or a benzo-fused derivative of these radicals.

If necessary to prevent side reactions or for the synthesis of specific peptides, the functional groups in the side chain of amino, aza-amino and imino acids are additionally protected by suitable protective groups (see, for example, T.W. Greene, "Protective Groups in Organic Synthesis", New York, John Wiley & Sons, 1981; Hubbuch, Kontakte (Merck) 1979, No. 3, pages 14-23; Büllesbach, Kontakte (Merck) 1980, No. 1, pages 23-35), employing primarily Arg(Tos), Arg(Mts), Arg(Mtr), Arg(Pmc), Asp(OBzl), Asp(OtBu), Cys(4-MeBzl), Cys(Acm), Cys(StBu), Glu(OBzl), Glu(OtBu), His(Tos), His(Fmoc), His(Dnp), His(Trt), Lys(Cl-2), Lys(Boc), Met(O), Ser(Bzl), Ser(tBu), Thr(Bzl), Thr(tBu). It is also possible for the functional groups in the side chain to be glycosylated as described, for example, in EP-A 263 521 (HOE 86/F 253).

The resins used as support material are commercially available or prepared by the user, such as, for example, alkoxybenzyl alcohol-resins, aminomethyl-resins or benzhydrylamino-resins. Aminomethyl-, benzhydrylamino- (BHA) and methylbenzhydrylamino-resins (MBHA) are preferred. The loading is determined by amino-acid analysis and/or elemental analysis.

By suitable spacers are meant, for example, the spacers described in Atherton, Shepperd in Perspectives in Peptide Chemistry, pages 101-117 (Karger, Basel 1981); EP-A 264 802 (HOE 86/F 259), EP-A 287 882 (HOE 87/F 101) and EP-A 322 348 (HOE 87/F 386K), and derivatives derived therefrom, such as, for example, those whose protective group has been eliminated. 4-Carboxylatopropoxy-4'-methoxybenzhydrylamine and 5-carboxylatoethyl-2,4-dimethoxy-4'-methoxybenzhydrylamine are preferred.

It is possible to use as coupling reagent for the spacer of the formula V and the other amino acid derivatives all the possible activating reagents used in peptide synthesis, see, for example, Houben-Weyl, Methoden der Organischen Chemie, Volume XV/2, Stuttgart 1974, but especially carbodiimides such as, for example, N,N'-dicyclohexylcarbodiimide, N,N'-diisopropylcarbodiimide or N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide. This coupling can be carried out directly by addition of amino acid derivative with the activating reagent and, where appropriate, an additive suppressing racemization, such as, for example, 4-dimethylaminopyridine, 1-hydroxybenzotriazole (HOBt) (W. König, R. Geiger, Chem. Ber. 103 (1970) 788 - 798) or 3-hydroxy-4-oxo-3,4-dihydrobenzotriazine (HOObt) (W. König, R. geiger, Chem. Ber. 103 (1970) 2034 - 2040) to the resin, or else the preactivation of the amino acid derivative as symmetrical anhydride or HOBt or HOObt ester can take place separately and the solution of the activated species in a suitable solvent can be added to the peptide-resin capable of coupling.

The coupling and activation of the spacer of the formula V and of the amino acid derivative with one of the abovementioned activating reagents can be carried out in dimethylformamide or methylene chloride or a mixture of the two. The activated amino acid derivative is normally employed in a 1.5- to 4-fold excess. In cases where incomplete coupling occurs, the coupling reaction is repeated without previously carrying out the deblocking of the α-amino group of the peptide-resin which is necessary for the coupling of the next amino acid in sequence.

The success of the coupling reaction can be checked using the ninhydrin reaction as described, for example, by E. Kaiser et al. (Anal. Biochem. 34 (1970) 595). The synthesis can also be carried out automatically, for example with a model 430A peptide synthesizer from Applied Biosystems, it being possible to use either the synthesis programs provided by the equipment manufacturer or else those drawn up by the user himself. The latter are particularly employed when using amino acid derivatives protected by the Fmoc group.

The peptide amides are cleaved off the resin by treatment with moderately strong acids customarily used in peptide synthesis (for example trifluoroacetic acid), adding as cation traps substances such as phenol, cresol, thiocresol, anisole, thioanisole, ethanedithiol, dimethyl sulfide, ethyl methyl sulfide or similar cation traps customary in solid-phase synthesis, singly or a mixture of two or more of these auxiliaries. In this connection the trifluoroacetic acid can also be used diluted by a suitable solvent such as, for example, methylene chloride. When the spacer is cleaved off the resin there is simultaneous elimination of the side-chain protective groups.

The crude peptides obtained in this way are purified by chromatography on •Sephadex, ion exchanger resins or by

HPLC.

