affinity for cell walls at the target site. However, PEG lacks functional groups except at the ends of the polymer chains, and the majority of the terminal groups are inevitably taken up by bonds to the other block copolymer component. For this reason, attachment of targeting moieties such as antibodies or cell-adhesion molecules to PEG block copolymers is generally limited to the non-PEG block, which unfortunately is not the part of the copolymer that is normally exposed in the corona of the self-assembled aggregate.

The phase separation phenomenon which results in the self-assembly of block copolymers into polymer aggregates is readily reversible, and attempts have been made to increase the stability of the aggregates by cross-linking the hydrophobic core (see European Patent No. EP 0552802). Covalent attachment of the drug to the hydrophobic component of a block copolymer has also been attempted (Park and Yoo, U.S. Patent No. 6,623,729; European Patent No. EP 0397307).

There remains a need for a drug delivery system that is stable, biocompatible, amenable to the attachment of targeting moieties to the exterior of the aggregates, and efficient at delivering drugs to the desired cellular targets.

SUMMARY ~~OF THE INVENTION~~

~~The present invention provides b~~Biocompatible comb-type polymer molecules, comprising a hydrophilic backbone having branch-point moieties, and hydrophobic branches attached at these branch-point moieties are disclosed herein. ~~The invention provides a~~Aqueous suspensions of polymer aggregates formed from such polymers, and ~~provides~~ methods for solubilizing insoluble or sparingly-soluble organic compounds, such as drugs, dyes, and vitamins~~, and the like~~, by incorporating such compounds in the hydrophobic cores of the polymer aggregates, are also disclosed. The method for solubilizing a water-insoluble organic species in an aqueous solvent basically comprises contacting the water-insoluble organic species with a polymer ~~of the invention~~ disclosed herein in an aqueous or mixed-aqueous solvent.

In particular embodiments, the branch point moieties further comprise reactive functional groups capable of serving as attachment points for targeting moieties. In particularly preferred embodiments, targeting moieties such as ligands or antibodies are covalently attached

to the branch-point moieties of the polymers ~~of the invention~~ disclosed herein, and a drug is incorporated into the core of the aggregates, so as to form a targeted drug complex.

~~The invention further provides m~~Methods for the preparation of the comb-type polymers, aggregates, and targeted drug complexes described herein are also disclosed. The polymers ~~of the invention~~ disclosed herein self-assemble into polymer aggregates that efficiently solubilize, distribute, and deliver drugs in vivo, are non-toxic and biocompatible, are stable, and are capable of bearing multiple cell targeting moieties on their exterior surfaces.

BRIEF DESCRIPTION OF THE DRAWINGS

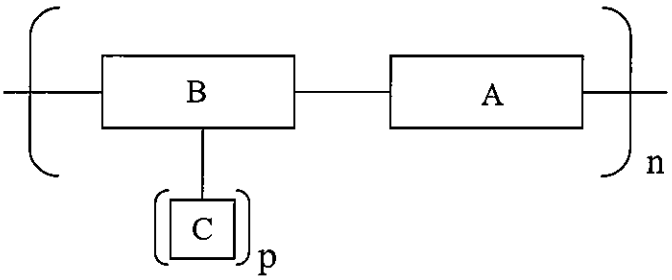
Fig. 1 shows 20 ul samples of saturated solutions of three lipophilic dyes (A, Sudan IV; B, dichlorofluorescein; C, spirit soluble Eosin Y) in deionized water, spotted onto a silica gel TLC plate. Upper row: with 50 mg/ml of the π-polymer of Example 1; lower row: without π-polymer.

Fig. 2 shows 50 ul samples of saturated solutions of four insoluble drugs (row 1, Purpurin; row 2, Camptothecin; row 3, Amphotericin B; row 4, Doxorubicin) in deionized water, spotted onto a silica get TLC plate. Column A, folated Polymer A; column B, Polymer A, column C, no polymer. Three pencil marks around each spot indicate the extent of spread of solvent on the plate. The central circles in columns A and B are uniformly dispersed into the silica, indicating clear solutions; the central circles in column C consist largely of solid deposits.

DETAILED DESCRIPTION ~~OF THE INVENTION~~

The polymers ~~of the invention~~disclosed herein, referred to herein as "π-polymers", have a comb-type architecture, with a backbone formed of alternating branch-point moieties B and hydrophilic, water-soluble polymer blocks A; and having a plurality of hydrophobic side chains C attached to each branch-point moiety. They consist essentially of the structure shown in Formula **1**. The side chains C are relatively short, hydrophobic moieties, which may be aliphatic molecules, chains or oligomers. The value of p is ideally an integer, either 2, 3, or 4. In practice the side chains are most often introduced with less-than-perfect efficiency via chemical reactions, resulting in an average value of p for the polymer preparation as a whole that is not the intended

integer. Non-integer average values can also be obtained by design, as discussed below. Thus, the average value of p in the polymers ~~of the invention~~ disclosed herein is greater than one and may be as high as four ($1 < p \leq 4$). In preferred embodiments, P ranges from about 2 to 4, and most preferably $1.5 < p \leq 2$.



1

The backbone polymer block A is ~~selected from~~ hydrophilic and/or water-soluble ~~polymer chains, including but not limited to~~, wherein A is selected from the group consisting of poly(ethylene glycol), poly(propylene glycol), poly(ethylene imine), poly(vinyl alcohol), poly(vinylpyrrolidone), polysaccharides, and ~~the like~~ co-polymers thereof. Preferably, the polymer units A are poly(ethylene glycol) chains of formula $-(CH_2CH_2O)_m-$ where m is between 1 and 10,000, preferably between 3 and 3,000. The terminal functional groups of the polymer chains have not been characterized, and are not relevant to the invention.

In the manufacture of poly(ethylene glycol) of various grades, it is known in the industry to couple a divalent linker moiety (*e.g.*, bisphenol A diglycidyl ether) to two poly(ethylene glycol) chains, effectively doubling the molecular weight of the polymer while retaining a relatively narrow molecular weight range. The resulting "poly(ethylene glycol)" molecules are consequently interrupted at the midpoint of the polymer chain by the non-glycol linker moiety (see, *e.g.*, the poly(ethylene glycol)-bisphenol A diglycidyl ether adduct, CAS registry No. 37225-26-6). Higher oligomers, *i.e.* those having three PEG chains separated by two bisphenol A diglycidyl ether moieties, are also known, see for example international patent application WO 00/24008. As used herein, therefore, the terms "poly(ethylene glycol)" and "poly(propylene glycol)" encompass poly(ethylene glycol) and poly(propylene glycol) polymer chains that incorporate non-glycol linker units, including but not limited to bisphenol A diglycidyl ether, bisphenol B diglycidyl ether, bisphenol S diglycidyl ether, hydroquinone

diglycidyl ether, and the like. For purposes of this specification, any such linker moieties are not counted as "monomer units".

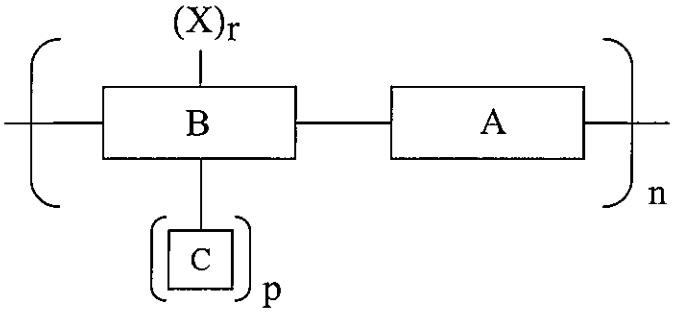
The polymer block A most preferably has an average length of between twenty and fifty monomer units. The polyethylene glycol chains may be end-substituted with functional groups suitable for use as linkers to other moieties, including but not limited to amino, mercapto, acrylate, acrylamide, maleate, and maleimide~~, and the like~~, at one or both ends. The value of n ranges from ~~1 to 1000 and is preferably between~~ 3 ~~and~~ to about 100. The overall molecular weight of the π-polymer may range from 1000 to 100,000 daltons or more; it is preferably above 2,000 daltons, and more preferably above 7,000 daltons.

Hydrophobic moieties C may be the same or different, and are independently selected from the group consisting of ~~may be for example~~ linear hydrocarbons (optionally substituted with one or more hydrophilic substituents), polycyclic hydrocarbons (optionally substituted with one or more hydrophilic substituents), hydrophobic amino acids, peptides and polymers. Suitable hydrophilic substituents include, but are not limited to, hydroxyl, ether, cyano, and amide functional groups. Specifically contemplated are C_8 to C_{20} alkyl groups bearing ω-hydroxy, ω-cyano, ω-amido, or ω-alkoxy substituents. In this context, the term "substituent" includes the substitution of a heteroatom, such as O, N, or S, for a carbon atom in the hydrocarbon chain or ring system of the moiety C. Thus, ether and amide linkages, and heterocyclic rings, may be incorporated into C.

Hydrophobic moieties C are preferably relatively short (C_8-C_{20}) aliphatic chains, but may also be short oligomers. Suitable oligomers include oligo hydroxy acids such as poly(glycolic acid), poly(DL-lactic acid), poly(L-lactic acid), and copolymers of poly(glycolic acid) and poly(lactic acid)hydroxy acids, and poly(amino acids), poly(anhydrides), poly(orthoesters), and poly(phosphoesters), polylactones such as poly(epsilon-caprolactone) poly(delta-valerolactone) poly(gamma-butyrolactone) and poly(beta-hydroxybutyrate). C moieties may also be selected from hydrophobic molecules, such as cholesterol, cholic acid, lithocholic acid, and hydrophobic peptides~~, and the like~~. The molecular weight of each moiety C is greater than 40, preferably between 50 and 1,000, and most preferably between 100 and 500. In general, any moiety C which is not appreciably soluble in water when in the molecular form C-H is thought to be suitable for use ~~in the present invention~~.

It is a distinguishing feature of the comb polymers ~~of this invention~~ disclosed herein that the side chains C are not regularly and uniformly distributed along the polymer chain, but rather occur in clusters $[C]_p$. These clusters are spaced more or less regularly along the polymer chain, depending on the degree of monodispersity of the polymer units A. Thus, the distance between two side chains C attached to a common branching moiety B is different from the distance between two side chains attached to different branching moieties.

In ~~a second~~ another embodiment ~~of the invention~~, the branch-point moieties B further comprise one or more reactive functional groups X, and the polymers consist essentially of the structure -shown in Formula **2**.



2

In Formula **2**, the individual reactive groups X may be the same or may be different from one another, and may optionally be blocked or protected as may be necessary during assembly of the polymer **2.** The average value of r will range from 0 (no X groups) to about 4. Typically, the reactive groups will be selected from functional groups known in the art to be useful for forming covalent linkages between molecular species. The groups X serve as attachment points for drug molecules, tissue- or cell-targeting moieties, virus-targeting moieties, or matrix attachment moieties (such as for the purpose of coating the surface of a stent or other medical device). In certain embodiments, there may be a single attachment point X. In other embodiments, there may be three or four different types of reactive groups. The matrix attachment moiety may attach to a matrix via covalent bonds, specific non-covalent interactions (e.g., antibody-antigen, or non-specific interactions (e.g., via ionic pairing or "hydrophobic" interaction). Suitable reactive groups X include but are not limited to -OH, $-NH_2$, -SH, -CHO, -

$NHNH_2$, -COOH, $-CONHNH_2$, haloacyl, acetoacetyl, -CN, -OCN, -SCN, -NCO, and -NCS~~, and the like~~; reactive double bonds such as vinylic, acrylic, allylic, maleic, and cinnamic, ~~and the like,~~ and groups with reactive triple bonds such as acetylenecarboxy and acetylenecarboxamido (suitable for Michael additions, Diels-Alder reactions, and free radical addition reactions).

Exemplary cell-targeting moieties include but are not limited to receptor-specific ligands, antibodies, and other targeting moieties, such as peptides possessing an Arginine-Glycine-Aspartic acid (RGD) amino acid sequence or a Tyrosine-Isoleucine-Serine-Arginine-Glycine (YISRG) motif; growth factors including epidermal growth factor, vascular endothelial growth factor and fibroblast growth factor; viral surface ligands such as sialic acid and N-acetylneuraminic acid derivatives; cell receptor ligands such as folate, methotrexate, pteroic acid, estradiol, estratriol, ~~testosterone~~ testosterone, and other hormones; mannose-6-phosphate, sugars, vitamins, and tryptophan~~, and the like~~. Antibodies are preferably monoclonal antibodies directed at cell-specific surface antigens; suitable targeting moieties include not only complete antibodies but also antibody fragments containing the active antigen-binding sequences, such as Fab'2 fragments, Fab' fragments, or short chain peptide analogues of the active antigen binding sequences of such antibodies.

Examples of virus-targeting moieties include small molecule ligands that bind to a virus, such as aminoalkyladamantanes, Fuzeon™ (enfuvirtide), PRO-542, BMS-488043, sialic acid, 2-deoxy-2,3-didehydro-N-acetylneuraminic acid, 4-guanidino-Neu5Ac2en (zanamivir), oseltamivir, and RWJ-270201~~, and the like~~; oligopeptides, oligosaccharides, and glycopeptides that bind to viral surfaces, and antibodies and antibody fragments directed at virus-specific surface antigens. In preferred embodiments, ~~the present invention provides~~ π-polymers bearing ligands for viral neuraminidase or hemagglutinin are provided. It is well-established that such polymers have antiviral properties in their own right; see for example T. Masuda et al., *Chemical & Pharmaceutical Bulletin* **51**:1386-98 (2003); M. Itoh et al., *Virology* **212**:340-7 (1995), and Reece et al., U.S. Patent No. 6,680,054 (2004). The hydrophobic cores of the antiviral polymers and polymer aggregates ~~of the present invention~~ disclosed herein may optionally be loaded with one or more conventional antiviral drugs, which are advantageously released in the vicinity of the viral particle.

