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**(54) Title:** SYNTHETIC POLYAMINO ACIDS, METHOD OF THEIR PRODUCTION AND USE THEREOF

**(57) Abstract:** The invention relates to a novel method for the production of polyamino acids with a high rate of glutamine, which have a better solubility in water and a method the formation of gels of these polyamino acids. An excess of an γ-ester of N-carboxyanhydride (NCA) of glutamic acid is mixed with one of the N-carboxyanhydrides of L-proline, lysine or serine, following an amidation of the -γester, preferably in liquid ammonia and accelerated by microwaves. The polyamino acids may be used in biotechnology.

# Synthetic polyamino acids, method of their production and use thereof

## Description

**[0001]** The invention relates to a novel method for the production of polyamino acids which have a better solubility in water and a method the formation of gels of these polyamino acids. The polyamino acids may be used in biotechnology.

**[0002]** Polymers play an important role in pharmaceutical biotechnology and are used as carrier substances, cryoprotectives, emulsifiers etc. Polymers from amino acids have major archetypes in peptides and proteins. The amino acid sequence is defined in order to meet specializations in regard to biological functions. Less stringent demands in this specialization no longer require defined amino acid sequences. Rather, universal application fields can be developed with "random" polyamino acids.

**[0003]** Polyamino acids, comprising the "building blocks of life", offer numerous advantages for pharmaceutical biotechnology. Important physical, chemical and biological properties can be standardized, thus for example solubility, diffuseness, hydrophobia, charge, affinity, activity and compatibility. Amino acids as degradation products can be "recycled" in-situ by other bioprocesses. In this way, for example, cell culture media made viscous with polyamino acids are suitable for influencing extracellular diffusion processes, enabling very low start cell densities and expanding into high cell densities (see also PCT/DE03/01826). Polyamino acids are also produced in order to test them as anti-cancer agents in clinical trials. At the same time, active substances are immobilized in the polyamino acid. Moreover, modified polyamino acids are also available on the market as carrier substances, whereby the modification contributes towards stabilization and delayed release of peptide pharmaceuticals.

**[0004]** In the present invention, we have concentrated on the use of glutamine as an essential amino acid for the synthesis of polymers. The particular advantages in the use of glutamine for the synthesis of polyamino acids lie in the polar yet uncharged properties, the chemical stability of the amide groups in comparison to amino or hydroxy groups and the ubiquity in biotechnical processes. In addition to this, glutamine-glutamine-peptide bonds are proteolytically stable, which forms the essential prerequisite for synthesis of a stable polymer framework. Free glutamine occurring in degradation processes does not represent any restriction in biotechnical processes, as it directly enters the cell metabolism as an energy source.

[0005] If these polyamino acids are cross-linked, gels may result, which combine the properties of the polymers with the requirements for room-filling materials (see also US Patent 5,955,549. Chang, et al. "Cross-linked poly(amino acids) and methods of preparation"). Previously soluble polymers become insoluble through cross-linking and are then determined as a new property through their swelling capacity. Polymer solutions can be cross-linked in any vessel forms. Taking the swelling capacity into account, dried gels can also be trimmed and hence end forms of the swollen gel specified.

[0006] To be able to utilize the above advantages, however, the following problems have to be solved.

[0007] The production of polyamino acids must occur purely synthetically, as raw materials of a biological origin represent a safety and quality risk in pharmaceutical biotechnology. Polyglutamine, irrespective of the production process (biotechnical, solid-phase chemical etc.), is known for its very poor solubility in water and thus cannot be used in this form. The swelling and shrinkage of the gels from polyamino acids must be as reversible as possible, so that dried gels can be trimmed and shaped. Finally, the polymers must be sterile as a raw material, allowing them to be used in biotechnical processes.

[0008] The following will describe the structure which polyamino acids from glutamine need to have so that they are water soluble, how these polyamino acids are produced and how they can be cross-linked and sterilized.

[0009] According to the invention a new method is disclosed for producing water soluble polyamino acids with a high rate of glutamine, comprising the steps of:

- Mixing an excess of an γ-ester of N-carboxyanhydride (NCA) of glutamic acid with one of the N-carboxyanhydrides of
  a) L-proline or
  b) lysine, containing a protective group or
  c) serine, containing a protective group
- Copolymerization of the mixed N-carboxyanhydrides
- Washing of copolymerizate
- Amidation of the ester groups
- if necessary, removal of the protective groups.

[0010] Preferably, the γ-ester of NCA of glutamic acid is NCA-Me-glutamate. The ratio between the γ-ester of the N-carboxyanhydride of the glutamic acid and the other N-carboxyanhydride is between 4:1 to 11:1, preferably 6:1.

[0011] As a third component NCAs of cysteine, S-trityl cysteine or of ε-Boc-lysine ca be added and thus the copolymerization is realized with three NCAs.

