(19) DANMARK

(10) DK/EP 1259563 T4

Patent- og Varemærkestyrelsen

(12) Oversættelse af ændret europæisk patentskrift

(51) Int.Cl.: *C 08 G 65/333 (2006.01)* *A 61 K 47/48 (2006.01)* *C 08 G 65/329 (2006.01)*
C 08 G 65/332 (2006.01)

(45) Oversættelsen bekendtgjort den: **2016-11-21**

(80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om opretholdelse af patentet i ændret form: **2016-08-10**

(86) Europæisk ansøgning nr.: **00986602.1**

(86) Europæisk indleveringsdag: **2000-12-18**

(87) Den europæiske ansøgnings publiceringsdag: **2002-11-27**

(86) International ansøgning nr.: **US2000034590**

(87) Internationalt publikationsnr.: **WO2001045796**

(30) Prioritet: **1999-12-22 US 171834 P**

(84) Designerede stater: **AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE TR**

(73) Patenthaver: **Nektar Therapeutics, 150 Industrial Road, San Carlos, CA 94070, USA**

(72) Opfinder: **KOZLOWSKI, Antoni, 4433 Torrance Drive, Huntsville, AL 35816, USA**

(74) Fuldmægtig i Danmark: **Zacco Denmark A/S, Arne Jacobsens Allé 15, 2300 København S, Danmark**

(54) Benævnelse: **FREMGANGSMÅDE TIL FREMSTILLING AF 1-BENZOTRIAZOLYLCARBONAT-ESTRE AF VANDOPLØSELIGE POLYMERER**

(56) Fremdragne publikationer:
DD-A- 287 951
US-A- 5 629 384
US-A- 5 650 234
US-A- 5 985 263
MONFARDINI C ET AL: "A BRANCHED MONOMETHOXYPOLY(ETHYLENE GLYCOL) FOR PROTEIN MODIFICATION" BIOCONJUGATE CHEMISTRY, AMERICAN CHEMICAL SOCIETY, WASHINGTON, US, vol. 6, no. 1, 1995, pages 62-69, XP000494804 ISSN: 1043-1802
DD-A5- 287 951
US-A- 5 281 698
MONFARDINI C. ET AL: 'A Branched Monomethoxypoly(ethylene glycol) for Protein Modification' BIOCONJUGATE CHEMISTRY vol. 6, no. 1, 1995, pages 62 - 69
ZALIPSKY S.: 'Functionalized Poly(ethylene glycol) for Preparation of Biologically Relevant Conjugates' BIOCONJUGATE CHEMISTRY vol. 6, 1995, pages 150 - 165
Fluka Catalogue, 1195/96, Cover and page 490
MIRON T. ET AL: 'A Simplified Method for the Preparation of Succinimidyl Carbonate Polyethylene Glycol for Coupling to Proteins' BIOCONJUGATE CHEMISTRY vol. 4, 1993, pages 568 - 569

DESCRIPTION

[0001] This invention relates to a method for the preparation of an amino acid derivative of certain water-soluble and non-peptidic polymers.

[0002] Covalent attachment of the hydrophilic polymer poly(ethylene glycol), abbreviated PEG, also known as poly (ethylene oxide), abbreviated PEO, to molecules and surfaces is of considerable utility in biotechnology and medicine. In its most common form, PEG is a linear polymer terminated at each end with hydroxyl groups:

$$Ho\text{-}CH_2CH_2O\text{-}(CH_2CH_2O)_n\text{-}CH_2CH_2\text{-}OH$$

[0003] The above polymer, alpha-,omega-dihydroxylpoly(ethylene glycol), can be represented in brief form as HO-PEG-OH where it Is understood that the -PEG-symbol represents the following structural unit:

$$-CH_2CH_2O\text{-}(CH_2CH_2O)_n\text{-}CH_2CH_2-$$

where n typically ranges from about 3 to about 4000.

[0004] PEG is commonly used as methoxy-PEG-OH, or mPEG in brief, in which one terminus is the relatively inert methoxy group, while the other terminus is a hydroxyl group that is subject to ready chemical modification. The structure of mPEG is given below.

$$CH_3O\text{-}(CH_2CH_2O)_n\text{-}CH_2CH_2\text{-}OH$$

Random or block copolymers of ethylene oxide and propylene oxide, shown below, are closely related to PEG in their chemistry, and they can be substituted for PEG in many of its applications.

$$HO\text{-}CH_2CHRO(CH_2CHRO)_nCH_2CHR\text{-}OH$$

wherein each R is independently H or CH_3.

