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(54) Title: RADIANT STAR NANOPARTICLE PRODRUGS AND RELATED METHODS

a. Synthesis of radiant star nanoparticles

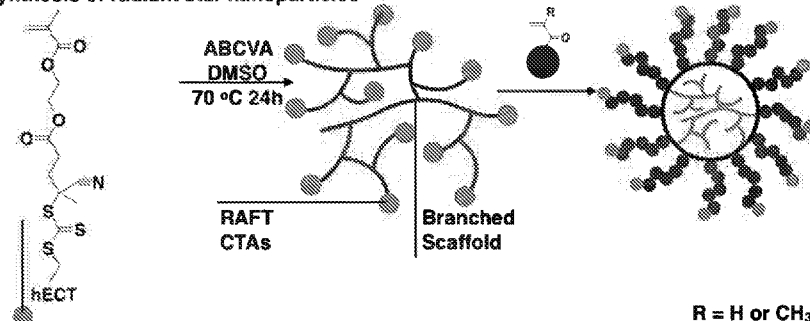


Fig. 1A

(57) Abstract: Radiant star nanoparticle prodrugs, methods for making the nanoparticle prodrugs, and methods for using the nanoparticle prodrugs for the delivery of therapeutic agents.

Declarations under Rule 4.17:

- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
- *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*
- *as to non-prejudicial disclosures or exceptions to lack of novelty (Rule 4.17(v))*

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RADIANT STAR NANOPARTICLE PRODRUGS AND RELATED METHODS

CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit of US Application No. 62/629,815, filed
5 February 13, 2018, expressly incorporated herein by reference in its entirety.

STATEMENT OF GOVERNMENT LICENSE RIGHTS

This invention was made with government support under Grant
No. HDTRA-13-1-0047 DTR awarded by the Defense Threat Reduction Agency. The
government has certain rights in the invention.

10 BACKGROUND OF THE INVENTION

Polymeric prodrugs are a strategy for overcoming the limitations typically
associated with physically encapsulating drug delivery systems. These prodrugs consist
of therapeutic agents that have been covalently conjugated to a macromolecular scaffold
via a hydrolytic or enzymatically degradable linkage. This strategy has been shown to
15 substantially increase the solubility and stability of the parent drug while also enhancing
drug circulation half-lives and reducing immunogenicity. An attractive route for the
synthesis of polymeric prodrugs is the direct reversible deactivation radical
polymerization (RDRP) of therapeutic agents that have been reversibly modified with
suitable vinyl functionality. These polymerizable prodrug monomers (PPMs) allow for
20 the facile incorporation of drug moieties into the final polymer at predetermined ratios
without the need for additional conjugation and purification steps.

Recently, the synthesis of polymeric prodrugs using PPMs based on derivatives of
the antibiotic ciprofloxacin has been reported. Drug release studies conducted in human
serum showed that the phenyl ester-linked antibiotic was cleaved from the polymer
25 scaffold at higher rates relative to the aliphatic ester-linked drug. These differences in the
relative antibiotic release rates were found to strongly influence the antimicrobial activity
of the polymeric prodrugs with ciprofloxacin linked via phenyl esters showing
significantly lower minimum inhibitory concentrations than the aliphatic ester linked
prodrugs. Subsequent animal studies confirmed the therapeutic effectiveness of the this
30 system with the phenyl ester-linked polymeric prodrugs showing high cure efficiencies in
completely lethal murine *Francisella tularensis* subsp. *novacida* pulmonary challenge
models, while mice treated with free ciprofloxacin succumbed to the bacterial infection
within a few days of exposure.

Diblock copolymer-based prodrugs that self-assemble under physiological conditions to form nanoparticles are desirable from a drug delivery perspective, as they can contain the covalently linked prodrugs at a significant fraction of their total mass. These systems also typically show longer in vivo circulation times relative to analogous
5 linear polymers and can provide preferential uptake in some cell populations, such as macrophages, because of their size and ability to display multivalent receptor-specific targeting ligands.

Despite these advantages, diblock copolymer based nanoparticles, where the covalently linked prodrugs are localized in the hydrophobic core, typically show low
10 rates of drug release. Additionally, self-assembled structures such as micelles and liposomes can interact with serum proteins in vivo resulting in partial or complete disassociation of the constituent polymer or lipids. Interaction of these structures with serum proteins can also cause them to be eliminated by the mononuclear phagocytic system or, in the case of encapsulation-based drug delivery, result in extraction of the
15 physically bound drugs.

The incorporation of PPMs into advanced polymer nanostructures has the potential to provide further enhancements in therapeutic activity while substantially reducing the cost and complexity of preparing multifunctional drug delivery systems. To date a variety of sophisticated polymeric architectures have been prepared by RAFT
20 polymerization methodology including stars, brushes, brushed-brushes, and bottlebrushes. Hyperbranched polymers have also been investigated for drug delivery applications. Hyperbranched polymers can be conveniently prepared by nitroxide-mediated polymerization (NMP) and atom transfer radical polymerization (ATRP) via the use of vinyl-functionalized initiators (inimers) or by RAFT using vinyl-functionalized
25 CTAs (transmers).

Hyperbranched polymers synthesized with inimers/transmers typically have larger molar mass dispersities. However, their constituent segments are still somewhat controlled. In this approach the degree of branching can be manipulated by simple adjustment of the inimer/transmer to monomer ratio and overall monomer conversion.
30 For example, transmers have been employed to prepare architecturally distinct antigen carriers with pH-responsive endosomal-releasing segments. In these studies, dendritically branched copolymers were synthesized using a methacrylate-functionalized RAFT CTA. Antigen delivery with the hyperbranched and cross-linked polymer

architecture enhanced in vitro MHC-I antigen presentation relative to free antigen, whereas the linear construct did not have a discernible effect.

There have been few literature reports detailing the homopolymerization of transmers. Homopolymerization of transmers using thermal initiators generally yield
5 hyperbranched polymers with relatively low molecular weights. In contrast, concurrent ATRP/RAFT polymerization of transmers have been shown to yield hyperbranched polymers over 500 kDa. Unimolecular hyperstar polymer for siRNA delivery have also been synthesized by homopolymerization of ATRP inimers in microemulsion followed by solution polymerization of DMAEMA from the multifunctional core. The resultant
10 hyperstar-siRNA complexes showed in vitro transfection efficiencies higher than the Lipofectamine control.

Despite the advances in the development of polymeric prodrugs, a need exists for new polymeric prodrug architectures for the safe, efficient, and effective delivery of therapeutic agents. The present invention seeks to fulfill this need and provides further
15 related advantages.

SUMMARY OF THE INVENTION

This invention relates to radiant star nanoparticle prodrugs, methods for making the nanoparticle prodrugs, and methods for using the nanoparticle prodrugs for the delivery of therapeutic agents.

20 In one aspect, the invention provides a radiant star nanoparticle prodrug useful for the delivery of therapeutic agents. In one embodiment, the invention provides a radiant star nanoparticle comprising (a) a hyperbranched polymeric core comprising a homopolymerized transmer product, wherein the core comprises polymeric branches covalently attached to a polymer backbone at branch points, and wherein at least a
25 portion of the polymeric branches comprise degradable groups; and (b) a plurality of polymeric arms extending from and covalently attached to the core, wherein the polymeric arms comprises repeating units, wherein at least a portion of the repeating units include therapeutic agent moieties, and wherein the portion of repeating units that include therapeutic moieties include degradable groups effective to release a therapeutic agent
30 from the nanoparticle.

In another aspect, the invention provides methods for making the radiant star nanoparticle prodrug. In one embodiment, the method for making a radiant star nanoparticle having a hyperbranched polymeric core and a plurality of polymeric arms

extending from and covalently coupled to the core, comprises (a) polymerizing one or more transmers to provide a hyperbranched polymeric core in which at least portions of the core terminate with a charge transfer agent, wherein the transmer comprises a degradable group intermediate a polymerizable group and a chain transfer agent; and
5 (b) reverse addition fragment transfer (RAFT) polymerizing a prodrug monomer from the polymeric core to provide polymeric arms extending from and covalently coupled to the core.

In a further aspect, the invention provides a composition that includes the radiant star nanoparticle of the invention and a pharmaceutically acceptable carrier or diluent.

10 In another aspect of the invention, a method for intracellular delivery of a therapeutic agent is provided. In certain embodiments, the method comprises contacting a cell with the radiant star nanoparticle of the invention, which ultimately results in release of the therapeutic agent into the cell.

In a further aspect, the present invention provides a method for treating or
15 preventing or treating a disease, disorder, or condition treatable by a specific therapeutic agent. In certain embodiments, the method comprises administering to subject in need thereof an effective amount of the radiant star nanoparticle of the invention, wherein the therapeutic agent released from the radiant star nanoparticle is the specific therapeutic agent effective to treat or prevent the disease, disorder, or condition treatable by the
20 therapeutic agent released from the nanoparticle.

DESCRIPTION OF THE DRAWINGS

The foregoing aspects and many of the attendant advantages of this invention will become more readily appreciated as the same become better understood by reference to the following detailed description, when taken in conjunction with the accompanying
25 drawings.

FIGURE 1A is a schematic illustration of the preparation of a representative radiant star nanoparticle prodrug of the invention prepared from an alkyl ester linked transmer FIGURE 1B is a schematic illustration of the preparation of a representative radiant star nanoparticle prodrug of the invention prepared from a pH-sensitive acetal
30 linked transmer (starburst nanoparticle).

FIGURE 2B is the ^1H NMR spectrum in d_6 -benzene for the acetal linked RAFT transmer aECT.

FIGURE 2A presents the synthetic schemes for the synthesis of alkyl ester (hECT) and acetal linked (aECT) RAFT transmers.

FIGURE 2B is the ^1H NMR spectrum in d_6 -benzene for the acetal linked RAFT transmer aECT.

5 FIGURES 3A-3D illustrate the ^1H NMR spectra (3A and 3B) and molecular weight distributions (RI response as a function of elution volume) (3C and 3D) for the poly(hECT) (3A and 3C) and poly(aECT) (3B and 3D) hyperbranched transmer cores synthesized via homopolymerization of hECT and aECT.

10 FIGURES 4A-4G compare molecular weight distributions (RI response as a function of elution volume) for polymeric transmer cores as well as the corresponding RSN for dimethyl acrylamide (DMA) from p(hECT) (4A), DMA from p(aECT) (4B), DMA from p(hECT) targeting multiple degrees of polymerization (4C), N,N-dimethylaminoethyl methacrylate (DMAEMA) (4D), 2-hydroxyethyl methacrylate (HEMA) (4E), polyethyleneglycol monomethyl ether methacrylate (FW about 300 Da) (O300) (4F), and polyethyleneglycol monomethyl ether methacrylate (FW about 950 Da) (O950) (4G). Molecular weight, molar mass dispersity ($\bar{M}_w/\bar{M}_n$), and % monomer conversion values were determined to be: (poly(hECT) 8700:1.42:99.9, poly(aECT) 9300:1.43:99.9, DMA (DP200) poly(hECT) 364,000:1.32:85%, DMA poly(aECT) (DP200) 275 000:1.36:79%, DMAEMA (DP150) 186 300:1.39:48%, HEMA(DP150) 401 20 400:1.57:63%, O300 (DP80) 924,000:3.40:79%, O950 (DP25) 417,600:2.30:71%.

FIGURE 5A shows the TEM analysis of poly(mono-2-(methacryloyloxy)ethyl succinate) radiant star nanoparticles prepared from poly(hECT) core targeting a DP of 200.

25 FIGURES 5B and 5C compare molecular weight distributions (RI response as a function of time) for poly(DMA) polymerized from poly(hECT) (5B) and poly(aECT) (5C) transmer cores following incubation in pH 5.0 acetate buffer at 37 °C.

FIGURE 6 shows the synthetic scheme for the preparation of a representative mannose targeted RSN prodrug of the invention via direct RAFT copolymerization of glycan functionalize monomer (Man) with the phenyl ester linked ciprofloxacin monomer (CTM) from polymeric transmer cores (top panel), and the representative mannose 30 targeted RSN prodrug being internalized by alveolar macrophages following binding to the mannose receptor (lower panel).

FIGURES 7A-7D compare characteristics of representative radiant star nanoparticle prodrugs of the invention: poly(MEM-co-CTM) and poly(DMA-co-CTM). ¹H NMR spectra in d₆ DMSO for poly(MEM-co-CTM) (7A) and poly(DMA-co-CTM) (7B) as well as the corresponding molecular weight distributions (RI response as a function of time) (7C and 7D, respectively). Molecular weight, molar mass dispersity ($\bar{M}_w/\bar{M}_n$) values were determined to be 490,000/1.38 and 452,000/1.43 respectively.

FIGURE 8 compares ciprofloxacin release from polymers incubated in 100 % human serum at 37 °C. Representative radiant star nanoparticles of the invention containing CTM copolymerized with DMA (open circles) show significantly faster ciprofloxacin release kinetic relative to diblock copolymer micelles with CTM core segments (solid black circles) but somewhat slower than soluble copolymers with DMA and mannose (solid light and darker circles).

FIGURES 9A-9D compare flow cytometry results for studies conducted in IL4 transformed RAW264.7 cells comparing the cell binding properties of representative mannose-targeted and untargeted RSN prodrugs as well as the linear controls.

FIGURES 9E-9H compare pulmonary toxicity of a representative poly(man-co-CTM) RSN prodrug of the invention endotracheally delivered dosed at 20 mg/kg ciprofloxacin every 24 h over three consecutive days

DETAILED DESCRIPTION OF THE INVENTION

The invention provides radiant star nanoparticle prodrugs, methods for making the nanoparticle prodrugs, and methods for using the nanoparticle prodrugs for the delivery of therapeutic agents.

Radiant Star Nanoparticles and Methods for their Preparation

In one aspect, the invention provides a radiant star nanoparticle prodrug useful for the delivery of therapeutic agents.

In one embodiment, the invention provides a radiant star nanoparticle comprising (a) a hyperbranched polymeric core comprising a homopolymerized transmer product, wherein the core comprises polymeric branches covalently attached to a polymer backbone at branch points, and wherein at least a portion of the polymeric branches comprise degradable groups; and (b) a plurality of polymeric arms extending from and covalently attached to the core, wherein the polymeric arms comprises repeating units, wherein at least a portion of the repeating units include therapeutic agent moieties, and

wherein the portion of repeating units that include therapeutic moieties include degradable groups effective to release a therapeutic agent from the nanoparticle.

As used herein, a "radiant star nanoparticle" refers to a macromolecule containing a branched constitutional unit from which a plurality of polymeric chains (or polymeric arms) emanate. The branched constitutional unit is the core of the nanoparticle and the plurality of polymeric chains or polymeric arms emanating (radiating) from the nanoparticle core are covalently attached (i.e., formed by polymerization from) to the core.

The core of the radiant star nanoparticle is a highly branched polymeric unit (e.g., hyperbranched polymeric core). As used herein, the term "branched" refers to a polymeric branch covalently attached to a polymer backbone at a branch point. The term "branched polymeric unit" refers to a polymeric unit with at least one branch point intermediate the boundary units (i.e., the end-groups or other branch points). The term "branch point" refers to a point on a chain at which a branch is attached. The term "branch" refers to an oligomeric or polymeric offshoot or extension from a chain (e.g., backbone).

As used herein, the term "hyperbranched polymeric core" refers to a branched polymeric core that is the product of transmer homopolymerization (i.e., a homopolymerized transmer product).

The radiant star nanoparticle has a core that is the product of homopolymerization of a transmer, as described herein. The transmer homopolymerization product is a branched polymeric unit (i.e., a highly branched or hyperbranched polymeric unit). The core includes polymeric branches covalently attached to a polymer backbone at branch points. The core is advantageously degradable having at least a portion of the polymeric branches that include degradable groups. In certain embodiments, each polymeric branch includes a degradable group. Suitable degradable groups are described below. In certain embodiments, the degradable group is selected from alkyl ester, acetal, hemiacetal, phenyl ester, thioester, disulfide, and enzyme cleavable peptide groups.

