Office de la Propriété Intellectuelle du Canada
Un organisme d'Industrie Canada

Canadian Intellectual Property Office
An agency of Industry Canada

CA 2372841 A1 2000/11/09

(21) 2 372 841

(12) DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION

(13) A1

(86) Date de dépôt PCT/PCT Filing Date: 2000/05/04
(87) Date publication PCT/PCT Publication Date: 2000/11/09
(85) Entrée phase nationale/National Entry: 2001/11/02
(86) N° demande PCT/PCT Application No.: AU 2000/000406
(87) N° publication PCT/PCT Publication No.: 2000/066091
(30) Priorité/Priority: 1999/05/04 (PQ 0147) AU

(51) Cl.Int.^{7}/Int.Cl.^{7} A61K 9/51, A61K 31/519, A61K 47/48, A61P 35/00, C08B 37/02, C08G 69/36, C08F 120/56, C08G 65/34, C08B 37/08, C08B 11/08, C08G 71/04

(71) Demandeur/Applicant:
BIOTECH AUSTRALIA PTY. LIMITED, AU

(72) Inventeurs/Inventors:
MCEWAN, JOHN FERGUS, AU;
RUSSELL-JONES, GREGORY JOHN, AU

(74) Agent: BERESKIN & PARR

(54) Titre : AMPLIFICATION DU CIBLAGE DES CELLULES TUMORALES, INDUIT PAR LES FOLATES A L'AIDE DE POLYMERES

(54) Title: AMPLIFICATION OF FOLATE-MEDIATED TARGETING TO TUMOR CELLS USING POLYMERS

(57) Abrégé/Abstract:
The invention relates to the delivery of drug, peptide and protein pharmaceuticals using the folate-mediated uptake system. More particularly the invention relates to the amplification of drug/pharmaceutical delivery with the folate uptake system using a folate-polymer complex. The invention also relates to processes for preparing the complexes, pharmaceutical compositions containing same, methods of treatment involving the complexes and uses of the complexes in the manufacture of medicaments.

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification [7]: A61K 9/51, 47/48, 31/519, A61P 35/00, C08G 69/36, C08B 37/02, 37/08, 11/08, C08F 120/56, C08G 71/04, 65/34	A1	(11) International Publication Number: **WO 00/66091** (43) International Publication Date: 9 November 2000 (09.11.00)

(21) International Application Number: PCT/AU00/00406

(22) International Filing Date: 4 May 2000 (04.05.00)

(30) Priority Data:

PQ 0147	4 May 1999 (04.05.99)	AU

(71) Applicant *(for all designated States except US)*: BIOTECH AUSTRALIA PTY LIMITED [AU/AU]; 28 Barcoo Street, P.O. Box 20, Roseville, NSW 2069 (AU).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: RUSSELL–JONES, Gregory, John [AU/AU]; 23 Greenfield Avenue, Middle Cove, NSW 2068 (AU). MCEWAN, John, Fergus [NZ/AU]; 241A West Street, Blakehurst, NSW 2221 (AU).

(74) Agents: STEARNE, Peter, Andrew et al.; Davies Collison Cave, Level 10, 10 Barrack Street, Sydney, NSW 2000 (AU).

(81) Designated States: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

With international search report.

(54) Title: AMPLIFICATION OF FOLATE–MEDIATED TARGETING TO TUMOR CELLS USING POLYMERS

(57) Abstract

The invention relates to the delivery of drug, peptide and protein pharmaceuticals using the folate–mediated uptake system. More particularly the invention relates to the amplification of drug/pharmaceutical delivery with the folate uptake system using a folate–polymer complex. The invention also relates to processes for preparing the complexes, pharmaceutical compositions containing same, methods of treatment involving the complexes and uses of the complexes in the manufacture of medicaments.

AMPLIFICATION OF FOLATE-MEDIATED TARGETING TO TUMOR CELLS USING POLYMERS

Technical Field

The invention relates to the delivery of drug, peptide and protein pharmaceuticals using the folate-mediated uptake system. More particularly the invention relates to the amplification of drug/pharmaceutical delivery with the folate uptake system using a folate-polymer complex. The invention also relates to processes for preparing the complexes, pharmaceutical compositions containing same, methods of treatment involving the complexes and uses of the complexes in the manufacture of medicaments.

Background Art

In conventional cancer chemotherapy, in order to obtain a linear increase in killing of cancer cells it is often necessary to increase the quantity of cytotoxic drugs present in the system in an exponential fashion. This in turn leads to an undesirable increase in non-specific cytotoxicity of bistander, healthy cells. Hence it is often necessary to repeatedly deliver a smaller dose of cytotoxin, which inevitably leads to the survival of a small fraction of drug-resistant cells. In an attempt to increase the dose of cytotoxic agent delivered to the tumor cell, specific targeting agents such as monoclonal antibodies to "tumor-specific antigens" have been employed. In many cases the resultant antibody-drug conjugate may be highly immunogenic, and thus lead to an antibody response against the conjugate thereby precluding further use. For this reason small, poorly immunogenic, tumor-specific molecules have been sought as alternatives to antibody molecules. Recently focus has switched to the use of molecules essential for growth to be used as targeting agents for drug delivery. The use of one of these, folic acid, is the subject of the current application.

Folic acid enters cells either through a carrier protein, termed the reduced folate carrier, or via receptor-mediated endocytosis facilitated by the folate receptor. The folate receptor is significantly over-expressed on a large fraction of human cancer cells including ovarian, breast, lung, endometrial, renal, colon, and cancers of myeloid hematopoietic cells. There are two folate receptors FR-α, and FR-β. In general FR-α, is upregulated in malignant tissues of epithelial origin such as ovarian carcinoma, while FR-β is overexpressed in malignant tissues of nonepithelial origin. While the FR have been detected in normal tissues involved in the retention and uptake of the vitamin, these tissues are in protected sites and generally not accessible following blood-borne delivery of folate conjugates. Thus there is expression in the choroid plexus, the intestinal brush border apical membrane surface and the proximal tubules of the kidney. In the latter case the receptor probably functions to scavenge excreted folate, and as such would not be accessible to large molecule weight folate complexes. Folate-mediated tumor targeting has been exploited to date for delivery of the following molecules and molecular complexes (i) protein toxins, (ii) low-molecular-weight chemotherapeutic agents, (iii) radio-imaging agents (iv) MRI contrast agents, (v) radiotherapeutic agents, (vi) liposomes with entrapped drugs, (vii) genes, (viii) antisense oligonucleotides, (ix) ribozymes, and (x) immunotherapeutic agents.

Two major limitations to the use of folate to target to tumor cells is that the dose deliverable is small, *i.e.* one molecule of drug for each molecule of folate, and that the majority of the folate-drug complexes are very small and as such are excreted in the kidneys and re-absorbed in the proximal tubules, thus leading to undesirable accumulation of folate-drug complexes in the kidney.

It is an object of the present invention to overcome or at least alleviate one or more of the above-mentioned disadvantages of the prior art.

Summary of the Invention

Surprisingly it has been found by the present inventors that both of the above-mentioned limitations could be addressed by producing large molecular weight polymer complexes incorporating folic acid or analogues thereof and a

pharmaceutically active substance to be delivered. Thus, amplification can occur by linkage of the pharmaceutical to a polymer backbone to which a small number of folate molecules are linked, either subsequently, previously or concurrently. The large size of the polymer also minimises accumulation of the folate-drug polymers in the kidneys.

Accordingly this invention provides a new set of drug/pharmaceutical-polymer conjugates, to which a folate molecule, or analogue thereof, has been conjugated. These folate-polymer-drug conjugates are suitable for parenteral delivery to tumors as they can utilise the aforementioned folate receptor system for uptake binding and uptake, and have the added advantage of increasing the amount of pharmaceutical agent which can be delivered via the folate uptake mechanism, as well as minimising or avoiding targeting to the kidneys by virtue of their size.

According to a first aspect of the present invention there is provided a polymer complex having the general formula:

$$(\text{folate-Q})_n\text{-P-(Q'-A)}_m$$

wherein, folate, or an analogue thereof, is a targeting molecule which will bind to a natural folate receptor, preferably a surface folate receptor on a tumor or cancer cell;

n, the molar substitution ration of folate in the complex, is in the range from 1.0 to 50.0, preferably from 1.0 to 1.5;

P is a pharmaceutically acceptable polymer;

A is a pharmaceutically active substance;

m, the molar substitution ratio of A in the complex, is in the range from 1.0 to 1000, preferably from 2 to 200, more preferably from 10 to 100; and

Q and Q' are independently a covalent bond, or a spacer compound linking folate, P and A by covalent bonds.

