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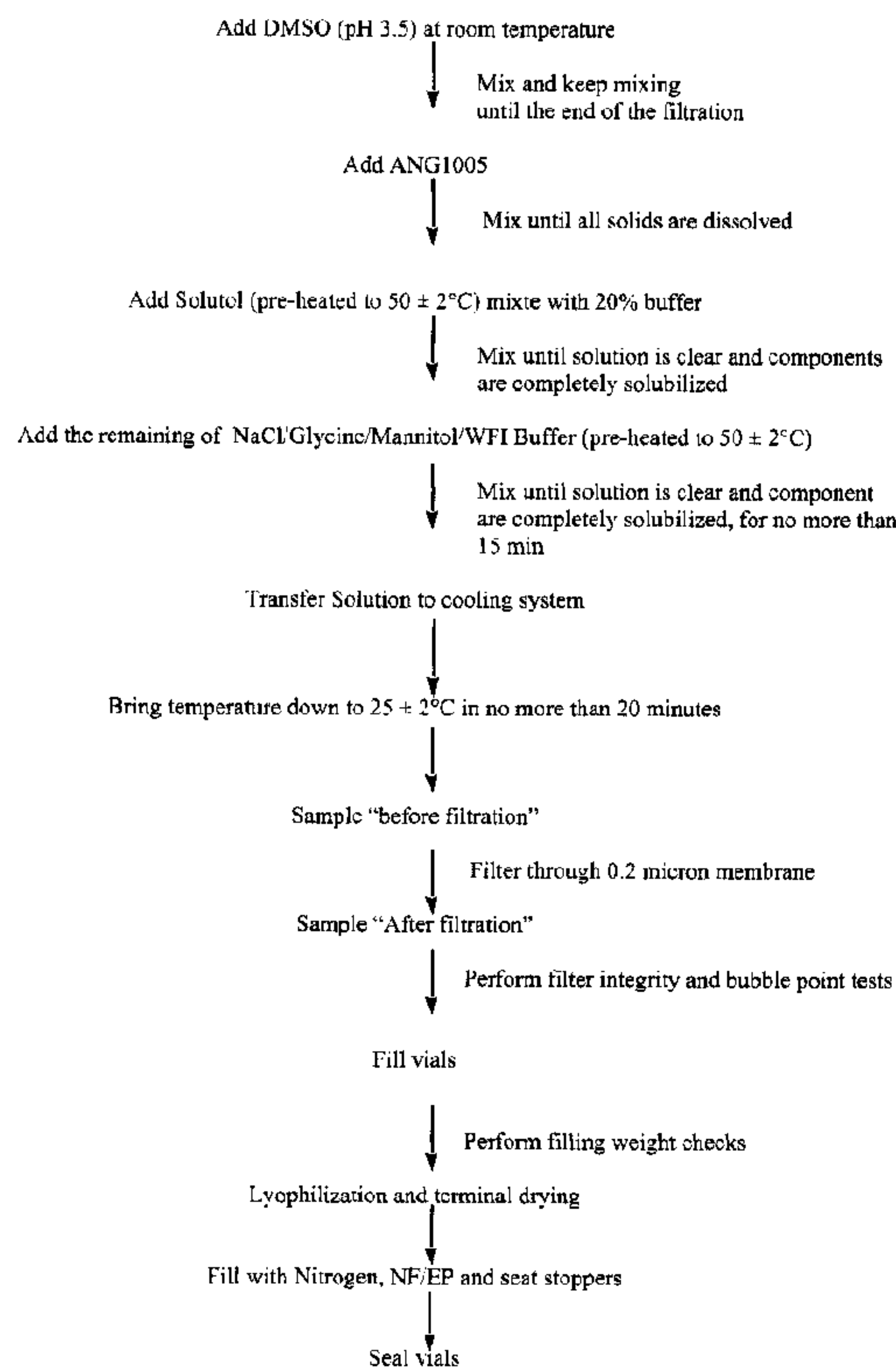
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 (54) Title: PHARMACEUTICAL COMPOSITIONS OF PACLITAXEL, PACLITAXEL ANALOGS OR PACLITAXEL CONJUGATES AND RELATED METHODS OF PREPARATION AND USE

Flow Chart of the Drug Product Manufacturing Process



(57) **Abrégé/Abstract:**

Pharmaceutical compositions useful for hydrophobic agents paclitaxel, paclitaxel analogs and conjugates thereof (e.g. ANG1005) which do not contain Cremophor™. The compositions further comprise an optional tonicity agent, a buffering agent, a bulking agent and a solubilizing agent which is not Cremophor™. Methods of preparing said compositions and of said compositions in the treatment of cancer are also included.

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(54) Title: PHARMACEUTICAL COMPOSITIONS OF PACLITAXEL, PACLITAXEL ANALOGS OR PACLITAXEL CONJUGATES AND RELATED METHODS OF PREPARATION AND USE

Flow Chart of the Drug Product Manufacturing Process

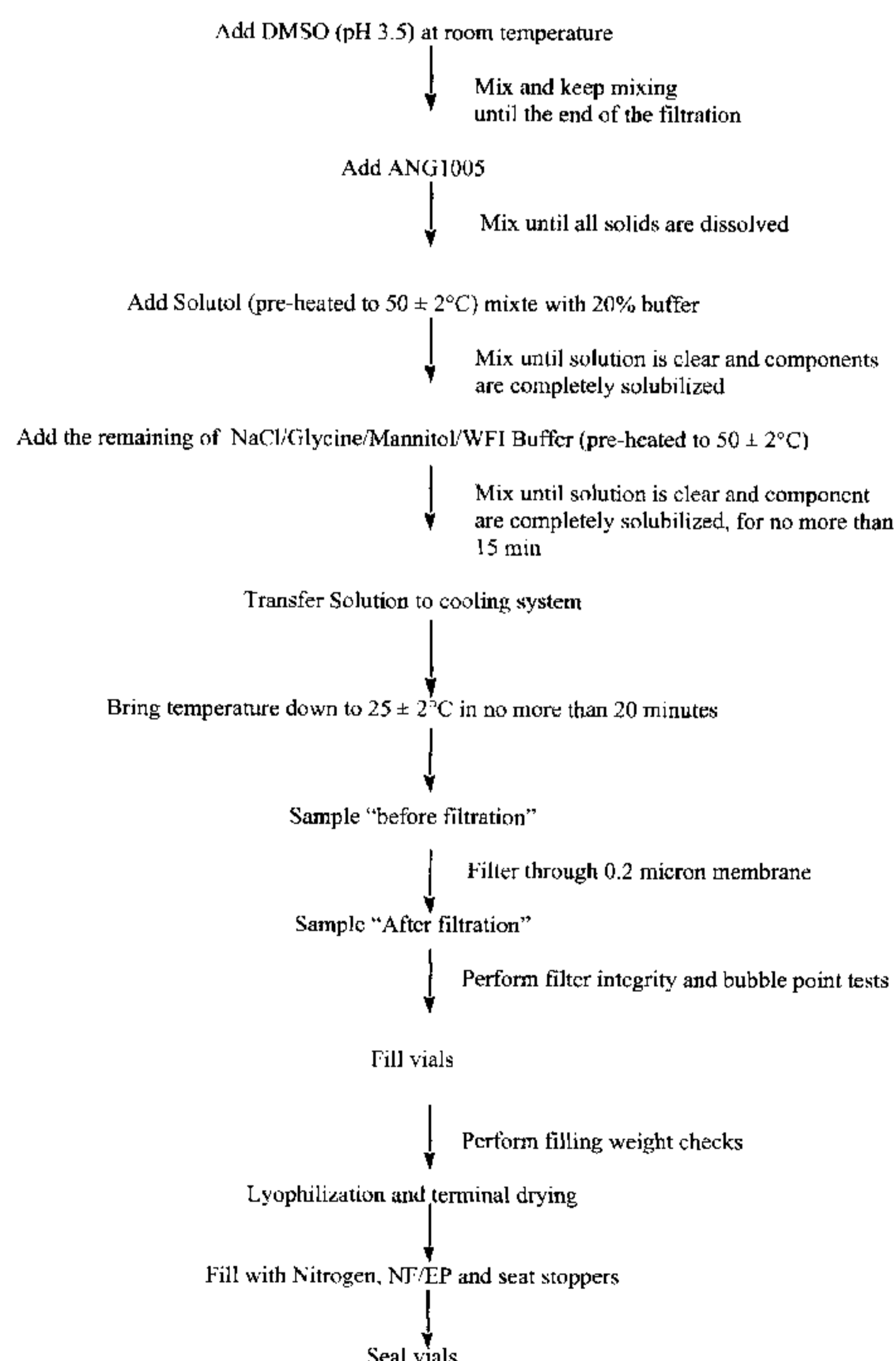


Figure 1

(57) Abstract: Pharmaceutical compositions useful for hydrophobic agents paclitaxel, paclitaxel analogs and conjugates thereof (e.g. ANG1005) which do not contain Cremophor™. The compositions further comprise an optional tonicity agent, a buffering agent, a bulking agent and a solubilizing agent which is not Cremophor™. Methods of preparing said compositions and of said compositions in the treatment of cancer are also included.

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Pharmaceutical Compositions of Paclitaxel, Paclitaxel Analogs or Paclitaxel Conjugates and Related Methods of Preparation and Use

5 **Background of the Invention**

The invention relates to formulations of paclitaxel and paclitaxel analogs, or conjugates thereof, as well as other hydrophobic agents.

