

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
24 August 2006 (24.08.2006)

PCT

(10) International Publication Number
WO 2006/089007 A2

(51) International Patent Classification:
A61K 47/48 (2006.01) **A61K 41/00** (2006.01)

[US/US]; 575 Laguna Road, Pasadena, California 91125 (US).

(21) International Application Number:
PCT/US2006/005448

(74) Agents: **VINCENT, Matthew P.** et al.; ROPES & GRAY LLP, One International Place, Boston, Massachusetts 02110-2624 (US).

(22) International Filing Date:
15 February 2006 (15.02.2006)

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

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/653,409 16 February 2005 (16.02.2005) US

(71) Applicant (for all designated States except US): **INSERT THERAPEUTICS, INC.** [US/US]; 2585 Nina Street, Pasadena, California 91107 (US).

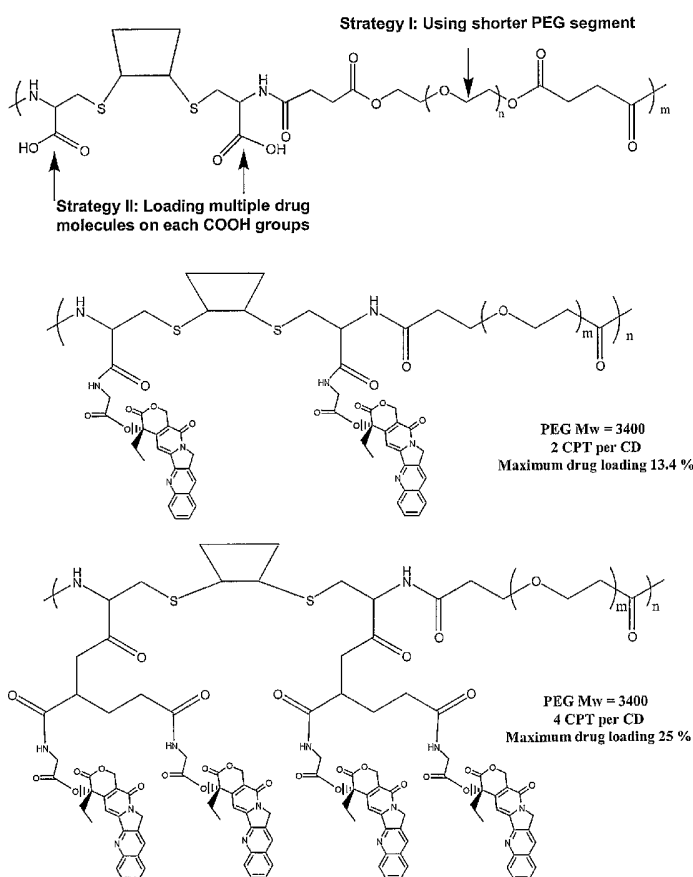
(72) Inventor; and

(75) Inventor/Applicant (for US only): **DAVIS, Mark E.**

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),

[Continued on next page]

(54) Title: CYCLODEXTRIN-BASED POLYMERS FOR THERAPEUTICS DELIVERY



(57) Abstract: The present invention relates to novel compositions of therapeutic polymeric compounds designed as carriers for small molecule therapeutics delivery and pharmaceutical compositions thereof. In some embodiments, the small molecule therapeutic is attached to the polymer by a photocleavable linker. The polymeric compounds may also employ targeting agents. By selecting from a variety of linker groups and targeting ligands the polymers present methods for controlled delivery of the therapeutic agents. On reaching a targeted site in the body of a patient, the linker can then be cleaved by the shining of ultraviolet, visible, or infrared wavelength light onto the site. The methods provide reduced toxicity and local delivery of therapeutics. The invention also relates to methods of treating subjects with the therapeutic compositions described herein. The invention further relates to methods for conducting a pharmaceutical business comprising manufacturing, licensing, or distributing kits containing or relating to the polymeric compounds described herein.



European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

Published:

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

Cyclodextrin-Based Polymers for Therapeutics Delivery

Related Applications

This application claims the benefit of U.S. Provisional Patent Application
5 60/653,409, filed February 16, 2005, the specification of which is incorporated by reference herein.

Background of the Invention

Drug delivery of some small molecule therapeutic agents, such as
camptothecin, has been problematic due to their poor pharmacological profiles.
10 These therapeutic agents often have low aqueous solubility, their bioactive forms exist in equilibrium with an inactive form, or high systemic concentrations of the agents lead to toxic side-effects. Some approaches to circumvent the problem of their delivery have been to conjugate the agent directly to a water-soluble polymer such as hydroxypropyl methacrylate (HPMA), polyethyleneglycol, and poly-L-
15 glutamic acid. In some cases, such conjugates have been successful in solubilizing or stabilizing the bioactive form of the therapeutic agent, or achieving a sustained release formulation which circumvents complications associated with high systemic concentrations of the agent.

Another approach to the drug delivery problem has been to form host/guest
20 inclusion complexes between the therapeutic agent and cyclodextrins or derivatives thereof. Cyclodextrins (α , β , γ) and their oxidized forms have unique physico-chemical properties such as good water solubility, low toxicity and low immune response. To date, most of the drug delivery studies with cyclodextrins have focused on their ability to form supra-molecular complexes, wherein cyclodextrins
25 form host/guest inclusion complexes with therapeutic molecules and thus alter the physical, chemical, and/or biological properties of these guest molecules.

U.S. Patent 5,276,088 describes a method for synthesizing cyclodextrin-containing polymers by either reacting polyvinyl alcohol or cellulose or derivatives thereof with cyclodextrin derivatives, or by copolymerization of a cyclodextrin
30 derivative with vinyl acetate or methyl methacrylate.

U.S. Patent No. 5,855,900 describes a biodegradable cyclodextrin-containing polymer. The patent discloses a supramolecular-structured biodegradable polymeric assembly comprising a plurality of drug-modified α , β , γ -cyclodextrins and a linear polymeric chain threading through the structural cavity of the
5 cyclodextrins.

There is an ongoing need for new approaches to the delivery of small therapeutic agents that have poor pharmacological profiles such as camptothecin, paclitaxel, doxorubicin, and cyclosporine A.

Summary of the Invention

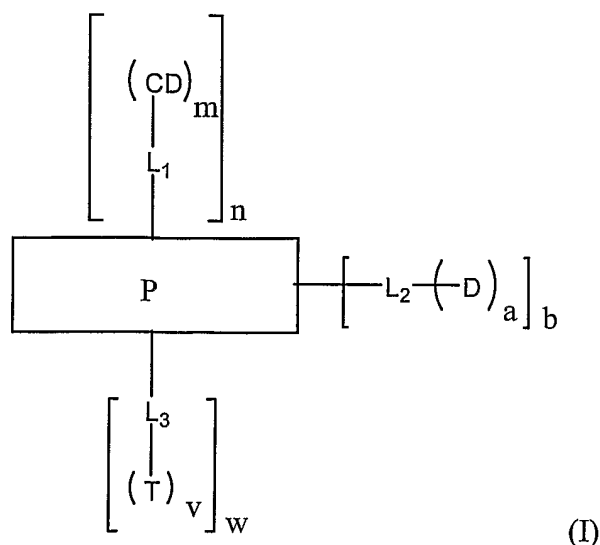
10 The present invention relates to novel compositions of polymer conjugates, defined as polymeric materials covalently coupled to therapeutic/bioactive agents or drugs, as carriers for therapeutics delivery. In one aspect, the present invention provides water-soluble, biocompatible polymer conjugates comprising a water-soluble, biocompatible polymer covalently attached to bioactive moieties through
15 attachments that are cleaved under biological or photolytic conditions to release the bioactive moieties. In certain such embodiments, the polymer comprises cyclic moieties alternating with linker moieties that connect the cyclic structures, e.g., into linear or branched polymers, preferably linear polymers. The polymer may be a polycation, polyanion, a combination thereof, or non-ionic polymer. The bioactive
20 agent, which may be a therapeutic agent, a diagnostic agent, or an adjuvant, preferably makes up at least 5%, 10%, 15%, 20%, 25%, 30%, or even 35% by weight of the conjugate. In certain embodiments, the rate of drug release is dependent primarily upon the rate of cleavage of the attachments, for example by hydrolysis. In certain other embodiments, the rate of drug release is dependent
25 primarily on enzymatic cleavage.

The present invention provides cyclodextrin-containing polymeric compounds for use in drug delivery of these therapeutic agents. The invention also provides compounds for use in controlled drug delivery which are capable of releasing a therapeutic agent in a targeted, predictable, and controlled rate.

Accordingly, one aspect of the present invention is a polymer conjugate comprising cyclodextrin moieties, a therapeutic agent, and an optional ligand targeting agent. The polymer may be linear or branched, and may be formed via polycondensation of cyclodextrin-containing monomers and/or copolymerization
5 between one or more cyclodextrin-containing monomers and one or more comonomers which do not contain cyclodextrin moieties. Furthermore, the present invention also contemplates cyclodextrin-containing polymers formed by grafting cyclodextrin moieties to an already formed polymer. The cyclodextrin moieties contemplated by the present invention include, but are not limited to, α , β , and γ
10 cyclodextrins and oxidized forms thereof.

The therapeutic agent may be attached to a monomer via an optional linker prior to the polymerization step, or may be subsequently grafted onto the polymer via an optional linker. Likewise, the targeting ligand may be attached to a monomer via an optional linker prior to the polymerization step, or may be subsequently
15 grafted onto the polymer via an optional linker, or may be attached to the polymer as an inclusion complex or host-guest interactions. The above linkers may be the same or different and may be cleaved by any method known in the art, particularly by photolytic methods. Cleavage of the linker by photolysis permits release of the therapeutic agent to sites that can be targeted by light energy, especially ultraviolet,
20 visible, and/or infrared wavelengths.

To illustrate further, one embodiment of the invention is a polymeric compound represented by Formula I:



wherein

P represents a linear or branched polymer chain;

CD represents a cyclic moiety such as a cyclodextrin moiety;

5 L_1 , L_2 and L_3 , independently for each occurrence, represent a direct bond or the same or different linker groups;

D, independently for each occurrence, represents a therapeutic agent or a prodrug thereof;

10 T, independently for each occurrence, represents a targeting ligand or precursor thereof;

a, m, and v, independently for each occurrence, represent integers in the range of 1 to 10 (preferably 1 to 8, 1 to 5, or even 1 to 3);

b represents an integer in the range of 1 to about 30,000 (preferably <25,000, <20,000, <15,000, <10,000, <5,000, <1,000, <500, <100, <50, <25, <10, or even <5); and

15 n and w, independently for each occurrence, represents an integer in the range of 0 to about 30,000 (preferably <25,000, <20,000, <15,000, <10,000, <5,000, <1,000, <500, <100, <50, <25, <10, or even <5,

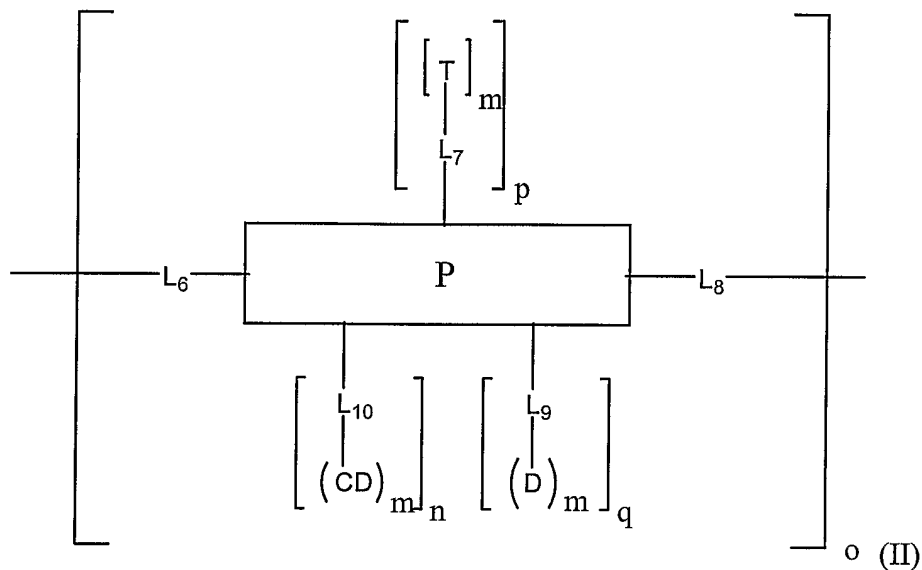
20 wherein either the polymer chain comprises cyclodextrin moieties or n is at least 1.

In certain embodiments, one or more occurrences of L_1 , L_2 , or L_3 , independently represent a photocleavable linker. In certain embodiments, one or

more occurrences of L_2 or L_1 , independently represent a photocleavable linker. In certain embodiments, one or more occurrences of L_2 represent a photocleavable linker.

Another embodiment of the present invention is a compound represented by

5 Formula II:



wherein

P represents a monomer unit of a polymer;

10 T, independently for each occurrence, represents a targeting ligand or a precursor thereof;

L_6 , L_7 , L_8 , L_9 , and L_{10} , independently for each occurrence, represent a direct bond or the same or different linker groups;

CD, independently for each occurrence, represents a cyclic moiety such as a cyclodextrin moiety or a derivative thereof;

15 D, independently for each occurrence, represents a therapeutic agent or a prodrug form thereof;

m, independently for each occurrence, represents an integer in the range of 1 to 10 (preferably 1 to 8, 1 to 5, or even 1 to 3);

o represents an integer in the range of 1 to about 30,000 (preferably <25,000, <20,000, <15,000, <10,000, <5,000, <1,000, <500, <100, <50, <25, <10, or even <5); and

20

p, n, and q, independently for each occurrence, represent an integer in the range of 0 to 10 (preferably 0 to 8, 0 to 5, 0 to 3, or even 0 to about 2),

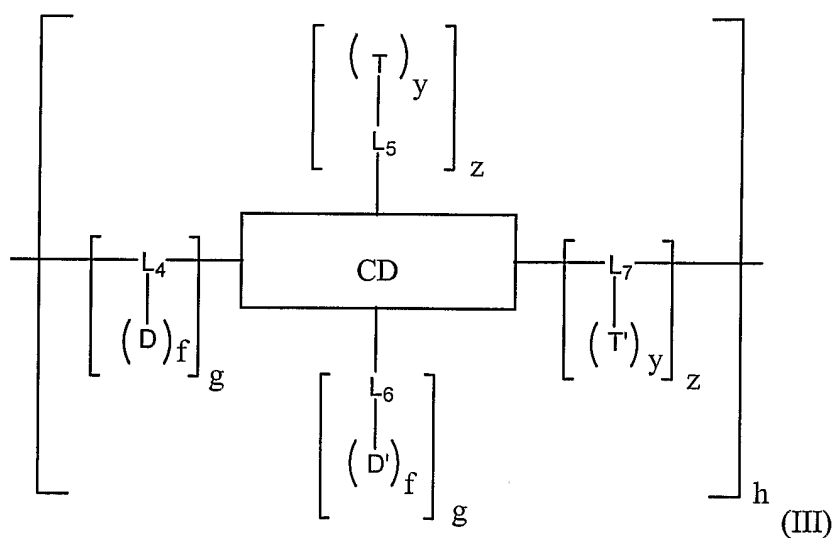
wherein CD and D are preferably each present at least once (preferably at least 5, 10, 25, 50 or even >100 times) in the compound.

5

In certain embodiments, one or more occurrences of L₆, L₇, L₈, or L₁₀, independently represent a photocleavable linker. In certain embodiments, one or more occurrences of L₇, L₉, or L₁₀, independently represent a photocleavable linker. In certain embodiments, one or more occurrences of L₉ or L₁₀, independently represent a photocleavable linker. In certain embodiments, one or more occurrences of L₉ represent a photocleavable linker.

10

Another embodiment of the present invention is a compound represented by Formula III:



15

wherein

CD represents a cyclic moiety such as a cyclodextrin moiety, or derivative thereof;

L₄, L₅, L₆, and L₇, independently for each occurrence, represent a direct bond or the same or different a linker groups;

20

D and D', independently for each occurrence, represent the same or different therapeutic agents or prodrugs thereof;

T and T', independently for each occurrence, represent the same or different targeting ligands or precursors thereof;

f and y, independently for each occurrence, represent an integer in the range of 1 and 10 (preferably 1 to 8, 1 to 5, or even 1 to 3);

5 g and z, independently for each occurrence, represent an integer in the range of 0 and 10 (preferably 0 to 8, 0 to 5, 0 to 3, or even 0 to about 2); and

h represents an integer in the range of 1 and 30,000 (preferably <25,000, <20,000, <15,000, <10,000, <5,000, <1,000, <500, <100, <50, <25, <10, or even <5),

10 wherein at least one occurrence (and preferably at least 5, 10, or even at least 20, 50, or >100 occurrences) of g represents an integer greater than 0.

In certain embodiments, one or more occurrences of L₄, L₅, L₆, or L₇, independently represent a photocleavable linker. In certain embodiments, one or
15 more occurrences of L₄, L₆, or L₇, independently represent a photocleavable linker. In certain embodiments, one or more occurrences of L₄ or L₆, particularly L₆, independently represent a photocleavable linker.

Another aspect of the present invention is a method for preparing the therapeutic cyclodextrin-containing polymeric conjugates described herein.

20 Another aspect of the present invention is a pharmaceutical composition comprising a compound or polymer as discussed above.

Another aspect of the present invention is a pharmaceutical dosage form comprising a polymeric conjugate as described herein.

25 Another aspect of the present invention is a method for treating a subject comprising administering a therapeutically effective amount of any of the polymeric conjugates described herein.

Another aspect of the present invention is a method of conducting a pharmaceutical business comprising manufacturing, licensing, or distributing kits containing or relating to any of the polymeric conjugates described herein.

In certain embodiments, these therapeutic polymer conjugates improve drug stability and/or solubility of the therapeutic agent when used in vivo. Furthermore, by selecting from a variety of linker groups, the polymer conjugates present methods for controlled release of the therapeutic/bioactive agents, or improve the in vivo safety and/or therapeutic efficacy of the therapeutic/bioactive agent. In certain embodiments, the polymer conjugates are bioerodable or biodegradable.

Brief Description of the Figures

Figure 1 shows strategies for varying polymer conjugates to tune their characteristics.

Figure 2 demonstrates the effect of peptide tether length on drug release rate for drug-loaded CD polymer.

Figure 3 presents the effect that tethering camptothecin has on enhancing camptothecin stability, e.g., inhibiting lactone ring-opening.

Figure 4 shows lactone ring opening studies in pH 7.4 KH_2PO_4 buffer.

Figure 5a and 5b show polymerization control by adjusting polymerization time.

Figures 6 illustrates CPT release from **HG6** and **HGGG6** at 37 °C after 24 h in buffer solutions with pHs ranging from 1.1 to 13.1.

Figure 7 Displays HPLC analysis of degradation of CD-BisCys-SS-Peg3400 Polymer

Figure 8 Shows the tumor growth curve as a function of time for the D5W, CPT, irinotecan, **LGGG10** at its highest non-toxic dose tested (18 mg CPT/kg), and the other three conjugates with high MW polymer (**HGGG6**, **HG6**, **HGGG10**) at their MTDs in xenograft mice.

Figure 9 presents the median tumor growth curves for **HGGG6**, **HG6** and **HGGG10** in xenograft mice.

Figure 10 presents the medium tumor growth curves for **LGGG10** and **HGGG10** each dosed at 9 mg CPT/kg in xenograft mice.

Figure 11 presents the mean body weight (MBW) losses as a function of time plotted for D5W, CPT, irinotecan and the three conjugates containing high MW polymer at their MTDs in xenograft mice.

Figure 12 shows the correlation of CPT concentration (ng/mg tissue) to tumor size (in mg) in xenograft mice.

Detailed Description of the Invention

5 I. Overview

The present invention relates to novel compositions of therapeutic cyclodextrin-containing polymeric compounds designed for drug delivery of therapeutic agents. In certain embodiments, these cyclodextrin-containing polymers improve drug stability and/or solubility, and/or reduce toxicity, and/or improve
10 efficacy of the small molecule therapeutic when used in vivo. In certain embodiments, the polymers can be used for delivery of therapeutics such as camptothecin, taxol, doxorubicin, and amphotericin. Furthermore, by selecting from a variety of linker groups, and/or targeting ligands, the rate of drug release from the polymers can be attenuated for controlled delivery. The invention also relates to
15 methods of treating subjects with the therapeutic compositions described herein. The invention further relates to methods for conducting a pharmaceutical business comprising manufacturing, licensing, or distributing kits containing or relating to the polymeric compounds described herein.

More generally, the present invention provides water-soluble, biocompatible
20 polymer conjugates comprising a water-soluble, biocompatible polymer covalently attached to bioactive moieties through attachments that are cleaved under biological or photolytic conditions to release the bioactive moieties. In certain such embodiments, the polymer comprises cyclic moieties alternating with linker moieties that connect the cyclic structures, e.g., into linear or branched polymers,
25 preferably linear polymers. In some instances, the linker moieties may be cleaved under biological or photolytic conditions. The cyclic moieties may be any suitable cyclic structures, such as cyclodextrins, crown ethers (e.g., 18-crown-6, 15-crown-5, 12-crown-4, etc.), cyclic oligopeptides (e.g., comprising from 5 to 10 amino acid residues), cryptands or cryptates (e.g., cryptand [2.2.2], cryptand-2,1,1, and
30 complexes thereof), calixarenes, or cavitands, or any combination thereof. Preferably, the cyclic structure is (or is modified to be) water-soluble. In certain embodiments, e.g., where a linear polymer is desired, the cyclic structure is selected

such that under polymerization conditions, exactly two moieties of each cyclic structure are reactive with the linker moieties, such that the resulting polymer comprises (or consists essentially of) an alternating series of cyclic moieties and linker moieties, such as at least four of each type of moiety. Suitable
5 difunctionalized cyclic moieties include many that are commercially available and/or amenable to preparation using published protocols. In certain embodiments, conjugates are soluble in water to a concentration of at least 0.1 g/mL, preferably at least 0.25 g/mL.

The polymer may be a polycation, polyanion, a combination thereof, or non-
10 ionic polymer. A polycationic or polyanionic polymer has at least one site that bears a positive or negative charge, respectively. In certain such embodiments, at least one of the linker moiety and the cyclic moiety comprises such a charged site, so that every occurrence of that moiety includes a charged site.

The bioactive agent, which may be a therapeutic agent, a diagnostic agent, or
15 an adjuvant (such as a radiosensitizer, or a compound that lacks significant activity administered alone but that potentiates the activity of another therapeutic agent), preferably makes up at least 5%, 10%, 15%, 20%, 25%, 30%, or even 35% by weight of the conjugate. In preferred embodiments, administration of the polymer to a patient results in release of the therapeutic/bioactive agent over a period of at least
20 6 hours, preferably at least 12 or 18 hours. For example, the agent may be released over a period of time ranging from 6 hours to a month, 6 hours to two weeks, 6 hours to 3 days, etc. In certain embodiments, the rate of drug release is dependent primarily upon the rate of cleavage of linker moieties, such as by hydrolysis (as opposed to enzymatic cleavage), e.g., the rate of release changes by less than a
25 factor of 5, preferably less than a factor of 2, in the presence of hydrolytic enzymes. In other embodiments, the rate of drug release may be dependent primarily on the rate of enzymatic cleavage.

Polymeric conjugates of the present invention may be useful to improve solubility and/or stability of a bioactive/therapeutic agent, reduce drug-drug
30 interactions, reduce interactions with blood elements including plasma proteins, reduce or eliminate immunogenicity, protect the agent from metabolism, modulate

drug-release kinetics, improve circulation time, improve drug half-life (e.g., in the serum, or in selected tissues, such as tumors), attenuate toxicity, improve efficacy, normalize drug metabolism across subjects of different species, ethnicities, and/or races, and/or provide for targeted delivery into specific cells or tissues. Poorly
5 soluble and/or toxic compounds may benefit particularly from incorporation into polymeric compounds of the invention. Incorporation can include association of the compounds with the polymers preferably by covalent interactions. Alternatively, incorporation can also include association by non-covalent interactions, for example by host-guest interactions, dative interactions, van der Waals forces, hydrogen
10 bonding, or simple mixing. Combinations of covalent and non-covalent interactions are also contemplated.

II. Definitions

(a) General Terms

An 'adjuvant', as the term is used herein, is a compound that has little or no
15 therapeutic value on its own, but increases the effectiveness of a therapeutic agent. Exemplary adjuvants include radiosensitizers, transfection-enhancing agents (such as chloroquine and analogs thereof), chemotactic agents and chemoattractants, peptides that modulate cell adhesion and/or cell mobility, cell permeabilizing agents, inhibitors of multidrug resistance and/or efflux pumps, etc.

20 The term "agonist", as used herein, is meant to refer to an agent that mimics or up-regulates (e.g., potentiates or supplements) the bioactivity of a protein of interest, or an agent that facilitates or promotes (e.g., potentiates or supplements) an interaction among polypeptides or between a polypeptide and another molecule (e.g., a steroid, hormone, nucleic acids, small molecules etc.). An agonist can be a
25 wild-type protein or derivative thereof having at least one bioactivity of the wild-type protein. An agonist can also be a small molecule that up-regulates the expression of a gene or which increases at least one bioactivity of a protein. An agonist can also be a protein or small molecule which increases the interaction of a polypeptide of interest with another molecule, e.g., a target peptide or nucleic acid.

30 "Antagonist" as used herein is meant to refer to an agent that down-regulates (e.g., suppresses or inhibits) the bioactivity of a protein of interest, or an agent that

inhibits/suppresses or reduces (e.g., destabilizes or decreases) interaction among polypeptides or other molecules (e.g., steroids, hormones, nucleic acids, etc.). An antagonist can also be a compound that down-regulates the expression of a gene of interest or which reduces the amount of the wild-type protein present. An antagonist
5 can also be a protein or small molecule which decreases or inhibits the interaction of a polypeptide of interest with another molecule, e.g., a target peptide or nucleic acid.

The terms "biocompatible polymer" and "biocompatibility" when used in relation to polymers are art-recognized. For example, biocompatible polymers include polymers that are neither themselves toxic to the host (e.g., an animal or
10 human), nor degrade (if the polymer degrades) at a rate that produces monomeric or oligomeric subunits or other byproducts at toxic concentrations in the host. In certain embodiments of the present invention, biodegradation generally involves degradation of the polymer in an organism, e.g., into its monomeric subunits, which may be known to be effectively non-toxic. Intermediate oligomeric products
15 resulting from such degradation may have different toxicological properties, however, or biodegradation may involve oxidation or other biochemical reactions that generate molecules other than monomeric subunits of the polymer. Consequently, in certain embodiments, toxicology of a biodegradable polymer intended for in vivo use, such as implantation or injection into a patient, may be
20 determined after one or more toxicity analyses. It is not necessary that any subject composition have a purity of 100% to be deemed biocompatible. Hence, a subject composition may comprise 99%, 98%, 97%, 96%, 95%, 90% 85%, 80%, 75% or even less of biocompatible polymers, e.g., including polymers and other materials and excipients described herein, and still be biocompatible.

25 To determine whether a polymer or other material is biocompatible, it may be necessary to conduct a toxicity analysis. Such assays are well known in the art. One example of such an assay may be performed with live carcinoma cells, such as GT3TKB tumor cells, in the following manner: the sample is degraded in 1 M NaOH at 37 °C until complete degradation is observed. The solution is then
30 neutralized with 1 M HCl. About 200 µL of various concentrations of the degraded sample products are placed in 96-well tissue culture plates and seeded with human

gastric carcinoma cells (GT3TKB) at 104/well density. The degraded sample products are incubated with the GT3TKB cells for 48 hours. The results of the assay may be plotted as % relative growth vs. concentration of degraded sample in the tissue-culture well. In addition, polymers and formulations of the present invention
5 may also be evaluated by well-known in vivo tests, such as subcutaneous implantations in rats to confirm that they do not cause significant levels of irritation or inflammation at the subcutaneous implantation sites.

The term "biodegradable" is art-recognized, and includes polymers, compositions and formulations, such as those described herein, that are intended to
10 degrade during use. Biodegradable polymers typically differ from non-biodegradable polymers in that the former may be degraded during use. In certain embodiments, such use involves in vivo use, such as in vivo therapy, and in other certain embodiments, such use involves in vitro use. In general, degradation attributable to biodegradability involves the degradation of a biodegradable polymer
15 into its component subunits, or digestion, e.g., by a biochemical process, of the polymer into smaller, non-polymeric subunits. In certain embodiments, two different types of biodegradation may generally be identified. For example, one type of biodegradation may involve cleavage of bonds (whether covalent or otherwise) in the polymer backbone. In such biodegradation, monomers and oligomers typically
20 result, and even more typically, such biodegradation occurs by cleavage of a bond connecting one or more of subunits of a polymer. In contrast, another type of biodegradation may involve cleavage of a bond (whether covalent or otherwise) internal to sidechain or that connects a side chain to the polymer backbone. For example, a therapeutic agent or other chemical moiety attached as a side chain to the
25 polymer backbone may be released by biodegradation. In certain embodiments, one or the other or both general types of biodegradation may occur during use of a polymer.

As used herein, the term "biodegradation" encompasses both general types of biodegradation. The degradation rate of a biodegradable polymer often depends in
30 part on a variety of factors, including the chemical identity of the linkage responsible for any degradation, the molecular weight, crystallinity, biostability, and

degree of cross-linking of such polymer, the physical characteristics (e.g., shape and size) of an implant, and the mode and location of administration. For example, the greater the molecular weight, the higher the degree of crystallinity, and/or the greater the biostability, the biodegradation of any biodegradable polymer is usually
5 slower. The term "biodegradable" is intended to cover materials and processes also termed "bioerodible".

In certain embodiments wherein the biodegradable polymer also has a therapeutic agent or other material associated with it, the biodegradation rate of such polymer may be characterized by a release rate of such materials. In such
10 circumstances, the biodegradation rate may depend on not only the chemical identity and physical characteristics of the polymer, but also on the identity of material(s) incorporated therein. Degradation of the subject compositions includes not only the cleavage of intramolecular bonds, e.g., by oxidation and/or hydrolysis, but also the disruption of intermolecular bonds, such as dissociation of host/guest complexes by
15 competitive complex formation with foreign inclusion hosts.

In certain embodiments, polymeric formulations of the present invention biodegrade within a period that is acceptable in the desired application. In certain embodiments, such as in vivo therapy, such degradation occurs in a period usually less than about five years, one year, six months, three months, one month, fifteen
20 days, five days, three days, or even one day on exposure to a physiological solution with a pH between 6 and 8 having a temperature of between 25 and 37 °C. In other embodiments, the polymer degrades in a period of between about one hour and several weeks, depending on the desired application.

As used herein the term "bioerodable" refers to polymers which deliver
25 sustained effective amounts of therapeutic agent to target tissue over desired extended periods of time. Thus, a polymer according to the invention in the biological environment of host tissue and the like, in one aspect, is subjected to hydrolytic enzymes and oxidative species under, and in proportion to, the host's inflammatory response. This results in release of the therapeutic agent via the
30 breaking of the covalent linked bonds. Thus, in certain embodiments, the materials

of the invention utilize the mammal's own wound-healing repair process in being degraded thereby, as hereinbefore described.

The biodegradable polymers polylactic acid, polyglycolic acid, and polylactic-glycolic acid copolymer (PLGA), have been investigated extensively for nanoparticle formulation. These polymers are polyesters that, upon implantation in the body, undergo simple hydrolysis. The products of such hydrolysis are biologically compatible and metabolizable moieties (e.g., lactic acid and glycolic acid), which are eventually removed from the body by the citric acid cycle. Polymer biodegradation products are formed at a very slow rate, and hence do not affect normal cell function. Several implant studies with these polymers have proven safe in drug delivery applications, used in the form of matrices, microspheres, bone implant materials, surgical sutures, and also in contraceptive applications for long-term effects. These polymers are also used as graft materials for artificial organs, and recently as basement membranes in tissue engineering investigations. Nature Med. 824-826 (1996). Thus, these polymers have been time-tested in various applications and proven safe for human use. Most importantly, these polymers are FDA-approved for human use.

When polymers are used for delivery of pharmacologically active agents in vivo, it is essential that the polymers themselves be nontoxic and that they degrade into non-toxic degradation products as the polymer is eroded by the body fluids. Many synthetic biodegradable polymers, however, yield oligomers and monomers upon erosion in vivo that adversely interact with the surrounding tissue. D. F. Williams, J. Mater. Sci. 1233 (1982). To minimize the toxicity of the intact polymer carrier and its degradation products, polymers have been designed based on naturally occurring metabolites. Probably the most extensively studied examples of such polymers are the polyesters derived from lactic or glycolic acid and polyamides derived from amino acids.

A number of bioerodable or biodegradable polymers are known and used for controlled release of pharmaceuticals. Such polymers are described in, for example, U.S. Pat. No. 4,291,013; U.S. Pat. No. 4,347,234; U.S. Pat. No. 4,525,495; U.S. Pat. No. 4,570,629; U.S. Pat. No. 4,572,832; U.S. Pat. No. 4,587,268;

U.S. Pat. No. 4,638,045; U.S. Pat. No. 4,675,381; U.S. Pat. No. 4,745,160; and U.S. Pat. No. 5,219,980, each of which is incorporated by reference herein.

A biohydrolyzable bond (e.g., ester, amide, carbonate, carbamates, or imide) refers to a bond that is cleaved (e.g., an ester is cleaved to form a hydroxyl and a
5 carboxylic acid) under physiological conditions. Physiological conditions include the acidic and basic environments of the digestive tract (e.g., stomach, intestines, etc.), acidic environment of a tumor, enzymatic cleavage, metabolism, and other biological processes, and preferably refer to physiological conditions in a vertebrate, such as a mammal.

10 As used herein the terms “comonomer A precursor”, “linker”, “linker group”, and “linker moiety” refer to any straight chain or branched, symmetric or asymmetric compound which upon reaction with a cyclodextrin monomer precursor or other suitable cyclic moiety links two such moieties together. In certain
15 embodiments, a comonomer A precursor is a compound containing at least two functional groups through which reaction and thus linkage of the cyclodextrin monomers can be achieved. Examples of functional groups, which may be the same or different, terminal or internal, of each comonomer A precursor include, but are not limited, to amino, acid, imidazole, hydroxyl, thio, acyl halide, $-C=C-$, or $-C\equiv C-$ groups and derivatives thereof. In preferred embodiments, the two functional groups
20 are the same and are located at termini of the comonomer. In certain embodiments, a comonomer A precursor contains one or more pendant groups with at least one functional group through which reaction and thus linkage of therapeutic agent or targeting ligand can be achieved, or branched polymerization can be achieved. Examples of functional groups, which may be the same or different, terminal or
25 internal, of each comonomer A precursor pendant group include, but are not limited, to amino, acid, imidazole, hydroxyl, thiol, acyl halide, ethylene, and ethyne groups and derivatives thereof. In certain embodiments, the pendant group is a (un)substituted branched, cyclic or straight chain C1-C10 (preferably C1-C6) alkyl, or arylalkyl optionally containing one or more heteroatoms, e.g., N, O, S, within the
30 chain or ring.

Upon copolymerization of a comonomer A precursor with a cyclodextrin monomer precursor, two cyclodextrin monomers may be linked together by joining the primary hydroxyl side of one cyclodextrin monomer with the primary hydroxyl side of another cyclodextrin monomer, by joining the secondary hydroxyl side of one cyclodextrin monomer with the secondary hydroxyl side of another cyclodextrin monomer, or by joining the primary hydroxyl side of one cyclodextrin monomer with the secondary hydroxyl side of another cyclodextrin monomer. Accordingly, combinations of such linkages may exist in the final copolymer. Both the comonomer A precursor and the comonomer A of the final copolymer may be neutral, cationic (e.g., by containing protonated groups such as, for example, quaternary ammonium groups), or anionic (e.g., by containing deprotonated groups, such as, for example, sulfate, phosphate, borinate or carboxylate). The charge of comonomer A of the copolymer may be adjusted by adjusting pH conditions. Examples of suitable comonomer A precursors include, but are not limited to succinimide (e.g., dithiobis(succinimidyl propionate) DSP, and dissuccinimidyl suberate (DSS)), glutamates, and aspartates).

The cyclodextrin-containing polymers of the present invention may be linear, branched or grafted. As used herein, the term "linear cyclodextrin-containing polymer" refers to a polymer comprising (α , β , or γ) cyclodextrin molecules, or derivatives thereof which are inserted within a polymer chain. As used herein, the term "grafted cyclodextrin-containing polymer" refers to a polymer comprising (α , β , or γ) cyclodextrin molecules, or derivatives thereof which are pendant off of the polymer chain. The term "graft polymer" as used herein refers to a polymer molecule which has additional moieties attached as pendent groups along a polymer backbone. The term "graft polymerization" denotes a polymerization in which a side chain is grafted onto a polymer chain, which side chain consists of one or several other monomers. The properties of the graft copolymer obtained such as, for example, solubility, melting point, water absorption, wettability, mechanical properties, adsorption behavior, etc., deviate more or less sharply from those of the initial polymer as a function of the type and amount of the grafted monomers. The term "grafting ratio", as used herein, means the weight percent of the amount of the monomers grafted based on the weight of the polymer. As used herein, a branched

cyclodextrin-containing polymer refers to a polymer backbone with a plurality of branch points, wherein each branch point is a starting point of yet another strand of the polymer backbone, and each section of polymer backbone may have a plurality of (α , β , or γ) cyclodextrin molecules, or derivatives thereof, inserted into or grafted
5 onto the chain.

The term "cyclodextrin moiety" refers to (α , β , or γ) cyclodextrin molecules or derivatives thereof, which may be in their oxidized or reduced forms, for example, oxidized to dicarbonyl forms. Cyclodextrin moieties may be substituted or unsubstituted and may further comprise optional linkers. Optional therapeutic
10 agents and/or targeting ligands may be further linked to these moieties via an optional linker. The linkage may be covalent (optionally via biohydrolyzable bonds, e.g., esters, amides, carbamates, and carbonates) or may be a host-guest complex between the cyclodextrin derivative and the therapeutic agent and/or targeting ligand or the optional linkers of each. Cyclodextrin moieties may further include one or
15 more carbohydrate moieties, preferably simple carbohydrate moieties such as galactose, attached to the cyclic core, either directly (i.e., via a carbohydrate linkage) or through a linker group.

The term "ED₅₀" means the dose of a drug that produces 50% of its maximum response or effect.

20 An 'effective amount' of a subject compound, with respect to the subject method of treatment, refers to an amount of the therapeutic in a preparation which, when applied as part of a desired dosage regimen provides a benefit according to clinically acceptable standards for the treatment or prophylaxis of a particular disorder.

25 The term "healthcare providers" refers to individuals or organizations that provide healthcare services to a person, community, etc. Examples of "healthcare providers" include doctors, hospitals, continuing care retirement communities, skilled nursing facilities, subacute care facilities, clinics, multispecialty clinics, freestanding ambulatory centers, home health agencies, and HMO's.

“Instruction(s)” as used herein means documents describing relevant materials or methodologies pertaining to a kit. These materials may include any combination of the following: background information, list of components and their availability information (purchase information, etc.), brief or detailed protocols for using the kit, trouble-shooting, references, technical support, and any other related documents. Instructions can be supplied with the kit or as a separate member component, either as a paper form or an electronic form which may be supplied on computer readable memory device or downloaded from an internet website, or as recorded presentation. Instructions can comprise one or multiple documents, and are meant to include future updates.

“Kit” as used herein means a collection of at least two components constituting the kit. Together, the components constitute a functional unit for a given purpose. Individual member components may be physically packaged together or separately. For example, a kit comprising an instruction for using the kit may or may not physically include the instruction with other individual member components. Instead, the instruction can be supplied as a separate member component, either in a paper form or an electronic form which may be supplied on computer readable memory device or downloaded from an internet website, or as recorded presentation.

The term “LD₅₀” means the dose of a drug that is lethal in 50% of test subjects.

A “patient” or “subject” to be treated by the subject method can mean either a human or non-human subject.

“Photocleavable” or “photolytically cleavable” as used herein is art recognized and refers to groups or moieties which possess chemical bonds whose cleavage may be induced by light or other forms of radiant energy, i.e., cleavage by photolysis. Such groups and bonds are often referred to as “photosensitive,” “photocleavable,” or “photolabile.” The present invention contemplates all such forms of energy capable of inducing photolysis, including, but not limited to, energy of X-ray, ultraviolet, visible, and infrared wavelengths.

The "polymerizations" of the present invention include radical, anionic, and cationic mechanisms, as well as reactions of bifunctional molecules (analogous to the formation of nylon, e.g., reacting molecules each of which bears two or more different reactive moieties that react with each other (but, preferably, are disfavored from reacting intramolecularly by steric, conformational, or other constraints), or
5 reacting two or more different compounds, each compound bearing two or more reactive moieties that react only with reactive moieties of different compounds (i.e., intermolecularly)), as well as metal-catalyzed polymerizations such as olefin metathesis, and other polymerization reactions known to those of skill in the art.

10 The term "preventing" is art-recognized, and when used in relation to a condition, such as a local recurrence (e.g., pain), a disease such as cancer, a syndrome complex such as heart failure or any other medical condition, is well understood in the art, and includes administration of a composition which reduces the frequency of, or delays the onset of, symptoms of a medical condition in a
15 subject relative to a subject which does not receive the composition. Thus, prevention of cancer includes, for example, reducing the number of detectable cancerous growths in a population of patients receiving a prophylactic treatment relative to an untreated control population, and/or delaying the appearance of detectable cancerous growths in a treated population versus an untreated control
20 population, e.g., by a statistically and/or clinically significant amount. Prevention of an infection includes, for example, reducing the number of diagnoses of the infection in a treated population versus an untreated control population, and/or delaying the onset of symptoms of the infection in a treated population versus an untreated control population. Prevention of pain includes, for example, reducing the
25 frequency of, or alternatively delaying, pain sensations experienced by subjects in a treated population versus an untreated control population.

