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(54) Title: PROCESS FOR OCTREOTIDE SYNTHESIS

(57) Abstract: A hybrid solid phase-liquid phase method of octreotide synthesis is described, along with methods for preparation of intermediate compounds useful in the synthesis of Octreotide. The method comprises liquid phase condensation of two or three peptide blocks, of which at least one is synthesized by solid-phase method. Each block contains two or more amino acid residues. The method combines the time and labor effectiveness of the solid-phase method with relative cheapness and easiness of purification of the product, characteristic of the liquid-phase method.

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PROCESS FOR OCTREOTIDE SYNTHESIS

This application claims priority to and all benefit of Canadian Patent Application No. 2,458,084, filed March 12, 2004, the entirety of which is incorporated herein by reference.

Background of the invention

Octreotide (OCT) is the active pharmaceutical ingredient of the drug Sandostatin, using in treatment of some cancer diseases, especially acromegaly etc. It has the following chemical structure:

(D)-Phe-Cys-Phe-(D)-Trp-Lys-Thr-Cys-Thr-ol.

Conventional syntheses of OCT may be divided two main approaches, direct solid-phase synthesis and liquid-phase synthesis.

Direct solid-phase synthesis comprises attachment of a C-terminal amino acid to a resin, and step-by step elongation of the peptide chain, with pre-activated amino acids. This route is expensive because it requires large excesses of starting amino acids and additionally is quite labor consuming as the peptide size increases, necessitating complex purification procedures to separate the product from the impurities since they are very similar to the final product. These shortcomings are especially important for large scale industrial production of the product. For example, see Canadian Patent Application 2,309,312 and U.S. Patent No. 6,476,186. With each successive condensation reaction required to add an amino acid, waste of starting materials increases, and purification steps are repeated.

Liquid-phase synthesis comprises condensation of amino acids in solution. Several blocks, containing from 2 to 5 amino acids may be synthesized independently, followed by condensation of these synthons to each other in the required sequence. For example, see WO 03/097668; U.S. Patent No. 4,395,403; and RU 2196144 C1. The advantage of this kind of processes is that it is less expensive than the previous one and the product is easier to purify. This method is also more effective for scale-up. However, liquid phase synthesis of lengthy peptide blocks, for example having more than 3 amino acids, is

inefficient. Liquid-phase octreotide synthesis has the drawback is that the method is extremely labor-intensive and time consuming.

U.S. Patent No. 6,346,601 describes a method for octreotide synthesis where a solid-phase method is used to obtain a 7-mer, followed by condensation in solution with the modified amino acid threoninol. However, by using solid-phase synthesis to produce a 7-mer, only one less condensation is required compared to the solid-phase process for forming octreotide itself. Thus, only a marginal efficiency is introduced.

Summary of the invention

According to an embodiment of the invention, there is provided a process for obtaining octreotide or a pharmaceutically acceptable salt thereof by hybrid solid-phase – liquid-phase synthesis. The synthesis comprises the steps of condensing two or three peptide blocks using liquid phase condensation to form a condensation product followed by cyclizing the product. Each peptide block contains two or more amino acid residues, and at least one of the blocks is synthesized by solid-phase synthesis. The condensation product comprises in sequence the amino acids residues of octreotide. In the step of cyclizing, the condensation product is cyclized to form a disulfide bridge between the two cysteine residues, thereby forming octreotide.

Further, according to another embodiment of the invention, a process is provided for obtaining an intermediate in octreotide synthesis by hybrid solid-phase – liquid-phase synthesis. The synthesis of the intermediate comprises the steps of obtaining two or three peptide blocks, each peptide block containing two or more amino acid residues, and at least one of the blocks is synthesized by solid-phase synthesis. Subsequently, the peptide blocks are condensed using liquid phase condensation to form a condensation product, wherein the condensation product comprises in sequence the amino acids residues of octreotide.

This invention provides a more cost-effective and labor-saving method for obtaining OCT and its pharmaceutically acceptable salts by means of hybrid solid-phase – liquid-phase synthesis. The invention involves liquid phase condensation of two peptide blocks, at least one of which is obtained by solid-

phase synthesis, the blocks containing more two or more amino acid residue in every block, followed by formation of a disulfide bridge from the two cysteine groups. Optionally, three blocks may be condensed.

