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E S D

07
SUPERINTENDENCIA Y COMERCIO
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Referencia Expediente No 02 061 740
Asunto Solicitud de patente para "PROCEDIMIENTO PARA LA PREPARACIÓN DE COMPUESTOS QUE CONTIENEN UNO O MAS ENLACES AMIDA, EN PARTICULAR PÉPTIDOS"
Solicitante AKZO NOBEL N V
Fecha de Solicitud 26 de Julio de 2000
Publicada en Gaceta 536 del 30 de Enero de 2004

1 Yo, Emilio FERRERO, identificado como aparece al pie de mi firma, actuando como apoderado en el asunto de la referencia, atentamente, solicito se efectúe el examen de patentabilidad de la solicitud en referencia segun lo establecido en el artículo 44 de la Decisión 486 de la Comisión de la Comunidad Andina Para tal efecto presento junto con este escrito el recibo No 04 – 10,031 por valor de \$353 000 00, que corresponde a la tasa fijada según lo dispuesto en la resolución No 37323 del 30 de diciembre de 2003

2 Ruego atentamente a la Señora Jefe se sirva ordenar que se anexe este recibo, para que se efectue dicho examen y se continúe con el trámite de la solicitud

Bogotá, 24 MAR 2004
Señora Jefe,


EMILIO FERRERO
T P A No 31 929

 COLO 11214-801-028-6
PRP/PRP/ID-000304/WPC11214

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***** CONSIGNACION *****

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CAVELIER ARBOREDO	CONSIGNACION	BANCO POPULAR	050-00110 6	10407410	03/02/2004	32,025,000 00

***** C O N C E P T O *****

CANT	CONCEPTO	TOTAL CONCEPTO
50005 01-01	SOLICITUDES	
	05 EXAMEN DE PATENTABILIDAD DE INVEN	353 000 00
	TOTAL	353,000 00

SOM TRESCIENTOS CINCUENTA Y TRES MIL PESOS

RESPONSABLE

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RECIBO DE CAJA APLICADO AL EXPEDIENTE N° _____

Cdo 11214-801-028-6

DIVISION DE NUEVAS CREACIONES

RADICACIÓN EXPEDIENTE No 02-61740

**EL EXTRACTO DE LA PRESENTE SOLICITUD DE PATENTE DE INVENCION
FUE PUBLICADO EN LA GACETA DE PROPIEDAD INDUSTRIAL No 536, DE
FECHA 30 DE ENERO DE 2004, EL TERMINO HABIL SEÑALADO POR LA LEY
EXPIRA EL 28 DE ABRIL DE 2004**

NOTA Dentro del plazo de los seis (6) meses siguientes a partir de la fecha de publicación en la gaceta de Propiedad Industrial, deberá solicitar y cancelar el examen de patentabilidad so pena de que la solicitud calga en abandono, de acuerdo con lo establecido en el Artículo 44 de la Decisión 486/2000

SE PRESENTARON OPOSICIONES POR PARTE DE TERCEROS

SI

NO **X**

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**SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISIÓN DE NUEVAS CREACIONES**

2020

Bogotá, DC, 07 de septiembre de 2006

Apoderado Emilio Ferrero

Oficio J J J 10660

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 Evento 001
 Actuación 430
 Folios

Como resultado del examen de fondo, realizado con fundamento en el artículo 45 de la decisión 486 de la Comisión de la Comunidad Andina, se le comunica

1 Documentos estudiados

- ◆Capítulo descriptivo, folios 03 a 15,
- ◆Capítulo reivindicatorio, folios 16 a 18,
- ◆1º examen de forma, folio 43,
- ◆Solicitud de la cual se reivindica prioridad, junto con su traducción, folios 31 a 64,
- ◆2º examen de forma, folio 65,
- ◆Solicitud del examen de patentabilidad 67 y 68

Objeto de la invención

El título de la invención es "PROCEDIMIENTO PARA LA PREPARACIÓN DE COMPUESTOS QUE CONTIENEN UNO O MÁS ENLACES AMIDA, EN PARTICULAR PÉPTIDOS"

El objeto de la presente solicitud se refiere a un procedimiento para la síntesis de péptidos especialmente en solución

Determinación del estado de la técnica

Considerando que la fecha de presentación de la primera solicitud de patente de la cual se reivindica prioridad es 19-jul-2001, se buscaron documentos del estado de la técnica relacionados, con fecha de publicación anterior a esta

Se hizo la búsqueda de documentos relevantes en el estado de la técnica, entendiendo como estado de la técnica la definición provista en el artículo 16

de la Decisión 486 de la Comisión de la Comunidad Andina y las excepciones establecidas en el artículo 17 de la misma

Consultadas la fuente de información con que cuenta este despacho se encontraron los siguientes documentos, anteriores a la fecha de la prioridad invocada

D1	WO0071569	30/nov/2000	Minimal isolation peptide síntesis process using ion-exchange resins as scavenging agents
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Evaluación de Novedad

