MANUFACTURE OF POLYGLUTAMATE-THERAPEUTIC AGENT CONJUGATES

Field of the Invention

This invention relates to a process for scaled-up manufacture of polyglutamate-therapeutic agent conjugates for clinical development.

BACKGROUND OF THE INVENTION

The antitumor agent paclitaxel shows increased efficacy and decreased toxicity when administered to tumor-bearing hosts as a polyglutamic acid conjugate compared with the unconjugated form of the drug (U.S. Patent No. 5,977,163; Li *et al.*, *Cancer Res.*, 58:2404, 1998). The polyglutamic acid-paclitaxel conjugate shows increased water solubility, a slower clearance from the body, and an increased accumulation in the tumor. Conjugates of polyglutamic acid and various other therapeutic agents are expected to provide clinically useful alternatives to the presently available formulations.

For research purposes, the polyglutamic acid-therapeutic agent conjugates can be produced by the method disclosed in Li *et al.*, *ibid.* In that method, the conjugate is prepared as a sodium salt, dialyzed to remove low molecular weight contaminants and excess salt and then lyophilized. The method is not well-suited for large-scale manufacture of quantities of conjugates for clinical development and use, however. In particular, the use of dialysis to remove impurities is time-consuming and lowers final product yield. In addition, although many pharmaceuticals have more favorable properties when prepared as salts (*e.g.*, improved solubility, storage, and handling), this is not true of the polyglutamate-therapeutic agent conjugates of the present invention. The salt forms of the conjugates are electrostatic solids, not free flowing powders. They are more

(b) protonating said polymer by the addition of an anhydrous acid to said suspension to form a soluble salt of the conjugate base;

(c) providing a therapeutic agent and covalently linking said agent to said polyglutamic acid polymer to form a polyglutamic acid-therapeutic agent conjugate;

(d) precipitating said polyglutamic acid-therapeutic agent conjugate from solution by the addition of an excess volume of aqueous salt solution; and

(e) collecting said conjugate as a protonated solid.

In another embodiment, the process for the large-scale manufacture of polyglutamic acid-2'paclitaxel conjugate comprises:

(a) providing an aqueous solution of the sodium salt of poly-L-glutamic acid;

(b) acidifying the solution to a pH of about 2 to 4, thereby converting the sodium salt of poly-L-glutamic acid to the protonated form and precipitating it from solution;

(c) collecting the poly-L-glutamic acid precipitate and washing with water;

(d) drying the poly-L-glutamic acid to a water content of between about 2% to about 7%, preferably between 7% and 21% and most preferably between about 7% to about 21 % by weight;

(e) contacting the poly-L-glutamic acid with paclitaxel under standard coupling conditions for sufficient time to conjugate paclitaxel to the polyglutamic acid polymer via an ester linkage formed between the 2'-OH group of paclitaxel and a carboxy group of poly-L-glutamic acid;

(f) cooling the reaction mixture from between 0° C to 10° C or between about 0° C to 10° C while slowly adding aqueous salt solution to the reaction mixture;

floxuridine (FUDR); cyclosporin; retinoic acids; 7-dimethyl-*tert*-butylsilyloxy)-10-hydroxy camptothecin and others.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1. Exemplary conjugates

Fig. 2. Manufacturing Scheme for poly-L-glutamic acid-paclitaxel conjugate

Fig. 3. Proton NMR scan of poly-L-glutamic acid paclitaxel conjugate

Fig. 4. Preparation of poly-L-glutamic acid-glycyl-20(S)camptothecin

Figs. 5-7. Reaction Schemes I-III.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

As used herein, "a polyglutamic acid" or "polyglutamic acid polymer " includes poly (l-glutamic acid), poly (d-glutamic acid) and poly (dl-glutamic acid). Preferably the polyglutamic acid polymer comprises at least 50% of its amino acid residues as glutamic acid, and more preferably, 100%. The polyglutamic acid polymer can be substituted up to 50% by naturally occurring or chemically modified amino acids, preferably hydrophilic amino acids, provided that when conjugated to a therapeutic agent, the substituted polyglutamic acid polymer has improved aqueous solubility and/or improved efficacy relative to the unconjugated therapeutic agent, and is preferably nonimmunogenic.

