

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
14 May 2009 (14.05.2009)

PCT

(10) International Publication Number  
**WO 2009/061854 A2**

(51) International Patent Classification:  
C08L 63/08 (2006.01)

(21) International Application Number:

PCT/US2008/082529

(22) International Filing Date:

5 November 2008 (05.11.2008)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

|            |                                |    |
|------------|--------------------------------|----|
| 60/985,608 | 5 November 2007 (05.11.2007)   | US |
| 61/038,041 | 19 March 2008 (19.03.2008)     | US |
| 61/100,752 | 28 September 2008 (28.09.2008) | US |
| 61/101,039 | 29 September 2008 (29.09.2008) | US |

(71) Applicant (for all designated States except US): **VAN-  
DERBILT UNIVERSITY** [US/US]; Office Of Technol-  
ogy Transfer, 305 Kirkland Hall, Nashville, TN 37240  
(US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **HARTH, Eva,  
M.** [DE/US]; 415 Church Street, #2402, Nashville, TX  
37219 (US). **VAN DER ENDE, Alice, E.** [CA/US]; 477  
Claircrest Drive, Antioch, TN 37013 (US). **HAMILTON,**

**Sharon, K.** [US/US]; 342 24th Avenue, Nashville, TN  
37223 (US). **CROCE, Teresa, A.** [US/US]; 456 Hickory  
Blvd., Nashville, TX 37342 (US).

(74) Agents: **SHORTELL, D., Brian** et al.; Ballard Spahr An-  
drews & Ingersoll, LLP, Suite 1000, 999 Peachtree Street,  
Atlanta, GA 30309-3915 (US).

(81) Designated States (unless otherwise indicated, for every  
kind of national protection available): AE, AG, AL, AM,  
AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA,  
CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE,  
EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID,  
IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK,  
LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW,  
MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT,  
RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ,  
TM, TN, TR, TT, TZ, UA, UG, US (patent), UZ, VC, VN,  
ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every  
kind of regional protection available): ARIPO (BW, GH,  
GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM,  
ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),  
European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI,  
FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL,

[Continued on next page]

(54) Title: MULTIFUNCTIONAL DEGRADABLE NANOPARTICLES WITH CONTROL OVER SIZE AND FUNCTIONALI-  
TIES

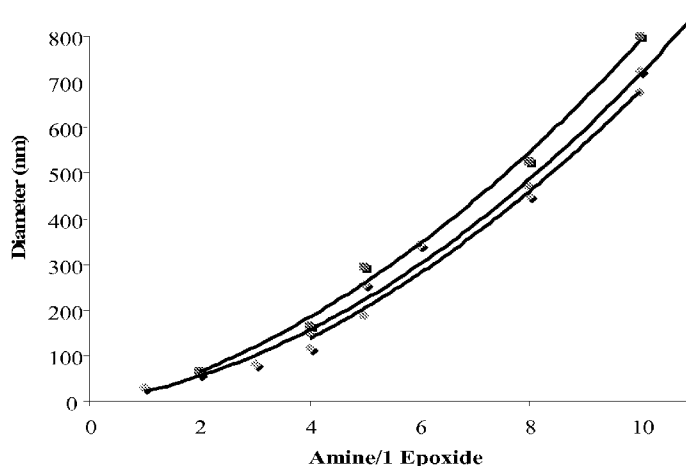


FIGURE 3

(57) Abstract: In one aspect, the invention relates to polymers, crosslinked polymers, functionalized polymers, nanoparticles, and functionalized nanoparticles and methods of making and using same. In one aspect, the invention relates to degradable polymer and degradable nanoparticles. In one aspect, the invention relates to methods of preparing degradable nanoparticles and, more specifically, methods of controlling particle size during the preparation of degradable nanoparticles. In one aspect, the degradable nanoparticles are useful for complexing, delivering, and releasing payloads, including pharmaceutically active payloads. This abstract is intended as a scanning tool for purposes of searching in the particular art and is not intended to be limiting of the present invention.

WO 2009/061854 A2



NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG,  
CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Published:**

- *without international search report and to be republished  
upon receipt of that report*

**MULTIFUNCTIONAL DEGRADABLE NANOPARTICLES WITH CONTROL  
OVER SIZE AND FUNCTIONALITIES**

5

**CROSS-REFERENCE TO RELATED APPLICATIONS**

[0001] This application claims benefit of U.S. Provisional Application No. 60/985,608, filed November 5, 2007, U.S. Provisional Application No. 61/038,041, filed March 19, 2008, U.S. Provisional Application No. 61/100,752, filed September 28, 2008, and U.S. Provisional Application No. 61/101,039, filed September 29, 2008, each of which is incorporated herein in its entirety by reference.

10

**ACKNOWLEDGMENT**

[0002] This invention was made with government support under a CAREER Award CHE-0645737 awarded by the National Science Foundation. The United States government has certain rights in the invention.

15

**BACKGROUND**

[0003] Biodegradable nanoparticles have received increasing attention as versatile drug delivery scaffolds to enhance the efficacy of therapeutics. Effectiveness of delivery, however, can be influenced by the particle size and morphology, as these parameters can greatly affect the biological function and fate of the material. [Zweers, M. L. T.; Grijpma, D. W.; Engbers, G. H. M.; Feijen, J., J. Controlled Release 2003, 87, 252-254.] Narrowly dispersed particles are highly preferred for use in delivery or sensing applications with respect to monitoring and predicting their behavior as they exhibit a more constant response to external stimuli.

20

[Lubetkin, S.; Mulqueen, P.; Paterson, E. Pesti. Sci. 1999, 55, 1123-1125.]

25

[0004] One disadvantage of conventional methods is the irreproducibility in the size and shape of the particles, since these can be profoundly influenced by the stabilizer and the solvent used. [Kumar, M. N. V. R.; Bakowsky, U.; Lehr, C. M., Biomaterials 2004, 25, 1771-

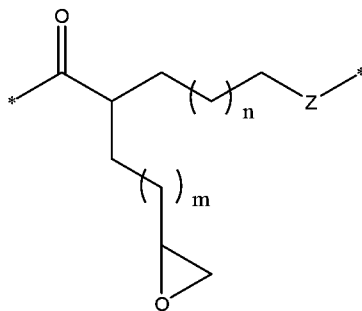
1777.] Another major drawback of conventional biodegradable nanoparticles, based on poly( $\epsilon$ -caprolactone) and other aliphatic polyesters, is the lack of pendant functional groups, which can make physiochemical, mechanical, and biological properties difficult to modify. [(a) Riva, R.; Lenoir, S.; Jerome, R.; Lecomte, P. *Polymer* 2005, 46, 8511-8518. (b) Sasatsu, M.; Onishi, H.; Machida, Y. *Inter. J. Pharm.* 2006, 317, 167-174.] The availability of functional groups is a desirable means of tailoring the properties of a particle, including hydrophilicity, biodegradation rate, and bioadhesion.

[0005] Therefore, there remains a need for methods and compositions that overcome these deficiencies and that effectively provide functionalized, degradable nanoparticles with reproducibility in particle size and shape.

#### SUMMARY

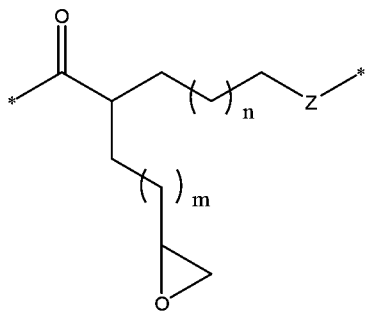
[0006] In accordance with the purpose(s) of the invention, as embodied and broadly described herein, the invention, in one aspect, relates to polymers, crosslinked polymers, functionalized polymers, nanoparticles, and functionalized nanoparticles and methods of making and using same.

[0007] Disclosed are polymers comprising at least one monomer residue having an optionally substituted structure represented by a formula:

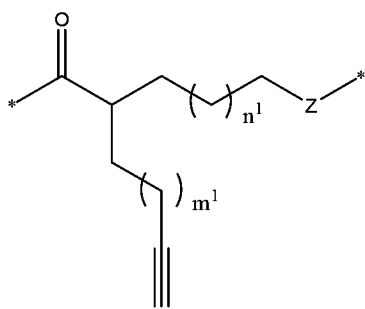


wherein Z is O or NR, wherein R is H or C1 to C6 alkyl; wherein m is an integer from 0 to 6; wherein n is an integer from 0 to 2; and wherein the monomer residue comprises less than about 10% by weight of the monomer residue of halogen selected from chlorine, bromine, and iodine.

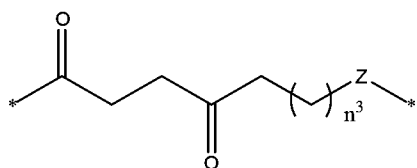
[0008] Also disclosed are polymers comprising monomer residues selected from two or more of an epoxide-functionalized monomer residue having an optionally substituted structure represented by a formula:



- 5 wherein Z is O or NR, wherein R is H or C1 to C6 alkyl; wherein m is an integer from 0 to 6; wherein n is an integer from 0 to 2; and a propargyl-functionalized monomer residue having an optionally substituted structure represented by a formula:

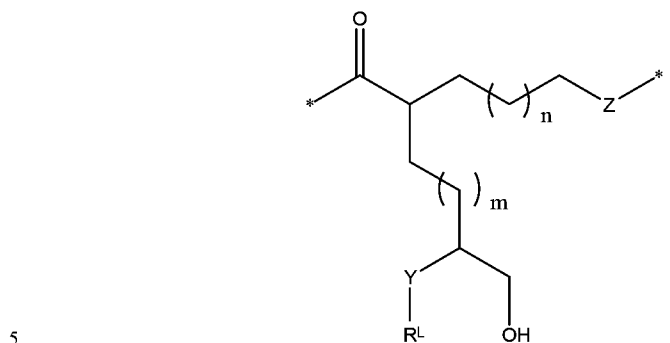


- 10 wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, wherein m<sup>1</sup> is an integer from 0 to 6, and wherein n<sup>1</sup> is an integer from 0 to 2; and a keto-functionalized monomer residue having an optionally substituted structure represented by a formula:



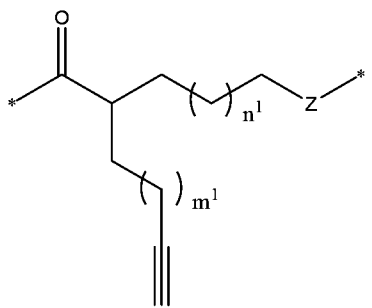
wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, and wherein  $n^3$  is an integer from 0 to 2.

[0009] Also disclosed are polymers comprising at least one monomer residue having an optionally substituted structure represented by a formula:

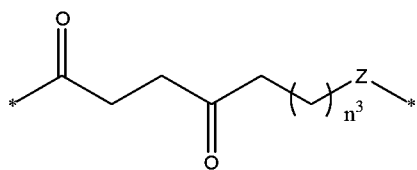


wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, wherein Y is O, S, or NR, wherein R is H or C1 to C6 alkyl, wherein  $R^L$  is selected from optionally substituted alkyl and optionally substituted alkoxy, wherein m is an integer from 0 to 6, and wherein n is an integer from 0 to 2; and one or more of: a propargyl-functionalized monomer residue having an optionally substituted structure represented by a formula:

10

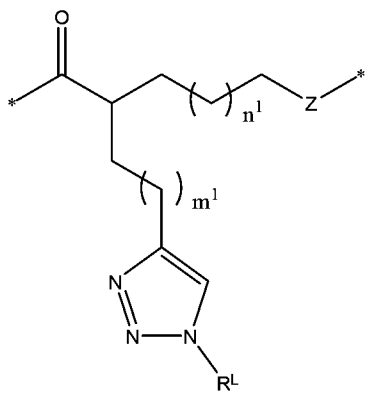


wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, wherein  $m^1$  is an integer from 0 to 6, and wherein  $n^1$  is an integer from 0 to 2; and a keto-functionalized monomer residue having an optionally substituted structure represented by a formula:

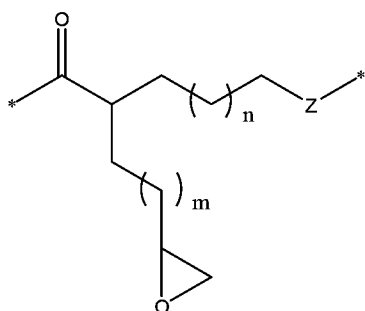


wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, and wherein  $n^3$  is an integer from 0 to 2.

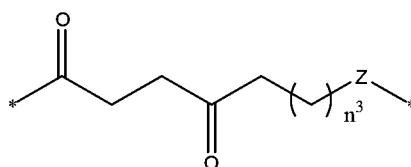
[0010] Also disclosed are polymers comprising at least one monomer residue having an optionally substituted structure represented by a formula:



wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, wherein R<sup>L</sup> is selected from optionally substituted alkyl and optionally substituted alkoxy, wherein  $m^1$  is an integer from 0 to 6, and wherein  $n^1$  is an integer from 0 to 2; and one or more of: an epoxide-functionalized monomer residue having an optionally substituted structure represented by a formula:



wherein Z is O or NR, wherein R is H or C1 to C6 alkyl; wherein m is an integer from 0 to 6;  
 wherein n is an integer from 0 to 2; and a keto-functionalized monomer residue having an  
 optionally substituted structure represented by a formula:

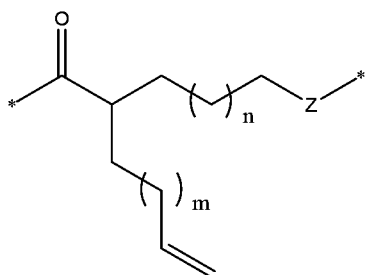


5

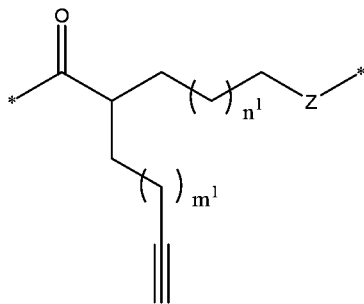
wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, and wherein  $n^3$  is an integer from 0  
 to 2.

[0011] Also disclosed are methods of preparing a polymer comprising the step of  
 copolymerizing a mixture of two or more of an alkene-functionalized monomer providing a  
 residue having an optionally substituted structure represented by a formula:

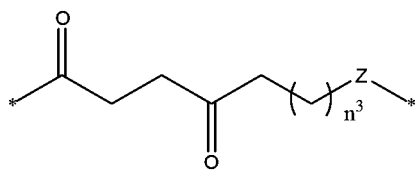
10



wherein Z is O or NR, wherein R is H or C1 to C6 alkyl; wherein m is an integer from 0 to 6;  
 wherein n is an integer from 0 to 2; a propargyl-functionalized monomer providing a residue  
 having an optionally substituted structure represented by a formula:

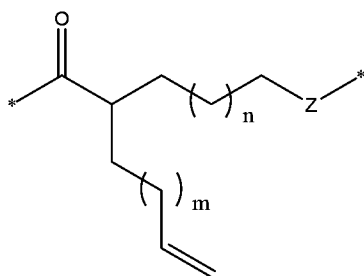


- 5 wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, wherein  $m^1$  is an integer from 0 to 6,  
 and wherein  $n^1$  is an integer from 0 to 2; and a keto-functionalized monomer providing a  
 residue having an optionally substituted structure represented by a formula:



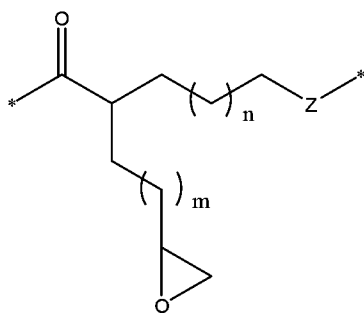
- 10 wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, and wherein  $n^3$  is an integer from 0  
 to 2.

**[0012]** Also disclosed are methods of preparing an epoxide-functionalized polymer  
 comprising the step of oxidizing a polymer having at least one monomer residue having an  
 optionally substituted structure represented by a formula:

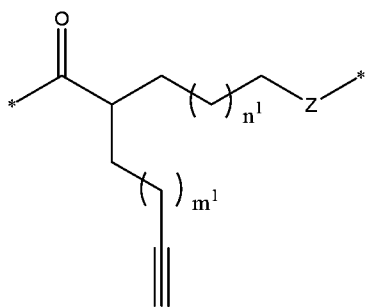


wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, wherein m is an integer from 0 to 6,  
and wherein n is an integer from 0 to 2.

[0013] Also disclosed are methods of crosslinking a polymer comprising the step of reacting  
5 a polymer comprising at least one monomer residue selected from an epoxide-functionalized  
monomer residue having an optionally substituted structure represented by a formula:

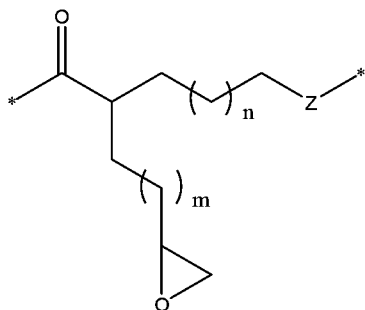


wherein Z is O or NR, wherein R is H or C1 to C6 alkyl; wherein m is an integer from 0 to 6;  
wherein n is an integer from 0 to 2; and a propargyl-functionalized monomer residue having  
10 an optionally substituted structure represented by a formula:



wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, wherein  $m^1$  is an integer from 0 to 6,  
 and wherein  $n^1$  is an integer from 0 to 2; with a cross-linker having a structure represented by  
 a formula  $X-R^L-X'$ , wherein X and X' are independently  $N_3$ , OH, SH,  $NH_2$ , or NHR, wherein  
 5 R is H or C1 to C6 alkyl, and wherein  $R^L$  is selected from optionally substituted alkyl and  
 optionally substituted alkoxyene.

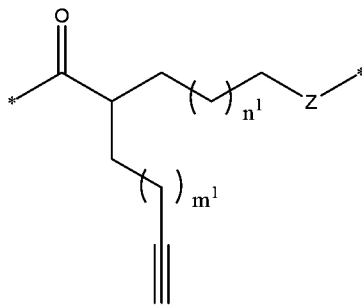
**[0014]** Also disclosed are methods of functionalizing a polymer comprising the step of  
 reacting an epoxide-functionalized monomer residue having an optionally substituted  
 structure represented by a formula:



10

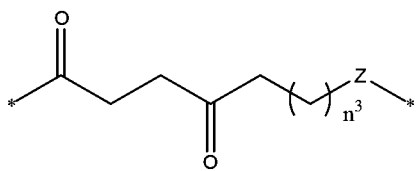
wherein Z is O or NR, wherein R is H or C1 to C6 alkyl; wherein m is an integer from 0 to 6;  
 wherein n is an integer from 0 to 2; with a nucleophile having a structure represented by a  
 formula  $X-R^1$ , wherein X is OH, SH,  $NH_2$ , or NHR, wherein R is H or C1 to C6 alkyl; and  
 wherein  $R^1$  is an optionally substituted organic radical comprising 1 to 24 carbon atoms.

[0015] Also disclosed are methods of functionalizing a polymer comprising the step of reacting a propargyl-functionalized monomer residue having an optionally substituted structure represented by a formula:



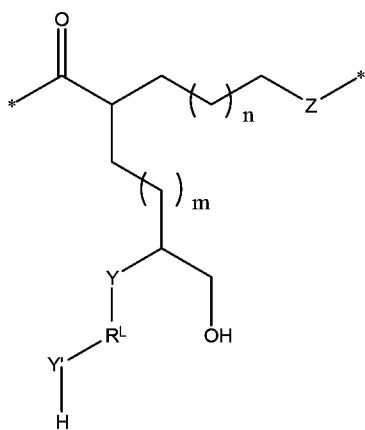
5 wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, wherein  $m^1$  is an integer from 0 to 6, and wherein  $n^1$  is an integer from 0 to 2; with an azide having a structure represented by a formula  $N_3-R^1$ , wherein  $R^1$  is an optionally substituted organic radical comprising 1 to 24 carbon atoms.

[0016] Also disclosed are methods of functionalizing a polymer comprising the steps of  
10 reacting a keto-functionalized monomer providing a residue having an optionally substituted structure represented by a formula:



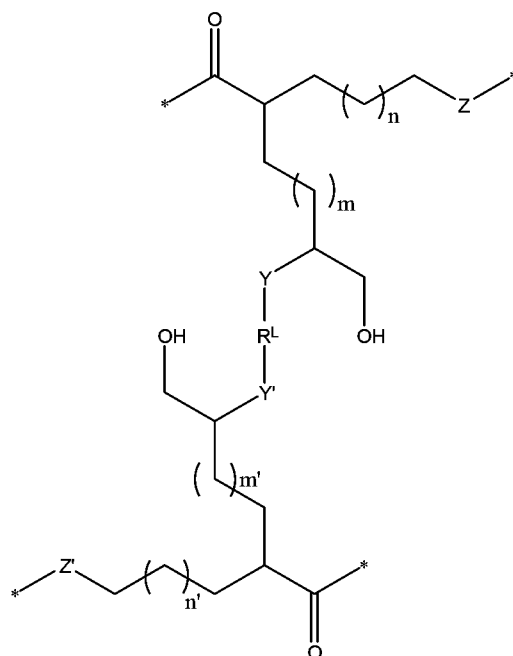
wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, and wherein  $n^3$  is an integer from 0 to 2; with an amine having a structure represented by a formula  $H_2N-R^1$ , wherein  $R^1$  is an  
15 optionally substituted organic radical comprising 1 to 24 carbon atoms; and reducing the resulting imine.

[0017] Also disclosed are methods of functionalizing a polymer comprising the step of reacting a nucleophile-functionalized monomer residue having an optionally substituted structure represented by a formula:



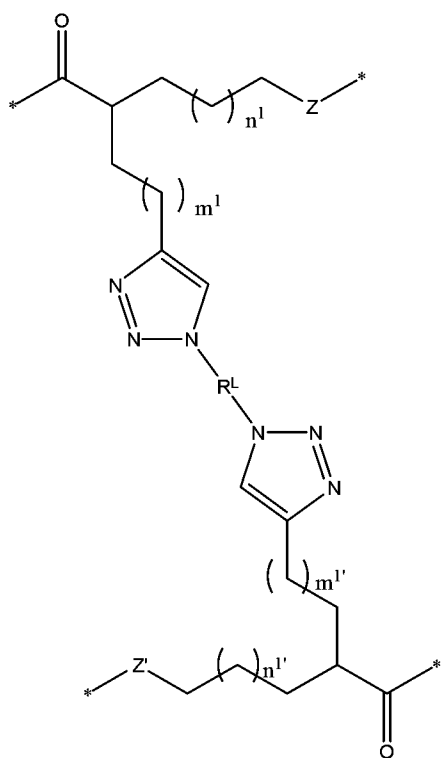
wherein Z is O or NR, wherein R is H or C1 to C6 alkyl; wherein m is an integer from 0 to 6;  
 wherein n is an integer from 0 to 2; wherein Y and Y' are independently O, S, or NR, wherein  
 R is H or C1 to C6 alkyl; and wherein R<sup>L</sup> is selected from optionally substituted alkyl and  
 5 optionally substituted alkoxyene; with an electrophile having a structure represented by a  
 formula E-R<sup>1</sup>, wherein E is an electrophilic moiety; and wherein R<sup>1</sup> is an optionally  
 substituted organic radical comprising 1 to 24 carbon atoms.

**[0018]** Also disclosed are degradable polymeric nanoparticles comprising at least one  
 monomer residue having an optionally substituted structure represented by a formula:



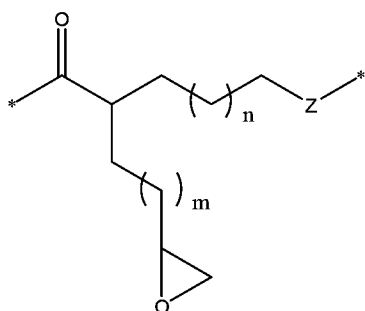
wherein m and m' are independently integers from 0 to 6; wherein n and n' are independently integers from 0 to 2; and wherein Z and Z' are independently O or NR, wherein R is H or C1 to C6 alkyl; wherein Y and Y' are independently O, S, or NR, wherein R is H or C1 to C6 alkyl; and wherein R<sup>L</sup> is selected from optionally substituted alkyl and optionally substituted alkoxylenes, wherein the nanoparticle has a particle size of from about 5 nm to about 850 nm.

**[0019]** Also disclosed are degradable polymeric nanoparticles comprising at least one monomer residue having an optionally substituted structure represented by a formula:



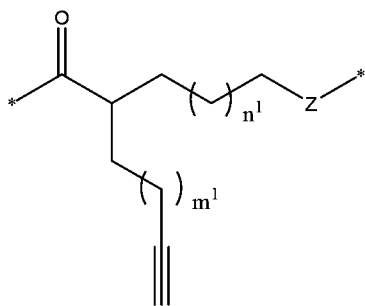
wherein  $m^1$  and  $m^{1'}$  are independently integers from 0 to 6; wherein  $n^1$  and  $n^{1'}$  are independently integers from 0 to 2; and wherein Z and Z' are independently O or NR, wherein R is H or C1 to C6 alkyl; and wherein  $R^L$  is selected from optionally substituted alkyl  
 5 and optionally substituted alkoxylenes, wherein the nanoparticle has a particle size of from about 5 nm to about 850 nm.

**[0020]** Also disclosed are methods of preparing a degradable nanoparticle comprising the step of adding a polymer comprising at least one monomer residue having a structure represented by a formula:



wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, wherein m is an integer from 0 to 6,  
 and wherein n is an integer from 0 to 2; to a solution of from about 1 to about 10 molar  
 equivalents of a dinucleophile (nucleophilic moiety:epoxide functionality) having a structure  
 5  $X-R^L-X'$ , wherein X and X' are independently OH, SH,  $NH_2$ , or NHR, wherein R is H or C1  
 to C6 alkyl, and wherein  $R^L$  is selected from optionally substituted alkyl and optionally  
 substituted alkoxylenes.

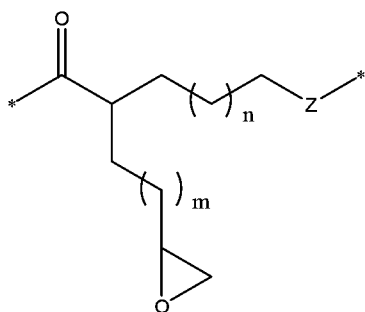
[0021] Also disclosed are methods of preparing a degradable nanoparticle comprising the  
 step of adding a polymer comprising at least one monomer residue having a structure  
 10 represented by a formula:



wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, wherein  $m^1$  is an integer from 0 to 6,  
 and wherein  $n^1$  is an integer from 0 to 2; to a solution of from about 1 to about 10 molar  
 equivalents of a bis-azide (azide moiety:alkyne functionality) having a structure  $N_3-R^L-N_3$ ,  
 15 wherein  $R^L$  is selected from optionally substituted alkyl and optionally substituted alkoxylenes.

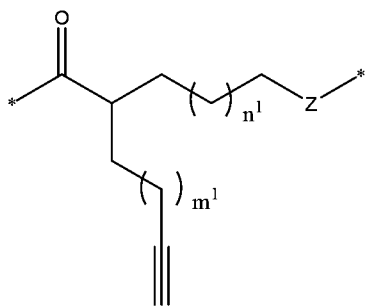
[0022] Also disclosed are methods of controlling particle size during the preparation of a degradable nanoparticle comprising the step of adding an epoxide-functionalized polymer to a solution of a dinucleophilic cross-linker, wherein the stoichiometry of the cross-linker is selected to provide a desired particle size.

- 5 [0023] Also disclosed are methods of functionalizing a nanoparticle comprising the step of reacting a nanoparticle comprising at least one epoxide-functionalized monomer residue having an optionally substituted structure represented by a formula:



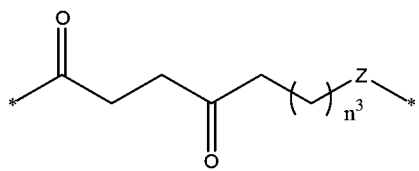
- wherein Z is O or NR, wherein R is H or C1 to C6 alkyl; wherein m is an integer from 0 to 6;  
 10 wherein n is an integer from 0 to 2; with a nucleophile having a structure represented by a formula X-R<sup>1</sup>, wherein X is OH, SH, NH<sub>2</sub>, or NHR, wherein R is H or C1 to C6 alkyl; and wherein R<sup>1</sup> is an optionally substituted organic radical comprising 1 to 24 carbon atoms.

- [0024] Also disclosed are methods of functionalizing a nanoparticle comprising the step of reacting a nanoparticle comprising at least one propargyl-functionalized monomer residue  
 15 having an optionally substituted structure represented by a formula:



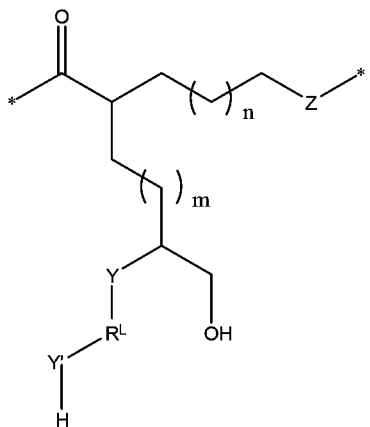
wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, wherein  $m^1$  is an integer from 0 to 6, and wherein  $n^1$  is an integer from 0 to 2; with an azide having a structure represented by a formula  $N_3-R^1$ , wherein  $R^1$  is an optionally substituted organic radical comprising 1 to 24 carbon atoms.

- 5 [0025] Also disclosed are methods of functionalizing a nanoparticle comprising the steps of reacting a nanoparticle comprising at least one keto-functionalized monomer providing a residue having an optionally substituted structure represented by a formula:



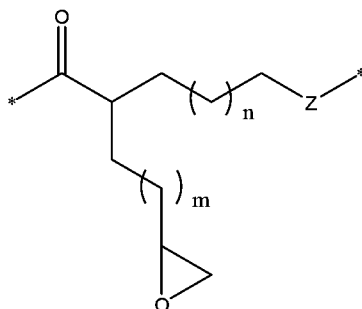
- 10 wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, and wherein  $n^3$  is an integer from 0 to 2; with an amine having a structure represented by a formula  $H_2N-R^1$ , wherein  $R^1$  is an optionally substituted organic radical comprising 1 to 24 carbon atoms; and reducing the resulting imine.

- 15 [0026] Also disclosed are methods of functionalizing a nanoparticle comprising the step of reacting a nanoparticle comprising at least one nucleophile-functionalized monomer residue having an optionally substituted structure represented by a formula:



wherein Z is O or NR, wherein R is H or C1 to C6 alkyl; wherein m is an integer from 0 to 6;  
 wherein n is an integer from 0 to 2; wherein Y and Y' are independently O, S, or NR, wherein  
 R is H or C1 to C6 alkyl; and wherein R<sup>L</sup> is selected from optionally substituted alkyl and  
 optionally substituted alkoxyene; with an electrophile having a structure represented by a  
 5 formula E-R<sup>1</sup>, wherein E is an electrophilic moiety; and wherein R<sup>1</sup> is an optionally  
 substituted organic radical comprising 1 to 24 carbon atoms.

[0027] Also disclosed are methods for preparing degradable nanoparticles comprising the  
 step of reacting a polymer comprising at least one monomer residue having a structure  
 represented by a formula:



10

wherein Z is O, S, or NR, wherein R is H, alkyl, or aryl; and wherein m and n are  
 independently non-negative integers; with a dinucleophile having a structure X-R<sup>L</sup>-X',  
 wherein X and X' are independently OH, SH, NH<sub>2</sub>, or NHR, wherein R is H, alkyl, or aryl,  
 and wherein R<sup>L</sup> is selected from optionally substituted alkyl, optionally substituted aryl,  
 15 optionally substituted alkoxyene, and optionally substituted esters, thereby producing  
 nanoparticles.

[0028] Also disclosed are functionalized polymers and functionalized nanoparticles and  
 methods for making and using same.

[0029] Also disclosed are the products of the disclosed methods.

20 [0030] Also disclosed are methods of intracellular delivery comprising administering an  
 effective amount of a disclosed nanoparticle to a subject.

[0031] Also disclosed are methods for the manufacture of a medicament for delivery of a biologically active agent, a pharmaceutically active agent, and/or an imaging moiety comprising combining at least one disclosed polymer or at least one disclosed nanoparticle with a pharmaceutically acceptable carrier.

5 [0032] Also disclosed are uses of a disclosed polymer or a disclosed nanoparticle to deliver a biologically active agent, a pharmaceutically active agent, and/or an imaging moiety.

[0033] Also disclosed are pharmaceutical compositions comprising a therapeutically effective amount of one or more disclosed polymer and/or one or more disclosed nanoparticle and a pharmaceutically acceptable carrier for administration in a subject, for example, a mammal.

10 [0034] Also disclosed are pharmaceutical compositions for diagnosing, treating, and/or preventing ophthalmic disorders, the compositions comprising a therapeutically effective amount of one or more disclosed polymer and/or one or more disclosed nanoparticle and a pharmaceutically acceptable carrier for administration in a subject, for example, a mammal. In one aspect, the compositions can be administered transcorneally.

15 [0035] While aspects of the present invention can be described and claimed in a particular statutory class, such as the system statutory class, this is for convenience only and one of skill in the art will understand that each aspect of the present invention can be described and claimed in any statutory class. Unless otherwise expressly stated, it is in no way intended that any method or aspect set forth herein be construed as requiring that its steps be performed in a  
20 specific order. Accordingly, where a method claim does not specifically state in the claims or descriptions that the steps are to be limited to a specific order, it is no way intended that an order be inferred, in any respect. This holds for any possible non-express basis for interpretation, including matters of logic with respect to arrangement of steps or operational flow, plain meaning derived from grammatical organization or punctuation, or the number or  
25 type of aspects described in the specification.

#### BRIEF DESCRIPTION OF THE FIGURES

[0036] The accompanying figures, which are incorporated in and constitute a part of this specification, illustrate several aspects and together with the description serve to explain the principles of the invention.

[0037] Figure 1 shows multifunctional linear polyester precursors with epoxide cross-linking entity.

[0038] Figure 2 shows TEM images of AB nanoparticles; (1) 2 equivalents of amine; (2) 5 equivalents of amine; (3) 8 equivalents of amine.

5 [0039] Figure 3 shows polynomial increase of nanoparticle diameter (nm) with increase of diamine cross-linker; (■) ABD nanoparticles; (♦) AB nanoparticles; (●) ABC nanoparticles.

[0040] Figure 4 shows <sup>1</sup>H NMR overlay for poly(vl-evl) nanoparticles with increasing cross-linking.

10 [0041] Figure 5 shows polynomial increase of nanoparticle diameter (nm) with increase of diamine cross-linker for (♦) AB nanoparticles from Figure 3.

[0042] Figure 6 shows polynomial increase of nanoparticle diameter (nm) with increase of diamine cross-linker for (■) ABD nanoparticles from Figure 3.

[0043] Figure 7 shows polynomial increase of nanoparticle diameter (nm) with increase of diamine cross-linker for (●) ABC nanoparticles from Figure 3.

15 [0044] Figure 8 shows polynomial increase of nanoparticle diameter (nm) with increase of diamine cross-linker for AB nanoparticles from poly(vl-evl) (2% evl) (■).

[0045] Figure 9 shows polynomial increase of nanoparticle diameter (nm) with increase of diamine cross-linker for AB nanoparticles from poly(vl-evl) (7% evl) (♦).

20 [0046] Figure 10 shows polynomial increase of nanoparticle diameter (nm) with increase of diamine cross-linker for AB nanoparticles from poly(vl-evl) (19% evl) (♦).

[0047] Figure 11 shows a schematic representation of the structures for FD-1 and FD-2.

[0048] Figure 12 shows time course of internalization of (a) FD-1 and (b) FD-2 into NIH-3T3 Fibroblasts at 37 °C. The conjugate concentration was 10 μM.

25 [0049] Figure 13 shows the effect of temperature on (a) FD-1 and (b) FD-2 internalization. The human microvascular endothelial cells (HMEC) cells were incubated with conjugates (10uM) for 2.5 min at 4 °C or at 37 °C.

[0050] Figure 14 shows the effect of temperature on (a) FD-1 and (b) FD-2 internalization. The HMEC cells were incubated with conjugates (1uM) for 30 min at 4 °C or at 37 °C.

[0051] Figure 15 shows control experiments: (a) The HMEC cells were incubated with free FITC conjugates (10uM) for 60 min at 37 °C. (b) The HMEC cells were incubated with Boc-protected guanidinylated FD-2 (10uM) for 60 min at 37 °C.

[0052] Figures 6A and 6B show an exemplary synthetic scheme for the preparation of FD-1, FD-2, and intermediates thereof.

[0053] Figure 17 shows a micrograph of a collection of organic quantum dots formed via intramolecular chain collapse. The various dots are imaged having different colors as a function of the selected functional moiety.

[0054] Figure 18 shows a schematic representation of a synthetic scheme for preparing the various types of disclosed organic quantum dots.

[0055] Figure 19 shows a schematic representation of a synthetic scheme for preparing ABA-type organic quantum dots.

[0056] Figure 20 shows a schematic representation of the composition of an ABA-type organic quantum dot precursor, an ABA triblock copolymer.

[0057] Figure 21 shows a schematic representation of an organic quantum dot and its applicability as a biological probe and/or a drug-delivery vehicle.

[0058] Figure 22 shows a schematic for coupling reactions of benzocyclobutene derivatives.

[0059] Figure 23 shows an exemplary synthesis of benzocyclobutene monomer Q4.

[0060] Figure 24 shows an exemplary synthesis of benzocyclobutene functionalized linear polystyrene Q8.

[0061] Figure 25 shows a graph of the variation in molecular weight of final macromolecule, Mw, with total concentration of BCB groups in solution (circles represent ultra-high dilution strategy; squares represent continuous addition strategy; I represents that an insoluble gel was produced) (starting linear polymer, mw = 95,000, PDI = 1.11).

[0062] Figure 26 shows a schematic representation of the intramolecular collapse of the linear polymer Q8 to give the nanoparticle Q9.

[0063] Figure 27 shows a comparison of  $^1\text{H}$  NMR spectrum for (a) the starting linear polymer, 8, 80/20 Sty/BCB,  $M_w = 95,000$ , PDI = 1.12; and the resulting nanoparticle 9,  $M_w = 65,000$ , PDI = 1.10.

[0064] Figure 28 shows an overlay of GPC traces for (a) the starting linear polymer, Q8,  $M_w = 105000$ , PDI = 1.12; and nanoparticles Q9 with (a) 0 mol % BCB incorporation, (b) 5 mol % BCB incorporation, (c) 10 mol % BCB incorporation, (d) 20 mol % BCB incorporation, and (e) 25 mol % BCB incorporation.

[0065] Figure 29 shows variation in the percent reduction in molecular weight for the nanoparticles Q9 with the mol % of BCB units in the starting linear polymer Q8, for 44 K (b), 110 K (9), and 230 K (b) series.

[0066] Figure 30 shows a schematic representation of the intramolecular collapse of a random n-butyl acrylate-based linear polymer, Q10, to give the nanoparticle, Q11.

[0067] Figure 31 shows a schematic of the formation and intramolecular collapse of the PEG-*b*-PSt/BCB block copolymer, Q15, to give a hybrid linear-nanoparticle copolymer, Q16.

[0068] Figure 32 shows GPC traces for (a) the starting poly(ethylene glycol)-*b*-poly(styrene-*co*-benzocyclobutene), Q15, ( $M_w = 95,000$ , PDI = 1.11) and (b) the final hybrid nanoparticle-linear block copolymer, Q16, ( $M_w = 52,000$ , PDI = 1.09).

[0069] Figure 33 shows a schematic of exemplary multimodal nanoparticles.

[0070] Figure 34 shows a schematic of an exemplary synthesis of ABA-triblock copolymer linear precursors before and after chain-collapse.

[0071] Figure 35 shows a schematic illustrating conjugation of the disclosed dendrimeric materials with the disclosed organic quantum dot materials (conjugation with cleavable linker).

[0072] Figure 36 shows an exemplary preparation of a cross-linked organic nanoparticle.

[0073] Figure 37 shows an exemplary conjugation of a disclosed dendrimeric material with a disclosed cross-linked organic nanoparticle.

[0074] Figure 38 shows a schematic illustrating a disclosed delivery system (e.g., gene delivery).

5 [0075] Figure 39 illustrates preparation of a disclosed delivery system (e.g., gene delivery).

[0076] Figure 40 shows micrographs demonstrating mitochondrial localization of the disclosed delivery systems (e.g., gene delivery).

[0077] Figure 41 shows micrographs demonstrating uptake of a disclosed delivery system (e.g., gene delivery) in ciEndothelial cells.

10 [0078] Figure 42 demonstrates the flexibility of assembly of the disclosed delivery systems.

[0079] Figure 43 shows micrographs of HeLa cells exposed 10  $\mu$ M **FD-1** for 1h, fixed with 3.3% paraformaldehyde, stained with 100nM Mitotracker® Red 580 FM. The illuminated regions show cell penetration (left), mitochondria location (center), and overlap (right).

15 [0080] Figure 44 shows micrographs of HeLa cells exposed 20  $\mu$ M **FD-2** for 1h, fixed with 3.3% paraformaldehyde, stained with 100nM Mitotracker® Red 580 FM. The illuminated regions show cell penetration (left), mitochondria location (center), and overlap (right).

[0081] Figure 45 shows micrographs demonstrating intercellular transport of an aprotinin-fluorophore-transporter conjugate (**FD-1**, illustrated) into HAEC cells.

20 [0082] Figure 46 shows micrographs demonstrating intercellular transport of an aprotinin-fluorophore-transporter conjugate (**FD-2**) into HAEC cells.

[0083] Figure 47 shows micrographs demonstrating no uptake (i.e., no intercellular transport into HAEC cells) of a control aprotinin-fluorophore conjugate (illustrated).

[0084] Figure 48 illustrates several chemical strategies for binding transporter moieties to various protein functional groups (e.g., amine, thiol, carbonyl).

[0085] Figure 49 presents strategies for vaccine development by incorporation of aprotinin through conjugation to carbonyl-functionalized proteins (e.g., tyrosine residues) by Mannich reaction.

[0086] Figure 50 illustrates incorporation of fluorophores through conjugation to carbonyl-  
5 functionalized proteins (e.g., tyrosine residues) by Mannich reaction.

[0087] Figure 51 illustrates incorporation of transporter moieties through conjugation to carbonyl-functionalized proteins.

[0088] Figure 52 shows TEM analysis of the nanoparticles (225.6 nm) produced from crosslinking of poly(vl-evl-avl-opd) (ABbD).

10 [0089] Figure 53 shows the particle size distribution measured by dynamic light scatter analysis of “one-pot” nanoparticles ( $272.3 \pm 23.3$  nm) produced from crosslinking of poly(vl-evl-avl-opd) (ABbD).

[0090] Figure 54 shows a scheme for a thiol exchange reaction with an IgG antibody to form an IgGMT bioconjugate.

15 [0091] Figure 55 shows microscopy images of uptake of IgGMT into HEp-2 cells for 10 min, 30 min, 1 h, 2 h, 6 h and negative control experiment (NC) with Alexa Fluor® 568 labeled IgG.

[0092] Figure 56 shows microscopy images of HEp-2 cells infected with RSV for 24 h, washed and imaged 48 h after infection for the fluorescence of GFP (c). HEp-2 cells infected  
20 with RSV for 24 h, incubated for 30 min with IgGMT and imaged after 48 h for the fluorescence of GFP (a) and Alexa Fluor® 568 of the IgGMT (b), merged images (a) and (b) (merged a + b).

[0093] Figure 57 shows microscopy images of HEp-2 cells infected with RSV for 24 h, incubated for 30 min with IgGMT and imaged immediately for the green fluorescence of the  
25 GFP (a) and the red fluorescence of the IgGMT conjugate (b), merged images of (a) and (b) (a + b merged). HEp-2 cells infected with RSV for 24 h, incubated for 30 min with IgGMT and imaged after 48 h for the fluorescence of GFP (a) and Alexa Fluor® 568 of the IgGMT conjugate (b), merged images (a) and (b) (merged a + b).

[0094] Figure 58 shows results for a radiation guided Nanoparticle-peptide targeting in a Lewis-Lung Carcinoma Tumor Model.

[0095] Figure 59 shows a scheme for delivery of a biological active substance.

[0096] Figure 60 shows fluorescence microscopy images of portions of the eye of a rat after  
5 administration of a nanoparticle bioconjugate comprising an imaging agent.

[0097] Additional advantages of the invention will be set forth in part in the description which follows, and in part will be obvious from the description, or can be learned by practice of the invention. The advantages of the invention will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims. It is to be  
10 understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed.

#### DESCRIPTION

[0098] The present invention can be understood more readily by reference to the following detailed description of the invention and the Examples included therein.

15 [0099] Before the present compounds, compositions, articles, systems, devices, and/or methods are disclosed and described, it is to be understood that they are not limited to specific synthetic methods unless otherwise specified, or to particular reagents unless otherwise specified, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular aspects only and is not intended to be  
20 limiting. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, example methods and materials are now described.

[00100] All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications  
25 are cited. The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided herein can be different from the actual publication dates, which can require independent confirmation.

**A. DEFINITIONS**

[00101] As used in the specification and the appended claims, the singular forms “a,” “an” and “the” include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to “a functional group,” “an alkyl,” or “a residue” includes mixtures of  
5 two or more such functional groups, alkyls, or residues, and the like.

[00102] Ranges can be expressed herein as from “about” one particular value, and/or to “about” another particular value. When such a range is expressed, another aspect includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent “about,” it will be understood that the  
10 particular value forms another aspect. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint. It is also understood that there are a number of values disclosed herein, and that each value is also herein disclosed as “about” that particular value in addition to the value itself. For example, if the value “10” is disclosed, then “about 10” is also disclosed. It is also  
15 understood that each unit between two particular units are also disclosed. For example, if 10 and 15 are disclosed, then 11, 12, 13, and 14 are also disclosed.

[00103] As used herein, the terms “optional” or “optionally” means that the subsequently described event or circumstance can or can not occur, and that the description includes instances where said event or circumstance occurs and instances where it does not.

20 [00104] As used herein, the term “treatment” refers to the medical management of a patient with the intent to cure, ameliorate, stabilize, or prevent a disease, pathological condition, or disorder. This term includes active treatment, that is, treatment directed specifically toward the improvement of a disease, pathological condition, or disorder, and also includes causal treatment, that is, treatment directed toward removal of the cause of the  
25 associated disease, pathological condition, or disorder. In addition, this term includes palliative treatment, that is, treatment designed for the relief of symptoms rather than the curing of the disease, pathological condition, or disorder; preventative treatment, that is, treatment directed to minimizing or partially or completely inhibiting the development of the associated disease, pathological condition, or disorder; and supportive treatment, that is,

treatment employed to supplement another specific therapy directed toward the improvement of the associated disease, pathological condition, or disorder.

[00105] As used herein, the term “prevent” or “preventing” refers to precluding, averting, obviating, forestalling, stopping, or hindering something from happening, especially  
5 by advance action. It is understood that where reduce, inhibit or prevent are used herein, unless specifically indicated otherwise, the use of the other two words is also expressly disclosed.

[00106] As used herein, the terms “administering” and “administration” refer to any method of providing a pharmaceutical preparation to a subject. Such methods are well known  
10 to those skilled in the art and include, but are not limited to, oral administration, transdermal administration, administration by inhalation, nasal administration, topical administration, intravaginal administration, ophthalmic administration, intraaural administration, intracerebral administration, rectal administration, and parenteral administration, including injectable such as intravenous administration, intra-arterial administration, intramuscular  
15 administration, and subcutaneous administration. Administration can be continuous or intermittent. In various aspects, a preparation can be administered therapeutically; that is, administered to treat an existing disease or condition. In further various aspects, a preparation can be administered prophylactically; that is, administered for prevention of a disease or condition.

20 [00107] As used herein, the term “subject” refers to a target of administration. The subject of the herein disclosed methods can be a vertebrate, such as a mammal, a fish, a bird, a reptile, or an amphibian. Thus, the subject of the herein disclosed methods can be a human, non-human primate, horse, pig, rabbit, dog, sheep, goat, cow, cat, guinea pig or rodent. The term does not denote a particular age or sex. Thus, adult and newborn subjects, as well as  
25 fetuses, whether male or female, are intended to be covered. A patient refers to a subject afflicted with a disease or disorder. The term “patient” includes human and veterinary subjects.

[00108] As used herein, the term “effective amount” refers to an amount that is sufficient to achieve a desired result or to have an effect on undesired symptoms, but is  
30 generally insufficient to cause adverse side affects. The specific effective dose level for any

particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration; the route of administration; the rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed and like factors well known in the medical arts. For example, it is well within the skill of the art to start doses of a compound at levels lower than those required to achieve the desired effect and to gradually increase the dosage until the desired effect is achieved. If desired, the effective daily dose can be divided into multiple doses for purposes of administration.

Consequently, single dose compositions can contain such amounts or submultiples thereof to make up the daily dose. The dosage can be adjusted by the individual physician in the event of any contraindications. Dosage can vary, and can be administered in one or more dose administrations daily, for one or several days. Guidance can be found in the literature for appropriate dosages for given classes of pharmaceutical products. In a further aspect, a preparation can be administered in a “diagnostically effective amount”; that is, an amount effective for diagnosis of a disease or condition. In a further aspect, a preparation can be administered in a “therapeutically effective amount”; that is, an amount effective for treatment of a disease or condition. In a further aspect, a preparation can be administered in a “prophylactically effective amount”; that is, an amount effective for prevention of a disease or condition.

**[00109]** As used herein, the term “pharmaceutically acceptable carrier” refers to sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, as well as sterile powders for reconstitution into sterile injectable solutions or dispersions just prior to use. Examples of suitable aqueous and nonaqueous carriers, diluents, solvents or vehicles include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol and the like), carboxymethylcellulose and suitable mixtures thereof, vegetable oils (such as olive oil) and injectable organic esters such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials such as lecithin, by the maintenance of the required particle size in the case of dispersions and by the use of surfactants. These compositions can also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms can be ensured by the inclusion of various antibacterial and antifungal agents such as paraben, chlorobutanol, phenol, sorbic acid

and the like. It can also be desirable to include isotonic agents such as sugars, sodium chloride and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the inclusion of agents, such as aluminum monostearate and gelatin, which delay absorption. Injectable depot forms are made by forming microencapsule matrices of the drug in biodegradable polymers such as polylactide-polyglycolide, poly(orthoesters) and poly(anhydrides). Depending upon the ratio of drug to polymer and the nature of the particular polymer employed, the rate of drug release can be controlled. Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissues. The injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable media just prior to use. Suitable inert carriers can include sugars such as lactose. Desirably, at least 95% by weight of the particles of the active ingredient have an effective particle size in the range of 0.01 to 10 micrometers.

**[00110]** As used herein, the term “biologically active agent” or “bioactive agent” means an agent that is capable of providing a local or systemic biological, physiological, or therapeutic effect in the biological system to which it is applied. For example, the bioactive agent can act to control infection or inflammation, enhance cell growth and tissue regeneration, control tumor growth, act as an analgesic, promote anti-cell attachment, and enhance bone growth, among other functions. Other suitable bioactive agents can include anti-viral agents, hormones, antibodies, or therapeutic proteins. Other bioactive agents include prodrugs, which are agents that are not biologically active when administered but, upon administration to a subject are converted to bioactive agents through metabolism or some other mechanism. Additionally, any of the compositions of the invention can contain combinations of two or more bioactive agents. It is understood that a biologically active agent can be used in connection with administration to various subjects, for example, to humans (*i.e.*, medical administration) or to animals (*i.e.*, veterinary administration).

**[00111]** As used herein, the term “pharmaceutically active agent” includes a “drug” or a “vaccine” and means a molecule, group of molecules, complex or substance administered to an organism for diagnostic, therapeutic, preventative medical, or veterinary purposes. This term include externally and internally administered topical, localized and systemic human and animal pharmaceuticals, treatments, remedies, nutraceuticals, cosmeceuticals, biologicals,

devices, diagnostics and contraceptives, including preparations useful in clinical and veterinary screening, prevention, prophylaxis, healing, wellness, detection, imaging, diagnosis, therapy, surgery, monitoring, cosmetics, prosthetics, forensics and the like. This term may also be used in reference to agricultural, workplace, military, industrial and

5 environmental therapeutics or remedies comprising selected molecules or selected nucleic acid sequences capable of recognizing cellular receptors, membrane receptors, hormone receptors, therapeutic receptors, microbes, viruses or selected targets comprising or capable of contacting plants, animals and/or humans. This term can also specifically include nucleic acids and compounds comprising nucleic acids that produce a bioactive effect, for example

10 deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). Pharmaceutically active agents include the herein disclosed categories and specific examples. It is not intended that the category be limited by the specific examples. Those of ordinary skill in the art will recognize also numerous other compounds that fall within the categories and that are useful according to the invention. Examples include a radiosensitizer, the combination of a radiosensitizer and

15 a chemotherapeutic, a steroid, a xanthine, a beta-2-agonist bronchodilator, an anti-inflammatory agent, an analgesic agent, a calcium antagonist, an angiotensin-converting enzyme inhibitors, a beta-blocker, a centrally active alpha-agonist, an alpha-1-antagonist, an anticholinergic/antispasmodic agent, a vasopressin analogue, an antiarrhythmic agent, an antiparkinsonian agent, an antiangina/antihypertensive agent, an anticoagulant agent, an

20 antiplatelet agent, a sedative, an anxiolytic agent, a peptidic agent, a biopolymeric agent, an antineoplastic agent, a laxative, an antidiarrheal agent, an antimicrobial agent, an antifungal agent, a vaccine, a protein, or a nucleic acid. In a further aspect, the pharmaceutically active agent can be coumarin, albumin, bromolidine, steroids such as betamethasone, dexamethasone, methylprednisolone, prednisolone, prednisone, triamcinolone, budesonide,

25 hydrocortisone, and pharmaceutically acceptable hydrocortisone derivatives; xanthines such as theophylline and doxophylline; beta-2-agonist bronchodilators such as salbutamol, fenterol, clenbuterol, bambuterol, salmeterol, fenoterol; antiinflammatory agents, including antiasthmatic anti-inflammatory agents, antiarthritis antiinflammatory agents, and non-steroidal antiinflammatory agents, examples of which include but are not limited to sulfides,

30 mesalamine, budesonide, salazopyrin, diclofenac, pharmaceutically acceptable diclofenac salts, nimesulide, naproxene, acetaminophen, ibuprofen, ketoprofen and piroxicam; analgesic agents such as salicylates; calcium channel blockers such as nifedipine, amlodipine, and nicardipine; angiotensin-converting enzyme inhibitors such as captopril, benazepril

hydrochloride, fosinopril sodium, trandolapril, ramipril, lisinopril, enalapril, quinapril hydrochloride, and moexipril hydrochloride; beta-blockers (i.e., beta adrenergic blocking agents) such as sotalol hydrochloride, timolol maleate, esmolol hydrochloride, carteolol, propranolol hydrochloride, betaxolol hydrochloride, penbutolol sulfate, metoprolol tartrate, metoprolol succinate, acebutolol hydrochloride, atenolol, pindolol, and bisoprolol fumarate; centrally active alpha-2-agonists such as clonidine; alpha-1-antagonists such as doxazosin and prazosin; anticholinergic/antispasmodic agents such as dicyclomine hydrochloride, scopolamine hydrobromide, glycopyrrolate, clidinium bromide, flavoxate, and oxybutynin; vasopressin analogues such as vasopressin and desmopressin; antiarrhythmic agents such as quinidine, lidocaine, tocainide hydrochloride, mexiletine hydrochloride, digoxin, verapamil hydrochloride, propafenone hydrochloride, flecainide acetate, procainamide hydrochloride, moricizine hydrochloride, and disopyramide phosphate; antiparkinsonian agents, such as dopamine, L-Dopa/Carbidopa, selegiline, dihydroergocryptine, pergolide, lisuride, apomorphine, and bromocryptine; antiangina agents and antihypertensive agents such as isosorbide mononitrate, isosorbide dinitrate, propranolol, atenolol and verapamil; anticoagulant and antiplatelet agents such as coumadin, warfarin, acetylsalicylic acid, and ticlopidine; sedatives such as benzodiazapines and barbiturates; ansiolytic agents such as lorazepam, bromazepam, and diazepam; peptidic and biopolymeric agents such as calcitonin, leuprolide and other LHRH agonists, hirudin, cyclosporin, insulin, somatostatin, protirelin, interferon, desmopressin, somatotropin, thymopentin, pidotimod, erythropoietin, interleukins, melatonin, granulocyte/macrophage-CSF, and heparin; antineoplastic agents such as etoposide, etoposide phosphate, cyclophosphamide, methotrexate, 5-fluorouracil, vincristine, doxorubicin, cisplatin, hydroxyurea, leucovorin calcium, tamoxifen, flutamide, asparaginase, altretamine, mitotane, and procarbazine hydrochloride; laxatives such as senna concentrate, casanthranol, bisacodyl, and sodium picosulphate; antidiarrheal agents such as difenoxine hydrochloride, loperamide hydrochloride, furazolidone, diphenoxylate hydrochloride, and microorganisms; vaccines such as bacterial and viral vaccines; antimicrobial agents such as penicillins, cephalosporins, and macrolides, antifungal agents such as imidazolic and triazolic derivatives; and nucleic acids such as DNA sequences encoding for biological proteins, and antisense oligonucleotides. It is understood that a pharmaceutically active agent can be used in connection with administration to various subjects, for example, to humans (*i.e.*, medical administration) or to animals (*i.e.*, veterinary administration).

[00112] As used herein, the term “ophthalmic disorders” and/or “ophthalmic conditions” refers to ophthalmic diseases, conditions, and/or disorders including, without limitation, those associated with the anterior chamber of the eye (i.e., hyphema, synechia); the choroid (i.e., choroidal detachment, choroidal melanoma, multifocal choroidopathy syndromes); the conjunctiva (i.e., conjunctivitis, cicatricial pemphigoid, filtering Bleb complications, conjunctival melanoma, Pharyngoconjunctival Fever, pterygium, conjunctival squamous cell carcinoma); connective tissue disorders (i.e., ankylosing spondylitis, pseudoxanthoma elasticum, corneal abrasion or edema, limbal dermoid, crystalline dystrophy keratitis, keratoconjunctivitis, keratoconus, keratopathy, megalocornea, corneal ulcer); dermatologic disorders (i.e., eczematitis enteropathica, atopic dermatitis, ocular rosacea, psoriasis, Stevens-Johnson syndrome); endocrine disorders (i.e., pituitary apoplexy); extraocular disorders (i.e., Abducens Nerve Palsy, Brown syndrome, Duane syndrome, esotropia, exotropia, oculomotor nerve palsy); genetic disorders (i.e., albinism, Down syndrome, Peters Anomaly); the globe (i.e., anophthalmos, endophthalmitis); hematologic and cardiovascular disorders (i.e., Giant Cell Arteritis, hypertension, leukemias, Ocular Ischemic syndrome, sickle cell disease); infectious diseases (i.e., actinomycosis, botulism, HIV, diphtheria, Escherichia coli, Tuberculosis, ocular manifestations of syphilis); intraocular pressure (i.e., glaucoma, ocular hypotony, Posner-Schlossman syndrome), the iris and ciliary body (i.e., aniridia, iris prolaps, juvenile xanthogranuloma, ciliary body melanoma, iris melanoma, uveitis); the lacrimal system (i.e., alacrima, Dry Eye syndrome, lacrimal gland tumors); the lens (i.e., cataract, ectopia lentis, intraocular lens decentration or dislocation); the lid (i.e., blepharitis, dermatochalasis, distichiasis, ectropion, eyelid coloboma, Floppy Eye syndrome, trichiasis, xanthelasma); metabolic disorders (i.e., gout, hyperlipoproteinemia, Oculocerebrorenal syndrome); neurologic disorders (i.e., Bell Palsy, diplopia, multiple sclerosis); general ophthalmologic (i.e., red eye, cataracts, macular degeneration, red eye, macular degeneration); the optic nerve (i.e., meningioma, optic neuritis, optic neuropathy, papilledema); the orbit (i.e., orbital cellulitis, orbital dermoid, orbital tumors); phakomatoses (i.e., ataxia-telangiectasia, neurofibromatosis-1); presbyopia; the pupil (i.e., anisocoria, Homer syndrome); refractive disorders (i.e., astigmatism, hyperopia, myopia); the retina (i.e., Coats disease, Eales disease, macular edema, retinitis, retinopathy); and the sclera (i.e., episcleritis, scleritis).

[00113] As used herein, the term “imaging moiety” refers to any chemical groups or substance useful for imaging applications, as known to those of skill in the art. Examples of imaging agents include radioconjugate, cytotoxin, cytokine, Gadolinium-DTPA or a quantum dot, iron oxide, manganese oxide. In one aspect, an imaging agent can be provided in  
5 nanoparticular form or in microparticular form. In a further aspect, an imaging agent comprises Gadolinium-DTPA and iron oxide nanoparticles (magnetite), as specific MRI contrast agents. In a yet further aspect, an imaging agent comprises at least one near infrared dye, for example near infrared dyes based on a porphyrin and/or a phthalocyanine. See Ghoroghchian *et al.*, Near-infrared-emissive polymersomes: Self-assembled soft matter for in  
10 vivo optical imaging, *PNAS*, 2005, vol. 102, no. 8, 2922–2927.

[00114] As used herein, the term “organic quantum dot” refers to a generally carbon based compound having a generally particle-like overall structure and comprising a generally central functional moiety. In one aspect, an organic quantum dot is prepared via intramolecular chain collapse. In a further aspect, an organic quantum dot can be prepared  
15 from a block copolymer comprising at least two reactive moieties and at least one functional moiety.

[00115] As used herein, the term “polymer” refers to a relatively high molecular weight organic compound, natural or synthetic, whose structure can be represented by a repeated small unit, the monomer (e.g., polyethylene, rubber, cellulose). Synthetic polymers are  
20 typically formed by addition or condensation polymerization of monomers.

[00116] As used herein, the term “copolymer” refers to a polymer formed from two or more different repeating units (monomer residues). By way of example and without limitation, a copolymer can be an alternating copolymer, a random copolymer, a block copolymer, or a graft copolymer. It is also contemplated that, in certain aspects, various  
25 block segments of a block copolymer can themselves comprise copolymers.

[00117] As used herein, the term “oligomer” refers to a relatively low molecular weight polymer in which the number of repeating units is between two and ten, for example, from two to eight, from two to six, or from two to four. In one aspect, a collection of oligomers can have an average number of repeating units of from about two to about ten, for

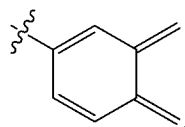
example, from about two to about eight, from about two to about six, or from about two to about four.

[00118] As used herein, the term “reactive residue” refers to a moiety (*e.g.*, a monomer residue) capable of undergoing chemical reaction at a reaction temperature and/or in response to a stimulus to form a reactive intermediate. In one aspect, a reactive residue is a moiety capable undergoing an intramolecular cross-linking reaction to provide intramolecular chain collapse.

[00119] As used herein, the term “polymerizable group” refers to a group (*i.e.*, a chemical functionality) capable of undergoing a polymerization reaction at a polymerization temperature and/or in response to a polymerization initiator to form a polymer or an oligomer. In one aspect, the polymerization reaction is a radical polymerization (*e.g.*, a vinyl polymerization). It is understood that catalysts can be employed in connection with the polymerization reaction. It is contemplated that, in various aspects, polymerizable groups can be used in step-growth or chain growth reactions. Exemplary polymerizable groups include residues of vinyl, styryl, acryloyl, methacryloyl, aryl, and heteroaryl compounds.

[00120] As used herein, the term “reactive intermediate” refers to a chemical species formed from a reactive moiety and in response to a stimulus and/or at a reaction temperature and capable of undergoing further chemical reaction (*e.g.*, cross-linking) with another reactive intermediate. In one aspect, two reactive intermediates can undergo a cross-linking reaction to provide intramolecular chain collapse.

[00121] As used herein, the term “*o*-quinoid moiety” refers to a reactive intermediate formed from, for example, a benzocyclobutene moiety a reaction temperature and/or in response to a stimulus. In one aspect, an *o*-quinoid moiety can have the general structure:



It is understood that a reactive moiety capable of forming an *o*-quinoid moiety can be provided as, for example, a residue or as a pendant group in a copolymer. In a further aspect, an *o*-quinoid moiety can be further substituted.

[00122] As used herein, the term “functional moiety” refers to a moiety (*e.g.*, a monomeric residue) capable of performing a function, for example an imaging function or a drug-delivery function. In one aspect, a functional moiety can be provided in a generally central portion of an organic quantum dot. In various further aspects, a functional moiety can  
5 comprise a semiconducting moiety, an imaging moiety, and/or a drug-delivery moiety.

[00123] As used herein, the term “stimulus” refers to an external condition or event that is capable of acting upon a reactive species, for example a reactive moiety, to produce a reactive intermediate, for example an *o*-quinoid moiety. In various aspects, a stimulus can refer to temperature (*i.e.*, thermal stimulus), a chemical species (*i.e.*, a chemical stimulus) such  
10 as a radical or an increase or decrease in pH, or light (*i.e.*, an electromagnetic stimulus) or a mixture thereof).

[00124] A residue of a chemical species, as used in the specification and concluding claims, refers to the moiety that is the resulting product of the chemical species in a particular reaction scheme or subsequent formulation or chemical product, regardless of whether the  
15 moiety is actually obtained from the chemical species. Thus, an ethylene glycol residue in a polyester refers to one or more -OCH<sub>2</sub>CH<sub>2</sub>O- units in the polyester, regardless of whether ethylene glycol was used to prepare the polyester. Similarly, a sebacic acid residue in a polyester refers to one or more -CO(CH<sub>2</sub>)<sub>8</sub>CO- moieties in the polyester, regardless of whether the residue is obtained by reacting sebacic acid or an ester thereof to obtain the  
20 polyester.

[00125] As used herein, the term “substituted” is contemplated to include all permissible substituents of organic compounds. In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, and aromatic and nonaromatic substituents of organic compounds. Illustrative  
25 substituents include, for example, those described below. The permissible substituents can be one or more and the same or different for appropriate organic compounds. For purposes of this disclosure, the heteroatoms, such as nitrogen, can have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valences of the heteroatoms. This disclosure is not intended to be limited in any manner by the  
30 permissible substituents of organic compounds. Also, the terms “substitution” or “substituted with” include the implicit proviso that such substitution is in accordance with permitted

valence of the substituted atom and the substituent, and that the substitution results in a stable compound, *e.g.*, a compound that does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, etc.

[00126] In defining various terms, “A<sup>1</sup>,” “A<sup>2</sup>,” “A<sup>3</sup>,” and “A<sup>4</sup>” are used herein as  
5 generic symbols to represent various specific substituents. These symbols can be any substituent, not limited to those disclosed herein, and when they are defined to be certain substituents in one instance, they can, in another instance, be defined as some other substituents.

[00127] The term “alkyl” as used herein is a branched or unbranched saturated  
10 hydrocarbon group of 1 to 24 carbon atoms, such as methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, isobutyl, *s*-butyl, *t*-butyl, *n*-pentyl, isopentyl, *s*-pentyl, neopentyl, hexyl, heptyl, octyl, nonyl, decyl, dodecyl, tetradecyl, hexadecyl, eicosyl, tetracosyl, and the like. The alkyl group can also be substituted or unsubstituted. The alkyl group can be substituted with one or more groups including, but not limited to, optionally substituted alkyl, cycloalkyl, alkoxy, amino,  
15 ether, halide, hydroxy, nitro, silyl, sulfo-oxo, or thiol, as described herein. A “lower alkyl” group is an alkyl group containing from one to six carbon atoms.

[00128] Throughout the specification “alkyl” is generally used to refer to both unsubstituted alkyl groups and substituted alkyl groups; however, substituted alkyl groups are also specifically referred to herein by identifying the specific substituent(s) on the alkyl  
20 group. For example, the term “halogenated alkyl” specifically refers to an alkyl group that is substituted with one or more halide, *e.g.*, fluorine, chlorine, bromine, or iodine. The term “alkoxyalkyl” specifically refers to an alkyl group that is substituted with one or more alkoxy groups, as described below. The term “alkylamino” specifically refers to an alkyl group that is substituted with one or more amino groups, as described below, and the like. When “alkyl”  
25 is used in one instance and a specific term such as “alkylalcohol” is used in another, it is not meant to imply that the term “alkyl” does not also refer to specific terms such as “alkylalcohol” and the like.

[00129] This practice is also used for other groups described herein. That is, while a term such as “cycloalkyl” refers to both unsubstituted and substituted cycloalkyl moieties, the  
30 substituted moieties can, in addition, be specifically identified herein; for example, a

particular substituted cycloalkyl can be referred to as, *e.g.*, an “alkylcycloalkyl.” Similarly, a substituted alkoxy can be specifically referred to as, *e.g.*, a “halogenated alkoxy,” a particular substituted alkenyl can be, *e.g.*, an “alkenylalcohol,” and the like. Again, the practice of using a general term, such as “cycloalkyl,” and a specific term, such as “alkylcycloalkyl,” is not meant to imply that the general term does not also include the specific term.

**[00130]** The term “cycloalkyl” as used herein is a non-aromatic carbon-based ring composed of at least three carbon atoms. Examples of cycloalkyl groups include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, norbornyl, and the like. The term “heterocycloalkyl” is a type of cycloalkyl group as defined above, and is included within the meaning of the term “cycloalkyl,” where at least one of the carbon atoms of the ring is replaced with a heteroatom such as, but not limited to, nitrogen, oxygen, sulfur, or phosphorus. The cycloalkyl group and heterocycloalkyl group can be substituted or unsubstituted. The cycloalkyl group and heterocycloalkyl group can be substituted with one or more groups including, but not limited to, optionally substituted alkyl, cycloalkyl, alkoxy, amino, ether, halide, hydroxy, nitro, silyl, sulfo-oxo, or thiol as described herein.

**[00131]** The term “polyalkylene group” as used herein is a group having two or more  $\text{CH}_2$  groups linked to one another. The polyalkylene group can be represented by the formula  $-(\text{CH}_2)_a-$ , where “a” is an integer of from 2 to 500.

**[00132]** The terms “alkoxy” and “alkoxyl” as used herein to refer to an alkyl or cycloalkyl group bonded through an ether linkage; that is, an “alkoxy” group can be defined as  $-\text{OA}^1$  where  $\text{A}^1$  is alkyl or cycloalkyl as defined above. “Alkoxy” also includes polymers of alkoxy groups as just described; that is, an alkoxy can be a polyether such as  $-\text{OA}^1-\text{OA}^2$  or  $-\text{OA}^1-(\text{OA}^2)_a-\text{OA}^3$ , where “a” is an integer of from 1 to 200 and  $\text{A}^1$ ,  $\text{A}^2$ , and  $\text{A}^3$  are alkyl and/or cycloalkyl groups.

**[00133]** The term “alkenyl” as used herein is a hydrocarbon group of from 2 to 24 carbon atoms with a structural formula containing at least one carbon-carbon double bond. Asymmetric structures such as  $(\text{A}^1\text{A}^2)\text{C}=\text{C}(\text{A}^3\text{A}^4)$  are intended to include both the *E* and *Z* isomers. This can be presumed in structural formulae herein wherein an asymmetric alkene is present, or it can be explicitly indicated by the bond symbol  $\text{C}=\text{C}$ . The alkenyl group can be substituted with one or more groups including, but not limited to, optionally substituted alkyl,

cycloalkyl, alkoxy, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, heteroaryl, aldehyde, amino, carboxylic acid, ester, ether, halide, hydroxy, ketone, azide, nitro, silyl, sulfo-oxo, or thiol, as described herein.

[00134] The term “cycloalkenyl” as used herein is a non-aromatic carbon-based ring  
5 composed of at least three carbon atoms and containing at least one carbon-carbon double  
bound, *i.e.*, C=C. Examples of cycloalkenyl groups include, but are not limited to,  
cyclopropenyl, cyclobutenyl, cyclopentenyl, cyclopentadienyl, cyclohexenyl, cyclohexadienyl,  
norbornenyl, and the like. The term “heterocycloalkenyl” is a type of cycloalkenyl group as  
defined above, and is included within the meaning of the term “cycloalkenyl,” where at least  
10 one of the carbon atoms of the ring is replaced with a heteroatom such as, but not limited to,  
nitrogen, oxygen, sulfur, or phosphorus. The cycloalkenyl group and heterocycloalkenyl  
group can be substituted or unsubstituted. The cycloalkenyl group and heterocycloalkenyl  
group can be substituted with one or more groups including, but not limited to, optionally  
substituted alkyl, cycloalkyl, alkoxy, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl,  
15 heteroaryl, aldehyde, amino, carboxylic acid, ester, ether, halide, hydroxy, ketone, azide,  
nitro, silyl, sulfo-oxo, or thiol as described herein.

[00135] The term “alkynyl” as used herein is a hydrocarbon group of 2 to 24 carbon  
atoms with a structural formula containing at least one carbon-carbon triple bond. The  
alkynyl group can be unsubstituted or substituted with one or more groups including, but not  
20 limited to, optionally substituted alkyl, cycloalkyl, alkoxy, alkenyl, cycloalkenyl, alkynyl,  
cycloalkynyl, aryl, heteroaryl, aldehyde, amino, carboxylic acid, ester, ether, halide, hydroxy,  
ketone, azide, nitro, silyl, sulfo-oxo, or thiol, as described herein.

[00136] The term “cycloalkynyl” as used herein is a non-aromatic carbon-based ring  
composed of at least seven carbon atoms and containing at least one carbon-carbon triple  
25 bound. Examples of cycloalkynyl groups include, but are not limited to, cycloheptynyl,  
cyclooctynyl, cyclononyl, and the like. The term “heterocycloalkynyl” is a type of  
cycloalkenyl group as defined above, and is included within the meaning of the term  
“cycloalkynyl,” where at least one of the carbon atoms of the ring is replaced with a  
heteroatom such as, but not limited to, nitrogen, oxygen, sulfur, or phosphorus. The  
30 cycloalkynyl group and heterocycloalkynyl group can be substituted or unsubstituted. The  
cycloalkynyl group and heterocycloalkynyl group can be substituted with one or more groups

including, but not limited to, optionally substituted alkyl, cycloalkyl, alkoxy, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, heteroaryl, aldehyde, amino, carboxylic acid, ester, ether, halide, hydroxy, ketone, azide, nitro, silyl, sulfo-oxo, or thiol as described herein.

**[00137]** The term “aryl” as used herein is a group that contains any carbon-based aromatic group including, but not limited to, benzene, naphthalene, phenyl, biphenyl, phenoxybenzene, and the like. The term “aryl” also includes “heteroaryl,” which is defined as a group that contains an aromatic group that has at least one heteroatom incorporated within the ring of the aromatic group. Examples of heteroatoms include, but are not limited to, nitrogen, oxygen, sulfur, and phosphorus. Likewise, the term “non-heteroaryl,” which is also included in the term “aryl,” defines a group that contains an aromatic group that does not contain a heteroatom. The aryl group can be substituted or unsubstituted. The aryl group can be substituted with one or more groups including, but not limited to, optionally substituted alkyl, cycloalkyl, alkoxy, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, heteroaryl, aldehyde, amino, carboxylic acid, ester, ether, halide, hydroxy, ketone, azide, nitro, silyl, sulfo-oxo, or thiol as described herein. The term “biaryl” is a specific type of aryl group and is included in the definition of “aryl.” Biaryl refers to two aryl groups that are bound together *via* a fused ring structure, as in naphthalene, or are attached *via* one or more carbon-carbon bonds, as in biphenyl.

**[00138]** The term “aldehyde” as used herein is represented by the formula  $\text{—C(O)H}$ . Throughout this specification “C(O)” is a short hand notation for a carbonyl group, *i.e.*,  $\text{C=O}$ .

**[00139]** The terms “amine” or “amino” as used herein are represented by the formula  $\text{NA}^1\text{A}^2\text{A}^3$ , where  $\text{A}^1$ ,  $\text{A}^2$ , and  $\text{A}^3$  can be, independently, hydrogen or optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, or heteroaryl group as described herein.

**[00140]** The term “carboxylic acid” as used herein is represented by the formula  $\text{—C(O)OH}$ .

**[00141]** The term “ester” as used herein is represented by the formula  $\text{—OC(O)A}^1$  or  $\text{—C(O)OA}^1$ , where  $\text{A}^1$  can be an optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, or heteroaryl group as described herein. The term “polyester” as used herein is represented by the formula  $\text{—(A}^1\text{O(O)C—A}^2\text{—C(O)O)}_a\text{—}$  or —

(A<sup>1</sup>O(O)C-A<sup>2</sup>-OC(O))<sub>a</sub>—, where A<sup>1</sup> and A<sup>2</sup> can be, independently, an optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, or heteroaryl group described herein and “a” is an interger from 1 to 500. “Polyester” is as the term used to describe a group that is produced by the reaction between a compound having at least two  
 5 carboxylic acid groups with a compound having at least two hydroxyl groups.

**[00142]** The term “ether” as used herein is represented by the formula A<sup>1</sup>OA<sup>2</sup>, where A<sup>1</sup> and A<sup>2</sup> can be, independently, an optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, or heteroaryl group described herein. The term “polyether” as used herein is represented by the formula —(A<sup>1</sup>O-A<sup>2</sup>O)<sub>a</sub>—, where A<sup>1</sup> and A<sup>2</sup>  
 10 can be, independently, an optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, or heteroaryl group described herein and “a” is an integer of from 1 to 500. Examples of polyether groups include polyethylene oxide, polypropylene oxide, and polybutylene oxide.

**[00143]** The term “halide” as used herein refers to the halogens fluorine, chlorine,  
 15 bromine, and iodine.

**[00144]** The term “heterocycle,” as used herein refers to single and multi-cyclic aromatic or non-aromatic ring systems in which at least one of the ring members is other than carbon. Heterocycle includes pyridinde, pyrimidine, furan, thiophene, pyrrole, isoxazole, isothiazole, pyrazole, oxazole, thiazole, imidazole, oxazole, including, 1,2,3-oxadiazole,  
 20 1,2,5-oxadiazole and 1,3,4-oxadiazole,thiadiazole, including, 1,2,3-thiadiazole, 1,2,5-thiadiazole, and 1,3,4-thiadiazole, triazole, including, 1,2,3-triazole, 1,3,4-triazole, tetrazole, including 1,2,3,4-tetrazole and 1,2,4,5-tetrazole, pyridine, pyridazine, pyrimidine, pyrazine, triazine, including 1,2,4-triazine and 1,3,5-triazine, tetrazine, including 1,2,4,5-tetrazine, pyrrolidine, piperidine, piperazine, morpholine, azetidine, tetrahydropyran, tetrahydrofuran,  
 25 dioxane, and the like.

**[00145]** The term “hydroxyl” as used herein is represented by the formula —OH.

**[00146]** The term “ketone” as used herein is represented by the formula A<sup>1</sup>C(O)A<sup>2</sup>, where A<sup>1</sup> and A<sup>2</sup> can be, independently, an optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, or heteroaryl group as described herein.

[00147] The term “azide” as used herein is represented by the formula  $\text{—N}_3$ .

[00148] The term “nitro” as used herein is represented by the formula  $\text{—NO}_2$ .

[00149] The term “nitrile” as used herein is represented by the formula  $\text{—CN}$ .

[00150] The term “silyl” as used herein is represented by the formula  $\text{—SiA}^1\text{A}^2\text{A}^3$ ,  
 5 where  $\text{A}^1$ ,  $\text{A}^2$ , and  $\text{A}^3$  can be, independently, hydrogen or an optionally substituted alkyl, cycloalkyl, alkoxy, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, or heteroaryl group as described herein.

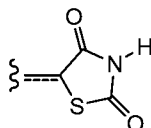
[00151] The term “sulfo-oxo” as used herein is represented by the formulas  $\text{—S(O)A}^1$ ,  
 $\text{—S(O)}_2\text{A}^1$ ,  $\text{—OS(O)}_2\text{A}^1$ , or  $\text{—OS(O)}_2\text{OA}^1$ , where  $\text{A}^1$  can be hydrogen or an optionally  
 10 substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, or heteroaryl group as described herein. Throughout this specification “S(O)” is a short hand notation for  $\text{S=O}$ . The term “sulfonyl” is used herein to refer to the sulfo-oxo group represented by the formula  $\text{—S(O)}_2\text{A}^1$ , where  $\text{A}^1$  can be hydrogen or an optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, or heteroaryl group as described herein.  
 15 The term “sulfone” as used herein is represented by the formula  $\text{A}^1\text{S(O)}_2\text{A}^2$ , where  $\text{A}^1$  and  $\text{A}^2$  can be, independently, an optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, or heteroaryl group as described herein. The term “sulfoxide” as used herein is represented by the formula  $\text{A}^1\text{S(O)A}^2$ , where  $\text{A}^1$  and  $\text{A}^2$  can be, independently, an optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl,  
 20 or heteroaryl group as described herein.

[00152] The term “thiol” as used herein is represented by the formula  $\text{—SH}$ .

[00153] The term “organic residue” defines a carbon containing residue, i.e., a residue comprising at least one carbon atom, and includes but is not limited to the carbon-containing groups, residues, or radicals defined hereinabove. Organic residues can contain various  
 25 heteroatoms, or be bonded to another molecule through a heteroatom, including oxygen, nitrogen, sulfur, phosphorus, or the like. Examples of organic residues include but are not limited alkyl or substituted alkyls, alkoxy or substituted alkoxy, mono or di-substituted amino, amide groups, etc. Organic residues can preferably comprise 1 to 18 carbon atoms, 1 to 15, carbon atoms, 1 to 12 carbon atoms, 1 to 8 carbon atoms, 1 to 6 carbon atoms, or 1 to 4

carbon atoms. In a further aspect, an organic residue can comprise 2 to 18 carbon atoms, 2 to 15, carbon atoms, 2 to 12 carbon atoms, 2 to 8 carbon atoms, 2 to 4 carbon atoms, or 2 to 4 carbon atoms

[00154] A very close synonym of the term “residue” is the term “radical,” which as  
 5 used in the specification and concluding claims, refers to a fragment, group, or substructure of a molecule described herein, regardless of how the molecule is prepared. For example, a 2,4-thiazolidinedione radical in a particular compound has the structure



regardless of whether thiazolidinedione is used to prepare the compound. In some  
 10 embodiments the radical (for example an alkyl) can be further modified (i.e., substituted alkyl) by having bonded thereto one or more “substituent radicals.” The number of atoms in a given radical is not critical to the present invention unless it is indicated to the contrary elsewhere herein.

[00155] “Organic radicals,” as the term is defined and used herein, contain one or more  
 15 carbon atoms. An organic radical can have, for example, 1-26 carbon atoms, 1-18 carbon atoms, 1-12 carbon atoms, 1-8 carbon atoms, 1-6 carbon atoms, or 1-4 carbon atoms. In a further aspect, an organic radical can have 2-26 carbon atoms, 2-18 carbon atoms, 2-12 carbon atoms, 2-8 carbon atoms, 2-6 carbon atoms, or 2-4 carbon atoms. Organic radicals often have hydrogen bound to at least some of the carbon atoms of the organic radical. One  
 20 example, of an organic radical that comprises no inorganic atoms is a 5, 6, 7, 8-tetrahydro-2-naphthyl radical. In some embodiments, an organic radical can contain 1-10 inorganic heteroatoms bound thereto or therein, including halogens, oxygen, sulfur, nitrogen, phosphorus, and the like. Examples of organic radicals include but are not limited to an alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, mono-substituted amino, di-substituted  
 25 amino, acyloxy, cyano, carboxy, carboalkoxy, alkylcarboxamide, substituted alkylcarboxamide, dialkylcarboxamide, substituted dialkylcarboxamide, alkylsulfonyl, alkylsulfinyl, thioalkyl, thiohaloalkyl, alkoxy, substituted alkoxy, haloalkyl, haloalkoxy, aryl, substituted aryl, heteroaryl, heterocyclic, or substituted heterocyclic radicals, wherein the

terms are defined elsewhere herein. A few non-limiting examples of organic radicals that include heteroatoms include alkoxy radicals, trifluoromethoxy radicals, acetoxy radicals, dimethylamino radicals and the like.

[00156] “Inorganic radicals,” as the term is defined and used herein, contain no carbon  
5 atoms and therefore comprise only atoms other than carbon. Inorganic radicals comprise  
bonded combinations of atoms selected from hydrogen, nitrogen, oxygen, silicon,  
phosphorus, sulfur, selenium, and halogens such as fluorine, chlorine, bromine, and iodine,  
which can be present individually or bonded together in their chemically stable combinations.  
Inorganic radicals have 10 or fewer, or preferably one to six or one to four inorganic atoms as  
10 listed above bonded together. Examples of inorganic radicals include, but not limited to,  
amino, hydroxy, halogens, nitro, thiol, sulfate, phosphate, and like commonly known  
inorganic radicals. The inorganic radicals do not have bonded therein the metallic elements  
of the periodic table (such as the alkali metals, alkaline earth metals, transition metals,  
lanthanide metals, or actinide metals), although such metal ions can sometimes serve as a  
15 pharmaceutically acceptable cation for anionic inorganic radicals such as a sulfate, phosphate,  
or like anionic inorganic radical. Inorganic radicals do not comprise metalloids elements such  
as boron, aluminum, gallium, germanium, arsenic, tin, lead, or tellurium, or the noble gas  
elements, unless otherwise specifically indicated elsewhere herein.

[00157] Compounds described herein can contain one or more double bonds and, thus,  
20 potentially give rise to cis/trans (E/Z) isomers, as well as other conformational isomers.  
Unless stated to the contrary, the invention includes all such possible isomers, as well as  
mixtures of such isomers.

[00158] Unless stated to the contrary, a formula with chemical bonds shown only as  
solid lines and not as wedges or dashed lines contemplates each possible isomer, *e.g.*, each  
25 enantiomer and diastereomer, and a mixture of isomers, such as a racemic or scalemic  
mixture. Compounds described herein can contain one or more asymmetric centers and, thus,  
potentially give rise to diastereomers and optical isomers. Unless stated to the contrary, the  
present invention includes all such possible diastereomers as well as their racemic mixtures,  
their substantially pure resolved enantiomers, all possible geometric isomers, and  
30 pharmaceutically acceptable salts thereof. Mixtures of stereoisomers, as well as isolated  
specific stereoisomers, are also included. During the course of the synthetic procedures used

to prepare such compounds, or in using racemization or epimerization procedures known to those skilled in the art, the products of such procedures can be a mixture of stereoisomers.

[00159] Disclosed are the components to be used to prepare the compositions of the invention as well as the compositions themselves to be used within the methods disclosed  
5 herein. These and other materials are disclosed herein, and it is understood that when combinations, subsets, interactions, groups, etc. of these materials are disclosed that while specific reference of each various individual and collective combinations and permutation of these compounds can not be explicitly disclosed, each is specifically contemplated and described herein. For example, if a particular compound is disclosed and discussed and a  
10 number of modifications that can be made to a number of molecules including the compounds are discussed, specifically contemplated is each and every combination and permutation of the compound and the modifications that are possible unless specifically indicated to the contrary. Thus, if a class of molecules A, B, and C are disclosed as well as a class of molecules D, E, and F and an example of a combination molecule, A-D is disclosed, then  
15 even if each is not individually recited each is individually and collectively contemplated meaning combinations, A-E, A-F, B-D, B-E, B-F, C-D, C-E, and C-F are considered disclosed. Likewise, any subset or combination of these is also disclosed. Thus, for example, the sub-group of A-E, B-F, and C-E would be considered disclosed. This concept applies to all aspects of this application including, but not limited to, steps in methods of making and  
20 using the compositions of the invention. Thus, if there are a variety of additional steps that can be performed it is understood that each of these additional steps can be performed with any specific embodiment or combination of embodiments of the methods of the invention.

[00160] It is understood that the compositions disclosed herein have certain functions. Disclosed herein are certain structural requirements for performing the disclosed functions,  
25 and it is understood that there are a variety of structures that can perform the same function that are related to the disclosed structures, and that these structures will typically achieve the same result.

#### **B. DENDRIMERIC COMPOUNDS**

[00161] Dendrimers can be ideal building blocks for biomedical applications, because  
30 of their precise architecture, high loading capacity, tunable solubility, immunogenicity, and

bioconjugation capability. [Gillies, E. R.; Fréchet, J. M. J. Drug Discov. Today 2005, 10, 35.;  
Lee, C. C.; MacKay, J. A.; Fréchet, J. M. J.; Szoka, F. C. Nat. Biotechnol. 2005, 23, 1517.]

The combination of the unique properties of dendrimers with membrane-permeable guanidino  
groups can lead to a more efficient-synthesis of membrane-permeable carrier molecules

5 possessing high efficiency, for example, for bulk production.

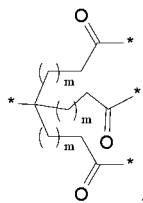
**[00162]** The compounds of the invention are desirably based upon a compact, high  
branching multiplicity dendrimer, for example, the classic Newkome-type dendrimer.

[Newkome, G. R.; Behera, R. K.; Moorefield, C. N.; Baker, G. R. J. Org. Chem. 1991, 56,  
7162.] Newkome type dendrimers are typically 1→3 C-branched polyamide macromolecules,

10 built from “Behera’s Amine” monomer or its derivatives, and can be attached to a variety of  
starting cores, surfaces, and polymers.

**[00163]** It is also understood that the compounds of the invention can be tailored to  
enhance accumulation in specific sublocations of cells, such as the nucleus, the cytosol, or the  
mitochondria. Tailoring can be the selection of chemical moieties or groups having an  
15 affinity for a targeted subcellular region of a cell, for example an organelle, and the  
functionalization of the compounds with the selected chemical moieties or groups. Such  
tailoring of the compound structure can be accomplished using organic synthetic  
methodology know to those of skill in the art.

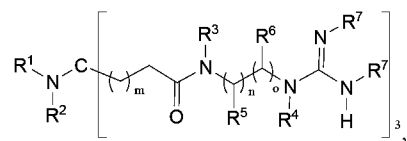
**[00164]** In one aspect, the invention relates to compounds comprising the structure:



20

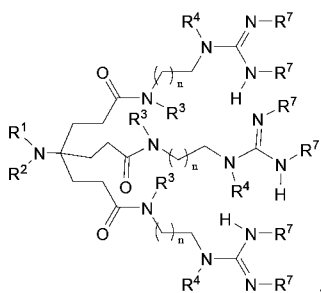
and at least one guanidinium residue, wherein m is zero or a positive integer. In certain  
aspects, m can be 0, 1, 2, 3, 4, 5, or 6 and each residue can be substituted or unsubstituted. In  
a further aspect, m is 1.

**[00165]** In one aspect, the invention relates to compounds comprising the structure:



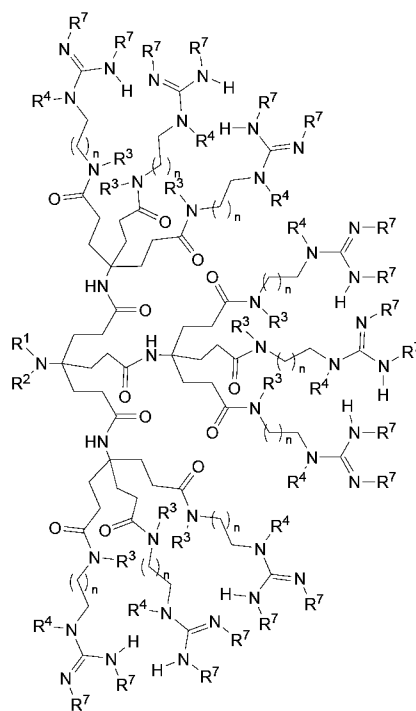
wherein n and o are, independently, zero or a positive integer; wherein R<sup>1</sup> and R<sup>2</sup> are, independently, hydrogen, oxygen, alkyl, acyl, thioacyl, or carbonyl; wherein R<sup>3</sup> is hydrogen, alkyloxycarbonyl, or alkyl; R<sup>4</sup> is hydrogen, or alkyloxycarbonyl; wherein R<sup>5</sup> and R<sup>6</sup> are, independently, hydrogen, or alkyl; and wherein R<sup>7</sup> is hydrogen or alkyloxycarbonyl.

