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(54) **Title:** PROCESS FOR THE SYNTHESIS OF AC-ARG-CYCLO(CYS-D-ALA-HIS-D-PHE-ARG-TRP-CYS)-NH₂

(57) **Abstract:** The present invention relates to a novel process for the synthesis of the melanocortin analog, Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂, using solution-phase peptide chemistry.

PROCESS FOR THE SYNTHESIS OF Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂BACKGROUND OF THE INVENTION

5 The present invention relates to a novel process for the synthesis of the melanocortin analog, Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂, using solution-phase peptide chemistry.

 Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂ is a highly potent and pharmacologically selective analog of melanocortin. Melanocortins are a family of regulatory peptides which are formed by post-translational processing of pro-hormone pro-opiomelanocortin (POMC; 131 amino acids in length). POMC is processed into three classes of hormones; the
10 (POMC; 131 amino acids in length). POMC is processed into three classes of hormones; the melanocortins, adrenocorticotropin hormone, and various endorphins (e.g. lipotropin) (Cone, et al., Recent Prog. Horm. Res., 51:287-317, (1996); Cone et al., Ann. N.Y. Acad. Sci., 31:342-363, (1993)).

 Melanocortins have been found in a wide variety of normal human tissues including the brain, adrenal, skin, testis, spleen, kidney, ovary, lung, thyroid, liver, colon, small intestine and pancreas
15 (Tatro, J. B. et al., Endocrinol. 121:1900-1907 (1987); Mountjoy, K. G. et al., Science 257:1248-1251 (1992); Chhajlani, V. et al., FEBS Lett. 309:417-420 (1992); Gantz, I. et al. J. Biol. Chem. 268:8246-8250 (1993) and Gantz, I. et al., J. Biol. Chem. 268:15174-15179 (1993)).

 Melanocortin peptides have been shown to exhibit a wide variety of physiological activities including the control of behavior and memory, affecting neurotrophic and antipyretic properties, as
20 well as affecting the modulation of the immune system. Aside from their well known effects on adrenal cortical functions (adrenocorticotropin hormone or "ACTH") and on melanocytes (melanocyte stimulating hormone or "MSH"), melanocortins have also been shown to control the cardiovascular system, analgesia, thermoregulation and the release of other neurohumoral agents including prolactin, luteinizing hormone and biogenic amines (De Wied, D. et al., Methods Achiev. Exp. Pathol. 15:167-
25 199 (1991); De Wied, D. et al., Physiol. Rev. 62:977-1059 (1982); Guber, K.A. et al., Am. J. Physiol. 257:R681-R694 (1989); Walker J.M. et al., Science 210:1247-1249 (1980); Murphy, M. T. et al., Science 221:192-193 (1983); Ellerkmann, E. et al., Endocrinol. 130:133-138 (1992) and Versteeg, D. H. G. et al., Life Sci. 38:835-840 (1986)).

 It has also been shown that binding sites for melanocortins are distributed in many different
30 tissue types including lachrymal and submandibular glands, pancreas, adipose, bladder, duodenum, spleen, brain and gonadal tissues as well as malignant melanoma tumors. Five melanocortin receptors have been characterized to date. These include melanocyte-specific receptor (MC1-R), corticoadrenal-specific ACTH receptor (MC2-R), melacortin-3 (MC3-R), melanocortin-4 (MC4-R) and melanocortin-5 receptor (MC5-R). All of the melanocortin receptors respond to the peptide
35 hormone class of melanocyte stimulating hormones (MSH) (Cone, R. D. et al., Ann. N.Y. Acad. Sci., 680:342-363 (1993); Cone, R. D. et al., Recent Prog. Horm. Res., 51:287-318 (1996)).

MC1-R, known in the art as Melanocyte Stimulating Hormone Receptor (MSH-R), Melanotropin Receptor or Melanocortin-1 Receptor, is a 315 amino acid transmembrane protein belonging to the family of G-Protein coupled receptors. MC1-R is a receptor for both MSH and ACTH. The activity of MC1-R is mediated by G-proteins which activate adenylate cyclase. MC1-R receptors are found in melanocytes and corticoadrenal tissue as well as various other tissues such as adrenal gland, leukocytes, lung, lymph node, ovary, testis, pituitary, placenta, spleen and uterus. MC2-R, also called Adrenocorticotrophic Hormone Receptor (ACTH-R), is a 297 amino acid transmembrane protein found in melanocytes and the corticoadrenal tissue. MC2-R mediates the corticotrophic effect of ACTH. In humans, MC3-R is a 360 AA protein found in brain tissue; in mice and rats MC3-R is a 323 AA protein. MC4-R is a 332 amino acid transmembrane protein which is also expressed in brain as well as placental and gut tissues. MC5-R is a 325 amino acid transmembrane protein expressed in the adrenals, stomach, lung and spleen and very low levels in the brain. MC5-R is also expressed in the three layers of adrenal cortex, predominantly in the aldosterone-producing zona glomerulosa cells.

The five known melanocortin receptors differ, however, in their functions. For example, MC1-R is a G-protein coupled receptor that regulates pigmentation in response to α -MSH, a potent agonist of MC1-R. Agonism of the MC1-R receptor results in stimulation of the melanocytes which causes eumelanin and increases the risk for cancer of the skin. Agonism of MC1-R can also have neurological effects. Stimulation of MC2-R activity can result in carcinoma of adrenal tissue. Recent pharmacological confirmation has established that central MC4-R receptors are the prime mediators of the anorexic and orexigenic effects reported for melanocortin agonists and antagonists, respectively. The effects of agonism of the MC3-R and MC5-R are not yet known.

There has been great interest in melanocortin receptors as targets for the design of novel therapeutics to treat disorders of body weight such as obesity and cachexia. Both genetic and pharmacological evidence points toward central MC4-R receptors as the principal target (Giraudou, S. Q. et al., *Brain Res.*, 809:302-306 (1998); Farooqi, I. S. et al., *NE J Med.*, 348:1085-1095 (2003); MacNeil, D. J. et al., *Eu. J. Pharm.*, 44:141-157 (2002); MacNeil, D. J. et al., *Eu. J. Pharm.*, 450:93-109 (2002); Kask, A. et al., *NeuroReport*, 10:707-711 (1999)). The current progress with receptor-selective agonists and antagonists evidences the therapeutic potential of melanocortin receptor activation, particularly MC4-R.

The solution-phase synthesis described in U.S. Patent No. 4,395,403 uses BTFA/TFA to remove the methoxybenzyl group protecting the thiol group of cysteine followed by cyclization. Decomposition of tryptophan residue, however, is known to frequently occur during such harsh acid treatment for removal of protecting groups. As such, there is a need for developing an efficient method for producing Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂.

SUMMARY OF THE INVENTION

In a first aspect of the present invention, there is provided a novel process for the synthesis of Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂, which comprises a fragment condensation
5 procedure, wherein protected amino acids, such as Boc protected amino acids, benzoyloxycarbonyl protected amino acids, Fmoc protected amino acids, and protected amino acid fluorides, such as Fmoc amino acid fluoride or Bsmoc amino acid fluoride, are used, wherein a mixed anhydride coupling method is employed, and wherein a protected peptide fragment Trp-Cys or Arg-Trp, such as Boc-Trp-Cys(Acm)-OMe or Boc-Trp(For)-Cys(Acm)-OMe, is provided.

10 In a preferred embodiment of the first aspect of the present invention, a peptide-hydrazide coupling method is employed, wherein ammonia is used to convert an ester functional group to an amide functional group.

The first aspect of the present invention may comprise the steps of:

- (a) synthesizing a fragment benzoyloxycarbonyl-D-Ala-His-OH from
15 benzoyloxycarbonyl-D-Ala-OH and H-His-OH in the presence of a coupling reagent;
or alternatively, synthesizing a fragment benzoyloxycarbonyl-D-Ala-His(Trt)-OH from benzoyloxycarbonyl-D-Ala-OH and H-His(Trt)-OH in the presence of a coupling reagent;
- (b-1) synthesizing a fragment benzoyloxycarbonyl-D-Phe-Arg(Pbf)-OMe from benzoyloxycarbonyl-D-Phe-OH and H-Arg(Pbf)-OMe in the presence of a coupling reagent;
- 20 (b-2) synthesizing a fragment H-D-Phe-Arg(Pbf)-OMe by hydrogenating the fragment benzoyloxycarbonyl-D-Phe-Arg(Pbf)-OMe obtained in the step (b-1);
- (c-1) synthesizing a fragment benzoyloxycarbonyl-D-Ala-His-D-Phe-Arg(Pbf)-OMe from benzoyloxycarbonyl-D-Ala-His-OH and the fragment H-D-Phe-Arg(Pbf)-OMe obtained in the step (b-2) in the presence of a coupling reagent;
- 25 or alternatively, synthesizing a fragment benzoyloxycarbonyl-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-OMe from benzoyloxycarbonyl-D-Ala-His(Trt)-OH and the fragment H-D-Phe-Arg(Pbf)-OMe obtained in the step (b-2) in the presence of a coupling reagent;
- (c-2) synthesizing a fragment H-D-Ala-His-D-Phe-Arg(Pbf)-OMe by hydrogenating the fragment benzoyloxycarbonyl-D-Ala-His-D-Phe-Arg(Pbf)-OMe obtained in the step (c-1);
- 30 or alternatively, synthesizing a fragment H-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-OMe by hydrogenating the fragment benzoyloxycarbonyl-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-OMe obtained in the step (c-1);
- (d-1) synthesizing a fragment Fmoc-Arg(Pbf)-Cys(Acm)-OH from Fmoc-Arg(Pbf)-OH and H-Cys(Acm)-OBzl in the presence of a coupling reagent, followed by hydrogenation;