[0005] PEG is a polymer having the properties of solubility in water and in many organic solvents, lack of toxicity, and lack of immunogenicity. One use of PEG is to covalently attach the polymer to insoluble molecules to make the resulting PEG-molecule 'conjugate" soluble. For example, it has been shown that the water-insoluble drug paclitaxel, when coupled to PEG, becomes water-soluble. Greenwald, et al., J. Org. Chem., 60:331-336 (1995).

[0006] To couple PEG to a molecule, such as a protein, it is often necessary to "activate" the PEG by preparing a derivative of the PEG having a functional group at a terminus thereof. The functional group can react with certain moieties on the protein, such as an amino group, thus forming a PEG-protein conjugate.

[0007] In U.S. Patent No. 5,650,234, which is incorporated by reference herein in its entirety, a 1-benzotriazolylcar-bonate ester of polyethylene glycol) is described. The multi-step process described in the '234 patent for forming the 1-benzotriazolylcarbonate ester of PEG includes reaction of a PEG molecule with the volatile and hazardous compound, phosgene, in order to form a PEG chloroformate intermediate. The use of phosgene in the process results in the formation of HCl, which can cause degradation of the PEG backbone. Due to the volatile nature of phosgene, and the resulting safety and quality problems associated with its use, there Is a need In the art for a method for preparing 1-benzotria-zolylcarbonate esters of PEG without using phosgene.

[0008] The invention provides a method for the preparation of an amino acid derivative of a water-soluble and non-peptidic polymer as defined in the claims. Using the invention, a 1-benzo-triazolylcarbonate ester can be formed in a single step and without using phosgene, thereby avoiding the safety and quality problems associated with that compound.

[0009] The method of the invention includes providing a water-soluble and non-peptidic polymer having at least one terminal hydroxyl group and reacting the terminal hydroxyl group of the water-soluble and non-peptidic polymer with di (1-benzotriazolyl)carbonate to form the 1-benzotriazolylcarbonate ester of the water-soluble and non-peptidic polymer. The water-soluble and non-peptidic polymer is selected from poly(alkylene glycol), poly(oxyethylated polyol), poly(olefinic alcohol),

poly(vinylpyrrolidone), poly(α-hydroxy acid), polyphosphazene, polyoxazoline, poly(N-acryloylmorpholine), and copolymers, terpolymers, and mixtures thereof. In one embodiment, the polymer is poly(ethylene glycol) having an average molecular weight from about 200 Da to about 100,000 Da.

[0010] The reaction step can be conducted in the presence of an organic solvent and a base. Examples of suitable organic solvents include methylene chloride, chloroform, acetonitrile, tetrahydrofuran, dimethylformamide, dimethyl sulfoxide, and mixtures thereof. The base can be, for example, pyridine, dimethylaminopyridine, quinoline, trialkylamines, and mixtures thereof.

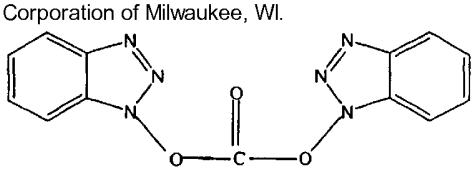
[0011] The 1-benzotriazolylcarbonate ester is reacted with an amino acid, such as lysine, to form a polymeric amino acid derivative.

[0012] The terms "functional group", "active moiety", "activating group", "reactive site", "chemically reactive group" and "chemically reactive moiety" are used in the art and herein to refer to distinct, definable portions or units of a molecule. The terms are somewhat synonymous in the chemical arts and are used herein to indicate that the portions of molecules that perform some function or activity and are reactive with other molecules. The term "active," when uses in conjunction with functional groups, is intended to include those functional groups that react readily with electrophilic or nucleophilic groups on other molecules, in contrast to those groups that require strong catalysts or highly impractical reaction conditions In orderto react. For example, as would be understood in the art, the term "active ester" would include those esters that react readily with nucleophilic groups such as amines. Typically, an active ester will react with an amine in aqueous medium In a matter of minutes, whereas certain esters, such as methyl or ethyl esters, require a strong catalyst in order to react with a nucleophilic group.

[0013] The term "linkage" or "linker" is used herein to refer to groups or bonds that normally are formed as the result of a chemical reaction and typically are covalent linkages. Hydrolytically stable linkages means that the linkages are substantially stable in water and do not react with water at useful pHs, e.g., under physiological conditions for an extended period of time, perhaps even indefinitely. Hydrolytically unstable or degradable linkages means that the linkages are degradable In water or in aqueous solutions, including for example, blood. Enzymatically unstable or degradable linkages means that the linkage can be degraded by one or more enzymes. As understood in the art, PEG and related polymers may include degradable linkages in the polymer backbone or in the linker group between the polymer backbone and one or more of the terminal functional groups of the polymer molecule.