Due to insolubility in aqueous solution, hydrophobic agents, such as paclitaxel and paclitaxel analogs, typically are either solubilized in non-aqueous or surfactant buffers or attached to hydrophilic moieties for increased solubility in aqueous solution prior to administration to a patient. Paclitaxel is commercially supplied in a formulation where each ml contains 6 mg paclitaxel, 527 mg of purified Cremophor[®] EL (polyoxyethylated castor oil) and 49.7% (v/v) dehydrated alcohol, USP and ethanol. Prior to administration, the formulated paclitaxel is diluted in a sodium chloride/dextrose or dextrose in Ringer's solution. Because Cremophor can cause hypersensitivity (e.g., anaphylactic) reactions, patients receiving paclitaxel are premedicated with dexamethasone to reduce the occurrence of these reactions. Because of these reactions, paclitaxel is administered over 4 hours to minimize the hypersensitivity effects.

20 Because of the high rate of side effects due to the inclusion of Cremophor in the standard paclitaxel formulations, alternate formulations have been created. These formulations rely upon association of paclitaxel with a soluble compound. Abraxane is paclitaxel formulation where paclitaxel is bound to albumin. Liposomal paclitaxel formulations have also been proposed.

25 Because the existing formulations of hydrophobic agents, such as paclitaxel, either contain undesirable excipients or can be difficult to manufacture, there is a need for new formulations of such agents.

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Summary of the Invention

In first aspect, the invention features a composition including (a) a hydrophobic agent, paclitaxel, a paclitaxel analog, or a conjugate (e.g., ANG1005) including (i) a polypeptide vector; and (ii) a therapeutic agent selected from the group consisting of paclitaxel and a paclitaxel analog, where the therapeutic agent is conjugated to a polypeptide, or any hydrophobic agent described herein); (b) an optional tonicity agent (e.g., sodium chloride or any tonicity agent described herein); (c) a buffering agent (e.g., glycine, lactic acid, or citric acid, or any buffering agent described herein); (d) a bulking agent (e.g., mannitol, sorbitol, or any bulking agent described herein); and (e) a solubilizing agent (e.g., polyoxyethylene ester of a fatty acid such as Solutol HS 15, or any solubilizing agent described herein), for example, where the solubilizing agent is not Cremophor. The polypeptide vector may include an amino acid sequence substantially identical (e.g., at least 70%, 80%, 90%, 95%, or 100% identical) to an amino acid sequence selected from the group consisting of SEQ ID NOS:1-105 and 107-116 (e.g., AngioPep-1 (SEQ ID NO:67); AngioPep-2 (SEQ ID NO:97), or AngioPep-7 (SEQ ID NO:112)). In certain embodiments, the buffering agent maintains the solution at a pH of less than 6 (e.g., pH 4-6). In certain embodiments, the composition further includes 0.01-10% (e.g., less than 8%, 6%, 5%, 4%, 3%, 2%, 1%, 0.75%, 0.5%, 0.2%, or 0.1%) DMSO. In certain embodiments, the composition is substantially free from Cremophor (e.g., free of Cremophor). The composition may be dissolved in water.

In certain embodiments, the composition comprises agents in the amounts shown in any of Tables 1-4.

Table 1

Compound	Percentage (by non-water weight)
ANG1005	0.1-5%
Tonicity agent	0-15%

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Buffering agent	1-10%
Bulking agent	0-15%
Solutol HS 15	40-75%
DMSO	0.01-10%

Table 2

Compound	Percentage (by non-water weight)
ANG1005	1.8-2.3%
Tonicity agent	9-11%
Buffer	4.5-6%
Bulking agent	8-10%
Solutol HS 15	69-75%
DMSO	0.2-1%

Table 3

Compound	Percentage (by non-water weight)
ANG1005	1.8-4.0%
Buffer	0.1-6%
Bulking agent	2-10%
Solutol HS 15	80-95%
DMSO	0.2-1%

Table 4

Compound	Percentage (by non-water weight)
ANG1005	2.0-3.0%
Buffer	0.5-6%
Bulking agent	4-7%
Solutol HS 15	85-95%
DMSO	0.2-0.6%

5 In these compositions, the tonicity agent, if present, may be sodium chloride, the buffering agent may be glycine, lactic acid, or citric acid, and/or the bulking agent may be mannitol. The composition may be made up of about 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1.0%, 1.1, 1.2, 1.3%, 1.4%, 1.5%, 1.6%, 1.7%, 1.8%, 1.9%, 2.0%, 2.1%, 2.2%, 2.3%, 2.4%, 2.5%, 2.6%, 2.7%,

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2.8%, 3.0%, 3.2%, 3.5%, 4.0%, or 5.0% ANG1005, or any range in between any of these values. The ANG1005 may be dissolved in a sufficient amount of Solutol HS 15, and/or DMSO, which may be further diluted in an aqueous solution.

The above compositions may be present in a container that may be sealed.

5 The container may be part of a kit that further includes instructions for use (e.g., for administering the composition for treatment of any disease such as those described herein).

In another aspect, the invention features a method of administering a composition of the above aspects to patient suffering from a disease, for example,
10 any disease described herein such as cancer (e.g., ovary, brain, lung, liver, spleen, or kidney cancer). The method includes administering to the patient the composition in an amount sufficient to treat or treat prophylactically the disease. In certain embodiments, the cancer is a brain cancer selected from the group consisting of glioblastoma, astrocytoma, glioma, meduloblastoma, and
15 oligodendroma, neuroglioma, ependymoma, and meningioma.

In another aspect, the invention features a method for preparing a pharmaceutical composition. The method includes (a) dissolving a hydrophobic agent in a first solubilizing agent (e.g., DMSO or any such agent described herein) to form a mixture; (b) adding a second solubilizing agent (e.g., a polyoxyethylene
20 ester of a fatty acid such as Solutol HS 15, or any such agent described herein) to the mixture of step (a); (c) optionally adding water and a buffering agent to the mixture; (d) lyophilizing mixture of step (c); where the lyophilization results in a reduction of at least 5% (e.g., 10%, 20%, 30%, 50%, 75%, 90%, 95%, or 99%) of the amount of the first solubilizing agent (e.g., to a final proportion of less than
25 0.2%, 0.4%, 0.6%, 0.8%, 1.0%, 1.5%, 2%, 3%, 4%, 5%, 8% of the total weight of the lyophilized product). In certain embodiments, the lyophilizing does not substantially reduce the amount of the second solubilizing agent. In certain embodiments, the hydrophobic agent includes paclitaxel or a paclitaxel analog.

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The hydrophobic agent may include or may be a conjugate including (a) a polypeptide vector and (b) an agent described herein (e.g., paclitaxel and analogs thereof), where the agent is conjugated to the vector. The polypeptide vector may be substantially identical to an amino acid sequence selected from the group consisting of SEQ ID NOS:1-105 and 107-116 (e.g., AngioPep-1 (SEQ ID NO:67); AngioPep-2 (SEQ ID NO:97), or AngioPep-7 (SEQ ID NO:112)). In particular embodiments, the conjugate is ANG1005. In certain embodiments, water and a buffering agent are added in step (c) and the step (d) lyophilizing includes (i) freezing the mixture; (ii) drying the frozen product at a first temperature and pressure sufficient to remove at least a portion (e.g., at least 50%, 60%, 70%, 80%, 90%, 95%, 99%, 99.5%, 99.9%, or 99.99%) of the water; and (iii) drying the product at a second temperature and pressure sufficient to remove at least a portion (e.g., at least 5% (e.g., 10%, 20%, 30%, 50%, 75%, 90%, 95%, or 99%) of the first solvent. The mixture of step (b) may be filtered prior to step (c) lyophilizing or may be placed into a vial or container prior to step (c) lyophilizing. The method may further include (c) resuspending the lyophilized product.

In another aspect, the invention features a method for producing a pharmaceutical composition including the steps (a) dissolving in DMSO a conjugate including paclitaxel or paclitaxel analog conjugated to a polypeptide vector, thereby forming a mixture; (b) adding Solutol HS 15 to the mixture; (c) adding water, a buffering agent, and optionally salt or a bulking agent to the mixture; and (d) lyophilizing the mixture under conditions which remove the water and the DMSO from the mixture. The Solutol HS 15 may be mixed with water, a buffering agent, and optionally a tonicity agent or a bulking agent prior to adding to the mixture, where the water, buffering agent, and optional tonicity agent are added in a amount which maintains solubility of the conjugate in the mixture. The buffering agent may maintain the solution at a pH between 4 and 6. The DMSO may be acidified between pH 3.5 and 4.5 prior to the step (a) dissolving. In certain

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embodiments, the lyophilization does not substantially reduce the amount of Solutol HS 15 in the mixture. The conjugate may include any of the polypeptides (e.g., AngioPep-2) described herein. In particular embodiments, the paclitaxel-polypeptide conjugate is ANG1005.

5 In another aspect, the invention features a pharmaceutical composition produced by any of the methods described above.

By “buffering agent” is meant any compound or group of compounds capable of maintaining the pH (e.g., between any of pH 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5, 11.0, 11.5, 12.0, 12.5, 13.0,
10 and 13.5) of a solution within a particular range upon addition of agents that can otherwise alter the pH. Exemplary buffering agents are described herein.

By “tonicity agent” is meant any agent that alters the osmolarity of an aqueous solution (e.g., any of or any range between 10, 20, 50, 75, 100, 150, 200, 250, 300, 400, 500, 750, 1000, 1500, or 2000 mM). Ionic salts, such as sodium
15 chloride, can be used to adjust tonicity. Additional tonicity agents are described herein.

By “bulking agent” is meant a compound that alters the physical form of a chemical composition following a dehydration or lyophilization procedure. Exemplary bulking agents are described herein.