(d-2) synthesizing a fragment H-Arg(Pbf)-Cys(Acm)-OH from the fragment Fmoc-Arg(Pbf)-Cys(Acm)-OH obtained in the step (d-1) in the presence of a base;

(d-3) synthesizing a fragment Ac-Arg(Pbf)-Cys(Acm)-OH from the fragment H-Arg(Pbf)-Cys(Acm)-OH obtained in the step (d-2);

5 or alternatively, synthesizing a fragment Ac-Arg(Pbf)-Cys(Acm)-OH from Ac-Arg(Pbf)-OH and H-Cys(Acm)-OMe in the presence of a coupling reagent, followed by hydrolysis by using a base;

(e-1) synthesizing a fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-OMe from Ac-Arg(Pbf)-Cys(Acm)-OH and H-D-Ala-His-D-Phe-Arg(Pbf)-OMe in the presence of a coupling reagent;

10 or alternatively, synthesizing a fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-OMe from Ac-Arg(Pbf)-Cys(Acm)-OH and H-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-OMe in the presence of a coupling reagent;

(e-2) synthesizing a fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-OH by hydrolyzing the fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-OMe obtained in the step (e-1) in the presence of a base;

15 or alternatively, synthesizing a fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-OH by hydrolyzing the fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-OMe obtained in the step (e-1) in the presence of a base;

(f) synthesizing a fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-NHNH₂ from the fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-OMe obtained in the step (e-1) in the presence of hydrazine;

20 or alternatively, synthesizing a fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-NHNH₂ from the fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-OMe obtained in the step (e-1) in the presence of hydrazine;

(g-1) synthesizing a fragment Boc-Trp-Cys(Acm)-OMe from Boc-Trp-OH and H-Cys(Acm)-OMe in the presence of a coupling reagent;

25 or alternatively, synthesizing a fragment Boc-Trp(For)-Cys(Acm)-OMe from Boc-Trp(For)-OH and H-Cys(Acm)-OMe in the presence of a coupling reagent;

(g-2) synthesizing H-Trp-Cys(Acm)-OMe from the fragment Boc-Trp-Cys(Acm)-OMe obtained in the step (g-1) in the presence of TFA;

30 or alternatively, synthesizing H-Trp(For)-Cys(Acm)-OMe from the fragment Boc-Trp(For)-Cys(Acm)-OMe obtained in the step (g-1) in the presence of TFA;

(h) synthesizing Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp(For)-Cys(Acm)-OMe from the fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-NHNH₂ obtained in the step (f) and H-Trp(For)-Cys(Acm)-OMe by using an acid and *tert*-butylnitrite;

5 or alternatively, synthesizing Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp(For)-Cys(Acm)-OMe from the fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-NHNH₂ obtained in the step (f) and H-Trp(For)-Cys(Acm)-OMe by using an acid and *tert*-butylnitrite;

10 or alternatively, synthesizing Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp-Cys(Acm)-OMe from the fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-NHNH₂ obtained in the step (f) and H-Trp-Cys(Acm)-OMe by using an acid and *tert*-butylnitrite;

or alternatively, synthesizing Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp-Cys(Acm)-OMe from the fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-NHNH₂ obtained in the step (f) and H-Trp-Cys(Acm)-OMe by using an acid and *tert*-butylnitrite;

15 or alternatively, synthesizing Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp-Cys(Acm)-OMe from Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-OH and H-Trp-Cys(Acm)-OMe in the presence of a coupling reagent;

or alternatively, synthesizing Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp(For)-Cys(Acm)-OMe from Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-OH and H-Trp(For)-Cys(Acm)-OMe in the presence of a coupling reagent;

20 or alternatively, synthesizing Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp(For)-Cys(Acm)-OMe from Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-OH and H-Trp(For)-Cys(Acm)-OMe in the presence of a coupling reagent;

25 or alternatively, synthesizing Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp-Cys(Acm)-OMe from Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-OH and H-Trp-Cys(Acm)-OMe in the presence of a coupling reagent;

or alternatively, synthesizing Ac-Arg-Cys(Acm)-D-Ala-His-D-Phe-Arg-Trp-Cys(Acm)-OMe from Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp-Cys(Acm)-OMe in the presence of TFA;

30 or alternatively, synthesizing Ac-Arg-Cys(Acm)-D-Ala-His-D-Phe-Arg-Trp-Cys(Acm)-OMe from Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp-Cys(Acm)-OMe in the presence of TFA;

or alternatively, synthesizing Ac-Arg-Cys(Acm)-D-Ala-His-D-Phe-Arg-Trp(For)-Cys(Acm)-OMe from Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp(For)-Cys(Acm)-OMe in the presence of TFA;

35 or alternatively, synthesizing Ac-Arg-Cys(Acm)-D-Ala-His-D-Phe-Arg-Trp(For)-Cys(Acm)-OMe from Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp(For)-Cys(Acm)-OMe in the presence of TFA;

(i) synthesizing Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp-Cys(Acm)-NH₂ from Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp-Cys(Acm)-OMe obtained in the step (h) in the presence of ammonia;

5 or alternatively, synthesizing Ac-Arg-Cys(Acm)-D-Ala-His-D-Phe-Arg-Trp-Cys(Acm)-NH₂ from Ac-Arg-Cys(Acm)-D-Ala-His-D-Phe-Arg-Trp-Cys(Acm)-OMe obtained in the step (h) in the presence of ammonia;

or alternatively, synthesizing Ac-Arg-Cys(Acm)-D-Ala-His-D-Phe-Arg-Trp-Cys(Acm)-NH₂ from Ac-Arg-Cys(Acm)-D-Ala-His-D-Phe-Arg-Trp(For)-Cys(Acm)-OMe obtained in the step (h) in the presence of ammonia;

10 or alternatively, synthesizing Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp-Cys(Acm)-NH₂ from Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp-Cys(Acm)-OMe obtained in the step (h) in the presence of ammonia;

15 or alternatively, synthesizing Ac-Arg-Cys(Acm)-D-Ala-His-D-Phe-Arg-Trp-Cys(Acm)-NH₂ from Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp-Cys(Acm)-NH₂ in the presence of TFA;

or alternatively, synthesizing Ac-Arg-Cys(Acm)-D-Ala-His-D-Phe-Arg-Trp-Cys(Acm)-NH₂ from Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp-Cys(Acm)-NH₂ in the presence of TFA; and

20 (j) synthesizing Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂ from Ac-Arg-Cys(Acm)-D-Ala-His-D-Phe-Arg-Trp-Cys(Acm)-NH₂ by using an oxidizing agent.

In the above-described aspect of the present invention, said oxidizing agent preferably is iodine, said coupling agent preferably is DCC, HBTU, HATU, DIC, EDC, or chloroformic acid isobutyl ester, and said base preferably is Et₂NH, TAEA, piperazine, sodium hydroxide, or potassium hydroxide.

25 The synthetic sequences summarized above are schematically diagrammed in FIG. 1A.

FIG. 1B shows another schematic diagram of the synthetic sequences summarized above.

In a second aspect of the present invention, there is provided a novel process for the synthesis of Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂ which is similar to the first aspect described above, except a protected peptide fragment Cys-D-Ala or Arg-Cys is provided.

30 In a preferred embodiment of the second aspect of the present invention, said protected peptide fragment Cys-D-Ala is Boc-Cys(Acm)-D-Ala-OH.

The second aspect of the present invention may comprise the steps of:

(a-1) synthesizing a fragment Boc-Cys(Acm)-D-Ala-OH from Boc-Cys(Acm)-OH and H-D-Ala-OH in the presence of a coupling reagent;

(a-2) synthesizing a fragment H-Cys(Acm)-D-Ala-OH from the fragment Boc-Cys(Acm)-D-Ala-OH obtained in the step (a-1) in the presence of TFA;

(b) synthesizing a fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-OH from Ac-Arg(Pbf)-OH and the fragment H-Cys(Acm)-D-Ala-OH obtained in the step (a-2) in the presence of a coupling reagent;

(c-1) synthesizing a fragment Boc-His-D-Phe-OMe from Boc-His-OH and H-D-Phe-OMe in the presence of a coupling reagent;

(c-2) synthesizing a fragment H-His-D-Phe-OMe from the fragment Boc-His-D-Phe-OMe obtained in the step (c-1) in the presence of TFA;

(d-1) synthesizing a fragment Boc-Trp-Cys(Acm)-OMe from Boc-Trp-OH and H-Cys(Acm)-OMe in the presence of a coupling reagent;

or alternatively, synthesizing a fragment Boc-Trp(For)-Cys(Acm)-OMe from Boc-Trp(For)-OH and H-Cys(Acm)-OMe in the presence of a coupling reagent;

(d-2) synthesizing H-Trp-Cys(Acm)-OMe from the fragment Boc-Trp-Cys(Acm)-OMe obtained in the step (d-1) in the presence of TFA;

or alternatively, synthesizing H-Trp(For)-Cys(Acm)-OMe from the fragment Boc-Trp(For)-Cys(Acm)-OMe obtained in the step (d-1) in the presence of TFA;

(e-1) synthesizing a fragment benzoyloxycarbonyl-Arg(Pbf)-Trp-Cys(Acm)-OMe from benzoyloxycarbonyl-Arg(Pbf)-OH and the fragment H-Trp-Cys(Acm)-OMe obtained in the step (d-2) in the presence of a coupling reagent;

or alternatively, synthesizing a fragment benzoyloxycarbonyl-Arg(Pbf)-Trp(For)-Cys(Acm)-OMe from benzoyloxycarbonyl-Arg(Pbf)-OH and the fragment H-Trp(For)-Cys(Acm)-OMe obtained in the step (d-2) in the presence of a coupling reagent;