The term "prophylactic or therapeutic" treatment is art-recognized and includes administration to the host of one or more of the subject compositions. If it is administered prior to clinical manifestation of the unwanted condition (e.g.,
30 disease or other unwanted state of the host animal) then the treatment is prophylactic, i.e., it protects the host against developing the unwanted condition,

whereas if it is administered after manifestation of the unwanted condition, the treatment is therapeutic, (i.e., it is intended to diminish, ameliorate, or stabilize the existing unwanted condition or side effects thereof).

The phrase "protecting group" or "protective group" as used herein means a
5 temporary substituent that protects a potentially reactive functional group from undesired chemical transformations. Examples of such protecting groups include esters of carboxylic acids, silyl ethers of alcohols, and acetals and ketals of aldehydes and ketones, respectively. The field of protecting group chemistry has been reviewed (Greene, T.W.; Wuts, P.G.M. *Protective Groups in Organic*
10 *Synthesis*, 2nd ed.; Wiley: New York, 1991). The instant invention contemplates the modification or derivitization of protecting groups, particularly photolabile protecting groups, already known in the art to provide linkers of the instant invention.

The term "targeting ligand" refers to any molecular structure which assists
15 the construct in localizing to a particular target area, entering a target cell(s), and/or binding to a target receptor. For example, lipids (including cationic, neutral, and steroidal lipids, virosomes, and liposomes), antibodies, lectins, ligands, sugars, steroids, hormones, nutrients, and proteins can serve as targeting ligands. Targeting ligands can direct compounds of the instant invention to a "target," such as an in
20 vivo or in vitro site. Non-limiting examples of targets include tumors (e.g., tumors of the brain, lung (small cell and non-small cell), ovary, prostate, breast and colon as well as other carcinomas and sarcomas). Another example of a target is a site of infection (e.g., by bacteria, viruses (e.g., HIV, herpes, hepatitis) and pathogenic fungi (Candida sp.). Other examples of targets include infectious organisms that are drug
25 resistant (e.g., Enterobacteriaceae, Enterococcus, Haemophilus influenza, Mycobacterium tuberculosis, Neisseria gonorrhoeae, Plasmodium falciparum, Pseudomonas aeruginosa, Shigella dysenteriae, Staphylococcus aureus, Streptococcus pneumoniae). A target may refer to a molecular structure to which a targeting ligand binds, such as a hapten, epitope, receptor, dsDNA fragment,
30 carbohydrate, or enzyme. Additionally, a target may be a type of tissue, e.g., neuronal tissue, intestinal tissue, pancreatic tissue, etc.

As used herein, the terms "therapeutic agent" include any synthetic or naturally occurring biologically active compound or composition of matter which, when administered to an organism (human or nonhuman animal), induces a desired pharmacologic, immunogenic, and/or physiologic effect by local and/or systemic action. The term therefore encompasses those compounds or chemicals traditionally regarded as drugs, vaccines, and biopharmaceuticals including molecules such as proteins, peptides, hormones, nucleic acids, gene constructs and the like. More particularly, the term "therapeutic agent" includes compounds or compositions for use in all of the major therapeutic areas including, but not limited to, adjuvants; anti-infectives such as antibiotics and antiviral agents; analgesics and analgesic combinations, anorexics, anti-inflammatory agents, anti-epileptics, local and general anesthetics, hypnotics, sedatives, antipsychotic agents, neuroleptic agents, antidepressants, anxiolytics, antagonists, neuron blocking agents, anticholinergic and cholinomimetic agents, antimuscarinic and muscarinic agents, antiadrenergics, antiarrhythmics, antihypertensive agents, hormones, and nutrients, antiarthritics, antiasthmatic agents, anticonvulsants, antihistamines, antinauseants, antineoplastics, antipruritics, antipyretics; antispasmodics, cardiovascular preparations (including calcium channel blockers, beta-blockers, beta-agonists and antiarrhythmics), antihypertensives, diuretics, vasodilators; central nervous system stimulants; cough and cold preparations; decongestants; diagnostics; hormones; bone growth stimulants and bone resorption inhibitors; immunosuppressives; muscle relaxants; psychostimulants; sedatives; tranquilizers; proteins, peptides, and fragments thereof (whether naturally occurring, chemically synthesized or recombinantly produced); and nucleic acid molecules (polymeric forms of two or more nucleotides, either ribonucleotides (RNA) or deoxyribonucleotides (DNA) including both double- and single-stranded molecules, gene constructs, expression vectors, antisense molecules and the like), small molecules (e.g., doxorubicin) and other biologically active macromolecules such as, for example, proteins and enzymes. The agent may be a biologically active agent used in medical, including veterinary, applications and in agriculture, such as with plants, as well as other areas. The term therapeutic agent also includes without limitation, medicaments; vitamins; mineral supplements; substances used for the treatment, prevention, diagnosis, cure or mitigation of

disease or illness; or substances which affect the structure or function of the body; or pro-drugs, which become biologically active or more active after they have been placed in a predetermined physiological environment.

As used herein the term "low aqueous solubility" refers to water insoluble compounds having poor solubility in water, that is <5 mg/ml at physiological pH (6.5-7.4). Preferably, their water solubility is <1 mg/ml, more preferably <0.1 mg/ml. It is desirable that the drug is stable in water as a dispersion; otherwise a lyophilized or spray-dried solid form may be desirable.

Examples of some preferred water-insoluble drugs include immunosuppressive agents such as cyclosporins including cyclosporine (cyclosporin A), immunoactive agents, antiviral and antifungal agents, antineoplastic agents, analgesic and anti-inflammatory agents, antibiotics, anti-epileptics, anesthetics, hypnotics, sedatives, antipsychotic agents, neuroleptic agents, antidepressants, anxiolytics, anticonvulsant agents, antagonists, neuron blocking agents, anticholinergic and cholinomimetic agents, antimuscarinic and muscarinic agents, antiadrenergic and antiarrhythmics, antihypertensive agents, hormones, and nutrients. A detailed description of these and other suitable drugs may be found in Remington's Pharmaceutical Sciences, 18th edition, 1990, Mack Publishing Co. Philadelphia, Pa

The term "therapeutic index" refers to the therapeutic index of a drug defined as LD_{50}/ED_{50} .

A "therapeutically effective amount" of a compound, with respect to a method of treatment, refers to an amount of the compound(s) in a preparation which, when administered as part of a desired dosage regimen (to a mammal, preferably a human) alleviates a symptom, ameliorates a condition, or slows the onset of disease conditions according to clinically acceptable standards for the disorder or condition to be treated or the cosmetic purpose, e.g., at a reasonable benefit/risk ratio applicable to any medical treatment.

A "therapeutically effective daily dosage" of a compound, with respect to a method of treatment, refers to an amount of the compound(s) in a preparation which, when administered as part of a desired daily dosage regimen (to a mammal, preferably a human) alleviates a symptom, ameliorates a condition, or slows the onset of disease conditions according to clinically acceptable standards for the disorder or condition to be treated or the cosmetic purpose, e.g., at a reasonable benefit/risk ratio applicable to any medical treatment.

(b) Chemical Terms

An aliphatic chain comprises the classes of alkyl, alkenyl and alkynyl defined below. A straight aliphatic chain is limited to unbranched carbon chain radicals. As used herein, the term "aliphatic group" refers to a straight chain, branched-chain, or cyclic aliphatic hydrocarbon group and includes saturated and unsaturated aliphatic groups, such as an alkyl group, an alkenyl group, and an alkynyl group.

Alkyl refers to a fully saturated branched or unbranched carbon chain radical having the number of carbon atoms specified, or up to 30 carbon atoms if no specification is made. For example, alkyl of 1 to 8 carbon atoms refers to radicals such as methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, and octyl, and those radicals which are positional isomers of these radicals. Alkyl of 10 to 30 carbon atoms includes decyl, undecyl, dodecyl, tridecyl, tetradecyl, pentadecyl, hexadecyl, heptadecyl, octadecyl, nonadecyl, eicosyl, heneicosyl, docosyl, tricosyl and tetracosyl. In preferred embodiments, a straight chain or branched chain alkyl has 30 or fewer carbon atoms in its backbone (e.g., C1-C30 for straight chains, C3-C30 for branched chains), and more preferably 20 or fewer. Likewise, preferred cycloalkyls have from 3-10 carbon atoms in their ring structure, and more preferably have 5, 6 or 7 carbons in the ring structure.

Moreover, the term "alkyl" (or "lower alkyl") as used throughout the specification, examples, and claims is intended to include both "unsubstituted alkyls" and "substituted alkyls", the latter of which refers to alkyl moieties having substituents replacing a hydrogen on one or more carbons of the hydrocarbon backbone. Such substituents can include, for example, a halogen, a hydroxyl, a

carbonyl (such as a carboxyl, an alkoxy carbonyl, a formyl, or an acyl), a thiocarbonyl (such as a thioester, a thioacetate, or a thioformate), an alkoxy, a phosphoryl, a phosphate, a phosphonate, a phosphinate, an amino, an amido, an amidine, a cyano, a nitro, a sulfhydryl, an alkylthio, a sulfate, a sulfonate, a sulfamoyl, a sulfonamido, a sulfonyl, a heterocyclyl, an aralkyl, or an aromatic or heteroaromatic moiety. It will be understood by those skilled in the art that the moieties substituted on the hydrocarbon chain can themselves be substituted, if appropriate. For instance, the substituents of a substituted alkyl may include substituted and unsubstituted forms of amino, azido, imino, amido, phosphoryl (including phosphonate and phosphinate), sulfonyl (including sulfate, sulfonamido, sulfamoyl and sulfonate), and silyl groups, as well as ethers, alkylthios, carbonyls (including ketones, aldehydes, carboxylates, and esters), -CF₃, -CN and the like. Exemplary substituted alkyls are described below. Cycloalkyls can be further substituted with alkyls, alkenyls, alkoxy, alkylthios, aminoalkyls, carbonyl-substituted alkyls, -CF₃, -CN, and the like.

Unless the number of carbons is otherwise specified, "lower alkyl", as used herein, means an alkyl group, as defined above, but having from one to ten carbons, more preferably from one to six carbon atoms in its backbone structure such as methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, and tert-butyl. Likewise, "lower alkenyl" and "lower alkynyl" have similar chain lengths. Throughout the application, preferred alkyl groups are lower alkyls. In preferred embodiments, a substituent designated herein as alkyl is a lower alkyl.

The term "alkylthio" refers to an alkyl group, as defined above, having a sulfur radical attached thereto. In preferred embodiments, the "alkylthio" moiety is represented by one of -(S)-alkyl, -(S)-alkenyl, -(S)-alkynyl, and -(S)-(CH₂)_m-R₁, wherein m and R₁ are defined below. Representative alkylthio groups include methylthio, ethylthio, and the like.

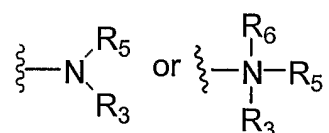
Alkenyl refers to any branched or unbranched unsaturated carbon chain radical having the number of carbon atoms specified, or up to 26 carbon atoms if no limitation on the number of carbon atoms is specified; and having 1 or more double bonds in the radical. Alkenyl of 6 to 26 carbon atoms is exemplified by hexenyl,

heptenyl, octenyl, nonenyl, decenyl, undecenyl, dodenyl, tridecenyl, tetradecenyl, pentadecenyl, hexadecenyl, heptadecenyl, octadecenyl, nonadecenyl, eicosenyl, heneicosoenyl, docosenyl, tricosenyl and tetracosenyl, in their various isomeric forms, where the unsaturated bond(s) can be located anywhere in the radical and can
 5 have either the (Z) or the (E) configuration about the double bond(s).

Alkynyl refers to hydrocarbyl radicals of the scope of alkenyl, but having 1 or more triple bonds in the radical.

The terms "alkoxyl" or "alkoxy" as used herein refers to an alkyl group, as defined below, having an oxygen radical attached thereto. Representative alkoxyl
 10 groups include methoxy, ethoxy, propoxy, tert-butoxy and the like. An "ether" is two hydrocarbons covalently linked by an oxygen. Accordingly, the substituent of an alkyl that renders that alkyl an ether is or resembles an alkoxyl, such as can be represented by one of -O-alkyl, -O-alkenyl, -O-alkynyl, -O-(CH₂)_m-R₁, where m and R₁ are described below.

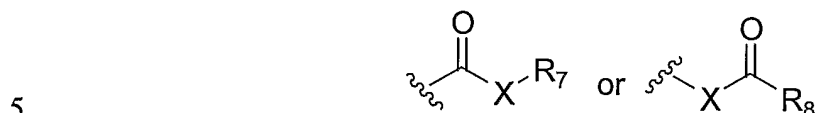
15 The terms "amine" and "amino" are art-recognized and refer to both unsubstituted and substituted amines, e.g., a moiety that can be represented by the general formulae:



wherein R₃, R₅ and R₆ each independently represent a hydrogen, an alkyl, an
 20 alkenyl, -(CH₂)_m-R₁, or R₃ and R₅ taken together with the N atom to which they are attached complete a heterocycle having from 4 to 8 atoms in the ring structure; R₁ represents an alkenyl, aryl, cycloalkyl, a cycloalkenyl, a heterocyclyl or a polycyclyl; and m is zero or an integer in the range of 1 to 8. In preferred embodiments, only one of R₃ or R₅ can be a carbonyl, e.g., R₃, R₅ and the nitrogen
 25 together do not form an imide. In even more preferred embodiments, R₃ and R₅ (and optionally R₆) each independently represent a hydrogen, an alkyl, an alkenyl, or -(CH₂)_m-R₁. Thus, the term "alkylamine" as used herein means an amine group, as defined above, having a substituted or unsubstituted alkyl attached thereto, i.e., at least one of R₃ and R₅ is an alkyl group. In certain embodiments, an amino group or

an alkylamine is basic, meaning it has a $pK_a > 7.00$. The protonated forms of these functional groups have pK_a s relative to water above 7.00.

The term "carbonyl" is art-recognized and includes such moieties as can be represented by the general formula:



wherein X is a bond or represents an oxygen or a sulfur, and R_7 represents a hydrogen, an alkyl, an alkenyl, $-(CH_2)_m-R_1$ or a pharmaceutically acceptable salt, R_8 represents a hydrogen, an alkyl, an alkenyl or $-(CH_2)_m-R_1$, where m and R_1 are as defined above. Where X is an oxygen and R_7 or R_8 is not hydrogen, the formula represents an "ester". Where X is an oxygen, and R_7 is as defined above, the moiety is referred to herein as a carboxyl group, and particularly when R_7 is a hydrogen, the formula represents a "carboxylic acid". Where X is an oxygen, and R_8 is hydrogen, the formula represents a "formate". In general, where the oxygen atom of the above formula is replaced by sulfur, the formula represents a "thiocarbonyl" group. Where X is a sulfur and R_7 or R_8 is not hydrogen, the formula represents a "thioester" group. Where X is a sulfur and R_7 is hydrogen, the formula represents a "thiocarboxylic acid" group. Where X is a sulfur and R_8 is hydrogen, the formula represents a "thioformate" group. On the other hand, where X is a bond, and R_7 is not hydrogen, the above formula represents a "ketone" group. Where X is a bond, and R_7 is hydrogen, the above formula represents an "aldehyde" group.

The term "derivatized" refers to chemically modifying molecules. The chemical modifications may be artificial such as formation of drugs, natural such as formation of metabolites. The skilled artisan would readily recognize the variety of ways molecules may be modified, such as oxidations, reductions, electrophilic/nucleophilic substitutions, alkylations, ester/amide formations and the like. For example, cyclodextrins of the present invention may be chemically modified by amination, tosylation, or iodination prior to covalently attaching them to monomers, polymers, or other compounds as described herein. Likewise, therapeutic agents may be chemically modified by preparing prodrugs (e.g., glycine-camptothecin).

The terms "heterocyclyl" or "heterocyclic group" refer to 3- to 10-membered ring structures, more preferably 3- to 7-membered rings, whose ring structures include one to four heteroatoms. Heterocycles can also be polycycles. Heterocyclyl groups include, for example, thiophene, thianthrene, furan, pyran, isobenzofuran, chromene, xanthene, phenoxathiin, pyrrole, imidazole, pyrazole, isothiazole, isoxazole, pyridine, pyrazine, pyrimidine, pyridazine, indolizine, isoindole, indole, indazole, purine, quinolizine, isoquinoline, quinoline, phthalazine, naphthyridine, quinoxaline, quinazoline, cinnoline, pteridine, carbazole, carboline, phenanthridine, acridine, pyrimidine, phenanthroline, phenazine, phenarsazine, phenothiazine, furazan, phenoxazine, pyrrolidine, oxolane, thiolane, oxazole, piperidine, piperazine, morpholine, lactones, lactams such as azetidinones and pyrrolidinones, sultams, sultones, and the like. The heterocyclic ring can be substituted at one or more positions with such substituents as described above, as for example, halogen, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, amino, nitro, sulfhydryl, imino, amido, phosphate, phosphonate, phosphinate, carbonyl, carboxyl, silyl, sulfamoyl, sulfinyl, ether, alkylthio, sulfonyl, ketone, aldehyde, ester, a heterocyclyl, an aromatic or heteroaromatic moiety, -CF₃, -CN, or the like.

As used herein, the term "substituted" is contemplated to include all permissible substituents of organic compounds. In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and nonaromatic substituents of organic compounds. Illustrative substituents include, for example, those described herein above. The permissible substituents can be one or more and the same or different for appropriate organic compounds. For purposes of this invention, the heteroatoms such as nitrogen may have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valences of the heteroatoms. This invention is not intended to be limited in any manner by the permissible substituents of organic compounds.

The term "hydrocarbyl" refers to a monovalent hydrocarbon radical comprised of carbon chains or rings of up to 26 carbon atoms to which hydrogen atoms are attached. The term includes alkyl, cycloalkyl, alkenyl, alkynyl and aryl

groups, groups which have a mixture of saturated and unsaturated bonds, carbocyclic rings and includes combinations of such groups. It may refer to straight chain, branched-chain, cyclic structures or combinations thereof.

The term "hydrocarbylene" refers to a divalent hydrocarbyl radical.

- 5 Representative examples include alkylene, phenylene, or cyclohexylene. Preferably, the hydrocarbylene chain is fully saturated and/or has a chain of 1-10 carbon atoms.

As used herein, the term "nitro" means -NO₂; the term "halogen" designates -F, -Cl, -Br or -I; the term "sulfhydryl" means -SH; the term "hydroxyl" means -OH; and the term "sulfonyl" means -SO₂-.

- 10 It will be understood that "substitution" or "substituted with" includes the implicit proviso that such substitution is in accordance with permitted valence of the substituted atom and the substituent, and that the substitution results in a stable compound, e.g., which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, etc.

- 15 Analogous substitutions can be made to alkenyl and alkynyl groups to produce, for example, aminoalkenyls, aminoalkynyls, amidoalkenyls, amidoalkynyls, iminoalkenyls, iminoalkynyls, thioalkenyls, thioalkynyls, carbonyl-substituted alkenyls or alkynyls.

- 20 As used herein, the definition of each expression, e.g., alkyl, m, n, etc., when it occurs more than once in any structure, is intended to be independent of its definition elsewhere in the same structure.

- 25 The terms triflyl, tosyl, mesyl, and nonafllyl are art-recognized and refer to trifluoromethanesulfonyl, p-toluenesulfonyl, methanesulfonyl, and nonafluorobutanesulfonyl groups, respectively. The terms triflate, tosylate, mesylate, and nonaflate are art-recognized and refer to trifluoromethanesulfonate ester, p-toluenesulfonate ester, methanesulfonate ester, and nonafluorobutanesulfonate ester functional groups and molecules that contain said groups, respectively.

The abbreviations Me, Et, Ph, Ms represent methyl, ethyl, phenyl, and methanesulfonyl, respectively. A more comprehensive list of the abbreviations utilized by organic chemists of ordinary skill in the art appears in the first issue of each volume of the Journal of Organic Chemistry; this list is typically presented in a table entitled Standard List of Abbreviations. The abbreviations contained in said list, and all abbreviations utilized by organic chemists of ordinary skill in the art are hereby incorporated by reference.

Certain compounds of the present invention may exist in particular geometric or stereoisomeric forms. The present invention contemplates all such compounds, including cis- and trans-isomers, (R)- and (S)-enantiomers, diastereomers, (d)-isomers, (l)-isomers, the racemic mixtures thereof, and other mixtures thereof, as falling within the scope of the invention. Additional asymmetric carbon atoms may be present in a substituent such as an alkyl group. All such isomers, as well as mixtures thereof, are intended to be included in this invention.

If, for instance, a particular enantiomer of a compound of the present invention is desired, it may be prepared by asymmetric synthesis, or by derivatization with a chiral auxiliary, where the resulting diastereomeric mixture is separated and the auxiliary group cleaved to provide the pure desired enantiomers. Alternatively, where the molecule contains a basic functional group, such as amino, or an acidic functional group, such as carboxyl, diastereomeric salts may be formed with an appropriate optically active acid or base, followed by resolution of the diastereomers thus formed by fractional crystallization or chromatographic means well known in the art, and subsequent recovery of the pure enantiomers.

Contemplated equivalents of the compounds described above include compounds which otherwise correspond thereto, and which have the same general properties thereof, wherein one or more simple variations of substituents are made which do not adversely affect the efficacy of the compound. In general, the compounds of the present invention may be prepared by the methods illustrated in the general reaction schemes as, for example, described below, or by modifications thereof, using readily available starting materials, reagents and conventional

synthesis procedures. In these reactions, it is also possible to make use of variants which are in themselves known, but are not mentioned here.

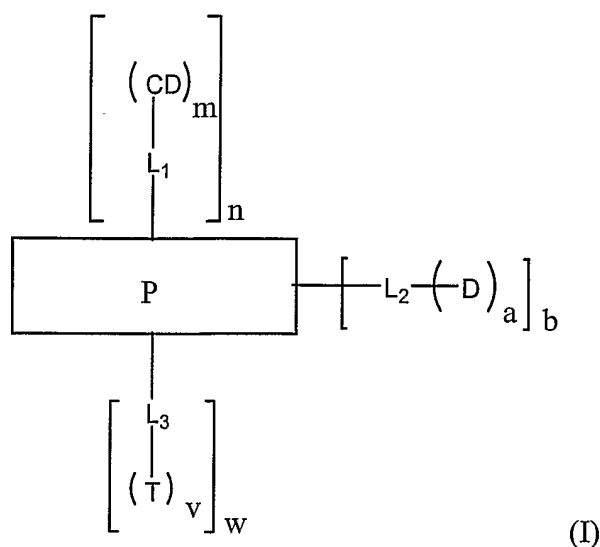
For purposes of this invention, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, Handbook of
5 Chemistry and Physics, 67th Ed., 1986-87, inside cover. Also for purposes of this invention, the term "hydrocarbon" is contemplated to include all permissible compounds having at least one hydrogen and one carbon atom. In a broad aspect, the permissible hydrocarbons include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and nonaromatic organic compounds which
10 can be substituted or unsubstituted.

III. Exemplary Applications of Method and Compositions

(a) Exemplary Compositions

The present invention includes polymer conjugates, such as cyclodextrin-containing polymer conjugates, wherein one or more therapeutic/bioactive agents
15 are covalently attached. In certain embodiments, the therapeutic agent is a small molecule, a macromolecule, an antibody, a peptide, a protein, an enzyme, a nucleic acid, or a polymer that has therapeutic function. The polymers include linear or branched cyclodextrin-containing polymers and polymers grafted with cyclodextrin. Exemplary cyclodextrin-containing polymers that may be modified as described
20 herein are taught in U.S. Patent No. 6,509,323, published U.S. application No. 20020151523, and U.S. patent application serial No.s 60/417373, and 10/372723, each of which is incorporated by reference herein. These polymers are useful as carriers for small molecule therapeutic delivery, and may improve drug stability and solubility when used in vivo.

25 Accordingly, one embodiment of present invention is a polymeric compound represented by Formula I:



wherein

P represents a linear or branched polymer chain;

CD represents a cyclic moiety such as a cyclodextrin moiety;

5 L_1 , L_2 and L_3 , independently for each occurrence, represent a direct bond or the same or different linker groups;

D, independently for each occurrence, represents a therapeutic agent or a prodrug thereof;

10 T, independently for each occurrence, represents a targeting ligand or precursor thereof;

a, m, and v, independently for each occurrence, represent integers in the range of 1 to 10 (preferably 1 to 8, 1 to 5, or even 1 to 3);

15 n and w, independently for each occurrence, represent an integer in the range of 0 to about 30,000 (preferably <25,000, <20,000, <15,000, <10,000, <5,000, <1,000, <500, <100, <50, <25, <10, or even <5); and

b represents an integer in the range of 1 to about 30,000 (preferably <25,000, <20,000, <15,000, <10,000, <5,000, <1,000, <500, <100, <50, <25, <10, or even <5),

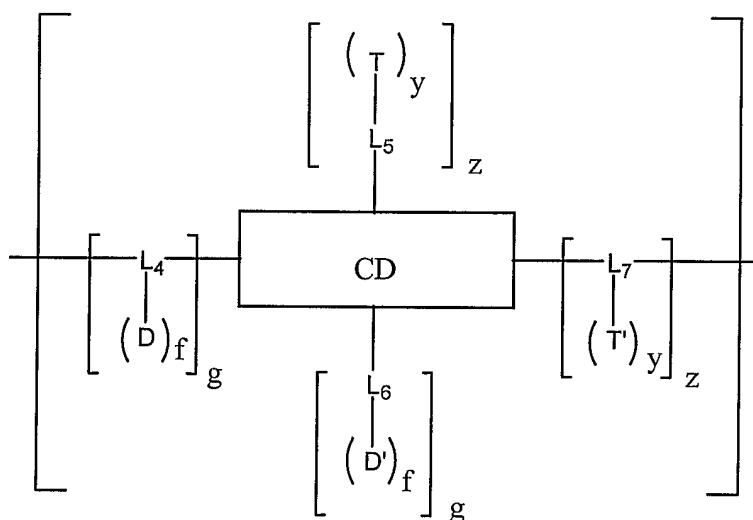
wherein either P comprises cyclodextrin moieties or n is at least 1.

20

In certain embodiments, one or more occurrences of L_1 , L_2 , or L_3 , independently represent a photocleavable linker. In certain embodiments, one or more occurrences of L_2 or L_1 , independently represent a photocleavable linker. In

certain embodiments, one or more occurrences of L_2 represent a photocleavable linker.

In certain embodiments, P contains a plurality of cyclodextrin moieties within the polymer chain as opposed to the cyclodextrin moieties being grafted on to pendant groups off of the polymeric chain. Thus in certain embodiments, the polymer chain of formula I further comprises n' units of U, wherein n' represents an integer in the range of 1 to about 30,000 (preferably <25,000, <20,000, <15,000, <10,000, <5,000, <1,000, <500, <100, <50, <25, <10, or even <5); and U is represented by the general formula:



wherein

CD represents a cyclic moiety, such as a cyclodextrin moiety, or derivative thereof;

L_4 , L_5 , L_6 , and L_7 , independently for each occurrence, represent a direct bond or the same or different linker groups;

D and D', independently for each occurrence, represent the same or different therapeutic agent or prodrug forms thereof;

T and T', independently for each occurrence, represent the same or different targeting ligand or precursor thereof;

f and y, independently for each occurrence, represent an integer in the range of 1 and 10; and

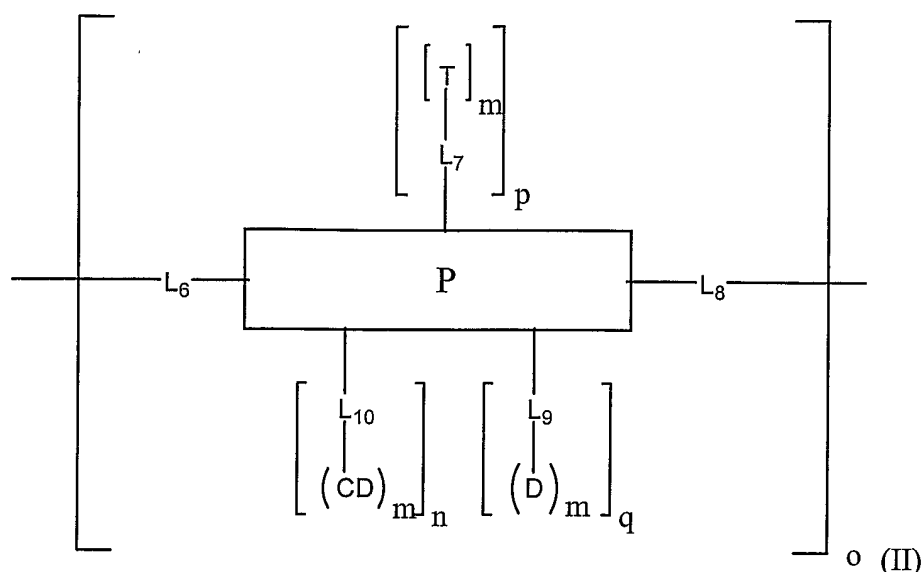
g and z, independently for each occurrence, represent an integer in the range of 0 and 10.

In some embodiments, L_4 and L_7 represent linker groups, such as
5 photocleavable linker groups..

In certain embodiments, one or more occurrences of L_4 , L_5 , L_6 , or L_7 , independently represent a photocleavable linker. In certain embodiments, one or more occurrences of L_4 , L_6 , or L_7 , independently represent a photocleavable linker. In certain embodiments, one or more occurrences of L_4 or L_6 , independently
10 represent a photocleavable linker.

In certain embodiments, the polymer may be selected from polysaccharides, and other non-protein biocompatible polymers, and combinations thereof, that contain at least one terminal hydroxyl group, such as polyvinylpyrrolidone, poly(oxyethylene)glycol (PEG), polysuccinic anhydride, polysebacic acid, PEG-
15 phosphate, polyglutamate, polyethylenimine, maleic anhydride divinylether (DIVMA), cellulose, pullulans, inulin, polyvinyl alcohol (PVA), N-(2-hydroxypropyl)methacrylamide (HPMA), dextran and hydroxyethyl starch (HES), and have optional pendant groups for grafting therapeutic agents, targeting ligands and/or cyclodextrin moieties. In certain embodiments, the polymer may be
20 biodegradable such as poly(lactic acid), poly(glycolic acid), poly(alkyl 2-cyanoacrylates), polyanhydrides, and polyorthoesters, or bioerodible such as polylactide-glycolide copolymers, and derivatives thereof, non-peptide polyaminoacids, polyiminocarbonates, poly alpha-amino acids, polyalkyl-cyanoacrylate, polyphosphazenes or acyloxymethyl poly aspartate and polyglutamate
25 copolymers and mixtures thereof.

Another embodiment of the invention is a polymeric compound represented by Formula II:



wherein

P represents a monomer unit of a polymer;

T, independently for each occurrence, represents a targeting ligand or a precursor thereof;

L_6 , L_7 , L_8 , L_9 , and L_{10} , independently for each occurrence, represent a direct bond or the same or different linker groups;

CD, independently for each occurrence, represents a cyclodextrin moiety or a derivative thereof;

D, independently for each occurrence, represents a therapeutic agent or a prodrug form thereof;

m, independently for each occurrence, represents an integer in the range of 1 to 10 (preferably 1 to 8, 1 to 5, or even 1 to 3);

o represents an integer in the range of 1 to about 30,000 (preferably <25,000, <20,000, <15,000, <10,000, <5,000, <1,000, <500, <100, <50, <25, <10, or even <5); and

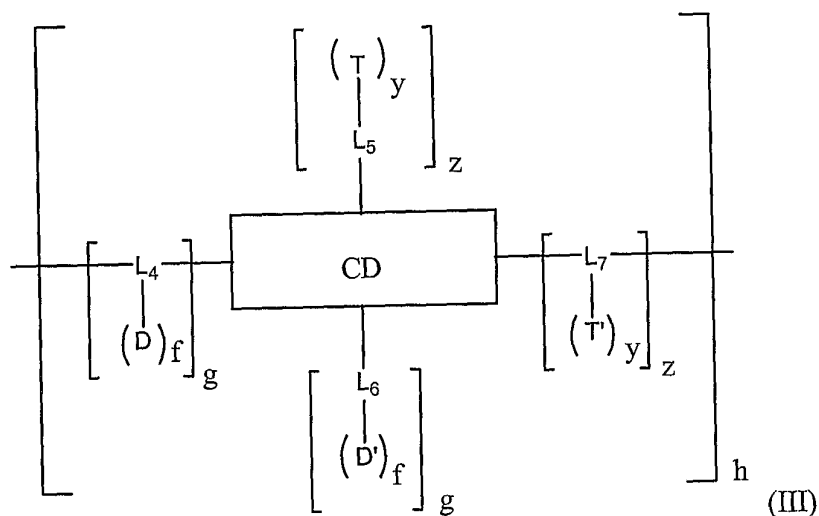
p, n, and q, independently for each occurrence, represent an integer in the range of 0 to 10 (preferably 0 to 8, 0 to 5, 0 to 3, or even 0 to about 2),

wherein CD and D are preferably each present at least once (preferably at least 5, 10, 25, or even 50 or 100 times) in the compound.

In certain embodiments, one or more occurrences of L_6 , L_7 , L_8 , or L_{10} , independently represent a photocleavable linker. In certain embodiments, one or

more occurrences of L_7 , L_9 , or L_{10} , independently represent a photocleavable linker. In certain embodiments, one or more occurrences of L_9 or L_{10} , independently represent a photocleavable linker. In certain embodiments, one or more occurrences of L_9 represent a photocleavable linker.

- 5 Another embodiment of the invention is a compound represented by Formula III:



wherein

- CD represents a cyclic moiety, such as a cyclodextrin moiety, or derivative thereof;

L_4 , L_5 , L_6 , and L_7 , independently for each occurrence, represent a direct bond or the same or different linker groups;

D and D' , independently for each occurrence, represent the same or different therapeutic agents or prodrugs thereof;

- 15 T and T' , independently for each occurrence, represent the same or different targeting ligands or precursors thereof;

f and y , independently for each occurrence, represent an integer in the range of 1 and 10 (preferably 1 to 8, 1 to 5, or even 1 to 3);

- 20 g and z , independently for each occurrence, represent an integer in the range of 0 and 10 (preferably 0 to 8, 0 to 5, 0 to 3, or even 0 to about 2); and

h represents an integer in the range of 1 and 30,000 (preferably <25,000, <20,000, <15,000, <10,000, <5,000, <1,000, <500, <100, <50, <25, <10, or even <5),

wherein at least one occurrence (and preferably at least 5, 10, or even at least
5 20, 50, or 100 occurrences) of g represents an integer greater than 0.

In some embodiments, L_4 and L_7 represent linker groups, such as photocleavable linker groups.

In certain embodiments, one or more occurrences of L_4 , L_5 , L_6 , or L_7 ,
10 independently represent a photocleavable linker. In certain embodiments, one or more occurrences of L_4 , L_6 , or L_7 , independently represent a photocleavable linker. In certain embodiments, one or more occurrences of L_4 or L_6 , particularly L_6 , independently represent a photocleavable linker.

In certain embodiments, the underlying polymers are linear cyclodextrin-
15 containing polymers, e.g., the polymer backbone includes cyclodextrin moieties. For example, the polymer may be a water-soluble, linear cyclodextrin polymer produced by providing at least one cyclodextrin derivative modified to bear one reactive site at each of exactly two positions, and reacting the cyclodextrin derivative with a linker having exactly two reactive moieties capable of forming a covalent bond with the
20 reactive sites under polymerization conditions that promote reaction of the reactive sites with the reactive moieties to form covalent bonds between the linker and the cyclodextrin derivative, whereby a linear polymer comprising alternating units of cyclodextrin derivatives and linkers is produced. Alternatively, the polymer may be a water-soluble, linear cyclodextrin polymer having a linear polymer backbone,
25 which polymer comprises a plurality of substituted or unsubstituted cyclodextrin moieties and linker moieties in the linear polymer backbone, wherein each of the cyclodextrin moieties, other than a cyclodextrin moiety at a terminus of a polymer chain, is attached to two of said linker moieties, each linker moiety covalently linking two cyclodextrin moieties, other than a linker moiety at a terminus of a
30 polymer chain. In yet another embodiment, the polymer is a water-soluble, linear cyclodextrin polymer comprising a plurality of cyclodextrin moieties covalently

linked together by a plurality of linker moieties, wherein each cyclodextrin moiety, other than a cyclodextrin moiety at the terminus of a polymer chain, is attached to two linker moieties to form a linear cyclodextrin polymer.

The linker group(s) may be an alkylene chain, a polyethylene glycol (PEG) chain, polysuccinic anhydride, poly-L-glutamic acid, poly(ethyleneimine), an oligosaccharide, an amino acid chain, or any other suitable linkage. In certain embodiments, the linker group itself can be stable under physiological conditions, such as an alkylene chain, or it can be cleavable under physiological conditions, such as by an enzyme (e.g., the linkage contains a peptide sequence that is a substrate for a peptidase), or by hydrolysis (e.g., the linkage contains a hydrolyzable group, such as an ester or thioester). The linker groups can be biologically inactive, such as a PEG, polyglycolic acid, or polylactic acid chain, or can be biologically active, such as an oligo- or polypeptide that, when cleaved from the moieties, binds a receptor, deactivates an enzyme, etc. Various oligomeric linker groups that are biologically compatible and/or bioerodible are known in the art, and the selection of the linkage may influence the ultimate properties of the material, such as whether it is durable when implanted, whether it gradually deforms or shrinks after implantation, or whether it gradually degrades and is absorbed by the body. The linker group may be attached to the moieties by any suitable bond or functional group, including carbon-carbon bonds, esters, ethers, amides, amines, carbonates, carbamates, sulfonamides, etc.

In certain embodiments, the linker group(s) of the present invention represent a hydrocarbylene group wherein one or more methylene groups is optionally replaced by a group Y (provided that none of the Y groups are adjacent to each other), wherein each Y, independently for each occurrence, is selected from, substituted or unsubstituted aryl, heteroaryl, cycloalkyl, heterocycloalkyl, or -O-, C(=X) (wherein X is NR₁, O or S), -OC(O)-, -C(=O)O-, -NR₁-, -NR₁CO-, -C(O)NR₁-, -S(O)_n- (wherein n is 0, 1, or 2), -OC(O)-NR₁-, -NR₁-C(O)-NR₁-, -NR₁-C(NR₁)-NR₁-, and -B(OR₁)_n-; wherein R₁, independently for each occurrence, represents H or a lower alkyl.

In certain embodiments, the linker group represents a derivatized or non-derivatized amino acid. In certain embodiments, linker groups with one or more terminal carboxyl groups may be conjugated to the polymer. In certain
5 embodiments, one or more of these terminal carboxyl groups may be capped by covalently attaching them to a therapeutic agent, a targeting moiety, or a cyclodextrin moiety via an (thio)ester or amide bond. In still other embodiments, linker groups with one or more terminal hydroxyl, thiol, or amino groups may be incorporated into the polymer. In preferred embodiments, one or more of these
10 terminal hydroxyl groups may be capped by covalently attaching them to a therapeutic agent, a targeting moiety, or a cyclodextrin moiety via an (thio)ester, amide, carbonate, carbamate, thiocarbonate, or thiocarbamate bond. In certain embodiments, these (thio)ester, amide, (thio)carbonate or (thio)carbamates bonds may be biohydrolyzable, i.e., capable of being hydrolyzed under biological conditions.

15 In certain embodiments the linker group may comprise one or more chemical bonds that may be cleaved by photolysis. Such linker groups, or linkers, are referred to herein as “photocleavable” or “photolabile.” Such groups or moieties may comprise any photocleavable bonds known in the art. Groups with chemical bonds whose cleavage can be reasonably predicted based on current knowledge in the art
20 are also contemplated. In some instances, photolabile groups may be derived from photolabile protecting groups already known in the art. Examples of photolabile linker groups include dimethoxy benzoin, photolabile phosphoramidites, 4-(2-chloropropionyl)phenyl]acetic acid (CPA) derivatives, nitroaromatics, 1-nitrobenzyls, α -substituted-1-nitrobenzyls, 3,5-dimethoxybenzyls,
25 methylnitropiperonyl systems (Menpoc and Menp, for example), 3-nitrobenzenesulfenyls, nitrophenyl-ethyleneglycol-based systems, nitroveratryls, phenacyls, alkoxybenzoin, NpSSM₂pact systems, pivaloylglycol based linkers, and chromium arene complexes.

Examples of photocleavable linkers and groups may be found in U.S. Patent
30 6,849,462; U.S. Patent 6,828,392; U.S. Patent 6,589,736; U.S. Patent 6,426,184; U.S. Patent 6,410,342; U.S. Patent 5,917,016; U.S. Patent 5,840,485; U.S. Patent

5,679,773; U.S. Patent 5,549,974; U.S. Patent 5,510,240; U.S. Patent 5,482,719; U.S. Patent 5,424,186; U.S. Patent 5,405,783; U.S. Patent 5,148,854; U.S. Patent 5,143,854; U.S. Patent 4,625,014; U.S. patent applications Ser. No. 07/624,120, filed Dec. 6, 1990, and 07/971,181, filed Nov. 2, 1992; PCT Publication No. WO 98/20967; PCT Publication No. WO 92/10092; PCT Publication No. WO 90/15070; Gullier, et al.; "Linkers and Cleavage Strategies in Solid-Phase Organic Synthesis and Combinatorial Chemistry," *Chem. Rev.*, **2000**, *100*, 2091-2157; McGall et al., *J. Am. Chem. Soc.*, **1997**, *119*, 5081-5090; Teague, *Tetrahedron Lett.*, **1996**, *37*, 5751-5754; McGall et al., *Proc. Natl. Acad. Sci. USA*, **1996**, *93*, 13555-13560; Pirrung et al., *J. Org. Chem.*, **1995**, *60*, 6270-6276; Holmes, C.P. et al., *J. Org. Chem.* **1995**, *60*, 2318-2319; Yoo et al., *J. Org. Chem.* **1995**, *60*, 3358-3364; Greene, T.W.; Wuts, P.G.M., *Protective Groups in Organic Synthesis*, 2nd ed.; Wiley: New York, 1991; Fodor, et al., *Science*, **1991**, *251*, 767-773; Baldwin, et al., "New Photolabile Phosphate Protecting Groups," *Tetrahedron*, **1990**, *46*, 6879-6884; Hammer, et al., *Int. J. Pept. Protein Res.*, **1990**, *36*, 31-45; Kreib-Cordonier, et al. in *Peptides--Chemistry, Structure and Biology*, Rivier and Marshall, eds. (1990) pp. 895-897; McCray et al., "Properties and Uses of Photoreactive Cage Compounds," *Annu. Rev. Biophys. Biophys. Chem.*, **1989**, *18*, 239-270; Haridasan et al., "Peptide Synthesis using Photolytically Cleavable 2-Nitrobenzyloxycarbonyl Protecting Group," *Proc. Indian Natl. Sci. Acad.*, **1987**, *53A*, 717-728; Hazum et al., "A Photocleavable Protecting Group for the Thiol Function of Cysteine," *Proc. 16^{sup}.th European Peptide Sym.*, **1987**, 105-110; Barany et al., *J. Am. Chem. Soc.*, **1985**, *107*, 4936-4942; Bellof, et al. *Chimia*, **1985**, *39*, 10; Pillai, "Photoremovable Protecting Groups in Organic Synthesis," *Synthesis*, **1980**, 1-26; Patchornik, *J. Am. Chem. Soc.* **1970** *92*, 6333; Wang et al., *J. Org. Chem.* **1976**, *41*, 32-58; Rich, et al. *J. Am. Chem. Soc.*, **1975**, *97*, 1575-1579; Amit et al., *J. Org. Chem.* **1974** *39*, 192; and Patchornik et al., *J. Am. Chem. Soc.* **1970**, *92*, 6333, which are all incorporated by reference.