20 By “solubilizing agent” is meant any solvent capable of dissolving a particular compound (e.g., a hydrophobic compound such a compound or conjugate containing paclitaxel or a paclitaxel analog). Exemplary solubilizing agents suitable for hydrophobic compounds are described herein.

By “vector” is meant a compound or molecule such as a polypeptide that
25 can be transported into a particular cell type (e.g., liver, lungs, kidney, spleen, or muscle) or across the BBB. The vector may be attached to (covalently or not) or conjugated to an agent and thereby may be able to transport the agent into a particular cell type or across the BBB. In certain embodiments, the vector may

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bind to receptors present on cancer cells or brain endothelial cells and thereby be transported into the cancer cell or across the BBB by transcytosis. The vector may be a molecule for which high levels of transendothelial transport may be obtained, without affecting the cell or BBB integrity. The vector may be a polypeptide or a
5 peptidomimetic and may be naturally occurring or produced by chemical synthesis or recombinant genetic technology.

By “conjugate” is meant a vector linked to an agent. The conjugation may be chemical in nature, such as via a linker, or genetic in nature for example by recombinant genetic technology, such as in a fusion protein with for example a
10 reporter molecule (e.g., green fluorescent protein, β -galactosidase, Histag, etc.).

By a vector or conjugate which is “efficiently transported to a particular cell type” is meant a vector or conjugate that is able to accumulate (e.g., either due to increased transport into the cell, decreased efflux from the cell, or a combination thereof) in that cell type at least 10% (e.g., 25%, 50%, 100%, 200%, 500%,
15 1,000%, 5,000%, or 10,000%) greater extent than either a control substance, or, in the case of a conjugate, as compared to the unconjugated agent.

By “substantially pure” or “isolated” is meant a compound (e.g., a polypeptide or conjugate) that has been separated from other chemical components. Typically, the compound is substantially pure when it is at least
20 30%, by weight, free from other components. In certain embodiments, the preparation is at least 50%, 60%, 75%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% by weight, free from other components. A purified polypeptide may be obtained, for example, by expression of a recombinant polynucleotide encoding such a polypeptide or by chemically synthesizing the polypeptide. Purity can be
25 measured by any appropriate method, for example, column chromatography, polyacrylamide gel electrophoresis, or by HPLC analysis.

A pharmaceutical composition which is “substantially free” from a substance means that the amount of a substance in the composition is less than 5%,

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4%, 3%, 2%, 1%, 0.5%, 0.3%, 0.2%, 0.1%, 0.05%, or 0.01% of the dry weight of a composition.

By “substantially identical” is meant a polypeptide or nucleic acid exhibiting at least 35%, 40%, 50%, 55%, 60%, 65%, 70%, 75%, 85%, 90%, 95%,
5 or even 99% identity to a reference amino acid or nucleic acid sequence. For polypeptides, the length of comparison sequences will generally be at least 4 (e.g., at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 50, or 100) amino acids. For nucleic acids, the length of comparison sequences will generally be at least 60 nucleotides, preferably at least 90 nucleotides, and more preferably at least
10 120 nucleotides, or full length. It is to be understood herein that gaps may be found between the amino acids of an analogs which are identical or similar to amino acids of the original polypeptide. The gaps may include no amino acids, one or more amino acids which are not identical or similar to the original polypeptide. Biologically active analogs of the vectors (polypeptides) of the
15 invention are encompassed herewith. Percent identity may be determined, for example, with n algorithm GAP, BESTFIT, or FASTA in the Wisconsin Genetics Software Package Release 7.0, using default gap weights.

By “fragment” is meant a polypeptide originating from a portion of an original or parent sequence or from an analogue of said parent sequence.
20 Fragments encompass polypeptides having truncations of one or more amino acids, wherein the truncation may originate from the amino terminus (N-terminus), carboxy terminus (C-terminus), or from the interior of the protein. A fragment may include the same sequence as the corresponding portion of the original sequence. Functional fragments of the vector (polypeptide) described herein are
25 encompassed by the invention. Fragments may be at least 5 (e.g., at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 25, 28, 30, 35, 40, 45, 50, 60, 75, 100, or 150) amino acids. Fragments of the invention may include, for example, a polypeptide of 7, 8, 9 or 10 amino acids to 18 amino acids. Fragments

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may contain any of the modifications described herein (e.g., acetylation, amidation, amino acid substitutions)

A “non-naturally occurring amino acid” is an amino acid that is not naturally produced or found in a mammal.

5 By “agent” is meant any compound, for example, an antibody, or a therapeutic agent, a marker, a tracer, or an imaging compound.

By “therapeutic agent” is meant an agent having a biological activity. In some cases, the therapeutic agent is used to treat the symptoms of a disease, a physical or mental condition, an injury, or an infection and includes anti-cancer
10 agents, antibiotics, anti-angiogenic agents, and molecules active at the level of the central nervous system.

By “small molecule drug” is meant a drug having a molecular weight of 1000 g/mol or less (e.g., less than 800, 600, 500, 400, or 200 g/mol).

By “subject” is meant a human or non-human animal (e.g., a mammal).

15 By “treating” a disease, disorder, or condition in a subject is meant reducing at least one symptom of the disease, disorder, or condition by administering a therapeutic agent to the subject.

By “treating prophylactically” a disease, disorder, or condition in a subject is meant reducing the frequency of occurrence of (e.g., preventing) a disease,
20 disorder or condition by administering a therapeutic agent to the subject.

By “cancer” is meant any cellular proliferation whose unique trait is the loss of normal controls which can result in unregulated growth, lack of differentiation, or ability to invade tissues and metastasize. Cancer can develop in any tissue or in any organ. Cancer is intended to include, without limitation, cancer of the brain,
25 liver, lungs, kidney, or spleen. Additional cancers are described herein.

By “administering” and “administration” is meant a mode of delivery including, without limitation, orally, intra-arterially, intra-nasally, intraperitoneally, intravenously, intramuscularly, subcutaneously, transdermally or

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per os. A daily dosage can be divided into one, two or more doses in a suitable form to be administered at one, two or more times throughout a time period.

By “therapeutically effective” or “effective amount” is meant an amount of a therapeutic agent sufficient to improve, decrease, prevent, delay, suppress, or
5 arrest any symptom of the disease or condition being treated. A therapeutically effective amount of an agent need not cure a disease or condition but will provide a treatment for a disease or condition such that the onset of the disease or condition is delayed, hindered, or prevented, or the disease or condition symptoms are ameliorated, or the term of the disease or condition is changed or, for example, is
10 less severe or recovery is accelerated in an individual.

If a “range” or “group of substances” is mentioned with respect to a particular characteristic (e.g., temperature, concentration, time and the like), the invention relates to and explicitly incorporates herein each and every specific member and combination of sub-ranges or sub-groups therein. Thus, for example,
15 with respect to a length of from 9 to 18 amino acids, is to be understood as specifically incorporating herein each and every individual length, e.g., a length of 18, 17, 15, 10, 9, and any number therebetween. Therefore, unless specifically mentioned, every range mentioned herein is to be understood as being inclusive. For example, in the expression from 5 to 19 amino acids long is to be as including
20 5 and 19. This similarly applies with respect to other parameters such as sequences, length, concentrations, elements, and the like.

The sequences, regions, portions defined herein each include each and every individual sequence, region, and portion described thereby as well as each and every possible sub-sequence, sub-region, and sub-portion whether such sub-
25 sequences, sub-regions, and sub-portions are defined as positively including particular possibilities, as excluding particular possibilities or a combination thereof. For example, an exclusionary definition for a region may read as follows: “provided that said polypeptide is no shorter than 4, 5, 6, 7, 8 or 9 amino acids. A

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further example of a negative limitation is the following; a sequence including SEQ ID NO:X with the exclusion of a polypeptide of SEQ ID NO:Y; etc. An additional example of a negative limitation is the following; provided that said polypeptide is not (does not include or consist of) SEQ ID NO:Z.

5 Other features and advantages of the invention will be apparent from the following Detailed Description, the drawings, and the claims.

Brief Description of the Drawings

Figure 1 is a schematic depiction of an exemplary method for preparing a pharmaceutical composition including ANG1005.

Figure 2 is a graph showing HPLC profiles of reconstituted ANG1005 for Injection diluted in D5W to 1.0 mg/ml under conditions of clinical use over time.

Figure 3 is a graph showing the HPLC profile of the centrifuged sediment collected from the sample at 2.0 mg/mL and ~6h storage, and solubilized in DMSO.

Detailed Description

We have developed pharmaceutical formulations useful for hydrophobic therapeutic agents, including paclitaxel and paclitaxel analogs, or conjugates thereof (e.g., ANG1005) and methods for making and administering pharmaceutical compositions with such formulations. Hydrophobic therapeutic agents (e.g., paclitaxel) are often solubilized in (and, indeed, often require) hydrophobic solvents. Commonly used solvents for paclitaxel include Cremophor and DMSO, which may not be well tolerated by patients. Cremophor, in particular, can cause anaphylactic reactions, thus requiring pretreatment with agents such as corticosteroids. To avoid using such poorly tolerated solvents, we have developed new formulations for the exemplary polypeptide-paclitaxel conjugate, ANG1005. The formulations described herein are advantageous in that

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they can be manufactured without the use of Cremophor, can be prepared to contain minimal DMSO concentrations, result in low degradation and high activity of the active agent, and can be manufactured using conventional methods. Compositions that do not contain poorly tolerated excipients can be administered to patients in
5 higher doses, can be administered more rapidly (e.g., in the case of intravenous administration), can be administered more frequently, or may avoid the need to for pretreatments with agents (e.g., corticosteroids) to increase tolerance to such excipients.