Other attachment groups of medical relevance may be small chemicals, peptides, antibodies or antibody fragments, enzymes, or active pharmaceutical ingredients, that may affect biological processes such as hormones or hormone agonists or antagonists, substances that interfere with virus binding, and substances that interfere with cell cycle or cellular processes after intracellular entry~~, and the like~~. Cells of unicellular and multicellular organisms, including bacteria, fungi, higher animals, and plants, may be targeted. Biotin may be attached to the π-polymer and used as an attachment point for avidin- and streptavidin-coupled proteins, peptides, and other targeting or pharmacologically active agents, such as antibodies, growth hormones, and imaging reagents~~, and the like.~~

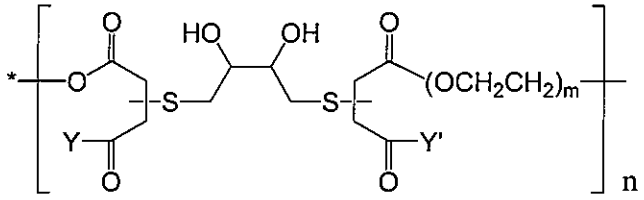
"Matrix" refers to organic or inorganic materials, surfaces, and deposits, such as glass, silica or metal surfaces, extracellular matrix, protein deposits such as amyloid plaques of various kinds, cell surface, virus surface, and general homogeneous or heterogeneous surfaces that may or may not be well characterized, including prions.

Examples of glass or silica matrix attachment moieties include various halosilanes, alkoxysilanes, acylsilanes, as well as chemicals exhibiting such functional groups including polymers. Other attachment groups can be devised based on the particular physico-chemical characteristics of the matrix. Suitable attachment moieties, for example those used in the coating of stents, are known to those skilled in the art.

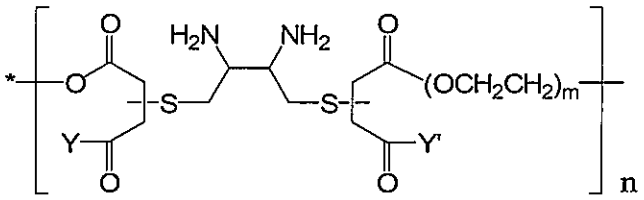
~~In a third aspect of the invention~~In embodiments disclosed herein, the branch point moieties B are connected to other branch point moieties elsewhere in the polymer chain, so as to form a crosslinked hydrogel structure. Such crosslinking may be effected by reacting the polymer with multifunctional moieties that contain homofunctional or heterofunctional groups, at least one of which reacts with X or a reactive group on C located on a first branch point moiety, and at least one of which reacts with X or with a reactive functional group present on C at a second branch point moiety. Cross-linking may also be made via a link to the terminal functional groups of the polymer chain A. Such crosslinked polymers may optionally contain reactive functional groups suitable for attachment of drug molecules or targeting moieties.

The branch-point moiety B is typically derived from a multifunctional molecule having a plurality of reactive groups, two of which are suitable for attachment to the hydrophilic polymer unit A, and two of which are suitable for attachment of the hydrophobic moieties C. Moiety B may optionally have additional reactive groups X as described above.

Particularly preferred branch-point moieties are the conjugates of dithiothreitol (DTT), dithioerythritol (DTE), or 2,3-diaminobutane-1,4-dithiol with two molecules of maleic acid. The combination of this branch-point moiety with polyethylene glycol as the moiety A generates the polymer backbone of Formulas **3** and **3a**



3



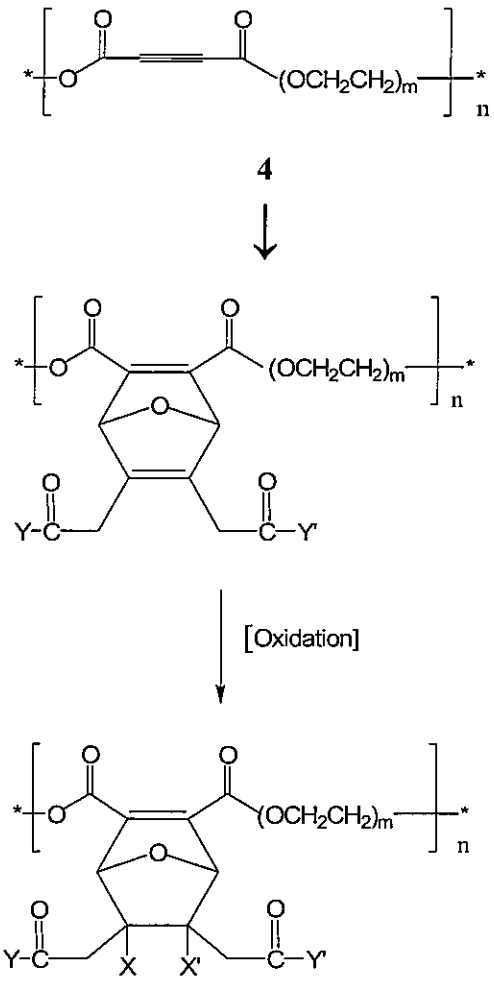
3a

wherein Y and Y' may be the same or different, and are preferably selected from OH, NH_2, ONH_2, NHOH, and $NHNH_2$. In a preferred embodiment, the hydroxyl or amino groups of the dithiol are the reactive groups X, serving as attachment points for targeting or drug moieties, while the functional groups Y and Y' serve as attachment points for C moieties. Alternatively, the groups Y and Y' may serve as attachment points, while the hydroxyl or amino groups are used to attach the C moieties.

Formulas **3** and **3a** are intended to convey that each sulfur atom may independently be attached alpha or beta to a PEG ester carbonyl group. ~~The invention encompasses s~~Single isomer

compositions as well as mixtures of regioisomers at one or both C-S bonds are encompassed. Furthermore, due to the four asymmetric carbons in Formula 1, ~~the invention encompasses~~ all chiral, meso, and diastereomeric isomers and mixtures thereof are encompassed.

The Diels-Alder adduct of acetylene dicarboxylic acid and a furan may also serve as a suitable branch point moiety. For example, the polyester **4** derived from PEG and acetylenedicarboxylic acid is known to undergo Diels-Alder reactions with furans (M. Delerba *et al., Macromol. Rapid Commun.* **18**(8):723-728 (1997)).



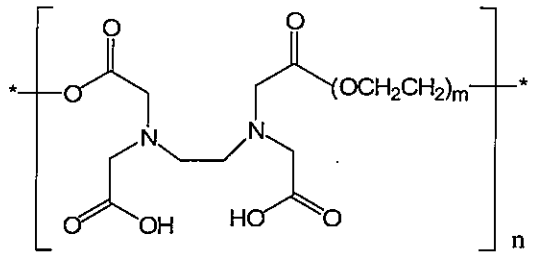
4

5

Scheme 1

Thus, it may be subjected to a Diels-Alder reaction with a 3,4-disubstituted furan to generate a species such as **5**, and polymer **5** can be modified by hydroxylation or epoxidation to provide reactive groups (e.g., X and X' in Scheme 1).

Similarly, reaction of PEG with ethylenediamine tetraacetic acid dianhydride will provide a polyester of formula **6**:

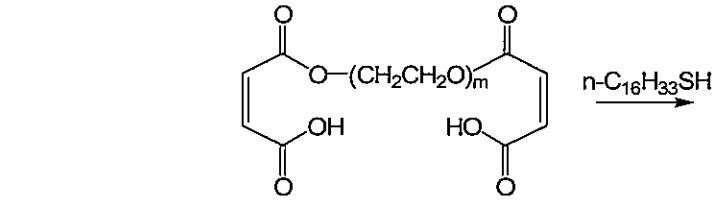


6

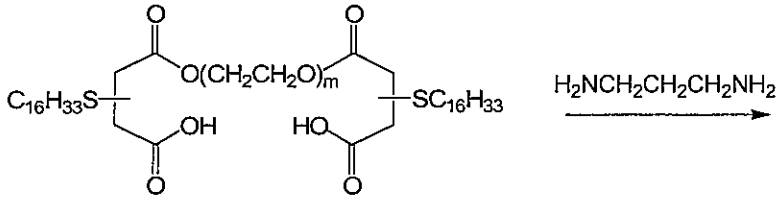
Other suitable branch point moieties may be derived from tartaric acid, acetylenedicarboxylic acid, nitrilotriacetic acid, 3,4,3',4'-diphenyl sulfone tetracarboxylic acid dianhydride, 3,4,3',4'-diphenyl ether tetracarboxylic acid dianhydride, pyromellitic dianhydride, alkanedithiols such as 1,2-ethanedithiol and 1,4-butanedithiol, bis(2-mercaptoethyl)ether, 2-mercaptoethylsulfide, dimercaptopropanol, dimercaptopurine, dimercaptothiadiazole, dimercaptosuccinic acid, benzenedimethanethiols, benzenedithiols, dihalogenated benzenedimethanethiols, and dihalogenated 4,4'-thiobisbenzenethiol~~, and the like~~.

Where Y and Y' are OH, hydrophobic groups C may be linked to the polymer by amidation or esterification of the carboxylic acid groups. The hydrophobic groups C are preferably relatively small (C_8-C_{20}) and predominantly hydrocarbon moieties, and may be linear or branched or contain one or more rings. Examples include but are not limited to covalently attached dodecylamine, pentadecylamine, cholesterol, and cholic acid moieties. Although the polymers ~~of the invention~~ disclosed herein are represented, for convenience, as having at most two different hydrophobic side chains, is should be understood that mixtures of two or more hydrophobic compounds may be used to introduce a variety of hydrophobic side chains into a particular polymer.

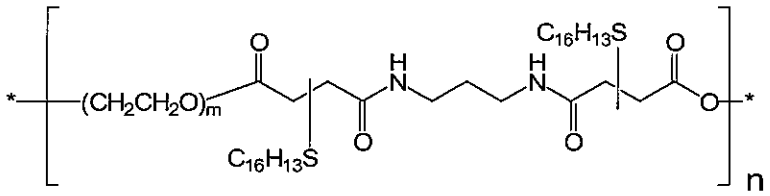
existence at the time of polymerization (Scheme 5).



7



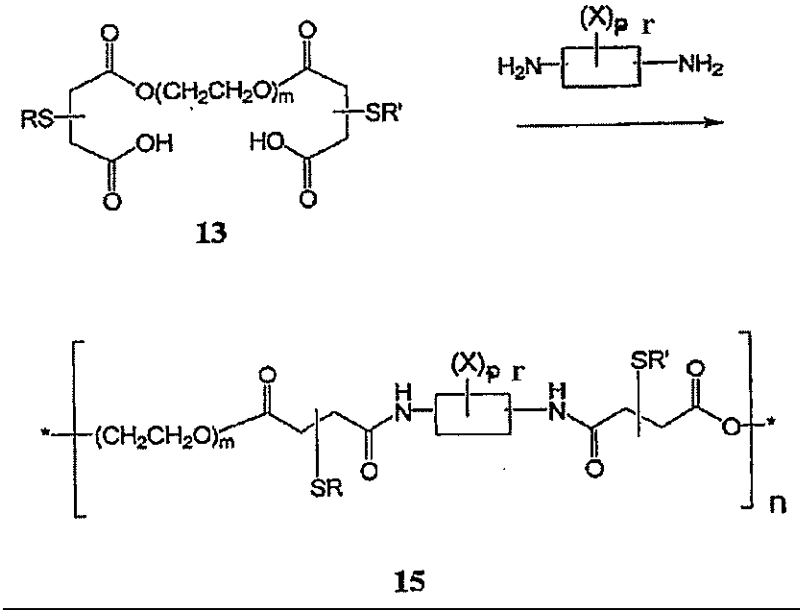
13



14

Scheme 5

In addition to simple diamines such as 1,3-diaminopropane, as shown in Scheme 5, diamines having (optionally masked) reactive functional groups X may be employed, leading to polymers **15** suitable for attachment of targeting moieties (Scheme 6). In the formulae below, ~~p~~ r may range from 0-4, and each X is independently the same or different from any other group X that may be present. A reactive group X need not be pendant, but may for example be an NH group within the chain of atoms that makes up the diamine, as in the monomer H_2N-$(CH_2)_3$-NH-$(CH_2)_3$-NH_2.



Scheme 6

Certain of the π-polymers prepared as above possess reactive groups X suitable for further derivatization, to attach targeting moieties such as small molecules, peptides, nucleotides, sugars, antibodies, etc., or to effect crosslinking of the polymer chains via bifunctional or multifunctional crosslinking agents. In particular embodiments, partial derivatization of the reactive groups on the polymer chain is carried out to generate π-polymers having a variety of different reactive groups, which permits attachment of a variety of targeting and drug moieties to a single polymer chain. Thus, addition of a sub-stoichiometric amount of acryloyl chloride (or maleic anhydride) to the π-polymer of Example 1 will provide a polymer with both acryloyl (or maleyl) groups and residual hydroxyl groups. Subsequent Michael addition of a sub-stoichiometric amount of a mercapto-carboxylic acid, for example HS-$(CH_2)_3$-COOH, would provide a polymer with hydroxyl, acryloyl, and carboxyl groups. Addition of cysteine introduces amino and carboxyl groups, in addition to any residual reactive groups left behind by sub-stoichiometric amounts of reagents.

Another approach to poly-functional π-polymers involves the deliberate omission of a fraction of the hydrophobic chains C. The π-polymer of Example 1, for example, can be

By performing partial reactions, one can introduce additional reactive functional groups to a polymer ~~of the invention~~disclosed herein, including but not limited to (1) thiol-reactive groups such as acrylic or maleic acid derivatives, (2) carboxylic-acid reactive groups such as amino or hydroxyl, (3) amine-reactive groups such as carboxyl, and (4) disulfide-reactive groups such as mercapto. The number of such added functional groups per polymer molecule may range from 1/r up to several multiples of r, depending on the reagent used and the quantity used.

Alternatively, two or more specific ligands can be attached to improve specificity of binding to say, a virus, or cell surface. Two or more specific ligands can also be used so as to cause an interaction between different cellular targets, for example, one ligand may target a virus particle, and another ligand may facilitate binding to a phagocyte, thereby bringing virus particle into proximity or contact with the phagocyte and promoting phagocytosis.