The molecular weight of the polyglutamic acid polymer used in the preparation of the conjugate by the methods described herein is typically greater than 5000 daltons, preferably from 15kd to 80kd, more preferably 20kd to 80kd, even more preferably from 20 kd to 60 kd, and most preferably from 30 kd to 60kd (as determined by viscosity). At the lower end of molecular weight, the

hydroxybutyric acid), diols, aminothiols, hydroxythiols, aminoalcohols, and combinations of these. A therapeutic agent can be linked to the polymer or linker by any linking method that results in a physiologically cleavable bond (*i.e.*, a bond that is cleavable by enzymatic or nonenzymatic mechanisms that pertain to conditions in a living animal organism). Examples of preferred linkages include ester, amide, carbamate, carbonate, acyloxyalkylether, acyloxyalkylthioether, acyloxyalkylester, acyloxyalkylamide, acyloxyalkoxycarbonyl, acyloxyalkylamine, acyloxyalkylamide, acyloxyalkylcarbamate, acyloxyalkylsulfonamide, ketal, acetal, disulfide, thioester, N-acylamide, alkoxycarbonyloxyalkyl, urea, and N-sulfonylimidate.

Methods for forming these linkages are well known to those skilled in synthetic organic chemistry, and can be found, for example, in standard texts, such as J. March, Advanced Organic Chemistry, Wiley Interscience, 4th Edition.

The degree of loading of bioactive, therapeutic or diagnostic agent on the polymer (*i.e.*, the" loading density") may be expressed as the number of molecules or average number of molecules per polyglutamic acid polymer chain or preferably as a percent (%) of total weight of the conjugate ("% loading"). A desired % loading can be obtained by adjusting the ratios of the therapeutic agent and polymer, and optimizing other reagents as necessary. The optimal loading density for a given conjugate and given use is determined empirically based on the desired properties of the conjugate (*e.g.*, water solubility, therapeutic efficacy, pharmacokinetic properties, toxicity and dosage requirements). The loading density ranges from between 1% to about 60% or from about 1% to about 60%, preferably from 5% to 55% or from about 5% to about 55%, and more preferably from 10% to 45% or from about 10% to about 45% for the conjugates that are specifically described herein.

relation to the peaks from the therapeutic agent. This is illustrated below for polyglutamic acid-paclitaxel conjugate.

The area between about 4.5 ppm to about 6.5 ppm, preferably 4.5 ppm and 6.5 ppm, is totalled and divided by the number of protons (7). This number is then compared to the area between about 3.8 ppm to about 4.4 ppm, preferably 3.8 ppm to 4.4 ppm, for the polymer backbone and is corrected for 2 protons from paclitaxel that overlap. The two areas per proton are compared taking into account the molecular weights of the paclitaxel and the polymer.

A= area per proton for polymer ÷area per proton for paclitaxel = 21.36/1.98=10.79.

MW paclitaxel = 837; MW of polyglutamic acid monomer is 129.

% loading = (837/(10.79 x 129) + 837) x 100 = 37.6%.

The processes described herein are generally useful for preparing conjugates of polyglutamic acid with any bioactive, therapeutic or diagnostic agent that is appropriately functionalized for linking to the polyglutamic acid, as described herein. The conjugates that are exemplified herein are intended to illustrate the invention, but not to limit its scope.

In one preferred embodiment, the therapeutic agents comprise drugs that are effective in treating cancerous conditions that are expected to benefit from the unique pharmacokinetic properties of these conjugate (*e.g.*, enhanced permeability and retention in tumor tissue, sustained release of active agent, long biological half life compared with the unconjugated agent, and others). Presently preferred agents include, by way of example, taxanes (*e.g.*, paclitaxel, docetaxel); etoposide; teniposide; epothilones, such as epothilone A, epothilone B, epothilone C, epothilone D, epothilone F and 12,13-disoxyepothilone F; gemcitabine; 20(S)(+) camptothecin; 9-aminocamptothecin; 9-nitrocamptothecin; 7-ethyl-10-hydroxycamptothecin; 9-dimethylaminomethyl-10-hydroxycamptothecin; 10,11-methylenedioxycamptothecin; 7-methylpiperizinomethyl-10,11-

methanesulphonyl chloride, pivaloyl chloride, bis(2-oxo-3-oxazolidinyl)phosphinic acid ("BOP-Cl"), 2-chloromethylpyridinium iodide ("CMPI"), and the like. Suitable catalysts for alcohol coupling include organic bases, *e.g.*, 4-N,N-dimethylaminopyridine and 4-pyrollidinopyridine.