**[00166]** In a further aspect, the compounds can comprise the structure:



wherein n is an integer from 1 to 9; wherein R<sup>1</sup> and R<sup>2</sup> are, independently, hydrogen, oxygen, nitrogen, alkyl, acyl, thioacyl, carbonyl, or amine; wherein R<sup>3</sup> is hydrogen or alkyl; and  
10 wherein R<sup>4</sup> is hydrogen, or alkyloxycarbonyl, alkyl, or acyl. In certain aspects, n can be 0, 1, 2, 3, 4, 5, 6, 7, 8, or 9. In a further aspect, n is 1 or 5. In a further aspect, R<sup>4</sup> can be hydrogen or alkyloxycarbonyl. In a further aspect, R<sup>7</sup> is Boc, for example, t-Boc.

**[00167]** In one aspect, the compound comprises the structure:



wherein  $n$  is an integer from 1 to 9; wherein  $R^1$  and  $R^2$  are, independently, hydrogen, amino, hydroxyl, alkyl, alkoxy, acyl, carbonyl, or thioacyl; wherein  $R^3$  is hydrogen or alkyl; and wherein  $R^4$  is hydrogen, or alkyloxycarbonyl.

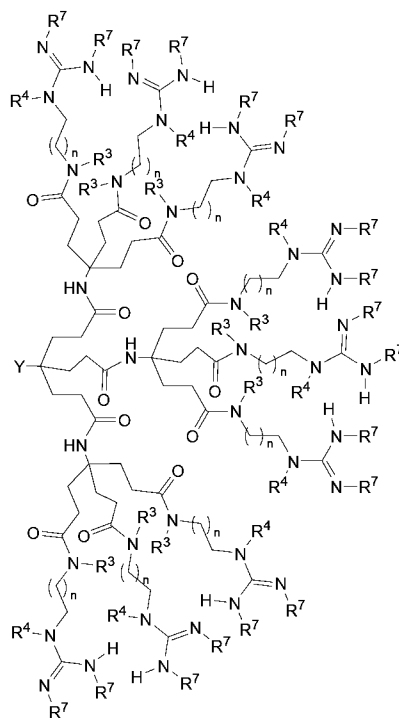
### 5 C. METHODS OF MAKING DENDRIMERIC COMPOUNDS

[00168] The disclosed methods typically employ a divergent method to prepare a G-1 dendrimer scaffold with nine end functionalities. Although the Newkome type dendrimer is well known, one of the drawbacks for a broader application of conventional methods is the elaborate synthesis of the monomer. In contrast, the “Behera’s amine” gives the most compact, low molecular weight polyamide dendrimer possible; achieving the necessary nine end functionalities in just one generation of dendritic growth. As set forth below and in the Experimental section, following synthesis of the monomer through improved hydrogenation and work-up procedures, the G-1 dendritic nona-acid scaffold can be prepared in high yields (see Figures 6A and 6B).

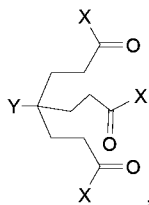
[00169] In order to introduce the guanidinium groups to the dendrimer exterior as shown in Figure 11, the nine carboxylic acid groups were first converted into nine protected amine groups, by reaction with, for example, N-Boc ethylenediamine and N-Boc-1,6-diaminehexane through amide coupling reactions. After removal of the protecting groups, the  
5 nine free amines can be reacted with a guanidylating reagent [Feichtinger, K.; Sings, H. L.; Baker, T. J.; Matthews, K.; Goodman, M. J. *Org. Chem.* 1998, 63, 8432.] to give a guanidinylated dendritic scaffold in high yield.

[00170] For uptake evaluation and imaging function, a fluorophore can be conjugated to the focal point of the molecular transporter. The attachment of a fluorescein isothiocyanate  
10 (FITC) moiety to the guanidinylated scaffold can be achieved with a reduction of the nitro group at the focal point to an amino group via hydrogenation at room temperature in quantitative yields, followed by direct reaction with FITC to form the Boc-protected FITC-labeled guanidino-dendrimer. After deprotection of the Boc-protected guanidine groups, FITC-labeled dendritic molecules can be obtained and further purified by dialysis or HPLC.

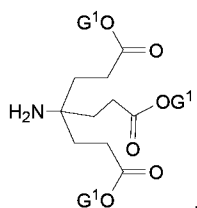
15 [00171] In one aspect, the invention relates to methods of preparing compounds having the structure:



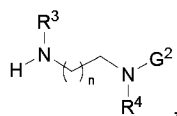
wherein  $n$  is an integer from 1 to 9, wherein  $R^3$  is hydrogen or alkyl, wherein  $R^4$  and  $R^7$  are, independently, hydrogen, alkyloxycarbonyl, alkyl, or acyl; wherein  $R^7$  is hydrogen, alkyl, or acyl; wherein  $Y$  comprises a nitro group, an amine group, an amide group, azide group, or an alkyloxycarbonyl protected amine group or a derivative thereof, the method comprising the steps of providing a first compound comprising the structure:



wherein  $X$  comprises OH, halogen, or OC(O)-alkyl; coupling the first compound with at least about three molar equivalents of a second compound comprising the structure:



wherein  $G^1$  is an ester-protecting group; removing the ester-protecting group; reacting the product of step (c) with at least about three molar equivalents of a third compound comprising the structure:

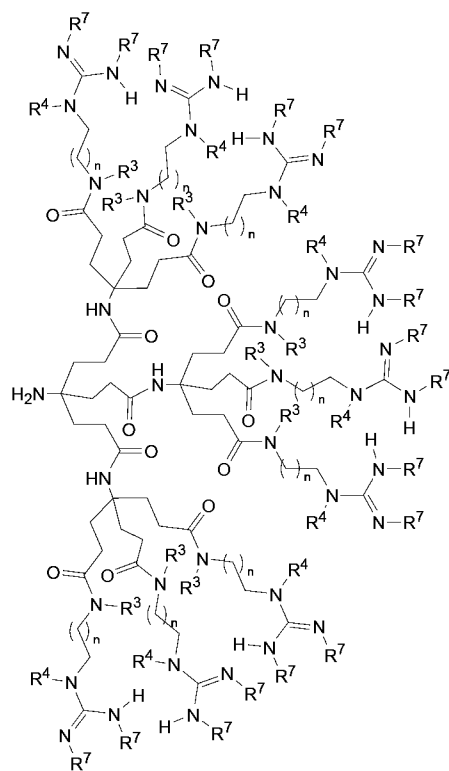


5

wherein  $G^2$  is an amine-protecting group; removing the amine-protecting group; and functionalizing the product of step (e) with at least three molar equivalents of a guanidine-providing agent.

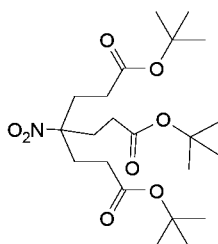
**[00172]** In a further aspect, the guanidine-providing agent comprises at least one of  
 10 N,N'-diBoc-N''-triflylguanidine, N,N'-diCbz-N''-triflylguanidine, N,N'-dialloc-N''-triflylguanidine, N,N'-ditroc-N''-triflylguanidine, 1,3-diboc-2-(2-hydroxyethyl)guanidine, N,N'-diBoc-1H-pyrazole-1-carboxamidine, N,N'-diCbz-1H-pyrazole-1-carboxamidine, 1H-pyrazole-1-carboxamidine hydrochloride, 1,3-diboc-2-(2-hydroxyethyl)guanidine, 2-(2-aminoethyl)-1,3-diboc-guandine, or 1,3-diboc-2-(carboxymethyl)guanidine

15 **[00173]** In a further aspect, the method further comprises the step of transforming Y into an amine to provide a compound comprising the structure:



**[00174]** In a further aspect, the method further comprises the step of removing  $R^7$ . The removing step can be, for example, treatment with one or more reagents known to those of skill in the art for removing protecting groups.

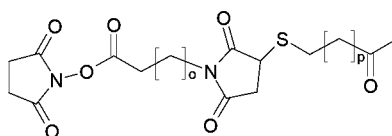
- 5 **[00175]** In one aspect, the providing step comprises synthesis of the starting materials. Each starting material can be obtained commercially and/or prepared by those of skill in the art from commercially available compounds. For example, the nitroester shown below can be prepared using methodology from Newkone, G. R.; Behera, R. K.; Moorefield, C. N.; Baker, G. R.; *J. Org. Chem.* **1991**, 56, 7162:



[00176] In a further aspect, the ester-protecting group comprises methyl, ethyl, or t-butyl.

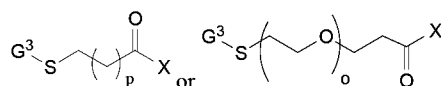
[00177] In a further aspect, the amine-protecting group comprises a butyloxycarbonyl group, a trifluoroacetyl group, a 9-fluorenylmethyloxycarbonyl group, an alloc group, or a carbobenzyloxy group.

[00178] In a further aspect, the method further comprises the step of acylating the amine with a compound comprising the structure:



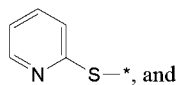
10 wherein o and p are, independently, zero or a positive integer. In a yet further aspect, the method further comprises the step of reacting the product of the acylating step with a payload compound comprising at least one amine group and at least one of a luminescent group, a biologically active group, or a pharmaceutically active group.

[00179] In a further aspect, the method further comprises the step of acylating the  
15 amine with a fourth compound comprising the structure:

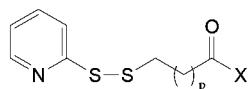


wherein o and p are, independently, zero or a positive integer, and wherein G<sup>3</sup> is an thiol-protecting group.

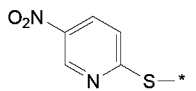
**[00180]** In a further aspect, the thiol protecting group comprises the structure:



wherein the fourth compound comprises the structure:



5   **[00181]**   In a further aspect, the thiol-protecting group comprises the structure:



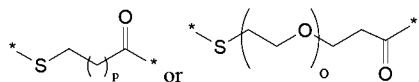
**[00182]** In a further aspect, the method further comprises the step of removing the thiol-protecting group, thereby providing a deprotected thiol. In a yet further aspect, the method further comprises the step of attaching the deprotected thiol to a thiol-functionalized 10 payload. In a still further aspect, the thiol-functionalized payload comprises at least one of a luminescent group, a biologically-active group, or a pharmaceutically-active group.

## D. COMPOSITIONS

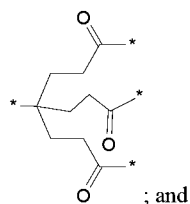
**[00183]** In one aspect, the invention relates to compositions comprising one or more compounds of the invention or one or more products of the methods of the invention.

15           **1. INTRACELLULAR DELIVERY COMPOSITIONS**

**[00184]** In one aspect, the invention relates to intracellular delivery compositions comprising the general structure P-L-B-F, wherein P is payload moiety; wherein L is a linking moiety comprising the structure:

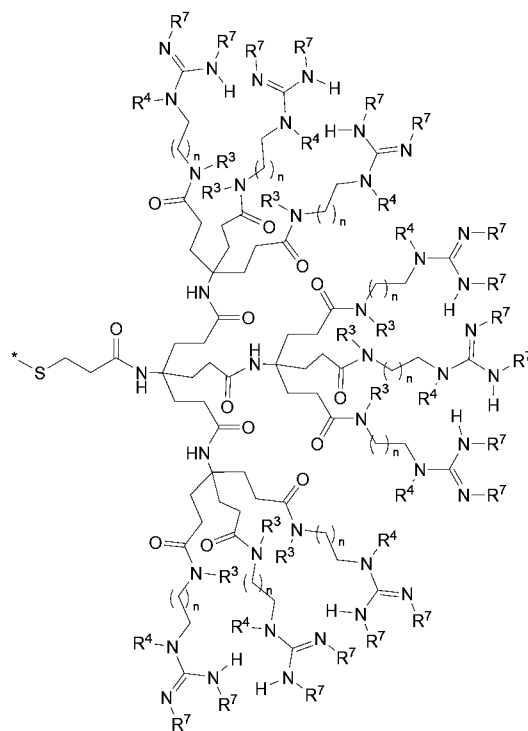


wherein o and p are, independently, zero or a positive integer; wherein B is a branching moiety comprising the structure:



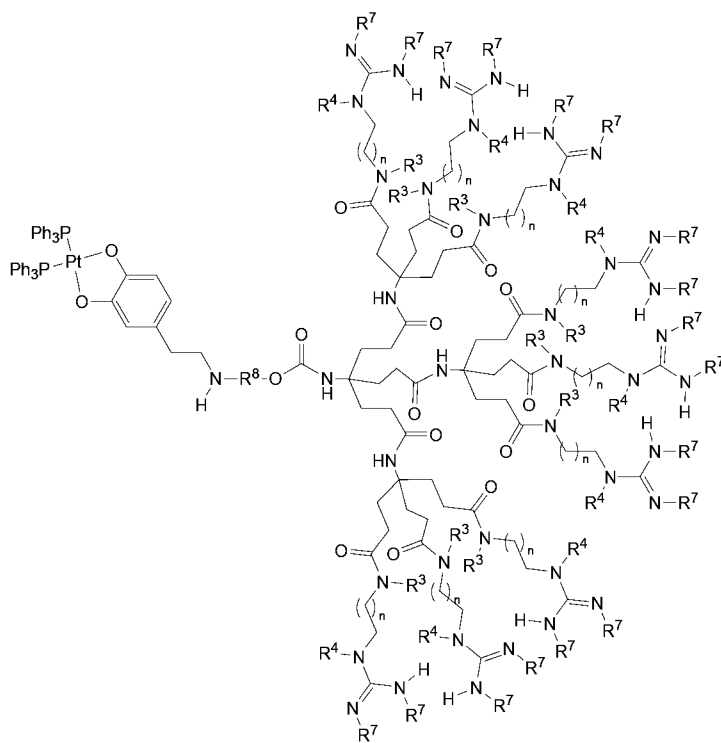
wherein F is a functional moiety comprising at least one guanidinium residue. In a further aspect, p is an integer from 0 to 6, for example, 0, 1, 2, 3, 4, 5, or 6. In a further aspect, the composition comprises at least six guanidinium residues, at least seven guanidinium residues, at least eight guanidinium residues, or at least nine guanidinium residues.

[00185] In one aspect, L-B-F comprises the structure:

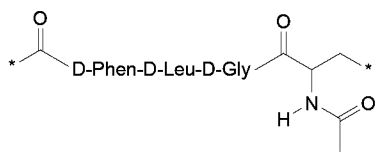


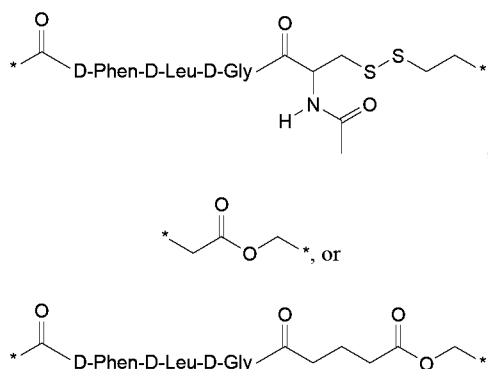
wherein  $n$  is an integer from 1 to 9; wherein  $R^3$  is hydrogen or alkyl; wherein  $R^4$  is hydrogen, alkyl or acyl; and wherein  $R^7$  is hydrogen, alkyl or acyl.

[00186] In a further aspect, P-L-B-F comprises the structure:



- 5 wherein  $n$  is an integer from 1 to 9; wherein  $R^3$  is hydrogen or alkyl; wherein  $R^4$  is hydrogen, alkyl or acyl; wherein  $R^7$  is hydrogen, alkyl or acyl; and wherein  $R^8$  comprises the structure:

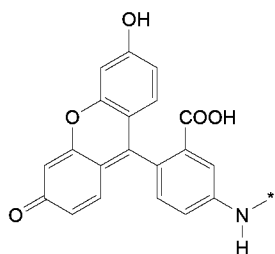




#### a. PAYLOADS

5 [00187] Typically, the compounds of the invention can be functionalized to carry a payload. In various aspects, a payload compound can be attached or associated with a compound of the invention by covalent bonding, by ionic bonding, by coordination bonding, or by hydrogen bonding. In further aspects, a payload compound can be associated with a compound of the invention by hydrophilic interactions or hydrophobic interactions. In  
 10 certain aspects, a payload compound is part of a compound of the invention, while in certain further aspects, payload compound is a separate compound from of a compound of the invention.

[00188] In one aspect, the payload moiety bears a thiol moiety. In a further aspect, the payload moiety is a luminescent group. For example, the luminescent group can comprise the  
 15 structure:



[00189] In certain aspects, the luminescent group is selected from a dansyl group, a coumarin group, an FITC group, a DOTA group, a catechol group, or a DPTA group. DOTA,

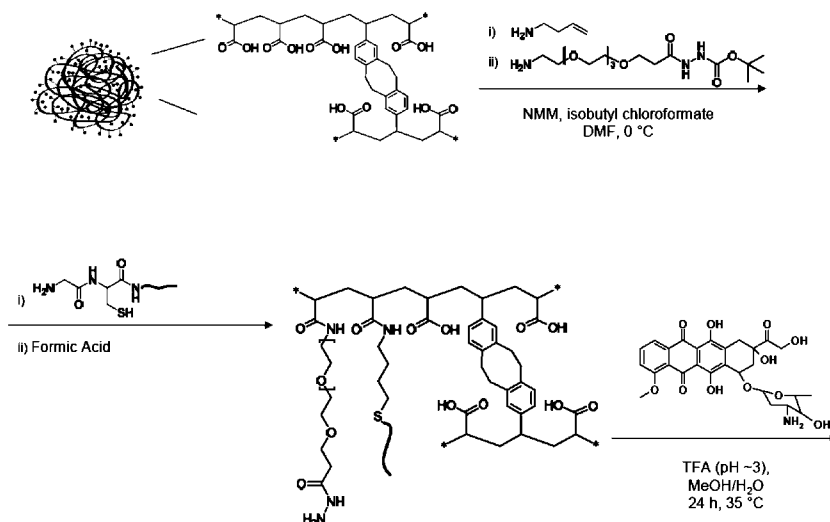
catechol, and/or DPTA groups can be used for complexing, for example, lanthanides.

Catechol can be used for complexing, for example, quantum dots, lanthanides, metals (such as iron or copper (*e.g.*, radioactive Cu)), ironoxides, metal oxides, and/or platinum (*e.g.*, cis-platinum).

5    **[00190]**       In a further aspect, the payload moiety is a biologically-active group. For example, the biologically-active group can be selected from one or more of an oligonucleotide, a plasmid DNA, a protein, an immunoglobulin, an antisense oligoDNA, a peptide nucleic acid (PNA), or a peptide. For example, in various aspects, the biologically-active group can comprise one or more of  $\beta$ -galactosidase, horseradish peroxidase, RNase, anti-apoptotic proteins Bcl-X(L)/PEA-15, catalase, green fluorescence protein, heat shock protein 70, human glutamate dehydrogenase, ovalbumin, neuroprotectant Bcl-xL, E2 protein, phosphorothioate antisense oligonucleotides, anti-tetanus F(ab')<sub>2</sub>, G protein, p16<sup>INK4a</sup>, caspase-3, p14<sup>INK4a</sup>, p27<sup>kip1</sup>, Bak BH3 domain peptide, cGPK-I $\alpha$  inhibitory peptide, IKK $\beta$  C-terminal peptide, PKA inhibitory peptide, MEK 1 N-terminal peptide, luciferin, RhoA, APO-15   BEC-1, Cre recombinase, H-Ras, Filmin-1, p16, HPC-1/syntaxin, Cdk2, E2f-1/p73/p53, influenza virus, antibodies, single chain antibodies, si-RNA, RNA derivatives, peptide 46, peptide 15, peptides that influence the immunresponse, mitochondrial DNA, bacteria, birdflu virus, and/or bacteria.

**[00191]**       In a further aspect, the payload moiety is a pharmaceutically-active group. For example, the pharmaceutically-active group is selected from a small molecular weight drug, a silica nanoparticle, a metal nanoparticle, a protein, a peptide, a linear polymer backbone, a hydrogel, a collapsed nanoparticle, a dendrimers, or a hyperbranched polymeric structure. For example, in various aspects, the pharmaceutically-active group can comprise one or more of superparamagnetic iron oxide particles, doxorubicin, methotrexate, liposome, multiple 25   sclerosis agents, cis-platinum, paclitaxel, hormones, antioxidants, antimicrobials, antibacterial agents, antidepressants, sedatives, antihypertensive drugs, antibodies, a carbohydrate-based drug, cardioprotective  $\epsilon$ PKC agonist peptide, Fab fragments of the anti-melanoma antibody NRML-05, pan-carcinoma antibody NRLU-10, anti-CEA immunotoxin, liposome drugs, bromonidine, fusogenic, dendritic cell vaccines, VHL tumor suppressor peptide, HER-2, Pro-30   apoptotic Smac peptide, viralcapsids, and/or bacteria.

[00192] A doxorubicin bioconjugate, for example, can be synthesized by the scheme shown below:



[00193] In a still further aspect, the payload is an antibody, an intrabody, DNA, RNA, siRNA, among other biologically significant conjugates. For example, an antibody can be attached to the core of a disclosed dendrimer, through disclosed methods. Such compositions can be used to treat viral related disorders, such as, for example, HIV or influenza, among others. A specific example of an antibody suitable for use with the disclosed dendrimers is an IgG antibody.

10 [00194] A disclosed dendrimer can also be attached to a protein is associated with a number of disorders, including cancer. For example, a disclosed protein-dendrimer conjugate can be used to treat a cancer. An example is a p53 (tumor suppressor protein) dendrimer conjugate which can be capable of restoration of a mutant p53 transcriptional activity, to trigger apoptosis and stop tumor progression through the cytoplasm. A further  
15 example is a dendrimer-Huntingtin (protein responsible of Huntington's disease) conjugate which can aid in the inhibition of aberrant protein aggregation in a cellular model of Huntington's disease, by targeting huntingtin to the nucleus, through the action of the dendritic molecular transporter.

[00195] Further examples of conjugates that can be used in combination with the disclosed dendritic transporters include M and N intrabodies for RSV, RV6-26 Fab Rotavirus, Tat ( HIV-1- transcription activator) for the inhibition of viral replication by sequestering Tat in the cytoplasm.

5                   **b. INTRACELLULAR DELIVERY**

[00196] In one aspect, the invention relates to methods of intracellular delivery comprising administering an effective amount of one or more compounds of the invention or one or more compositions of the invention to a subject. The subject is a mammal, for example, a human. In a further aspect, the subject is a cell. The delivery can be, for example,  
10 oral, transmucosal, rectal, or subcutaneous administration or, for example, intravenous, intrathecal, intramuscular, intranasal, intraperitoneal, or intraocular injection.

**2. PHARMACEUTICAL COMPOSITIONS**

[00197] A pharmaceutical composition comprising a therapeutically effective amount of one or more compounds of the invention or one or more compositions of the invention and  
15 a pharmaceutically acceptable carrier for administration in a mammal, for example, a human. The compositions can be, for example, granules, powders, tablets, or capsules.

**a. DOSAGE**

[00198] The specific therapeutically effective dose level for any particular patient will depend upon a variety of factors including the compound or composition being administered;  
20 the disorder being treated and the severity of the disorder; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration; the route of administration; the rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed and like factors well known in the medical arts. If desired, the effective  
25 daily dose can be divided into multiple doses for purposes of administration. Consequently, single dose compositions can contain such amounts or submultiples thereof to make up the daily dose. The dosage can be adjusted by the individual physician in the event of any contraindications. Dosage can vary, and can be administered in one or more dose administrations daily, for one or several days.

**b. CARRIERS**

[00199] A “pharmaceutically acceptable carrier” refers to sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, as well as sterile powders for reconstitution into sterile injectable solutions or dispersions just prior to use. Examples of  
 5 suitable aqueous and nonaqueous carriers, diluents, solvents or vehicles include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol and the like), carboxymethylcellulose and suitable mixtures thereof, vegetable oils (such as olive oil) and injectable organic esters such as ethyl oleate. Proper fluidity may be maintained, for example, by the use of coating materials such as lecithin, by the maintenance of the required particle  
 10 size in the case of dispersions and by the use of surfactants. These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents, and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents such as paraben, chlorobutanol, phenol, sorbic acid, and the like. It can also be desirable to include isotonic agents such as sugars, sodium  
 15 chloride, and the like. Prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents, such as aluminum monostearate and gelatin, which delay absorption. Injectable depot forms are made by forming microencapsule matrices of the drug in biodegradable polymers such as polylactide-polyglycolide, poly(orthoesters) and poly(anhydrides). Depending upon the ratio of drug to polymer and the nature of the  
 20 particular polymer employed, the rate of drug release can be controlled. Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissues. The injectable formulations may be sterilized, for example, by filtration through a bacterial-retaining filter or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or  
 25 other sterile injectable media just prior to use. Suitable inert carriers can include sugars such as lactose. Desirably, at least 95% by weight of the particles of the active ingredient have an effective particle size in the range of 0.01 to 10 micrometers.

**E. SYNTHESIS AND CHARACTERIZATION OF “BOW-TIE” DENDRITIC MOLECULAR TRANSPORTERS BY ORTHOGONAL AND CLICK APPROACH**

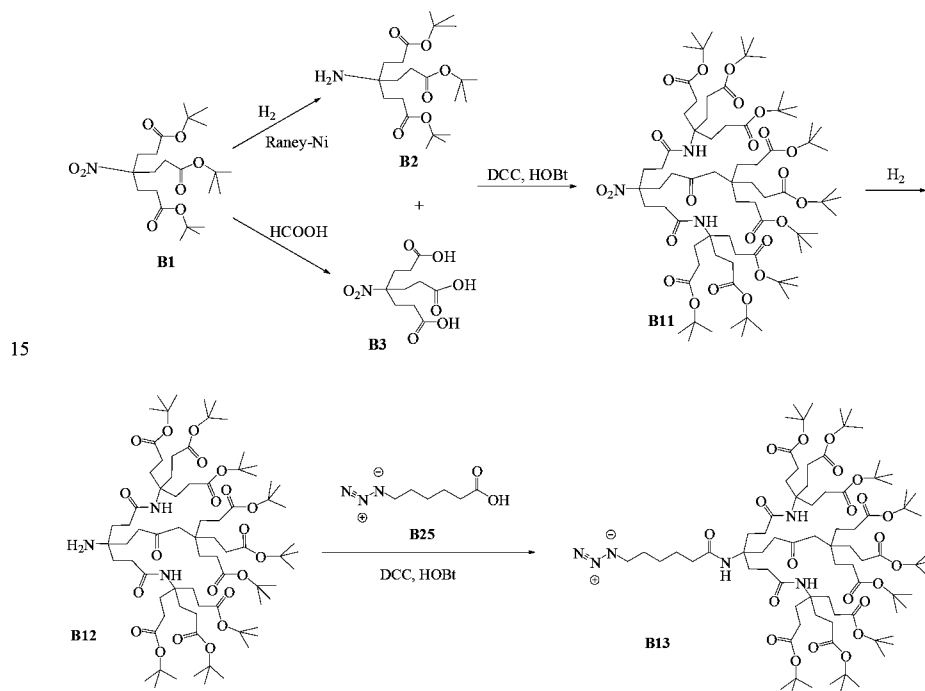
30 [00200] Disclosed is the synthesis and characterization of “Bow-Tie” dendritic architectures with orthogonally reactive groups, defined composition and functionality, which

can be used as multi-drug carries for specific intracellular delivery. Huisgen cycloadditions or so called “click” reactions have been shown to be extremely versatile tools for advanced macromolecular design. However, little attempt has been made to utilize this approach to prepare multifunctional dendritic structures. In the disclosed approach, two orthogonal

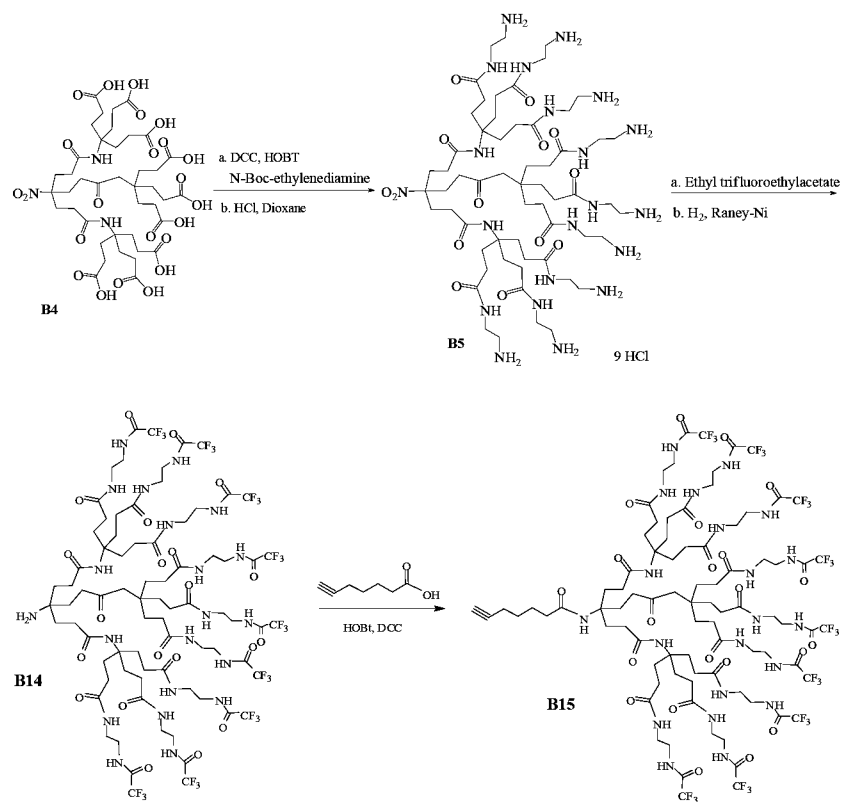
5 protected dendritic structures are combined by utilizing the “click” reaction. This strategy allows the controlled deprotection of the trifluoro protecting group to selectively attach the dithiopyridylpropionic acid the periphery of the macromolecule. In a further step, the BOC groups of the second dendritic scaffolds are deprotected to be guanydilated to the ethyl- or hexyl linker of the system. The bow-tie structure is the first of its kind that consists of a

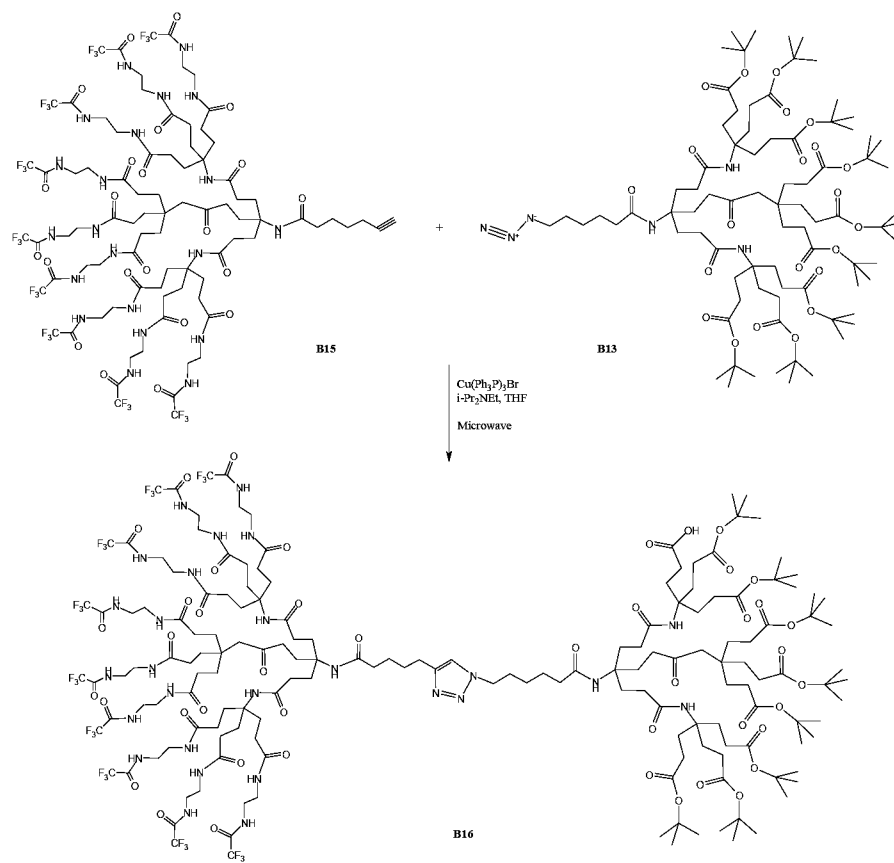
10 molecular transporter part and drug delivery entity on the other. The chemistry applied for the construction is high-yielding and, thus, gives the bow-tie delivery structure in the most straightforward approach. In this fashion, nine drug molecules, for example peptides, genes and oligonucleotides can be transported across cellular membranes.

**[00201]** Synthetic Pathway of Acid-labile Azide-linker-Dendron:

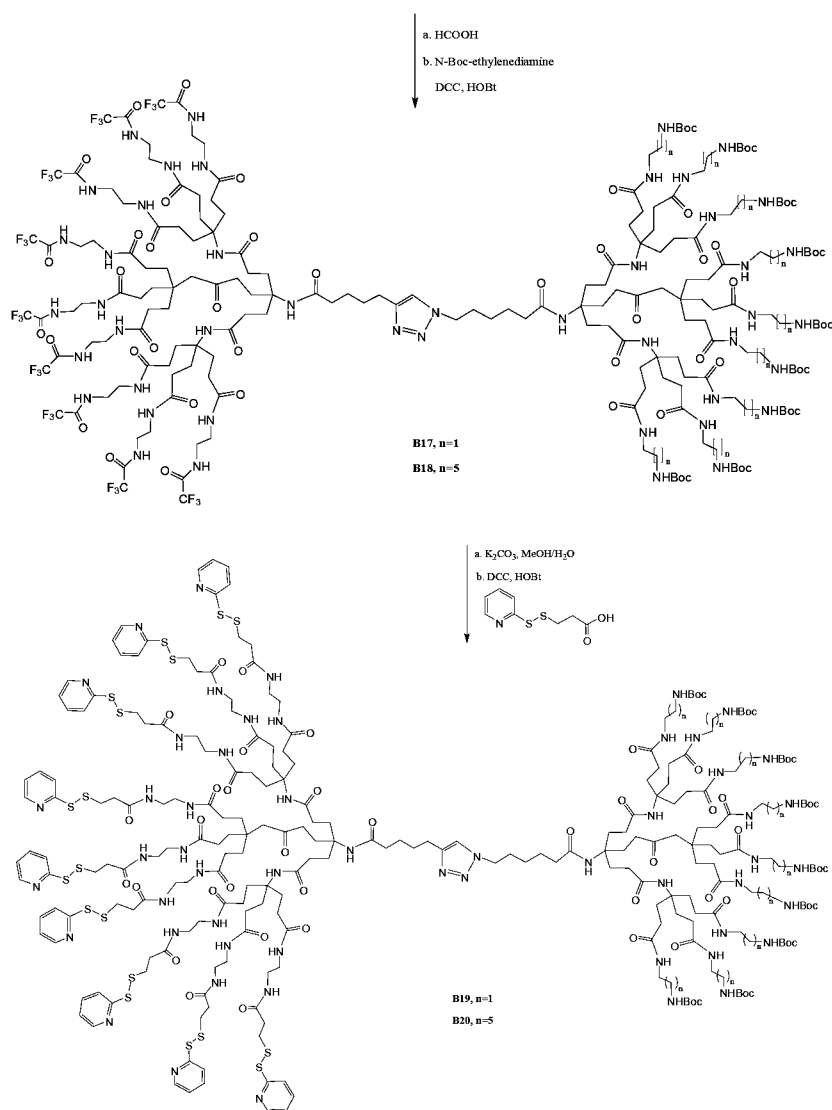


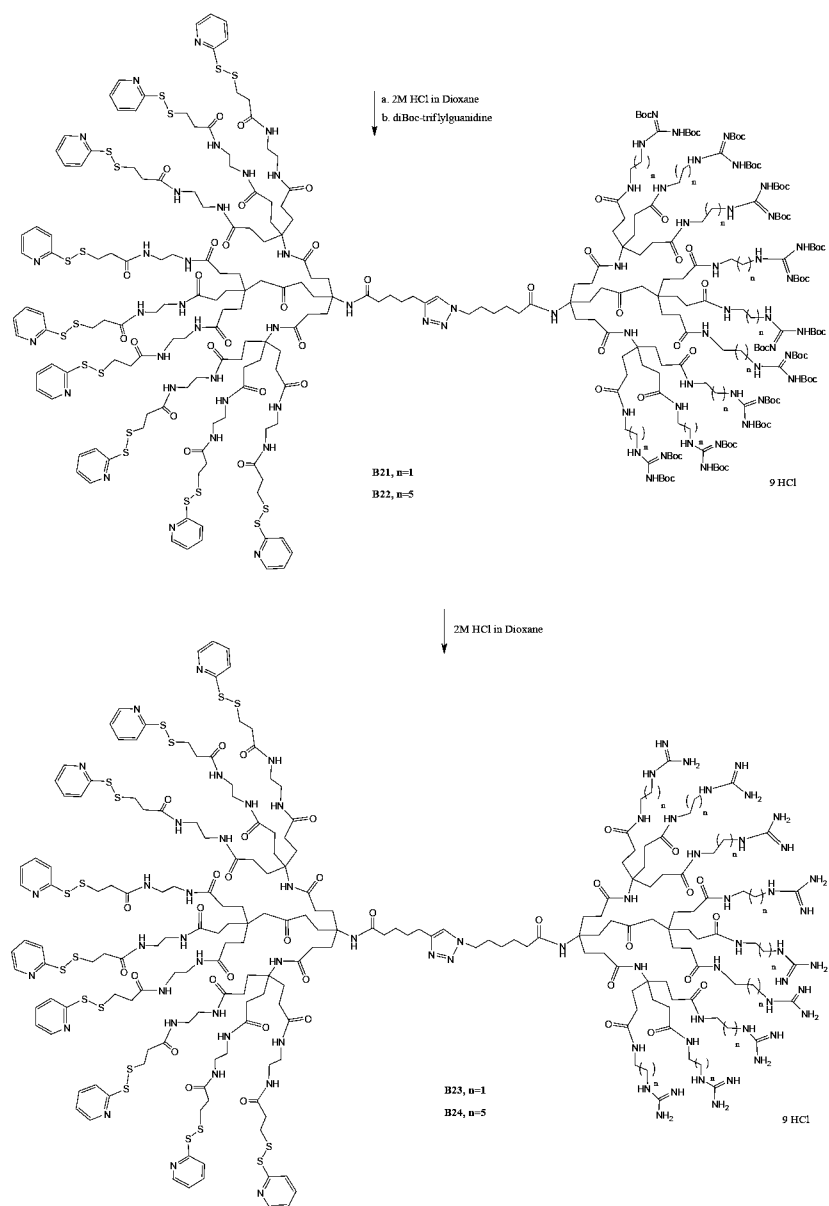
## [00202] Synthetic Pathway for Base-labile Alkyne-linker-Dendron:



**[00203]** Bifunctional Bow-Tie Synthesis by Click Reaction:

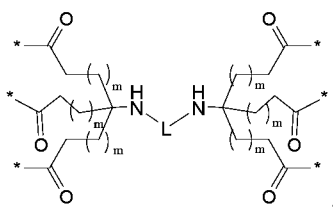
**[00204]** Further Functionalization for Synthesis of Cell-Permeable Multi-Drug Carrier Conjugates:





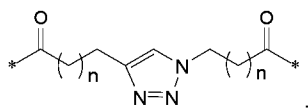
[00205]

In one aspect, the invention relates to compounds comprising the structure:



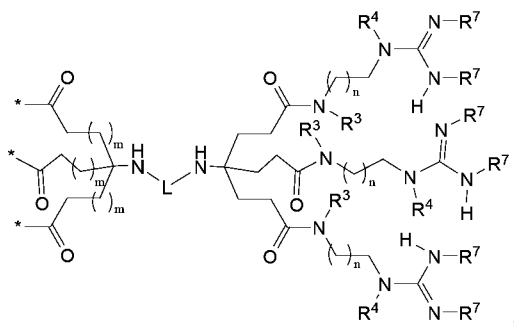
wherein each  $m$  is independently zero or a positive integer, and wherein  $L$  is a linking moiety comprising optionally substituted alkyl, optionally substituted alkoxy, optionally substituted heteroalkyl, or optionally substituted heteroaryl.

5 [00206] In a further aspect,  $L$  comprises a structure:



wherein each  $n$  is independently selected from 0-8. That is,  $L$  can comprise the reaction product of a “click” reaction.

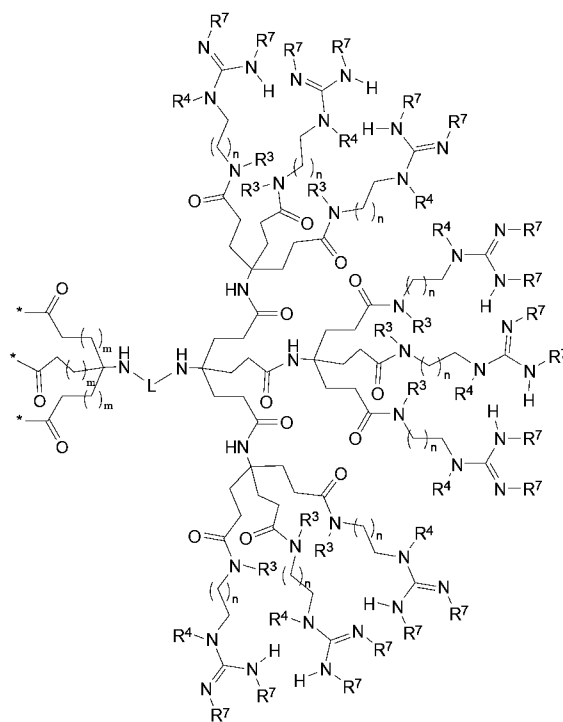
[00207] In a further aspect, the compound can comprise a structure



10

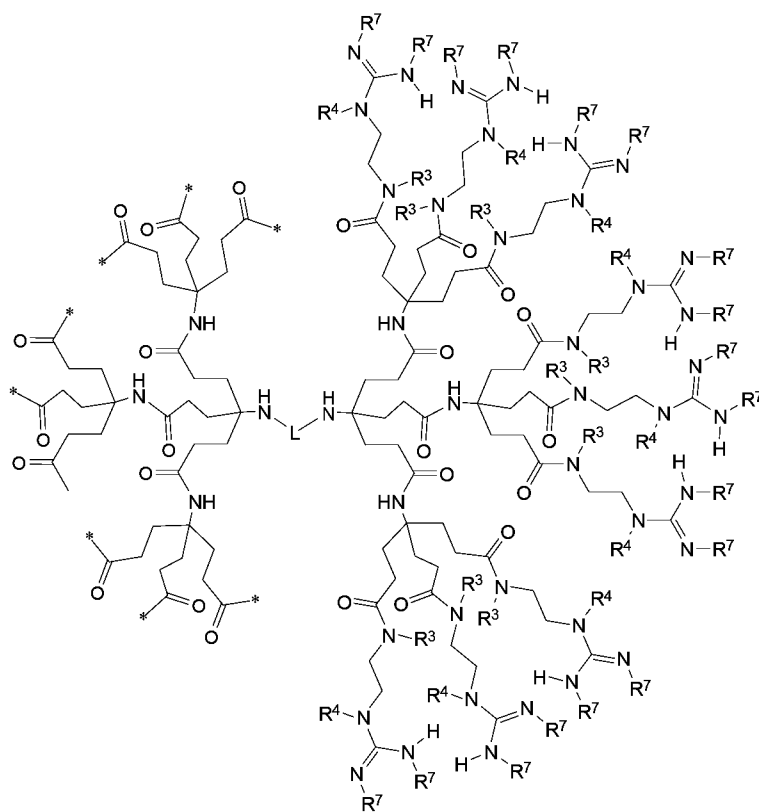
wherein  $n$  is an integer from 1 to 9; wherein  $R^3$  is hydrogen or alkyl; wherein  $R^4$  is hydrogen, or alkyloxycarbonyl, alkyl, or acyl; and wherein  $R^7$  is hydrogen or alkyloxycarbonyl.

[00208] In a yet further aspect, the compound can comprise the structure:



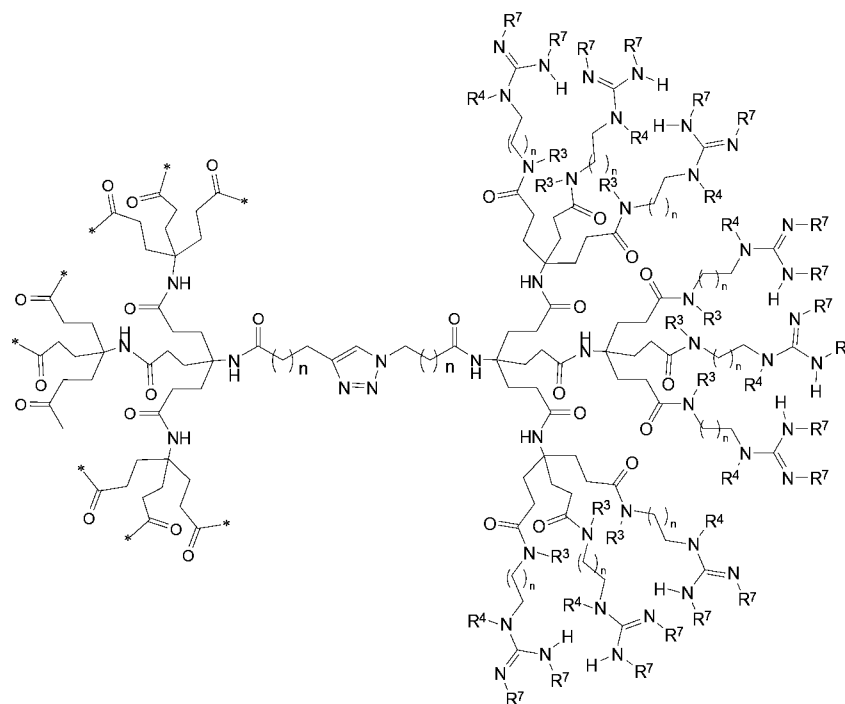
wherein  $n$  is an integer from 1 to 9; wherein  $R^3$  is hydrogen or alkyl; wherein  $R^4$  is hydrogen, or alkyloxycarbonyl, alkyl, or acyl; and wherein  $R^7$  is hydrogen or alkyloxycarbonyl.

[00209] In a still further aspect, the compound can comprise the structure:



wherein  $n$  is an integer from 1 to 9; wherein  $R^3$  is hydrogen or alkyl; wherein  $R^4$  is hydrogen, or alkyloxycarbonyl, alkyl, or acyl; and wherein  $R^7$  is hydrogen or alkyloxycarbonyl.

**[00210]** In an even further aspect, the compound can comprise the structure:



wherein each  $n$  is independently an integer from 0 to 9; wherein  $R^3$  is hydrogen or alkyl; wherein  $R^4$  is hydrogen, or alkyloxycarbonyl, alkyl, or acyl; and wherein  $R^7$  is hydrogen or alkyloxycarbonyl.

- 5 [00211] It is demonstrated that the disclosed transporter (e.g., FD-2, hexyl linker) shows selectivity towards the mitochondria of a cell. (see Figure 43) The FD-1 shows selectivity towards the cell nucleus (see Figure 42). A common obstacle in macromolecular drug delivery is the cellular uptake into cell compartments that do not release the drug delivery vector into the cytosol or mitochondria in which the drug becomes effective. Most
- 10 other delivery pathways into the cell end up in the lysosome and do not get released (endocytosis). The therapeutic efficacy of drug molecules typically depends on its ability to reach desired target tissues, cells and intracellular organelles.

[00212] The mitochondria play a key role in apoptosis (cancer therapy), familial amyotrophic lateral sclerosis (ALS, Lou Gehrig's disease), Leber hereditary optic neuropathy

(LHON), lactic acidosis, strokelike syndrome (MELAS) Huntington's disease, and Alzheimer's disease, Kearns-Sayre Syndrome (KSS), myoclonic epilepsy, ragged-red fibers (MERRF), cluster of metabolic diseases (SyndromeX), progressive external ophthalmoplegia (PEO) and antioxidants. By targeting the mitochondria, the disclosed  
5 compounds, compositions, and methods can play a role in therapy or prevention of disease processes relating to mitochondria function.

[00213] When the disclosed transporter is attached to the disclosed nanoparticle, it can enter the cell and also can achieve localization in the entire cell, including the mitochondria. The nanoparticle allows delivering a high drug load and, thus, can facilitate delivery of small  
10 and other molecules, such as peptides, nucleotides and such. The structures can be further modified with amines to allow complexation with plasmic DNA and covalent attachment through covalent approaches. (See Figures 34-39).

[00214] A nanoparticle with a number of molecular transporter moieties conjugated to the periphery crosses the plasmic membrane and localizes in the cytosol and, particularly, in  
15 the mitochondria of the cells. Techniques are disclosed that allow the attachment of the molecular transporter the scaffolds that increase the drug load significantly. Attachment to the "bow-tie" structure and/or the attachment to nanoparticles from intramolecular chain collapse techniques also increase the drug load significantly.

[00215] The dendritic transporter allows the conjugation of nine bioactive conjugates  
20 and the drug load is increased nine fold by attaching a dendric molecule to the focal point of the dendritic molecular transporter (bow-tie). A well-defined macromolecule is designed, that is "clicked" together in a Huisgen type reaction. The deprotection of the basic/acidic protecting groups allows the modification to a delivery system with a short ethyl linker or hexyl linker before guanidylation to maintain uptake into specific subcellular locations. The  
25 disulfide linker is only one of the examples of a linker chemistry attached to the drug part of the bow-tie structure. All other linkers presented can be applied as well.

[00216] The drug load can be increased nine-fold by attaching a dendric molecule to the focal point of the dendritic molecular transporter (bow-tie). A well-defined  
macromolecule is designed, that is "clicked" together in a Huisgen type reaction. The  
30 deprotection of the basic/acidic protecting groups allows the modification to a delivery

system with a short ethyl linker or hexyl linker before guanidylation to maintain uptake into specific subcellular locations.

[00217] Here, the drug load can be increased to a theoretical amount of 100-300 positions to conjugate small molecule drugs, peptides, oligonucleotides and more. The functionalization of the particle with a varied amount of amines allows together with the attachment of transporter allows the development of a gene delivery system. A “drug” can also be conjugated though a disulfide bond in a covalent conjugation approach. For example, proteins can be delivered. (See Figures 47, 48, 50, and 51).

#### F. ORGANIC QUANTUM DOTS VIA INTRAMOLECULAR CHAIN COLLAPSE

[00218] The synthesis and application of polymeric nanoparticles has attracted significant attention due to, in part, the realization that functionalized nanoparticles can be considered as building blocks for a variety of nanotechnological applications, ranging from vectors for drug and DNA delivery systems to templating agents for nanoporous microelectronic materials. Typical strategies for preparing nanoparticles can be broadly classified into two main approaches, top-down approaches – where emulsion polymerization techniques result in particles from 20 to 50 nm – and bottom-up approaches – which either rely on the synthesis of discrete spherical macromolecules such as dendrimers (1-10 nm) or the self-assembly of linear block copolymers into polymeric micelles followed by chemical cross-linking to give nanoparticles with typical dimensions ranging from 20 to 200 nm. As a consequence, the ability to routinely prepare nanoparticles in the 5-20 nm size range is limited.

[00219] To address this issue, strategies involving the collapse and intramolecular coupling of single-polymer chains to give discrete nanoparticles have been developed. However, typical intramolecular chain collapse strategies can fail to provide resultant nanoparticles with satisfactory functionality. Moreover, these strategies can also fail to provide reactive monomers capable of undergoing the requisite intramolecular chain collapse at desirable temperatures and/or under desirable conditions. In contrast, the disclosed methods and compounds address these shortcomings.

## 1. COPOLYMERS FOR INTRAMOLECULAR CHAIN COLLAPSE

- [00220] “Organic quantum dots” can be formed from AB-block, ABA-block, and graft-type copolymers via intramolecular chain collapse (*see* Figure 17 and Figure 18). Such organic quantum dots can comprise a functional moiety, *e.g.* a color-tunable semiconducting block (*see* Figure 19, Figure 20, and Figure 21). Methods and compositions for preparing copolymers capable of undergoing intramolecular chain collapse are known and described in, for example, Harth *et al.*, “A Facile Approach to Architecturally Defined Nanoparticles via Intramolecular Chain Collapse,” *J. Am. Chem. Soc.*, **2002**, 124, 8653-8660, which is incorporated herein by reference in its entirety.
- 10 [00221] The field of living free radical polymerizations, where the high degree of control is a result of equilibrium between dormant and reactive propagating radicals, can be useful for developing strategies for nanoparticle formation. In such polymerizations, the reactive radical chain ends are present in extremely low concentrations. This general concept, that is, only the reactive species need to be at ultra-dilute concentrations, can be applied to the formation of nanoparticles by intramolecular coupling. In this case, the linear polymer, which contains numerous latent coupling groups along the backbone, can be added slowly to a heated solvent in which the coupling groups are either thermally or chemically activated. As a consequence, the traditional conditions of ultrahigh dilution typically need only be met for the reactive intermediates and not for the polymers themselves. Following this coupling event, the nanoparticles are typically unreactive to further coupling reaction, which allows their concentration to increase to relatively high levels, (0.1-1.0 M) without intermolecular cross-linking reactions leading to gelation or coupling of individual nanoparticles. The ability to work at 0.1-1.0 M concentrations can be compared to the typically impractical concentration levels (ca.  $10^{-6}$  M) required for traditional ultrahigh dilution techniques.
- 25 [00222] The nature of the cross-linking group can be important; it can be selectively activated and react rapidly, leading to efficient intramolecular bond formation. It can also be important that this reaction is irreversible and leads to a coupled structure that is subsequently unreactive under the reaction conditions. To fulfill these goals, attention was directed to the benzocyclobutene group (BCB), which has found wide use as a latent Diels-Alder reagent in organic synthesis and in the formulation of thermosetting materials. Upon heating, the benzocyclobutene group, Q1, undergoes ring opening to give a relatively reactive *o*-quinoid
- 30

structure, Q2, which primarily reacts via an irreversible dimerization reaction to form the dibenzocyclooctadiene derivative, Q3, as well as a mixture of unidentified oligomeric materials (Figure 22). The direct result of this chemistry is the selective formation of cross-links from the coupling of two or more benzocyclobutene units. Having identified the

5 benzocyclobutene functionality as the critical coupling group for the preparation of nanoparticles, the desired monomer, 4-vinylbenzocyclobutene, Q4, was prepared from 4-bromobenzocyclobutene, Q5, by initial Grignard formation followed by reaction with *N,N*-dimethylformamide to give the aldehyde, Q6. Wittig coupling of Q6 with methyltriphenylphosphonium bromide afforded the desired styrene derivative, Q4, in high

10 yield (Figure 23). The incorporation of the cyclobutene group into the monomer, Q4, did not decrease its stability when compared to styrene, and Q4 proved to be stable to a wide variety of reaction conditions. One key to the success of this intramolecular chain collapse strategy is the minimization or elimination of intermolecular cross-linking between BCB groups on different chains. Since the balance between intramolecular coupling and intermolecular

15 cross-linking can be influenced by the number and placement of BCB units along the polymeric backbone, accurate control of the starting linear polymers structure also can be important. To achieve this and to permit a wide variety of linear polymers to be conveniently prepared, the polymerization of the desired monomer, 4-vinylbenzocyclobutene, Q4, was examined under living free radical conditions. Copolymerization of Q4 with vinyl monomers

20 such as styrene, methyl methacrylate, or *n*-butyl acrylate in the presence of the R-hydrido alkoxyamine, Q7, proved to be a controlled procedure, leading to random incorporation of the reactive BCB units and low polydispersities for the resulting copolymers, Q8 (Figure 24). At molecular weights less than 120000 amu the polydispersities for these random copolymers were 1.08-1.16, which increased to 1.19-1.26 for molecular weights above 200 000 amu.

25 This increased polydispersity is due, at least on part, to the significantly reduced concentration of initiating groups at these high molecular weights and has been observed previously for both ATRP- and nitroxide-mediated procedures. The BCB-functionalized polystyrene derivatives, Q8, can be readily characterized by standard techniques and incorporation of the BCB units monitored by <sup>1</sup>H NMR which showed characteristic aliphatic

30 resonances for the cyclobutene ring at 3.10 ppm. Intramolecular collapse was then examined under traditional ultrahigh dilution techniques. For this, a solution of a 80:20 styrene/BCB random copolymer, Q8 (*M*<sub>w</sub> = 95 000; PDI = 1.11) in dibenzyl ether was heated at a variety of concentrations under N<sub>2</sub> for 30 min at 250 °C. As can be seen in Figure 25, at very low

concentrations of BCB groups, ca.  $5.0 \times 10^{-5}$  M (see inset, Figure 25), intermolecular cross-linking becomes apparent as evidenced by the increase in molecular weight of the product from its base value of 65000 for a discrete nanoparticle due to chain-chain coupling. This ability to readily identify chain-chain coupling has been previously observed for dendrimer chemistry where even minor amounts of intermolecular coupling can be easily detected by gel permeation chromatography (GPC). This ability is due to the molecular weight doubling on chain-chain coupling, and the combination of this feature with the low polydispersity of the initial chains results in a lower limit of ca. 1-2% of intermolecular cross-linking being readily detected as a higher molecular shoulder under standard GPC conditions. At higher concentrations of ca.  $9.0 \times 10^{-3}$  M crosslinking to a swollen gel occurs very rapidly due to the large number of BCB functional groups along the polymeric backbone. While these concentrations are comparable to results obtained with other traditional ultra-high dilution techniques, the results are in stark contrast to the continuous addition strategy. In this approach, a concentrated solution ([BCB] = 0.2 M) of the same starting linear polymer, Q8, is continuously added via a peristaltic pump to a high-boiling solvent, such as dibenzyl ether, heated at 250 °C to give a final BCB concentration of 0.05 M. After addition, the solvent is removed, and the nanoparticles, Q9, are isolated using normal precipitation techniques (Figure 26). No gelation or intermolecular crosslinking is observed under these conditions, and only after increasing the final concentration of BCB groups to 0.12 M were minor amounts of nanoparticle coupling observed. The ability to successfully conduct these chain-collapse reactions at final BCB concentrations of 0.01-0.1 M represents an increase of 3-4 orders of magnitude when compared to the traditional ultrahigh dilution strategy (Figure 25). This permits multigram samples to be prepared on a routine basis with standard laboratory equipment, a dramatic improvement compared to previous approaches.

[00223] One significant feature of the above concentration studies is the reduction in hydrodynamic volume of the random coil linear polymer on intramolecular collapse to give the final nanoparticle. In the above example, the original linear polymer has a molecular weight,  $M_w$  of 95000 amu; however, upon reaction the macromolecule decreases in size to give a nanoparticle with an apparent or polystyrene equivalent  $M_w$  of 65000 amu. Dynamic light scattering was also employed to follow this decrease in size, and a reduction in the hydrodynamic radius,  $R_h$  from 8.7 to 6.6 nm, was observed upon intramolecular collapse. Since essentially no byproducts are produced during this reaction and no molecular weight

lost, this decrease can only be due to a change in the architecture of the macromolecule from a random coil to a nanoparticle. Again, this is consistent with dendrimer chemistry where the compact, three-dimensional dendritic structure leads to an apparent molecular weight which is significantly smaller than the actual molecular weight. Further confirmation of the structural change was obtained from NMR studies; of particular note is the observed absence of unreacted BCB units in the final nanoparticles, Q9. As shown in Figure 27, comparison of the  $^1\text{H}$  NMR spectra for the starting linear polymer, Q8, and the nanoparticle, Q9, shows the prominent resonance for the aliphatic protons of the cyclobutene group at 3.10 ppm in the former, which completely disappear after collapse, and a broad resonance at 2.0-3.0 ppm is observed. This is consistent with ring opening of the benzocyclobutene group and coupling to give cyclooctane derivatives and higher aliphatic coupled oligomers. A direct consequence of this ring opening is that the BCB groups undergo reaction to give dimers and oligomers that do not undergo any further coupling chemistry. This fulfils one of the requirements discussed above for a successful intramolecular chain collapse reaction and permits the substantial build-up of product in the final reaction mixture. The lack of reactivity can also be demonstrated by repeated thermal cycling of the isolated nanoparticles, which results in no observable change in physical properties such as molecular weight, NMR spectra, and so forth. Formation of the nanoparticles also leads to an increase in the glass transition temperature of the nanoparticles when compared to the starting linear polymers. The BCB functionalized linear polystyrenes, Q8, show  $T_g$ 's similar to that observed for polystyrene, ca. 100-105 °C, while the glass transition temperature for the nanoparticles, Q9, increase by ca. 20 °C to 120-130 °C at 20% BCB incorporation with an associated broadening of the transition. All of the above data is consistent with the intramolecular collapse of a random coil linear polymer to give a single, higher density nanoparticle.

[00224] One unique feature of being able to tailor the nanoparticle via the starting linear polymer was then examined in detail using three different series of polystyrene derivatives, ca. 44000; 110000; and 230000 amu containing varying levels of BCB incorporation from 1.25 to 30% (Table 1). Under the continuous addition technique described above, conversion of the linear polymers to nanoparticles was a facile process at all molecular weights and percent BCB incorporations studied. At concentrations of up to 0.01-0.1 M, no indication of intermolecular crosslinking was observed, and in each case the GPC trace shifted to lower hydrodynamic volumes. As can be seen in Figure 28, for the same

molecular weight of the starting linear polymer, Q8, a systematic decrease in the hydrodynamic volume of the nanoparticles is observed on increasing the percent of benzocyclobutene groups, which is consistent with an increase in the level of intramolecular coupling and a more globular, three-dimensional structure. A further pertinent feature of Figure 28 (b-e) is the symmetrical nature/low PDI of the GPC traces for the nanoparticles and the associated lack of higher-molecular weight shoulders. This demonstrates that even at high BCB loadings, ca. 25 mol %, no detectable amount of intermolecular cross-linking is occurring.

**Table 1.** Comparison of the Polystyrene Equivalent Molecular Weights and PDI for the Starting Linear Polymers, Q8, and the Final Nanoparticles, Q9

| composition | linear |      | nanoparticle |      | %BCB  |
|-------------|--------|------|--------------|------|-------|
|             | Mw     | PDI  | Mw           | PDI  |       |
| Sty/BCB     | 44000  | 1.09 | 41700        | 1.09 | 2.50  |
| Sty/BCB     | 44500  | 1.08 | 38200        | 1.12 | 5.00  |
| Sty/BCB     | 45500  | 1.09 | 29100        | 1.11 | 10.00 |
| Sty/BCB     | 43500  | 1.10 | 20700        | 1.12 | 15.00 |
| Sty/BCB     | 44000  | 1.07 | 18500        | 1.12 | 20.00 |
| Sty/BCB     | 110500 | 1.15 | 103000       | 1.17 | 1.25  |
| Sty/BCB     | 113000 | 1.12 | 94500        | 1.19 | 2.50  |
| Sty/BCB     | 109000 | 1.14 | 79300        | 1.18 | 5.00  |
| Sty/BCB     | 108000 | 1.15 | 59800        | 1.18 | 7.50  |
| Sty/BCB     | 112000 | 1.10 | 56000        | 1.19 | 10.00 |
| Sty/BCB     | 110000 | 1.16 | 44200        | 1.16 | 15.00 |
| Sty/BCB     | 111000 | 1.11 | 42800        | 1.15 | 20.00 |
| Sty/BCB     | 112000 | 1.12 | 40500        | 1.09 | 25.00 |
| Sty/BCB     | 231000 | 1.23 | 189800       | 1.26 | 1.25  |
| Sty/BCB     | 235000 | 1.25 | 174000       | 1.22 | 2.50  |
| Sty/BCB     | 228000 | 1.22 | 109000       | 1.26 | 5.00  |
| Sty/BCB     | 230000 | 1.19 | 98000        | 1.18 | 7.50  |
| Sty/BCB     | 233000 | 1.26 | 91500        | 1.16 | 10.00 |
| Sty/BCB     | 231000 | 1.24 | 81000        | 1.17 | 12.50 |
| Sty/BCB     | 235000 | 1.23 | 80300        | 1.19 | 15.00 |
| Sty/BCB     | 230000 | 1.21 | 66000        | 1.17 | 20.00 |
| Sty/BCB     | 229000 | 1.24 | 62000        | 1.25 | 25.00 |
| Sty/BCB     | 234000 | 1.23 | 63500        | 1.16 | 30.00 |

[00225] Analysis of the trends within each series showed that the percent reduction in hydrodynamic volume increases with both increasing molar percentage of BCB and the molecular weight of the starting linear polymer. In each case, the actual molecular weight of the cross-linked macromolecules is significantly greater than the apparent molecular weight.

5 For example, a 70/30 styrene/BCB random copolymer with an initial molecular weight,  $M_w = 234000$  (PDI = 1.23) gives a nanoparticle with a polystyrene equivalent molecular weight,  $M_w = 63500$  (PDI = 1.16) which represents a reduction in hydrodynamic volume of 73%. The actual molecular weight of the final nanoparticle was also determined by light scattering and found to be 230000, which is within experimental error of that of the starting linear

10 polymer which demonstrates that the actual molecular weights of the starting linear polymer and the final nanoparticles are approximately the same. This collapse and associated change in hydrodynamic volume is therefore due to the formation of up to 310 intramolecular links per nanoparticle, assuming that each activated BCB group reacts with one other activated BCB group. Interestingly, all plots are of a similar shape and seem to reach a plateau of

15 between 65 and 75% reduction in apparent molecular weight (Figure 29). It should also be noted that in the control experiments, heating polystyrene with 0% BCB incorporation resulted in no detectable change in the chromatographic or spectral properties of the polymers.

[00226] Examination of the data in Table 1 also demonstrates the inherent versatility of

20 this approach in controlling the size of the final nanoparticle. Not only can the size and cross-link density of the nanoparticle be controlled by the level of BCB incorporation, but the molecular weight of the starting linear polymer also plays a key role in determining the hydrodynamic volume of the final nanoparticle. For example, a polystyrene derivative with a 10% incorporation of BCB and a molecular weight,  $M_w$ , of 112000 gives a nanoparticle with

25 a  $R_h$  of 6.2 nm. Increasing the molecular weight,  $M_w$ , of the starting linear polymer to 233000 while still retaining the 10% incorporation of BCB gives a larger nanoparticle with a  $R_h$  of 9.5 nm. In turn, a polystyrene derivative with an analogously higher molecular weight of 229000 but with a 25% incorporation of BCB gives a nanoparticle with a  $R_h$  of 6.4 nm, very similar to the first example with a lower molecular weight (117000) and level of BCB

30 incorporation (10%). A consequence of this is that the size and physical characteristics of the final nanoparticle can be directly dictated by the structure and functionality of the starting linear polymer. The versatile nature of this intramolecular chain collapse approach to

nanoparticles coupled with the ability to prepare a wide variety of linear polymers by living free radical techniques also opens up the possibility of preparing well-defined nanoparticles incorporating functional groups, nonstyrenic monomers, or different macromolecular architectures. As shown in Table 2, starting linear polymers based on methyl methacrylate (MMA) or *n*-butyl acrylate (*n*-BuA) can be employed as the backbone polymer with no change in the efficiency of the intramolecular collapse process. For example, copolymerization of an 85:15 mixture of *n*-butyl acrylate and the vinyl BCB derivative, Q4, in the presence of the alkoxyamine initiator, 7, proceeds smoothly to give the well-defined random copolymer, Q10, with a molecular weight, *M<sub>w</sub>* of 75000 and a polydispersity of 1.09. Addition of a concentrated solution of Q10 (0.1 M) to dibenzyl ether, heated at 250 °C gives a poly(*n*-butyl acrylate) nanoparticle, Q11, with an apparent molecular weight, *M<sub>w</sub>* of 33 500 and a polydispersity of 1.09. (Figure 30). The relative selectivity of the thermal procedure used to activate the BCB group also allows other functional groups such as chloromethyl substituents to be introduced into the linear polymer, thereby leading to functionalized nanoparticles.

**Table 2.** Comparison of Molecular Weight (Linear Standard Equivalent) and PDI for the Starting Functionalized Linear Polymers, Q10, and the Final Nanoparticles, Q11

| composition                 | linear               |      | nanoparticle         |      | %BCB  |
|-----------------------------|----------------------|------|----------------------|------|-------|
|                             | <i>M<sub>w</sub></i> | PDI  | <i>M<sub>w</sub></i> | PDI  |       |
| MMA/BCB                     | 52500                | 1.17 | 36500                | 1.14 | 10.00 |
| MMA/BCB                     | 54500                | 1.12 | 28000                | 1.11 | 15.00 |
| MMA/BCB                     | 56000                | 1.13 | 26900                | 1.13 | 20.00 |
| <i>n</i> -BuA/BCB           | 74500                | 1.10 | 58100                | 1.12 | 5.00  |
| <i>n</i> -BuA/BCB           | 77500                | 1.12 | 45700                | 1.14 | 10.00 |
| <i>n</i> -BuA/BCB           | 75000                | 1.09 | 33500                | 1.09 | 15.00 |
| <i>n</i> -BuA/BCB           | 73000                | 1.09 | 27800                | 1.10 | 20.00 |
| Sty/Cl-Sty/BCB <sup>a</sup> | 101000               | 1.18 | 73500                | 1.20 | 5.00  |
| Sty/Cl-Sty/BCB <sup>a</sup> | 92000                | 1.14 | 48500                | 1.13 | 10.00 |
| Sty/Cl-Sty/BCB <sup>a</sup> | 85000                | 1.14 | 34000                | 1.17 | 20.00 |
| PEG-Sty/BCB <sup>b</sup>    | 92000                | 1.13 | 70500                | 1.10 | 5.0   |
| PEG-Sty/BCB <sup>b</sup>    | 95000                | 1.11 | 52000                | 1.09 | 10.0  |
| PEG-Sty/BCB <sup>b</sup>    | 89500                | 1.12 | 36500                | 1.14 | 20.0  |

<sup>a</sup> 10 mol % incorporation of *p*-chloromethylstyrene

<sup>b</sup> PEG block, *M<sub>n</sub>* = 5000; PDI = 1.06.

[00227] It should, however, be also noted that this intramolecular collapse procedure is not limited to simple linear random copolymers. Additional structural features can be built

into the starting materials, which are then translated into the nanoparticle structure. For example, block copolymers can potentially be used in such an approach, and if the reactive BCB groups are contained in only one of the blocks, novel macromolecular architectures can be prepared in which a controlled number of linear chains, one or two for AB and ABA block copolymers respectively, are attached to the nanoparticle. To test this hypothesis,

5 functionalized poly(styrene)-*b*-poly(ethylene glycol) AB block copolymers were prepared by living free radical procedures. The alkoxyamine substituted poly(ethylene glycol) macroinitiator, Q12, was obtained by reaction of the sodium salt of monomethylpoly(ethylene glycol), Q13 ( $M_n = 5000$ , PDI = 1.06), with the chloromethyl substituted alkoxyamine, Q14.

10 The macroinitiator, Q12, was then used to initiate the polymerization of a mixture of styrene and Q4 at 120 °C to give the desired AB block copolymer, Q15, which contains the cross-linking BCB units in the second block only (Figure 31). Reaction of Q15 under continuous addition conditions then results in the selective intramolecular collapse of the second block to give a novel hybrid linear-nanoparticle architecture, Q16, in which a single water soluble

15 PEG linear chain is attached to a three-dimensional cross-linked polystyrene nanoparticle, similar in structure to that of hybrid dendritic-linear block copolymers. As can be seen in Figure 32, the effect of intramolecular collapse is clearly evident in the shift of the GPC trace for the starting poly(ethylene glycol)-*b*-poly(styrene-*co*-benzocyclobutene), Q15, ( $M_w = 95000$ , PDI = 1.11) to that of the final hybrid nanoparticle-linear block copolymer, Q16, ( $M_w = 52000$ , PDI = 1.09) and demonstrates the controlled nature of this procedure. The solubility of these hybrid block copolymers were similar to that for the parent polystyrene nanoparticles, which can be due to the relatively small size of the PEG block.

## 2. APPROACHES IN THE DEVELOPMENT OF 3-D NANOSCOPIC, MULTIMODAL VECTORS

25 [00228] Nanoscopic particle design strategies (*see, e.g.*, Huang, H.; Remsen, E.E.; Kowalewski, T.; Wooley, K.L. *J. Am. Chem. Soc.* **1999**, *121*, 3805.) to develop multimodal architectures are still limited, and the understanding of key problems in biology and medicine will be one of the driving forces to implement materials to execute biological functions. *See* Lui, M.; Fréchet, J.M.J. *Pharm. Sci. Technol. Today.* **1999**, *2*, 393. The same care used for

30 these designs can be applied to polymeric nanostructures with applications in imaging and device technologies.

[00229] First, highly versatile, biocompatible, building blocks from vinyl polymers with pendant amino acids as linear precursors were prepared. The approach mimics and facilitates pathways mediated in nature and allows the construction and investigation of collapsed supramolecular objects as well as nanoparticles with distinct and permanent shapes.

5 These multifunctional nanoparticles are poised to become powerful tools for the targeted drug delivery with ScFv antibodies directed towards radiation-induced antigens within the microvasculature. See Hallahan, D.; Geng, L.; Qu, S.; Scafione, C.; Giorgio, T.; Donnelly, E.; Gao, X.; Claton, J. *Cancer Cell*. **2003**, 3, 63. Lu, S.X.; Cebe, P.; Capel, M. *Macromolecules* **1997**, 30(20), 6243. Multiple functionalities within the polymer backbone allow the

10 attachment of anticancer drugs and the complexation of imaging reagents which engage the catechol units, including MRI reagents and radioactive compounds. As mentioned above, one result of this work is the development of synthetic platforms which hold the potential to be multifaceted in their application. The chemistries, which were applied, provide the opportunities to make pioneering steps towards "organic quantum dots" in the nanoscopic

15 range of 5-15 nm and 60-100 nm. It is also contemplated that the incorporation of lanthanides can make these particles attractive objects to study in terms of their magnetic and fluorescent properties.

[00230] The synthesis of the multifunctional, biocompatible 3-D nanoparticles began with the preparation of random copolymers with pendant aminoacids. See Hatanaka, K.Y.;

20 Miyahara, S.; Sato, T.; Ono, F.; Uryu, T.; Kuzuhara, H. *J. Med. Chem.*, **1987**, 30, 810. Living free radical techniques were applied, such as nitroxide-mediated polymerization (NMP; see Smolders, W.; Monteiro, M.J. *Macromolecules*, **2004**, 12, 34. Hawker, C.J.; Bosman, A.; Harth, E. *Chem. Rev.* **2001**, 101, 12.) as well as Reversible Addition Chain Transfer Fragmentation (RAFT). N-Acyl amino acids gave polymers with narrow molecular weight

25 distributions with polydispersities in the range of 1.15-1.28. The most successful RAFT polymerizations were observed in the presence of 2-cyanopentanoic acid dithiobenzoate as a dithioester chain transfer reagent. See Croce, T.; Funk, M.; Adkins, C.; Harth, E. *Poly.Prep*, ACS Fall 2005. In experiments with NMP techniques, similar control was observed, but the polymerization appeared to be more sensitive towards the selected monomer conformation.

30 The formation of the 3-D structures can be realized with the new vinyl benzosulfone crosslinking units, which result in non-reversible C-C bonds and are powerful alternatives to the well-known benzocyclobutene precursor. See Harth, E.; van Horn, B.; Lee, V.Y.;

Germack, D.S.; Gonzales, C.P.; Miller, R.D.; Hawker, C.J. J. Am. Chem. Soc. **2002**, *123*, 8653. The nanoparticles in the range of 10-14 nm can be functionalized with spacer units to provide the optimum specific binding to expressed neo-antigens and show functionalities for the attachment of fluorophores for the first round of studies. The novel crosslinker was  
5 incorporated as well into vinyl polymer backbones growing from a fluorine-based (*see* McGehee, M.; Heeger, A. Adv. Materials, 200, 1655.) macroinitiator via NMP polymerization. The polystyrene blocks contained 10% crosslinking units and the resulting polymer could be prepared in the targeted MW of 20K (2:1:2).

### 3. O-CHINODIMETHANE CROSSLINKING PRECURSORS

10 **[00231]** Benzosulfone derivatives can serve as precursors for o-chinodimethane intermediates to form nanoparticles via an intra-molecular chain collapse process. In a five-step synthesis the sulfone functionality was introduced over the oxidation of sulfides formed by a nucleophilic substitution reaction of 3,4-dimethyl benzobromine. A Heck Reaction can serve as an excellent method to transform the arylbromine derivative into the final  
15 vinylbenzosulfone in one step. The compatibility of the novel crosslinker towards living free radical polymer procedures can be confirmed, and the formation of monodisperse nanoparticles demonstrated the feasibility of the new crosslinker.

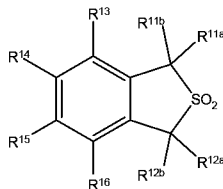
**[00232]** The number and diversity of techniques to create well defined polymeric architectures has led the foundation to built more refined structures with multifaceted, cross  
20 disciplinary applications. The fascination to prepare 3-D nanostructures stems from the desire to benefit from the shape and thereby the function in a biological environment or artificial system. In order to control a range of different size dimensions in the nanoscale a number of techniques have been recently successfully investigated and applied. Recently, the synthesis of nanoparticles in the size dimension of 5-10 nm was reported implementing an  
25 intramolecular chain collapse process of one linear polymer. The folded linear polymer is locked into the 3-D architecture by covalent crosslinking units incorporated into the polymer. The copolymerized crosslinking units are thermally activated and form a highly reactive o-chinodimethane unit from benzocyclobutene derivatives. However, the copolymerized crosslinking monomer, vinyl benzocyclobutene is not easily accessible and requires a  
30 multistep synthesis with over all low yields. In response to the demand to find alternative crosslinking units with the same features but more convenient synthesis, benzothiophen-

dioxide derivatives as potential *o*-chinodimethane precursors were identified. Benzothiophene derivatives are well known structures to form *o*-chinodimethanes upon heating at 250 °C under the loss of SO<sub>2</sub> and can be prepared in a number of different ways described in the literature. In contrast to known benzothiophene derivatives, a novel bromo substituted benzothiophen-dioxide derivative was converted into the desired vinylbenzodevivate crosslinking monomer. In order to overcome difficult synthetic steps, a one step procedure was developed in which a modified Heck coupling reaction provides the vinyl derivative in high yields. Additionally, this novel reaction can be applied to the preparation of other monomer and is poised to open up novel opportunities to combine the crucial vinyl functionality to perform living free radical polymerization procedures with demanding moieties for the further utilization of macromolecules. The novel benzosulfone crosslinking unit was proved to be compatible with standard living free radical polymerization conditions. The intramolecular chain collapse process of polystyrene polymers with a variety of crosslinking units gave well defined monodisperse nanoparticles in the 5-10 nm dimension.

#### 4. COMPOUNDS

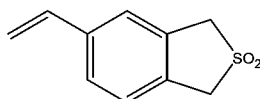
##### a. REACTIVE MOIETIES

[00233] In one aspect, the invention relates to a compound comprising the structure:



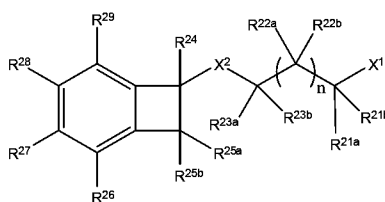
wherein R<sup>11a</sup>, R<sup>11b</sup>, R<sup>12a</sup>, and R<sup>12b</sup> are, independently, hydrogen, alkyl, alkoxy, acyl, or carbonyl; wherein R<sup>13</sup>, R<sup>14</sup>, R<sup>15</sup>, and R<sup>16</sup> are, independently, hydrogen, alkyl, alkenyl, alkynyl, alkoxy, acyl, or carbonyl, with the proviso that at least one of R<sup>13</sup>, R<sup>14</sup>, R<sup>15</sup>, or R<sup>16</sup> is a polymerizable group; and wherein the compound eliminates SO<sub>2</sub> to form a reactive intermediate at a reaction temperature. In a further aspect, at least one of R<sup>13</sup>, R<sup>14</sup>, R<sup>15</sup>, and R<sup>16</sup> is alkenyl or alkynyl. In a yet further aspect, R<sup>14</sup> is vinyl. In a still further aspect, R<sup>11a</sup>, R<sup>11b</sup>, R<sup>12a</sup>, and R<sup>12b</sup> are hydrogen. In a still further aspect, R<sup>14</sup> is vinyl and R<sup>13</sup>, R<sup>15</sup>, and R<sup>16</sup> are hydrogen.

[00234] In one aspect, the compound comprises the structure:



[00235] In a further aspect, the compound undergoes polymerization at a polymerization temperature. In one aspect, the polymerization temperature is less than the reaction temperature. In a further aspect, the reaction temperature is less than about 250 °C. In a still further aspect, the reaction temperature is less than about 225 °C. In a yet further aspect, the reaction temperature is less than about 200 °C. In a further aspect, the reaction temperature is less than about 175 °C. In a still further aspect, the reaction temperature is from about 175 °C to about 250 °C.

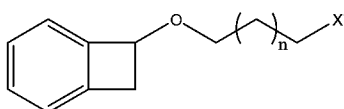
10 [00236] In one aspect, the invention relates to a compound comprising the structure:



wherein  $X^1$  is hydroxyl, primary amino, secondary amino, tertiary amino, thiol, or carboxyl; wherein  $n$  is zero or a positive integer; wherein  $R^{21a}$ ,  $R^{21b}$ ,  $R^{22a}$ , and  $R^{22b}$ ,  $R^{23a}$ , and  $R^{23b}$  are, independently, hydrogen, alkyl, alkoxy, acyl, or carbonyl; wherein  $X^2$  is oxygen, secondary nitrogen, tertiary nitrogen, or sulfur; wherein  $R^{24}$ ,  $R^{25a}$ ,  $R^{25b}$ ,  $R^{26}$ ,  $R^{27}$ ,  $R^{28}$ , and  $R^{29}$  are, independently, hydrogen, alkyl, alkenyl, alkynyl, alkoxy, acyl, or carbonyl; and wherein the cyclobutane ring undergoes ring opening to form a reactive intermediate at a reaction temperature.

[00237] In a further aspect,  $n$  is an integer from 0 to 4. In a yet further aspect,  $n$  is 0, 1, or 2. In a still further aspect,  $X^2$  is oxygen. In a further aspect,  $R^{24}$ ,  $R^{25a}$ , and  $R^{25b}$  are hydrogen. In a yet further aspect,  $R^{26}$ ,  $R^{27}$ ,  $R^{28}$ , and  $R^{29}$  are hydrogen.

[00238] In a further aspect, the compound comprises the structure:



[00239] In a further aspect,  $n$  is 0, 1, or 2. In a yet further aspect, the reaction temperature is less than about 150 °C. In a still further aspect, the reaction temperature is less than about 125 °C. In a yet further aspect, the reaction temperature is less than about 115 °C.

5 In a still further aspect, the reaction temperature is less than about 110 °C. In a further aspect, the reaction temperature is from about 100 °C to about 150 °C.

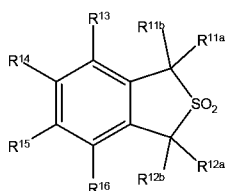
[00240] In a further aspect, the invention relates to a polymer or an oligomer comprising at least one residue of at least one disclosed compound.

#### b. COMPOUNDS CAPABLE OF FORMING AN *O*-QUINOID MOIETY

10 [00241] In a further aspect, the invention relates to a compound capable of forming a reactive intermediate comprising an *o*-quinoid moiety at a reaction temperature of less than about 175 °C. In a further aspect, the reaction temperature is less than about 150 °C. In a yet further aspect, the reaction temperature is less than about 125 °C. In a still further aspect, the reaction temperature is less than about 115 °C. In one aspect, the reaction temperature is less

15 than about 110 °C. In a further aspect, the reaction temperature is from about 100 °C to about 150 °C.

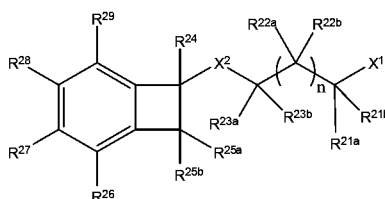
[00242] In a further aspect, the compound comprises the structure:



wherein  $R^{11a}$ ,  $R^{11b}$ ,  $R^{12a}$ , and  $R^{12b}$  are, independently, hydrogen, alkyl, alkoxy, acyl, or carbonyl; wherein  $R^{13}$ ,  $R^{14}$ ,  $R^{15}$ , and  $R^{16}$  are, independently, hydrogen, alkyl, alkenyl, alkynyl, alkoxy, acyl, or carbonyl, with the proviso that at least one of  $R^{13}$ ,  $R^{14}$ ,  $R^{15}$ , or  $R^{16}$  is a polymerizable group; and wherein the compound eliminates  $SO_2$  to form a reactive intermediate at a reaction temperature.

20

[00243] In a further aspect, the compound comprises the structure:



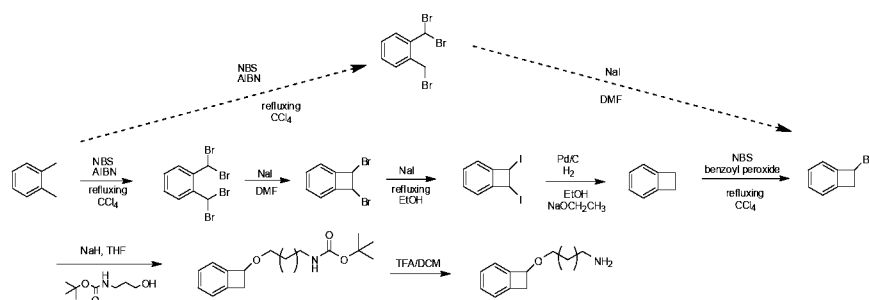
wherein  $X^1$  is hydroxyl, primary amino, secondary amino, tertiary amino, thiol, or carboxyl;  
 wherein  $n$  is zero or a positive integer; wherein  $R^{21a}$ ,  $R^{21b}$ ,  $R^{22a}$ , and  $R^{22b}$ ,  $R^{23a}$ , and  $R^{23b}$  are,  
 5 independently, hydrogen, alkyl, alkoxy, acyl, or carbonyl; wherein  $X^2$  is oxygen, secondary  
 nitrogen, tertiary nitrogen, or sulfur; wherein  $R^{24}$ ,  $R^{25a}$ ,  $R^{25b}$ ,  $R^{26}$ ,  $R^{27}$ ,  $R^{28}$ , and  $R^{29}$  are,  
 independently, hydrogen, alkyl, alkenyl, alkynyl, alkoxy, acyl, or carbonyl; and wherein the  
 cyclobutane ring undergoes ring opening to form a reactive intermediate at a reaction  
 temperature.

10 [00244] In a further aspect, the invention relates to a polymer or an oligomer  
 comprising at least one residue of at least one disclosed compound.

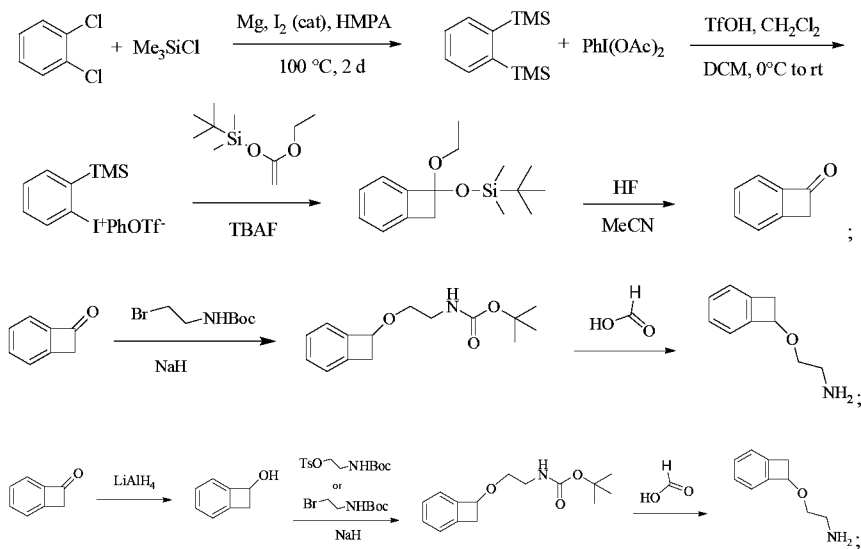
#### c. LOW TEMPERATURE CROSSLINKER

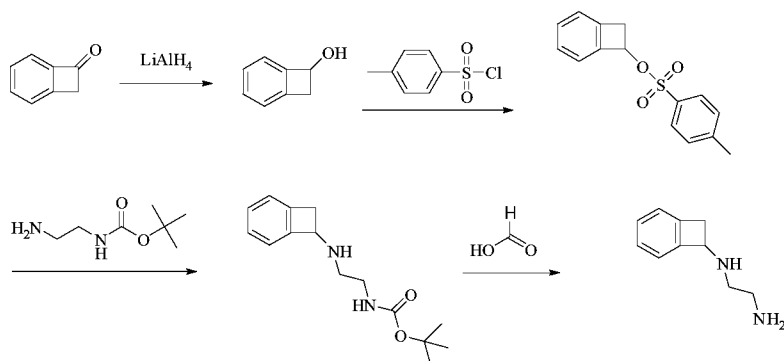
[00245] In one aspect, the invention relates to a low temperature crosslinker. That is,  
 the invention relates to a reactive moiety capable of undergoing reaction to form a reactive  
 15 intermediate and, thus, undergo intramolecular chain collapse at a relatively low temperature  
 and/or in response to a stimulus. In one aspect, the low temperature crosslinkers comprise  
 reactive moieties suitable for incorporation in polymers and/or copolymers as pendant groups.

[00246] Exemplary low temperature crosslinkers can be prepared, for example, with  
 the following reaction sequence.

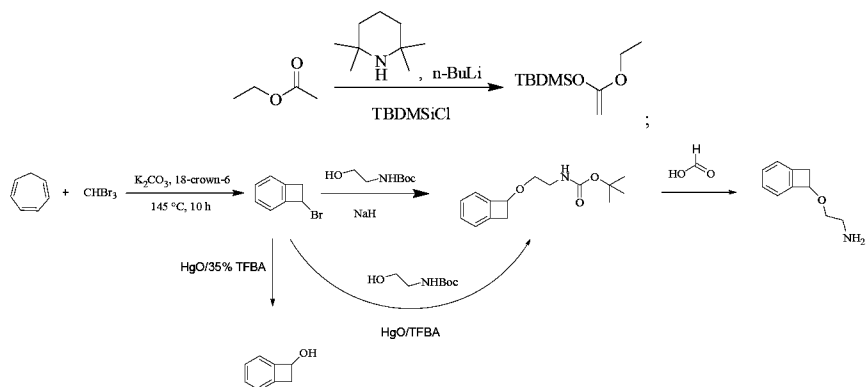


[00247] In a further aspect, exemplary low temperature crosslinkers can be prepared, for example, with one or more of the following reaction sequences:



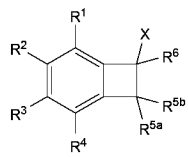


[00248] In a yet further aspect, exemplary low temperature crosslinkers can be prepared, for example, with the following reaction sequences.