10 **Development of a formulation for ANG1005**

In developing a new formulation of the exemplary hydrophobic agent, ANG1005, we first tested its solubility in various solvents and combinations of solvents. As outlined in Example 1, and as with paclitaxel, ANG1005 has low solubility in aqueous solutions but is highly soluble in DMSO (120 mg/ml).
15 ANG1005 was also soluble in Solutol HS 15 (BASF, Parsippany, N.J.) with ethanol at 75 °C (6 mg/ml). Because of its low toxicity and compatibility with the drug, Solutol HS 15 was selected as a solubilization agent. However, dissolving in Solutol HS 15 by itself resulted in significant degradation of the ANG1005. To dissolve ANG1005, Solutol HS 15 was heated to at least 65 °C. In addition, we
20 noted that heating unbuffered Solutol from 25 °C to 50 °C increased its pH from 6.0 to 9.0. Thus, the combination of high temperature and high pH likely contributes to the observed instability of ANG1005 under these conditions.

To avoid excessive degradation, ANG1005 was first dissolved in acidified DMSO (pH 3.5-4.0) prior to addition of 50 °C Solutol (see Example 2). To further
25 stabilize the ANG1005, we acidified the Solutol HS 15 by pre-mixing with glycine buffer at pH 5.0, which maintains solubility of the ANG1005. Doing so minimizes the degradation of the ANG1005. It is possible to add up to 20% (e.g., 1%, 5%, 10%, or 15%) of the buffer to the Solutol prior to the addition of ANG1005

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without effecting solubility of ANG1005, whereas mixing a larger amount of buffer with the Solutol results in incomplete solubilization.

To ensure ANG1005 stability, the formulation was diluted in aqueous solution buffered at pH 5 with glycine, as we have observed that ANG1005 becomes increasingly unstable at pH 6 and above. Other buffers in this pH range were evaluated, including acetate, and phosphate, but these were less compatible with the formulation. We also attempted to stabilize the ANG1005 by reducing the final pH to 4, but the resulting lyophilized cake did not reconstitute to a clear solution.

An exemplary ANG1005 composition is provided in Table 5 below.

Table 5: Component Function in ANG1005 for Injection

Component	Purpose	Target Amount/Batch	Target Amount/vial
ANG1005	API	60 g	120 mg
Sodium chloride, USP	Osmolarity	290 g	580 mg
Glycine	Buffer	150 g	300 mg
Mannitol	Bulking agent	260 g	520 mg
Solutol HS 15	Solubilization of API	2000 ml	4 ml
DMSO	Solubilization of API pre-lyophilization	500 ml ¹	1 ml ¹
HCl	pH adjustment	to adjust pH	
Water for Injection, USP	Solvent	QS to 10 L ¹	to 20 ml ¹

¹Removed by Lyophilization

Bulking agents were also added to facilitate the reconstitution of the lyophilized product. Both mannitol and sorbitol containing formulations were evaluated. Mannitol yielded a superior cake.

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Lyophilization

Because the DMSO/Solutol/buffer preparation contained undesirably high levels of DMSO and was not sufficiently stable, a lyophilization protocol designed to reduce the DMSO and to increase ANG1005 stability was developed. A number of alternative lyophilization cycles were evaluated to minimize DMSO content (i.e., increasing temperature and length of secondary drying; see Example 3). Lyophilization conditions are described in detail below. A first lyophilization protocol was attempted, and this procedure resulted in DMSO concentrations greater than 1%. Details of this procedure are shown in Table 6.

10 **Table 6**

Lyophilization Cycle for ANG1005 for Injection			
Step	Temperature	Vacuum	Hold Time
Loading	≤-40°C	Atmospheric	N/A
Freezing	≤-40°C	Atmospheric	3 ± 1 hour
Primary Drying	≥-25°C	50 mT	90 ± 1 hours
Secondary Drying	≥22°C	50 mT	9 ± 1 hour
Stoppering	≥22°C	5-10 mmHg	N/A

We have been able to reduce the DMSO concentration further, to less than 1%, by using an optimized two-step drying procedure. Briefly, following freezing of the product, lyophilization is carried out at a shelf temperature and for a time sufficient to remove most of the water from the product. The shelf temperature is raised, and the product is dried a temperature suitable for DMSO removal. The precise conditions will vary depending on the volume of the sample being dried, the pressure and the temperatures used, and the formulation and buffers used. Based on the procedure described herein, one of skill in the art would be able to determine appropriate drying conditions to generate the compositions described herein.

In one exemplary procedure, the formula is loaded at a temperature between -70 and +25 °C (e.g., -40 °C). The temperature is then ramped to a set temperature

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sufficient to freeze the solution (any temperature between 0 °C and -70 °C such as -40 °C) and the temperature is held at that temperature for a time sufficient to freeze the product, and preferably for a time sufficient to ensure that the lyophilization cake does not collapse. We determined that, at -40 °C, at least 12 hours (e.g., at least 15, 18, 20, 24, 36, or 48 hours) of freezing time was required to ensure the cake did not collapse. Following freezing, the vacuum was set to a pressure (e.g., 10-500 mT such as 20, 50, 100, 200, or 500 mT) and temperature (e.g., -15 to -35 °C such as -25 °C) sufficient to remove the water from the product for the primary drying cycle. To this end, pressures between 10-100 mT were tested with minimal variation in results. The drying time can be for a time sufficient (e.g., at least 6 hours, 12 hours, 1 day, 2 days, 4 days, 6 days, 8 days, 10 days, or 14 days) to remove a substantial portion (e.g., at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, 99.5%, 99.9%) of the water present in the product. Following the primary drying cycle, a secondary drying cycle to remove DMSO was performed. The product was ramped to a higher temperature between 10-30 °C (e.g., 18, 19, 20, 21, 22, 23, 24, 25, 26, or 27 °C) to remove the DMSO. In a preferred embodiment, the shelf temperature is ramped to 27 °C over 2 hours, and then held at 27 °C for one hour. The shelf temperature is then ramped (or maintained) between 23 and 27 °C over 30 minutes and then held at that temperature for at least another 10 hours (e.g., at least 15, 20, 25, 30, 40, 48, 60, or 72 hours). To prevent the residual DMSO from melting, the product can be kept below 25 °C. An exemplary protocol for this method is shown in Table 7. Lyophilization was performed using a Hull Freezer Dryer, Model 72FS100-SS20C.

Table 7

Segment	Operation	Temperature	Time
1	Load Set Point	-40 °C	N/A
FREEZE DOWN			
2	Ramp Shelf to	-40 °C	0 minutes
3	Hold Shelf at	-40 °C	420-1440 minutes

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PRIMARY DRYING			
Set vacuum control to 50mT & condenser control set to -50°C for step 4-7			
4	Ramp Shelf to	-25 °C	120 minutes
5	Hold Shelf at	-25 °C	5760 minutes
SECONDARY DRYING			
6	Ramp Shelf to	27 °C	120 minutes
7	Hold Shelf at	27 °C	60 minutes
8	Ramp Shelf to	23-27 °C	30 minutes
9	Hold Shelf at	23-27 °C	900-1200 minutes

Reconstitution of the product

Prior to injection into a patient or laboratory analysis of the product, the lyophilized product can be reconstituted. Any buffer, solvents, or combination of buffer(s) and solvent(s) suitable for reconstitution can be used; the precise buffer is not critical. It is, however, often desirable that the active agent is sufficiently stable in the solution and that the buffer(s) or solvent(s) used be sufficiently well tolerated by patients in solutions for administration to patients. In case of ANG1005, because the product is less stable at pH above 6.0, it is generally desirable to use a reconstitution solvent/buffer system which maintains a pH below 6.0. For ANG1005, one preferred solvent system is a combination of ethanol and lactated Ringers/5% Dextrose. In this system, ethanol is added to the vial containing the product, gently mixed, and then the lactated Ringers/5% Dextrose is added to dissolve the product. The use of conventional water for injection (WFI) or saline as diluents yielded high pH levels, leading to degradation of ANG1005. Following dissolution, the mixture may be further diluted in water or other buffer systems. Exemplary conditions for reconstitution of the lyophilized product are described further in Example 4 below.

Formulation Compositions

As described above, we have developed formulations of the exemplary hydrophobic agent, ANG1005 suitable for administration to patients. Prior to

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lyophilization, the formulation may, in certain embodiments, contain a significant proportion of DMSO. Such compositions may have the following components (e.g., dry weight) as show in Tables 8A and 8B. Table 8C shows exemplary concentrations of the various component in aqueous solution prior to
5 lyophilization.

Table 8A Percentage by weight not including water

Compound	Percentage (by weight)	Preferred percentages	Exemplary Percentages
ANG1005	0.1-5%	1-3%	1.8%
Tonicity agent (e.g., sodium chloride)	1-15%	5-12%	8.6%
Buffer (e.g., glycine)	1-10%	3-7%	4.5%
Bulking agent (e.g., mannitol)	0-15%	5-12%	7.7%
Solutol HS 15	40-75%	50-70%	61.1%
DMSO	3-20%	10-20%	16.3%

Table 8B Percentage in aqueous solution (prelyophilization)

Compound	Percentage (by weight)	Preferred percentages	More Preferred Percentages	Exemplary Percentages
ANG1005	0.05-1.5%	0.1-1.0%	0.2-0.8%	0.55%
Tonicity agent (e.g., sodium chloride)	0.1-10%	0.5-6%	1-3%	2.7%
Buffer (e.g., glycine)	0.05-5%-	0.1-4%	0.5-1.5%	1.4%
Bulking agent (e.g., mannitol)	0-5%	0.2-4%	1.0-3.0%	2.4%
Solutol HS 15	1-40%	3-30%	10-25%	19.0%
DMSO	0.5-15%	1-10%	2-8%	5.0%
Water	25-85%	50-80%	65-75%	69.0%

Table 8C: Concentrations (mg/ml) prelyophilization aqueous solution

Compound	Concentration (mg/ml)	Preferred Concentrations	More Preferred Concentrations	Exemplary Concentrations
ANG1005	0.1-10.0	2-7	4-6.5	6.0
Tonicity agent (e.g., sodium chloride)	5-200	10-100	10-50	29
Buffer (e.g., glycine)	1-200	3-100	5-25	15
Bulking agent (e.g., mannitol)	0-100	2-50	5-35	26

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Solutol HS 15	10-400	20-300	50-250	206
DMSO	1-200	15-200	30-100	55

The composition is typically diluted into in water prior to lyophilization (see below regarding lyophilization conditions). For most clinical applications, the solution is divided into appropriate amounts for single dose administration of ANG1005 (e.g., about 10, 20, 30, 60, 90, 120, 150, 200, 240, 300, 400, or 500 mg). Following lyophilization (e.g., under the conditions described herein), DMSO concentration can be reduced significantly. Following lyophilization, an ANG1005 composition of the invention may have the following characteristics (e.g., dry weight) as shown in Table 9.