A process of solid-phase synthesis for the preparation of Ac-D-Nal(2)-p-Cl-D-Phe-D-Trp-Ser-Tyr-D-Ser(α-L-Rha)-Leu-Arg-Pro-Azagly-NH_2 and pGlu-His-Trp-Ser-Tyr-D-Ser(tBu)-Leu-Arg-Pro-Azagly-NH_2 (Zoladex) is preferred.

List of abbreviations used:

BSA	bistrimethylsilylacetamide
Cha	cyclohexylalanine
Chg	cyclohexylglycine
DCC	dicyclohexylcarbodiimide
DIC	diisopropylcarbodiimide
DMAP	dimethylaminopyridine
Fmoc	9-fluorenylmethoxycarbonyl
HOBt	1-hydroxybenzotriazole
HOObt	3-hydroxy-4-oxo-3,4-dihydro-1,2,3-benzotriazine
Nal	naphthylalanine
Npg	neopentylglycine
Pmc	2,2,5,7,8-pentamethylchroman-6-sulfonyl
Tbg	tert.-butylglycine
THF	tetrahydrofuran
Thia	2-thienylalanine

The examples which follow serve to illustrate the present invention without intending to restrict it thereto.

Example 1

a) 5-Carboxylatoethyl-2,4-dimethoxy-4'-methoxybenzhydrylamine

17.5 g of 5-carboxylatoethyl-2,4-dimethoxy-4'-methoxybenzophenone oxime were dissolved in 450 ml of a 1:1 mixture of ethanol and DMF, and 2 ml of concentrated NH_3 were added. After addition of the Pt/C catalyst, hydrogenation was carried out under atmospheric pressure for 5 days. After completion of the reaction, the catalyst was filtered off with suction, the filtrate was

concentrated, and the product was precipitated with ether. It was employed as such without further purification.

1b) N-(p-Nitrophenyloxycarbonyl)-5-carboxylatoethyl-2,4-dimethoxy-4'-methoxybenzhydrylamine

10 g of the title compound from Example 1a) were introduced into 100 ml of a 4:1 THF/DMF mixture and, at room temperature, 2.1 equiv. of bistrimethylsilylacetamide (BSA) were added. The suspension became completely clear in a short time, and the clear solution was then stirred for 2 hours. 3 g of nitrophenyl chloroformate were then added and the mixture was stirred for a further hour. After completion of the reaction, the solvent was removed under high vacuum. The residue was mixed with 300 ml of water, and the resulting oil was extracted with ethyl acetate. The ethyl acetate phase was washed with a 1 N $KHSO_4$ solution and water. The organic phase was dried over $MgSO_4$ and evaporated to dryness. The residue (12 g) is characterized by NMR, IR and MS.

N-(p-Nitrophenyloxycarbonyl)-5-carboxylatoethyl-2,4-dimethoxy-4'-methoxybenzhydrylamine was then reacted with amino hydrazides to give the suitable substituted anchors.

1c) Benzyloxycarbonyl-4-prolylazaglycine (5-carboxylatoethyl-2,4-dimethoxy-4'-methoxybenzhydryl)amide.

3.27 g of benzyloxycarbonyl-prolyl hydrazide hydrochloride and 6.94 g of the title compound from Example 1b) were dissolved in 40 ml of dimethylformamide (DMF), and 3 equiv. of N-ethylmorpholine and a catalytic amount of dimethylaminopyridine (DMAP) were added. Reaction was allowed to take place for 16 hours. After completion of the reaction, the mixture was evaporated to dryness. The residue was taken up in ethyl acetate/butanol, and the organic phase was washed with saturated $NaHCO_3$ solution,

1 N $KHSO_4$ solution and water. The organic phase was dried over $MgSO_4$ and, after filtration, evaporated to dryness. It was possible to recrystallize the residue from pure ethyl acetate. 6.6 g of the title compound were obtained.

FAB-MS: 641 ($M+Li^+$)

IR: CO 1695 cm^{-1}

^1H-NMR (DMSO): δ = 3.7 s (6H, OCH_3) ppm

1d) 9-Fluorenylmethoxycarbonyl-L-prolylazaglycine (5-carboxylatoethyl-2,4-dimethoxy-4'methoxybenzhydryl)-amide

26.5 g of the title compound from Example 1c) were dissolved in 300 ml of methanol, and 2 g of Pd/C catalyst were added. The hydrogenation was complete after one hour. The catalyst was filtered off, and the filtrate was evaporated to dryness. The residue (17.5 g) was, without further purification, taken up in a mixture of 80 ml of water and 80 ml of dioxane and mixed with 8 g of sodium bicarbonate and 17 g of N-(9-fluorenylmethoxycarbonyl-oxy)succinimide (Fmoc-ONSu). Reaction was allowed to take place for one day. After the reaction was complete, the mixture was filtered through a clarifying filter. The filtrate was adjusted to pH 6 with 2 N H_2SO_4 and evaporated in vacuo to a volume of 80 ml. The mixture was diluted with 100 ml of water and extracted with a mixture of ethyl acetate and n-butanol (8.5:1.5). The organic phase was washed with 50 % saturated NaCl solution and then evaporated to dryness. The residue was filtered through 500 g of silica gel with ethyl acetate. 20 g of the title compound were obtained.