The polymer complexes of the invention may comprise more than one active substance linked to the polymer, which in turn is linked to at least one targeting molecule which is a folic acid molecule, or analogue thereof, wherein the ability of the targeting molecule to undergo the binding reactions necessary for uptake and

transport of folate in a vertebrate host and the activity of the active substance(s) are substantially maintained, following conjugation or following biological release of the active substance from the polymer.

According to a second aspect of the present invention there is provided a process for the production of a polymer complex of the invention incorporating one or more active substances, which process comprises one or more of the following steps:

a) reacting the active substance with the polymer to form said complex;

b) chemically modifying the active substance to provide at least one functional group capable of forming a chemical linkage, and reacting the active substance and polymer to form said complex;

c) chemically modifying the folate or folate analogue targeting molecule (herein after referred to as TM) to provide at least one functional group capable of forming a chemical linkage and reacting the TM and polymer to form said complex;

d) chemically modifying the active substance and the polymer to provide functional groups capable of forming a chemical linkage, and reacting the active substance and polymer to form said complex;

e) reacting the active substance with at least one cross-linking agent and reacting the active substance of polymer to form said complex;

f) reacting the TM with at least one cross-linking agent and reacting the polymer and TM to form said complex;

g) reacting the active substance and polymer with at least one cross-linking agent and reacting the active substance and polymer to form said complex;

h) reacting the active substance directly with a polymeric support to form an intermediate containing one or more molecules of the active substance linked to the polymer, and subsequently coupling the polymer-active substance intermediate to one or more targeting molecule;

i) coupling one or more TM molecules to a polymeric support and subsequently reacting the folate-polymer intermediate with one or more molecules of the active substance to give a final complex containing one or more molecules of the active substance.

According to a third aspect of the present invention there is provided a process for the production of a complex having the general formula:

$$(\text{folate-Q})_n\text{-P-(Q'-A)}_m$$

wherein folate, Q, P, Q', A, n and m are as defined above, said process selected from:

a) reacting A with P to form an intermediate complex, and thereafter reacting the intermediate complex with folate;

b) reacting folate with P to form an intermediate complex and thereafter reacting the intermediate complex with A;

c) the process of step a) or step b) wherein one or more of folate, P or A are modified to provide at least one functional group capable of forming a chemical linkage prior to coupling with the other reactants; or

d) reacting one or two of folate, P or A with Q and/or Q' prior to coupling with the other reactants.

The invention further provides a method for the modification of a polymeric support to introduce functional groups capable of reacting either directly with the active substance or with a chemically-modified form of the active substance. The resulting polymer-active substance intermediate contains one or more molecules of the active substance, said intermediate being suitable for coupling to the TM to give a complex capable of amplified delivery of the active substance.

According to a fourth aspect of the present invention there is provided a pharmaceutical composition which comprises a polymer complex of the present invention in association with a pharmaceutically acceptable carrier, excipient, diluent and/or adjuvant.

According to a fifth aspect of the present invention there is provided a method for the treatment, prophylaxis or amelioration of disease, in particular a tumor or cancer cell in a vertebrate host, which method comprises administering to said host a therapeutically effective amount of a polymer complex or composition of the present invention.

According to a sixth aspect of the present invention there is provided the use of a polymer complex of the invention in the preparation of a medicament for the treatment, prophylaxis or amelioration of disease, preferably cancer.

According to a seventh aspect of the present invention there is provided a method of delivering an active substance to a tumor or cancer cell comprising contacting said tumor or cancer cell with a polymer complex of the invention. The method of delivering the active substance may be achieved *in vivo* by administering the polymer complex to a host, preferably a vertebrate host, of said tumor or cancer cell.

The polymer complex of the invention has coupled to it one or more molecules of one or more active substances to be delivered, the polymer being coupled to the TM to give a complex capable of amplified delivery of the active substances.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

Brief Description of the Figure

The present invention will now be described by way of example only and with reference to the figure wherein:

Figure 1 represents the biodistribution of folate derivatized HMPA and CMC polymers in the presence and absence of the excess folate. The recovery of various folate-polymer complexes from Balb/C mice injected with hybridoma tumor cells are compared by plotting the percentage of recovered injected counts. The data shows that the level of polymer uptake by liver and tumor cells can be enhanced by folate derivatization of the polymers.

Detailed Description of the Invention

The polymer complexes of the present invention have been especially targeted to tumors and cancer cells using folic acid or analogues thereof as the targeting moiety. Once the drug-polymer has reached its target tissue the complex is endocytosed by the target cell and the pendant drug may be released by the action of lysosomal enzymes, by cleavage of a disulfide linked drug by intracellular glutathione or otherwise. While it is possible that these complexes could be used for oral delivery of the drug to the circulatory or lymphatic drainage system in general, the products of this invention and a co-pending nanoparticle delivery invention (Australian Provisional Patent Application No. PQ0146 entitled "Amplification of Folate-Mediated Targeting to Tumor Cells using Nanoparticles" filed on 4 May 1999 and incorporated herein in its entirety by reference) preferably relate to targeting the active substances to tumor/cancer cells.

The target molecules utilised in the invention are folate molecules or analogues thereof, which possess binding activity for the folic acid receptor, and in particular to surface folate receptors on tumor cells. Analogues contemplated herein include, but are not limited to, modification to the ring structure, functional groups or side chains of the folic acid molecule including the additional removal of protecting groups and salts and complexes thereof derived from any source such as being chemically synthesised or identified by screening process such as natural product screening provided that the analogue possesses some binding activity for the folic acid receptor.

The polymer, P, of the present invention can be any pharmaceutically acceptable polymer. The polymer is able to attach to at least one folate molecule and to at least one, but preferably a multiplicity of active substance molecules.

Suitable polymers for substitution with folate and modification according to the invention, include but are not limited to poly[N-(2-hydroxypropyl)-methacrylamide], dextran, chondroitan sulfate, water soluble polyurethanes formed by covalent linkage of PEG with lysine, poly(glutamic acid), poly(hydroxypropyl glutamine) and branched chain polypeptides formed by the dual modification of the α and ε-amino groups of lysine during the peptide synthesis, as well as dendrimers and PEG-

dendrimers. Such polymers may have multiple amino-termini, to which can be conjugated a plurality of the pharmaceutical or drug to be delivered. The polymers can also be formed with multiple cystines, to provide free thiols, or multiple glutamates or aspartates, to provide free carboxyls for conjugation using suitable carbodiimides. Similarly the polymer can contain multiple histidines or tyrosines for conjugation. Particularly suitable pharmaceutically acceptable polymers for use in the invention include those having the sequence of $[(NH_2\text{-}X_o)_4\text{-}Lys_2\text{-}Y_2\text{-}Lys]_n\text{-}Z_m\text{-}COOH$, wherein n = 1 to 85; m = 1 to 10; o = 1 to 10; X is any amino acid, Y is any amino acid, and Z is any amino acid, and more preferably polymers having the sequence of $[(NH_2\text{-}Gly)_4\text{-}Lys_2\text{-}Ser_2\text{-}Lys]_n\text{-}Ala\text{-}COOH$, where n = 1 to 85. Other suitable polymers include those having the sequence of $[(NH_2\text{-}X)_{16}\text{-}Lys_8\text{-}Lys_4\text{-}Y_4\text{-}Z_4\text{-}Lys_2\text{-}Lys]_n\text{-}AA_m\text{-}Cys\text{-}COOH$, wherein n = 1 to 85; m = 1 to 10; and X, Y, Z and AA independently represent any amino acid, and more preferably those having the sequence of $[(NH_2\text{-}Gly)_{16}\text{-}Lys_8\text{-}Lys_4\text{-}His_4\text{-}Glu_4\text{-}Lys_2\text{-}Lys]_n\text{-}Gly_m\text{-}Cys\text{-}COOH$, wherein n = 1 to 85; and m = 1 to 10.

Preferably the linkage to the polymer, or the polymer to which the pharmaceutical is linked, should be biodegradable. Potentially biodegradable polymers include dextran and its derivatives, and amino acid polymers such as poly-lysine, poly-glutamic acid.

Non-biodegradable polymers may also be employed in the present invention and include poly[N-(2-hydroxypropyl)-methacrylamide], to which is attached biodegradable side chains such as those containing ester linkages, or amino acid sequences cleavable within lysosomal vacuoles *i.e.* Gly-Phe-Leu-Gly (Rihova, B. and J. Kopecek., 1985 Biological properties of targetable poly[N-(2-hydroxypropyl)-methacrylamide]-antibody complexes. J. Control Rel., 2 :289-310). Other amino acid spacers cleavable by intracellular proteases include Gly-Phe-Ala; Gly-Phe-Ala-Gly; Gly-Phe-Tyr-Ala; and Gly-Phe-Tyr-Ala-Ala [Rejmanova, P., Obereigner, B., and Kopecek, J., 1981 Makromol. Chem. 182 : 1899-1915].