Such derivatization allows the attachment of three or more distinct targeting and/or therapeutic moieties to the polymer, through different functional group attachments (such as amine, carboxylate, and thiol). Thus, one may attach a tissue-specific targeting agent, an imaging agent, and a therapeutic agent to a single polymer chain, and subsequent self-assembly of the polymer will yield a targeted therapeutic whose distribution and efficiency of targeting can be monitored.

Attachment of ligands to the repeating units of the polymers ~~of the invention~~ disclosed herein affords multivalent display of the ligand on the polymer chain and on the nanoparticles surface. Multivalent display often leads to great increases in affinity for the target. For example, multivalent antibodies can be far more effective in clearance of their targets than the normal divalent antibodies. Carbohydrate-binding proteins and carbohydrates are known to be multivalent in nature, and ineffective if monovalent. Similarly, multivalent peptide and carbohydrate targeting moieties will be far more effective than the monomer alone. The increase in MW due to attachment to the polymer results in reduced renal clearance rates of peptides and other ligands. In addition, the PEG backbone affords to the peptide benefits similar to those of PEGylation, including evasion of immune surveillance.

Further, a multivalent targeting moiety will decorate a multivalent target (say, a virus particle) and neutralize it far more effectively than the monomeric targeting moiety. The ability to display multiple (different) peptides in multivalent format will lead to enhanced specificity. For example, a truly HIV-specific (HIV virus-binding) polymer can be built by attaching a peptide corresponding to the CD4 binding region, and another peptide corresponding to the CCR-5 or CXCR-4 binding region of the virus, and possibly a third peptide corresponding to the other receptor (CXCR-4 or CCR-5 respectively). Such a polymer could completely mask the virus's binding regions and render the virus unable to attach to cells and thereby non-infective. In addition, the surfactant properties of the polymer would lead to destabilization of the virus structure itself upon binding. Instead of peptides, small molecules that interfere with the same binding patterns (CD4, CCR-5, CXCR-4) or a mixture of peptides and small molecules, preferably with complementary activities, can be employed. The resulting polymers will render any free virus ineffective, and thus may be ideal for stopping spread of infection, by using them as components of condom lubricants ~~and the like~~. In addition, such polymers may be injected into patients to reduce the HIV burden.

Generally, when a polyfunctional reagent such as DTT is employed, there may be partial cross-linking of polymer chains via esterification of the carboxylic acid with DTT or similar side reactions. Secondary hydroxyl groups in the central region of the PEG chains, e.g. those associated with bisphenol A diglycidyl ether residues, may also contribute to cross-linking if they are present in the PEG starting material. The resulting crosslinked hydrogel structures are also useful materials. For example, by suitably increasing the extent of this crosslinking or by explicit crosslinking using alternative crosslinkers (such as bisoxiranes, for example), materials can be made that are flexible hydrogels which can serve as repository depots for drugs. By suitably modifying the materials (e.g. lower PEG length, greater open carboxylic groups, and incorporation of suitable acrylic groups) either linear or crosslinked hydrogel materials can be made that can serve as repositories that can be supported either immobilized on devices such as stents or absorbed in devices such as pads for adhesive patches or subdermal insertion patches. In general, such crosslinked materials will be suitable for controlled release rather than enhanced, targeted release.

The comb polymers ~~of the invention~~ are useful for solubilizing, in aqueous solvent systems, sparingly water-soluble materials. The method of solubilizing a substance in an aqueous solvent comprises contacting the sparingly-soluble substance with a comb-type polymer ~~of the invention~~ disclosed herein in the presence of water, so as to form a water-soluble complex of the substance and the polymer. Alternatively, the polymer and the substance to be solubilized may be combined in a two-phase aqueous-organic emulsion, and the organic solvent removed by evaporation. An exemplary process is described in U.S. Patent No. 6,838,089~~, incorporated herein by reference~~. It is believed that in most cases, the polymer self-assembles into nanoparticles having the sparingly-soluble substance dissolved among the hydrophobic C chains that coalesce at the core of the particles, while the A blocks form a hydrophilic corona that sufficiently lowers the interfacial free energy to permit an aqueous suspension of the particles to remain stable.

In some cases, the sparingly-soluble substance may not entirely dissolve in the core, but may exist as a solid nanoparticle surrounded by and suspended in the C chains at the core of the particles. For the purposes of this disclosure ~~of the present invention~~, this is a differences of degree, as the practice ~~of the invention~~ does not rely on any particular degree of mixing of the C chains with the sparingly-soluble substance. The substance may in some cases dissolve at the molecular level among the C chains, but in other cases it may exhibit any degree of phase separation from the C-chain environment. In some cases, it can be expected that the system will move from one state to the other as a function of temperature.

The solvating power of the hydrophobic core of the polymer particles can be modified by modifying the hydrophobic C moieties. Suitable modifications include but are not limited to the introduction of one or more hydrophilic substituents, such as hydroxyl, ether, amide, and cyano functional groups, in order to increase the polarity and/or polarizability of the hydrophobic core.

Sparingly-soluble materials that can be rendered soluble by these polymers include fat-soluble vitamins and nutrients, including but not limited to vitamins A, D, E and K, carotenes, cholecalciferol, and coenzyme Q; insoluble drugs such as docetaxel, amphotericin B, nystatin, paclitaxel, doxorubicin, epirubicin, rubitecan, teniposide, etoposide, daunomycin, methotrexate,

mitomycin C, cyclosporine, irinotecan metabolite (SN-38), statins, and steroids; dyes, photodynamic agents, and imaging agents, and nucleic acids, nucleic acid analogues, and nucleic acid complexes. Nucleic acid analogues include species such as thiophosphates and peptide nucleic acids; nucleic acid complexes are ionic complexes of oligonucleic acids with a substantially charge-neutralizing amount of cationic or polycationic species.

For the purposes of this disclosure, a drug that is insoluble at neutral pH is considered "sparingly soluble", because there is in many cases a need for a neutral pharmaceutical composition. For example, ciprofloxacin is reasonably soluble in water at a pH below 4.5, but this pH can be highly irritating when the drug is formulated for ocular administration. A polymer ~~of the present invention~~ disclosed herein will solubilize ciprofloxacin in normal saline at pH 7. Also, for the purposes of this disclosure, "sparingly soluble" should be understood to refer to any substance whose solubility in an aqueous vehicle is such that an increase in solubility would yield an improved or more-useful composition. Thus, a drug that is moderately soluble, e.g. to the extent of 2g/liter, is "sparingly soluble" if a unit dose for intravenous administration is 5 g.

As a result of the ability of the polymers ~~of the invention~~ disclosed herein to solubilize pharmacologically active species, ~~the present invention also provides~~ pharmaceutical compositions, which comprise one or more π-polymers ~~of the invention~~ disclosed herein in combination with a therapeutically effective amount of one or more pharmacologically active agents, are also disclosed. The polymers ~~of the invention~~ disclosed herein can render effective what would otherwise be an ineffective amount of a pharmacologically active agent. For purposes of this disclosure, therefore, a "therapeutically effective amount" is the amount of agent that renders the overall composition effective.

~~All patents, patent applications, and publications mentioned herein are hereby incorporated by reference in their entirety.~~

EXPERIMENTAL

1. General procedures.

~~The invention also provides p~~Processes for the preparation of the comb polymers ~~of the invention~~disclosed herein are also provided. Synthesis of these polymers is readily

carried out by one skilled in the art of organic synthesis, by following the procedures described below. The key starting material is

polyethylene glycol, which is preferably dried before use. This is conveniently done by stirring molten PEG under vacuum at an elevated temperature, until bubbles stop forming. This may take 8-12 hours, depending on the quality of the PEG. Once dried, the PEG can be stored under argon indefinitely. Commercially available industrial and research grades of PEG may be employed in making the polymers ~~of the invention~~ disclosed herein, for example the polydisperse "PEG 1500" of commerce having a molecular weight distribution of 1430 – 1570. Such material may incorporate bisphenol A diglycidyl ether, which introduces secondary hydroxyl groups at the center of the PEG chain. In order to ensure that the polymers ~~of the invention~~ disclosed herein have the most reproducible and consistent properties, the PEG is preferably free of bisphenol A, and of low dispersity. Most preferable are PEG polymers that are >95% monodisperse, such as are commercially available from Nektar Therapeutics (formerly Shearwater Polymers), Huntsville AL, and Polypure AS, Oslo, Norway. An example of a particularly preferred PEG is "PEG-28" from Polypure, which is >95% $HO(CH_2CH_2O)_{28}H$, molecular weight 1252.

All reactions are carried out under an inert atmosphere such as nitrogen or argon, with magnetic or preferably mechanical stirring.

In step A, dry PEG is melted, and maleic anhydride (2 moles per mole of PEG) is added with stirring. The quantity of maleic anhydride should match the number of PEG terminal hydroxyl groups as closely as possible. A shortage of maleic anhydride will result in hydroxyl-terminated polymer chains, whereas an excess of maleic anhydride will consume thiol groups in the next step, leading to premature chain termination and terminal carboxyl groups. The reaction temperature is not critical, and the process can conveniently be carried out at temperatures between 45°C and 100°C. The preferred temperature of the reaction is between 65°C and 90°C. If elevated temperatures are employed, the maleic anhydride tends to sublime, and steps should be taken to see to it that the maleic anhydride remains in solution. Minimizing headspace and submerging the reaction vessel in an oil bath are effective methods.

Depending on the temperature selected, the reaction may be completed in 2 hours or less or can be conducted overnight. The reaction may be monitored by TLC on silica gel plates, and is continued until after the disappearance of the maleic anhydride. Visual contrast, UV, and iodine staining can all be used to examine the TLC plates.

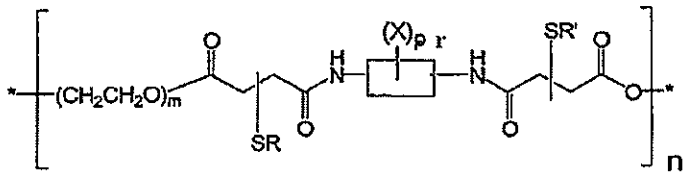
In step B, the crude PEG bis-maleate ester produced in step A is combined with dithiothreitol (DTT) and N,N,N',N'-tetramethylethylenediamine (TEMED) (with added water, if necessary for fluidity), and the mixture stirred at 70°C. The reaction is complete within 30 min, as indicated by the rapid increase in viscosity. The molecular weight of the product will be reduced if more or less than the optimal amount of DTT is employed. The molecular weight of the product can also be reduced, if desired, by replacing TEMED with a less effective tertiary amine base such as TEA.

In step C, sufficient water is added to the reaction mixture to reduce viscosity, and 0.1 mol N-hydroxysuccinimide (NHS) and 1.05 mol hexadecylamine per mol carboxylic acid groups in the polymer are added. (This amount of NHS appears to optimally minimize the extent of side-reactions.) An excess of N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (EDC) (1.4 mol EDC per mol of carboxylic acid groups) is then added in portions, with additional water ~~as~~ added as necessary to maintain stirring. The pH of the reaction mixture is maintained above 7, and preferably between 9- and 11, to optimize the reactivity of the alkylamine. With dodecylamine, this reaction can be conducted at about 40~45°C, whereas with octadecylamine, the temperature is ca. 55°C-57°C. The reaction is followed by TLC until a constant level of left-over alkylamine is observed, typically after running overnight.

The reaction mixture is acidified to a pH from about 3.0 to about 4.5 and stirred at room temperature for about 24 hours to destroy unreacted EDC, then titrated to a pH of 7.0 using 1N NaOH. The final reaction mixture is centrifuged at about 800 xg for 1 to 3 hours, to remove solid contaminants and by-products.

After centrifugation, the supernatant can be chromatographed on a GPC column (Toyopearl(TM), Sephadex(TM), Sephacryl(TM), Biogel(TM), etc.). The π polymers are amphipathic materials, and will exhibit affinity for most GPC column packings, thus complicating the removal of contaminants. Alternatively, the polymer may be chromatographed on a large-pore hydrophobic interaction column (e.g., TOYOPEARL™ Phenyl 650C, Toshoh Biosciences, Montgomeryville, PA, U.S.A.), eluting with a gradient of methanol in water. Preferably, the reaction mixture is dialyzed against several changes of acidified and neutral water to remove low-molecular-weight starting materials and reaction by-products.

Example 6B: PEG-diamine co-polymer with pendant thioethers.



The thiol adduct obtained in Example 6A is amidated with 1,4-diaminobutane (one equivalent of diamine per two COOH groups), using the same procedure used for dodecylamine in Example 1, with dilution with water is as necessary to maintain the fluidity of the reaction mixture. Additional aliquots of EDC are added as necessary to ensure complete polymerization. By this method, the thiol adducts of Example 6A and 6Aa through 6Ak are converted to a PEG-diaminobutane polyamide.

By this method, the following diamines may be converted to a PEG polyamide (BOC = *t*-butoxycarbonyl):

Example 6Ba: *2-(O*-BOC)-1,3-diamino-2-propanol

Example 6Bb: N',N"-di(BOC) hexaethylene tetraamine

Example 6Bc: N',N"-di(BOC) spermine

Example 6Bd: N'-BOC spermidine

Example 6Be: N',N",N'''-tri(BOC) pentaethylene hexamine

Example 6Bf: agmatine

Example 6Bg: lysine t-butyl ester

Example 6Bh: 1,6-diaminohexane

Example 6Bi: 1,4-phenylenediamine

Example 6Bj: 1,3-phenylenediamine

Example 6Bk: 1,4-diaminobutane-2,3-diol acetonide

4. Biocompatibility of π Polymers

Example 1: Suitability for topical emollients, creams or pastes

A concentrated oily wax of the polymer of Example 1 was rubbed on the inner wrist skin by the inventor and observed for uptake. The material appeared to be absorbed similarly to pharmacological waxy creams, with slight softening of the area. No immediate or delayed allergic responses, such as reddening, rash, or itching, were observed upon this single topical application.