(e-2) synthesizing a fragment H-Arg(Pbf)-Trp-Cys(Acm)-OMe by hydrogenating the fragment benzoyloxycarbonyl-Arg(Pbf)-Trp-Cys(Acm)-OMe obtained in the step (e-1);

or alternatively, synthesizing a fragment H-Arg(Pbf)-Trp(For)-Cys(Acm)-OMe by hydrogenating the fragment benzoyloxycarbonyl-Arg(Pbf)-Trp(For)-Cys(Acm)-OMe obtained in the step (e-1);

(f-1) synthesizing a fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-OMe from the fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-OH obtained in the step (b) and the fragment H-His-D-Phe-OMe obtained in the step (c-2) in the presence of a coupling reagent;

(f-2) synthesizing a fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-NHNH₂ from the fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-OMe obtained in the step (f-1) in the presence of hydrazine;

or alternatively, synthesizing a fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-OH by hydrolyzing the fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-OMe obtained in the step (f-1) in the presence of a base;

(g) synthesizing Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp-Cys(Acm)-OMe from the fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-NHNH₂ obtained in the step (f-2) and the fragment H-Arg(Pbf)-Trp-Cys(Acm)-OMe obtained in the step (e-2) using an acid and *tert*-butylnitrite;

or alternatively, synthesizing Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp(For)-Cys(Acm)-OMe from the fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-NHNH₂ obtained in the step (f-2) and the fragment H-Arg(Pbf)-Trp(For)-Cys(Acm)-OMe obtained in the step (e-2) using an acid and *tert*-butylnitrite;

or alternatively, synthesizing Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp-Cys(Acm)-OMe from the fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-OH obtained in the step (f-2) and the fragment H-Arg(Pbf)-Trp-Cys(Acm)-OMe obtained in the step (e-2) in the presence of a coupling agent;

or alternatively, synthesizing Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp(For)-Cys(Acm)-OMe from the fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-OH obtained in the step (f-2) and the fragment H-Arg(Pbf)-Trp(For)-Cys(Acm)-OMe obtained in the step (e-2) in the presence of a coupling agent;

(h) synthesizing Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp-Cys(Acm)-NH₂ from Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp-Cys(Acm)-OMe obtained in the step (g) in the presence of ammonia;

or alternatively, synthesizing Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp(For)-Cys(Acm)-NH₂ from Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp(For)-Cys(Acm)-OMe obtained in the step (g) in the presence of ammonia;

or alternatively, synthesizing Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp-Cys(Acm)-NH₂ from Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp-Cys(Acm)-OMe obtained in the step (g) in the presence of ammonia;

or alternatively, synthesizing Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp(For)-Cys(Acm)-NH₂ from Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp(For)-Cys(Acm)-OMe obtained in the step (g) in the presence of ammonia;

(i) synthesizing Ac-Arg-Cys(Acm)-D-Ala-His-D-Phe-Arg-Trp-Cys(Acm)-NH₂ from Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp-Cys(Acm)-NH₂ obtained in the step (h) in the presence of TFA; and

(j) synthesizing Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂ from Ac-Arg-Cys(Acm)-D-Ala-His-D-Phe-Arg-Trp-Cys(Acm)-NH₂ obtained in the step (i) by using an oxidizing agent.

FIG. 2 is a schematic diagram of the synthetic sequences summarized immediately above.

5 FIGS. 3-11 are schematic diagrams of various synthetic sequences employing fragment condensation steps and different peptide fragments which all result in Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂.

In a third aspect of the present invention, there is provided a novel process for the synthesis of Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂ which comprises a linear stepwise synthetic
10 procedure.

In a preferred embodiment of the third aspect of the present invention, protected amino acids, such as Boc protected amino acids, Fmoc protected amino acids, and protected amino acid fluorides, such as Fmoc amino acid fluoride or Bsmoc amino acid fluoride, are used, dimethylcyclopropylmethyl amine is used at the C-terminus of a protected peptide chain, and Fmoc-
15 Cys(Trt)-NH-CMe₂CP is used.

The second aspect of the present invention may comprise the steps of:

(a) synthesizing H-Cys(Trt)-NH-CMe₂CP from Fmoc-Cys(Trt)-NH-CMe₂CP in the presence of a base;

(b) synthesizing Fmoc-Trp(Boc)-Cys(Trt)-NH-CMe₂CP from Fmoc-Trp(Boc)-OH and
20 H-Cys(Trt)-NH-CMe₂CP obtained in the step (a) in the presence of a coupling reagent;

(c) synthesizing H-Trp(Boc)-Cys(Trt)-NH-CMe₂CP from Fmoc-Trp(Boc)-Cys(Trt)-NH-CMe₂CP obtained in the step (b) in the presence of a base;

(d) synthesizing Fmoc-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP from Fmoc-Arg(Pbf)-OH and H-Trp(Boc)-Cys(Trt)-NH-CMe₂CP obtained in the step (c) in the presence of a coupling
25 reagent;

(e) synthesizing H-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP from Fmoc-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP obtained in the step (d) in the presence of a base;

(f) synthesizing Fmoc-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP from Fmoc-D-Phe-OH and H-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP obtained in the step (e) in the presence of a
30 coupling reagent;

(g) synthesizing H-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP from Fmoc-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP obtained in the step (f) in the presence of a base;

(h) synthesizing Fmoc-His(Trt)-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP from Fmoc-His(Trt)-OH and H-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP obtained in the step (g) in the presence of a coupling reagent;

5 (i) synthesizing H-His(Trt)-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP from Fmoc-His(Trt)-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP obtained in the step (h) in the presence of a base;

(j) synthesizing Fmoc-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP from Fmoc-D-Ala-OH and H-His(Trt)-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP obtained in the step (i) in the presence of a coupling reagent;

10 (k) synthesizing H-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP from Fmoc-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP obtained in the step (j) in the presence of a base;

(l) synthesizing Fmoc-Cys(Trt)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP from Fmoc-Cys(Trt)-OH and H-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP obtained in the step (k) in the presence of a coupling reagent;

15 (m) synthesizing H-Cys(Trt)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP from Fmoc-Cys(Trt)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP obtained in the step (l) in the presence of a base;

20 (n) synthesizing Fmoc-Arg(Pbf)-Cys(Trt)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP from Fmoc-Arg(Pbf)-OH and H-Cys(Trt)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP obtained in the step (m) in the presence of a coupling reagent;

(o) synthesizing H-Arg(Pbf)-Cys(Trt)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP from Fmoc-Arg(Pbf)-Cys(Trt)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP obtained in the step (n) in the presence of a base;

25 (p) synthesizing Ac-Arg(Pbf)-Cys(Trt)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP from H-Arg(Pbf)-Cys(Trt)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP obtained in the step (o) and Ac₂O, AcCl or AcBr;

or alternatively, synthesizing Ac-Arg(Pbf)-Cys(Trt)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP from Ac-Arg(Pbf)-OH and H-Cys(Trt)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP obtained in the step (m) in the presence of a coupling reagent;

30 (q) synthesizing Ac-Arg-Cys-D-Ala-His-D-Phe-Arg-Trp-Cys-NH₂ from Ac-Arg(Pbf)-Cys(Trt)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP obtained in the step (p) in the presence of TFA; and

(r) synthesizing Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂ from Ac-Arg-Cys-D-Ala-His-D-Phe-Arg-Trp-Cys-NH₂ obtained in the step (q) in the presence of an oxidizing agent.

In the third aspect of the present invention, said oxidizing agent preferably is iodine, oxygen, air, or DMSO; said coupling agent preferably is DCC, HBTU, HATU, DIC, EDC, or chloroformic acid isobutyl ester, and said base preferably is Et₂NH, TAEA, or piperazine.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A is a schematic diagram of the first aspect of the present invention as summarized above.

FIG. 1B shows another schematic diagram of the first aspect of the present invention as summarized above.

FIG. 2 is a schematic diagram of the second aspect of the present invention as summarized above.

FIGS. 3-11 are schematic diagrams of various synthetic sequences employing fragment condensation steps and different peptide fragments, which all result in Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂, and which are within the scope of the present invention as claimed herein.

DETAILED DESCRIPTION OF THE INVENTION

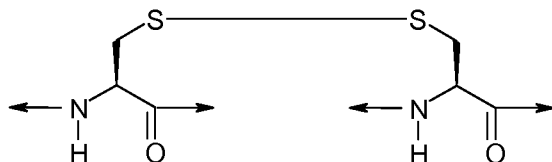
The application employs the following abbreviations:

Ac:	acetyl
20 Acm:	acetamidomethyl
AcOH:	acetic acid
Ala or A:	alanine
Arg or R:	arginine
Boc:	<i>tert</i> -butyloxycarbonyl
25 Bsmoc:	1,1-dioxobenzo[<i>b</i>]thiophene-2-ylmethyloxycarbonyl
BTFA:	boron-tris-trifluoroacetate
Bzl:	benzyl
Cys or C :	cysteine
DCCI:	<i>N,N'</i> -dicyclohexylcarbodiimide
30 DIC:	<i>N,N'</i> -diisopropylcarbodiimide
DMF:	dimethylformamide
EDC:	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
Fmoc:	9-Fluorenylmethyloxycarbonyl

	For:	formyl
	HATU:	O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
	HBTU:	2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
	His or H:	histidine
5	HOBt	1-hydroxybenzotriazole
	HPLC:	high performance liquid chromatography
	Me:	methyl
	MeOH:	methanol
	Mtr:	4-methoxy-2,3,6-trimethylbenzenesulfonyl
10	Mtt:	methyltrityl
	NH ₂ -CMe ₂ CP:	dimethylcyclopropylmethyl amine
	Pbf:	2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl
	Pmc:	2,2,5,7,8-pentamethylchroman-6-sulfonyl
	TAEA:	tris(2-aminoethyl)amine
15	TFA	trifluoroacetic acid
	THF:	tetrahydrofuran
	Trp or W:	tryptophan
	Trt:	trityl
	Z or Cbz:	benzyloxycarbonyl

20 The designation “NH₂” in Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂ indicates that the C-terminus of the peptide is amidated.

