Novel process for the preparation of peptides and N-carbamoyl-protected peptides

The invention relates to a process for the enzymatic preparation of protected di- or oligopeptides and removal of the protective group used by splitting off.

Synthetic short-chain peptides are increasingly used in pharmacology and in parenteral feeding. Kyotorphin (L-Tyr-L-Arg), which promotes the release of enkephalins, that is to say endogenous substances which have an analgesic and tranquillizing action in the brain (Hughes, 1975), may be mentioned as an example of a pharmacologically active dipeptide.

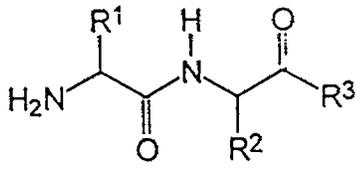
The enzymatic preparation of di- or oligopeptides is already known and generally makes use of protective group technology. The use of a formyl protective group is thus described in US-A-571,037, that of an acetyl protective group in JP 62074296, that of a benzyl protective group in US-A-4,935,355 or that of a phenacetyl protective group in Tetrahedron 1992, 48, 1115. These variants have the disadvantage that the protective groups in some cases are not inexpensive (benzyl, phenacetyl), can be split off again only with difficulty (acetyl, formyl) or can be removed (benzyl) only under quite specific conditions (hydrogenolysis).

The object of the invention was therefore to develop a process for the synthesis of peptides which is particularly simple and inexpensive, allows simple and gentle removal of the protective group by splitting off, and allows simple working up and separating-off of the enzyme.

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This object of the invention for the preparation of a peptide of the general formula I

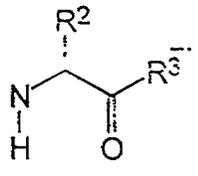


I

wherein

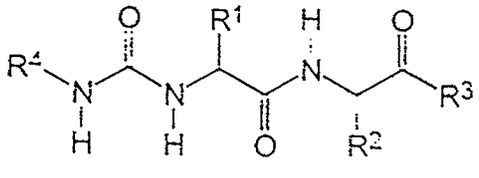
R^1 and R^2 independently of one another denote hydrogen, (C_1-C_6) alkyl, which can optionally be interrupted or substituted by heteroatoms, such as N, O or S, one [sic] or several times, it being possible for the heteroatoms in their turn to be substituted by hydrogen, (C_1-C_4) alkyl or benzyl or to be bonded to the alkyl group via a double bond, phenyl or benzyl, both of which can optionally be substituted by halogen or hydroxyl once or several times, heteroaralkyl, such as 3-indolylmethyl, 2-, 3- or 4-pyridylmethyl,

R^3 denotes (C_1-C_4) alkoxy, NH_2, hydroxyl, NR^1R^2, benzyloxy, which can optionally be substituted by halogen, nitro, NH_2, (C_1-C_4) alkyl, (C_1-C_4) alkoxy once or several times, or one or more units of the type II



II

is achieved by a process in which compounds of the type III or a salt form thereof,



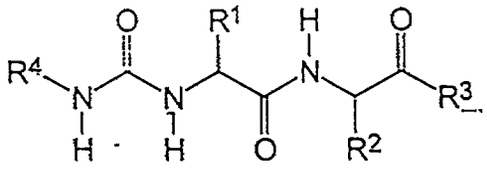
III

wherein R^1, R^2 and R^3 have the abovementioned meaning and

R^4 denotes hydrogen, (C_1-C_4) alkyl, phenyl, which can optionally be substituted by halogen, (C_1-C_4) alkyl, (C_1-C_4) alkoxy, nitro, CN, CF_3, (C_1-C_6) alkoxycarbonyl, COOH or $-NR^1R^2$ once or several times, aralkyl, such as benzyl, which can be substituted in its turn by halogen, (C_1-C_4) alkyl or (C_1-C_4) alkoxy, naphthyl, heteroaralkyl, such as 2-, 3- or 4-thienyl, 2-, 3- or 4-pyridyl or 2-quinolyl

are reacted with a carbamoylase, optionally in the presence of a solvent, or the carbamoyl protective group is split off chemically, optionally in the presence of a solvent and optionally in the presence of an acid.

It has furthermore been found that carbamoyl-protected peptides of the general structure III,



III

wherein

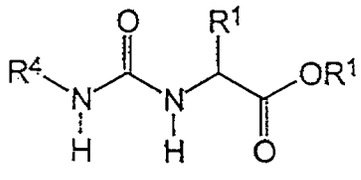
R^1 and R^2 independently of one another denote hydrogen, (C_1-C_6) alkyl, which can optionally be interrupted or substituted by heteroatoms, such as N, O or S, one [sic] or several times, it being possible for the heteroatoms in their turn to be substituted by hydrogen, (C_1-C_4) alkyl or benzyl or to be bonded to the alkyl group via a double bond, phenyl or benzyl, both of which can optionally be substituted by halogen

or hydroxyl once or several times, heteroaralkyl, such as 3-indolylmethyl, 2-, 3- or 4-pyridylmethyl,

R^3 denotes (C_1-C_4) alkoxy, NH_2, hydroxyl, NR^1R^2, benzyloxy, which can optionally be substituted by halogen, nitro, NH_2, (C_1-C_4) alkyl, (C_1-C_4) alkoxy once or several times,

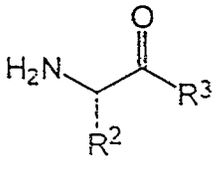
R^4 denotes hydrogen, (C_1-C_4) alkyl, phenyl, which can optionally be substituted by halogen, (C_1-C_4) alkyl, (C_1-C_4) alkoxy, nitro, CN, CF_3, (C_1-C_6) alkoxycarbonyl, COOH or $-NR^1R^2$ once or several times, aralkyl, such as benzyl, which can be substituted in its turn by halogen, (C_1-C_4) alkyl or (C_1-C_4) alkoxy, naphthyl, heteroaralkyl, such as 2-, 3- or 4-thienyl, 2-, 3- or 4-pyridyl or 2-quinolyl

are obtained by a process in which a compound of the type IV or a salt form of IV, in which R^1 and R^4 have the abovementioned meaning,



IV

is reacted with a compound of the type V, or of [sic] an acid addition salt thereof, wherein R^2 and R^3 have the abovementioned meaning,



V

optionally in the presence of a solvent and optionally in the presence of a base.

The term "alkyl groups" is to be understood as meaning both "straight-chain" and "branched" alkyl groups.

The term "straight-chain alkyl group" is to be understood as meaning, for example, radicals such as methyl, ethyl, n-propyl, n-butyl, n-pentyl, n-hexyl, and the term "branched alkyl group" is to be understood as meaning radicals such as, for example, isopropyl or tert.-butyl.