The molecular weight (number-average (M_n)) of the core of the radiant star nanoparticle may vary. In certain embodiments, the core has a number-average (M_n) from about 5000 to about 20,000 Da. In other embodiments, the core has a number-average (M_n) from about 7500 to about 15,000 Da. In further embodiments, the core has a number-average (M_n) from about 5000 to about 10,000 Da.

As used herein, the terms "plurality of polymeric chains" and "plurality of polymeric arms" refers to the number of polymeric chains or arms radiating from the nanoparticle core. The terms "polymeric chains" and "polymeric arms" are used herein interchangeably. In certain embodiments, the radiant star nanoparticle has from about
5 10 to about 100 polymeric arms extending from the core. In other embodiments, the radiant star nanoparticle has from about 20 to about 50 polymeric arms extending from the core. In further embodiments, the radiant star nanoparticle has from about 25 to about 40 polymeric arms extending from the core.

The polymeric arms are prepared by polymerization of a polymerizable prodrug
10 monomer, or copolymerizing a polymerizable prodrug monomer and a comonomer, from the nanoparticle core. The polymeric arms are linear polymers or linear copolymers. The polymeric arms can be homopolymers, random copolymers, block copolymers, or gradient block copolymers (e.g., each arm of a nanoparticle is a homopolymer, each arm of a nanoparticle is a random copolymer, each arm of a nanoparticle is a block
15 copolymer). Each polymeric arm can include from about 10 to about 500 repeating units. Representative polymeric arms have number-average molecular weights less than about 30 kDa, for example, from about 5 to about 30 kDa, or from about 10 to about 25 kDa.

Each repeating unit of the polymeric arm that includes a therapeutic agent moiety has a therapeutic agent moiety pendant from the backbone of the polymeric arm, and a
20 degradable group intermediate the backbone and the therapeutic agent moiety. Suitable degradable groups are described below. In certain embodiments, the degradable group is selected from alkyl ester, phenyl ester, acetal, and hemiacetal groups.

In certain embodiments of the radiant star nanoparticle, each polymeric arm further includes a plurality of repeating units, where each of these repeating units includes
25 a functional group effective to increase the water solubility of the nanoparticle. Suitable functional groups effective to increase the water solubility are described below. These embodiments are particularly useful when the therapeutic drug to be released from the nanoparticle is not highly water soluble.

In other embodiments of the radiant star nanoparticle, each polymeric arm further
30 includes a plurality of repeating units, where each of these repeating units includes a targeting group effective to accumulate the nanoparticle at a pre-determined site. Suitable targeting groups effective to accumulate the nanoparticle at a pre-determined site include ligands that target their cell surface receptors.

In further embodiments, each polymeric arm of the nanoparticle include repeating units that include functional groups effective to increase water solubility and repeating units that include targeting groups.

In certain embodiments, each polymer arm includes only repeating units that
5 include a therapeutic moiety.

In certain embodiments, the polymeric arms comprises repeating units, wherein each repeating unit includes a therapeutic agent moiety, and each repeating unit includes a degradable group effective to release the therapeutic agent from the nanoparticle.

The molecular weight (number-average (M_n)) and molar mass dispersity ($\mathcal{D}$) of
10 the radiant star nanoparticle may also vary. In certain embodiments, the nanoparticle has a number-average (M_n) molecular weight from about 0.3 to about 1.5 million Da. In other embodiments, the nanoparticle has a number-average (M_n) molecular weight from about 0.5 to about 1.0 million kDa. Dispersities ($\mathcal{D}$) for the nanoparticle may vary from about 1.3 to about 2.0. In certain embodiments, the molar mass dispersity is about 1.5.

15 In certain embodiments, the nanoparticle of has a hydrodynamic diameter from about 20 to about 50 nm, as measured by dynamic light scattering.

In another aspect, the invention provides methods for making the radiant star nanoparticle prodrug.

In one embodiment, the method for making a radiant star nanoparticle having a
20 hyperbranched polymeric core and a plurality of polymeric arms extending from and covalently coupled to the core, comprises:

(a) polymerizing one or more transmers to provide a hyperbranched polymeric core in which at least portions of the core terminate with a charge transfer agent, wherein the transmer comprises a degradable group intermediate a polymerizable
25 group and a chain transfer agent; and

(b) reverse addition fragment transfer (RAFT) polymerizing a prodrug monomer from the polymeric core to provide polymeric arms extending from and covalently coupled to the core.

The method for making the radiant star nanoparticle includes two steps. In the
30 first step, one or more transmers (e.g., a transmer) is polymerized to provide a hyperbranched polymeric core. In this step, the transmer is polymerized in the absence of a comonomer (i.e., transmer homopolymerization). The homopolymerization is responsible for the formation of a hyperbranched polymeric core having advantageous

dispersity and a molecular weight falling within the range from about 5000 to about 20,000 Da (e.g., about 5000 Da, about 7500 Da, about 10,000 Da, about 15,000 Da, about 20,000Da).

5 The hyperbranched cores serves as the scaffold from which the arms are polymerized. In the above method, the hyperbranched core includes portions that terminate with a charge transfer agent that facilitate polymerization in the second step. Thus, the hyperbranched core has a number of points from which polymerization can be extended to form the nanoparticles arms. As noted above, the nanoparticle of the invention can include from about 10 to about 100 arms (20-50 arms or 25-40 arms). The
10 hyperbranched core therefore has at least about 10 to about 100 points from which the arms can be prepared by polymerization.

Transmers useful in the method of the invention include a degradable group intermediate a polymerizable group and a chain transfer agent. The polymerizable group facilitates the formation of the polymeric core, the chain transfer agent imparts branching
15 and the functionality necessary for the arm-forming polymerization, and the degradable group facilitates degradation of the core and the nanoparticle itself. Representative useful transmers, chain transfer agents, and degradable groups are described below. To increase core branching, the transmer can include more than a single chain transfer agent group (e.g., a transmer including two chain transfer agent groups).

20 In a second step, a prodrug monomer is polymerized from the polymeric core (i.e., chain transfer via reverse addition fragment transfer (RAFT) polymerization to provide polymeric arms extending from and covalently coupled to the core.

In certain embodiments, the prodrug monomer is homopolymerized (i.e., in the absence of a comonomer). This embodiment is useful when the prodrug monomer is
25 sufficiently hydrophilic and imparts suitable water solubility to the nanoparticle for ready administration.

In other embodiments, polymerizing the prodrug monomer comprises copolymerizing the prodrug monomer with a comonomer. Suitable comonomers include water-solubilizing comonomers and targeting comonomers. Suitable comonomers
30 include glycerol methacrylate, DMAPS (a sulfobetaine monomer), PEGMA 300 or 950, DMAEMA, SMA (ampholyte form), sugar methacrylate monomers (mannose methacrylate), hydroxyethyl acrylamide (HEMA), and combinations thereof. In certain

of these embodiments, the ratio of prodrug monomer to comonomer is from about 1 to about 2.

In certain embodiments, the method for preparing the nanoparticle can be carried out in a single reaction vessel without isolating the product of the first step (i.e., polymerizing the transmer and polymerizing the prodrug monomer are conducted in a single reaction vessel). Alternatively, the hyperbranched polymeric product from the first step can be isolated from the reaction mixture for subsequent polymerization reaction in a separate vessel to provide the nanoparticle.

As used herein, the terms "radiant star nanoparticle", "RSN", "radiant star nanoparticle prodrug", and "RSN prodrug" are used interchangeably.

The radiant star nanoparticle of the invention is a radiant star nanoparticle prodrug. The radiant star nanoparticle is a prodrug because upon degradation (in situ) of the radiant star nanoparticle, a therapeutic agent is released.

In the RSNs of the invention, the polymeric arms emanating from the nanoparticle core include repeating units that include therapeutic agent moieties. These therapeutic agent moiety-containing repeating units effectively released the therapeutic agent in situ via degradation of the RSN.

As prodrugs, the RSNs of the invention include degradable groups (linkages) that result in the degradation of the RSN architecture into polymeric fragments, and ultimately, the released therapeutic agent. These degradable groups are present in the monomers used to prepare the RSN's branched core as well as the RSN's polymeric arms. The degradable groups are effective to degrade the RSN (core and/or polymeric arms) into fragments and ultimately release the therapeutic agent.

These degradable groups may be one or more functional groups selected from ester, acetal, hemiacetal, anhydride, carbonate, peroxide, peroxyester, phosphate, thioester urea, thiourethane, ether, disulfide, carbamate (urethane) and boronate ester. Degradation of a degradable group may be facilitated in the presence of an acid, a base, an enzyme and/or another endogenous biological compound, or external stimulus that can catalyze or at least assist in the bond cleavage process. For example, an ester, an acetal, or a hemiacetal may be hydrolytically cleaved to produce a carboxylic acid or other carbonyl group and an alcohol group, an amide may be hydrolytically cleaved to produce a carboxylic acid group and an amine group, and a disulfide may be reductively cleaved to produce thiol groups. In certain embodiments, the degradable group is an ester. In

other embodiments, the degradable group is an acetal. In further embodiments, the degradable group is a hemiacetal. In some embodiments, the degradable group is a disulfide.

5 The degradable groups are introduced into the RSN (i.e., core and/or polymeric arms) by selection of the appropriate monomer or comonomers.

Hydrophilic Monomers. To impart or increase the water solubility of the RSN's of the invention, the polymeric chains or arms emanating from the nanoparticle core may include hydrophilic repeating units that are derived from a hydrophilic monomer. Suitable hydrophilic monomers include monomers that include polar groups
10 (e.g., hydroxy, ether, polyether, amide, and sulfoxide), zwitterionic groups (e.g., sulfo-, carboxy-, and phosphobetaine), anionic groups or groups that become anionic at physiological pH (e.g., carboxylate, carboxylic acid), and cationic groups or groups that become cationic at physiological pH (e.g., amino and amine groups). Amine groups can only be used if they are protonated such as in an acidic buffer.

15 Hydrophilic repeating units include a residue selected from the group consisting of residues which are hydrophilic at physiologic pH and are substantially non-charged at physiologic pH (e.g., hydroxy, polyoxylated alkyl, polyethylene glycol, polypropylene glycol, protected thiol, or the like). In some embodiments, the hydrophilic repeating units can be derived from cationic monomers having a pKa ranging anywhere between about
20 6.0 and about 10.0, typically between about 6.2 and about 9.5, and in some embodiments between about 6.5 and about 8.5. Upon incorporation of the monomer into the polymer, the pKa of the residue tends to decrease relative to the unpolymerized monomer; in general, therefore, the pKa of the incorporated repeat units will be between about 6.0 and 10.0, typically between about 6.2 and 9.0, and in some embodiments, between about
25 6.5 and 8.0.

In certain embodiments, the hydrophilic monomer comprises a monomeric species comprising an acyclic amine (e.g., an amine, an alkyl amine, a dialkyl amine, or the like), an acyclic imine (e.g., an imine, an alkyl imine, or the like), a cyclic amine (e.g., piperidine), a nitrogen containing heterocycle (e.g., pyridine or quinoline), or the
30 like. In specific embodiments, a cationic species utilized herein includes a protonated acyclic amine (e.g., an amine, an alkyl amine, a dialkyl amine, or the like), an acyclic imine (e.g., an imine, an alkyl imine, or the like), a cyclic amine (e.g., piperidine), a nitrogen containing heterocycle (e.g., pyridine or quinoline), or the like.

Non-limiting examples of acyclic amines include methylamine, dimethylamine, ethylamine, diethylamine, propylamine, isopropylamine, diisopropylamine, diisopropylethylamine, n-butylamine, sec-butylamine, tert-butylamine, pentylamine, neo-pentylamine, iso-pentylamine, hexanamine or the like. Non-limiting examples of
5 acyclic imines include methylimine, ethylimine, propylimine, isopropylimine, n-butylimine, sec-butylimine, pentylimine, neo-pentylimine, iso-pentylimine, hexylimine or the like. Non-limiting examples of cyclic amines include cyclopropylamine, cyclobutylamine, cyclopentylamine, cyclohexylamine, cycloheptylamine, piperidine, pyrazine, pyrrolidine, homopiperidine, azabicycloheptane, diazabicycloundecane, or the
10 like. Non-limiting examples of cyclic imines include cyclopropylimine, cyclobutyylimine, cyclopentylimine, cyclohexylimine, cycloheptylimine, or the like. Non-limiting examples of nitrogen containing heteroaryls include imidazolyl, pyrrolyl, pyridyl, indolyl, or the like.

In some embodiments, the hydrophilic repeating units comprise monomeric
15 residues of optionally substituted, amino(C₁-C₆)alkyl-ethacrylate, amino(C₁-C₆)alkyl-methacrylate, amino(C₁-C₆)alkyl-acrylate, (N-(C₁-C₆)alkyl-amino(C₁-C₆)alkyl-ethacrylate, N-(C₁-C₆)alkyl-amino(C₁-C₆)alkyl-methacrylate, N-(C₁-C₆)alkyl-amino(C₁-C₆)alkyl-acrylate, (N,N-di(C₁-C₆)alkyl-amino(C₁-C₆)alkyl-ethacrylate, N,N-di(C₁-C₆)alkyl-amino(C₁-C₆)alkyl-methacrylate, N,N-di(C₁-C₆)alkyl-amino(C₁-C₆)alkyl-
20 acrylate, or a combination thereof. In specific embodiments, such monomeric residues constitute a cationic monomeric residue at neutral pH.

In certain embodiments, the hydrophilic repeating units comprise anionic monomeric residues. The anionic monomeric residues can have a species charged or chargeable to an anion, including a protonatable anionic species. The chargeable species
25 can preferably be anionic at serum physiological pH and substantially neutral or non-charged at the pH. In some embodiments, the carrier block comprises a plurality of anionic hydrophobic monomeric residues, monomeric residues comprising both hydrophobic species (e.g., a C₂-C₈ alkyl substituent) and species charged or chargeable to an anion.

30 In certain preferred embodiments of the present invention, the hydrophilic repeating units comprise constitutional units derived from *N,N*-dimethylacrylamide (DMA), diethylacrylamide (DEA), 2-hydroxypropyl methacrylamide (HPMA), hydroxyethylacrylamide, hydroxyethylmethacrylate, polyethylene glycol acrylate,

propylene glycol methacrylamide (PEGMA), and hydroxy- or methoxy-terminated diethylene glycol methacrylate (950 – 10 kDa).

Monomers. The RSNs of the invention are prepared in two polymerization steps: (i) core-forming transmer polymerization, and (ii) arm-forming polymerization, which
5 may include monomers other than the therapeutic agent-bearing monomer (PPM).

Monomers are selected for the preparation of the RSNs of the invention depending on the desired properties of the individual core and arms, and overall RSN.

In certain embodiments, ethylenically unsaturated monomers are used. The term "ethylenically unsaturated monomers" is defined as a compound having at least one
10 carbon double bond or triple bond. Suitable ethylenically unsaturated monomers include alkyl (alkyl)acrylates, methacrylates, acrylates, alkylacrylamides, methacrylamides, and acrylamides. Non-limiting examples of the ethylenically unsaturated monomers are: an alkyl (alkyl)acrylate, a methacrylate, an acrylate, an alkylacrylamide, a methacrylamide, an acrylamide, a styrene, an allylamine, an
15 allylammonium, a diallylamine, a diallylammonium, an N-vinyl formamide, a vinyl ether, a vinyl sulfonate, an acrylic acid, a sulfobetaine, a carboxybetaine, a phosphobetaine, or maleic anhydride.