Esta se evaluó por comparación entre lo reclamado como nuevo y el documento citado arriba y de acuerdo con la definición de novedad del Artículo 16 de la Decisión 486 de la Comisión de la Comunidad Andina, y se determinó la solicitud en estudio basa su novedad en que después de la acilación, una amina que comprende una unión libre o un anión latente es usada como agente barrido de las funciones carboxílicas activadas residuales

-La anterioridad D1 describe un proceso para la producción de péptidos de manera rápida con un aislamiento mínimo que se lleva a cabo en las mismas etapas que la presente solicitud, y se utiliza una amina con unión libre y también se utiliza un anión latente como agente barrido de las funciones carboxílicas activadas residuales, de las tal como puede verse en todo el documento D1, especialmente los ejemplos, las figuras 1, 2 y las reivindicaciones. Donde se puede observar que la anterioridad realmente se anticipa a describir la materia que se reclama como nueva en la solicitud en estudio

En conclusión queda plenamente establecido que el proceso reclamado era conocido en el estado de la técnica y una materia que tiene falta de novedad obviamente no tiene nivel inventivo

No	Documento	Reivindicaciones afectadas	Requisito afectado
D1	WO0071569	1 a 10	Novedad y <i>NI</i>

En cumplimiento del artículo 45 de la decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta días contados a partir de la fecha de la notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Notifíquese conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo 5° del título 1° de la Circular Única n° 10 de 2001.


ALIX CARMENZA CÉSPEDES DE VERGEL
Jefe de la División de Nuevas Creaciones

El anterior requerimiento fue notificado por fijación en lista de fecha 21 SET 2006'

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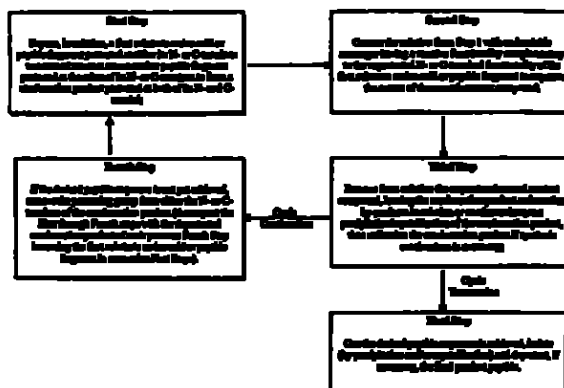
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- Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: MINIMAL ISOLATION PEPTIDE SYNTHESIS PROCESS USING ION-EXCHANGE RESINS AS SCAVENGING AGENTS



(57) Abstract: A process for the production of a polypeptide having a pre-determined number and sequence of amino acid residues, comprising the steps of first exposing a first substrate amino acid or peptide fragment to a stoichiometric excess of a second reactant amino acid or peptide fragment to form a condensation product; second, contacting the reaction solution from the first step with an insoluble scavenger to sequester the excess of the second reactant amino acid or peptide fragment; third, removing from the solution the sequestered excess second reactant amino acid or peptide fragment; fourth, subjecting the reaction solution to a reaction which removes the protecting group from either the N- or C-terminus of the condensation product of the first step; and fifth, if necessary repeating the first through fourth steps. The method is capable of large-scale production of peptides in solution, is not subject to the one-terminus-only limitation of the solid-phase method, possesses the "cleanliness" of the solid-phase method and, like the solid-phase method, is capable of automation. Most importantly, however, the method of the present invention does not require the frequent isolation of intermediates in a lengthy synthetic sequence nor, necessarily, the removal of all contaminating by-products from the reaction mixture prior to subsequent processing steps.

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**MINIMAL ISOLATION PEPTIDE SYNTHESIS PROCESS USING ION-EXCHANGE RESINS AS
SCAVENGING AGENTS**

This application is a continuation-in-part of U S Patent Application Serial No
09/322,762 filed May 26 1999

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Technical Field

The present invention relates to synthetic chemical processes More particularly the
present invention concerns a solution-phase process, particularly adapted to the production of
commercial-scale quantities of polypeptides, which minimizes the requirement of isolation of
intermediates

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Background of the Invention

Prior to the discovery by R Bruce Merrifield in 1963 of a solid-phase method for
synthesizing polypeptides (R B Merrifield, J Am Chem Soc, 85 2149-2154 (1963)), processes
for the preparation of peptides containing more than a small number of amino acid residues were
difficult and time-consuming. Since that time the so-called Merrifield solid-phase technique has
been used for the production of a large number of peptides

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The first stage of solid-phase peptide synthesis consists of the assembly of a peptide chain
on a supporting insoluble polymer or resin by sequential reactions of protected amino acid
derivatives In a subsequent stage, the peptide chain is cleaved from the solid resin support with
concurrent or subsequent cleavage of side-chain protecting groups to give the crude free peptide

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This technique has been adapted for use in two alternative methods In the stationary
solid-phase variant originally employed by Merrifield, the reagents and washing solvents are
passed through a column of resin beads to which the growing peptide chain is attached and upon
which it is supported This synthetic method is limited to adding new amino acid residues to the
growing peptide chain only at the N-terminus since the chain is typically bound to the resin at its
C-terminus In a typical stationary solid-phase synthesis, individual amino acids are added to the
N-terminus of the growing peptide chain until the desired polypeptide is obtained In a less
frequently used alternative, small peptide fragments consisting of multiple amino acid residues
are added to the growing peptide bound to the resin.