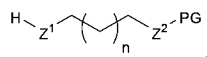
5

[00249] Thus, in one aspect, the invention relates to a method for preparing a nucleophile-functionalized benzocyclobutane comprising the steps of: providing a benzocyclobutane having a structure represented by a formula:

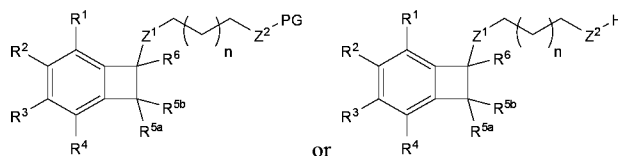


10

wherein  $R^1$ ,  $R^2$ ,  $R^3$ ,  $R^4$ ,  $R^{5a}$ ,  $R^{5b}$  and  $R^6$  are, independently, hydrogen, alkyl, alkene, alkyne, alkoxy, hydroxyl, or carboxyl and wherein X is a leaving group; reacting the benzocyclobutene with a nucleophile having a structure represented by a formula:



- 5 wherein  $Z^1$  and  $Z^2$  are, independently, hydroxyl, amino, or thiol, wherein n is an integer from 0 to 10, and wherein PG is a hydrogen or a protecting group; and optionally, removing the protecting group, if present, thereby preparing a nucleophile-functionalized benzocyclobutane having a structure represented by a formula:



- 10 In a further aspect,  $R^6$  is hydrogen. In a yet further aspect,  $R^1$ ,  $R^2$ ,  $R^3$ , and  $R^4$  are hydrogen.

**[00250]** In various aspects, n can be zero or a positive integer. For example, n can be from 0 to 10, from 0 to 4, or from 2 to 4. It is understood that, in one aspect, n is the average for a distribution of alkyl moieties and, thus, can be a non-integer.

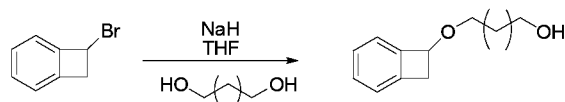
- [00251]** In a further aspect, the method further comprises the step of incorporating the nucleophile-functionalized benzocyclobutane into a polymer or oligomer. In a still further aspect, the incorporating step is grafting the nucleophile-functionalized benzocyclobutene onto a polymer or oligomer after polymerization.

**[00252]** In one aspect, the leaving group, X, is a halogen. In a further aspect, X is a sulfonyl-based leaving group, for example, tosylate, brosylate, or mesylate.

- 20 **[00253]** In one aspect, both  $Z^1$  and  $Z^2$  are oxygen. In a further aspect, both  $Z^1$  and  $Z^2$  are amino. In a yet further aspect, one of  $Z^1$  and  $Z^2$  is oxygen and one of  $Z^1$  and  $Z^2$  is amino, for example,  $Z^1=O$  and  $Z^2=NH$ . In a yet further aspect,  $Z^2$  is amino and PG is a protecting group selected from benzyl, *tert*-butoxycarbonyl, benzyloxycarbonyl, allyloxycarbonyl, and 2,2,2-trichloroethyloxycarbonyl. In a still further aspect,  $Z^2$  is oxygen and PG is a protecting

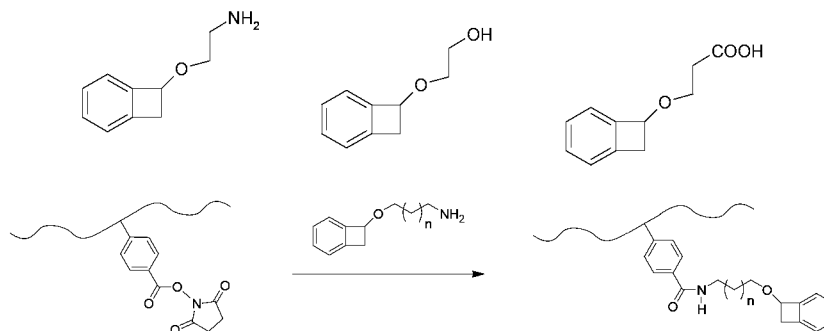
group selected from trimethylsilyl, *tert*-butyldimethylsilyl, triisopropylsilyl, acetyl, tetrahydropyran, methoxymethyl ether,  $\beta$ -methoxyethoxymethyl ether, *p*-methoxybenzyl ether, methylthiomethyl ether, and pivaloyl.

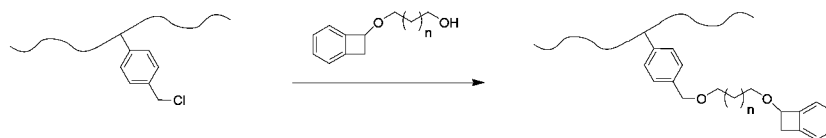
[00254] Note that the bromobenzocyclobutane intermediate can be conveniently prepared in two steps from *o*-xylene. The intermediate can then be readied for incorporation by conversion to a primary amine derivative. Alternatively, the intermediate can be readied for incorporation by conversion to a primary alcohol derivative.



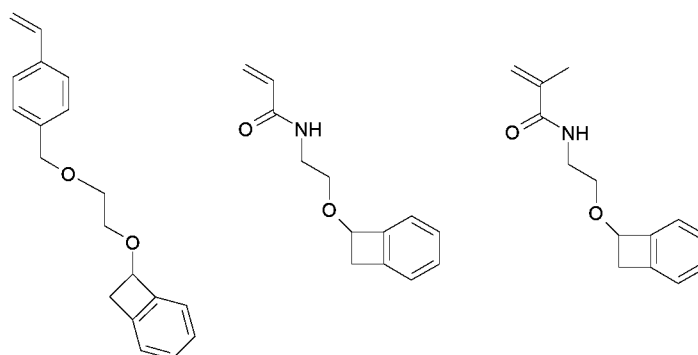
[00255] In one aspect, to avoid high temperatures during the intra-molecular crosslinking process, a crosslinking unit which can form an *o*-chinodimethane derivative at around 100-120 °C was developed. In contrast to other crosslinking units, this unit can be conjugated to a linear polymer after polymerization. This allows the polymerization at 124 °C, which is necessary for polymerization through NMP, without activating the low temperature crosslinker. Alternatively, by conjugating the unit to acrylmonomers (*e.g.*, via esterification, amidation, or etherification) and copolymerizing, a vinyl monomer can be prepared, suitable for use in with living free radical methods allowing, for example, polymerization temperatures of 80 °C or less.

[00256] Exemplary low-temperature crosslinking units for attachment onto polymers as pendant groups are illustrated below:





[00257] Exemplary low temperature crosslinking units synthesized as monomers for  
 5 copolymerization are illustrated below:

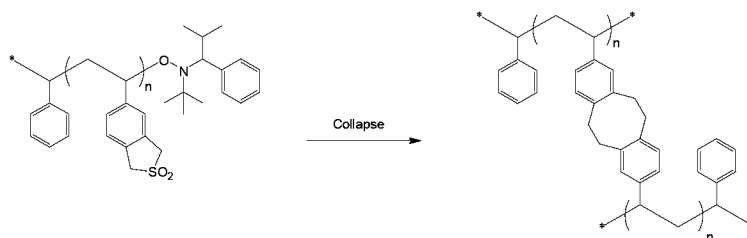


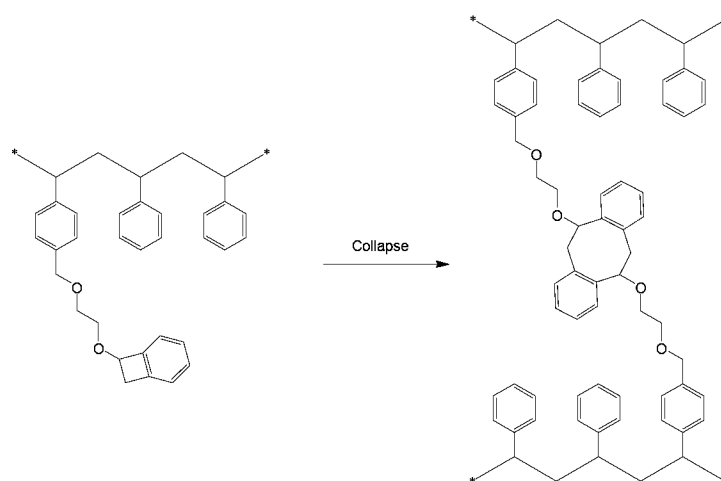
[00258] It is also contemplated that allyl crosslinking moieties can comprise known  
 polymeric chains, for example polyalkyl or polyoxalkyl.

10

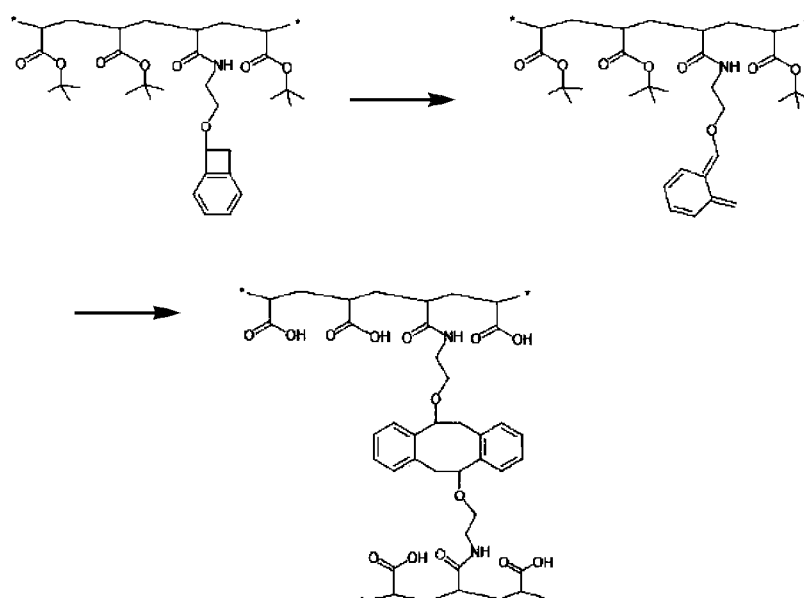
#### d. INTRAMOLECULAR CHAIN COLLAPSE

[00259] The disclosed crosslinking units can be imparted with a stimulus (*e.g.*,  
 increased temperature) to effect intramolecular chain collapse as shown below:





[00260] Other examples include the synthesis shown below:



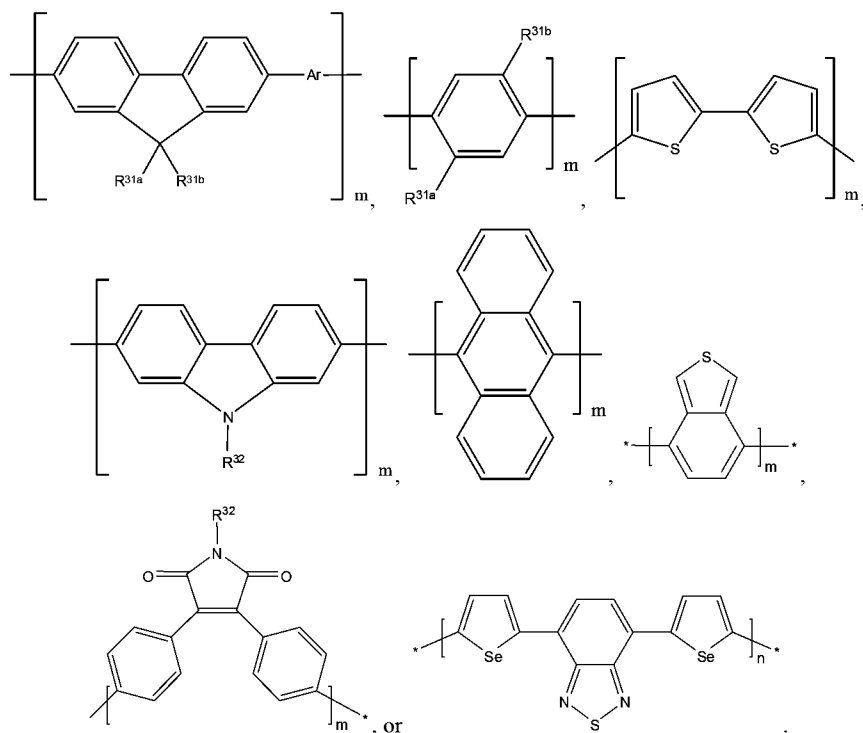
## 5. BLOCK COPOLYMERS

[00261] In one aspect, the invention relates to a block copolymer comprising the structure A-B, wherein A is an oligomeric block comprising at least two reactive residues, and wherein B is a block comprising at least one functional moiety. In one aspect, B  
5 comprises substantially no reactive residues. That is, reactive residues are essentially absent from B. In a further aspect, B further comprises at least one reactive residue.

[00262] In one aspect, the disclosed block copolymers relate to the disclosed compounds.

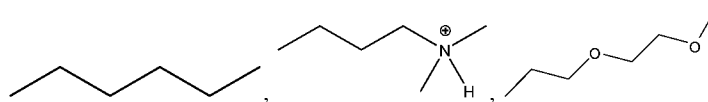
### a. FUNCTIONAL MOIETIES

10 [00263] In a further aspect, the functional moiety comprises a semiconducting moiety, an imaging moiety, or a drug-delivery moiety. In a yet further aspect, the functional moiety comprises a semiconducting moiety. In one aspect, the semiconducting moiety comprises one or more residues selected from:



wherein  $R^{31a}$  and  $R^{31b}$  are, independently, hydrogen or alkyl; wherein  $R^{32}$  is hydrogen, alkyl,  
 5 or polyalkylene glycol; and wherein  $m$  is zero or a positive integer.

**[00264]** In a further aspect,  $R^{31a}$  and  $R^{31b}$  are independently selected from



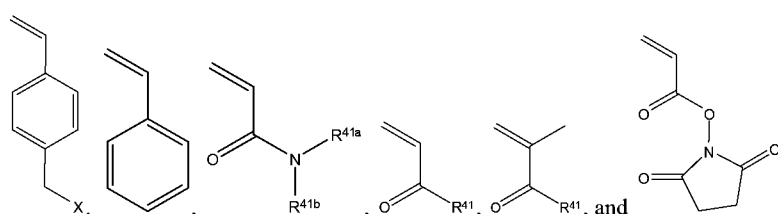
**[00265]** In one aspect,  $m$  is an integer from 1 to 20.

**[00266]** In one aspect, the functional moiety comprises an imaging moiety. In a further  
 10 aspect, the imaging moiety comprises at least one catechol unit for the complexation of  
 radioactive metals such as  $^{64}\text{Cu}$ ,  $^{111}\text{In}$ , lanthanides, rare earth metals, iron oxide, or  
 complexing units such as DOTA, DPTA, chromophores, nonlinear optical (NLO) materials,  
 fluorophores, and/or other electroactive materials.

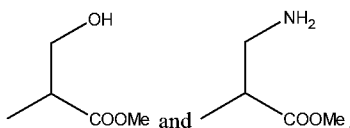
**[00267]** In one aspect, the functional moiety comprises a drug-delivery moiety. In a further aspect, the drug-delivery moiety bears at least one of a biologically active agent and/or a pharmaceutically active agent. In a yet further aspect, the drug-delivery moiety comprises cis-platinum, cancer drugs such as taxol, doxorubicin, abraxane, anastrozol paclitaxel, exemestane, cyclophosphamide, epirubicin, toremifene, letrozole, trastuzumab, megestrol, nolvadex, docetaxel, capecitabine, goserelin acetate, zoledronic acid, raloxifene, faslodex, methotrexate, multiple sclerosis agents, hormones, antioxidants, antimicrobials, antibacterial agents, antidepressants, sedatives, antihypertensive drugs, antibodies, a carbohydrate-based drug, Fab fragments of the anti-melanoma antibody NRML-05, pan-carcinoma antibody NRLU-10, anti-CEA immunotoxin, liposome drugs, fusogenic, dendritic cell vaccines, viral capsids, and/or bacteria.. These drugs can be directly connected to the backbone of a copolymer or conjugated with a linker. In a further aspect, the drug-delivery moiety can be a hypothermally-responsive metal, for example, gold. In a still further aspect, the drug-delivery moiety comprises at least one of a peptide and a glycoside, such as  $\alpha$ -mannose or other sugar molecules). It is also contemplated that further drug functionalities can be incorporated if capable of bonding to the disclosed compounds.

**[00268]** In one aspect, m is from about 5 to about 15. In a further aspect, A is from about 5 to about 15 residues in length. In a still further aspect, B is from about 5 to about 15 residues in length. In a yet further aspect, B is an oligomeric block.

20 **[00269]** In a further aspect, A further comprises at least one residue selected from styrene, acrylate, acrylamide, and methyl methacrylate and/or derivatives thereof, including:

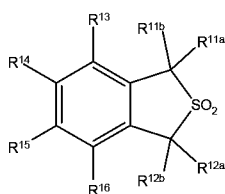


wherein X is halogen; wherein R<sup>41</sup>, R<sup>41a</sup>, and R<sup>41b</sup> are, independently, hydrogen, alkyl, or alkoxy. It is contemplated that, in certain aspects, other non-halogen leaving groups (e.g., 25 tosylate) can be substituted for halogen. In a still further aspect, R<sup>41</sup>, R<sup>41a</sup>, and R<sup>41b</sup> are independently selected from:



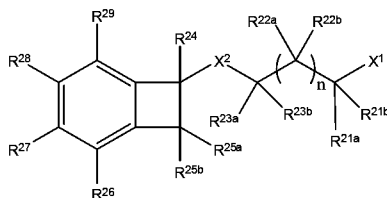
# b. REACTIVE RESIDUES

[00270] In one aspect, the at least two reactive residues comprise the structure:



5 wherein  $R^{11a}$ ,  $R^{11b}$ ,  $R^{12a}$ , and  $R^{12b}$  are, independently, hydrogen, alkyl, alkoxy, acyl, or carbonyl; wherein  $R^{13}$ ,  $R^{14}$ ,  $R^{15}$ , and  $R^{16}$  are, independently, hydrogen, alkyl, alkenyl, alkynyl, alkoxy, acyl, or carbonyl, with the proviso that at least one of  $R^{13}$ ,  $R^{14}$ ,  $R^{15}$ , or  $R^{16}$  is a polymerizable group; and wherein the compound eliminates  $SO_2$  to form a reactive intermediate at a reaction temperature.

10 [00271] In a further aspect, the at least two reactive residues comprise the structure:



15 wherein  $X^1$  is hydroxyl, primary amino, secondary amino, tertiary amino, thiol, or carboxyl; wherein  $n$  is zero or a positive integer; wherein  $R^{21a}$ ,  $R^{21b}$ ,  $R^{22a}$ , and  $R^{22b}$ ,  $R^{23a}$ , and  $R^{23b}$  are, independently, hydrogen, alkyl, alkoxy, acyl, or carbonyl; wherein  $X^2$  is oxygen, secondary nitrogen, tertiary nitrogen, or sulfur; wherein  $R^{24}$ ,  $R^{25a}$ ,  $R^{25b}$ ,  $R^{26}$ ,  $R^{27}$ ,  $R^{28}$ , and  $R^{29}$  are, independently, hydrogen, alkyl, alkenyl, alkynyl, alkoxy, acyl, or carbonyl; and wherein the cyclobutane ring undergoes ring opening to form a reactive intermediate at a reaction temperature.

[00272] In a further aspect, the at least two reactive residues are capable of forming a reactive intermediate comprising an *o*-quinoid moiety at a reaction temperature of less than about 175 °C. In a yet further aspect, the reaction temperature is less than about 150 °C. In a yet further aspect, the reaction temperature is less than about 125 °C. In a yet further aspect, the reaction temperature is less than about 115 °C. In a yet further aspect, the reaction temperature is less than about 110 °C. In a still further aspect, the reaction temperature is from about 100 °C to about 150 °C.

#### c. STRUCTURE

[00273] In one aspect, the block copolymer comprises the structure A-B-A. In a further aspect, the block copolymer comprises the structure A-B-C, wherein C is an oligomeric block comprising at least two reactive residues. In a yet further aspect, the at least two reactive residues of block A are different from the at least two reactive residues of block C. In a still further aspect, the block copolymer comprises the structure P-A-B, wherein P comprises a peptide moiety attached to block A. In various further aspects, the peptide moiety comprises cell penetrating peptides such as oligoarginines or Tat peptide, peptide ligands to irradiated tumor cells, G protein, p16<sup>INK4a</sup>, Bak BH3 domain peptide, cGPK-I $\alpha$  inhibitory peptide, IKK $\beta$  C-terminal peptide, PKA inhibitory peptide, MEK 1 N-terminal peptide, a peptide nucleic acid (PNA) cardioprotective  $\epsilon$ PKC agonist peptide, VHL tumor suppressor peptide, HER-2, Pro-apoptotic Smac peptide, oligonucleotides, a plasmid DNA, an immunoglobulin, and antisense oligoDNA. It is contemplated that proteins known for certain functions (*e.g.*, peptides for targeting, immunoresponse, etc.) can be used in connection with the invention. In one aspect, the block copolymer comprises the structure P-A-B-A. In a further aspect, the block copolymer comprises the structure P-A-B-C, wherein C is an oligomeric block comprising at least two reactive residues.

[00274] In a still further aspect, the block copolymer comprises the structure E-A-B, wherein E comprises an enzyme moiety attached to block A. In various further aspects, the enzyme moiety comprises  $\beta$ -galactosidase, horseradish peroxidase, RNase, anti-apoptotic proteins Bcl-X(L)/PEA-15, catalase, green fluorescence protein, heat shock protein 70, human glutamate dehydrogenase, ovalbumin, neurotactant Bcl-xL, E2 protein, phosphorothioate antisense oligonucleotides, anti-tetanus F(ab')<sub>2</sub>, caspase-3, p14<sup>INK4a</sup>, p27<sup>kpl</sup>, luciferin, RhoA, APO-BEC-1, Cre recombinase, H-Ras, Filmin-1, p16, HPC-1/syntaxin,

Cdk2, E2f-1/p73/p53, influenza virus, antibodies, single chain antibodies, si-RNA, RNA derivatives, peptide 46, peptide 15, peptides that influence the immunresponse, mitochondrial DNA, bacteria, birdflu virus, and/or bacteria. In a further aspect, the block copolymer comprises the structure E-A-B-A. In a still further aspect, the block copolymer comprises the structure E-A-B-C, wherein C is an oligomeric block comprising at least two reactive residues.

## 6. METHODS OF NANOPARTICLE FORMATION

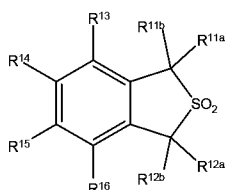
[00275] In one aspect, the invention relates to a method of nanoparticle formation comprising the steps of providing a reactor capable of imparting a stimulus and adding to the reactor a block copolymer comprising at least two reactive residues, thereby imparting the stimulus on at least a portion of the block copolymer; wherein the at least two reactive residues form reactive intermediates upon exposure to the stimulus, and wherein the reactive intermediates are capable of undergoing a bond-forming reaction.

[00276] In one aspect, the disclosed methods relate to the disclosed block copolymers.

[00277] In a further aspect, the adding step is performed at a first rate, wherein the reactive intermediates are formed at a second rate, wherein the reactive intermediates undergo the bond-forming reaction at a third rate, and wherein the third rate is greater than the first rate. In a yet further aspect, the third rate is greater than the second rate. In one aspect, the linear precursor concentration during the first rate is  $10^{-7}$  molar and is added to the solution with 12.6 ml/h.

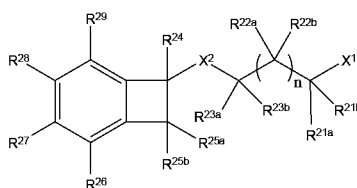
[00278] In one aspect, the stimulus is at least one of a thermal stimulus, a chemical stimulus, or an electromagnetic stimulus or a mixture thereof. In a further aspect, the stimulus is a thermal stimulus comprising increased heat, decreased heat, increased agitation, decreased agitation, increased kinetic energy, or decreased kinetic energy or a mixture thereof. In a yet further aspect, the stimulus is a chemical stimulus comprising an increase in pH, a decrease in pH, an addition of radical initiator, an initiation of radical scavenger, an increase in polarity, or a decrease in polarity or a mixture thereof. In a still further aspect, the stimulus is an electromagnetic stimulus comprising visible light, ultraviolet light, infrared light, microwaves, radio waves, or magnetic waves or a mixture thereof.

[00279] In one aspect, the at least two reactive residues comprise the structure:



wherein  $R^{11a}$ ,  $R^{11b}$ ,  $R^{12a}$ , and  $R^{12b}$  are, independently, hydrogen, alkyl, alkoxy, acyl, or carbonyl; wherein  $R^{13}$ ,  $R^{14}$ ,  $R^{15}$ , and  $R^{16}$  are, independently, hydrogen, alkyl, alkenyl, alkynyl, alkoxy, acyl, or carbonyl, with the proviso that at least one of  $R^{13}$ ,  $R^{14}$ ,  $R^{15}$ , or  $R^{16}$  is a polymerizable group; and wherein the compound eliminates  $SO_2$  to form a reactive intermediate at a reaction temperature.

[00280] In a further aspect, the at least two reactive residues comprise the structure:



wherein  $X^1$  is hydroxyl, primary amino, secondary amino, tertiary amino, thiol, or carboxyl; wherein  $n$  is zero or a positive integer; wherein  $R^{21a}$ ,  $R^{21b}$ ,  $R^{22a}$ , and  $R^{22b}$ ,  $R^{23a}$ , and  $R^{23b}$  are, independently, hydrogen, alkyl, alkoxy, acyl, or carbonyl; wherein  $X^2$  is oxygen, secondary nitrogen, tertiary nitrogen, or sulfur; wherein  $R^{24}$ ,  $R^{25a}$ ,  $R^{25b}$ ,  $R^{26}$ ,  $R^{27}$ ,  $R^{28}$ , and  $R^{29}$  are, independently, hydrogen, alkyl, alkenyl, alkynyl, alkoxy, acyl, or carbonyl; and wherein the cyclobutane ring undergoes ring opening to form a reactive intermediate at a reaction temperature.

[00281] In one aspect, the at least two reactive residues are capable of forming a reactive intermediate comprising an *o*-quinoid moiety at a reaction temperature of less than about 175 °C. In a further aspect, the reaction temperature is less than about 150 °C. In a further aspect, the reaction temperature is less than about 125 °C. In a further aspect, the reaction temperature is less than about 115 °C. In a further aspect, the reaction temperature is

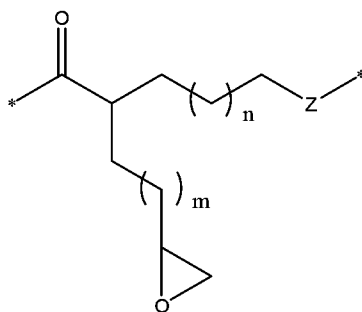
less than about 110 °C. In a further aspect, the reaction temperature is from about 100 °C to about 150 °C.

## G. POLYMERS

[00282] It is understood that the disclosed polymers can be used in connection with the disclosed nanoparticles and disclosed methods. Unless stated to the contrary, the disclosed structures can be used in connection with the disclosed methods, the disclosed polymers, and the disclosed nanoparticles.

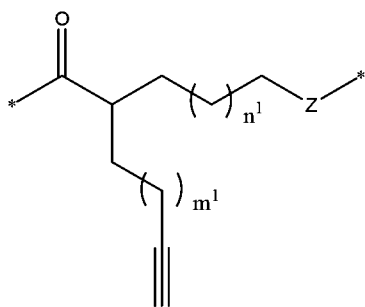
### 1. EPOXIDE-FUNCTIONALIZED POLYMERS

[00283] In one aspect, the invention relates to a polymer comprising at least one monomer residue having an optionally substituted structure represented by a formula:

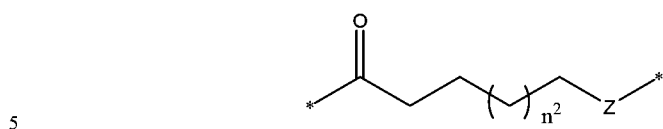


wherein Z is O or NR, wherein R is H or C1 to C6 alkyl; wherein m is an integer from 0 to 6; wherein n is an integer from 0 to 2; and wherein the monomer residue comprises less than about 10% by weight of the monomer residue of halogen selected from chlorine, bromine, and iodine. In further aspects, the monomer residue can comprise less than about 8%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, or less than about 1% of halogen selected from chlorine, bromine, and iodine, by weight of the monomer residue.

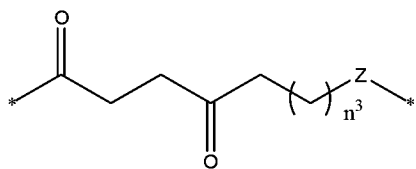
[00284] In a further aspect, an epoxide-functionalized polymer can further comprise at least one monomer residue selected from a propargyl-functionalized monomer residue having an optionally substituted structure represented by a formula:



wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, wherein  $m^1$  is an integer from 0 to 6, and wherein  $n^1$  is an integer from 0 to 2; a monomer residue having an optionally substituted structure represented by a formula:



wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, and wherein  $n^2$  is an integer from 0 to 2; and a keto-functionalized monomer residue having an optionally substituted structure represented by a formula:



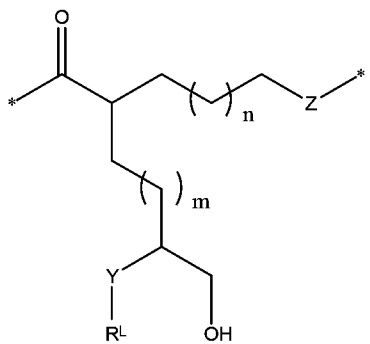
wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, and wherein  $n^3$  is an integer from 0 to 2.

**[00285]** In one aspect, Z is O. That is, the polymer residue can be a polyester residue. In a further aspect, the polymer is a polyester. In a further aspect, the polymer is a co-polyester.

**[00286]** In a further aspect, the Z is NR, wherein R is H or C1 to C6 alkyl. In one aspect, the polymer residue can be a polyamide residue. In a further aspect, the polymer is a

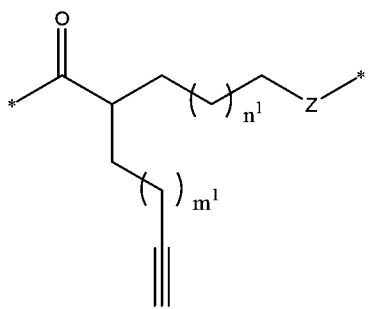
polyamide. In a further aspect, the polymer is a co- polyamide. The alkyl can be optionally further substituted. R can be C1 to C6, C2 to C6, C1 to C5, C2 to C5, C1 to C4, C2 to C4, C1, C2, C3, C4, C5, or C6 alkyl.

[00287] In one aspect, the polymer comprises at least one monomer residue having an optionally substituted structure represented by a formula:

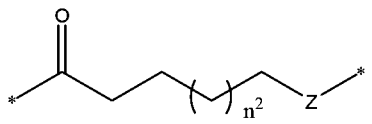


wherein Z is O or NR, wherein R is H or C1 to C6 alkyl; wherein Y is O, S, or NR, wherein R is H or C1 to C6 alkyl; wherein R<sup>L</sup> is selected from optionally substituted alkyl and optionally substituted alkoxy; wherein m is an integer from 0 to 6; and wherein n is an integer from 0 to 2.

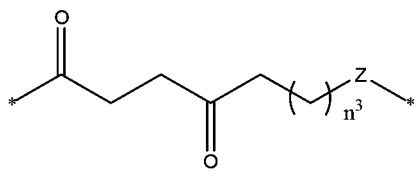
[00288] In a further aspect, the polymer further comprises at least one monomer residue selected from a propargyl-functionalized monomer residue having an optionally substituted structure represented by a formula:



wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, wherein  $m^1$  is an integer from 0 to 6, and wherein  $n^1$  is an integer from 0 to 2; a monomer residue having an optionally substituted structure represented by a formula:

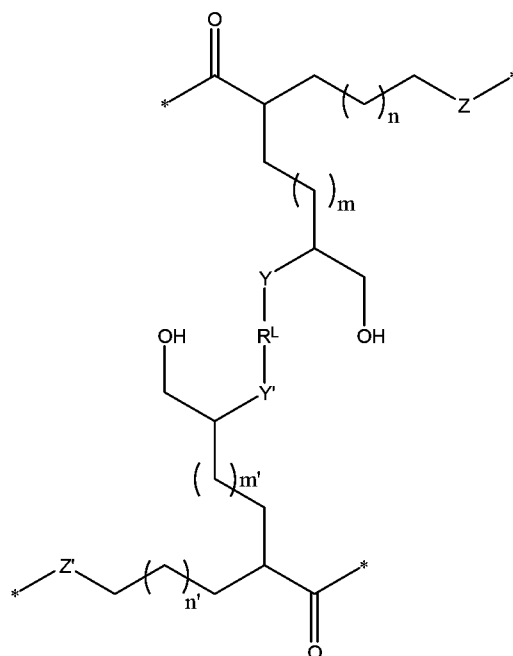


5 wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, and wherein  $n^2$  is an integer from 0 to 2; and a keto-functionalized monomer residue having an optionally substituted structure represented by a formula:



10 wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, and wherein  $n^3$  is an integer from 0 to 2.

**[00289]** In a further aspect, the polymer comprises an optionally substituted structure represented by a formula:



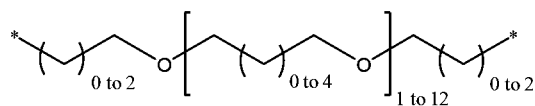
wherein m and m' are independently integers from 0 to 6; wherein n and n' are independently integers from 0 to 2; and wherein Z and Z' are independently O or NR, wherein R is H or C1 to C6 alkyl; wherein Y and Y' are independently O, S, or NR, wherein R is H or C1 to C6 alkyl; and wherein  $R^L$  is selected from optionally substituted alkyl and optionally substituted alkoxylenes.

**[00290]** In various aspects, m can be an integer from 0 to 6, from 1 to 6, from 0 to 5, from 1 to 5, from 0 to 4, from 1 to 4, from 0 to 3, from 1 to 3, from 0 to 2, from 1 to 2, 0, 1, 2, 3, 4, 5, or 6. In various aspects, m' can be an integer from 0 to 6, from 1 to 6, from 0 to 5, from 1 to 5, from 0 to 4, from 1 to 4, from 0 to 3, from 1 to 3, from 0 to 2, from 1 to 2, 0, 1, 2, 3, 4, 5, or 6. In various aspects,  $m^1$  can be an integer from 0 to 6, from 1 to 6, from 0 to 5, from 1 to 5, from 0 to 4, from 1 to 4, from 0 to 3, from 1 to 3, from 0 to 2, from 1 to 2, 0, 1, 2, 3, 4, 5, or 6.

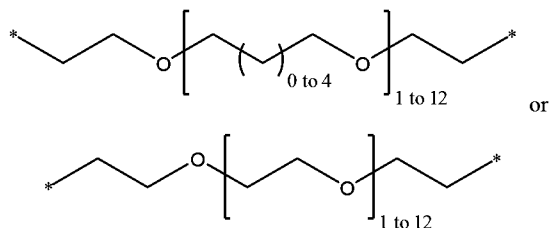
**[00291]** In various aspects, n can be an integer from 0 to 2, from 1 to 2, from 0 to 1, 0, 1, or 2. In various aspects, n' can be an integer from 0 to 2, from 1 to 2, from 0 to 1, 0, 1, or

2. In various aspects,  $n^1$  can be an integer from 0 to 2, from 1 to 2, from 0 to 1, 0, 1, or 2. In various aspects,  $n^2$  can be an integer from 0 to 2, from 1 to 2, from 0 to 1, 0, 1, or 2. In various aspects,  $n^3$  can be an integer from 0 to 2, from 1 to 2, from 0 to 1, 0, 1, or 2.

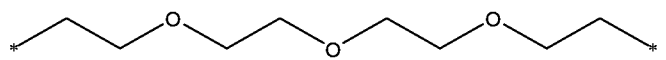
**[00292]**  $R^L$  can be selected from optionally substituted alkyl and optionally substituted alkoxyene. Suitable alkyls include divalent organic radicals selected from ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, decyl, dodecyl, hexadecyl, and higher alkyl. Suitable alkoxyene include divalent organic radicals selected from groups having a structure represented by a formula:



10 Further suitable alkoxyene include divalent organic radicals selected from groups having a structure represented by a formula:



Further suitable alkoxyene include a divalent organic radical having a structure represented by a formula:



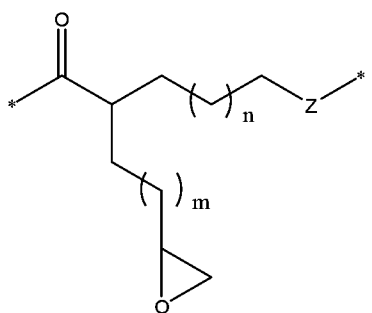
which can be derived from 2,2-(ethylenedioxy)bis(ethylamine).

**[00293]** The polymers and copolymers typically have a number average molecular weight ( $M_n$ ) of from about 3500-4800 Daltons with a narrow polydispersity of from about 1.17 to about 1.27. It is understood that the molecular weight can be higher or lower and that

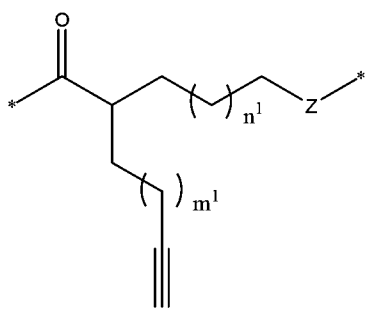
one of skill in the art can readily manipulate reaction conditions to achieve a different desired molecular weight.

## 2. MULTIFUNCTIONAL POLYMERS

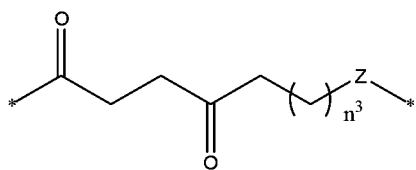
[00294] In one aspect, a polymer can be a multifunctional polymer. That is, the  
 5 polymer comprises monomer residues selected from two or more of an epoxide-  
 functionalized monomer residue having an optionally substituted structure represented by a  
 formula:



wherein Z is O or NR, wherein R is H or C1 to C6 alkyl; wherein m is an integer from 0 to 6;  
 10 wherein n is an integer from 0 to 2; and a propargyl-functionalized monomer residue having  
 an optionally substituted structure represented by a formula:



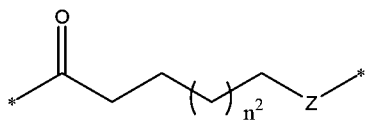
wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, wherein  $m^1$  is an integer from 0 to 6,  
 and wherein  $n^1$  is an integer from 0 to 2; and a keto-functionalized monomer residue having  
 15 an optionally substituted structure represented by a formula:



wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, and wherein  $n^3$  is an integer from 0 to 2.

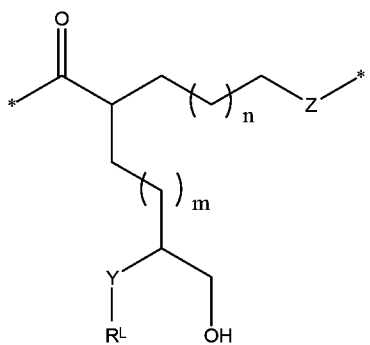
[00295] In one aspect, the epoxide-functionalized monomer residue is present and comprises less than about 10% by weight of the monomer residue of halogen selected from chlorine, bromine, and iodine. In further aspects, the monomer residue can comprise less than about 8%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, or less than about 1% of halogen selected from chlorine, bromine, and iodine, by weight of the monomer residue.

10 [00296] In a further aspect, the polymer further comprises at least one monomer residue having an optionally substituted structure represented by a formula:



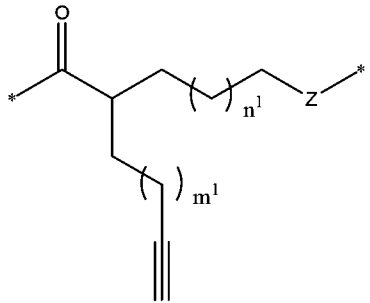
wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, and wherein  $n^2$  is an integer from 0 to 2.

15 [00297] In one aspect, a polymer comprises at least one monomer residue having an optionally substituted structure represented by a formula:



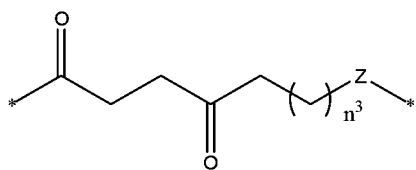
wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, wherein Y is O, S, or NR, wherein R is H or C1 to C6 alkyl, wherein  $R^L$  is selected from optionally substituted alkyl and optionally substituted alkoxy, wherein m is an integer from 0 to 6, and wherein n is an integer from 0 to 2; and one or more of:

a propargyl-functionalized monomer residue having an optionally substituted structure represented by a formula:

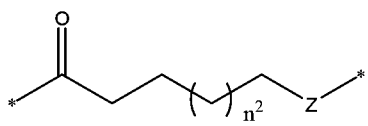


wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, wherein  $m^1$  is an integer from 0 to 6, and wherein  $n^1$  is an integer from 0 to 2; and

a keto-functionalized monomer residue having an optionally substituted structure represented by a formula:

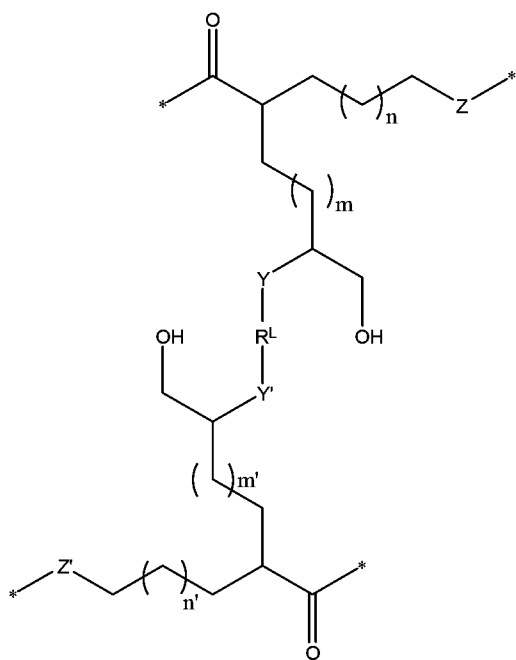


wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, and wherein  $n^3$  is an integer from 0 to 2. In a further aspect, the polymer further comprises at least one monomer residue having an optionally substituted structure represented by a formula:



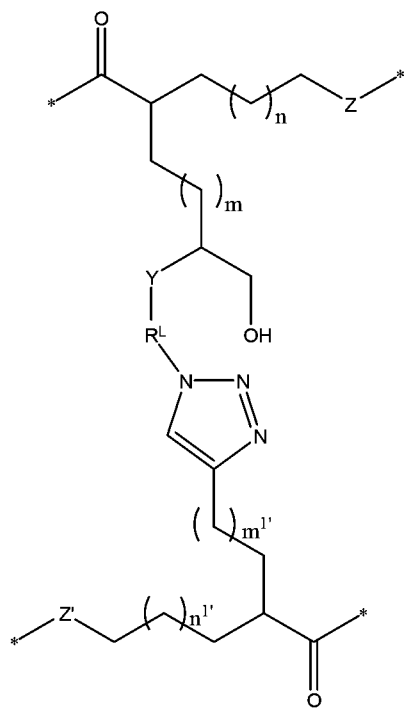
5

wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, and wherein  $n^2$  is an integer from 0 to 2. In one aspect, the at least one monomer residue has an optionally substituted structure represented by a formula:



wherein m and m' are independently integers from 0 to 6; wherein n and n' are independently integers from 0 to 2; and wherein Z and Z' are independently O or NR, wherein R is H or C1 to C6 alkyl; wherein Y and Y' are independently O, S, or NR, wherein R is H or C1 to C6 alkyl; and wherein R<sup>L</sup> is selected from optionally substituted alkyl and optionally substituted alkoxylenes.

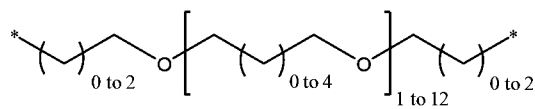
**[00298]** In one aspect, at least one monomer residue has an optionally substituted structure represented by a formula:



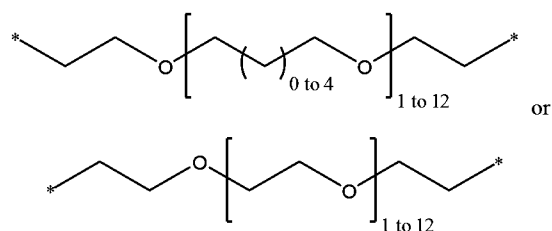
wherein m and m' are independently integers from 0 to 6; wherein n and n' are independently integers from 0 to 2; and wherein Z and Z' are independently O or NR, wherein R is H or C1 to C6 alkyl; wherein Y is O, S, or NR, wherein R is H or C1 to C6 alkyl; and wherein R<sup>L</sup> is selected from optionally substituted alkyl and optionally substituted alkoxylenes.

- [00299] In various aspects, m can be an integer from 0 to 6, from 1 to 6, from 0 to 5, from 1 to 5, from 0 to 4, from 1 to 4, from 0 to 3, from 1 to 3, from 0 to 2, from 1 to 2, 0, 1, 2, 3, 4, 5, or 6. In various aspects, m' can be an integer from 0 to 6, from 1 to 6, from 0 to 5, from 1 to 5, from 0 to 4, from 1 to 4, from 0 to 3, from 1 to 3, from 0 to 2, from 1 to 2, 0, 1, 2, 3, 4, 5, or 6. In various aspects, m<sup>1</sup> can be an integer from 0 to 6, from 1 to 6, from 0 to 5, from 1 to 5, from 0 to 4, from 1 to 4, from 0 to 3, from 1 to 3, from 0 to 2, from 1 to 2, 0, 1, 2, 3, 4, 5, or 6. In various aspects, m<sup>1'</sup> can be an integer from 0 to 6, from 1 to 6, from 0 to 5, from 1 to 5, from 0 to 4, from 1 to 4, from 0 to 3, from 1 to 3, from 0 to 2, from 1 to 2, 0, 1, 2, 3, 4, 5, or 6.
- 10 [00300] In various aspects, n can be an integer from 0 to 2, from 1 to 2, from 0 to 1, 0, 1, or 2. In various aspects, n' can be an integer from 0 to 2, from 1 to 2, from 0 to 1, 0, 1, or 2. In various aspects, n<sup>1</sup> can be an integer from 0 to 2, from 1 to 2, from 0 to 1, 0, 1, or 2. In various aspects, n<sup>1'</sup> can be an integer from 0 to 2, from 1 to 2, from 0 to 1, 0, 1, or 2. In various aspects, n<sup>2</sup> can be an integer from 0 to 2, from 1 to 2, from 0 to 1, 0, 1, or 2. In various aspects, n<sup>3</sup> can be an integer from 0 to 2, from 1 to 2, from 0 to 1, 0, 1, or 2.
- 15

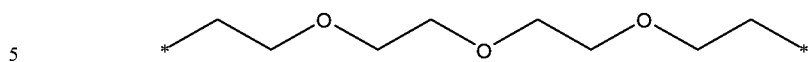
- [00301] R<sup>L</sup> can be selected from optionally substituted alkyl and optionally substituted alkoxyene. Suitable alkyls include divalent organic radicals selected from ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, decyl, dodecyl, hexadecyl, and higher alkyl. Suitable alkoxyene include divalent organic radicals selected from groups having a structure represented by a formula:
- 20



Further suitable alkoxyene include divalent organic radicals selected from groups having a structure represented by a formula:

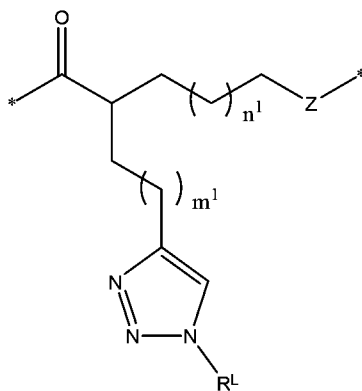


Further suitable alkoxylenes include a divalent organic radical having a structure represented by a formula:



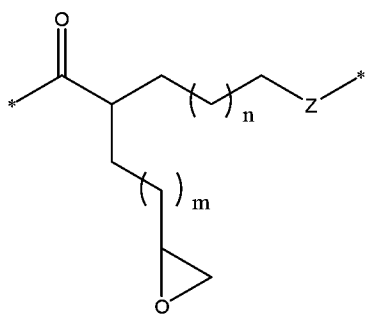
which can be derived from 2,2-(ethylenedioxy)bis(ethylamine) or 2,2-(ethylenedioxy)bis(ethylazide).

**[00302]** In one aspect, a polymer can comprise at least one monomer residue having an optionally substituted structure represented by a formula:



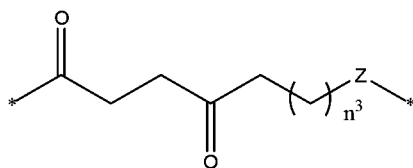
wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, wherein  $R^L$  is selected from optionally substituted alkyl and optionally substituted alkoxylenes, wherein  $m^1$  is an integer from 0 to 6, and wherein  $n^1$  is an integer from 0 to 2; and one or more of:

an epoxide-functionalized monomer residue having an optionally substituted structure represented by a formula:



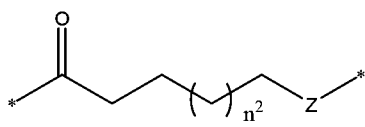
wherein Z is O or NR, wherein R is H or C1 to C6 alkyl; wherein m is an integer from 0 to 6;  
wherein n is an integer from 0 to 2; and

a keto-functionalized monomer residue having an optionally substituted structure represented  
5 by a formula:



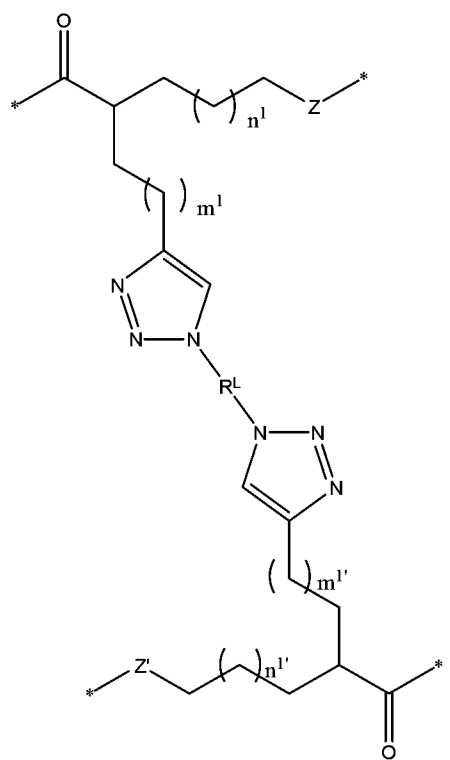
wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, and wherein n<sup>3</sup> is an integer from 0  
to 2. In a further aspect, the epoxide-functionalized monomer residue is present and  
comprises less than about 10% by weight of the monomer residue of halogen selected from  
10 chlorine, bromine, and iodine.

**[00303]** In one aspect, the polymer further comprises at least one monomer residue  
having an optionally substituted structure represented by a formula:



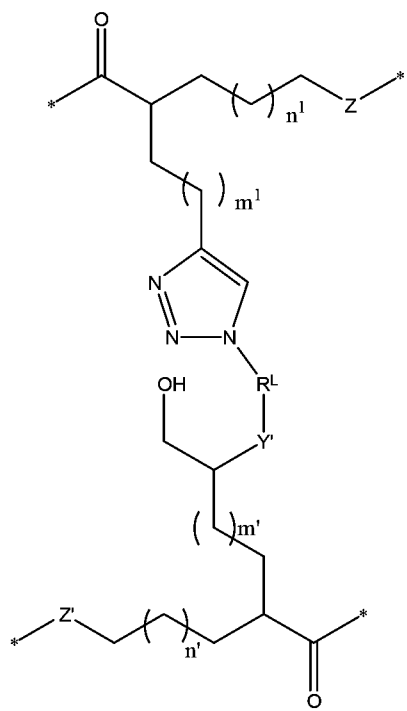
wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, and wherein n<sup>2</sup> is an integer from 0  
15 to 2.

[00304] In a further aspect, at least one monomer residue has an optionally substituted structure represented by a formula:



wherein  $m^1$  and  $m^{1'}$  are independently integers from 0 to 6; wherein  $n^1$  and  $n^{1'}$  are  
 5 independently integers from 0 to 2; and wherein Z and Z' are independently O or NR,  
 wherein R is H or C1 to C6 alkyl; and wherein  $R^L$  is selected from optionally substituted alkyl  
 and optionally substituted alkoxylenes.

[00305] In one aspect, at least one monomer residue has an optionally substituted structure represented by a formula:

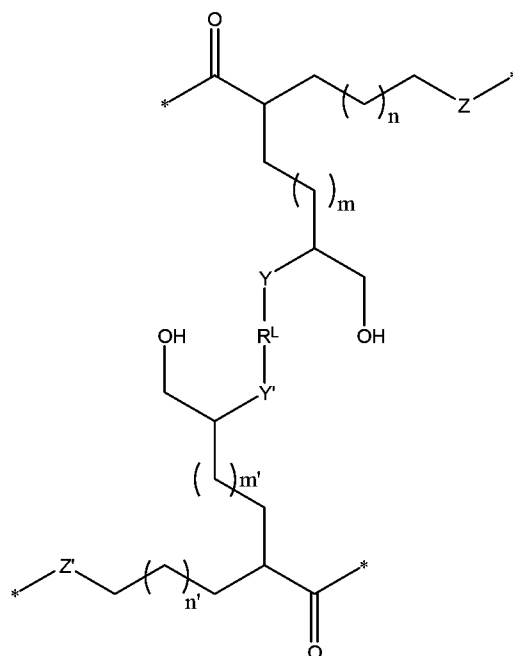


wherein  $m^1$  and  $m'$  are independently integers from 0 to 6; wherein  $n^1$  and  $n'$  are independently integers from 0 to 2; and wherein Z and Z' are independently O or NR, wherein R is H or C1 to C6 alkyl; and wherein Y' is O, S, or NR, wherein R is H or C1 to C6 alkyl; wherein  $R^L$  is selected from optionally substituted alkyl and optionally substituted alkoxylenes.

#### H. DEGRADABLE NANOPARTICLES

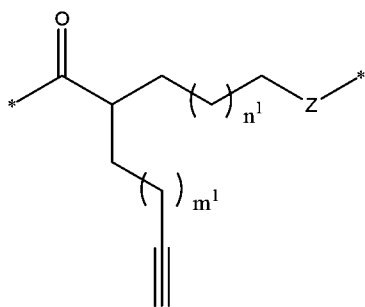
**[00306]** It is understood that the disclosed nanoparticles can be used in connection with the disclosed polymers and disclosed methods. Unless stated to the contrary, the disclosed structures can be used in connection with the disclosed methods, the disclosed polymers, and the disclosed nanoparticles.

**[00307]** In one aspect, the invention relates to a degradable polymeric nanoparticle comprising at least one monomer residue having an optionally substituted structure represented by a formula:

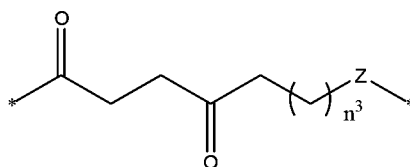


wherein  $m$  and  $m'$  are independently integers from 0 to 6; wherein  $n$  and  $n'$  are independently integers from 0 to 2; and wherein  $Z$  and  $Z'$  are independently O or NR, wherein R is H or C1 to C6 alkyl; wherein Y and Y' are independently O, S, or NR, wherein R is H or C1 to C6 alkyl; and wherein  $R^L$  is selected from optionally substituted alkyl and optionally substituted alkoxylenes, wherein the nanoparticle has a particle size of from about 5 nm to about 850 nm.

**[00308]** In one aspect, the nanoparticle further comprises at least one monomer residue selected from a propargyl-functionalized monomer residue having an optionally substituted structure represented by a formula:

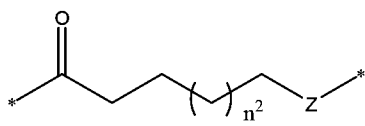


wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, wherein  $m^1$  is an integer from 0 to 6, and wherein  $n^1$  is an integer from 0 to 2; a keto-functionalized monomer residue having an optionally substituted structure represented by a formula:



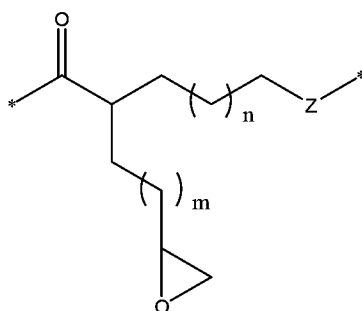
5

wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, and wherein  $n^3$  is an integer from 0 to 2; and a monomer residue having an optionally substituted structure represented by a formula:

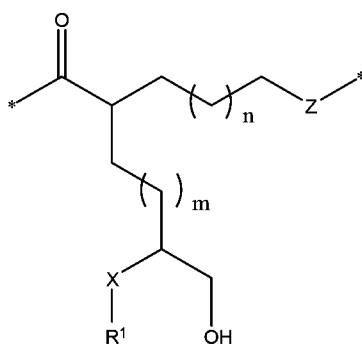


10 wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, and wherein  $n^2$  is an integer from 0 to 2. In a further aspect, Z and Z' are O.

**[00309]** In one aspect, the nanoparticle further comprises at least one epoxide-functionalized monomer residue having an optionally substituted structure represented by a formula:



**[00310]** In a further aspect, the nanoparticle further comprises at least one functionalized monomer residue having an optionally substituted structure represented by a formula:

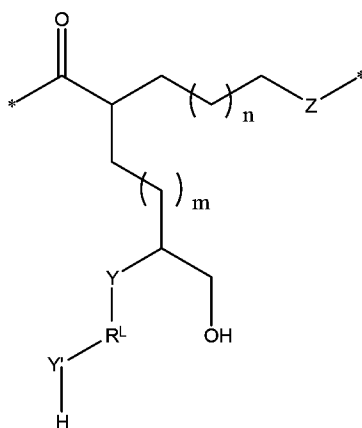


5

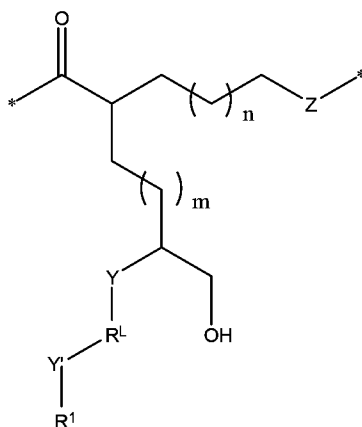
wherein X is OH, SH, NH<sub>2</sub>, or NHR, wherein R is H or C1 to C6 alkyl; and wherein R<sup>1</sup> is an optionally substituted organic radical comprising 1 to 24 carbon atoms.

**[00311]** In a further aspect, R<sup>1</sup> is further substituted with at least one biologically active agent, at least one pharmaceutically active agent, and/or at least one imaging moiety.

10 **[00312]** In one aspect, the nanoparticle further comprises at least one nucleophile-functionalized monomer residue having an optionally substituted structure represented by a formula:



**[00313]** In a further aspect, the nanoparticle further comprises at least one functionalized monomer residue having an optionally substituted structure represented by a formula:

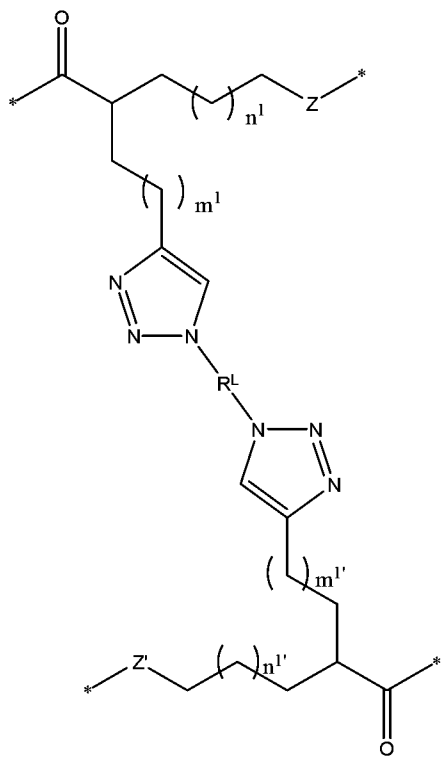


5

wherein  $R^1$  is an optionally substituted organic radical comprising 1 to 24 carbon atoms.

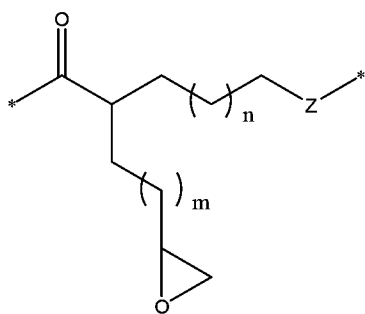
**[00314]** In a further aspect,  $R^1$  is further substituted with at least one biologically active agent, at least one pharmaceutically active agent, and/or at least one imaging moiety.

[00315] In one aspect, the invention relates to a degradable polymeric nanoparticle comprising at least one monomer residue having an optionally substituted structure represented by a formula:

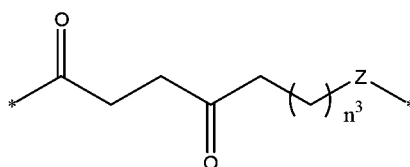


5 wherein  $m^1$  and  $m^{1'}$  are independently integers from 0 to 6; wherein  $n^1$  and  $n^{1'}$  are independently integers from 0 to 2; and wherein Z and Z' are independently O or NR, wherein R is H or C1 to C6 alkyl; and wherein  $R^L$  is selected from optionally substituted alkyl and optionally substituted alkoxylenes, wherein the nanoparticle has a particle size of from about 5 nm to about 850 nm.

10 [00316] In a further aspect, the nanoparticle further comprises at least one monomer residue selected from: an epoxide-functionalized monomer residue having an optionally substituted structure represented by a formula:

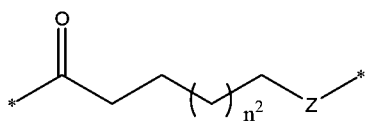


wherein Z is O or NR, wherein R is H or C1 to C6 alkyl; wherein m is an integer from 0 to 6;  
 wherein n is an integer from 0 to 2; a keto-functionalized monomer residue having an  
 optionally substituted structure represented by a formula:



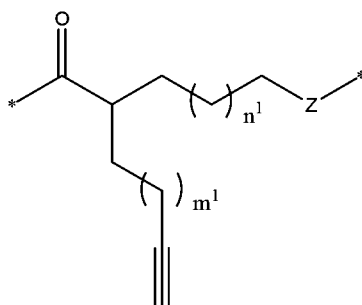
5

wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, and wherein  $n^3$  is an integer from 0  
 to 2; and a monomer residue having an optionally substituted structure represented by a  
 formula:

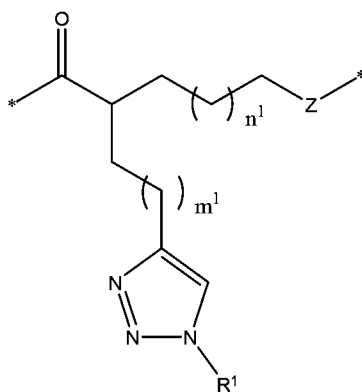


10 wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, and wherein  $n^2$  is an integer from 0  
 to 2. In a further aspect, Z and Z' are O.

**[00317]** In a further aspect, the nanoparticle further comprises at least one propargyl-  
 functionalized monomer residue having an optionally substituted structure represented by a  
 formula:



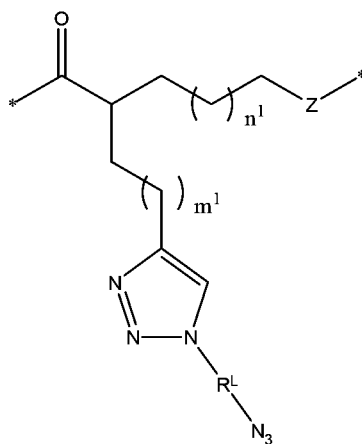
[00318] In a further aspect, the nanoparticle further comprises at least one functionalized monomer residue having an optionally substituted structure represented by a formula:



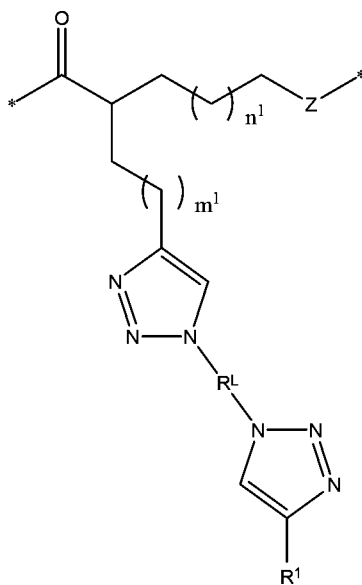
5

wherein  $R^1$  is an optionally substituted organic radical comprising 1 to 24 carbon atoms. In a further aspect,  $R^1$  is further substituted with at least one biologically active agent, at least one pharmaceutically active agent, and/or at least one imaging moiety.

[00319] In a further aspect, the nanoparticle further comprises at least one azide-  
 10 functionalized monomer residue having an optionally substituted structure represented by a formula:



**[00320]** In a further aspect, the nanoparticle further comprises at least one functionalized monomer residue having an optionally substituted structure represented by a formula:



wherein  $R^1$  is an optionally substituted organic radical comprising 1 to 24 carbon atoms. In a further aspect,  $R^1$  is further substituted with at least one biologically active agent, at least one pharmaceutically active agent, and/or at least one imaging moiety.

## I. METHODS

- 5    **[00321]**       It is understood that the disclosed methods can be used in connection with the disclosed polymers and disclosed nanoparticles. Unless stated to the contrary, the disclosed structures can be used in connection with the disclosed methods, the disclosed polymers, and the disclosed nanoparticles.

### 1. METHODS OF MAKING POLYMER

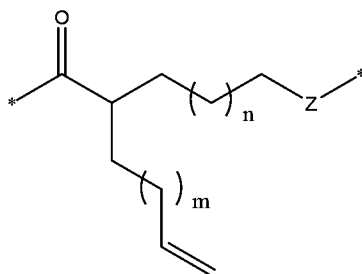
- 10   **[00322]**       To address the deficiencies of conventional nanoparticle compositions and methods, the availability of novel functional polyesters that allow orthogonal modification approaches was addressed. Additionally, controlled chain cross-linking strategies for obtaining distinct nanoparticles in a variety of nanoscopic dimensions are disclosed. In contrast to investigating emulsification-solvent techniques [Hans, M. L.; Lowman, A. M. Curr. Opin. Solid State Mater. Sci. 2002, 6, 319-327.] or emulsion diffusion methods  
15   [Kallinteri, P.; Higgins, S.; Hutcheon, G. A.; St. Pourcain, C. B.; Garnett, M. C. Biomacromolecules 2005, 6, 1885-1894.] that need surfactants or salts, the disclosed methods and compositions involve controlled cross-linking techniques.

- [00323]**       A clean and non-toxic cross-linking entity can be provided from epoxide  
20   groups that react with dinucleophiles (e.g., diamines) to form alkane -OH groups. While this crosslinking unit has been employed to form acrylate based microparticles [Burke, S. K.; Slatopolsky, E. A.; Goldberg, D. I., Nephrol. Dial. Transplant. 1997, 12, (8), 1640-1644.], it has been never investigated in the formation of degradable nanoparticles due to the lack of suitable linear precursors.

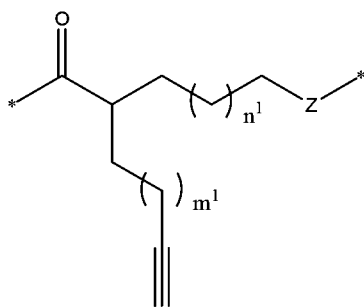
- 25   **[00324]**       The epoxide entity for the formation of discrete cross-linked nanoparticles can be integrated by polymerization of a low molecular weight linear copolymer, **Ab**, with pendant allyl groups. See Figure 1. Pendant allyl groups represent valuable intermediates to many functional groups and can be incorporated into the polymer backbone by copolymerizing  $\alpha$ -allyl- $\delta$ -valerolactone, (b), and commercially available  $\delta$ -valerolactone, (A),

via ring-opening polymerization (ROP). [Parrish, B.; Quansah, J. K.; Emrick, T. J. Polym. Sci. Part A: Polym. Chem. 2002, 40, 1983-1990.] Upon copolymerization, the pendant allyl groups can be oxidized by a Baeyer-Villiger oxidation with meta-chloroperoxybenzoic acid (m-CPBA) to convert the double bonds to epoxide rings, which then became a coupling group in the preparation of the nanoparticles. [(a) Mecerreyes, D.; Miller, R. D.; Hedrick, J. L.; Detrembleur, C.; Jerome, R. J. Polym. Sci. Part A: Polym. Chem. 2000, 38, 870-875. (b) Latere, J.P.; Lecomte, P.; Dubois, P.; Jérôme, R. Macromolecules 2002, 35, 7857-7859.] To introduce additional functional groups into the nanoparticle, additional monomers can be synthesized, for example  $\alpha$ -propargyl- $\delta$ -valerolactone, (C), and 2-oxepane-1,5-dione, (D). These monomers can then be individually copolymerized with (B) and  $\delta$ -valerolactone, (A), in a similar manner as **Ab**, to give rise to linear polyesters with additional propargyl or keto functionalities respectively. To increase the number of functionalities that allow orthogonal modification approaches, (C) and (D) were copolymerized together with (b) and  $\delta$ -valerolactone (A), as summarized in Figure 1. The copolymers were typically obtained in molecular weight ranges of 3500-4800 Da with narrow polydispersities of 1.17-1.27.

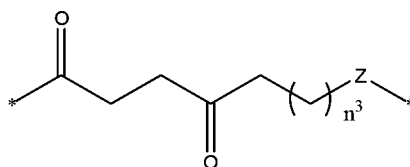
**[00325]** In one aspect, the invention relates to a method of preparing a polymer comprising the step of copolymerizing a mixture of two or more of an alkene-functionalized monomer providing a residue having an optionally substituted structure represented by a formula:



wherein Z is O or NR, wherein R is H or C1 to C6 alkyl; wherein m is an integer from 0 to 6; wherein n is an integer from 0 to 2; a propargyl-functionalized monomer providing a residue having an optionally substituted structure represented by a formula:



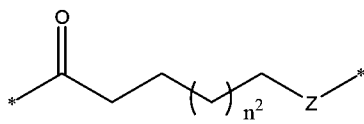
wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, wherein  $m^1$  is an integer from 0 to 6, and wherein  $n^1$  is an integer from 0 to 2; and a keto-functionalized monomer providing a residue having an optionally substituted structure represented by a formula:



5

wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, and wherein  $n^3$  is an integer from 0 to 2.

**[00326]** In a further aspect, the mixture further comprises at least one monomer providing a residue having an optionally substituted structure represented by a formula:

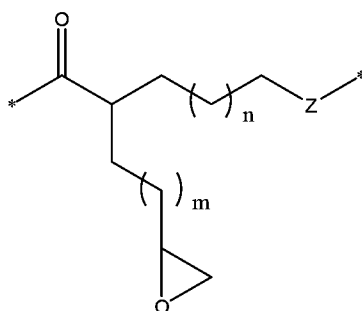


10

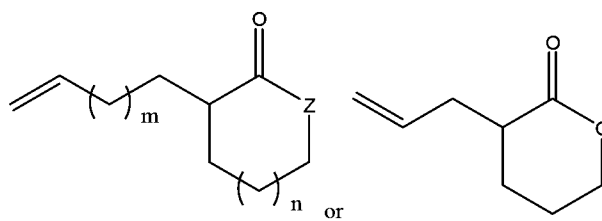
wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, and wherein  $n^2$  is an integer from 0 to 2.

**[00327]** In one aspect, the alkene-functionalized monomer is present and the method further comprises the step of oxidizing the resultant polymer to provide an epoxide-functionalized monomer residue having an optionally substituted structure represented by a formula:

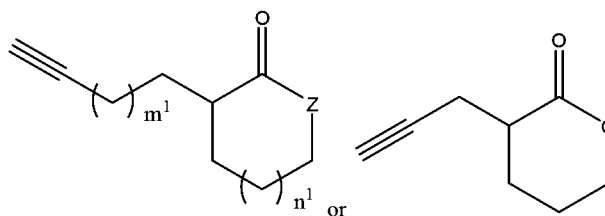
15



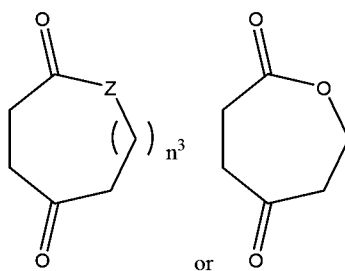
[00328] In a further aspect, the alkene-functionalized monomer is present and has an optionally substituted structure represented by a formula:



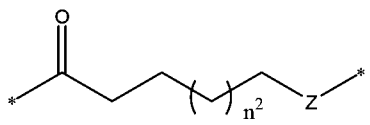
5 [00329] In a further aspect, the propargyl-functionalized monomer is present and has an optionally substituted structure represented by a formula:



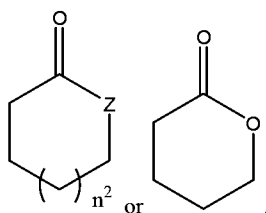
[00330] In a further aspect, the keto-functionalized monomer is present and has an optionally substituted structure represented by a formula:



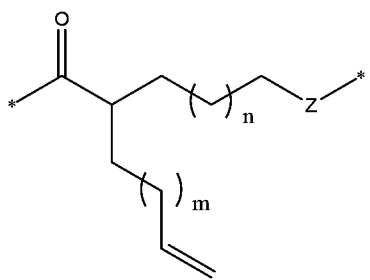
[00331] In a further aspect, the monomer providing a residue having an optionally substituted structure represented by a formula:



5 has an optionally substituted structure represented by a formula:

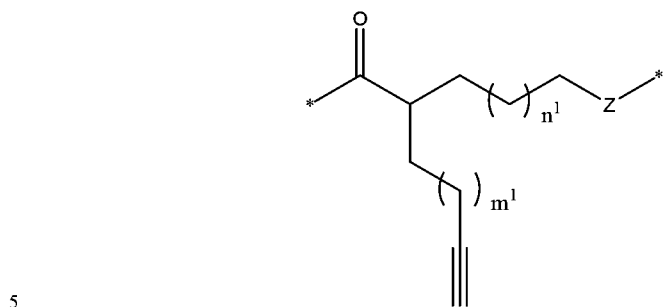


[00332] In one aspect, the invention relates to a method of preparing an epoxide-functionalized polymer comprising the step of oxidizing a polymer having at least one monomer residue having an optionally substituted structure represented by a formula:

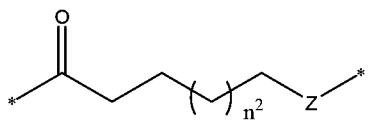


wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, wherein m is an integer from 0 to 6,  
and wherein n is an integer from 0 to 2.

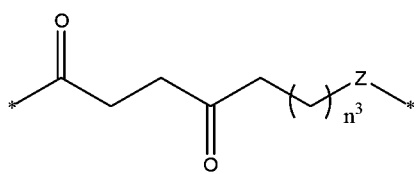
**[00333]** In a further aspect, the polymer further comprises at least one monomer  
residue selected from:



wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, wherein m<sup>1</sup> is an integer from 0 to 6,  
and wherein n<sup>1</sup> is an integer from 0 to 2;

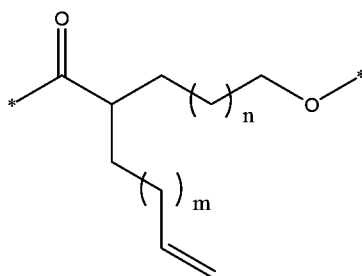


wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, wherein n<sup>2</sup> is an integer from 0 to 2;  
10 and

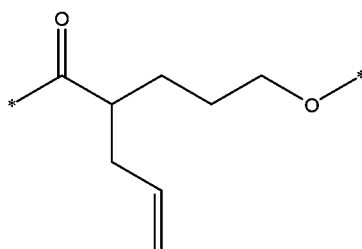


wherein n<sup>3</sup> is an integer from 0 to 2.

**[00334]** In a further aspect, at least one monomer residue has an optionally substituted  
structure represented by a formula:

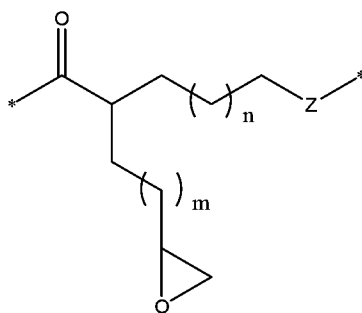


wherein m is an integer from 0 to 6, and wherein n is an integer from 0 to 2. For example, in one aspect, m is 1, and n is 0, providing an optionally substituted structure represented by a formula:



5

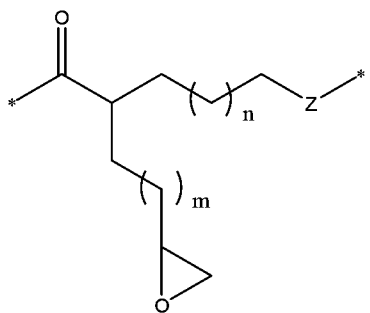
**[00335]** In a further aspect, the epoxide-functionalized polymer has an optionally substituted structure represented by a formula:



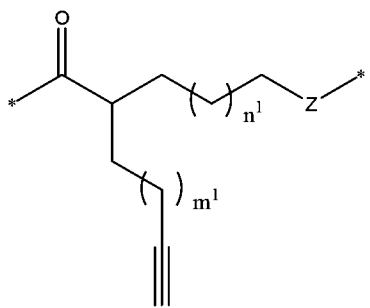
## 2. METHODS OF CROSSLINKING

10 **[00336]** In one aspect, the invention relates to a method of crosslinking a polymer comprising the step of reacting a polymer comprising at least one monomer residue selected

from an epoxide-functionalized monomer residue having an optionally substituted structure represented by a formula:



wherein Z is O or NR, wherein R is H or C1 to C6 alkyl; wherein m is an integer from 0 to 6;  
 5 wherein n is an integer from 0 to 2; and a propargyl-functionalized monomer residue having an optionally substituted structure represented by a formula:

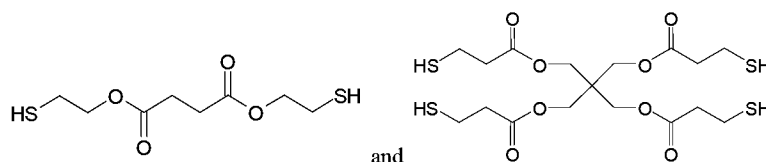


wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, wherein  $m^1$  is an integer from 0 to 6,  
 and wherein  $n^1$  is an integer from 0 to 2; with a cross-linker having a structure represented by  
 10 a formula  $X-R^L-X'$ , wherein X and X' are independently  $N_3$ , OH, SH,  $NH_2$ , or  $NHR$ , wherein  
 R is H or C1 to C6 alkyl, and wherein  $R^L$  is selected from optionally substituted alkyl and  
 optionally substituted alkoxylenes.

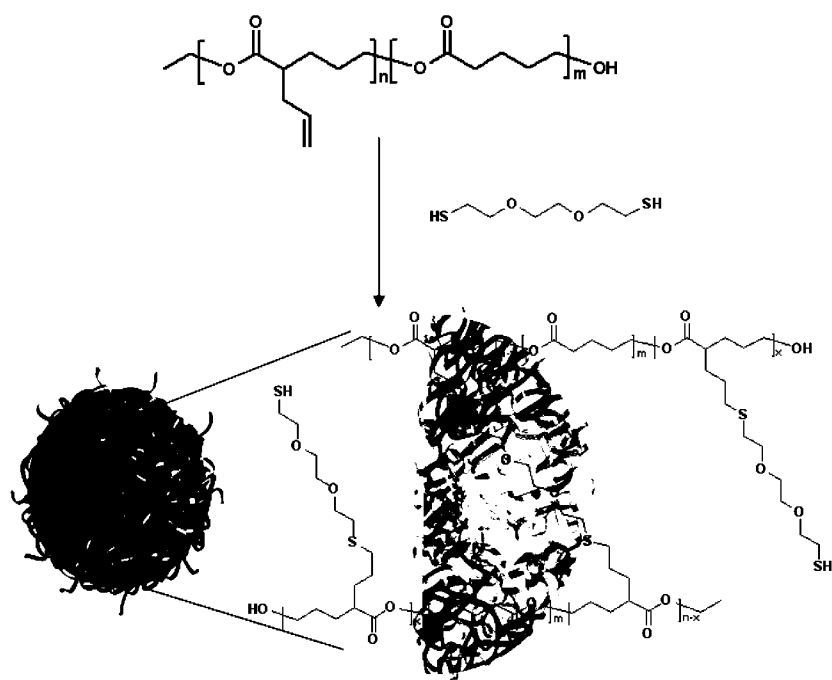
**[00337]** In one aspect, the linker groups can be bis-nucleophilic (e.g., diamine)  
 compounds derived from from alkylene oxides (e.g., diamino poly(ethylene oxides)) and/or  
 15 alkyls (e.g., 1,8-diaminooctane; Jeffamines) and their derivatives.

[00338] In a further aspect, the linker groups can be thiols. For example, the dinucleophile can have a structure  $X-R^L-X'$ , wherein X and X' are each SH, wherein R is H or C1 to C6 alkyl, and wherein  $R^L$  is selected from optionally substituted alkyl, optionally substituted alkoxy, and optionally substituted esters.

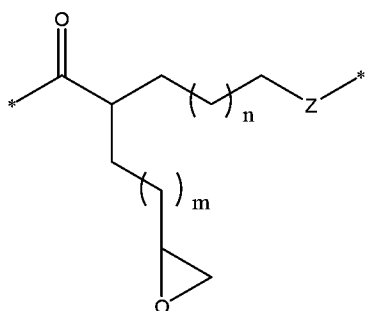
5 [00339] Thiols suitable for crosslinking include mono- and di-thiol analogues of compounds derived from from alkylene oxides (e.g., diamino poly(ethylene oxides)) and/or alkyls (e.g., 1,8-diaminooctane; Jeffamines) and their derivatives. Other suitable dithiols for cross-linking include:



10 [00340] An example crosslinking reaction, and example product thereof, is shown below:

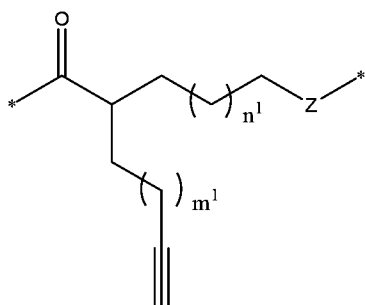


- [00341] In one aspect, the cross-linker reacts with two polymer strands. In a further aspect,  $X-R^L-X'$  reacts with two epoxide-functionalized monomer residues. In a further aspect,  $X-R^L-X'$  reacts with two propargyl-functionalized monomer residues. In a further aspect,  $X-R^L-X'$  reacts with one epoxide-functionalized monomer residue and one propargyl-functionalized monomer residue. In a further aspect,  $X=X'$ . In a further aspect,  $X=X'=NH_2$ . In a further aspect,  $R^L$  comprises two or more residues of ethylene oxide or trimethylene oxide. In a further aspect,  $X-R^L-X'$  is 2,2-(ethylenedioxy)bis(ethylamine). In a further aspect,  $X=X'=N_3$ .
- 10 [00342] In one aspect, the polymer comprises at least one monomer residue having an optionally substituted structure represented by a formula:



and wherein  $X=X'=NH_2$ . In one aspect, the polymer and the crosslinker are reacted in a ratio of about 1:1 (polymer:cross-linker). In a further aspect, the polymer and the crosslinker are reacted in a ratio of about >1:1 (polymer:cross-linker) to provide a polymer with excess epoxide-functionalization. In a further aspect, the polymer and the crosslinker are reacted in a ratio of about <1:1 (polymer:cross-linker) to provide a polymer with excess amino-functionalization.

**[00343]** In one aspect, the polymer comprises at least one monomer residue having an optionally substituted structure represented by a formula:

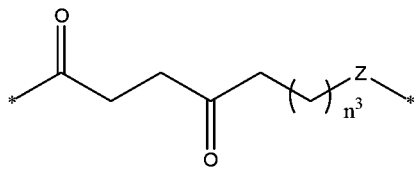


10

and wherein  $X=X'=N_3$ . In a further aspect, the polymer and the crosslinker are reacted in a ratio of about 1:1 (polymer:cross-linker). In a further aspect, the polymer and the crosslinker are reacted in a ratio of about >1:1 (polymer:cross-linker) to provide a polymer with excess alkyne-functionalization. In a further aspect, the polymer and the crosslinker are reacted in a ratio of about <1:1 (polymer:cross-linker) to provide a polymer with excess azide-functionalization.

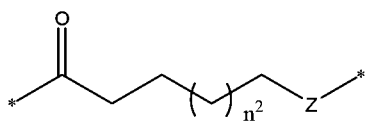
15

[00344] In a further aspect, the polymer further comprises a keto-functionalized monomer providing a residue having an optionally substituted structure represented by a formula:



5 wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, and wherein  $n^3$  is an integer from 0 to 2.

[00345] In a further aspect, the polymer further comprises at least one monomer providing a residue having an optionally substituted structure represented by a formula:

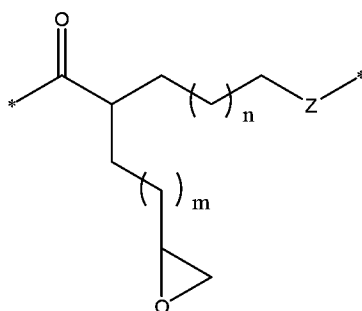


10 wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, and wherein  $n^2$  is an integer from 0 to 2.

### 3. METHODS OF FUNCTIONALIZING POLYMERS

[00346] In one aspect, the invention relates to a method of functionalizing a polymer comprising the step of reacting an epoxide-functionalized monomer residue having an optionally substituted structure represented by a formula:

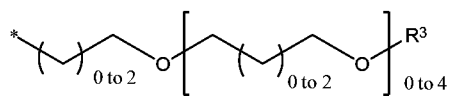
15



wherein Z is O or NR, wherein R is H or C1 to C6 alkyl; wherein m is an integer from 0 to 6;  
 wherein n is an integer from 0 to 2; with a nucleophile having a structure represented by a  
 formula  $X-R^1$ , wherein X is OH, SH,  $NH_2$ , or NHR, wherein R is H or C1 to C6 alkyl; and  
 5 wherein  $R^1$  is an optionally substituted organic radical comprising 1 to 24 carbon atoms.

**[00347]** Organic radicals suitable for use as  $R^1$  include substituted or unsubstituted  
 monovalent organic radicals selected from ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl,  
 decyl, dodecyl, hexadecyl, and higher alkyl. The alkyl can be linear or branched and can be  
 cyclic or acyclic. In a further aspect,  $R^1$  can comprise an optionally substituted alkoxylenes.

10 Suitable alkoxylenes include substituted or unsubstituted monovalent organic radicals selected  
 from groups having a structure represented by a formula:

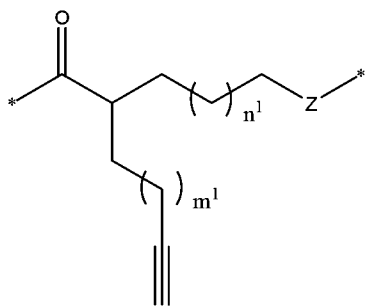


wherein  $R^3$  comprises C1 to C6 alkyl.

**[00348]** In a further aspect,  $R^1$  is further substituted with at least one biologically active  
 15 agent, at least one pharmaceutically active agent, and/or at least one imaging moiety, thus  
 providing a convenient method for functionalizing the polymer with one or more biologically  
 active agents, pharmaceutically active agents, and/or imaging moieties via a nucleophilic  
 substitution reaction. That is,  $R^1$  can comprise at least one biologically active agent, at least  
 one pharmaceutically active agent, and/or at least one imaging moiety. In a further aspect,  $R^1$   
 20 can comprise a portion of the at least one biologically active agent, at least one  
 pharmaceutically active agent, and/or at least one imaging moiety. In a further aspect,  $R^1$  can

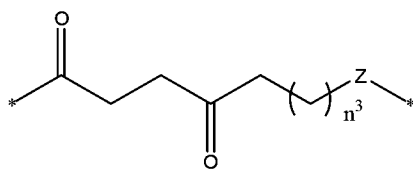
be covalently bonded to at least one biologically active agent, at least one pharmaceutically active agent, and/or at least one imaging moiety.

[00349] In one aspect, the invention relates to a method of functionalizing a polymer comprising the step of reacting a propargyl-functionalized monomer residue having an  
5 optionally substituted structure represented by a formula:



wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, wherein  $m^1$  is an integer from 0 to 6,  
and wherein  $n^1$  is an integer from 0 to 2; with an azide having a structure represented by a  
10 formula  $N_3-R^1$ , wherein  $R^1$  is an optionally substituted organic radical comprising 1 to 24  
carbon atoms. In a further aspect,  $R^1$  is further substituted with at least one biologically  
active agent, at least one pharmaceutically active agent, and/or at least one imaging moiety.

[00350] In one aspect, the invention relates to a method of functionalizing a polymer comprising the steps of reacting a keto-functionalized monomer providing a residue having  
an optionally substituted structure represented by a formula:

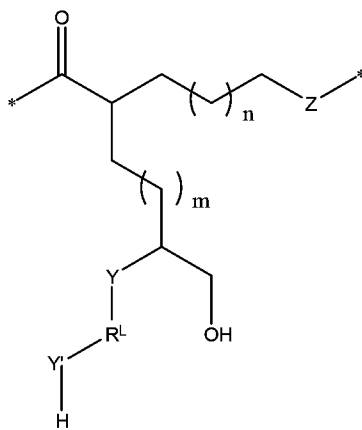


15

wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, and wherein  $n^3$  is an integer from 0  
to 2; with an amine having a structure represented by a formula  $H_2N-R^1$ , wherein  $R^1$  is an  
optionally substituted organic radical comprising 1 to 24 carbon atoms; and reducing the  
resulting imine. In a further aspect, the reacting step and the reducing step are performed

simultaneously. In a further aspect,  $R^1$  is further substituted with at least one biologically active agent, at least one pharmaceutically active agent, and/or at least one imaging moiety.

[00351] In one aspect, the invention relates to a method of functionalizing a polymer comprising the step of reacting a nucleophile-functionalized monomer residue having an optionally substituted structure represented by a formula:



wherein Z is O or NR, wherein R is H or C1 to C6 alkyl; wherein m is an integer from 0 to 6; wherein n is an integer from 0 to 2; wherein Y and Y' are independently O, S, or NR, wherein R is H or C1 to C6 alkyl; and wherein  $R^L$  is selected from optionally substituted alkyl and optionally substituted alkoxy; with an electrophile having a structure represented by a formula  $E-R^1$ , wherein E is an electrophilic moiety; and wherein  $R^1$  is an optionally substituted organic radical comprising 1 to 24 carbon atoms.

[00352] In a further aspect, Y' is  $NH_2$  or NHR. In a further aspect, wherein  $Y=Y'$ . In a further aspect, the electrophilic moiety is selected from alkyl halide, alkyl pseudohalide, and carboxyl derivative. In a further aspect,  $R^1$  is further substituted with at least one biologically active agent, at least one pharmaceutically active agent, and/or at least one imaging moiety.

#### 4. METHODS OF MAKING NANOPARTICLES

[00353] The formation of nanoparticles in controlled size dimensions can proceed from linear polymers containing pendant epoxide groups which crosslink with 2,2'-

(ethylenedioxy)bis(ethylamine). To evaluate the particle formation under controlled conditions, reactions in which the equivalents of diamine cross-linker were linearly increased with respect to the reactive epoxide groups of the polymers were studied.