[0012] The amidation is made in liquid ammonia and the reaction can be accelerated by microwaves.

[0013] Also disclosed in this invention is a new method for the production of gels, wherein a cross-linking agent, preferably poly(ethyleneglycol)diacrylate, poly(ethyleneglycol)-bis-mercaptoacetate or hexamethylene-diisocyanate), is added to the above mentioned polyamino acids. Following the gel is washed and either dried and be trimmed and hence end forms or the gel formed for further applications and then dried. As a last step, a γ-sterilization is realized.

[0014] Matter for which protection is sought are - according to the invention - the synthetic polyamino acids of the formula described in the embodiments and in the claims.

[0015] The synthetic polyamino acids according to the invention can be used in pharmaceutical biotechnology, for producing semi solid media in cell cultivation, cell selection or cell manipulation, for gel formation or immobilization, for enabling very low start cell densities and expanding into high cell densities in cell cultivation, for immobilization of active substances and /or for controlled release of active substances or for a gentle in situ formation of gels in presence of vital cells for tissue engineering.

[0016] Polyamino acids can be produced through polymerization of N-carboxyanhydrides (NCA), whereby the ring-opening polymerization through addition of a protic nucleophile is initiated via elimination of carbon dioxide. The production of special NCAs has been the known for many years. The synthesis of polyglutamine occurs through polymerization of the γ-methyl ester of the NCA of glutamic acid (γ-Me-L-Glu-NCA) and a subsequent polymer-analogous amidation.

[0017] The polyglutamine (PGIn) produced in this manner is insoluble in most solvents, also in water, if a degree of polymerization greater than 20 has been reached. We see the cause of this aggregation in the synthesis of intermolecular hydrogen bridging bonds, which result both between the polymer main chains as well as between the amide groups in the side chains. These interactions are greater the closer the donor and acceptor can position themselves. As a result, measures for restricting the molecular flexibility and introducing repellent groups or measures for changing the donor-acceptor relation should contribute towards improving solubility.

[0018] Proline is an amino acid which significantly restricts the flexibility of the polymer chain. Moreover, rejection forces can be generated through identically charged groups, thus for example if the amino group of the lysine can be protonated. The relation of hydrogen donors to acceptors can be altered by serine to the benefit of the donors. These considerations were initially examined through targeted peptide synthesis. Undecapeptides from glutamine were produced in synthesis instruments, whereby the glutamine in position 6 is optionally replaced by proline, lysine, serine, glutamate and tryptophan. The effect on the solubility was examined with the RP-HPLC. Surprisingly, the peptides which were substituted with proline, lysine or serine, exhibited short retention times with a simultaneously low peak width while, on the other hand, unsubstituted glutamine peptide revealed very long retention times and broad peaks. The chromatographic behavior observed in the substituted peptides was explained by the absence of intermolecular interactions and hence confirmed the considerations in regard to solubility.

[0019] The concept for improving the solubility was transferred to the polymerization, i.e. so that soluble polyglutamines are obtained at substitution degrees, the replacement of glutamine by a solubility-improving amino acid, of approx. 9 mol-% or more. The copolymerization of various NCAs is possible and leads to statistical copolymers. In this way, for example, monomers of the NCAs of methyl glutamate, of proline and of lysine were mixed in a ratio of 60:10:6 and induced to polymerization. With the aid of the IH-NMR, the ratios of the copolymerized amino acids can be determined. If the polymerization with N-butyl amine has been initiated, the highly intensive methyl group is available in the spectrogram which, as a polymer head group, can also be used for estimating the molecular weight. In conjunction with MALDI experiments, molecular weights greater than 20 000 g/mol are determined.

[0020] The polymerization was followed by polymer-analogous amidation in liquid ammonia. This reaction step is kinetically controlled and requires corresponding time. The application

of microwaves for accelerating the reaction is known from peptide chemistry in aqueous solutions. As ammonia exhibits a similar dipole moment to water, an acceleration of the reaction should also be possible in ammonia. Surprisingly, the application of microwaves could also accelerate the reaction speed of the amidation in liquid ammonia by around 40-fold. The conversion is quantitative and can be tracked via an IR-spectrometer at approx. 1750 cm-1. The polyamino acids produced in this way reveal a solubility of minimum 30 g/L.

[0021] The partial substitution of glutamine by proline allows water solubility to be attained while the proteolytic resistance is maintained. At the present time, no proteases are known which exhibit a decomposition activity in respect to these two amino acids. Starting from this stable basic framework, "target fracture points" can be incorporated through the use of amino acids such as lysine, which enable a proteolytic degradation. Thus, for example, the proteases split trypsin, LysC and LysN if glutamine is substituted by lysine, glutamyl endopeptidase if glutamine is substituted by glutamic acid, and pepsin or proteinase K if glutamine is substituted by tryptophan. The speed and degree of degradation can therefore be adjusted.