[0014] The term "biologically active molecule", "biologically active moiety" or "biologically active agent" when used herein means any substance which can affect any physical or biochemical properties of a biological organism, including but not limited to viruses, bacteria, fungi, plants, animals, and humans. In particular, as used herein, biologically active molecules include any substance intended for diagnosis, cure mitigation, treatment, or prevention of disease In humans or other animals, or to otherwise enhance physical or mental well-being of humans or animals. Examples of biologically active molecules include, but are not limited to, peptides, proteins, enzymes, small molecule drugs, dyes, lipids, nucleosides, oligonucleotides, cells, viruses, liposomes, microparticles and micelles. Classes of biologically active agents that are suitable for use with the invention include, but are not limited to, antibiotics, fungicides, anti-viral agents, anti-inflammatory agents, anti-tumor agents, cardiovascular agents, anti-anxiety agents, hormones, growth factors, steroidal agents, and the like.

[0015] A method for the preparation of a 1-benzotriazolylcarbonate ester (also referred to as a BTC ester) of a water-soluble and non-peptidic polymer is described, wherein a terminal hydroxyl group of a water-soluble and non-peptidic polymer is reacted with di(1-benzotriazolyl)carbonate, the structure of which is shown below, to form the 1-benzotriazolylcarbonate ester.
Di(1-benzotriazolyl)carbonate, which should not pose significant safety or handling problems as a reagent and should not cause degradation of the polymer backbone, can be purchased as a 70% (w/w) mixture with 1,1,2-trichloroethane from Fluka Chemical Corporation of Milwaukee, WI.



di(1-benzotriazolyl)carbonate (diBTC)

[0016] The polymer backbone of the water-soluble and non-peptidic polymer can be poly(ethylene glycol) (i.e. PEG). However, it should be understood that other related polymers are also suitable for use in the practice of this invention and that the use of the term PEG or poly(ethylene glycol) is intended to be inclusive and not exclusive in this respect. The term PEG includes

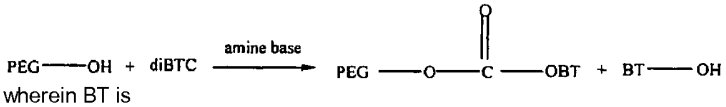
poly(ethylene glycol) in any of its forms, including alkoxy PEG, difunctional PEG, multiarmed PEG, forked PEG, branched PEG, pendent PEG (i.e. PEG or related polymers having one or more functional groups pendent to the polymer backbone), or PEG with degradable linkages therein.

[0017] PEG is typically clear, colorless, odorless, soluble in water, stable to heat, inert to many chemical agents, does not hydrolyze or deteriorate, and is generally non-toxic. Poly(ethylene glycol) is considered to be biocompatible, which is to say that PEG is capable of coexistence with living tissues or organisms without causing harm. More specifically, PEG is substantially non-immunogenic, which is to say that PEG does not tend to produce an immune response in the body. When attached to a molecule having some desirable function in the body, such as a biologically active agent, the PEG tends to mask the agent and can reduce or eliminate any immune response so that an organism can tolerate the presence of the agent. PEG conjugates tend not to produce a substantial immune response or cause clotting or other undesirable effects. PEG having the formula $-CH_2CH_2O-(CH_2CH_2O)_n-CH_2CH_2-$, where n is from about 3 to about 4000, typically from about 3 to about 2000, is one useful polymer in the practice of the invention. PEG having a molecular weight of from about 200 Da to about 100,000 Da are particularly useful as the polymer backbone.

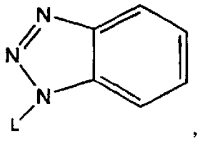
[0018] The polymer backbone can be linear or branched. Branched polymer backbones are generally known In the art. Typically, a branched polymer has a central branch core moiety and a plurality of linear polymer chains linked to the central branch core. PEG is commonly used in branched forms that can be prepared by addition of ethylene oxide to various polyols, such as glycerol, pentaerythritol and sorbitol. The central branch moiety can also be derived from several amino acids, such as lysine. The branched poly(ethylene glycol) can be represented in general form as $R(-PEG-OH)_m$ in which R represents the core moiety, such as glycerol or pentaerythritol, and m represents the number of arms. Multi-armed PEG molecules, such as those described in U.S. Patent No. 5,932,462, which is incorporated by reference herein in its entirety, can also be used as the polymer backbone.

