prepared by a Michael addition of primary aminoalcohols, disulfide-containing N,N'-cystamine bisacrylamide and 1,2-diaminoethane.

R. Namgung *et al.*, Biotechnol. Lett. *32*, 755, **2010**, incorporated by reference, discloses poly(amido amine)s containing disulfide linkages which are prepared by a Michael addition of 4-amino-1-butanol and disulfide-containing N,N'-cystamine bisacrylamide.

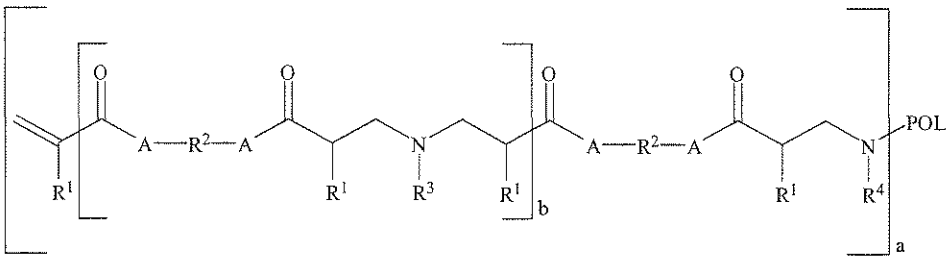
P. vader et al., Pharm. Res. *28*, 1013, **2011**, incorporated by reference, discloses poly(amido amine)s containing disulfide linkages which are prepared by a Michael addition of 4-amino-1-butanol, disulfide-containing N,N'-cystamine bisacrylamide and 1,2-diaminoethane.

D. Vercauteren et al., Biomaterials *32*, 3072, **2011**, incorporated by reference, discloses poly(amido amine)s containing disulfide linkages which are prepared by a Michael addition of 4-amino-1-butanol and disulfide-containing N,N'-cystamine bisacrylamide.

The cationic polymeric systems known from the prior art have still several disadvantages. In particular, the need for the presence of excess of (cytotoxic) polymer in the therapeutic formulation, the frequently low handling and storage stability of the therapeutically loaded nanogels, as well as limited efficiencies in endosomal escape and cytosolic unpacking of the therapeutic payload (nucleotide) from the complex are still major challenges in this field.

Summary of the invention

One aspect of t~~T~~he present invention relates to a PA (PolyAcryl) polymer according to the general Formulas (1) and (2):



(1)

heteroatoms, wherein the heteroatoms are independently selected from O, N and S; and

(f) C_7 - C_{12} alkylheteroarylene, wherein the alkylheteroarylene group comprises one, two or three heteroatoms independently selected from O, N and S and/or wherein the alkylheteroarylene group is optionally substituted, and/or wherein an alkyl part of the alkylheteroarylene group is linear or branched and is optionally (partly) unsaturated and/or is optionally interrupted by one or more heteroatoms, wherein the heteroatoms are independently selected from O, N and S;

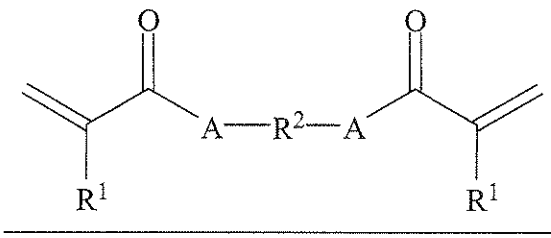
POL represents a polymeric core having a weight average molecular weight M_w of about 300 to about 25000;

a = 2 - 64; and

b = 1 - 50.

In another aspect, there is provided a process for the preparation of a PA polymer according to general Formulas (1) or (2), said process comprising the steps of:

(1) a monomer (I) according to general Formula (12):



(12)

wherein R^1, A and R^2 are as defined herein, is reacted with a monomer (II) according to general Formula (13) or a monomer (III) according to general Formula (14):

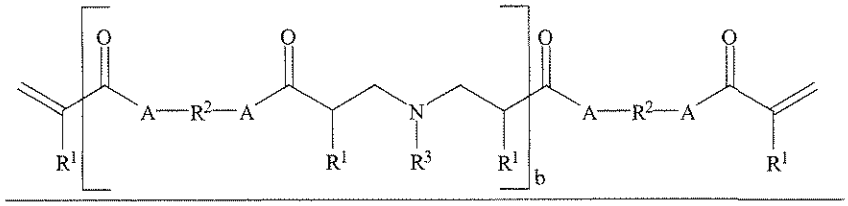
(H_2N)-R^3

(13)