FAB-MS: 729 ($M+Li^+$)

IR: CO 1695 cm^{-1}

1e) Coupling of the title compound from Example 1d) to a polystyrene resin

1.0 g of aminomethylpolystyrene resin (loading 1.07 mmol) and 1.2 g of the title compound from Example 1d) were

suspended in 10 ml of dimethylformamide, and 216 mg of 1-hydroxybenzotriazole (HOBt) and 0.75 ml of diisopropylcarbodiimide (DIC) were added. Reaction was allowed to take place overnight until the ninhydrin test indicated complete reaction. The resin was filtered off and washed with dimethylformamide and methylene chloride and thoroughly dried in vacuo. The loading of the resin with proline was 0.51 mmol/g.

1f) Ac-D-NaI(2)-p-Cl-D-Phe-D-Trp-Ser-Tyr-D-Ser(α-L-Rha)-Leu-Arg-Pro-Azagly-NH_2

The 9-fluorenylmethoxycarbonyl-N_α-amino-protective group of the compound from Example 1e) was eliminated with a 20 % strength piperidine/dimethylformamide solution (2×3 min, 2×8 ml). The resin was then washed with N-methylpyrrolidinone (5×10 ml) and the peptide was synthesized on the resin (785 mg of resin from Example 1c)), carrying out the following steps in cycles:

- elimination of the Fmoc protective group with 20 % piperidine in DMF
- washing of the resin with DMF/N-methylpyrrolidinone
- coupling on of the Fmoc-amino acid with in situ activation as HOBt ester using diisopropylcarbodiimide as activating reagent (1.5 mmol of amino acid, 2.25 mmol of HOBt, 1.6 mmol of diisopropylcarbodiimide)

If the coupling was incomplete (Kaiser test), the coupling step was repeated. The last amino acid employed was Fmoc-D-Nal(2)-OH. The N-terminal acetyl group was introduced by reaction with acetic anhydride.

After completion of the solid-phase synthesis, the resin was washed (DMF, CH_2Cl_2) and thoroughly dried. 1.35 g of substituted resin were obtained.

The dried resin was suspended at room temperature in 0.75 ml of ethanedithiol. After 15 minutes, 7.5 ml of trifluoroacetic acid were added and the suspension was stirred for 1.5 hours. After this time, the resin was filtered off and thoroughly washed with 80 % strength trifluoroacetic acid. The filtrate was evaporated in vacuo and taken up in 30 ml of water. $NaHCO_3$ was added to adjust to pH 6-7, and the peptide was extracted by shaking with n-pentanol (4×30 ml). The n-pentanol phase was evaporated and taken up in 10 ml of methanol/H_2O (9:1), and 0.5 g of K_2CO_3 was added. The mixture was stirred for 30 minutes and filtered, and the filtrate was concentrated. The residue was taken up in 100 ml of n-pentanol, and the organic phase was washed with water. The organic phase was dried with $MgSO_4$ and filtered and then evaporated. 740 mg of crude product were obtained. Chromatography on •Sephadex G 25 (1 M acetic acid) and on silica gel resulted in 185 mg of the pure title compound. FAB-MS: 1531 ($M+H^+$)

Patent claims

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A process for the preparation of peptides of the formula I

$$(X)_n - A - NH_2 \qquad (I)$$

in which

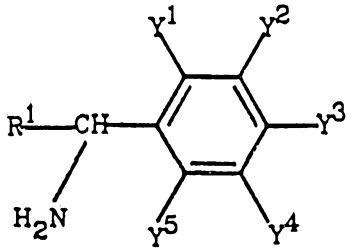
X is a natural or unnatural amino acid, aza-amino acid or imino acid,

n is an integer from 1 to 50, preferably 1 to 30, and

A is an aza-amino acid,

and the physiologically tolerated salts thereof, which comprises converting a spacer into a form capable of acylation, reacting the latter with a suitable formic acid derivative and subsequently with an appropriate amino hydrazide, where appropriate converting the protective group into a protective group which is base-labile or labile to weak acids, coupling the spacer obtained in this way to a resin, synthesizing the required peptide stepwise from the C-terminal end, subsequently cleaving the peptide off the resin and, where appropriate, converting it into physiologically tolerated salts thereof.