The term halogen represents fluorine, chlorine, bromine or iodine. The term "alkoxy group" represents radicals such as, for example, methoxy, ethoxy, propoxy, butoxy, isopropoxy, isobutoxy or pentoxy.

Dipeptides, such as, for example, aspartam, can preferably be prepared by this process.

Another advantage of the process is that the peptide coupling can be carried out in a highly dense suspension, it being possible for the substrate and product to be present in partial [sic] solid form.

The novel process for the synthesis of peptides using, as the intermediate product, the carbamoyl-protected peptides described comprises three reaction steps.

A) Preparation of the N-carbamoyl-amino acid or of the N-carbamoyl-amino acid derivative

B) Linking of the peptide bond between the carbamoyl-protected electrophile and the nucleophile

C) Removal of the carbamoyl protective group by splitting off

The preparation of the N-carbamoyl-amino acid or of the N-carbamoyl-amino acid derivative can be carried out in a manner known per se from the literature. Preferably, the reaction of the amino acid or of the amino acid derivative with an isocyanate of the general structure VI,

$$R^1—NCO \qquad VI$$

wherein R^1 has the meaning given above, in a two-phase system of H_2O/organic solvent, it being possible for the organic solvent to be, for example, toluene, chlorobenzene or tert.butyl methyl ether, is carried out. The reaction is carried out at temperatures between 0 °C and 120 °C, preferably between 20 °C and 70 °C. If appropriate, an inorganic base, such as, for example, NaOH, KOH, K_2CO_3, Na_2CO_3, potassium bicarbonate or sodium bicarbonate, or an organic base, such as, for example, triethylamine or tributylamine, can be added to the reaction mixture. The phenyl isocyanate of the structure III is advantageously used in a slight excess.

Reactions to give the N-carbamoyl-protected amino acids or amino acid derivatives with a cyanate of the structure VII,

$$M^{\oplus}\,{}^{\ominus}OCN \qquad VII$$

wherein $M^{\oplus}$ represents $Na^{\oplus}$, $K^{\oplus}$, $Ag^{\oplus}$, $Pb^{\oplus}$ (OCN) or ammonium ions, such as, for example, tetraethylammonium, tetrabutylammonium or benzyltriethylammonium, are particularly preferred.

The reaction is carried out in a manner known per se in aqueous solution at temperatures between 0 °C - 120 °C, preferably between 60 °C - 100 °C, and if appropriate with

the addition of an inorganic base, such as, for example, NaOH, KOH, K_2CO_3 or Na_2CO_3. It has proved favourable if the reaction is carried out in the presence of slight excesses of 1.01 - 2 equivalents, preferably 1.1 - 1.2 equivalents, of cyanate.

The linking of the peptide bond is carried out in a manner known per se with the aid of a hydrolase (Jakubke, Kuhl and Könnecke; Angew. Chem. 1985, vol. 97, p.p. 79 - 87). It is irrelevant for the reaction conditions here whether the peptide linking proceeds under kinetic or thermodynamic control. The process according to the invention can be applied to both process variants. The term "salt form" of the compound type IV is to be understood generally as meaning ionic structures. Thus, a possible cation, such as, for example, $Na^{\oplus}$, $K^{\oplus}$, $Ag^{\oplus}$, $Pb^{2\oplus}$ or an ammonium ion, such as, for example, tetraethylammonium, tetrabutylammonium or benzyltriethylammonium, can neutralize a negative charge on the compound type IV. The negative charge here can be distributed over the molecule (compound type IV) or localized on a nitrogen atom or the carboxyl group in the form of a carboxylate group.

Suprisingly, this reaction with the carbamoyl group, which is novel for this reaction sequence, as the protective group proceeds in good space/time yields and allows easy isolation of the products. The broad applicability of the reaction is also surprising.

An acid addition salt of V is to be understood as meaning, for example, an HCl salt, HBr salt or an H_2SO_4 salt.

The carbamoyl protective group is split off, for example, under mild reaction conditions with the aid of a

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carbamoylase. This type of removal of carbamoyl groups from peptides by splitting off is novel. It is particularly advantageous because, in addition to the enzyme, it requires only water as a substance used for the cleavage and because, in addition to the peptide, only carbon dioxide and ammonia are formed as cleavage products, from which the peptide can be separated off very easily. The carbamoylase can be employed for this reaction optionally in the partly purified, purified, isolated or immobilized state. The reaction is irreversible, and up to 100 % conversion are [sic] observed. The reaction is carried out in aqueous solution at temperatures between 10 - 50 °C, preferably 20 - 35 °C, especially preferably 25 - 30 °C, and pH values between 5 and 11, preferably 6.5 - 8.5, and especially preferably between 7 - 8.

Carbon dioxide and ammonia, which optionally escape from the reaction mixture by gassing out, are formed during the hydrolysis with carbamoylase.

From the peptide solution treated with carbamoylase, the now unprotected peptide can be precipitated out and isolated by crystallization by means of evaporation, cooling or, if appropriate, addition of organic solvents.

Carbamoylases which are deposited at the Deutsche Sammlung für Mikroorganismen [German Collection of Microorganisms] under the following numbers have proved suitable: DSM 7329, DSM 7330, DSM 9771. However, the novel process does not exclude microorganisms other than those mentioned above.

The removal of the carbamoyl protective group by splitting off can also be carried out analogously to that described above in the presence of an additional enzyme (in addition

to the carbamoylases mentioned), such as, for example, thermolysin.

The carbamoyl protective group can also be split off chemically. This type of removal of carbamoyl groups from peptides by splitting off is likewise novel. The removal by splitting off can in principle be carried out with any $NO^{\oplus}$ donor. $NaNO_2$ or $NO^{\oplus}BF_4^{\ominus}$ are particularly suitable. The reaction is optionally carried out in an aqueous medium and/or an inert organic solvent, such as an aromatic hydrocarbon, such as, for example, toluene or chlorobenzene, in an aliphatic hydrocarbon, a halogenated hydrocarbon, such as, for example, methylene chloride, or an ether, such as, for example, methyl tert.butyl ether or tetrahydrofuran. Depending on the $NO^{\oplus}$ donor, the reaction is carried out in the presence of an acid, such as, for example, HCl or H_2SO_4. Particularly suitable reaction temperatures are temperatures between +120 °C and -30 °C, particularly preferably +60 °C and -20 °C, especially preferably +25 °C and 0 °C. If an acid is added, the pH should be between -0.5 and 5, preferably 0 and 2. The reaction is advantageous since, in addition to nitrogen, CO_2 and, where appropriate, a salt, it produces no other by-products at all and the conversion proceeds with high yields and the desired formation of product without cleavage products. Another advantage of the reaction is the easy working up.