Reference to the term "folate" as used herein is to be considered in its broadest context and refers to the carboxylic acid anion of folic acid and, where not stated, the

counter cation may be any suitable cation including pharmaceutically acceptable cations and may also include a proton, *i.e.* folic acid. The term "folate" may be taken to include reference to analogues of the folate molecule, such as methotrexate, and preferably where the analogue possesses some binding activity for the folic acid receptor.

Folate is most easily covalently attached to a ligand, or the polymer, via either its α or γ- carboxylic acid moiety. It has been shown, however, that the α-carboxyl derivatives have low avidity for the folate receptor, whereas the γ-carboxyl derivatives have similar affinity to native folate. Other functional groups may be employed as required.

In one embodiment of the invention the linkage joining the pharmaceutical, or the folate to the polymer is a disulfide bond. In a further embodiment of the invention the linkage joining the pharmaceutical, or the folate to the polymer is an ester linkage. In yet another embodiment of the invention the linkage joining the pharmaceutical or the folate to the polymer is a γ-glutamyl-ε-lysine bond. In yet another embodiment of the invention the linkage joining the pharmaceutical or the folate to the polymer is a diazo-linkage.

The spacer compounds Q and Q' are optional. When they are absent the TM folate, and/or the active substance A are linked to polymer P by a direct covalent bond. They are introduced either to improve the folate receptor affinity of the folate complex or to overcome problems in the coupling of the carrier, folate, and/or the active substance A arising from unfavourable steric interactions between the folate and A with the polymer P, or to increase the bioactivity of A in the complex. The spacer compounds may also act as linking agents, being bi-functional compounds with selected functional groups on each end to react with suitable functional groups located on the polymer, and also on the folate targeting molecule and/or on the pharmaceutically active substances.

Suitable extended spacers for the conjugation of the pharmaceutical or folate to the polymer matrix preferably have from 1 to 50 atoms in its backbone. Such diradical spacers may be optionally substituted and contain within the chain double bonds, triple bonds, aryl groups and/or hetero atoms. Preferably the spacer compounds Q or Q' comprise optionally substituted saturated or unsaturated, branched or linear, C_{1-50} alkylene, cycloalkylene or aromatic group, optionally with one or more carbons within the chain being replaced with N, O or S, and wherein the optional substituents are selected from carbonyl, carboxy, hydroxy, amino and other groups. More preferably the extended spacers include: disuccinimidyl suberate (DSS), *bis*(sulfosuccinimidyl) suberate (BSS), ethylene glycol*bis*(succinimidylsuccinate) (EGS), ethylene glycol*bis*(sulfosuccinimidylsuccinate) (Sulfo-EGS), p-amino-phenylacetic acid, dithio*bis*(succinimidylpropionate) (DSP), 3,3'-dithio*bis*(sulfosuccinimidylpropionate) (DTSSP), disuccinimidyl tartarate (DST), disulfosuccinimidyl tartarate (Sulfo-DST), *bis*[2-(succinimidyloxycarbonyloxy)-ethylene]sulfone (BSOCOES), *bis*[2-(sulfosuccinimidooxycarbonyloxy)-ethylene]sulfone (Sulfo-BSOCOES), dimethyl adipimidate.2 HCl (DMA), dimethyl pimelimidate.2 HCl (DMP), dimethyl suberimidate.2 HCl (DMS), *N*-succinimidyl(4-iodoacetyl)aminobenzoate (SIAB) and succinimidyl 4-(p-maleimidophyl)butyrate (SMPB).

Suitable cross-linking agents for use in the preparation of thiol-cleavable biodegradable linkers include *N*-succinimidyl 3-(2-pyridyldithio)propionate (SPDP), iminothiolane, sulfosuccinimidyl 6-[3-(2-pyridyldithio) propionamido] hexanoate (Sulfo-LC-SPDP), succinimidyl 6-[3-(2-pyridyldithio) propionamido] hexanoate (LC-SPDP), sulfosuccinimidyl 6-[α-methyl-α-(2-pyridyldithio) toluamido]hexanoate (Sulfo-LC-SMPT), 1,4-di[3'-(2'-pyridyldithio)propionamido]butane (DPDPB), 4-succinimidyloxycarbonyl-α-methyl-α-(2-pyridyldithio)-toluene (SMPT) and dimethyl 3,3'dithiobispropionimidate.2 HCl (DTBP).

The active substance to be delivered is preferably a hormone, drug, prodrug, toxin, pharmaceutically active protein, immunogen, or DNA or RNA analogue.

Suitable toxins, according to the invention, include, but are not limited to, ricin, abrin, diphtheria toxin, modecin, tetanus toxin, mycotoxins, mellitin, α-amanitin, pokeweed antiviral protein, ribosome inhibiting proteins, especially those of wheat, barley, corn, rye, gelonin and maytansinoid.

Suitable cytotoxic agents, according to the invention, include, but are not limited to alkylating agents such as chlorambucil, cyclophosphamide, melphalan, cyclopropane; anthracycline antitumor antibiotics such as doxorubicin, daunomycin, adriamycin, mitomycin C, 2-(hydroxymethyl)anthraquinone; antimetabolites such as methotrexate, dichloromethatrexate: cisplatin, carboplatin, and metallopeptides containing platinum, copper, vanadium, iron, cobalt, gold, cadmium, zinc and nickel. Other agents include DON, thymidine, pentamethylmelamin, dianhydrogalactitol, 5-Methyl-THF, anguidine, maytansine, neocarzinostatin, chlorozotocin, AZQ, 2'deoxycoformycin, PALA, AD-32, *m*-AMSA and misonidazole.

Other active substances which may be delivered by the folate polymers of the invention include but are not limited to hormones and bioactive peptides and polypeptides, antibiotics, antipyretics, analgesics and antiinflammatory drugs, expectorants, sedatives, muscle relaxants, antiepileptics, antiulcer drugs, antidepressants, antiallergic drugs, cardiotonic drugs, antiarrythmic agents, vasodilators, antihypertensives, anticoagulants and haemostatic agents as known in the art.

Reference herein to "treatment" and "prophylaxis" is to be considered in its broadest context. The term "treatment" does not necessarily imply that a host is treated until total recovery. Similarly, "prophylaxis" does not necessarily mean that the subject will not eventually contract a disease condition. Accordingly, treatment and prophylaxis include amelioration of the symptoms of a particular condition or preventing or otherwise reducing the risk of developing a particular condition. The term "prophylaxis" may be considered as reducing the severity of onset of a particular condition. "Treatment" may also reduce the severity of an existing condition.

The subject of the treatment or prophylaxis is preferably a mammal such as but not limited to human, primate, livestock animal (e.g. sheep, cow, horse, donkey, pig) companion animal (e.g. dog, cat) laboratory test animal (e.g. mouse, rabbit, rat, guinea pig, hamster) captive wild animal (e.g. fox, deer). Preferably the mammal is a human or primate. Most preferably the mammal is a human.

It will be understood that those skilled in the art will be able to employ methods commonly known in the art for preparing suitable medicaments in concentrations and presented in forms appropriate to the administration of the folate complexes of the invention, optionally with other active agents as required, in suitable treatment regimes to achieve the desired physiological effects on the vertebrate host to be treated.

In accordance with these methods, the agents herein defined may be coadministered with one or more other compounds or molecules. For example, the polymer complex of the invention may be administered in combination with folate nanoparticle complexes, other chemotherapeutic agents or other ameliorative active substances. By "administered in combination" is meant simultaneous administration in the same formulation or in two different formulations via the same or different routes or sequential administration by the same or different routes. By "sequential" administration is meant a time difference of from seconds, minutes, hours or days between the administration of the formulations. These agents may be administered in any order.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions (where water soluble) and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained,

for example, by the use of a coating such as licithin, by the maintenance of the required particle size in the case of dispersion and by the use of superfactants. The preventions of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimersal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminium monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilisation. Generally, dispersions are prepared by incorporating the various sterilised active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

When the active ingredients are suitably protected they may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsule, or it may be compressed into tablets, or it may be incorporated directly with the food of the diet. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, gels, pastes, viscous colloidal dispersions, syrups, wafers, and the like. Such compositions and preparations should contain at least 1% by weight of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 5 to about 80% of the weight of the unit. The amount of active compound in such therapeutically useful compositions in such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit form

contains between about 0.1 μg and 2000 mg of active compound. In the case of compositions containing supplementary active ingredients, the dosages are determined by reference to the usual dose and manner of administration of the said ingredients.