[0019] Many other polymers selected from those mentioned above are also suitable for the invention. Polymer backbones that are non-peptidic and water-soluble, with from 2 to about 300 termini, are particularly useful in the invention. Suitable polymers are other poly(alkylene glycols), such as poly(propylene glycol) ("PPG"), copolymers of ethylene glycol and propylene glycol and the like, poly(oxyethylated polyol), poly(olefinic alcohol), poly(vinylpyrrolidone), poly(α-hydroxy acid), polyphosphazene, polyoxazoline, poly(N-acryloylmorpholine), such as described in U.S. Patent No. 5,629,384, and copolymers, terpolymers, and mixtures thereof. Although the molecular weight of each chain of the polymer backbone can vary, it is typically in the range of from about 100 Da to about 100,000 Da, often from about 6,000 Da to about 80,000 Da.

[0020] For purposes of illustration, a simplified reaction scheme is shown below.



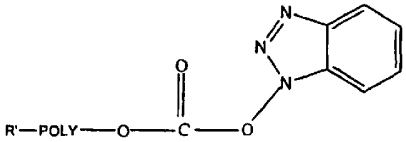
wherein BT is



L being the point of bonding to the oxygen atom.

[0021] In one embodiment, the reaction between the polymer and diBTC takes place in an organic solvent and in the presence of a base. Examples of suitable organic solvents include methylene chloride, chloroform, acetonitrile, tetrahydrofuran, dimethylformamide, dimethyl sulfoxide, and mixtures thereof. Amine bases, such as pyridine, dimethylaminopyridine, quinoline, trialkylamines, including triethylamine, and mixtures thereof, are examples of suitable bases. In one aspect of the invention, the molar ratio of di(1-benzotriazolyl) carbonate to the water-soluble and non-peptidic polymer is about 30:1 or less.

[0022] In one embodiment, the water-soluble and non-peptidic polymer has the structure R'-POLY-OH and the 1-benzotriazolylcarbonate ester of the water-soluble and non-peptidic polymer has the structure

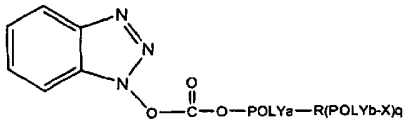


wherein POLY Is a water-soluble and non-peptidic polymer backbone, such as PEG, and R' Is a capping group. R' can be any suitable capping group known in the art for polymers of this type. For example, R' can be a relatively inert capping group, such as an alkoxy group (e.g. methoxy). Alternatively, R' can be a functional group. Examples of suitable functional groups include

hydroxyl, protected hydroxyl, active ester, such as N-hydroxysuccinimidyl esters and 1-benzotriazolyl esters, active carbonate, such as N-hydroxysuccinimidyl carbonates and 1-benzotriazolyl carbonates, acetal, aldehyde, aldehyde hydrates, alkenyl, acrylate, methacrylate, acrylamide, active sulfone, protected amine, protected hydrazide, thiol, protected thiol, carboxylic acid, protected carboxylic acid, isocyanate, isothiocyanate, maleimide, vinylsulfone, dithiopyridine, vinylpyridine, iodoacetamide, epoxide, glyoxals, diones, mesylates, tosylates, andtresylate. The functional group is typically chosen for attachment to a functional group on a biologically active agent.

[0023] As would be understood in the art, the term "protected" refers to the presence of a protecting group or moiety that prevents reaction of the chemically reactive functional group under certain reaction conditions. The protecting group will vary depending on the type of chemically reactive group being protected. For example, if the chemically reactive group is an amine or a hydrazide, the protecting group can be selected from the group of tert-butyloxycarbonyl (t-Boc) and 9-fluorenylmethoxycarbonyl (Fmoc). If the chemically reactive group is a thiol, the protecting group can be orthopy-ridyldisulfide. If the chemically reactive group is a carboxylic acid, such as butanoic or propionic acid, or a hydroxyl group, the protecting group can be benzyl or an alkyl group such as methyl or ethyl. Other protecting groups known in the art may also be used in the invention.

[0024] In another embodiment, the water-soluble and non-peptidic polymer has the structure HO-POLYa-R(POLYb-X)q and the 1-benzotriazolylcarbonate ester of the water-soluble and non-peptidic polymer has the structure



wherein POLYa and POLYb are water-soluble and non-peptidic polymer backbones, such as PEG, that may be the same or different;

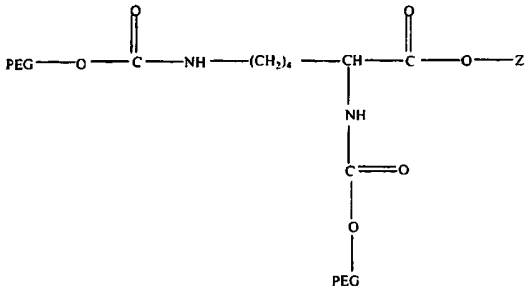
R is a central core molecule, such as glycerol or pentaerythritol;

q is an integer from 2 to about 300; and

each X is a capping group.