As used herein, the term "inert solvent" means a solvent inert under the conditions of the reaction being described in conjunction therewith [including, for example, benzene, toluene, acetonitrile, tetrahydrofuran ("THF"), dimethylformamide ("DMF"), chloroform ("$CHCl_3$"), methylene chloride (or dichloromethane or "CH_2Cl_2"), diethyl ether, ethyl acetate, acetone, methylethyl ketone, dioxane, pyridine, dimethoxyethane, t-butyl methyl ether, and the like]. Unless specified to the contrary, the solvents used in the reactions of the present invention are inert solvents.

If multiple functional groups are present on the therapeutic agent, selective attachment of a particular group of the agent to the polyglutamic acid polymer will require the use of a suitable protecting group. The term "protecting group" or "blocking group" refers to any group which when bound to one or more hydroxyl, thiol, amino or carboxyl groups of the compounds prevents reactions from occurring at these groups and which protecting group can be removed by conventional chemical or enzymatic steps to reestablish the hydroxyl, thiol, amino or carboxyl group. *See*, generally, T.W. Greene & P.G.M. Wuts, "Protective Groups in Organic Synthesis," 3rd Ed, 1999 , John Wiley and Sons, N.Y.

The particular removable blocking group employed is not critical and preferred removable hydroxyl blocking groups include conventional substituents, such as allyl, benzyl, acetyl, chloroacetyl, thiobenzyl, benzylidine, phenacyl, t-butyl-diphenylsilyl ,
t-butyldimethylsilyl, triethylsilyl, MOM (methoxymethyl), MEM (2-methoxyethoxymethyl), t-BOC (*tert*-butyloxycarbonyl), CBZ (benzyloxycarbonyl) and any other group that can be introduced chemically onto a hydroxyl

(c) precipitating said polyglutamic acid-therapeutic agent conjugate from solution by addition of an excess volume of aqueous salt solution; and

(d) collecting said conjugate as a protonated solid.

The protonated form of the polyglutamic acid polymer in step (a) is obtained by acidifying a solution containing the salt of the polyglutamic acid to be used as a starting material, and converting the salt to its acid form. After separating the solid by centrifugation, the solid is washed with water. (When dimethylaminopyridine ("DMAP") is to be used in step (b), it is preferred to wash the solid until the aqueous phase is pH 3 or greater). The polyglutamic acid is then dried, preferably by lyophilization and preferably to a constant weight comprising between about 2% to about 21% water, preferably between about 7% to about 21% water, more preferably between 7% and 21% of water, prior to conjugation to a desired therapeutic agent (step (b)).

The therapeutic agent of step (b) may require modification prior to conjugation, *e.g.*, the introduction of a new functional group, the modification of a preexisting functional group or the attachment of a spacer molecule. Such modifications may require the use of protecting groups, which are described above.

Reaction schemes I-III illustrate methods that were used for linking various exemplary therapeutic agents to poly-L-glutamic acid (PG), either directly or through glycine spacer molecules. The conditions shown in these schemes and described in the Examples may be varied, as will be readily appreciated by those skilled in synthetic organic chemistry. The exact conditions that are used for conjugating a particular therapeutic agent to polyglutamic acid may be based on the stability of the therapeutic agent to the reaction conditions, the reactivity of the linking groups, other factors pertinent to the manufacturing process (*e.g.*, safety and regulatory issues), and the like. As described above, various types of linkages may be used in preparing the conjugates, depending on the available functional

Unreacted starting materials, byproducts and other impurities can be removed prior to, or after acidification to yield the final protonated conjugate (illustrated in Examples 2 and 3 below, and Figs. 2 and 4). For example, after addition of the salt solution, the solid can be collected and resolubilized, then either filtered or extracted with an appropriate solvent in which the contaminants are soluble but the conjugate is not (*e.g.*, ethyl acetate, methylene chloride, chloroform, hexanes, heptane, diethyl ether and dioxane). The solution is then acidified and the protonated form of the conjugate is collected as described above.

Alternatively, the solid can be lyophilized, then slurried with an appropriate solvent or mixtures thereof, *e.g.*, acetonitrile (MeCN); ethers, such as diethyl ether, dioxane, tetrahydrofuran; halogenated solvents, such as choloform, methylene chloride; ketones, such as acetone and methylethyl ketone (MEK); C_1 to C_{10} alcohols, such as *tert*-butyl alcohol, isopropyl alcohol, ethyl alcohol or methanol; to remove impurities from the final protonated conjugate product.