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In an alternative solid-phase peptide synthesis method, resin beads are slurried in a
container and are exposed to successive reagent solutions or washing solvents with each being
removed, typically through a filter in the bottom of the container before the next is added The
slurry method of solid-phase peptide synthesis is likewise limited to one terminus-only synthesis
of peptides Both of the above-described variants of the solid-phase peptide synthesis method are

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capable of being automated and are well suited to the preparation of milligram to multi-gram quantities of peptides. A number of automated solid-phase peptide synthesizers are commercially available which employ a microcomputer to open and close valves which control the sequence and duration of flow of various reagents and washing solvents delivered to a resin on which the growing peptide is supported. When quantities of peptides exceeding hundreds of grams are required, the solid-phase synthetic method is generally inadequate and resort is made to the solution-phase method.

In the solution-phase method, polypeptides are pieced together by classical solution chemistry which facilitates joining together individual amino acids or di- tri- tetra- or oligopeptide fragments of the final polypeptide in which sites of unwanted reaction have been appropriately protected. The smaller fragments are themselves similarly prepared by piecing together individual amino acids or smaller protected fragments, etc. By the judicious mapping of such a synthesis, it is possible to minimize the number of steps required for the production of a desired end-product. Unlike the solid-phase method, the solution-phase method is free of the one-terminus-only limitation on the synthesis of a peptide. In the solution-phase method, a fragment formed in a previous step by reaction of an unprotected N-terminal amino function can be reacted in a subsequent step at its unprotected C-terminal carboxyl function. This possibility is not open to the solid-phase method since, as stated above, the growing peptide is "blocked" at C-terminus by attachment to the supporting resin.

Solution-phase peptide synthesis, despite being free of the one-terminus-only synthesis limitation of solid-phase peptide synthesis, suffers from a shortcoming: the need to frequently isolate and purify the growing peptide. All reactions are carried out in solution, resulting in a mixture which contains the desired product as well as unwanted unreacted reagents and by-products. After several steps of the synthesis, the solution would become burdened with appreciable amounts of these contaminants which, if left in the solution, would affect subsequent steps of the synthesis or burden the isolation and purification of the desired end-product peptide. As a consequence, it is prudent and often necessary to isolate intermediate products as the peptide synthesis goes forward. Each such isolation adds to the cost and time of the synthesis and lowers the overall yield of the desired peptide.

Methods of preparing small amounts of large libraries of compounds have become available with the advent in recent years of combinatorial chemistry techniques. Recently, the use of resins to sequester excess reagents and by-products from combinatorial chemistry steps has been described by D. L. Flynn *et al.*, J. Am. Chem. Soc., 119, 4874-4881 (1997). In the method described there, the authors use so-called CMR/R, or complementary molecular

recognition/reactivity resins to purify products of each parallel step in a combinatorial chemistry scheme for preparing compound libraries. The process involves the synthesis of library members by solution-phase methods, followed by the removal of the solution-phase excess reactants, reagents, by-products and/or catalysts by incubation of the reaction mixture through CMR/R resins

R J Booth *et al*, J. Am. Chem. Soc., 119 4882-4886 (1997) likewise describe the use of polystyrene-divinylbenzene-supported derivatives of *tris*-(2-aminoethyl)amine to quench excess reagents from crude reaction products obtained from solution-phase, parallel syntheses of amides in the construction of combinatorial libraries

L M Gayo, *et al* Tetrahedron Letters, 38(4) 513 516 (1997) describe the production of a combinatorial library of amides by first reacting, in parallel a number of amines with a slight excess of an acid chloride to produce the desired amides. Following the reaction, the mixture is treated with water to convert the excess acid chloride to the corresponding carboxylic acid which, together with the HCl by-product of amide formation and acid chloride hydrolysis, is removed by a scavenging resin

S D Brown, *et al*, J. Am. Chem. Soc., 118 6331-6332 (1996) describe a "resin capture" technique for the combinatorial synthesis of tetra-substituted ethylene compounds using the reaction between *bis*(boryl)alkenes and alkyl halides. An intermediate product in the reaction is captured by a resin-bound aryl iodide to produce a desired product

While these techniques describe creative ways of using functionalized resins to quench reactions or to capture excess reagents, by-products or desired products in combinatorial chemistry schemes, none has provided an effective method of preparing commercial-scale quantities of therapeutically useful compounds, particularly peptides. The particular problems of large-scale production characteristic of commercial synthesis, are of little concern in the construction of a combinatorial library at the initial stages of drug discovery

There is thus a need for improved methods of synthesizing commercial-scale quantities of peptides which makes the most of the advantages of both the solid-phase and the solution-phase methods while minimizing the disadvantages of each

Summary of the Invention

In accordance with the present invention, an improved method of peptide synthesis is provided which capitalizes on the principal advantages of both the solid-phase and solution-phase methods of peptide synthesis. The method disclosed is capable of large-scale production of peptides in solution, is not subject to the one-terminus-only limitation of the solid-phase method

possesses the "cleanliness" of the solid-phase method and like the solid-phase method, is capable of automation. Most importantly, the method of the present invention does not require the frequent isolation of intermediates in a lengthy synthetic sequence nor necessarily the removal of all contaminants from the reaction mixture prior to subsequent processing steps.