[0025] The X capping groups may be the same as discussed above for R'.

[0026] The invention involves the reaction of BTC esters of water-soluble and non-peptidic polymers with amino acids to form amino acid derivatives. In one embodiment, PEG-BTC esters are reacted with lysine to form a polymeric lysine derivative. For example, one such lysine derivative is a doubly PEGylated lysine, wherein the two PEGs are linked to the lysine amines by carbamate bonds, as shown below.



wherein PEG is poly(ethylene glycol) and Z is selected from the group consisting of H, N-succinimidyl, or 1-benzotriazolyl.

[0027] Such PEG derivative; of lysine are useful as reagents for preparation of PEG derivatives of proteins. These PEG derivatives often offer advantages overnon-PEGylated proteins, such as ionger circulating life-times *in vivo,* reduced rates of proteolysis, and lowered immunogenicity. In another aspect, PEG BTC derivatives are used directly in attaching PEG to proteins through carbamate linkages and may offer advantages similar to those described for the lysine PEG derivatives.

[0028] BTC esters of water-soluble and non-peptidic polymers can also be reacted with biologically active agents to form biologically active polymer conjugates. Examples of biologically active agents include peptides, proteins, enzymes, small molecule drugs, dyes, lipids, nucleosides, oligonucleotides, cells, viruses, liposomes, microparticles and micelles.

[0029] As noted above, it is believed that polymer derivatives prepared according to the invention exhibit higher quality because

degradation of the polymer backbone caused by phosgene is avoided. Further, since the method of the invention requires only one step and fewer reactants, process efficiency is enhanced and cost is reduced.

[0030] The following examples are given to illustrate the invention, but should not be considered in limitation of the invention.

EXPERIMENTAL

Example 1

Preparation of mPEG$_{5000}$BTC (Reference example)

[0031] A solution of mPEG$_{5000}$-OH (MW5000,15 g, 0.003 moles), di(1-benzotriazolyl) carbonate (4.0 g of 70% mixture, 0.000945 moles), and pyridine (2.2 ml) in acetonitrile (30 ml) was stirred at room temperature under nitrogen overnight. The solvent was removed by distillation, the residue was dissolved in 80 ml of methylene chloride, and the resulting solution was added to 850 ml of ethyl ether. The mixture was cooled to 0-5°C and the precipitate was collected by filtration. The precipitation process was then repeated to obtain a white solid which was dried under vacuum at room temperature to yield 13.5 g of product which was shown by ^{1}H nmr to be 100% substituted. ^{1}H nmr (dmso d-6): 3.23 ppm, CH_3O; 3.51 ppm, O-CH_2CH_2-O; 4.62 ppm, m, mPEG-O-CH_2-OCO_2-; 7.41-8.21, complex mult., benzotriazole protons.

Example 2

Preparation of mPEG$_{20,000}$BTC (Reference example)

[0032] A solution of mPEG$_{20,000}$-OH (MW 20,000, 20 g, 0.001 moles), di(1-benzotriazolyl) carbonate (3.4 g of 70% mixture, 0.00803moles), and pyridine (3.0 ml) in acetonitrile (40 ml) was stirred at room temperature under nitrogen overnight. The solvent was removed by distillation and the residue was dissolved in 80 ml of methylene chloride and the resulting solution was added to 800 ml of ethyl ether. The precipitate was collected by filtration and was dried under vacuum at room temperature to yield 16.8 g of product which was shown by ^{1}H nmr to be 100% substituted. ^{1}H nmr (dmso d-6): 3.23 ppm, CH_3O; 3.51 ppm, O-CH_2CH_2-O; 4.62 ppm, m, mPEG-O-CH_2-OCO_2-; 7.41-8.21, complex mult., benzotriazole protons.

Example 3

Derivatization of lysine with mPEG$_{20,000}$ BTC

[0033] Lysine.HCl (0.0275 g, 0.000151 moles) was dissolved in 26 ml of 0.1 M borate buffer and the pH was adjusted to 8.0 with 0.1 M NaOH. To the resulting solution was added mPEG $_{20,000}$ BTC (7.0 g, 0.00350 moles) over 15 minutes and the pH was kept at 8 by addition of 0.1 M NaOH. After stirring the resulting solution for 3 h, 15 g of H_2O and 4 g of NaCl were added and the pH was adjusted to 3.0 with 10% phosphoric acid. The product was extracted with methylene chloride and the extract dried over $MgSO_4$. After concentrating the solution to 30 ml, the solution was poured Into 300 ml of ethyl ether and the product collected by filtration and dried under vacuum at room temperature to yield 5.9 g of product as a white solid. Analysis by gel permeation chromatography (Ultrahydrogel 250, column temperature 75°C, aqueous buffer pH 7.2) showed the product to be a mixture of di-N-PEGylated lysine (MW - 40 KDa, 63.05%), mono-N-PEGylated lysine (MW-20 KDa,36.95%), and mPEG$_{20,000}$.