[0022] The partial substitution of glutamine with lysine enables cross-linking points to be provided, which can be used either for gel formation or immobilization. An especially mild reaction, which can also be carried out under physiological conditions (pH = 7.4; 300 mosmol/kg), is the Michael-Addition of acrylates to thiol compounds. The perquisites for this reaction were created in a such a way that NCA of S-trityl cysteine is synthesized as a new compound, together with further NCAs such as γ-Me-L-Glu-NCA, etc., then copolymerized and subsequently deprotected.

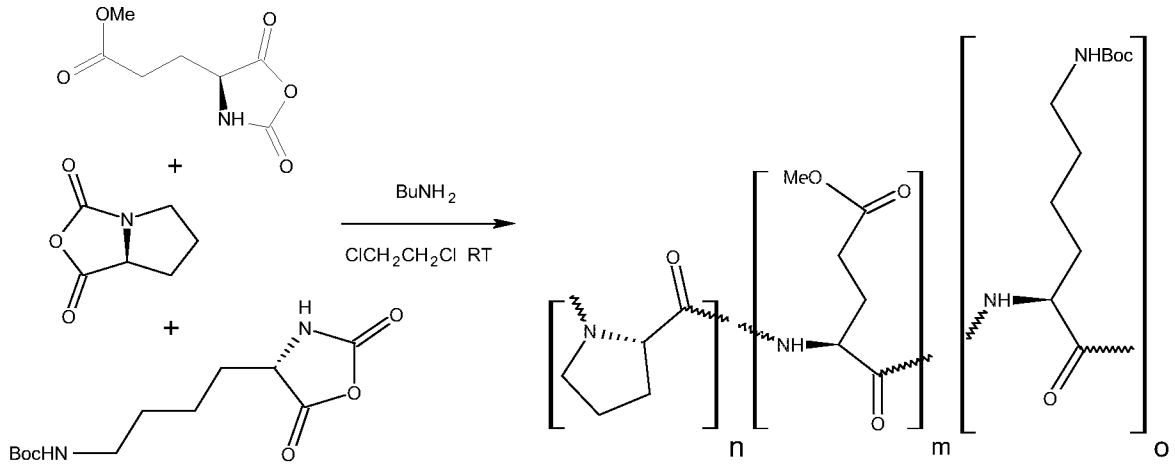
[0023] In order to obtain the sterile polyamino acids necessary for the biotechnical processes, sterilization processes with heat, gas and radiation were examined. The γ-sterilization proved to be the only suitable method. As the effectiveness of this process depends greatly on the absorption capacity of the sterile material, an irradiation of dried gels is always more efficient than the irradiation of swollen gels. Surprisingly, the irradiation of dried gels with a dosage of 25 to 40 kGy led to sterile materials for cell culture applications, which do not exhibit any cytotoxic effect in in-vitro experiments and which, in contrast to the non-irradiated material, do not reveal any loss of swelling capacity or loss of stability in the swollen gel.

[0024] The invention will now be described in more detail by means of exemplary embodiments, without being restricted to these examples.

**Embodiments**

Example 1: [AIn$_1$[BL[CI n- [Polvfamino acid) Al

**[0025]** PoIy(L-MeGIu-CO-L-PrO-Co-BoC-L-LyS). To a stirred solution of NCA L-Me-glutamate (11.2 g, 60 mmol), NCA L-proline (1.41 g, 10 mmol), and NCA ε-Boc-L-lysine (1.63 g, 6 mmol) in dry dichloromethane (150 mL) under nitrogen atmosphere was added a solution of n-butyl amine (50 µL, 0.5 mmol) in dry dichloromethane (1 mL) at rt. After several minutes gas evolution was observed. The reaction mixture was left stirring for 5 d at rt, after which diethyl ether (300 mL) was added. The resulting precipitate was filtered off via sintered filtration, washed with diethyl ether and 2% HCl and dried to afford the crude copolymer. This was used without further purification in the next step. Amino acid composition of poly(amino acid) was confirmed from a peak intensity of each proton in an $^1$H NMR spectrum of the poly(amino acid) A in solution ($CF_3COOD$) and by an amino acid analysis of the hydrolyzates of the copolymer. By means of $^1$H-NMR 81 mol-% MeGIu, 14 mol-% proline and 5 mol-% Boc-lysine were found according to the employed NCAs (79 mol-%, 13 mol-% and 8 mol-%, respectively). The chains were between 140 and 300 amino acid residues long (molecular weight 20-45 kDa was determined by MALDI-TOF mass spectrometry).