In some embodiments, monomers suitable for use in the preparation of the RSNs provided herein include, by way of non-limiting example, one or more of the following
20 monomers: methyl methacrylate, ethyl acrylate, propyl methacrylate (all isomers), butyl methacrylate (all isomers), 2-ethylhexyl methacrylate, isobornyl methacrylate, methacrylic acid, benzyl methacrylate, phenyl methacrylate, methacrylonitrile, alpha-methylstyrene, methyl acrylate, ethyl acrylate, propyl acrylate (all isomers), butyl acrylate (all isomers), 2-ethylhexyl acrylate, isobornyl acrylate, acrylic acid, benzyl acrylate,
25 phenyl acrylate, acrylonitrile, styrene, acrylates and styrenes selected from glycidyl methacrylate, 2-hydroxyethyl methacrylate, hydroxypropyl methacrylate (all isomers), hydroxybutyl methacrylate (all isomers), N,N-dimethylaminoethyl methacrylate, N,N-diethylaminoethyl methacrylate, triethyleneglycol methacrylate, itaconic anhydride, itaconic acid, glycidyl acrylate, 2-hydroxyethyl acrylate, hydroxypropyl acrylate (all
30 isomers), hydroxybutyl acrylate (all isomers), N,N-dimethylaminoethyl acrylate, N,N-diethylaminoethyl acrylate, triethyleneglycol acrylate, methacrylamide, N-methylacrylamide, N,N-dimethylacrylamide, N-tert-butylmethacrylamide, N-n-butylmethacrylamide, N-methylolacrylamide, N-ethylolacrylamide, vinyl benzoic

acid (all isomers), diethylaminostyrene (all isomers), alpha-methylvinyl benzoic acid (all isomers), diethylamino alpha-methylstyrene (all isomers), p-vinylbenzenesulfonic acid, p-vinylbenzene sulfonic sodium salt, trimethoxysilylpropyl methacrylate, triethoxysilylpropyl methacrylate, tributoxysilylpropyl methacrylate, 5 dimethoxymethylsilylpropyl methacrylate, diethoxymethylsilylpropylmethacrylate, dibutoxymethylsilylpropyl methacrylate, diisopropoxymethylsilylpropyl methacrylate, dimethoxysilylpropyl methacrylate, diethoxysilylpropyl methacrylate, dibutoxysilylpropyl methacrylate, diisopropoxysillpropyl methacrylate, trimethoxysilylpropyl acrylate, triethoxysilylpropyl acrylate, tributoxysilylpropyl 10 acrylate, dimethoxymethylsilylpropyl acrylate, diethoxymethylsilylpropyl acrylate, dibutoxymethylsilylpropyl acrylate, diisopropoxymethylsilylpropyl acrylate, dimethoxysilylpropyl acrylate, diethoxysilylpropyl acrylate, dibutoxysilylpropyl acrylate, diisopropoxysilylpropyl acrylate, vinyl acetate, vinyl butyrate, vinyl benzoate, vinyl chloride, vinyl fluoride, vinyl bromide, maleic anhydride, N-arylmaleimide, 15 N-phenylmaleimide, N-alkylmaleimide, N-butyilmaleimide, N-vinylpyrrolidone, N-vinylcarbazole, butadiene, isoprene, chloroprene, ethylene, propylene, 1,5-hexadienes, 1,4-hexadienes, 1,3-butadienes, 1,4-pentadienes, vinylalcohol, vinylamine, N-alkylvinylamine, allylamine, N-alkylallylamine, diallylamine, N-alkyldiallylamine, alkylenimine, acrylic acids, alkylacrylates, acrylamides, methacrylic acids, 20 alkylmethacrylates, methacrylamides, N-alkylacrylamides, N-alkylmethacrylamides, styrene, vinylnaphthalene, vinyl pyridine, ethylvinylbenzene, aminostyrene, vinylpyridine, vinylimidazole, vinylbiphenyl, vinylanisole, vinylimidazolyl, vinylpyridinyl, vinylpolyethyleneglycol, dimethylaminomethylstyrene, trimethylammonium ethyl methacrylate, trimethylammonium ethyl acrylate, 25 dimethylamino propylacrylamide, trimethylammonium ethylacrylate, trimethylammonium ethyl methacrylate, trimethylammonium propyl acrylamide, dodecyl acrylate, octadecyl acrylate, or octadecyl methacrylate monomers, or combinations thereof.

In some embodiments, monomers suitable for use in the preparation of the RSNs 30 include, for example, one or more of the following monomers: methyl methacrylate, ethyl acrylate, propyl methacrylate (all isomers), butyl methacrylate (all isomers), 2-ethylhexyl methacrylate, methacrylic acid, benzyl methacrylate, phenyl methacrylate, methyl acrylate, ethyl acrylate, propyl acrylate (all isomers), butyl acrylate (all isomers),

2-ethylhexyl acrylate, acrylic acid, benzyl acrylate, phenyl acrylate, acrylates selected from glycidyl methacrylate, 2-hydroxyethyl methacrylate, hydroxypropyl methacrylate (all isomers), hydroxybutyl methacrylate (all isomers), or combinations thereof.

Where ethylenically unsaturated monomers are to be polymerized by RAFT
5 polymerization technique, a RAFT chain transfer agent will generally be necessary. RAFT agents suitable for use in accordance with the invention comprise a thiocarbonylthio group (which is a divalent moiety represented by: $-C(=S)S-$). RAFT polymerization and RAFT agents are described in numerous publications (see, for example, WO 98/01478, Moad G.; Rizzardo, E; Thang S, H. Polymer 2008, 49,
10 1079-1131; Aust. J. Chem., 2005, 58, 379-410; Aust. J. Chem., 2006, 59, 669-692; and Aust. J. Chem., 2009, 62, 1402-1472, each expressly incorporated herein by reference in its entirety). Suitable RAFT agents for use in preparing the RSNs include xanthate, dithioester, dithiocarbamate and trithiocarbonate compounds.

RSN formation by living polymerization will usually require initiation from a
15 source of free radicals. A source of initiating radicals can be provided by any suitable means of generating free radicals, such as the thermally induced homolytic scission of suitable compound(s) (thermal initiators such as peroxides, peroxyesters, or azo compounds), the spontaneous generation from monomers (e.g. styrene), redox initiating systems, photochemical initiating systems or high energy radiation such as electron beam,
20 X- or gamma-radiation.

Polymer Definitions

The following definitions relate polymers in general and are useful in understanding the nonlinear copolymers of the invention.

The term "constitutional unit" of a polymer refers to an atom or group of atoms in
25 a polymer, comprising a part of the chain together with its pendant atoms or groups of atoms, if any. The constitutional unit can refer to a repeat unit. The constitutional unit can also refer to an end group on a polymer chain. For example, the constitutional unit of polyethylene glycol can be $-CH_2CH_2O-$ corresponding to a repeat unit, or $-CH_2CH_2OH$ corresponding to an end group.

30 The term "repeat unit" corresponds to the smallest constitutional unit, the repetition of which constitutes a regular macromolecule (or oligomer molecule or block).

The term "end group" refers to a constitutional unit with only one attachment to a polymer chain, located at the end of a polymer. For example, the end group can be

derived from a monomer unit at the end of the polymer, once the monomer unit has been polymerized. As another example, the end group can be a part of a chain transfer agent or initiating agent that was used to synthesize the polymer.

5 The term "monomer" is a polymerizable compound that, on polymerization, contributes one or more constitutional units in the structure of the polymer.

The term "polymer" refers to the product that is the result of polymerization of a single monomer.

10 The term "copolymer" refers to a polymer that is the result of polymerization of two or more different monomers. The number and the nature of each constitutional unit can be separately controlled in a copolymer. The constitutional units can be disposed in a purely random, an alternating random, a regular alternating, a regular block, a gradient block, or a random block configuration unless expressly stated to be otherwise. A purely random configuration can, for example, be: x-x-y-z-x-y-y-z-y-z-z-z... or y-z-x-y-z-y-z-x-x.... An alternating random configuration can be: x-y-x-z-y-x-y-z-y-x-z..., and a regular
15 alternating configuration can be: x-y-z-x-y-z-x-y-z....

The term "block copolymer" refers to a polymer formed of two or more covalently joined segments of polymers. A regular block configuration has the following general configuration: ...x-x-x-y-y-y-z-z-z-x-x-x..., A random block configuration has the general configuration: ...x-x-x-z-z-z-x-x-y-y-y-z-z-z-x-x-z-z-z-....

20 Pharmaceutical Compositions

In another aspect, the invention provides a composition that includes the radiant star nanoparticle of the invention and a pharmaceutically acceptable carrier or diluent. Suitable carriers and diluents include those known in the art, such as saline and dextrose.

Methods for Intracellular Delivery

25 In a further aspect of the invention, a method for intracellular delivery of a therapeutic agent is provided. In certain embodiments, the method comprises contacting a cell with the radiant star nanoparticle of the invention, which ultimately results in release of the therapeutic agent into the cell.

Methods for Treating or Preventing a Disease, Disorder, or Condition

30 In another aspect, the present invention provides a method for treating or preventing or treating a disease, disorder, or condition treatable by a specific therapeutic agent. In certain embodiments, the method comprises administering to subject in need thereof an effective amount of the radiant star nanoparticle of the invention, wherein the

therapeutic agent released from the radiant star nanoparticle is the specific therapeutic agent effective to treat or prevent the disease, disorder, or condition treatable by the therapeutic agent released from the nanoparticle.

5 The following describes the preparation, characterization, and properties of a representative radiant star nanoparticle prodrug of the invention.

The following describes a mannose-targeted radiant star nanoparticle (RSN) prodrugs prepared by the copolymerization of PPMs with glycan monomers from homopolymerized transmer cores linked via ester or acetal bonds.

Synthesis and characterization of transmers

10 In these studies a hydrophilic single polymer nanoparticle prodrug was developed that combines both the favorable uptake and circulation properties of nanoparticle-based systems with the higher drug release rates often observed for molecularly soluble polymeric prodrugs. In order to achieve this objective, RAFT transmers were first homopolymerized to yield a hyperbranched polymer core containing multiple chain
15 transfer agents (CTAs) from which linear polymeric prodrugs could then be grown using PPMs. As shown in FIGURES 1A and 1B, trithiocarbonate-based transmers containing cyanovaleric acid R-groups were employed in these studies because of the ability of these CTAs to effectively control the polymerization of methacrylate monomers while also allowing relatively high $[CTA]_0/[I]_0$ ratios (e.g. 100:1) to be employed. The latter
20 consideration is important for minimizing star-star coupling during the second polymerization step. The transmers shown in FIGURES 1A and 1B also contain a polymerizable methacrylate residue covalently bound to the CTA by either an alkyl ester or an acetal linkage. These linkages yield hyperbranched cores that degrade at different rates under aqueous conditions.

25 As shown in FIGURE 2A, the alkyl ester-linked transmer (hECT) was prepared by conjugation of the RAFT agent ECT to HEMA via standard carbodiimide coupling chemistry using DMAP catalyst. In order to synthesize the acetal-linked transmer (aECT) a two-step synthesis was employed where the CTA was first esterified with ethylene glycol vinyl ether, which was then reacted with HEMA in the presence of an
30 acid catalyst to yield the desired product. Shown in FIGURE 2B, is the ^1H NMR confirming the formation of the desired acetal-linked transmer with labeled peaks including the characteristic acetal CH and CH_3 resonances at 1.1 and 4.5 respectively. The resultant transmers were then homopolymerized in dry DMSO at 70 °C for 18 h

using an initial transmer to initiator ratio of 20. ^1H NMR analysis of the crude polymerization mixture indicated that these conditions led to quantitative conversion of the vinyl resonances at 6.1 and 5.2 ppm respectively. Following purification the resultant poly(hECT) and poly(aECT) cores were then analyzed using a combination of ^1H NMR and GPC (FIGURES 3A-3D).

Homopolymerization of transmers

Shown in FIGURES 3A and 3B are the ^1H NMR spectra for the homopolymerized transmers with the resonances labeled. Notably the peaks have broadened considerably relative to the low molecular weight precursors with the disappearance of the vinyl resonances and the concurrent appearance of methylene and methyl resonances in the backbone region. GPC analysis of poly(hECT) and poly(aECT) (FIGURES 3C and 3D) show molecular weight distributions that are unimodal and symmetric with molar mass dispersities around 1.30. The homopolymerized transmers have relatively low molecular weights of 8700 Da and 9300 Da for the alkyl ester and acetal ester containing structures, respectively. This corresponds to approximately 23 and 20 RAFT CTAs, respectively, per polymeric transmer core assuming a homogenous composition composed exclusively of the precursor transmers.

Polymerization from polymeric transmer cores

In order to prepare nanoparticle prodrugs from the homopolymerized transmer cores, the RAFT polymerization of a range of methacrylate and acrylamide based monomers was investigated (FIGURE 4). As can be seen in FIGURES 4A and 4B, the RAFT polymerization of DMA from both transmer cores yields unimodal and symmetric molecular weight distributions that elute at much lower elution volumes. In these polymerizations a $[\text{M}]_0:[\text{CTA}]_0:[\text{I}]_0$ ratio of 200:1:0.05 was targeted resulting in molecular weights and molar mass dispersities of 364 000/1.32 and 275 000/1.36 for the alkyl ester and acetal linked radiant star nanoparticles. The effect of $[\text{M}]_0/[\text{CTA}]_0$ at a fixed $[\text{CTA}]_0/[\text{I}]_0$ of 0.05 was next evaluated. As can be seen in FIGURE 4C, a clean progression of molecular weights towards lower elution volumes is observed with increasing $[\text{M}]_0/[\text{CTA}]_0$ suggesting the ability to prepare RSNs with different molecular weights simply by controlling the initial reaction stoichiometry. In all cases the molecular weight distributions remained symmetric and unimodal with moderate molar mass dispersities around 1.45. The ability to prepare methacrylate based RSNs was next evaluated by conducting RAFT polymerizations from the poly(hECT) core low molecular

weight methacrylate monomers (HEMA and DMAEMA) and high molecular weight methacrylate monomers (PEGMA FW_{avg} about 300 and 950, O300 and O900, respectively). As shown in FIGURES 4D-4F, a higher degree of control was observed for the polymerization of the smaller methacrylate monomers DMAEMA and HEMA from the polymeric transmer core. Here, relatively narrow and symmetric molecular weight distributions were observed with molecular weight and molar mass dispersities of 186 300/1.39 and 401 400/1.57 respectively. In contrast, the RAFT polymerization of the sterically bulky PEGMA monomers yielded somewhat asymmetric molecular weight distributions with the presence of a high molecular weight star-star coupling peak observed for both the O300 and O950 polymerizations. Nonetheless minimal low molecular weight contamination was observed in these polymerizations suggesting that the PEGMA based RSNs might still prove to be versatile nanoparticle prodrugs despite the presence of some heterogeneity.

RSN morphology and hydrolytic stability

Evaluation of the RSN morphology via TEM (FIGURE 5A) shows that the these structures seem to form somewhat irregular branchy structures that are consistent with the RSN morphology depicted in FIGURES 1A and 1B. Although aggregation of the RSNs was observed during TEM analysis, it is possible to identify individual particles that show hydrodynamic diameters that are consistent with the 24 nm determined via dynamic light scattering

Next the hydrolytic stability of DMA-based RSNs derived from both the alkyl ester linked poly(hECT) and acetal poly(aECT) linked cores was evaluated. Acetals have been shown to hydrolyze rapidly under acidic conditions such as those found in the intracellular compartment of macrophages while polymeric alkyl ester remain more stable under these conditions. Shown in FIGURES 5B and 5C are the GPC chromatograms for poly(DMA) polymerized from poly(hECT) and poly(aECT) cores. Here, the polymers were incubated at 37 °C in 150 mM acetate buffer at pH 5.0 for the indicated time period before being diluted into DMF and analyzed via GPC. As can be seen in FIGURE 5A, an overlay of the molecular weight distributions for the alkyl ester linked RSN shows no visible change between the initial polymeric nanoparticle and those incubated in buffer for 30 days. In contrast, a progressive degradation of the high molecular weight peak is observed for the acetal linked RSN over the course of the 30 day incubation period (see FIGURE 5C). Here, the relatively broad RSN peak is seen to reduce in area with the

appearance of a narrow lower molecular species that is consistent with linear polymers liberated from the central core following acetal hydrolysis. The slower rate of acetal hydrolysis observed relative to low molecular weight acetal species is hypothesized to arise from the hydrophobic environment of the central core.