Table 9

Compound	Percentage (by weight)	Preferred percentages	More preferred percentages	Exemplary percentages (0.5% DMSO)
ANG1005	0.1-5%	1.5-3%	1.8-2.3%	2.11
Tonicity agent (e.g., sodium chloride)	0-15%	0-12%	9-11%	10.18
Buffer (e.g., glycine)	1-10%	3-7%	4.5-6%	5.27
Bulking agent (e.g., mannitol)	0-15%	5-12%	8-10%	9.13
Solutol HS 15	40-75%	50-70%	69-75%	72.32
DMSO	0.01-10%	0.1-5%	0.2-0.5	0.50

10

Hydrophobic agents

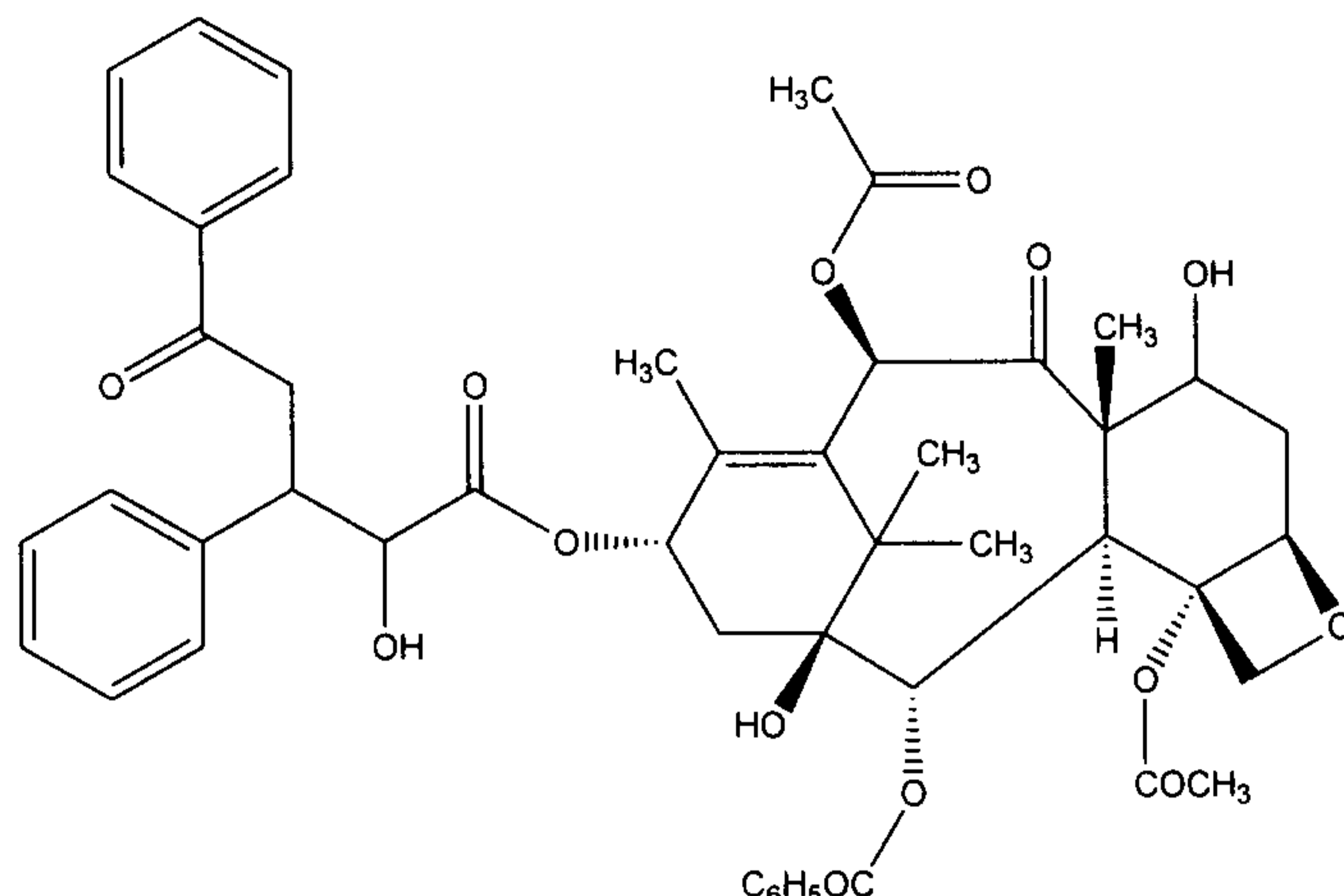
Any hydrophobic agents may be used in the compositions and methods of the present invention. Exemplary compounds are described below.

15

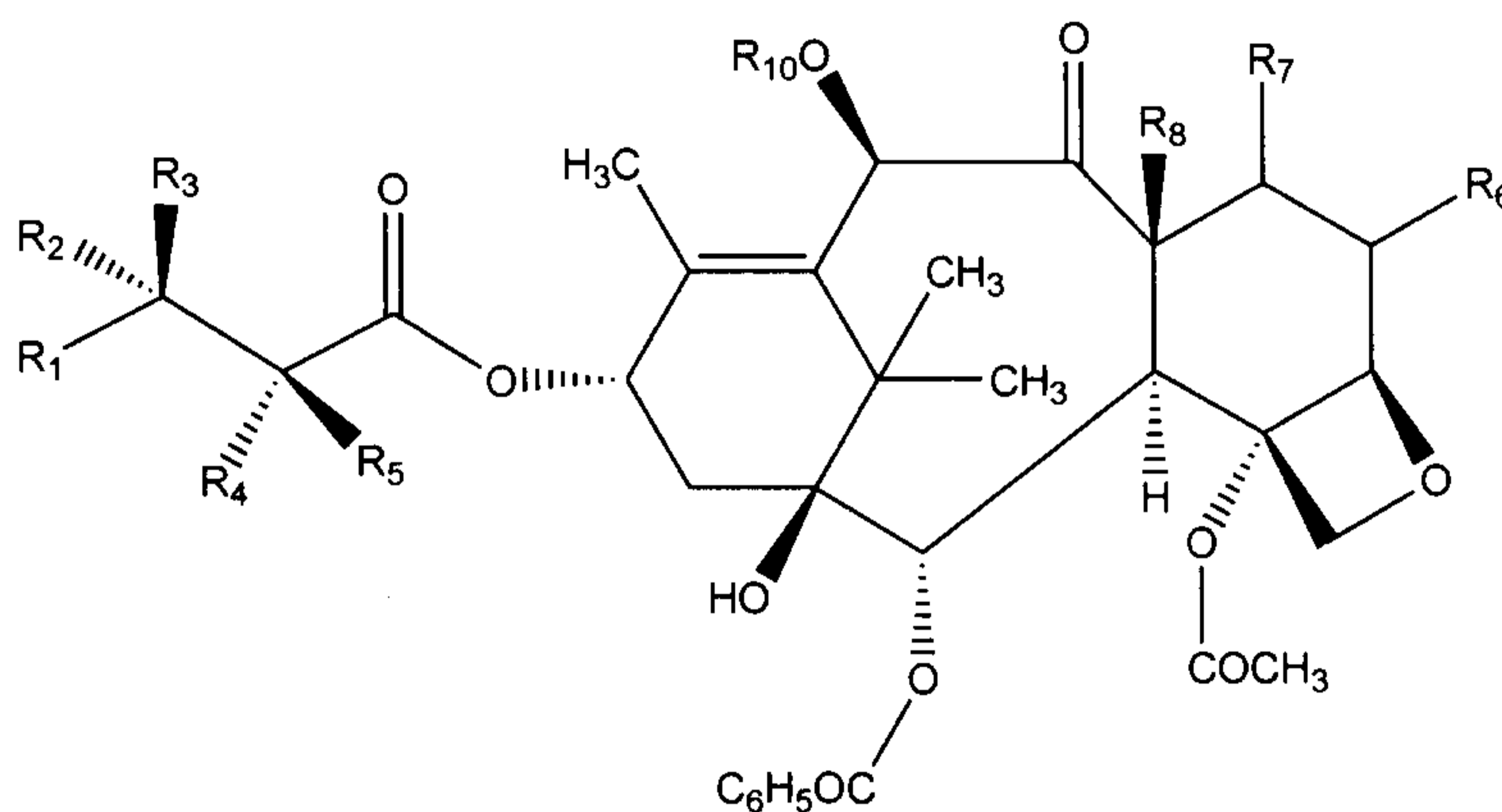
Paclitaxel and related compounds

While the invention has been exemplified using ANG1005, an AngioPep2-paclitaxel conjugate, the formulations described herein may be used with paclitaxel, paclitaxel analogs, or conjugates thereof. Paclitaxel has the formula:

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Structural analogs of paclitaxel are described in U.S. Patent No. 6,911,549, and can be described by the formula:



- 5 wherein R₁ is selected from the group consisting of -CH₃; -C₆H₅, or phenyl substituted with one, 2 or 3 C₁-C₄ alkyl, C₁-C₃ alkoxy, halo, C₁-C₃ alkylthio, trifluoromethyl, C₂-C₆, dialkylamino, hydroxyl, or nitro; and -2-furyl, 2-thienyl, 1-naphthyl, 2-naphthyl or 3,4-methylenedioxyphenyl; R₂ is selected from the group consisting of -H, -NHC(O)H, -NHC(O)C₁-C₁₀ alkyl (preferably -NHC(O)C₄-C₆ alkyl), -NHC(O)phenyl, -NHC(O)phenyl substituted with one, 2, or 3 C₁-C₄ alkyl,
- 10 C₁-C₃ alkoxy, halo, C₁-C₃ alkylthio, trifluoromethyl, C₂-C₆ dialkylamino, hydroxy or nitro, -NHC(O)C(CH₃)=CHCH₃, -NHC(O)OC(CH₃)₃, -NHC(O)OCH₂ phenyl, -NH₂, -NHSO₂-4-methylphenyl, -NHC(O)(CH₂)₃COOH, -NHC(O)-4-
- 15 NHC(O)O-4-tetrahydropyranyl, -NHC(O)CH₂C(CH₃)₃, -NHC(O)C(CH₃)₃, -NHC(O)OC₁-C₁₀ alkyl, -NHC(O)NHC₁-C₁₀ alkyl, -NHC(O)NHPh, -

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NHC(O)NHPH substituted with one, 2, or 3 C₁-C₄ alkyl, C₁-C₃ alkoxy, halo, C₁-C₃ alkylthio, trifluoromethyl, C₂-C₆ dialkylamino, or nitro, -NHC(O)C₃-C₈ cycloalkyl, -NHC(O)C(CH₂CH₃)₂CH₃, -NHC(O)C(CH₃)₂CH₂Cl, -NHC(O)C(CH₃)₂CH₂CH₃, phthalimido, -NHC(O)-1-phenyl-1-cyclopentyl, -NHC(O)-1-methyl-1-cyclohexyl, -NHC(S)NHC(CH₃)₃, -NHC(O)NHCC(CH₃)₃ or -NHC(O)NHPH; R₃ is selected from the group consisting of -H, -NHC(O)phenyl or -NHC(O)OC(CH₃)₃, with the overall proviso that one of R₂ and R₃ is -H but R₂ and R₃ are not both -H; R₄ is -H or selected from the group consisting of -OH, -OAc (-OC(O)CH₃), -OC(O)OCH₂ C(Cl)₃, -OCOCH₂ CH₂ NH₃⁺ HCOO⁻, -NHC(O)phenyl, -NHC(O)OC(CH₃)₃, -OCOCH₂ CH₂ COOH and pharmaceutically acceptable salts thereof, -OCO(CH₂)₃COOH and pharmaceutically acceptable salts thereof, and -OC(O)-Z-C(O)-R' [where Z is ethylene (-CH₂CH₂-), propylene (-CH₂CH₂CH₂-), -CH=CH-, 1,2-cyclohexane or 1,2-phenylene, R' is -OH, -OH base, -NR'₂R'₃, -OR'₃, -SR'₃, -OCH₂C(O)NR'₄R'₅ where R'₂ is -H or -CH₃, R'₃ is -(CH₂)_nNR'₆R'₇ or (CH₂)_nN⁺R'₆R'₇R'₈X⁻ where n is 1-3, R'₄ is -H or -C₁-C₄ alkyl, R'₅ is -H, -C₁-C₄ alkyl, benzyl, hydroxyethyl, -CH₂CO₂H or dimethylaminoethyl, R'₆ and R'₇ are -CH₃, -CH₂CH₃, benzyl or R'₆ and R'₇ together with the nitrogen of NR'₆R'₇ form a pyrrolidino, piperidino, morpholino, or N-methylpiperizino group; R'₈ is -CH₃, -CH₂CH₃ or benzyl, X⁻ is halide, and base is NH₃, (HOC₂H₄)₃N, N(CH₃)₃, CH₃N(C₂H₄)₂NH, NH₂(CH₂)₆NH₂, N-methylglucamine, NaOH or KOH], -OC(O)(CH₂)_n NR² R³ [where n is 1-3, R² is -H or -C₁-C₃ alkyl and R³ is -H or -C₁-C₃ alkyl], -OC(O)CH(R'')NH₂ [where R'' is selected from the group consisting of -H, -CH₃, -CH₂ CH(CH₃)₂, -CH(CH₃)CH₂CH₃, -CH(CH₃)₂, -CH₂ phenyl, -(CH₂)₄NH₂, -CH₂CH₂ COOH, -(CH₂)₃NHC(=NH)NH₂], the residue of the amino acid proline, -OC(O)CH=CH₂, -C(O)CH₂CH₂C(O)NHCH₂CH₂SO₃⁻Y⁺, -OC(O)CH₂CH₂C(O)NHCH₂CH₂CH₂SO₃⁻Y⁺ wherein Y⁺ is Na⁺ or N⁺(Bu)₄, -OC(O)CH₂CH₂C(O)OCH₂CH₂OH; R₅ is -H or -OH, with the overall proviso that

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when R_5 is $-OH$, R_4 is $-H$ and with the further proviso that when R_5 is $-H$, R_4 is not $-H$; R_6 is $-H:-H$ when R_7 is $\alpha-R_{71}:\beta-R_{72}$ where one of R_{71} and R_{72} is $-H$ and the other of R_{71} and R_{72} is $-X$ where X is halo and R_8 is $-CH_3$; R_6 is $-H:-H$ when R_7 is $\alpha-H:\beta-R_{74}$ where R_{74} and R_8 are taken together to form a cyclopropyl ring;
 5 R_{10} is $-H$ or $-C(O)CH_3$; and pharmaceutically acceptable salts thereof when the compound contains either an acidic or basic functional group.

Particular paclitaxel analogs include ((azidophenyl)ureido)taxoid,
 (2 α ,5 α ,7 β ,9 α ,10 β ,13 α)-5,10,13,20-tetraacetoxytax-11-ene-2,7,9-triol,
 (2 α ,5 α ,9 α ,10 β)-2,9,10-triacetoxy-5-((β -D-glucopyranosyl)oxy)-3,11-cyclotax-11-
 10 en-13-one, 1 β -hydroxybaccatin I, 1,7-dihydroxytaxinine, 1-acety-5,7,10-deacetyl-
 baccatin I, 1-dehydroxybaccatin VI, 1-hydroxy-2-deacetoxy-5-decinnamoyl-
 taxinine j, 1-hydroxy-7,9-dideacetyl baccatin I, 1-hydroxybaccatin I, 10-acetyl-4-
 deacetyl taxotere, 10-deacetoxypaclitaxel, 10-Deacetyl baccatin III dimethyl
 sulfoxide disolvate, 10-deacetyl-10-(3-aminobenzoyl)paclitaxel, 10-deacetyl-10-
 15 (7-(diethylamino)coumarin-3-carbonyl)paclitaxel, 10-deacetyl-9-dihydrotaxol, 10-
 deacetyl baccatine III, 10-deacetylpaclitaxel, 10-deacetyltaxinine, 10-deacetyl taxol,
 10-deoxy-10-C-morpholinoethyl docetaxel, 10-O-acetyl-2-O-
 (cyclohexylcarbonyl)-2-debenzoyl taxotere, 10-O-sec-aminoethyl docetaxel, 11-
 desmethyllaulimalide, 13-deoxo-13-acetyloxy-7,9-diacetyl-1,2-dideoxytaxine, 13-
 20 deoxybaccatin III, 14-hydroxy-10-deacetyl-2-O-debenzoyl baccatin III, 14-hydroxy-
 10-deacetyl baccatin III, 14 β -benzoyloxy-13-deacetyl baccatin IV, 14 β -benzoyloxy-
 2-deacetyl baccatin VI, 14 β -benzoyloxy baccatin IV, 19-hydroxybaccatin III, 2',2''-
 methylenedocetaxel, 2',2''-methylenepaclitaxel, 2'-(valyl-leucyl-lysyl-
 PABC)paclitaxel, 2'-acetyl taxol, 2'-O-acetyl-7-O-(N-(4'-
 25 fluoresceincarbonyl)alanyl)taxol, 2,10,13-triacetoxy-taxa-4(20),11-diene-5,7,9-
 triol, 2,20-O-diacetyltaxumairol N, 2-(4-azidobenzoyl)taxol, 2-deacetoxytaxinine
 J, 2-debenzoyl-2-m-methoxybenzoyl-7-triethylsilyl-13-oxo-14-hydroxybaccatin III
 1,14-carbonate, 2-O-(cyclohexylcarbonyl)-2-debenzoyl baccatin III 13-O-(N-

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(cyclohexylcarbonyl)-3-cyclohexylisoserinate), 2 α , 7 β , 9 α , 10 β , 13 α -pentaacetoxyltaxa-4 (20), 11-dien-5-ol, 2 α , 5 α , 7 β , 9 α , 13 α -pentahydroxy-10 β -acetoxyltaxa-4(20), 11-diene, 2 α , 7 β , 9 α , 10 β , 13-pentaacetoxy-11 β -hydroxy-5 α -(3'-N,N-dimethylamino-3'-phenyl)-propionyloxytaxa-4(20), 12-diene, 2 α , 7 β -