The novel process is explained in more detail with the aid of the following embodiment examples, but is not limited to these.

Example 1

Example of the preparation of N-carbamoyl-amino acids and derivatives using the example of N-carbamoyl-L-aspartic acid

12 g (0.3 mol) NaOH lozenges are initially introduced into approx. 200 ml H_2O and are dissolved. Aspartic acid (33.3 g, 0.25 mol) is added as a solution in 50 ml water and the reaction mixture is heated to 80 °C. The pH is 8.7. 16.6 g (0.255 mol) sodium cyanate are added in the course of 5 min (pH rises to 9.3, T to 86 °C). After a reaction time of 1 h, aspartic acid is no longer detectable.

Examples of the synthesis of the peptides:

The amino acids and peptides are abbreviated according to the internationally applicable rules (IUPAC-JUB Joint Commission on Biochemical Nomenclature (JCBN); Nomenclature and Symbolism for Amino Acids and Peptides. Eur. J. Biochem. 158, 9 - 37 (1984)).

AC-: aminocarbonyl- (carbamoyl-, NH_2CO-)

Example 2: Synthesis of AC-Asp-Phe-OMe

80 µl buffer (0.5 M Hepes/Na^+; pH 7) are added to 50 mg (0.2 mmol) AC-Asp(OK)-OK and 83 mg (0.4 mmol) H-Phe-OME•HCl in a closable polypropylene vessel and the components are immediately mixed intensively with a spatula. The reaction mixture is thermostatically controlled at 40 °C on a water-bath and the reaction is then started with 20 µl buffer comprising 4 mg thermolysin preparation (Sigma P 1512).

After 1 h, 2.5 h, 16 h, 22 h, 29 h and 39 h, samples are taken and the reaction is stopped in 0.75 ml 4 % aqueous acetic acid. The analytical evaluation is carried out here, as in the following examples, by means of HPLC and comparison with an authentic sample.

Yield:

Time (h)	Yield (% of th.)	H-Phe-OH/H-Phe-OMe (t=0) (-/-)
1	7	0.023
2.5	14	0.030
16	55	0.083
22	69	0.119
29	77	0.12
39	81	0.125

Example 3 a - e

Analogously to example 2, instead of 80 µl buffer, 120 (a), 160 (b), 200 (c), 240 (d) and 280 µl buffer are used to suspend the reactants. Sampling is carried out after 29 and 39 h.

Yield:

Example	Yield (% of th.)		H-Phe-OH/H-Phe-OMe (t=0) (-/-)	
	29 h	39 h	29 h	39 h
3a	79	82	0.139	0.136
3b	79	76	0.144	0.165
3c	73	75	0.169	0.169
3d	64	64	0.216	0.22
3e	59	61	0.215	0.224

Example 4

80 µl buffer (0.5 M Hepes/Na^+; pH 7) are added to 44 mg (0.175 mmol) AC-Asp(OK)-OK and 41.5 mg (0.2 mmol) H-Phe-OMe•HCl in a closable polypropylene vessel and the components are immediately suspended intensively with a spatula. After transfer of the reaction vessel into a water-bath thermostatically controlled at 40 °C, the reaction is started by addition of 15 µl buffer comprising 1.5 mg thermolysin preparation (Sigma P 1512). After 1 h, 2.5 h, 5 h, 6.5 h and 22 h, samples are taken and the reaction is stopped in 0.75 ml 4 % aqueous acetic acid and the product is analysed by means of HPLC.

Yield:

Time (h)	Yield (% of th.)	H-Phe-OH/H-Phe-OMe(t=0) (-/-)
1	7	0.023
2.5	16	0.039
5	23	0.064
6.5	28	0.078
22	48	0.136

Example 5

Analogously to example 4, instead of 80 µl buffer, 10 (a), 30 (b), 50 (c), 130 (d) and 180 µl (e) buffer are added to the reactants. Sampling is carried out after 22 h.

Yield:

Example	Yield (% of th.)	H-Phe-OH/H-Phe-OMe(t=0) (-/-)
5a	7	0.034
5b	31	0.072
5c	29	0.105
5d	39	0.319
5e	41	0.249

Example 6: Synthesis of AC-Asp-Phe-NH_2

400 µl buffer (0.5 M Hepes/Na^+; ph [sic] 7) are added to 250 mg (1.0 mmol) AC-Asp(OK)-OK and 400 mg (2.0 mmol) H-Phe-NH_2•HCl in a closable polypropylene vessel and the components are immediately suspended intensively with a spatula. After transfer of the reaction vessel into a water-bath thermostatically controlled at 40 °C, the reaction is started by addition of 50 µl buffer comprising 10 mg thermolysin preparation (Sigma P 1512). After 44 h, samples are taken and the reaction is stopped in 0.75 ml 4 % aqueous acetic acid and the product is analysed by means of HPLC. The reaction is ended by addition of 1 ml 1 N aqueous HCl and left overnight at 4 °C. The precipitate is then filtered off with suction and washed with ice-cold water.

Yield: 62 % of th.

Melting range: 221 - 223 °C

^{1}H-NMR (D_6-DMSO, 300 MHz); δ=2.38-2.6 (m, 2 H, $-^{\beta}CH_2-$), δ=2.8-2.88 (m, 1 H, $-^{\beta}CH_2-$), δ=5.72 (s, 2 H, $-NH_2$), δ=6.30 (d, 1 H, $-^{\alpha}NH-$, J = 7.69), δ=7.13 (s, 1 H, Phe-NH_2), δ=7.26-

7.28 (m, 5 H, Phe-C_6H_5), δ=7.33 (s, 1 H, Phe-NH_2), δ=7.83 (d, 1 H, -$^{\alpha}$NH-, J = 8.4)

Example 7: Synthesis of AC-Asp-Phe-Leu-NH_2

67.4 mg (0.2 mmol) AC-Asp-Phe-OMe and 26 mg (0.2 mmol) H-Leu-NH_2 (Bachem Biochemica GmbH) are dissolved in 180 µl buffer (0.5 M Hepes/Na^+, pH 7.9) in a polypropylene reaction vessel. After addition of 10 µl 10 M aqueous NaOH, the reaction is started with 10 µl α-chymotrypsin solution (10 mg/ml buffer; Serva, 3 x cryst.). After 2, 5, 10, 20, 30 and 60 min, samples are taken, dissolved in 1 ml 4 % aqueous acetic acid and analysed by means of HPLC. The reaction mixture which remains is taken up in 1 ml methanol, filtered and 1 ml 1 N aqueous HCl and 4 ml water are added to the filtrate. AC-Asp-Phe-Leu-NH_2 crystallized [sic] out within 48 h. The crystals were [sic] separated off and washed 2 times with ice-cold water (approx. 1.5 ml each time) and then dried over P_4O_{10} in vacuo.