Photocleavage of the photocleavable linker groups of the instant invention may be induced by the application of various forms of radiation. The radiation applied may comprise one or more wavelengths from the electromagnetic spectrum including X-rays (about 0.1 nm to about 10.0 nm; or about 10¹⁸ to about 10¹⁶ Hz),

ultraviolet (UV) rays (about 10.0 nm to about 380 nm; or about 8×10^{16} to about 10^{15} Hz), visible light (about 380 nm to about 750 nm; or about 8×10^{14} to about 4×10^{11} Hz), infrared light (about 750 nm to about 0.1 cm; or about 4×10^{14} to about 5×10^{14} Hz), such as near infrared (700 nm to 1000 nm), microwaves (about 0.1 cm to about 100 cm; or about 10^8 to about 5×10^{11} Hz), and radio waves (about 100 cm to about 10^4 m; or about 10^4 to about 10^8 Hz). Multiple forms of radiation may also be applied simultaneously, in combination, or coordinated in a step-wise fashion. Radiation exposure may be constant over a period of seconds, minutes or hours, or varied with pulses at predetermined intervals.

Typically, the radiation source is placed at a specified distance from the photocleavable group to be irradiated. That distance may be empirically determined or calculated from the energy loss produced between the source and the target and the amount of energy emitted by the source. Preferably, the radiation applied is UV, visible or IR radiation of the wavelength between about 200 nm to about 1,000 nm. Radiation may be administered continuously or as pulses for hours, minutes or seconds, and preferably for the shortest amount of time possible to minimize any risk of damage to the surrounding tissue and for convenience. Radiation may be administered for less than about one hour, preferably less for than about 30 minutes, more preferably for less than about ten minutes, and still more preferably for less than about one minute. Visible, UV and IR radiation are also preferred as all three of these forms of radiation can be conveniently and inexpensively generated from commercially available sources.

The power density or intensity of light per area necessary to selectively cleave the chemical bond of the photolabile group in certain embodiments may be very small, making the photocleavable process practical as a means for releasing drug in the desired area of the patient. Maximization of efficiency also may minimize exposure time necessary to achieve selective cleavage and provide a minimum of undesirable background effects.

Photocleavable linkers may be used to attach therapeutic/bioactive agents or prodrugs thereof to polymers as described herein. Such linkers may release the agents or prodrugs on photolysis. Photocleavable linkers may also be used to attach

targeting ligands or moieties to polymers of the instant invention. Such linkers may release the targeting ligands or moieties on photolysis. Photocleavable linkers may also be used to attach cyclodextrin moieties or derivatives thereof to polymers of the instant invention. Such linkers may release the cyclodextrin moieties or derivatives thereof on photolysis. Photocleavable linkers may also be employed in the backbone of the polymer as described herein, for example, or in side chains of the polymer. Photocleavable linkers in the backbone of the polymer may break the polymer up into smaller units on photolysis, possibly causing a change in the physical and chemical properties of the polymer. Changes to the polymer backbone resulting from photolysis may be selected to tune the ability of the polymer to deliver and/or release one or more therapeutic/bioactive agents. Such linkers on the side chains of the polymer may release the moieties attached thereto on photolysis, or cleave the side chains from the polymer backbone, possibly altering the physical and/or chemical properties of the polymer. In any given instance, one or more different types of photocleavable linkers may be employed, and these may be optimized for a desired photolysis; that is, to cleave a desired chemical bond releasing a desired moiety or group.

In certain embodiments, the polymers as described above have polydispersities less than about 3, or even less than about 2.

In certain embodiments, the therapeutic agent is a small molecule, a peptide, a protein, or a polymer that has therapeutic function. In certain embodiments, the agent is an anti-cancer (such as camptothecin or related derivatives), anti-fungal, anti-bacterial, anti-mycotic, or anti-viral therapeutic. In certain embodiments, the agent is a receptor agonist. In certain embodiments, the agent is a receptor antagonist. In certain embodiments, the therapeutic agent is a protease inhibitor. Furthermore, a polymer of the present invention may contain one kind of therapeutic agent, or may contain more than one kind of therapeutic agent. For instance, two or more different cancer drugs, or a cancer drug and an immunosuppressant, or an antibiotic and an anti-inflammatory agent may be grafted on to the polymer via optional linkers. By selecting different linkers for different drugs, the release of each drug may be attenuated to achieve maximal dosage and efficacy.

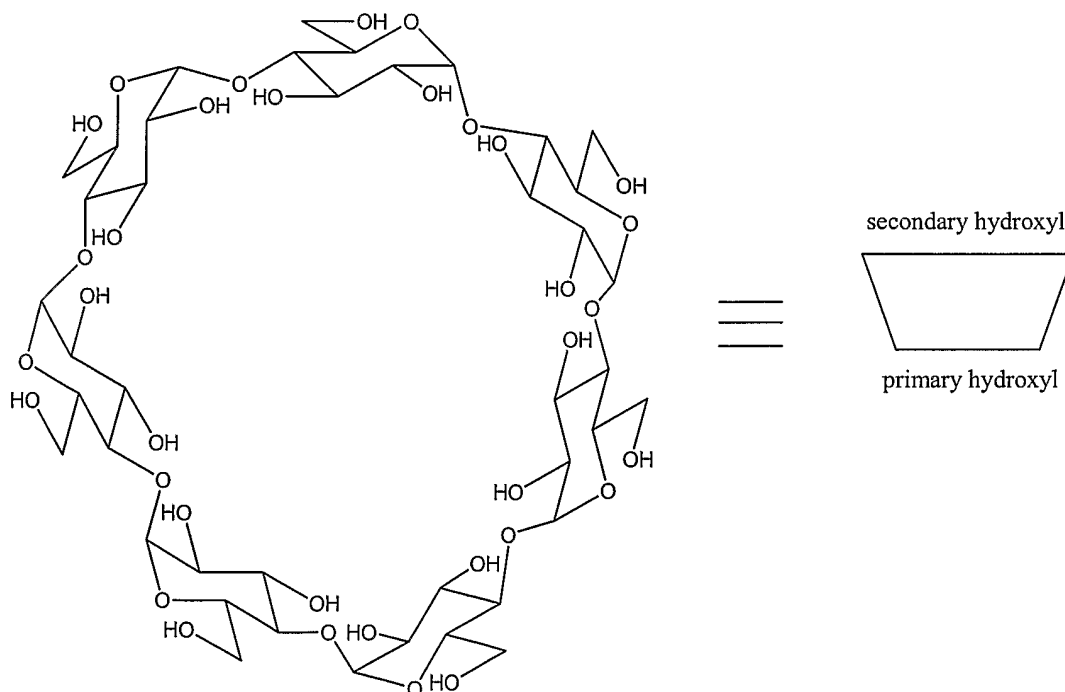
One embodiment of the present invention provides an improved delivery of certain hydrophobic small molecule therapeutics by covalently conjugating them to cyclodextrin containing polymers. Such conjugation improves the aqueous solubility and hence the bioavailability of the therapeutic agents. Accordingly, in one embodiment of the invention, the therapeutic agent is a hydrophobic compound with a log P >0.4, >0.6, >0.8, >1, >2, >3, >4, or even >5. In other embodiments, a hydrophobic therapeutic agent, such as camptothecin, may be conjugated to another compound, such as an amino acid, prior to covalently attaching the conjugate on to the polymer. Examples of amino acid derivatized camptothecin molecules are illustrated in the following schemes and also in U.S. application 10/656838 (now U.S. published application 2004-0077595), which is hereby incorporated by reference herein in its entirety.

The polymer conjugates of the present invention preferably have molecular weights in the range of 10,000 to 500,000; 30,000 to 200,000; or even 70,000 to 150,000 amu.

In certain embodiments, the cyclodextrin moieties make up at least about 2%, 5% or 10% by weight, up to 20%, 30%, 50% or even 80% of the cyclodextrin-modified polymer by weight. In certain embodiments, the therapeutic agents, or targeting ligands make up at least about 1%, 5%, 10% or 15%, 20%, 25%, 30% or even 35% of the cyclodextrin-modified polymer by weight. Number-average molecular weight (M_n) of the polymer may also vary widely, but generally falls in the range of about 1,000 to about 500,000 daltons, preferably from about 5000 to about 200,000 daltons and, even more preferably, from about 10,000 to about 100,000. Most preferably, M_n varies between about 12,000 and 65,000 daltons. In certain other embodiments, M_n varies between about 3000 and 150,000 daltons. Within a given sample of a subject polymer, a wide range of molecular weights may be present. For example, molecules within the sample may have molecular weights that differ by a factor of 2, 5, 10, 20, 50, 100, or more, or that differ from the average molecular weight by a factor of 2, 5, 10, 20, 50, 100, or more. Exemplary cyclodextrin moieties include cyclic structures consisting essentially of from 7 to 9 saccharide moieties, such as cyclodextrin and oxidized cyclodextrin. A cyclodextrin

moiety is optionally substituted and optionally comprises a linker moiety that forms a covalent linkage between the cyclic structure and the polymer backbone, preferably having from 1 to 20 atoms in the chain, such as alkyl chains, including dicarboxylic acid derivatives (such as glutaric acid derivatives, succinic acid derivatives, and the like), and heteroalkyl chains, such as oligoethylene glycol chains.

Cyclodextrins are cyclic polysaccharides containing naturally occurring D-(+)-glucopyranose units in an α -(1,4) linkage. The most common cyclodextrins are alpha (α)-cyclodextrins, beta (β)-cyclodextrins and gamma (γ)-cyclodextrins which contain, respectively six, seven, or eight glucopyranose units. Structurally, the cyclic nature of a cyclodextrin forms a torus or donut-like shape having an inner apolar or hydrophobic cavity, the secondary hydroxyl groups situated on one side of the cyclodextrin torus and the primary hydroxyl groups situated on the other. Thus, using (β)-cyclodextrin as an example, a cyclodextrin is often represented schematically as follows.



The side on which the secondary hydroxyl groups are located has a wider diameter than the side on which the primary hydroxyl groups are located. The present invention contemplates covalent linkages to cyclodextrin moieties on the

primary and/or secondary hydroxyl groups. The hydrophobic nature of the cyclodextrin inner cavity allows for host-guest inclusion complexes of a variety of compounds, e.g., adamantane. (Comprehensive Supramolecular Chemistry, Volume 3, J.L. Atwood et al., eds., Pergamon Press (1996); T. Cserhati, Analytical
5 Biochemistry, 225:328-332(1995); Husain et al., Applied Spectroscopy, 46:652-658 (1992); FR 2 665 169). Additional methods for modifying polymers are disclosed in Suh, J. and Noh, Y., *Bioorg. Med. Chem. Lett.* **1998**, 8, 1327-1330.

In certain embodiments, the present invention contemplates linear, water-soluble, cyclodextrin-containing polymer, wherein a plurality of
10 therapeutic/bioactive agents are covalently attached to the polymer through attachments that are cleaved under biological or photolytic conditions to release the therapeutic/bioactive agents, wherein administration of the polymer to a patient results in release of the therapeutic/bioactive agent over a period of at least 2, 3, 5, 6, 8, 10, 15, 20, 24, 36, 48 or even 72 hours.

15 In certain embodiments, the present invention contemplates attenuating the rate of release of the therapeutic/bioactive agent by introducing various linking groups between the therapeutic agent and/or targeting ligand and the polymer. Thus, in certain embodiments, the polymeric therapeutics of the present invention are compositions for controlled delivery of therapeutic agents. One skilled in the art
20 would also recognize that by labeling the therapeutic agent and/or targeting ligand with radionuclei, or by forming complexes of NMR active nuclei, e.g., technetium, gadolinium, or dysprosium, the polymers of the present invention can achieve a dual diagnostic/therapeutic utility.

In other embodiments, the polymeric compounds stabilize the bioactive form
25 of a therapeutic agent which exists in equilibrium between an active and inactive form. For instance, conjugating the therapeutic agent to the polymers of the present invention may shift the equilibrium between two tautomeric forms of the agent to the bioactive tautomer. In other embodiments, the polymeric compounds may attenuate the equilibrium between lactonic and acid forms of a therapeutic agent.

One method to determine molecular weight is by gel permeation chromatography ("GPC"), e.g., mixed bed columns, CH₂Cl₂ solvent, light scattering detector, and off-line dn/dc. Other methods are known in the art.

5 In other embodiments, the polymer conjugate of the invention may be a flexible or flowable material. When the polymer used is itself flowable, the polymer composition of the invention, even when viscous, need not include a biocompatible solvent to be flowable, although trace or residual amounts of biocompatible solvents may still be present.

10 In some embodiments, the biodegradable polymer or the biologically active agent may be dissolved in a small quantity of a solvent that is non-toxic to more efficiently produce an amorphous, monolithic distribution or a fine dispersion of the biologically active agent in the flexible or flowable composition. In other embodiments, no solvent is needed to form a flowable composition.

15 When a solvent is used to facilitate mixing or to maintain the flowability of the polymer conjugate of the invention, it is often non-toxic and/or biocompatible, and sometimes used in relatively small amounts.

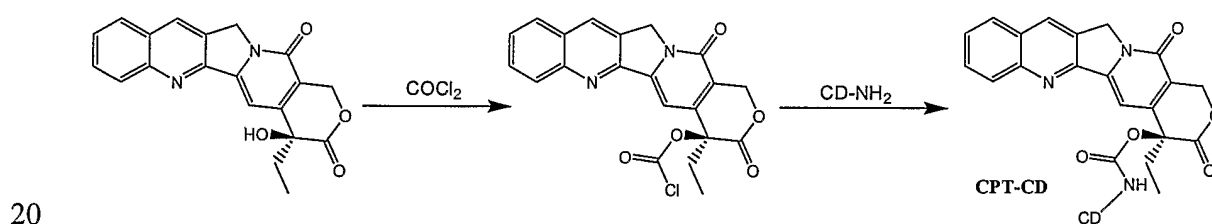
Examples of suitable biocompatible solvents, when used, include N-methyl-2-pyrrolidone, 2-pyrrolidone, ethanol, propylene glycol, acetone, methyl acetate, ethyl acetate, methyl ethyl ketone, dimethylformamide, dimethylsulfoxide, 20 tetrahydrofuran, caprolactam, oleic acid, or 1-dodecylazacylcoheptanone. Preferred solvents include N-methylpyrrolidone, 2-pyrrolidone, dimethylsulfoxide, and acetone because of their solvating ability and their biocompatibility.

25 In certain embodiments, the subject polymer conjugates are soluble in one or more common organic solvents for ease of fabrication and processing. Common organic solvents include such solvents as chloroform, dichloromethane, dichloroethane, 2-butanone, butyl acetate, ethyl butyrate, acetone, ethyl acetate, dimethylacetamide, N-methylpyrrolidone, dimethylformamide, and dimethylsulfoxide.

One aspect of the present invention contemplates attaching a hydrophobic therapeutic agent such as (*S*)-20-camptothecin, and/or one or more analogs thereof, to linear or branched cyclodextrin-containing polymers for better delivery of the drug. (*S*)-20-camptothecin (CPT), an alkaloid isolated from *Camptithec*
5 *accuminata* in the late 1950's, was found to exhibit anticancer activity by inhibiting the action of topoisomerase I during the S-phase of the cell cycle. Its application in human cancer treatment, however, is limited due to several factors, especially its undesirable interactions with human serum albumin, instability of the bioactive lactone form, and poor aqueous solubility. In order to circumvent this problem,
10 many CPT analogs have been developed to improve lactone stability and aqueous solubility. Topotecan and irinotecan are analogs of CPT that have already been approved by FDA for human cancer treatment. The present invention discloses various types of linear, branched, or grafted cyclodextrin-containing polymers wherein (*S*)-20-camptothecin or one or more analogs thereof is covalently bound to
15 the polymer. In certain embodiments, the drug is covalently linked via a biohydrolyzable bond selected from an ester, amide, carbamates, or carbonate.

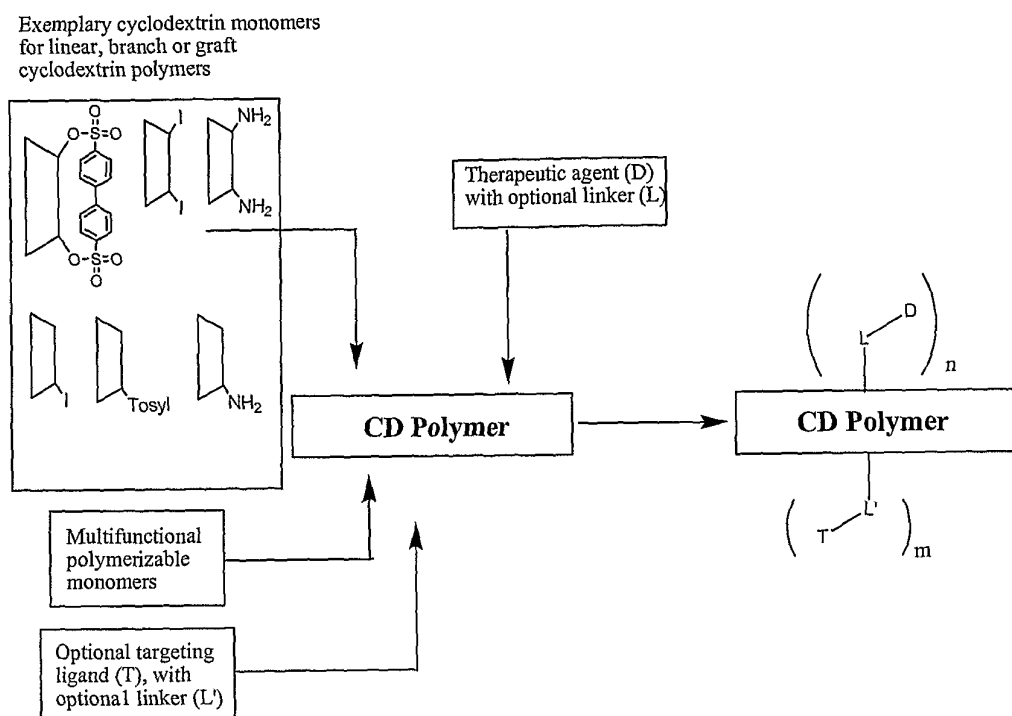
An exemplary synthetic scheme for covalently bonding a derivatized CD to 20(*S*)-camptothecin is shown in Scheme 1.

Scheme 1



Without intending to limit the scope of the invention, a general strategy for synthesizing linear, branched or grafted cyclodextrin-containing polymers (CD Polymer) for loading a therapeutic agent such as camptothecin, and an optional
25 targeting ligand is shown in Scheme 2.

Scheme 2



To illustrate further, without intending to be limiting, comonomer A

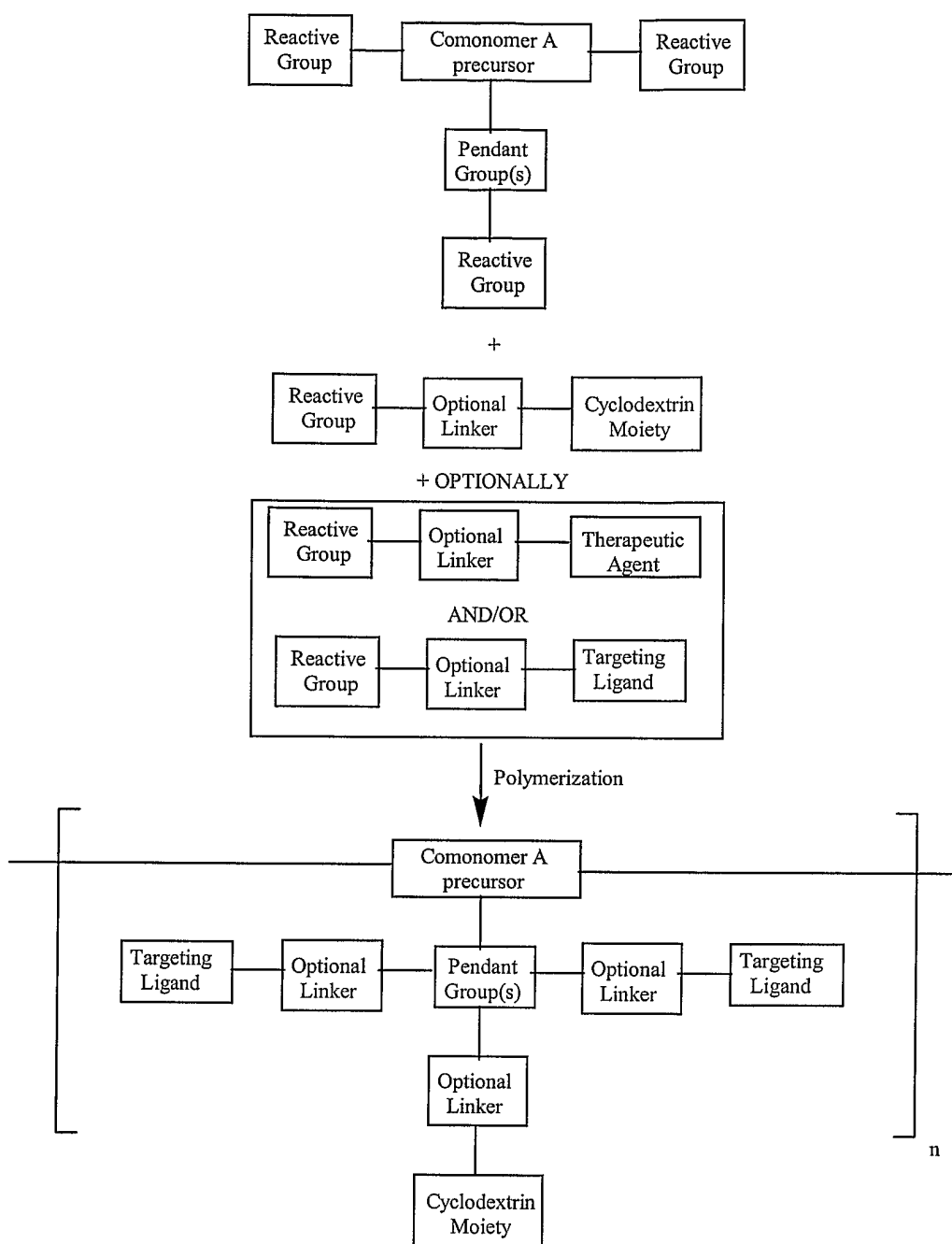
5 precursors, cyclodextrin moieties, therapeutic agents, and/or targeting ligands may be assembled as shown in Schemes 2a-b. Note that in schemes 2a-b, in any given reaction there may be more than one comonomer A precursor, cyclodextrin moiety, therapeutic agent or targeting ligand that is of the same type or different.

Furthermore, prior to polymerization, one or more comonomer A precursor,

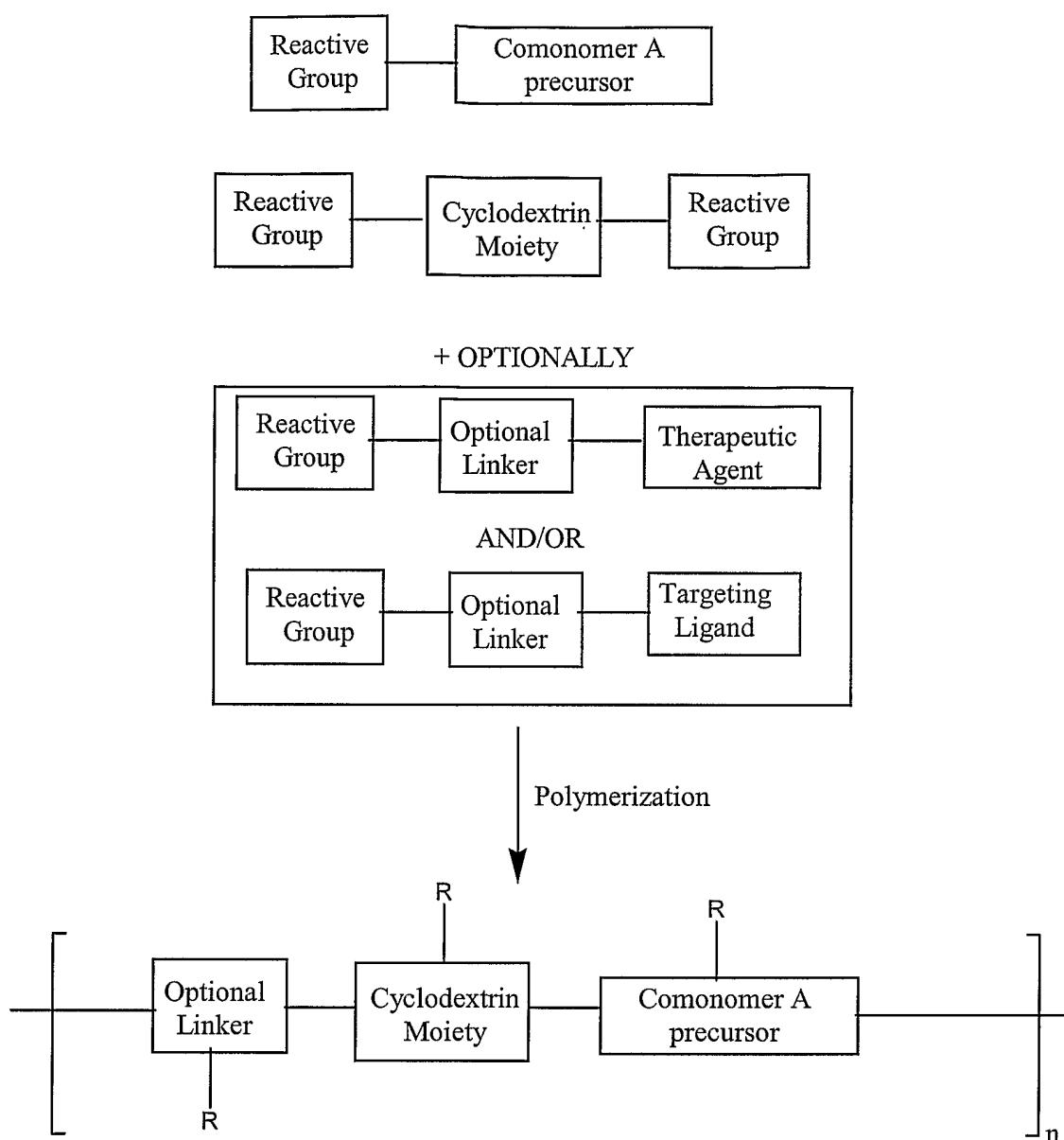
10 cyclodextrin moiety, therapeutic agent or targeting ligand may be covalently linked with each other in one or more separate step.

Scheme 2a: General scheme for graft polymers. The comonomer A precursor, cyclodextrin moiety, therapeutic agent and targeting ligand are as defined above. Furthermore, one skilled in the art may choose from a variety of reactive

15 groups, e.g., hydroxyls, carboxyls, halides, amines, and activated ethenes, ethynes, or aromatic groups in order achieve polymerization. For further examples of reactive groups are disclosed in Advanced Organic Chemistry: Reactions, Mechanisms, and Structure, 5th Edition, 2000.

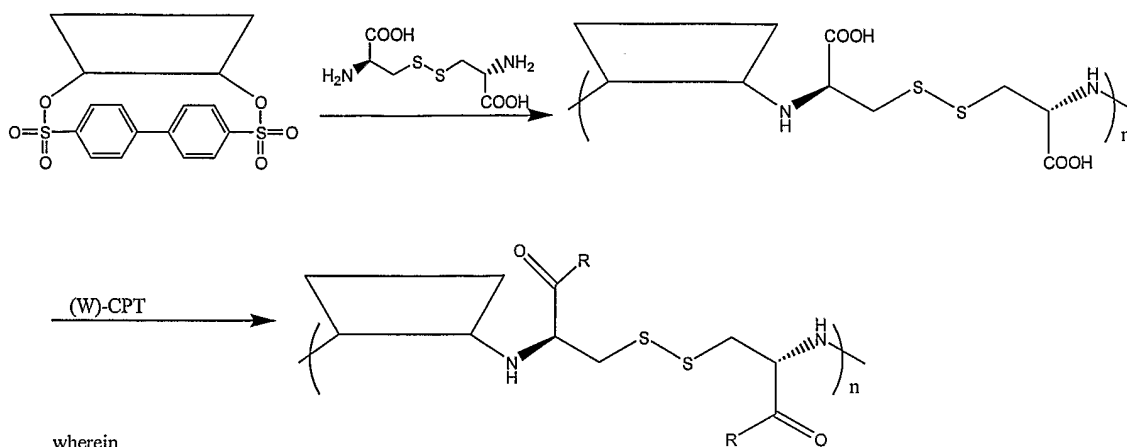


Scheme 2b: General scheme of preparing linear cyclodextrin-containing polymers. One skilled in the art would recognize that by choosing a comonomer A precursor that has multiple reactive groups polymer branching can be achieved.

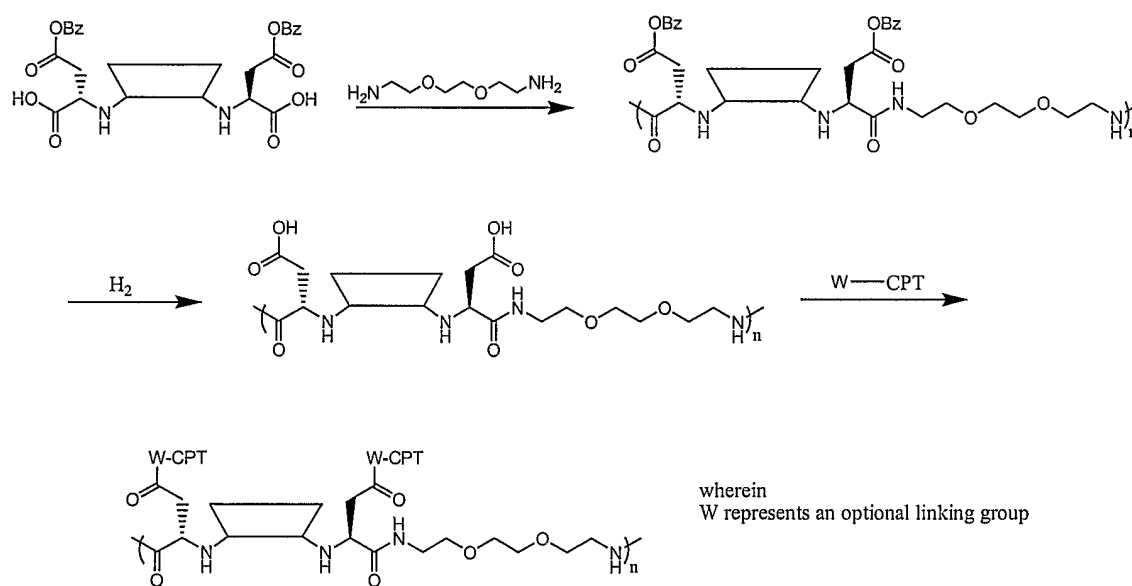


Examples for different ways of synthesizing linear cyclodextrin-CPT polymers are shown in Schemes 3-8.

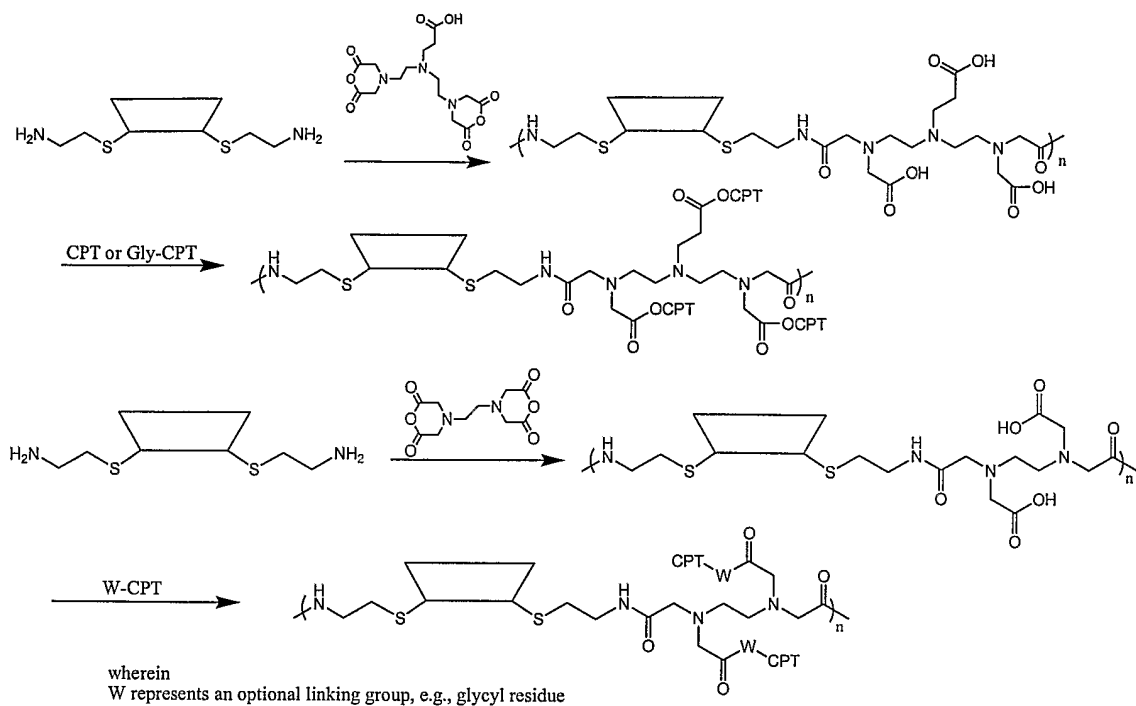
Scheme 3



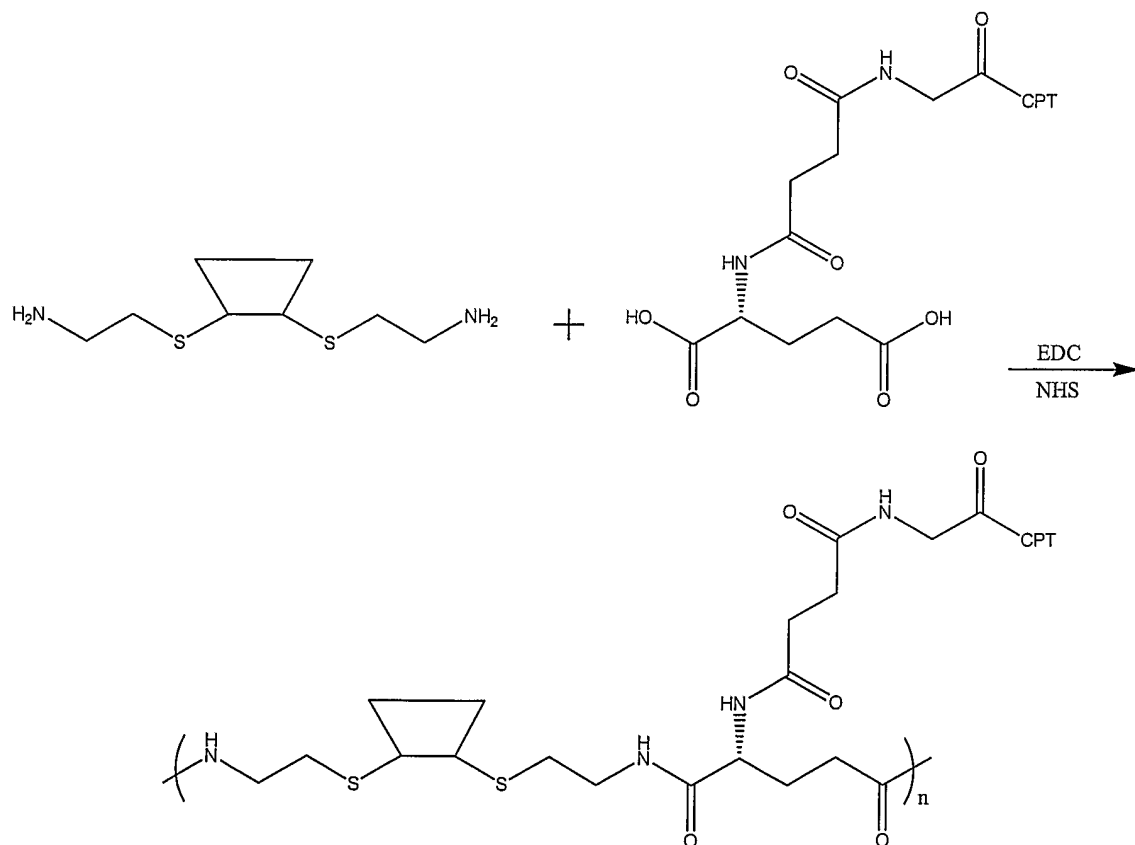
Scheme 4



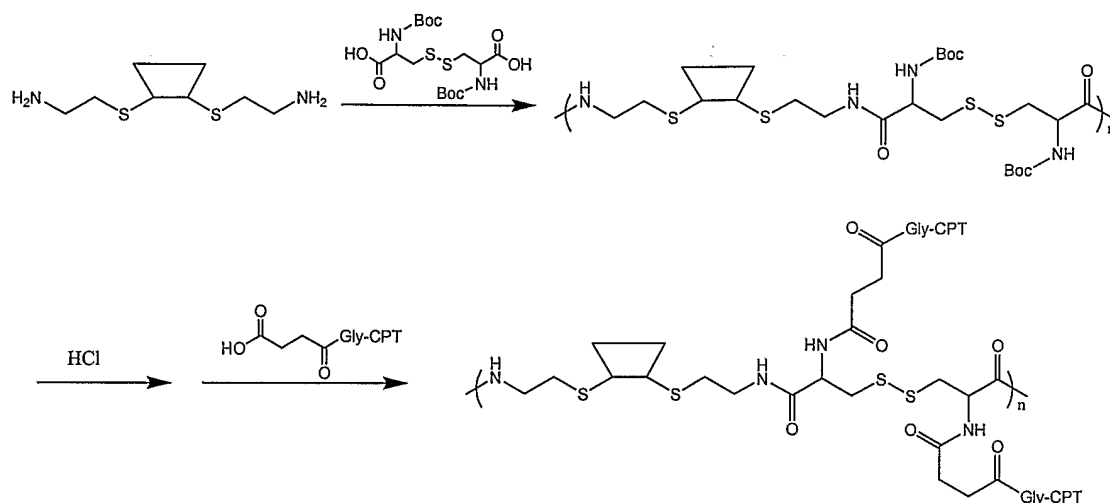
Scheme 5



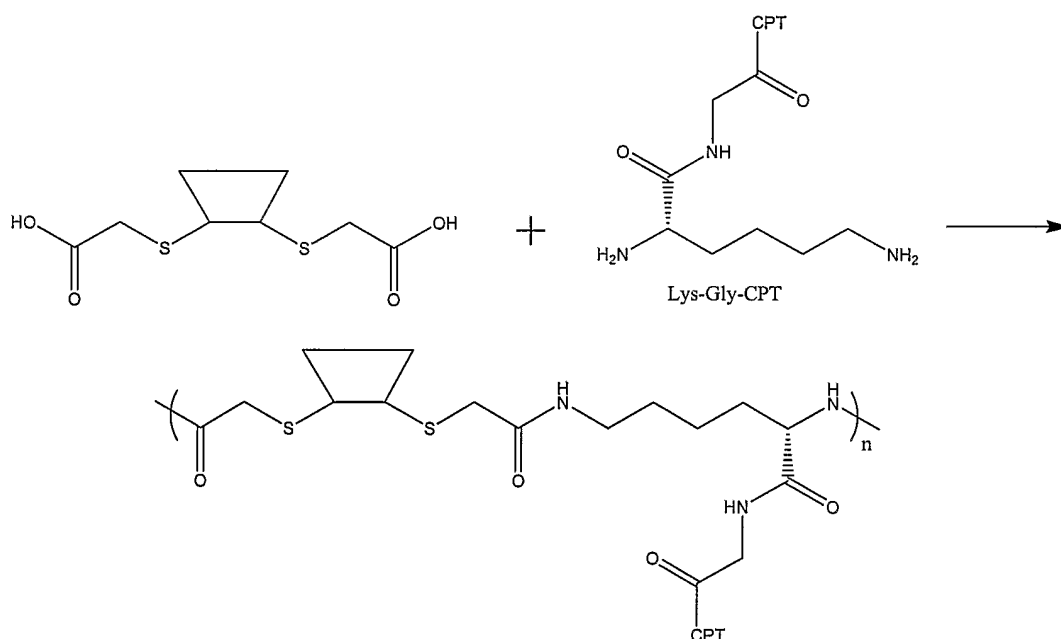
Scheme 6



Scheme 7



Scheme 8



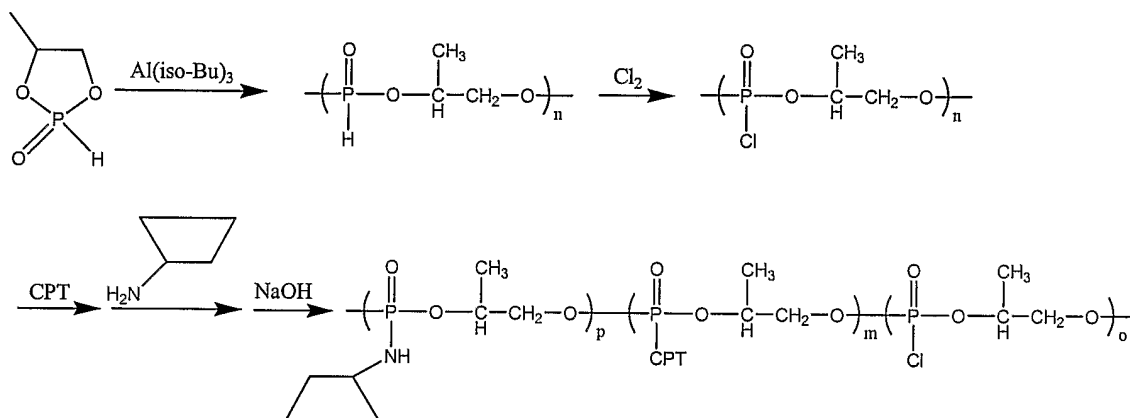
5

Examples for grafting cyclodextrins on to side-chains of CPT-containing polymers subunits are shown in Schemes 9-12. Each subunit may repeat any number of times, and one subunit may occur with substantially the same frequency, more often, or less often than another subunit, such that both subunits may be present in approximately the same amount, or in differing amounts, which may differ slightly or be highly disparate, e.g., one subunit is present nearly to the exclusion of the other.