5 Synthesis of mannose-targeted RSN prodrugs

Therapeutic RSN prodrugs were next synthesized, as shown in FIGURE 6, by copolymerizing the ciprofloxacin prodrug monomer (CTM) with a hydrophilic mannose monomer (MEM) from homopolymerized transmer cores containing either alkyl ester or acetal linkages. Phenyl ester linked prodrug polymerizable prodrug monomers were employed in these studies as it has been shown previously that these species hydrolyze at significantly higher rates in human serum than the analogous alkyl ester analogue. High cure rates for mice treated with phenyl ester linked ciprofloxacin based polymeric prodrugs have been observed while alkyl ester linked derivatives as well as the free ciprofloxacin controls show no protection against the lethal mouse model of *Francisella tularensis* infection. The mannose comonomer employed in these polymerizations functions as a biocompatible hydrophilic stabilizer while also efficiently targeting alveolar macrophages where many pathogens such as *Burkholderia pseudomallei* and *Francisella tularensis* are known to reside. Polymerizations were conducted with an initial mol fraction of CTM and MEM of 16 % and 84 % (33 wt. % CTM) with an [M]₀/[CTA]₀ and [CTA]₀:[I]₀ ratio of 100 and 0.05 respectively in DMSO. In order to suppress the reaction of the secondary amine present on ciprofloxacin with the polymeric chain transfer agents, polymerizations were conducted at 30 °C. In addition to these mannose based RSNs, materials containing DMA as the hydrophilic stabilizer as well as the analogous linear copolymers were also synthesized to serve as untargeted controls (vide infra).

Shown in FIGURES 7A-7D are the ¹H NMR spectra and molecular weight distributions for the poly(MEM-co-CTM) and poly(DMA-co-CTM) RSNs, respectively. Resonances associated with residues from CTM as well as the respective MEM and DMA comonomers can be clearly observed in FIGURES 7A and 7B, respectively. Because of the complex nature of the poly(Man-co-CTM) spectrum, copolymer composition was determined by acid hydrolysis followed by reverse phase HPLC analysis of the release ciprofloxacin relative to standard curves for both materials. Based on this analysis a molar copolymer composition of 20 % CTM / 80 % MEM and 19.7 %

CTM / 80.3 % DMA was determined, which is in good agreement with the feed. These values yield RSN prodrugs with 40 wt. % CTM (17.5 % ciprofloxacin drug) and 39 wt. % CTM (17 % ciprofloxacin drug), respectively.

Ciprofloxacin release studies

5 Shown in FIGURE 8 is the percentage ciprofloxacin release as a function of time for polymeric prodrugs incubated in human serum. In this study the rate of antibiotic released via cleavage of the phenyl ester-linked drugs was evaluated as a function of polymer architecture and hydrophilic comonomer. Here, the highest rates of ciprofloxacin release was observed for the poly(DMA-co-CTM) and poly(MEM-co-
10 CTM), where the amount of the hydrophobic prodrug monomer in the copolymer was limited to approximately 33 wt. % (feed). This composition has been observed to yield copolymers that are easily dispersed in phosphate buffer at concentrations as high as 200 mg/mL with sizes that are consistent with molecularly dissolved unimers. Comparable rates of drug release were observed for both the DMA and Man based
15 copolymers where 50 % drug release was observed at 125 and 110 hours respectively. In contrast, ciprofloxacin release from poly(O950-b-CTM), where the hydrophobic prodrug residues are localized in a discrete block copolymer segment, was observed to be quite slow with less than 4 % drug release over the same time period. This result is consistent with previous studies, where molecularly soluble copolymers of CTM and polyethylene
20 glycol methyl ether methacrylate (FW about 950 Da) (O950) showed considerably higher hydrolysis rates than those observed for diblock copolymers where the hydrophobic prodrug monomer was localized in the interior of micelles under aqueous conditions at pH 7.4. Evaluation of the DMA and mannose based RSNs show hydrolysis rates that are slower than the linear copolymers at similar compositions but significantly faster than the
25 hydrolysis rates observed for diblock copolymer micelles. For example, 31.4 % hydrolysis was observed for the DMA based RSN polymerized from a poly(hECT) transmer following 120 h incubation in human serum. This corresponds to 7.3 times more ciprofloxacin released than the poly(PEGMA-b-CTM) micelles and only 12.7 % less ciprofloxacin released than the linear copolymer. No dramatic difference in drug
30 release rates were observed between RSN prodrugs prepared from alkyl ester and acetal cores. This result likely arises from the slow rate of RSN degradation relative to the time scale of the hydrolysis experiment and moderately low differences in ciprofloxacin release between the linear and RSN prodrugs. This finding also suggests that more

hydrolytically unstable transmer linkages such as hemiacetal esters may provide further enhancements in drug release from RSNs.

Macrophage binding studies and in vitro co-culture activity using a B. thailandensis infection mode

5 Previously, it has been shown that primary and immortalized macrophages show distinct differences in mannose receptor expression levels with the primary cells showing significantly higher levels of the receptor. In order to evaluate the ability of the RSN prodrugs to target the macrophage mannose receptor, self-renewing, non-transformed MPI cells were first treated with 30 ng/mL murine GM-CSF to induce macrophage
10 differentiation. Functionally, these cells have been known to closely resemble alveolar macrophages more so than immortalized cell-lines. The cells were then treated with rhodamine B labeled poly(MEM-co-CTM) and poly(DMA-co-CTM) RSNs as well as the analogous linear controls for 30, 60, and 120 minutes. To make sure equivalent fluorescence was dosed for all treatment groups, standard curves were produced as a
15 function of polymer concentration. Flow cytometry was then employed to evaluate the amount of fluorescence for each treatment group. As shown in FIGURE 9A, cells treated for 30 minutes show an initial but modest increase in fluorescence for both the linear and RSN containing mannose-targeting groups (444 and 2037, respectively) while the untargeted materials show low fluorescence close to untreated controls. Evaluation of the
20 histograms following 120 minutes of treatment (FIGURE 9B) shows a significant increase in fluorescence for mannose-targeted RSN compared to the remaining treatment groups. These differences were observed to be the most apparent at 300 minutes (FIGURE 9C) where the mannose targeted RSN prodrugs showed approximately 6 times the fluorescence as the other treatment groups. These results, which are plotted in
25 FIGURE 9D, suggest that the combination of mannose targeting groups with nanoparticle dimensions provide substantially enhanced macrophage binding relative to both mannose targeted linear copolymers and untargeted RSNs.

The ability of the mannose-targeted RSNs to effectively release ciprofloxacin in an active form within intracellular compartments was evaluated using a co-cultured
30 model of B. thailandensis. In these studies a near quantitative elimination of colony forming units was observed at a polymeric ciprofloxacin concentration of $10 \mu\text{g mL}^{-1}$ (CFUs = 73 ± 61) while at a concentration of $1.0 \mu\text{g mL}^{-1}$ bacteria levels were similar to negative controls with CFUs of $300,000 \pm 4,900$ and $39,000 \pm 1,680$. While free

ciprofloxacin showed complete antibiotic activity at a concentration of $1.0 \mu\text{g mL}^{-1}$, it was observed previously that it is eliminated rapidly from circulation (k_{el} of 0.7 h^{-1}) and shows negligible in vivo activity while the polymeric prodrugs with phenyl ester linked ciprofloxacin have higher retention times and show high cure rates. Given the higher rate of macrophage binding observed via flow cytometry for mannose-targeted RSNs relative to the linear copolymers, their in vivo activity may be superior despite showing slightly lower drug release kinetics.

In vivo biocompatibility of RSN prodrugs

In order to determine the in vivo biocompatibility of the RSN prodrugs following pulmonary administration, the lung toxicity of endotracheally delivered poly(MEM-co-CTM) prepared from a poly(hECT) transmer core was evaluated using the metrics of animal weight change (FIGURE 9E), and neutrophil infiltration into the lungs (FIGURE 9F), tumor necrosis factor alpha (TNF- α) concentration in lung tissue homogenate (LTH) (FIGURE 9G), and bronchoalveolar lavage fluid (BALF) (FIGURE 9H). Here, mice were dosed with the RSN at 20 mg/kg ciprofloxacin once every 24 h for three-consecutive days. These data demonstrate no statistical differences ($P \leq 0.1$) across all observed toxicity markers at either drug dose for the poly(MEM-co-CTM) RSN prodrug compared to PBS controls. As shown in FIGURES 9D-9G, three consecutive doses resulted in lavage fluids containing $5.7 \pm 1.8 \%$ neutrophils, respectively, whereas PBS controls exhibited $3.7 \pm 7.0 \%$ neutrophils. Similarly, TNF- α concentrations in both the LTH and BALF remained low and comparable to PBS control administrations.

Conclusion

Homopolymerization of alkyl ester and acetal linked transmers yielded hyperbranched polymer cores with relatively low molecular weights and unimodal molecular weight distributions. Subsequent RAFT copolymerizations from the hyperbranched transmer cores enabled the synthesis of RSNs with high molecular weights, symmetric molecular weight distributions, and low amounts of homopolymer impurity. Hydrolysis studies conducted in acetate buffer from the alkyl ester and acetal linked cores demonstrated the high aqueous stability of the former while the latter showed a progressive degradation into unimeric species over the same period. Drug release studies conducted directly in 100 % human serum showed that the RSN prodrugs provided a substantial increase in ciprofloxacin release relative to diblock copolymer

micelles with only a modest reduction in release relative to linear copolymer controls. Flow cytometry studies conducted in RAW264.7 cells induced to express the mannose receptor show significantly higher levels of cell binding for mannose-targeted RSN prodrugs relative to untargeted RSNs as well as both targeted and untargeted linear control polymers. In vivo biocompatibility studies showed no statistical differences between mice treated with mannose-targeted RSNs and phosphate buffer negative control mice. These results taken together indicate that RSNs provide a promising and simple strategy for preparing biocompatible nanoparticle prodrugs with enhanced drug release and receptor binding properties.

As used herein, the term "about" refers to $\pm 5\%$ of the specified value.

EXPERIMENTAL

Materials

Chemicals and all materials were supplied by Sigma-Aldrich unless otherwise specified. 4-((((2-Carboxyethyl)thio)carbonothioyl)thio)-4-cyanopentanoic acid (CCC) was kindly donated by Boron Molecular. Hydroxyethyl methacrylate (HEMA) and *N,N'*-dimethylacrylamide (DMA) were distilled under reduced pressure. Spectra/Por regenerated cellulose dialysis membranes (6-8 kDa cutoff) were obtained from Fisher Scientific. 4-Cyano-4-((ethylsulfanylthiocarbonyl)sulfanyl)pentanoic acid (ECT) was synthesized as described previously (A. J. Convertine, D. S. W. Benoit, C. L. Duvall, A. S. Hoffman and P. S. Stayton, *J Control Release*, 2009, 133, 221–229). Sephadex G-25 prepacked PD10 columns were obtained from GE Life Sciences. 4-Cyano-4-ethylsulfanylthiocarbonylsulfanyl-4-methyl-butyric acid 1-(2-methyl-acryloyloxy)-ethyl ester (hECT) (J. T. Wilson, A. Postma, S. Keller, A. J. Convertine, G. Moad, E. Rizzardo, L. Meagher, J. Chiefari and P. S. Stayton, *AAPS J*, 2015, 17, 358–369), mannose ethyl methacrylate (MEM) (E.-H. Song, M. J. Manganiello, Y.-H. Chow, B. Ghosn, A. J. Convertine, P. S. Stayton, L. M. Schnapp and D. M. Ratner, *Biomaterials*, 2012, 33, 6889–6897), rhodamine B methacrylate (REMA) (J. Chen, H.-N. Son, J. J. Hill, S. Srinivasan, F.-Y. Su, P. S. Stayton, A. J. Convertine and D. M. Ratner, *Nanomedicine: Nanotechnology, Biology and Medicine*, 2016, 12, 2031–2041) and ciprofloxacin (tyramine) methacrylate (CTM) (D. Das, S. Srinivasan, A. M. Kelly, D. Y. Chiu, B. K. Daugherty, D. M. Ratner, P. S. Stayton and A. J. Convertine, *Polymer Chemistry*, 2016, 7, 826–837) were synthesized as described previously.

Synthesis of 4-Cyano-4-ethylsulfanylthiocarbonylsulfanyl-4-methyl-butyrac acid 2-{1-[2-(2-methyl-acryloyloxy)ethoxy]ethoxy}ethyl ester (aECT). To a 50 mL round bottom flask containing a magnetic stir bar was added ECT (5.0 g, 19.0 mmol, 1.0 equivalent), 4-dimethylaminopyridine (4.64 g, 38.0 mmol, 2 equivalents),
 5 *N,N'* dicyclohexylcarbodiimide (4.7 g, 22.8 mmol, 1.2 equivalents, and 28 mL methylene chloride. To the solution was then added ethyleneglycol vinyl ether (4.01 g, 45.5 mmol, 2.4 equivalents). The round bottom flask was then capped with a rubber septa and allowed to react over ice for 1 hour and then at room temperature overnight. The solution was then transferred to a separation funnel and was washed 5 times with saturated sodium
 10 bicarbonate solution. The organic phase was then collected, dried over anhydrous sodium sulfate, filtered through a plug of cotton, and then isolated via rotary evaporation. The product was then used for the synthesis of aECT without further purification. To a 10 mL round bottom flask was added ethylene glycol vinyl ether esterified ECT (EGVE-ECT) (0.5 g, 1.5 mmol), HEMA (0.195 g, 1.5 mmol), trifluoroacetic acid (TFA) (50 μ L,
 15 653 μ mol), and methylene chloride 1.0 mL. The reaction was then sealed with a septa and allowed to react overnight. The product was then isolated via silica gel column chromatography using an eluent consisting of ethyl acetate/hexanes (75:25) with 1 % triethylamine. ^1H NMR (500 MHz, benzene- d_6 , ppm) δ = 6.16 (1H, singlet, vinyl), 5.24 (1H, vinyl), 4.52 (1H, quartet, acetal), 4.19 (2H, triplet, ester), 4.08 (2H, triplet, ester),
 20 3.28-3.56 (4H, multiplet, $\text{OCH}_2\text{CHCH}_3\text{OCH}_2$), 2.79 (2H, quartet, SCH_2), 2.0-2.4 (4H, multiplet, $\text{C}(\text{CH}_3)(\text{CN})\text{CH}_2\text{CH}_2$), 1.85 (3H, singlet, vinyl-methyl), 1.32(3H, singlet, $\text{C}(\text{CN})\text{CH}_3$), 1.13 (3H, doublet, acetal-methyl), and 0.82 (3H, triplet, SCH_2CH_3) (for the labeled ^1H NMR spectrum see supporting information). ^{13}C NMR (125 MHz, benzene- d_6 , ppm) δ = 216.9, 170.8, 136.6, 125.1, 118.7, 99.3, 63.9, 63.7, 62.6, 62.3, 33.8, 31.0,
 25 29.5, 24.1, 19.2, 18.1, 12.2.

Synthesis of poly(hECT) and poly(aECT). To a 5 mL conical bottom flask was added either hECT (6.52 g, 17.36 mmol) or aECT (8.04 g, 17.36 mmol), Azobiscyanovaleric acid (ABCVA) (486 mg, 1.76 mmol), and 15.2 mL of anhydrous DMSO. The flask was then sealed with a rubber septa and then purged with nitrogen for
 30 30 minutes. The solution was then transferred to a preheated oil bath at 70 $^\circ\text{C}$ and allowed to polymerize for 18 h. The polymers were then isolated by precipitating the polymerization solution to 45 mL of diethyl ether in 50 ml conical tubes. After vortexing, the solutions were centrifuged at 4200 rpm for 5 minutes, the ether supernatant

was decanted and yellow polymer oil was diluted 1 to 1 with acetone and re-precipitated into ether as described above (x6).

Synthesis of DMA RSNs from poly(hECT) and poly(aECT). Polymerization of DMA was conducted in DMSO in the presence of either poly(hECT) or poly(aECT) and ABCVA. The initial molar feed percentages of the dasatinib esterified SMA (Dt-SMA) and O950 monomers were both 50 %. The $[M]_0:[CTA]_0:[I]_0$ was 200:1:0.025 at an initial monomer concentration of 25 wt. %. To a 10 mL conical bottom flask was added poly(hECT) (20 mg, 53 mmol) or poly(aECT) (25 mg, 53 mmol), DMA (1.06 g, 10.7 mmol), ABCVA (0.373 mg, 1.3 mmol) and 3.17 mL DMSO. The polymerization solutions were then septa sealed and then purged with nitrogen for 30 minutes. After this time, the polymerization solution was transferred to a preheated oil bath at 70 °C and allowed to polymerize for 4 hours. The copolymer was isolated by precipitating the polymerization solution into 45 mL of diethyl ether in 50 mL conical tubes. After vortexing, the solutions were centrifuged at 4200 rpm for 5 minutes. The clear ether supernatant was decanted and yellow polymer oil was diluted 1 to 1 with acetone and reprecipitated into ether as described above (x6).