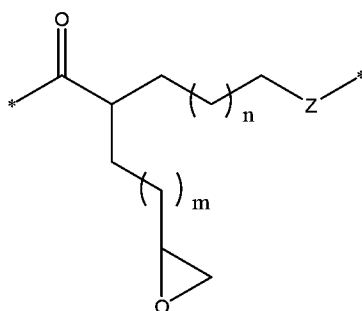
**[00354]** To achieve a high degree of cross-linking between the individual polyester chains, the polymer solution with the pendant epoxide entities can be added in a dropwise fashion to a refluxing solution of different equivalents of dinucleophile (e.g., diamine) in dichloromethane. In this strategy, the difunctional amine is in high excess during the addition (13 mL/min) of the linear polymer solution (0.5 M) and thus provides optimum cross-linking reactions (Table 1; particle size reported in nm diameter by dynamic light scattering (DLS) in relation to varying amine ratios).

**Table 1:** Nanoparticle Size Dimensions (nm)

| Amine/ 1 Epoxide | Diameter (nm)<br>Poly(vl-evl)<br><b>AB</b> | Diameter (nm)<br>Poly(vl-evl-opd)<br><b>ABD</b> | Diameter (nm)<br>Poly(vl-evl-pvl)<br><b>ABC</b> |
|------------------|--------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| 1                | 30.71 ± 2.21                               | 34.29 ± 3.22                                    | 21.40 ± 2.90                                    |
| 2                | 58.06 ± 6.20                               | 63.46 ± 7.68                                    | 41.70 ± 5.36                                    |
| 3                | 82.1 ± 5.73                                | 118.3 ± 13.6                                    | 114.9 ± 8.9                                     |
| 4                | 115.6 ± 25.4                               | 164.9 ± 65.7                                    | 148.3 ± 25.2                                    |
| 5                | 255.7 ± 60.3                               | 292.7 ± 80.3                                    | 186.1 ± 37.5                                    |
| 6                | 342.2 ± 52.2                               | 341.0 ± 86.6                                    | 253.9 ± 41.4                                    |
| 8                | 425.1 ± 100                                | 525.0 ± 100                                     | 472.1 ± 103.1                                   |
| 10               | 725.1 ± 94.3                               | 800.0 ± 135                                     | 675.0 ± 126.1                                   |

| Amine/ 1 Epoxide | Diameter (nm)<br><b>AB<sub>1</sub> nanoparticles<sup>a</sup></b> | M <sub>w, RI</sub><br>(g/mol) <sup>b</sup> | PDI <sup>c</sup> | M <sub>w</sub><br>(kg/mol) <sup>d</sup> |
|------------------|------------------------------------------------------------------|--------------------------------------------|------------------|-----------------------------------------|
| 1                | 30.71 ± 2.21                                                     | 3403                                       | 1.16             | 60.5 ± 3.5                              |
| 2                | 58.06 ± 6.20                                                     | 3445                                       | 1.16             | 81.5 ± 4.6                              |
| 3                | 82.61 ± 5.73                                                     | 3544                                       | 1.17             | 96.1 ± 4.9                              |
| 4                | 115.6 ± 12.5                                                     | 3860                                       | 1.18             | 112 ± 6                                 |
| 5                | 255.7 ± 26.9                                                     | 4005                                       | 1.18             | 187 ± 8                                 |
| 6                | 342.2 ± 42.2                                                     | 4267                                       | 1.21             | 222 ± 11                                |
| 8                | 425.1 ± 44.6                                                     | 4470                                       | 1.21             | 328 ± 15                                |
| 10               | 725.1 ± 94.3                                                     | 4887                                       | 1.22             | 525 ± 28                                |

- 5     **[00355]**       The first trial was employed with polymer (**AB**) and implemented 1 to 10 equivalents of amine functionalities to the pendant epoxide cross-linking entity. The resulting particles were characterized by transmission electron microscopy (TEM) that provides the actual size, and by dynamic light scattering (DLS), to obtain the hydrodynamic diameter as a representative measure of the particle under physiological conditions. Micrographs of representative nanoparticles are shown in Figure 2. It is also contemplated that reaction stoichiometry can be selected to utilize in excess of ten (10) equivalents, thereby providing microparticles, materials for use in tissue engineering and biogels in biomedical applications and devices.
- 10   **[00356]**       As illustrated in Figure 3, the particle size increase with a polynomial trend as the equivalents of amine rises. For example, two equivalents of amine yielded 58 nm particles, and five equivalents produced particles with 255 nm dimensions (Table 1). Synthesized linear polymers containing additional functionalities (**ABC** and **ABD**) were found to respond in the same way to the controlled intermolecular chain crosslinking conditions, as with polymer (**AB**) from the original trial, and well-defined nanoparticles were obtained (Table 1). As shown in Figure 4, characterization of the particles with <sup>1</sup>H NMR confirmed the nanoparticle formation for each trial with an increase of signals at 3.5 and 2.89 ppm corresponding to protons neighboring the secondary amine of the polyethylene glycol (PEG)-linker after successful crosslinking event. In particular, a shift in resonance from 2.86 to 2.89 ppm was observed due to the change of the primary amine to the secondary amine after cross-linking. As a consequence, the continuous increase in amine cross-linker equivalents not only extends the particle size, but it also introduces additional amine functionalities connected to short PEG linker that are available for further modification strategies.
- 20   **[00357]**       In one aspect, the invention relates to a method of preparing a degradable nanoparticle comprising the step of adding a polymer comprising at least one monomer residue having a structure represented by a formula:

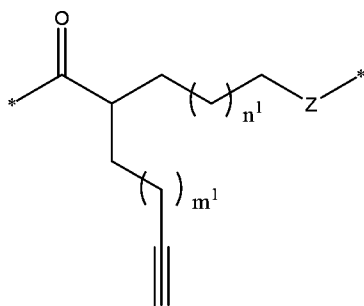


wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, wherein m is an integer from 0 to 6, and wherein n is an integer from 0 to 2; to a solution of from about 1 to about 10 molar equivalents of a dinucleophile (nucleophilic moiety:epoxide functionality) having a structure  
 5 X-R<sup>L</sup>-X', wherein X and X' are independently OH, SH, NH<sub>2</sub>, or NHR, wherein R is H or C1 to C6 alkyl, and wherein R<sup>L</sup> is selected from optionally substituted alkyl and optionally substituted alkoxylenes. In a further aspect, the monomer residue comprises less than about 10% by weight of the monomer residue of halogen selected from chlorine, bromine, and iodine. In a further aspect, Z is O.

- 10 **[00358]** In one aspect, the solution comprises from about 1 molar equivalent of a dinucleophile (nucleophilic moiety:epoxide functionality) and the resultant nanoparticle has a particle size of from about 5 nm to about 55 nm. In a further aspect, the solution comprises from about 1 molar equivalent of a dinucleophile (nucleophilic moiety:epoxide functionality) and the resultant nanoparticle has a particle size of from about 5 nm to about 55 nm. In a  
 15 further aspect, the solution comprises from about 2 molar equivalents of a dinucleophile (nucleophilic moiety:epoxide functionality) and the resultant nanoparticle has a particle size of from about 30 nm to about 80 nm. In a further aspect, the solution comprises from about 3 molar equivalents of a dinucleophile (nucleophilic moiety:epoxide functionality) and the resultant nanoparticle has a particle size of from about 70 nm to about 120 nm. In a further  
 20 aspect, the solution comprises from about 4 molar equivalents of a dinucleophile (nucleophilic moiety:epoxide functionality) and the resultant nanoparticle has a particle size of from about 110 nm to about 170 nm. In a further aspect, the solution comprises from about 5 molar equivalents of a dinucleophile (nucleophilic moiety:epoxide functionality) and the resultant nanoparticle has a particle size of from about 175 nm to about 300 nm. In a  
 25 further aspect, the solution comprises from about 6 molar equivalents of a dinucleophile

(nucleophilic moiety:epoxide functionality) and the resultant nanoparticle has a particle size of from about 250 nm to about 350 nm. In a further aspect, the solution comprises from about 8 molar equivalents of a dinucleophile (nucleophilic moiety:epoxide functionality) and the resultant nanoparticle has a particle size of from about 400 nm to about 550 nm. In a further aspect, the solution comprises from about 10 molar equivalents of a dinucleophile (nucleophilic moiety:epoxide functionality) and the resultant nanoparticle has a particle size of from about 650 nm to about 850 nm. It is also contemplated that reaction stoichiometry can be selected to utilize in excess of ten (10) molar equivalents, thereby providing higher particle sizes.

10 [00359] In one aspect, the invention relates to a method of preparing a degradable nanoparticle comprising the step of adding a polymer comprising at least one monomer residue having a structure represented by a formula:



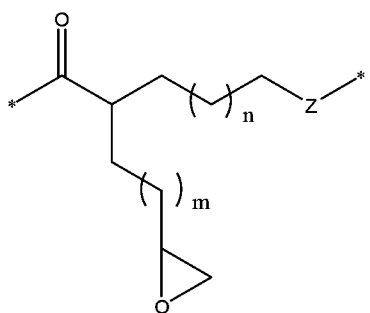
wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, wherein  $m^1$  is an integer from 0 to 6, and wherein  $n^1$  is an integer from 0 to 2; to a solution of from about 1 to about 10 molar equivalents of a bis-azide (azide moiety:alkyne functionality) having a structure  $N_3-R^L-N_3$ , wherein  $R^L$  is selected from optionally substituted alkyl and optionally substituted alkoxy. In a further aspect, the monomer residue comprises less than about 10% by weight of the monomer residue of halogen selected from chlorine, bromine, and iodine. In a further aspect, Z is O.

[00360] In one aspect, the invention relates to a method of controlling particle size during the preparation of a degradable nanoparticle comprising the step of adding an epoxide-functionalized polymer to a solution of a dinucleophilic cross-linker, wherein the stoichiometry of the cross-linker (ratio of nucleophilic moiety:epoxide functionality) is

selected to provide a desired particle size according to one or more of the graphs shown in Figure 5 – Figure 10.

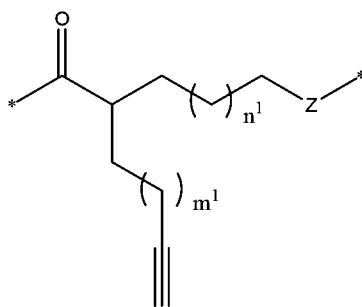
## 5. METHODS OF FUNCTIONALIZING NANOPARTICLES

[00361] In one aspect, the invention relates to a method of functionalizing a  
 5 nanoparticle comprising the step of reacting a nanoparticle comprising at least one epoxide-  
 functionalized monomer residue having an optionally substituted structure represented by a  
 formula:



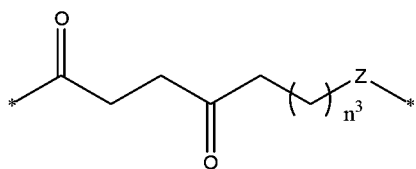
wherein Z is O or NR, wherein R is H or C1 to C6 alkyl; wherein m is an integer from 0 to 6;  
 10 wherein n is an integer from 0 to 2; with a nucleophile having a structure represented by a  
 formula X-R<sup>1</sup>, wherein X is OH, SH, NH<sub>2</sub>, or NHR, wherein R is H or C1 to C6 alkyl; and  
 wherein R<sup>1</sup> is an optionally substituted organic radical comprising 1 to 24 carbon atoms. In a  
 further aspect, R<sup>1</sup> is further substituted with at least one biologically active agent, at least one  
 pharmaceutically active agent, and/or at least one imaging moiety.

15 [00362] In one aspect, the invention relates to a method of functionalizing a  
 nanoparticle comprising the step of reacting a nanoparticle comprising at least one propargyl-  
 functionalized monomer residue having an optionally substituted structure represented by a  
 formula:



wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, wherein  $m^1$  is an integer from 0 to 6,  
and wherein  $n^1$  is an integer from 0 to 2; with an azide having a structure represented by a  
formula  $N_3-R^1$ , wherein  $R^1$  is an optionally substituted organic radical comprising 1 to 24  
5 carbon atoms. In a further aspect,  $R^1$  is further substituted with at least one biologically  
active agent, at least one pharmaceutically active agent, and/or at least one imaging moiety.

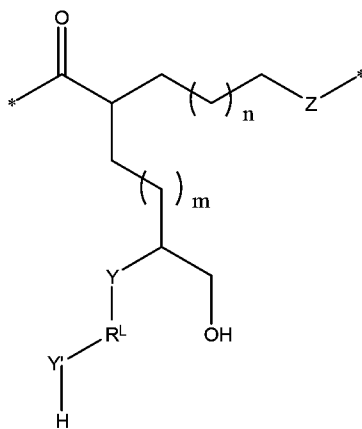
**[00363]** In one aspect, the invention relates to a method of functionalizing a  
nanoparticle comprising the steps of reacting a nanoparticle comprising at least one keto-  
functionalized monomer providing a residue having an optionally substituted structure  
10 represented by a formula:



wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, and wherein  $n^3$  is an integer from 0  
to 2; with an amine having a structure represented by a formula  $H_2N-R^1$ , wherein  $R^1$  is an  
optionally substituted organic radical comprising 1 to 24 carbon atoms; and reducing the  
15 resulting imine. In a further aspect, the reacting step and the reducing step are performed  
simultaneously. In a further aspect,  $R^1$  is further substituted with at least one biologically  
active agent, at least one pharmaceutically active agent, and/or at least one imaging moiety.

**[00364]** In one aspect, the invention relates to a method of functionalizing a  
nanoparticle comprising the step of reacting a nanoparticle comprising at least one

nucleophile-functionalized monomer residue having an optionally substituted structure represented by a formula:



wherein Z is O or NR, wherein R is H or C1 to C6 alkyl; wherein m is an integer from 0 to 6;  
 5 wherein n is an integer from 0 to 2; wherein Y and Y' are independently O, S, or NR, wherein R is H or C1 to C6 alkyl; and wherein R<sup>L</sup> is selected from optionally substituted alkyl and optionally substituted alkoxy; with an electrophile having a structure represented by a formula E-R<sup>1</sup>, wherein E is an electrophilic moiety; and wherein R<sup>1</sup> is an optionally substituted organic radical comprising 1 to 24 carbon atoms. In a further aspect, Y' is NH<sub>2</sub> or  
 10 NHR. In a further aspect, Y=Y'. In a further aspect, the electrophilic moiety is selected from alkyl halide, alkyl pseudohalide, and carboxyl derivative. In a further aspect, R<sup>1</sup> is further substituted with at least one biologically active agent, at least one pharmaceutically active agent, and/or at least one imaging moiety.

## 6. METHODS OF DEGRADING NANOPARTICLES

15 [00365] In one aspect, the invention relates to a method of degrading a degradable nanoparticle comprising subjecting the nanoparticle to reaction conditions sufficient to hydrolyze an ester. In a further aspect, the conditions are biological conditions. In a further aspect, the conditions involve exposure to an esterase. In a further aspect, the conditions exist within an organism.

[00366] In one aspect, the invention relates to a method of degrading a degradable polymer comprising subjecting the polymer to reaction conditions sufficient to hydrolyze an ester. In certain aspects, the degradable polymer is a disclosed polymer or a product of a disclosed method.

5                   **7. METHODS OF INTRACELLULAR DELIVERY**

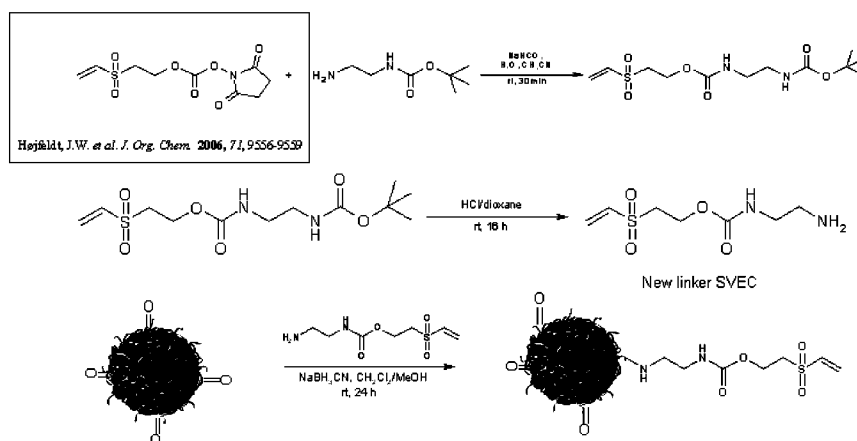
[00367] In one aspect, the invention relates to a method of intracellular delivery comprising administering an effective amount of a disclosed nanoparticle to a subject. In a further aspect, the nanoparticle is further substituted with at least one biologically active agent, at least one pharmaceutically active agent, and/or at least one imaging moiety. In a  
10 further aspect, the method further comprises the step of degrading the nanoparticle.

[00368] In a further aspect, the invention relates to a method of intracellular delivery comprising administering an effective amount of a disclosed polymer or product of a disclosed method to a subject. In a further aspect, the polymer or product of a disclosed method is further substituted with at least one biologically active agent, at least one  
15 pharmaceutically active agent, and/or at least one imaging moiety. In a further aspect, the method further comprises the step of degrading the polymer or product of a disclosed method.

**J. FUNCTIONALIZED POLYMERS AND NANOPARTICLES**

[00369] In one aspect, the disclosed nanoparticles can be functionalized with, for example, the disclosed dendrimeric compounds. That is, in one aspect, the invention relates  
20 to a nanoparticle-dendrimer conjugate. In a further aspect, the nanoparticle can be a disclosed organic quantum dots via intramolecular chain collapse. In a further aspect, the nanoparticle can be a disclosed degradable nanoparticle. In a further aspect, the dendrimer can be a disclosed intracellular delivery composition.

[00370] As disclosed herein, certain nanoparticles can bear electrophilic (e.g., ketone)  
25 functionalities. Vinylsulfonyl functionality can be introduced to the disclosed nanoparticles. Thus, a vinylsulfonyl linker moiety was prepared that can be attached in a reductive amination procedure to a keto groups of the particle. The synthesis of such a linker appears in Scheme 1. It is understood that the alkyl chain can be homologated by selection of appropriate reagents.

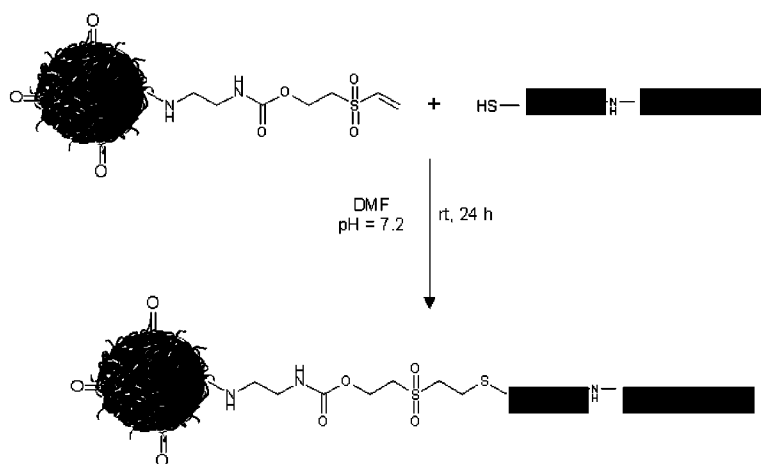


**Scheme 1.** Synthesis of SVEC (vinylsulfonyl-ethyl carbonate) linker and attachment to the particle

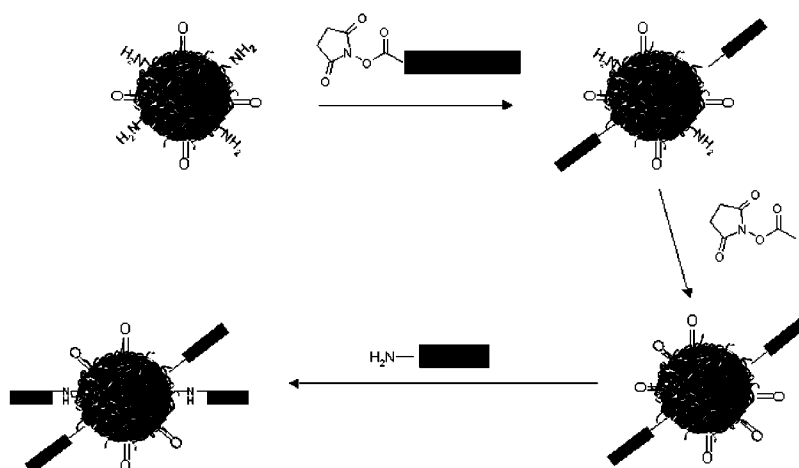
[00371] The vinylsulfonyl moiety readily reacts with a nucleophile (e.g., a thiol) to form a covalent bond, thereby further functionalizing a nanoparticle. These linkers can be used to attach peptides that are labeled with dye molecules at the focal point of the peptide or other amines groups of the peptide. The thiol groups of cysteines can be used to attach to the vinyl function of the vinyl sulfonyl linker. Also, the thiol group in the focal point of the disclosed dendritic molecular transporters can be attached to the vinyl sulfonyl (or allyl) group, thus allowing a transporter to be attached to any post-modified nanoparticle.

[00372] The same reaction can be used to attach peptides that are not labeled with dye. In such cases, the particle can be labeled with dye or not labeled.

[00373] Peptides (or other amines) can also be attached directly through the amine terminus of the peptide to the keto group through reductive amination. See Scheme 2. Here, it is preferred that the peptide contains only one amine group. Before the reductive amination is performed, the particle can be labeled with a dye that adds to the amine functionality of the particle. After the reaction, residual dyes can be quenched so as to not interfere with the following reductive amination.



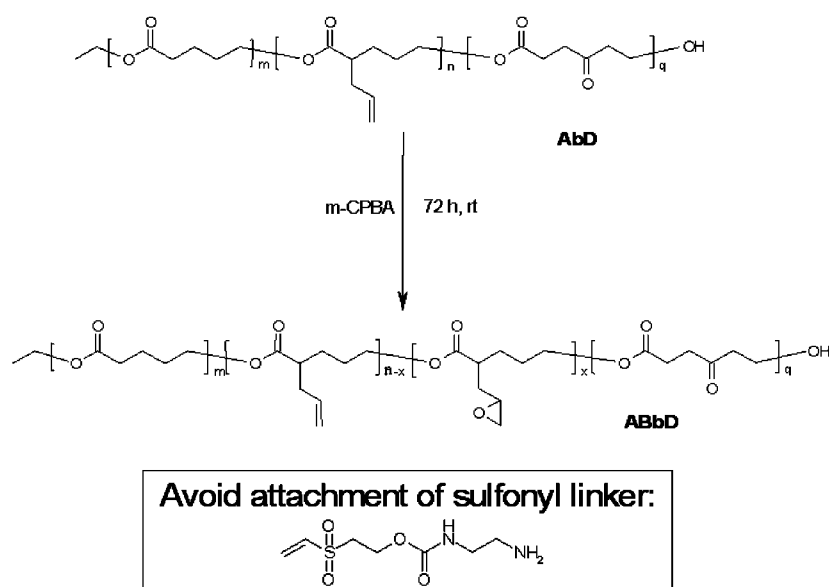
**Scheme 2.** Attachment of peptide with integrated thiol group from cysteins to linker modified particle



- 5 **Scheme 3.** Attachment of Alexa Fluor dye to free amine groups of the particle (NHS ester to amine) and quenching of the residual amines before reductive amination of amines of peptides (bioactive compounds) to the keto groups of the particle.

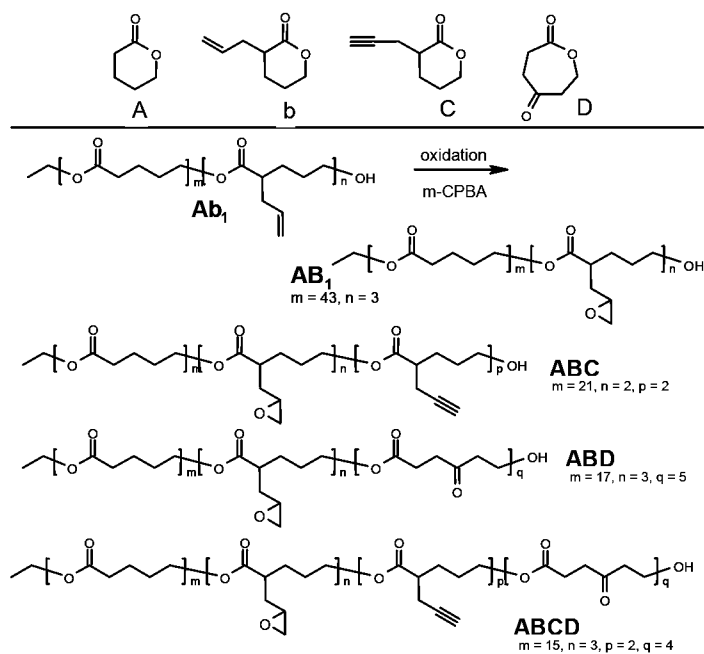
[00374] Similar systems can be constructed with particles from intramolecular cross-linking reactions. Replacing N-BED with an ethylenoxide equivalent enhances the solubility of the system.

[00375] Another approach that can enable formation of nanoparticle-dendrimer conjugate involves direct attachment of nucleophile-functionalized moieties (e.g., peptides or disclosed intracellular delivery compositions) to an allylic function on disclosed degradable nanoparticles. As shown in Scheme 4, direct attachment of a thiol with an allyl functionalized polymer or nanoparticle can bypass use of the disclosed SVEC linker.



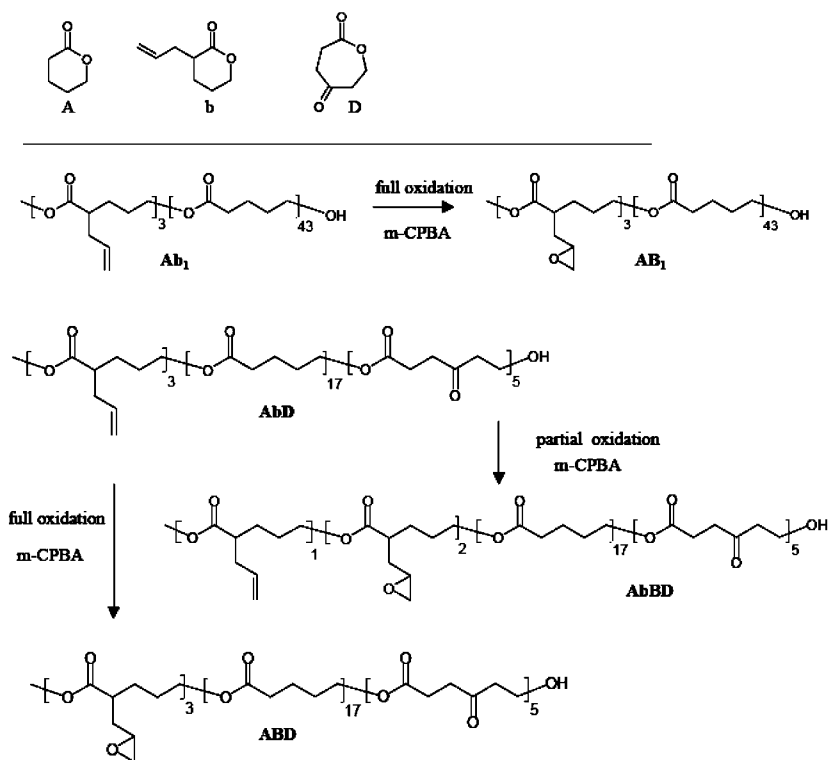
10 **Scheme 4.** Synthesis of allyl functionalized ABbD linear precursor

[00376] In one aspect, an allylic function on disclosed degradable nanoparticles can be provided via incomplete oxidation of epoxide functionalities, as shown in Scheme 5a.



Scheme 5a. Linear precursor.

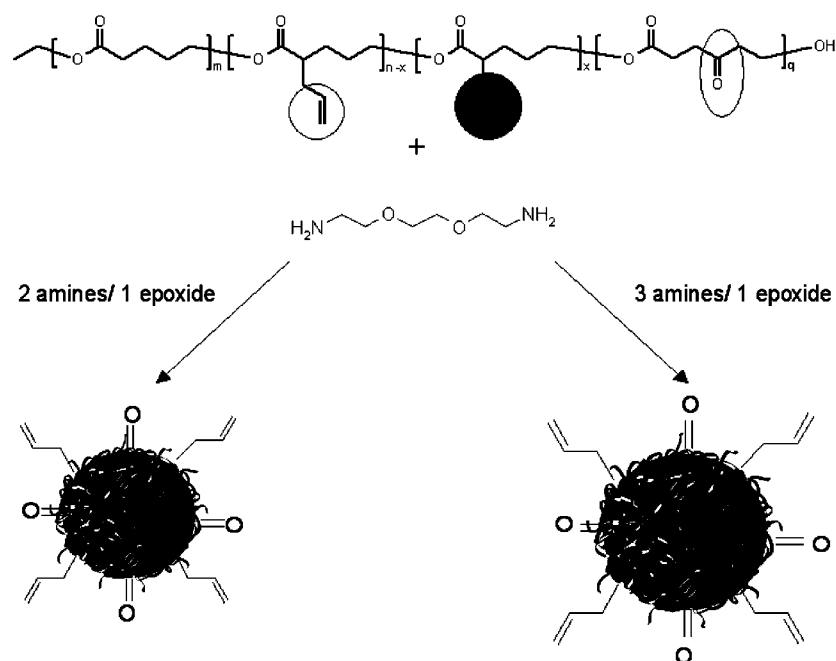
[00377] Still other examples of linear precursors can be prepared according to Scheme 5b.



**Scheme 5b.** Linear precursor.

[00378] An allyl functionality is thus available for functionalization and allows very mild conditions for the attachment of peptides and other moieties that contain nucleophilic (e.g., thiol) groups. The allyl groups from **Ab** linear precursors can be partially preserved by partial oxidation to the epoxide that is needed for cross-linking to the nanoparticle to form **AbB** linear polymer. This chemistry is also compatible with the keto-group-containing **ABD** linear precursor to form **AbBD**.

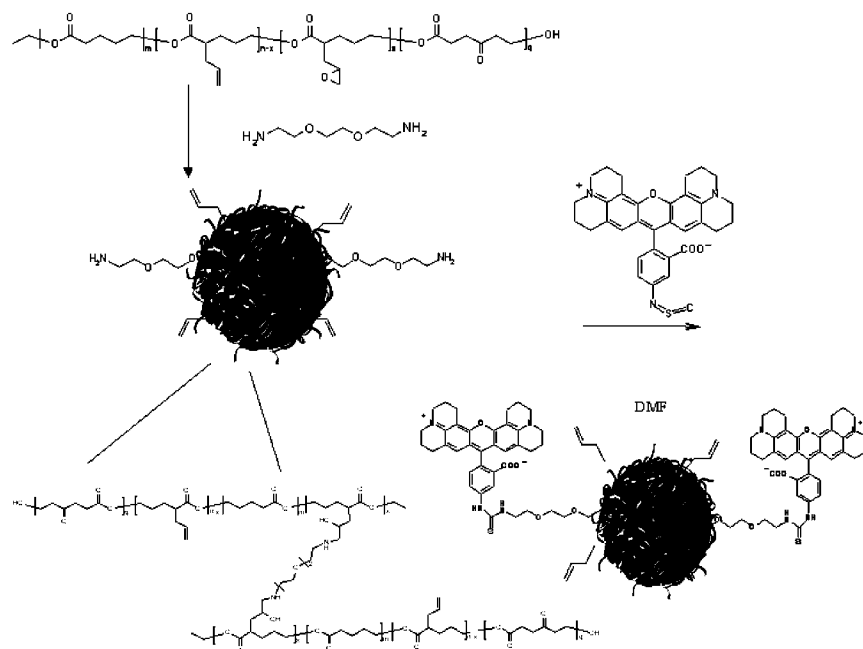
[00379] The nanoparticle formation does not take part in the cross-linking reaction and is therefore available for further modification. The allyl group is inert under the conditions used during the cross-linking process. The crosslinking reaction is illustrated in Scheme 6.



**Scheme 6.** Nanoparticle formation from allyl functionalized **ABbD** linear precursor with diamines.

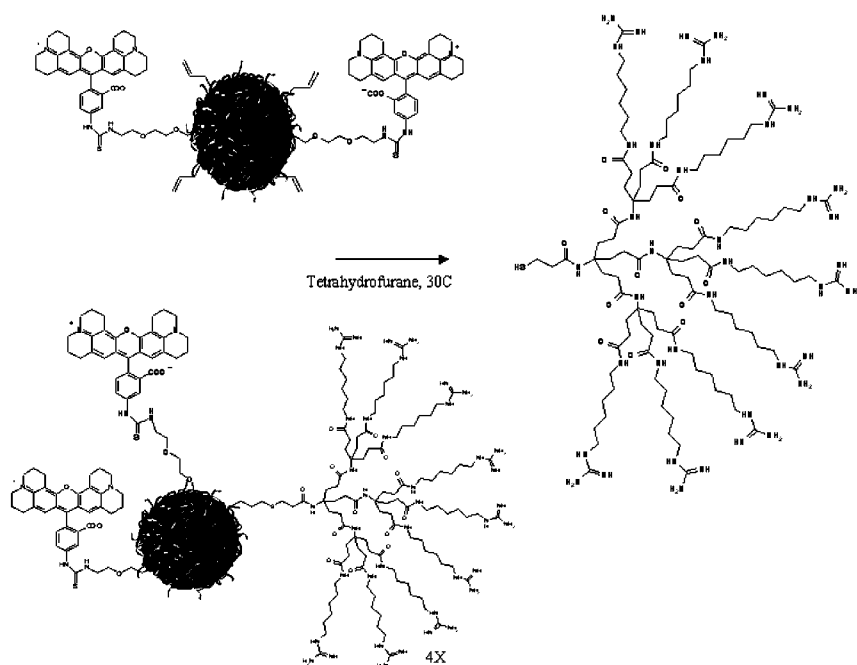
**[00380]** Again, the thiol group of the focal point of the dendritic molecular transporter  
 5 can be attached to the ally group. One advantage of such attachment is that it requires no  
 other reagent. This can permit the transporter to be attached to any already post-modified  
 nanoparticle because of the mild reaction conditions.

**[00381]** In order to track the drug delivery system and study the uptake into tissues, an  
 imaging moiety (e.g., a dye molecule such as rhodamine or other dye) that has functionality to  
 10 react with amines such as NHS- ester or isothiocyanates can be attached to the free amine  
 groups that result from the cross-linking reaction, as shown in Scheme 7. The allyl groups or  
 all other groups introduced are not affected.



**Scheme 7.** Nanoparticle formation from allyl functionalized ABbD linear precursor with diamines

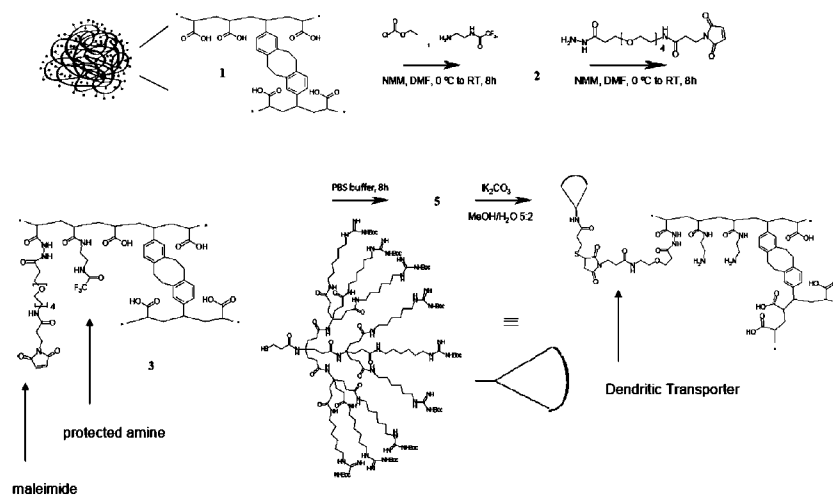
[00382] The allyl groups can then be reacted with thiol groups of the focal point of the  
 5 dendritic transporter, as illustrated in Scheme 8, thereby providing multiply functionalized  
 degradable nanoparticles.



**Scheme 8a.** Nanoparticle formation from allyl functionalized **ABbD** linear precursor with diamines

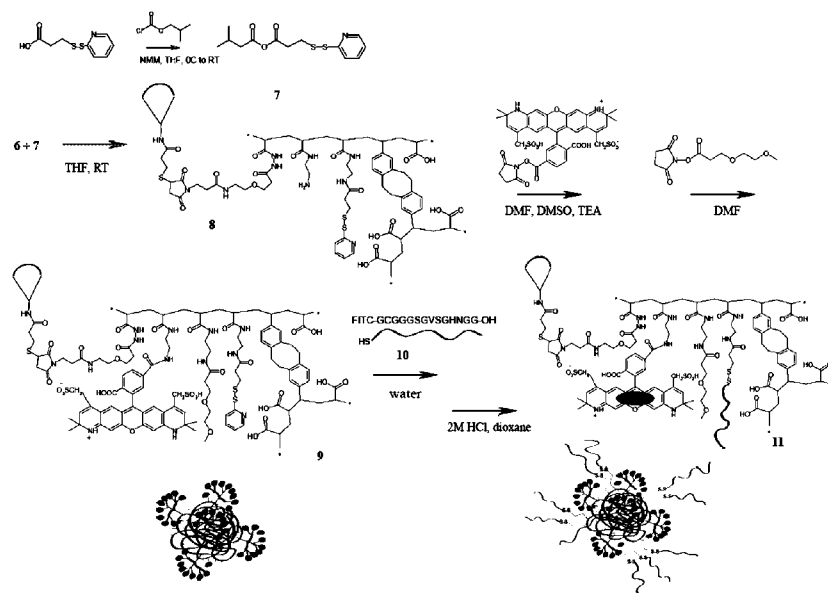
**[00383]** The number of molecular transporter(s) bonded to the nanoparticle can be selected by varying the stoichiometry of the reagents added to the allyl groups. The same reaction can be performed with thiol groups attached to peptides. It was found that elevated temperatures such as 37 °C speed up the reaction but do not destroy the peptide.

**[00384]** In a further aspect, a nanoparticle can be attached to a disclosed dendritic molecular transporter through an exemplary strategy shown in Scheme 8b.



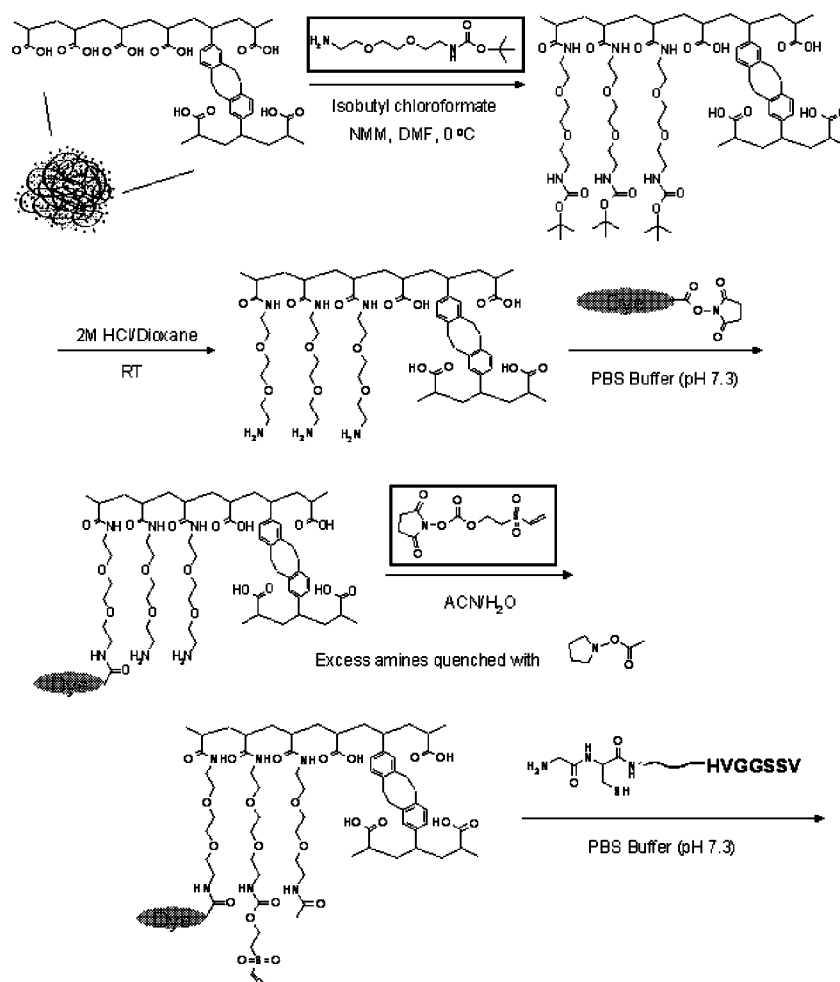
**Scheme 8b.** Strategy for attaching dendritic transporter to nanoparticle.

[00385] The dendritic transporter shown in Scheme 8 can be further functionalized according to Scheme 8c.



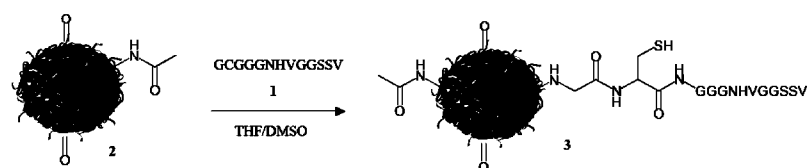
**Scheme 8c.** Sequential modification of collapsible nanoparticle.

[00386] The nanoparticle of the intramolecular chain collapse reaction can be reacted with the commercially available N-Boc ethylenoxide amine. The amine can be deprotected via acid cleavage with HCl or formic acid. Some of the free amines can be labeled with dye via NHS-ester reaction or thioisocyanide reaction. An SVEC moiety can then be connected through an NHS ester reaction. After the reaction the residual amine groups are being quenched. The thiol groups are attached to the vinylsulfone groups of the SVEC. The thiol groups of the molecular transporter can also be attached in the same fashion as the peptides, as shown in Scheme 9a.



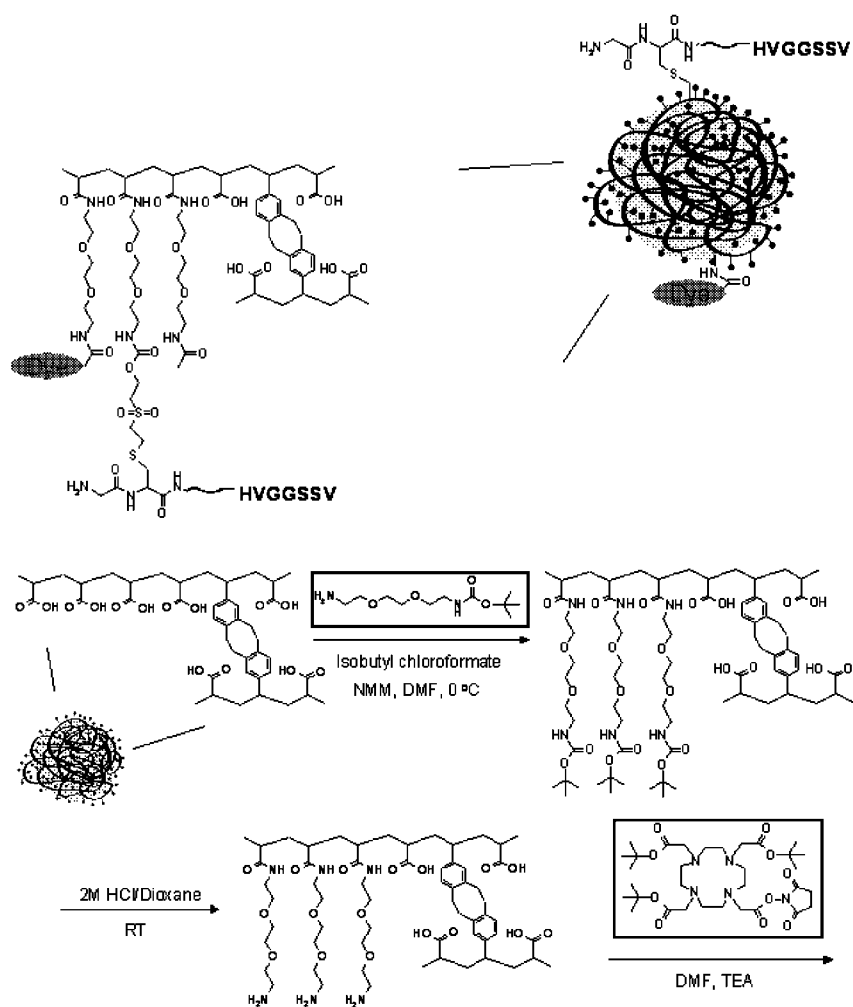
**Scheme 9a.** Attachment of the targeting peptide to the SVEC system.

[00387] Another example of attaching a peptide to a nanoparticle core is shown in Scheme 9b.

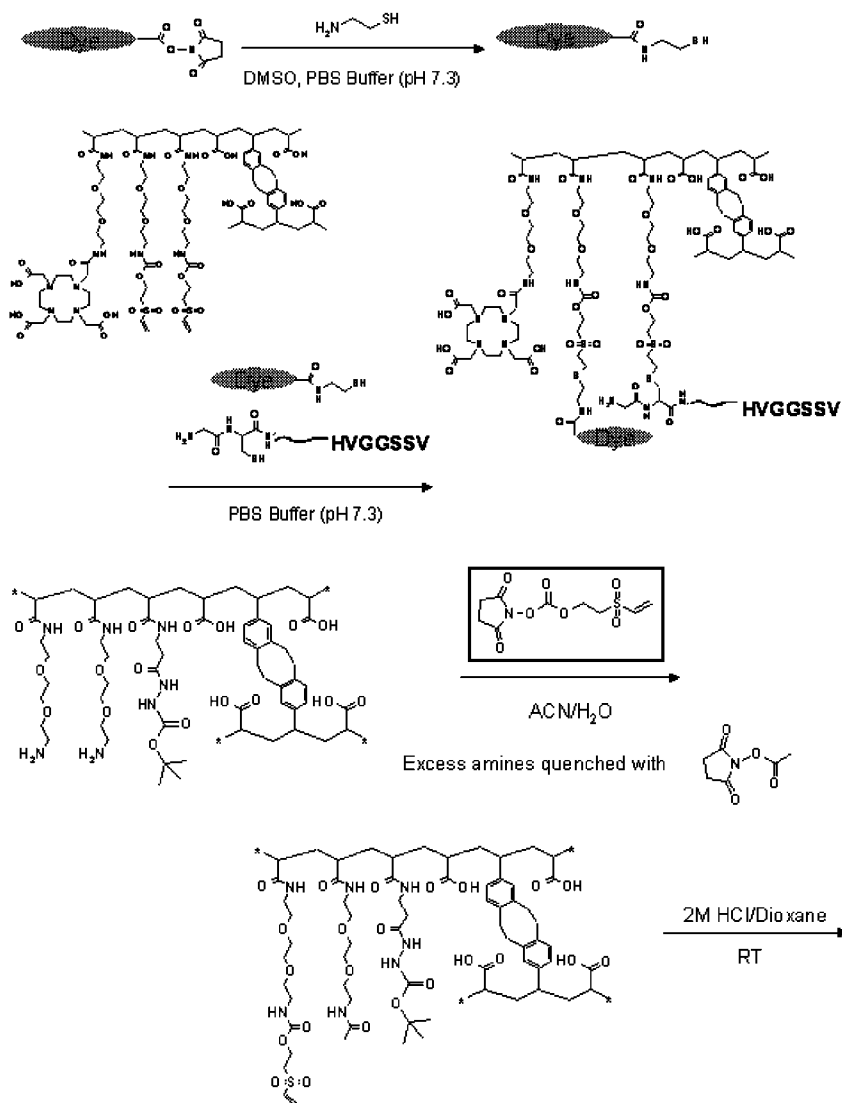


**Scheme 9b.** Attachment of the targeting peptide to a nanoparticle system.

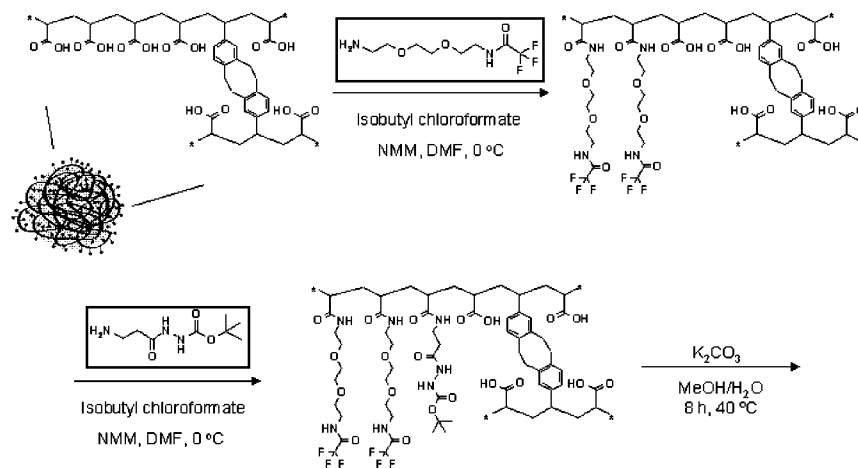
[00388] Imaging moieties (e.g., dyes or DOTA moieties) that can function as  
 5 therapeutic and tracking units can also be attached via a nucleophilic functionality, as shown  
 below.



**Scheme 10.** Functionalization of organic quantum dots via intramolecular chain collapse.



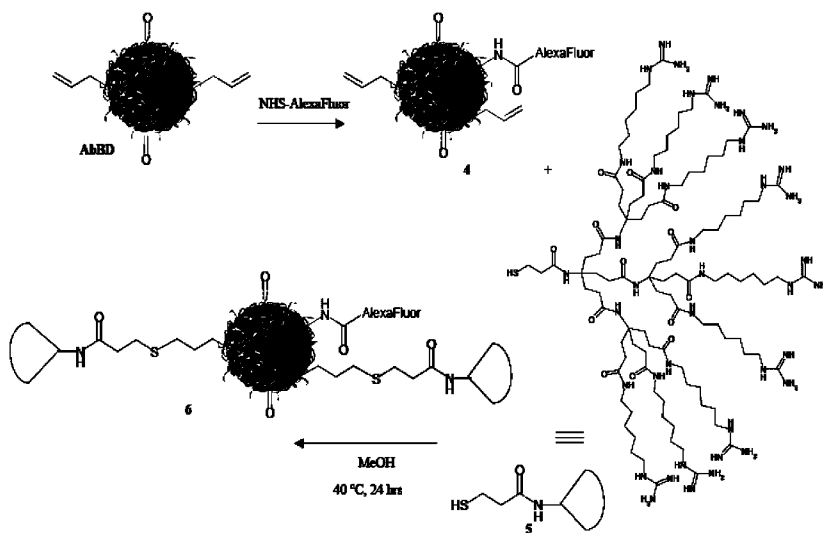
**Scheme 11.** Deprotection of triflate with base and attachment of SVEC followed by the deprotection of acylhydrazone linker.



Scheme 12a. DOTA instead of Dye.

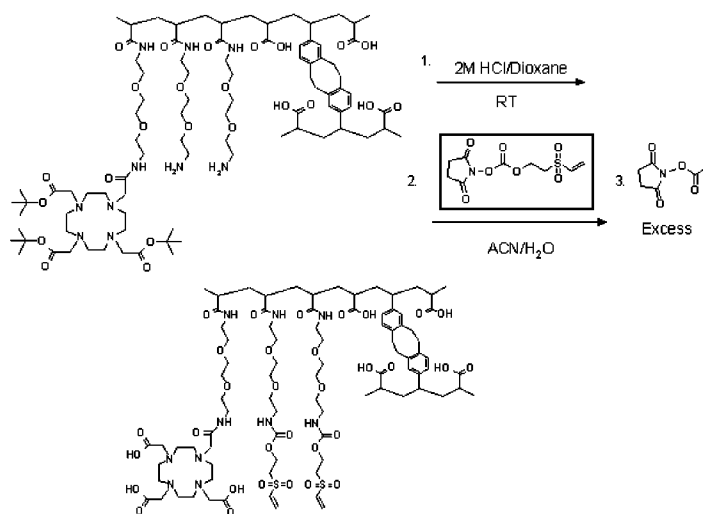
[00389] In a further aspect, a disclosed nanoparticle can be functionalized with a dye for imaging the eye in a subject. For example, such a method can be accomplished

5 conveniently by Scheme 12b.

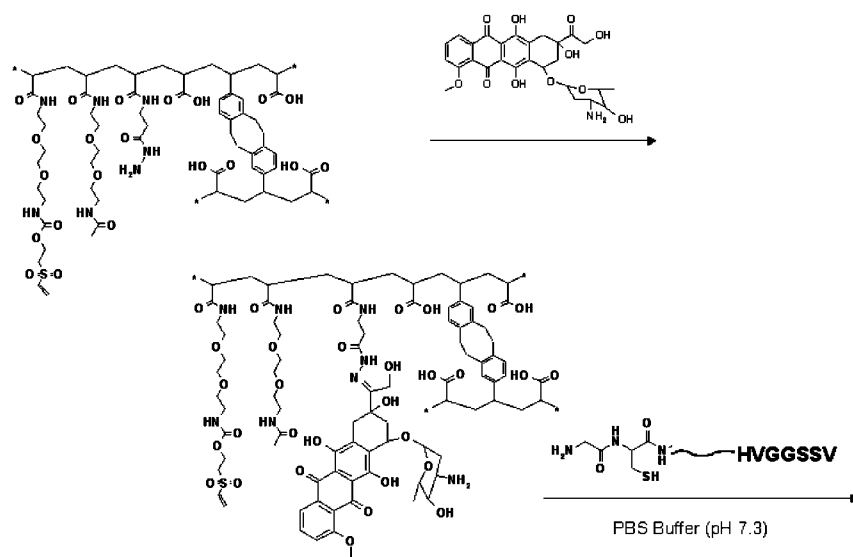


Scheme 12b. Synthesis of delivery of Imaging Reagents to the Eye for Testing.

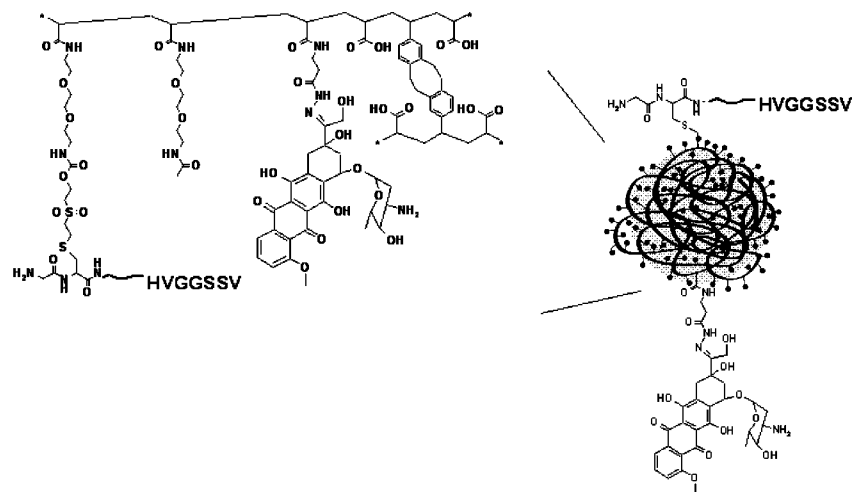
[00390] In a further aspect, analogous chemistry can be used to prepare a drug delivery system comprising a drug molecule that is attached to a pH sensitive linker and includes a hydrazide linker and doxorubicin. The synthesis is illustrated in Schemes 13-15.



5 **Scheme 13.** Attachment of two types of  $\beta$ -alanyl (Boc)hydrazide; and N-Tfa-ethyleneoxide diamine.

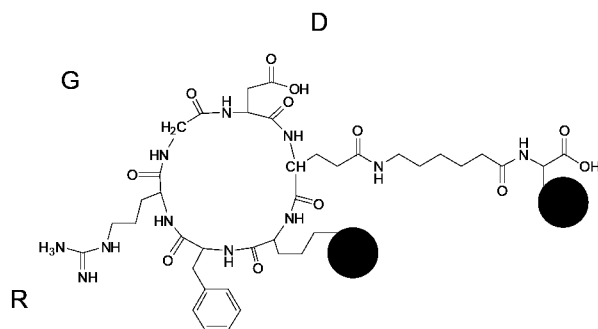


**Scheme 14.** Hydrazide linker formation of doxorubicine



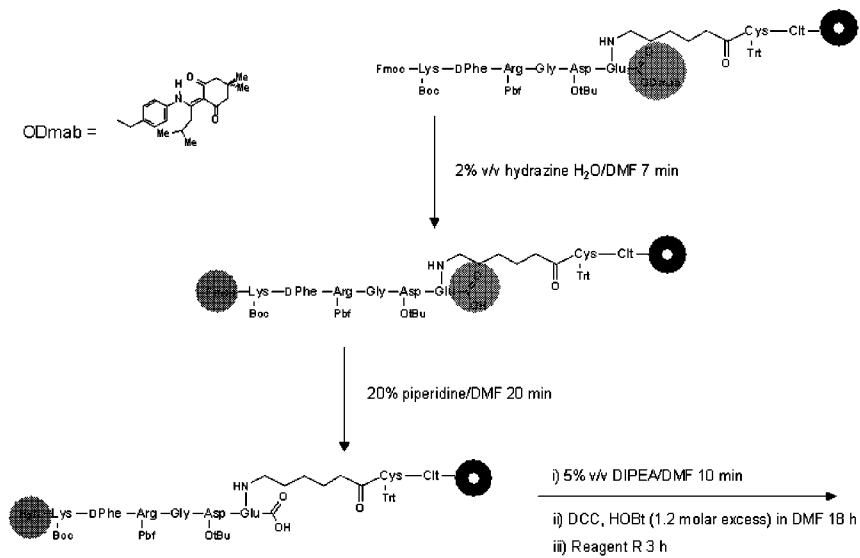
**Scheme 15.** Attachment of targeting unit; also c-RGD

[00391] A novel c-RGD has been prepared and can be attached to the nanoparticles and used for targeting of the disclosed delivery systems (See Scheme 16).



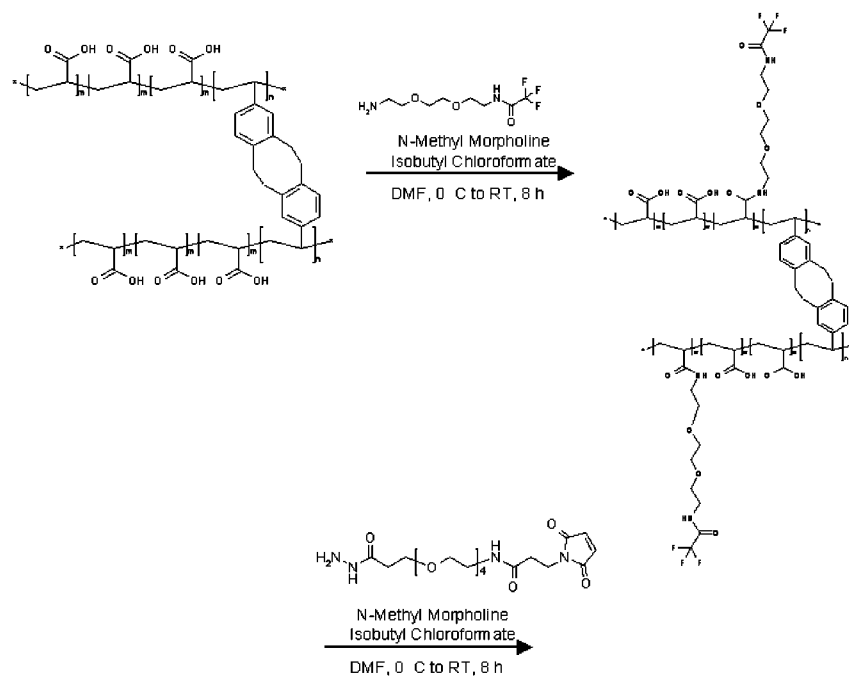
Scheme 16. c-RGD

5 [00392] The synthesis of the c-RGD that contains free amine and thiol unit for attachment to SVEC of the particle from the intra-molecular chain collapse and the SVEC or the allyl group of the polyester particles is detailed in Scheme 17.



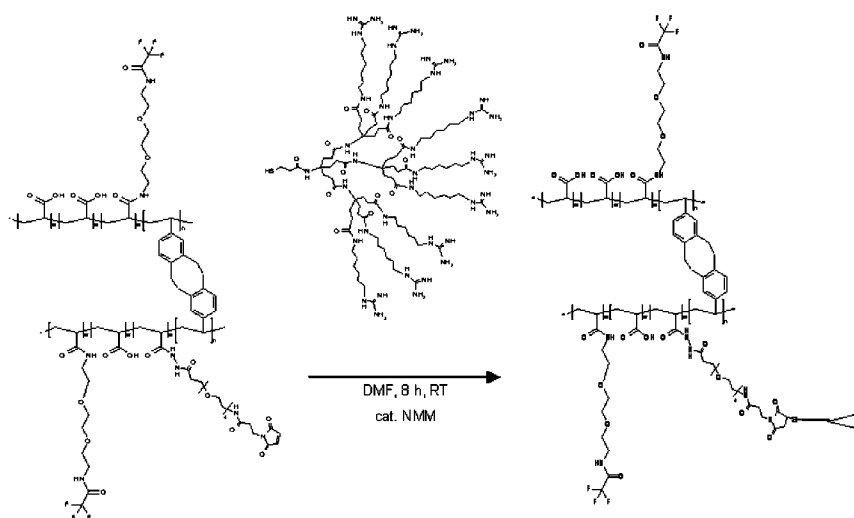
Scheme 17. Synthesis of C-RGD

[00393] The attachment of the molecular transporter to the maleimide of the intra-molecular chain collapse particle has also been investigated to create a system that transports peptides to intracellular location and across biological barriers. See Scheme 18.

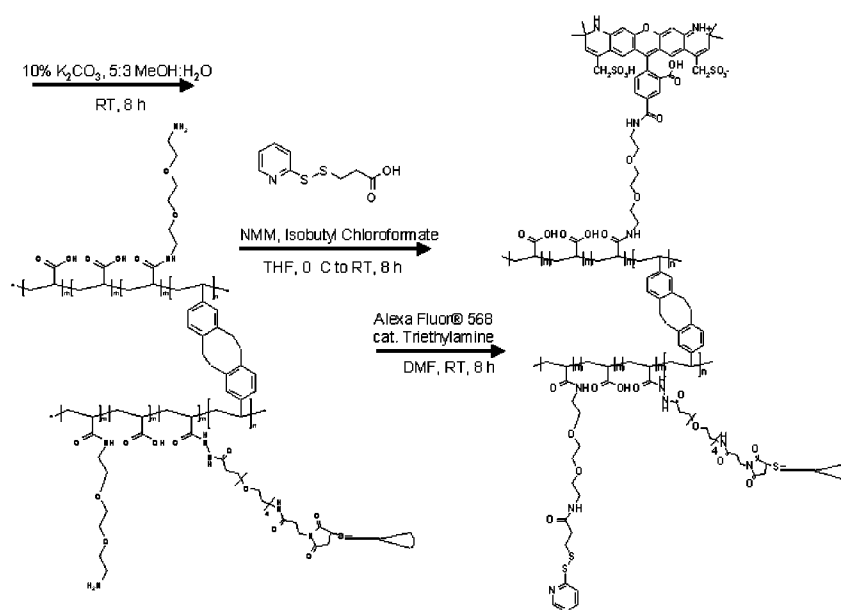


5 **Scheme 18.** Attachment of N-Tfa-ethylenedioxiide diamine linker and maleimide hydrazide to the nanoparticle.

[00394] Further modifications of the nanoparticle-dendrimer conjugate systems have also been investigated. See Schemes 19-20. The disclosed modifications, as well as analogous transformations, results in a collection of compounds available for use in  
10 intracellular transport.



**Scheme 19.** Attachment of dendritic moieties to organic quantum dots.



**Scheme 20.** Further modification of dendrimer functionalized organic quantum dots.

**K. MANUFACTURE OF A MEDICAMENT**

[00395] Also provided is a method for the manufacture of a medicament. In one aspect, the invention relates to a method for the manufacture of a medicament for delivery of a biologically active agent, a pharmaceutically active agent, and/or an imaging moiety comprising combining at least one disclosed polymer or at least one disclosed nanoparticle with a pharmaceutically acceptable carrier.

[00396] In a further aspect, the pharmaceutical composition relates to a composition for preventing and/or treating ophthalmic disorders.

**L. USES**

10 [00397] Also provided are uses of the disclosed polymers, nanoparticles, and products. In one aspect, the invention relates to a use of a disclosed polymer or a disclosed nanoparticle to deliver a biologically active agent, a pharmaceutically active agent, and/or an imaging moiety. The disclosed compounds, compositions, and conjugates and practical synthesis of same provide approaches for applications in cancer treatment and drug delivery across biological barriers such as the cornea, tissues, skin, and the blood brain barrier.

[00398] These degradable polymers find application in controlled release technologies that have to penetrate tissues and cellular membranes. Thus, the nanoparticle-dendrimer conjugates comprising a disclosed degradable nanoparticle and a disclosed intracellular delivery composition can hold and deliver therapeutics ranging from small molecules to larger peptides, proteins, and antibodies.

[00399] In a further aspect, the invention relates to a use of a disclosed polymer or a disclosed nanoparticle for transepithelial delivery of a biologically active agent, a pharmaceutically active agent, and/or an imaging moiety.

25 [00400] Many regions of the eye are relatively inaccessible to systemically administered agents. For example, orally administered agents pass through the liver before reaching estrogen sensitive tissues. Because the liver contains enzymes that can inactivate the agent, the agent that eventually reaches tissue targeted for treatment can be virtually ineffective. Moreover, systemic administration risks production of undesirable side effects. It can also be problematic to deliver a biologically active agent, a pharmaceutically active

agent, and/or an imaging moiety into the eye via invasive procedures such as injection. Further still, patient compliance can be low in cases of invasive administration.

[00401] As a result, topical drug delivery remains the preferred route of administration to the eye. There are a variety of factors that affect the absorption of drugs into the eye.

5 These factors include: the instillation volume of the drug, the frequency of instilled drug administration, the structure and integrity of the cornea, the protein level in tears, the level of enzymes in tears, lacrimal drainage and tear turnover rate, as well the rate of adsorption and absorption of a drug by the conjunctiva, sclera, and eyelids. A potential way of reducing or even eliminating systemic side effects is to improve ocular targeting that would allow for the  
10 use of reduced doses of the biologically active agent in the ophthalmic drug formation.

[00402] A major barrier to ocular drug penetration is the cornea. The cornea is composed of three layers: a lipid-rich epithelium, a lipid-poor soma, and a lipid-rich endothelium. Therefore, an agent must possess both lipophilic-hydrophilic balance for adequate transcorneal penetration and, thus, ocular bioavailability (Akers, H.J., "Ocular  
15 bioavailability of topically applied ophthalmic drugs," Am Pharm, NS23:33-36 (1983)).

[00403] Thus, in one aspect, the disclosed compounds provide improved physicochemical properties including, but not limited to, favorable ocular bioavailability and facile transcorneal penetration.

[00404] In another aspect, the disclosed compounds treat and/or protect against various  
20 ocular diseases. That is, the disclosed compounds can be used to diagnose, prevent, and/or treat ophthalmic disorders. Preferred disclosed compounds can be effective in treating and/or preventing maladies associated with vision-threatening intraocular damage due to pathophysiological predispositions. Preferred disclosed compounds include those which treat retinal infection, glaucoma, and/or macular degeneration.

#### 25 M. PHARMACEUTICAL COMPOSITIONS

[00405] In one aspect, the invention relates to pharmaceutical compositions comprising the disclosed compositions. That is, a pharmaceutical composition can be provided comprising a therapeutically effective amount of one or more disclosed polymer and/or one or more products of a disclosed method and/or one or more disclosed nanoparticle and a

pharmaceutically acceptable carrier for administration in a mammal. In a further aspect, the one or more disclosed polymer and/or one or more products of a disclosed method and/or the one or more disclosed nanoparticle is further substituted with at least one biologically active agent, at least one pharmaceutically active agent, and/or at least one imaging moiety.

- 5 [00406] The disclosed pharmaceutical compositions can further comprise other therapeutically active compounds, which are usually applied in the treatment of the above mentioned pathological conditions. It is understood that the disclosed compositions can be employed in the disclosed methods of using.

#### N. KITS

- 10 [00407] Also provided are kits related to the disclosed compositions. In one aspect, the invention relates to a kit comprising at least one disclosed polymer, at least one disclosed nanoparticle or at least one product of a disclosed method. It is understood that the disclosed kits can be used in connection with the disclosed methods of using.

- [00408] Also provided are microparticles, and/or larger networks, for use as materials  
15 for tissue engineering and biogels in biomedical devices.

#### O. EXPERIMENTAL

- [00409] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how the compounds, compositions, articles, devices and/or methods claimed herein are made and evaluated, and are intended to  
20 be purely exemplary of the invention and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers (e.g., amounts, temperature, etc.), but some errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, temperature is in °C or is at ambient temperature, and pressure is at or near atmospheric.

#### 25 1. CHARACTERIZATION METHODS

- [00410] <sup>1</sup>H NMR spectra were obtained from a Bruker AC300 Fourier Transform Spectrometer, with CDCl<sub>3</sub> in TMS as the solvent. <sup>13</sup>C NMR spectra were obtained from a Bruker AC400 Fourier Transform Spectrometer with CDCl<sub>3</sub> as the solvent.

[00411] Gel-permeation chromatography (GPC) was performed on a Waters chromatograph equipped with a Waters 2414 refractive index detector, a Waters 2481 dual  $\lambda$  absorbance detector, a Waters 1525 binary HPLC pump, and four 5 mm Waters columns (300 mm x 7.7 mm), connected in series with increasing pore size (100, 1000, 100,000 and  
5 1,000,000 Å respectively). All runs were performed with tetrahydrofuran (THF) as the eluent at a flow rate of 1 mL/min.

[00412] For dynamic light scattering (DLS) a Zetasizer Nano Series instrument with a CGS-3 compact goniometer system by Malvern Instruments (Malvern Zetasizer Nanoseries, Malvern, UK) was employed at a fixed angle of 90° at 25 °C, taking the average of three  
10 measurements. The particles were diluted with toluene to a concentration of 5-6 mg/mL, which gave the desired number of counts in order to obtain a good signal-to-noise ratio.

[00413] Samples for transmission electron microscopy (TEM) imaging were prepared by dissolving 0.5 mg nanoparticles in 1 mL isopropanol and 0.3 mL acetonitrile. The samples were sonicated for 5 min and were stained with 2 drops of 3% phosphotungstic acid. The  
15 carbon grids were prepared by placing a drop of dispersed particles onto an Ultrathin Carbon Type-A 400 Mesh Copper Grid (Ted Pella, Inc., Redding, CA) and drying at ambient temperature. A Philips CM20T transmission electron microscope operating at 200 kV in bright-field mode was used to obtain TEM micrographs of the polymeric nanoparticles.

[00414] Samples were centrifuged at 600 rpm on a Model CS International Centrifuge  
20 from International Equipment Company (Boston, MA).

## 2. MATERIALS

[00415] Reagent chemicals were purchased from Aldrich (Milwaukee, WI), EMD, Alfa-Aesar, Fisher Scientific, and Acros and used as received, unless otherwise stated. Spectra/Por® Dialysis membrane and SnakeSkin® Pleated Dialysis Tubing, regenerated  
25 cellulose, were purchased from Spectrum Laboratories Inc. and Pierce Biotechnology, respectively. Analytical TLC was performed on commercial Merck plates coated with silica gel GF254 (0.24 mm thick). Silica gel for flash chromatography was Merck Kieselgel 60 (230-400 mesh, ASTM) or Sorbent Technologies 60 Å (40-63µm, technical grade). *MAL-dPeg<sup>TM</sup><sub>4</sub>-t-boc-hydrazide* was obtained from Quanta Biodesign, Ltd. (Powell, OH) and used  
30 as received. Cy3 NHS dye and PD-10 Desalting columns were received from GE Healthcare

(Piscataway, NJ). Spectra/Por® Biotech Cellulose Ester (CE) Dialysis Membranes (1,000 MWCO) obtained from Spectrum Laboratories, Inc. (Rancho Dominguez, CA). SnakeSkin® Pleated Dialysis Tubing (10,000 MWCO) was obtained from Pierce Biotechnology, Inc. (Rockford, IL). Absolute molecular weight was determined with static light scattering.

### 5 3. SYNTHESIS OF A-ALLYL-A-VALEROLACTONE (AVL) (B)

[00416] A 500 mL round bottom flask, equipped with stir bar, was sealed with a septum, purged with nitrogen for 30 min and cooled in a dry ice/acetone bath. A solution of lithium diisopropylamine (2.0 M in THF/heptane/ethyl benzene, 33 mL, 66 mmol) was added to the round bottom flask. A nitrogen purged solution of  $\delta$ -valerolactone (5.43 mL, 60 mmol) in THF (60 mL) was added dropwise *via* syringe over 1.5 h. After an additional 30 min of stirring, a solution of allyl bromide (6.21 mL, 72 mmol) in hexamethylphosphoramide (12.51 mL, 72 mmol) was added dropwise *via* syringe over 30 min. The reaction mixture was warmed up to  $-40^{\circ}\text{C}$  using a dry ice/acetone bath and stirred for 3 h. The reaction was quenched with excess  $\text{NH}_4\text{Cl}$  solution and warmed to room temperature. The crude product was washed twice with brine, dried with anhydrous magnesium sulfate and concentrated via rotary evaporator. Column chromatography using  $\text{CH}_2\text{Cl}_2$  gave a viscous yellow product. Yield: 3.4262 g (41%).  $^1\text{H}$  NMR (300MHz,  $\text{CDCl}_3/\text{TMS}$ , ppm)  $\delta$ : 5.7 (m, 1H,  $\text{H}_2\text{C}=\text{CH}-$ ), 5.08 (m, 2H,  $\text{H}_2\text{C}=\text{CH}-$ ), 4.28 (m, 2H,  $-\text{C}(\text{O})\text{OCH}_2-$ ), 2.53-2.58 (m, 2H,  $\text{H}_2\text{C}=\text{CHCH}_2-$ ), 2.27 (m, 1H,  $\text{H}_2\text{C}=\text{CHCH}_2\text{CH}-$ ), 2.06 (m, 1H,  $\text{H}_2\text{C}=\text{CHCH}_2\text{CHCH}_2-$ ), 1.89 (m, 2H,  $\text{C}(\text{O})\text{OCH}_2\text{CH}_2-$ ), 1.55 (m, 1H,  $\text{H}_2\text{C}=\text{CHCH}_2\text{CHCH}_2-$ );  $^{13}\text{C}$  NMR (400MHz,  $\text{CDCl}_3$ , ppm)  $\delta$ : 173.8 ( $-\text{C}(\text{O})\text{O}-$ ), 135.0 ( $\text{H}_2\text{C}=\text{CH}-$ ), 117.4 ( $\text{H}_2\text{C}=\text{CH}-$ ), 68.4 ( $-\text{C}(\text{O})\text{OCH}_2-$ ), 39.2 ( $\text{H}_2\text{C}=\text{CHCH}_2\text{CH}-$ ), 35.4 ( $\text{H}_2\text{C}=\text{CHCH}_2-$ ), 24.0 ( $-\text{CH}_2\text{CH}_2\text{CH}_2-$ ), 21.9 ( $-\text{CH}_2\text{CH}_2\text{CH}_2-$ ).

### 4. SYNTHESIS OF COPOLYMER POLY(VL-AVL) (AB)

[00417] A 50 mL 3-necked round bottom flask, equipped with stir bar, was sealed with two septa and a gas inlet. The flask was evacuated and refilled with nitrogen three times. Stock solutions of 1.7 M ethanol (EtOH) in THF and  $3.7 \times 10^{-2}$  M tin(II) 2-ethylhexanoate ( $\text{Sn}(\text{Oct})_2$ ) in THF were made in sealed  $\text{N}_2$  purged flasks. Solutions of EtOH (0.32 mL,  $5.410 \times 10^{-1}$  mmol) and  $\text{Sn}(\text{Oct})_2$  (0.30 mL,  $1.12 \times 10^{-2}$  mmol) were combined in the nitrogen purged 50 mL flask. After stirring the mixture for 30 min,  $\alpha$ -allyl- $\delta$ -valerolactone (1.16 g, 8.32 mmol) and  $\delta$ -valerolactone (vl, 2.5 g, 24.97 mmol) were added. The reaction vessel

stirred in a 105°C oil bath for 48 h. Residual monomer and catalyst were removed by dialyzing with Spectra/Por® dialysis membrane (MWCO = 1000) against CH<sub>2</sub>Cl<sub>2</sub> to give a golden brown polymer. Yield: 3.2398 g (88%).  $M_w$  = 4834 Da, PDI = 1.17; <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>/TMS, ppm) δ: 5.7 (m, H<sub>2</sub>C=CH-), 5.09 (m, H<sub>2</sub>C=CH-), 4.09 (m, -CH<sub>2</sub>-O-), 3.65 (m, CH<sub>3</sub>CH<sub>2</sub>O-), 2.35 (m, v1, -CH<sub>2</sub>CH<sub>2</sub>C(O)O-, av1, H<sub>2</sub>C=CHCH<sub>2</sub>CH-, H<sub>2</sub>C=CHCH<sub>2</sub>CH-), 1.68 (m, av1 & v1, -CHCH<sub>2</sub>CH<sub>2</sub>-), 1.25 (t, CH<sub>3</sub>CH<sub>2</sub>O-); <sup>13</sup>C NMR (400MHz, CDCl<sub>3</sub>, ppm) δ: 174.6 (av1, -C(O)-), 172.7 (v1, -C(O)-), 134.6 (H<sub>2</sub>C=CH-), 116.4 (H<sub>2</sub>C=CH-), 63.3, 44.3, 35.9, 33.1, 27.5, 25.9, 23.6, 20.9.

#### 5. SYNTHESIS OF A-PROPARGYL-A-VALEROLACTONE (PVL) (C)

10 [00418] A 250 mL round bottom flask, equipped with stir bar, was sealed with a septum, purged with nitrogen for 30 min and cooled in a dry ice/acetone bath. A solution of lithium diisopropylamine (2.0 M in THF/heptane/ethyl benzene, 22 mL, 44 mmol) was added to the flask. A nitrogen purged solution of δ-valerolactone (3.62 mL, 40 mmol) in THF (40 mL) was added dropwise via syringe over 1.5 h. After an additional 30 min of stirring, a  
15 solution of propargyl bromide (4.34 mL, 48 mmol) in hexamethylphosphoramide (8.4 mL, 48 mmol) was added dropwise via syringe over 20 min. The reaction mixture was warmed up to -30°C using a dry ice/acetone bath and stirred for 3 h. The reaction was quenched with excess NH<sub>4</sub>Cl solution and warmed to room temperature. The crude product was washed twice with brine, dried with anhydrous magnesium sulfate and concentrated via rotary evaporator.  
20 Column chromatography with CH<sub>2</sub>Cl<sub>2</sub> gave a viscous yellow product. Yield: 2.8194 g (50.6%). <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>/TMS, ppm) δ: 4.35 (m, 2H, -C(O)OCH<sub>2</sub>-), 2.69 (m, 2H, -C(O)CHCH<sub>2</sub>C≡CH), 2.53 (m, 1H -C(O)CHCH<sub>2</sub>C≡CH), 2.29 (m, 1H, -CHCH<sub>2</sub>CH<sub>2</sub>-), 2.05 (s, 1H, HC≡CCH<sub>2</sub>-), 1.96 (m, 2H, -CHCH<sub>2</sub>CH<sub>2</sub>-), 1.74 (m, 1H, -CHCH<sub>2</sub>CH<sub>2</sub>-); <sup>13</sup>C NMR (400MHz, CDCl<sub>3</sub>, ppm) δ: 172.8, 80.8, 70.1, 68.5, 38.5, 23.8, 21.7, 20.4.

#### 25 6. SYNTHESIS OF COPOLYMER POLY (VL-AVL-PVL) (ABC)

[00419] A 50 mL 3-necked round-bottom flask, equipped with stir bar, was sealed with two septa and a gas inlet. The flask was evacuated and refilled with nitrogen three times. Stock solutions of 1.7 M ethanol in THF and 3.7x10<sup>-2</sup> M Sn(Oct)<sub>2</sub> in THF were made in sealed N<sub>2</sub> purged flasks. Solutions of ethanol (0.21 mL, 3.69x10<sup>-1</sup> mmol) and Sn(Oct)<sub>2</sub> (0.20  
30 mL, 5.41x10<sup>-3</sup> mmol) were combined in the nitrogen purged 50 mL flask. After stirring the

mixture for 30 min,  $\alpha$ -allyl- $\delta$ -valerolactone (0.8 g, 5.7 mmol),  $\delta$ -valerolactone (1.26 g, 12.6 mmol) and  $\alpha$ -propargyl- $\delta$ -valerolactone (0.63 g, 4.6 mmol) were added. The reaction vessel stirred in a 105°C oil bath for 48 h. Residual monomer and catalyst were removed by dialyzing with Spectra/Por<sup>®</sup> dialysis membrane (MWCO = 1000) against CH<sub>2</sub>Cl<sub>2</sub> to give a golden brown polymer. Yield: 2.25 g (84%).  $M_w$  = 3500 Da, PDI = 1.26; <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>/TMS, ppm)  $\delta$ : 5.71 (m, H<sub>2</sub>C=CH-), 5.03 (m, H<sub>2</sub>C=CH-), 4.08 (m, -CH<sub>2</sub>O-), 3.65 (m, CH<sub>3</sub>CH<sub>2</sub>O-), 2.55 (m, pvl, -C(O)CH-, -CHCH<sub>2</sub>C $\equiv$ CH), 2.45 (m, -CH<sub>2</sub>C $\equiv$ CH), 2.34 (m, vl, -CH<sub>2</sub>CH<sub>2</sub>C(O)O-, avl, H<sub>2</sub>C=CHCH<sub>2</sub>CH-, H<sub>2</sub>C=CHCH<sub>2</sub>CH-), 2.02 (m, pvl, -C $\equiv$ CH), 1.68 (m, pvl, avl & vl, -CHCH<sub>2</sub>CH<sub>2</sub>-), 1.259 (t, CH<sub>3</sub>CH<sub>2</sub>O-); <sup>13</sup>C NMR (400MHz, CDCl<sub>3</sub>, ppm)  $\delta$ : 174.6, 172.7, 133.6, 117.2, 80.7, 69.9, 63.3, 44.3, 35.9, 33.1, 27.5, 25.9, 23.6, 20.9.

#### 7. SYNTHESIS OF 2-OXEPANE-1,5-DIONE (OPD) (D)

[00420] A 100 mL round bottom flask, equipped with stir bar, was charged with 1,4-cyclohexanedione (2.0 g, 17.84 mmol) and 3-chloroperoxybenzoic acid (4.5 g, 26.08 mmol). Dichloromethane (22 mL) was added and the reaction mixture stirred and refluxed for 3 h at 40 °C. The reaction mixture was cooled to room temperature and dried with anhydrous MgSO<sub>4</sub>. Solvent was removed via rotary evaporation. The crude product was washed three times with cold diethyl ether (100 mL for each wash) and dried *in vacuo* at room temperature. Yield: 1.4814 g (64.7%). <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>/TMS, ppm)  $\delta$ : 4.4 (t, 2H, -C(O)OCH<sub>2</sub>CH<sub>2</sub>C(O)-), 2.84 (dd, 2H, -CH<sub>2</sub>C(O)O-), 2.72 (m, 4H, -CH<sub>2</sub>C(O)CH<sub>2</sub>-); <sup>13</sup>C NMR (400MHz, CDCl<sub>3</sub>, ppm)  $\delta$ : 204.9 (-C(O)-), 173.3 (-C(O)O-), 63.3 (-CH<sub>2</sub>O-), 44.7(-OCH<sub>2</sub>CH<sub>2</sub>C(O)-), 38.6 (-C(O)CH<sub>2</sub>CH<sub>2</sub>C(O)-), 27.9 (-CH<sub>2</sub>C(O)O-).

#### 8. SYNTHESIS OF COPOLYMER POLY(VL-AVL-OPD) (ABD)

[00421] To a 50 mL 3-necked round bottom flask, equipped with stir bar, condenser, nitrogen purge and septa, 2-oxepane-1,5-dione (0.6987 g, 5.45 mmol) and dry toluene (4 mL) was added. The mixture stirred in an oil bath at 70 °C to dissolve the monomer. Upon dissolving,  $\delta$ -valerolactone (1.5 g, 14.98 mmol),  $\alpha$ -allyl- $\delta$ -valerolactone (0.9546 g, 6.81 mmol), absolute ethanol (0.0205 g, 4.4 x 10<sup>-1</sup> mmol) and Sn(Oct)<sub>2</sub> (0.0119 g, 2.73x10<sup>-2</sup> mmol) were then added to the reactor and the mixture was heated for 48 h at 110 °C. Residual monomer and catalyst were removed by dialyzing with Spectra/Por<sup>®</sup> dialysis membrane (MWCO = 1000) against CH<sub>2</sub>Cl<sub>2</sub> to give a golden brown polymer. Yield: 2.6894 g (85%).  $M_w$

= 4858 Da, PDI = 1.27; <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>/TMS, ppm) δ: 5.72 (m, H<sub>2</sub>C=CH-), 5.06 (m, H<sub>2</sub>C=CH-), 4.34 (m, -CH<sub>2</sub>CH<sub>2</sub>C(O)CH<sub>2</sub>CH<sub>2</sub>O-), 4.08 (m, -CH<sub>2</sub>O-), 3.67 (m, -OCH<sub>2</sub>CH<sub>3</sub>), 2.78 (m, opd, -OC(O)CH<sub>2</sub>CH<sub>2</sub>C(O)CH<sub>2</sub>-), 2.58 (m, opd, -OC(O)CH<sub>2</sub>CH<sub>2</sub>C(O)CH<sub>2</sub>-), 2.34 (m, vl, -CH<sub>2</sub>CH<sub>2</sub>C(O)O-, avl, H<sub>2</sub>C=CHCH<sub>2</sub>CH-, H<sub>2</sub>C=CHCH<sub>2</sub>CH-), 1.66 (m, avl & vl, -CHCH<sub>2</sub>CH<sub>2</sub>-), 1.25 (t, -CH<sub>2</sub>CH<sub>3</sub>); <sup>13</sup>C NMR (400MHz, CDCl<sub>3</sub>, ppm) δ: 204.9, 175.2, 173.7, 173.2, 135.0, 117.0, 63.9, 44.8, 36.4, 33.6, 28.0, 26.3, 21.3.

### 9. SYNTHESIS OF COPOLYMER POLY(VL-AVL-PVL-OPD) (ABCD)

[00422] To a 25 mL 3-necked round bottom flask, equipped with stir bar, 2-oxepane-1,5-dione (0.2626 g, 2.05 mmol) was added and the flask was sealed with two septa and a gas inlet. The flask was evacuated and refilled with argon three times. Dry toluene (1.25 mL) was added and the mixture stirred in an oil bath at 70 °C to dissolve the monomer. Upon dissolving, Sn(Oct)<sub>2</sub> (0.0018 g, 4.41x10<sup>-3</sup> mmol in 0.15 mL dry toluene), absolute ethanol (12.8 μL, 2.22x10<sup>-1</sup> mmol), δ-valerolactone (0.62 g, 6.2 mmol), α-allyl-δ-valerolactone (0.38 g, 2.69 mmol), and α-propargyl-δ-valerolactone (0.38 g, 2.73 mmol) were added. The temperature of the oil bath was increased to 105 °C and the mixture stirred for 50 h. Residual monomer and catalyst were removed by dialyzing with Spectra/Por<sup>®</sup> dialysis membrane (MWCO = 1000) against CH<sub>2</sub>Cl<sub>2</sub> to give a golden brown polymer. Yield: 1.31 g (80%). M<sub>w</sub> = 3525 Da, PDI = 1.27; <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>/TMS, ppm) δ: 5.86 (m, H<sub>2</sub>C=CH-), 5.09 (m, H<sub>2</sub>C=CH-), 4.34 (m, opd, -CH<sub>2</sub>CH<sub>2</sub>C(O)CH<sub>2</sub>CH<sub>2</sub>O-), 4.08 (m, avl, pvl & vl, -CH<sub>2</sub>O-), 3.65 (m, -OCH<sub>2</sub>CH<sub>3</sub>), 2.74 (m, opd, -OC(O)CH<sub>2</sub>CH<sub>2</sub>C(O)-), 2.60 (m, opd, -CH<sub>2</sub>CH<sub>2</sub>C(O)CH<sub>2</sub>CH<sub>2</sub>-, pvl, -OC(O)CH-, -CHCH<sub>2</sub>C=CH), 2.50 (m, CHCH<sub>2</sub>C=CH), 2.34 (m, vl, -CH<sub>2</sub>CH<sub>2</sub>C(O)O-, avl, H<sub>2</sub>C=CHCH<sub>2</sub>CH-, H<sub>2</sub>C=CHCH<sub>2</sub>CH-), 2.02 (m, HC=C-), 1.68 (m, pvl, avl & vl, -CHCH<sub>2</sub>CH<sub>2</sub>-), 1.25 (m, -CH<sub>2</sub>CH<sub>3</sub>).

### 10. GENERAL PROCEDURE FOR OXIDATION OF COPOLYMERS

[00423] In a 200 mL round bottom flask, equipped with stir bar, poly(vl-avl) (2.7389 g, 6.12 mmol) was dissolved in 37 mL of CH<sub>2</sub>Cl<sub>2</sub>. To this solution, 3-chloroperoxybenzoic acid (2.0903 g, 12.11 mmol) was added slowly. The mixture was stirred for 72 h at room temperature and then concentrated via rotary evaporator. The crude product was dissolved in a minimal amount of THF (5 mL) and poured into a round-bottomed flask containing 1L diethyl ether. The solution was kept overnight at 0 °C and a white solid was obtained. The

solution was decanted off and the solid was dried *in vacuo* to obtain poly(vl-evl). Yield: 1.9467 g (71%). <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>/TMS, ppm) δ: The significant change is the disappearance of the allylic protons at 5.7 and 5.09 ppm and the appearance of small broad resonance peaks at 2.96, 2.75 and 2.47 ppm due to the formation of the epoxide ring. All other aspects of the spectrum are similar.

#### 11. GENERAL PROCEDURE FOR NANOPARTICLE FORMATION

[00424] In a 100 mL three-necked round bottom flask equipped with stir bar, condenser and septa, a solution of 2,2'-(ethylenedioxy)diethylamine (39.3 μL, 2.68x10<sup>-4</sup> mol) in 27.6 mL CH<sub>2</sub>Cl<sub>2</sub>. A solution of poly(vl-evl) (0.1330 g, M<sub>w</sub>= 4834 Da, PDI = 1.17) dissolved in CH<sub>2</sub>Cl<sub>2</sub> (0.18 mL) was added dropwise via a peristaltic pump at 13 mL/min with vigorous stirring. The mixture was heated at reflux for a total of 12 h. Residual diamine was removed by dialyzing with SnakeSkin<sup>®</sup> Pleated Dialysis Tubing (MWCO = 10,000) against dichloromethane. <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>/TMS, ppm) δ: The significant change is the disappearance of the epoxide protons at 2.96, 2.75 and 2.47 ppm and the appearance of signals at 3.5 and 2.89 ppm corresponding to the protons neighboring the secondary amine of the PEG linker after cross-linking. All other aspects of the spectrum are similar.

#### 12. DETERMATION OF AMINE CONTENT

[00425] Nanoparticles can be titrated with a strong acid to determine amine content. As shown in Table 2, several poly(vl-evl) (AB) nanoparticle samples were titrated with perchloric acid to determine the weight percentages (wt %) of primary amine and secondary amine in the three samples that we analyzed with transmission electron microscopy. The three samples (shown in Table 2) titrated have the following size dimensions by DLS: 58.06, 255.7 and 425.1 nm.

