The present invention provides a process for the production of desmopressin. In particular, the process provides a process for solid phase synthesis of desmopressin using cyclization of the peptide on the resin.
SYNTHESIS OF DESMOPRESSIN

FIELD OF THE INVENTION

[0001] The invention provides a process for the production of desmopressin using solid phase peptide synthesis.

BACKGROUND OF THE INVENTION

[0002] Desmopressin, also known as Stimate and Minirin, is a synthetic analog of vasopressin (VP), the hormone that reduces urine production during sleep. Desmopressin possesses a high anti-diuretic activity, and is prescribe most frequently for treatment of diabetes insipidus or bedwetting. It is described as 1-deamino-8-D-arginine vasopressin (dDAVP), and its chemical structure is depicted in the diagram below. Desmopressin is a cyclic nonapeptide with a mercaptopropionyl moiety (mpr or mpa) at the N-terminus, and a disulfide bridge between the mpr and cys residues. It has traditionally been manufactured by solid phase peptide synthesis method (SPPS) using BOC/Benzyl chemistry, which requires the toxic corrosive hydrogen fluoride (HF) during its last step of synthesis to release the peptide from the support. In addition, currently known methods for cyclization of the linear peptide requires excessive separate purification steps before purifying the final product, which increases the complexity and the expense of synthesizing the peptide, and inevitably affect the final product quality and quantity. In addition, purification is costly in terms of manpower hours and the obvious loss of profits from reduced product yield,

\[
\text{CH}_2\text{S}---\text{S}---\text{CH}_2
\]

\[
\text{CH}_2\text{CO-NH-Tyr-Phe-Gln-Asn-Cys-Pro-D.Arg-Gly-CO NH}_2
\]

[0003] Thus, there is a need in the art for synthetic routes for producing desmopressin that would allow the use of the more benign Fmoc chemistry for SPPS, and wherein the yield and quality achieved are increased. This will reduce the need for extensive purification of intermediates and product, and provide a superior product in improved yield.
SUMMARY OF THE INVENTION

[0004] Briefly, therefore, the present invention provides a process for solid phase synthesis of desmopressin using cyclization of the peptide on the resin. In one aspect, the invention encompasses a process for solid phase synthesis of desmopressin, the process comprising:

(a) providing a solid support coupled with a glycine residue;

(b) activating the carboxy group of a D-arginine residue that has its amine protected by an Fmoc group and its side chain protected by an acid labile group, followed by coupling the D-arginine residue to the glycine residue on the solid support of (a), and treatment of the solid support with a base to deprotect the amine group of the D-arginine residue;

(c) activating the carboxy group of a proline residue that has its amine protected by a Fmoc group, followed by coupling the proline residue to the D-arginine residue on the solid support of (b), and treatment of the solid support with a base to deprotect the amine group of the proline residue;

(d) activating the carboxy group of a cysteine residue that has its amine protected by a Fmoc group and its thiol group protected by a group labile to an oxidizing agent, followed by coupling the cysteine residue to the proline residue on the solid support of (c), and treatment of the solid support with an agent to deprotect the amine group of the cysteine residue;

(e) activating the carboxy group of an asparagine residue that has its amine protected by a Fmoc group and its side chain protected by an acid labile group, followed by coupling the asparagine residue to the cysteine residue on the solid support of (d), and treatment of the solid support with a base to deprotect the amine group of the asparagine residue;

(f) activating the carboxy group of a glutamine residue that has its amine protected by a Fmoc group and its side chain protected by an acid labile group, followed by coupling the glutamine residue to the asparagine residue on the solid support of (e), and treatment of the solid support with a base to deprotect the amine group of the glutamine residue;

(g) activating the carboxy group of a phenylalanine residue that has its amine protected by a Fmoc group, followed by coupling the phenylalanine residue to the glutamine residue on the solid support of (f), and treatment of the solid support with a base to deprotect the
(h) activating the carboxy group of a tyrosine residue that has its amine protected by a Fmoc group and its side chain protected by an acid labile group, followed by coupling the tyrosine residue to the phenylalanine residue on the solid support of (g), and treatment of the solid support with a base to deprotect the amine group of the tyrosine residue;

(i) activating the carboxy group of a mercaptopropionic acid residue that has its thiol group protected by a group labile to an oxidizing agent, followed by coupling the mercaptopropionic acid residue to the tyrosine residue on the solid support of (h);

(j) circularizing the peptide formed in (i) by simultaneously deprotecting and oxidizing the thiol groups of the cysteine residue and the mercaptopropionic acid residue, by contacting the solid support of (i) with an oxidizing agent in a manner such that a disulfide bond is formed between the cysteine residue and the mercaptopropionic acid residue to form a cyclic peptide; and

(k) contacting the solid support of (j) with an acid in a manner such that the amino acid side chains are deprotected, and desmopressin is released from the solid support.

[0005] In an additional aspect, the invention encompasses a process for solid phase synthesis of desmopressin, the process comprising:

(a) providing a solid support coupled with a glycine residue;

(b) activating the carboxy group of a D-arginine residue that has its amine protected by an Fmoc group and its side chain protected by an acid labile group, followed by coupling the D-arginine residue to the glycine residue on the solid support of (a), and treatment of the solid support with a base to deprotect the amine group of the D-arginine residue;

(c) activating the carboxy group of a proline residue that has its amine protected by a Fmoc group, followed by coupling the proline residue to the D-arginine residue on the solid support of (b), and treatment of the solid support with a base to deprotect the amine group of the proline residue;

(d) activating the carboxy group of a cysteine residue that has its amine protected by a Fmoc group and its thiol group protected by a group labile to an oxidizing agent, followed by coupling the cysteine residue to the proline residue on the solid support of (c), and treatment of the solid support with an agent to deprotect the amine group of the cysteine
(e) activating the carboxy group of an asparagine residue that has its amine protected by a Fmoc group and its side chain protected by an acid labile group, followed by coupling the asparagine residue to the cysteine residue on the solid support of (d), and treatment of the solid support with a base to deprotect the amine group of the asparagine residue;

(f) activating the carboxy group of a glutamine residue that has its amine protected by a Fmoc group and its side chain protected by an acid labile group, followed by coupling the glutamine residue to the asparagine residue on the solid support of (e), and treatment of the solid support with a base to deprotect the amine group of the glutamine residue;

(g) activating the carboxy group of a phenylalanine residue that has its amine protected by a Fmoc group, followed by coupling the phenylalanine residue to the glutamine residue on the solid support of (f), and treatment of the solid support with a base to deprotect the amine group of the phenylalanine residue;

(h) activating the carboxy group of a tyrosine residue that has its amine protected by a Fmoc group and its side chain protected by an acid labile group, followed by coupling the tyrosine residue to the phenylalanine residue on the solid support of (g), and treatment of the solid support with a base to deprotect the amine group of the tyrosine residue;

(i) activating the carboxy group of a mercaptopropionic acid residue that has its thiol group protected by a group labile to an oxidizing agent, followed by coupling the mercaptopropionic acid residue to the tyrosine residue on the solid support of (h);

(j) circularizing the peptide formed in (i) by simultaneously deprotecting and oxidizing the thiol groups of the cysteine residue and the mercaptopropionic acid residue, by contacting the solid support of (i) with an oxidizing agent in a manner such that a disulfide bond is formed between the cysteine residue and the mercaptopropionic acid residue to form a cyclic peptide;

(k) contacting the solid support of (j) with a first acid in a manner such that a protected cyclic peptide acid is released from the solid support;

(l) amidating the protected cyclic peptide acid of (k) to produce protected desmopressin; and

(m) contacting the protected desmopressin of (j) with a second acid in a manner such that the amino acid side chains are deprotected and desmopressin is produced.
DETAILED DESCRIPTION OF THE INVENTION

[0007] A process that simplifies solid phase synthesis of desmopressin has been developed. The process uses the safer, more environmentally friendly Fmoc chemistry for SPPS, and allows for the formation of the ring structure of desmopressin using fewer synthetic and purification steps. As illustrated in the examples, the process of the invention generally produces desmopressin in higher yield and purity compared to other methods currently used to synthesize desmopressin.