The designation “-cyclo(Cys-Cys)-” indicates the structure:



25 The term “about” as used herein, in associations with parameters and amounts, means that the parameter or amount is within $\pm 5\%$ of the stated parameter or amount.

Synthesis

- Synthesis of Example 1, i.e., Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂
 - Step 1: Preparation of Z-D-Ala-His-OH

30 30 mmol of Z-D-Ala-OH in 150 mL of acetonitrile was cooled to -18°C . To the cooled solution, 3.4 mL of N-methyl-morpholine followed by 4.1 mL of chloroformic acid isobutyl ester were added. The solution was stirred for 15 minutes at -15°C and a cold solution of 30.3 mmol of H-

His-OH in 30 mL of 1N NaOH and 100 mL of acetonitrile was added. The resulting solution was stirred for additional 18 hours at a temperature of -5°C to 0°C. The reaction mixture was concentrated under vacuum, diluted with water, and extracted 3 times with a small quantity of ether. The aqueous phase was adjusted to about pH 2 by the addition of 4N H₂SO₄. A precipitate formed which was
5 extracted with ether/acetic acid. The organic phase was washed with water and dried over Na₂SO₄. After evaporation under vacuum, the residue was crystallized from ether/petrolether to yield the title compound.

○ Step 2: Preparation of Z-D-Phe-Arg(Pbf)-OMe

13.5 mL of chloroformic acid isobutyl ester was added, with stirring, to 147 mmol of Z-
10 DPhe-OH dissolved in 1200 mL of THF which had been pre-cooled to -20°C. Stirring was continued for additional 20 minutes at -15° C and a cold solution (-21°C) of 158 mmol of HCl·H-Arg(Pbf)-OMe in 600 mL of THF was added. 17 mL of triethylamine was added dropwise and the temperature of the reaction vessel was maintained at a temperature of -15° C. The reaction mixture was stirred for 18
15 hours at 0°C and the product was subjected to concentration under vacuum. Dilution was effected with ether/ethylacetate (1:1) and filtration was performed. The filtrate was washed with 2 N citric acid, 10% KHCO₃, and water. The organic phase was dried over Na₂SO₄ and evaporated to dryness to yield the title compound.

○ Step 3: Preparation of Z-D-Ala-His-D-Phe-Arg(Pbf)-OMe

131 mmol of Z-D-Phe-Arg(Pbf)-OMe in 150 mL of MeOH was hydrogenated in the presence
20 of Pd/C. The product was filtered and washed with MeOH. The filtrate was evaporated under vacuum. The residue that formed was dissolved together with 99 mmol of Z-D-Ala-His-OH and 2.0 g HOBt in 50 mL of DMF. The solution was cooled to -15° C. 2.1 g of DCCI in 15 mL of DMF was added with stirring. The obtained solution was stirred for additional 2 days at 0°C, evaporated under vacuum, diluted with acetic acid/ether (1:1), and filtered. The filtrate was washed with 2 N citric acid,
25 10% KHCO₃ and water. The organic phase was dried over Na₂SO₄ and evaporated. The residue was purified by chromatography over silica gel with CH₂Cl₂/MeOH as an eluant. The fractions containing the desired product were collected and evaporated under vacuum to yield the title compound.

○ Step 4: Preparation of H-D-Ala-His-D-Phe-Arg(Pbf)-OMe

1.3 mmol of Z-D-Ala-His-D-Phe-Arg(Pbf)-OMe in 50 mL of MeOH was hydrogenated in the
30 presence of 10% Pd/C and the solution was filtered and evaporated under vacuum to yield the title compound.

○ Step 5: Preparation of Ac-Arg(Pbf)-Cys(Acm)-OH

8.4 mL of NEt₃, followed by 8.3 mL of chloroformic acid isobutyl ester, were added to 60 mmol of Ac-Arg(Pbf)-OH in 300 mL of THF that was pre-cooled to -20°C. The obtained mixture

was stirred for 10 minutes at -15°C. A cold solution of H-Cys(Acm)-OH and 11.5 mL of NET_3 in 400 mL of THF/water (5:1) was subsequently added dropwise. The reaction mixture was stirred for about 2 days at 0°C, concentrated under vacuum, diluted with 1.3 L of water, and extracted with ether. The aqueous phase was adjusted to pH 2.0 by the addition of 4 N H_2SO_4 , and the precipitate was extracted with ethyl acetate. The ethyl acetate extract was washed with water, dried over Na_2SO_4 and evaporated under vacuum. The residue was purified by chromatography over silica gel with ether/1% AcOH as an eluant. The fractions containing the desired product were combined and evaporated under vacuum to yield the title compound.

○ Step 6: Preparation of Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-OMe

0.30 g of DCCI was added with stirring to 1.42 mmol of Ac-Arg(Pbf)-Cys(Acm)-OH, 1.3 mmol of H-D-Ala-His-D-Phe-Arg(Pbf)-OMe and 0.5 g of HOBt dissolved in 30 mL of DMF pre-cooled to -20°C. The reaction mixture was stirred for about 12 hours at a constant temperature of -5°C to 0°C and then for about 4 hours at room temperature. The precipitated dicyclohexyl urea was filtered off and the filtrate was washed with 2 N citric acid, 10% KHCO_3 and water. The organic phase was dried over Na_2SO_4 , purified and concentrated. The product was precipitated by the addition of ether, filtered and dried to yield the title compound.

○ Step 7: Preparation of Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-NHNH₂

1.5 mL of hydrazine hydrate was added to 0.07 mmol of Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-OMe dissolved in 15 mL of DMF. The solution was allowed to stand for 5 hours at room temperature. Aqueous MeOH was then added. The precipitate that formed was filtered off, washed with MeOH/water (1:9), and dried to yield the title compound.

○ Step 8: Preparation of H-Trp-Cys(Acm)-OMe

A solution of 17.2 mmol of Boc-Trp-Cys(Acm)-OMe and 5 mL of thioanisole in 25 mL of methylenechloride was added to 50 mL of TFA and allowed to stand for 20 minutes at room temperature. The solution was diluted with about 1.5 L of ether, and the precipitate that formed was filtered off, washed with ether and dried to yield the title compound.

○ Step 9: Preparation of Boc-Trp-Cys(Acm)-OMe

2.1 mL of N-methylmorpholine was added with stirring to 19.4 mmol of Boc-Trp-OH in 50 mL of THF pre-cooled to -20°C followed by dropwise addition at -15°C of 2.4 mL of chloroformic acid isobutyl ester. After stirring for 5 minutes at -15°C, a cold solution (-10°C) comprising 24 mmol of H-Cys(Acm)-OMe and 4.1 mL of N-methylmorpholine in 30 mL of DMF was added. The mixture was stirred for 2 hours at a constant temperature of 0°C and then for an additional 2 hours at room temperature. 50 mL of 10% KHCO_3 was added. The reaction mixture was concentrated under vacuum, diluted with ethyl acetate and washed 3 times with 2 N citric acid, then 3 times with 10%

KHCO₃, and then with a 30% NaCl solution. The organic phase was dried over Na₂SO₄ and evaporated under vacuum to yield the title compound.

○ Step 10: Preparation of Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp-Cys(Acm)-OMe

5 0.7 mL of 5 N HCl in ether was added with stirring to 0.63 mmol of Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-NHNH₂ in 30 mL of DMF pre-cooled to -20°C. 0.8 mL of 10% tert-butyl nitrite in DMF was then added. The mixture was stirred for 15 minutes at -20°C to -15°C. 0.56 mL of NEt₃ was added at -25°C followed by a solution of 1.1 mmol of H-Trp-Cys(Acm)-OMe in 3 mL of DMF pre-cooled to -15°C and the resulting mixture was stirred for 20 hours at -5°C to 0°C and
10 then for additional 3 hours at room temperature. The reaction mixture was diluted with about 100 mL of methanol and the product was precipitated by the addition of approximately 40 mL of water. The precipitated that formed was filtered off, washed with aqueous MeOH and dried to yield the title compound.

○ Step 11: Preparation of Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp-Cys(Acm)-NH₂

15 0.5 mmol of Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp-Cys(Acm)-OMe was treated with liquid ammonia for several hours. The ammonia was then slowly evaporated. The residue was diluted with about 100 mL of methanol and the product was precipitated out by the addition of approximately 40 mL of water. The precipitate that formed was filtered off, washed with
20 aqueous MeOH and dried to yield the title compound.

○ Step 12: Preparation of Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂

 0.4 mmol of Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp-Cys(Acm)-NH₂ and 6.0 mL of thioanisole were dissolved in 30 mL of TFA at 0°C. Stirring was continued for 1.5 hours maintaining a temperature of -5°C to -10°C and subsequently 50 mL of cold MeOH (-70°C) was
25 added. After additional 5 minutes, 1 L of ether and 5 mL of approximately 5 N HCl in ether were added with stirring. The precipitated product was filtered off and washed briefly with ether. The residue was dissolved immediately in 2.5 L of MeOH/H₂O. The pH was adjusted to 7-7.5 by the addition of a dilute ammonia solution. To the mixture was added iodine solution in MeOH dropwise until a persistent color was achieved. The reaction was stirred at room temperature until it tested
30 negative for -SH groups (e.g., the Ellmann test). The reaction was quenched with 10% Na₂S₂O₃. The pH was adjusted to 3-4 by the addition of HCl. The obtained solution was concentrated under vacuum and the product was lyophilized. The lyophilisate was dissolved in a small quantity of 10% AcOH and purified by HPLC. Fractions containing the desired product were collected and lyophilized to yield the title compound.