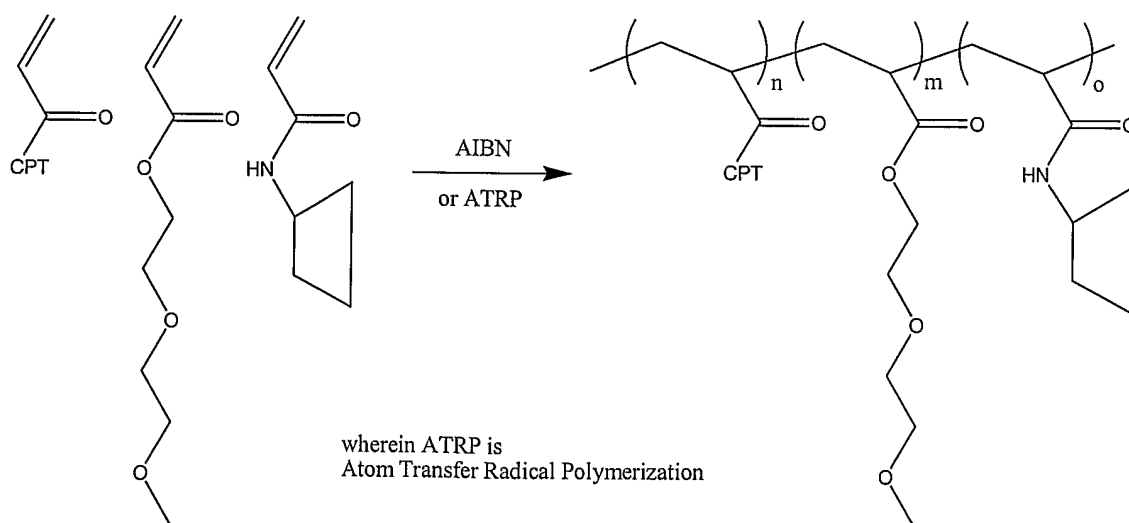
10

In certain instances, the polymers are random copolymers, in which the different subunits and/or other monomeric units are distributed randomly throughout the polymer chain. Thus, where the formula $X_m-Y_n-Z_o$ appears, wherein X, Y and Z are polymer subunits, these subunits may be randomly interspersed throughout the polymer backbone. In part, the term "random" is intended to refer to the situation in which the particular distribution or incorporation of monomeric units in a polymer that has more than one type of monomeric units is not directed or controlled directly by the synthetic protocol, but instead results from features inherent to the polymer system, such as the reactivity, amounts of subunits and other characteristics of the synthetic reaction or other methods of manufacture, processing, or treatment.

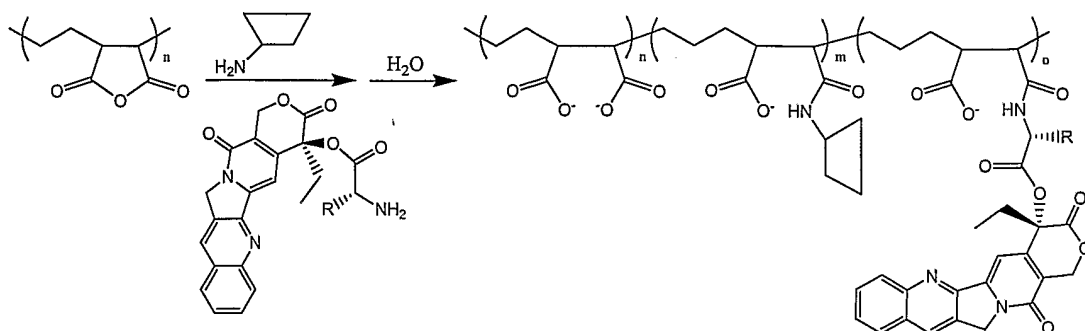
Scheme 9



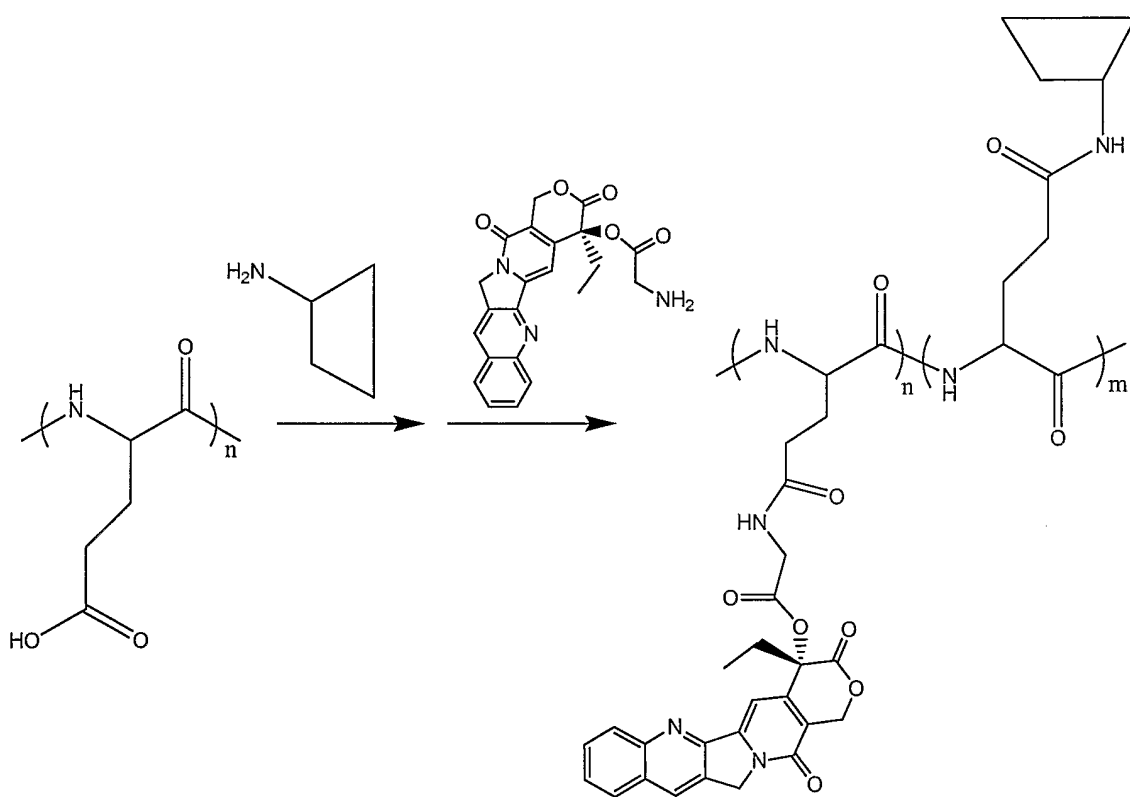
Scheme 10



Scheme 11



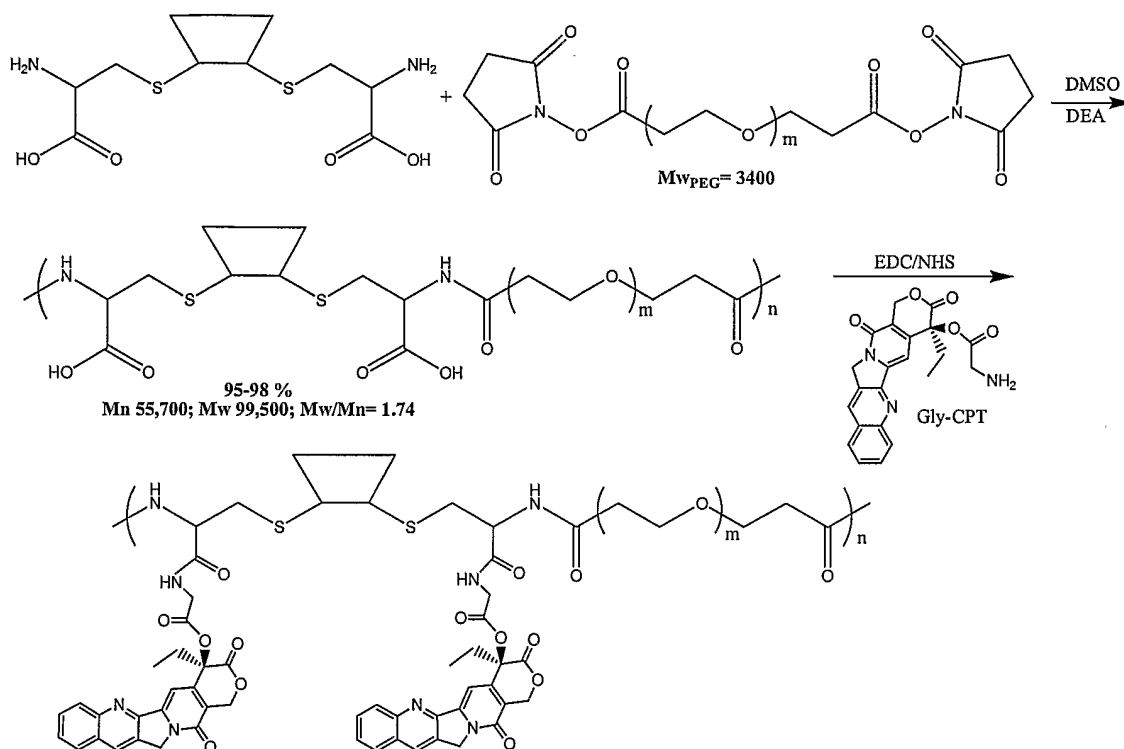
Scheme 12



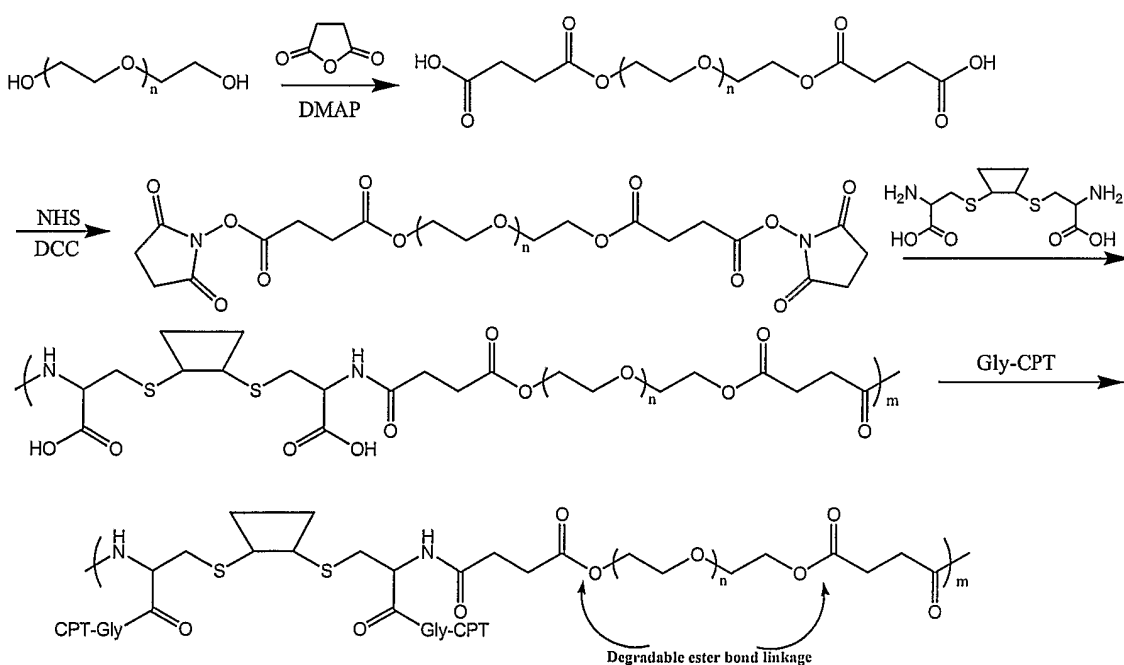
5

The present invention further contemplates CD-polymers synthesized using CD-biscysteine monomer and a di-NHS ester such as PEG-DiSPA or PEG-BTC as shown in Schemes 13-14.

Scheme 13



Scheme 14



5

In certain embodiments, the present invention discloses several strategies to increase drug loading as shown in Figure 1.

(b) Targeting Ligand

As mentioned above, one aspect of the present invention contemplates attaching a therapeutic agent to the polymer conjugates described herein.

In certain embodiments, the polymer conjugate further comprises a targeting
5 ligand. Thus in certain embodiments, a receptor, cell, and/or tissue-targeting ligand, or a precursor thereof is coupled to a polymer conjugate. As used herein the term "targeting ligand" refers to any material or substance which may promote targeting of receptors, cells, and/or tissues in vivo or in vitro with the compositions of the present invention. Many targeting ligands are known or are predicted based on
10 current knowledge in the art and are contemplated by the present invention. The targeting ligand may be synthetic, semi-synthetic, or naturally-occurring. Materials or substances which may serve as targeting ligands include, for example, proteins, including antibodies, antibody fragments, hormones, hormone analogues, glycoproteins and lectins, peptides, polypeptides, amino acids, sugars, saccharides,
15 including monosaccharides and polysaccharides, carbohydrates, small molecules, vitamins, steroids, steroid analogs, hormones, cofactors, bioactive agents, and genetic material, including nucleosides, nucleotides, nucleotide acid constructs and polynucleotides. The targeting ligand may be any molecular structure which assists the construct in localizing to a particular target area, entering a target cell(s), and/or
20 binding to a target receptor. The targeting ligand may localize to a site in an organism. The site in the organism may be the location of an abnormal physiological condition, or a particular tissue type. The targeting ligand may be a small molecule, lipid, antibody, lectin, sugar, steroid, hormone, nutrient, or protein.

As used herein, the term "precursor" to a targeting ligand refers to any
25 material or substance which may be converted to a targeting ligand. Such conversion may involve, for example, anchoring a precursor to a targeting ligand. Exemplary targeting precursor moieties include maleimide groups, disulfide groups, such as ortho-pyridyl disulfide, vinylsulfone groups, azide groups, and α -iodo acetyl groups.

30 The attachment of the targeting ligand or precursor thereof to the polymer may be accomplished in various ways including but not limited to chelation,

covalent attachment, or formation of host-guest complexes. In certain embodiments, an optional linker group may be present between the targeting ligand or precursor thereof and the polymer, wherein the linker group is attached to the polymer via chelation, covalent attachment or form host guest complexes. For example, the one
5 terminal end of a linker group may be attached to the targeting ligand while the other may be attached to an adamantane group, or other such hydrophobic moiety, which forms a host guest complex with a cyclodextrin moiety. Thus the targeting ligand may be attached to a grafted cyclodextrin moiety, to a cyclodextrin moiety within the polymeric chain, or to the polymeric chain itself. The number of targeting
10 ligands per polymeric chain may vary according to various factors including but not limited to the identity of the therapeutic agent, nature of the disease, type of polymer chain. Structures of possible linker groups are the same as linker groups defined elsewhere in this application.

The targeting ligand may be selected on the basis of the particular condition
15 or site to be targeted. The targeting ligand may further comprise any of a number of different chemical entities. In one embodiment, the targeting moiety is a small molecule.

Receptor mediated endocytotic activity has been utilized for delivering exogenous molecules such as proteins and nucleic acids to cells. Generally, a
20 specified ligand is chemically conjugated by covalent, ionic, or hydrogen bonding to an exogenous molecule of interest (i.e., the exogenous compound), forming a conjugate molecule having a moiety (the ligand portion) that is still recognized in the conjugate by a target receptor. Using this technique, the phototoxic protein psoralen has been conjugated to insulin and internalized by the insulin receptor
25 endocytotic pathway (Gasparro, Biochem. Biophys. Res. Comm. 141(2), pp. 502-509, Dec. 15, 1986); the hepatocyte-specific receptor for galactose terminal asialoglycoproteins has been utilized for the hepatocyte-specific transmembrane delivery of asialoorosomucoid-poly-L-lysine non-covalently complexed to a DNA
plasmid (Wu, G. Y., J. Biol. Chem., 262(10), pp. 4429-4432, 1987); the cell receptor
30 for epidermal growth factor has been utilized to deliver polynucleotides covalently linked to EGF to the cell interior (Myers, European Patent Application 86810614.7,

published Jun. 6, 1988); the intestinally situated cellular receptor for the organometallic vitamin B¹²-intrinsic factor complex has been used to mediate delivery to the circulatory system of a vertebrate host a drug, hormone, bioactive peptide or immunogen complexed with vitamin B¹² and delivered to the intestine
5 through oral administration (Russell-Jones et al., European patent Application 86307849.9, published Apr. 29, 1987); the mannose-6-phosphate receptor has been used to deliver low density lipoproteins to cells (Murray, G. J. and Neville, D. M., Jr., J.Bio.Chem, Vol. 255 (24), pp. 1194-11948, 1980); the cholera toxin binding subunit receptor has been used to deliver insulin to cells lacking insulin receptors
10 (Roth and Maddox, J. Cell. Phys. Vol. 115, p. 151, 1983); and the human chorionic gonadotropin receptor has been employed to deliver a ricin a-chain coupled to HCG to cells with the appropriate HCG receptor in order to kill the cells (Oeltmann and Heath, J. Biol. Chem, vol. 254, p. 1028 (1979)).

Another example of a targeting ligand is biotin, a naturally occurring
15 vitamin, which has been shown to localize effectively to tumors and sites of infection. Furthermore, as described in U.S. Pat. No. 5,716,594, which is incorporated by reference herein, imaging agents and therapeutics have been successfully delivered to such sites when coupled to biotin. Another small molecule targeting ligand is folate (see U.S. Pat. No. 5,820,847, which is incorporated by
20 reference herein). Folates are particularly useful in targeting cancer cells, since a variety of carcinomas overexpress folate receptors. See Ladino et al. (Int J Cancer 1997, 73(6):859-6). Riboflavin and its derivatives are other small molecule targeting moieties for targeting delivery of constructs to cancer cells (see, for example, U.S. Pat. No. 5,688,488, which is incorporated by reference herein). Additional nutrients
25 believed to trigger receptor-mediated endocytosis and therefore useful targeting ligands of the instant invention include carnitine, inositol, lipoic acid, niacin, pantothenic acid, thiamin, pyridoxal, ascorbic acid, and the lipid soluble vitamins A, D, E and K. Another type of small molecule targeting ligand includes steroidal lipids, such as cholesterol, and steroidal hormones, such as estradiol, testosterone,
30 etc.

In another embodiment, the targeting ligand may comprise a protein. Particular types of proteins may be selected based on known characteristics of the target site or target cells. For example, the targeting ligand can be an antibody either monoclonal or polyclonal, where a corresponding antigen is displayed at the target
5 site. In situations wherein a certain receptor is expressed by the target cells, the targeting ligand may comprise a protein or peptidomimetic ligand capable of binding to that receptor. Protein ligands of known cell surface receptors include low density lipoproteins, transferrin, insulin, fibrinolytic enzymes, anti-HER2, platelet binding proteins such as annexins, and biological response modifiers (including interleukin,
10 interferon, erythropoietin and colony-stimulating factor). Also, anti-EGF receptor antibodies, which internalize following binding to the receptor and traffic to the nucleus to an extent, are examples targeting ligands for use in the present invention for delivery of Auger emitters and nucleus binding drugs to target cell nuclei.

A number of monoclonal antibodies that bind to a specific type of cell have
15 been developed, including monoclonal antibodies specific for tumor-associated antigens in humans. Among the many such monoclonal antibodies that may be used are anti-TAC, or other interleukin-2 receptor antibodies; 9.2.27 and NR-ML-05 to the 250 kilodalton human melanoma-associated proteoglycan; and NR-LU-10 to a pancreatic carcinoma glycoprotein. An antibody employed in the present invention as a
20 targeting ligand may be an intact (whole) molecule, a fragment thereof, or a functional equivalent thereof. Examples of antibody fragments are F(ab')₂, Fab', Fab, and Fv fragments, which may be produced by conventional methods or by genetic or protein engineering.

Other examples of targeting ligands include sugars (e.g., glucose, fucose,
25 galactose, mannose) that are recognized by target-specific receptors. For example, instant claimed constructs can be glycosylated with mannose residues (e.g., attached as C-glycosides to a free nitrogen) to yield targeted constructs having higher affinity binding to tumors expressing mannose receptors (e.g., glioblastomas and gangliocytomas), and bacteria, which are also known to express mannose receptors
30 (Bertozzi, C R and M D Bednarski Carbohydrate Research 223:243 (1992); J. Am. Chem. Soc. 114:2242,5543 (1992)), as well as potentially other infectious agents.

Certain cells, such as malignant cells and blood cells (e.g., A, AB, B, etc.) display particular carbohydrates, for which a corresponding lectin may serve as a targeting ligands.

Additional structures which may be suitable for use as targeting ligands in the present invention include haptens, epitopes, and dsDNA fragments and analogs and derivatives thereof. Such ligands bind specifically to antibodies, fragments or analogs thereof, including mimetics (for haptens and epitopes), and zinc finger proteins (for dsDNA fragments).

Antibodies are effective ways of targeting cells that express particular antigens on the cell surface, and thus can be used as targeting ligands to selectively target particular cells, such as cancer cells or cells from a particular tissue. Furthermore, antibodies may be made by using standard protocols (See, for example, *Antibodies: A Laboratory Manual*, ed. Harlow and Lane (Cold Spring Harbor Press: 1988)). A mammal, such as a mouse, a hamster or rabbit can be immunized with an immunogenic form of the peptide (e.g., a polypeptide or an antigenic fragment which is capable of eliciting an antibody response, or a fusion protein as described above).

In one exemplary technique, following immunization of an animal with an antigenic preparation of a polypeptide, antisera can be obtained and, if desired, polyclonal antibodies isolated from the serum. To produce monoclonal antibodies, antibody-producing cells (lymphocytes) can be harvested from an immunized animal and fused by standard somatic cell fusion procedures with immortalizing cells such as myeloma cells to yield hybridoma cells. Such techniques are well known in the art, and include, for example, the hybridoma technique (originally developed by Kohler and Milstein, (1975) *Nature*, 256: 495-497), the human B cell hybridoma technique (Kozbar et al., (1983) *Immunology Today*, 4: 72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., (1985) *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc. pp. 77-96). Hybridoma cells can be screened immunochemically for production of antibodies specifically reactive with a polypeptide of the present invention and monoclonal antibodies isolated from a culture comprising such hybridoma cells.

The term antibody as used herein is intended to include fragments thereof which are also specifically reactive with one of the subject mammalian polypeptides. Antibodies can be fragmented using conventional techniques and the fragments screened for utility in the same manner as described above for whole antibodies. For example, F(ab)2 fragments can be generated by treating antibody with pepsin. The resulting F(ab)2 fragment can be treated to reduce disulfide bridges to produce Fab fragments. The antibody of the present invention is further intended to include bispecific, single-chain, and chimeric and humanized molecules having affinity for a subject protein conferred by at least one CDR region of the antibody.

In certain embodiments targeting ligands facilitate binding of therapeutic/bioactive agents of the instant invention to desired targets with an affinity of at least about k_D 10^{-6} M, preferably 10^{-7} M, more preferably 10^{-8} M, and most preferably 10^{-9} M. Binding of the targeting ligand to a receptor should be sufficient to allow a significant amount of the targeting ligand to bind sufficiently long to allow the targeting ligand and/or polymers and/or therapeutic/bioactive agents of the instant invention to be taken into the cell. The affinity of a targeting ligand for a receptor can be determined according to methods well known in the art.

Some embodiments of the instant invention exhibit a high target to non-target ratio when administered in vivo. Preferably the ratio is at least of 2:1, even more preferably at least 3:1; and most preferably at least 5:1 (i.e., it is 2, 3, or 5 times more likely that the target ligand will bind to its specific receptor, relative to other receptors). In certain embodiments, the instant compounds will be administered locally to a subject, or to specific cells in vitro. In such embodiments, it may not be of consequence that the targeting ligand may also interact with cell surface molecules that are located on different tissues, since the compound will not reach that site. Thus, the level of specificity of the targeting ligand depends on factors, such as the type of administration of the compound.

The reactivity of a targeting ligand towards structures other than the targeted receptor can be determined by assays, e.g., by labeling the targeting ligand or using a labeled compound; incubating it with tissue slices; and determining the location of the label. Assays can also be done in animals, such as mice or rats. For example, a

compound can be administered to a non-human animal and the amount of compound present at various locations is determined.

In certain embodiments, the compound may comprise an internalizing polypeptide sequence, such as antepennepedia protein, HIV transactivating (Tat) protein, mastoparan (T. Higashijima et al. (1990) J. Biol. Chem. 265:14176), melittin, bombolittin, delta hemolysin, pardaxin, Pseudomonas exotoxin A, clathrin, Diphtheria toxin, C9 complement protein, or a fragment of one of the preceding proteins. An internalizing peptide is capable of crossing a cellular membrane by, e.g., transcytosis, at a relatively high rate, and thereby promote cellular uptake of molecules to which they are attached. Certain internalizing polypeptides, such as Tat, are also known to localize to the nucleus or other cellular structures. Thus a compound of the present invention which includes such an internalizing peptide sequence may exhibit increased uptake by target cells relative to constructs that lack such a sequence.

The internalizing polypeptide may be part of the targeting ligand or a separate element of the compound. In one embodiment of the invention, the internalizing polypeptide serves as the targeting. In another embodiment, the internalizing polypeptide is covalently linked to one or more of the other elements of the compound. A preferred location of an internalizing polypeptide in a compound can be determined, e.g., by conduction in vitro assays using target cells, labeled compound, and determining the amount of label that is incorporated into the cells.

In one embodiment, the internalizing peptide is derived from the drosophila antepennepedia protein, or homologs thereof. The 60 amino acid long homeodomain of the homeo-protein antepennepedia has been demonstrated to translocate through biological membranes and can facilitate the translocation of heterologous polypeptides to which it is coupled. See for example Derossi et al. (1994) J Biol Chem 269:10444-10450; Perez et al. (1992) J Cell Sci 102:717-722. Recently, it has been demonstrated that fragments as small as 16 amino acids long of this protein are sufficient to drive internalization. See Derossi et al. (1996) J Biol Chem 271:18188-18193. The present invention contemplates a compound comprising at least a

portion of the antepennepedia protein (or homolog thereof) sufficient to increase the transmembrane transport of the compound, relative to the compound alone, by a statistically significant amount.

Another example of an internalizing peptide is the HIV transactivator (TAT) protein. This protein appears to be divided into four domains (Kuppuswamy et al. (1989) Nucl. Acids Res. 17:3551-3561). Purified TAT protein is taken up by cells in tissue culture (Frankel, et al. (1989) Cell 55:1189-1193), and peptides, such as the fragment corresponding to residues 37-62 of TAT, are rapidly taken up by cell in vitro (Green, et al. (1989) Cell 55:1179-1188). The highly basic region mediates internalization and targeting of the internalizing moiety to the nucleus (Ruben et al. (1989) J. Virol. 63:1-8). Peptides or analogs that include a sequence present in the highly basic region, such as CFITKALGISYGRKKRRQRRPPQGS (SEQ ID NO: 1), are conjugated to a compound to aid in internalization and targeting those compounds to the intracellular milieu.

While not wishing to be bound by any particular theory, it is noted that hydrophilic polypeptides may be also be physiologically transported across the membrane barriers by coupling or conjugating a targeting construct to a transportable peptide which is capable of crossing the membrane by receptor-mediated transcytosis. Suitable internalizing peptides of this type can be generated using all or a portion of, e.g., a histone, insulin, transferrin, basic albumin, prolactin and insulin-like growth factor I (IGF-I), insulin-like growth factor II (IGF-II) or other growth factors. For instance, it has been found that an insulin fragment, showing affinity for the insulin receptor on capillary cells, and being less effective than insulin in blood sugar reduction, is capable of transmembrane transport by receptor-mediated transcytosis. Examples of growth factor-derived internalizing peptides include EGF (epidermal growth factor)-derived peptides, such as CMHIESLDSYTC (SEQ ID NO: 2) and CMYIEALDKYAC (SEQ ID NO: 3); TGF-beta (transforming growth factor beta)-derived peptides; peptides derived from PDGF (platelet-derived growth factor) or PDGF-2; peptides derived from IGF-I (insulin-like growth factor) or IGF-II; and FGF (fibroblast growth factor)-derived

peptides. Hydrophilic polypeptides can be included in a compound, or they can constitute the targeting ligand.

Another class of translocating/internalizing peptides exhibits pH-dependent membrane binding. For an internalizing peptide that assumes a helical conformation at an acidic pH, the internalizing peptide acquires the property of amphiphilicity, e.g., it has both hydrophobic and hydrophilic interfaces. More specifically, within a pH range of approximately 5.0-5.5, an internalizing peptide forms an alpha-helical, amphiphilic structure that facilitates insertion of the moiety into a target membrane. An alpha-helix-inducing acidic pH environment may be found, for example, in the low pH environment present within cellular endosomes. Such internalizing peptides can be used to facilitate transport of targeting constructs, taken up by an endocytic mechanism, from endosomal compartments to the cytoplasm.

An example of a pH-dependent membrane-binding internalizing peptide includes a high percentage of helix-forming residues, such as glutamate, methionine, alanine and leucine. In addition, a preferred internalizing peptide sequence includes ionizable residues having pKa's within the range of pH 5-7, so that a sufficient uncharged membrane-binding domain will be present within the peptide at pH 5 to allow insertion into the target cell membrane.

An example of a pH-dependent membrane-binding internalizing peptide in this regard is aa1-aa2-aa3-EAALA(EALA)4-EALEALAA- -amide (SEQ ID NO: 4), which represents a modification of the peptide sequence of Subbarao et al. (Biochemistry 26:2964 (1987)). Within this peptide sequence, the first amino acid residue (aa1) is preferably a unique residue, such as cysteine or lysine, that facilitates chemical conjugation of the internalizing peptide to a targeting protein conjugate. Amino acid residues 2-3 may be selected to modulate the affinity of the internalizing peptide for different membranes. For instance, if both residues 2 and 3 are lys or arg, the internalizing peptide will have the capacity to bind to membranes or patches of lipids having a negative surface charge. If residues 2-3 are neutral amino acids, the internalizing peptide will insert into neutral membranes.

Yet other preferred internalizing peptides include peptides of apo-lipoprotein A-1 and B; peptide toxins, such as melittin, bombolittin, delta hemolysin and the pardaxins; antibiotic peptides, such as alamethicin; peptide hormones, such as calcitonin, corticotrophin releasing factor, beta endorphin, glucagon, parathyroid hormone, pancreatic polypeptide; and peptides corresponding to signal sequences of numerous secreted proteins. In addition, exemplary internalizing peptides may be modified through attachment of substituents that enhance the alpha-helical character of the internalizing peptide at acidic pH.

Yet another class of internalizing peptides suitable for use within the present invention include hydrophobic domains that are "hidden" at physiological pH, but are exposed in the low pH environment of the target cell endosome. Upon pH-induced unfolding and exposure of the hydrophobic domain, the ligand binds to lipid bilayers and effects translocation of a covalently linked targeting construct into the cell cytoplasm. Such internalizing peptides may be modeled after sequences identified in, e.g., *Pseudomonas* exotoxin A, clathrin, or Diphtheria toxin.

Pore-forming proteins or peptides may also serve as internalizing peptides herein. Pore forming proteins or peptides may be obtained or derived from, for example, C9 complement protein, cytolytic T-cell molecules or NK-cell molecules. These ligands are capable of forming ring-like structures in membranes, thereby allowing transport of attached targeting construct through the membrane and into the cell interior.

Mere membrane intercalation of an internalizing peptide may be sufficient for translocation of a targeting construct across cell membranes. However, translocation may be improved by attaching to the internalizing peptide a substrate for intracellular enzymes (i.e., an "accessory peptide"). It is preferred that an accessory peptide be attached to a portion(s) of the internalizing peptide that protrudes through the cell membrane to the cytoplasmic face. The accessory peptide may be advantageously attached to one terminus of a translocating/internalizing moiety or anchoring peptide. An accessory moiety of the present invention may contain one or more amino acid residues. In one embodiment, an accessory moiety

may provide a substrate for cellular phosphorylation (for instance, the accessory peptide may contain a tyrosine residue).

An exemplary accessory moiety in this regard would be a peptide substrate for N-myristoyl transferase, such as GNAAAARR (SEQ ID NO: 5) (Eubanks et al. (1988) Peptides. Chemistry and Biology, Garland Marshall (ed.), ESCOM, Leiden 566-69). In this construct, an internalizing, peptide would be attached to the C-terminus of the accessory peptide, since the N-terminal glycine is critical for the accessory moiety's activity. This hybrid peptide, upon attachment to a targeting cosntruct is N-myristylated and will be translocated across the cell membrane.

While not intended to be limiting, additional examples of targeting ligands include those described in U.S. Patent Application Publication No. 2003-0049203, which is incorporated by reference herein.

In certain embodiments, a therapeutic/bioactive agent or targeting moiety may be covalently coupled to a polymeric particle of the invention. In such embodiments, the therapeutic/bioactive agent may preferably be a hydrophilic or charged agent, such that its presence on the surface of the particle increases charge or hydrophilicity of the particle or otherwise increases the particle's mucus resistance. The covalent linkage may be selected to be cleaved under biological conditions, e.g., by chemical or enzymatic hydrolysis or other cleavage processes.

In certain embodiments, a polymeric particle of the invention may further comprise a targeting moiety or molecule. The targeting molecule may be covalently linked to any other component of the polymeric particle, such as the polymer or a surface-altering agent. The targeting molecule may also be co-localized with a polymeric particle, using methods known in the art. The targeting molecule may direct the particle, and thus the included therapeutic/bioactive agent, to a desirable target or location in a patient.

(c) Pharmaceutical Compositions, Formulations and Dosages

In part, a biocompatible polymer composition of the present invention includes a biocompatible and optionally biodegradable polymer, such as one having the recurring monomeric units shown in one of the foregoing formulas, optionally

including any other biocompatible and optionally biodegradable polymer mentioned above or known in the art. In certain embodiments, the compositions are non-pyrogenic, e.g., do not trigger elevation of a patient's body temperature by more than a clinically acceptable amount.

5 The subject compositions may contain a "drug", "therapeutic agent," "medicament," or "bioactive substance," which are biologically, physiologically, or pharmacologically active substances that act locally or systemically in the human or animal body. For example, a subject composition may include any of the other compounds discussed above.

10 Various forms of the medicaments or biologically active materials may be used which are capable of being released from the polymer conjugate into adjacent tissues or fluids. They may be hydrophobic molecules, neutral molecules, polar molecules, or molecular complexes capable of hydrogen bonding. They may be in the form of ethers, esters, amides and the like, including prodrugs which are
15 biologically activated when injected into the human or animal body, e.g., by cleavage of an ester or amide. A therapeutic agent in a subject composition may vary widely with the purpose for the composition.

 Plasticizers and stabilizing agents known in the art may be incorporated in polymers of the present invention. In certain embodiments, additives such as
20 plasticizers and stabilizing agents are selected for their biocompatibility. In certain embodiments, the additives are lung surfactants, such as 1,2-dipalmitoylphosphatidylcholine (DPPC) and L- α -phosphatidylcholine (PC).

 A composition of this invention may further contain one or more adjuvant substances, such as fillers, thickening agents or the like. In other embodiments,
25 materials that serve as adjuvants may be associated with the polymer conjugate. Such additional materials may affect the characteristics of the polymer conjugate that results.

 For example, fillers, such as bovine serum albumin (BSA) or mouse serum albumin (MSA), may be associated with the polymer conjugate. In certain

embodiments, the amount of filler may range from about 0.1 to about 50% or more by weight of the polymer conjugate, or about 2.5, 5, 10, 25, or 40 percent.

Incorporation of such fillers may affect the biodegradation of the polymeric material and/or the sustained release rate of any encapsulated substance. Other fillers known
5 to those of skill in the art, such as carbohydrates, sugars, starches, saccharides, celluloses and polysaccharides, including mannitolose and sucrose, may be used in certain embodiments of the present invention.

In other embodiments, spheronization enhancers facilitate the production of subject polymeric matrices that are generally spherical in shape. Substances such as
10 zein, microcrystalline cellulose or microcrystalline cellulose co-processed with sodium carboxymethyl cellulose may confer plasticity to the subject compositions as well as implant strength and integrity. In particular embodiments, during spheronization, extrudates that are rigid, but not plastic, result in the formation of dumbbell shaped implants and/or a high proportion of fines, and extrudates that are
15 plastic, but not rigid, tend to agglomerate and form excessively large implants. In such embodiments, a balance between rigidity and plasticity is desirable. The percent of spheronization enhancer in a formulation typically range from 10 to 90% (w/w).

In certain embodiments, a subject composition includes an excipient. A
20 particular excipient may be selected based on its melting point, solubility in a selected solvent (e.g., a solvent that dissolves the polymer and/or the therapeutic agent), and the resulting characteristics of the microparticles.

Excipients may comprise a few percent, about 5%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, or higher percentage of the subject compositions.

25 Buffers, acids and bases may be incorporated in the subject compositions to adjust their pH. Agents to increase the diffusion distance of agents released from the polymer conjugate may also be included.

Disintegrants are substances that, in the presence of liquid, promote the disruption of the subject compositions. Disintegrants are most often used in

implants, in which the function of the disintegrant is to counteract or neutralize the effect of any binding materials used in the subject formulation. In general, the mechanism of disintegration involves moisture absorption and swelling by an insoluble material.

5 Examples of disintegrants include croscarmellose sodium and crospovidone which, in certain embodiments, may be incorporated into polymer conjugates in the range of about 1-20% of total polymer conjugate weight. In other cases, soluble fillers such as sugars (mannitol and lactose) may also be added to facilitate disintegration of implants.

10 Other materials may be used to advantage or to control the desired release rate of a therapeutic agent for a particular treatment protocol. For example, if the sustained release is too slow for a particular application, a pore-forming agent may be added to generate additional pores in the polymer conjugate and/or between polymer conjugates. Any biocompatible water-soluble material may be used as the
15 pore-forming agent. They may be capable of dissolving, diffusing or dispersing out of the formed polymer system whereupon pores and microporous channels are generated in the system. The amount of pore-forming agent (and size of dispersed particles of such pore-forming agent, if appropriate) within the composition should affect the size and number of the pores in the polymer system.

20 Pore-forming agents include any pharmaceutically acceptable organic or inorganic substance that is substantially miscible in water and body fluids and will dissipate from the forming and formed polymer into aqueous medium or body fluids or water-immiscible substances that rapidly degrade to water-soluble substances.

 Suitable pore-forming agents include, for example, sugars such as sucrose
25 and dextrose, salts such as sodium chloride and sodium carbonate, and polymers such as hydroxylpropylcellulose, carboxymethylcellulose, polyethylene glycol, and PVP. The size and extent of the pores may be varied over a wide range by changing the molecular weight and percentage of pore-forming agent incorporated into the polymer system.

The charge, lipophilicity or hydrophilicity of any subject polymer conjugate may be modified by attaching in some fashion an appropriate compound to the surface of the polymer. For example, surfactants may be used to enhance wettability of poorly soluble or hydrophobic compositions. Examples of suitable surfactants
5 include dextran, polysorbates and sodium lauryl sulfate. In general, surfactants are used in low concentrations, generally less than about 5%.

Binders are adhesive materials that may be incorporated in polymeric formulations to bind and maintain polymer or aggregate polymer integrity. Binders may be added as dry powder or as solution. Sugars and natural and synthetic
10 polymers may act as binders.

Materials added specifically as binders are generally included in the range of about 0.5%-15% w/w of the polymer formulation. Certain materials, such as microcrystalline cellulose, also used as a spheronization enhancer, also have additional binding properties.

15 Various coatings may be applied to modify the properties of the matrices.

Three exemplary types of coatings are seal, gloss and enteric coatings. Other types of coatings having various dissolution or erosion properties may be used to further modify subject matrices behavior, and such coatings are readily known to one of ordinary skill in the art.

20 The seal coat may prevent excess moisture uptake by the matrices during the application of aqueous based enteric coatings. The gloss coat generally improves the handling of the finished matrices. Water-soluble materials such as hydroxypropylcellulose may be used to seal coat and gloss coat implants. The seal coat and gloss coat are generally sprayed onto the matrices until an increase in
25 weight between about 0.5% and about 5%, often about 1% for a seal coat and about 3% for a gloss coat, has been obtained.