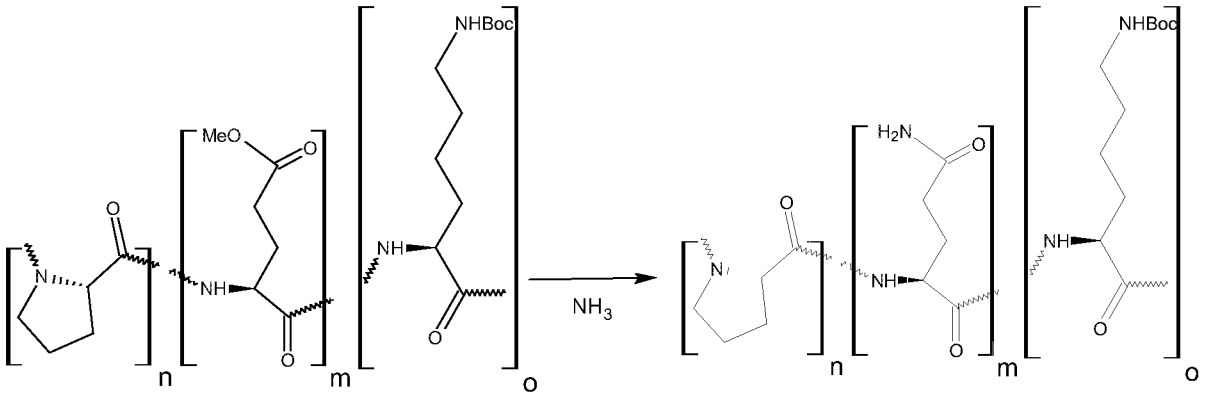



*Scheme 1: Reaction scheme corresponding to Example 1 - Copolymerization reaction*

Example 2: [AIn$_1$[BL[CL - [Polyfamino acid) Bl

**[0026]** PoIy(L-GIn-CO-L-PrO-Co-BoC-L-LyS). In a glass reactor of the Miniclave 300 (Buchi) were placed [Poly(amino acid) A] (11.0 g) and a magnetic stir bar. The Miniclave was sealed and cooled with liquid nitrogen. Ammonia was allowed to condense to the 200 mL mark in the glass reactor before warming slowly to rt. The contents of the Miniclave 300 were

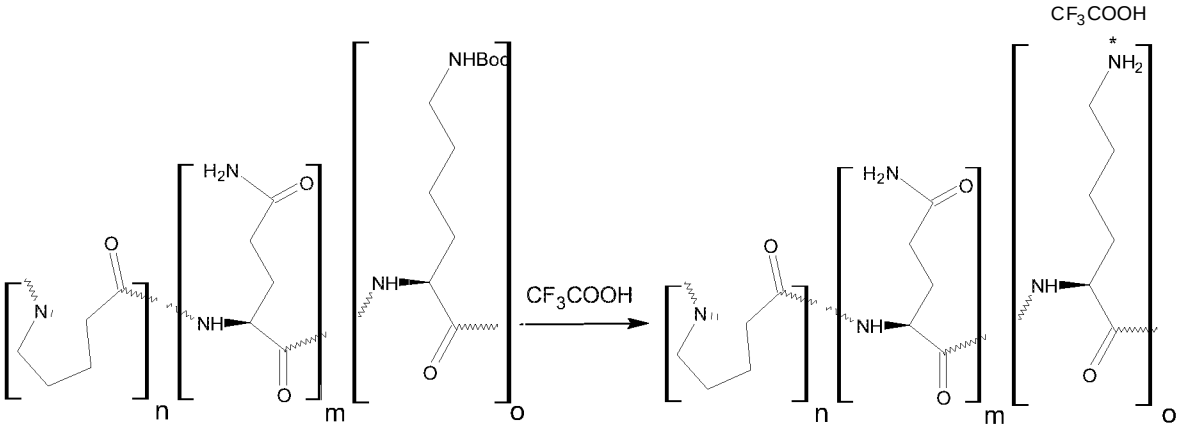
carefully stirred using a magnetic stirrer in a thermostat-controlled vessel at 17°C in a water bath behind a protective shield. The pressure in the reactor was approximately 8 bar at this temperature. After 14 days ammonia was slowly released from the vessel. After warming to rt, the polymer was taken up in 2% HCl, washed with diethyl ether, and dried to yield the crude product. NMR- and IR-Analysis of the synthesis product shows a complete conversion to glutamine from methyl glutamate (absence of -OCH3 signal by 3.77 ppm in CF3COOD solution and -COOMe signal by 1748cm$^{-1}$ in KBr).