This hybrid solid phase-liquid phase method involves formation of one or more blocks of the octreotide amino acid sequence by solid-phase synthesis, followed by liquid phase condensation of the block(s) with required supplementary amino acids or other block(s) of amino acids. This method is a blend of solid-phase and liquid-phase synthesis methods, combining the efficiencies of preparing shorter (6-mer or less) peptides using a solid-phase method with relative cheapness and easiness of purification of the product, characteristic of the liquid-phase method.

Generally, the methods of invention comprise synthesizing specific side-chain protected peptide fragment intermediates of OCT on a solid support or in solution, coupling of the protected fragments in solution to form a protected OCT, followed by deprotection of the side chains and oxidation to yield the final OCT.

The present invention further relates to individual peptide fragments which act as intermediates in the synthesis of the OCT.

Detailed description of the invention

The invention provides a method for synthesis of octreotide, its salts, and intermediate compounds for preparation of octreotide. Octreotide is been synthesized by condensation of two or three fragments in solution. At least one of these fragments is synthesized by solid phase synthesis, whereas the remaining one or two fragments could be synthesized by a liquid-phase or solid-phase method. Examples of such fragments are shown below.

Fmoc-(D)-Phe-Cys(Trt)-Phe-(D)-Trp(Boc)-OH (I)

Boc-(D)-Phe-Cys(Trt)-Phe-(D)-Trp(Boc)-OH (II)

NH_2-Lys(Boc)-Thr(tBu)-Cys(Trt)-Thr(tBu)-ol (III)

Fmoc-(D)-Phe-Cys(Trt)-Phe-(D)-Trp(Boc)-Lys(Boc)-OH (IV)

Boc-(D)-Phe-Cys(Trt)-Phe-(D)-Trp(Boc)-Lys(Boc)-OH (V)

NH_2-Thr(tBu)-Cys(Trt)-Thr(tBu)-ol (VI)

Fmoc-(D)-Phe-Cys(Trt)-Phe-(D)-Trp(Boc)-Lys(Boc)-Thr(tBu)-OH (VII)

Boc-(D)-Phe-Cys(Trt)-Phe-(D)-Trp(Boc)-Lys(Boc)-Thr(tBu)-OH (VIII)

NH_2-Cys(Trt)-Thr(tBu)-ol (IX)

Fmoc-(D)-Phe-Cys(Trt)-Phe-OH (X)

Boc-(D)-Phe-Cys(Trt)-Phe-OH (XI)

NH_2-Trp(Boc)-Lys(Boc)-Thr(tBu)-Cys(Trt)-Thr(tBu)-ol (XII)

Optimally, two peptide blocks are condensed in the step of condensing. At least one peptide block synthesized by solid-phase synthesis preferably contains at least three amino acid residues, and more preferably contains at least four amino acid residues.

Solid-phase synthesis may be performed on a super acid sensitive resin, such as CTC resin, or any other resin acceptable to those skilled in the art. The step of condensing may be accomplished by use of HBTU, TBTU or HATU as a condensation agent, or by employing any other agent as would be acceptable to those skilled in the art. An α-amino group on at least one peptide block may be protected with a Boc or Fmoc protecting group. Further, a side-chain of at least one amino acid residue of at least one peptide block may be protected with a t-Butyl, Trityl, Boc, or Acm protecting group.

Solid-phase synthesis of short fragments containing 2 amino acid residues is economically not expedient, therefore the preferred embodiment of the invention comprises the fragments synthesized by solid-phase method containing 3 or more amino acid residues. For example, two tetramers may be used.

The side-chains of the amino acid residues of peptide fragments may be protected with standard protecting groups such as t-Butyl, trityl, Boc, Acm. The tBu group is preferred side-chain protecting group for amino acid residues Thr and threoninol; the Trt and Acm groups are preferred side-chain protecting groups for Cys; the Boc group is preferred side-chain protecting group for amino acid residues Lys and Trp.

Resin loading can be performed via reaction of excess of an amino acid or an amino alcohol and super acid sensitive resin (for example, 2-chlorotrityl chloride resin) in the presence of amine.

Standard Fmoc protocols can be used for synthesis of the fragments. Removal of the Fmoc protecting group from the terminal amine group can be

accomplished by treating the resin with piperidine solution in DMF. The protected amino acid may be activated through the reaction with condensing agents well-known to those skilled in the art (such as HBTU or TBTU). Coupling completion may be monitored with a qualitative ninhydrin test.