Additional embodiments of the present invention will be apparent from the foregoing disclosure and are intended to be encompassed by the invention as described fully herein and defined in the following claims.

CLAIMS

What is claimed is:

1. A process for the solution-phase synthesis of Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂ comprising a fragment condensation procedure.
5
2. A process for the solution-phase synthesis of Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂ according to claim 1, wherein protected amino acids are used.
3. A process for the solution-phase synthesis of Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂ according to claim 2, wherein said protected amino acids are selected from the group consisting of Boc protected amino acids, benzoyloxycarbonyl protected amino acids, Fmoc protected amino acids, and protected amino acid fluorides.
10
4. A process for the solution-phase synthesis of Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂ according to claim 3, wherein said protected amino acid fluoride is Fmoc amino acid fluoride or Bsmoc amino acid fluoride.
15
5. A process for the solution-phase synthesis of Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂ according to any one of the preceding claims, wherein a mixed anhydride coupling method is employed.
20
6. A process for the solution-phase synthesis of Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂ according to any one of the preceding claims, wherein a peptide-hydrazide coupling method is employed.
25
7. A process for the solution-phase synthesis of Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂ according to any one of the preceding claims, wherein ammonia is used to convert an ester functional group to an amide functional group.
30
8. A process for the solution-phase synthesis of Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂ according to any one of the preceding claims, wherein a protected peptide fragment Trp-Cys or Arg-Trp is provided.
9. A process for the solution-phase synthesis of Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂ according to claim 8, wherein said protected peptide fragment Trp-Cys is Boc-Trp-Cys(Acm)-OMe or Boc-Trp(For)-Cys(Acm)-OMe.
35

10. A process for the solution-phase synthesis of Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂ according to claim 8 or claim 9, wherein a protected peptide fragment D-Ala-His or His-D-Phe is provided.

5 11. A process for the solution-phase synthesis of Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂ according to claim 10, wherein said protected peptide fragment D-Ala-His is benzoyloxycarbonyl-D-Ala-His-OH or benzoyloxycarbonyl-D-Ala-His(Trt)-OH.

10 12. A process for the solution-phase synthesis of Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂ according to claims 8-11, comprising the steps of:

(a) synthesizing a fragment benzoyloxycarbonyl-D-Ala-His-OH from benzoyloxycarbonyl-D-Ala-OH and H-His-OH in the presence of a coupling reagent; or alternatively, synthesizing a fragment benzoyloxycarbonyl-D-Ala-His(Trt)-OH from benzoyloxycarbonyl-D-Ala-OH and H-His(Trt)-OH in the presence of a coupling reagent;

15 (b-1) synthesizing a fragment benzoyloxycarbonyl-D-Phe-Arg(Pbf)-OMe from benzoyloxycarbonyl-D-Phe-OH and H-Arg(Pbf)-OMe in the presence of a coupling reagent;

(b-2) synthesizing a fragment H-D-Phe-Arg(Pbf)-OMe by hydrogenating the fragment benzoyloxycarbonyl-D-Phe-Arg(Pbf)-OMe obtained in the step (b-1);

20 (c-1) synthesizing a fragment benzoyloxycarbonyl-D-Ala-His-D-Phe-Arg(Pbf)-OMe from benzoyloxycarbonyl-D-Ala-His-OH and the fragment H-D-Phe-Arg(Pbf)-OMe obtained in the step (b-2) in the presence of a coupling reagent;

or alternatively, synthesizing a fragment benzoyloxycarbonyl-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-OMe from benzoyloxycarbonyl-D-Ala-His(Trt)-OH and the fragment H-D-Phe-Arg(Pbf)-OMe obtained in the step (b-2) in the presence of a coupling reagent;

25 (c-2) synthesizing a fragment H-D-Ala-His-D-Phe-Arg(Pbf)-OMe by hydrogenating the fragment benzoyloxycarbonyl-D-Ala-His-D-Phe-Arg(Pbf)-OMe obtained in the step (c-1);

or alternatively, synthesizing a fragment H-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-OMe by hydrogenating the fragment benzoyloxycarbonyl-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-OMe obtained in the step (c-1);

30 (d-1) synthesizing a fragment Fmoc-Arg(Pbf)-Cys(Acm)-OH from Fmoc-Arg(Pbf)-OH and H-Cys(Acm)-OBzl in the presence of a coupling reagent, followed by hydrogenation;

(d-2) synthesizing a fragment H-Arg(Pbf)-Cys(Acm)-OH from the fragment Fmoc-Arg(Pbf)-Cys(Acm)-OH obtained in the step (d-1) in the presence of a base;

35 (d-3) synthesizing a fragment Ac-Arg(Pbf)-Cys(Acm)-OH from the fragment H-Arg(Pbf)-Cys(Acm)-OH obtained in the step (d-2);

or alternatively, synthesizing a fragment Ac-Arg(Pbf)-Cys(Acm)-OH from Ac-Arg(Pbf)-OH and H-Cys(Acm)-OMe in the presence of a coupling reagent, followed by hydrolysis by using a base;

(e-1) synthesizing a fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-OMe from Ac-Arg(Pbf)-Cys(Acm)-OH and H-D-Ala-His-D-Phe-Arg(Pbf)-OMe in the presence of a
5 coupling reagent;

or alternatively, synthesizing a fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-OMe from Ac-Arg(Pbf)-Cys(Acm)-OH and H-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-OMe in the presence of a coupling reagent;

(e-2) synthesizing a fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-OH by
10 hydrolyzing the fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-OMe obtained in the step (e-1) in the presence of a base;

or alternatively, synthesizing a fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-OH by hydrolyzing the fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-OMe obtained in the step (e-1) in the presence of a base;

(f) synthesizing a fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-NHNH₂ from the fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-OMe obtained in the step (e-1) in the presence of hydrazine;

or alternatively, synthesizing a fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-NHNH₂ from the fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-OMe
20 obtained in the step (e-1) in the presence of hydrazine;

(g-1) synthesizing a fragment Boc-Trp-Cys(Acm)-OMe from Boc-Trp-OH and H-Cys(Acm)-OMe in the presence of a coupling reagent;

or alternatively, synthesizing a fragment Boc-Trp(For)-Cys(Acm)-OMe from Boc-Trp(For)-OH and H-Cys(Acm)-OMe in the presence of a coupling reagent;

(g-2) synthesizing H-Trp-Cys(Acm)-OMe from the fragment Boc-Trp-Cys(Acm)-OMe obtained in the step (g-1) in the presence of TFA;

or alternatively, synthesizing H-Trp(For)-Cys(Acm)-OMe from the fragment Boc-Trp(For)-Cys(Acm)-OMe obtained in the step (g-1) in the presence of TFA;

(h) synthesizing Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp(For)-Cys(Acm)-OMe from the fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-NHNH₂ obtained in the step (f) and H-Trp(For)-Cys(Acm)-OMe by using an acid and *tert*-butylnitrite;

or alternatively, synthesizing Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp(For)-Cys(Acm)-OMe from the fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-NHNH₂ obtained in the step (f) and H-Trp(For)-Cys(Acm)-OMe by using an acid and *tert*-butylnitrite;
35

or alternatively, synthesizing Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp-Cys(Acm)-OMe from the fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-NHNH₂ obtained in the step (f) and H-Trp-Cys(Acm)-OMe by using an acid and *tert*-butylnitrite;

5 or alternatively, synthesizing Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp-Cys(Acm)-OMe from the fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-NHNH₂ obtained in the step (f) and H-Trp-Cys(Acm)-OMe by using an acid and *tert*-butylnitrite;

or alternatively, synthesizing Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp-Cys(Acm)-OMe from Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-OH and H-Trp-Cys(Acm)-OMe in the presence of a coupling reagent;

10 or alternatively, synthesizing Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp(For)-Cys(Acm)-OMe from Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-OH and H-Trp(For)-Cys(Acm)-OMe in the presence of a coupling reagent;

15 or alternatively, synthesizing Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp(For)-Cys(Acm)-OMe from Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-OH and H-Trp(For)-Cys(Acm)-OMe in the presence of a coupling reagent;

or alternatively, synthesizing Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp-Cys(Acm)-OMe from Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-OH and H-Trp-Cys(Acm)-OMe in the presence of a coupling reagent;

20 or alternatively, synthesizing Ac-Arg-Cys(Acm)-D-Ala-His-D-Phe-Arg-Trp-Cys(Acm)-OMe from Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp-Cys(Acm)-OMe in the presence of TFA;

or alternatively, synthesizing Ac-Arg-Cys(Acm)-D-Ala-His-D-Phe-Arg-Trp-Cys(Acm)-OMe from Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp-Cys(Acm)-OMe in the presence of TFA;

25 or alternatively, synthesizing Ac-Arg-Cys(Acm)-D-Ala-His-D-Phe-Arg-Trp(For)-Cys(Acm)-OMe from Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp(For)-Cys(Acm)-OMe in the presence of TFA;

30 or alternatively, synthesizing Ac-Arg-Cys(Acm)-D-Ala-His-D-Phe-Arg-Trp(For)-Cys(Acm)-OMe from Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp(For)-Cys(Acm)-OMe in the presence of TFA;