Enteric coatings consist of polymers which are insoluble in the low pH (less than 3.0) of the stomach, but are soluble in the elevated pH (greater than 4.0) of the small intestine. Polymers such as EUDRAGIT™, RohmTech, Inc., Malden, Mass.,

and AQUATERIC™, FMC Corp., Philadelphia, Penn., may be used and are layered as thin membranes onto the implants from aqueous solution or suspension or by a spray drying method. The enteric coat is generally sprayed to a weight increase of about 1% to about 30%, preferably about 10 to about 15% and may contain coating
5 adjuvants such as plasticizers, surfactants, separating agents that reduce the tackiness of the implants during coating, and coating permeability adjusters.

The present compositions may additionally contain one or more optional additives such as fibrous reinforcement, colorants, perfumes, rubber modifiers, modifying agents, etc. In practice, each of these optional additives should be
10 compatible with the resulting polymer and its intended use. Examples of suitable fibrous reinforcement include PGA microfibrils, collagen microfibrils, cellulosic microfibrils, and olefinic microfibrils. The amount of each of these optional additives employed in the composition is an amount necessary to achieve the desired effect.

15 The therapeutic polymer conjugates as described herein can be administered in various pharmaceutical formulations, depending on the disorder to be treated and the age, condition and body weight of the patient, as is well known in the art. For example, where the compounds are to be administered orally, they may be formulated as tablets, capsules, granules, powders or syrups; or for parenteral
20 administration, they may be formulated as injections (intravenous, intramuscular or subcutaneous), drop infusion preparations or suppositories. For application by the ophthalmic mucous membrane route, they may be formulated as eyedrops or eye ointments. These formulations can be prepared by conventional means, and, if desired, the active ingredient may be mixed with any conventional additive, such as
25 an excipient, a binder, a disintegrating agent, a lubricant, a corrigent, a solubilizing agent, a suspension aid, an emulsifying agent or a coating agent. Although the dosage will vary depending on the symptoms, age and body weight of the patient, the nature and severity of the disorder to be treated or prevented, the route of administration and the form of the drug, in general, a daily dosage of from 0.01 to
30 2000 mg of the therapeutic agent is recommended for an adult human patient, and this may be administered in a single dose or in divided doses.

The precise time of administration and/or amount of therapeutic polymer conjugate that will yield the most effective results in terms of efficacy of treatment in a given patient will depend upon the activity, pharmacokinetics, and bioavailability of a particular compound, physiological condition of the patient
5 (including age, sex, disease type and stage, general physical condition, responsiveness to a given dosage and type of medication), route of administration, etc. However, the above guidelines can be used as the basis for fine-tuning the treatment, e.g., determining the optimum time and/or amount of administration, which will require no more than routine experimentation consisting of monitoring
10 the subject and adjusting the dosage and/or timing.

The phrase “pharmaceutically acceptable” is employed herein to refer to those therapeutic polymer conjugates, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation,
15 allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The phrase “pharmaceutically acceptable carrier” as used herein means a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying
20 or transporting the subject chemical from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be “acceptable” in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically acceptable carriers include: (1) sugars, such as lactose, glucose and
25 sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as
30 propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering

agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) phosphate buffer solutions; and (21) other non-toxic compatible substances employed in pharmaceutical formulations.

5 The term "pharmaceutically acceptable salts" refers to the relatively non-toxic, inorganic and organic acid addition salts of the therapeutic polymer conjugates. These salts can be prepared in situ during the final isolation and purification of the therapeutic polymer conjugates, or by separately reacting a purified polymer in its free base form with a suitable organic or inorganic acid, and
10 isolating the salt thus formed. Representative salts include the hydrobromide, hydrochloride, sulfate, bisulfate, phosphate, nitrate, acetate, valerate, oleate, palmitate, stearate, laurate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, naphthylate, mesylate, glucoheptonate, lactobionate, and laurylsulphonate salts and the like. (See, for example, Berge et al. *J. Pharm.*
15 *Sci.*, **1977**, *66*, 1-19).

 In other cases, the therapeutic polymer conjugates useful in the methods of the present invention may contain one or more acidic functional groups and, thus, are capable of forming pharmaceutically acceptable salts with pharmaceutically acceptable bases. The term "pharmaceutically acceptable salts" in these instances
20 refers to the relatively non-toxic, inorganic and organic base addition salts of the polymer(s). These salts can likewise be prepared in situ during the final isolation and purification of the polymer(s), or by separately reacting the purified polymer(s) in its free acid form with a suitable base, such as the hydroxide, carbonate or bicarbonate of a pharmaceutically acceptable metal cation, with ammonia, or with a
25 pharmaceutically acceptable organic primary, secondary or tertiary amine. Representative alkali or alkaline earth salts include the lithium, sodium, potassium, calcium, magnesium, and aluminum salts and the like. Representative organic amines useful for the formation of base addition salts include ethylamine, diethylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine and the
30 like (see, for example, Berge et al., *supra*).

Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

- 5 Examples of pharmaceutically acceptable antioxidants include: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal
10 chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

- Formulations useful in the methods of the present invention include those suitable for oral, nasal, topical (including ophthalmic, otic, buccal and sublingual), rectal, vaginal, aerosol and/or parenteral administration. The formulations may
15 conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the host being treated, the particular mode of administration. The amount of active ingredient which can be combined with a carrier material to
20 produce a single dosage form will generally be that amount of the compound which produces a therapeutic effect. Generally, out of one hundred per cent, this amount will range from about 1 per cent to about ninety-nine percent of active ingredient, preferably from about 5 per cent to about 70 per cent, most preferably from about 10 per cent to about 30 per cent.

- 25 Methods of preparing these formulations or compositions include the step of bringing into association a therapeutic polymer conjugate(s) with the carrier and, optionally, one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association a therapeutic polymer conjugate with liquid carriers, or finely divided solid carriers, or both, and
30 then, if necessary, shaping the product.

Formulations suitable for oral administration may be in the form of capsules, cachets, pills, tablets, gums, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and/or as mouthwashes and the like, each containing a predetermined amount of a therapeutic polymer conjugate(s) as an active ingredient. A compound may also be administered as a bolus, electuary or paste.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared using binder (for example, gelatin or hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered peptide or peptidomimetic moistened with an inert liquid diluent.

Tablets, and other solid dosage forms, such as dragees, capsules, pills and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile, other polymer matrices, liposomes and/or microspheres. They may be sterilized by, for example, filtration through a bacteria-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved in sterile water, or some other sterile injectable medium immediately before use. These compositions may also optionally contain opacifying agents and may be of a composition that they release the active ingredient(s) only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes. The active ingredient can also be in micro-encapsulated form, if appropriate, with one or more of the above-described excipients.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active ingredient, the liquid dosage forms may contain inert diluents commonly used in the art, such as, for example, water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

10 Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents.

Suspensions, in addition to the active therapeutic polymer conjugates may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

Formulations for rectal or vaginal administration may be presented as a suppository, which may be prepared by mixing one or more therapeutic polymer conjugates with one or more suitable nonirritating excipients or carriers comprising for example, cocoa butter, polyethylene glycol, a suppository wax or a salicylate, and which is solid at room temperature, but liquid at body temperature and, therefore, will melt in the rectum or vaginal cavity and release the active agent.

Formulations which are suitable for vaginal administration also include pessaries, tampons, creams, gels, pastes, foams or spray formulations containing such carriers as are known in the art to be appropriate.

Dosage forms for the topical or transdermal administration of a therapeutic polymer conjugate(s) include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active component may be mixed under

sterile conditions with a pharmaceutically acceptable carrier, and with any preservatives, buffers, or propellants which may be required.

The ointments, pastes, creams and gels may contain, in addition to ligand(s), excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

Powders and sprays can contain, in addition to a therapeutic polymer conjugate(s), excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants, such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane.

The therapeutic polymer conjugate(s) can be alternatively administered by aerosol. This is accomplished by preparing an aqueous aerosol, liposomal preparation or solid particles containing the compound. A nonaqueous (e.g., fluorocarbon propellant) suspension could be used. Sonic nebulizers are preferred because they minimize exposing the agent to shear, which can result in degradation of the compound.

Ordinarily, an aqueous aerosol is made by formulating an aqueous solution or suspension of the agent together with conventional pharmaceutically acceptable carriers and stabilizers. The carriers and stabilizers vary with the requirements of the particular compound, but typically include nonionic surfactants (Tweens, Pluronic, or polyethylene glycol), innocuous proteins like serum albumin, sorbitan esters, oleic acid, lecithin, amino acids such as glycine, buffers, salts, sugars or sugar alcohols. Aerosols generally are prepared from isotonic solutions.

Transdermal patches have the added advantage of providing controlled delivery of a therapeutic polymer conjugate(s) to the body. Such dosage forms can be made by dissolving or dispersing the agent in the proper medium. Absorption enhancers can also be used to increase the flux of the ligand across the skin. The

rate of such flux can be controlled by either providing a rate controlling membrane or dispersing the peptidomimetic in a polymer matrix or gel.

Ophthalmic formulations, eye ointments, powders, solutions and the like, are also contemplated as being within the scope of this invention.

5 Pharmaceutical compositions of this invention suitable for parenteral administration comprise one or more therapeutic polymer conjugate(s) in combination with one or more pharmaceutically acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior
10 to use, which may contain antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

 Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the invention include water,
15 ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

20 These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium
25 chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

 In some cases, in order to prolong the effect of a drug, it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may

be accomplished by the use of a liquid suspension of crystalline or amorphous material having poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally
5 administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle.

Injectable depot forms are made by forming microencapsule matrices of therapeutic polymer conjugate(s) in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of drug to polymer, and the nature of the
10 particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissue.

When the therapeutic polymer conjugate(s) of the present invention are
15 administered as pharmaceuticals, to humans and animals, they can be given per se or as a pharmaceutical composition containing, for example, 0.1 to 99.5% (more preferably, 0.5 to 90%) of active ingredient in combination with a pharmaceutically acceptable carrier.

The preparations of agents may be given orally, parenterally, topically, or
20 rectally. They are of course given by forms suitable for each administration route. For example, they are administered in tablets or capsule form, by injection, inhalation, eye lotion, ointment, suppository, infusion; topically by lotion or ointment; and rectally by suppositories. Oral administration is preferred.

The phrases "parenteral administration" and "administered parenterally" as
25 used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal and intrasternal injection and infusion.

The phrases “systemic administration,” “administered systemically,” “peripheral administration” and “administered peripherally” as used herein mean the administration of a therapeutic polymer conjugate, drug or other material other than directly into the central nervous system, such that it enters the patient’s system and, thus, is subject to metabolism and other like processes, for example, subcutaneous administration.

These therapeutic polymer conjugate(s) may be administered to humans and other animals for therapy by any suitable route of administration, including orally, nasally, as by, for example, a spray, rectally, intravaginally, parenterally, intracisternally and topically, as by powders, ointments or drops, including buccally and sublingually.

Regardless of the route of administration selected, the therapeutic polymer conjugate(s), which may be used in a suitable hydrated form, and/or the pharmaceutical compositions of the present invention, are formulated into pharmaceutically acceptable dosage forms by conventional methods known to those of skill in the art.

Actual dosage levels of the active ingredients in the pharmaceutical compositions of this invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient.

(d) Physical Structures Of The Subject Compositions

The subject polymers may be formed in a variety of shapes. For example, in certain embodiments, subject polymer conjugates may be presented in the form of microparticles or nanoparticles. Microspheres typically comprise a biodegradable polymer conjugate incorporating a drug. Microspheres can be formed by a wide variety of techniques known to those of skill in the art. Examples of microsphere forming techniques include, but are not limited to, (a) phase separation by emulsification and subsequent organic solvent evaporation (including complex emulsion methods such as oil in water emulsions, water in oil emulsions and water-

oil-water emulsions); (b) coacervation-phase separation; (c) melt dispersion; (d) interfacial deposition; (e) in situ polymerization; (f) spray drying and spray congealing; (g) air suspension coating; and (h) pan and spray coating. These methods, as well as properties and characteristics of microspheres are disclosed in, 5 for example, U.S. Pat. No. 4,652,441; U.S. Pat. No. 5,100,669; U.S. Pat. No. 4,526,938; WO 93/24150; EPA 0258780 A2; U.S. Pat. No. 4,438,253; and U.S. Pat. 5,330,768, the entire disclosures of which are incorporated by reference herein.

To prepare microspheres of the present invention, several methods can be employed depending upon the desired application of the delivery vehicles. Suitable 10 methods include, but are not limited to, spray drying, freeze drying, air drying, vacuum drying, fluidized-bed drying, milling, co-precipitation and critical fluid extraction. In the case of spray drying, freeze drying, air drying, vacuum drying, fluidized-bed drying and critical fluid extraction; the components (stabilizing polyol, bioactive material, buffers, etc.) are first dissolved or suspended in aqueous 15 conditions. In the case of milling, the components are mixed in the dried form and milled by any method known in the art. In the case of co-precipitation, the components are mixed in organic conditions and processed as described below. Spray drying can be used to load the stabilizing polyol with the bioactive material. The components are mixed under aqueous conditions and dried using precision 20 nozzles to produce extremely uniform droplets in a drying chamber. Suitable spray drying machines include, but are not limited to, Buchi, NIRO, APV and Lab-plant spray driers used according to the manufacturer's instructions.

The shape of microparticles and nanoparticles may be determined by scanning electron microscopy. Spherically shaped nanoparticles are used in certain 25 embodiments, for circulation through the bloodstream. If desired, the particles may be fabricated using known techniques into other shapes that are more useful for a specific application.

In addition to intracellular delivery of a therapeutic agent, it also possible that particles of the subject compositions, such as microparticles or nanoparticles, 30 may undergo endocytosis, thereby obtaining access to the cell. The frequency of such an endocytosis process will likely depend on the size of any particle.

In certain embodiments, solid articles useful in defining shape and providing rigidity and structural strength to the polymeric matrices may be used. For example, a polymer may be formed on a mesh or other weave for implantation. A polymer may also be fabricated as a stent or as a shunt, adapted for holding open areas within
5 body tissues or for draining fluid from one body cavity or body lumen into another. Further, a polymer may be fabricated as a drain or a tube suitable for removing fluid from a post-operative site, and in some embodiments adaptable for use with closed section drainage systems such as Jackson-Pratt drains and the like as are familiar in the art.

10 The mechanical properties of the polymer may be important for the processability of making molded or pressed articles for implantation. For example, the glass transition temperature may vary widely but must be sufficiently lower than the temperature of decomposition to accommodate conventional fabrication techniques, such, as compression molding, extrusion, or injection molding.

15 (e) Biodegradability And Release Characteristics

In certain embodiments, the polymers and blends of the present invention, upon contact with body fluids, undergo gradual degradation. The life of a biodegradable polymer in vivo depends upon, among other things, its molecular weight, crystallinity, biostability, and the degree of crosslinking. In general, the
20 greater the molecular weight, the higher the degree of crystallinity, and the greater the biostability, the slower biodegradation will be.

If a subject composition is formulated with a therapeutic agent or other material, release of such an agent or other material for a sustained or extended period as compared to the release from an isotonic saline solution generally results.
25 Such release profile may result in prolonged delivery (over, say 1 to about 2,000 hours, or alternatively about 2 to about 800 hours) of effective amounts (e.g., about 0.0001 mg/kg/hour to about 10 mg/kg/hour) of the agent or any other material associated with the polymer.

A variety of factors may affect the desired rate of hydrolysis of polymers of
30 the subject invention, the desired softness and flexibility of the resulting solid

aggregate polymer, rate and extent of bioactive material release. Some of such factors include the selection/identity of the various subunits, the enantiomeric or diastereomeric purity of the monomeric subunits, homogeneity of subunits found in the polymer, and the length of the polymer. For instance, the present invention
5 contemplates heteropolymers with varying linkages, and/or the inclusion of other monomeric elements in the polymer, in order to control, for example, the rate of biodegradation of the polymer.

To illustrate further, a wide range of degradation rates may be obtained by adjusting the hydrophobicities of the backbones or side chains of the polymers while
10 still maintaining sufficient biodegradability for the use intended for any such polymer. Such a result may be achieved by varying the various functional groups of the polymer. For example, the combination of a hydrophobic backbone and a hydrophilic linkage produces heterogeneous degradation because cleavage is encouraged whereas water penetration is resisted.

15 One protocol generally accepted in the field that may be used to determine the release rate of any therapeutic agent or other material loaded in the polymer matrices of the present invention involves degradation of any such polymer in a 0.1 M PBS solution (pH 7.4) at 37 °C, an assay known in the art. For purposes of the present invention, the term "PBS protocol" is used herein to refer to such protocol.

20 In certain instances, the release rates of different polymer systems of the present invention may be compared by subjecting them to such a protocol. In certain instances, it may be necessary to process polymeric systems in the same fashion to allow direct and relatively accurate comparisons of different systems to be made. For example, the present invention teaches several different means of
25 formulating the polymeric, matrices of the present invention. Such comparisons may indicate that any one polymeric system releases incorporated material at a rate from about 2 or less to about 1000 or more times faster than another polymeric system.

Alternatively, a comparison may reveal a rate difference of about 3, 5, 7, 10, 25, 50, 100, 250, 500 or 750 times. Even higher rate differences are contemplated by the present invention and release rate protocols.

5 In certain embodiments, when formulated in a certain manner, the release rate for polymer systems of the present invention may present as mono- or bi-phasic.

Release of any material incorporated into the polymer conjugate, which is often provided as a microsphere, may be characterized in certain instances by an initial increased release rate, which may release from about 5 to about 50% or more of any incorporated material, or alternatively about 10, 15, 20, 25, 30 or 40%,
10 followed by a release rate of lesser magnitude.

The release rate of any incorporated material may also be characterized by the amount of such material released per day per mg of polymer conjugate. For example, in certain embodiments, the release rate may vary from about 1 ng or less of any incorporated material per day per mg of polymeric system to about 500 or
15 more ng/day/mg. Alternatively, the release rate may be about 0.05, 0.5, 5, 10, 25, 50, 75, 100, 125, 150, 175, 200, 250, 300, 350, 400, 450, or 500 ng/day/mg. In still other embodiments, the release rate of any incorporated material may be 10,000 ng/day/mg, or even higher. In certain instances, materials incorporated and characterized by such release rate protocols may include therapeutic agents, fillers,
20 and other substances.

In another aspect, the rate of release of any material from any polymer conjugate of the present invention may be presented as the half-life of such material in the conjugate.

In addition to the embodiment involving protocols for in vitro determination
25 of release rates, in vivo protocols, whereby in certain instances release rates for polymeric systems may be determined in vivo, are also contemplated by the present invention. Other assays useful for determining the release of any material from the polymers of the present system are known in the art.

(f) Implants and Delivery Systems

In its simplest form, a biodegradable delivery system for a therapeutic agent consists of incorporation of such a therapeutic agent in a polymer conjugate.

Incorporation can include association of the therapeutic agent with the polymer preferably by covalent interactions. Alternatively, incorporation can also include association by non-covalent interactions, for example by host-guest interactions, dative interactions, van der Waals forces, hydrogen bonding, or simple mixing. Combinations of covalent and non-covalent interactions are also contemplated.

In other embodiments, an article is used for implantation, injection, or otherwise placed totally or partially within the body, the article comprising the subject compositions.

Biodegradable delivery systems, and articles thereof, may be prepared in a variety of ways known in the art. The subject polymer may be melt-processed using conventional extrusion or injection molding techniques, or these products may be prepared by dissolving in an appropriate solvent, followed by formation of the device, and subsequent removal of the solvent by evaporation or extraction.

Once a system or implant article is in place, it may remain in at least partial contact with a biological fluid, such as blood, internal organ secretions, mucus membranes, cerebrospinal fluid, and the like to allow for sustained release of any encapsulated therapeutic agent.

In certain delivery systems of the instant invention, the delivery of the drug or agent takes advantage of one or more photocleavable linker groups incorporated in the polymer. A composition of the instant invention, comprising one or more compounds disclosed herein, may be introduced systemically into the body of a patient. The compound may spread and/or accumulate in different tissues or parts of the body. The compound may also accumulate in certain tissues more than others through the use of targeting ligands as described herein. Following introduction of the compound to the body, certain areas of the body, the eye or parts near the surface of the skin, for example, may then be subjected to radiant energy of corresponding wavelength to promote photolysis of one or more photocleavable linker groups of

the compounds present in these tissues or parts of the body. Such photolysis may result in the release one or more groups or moieties of the therapeutic/bioactive agent from the polymeric compound to the tissue or part of the body subject to the radiation. In this way, the release of the drug or therapeutic agent to a desired tissue
5 or body part can be controlled – in terms of dose and/or localization – by selectively subjecting the tissue or body part to radiant energy, such as UV, visible, or infrared light.

In one embodiment, the eye is subjected to radiant energy such that polymeric compounds of the instant invention which are present or have
10 accumulated in the eye following systemic introduction to the patient undergo photolysis of one or more photocleavable linker groups, releasing a drug or therapeutic agent to the eye. The cleavage of the linker group may be induced by UV, visible, or infrared light, preferably visible light. Such a method of delivery to the eye may employ targeting ligands as described herein. Such ligands may target
15 receptors which may be specific to the eye, such as insulin and transferrin. These ligands may impart medium to high selectivity for directing compounds of the instant invention to the eye for delivering a drug or therapeutic agent following photolysis of a linker group.

In another embodiment, tissues or parts of the body on or near the skin may
20 be subjected to radiant energy such that compounds of the instant invention which are present or have accumulated in these tissues or parts of the body following systemic introduction to the patient undergo photolysis of one or more photocleavable linker groups, releasing a drug or therapeutic agent to the tissue or body part. The cleavage of the linker group may be induced by UV, visible, or
25 infrared light, preferably visible or infrared light. Infrared light includes light of wavelengths 680 nm to 100,000 nm of the spectrum, from near infrared (700 nm to 1000 nm) to mid infrared (1000 nm to 20,000 nm) to far infrared (20,000 nm to 100,000 nm). Energy of certain wavelengths, such as 700-1000 nm, can be used to penetrate tissues, such as soft tissue, and blood to at least 15 cm. Radiant energy of
30 these wavelengths can be used to induce photolysis of photocleavable linker groups of compounds of the instant invention that are present or have accumulated in these

tissues, which may result in release and delivery of a drug or therapeutic agent to the targeted tissue. Such compounds may comprise targeting ligands as described herein to direct delivery of a drug or targeting agent to a desired tissue or body part.

(g) Business Methods

5 Other aspects of the invention provides for certain methods of doing business. In particular, practicing the methods of the invention may enable novel therapeutic compositions and improved formulations thereof. This technical step, when combined with one or more additional steps, provides for novel approaches to
10 conduct a pharmaceutical, or preferably a life-science business. For example, such therapeutic prepared by the method of the invention may be tested for efficacy as therapeutics in a variety of disease models, the potential therapeutic compositions then tested for toxicity and other safety-profiling before formulating, packaging and subsequently marketing the resulting formulation for the treatment of disease. Alternatively, the rights to develop and market such formulations or to conduct such
15 steps may be licensed to a third party for consideration.

Accordingly, in certain embodiments, the present invention provides a method for conducting a pharmaceutical business, comprising:

- a. manufacturing a formulation or kit including a pharmaceutical composition of any of the compounds of claims 1-4; and
- 20 b. marketing to healthcare providers the benefits of using the formulation or kit in the treatment of a disease or disorder.

In other embodiments, the present invention discloses a method for conducting a pharmaceutical business, comprising:

- a. providing a distribution network for selling a pharmaceutical
25 composition of any of the compounds of claims 1-4; and
- b. providing instruction material to patients or physicians for using the preparation in the treatment of a disease or disorder.

In certain embodiments, the present invention provides a method for conducting a pharmaceutical business, comprising:

- a. determining an appropriate formulation and dosage of a pharmaceutical composition of any of the compounds of claims 1-4;
- b. conducting therapeutic profiling of formulations identified in step (a), for efficacy and toxicity in animals; and
- 5 c. providing a distribution network for selling a preparation or preparations identified in step (b) as having an acceptable therapeutic profile.

An additional step of the embodiment comprises providing a sales group for marketing the preparation to healthcare providers.

- 10 In still other embodiments, the present invention provides a method for conducting a pharmaceutical business, comprising:

- a. determining an appropriate formulation and dosage of a pharmaceutical composition of any of the compounds of claims 1-4; and
- b. licensing, to a third party, the rights for further development and sale of the formulation.

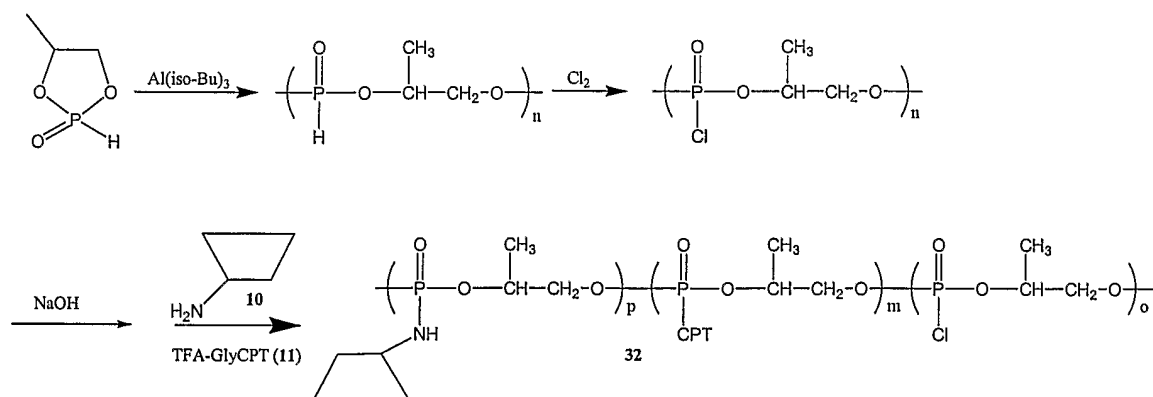
15

Exemplification

- The invention now being generally described, it will be more readily understood by reference to the following examples which are included merely for purposes of illustration of certain aspects and embodiments of the present invention,
- 20 and are not intended to limit the invention.

Example 1 Synthesis of biodegradable CD-Polyphosphoester Polymer **31** and its CPT conjugates **32**

Scheme 15



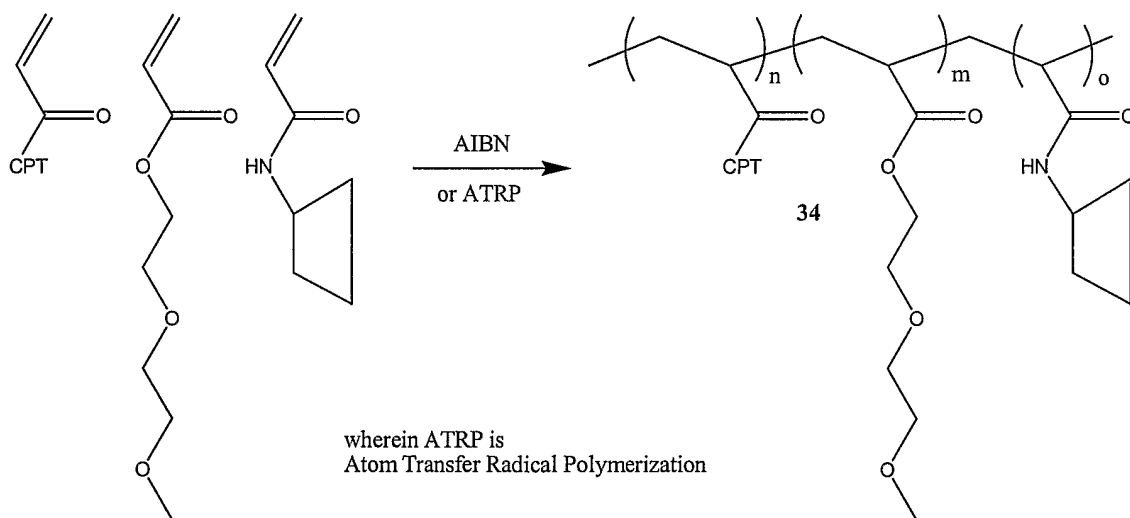
25

Synthesis of the biodegradable polyphosphoester can be found in Wang J et al, *JACS*, **2001**, *123*, 9480-9481.

Polyphosphoester is mixed with **10** (0.5 eq of repeat unit) in DMSO. EDC (2 eq), NHS (1 eq), and DIEA (1.0 eq) are added to the solution. The solution is stirred for 16 hrs and then precipitated with ether. The obtained CD-polyphosphoester **31** is dissolved in DMSO. To the solution is added **11** (0.5 eq of repeat unit), EDC (2 eq), NHS (1 eq), and DIEA (1.0 eq). The solution is stirred for 16 h and then precipitated with ether. The precipitate is washed with ether extensively until no free drug is observed in the washing solution. Compound **32** is obtained after drying under high vacuum.

Example 2 Synthesis of CD Copolymer–CPT conjugate **33** with polyethylene backbone via radical polymerization

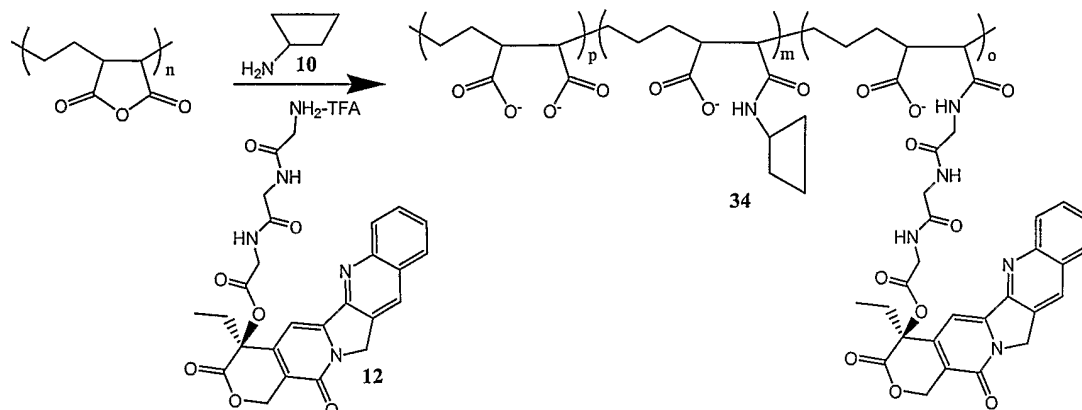
Scheme 16



Acrylate monomers of CPT, triethyleneglycol monomethylether, and CD-monocystamine can be synthesized from N-Acryloxysuccinimide (Polysciences, Inc.). These monomers are mixed in 1:1:1 ratio in dry DMSO. AIBN is added to the mixture under argon. The solution is stirred at rt for 24-48 hrs until the solution becomes viscous. Polymer-CPT conjugate **33** is precipitated with ether and dried under vacuum.

Example 3 Synthesis of CD-graft-poly(ethylene-*alt*-maleic anhydride)-GlyGlyGlyCPT **34**

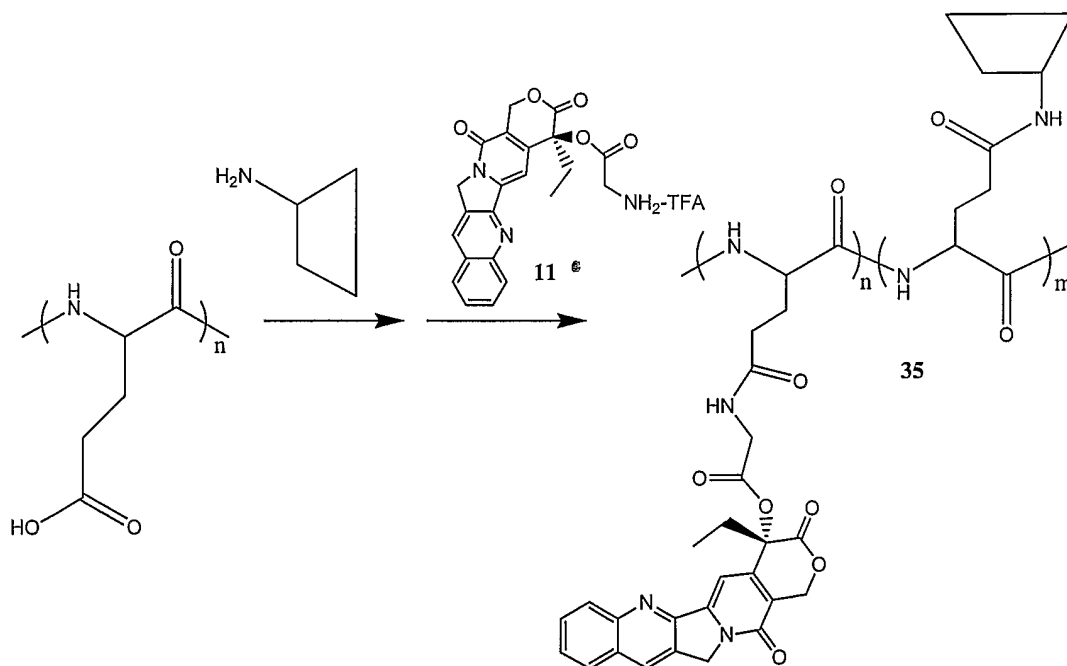
Scheme 17



- 5 Poly(ethylene-*alt*-maleic anhydride) (Aldrich) is dissolved in DMSO. **10** (0.4 eq of repeat unit) and **12** (0.4 eq of repeat) are added. The solution is heated at 70 °C for 16 hrs and then precipitated with ether. The obtained CD-graft-poly(ethylene-*alt*-maleic anhydride)-GlyGlyGlyCPT **34** is dried under high vacuum.

10 **Example 4** Synthesis of Polyglutamate-CD-CPT Conjugate **35**

Scheme 18



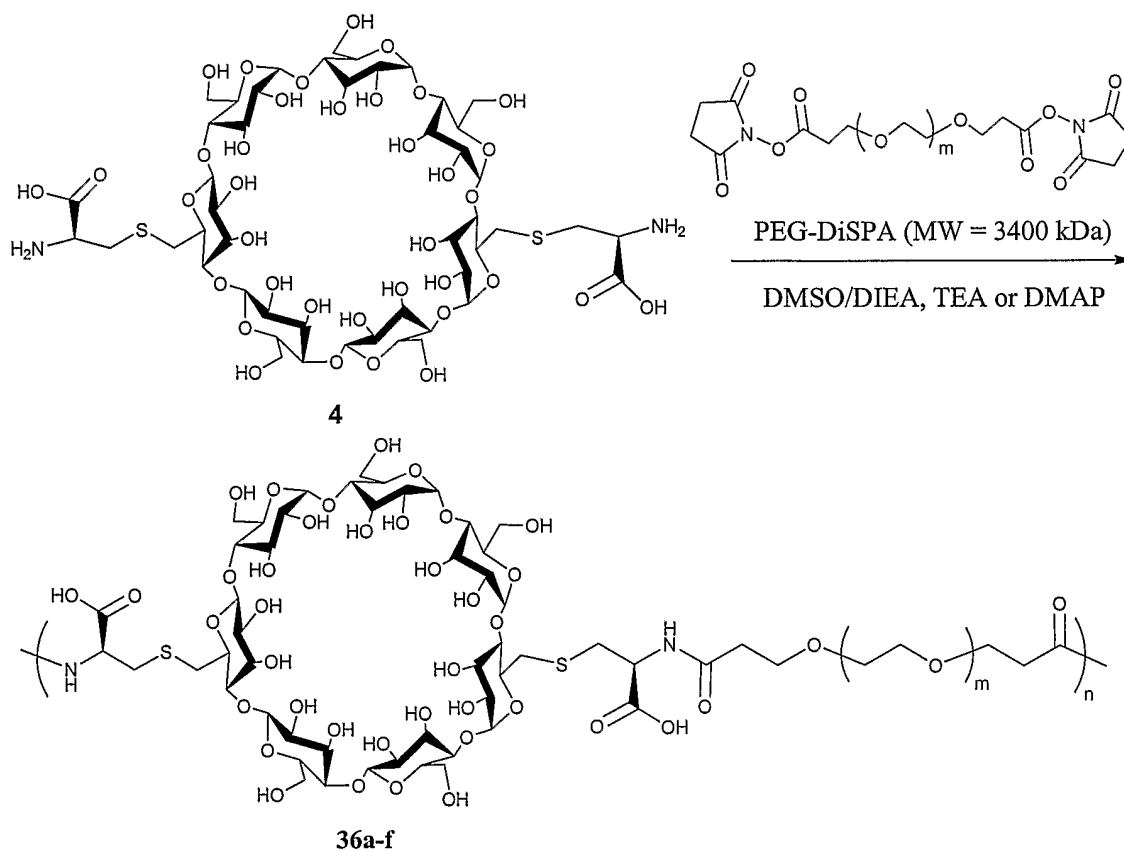
- Polyglutamate (from Sigma-Aldrich) is mixed with **10** (0.5 eq of repeat unit) and **11** (0.5 eq of repeat unit) in DMSO. EDC (3 eq), NHS (2 eq), and DIEA (1.0

eq) are added to the solution. The solution is stirred for 16 hr and then precipitated with ether. After drying under high vacuum, polyglutamate-CD-CPT conjugate **35** is obtained.

5 Example 5: Synthesis and Characterization of CD-BisCys-Peg3400 Copolymers 36 and their CPT Conjugates 37.

A. Synthesis and Characterization of CD-BisCys-Peg3400 Copolymers 36

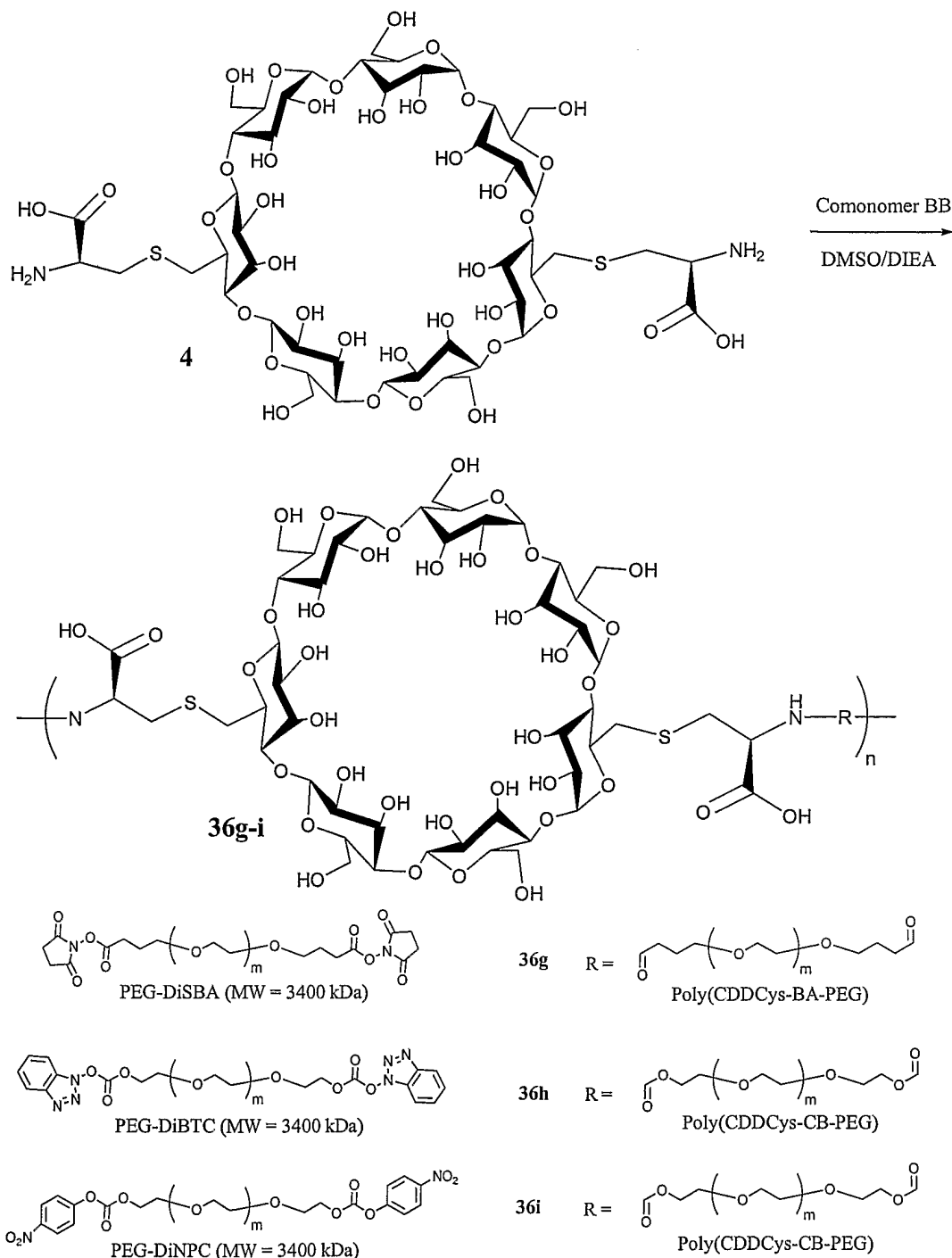
Scheme 19a



Poly(CDDCys-PA-PEG)

Abbr: PA = propanoicamide bond between PEG and CD

Scheme 19b



Abbr: BA = butanoicamide bond; CB = carbamate bond

Synthesis of Poly(CDDCys-PA-PEG), 36a

- 4 (after precipitation with acetone, 63 mg, 0.047 mmol) and PEG-DiSPA (MW 3400, 160 mg, 0.047 mmol) were dried under vacuum for 8 hours. Anhydrous DMSO (1.26 mL) was added to the mixture under argon. After 10 minutes of stirring, anhydrous

diisopropylethylamine (DIEA, 19 μ L, 2.3 eq.) was added under argon. The reaction mixture was stirred under argon for 120 h. The polymer containing solution was dialyzed using a 10,000 MWCO membrane (Spectra/Por 7) against water for 48 h and lyophilized to yield 196 mg **36a** (90%, Table 1). $M_w = 57.4$ kDa, $M_n = 41.7$ kDa, $M_w/M_n = 1.38$. ^1H NMR (D_2O) δ 5.08 (m, CD-2-H), 4.27 (m, Cys-CH), 2.72-3.76 (m, CD-3,4,5,6-CH, CD-CH₂, PEG-CH₂), 2.44 (m, Cys-CH₂).