5 In accordance with the principal embodiment of the present invention, there is provided a process for the synthesis of a polypeptide having a pre-determined number and sequence of amino acid residues. In its most general aspect, the process comprises sequentially the steps of first exposing, in solution, a first substrate amino acid or peptide fragment of the desired polypeptide product, the first substrate amino acid or peptide fragment being protected at either its
10 N- or C-terminus, to a stoichiometric excess of a second reactant amino acid or peptide fragment of the desired polypeptide, the second reactant amino acid or peptide fragment being protected at the other of its N- or C-terminus, to form a condensation product of the substrate and reactant. The resulting condensation product is protected at both its N- and C-termini. In the second step of the process, the reaction solution is contacted with an insoluble scavenger having a reactive
15 functionality complementary to the unprotected N- or C-terminal functionality of the first amino acid or peptide fragment, to sequester the excess of the second reactant amino acid or peptide fragment.

In the third step, the sequestered excess second reactant amino acid or peptide fragment is removed from the reaction solution, leaving the condensation product and reaction by-products in
20 solution. Should it become necessary or desirable to decrease the volume of the reaction solution, which increases during the process of the present invention, the condensation product may be precipitated or crystallized, then placed back in a solution of lesser volume. This solution, in the fourth step, is subjected to a reaction which removes the protecting group from either the N- or C-terminus of the condensation product of the first step. If at this point, the desired polypeptide
25 sequence is not yet achieved, the first through fourth steps are repeated as a cycle, with the deprotected condensation product of each previous fourth step becoming the substrate peptide fragment of each successive first step, until the desired peptide is produced. At the point at which the desired polypeptide sequence has been produced, the product is isolated and deprotected, if needed, of any terminal or side-chain protecting groups.

Description of the Drawing Figures

In the Drawing, which forms a part of the disclosure of the present invention

FIGURE 1 is a schematic representation of the process steps for preparing polypeptides according to the method of the present invention

FIGURE 2 is a schematic representation of a commercial-scale semi-automatic process apparatus for preparing polypeptides in accordance with the process of the present invention

Detailed Description of the Invention

As used throughout this specification and the appended claims, the terms "protected" or "blocked," as it is applied to amino acids and peptides, have the meanings commonly accepted in the art. That is, a protected or blocked amino acid or peptide is one in which the reactive functionality of either or both the N-terminal amino group and/or the C-terminal carboxyl group have been blocked by reaction with a "blocking group" to prevent their reactivity. Additionally, other functional groups of the amino acid or peptide such as side-chain amino groups in lysine or hydroxy groups in serine, threonine or tyrosine, and carboxyl groups in aspartic or glutamic acid residues may be blocked by appropriate blocking groups to prevent unwanted reactions.

Blocking groups suitable for the protection of amine, hydroxyl and carboxyl functions are well known in the art. Blocking groups and methods for their attachment and cleavage are fully set out in "Protective Groups in Organic Synthesis," 2d Edition, by T. W. Greene, *et al.* John Wiley & Sons, Inc. New York 1991.

In referring to various blocking groups, the acronym short-hand designations commonly employed by chemists is used throughout this specification. The definitions of the acronyms can be found, for example, in T. W. Greene *et al.*, *op. cit.* at pages XI-XVI. The acronym "OSu" refers to the moiety derived by removal of the hydroxyl hydrogen atom from N-hydroxysuccinimide. The blocking group known alternatively as "Cbz" or simply "Z" is the benzyloxycarbonyl protecting group. The blocking group known alternatively as "tBoc" or simply "Boc" is the tert-butyloxycarbonyl protecting group.

Referring to Figure 1, the general process of the present invention is depicted in schematic block diagram. In the first step of the process, the first amino acid or peptide fragment substrate, suitably protected except for either its N-terminal amino group or its C-terminal carboxyl function, is exposed in a suitable solvent, to the reactant amino acid or peptide fragment. The reactant may be a single amino acid or some peptide fragment of the end-product polypeptide, suitably protected at the other of its N-terminal amino group or C-terminal carboxyl group as well as at reactive side-chain functional groups. The carboxyl terminus of the N-terminal protected amino acid or peptide fragment is activated by conversion to an active ester of the types well known in the art. A preferred ester activating group for the process of the present invention is the N-hydroxysuccinimide ester group.