The peptide fragments synthesized via solid phase synthesis techniques can be cleaved by acidic treatment of the peptidyl-resin with dilute solution of acid. The preferred acids for this purpose are TFA or HCl.

The cleaved peptide fragments can be coupled in the solution through activation of the N-terminal fragment carrying alpha-amino protecting group (Boc or Fmoc for example) with an appropriate coupling agent. The preferred coupling agents are HBTU or TBTU, though other usually used agents might be chosen for this transformation.

The protected octapeptide can be precipitated by the addition of water and collected by vacuum filtration. The final deprotection of the OCT can be done by treatment of the protected peptide with the solution of acid, and the preferred acid is TFA.

The cyclic product can be obtained by reacting the linear peptide with an appropriate reagent (for example hydrogen peroxide or iodine) in a buffer solution. Crude product can be purified by using any acceptable methodology in the art, such as reverse phase, normal-phase or ion-exchange chromatography.

Examples

Example 1: Fmoc-Thr(tBu)-ol-CTC-resin synthesis

The 2-chlorotrityl chloride resin (25g, 1 eq) was charged to a 500 ml SPS reactor and washed with 250 ml of DCM. The bed was drained and a solution of Fmoc-Thr(tBu)-ol (1.5 eq) and DIPEA (3 eq) in 250 ml of DCM was added. The mixture was agitated with nitrogen bubbling for 2 hrs.

The bed was drained and washed with 250 ml of DCM. The active sites on the resin were end-capped with 200 ml of a 5:4:1 MeOH:DCM:DIPEA solution for 20 min. The bed was drained, washed with 4x250 ml of DCM, dried with an argon purge to give 34.4 g of loaded resin.

Example 2: Fmoc-(D)-Trp(Boc)-CTC-resin synthesis

The 2-chlorotrityl chloride resin (25g, 1 eq) was charged to a 500 ml SPS

reactor and washed with 250 ml of DCM. The bed was drained and a solution of Fmoc-(D)Trp(Boc)-OH (1.5 eq) and DIPEA (3 eq) in 250 ml of DCM was added. The mixture was agitated with nitrogen bubbling for 2 hrs.

The bed was drained and washed with 250 ml of DCM. The active sites on the resin were end-capped with 200 ml of a 5:4:1 MeOH:DCM:DIPEA solution for 20 min. The bed was drained, washed with 4x250 ml of DCM, dried with an argon purge to give 34.4 g of loaded resin.

Example 3: Solid phase synthesis of the peptide fragment Lys(Boc)-Thr(tBu)-Cys(Trt)-Thr(tBu)-ol

The 500 ml SPS reactor was charged 20.0 g of Fmoc-Thr(tBu)-ol-2-chlorotrityl resin. The resin was conditioned in 200ml of DCM with nitrogen agitation for about 15 min to swell the beads, and then drained. Removal of the Fmoc protecting group from the terminal amine was accomplished by treating the resin with 2 aliquots of 20% solution piperidine in DMF. The resin is then washed 5-7 times with aliquots of DMF to remove the Fmoc by-products.

Fmoc-Cys(Trt)-OH was activated as follows. The Fmoc-protected amino acid (2 eq), 1-hydroxybenzotriazole hydrate (HOBT) (2 eq) and DIPEA (4 eq) were dissolved in NMP (about 10 volumes) at rt. The solution is chilled to 0-5°C and then HBTU (2 eq) is added followed by stirring for 5-15 min to dissolve. The solution of activated amino acid was added to the drained resin and the reaction was agitated with argon bubbling for about 1 hr. Coupling completion may be monitored with a qualitative ninhydrin test.

The cycle was repeated for Fmoc-Thr(tBu)-OH and Fmoc-Lys(Boc)-OH. Following the final coupling reaction and washings the deprotection step was performed and the resin was washed with DMA, DCM. The resin was dried with an argon purge to give 36 g resin-bond peptide.

The peptide was cleaved from 21 g of the resin using 300 ml of 1% TFA in DCM for about 2 min, followed by 200 ml of 0.5% TFA in DCM. The cleavage fractions were collected onto pyridine (1:1 ratio to TFA). The cleavage washes were concentrated under vacuum to a volume 50 ml. The product was precipitated with addition of 200 ml of water. The slurry was stirred for 30 min. The solids were collected by vacuum filtration and washed with 100 ml of water.