Synthesis of other poly(CDDCys-PA-PEG) (**36b-f**), Poly(CDDCys-BA-PEG) (**36g**) Poly(CDDCys-CB-PEG) (**36h-i**) were achieved under polymerization condition similar to that of **36a**. Details for the polymerization conditions, monomer selection, polymer molecular weight, polydispersity and yields are listed in Table 1. **36g**: ^1H NMR (D_2O) δ 5.10 (m, CD-2-H), 4.25-4.37 (m, Cys-CH), 2.72-3.86 (m, CD-3,4,5,6-CH, CD-CH₂, PEG-CH₂), 2.21 (m, Cys-CH₂). **36h-i**: ^1H NMR (D_2O) δ 5.05 (m, CD-2-H), 4.56 (m, Cys-CH), 2.70-3.93 (m, CD-3,4,5,6-CH, CD-CH₂, PEG-CH₂), 2.38 (m, -OCH₂CH₂CH₂C(O)-NH-), 2.34 (m, Cys-CH₂), 1.90 (m, -OCH₂CH₂CH₂C(O)-NH-).

Addition of a non-nucleophilic organic base (such as DIEA) was essential for this polymerization as no viscosity changes of the polymerization solutions were observed after 48 hours if no base was added. When 2.3 eq. of DIEA were added, the viscosity of the polymerization solution increased dramatically after 4-6 hours of reaction. DIEA deprotonates the amino groups of **4** to render them more nucleophilic for coupling with PEG-DiSPA. There were essentially no differences in the polymerizations if other bases, such as TEA or DMAP, were used (**36b-c**, Table 1). Polymerization using **4** recovered by the two different precipitation methods (acetone and methanol) produced polymers with different MWs. **4** that was purified by the methanol-precipitation method (contains no free cystine) gave higher MW polymer (**36d-e**) as compared to the less pure **4** that was obtained from the acetone-precipitation method (**36a**). Polymerization of **4** with PEG-DiSPA typically produced polymer yields greater than 90%.

30

4 was polymerized with other activated monomers such as PEG-DiSBA, PEG-DiBTC, and PEG-DiNPC. Reaction of **4** with PEG-DiSBA gave polymer **36g** with

similar linkages as **36a-f** (amide bond, but one more $-\text{CH}_2$ group than **36a-f** at the linker) with M_w over 100 kDa, while reaction of **4** with PEG-DiBTC and PEG-DiNPC generated polymers **36h** and **36i**, respectively, with connecting carbamate moiety and M_w 's over 50 kDa (Table 1).

5

Table 1. Polymerization of **4** with difunctionalized PEG

CDP	PEG Comonomer	Base	Polymerization time (h)	M_w (kDa)	M_n (kDa)	M_w/M_n	Yield (%)
36a^a	PEG-DiSPA	DIEA	120	57.4	41.7	1.38	90
36b^a	PEG-DiSPA	DMAP	120	54.2	38.1	1.42	91
36c^a	PEG-DiSPA	TEA	120	57.4	42.6	1.35	91
36d^b	PEG-DiSPA	DIEA	120	93.6	58.0	1.48	96
36e^b	PEG-DiSPA	DIEA	144	97.3	58.0	1.67	94
36f^b	PEG-DiSPA	DIEA	2	35.3	25.6	1.38	95
36g	PEG-DiSBA	DIEA	120	114.7	77.9	1.47	96
36h	PEG-DiBTC	DIEA	120	67.6	39.4	1.47	95
36i	PEG-DiNPC	DIEA	120	86.5	57.2	1.51	96

^a **4** was washed with acetone before polymerization.

^b **4** was washed with methanol before polymerization.

10 Polymers **36a-i** are highly soluble in aqueous solution. They can be easily dissolved in water or phosphate buffered saline (PBS) solution at concentrations of at least 200 mg/mL. Solubility of these polymers in aqueous solution at concentrations higher than 200 mg/mL was not attempted due to the high viscosity. These polymers were also soluble in DMF, DMSO and methanol, slightly soluble in
15 CH_3CN and CHCl_3 , but insoluble in THF and ethyl ether.

Molecular Weight Control of CD Polymers **4** (after precipitation with methanol) (56.2 mg, 0.0419 mmol) and PEG-DiSPA (147 mg, 0.0419 mmol) were dried under vacuum for 4-8 hours. To the mixture was added dry DMSO (1.1 mL)
20 under argon. After 10 minutes stirring, DIEA (16 μL , 2.2 eq) was added under argon. A portion of polymerization solution (150 μL) was removed and precipitated

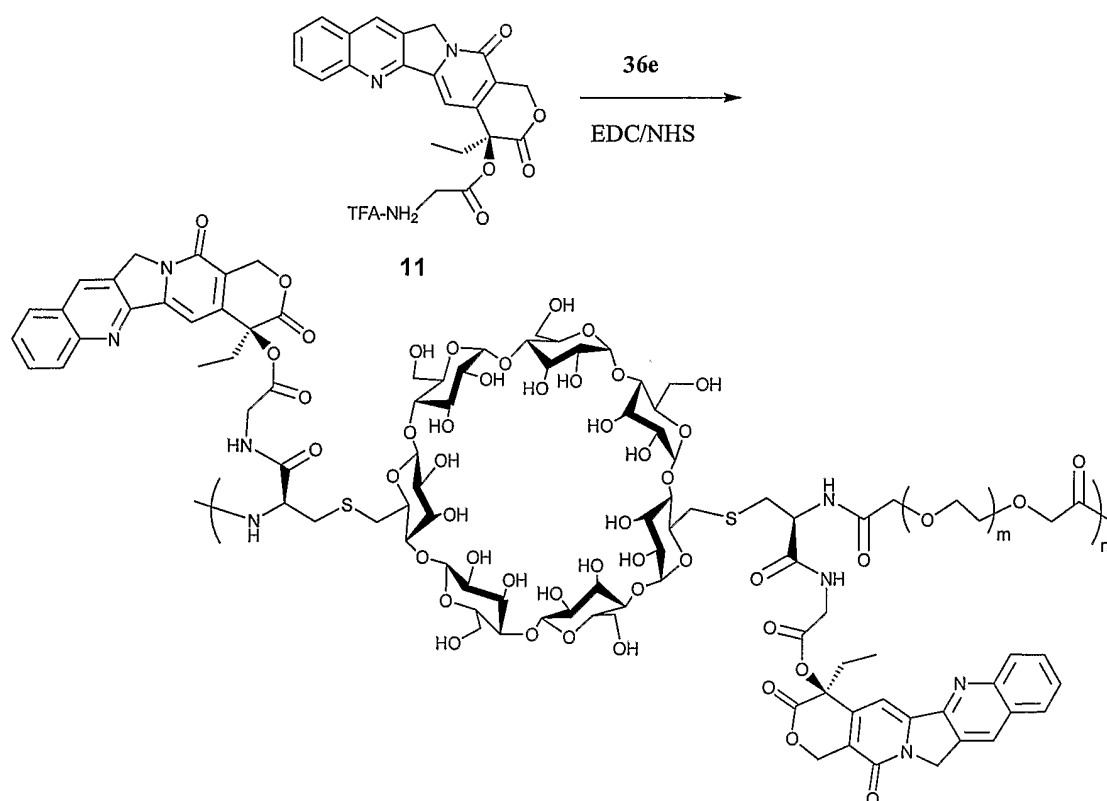
with ether at selected times (2 h, 18 h, 43 h, 70 h, 168 h and 288 h). MWs of the precipitated polymers were determined as described above.

As shown in Figures 5a and 5b, molecular weights of **36** can be controlled by adjusting polymerization time.

5

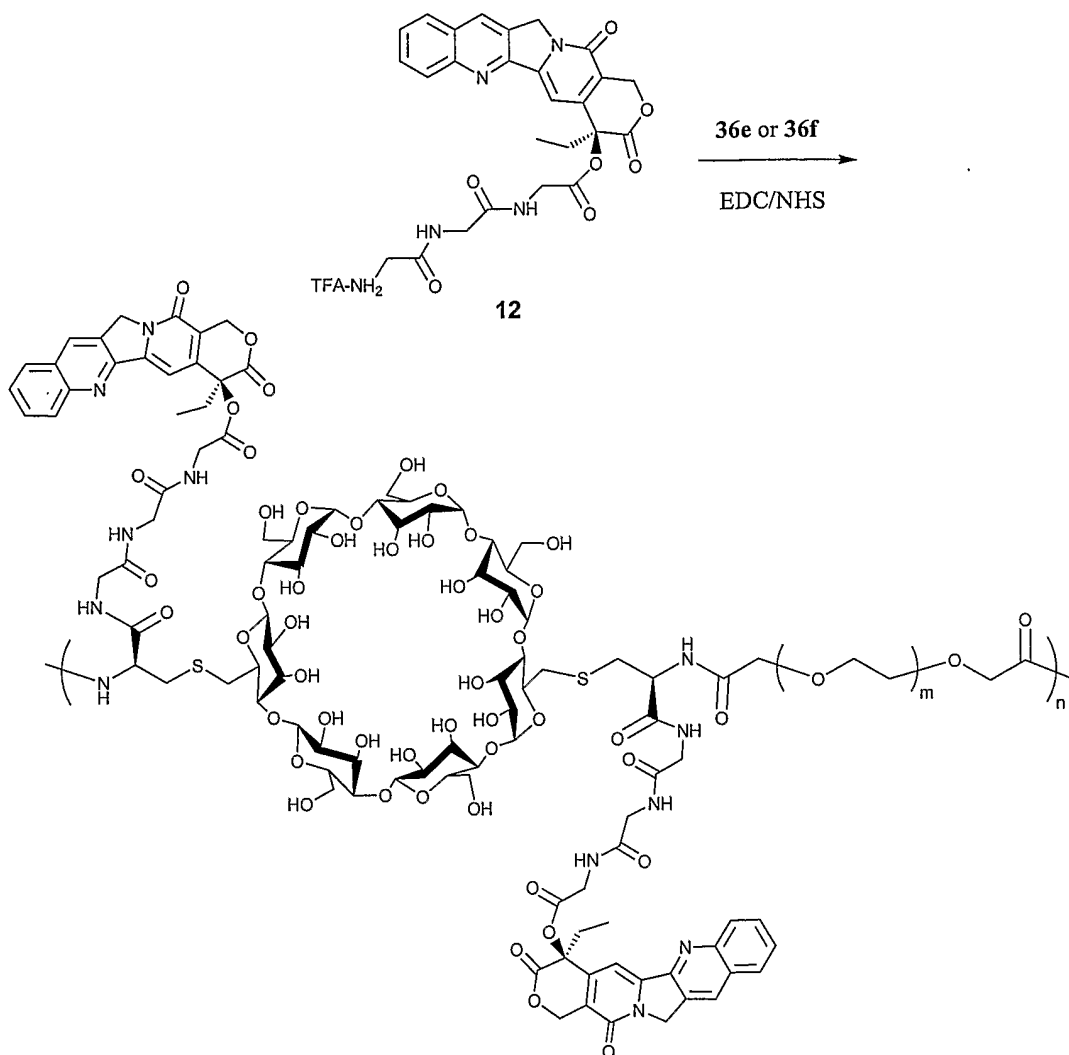
B. Synthesis of Poly(CDDCys-PA-PEG)-CPTConjugates (HGGG6, LGGG10, HG6, HGGG10).

Scheme 20a



10

Scheme 20b



Synthesis of Poly(CDDCys-PA-PEG)-GlyGlyGly-CPT (**HGGG6**) **36e** (1.37 g, 0.30 mmol of repeat unit) was dissolved in dry DMSO (136 mL). The mixture was stirred for 10 minutes. **12** (419 mg, 0.712 mmol, 2.36 eq), DIEA (0.092 mL, 0.712 mmol, 2.36 eq), EDC (172 mg, 0.903 mmol, 3 eq), and NHS (76 mg, 0.662 mmol, 2.2 eq) were added to the polymer solution and stirred for ca. 15 hours. The polymer was precipitated with ethyl ether (1 L). The ether was poured out and the precipitate was washed with CH₃CN (3 × 100 mL). The precipitate was dissolved in water 600 mL. Some insoluble solid was filtered through 0.2 μm filters. The solution was dialyzed using 25,000 MWCO membrane (Spectra/Por 7) for 10 h at 10-15 °C in DI water. Dialysis water was changed every 60 minutes. The polymer-

drug conjugate solution was sterilized by passing it through 0.2 μ M filters. The solution was lyophilized to yield a yellow solid **HGGG6** (1.42 g, 85% yield).

Synthesis of Poly(CDDCys-PA-PEG)-GlyGlyGly-CPT (**LGGG10**)

Conjugation of **12** to **36f** was performed in a manner similar to that used to produce **HGGG6** except that this conjugate was dialyzed with 10,000 MWCO membrane (Spectra/Por 7) instead of with 25,000 MWCO membrane. The yield of **LGGG10** was 83%.

Synthesis of Poly(CDDCys-PA-PEG)-Gly-CPT (**HG6**) Conjugation of **11** to **36e** was performed in a manner similar to that used to produce **HGGG6**. The yield of **HG6** was 83%.

Synthesis of Poly(CDDCys-PA-PEG)-GlyGlyGly-CPT (**HGGG10**) **36e** (1.5 g, 0.33 mmol of repeat unit) was dissolved in dry DMSO (150 mL). The mixture was stirred for 10 minutes. **12** (941 mg, 1.49 mmol, 4.5 eq), DIEA (0.258 mL, 1.49 mmol, 4.5 eq), EDC (283 mg, 1.49 mmol, 4.5 eq), and NHS (113 mg, 0.99 mmol, 3 eq) was added to the polymer solution and stirred for ca. 24 hours. Another portion of EDC (142 mg, 0.75 mmol, 2.3 eq) and NHS (56 mg, 0.5 mmol, 1.5 eq) were added to the conjugation solution. The polymer was stirred for an additional 22 hours. The workup procedure was the same as that for the synthesis of **HGGG6**. The yield of **HGGG10** was 77%.

Determination of wt% CPT on the Conjugates Stock solutions of **HGGG6**, **LGGG10**, **HG6** and **HGGG10** were prepared at a concentration of 10 mg/mL in DMSO. An aliquot of corresponding stock solution was diluted to 100 μ g/mL using 1 N NaOH. CPT was completely hydrolyzed in this basic solution and transformed to its carboxylate form within 2 h at room temperature. An aliquot of this solution was diluted to 10 μ g/mL using 8.5% H_3PO_4 , and the CPT carboxylate form was transformed to its lactone form. 30 μ L of this solution was injected into the HPLC. The peak area from the CPT lactone form was integrated and compared to a standard curve.

11 and **12** were conjugated to **36e** or **36f** (Table 2) using conventional coupling methods. Due to the instability of the ester linker of **11** and **12** in aqueous solution, the conjugation was conducted in anhydrous DMSO under argon. An organic base was required to deprotonate the TFA salts of **11** and **12** to facilitate the

coupling. For polymer conjugation with **12**, the weight percent (wt%) drug loading was around 6-10%. The theoretical maximum drug loading is around 13% using PEG with MW of 3400 Da; maximum values can be increased by decreasing the MW of the PEG segments. Solubilities of all conjugates in water or PBS were more than 200 mg/mL (equivalent to a 12-20 mg CPT/mL for 6-10 wt% drug loading, respectively). Details for the **HGGG6**, **LGGG10**, **HG6**, and **HGGG10** are summarized in Table 2.

Table 2. Properties of polymer-CPT conjugates.

Conjugate ^a	M_w of parent polymer ($\times 10^{-3}$)	M_w/M_n ^b	Linker	CPT (wt%)
HGGG6	97	1.7	triglycine	6.1
LGGG10	35	1.6	triglycine	10.2
HG6	97	1.7	glycine	6.8
HGGG10	97	1.7	triglycine	9.6

^aAbbreviations: **H** = High M_w polymer (97 kDa), **L** = Low M_w polymer (35 kDa), **GGG** = triglycine linker, **G** = glycine linker, **6** = drug loading around 6 wt%, **10** = drug loading around 10 wt%.

^b Polymer polydispersity as measured by light scattering techniques(26)

15 C. Release of CPT from **HGGG6** and **HG6**

Release of CPT in PBS **HGGG6** and **HG6** were prepared at 1 mg/mL in PBS (1×, pH 7.4). A 100 μ L aliquot of the solution was transferred to a 1.5 mL Eppendorf tube and incubated at 37 °C. The incubated samples were quenched at selected time intervals and stored at -80 °C until the analysis. Each solution was diluted with 8.5% H_3PO_4 to a 5 mL total volume in a volumetric flask. 30 μ L of such solution was injected into the HPLC. The peak area from the CPT lactone form was integrated and compared to a standard curve.

Analysis for the release of CPT from **HGGG6** and **HG6** in PBS containing acetyl cholinesterase (an esterase, 100 units/mL), in KH_2PO_4 buffer (pH 6.1, 0.1 M) and in the KH_2PO_4 buffer (pH 6.1, 0.1 M) containing cathepsin B (a cysteine proteinase, 200 μM , preactivated on ice for 30 minutes in this buffer containing 2 mM DTT and 1 mM EDTA) were performed in a manner similar to that described above for PBS alone.

Release of CPT in Human Plasma An aliquot of **HGGG6** and **HG6** stock solution were diluted to give final concentration of 0.5 mg/mL in PBS (1 $\times$, pH 7.4). This solution was added to a lyophilized powder of human plasma to reconstitute 100% human plasma by the recommended amount. The solution was divided into equal volume (250 μL) to 1.5 mL Eppendorf tubes, incubated at 37 $^{\circ}\text{C}$, and stopped at selected time point. Samples were stored at -80°C until the analysis. Samples were separated from plasma by solid phase extraction columns. The solid phase extraction cartridge (Oasis HLB 1cc cartridge from Waters) was pre-conditioned with 1 mL of acetonitrile and then with 1 mL of 8.5% H_3PO_4 before loading. Samples were acidified with equal volume of 8.5% H_3PO_4 prior to loading. After the acidified solution was loaded on the cartridge, the bed was washed with $3 \times 1\text{ mL}$ of water. Released CPT and polymer conjugate were eluted with $3 \times 1\text{ mL}$ of a solution mixture of acetonitrile and potassium phosphate buffer (pH 4.1) (60/40 v/v). The eluted solution was diluted to 5 mL total volume in a 5 mL volumetric flask. 30 μL of such solution was injected into the HPLC. The peak area from the CPT lactone form was integrated and compared to a standard curve.

Release of CPT from **HGGG6** and **HG6** in PBS containing 4% human plasma (PBS/reconstituted human plasma solution = 96/4 (v/v)), in mouse plasma and in reconstituted human albumin (PBS solution) were performed in a manner similar to that described above for pure human plasma.

In PBS (1 $\times$, pH 7.4), the half-lives ($t_{1/2}$) for releasing CPT from **HG6** and **HGGG6** were 59h and 32h, respectively. The half-lives decreased to 25h and 22h, respectively, in the presence of 4% human plasma, and to 1.7h and 1.6h, respectively, in 100% human plasma ("HP") and 2.6h and 2.2h, respectively, in 100% mouse plasma ("MP"). CPT release rates for both **HG6** and **HGGG6** in the presence of albumin ("Alb") or acetyl cholinesterase ("Ac Cho") were on the same

order of magnitude as in PBS. In a buffer solution at a pH lower than PBS (pH 6.1) with or without the enzyme cathepsin B (active at pH 6.1), less than 50% of total conjugated CPT was released from both **HG6** and **HGGG6** for times up to 144 h (Table 3).

5

Table 3. Half-life ($t_{1/2}$, in hour) of the release of CPT from **HG6** and **HGGG6**^a

Conjugate	PBS ^b	4% HP ^c	HP ^d	MP ^e	Alb ^f	Ac Cho ^g	pH 6.1 buffer ^h	Cath B (pH 6.1) ⁱ
HG6	59	25	1.7	2.6	62	33	>144	>144
HGGG6	32	22	1.6	2.2	73	43	>144	>144

^a $t_{1/2}$ is defined as time (hours) for the release of half of the total conjugated CPT.

Abbreviations: HP means human plasma, MP means mouse plasma.

10 ^b pH 7.4 PBS 1x buffer.

^c Reconstituted human plasma mixed with PBS (v/v = 4/96).

^d Reconstituted human plasma

^e Fresh mouse plasma

^f In reconstituted human albumin PBS buffer

15 ^g In the presence of acetyl cholinesterase PBS solution (100 units/mL).

^h pH 6.1 phosphate buffer (0.1M)

ⁱ pH 6.1 phosphate buffer in the presence of Cathepsin B

Release of CPT in Solution at Different pH.

20 **HGGG6** and **HG6** were prepared at 1 mg/mL in buffer solution with pHs ranging from acidic (pH = 1.2) to basic (pH = 13.1) and incubated at 37 °C for 24h. An aliquot of each solution was diluted with 8.5% H₃PO₄ to about 100 µg/mL. 30 µL of such solution was injected into HPLC. The peak area from the CPT lactone form was integrated and compared to a standard curve.

25 The pH of aqueous solution has a significant effect on the CPT release rates from both **HG6** and **HGGG6**. The amounts of CPT released from **HG6** and **HGGG6** at 37 °C after 24 h in buffer solutions with pHs ranging from 1.1 to 13.1 are illustrated in Figure 6. The glyciny-CPT ester bonds of both **HG6** and **HGGG6**

were very stable in acidic pH (1.1 to 6.4) as less than 7% of CPT were released in 24 h.

IC₅₀ via MTT Assay The human ovarian carcinoma A2780 cell line was
 5 obtained from the European Collection of Cell Cultures (Salisbury, Wiltshire, UK).
 The human colorectal adenocarcinoma HT29, human prostate carcinoma PC-3, and
 human colonic adenocarcinoma LS174T cell lines were obtained from the American
 Type Culture Collection (Rockville, MD). Cells were seeded in 96-well plates at
 5000 cells/well and grown in medium containing 10% fetal bovine serum at 37 °C
 10 for 24 h in a humidified 5% CO₂ atmosphere. The medium was replaced with fresh
 medium containing CPT, **36e**, **HGGG6** or **HG6** in concentrations ranging from 1
 nM to 10 μ M of CPT and **36e** (CPT equivalent for **HGGG6** and **HG6**). At each
 concentration three wells per plate were treated. The effect of the compounds on
 cell growth was measured by the MTT assay after 72 h. The medium was removed,
 15 the cells were rinsed with PBS, MTT solution was added at a concentration of 0.5
 mg/mL, and the plates were incubated for 4 h at 37 °C. The medium was removed
 and the formazan crystals were solubilized in DMSO. Absorbance was measured at
 560 nm using a SPECTRAFluor Plus plate reader (Tecan, Durham, NC). The
 percentage of cell survival was calculated relative to untreated cells, and *IC₅₀*'s were
 20 determined from plots of dose versus cell survival. *IC₅₀* data of CPT, **36e**, **HGGG6**
 or **HG6** are listed in Table 4.

Table 4. *IC₅₀* of CPT, unconjugated polymer **36e** and CPT conjugates **HG6** and **HGGG6** in various cell lines

Cell Line	36e (μ M)	CPT (μ M)	HG6 (μ M)	HGGG6 (μ M)
LS174T	>300	0.005	0.050	0.010
HT29	300	0.020	0.050	0.030
A2780	100	0.007	0.025	0.020
PC3	>300	0.075	0.25	0.15

25

Example 6: Poly-CD-BisCys-Peg3400-Ala-CPT 37

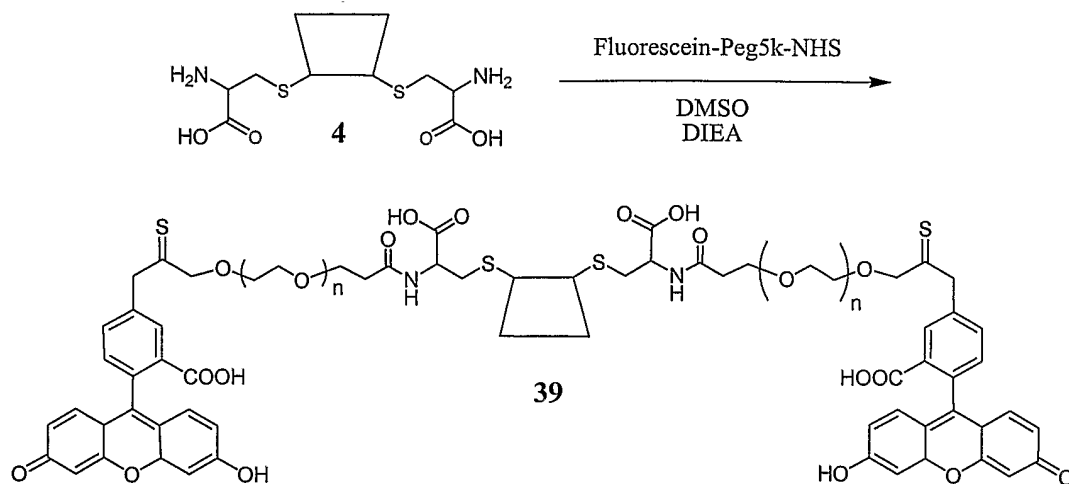
36e (54 mg, 0.012 mmol of repeat unit) was dissolved in dry DMSO (226 mL) and stirred for 10 minutes. TFA-Ala-CPT which is prepared similar to **11** (15 mg, 0.028 mmol, 2.36 eq), DIEA (4.88 mL, 0.028 mmol, 2.36 eq), DCC (24.52 mg, 0.12 mmol, 10 eq), and NHS (13.6 mg, 0.12 mmol, 10 eq) were added to the polymer solution. The mixture was stirred for about 16 hours. The polymer was precipitated with ether (40 mL) and washed with ether (2 x 30 mL) and with CH₃CN (2 x 10 mL). It was then redissolved in pH 4 aqueous solution (10 mL) and dialyzed at room temperature for 48h using 25,000 MWCO membrane. The solution was then passed through a sterilized 0.2 µm filter and then lyophilized to yield **37** (46 mg, 85%). Weight percent of drug loading was calculated to be 5.5% using HPLC equipped with a fluorescence detector after releasing CPT from **37** using base. Free CPT in **37** is <1%.

Example 7: Poly-CD-BisCys-Peg3400-Leu-CPT 38

36e (54 mg, 0.012 mmol of repeat unit) was dissolved in dry DMSO (226 mL) and stirred for 10 minutes. TFA-Leu-CPT which is prepared similar to **11** (16 mg, 0.028 mmol, 2.36 eq), DIEA (4.88 mL, 0.028 mmol, 2.36 eq), DCC (24.52 mg, 0.12 mmol, 10 eq), and NHS (13.6 mg, 0.12 mmol, 10 eq) were added to the polymer solution. The mixture was stirred for about 16 hours. The polymer was precipitated with ether (40 mL) and washed with ether (2 x 30 mL) and with CH₃CN (2 x 10 mL). It was then redissolved in pH 4 aqueous solution (10 mL) and dialyzed at room temperature for 48 h using 25,000 MWCO membrane. The solution was then passed through a sterilized 0.2 µm filter and then lyophilized to yield **38** (42 mg, 78%). Weight percent of drug loading was calculated to be 5.0% using HPLC equipped with a fluorescence detector after releasing CPT from **38** using base. Free CPT in **38** is <1%.

Example 8: Synthesis of CD-BisCys-BisPeg-FITC 39

Scheme 21

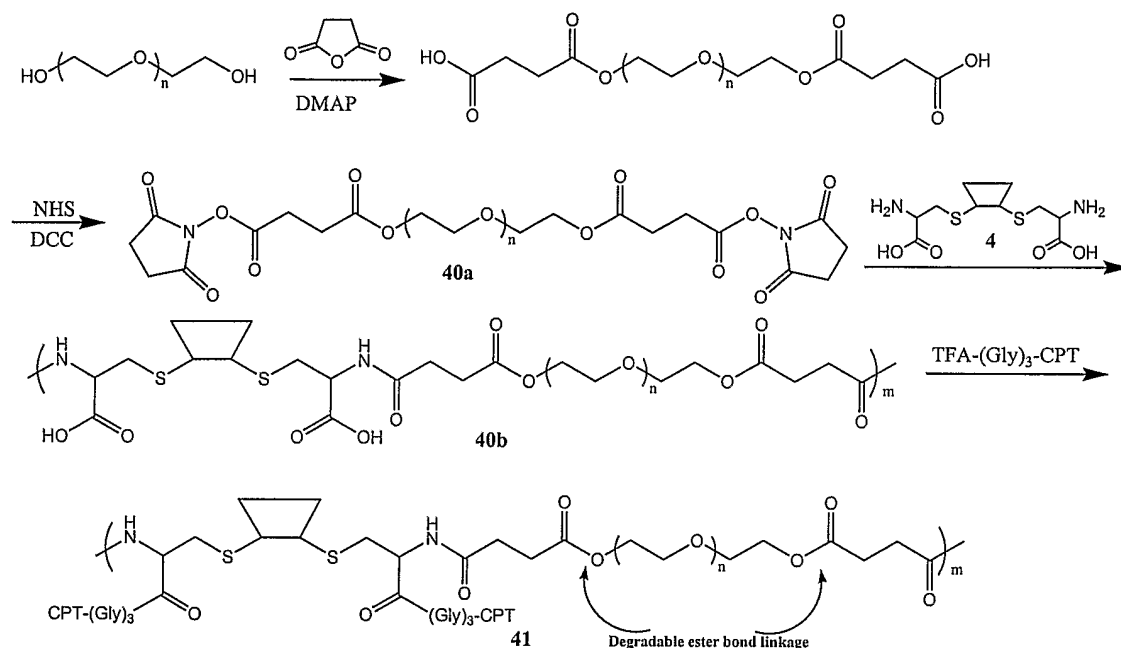


4 (25 mg, 0.0186 mmol) and FITC-Peg5000-NHS (Shearwater, 186 mg, 0.0373 mmol) were dissolved in dry DMSO (2 mL). DIEA (0.0094 mL, 0.056 mmol, 3 eq) was added to the mixture. The mixture was kept in dark and stirred for 24 hours. Water (10 mL) was then added and the solution was dialyzed in dark using 10,000 MWCO for about 48 hours. After lyophilization a yellow polymer 39 was obtained. Polymer was characterized by MS and ^1H NMR.

10

Example 9: Synthesis of Bis-succinimidyl succinatePeg3400 (Bis-SS-PEG) (40a) and biodegradable CD-BisCys-SS-Peg3400 (40b) and its CPT conjugate 41

Scheme 22



A 100 mL round bottom flask equipped with a magnetic stirbar and a septum
 5 was charged with 10 g (2.99 mmol) of polyethylene glycol Mw 3350, 2.0 g (20
 mmol) of succinic anhydride and 50 mL of anhydrous pyridine. After stirring the
 solution at 50 °C for 16 h, the solvent was removed by rotary evaporator. The
 residue was redissolved in 30 mL of water and extracted with 10 mL of chloroform
 three times. The organic layer was dried over MgSO₄ and filtered. The solvent was
 10 then concentrated and precipitated out in diethyl ether. This resulted in 9.6 g of bis-
 succinimidyl Peg3400 at a yield of 90.6%. The product was analyzed by reverse-
 phase columned High Performance Liquid Chromatography.

A 100 mL round bottom flask equipped with a magnetic stirbar and a septum
 was charged with 2 g (0.56 mmol) of bis-succinimidyl Peg3400 and 10 mL of
 15 anhydrous dichloromethane. To this solution was added 0.324 g (2.82 mmol) of N-
 hydroxyl succinimide. The solution mixture was then cooled in an ice bath and
 added 0.58 g (2.82 mmol) of 1,3-dicyclohexylcarbodiimide. After leaving at room
 temperature for 24 h, the solution was filtered and precipitated out in 150 mL of
 diethyl ether. Dissolution in 10 mL dichloromethane and precipitation in diethyl
 20 ether was repeated two times. This afforded 1.74 g (82.9 %) of Bis-SS-PEG 40a. It

was analyzed by reverse-phase columned High Performance Liquid Chromatography.

CD-BisCys-SS-Peg3400 Polymer 40b

- 5 A 50-mL pearl shaped flask was charged with 100 mg (0.0746 mmol) of **4** and 254 mg (0.0746 mmol) of **40a**. The combined solids were dried under vacuum for 24 hours before the addition of 1mL of anhydrous DMSO and 2.2 equivalents (0.164 mmol) of DIEA. The solution mixture was stirred at room temperature for 3 days and then precipitated out in diethyl ether. This yielded 100% of **40b**.
- 10 Molecular weight was analyzed on a Hitachi HPLC system equipped with an Anspec RI detector, a Precision Detectors DLS detector, and a Progel-TSK G3000_{PWXL} column using 0.1 M PBS as eluant at a 0.7 mL·min⁻¹ flow rate. $M_w = 93,000$, $M_n = 61,000$ and $M_w/M_n = 1.5$.

15 CD-BisCys-SS-Peg3400-GlyGlyGly-CPT conjugate 41

- 40b** (201.8 mg, 0.044 mmol of repeat unit), TFA-GlyGlyGly-CPT **12** (66 mg, 0.105 mmol, 2.36 eq), EDC (25.5 mg, 0.133 mmol, 3 eq), and NHS (11 mg, 0.0977 mmol, 2.2 eq) were dissolved in dry DMSO (6 mL) and stirred for 30 minutes. DIEA (19 μ L, 0.105 mmol, 2.36 eq), added to the polymer solution. The mixture
- 20 was stirred for about 41 hours. The polymer was crashed out with diethyl ether (250 mL) and washed with acetonitrile (3x 25 mL). It was then re-dissolved in pH 4 water (10mg/mL) and dialyzed at room temperature for 24 hours using 10,000 MWCO membrane. The solution was then passed through a sterilized 0.2 μ m filter and then lyophilized to yield **41** (128 mg, 52%). Weight percent of drug loading
- 25 was calculated to be 6.95% using HPLC equipped with a fluorescence detector after releasing CPT from **41** using base.

- Hydrolysis of **41** was set up in human plasma (100% solution) at 1mg/mL. Aliquot solutions (100 μ L) were placed in 1.5mL eppendorf tubes and incubated in 37 °C water bath. Then, the samples were acidified with 100 μ L of 8.5% H₃PO₄ and
- 30 loaded on pre-conditioned solid phase extraction cartridge. It was eluted with 60:40 (v/v) acetonitrile: KH₂PO₄ buffer. Free CPT (lactone form) was analyzed on

HPLC/Fluorescence detector using acetonitrile/ KH_2PO_4 buffer. Half-life was determined to be 3 h.

Degradation of CD-BisCys-SS-Peg3400 Polymer 40b

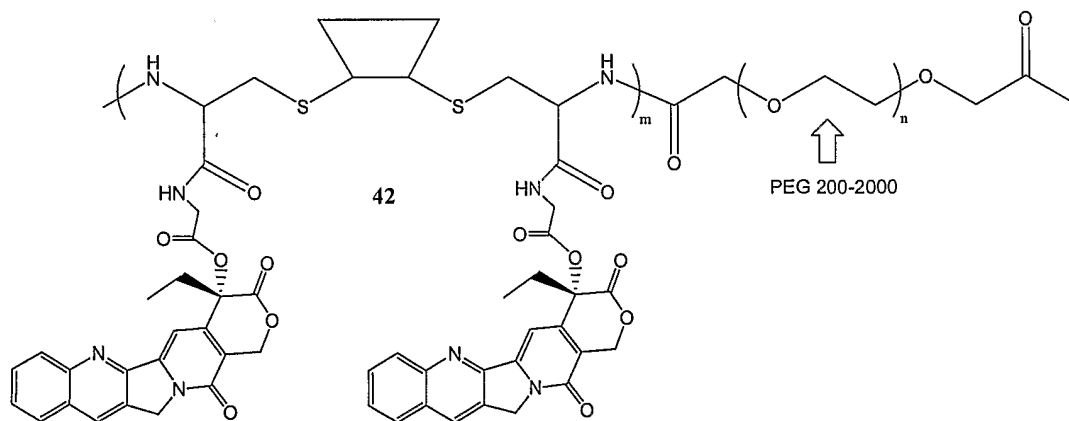
50mg/mL of **40b** solution was prepared in human plasma reconstituted in PBS (pH 7.4) solution. 100 μL aliquots were incubated at 37 °C. Each sample tube was taken out at a specific time point and crashed out in 900 μL cold methanol. The solution was centrifuged and the supernatant was analyzed on a HPLC/ELS detector. The resulting spectrum is shown in Figure 7.

Methods for increasing drug weight percent loading

Method I. Synthesis of CD-BisCys-Peg Copolymer with a short Peg linkage and its GlyCPT conjugate

Example 10: Synthesis of CD-BisCys-Peg (short PEG, e.g., Peg200-Peg2000) and its CPT Conjugate **42**

Scheme 23

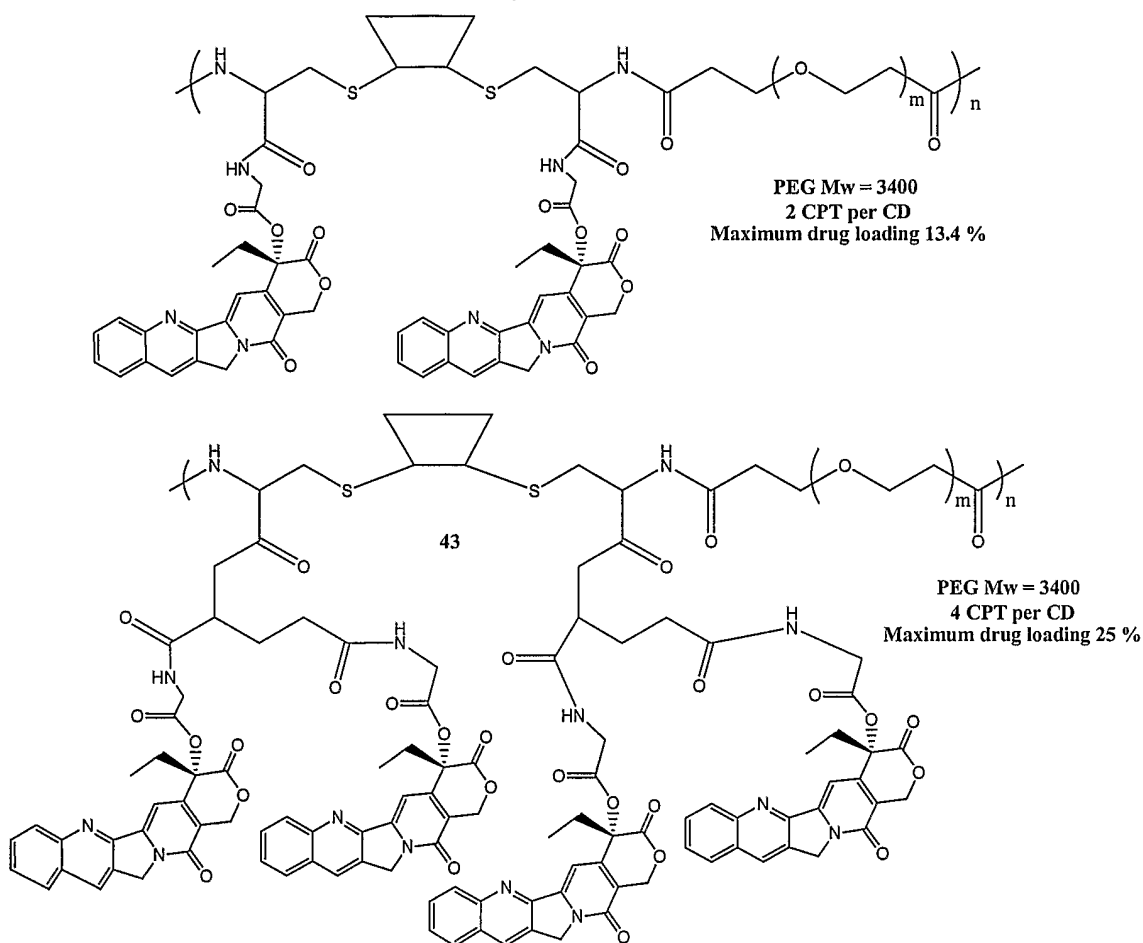


Synthesis of polymer and drug conjugate **42** are same as **36**, **37**, and **38** in Example 5.

Method II. Synthesis of CD-BisCys-Peg Copolymer with multiple drug molecules on each loading site.

Example 11: Synthesis of CD-BisCys-Peg and its GluBis(GlyCPT) Conjugate 43.

Scheme 24

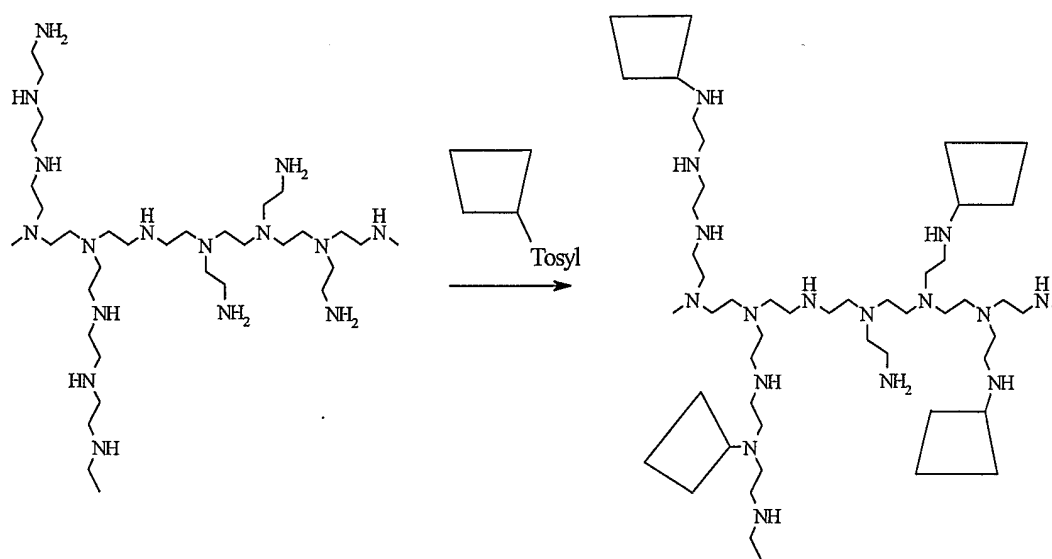


- 5 **36** and Glu-Bis(Gly-CPT) **17** are dissolved in DMSO. EDC (3 eq), NHS (2.2 eq), and DIEA (2.2 eq) are added to the solution. CD-BisCys-Peg-GluBis(GlyCPT) **43** is precipitated with CH₃CN and washed with the same solvent until no free drug is detected using UV or TLC. **43** is dried under high vacuum.