(-OSu)

The protecting group for the N-terminus of the reactant or substrate amino acid or peptide fragment is a group which is "orthogonal" to protecting groups which are employed in protecting side-chain amino, hydroxyl and carboxyl groups in either the reactant or substrate. Moreover the N-terminal protecting group on the reactant or substrate amino acid or peptide fragment should be one which is easily removed under conditions which do not remove the side-chain protecting groups or the C-terminal blocking group of the other of the reactant or substrate. Two protecting groups are said to be "orthogonal" if chemical processes which are employed to remove one do not remove the other. A preferred N-terminal protecting group for the reactant or substrate amino acid or peptide fragment is one which is easily cleaved under conditions of hydrogenolysis, or catalytic hydrogenation. As sulfur is known to "poison" or inactivate hydrogenation catalysts, the preferred embodiment of the process of the present invention is limited to the synthesis of peptides which do not contain side-chain sulfhydryl or thioether groups, i.e., to the synthesis of non-cysteine-containing and non-methionine-containing peptides. The side-chain amino, hydroxyl and carboxyl blocking groups are selected from blocking groups well known in the art which are not cleaved under hydrogenolysis conditions. A preferred N-terminal amino protecting group for the reactant or substrate amino acids and peptide fragments utilized in the process of the present invention is the benzyloxycarbonyl group, sometimes termed the carbobenzoxy group and denoted "Cbz" or simply "Z" in chemical shorthand. The Z group is easily cleaved by hydrogenolysis under mild conditions from the N-terminal amino groups of protected amino acids or peptides while leaving unaffected the less reactive protecting groups which have been used to protect side-chain functional groups. Preferred blocking groups for the C-terminus of the reactant or substrate amino acid or peptide fragment are simple ester groups such as the *tert*-butyl ester group and the like.

The substrate (unprotected at either its N-terminus or C-terminus) is allowed to react with the reactant (unprotected at the other of its N- or C-terminus) until analysis of aliquot samples periodically taken from the reaction mixture indicate substantially complete reaction. In the process of the present invention the reactant is employed in stoichiometric excess to the amount of substrate in order to drive the condensation reaction to completion. Preferably an amount greater than 1.0 up to about 1.1 moles of reactant amino acid or peptide fragment is employed per mole of substrate amino acid or peptide fragment. The amount of excess required in each particular coupling reaction will vary according to the chemical nature of the substrate and reactant to be coupled. However it is within the skill of the process chemist to determine with a small-scale bench reaction the required molar ratio of reactant to substrate prior to committing to the cost of a large-scale preparation.

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When the initial reaction between the substrate amino acid or peptide fragment and the reactant amino acid or peptide fragment is essentially complete, the reaction solution is contacted with a scavenger. The scavenger is both insoluble in the solvent system employed, and possesses a functionality which is complementary to that of the unprotected terminus of the substrate amino acid or peptide fragment. In the preferred embodiment of the process of the present invention, the substrate amino acid or peptide fragment is unprotected at its N-terminus and protected against reaction at its C-terminus. Correspondingly the reactant amino acid or peptide fragment is protected at its N-terminus, preferably by a Z group, and is activated at its C-terminus. In this preferred embodiment of the process, the scavenger possesses an active amine functionality. The preferred scavenger is an amine-functionalized resin, such as aminomethyl-functionalized resins known in the art, particularly commercially available aminomethyl-modified styrene-divinylbenzene copolymers.

Example

The method of contacting the reaction solution with the scavenger resin may be by either adding resin to the reaction vessel, or *vice versa* with circulation of the reaction solution through a column of the resin being the preferred method. This method permits the repeated recirculation of the reaction solution through the resin column to ensure that the removal of excess reactant amino acid or peptide fragment is complete. Should it become necessary or desirable to decrease the reaction volume, which increases during each cycle of the process of the present invention, the condensation product may be precipitated or crystallized, then redissolved in a smaller volume of a suitable organic solvent.

In the next step of the process, the desired N-terminal or C-terminal blocking group of the condensation product of the first step of the process is removed. If in a subsequent step, the reactant amino acid or peptide fragment is one in which the carboxyl terminus is unprotected, then the N-terminal protecting group of the condensation product is removed, and *vice versa*. In the preferred embodiment of the process of this invention, new segments, either single amino acids or short peptide fragments, are added to the growing polypeptide by utilizing a C-terminally activated reactant and an N-terminally unprotected substrate. This is because of the preferred N-terminal Z protecting group and the preferred C-terminal OSu activating group. Hence in this step of the preferred embodiment of the process, the N-terminal Z group is removed from the condensation product.

The preferred method for this deprotection step is catalytic hydrogenolysis in the presence of a palladium catalyst at pressures ranging between atmospheric pressure and about 30 to 50 psi (206.8 kPa to 344.7 kPa). Preferred catalysts are those which can readily be removed from solution by filtration such as palladium supported on Deloxan® (organofunctional polysiloxane).

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polymers manufactured by DEGUSSA AG Weissfrauenstrasse 9 Frankfurt am Main, Germany), and palladium supported on carbon, alumina (Al_2O_3), or silica (SiO_2). The hydrogenolysis leaves in the solution, once the catalyst has been removed by filtration only the N-hydroxysuccinimide by-product of the initial coupling reaction, the N-terminally deprotected condensation product, and toluene which, together with carbon dioxide is the by-product of the Z-group hydrogenolysis. (It should be noted that the N-hydroxysuccinimide and toluene by-products interfere with neither the catalytic hydrogenation deprotection step, nor subsequent condensations. The absence of any need for their removal in multi-step syntheses is a particular advantage of the preferred process of the present invention.)