*Scheme 2: Reaction scheme corresponding to Example 2 - Amidation reaction*

Example 3: [AIn$_1$[BlJCI n- [Polvfamino acid) Cl

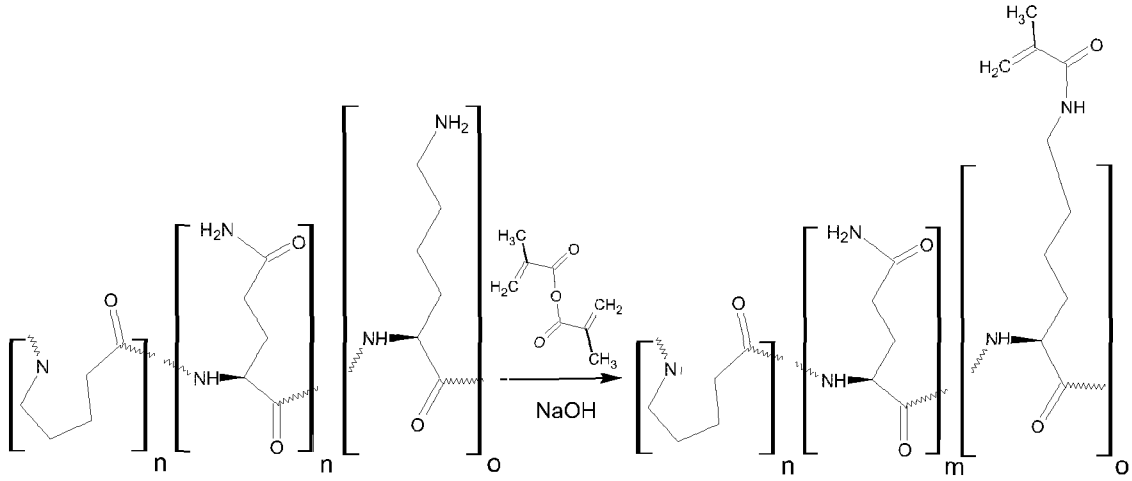
**[0027]** PoIy(L-GIn-CO-L-PrO-Co-L-LyS(TFA)). A 25OmL Schlenk flask containing Boc-protected [Poly(amino acid) B] (4.Og) was charged with trifluoroacetic acid (80 mL) and stirred for 2 h under an inert atmosphere. The mixture was diluted with diethyl ether (400 mL), and the resulting precipitate was filtered off, repeatedly washed with petroleum ether and diethyl ether, and dried in vacuo to yield the pure title compound as hygroscopic light yellow solid.



*Scheme 3: Reaction scheme corresponding to Example 3 - Deprotection of crosslinkage site in polymer*

Example 4: [AIm[Bl$_n$[CIn- [Poly(amino acid) DI

[0028] Poly(L-Gln-co-L-Pro-co-L-Lys)-methacrylamide. The TFA-salt of [Poly(amino acid) C] (3.Og) was dissolved in 1% NaOH (100 mL) and the pH of the solution was adjusted to 7.5. To this solution of polypeptide was added a solution of methacrylic acid anhydride (1.5 g, 1 mmol) in acetone (20 mL). The reaction was stirred for 5 h at it. The solvent was evaporated, and the dry residue was dissolved in Milli-Q-Water (200 mL), before being passed over a 1OkDa-Roth-tube dialysis machine. The pH of the solution isolated from the dialysis was adjusted with NaHC θ 3 to 7.0. Removal of the solvent afforded the dry residue (light yellow solid), which was γ-sterilized to 25 - 4OkGy.




*Scheme 4: Reaction scheme corresponding to Example 4 - Modification of crosslinkage site in polymer*

Example 5: [AIm[B]$_1$[CIn- [Polv(amino acid) E]

[0029] PoIy(L-GIn-CO-L-PrO-CO-BoC-L-LyS). [Poly(amino acid) Example A] (2.Og) was placed in the Teflon-pressure vessel of the MARS Microwave synthesis (CEM) machine. The pressurized vessel was cooled with liquid nitrogen resulting in a condensation of ammonia (40 mL), after which it was tightly sealed and slowly warmed to rt. The vessel was warmed to $70^0$C and 25 bar pressure was applied to allow the amidation reaction to occur. After 8 h the pressurized vessel was cooled down, the ammonia was slowly released, yielding the crude polymer upon warming to rt. The polymer was washed with 2% HCl and diethyl ether and dried in vacuo. NMR- and IR-Analysis of the synthesis product shows a complete conversion to glutamine from methyl glutamate (absence of -OCH3 signal by 3.77 ppm in CF3COOD solution and -COOMe signal by 1748cm$^{-1}$ in KBr).