2. The process as claimed in claim 1, wherein a compound of the formula II



(II)

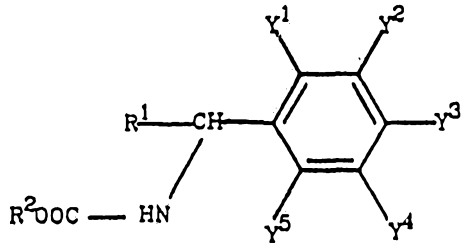
in which

Y^1, Y^2, Y^3, Y^4 and Y^5 are hydrogen, C_1-C_4-alkyl, C_1-C_4-alkoxy or $-O-(CH_2)_n-COOH$, $-(CH_2)_n-COOH$ or $-NH-CO-(CH_2)_n-COOH$,

where the radicals can be identical or different, but at least one radical is $-O-(CH_2)_n-COOH$, $-(CH_2)_n-COOH$ or $-NH-CO-(CH_2)_n-COOH$, n is an integer from 1 to 6, preferably 1 to 3, and

R^1 is hydrogen or C_1-C_6-alkoxy-C_6-C_{12}-aryl,

is reacted with a silylating reagent in a solvent suitable for this purpose, and subsequently the silylated compound is converted with a chloroformic acid derivative into compounds of the formula III



(III)

in which

R^1, Y^1, Y^2, Y^3, Y^4 and Y^5 are as defined above, and

R^2 is a C_6-C_{12}-aryl radical which is substituted by electron-attracting substituents, preferably nitro and halogen,

the compounds of the formula III obtained in this way are reacted with an amino hydrazide of the formula IV

$$R^3 \cdot X \cdot CO \cdot NH \cdot NH \cdot R^4 \qquad (IV)$$

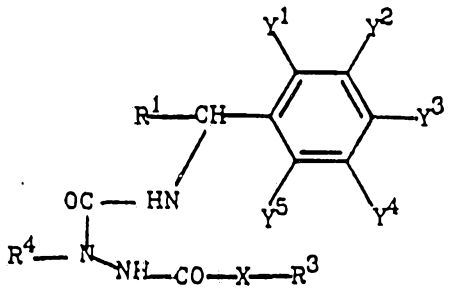
in which

X is a natural or unnatural amino acid or imino acid and is as defined above,

R^3 is a protective group which is base-labile or labile to weak acids or hydrogenation, and

R^4 is C_1-C_8-alkyl, C_3-C_8-cycloalkyl, C_6-C_{12}-aryl, C_6-C_{12}-aryl-C_1-C_8-alkyl, heteroaryl or heteroaryl-C_1-C_8-alkyl or hydrogen,

in a suitable solvent to give the compounds of the formula V



(V)

in which R^1, R^3, R^4 and Y^1, Y^2, Y^3, Y^4 and Y^5 have the abovementioned meanings, if R^3 is a protective group labile to hydrogenation this protective group is removed by hydrogenation on a Pd catalyst and, before the subsequent reaction, converted into a urethane protective group which is base-labile or labile to weak acids, subsequently the compound of the formula V in which R^1, R^4 Y^1, Y^2, Y^3, Y^4 and Y^5 have the abovementioned meanings, and R^3 is a urethane protective group which is labile to bases and weak acids, is coupled with the coupling reagents customary in peptide chemistry via the $-O-(CH_2)_n-COOH$, $-(CH_2)_n-COOH$ or $-NH-CO-(CH_2)_n-COOH$ group to a resin, the protective group R^3 is eliminated, natural or unnatural amino, imino or aza-amino acids which have been temporarily protected by amino-protective groups which are base-labile or labile to weak acids and which are optionally in the form of their activated derivatives are coupled on stepwise, and, after the synthesis is complete, the peptides of the formula I are liberated from the resin by treatment with a moderately strong acid, with elimination again, simultaneously or by suitable measures subsequent thereto, of temporarily introduced side-chain protective groups.

3. The process as claimed in either of claims 1 and 2, wherein Ac-D-Nal(2)-p-Cl-D-Phe-D-Trp-Ser-Tyr-D-Ser(α-L-Rha)-Leu-Arg-Pro-Azagly-NH_2 is prepared.

4. The process as claimed in either of claims 1 and 2,

wherein pGlu-His-Trp-Ser-Tyr-D-Ser(tBu)-Leu-Arg-Pro-Azagly-NH_2 is prepared.

DATED this 28th day of August 1991.

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