Yield:

Time (min)	Ac-Asp-Phe-OH (% of th [sic])	AC-Asp-Phe-Leu-NH_2 (% of th.)
2	18.3	11.6
5	19	23.9
10	19.8	41.5
20	21.2	60.5
30	21.6	70.2
60	20.8	79.1

^{1}H-NMR; COSY (DMSO, 300 MHz): δ=0.82 (d, 3 H, Leu-$^{\delta}CH_3$), δ=0.87 (d, 3 H, Leu-$^{\delta}CH_3$), δ=1.41-1.6 (m, 3 H, Leu-$^{\gamma}$CH-, Leu-$^{\beta}CH_2$-), δ=2.35-2.58 (m, 2 H, Asp-$^{\beta}CH_2$-), δ=2.81-3.06 (m,

2 H, Phe-$^{\beta}CH_2$-), δ=4.18 (dt, 1 H, Leu-$^{\alpha}$CH-), δ=4.33 (dt, 1 H, Asp-$^{\alpha}$CH-), δ=4.44 (dt, 1 H, Phe-$^{\alpha}$CH-), δ=5.70 (s, 2 H, AC-NH_2), δ=6.30 (d, 1 H, Asp-$^{\alpha}$NH-), δ=6.96 (s, 1 H, Leu-NH_2), δ=7.05 (s, 1 H, Leu-NH_2), δ=7.14-7.27 (m, 5 H, Phe-C_6H_5), δ=7.85-7.96 (m, 2 H, Leu-$^{\alpha}$NH, Phe-$^{\alpha}$NH)

Example 8: Synthesis of AC-Asp-Phe-Ile-Gly-OMe

Analogously to example 7, instead of H-Leu-NH_2, 0.2 mmol (47.8 mg) H-Ile-Gly-OMe•HCl are employed. To establish the pH, 35 µl of the buffer are replaced by 10 M aqueous NaOH.

Yield after 70 min:

AC-Asp-Phe-OH (% of th.)	AC-Asp-Phe-Ile-Gly-OH (% of th.)	AC-Asp-Phe-Ile-Gly-OMe (% of th.)
28.4	3.9	67.7

^{1}H-NMR; COSY(DMSO, 300 MHz): δ=0.77-0.88 (m, 6 H, Ile-$^{\delta}CH_3$), δ=1.00-1.15 (m, 1 H, Ile-$^{\gamma}CH_2$-), δ=1.38-1.52 (m, 1 H, Ile-$^{\gamma}CH_2$-), δ=1.65-1.78 (m, 1 H, Ile-$^{\beta}$CH-), δ=2.34-2.58 (m, 2 H, Asp-$^{\beta}CH_2$-), δ=2.78-2.88 (m, 1 H, Phe-$^{\beta}CH_2$-), δ=2.94-3.2 (m, 1 H, Phe-$^{\beta}CH_2$-), δ=3.62 (s, 3 H, OCH_3), δ=3.75-3.95 (m, 2 H, Gly-$^{\alpha}CH_2$-), δ=4.17 (t, 1 H, Ile-$^{\alpha}$CH-), δ=4.30-4.40 (m, 1 H, Asp-$^{\alpha}$CH-), δ=4.48-4.58 (m, 1 H, Phe-$^{\alpha}$CH-), δ=5.7 (s-broad, AC-NH_2), δ=6.30 (d, 1 H, Asp-$^{\alpha}$NH-), δ=7.12-7.26 (m, 5 H, Phe-C_6H_5-), δ=7.80 (d, 1 H, Phe-$^{\alpha}$NH-), δ=7.92 (d, 1 H, Leu-$^{\alpha}$NH-), δ=8.32 (t, 1 H, Gly-$^{\alpha}$NH-).

Example 9: Synthesis of AC-Tyr-Ile-Ala-NH_2

a) 45 µl buffer (0.5 M Hepes/Na^+; pH 7) are added to

44.8 mg (0.2 mmol) AC-Tyr-OH and 47.4 mg (0.2 mmol) H-Ile-Ala-NH_2•HCl in a closable polypropylene vessel and the components are immediately suspended intensively with a spatula. The pH is established by addition of 25 µl 10 M aqueous NaOH. After transfer of the reaction vessel into a water-bath thermostatically controlled at 40 °C, the reaction is started by addition of 10 µl buffer comprising 1 mg thermolysin preparation (Sigma P 1512). After 3 h, a sample is taken and the reaction is stopped in 1 ml 4 % aqueous acetic acid and 200 µl acetonitrile and the product is analysed by means of HPLC. The reaction mixture which remains is worked up as in example 7, with the difference that instead of 1 N HCl, 4 % aqueous acetic acid is used.

Yield of AC-Tyr-Ile-Ala-NH_2 after 3 h: 76 % of th.

^{1}H-NMR;COSY(DMSO), 300 MHz): δ=0.76-0.84 (m, 6 H, Ile-$^{\delta}CH_3$, Ile-$^{\gamma}CH_3$), δ=1.00-1.12 (m, 1 H, Ile-$^{\gamma}CH_2$-), δ=1.22 (d, 3 H, Ala-$^{\beta}CH_3$), δ=1.36-1.42 (m, 1 H, Ile-$^{\gamma}CH_2$-), δ=1.68-1.78 (m, 1 H, Ile-$^{\beta}CH$-), δ=2.56-2.68 (m, 1 H, Tyr-$^{\beta}CH_2$-), δ=2.80-2.90 (m, 1 H, Tyr-$^{\beta}CH_2$-), δ=4.12-4.24 (m, 2 H, Ala-$^{\alpha}CH$-, Ile-$^{\alpha}CH$-), δ=4.28-4.38 (m, 1 H, Tyr-$^{\alpha}CH$-), δ=5.58 (s, 2 H, AC-NH_2), δ=6.03 (d, 1 H, Tyr-$^{\alpha}NH$-), δ=6.61 (d, 2 H, Tyr-ArH), δ=6.92-6.98 (m, 3 H, 2 Tyr-ArH, 1 Ala-NH_2), δ=7.20 (s, 1 H, Ala-NH_2), δ=7.84 (d, 1 H, Ala-$^{\alpha}NH$-), δ=7.92 (d, 1 H, Ile-$^{\alpha}NH$-), δ=9.12 (s, 1 H, Tyr-OH)

b) Analogously to example 9a, 224 mg (1.0 mmol) AC-Tyr-OH, 237.7 mg (1.0 mmol) H-Ile-Ala-NH_2•HCl, 225 µl buffer, 125 µl 10 M NaOH and 50 µl thermolysin suspension comprising 5 mg thermolysin (Sigma P1512) are employed.