Many of these polymers are hygroscopic waxes at room temperature, with an expected mp of about 45 °C to 60 °C or greater, depending upon the composition. Polymers made with lower MW PEG²s may even be liquid at room temperature. Some polymers may be solid at room temperature, melting at body temperature. Thus the properties of these π polymers make them excellent substrates for making lotions, creams, ointments, emollients, and other delivery forms, either by themselves, or in mixture with various substances, including active pharmaceutical agents.

Example 2: Suitability for parenteral administration

An aqueous solution of the polymer of Example 1 was prepared in phosphate-buffered saline and then filtered into sterile tubes through 0.22um filters.

A maximum tolerated dose protocol was employed, wherein CD-1 mice were subjected to a dose of 10ml per kg body weight tail vein injection of up to 5% w/v aqueous solution of the polymer. The mice were observed for 12 hours continuously and every 2hr thereafter until 48 to 72 hrs, depending upon the group. Blood samples were taken and analyzed. Some mice were sacrificed and first examined for gross histology. Microscopic histology was then performed on selected sections.

No observable differences were found in the blood chemistry between the control mice and the treated mice. No observable differences or lesions were found compared to control animals in the gross histology of various organs including heart, lungs, kidneys, spleen, liver, intestines, stomach, bladder, skin, muscles, bones, brain, and lymph nodes. Multiple specimens from different groups of animals were studied with the same results being observed. No

observable differences were found in cellular tissue structure of examined tissues. Some of the kidneys showed some casting that diminished with exposure time to the polymer. This implies that the casting is a temporary phase and as the time progresses it will become normal.

It is concluded that the polymer is safe for medical use as a pharmaceutical agent in injectable preparations and other parenteral formulations. It is reasonable to expect that the polymer is safe in oral solutions, caplets, and tablets, nasal spray, oral/bronchial aerosols, sublingual, skin cream/lotion/patch, eyedrops, other topical routes, and other routes of administration.

5. Attachment of Targeting Moieties to π-Polymers

Example 1: Attachment of galactosamine to C-16 π-Polymer B via amide bond formation.

Galactosamine (GA) targets the hepatic asialoglycoprotein receptor (ASGPR), and polymers bearing covalently-bonded glactosamine are delivered to the liver; see L. Seymour et al., "Hepatic Drug Targeting: Phase I Evaluation of Polymer-Bound Doxorubicin" *J. Clin. Oncology*, **20**(6): 1668-1676 (2002) and references therein.

C16-π-Polymer B (Example 10 in synthetic method section above) (461 mg, 0.2 mmols equivalent COOH per repeating unit) was dispersed in 14 mL water, and to this dispersion was added EDC HCl (0.485 mmols) and N-hydroxysuccinimide (0.464 mmols). The mixture was stirred at ambient temperature for 15 minutes and a solution of galactosamine HCl (0.386 mmols) and TEMED (0.387 mmols) in 1 ml water was added. The solution was stirred and the reaction was followed by TLC on silica gel and development in 1-butanol-acetic acid-water (3:1:1). An additional amount of TEMED (0.079 mmols), NHS (0.078 mmols) and EDC HCl (0.193 mmols) were added to force the reaction to completion. When TLC showed a steady state with respect to consumption of GA, the reaction mixture was dialyzed (3500 Da cut-off membrane) against 3 x 1000 ml deionized water to remove the low molecular weight reactants and by-products. The retentate was removed and dried at 60°C to constant weight (348 mg).

TLC of the product showed no free GA (ninhydrin negative). A sample of the product was hydrolyzed with 6 N HCl at 100°C to hydrolyze bound GA. TLC analysis showed the presence of GA (ninhydrin positive) at the same Rf as reference GA.

Example 2: Attachment of folic acid to C18-π-Polymer A

BDDC (2.44 g, 8.56 mmols) was weighed out in a 125 mL round-bottom flask flushed with argon (BDDC is very viscous with honey like consistency and difficult to handle). C18-π-Polymer A (10 g, 4.28 mmols) was added to the flask, the mixture was heated to 70 °C, and the reactants were stirred together for about 30 minutes. Folic acid (3 g) was added followed by sufficient THF to make stirring possible. The reactants were stirred at 40-70°C overnight, protected from moisture. The THF was then allowed to evaporate and water (80 mL) was added, and the mixture was stirred at 50 °C for an additional 2 h. After cooling to room temperature, the mixture was transferred to a section of dialysis tubing with a 3500 Dalton cut off, and dialyzed against 0.1 N HCl (2 x 2000 ml), water (2000 ml), 5% sodium carbonate (2 x 2000 ml) and water (4 x 2000 ml), to remove unreacted reagents and by-products. The bright yellow-orange retentate was ~~was~~ removed. A portion was evaporated to constant weight to determine the solid concentration, and was used for the solubilization experiments described above.

Example 3: Attachment of N-acetyl neuraminic acid (NANA) to C16-π-Polymer B.

Neuraminic acid derivatives are expected to be targeting moieties for influenza viruses because of the hemagglutinin and neuraminidase coat proteins, both of which are known to bind to sialic acid.

BDDC (2.44 g, 8.56 mmols) and C18-π-Polymer A (10 g, 4.28 mmols) are combined and heated to 70 °C, and stirred together under argon for about 30 minutes. N-acetyl neuraminic acid (3 g) is added, followed by THF as necessary to maintain fluidity. The reactants are stirred at 40-70°C overnight, protected from moisture. Water (80 mL) is added, and the mixture is stirred at 50 °C for an additional 2 h. After cooling to room temperature, the mixture is dialyzed against 0.1 N HCl, 5% $NaHCO_3$, and water (2 x 2000 ml each) with a 3.5 kDa cutoff membrane.

Example 4: Attachment of β-O-methyl neuraminic acid (MNA) to C16-π-Polymer B.

C16-π-Polymer B, 43 micromoles COOH basis, in 1 ml water, and neuraminic acid β-methyl glycoside (Toronto Research Chemicals), 40 micromoles, were mixed together, and 40 micromoles NHS in 0.1 ml water was added, followed by 40 micromoles EDC hydrochloride in 0.1 ml water. The reaction mixture was shaken at ambient temperature for 48 hours, and analyzed by TLC on silica gel with isopropanol-ethyl acetate-water (4:3:2). Detection with 0.2% orcinol in 70% sulfuric acid, at 130°C, does not generate a color reaction with the starting polymer, but TLC of the reaction mixture gave a purple spot co-migrating with the polymer.

Example 5: Attachment of zanamivir to C16-π-Polymer B.

Zanamivir (GG167) is a potent inhibitor of viral neuraminidase, and polymers bearing this molecule as a multivalent ligand are inhibitors of influenza virus replication.

C16-π-Polymer B (920 mg) is dispersed in 30 mL water, and to this is added EDC HCl (1.2 mmol) and N-hydroxysuccinimide (1.1 mmol). The mixture is stirred at ambient temperature for 20 minutes, and a solution of the trifluoroacetic acid salt of 5-acetamido-7-(6'-aminohexyl)-carbamyloxy-4-guanidino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonic acid (U.S. Patent Nos. 6,242,582 and 6,680,054) (0.39 g, 0.67 mmol) and TEMED (0.67 mmols) in 1 ml water is added. The solution is stirred at room temperature, and the reaction is followed by TLC. The reaction mixture is dialyzed (3500 kDa cut-off membrane) against 3 x 1000 ml deionized water to remove the low molecular weight reactants and by-products. The retentate is removed and dried at 60°C to constant weight. The level of sugar incorporation may be determined by a colorimetric assay for the guanidine group (*Can. J. Chem.*, **36**:1541 (1958)). A neuraminidase assay may be carried out following the procedure of Potier *et al*, *Anal. Biochem.*, **29** 287 (1979).

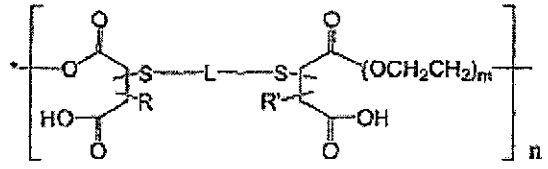
Example 6: Attachment of Fab fragment to C16-π-Polymer A Dimaleate:

A single-chain variable fragment antibody (scFv) directed against the surface glycoprotein high-molecular-weight melanoma-associated antigen (HMW-MAA) targets melanoma cells; see F. Martin *et al.*, *J. Virology*, **73**:6923-6929 (1999).

Disulfide bonds in this antibody fragment are reduced with Immobilized TCEP Disulfide Reducing Gel (Pierce Biotechnology, Rockford, IL) according to the manufacturer's protocol, and reacted with C16-π-Polymer A dimaleate by the method of Example 12 in the Synthetic Methods section.

The scope of the invention is defined by the appended claims.

11. The polymer of claim 4, having the structure

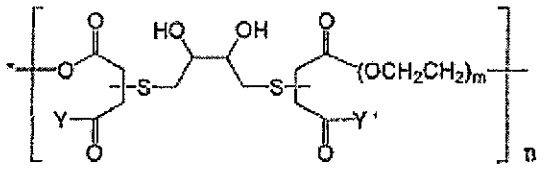


wherein m is 4-700, L is phenylene, C_2-C_6 alkylene, or benzenedimethylene, and R and R' are independently selected from the group consisting of linear hydrocarbons optionally substituted with one or more hydrophilic substituents, polycyclic hydrocarbons optionally substituted with one or more hydrophilic substituents, hydrophobic amino acids, peptides and polymers.

~~12. The composition resulting from the chemical reaction of polyethylene glycol and maleic anhydride, resulting in the substantially complete esterification of the terminal hydroxyl groups of the polyethylene glycol by the maleic anhydride, and chemical reaction of the resulting composition with dithiothreitol.~~

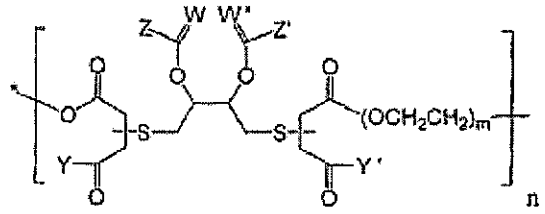
~~13. The composition resulting from the chemical reaction of polypropylene glycol and maleic anhydride, resulting in the substantially complete esterification of the terminal hydroxyl groups of the polypropylene glycol by the maleic anhydride, and chemical reaction of the resulting composition with dithiothreitol.~~

~~14.~~12. A polymer having the structure



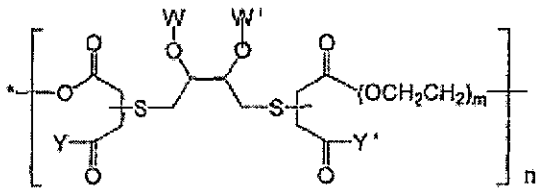
wherein m is 4 to about 700 and n is 3 to about 100, and wherein, in each occurrence of the monomer unit having the structure shown, Y and Y' are independently selected from the group consisting of OH, COOH, SH, NH_2, NHR, ONH_2, NHOH, $NHNH_2$, and $NRNH_2$, wherein R is selected from the group consisting of C_1 to C_5 alkyl, $(CH_2)_kOH$, $(CH_2)_kCOOH$, $(CH_2)_kSH$, $(CH_2)_kNH_2$, $(CH_2)_kONH_2$, $(CH_2)_kNHOH$, and $(CH_2)_kNHNH_2$, where k is from 2 to 5.

~~15.~~13. A polymer having the structure



wherein m is 4 to about 700 and n is 3 to about 100, and wherein, in each occurrence of the monomer unit having the structure shown, Y and Y' are independently selected from the group consisting of OH, COOH, SH, NH_2, NHR, ONH_2, NHOH, $NHNH_2$, and $NRNH_2$, wherein R is selected from the group consisting of C_1 to C_5 alkyl, $(CH_2)_kOH$, $(CH_2)_kCOOH$, $(CH_2)_kSH$, $(CH_2)_kNH_2$, $(CH_2)_kONH_2$, $(CH_2)_kNHOH$, and $(CH_2)_kNHNH_2$, where k is from 2 to 5; W and W' are independently O or H_2, and wherein, in each occurrence of the monomer unit, Z and Z' are independently selected from the group consisting of linear hydrocarbons optionally substituted with one or more hydrophilic substituents, polycyclic hydrocarbons optionally substituted with one or more hydrophilic substituents, hydrophobic amino acids, peptides and polymers.

~~16.~~14. A polymer having the structure



wherein m is 4-700, and Y and Y' are independently selected from the group consisting of R, OR, COOR, SR, NHR, NRR', ONHR, NHOR, $NRNH_2$, NHNHR, NRNHR', and NHNRR', wherein R and R' are independently selected from the group consisting of linear hydrocarbons optionally substituted with one or more hydrophilic substituents, polycyclic hydrocarbons optionally substituted with one or more hydrophilic substituents, hydrophobic amino acids, peptides and polymers; and wherein, in each occurrence of the monomer unit, W and W' are independently selected from the group consisting of H, -$COCH=CH_2$, -$COC(CH_3)=CH_2$, $COCH=CHCO_2H$, and -$COC(CH_3)=CHCO_2H$.

~~17.~~15. A pharmaceutical composition comprising a polymer according to any of claims 1 -8, and further comprising an effective amount of a pharmacologically active agent.

~~18.~~16. A method of increasing the solubility of a substance in an aqueous solvent, which comprises contacting the substance with a polymer according to claim 1 or claim 2 so as to form a water-soluble complex of the substance and the polymer.

~~19.~~17. A method of increasing the solubility of a substance in a non-aqueous solvent, which comprises contacting the substance with a polymer according to claim 1 or claim 2 so as to form a complex of the substance and the polymer that is soluble in the non-aqueous solvent.

~~20.~~18. The method of claim ~~18~~ 16 or claim ~~19~~17, wherein the substance is selected from the group consisting of vitamins, nutrients, drugs, dyes, nucleic acid complexes, and imaging agents.

~~21.~~19. The method of claim ~~20~~18, wherein the substance is a drug.