5 diacetoxy-5 α , 10 β , 13 β -trihydroxy-2(3-20)abeotaxa-4(20), 11-dien-9-one, 2 α , 9 α -dihydroxy-10 β , 13 α -diacetoxy-5 α -(3'-methylamino-3'-phenyl)-propionyloxytaxa-4(20), 11-diene, 2 α -hydroxy-7 β , 9 α , 10 β , 13 α -tetraacetoxy-5 α -(2'-hydroxy-3'-N,N-dimethylamino-3'-phenyl)-propionyloxytaxa-4(20), 11-diene, 3'-(4-azidobenzamido)taxol, 3'-N-(4-benzoyldihydrocinnamoyl)-3'-N-

10 debenzoylpaclitaxel, 3'-N-m-aminobenzamido-3'-debenzamidopaclitaxel, 3'-p-hydroxypaclitaxel, 3,11-cyclotaxinine NN-2, 4-deacetyltaxol, 5,13 -diacetoxytaxa-4(20), 11-diene-9,10-diol, 5-O-benzoylated taxinine K, 5-O-phenylpropionyloxytaxinine A, 5 α , 13 α -diacetoxy-10 β -cinnamoyloxy-4(20), 11-taxadien-9 α -ol, 6,3'-p-dihydroxypaclitaxel, 6- α -hydroxy-7-deoxy-10-

15 deacetylbaaccatin-III, 6-fluoro-10-acetyldocetaxel, 6-hydroxytaxol, 7,13-diacetoxy-5-cinnamyloxy-2(3-20)-abeo-taxa-4(20), 11-diene-2,10-diol, 7,9-dideacetylbaaccatin VI, 7-(5'-Biotinylamidopropanoyl)paclitaxel, 7-acetyltaxol, 7-deoxy-10-deacetylbaaccatin-III, 7-deoxy-9-dihdropaclitaxel, 7-epipaclitaxel, 7-methylthiomethylpaclitaxel, 7-O-(4-benzoyldihydrocinnamoyl)paclitaxel, 7-O-(N-

20 (4'-fluoresceincarbonyl)alanyl)taxol, 7-xylosyl-10-deacetyltaxol, 8,9-single-epoxy brevifolin, 9-dihydrobaaccatin III, 9-dihydrotaxol, 9 α -hydroxy-2 α , 10 β , 13 α -triacetoxy-5 α -(3'-N,N-dimethylamino-3'-phenyl)-propionyloxytaxa-4(20), 11-diene, baaccatin III, baaccatin III 13-O-(N-benzoyl-3-cyclohexylisoserinate), BAY59, benzoyltaxol, BMS 181339, BMS 185660, BMS 188797, brevifoliol,

25 butitaxel, cephalomannine, dantaxusin A, dantaxusin B, dantaxusin C, dantaxusin D, dibromo-10-deacetylcephalomannine, DJ927, docetaxel, Flutax 2, glutarylpaclitaxel 6-aminohexanol glucuronide, IDN 5109, IDN 5111, IDN 5127, IDN 5390, isolaulimalide, laulimalide, MST 997, N-(paclitaxel-2'-O-(2-

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amino)phenylpropionate)-O-(β -glucuronyl)carbamate, N-(paclitaxel-2'-O-3,3-
 dimethyl butanoate)-O-(β -glucuronyl)carbamate, N-debenzoyl-N-(3-
 (dimethylamino)benzoyl)paclitaxel, nonataxel, octreotide-conjugated paclitaxel,
 Paclitaxel, paclitaxel-transferrin, PNU 166945, poly(ethylene glycol)-conjugated
 5 paclitaxel-2'-glycinate, polyglutamic acid-paclitaxel, protax, protaxel, RPR
 109881A, SB T-101187, SB T-1102, SB T-1213, SB T-1214, SB T-1250, SB T-
 12843, tasumatrol E, tasumatrol F, tasumatrol G, taxa-4(20),11(12)-dien-5-yl
 acetate, taxa-4(20),11(12)-diene-5-ol, taxane, taxchinin N, taxcultine, taxezopidine
 M, taxezopidine N, taxine, taxinine, taxinine A, taxinine M, taxinine NN-1,
 10 taxinine NN-7, taxol C-7-xylose, taxol-sialyl conjugate, taxumairol A, taxumairol
 B, taxumairol G, taxumairol H, taxumairol I, taxumairol K, taxumairol M,
 taxumairol N, taxumairol O, taxumairol U, taxumairol V, taxumairol W,
 taxumairol-X, taxumairol-Y, taxumairol-Z, taxusin, taxuspinanane A,
 taxuspinanane B, taxuspine C, taxuspine D, taxuspine F, taxuyunnanine C,
 15 taxuyunnanine S, taxuyunnanine T, taxuyunnanine U, taxuyunnanine V, tRA-
 96023, and wallifoliol. Other paclitaxel analogs include 1-deoxypaclitaxel, 10-
 deacetoxy-7-deoxypaclitaxel, 10-O-deacetylpaclitaxel 10-monosuccinyl ester, 10-
 succinyl paclitaxel, 12b-acetyloxy-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-
 4,11-dihydroxy-12-(2,5-dimethoxybenzyloxy)-4a,8,13,13-tetramethyl-5-oxo-7,11-
 20 methano-1H-cyclodeca(3,4)benz(1,2-b)oxet-9-yl 3-(tert-butyloxycarbonyl)amino-
 2-hydroxy-5-methyl-4-hexaenoate, 130-nm albumin-bound paclitaxel, 2'-
 paclitaxel methyl 2-glucopyranosyl succinate, 3'-(4-azidophenyl)-3'-
 dephenylpaclitaxel, 4-fluoropaclitaxel, 6,6,8-trimethyl-4,4a,5,6,7,7a,8,9-
 octahydrocyclopenta(4,5)cyclohepta(1,2-c)-furan-4,8-diol 4-(N-acetyl-3-
 25 phenylisoserinate), 6,6,8-trimethyl-4,4a,5,6,7,7a,8,9-
 octahydrocyclopenta(4,5)cyclohepta(1,2-c)-furan-4,8-diol 4-(N-tert-
 butoxycarbonyl-3-phenylisoserinate), 7-(3-methyl-3-nitrosothiobutyryl)paclitaxel,
 7-deoxypaclitaxel, 7-succinylpaclitaxel, A-Z-CINN 310, AI-850, albumin-bound

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paclitaxel, AZ 10992, isotaxel, MAC321, MBT-0206, NK105, Pacliex, paclitaxel
 poliglumex, paclitaxel-EC-1 conjugate, polilactofate, and TXD 258. Other
 paclitaxel analogs are described in U.S. Patents Nos. 4,814,470, 4,857,653,
 4,942,184, 4,924,011, 4,924,012, 4,960,790; 5,015,744; 5,157,049; 5,059,699;
 5 5,136,060; 4,876,399; and 5,227,400

Other hydrophobic agents

Other hydrophobic agents include analgesics and antiinflammatory agents
 (e.g., aloxiprin, auranofin, azapropazone, benorylate, diflunisal, etodolac,
 fenbufen, fenoprofen calcim, flurbiprofen, ibuprofen, indomethacin, ketoprofen,
 10 meclofenamic acid, mefenamic acid, nabumetone, naproxen, oxyphenbutazone,
 phenylbutazone, piroxicam, sulindac), antihelmintics (e.g., albendazole,
 bephenium hydroxynaphthoate, cambendazole, dichlorophen, ivermectin,
 mebendazole, oxamniquine, oxfendazole, oxantel embonate, praziquantel, pyrantel
 embonate, thiabendazole), anti-arrhythmic agents (e.g., amiodarone HCl,
 15 disopyramide, flecainide acetate, quinidine sulphate, anti-bacterial agents (e.g.,
 benethamine penicillin, cinoxacin, ciprofloxacin HCl, clarithromycin, clofazimine,
 cloxacillin, demeclocycline, doxycycline, erythromycin, ethionamide, imipenem,
 nalidixic acid, nitrofurantoin, rifampicin, spiramycin, sulphabenzamide,
 sulphadoxine, sulphamerazine, sulphacetamide, sulphadiazine, sulphafurazole,
 20 sulphamethoxazole, sulphapyridine, tetracycline, trimethoprim), anti-coagulants
 (e.g., dicoumarol, dipyridamole, nicoumalone, phenindione), antidepressants (e.g.,
 amoxapine, maprotiline HCl, mianserin HCl, nortriptyline HCl, trazodone HCl,
 trimipramine maleate), antidiabetics (e.g., acetohexamide, chlorpropamide,
 glibenclamide, gliclazide, glipizide, tolazamide, tolbutamide), anti-epileptics (e.g.,
 25 beclamide, carbamazepine, clonazepam, ethotoin, methoin, methsuximide,
 methylphenobarbitone, oxcarbazepine, paramethadione, phenacemide,
 phenobarbitone, phenytoin, phensuximide, primidone, sulthiame, valproic acid),
 antifungal agents (e.g., amphotericin, butoconazole nitrate, clotrimazole, econazole