(I) Synthesis of the linear protected peptide resin

[0008] The peptide resin is synthesized using methods commonly used in the art. In essence, a solid support coupled with a glycine residue is provided. This is followed by activating the carboxy group of a D.arginine residue that has its amine protected by a Fmoc group and its side chain protected by an acid labile group, followed by coupling the D.arginine residue to the glycine residue on the solid support, and treatment of the solid support with an agent to deprotect the amine group of the D.arginine residue. This process is repeated for a proline residue that has its amine protected by a Fmoc group, a cysteine residue that has its amine protected by a Fmoc group and its thiol group protected by a group labile to an oxidizing agent, an asparagine residue that has its amine protected by a Fmoc group and its side chain protected by an acid labile group, a glutamine residue that has its amine protected by a Fmoc group and its side chain protected by an acid labile group, a phenylalanine residue that has its amine protected by a Fmoc group, a tyrosine residue that has its amine protected by a Fmoc group and its side chain protected by an acid labile group, a mercaptopropionic acid residue that has its thiol group protected by a group labile to an oxidizing agent.

(A) Solid support coupled with glycine:

[0009] In essence, a solid support coupled with glycine is first provided. In one aspect of the invention, the solid support comprises an amide group that will become part of the peptide upon cleavage to produce a peptide amide. A non-limiting list of suitable solid supports that may be used in the preparation of peptide amides may include NovaSyn® TGR resin, Rink amide resin, Rink amide MBHA
amide MBHA resin. In another preferred embodiment, the solid support is Sieber amide resin. In yet another preferred embodiment, the solid support is Rink amide-AM resin. As is commonly known in the art, an Fmoc group may be covalently attached to the solid support. If an Fmoc group is covalently attached to the solid support, the Fmoc group may be removed using methods described further below.

[0010] In another aspect of the invention, the solid support of the invention may be any solid support that may be used in the preparation of peptide acids. Non-limiting examples of suitable solid supports that may be used in the preparation of peptide acids may include chlorotrityl resin, trityl resin, methyltrityl resins, methoxytrityl resins, NovaSyn® TGT resin, HMPB-AM resin, HMPB-BHA resin, HMPB-MBHA resin, Wang resin, NovaSyn-TGA resin, HMPA-PEGA resin, HMPA-NovaGel resin, PAM resin, and Merrifield resin. In a preferred embodiment, the solid support may be 2-chlorotrityl chloride resin.

[0011] The solid support is coupled with a glycine residue. The glycine residue may be coupled to the solid support by methods known in the art. Methods of loading the first amino acid are known to those skilled in the art and can be found in, for example, Fmoc Solid Phase Peptide Synthesis: A Practical Approach (Practical Approach Series) Oxford University Press, USA; 1 edition (March 2, 2000), which is incorporated herein by reference in its entirety. Non-limiting examples of methods for attaching the first amino acid to the solid support include the symmetrical anhydride method, the dichlorobenzoyl chloride method, DIC-HOBt method, and the MSNT/Melm method. In one embodiment, the glycine residue coupled to the solid support may be protected with an Fmoc-protecting group.

[0012] Upon loading the Fmoc-Gly onto the solid support, the Fmoc group may be removed using methods described in below.

(B) Peptide synthesis:

[0013] As detailed herein, after the solid support is loaded with the glycine moiety, peptide elongation may be conducted using methods of solid phase peptide synthesis known in the art. In general, solid phase peptide synthesis methods known in the art involve the sequential coupling of amino acids that have their amines protected. After each coupling step, the terminal amino acid protecting group is then cleaved to provide a free amine group ready for coupling the next amino acid in the next addition cycle. Commonly used amine protecting groups may include tert-Butyloxycarbonyl (Boc) and 9H-fluoren-9-yl-methoxycarbonyl (Fmoc) protecting groups. In an exemplary embodiment, the amine protecting group is
Accordingly, the synthesis of desmopressin involves the following steps: activating the carboxy group of a D-arginine residue that has its amine protected by a Fmoc group and its side chain protected by an acid labile group, followed by coupling the D-arginine residue to the glycine residue on the solid support, and treatment of the solid support with a base to deprotect the amine group of the D-arginine residue; activating the carboxy group of a proline residue that has its amine protected by a Fmoc group, followed by coupling the proline residue to the D-arginine residue on the solid support, and treatment of the solid support with a base to deprotect the amine group of the proline residue; activating the carboxy group of a cysteine residue that has its amine protected by a Fmoc group and its thiol group protected by a group labile to an oxidizing agent, followed by coupling the cysteine residue to the proline residue on the solid support, and treatment of the solid support with an agent to deprotect the amine group of the cysteine residue; activating the carboxy group of an asparagine residue that has its amine protected by a Fmoc group and its side chain protected by an acid labile group, followed by coupling the asparagine residue to the cysteine residue on the solid support, and treatment of the solid support with a base to deprotect the amine group of the asparagine residue; activating the carboxy group of a glutamine residue that has its amine protected by a Fmoc group and its side chain protected by an acid labile group, followed by coupling the glutamine residue to the asparagine residue on the solid support, and treatment of the solid support with a base to deprotect the amine group of the glutamine residue; activating the carboxy group of a phenylalanine residue that has its amine protected by a Fmoc group, followed by coupling the phenylalanine residue to the glutamine residue on the solid support, and treatment of the solid support with a base to deprotect the amine group of the phenylalanine residue; activating the carboxy group of a tyrosine residue that has its amine protected by a Fmoc group and its side chain protected by an acid labile group, followed by coupling the tyrosine residue to the phenylalanine residue on the solid support, and treatment of the solid support with a base to deprotect the amine group of the tyrosine residue; activating the carboxy group of a mercaptopropionic acid residue that has its thiol group protected by a group labile to an oxidizing agent, followed by coupling the mercaptopropionic acid residue to the tyrosine residue on the solid support.

Amine groups protected with Fmoc may be deprotected by treatment with an organic base. Suitable organic bases include piperidine, cyclohexyamine, 1,5-diazabicyclo [5.4.0] undec-5-ene, ethanolamine, pyrrolidine 1,8-diazabicyclo[5.4.0]undec-7-ene, diethylamine, morpholine, and
The Fmoc deprotection reaction is carried out in the presence of a solvent at approximately room temperature. Non-limiting examples of suitable solvents include anisole, dimethylformamide, dimethylsulfoxide, dimethyl acetamide, dichloromethane, N-methyl pyrrolidine, and mixtures thereof. A list of additional suitable solvents can be found in Tetrahedron Letters 39:8451-54 (1998), which is incorporated herein by reference in its entirety.

For the coupling reaction, the carboxyl group of the incoming amino acid is usually activated. Suitable activating compounds include carbodiimides, or those belonging to the aromatic oximes class or combinations thereof. In one embodiment, the carbodiimide is selected from dicyclohexylcarbodiimide (DCC), or diisopropylcarbodiimide (DIC). In another embodiment, the aromatic oxime is selected from i-hydroxy-benzotriazole (HOBT), and 1-hydroxy-7-aza-benzotriazole (HOAt). In an exemplary embodiment, the activating compounds are DIC and HOBT. Other suitable activating compounds include HATU/HOBT, PyBOP/HOBT, or OPFP preactivated amino acids/HOBT.