**Table 2:** Correlation of particle size and amine content

| AB Nanoparticle size (nm) | Primary amine wt% | Secondary amine wt% |
|---------------------------|-------------------|---------------------|
| 58.06                     | 0.008%            | 0.031%              |
| 255.7                     | 0.025%            | 0.098%              |
| 425.1                     | 0.055%            | 0.20%               |

25

### 13. NANOPARTICLES FORMED BY CO-POLYMERIZATION

[00426] While nanoparticles are typically prepared with a single type of polymer or copolymer, nanoparticles have also been successfully produced from a mixture of poly(vl-evl-pvl) and poly(vl-evl-opd). Such nanoparticles are tabulated in Table 3.

**Table 3:** Nanoparticles formed from two polymers

| Amine/1 Epoxide | Diameter (nm)<br>Poly(vl-evl-pvl) with<br>poly(vl-evl-opd) |
|-----------------|------------------------------------------------------------|
| 4               | $43.7 \pm 4.50$                                            |
| 8               | $94.15 \pm 6.85$                                           |

### 14. VARYING COMONOMER CONTENT

[00427] The properties of nanoparticles can be further tailored by incorporating different percentages of epoxy- $\delta$ -valerolactone (evl) into the polymer backbone. The data summarized in Table 4, below, shows the nanoparticles made from the linear poly(vl-evl) with 2% evl, 7% evl, and 19% evl. These data show that, as the % evl is decreased to 2% in the linear polymer, smaller nanoparticles can be obtained. As the % evl is increase to 19%, the resulting nanoparticles are larger but have a small deviation in comparison to the larger nanoparticles made from poly(vl-evl) with 7% evl.

**Table 4:** Effect of varying comonomer content

| Amine/ 1 Epoxide | Diameter (nm)<br>Poly(vl-evl)<br>2% evl | Diameter (nm)<br>Poly(vl-evl)<br>7% evl | Diameter (nm)<br>Poly(vl-evl)<br>19% evl |
|------------------|-----------------------------------------|-----------------------------------------|------------------------------------------|
| <b>AB</b>        |                                         |                                         |                                          |
| 3                | $7.02 \pm 1.05$                         | $82.1 \pm 5.73$                         | $179.9 \pm 18.0$                         |
| 4                | $19.04 \pm 1.32$                        | $115.6 \pm 25.4$                        | $225.6 \pm 22.5$                         |
| 5                | $33.55 \pm 1.93$                        | $255.7 \pm 60.3$                        | $299.0 \pm 31.2$                         |
| 6                | $48.66 \pm 3.18$                        | $342.2 \pm 52.2$                        | $409.1 \pm 42.7$                         |
| 8                | $84.89 \pm 10.47$                       | $425.1 \pm 100$                         | $843.3 \pm 88.0$                         |

[00428] The relationship between reaction stoichiometry and particle size for varying comonomer content is further illustrated in Figure 8 – Figure 10.

**15. ADDITION OF ETHYLENEDIAMINE 2-VINYLSULFONYL-ETHYL CARBONATE TO ABD (POLY(VL-EVL-OPD)) NANOPARTICLES**

5 [00429] In a 100 mL round bottom flask, equipped with stir bar, ABD nanoparticles (0.0846 g,  $2.45 \times 10^{-4}$  mmol) were dissolved in 12.5 mL of  $\text{CH}_2\text{Cl}_2$ . To this solution, ethylenediamine 2-(vinylsulfonyl)-ethyl carbonate in methanol (0.0152 g in 69  $\mu\text{L}$  methanol,  $5.89 \times 10^{-2}$  mmol) was added. Sodium cyanoborohydride (0.0111 g,  $1.76 \times 10^{-1}$  mmol) was dissolved in 12.5 mL methanol and added to the round bottom flask. The pH of the reaction  
10 mixture was adjusted to a pH of 6.5 with aqueous 1 M NaOH and 1 M HCl. The mixture was stirred for 25 h at room temperature and then dialyzed with SnakeSkin® Pleated Dialysis Tubing (MWCO = 10,000) against 1:1 dichloromethane/methanol. Successful attachment of the linker was observed by the appearance of signals 6.7 ppm and 6.9 ppm ( $^1\text{H}$  NMR, 300MHz,  $\text{CDCl}_3/\text{TMS}$ ) due to the vinyl protons of the linker.

15 **16. ATTACHMENT OF GV-13-ALEXAFLUOR 750 TO ABD NANOPARTICLES**

[00430] In a small vial, equipped with a stir bar, linker modified nanoparticles (L-ABD) (29.9 mg) were dissolved in 800  $\mu\text{L}$  PBS buffer (pH 7.2) and 700  $\mu\text{L}$  dimethylformamide. To this solution, 251  $\mu\text{L}$  GV-13-Alexafluor (0.44 mg in 150  $\mu\text{L}$  PBS buffer and 26.5  $\mu\text{L}$  DMF) was added to the vial via micropipette. After 45 min of stirring at  
20 room temperature, GV-13 (2.08 mg,  $1.9 \times 10^{-3}$  mmol) dissolved in 200  $\mu\text{L}$  PBS buffer was added. The reaction mixture stirred for 24 h in aluminum covered beaker. The resulting mixture was purified with concentrating tubes (MWCO = 10,000) to remove excess GV-13 and GV-13-Alexafluor. The purified product was concentrated via rotary evaporator. Successful attachment of peptide and dye was observed by the presence of a bright blue color  
25 due to the dye.  $^1\text{H}$  NMR also shows the presence of the peptide.

[00431] **General.** Commercial reagents were obtained from commercial sources (Aldrich, EMD, Alfa-Aesar, Fisher Scientific, and Acros) and used without further purification. Analytical TLC was performed on commercial Merck plates coated with silica gel GF254 (0.24 mm thick) and spots located by UV light (254 and 366nm). Silica gel for  
30 flash chromatography was Merck Kieselgel 60 (230-400 mesh, ASTM) or Sorbent

Technologies 60 Å (40-63µm, technical grade). *MAL-dPeg*<sup>TM</sup><sub>4</sub>-*t-boc-hydrazide* was obtained from Quanta Biodesign, Ltd. (Powell, OH) and used as received. Cy3 NHS dye and PD-10 Desalting columns were received from GE Healthcare (Piscataway, NJ). Spectra/Por<sup>®</sup> Biotech Cellulose Ester (CE) Dialysis Membranes (1,000 MWCO) obtained from Spectrum Laboratories, Inc. (Rancho Dominguez, CA). SnakeSkin<sup>®</sup> Pleated Dialysis Tubing (10,000 MWCO) was obtained from Pierce Biotechnology, Inc. (Rockford, IL).

[00432] **Instrumentation:** Samples were centrifuged at 600 rpm on a Model CS International Centrifuge from International Equipment Company (Boston, MA). Reverse-phase high performance liquid chromatography (RP-HPLC) was carried out with a Varian Prostar HPLC. The products were eluted using a solvent gradient (solvent A = 0.05% TFA/H<sub>2</sub>O; solvent B = 0.05% TFA/CH<sub>3</sub>CN). Nuclear magnetic resonance was performed on Bruker AC300 and AC400 Fourier Transform Spectrometers using deuterated solvents and the solvent peak as a reference. Gel permeation chromatography was performed in tetrahydrofuran (THF) with the eluent at a flow rate of 1 mL/min on a Waters chromatograph equipped with four 5 mm Waters columns (300 mm x 7.7 mm) connected in series with increasing pore size (100, 1000, 100,000 and 1,000,000 Å respectively). A Waters 2487 Dual λ Absorbance Detector and a 2414 Refractive Index Detector were employed. Dynamic light scattering was performed on a Malvern Zetasizer Nanoseries instrument with a CGS-3 compact goniometer system.

## 17. SYNTHESIS OF COMPOUND 1

[00433] To a solution of dimethoxyethane (40 mL) was added MeNO<sub>2</sub> (11.37 mL, 200 mmol) followed by Triton B (2 mL). The mixture was heated to 67 °C and then *tert*-butyl acrylate (91.83 mL, 620 mmol) was added to maintain the temperature at 75 °C. When the temperature started to decrease, additional Triton B (1 mL) was added. After the addition was completed, the solution was heated to maintain at 75 °C for 2 hours. The solvent was removed *in vacuo* and the residue was dissolved in CHCl<sub>3</sub> and the resulting organic solution was washed with 10% HCl, brine, and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. Removal of the solvent *in vacuo* gave a crude solid that was further purified by recrystallization from EtOH to obtain a colorless crystal (95% yield). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ1.44 (s, CH<sub>3</sub>, 27H), 2.21 (m, CH<sub>2</sub>, 12H). <sup>13</sup>C NMR (CDCl<sub>3</sub>): 27.93 (CH<sub>3</sub>), 29.68 (CH<sub>2</sub>CO), 30.22 (CCH<sub>2</sub>), 81.02 (CCH<sub>3</sub>), 92.09 (CNH<sub>2</sub>), 170.97 (CO<sub>2</sub>).

**18. SYNTHESIS OF COMPOUND 2**

[00434] A solution of compound 1 (6.0 g, 0.0135 mol) in a mixture of ethanol (140 mL) and dichloromethane (20 mL) was added to a Parr hydrogenation bottle. Then, 4 grams of Raney-nickel was added. The mixture was hydrogenated at 50 psi and room temperature.

5 The reaction was monitored by thin-layer chromatography (TLC) until the starting material disappeared. The catalyst was carefully filtered through Celite, and the solvent was removed in vacuo yielding a crude solid. The residue was dissolved in dichloromethane and washed with saturated NaHCO<sub>3</sub> and water, and then dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. Removal of dichloromethane gave a white solid (93%). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 1.44 (s, CH<sub>3</sub>, 27H), 1.95 (t, CH<sub>2</sub>, 6H), 2.43 (t, CH<sub>2</sub>, 6H); <sup>13</sup>C NMR (CDCl<sub>3</sub>): 27.98 (CH<sub>3</sub>), 29.46 (CH<sub>2</sub>CO), 31.47 (CCH<sub>2</sub>), 56.99 (CNH<sub>2</sub>), 80.96 (CCH<sub>3</sub>), 172.30 (CO<sub>2</sub>).

10

**19. SYNTHESIS OF COMPOUND 4**

[00435] To a solution of compound 3 (0.65 g, 2.35 mmol) in 50 mL dry THF the following reagents were added 1-hydrobenzotriazole (HOBt) (0.96 g, 7.10 mmol), DCC (1.46 g, 7.10 mmol) and then 2 (3.54 g, 8.5 mmol). The solution was stirred at room temperature

15 and the reaction was monitored by TLC. After 40 hrs, the white precipitate was filtered and the solution was concentrated to yield a crude residue. The product was purified by column chromatography (silica gel, hexane : ethyl acetate = 3:2) yielding a white solid (85%). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 1.44 (m, CH<sub>3</sub>, 81H), 1.95 (m, CH<sub>2</sub>, 18 H), 2.21 (m, CH<sub>2</sub>, 30H), 6.20 (s, NH, 3H); <sup>13</sup>C NMR (CDCl<sub>3</sub>): 28.04, 29.74, 29.85, 31.28, 57.56, 80.69, 92.47, 170.46, 172.76.

20

**20. SYNTHESIS OF COMPOUND 5**

[00436] A solution of compound 4 (1.47 g, 1 mmol) in 15 mL of formic acid was stirred at room temperature overnight. After the solution was concentrated, toluene was added and the solution was evaporated to remove any residue of formic acid to give a white

25 solid (100%). <sup>1</sup>H NMR (DMSO): δ 1.81 (m, CH<sub>2</sub>, 18 H), 2.11 (m, CH<sub>2</sub>, 30H), 7.29 (s, NH, 3H), 12.10 (br, COOH); <sup>13</sup>C NMR (DMSO): 28.03, 29.03, 30.08, 56.41, 93.31, 170.43, 174.42.

## 21. SYNTHESIS OF COMPOUND 6

[00437] To a solution of compound 5 (2.12 g, 0.0022 mol) in DMF (30 mL), HOBt (2.68 g, 0.0198 mol) and DCC (4.09 g, 0.0198 mol) were added. The mixture was chilled to 0°C with ice-water bath. Then, a solution of *N*-Boc-ethylenediamine (3.49g, 0.0218 mol) in 5 DMF (5 mL) was added dropwise at 0 °C. The reaction mixture was stirred at room temperature for 48 hrs. The solution was then filtered and 200 mL of dichloromethane was added, and washed with 1N HCl, saturated NaHCO<sub>3</sub>, and water. The organic phase was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated to yield a crude residue. The product was purified by column chromatography (eluted first with 2% methanol in dichloromethane, then with 6% 10 methanol in dichloromethane, followed by 10% methanol in dichloromethane) to obtain a white solid (51%). <sup>1</sup>H NMR (CD<sub>3</sub>OD): δ 1.44 (m, CH<sub>3</sub>, 81H), 1.80-2.10 (m, CH<sub>2</sub>, 48 H), 3.0-3.2 (m, CH<sub>2</sub>, 36H), 6.20 (m, NH, 3H), 6.46 (m, NH, 8H), 7.71(m, NH, 8H); <sup>13</sup>C NMR (CD<sub>3</sub>OD): 28.40, 31.24, 31.44, 31.80, 32.09, 40.66, 40.97, 59.14, 80.13, 94.42, 158.48, 173.48, 175.91. This white solid was then dissolved in 40 mL of 1, 4-dioxane. At 0 °C, 40 15 mL of 4 M HCl in dioxane was added to the solution under Ar atmosphere and stirred at room temperature for 1 hr. Removal of the solvent gave a white solid as the deprotected HCl salt (100%). <sup>1</sup>H NMR (D<sub>2</sub>O): δ 1.70-2.15 (m, CH<sub>2</sub>, 48 H), 3.30 (m, CH<sub>2</sub>, 18H), 3.36 (m, CH<sub>2</sub>, 18H); <sup>13</sup>C NMR (D<sub>2</sub>O): 27.61, 27.98, 28.86, 35.11, 37.41, 56.29, 92.01, 171.84, 174.98. 1.53 g (0.92 mmol) of the resulting HCl salt was dissolved in 80 mL of methanol. At 0°C, 3.5 mL 20 of Et<sub>3</sub>N was added to the solution, followed by the addition of N,N'-diBoc-N"-triflylguanidine (4.2 g, 10.73 mmol). The solution was stirred at room temperature for 24 hr. After removal of the solvent, the residue was dissolved in dichloromethane and washed with water, 1N HCl, saturated NaHCO<sub>3</sub>, and water. The organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and removed in vacuo. The residue product was purified by column chromatography 25 (eluted with 2% methanol in dichloromethane, then 10% methanol in dichloromethane) to give a white solid (90%) as compound 6. <sup>1</sup>H NMR (CD<sub>3</sub>OD): δ 1.45 (m, CH<sub>3</sub>, 81H), 1.51 (m, CH<sub>3</sub>, 81H), 1.90-2.25 (m, CH<sub>2</sub>, 48 H), 3.30-3.52 (m, CH<sub>2</sub>, 36H); <sup>13</sup>C NMR (CD<sub>3</sub>OD): 28.37, 28.67, 31.32, 31.67, 32.06, 39.74, 41.24, 59.02, 80.23, 84.35, 94.31, 153.91, 157.737, 164.38, 173.33, 175.87.

## 22. SYNTHESIS OF COMPOUND 7

[00438] To a solution of compound 5 (1.2, 0.001245 mol), HOBt (1.514 g, 0.0112 mol) and DCC (2.311 g, 0.0112 mol) were added in 20 mL of DMF. Then, N-Boc-1,6-diaminohexane (2.66g, 0.0123 mol) was dissolved in 5 mL of DMF dropwise at 0 °C. The solution was then stirred at room temperature for 48 hrs. The solution was then filtered and 200 mL of dichloromethane was added, and washed with 1N HCl, saturated NaHCO<sub>3</sub>, and water. The organic phase was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated to yield a crude residue. The product was purified by column chromatography (eluted first with 2% methanol in dichloromethane, then with 5% methanol in dichloromethane, followed by 10% methanol in dichloromethane) to obtain a white solid (45%). <sup>1</sup>H NMR (CD<sub>3</sub>OD): δ 1.2 – 1.6 (m, CH<sub>3</sub>, CH<sub>2</sub>, 153H), 1.80-2.10 (m, CH<sub>2</sub>, 48 H), 3.0-3.2 (m, CH<sub>2</sub>, 36H); <sup>13</sup>C NMR (CD<sub>3</sub>OD): 27.54, 28.85, 30.37, 30.90, 31.28, 31.60, 32.14, 40.58, 41.24, 59.13, 79.30, 94.30, 158.49, 173.50, 175.56. This white solid was then dissolved in 40 mL of 1, 4-dioxane. At 0°C, 40 mL of 4 M HCl in dioxane was added to the solution under Ar atmosphere and stirred at room temperature for 1 hr. Removal of the solvent gave a white solid as the deprotected HCl salt (100%). <sup>1</sup>H NMR (D<sub>2</sub>O): δ 1.10-1.60 (m, CH<sub>2</sub>, 72 H), 1.7–2.2 (m, CH<sub>2</sub>, 48H), 3.30 (m, CH<sub>2</sub>, 18H), 3.36 (m, CH<sub>2</sub>, 18H). 0.838 g (0.385 mmol) of the resulting HCl salt was dissolved in 80 mL of methanol. At 0 °C, 1.45 mL of Et<sub>3</sub>N was added to the solution, followed by the addition of N,N'-diBoc-N"-triflylguanidine (1.765 g, 4.51mmol). The solution was stirred at room temperature for 24 hr. After removal of the solvent, the residue was dissolved in dichloromethane and washed with water, 1N HCl, and water. The organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and removed in vacuo. The residue product was purified by column chromatography (eluted with 2% methanol in dichloromethane, then 10% methanol in dichloromethane) to give a white solid (90%) as compound 7. <sup>1</sup>H NMR (CD<sub>3</sub>OD): <sup>1</sup>H NMR of 9 (CD<sub>3</sub>OD): δ 1.15 - 1.55 (m, 234H), 1.70-2.15 (m, CH<sub>2</sub>, 48 H), 3.29-3.30 (m, CH<sub>2</sub>, 36H); <sup>13</sup>C NMR (CD<sub>3</sub>OD): 27.70, 27.62, 28.33, 28.67, 30.08, 30.33, 31.30, 31.60, 40.48, 40.62, 41.27, 54.5, 59.14, 80.25, 84.40, 154.22, 157.49, 164.53, 173.50, 175.53.

## 23. SYNTHESIS OF COMPOUND 8 AND 9

[00439] Compound 6 (or 7, 0.10 mmol) was dissolved in 40 mL of ethanol and transferred into a hydrogenation bottle containing 5 g of Raney-Nickel catalyst. The solution was hydrogenated at room temperature at 65 psi and monitored by TLC. The catalyst was

filtered through Celite. The solvent was removed in vacuo to give a white solid **8** or **9** (80%).

<sup>1</sup>H NMR of **8** (CD<sub>3</sub>OD): δ 1.46 (m, CH<sub>3</sub>, 81H), 1.51 (m, CH<sub>3</sub>, 81H), 1.90-2.25 (m, CH<sub>2</sub>, 48 H), 3.30-3.55 (m, CH<sub>2</sub>, 36H); <sup>13</sup>C NMR (CD<sub>3</sub>OD): 28.37, 28.67, 31.40, 31.76, 39.76, 41.27,

54.0, 58.86, 80.32, 84.37, 153.97, 157.81, 164.4, 175.61, 176.02. <sup>1</sup>H NMR of **9** (CD<sub>3</sub>OD):

5 δ 1.20 - 1.70 (m, 234H), 1.85-2.40 (m, CH<sub>2</sub>, 48 H), 3.10-3.50 (m, CH<sub>2</sub>, 36H); <sup>13</sup>C NMR (CD<sub>3</sub>OD): 27.01, 27.18, 28.27, 28.53, 29.42, 29.71, 30.15, 30.88, 31.19, 40.03, 41.23, 54.3, 58.21, 79.93, 83.84, 153.62, 156.65, 163.83, 175.77.

#### 24. SYNTHESIS OF COMPOUND FD-1

[00440] FITC (0.14 g, 0.36 mmol), dissolved in 1 mL of DMF, was added to a solution of compound **8** (0.23g, 0.066 mmol) in a mixture of DMF and dichloromethane. The solution was chilled to 0 °C, to which Et<sub>3</sub>N (0.092 mL, 0.66 mmol) was added. The mixture was stirred overnight at room temperature. After removal of DMF in vacuo, the residue was dissolved in dichloromethane and washed with 1N HCl and water. The dichloromethane layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated to obtain a yellow solid. <sup>1</sup>H NMR (CD<sub>3</sub>OD): δ 1.46 (m, CH<sub>3</sub>, 81H), 1.51 (m, CH<sub>3</sub>, 81H), 1.90-2.25 (m, CH<sub>2</sub>, 48 H), 3.30-3.55 (m, CH<sub>2</sub>, 36H), 6.52-6.72 (br, 4H), 7.15 (br, 1H), 7.5 (br, 2H), 7.72 (br, 1H), 8.4 (br, 1H). The resulting yellow solid (200 mg, 0.052 mmol) was dissolved in 10 mL of 1, 4-dioxane. At 0°C, 10 mL of 4 M HCl in dioxane was added to the solution under Ar protection and stirred at room temperature overnight. After evaporation of the solvent in vacuo, the product was dissolved in water and the insoluble precipitate was filtered. Removal of water yielded a crude yellow solid, which was further purified by RP-HPLC using a solvent gradient (solvent A = 0.05% TFA/H<sub>2</sub>O; solvent B = 0.05% TFA/CH<sub>3</sub>CN) to obtain compound 10. <sup>1</sup>H NMR (D<sub>2</sub>O): δ 1.85-2.30 (m, CH<sub>2</sub>, 48 H), 3.10-3.30 (m, CH<sub>2</sub>, 36H), 6.9 (br, 2H), 7.10-7.2 (m, 3H), 7.4 (s, 2H), 7.5 (br, 1H), 8.1 (s, 1H).

#### 25. SYNTHESIS OF COMPOUND FD-2

[00441] FITC (0.016 g, 0.0376 mmol), dissolved in 1 mL of DMF, was added to a solution of compound **9** (0.050g, 0.0125 mmol) in a mixture of DMF and dichloromethane (1:1). The solution was chilled to 0°C, to which Et<sub>3</sub>N (12 µL) was added. The mixture was stirred overnight at room temperature. After removal of DMF in vacuo, the residue was dissolved in dichloromethane and washed with 1N HCl and water. The dichloromethane

layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated to obtain a solid. The product was dissolved in methanol and purified by dialysis with Spectro<sup>®</sup> Por Biotech RC membranes (MWCO 3500). After removal of the methanol, a yellow solid was obtained. <sup>1</sup>H NMR (CD<sub>3</sub>OD): δ 1.20 – 1.7 (m, CH<sub>3</sub>, CH<sub>2</sub>, 234H), 1.89–2.30 (m, CH<sub>2</sub>, 48 H), 3.10–3.40 (m, CH<sub>2</sub>, 36H), 6.52–6.72 (br, 4H), 7.15 (br, 1H), 7.5–7.72 (br, 3H), 8.1 (br, 1H). The resulting yellow solid (200 mg, 0.052 mmol) was dissolved in 10 mL of 1, 4-dioxane. At 0 °C, 10 mL of 4 M HCl in dioxane was added to the solution under Ar protection and stirred at room temperature overnight. The precipitate was filtered out and dried in vacuo. The obtained yellow solid was dissolved in water and lyophilized to yield compound **11**. <sup>1</sup>H NMR (D<sub>2</sub>O): δ 1.1 – 1.50, (m, CH<sub>2</sub>, 72H), 1.50–2.20 (m, CH<sub>2</sub>, 48 H), 3.10–3.30 (m, CH<sub>2</sub>, 36H), 6.5–6.7 (br, 6H), 7.10 (m, 1H), 7.5 (br, 3H).

## 26. EXAMPLES FD-1 AND FD-2

[00442] As examples of the compounds of the invention, two non-peptidic fluorescently labeled Newkome-type dendrimers, differentiated over a varied alkylspacer with guanidine end moieties, were designed and synthesized. The assessment of internalization into mammalian cells using NIH-3T3 fibroblasts and human microvascular endothelial cells (HMEC) showed that the spacer length at the terminal generation of the dendrimers can affect direction of cargo molecules precisely into specific subcellular compartments (e.g., nucleus or cytosol). Such direction can be particularly advantageous for the controlled intracellular delivery of bioactive cargo molecules into targeted locations.

[00443] The two exemplary FITC-dendrimer conjugates were found to be highly water soluble and were further investigated for their capability to translocate through the cell membrane. Internalization of FD-1 and FD-2 in mammalian cells was assessed using two different cell lines and a previously described method [Futaki, S.; Nakase, I.; Suzuki, T.; Youjun, Y.; Sugiura, Y. Biochemistry 2002, 41, 7925.] with NIH-3T3 fibroblasts and HMEC (human microvascular endothelial cells) and a Zeiss LSM 510 confocal microscope. Figure 12 shows the time course of uptake of FD-1 and FD-2 into NIH-3T3 Fibroblasts at 37 °C. The fluorescence was clearly observed within the cells 2.5 min after the addition of conjugates to the medium, which is comparable to the uptake rate of Tat-peptide. [Futaki, S.; Nakase, I.; Suzuki, T.; Youjun, Y.; Sugiura, Y. Biochemistry 2002, 41, 7925.; Vivès, E.; Brodin, P.; Lebleu, B. J. Biol. Chem. 1997, 272, 16010.] Furthermore, the extent of

internalization increased in an incubation time-dependent manner, and it was observed that after just 10 min, the fluorescence intensity of cells treated with FD-2 was near saturation. However, the fluorescence intensity of cells treated with FD-1 did not approach saturation until the longer time points (45 min ~ 2 hr). Additionally, FD-1 and FD-2 exhibited

5 differential patterns of subcellular localization, as FD-1 appeared to concentrate in the nucleus while FD-2 appeared to concentrate in the cytosol. Without wishing to be bound by theory, it is believed that the length of the spacer at the terminal generation of the dendrimer can not only control the uptake rate, [Wender, P. A.; Kreider, E.; Pelkey, E. T.; Steinman, L.; Rothbard, J. B.; VanDeusen, C. L. *Org. Lett.* 2005, 7, 4815.] but also regulate the subcellular

10 localization of the molecule and its putative cargo. For instance, the uptake levels of FD-2 appeared to be generally stronger than those of FD-1 after the same incubation time at the same concentration. Therefore, the dendrimer with a hexyl spacer crosses the cell membrane faster than the molecule with an ethyl chain. On the other hand, the localization patterns can also be controlled by the length of the spacer. FD-1 with the short spacer appeared to be

15 localized everywhere in the cell, but highly concentrated in the nucleus. However, FD-2, with its longer spacer, was observed to reside mainly in the cytosol. These translocation features of guanidinylated dendritic scaffolds as carriers can be important for intracellular delivery of cargo molecules to specific subcellular compartments (e.g., cytosol or nucleus). For example, a translocation approach that does not saturate the nucleus can be highly

20 attractive as it can be both less cytotoxic and could afford cytosolic-targeted cargos with greater accuracy in delivery, and therefore higher efficacy. Without wishing to be bound by theory, it is believed that the differential uptake patterns by FD-1 and FD-2 are due to the presence of a hexyl spacing chain in FD-2, resulting in a greater hydrophobicity of the entire conjugate as compared with FD-1. Additionally, the uptake of FD-1 and FD-2 conjugates by

25 HMEC was also conducted. Entry of the two conjugates into HMEC shows a similar internalization pattern to that seen in fibroblasts.

[00444] In control experiments, cells treated with free FITC and Boc-protected guanidinylated FITC-dendrimer showed no or extremely weak fluorescence, respectively. Therefore, the guanidino groups play an important role in the cell permeability of these

30 molecules, while the length of the spacing chain determines both the differential rate of uptake and subcellular localization patterns. Although the mechanism of Tat translocation remains to be understood, it has been demonstrated that the rate of uptake is not temperature

dependent. [Futaki, S.; Nakase, I.; Suzuki, T.; Youjun, Y.; Sugiura, Y. Biochemistry 2002, 41, 7925.; Vivès, E.; Brodin, P.; Lebleu, B. J. Biol. Chem. 1997, 272, 16010.] This indicates that endocytosis does not play a crucial role in the translocation process. Evaluation of the effect of temperature on the internalization of FD-1 and FD-2 indicated that the two  
5 conjugates are able to get into cells not only at 37 °C, but also at 4 °C, even at a lower dendrimer concentration (1 μM) (see Figures 3 and 4 in contrast to control experiments, as shown in Figure 15). No significant decrease in fluorescence intensity of cells treated with FD-1 or FD-2 was observed, indicating that the uptake process does not occur via endocytosis.

10           **27. SYNTHESIS OF DENDRIMER B11.**

**[00445]**       A three-neck round bottom flask was flame-dried under argon, to which nitrotriacid B3 (3.192 g, 0.0115 mmol), 1-hydrobenzotriazole (HOBt) (5.609 g, 0.0415 mol), DCC (8.560 g, 0.0415 mol) and 100 mL THF were added sequentially. After 2 hours activation, aminotriester B2 (17.216 g, 0.0415 mol) was added. The solution was stirred at  
15 room temperature for 40 h, and the crude product was purified by flash column chromatography, eluting first with hexane/ethyl acetate (10:1) and then hexane/ethyl acetate (3:2) to yield dendrimer B11 (15.91 g, 94.1%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ = 1.44 (*m*, CH<sub>3</sub>, 81 H), 1.95 (*m*, CH<sub>2</sub>, 18 H), 2.21 (*m*, CH<sub>2</sub>, 30 H), 6.20 (*s*, NH, 3 H); <sup>13</sup>C NMR (400 MHz, CDCl<sub>3</sub>): δ = 28.04, 29.74, 29.85, 31.28, 57.56, 80.69, 92.47, 170.46, 172.76.

20           **28. SYNTHESIS OF DENDRIMER B12.**

**[00446]**       A solution of B11 (10.0 g, 0.0 mol) in 150 mL of absolute ethanol in the presence of 8 grams of Raney-Nickel was hydrogenated at 60 psi of hydrogen at room temperature for 24 h. The suspension was carefully filtered through Celite and removal of the solvent under reduced pressure yielded B12 (9.86 g, 98.5%). <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD):  
25 δ = 1.44 (*m*, CH<sub>3</sub>, 81 H), 1.61 (*m*, CH<sub>2</sub>, 6 H), 1.95 (*m*, CH<sub>2</sub>, 12 H), 2.21 (*m*, CH<sub>2</sub>, 30 H); <sup>13</sup>C NMR (400 MHz, CD<sub>3</sub>OD): δ = 28.42, 30.24, 30.47, 32.02, 36.24, 53.53, 58.37, 81.18, 173.96, 175.39.

**29. SYNTHESIS OF B25.**

[00447] To a room temperature stirred solution of 6-bromohexanoic acid (2.0 g, 0.0102 mol) in 7 mL of DMF was added NaN<sub>3</sub> (1.30 g, 0.020 mol). The reaction mixture was heated and stirred at 85°C for 5 h. After DMF was removed, DCM was added to dissolve the residue. The mixture was washed with 0.1 N HCl and dried over anhydrous NaSO<sub>4</sub>. Removal of the solvent gave a crude oil that was purified by flash column chromatography, eluting first with DCM and then ethyl acetate/DCM (3:7) to yield B25 (1.67 g, 69.07%). <sup>1</sup>H NMR (400 MHz, MeOD): δ = 1.38-1.49 (m, CH<sub>2</sub>, 2H), 1.54-1.70 (m, CH<sub>2</sub>, 4 H), 2.32 (t, CH<sub>2</sub>, 2 H), 3.30 (t, CH<sub>2</sub>, 2 H); <sup>13</sup>C NMR (400 MHz, MeOD): δ = 25.57, 27.32, 29.62, 34.72, 52.27, 177.38.

**30. SYNTHESIS OF DENDRIMER B13.**

[00448] To a stirred solution of B25 (1.29 g, 8.22 mmol) in anhydrous THF (50 mL) were added DCC (1.70 g, 8.22 mmol) and HOBt (1.112 g, 8.22 mmol) at room temperature. The mixture was stirred for 2 h, then dendrimer B12 (9.86 g, 6.85 mmol) was added and the resulting solution was stirred for 40 h. After filtration and removal of THF, the product was purified by flash column chromatography, eluting with hexane/ethyl acetate (1:1) to yield B13 (8.50 g, 78.53%). <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD): δ = 1.44 (m, CH<sub>3</sub>, CH<sub>2</sub>, 83 H), 1.95 (m, CH<sub>2</sub>, 18 H), 2.21 (m, CH<sub>2</sub>, 32 H), 3.30 (m, CH<sub>2</sub>, 2 H); <sup>13</sup>C NMR (400 MHz, CD<sub>3</sub>OD): δ = 26.47, 27.47, 28.43, 29.62, 30.35, 30.61, 32.07, 32.23, 37.56, 52.28, 58.63, 58.77, 81.54, 174.21, 175.35, 175.66.

**31. SYNTHESIS OF DENDRIMER B14.**

[00449] To a 0 °C stirred solution of nona-amine B5 (4.06 g, 2.43 mmol) in a methanol/acetonitrile (25 mL/15 mL) were added triethylamine (6.87 g, 68.0 mmol) and ethyl trifluoroacetate (9.32 g, 65.6 mmol) and the reaction mixture was stirred at 0 °C for 1 h and then at room temperature overnight. The solvent was removed *in vacuo* and the residue was taken up in ethyl acetate, and the resulting organic solution was washed with 1N HCl and brine and dried over anhydrous NaSO<sub>4</sub>. Removal of the solvent *in vacuo* gave a crude solid that was purified by flash chromatography (EtOAc/Methanol gradient) to yield a solid (3.02 g, 56.3 %). <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD): δ = 1.85-2.10 (m, CH<sub>2</sub>, 18 H), 2.11-2.35 (m, CH<sub>2</sub>, 30 H), 3.24-3.48 (m, CH<sub>2</sub>, 36 H); <sup>13</sup>C NMR (400 MHz, CD<sub>3</sub>OD): δ = 31.08, 31.26, 31.75,

32.01, 39.42, 40.42, 58.93, 94.33, 111.74, 115.54, 119.33, 123.13, 158.57, 159.06, 159.55, 160.04, 173.57, 176.14. The resulting white solid (1.0 g, 0.453 mmol) was dissolved in ethanol (45 mL) and transferred into a hydrogenation vessel containing Raney-Nickel catalyst (5 g) and the suspension was stirred at 80 psi of hydrogen at 50°C for 48 h. After filtration  
 5 through Celite, the solvent was removed under reduced pressure to give a B14 as a white solid (0.964 g, 97.7%). <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD): δ = 1.67(m, CH<sub>2</sub>, 6 H), 1.986 (m, CH<sub>2</sub>, 12 H), 2.188 (m, CH<sub>2</sub>, 30 H), 3.30-3.55 (m, CH<sub>2</sub>, 36 H); <sup>13</sup>C NMR (400 MHz, CD<sub>3</sub>OD) δ = 31.20, 32.11, 36.17, 39.38, 40.52, 54.06, 58.80, 111.79, 115.53, 119.36, 123.10, 158.58, 159.04, 159.50, 160.11, 175.55, 176.24.

10 **32. SYNTHESIS OF DENDRIMER B15.**

[00450] To a stirred solution of 6-heptynoic acid (0.3022 g, 2.40 mmol) in anhydrous THF (50 mL) were added DCC (0.4952 g, 2.40 mmol) and HOBt (0.3245 g, 2.40 mmol) at room temperature. The mixture was stirred for 2 h, then dendrimer B14 (1.0432 g, 0.48 mmol) was added and the resulting solution was stirred for 40 h. After filtration and removal  
 15 of THF, the product was purified by flash column chromatography, eluting with ethyl acetate/methanol gradient to yield B15 (0.620 g, 56.57%). <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD): δ = 1.53 (m, CH<sub>2</sub>, 2 H), 1.71 (m, CH<sub>2</sub>, 3 H), 1.890-2.5 (m, CH<sub>2</sub>, 50 H), 3.30 (m, CH<sub>2</sub>, 36 H); <sup>13</sup>C NMR (400 MHz, CD<sub>3</sub>OD): δ = 18.81, 26.14, 29.43, 31.27, 31.80, 37.37, 39.35, 40.43, 58.83, 59.05, 69.95, 83.4, 111.74, 115.57, 119.37, 123.13, 158.55, 159.07, 159.53, 159.99,  
 20 175.60, 176.25.

**33. SYNTHESIS OF DENDRIMER B16.**

[00451] Azide dendron B13 (100 mg, 0.044 mmol) and alkyne dendron B15 (70 mg, 0.044 mmol) were dissolved in THF/H<sub>2</sub>O (4:1) and DIPEA (0.017 g, 0.132 mmol, 3 equiv) followed by Cu(PPh<sub>3</sub>)<sub>3</sub>Br (0.0042g, 0.0044 mmol) were added. The reaction mixture was  
 25 placed in the microwave reactor (Biotage) and irradiated at 120 °C for 20 min. After completion of the reaction, THF was removed and the residue was taken up in DCM. The organic layer was washed with water once and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. <sup>1</sup>H NMR of B16 (400 MHz, CD<sub>3</sub>OD): δ = 1.43 (m, CH<sub>3</sub>, 81 H), 1.71 (m, CH<sub>2</sub>, 8 H), 1.890-2.5 (m, CH<sub>2</sub>, 96 H), 2.71 (m, CH<sub>2</sub>, 2 H), 3.30 (m, CH<sub>2</sub>, 36 H), 4.38 (m, CH<sub>2</sub>, 2 H), 7.75 (s, 1 H).

**34. SYNTHESIS OF B17 AND B18.**

[00452] The "Bow-Tie" B16 was stirred in formic acid overnight at room temperature. After the solvent was evaporated under reduced pressure, toluene was added and concentrated in vacuo to remove any residue of formic acid to give a white nonacid (100%). To a solution  
5 of the above resulting solid in DMF, HOBt and DCC were added and the solution was cooled to 0 °C. *N*-Boc-ethylenediamine or *N*-Boc-hexyldiamine was added dropwise and the mixture was stirred for 48 h at room temperature, filtered and concentrated under reduced pressure. The residue was dissolved in dichloromethane and the resulting organic solution was washed sequentially with 1N HCl, water and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The solvent  
10 was evaporated under reduced pressure and the crude residue was purified by flash column chromatography to yield B17 or B18.

**35. SYNTHESIS OF B19 AND B20.**

[00453] Potassium carbonate was added to B17 or B18 in methanol/water, the mixture was stirred at room temperature for 6 h. The crude product was purified by dialysis against  
15 methanol with Spectra® Por Biotech regenerated cellulose membranes (MWCO = 3500) for 24 h to give B19 or B20.

**36. SYNTHESIS OF B21 AND B22.**

[00454] The above B19 or B20 was then dissolved in 1, 4-dioxane and the solution cooled 0 °C, 4 M HCl in dioxane was added and stirred for 1 hr at room temperature.  
20 Removal of the solvent under reduced pressure gave a white solid. The resulting HCl salt was dissolved in methanol and the solution was cooled to 0 °C. Et<sub>3</sub>N was added, followed by *N,N'*-diBoc-*N''*-triflylguanidine and the mixture was stirred for 24 h at room temperature. After the solvent was evaporated under reduced pressure, the residue was dissolved in dichloromethane and the solution was washed with 1N HCl water, and dried over anhydrous  
25 Na<sub>2</sub>SO<sub>4</sub>. After removal of the solvent under reduced pressure, the crude product was purified by dialysis against methanol with Spectra® Por Biotech regenerated cellulose membranes (MWCO = 3500) for 24 h to give B21 or B22.

**37. SYNTHESIS OF B23 AND B24.**

[00455] The resulting solid B21 or B22 was dissolved in 1,4-dioxane and the solution cooled to 0 °C, 4 M HCl in dioxane was added and the solution stirred overnight at room temperature. The precipitate was filtered off and dried to give a crude product. The solid was  
5 re-dissolved in water and insoluble precipitate was filtered off and the filtrate was dialyzed against water with Spectra® Por Biotech cellulose ester membranes (MWCO =1000) for 48 hrs and lyophilized to yield a water-soluble B23 or B24.

**38. COMPOUND Q6**

[00456] 3-Carboxaldehydebicyclo[4,2,0]octa-1,3,5-triene or 4-Carboxaldehydebenzocyclobutene, Q6. To a 500-mL flask was added 50 mL dry of THF, Mg turnings (2.88 g, 120 mmol), and 1,2-dibromoethane (4 drops). The reaction mixture was then heated under reflux for 15 min, 4-Bromobenzocyclobutene, 5,11 (20.0 g, 109 mmol) in 25 mL THF was added via a dropping funnel to form the Grignard reagent. After addition and rinsing the dropping funnel with 25 mL of dry THF, the reaction mixture was heated for an  
15 additional 45 min under reflux to give a green brown solution. The reaction mixture was then cooled to 0 °C, DMF (15 mL, 210 mmol) was added dropwise to the solution, and the reaction mixture was heated under reflux for 15 min. The reaction mixture was poured onto 150 g of ice, acidified to pH), and neutralized with saturated NaHCO<sub>3</sub> solution. The crude product was extracted with ethyl acetate, the organic phase was filtered over Celite, and  
20 evaporation of the solvent gave the crude product. The product was purified by column chromatography using 10% diethyl ether/hexane as eluting solvents and was finally purified by Kugelrohr distillation (145 °C, 0.5 mm) to give the aldehyde Q6 (11.7 g, 81.2%) as a colorless liquid; IR 3000-2800, 1690, 1598, 1216, 1067 and 827 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 9.9 (s, 1H, CHO), 7.65 (dd, 1H, *J* = 7.4 Hz, *J'* = 1.2 Hz, ArH), 7.50 (s, 1H, ArH),  
25 7.14 (dd, 1H, *J* = 7.4 Hz, *J'* = 1.2 Hz, ArH), 3.15 (s, 4H, CH<sub>2</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 192.28, 153.69, 146.57, 135.4, 130.26, 122.89, 122.81, 29.97, and 29.23. Anal. Calcd for C<sub>9</sub>H<sub>8</sub>O; C, 81.8; H 6.10. Found: C, 81.7; H, 5.94.

**39. COMPOUND Q4**

[00457] 3-Ethenylbicyclo[4,2,0]octa-1,3,5-triene or 4-Vinylbenzocyclobutene, Q4. To  
30 a 500-mL round-bottom neck flask was added (Ph)<sub>3</sub>PCH<sub>3</sub>Br (24.3 g, 68.1 mmol), 110 mL of

dry THF, and the solution was cooled to -78 °C. *n*-BuLi (2.5 M in hexane, 26.4 mL, 66 mmol) was added dropwise, and the reaction mixture was allowed to warm to room temperature. The yellow-orange solution was cooled to -78 °C, and the aldehyde 6 (7.16 g, 54.2 mmol), diluted in 34 mL of dry THF, was added slowly. The mixture warmed to room temperature, and stirring continued for 2 h. The reaction was treated sequentially with saturated NH<sub>4</sub>Cl and saturated NaHCO<sub>3</sub> solution, and the crude product was filtered over Celite, washed with diethyl ether/hexane (1:1), and evaporated to dryness (no heat) to give the crude product. Further purification by column chromatography using 5% diethyl ether/hexane as an eluting solvent followed by Kugelrohr distillation (75 °C, 1.0 mm) gave the pure styrene derivative Q4 as a colorless liquid (5.50 g, 78%); IR 2925, 1627, 1473, 989, 901, and 829 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.26 (d, 1H, *J* = 7.4 Hz, ArH), 7.20 (s, 1H, ArH), 7.04 (d, 1H, *J* = 7.4 Hz, ArH), 6.74 (dd, 1H, *J* = 17.5 Hz, *J'* = 10.8 Hz, CH), 5.70 (d, 1H, *J* = 17.5 Hz, CH<sub>2</sub>), 5.20 (d, 1H, *J* = 10.8 Hz, CH<sub>2</sub>), 3.19 (s, 4H, CH<sub>2</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 146.09, 145.75, 137.94, 136.69, 125.71, 122.58, 119.90, 112.38, 29.52, and 29.35. Anal. Calcd for C<sub>10</sub>H<sub>10</sub>; C, 92.2; H 7.80. Found: C, 92.0; H, 8.03.

#### 40. RANDOM COPOLYMER OF Q4 AND STYRENE, Q8.

[00458] The alkoxyamine initiator, Q7 (32.5 mg, 0.1 mmol), Q12 dissolved in styrene (10.4 g, 100 mol) and 4-vinylbenzocyclobutene, Q4 (3.25 g, 25.0 mmol) were added to a glass ampule with a stir bar. After three freeze and thaw cycles the ampule was sealed under argon and heated for 6 h at 120 °C. The resulting polymer was dissolved in dichloromethane and purified by precipitation into a 1:1 mixture of 2-propanol/acetone followed by reprecipitation into methanol to give Q8 as a colorless powder (12.1 g, 88%), *M*<sub>w</sub> = 111 000; PDI ) 1.11; IR 3100-2850, 1601, 1492, 1452, 909, and 699 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.24-6.57 (m, ArH), 3.05 (br s, CH<sub>2</sub>), 1.83-1.26 (m, CH<sub>2</sub>, CH); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 145.0-146.4, 1127.9, 125.5, 121.8, 42.0-44.0, 40.4, and 29.2.

#### 41. RANDOM COPOLYMER OF Q4 AND *N*-BUTYLACRYLATE, Q10.

[00459] The alkoxyamine initiator Q7 (32.5 mg, 0.1 mmol) was dissolved in *n*-butyl acrylate (10.2 g, 72.0 mmol) and Q4 (1.04 g, 8.0 mmol) and placed in a glass ampule with a stir bar. After three freeze and thaw cycles the ampule was sealed under argon and heated for 15 h at 125 °C. The resulting polymer was dissolved in dichloromethane and precipitated in

MeOH/H<sub>2</sub>O (3:1) to give Q10 as a colorless gum (10.2 g, 91%), *M<sub>w</sub>* = 77 500; PDI ) 1.12; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 6.83-6.63 (m, ArH), 4.10-3.83 (m, CH<sub>2</sub>,CH), 3.05 (bs, CH<sub>2</sub>), 2.22-1.01 (m, CH<sub>2</sub>, CH<sub>3</sub> ).

#### 42. COMPOUND Q12

5 [00460] Methyl(2,2,5-Trimethyl-3-(benzylethoxy)-4-phenyl-3-azahexane)-poly(ethylene Glycol), Q12. NaH (0.23 g, 6.3 mmol) was slowly added to a mixture of monomethylpoly(ethylene glycol), Q14 (7.85 g, 1.57 mmol), and 18-crown-6 (10 mg) dissolved in 10 mL of THF under a constant argon flow. After 15 min, the chloromethyl-substituted alkoxyamine, 13 (1.16 g, 3.14 mmol) was added to the reaction mixture, which  
10 was subsequently heated at reflux for 16 h. After the addition of a few drops of water to neutralize the excess NaH, the reaction mixture was concentrated, dissolved in dichloromethane, filtered, and evaporated to dryness. The crude product was obtained after flash chromatography eluting with dichloromethane gradually increasing to 10% methanol/dichloromethane to give the PEG-macroinitiator, Q12, as a colorless solid (8.03 g,  
15 89%); IR (KBr) 3439 cm<sup>-1</sup> (NH), 1693 cm<sup>-1</sup> (amide). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.4-7.1 (m, ArH), 5.10 (d, CH), 4.92 (d, CH<sub>2</sub>OAr), 3.65 (s, OCH<sub>2</sub>), 3.41 (d, CH), 3.28 (d, CH), 2.43 (m, CH), 1.65 (d, CH<sub>3</sub>), 1.52 (d, CH<sub>3</sub>), 1.40 (m, CH), 1.33 (d, CH<sub>3</sub>), 1.05 (s, *t*-Bu), 0.89 (d, CH<sub>3</sub>), 0.80 (s, *t*-Bu), 0.61 (d, CH<sub>3</sub>), and 0.22 (d, CH<sub>3</sub>).

#### 43. COMPOUND Q15

20 [00461] Poly(ethylene glycol)-*b*-(styrene-*co*-benzocyclobutene), Q15. The poly(ethylene glycol) terminated alkoxyamine, Q12 (500 mg, 0.1 mmol) (*M<sub>n</sub>* = 5 000, PDI ) 1.06) was dissolved in styrene (10.4 g, 100 mol) and 4-vinylbenzocyclobutene, Q4 (3.25 g, 25.0 mmol) in a glass ampule with a stir bar. After three freeze and thaw cycles the ampule was sealed under argon and heated for 6 h at 125 °C. The resulting polymer was dissolved in  
25 dichloromethane and purified by precipitation into a 1:1 mixture of isopropanol/acetone followed by reprecipitation into methanol to give Q15 as a colorless powder (10.7 g, 76.1%), *M<sub>w</sub>* = 89 500; PDI = 1.12; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.24-6.57 (m, ArH), 3.65 (s, OCH<sub>2</sub>), 3.05 (br s, CH<sub>2</sub>), 1.83-1.26 (m, CH<sub>2</sub>, CH).

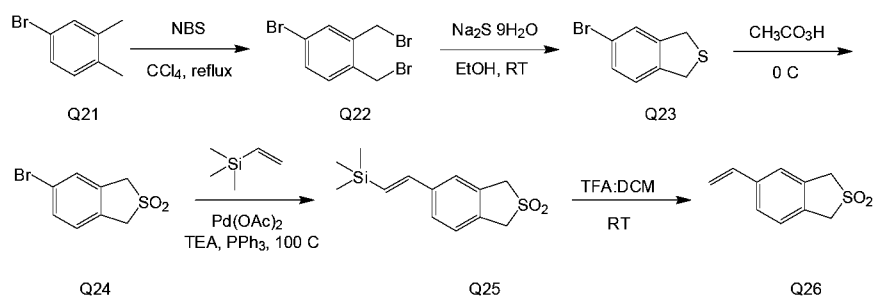
**44. GENERAL PROCEDURE FOR NANOPARTICLE FORMATION, Q9.**

[00462] In a 500-mL three-necked flask equipped with a internal thermometer, condenser, and septum, 120 mL of benzyl ether was heated at 250 °C under argon. A solution of the benzocyclobutene (BCB)-functionalized linear polymer, Q8 (4.00 g,  $M_n$  = 108000; PDI = 1.15, 7.5 mol % BCB), dissolved in benzyl ether (40 mL) was added dropwise via a  
 5 peristaltic pump at ca. 12.8 mL/h with vigorously stirring under argon. After addition the reaction mixture was heated for an additional 1 h, the solvent was distilled under reduced pressure, and the remaining crude product was dissolved in dichloromethane and precipitated into methanol. This gave the nanoparticles, Q9, as a colorless solid (3.76 g, 94% yield),  $^1\text{H}$   
 10 NMR (400 MHz,  $\text{CDCl}_3$ ). The significant change is the disappearance of the aliphatic benzocyclobutene protons at 3.05 on formation of the cross-linked nanoparticles; all other aspects of the spectrum are similar.

**45. COMPOUND Q20**

[00463] Synthesis of 5-Vinyl-1,3-dihydro-benzo(c)thiophene 2,2-dioxide, Q20. Under  
 15 classical Heck reaction conditions, 5-Bromo-1,3-dihydro-benzo(c)thiophene 2,2 -dioxide was transformed with  $\text{PPh}_3$ ,  $\text{Pd}(\text{OAc})_2$ , TEA, and vinyltrimethylsilane at 90 °C in DMF. The crude product was extracted with  $\text{CH}_2\text{Cl}_2$  and concentrated. Deprotection in  $\text{CH}_2\text{Cl}_2/\text{TFA}$  gave the pure product after purification with flash chromatography ( $\text{CH}_2\text{Cl}_2$ ) in high yields.

[00464] Compounds Q22 to Q26 were prepared according to the following procedures,  
 20 as set forth schematically below.



**46. COMPOUND Q22**

[00465] 4-bromo-1,2-bis(bromomethyl)benzene (Q22). To a 1000 mL round bottom flask was added 4-bromo-*o*-xylene (21) (0.0811 mol, 15.00g), NBS (0.0426 mol, 7.580 g), 2,2'-azobisisobutyronitrile (0.00405 mol, 0.6650 g) and 500 mL of CCl<sub>4</sub>. The reaction mixture was then heated under reflux for 1h. After this time another amount of NBS and AIBN was added and repeated over four times in total. The precipitate was filtered off from the warm reaction mixture and the filtrate concentrated. Crude material was crystallized from hexanes to form white crystals (23 g, 93%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.53 (d, 1H, ArH, *J*=2.0 Hz), 7.44 (dd, 1H, ArH, *J*=8.1 Hz, *J'*=2.0 Hz), 7.24 (d, 1H, ArH, *J*=8.10), 4.60 (d, 4H, CH<sub>2</sub>, *J*=4.20 Hz)

**47. COMPOUND Q23**

[00466] 5-bromo-1,3-dihydro-2-benzo[*c*]thiophene (Q23). To the stirring solution of Na<sub>2</sub>S·9H<sub>2</sub>O (0.0502 mol, 12.043g) in 800 mL of ethanol was added dropwise a solution of 4-bromo-1,2-bis(bromomethyl)benzene (0.0418 mol, 14.334g) in 200 mL of ethanol in room temperature. After 20h a cloudy white reaction mixture was filtered and concentrated down to a 200 mL volume. Then 250 mL of ethyl acetate was added and the organic solution was washed 3x with water (125 mL) and 1x with brine (125ml). The organic phase was dried over anhydrous MgSO<sub>4</sub> and concentrated. The crude product gave a yellow oil and was used without further purification. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.38 (s, 1H, ArH) ppm 7.32 (dd, 1H, *J*=1.8Hz, *J*=8.1Hz, ArH) ppm 7.11 (d, 1H, *J*=8.1Hz, ArH) ppm 4.21 (dd, 1H, *J*=1.8Hz, *J*=8.5Hz)

**48. COMPOUND Q24**

[00467] 5-bromo-1,3-dihydro-2-benzo[*c*]thiophene-2,2 dioxide (Q24). The crude 5-bromo-1,3-dihydrobenzo[*c*]thiophene (Q21) from previous reaction 7.815 g was dissolved in 5.00 mL of glacial acetic acid and cooled in an ice bath. This suspension was added dropwise to 11ml of cold peracetic acid cooled with an ice bath. After complete addition, the reaction was allowed to warm up to RT and a white precipitate formed over night. The reaction mixture was filtered and the precipitated washed with cold ethanol. The crude product was a light yellow precipitate and was used without any further purification. A typical yield of 3.749 g was observed for the reaction. <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>) ppm 7.36 (dd, 1H, *J*=9.8Hz,

$J=92.9\text{Hz}$  (m, ArH), 4.34 (d, 1H,  $J=13.0\text{Hz}$ ) (d,  $\text{CH}_2$ ).  $^{13}\text{C}$ -NMR (400 MHz,  $\text{CDCl}_3$ ) ppm  
133.604, 132.549, 130.559, 129.570, 127.999, 123.088, 57.017, 56.914

#### 49. COMPOUND Q25

[00468] 5-Trimethylsilylvinyl-1,3-dihydro-benzo[c]thiophene 2,2-dioxide (Q25). A  
5 250 mL 3-neck round bottom flask was purged with  $\text{N}_2$  for 10 minutes. Compound Q23 (5 g,  
20.2 mmol), vinyl trimethylsilane (10.10 g, 100.77 mmol), triethylamine (5.562 g, 54.97  
mmol), triphenyl phosphine (0.433 g, 1.65 mmol), and palladium (II) acetate (0.185 g, 0.824  
mmol) were then added to the flask with 50 mL of anhydrous DMF. Nitrogen was then  
bubbled through the mixture for 10 min. The round bottom flask was then sealed via a rubber  
10 septum and was charged with  $\text{N}_2$  the mixture was yellow-orange in color. The reaction  
mixture heated to  $100^\circ\text{C}$  and additional vinyl trimethylsilane (5.005 g, 50.39 mmol),  
triethylamine (2.781 g, 27.48 mmol), triphenylphosphine (0.216 g, 0.824 mmol), and  
palladium (II) acetate (0.0925 g, 0.413 mmol) were added to the mixture followed by a  $\text{N}_2$   
purge of the reaction and charge of the vessel after 1 hour. After reacting a total of 2 hours,  
15 another equivalent vinyl trimethylsilane (5.005 g, 50.39 mmol), triethylamine (2.781 g, 27.48  
mmol), triphenylphosphine (0.216 g, 0.824 mmol), and palladium (II) acetate (0.0925 g,  
0.413 mmol) was added and the reaction mixture was again purged then charged with  $\text{N}_2$  and  
was allowed to react a total of 18 h. The reaction mixture was removed from the oil bath and  
allowed to cool to room temperature. The mixture was diluted with methylene chloride,  
20 washed 3x with water (500ml), dried over  $\text{MgSO}_4$ , filtered, and concentrated to give the crude  
product, Q25.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.43-7.26 (m, 3H, ArH), 6.83 (d, 1H, CH),  
6.51 (d, 1H, CH), 4.37 (s, 4H,  $\text{CH}_2$ ), 0.16 (s, 9H,  $\text{CH}_3$ );  $^{13}\text{C}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$   
142.03, 139.20, 131.84, 131.61, 130.59, 126.95, 126.14, 123.59, 56.95, 56.86, -1.03, -1.36.

#### 50. COMPOUND Q26

25 [00469] 5-vinyl-1,3-dihydro-benzo[c]thiophene 2,2-dioxide (Q26). A 60% (wt)  
solution of trifluoroacetic acid in methylene chloride was prepared and added to the crude  
product Q25. The reaction was allowed to stir for 14 h at which time it was diluted with  
 $\text{CH}_2\text{Cl}_2$  and washed 3x with 500ml water with  $\text{H}_2\text{O}$ . The organic phase was dried over  
 $\text{MgSO}_4$ , filtered and concentrated. The crude product was then purified by column  
30 chromatography using a gradient system of 4:1 hexanes:ethyl acetate followed by 3:2

hexanes:ethyl acetate. The pure product Q26 was isolated as a light yellow powder (0.91 g, 93.2%). <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>) ppm 7.39 (d, 1H, J=8.1Hz, ArH) ppm 7.33 (s, 1H, ArH) ppm 7.26 (d, 1H, J=8.1Hz, ArH) ppm 6.69 (dd, 1H, J=10.9Hz, J=17.6Hz, =CH) ppm 5.77 (d, 1H, J=17.6Hz, =CH<sub>2</sub>) ppm 5.32 (d, 1H, J=10.9Hz, =CH<sub>2</sub>) ppm 4.35 (s, 1H, CH<sub>2</sub>) <sup>13</sup>C NMR (400 MHz, CDCl<sub>3</sub>): δ 138.11, 130.14, 126.47, 125.90, 123.26, 115.21, 56.63, 56.54

#### 51. SYNTHESIS OF BENZENE DIAZONIUM CARBOXYLATE

[00470] Anthranilic acid (10.0 g, 72.9 mmol) and a solution of trifluoroacetic acid (60.7 mg, 0.532 mmol) in 6 mL of THF were dissolved with stirring in 73.1 mL tetrahydrofuran in a plastic beaker. The solution was cooled to 0 °C and isoamyl nitrite (16.0 mL, 119.6 mmol) added dropwise over a period of 1-2 minutes. The reaction mixture was allowed to warm to room temperature and stirred 1-1.5 h. A brick-red precipitate formed and was slowly converted to the tan product. Upon completion, the product was collected by suction filtration on a plastic Buchner funnel and washed on the funnel with cold tetrahydrofuran until the washings were colorless. The product was then washed with 1,2-dichloroethane to displace the tetrahydrofuran. The solvent-wet product was used without further purification.

#### 52. SYNTHESIS OF BENZOCYCLOBUTENYL ACETATE

[00471] In a 1000 mL round bottomed flask, benzenediazonium-2-carboxylate was dissolved with stirring in a minimal amount 1,2-dichloroethane. Vinyl acetate (40.4 mL, 437.4 mmol) was added dropwise to the solution and the reaction heated to 80 °C. The reaction was refluxed for 4 hrs and yielded an orange oil which was purified via column chromatography eluting with a 1:1 dichloromethane:hexanes solvent system (0.515 g, 4.36 %). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.35-7.14 (m, 4H, ArH), 5.91 (d, 1H, J=4.4Hz, CH), 3.66 (dd, 1H, J=4.6Hz, J=14.5Hz, CH<sub>2</sub>), 3.22 (d, 1H, J=14.5Hz, CH<sub>2</sub>).

#### 53. HYDROLYSIS OF BENZOCYCLOBUTENYL ACETATE

[00472] A mixture of benzocyclobutenyl acetate (0.515 g, 3.18mmol), Na<sub>2</sub>CO<sub>3</sub> (0.337 g, 3.18 mmol), methanol (2.79 mL), and water (5.58 mL) was vigorously stirred overnight. The solution was extracted with diethyl ether (3x, 200 mL) and the organic phase washed with water (3x, 400 mL) and dried with MgSO<sub>4</sub> and concentrated. The resulting orange oil

was recrystallized from pentane to yield white crystals. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.33-7.14 (m, 4H, ArH), 5.30 (ddd, 1H, J=1.7Hz, J=4.4Hz, J=8.8Hz, CH), 3.63 (dd, 1H, J=4.4Hz, J=14.4Hz, CH<sub>2</sub>), 3.05 (dd, 1H, J=1.3Hz, J=14.1Hz, CH<sub>2</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.33-7.14 (m, 4H, ArH), 5.30 (dd, 1H, J=4.4Hz, J=6.6Hz, CH), 3.63 (dd, 1H, J=4.5Hz, J=14.3Hz, CH<sub>2</sub>), 3.05 (d, 1H, J=14.3Hz, CH<sub>2</sub>)

#### 54. ATTACHMENT OF 2-(BOC-AMINO) ETHYL BROMIDE TO BENZOCYCLOBUTENOL

[00473] Sodium hydride (143.15 mg, 3.58 mmol) was placed in a dried flask which was then sealed and charged with N<sub>2</sub>. A solution of benzocyclobutenol (400 mg, 3.33 mmol) in a minimal amount of THF was added to the flask and the solution stirred at room temperature for 30 minutes. The solution was then chilled to 0 °C and a solution 2-(boc-amino) ethyl bromide (1.0 g, 4.46 mmol) in a minimal amount of THF was added dropwise to the reaction and the solution was stirred at room temperature for an hour. The precipitate was filtered off and the filtrate diluted with ether and washed with water (3x, 50 mL). The organic phase was dried and concentrated to yield an orange oil that was purified via column chromatography using a 5:2 Hex:EtOAc solvent system (0.44 g, 50.2 %).

#### 55. DEPROTECTION OF T-BUTYL 2-(1,2-DIHYDROCyclobutabenzene-1-yl)ethoxycarbamate

[00474] In a 200 mL round bottomed flask, t-Butyl 2-(1,2-dihydrocyclobutabenzene-1-yl)ethoxycarbamate (275.0 mg, 1.044 mmol) was dissolved in formic acid and CH<sub>2</sub>Cl<sub>2</sub> and allowed to stir for 48 h at room temperature. After the solvent was evaporated under reduced pressure, toluene was added and concentrated to remove any residual formic acid yielding a green oil which was purified via column chromatography using a 5:2 Hex:EtOAc solvent system to yield the product (0.023 g, 13.5 %).

#### 56. SYNTHESIS OF 1,2-BIS(TRIMETHYLSILYL)BENZENE

[00475] Magnesium (9.72 g, 400 mmol), hexamethylphosphoramide (HMPA) (80 mL, 460 mmol), 1,2-dichlorobenzene (14.76 g, 100 mmol), and a catalytic amount of I<sub>2</sub> were combined in a 500 mL round bottomed flask and heated to 70 °C with stirring. Chlorotrimethylsilane was added dropwise to the solution at 70 °C. The solution was stirred for an additional 30 minutes and then heated to 100 °C for 48 h. After cooling, the reaction

mixture was poured over ice and  $\text{NaHCO}_3$ . The Mg and precipitate were filtered off and the filtrate was extracted with ether (3x, 200 mL). The organic phase was washed with water (2x, 400 mL) and brine (1x, 400 mL) and dried with  $\text{Na}_2\text{SO}_4$  and concentrated. The product was distilled under reduced pressure at 128-133 °C to yield a light yellow oil (12.0426 g, 54.13%).

5  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.85 (dd, 2H,  $J=3.4\text{Hz}$ ,  $J=5.5\text{Hz}$ , ArH), 7.49 (dd, 2H,  $J=3.4\text{Hz}$ ,  $J=5.5\text{Hz}$ , ArH), 0.55 (s, 18H,  $\text{CH}_3$ );  $^{13}\text{C}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  145.98, 135.17, 127.76, 1.96

#### 57. SYNTHESIS OF (PHENYL)[2-(TRIMETHYLSILYL)PHENYL]IODONIUM TRIFLATE

[00476] Iodobenzene diacetate (9.66 g, 30 mmol) was dissolved in  $\text{CH}_2\text{Cl}_2$  (60 mL) with stirring and the solution chilled to 0 °C. Triflic acid (5.1 mL, 58 mmol) was added dropwise to the solution and the reaction mixture was stirred for 2 h. A solution of 1,2-bis(trimethylsilyl)benzene (6.67 g, 30 mmol) in  $\text{CH}_2\text{Cl}_2$  (10 mL) was added at 0 °C and the reaction was allowed to stir at room temperature for 2 h. Concentration of the reaction mixture gave crystals which were triturated in ether and collected by filtration to yield white crystals (9.82 g, 65.14 %).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.11-7.47 (m, 9H, ArH), 0.42 (s, 9H,  $\text{CH}_3$ );  $^{13}\text{C}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  139.0, 138.4, 133.3, 133.2, 132.4, 132.3, 132.1, 109.6, 0.1.

#### 58. SYNTHESIS OF T-BUTYL(1-ETHOXYVINYLOXY)DIMETHYLSILANE

[00477] To a solution of 2,2,6,6-tetramethylpiperidine (17.34 mL, 102.14 mmol) in THF (63.4 mL) under argon, 2.9 M BuLi in hexanes (34.05 mL, 98.74 mmol) was added dropwise at 0 °C. The reaction was stirred for 15 min. and subsequently cooled to -78 °C. t-Butyldimethylchlorosilane (TBDMSCl) (16.94 g, 112.36 mmol) in THF (25.4 mL) was added and then a solution of anhydrous ethyl acetate (8.38 mL, 85.12 mmol) in THF (57.2 mL) was slowly added over 1 h. The mixture was stirred for an additional 10 min at -78 °C and then stirred at r.t. for 1 h. The solution was then diluted with hexanes (285.9 mL), filtered through a *Celite* pad, and the filtrate concentrated. Distillation under vacuum at 42-50 °C gave an orange oil (g, 2.0 %).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  3.74 (q, 2H,  $J=7.0\text{Hz}$ ,  $\text{CH}_2$ ), 3.22 (d, 1H,  $J=2.0\text{Hz}$ ,  $\text{CH}_2$ ), 3.05 (d, 1H,  $J=2.0\text{Hz}$ ,  $\text{CH}_2$ ), 1.29 (t, 3H,  $J=7.1\text{Hz}$ ,  $\text{CH}_3$ ), 0.94 (s, 9H,  $\text{CH}_3$ ), 0.17 (s, 6H,  $\text{CH}_3$ );  $^{13}\text{C}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  160.3, 62.4, 59.6, 24.79, 17.3, 13.5, -

30 5.4

**59. SYNTHESIS OF T-BUTYL(1-ETHOXY-1,2-DIHYDROCyclobutabenzene-1-YLOXY)DIMETHYLSILANE**

[00478] A stirring solution of (phenyl)[2-(trimethylsilyl)phenyl]iodonium triflate (1.0 g, 2.0 mmol) and t-butyl(1-ethoxyvinyl)dimethylsilane (1.85 g, 9.96 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (14 mL) was chilled to 0 °C. Bu<sub>4</sub>NF (2.39 mL) was added dropwise to the solution and the reaction allowed to come to r.t. After 3 h., water (75 mL) was added to the reaction and the solution was extracted with EtOAc (3x, 50 mL). The organic phases were dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated to give the crude cycloadduct. <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>) δ 0.06 (s, 6H, CH<sub>3</sub>), 0.89 (s, 9H, CH<sub>3</sub>), 0.97 (s, 3H, CH<sub>3</sub>), 3.19 (m, 2H, CH<sub>2</sub>), 4.09 (q, 2H, J=7.1Hz, CH<sub>2</sub>), 7.08 (t, 1H, J=7.7Hz, ArH), 7.18 (d, 1H, J=2.3Hz, ArH), 7.30 (dd, 1H, J=5.0Hz, J=8.7Hz, ArH), 7.67 (d, 1H, J=7.2Hz, ArH).

**60. SYNTHESIS OF BENZOCYCLOBUTENONE OR CYCLOBUTABENZENE-1(2H)-ONE**

[00479] To a solution of t-butyl(1-ethoxy-1,2-dihydrocyclobutabenzene-1-yloxy)dimethylsilane (0.56 g, 2.0 mmol) in acetonitrile (2.3 mL) was added 48-50% aq. HF (0.23 mL) at 0 °C. The solution was allowed to warm to room temperature and stirred for 15 hrs. The reaction was then diluted with water and washed with ether (3x, 50 mL). The combined organic extracts were washed with brine (3x, 100 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), and concentrated. This gives a brown residue that was purified via column chromatography eluting with hexanes/EtOAc (9:1). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 3.99 (s, 2H, CH<sub>2</sub>), 7.40-7.24 (m, 4H, ArH).

**61. SYNTHESIS OF 1-BROMOBENZOCYCLOBUTENE**

[00480] In a 250 mL round bottomed flask equipped with a drying tube and a condenser, a solution of cycloheptatriene (30.7 g, 300 mmol), bromoform (25.3 g, 100 mmol), anhydrous K<sub>2</sub>CO<sub>3</sub> (15.0 g, 109 mmol), and 18-crown-6 (0.75 g) was heated with stirring at 145 °C for 9-10 h. The solution was allowed to cool and diluted with an equal volume of acetone. Silica gel (15.0 g) was added to reaction mixture and the insoluble solid residue was separated via vacuum filtration and the filter cake washed with acetone until the washings were colorless. The filtrate was concentrated and distilled to remove residual cycloheptatriene. The viscous, brown residue was precipitated into hot petroleum ether. After filtration to remove the precipitate, the filtrate was concentrated and distilled in vacuo

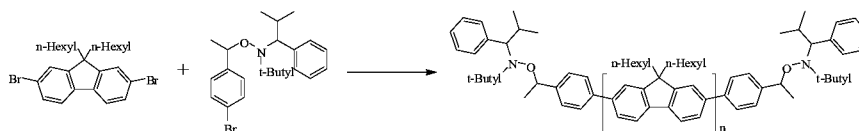
through a Vigreux column to give slightly impure product. Pure 1-bromobenzocyclobutene was obtained as a light yellow liquid by redistillation yield (2.95 g, 5.94 %).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.28 (m, 1H, ArH), 7.16 (d, 1H,  $J=7.0\text{Hz}$ , ArH), 7.07 (d, 1H,  $J=6.4\text{Hz}$ , ArH), 5.39 (m, 1H, CH), 3.85 (dd, 1H,  $J=4.4\text{Hz}$ ,  $J=14.7\text{Hz}$ ,  $\text{CH}_2$ ), 3.45 (d, 1H,  $J=14.7\text{Hz}$ ,  $\text{CH}_2$ ).

## 5 62. SYNTHESIS OF BENZOCYCLOBUTENOL

[00481] To a stirred solution of mercury(II) oxide (591.3 mg, 2.73 mmol) and 35 % aqueous tetrafluoroboric acid (TFBA) (999.4 mg, 5.46 mmol) in 1,4-dioxane (10.9 mL), 1-bromobenzocyclobutene (1.0 g, 5.46 mmol) was added. The reaction stirred at room temperature for 2 h and was then treated successively with  $\text{NaHCO}_3$  and 3N KOH until the solution remained basic. The precipitated mercury(II) oxide was filtered off and the filtrate extracted with  $\text{CH}_2\text{Cl}_2$  (3 x 300 mL), dried with anhydrous  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated to yield white crystals that were purified via column chromatography using a 3:2 Hexanes:EtOAc solvent system.

## 63. STYRENE-POLYFLUORENE-STYRENE TRIBLOCKCOPOLYMER

### 15 a. SYNTHESIS OF MACROINITIATOR



[00482] To the 3-necked round bottom flask, flushed with argon (30 min) added Nickel (5.080 mmol, 1.4000 g), 2,2'-bipyridyl (5.800 mmol, 0.9000 g), dry toluene (10 mL), 1,5-cyclooctadiene (0.5 mL), and dry DMF (12 mL). The mixture was heated to 80 °C under argon. After 30 minutes, brominated alkoxyamine initiator (0.7750 mmol, 0.3134 g) and 2,7-dibromo-9,9'-di-n-hexylfluorene (2.250 mmol, 1.1080 g) dissolved in dry toluene (13 mL) via syringe. The mixture stirred in 80 °C for 24 hours in the dark. After 24 hours, hot solution precipitated into 600 mL of solution of  $\text{HCl}_{(\text{conc})}$ :acetone:methanol in ratio 1:1:1. The resulting dark brown precipitate was then filtered, dissolved in dichloromethane, concentrated *in vacuo* and reprecipitated with 200 mL solution of acetone:methanol, 1:1.

**b. SYNTHESIS OF A-B-A COPOLYMER WITH 10% CROSSLINKER**

[00483] Macromolecular initiator (M=3000, n=0.060 mmol, 180 mg), styrene (90 eq per chain end, 9.72 mmol, 1.0123 g), and crosslinker (5-Vinyl-1,3-dihydro-benzo[c]thiophene 2,2-dioxide) (10%, 1.08 mmol, 0.2098 g) were dissolved in 0.5 mL of chlorobenzene in 10 mL ampule. The ampule was degassed, sealed, and heated at 124 °C for 7-10 hours. After this time, the polymer was precipitated from methanol. The resulting brown precipitate was filtered, washed with methanol, and dried.

**c. INTRAMOLECULAR CHAIN COLLAPSE PROCEDURE FOR A-B-A**

[00484] 0.300 g of polymer was dissolved in 50 mL of benzyl ether. The solution of polymer was added dropwise 12.4 ml/hr to benzyl ether in 260 °C under nitrogen. After adding all of the polymer, the reaction was cooled down, and the benzyl ether was distilled from the reaction mixture, and the residue was precipitated from methanol.

**64. SYNTHESIS OF N-BOC-N-TFA-ETHYLENEDIAMINE.**

[00485] To a solution of N-boc-ethylenediamine (5.0 g, 31.2 mmol) in 20 mL THF, ethyl trifluoroacetate (3.72 mL, 31.2 mmol) was added dropwise and the reaction stirred overnight. The reaction solution was concentrated to yield a white crystalline product (8.0 g, 100%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 1.44 (s, 9 H, CH<sub>3</sub>), 3.37 (dd, 2 H, J=5.4Hz, J=10.2Hz, CH<sub>2</sub>), 3.46 (dd, 2 H, J=5.1Hz, J=10.4Hz, CH<sub>2</sub>), 5.01 (s, 1 H, NH), 7.85 (s, 1 H, NH); <sup>13</sup>C NMR (400 MHz, CDCl<sub>3</sub>) δ 28.2, 39.1, 42.2, 80.6, 140.6, 151.2, 157.7.

**65. BOC DEPROTECTION OF N-BOC-N-TFA-ETHYLENEDIAMINE.**

[00486] N-Boc-N-Tfa-ethylenediamine (8.0 g, 31.5 mmol) was dissolved in 50 mL formic acid and stirred for 14 h at r.t.. After the solvent was evaporated under reduced pressure, toluene was added and concentrated to remove any residual formic acid, yielding an orange oil (4.90 g, 99.7%). <sup>1</sup>H NMR (400 MHz, MeOD) δ 2.31 (s, 2 H, NH<sub>2</sub>), 3.15 (t, 2 H, J=6.1Hz, CH<sub>2</sub>), 3.61 (t, 2 H, J=6.1Hz, CH<sub>2</sub>), 8.35 (s, 1 H, NH); <sup>13</sup>C NMR (400 MHz, MeOD) δ 38.5, 39.7, 113.1, 115.9, 118.8, 121.6, 159.7, 160.1.

**66. ATTACHMENT OF N-TFA-ETHYLENEDIAMINE.**

[00487] The deprotected nanoparticles (77 mg, 0.00194 mmol) in DriSolv DMF (9.0 mL) were stirred under argon at 0 °C with N-methylmorpholine (6.9 mg, 0.0680 mmol) followed by dropwise addition of isobutyl chloroformate (10.2 mg, 0.0748 mmol) in DMF (0.5 mL). After 1.5 h, a solution of N-Tfa-ethylenediamine (10.6 mg, 0.0680 mmol) was added dropwise. The reaction was allowed to warm to room temperature and stirred overnight. After removal of DMF in vacuo, the product was dissolved in methanol and dialyzed against methanol with SnakeSkin® Pleated Dialysis Tubing (MWCO = 10,000).

**67. DEPROTECTION OF MAL-DPEG<sup>TM</sup><sub>4</sub>-T-BOC-HYDRAZIDE.**

10 [00488] In a 100 mL round bottomed flask, *MAL-dPeg<sup>TM</sup><sub>4</sub>-t-boc-hydrazide* (8.8 mg, 135.0 nmol) was dissolved in 10.0 mL of formic acid and stirred over night at room temperature. After the solvent was evaporated under reduced pressure, toluene was added and concentrated to remove any residual formic acid to give *MAL-dPeg<sup>TM</sup><sub>4</sub>-hydrazide* (58.1 mg, 100%).

**68. ATTACHMENT OF MAL-DPEG<sup>TM</sup><sub>4</sub>-HYDRAZIDE.**

[00489] The deprotected nanoparticles (77.5 mg, 0.00186 mmol) in DriSolv DMF (9.0 mL) were stirred under argon at 0 °C with N-methylmorpholine (1.5 mg, 0.0149 mmol) followed by dropwise addition of isobutyl chloroformate (2.2 mg, 0.0164 mmol) in DMF (0.1 mL). After 1.5 h, a solution of *MAL-dPeg<sup>TM</sup><sub>4</sub>-hydrazide* (8.8 mg, mol) was added dropwise. The reaction was allowed to warm to room temperature and stirred overnight. After removal of DMF in vacuo, the product was dissolved in methanol and dialyzed against methanol with SnakeSkin® Pleated Dialysis Tubing (MWCO = 10,000).

**69. ATTACHMENT OF DISULFIDE LINKER TO BOC-PROTECTED HEXYL MOLECULAR TRANSPORTER.**

25 [00490] A solution of boc-protected hexyl molecular transporter (500.0 mg, 0.13 mmol), 3-(pyridine-2-yl disulfanyl)propanoic acid (269.5 mg, 1.25 mmol), 1-hydroxybenzotriazole (HOBt) (169.0 mg, 1.25 mmol), triethylamine (TEA) (126.7 mg, 1.25 mmol), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC HCl) (240.0 mg, 1.25 mmol) in 10 mL DriSolv DMF was stirred for 48 h. The DMF was removed under

vacuum and the resulting brown oil purified via column chromatography eluting with a 2-10 % methanol in dichloromethane gradient system (160.5 mg, 30.6%). <sup>1</sup>H NMR (300 MHz, MeOD) δ 1.33-1.47 (m, 246 H, CH<sub>2</sub>, CH<sub>3</sub>), 2.03 (d, 48 H, J=65.3Hz, CH<sub>2</sub>), 3.15 (td, 30 H, J=6.3Hz, J=12.7Hz, CH<sub>2</sub>), 3.29 (m, 45 H, CH<sub>2</sub>), 7.42 (m, 1 H, ArH), 7.67 (d, 1 H, J=8.2Hz, ArH), 7.79 (d, 1 H, J=8.1Hz, ArH), 8.02 (s, 1 H, ArH).

#### 70. CLEAVAGE OF DISULFIDE BRIDGE ON MOLECULAR TRANSPORTER.

[00491] A solution of the disulfide linker hexyl molecular transporter (118.2 mg,  $2.82 \times 10^{-5}$  mol) in DriSolv DMF (2.88 mL) was stirred under argon. A solution of DL-dithiothreitol (339.5 mg,  $2.2 \times 10^{-3}$  mol) in DMF (71.4 mL) was added dropwise to transporter and the reaction proceeded for 2 h at room temperature. After removal of DMF in vacuo, the reaction was purified using a Sephadex LH-20 column, eluting with DMF and concentrating the fractions in vacuo again.

#### 71. ATTACHMENT OF MOLECULAR TRANSPORTER TO NANOPARTICLES.

[00492] The nanoparticles (77.5 mg,  $1.76 \times 10^{-6}$  mol) in DriSolv DMF (mL) were stirred under argon. The free thiol molecular transporter in DMF was added dropwise followed by the addition of a catalytic amount of N-methylmorpholine. After removal of DMF in vacuo, the product was dissolved in methanol and dialyzed against a 1:1 methanol:water solution, eventually dialyzing against pure methanol with SnakeSkin<sup>®</sup> Pleated Dialysis Tubing (MWCO = 10,000).

#### 72. DEPROTECTION OF TRIFLUOROACETYL PROTECTED AMINES ON MODIFIED PARTICLES.

[00493] The nanoparticles (142.0 mg, x mol) were dissolved in a 10% K<sub>2</sub>CO<sub>3</sub> solution of 5:3 methanol:water. Methanol was added as needed to completely dissolve the particles. The reaction proceeded overnight at room temperature. The reaction was purified by dialysis with SnakeSkin<sup>®</sup> Pleated Dialysis Tubing (MWCO = 10 000) against a 5:3 methanol:water solution, eventually dialyzing against pure methanol.

**73. ATTACHMENT OF FITC.**

[00494] Nanoparticles (30.0 mg,  $4.75 \times 10^{-7}$  mol) in DriSolv DMF (1.0 mL) were stirred under argon. A solution of FITC (2.8 mg,  $7.13 \times 10^{-6}$  mol) in DriSolv DMF (1.0 mL) was added dropwise and the reaction chilled to 0 °C. Triethylamine (1.14 µL,  $8.17 \times 10^{-6}$  mol) was added to the solution and the reaction proceeded in the dark, overnight at room temperature.

**74. CAPPING OF THE REMAINING AMINES.**

[00495] Upon completion of the FITC addition to the nanoparticles, a solution of N-acetoxysuccinimide (47.1 mg,  $3.00 \times 10^{-4}$  mol) in DriSolv DMF (1.0 mL) was added to the reaction solution. The reaction was allowed to proceed for 3 h at RT. After removal of DMF in vacuo, the product was dissolved in methanol and dialyzed against methanol with SnakeSkin® Pleated Dialysis Tubing (MWCO = 10,000).

**75. BOC DEPROTECTION OF MODIFIED NANOPARTICLES.**

[00496] Modified nanoparticles were dissolved in anhydrous 1,4-dioxane (10 mL) and chilled to 0 °C. A solution of 4 M HCl in 1,4-dioxane (10 mL) was added dropwise to the stirring nanoparticles and the reaction was allowed to proceed overnight at room temperature. The nanoparticle solution was diluted to three times the original volume with water and dialyzed against water with SnakeSkin® Pleated Dialysis Tubing (MWCO = 10,000). Upon completion of dialysis, the aqueous solution was lyophilized to yield a yellow solid.

**76. G<sub>13</sub> PEPTIDE ATTACHMENT TO MULTIFUNCTIONAL NANOPARTICLE.**

[00497] A solution of G<sub>13</sub> peptide (1.2 mg,  $8.22 \times 10^{-7}$  mol) in a solution of DriSolv DMF (1.0 mL) was added to a solution of MFNP (1.5 mg,  $2.28 \times 10^{-8}$  mol) in DriSolv DMF (0.75 mL) and allowed to stir overnight. The reaction was then diluted with water and dialyzed against water with SnakeSkin® Pleated Dialysis Tubing (MWCO = 10,000). Upon completion of dialysis, the aqueous solution was lyophilized to yield a light yellow solid.

**77. G<sub>1R</sub> PEPTIDE ATTACHMENT TO MULTIFUNCTIONAL NANOPARTICLE.**

[00498] A solution of G<sub>1R</sub> peptide (1.2 mg,  $8.22 \times 10^{-7}$  mol) in a solution of DriSolv DMF (0.5 mL) was added to a solution of MFNP (1.5 mg,  $2.28 \times 10^{-8}$  mol) in DriSolv DMF (0.75 mL) and allowed to stir overnight. The reaction was then diluted with water and

dialyzed against water with SnakeSkin<sup>®</sup> Pleated Dialysis Tubing (MWCO = 10,000). Upon completion of dialysis, the aqueous solution was lyophilized to yield a light yellow solid.

**[00499]      Synthesis of copolymer poly(vl-avl-opd) (AbD).** To a 25 mL 3-necked round bottom flask, equipped with stir bar, gas inlet and 2 rubber septa, 2-oxepane-1,5-dione (0.7000 g, 5.46 mmol) was added. The round bottom flask was purged with argon. After purging for 30 min, dry toluene (4 mL) was added. The mixture stirred in an oil bath at 80 °C to dissolve the monomer. Upon dissolving, Sn(Oct)<sub>2</sub> (0.011 g, 2.73x10<sup>-2</sup> mmol) in 0.5 mL dry toluene, absolute ethanol (0.020 g, 4.4 x10<sup>-1</sup> mmol), α-allyl-δ-valerolactone (1.15 g, 8.2 mmol) and δ-valerolactone (1.37 g, 13.7 mmol) were then added to the reactor and the mixture was heated for 48 h at 105 °C. Residual monomer and catalyst were removed by dialyzing with Spectra/Por<sup>®</sup> dialysis membrane (MWCO = 1000) against CH<sub>2</sub>Cl<sub>2</sub> to give a golden brown polymer. Yield: 1.7 g. M<sub>w</sub> = 3287 Da, PDI = 1.17; <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>/TMS, ppm) δ: 5.72 (m, H<sub>2</sub>C=CH-), 5.06 (m, H<sub>2</sub>C=CH-), 4.34 (m, -CH<sub>2</sub>CH<sub>2</sub>C(O)CH<sub>2</sub>CH<sub>2</sub>O-), 4.08 (m, -CH<sub>2</sub>O-), 3.67 (m, -OCH<sub>2</sub>CH<sub>3</sub>), 2.78 (m, opd, -OC(O)CH<sub>2</sub>CH<sub>2</sub>C(O)CH<sub>2</sub>-), 2.58 (m, opd, -OC(O)CH<sub>2</sub>CH<sub>2</sub>C(O)CH<sub>2</sub>-), 2.34 (m, vl, -CH<sub>2</sub>CH<sub>2</sub>C(O)O-, avl, H<sub>2</sub>C=CHCH<sub>2</sub>CH-, H<sub>2</sub>C=CHCH<sub>2</sub>CH-), 1.66 (m, avl & vl, -CHCH<sub>2</sub>CH<sub>2</sub>-), 1.25 (t, -CH<sub>2</sub>CH<sub>3</sub>); <sup>13</sup>C NMR (400MHz, CDCl<sub>3</sub>, ppm) δ: 204.9, 175.2, 173.7, 173.2, 135.0, 117.0, 63.9, 44.8, 36.4, 33.6, 28.0, 26.3, 21.3. (10.39% avl, 7.97% evl, 6.42% opd and 75.21% vl).

#### 20                    78. SYNTHESIS OF POLY(VL-EVL-AVL-OPD) (ABBD).

**[00500]**      In a 200 mL round bottom flask, equipped with stir bar, poly(vl-avl-opd) (1.7 g, 1.56 mmol) was dissolved in 30 mL CH<sub>2</sub>Cl<sub>2</sub>. To this solution, 3-chloroperoxybenzoic acid (0.2210 g, 1.28 mmol) was added slowly. The mixture was stirred for 72 h at room temperature and then concentrated via rotary evaporator. The crude product was dissolved in a minimal amount of THF (5 mL) and poured into a round-bottomed flask containing 1L diethyl ether. The solution was kept overnight at 0 °C and a white solid was obtained. The solution was decanted off and the solid was dried *in vacuo* to obtain poly(avl-evl-vl-opd). Yield: 1.2 g (71%). <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>/TMS, ppm) δ: 5.72 (m, H<sub>2</sub>C=CH-), 5.06 (m, H<sub>2</sub>C=CH-), 4.34 (m, -CH<sub>2</sub>CH<sub>2</sub>C(O)CH<sub>2</sub>CH<sub>2</sub>O-), 4.08 (m, -CH<sub>2</sub>O-), 3.67 (m, -OCH<sub>2</sub>CH<sub>3</sub>), 2.96 (m, epoxide proton), 2.78 (m, evl epoxide proton, opd, -OC(O)CH<sub>2</sub>CH<sub>2</sub>C(O)CH<sub>2</sub>-), 2.58 (m, opd, -OC(O)CH<sub>2</sub>CH<sub>2</sub>C(O)CH<sub>2</sub>-), 2.47 (epoxide proton), 2.34 (m, vl, -CH<sub>2</sub>CH<sub>2</sub>C(O)O-,

av1,  $\text{H}_2\text{C}=\text{CHCH}_2\text{CH}-$ ,  $\text{H}_2\text{C}=\text{CHCH}_2\text{CH}-$ ), 1.66 (m, av1 & v1,  $-\text{CHCH}_2\text{CH}_2-$ ), 1.25 (t,  $-\text{CH}_2\text{CH}_3$ ).

#### 79. NANOPARTICLE FORMATION FROM POLY(VL-EVL-AVL-OPD).

[00501] In a 250 mL three-necked round bottom flask equipped with stir bar,  
 5 condenser and septa, a solution of 2,2'-(ethylenedioxy)diethylamine (26.4  $\mu\text{L}$ , 0.18 mmol) in  
 55.6 mL  $\text{CH}_2\text{Cl}_2$  was heated at 44 °C. A solution of poly(av1-ev1-vl-opd) (0.2500 g,  $M_w$  = 3287  
 Da, PDI = 1.17) dissolved in  $\text{CH}_2\text{Cl}_2$  (0.36 mL) was added dropwise via a peristaltic pump at  
 13 mL/min with vigorous stirring. The reaction mixture was heated for 12 h. Residual  
 diamine was removed by dialyzing with SnakeSkin® Pleated Dialysis Tubing (MWCO =  
 10,000) against dichloromethane.  $^1\text{H}$  NMR (300MHz,  $\text{CDCl}_3/\text{TMS}$ , ppm)  $\delta$ : The significant  
 change is the disappearance of the epoxide protons at 2.96, 2.75 and 2.47 ppm and the  
 appearance of signals at 3.5 and 2.89 ppm corresponding to the protons neighboring the  
 secondary amine of the PEG linker after cross-linking. All other aspects of the spectrum are  
 similar. To demonstrate the reactivity of the ally groups to thiols, in a model reaction we  
 15 added benzyl mercaptan to the allyl groups. We found a high reactivity using no other reactant.  
 We also added the molecular transporter in the same fashion.

#### 80. ATTACHMENT OF BENZYL MERCAPTAN TO POLY(VL-EVL-AVL-OPD)

##### NANOPARTICLES (GENERAL PROCEDURE TO ATTACH THIOL FUNCTIONALIZED COMPOUNDS INCLUDING "MOLECULAR TRANSPORTER" AND PEPTIDES)

20 [00502] In a vial equipped with a stir bar, poly(av1-ev1-vl-opd) nanoparticles (0.030 g,  
 0.0268 mmol) and benzyl mercaptan (9.48 mg, 0.0764 mmol) were dissolved in 0.6 mL  
 toluene. The reaction mixture was heated for 72 h at 30 °C. The remaining toluene was  
 removed *in vacuo* and residual benzyl mercaptan was removed by dialyzing with SnakeSkin®  
 Pleated Dialysis Tubing (MWCO = 10,000) against dichloromethane.  $^1\text{H}$  NMR (300MHz,  
 25  $\text{CDCl}_3/\text{TMS}$ , ppm)  $\delta$ : The significant change is the disappearance of the allyl protons at 5.72  
 and 5.06 ppm and the appearance of signals at 3.52 ppm and 7.30 ppm corresponding to the  
 methylene and benzene protons respectively of the attached benzyl mercaptan. All other  
 aspects of the spectrum are similar.