Example 6: S-Trityl-L-Cysteine N-carboxyanhydride

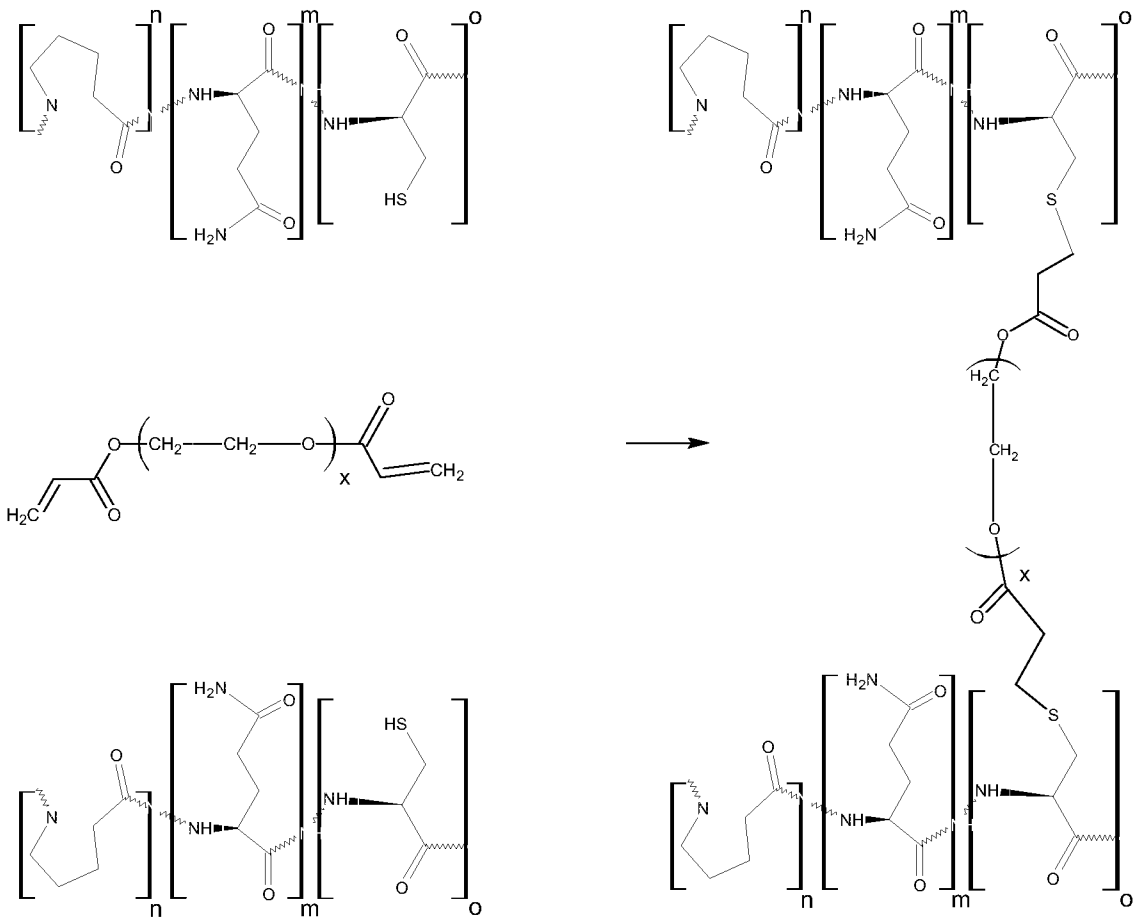
[0030] To a stirred suspension of S-Trityl-L-Cysteine (5.Og, 13mmol) in 5O mL of dry tetrahydrofurane was added drop wise at $55^0$C under nitrogen atmosphere a solution of triphosgene (1.60 g, 5 mmol) in 15 mL of dry tetrahydrofurane. The reaction mixture was left stirring for 4h at $55^0$C, cooled down and the solvent was removed. The resulting precipitate was dissolved in the mixture of ethyl acetate and petroleum ether and the solution was cooled at $-2O^0$C. The resulting precipitate was filtered off via sintered filtration, washed with diethyl ether and dried to afford the crude NCA. This was used without further purification in the next step. Structure of this compound was confirmed by NMR-Analysis (7.17-7.40 m, 15H, 5.62 s, IH, 3.44 dd, IH, 2.71 m, 2H, in $CDCl_3$).

Example 7: $[A]_m[B]_n[C]_o[E]_q$ – [Poly(amino acid) E]

[0031] a) PoIy(L-GIn-CO-L-PrO-Co-L-CyS). To a stirred solution of NCA L-Me-glutamate (11.2 g, 60 mmol), NCA L-proline (1.41 g, 10 mmol), and NCA S-trityl-L-cysteine (2.33 g, 6 mmol) in dry dichloromethane (150 mL) under nitrogen atmosphere was added a solution of n-butyl amine (50 µL, 0.5 mmol) in dry dichloromethane (1 mL) at rt. After several minutes gas evolution was observed. The reaction mixture was left stirring for 5 d at rt, after which diethyl ether (300 mL) was added. The resulting precipitate was filtered off via sintered filtration, washed with diethyl ether and 2% HCl and dried to afford the crude copolymer. This was used without further purification in the next step. Amino acid composition of poly(amino acid) was confirmed from a peak intensity of each proton in an $^1$H NMR spectrum in solution ($CF_3COOD$) and by an amino acid analysis of the hydrolyzates of the copolymer. The chains were between 120 and 250 amino acid residues long (molecular weight 20-42 kDa was determined by MALDI-TOF mass spectrometry). 3.Og of resulted poly(amino acid) was placed in the Teflon-pressure vessel of the MARS Microwave synthesis (CEM) machine. The pressurized vessel was cooled with liquid nitrogen resulting in a condensation of ammonia (40 mL), after which it was tightly sealed and slowly warmed to rt. The vessel was warmed to $70^0$C and 25 bar pressure was applied to allow the amidation reaction to occur. After 8 h the pressurized vessel was cooled down, the ammonia was slowly released, yielding the crude polymer upon warming to rt. The polymer was washed with 2% HCl and diethyl ether and dried in vacuo. NMR- and IR-Analysis of the synthesis product shows a complete conversion to glutamine from methyl glutamate (absence of —$OCH_3$ signal by 3.77 ppm in $CF_3COOD$