The amount of the various reactants in the coupling reaction can and will vary greatly. Typically the molar ratio of the solid support to the Fmoc-amino acid to the activating compound will range from about 1:1:1 to about 1:5:5. In one embodiment, the molar ratio of the solid support to the Fmoc-amino acid to the activating compound may be about 1:1.5:1.5.

The coupling reaction is carried out in the presence of an aprotic solvent, an organic solvent, and combinations thereof. Non-limiting examples of suitable aprotic solvents include, acetonitrile, diethoxymethane, N,N-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), N,N-dimethylpropionamide, 1,3-dimethyl-3,4,5,6-tetrahydro-2(1 H)-pyrimidinone (DMPU), 1,3-dimethyl-2-imidazolidinone (DMI), 1,2-dimethoxyethane (DME), dimethoxymethane, bis(2-methoxyethyl)ether, N,N-dimethylacetamide (DMAC), 1,4-dioxane, N-methyl-2-pyrrolidinone (NMP), ethyl acetate, ethyl formate, ethyl methyl ketone, formamide, hexachloroacetone, hexamethylphosphoramidite, methyl acetate, N-methylacetamide, N-methylformamide, methylene chloride, nitrobenzene, nitromethane, propionitrile, sulfolane, tetramethyleurea, tetrahydrofuran (THF), 2-methyl tetrahydrofuran, trichloromethane, and combinations thereof. Suitable organic solvents include, but are not limited to, alkane and substituted alkane solvents (including cycloalkanes), aromatic hydrocarbons, esters, ethers, ketones, combinations thereof, and the like. Specific organic solvents that may be employed, include, for example, acetonitrile, benzene, butyl acetate, t-butyl methylether, t-butyl methylketone, chlorobenzene, chloroform,
methylethylketone, methyltetrahydrofuran, propyl acetate, tetrahydrofuran, toluene, and combinations thereof. In an exemplary embodiment, the coupling reaction is carried out in a 3:1 volume ratio of DMF and DCM.

The progress of amino acid couplings may be followed using a ninhydrin reaction, as described in the examples. The ninhydrin solution turns dark blue (positive result) in the presence of a free primary amine but is otherwise colorless (negative result).

Acid-labile side chain protecting groups generally protect the side chains of the D-arginine, asparagine, glutamine, and tyrosine amino acids. The acid-labile protecting groups may be selected from the group consisting of Boc, Mtr (methoxytrimethylbenzenesulphonyl), OtBu (t-butyl ester), Pbf (2,2A6,7-pentamethyl-dihydrobenzofuran-5-sulphonyl), Pmc (2,2,5,7,8-pentamethylchroman-6-sulphonyl), tBu (t-butyl), and Trt (trityl). In a preferred embodiment, the acid-labile protecting group for tyrosine is tBu, the acid-labile protecting group for glutamine is Trt, the acid-labile protecting group for asparagine is Trt, and the acid-labile protecting group for D-arginine is Pbf.

Side chain protecting groups labile to an oxidizing agent protect the thiol groups of cysteine and mercaptopropionic acid. The side chain protecting groups labile to an oxidizing agent may be selected from the group consisting of Acm (acetamidomethyl), and Trt (trityl). In a preferred embodiment, the side chain protecting groups labile to an oxidizing agent for cysteine and mercaptopropionic acid is Trt.

(II) Production of desmopressin from the peptide resin

In one aspect of the invention, the peptide-solid support formed in Section (I) is first cyclized by simultaneously deprotecting and oxidizing the thiol groups of the cysteine residue and the mercaptopropionic acid residue by contacting the solid support with an oxidizing agent in a manner such that a disulfide bond is formed between the cysteine residue and the mercaptopropionic acid residue to form a cyclic peptide. The solid support is then contacted with an acid in a manner such that the amino acid side chains are deprotected, and desmopressin is released from the solid support.

In another aspect, the peptide-solid support formed in Section (I) is cyclized by simultaneously deprotecting and oxidizing the thiol groups of the cysteine residue and the mercaptopropionic acid residue, by contacting the solid support with an oxidizing agent in a manner such that a disulfide bond is formed between the cysteine residue and the mercaptopropionic acid residue to
peptide acid is amidated to produce protected desmopressin, and the protected desmopressin is contacted with a second acid in a manner such that the amino acid side chains are deprotected.

[0025] In yet another aspect of the invention, the peptide-solid support formed in Section (I) is contacted with an acid in a manner such that the amino acid side chains are deprotected and a deprotected linear peptide amide is released from the solid support. The linear peptide amide is then cyclized by oxidizing the thiol groups of the cysteine residue and the mercaptopropionic acid residue with an oxidizing agent in a manner such that a disulfide bond is formed between the cysteine residue and the mercaptopropionic acid residue in a manner such that desmopressin is produced.

[0026] In another aspect of the invention, the peptide-solid support formed in Section (I) is contacted with a first acid in a manner such that a protected linear peptide amide is released from the solid support. The linear protected peptide amide is then cyclized by simultaneously deprotecting and oxidizing the thiol groups of the cysteine residue and the mercaptopropionic acid residue with an oxidizing agent in a manner such that a disulfide bond is formed between the cysteine residue and the mercaptopropionic acid residue to form protected desmopressin. The protected desmopressin is contacted with a second acid in a manner such that the amino acid side chains are deprotected and desmopressin is produced.

[0027] In yet another aspect of the invention, the peptide-solid support formed in Section (I) is contacted with a first acid in a manner such that a protected linear peptide acid is released from the solid support. The linear protected peptide acid is then cyclized by simultaneously deprotecting and oxidizing the thiol groups of the cysteine residue and the mercaptopropionic acid residue by contacting the solid support with an oxidizing agent in a manner such that a disulfide bond is formed between the cysteine residue and the mercaptopropionic acid residue to form protected cyclic peptide acid. To produce desmopressin, the protected cyclic peptide acid is amidated to produce protected desmopressin, and the protected desmopressin is contacted with a second acid in a manner such that the amino acid side chains are deprotected.

[0028] In an alternative of this aspect, the linear protected peptide acid is amidated to produce a linear protected peptide amide. The linear protected peptide amide is then cyclized by simultaneously deprotecting and oxidizing the thiol groups of the cysteine residue and the mercaptopropionic acid residues by contacting the solid support with an oxidizing agent in a manner such that a disulfide bond is formed between the cysteine residue and the mercaptopropionic acid residue to
(A) **Cyclization of the peptide**

[0029] Methods of cyclizing peptides comprising two thiol groups are known in the art, and can be found in, for example, Chan and White, 'FMOC Solid-phase Peptide Synthesis', Oxford University Press (2000), which is incorporated herein by reference in its entirety, in general, cyclization of peptides comprising two thiol groups may include oxidation in the presence of air/oxygen, and oxidizing in the presence of an oxidizing agent. In preferred embodiments of the invention, the thiol groups are oxidized in the presence of an oxidizing agent. Non limiting examples of oxidizing agents that may be suitable for formation of the disulfide bond of desmopressin include salts of thallium, Ellman's reagent (5,5'-dithiobis(2-nitrobenzoic acid)), alkyltrichlorosilane-sulphoxide, silver trifluoromethanesulphonate, iodine, cyanogen iodide, and thallium trifluoroacetate (Tl(CF3COO)3).

[0030] As it is recognized in the art, when the cysteine and mercaptopropionyl residues are protected with a side chain protecting group labile to an oxidizing agent, the side chain protecting groups may be simultaneously deprotected, and the thiol groups of the cysteine residue and the mercaptopropionic acid residue oxidized by contacting the solid support with an oxidizing agent. In general, acetamidomethyl and trityl side chain protecting groups are deprotected when contacted with an oxidizing agent. Non limiting examples of oxidizing agents that may be suitable for simultaneously deprotecting and oxidizing the thiol groups of the cysteine and mercaptopropionic acid residues include, iodine, cyanogen iodide, and thallium trifluoroacetate (Tl(CF3COO)3).