The product was air dried to give 15 g of the peptide fragment.

Example 4: Solid phase synthesis of the peptide fragment Boc-(D)-Phe-Cys(Trt)-Phe-(D)-Trp(Boc)-OH

The 500 ml SPS reactor was charged with 20.0 g of Fmoc-(D)-Trp(Boc)-2-chlorotrityl resin. The resin was conditioned in 200ml of DCM with nitrogen agitation for about 15 min to swell the beads, and then drained. After Fmoc removal, Fmoc-Phe-OH was activated as described above.

The cycle was repeated for Fmoc-Cys(Trt)-OH and Boc-(D)-Phe-OH. Following the final coupling reaction and washings the resin was dried with an argon purge to give 38 g resin-bond peptide.

The peptide was cleaved from 21 g of the resin using 300 ml of 1% TFA in DCM for about 2 min, followed by 200 ml of 0.5% TFA in DCM. The cleavage fractions were collected onto pyridine (1:1 ratio to TFA). The cleavage washes were concentrated under vacuum to a volume 50 ml. The product was precipitated with addition of 200 ml of water. The slurry was stirred for 30 min. The solids were collected by vacuum filtration and washed with 100 ml of water. The product was air dried to give 17 g of the peptide fragment.

Example 5: Synthesis of Boc-(D)-Phe-Cys(Trt)-Phe-(D)-Trp(Boc)-Lys(Boc)-Thr(tBu)-Cys(Trt)-Thr(tBu)-ol

A 25 ml round bottom flask containing a magnetic stir bar was charged with Lys(Boc)-Thr(tBu)-Cys(Trt)-Thr(tBu)-ol, Boc-(D)-Phe-Cys(Trt)-Phe-(D)-Trp(Boc)-OH and HOBt. The solids were dissolved in 9:1 DMA:DMSO (5 ml) containing DIPEA, and then cooled to 0-5°C under nitrogen. To the cool solution HBTU was added. The reaction mixture was stirred for 15 min, then warmed up to room temperature and stirred an additional 60 min. The peptide was precipitated from the solution by addition of water, 7 ml. The solid was collected by filtration, washed with water and dried give 0.30 g of Boc-(D)-Phe-Cys(Trt)-Phe-(D)-Trp(Boc)-Lys(Boc)-Thr(tBu)-Cys(Trt)-Thr(tBu)-ol.

Example 6: Synthesis of (D)-Phe-Cys-Phe-(D)-Trp-Lys-Thr-Cys-Thr-ol.

Boc-(D)-Phe-Cys(Trt)-Phe-(D)-Trp(Boc)-Lys(Boc)-Thr(tBu)-Cys(Trt)-Thr(tBu)-ol was cleaved with the mixture 95%TFA-2.5%EDT-2.5%H_2O at room

temperature for 2 hours. The cleavage solution was filtered, the resin was washed with TFA. The combined washings were concentrated under vacuum to volume 0.5-1 ml followed by addition of 10 volumes of cold ether. The pellets were collected by centrifugation and washed with cold ether. The product was extracted with 50% ACN and dried by lyophilization.

Example 7: Synthesis of Octreotide

(D)-Phe-Cys-Phe-(D)-Trp-Lys-Thr-Cys-Thr-ol was dissolved in water at concentration 100 mg/L. The pH of the solution was adjusted with 10 % NaOH to 8.0. 0.5 ml of H_2O_2 was added to the solution. The reaction mixture was stirred for 2 hrs, lyophilized. The crude product was purified by reverse phase HPLC and ion-exchange LC.

Claims

1. A process for obtaining octreotide or a pharmaceutically acceptable salt thereof by hybrid solid-phase – liquid-phase synthesis, comprising the steps of:

condensing two or three peptide blocks using liquid phase condensation to form a condensation product, each peptide block containing two or more amino acid residues, at least one of the blocks being synthesized by solid-phase synthesis, and wherein the condensation product comprises in sequence the amino acids residues of octreotide; and

cyclizing the condensation product to form a disulfide bridge between two cysteine residues.