**81. ATTACHMENT OF N-BOC-ETHYLENEDIAMINE TO SUCCINIMIDYL 2-VINYLSULFONYLETHYL CARBONATE (SVEC).**

[00503] To a solution of SVEC (1.03 g, 3.72 mmol) in acetonitrile (50 mL), N-boc-ethylenediamine (0.77 mL, 4.86 mmol) and water (50 mL) were added. Sodium bicarbonate (0.4066 g, 4.84 mmol) was added and the reaction stirred for 4 h at room temperature. The acetonitrile was removed *in vacuo* and the remaining aqueous phase was diluted with brine (45 mL). The aqueous phase was extracted three times with dichloromethane (90 mL). The organic phases were combined, washed with brine, dried with  $\text{MgSO}_4$ , and concentrated *in vacuo*. The crude product was purified by flash chromatography (eluent: ethyl acetate) to give a white solid in 90% yield.  $^1\text{H}$  NMR (300MHz,  $\text{CDCl}_3/\text{TMS}$ , ppm)  $\delta$ : 6.6 (m,  $\text{H}_2\text{C}=\text{CH}-$ ), 6.4 & 6.17 (m,  $\text{H}_2\text{C}=\text{CH}-$ ), 4.43 (t,  $-\text{CH}_2\text{CH}_2\text{OC}(\text{O})-$ ), 3.3 (t,  $-\text{CH}_2\text{CH}_2\text{OC}(\text{O})-$ ), 3.24 (m,  $-\text{NHCH}_2\text{CH}_2\text{NHC}(\text{O})-$ ), 1.41 (s,  $-\text{NHC}(\text{O})\text{OC}(\text{CH}_3)_3$ ).

**82. ATTACHMENT OF SULFONYL LINKER TO NANOPARTICLES FROM POLY(VL-EVL-OPD).**

[00504] In a 100 mL round bottom flask, equipped with stir bar, poly(vl-evl-opd) (ABD) nanoparticles (84.6 mg,  $2.45 \times 10^{-7}$  mol) were dissolved in 12.5 mL  $\text{CH}_2\text{Cl}_2$ . To this solution, sulfonyl linker (69  $\mu\text{L}$  of 0.85 M linker in methanol,  $5.89 \times 10^{-5}$  mol),  $\text{NaCNBH}_3$  (0.0111 g in 0.1 mL methanol,  $1.77 \times 10^{-4}$  mol) and methanol (12.4 mL) were added. The pH was adjusted to 6.5 using 0.1 M hydrochloric acid aqueous solution and 0.1 M sodium hydroxide aqueous solution. The reaction mixture stirred for 25 h at room temperature and was purified by dialyzing with SnakeSkin<sup>®</sup> Pleated Dialysis Tubing (MWCO = 10,000) against 1:1 dichloromethane/methanol.  $^1\text{H}$  NMR (300MHz,  $\text{CDCl}_3/\text{TMS}$ , ppm)  $\delta$ : The significant change is the appearance of the following peaks: 6.8 (m,  $\text{CH}_2=\text{CH}-$ ), 6.5 & 6.3 (m,  $\text{CH}_2=\text{CH}-$ ), 4.5 (m,  $\text{CH}_2=\text{CHSO}_2\text{CH}_2\text{CH}_2-$ ), 3.3 (m,  $-\text{NHCH}_2\text{CH}_2\text{NH}-$ ), 3.1 (m,  $\text{CH}_2=\text{CHSO}_2\text{CH}_2\text{CH}_2-$ ). All other aspects of the spectrum are similar.

**83. GENERAL PROCEDURE FOR ATTACHMENT OF PEPTIDE-ALEXA FLUOR<sup>®</sup> 750 TO LINKER CONJUGATED NANOPARTICLES.**

[00505] In a small vial, equipped with stir bar, peptide (33  $\mu\text{L}$  of 0.013 mg/mL peptide in phosphate buffer- pH 7.2) and Alexa Fluor<sup>®</sup> 750 (26.5  $\mu\text{L}$  of 20 mg/mL Alexa Fluor<sup>®</sup> in dimethylformamide) were added. The reaction stirred for 24 h in an aluminum foiled. In a

small vial, poly(vl-evl-opd) (ABD) nanoparticles (29.9 mg) were dissolved in 800  $\mu$ L phosphate buffer (pH = 7.2) and 700  $\mu$ L dimethylformamide. To the peptide-Alexa Fluor® solution, 251  $\mu$ L of dissolved nanoparticles was added. After stirring for 45 min at room temperature, additional peptide (2 mg,  $1.84 \times 10^{-6}$  mol) was added. The reaction mixture was  
5 purified using concentrator tubes with a molecular weight cut-off of 10,000 Da.

#### 84. ATTACHMENT OF ALEXA FLUOR® 750 TO POLY(VL-EVL-OPD) NANOPARTICLES.

[00506] In a 25 mL round bottom flask, poly(vl-evl-opd) nanoparticles (63.55 mg,  $1.92 \times 10^{-7}$  mol) was dissolved in 6.4 mL tetrahydrofuran. The round bottom flask was sealed with a rubber septum and purged with argon. To the purged solution, Alexa Fluor® 750 (5  
10 mg in 0.5 mL anhydrous dimethylformamide was added. The reaction mixture stirred for 24 h at room temperature. After 24 h, N-acetoxysuccinimide (50 mg, 0.3 mmol) was added to quench the remaining unreacted amines.  $^1\text{H}$  NMR (300MHz,  $\text{CDCl}_3/\text{TMS}$ , ppm)  $\delta$ : The significant change is the appearance of the following peaks: 7.12, 5.6, 5.5, 5.1, 3.81, 1.90 ppm. The structure of Alexa Fluor® 750 is not publicly known. All other aspects of the  
15 spectrum are similar.

#### 85. GENERAL REDUCTIVE AMINATION FOR THE ATTACHMENT OF PEPTIDES TO ALEXA FLUOR® CONJUGATED NANOPARTICLES.

[00507] In a small vial, equipped with stir bar, peptide (2.6 mg,  $2.4 \times 10^{-6}$  mol) was dissolved in 2 mL tetrahydrofuran. To this solution, dye conjugated nanoparticles (0.0923 g,   
20  $2.8 \times 10^{-8}$  mol, in 0.5 mL tetrahydrofuran) and  $\text{NaCNBH}_3$  (2.23  $\mu$ L of 1.0 M  $\text{NaCNBH}_3$  in tetrahydrofuran) were added. The reaction mixture stirred for 12 h at room temperature. The reaction mixture was purified by dialyzing with SnakeSkin® Pleated Dialysis Tubing (MWCO = 10,000) against tetrahydrofuran.  $^1\text{H}$  NMR (300MHz,  $\text{CDCl}_3/\text{TMS}$ , ppm)  $\delta$ : The significant change is the appearance of the following peaks: 5.2, 5, 4.8, 2.6, 2.45, 2.0, 1.22 and 0.89 ppm.

#### 86. ATTACHMENT OF N-(BOC)-2,2(ETHYLENEDIOXY)DIETHYLAMINE.

[00508] A 50 mL 3-neck round bottom flask was flame-dried under argon. The deprotected nanoparticles (27.6 mg, 0.79  $\mu$ mol) were dissolved in DriSolv DMF and transferred to the sealed flask, which was then cooled to 0 °C via an ice bath. N-methylmorpholine (6.37 mg, 0.063 mmol) followed by isobutyl chloroformate (9.46 mg,

0.0693 mmol) was added to the cooled solution and allowed to activate for 1.5 h. Next, N-(boc)-2,2(ethylenedioxy)diethylamine (15.6 mg, 0.063 mmol) was added, the ice bath was removed and the reaction was allowed to stir overnight. The reaction was concentrated in vacuo, the residue was dissolved in MeOH, transferred to SnakeSkin® Pleated Dialysis  
5 Tubing (MWCO = 10 000), and was dialysed against MeOH.

**87. DEPROTECTION OF NANOPARTICLES CONTAINING N-(BOC)-  
2,2(ETHYLENEDIOXY)DIETHYLAMINE.**

[00509] The nanoparticles were dissolved in 2 M HCl/Dioxane (15 mL). The reaction was allowed to stir overnight. The reaction was dissolved in MeOH/H<sub>2</sub>O and transferred to  
10 SnakeSkin® Pleated Dialysis Tubing (MWCO = 10 000), and was dialysed against MeOH/H<sub>2</sub>O.

**88. ATTACHMENT OF ALEXA FLUOR 750®.**

[00510] PBS Buffer (pH 7.3) was purged with argon for 1 h. The Alexa Fluor® 750 (3 mg, 2.3 µmol) in 0.3 mL DMF was added to a solution of deprotected nanoparticles (15.8  
15 mg) in PBS Buffer (1.2 mL) and was allowed to stir for 24 h. The reaction was diluted with H<sub>2</sub>O, was transferred to SnakeSkin® Pleated Dialysis Tubing (MWCO = 10 000), and was dialyzed against H<sub>2</sub>O.

**89. ATTACHMENT OF SVEC.**

[00511] The nanoparticles were dissolved in 4 mL of H<sub>2</sub>O followed by the addition of  
20 sodium bicarbonate (2.7 mg, 0.0318 mmol). Next, the SVEC was added in 1 mL of ACN followed by an additional 3 mL of ACN. The reaction was allowed to proceed for 2 h at which time acetoxysuccinimide (127 mg, 0.79 mmol) was added in order to quench any remaining amines. This reaction was allowed to proceed for 2 h. The reaction was diluted with H<sub>2</sub>O and was transferred to SnakeSkin® Pleated Dialysis Tubing (MWCO = 10 000),  
25 and was dialyzed against H<sub>2</sub>O (pH 4.5).

**90. TARGETING PEPTIDE ATTACHMENT.**

[00512] The modified nanoparticles (2 mg) were dissolved in 0.2 mL of PBS Buffer (pH 7.3) and to that a solution of GCGGGNHVGGSSV (11.4 mg, 0.0105 mmol) in 0.4 mL of

PBS Buffer (pH 7.3) was added. This reaction was allowed to proceed for 24 h. The reaction was diluted with H<sub>2</sub>O and was transferred to SnakeSkin® Pleated Dialysis Tubing (MWCO = 10 000), and was dialyzed against H<sub>2</sub>O.

#### 91. CONTROL PEPTIDE ATTACHMENT.

5 [00513] The modified nanoparticles (2 mg) were dissolved in 0.2 mL of PBS Buffer (pH 7.3) and to that a solution of GCGGGSGVSGHNG (11.0 mg, 0.0105 mmol) in 0.4 mL of PBS Buffer (pH 7.3) was added. This reaction was allowed to proceed for 24 h. The reaction was diluted with H<sub>2</sub>O and was transferred to SnakeSkin® Pleated Dialysis Tubing (MWCO = 10 000), and was dialyzed against H<sub>2</sub>O.

#### 10 92. ATTACHMENT OF N-(BOC)-2,2(ETHYLENEDIOXY)DIETHYLAMINE.

[00514] A 50 mL 3-neck round bottom flask was flame-dried under argon. The deprotected nanoparticles (27.6 mg, 0.79 µmol) were dissolved in DriSolv DMF and transferred to the sealed flask, which was then cooled to 0 °C via an ice bath. N-methylmorpholine (6.37 mg, 0.063 mmol) followed by isobutyl chloroformate (9.46 mg, 0.0693 mmol) was added to the cooled solution and allowed to activate for 1.5 h. Next, N-(15 (boc)-2,2(ethylenedioxy)diethylamine (15.6 mg, 0.063 mmol) was added, the ice bath was removed and the reaction was allowed to stir overnight. The reaction was concentrated in vacuo, the residue was dissolved in MeOH, transferred to SnakeSkin® Pleated Dialysis Tubing (MWCO = 10 000), and was dialysed against MeOH.

#### 20 93. DEPROTECTION OF NANOPARTICLES CONTAINING N-(BOC)-2,2(ETHYLENEDIOXY)DIETHYLAMINE.

[00515] The nanoparticles were dissolved in 2 M HCl/Dioxane (15 mL). The reaction was allowed to stir overnight. The reaction was dissolved in MeOH/H<sub>2</sub>O, was transferred to SnakeSkin® Pleated Dialysis Tubing (MWCO = 10 000), and was dialysed against 25 MeOH/H<sub>2</sub>O.

**94. ATTACHMENT OF 1,4,7,10-TETRAAZACYCLODODECANE-1,4,7-TRIS(*T*-BUTYL ACETATE)-10-SUCCINIMIDYL ACETATE (DOTA).**

[00516] The nanoparticles were dissolved in DMF followed by the addition of triethylamine (TEA). To this solution, DOTA was added and the reaction was allowed to stir  
5 overnight. The reaction was concentrated in vacuo, the residue was dissolved in MeOH/H<sub>2</sub>O, transferred to SnakeSkin® Pleated Dialysis Tubing (MWCO = 10 000), and dialyzed against MeOH/H<sub>2</sub>O.

**95. DEPROTECTION OF NANOPARTICLES CONTAINING *T*-BUTYL PROTECTED DOTA.**

[00517] The nanoparticles were dissolved in 2 M HCl/Dioxane (15 mL). The reaction  
10 was allowed to stir overnight. The reaction was dissolved in MeOH/H<sub>2</sub>O, transferred to SnakeSkin® Pleated Dialysis Tubing (MWCO = 10 000), and dialyzed against MeOH/H<sub>2</sub>O.

**96. ATTACHMENT OF SVEC.**

[00518] The nanoparticles were dissolved in H<sub>2</sub>O followed by the addition of sodium bicarbonate. Next, the SVEC was added in ACN followed by an additional ACN. The  
15 reaction was allowed to proceed for 2 h at which time acetoxysuccinimide was added in order to quench any remaining amines. This reaction was allowed to proceed for 2 h. The reaction was diluted with H<sub>2</sub>O, was transferred to SnakeSkin® Pleated Dialysis Tubing (MWCO = 10 000), and was dialyzed against H<sub>2</sub>O (pH 4.5).

**97. MODIFICATION OF ALEXA FLUOR 750®.**

20 [00519] Alexa Fluor 750® (1.43 mg, 1.1 µmol) was dissolved in 143 µL DMSO and added to cysteamine (0.077 mg, 1.0 µmol) in 30.9 µL of PBS Buffer (pH 7.5). The reaction was allowed to proceed overnight.

**98. SIMULTANEOUS ATTACHMENT OF MODIFIED ALEXA FLUOR 750® AND PEPTIDE.**

[00520] The modified nanoparticles were dissolved in PBS Buffer (pH 7.3), which had  
25 been purged with argon for 20 min. Next, the modified Alexa Fluor 750® and one equivalent of GCGGGNHVGGSSV was added and allowed to react for 2 h. An additional 4 equivalents of peptide was then added and the reaction stirred overnight. The solution was diluted in

H<sub>2</sub>O, transferred to SnakeSkin® Pleated Dialysis Tubing (MWCO = 10 000), and dialyzed against H<sub>2</sub>O.

#### 99. SYNTHESIS OF LINEAR RGD.

[00521] A typical Fmoc solid phase peptide synthesis was performed to synthesize the linear peptide. A cysteine preloaded 2-chlorotrityl resin was employed. HOBt: HBTU: DIPEA (1:1:2) in DMF was used as the coupling reagent and amino acids were double coupled. A 20% piperidine (v/v) in DMF employed to deprotect the Fmoc. An amino-hexyl spacer was coupled to the cystine on the resin, followed by glutamic acid, aspartic acid, glycine, arginine, phenylalanine, and finally lysine.

#### 100. CYCLIZATION OF RGD.

[00522] The peptide was cyclized by utilizing an ODmab group, which allows for the selective deprotection carboxylic acid side chain of the glutamic acid, which can then be coupled to the N-terminus. The ODmab was deprotected using 2% v/v hydrazine-H<sub>2</sub>O/DMF added to the resin and allowed to react for 7 min. Next it was washed with 20 mL of DMF followed by 10 mL of a 5% v/v DIPEA/DMF solution which was allowed to shake for 10 min. Carboxy activation was achieved through the use of DCC (44.6 mg, mmol) and HOBt (29.2 mg, mmol) was added to 10 mL of DMF and then added to the resin and allowed to shake for 18 h.

[00523] Reagent R was used to deprotect all side groups and cleave the cyclic peptide from the resin. Reagent R was prepared by combining 5.4 mL TFA, 0.3 mL thioanisole, 0.18 mL anisole, and 0.12 mL ethanedithiol. This was allowed to react for 3 hours at which time the resin was filtered off. The supernatant was cooled to 0 °C and the peptide was precipitated using cold diethyl ether. It was collected through centrifugation and then washed three times using diethyl ether. The pellet was dissolved in 0.6 mL H<sub>2</sub>O and 0.4 mL ACN with 0.3% TFA and purified using HPLC.

#### 101. SYNTHESIS OF N-BOC-N-TFA-ETHYLENEDIAMINE.

[00524] To a solution of N-boc-ethylenediamine (5.0 g, 31.2 mmol) in 20 mL THF, ethyl trifluoroacetate (3.72 mL, 31.2 mmol) was added dropwise and the reaction stirred overnight. The reaction solution was concentrated to yield a white crystalline product (8.0 g,

100%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 1.44 (s, 9 H, CH<sub>3</sub>), 3.37 (dd, 2 H, J=5.4Hz, J=10.2Hz, CH<sub>2</sub>), 3.46 (dd, 2 H, J=5.1Hz, J=10.4Hz, CH<sub>2</sub>), 5.01 (s, 1 H, NH), 7.85 (s, 1 H, NH); <sup>13</sup>C NMR (400 MHz, CDCl<sub>3</sub>) δ 28.2, 39.1, 42.2, 80.6, 140.6, 151.2, 157.7.

#### 102. BOC DEPROTECTION OF N-BOC-N-Tfa-ETHYLENEDIAMINE.

5 [00525] N-Boc-N-Tfa-ethylenediamine (8.0 g, 31.5 mmol) was dissolved in 50 mL formic acid and stirred for 14 h at room temperature. After the solvent was evaporated under reduced pressure, toluene was added and concentrated to remove any residual formic acid, yielding an orange oil (4.90 g, 99.7%). <sup>1</sup>H NMR (400 MHz, MeOD) δ 2.31 (s, 2 H, NH<sub>2</sub>), 3.15 (t, 2 H, J=6.1Hz, CH<sub>2</sub>), 3.61 (t, 2 H, J=6.1Hz, CH<sub>2</sub>), 8.35 (s, 1 H, NH); <sup>13</sup>C NMR (400  
10 MHz, MeOD) δ 38.5, 39.7, 113.1, 115.9, 118.8, 121.6, 159.7, 160.1.

#### 103. ATTACHMENT OF N-Tfa-ETHYLENEDIAMINE.

[00526] The deprotected nanoparticles (162.3 mg, 4.58 μmol) in DriSolv DMF (10.0 mL) were stirred under argon at 0 °C with N-methylmorpholine (47.8 mg, 472.6 μmol) followed by dropwise addition of isobutyl chloroformate (71.0 mg, 519.8 μmol) in DriSolv  
15 DMF (0.75 mL). After 1.5 h, a solution of N-Tfa-ethylenediamine (73.8 mg, 472.6 μmol) in DriSolv DMF (2.5 mL) was added dropwise. The reaction was allowed to warm to room temperature and stirred overnight. After removal of DMF in vacuo, the product was dissolved in methanol and dialyzed against methanol with SnakeSkin<sup>®</sup> Pleated Dialysis Tubing (MWCO = 10,000). <sup>1</sup>H NMR (400 MHz, MeOD) δ 7.61-6.45 (br m, aromatic from  
20 crosslinker), 3.15-3.00 (br m, N-Tfa-ethylenediamine), 3.00-2.69 (br m, backbone and N-Tfa-ethylenediamine), 2.69-1.34 (br m, backbone).

#### 104. DEPROTECTION OF MAL-DPEG<sup>TM</sup><sub>4</sub>-T-BOC-HYDRAZIDE.

[00527] In a 100 mL round bottomed flask, MAL-dPeg<sup>TM</sup><sub>4</sub>-t-boc-hydrazide (127.1 mg, 239.5 μmol) was dissolved in 80.0 mL of formic acid and stirred over night at room  
25 temperature. After the solvent was evaporated under reduced pressure, toluene was added and concentrated to remove any residual formic acid to give MAL-dPeg<sup>TM</sup><sub>4</sub>-hydrazide (103.1 mg, 100%).

**105. ATTACHMENT OF MAL-DPEG<sup>TM</sup><sub>4</sub>—HYDRAZIDE.**

[00528] The deprotected nanoparticles (141.1 mg, 3.13  $\mu$ mol) in DriSolv DMF (10.0 mL) were stirred under argon at 0 °C with N-methylmorpholine (17.1 mg, 169.1  $\mu$ mol) followed by dropwise addition of isobutyl chloroformate (25.4 mg, 86.0  $\mu$ mol) in DriSolv DMF (0.7 mL). After 1.5 h, a solution of MAL-dPeg<sup>TM</sup><sub>4</sub>--hydrazide (103.1 mg, 239.5  $\mu$ mol) was added dropwise. The reaction was allowed to warm to room temperature and stirred overnight. After removal of DMF in vacuo, the product was dissolved in methanol and dialyzed against methanol with SnakeSkin<sup>®</sup> Pleated Dialysis Tubing (MWCO = 10,000). <sup>1</sup>H NMR (400 MHz, MeOD)  $\delta$  7.67-6.47 (br m, aromatic from crosslinker and maleimide linker), 3.89-3.48 (br t, maleimide linker), 3.21-3.02 (br m, N-Tfa-ethylenediamine), 3.02-2.69 (br m, backbone and N-Tfa-ethylenediamine), 2.69-1.01 (br m, backbone).

**106. HYDROGENATION OF G1.**

[00529] A solution of G1 (8.36 g, 5.69 mmol) in ethanol (214 mL) in a Parr hydrogenation bottle with Raney-Nickel (3.49 g) was shaken at 65 psi for 3 days at room temperature. Another 1 g of Raney-Nickel was added to the reaction and it was again shaken at 65 psi for 3 days at room temperature. The reaction was filtered through Celite, and the removal of the solvent under reduced pressure gave the crude product. The residue was dissolved in ethyl acetate and subsequently washed with saturated sodium bicarbonate solution (2x, 100 mL) and brine (2x, 100 mL) then the organic layer was dried over anhydrous sodium sulfate. The solution was filtered and concentrated under reduced pressure to yield amine G1 (8.19 g, 93.7 %).

**107. PDPOH ATTACHMENT TO G1.**

[00530] PDPOH (91.46 mg, 4.25 mmol) in dry THF (100 mL) was stirred under argon at room temperature with 1-hydrobenzotriazole (HOBt) (68.90 mg, 5.10 mmol) and DCC (1.05 g, 5.10 mmol). After 1 h, amine G1 (7.34 g, 5.10 mmol) was added to the solution and the reaction proceeded for 48 h, after which, it was filtered and concentrated under reduced pressure. The crude material was purified via flash column chromatography eluting with 10:1 hexanes:ethyl acetate increasing to 100% ethyl acetate to give white SS-G1 (4.67 g, 67.1 %).

**108. SSG1 DEPROTECTION VIA FORMIC ACID.**

[00531] SSG1 (4.67 g, 2.85 mmol) was dissolved with stirring in formic acid (100 mL) and the reaction proceeded at room temperature overnight. Upon completion, the formic acid was removed azeotropically with toluene under reduced pressure to yield the product (3.29 g,  
5 100.0 %).

**109. N-Boc-1,6-DIAMINOHEXANE ATTACHMENT TO SSG1.**

[00532] SSG1OH (3.29 g, 2.91 mmol) in anhydrous THF (100 mL) was stirred under argon at room temperature with HOBt (4.23 g, 31.25 mmol) and DCC (6.45 g, 31.25 mmol). After one hour, N-boc-1,6-diaminohexane (6.76 g, 31.25 mmol) was added to the solution  
10 and the reaction proceeded for 48 h at room temperature. Upon completion, the reaction solution was filtered to remove the DCC salt and the filtrate concentrated and purified via flash column chromatography eluting with 1% methanol in dichloromethane and gradually increasing to 10% methanol in dichloromethane to yield a white solid (4.42 g, 52.0 %).

[00533] The resulting solid was dissolved in 1,4-dioxane (20 mL), the solution was  
15 cooled to 0 °C, and 4 M HCl in 1,4-dioxane (20 mL) was added and the reaction stirred for 24 h at room temperature. Removal of the solvent under pressure gave a white solid (3.55 g, 100.0%).

**110. ATTACHMENT OF GOODMAN'S REAGENT TO SSG1LL.**

[00534] The resulting SSG1LL HCl salt (3.55 g, 1.51 mol) was dissolved in methanol  
20 (50 mL), and the solution was cooled to 0 °C. Triethylamine (TEA) (3.41 mL, 24.56 mmol) was added followed by N,N'-diboc-N''-triflylguanidine (6.94 g, 17.74 mmol) and the reaction was stirred 24 h at room temperature. After removal of the solvent under reduced pressure, the crude product was purified via flash column chromatography eluting with 1% methanol in dichloromethane and gradually increasing to 10% methanol in dichloromethane to yield a  
25 white solid (838.2 mg, 13.13 %). <sup>1</sup>H NMR (300 MHz, MeOD) δ 1.33-1.47 (m, 246 H, CH<sub>2</sub>, CH<sub>3</sub>), 2.03 (d, 48 H, J=65.3 Hz, CH<sub>2</sub>), 3.15 (td, 30 H, J=6.3 Hz, J=12.7 Hz, CH<sub>2</sub>), 3.29 (m, 45 H, CH<sub>2</sub>), 7.42 (m, 1 H, ArH), 7.67 (d, 1 H, J=8.2 Hz, ArH), 7.79 (d, 1 H, J=8.1 Hz, ArH), 8.02 (s, 1 H, ArH).

**111. CLEAVAGE OF DISULFIDE BRIDGE ON MOLECULAR TRANSPORTER.**

[00535] The disulfide linker hexyl molecular transporter (257.8 mg, 61.41  $\mu\text{mol}$ ) in DriSolv DMF (5 mL) was stirred under argon and a solution of DL-dithiothreitol (740.0 mg, 4.80 mmol) in DMF (5 mL) was added dropwise and the reaction proceeded for 2 h at room temperature. After removal of DMF in vacuo, the reaction was purified using a Sephadex LH-20 column, eluting with DMF and concentrating the fractions in vacuo again yielding the product (251.0 mg, 100%).

**112. ATTACHMENT OF MOLECULAR TRANSPORTER TO NANOPARTICLES.**

[00536] The nanoparticles (147.4 mg, 3.07  $\mu\text{mol}$ ) in DriSolv DMF (10.0 mL) were stirred under argon and the free thiol hexyl molecular transporter (251.0 mg, 61.41  $\mu\text{mol}$ ) in DriSolv DMF (10.0 mL) was added dropwise followed by the addition of a catalytic amount of N-methylmorpholine. After removal of DMF in vacuo, the product was dissolved in methanol and dialyzed against a 1:1 methanol:water solution, eventually dialyzing against pure methanol with SnakeSkin® Pleated Dialysis Tubing (MWCO = 10,000).  $^1\text{H}$  NMR (400 MHz, MeOD)  $\delta$  7.55-6.21 (br m, aromatic from crosslinker), 3.85-3.49 (br t, maleimide linker), 3.22-3.00 (br m, N-Tfa-ethylenediamine and molecular transporter), 3.00-2.70 (br m, backbone, N-Tfa-ethylenediamine, and molecular transporter), 2.70-1.00 (br m, backbone and molecular transporter).

**113. DEPROTECTION OF TRIFLUOROACETYL PROTECTED AMINES ON MODIFIED PARTICLES.**

[00537] The nanoparticles (142.0 mg, 1.54  $\mu\text{mol}$ ) were dissolved in methanol (5.0 mL) and a 10% K<sub>2</sub>CO<sub>3</sub> solution of 5:3 methanol:water (13.0 mL) was added to the solution and the reaction proceeded overnight at room temperature. The reaction was purified by dialysis with SnakeSkin® Pleated Dialysis Tubing (MWCO = 10 000) against a 5:3 methanol:water solution, eventually dialyzing against pure methanol then dialyzing against a 1:1 methanol THF solution, eventually dialyzing against pure THF.  $^1\text{H}$  NMR (400 MHz, THF d<sub>8</sub>)  $\delta$  8.26-6.53 (br m, aromatic from crosslinker), 3.94-3.52 (br m, maleimide linker), 3.28-3.12 (br t, ethylenediamine and molecular transporter), 3.12-2.68 (br m, backbone, ethylenediamine, and molecular transporter), 2.68-1.05 (br m, backbone and molecular transporter).

**114. ATTACHMENT OF 3-(PYRIDINE-2-YL DISULFANYL)PROPANOIC ACID NANOPARTICLES.**

[00538] A solution of 3-(pyridine-2-yl disulfanyl)propanoic acid (16.8 mg, 77.9  $\mu\text{mol}$ ) in anhydrous THF (2.5 mL) was stirred under argon at 0 °C with N-methylmorpholine (7.88 mg, 77.9  $\mu\text{mol}$ ) followed by dropwise addition of isobutyl chloroformate (11.7 mg, 85.7  $\mu\text{mol}$ ). After 1.5 h, a solution of the deprotected nanoparticles (111.0 mg, 1.30  $\mu\text{mol}$ ) in anhydrous THF (35.0 mL) was added dropwise. The reaction was allowed to warm to room temperature and stirred for 24 h. The reaction was diluted and purified by dialysis with SnakeSkin® Pleated Dialysis Tubing (MWCO = 10,000) against methanol, eventually dialyzing against a 3:1 THF:MeOH solution.  $^1\text{H}$  NMR (400 MHz, THF  $d_8$ )  $\delta$  7.58-6.22 (br m, aromatic from crosslinking and disulfide linker), 3.87-3.67 (br m, maleimide linker), 3.24-3.16 (br m, disulfide linker), 3.15-3.04 (br m, diamine and molecular transporter), 2.93-2.83 (br m, diamine and molecular transporter), 2.78-2.62 (br m, disulfide linker), 2.62-1.06 (br m, backbone).

**115. ATTACHMENT OF ALEXA FLUOR® 568.**

[00539] To a solution of multifunctional nanoparticles (10.0 mg, 106.0 nmol) in DriSolv DMF (3.0 mL), a solution of Alexa Fluor® 568 (3.78 mg, 4.77  $\mu\text{mol}$ ) in anhydrous DMSO (377.7  $\mu\text{L}$ ) and triethylamine (50.0  $\mu\text{L}$ , 358.7  $\mu\text{mol}$ ) was added to the solution and the reaction proceeded in the dark for 24 h at room temperature. The reaction was diluted with THF and purified by dialysis with SnakeSkin® Pleated Dialysis Tubing (MWCO = 10,000) against 1%  $\text{H}_2\text{O}$  in THF eventually dialyzing against pure THF.

**116. CAPPING OF THE REMAINING AMINES.**

[00540] Upon completion of the Alexa Fluor 568 addition to the nanoparticles, a solution of N-acetoxysuccinimide (47.1 mg, 299.5  $\mu\text{mol}$ ) in DriSolv DMF (1.0 mL) was added to the reaction solution. The reaction was allowed to proceed for 3 h at RT. After removal of DMF in vacuo, the product was dissolved in methanol and dialyzed against methanol with SnakeSkin® Pleated Dialysis Tubing (MWCO = 10,000).  $^1\text{H}$  NMR (400 MHz, MeOD)  $\delta$  1.29-2.43 (br m, backbone and molecular transporter), 2.59-2.83 (br m, disulfide linker), 2.84-2.95 (br m, disulfide linker), 2.98-3.02 (br m, disulfide linker), 3.04-3.09 (br m,

disulfide linker), 3.16 (br t, diamine and molecular transporter), 3.67 (br t, maleimide linker), 6.53-7.98 (br m, aromatic from crosslinking, disulfide linker, and FITC).

#### 117. BOC DEPROTECTION OF MODIFIED NANOPARTICLES.

[00541] Modified nanoparticles (30.0 mg, 434.0 nmol) were dissolved in anhydrous  
 5 1,4-dioxane (10 mL) and chilled to 0 °C. A solution of 4 M HCl in 1,4-dioxane (10 mL) was  
 added dropwise to the stirring nanoparticles and the reaction was allowed to proceed  
 overnight at room temperature. The nanoparticle solution was diluted to three times the  
 original volume with water and dialyzed against water with SnakeSkin® Pleated Dialysis  
 Tubing (MWCO = 10,000). Upon completion of dialysis, the aqueous solution was  
 10 lyophilized to yield a yellow solid. <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O) δ 1.18-2.37 (br m, backbone  
 and molecular transporter), 2.71-2.79 (br m, disulfide linker), 2.81-2.86 (br m, disulfide  
 linker), 2.89-2.93 (br m, disulfide linker), 2.94-2.99 (br m, disulfide linker), 3.12 (br t,  
 diamine and molecular transporter), 3.69 (br t, maleimide linker), 6.53-8.41 (br m, aromatic  
 from crosslinking, disulfide linker, and FITC).

#### 15 118. SYNTHESIS OF COPOLYMER POLY(VL-AVL) (AB).

[00542] A 50 mL 3-necked round bottom flask, equipped with stir bar, was sealed  
 with two septa and a gas inlet. The flask was evacuated and refilled with nitrogen three times.  
 Stock solutions of 1.7 M ethanol (EtOH) in THF and 3.7x10<sup>-2</sup> M tin(II) 2-ethylhexanoate  
 (Sn(Oct)<sub>2</sub>) in THF were made in sealed N<sub>2</sub> purged flasks. Solutions of EtOH (0.32 mL,  
 20 5.41x10<sup>-1</sup> mmol) and Sn(Oct)<sub>2</sub> (0.30 mL, 1.12x10<sup>-2</sup> mmol) were combined in the nitrogen  
 purged 50 mL flask. After stirring the mixture for 30 min, α-allyl-δ-valerolactone (1.16 g,  
 8.32 mmol) and δ-valerolactone (vl, 2.50 g, 24.97 mmol) were added. The reaction vessel  
 stirred at 105 °C for 48 h. Residual monomer and catalyst were removed by dialyzing with  
 Spectra/Por® dialysis membrane (MWCO = 1000) against CH<sub>2</sub>Cl<sub>2</sub> to give a golden brown  
 25 polymer. Yield: 3.24 g (88%). M<sub>w</sub> = 3042Da, PDI = 1.18; <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>/TMS,  
 ppm) δ: 5.7 (m, H<sub>2</sub>C=CH-), 5.09 (m, H<sub>2</sub>C=CH-), 4.09 (m, -CH<sub>2</sub>-O-), 3.65 (m, CH<sub>3</sub>CH<sub>2</sub>O-),  
 2.35 (m, vl, -CH<sub>2</sub>CH<sub>2</sub>C(O)O-, avl, H<sub>2</sub>C=CHCH<sub>2</sub>CH-, H<sub>2</sub>C=CHCH<sub>2</sub>CH-), 1.68 (m, avl & vl, -  
 CHCH<sub>2</sub>CH<sub>2</sub>-), 1.25 (t, CH<sub>3</sub>CH<sub>2</sub>O-); <sup>13</sup>C NMR (400MHz, CDCl<sub>3</sub>, ppm) δ: 174.6 (avl, -C(O)-),  
 172.7 (vl, -C(O)-), 134.6 (H<sub>2</sub>C=CH-), 116.4 (H<sub>2</sub>C=CH-), 63.3, 44.3, 35.9, 33.1, 27.5, 25.9,  
 30 23.6, 20.9.

**119. NANOPARTICLE FORMATION FROM AB.**

**[00543]** A solution of **Ab** (0.0804 g,  $M_w$  = 3042 Da, PDI = 1.18) dissolved in  $\text{CH}_2\text{Cl}_2$  (0.18 mL) was added to a solution of 3,6-dioxa-1,8-octanedithiol (30.0  $\mu\text{L}$ , 0.18 mmol) in  $\text{CH}_2\text{Cl}_2$  (28.4 mL) at 44 °C. The reaction mixture was heated for 12 h. Residual dithiol was removed by dialyzing with SnakeSkin<sup>®</sup> Pleated Dialysis Tubing (MWCO = 10,000) against dichloromethane. Yield: 0.078 g.  $^1\text{H}$  NMR (300MHz,  $\text{CDCl}_3/\text{TMS}$ )  $\delta$ : The significant change is the reduction of the allyl protons at 5.06 and 5.77 ppm and the appearance of signals at 3.65 and 2.71 ppm corresponding to the protons neighboring the thiols of the PEG linker after cross-linking. All other aspects of the spectrum are similar to that of **Ab**. The reaction can also be conducted with photoinitiators at RT in organic solvents. The particle sizes of the resulting particles correspond to those produced in analogous epoxide/amine procedures.

**[00544]** When reaction times are increased to from about 24h to about 48h, the particle sizes increase due to the total consumption of allyl moieties. Reaction at room temperature was found to be sufficient. Addition of radical starters or other photoinitiators does not significantly increase the quality of the particles.

**120. ONE POT SYNTHESIS OF NANOPARTICLES FROM POLY(VL-EVL-AVL-OPD) (ABbD).**

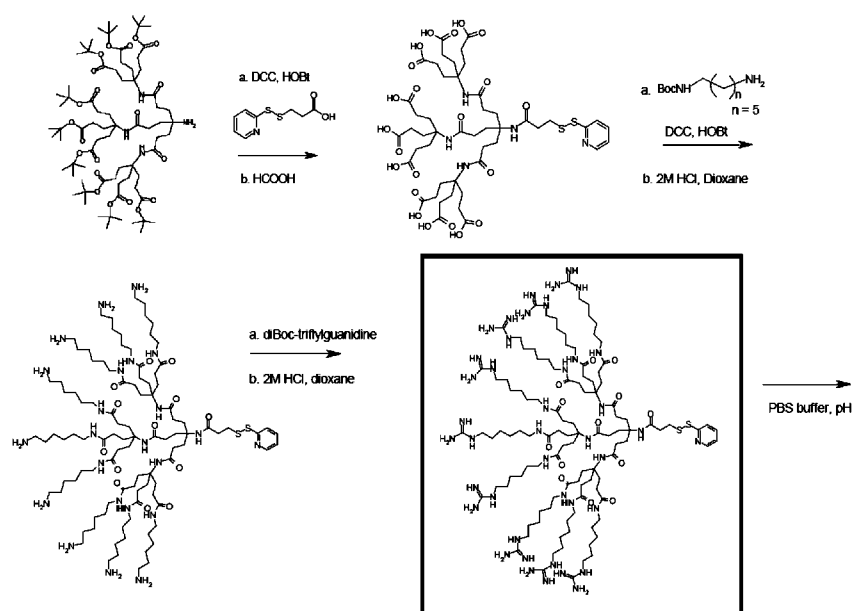
**[00545]** In a 25 mL three-necked round bottom flask equipped with stir bar, condenser and septa, 2,2'-(ethylenedioxy)diethylamine (18.3  $\mu\text{L}$ ,  $1.25 \times 10^{-4}$  mol), 17.1 mL  $\text{CH}_2\text{Cl}_2$  and a solution of poly(vl-evl- were added. A solution of poly(vl-evl-avl-opd), **ABbD**, (0.0781 g,  $M_w$  = 3500 Da, PDI = 1.29). The mixture was heated at 44 °C for 12 h. Residual diamine was removed by dialyzing with SnakeSkin<sup>®</sup> Pleated Dialysis Tubing (MWCO = 10,000) against dichloromethane.  $^1\text{H}$  NMR (300MHz,  $\text{CDCl}_3/\text{TMS}$ , ppm)  $\delta$ : The significant change is the disappearance of the epoxide protons at 2.94, 2.75 and 2.47 ppm and the appearance of signals at 3.64 and 2.97 ppm corresponding to the protons neighboring the secondary amine of the PEG linker after cross-linking. All other aspects of the spectrum are similar. TEM analysis of the resulting nanoparticles is shown in Figure 52. The particle size distribution, with unusually narrow polydispersity, of the resulting nanoparticles is shown in Figure 53. To increase particle sizes, reaction times can be increased to from about 24h to about 48h.

**121. UPTAKE EXPERIMENT PROTOCOL.**

**[00546]** Fluorescent multifunctional nanoparticle, negative control particle, FD-1, and FD-2 uptake by mammalian cells was assessed using HeLa cells, cancer cells, grown in uncoated, 14 mm diameter Microwell, No. 1.5 MatTek Dishes and a Zeiss LSM 510 META  
5 confocal microscope. HeLa cells were grown in Dulbecco's Modified Eagle's Medium-Low Glucose (DMEM) (Sigma Aldrich) supplemented with 10% (v/v) fetal bovine serum (Gibco) and 1% (v/v) antibiotic-antimycotic (Gibco). The cells were treated with the multifunctional nanoparticles, negative control particles, FD-1, or FD-2 for one hour, washed three times with  $\text{Ca}^{2+}/\text{Mg}^{2+}$  free Phosphate Buffered Saline with EDTA (PBS), fixed with 3.3%  
10 paraformaldehyde at room temperature for 10 minutes, and analyzed using confocal microscopy.

**122. BIOCONJUGATE MOLECULAR TRANSPORTER.**

**[00547]** To prepare an exemplary antibody conjugated molecular transporter, a G1-Newkome dendrimer that contains nine t-butyl end functionalities and a primary amine group  
15 at the focal point was prepared using disclosed methods (Figure 54). The amine functionality was reacted with 3-(2-pyridinyldithio)propanoic acid via amide coupling reactions with DCC/HOBt to form a protected dendimer with a reactive core. The t-butyl ester groups on the periphery of the dendritic scaffold were deprotected with formic acid to give free carboxylic acid groups that were coupled with N-Boc-1,6-diaminohexane. After deprotection of the Boc  
20 protecting groups with 2M HCl in dioxane the free amines were transformed into guanidine groups with N,N-diBoc-N-triflylguanidine and the subsequent deprotection of the Boc groups using 2 M HCl in dioxane gave the desired compound (Scheme 21).



**Scheme 21.** Synthesis of dendrimer bioconjugate precursor.

[00548] The final compound is designed to localize in the cytoplasm of the cells, as an integrated a hexyl alkyl spacer is present at the periphery of the dendrimer scaffold that was previously found to be a feature for the specificity of its subcellular location. Furthermore, the pyridyldithio linker at the focal point allows for the exchange with sulfhydryl groups to form bioconjugates that are connected over a disulfide bond to afford a reductive cleavable linker that can maintain activity of the biomolecule in cells. The IgG molecular transporter conjugate (IgGMT) was formed by the mild reaction of Alexa Fluor® 568 labeled IgG antibody in PBS buffer at RT with the dendrimer (Figure 54). Five transporter dendrimers were attached to the IgG structure which has a molecular weight of 148 kDa. The conjugate was dialyzed against PBS buffer to remove any unreacted dendrons and the concentration in the dialysis tubing was chosen to be 1 mg ml<sup>-1</sup> IgGMT, that allowed for the use of the solution directly for the uptake and neutralization experiments.

[00549] First, the uptake efficiency of the IgGMT conjugate into human epithelial cells (HEp-2) was tested. The 60% confluent cells were incubated with IgGMT initially

for 10 min then for 30 min, 1, 2 and 6 h. The uptake efficiency was studied with confocal microscopy and the uptake of the bioconjugate could be observed as early as 10 min. Later time points showed an increase of red fluorescence of the IgGMT conjugate, progressing from the cell surface membrane to localize intracellularly in the perinuclear area at time

5 points of 2 and 6 h. Contrary to the affinity and uptake of the IgGMT bioconjugate, the Alexa Fluor® 568 labeled, unmodified IgG did not enter the cell at all times points investigated (Figure 55). After the uptake into HEp-2 cells was confirmed with no evidence of cellular damage, the activity of the conjugate in RSV infected cells that expressed green fluorescent protein GFP as a result of RSV infection was examined. First, HEp-2 cells were infected for

10 24 h with recombinant RSV-GFP, washed and allowed to incubate for an additional 48 h. The cells were then imaged with confocal microscopy at a total of 72 h after initial infection (Figure 56). The typical syncytia formation was observed, a combination and fusion of the infected cells, together with the expression of the green fluorescent protein (GFP). To study the neutralization effect of the IgGMT, HEp-2 cells infected for 24 h with RSV-GFP were

15 incubated for 30 min with a solution of IgGMT in PBS buffer, washed and imaged 48 h later (Figure 56a and b). By confocal microscopy it was observed that a significant reduction of the green fluorescence of GFP (a) and a strong red fluorescence of the Alexa Fluor® 568 labeled IgGMT conjugate (b). The merged images of (a) and (b) combined with differential interference contrast (DIC) also gave evidence of healthier cells with significantly less

20 syncytia formation than the untreated infected cells at the same time period (Figure 56c). This result illustrated the significant reduction of GFP in treated cells in contrast to the untreated cells observed at a total incubation time of 72 h after infection with RSV for 24 h. Besides the presence of neutralized cells that showed only the red fluorescence of the conjugate, cells that showed the coexistence of RSV-GFP and the red fluorescence of the IgGMT, appearing in

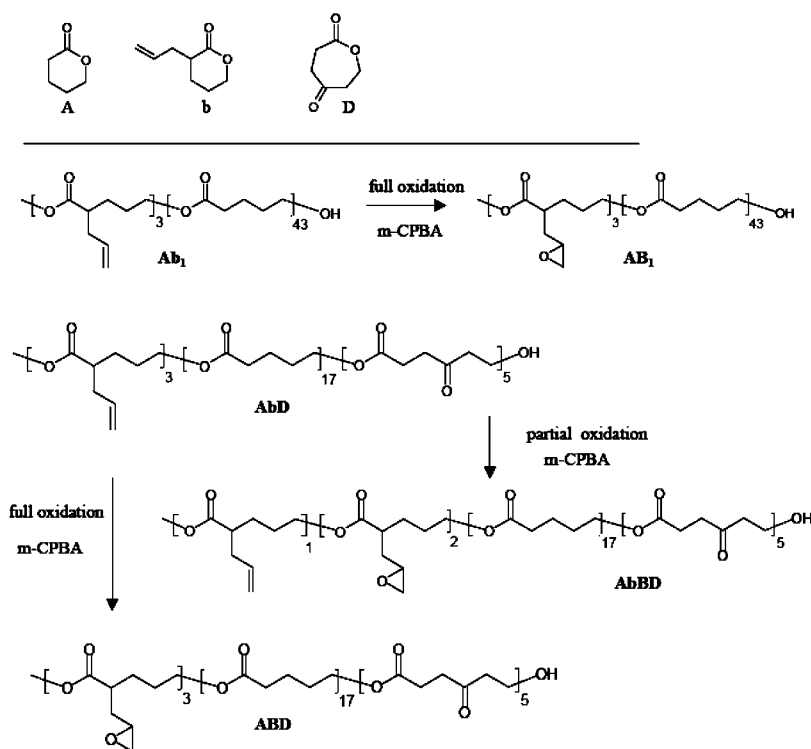
25 the center of the cells (Figure 56 and 57, a + b merged) were also observed. Imaging directly after the 30 min incubation of the RSV infected cells with the IgGMT, showed the green fluorescence localized intracellularly, whereas the red fluorescence was observed on the cell-surface membranes (Figure 57, a + b merged). These images documented the high affinity of the conjugate with the cells surface directly after the incubation period followed by the uptake

30 into the Hep-2 cells after an additional incubation time of 48 h. Parallel investigations of the RSV titres of the supernatant showed a significant reduction by 80–90% in viral replication when compared to cells not exposed to the IgGMT conjugate. Without wishing to be bound by theory, the intracellular delivery of IgG antibody directed to the surface protein inhibits the

syncytial formation mediated by the F protein and has an effect on total virus production when added 24 h after the initiation of RSV infection.

### 123. TAILORED POLYESTER NANOPARTICLES.

[00550] In this example, polyester nanoparticles in controlled nanoscopic dimensions have been prepared through a one-pot procedure that contains amine, keto, and allyl groups and is tailored towards the conjugation of bioactive building blocks, such as a dendritic molecular transporter to facilitate cellular uptake, or peptides and dyes to accomplish targeting and imaging. In several examples of bioconjugate synthesis, demonstrated is the versatility and the orthogonal attachment strategies involving high yielding thiol-ene reactions under mild conditions and reductive amination reactions, circumventing the integration of linker and multi-step post-modification pathways. Several linear nanoparticle precursors were prepared according to Scheme 22.



**Scheme 22.** Linear precursor.

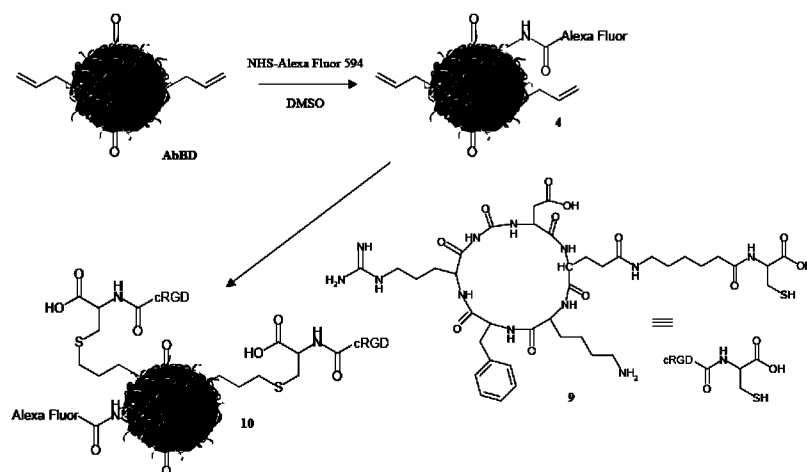
**[00551]** After the collapse of the above linear precursors using disclosed methods, the nanoparticle can be functionalized with a desired moiety. In contrast to reported strategies that form amide bonds with polyester scaffolds using EDC activation that are typically not very high yielding and require a high excess of expensive peptides, in this example the N-terminus of the targeting unit (e.g., a peptide) was reacted with the keto group, integrated in the polymer backbone of the developed polyester particle. In a model reaction, the successful reductive amination of N-Boc-ethylenediamine with keto groups of the particle has been shown and applied these reaction conditions to test the addition of peptidic units. Here, the targeting peptide sequence GCGGGNHVGGSSV was tested and chosen for the reaction with the ABD nanoparticle, with the nanoscopic dimension of 118 nm prepared from the ABD linear precursor polymer with 1.5 equivalents of 2,2'-(ethylenedioxy)bis(ethylamine) cross-linking units with the conditions as described above (Scheme 22). The amine groups of the nanoparticle were first capped with N-acetoxysuccinimide and the modified nanoparticle and the peptide were solubilized in tetrahydrofuran with NaCNBH<sub>3</sub> as the reducing reagent.

**[00552]** After purification through dialysis the modified particles,<sup>3</sup> were characterized with <sup>1</sup>H NMR and DLS. The increase in hydrodynamic diameter from 118 ± 10 nm to 120 ± 10 nm indicated the addition of peptides to the polyester backbone and further investigations with <sup>1</sup>H NMR showed the conjugation of peptides with the characteristic resonance peaks at 4.39 and 7.42 ppm. With additional analysis through static light scattering (SLS) we could determine the amount of peptide attached to the nanoparticle that was estimated to be between 36 of the intended attachment of 40 peptides per particle.

**[00553]** This result confirmed the efficiency of the reductive amination reactions with the N-terminus of the selected peptidic units. Targeting units, however, that contain more than one amine group give mixed conjugation products and an alternative strategy has to be developed. For this reason, we wanted to pursue thiol-ene type reactions that will be performed between cysteine units, integrated into the sequence close to the N-terminus, and double bonds that we find in maleimides, vinylsulfones or allyl groups. To integrate the reaction partner for the thiol/cysteine containing units, such as peptides, or oligonucleotides, into the nanoparticles, either a suitable linker that would be attached to the prepared nanoparticle was synthesized or a method that would circumvent the conjugation of a linker

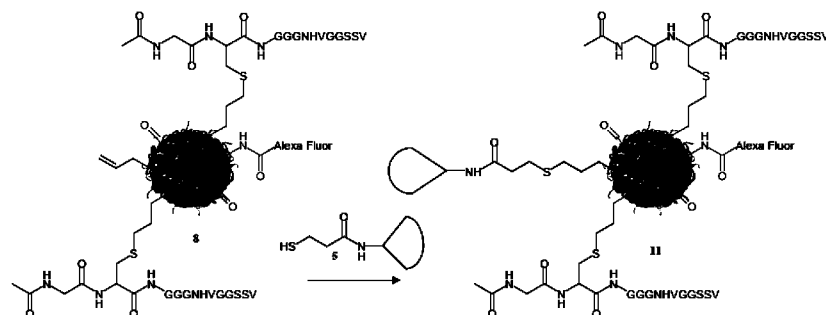
molecule to facilitate the attachment of thiol containing entities was found. Therefore, the integration of allyl groups in the polyester backbone as pendant functional units that would be already present in the linear polyester precursor before nanoparticle formation was studied. The available allyl groups that stem from the  $\alpha$ -allyl- $\delta$ -valerolactone of the linear polyester precursors were oxidized and converted entirely into epoxide groups to provide units that would cross-link with the diamine. However, with partial oxidation of the allyl group, linear polyester precursors containing epoxide units and remaining allyl groups, could be accomplished. In the next step, a linear polyester AbD that was partially oxidized to comprise 16 % of allyl units and 11% of epoxide units was cross-linked with 1.5 equivalents of diamine, using the novel one-pot reaction procedure to examine the compatibility of the allyl groups to the conditions of nanoparticle formation. The investigation of the resulting particles with DLS showed that hydrodynamic diameters corresponded to the size and solubility of the particles that did not contain any allyl groups. The allyl resonance peaks were still present in the  $^1\text{H}$  NMR spectra of the particles and were found to be analogous to the resonances of the allyl functionalities in the linear precursor.

[00554] After attaching a fluorescent probe, a disclosed cyclic peptide was attached, as shown in Scheme 23.



**Scheme 23.** Attachment of c-RGD

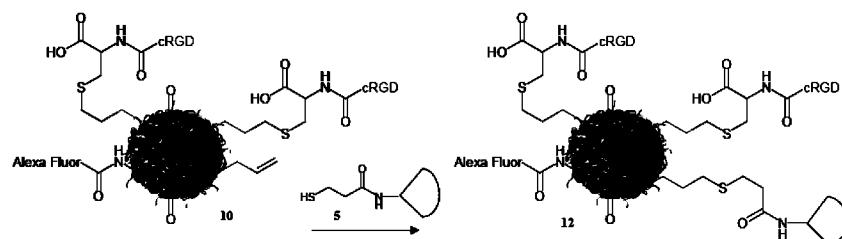
[00555] In the next step, a combined dendritic, peptidic, nanoparticle scaffold was synthesized according to Scheme 24.



**Scheme 24.** Combination of dendritic and peptidic scaffold.

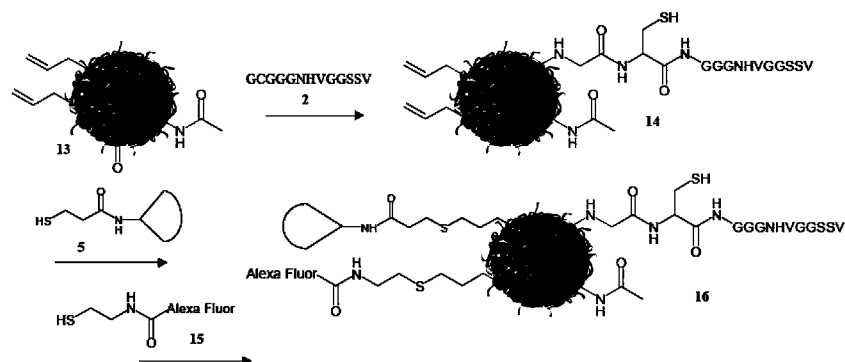
5 [00556] For the first approach, linear peptides GCGGGNHVGSSV with the recognition unit HVGGSSV with protected amines after capping with N-acetoxysuccinimide, were conjugated to the allyl functionality of a ABbD nanoparticle of 126.6 nm through the thiol of the cysteine unit as discussed above. In a following reaction, the imaging reagent Alexa Fluor®594 was introduced to label around 20 of the incorporated amine units of the nanoparticle. In a sequential thiolene reaction, the conjugation of 30 dendritic transporter molecules was achieved (Scheme 7), as was confirmed via <sup>1</sup>H NMR spectroscopy. The sequential conjugation of the bioactive compounds can be followed with an overlay of the <sup>1</sup>H NMR spectra that show the addition of first the peptide and the remaining allyl groups of the nanoparticle and the characteristic peaks of the molecular transporter molecule at 2.0 and 3.2 ppm.

[00557] The reaction sequence was changed to obtain a similar bioconjugate product that was only differentiated by the peptidic targeting unit. The amine groups of the c-RGD unit were not capped to avoid inactivation of the arginine recognition unit. Therefore the conjugation strategy included that the amine groups of the nanoparticle were first labeled with the NHS Alexa Fluor dye followed by the thiol-ene reaction with the targeting unit as shown in Scheme 6. In the last step, same as in the previous reaction, the dendritic transporter unit was added in a sequential thiol-ene reaction (Scheme 25).



**Scheme 25.** Synthesis of NP-P-MT-dye, ABbD-NP-594-cRGD-MT (12), utilizing thiol-ene chemistry.

[00558] In a third and last reaction sequence, we could demonstrate the versatility of the provided functional units of the nanoparticle and proceeded with an orthogonal conjugation approach. The free amine groups of the nanoparticle are capped with N-acetoxysuccinimide to not interfere with the following reductive amination reaction between the keto group of the polyester backbone and the N-terminus of the unmodified targeting peptide HVGGSV. After the reductive amination reaction was completed in the same fashion as described for compound 3, a thiolene reaction between the allyl groups of the nanoparticle and the thiol group of the molecular transporter could achieve the attachment of 30 units according to <sup>1</sup>H NMR spectroscopy analysis. The additional final characterization of the modified particles with static light scattering (SLS) the number of conjugated peptides peptides could determine the addition of 36 peptides to the particle. In a last step, the NHS ester Alexa Fluor dye was modified with thioethylamine (Scheme 26) to label exclusively the particle through a thiol-ene reaction to image the system *in vitro*. The Alexa Fluor 594 dye proved to be stable under the conditions and another example of the chemical versatility of the system was given.



**Scheme 26.** Synthesis of NP-P-MT-dye conjugate, ABbD-NP-594-MT utilizing reductive amination and thiol-ene chemistry.

**Table 6.** Summary of nanoparticle conjugates with definition of particle type depending on linear polymer precursors and connected targeting peptide: 'c' for capped N-terminus of peptide with HVGGSSV recognition unit via N-acetoxysuccinimide and 'c' for cyclic RGD. c Dendritic molecular transporter is abbreviated as MT, and the compound name is given in the order of the attachment d.

| Particle Type <sup>a</sup> | Targeting Peptides <sup>b</sup> | Alexa Fluor <sup>®</sup> Dye | Dendritic Molecular Transporter <sup>c</sup> | Compound Name <sup>d</sup>  | Compound Class |
|----------------------------|---------------------------------|------------------------------|----------------------------------------------|-----------------------------|----------------|
| ABD                        | HVGGSSV                         | -                            | -                                            | ABD-NP-HVGGSSV (3)          | NP-P           |
| ABbD                       | HVGGSSV                         | -                            | -                                            | ABbD-NP-HVGGSSV (14)        | NP-P           |
| ABbD                       | cHVGGSSV                        | 594                          | -                                            | ABbD-NP-cHVGGSSV-594 (8)    | NP-P-dye       |
| ABbD                       | cRGD                            | 594                          | -                                            | ABbD-NP-594-cRGD (10)       | NP-P-dye       |
| ABbD                       | -                               | 594                          | MT                                           | ABbD-NP-594-MT (6)          | NP-MT-dye      |
| ABbD                       | cHVGGSSV                        | 594                          | MT                                           | ABbD-NP-cHVGGSSV-594-MT(11) | NP-P-MT-dye    |
| ABbD                       | cRGD                            | 594                          | MT                                           | ABbD-NP-594-cRGD-MT (12)    | NP-P-MT-dye    |
| ABbD                       | HVGGSSV                         | 594                          | MT                                           | ABbD-NP-594-MT (16)         | NP-P-MT-dye    |

10 [00559] Below are the experimental procedures relevant to Example 123.

**[00560] Synthesis of copolymer poly(vl-avl-opd) (AbD).** To a 25 mL 3-necked round bottom flask, equipped with stir bar, gas inlet and 2 rubber septa, 2-oxepane-1,5-dione (0.70 g, 5.46 mmol) was added. The round bottom flask was purged with argon. After purging for 30 min, dry toluene (4 mL) was added. The mixture stirred in an oil bath at 80 °C to dissolve the monomer. Upon dissolving, Sn(Oct)<sub>2</sub> (11.1 mg, 27.3 μmol) in 0.5 mL dry toluene,  
 5 absolute ethanol (20.5 mg, 440 μmol), α-allyl-δ-valerolactone (1.15 g, 8.19 mmol) and δ-valerolactone (1.37 g, 13.7 mmol) were then added to the reactor and the mixture was heated for 48 h at 105 °C. Residual monomer and catalyst were removed by dialyzing with Spectra/Por® dialysis membrane (MWCO = 1000) against CH<sub>2</sub>Cl<sub>2</sub> to give a golden brown polymer. Yield: 2.70 g (85%). Mw = 3287 Da, PDI = 1.17; <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>/TMS, ppm) δ: 5.72 (m, H<sub>2</sub>C=CH-), 5.06 (m, H<sub>2</sub>C=CH-), 4.34 (m, -CH<sub>2</sub>CH<sub>2</sub>C(O)CH<sub>2</sub>CH<sub>2</sub>O-), 4.08 (m, -CH<sub>2</sub>O-), 3.67 (m, -OCH<sub>2</sub>CH<sub>3</sub>), 2.78 (m, opd, -OC(O)CH<sub>2</sub>CH<sub>2</sub>C(O)CH<sub>2</sub>-), 2.58 (m, opd, -OC(O)CH<sub>2</sub>CH<sub>2</sub>C(O)CH<sub>2</sub>-), 2.34 (m, vl, -CH<sub>2</sub>CH<sub>2</sub>C(O)O-, avl, H<sub>2</sub>C=CHCH<sub>2</sub>CH<sub>2</sub>-, H<sub>2</sub>C=CHCH<sub>2</sub>CH<sub>2</sub>-), 1.66 (m, avl & vl, -CHCH<sub>2</sub>CH<sub>2</sub>-), 1.25 (t, -CH<sub>2</sub>CH<sub>3</sub>); <sup>13</sup>C NMR  
 10 (400MHz, CDCl<sub>3</sub>, ppm) δ: 204.9, 175.2, 173.7, 173.2, 135.0, 117.0, 63.9, 44.8, 36.4, 33.6, 28.0, 26.3, 21.3.

**[00561] Synthesis of poly(vl-evl-opd) (ABD).** To a solution of AbD (2.70 g, 4.67 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (37 mL), 3-chloroperoxybenzoic acid (1.46 g, 8.48 mmol) was added. The mixture stirred for 72 h at room temperature and then concentrated via rotary evaporator. The crude product was dissolved in a minimal amount of tetrahydrofuran (THF) (5 mL) and  
 20 dropped into a round bottom flask containing 1L diethyl ether. The solution was kept overnight at 0 °C and a white solid was obtained. The solution was decanted off and the solid was dried in vacuo to obtain ABD. Yield: 1.95 g (72 %). Mw = 3392 Da, PDI = 1.19. <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>/TMS) δ: The significant change is the disappearance of the allylic protons at 5.74 and 5.09 ppm and the appearance of small broad resonance peaks at 2.94, 2.75 and 2.47 ppm due to the formation of the epoxide ring. All other aspects of the spectrum are similar.

**[00562] Nanoparticle formation from poly(vl-evl-opd) (ABD).** A solution of ABD (0.11 g, Mw= 3392 Da, PDI = 1.19) dissolved in CH<sub>2</sub>Cl<sub>2</sub> (0.26 mL) was added dropwise via a peristaltic pump at 13 mL/min with vigorous stirring to a solution of 2,2'-(ethylenedioxy)diethylamine (76.4 μL, 0.52 μmol) in CH<sub>2</sub>Cl<sub>2</sub> (40.3 mL) at 44 °C. The  
 30

mixture was heated for 12 h. Residual diamine was removed by dialyzing with SnakeSkin® Pleated Dialysis Tubing (MWCO = 10,000) against dichloromethane. Yield: 0.17 g (91%). DLS: DH =  $118.3 \pm 9.6$  nm. SLS: Mw = 323,000. <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>/TMS) δ: The significant change is the disappearance of the epoxide protons at 2.94, 2.75 and 2.47 ppm and the appearance of signals at 3.54 and 2.97 ppm corresponding to the protons neighboring the secondary amine of the PEG linker after cross-linking. All other aspects of the spectrum are similar.

**[00563] N-Boc-ethylenediamine (NBED) conjugated ABD nanoparticles.** To a solution of ABD nanoparticles (20 mg, 0.06 μmol) in THF (2 mL), N-acetoxysuccinimide (0.02 g, 0.13 mmol) was added. The reaction mixture stirred for 3 h. Residual N-acetoxysuccinimide was removed by dialyzing with SnakeSkin® Pleated Dialysis Tubing (MWCO = 10,000) against THF. Once the product was concentrated and dried, the nanoparticles (18 mg, 0.05 μmol) were dissolved in a mixture of CH<sub>2</sub>Cl<sub>2</sub> and CH<sub>3</sub>OH (1:1, v/v, 2 mL). To this solution, N-Boc-ethylenediamine (4.6 μL of 1.59 M NBED in CH<sub>3</sub>OH) and NaCNBH<sub>3</sub> (21.8 μL of 1.0 M NaCNBH<sub>3</sub> in THF) were added. The reaction mixture stirred for 12 h at room temperature and then was purified by dialyzing with SnakeSkin® Pleated Dialysis Tubing (MWCO = 10,000) against 1:1 CH<sub>2</sub>Cl<sub>2</sub>/CH<sub>3</sub>OH. Yield: 18 mg (88%). DLS: DH =  $119.5 \pm 10.3$  nm; original particle DH =  $118.3 \pm 9.6$  nm. <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>/TMS) δ: The significant change is the appearance of the peak at 1.43 ppm due to the Boc protecting group. All other aspects of the spectrum are similar to that of the ABD nanoparticles.

**[00564] General procedures for the synthesis of HVGGSSV peptide (1).** The HVGGSSV peptide was synthesized by solid-phase peptide synthesis using standard Fmoc chemistry on a Model 90 Peptide Synthesizer (Advanced ChemTech). General procedure: Attachment of N-Fmoc amino acids to resin. After swelling with dichloromethane (20 mL) for 20 min, H-val-2-Cl-Trt resin (0.20 g, 1.03 mmol/g, 0.21 mmol surface amino acids) was treated with a solution of Fmoc-protected amino acids (4.4 equiv, 0.9 mmol) in dimethylformamide (DMF) (9 mL). The amino acids were attached to the resin using double coupling with a solution (9 mL) consisting of N-hydroxybenzotriazole monohydrate (HOBt) (0.9 mmol, 0.14 g) o-(benzotriazole-N,N',N'-tetramethyluronium hexafluorophosphate (0.9 mmol, 0.34 g), N,N'-diisopropylethylamine (DIPEA) (1.8 mmol, 0.31 mL) in 9 mL DMF. The reaction mixture was shaken for 60 min and washed with DMF (4 x 10 mL), methanol (4

x 10 mL) and DMF (4 x 10 mL). The end of the coupling was controlled by the Ninhydrin test. A 20% (v/v) piperidine in DMF solution was used to deprotect the Fmoc groups. The amino acids were attached to the resin in the following sequence: Ser, Ser, Gly, Gly, Val, His, Asn, Gly, Gly, Gly, Cys, and Gly.

5 **[00565] General procedure: Cleavage from resin.** The resin was treated with Reagent R, a solution of TFA, thioanisole, anisole, and ethanedithiol (90:5:3:2, 6 mL), for 4 h. After removal of the resin by filtration, the filtrate was concentrated to precipitate the peptide with cold diethyl ether. Crude peptides were purified by RP-HPLC and lyophilized. Peptide identity was confirmed by MALDI-MS (m/z: 1087.1).

10 **[00566] HVGGSSV conjugated ABD nanoparticles (3).** To a solution of ABD nanoparticles (20.0 mg, 0.06  $\mu$ mol) in THF (2 mL), N-acetoxysuccinimide (3 mg, 18.1  $\mu$ mol) was added. The reaction mixture stirred for 3 h. Residual N-acetoxysuccinimide was removed by dialyzing with SnakeSkin® Pleated Dialysis Tubing (MWCO = 10,000) against 1:1 THF/CH<sub>3</sub>OH to give amine capped ABD nanoparticles, 2. To a solution of 2 (0.0174 g, 0.05  $\mu$ mol, in 3 mL THF), 1 (3.5 mg, 3.18  $\mu$ mol) dissolved in DMSO (2 mL) and NaCNBH<sub>3</sub> (6.36  $\mu$ L 1.0 M NaCNBH<sub>3</sub> in THF) were added. The reaction mixture stirred for 12 h at room temperature. The reaction mixture was purified by dialyzing with SnakeSkin® Pleated Dialysis Tubing (MWCO = 10,000) against 1:1 THF/CH<sub>3</sub>CN. Yield: 19 mg (88%) DLS: DH = 120.5  $\pm$  10.2 nm; original particle DH = 118.3  $\pm$  9.6 nm. SLS: Mw = 362,000; original particle Mw = 323,000. <sup>1</sup>H NMR (600MHz, (CD<sub>3</sub>)<sub>2</sub>SO)  $\delta$ : The significant change is the appearance of the following peaks: 8.26-7.87, 7.42, 6.90, 4.39, and 4.25 ppm due to the attachment of the peptide. All other aspects of the spectrum are similar to that of the ABD nanoparticles.

25 **[00567] Synthesis of poly(vl-evl-avl-opd) (ABbD).** To a solution of AbD (1.70 g, 1.56 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (30 mL), 3-chloroperoxybenzoic acid (0.22 g, 1.28 mmol) was added. The mixture stirred for 72 h at room temperature and then was concentrated via rotary evaporator. The crude product was dissolved in a minimal amount of THF (5 mL) and poured into a round bottom flask containing 1L diethyl ether. The solution was kept overnight at 0 °C and a white solid was obtained. The solution was decanted off and the solid was dried in vacuo to obtain ABbD. Yield: 1.2 g (71%). Mw = 3356 Da, PDI = 1.18. <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>/TMS, ppm)  $\delta$ : 5.72 (m, H<sub>2</sub>C=CH-), 5.06 (m, H<sub>2</sub>C=CH-), 4.34 (m, -

CH<sub>2</sub>CH<sub>2</sub>C(O)CH<sub>2</sub>CH<sub>2</sub>O-), 4.08 (m, -CH<sub>2</sub>O-), 3.67 (m, -OCH<sub>2</sub>CH<sub>3</sub>), 2.96 (m, epoxide proton), 2.78 (m, v1 epoxide proton, opd, -OC(O)CH<sub>2</sub>CH<sub>2</sub>C(O)CH<sub>2</sub>-), 2.58 (m, opd, -OC(O)CH<sub>2</sub>CH<sub>2</sub>C(O)CH<sub>2</sub>-), 2.47 (epoxide proton), 2.34 (m, v1, -CH<sub>2</sub>CH<sub>2</sub>C(O)O-, av1, H<sub>2</sub>C=CHCH<sub>2</sub>CH-, H<sub>2</sub>C=CHCH<sub>2</sub>CH-), 1.66 (m, av1 & v1, -CHCH<sub>2</sub>CH<sub>2</sub>-), 1.25 (t, -CH<sub>2</sub>CH<sub>3</sub>).

**[00568] Nanoparticle formation from ABbD.** A solution of ABbD (0.21 g, Mw = 3356 Da, PDI = 1.18) dissolved in CH<sub>2</sub>Cl<sub>2</sub> (0.39 mL) was added dropwise via a peristaltic pump at 13 mL/min with vigorous stirring to a solution of 2,2'-(ethylenedioxy)diethylamine (42.6 μL, 0.29 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (60 mL) at 44 °C. The reaction mixture was heated for 12 h. Residual diamine was removed by dialyzing with SnakeSkin® Pleated Dialysis Tubing (MWCO = 10,000) against dichloromethane. Yield: 0.24 g (96%). DLS: DH = 123.4 ± 9.22 nm. SLS: Mw = 345,000. <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>/TMS) δ: The significant change is the disappearance of the epoxide protons at 2.96, 2.75 and 2.47 ppm and the appearance of signals at 3.56 and 2.98 ppm corresponding to the protons neighboring the secondary amine of the PEG linker after crosslinking. All other aspects of the spectrum are similar to that of ABbD.

**[00569] One pot synthesis of nanoparticles from ABbD.** To a solution of 2,2'-(ethylenedioxy)diethylamine (26.2 μL, 0.18 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (34.6 mL), a solution of ABbD (0.13 g, Mw = 3356 Da, PDI = 1.18) in CH<sub>2</sub>Cl<sub>2</sub> (0.24 mL) was added. The mixture was heated at 44 °C for 12 h. Residual diamine was removed by dialyzing with SnakeSkin® Pleated Dialysis Tubing (MWCO = 10,000) against CH<sub>2</sub>Cl<sub>2</sub>. Yield: 0.15 g (94%). DLS: DH = 126.6 ± 9.3 nm. SLS: Mw = 350,000. <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>/TMS) δ: The significant change is the disappearance of the epoxide protons at 2.94, 2.75 and 2.47 ppm and the appearance of signals at 3.54 and 2.97 ppm corresponding to the protons neighboring the secondary amine of the PEG linker after cross-linking. All other aspects of the spectrum are similar to that of ABbD.

**[00570] General procedure for the attachment of benzyl mercaptan to ABbD nanoparticles.** To a solution of ABbD nanoparticles (15 mg, 0.04 μmol) in toluene (0.5 mL), benzyl mercaptan (3.5 μL, 29 μmol) was added. The reaction mixture was heated for 72 h at 35 °C. The remaining toluene was removed in vacuo and residual benzyl mercaptan was removed by dialyzing with SnakeSkin® Pleated Dialysis Tubing (MWCO = 10,000) against

CH<sub>2</sub>Cl<sub>2</sub>. <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>/TMS) δ: The significant change is the reduction of the allyl protons at 5.72 and 5.06 ppm and the appearance of signals at 3.73 and 7.30 ppm corresponding to the methylene and benzene protons respectively of the attached benzyl mercaptan. All other aspects of the spectrum are similar to that of ABbD nanoparticles.

5    **[00571]**        Deprotection of molecular transporter (MT) (5) (contribution of Sharon Hamilton). To a solution of LL-MT (15 mg, 4.56 μmol) in CH<sub>3</sub>OH (0.4 mL), a solution of D,L-dithiothreitol in CH<sub>3</sub>OH (0.2 mL) was added. The reaction mixture stirred for 3 h at room temperature. Residual dithiothreitol was removed by purification with Sephadex LH-20. The product was immediately attached to ABbD nanoparticles.

10   **[00572]**        Model reaction of attachment of MT to ABbD nanoparticles. To a solution of ABbD nanoparticles (15 mg, 0.04 μmol) in CH<sub>3</sub>OH (0.2 mL), 5 (11 mg, 3.35 μmol) in CH<sub>3</sub>OH (0.4 mL) was added. The reaction mixture was heated for 72 h at 37 °C. Residual 5 was removed by dialyzing with SnakeSkin® Pleated Dialysis Tubing (MWCO = 10,000) against methanol. Yield: 31.3 mg (89%). DLS: DH = 128.9 ± 10.2 nm; original particle DH =  
15   126.6 ± 9.3 nm. <sup>1</sup>H NMR (300MHz, CD<sub>3</sub>OD) δ: The significant change is the reduction of the allyl protons at 5.72 and 5.06 ppm and the appearance of signals at 2.20-1.98 (CH<sub>2</sub>), 1.57 (CH<sub>2</sub>) and 1.39 (CH<sub>2</sub>) ppm due to the dendritic backbone of the MT. All other aspects of the spectrum are similar to that of ABbD nanoparticles.

**[00573]**        Alexa Fluor® 594 conjugated ABbD nanoparticles (4). To a solution of ABbD nanoparticles (0.021 g, 0.06 μmol) in dry THF (1.5 mL), Alexa Fluor® 594 (0.14 mL of 10 mg/mL Alexa Fluor® 594 in DMF, 1.7 μmol) was added. The reaction mixture stirred for 24 h at room temperature. Residual Alexa Fluor® 594 was removed by dialyzing with SnakeSkin® Pleated Dialysis Tubing (MWCO = 10,000) against CH<sub>3</sub>OH. Yield: 15.2 mg (88%). <sup>1</sup>H NMR (300MHz, CD<sub>3</sub>OD) δ: The significant change is the appearance of the  
20   following peaks due to Alexa Fluor® 594: 7.14-7.20, 6.78, 5.48, 4.48, 3.62, 3.43, and 1.24 ppm. <sup>1</sup>H NMR (600MHz, (CD<sub>3</sub>)<sub>2</sub>SO) δ: The significant change is the appearance of the following peaks due to Alexa Fluor® 594: 7.52, 7.47, 7.08, 5.32, 4.44, 4.35, 3.58, 3.16, 2.03, and 1.25 ppm. All other aspects of the spectrum are similar to that of ABbD nanoparticles.

**[00574]**        Attachment of MT to Alexa Fluor® 594 conjugated ABbD nanoparticles,  
30   **NP-594-MT (6)**. To a solution of 4 (8 mg, 0.89 μmol) in CH<sub>3</sub>OH (0.2 mL), 5 (7.5 mg, 2.27

5  $\mu\text{mol}$ ) in  $\text{CH}_3\text{OH}$  (0.4 mL) was added. The reaction mixture was heated for 72 h at 37 °C. Residual 5 was removed by dialyzing with SnakeSkin® Pleated Dialysis Tubing (MWCO = 10,000) against  $\text{CH}_3\text{OH}$ . Yield: 10.0 mg (91%). DLS:  $\text{DH} = 129.4 \pm 9.8 \text{ nm}$ ; original particle  $\text{DH} = 126.6 \pm 9.3 \text{ nm}$ .  $^1\text{H NMR}$  (300MHz,  $\text{CD}_3\text{OD}$ )  $\delta$ : The significant change is the  
 reduction of the allyl protons at 5.72 and 5.06 ppm and the appearance of signals at 2.20-1.98 (CH<sub>2</sub>), 1.57 (CH<sub>2</sub>) and 1.39 (CH<sub>2</sub>) ppm due to the dendritic backbone of the MT. All other aspects of the spectrum are similar to that of 4.

**[00575] N-acetoxysuccinimide conjugated HVGGSSV peptide, cHVGGSSV (7).**  
 To a solution of 1 (29.4 mg,  $2.7 \times 10^{-5} \text{ mol}$ ) dissolved in  $\text{CH}_3\text{CN}$  (3 mL), N-  
 10 acetoxysuccinimide (0.42 g,  $2.7 \times 10^{-3} \text{ mol}$ ) was added. The reaction mixture stirred for 3 h at room temperature. After removal of the solvent under reduced pressure, the crude product was purified by RP-HPLC. MALDI-MS:  $m/z = (\text{M}+\text{H}^+)$  1174.2.

**[00576] Capped HVGGSSV conjugated Alexa Fluor® 594-ABbD nanoparticles, NP-cHVGGSSV-594.** To a solution of ABbD nanoparticles (0.021 g, 0.06  $\mu\text{mol}$ ) in  
 15 dimethylsulfoxide (0.7 mL), 7 (6.4 mg, 5.46  $\mu\text{mol}$ ) was added. The reaction mixture was heated for 72 h at 33 °C. To this solution, Alexa Fluor® 594 (0.14 mL of 10 mg/mL Alexa Fluor® 594 in DMF, 1.7  $\mu\text{mol}$ ) was added. Residual Alexa Fluor® 594 and peptide were removed by dialyzing with SnakeSkin® Pleated Dialysis Tubing (MWCO = 10,000) against 1:1  $\text{CH}_3\text{OH}/\text{CH}_3\text{CN}$ . Yield: 20.1 mg (80%). DLS:  $\text{DH} = 128.9 \pm 10.9 \text{ nm}$ ; original particle  
 20  $\text{DH} = 126.6 \pm 9.3 \text{ nm}$ .  $^1\text{H NMR}$  (600MHz,  $(\text{CD}_3)_2\text{SO}$ )  $\delta$ : The significant change is the reduction of the allyl protons at 5.72 and 4.97 ppm and the appearance of the following sets of significant signals: 8.21, 7.83, 4.55, 3.73 and 0.80 ppm due to the peptide, and 7.25, 7.16, 6.53, 5.32, 4.44, 4.37, and 1.25 ppm due to the Alexa Fluor® 594. All other aspects of the spectrum are similar to that of ABbD nanoparticles.

25 **[00577] Attachment of MT to cHVGGSSV conjugated Alexa Fluor® 594-ABbD nanoparticles, NP-cHVGGSSV-594-MT.** To a solution of 8 (6 mg, 0.02  $\mu\text{mol}$ ) in DMSO (0.1 mL), 5 (2 mg, 0.88  $\mu\text{mol}$ ) in  $\text{CH}_3\text{OH}$  (0.3 mL) was added. The reaction mixture was heated for 48 h at 33°C. Residual 5 was removed by dialyzing with SnakeSkin® Pleated Dialysis Tubing (MWCO = 10,000) against 1:1  $\text{CH}_3\text{OH}/\text{CH}_3\text{CN}$ . Yield: 7.4 mg (93%). DLS:  
 30  $\text{DH} = 130.7 \pm 9.4 \text{ nm}$ ; original particle  $\text{DH} = 126.6 \pm 9.3 \text{ nm}$ .  $^1\text{H NMR}$  (600MHz,  $(\text{CD}_3)_2\text{SO}$ )  $\delta$ : The significant change is the reduction of the allyl protons at 5.72 and 4.97

ppm and the appearance of signals at 3.06 (CH<sub>2</sub>), 2.96 (CH<sub>2</sub>), 1.97 (CH<sub>2</sub>), 1.77 (CH<sub>2</sub>), 1.41 (CH<sub>2</sub>) and 1.35 (CH<sub>2</sub>) ppm due to the dendritic backbone of the MT. All other aspects of the spectrum are similar to that of 8.

**[00578]      Synthesis of cyclic RGD, cRGD (9)** The RGD peptide was synthesized by solid-phase peptide synthesis using standard Fmoc chemistry on a Model 90 Peptide Synthesizer (Advanced ChemTech).

**[00579]      Synthesis of Linear RGD.** After swelling with dichloromethane (20 mL), Fmoc-Cys-2-Cl-Trt resin (0.20 g, 0.9 mmol/g, 0.18 mmol surface amino acids) was deprotected with a 20% (v/v) piperidine in DMF solution and treated with a solution of Fmoc-protected amino acid (4.4 equiv, 0.9 mmol) in dimethylformamide (DMF) (9 mL). The amino acids were attached to the resin using double coupling with a solution (9 mL) consisting of N-hydroxybenzotriazole monohydrate (0.9 mmol, 0.14 g) o-(benzotriazole-N,N,N',N'-tetramethyluronium hexafluorophosphate (0.9 mmol, 0.34 g), N,N'-diisopropylethylamine (1.8 mmol, 0.31 mL) in 9 mL DMF. The reaction mixture was shaken for 60 min and washed with DMF (4 x 10 mL), methanol (4 x 10 mL) and DMF (4 x 10 mL). A 20% (v/v) piperidine in DMF solution was used to deprotect the Fmoc groups. An amino-hexyl spacer was coupled to the cystine on the resin, followed by glutamic acid, aspartic acid, glycine, arginine, phenylalanine, and finally lysine.

**[00580]      Cyclization of RGD.** The peptide was cyclized by utilizing an ODmab group, which allows for the selective deprotection carboxylic acid side chain of the glutamic acid, which can then be coupled to the N-terminus. The ODmab was deprotected using 2% v/v hydrazine monohydrate/DMF added to the resin and shaken for 7 min. Next it was washed with 20 mL of DMF followed by 10 mL of a 5% v/v DIPEA/DMF solution which was allowed to shake for 10 min. Carboxy activation was achieved through the use of N,N'-dicyclohexylcarbodiimide (DCC) (44.6 mg, 0.22 mmol) and hydroxybenzotriazole (HOBt) (29.2 mg, 0.22 mmol) which was added to 10 mL of DMF and then added to the resin and allowed to shake for 18 h.

**[00581]      General procedure: Cleavage from resin.** The resin was treated with Reagent R, a solution of TFA, thioanisole, anisole, and ethanedithiol (90:5:3:2, 6 mL), for 3 h. After removal of the resin by filtration, the filtrate was concentrated to precipitate the

peptide with cold diethyl ether. The crude peptide was collected by centrifugation, purified by RP-HPLC and lyophilized. Peptide identity was confirmed by MALDI-MS ( $m/z$ : 945).

**[00582] Attachment of cRGD to Alexa Fluor® 594 conjugated ABbD**

**nanoparticles, NP-594-cRGD (10).** To a solution of ABbD nanoparticles (23.0 mg, 0.07  $\mu$ mol) in THF (2.3 mL), Alexa Fluor® 594 (0.15 mL of 10 mg/mL Alexa Fluor® 594 in DMF, 1.83  $\mu$ mol) was added. After stirring the reaction mixture for 24 h at room temperature, the solvent was removed via rotary evaporator. To the Alexa Fluor® 594 conjugated nanoparticles, methanol (0.35 mL) and 9 (5.7 mg, 6.0  $\mu$ mol), dissolved in DMSO (0.35 mL), were added. The reaction mixture was heated for 72 h at 33 °C. Residual Alexa Fluor® 594 and peptide were removed by dialyzing with SnakeSkin® Pleated Dialysis Tubing (MWCO = 10,000) against 1:1 CH<sub>3</sub>OH/CH<sub>3</sub>CN. Yield: 22.0 mg (81%). DLS: DH = 129.8 ± 9.6 nm; original particle DH = 126.6 ± 9.3 nm. <sup>1</sup>H NMR (600MHz, (CD<sub>3</sub>)<sub>2</sub>SO)  $\delta$ : The significant change is the reduction of the allyl protons at 5.72 and 4.97 ppm and the appearance of the following sets of significant signals: 7.37, 4.79, 2.23 and 1.66 ppm due to cRGD, and 7.25, 6.55, 5.31, 4.44, and 1.23 ppm due to the Alexa Fluor® 594. All other aspects of the spectrum are similar to that of ABbD nanoparticles.

**[00583] Attachment of MT to cRGD conjugated Alexa Fluor® 594-ABbD**

**nanoparticles, NP-594- cRGD-MT (12).** To a solution of 10 (7.8 mg, 0.02  $\mu$ mol) in DMSO (0.1 mL), 5 (1.4 mg, 0.67  $\mu$ mol) in CH<sub>3</sub>OH (0.3 mL) was added. The reaction mixture was heated for 48 h at 33°C. Residual 5 was removed by dialyzing with SnakeSkin® Pleated Dialysis Tubing (MWCO = 10,000) against 1:1 CH<sub>3</sub>OH/CH<sub>3</sub>CN. Yield: 7.6 mg (83%). DLS: DH = 131.9 ± 10.6 nm; original particle DH = 126.6 ± 9.3 nm. <sup>1</sup>H NMR (600MHz, (CD<sub>3</sub>)<sub>2</sub>SO)  $\delta$ : The significant change is the reduction of the allyl protons at 5.72 and 4.97 ppm and the appearance of signals at 3.04 (CH<sub>2</sub>), 2.98 (CH<sub>2</sub>), 1.98 (CH<sub>2</sub>), 1.75 (CH<sub>2</sub>), 1.41 (CH<sub>2</sub>), and 1.35 (CH<sub>2</sub>) ppm due to the dendritic backbone of the MT. All other aspects of the spectrum are similar to that of 11.

**[00584] HVGGSSV conjugated ABbD nanoparticles, NP-HVGGSSV (14).** To a

solution of ABbD nanoparticles (50.0 mg, 0.14  $\mu$ mol) in THF (2 mL), N-acetoxysuccinimide (7 mg, 44.5  $\mu$ mol) was added. The reaction mixture stirred for 3 h. Residual N-acetoxysuccinimide was removed by dialyzing with SnakeSkin® Pleated Dialysis Tubing (MWCO = 10,000) against 1:1 THF/CH<sub>3</sub>OH to give amine capped ABbD nanoparticles, 13.

To a solution of 13 (50.0 mg, 0.14  $\mu$ mol, in 3 mL THF), 1 (9.3 mg, 8.57  $\mu$ mol) dissolved in DMSO (2 mL) and NaCNBH<sub>3</sub> (17.1  $\mu$ L 1.0 M NaCNBH<sub>3</sub> in THF) were added. The reaction mixture stirred for 12 h at room temperature. The reaction mixture was purified by dialyzing with SnakeSkin® Pleated Dialysis Tubing (MWCO = 10,000) against 1:1 THF/CH<sub>3</sub>CN.

- 5 Yield: 43.2 mg (83%). DLS: DH = 129.7  $\pm$  9.5 nm; original particle DH = 126.6  $\pm$  9.3 nm. SLS: Mw = 391,000; original particle Mw = 350,000. <sup>1</sup>H NMR (600MHz, (CD<sub>3</sub>)<sub>2</sub>SO, ppm)  $\delta$ : The significant change is the appearance of the following peaks: 8.21, 7.85, 4.55, 3.73 and 0.80 ppm due to the peptide. All other aspects of the spectrum are similar to that of ABbD nanoparticles.

- 10 **[00585] Thiolated Alexa Fluor® 594 (15).** To a solution of Alexa Fluor® 594 (0.2 mL of 10 mg/mL Alexa Fluor® 594 in DMF, 2.4  $\mu$ mol), cysteamine (68.4  $\mu$ L of 2.5 mg/mL cysteamine in DMSO, 2.2  $\mu$ mol) was added. The reaction mixture stirred for 3 h at room temperature. The product was immediately attached to 14.

- [00586]** Attachment of MT to HVGGSV conjugated Alexa Fluor® 594-ABbD nanoparticles, NPHVGGSV- 594-MT (16). To a solution of 14 (16 mg, 0.04  $\mu$ mol) in DMSO (0.2 mL), 15 (2 mg, 1.95  $\mu$ mol) in DMSO (0.2 mL) and 5 (2.7 mg, 1.2  $\mu$ mol) in CH<sub>3</sub>OH (0.4 mL) were added. The reaction mixture was heated for 48 h at 33°C. Residual 5 and 15 were removed by dialyzing with SnakeSkin® Pleated Dialysis Tubing (MWCO = 10,000) against CH<sub>3</sub>OH. Yield: 18.5 mg (86%). DLS: DH = 132.1  $\pm$  9.3 nm; original particle DH = 126.6  $\pm$  9.3 nm. <sup>1</sup>H NMR (600MHz, (CD<sub>3</sub>)<sub>2</sub>SO)  $\delta$ : The significant change is the reduction of the allyl protons at 5.72 and 4.97 ppm and the appearance of the following sets of significant signals: 3.08, 2.99, 1.97, 1.79, 1.43 and 1.34 ppm due to the dendritic backbone of the MT, and 7.27, 7.07, 6.53, 5.32, 4.46, 4.37, and 1.24 ppm due to the Alexa Fluor® 594. All other aspects of the spectrum are similar to that of 14.
- 20

- 25 **124. PACLITAXEL ENCAPSULATION IN POLY(VL-EVL-AVL-OPD) (ABbD) NANOPARTICLES.**

- [00587]** To a 150 mL beaker containing D- $\alpha$ -tocopherol polyethylene glycol 1000 succinate (0.39 g) dissolved in Lonza cell culture water (78 mL), poly(vl-evl-avl-opd), ABbD, nanoparticles (0.17 g) and paclitaxel (34.0 mg) dissolved in dimethyl sulfoxide (0.75 mL) was added slowly with vigorous stirring. The solution was split into two 50 mL centrifuge
- 30

tubes. The paclitaxel loaded nanoparticles were purified by applying two cycles of centrifugation (8000 rpm for 1 h) and reconstitution with cell culture water. The nanoparticle suspension was then lyophilized. The loading ratio of paclitaxel for the encapsulation was determined by NanoDrop UV/Vis and was found to be 11.34%.