[0031] In some embodiments, the cysteine and mercaptopropionyl residues are not protected with a side chain protecting group labile to an oxidizing agent. In exemplary iteration of these embodiments, the thiol groups of the peptide are cyclized using thallium trifluoroacetate. In general, the peptide may be treated with Tl(CF3COO)3 in a solution of acetic acid in water as detailed in the examples.

[0032] In preferred embodiments, the cysteine and mercaptopropionyl residues are protected with a side chain protecting group labile to an oxidizing agent. In exemplary alternatives of this embodiment, the thiol groups are oxidized using iodine. In general, the peptide may be treated with iodine in the presence of a base in an aprotic solvent, an organic solvent, or combinations thereof. Non limiting examples of bases that may be suitable for formation of the disulfide bond of desmopressin include pyridine, cyclohexylamine, 1,5-diazabicyclo [5,4,0] undec-5-ene, ethanolamine, pyrrolidine 1,8-
the presence of pyridine in DCM and DMF. The amount of the various reactants in the reaction can and will vary. Typically, the molar ratio of the peptide to iodine to pyridine may range from about 1:1:1 to about 1:5:5. In one embodiment, the molar ratio of the peptide to iodine to pyridine may be about 1:4:4. The reaction conditions for cyclizing the peptide using iodine, such as reaction time and temperature, may also vary without departing from the scope of the invention. By way of non-limiting example, the reaction time may range from several hours to several days, and the reaction temperature may range from approximately room temperature to about 0°C. Exemplary reaction parameters of the process are detailed in the examples.

(B) Release and deprotection of peptide from the solid support:

[0033] In one aspect of the invention, the peptide-solid support may be contacted with an acid in such a manner that the amino acid side chains are deprotected, and the peptide is released from the solid support. The acid may be selected from the group consisting of acetic acid (AcOH), TFA, hydrochloric acid (HCl), and trifluoroethanol (TFE) or combinations thereof. In general, the solid support may be treated with trifluoroacetic acid (TFA) in the presence of appropriate scavengers. Scavengers that may be used to release and deprotect the peptide may include phenol, water, 1,2-ethanediithiol (EDT), and triisopropylsilane (TIS) or combinations thereof. In an exemplary embodiment, the amino acid side chains are deprotected, and the peptide is released from the solid support by contacting the solid support with a solution of TFA, EDT, TIS, and water as described for the second acid in Table 1.

[0034] The reaction conditions for deprotecting the amino acid side chains and releasing the peptide from the solid support, such as reaction time, and temperature may vary without departing from the scope of the invention. By way of non-limiting example, the reaction time may range from several hours to several days, and the reaction temperature may range from about 0°C to approximately room temperature. Exemplary reaction parameters are detailed in the examples.

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<td>Type</td>
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<tr>
<td>First acid</td>
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<td>Second acid</td>
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The first acid may be selected from the group consisting of acetic acid (AcOH), TFA, hydrochloric acid (HCl), and trifluoroethanol (TFE) or combinations thereof. In general, the first acid is TFA in an organic solvent. The amount of TFA typically used for releasing the protected peptide from the solid support may range from about 0.1% to about 5% (v/v) in an organic solvent. Specific organic solvents that may be employed, include, for example, acetonitrile, benzene, butyl acetate, t-butyl methylketone, chlorobenzene, chloroform, chloromethane, cyclohexane, dichloromethane (DCM), dichloroethane, dichloroethene, fluoro pyridine, heptane, hexane, isobutylmethylketone, isopropyl acetate, methyl ethyl ketone, methyl tetrahydrofuran, pentyl acetate, n-propyl acetate, tetrahydrofuran, toluene, and combinations thereof. In an exemplary embodiment, the protected peptide is released from the solid support in the presence of dichloromethane by contacting the solid support with a solution of TFA and DCM as described in Table 1.

(C) Amidation of peptide acid

Methods of amidating peptides are known in the art and include enzymatic amidation systems, as well as chemical methods of amidating peptide acids. Non-limiting examples of enzymatic amidation methods include the use of α-amidating enzyme, peptide amidase, carboxypeptidase II, and carboxypeptidase Y.

In a preferred embodiment, the peptide acid is converted to peptide amide using di-tert-butyl pyrocarbonate (di-tert-butyl dicarbonate, Boc-anhydride) as activating agent in the presence of pyridine and ammonium hydrogen carbonate in the presence of an aprotic solvent. Suitable aprotic solvents include, but are not limited to, acetonitrile, diethoxymetha pyridine, N,N-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), N,N-dimethylpropiolamide, 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (DMPU), 1,3-dimethyl-2-imidazolidinone (DMI), 1,2-dimethoxyethane (DME), dimethoxymethane, bis(2-methoxyethyl)ether, N,N-dimethylacetamide (DMAC), 1,4-dioxane, N-methyl-2-pyrrolidinone (NMP), ethyl acetate, ethyl formate, ethyl methyl ketone, formamide, hexachloroacetone, hexamethylphosphoramide, methyl acetate, N-methylacetamide, N-methylformamide, methylene chloride, nitrobenzene, nitromethane, propionitrile, sulfolane, tetramethyleurea, tetrahydrofuran (THF), 2-methyl tetrahydrofuran, trichloromethane, and combinations thereof. In certain alternatives of this embodiment, the peptide acid is contacted with pyridine, pyrocarbonate, and ammonium bicarbonate in the presence of DMF. Generally speaking, the
peptide acid is amidated at approximately room temperature with the amount of peptide acid to pyridine, to pyrocarbonate to ammonium bicarbonate is a molar ratio of about 1:2:1.3:1.3. Exemplary reaction parameters of the process are detailed in the examples.

(D) Desmopressin yields

[0039] The yield of desmopressin from the process may vary within the scope of the present invention. However, in certain highly preferred aspects, particularly and especially those in which the desmopressin is cyclized on the solid support, the yield of desmopressin is at least about 20%, at least about 30%, at least about 35%, at least about 50%, or at least about 80%. In one embodiment, the yield of desmopressin is at least 30%. In another embodiment, the yield of desmopressin is at least 30%. In yet another embodiment, the yield of desmopressin is at least 30%. In still another embodiment, the yield of desmopressin is at least 35%.

DEFINITIONS

[0040] "SPPS" as used herein stands for solid phase peptide synthesis.
[0041] "DIC" as used herein stands for diisopropylcarbodiimide.
[0042] "DIEA" as used herein stands for diisopropylethylamine.
[0043] "DCM" as used herein stands for dichloromethane.
[0044] "DiVIF" as used herein stands for dimethylformamide.
[0045] "Fmoc" as used herein stands for 9-fluorenyl-methoxy-carbonyl.
[0046] "HOBT" as used herein stands for 1-hydroxybenzotriazole.
[0047] "TIS" as used herein stands for trisopropylsilane.
[0048] "TFA" as used herein stands for trifluoroacetic acid.
[0049] "AcOH" as used herein stands for acetic acid.
[0050] "ACN" as used herein stands for acetonitrile.
[0051] "DTE" as used herein stands for dithioerythretol.
[0052] "EDT" as used herein stands for ethanedithiol.
[0053] "IPA" as used herein stands for isopropanol.
[0054] "MeOH" as used herein stands for methanol.
"TFA" as used herein stands for trifluoroacetic acid.

"But (tBu)" as used herein stands for tert. Butyl.

"Trt" as used herein stands for trityl.