The tablets, troches, pills, capsules and the like may also contain the following: A binder such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; buffering agents such as sodium bicarbonate to neutralise or buffer stomach acid; and a sweetening agent such a sucrose, lactose or saccharin may be added or a flavouring agent such as peppermint, oil of wintergreen, or cherry flavouring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavouring such as cherry or orange flavour. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound may be incorporated into sustained-release preparations and formulations.

Pharmaceutically acceptable carriers and/or diluents include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, use thereof in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active

material calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the novel dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active material and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active material for the treatment of disease in living subjects having a diseased condition in which bodily health is impaired.

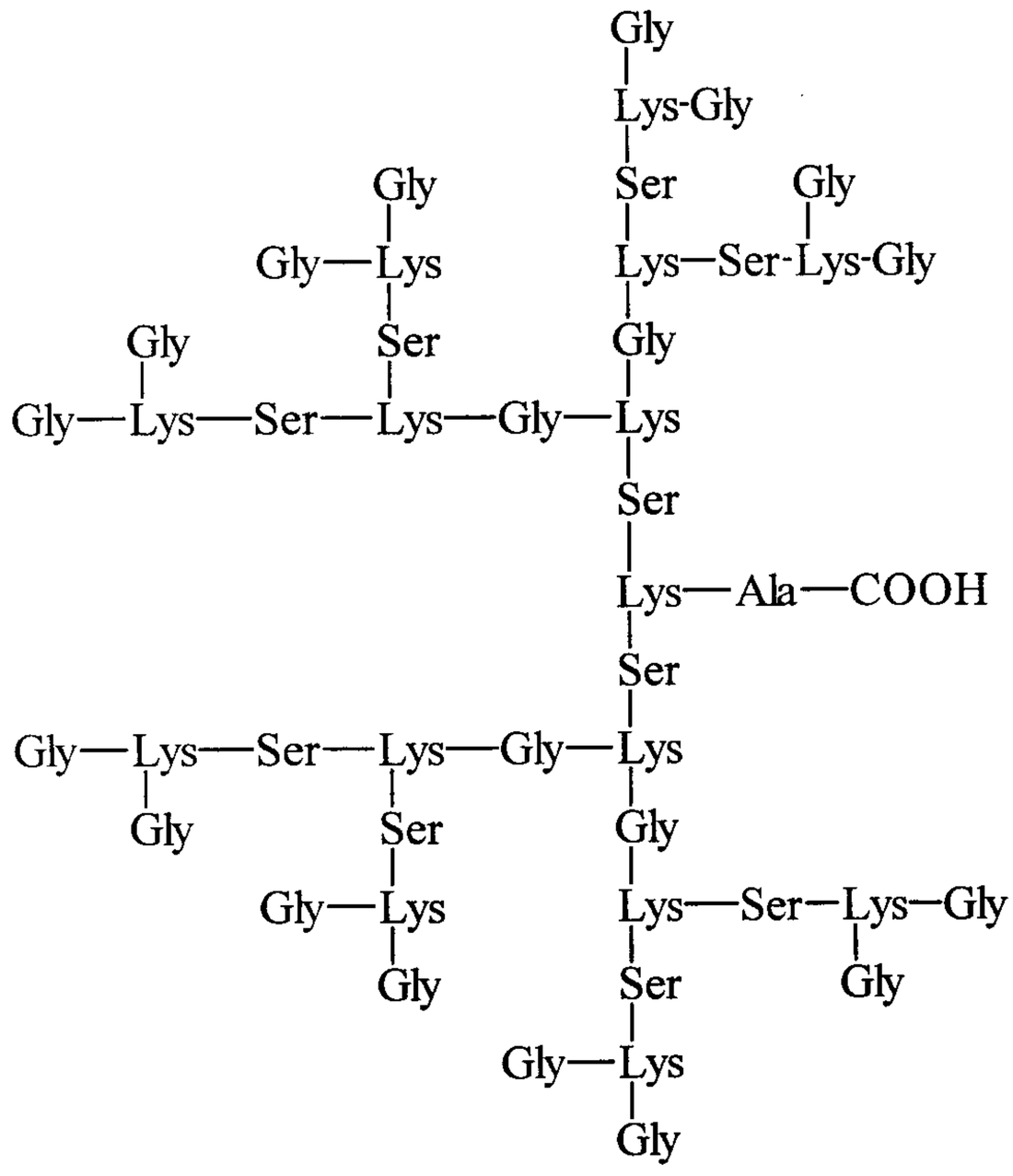
Administration of the agent in the form of a pharmaceutical composition may be performed by any convenient means. The agent of the pharmaceutical composition is contemplated to exhibit therapeutic activity when administered in an amount which depends on the particular case. Variation depends for example, on the human or animal and the agent chosen. Dosage regimes may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily, weekly, monthly or other suitable time intervals or the dose may be proportionally reduced as indicated by the exigencies of the situation. The agent may be administered in any suitable manner. Routes of administration include, but are not limited to, respiratorally, intratracheally, nasopharyngeally, intravenously, intraperitoneally, subcutaneously, intracranially, intradermally, intramuscularly, intraoccularly, intrathecally, intracereberally, intranasally, infusion, orally, rectally, *via* IV drip, patch and implant. With respect to intravenous routes, particularly suitable routes are via injection into vessels which supply the tumour or diseased organs. Peptides may also be installed into cavities for example the pleural or peritoneal cavity or injected directly into tumour tissues.

The present invention is further described with reference to the following examples which are in no way limiting on the scope of the invention.

Example 1. Synthesis of Multi-Lysine polymer 1 (MLP1)

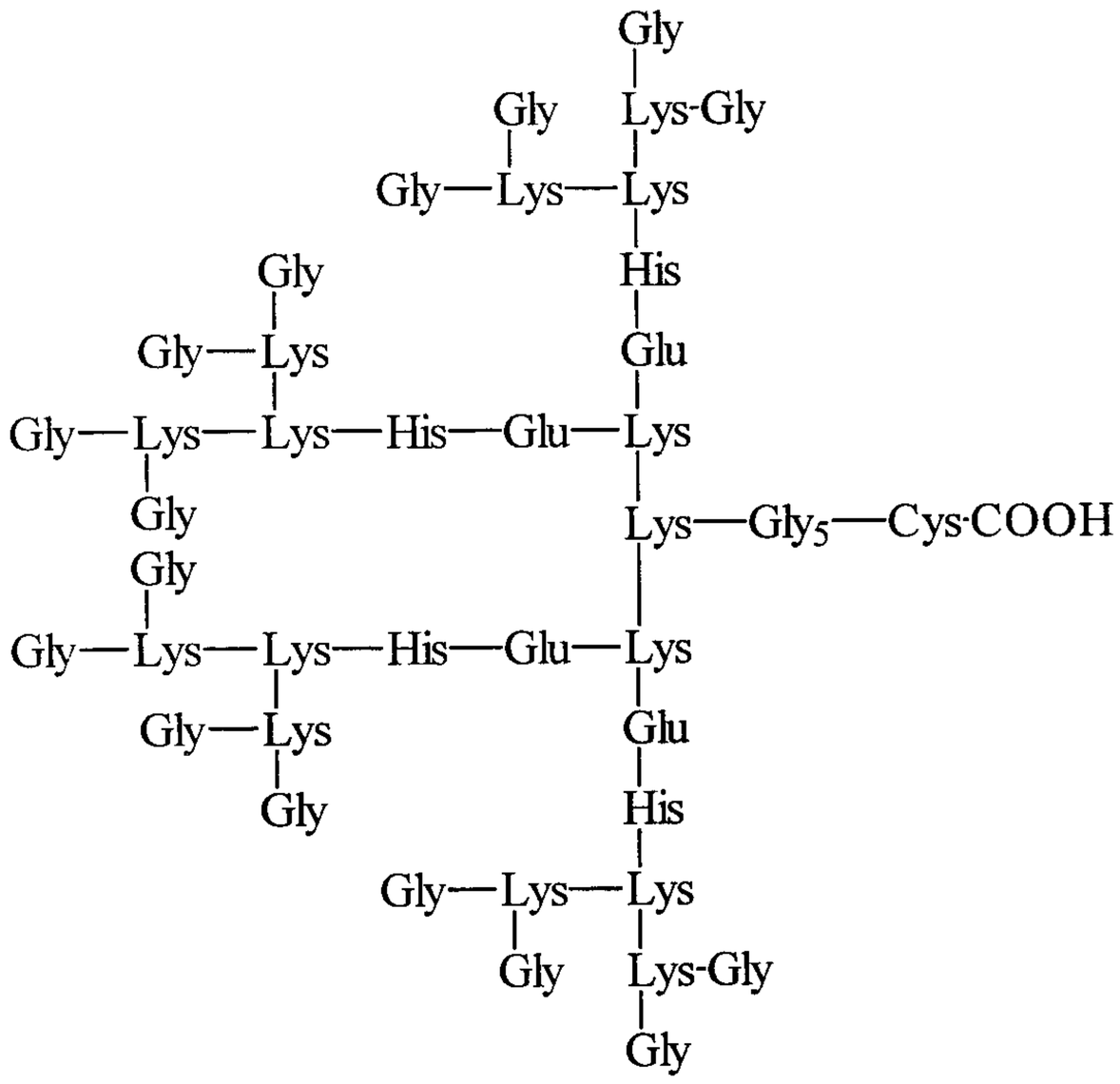
A multi-lysine polymer (MLP1) of the formula $[(NH_2\text{-Gly})_4\text{-Lys}_2\text{-Ser}_2\text{-Lys}]_5\text{-Ala-COOH}$, was synthesized on an Applied Biosystems peptide synthesiser. More