~~22.~~20. A method of inducing binding affinity for a biological target in a polymer according to claim 4, comprising the step of attaching a targeting moiety to one or more of the reactive functional groups X present on the polymer.

~~23.~~21. The method of claim ~~22~~20, wherein the biological target is the surface of a cell or virus.

~~24.~~22. The method of claim ~~23~~21, wherein the targeting moiety is selected from the group consisting of receptor-specific ligands, antibodies, antibody fragments, peptides comprising an RGD amino acid sequence, peptides comprising a YISRG motif, growth factors, sialic acid derivatives, N-acetylneuraminic acid derivatives; folate, methotrexate, pteroic acid, estradiol, estratriol, testosterone, mannose-6-phosphate, sugars, vitamins, tryptophan, aminoalkyladamantanes, ~~Fuzeon™~~enfuvirtide, PRO-542, BMS-488043, sialic acid, 2-deoxy-2,3-didehydro-N-acetylneuraminic acid, 4-guanidino-Neu5Ac2en (zanamivir), oseltamivir, and RWJ-270201.

~~25.~~23. The method of claim ~~24~~22, wherein the targeting moiety is a monoclonal antibody or an antibody fragment.

affinity for cell walls at the target site. However, PEG lacks functional groups except at the ends of the polymer chains, and the majority of the terminal groups are inevitably taken up by bonds to the other block copolymer component. For this reason, attachment of targeting moieties such as antibodies or cell-adhesion molecules to PEG block copolymers is generally limited to the non-PEG block, which unfortunately is not the part of the copolymer that is normally exposed in the corona of the self-assembled aggregate.

The phase separation phenomenon which results in the self-assembly of block copolymers into polymer aggregates is readily reversible, and attempts have been made to increase the stability of the aggregates by cross-linking the hydrophobic core (see European Patent No. EP 0552802). Covalent attachment of the drug to the hydrophobic component of a block copolymer has also been attempted (Park and Yoo, U.S. Patent No. 6,623,729; European Patent No. EP 0397307).

There remains a need for a drug delivery system that is stable, biocompatible, amenable to the attachment of targeting moieties to the exterior of the aggregates, and efficient at delivering drugs to the desired cellular targets.

SUMMARY

Biocompatible comb-type polymer molecules, comprising a hydrophilic backbone having branch-point moieties, and hydrophobic branches attached at these branch-point moieties are disclosed herein. Aqueous suspensions of polymer aggregates formed from such polymers, and methods for solubilizing insoluble or sparingly-soluble organic compounds, such as drugs, dyes, and vitamins, by incorporating such compounds in the hydrophobic cores of the polymer aggregates, are also disclosed. The method for solubilizing a water-insoluble organic species in an aqueous solvent basically comprises contacting the water-insoluble organic species with a polymer disclosed herein in an aqueous or mixed-aqueous solvent.

In particular embodiments, the branch point moieties further comprise reactive functional groups capable of serving as attachment points for targeting moieties. In particularly preferred embodiments, targeting moieties such as ligands or antibodies are covalently attached

to the branch-point moieties of the polymers disclosed herein, and a drug is incorporated into the core of the aggregates, so as to form a targeted drug complex.

Methods for the preparation of the comb-type polymers, aggregates, and targeted drug complexes described herein are also disclosed. The polymers disclosed herein self-assemble into polymer aggregates that efficiently solubilize, distribute, and deliver drugs in vivo, are non-toxic and biocompatible, are stable, and are capable of bearing multiple cell targeting moieties on their exterior surfaces.

BRIEF DESCRIPTION OF THE DRAWINGS

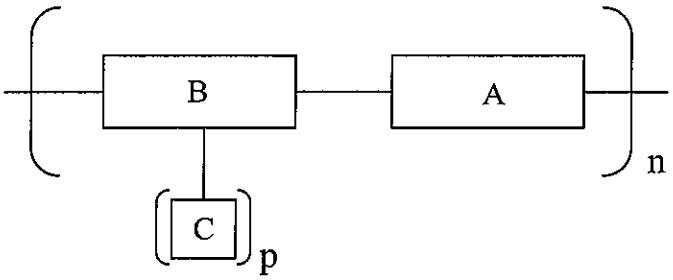
Fig. 1 shows 20 ul samples of saturated solutions of three lipophilic dyes (A, Sudan IV; B, dichlorofluorescein; C, spirit soluble Eosin Y) in deionized water, spotted onto a silica gel TLC plate. Upper row: with 50 mg/ml of the π-polymer of Example 1; lower row: without π-polymer.

Fig. 2 shows 50 ul samples of saturated solutions of four insoluble drugs (row 1, Purpurin; row 2, Camptothecin; row 3, Amphotericin B; row 4, Doxorubicin) in deionized water, spotted onto a silica get TLC plate. Column A, folated Polymer A; column B, Polymer A, column C, no polymer. Three pencil marks around each spot indicate the extent of spread of solvent on the plate. The central circles in columns A and B are uniformly dispersed into the silica, indicating clear solutions; the central circles in column C consist largely of solid deposits.

DETAILED DESCRIPTION

The polymers disclosed herein, referred to herein as "π-polymers", have a comb-type architecture, with a backbone formed of alternating branch-point moieties B and hydrophilic, water-soluble polymer blocks A; and having a plurality of hydrophobic side chains C attached to each branch-point moiety. They consist essentially of the structure shown in Formula 1. The side chains C are relatively short, hydrophobic moieties, which may be aliphatic molecules, chains or oligomers. The value of p is ideally an integer, either 2, 3, or 4. In practice the side chains are most often introduced with less-than-perfect efficiency via chemical reactions, resulting in an average value of p for the polymer preparation as a whole that is not the intended

integer. Non-integer average values can also be obtained by design, as discussed below. Thus, the average value of p in the polymers disclosed herein is greater than one and may be as high as four ($1 < p \leq 4$). In preferred embodiments, P ranges from about 2 to 4, and most preferably $1.5 < p \leq 2$.



1

The backbone polymer block A is hydrophilic and/or water-soluble , wherein A is selected from the group consisting of poly(ethylene glycol), poly(propylene glycol), poly(ethylene imine), poly(vinyl alcohol), poly(vinylpyrrolidone), polysaccharides, and co-polymers thereof. Preferably, the polymer units A are poly(ethylene glycol) chains of formula $-(CH_2CH_2O)_m-$ where m is between 1 and 10,000, preferably between 3 and 3,000. The terminal functional groups of the polymer chains have not been characterized, and are not relevant to the invention.

In the manufacture of poly(ethylene glycol) of various grades, it is known in the industry to couple a divalent linker moiety (*e.g.*, bisphenol A diglycidyl ether) to two poly(ethylene glycol) chains, effectively doubling the molecular weight of the polymer while retaining a relatively narrow molecular weight range. The resulting "poly(ethylene glycol)" molecules are consequently interrupted at the midpoint of the polymer chain by the non-glycol linker moiety (see, *e.g.*, the poly(ethylene glycol)-bisphenol A diglycidyl ether adduct, CAS registry No. 37225-26-6). Higher oligomers, *i.e.* those having three PEG chains separated by two bisphenol A diglycidyl ether moieties, are also known, see for example international patent application WO 00/24008. As used herein, therefore, the terms "poly(ethylene glycol)" and "poly(propylene glycol)" encompass poly(ethylene glycol) and poly(propylene glycol) polymer chains that incorporate non-glycol linker units, including but not limited to bisphenol A diglycidyl ether, bisphenol B diglycidyl ether, bisphenol S diglycidyl ether, hydroquinone

diglycidyl ether, and the like. For purposes of this specification, any such linker moieties are not counted as "monomer units".

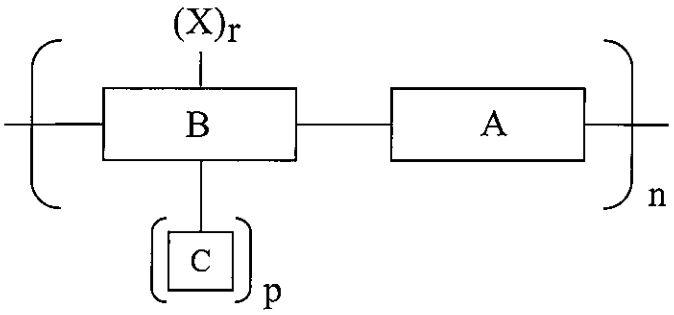
The polymer block A most preferably has an average length of between twenty and fifty monomer units. The polyethylene glycol chains may be end-substituted with functional groups suitable for use as linkers to other moieties, including but not limited to amino, mercapto, acrylate, acrylamide, maleate, and maleimide, at one or both ends. The value of n ranges from 3 to about 100. The overall molecular weight of the π-polymer may range from 1000 to 100,000 daltons or more; it is preferably above 2,000 daltons, and more preferably above 7,000 daltons.

Hydrophobic moieties C may be the same or different, and are independently selected from the group consisting of linear hydrocarbons (optionally substituted with one or more hydrophilic substituents), polycyclic hydrocarbons (optionally substituted with one or more hydrophilic substituents), hydrophobic amino acids, peptides and polymers. Suitable hydrophilic substituents include, but are not limited to, hydroxyl, ether, cyano, and amide functional groups. Specifically contemplated are C_8 to C_{20} alkyl groups bearing ω-hydroxy, ω-cyano, ω-amido, or ω-alkoxy substituents. In this context, the term "substituent" includes the substitution of a heteroatom, such as O, N, or S, for a carbon atom in the hydrocarbon chain or ring system of the moiety C. Thus, ether and amide linkages, and heterocyclic rings, may be incorporated into C.

Hydrophobic moieties C are preferably relatively short (C_8-C_{20}) aliphatic chains, but may also be short oligomers. Suitable oligomers include oligo hydroxy acids such as poly(glycolic acid), poly(DL-lactic acid), poly(L-lactic acid), and copolymers of poly(glycolic acid) and poly(lactic acid)hydroxy acids, and poly(amino acids), poly(anhydrides), poly(orthoesters), and poly(phosphoesters), polylactones such as poly(epsilon-caprolactone) poly(delta-valerolactone) poly(gamma-butyrolactone) and poly(beta-hydroxybutyrate). C moieties may also be selected from hydrophobic molecules, such as cholesterol, cholic acid, lithocholic acid, and hydrophobic peptides. The molecular weight of each moiety C is greater than 40, preferably between 50 and 1,000, and most preferably between 100 and 500. In general, any moiety C which is not appreciably soluble in water when in the molecular form C-H is thought to be suitable for use.

It is a distinguishing feature of the comb polymers disclosed herein that the side chains C are not regularly and uniformly distributed along the polymer chain, but rather occur in clusters $[C]_p$. These clusters are spaced more or less regularly along the polymer chain, depending on the degree of monodispersity of the polymer units A. Thus, the distance between two side chains C attached to a common branching moiety B is different from the distance between two side chains attached to different branching moieties.

In another embodiment, the branch-point moieties B further comprise one or more reactive functional groups X, and the polymers consist essentially of the structure shown in Formula **2**.



2

In Formula **2**, the individual reactive groups X may be the same or may be different from one another, and may optionally be blocked or protected as may be necessary during assembly of the polymer **2.** The average value of r will range from 0 (no X groups) to about 4. Typically, the reactive groups will be selected from functional groups known in the art to be useful for forming covalent linkages between molecular species. The groups X serve as attachment points for drug molecules, tissue- or cell-targeting moieties, virus-targeting moieties, or matrix attachment moieties (such as for the purpose of coating the surface of a stent or other medical device). In certain embodiments, there may be a single attachment point X. In other embodiments, there may be three or four different types of reactive groups. The matrix attachment moiety may attach to a matrix via covalent bonds, specific non-covalent interactions (e.g., antibody-antigen, or non-specific interactions (e.g., via ionic pairing or "hydrophobic" interaction). Suitable reactive groups X include but are not limited to -OH, $-NH_2$, -SH, -CHO, -

$NHNH_2$, -COOH, $-CONHNH_2$, haloacyl, acetoacetyl, -CN, -OCN, -SCN, -NCO, and -NCS; reactive double bonds such as vinylic, acrylic, allylic, maleic, and cinnamic, and groups with reactive triple bonds such as acetylenecarboxy and acetylenecarboxamido (suitable for Michael additions, Diels-Alder reactions, and free radical addition reactions).

Exemplary cell-targeting moieties include but are not limited to receptor-specific ligands, antibodies, and other targeting moieties, such as peptides possessing an Arginine-Glycine-Aspartic acid (RGD) amino acid sequence or a Tyrosine-Isoleucine-Serine-Arginine-Glycine (YISRG) motif; growth factors including epidermal growth factor, vascular endothelial growth factor and fibroblast growth factor; viral surface ligands such as sialic acid and N-acetylneuraminic acid derivatives; cell receptor ligands such as folate, methotrexate, pteroic acid, estradiol, estratriol, testosterone, and other hormones; mannose-6-phosphate, sugars, vitamins, and tryptophan. Antibodies are preferably monoclonal antibodies directed at cell-specific surface antigens; suitable targeting moieties include not only complete antibodies but also antibody fragments containing the active antigen-binding sequences, such as Fab'2 fragments, Fab' fragments, or short chain peptide analogues of the active antigen binding sequences of such antibodies.

Examples of virus-targeting moieties include small molecule ligands that bind to a virus, such as aminoalkyladamantanes, Fuzeon™ (enfuvirtide), PRO-542, BMS-488043, sialic acid, 2-deoxy-2,3-didehydro-N-acetylneuraminic acid, 4-guanidino-Neu5Ac2en (zanamivir), oseltamivir, and RWJ-270201; oligopeptides, oligosaccharides, and glycopeptides that bind to viral surfaces, and antibodies and antibody fragments directed at virus-specific surface antigens. In preferred embodiments, π-polymers bearing ligands for viral neuraminidase or hemagglutinin are provided. It is well-established that such polymers have antiviral properties in their own right; see for example T. Masuda et al., *Chemical & Pharmaceutical Bulletin* **51**:1386-98 (2003); M. Itoh et al., *Virology* **212**:340-7 (1995), and Reece et al., U.S. Patent No. 6,680,054 (2004). The hydrophobic cores of the antiviral polymers and polymer aggregates disclosed herein may optionally be loaded with one or more conventional antiviral drugs, which are advantageously released in the vicinity of the viral particle.