Kinetic evaluation of the RAFT polymerization of DMA from poly(hECT). The initial monomer ($[M]_0$) to CTA equivalents to initiator ($[I]_0$) ratio was 350:1:0.025, respectively. Individual polymerization solutions were transferred to a septa-sealed vial and purged with nitrogen for 30 minutes. After this time, the polymerization vials were transferred to a preheated oil bath at 90 °C and allowed to polymerize for the prescribed time period. In order to determine the monomer conversion 50 μ L of the polymerization solutions were first diluted into 900 μ L of $CDCl_3$ and then ^1H NMR spectra were recorded. The molar fraction of DMA converted to polymer was then determined by comparison of the total vinyl resonances (3H) (A) between 5.0-6.5 ppm to the total backbone resonances between 0.5-1.5 ppm (B) using the equation: % DMA conversion = $[(A+B)-(A)]/(A+B)*100$.

Synthesis of poly(mannose-co-CTM) RSN from poly(hECT). Copolymerization of MEM and CTM from poly(hECT) was conducted with an initial monomer $[M]_0:[CTA]_0:[I]_0$ ratio was 100:1:0.05 at an initial overall monomer concentration of 20 % m/v. To a 5 mL conical bottom flask was added poly(hECT) (3.06 mg, 8.15 μ mol), MEM (0.2 g, 0.684 mmol), CTM (0.1 g, 0.131 mmol), REMA (7.75 mg, 13.1 μ mol), 2,2'-azobis(4-methoxy-2,4-dimethylvaleronitrile) (V70) (0.125 mg, 0.408 μ mol) as

100 μL of $1.25 \text{ mg}\cdot\text{mL}^{-1}$ solution in dioxane, and 1.2 mL of DMSO. The polymerization solution was then septa-sealed purged with nitrogen for 30 minutes. After this time, the polymerization vials were transferred to a preheated oil bath at 30°C and allowed to polymerize for 18 hours. After this time, the polymerization solution was precipitated
5 into 45 mL of diethyl ether in 50 mL conical tubes. After vortexing, the solution was centrifuged at 4200 rpm for 5 minutes. The ether supernatant was decanted and yellow polymer oil was diluted to a final volume of 25 mL in 0.5 M phosphate buffer pH 7.4. The aqueous solution was dialyzed against deionized water at 5°C using spectra/Por regenerated cellulose dialysis membranes (6-8 kDa cutoff) and isolated by lyophilisation.
10 The lyophilized polymer was then redissolved in deionized water and then purified by double Sephadex G-25 prepacked PD10 columns according to the manufacturer's instructions.

Synthesis of poly(DMA-co-CTM) RSN from poly(hECT). Copolymerization of DMA and CTM from poly(hECT) was conducted with an initial monomer
15 $[\text{M}]_0:[\text{CTA}]_0:[\text{I}]_0$ ratio was 100:1:0.05 at an initial overall monomer concentration of 20 % m/v. To a 5 mL conical bottom flask was added poly(hECT) (8.06 mg, $21.5 \mu\text{mol}$), DMA (0.2 g, 2.02 mmol), CTM (0.1 g, 0.131 mmol), REMA (7.75 mg, $13.1 \mu\text{mol}$), V70 (0.331 mg , $1.07 \mu\text{mol}$) as 100 μL of 3.31 mg mL^{-1} solution in dioxane, and 1.2 mL of DMSO. The polymerization solution was then septa-sealed and purged with nitrogen for
20 30 minutes. After this time, the polymerization vials were transferred to a preheated oil bath at 30°C and allowed to polymerize for 18 hours. After this time, the polymerization solution was precipitated into 45 mL of diethyl ether in 50 mL conical tubes. After vortexing, the solution was centrifuged at 4200 rpm for 5 minutes. The ether supernatant was decanted and yellow polymer oil was diluted to a final volume of 25 mL in 0.5 M
25 phosphate buffer pH 7.4. The aqueous solution was then dialyzed against deionized water at 5°C using spectra/Por regenerated cellulose dialysis membranes (6-8 kDa cutoff) and then isolated by lyophilisation. The lyophilized polymer was then redissolved in deionized water and then purified by double Sephadex G-25 prepacked PD10 columns according to the manufacturer's instructions.

30 Synthesis of linear poly(MEM-co-CTM). Copolymerization of MEM and CTM in the presence of the trithiocarbonate-based RAFT agent CCC was conducted with an initial monomer $[\text{M}]_0:[\text{CTA}]_0:[\text{I}]_0$ ratio was 100:1:0.05 at an initial overall monomer concentration of 20 % m/v. To a 5 mL conical bottom flask was added CCC (2.50 mg,

8.15 μmol), MEM (0.2 g, 0.684 mmol), CTM (0.1 g, 0.131 mmol), REMA (7.75 mg, 13.1 μmol), V70 (0.125 mg, 0.408 μmol) as 100 μL of 1.25 mg mL^{-1} solution in dioxane, and 1.2 mL of DMSO. The polymerization solution was then septa-sealed and purged with nitrogen for 30 minutes. After this time, the polymerization vials were transferred to a preheated oil bath at 30 $^{\circ}\text{C}$ and allowed to polymerize for 18 hours. After this time, the polymerization solution was precipitated into 45 mL of diethyl ether in 50 mL conical tubes. After vortexing, the solution was centrifuged at 4200 rpm for 5 minutes. The ether supernatant was decanted and yellow polymer oil was diluted to a final volume of 25 mL in 0.5 M phosphate buffer pH 7.4. The aqueous solution was then dialyzed against deionized water at 5 $^{\circ}\text{C}$ using spectra/Por regenerated cellulose dialysis membranes (6-8 kDa cutoff) and then isolated by lyophilisation. The lyophilized polymer was then redissolved in deionized water and then purified by double Sephadex G-25 prepacked PD10 columns according to the manufacturer's instructions.

Synthesis of linear poly(DMA-co-CTM). Copolymerization of DMA and CTM in the presence of the trithiocarbonate-based RAFT agent CCC was conducted with an initial monomer $[\text{M}]_0:[\text{CTA}]_0:[\text{I}]_0$ ratio was 100:1:0.05 at an initial overall monomer concentration of 20 % m/v. To a 5 mL conical bottom flask was added CCC (6.60 mg, 21.5 μmol), DMA (0.2 g, 2.02 mmol), CTM (0.1 g, 0.131 mmol), REMA (7.75 mg, 13.1 μmol), V70 (0.331 mg, 1.07 μmol) as 100 μL of 3.31 mg mL^{-1} solution in dioxane, and 1.2 mL of DMSO. The polymerization solution was then septa-sealed and purged with nitrogen for 30 minutes. After this time, the polymerization vials were transferred to a preheated oil bath at 30 $^{\circ}\text{C}$ and allowed to polymerize for 18 hours. After this time, the polymerization solution was precipitated into 45 mL of diethyl ether in 50 mL conical tubes. After vortexing, the solution was centrifuged at 4200 rpm for 5 minutes. The ether supernatant was decanted and yellow polymer oil was diluted to a final volume of 25 mL in 0.5 M phosphate buffer pH 7.4. The aqueous solution was then dialyzed against deionized water at 5 $^{\circ}\text{C}$ using spectra/Por regenerated cellulose dialysis membranes (6-8 kDa cutoff) and then isolated by lyophilisation. The lyophilized polymer was then redissolved in deionized water and then purified by double Sephadex G-25 prepacked PD10 columns according to the manufacturer's instructions.

Methods

Gel permeation chromatography (GPC). Absolute molecular weights and polydispersity indices were determined using Tosoh SEC TSK-GEL α -3000 and α -e4000 columns (Tosoh Bioscience, Montgomeryville, PA) connected in series to an Agilent 1200 Series Liquid Chromatography System (Santa Clara, CA) and Wyatt Technology miniDAWN TREOS, 3 angle MALS light scattering instrument and Optilab TrEX, refractive index detector (Santa Barbara, CA). HPLC-grade DMF containing 0.1 wt.% LiBr at 60 °C was used as the mobile phase for p(hECT), p(aECT), and poly(DMA) at a flow rate of 1 mL/min. Copolymers containing mannose were evaluated in an aqueous eluent consisting of 150 mM sodium acetate buffer at pH 4.4.

Dynamic Light Scattering. Particle sizes of the polymers were measured by dynamic light scattering (DLS) using a Malvern Zetasizer Nano ZS. Samples were prepared by dissolving lyophilized polymer in phosphate buffer saline (150 mM NaCl, 20 mM phosphates, pH 7.4), at a concentration of 1 mg mL⁻¹.

Transmission Electron Microscopy (TEM). 0.5 mg mL⁻¹ samples were applied to glow-discharged continuous carbon film EM grids and negatively stained using 1% uranyl formate. Grids were imaged by transmission electron microscopy using an FEI Morgagni operating at 100 kV and a Gatan Orius Camera.

Polymer hydrolysis studies. The hydrolytic stability of poly(DMA) RSNs polymerized from p(hECT) and p(aECT) cores was evaluated at pH 4.8 and 7.4 in 150 mM acetate and phosphate buffer respectively. Polymers were dissolved in the buffers at a concentration of 25 mg mL⁻¹ and then incubated at 37 °C for the appropriate time period. Prior to analysis, a 200 μ L aliquot was diluted into 800 μ L of DMF. The sample was analyzed directly via gel permeation chromatography as described above.

Analysis of ciprofloxacin by high-performance liquid chromatography (HPLC). The HPLC analysis of ciprofloxacin was carried out with an Agilent 1260 Quaternary HPLC Pump, Agilent 1260 Infinity Standard Automatic Sampler, Agilent 1260 Infinity Programmable Absorbance Detector, and Agilent ChemStation software for LC system (Palo Alto, CA). Both ciprofloxacin hydrochloride and liquid Sera Human from AB blood donor were purchased and used as received. The analyte was separated at ambient temperature using a Zorbax RX-C18 (4.6 $\times$ 150 mm; 5 μ m) analytical column (Agilent Technologies, CA).

The UV detector was operated at 277 nm, and the mobile phase consisted of 2% aqueous acetic acid and acetonitrile (84:16) v/v, as described elsewhere. The flow rate was set at 1.0 mL min⁻¹ and sample injection volume at 20 µL. A stock solution of ciprofloxacin was prepared in deionized water at 10 mg mL⁻¹. Working solutions of ciprofloxacin for standard curves were diluted from stock solution using the mobile phase to the listed concentrations of 200 µg mL⁻¹, 100 µg mL⁻¹, 50 µg mL⁻¹, 25 µg mL⁻¹, 12.5 µg mL⁻¹, 6.25 µg mL⁻¹, 3.12 µg mL⁻¹, and 1.56 µg mL⁻¹. Each listed solution above was diluted with a 1:1 v/v ratio of either mobile phase:deionized water or mobile phase: human serum to create a final ciprofloxacin standards of 100 µg mL⁻¹, 50 µg mL⁻¹, 25 µg mL⁻¹, 12.5 µg mL⁻¹, 6.25 µg mL⁻¹, 3.12 µg mL⁻¹, 1.56 µg mL⁻¹, and 0.78 µg mL⁻¹ for pharmaceutical and biological analysis, respectively. Both non-serum (mobile phase:deionized water) and serum standards were subsequently diluted to 50% (v/v) with acetonitrile to promote protein precipitation. Serum standards were centrifuged at 12 000g for 15 minutes and supernatants were collected and filtered using a 0.45 µm low protein binding filter before HPLC analysis. Non-serum standards were analyzed without the need for centrifugation. All standards were processed using a gradient HPLC elution profile, where the mobile phase transitioned to 100% acetonitrile over 15 minutes, followed by 10 minutes of column washing with acetonitrile and water and 5 minutes of equilibration with mobile phase.

Drug release from polymeric prodrugs. Drug release from polymeric prodrugs was carried out in human serum at 37 °C at a polymer concentration of 6 mg mL⁻¹. Sample time points were collected on a regular basis. Quantification of total ciprofloxacin in the polymeric prodrugs was measured by taking 6 mg mL⁻¹ of polymer and dissolving it in 10% aq. H₂SO₄ for 48 h at 25 °C. The HPLC with a gradient elution profile was used to quantify amount of drug released using the same instrument parameters set forth for drug standards. A 1:1 dilution of serum sample to 2% aqueous acetic acid and acetonitrile (84:16) v/v was conducted, followed by another 1:1 dilution with acetonitrile. The resulting samples were vortexed and centrifuged at 12 000g for 15 minutes. Supernatants were collected and filtered using a 0.45 µm low protein-binding filter before running on the HPLC.

In vitro uptake studies. Experiments were conducted with MPI cells (passaged with 30 ng mL⁻¹ murine GM-CSF) seeded at 200,000 cells/well. The cells were treated with 20 µg mL⁻¹ of rhodamine labeled polymers (for 0.5 h, 2h, and 5h). To make sure

equivalent fluorescence was dosed for all treatment groups, standard curves were calculated as a function of polymer concentration. At each time point, the cells were collected, washed, and re-suspended with cold PBS containing 0.2% fetal bovine serum (FBS) to remove unbound polymer. All samples were kept on ice and uptake/association was detected using the Y1 channel (rhodamine B). Statistical analysis was performed by Student's paired t test. (*) denotes a P-value of ≤ 0.05 . (**) denotes a P-value of ≤ 0.005 . Error bars are reported as standard deviations (SDs). All samples were performed in triplicate unless noted otherwise.

In vitro co-culture activity using a *B. thailandensis* infection model. RAW 264.7 cells were seeded (700,000 cells/mL, 250 μ L/well) into a 48 well plate with antibiotic free Dulbecco's modified eagle medium (DMEM) containing 10% FBS, and incubated at 37°C with 5% CO₂. After 18 hours, cells were infected with *F. novicida* U112 at early log phase of growth (OD₆₀₀=0.2) at a multiplicity of infection of 50, and then incubated for 1 hour. Subsequently, growth media was replaced with fresh DMEM containing 10% FBS and 250 μ g/mL kanamycin to eliminate extracellular bacteria not internalized by the cells; cells were then incubated for another hour. Growth media was then replaced with fresh DMEM containing free ciprofloxacin or mannose-targeted RSN (equivalent to 1 or 10 μ g/mL ciprofloxacin). Cells were incubated for another 22 hours (24 hours post-infection). After incubation, cells were washed three times with 1x PBS and lysed with 100 μ L of PBS containing 0.1% [v/v] Triton X-100. Lysates were serially diluted and plated onto triplicate TSB agar plates and incubated for 24 hours. CFUs were counted when individual bacterial colonies were distinguishable.

Acute lung safety of ciprofloxacin delivery systems. In order to determine the in vivo biocompatibility of the polymeric prodrugs after pulmonary administration, the lung toxicity profiles of endotracheally delivered poly(MEM-co-CTM) RSNs were evaluated using the metrics of animal weight change, tumor necrosis factor alpha (TNF- α) concentration in lung tissue homogenate (LTH) and bronchoalveolar lavage fluid (BALF), and neutrophil infiltration into the lungs. The mice were anesthetized with 5% isoflurane for 5 min before administration of 50 μ L per mouse of PBS only (Corning 21-040-CV), or 20 mg/kg ciprofloxacin (n = 5) as polymer formulations in PBS, pH 7.4. All solutions were filtered (0.2 μ m) and administered via endotracheal delivery using a Microsprayer® aerosolizer designed for use on mice (Penn-Century MSA-250-M, PA, USA). Mice were dosed as above once every 24 hours for three consecutive days.