(i) synthesizing Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp-Cys(Acm)-NH₂ from Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp-Cys(Acm)-OMe obtained in the step (h) in the presence of ammonia;

35 or alternatively, synthesizing Ac-Arg-Cys(Acm)-D-Ala-His-D-Phe-Arg-Trp-Cys(Acm)-NH₂ from Ac-Arg-Cys(Acm)-D-Ala-His-D-Phe-Arg-Trp-Cys(Acm)-OMe obtained in the step (h) in the presence of ammonia;

or alternatively, synthesizing Ac-Arg-Cys(Acm)-D-Ala-His-D-Phe-Arg-Trp-Cys(Acm)-NH₂ from Ac-Arg-Cys(Acm)-D-Ala-His-D-Phe-Arg-Trp(For)-Cys(Acm)-OMe obtained in the step (h) in the presence of ammonia;

5 or alternatively, synthesizing Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp-Cys(Acm)-NH₂ from Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp-Cys(Acm)-OMe obtained in the step (h) in the presence of ammonia;

or alternatively, synthesizing Ac-Arg-Cys(Acm)-D-Ala-His-D-Phe-Arg-Trp-Cys(Acm)-NH₂ from Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp-Cys(Acm)-NH₂ in the presence of TFA;

10 or alternatively, synthesizing Ac-Arg-Cys(Acm)-D-Ala-His-D-Phe-Arg-Trp-Cys(Acm)-NH₂ from Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp-Cys(Acm)-NH₂ in the presence of TFA; and

(j) synthesizing Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂ from Ac-Arg-Cys(Acm)-D-Ala-His-D-Phe-Arg-Trp-Cys(Acm)-NH₂ by using an oxidizing agent.

15

13. A process for the solution-phase synthesis of Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂ according to claim 12, wherein:

said oxidizing agent is iodine;

said coupling reagent is DCC, HBTU, HATU, DIC, EDC, or chloroformic acid isobutyl ester;

20 and

said base is Et₂NH, TAEA, piperazine, sodium hydroxide, or potassium hydroxide.

14. A process for the solution-phase synthesis of Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂ according to claim 8 or claim 9, wherein a protected peptide fragment Cys-D-Ala or Arg-Cys is provided.

25

15. A process for the solution-phase synthesis of Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂ according to claim 14, wherein said protected peptide fragment Cys-D-Ala is Boc-Cys(Acm)-D-Ala-OH.

30

16. A process for the solution-phase synthesis of Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂ according to claim 14 or claim 15, comprising the steps of:

(a-1) synthesizing a fragment Boc-Cys(Acm)-D-Ala-OH from Boc-Cys(Acm)-OH and H-D-Ala-OH in the presence of a coupling reagent;

35 (a-2) synthesizing a fragment H-Cys(Acm)-D-Ala-OH from the fragment Boc-Cys(Acm)-D-Ala-OH obtained in the step (a-1) in the presence of TFA;

(b) synthesizing a fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-OH from Ac-Arg(Pbf)-OH and the fragment H-Cys(Acm)-D-Ala-OH obtained in the step (a-2) in the presence of a coupling reagent;

5 (c-1) synthesizing a fragment Boc-His-D-Phe-OMe from Boc-His-OH and H-D-Phe-OMe in the presence of a coupling reagent;

(c-2) synthesizing a fragment H-His-D-Phe-OMe from the fragment Boc-His-D-Phe-OMe obtained in the step (c-1) in the presence of TFA;

(d-1) synthesizing a fragment Boc-Trp-Cys(Acm)-OMe from Boc-Trp-OH and H-Cys(Acm)-OMe in the presence of a coupling reagent;

10 or alternatively, synthesizing a fragment Boc-Trp(For)-Cys(Acm)-OMe from Boc-Trp(For)-OH and H-Cys(Acm)-OMe in the presence of a coupling reagent;

(d-2) synthesizing H-Trp-Cys(Acm)-OMe from the fragment Boc-Trp-Cys(Acm)-OMe obtained in the step (d-1) in the presence of TFA;

15 or alternatively, synthesizing H-Trp(For)-Cys(Acm)-OMe from the fragment Boc-Trp(For)-Cys(Acm)-OMe obtained in the step (d-1) in the presence of TFA;

(e-1) synthesizing a fragment benzoyloxycarbonyl-Arg(Pbf)-Trp-Cys(Acm)-OMe from benzoyloxycarbonyl-Arg(Pbf)-OH and the fragment H-Trp-Cys(Acm)-OMe obtained in the step (d-2) in the presence of a coupling reagent;

20 or alternatively, synthesizing a fragment benzoyloxycarbonyl-Arg(Pbf)-Trp(For)-Cys(Acm)-OMe from benzoyloxycarbonyl-Arg(Pbf)-OH and the fragment H-Trp(For)-Cys(Acm)-OMe obtained in the step (d-2) in the presence of a coupling reagent;

(e-2) synthesizing a fragment H-Arg(Pbf)-Trp-Cys(Acm)-OMe by hydrogenating the fragment benzoyloxycarbonyl-Arg(Pbf)-Trp-Cys(Acm)-OMe obtained in the step (e-1);

25 or alternatively, synthesizing a fragment H-Arg(Pbf)-Trp(For)-Cys(Acm)-OMe by hydrogenating the fragment benzoyloxycarbonyl-Arg(Pbf)-Trp(For)-Cys(Acm)-OMe obtained in the step (e-1);

(f-1) synthesizing a fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-OMe from the fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-OH obtained in the step (b) and the fragment H-His-D-Phe-OMe obtained in the step (c-2) in the presence of a coupling reagent;

30 (f-2) synthesizing a fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-NHNH₂ from the fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-OMe obtained in the step (f-1) in the presence of hydrazine;

or alternatively, synthesizing a fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-OH by hydrolyzing the fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-OMe obtained in the step (f-1) in the presence of a base;

5 (g) synthesizing Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp-Cys(Acm)-OMe from the fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-NHNH₂ obtained in the step (f-2) and the fragment H-Arg(Pbf)-Trp-Cys(Acm)-OMe obtained in the step (e-2) using an acid and *tert*-butylnitrite;

10 or alternatively, synthesizing Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp(For)-Cys(Acm)-OMe from the fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-NHNH₂ obtained in the step (f-2) and the fragment H-Arg(Pbf)-Trp(For)-Cys(Acm)-OMe obtained in the step (e-2) using an acid and *tert*-butylnitrite;

15 or alternatively, synthesizing Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp-Cys(Acm)-OMe from the fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-OH obtained in the step (f-2) and the fragment H-Arg(Pbf)-Trp-Cys(Acm)-OMe obtained in the step (e-2) in the presence of a coupling agent;

or alternatively, synthesizing Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp(For)-Cys(Acm)-OMe from the fragment Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-OH obtained in the step (f-2) and the fragment H-Arg(Pbf)-Trp(For)-Cys(Acm)-OMe obtained in the step (e-2) in the presence of a coupling agent;

20 (h) synthesizing Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp-Cys(Acm)-NH₂ from Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp-Cys(Acm)-OMe obtained in the step (g) in the presence of ammonia;

25 or alternatively, synthesizing Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp(For)-Cys(Acm)-NH₂ from Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp(For)-Cys(Acm)-OMe obtained in the step (g) in the presence of ammonia;

or alternatively, synthesizing Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp-Cys(Acm)-NH₂ from Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp-Cys(Acm)-OMe obtained in the step (g) in the presence of ammonia;

30 or alternatively, synthesizing Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp(For)-Cys(Acm)-NH₂ from Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp(For)-Cys(Acm)-OMe obtained in the step (g) in the presence of ammonia;

(i) synthesizing Ac-Arg-Cys(Acm)-D-Ala-His-D-Phe-Arg-Trp-Cys(Acm)-NH₂ from Ac-Arg(Pbf)-Cys(Acm)-D-Ala-His-D-Phe-Arg(Pbf)-Trp-Cys(Acm)-NH₂ obtained in the step (h) in the presence of TFA; and

(j) synthesizing Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂ from Ac-Arg-Cys(Acm)-D-Ala-His-D-Phe-Arg-Trp-Cys(Acm)-NH₂ obtained in the step (i) by using an oxidizing agent.

5 17. A process for the solution-phase synthesis of Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂ according claim 16, wherein:
said oxidizing agent is iodine;
said coupling reagent is DCC, HBTU, HATU, DIC, EDC, or chloroformic acid isobutyl ester;
and
10 said base is Et₂NH, TAEA, piperazine, sodium hydroxide, or potassium hydroxide.

18. A process for the solution-phase synthesis of Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂ comprising a linear stepwise synthetic procedure.

15 19. A process for the solution-phase synthesis of Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂, according to claim 12, wherein protected amino acids are used.

20. A process for the solution-phase synthesis of Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂, according to claim 19, wherein said protected amino acids are selected from the
20 group consisting of Boc protected amino acids, Fmoc protected amino acids, and protected amino acid fluorides.

21. A process for the solution-phase synthesis of Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂, according to claim 20, wherein said protected amino acid fluoride is Fmoc amino
25 acid fluoride or Bsmoc amino acid fluoride.

22. A process for the solution-phase synthesis of Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂, according to any one of claims 18-21, wherein dimethylcyclopropylmethyl amine
is used at the C-terminus of a protected peptide chain.

30 23. A process for the solution-phase synthesis of Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂, according to claim 22, wherein Fmoc-Cys(Trt)-NH-CMe₂CP is used.