In an alternative preferred embodiment, step (c) above is replaced by step (c'), which comprises:

(c') separating said polyglutamic acid-therapeutic agent conjugate from unreacted starting materials and byproducts and precipitating said polyglutamic acid-therapeutic agent conjugate from solution by addition of an organic solvent in which unreacted starting materials and byproducts are soluble.

In addition to ethyl acetate and acetonitrile, examples of other solvents that can be used to purify the conjugate include chloroform, tetrahydrofuran, dioxane, toluene, 2-butylmethyl ether, and the like.

An alternative procedure is presently most preferred, wherein *in situ* generation of a protonated polyglutamic acid-therapeutic agent conjugate is carried out by a process comprising:

(a) suspending a salt of a polyglutamic acid polymer in an inert organic solvent;

(b) protonating said polymer by the addition of an anhydrous acid to said

TABLE 1. Analytical data

mass %[a]	total output[b] (g)	% loading (UV)[c]	% loading (NMR)[d]	% free paclitaxel[e]	% residual MeCN[f]	% residual DMF[g]	% DIPU[h]	ROI[i]
93.6	87.80	42.0	34.0	0.128	0.15	0.27	0.160	0.87

[a] % yield; [b] grams of conjugate; [c] grams paclitaxel/grams conjugate determined by UV method; [d] grams paclitaxel/grams conjugate determined by NMR method; [e] wt. % free paclitaxel relative to conjugate; [f] wt % residual acetonitrile relative to conjugate; [g] wt % residual dimethylformamide relative to conjugate; [h] wt % diisopropylurea relative to conjugate; [i] wt % residue on ignition.

TABLE 2. Analytical data

mass %[a]	total output[b] (g)	% loading (NMR)[d]	% residual MeCN[f]	% residual DMF[g]	% DIPU[h]
95.1	0.485	36.0	0-0.01	0.01-0.45	0

[a] % yield; [b] grams of conjugate; [d] grams paclitaxel/grams conjugate determined by NMR method; [f] wt % residual acetonitrile relative to conjugate; [g] wt % residual dimethylformamide relative to conjugate; [h] wt % diisopropylurea relative to conjugate.

The invention is illustrated by the following examples which should not be regarded as limiting the scope of the invention in any way.

EXAMPLES

In the following examples, the intermediates in the production of the conjugates were characterized by ^{1}HNMR. The molecular weights of the polyglutamic acid (Na salt) used to prepare the conjugates exemplified below range from 20 kd to 50 kd, as specified by the supplier (Sigma Chemical Co., Milwaukee, WI) based on viscosity measurements. The average loading density of the conjugates was 37%

to bring the pH of the solution to pH 7. The reaction was stirred for an hour and filtered through a 0.2 micron filter to remove impurities. The filtrate was cooled to 0°C-5°C and HCl (1N) was added slowly with vigorous stirring until the pH of the solution was brought to pH 3. Stirring was continued for 30 minutes. The precipitated solid was centrifuged at 1500 g, and the wet solid was washed twice by suspending in water (150 mL) and centrifuging. The product was lyophilized to yield 24 g of poly-L-glutamic acid-paclitaxel conjugate (90% yield).

In the above procedure, the filtration step can be omitted by washing the solution with ethyl acetate (250 mL, 2x) to remove impurities.

Figure 3 shows a representative proton NMR scan for poly-L-glutamic acid-2'- paclitaxel conjugate prepared by same procedure as described above, but having a higher paclitaxel loading (*i.e.*, 55%).

Example 3. Preparation of poly-L-glutamic acid-2'-paclitaxel conjugate (manufacturing process)

Poly-L-glutamic acid (42 g), prepared as described in Example 1 above, was added to a three-necked three liter round bottom flask equipped with a mechanical stirrer, addition funnel and a thermo probe. N,N-dimethylformamide (350 mL) was added and stirred for 10 minutes. Paclitaxel (24.66 g) and N,N-dimethylaminopyridine (0.70 g) was added and stirred for 10 minutes. A solution of N,N-diisopropylcarbodiimide (4.73 g) in N,N-dimethylformamide (143 mL) was added at room temperature over a period of 1 hr using the addition funnel and was stirred for four hours. The reaction mixture was cooled to 5°C-10°C and a cooled solution of 10% sodium chloride (1.2 L) was added dropwise using an addition funnel and keeping the temperature at 5°C-10°C by cooling the flask in an ice-salt mixture. After the addition of sodium chloride solution, 1N solution of hydrochloric acid (35 mL) was added dropwise until the pH of the reaction reached 2.5. The reaction mixture was stirred for 30 minutes at 5°C-10°C and the precipitated poly-L-glutamic acid-paclitaxel conjugate was collected by filtration.

with dichloromethane (2x 40 mL). The combined dichloromethane extracts were dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by silica gel flash chromatography eluting with 4% methanol/dichloromethane to yield 2'-(glycyl)paclitaxel (161 mg, 73 % yield) as a white powder.