If, following removal of excess reactant amino acid or peptide fragment, the desired end-product polypeptide sequence has been completely constructed, the process is over, except for isolation of the polypeptide and deprotection by conventional methods, if needed or desired. The isolation is further simplified in the preferred embodiment of the process of the present invention, since the only contaminants remaining in the reaction solution are N-hydroxysuccinimide, tertiary amine salt (if the substrate or reactant amino acid or peptide fragment is employed in salt form, requiring neutralization prior to coupling), and toluene, all of which are easily removed by simple precipitation or crystallization of the desired end-product polypeptide. Those skilled in the art will realize that, when a polypeptide bearing, for example, a free N-terminus is the desired end-product or when recrystallization alone can serve to remove excess reactant amino acid or peptide fragment, isolation may be carried out following completion of a particular fourth or first step, respectively.

If, however, as is the more general case, the desired end-product polypeptide synthesis is incomplete following removal of excess reactant amino acid or peptide fragment, the deprotection, coupling, and scavenging steps are repeated using the deprotected condensation product of each cycle as the substrate material of each subsequent cycle until the desired polypeptide sequence is achieved.

Referring to Figure 2, one embodiment of a semi-automatic commercial scale processing system is shown for use in carrying out the process of the present invention. The system 100 comprises a first reactor vessel 102 for carrying out amino acid/peptide fragment condensation or coupling reactions, a second reactor 106 for carrying out de-protecting reactions, a resin column 104, a filter 108, a product holding tank 110, first and second solvent tanks, 112 and 114, and a numeric processor control unit 116. While stainless steel reaction vessels may be used, it is preferred that all reactor vessels and tanks be glass-lined. It should be noted that the process layout in Figure 2 is schematic only. Representation of the filter as a plate-and-frame filter press

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WE CLAIM.

- 1 **A process for the synthesis of a polypeptide having a pre-determined number and sequence of amino acid residues comprising sequentially the steps of**
- 5
- a) exposing, in solution, a first substrate amino acid or peptide fragment of said polypeptide said first substrate amino acid or peptide fragment being protected at either of its N- or C-terminus, to a stoichiometric excess of a second reactant amino acid or peptide fragments of said polypeptide, said**
- 10 **second reactant amino acid or peptide fragment being protected at the other of its N- or C-terminus, to form a condensation product of said substrate and reactant, said condensation product being protected at both its N- and C-termini**
- 15 **b) contacting the solution from step a) with an insoluble scavenger having a reactive functionality complementary to the unprotected N- or C-terminal functionality of said first substrate amino acid or peptide fragment, to sequester the excess of said second reactant amino acid or peptide fragment;**
- 20 **c) removing the sequestered excess second reactant amino acid or peptide fragment, leaving the condensation product and reaction by-products in solution or conducting subsequent precipitation or crystallization of the condensation product, then redissolving the condensation product if synthesis continuation is necessary or desired**
- 25
- d) removing the protecting group from either the N- or C-terminus of the condensation product of step a),**
- e) repeating steps a) through d) if the desired polypeptide sequence is not yet**
- 30 **achieved, with the deprotected condensation product of each previous step d) becoming the substrate peptide fragment of each successive step a) and**
- f) isolating and deprotecting, if needed the final polypeptide product once the desired peptide sequence is achieved**

- 2 The process according to Claim 1 wherein the protecting group removed in step d) is removed from the N-terminus
- 5 3 The process according to Claim 1 wherein the protecting group removed in step d) is removed from the C-terminus
- 4 The process according to Claim 1 wherein the insoluble scavenger employed in step b) is selected from amine- and carboxyl-functionalized resins
- 10 5 The process according to Claim 2 wherein the insoluble scavenger employed in step b) is an amine-functionalized resin
- 6 The process according to Claim 3 wherein the insoluble scavenger employed in step b) is a carboxyl-functionalized resin
- 15 7 The process according to Claim 5 wherein said resin is an aminomethyl-functionalized polystyrene-divinylbenzene resin
- 20 8 A process for the synthesis of a polypeptide having a pre-determined number and sequence of amino acid residues comprising the steps of
- 25 a) exposing a solution of a substrate amino acid or fragment of said polypeptide, said substrate having a C-terminal protecting group not removable by hydrogenolysis, to a stoichiometric excess of a reactant amino acid or a fragment of said polypeptide said reactant having an N-terminal protecting group removable by hydrogenolysis to form a condensation product;
- 30 b) contacting the solution from step a) with an amine-functionalized resin to sequester the excess of said reactant amino acid or peptide fragment
- c) removing from the solution the sequestered excess of said reactant amino acid or peptide fragment leaving the condensation product and reaction

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by-products in solution or conducting subsequent precipitation or crystallization of the condensation product, then redissolving the condensation product if synthesis continuation is necessary or desired

- 5 d) subjecting the solution from step c) to hydrogenolysis conditions to remove the protecting group from the N-terminus of said condensation product,
- e) repeating steps a) through d) utilizing as the substrate peptide fragment for subsequent steps a) the deprotected condensation product of subsequent
- 10 steps d) until the desired polypeptide has been produced

9 The process according to Claim 8 wherein said amine-functionalized resin is an aminomethyl-modified resin

15 10 The process according to Claim 8 wherein the C-terminus of the reactant amino acid or a peptide fragment is activated for reaction with amino functional groups.