solution and —COOMe signal by 1748cm$^{-1}$ in KBr). 2g of this product was charged in a 250 mL Schlenk flask containing with 60 ml of trifluoroacetic acid and stirred for 2 h under an inert atmosphere. The mixture was diluted with diethyl ether (300 mL), and the resulting precipitate was filtered off, repeatedly washed with petroleum ether and diethyl ether, and dried in vacuo to yield the pure title compound as hygroscopic light yellow solid.

**[0032]** b) To a solution of [Poly(amino acid) E], 1 g in 25 mL of PBS buffer (4% solution) in a 50 mL Schott bottle was added under stirring poly(ethylene glycol)diacrylate (1 g). A light-yellow gel was formed after 5 h at rt and shown a swelling ratio of 20. Excess cross-linking agent may be removed via dialysis if desired.



*Scheme 5: Reaction scheme corresponding to example 7b - Cross-linking with cysteine*

Example 8: [A L [B]$_1$[CyDyEl$_1$

**[0033]** The TFA-salt of [Poly(amino acid) C] (12g) was dissolved in 1% NaOH (300 mL) and the pH of the solution was adjusted to 7.0 and the solvent was removed. To a solution of Poly(Gln-co-Pro-co-Lys) (10 g in 250 mL of DMSO) in a 500 mL beaker was added hexamethylene diisocyanate (1 g) in 10 mL von DMSO. A colourless and nearly transparent gel was formed after 2 h intense stirring at rt shown a swelling ratio of 50. Excess cross-linking agent may be removed via washing of the gel if desired.

Example 9: Proteolytic digestion

**[0034]** The TFA-salt of [Poly(amino acid) C] (0.3 g) was dissolved in 1% NaOH (7.5 mL) and the pH of the solution was adjusted to 7.0 and the solvent was removed. 16 µg proteinase K, dissolved in 0.4 mL 20 mM $CaCl_2$, were added to 0.3 g of Poly(Gln-co-Pro-co-Lys) dissolved in 20 mL phosphate buffered saline (0.1 mol/L, pH 7.5, containing 20 mM $CaCl_2$). The solution was transferred into a 10kDa-Roth dialysis tube and was dialyzed against PBG at 25 $^0$C. After 12h no product was found within the tube because all chains of [Poly(amino acid) C] were degraded and passed the dialysis tube whereas Poly(L-Gln-co-L-Pro) was totally rejected.

Example 9: Semi-Solid Media

**[0035]** The gel particles, obtained from example 8 were autoclaved. In one cell culture trial, 900 µl of the comminuted gel were blended with 100 µl of a cell suspension that had a concentration of 1-10$^4$ cells/mL. The resulting cell density was thus 1-10$^3$ cells/mL.

**[0036]** 500 µl of this suspension are added to a 24-well cell culture plate. This corresponds to a gel bed height of approx. 3 mm. The cell-containing gel in the cell culture plate is coated with 50 µl fresh medium and incubated at 37° C. in the incubator (5 vol. % $CO_2$). The cell count is performed daily. The cell count in the gel increases with culture duration.

**We claim:**

1. A method for producing water-soluble polyamino acids with a high rate of glutamine, comprising the steps of:
   - Mixing an excess of an γ-ester of N-carboxyanhydride (NCA) of glutamic acid with one of the N-carboxyanhydrides of
     a) L-proline or
     b) lysine, containing a protective group or
     c) serine, containing a protective group
   - Copolymerization of the mixed N-carboxyanhydrides
   - Washing of copolymerizate
   - Amidation of the ester groups
   - if necessary, removal of the protective groups.

2. A method according to claim 1, wherein the γ-ester of the N-carboxyanhydride of the glutamic acid is NCA-Me-glutamate.

3. A method according to claim 1, wherein the ratio between the γ-ester of the N-carboxyanhydride of the glutamic acid and the other N-carboxyanhydride is between 4:1 to 11:1, preferably 6:1.

4. A method according to claim 1, wherein in case of Ia) or Ic) additionally the N-carboxyanhydride (NCA) of cysteine or S-trityl cysteine or the N-carboxyanhydride (NCA) of ε-Boc-lysine is added as a third component and the copolymerization is realized with three NCAs.