Yield of AC-Tyr-Ile-Ala-NH_2 after 16 h:

228 mg (= 56 % of th.)

Example 10: Synthesis of AC-Tyr-Val-NH_2

Analogously to example 9a, instead of H-Ile-Ala-NH_2•HCl, 31.2 mg (0.2 mmol) H-Val-NH_2•HCl are employed.

Yield of AC-Tyr-Val-NH_2 after 18 h: 65 % of th.

^{1}H-NMR (D_6-DMSO, 300 MHz): δ=0.78-0.88 (m, 6 H, Val-$^{\gamma}CH_3$), δ=1.92-2.00 (m, 1 H, Val-$^{\beta}CH$-), δ=2.58-2.68 (m, 1 H, Tyr-$^{\beta}CH_2$-), δ=2.80-2.90 (m, 1 H, Tyr-$^{\beta}CH_2$-), δ=4.11 (dd, 1 H, Val-$^{\alpha}CH$-), δ=4.28-4.38 (m, 1 H, Tyr-$^{\alpha}CH$-), δ=5.57 (s, 2 H, AC-NH_2), δ=6.08 (d, 1 H, Tyr-$^{\alpha}NH$-), δ=6.62 (d, 2 H, Tyr-ArH), δ=6.97 (d, 2 H, Tyr-ArH), δ=7.03 (s, 1 H, Val-NH_2), δ=7.31 (s, 1 H, Val-NH_2), δ=7.67 (d, 1 H, Val-$^{\alpha}NH$-), δ=9.12 (s, 1 H, Tyr-OH)

Example 11: Synthesis of AC-Tyr-Met-NH_2

Analogously to example 9a, instead of H-Ile-Ala-NH_2•HCl, 37 mg (0.2 mmol) H-Met-NH_2•HCl are employed.

Yield of AC-Tyr-Met-NH_2 after 14 h: 75 % of th.

^{1}H-NMR (D_6-DMSO, 300 MHz); δ=1.75-1.85 (m, 1 H, Met-$^{\beta}CH_2$-), δ-1.88-2.00 (m, 1 H, Met-$^{\beta}CH_2$-), δ=2.02 (s, 3 H, Met-S-CH_3), δ=2.30-2.45 (m, 2 H, Met-$^{\beta}CH_2$-), δ=2.60-2.85 (m, 2 H, Tyr-$^{\beta}CH_2$-), δ=4.15-4.28 (m, 2 H, Tyr-$^{\alpha}CH$-, Met-$^{\alpha}CH$-), δ=5.65 (s, 2 H, AC-NH_2), δ=6.28 (d, 1 H, Tyr-$^{\alpha}NH$-), δ=6.63 (d, 2 H, Tyr-ArH), δ=6.97 (d, 2 H, Tyr-ArH), δ=7.04 (s, 1 H, Met-

NH_2), δ=7.25 (s, 1 H, Met-NH_2), δ=8.08 (d, 1 H, Met-$^{\alpha}$NH-), δ=8.54 (s, 1 H, Tyr-OH)

Example 12: Synthesis of AC-Tyr-Nvl-NH_2

Analogously to example 9a, instead of H-Ile-Ala-NH_2•HCl, 31.2 mg (0.2 mmol) H-Hvl-NH_2•HCl [sic] are employed.

Yield of AC-Tyr-Nvl-NH_2 after 14 h: 88 % of th.

^{1}H-NMR (D_6-DMSO, 300 MHz): δ=0.85 (t, 3 H, Nvl-$^{\delta}CH_3$), δ=1.18-1.32 (m, 2 H, Nvl-$^{\gamma}CH_2$-), δ=1.45-1.68 (m, 2 H, Nvl-$^{\beta}CH_2$-), δ=2.55-2.68 (m, 1 H, Tyr-$^{\beta}CH_2$-), δ=2.78-2.88 (m, 1 H, Tyr-$^{\beta}CH_2$-), δ=4.12-4.30 (m, 2 H, Tyr-$^{\alpha}$CH-, Nvl-$^{\alpha}$CH-), δ=5.59 (s, 2 H, AC-NH_2), δ=6.02 (d, 1 H, Tyr-$^{\alpha}$NH-), δ=6.62 (d, 2 H, Tyr-ArH), δ=6.94-6.99 (m, 3 H, 2 Tyr-ArH, 1 Nvl-NH_2), δ=7.21 (s, 1 H, Nvl-NH_2), δ=7.79 (d, 1 H, Met-$^{\alpha}$NH-), δ=9.12 (s, 1 H, Tyr-OH)

Example 13: Synthesis of AC-Tyr-Phe-NH_2

Analogously to example 9a, instead of H-Ile-Ala-NH_2•HCl, 40 mg (0.2 mmol) H-Phe-NH_2•HCl are employed.

Yield of AC-Tyr-Phe-NH_2 after 1 h: 86 % of th.
^{1}H-NMR (D_6-DMSO, 300 MHz): δ=2.72-2.88 (m, 3 H, -$^{\beta}CH_2$-), δ=1.98-3.06 (m, 1 H, -$^{\beta}CH_2$-), δ=4.12-4.20 (m, 1 H, -$^{\alpha}$CH-), δ=4.38-4.46 (m, 1 H, -$^{\alpha}$CH-), δ=5.59 (s, 2 H, AC-NH_2), δ=5.97 (d, 1 H, Tyr-$^{\alpha}$NH-), δ=6.61 (d, 2 H, Tyr-ArH), δ=6.92 (d, 2 H, Tyr-ArH), δ=7.08 (s, 1 H, Phe-NH_2), δ=7.15-7.28 (m, 5 H, Phe-C_6H_5), δ=7.32 (s, 1 H, Phe-NH_2), δ=7.92 (d, 1 H, Phe-$^{\alpha}$NH-), δ=9.12 (s, 1 H, Tyr-OH)

Example 14: Synthesis of AC-Tyr-Phe-OMe

Analogously to example 9a, instead of H-Ile-Ala-NH_2•HCl, 43 mg (0.2 mmol) H-Phe-OMe•HCl are employed. The amount of thermolysin used is 2 mg.

Yield of AC-Tyr-Phe-OMe after 14 h: 81 % of th.