2. The process of claim 1 wherein two peptide blocks are condensed in the step of condensing.

3. The process of claim 1 or 2, wherein one peptide block synthesized by solid-phase synthesis contains at least three amino acid residues.

4. The process of claim 3, wherein one peptide block synthesized by solid-phase synthesis contains at least four amino acid residues.

5. The process of any one of claims 1 to 4, wherein solid phase synthesis is performed on super acid sensitive resin.

6. The process of claim 5, wherein the super acid sensitive resin is CTC resin.

7. The process of any one of claims 1 to 6, wherein the step of condensing is accomplished by use of HBTU, TBTU or HATU as a condensation agent.

8. The process of any one of claims 1 to 7, wherein an α-amino group on at least one peptide block is protected with a Boc or Fmoc protecting group.

9. The process of any one of claims 1 to 8, wherein a side-chain of at least one amino acid residue of at least one peptide block is protected with a t-Butyl, Trityl, Boc, or Acm protecting group.

10. The process of any one of claims 1 to 19, wherein the step of cyclizing is accomplished by action of an oxidative reagent.

11. The process of claim 10, wherein the oxidative reagent is hydrogen peroxide or iodine.

12. The process of any one of claims 1 to 11, wherein at least one of the peptide blocks is selected from the group consisting of:
Fmoc-(D)-Phe-Cys(Trt)-Phe-(D)-Trp(Boc)-OH (I)
Boc-(D)-Phe-Cys(Trt)-Phe-(D)-Trp(Boc)-OH (II)
NH_2-Lys(Boc)-Thr(tBu)-Cys(Trt)-Thr(tBu)-ol (III)
Fmoc-(D)-Phe-Cys(Trt)-Phe-(D)-Trp(Boc)-Lys(Boc)-OH (IV)
Boc-(D)-Phe-Cys(Trt)-Phe-(D)-Trp(Boc)-Lys(Boc)-OH (V)
NH_2-Thr(tBu)-Cys(Trt)-Thr(tBu)-ol (VI)
Fmoc-(D)-Phe-Cys(Trt)-Phe-(D)-Trp(Boc)-Lys(Boc)-Thr(tBu)-OH (VII)
Boc-(D)-Phe-Cys(Trt)-Phe-(D)-Trp(Boc)-Lys(Boc)-Thr(tBu)-OH (VIII)
NH_2-Cys(Trt)-Thr(tBu)-ol (IX)
Fmoc-(D)-Phe-Cys(Trt)-Phe-OH (X)
Boc-(D)-Phe-Cys(Trt)-Phe-OH (XI) and
NH_2-Trp(Boc)-Lys(Boc)-Thr(tBu)-Cys(Trt)-Thr(tBu)-ol (XII).

13. The process of any one of claims 1 to 11 wherein the step of condensing comprises condensing two peptide blocks formed using solid phase synthesis, the peptide blocks having the following amino acid residues:
(D)-Phe-Cys-Phe-(D)-Trp and Lys-Thr-Cys-Thr-ol.

14. The process of claim 12 wherein the two peptide blocks are:
Fmoc-(D)-Phe-Cys(Trt)-Phe-(D)-Trp(Boc)-OH (I)
and
NH_2-Lys(Boc)-Thr(tBu)-Cys(Trt)-Thr(tBu)-ol (III).

15. The process of claim 14 wherein the peptide block Fmoc-(D)-Phe-Cys(Trt)-Phe-(D)-Trp(Boc)-OH (I) is prepared by forming a protected resin: Fmoc-(D)-Trp(Boc)-CTC-resin; deprotecting the resin; successively coupling amino acids to the resin to form Fmoc-(D)-Phe-Cys(Trt)-Phe-(D)-Trp(Boc)-CTC-resin; and cleaving the peptide block from the resin.

16. The process of claim 14 wherein the peptide block NH_2-Lys(Boc)-Thr(tBu)-Cys(Trt)-Thr(tBu)-ol (III) is prepared by forming a protected resin: Fmoc-Thr(tBu)-ol-CTC-resin; deprotecting the resin; successively coupling amino acids to the resin; and cleaving the peptide block from the resin.

17. The process of claim 2 wherein the two peptide blocks are prepared using solid-phase synthesis.

18. A process for obtaining an intermediate in octreotide synthesis by hybrid solid-phase – liquid-phase synthesis, comprising the steps of:

obtaining two or three peptide blocks, each peptide block containing two or more amino acid residues, at least one of the blocks being synthesized by solid-phase synthesis;

condensing the peptide blocks using liquid phase condensation to form a condensation product, wherein the condensation product comprises in sequence the amino acids residues of octreotide.