Other attachment groups of medical relevance may be small chemicals, peptides, antibodies or antibody fragments, enzymes, or active pharmaceutical ingredients, that may affect biological processes such as hormones or hormone agonists or antagonists, substances that interfere with virus binding, and substances that interfere with cell cycle or cellular processes after intracellular entry. Cells of unicellular and multicellular organisms, including bacteria, fungi, higher animals, and plants, may be targeted. Biotin may be attached to the π-polymer and used as an attachment point for avidin- and streptavidin-coupled proteins, peptides, and other targeting or pharmacologically active agents, such as antibodies, growth hormones, and imaging reagents.

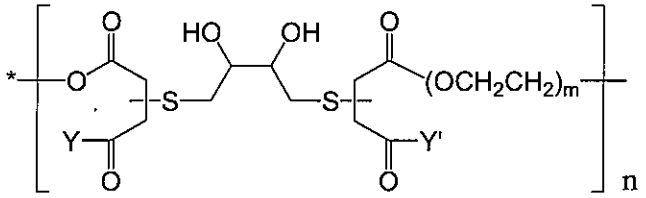
"Matrix" refers to organic or inorganic materials, surfaces, and deposits, such as glass, silica or metal surfaces, extracellular matrix, protein deposits such as amyloid plaques of various kinds, cell surface, virus surface, and general homogeneous or heterogeneous surfaces that may or may not be well characterized, including prions.

Examples of glass or silica matrix attachment moieties include various halosilanes, alkoxysilanes, acylsilanes, as well as chemicals exhibiting such functional groups including polymers. Other attachment groups can be devised based on the particular physico-chemical characteristics of the matrix. Suitable attachment moieties, for example those used in the coating of stents, are known to those skilled in the art.

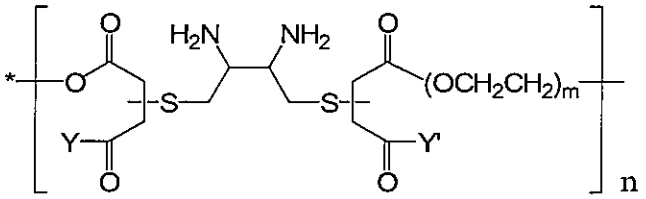
In embodiments disclosed herein, the branch point moieties B are connected to other branch point moieties elsewhere in the polymer chain, so as to form a crosslinked hydrogel structure. Such crosslinking may be effected by reacting the polymer with multifunctional moieties that contain homofunctional or heterofunctional groups, at least one of which reacts with X or a reactive group on C located on a first branch point moiety, and at least one of which reacts with X or with a reactive functional group present on C at a second branch point moiety. Cross-linking may also be made via a link to the terminal functional groups of the polymer chain A. Such crosslinked polymers may optionally contain reactive functional groups suitable for attachment of drug molecules or targeting moieties.

The branch-point moiety B is typically derived from a multifunctional molecule having a plurality of reactive groups, two of which are suitable for attachment to the hydrophilic polymer unit A, and two of which are suitable for attachment of the hydrophobic moieties C. Moiety B may optionally have additional reactive groups X as described above.

Particularly preferred branch-point moieties are the conjugates of dithiothreitol (DTT), dithioerythritol (DTE), or 2,3-diaminobutane-1,4-dithiol with two molecules of maleic acid. The combination of this branch-point moiety with polyethylene glycol as the moiety A generates the polymer backbone of Formulas **3** and **3a**



3



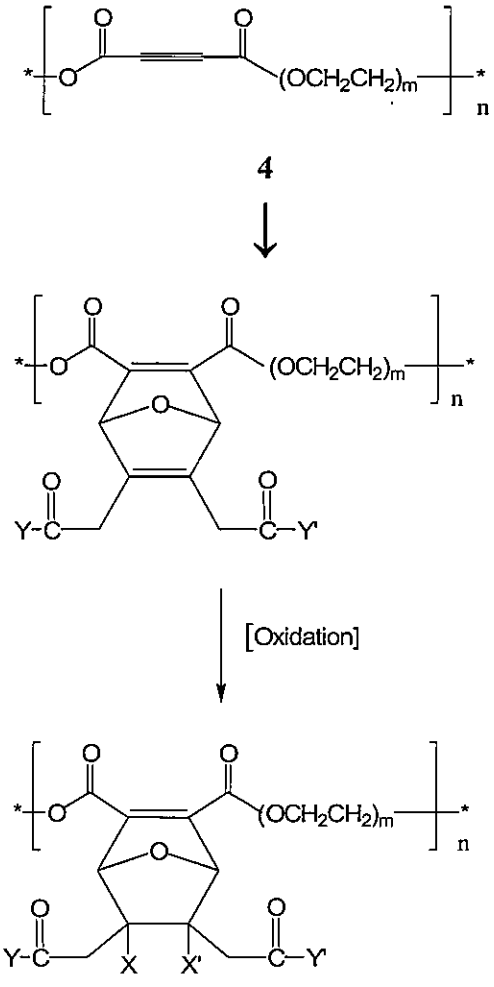
3a

wherein Y and Y' may be the same or different, and are preferably selected from OH, NH_2, ONH_2, NHOH, and $NHNH_2$. In a preferred embodiment, the hydroxyl or amino groups of the dithiol are the reactive groups X, serving as attachment points for targeting or drug moieties, while the functional groups Y and Y' serve as attachment points for C moieties. Alternatively, the groups Y and Y' may serve as attachment points, while the hydroxyl or amino groups are used to attach the C moieties.

Formulas **3** and **3a** are intended to convey that each sulfur atom may independently be attached alpha or beta to a PEG ester carbonyl group. Single isomer

compositions as well as mixtures of regioisomers at one or both C-S bonds are encompassed. Furthermore, due to the four asymmetric carbons in Formula 1, all chiral, meso, and diastereomeric isomers and mixtures thereof are encompassed.

The Diels-Alder adduct of acetylene dicarboxylic acid and a furan may also serve as a suitable branch point moiety. For example, the polyester **4** derived from PEG and acetylenedicarboxylic acid is known to undergo Diels-Alder reactions with furans (M. Delerba *et al., Macromol. Rapid Commun.* **18**(8):723-728 (1997)).



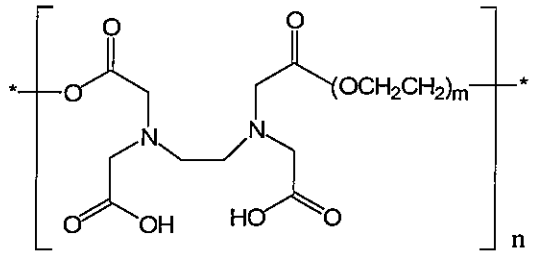
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5

Scheme 1

Thus, it may be subjected to a Diels-Alder reaction with a 3,4-disubstituted furan to generate a species such as **5**, and polymer **5** can be modified by hydroxylation or epoxidation to provide reactive groups (e.g., X and X' in Scheme 1).

Similarly, reaction of PEG with ethylenediamine tetraacetic acid dianhydride will provide a polyester of formula **6**:

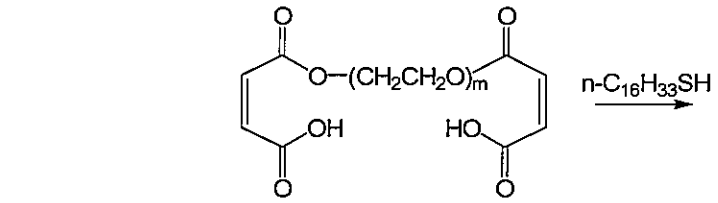


6

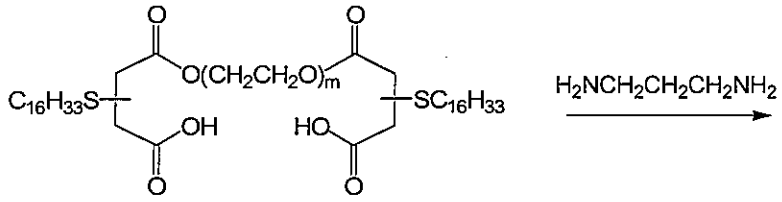
Other suitable branch point moieties may be derived from tartaric acid, acetylenedicarboxylic acid, nitrilotriacetic acid, 3,4,3',4'-diphenyl sulfone tetracarboxylic acid dianhydride, 3,4,3',4'-diphenyl ether tetracarboxylic acid dianhydride, pyromellitic dianhydride, alkanedithiols such as 1,2-ethanedithiol and 1,4-butanedithiol, bis(2-mercaptoethyl)ether, 2-mercaptoethylsulfide, dimercaptopropanol, dimercaptopurine, dimercaptothiadiazole, dimercaptosuccinic acid, benzenedimethanethiols, benzenedithiols, dihalogenated benzenedimethanethiols, and dihalogenated 4,4'-thiobisbenzenethiol.

Where Y and Y' are OH, hydrophobic groups C may be linked to the polymer by amidation or esterification of the carboxylic acid groups. The hydrophobic groups C are preferably relatively small (C_8-C_{20}) and predominantly hydrocarbon moieties, and may be linear or branched or contain one or more rings. Examples include but are not limited to covalently attached dodecylamine, pentadecylamine, cholesterol, and cholic acid moieties. Although the polymers disclosed herein are represented, for convenience, as having at most two different hydrophobic side chains, is should be understood that mixtures of two or more hydrophobic compounds may be used to introduce a variety of hydrophobic side chains into a particular polymer.

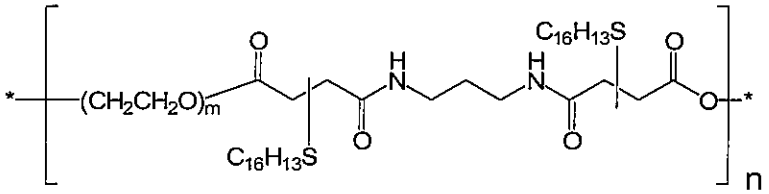
existence at the time of polymerization (Scheme 5).



7



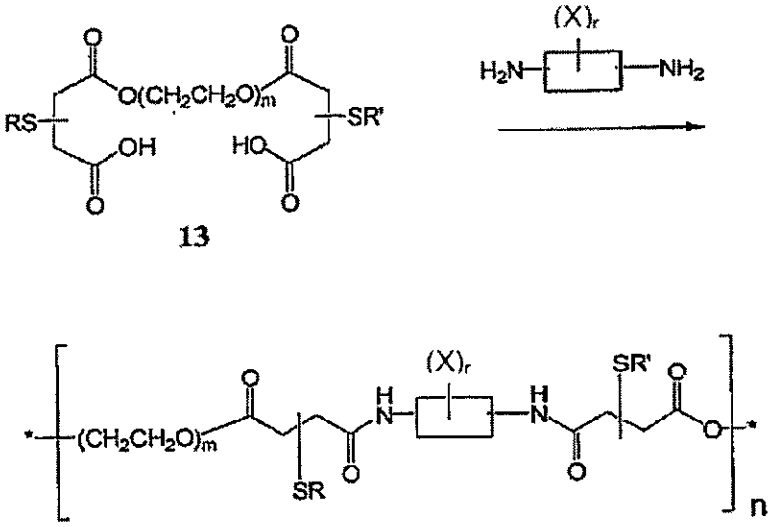
13



14

Scheme 5

In addition to simple diamines such as 1,3-diaminopropane, as shown in Scheme 5, diamines having (optionally masked) reactive functional groups X may be employed, leading to polymers **15** suitable for attachment of targeting moieties (Scheme 6). In the formulae below, r may range from 0-4, and each X is independently the same or different from any other group X that may be present. A reactive group X need not be pendant, but may for example be an NH group within the chain of atoms that makes up the diamine, as in the monomer H_2N-$(CH_2)_3$-NH-$(CH_2)_3$-NH_2.



Scheme 6

Certain of the π-polymers prepared as above possess reactive groups X suitable for further derivatization, to attach targeting moieties such as small molecules, peptides, nucleotides, sugars, antibodies, etc., or to effect crosslinking of the polymer chains via bifunctional or multifunctional crosslinking agents. In particular embodiments, partial derivatization of the reactive groups on the polymer chain is carried out to generate π-polymers having a variety of different reactive groups, which permits attachment of a variety of targeting and drug moieties to a single polymer chain. Thus, addition of a sub-stoichiometric amount of acryloyl chloride (or maleic anhydride) to the π-polymer of Example 1 will provide a polymer with both acryloyl (or maleyl) groups and residual hydroxyl groups. Subsequent Michael addition of a sub-stoichiometric amount of a mercapto-carboxylic acid, for example HS-$(CH_2)_3$-COOH, would provide a polymer with hydroxyl, acryloyl, and carboxyl groups. Addition of cysteine introduces amino and carboxyl groups, in addition to any residual reactive groups left behind by sub-stoichiometric amounts of reagents.

Another approach to poly-functional π-polymers involves the deliberate omission of a fraction of the hydrophobic chains C. The π-polymer of Example 1, for example, can be

By performing partial reactions, one can introduce additional reactive functional groups to a polymer disclosed herein, including but not limited to (1) thiol-reactive groups such as acrylic or maleic acid derivatives, (2) carboxylic-acid reactive groups such as amino or hydroxyl, (3) amine-reactive groups such as carboxyl, and (4) disulfide-reactive groups such as mercapto. The number of such added functional groups per polymer molecule may range from 1/r up to several multiples of r, depending on the reagent used and the quantity used.

Alternatively, two or more specific ligands can be attached to improve specificity of binding to say, a virus, or cell surface. Two or more specific ligands can also be used so as to cause an interaction between different cellular targets, for example, one ligand may target a virus particle, and another ligand may facilitate binding to a phagocyte, thereby bringing virus particle into proximity or contact with the phagocyte and promoting phagocytosis.