35 24. A process for the solution-phase synthesis of Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂, according to claim 23, comprising the steps of:

(a) synthesizing H-Cys(Trt)-NH-CMe₂CP from Fmoc-Cys(Trt)-NH-CMe₂CP in the presence of a base;

(b) synthesizing Fmoc-Trp(Boc)-Cys(Trt)-NH-CMe₂CP from Fmoc-Trp(Boc)-OH and H-Cys(Trt)-NH-CMe₂CP obtained in the step (a) in the presence of a coupling reagent;

(c) synthesizing H-Trp(Boc)-Cys(Trt)-NH-CMe₂CP from Fmoc-Trp(Boc)-Cys(Trt)-NH-CMe₂CP obtained in the step (b) in the presence of a base;

(d) synthesizing Fmoc-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP from Fmoc-Arg(Pbf)-OH and H-Trp(Boc)-Cys(Trt)-NH-CMe₂CP obtained in the step (c) in the presence of a coupling
5 reagent;

(e) synthesizing H-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP from Fmoc-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP obtained in the step (d) in the presence of a base;

(f) synthesizing Fmoc-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP from Fmoc-D-Phe-OH and H-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP obtained in the step (e) in the presence of a
10 coupling reagent;

(g) synthesizing H-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP from Fmoc-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP obtained in the step (f) in the presence of a base;

(h) synthesizing Fmoc-His(Trt)-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP from Fmoc-His(Trt)-OH and H-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP obtained in the step (g)
15 in the presence of a coupling reagent;

(i) synthesizing H-His(Trt)-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP from Fmoc-His(Trt)-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP obtained in the step (h) in the
presence of a base;

(j) synthesizing Fmoc-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP
20 from Fmoc-D-Ala-OH and H-His(Trt)-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP obtained in the step (i) in the presence of a coupling reagent;

(k) synthesizing H-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP
from Fmoc-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP obtained in the step (j)
in the presence of a base;

(l) synthesizing Fmoc-Cys(Trt)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-
25 NH-CMe₂CP from Fmoc-Cys(Trt)-OH and H-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-
NH-CMe₂CP obtained in the step (k) in the presence of a coupling reagent;

(m) synthesizing H-Cys(Trt)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-
30 CMe₂CP from Fmoc-Cys(Trt)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP
obtained in the step (l) in the presence of a base;

(n) synthesizing Fmoc-Arg(Pbf)-Cys(Trt)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp(Boc)-
Cys(Trt)-NH-CMe₂CP from Fmoc-Arg(Pbf)-OH and H-Cys(Trt)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-
Trp(Boc)-Cys(Trt)-NH-CMe₂CP obtained in the step (m) in the presence of a coupling reagent;

(o) synthesizing H-Arg(Pbf)-Cys(Trt)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP from Fmoc-Arg(Pbf)-Cys(Trt)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP obtained in the step (n) in the presence of a base;

(p) synthesizing Ac-Arg(Pbf)-Cys(Trt)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP from H-Arg(Pbf)-Cys(Trt)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP obtained in the step (o) and Ac₂O, AcCl or AcBr;

or alternatively, synthesizing Ac-Arg(Pbf)-Cys(Trt)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP from Ac-Arg(Pbf)-OH and H-Cys(Trt)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP obtained in the step (m) in the presence of a coupling reagent;

(q) synthesizing Ac-Arg-Cys-D-Ala-His-D-Phe-Arg-Trp-Cys-NH₂ from Ac-Arg(Pbf)-Cys(Trt)-D-Ala-His(Trt)-D-Phe-Arg(Pbf)-Trp(Boc)-Cys(Trt)-NH-CMe₂CP obtained in the step (p) in the presence of TFA; and

(r) synthesizing Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂ from Ac-Arg-Cys-D-Ala-His-D-Phe-Arg-Trp-Cys-NH₂ obtained in the step (q) in the presence of an oxidizing agent.

25. A process for the solution-phase synthesis of Ac-Arg-cyclo(Cys-D-Ala-His-D-Phe-Arg-Trp-Cys)-NH₂, according to claim 24, wherein:

said oxidizing agent is iodine, oxygen, air, or DMSO;

said coupling reagent is DCC, HBTU, HATU, DIC, EDC, or chloroformic acid isobutyl ester;

and

said base is Et₂NH, TAEA, or piperazine.

25

FIG. 1B

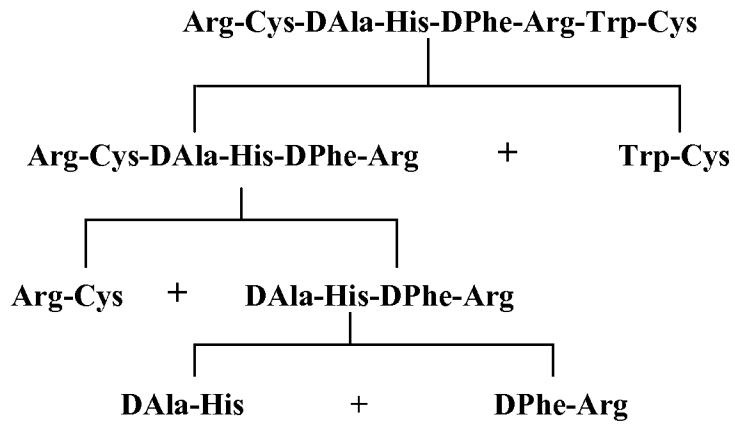


FIG. 2

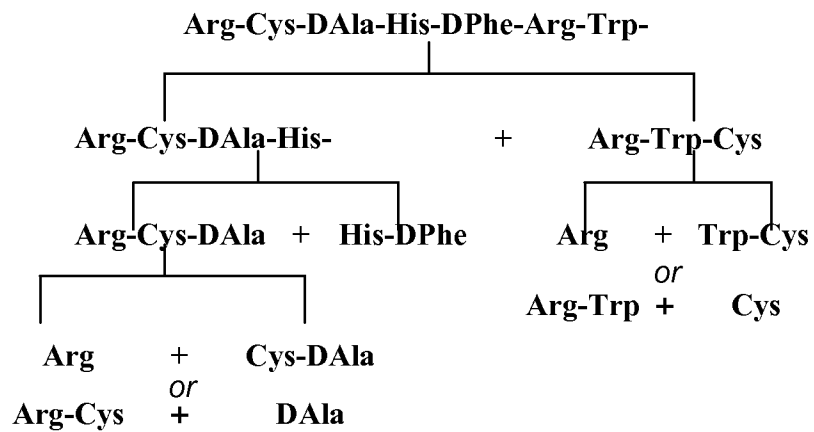


FIG. 3

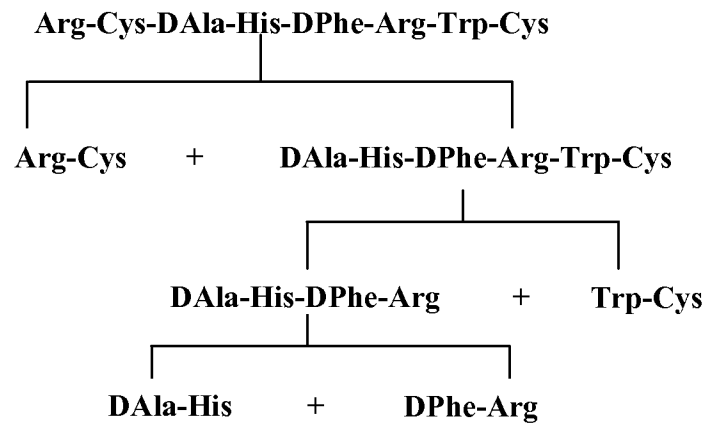


FIG. 4

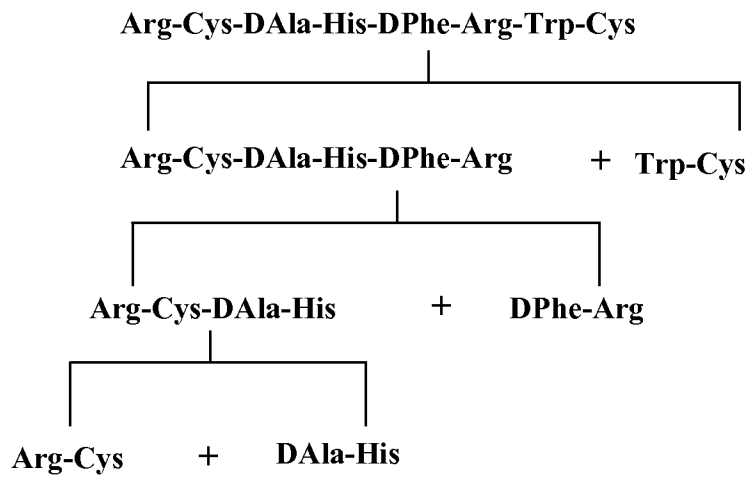


FIG. 5

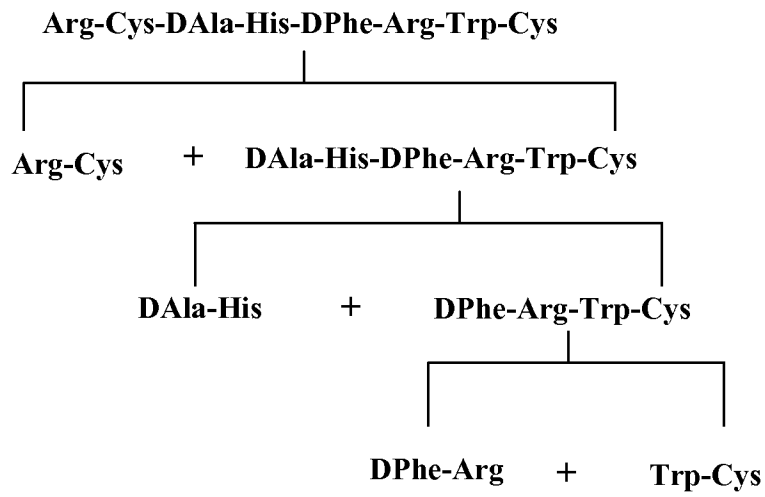
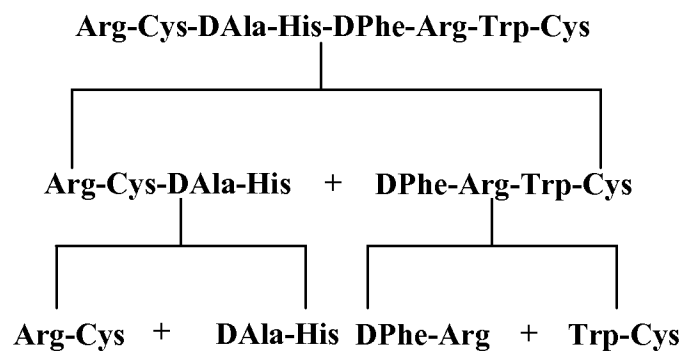