Example 4 (not within the claims)

Derivatization of lysozyme with $mPEG_{5000}BTC$

[0034] To 4 ml of lysozyme solution (3 mg/ml in 50 mM sodium phosphate buffer, pH 7.2) was added 20.3 mg of mPE_{5000} BTC (5-fold excess of mPEG5000 BTC) and the mixture was continually mixed at room temperature. Analysis by capillary electrophoresis (57cm x 76um column; 30mM phosphate buffer; operating voltage 25 kV) after 4 hours showed that 6.94% of unreacted lysozyme remained, while 33.99% of mono-PEGylated lysozyme, 43.11 % di-PEGylated lysozyme, 13.03% tri-PEGylated lysozyme, and 2.92% of tetra-PEGylated lysozyme had formed.

Example 5 (not within the claims)

PEG_{2KDa}-α-hydroxy-ω-propionic acid, benzyl ester

[0035] To a solution of PEG_{2KDa}-α-hydroxy-ω-propionic acid (10g, 0.0050 moles)(Shearwater Corp.) in anhydrous methylene chloride (100 ml) 1-hydroxybenzotriazole (0.30g), 4-(dimethylamino)pyridine (1.0g), benzyl alcohol (10.8g, 0.100 moles) and 1,3-dicyclohexylcarbodiimide (1.0 M solution in methylene chloride, 7.5 ml, 0.0075 moles) were added. The reaction mixture was stirred overnight at room temperature under argon. The mixture was then concentrated to about 50 ml, filtered and added to 800 ml cold diethyl ether. The precipitated product was filtered off and dried under reduced pressure. Yield 8.2g. NMR (d6-DMSO): 2.60 ppm (t, $-CH_2$ -COO-), 3.51 ppm (s, PEG backbone), 4.57 ppm (t, -OH-), 5.11 ppm (s, $-CH_2$- (benzyl)), 7.36 ppm (m, $-C_6H_5$ (benzyl)).

Example 6 (not within the claims)

PEG_{2KDa}-α-benzotriazole carbonate-ω-propionic acid, benzyl ester

[0036] To a solution of PEG_{2KDa}-α-hydroxy-α-propionic acid, benzyl ester (8.2g, 0.0025 moles) in acetonitrile (82 ml), pyridine (0.98 ml) and di(1-benzotriazolyl)carbonate (1.48g) were added and the reaction mixture was stirred overnight at room temperature under argon atmosphere. The mixture was then filtered and solvent was evaporated to dryness. The crude product was dissolved in methylene chloride and precipitated with isopropyl alcohol. The wet product was dried under reduced pressure. Yield 6.8g. NMR (d6-DMSO): 2.60 ppm (t, $-CH_2$-COO-), 3.51 ppm (s, PEG backbone), 4.62 ppm (m, $-CH_2$-O(C=O)-), 5.11 ppm (s, $-CH_2$-(benzyl)), 7.36 ppm (m, $-C_6H_5$ (benzyl)), 7.60 - 8.50 ppm (4m, aromatic protons of benzotriazole).

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- US5650234A [0007]
- US5932462A [0018]
- US5629384A [0019]

Non-patent literature cited in the description

- **GREENWALD et al.** J. Org. Chem., 1995, vol. 60, 331-336 [0005]

Patentkrav

1. Fremgangsmåde til fremstilling af et aminosyrederivat af en vandopløselig og ikke-peptidisk polymer, hvilken fremgangsmåde omfatter:
at tilvejebringe en vandopløselig og ikke-peptidisk polymer med mindst en terminal hydroxylgruppe valgt fra gruppen bestående af poly(alkylenglycol), poly(oxyethyleret polyol), poly(olefin alkohol), poly(vinylpyrrolidon), poly(α-hydroxy-syre), polyphosphazen, polyoxazolin, poly(N-acryloylmorpholin) og copolymerer, terpolymerer, og blandinger deraf, og
at reagere den terminale hydroxylgruppe af den vandopløselige og ikke-peptidiske polymer med di(1-benzotriazolyl)carbonat for at danne 1-benzotriazolylcarbonat-ester af den vandopløselige og ikke-peptidiske polymer, yderligere omfattende trinnet med at reagere 1-benzotriazdylcarbonat-esteren af den vandopløselige og ikke-peptidiske polymer med en aminosyre for at danne et aminosyrederivat.