Step 3. Preparation of poly-L-glutamic acid-2'-(glycyl) paclitaxel conjugate

To a stirred suspension of poly-L-glutamic acid (275 mg, 1.87 mmol) in anhydrous dimethylformamide (6 mL) was added 2'-(glycyl)paclitaxel (161 mg, 0.177 mmol). A solution of 1,3-diisopropylcarbodimide (29 mg, 0.23 mmol) in dimethylformamide (1.4 mL) was added to the stirred suspension over a period of 30 minutes. After stirring at room temperature for 3 hours, the mixture was cooled in an ice bath to bring the bath temperature to 0°C – 5°C and then 10 % aqueous sodium chloride solution (7 mL) was added over a period of 30 minutes to precipitate the poly-L-glutamic acid-2'-(glycyl)paclitaxel conjugate. The resulting white suspension was centrifuged at 1500 g, 15 minutes. After filtration, the solid was washed twice by suspension in water (10 mL) and centrifugation. The crude product was suspended in water (6 mL) and a 1 M aqueous sodium bicarbonate solution (2.3 mL) was added slowly with stirring to bring the contents of the flask to pH 7.6. After stirring for an additional 2 hours, the aqueous layer was washed with ethyl acetate (3 x 6 mL) and then acidified by addition of 1 N hydrochloric acid to pH 2.8. The precipitated solid was separated by centrifugation and washed with water (2 x 6 mL). The wet solid was lyophilized to give poly-L-glutamic acid-2'-(glycyl)paclitaxel conjugate (315 mg, 72 % yield) as a white powder.

Using a similar procedure, the above conjugate can be substituted by amino acids other than glycine.

Step 3. Preparation of 2',7-bis(triethylsilyl)-10-deacetylpaclitaxel imine

2',7-Bis(triethylsilyl)-10-deacetylpaclitaxelimine was prepared as described in U.S. Patent No. 5, 629,433.

To a solution of 2',7-bis(triethylsilyl)-10-deacetylpaclitaxel (730 mg, 0.70 mmol) in tetrahydrofuran (7.3 mL) was added zirconocene chloride hydride (543 mg, 2.11 mmol). After stirring at room temperature under an agron atmosphere for 15 hours, the mixture was poured into cold hexanes (75 mL). The precipitated zirconium complexes were removed by filtration. The filtrate was concentrated under reduced pressure to give 2',7-bis(triethylsilyl)-10-deacetylpaclitaxelimine (636 mg, 92% yield) as a white powder.

Step 4. Preparation of 10-deacetylpaclitaxel primary amine

10-Deacetylpaclitaxel primary amine was prepared according to U.S. Patent No. 5,629,433.

A solution of 2',7-bis(triethylsilyl)-10-deacetylpaclitaxel imine (636 mg, 0.621 mmol) in 1% (w/w) conc. hydrochloric acid/95% ethanol (25 mL) was stirred for 15 hours, treated with water (65 mL), and washed with hexanes (2 x 30 mL). The aqueous layer was neutralized (pH 7) by addition of saturated aqueous sodium bicarbonate solution and extracted with dichloromethane (2 x 40 mL). The combined extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure to yield the crude primary amine product (405 mg, 92% yield) as a white powder. This product was used for the next step without further purification.

Step 5. Preparation of docetaxel

Docetaxel was prepared according to U.S. Patent No. 5,629,433.