precisely this represents $[(NH_2\text{-Gly})_4\text{-Lys}_2\text{-Ser}_2\text{-Lys}]_4[\text{Gly}_4\text{-Lys}_2\text{-Ser}_2\text{-Lys}]$-Ala-COOH which can be represented as follows:



Example 2. Synthesis of Multi-Lysine polymer 2 (MLP2)

A multi-Lysine polymer (MLP2) of the general formula $[(NH_2\text{-Gly})_{16}\text{-Lys}_8\text{-Lys}_4\text{-His}_4\text{-Glu}_4\text{-Lys}_2\text{-Lys}]\text{-Gly}_5\text{-Cys-COOH}$ was synthesized on an Applied Biosystems peptide synthesiser. More precisely the structure can be represented as follows:



Example 3. Preparation of NHS-folate.

Folic acid (5g) was dissolved in 100 ml dry DMSO, plus 2.5 ml triethylamine. *N*-hydroxysuccinimide (2.6 g) was added as a powder to the folic acid and reacted overnight with 4.7 g dicyclohexylcarbodiimide at room temperature. The dicyclohexylurea was removed by filtration. The DMSO was concentrated under reduced pressure and heating, and NHS-folate precipitated with diethylether. The product, was washed several times with anhydrous ether, dried under vacuum and stored as a yellow powder.

Example 4. Formation of MLP-toxin conjugates using biodegradable cross-linkers.

There are many toxins which could be used for formation of folate-MLP-toxin conjugates, including momordin, Pseudomonas exotoxin A, ricin and abrin. A general method for the formation of folate-MLP-toxin conjugates is described below. Conjugates are prepared in which the covalent linker contains a biodegradable disulfide bond, which would be reduced *in vivo*, presumably by intracellular glutathione in the tumor cell, thereby releasing the active substance after transport from the serum into the tumor cell. Briefly, MLP1 or MLP2 was reacted with N-

succinimidyl 3-(2-pyridyldithio)propionate (SPDP). The dithiopyridyl-MLP (DTP-MLP) product was purified by RP-HPLC. A free thiol was introduced onto the toxin by a two step procedure in which the toxin was firstly reacted with SPDP, after which the thiopyridyl group was reduced with mercapto-ethanol. The product was purified by RP-HPLC. Alternatively free thiol was introduced into the toxin directly by reaction with iminothiolane. The thiolated product (SH-HN^{+}toxin) was purified by RP-HPLC. Formation of the disulfide linked MLP-toxin conjugates was achieved by reaction of the thiolated toxin derivative with DTP-MLP in 2.5% acetic acid for 24 hours. The conjugated material was purified by Sephadex G-25 chromatography, followed by RP-HPLC.

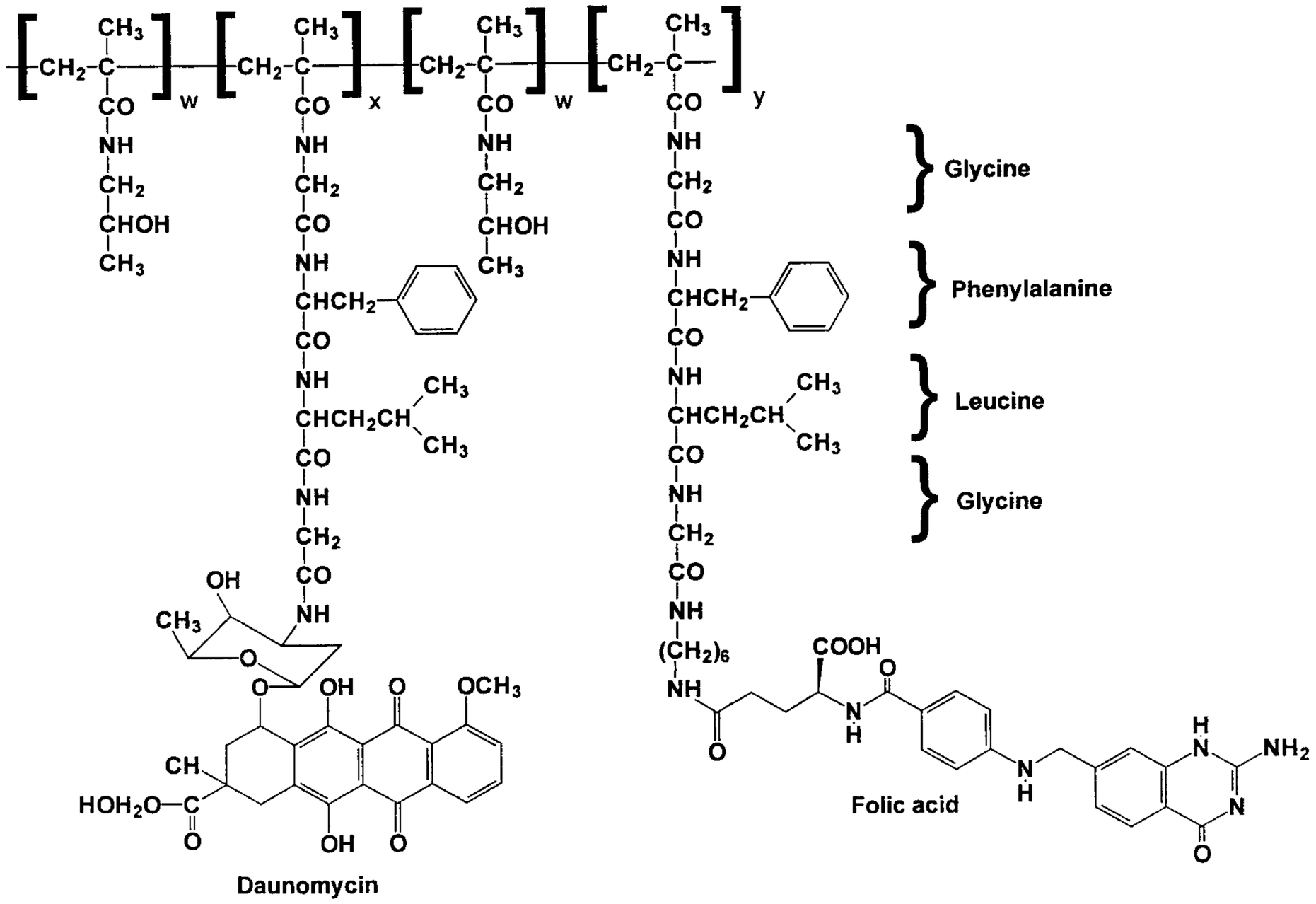
Example 5 Preparation of poly-drug-HPMA-folate complex.

Two N-(2-Hydroxypropyl)methacrylamide (HPMA) copolymers were synthesised as polymer backbones for the incorporation and derivatization with cytotoxic drugs and folate. A non-biodegradable polymer backbone (HPMA-GG) was synthesised by the free radical copolymerisation of HPMA with N-methacryloylglycylglycine p-nitrophenyl ester. A biodegradable polymer (HPMA-GFALG) was synthesised by the free radical copolymerisation of HPMA with N-methacryloylglycylphenylalanylleucylglycine p-nitrophenol ester by the method of Rejmanova and co-workers [Rejmanova,P., Obereigner, B., and Kopecek, J., 1981 Makromol. Chem. 182 : 1899-1915]. In order to incorporate ricin A chain and folate onto the polymers, they were reacted with a ten molar excess of a mixture of aminohexyl-folate and Dithiopyridyldodecylsuberyl-hexylamine (1:10 mole:mole) overnight. Unreacted nitrophenyl esters were subjected to aminolysis by the addition of 1-amino-2-propanol. The modified polymers were purified by chromatography on Sepharose 6B. A solution of the dithiopyridyldodecylsuberylhexyl modified folate-substituted polymers was dissolved in 2.5% acetic acid and reacted with ricin A chain. The reaction mixture was left for 144 hours at 4^{o}C, after which the ricin-folate-substituted polymers were purified by chromatography on Sepharose 6B.