19. The process of claim 18 wherein two peptide blocks are condensed in the step of condensing.

20. The process of claim 18 or 19, wherein one peptide block synthesized by solid-phase synthesis contains at least three amino acid residues.

21. The process of claim 20, wherein one peptide block synthesized by solid-phase synthesis contains at least four amino acid residues.

22. The process of any one of claims 18 to 21, wherein solid phase synthesis is performed on super acid sensitive resin.

23. The process of claim 22, wherein the super acid sensitive resin is CTC resin.

24. The process of any one of claims 18 to 23, wherein the step of condensing is accomplished by use of HBTU, TBTU or HATU as a condensation agent.

25. The process of any one of claims 18 to 24, wherein an α-amino group on at least one peptide block is protected with a Boc or Fmoc protecting group.

26. The process of any one of claims 18 to 25, wherein a side-chain of at least one amino acid residue of at least one peptide block is protected with a t-Butyl, Trityl, Boc, or Acm protecting group.

27. The process of any one of claims 18 to 26, wherein at least one of the peptide blocks is selected from the group consisting of:
Fmoc-(D)-Phe-Cys(Trt)-Phe-(D)-Trp(Boc)-OH (I)
Boc-(D)-Phe-Cys(Trt)-Phe-(D)-Trp(Boc)-OH (II)
NH_2-Lys(Boc)-Thr(tBu)-Cys(Trt)-Thr(tBu)-ol (III)
Fmoc-(D)-Phe-Cys(Trt)-Phe-(D)-Trp(Boc)-Lys(Boc)-OH (IV)
Boc-(D)-Phe-Cys(Trt)-Phe-(D)-Trp(Boc)-Lys(Boc)-OH (V)
NH_2-Thr(tBu)-Cys(Trt)-Thr(tBu)-ol (VI)

Fmoc-(D)-Phe-Cys(Trt)-Phe-(D)-Trp(Boc)-Lys(Boc)-Thr(tBu)-OH (VII)

Boc-(D)-Phe-Cys(Trt)-Phe-(D)-Trp(Boc)-Lys(Boc)-Thr(tBu)-OH (VIII)

NH_2-Cys(Trt)-Thr(tBu)-ol (IX)

Fmoc-(D)-Phe-Cys(Trt)-Phe-OH (X)

Boc-(D)-Phe-Cys(Trt)-Phe-OH (XI) and

NH_2-Trp(Boc)-Lys(Boc)-Thr(tBu)-Cys(Trt)-Thr(tBu)-ol (XII).

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2005/000378

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7): C07K 7/06, C07K 1/00

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(7): C07K 7/06, C07K 1/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Delphion, STN (biosis, caplus, medline), CPD, Internet search engine; Keywords: sandostatin, octreotide, peptide synthesis, solid-phase, liquid phase, hybrid

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 6,346,601 A (LIPOTEC S.A. (ES)) 12 February 2002 - the entire document, cited in the application	1-27
Y	BRUCKDORFER, T. et al. From production of peptides in milligram amounts for research to multi-tons quantities for drugs of the future. Current Pharmaceutical Biotechnology, February 2004, Volume 5, No. 1, pages 29-43, ISSN 1389-2010. - Figures 9, 10; page 35	1-27
A	FISCHER, P.M. and ZHELEVA, D.I. Liquid-phase peptide synthesis on polyethylene glycol (PEG) supports using strategies based on the 9-fluorenylmethoxycarbonyl amino protecting group: application of pegylated peptides in biochemical assays. Journal of Peptide Science, August 2002, Volume 8, No. 9, pages 529-542, ISSN 1075-2617.	1-27

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
23 June 2005 (23-06-2005)	12 July 2005 (12-07-2005)

Name and mailing address of the ISA/CA	Authorized officer
Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001(819)953-2476	Michael W. De Vouge (819) 997-2952

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2005/000378

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6,476,186 B1 (INSTITUTE OF NUCEAR ENERGY RESEARCH (TW)) 5 November 2002 - cited in the application	1-27
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INTERNATIONAL SEARCH REPORT

Information on patent family members

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