^{1}H-NMR (D_6-DMSO, 300 MHz): δ=2.50-2.62 (m, 1 H, $-^{\beta}CH_2-$), δ=2.72-2.85 (m, 1 H, $-^{\beta}CH_2-$), δ=2.90-3.08 (m, 2 H, $-^{\beta}CH_2-$), δ=3.58 (s, 3 H, $-O-CH_3$), δ=4.25-4.35 (m, 1 H, $-^{\alpha}CH-$), δ=4.44-4.52 (m, 1 H, $-^{\alpha}CH-$), δ=5.52 (s, 2 H, AC-NH_2), δ=5.99 (d, 1 H, Tyr-$^{\alpha}NH-$), δ=6.62 (d, 2 H, Tyr-ArH), δ=6.94 (d, 2 H, Tyr-ArH), δ=7.15-7.30 (m, 5 H, Phe-C_6H_5), δ=8.36 (d, 1 H, Phe-$^{\alpha}NH-$), δ=9.14 (s, 1 H, Tyr-OH)

Example 15: Synthesis of AC-Tyr-Ile-NH_2

Analogously to example 9a, instead of H-Ile-Ala-NH_2•HCl, 33.2 mg (0.2 mmol) H-Ile-NH_2•HCl are employed.

Yield of AC-Tyr-Ile-NH_2 after 18 h: 68.9 % of th.

^{1}H-NMR (D_6-DMSO, 300 MHz): δ=0.76-0.86 (m, 6 H, Ile-$^{\beta}CH_3$, Ile-$^{\gamma}CH_3$), δ=0.99-1.12 (m, 1 H, Ile-$^{\gamma}CH_2-$), δ=1.35-1.46 (m, 1 H, Ile-$^{\gamma}CH_2-$), δ=1.62-1.76 (m, 1 H, Ile-$^{\beta}CH-$), δ=2.58-2.68 (m, 1 H, Tyr-$^{\beta}CH_2-$), δ=2.79-2.88 (m, 1 H, Tyr-$^{\beta}CH_2-$), δ=4.12 (dd, 1 H, Ile-$^{\alpha}CH-$), δ=4.26-4.37 (m, 1 H, Tyr-$^{\alpha}CH-$), δ=5.58 (s, 2 H, AC-NH_2), δ=6.06 (d, 1 H, Tyr-$^{\alpha}NH-$), δ=6.62 (d, 2 H, Tyr-ArH), δ=6.96 (d, 2 H, Tyr-ArH), δ=7.02 (s, 1 H, Ile-

NH_2), δ=7.31 (s, 1 H, Ile-NH_2), δ=7.68 (d, 1 H, Ile-$^{\alpha}$NH-), δ=9.12 (s, 1 H, Tyr-OH)

Example 16: Synthesis of AC-Tyr-Leu-NH_2

Analogously to example 9a, 224 mg (1.0 mmol) AC-Tyr-OH, 130 mg (1.0 mmol) H-Leu-NH_2 (Bachem Feinchemikalien AG), 225 µl buffer, no NaOH and 50 µl thermolysin suspension comprising 5 mg thermolysin (Sigma P1512) are employed.

Yield of AC-Tyr-Leu-NH_2 after 16 h:
127.8 mg (= 38 % of th.)

^{1}H-NMR (D_6-DMSO, 300 MHz): δ=0.80-0.92 (m, 6 H, Leu-$^{\delta}CH_3$), δ=1.42-1.62 (m, 3 H, Leu-$^{\gamma}$CH-, Leu-$^{\beta}CH_2$-), δ=2.58-2.70 (m, 1 H, Tyr-$^{\beta}CH_2$-), δ=2.79-2.88 (m, 1 H, Tyr-$^{\beta}CH_2$-), δ=4.16-4.30 (m, 2 H, Leu-$^{\alpha}$CH-, Tyr-$^{\alpha}$CH-), δ=5.60 (s, 2 H, AC-NH_2), δ=6.02 (d, 1 H, Tyr-$^{\alpha}$NH-), δ=6.63 (d, 2 H, Tyr-ArH), δ=6.93-7.00 (m, 2 H, Tyr-ArH, 1 H, Leu-NH_2), δ=7.19 (s, 1 H, Leu-NH_2), δ=7.83 (d, 1 H, Leu-$^{\alpha}$NH-), δ=9.15 (s, 1 H, Tyr-OH)

Example 17: Synthesis of AC-Tyr-Ile-Gly-OMe

Analogously to example 9a, instead of H-Ile-Ala-NH_2•HCl, 47.8 mg (0.2 mmol) H-Ile-Gly-OMe•HCl are employed.

Yield of AC-Tyr-Ile-Gly-OMe after 3 h: 89 % of th.

^{1}H-NMR;COSY(D_6-DMSO, 300 MHz): δ=0.76-0.88 (m, 6 H, Ile-$^{\delta}CH_3$, Ile-$^{\gamma}CH_3$), δ=1.00-1.14 (m, 1 H, Ile-$^{\gamma}CH_2$-), δ=1.36-1.52 (m, 1 H, Ile-$^{\gamma}CH_2$-), δ=1.65-1.78 (m, 1 H, Ile-$^{\beta}$CH-), δ=2.55-2.68 (m, 1 H, Tyr-$^{\beta}CH_2$-), δ=2.75-2.86 (m, 1 H, Tyr-$^{\beta}CH_2$-),

δ=3.62 (s, 3 H, -O-CH_3), δ=3.84 (t, 2 H, Gly-$^{\alpha}CH_2$-), δ=4.27-4.35 (m, 1 H, Ile-$^{\alpha}$CH-), δ=4.38-4.48 (m, 1 H, Tyr-$^{\alpha}$CH-), δ=5.57 (s, 2 H, AC-NH_2), δ=6.05 (d, 1 H, Tyr-$^{\alpha}$NH-), δ=6.61 (d, 2 H, Tyr-ArH), δ=6.95 (d, 2 H, Tyr-ArH), δ=7.79 (d, 1 H, Ile-$^{\alpha}$NH), δ=8.32 (t, 1 H, Gly-$^{\alpha}$NH-), δ=9.11 (s, 1 H, Tyr-OH)

Example 18: Synthesis of AC-Tyr-Val-OBzl

Analogously to example 16, instead of H-Leu-NH_2, 379.4 mg (1.0 mmol) H-Val-OBzl•pTos (Bachem Feinchemikalien AG), 175 µl buffer, 125 µl 10 M aqueous NaOH and 100 µl thermolysin suspension comprising 10 mg thermolysin (Sigma P1512) are employed.

Yield of AC-Tyr-Val-OBzl after 88 h:
230 mg (=55 % of th.)