Such derivatization allows the attachment of three or more distinct targeting and/or therapeutic moieties to the polymer, through different functional group attachments (such as amine, carboxylate, and thiol). Thus, one may attach a tissue-specific targeting agent, an imaging agent, and a therapeutic agent to a single polymer chain, and subsequent self-assembly of the polymer will yield a targeted therapeutic whose distribution and efficiency of targeting can be monitored.

Attachment of ligands to the repeating units of the polymers disclosed herein affords multivalent display of the ligand on the polymer chain and on the nanoparticles surface. Multivalent display often leads to great increases in affinity for the target. For example, multivalent antibodies can be far more effective in clearance of their targets than the normal divalent antibodies. Carbohydrate-binding proteins and carbohydrates are known to be multivalent in nature, and ineffective if monovalent. Similarly, multivalent peptide and carbohydrate targeting moieties will be far more effective than the monomer alone. The increase in MW due to attachment to the polymer results in reduced renal clearance rates of peptides and other ligands. In addition, the PEG backbone affords to the peptide benefits similar to those of PEGylation, including evasion of immune surveillance.

Further, a multivalent targeting moiety will decorate a multivalent target (say, a virus particle) and neutralize it far more effectively than the monomeric targeting moiety. The ability to display multiple (different) peptides in multivalent format will lead to enhanced specificity. For example, a truly HIV-specific (HIV virus-binding) polymer can be built by attaching a peptide corresponding to the CD4 binding region, and another peptide corresponding to the CCR-5 or CXCR-4 binding region of the virus, and possibly a third peptide corresponding to the other receptor (CXCR-4 or CCR-5 respectively). Such a polymer could completely mask the virus's binding regions and render the virus unable to attach to cells and thereby non-infective. In addition, the surfactant properties of the polymer would lead to destabilization of the virus structure itself upon binding. Instead of peptides, small molecules that interfere with the same binding patterns (CD4, CCR-5, CXCR-4) or a mixture of peptides and small molecules, preferably with complementary activities, can be employed. The resulting polymers will render any free virus ineffective, and thus may be ideal for stopping spread of infection, by using them as components of condom lubricants. In addition, such polymers may be injected into patients to reduce the HIV burden.

Generally, when a polyfunctional reagent such as DTT is employed, there may be partial cross-linking of polymer chains via esterification of the carboxylic acid with DTT or similar side reactions. Secondary hydroxyl groups in the central region of the PEG chains, e.g. those associated with bisphenol A diglycidyl ether residues, may also contribute to cross-linking if they are present in the PEG starting material. The resulting crosslinked hydrogel structures are also useful materials. For example, by suitably increasing the extent of this crosslinking or by explicit crosslinking using alternative crosslinkers (such as bisoxiranes, for example), materials can be made that are flexible hydrogels which can serve as repository depots for drugs. By suitably modifying the materials (e.g. lower PEG length, greater open carboxylic groups, and incorporation of suitable acrylic groups) either linear or crosslinked hydrogel materials can be made that can serve as repositories that can be supported either immobilized on devices such as stents or absorbed in devices such as pads for adhesive patches or subdermal insertion patches. In general, such crosslinked materials will be suitable for controlled release rather than enhanced, targeted release.

The comb polymers are useful for solubilizing, in aqueous solvent systems, sparingly water-soluble materials. The method of solubilizing a substance in an aqueous solvent comprises contacting the sparingly-soluble substance with a comb-type polymer disclosed herein in the presence of water, so as to form a water-soluble complex of the substance and the polymer. Alternatively, the polymer and the substance to be solubilized may be combined in a two-phase aqueous-organic emulsion, and the organic solvent removed by evaporation. An exemplary process is described in U.S. Patent No. 6,838,089. It is believed that in most cases, the polymer self-assembles into nanoparticles having the sparingly-soluble substance dissolved among the hydrophobic C chains that coalesce at the core of the particles, while the A blocks form a hydrophilic corona that sufficiently lowers the interfacial free energy to permit an aqueous suspension of the particles to remain stable.

In some cases, the sparingly-soluble substance may not entirely dissolve in the core, but may exist as a solid nanoparticle surrounded by and suspended in the C chains at the core of the particles. For the purposes of this disclosure, this is a difference of degree, as the practice does not rely on any particular degree of mixing of the C chains with the sparingly-soluble substance. The substance may in some cases dissolve at the molecular level among the C chains, but in other cases it may exhibit any degree of phase separation from the C-chain environment. In some cases, it can be expected that the system will move from one state to the other as a function of temperature.

The solvating power of the hydrophobic core of the polymer particles can be modified by modifying the hydrophobic C moieties. Suitable modifications include but are not limited to the introduction of one or more hydrophilic substituents, such as hydroxyl, ether, amide, and cyano functional groups, in order to increase the polarity and/or polarizability of the hydrophobic core.

Sparingly-soluble materials that can be rendered soluble by these polymers include fat-soluble vitamins and nutrients, including but not limited to vitamins A, D, E and K, carotenes, cholecalciferol, and coenzyme Q; insoluble drugs such as docetaxel, amphotericin B, nystatin, paclitaxel, doxorubicin, epirubicin, rubitecan, teniposide, etoposide, daunomycin, methotrexate,

mitomycin C, cyclosporine, irinotecan metabolite (SN-38), statins, and steroids; dyes, photodynamic agents, and imaging agents, and nucleic acids, nucleic acid analogues, and nucleic acid complexes. Nucleic acid analogues include species such as thiophosphates and peptide nucleic acids; nucleic acid complexes are ionic complexes of oligonucleic acids with a substantially charge-neutralizing amount of cationic or polycationic species.

For the purposes of this disclosure, a drug that is insoluble at neutral pH is considered "sparingly soluble", because there is in many cases a need for a neutral pharmaceutical composition. For example, ciprofloxacin is reasonably soluble in water at a pH below 4.5, but this pH can be highly irritating when the drug is formulated for ocular administration. A polymer disclosed herein will solubilize ciprofloxacin in normal saline at pH 7. Also, for the purposes of this disclosure, "sparingly soluble" should be understood to refer to any substance whose solubility in an aqueous vehicle is such that an increase in solubility would yield an improved or more-useful composition. Thus, a drug that is moderately soluble, e.g. to the extent of 2g/liter, is "sparingly soluble" if a unit dose for intravenous administration is 5 g.

As a result of the ability of the polymers disclosed herein to solubilize pharmacologically active species, pharmaceutical compositions, which comprise one or more π-polymers disclosed herein in combination with a therapeutically effective amount of one or more pharmacologically active agents, are also disclosed. The polymers disclosed herein can render effective what would otherwise be an ineffective amount of a pharmacologically active agent. For purposes of this disclosure, therefore, a "therapeutically effective amount" is the amount of agent that renders the overall composition effective.

EXPERIMENTAL

1. General procedures.

Processes for the preparation of the comb polymers disclosed herein are also provided. Synthesis of these polymers is readily carried out by one skilled in the art of organic synthesis, by following the procedures described below. The key starting material is

polyethylene glycol, which is preferably dried before use. This is conveniently done by stirring molten PEG under vacuum at an elevated temperature, until bubbles stop forming. This may take 8-12 hours, depending on the quality of the PEG. Once dried, the PEG can be stored under argon indefinitely. Commercially available industrial and research grades of PEG may be employed in making the polymers disclosed herein, for example the polydisperse "PEG 1500" of commerce having a molecular weight distribution of 1430 – 1570. Such material may incorporate bisphenol A diglycidyl ether, which introduces secondary hydroxyl groups at the center of the PEG chain. In order to ensure that the polymers disclosed herein have the most reproducible and consistent properties, the PEG is preferably free of bisphenol A, and of low dispersity. Most preferable are PEG polymers that are >95% monodisperse, such as are commercially available from Nektar Therapeutics (formerly Shearwater Polymers), Huntsville AL, and Polypure AS, Oslo, Norway. An example of a particularly preferred PEG is "PEG-28" from Polypure, which is >95% $HO(CH_2CH_2O)_{28}H$, molecular weight 1252.

All reactions are carried out under an inert atmosphere such as nitrogen or argon, with magnetic or preferably mechanical stirring.

In step A, dry PEG is melted, and maleic anhydride (2 moles per mole of PEG) is added with stirring. The quantity of maleic anhydride should match the number of PEG terminal hydroxyl groups as closely as possible. A shortage of maleic anhydride will result in hydroxyl-terminated polymer chains, whereas an excess of maleic anhydride will consume thiol groups in the next step, leading to premature chain termination and terminal carboxyl groups. The reaction temperature is not critical, and the process can conveniently be carried out at temperatures between 45°C and 100°C. The preferred temperature of the reaction is between 65°C and 90°C. If elevated temperatures are employed, the maleic anhydride tends to sublime, and steps should be taken to see to it that the maleic anhydride remains in solution. Minimizing headspace and submerging the reaction vessel in an oil bath are effective methods.

Depending on the temperature selected, the reaction may be completed in 2 hours or less or can be conducted overnight. The reaction may be monitored by TLC on silica gel plates, and is continued until after the disappearance of the maleic anhydride. Visual contrast, UV, and iodine staining can all be used to examine the TLC plates.

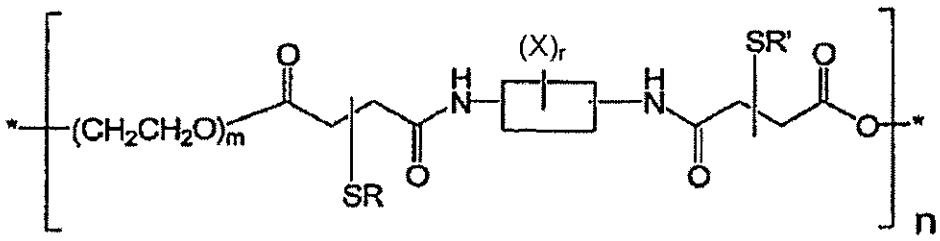
In step B, the crude PEG bis-maleate ester produced in step A is combined with dithiothreitol (DTT) and N,N,N',N'-tetramethylethylenediamine (TEMED) (with added water, if necessary for fluidity), and the mixture stirred at 70°C. The reaction is complete within 30 min, as indicated by the rapid increase in viscosity. The molecular weight of the product will be reduced if more or less than the optimal amount of DTT is employed. The molecular weight of the product can also be reduced, if desired, by replacing TEMED with a less effective tertiary amine base such as TEA.

In step C, sufficient water is added to the reaction mixture to reduce viscosity, and 0.1 mol N-hydroxysuccinimide (NHS) and 1.05 mol hexadecylamine per mol carboxylic acid groups in the polymer are added. (This amount of NHS appears to optimally minimize the extent of side-reactions.) An excess of N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (EDC) (1.4 mol EDC per mol of carboxylic acid groups) is then added in portions, with additional water added as necessary to maintain stirring. The pH of the reaction mixture is maintained above 7, and preferably between 9 and 11, to optimize the reactivity of the alkylamine. With dodecylamine, this reaction can be conducted at about 40~45°C, whereas with octadecylamine, the temperature is ca. 55°C-57°C. The reaction is followed by TLC until a constant level of left-over alkylamine is observed, typically after running overnight.

The reaction mixture is acidified to a pH from about 3.0 to about 4.5 and stirred at room temperature for about 24 hours to destroy unreacted EDC, then titrated to a pH of 7.0 using 1N NaOH. The final reaction mixture is centrifuged at about 800 xg for 1 to 3 hours, to remove solid contaminants and by-products.

After centrifugation, the supernatant can be chromatographed on a GPC column (Toyopearl(TM), Sephadex(TM), Sephacryl(TM), Biogel(TM), etc.). The π polymers are amphipathic materials, and will exhibit affinity for most GPC column packings, thus complicating the removal of contaminants. Alternatively, the polymer may be chromatographed on a large-pore hydrophobic interaction column (e.g., TOYOPEARL™ Phenyl 650C, Toshoh Biosciences, Montgomeryville, PA, U.S.A.), eluting with a gradient of methanol in water. Preferably, the reaction mixture is dialyzed against several changes of acidified and neutral water to remove low-molecular-weight starting materials and reaction by-products.

Example 6B: PEG-diamine co-polymer with pendant thioethers.



The thiol adduct obtained in Example 6A is amidated with 1,4-diaminobutane (one equivalent of diamine per two COOH groups), using the same procedure used for dodecylamine in Example 1, with dilution with water is as necessary to maintain the fluidity of the reaction mixture. Additional aliquots of EDC are added as necessary to ensure complete polymerization. By this method, the thiol adducts of Example 6A and 6Aa through 6Ak are converted to a PEG-diaminobutane polyamide.

By this method, the following diamines may be converted to a PEG polyamide (BOC = *t*-butoxycarbonyl):

Example 6Ba: *2-(O*-BOC)-1,3-diamino-2-propanol

Example 6Bb: N',N"-di(BOC) hexaethylene tetraamine

Example 6Bc: N',N"-di(BOC) spermine

Example 6Bd: N'-BOC spermidine

Example 6Be: N',N",N'''-tri(BOC) pentaethylene hexamine

Example 6Bf: agmatine

Example 6Bg: lysine t-butyl ester

Example 6Bh: 1,6-diaminohexane

Example 6Bi: 1,4-phenylenediamine

Example 6Bj: 1,3-phenylenediamine

Example 6Bk: 1,4-diaminobutane-2,3-diol acetonide

4. Biocompatibility of π Polymers

Example 1: Suitability for topical emollients, creams or pastes

A concentrated oily wax of the polymer of Example 1 was rubbed on the inner wrist skin by the inventor and observed for uptake. The material appeared to be absorbed similarly to pharmacological waxy creams, with slight softening of the area. No immediate or delayed allergic responses, such as reddening, rash, or itching, were observed upon this single topical application.

Many of these polymers are hygroscopic waxes at room temperature, with an expected mp of about 45 °C to 60 °C or greater, depending upon the composition. Polymers made with lower MW PEGs may even be liquid at room temperature. Some polymers may be solid at room temperature, melting at body temperature. Thus the properties of these π polymers make them excellent substrates for making lotions, creams, ointments, emollients, and other delivery forms, either by themselves, or in mixture with various substances, including active pharmaceutical agents.