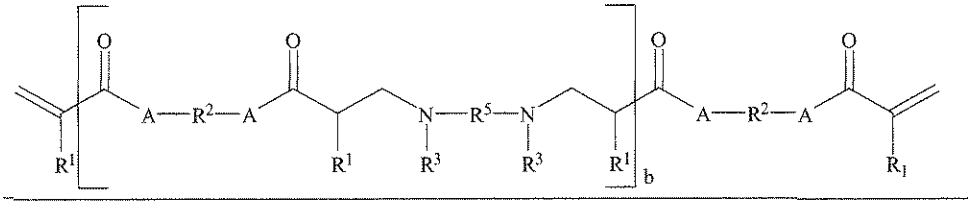
HR^3N-R^5-NR^3H

(14)

wherein R^3 and R^5 are as defined herein, in a molar ratio of monomer (I) : monomer (II) of from about 1.5 : 1 to about 10 : 1 to form a macromer according to general Formulas (15) or (16):



(15)



(16)

wherein b is as defined herein; and

(2) reacting the macromer according to general Formulas (15) or (16) with a polymer according to Formula (17):

$$[(R^4{}_2)N]_a\text{-POL}$$

(17)

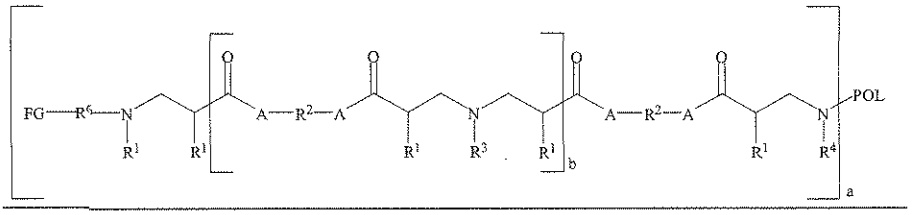
wherein a and R^4 are as defined herein and wherein at least one R^4 is H.

In yet another aspect, there is provided a PA polymer according to general Formulas (1) or (2) obtainable by the process as described herein.

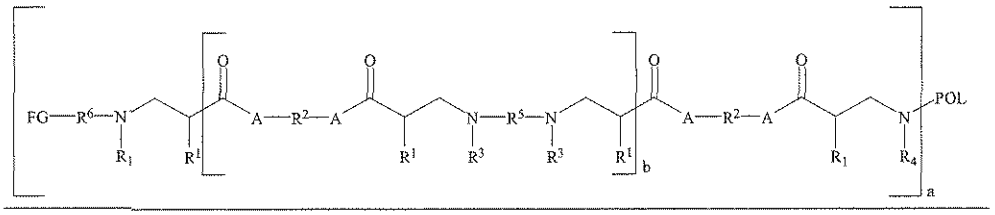
In a further aspect, there is provided a nanogel comprising the PA polymer as described herein.

In another aspect, there is provided a process for preparing the nanogel as described herein, wherein a PA polymer according to general Formulas (1) or (2) is cross-linked.

In yet another aspect, there is provided a nanoparticle the general Formulas (3) and (4):



(3)



(4)

wherein R^1, R^2, R^3, R^4, R^5, A, POL, a and b are as defined herein;

R^6 is selected from the group consisting of:

(a) C_1 - C_{40} alkylene, wherein the alkylene group may be linear or branched and is optionally substituted and/or is optionally (partly) unsaturated and/or is optionally interrupted by one or more heteroatoms, wherein the heteroatoms are independently selected from O, N and S, and/or wherein the alkylene group is interrupted by one or more -S-S- groups; or

(b) C_3 - C_{40} cycloalkylene, wherein the cycloalkylene group is optionally substituted and/or is optionally (partly) unsaturated and/or optionally comprises one or more heteroatoms in the ring, wherein the heteroatoms are independently selected from O, N and S, and/or wherein the cycloalkylene group is interrupted by one or more -S-S- groups outside the ring; and

FG is a Functional Group which is selected from the group consisting of -OH, -OR^7, -NH_2, -$NH(R^7)$, -$N(R^7)_2$, -C(O)OR^7, -C(O)R^7, -C(O)NHR^7, and -C(O)NR^7_2, wherein R^7 is independently selected from the group consisting of:

(a") H;

(b") C_1 - C_{10} alkyl, wherein the alkyl group may be linear or branched and is optionally substituted and/or is optionally (partly) unsaturated and/or is optionally interrupted by one, two or three heteroatoms, wherein the heteroatoms are independently selected from O, N and S;

(c") C_3 - C_{12} cycloalkyl, wherein the cycloalkyl group is optionally substituted and/or is optionally (partly) unsaturated and/or optionally comprises one, two or three heteroatoms in the ring, wherein the heteroatoms are independently selected from O, N and S;

(d") C_6 - C_{12} aryl, wherein the aryl group is optionally substituted;

(e") C_6 - C_{12} heteroaryl, wherein the heteroaryl group comprises one, two or three heteroatoms independently selected from O, N and S and/or wherein the heteroaryl group is optionally substituted;

(f") C_7 - C_{14} alkylaryl wherein the alkylaryl group is optionally substituted and/or wherein an alkyl part of the alkylarylene group is linear or branched and is optionally (partly) unsaturated and/or is optionally interrupted by one, two or three heteroatoms, wherein the heteroatoms are independently selected from O, N and S; and

(g") C_7 - C_{14} alkylheteroaryl, wherein the alkylheteroaryl group comprises one, two or three heteroatoms independently selected from O, N and S and/or wherein the alkylheteroarylene group is optionally substituted, and/or wherein an alkyl part of the alkylheteroarylene group is linear or branched and is optionally (partly) unsaturated and/or is optionally interrupted by one, two or three heteroatoms, wherein the heteroatoms are independently selected from O, N and S.

In a further aspect, there is provided a process for preparing a nanoparticle comprising the step of reacting a PA polymer according to general Formulas (1) or (2) with a reagent according to Formulas (26a) or (26b):

$$FG\text{-}R^6\text{-}NHR^{30}$$

(26a)

7d

$FG\text{-}R^6\text{-}SH$

(26b)

wherein R^6 and FG are as defined herein, FG being optionally H, and R^{30} is independently selected from the group consisting of:

(a) H;

(b) C_1 - C_{10} alkyl, wherein the alkyl group may be linear or branched and is optionally substituted and/or is optionally (partly) unsaturated and/or is optionally interrupted by one, two or three heteroatoms, wherein the heteroatoms are independently selected from O, N and S;

(c) C_3 - C_{12} cycloalkyl, wherein the cycloalkyl group is optionally substituted and/or is optionally (partly) unsaturated and/or optionally comprises one, two or three heteroatoms in the ring, wherein the heteroatoms are independently selected from O, N and S;

(d) C_6 - C_{12} aryl, wherein the aryl group is optionally substituted;

(e) C_6 - C_{12} heteroaryl, wherein the heteroaryl group comprises one, two or three heteroatoms independently selected from O, N and S and/or wherein the heteroaryl group is optionally substituted;

(f) C_7 - C_{14} alkylaryl wherein the alkylaryl group is optionally substituted and/or wherein an alkyl part of the alkylarylene group is linear or branched and is optionally (partly) unsaturated and/or is optionally interrupted by one, two or three heteroatoms, wherein the heteroatoms are independently selected from O, N and S; and

(g) C_7 - C_{14} alkylheteroaryl, wherein the alkylheteroaryl group comprises one, two or three heteroatoms independently selected from O, N and S and/or wherein the alkylheteroarylene group is optionally substituted, and/or wherein an alkyl part of the alkylheteroarylene group is linear or branched and is optionally (partly) unsaturated and/or is optionally interrupted by one, two or three heteroatoms, wherein the heteroatoms are independently selected from O, N and S.

In another aspect, there is provided a surface modified nanogel obtainable by a process comprising the steps of:

(1) cross-linking a PA polymer according to general Formulas (1) or (2);

(2) optionally loading the nanogel with a biologically active component, preferably selected from the group of RNA (in particular siRNA) or derivatives or fragments hereof, DNA or derivatives or fragments hereof, (oligo)peptides and derivatives thereof, and proteins and derivatives thereof; and