24 hours after the final administration, mice were weighed, and then sacrificed by CO₂ asphyxiation and lavaged by cannulating tracheas with a 22G soft catheter (Exel International 14-841-10) prior to a 1 mL PBS flush and followed by three 0.8 mL flushes. Approximately 3 mL of lavage fluid was recovered per mouse. Lungs were removed,
5 weighed, and placed into 1 mL PBS on ice. Lung tissues were mechanically homogenized with a Qiagen TissueRuptor (9001271) before addition of 1 mL of lysis buffer [PBS+1% Triton X-100 and 1 protease tablet/10 mL (Roche 1836153001)]. BALF was spun at 1,000 g for 15 min to pellet lavage cells. BAL cells were re-suspended into 0.5 mL RPMI 1640+10% FBS, mounted onto microscopy slides with a Cytospin centrifuge at
10 46 g for 5 min, and then stained with Hemacolor (EMD Millipore 65044) prior to cytology analysis. Stained slides were analyzed for macrophage to neutrophil ratios with a minimum of 200 cells counted per slide. Cell-free BALF and LTH TNF- α concentration was assayed using Biolegend's paired TNF- α ELISA kit (430902).

15 While illustrative embodiments have been illustrated and described, it will be appreciated that various changes can be made therein without departing from the spirit and scope of the invention.

CLAIMS

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A radiant star nanoparticle, comprising:
 - (a) a hyperbranched polymeric core comprising a homopolymerized transmer product,
wherein the core comprises polymeric branches covalently attached to a polymer backbone at branch points, and
wherein at least a portion of the polymeric branches comprise degradable groups;
and
 - (b) a plurality of polymeric arms extending from and covalently attached to the core,
wherein the polymeric arms comprises repeating units,
wherein at least a portion of the repeating units include therapeutic agent moieties,
and
wherein the portion of repeating units that include therapeutic moieties include degradable groups effective to release a therapeutic agent from the nanoparticle.
2. The nanoparticle of Claim 1, wherein the core's degradable group is selected from the group consisting of alkyl ester, acetal, hemiacetal, phenyl ester, thioester, disulfide, and enzyme cleavable peptide groups.
3. The nanoparticle of Claim 1, wherein each repeating unit that includes a therapeutic agent moiety has the therapeutic agent moiety pendant from the backbone of the polymeric arm, and a degradable group intermediate the backbone and the therapeutic agent moiety.
4. The nanoparticle of Claim 3, wherein the degradable group is selected from the group consisting of alkyl ester, phenyl ester, acetal, and hemiacetal groups.
5. The nanoparticle of Claim 1, wherein each polymeric arm further comprises a plurality of repeating units, each repeating unit comprising a functional group effective to increase the water solubility of the nanoparticle.

6. The nanoparticle of Claim 1, wherein each polymeric arm further comprises a plurality of repeating units, each repeating unit comprising a targeting group effective to accumulate the nanoparticle at a pre-determined site.

7. The nanoparticle of any one of Claims 1-6, having from about 10 to about 100 polymeric arms.

8. The nanoparticle of any one of Claims 1-6, wherein each polymeric arm is a homopolymer, a random copolymer, a block copolymer, or a gradient block copolymer.

9. The nanoparticle of any one of Claims 1-6, wherein each polymeric arm has from about 10 to about 500 repeating units.

10. The nanoparticle of any one of Claims 1-6, having a number-average molecular weight from about 0.3 to about 1.5 million Da.

11. The nanoparticle of any one of Claims 1-6, having a molar mass dispersity from about 1.3 to about 2.0.

12. The nanoparticle of any one of Claims 1-6, having a hydrodynamic diameter from about 20 to about 50 nm, as measured by dynamic light scattering.

13. A method for making a radiant star nanoparticle having a hyperbranched polymeric core and a plurality of polymeric arms extending from and covalently coupled to the core, the method comprising:

(a) polymerizing one or more transmers to provide a hyperbranched polymeric core in which at least portions of the core terminate with a charge transfer agent, wherein the transmer comprises a degradable group intermediate a polymerizable group and a chain transfer agent; and

(b) reverse addition fragment transfer (RAFT) polymerizing a prodrug monomer from the polymeric core to provide polymeric arms extending from and covalently coupled to the core.

14. The method of Claim 13, wherein the polymerizing the prodrug monomer comprises copolymerizing the prodrug monomer with a comonomer.

15. The method of Claim 14, wherein the ratio of prodrug monomer to comonomer is from about 1 to about 2.

16. The method of Claim 14, wherein the comonomer is selected from the group consisting of water-solubilizing comonomers and targeting comonomers.

17. The method of Claim 16, wherein the comonomer is selected from the group consisting of glycerol methacrylate, DMAPS, PEGMA 300 or 950, DMAEMA, SMA, mannose methacrylate, hydroxyethyl acrylamide, and combinations thereof.

18. The method of Claim 13, wherein polymerizing the transmer and polymerizing the prodrug monomer are conducted in a single reaction vessel.

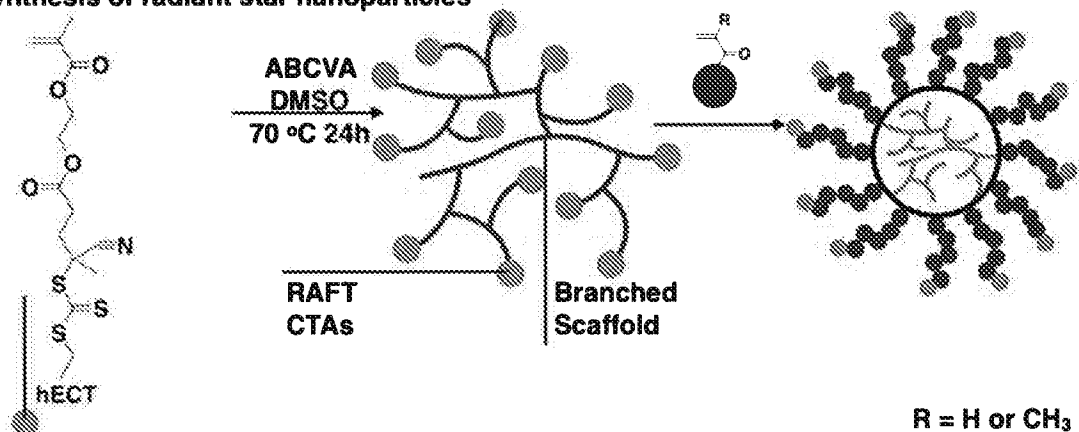
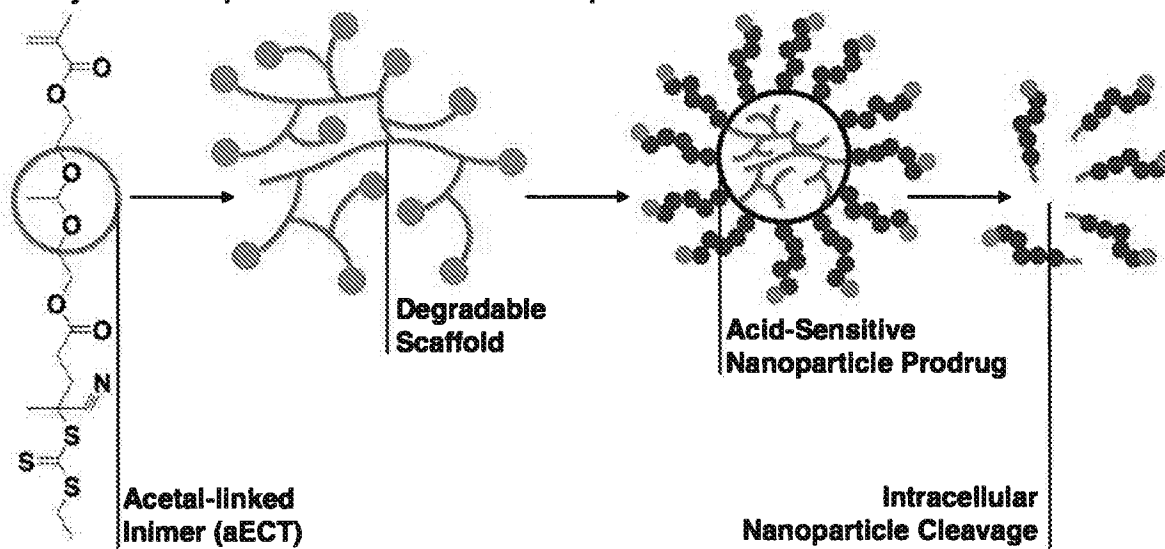
19. The method of Claim 13, wherein the branched polymeric core having at least a portion of the branched polymeric core terminating with a charge transfer agent is isolated prior to polymerizing the prodrug monomer from the polymeric core.

20. A pharmaceutical composition, comprising the radiant star nanoparticle of any one of Claims 1-12 and a pharmaceutically acceptable carrier.

21. A method for the intracellular delivery of a therapeutic agent, comprising contacting a cell with the radiant star nanoparticle of any one of Claims 1-12.

22. A method for treating or preventing a disease, disorder, or condition treatable by a specific therapeutic agent, the method comprising administering an effective amount of the radiant star nanoparticle of any one of Claims 1-12 to subject in need thereof, wherein the therapeutic agent released from the radiant star nanoparticle is the specific therapeutic agent effective to treat the disease, disorder, or condition.

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a. Synthesis of radiant star nanoparticles*Fig. 1A***b. Synthesis of pH-sensitive starburst nanoparticles***Fig. 1B*

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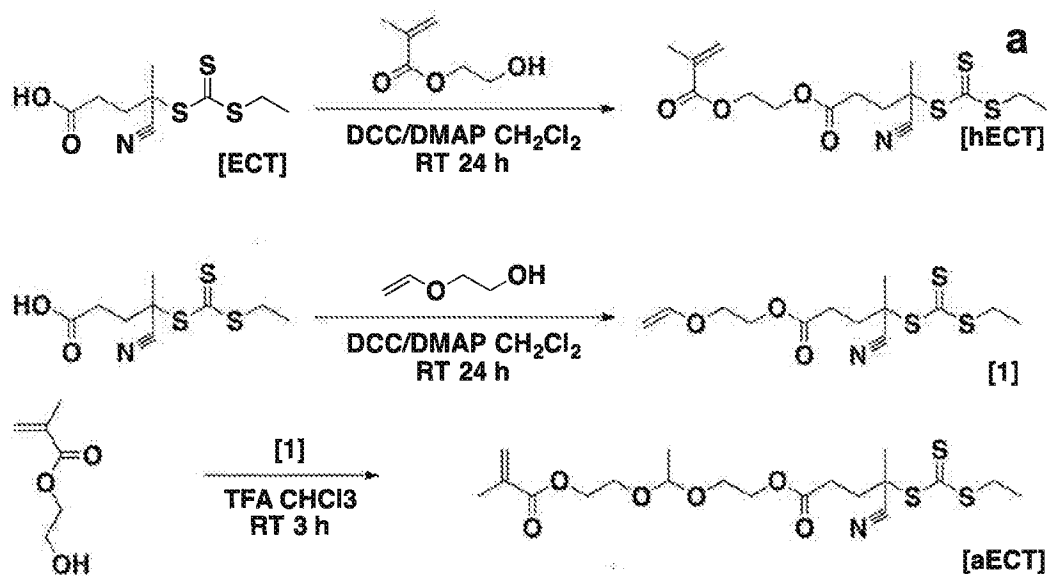


Fig. 2A

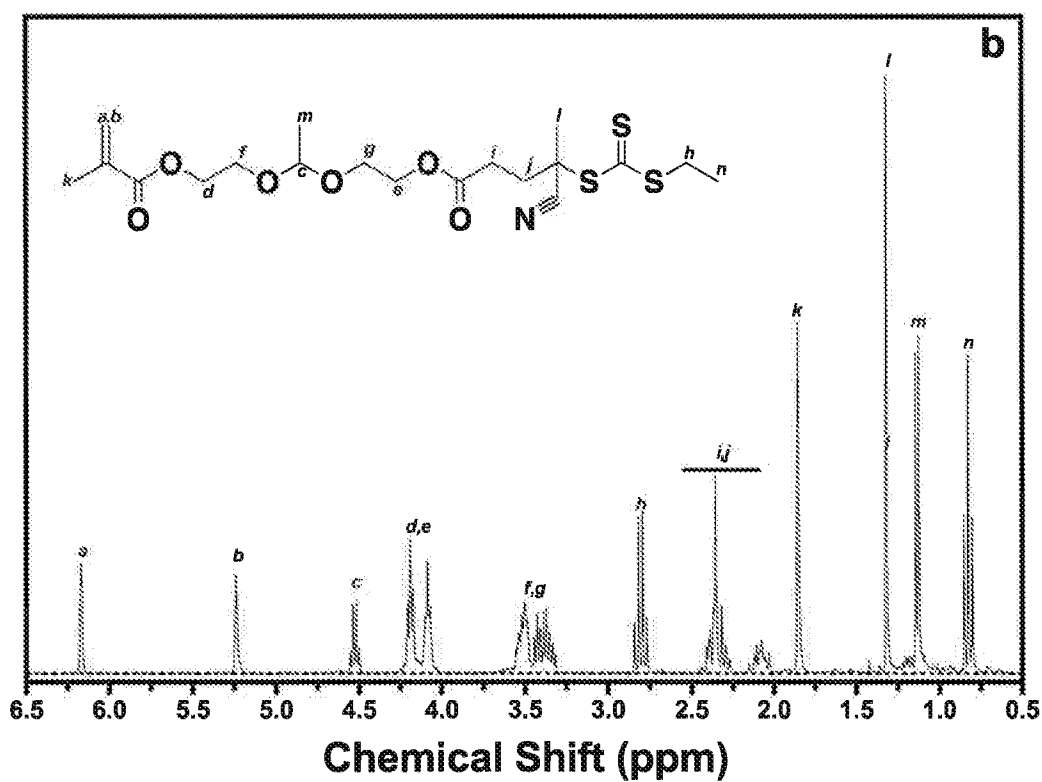
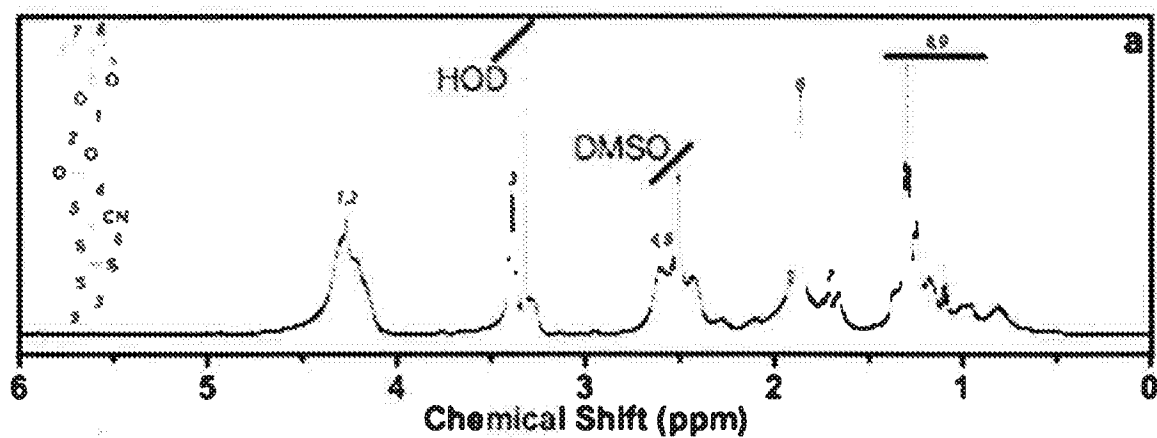
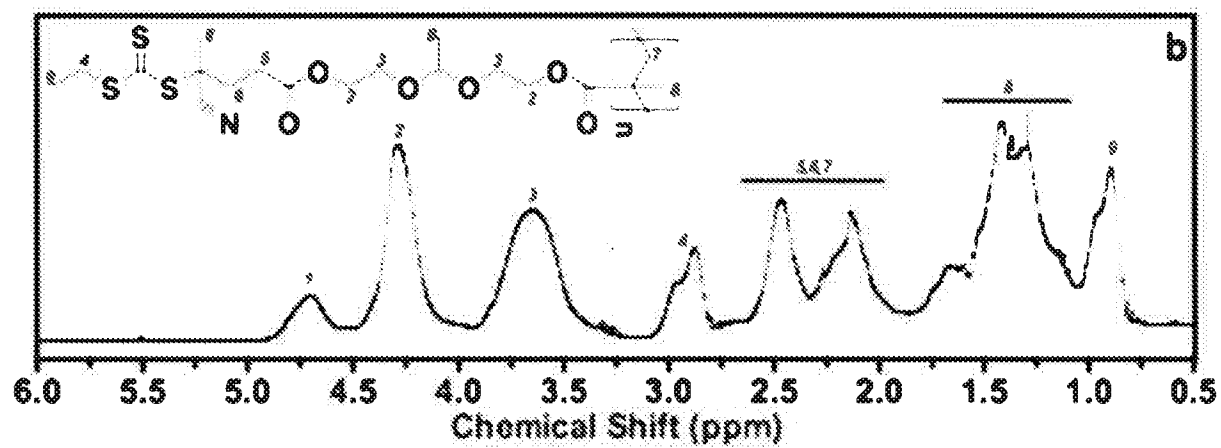
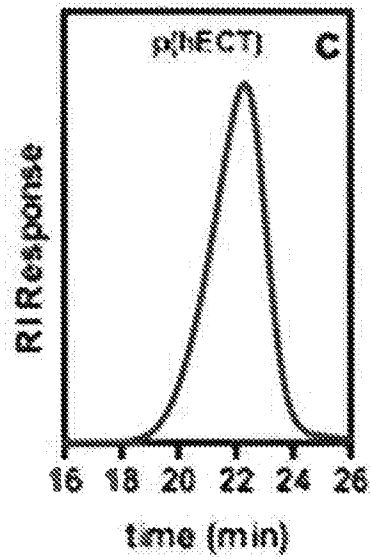
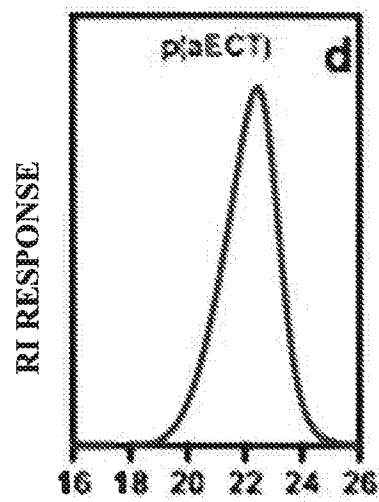
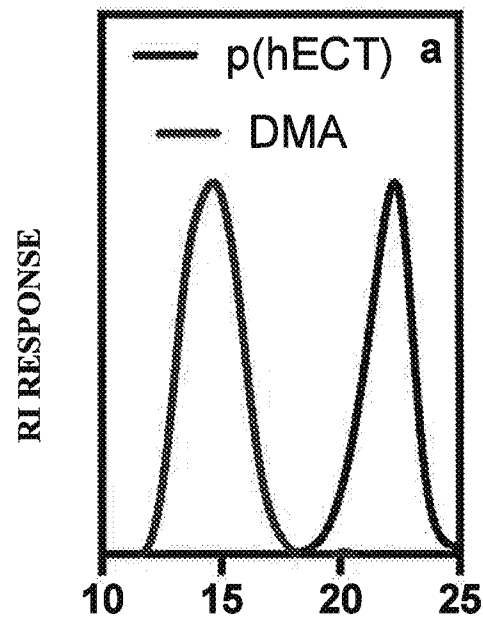
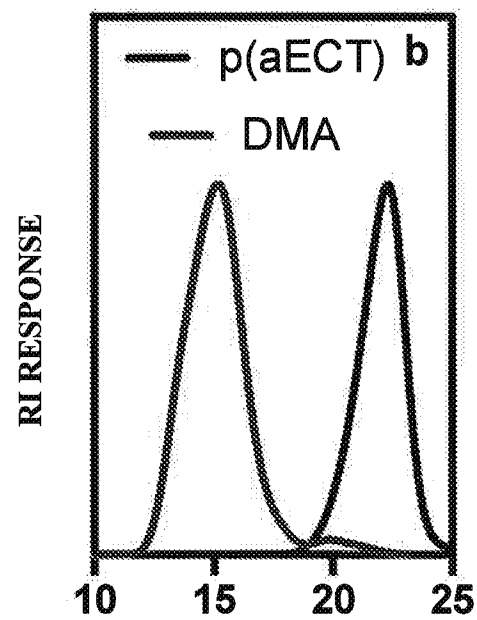