5           **125. IN VIVO ADMINISTRATION OF NANOPARTICLE-BIOCONJUGATE.**

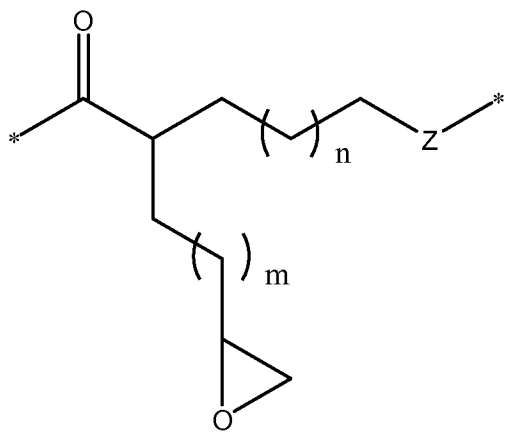
[00588]       Five adult Sprague-Dawley rats were sacrificed by lethal inhalation of CO<sub>2</sub>. At the moment of euthanasia, eight eyes of four rats were treated with a solution of  $2 \times 10^{-2}$  M nanoparticle conjugate in a molar ratio of 5:1 (dye: transporter) up to 15 minutes, one rat served as the no treatment control. The solution was dropped with a micropipette on to the  
10 cornea and multiple drops were instilled in series to maintain a tear meniscus over the cornea. The rats were kept in the dark in a cold room for two hours after the treatment and underwent enucleation of the globe with optic nerve stump attached. The eye globes with attached optic nerves were placed in 4% paraformaldehyde until paraffin embedding. The paraffin blocks were cut into 4- $\mu$ m sections and were stained with traditional DAPI dye. Slides were viewed  
15 at 40x's magnification using a digital fluorescent microscope Olympus Provis AX70 digitally interfaced with a semi-cooled CCD camera to visualize Alexa Fluor 594-labeled transporter. Background autofluorescence was subtracted and the settings were held constant for both the control and the treatment eyes. To proof and image the intended eye region, images of the same location were measured under the DAPI and Alexafluor wavelength with the  
20 microscope-mounted camera (*see* Figure 60, A-D).

[00589]       It will be apparent to those skilled in the art that various modifications and variations can be made in the present invention without departing from the scope or spirit of the invention. Other embodiments of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. It is  
25 intended that the specification and examples be considered as exemplary only, with a true scope and spirit of the invention being indicated by the following claims.

## CLAIMS

**What is claimed is:**

1. A polymer comprising at least one monomer residue having an optionally substituted structure represented by a formula:



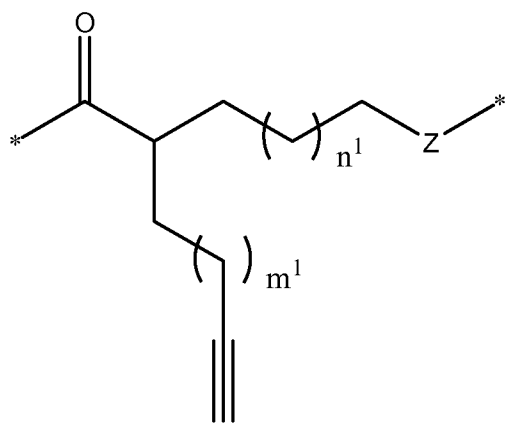
wherein Z is O or NR, wherein R is H or C1 to C6 alkyl;

wherein m is an integer from 0 to 6;

wherein n is an integer from 0 to 2; and

wherein the monomer residue comprises less than about 10% by weight of the monomer residue of halogen selected from chlorine, bromine, and iodine.

2. The polymer of claim 1, further comprising at least one monomer residue selected from:
  - a. a propargyl-functionalized monomer residue having an optionally substituted structure represented by a formula:

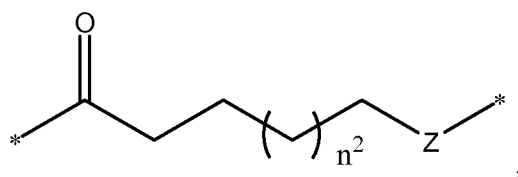


wherein Z is O or NR, wherein R is H or C1 to C6 alkyl,

wherein  $m^1$  is an integer from 0 to 6, and

wherein  $n^1$  is an integer from 0 to 2;

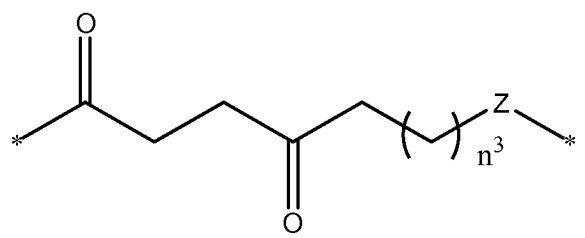
- b. a monomer residue having an optionally substituted structure represented by a formula:



wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, and

wherein  $n^2$  is an integer from 0 to 2; and

- c. a keto-functionalized monomer residue having an optionally substituted structure represented by a formula:

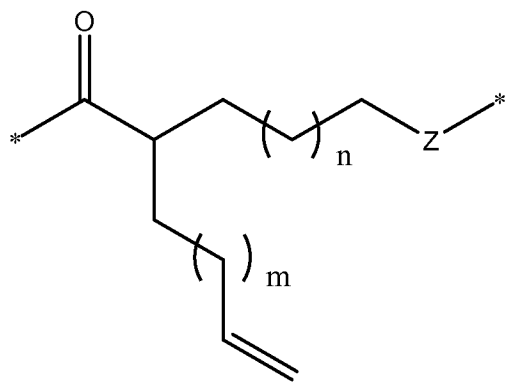


wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, and

wherein  $n^3$  is an integer from 0 to 2.

3. A method of preparing a polymer comprising the step of copolymerizing a mixture of two or more of:

- a. an alkene-functionalized monomer providing a residue having an optionally substituted structure represented by a formula:

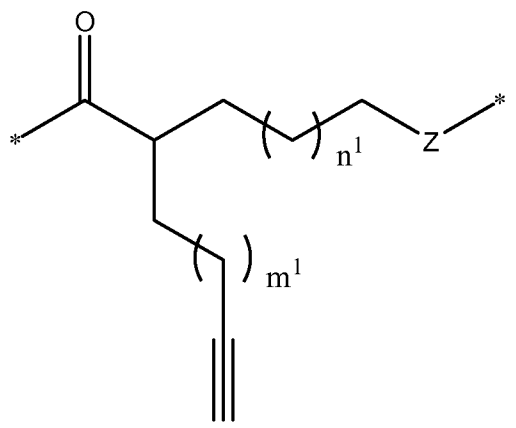


wherein Z is O or NR, wherein R is H or C1 to C6 alkyl;

wherein m is an integer from 0 to 6;

wherein n is an integer from 0 to 2;

- b. a propargyl-functionalized monomer providing a residue having an optionally substituted structure represented by a formula:

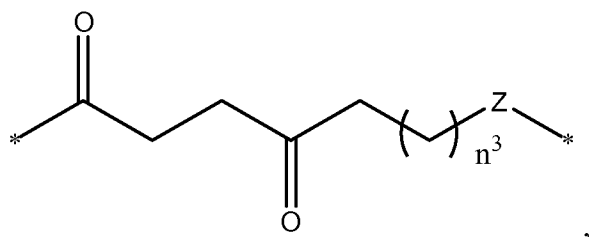


wherein Z is O or NR, wherein R is H or C1 to C6 alkyl,

wherein m<sup>1</sup> is an integer from 0 to 6, and

wherein n<sup>1</sup> is an integer from 0 to 2; and

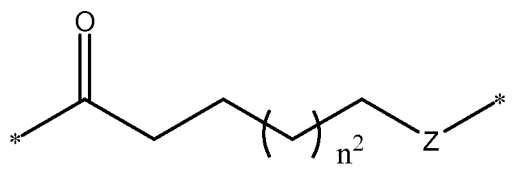
- c. a keto-functionalized monomer providing a residue having an optionally substituted structure represented by a formula:



wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, and

wherein  $n^3$  is an integer from 0 to 2.

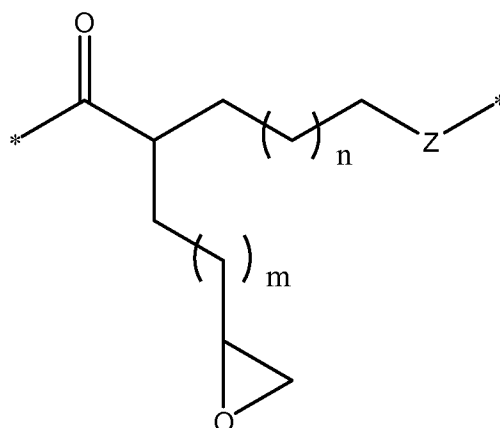
4. The method of claim 3, wherein the mixture further comprises at least one monomer providing a residue having an optionally substituted structure represented by a formula:



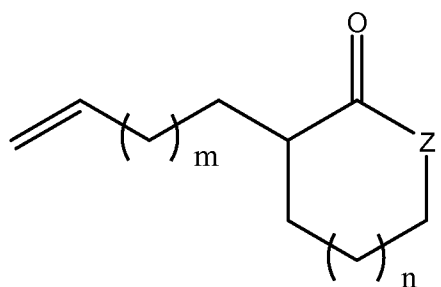
wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, and

wherein  $n^2$  is an integer from 0 to 2.

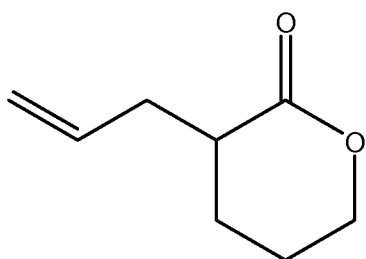
5. The method of claim 3, wherein the alkene-functionalized monomer is present and the method further comprises the step of oxidizing the resultant polymer to provide an epoxide-functionalized monomer residue having an optionally substituted structure represented by a formula:



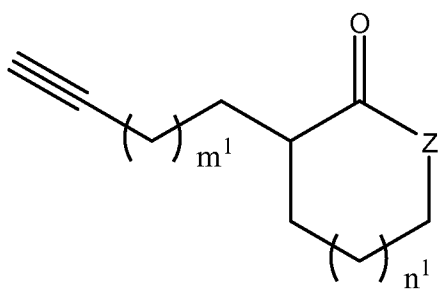
6. The method of claim 3, wherein the alkene-functionalized monomer is present and has an optionally substituted structure represented by a formula:



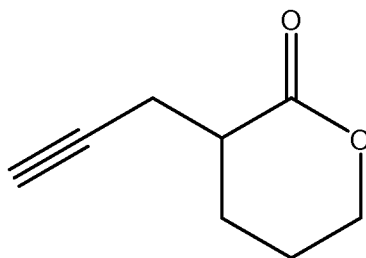
7. The method of claim 6, wherein the alkene-functionalized monomer is present and has an optionally substituted structure represented by a formula:



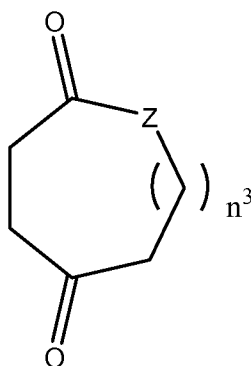
8. The method of claim 3, wherein the propargyl-functionalized monomer is present and has an optionally substituted structure represented by a formula:



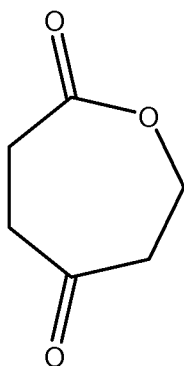
9. The method of claim 8, wherein the propargyl-functionalized monomer is present and has an optionally substituted structure represented by a formula:



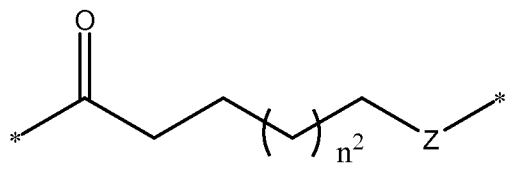
10. The method of claim 3, wherein the keto-functionalized monomer is present and has an optionally substituted structure represented by a formula:



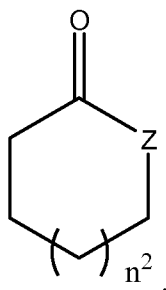
11. The method of claim 10, wherein the keto-functionalized monomer is present and has an optionally substituted structure represented by a formula:



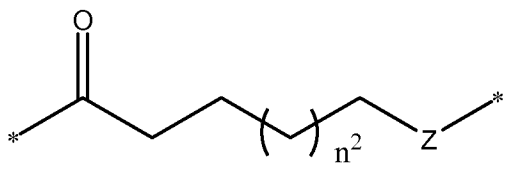
12. The method of claim 4, wherein the monomer providing a residue having an optionally substituted structure represented by a formula:



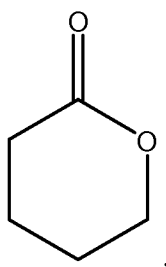
has an optionally substituted structure represented by a formula:



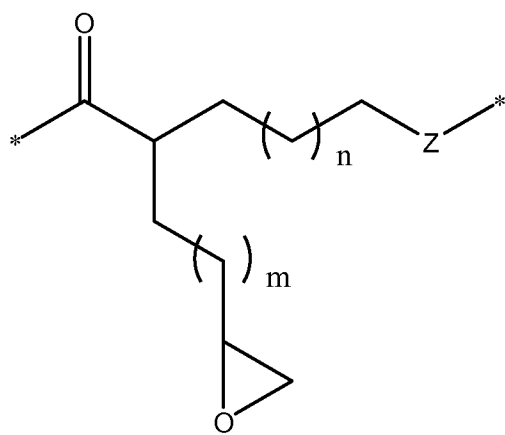
13. The method of claim 12, wherein the monomer providing a residue having an optionally substituted structure represented by a formula:



has an optionally substituted structure represented by a formula:



14. The method of claim 3, further comprising the step of functionalizing the polymer.
15. The method of claim 14, wherein functionalizing comprises the step of reacting:
- an epoxide-functionalized monomer residue having an optionally substituted structure represented by a formula:



wherein Z is O or NR, wherein R is H or C1 to C6 alkyl;

wherein m is an integer from 0 to 6;

wherein n is an integer from 0 to 2;

with

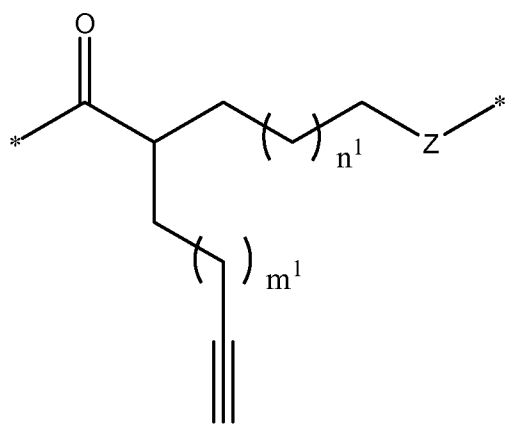
b. a nucleophile having a structure represented by a formula  $X-R^1$ ,

wherein X is OH, SH,  $NH_2$ , or NHR, wherein R is H or C1 to C6 alkyl; and

wherein  $R^1$  is an optionally substituted organic radical comprising 1 to 24 carbon atoms.

16. The method of claim 14, wherein functionalizing comprises the step of reacting:

a. a propargyl-functionalized monomer residue having an optionally substituted structure represented by a formula:



wherein Z is O or NR, wherein R is H or C1 to C6 alkyl,

wherein  $m^1$  is an integer from 0 to 6, and

wherein  $n^1$  is an integer from 0 to 2;

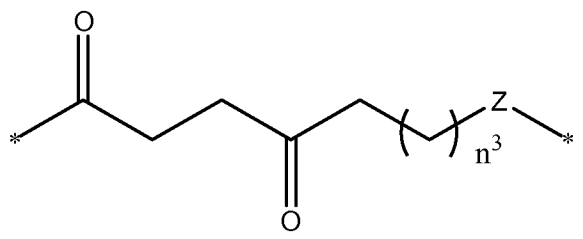
with

- b. an azide having a structure represented by a formula  $N_3-R^1$ ,

wherein  $R^1$  is an optionally substituted organic radical comprising 1 to 24 carbon atoms.

17. The method of claim 14, wherein functionalizing comprises the steps of reacting:

- a. a keto-functionalized monomer providing a residue having an optionally substituted structure represented by a formula:



wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, and

wherein  $n^3$  is an integer from 0 to 2;

with

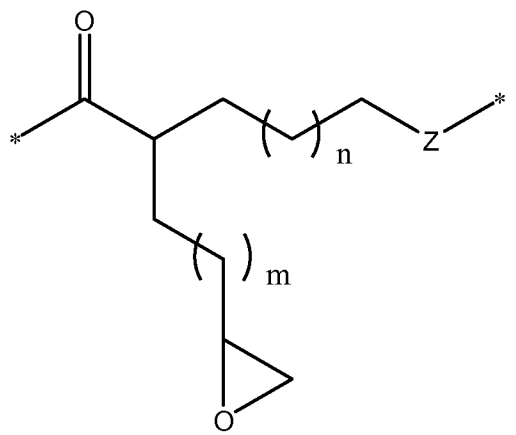
- b. an amine having a structure represented by a formula:



wherein  $R^1$  is an optionally substituted organic radical comprising 1 to 24 carbon atoms; and

- c. reducing the resulting imine.

18. A method of preparing a degradable nanoparticle comprising the step of reacting a polymer comprising at least one monomer residue having a structure represented by a formula:



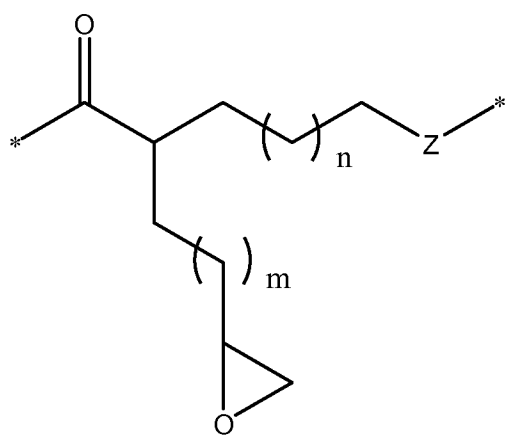
wherein Z is O, S, or NR, wherein R is H, alkyl, or aryl; and

wherein m and n are independently non-negative integers;

with a dinucleophile having a structure  $X-R^L-X'$ , wherein X and X' are independently OH, SH,  $NH_2$ , or NHR, wherein R is H, alkyl, or aryl, and wherein  $R^L$  is selected from optionally substituted alkyl, optionally substituted aryl, optionally substituted alkoxy, and optionally substituted esters,

thereby producing nanoparticles.

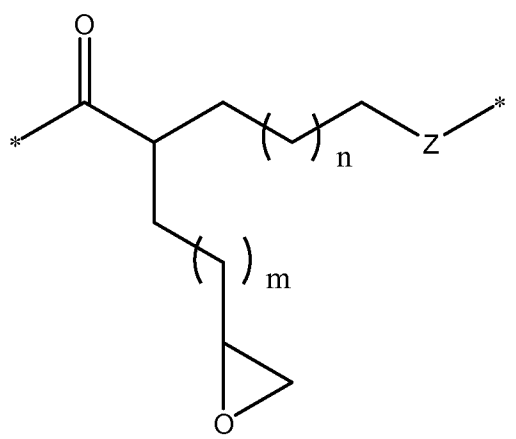
19. The method of claim 18, wherein reacting is adding a polymer comprising at least one monomer residue having a structure represented by a formula:



wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, wherein m is an integer from 0 to 6, and wherein n is an integer from 0 to 2;

to a solution of from about 1 to about 10 molar equivalents of a dinucleophile (nucleophilic moiety:epoxide functionality) having a structure  $X-R^L-X'$ , wherein X and X' are independently OH, SH,  $NH_2$ , or NHR, wherein R is H or C1 to C6 alkyl, and wherein  $R^L$  is selected from optionally substituted alkyl and optionally substituted alkoxylenes.

20. The method of claim 18, wherein reacting is contacting a polymer comprising at least one monomer residue having a structure represented by a formula:



wherein Z is O or NR, wherein R is H or C1 to C6 alkyl, wherein m is an integer from 0 to 6, and wherein n is an integer from 0 to 2;

with a solution of at least one molar equivalent of the dinucleophile.