- 10 **Example 12: Synthesis of PEI-CD-CPT conjugate 44 (Branched CD-Polymer with CPT conjugates)**

A: Synthesis of branched PEI-cyclodextrin polymer

Scheme 25



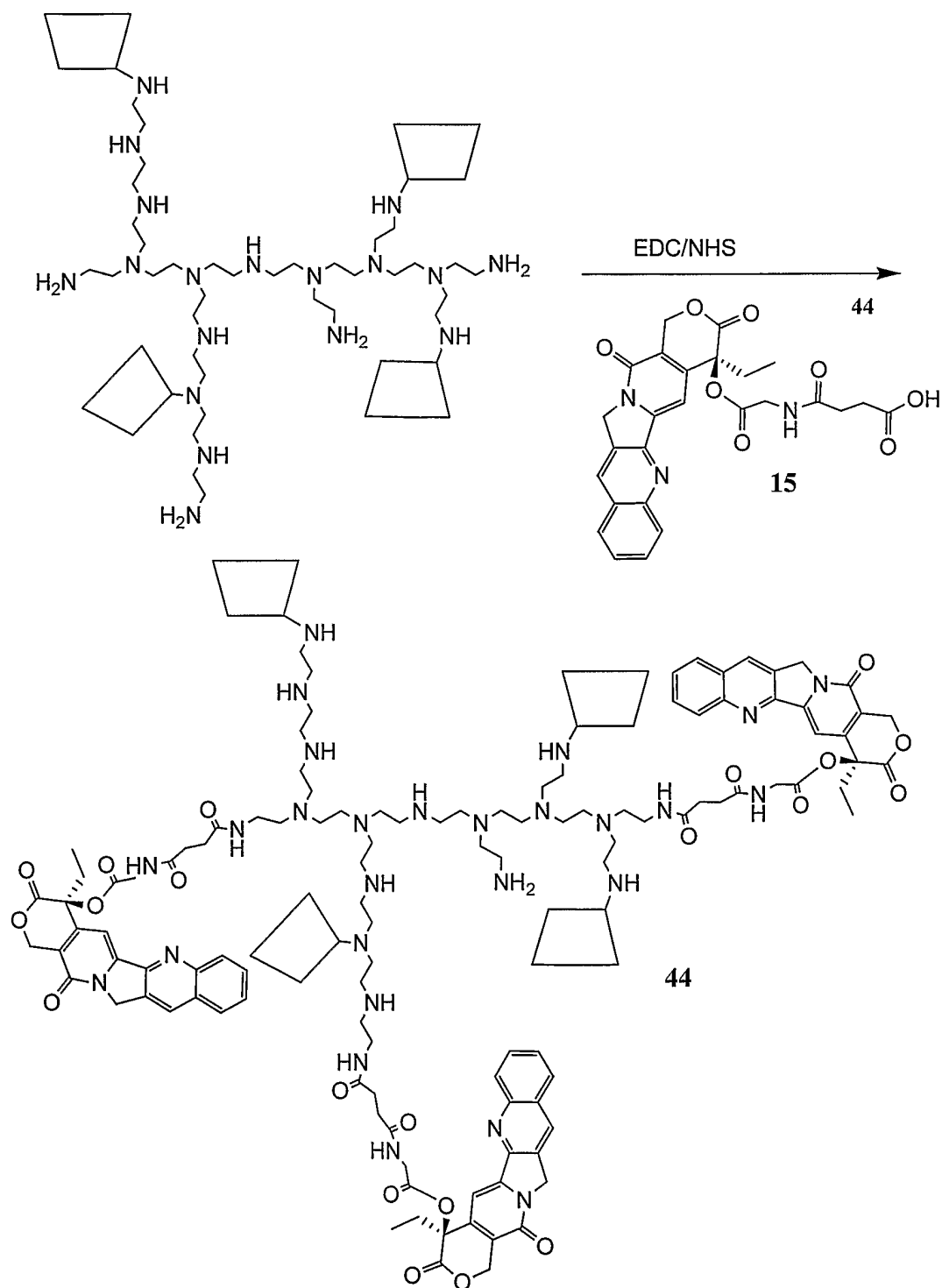
PEI (29 mg, Aldrich Mw 25,000) was dissolved in dry DMSO (2 mL).

- 5 Cyclodextrin monotosylate (448 mg, Cyclodextrin Technologies Development, Inc.) was added to the solution under N_2 . The cloudy solution turned clear after the mixture was stirred at 70 °C for about 1 hour. The solution turned slightly yellow after 48 hours at such temperature under N_2 .

- The solution was transferred to a Spectra/Por MWCO 10,000 membrane and
- 10 dialyzed against water for 4 days. Water was then removed by lyophilization. A white powder was obtained (120-140 mg) after the solution was lyophilized. Cyclodextrin/PEI ratio was calculated based on the proton integration of 1H NMR.

B: Synthesis of branched PEI-CD-CPT conjugate

Scheme 26

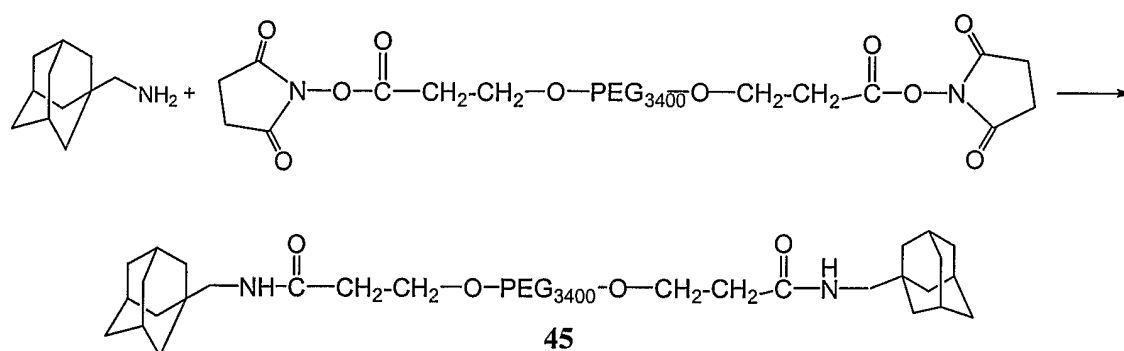


PEI-CD and Suc-Gly-CPT **15** (1.0 eq) are dissolved in DMSO. EDC (3 eq),
 5 NHS (2.2 eq), and DIEA (1 eq) are added to the solution. PEI-CD-Gly-CPT **44** is

precipitated with ether, washed extensively with this solvent, and dried under high vacuum.

Example 13: Synthesis of Ad-PEG₃₄₀₀-Ad 45

5 Scheme 27



240 mg of 1-aminoadamantane (1.60 mmol, Aldrich) and 272 mg of PEG₃₄₀₀(SPA)₂ (0.080 mmol, Shearwater Polymers) was added to a glass vial equipped with a stirbar. To this was added 5 mL of dichloromethane, and the solution was stirred overnight. The next day, the solution was filtered to remove the n-hydroxysuccinimide byproduct and the dichloromethane was removed *in vacuo*. The residue was dissolved in water and centrifuged to remove excess 1-aminoadamantane. The supernatant was then dialyzed overnight in Pierce's Slide-A-Lyzer with MWCO=3500. The solution was then lyophilized to afford 248 mg of a white fluffy solid of Ad-PEG₃₄₀₀-Ad **45**.

Example 14: Synthesis of DiCyclodextrin PEG 46

362 mg of CD-NH₂ (0.32 mmol, Cyclodextrin Technology, Inc.) and 436 mg of PEG₃₄₀₀(SPA)₂ (0.128 mmol, Shearwater Polymers) were added to a glass vial equipped with a stirbar. To this vial was added 4.36 mL of DMSO, and the solution was stirred for 72 hrs. The solution was dialyzed using 2000 MWCO membrane for 4 days in water. **46** (603 mg, 86%) was obtained as a white powder after lyophilization.

25

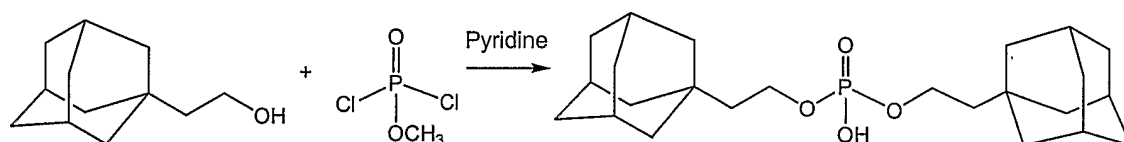
Example 15: Synthesis of Inclusion polymer 47 using DiAD-Peg 45 and DiCD-PEG 46

46 (54.6 mg, 0.01 mmol) and **45** (34 mg, 0.01 mmol) were mixed in water (0.27 mL) and stirred for overnight. The solution is very viscous. Polymer **47** was crashed out with ether and dried under vacuum

- 5 **Example 16:** Synthesis of Inclusion Polymer CPT-Conjugate **48** between DiCD-PEG **46** and a CPT-Di-AD compound

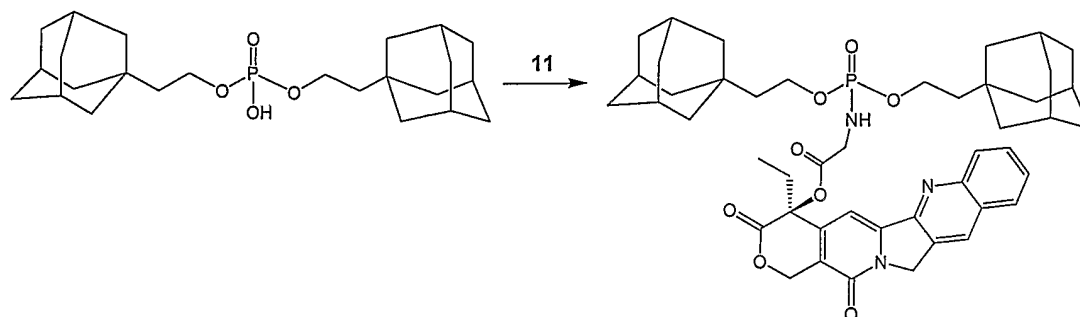
C: Synthesis of diadamantane crosslinker: Bis-(2(1-adamantyl)ethyl)phosphate
(Zhang et al., *J. Am. Chem. Soc.*, **1997**, *119*, 1676-1681)

- 10 Scheme 28



- Anhydrous pyridine (10 mL, Aldrich, Milwaukee, WI) was cooled in an ice bath and methyl dichlorophosphate (1.488 g, 10 mmol, Aldrich, Milwaukee, WI) was added dropwise. The mixture was kept cold for a further 15 min. During this period a precipitate of N-methylpyridinium dichlorophosphate formed. 1-Adamantane ethanol (4.758 g, 26.4 mmol, Aldrich, Milwaukee, WI) was added, and the sealed mixture was stirred overnight at room temperature. It was then poured into 10% NaHCO₃ solution (50 mL) and the pyridine was evaporated under vacuum.
- 20 The slightly yellow solid was dissolved in 1 L of water and extracted with ether (three 150 mL portions). The aqueous phase was acidified with 2 N HCl to pH 1, and then extracted with three 150 mL portions of CHCl₃:*n*-BuOH (7:3). The combined organic layer (ether and CHCl₃:*n*-BuOH) was washed with water and a slightly yellow precipitate was formed in the mixed solvents, at which point the solvents were evaporated under vacuum. A slightly yellow solid was formed and was recrystallized from acetone/hexane. The solid was dried under vacuum, yield 60 %.
- 25

Scheme 29



Bis-(2(1-adamantyl)ethyl)phosphate and **11** are mixed in DMSO. EDC (3
 5 eq), NHS (2.2 eq), and DIEA (1 eq) are added to the solution. Solution is stirred
 under argon for 16 hours. Bis-(2(1-adamantyl)ethyl)phosphate-Gly-CPT is
 precipitated with ether, washed extensively with this solvent, and dried under high
 vacuum. This compound and Di-CD-PEG **46** are mixed in DMSO to form inclusion
 polymer-CPT conjugate **48**.

10

Example 17: Synthesis of AD-Peg-Folate **49**

The following procedure is the synthesis of AD-Peg₅₀₀₀-Folate. This method
 can be adapted for the synthesis of any Guest molecule-Linker-Ligand tri-
 component molecules.

15

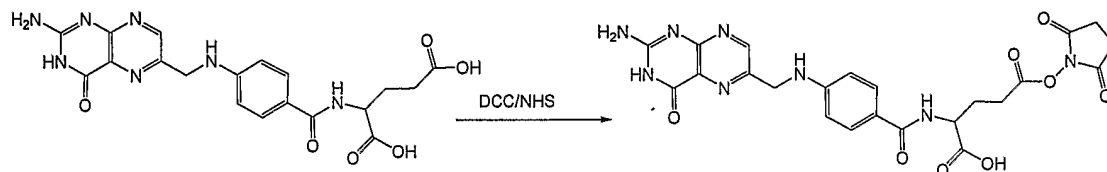
1. Synthesis of AD-Peg-NH₂

266 mg of Fmoc-PEG₅₀₀₀-NHS (78.2 μ mol, Shearwater Polymers,
 Huntsville AL) were added to a glass vial equipped with a magnetic stirbar. 10 eq.
 of 1-adamantane-methylamine (1.5 mmol, Aldrich) dissolved in 3 mL of
 20 dichloromethane were then added and the solution stirred overnight at room
 temperature. The solvent was removed *in vacuo* and water was added to the
 remaining solution to dissolve the PEG product. The solution was centrifuged at
 20K rcf for 10 minutes, whereupon the adamantane-methylamine phase-separated as
 a denser liquid. The aqueous portion was collected and water removed *in vacuo*.
 25 The remaining viscous liquid was redissolved in 20% piperidine in DMF for Fmoc
 deprotection and stirred for 30 minutes at room temperature. The solvent was
 removed *in vacuo*, washed several times with DMF, redissolved in water, and run on

an anionic exchange column to remove unreacted PEG. The first fractions were collected and lyophilized to yield 222 mg of a white, fluffy powder (76% yield) of the desired product which was confirmed by MALDI-TOF analysis.

5 2. Synthesis of N-Hydroxysuccinimide-folate (NHS-folate)

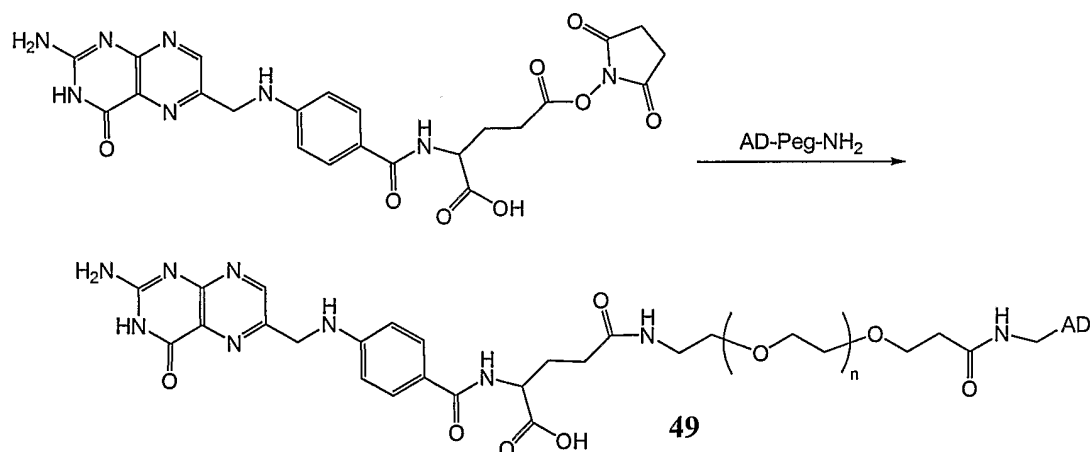
Scheme 30



NHS-folate is synthesized according to the method of Lee and Low (Lee, R. J; Low, P. S. *J. Biol. Chem.* **1994**, 269, 3198-3204). Folic acid (5 g, 11.3 mmol; Sigma) is dissolved in DMSO (100 ml) and triethylamine (2.5 ml) and reacted with N-hydroxysuccinimide (2.6 g, 22.6 mmol) and dicyclohexylcarbodiimide (4.7 g, 22.7 mmol) over-night at room temperature. The solution is filtered and concentrated under reduced pressure. NHS-folate is precipitated using diethyl ether (yellow-orange precipitate), and washed 2-3 times in anhydrous ether, dried under vacuum, and stored at -20°C .

3. AD-Peg₅₀₀₀-Folate

Scheme 31



20

AD-Peg5000-NH₂ and NHS-Folate are mixed at 1:1 eq. in DMSO solution. DIEA (2 eq) is added. The mixture is allowed to stir at room temperature for overnight. The DMSO solution is then dialyzed against 3500 MWCO (Spectra/Por 7) membrane for 48 hours. AD-Peg5000-Folate **49** is obtained after lyophilization.

5

Example 18: Formulation of the AD-Peg-Folate and a CD-Polymer-CPT conjugate

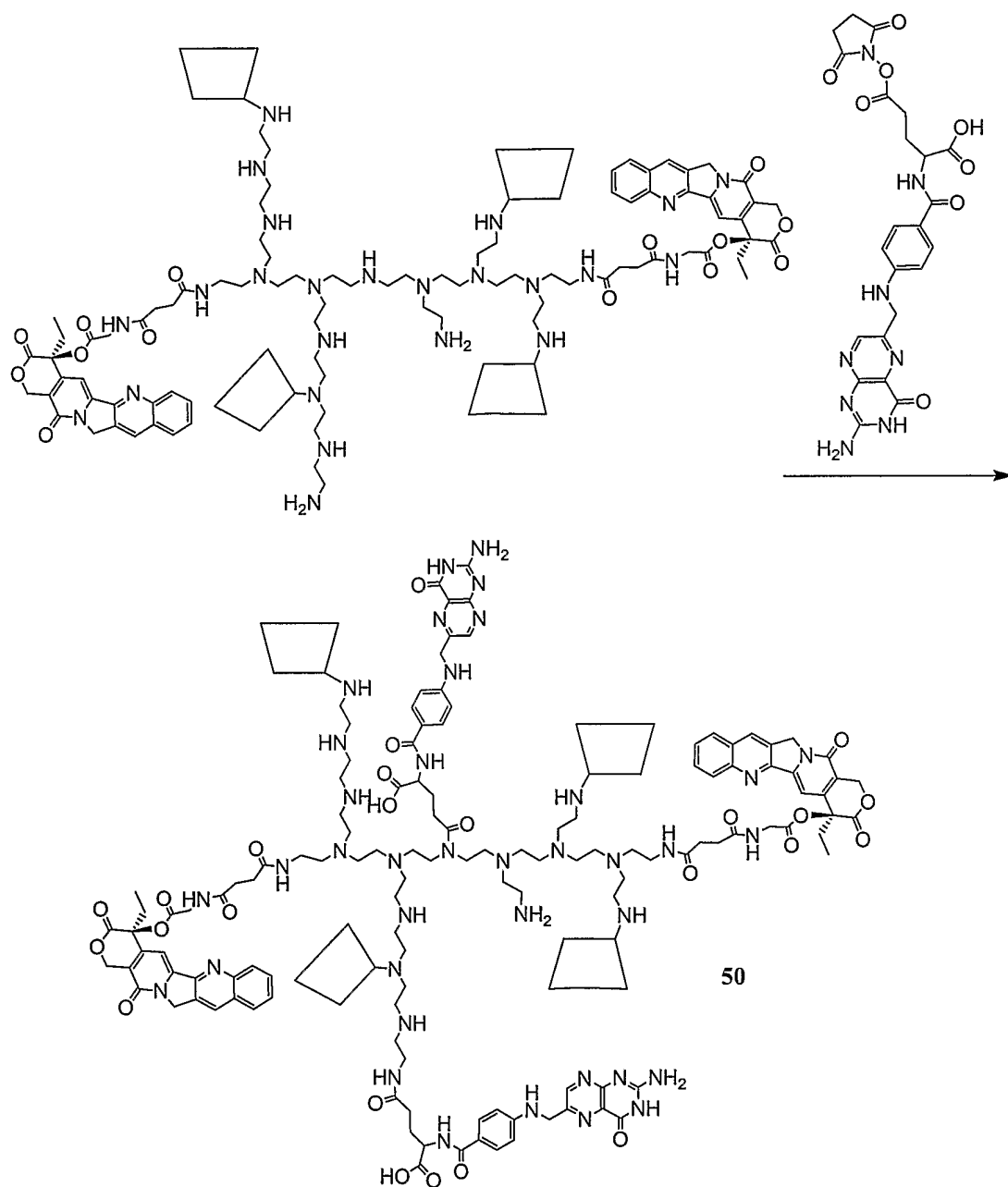
A typical procedure: A CD-polymer CPT conjugate is dissolved in a D5W buffer.

A D5W solution containing 0.1-1 eq (mols of repeat unit) of AD-Peg-Folate solution is added to polymer solution. Particle sizes of polymer are measured before and

10 after adding of AD-Peg-Folate using light scattering. This solution is used for either *in vitro* or *in vivo* test for the analysis of folate targeting effects.

Example 19: Covalent linking a targeting ligand (e.g., folate) to a CD-CPT polymer conjugate (eg PEI-CD-GlyCPT) **50**

Scheme 32



- 5 PEI-CD-GlyCPT **44** and Folate-NHS (0.1-0.5 eq) are mixed in DMSO and stirred for 24 hours. The polymer is crashed out with ether and washed extensively with this solvent until no free folate molecule can be detected. The resulting CD-polymer-CPT-Folate conjugate **50** is dried under vacuum.

Example 20: Crosslinking of CD-CPT polymer using Ad-Peg-Ad. 51

A typical procedure: AD-Peg-AD 45 (0.01-0.1 eq of CD-polymer repeat unit) and CD-polymer CPT conjugate are mixed in a minimum amount of DMSO. The resulting viscous solution is precipitated with ether and dried under vacuum to yield
5 lightly crosslinked polymer 51. 51 should still maintain small particle size in solution, have good water solubility, and have higher molecular weight than parent CD-polymer CPT conjugate.

Example 21: In vivo tests of camptothecin polymer conjugates HGGG6, LGGG10, HG6, and HGGG10 (Synthesized according to Example 5).
10

A. In Vivo Toxicity and Blood Chemistry Analysis from Dosing With Parent Polymer 36e

The toxicity of 36e and its effects on blood chemistry were evaluated in
15 female Charles River nude mice (13-14 weeks old). Four treatment groups of six mice each were treated with a D5W solution of 36e at a dose of 240 mg/kg, 160 mg/kg, 80 mg/kg or D5W alone by i.v. tail vein injection on Days 1, 5, and 9, respectively. Dosing volume was determined based upon a ratio of 200 μ l for a 20 g mouse, and was scaled appropriately according to the actual BW of the mice. The
20 BWs of the mice were followed daily for the first 5 days and then twice a week, thereafter. Blood samples (150-200 μ l) were collected from each mouse by retro-orbital bleeding under isoflourane on Day 12. Samples from three mice in each group were used for complete blood count (CBC) analyses, while blood samples from the remaining three mice in each group were processed for blood chemistry
25 analyses. The study was stopped at Day 23. All mice were euthanized by cardiac puncture under CO₂, and blood from each mouse was collected for CBC and blood chemistry analysis in the same manner as on Day 12.

There was no significant difference in BW loss, CBC or blood chemistry data between any of the 36e treated groups and D5W control group throughout the study,
30 and no time dependent effects were observed over 23 days for all the treated groups. 36e was well tolerated by mice at the maximum dose treated (240 mg/kg).

B. Determination of Maximum Tolerable Dose (MTD) for CDP-CPT**Conjugates.**

The MTD was determined using female Charles River nude mice (15–16 weeks old) for **HG6**, **LGGG10**, **HGGG10**. A 5% (w/v) of dextrose solution (D5W) of the polymer-CPT conjugates was freshly prepared before each injection. Doses for the treatment groups ranged from 2.25 mg CPT/kg to 54 mg CPT/kg. Dosing was administered intravenously (i.v.) by tail vein injection on Days 1, 5, and 9. The dosing volume was determined based upon a ratio of 200 μ l for a 20 g mouse, and was scaled appropriately according to actual body weight (BW) of the mice. Three to five mice were used in each treatment group. The BWs of the mice were followed daily for the first 5 days and then twice a week, thereafter. The MTD was defined as the highest administered dose that resulted in a decrease of mean group BW of less than 20% or the highest administered dose that did not result in death of any animal in that group. The maximum mean body weight loss and treatment related deaths for all treated groups are listed in Table 5.

Table 5. Treatment response for the MTD study. Nude mice (n= 3-6) were treated i.v. by tail vein injection.

Agent	mg/kg ^a	Max %BW loss; Day ^b	N _{TR} /N ^c
D5W	-	-2.5%; Day 3	0/6
36e	240	-2.0%; Day 3	0/6
36e	160	-3.5%; Day 13	0/6
36e	80	-2.3%; Day 3	0/6
LGGG10	54	-20.6%; Day 3	3/3
LGGG10	36	-9.3%; Day 13	0/3
LGGG10	18	0	0/3
LGGG10	9	0	0/5
LGGG10	4.5	-0.8%; Day 13	0/5
HG6	54	-28.5%; Day 3	3/3
HG6	36	-23.9%; Day 3	3/3
HG6	18	-22.1%; Day 3	3/3

HG6	9	-6.1%; Day 9	0/5
HG6	4.5	-4.4%; Day 5	0/5
HG6	2.25	-2.9%; Day 9	0/5
HGGG10	54	---	3/3
HGGG10	36	-34%; Day 5	3/3
HGGG10	18	-16%; Day 3	1/3
HGGG10	9	-3.3%; Day 9	0/5
HGGG10	4.5	-2.5%; Day 9	0/5

^a Mg CDP /kg for the CDP polymer and mg CPT/kg for the three conjugates tested.

^b Maximum body weight (BW) loss observed post injection

^c Number of treatment-related deaths (N_{TR}) to the number of mice treated (N)

5

The MTD of **LGGG10**, **HG6**, and **HGGG10** were determined to be 36 mg CPT/kg, 9 mg CPT/kg, and 9 mg CPT/kg, respectively. Based on the structural similarities between **HGGG6** and **HGGG10**, it is expected that the MTD for these two groups are similar. Therefore, the MTD of **HGGG6** (not tested) was assumed to be 9 mg CPT/kg.

10

C. Antitumor Efficacy Study.

The antitumor efficacy study was performed using female Charles River nude mice (15–16 weeks old). A fragment (1 mm³) of human LS174T colon carcinoma tissue was implanted subcutaneously (s.c.) into the right flank of each test mouse approximately 14–18 days before dosing. The tumor volume was determined by measuring the tumor in two dimensions with calipers and calculated using the formula: tumor volume = (length x width²)/2. Tumor volume was converted to tumor weight assuming 1 mm³ is equal to 1 mg tumor in weight. Treatment was initialized when mean tumor size reached approximately 60–100 mg (Day 1). The animals were sorted into twelve groups. Each group consisted of seven mice with tumor sizes ranging from 62.5–144.0 mg with group mean tumor sizes of 88.6–90.7 mg. Mice in each group were treated according to the protocol listed in Table 6. All conjugate treatments were administered intravenously by tail vein injection. Tumor

15

20

sizes were measured twice a week for the duration of the experiment. At the end of study, tumors from each euthanized mouse were harvested and frozen at -80°C .

Table 6. Dosing protocol for efficacy study.^a

Group	Agent	Dose (mg CPT/kg) ^b	Route ^c	Schedule ^d
1	D5W	-	i.v.	Q4D x 3
2	CPT	9	i.p.	Q4D x 2 ^e
3	Irinotecan	100 ^f	i.p.	Qwk x 3
4	HGGG6	9	i.v.	Q4D x 3
5	HGGG6	4.5	i.v.	Q4D x 3
6	LGGG10	36	i.v.	Q4D x 3
7	LGGG10	18	i.v.	Q4D x 3
8	LGGG10	9	i.v.	Q4D x 3
9	HG6	9	i.v.	Q4D x 3
10	HG6	4.5	i.v.	Q4D x 3
11	HGGG10	9	i.v.	Q4D x 3
12	HGGG10	4.5	i.v.	Q4D x 3

5 ^a Seven mice were used in each group

^b Doses are equivalent of CPT except for group 3

^c i.p. = intraperitoneal, i.v. = intravenous

^d Administration schedules were abbreviated as: Q4D x 3 = three injection with four-day

10 intervals, Qwk x 3 = three injection with one-week interval, first dose was initialized on day 1 for all groups.

^e The scheduled third dose was not given due to the emerging toxicity

^f 100 mg irinotecan/kg

Each animal was euthanized when tumor weight reached the predetermined endpoint size (1,500 mg). The time-to-endpoint (TTE) for each mouse was calculated from the equation: $TTE = (\log(\text{endpoint}-b))/m$, where b and m are the intercept and the slope, respectively, of the line obtained by linear regression of a log-transformed tumor growth data set comprised of the first observation that exceeded the study endpoint volume and the three consecutive observations that immediately preceded the attainment of the endpoint volume. Animals that do not reach the endpoint were assigned a TTE value equal to the last day of the study (114 days). Animals classified as treatment-related deaths (TR) were assigned a TTE value equal to the day of death. Animals classified as non-treatment-related death (NTR) are excluded from TTE calculations. Tumor growth delay (TGD), defined as the increase in the median time to endpoint (TTE) in a treatment group compared to the control group, was one parameter investigated to evaluate treatment efficacy. TGD is calculated as the difference between the median TTE for a treatment group and the median TTE of the control group ($TGD = T - C$) and is expressed in days, and as a percentage of the median TTE of the control group; $\%TGD = (T-C)/C$ where T is equal to median TTE for a treatment groups and C is equal to median TTE for the control, Group 1.

Toxicity. Animals were weighed daily on Days 1–5, then twice weekly thereafter. Mice were examined frequently for overt signs of any adverse, drug-related side effects. Acceptable toxicity for cancer drugs in mice is defined by the NCI as a group mean body-weight loss of less than 20% during the study, and not more than one toxic death among seven treated animals.

Results for this efficacy study that include median TTE values, median tumor burden on day 114, treatment response and deaths are summarized in Table 7.

Table 7.

Group	Median TTE ^a	T-C ^b	%TGD ^c	Median Tumor Burden in mg (N _s ^d)	N _{TR} ^e / N _{NTR} ^f / N _{EU} ^g	P vs D5W ^h	P vs CPT ⁱ
1	34.9	-	-	-(0)	0/1/6	-	-

2	51.4	16.5	47%	- (0)	2/0/5	0.2128	-
3	68.7	33.8	97%	1152 (3)	0/0/4	0.0002	0.0253
4	114.0	79.1	227%	256 (5)	1/0/1	0.0040	0.0115
5	65.6	30.7	88%	566 (2)	0/1/4	0.0046	0.1369
6	100.0	65.1	187%	666 (3)	4/0/0	0.0272	0.0289
7	75.6	40.7	117%	221 (3)	0/0/4	0.0018	0.0601
8	63.2	28.3	81%	700 (1)	1/0/5	0.0006	0.1064
9	114.0	79.1	227%	394 (4)	0/0/3	0.0002	0.0028
10	74.2	39.3	113%	668(2)	1/1/3	0.0016	0.0673
11	114.0	79.1	227%	500 (5)	1/0/1	0.0040	0.0050
12	78.0	43.1	123%	1010 (2)	0/0/6	0.0006	0.0392

^a TTE = Time (Days) to endpoint (1500 mg)

^b T-C = Difference between TTE (Days) of treated versus control group

^c %TGD = [(T-C)/C]

^d Mice surviving

5 ^e N_{TR} = Number of treatment-related death

^f N_{NTR} = Number of non-treatment-related death

^g N_{EU} = Number of mice euthanized after reaching endpoint

^h P value versus the D5W treatment group (Group 1)

ⁱ P value versus the CPT treatment group (Group 2)

10

One NTR death on day 72 was observed in the control animals treated with D5W. Tumors in the other six control mice grew to the 1500 mg endpoint size, yielding a median TTE of 34.9 days (Table 7).

Two treatment-related deaths were reported on Day 9 for CPT at 9 mg/kg. Thus, CPT must be considered to be toxic at this dose in this experiment. The median TTE for this group was 51.4 days, corresponding to a 16.5 day T-C and a 47% TGD, relative to untreated control mice (*not significant*). No animal in Group 2 survived to Day 114.

Group 3 received irinotecan i.p. at 100 mg/kg (Qwk x 3). The median TTE for Group 3 was 68.7 days, corresponding to a significant 33.8 day T-C and a 97%

TGD, relative to control mice ($P < 0.01$). Three animals survived to Day 114 with a median tumor burden of 1,152 mg. No regressions were recorded.

Groups 4 and 5 received **HGGG6** i.v. Q4D x 3 at 9 and 4.5 mg CPT/kg, respectively. One treatment-related death was observed on Day 16 in Group 4, and one NTR death was recorded on Day 37 in Group 5. The median TTE for Group 4 was 114 days, the maximum possible value in this study. This TTE value corresponds to a significant 79.1 day T-C and a 227% TGD, relative to control ($P < 0.01$). Tumors in five mice of Group 4 did not reach the 1,500 mg endpoint. These five mice had a median tumor burden of 256 mg on Day 114. The median TTE for Group 5 was 65.6 days, and corresponds to a significant 30.7 day T-C and an 88% TGD, relative to control ($P < 0.01$).

Groups 6–8 were treated with **LGGG10** i.v. Q4D x 3 at 36, 18, and 9 mg CPT/kg, respectively. Although no death was observed in MTD study using this conjugate in non-tumor bearing mice at 36 mg CPT/kg (Table 5), four treatment-related deaths were recorded in Group 6 when tumor-bearing mice were given at this dose, two on Day 16 and one each on Days 75 and 100. These results indicate that 36 mg CPT/kg is probably over the MTD of **LGGG10**. As shown in Table 5, no significant body weight loss was recorded in the MTD study when the mice were dosed at 18 mg CPT/kg, indicating that this dose is below the MTD. Therefore, the MTD of **LGGG10** lies somewhere between 18 to 36 mg CPT/kg. The median TTE for Group 7 (18 mg CPT/kg) was 75.6 days. This TTE value corresponds to a significant 40.7 day T-C and a 117% TGD, relative to control mice ($P < 0.01$). Three mice in this group had a median tumor burden of 221 mg on Day 114. One late TR death was recorded on Day 103 in Group 8 (9 mg CPT/kg). The median TTE for Group 8 was 63.2 days. This TTE value corresponds to a significant 28.3 day T-C and an 81% TGD, relative to untreated control mice ($P < 0.01$). The remaining mouse in this group had a tumor burden of 700 mg on Day 114.

Groups 9 and 10 were dosed with **HG6** i.v. Q4D x 3 at 9 and 4.5 mg CPT/kg, respectively. One TR and one NTR death were recorded in Group 10 on Days 47 and 84, respectively. The median TTE for Group 9 was the maximum, 114 days. This TTE value corresponds to a significant 79.1 day T-C and a 227% TGD, relative to untreated control mice ($P < 0.01$). Four mice in Group 9 had a median

tumor burden of 394 mg on Day 114. The median TTE for Group 10 was 74.2 days. This TTE value corresponds to a significant 39.3 day T–C and a 113% TGD, relative to control mice ($P < 0.01$). The remaining two mice in Group 10 had a median tumor burden of 668 mg on Day 114.

5 Groups 11 and 12 were dosed with **HGGG10** i.v. Q4D x 3 at 9 and 4.5 mg CPT/kg, respectively. One treatment-related death was recorded on Day 16 in Group 11. The median TTE for Groups 11 and 12 were 114 days and 78 days, respectively. The TTE value for Group 11 corresponds to a significant 79.1 day T–C and a 227% TGD, relative to control mice ($P < 0.01$). Tumors in five mice in Group 11 did not
10 reach the endpoint; these five mice had a median tumor burden of 500 mg on Day 114. The TTE value of Group 12 corresponds to a significant 43.1 day T–C and a 123% TGD, relative to control mice ($P < 0.01$). The remaining two mice in this group had a median tumor burden of 1,010 mg on Day 114.

The tumor growth curve as a function of time for the D5W, CPT, irinotecan,
15 **LGGG10** at its highest non-toxic dose tested (18 mg CPT/kg), and the other three conjugates with high MW polymer (**HGGG6**, **HG6**, **HGGG10**) at their MTDs are shown in Figure 8. The three high MW conjugates administered at their MTDs displayed more prolonged tumor growth inhibition compared to D5W, CPT and irinotecan. The median tumor growth curves for **HGGG6**, **HG6** and **HGGG10** that
20 are illustrated in Figure 9 show that there is a distinct dose response for all three of these polymers when dosed as their MTD and at half of their MTD. The medium tumor growth curves for **LGGG10** and **HGGG10** each dosed at 9 mg CPT/kg as illustrated in Figure 10 demonstrate that high MW polymer-drug conjugate has greater antitumor effect when compared to the low MW conjugates presumably due
25 to the enhanced accumulation (EPR effect) and reduced renal clearance.

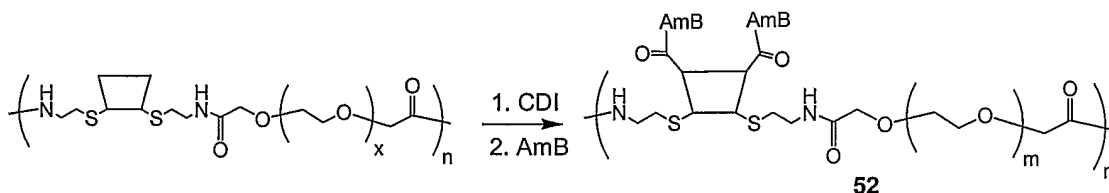
Mean Body Weight Loss of Mice. Mean body weight (MBW) losses as a function of time are plotted for D5W, CPT, irinotecan and the three conjugates containing high MW polymer at their MTDs (Fig. 11). Maximum MBW losses observed in Group 2 (CPT) and the two conjugates with the triglycine linker dosed
30 at their MTDs (Groups 4 and 11) were 13.1%, 18.3%, and 12.6%, respectively. Maximum MBW loss of **HG6** (3.4%), the only conjugate with a glycine linker, was similar to the maximum MBW loss recorded for irinotecan (5.0%). Negligible

(<5%) maximum group mean body-weight losses were recorded in all the other treatment groups and in the D5W group. Mean body weight returned to baseline levels for all treatment groups following cessation of therapy.

5 D Correlation of Tumor size of euthanized mouse and the CPT concentration in corresponding tumor.

Each tumor harvested from mice at the completion of the LS174t xenograft mouse study was thawed and placed in a 2 ml lysis tube (Lysing Matrix D, Qbiogen). 300 μ L of lysis reagent (Cellytic –MT Mommalian Tissue Lysis/Extraction reagent) was added to each tube. The tissue was homogenized on a FastPrep FP12 homogenizer (Qbiogen) at 5 m/s for 40 sec. Homogenization was repeated six times with a 10 min interval between successive homogenization. The homogenized solution was centrifuged at 14000g for 15 min at 10°C. 90 μ L of the solution was syringed out to which 10 μ L 1N NaOH was added. An aliquot of 400 μ L MeOH was added to this solution after allowing the homogenized solution to stand for 2h at room temperature. The solution was centrifuged for 15 min at 14000 g. The supernatant (270 μ L) was mixed with 30 μ L 1N HCl and injected into an HPLC for analysis. The correlation of CPT concentration (ng/mg tissue) to tumor size (in mg) is illustrated in Figure 12. CPT concentration was inversely correlated to tumor size.

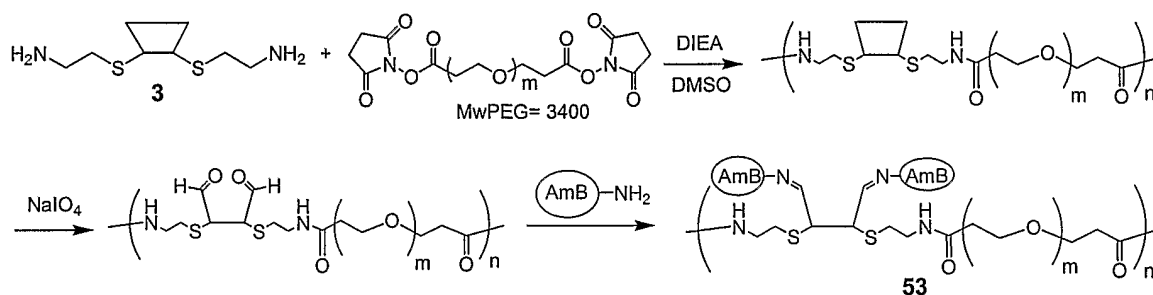
Example 22: Synthesis of Poly(CDDC-PEG)-Amphotericin B **52** via amide linker Scheme 33



Poly(CDDC-PEG) (788 mg) and 1,1'-carbonyl diimidazole (CDI, 1.45 g, 50 eq.) were stirred in anhydrous DMSO (10 mL) in the presence of DMAP (429 mg, 20 eq) for 16 h. Ether (200 mL) was added to the mixture to precipitate poly(CDDC-PEG)-carbonyl-imidazole. The resulting yellow solid was washed with

ether 2 x 200 mL and dried under vacuum. The solid was dissolved in anhydrous DMSO (15 mL), followed by adding Amphotericin B (AmB) (332 mg, 2 eq) and DMAP (43.0 mg, 2eq). The solution was stirred in dark for 48 h and dialyzed in water using 25000 MWCO membrane for 3 days. The solution was then filtered
 5 using 0.2 μ m filter and lyophilized. A yellow solid (920 mg) **52** was obtained. The wt% of AmB is around 13%.