5. A method according to claim 1, wherein in case of Ib) additionally the N-carboxyanhydride (NCA) of cysteine or S-trityl cysteine is added as a third component and the copolymerization is realized with three NCAs.

6. A method according to claim 1, wherein the amidation is made in liquid ammonia and the reaction is accelerated by microwaves.

7. A method for the production of gels, wherein a cross-linking agent, preferably poly(ethyleneglycol)diacrylate, poly(ethyleneglycol)-bis-mercaptoacetate or hexamethylene-diisocyanate), is added to the polyamino acids according to claim 1, following the gel is washed and either dried and be trimmed and hence end forms or formed for further applications and then dried.

8. A method according to claim 7, wherein a γ-sterilization is realized as a last step.

9. Synthetic, water soluble polyamino acids of the formula $[A]_m[B]_n$ , obtainable according to claim 1, wherein [A] is glutamine and [B] is an amino acid, which effects a better solubility in water, preferably proline, lysine or serine.

10. Synthetic, water soluble polyamino acids according to claim 9, wherein glutamine is the main component (m > n) and the sequence of the polyamino acid
    a) is not defined, but leads to a statistical arrangement or
    b) has a defined sequence.

11. Synthetic, water soluble polyamino acids of the formula $[A]_m[B]_n[C]_0$, obtainable according to claim 1, wherein [A] = glutamine, [B] = proline, lysine or serine and [C] is an amino acid, enables cross-linking, in case [B] = proline or serine, preferably lysine, cysteine or S-trityl cysteine, in case [B] = lysine, preferably cysteine or S-trityl cysteine and glutamine is the main component (m > n + o).

12. Synthetic, water soluble polyamino acids of the formula $[A]_m[B]_n[C]_0[D]_p$ obtainable according to claim 1, wherein [D] is an amino acid, which enables a proteolytic degradation, preferably lysine, arginine or tryptophane and glutamine is the main component (m > n + o + p).

13. Synthetic, cross-linked polyamino acids of the formula $[A]_m[B]_n[C]_0[D]_p[E]_q$ obtainable according to claim 7, wherein glutamine is the main component (m > n + o + p + q), and [E] is a cross-linking agent, which connects two polymer chains, preferably N,N''-hexane-1,6-diyldiurea or polyethyleneglycol-bis-diyl-3-mercaptopropanoate, between the polymer chains and its rate is $q \leq o$.

14. Use of synthetic polyamino acids according to one of the claims 9 to 13 in pharmaceutical biotechnology.

15. Use according to claim 14 for producing semi solid media in cell cultivation, cell selection or cell manipulation.

16. Use according to claim 14 for gel formation or immobilization.

17. Use according to claim 14 for enabling very low start cell densities and expanding into high cell densities in cell cultivation.

18. Use according to claim 14 for immobilization of active substances and /or for controlled release of active substances.

19. Use according to claim 14 for a gentle in situ formation of gels in presence of vital cells for tissue engineering.

# INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2007/051562

**A. CLASSIFICATION Of SUBJECT MATTER**
INV. C08G69/10

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)
C08G

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

Electronic data base consulted during the international search (name of data base and, where practical search terms used)
EPO-Internal , CHEM ABS Data

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of tha relevant passages | Relevant to claim No |
|---|---|---|
| X | US 2002/128177 Al (KIRK RANDAL J [US] ET AL) 12 September 2002 (2002-09-12) claims 1,24 examples | 9-19 |
| A | US 2002/032309 Al (DEMING TIMOTHY J CUS] ET AL) 14 March 2002 (2002-03-14) claims | 1 |
| A | WO 03/102123 A (PROBIOGEN AG [DE]; RIEDEL MARCO [DE]; MARX UWE [DE]; BUSHNAQ-JOSTING H) 11 December 2003 (2003-12-11) cited in the application claims | 1 |

**D** Further documents are listed in the continuation of Box C

[X] See patent family annex

* Special categories of cited documents

"A" document defining the general state of the art which is not considered to be of particular relevance

[1]E[1] earlier document but published on or after the international filing date

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"X" document of particular relevance, the claimed invention cannot be considered novel or cannot bθ considered to involve an inventive step when the document is taken alone

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| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 19 June 2007 | 04/07/2007 |

| Name and mailing address of the ISA/ | Authorized officer |
|---|---|
| European Patent Office, P B 5818 Patentlaan 2<br>NL - 2280 HV Rljswljk<br>Tel (+31-70) 340-2040, Tx 31 651 epo nl,<br>Fax (+31-70) 340-3016 | West , Nuki |

# INTERNATIONAL SEARCH REPORT

**Information on patent family members"**

**International application No**
PCT/EP2007/051562

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| | | DE 10325148 A1 | 11-12-2003 |
| | | DE 10393303 D2 | 23-06-2005 |
| | | EP 1513920 A2 | 16-03-2005 |
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