^{1}H-NMR (D_6-DMSO, 300 MHz): δ=0.81-0.92 (m, 6 H, Val-$^{\gamma}CH_3$), δ=2.01-2.14 (m, 1 H, Val-$^{\beta}$CH-), δ=2.50-2.64 (m, 1 H, Tyr-$^{\beta}CH_2$-), δ=2.75-2.85 (m, 1 H, Tyr-$^{\beta}CH_2$-), δ=4.23 (dd, 1 H, Val-$^{\alpha}$CH-), δ=4.36-4.45 (m, 1 H, Tyr-$^{\alpha}$CH-), δ=5.13 (s, 2 H, -O-CH_2-), δ=5.52 (s, 2 H, AC-NH_2), δ=6.04 (d, 1 H, Tyr-$^{\alpha}$NH-), δ=6.62 (d, 2 H, Tyr-ArH), δ=6.96 (d, 2 H, Tyr-ArH), δ=7.30-7.40 (m, 5 H, -C_6H_5), δ=8.20 (d, 1 H, Val-$^{\alpha}$NH-), δ=9.13 (s, 1 H, Tyr-OH)

Example 19: Synthesis of AC-Tyr-Phe-Ala-OBzl

Analogously to example 9a, instead of H-Ile-Ala-NH_2•HCl, 88 mg (0.2 mmol) H-Phe-Ala-OBzl•TFA are employed.

Yield of AC-Tyr-Phe-Ala-OBzl after 3 h: 72 % of th.

^{1}H-NMR;COSY(D_6-DMSO, 300 MHz): δ=1.33 (d, 3 H, Ala-$^{\beta}CH_3$), δ=2.48-2.58 (m, 1 H, Tyr-$^{\beta}CH_2$-), δ=2.70-2.85 (m, 2 H, Tyr-$^{\beta}CH_2$-), δ=2.95-3.05 (m, 1 H, Phe-$^{\beta}CH_2$-), δ=4.14-4.24 (m, 1 H, Tyr-$^{\alpha}CH$-), δ=4.32-4.41 (m, 1 H, Ala-$^{\alpha}CH$-), δ=4.51-4.62 (m, 1 H, Phe-$^{\alpha}CH$-), δ=5.13 (s, 2 H, O-CH_2-), δ=5.56 (s, 2 H, AC-NH_2), δ=5.94 (d, 1 H, Tyr-$^{\alpha}NH$-), δ=6.60 (d, 2 H, Tyr-ArH), δ=6.90 (d, 2 H, Tyr-ArH), δ=7.14-7.28 (m, 5 H, OBzl-C_6H_5), δ=7.30-7.40 (m, 5 H, Phe-C_6H_5), δ=8.01 (d, 1 H, Phe-$^{\alpha}NH$), δ=8.46 (d, 1 H, Ala-$^{\alpha}NH$-), δ=9.12 (s, 1 H, Tyr-OH)

Example 20: Synthesis of AC-Met-Phe-NH_2

Analogously to example 9a, instead of AC-Tyr-OH, 76.8 mg (0.2 mmol) AC-Met-OH [sic] 80 mg (0.4 mmol) H-Phe-NH_2•HCl (Degussa AG), 90 µl buffer, 50 µl 10 M aqueous NaOH and 20 µl thermolysin suspension comprising 2 mg thermolysin (Sigma P1512) are employed.

Yield of AC-Met-Phe-NH_2 after 3 h: 96 % of th.

^{1}H-NMR (D_6-DMSO, 300 MHz); δ=1.58-1.80 (m, 2 H, Met-$^{\beta}CH_2$-), δ=1.99 (s, 3 H, Met-S-CH_3), δ=2.25-2.36 (m, 2 H, Met-$^{\gamma}CH_2$-), δ=2.76-2.88 (m, 1 H, Phe-$^{\beta}CH_2$-), δ=2.98-2.08 (m, 1 H, Phe-$^{\beta}CH_2$-), δ=3.98-4.08 (m, 1 H, -$^{\alpha}CH$-), δ=4.36-4.47 (m, 1 H, -$^{\alpha}CH$-), δ=5.63 (s, 2 H, AC-NH_2), δ=6.23 (d, 1 H, Met-$^{\alpha}NH$-), δ=7.09 (s, 1 H, Phe-NH_2), δ=7.12-7.28 (m, 5 H, Phe-C_6H_5), δ=7.42 (s, 1 H, Phe-NH_2), δ=7.96 (d, 1 H, Phe-$^{\alpha}NH$-)

Example 21: Synthesis of AC-Leu-Phe-NH_2

Analogously to example 20, instead of AC-Met-OH, 69.6 mg (0.4 mmol) AC-Leu-OH are employed.

Yield of AC-Leu-Phe-NH_2 after 70 h: 97 % of th.

^{1}H-NMR (D_6-DMSO, 300 MHz): δ=0.78-0.88 (m, 6 H, Leu-$^{\delta}CH_2$-), δ=1.16-1.35 (m, 2 H, Leu-$^{\beta}CH_2$-, Leu-$^{\gamma}CH$-), δ=1.45-1.56 (m, 1 H, Leu-$^{\beta}CH_2$-), δ=2.78-2.90 (m, 1 H, Phe-$^{\beta}CH_2$-), δ=2.99-3.08 (m, 1 H, Phe-$^{\beta}CH_2$-), δ=3.90-4.00 (m, 1 H, -$^{\alpha}CH$-), δ=4.36-4.45 (m, 1 H, -$^{\alpha}CH$-), δ=5.58 (s, 2 H, AC-NH_2), δ=6.08 (d, 1 H, Leu-$^{\alpha}NH$-), δ=7.06 (s, 1 H, Phe-NH_2), δ=7.12-7.28 (m, 5 H, Phe-C_6H_5), δ=7.34 (s, 1 H, Phe-NH_2), δ=7.82 (d, 1 H, Phe-$^{\alpha}NH$-)

Example 22

Removal of the carbamoyl protective group by splitting off (by the example of carbamoyl-aspartam as an example) by means of the enzyme technique

16 ml (65.6 U) carbamoylase and 50.6 mg thermolysin are initially introduced together into water and are stirred overnight (approx. 18 h). Carbamoyl-aspartam (470 mg) [sic], 1.25 mmol) is added. Total volume: ≈ 16 ml. The pH is between 6.5 and 7.0. After 24 h, carbamoyl-aspartam is no longer detectable. According to HPLC calibration, the conversion to aspartam is 98.3%.

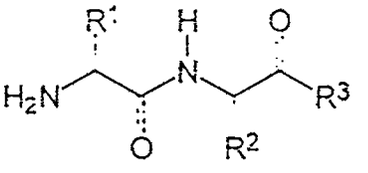
Example 23

Removal of the carbamoyl protective group by splitting off (by the example of carbamoyl-L-aspartam by way of example) by addition of sodium nitrite

4.72 g (12.6 mmol) carbamoyl-L-aspartam potassium salt are initially introduced into 60 ml H_2O / 40 ml HCl (conc., technical grade), the mixture is cooled to 5 °C (pH = 0.80) and 15.1 ml (15.1 mmol) $NaNO_2$ solution are slowly metered in (1 ml/10 min). The mixture is allowed to after-react for approx. 1 h, before the reaction mixture is neutralized with 50 wt.% sodium hydroxide solution. The reaction mixture is left overnight at RT, before the precipitate which has separated out is separated off and discarded. The mother liquor is then concentrated until aspartam precipitates out. The aspartam thus obtained is dried to constant weight in vacuo at 60 °C. The reaction proceeds quantitatively.