 11 The process according to Claim 10 wherein the C-terminus of the reactant amino acid or a peptide fragment is activated by esterification with N-hydroxysuccinimide

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 12 The process according to Claim 8 wherein the N-terminal protecting group of the reactant amino acid or peptide fragment is benzyloxycarbonyl

 13 A process for the synthesis of a polypeptide having a pre-determined number and

25 sequence of amino acid residues comprising the steps of

- a) exposing a solution of a substrate amino acid or fragment of said polypeptide said substrate having a C-terminal protecting group to a stoichiometric excess of a reactant amino acid or a fragment of
- 30 said polypeptide said reactant having an N-terminal benzyloxycarbonyl protecting group and being activated by esterification with N-hydroxy-succinimide to form a condensation product until reaction between said substrate and reactant is substantially complete

- 5

b) contacting the solution from step a) with an amine-functionalized resin to sequester and to remove from solution the excess of the reactant amino acid or peptide fragment leaving the condensation product and reaction by-products in solution or conducting subsequent precipitation or crystallization of the condensation product, then redissolving the condensation product if synthesis continuation is necessary or desired,
- 10

c) subjecting the solution from step b) to catalytic hydrogenation conditions to remove the benzyloxycarbonyl protecting group from the N-terminus of said condensation product,
- d) removing, by filtration, the hydrogenation catalyst from the solution,
- 15

e) repeating steps a) through d) until the desired polypeptide has been produced, and
- f) isolating and deprotecting, if needed or desired, the product polypeptide
- 20

14 A process according to claim 13 wherein the polypeptide synthesized is Benzyloxycarbonyl-Lysyl(*tert*-butyloxycarbonyl)-Alanyl-Phenylalanyl-Valyl-Lysyl(*tert*-butyloxycarbonyl)-Isoleucyl-Leucyl-Lysyl(*tert*-butyloxycarbonyl)-Lysine(*tert*-butyloxycarbonyl)-methyl Ester
- 25

15 A process according to claim 13 wherein the polypeptide synthesized is 9-Fluorenylmethoxycarbonyl-*beta*-Alanyl-Leucyl-Alanyl-Leucine-*tert*-butyl Ester