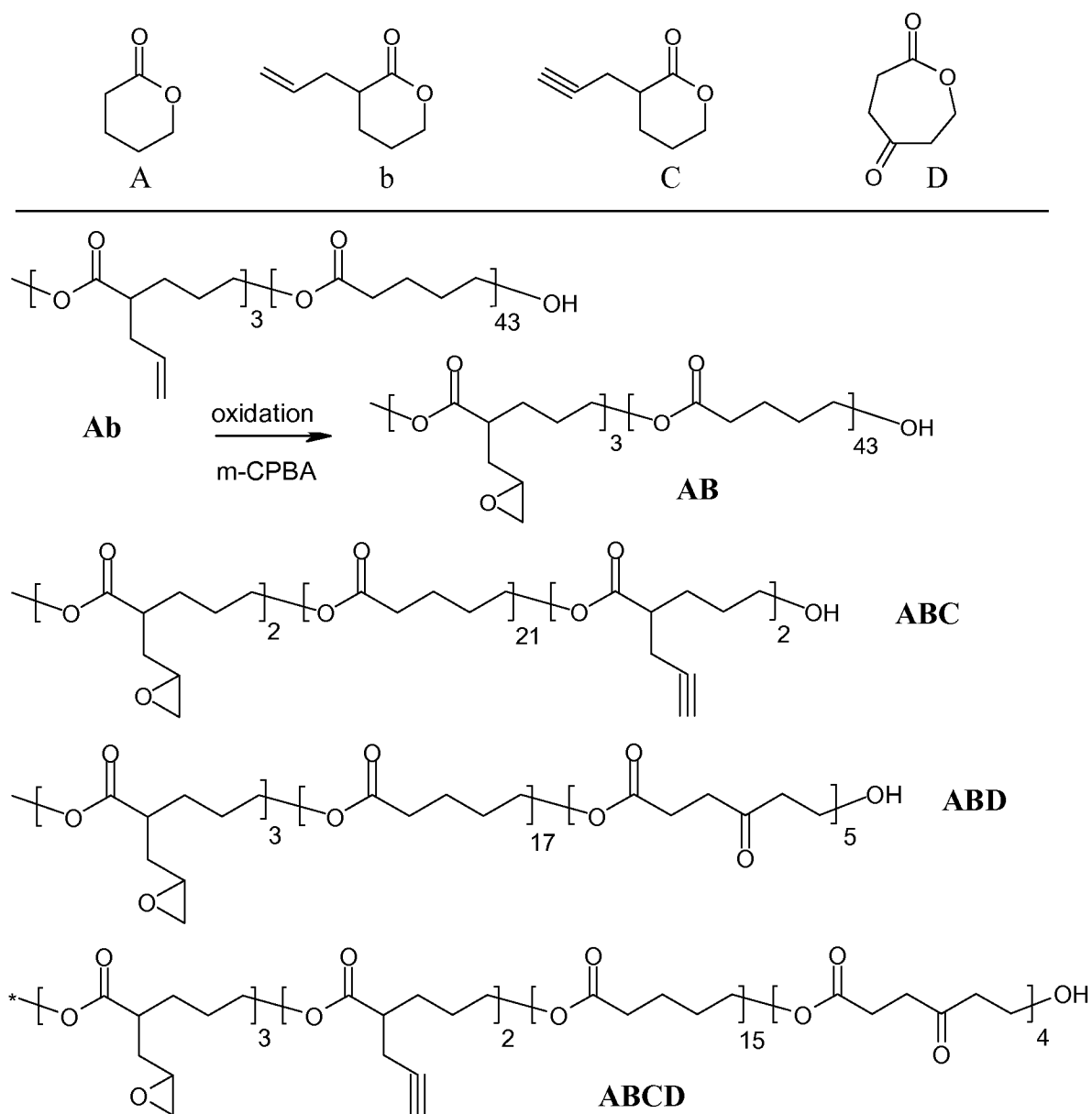


FIGURE 1

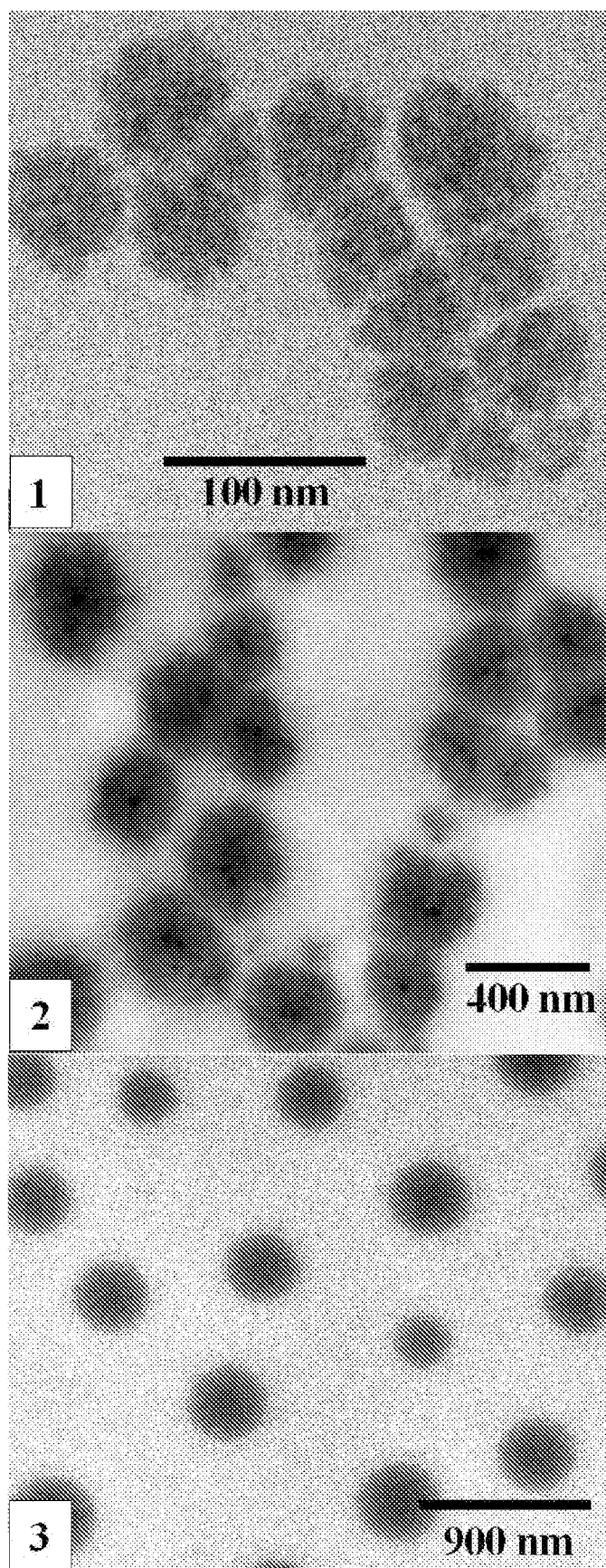


FIGURE 2

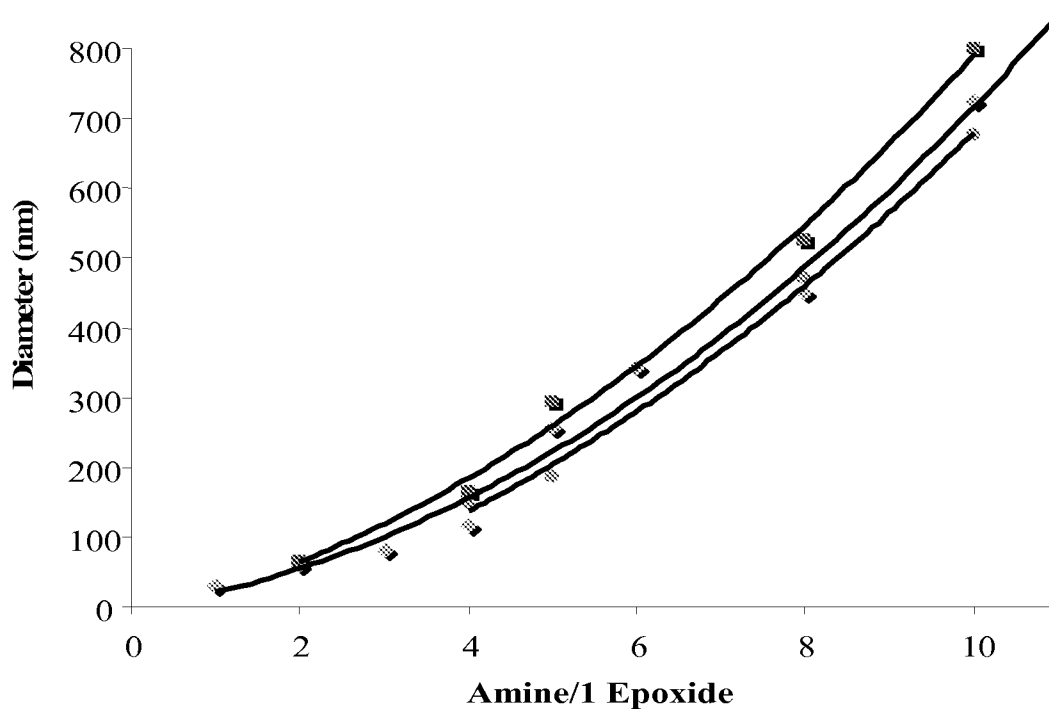


FIGURE 3

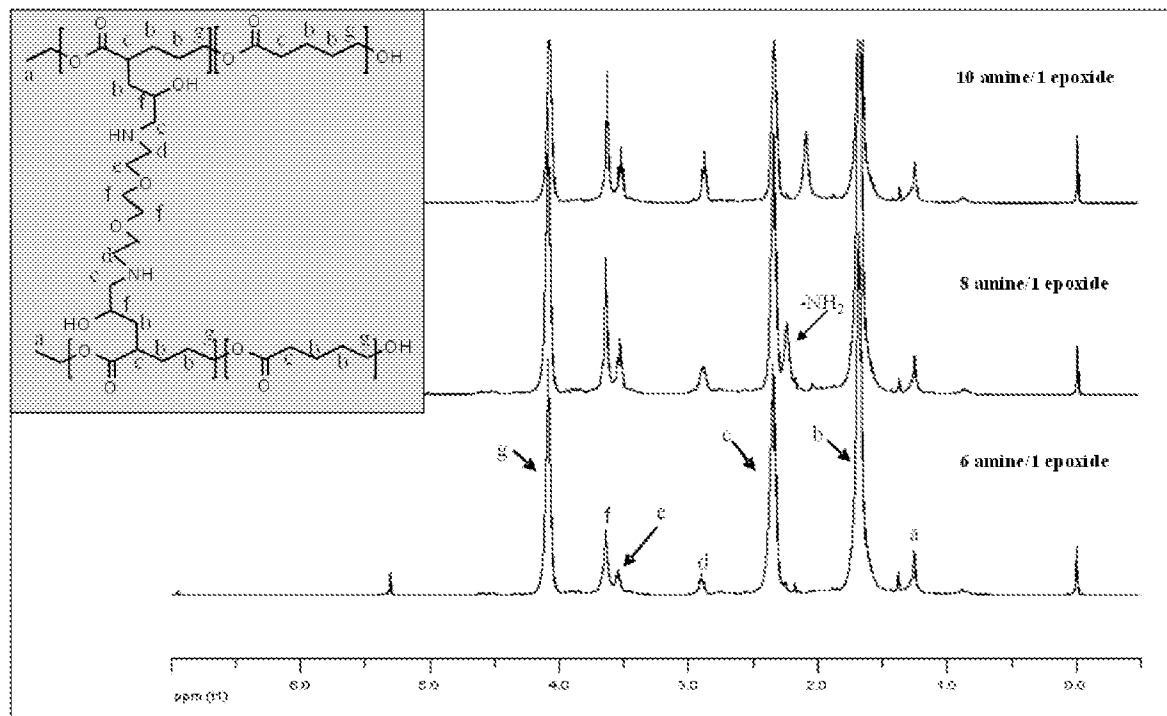


FIGURE 4

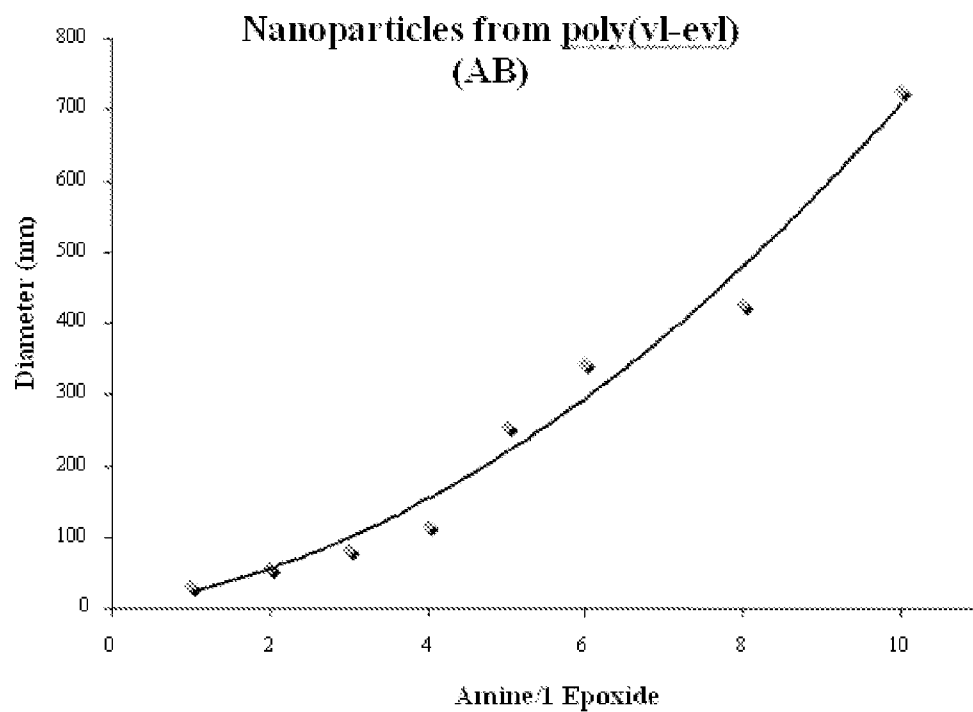


FIGURE 5

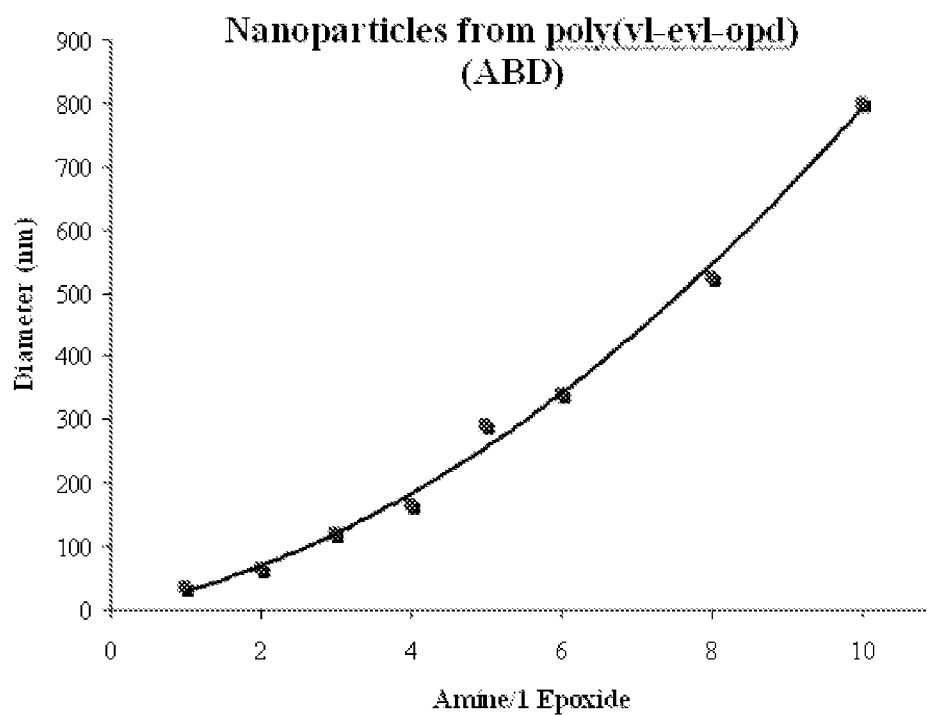


FIGURE 6

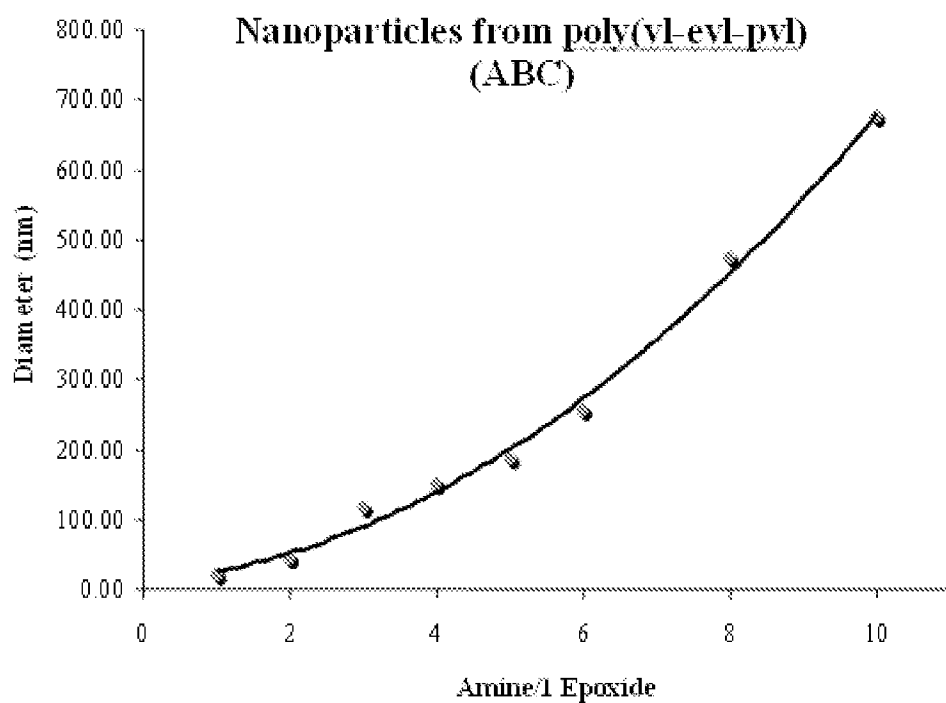


FIGURE 7

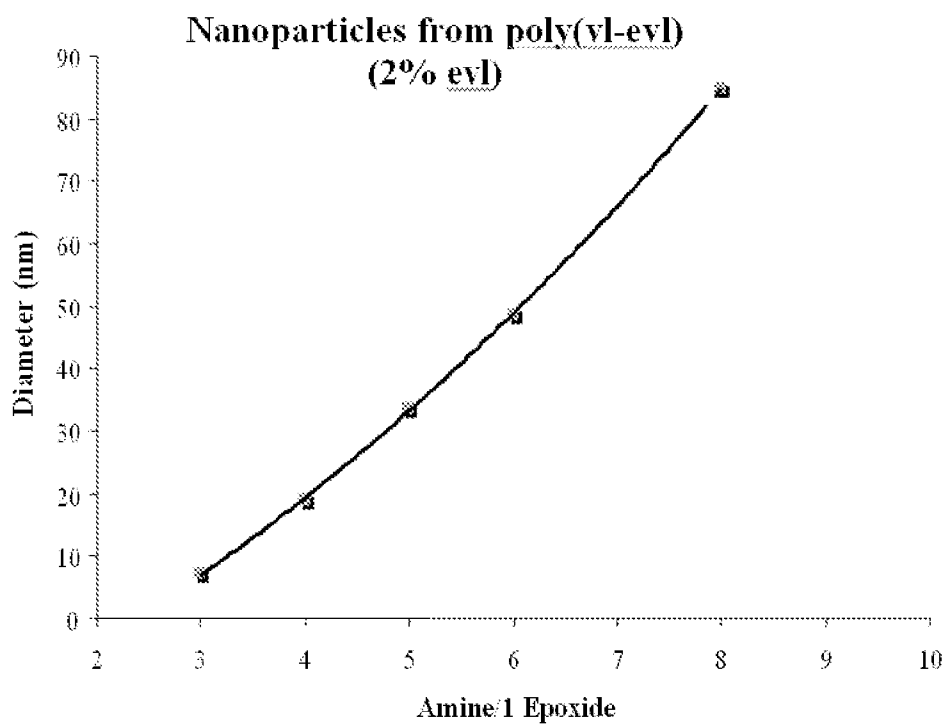


FIGURE 8

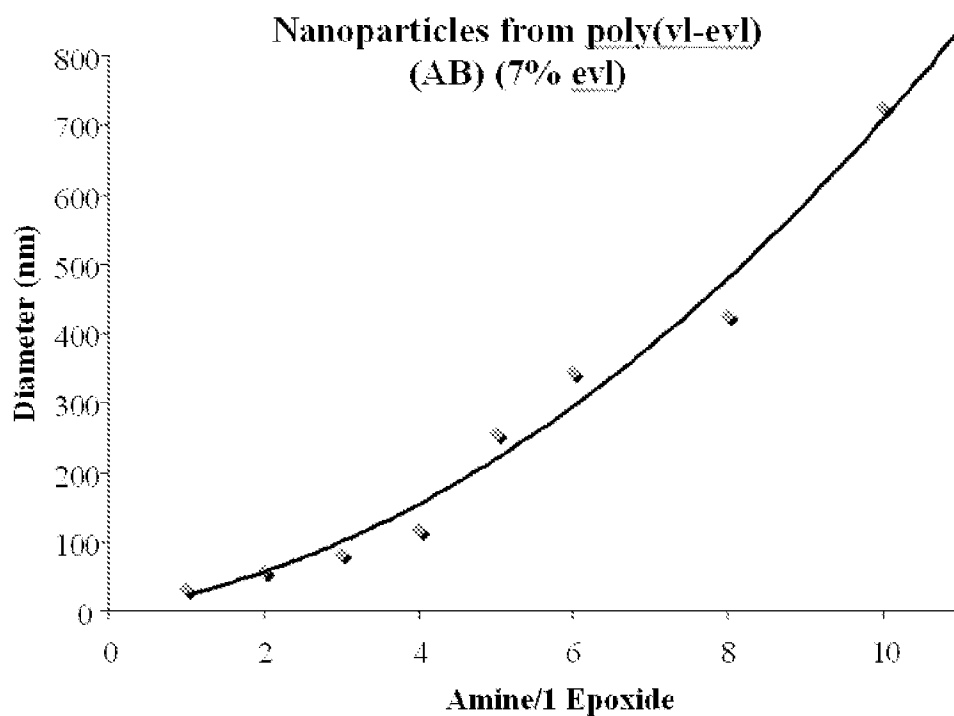


FIGURE 9

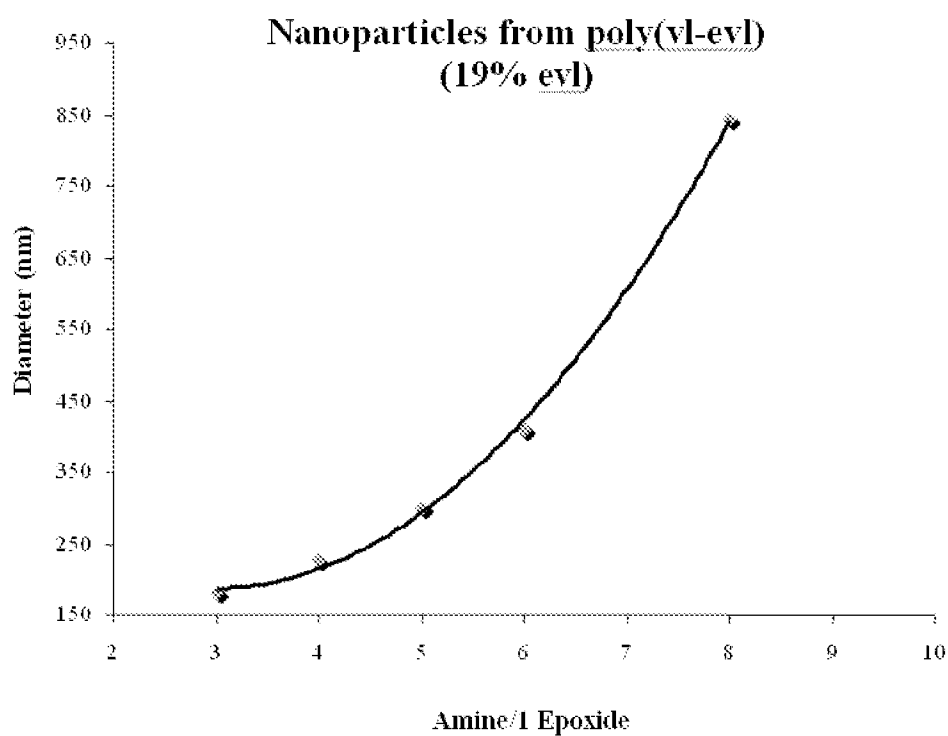


FIGURE 10

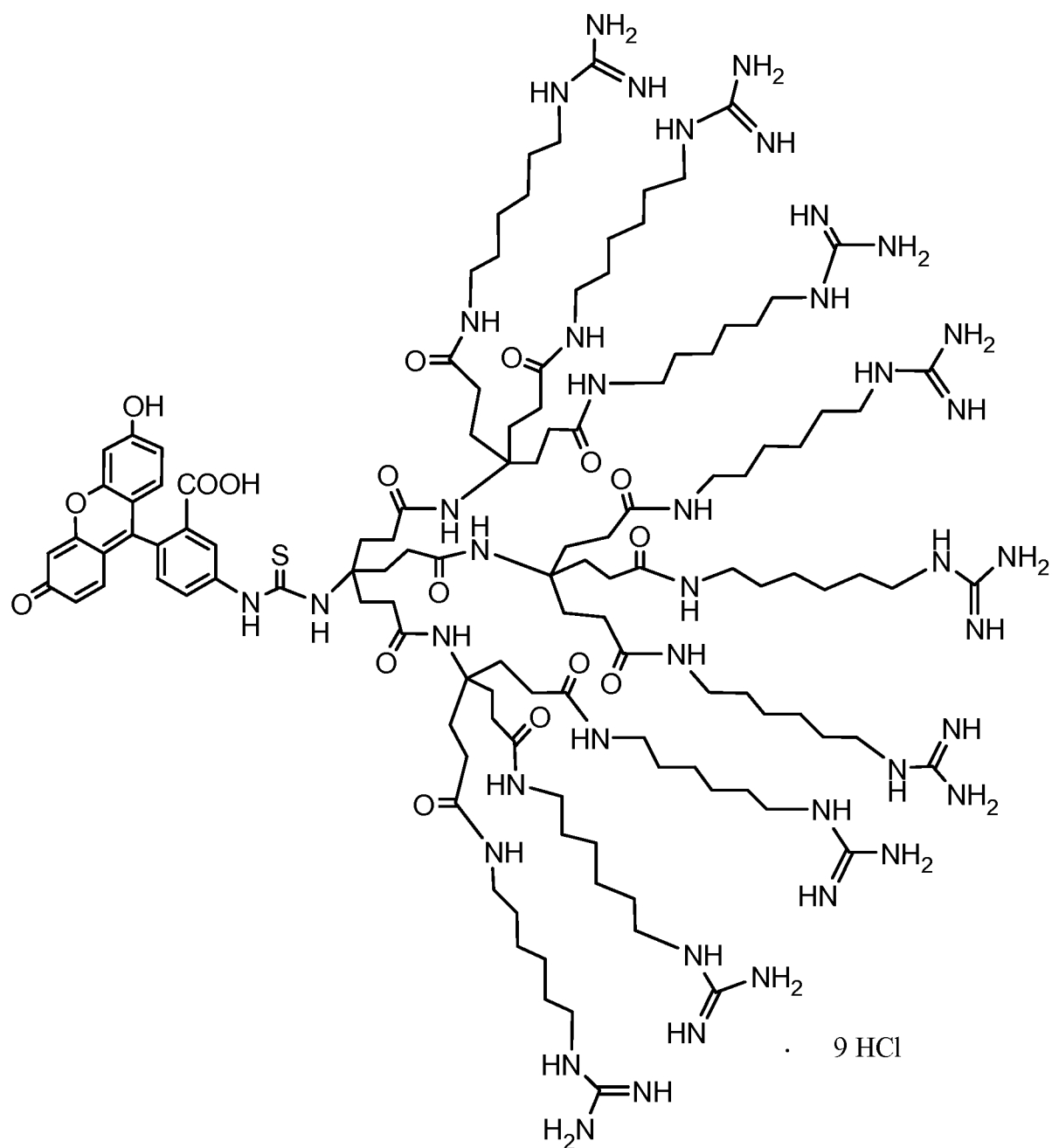


FIGURE 11

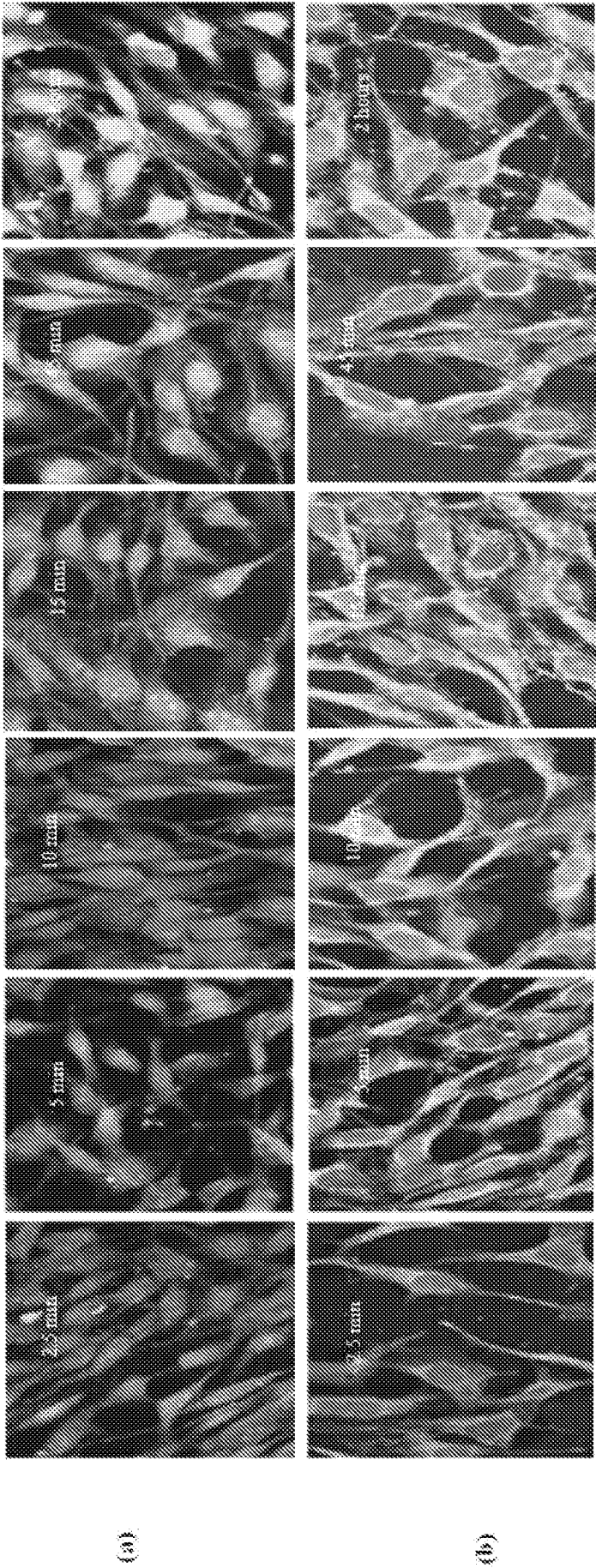


FIGURE 12

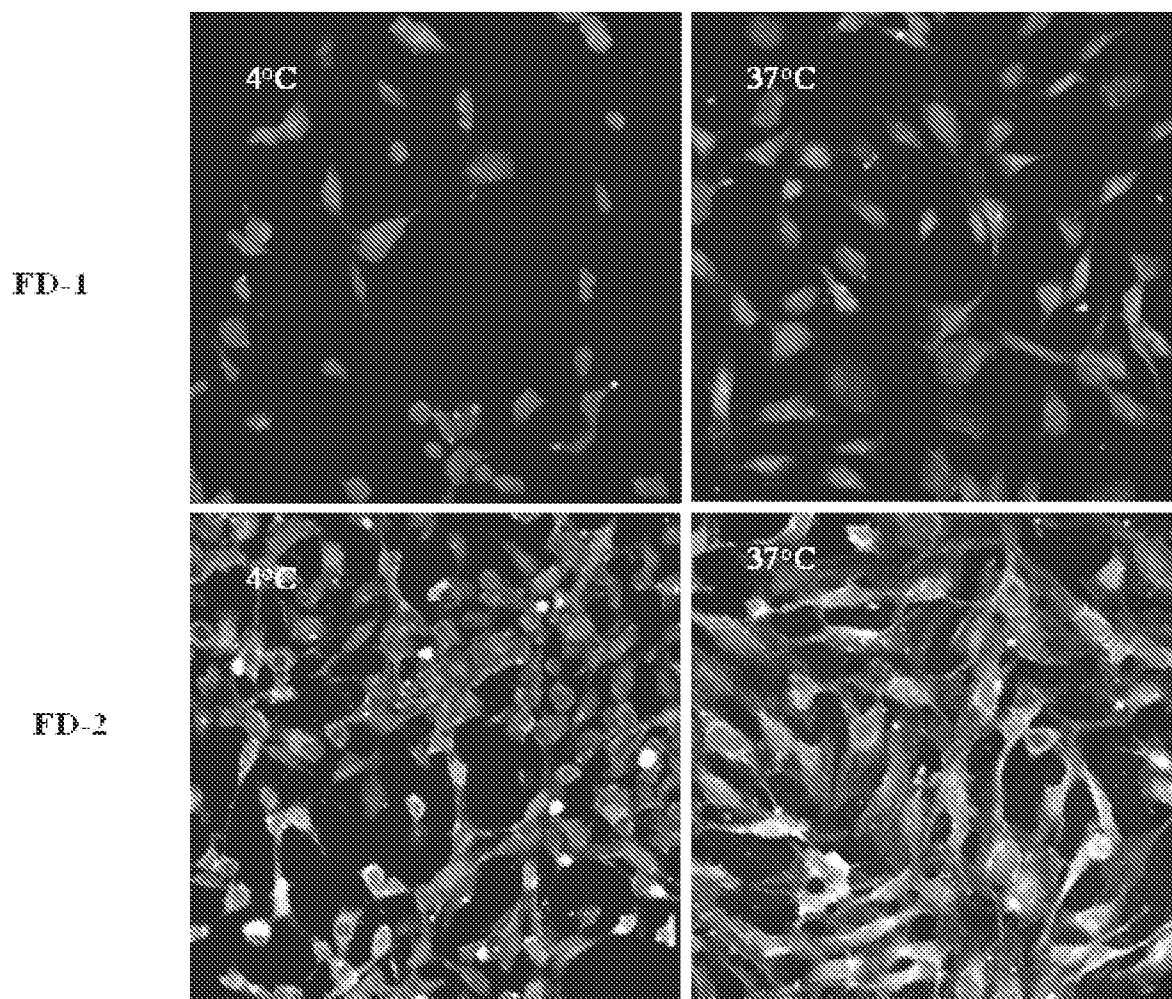


FIGURE 13

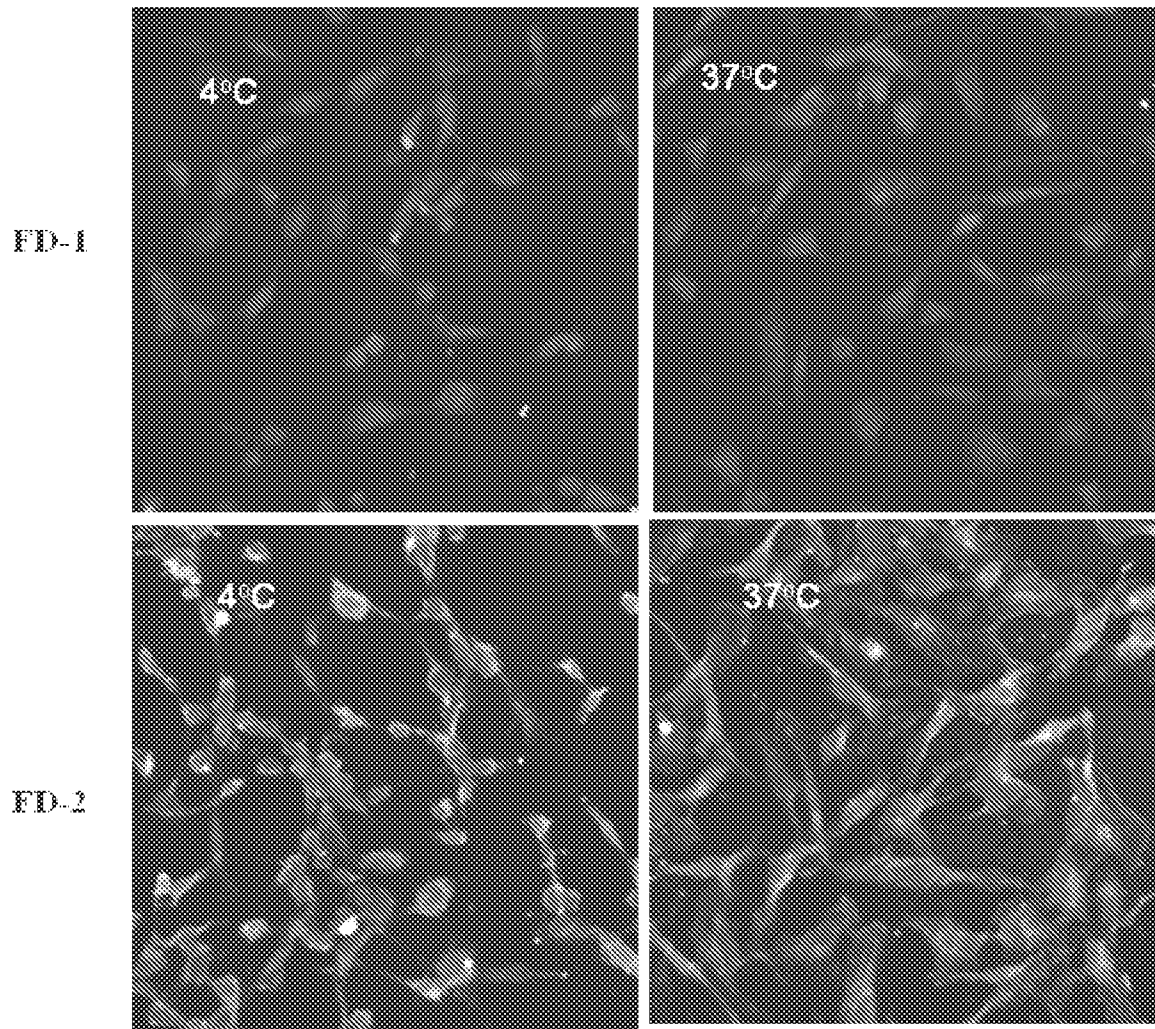


FIGURE 14

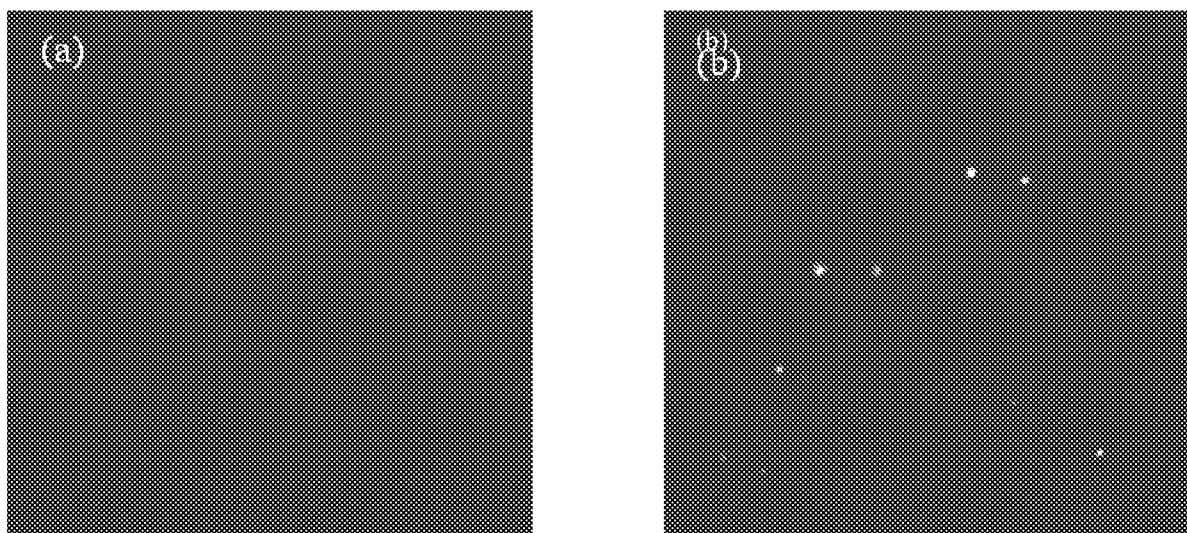


FIGURE 15

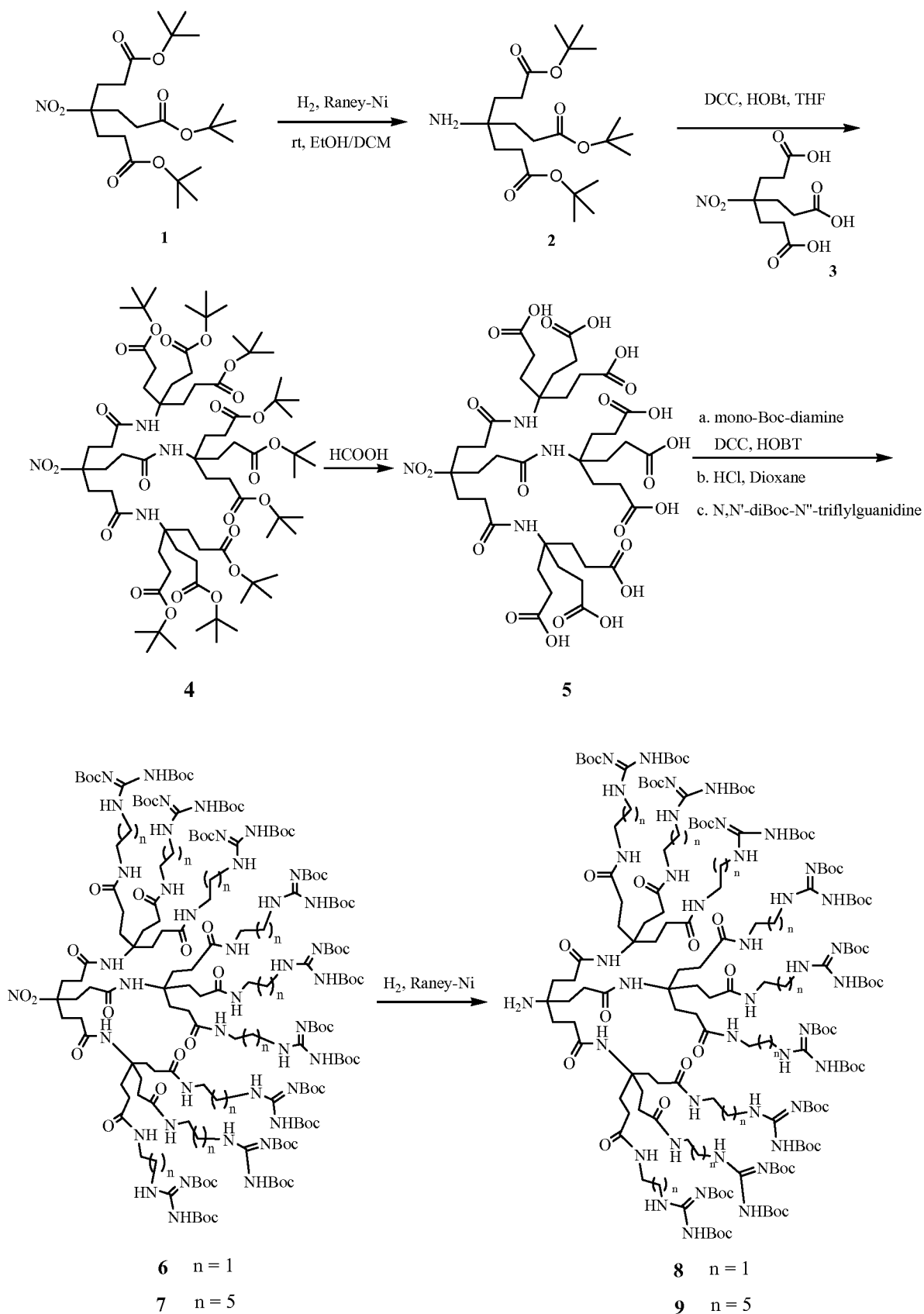


FIGURE 16A

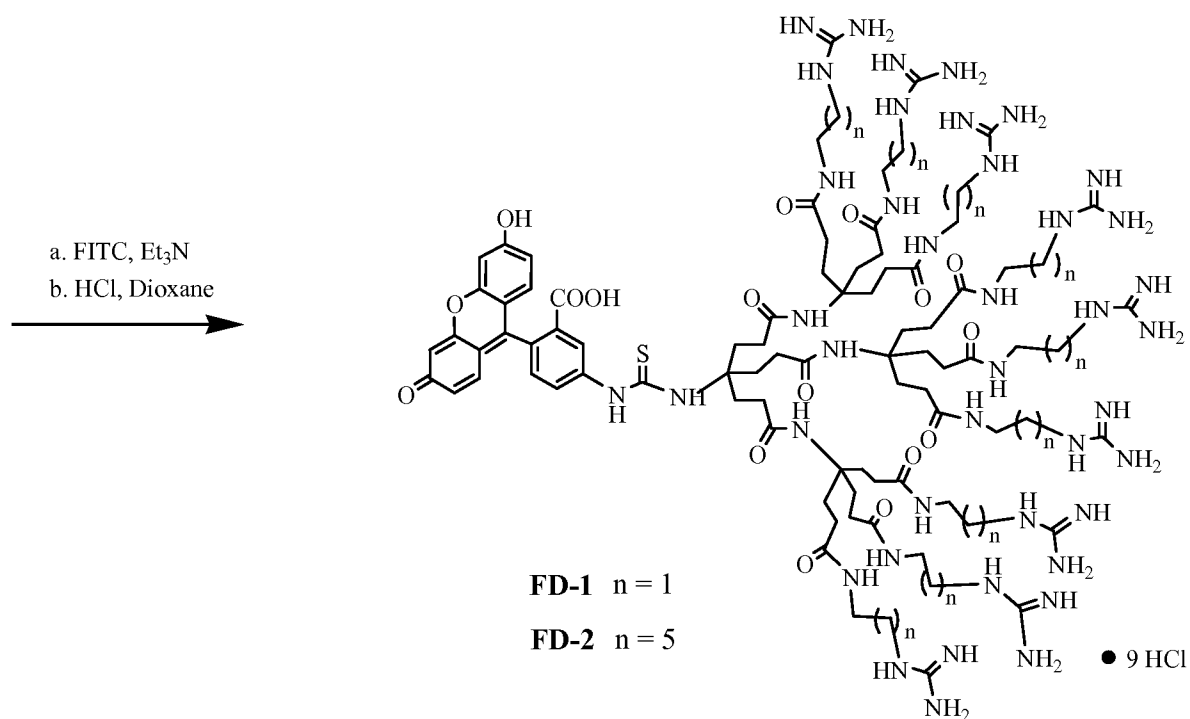


FIGURE 16B

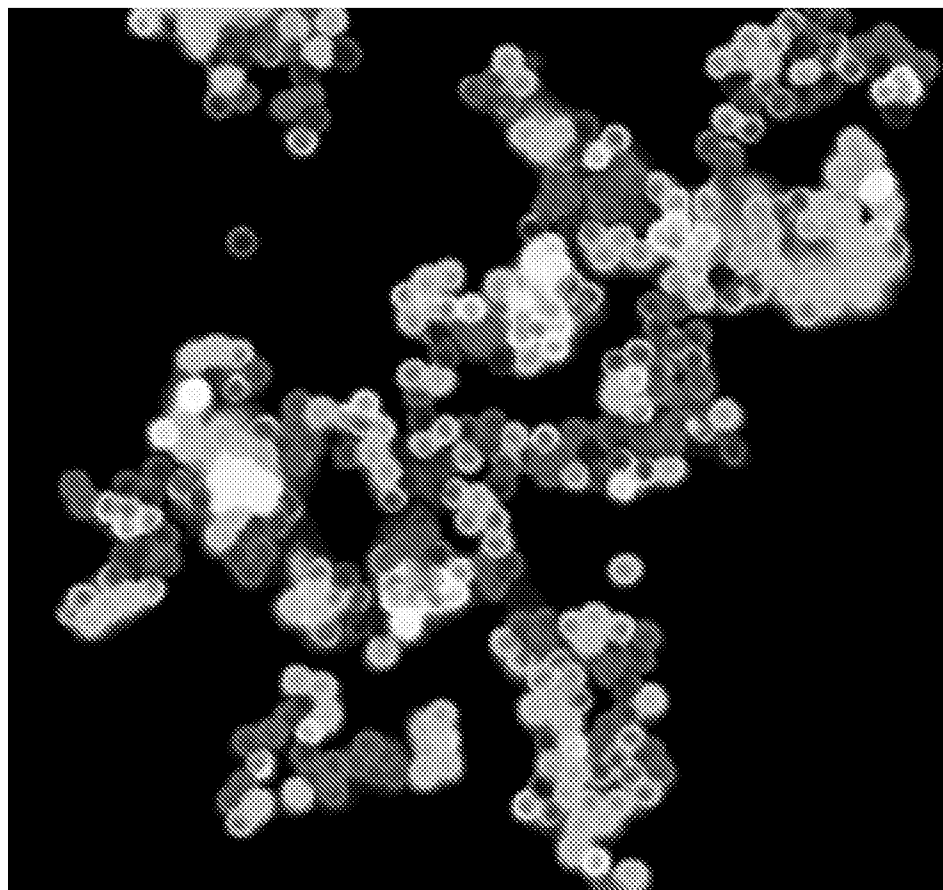


FIGURE 17

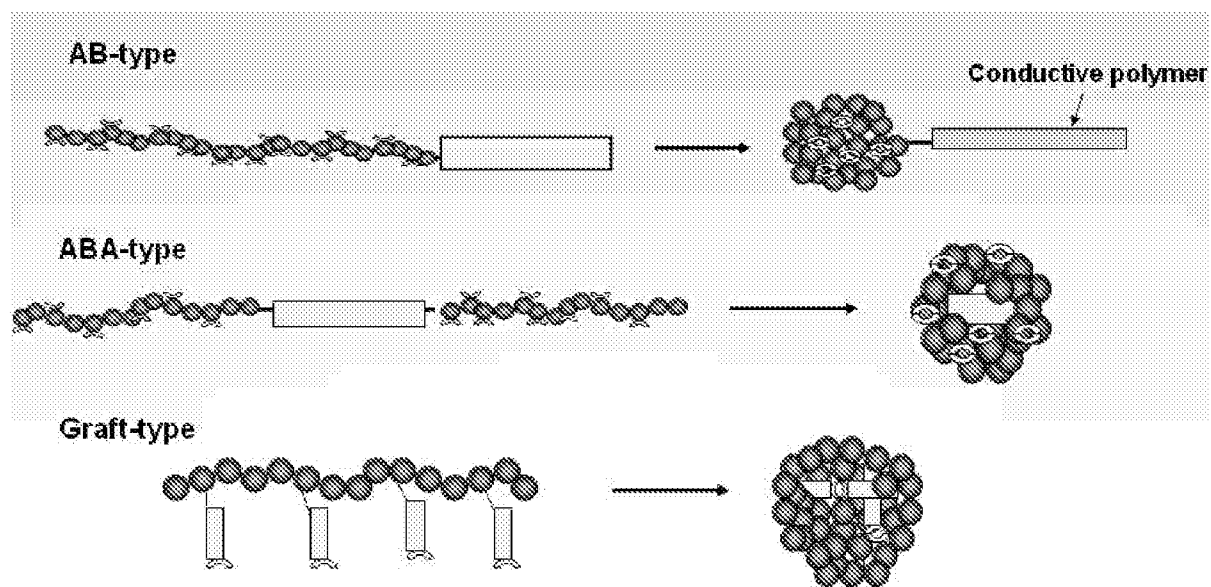


FIGURE 18

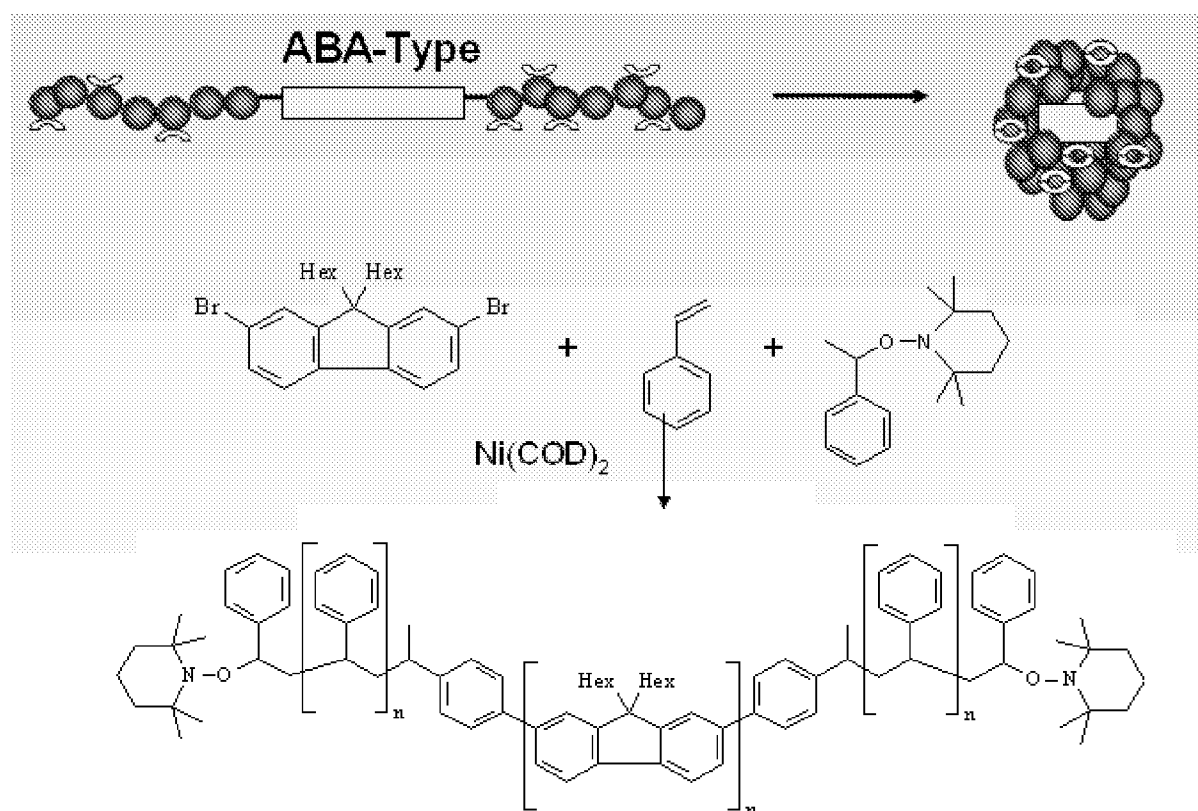


FIGURE 19

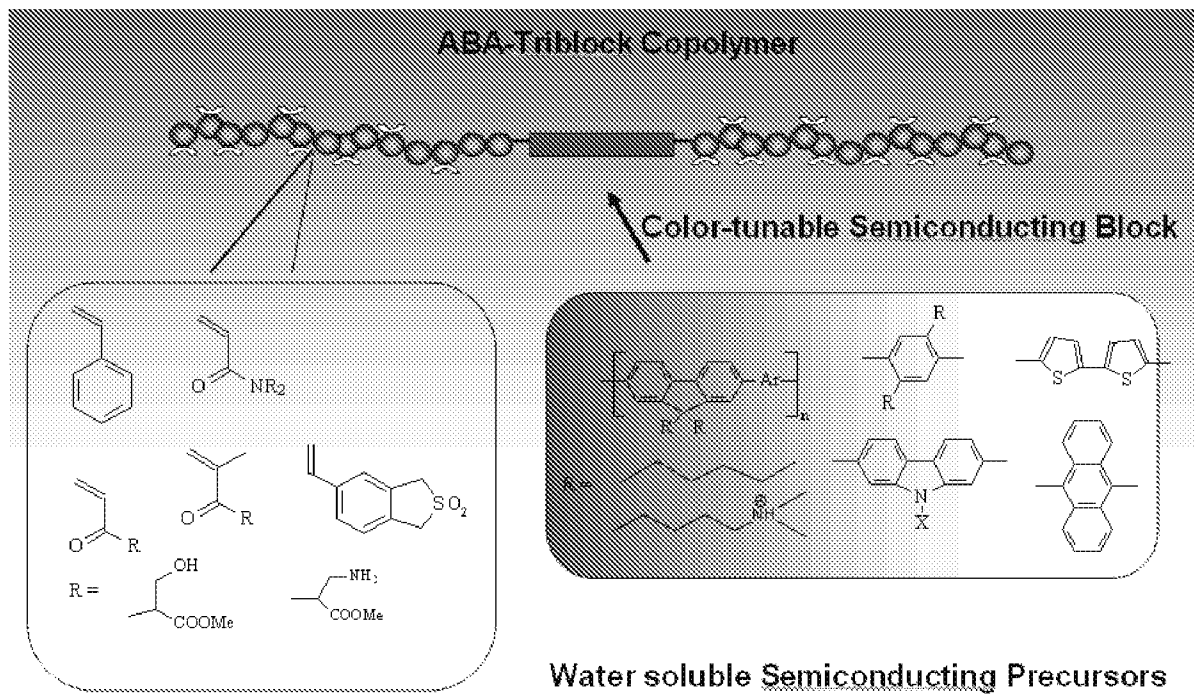


FIGURE 20

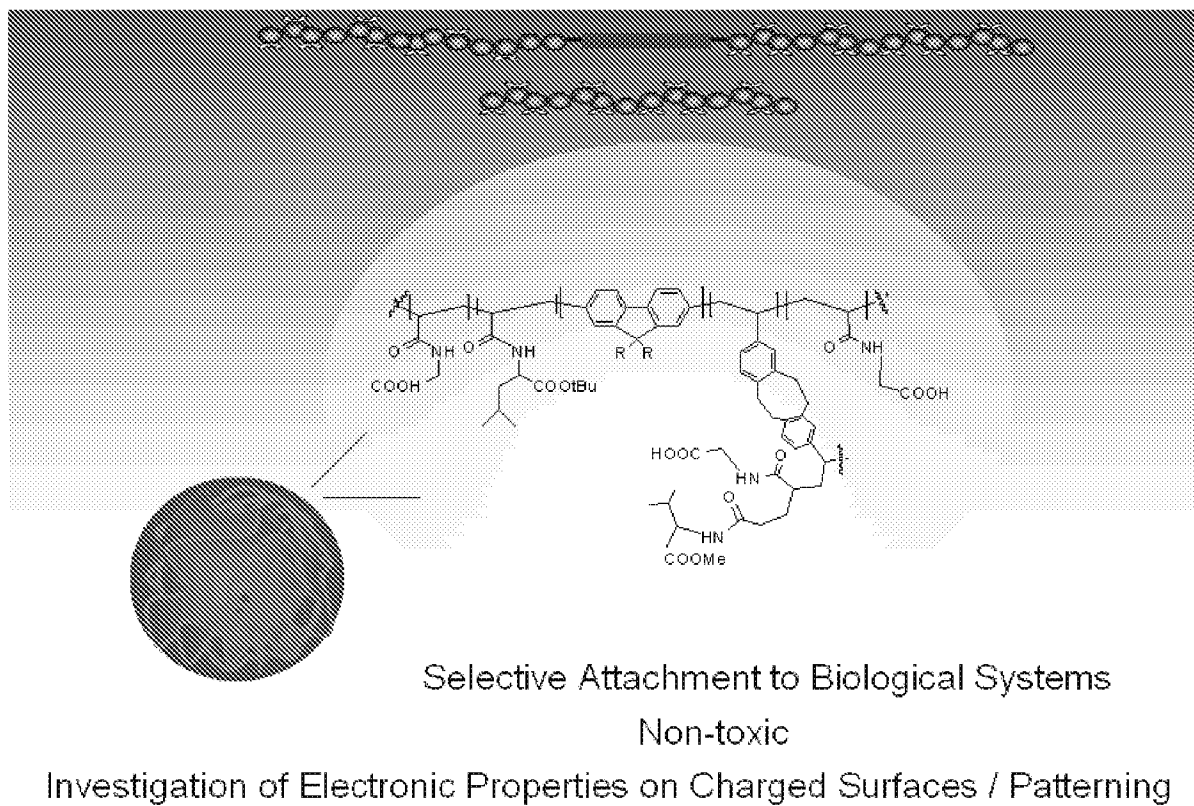


FIGURE 21

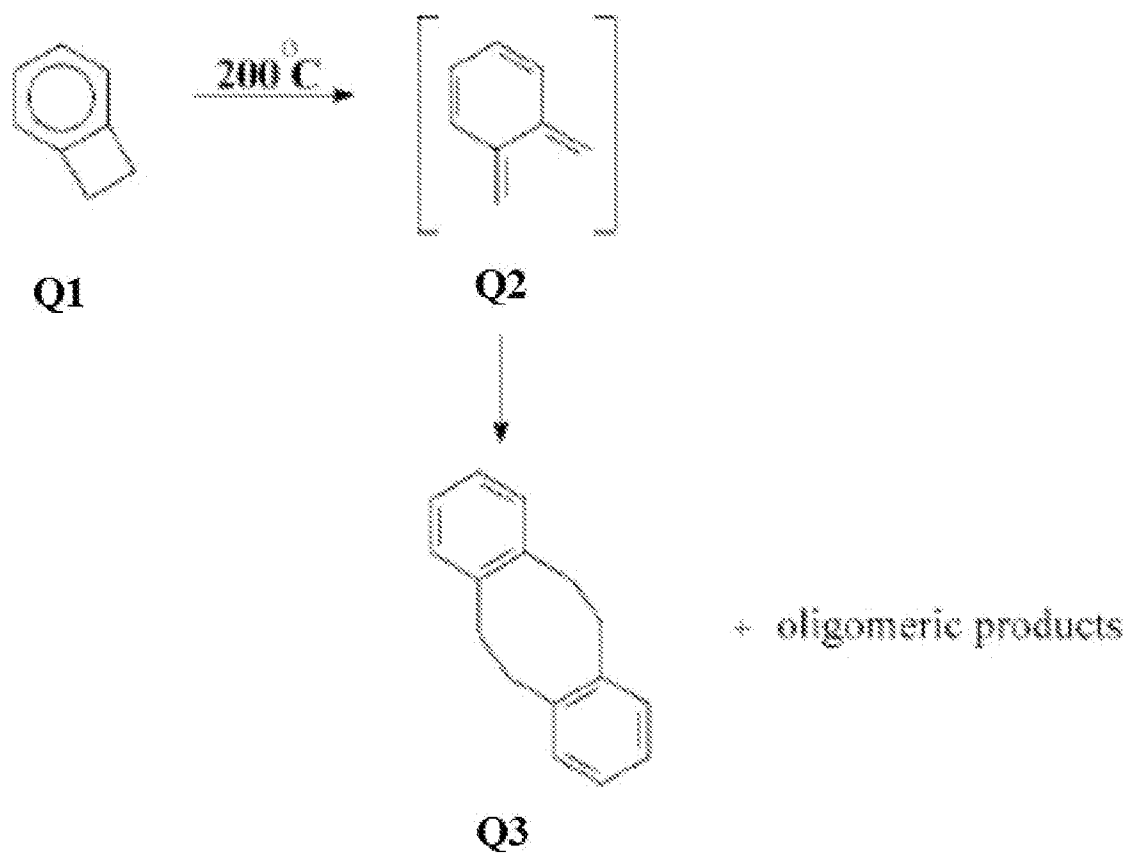


FIGURE 22

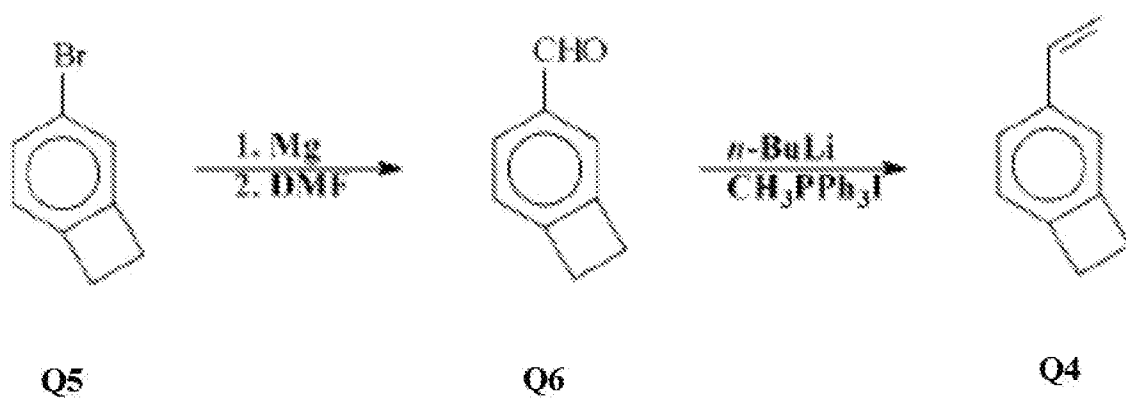


FIGURE 23

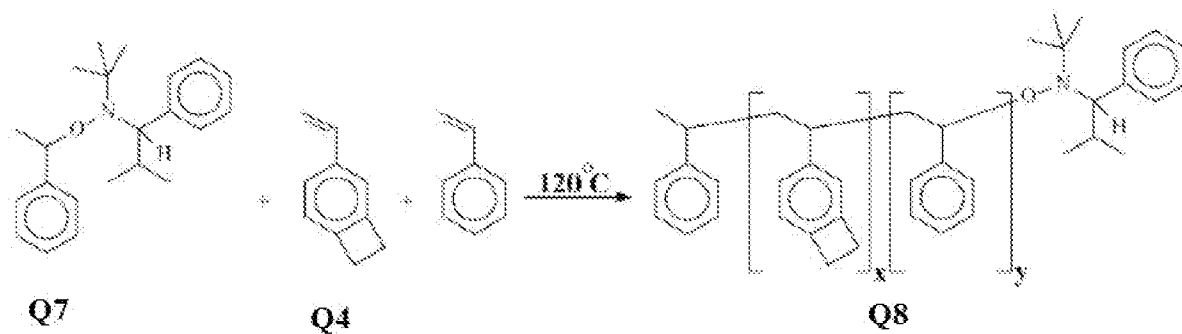


FIGURE 24

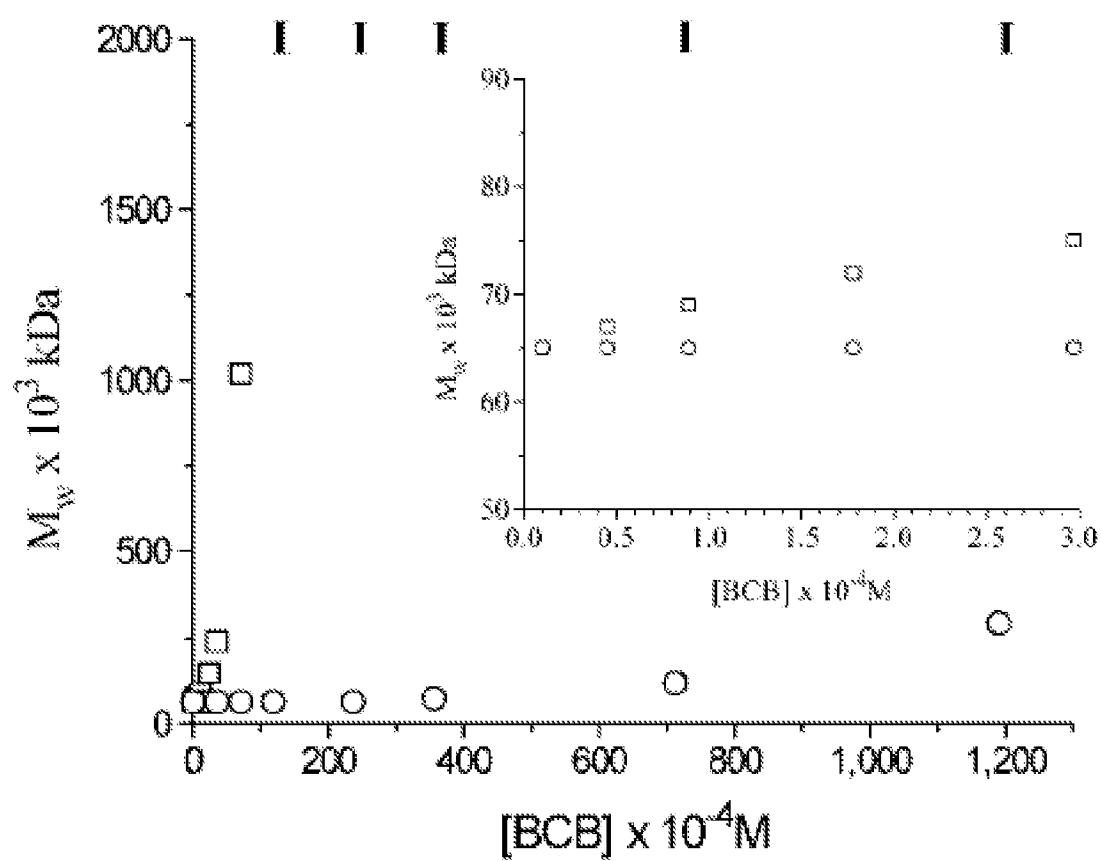


FIGURE 25

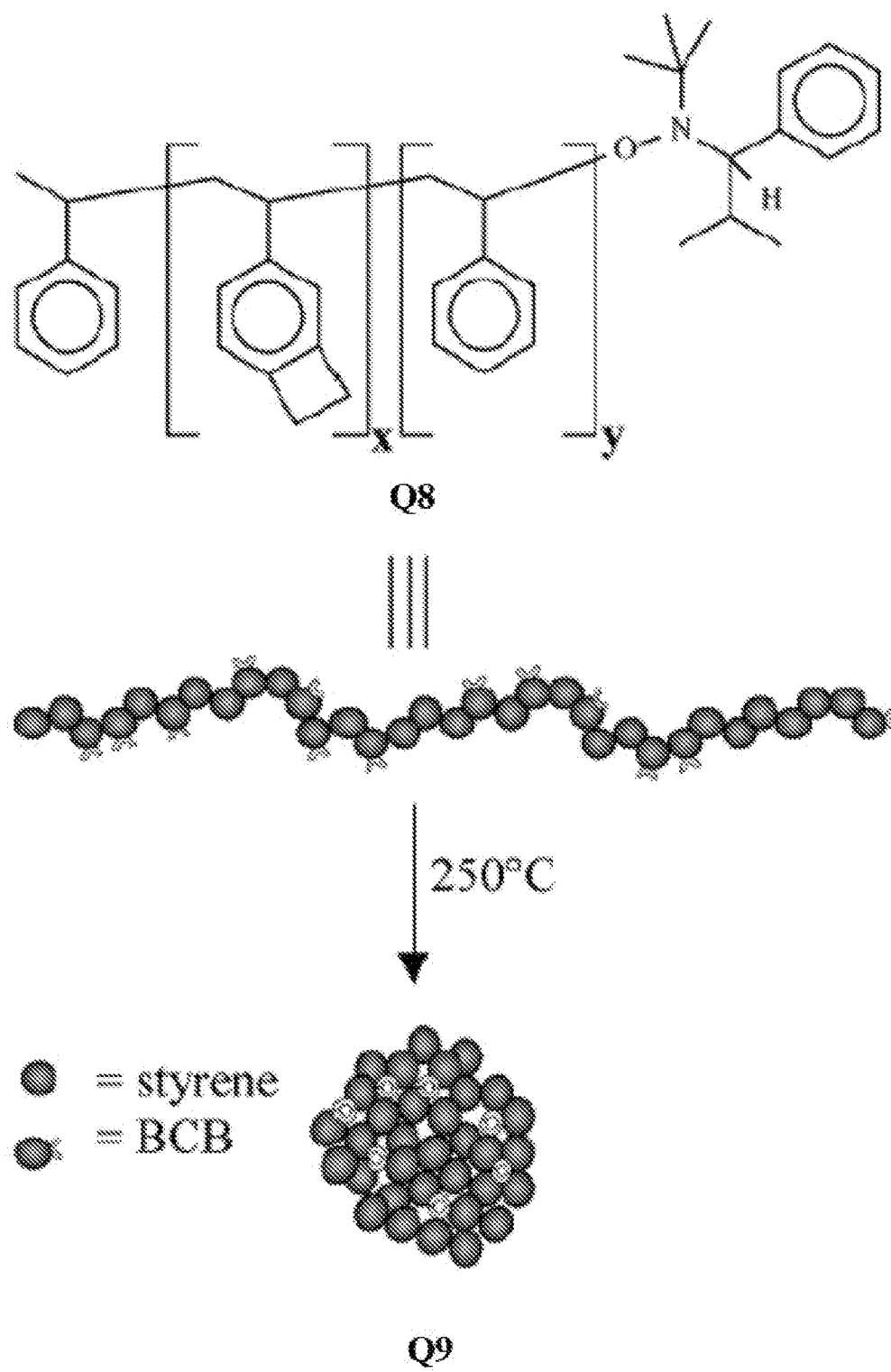


FIGURE 26

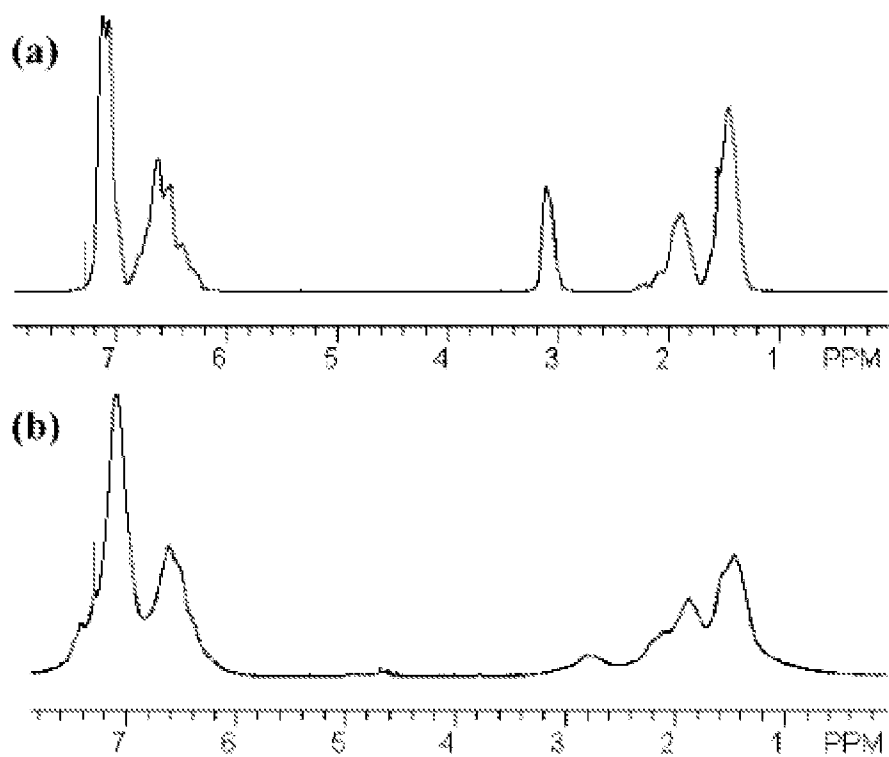


FIGURE 27

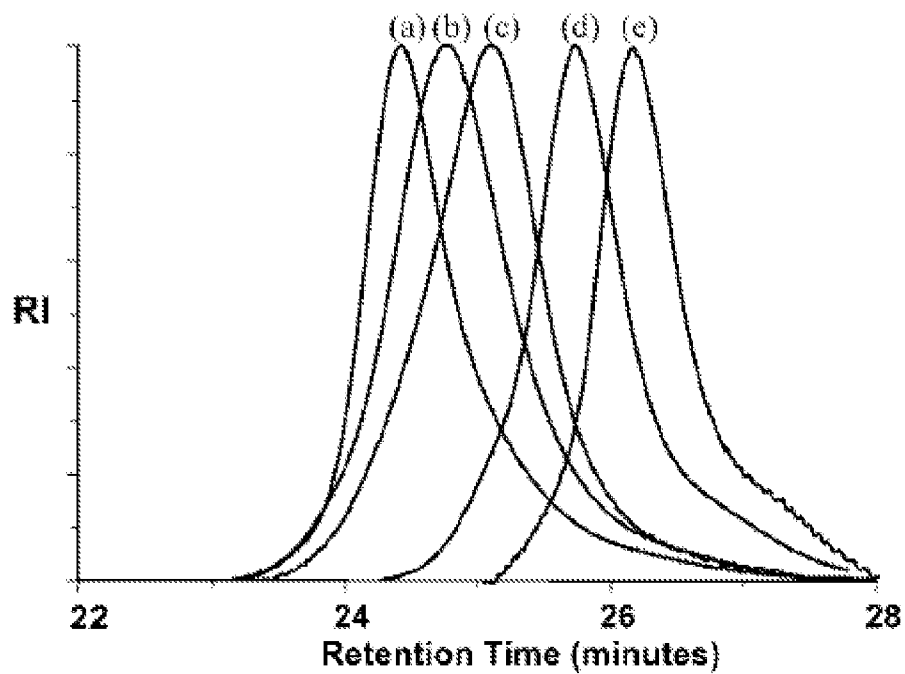


FIGURE 28

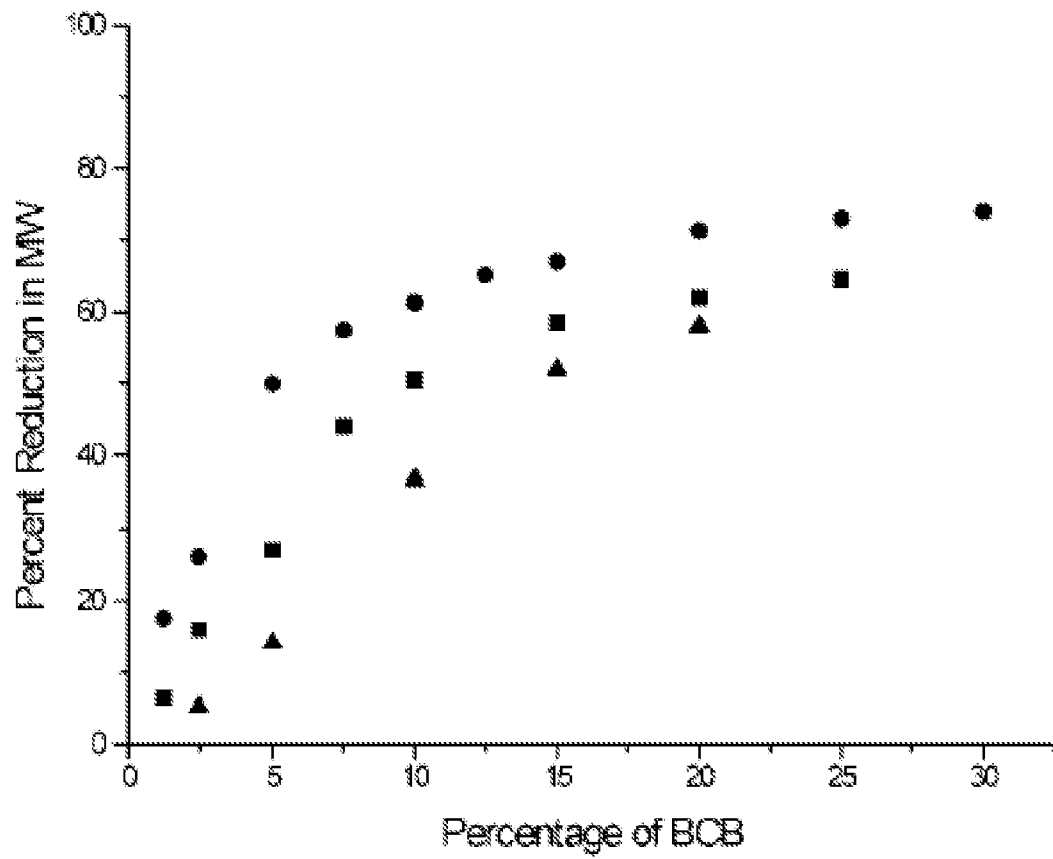


FIGURE 29

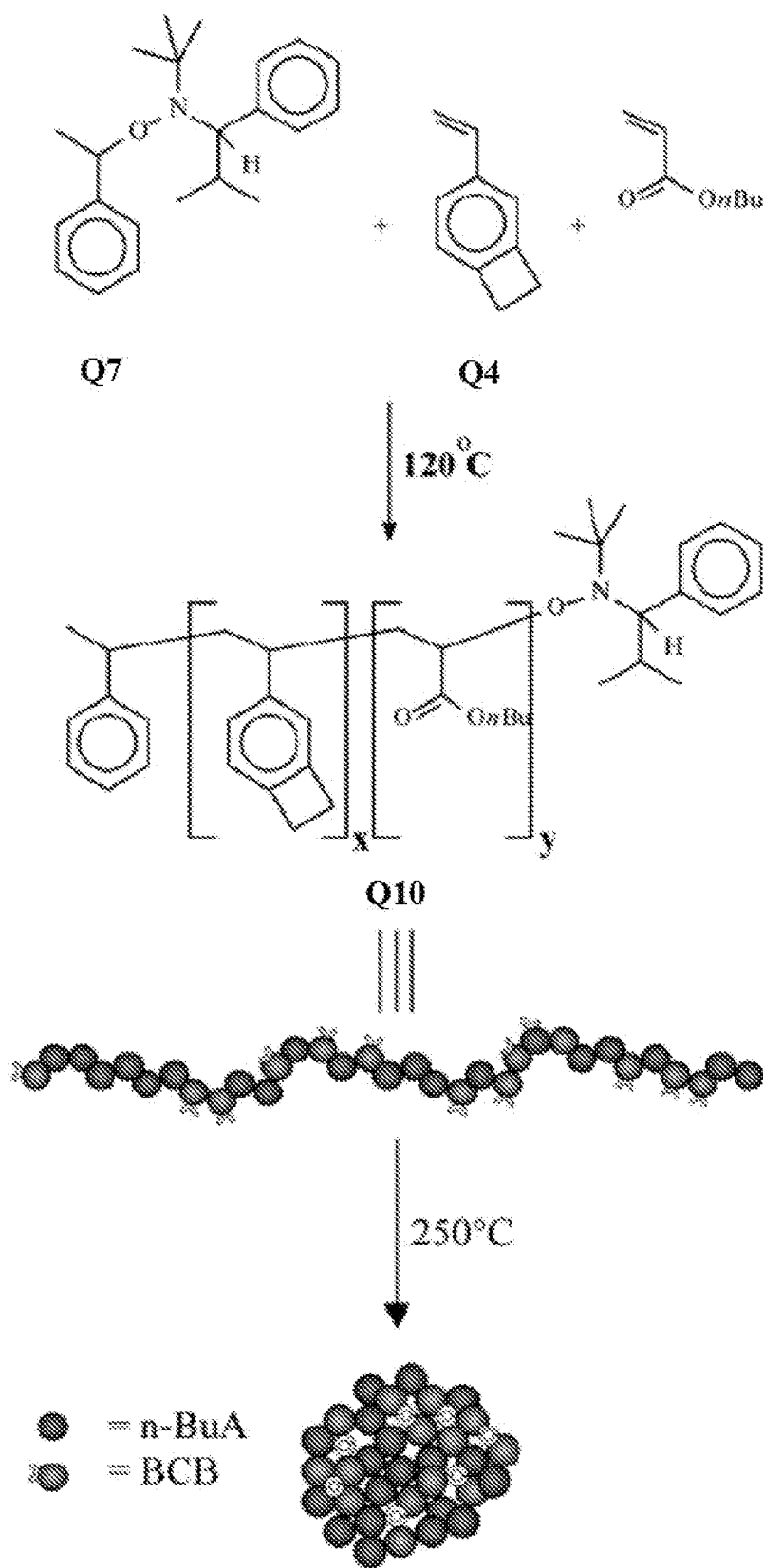


FIGURE 30

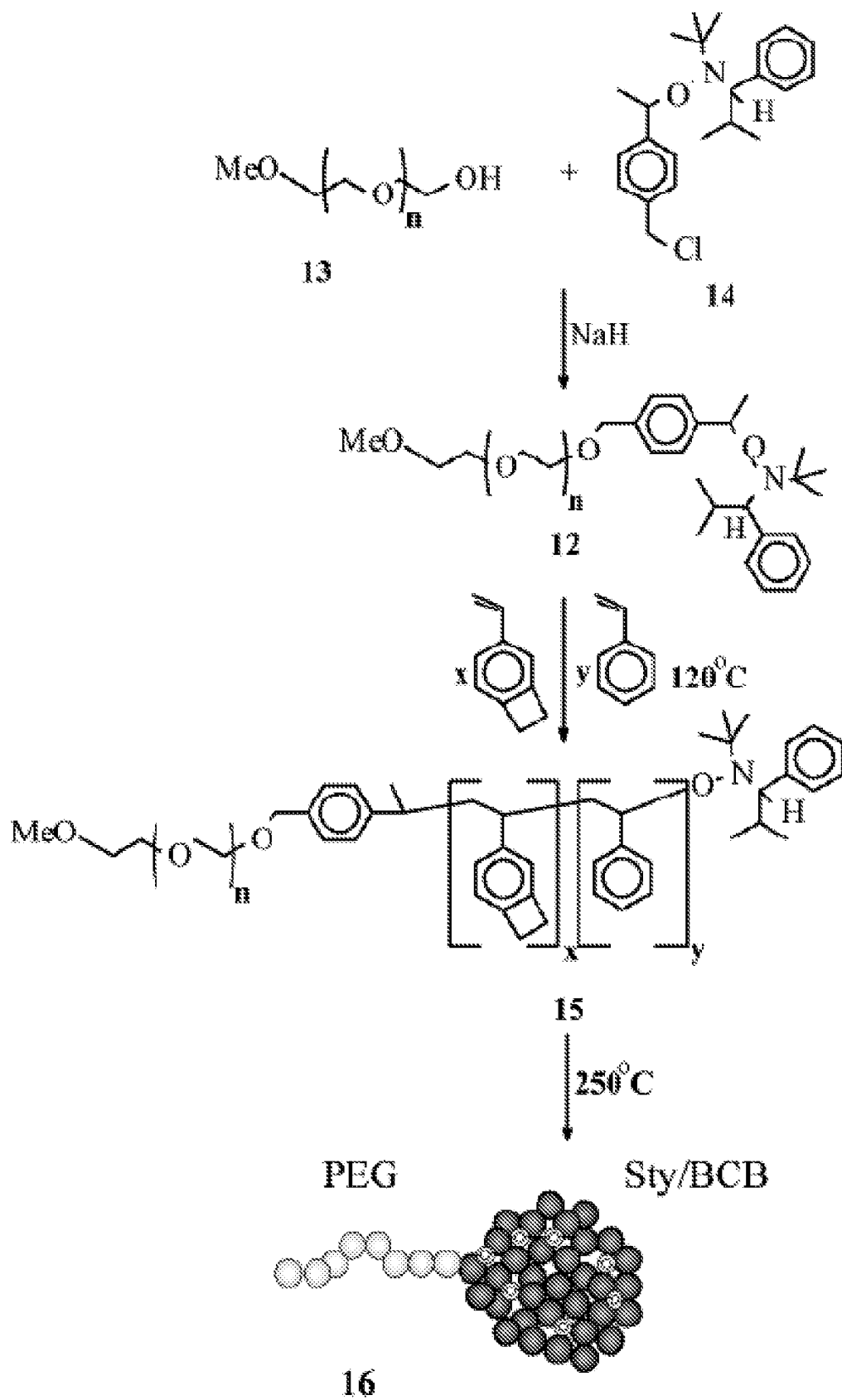


**FIGURE 31**

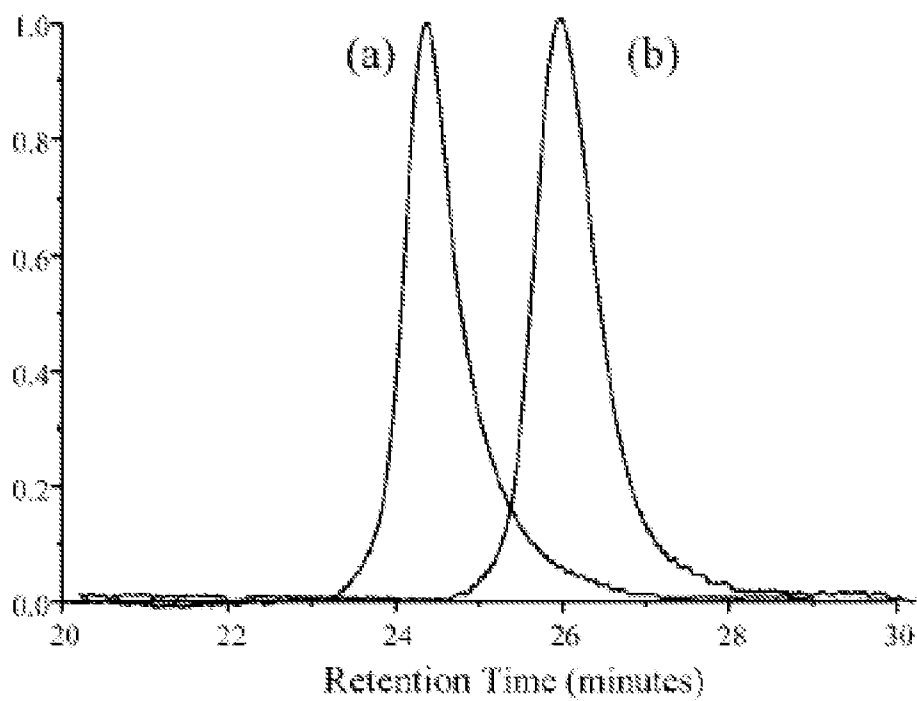


FIGURE 32

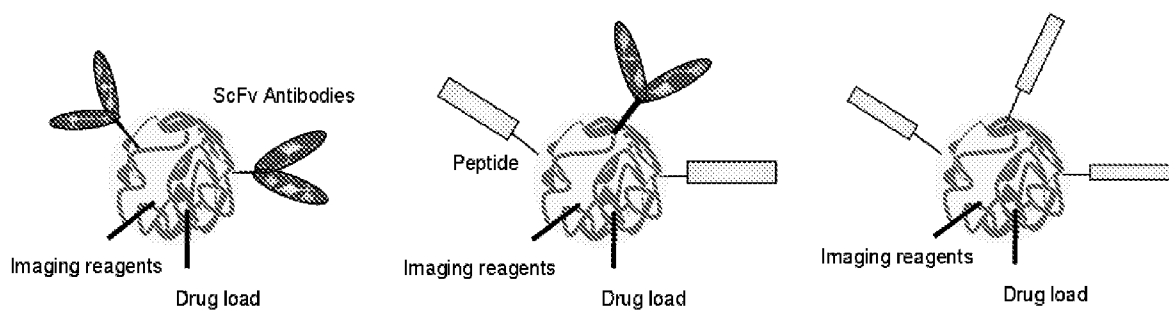


FIGURE 33

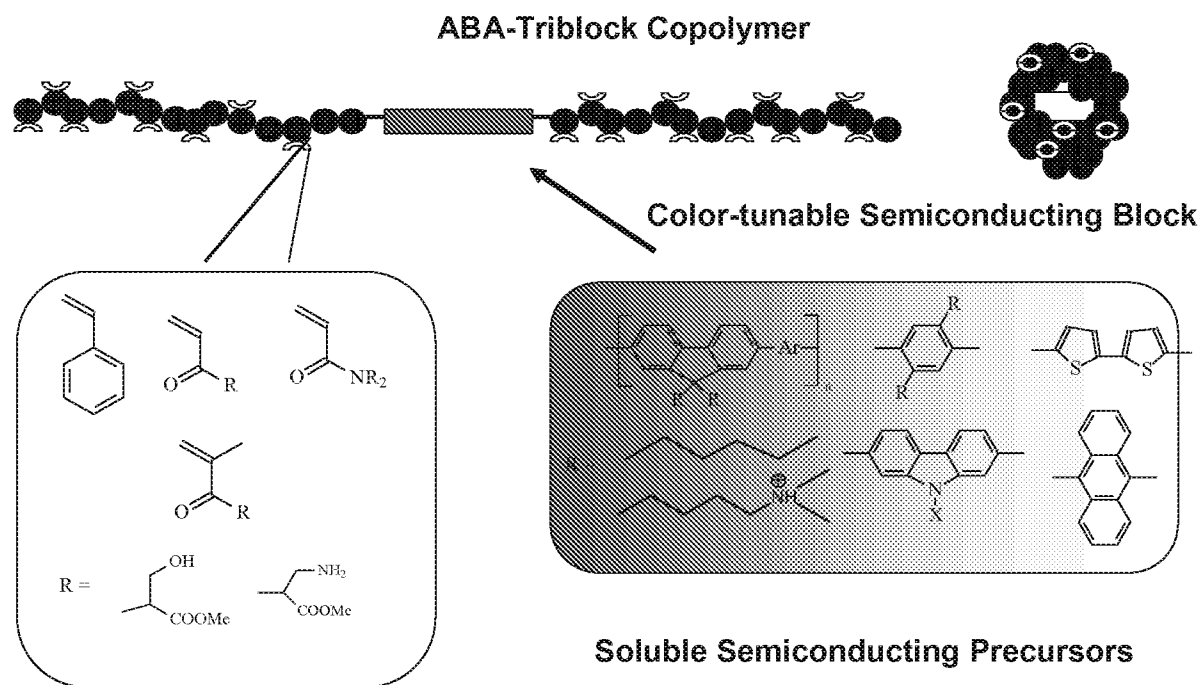


FIGURE 34

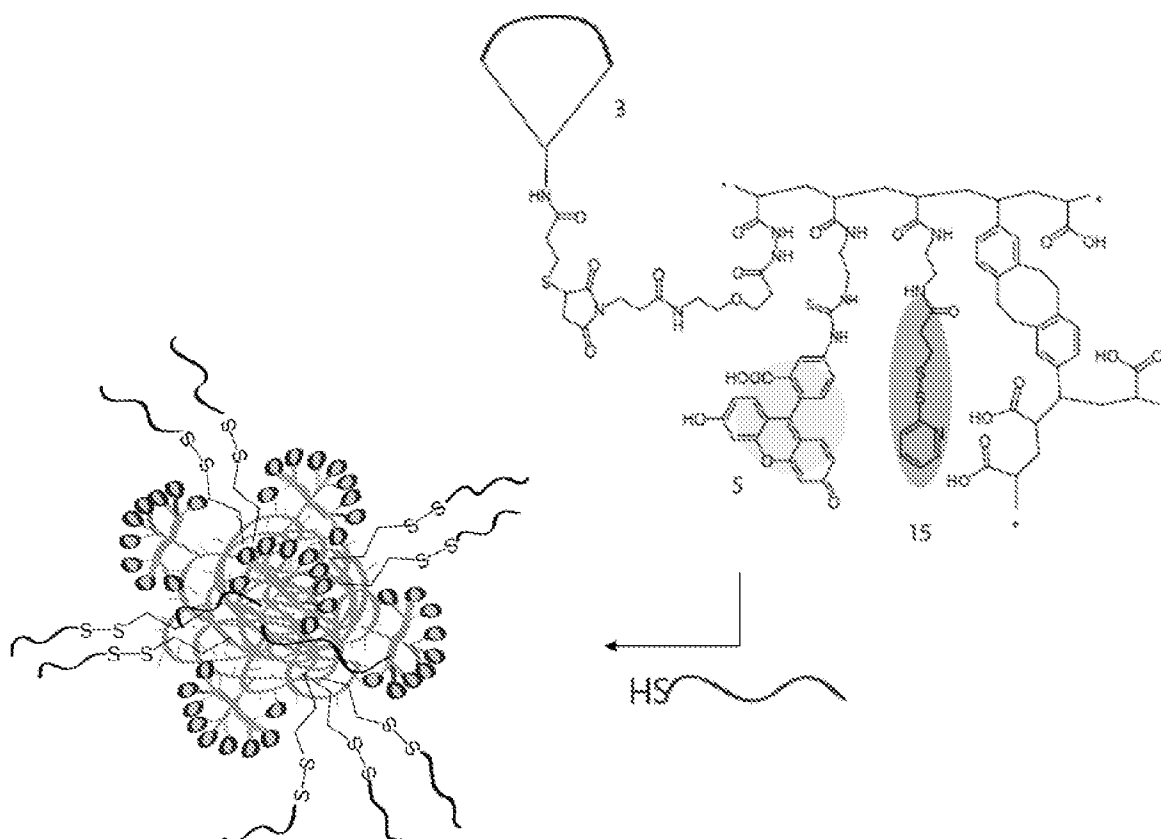


FIGURE 35

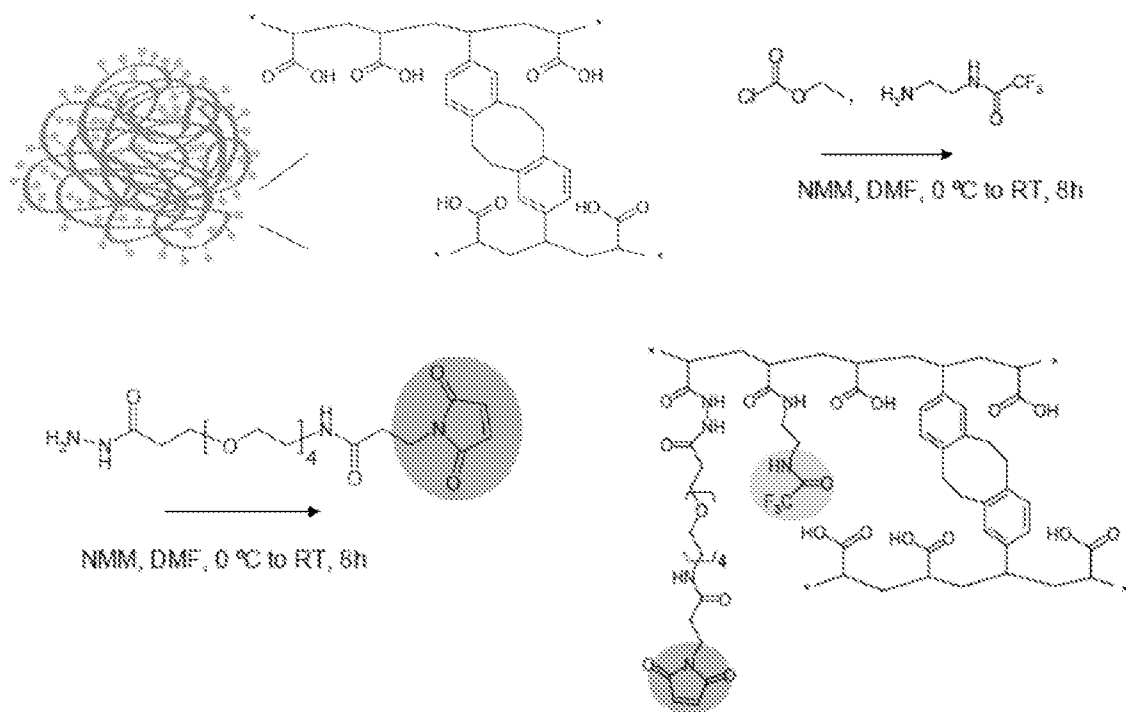


FIGURE 36

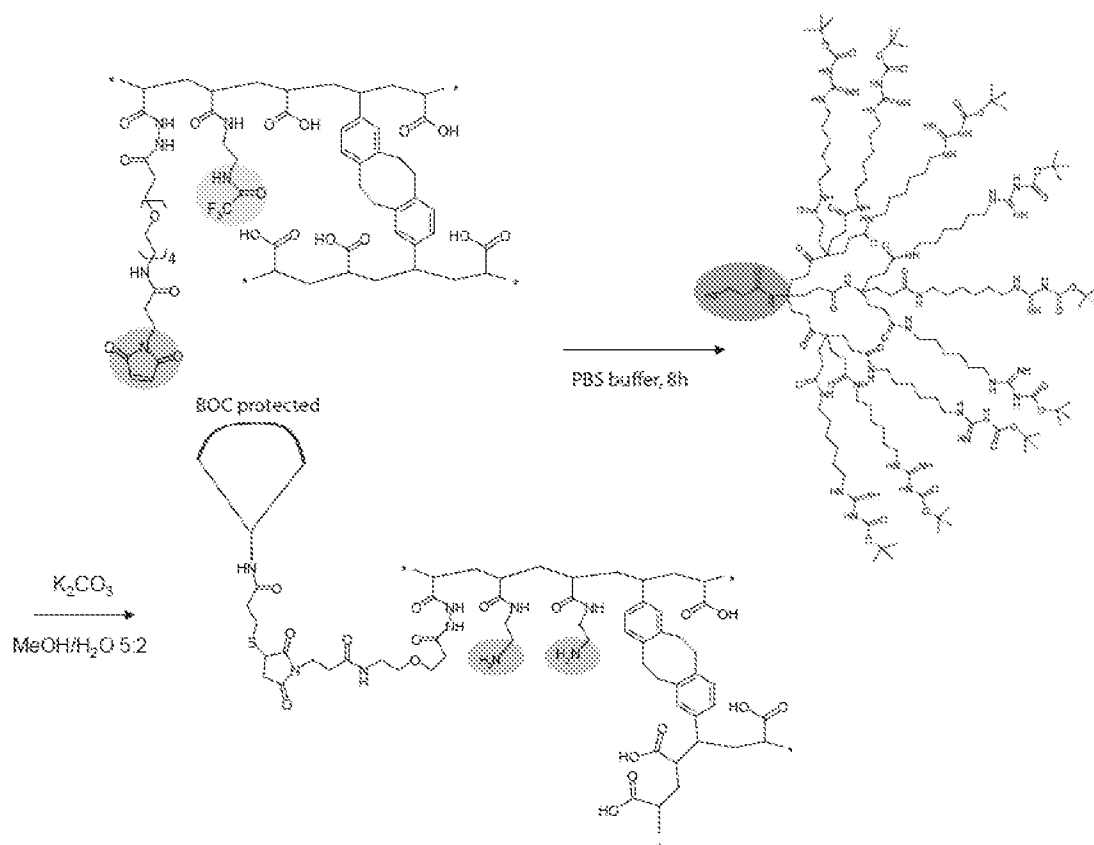


FIGURE 37

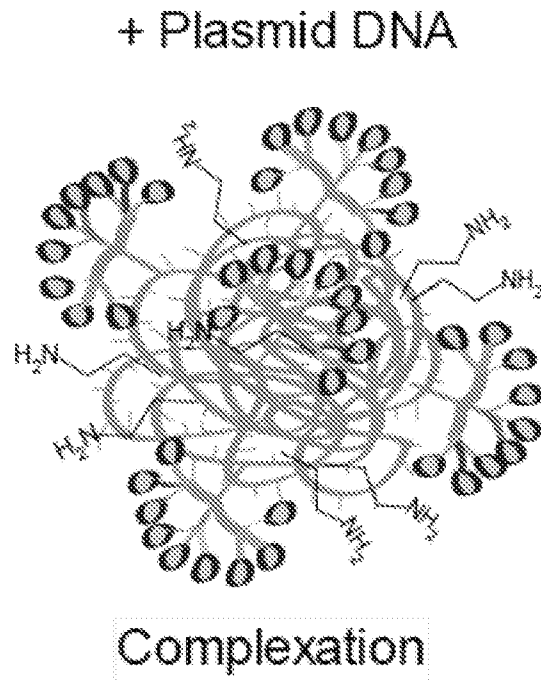


FIGURE 38

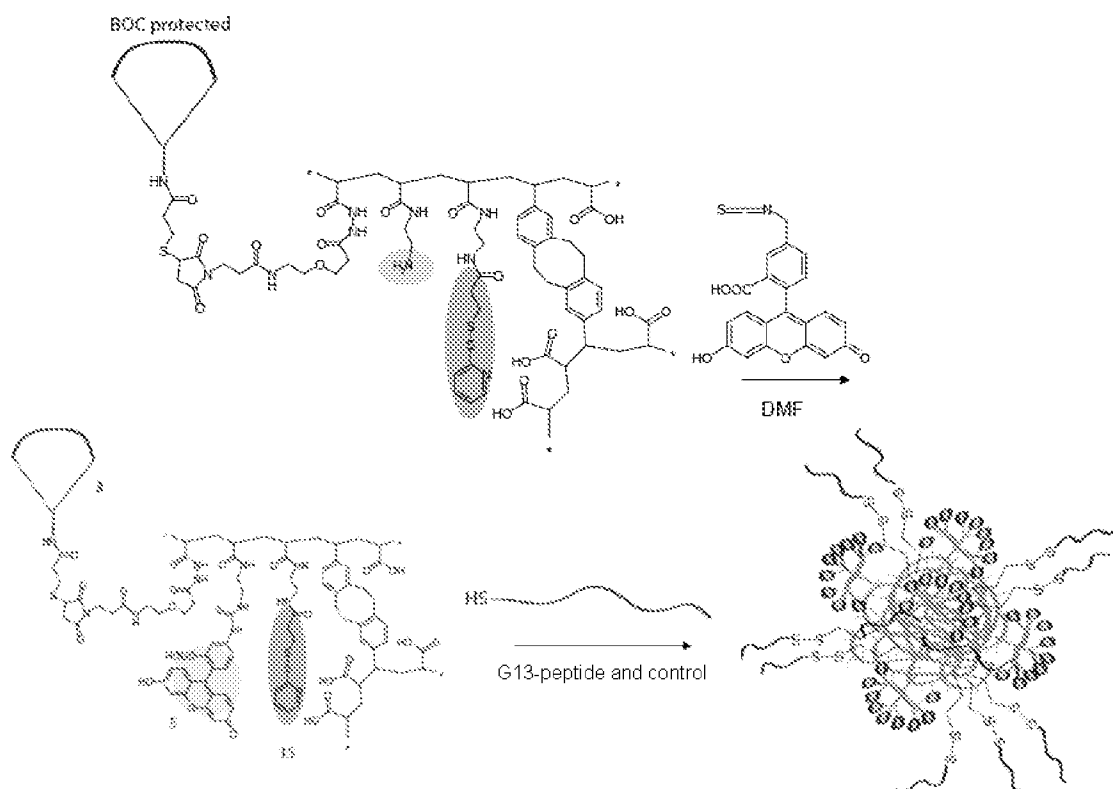


FIGURE 39

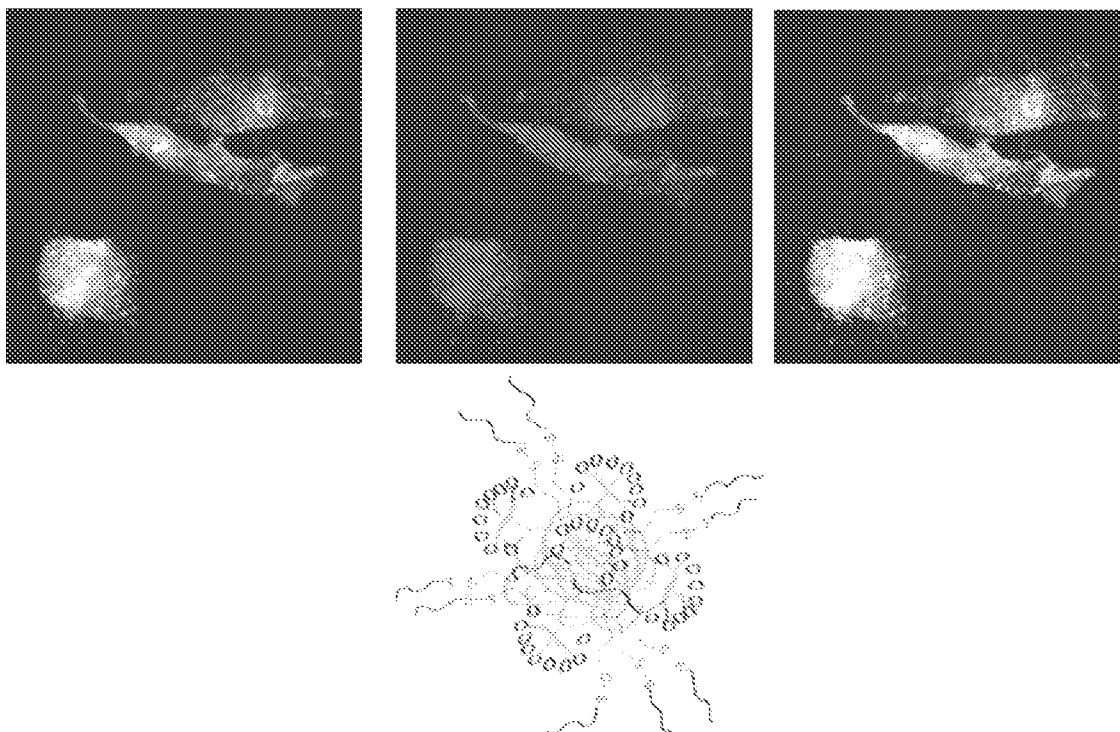


**FIGURE 40**

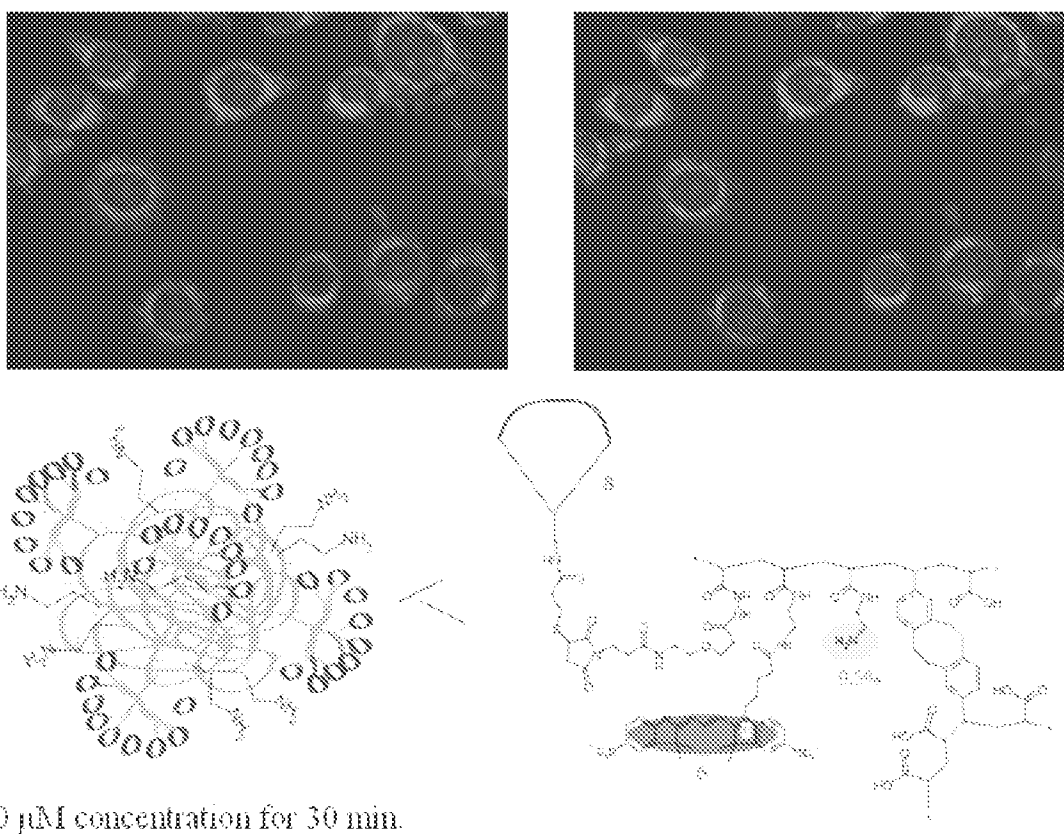
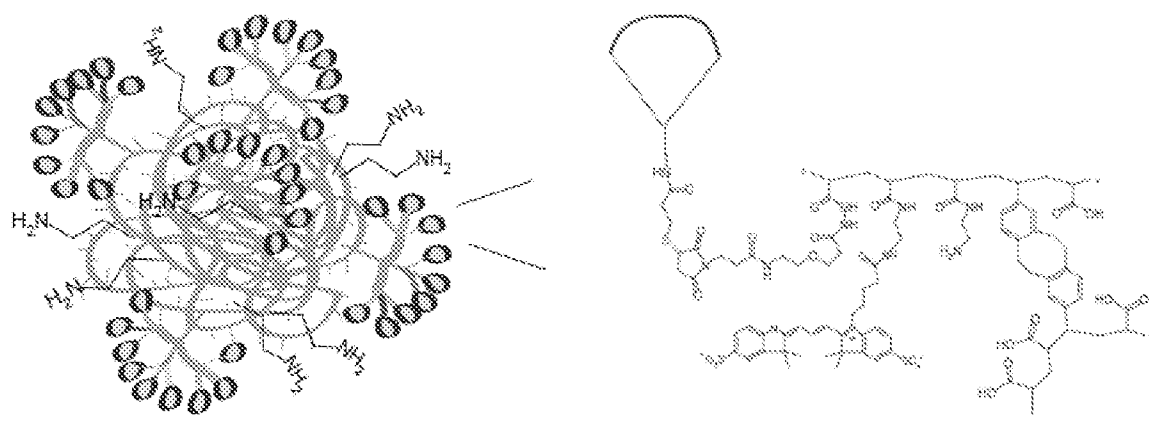


FIGURE 41



|      | <u>% <math>\text{NH}_2</math></u> | <u>% Maleimide Linker</u> | <u>Molecular<br/>Transporter<br/>FD-2</u> | <u>Cy3 Dye</u> |
|------|-----------------------------------|---------------------------|-------------------------------------------|----------------|
| NP 1 | 26.9                              | 0                         | —                                         | —              |
| NP 2 | 30.1                              | 0                         | —                                         | —              |
| NP 3 | 9.7                               | 0                         | —                                         | —              |
| NP 4 | 3.3                               | 12                        | 10                                        | 3              |
| NP 5 | 18.7                              | 19.5                      | 8                                         | 9              |
| NP 6 | 9.5                               | 9.7                       | 8                                         | 6              |

FIGURE 42

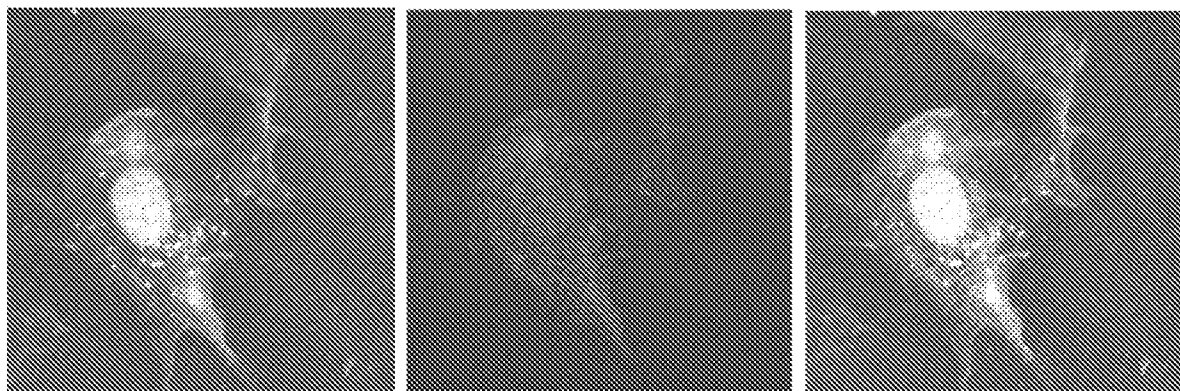


FIGURE 43

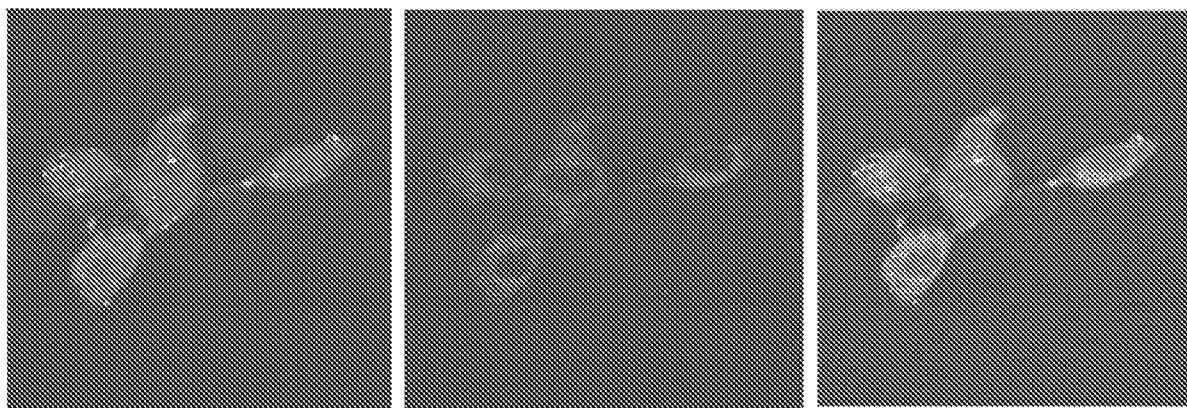
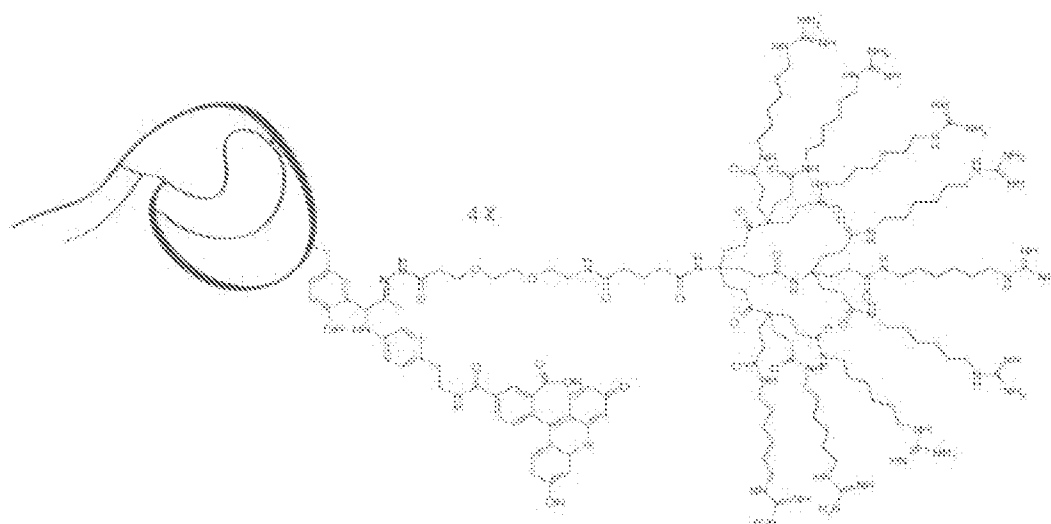


FIGURE 44



Aprotinin-Fluorophore-Transporter Conjugate

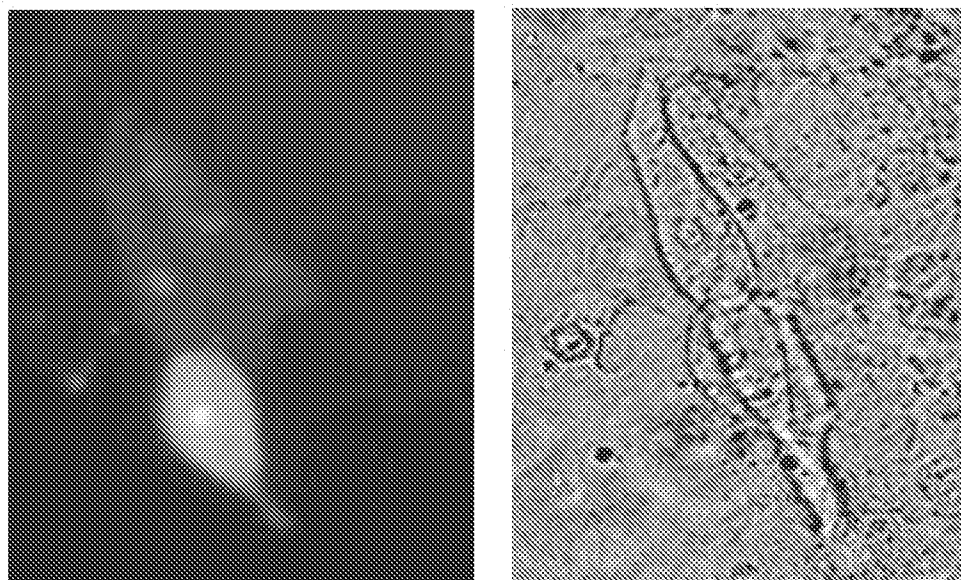


FIGURE 45

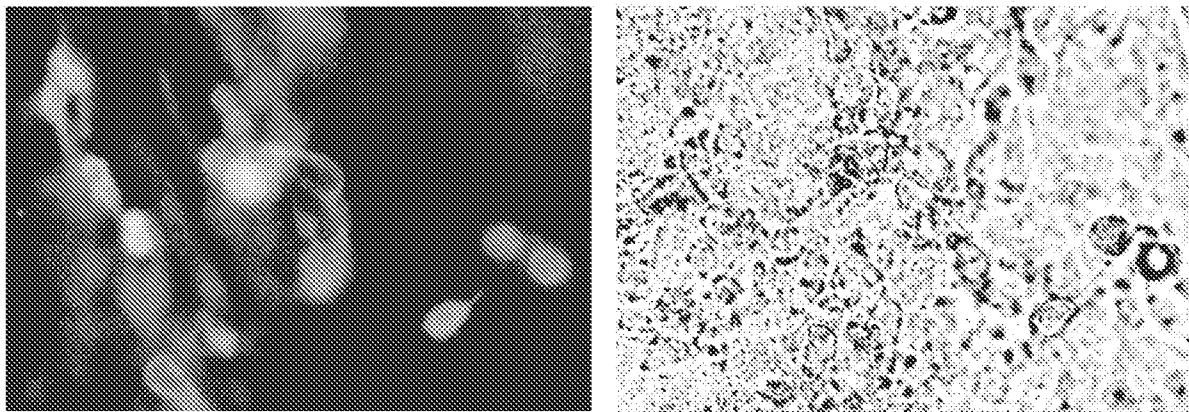


FIGURE 46

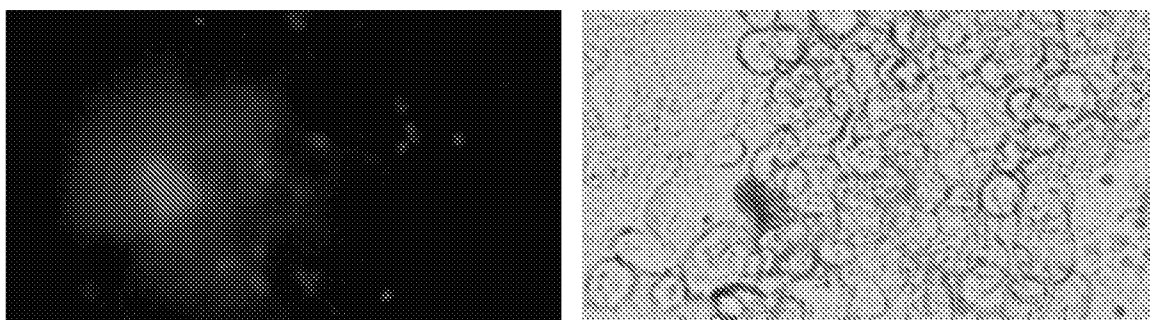
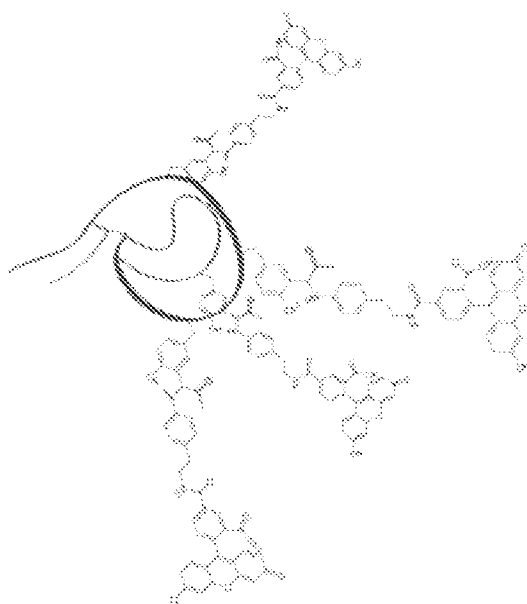


FIGURE 47

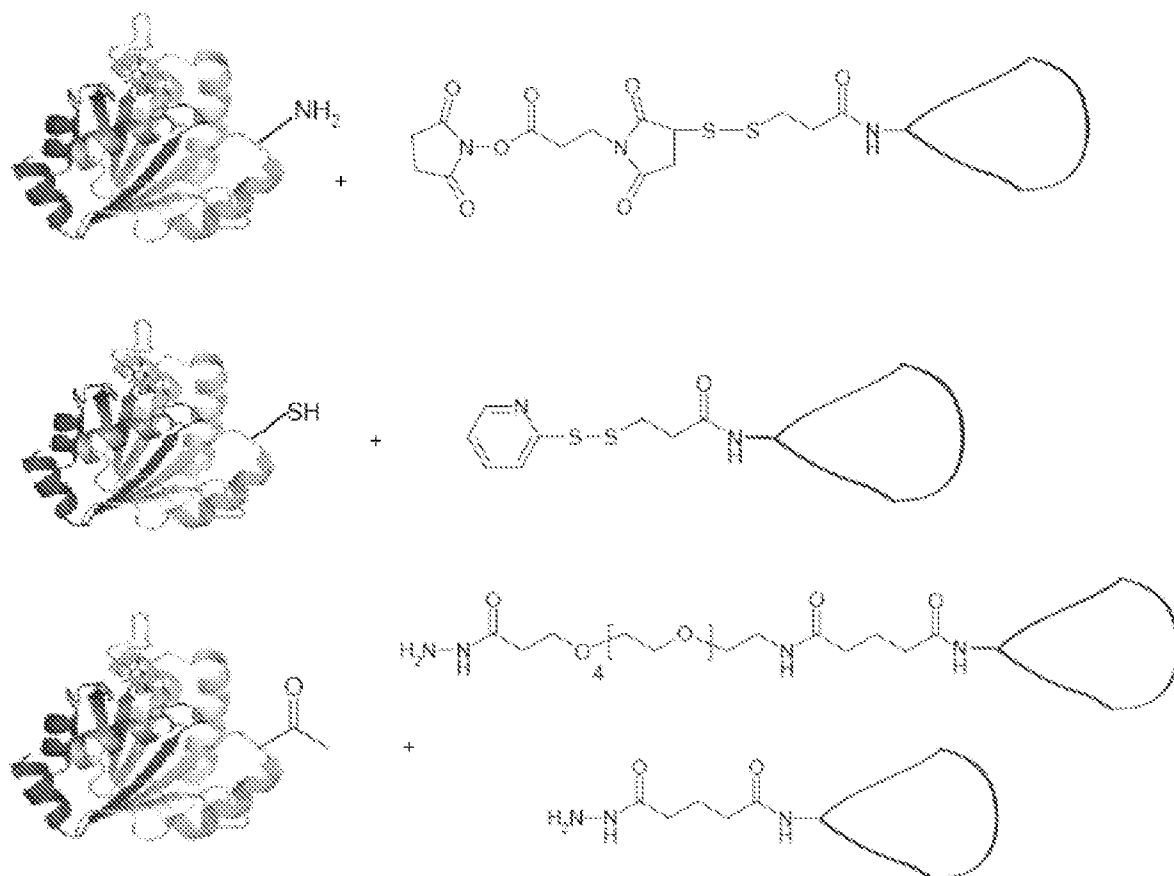
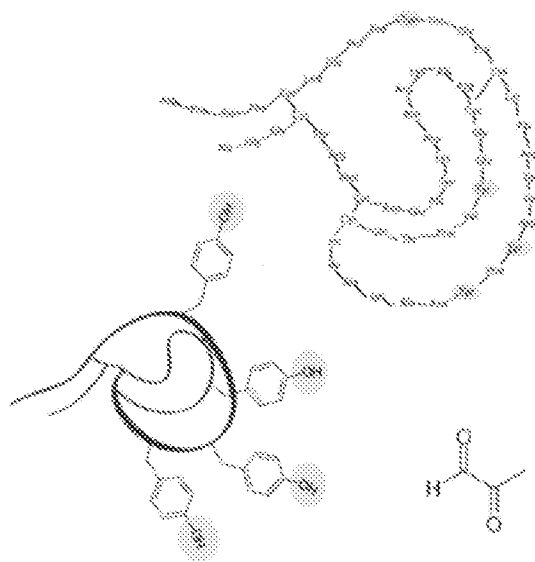


FIGURE 48

## Vaccine Development

### Protein Delivery

Serine Protease Inhibitor: Aprotinin



### Blocking Virus Reproduction

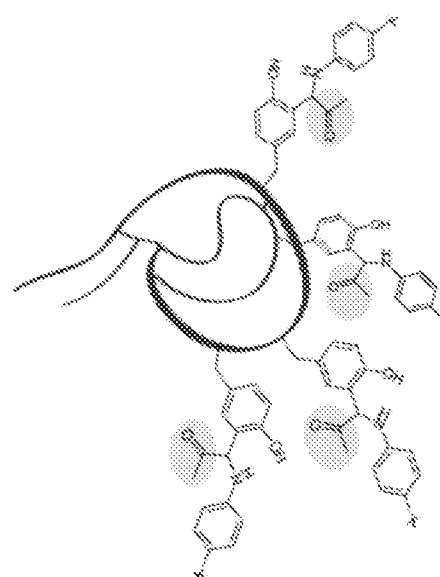


FIGURE 49

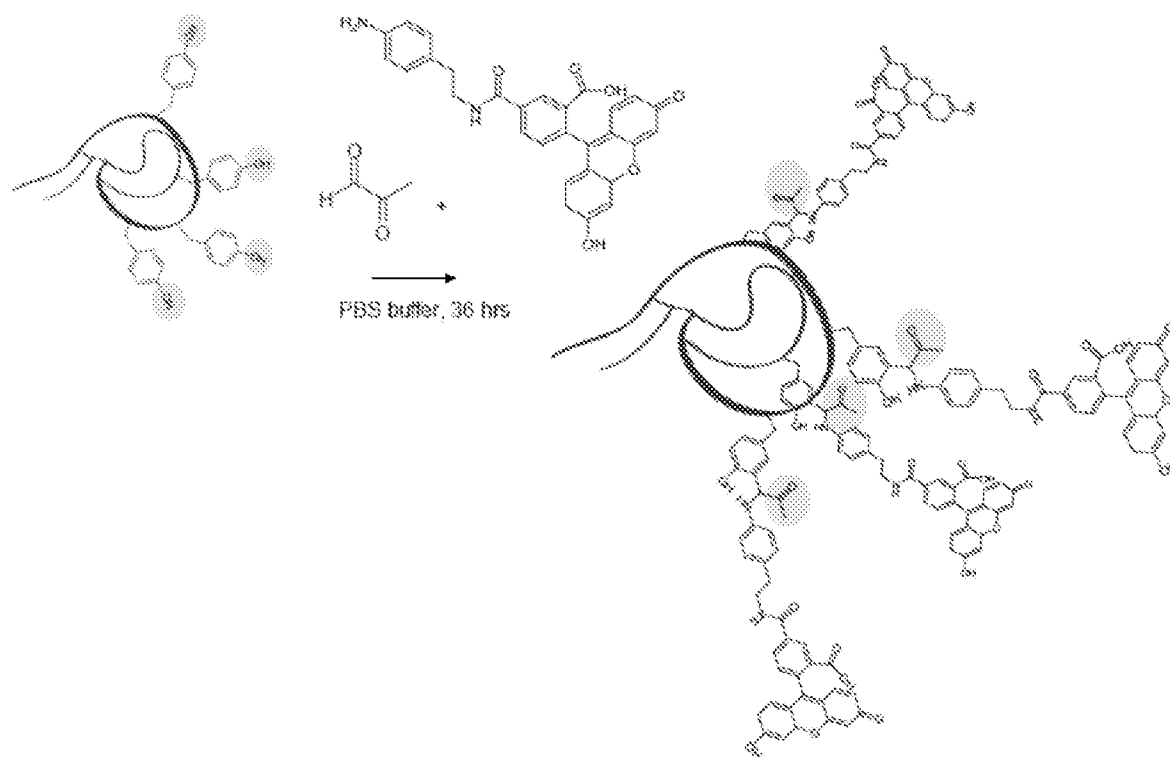


FIGURE 50

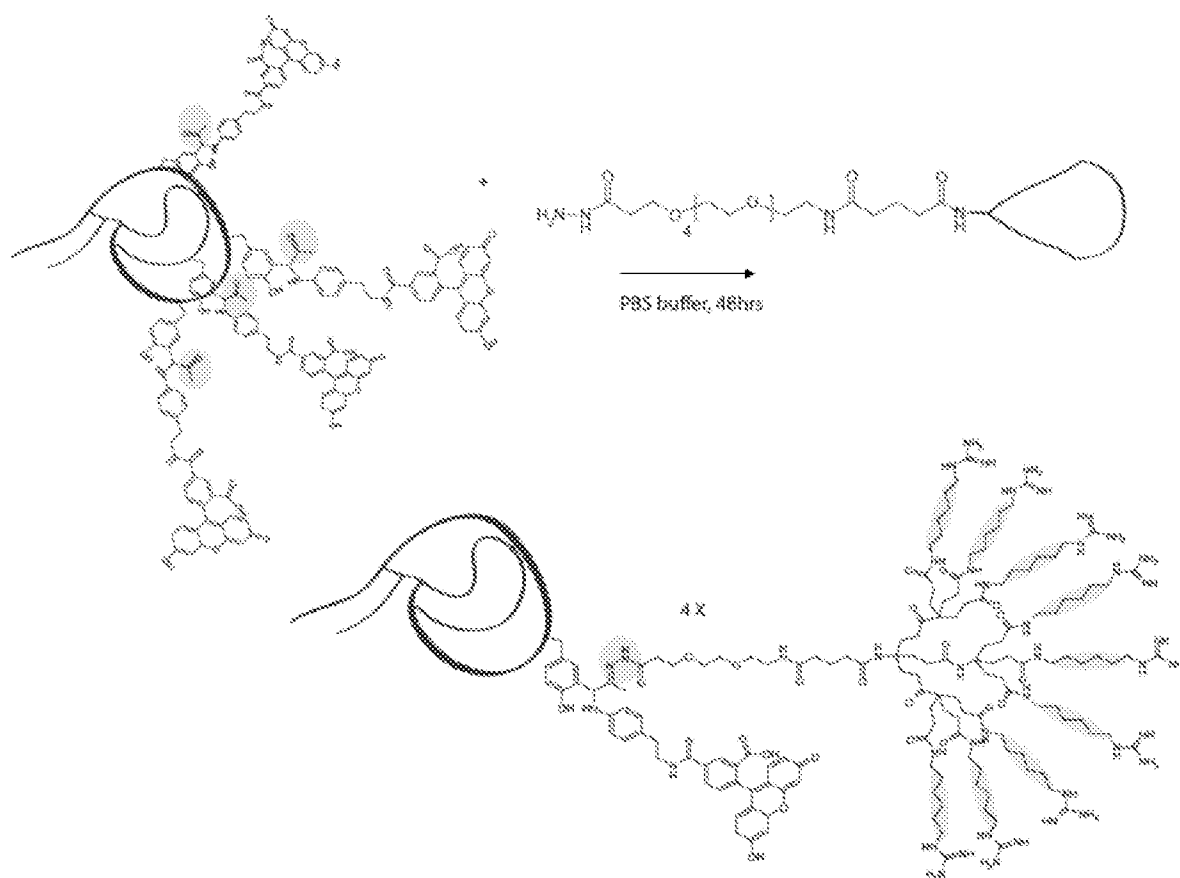


FIGURE 51

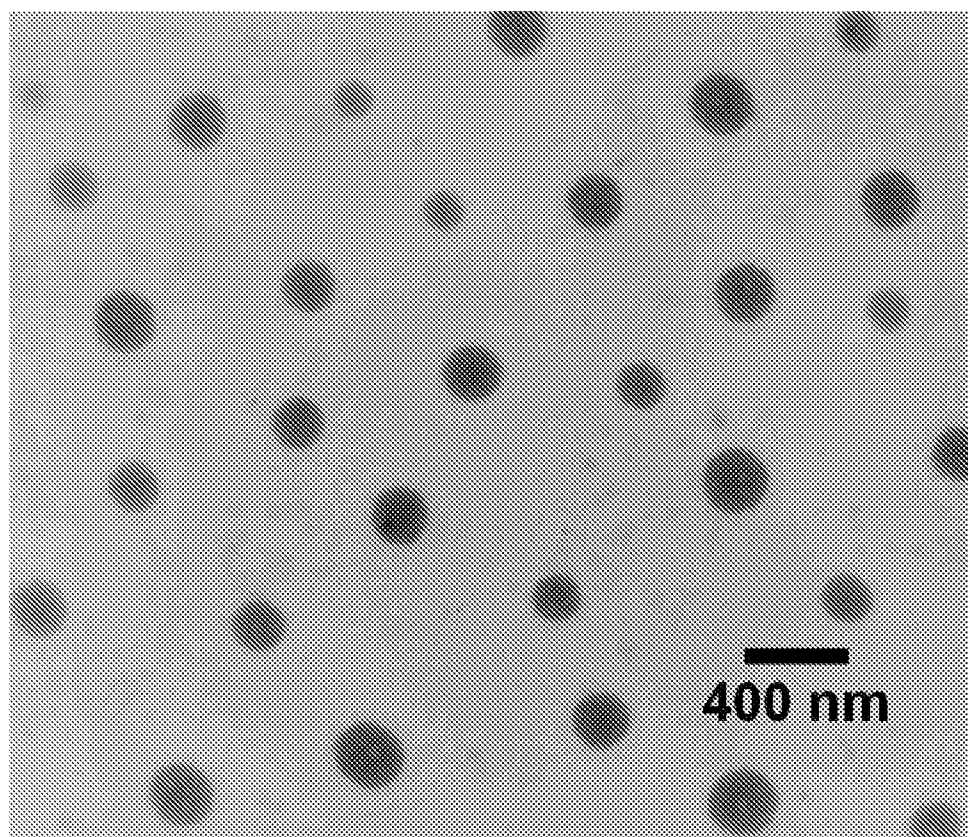


FIGURE 52

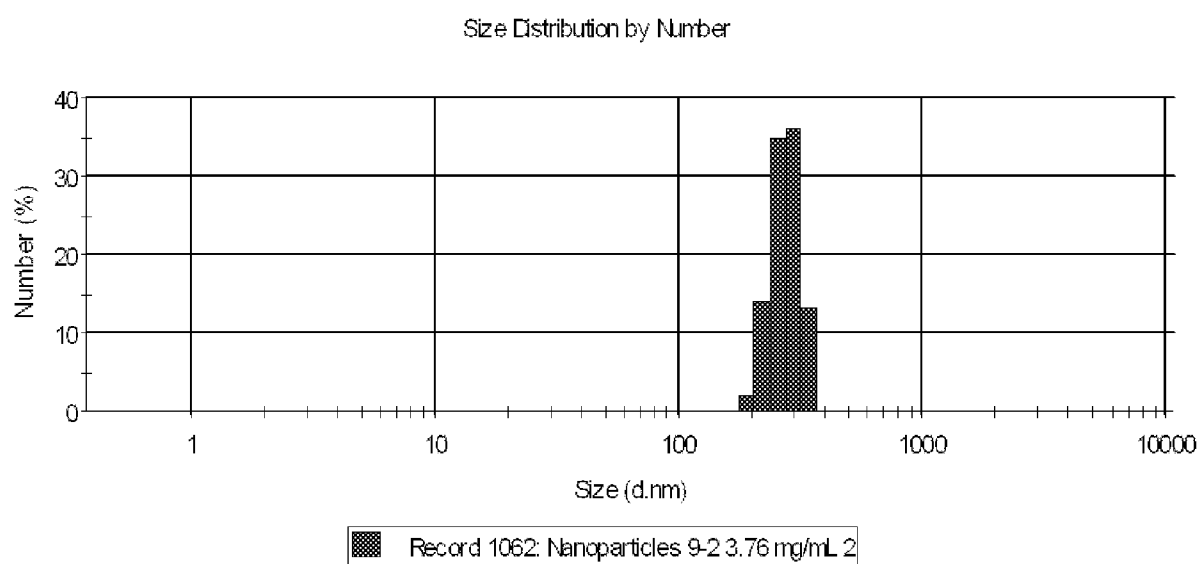


FIGURE 53

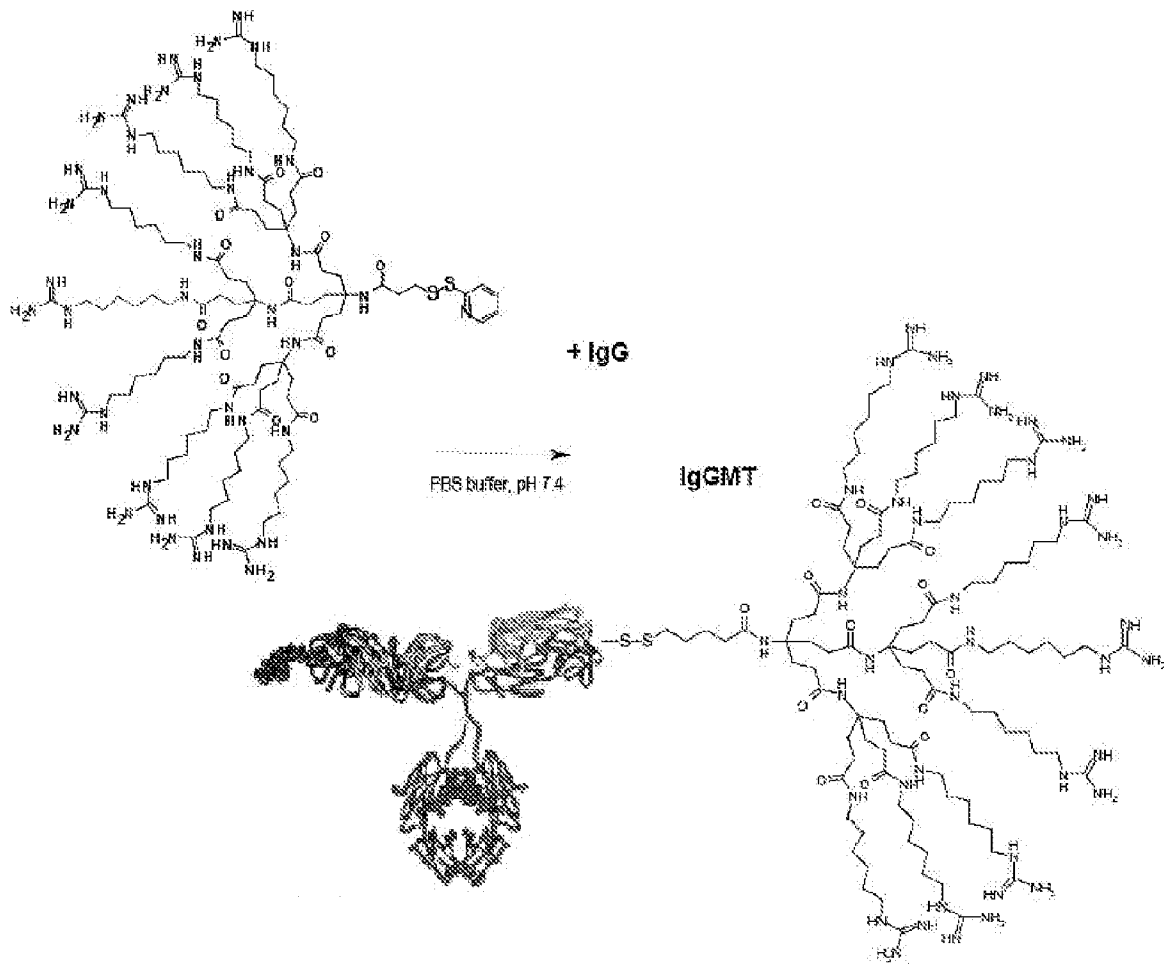


FIGURE 54

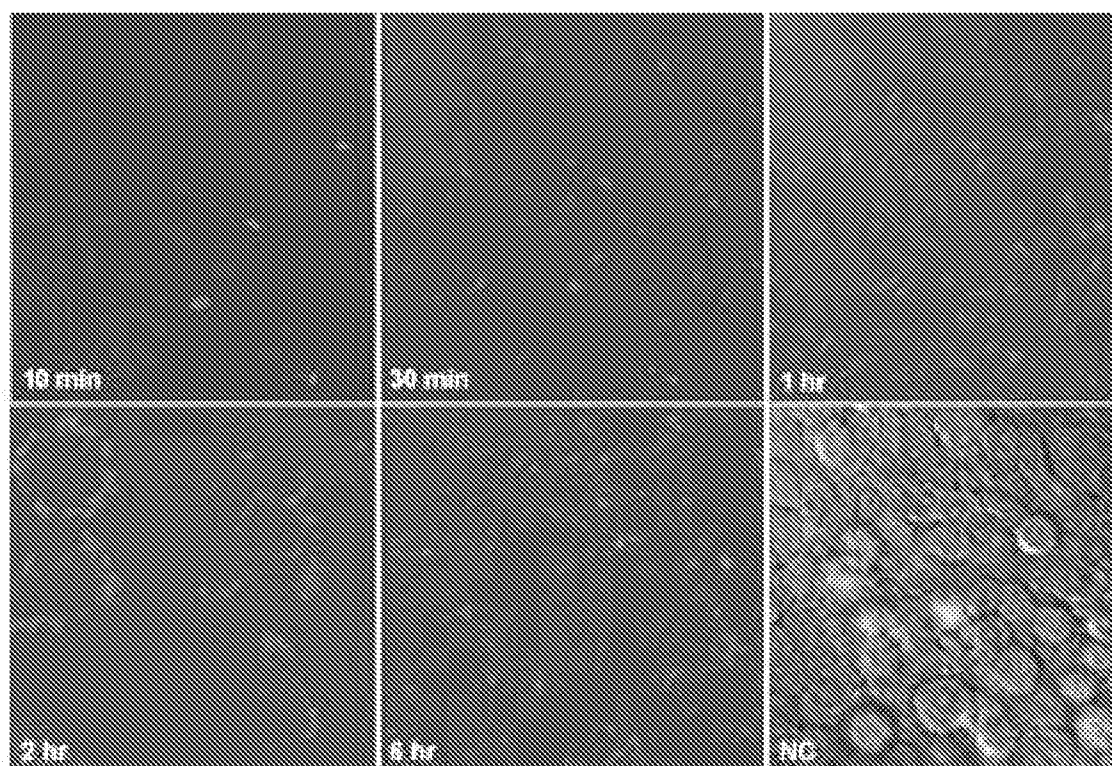
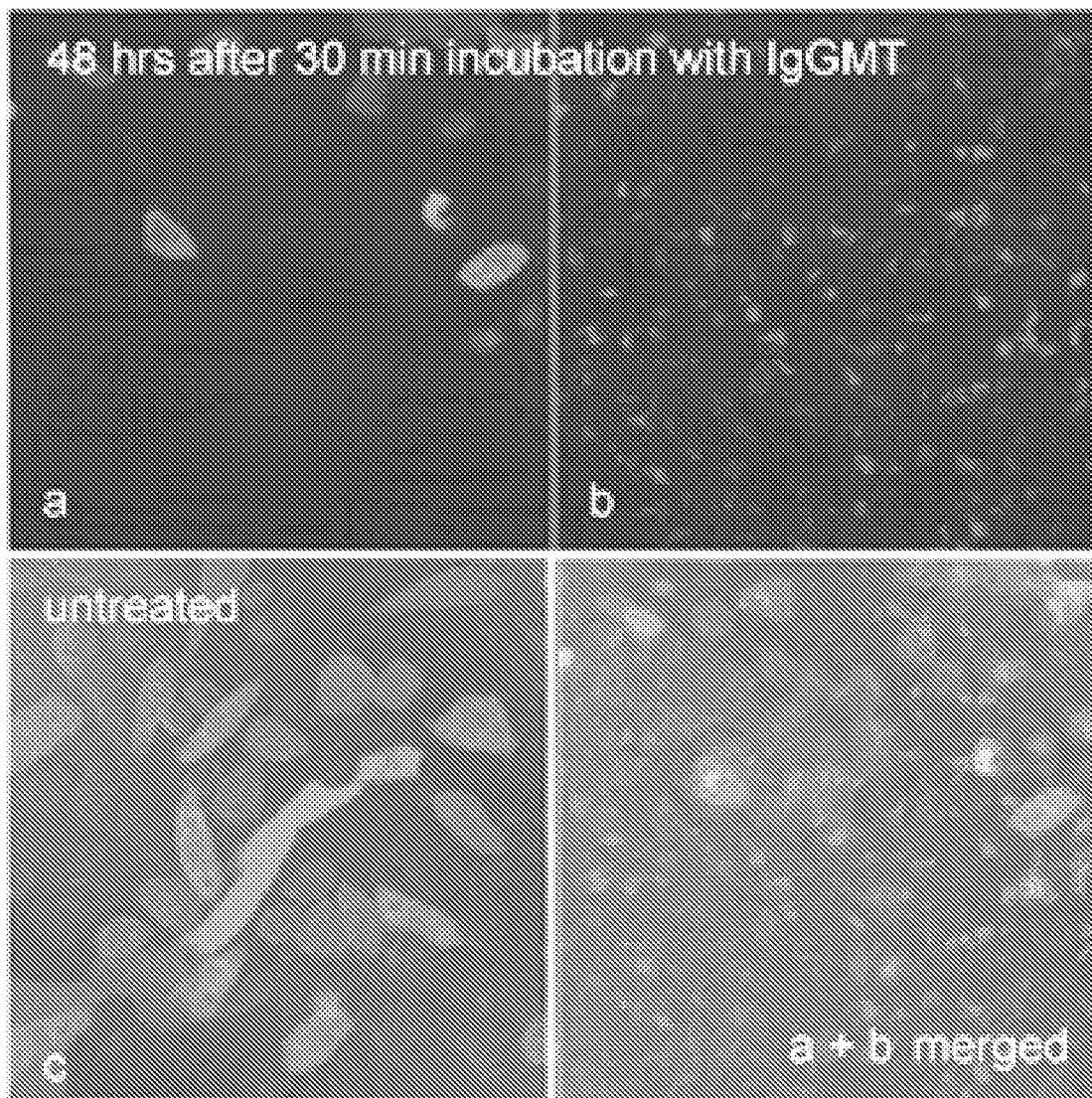


FIGURE 55

**FIGURE 56**

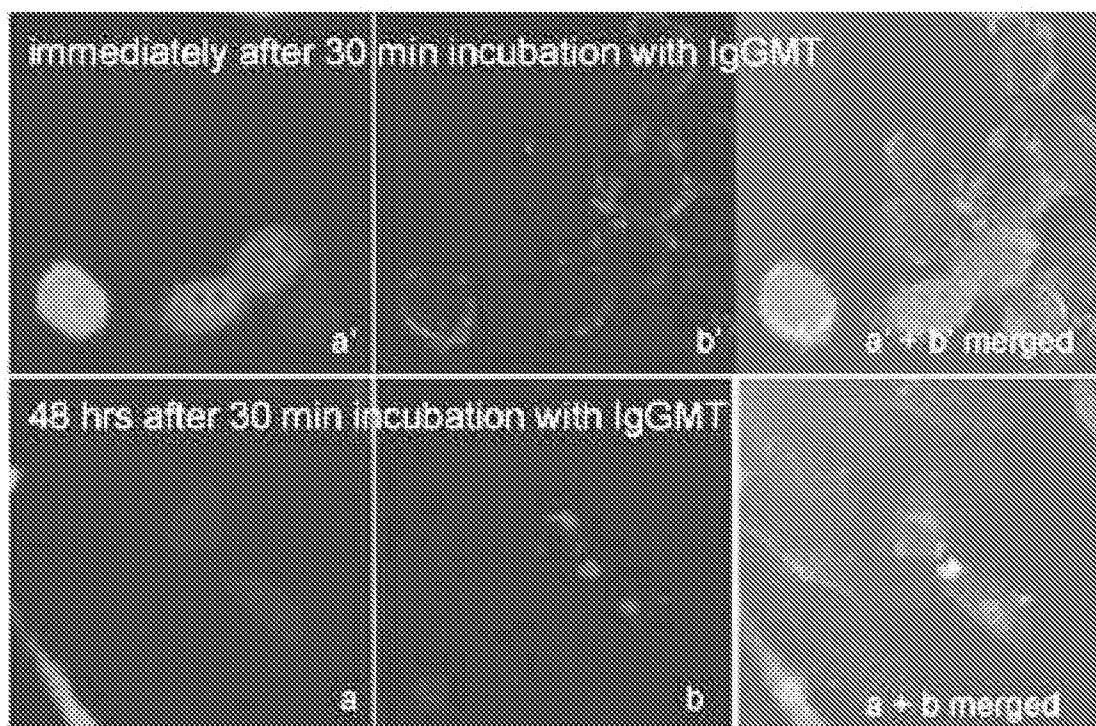
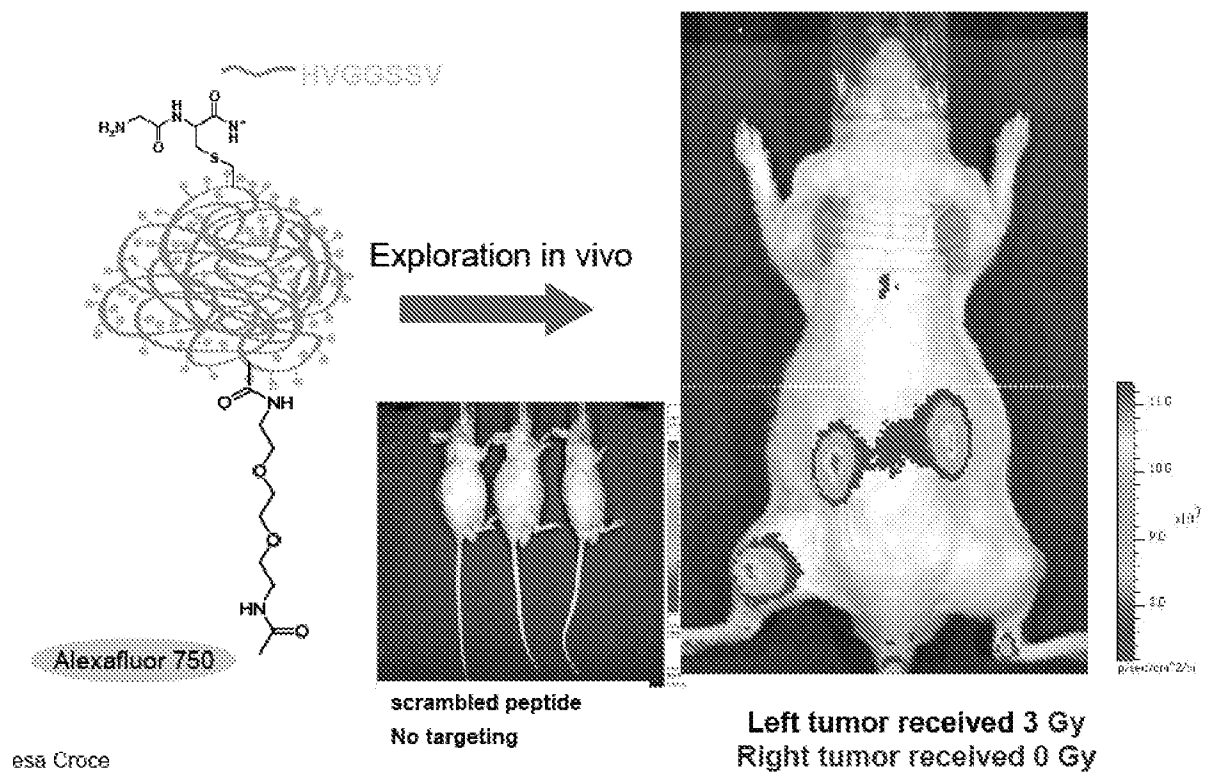
**FIGURE 57**

**FIGURE 58**

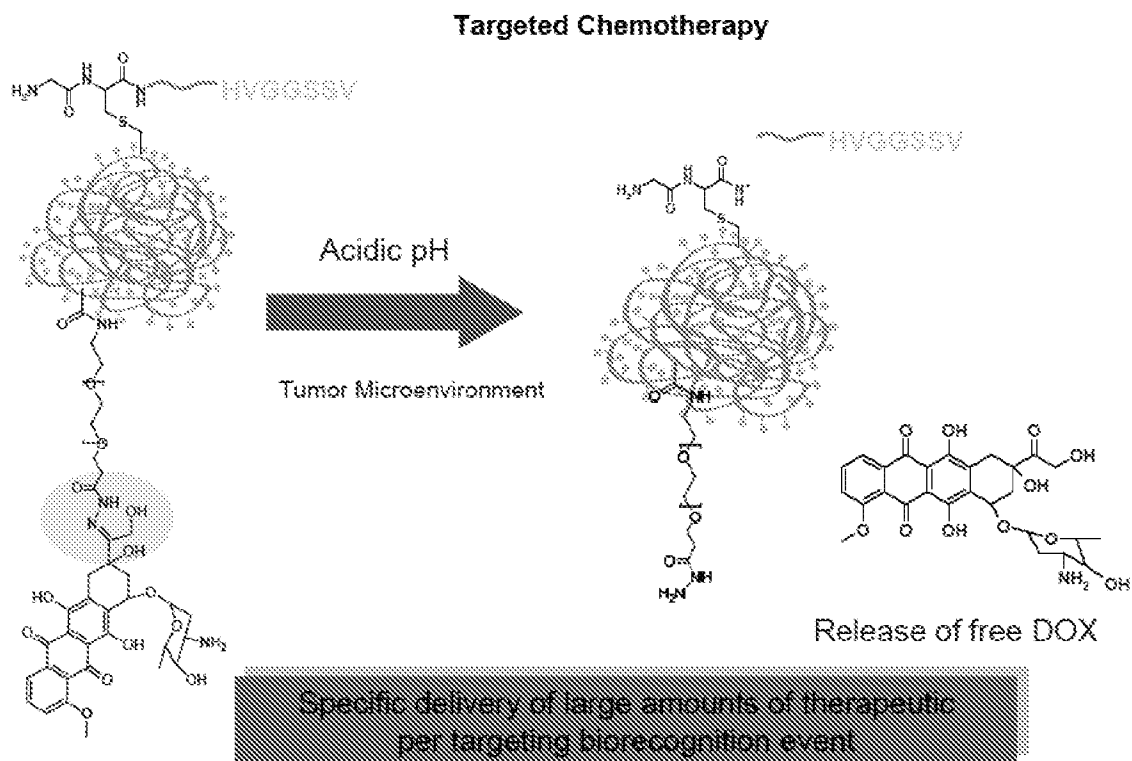


FIGURE 59

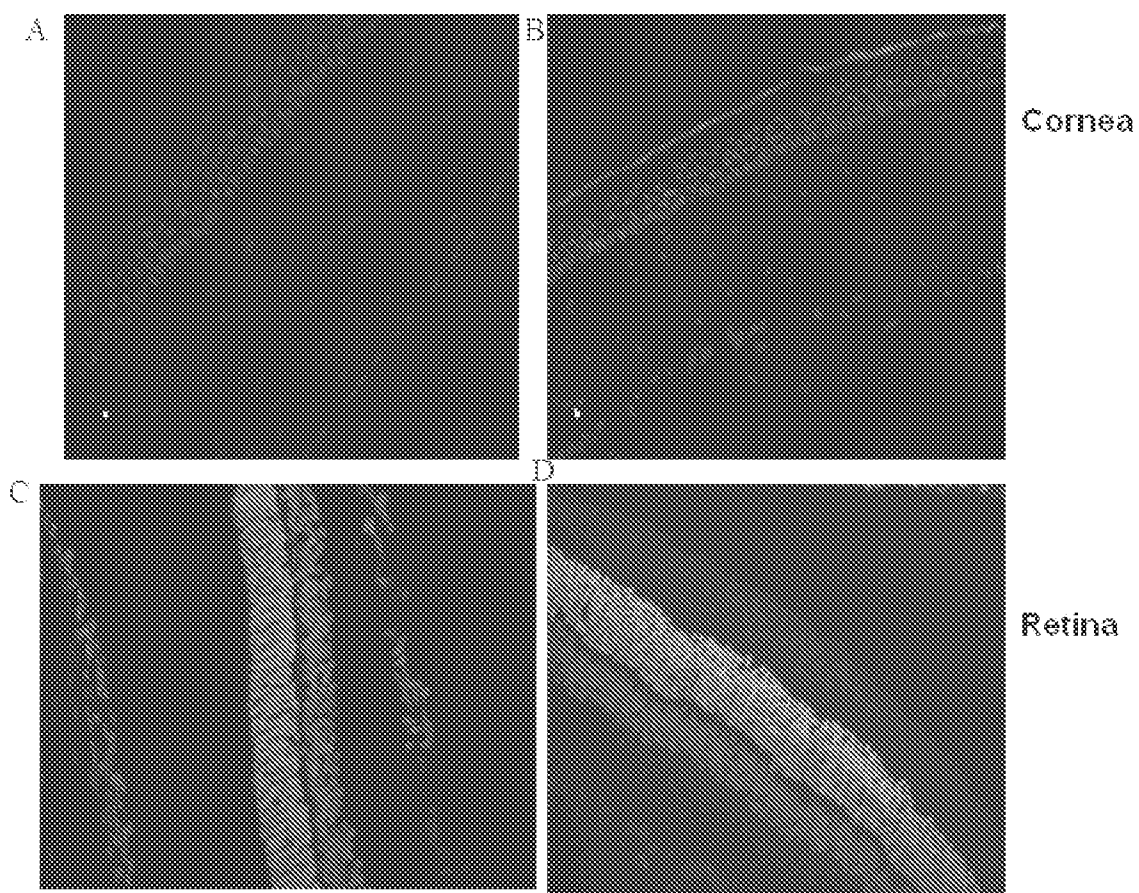


FIGURE 60