Example 6 Preparation of poly-daunomycin-HPMA-folate complex.

An N-(2-Hydroxypropyl)methacrylamide (HPMA) copolymer was synthesised as a polymer backbone for the incorporation and derivatization with both the cytotoxic drug, daunomycin and folate. A biodegradable polymer (HPMA-GFLG) was synthesised by the free radical copolymerisation of HPMA with N-methacryloylglycylphenylleucinylglycine p-nitrophenol ester by the method of Rejmanova and co-workers [Rejmanova,P., Obereigner, B., and Kopecek, J., 1981 Makromol. Chem. 182 : 1899-1915]. In order to incorporate daunomycin and folate onto the polymers, they were reacted with a ten molar excess of a mixture of aminohexyl-folate and daunomycin (1:10 mole:mole) overnight. Unreacted nitrophenyl esters were subjected to aminolysis by the addition of 1-amino-2-propanol. The modified polymers were purified by chromatography on Sepharose 6B.

HPMA-Daunomycin-folate polymer



Example 7 Preparation of ^{125}I Labelled Polymers

Bolton-Hunter reagent was dissolved at 1 mg/ml in DMSO. The amino-derivatized polymer was dissolved at 5 mg/ml in DMSO or DW containing 25 µl/ml DIEA. A 3 µl aliquot of Bolton-Hunter was added to 20 µl of the polymer solution. The reaction was allowed to proceed for 3 hours. Unreacted Bolton-Hunter was extracted with DCM (5 × 100 µl) after addition of 50 µl water. ^{125}I (1 µl) was added to the derivatized polymer, followed by the addition of 4 µl Chloramine-T dissolved @ 20 mg/ml in PBS. The reaction proceeded for 15 secs, at which time the radioactive polymer was purified on PD10 column which had been equilibrated with 2.5% AcOH.

Example 8 Alternative Method of Preparation of Hydroxypropyl methacrylamide (HPMA)

1-Amino-2-propanol (58 g) was dissolved in acetonitrile (225 ml). The solution was cooled to −10 °C using an ethanol/dry ice bath. Methacryloyl chloride (40 g) in acetonitrile (170 ml) was added dropwise with vigorous stirring from a pressure equalising dropping funnel. The mixture was then allowed to warm slowly to room temperature overnight. The hydrochloride salt of 1-amino-2-propanol was removed by filtration through Celite filter aid. The solvent was removed at reduced pressure with a bath temperature of 50 °C. The product was isolated by dissolving in methanol and precipitation using acetone. The product was then dissolved in DW and dialysed extensively against DW.

Example 9 Preparation of Amino-HPMA

HPMA (4.0 g) was dissolved in DMSO (100 ml). A 1.5 ml aliquot of DIEA was added followed by 1.26 g of solid CDI (1,1'-carbonyldiimidazole). The HPMA was activated for 45 min, whereupon an excess of 1,6-diaminohexane (4.0 g) was added. The reaction proceeded for 2 h, at which time the product was dialysed to remove unreacted amines. The final product was lyophilised.

Example 10 Preparation of Aminohexyl-carboxymethyl cellulose (CMC)

CMC (low viscosity) was dissolved at 25 mg/ml in DW (2 g/40 ml). NHS (150 mg dissolved @ 100 mg/ml in acetone) was added followed by 300 mg dry EDAC. The

CMC was reacted for 15 minutes, whereupon 5 ml 1 M diaminohexane pH 9.5 was added and allowed to react O/WE. The product was dialysed exhaustively against DW. The product was then filter sterilised.

Example 11 Folate Derivatisation of Polymers

Folate (90 mg) was dissolved in DMSO (5.0 ml). DIEA (75 μL) was added, followed by TSTU ((O-(N-Succinimidyl)-N,N,N',N'-bis(tetramethylene)uronium hexafluorophosphate) (180 mg). The folate was activated for 10 min, then 1.0 g Polymer (amino-HPMA, or amino-hexyl-CMC) dissolved in DMSO (50 ml) was added to the activated folate solution and reacted overnight. The product was dialysed extensively to ensure removal of unreacted acid. The product was lyophilised.

Example 12 Preparation of methotrexate-GFLG-HPMA-Folate

Methotrexate-GFLG-OH (FW 828, 36 mg, 3 x folate) was dissolved in DMSO (5 ml). DIEA (20 μL) was added, followed by TSTU (35 mg). The methotrexate was activated for10 min. The polymer (100 mg) (Aminohexyl-HPMA or FA-hexyl-HPMA) dissolved in DMSO (15 ml) was added to the activated Drug-GFLG-acid solution and reacted 60 min. The product was dialysed extensively to ensure removal of unreacted acid and lyophilised.

Example 13 Preparation of methotrexate-GFLG-CMC-Folate

Methotrexate-GFLG-OH (FW 828, 36 mg, 3 x folate) was dissolved in DMSO (5 ml). DIEA (20 μL) was added, followed by TSTU (35 mg). The methotrexate was activated for10 min. The polymer (100 mg) (Aminohexyl-CMC or FA-hexyl-CMC) dissolved in DMSO (15 ml) was added to the activated Drug-GFLG-acid solution and reacted 60 min. The product was dialysed extensively to ensure removal of unreacted acid and lyophilised.

Example 14 Preparation of Clorambucil-GFLG-HPMA-Folate

Chlorambucil-GFLG-OH (FW 678, 29 mg, 3 x folate) was dissolved in DMSO (5 ml). DIEA (20 μL) was added, followed by TSTU (35 mg). The chlorambucil was

activated for10 min. The polymer (100 mg) (Aminohexyl-HPMA or FA-hexyl-HPMA) dissolved in DMSO (15 ml) was added to the activated Drug-GFLG-acid solution and reacted 60 min. The product was dialysed extensively to ensure removal of unreacted acid and lyophilised.

Example 15 Preparation of Chlorambucil-GFLG-CMC-Folate

Chlorambucil-GFLG-OH (FW 678, 29 mg, 3 x folate) was dissolved in DMSO (5 ml). DIEA (20 μL) was added, followed by TSTU (35 mg). The chlorambucil was activated for10 min. The polymer (100 mg) (Aminohexyl-CMC or FA-hexyl-CMC) dissolved in DMSO (15 ml) was added to the activated Drug-GFLG-acid solution and reacted 60 min. The product was dialysed extensively to ensure removal of unreacted acid and lyophilised.

Example 16 Preparation of HPMA-hexylaminosuccinate

Aminohexyl-HPMA (300 mg) was dissolved in DMSO (5 ml) and succinic anhydride (100 mg) and DIEA (100 μL) added. The polymer was reacted overnight then dialysed extensively against DW and lyophilised.

Example 17 Preparation of Daunomycin-GLFG-HPMA-folate

HPMA-hexylaminosuccinic acid (35 mg) was dissolved in DMSO (2.0 ml). TSTU (18 mg) was added and activated for 10 min. H_2N-GFLG-Daunomycin (FW 938, 3 x folate, 4.4 mg) was added and allowed to react for 5 min. For targeted polymers 6-aminohexyl FA (3 mg, designed to give 20% loading) was added and reacted for 1 h. The product was dialysed to remove unconjugated reagents. The final product was concentrated using an AMICON positive pressure stirred cell with 10K membrane.

Example 18 Preparation of MTX-GFLG-MLP-folate

MTX-GFLG-OH (FW 828, 25 mg) was dissolved in DMSO (2 ml). TEA (5 μl) was added, followed by TSTU (15 mg, 1.2 equiv.). The reaction was allowed to proceed for10 min, after which 13 mg MLP Polymer dissolved in DMSO (0.5 ml) was added and reacted for 60 min. For preparation of targeted polymers folate (8 mg) dissolved in DMSO (0.8 ml) was activated with TSTU (8.5 mg) for 10 min and then the

activated targeting agent was added to MTX-GFLG-MLP mixture. The reaction proceeded for 60 min. 0.1 M Tris pH 7.5 (5 ml) was added and stirred 1 h. The product was dialysed extensively and lyophilised.

Example 19 Demonstration of folate-mediated targeting of polymers.