Example 23: Synthesis of Poly(CDDC-PEG)-Amphotericin B **53 via imine linker**



3 and PEG-DiSPA (1:1 ratio) were dried under vacuum at room temperature. DMSO (10 mg of **3**/mL DMSO) was added to the solid and followed by adding of DIEA (2 eq) to the mixture. Polymer was crashed out with excess ether 5 days later and dialyzed using 25000 MWCO membrane for 48 h. The yield of poly(CDDC-PEG) is 80-95%. The M_w of polymer was determined using GPC to be 70-100
 15 kDa.

Poly(CDDC-PEG) (1.124g, 0.25 mmol) was dissolved in water (55 mL). NaIO_4 (0.264g, 5 eq.) was added. The solution was stirred in dark at room temperature for 20 min and stored at 4 $^{\circ}\text{C}$ for 24h in dark. BaCl_2 solution was added (5.05 eq) to the solution to give immediate precipitation of $\text{Ba}(\text{IO}_4)_2$. The precipitate
 20 was filtered. Saturated Na_2CO_3 solution was added to adjust pH to 11. Amphotericin B (343mg, 1.5 eq) was then added to solution and stirred at rt in dark for 48h. The pH of solution was maintained to be 11 by adding NaOH (0.1N) throughout the reaction. The solution was dialyzed at 4 $^{\circ}\text{C}$ for 48 h using 25000 MWCO and lyophilized to give 1.03 g polymer-AmB conjugate **53** as a yellow
 25 powder. The wt% of AmB is determined to be 18 using UV spectrometer at 405 nm.

References

Additional cyclodextrin-containing polymers that can be modified according to the teachings of the present invention, as well as methods of preparing such polymers, are disclosed in U.S. Patent applications 09/203,556, 09/339,818, 5 09/453,707, 10/021,294, 10/021,312, and 10/656838 (now U.S. published application 2004-0077595) as well as WO 2004/022099 all of which are hereby incorporated herein by reference in their entirety.

All of the references, patents, and publications cited herein are hereby incorporated by reference.

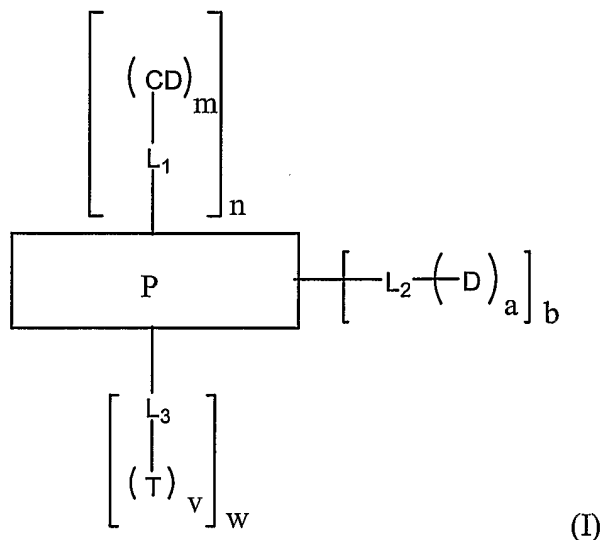
10

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the compounds and methods of use thereof described herein. Such equivalents are considered to be within the 15 scope of this invention and are covered by the following claims.

CLAIMS:

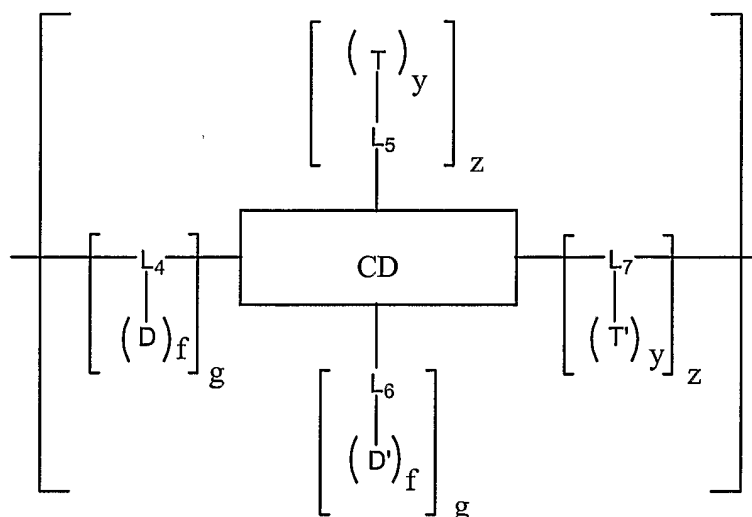
1. A compound represented by Formula I:



wherein

- 5 P represents a linear or branched polymer chain;
 CD represents a cyclodextrin moiety;
 L₁, L₂ and L₃, independently for each occurrence, represent a direct bond or the
 same or different linker groups;
 D, independently for each occurrence, represents a therapeutic agent or a prodrug
 10 thereof;
 T, independently for each occurrence, represents a targeting ligand or precursor
 thereof;
 a, m and v, independently for each occurrence, represent integers in the range of 1 to
 10;
 15 n and w, independently for each occurrence, represent an integer in the range of 0 to
 about 30,000;
 b represents an integer in the range of 1 to about 30,000; and
 one or more occurrences of L₁, L₂, or L₃, independently, represent a photocleavable
 linker;
 20 wherein either P comprises cyclodextrin moieties or n is at least 1.
2. The compound of claim 1, wherein one or more occurrences of L₂ or L₁,
 independently represent a photocleavable linker.

3. The compound of any one of claims 1-2, wherein one or more occurrences of L_2 represent a photocleavable linker.
4. The compound of any one of claims 1-3, wherein the polymer chain comprises n' units of U, wherein n' represents an integer in the range of 1 to about
- 5 30,000; and U is represented by the general formula:



wherein

CD represents a cyclodextrin molecule, or derivative thereof;

10 L_4 , L_5 , L_6 , and L_7 , independently for each occurrence, represent a direct bond or the same or different linker groups;

D and D', independently for each occurrence, represent the same or different therapeutic agents or prodrugs thereof;

T and T', independently for each occurrence, represents the same or different targeting ligands or precursor thereof;

15 f and y, independently for each occurrence, represent an integer in the range of 1 and 10; and

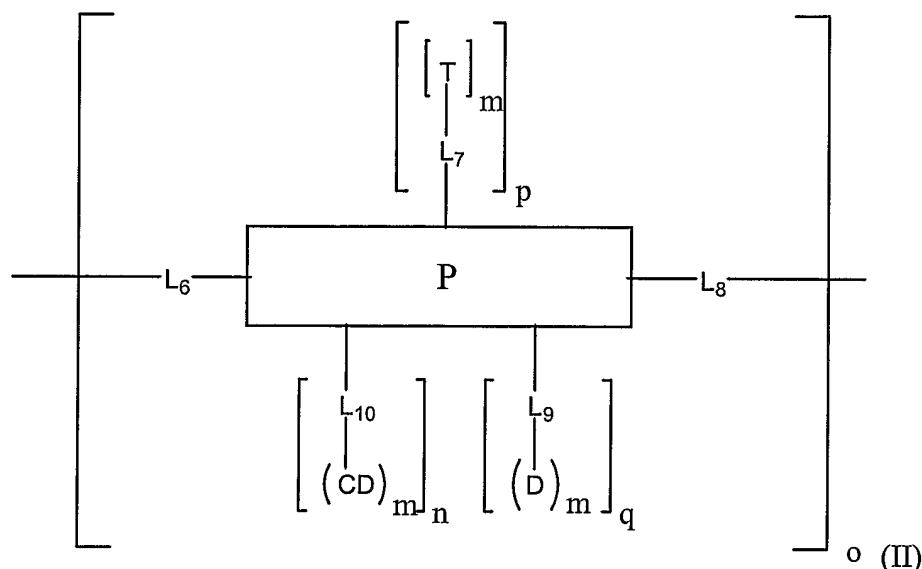
g and z, independently for each occurrence, represent an integer in the range of 0 and 10,

20 wherein one or more occurrences of L_4 , L_5 , L_6 , or L_7 , independently, represent a photocleavable linker.

5. The compound of claim 4, wherein one or more occurrences of L_4 , L_6 , or L_7 , independently, represent a photocleavable linker.

6. The compound of any one of claims 4-5, wherein one or more occurrences of L_4 or L_6 , independently represent a photocleavable linker.

7. A compound represented by Formula II:



5 wherein

P represents a monomer unit of a polymer;

T, independently for each occurrence, represents a targeting ligand or a precursor thereof;

10 L_6 , L_7 , L_8 , L_9 , and L_{10} , independently for each occurrence, represent a direct bond or the same or different linker groups;

CD, independently for each occurrence, represents a cyclodextrin moiety or a derivative thereof;

D, independently for each occurrence, represents a therapeutic agent or a prodrug form thereof;

15 m, independently for each occurrence, represents an integer in the range of 1 to 10;

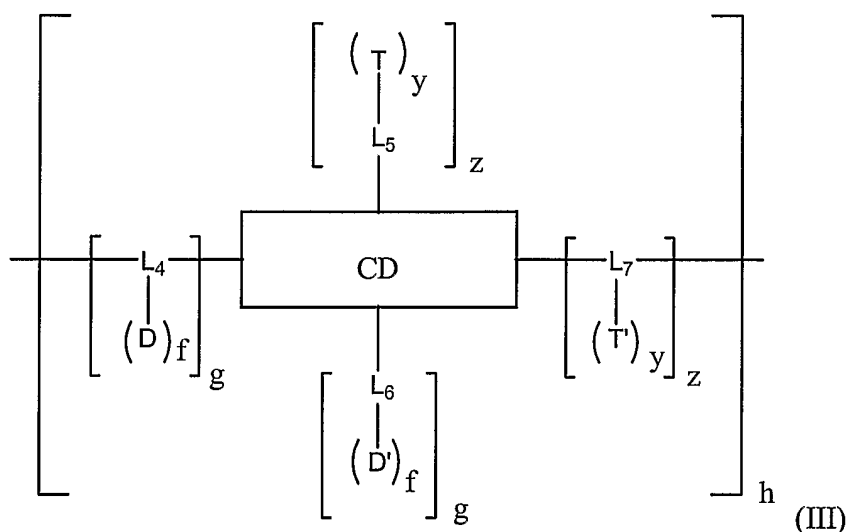
o represents an integer in the range of 1 to about 30,000; and

p, n, and q, independently for each occurrence, represent an integer in the range of 0 to 10;

20 one or more occurrences of L_6 , L_7 , L_8 , L_9 , or L_{10} , independently represent a photocleavable linker;

wherein CD and D are each present at least once in the compound.

8. The compound of claim 7, wherein one or more occurrences of L_7 , L_9 , or L_{10} , independently represent a photocleavable linker.
9. The compound of any one of claims 7-8, wherein one or more occurrences of L_9 or L_{10} , independently represent a photocleavable linker.
- 5 10. The compound of any one of claims 7-9, wherein one or more occurrences of L_9 represent a photocleavable linker.
11. A compound represented by Formula III:



wherein

- 10 CD represents a cyclodextrin molecule, or derivative thereof;
 L_4 , L_5 , L_6 , and L_7 , independently for each occurrence, represent a direct bond or the same or different linker groups;
 D and D' , independently for each occurrence, represent the same or different therapeutic agents or prodrugs thereof;
- 15 T and T' , independently for each occurrence, represent the same or different targeting ligands or precursors thereof;
 f and y , independently for each occurrence, represent an integer in the range of 1 and 10;
 h represents an integer in the range of 1 to about 30,000; and
- 20 g and z , independently for each occurrence, represent an integer in the range of 0 and 10;

one or more occurrences of L₄, L₅, L₆, or L₇, independently represent a photocleavable linker;
wherein at least one occurrence of g represents an integer greater than 0.

12. The compound of claim 11, wherein one or more occurrences of L₄, L₆, or L₇,
5 independently represent a photocleavable linker.

13. The compound of any one of claims 11-12, wherein one or more occurrences of L₄ or L₆, independently represent a photocleavable linker.

14. The compound of any one of claims 1-13, wherein one or more photocleavable linkers undergo photolysis on subjecting the compound to radiant
10 energy of UV, visible, or infrared wavelength.

15. The compound of claim 1-13, wherein one or more photocleavable linkers undergo photolysis on subjecting the compound to radiant energy of visible wavelength.

16. The compound of claim 1-13, wherein one or more photocleavable linkers
15 undergo photolysis on subjecting the compound to radiant energy of near infrared wavelength

17. The compound of any one of claims 1-13, wherein one or more linker groups, independently for each occurrence, represents a hydrocarbylene group wherein one or more methylene groups is optionally replaced by a group Y
20 (provided that none of the Y groups are adjacent to each other), wherein each Y, independently for each occurrence, is selected from, substituted or unsubstituted aryl, heteroaryl, cycloalkyl, heterocycloalkyl, or -O-, C(=X) (wherein X is NR₁, O or S), -OC(O)-, -C(=O)O-, -NR₁-, -NR₁CO-, -C(O)NR₁-, -S(O)_n- (wherein n is 0, 1, or 2), -OC(O)-NR₁-, -NR₁-C(O)-NR₁-, -NR₁-C(NR₁)-NR₁-, and -B(OR₁)_n-; wherein R₁,
25 independently for each occurrence, represents H or a lower alkyl.

18. The compound of any one of claims 1-13, wherein one or more linker groups, independently for each occurrence, represents an amino acid or peptide, or derivative thereof.
19. The compound of any one of claims 1-18, wherein said therapeutic agent is a
5 small molecule, a peptide, a protein or a polymer that has therapeutic activity.
20. The compound of any one of claims 1-18, wherein the therapeutic agent is hydrophobic ($\log P > 0.4, 0.6, 0.8, 1$).
21. The compound of any one of claims 1-18, wherein the therapeutic agent has low aqueous solubility.
- 10 22. The compound of any one of claims 1-21, wherein the therapeutic agent or targeting ligand is covalently-bonded to a photocleavable linker group.
23. The compound of any one of claims 1-22, wherein the the therapeutic agent is covalently-bonded to a photocleavable linker group
24. The compound of claim 23, wherein the photocleavable linker group is
15 selected from a dimethoxy benzoin, a photolabile phosphoramidite, 4-(2-chloropropionyl)phenyl]acetic acid (CPA) or a CPA derivative, a nitroaromatic, a 1-nitrobenzyl, an α -substituted-1-nitrobenzyl, a 3,5-dimethoxybenzyl, a methylnitropiperonyl, a 3-nitrobenzenesulfenyl, a nitrophenyl-ethyleneglycol-based
system, a nitroveratryl, a phenacyl, an alkoxybenzoin, a NpSSMpact system, a
20 pivaloylglycol based system, or a chromium arene.
25. The compound of claim 24, wherein the photocleavable linker group is selected from a photolabile phosphoramidite, a 1-nitrobenzyl, a α -substituted-1-nitrobenzyl, Menpoc, Menp, a methylnitropiperonyl, a nitroveratryl, or NoSSMpact.

26. The compound of any one of claims 1-18, wherein the therapeutic agent is selected from an anti-cancer, anti-fungal, anti-bacterial, anti-mycotic, or anti-viral therapeutic.
27. The compound of any one of claims 1-18, wherein the therapeutic agent is a
5 receptor agonist.
28. The compound of any one of claims 1-27, wherein the compound is biodegradable or bioerodable.
29. The compound of any one of claims 1-28, wherein the compound has a number average (M_n) molecular weight between 1,000 to 500,000 amu.
- 10 30. The compound of any one of claims 1-10, wherein the polymer has a number average (M_n) molecular weight between 5,000 to 200,000 amu.
31. The compound of any one of claims 1-10, wherein the polymer has a number average (M_n) molecular weight between 10,000 to 100,000 amu.
32. A pharmaceutical preparation comprising a pharmaceutical excipient and a
15 compound of any of claims 1-31, or a pharmaceutically acceptable ester, salt, or hydrate thereof.
33. A linear, water-soluble, cyclodextrin-containing polymer, wherein a plurality of bioactive agents are covalently attached to the polymer through attachments that are cleaved under photolytic conditions to release the bioactive agents, wherein
20 administration of the polymer to a patient results in release of the bioactive agent.
34. The polymer of claim 33, wherein the photolytic conditions comprise subjecting the polymer to radiant energy of X-ray, UV, visible, or infrared wavelength.

35. The polymer of any one of claims 33-34, wherein the photolytic conditions comprise subjecting the polymer to radiant energy of UV wavelength.

36. The polymer of any one of claims 33-34, wherein the photolytic conditions comprise subjecting the polymer to radiant energy of visible wavelength.

5 37. The polymer of any one of claims 33-34, wherein the photolytic conditions comprise subjecting the polymer to radiant energy of infrared wavelength.

38. The polymer of any one of claims 33-34 or 37, wherein the photolytic conditions comprise subjecting the polymer to radiant energy of near infrared wavelength.

10 39. The compound of any one of claims 1-18, wherein therapeutic agent is selected from anorexics, antiarthritics, antiasthmatic agents, anticonvulsants, antidepressants; antihistamines, anti-inflammatory agents, antinauseants, antineoplastics, antipruritics, antipsychotics, antipyretics, antispasmodics, cardiovascular preparations, antihypertensives, diuretics, vasodilators, central
15 nervous system stimulants, cough and cold preparations, decongestants, diagnostics, hormones, bone growth stimulants and bone resorption inhibitors, immunosuppressives, muscle relaxants, psychostimulants, sedatives, tranquilizers, anti-inflammatory agents, anti-epileptics, anesthetics, hypnotics, sedatives, neuroleptic agents, antidepressants, anxiolytics, anticonvulsant agents, neuron
20 blocking agents, anticholinergic and cholinomimetic agents, antimuscarinic and muscarinic agents, antiadrenergics, antiarrhythmics, and antihypertensive agents.

40. A method of preparing the compound of any one of claims 1-18.

25

9936944v1

FIG. 1

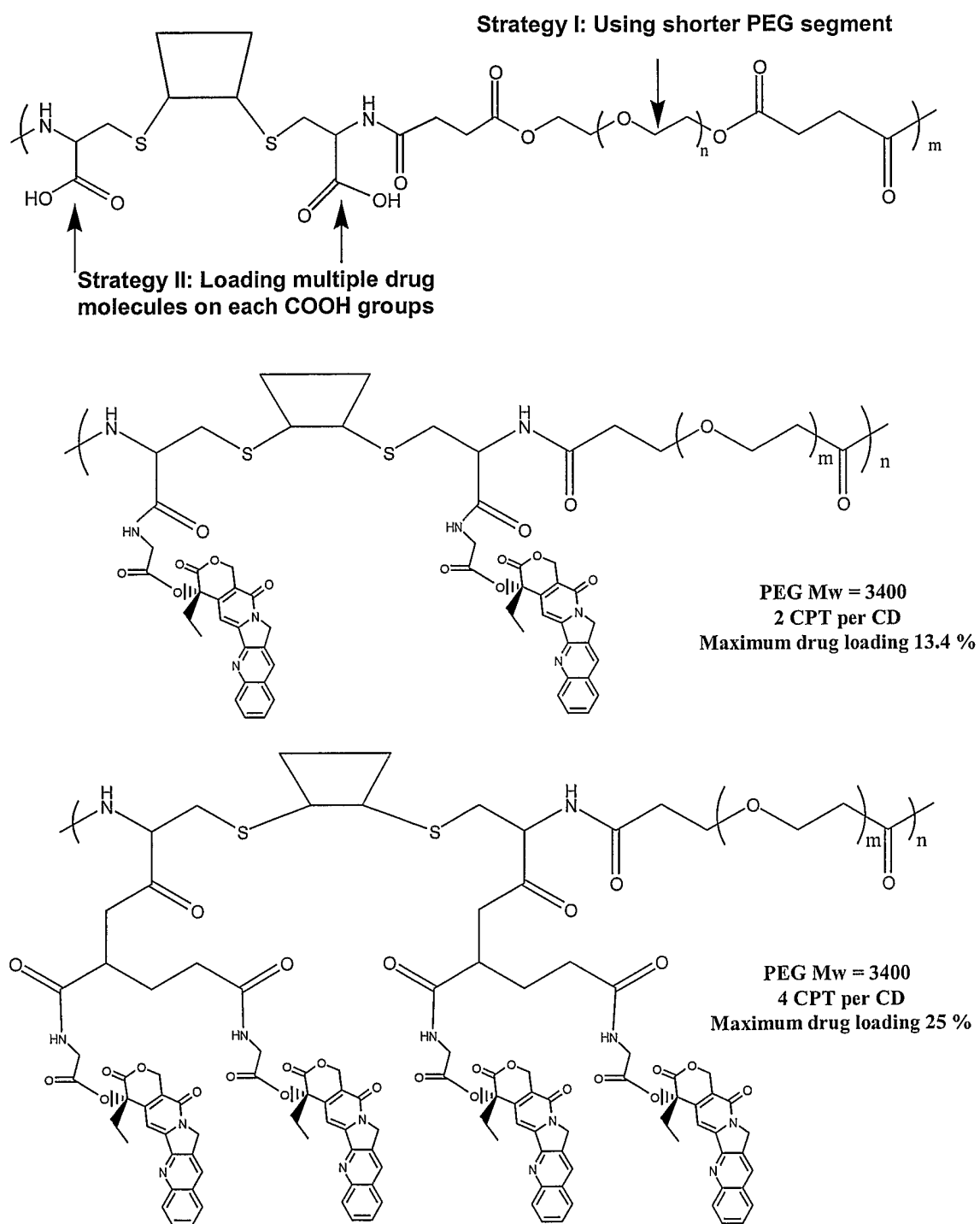


FIG. 2

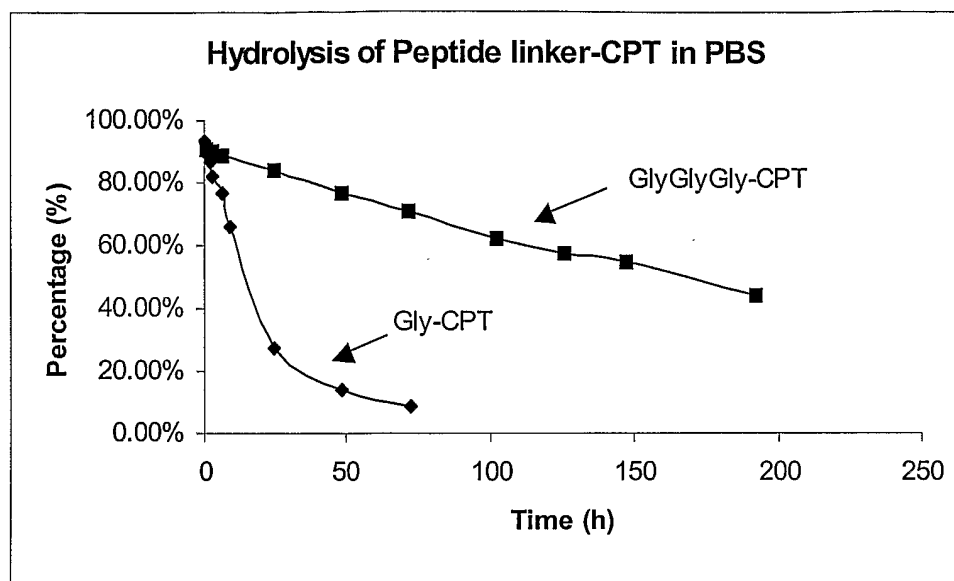


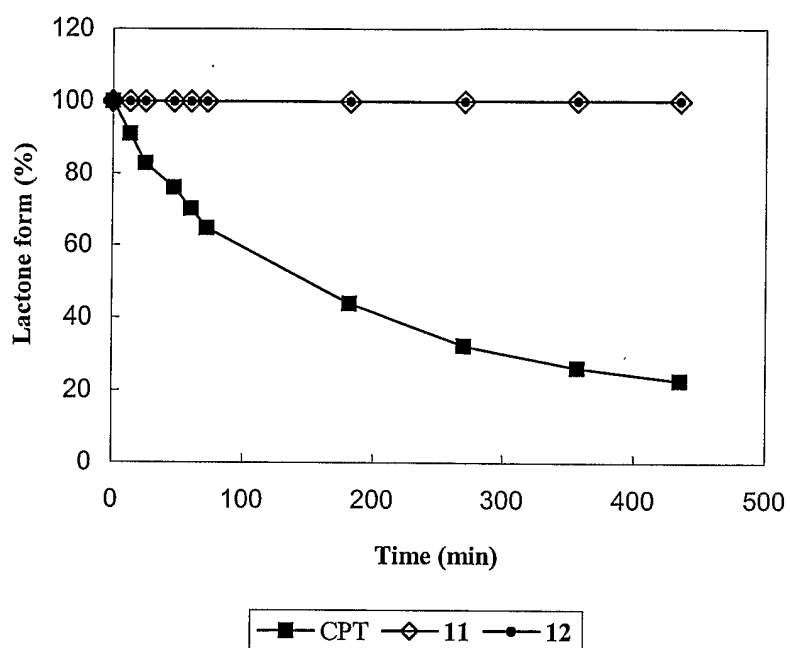
FIG. 3: Lactone Ring Stability of CPT, 11 and 12 in PBS (pH 7.4)

FIG. 4

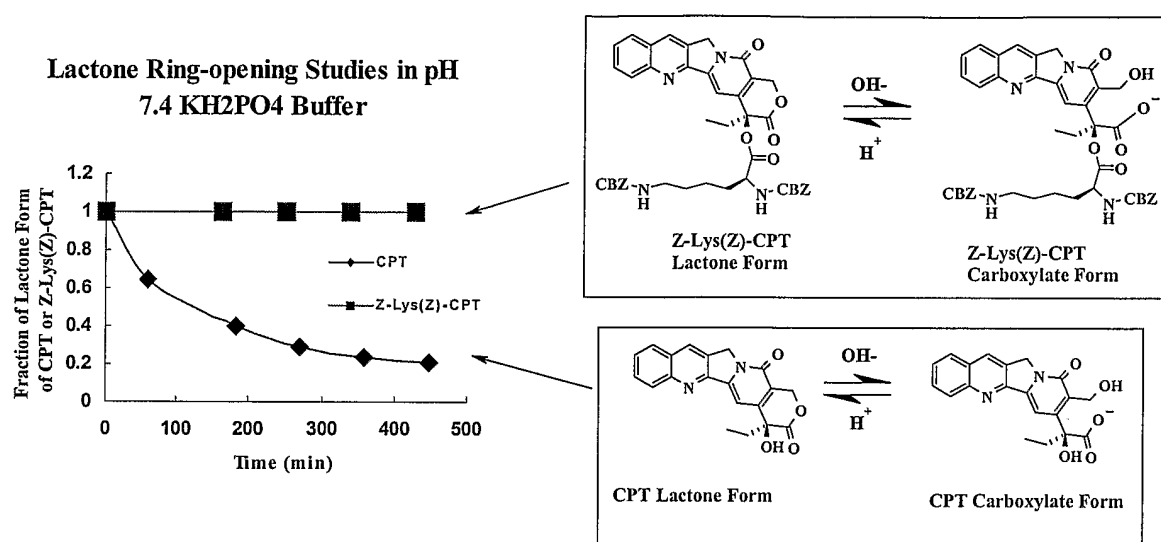


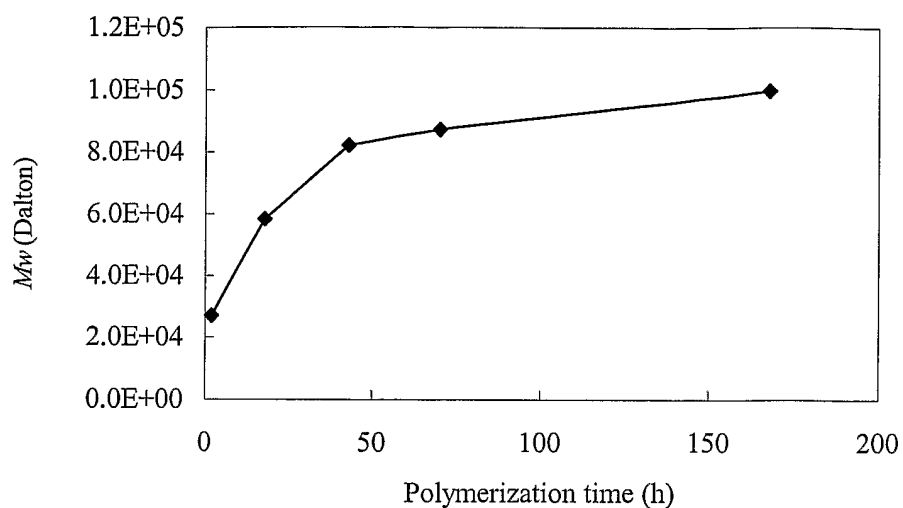
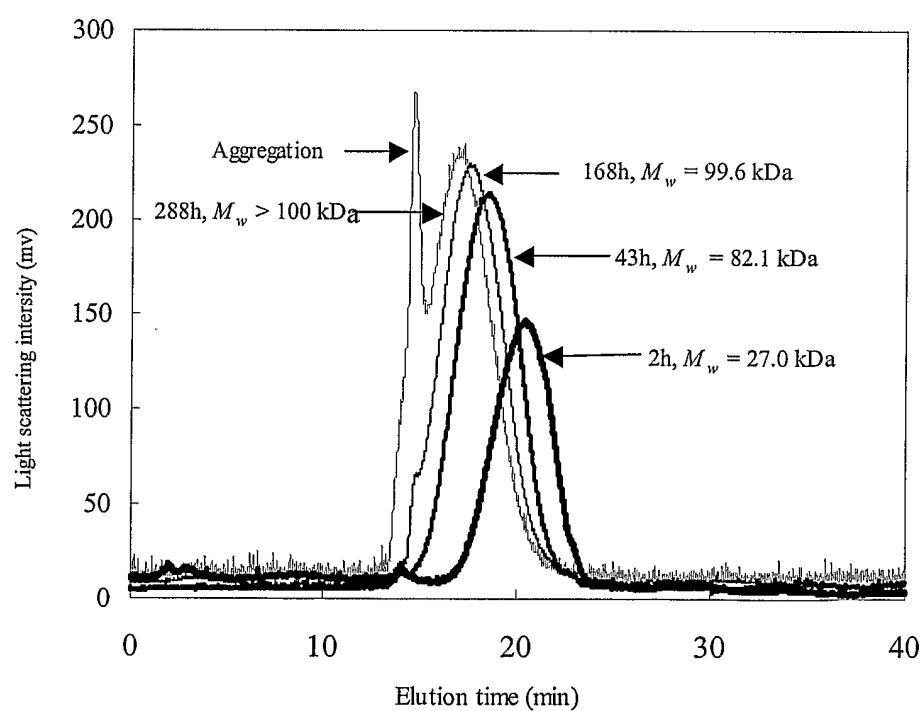
FIG. 5a: Polymerization control by adjusting polymerization time.**FIG. 5b:** Polymerization control by adjusting polymerization time.

FIG. 6: CPT release from **HG6** and **HGGG6** at 37 °C after 24 h in buffer solutions with pHs ranging from 1.1 to 13.1

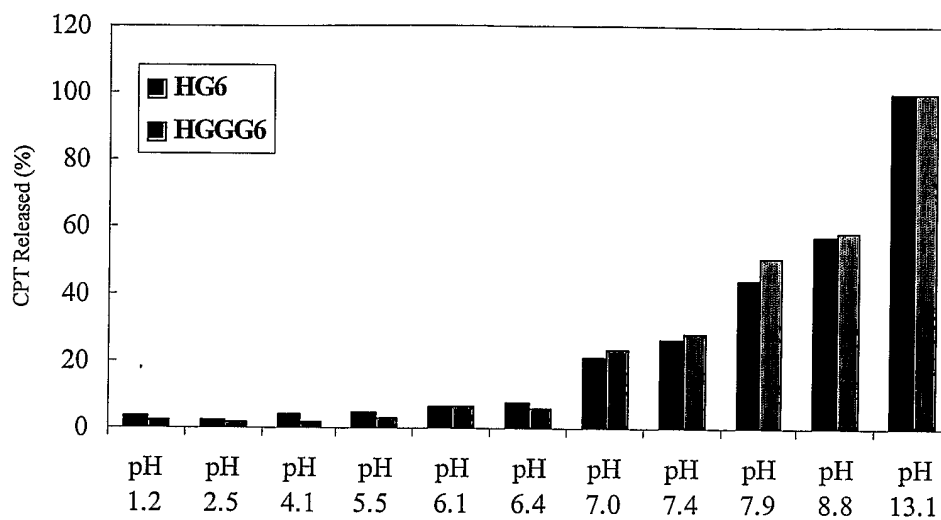


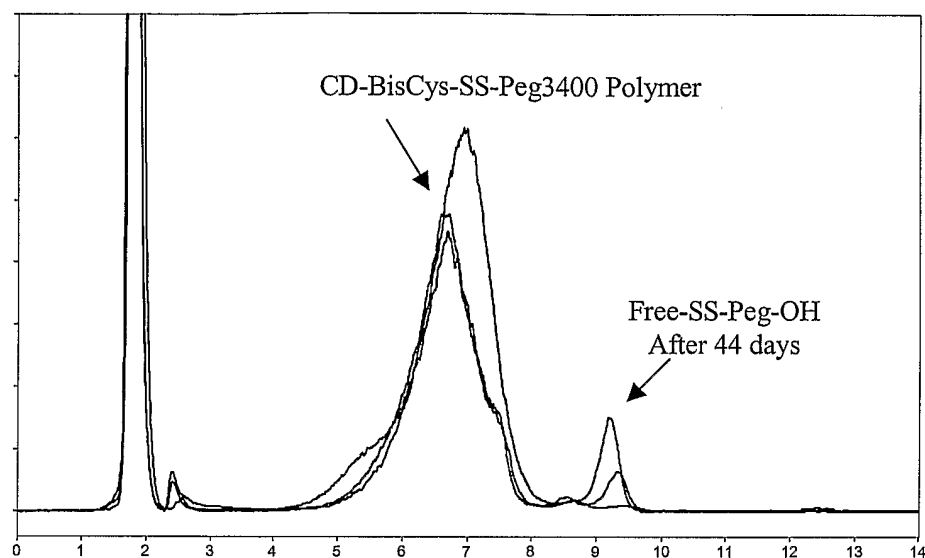
FIG. 7: HPLC analysis of degradation of CD-BisCys-SS-Peg3400 Polymer

FIG. 8: The tumor growth curve as a function of time for the D5W, CPT, irinotecan, LGGG10 at its highest non-toxic dose tested (18 mg CPT/kg), and the other three conjugates with high MW polymer (HGGG6, HG6, HGGG10) at their MTDs.

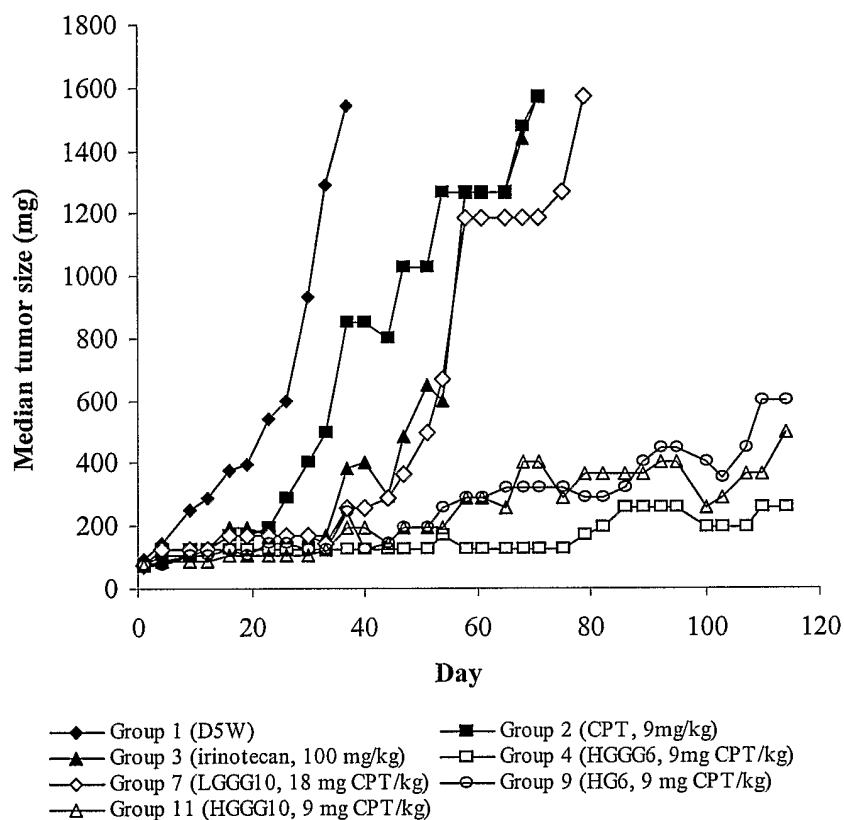


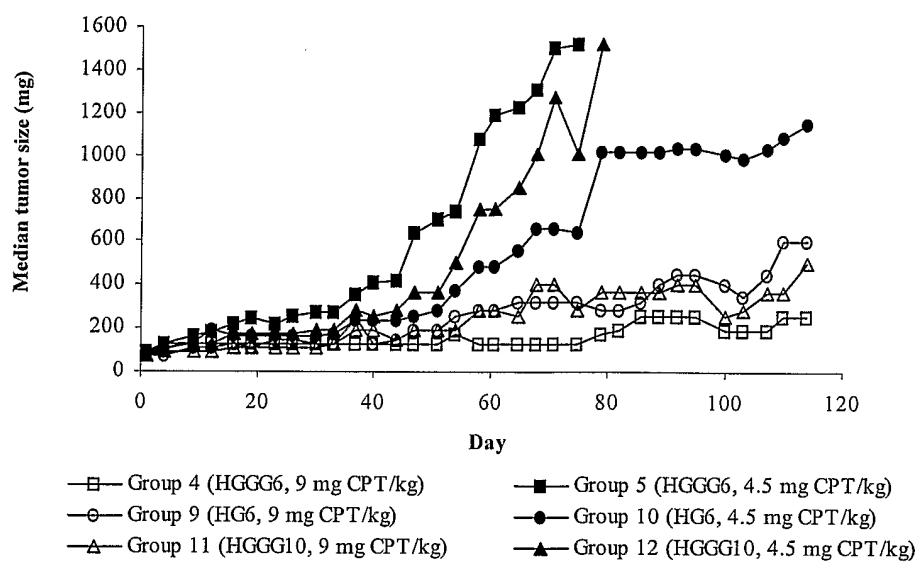
FIG. 9. The median tumor growth curves for **HGGG6**, **HG6** and **HGGG10**

FIG. 10: The medium tumor growth curves for LGGG10 and HGGG10 each dosed at 9 mg CPT/kg

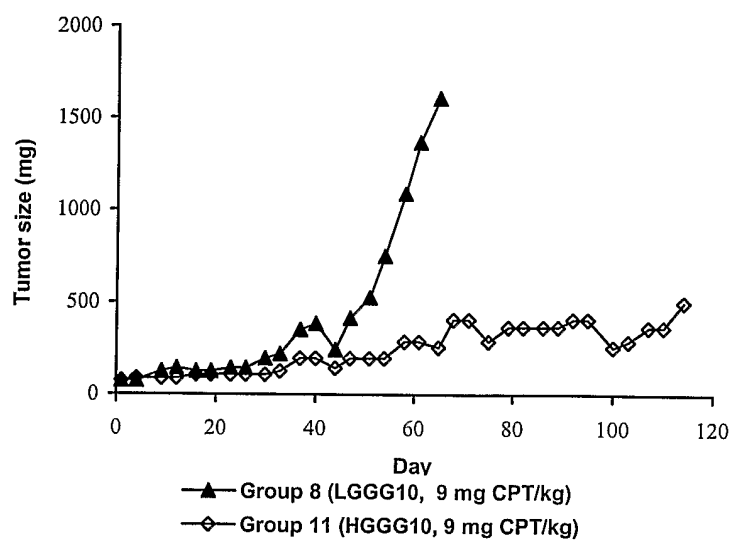


FIG. 11: Mean body weight (MBW) losses as a function of time plotted for D5W, CPT, irinotecan and the three conjugates containing high MW polymer at their MTDs

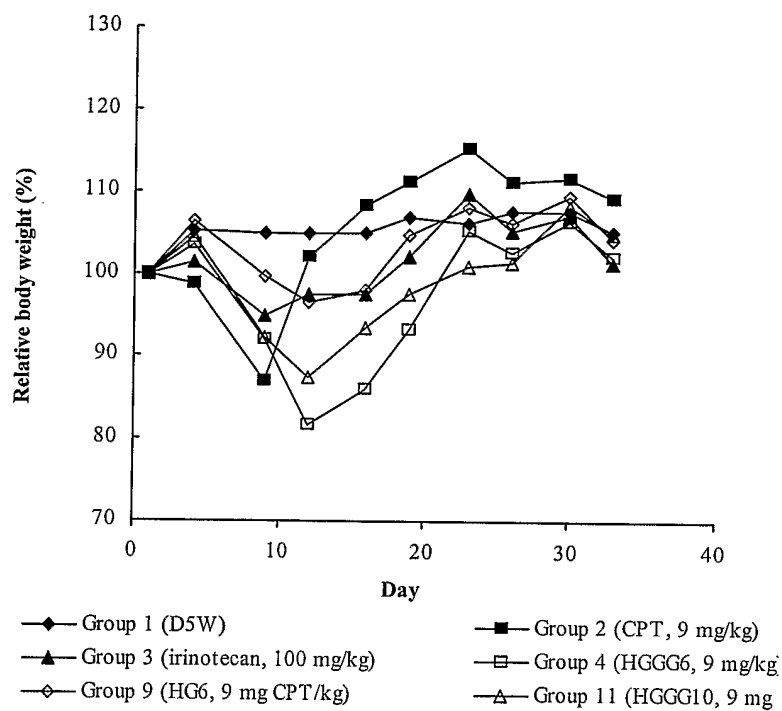


FIG. 12. The correlation of CPT concentration (ng/mg tissue) to tumor size (in mg)