2. Fremgangsmåde ifølge krav 1, hvor den vandopløselige og ikke-peptidiske polymer er poly(ethylenglycol).

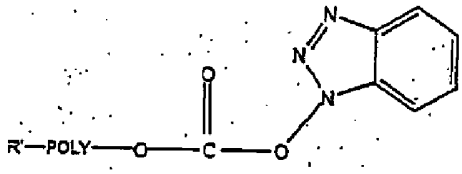
3. Fremgangsmåde ifølge krav 2, hvor poly(ethylenglycol) har en gennemsnitsmolekylvægt fra ca. 200 Da til ca. 100.000 Da.

4. Fremgangsmåde ifølge krav 2, hvor poly(ethylenglycol) har et antal af subenheder på 3-4.000.

5. Fremgangsmåde ifølge krav 2, hvor poly(ethylenglycol) har et antal af subenheder på 3-2.000.

6. Fremgangsmåde ifølge krav 1, hvor den vandopløselige og ikke-peptidiske polymer har fra ca. 2 til ca. 300 termini.

7. Fremgangsmåde ifølge krav 1, hvor den vandopløselige og ikke-peptidiske polymer har strukturen R'-POLY-OH, og 1-benzotriazofylcarbonat-esteren af den vandopløselige og ikke-peptidiske polymer har strukturen



hvor POLY er et vandopløseligt og ikke-peptidisk polymerskelet, og R' er beskyttelses- eller funktionel gruppe.

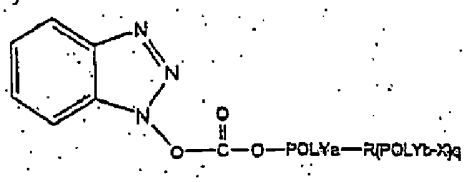
8. Fremgangsmåde ifølge krav 7, hvor POLY er poly(ethylenglycol).

9. Fremgangsmåde ifølge krav 8, hvor poly(ethylenglycol) har en gennemsnitsmolekylvægt fra ca. 200 Da til ca. 100.000 Da.

10. Fremgangsmåde ifølge et hvilket som helst af kravene 7 til 9, hvor R' er methoxy.

11. Fremgangsmåde ifølge et hvilket som helst af kravene 7 til 9, hvor R' er en funktionel gruppe valgt fra gruppen bestående af hydroxyl, beskyttet hydroxyl, aktiv ester, aktiv carbonat, acetal, aldehyd aldehyd hydrater, alkenyl, acrylat, methacrylat, acrylamid, aktiv sulfon, beskyttet amin, beskyttet hydrazid, thiol, beskyttet thiol, carboxylsyre, beskyttet carboxylsyre, isocyanat, isothiocyanat, maleimid, vinylsulfon, dithiopyridin, vinylpyridin, iodoacetamid, epoxid, glyoxaler, dioner, mesylater, tosylater, og tresylat.

12. Fremgangsmåde ifølge krav 1, hvor den vandopløselige og ikke-peptidiske polymer har strukturen HO-POLYa-R(POLYb-X)q, og 1-benzotriazolylcarbonat-esteren af den vandopløselige og ikke-peptidiske polymer har strukturen



hvor POLYa og POLYb er vandopløselige og ikke-peptidiske polymerskeletter, som kan være ens eller forskellige,
R er et centralt kernemolekyle;
q er et helt tal fra 2 til ca. 300; og
hver X er en beskyttelses eller funktionel gruppe.

13. Fremgangsmåde ifølge krav 12, hvor POLYa og POLYb er poly(ethylenglycol).

14. Fremgangsmåde ifølge krav 13, hvor POLYa og POLYb hver har en gennemsnitsmolekylvægt fra ca. 200 Da til ca. 100.000 Da.

15. Fremgangsmåde ifølge et hvilket som helst af kravene 12 til 14, hvor hver X uafhængigt er valgt fra gruppen bestående af alkoxy, hydroxyl, beskyttet hydroxyl, aktiv ester, aktiv carbonat, acetal, aldehyd, aldehyd hydrater, alkenyl, acrylat, methacrylat, acrylamid, aktiv sulfon, beskyttet amin, beskyttet hydrazid, thiol, beskyttet thiol, carboxylsyre, beskyttet carboxylsyre, isocyanat, isothiocyanat, maleimid, vinylsulfon, dithiopyridin, vinylpyridin, iodoacetamid, epoxid, glyoxaler, dioner, mesylater, tosylater, og tresylat.