FIG. 6



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FIG. 7

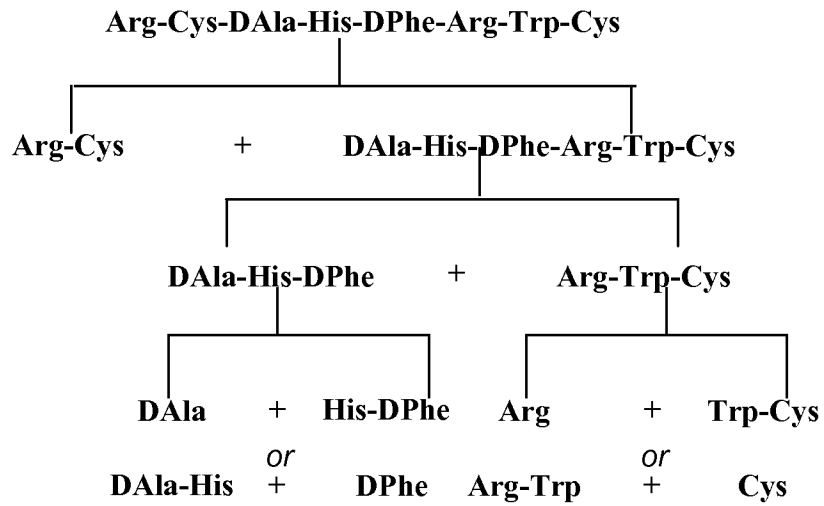


FIG. 8

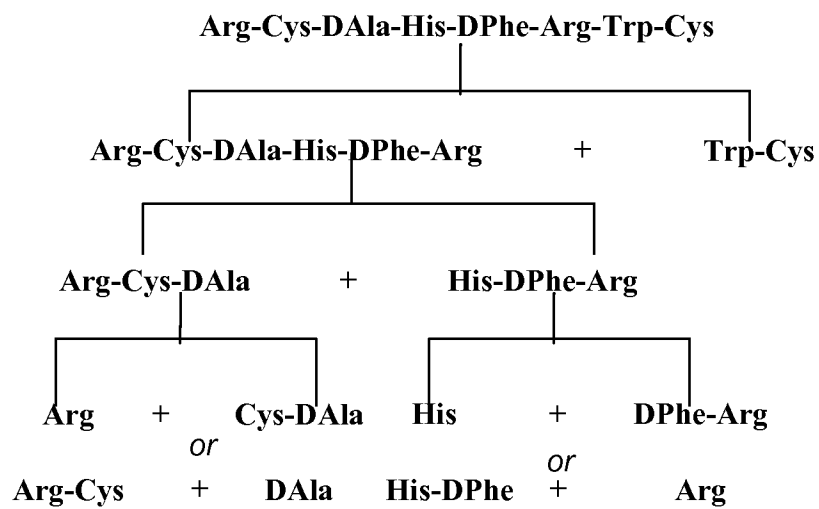


FIG. 9

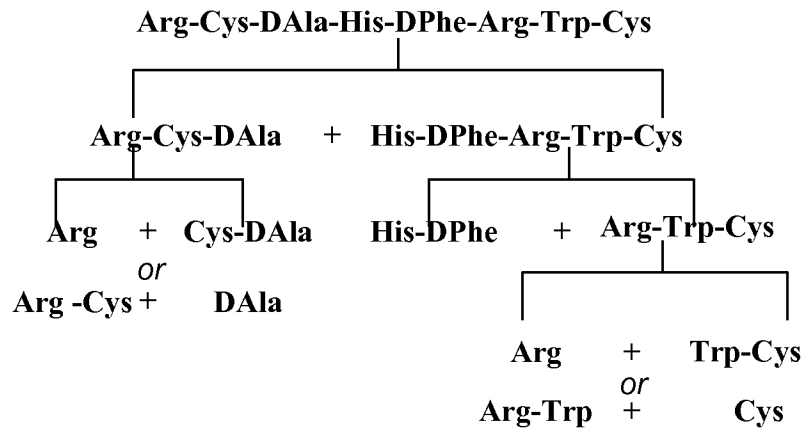


FIG. 10

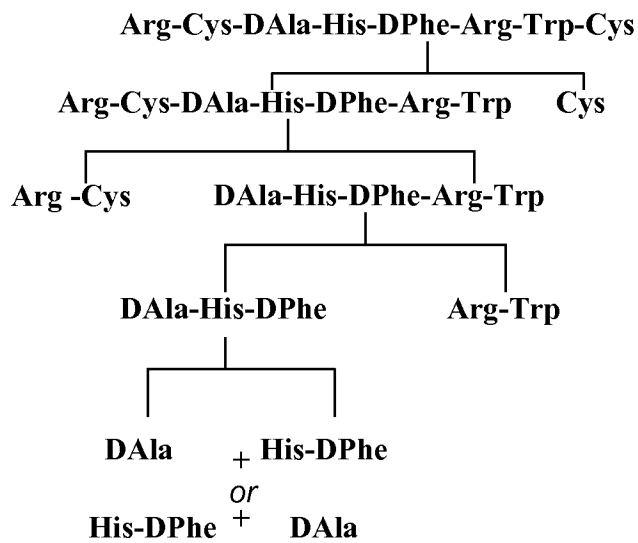
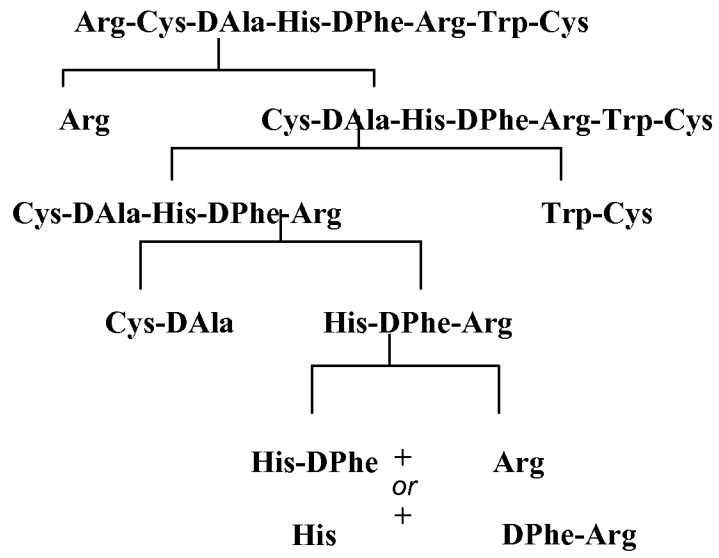


FIG. 11



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 10/56696

A. CLASSIFICATION OF SUBJECT MATTER IPC(8) - C07K 1/00; A61K 38/12; A61K 38/04 (2011.01) USPC - 530/338; 530/317; 530/328 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC(8): C07K 1/00; A61K 38/12; A61K 38/04 (2011.01) USPC: 530/338; 530/317; 530/328 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) PubWest;PGPB, USPT, EPAB, JPAB, GoogleScholar, Dialog: melanocortin analog, solution-phase peptide chemistry, fragment condensation, Boc protected amino acids, benzoyloxycarbonyl protected amino acids, Fmoc protected amino acids, protected amino acid fluorides, mixed anhydride coupling, linear stepwise synthetic		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2007/008704 A2 (DONG et al.) 18 January 2007 (18.01.2007) page 19, ln 8, page 43- page 44; Synthesis, page 44-page 45; Example 1, page 46, paragraph 2	1-5 and 18
Y	Kent. CHEMICAL SYNTHESIS OF PEPTIDES AND PROTEINS. Ann. Rev. Biochem. 1988. 57:957-89; page 960, Fig. 1A-1B, page 961	1-5 and 18
Y	US 7,169,777 B2 (BACKER et al.) 30 January 2007 (30.01.2007) Abstract, col 145, ln 40-60	1-4
Y	US 2008/0171849 A1 (GIRAUD et al) 17 July 2008 (17.07.2008) para [0028], [0044]	5
A	Yang et al. Characterization of Agouti-Related Protein Binding to Melanocortin Receptors. Molecular Endocrinology 1999, 13:148-155	1-5 and 18
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/>		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 21 January 2011 (21.01.2011)		Date of mailing of the international search report 09 FEB 2011
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201		Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 10/56696

Box No. 1 Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

a. (means)

on paper

in electronic form

b. (time)

in the international application as filed

together with the international application in electronic form

subsequently to this Authority for the purposes of search

2. In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 10/56696

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

- 1. Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

- 2. Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

- 3. Claims Nos.: 6-17, 19-25
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

- 1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
- 2. As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
- 3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

- 4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.