"pbf" as used herein stands for 2, 2, 4, 6, 7-pentamethyldihydrobenzofurane-5-sulfonyl.

"Mpa (mpr)" as used herein stands for Mercaptopropionic acid.

"Fmoc-RA-MBHAR" as used herein stands for 9-fluoromethyloxy carbonyl-Rinkamide-p-methyl benzhydrylamine resin.

"Fmoc-SR" as used herein stands for Fmoc-Sieberamide resin.

"CTC (CTR)" as used herein stands for 2-chlorotryptophyl chloride resin.

As various changes could be made in the above compounds, products and methods without departing from the scope of the invention, it is intended that all matter contained in the above description and in the examples given below, shall be interpreted as illustrative and not in a limiting sense.

**EXAMPLES**

**Example 1: Synthesis of the protected peptide resin**

Desmopressin was prepared by linear Fmoc-solid phase peptide synthesis on acid labile solid support using the DIC/HOBT, following standard procedure described in the art. The synthesis protocol is shown in **Table 2**. The synthesis of desmopressin was carried out manually (in a mechanically stirred 250ml reactor) on a solid support or on a c-terminal pre-loaded Fmoc-glycine resin.

**Table 2. Peptide Synthesis Protocol.**

<table>
<thead>
<tr>
<th>Step No.</th>
<th>Reagents/Solvents*</th>
<th>Times x Minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DMF Wash</td>
<td>1 x 5-10 minutes</td>
</tr>
<tr>
<td>2</td>
<td>20% Piperidine in DMF</td>
<td>2 x 20 minutes</td>
</tr>
<tr>
<td>3</td>
<td>DMF Wash</td>
<td>2 x 3 minutes</td>
</tr>
<tr>
<td>4</td>
<td>IPA wash</td>
<td>2 x 3 minutes</td>
</tr>
<tr>
<td>5</td>
<td>DMF Wash</td>
<td>3 x 3 minutes (until chloranil test is negative)</td>
</tr>
<tr>
<td>6</td>
<td>Coupling</td>
<td>1 hr to overnight or until ninhydrin test is negative.</td>
</tr>
<tr>
<td>7</td>
<td>DMF wash</td>
<td>1 x 3 minutes</td>
</tr>
<tr>
<td>8</td>
<td>IPA wash</td>
<td>1 x 3 minutes</td>
</tr>
</tbody>
</table>

* DMF = N,N-Dimethylformamide, IPA = Isopropyl alcohol, CAC = Coupling agent (such as HBTU), DMF = N,N-Dimethylformamide, and TFA = Trifluoroacetic acid.
In process controls included the chloranii test for testing the complete removal of piperidine after step 5, and the ninhydrin test to make sure the coupling is complete. These tests are described in Table 3.

<table>
<thead>
<tr>
<th>Table 3. In process controls.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloranii test</td>
</tr>
<tr>
<td>Ninhydrin test</td>
</tr>
</tbody>
</table>

The Fmoc resin or Fmoc glycline-loaded resin was charged to a 250 ml reactor and was agitated with DMF (5-6 volume) for approx. 5-10 min., and then the solvent was drained. A 20% piperidine solution in DMF was added and the mixture was agitated for 20 min., and then drained. This process was repeated one additional time. The resin was washed as described in the synthesis protocol in Table 2 to remove liberated dibenzofulvene and piperidine as determined by a chloranii test (process control A.). The resin was treated with a solution of 1.5 mole equivalent of Fmoc-amino acid and HOBT in DMF + DCM (3:1) and DIC and was agitated for one hour to overnight. An aliquot of the resin was removed from time to time, washed and subjected to a qualitative ninhydrin test. If the test was negative, then the resin was drained and washed with DMF, IPA, and DMF again (1 time each). If the test was weak positive, it was re-coupled: the Fmoc-amino acid and HOBT (1 mole equivalent) dissolved in DMF + DCM (3:1) was added in the reactor followed by the addition of 1 mole equivalent of DIC and was coupled as usual. If the test was slightly to very slightly positive, it was acetylated (5 equivalent of acetic anhydride and pyridine) in DMF: the resin was stirred in DMF + DCM (3:1), treated with five equivalents of acetic anhydride and pyridine for 20-30 min., and washed with DMF (5 times). The synthesis cycle was repeated until all the
methods are described below. Conditions used for synthesis of the protected peptide using the various resins are described below.

[0069] **(A) Synthesis of pre-desmopressin on Fmoc-Rinkamide-MBHA resin:** Weight of Fmoc-Rinkamide-MBHA resin = 12.2 g (sub. = 0.41 mm/g, total sub. = 5mm or 0.005 mole). Fmoc-amino acid = 0.005 mole x 1.5 eq. = 0.0075 mole. HOBT.H2O = 0.005 mole x 1.5 eq. = 0.0075 x 153.14 g = 1.15 g. DIC = 0.005 mole x 1.5 eq = 0.0075 mole x 156.6 ml = 1.18 ml. DMF + DCM (3: 1) = 45 ml + 15 ml = 60 ml (for coupling). Weight of pre-desmopressin resin = 24.6 g, equivalent to 103% of the theoretical yield = 23.6 g. Net gain in weight = 12.4 g, equivalent to 106.3% of the theoretical yield = 23.86g.

[0070] **(B) Synthesis of pre-desmopressin on Fmoc-Sieberamide resin:** Weight of Fmoc-Sieberamide resin = 6.9g (sub. = 0.58 mm/g, total sub. = 4.0 mm or 0.004 mole). Fmoc-amino acid = 0.004 mole x 1.5 eq. = 0.006 mole. HOBT.H2O = 0.006 mole x 153.14g = 0.92g. DIC = 0.006 mole x 156.6ml = 0.94 ml. Weight of pre-desmopressin = 15.448, equivalent to 95.1% of the theoretical yield = 16.238. Net gain in weight = 8.54 g, equivalent to 91.5% of the theory yield = 9.33g.

[0071] **(C) Synthesis of pre-desmopressin on 2-chlorotrityl chloride resin:**

[0072] 1. **Loading of Fmoc-glycine on chlorotrityl chloride resin (CTR):** preparation of Fmoc-Gly-CTR: 10g of CTC resin (chloride sub. = 1 .5mm/g or 15mm/total) was agitated with 70 ml of DCM for 15 minutes and drained. A solution of Fmoc-Gly (2.7g = 9mm, 0.6 eq. relative to acid) and DIEA (6.3ml, 36mm) in 50ml DCM was added to the resin, stirred for 2 hours and drained. The resin was then stirred with 50 ml of MeOH + DIEA (9:1) for 15-20 minutes and drained, followed by washing with DCM, DMF, DCM (1 time/each). A sample was taken out for substitution determination (sub = 0.5 mm/g), and then it was de-Fmoced for the coupling of the next amino acid following the synthesis protocol.

[0073] 2. **Synthesis of peptide on Gly-CTR:** Weight of Fmoc-amino acid = 0.005 mole x 1.5 eq = 0.0075 mole. HOBT = 0.0075 mole x 153.14 g = 1.15 g. DIC = 0.0075 mole x 156.6 ml = 1.18 ml. Weight of pre-desmopressin resin = 15.6 g, equivalent to 65.4% of a theoretical yield = 23.868. Net gain in weight = 5.6 g, equivalent to 48% of the theoretical yield = 11.66g.