To a solution of 10-deacetylpaclitaxel primary amine (405 mg, 0.57 mmol) in ethyl acetate (40 mL) was added saturated aqueous sodium bicarbonate solution

Step 1. Preparation of 20-(N-t-BOC-glycyl)-20(S)camptothecin

To a solution of N-t-BOC-glycine (530 mg, 3.0 mmol) in anhydrous dichloromethane (240 mL) was added 1,3-diisopropylcarbodiimide (379 mg, 3.0 mmol), N,N-dimethylaminopyridine (244 mg, 2 mmol), and 20(S)camptothecin (348 mg, 1.0 mmol) at 0^0C. The reaction mixture was allowed to warm to room temperature. After stirring for 18 hours, the mixture was washed sequentially with 0.1N aqueous hydrochloric acid solution (2 x 50 mL), with water (2 x 50 mL), with 0.1M aqueous sodium bicarbonate solution (2 x 25 mL), and with water (2 x 50 mL). The organic phase was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was crystallized from methanol (7 mL) to provide 20-(N-t-BOC-glycyl)-20(S)camptothecin (424 mg, 84% yield) as a yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.35 (s, 1H), 8.22 (d, J = 8.38 Hz 1H), 7.91(d, J = 8.07, 1H), 7.76-7.85 (m, 1H), 7.65 (t, J = 7.4 Hz, 1H), 7.26 (s, 1H), 5.70 (d, J = 17.25 Hz, 1H), 5.40 (d, J = 17.25 Hz, 1H), 5.25 (s, 2H), 4.95 (br s, 1H), 3.98-4.25 (m, 2H), 2.18-2.26 (m, 2H), 1.38 (s, 9H), 0.95 (t, J = 7.47 Hz, 3H).

Step 2. Preparation of 20-Glycyl-20(S)camptothecin trifluoroacetic acid salt

A solution of 20-(N-t-BOC-glycyl)-20(S)camptothecin (424 mg, 0.84 mmol) in a 1:1 (v/v) mixture of dichloromethane/trifluoroacetic acid (21 mL) was stirred at room temperature for 1 hour. The solvents were evaporated under reduced pressure. The yellow solid was crystallized from dichloromethane/diethyl ether (3:7 (v/v), 50 mL) to provide 20-glycyl-20(S)camptothecin trifluoroacetic acid salt (361 mg, 83 % yield) as a light yellow powder. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.78 (s, 1H), 8.45 (br s, 2H), 8.20 (d, J = 8.2 Hz 1H), 7.70-7.95 (m, 2H), 7.30 (s, 1H), 5.55 (s, 2H), 5.30 (s, 2H), 4.35 (d, J = 17.9 Hz, 1H), 4.15 (d, J = 17.9 Hz, 1H), 2.10-2.30 (m, 2H), 1.00 (t, J= 7.4 Hz, 3H).

stirring, resulting in precipitation of a fine white solid. The pH was adjusted to 2.5 with dilute hydrochloric acid and the suspension transferred to a 50 mL centrifuge tube. The solid was spun, the supernatant was decanted, and the resulting material was suspended in 35 mL of water. The suspension was again spun, decanted, and resuspended in 35 mL water. After this final rinse, the remaining material was lyophilized to obtain a dry powder. The powder was rinsed with 3 x 15 mL of acetonitrile and then the remaining solvent was removed under high vacuum, affording 485 mg of a white powder. ^{1}H NMR (d_6 DMSO) indicated a paclitaxel loading of 38% by weight.

Example 8. Biological Assay

Antitumor activity was assayed in mice implanted subcutaneously with Lewis lung carcinoma cells (LL/2). Tumors were produced in the muscle of the right interscapular region by subcutaneously injecting 2.5 x 10^5 murine Lewis Lung (LL/2) carcinoma cells (ATTC CRL-1642) in a volume of 0.25 ml PBS + 2% FBS. Test compounds and vehicle control were injected ip 7 days after tumor cell implantation when the tumors had grown to 20 ± 20 mm^3 (average of 230 tumors). A single dose of polyglutamic acid-therapeutic agent conjugate in 0.1N Na_2HP0_4 was administered at a 1x-4x the maximum tolerated equivalent dose of the unconjugated agent, which was typically administered in 8.3% cremophore EL/8.3% ethanol in 0.75% saline. Each treatment group consisted of 10 mice randomly allocated to each group. Initially, tumor growth was monitored every 3 to 4 days. When tumor sizes approached the arbitrarily set upper limit of 2500 mm^3, tumor sizes were determined daily. Tumor volume was calculated according to the formula (length x width x height)/2. Mice with tumors equal to or greater than 2500 mm^3 were euthanized by cervical dislocation. Efficacy of the various treatments was expressed in terms of days for tumor to reach a volume of 2500 mm^3 (*i.e.*, TGD, tumor growth delay) compared with maximum tolerated dose of the unconjugated therapeutic agent.