Fig. 2B

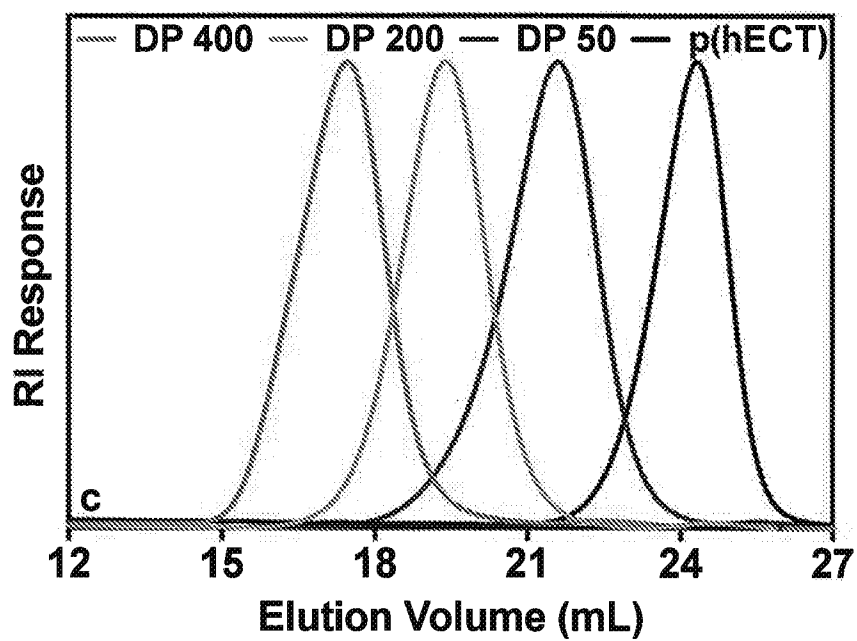
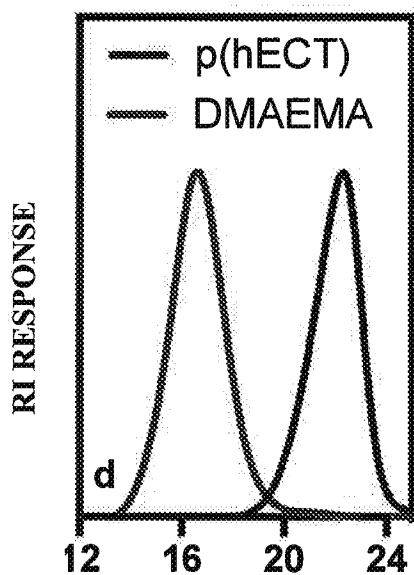
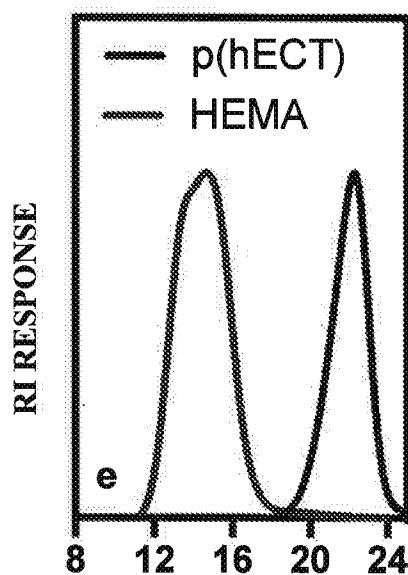
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*Fig. 3A**Fig. 3B*

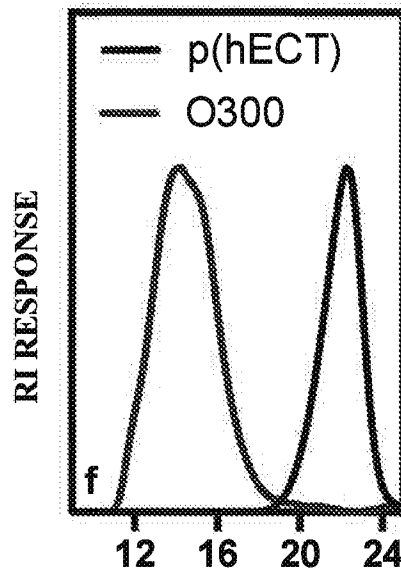
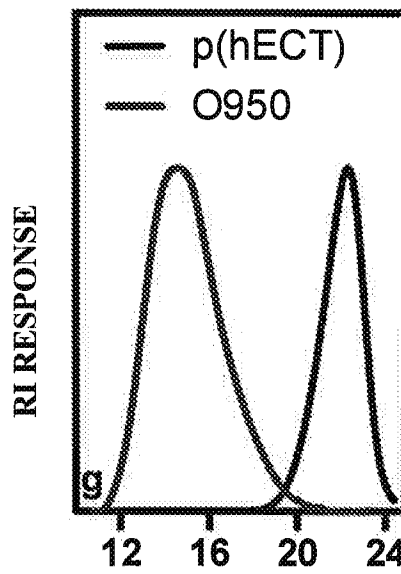
4/15*Fig. 3C**Fig. 3D*

5/15*Fig. 4A**Fig. 4B*

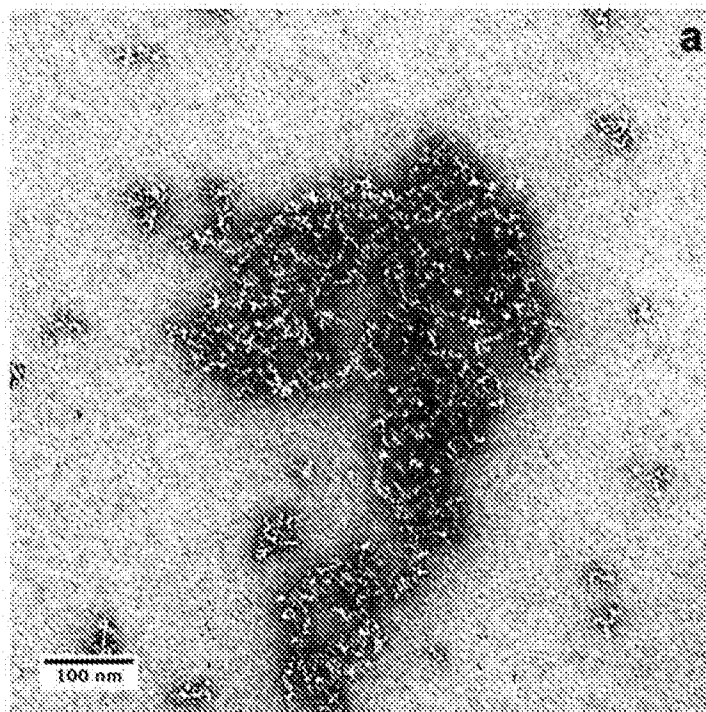
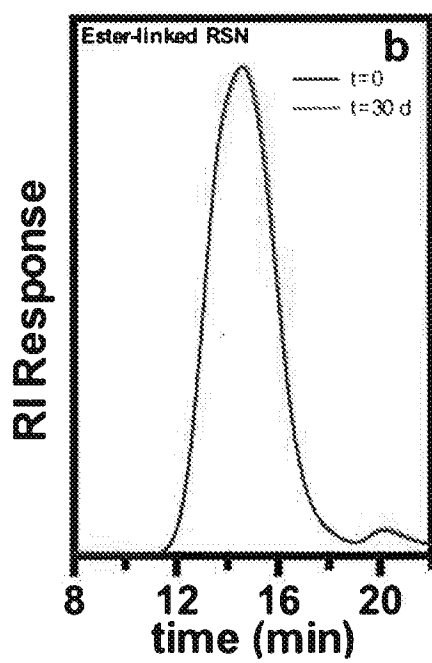
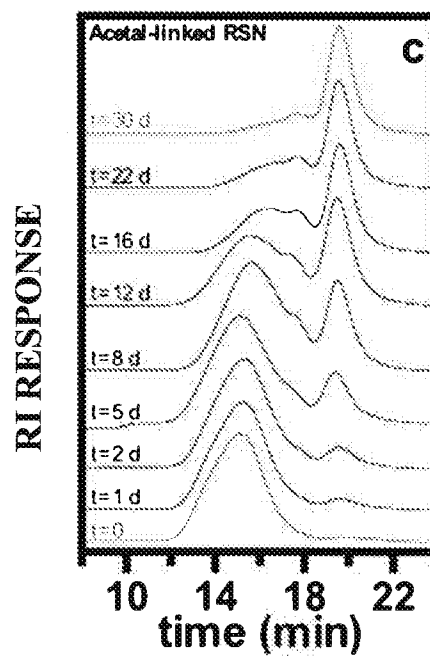
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*Fig. 4C**Fig. 4D**Fig. 4E*

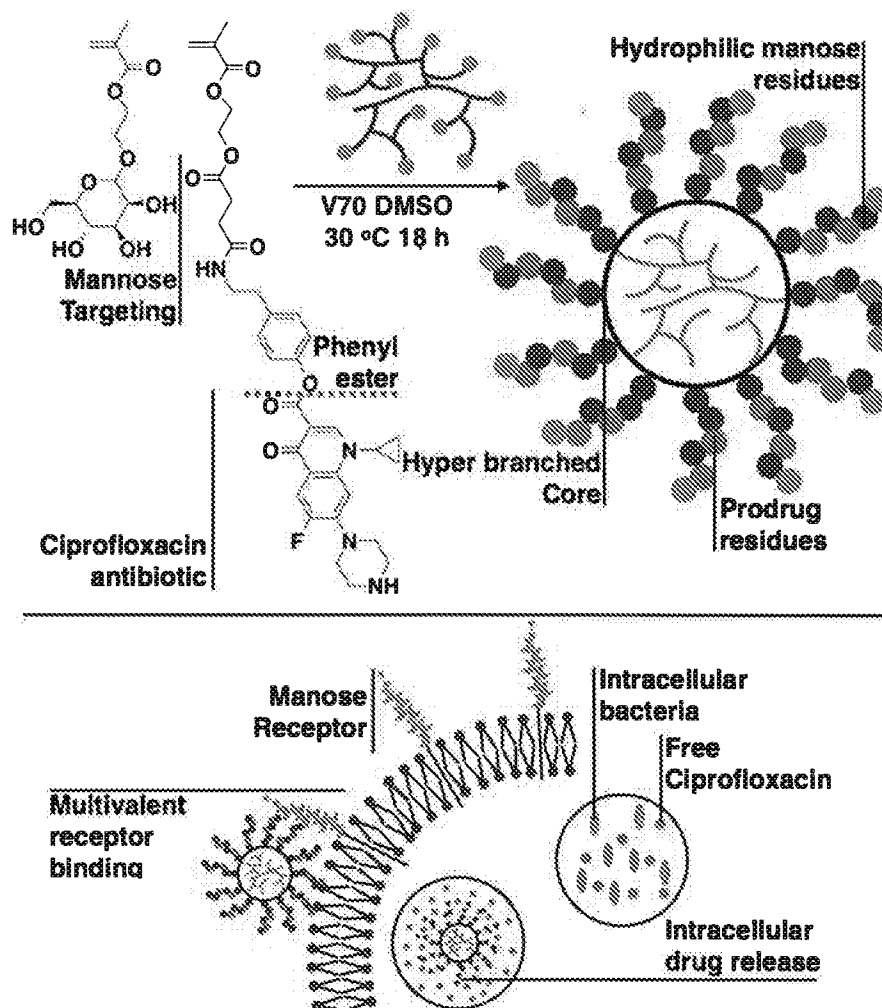
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*Fig. 4F**Fig. 4G*