In order to examine the potential utility of folate as a targeting agent for polymer-drug conjugates, folate substituted-polymers were substituted with ^{125}I-labelled Bolton-Hunter reagent. Control polymers were prepared without folate. For biodistribution studies, Balb/C mice were injected subcutaneously with $2x10^6$ hybridoma tumour cells. Two weeks after tumour injection, the radio-iodinated polymers were injected intravenously into the mice. At various time-points the mice were bled from the retro-orbital plexus, euthanased and their tissues removed for determination of radioactivity. Data is presented as the percentage of injected counts that were injected in the mice. As can be seen from the graph there was good evidence of folate-mediated targeting, particularly with HPMA, to the liver. Targeting was inhibited with excess folate. There was smaller, folate-enhanced targeting of both HPMA and CMC polymers to the tumour.

The data shows that the level of polymer uptake by liver and tumour cells can be enhanced by folate derivatization of the polymers.

Industrial Applications

The present invention provides a simple and novel technique for the amplification of the folate uptake system thus enabling the amplified delivery of a wide range of active agents to tumor and cancer cells in particular.

The claims defining the invention are as follows:

1. A complex having the general formula

$(folate\text{-}Q)_n\text{-}P\text{-}(Q'\text{-}A)_m$

wherein, folate, or an analogue thereof, is a targeting molecule, which will bind to a natural folate receptor;

n is a number from 1.0 to about 50;

P is a pharmaceutically acceptable polymer;

A is a pharmaceutically active substance;

m is a number greater than 1.0 to about 1000; and

Q and Q' are independently a covalent bond, or a spacer compound linking folate, P and A by covalent bonds.

2. The complex according to claim 1, wherein at least one of Q and Q' is a spacer compound which contains a biodegradable portion.

3. The complex according to claim 2 wherein said biodegradable portion is selected from a disulfide bond, ester linkage, a γ-glutamyl-ε-lysine linkage and a diazo bond, and Gly-Phe-Leu-Gly.

4. The complex according to claim 1, wherein n is from 1.0 to 1.5 and m is from 2 to about 200, more preferably from 10 to 100.

5. A complex according to claim 1 wherein P is a biodegradable polymer.

6. A complex according to claim 5 wherein said biodegradable polymer is selected from a biodegradable carbohydrate polymer or a polymer of amino acids.

7. A complex according to claim 5 wherein said polymer is a non-biodegradable.

8. A complex according to claim 7 wherein said non-biodegradable polymer comprises biodegradable side chains for covalent linkage to an active substance.

9. A complex according to claim 1 wherein said polymer is selected from poly[N-(2-hydroxypropyl)-methacrylamide], dextran or dextran derivatives, chondroitan sulfate, water soluble polyurethanes formed by covalent linkage of PEG with lysine, poly(glutamic acid), poly(hydroxypropyl glutamine), branched chain polypeptides, carboxymethyl cellulose, dendrimers and PEG-dendrimers.

10. A polymer according to claim 9 wherein said polymer is a branched chain polypeptide optionally modified to provide multiple functional groups for coupling of an active substance.

11. A complex according to claim 2 wherein said spacer compound Q or Q' comprises optionally substituted saturated or unsaturated, branched or linear, C_{1-50} alkylene, cycloalkylene or aromatic group, optionally with one or more carbons within the chain being replaced with N, O or S, and wherein the optional substituents are selected from carbonyl, carboxy, hydroxy, amino and other groups.

12. A complex according to claim 11 wherein said spacer compound is derived from disuccinimidyl suberate (DSS), *bis*(sulfosuccinimidyl) suberate (BSS), ethylene glycol*bis*(succinimidylsuccinate) (EGS), ethylene glycol*bis*(sulfosuccinimidylsuccinate) (Sulfo-EGS), p-amino-phenylacetic acid, dithio*bis*(succinimidylpropionate) (DSP), 3,3'-dithio*bis*(sulfosuccinimidylpropionate) (DTSSP), disuccinimidyl tartarate (DST), disulfosuccinimidyl tartarate (Sulfo-DST), *bis*[2-(succinimidyloxycarbonyloxy)-ethylene]sulfone (BSOCOES), *bis*[2-(sulfosuccinimidooxycarbonyloxy)-ethylene]sulfone (Sulfo-BSOCOES), dimethyl adipimidate.2 HCl (DMA), dimethyl pimelimidate.2 HCl (DMP), dimethyl suberimidate.2 HCl (DMS).

13. A complex according to claim 11 wherein said spacer compound is thiol cleavable.

14. A complex according to claim 13 wherein said thiol-cleavable spacer is derived from *N*-succinimidyl 3-(2-pyridyldithio)propionate (SPDP), iminothiolane, sulfosuccinimidyl 6-[3-(2-pyridyldithio) propionamido] hexanoate (Sulfo-LC-SPDP), succinimidyl 6-[3-(2-pyridyldithio) propionamido] hexanoate (LC-SPDP), sulfosuccinimidyl 6-[α-methyl-α-(2-pyridyldithio) toluamido]hexanoate (Sulfo-LC-SMPT), 1,4-di[3'-(2'-pyridyldithio)propionamido]butane (DPDPB), 4-succinimidyloxycarbonyl-α-methyl-α-(2-pyridyldithio)-toluene (SMPT) or dimethyl 3,3'dithiobispropionimidate.2 HCl (DTBP).

15. A complex according to claim 1 wherein said active substance A is a hormone, drug, prodrug, toxin, pharmaceutically active protein, immunogen, or DNA or RNA analogue

16. A complex according to claim 15 wherein said toxin is selected from ricin, abrin, diphtheria toxin, modecin, tetanus toxin, mycotoxins, mellitin, α-amanitin, pokeweed antiviral protein, ribosome inhibiting proteins, especially those of wheat, barley, corn, rye, gelonin and maytansinoid.

17. A complex according to claim 15 wherein said toxin is a cytotoxin selected from the group consisting of chlorambucil, cyclophosphamide, melphalan, cyclopropane, doxorubicin, daunomycin, adriamycin, mitomycin C, [2-(hydroxymethyl)anthraquinone], methotrexate, dichloromethatrexate: cisplatin, carboplatin, metallopeptides containing platinum, copper, vanadium, iron, cobalt, gold, cadmium, zinc and nickel, DON, thymidine, pentamethylmelamin, dianhydrogalactitol, 5-Methyl-THF, anguidine, maytansine, neocarzinostatin, chlorozotocin, AZQ, 2'deoxycoformycin, PALA, AD-32, *m*-AMSA and misonidazole

18. A complex according to claim 1 in which the pharmaceutically acceptable polymer has the sequence of $[(NH_2\text{-}X_o)_4\text{-}Lys_2\text{-}Y_2\text{-}Lys]_n\text{-}Z_m\text{-}COOH$, wherein n = 1 to 85; m = 1 to 10; o = 1 to 10; X is any amino acid, Y is any amino acid, and Z is any amino acid.

19. A complex according to claim 18 in which the pharmaceutically acceptable polymer has the sequence of $[(NH_2\text{-}Gly)_4\text{-}Lys_2\text{-}Ser_2\text{-}Lys]_n$-Ala-COOH, where n = 1 to 85.

20. A complex according to claim 1 in which the pharmaceutically acceptable polymer has the sequence of $[(NH_2\text{-}X)_{16}\text{-}Lys_8\text{-}Lys_4\text{-}Y_4\text{-}Z_4\text{-}Lys_2\text{-}Lys]_n\text{-}AA_m$-Cys-COOH, wherein n = 1 to 85; m = 1 to 10; and X, Y, Z and AA independently represent any amino acid.

21. A complex according to claim 20 in which the pharmaceutically acceptable polymer has the sequence of $[(NH_2\text{-}Gly)_{16}\text{-}Lys_8\text{-}Lys_4\text{-}His_4\text{-}Glu_4\text{-}Lys_2\text{-}Lys]_n\text{-}Gly_m$-Cys-COOH, wherein n= 1 to 85; and m = 1 to 10.

22. A complex according to claim 1 in which the pharmaceutically acceptable polymer is poly[N-(2-hydroxypropyl)-methacrylamide].

23. A process for the production of a complex having the general formula

$$(folate\text{-}Q)_n\text{-}P\text{-}(Q'\text{-}A)_m$$

wherein folate, Q, P, Q', A, n and m are as defined in claim 1, said process being selected from any one or more of the following steps:

a) reacting A with P to form an intermediated complex, and thereafter reacting the intermediate complex with folate;

b) reacting folate with P to form an intermediate complex and thereafter reacting the intermediate complex with A;

c) the process of step a) or step b) wherein one or more of folate, P or A are modified to provide at least one functional group capable of forming a chemical linkage prior to coupling with the other reactants; and

d) reacting one or two of folate, P or A with Q and/or Q' prior to coupling with the other reactants.