**Example 2: Synthesis of desmopressin by cyclization on RA-MBHA, and Sieber amide resins**

[0074] Pre-desmopressin peptides synthesized using RA-MBHA and Sieber amide resins described in **Example 1** were cyclized, cleaved and deprotected to produce desmopressin using similar
SYNTHESIS SCHEME 1

N-α-Fmoc-Gly-resin 1, 2, 3

1. Deprotection (20% piperidine/DMF)
2. Coupling (DIC/HOBt)
3. Elongation

Mpa(Trt)-Tyr(OtBu)-Phe-Gln(Trt)-Asn(Trt)-Cys(Trt)-Pro-D.Arg(pbf)-Gly-resin 1, 2, 3

↓

iodine/DCM

Mpa-Tyr(OtBu)-Phe-Gln(Trt)-Asn(Trt)-Cys-Pro-D.Arg(pbf)-Gly-resin 1, 2, 3

(A) Cleavage
(TFA/scavengers)
Resins 1, 2

(B) Cleavage (1% TFA/DCM)
Resin 3

Desmopressin

 protected cyclic peptide-acid

TFA/scavengers

Protected desmopressin

[0075] (A) Synthesis of desmopressin by cyclization on RA-MBHA resin:

On-resin oxidation of pre-desmopressin RA-MBHA resin with iodine in DCM at RT and at O°C. Stirred 4.8 g (1mm) of pre-desmopressin-RA-MBHAR in 40ml DCM containing 0.32 ml (4mm) of pyridine. Added a solution of iodine (1g, 4mm) dissolved in 32 ml of DCM + DMF (3:1), continued stirring for one hour and filtered followed by washing with DCM (2 times), DMF (1x), and DCM (1x). Iodine/DCM oxidation was repeated again for 1 hour as mentioned above. It was filtered and washed with DCM, DMF, and DCM (2 times/each) and was dried. Weight of cyclic peptide resin = 4.13 g.

[0077] Cleavage of Desmopressin from RA-MBHA support. 3.0 g (0.726 mm) of cyclized peptide-resin was stirred with 30 ml of cleavage reagent TFA + H2O + TIS + EDT (94 + 2.5 + 2.5 mm). After 1 hour, filtered follow by washing with DCM (2 times), DMF (1x), and DCM (1x) and was dried. Weight of protected desmopressin = 3.10 g.
to yield 0.78g, equivalent to 100% of the theoretical yield of desmopressin. HPLC analysis revealed an estimated yield and purity of 51.51% and >73.23%, respectively.

[0078] Result of on-resin oxidation at 0°C: When on-resin oxidation was performed at 0°C (0.5 mm scale) followed by its cleavage (as described above in section 5.3, it yielded desmopressin in 101% yield (0.54g) compared to a theory yield of 0.54g. According to HPLC, the estimated yield was 37.7% of the theoretical yield, and the purity was 54.6%.

[0079] (B) Synthesis of desmopressin by cyclization on Sieber amide resin

[0080] On resin oxidation of pre-desmopressin-sieberamide resin with h/DCM at RT

Stirred 4.06 g (1 mm) of pre-desmopressin-Sieber amide resin in 30 ml DCM for 20 min and drained. It was then agitated with 30 ml DCM containing 0.32 ml (4 mm) of pyridine and added a solution of I2 (1.01 g, 4 mm) in 12 ml of DCM and DMF (3:1) and continued agitating for 2 hours. The resin was filtered, then washed once with DCM and then treated again with a second batch of I2/DCM and stirred for 1 hr. Filtered it, washed with DCM (lx), DMF (1x), MeOH (2x), and dried it. Weight of the peptide-resin after oxidation was 3.43 g.

[0081] Cleavage of desmopressin from Sieberamide resin. 2.0 g (0.583 mm) of the oxidized resin was stirred with 20 ml of TFA + H2O + TIS + EDT (94 + 2.5 + 2.5 + 1.0) for 4 hrs and was filtered followed by washing with TFA (2x) to remove the cleaved resin. The filtrate was evaporated and the residue left was treated with ether and then solid obtained was filtered followed by washing with ether and was dried. The yield of the crude peptide was 0.46 g, equivalent to 73.83% compared to a theory yield of 0.62 g. According to HPLC, the estimated yield was 38.2% and the purity was > 74.6%.

[0082] On-resin oxidation at 0°C: When on-resin oxidation was performed at 0°C (0.5 mm scale) followed by its cleavage (as described above), it yielded 0.44 g desmopressin, equivalent to 82.3% of the theoretical yield. The HPLC estimated yield and purity was 22.35% and >45.4% respectively.

[0083] Example 3: Synthesis of desmopressin by cyclization on CTC resin

[0084] Pre-desmopressin synthesized using CTC resin was cyclized as described in Example 2 above, using 2.4 g (0.5 mm) of peptide-CTR. A protected cyclized peptide acid was then cleaved, amidated and deprotected to produce desmopressin using similar procedure as described in Synthesis Scheme 1.

[0085] Release of protected cyclic peptide-acid from its support with 1% TFA/DCM. 1.91
[0086] Conversion of protected cyclic peptide-acid into its amide (protected desmopressin): A stirred solution of protected cyclic peptide acid (0.5 g, 0.27 mm) in 5 ml DMF was treated with pyridine (0.015 ml, 0.62 eq), BOC-anhydride (0.08 g, 30% excess) and ammonium bicarbonate (0.03 g, 30% excess) and stirring was continued overnight. It was diluted with water and precipitated solid was filtered followed by washing with water and dried. The dried solid was washed with hexane and dried again to yield 0.12 g, equivalent to 24% of the theoretical yield of protected desmopressin.

[0087] Conversion of protected desmopressin into desmopressin. Protected desmopressin (0.12 g, 0.064 mm) was cleaved with 5 ml of cleavage reagent TFA + H2O + TIS + EDT (94 + 2.5 + 2.5 + 1.0) for 4 hours and then evaporated residue was worked up with ether to yield 0.06 g (87.7%) of desmopressin. According to HPLC, the estimated yield was 26.5% of the theoretical yield, and purity was 59.6%.

Example 4: Synthesis of desmopressin by cyclizing after cleavage from RA-MBHA resin

[0088] In this example, a linear deprotected peptide amide (reduced desmopressin) is...
Cleavage and deprotection of peptide-RA-MBHAR; 1.0 g (0.2 mm) of pre-desmopressin resin was stirred with 10 ml of TFA + H2O + TIS + EDT (94 + 2.5 + 2.5 + 1.0) for 4 hours and filtered, followed by washing with TFA (2 times) to remove the cleaved resin. The filtrate was evaporated at low temp (40°C) and the residue was treated with ether to precipitate the reduced peptide. It was filtered followed by washing with ether (2 times) and dried to yield 3.67 g (78.6%) of the protected peptide compared to a theory yield of 4.67 g.

Example 5: Synthesis of desmopressin by cyclizing after cleavage from Sieberamide resin

In this example, a protected peptide amide is first cleaved from the Sieberamide resin, cyclized, and then deprotected to produce desmopressin as shown in Synthesis Scheme 2, and described in detail below.

Cleavage of protected peptide amide from the resin. Stirred 8.1 g (2 mm) of the peptide resin with 40 ml of DCM for 20-30 min and drained it. Stirred it with 40 ml of 1% TFA/DCM for 2 min and filtered. Repeated it 15 times monitoring the filtrate with TLC (the filtrate was collected in a flask containing 50 ml of 10% pyridine in methanol). Washed the resin with DCM, MeOH and DCM (2 times/each). Evaporated the combined filtrate and washes to dryness and stirred the residue with water for an hour and then filtered the solid followed by washing with water. The colorless solid was dried to yield 3.67 g (78.6%) of the protected peptide compared to a theory yield of 4.67 g.
stirred solution of iodine (1.78 g, 7 mm) in 220 ml of DCM + MeOH (10:1) and was stirred for 30 minutes. It was treated with 5% ascorbic acid solution at 0% to neutralize excess of iodine (dark solution became colorless) and stirring was continued for 20 minutes. The organic phase was separated, washed with water (2 times), dried over sodium sulfate, filtered and evaporated to dryness. The solid obtained was filtered from ether and was dried to yield 1.72 g (92.97%) of protected desmopressin, compared to a theoretical yield of 1.85 g.