(3) reacting the nanogel with a reagent R-CFG or R'-SH,

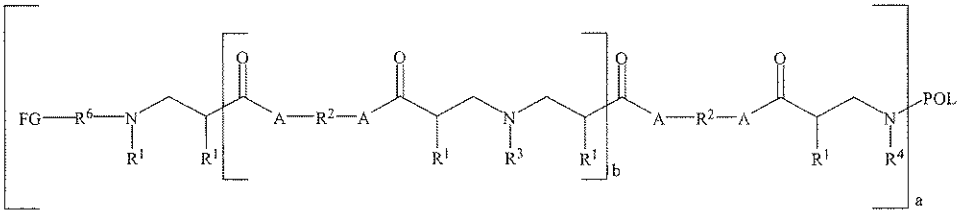
wherein:

R and R' are selected from the group consisting of 2-thioethyl, 2-hydroxyethyl, PEG (polyethylene oxide) residues and PPO (polypropylene oxide) residues, wherein the PEG residues and the PPO residues have a number average molecular weight M_n of about 500 to about 10000;

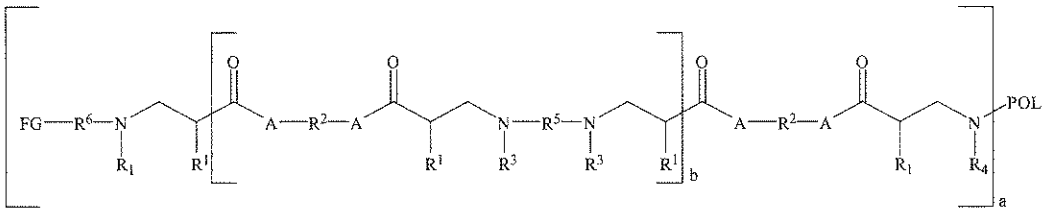
CFG is a group capable of forming a covalent bond with a group FG, wherein FG is as defined herein; and

wherein the sequence of steps (1), (2) and (3) is: (1) - (2) - (3), .(1) - (3) - (2), or (3) - (1) - (2).

The present ~~invention~~ disclosure further relates to a nanoparticle according to the general Formulas (3) and (4):



(3)



(4)

wherein R^1, R^2, R^3, R^4, R^5, A, POL, a and b are as defined above, and wherein R^6 is selected from the group consisting of:

(a) C_1 - C_{40} alkylene, wherein the alkylene group may be linear or branched and is optionally substituted and/or is optionally (partly) unsaturated and/or is optionally interrupted by one or more heteroatoms, wherein the heteroatoms are independently selected from O, N and S, and/or wherein the alkylene group is interrupted by one or more -S-S- groups; or

(b) C_3 - C_{40} cycloalkylene, wherein the cycloalkylene group is optionally substituted and/or is optionally (partly) unsaturated and/or optionally comprises one or more heteroatoms in the ring, wherein the heteroatoms are independently selected from O, N and S, and/or wherein the cycloalkylene group is interrupted by one or more -S-S- groups outside the ring; and

FG is a Functional Group.

The present ~~invention~~ disclosure further relates to a nanogel comprising a PA (PolyAcryl) polymer according to the general Formulas (1) or (2), wherein the nanogel is formed by cross-linking the PA polymer.

The present disclosure ~~invention~~ further relates to nanogels having a modified surface.

The present disclosure ~~invention~~ further relates to nanogels comprising a biologically active component.

The present disclosure ~~invention~~ further relates to the use of the nanogels and the use of the nanogels in the delivery of a biologically active component to a mammal.

The present disclosure ~~invention~~ further relates to pharmaceutical compositions comprising the nanogel and a pharmaceutically acceptable carrier.

The present disclosure ~~invention~~ further relates to a method for delivering a biologically active component to a mammal, wherein a nanogel or a composition comprising such a nanogel is administered to a mammal.

Description of the figures

Figure 1 shows the ability of PA polymers to bind siRNA as established in an EtBr (ethidium bromide) assay. A low fluorescence signal is indicative for efficient shielding (protection) of siRNA.

Figure 2 shows the ability of nanogels comprising a PA polymer to bind and shield siRNA as established in an EtBr (ethidium bromide) assay.

Figure 3 shows that the nanogels have a higher ability to bind and shield siRNA than the PA polymers (compare Sample Nos. 12 vs. 4, and 13 vs. 3 at higher polymer/siRNA ratio's).

Figure 4 shows the gene silencing efficiency in H1299 cells of PA polymers. The dark grey bars show the percentage of luciferase expression. The light grey bars are the controls with non-sensitive siRNA.