16. Fremgangsmåde ifølge krav 13, hvor R er afledt fra en polyol.

17. Fremgangsmåde ifølge krav 16, hvor polyolen er valgt fra gruppen bestående af glycerol, pentaerythritol, og sorbitol.

18. Fremgangsmåde ifølge et hvilket som helst af kravene 1 til 17, hvor nævnte første reaktionstrin udføres i et organisk solvent.

19. Fremgangsmåde ifølge krav 18, hvor det organiske solvent er valgt fra gruppen bestående af methylenchlorid, chloroform, acetonitril, tetrahydrofuran, dimethylformamid, dimethyl sulfoxid, og blandinger deraf.

20. Fremgangsmåde ifølge et hvilket som helst af kravene 1 til 19, hvor nævnte første reaktionstrin udføres i nærvær af en base.

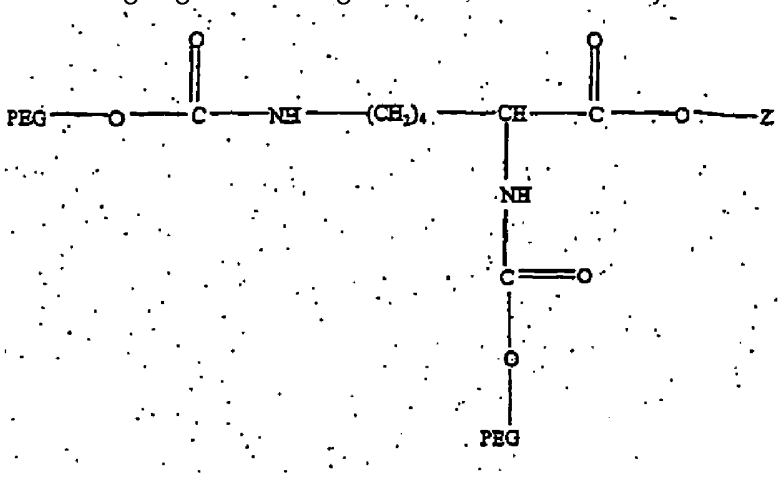
21. Fremgangsmåde ifølge krav 20, hvor basen vælges fra gruppen bestående af pyridin, dimethylaminopyridin, quinolin, trialkylaminer, og blandinger deraf.

22. Fremgangsmåde ifølge et hvilket som helst af kravene 1 til 21, hvor den molære ratio af di(1-benzotriazolyl)carbonat til den vandopløselige og ikke-

peptidiske polymer er ca. 30:1 eller mindre.

23. Fremgangsmåde ifølge krav 1, hvor aminosyren er lysin.

24. Fremgangsmåde ifølge krav 1, hvor aminosyrederivatet har strukturen



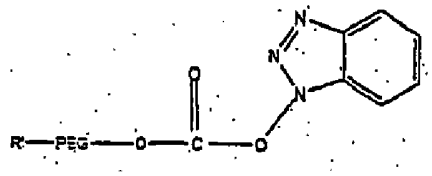
hvor PEG er poly(ethylenglycol), og Z vælges fra gruppen bestående af H, N-succinimidyl, eller 1-benzo-triazolyl.

25. Fremgangsmåde ifølge krav 24, yderligere omfattende at konjugere aminosyrederivatet til et protein for at danne et polymerderivat af proteinet.

26. Fremgangsmåde ifølge krav 1 omfattende:
at tilvejebringe et poly(ethylenglycol)-molekyle med en terminal hydroxylgruppe og med en gennemsnitsmolekylvægt fra ca. 200 Da til ca. 100.000 Da og med strukturen

R'-PEG-OH

hvor R' er en beskyttelsesgruppe eller en funktionel gruppe; og at reagere den terminale hydroxylgruppe med di(1-benzotriazolyl)-carbonat for at danne en 1-benzotriazolylcarboriat-ester af nævnte poly(ethylene glycol) med strukturen



hvor R' er som defineret ovenfor.

27. Fremgangsmåde ifølge krav 26, hvor R' er methoxy.

28. Fremgangsmåde ifølge krav 26, hvor R' er en funktionel gruppe udvalgt fra gruppen bestående af hydroxyl, beskyttet hydroxyl, aktiv ester, aktiv carbonat, acetal, aldehyd, aldehyd hydrat, alkenyl, acrylat, methacrylat, acrylamid, aktiv sulfon, beskyttet amin, beskyttet hydrazid, thiol, beskyttet thiol, carboxylsyre, beskyttet carboxylsyre, isocyanat, isothiocyanat, maleimid, vinylsulfon, dithiopyridin, vinylpyridin, iodoacetamid, epoxid, glyoxal, dion, mesylat, tosylat, og tresylat.