[0095] **Cleavage of protected desmopressin into desmopressin.** 0.92 g (0.5 mm) of protected desmopressin was stirred with 10 ml of cleavage reagent TFA + H2O + TIS + EDT (94 + 2.5 + 2.5 + 1.0) for 3.5 hrs and was then evaporated to dryness. The residue was treated with ether and the precipitated solid was filtered followed by washing with ether and was dried to yield 0.6 g, equivalent to 111% of desmopressin, compared to a theoretical yield of 0.54 g. According to HPLC, the estimated yield was 64.95% and purity was 86.67%.
Example 6: Synthesis of desmopressin by cyclizing after cleavage from CTC resin (Scheme 3)

[0096] In this example, a protected peptide acid was first cleaved from the CTC resin and then processed into desmopressin using two synthesis schemes as described in Synthesis Scheme 3: (A)

SYNTHESIS SCHEME 3

N-α-Fmoc-Gly-CTC resin
1. Deprotection (20% piperidine/DMF)
2. Coupling (DIC/HOBT)
3. Elongation
Mpa(Trt)-Tyr(OtBu)-Phe-Gln(Trt)-Asn(Trt)-Cys(Trt)-Pro-D.Arg(pbf)-Giy-CTC resin

Cleavage (1% TFA/DCM)

Protected peptide-acid

I₂/DCM OXI

Protected cyclic peptide-acid

[VWNH₄HCO₃]

Protected cyclic peptide-amide

Cleavage (TFA/scavengers)

Protected peptide-amide

Desmopressin

The protected peptide acid was cyclized, amidated to produce protected desmopressin, and then deprotected to produce desmopressin, or (B) amidated to produce a linear peptide amide, cyclized to produce protected desmopressin, and then deprotected to produce desmopressin.

[0097] Cleavage of protected peptide-acid from its CTR. 10.92 g (3.5 mm) of peptide-CTR was treated with 30 ml of 1% TFA/DCM (15 times) with agitation for 2 minutes to release the protected peptide from the support and was worked up as described above. Yield was 8.28g (100%).
(A) Cyclization before amidation.

[C0098] Cyclization of protected peptide-acid with I2/DCM. A solution of protected peptide (3.5 g, 1.5 mm) in 60 ml of DCM + MeOH (5:1) containing pyridine (0.85 ml, 7 fold) was added to a stirred solution of iodine (2.7 g, 7-fold) in 330 ml of DCM + MeOH (10:1) and was stirred for 30 min. and was worked as usual (see section 5.8) to yield 1.1 g (39.7%) of the cyclic peptide.

[C0099] Conversion of cyclic peptide-acid into its amide. 1.1 g (0.6 mm) of cyclic peptide-acid (4228-198-2) was reacted with BOC anhydride and ammonium bicarbonate in presence of pyridine and was worked up with water as described under section 6.2 to yield 0.94 g (85.5%) of protected desmopressin.

[C0100] Cleavage of protected desmopressin into desmopressin. 0.92 g (0.5 mm) of the protected peptide, was stirred with 10ml of TFA + H2O + TIS + EDT (94 + 2.5 + 2.5 + 1.0) for 4 hrs and was evaporated to almost dryness. The residue was treated with ether and precipitated solid was filtered, washed with ether, and was dried to yield 0.55 g (101.8%) of crude desmopressin. According to HPLC, the estimated yield was 52.2%, and purity was > 74.8%

(B) Amidation before cyclization,

[C0101] Conversion of protected peptide-acid into its amide. A solution of linear peptide-acid (2.3 g, 1 mm) in 8ml DMF was stirred below -25°C and NMM (0.14 ml, 1.3 mm) followed by IBCF (0.17 ml, 1.3 mm) were added. After stirring for 10 min at or below -25°C, ammonium bicarbonate (0.1 1 g, 1.4 mm) was added and stirring was continued overnight. It was diluted with water and solid obtained, was filtered followed by washing with water and dried. The solid was washed again with hexane to yield 0.92 g (39.5%) of the acyclic peptide-amide.

[C0102] Cyclization of protected peptide-amide with I2/DCM. A solution of acyclic peptide, (0.82 g, 0.35 mm) in 18 ml DCM + MeOH (5:1) containing pyridine (0.2ml, 7-fold) was added to a stirred solution of I2 (0.62 g, 7-fold) in 66 ml of DCM + MeOH (10:1) and was stirred for 20 minutes. It was treated with ascorbic acid at 0°C to neutralize the excess iodine and then organic phase was separated, washed with water, dried over sodium sulfate, filtered and was evaporated into a solid. It was filtered from ether and hexane (1:1) and was dried to yield 0.58 g (89.2%) of protected desmopressin.

[C0103] Cleavage of protected desmopressin into desmopressin. 0.55 g (0.3 mm) of protected peptide, was stirred with 8 ml of TFA + H2O + TIS + EDT (94 + 2.5 + 2.5 + 1.0) for 4 hours and
the estimated yield was 32% and purity was >58.97%.
What is Claimed is:

1. A process for solid phase synthesis of desmopressin, the process comprising:
   (a) providing a solid support coupled with a glycine residue;
   (b) activating the carboxy group of a D-arginine residue that has its amine protected by an
       Fmoc group and its side chain protected by an acid labile group, followed by coupling the
       D-arginine residue to the glycine residue on the solid support of (a), and treatment of the
       solid support with a base to deprotect the amine group of the D-arginine residue;
   (c) activating the carboxy group of a proline residue that has its amine protected by a Fmoc
       group, followed by coupling the proline residue to the D-arginine residue on the solid
       support of (b), and treatment of the solid support with a base to deprotect the amine group
       of the proline residue;
   (d) activating the carboxy group of a cysteine residue that has its amine protected by a Fmoc
       group and its thiol group protected by a group labile to an oxidizing agent, followed by
       coupling the cysteine residue to the proline residue on the solid support of (c), and
       treatment of the solid support with an agent to deprotect the amine group of the cysteine
       residue;
   (e) activating the carboxy group of an asparagine residue that has its amine protected by a
       Fmoc group and its side chain protected by an acid labile group, followed by coupling the
       asparagine residue to the cysteine residue on the solid support of (d), and treatment of the
       solid support with a base to deprotect the amine group of the asparagine residue;
   (f) activating the carboxy group of a glutamine residue that has its amine protected by a Fmoc
       group and its side chain protected by an acid labile group, followed by coupling the
       glutamine residue to the asparagine residue on the solid support of (e), and treatment of
       the solid support with a base to deprotect the amine group of the glutamine residue;
   (g) activating the carboxy group of a phenylalanine residue that has its amine protected by a
       Fmoc group, followed by coupling the phenylalanine residue to the glutamine residue on
       the solid support of (f), and treatment of the solid support with a base to deprotect the
       amine of the phenylalanine residue:
tyrosine residue to the phenylalanine residue on the solid support of (g), and treatment of
the solid support with a base to deprotect the amine group of the tyrosine residue;
(i) activating the carboxy group of a mercaptopropionoic acid residue that has its thiol group
protected by a group labile b an oxidizing agent, followed by coupling the
mercaptopropionoic acid residue to the tyrosine residue on the solid support of (h);
(j) circularizing the peptide formed in (i) by simultaneously deprotecting and oxidizing the thiol
groups of the cysteine residue and the mercaptopropionoic acid residue, by contacting the
solid support of (i) with an oxidizing agent in a manner such that a disulfide bond is formed
between the cysteine residue and the mercaptopropionoic acid residue to form a cyclic
peptide; and
(k) contacting the solid support of (j) with an acid in a manner such that the amino acid side
chains are deprotected, and desmopressin is released from the solid support.