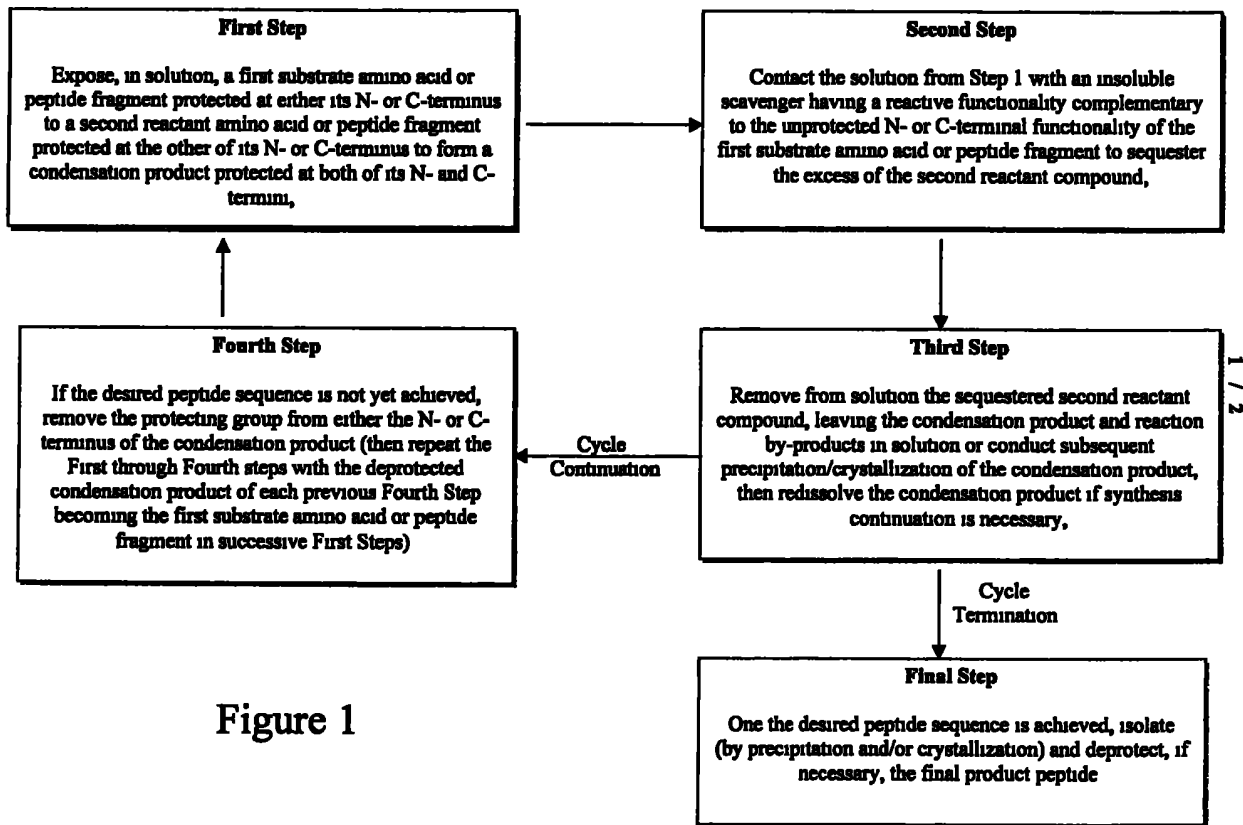


Figure 1

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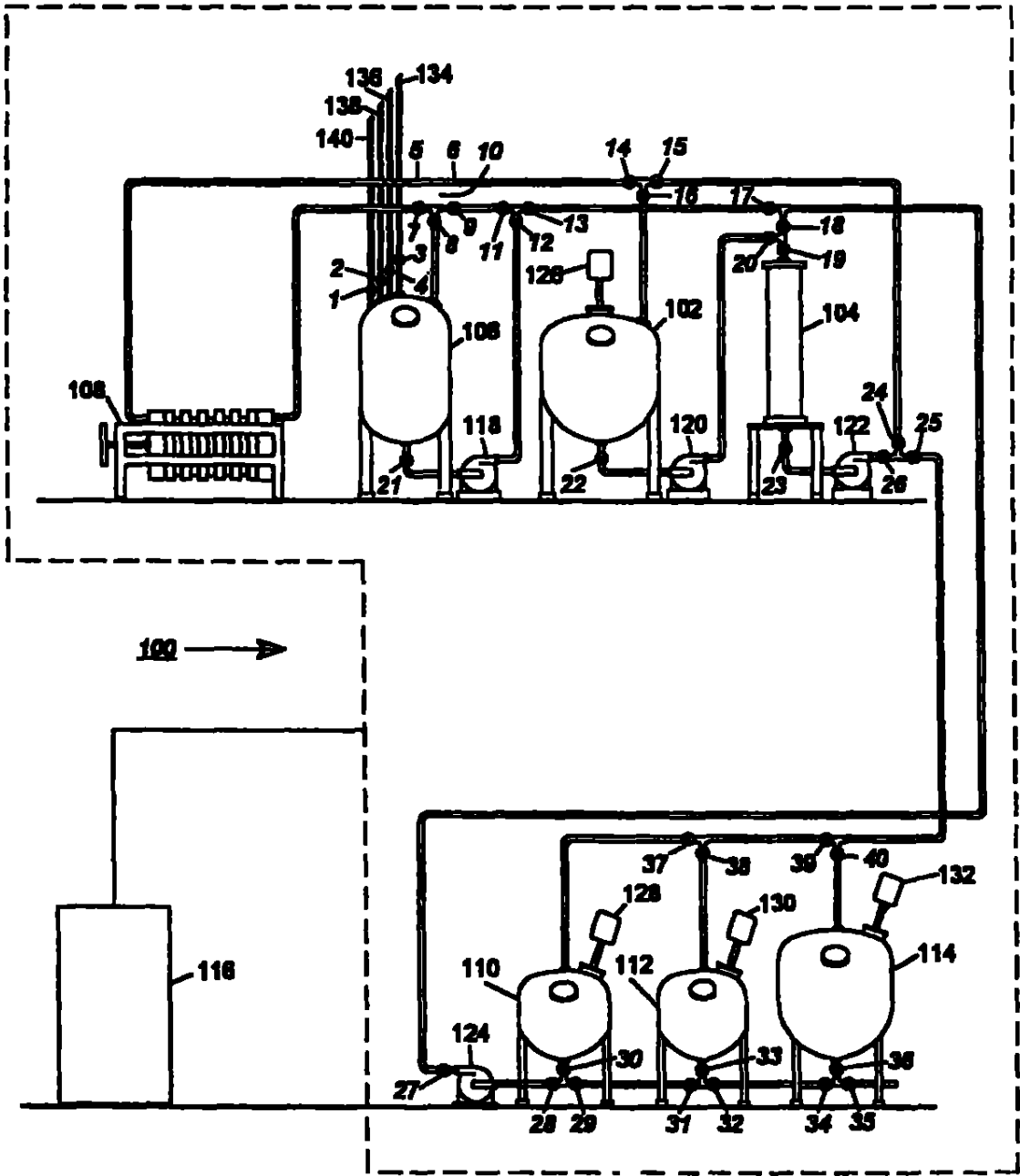


Figure 2