reaction mixture was allowed to warm to room temperature overnight. After 4 days, the reaction flask was again cooled to 0°C and 10% sodium chloride solution (35 mL) was slowly added during 25 minutes. This mixture was acidified to pH 2.5 using 0.5N HCl (3.5 mL) and then stirred at room temperature for an additional hour. The yellow precipitate which formed was filtered, washed with water (4 x 30 mL), then dried under vacuum for 12 hours. The dry yellow cake obtained was ground to a fine powder, resuspended in 2% MeOH/CH_2Cl_2 (10 mL) and stirred for 3 hours. The solid was separated by centrifugation. This process was repeated four times to remove any unreacted camptothecin. The resulting solid was dried under vacuum for 2 days, to yield PG-20(S)-camptothecin (295 mg, 97% yield, determined by weight balance on the basis of the recovered camptothecin (13 mg)). ^{1}H NMR (300 MHz in DMSO-d_6): δ 12.10 (s, -COOH), 6.90-8.80 (m), 5.15-5.8 (m), 3.10-4.35 (m), 1.42-2.62 (m), 0.90 (br s, 19-CH_3). The paclitaxel % loading was 16% by weight.

While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

All of the publications, patent applications and patents cited in this application are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent application or patent was specifically and individually indicated to be incorporated by reference in its entirety.



4. The process of claim 3, wherein the antitumor agent is selected from paclitaxel, docetaxel, etoposide, teniposide, epothilones, gemcitabine, 20(S)(+) camptothecin, 9-aminocamptothecin, 9-nitrocamptothecin, 7-ethyl-10-hydroxycamptothecin, 9-dimethylaminomethyl-10-hydroxycamptothecin, 10,11-methylenedioxycamptothecin, 7-methylpiperizinomethyl-10,11-ethylenedioxycamptothecin, flavopiridol, geldanamycin, 17-(allylamino)-17-demethoxygeldanamycin, ecteinascidin 743, phthalascidin, CT-2584 (1-(11-(dodecylamino)-10-hydroxyundecyl)-3,7-dimethylxanthine, CT-4582 (1-(11-(N-methyl N-dodecyl amino)-10-hydroxyundecyl)-3,7-dimethylxanthine), doxorubicin, 7-(dimethyl-*tert*-butylsilyloxy)-10-hydroxycamptothecin, or adriamycinone.

5. The process of claim 4, wherein the epothilone is epothilone A, epothilone B, epothilone C, epothilone D, epothilone F or 12,13-dioxyepothilone F.

6. The process of claim 4, wherein the therapeutic agent is paclitaxel or docetaxel.

7. The process of claim 1, step (a) wherein said polyglutamic acid has a molecular weight of from 20 kd to 80 kd as determined by viscosity.

8. The process of claim 1, step (b), wherein said agent is linked directly to a carboxy group of said polyglutamic acid by a physiologically cleavable linkage.

9. The process of claim 8, wherein said linkage is an ester linkage or an amide linkage.



17. The process of claim 1, step (c), further comprising the step of acidifying the reaction mixture.

18. The process of claim 1, further comprising the act of removing low molecular weight impurities from the conjugate, wherein said removing can be carried out between steps (c) and (d) or after step (d).

19. A process of preparing a conjugate of polyglutamic acid and a therapeutic agent, comprising:

(a) suspending a salt of a polyglutamic acid polymer in an inert organic solvent;

(d) protonating said polymer by the addition of an anhydrous acid to said suspension to form a soluble salt of the conjugate base;

(e) providing a therapeutic agent and covalently linking said agent to said polyglutamic acid polymer to form a polyglutamic acid-therapeutic agent conjugate;

(d) precipitating said polyglutamic acid-therapeutic agent conjugate from solution by the addition of an excess volume of aqueous salt solution; and

(e) collecting said conjugate as a protonated solid.

20. A process of preparing a poly-L-glutamic acid-2'-paclitaxel conjugate from the sodium salt of poly-L-glutamic acid and paclitaxel, said process comprising the steps of:

(a) providing an aqueous solution of the sodium salt of poly-L-glutamic acid;



22. A polyglutamic acid-therapeutic agent conjugate prepared by the process of claim 1.

23. A polyglutamic acid-therapeutic agent conjugate prepared by the process of claim 19.