Example 2: Suitability for parenteral administration

An aqueous solution of the polymer of Example 1 was prepared in phosphate-buffered saline and then filtered into sterile tubes through 0.22um filters.

A maximum tolerated dose protocol was employed, wherein CD-1 mice were subjected to a dose of 10ml per kg body weight tail vein injection of up to 5% w/v aqueous solution of the polymer. The mice were observed for 12 hours continuously and every 2hr thereafter until 48 to 72 hrs, depending upon the group. Blood samples were taken and analyzed. Some mice were sacrificed and first examined for gross histology. Microscopic histology was then performed on selected sections.

No observable differences were found in the blood chemistry between the control mice and the treated mice. No observable differences or lesions were found compared to control animals in the gross histology of various organs including heart, lungs, kidneys, spleen, liver, intestines, stomach, bladder, skin, muscles, bones, brain, and lymph nodes. Multiple specimens from different groups of animals were studied with the same results being observed. No

observable differences were found in cellular tissue structure of examined tissues. Some of the kidneys showed some casting that diminished with exposure time to the polymer. This implies that the casting is a temporary phase and as the time progresses it will become normal.

It is concluded that the polymer is safe for medical use as a pharmaceutical agent in injectable preparations and other parenteral formulations. It is reasonable to expect that the polymer is safe in oral solutions, caplets, and tablets, nasal spray, oral/bronchial aerosols, sublingual, skin cream/lotion/patch, eyedrops, other topical routes, and other routes of administration.

5. Attachment of Targeting Moieties to π-Polymers

Example 1: Attachment of galactosamine to C-16 π-Polymer B via amide bond formation.

Galactosamine (GA) targets the hepatic asialoglycoprotein receptor (ASGPR), and polymers bearing covalently-bonded glactosamine are delivered to the liver; see L. Seymour et al., "Hepatic Drug Targeting: Phase I Evaluation of Polymer-Bound Doxorubicin" *J. Clin. Oncology*, **20**(6): 1668-1676 (2002) and references therein.

C16-π-Polymer B (Example 10 in synthetic method section above) (461 mg, 0.2 mmols equivalent COOH per repeating unit) was dispersed in 14 mL water, and to this dispersion was added EDC HCl (0.485 mmols) and N-hydroxysuccinimide (0.464 mmols). The mixture was stirred at ambient temperature for 15 minutes and a solution of galactosamine HCl (0.386 mmols) and TEMED (0.387 mmols) in 1 ml water was added. The solution was stirred and the reaction was followed by TLC on silica gel and development in 1-butanol-acetic acid-water (3:1:1). An additional amount of TEMED (0.079 mmols), NHS (0.078 mmols) and EDC HCl (0.193 mmols) were added to force the reaction to completion. When TLC showed a steady state with respect to consumption of GA, the reaction mixture was dialyzed (3500 Da cut-off membrane) against 3 x 1000 ml deionized water to remove the low molecular weight reactants and by-products. The retentate was removed and dried at 60°C to constant weight (348 mg).

TLC of the product showed no free GA (ninhydrin negative). A sample of the product was hydrolyzed with 6 N HCl at 100°C to hydrolyze bound GA. TLC analysis showed the presence of GA (ninhydrin positive) at the same Rf as reference GA.

Example 2: Attachment of folic acid to C18-π-Polymer A

BDDC (2.44 g, 8.56 mmols) was weighed out in a 125 mL round-bottom flask flushed with argon (BDDC is very viscous with honey like consistency and difficult to handle). C18-π-Polymer A (10 g, 4.28 mmols) was added to the flask, the mixture was heated to 70 °C, and the reactants were stirred together for about 30 minutes. Folic acid (3 g) was added followed by sufficient THF to make stirring possible. The reactants were stirred at 40-70°C overnight, protected from moisture. The THF was then allowed to evaporate and water (80 mL) was added, and the mixture was stirred at 50 °C for an additional 2 h. After cooling to room temperature, the mixture was transferred to a section of dialysis tubing with a 3500 Dalton cut off, and dialyzed against 0.1 N HCl (2 x 2000 ml), water (2000 ml), 5% sodium carbonate (2 x 2000 ml) and water (4 x 2000 ml), to remove unreacted reagents and by-products. The bright yellow-orange retentate was removed. A portion was evaporated to constant weight to determine the solid concentration, and was used for the solubilization experiments described above.

Example 3: Attachment of N-acetyl neuraminic acid (NANA) to C16-π-Polymer B.

Neuraminic acid derivatives are expected to be targeting moieties for influenza viruses because of the hemagglutinin and neuraminidase coat proteins, both of which are known to bind to sialic acid.

BDDC (2.44 g, 8.56 mmols) and C18-π-Polymer A (10 g, 4.28 mmols) are combined and heated to 70 °C, and stirred together under argon for about 30 minutes. N-acetyl neuraminic acid (3 g) is added, followed by THF as necessary to maintain fluidity. The reactants are stirred at 40-70°C overnight, protected from moisture. Water (80 mL) is added, and the mixture is stirred at 50 °C for an additional 2 h. After cooling to room temperature, the mixture is dialyzed against 0.1 N HCl, 5% $NaHCO_3$, and water (2 x 2000 ml each) with a 3.5 kDa cutoff membrane.

Example 4: Attachment of β-O-methyl neuraminic acid (MNA) to C16-π-Polymer B.

C16-π-Polymer B, 43 micromoles COOH basis, in 1 ml water, and neuraminic acid β-methyl glycoside (Toronto Research Chemicals), 40 micromoles, were mixed together, and 40 micromoles NHS in 0.1 ml water was added, followed by 40 micromoles EDC hydrochloride in 0.1 ml water. The reaction mixture was shaken at ambient temperature for 48 hours, and analyzed by TLC on silica gel with isopropanol-ethyl acetate-water (4:3:2). Detection with 0.2% orcinol in 70% sulfuric acid, at 130°C, does not generate a color reaction with the starting polymer, but TLC of the reaction mixture gave a purple spot co-migrating with the polymer.

Example 5: Attachment of zanamivir to C16-π-Polymer B.

Zanamivir (GG167) is a potent inhibitor of viral neuraminidase, and polymers bearing this molecule as a multivalent ligand are inhibitors of influenza virus replication.

C16-π-Polymer B (920 mg) is dispersed in 30 mL water, and to this is added EDC HCl (1.2 mmol) and N-hydroxysuccinimide (1.1 mmol). The mixture is stirred at ambient temperature for 20 minutes, and a solution of the trifluoroacetic acid salt of 5-acetamido-7-(6'-aminohexyl)-carbamyloxy-4-guanidino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonic acid (U.S. Patent Nos. 6,242,582 and 6,680,054) (0.39 g, 0.67 mmol) and TEMED (0.67 mmols) in 1 ml water is added. The solution is stirred at room temperature, and the reaction is followed by TLC. The reaction mixture is dialyzed (3500 kDa cut-off membrane) against 3 x 1000 ml deionized water to remove the low molecular weight reactants and by-products. The retentate is removed and dried at 60°C to constant weight. The level of sugar incorporation may be determined by a colorimetric assay for the guanidine group (*Can. J. Chem.*, **36**:1541 (1958)). A neuraminidase assay may be carried out following the procedure of Potier *et al*, *Anal. Biochem.*, **29** 287 (1979).

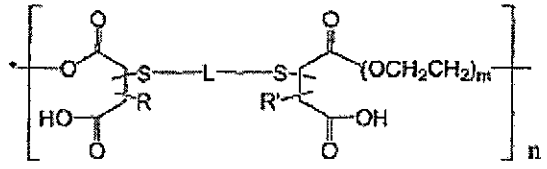
Example 6: Attachment of Fab fragment to C16-π-Polymer A Dimaleate:

A single-chain variable fragment antibody (scFv) directed against the surface glycoprotein high-molecular-weight melanoma-associated antigen (HMW-MAA) targets melanoma cells; see F. Martin *et al.*, *J. Virology*, **73**:6923-6929 (1999).

Disulfide bonds in this antibody fragment are reduced with Immobilized TCEP Disulfide Reducing Gel (Pierce Biotechnology, Rockford, IL) according to the manufacturer's protocol, and reacted with C16-π-Polymer A dimaleate by the method of Example 12 in the Synthetic Methods section.

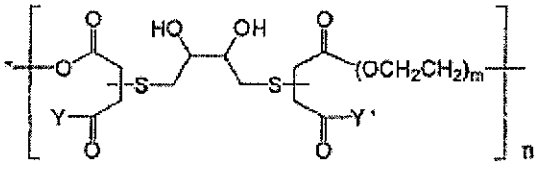
The scope of the invention is defined by the appended claims.

11. The polymer of claim 4, having the structure



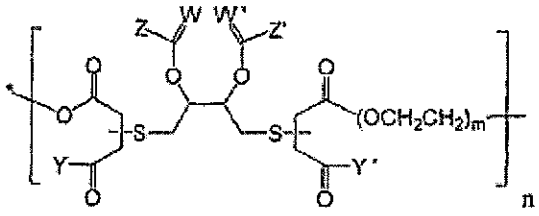
wherein m is 4-700, L is phenylene, C_2-C_6 alkylene, or benzenedimethylene, and R and R' are independently selected from the group consisting of linear hydrocarbons optionally substituted with one or more hydrophilic substituents, polycyclic hydrocarbons optionally substituted with one or more hydrophilic substituents, hydrophobic amino acids, peptides and polymers.

12. A polymer having the structure



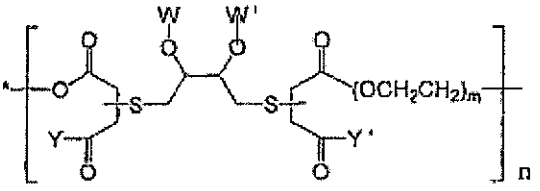
wherein m is 4 to about 700 and n is 3 to about 100, and wherein, in each occurrence of the monomer unit having the structure shown, Y and Y' are independently selected from the group consisting of OH, COOH, SH, NH_2, NHR, ONH_2, NHOH, $NHNH_2$, and $NRNH_2$, wherein R is selected from the group consisting of C_1 to C_5 alkyl, $(CH_2)_kOH$, $(CH_2)_kCOOH$, $(CH_2)_kSH$, $(CH_2)_kNH_2$, $(CH_2)_kONH_2$, $(CH_2)_kNHOH$, and $(CH_2)_kNHNH_2$, where k is from 2 to 5.

13. A polymer having the structure



wherein m is 4 to about 700 and n is 3 to about 100, and wherein, in each occurrence of the monomer unit having the structure shown, Y and Y' are independently selected from the group consisting of OH, COOH, SH, NH_2, NHR, ONH_2, NHOH, $NHNH_2$, and $NRNH_2$, wherein R is selected from the group consisting of C_1 to C_5 alkyl, $(CH_2)_kOH$, $(CH_2)_kCOOH$, $(CH_2)_kSH$, $(CH_2)_kNH_2$, $(CH_2)_kONH_2$, $(CH_2)_kNHOH$, and $(CH_2)_kNHNH_2$, where k is from 2 to 5; W and W' are independently O or H_2, and wherein, in each occurrence of the monomer unit, Z and Z' are independently selected from the group consisting of linear hydrocarbons optionally substituted with one or more hydrophilic substituents, polycyclic hydrocarbons optionally substituted with one or more hydrophilic substituents, hydrophobic amino acids, peptides and polymers.

14. A polymer having the structure



wherein m is 4-700, and Y and Y' are independently selected from the group consisting of R, OR, COOR, SR, NHR, NRR', ONHR, NHOR, $NRNH_2$, NHNHR, NRNHR', and NHNRR', wherein R and R' are independently selected from the group consisting of linear hydrocarbons optionally substituted with one or more hydrophilic substituents, polycyclic hydrocarbons optionally substituted with one or more hydrophilic substituents, hydrophobic amino acids, peptides and polymers; and wherein, in each occurrence of the monomer unit, W and W' are independently selected from the group consisting of H, -$COCH=CH_2$, -$COC(CH_3)=CH_2$, $COCH=CHCO_2H$, and -$COC(CH_3)=CHCO_2H$.

15. A pharmaceutical composition comprising a polymer according to any of claims 1 -8, and further comprising an effective amount of a pharmacologically active agent.

16. A method of increasing the solubility of a substance in an aqueous solvent, which comprises contacting the substance with a polymer according to claim 1 or claim 2 so as to form a water-soluble complex of the substance and the polymer.

17. A method of increasing the solubility of a substance in a non-aqueous solvent, which comprises contacting the substance with a polymer according to claim 1 or claim 2 so as to form a complex of the substance and the polymer that is soluble in the non-aqueous solvent.

18. The method of claim 16 or claim 17, wherein the substance is selected from the group consisting of vitamins, nutrients, drugs, dyes, nucleic acid complexes, and imaging agents.

19. The method of claim 18, wherein the substance is a drug.

20. A method of inducing binding affinity for a biological target in a polymer according to claim 4, comprising the step of attaching a targeting moiety to one or more of the reactive functional groups X present on the polymer.

21. The method of claim 20, wherein the biological target is the surface of a cell or virus.

22. The method of claim 21, wherein the targeting moiety is selected from the group consisting of receptor-specific ligands, antibodies, antibody fragments, peptides comprising an RGD amino acid sequence, peptides comprising a YISRG motif, growth factors, sialic acid derivatives, N-acetylneuraminic acid derivatives; folate, methotrexate, pteroic acid, estradiol, estratriol, testosterone, mannose-6-phosphate, sugars, vitamins, tryptophan, aminoalkyladamantanes, enfuvirtide, PRO-542, BMS-488043, sialic acid, 2-deoxy-2,3-didehydro-N-acetylneuraminic acid, 4-guanidino-Neu5Ac2en (zanamivir), oseltamivir, and RWJ-270201.

23. The method of claim 22, wherein the targeting moiety is a monoclonal antibody or an antibody fragment.