Claims

1. Process for the preparation of peptides of the general structure I,

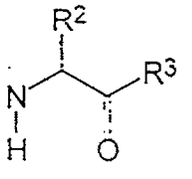


I

wherein

R^1 and R^2 independently of one another denote hydrogen, (C_1-C_6) alkyl, which can optionally be interrupted or substituted by heteroatoms, such as N, O or S, one [sic] or several times, it being possible for the heteroatoms in their turn to be substituted by hydrogen, (C_1-C_4) alkyl or benzyl or to be bonded to the alkyl group via a double bond, phenyl or benzyl, both of which can optionally be substituted by halogen or hydroxyl once or several times, heteroaralkyl, such as 3-indolylmethyl, 2-, 3- or 4-pyridylmethyl,

R^3 denotes (C_1-C_4) alkoxy, NH_2, hydroxyl, NR^1R^2, benzyloxy, which can optionally be substituted by halogen, nitro, NH_2, (C_1-C_4) alkyl, (C_1-C_4) alkoxy once or several times, or one or more units of the type II

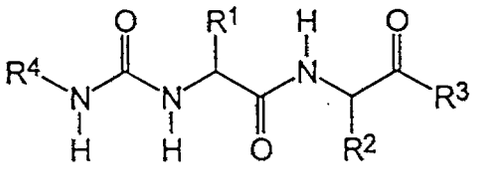


II

characterized in that

compounds of the type III or a salt form thereof,

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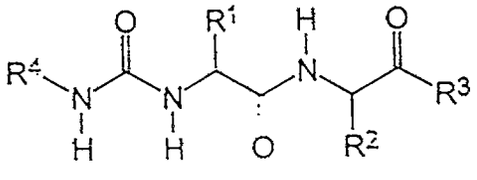
III

wherein R^1, R^2 and R^3 have the abovementioned meaning and

R^4 denotes hydrogen, (C_1-C_4) alkyl, phenyl, which can optionally be substituted by halogen, (C_1-C_4) alkyl, (C_1-C_4) alkoxy, nitro, CN, CF_3, (C_1-C_6) alkoxycarbonyl, COOH or $-NR^1R^2$ once or several times, aralkyl, such as benzyl, which can be substituted in its turn by halogen, (C_1-C_4) alkyl or (C_1-C_4) alkoxy, naphthyl, heteroaralkyl, such as 2-, 3- or 4-thienyl, 2-, 3- or 4-pyridyl or 2-quinolyl

are reacted with a carbamoylase, optionally in the presence of a solvent, or the carbamoyl protective group is split off chemically, optionally in the presence of a solvent and optionally in the presence of an acid.

2. Process for the preparation of peptides of the general structure III,



III

wherein

R^1 and R^2 independently of one another denote hydrogen, (C_1-C_6) alkyl, which can optionally be interrupted

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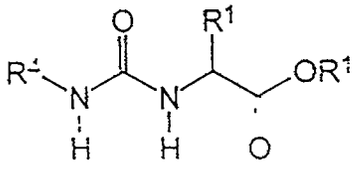
or substituted by heteroatoms, such as N, O or S, one [sic] or several times, it being possible for the heteroatoms in their turn to be substituted by hydrogen, (C_1-C_4) alkyl or benzyl or to be bonded to the alkyl group via a double bond, phenyl or benzyl, both of which can optionally be substituted by halogen or hydroxyl once or several times, heteroaralkyl, such as 3-indolylmethyl, 2-, 3- or 4-pyridylmethyl,

R^3 denotes (C_1-C_4) alkoxy, NH_2, hydroxyl, NR^1R^2, benzyloxy, which can optionally be substituted by halogen, nitro, NH_2, (C_1-C_4) alkyl, (C_1-C_4) alkoxy once or several times,

R^4 denotes hydrogen, (C_1-C_4) alkyl, phenyl, which can optionally be substituted by halogen, (C_1-C_4) alkyl, (C_1-C_4) alkoxy, nitro, CN, CF_3, (C_1-C_6) alkoxycarbonyl, COOH or $-NR^1R^2$ once or several times, aralkyl, such as benzyl, which can be substituted in its turn by halogen, (C_1-C_4) alkyl or (C_1-C_4) alkoxy, naphthyl, heteroaralkyl, such as 2-, 3- or 4-thienyl, 2-, 3- or 4-pyridyl or 2-quinolyl

characterized in that

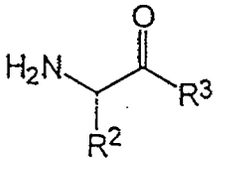
a compound of the type IV or a salt form of IV, in which R^1 and R^4 have the abovementioned meaning,



IV

is reacted with a compound of the type V or of [sic] an acid addition salt thereof, wherein R^2 and R^3 have the abovementioned meaning

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V

optionally in the presence of a solvent and optionally in the presence of a base.

3. Process according to claim 1, characterized in that, the reaction with carbamoylase is carried out in a temperature range between 10 °C and 50 °C, preferably 20 °C and 35 °C, especially preferably between 25 °C and 30 °C.

4. Process according to claim 1, characterized in that the reaction with carbamoylase is carried out in a pH range between 5 and 11, preferably between 6.5 and 8.

5. Process according to one of the preceding claims, characterized in that the carbamoylase originates from the microorganisms DSM 7329, DSM 7330 or DSM 9771.

6. Process according to one of the preceding claims, characterized in that the carbamoylase is employed optionally in a partly purified or purified, isolated or immobilized state.

7. Process according to claim 1, characterized in that

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the carbamoyl protective group is split off with an $NO^{\oplus}$ donor, preferably with $NaNO_2$ or $NO^{\oplus}BF_4^{\ominus}$.

8. Process according to claim 7, characterized in that the removal by splitting off is carried out in a temperature range between +120 °C and -30 °C, preferably between +60 °C and -20 °C, especially preferably between 25 °C and 0 °C.

9. Process according to claim 7, characterized in that the reaction is carried out with addition of an acid, in a pH range between -0.5 and 5, preferably between 0 and 2.

10. Process according to claim 3 and 7, characterized in that the reactions take place in an aqueous medium.

11. Process according to one of the preceding claims, characterized in that the peptide derivative cleaved is aspartam.

12. Process according to claim 2, characterized in that the peptide coupling is carried out in a highly dense suspension.

13. Process according to claim 12, characterized in that the substrate and product are present in partly solid form.

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14. Use of aspartam as a sweetener.

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