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*Fig. 5A**Fig. 5B**Fig. 5C*

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*Fig. 6*

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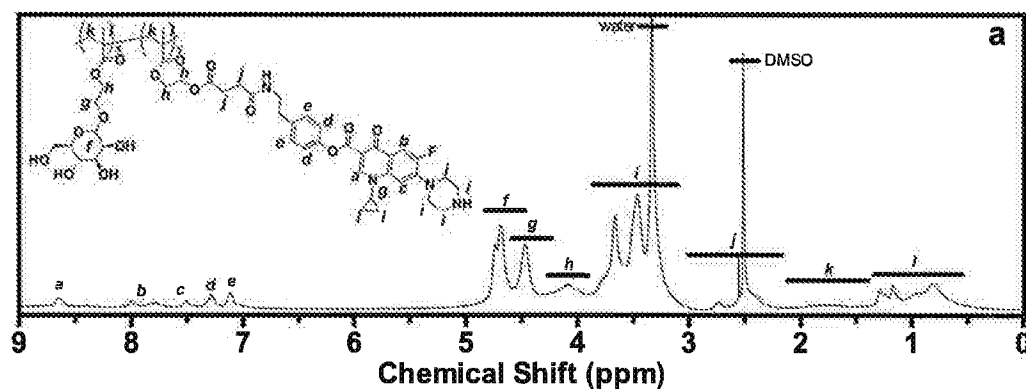


Fig. 7A

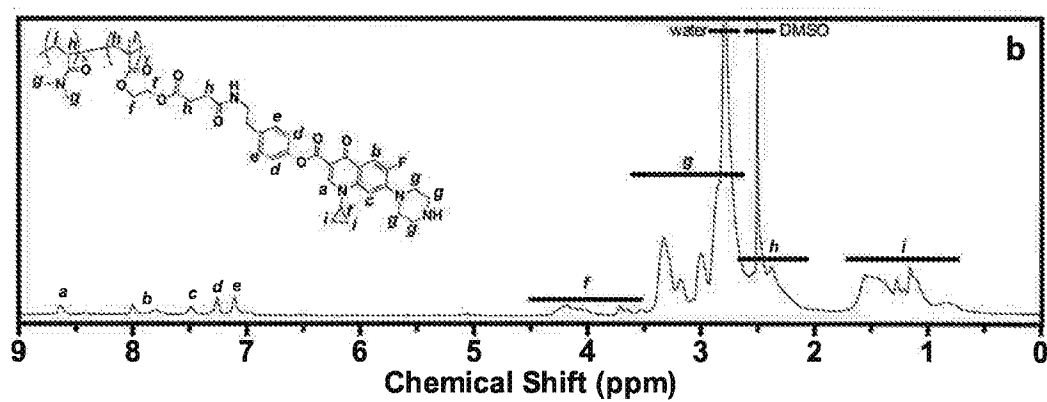


Fig. 7B

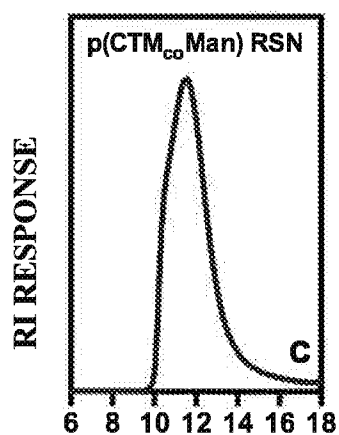


Fig. 7C

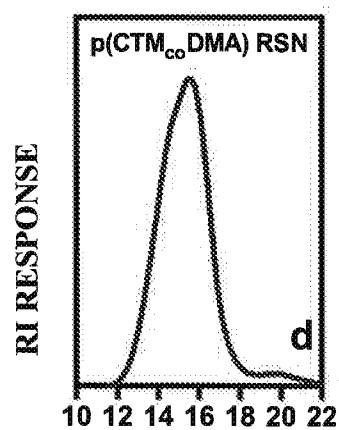
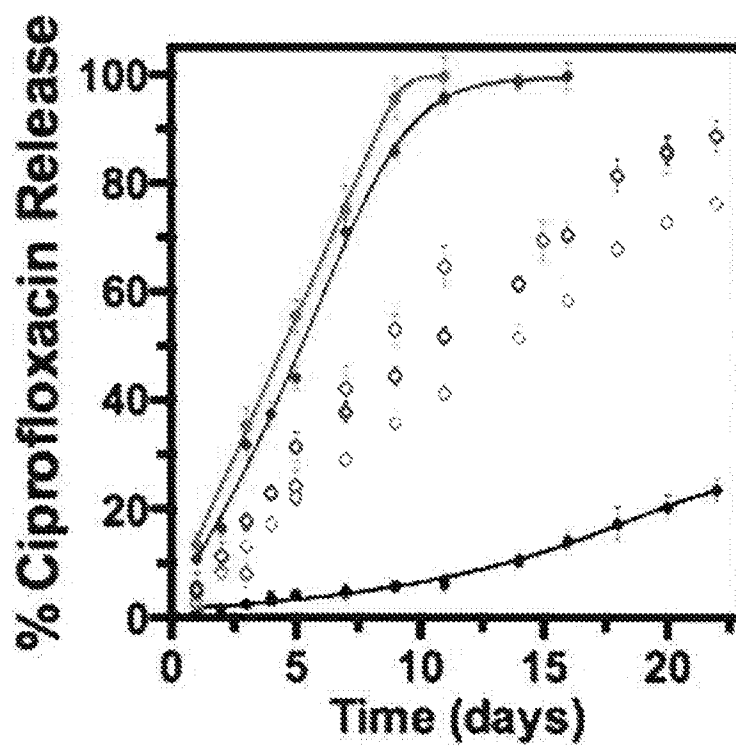
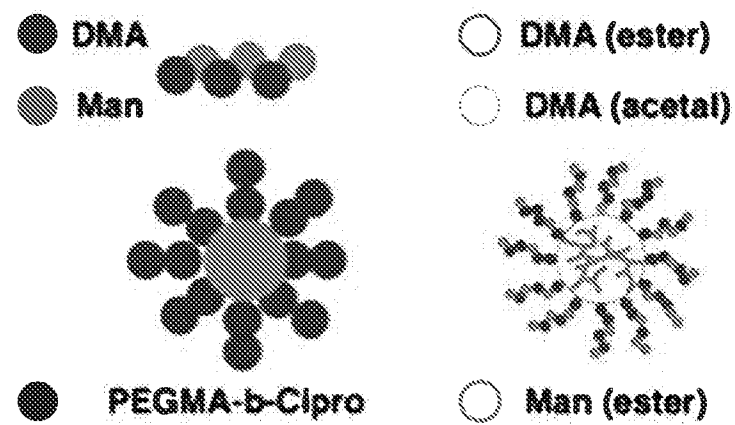
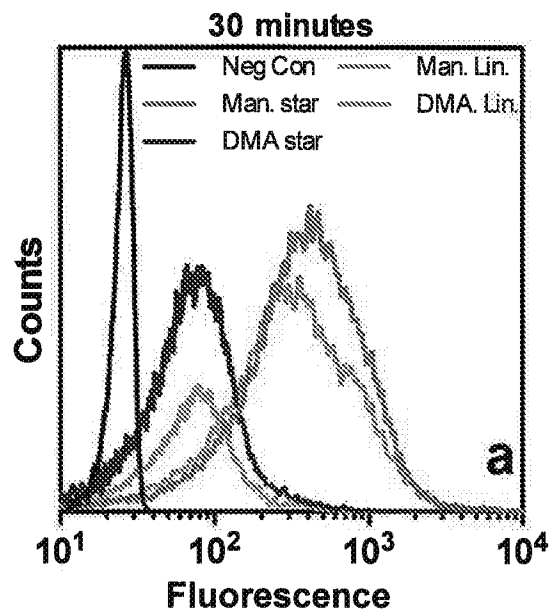
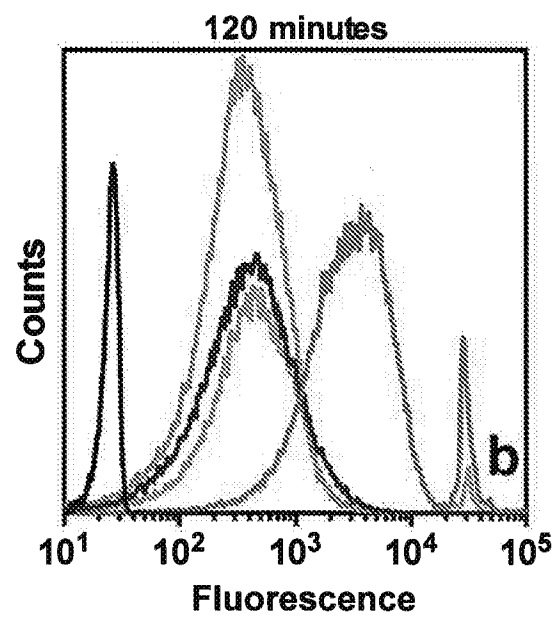
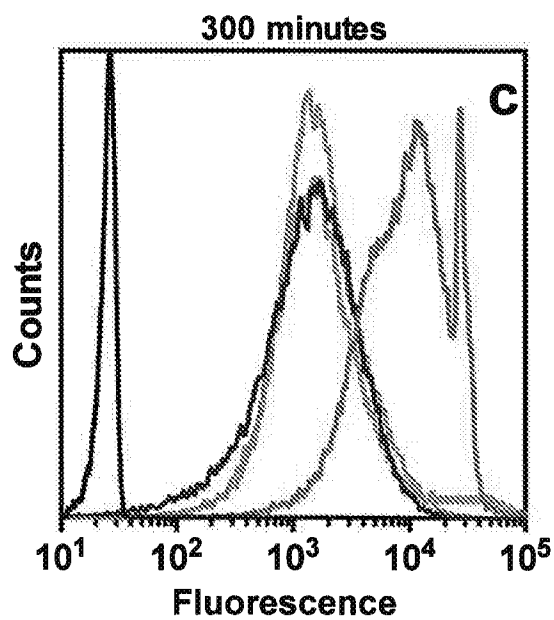
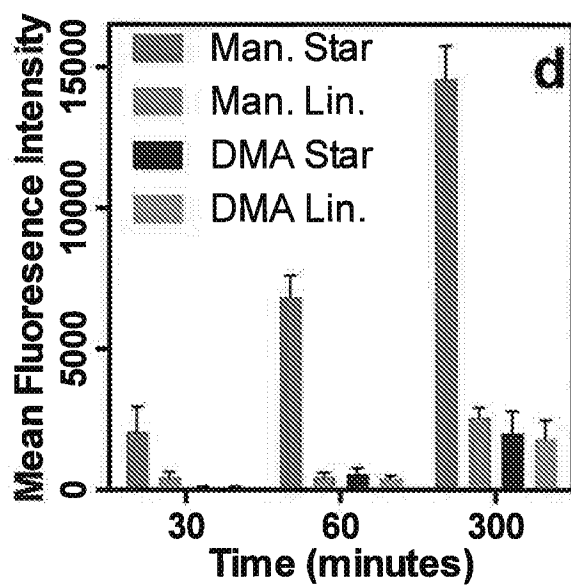


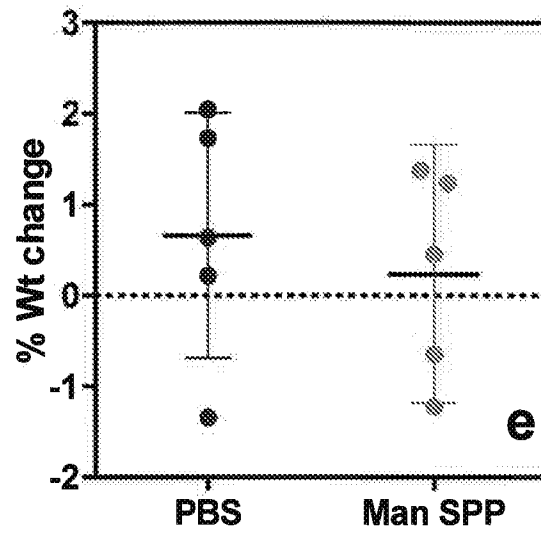
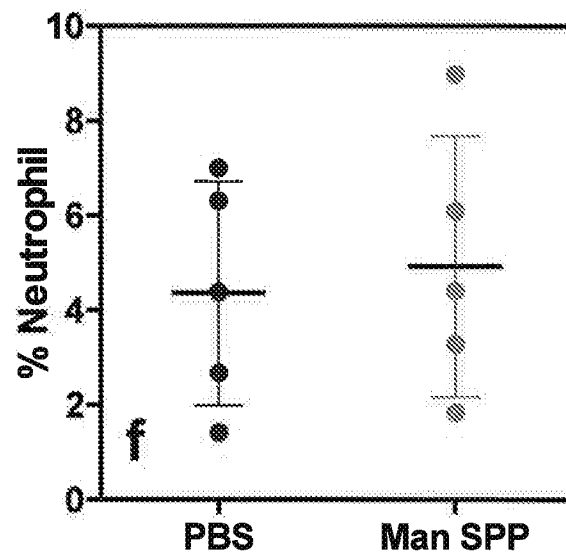
Fig. 7D

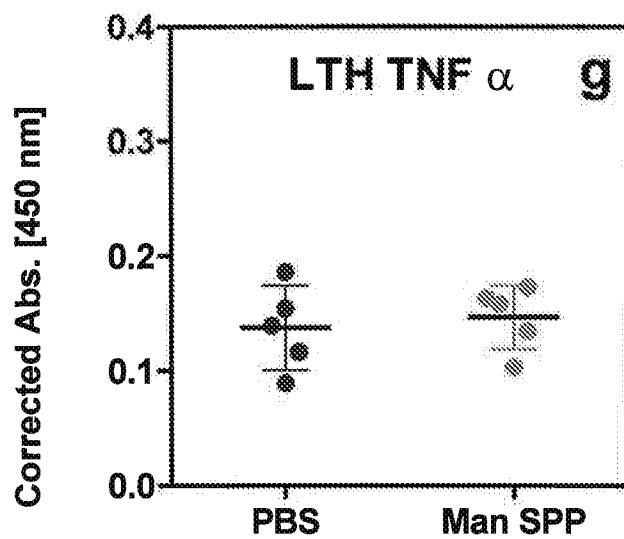
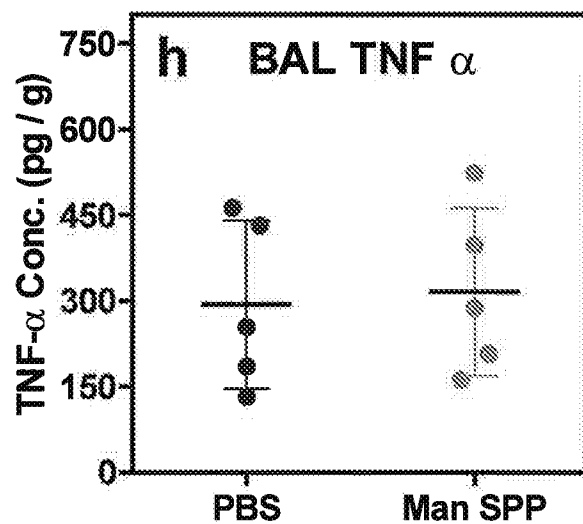
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*Fig. 8*

12/15*Fig. 9A**Fig. 9B*

13/15*Fig. 9C**Fig. 9D*

14/15*Fig. 9E**Fig. 9F*

15/15*Fig. 9G**Fig. 9H*

INTERNATIONAL SEARCH REPORT

PCT/US19/17888

International application No.

PCT/US19/17888

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 9/14, 47/56, 47/58, 47/59; B82Y 5/00; C08F 293/00; C08G 83/00 (2019.01)

CPC - A61K 9/14, 47/56, 47/58, 47/59; B82Y 5/00; C08F 293/00; C08G 83/00, 83/002, 83/003

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

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

See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DAS, D. Inhalable Macromolecular Prodrugs of Ciprofloxacin for the Treatment of Pulmonary Intracellular Bacterial Infections (Doctoral dissertation) CHAPTER 6. Radiant star nanoparticle prodrugs for the treatment of intracellular alveolar infections. 2017 [oline], [retrieved on 2019-03-21]. Retrieved from the Internet <URL: https://digital.lib.washington.edu/researchworks/bitstream/handle/1773/40844/Das_washington_0250E_17963.pdf?sequence=1&isAllowed=y >; entire document, in particular pages 138, 145, 152-163; figures 6.1 and 6.3	1-6, 7/1-6, 8/1-6, 9/1-6, 10/1-6, 11/1-6, 12/1-6, 13-19

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

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

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

21 March 2019 (21.03.2019)

Date of mailing of the international search report

24 APR 2019

Name and mailing address of the ISA/

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-8300

Authorized officer

Shane Thomas

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 20-22
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.