24. A process according to claim 23 wherein said spacer compound Q or Q' comprises optionally substituted saturated or unsaturated, branched or linear, C_{1-50} alkylene, cycloalkylene or aromatic group, optionally with one or more carbons within the chain being replaced with N, O or S, and wherein the optional substituents are selected from carbonyl, carboxy, hydroxy, amino and other groups

25. A process according to claim 23 wherein Q' is a cleavable cross-linking agent containing a disulfide bond.

26. A process according to claim 25 wherein the cross-linking agent is selected from disuccinimidyl suberate (DSS), *bis*(sulfosuccinimidyl) suberate (BSS), ethylene glycol*bis*(succinimidylsuccinate) (EGS), ethylene glycol*bis*(sulfosuccinimidylsuccinate) (Sulfo-EGS), p-amino-phenylacetic acid, dithio*bis*(succinimidylpropionate) (DSP), 3,3'-dithio*bis*(sulfosuccinimidylpropionate) (DTSSP), disuccinimidyl tartarate (DST), disulfosuccinimidyl tartarate (Sulfo-DST), *bis*[2-(succinimidyloxycarbonyloxy)-ethylene]sulfone (BSOCOES), *bis*[2-(sulfosuccinimidooxycarbonyloxy)-ethylene]sulfone (Sulfo-BSOCOES), dimethyl adipimidate.2 HCl (DMA), dimethyl pimelimidate.2 HCl (DMP) and dimethyl suberimidate.2 HCl (DMS).

27. A process according to claim 23 wherein said spacer is selected from disuccinimidyl suberate (DSS), *bis*(sulfosuccinimidyl) suberate (BSS), ethylene glycol*bis*(succinimidylsuccinate) (EGS), ethylene glycol*bis*(sulfosuccinimidylsuccinate) (Sulfo-EGS), p-amino-phenylacetic acid, dithio*bis*(succinimidylpropionate) (DSP), 3,3'-dithio*bis*(sulfosuccinimidylpropionate) (DTSSP), disuccinimidyl tartarate (DST), disulfosuccinimidyl tartarate (Sulfo-DST), *bis*[2-(succinimidyloxycarbonyloxy)-ethylene]sulfone (BSOCOES), *bis*[2-(sulfosuccinimidooxycarbonyloxy)-ethylene]sulfone (Sulfo-BSOCOES), dimethyl adipimidate.2 HCl (DMA), dimethyl pimelimidate.2 HCl (DMP), dimethyl suberimidate.2 HCl (DMS).

28. A process according to claim 23 wherein said spacer is selected from *N*-succinimidyl 3-(2-pyridyldithio)propionate (SPDP), iminothiolane, sulfosuccinimidyl 6-[3-(2-pyridyldithio) propionamido] hexanoate (Sulfo-LC-SPDP), succinimidyl 6-[3-(2-pyridyldithio) propionamido] hexanoate (LC-SPDP), sulfosuccinimidyl 6-[α-methyl-α-(2-pyridyldithio) toluamido]hexanoate (Sulfo-LC-SMPT), 1,4-di[3'-(2'-pyridyldithio)propionamido]butane (DPDPB), 4-succinimidyloxycarbonyl-α-methyl-α-(2-pyridyldithio)-toluene (SMPT) and dimethyl 3,3'dithiobispropionimidate.2 HCl (DTBP).

29. A process according to claim 25 wherein the cross-linking agent is selected from *N*-succinimidyl 3-(2-pyridyldithio)propionate (SPDP), iminothiolane, sulfosuccinimidyl 6-[3-(2-pyridyldithio) propionamido] hexanoate (Sulfo-LC-SPDP), succinimidyl 6-[3-(2-pyridyldithio) propionamido] hexanoate (LC-SPDP), sulfosuccinimidyl 6-[α-methyl-α-(2-pyridyldithio) toluamido]hexanoate (Sulfo-LC-SMPT), 1,4-di[3'-(2'-pyridyldithio)propionamido]butane (DPDPB), 4-succinimidyloxycarbonyl-α-methyl-α-(2-pyridyldithio)-toluene (SMPT) and dimethyl 3,3'dithiobispropionimidate.2 HCl (DTBP).

30. A complex prepared by a process of claim 23.

31. A pharmaceutical composition comprising a complex of claim 1 or claim 30 in association with a pharmaceutically acceptable carrier, excipient, diluent, or adjuvant.

32. A method for the treatment or prophylaxis of disease in a vertebrate host, including a human, which method comprises the administration to said host a therapeutically effective amount of a complex of claim 1 or 30 or a pharmaceutical composition of claim 31.

33. A method of claim 32 wherein the disease is cancer.

34. A method of claim 32 wherein the administration is parenteral administration.

35. Use of a polymer complex of claim 1 or claim 30 in the preparation of a medicament for the treatment, prophylaxis or amelioration of disease.

36. Use of claim 35 wherein the disease is cancer.

37. A method of delivering one or more active substances to a tumor or cancer cell comprising contacting said tumor or cancer cell with a polymer complex according to claim 1.

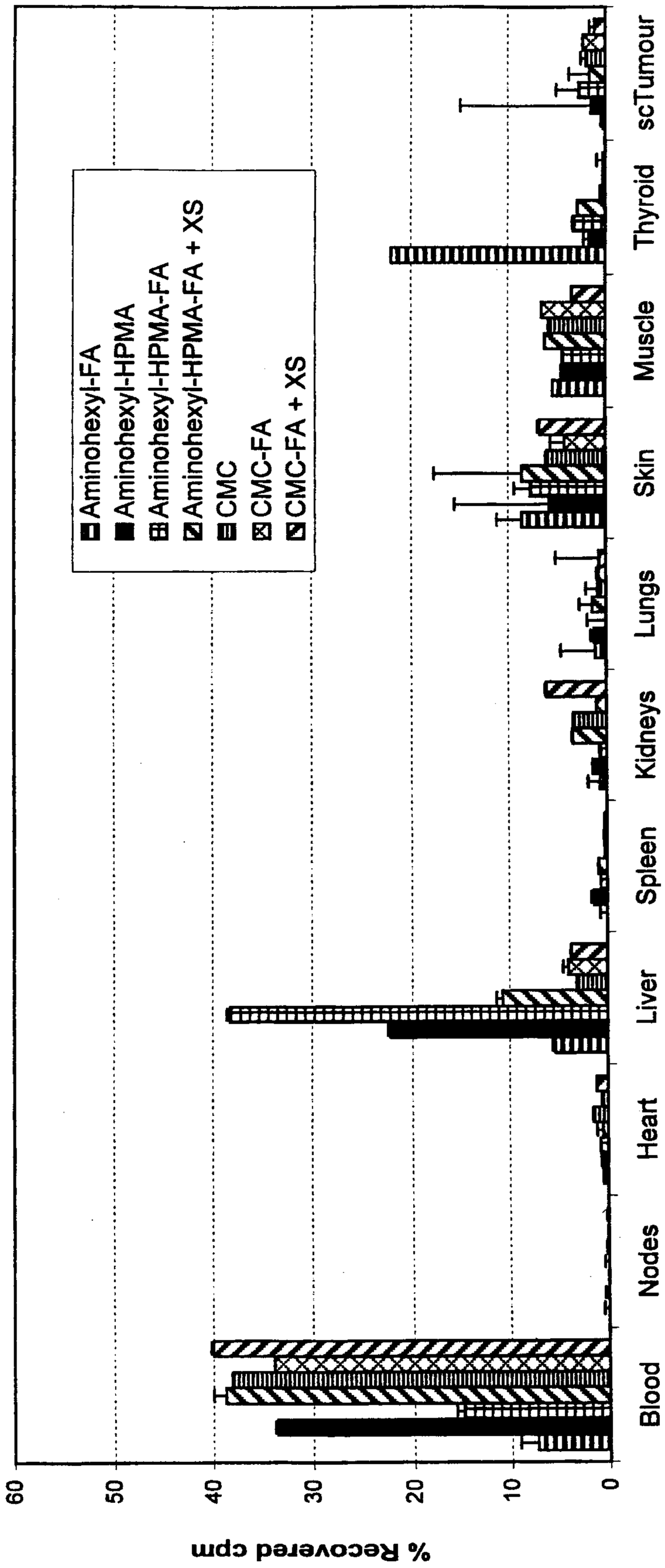

Distribution of Folate-Polymers in tumour mice, iv, T360
Aminohexyl-FA
Aminohexyl-HPMA
Aminohexyl-HPMA-FA
Aminohexyl-HPMA-FA + XS
CMC
CMC-FA
CMC-FA + XS
Thyroid
scTumour
Kidneys
Lungs
Skin
Muscle
Nodes
Heart
Liver
Spleen
Blood
Organ
60
50
40
30
20
10
0
% Recovered cpm

Figure 1