2. The process of claim 1, wherein the solid support is chosen from the group consisting of
NovaSyn® TGR resin, Rink amide resin, Rink amid MBHA resin, Rink amide AM resin, Rink
amide PEGA resin, Rink amide NovaGel® resin, Sieber amide resin, and NovaSyn® TG
Sieber resin and PEG resin.

3. The process of any of claims 1 to 2, wherein the carboxylic acid group of the amino acid
residues is activated by contacting the amino acid residue with a compound selected from the
group consisting of HOBt, DCC, DIEA, and DIC.

4. The process of any of claims 1 to 3, wherein the base used to deprotect the amine group is
chosen from piperidine, cyclohexylamine, 1,5-diazabicyclo [5,4,0] undec-5-ene, ethanolamine,
pyrrolidine 1,8-diazabicyclo[5,4,0]undec-7-ene, diethylamine, morpholine, and mixtures
thereof.

5. The process of any of claims 1 to 4, wherein the group labile to an oxidizing agent is chosen
from the group consisting of acetamidomethyl and trityl.

6. The process of any of claims 1 to 5, wherein the oxidizing agent is chosen from the group
consisting of iodine and ferricyanate salt.

7. The process of any of claims 1 to 6, wherein the oxidizing agent comprises iodine in an organic
solvent.
2,2,4,6,7-pentamethyl-dihydrobenzofuran-5-sulfonyl, 2,2,5,7,8-pentamethylchroman-6-sulphonyl, t-butyl, and trityl.

9. The process of any of claims 1 to 8, wherein the acid is chosen from the group consisting of trifluoroacetic acid, hydrochloric acid, acetic acid and trifluoroethanol.

10. The process of any of claims 1 to 9, wherein the acid comprises trifluoroacetic acid and appropriate scavengers.

11. The process of any of claims 1 to 10, wherein the yield of desmopressin is at least 30%.

12. The process of any of claims 1 to 10, wherein the yield of desmopressin is at least 50%.
A process for solid phase synthesis of desmopressin, the process comprising:

(a) providing a solid support coupled with a glycine residue;

(b) activating the carboxy group of a D-arginine residue that has its amine protected by an Fmoc group and its side chain protected by an acid labile group, followed by coupling the D-arginine residue to the glycine residue on the solid support of (a), and treatment of the solid support with a base to deprotect the amine group of the D-arginine residue;

(c) activating the carboxy group of a praline residue that has its amine protected by a Fmoc group, followed by coupling the proline residue to the D-arginine residue on the solid support of (b), and treatment of the solid support with a base to deprotect the amine group of the proline residue;

(d) activating the carboxy group of a cysteine residue that has its amine protected by a Fmoc group and its thiol group protected by a group labile to an oxidizing agent, followed by coupling the cysteine residue to the praline residue on the solid support of (c), and treatment of the solid support with an agent to deprotect the amine group of the cysteine residue;

(e) activating the carboxy group of an asparagine residue that has its amine protected by a Fmoc group and its side chain protected by an acid labile group, followed by coupling the asparagine residue to the cysteine residue on the solid support of (d), and treatment of the solid support with a base to deprotect the amine group of the asparagine residue;

(f) activating the carboxy group of a glutamine residue that has its amine protected by a Fmoc group and its side chain protected by an acid labile group, followed by coupling the glutamine residue to the asparagine residue on the solid support of (e), and treatment of the solid support with a base to deprotect the amine group of the glutamine residue;

(g) activating the carboxy group of a phenylalanine residue that has its amine protected by a Fmoc group, followed by coupling the phenylalanine residue to the glutamine residue on the solid support of (f), and treatment of the solid support with a base to deprotect the amine group of the phenylalanine residue;

(h) activating the carboxy group of a tyrosine residue that has its amine protected by a Fmoc
tyrosine residue to the phenylalanine residue on the solid support of (g), and treatment of the solid support with a base to deprotect the amine group of the tyrosine residue;
i) activating the carboxy group of a mercaptopropionic acid residue that has its thiol group protected by a group labile to an oxidizing agent, followed by coupling the mercaptopropionic acid residue to the tyrosine residue on the solid support of (h);
j) circularizing the peptide formed in (i) by simultaneously deprotecting and oxidizing the thiol groups of the cysteine residue and the mercaptopropionic acid residue, by contacting the solid support of (i) with an oxidizing agent in a manner such that a disulfide bond is formed between the cysteine residue and the mercaptopropionic acid residue to form a cyclic peptide;
k) contacting the solid support of (j) with a first acid in a manner such that a protected cyclic peptide acid is released from the solid support;
i) amidating the protected cyclic peptide acid of (k) to produce protected desmopressin; and
(m) contacting the protected desmopressin of (j) with a second acid in a manner such that the amino acid side chains are deprotected and desmopressin is produced.

14. The process of claim 13, wherein the solid support is chosen from the group consisting of chlorotrityl resins, trityl resins, methyltrityl resins, methoxytrityl resins, NovaSyn® TGT resin, HMPB-AM resin, HMPB-BHA resin, HMPB-MBHA resin, Wang resin, NovaSyn-TGA resin, HMPA-PEGA resin, HMPA-NovaGel resin, PAM resin, and Merrifield resin.

15. The process of any of claims 13 to 14, wherein the carboxylic acid group of the amino acid residues is activated by contacting the amino acid residue with a compound selected from the group consisting of HOBr, DCC, DIEA, and DIC.

16. The process of any of claims 13 to 15, wherein the base used to deprotect the amine group is chosen from piperidine, cyclohexylamine, 1,5-diazabicyclo[5,4,0]undec-5-ene, ethanolamine, pyrrolidine 1,8-diazabicyclo[5.4.0]undec-7-ene, diethylamine, morpholine, and mixtures thereof.

17. The process of any of claims 13 to 16, wherein the group labile to an oxidizing agent is chosen from the group consisting of acetamidomethyl and trityl.

18. The process of any of claims 13 to 17, wherein the oxidizing agent is chosen from the group
19. The process of any of claims 13 to 18, wherein the oxidizing agent comprises iodine in an organic solvent.

20. The process of any of claims 13 or 19, wherein the acid liable protecting group is chosen from the group consisting of acetamidomethyl, Boc, methoxytrimethylbenzenesulphonyl, t-butyl ester, 2,2,4,6,7-pentamethyl-dihydrobenzofuran-5-sulfonyl, 2,2,5,7,8-pentamethylchroman-6-sulphonyl, t-butyl, and trityl.

21. The process of any of claims 13 to 20, wherein the first acid is chosen from the group consisting of trifluoroacetic acid, hydrochloric acid, acetic acid and trifluoroethanol.

22. The process of claim 21, wherein the first acid comprises trifluoroacetic acid in an organic solvent.

23. The process of any of claims 13 to 22, wherein the second acid is chosen from the group consisting of trifluoroacetic acid, hydrochloric acid, acetic acid and trifluoroethanol.

24. The process of any of claims 13 to 23, wherein the second acid comprises trifluoroacetic acid and appropriate scavengers.

25. The process of any of claims 13 to 24, wherein the yield of desmopressin is at least 25%.

26. The process of any of claims 13 to 24, wherein the yield of desmopressin is at least 35%.
A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K7/16
ADD.

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)

C07K

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 practical, search terms used)

EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<th>Relevant to claim No</th>
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Further documents are listed in the continuation of Box C

See patent family annex

Date of the actual completion of the international search: 15 October 2010

Date of mailing of the international search report: 08/11/2010

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