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nitrate, fluconazole, flucytosine, griseofulvin, itraconazole, ketoconazole, miconazole, natamycin, nystatin, sulconazole nitrate, terbinafine HCl, terconazole, tioconazole, undecenoic acid), antigout agents (e.g., allopurinol, probenecid, sulphin-pyrazone), antihypertensive agents (e.g., amlodipine, benidipine, darodipine, diltiazem HCl, diazoxide, felodipine, guanabenz acetate, isradipine, minoxidil, nicardipine HCl, nifedipine, nimodipine, phenoxybenzamine HCl, prazosin HCl, reserpine, terazosin HCl), antimalarials (e.g., amodiaquine, chloroquine, chlorproguanil HCl, halofantrine HCl, mefloquine HCl, proguanil HCl, pyrimethamine, quinine sulphate), anti-migraine agents (e.g., dihydroergotamine mesylate, ergotamine tartrate, methysergide maleate, pizotifen maleate, sumatriptan succinate), anti-muscarinic agents (e.g., atropine, benzhexol HCl, biperiden, ethopropazine HCl, hyoscyamine, mepenzolate bromide, oxyphencylmine HCl, tropicamide), anti-neoplastic agents and immunosuppressants (e.g., aminoglutethimide, amsacrine, azathioprine, busulphan, chlorambucil, cyclosporin, dacarbazine, estramustine, etoposide, lomustine, melphalan, mercaptopurine, methotrexate, mitomycin, mitotane, mitozantrone, procarbazine HCl, tamoxifen citrate, testolactone), anti-protazoal agents (e.g., benznidazole, clioquinol, decoquinate, diiodohydroxyquinoline, diloxanide furoate, dinitolmide, furzolidone, metronidazole, nimorazole, nitrofurazone, ornidazole, tinidazole), anti-thyroid agents (e.g., carbimazole, propylthiouracil), anxiolytic, sedatives, hypnotics and neuroleptics (e.g., alprazolam, amylobarbitone, barbitone, bentazepam, bromazepam, bromperidol, brotizolam, butobarbitone, carbromal, chlordiazepoxide, chlormethiazole, chlorpromazine, clobazam, clotiazepam, clozapine, diazepam, droperidol, ethinamate, flunarisone, flunitrazepam, fluopromazine, flupenthixol decanoate, fluphenazine decanoate, flurazepam, haloperidol, lorazepam, lormetazepam, medazepam, meprobamate, methaqualone, midazolam, nitrazepam, oxazepam, pentobarbitone, perphenazine pimozone, prochlorperazine, sulphiride, temazepam, thioridazine, triazolam,

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zopiclone), β -Blockers (e.g., acebutolol, alprenolol, atenolol, labetalol, metoprolol, nadolol, oxprenolol, pindolol, propranolol), cardiac inotropic agents (e.g., amrinone, digitoxin, digoxin, enoximone, lanatoside C, medigoxin), corticosteroids (e.g., beclomethasone, betamethasone, budesonide, cortisone acetate, desoxymethasone, dexamethasone, fludrocortisone acetate, flunisolide, flucortolone, fluticasone propionate, hydrocortisone, methylprednisolone, prednisolone, prednisone, triamcinolone), diuretics: acetazolamide, amiloride, bendrofluazide, bumetanide, chlorothiazide, chlorthalidone, ethacrynic acid, frusemide, metolazone, spironolactone, triamterene), anti-parkinsonian agents (e.g., bromocriptine mesylate, lysuride maleate), gastrointestinal agents (e.g., bisacodyl, cimetidine, cisapride, diphenoxylate HCl, domperidone, famotidine, loperamide, mesalazine, nizatidine, omeprazole, ondansetron HCl, ranitidine HCl, sulphasalazine), histamine H₁-receptor antagonists (e.g., acrivastine, astemizole, cinnarizine, cyclizine, cyproheptadine HCl, dimenhydrinate, flunarizine HCl, loratadine, meclozine HCl, oxatomide, terfenadine), lipid regulating agents (e.g., bezafibrate, clofibrate, fenofibrate, gemfibrozil, probucol), nitrates and other anti-anginal agents (e.g., amyl nitrate, glyceryl trinitrate, isosorbide dinitrate, isosorbide mononitrate, pentaerythritol tetranitrate), opioid analgesics (e.g., codeine, dextropropoxyphene, diamorphine, dihydrocodeine, meptazinol, methadone, morphine, nalbuphine, pentazocine), sex hormones (e.g., clomiphene citrate, danazol, ethinyl estradiol, medroxyprogesterone acetate, mestranol, methyltestosterone, norethisterone, norgestrel, estradiol, conjugated oestrogens, progesterone, stanozolol, stibestrol, testosterone, tibolone), and stimulants (e.g., amphetamine, dexamphetamine, dexfenfluramine, fenfluramine, mazindol).

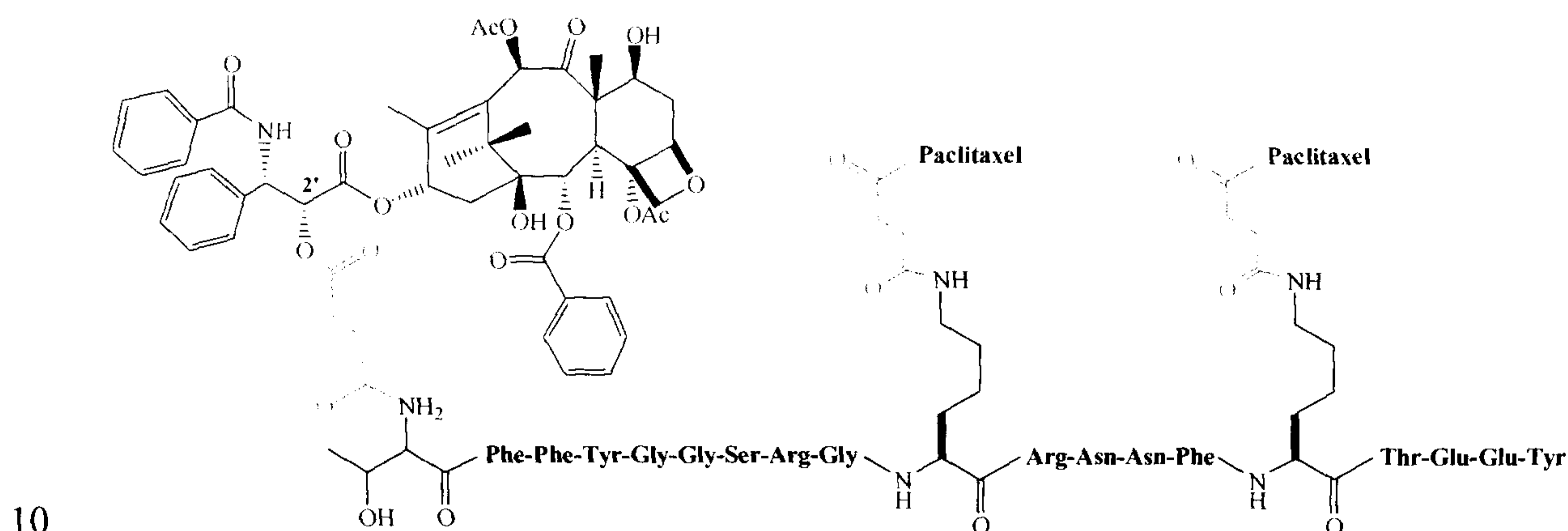
25

Polypeptide conjugates

Conjugates including an active agent a polypeptide may be used in the formulation described herein. As described in U.S. Patent Applications

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Publication Nos. 2006/0182684, and 2006/0189515, and U.S. Provisional Application No. 61/008,880, filed December 20, 2007, we have developed polypeptide-agent conjugates. Such conjugates may include any polypeptide described herein, a hydrophobic agent such as paclitaxel or a paclitaxel analog (e.g., those described herein), and a linker (e.g., those described herein). Paclitaxel conjugates are exemplified by ANG1005, which includes the AngioPep-2 peptide (SEQ ID NO:97) conjugated to three paclitaxel molecules through ester linkages at the N-terminus, and through lysines at positions 10 and 15. The structure of ANG1005 is:



The conjugates, in certain embodiments, can cross the blood-brain barrier (BBB) or can be preferentially targeted to certain cell types, such as liver, lung, kidney, muscle cells or may be targeted to tumor cells (of any cell type described herein). These agents conjugated to these peptides can exhibit increased uptake into the targeted cells, for example, by receptor-mediated endocytosis (e.g., through an LRP receptor). The conjugated agents may, either alternatively or in addition, exhibit increased stability or reduced expulsion from the cell (e.g., due to P-glycoprotein mediated efflux).

20 **Polypeptides**

The compositions and methods of the invention may include any polypeptide described herein, for example, any of the polypeptides described in

Table 10 (e.g., a polypeptide defined in any of SEQ ID NOS:1-105 and 107-112 such as SEQ ID NOS:1-97, 99, 100, 101, or 107-112), or any fragment, analog, derivative, or variant thereof. In certain embodiments, the polypeptide may have at least 35%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, or even 100% identity to a polypeptide described herein. The polypeptide may have one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15) substitutions relative to one of the sequences described herein. Other modifications are described in greater detail below.

The invention can also feature fragments of these polypeptides (e.g., a functional fragment). In certain embodiments, the fragments are capable of entering or accumulating in a particular cell type (e.g., liver, lung, kidney, spleen, or muscle) or capable of crossing the BBB. Truncations of the polypeptide may be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or more amino acids from either the N-terminus of the polypeptide, the C-terminus of the polypeptide, or a combination thereof. Other fragments include sequences where internal portions of the polypeptide are deleted.

Additional polypeptides may be identified by using one of the assays or methods described in U.S. Patent Application Publication No. 2006/0189515, or by any method known in the art. For example, a candidate vector may be produced by conventional polypeptide synthesis, conjugated with Taxol and administered to a laboratory animal. A biologically active vector may be identified, for example, based on its efficacy to increase survival of an animal injected with tumor cells and treated with the conjugate as compared to a control which has not been treated with a conjugate (e.g., treated with the unconjugated agent).

In another example, a biologically active polypeptide may be identified based on its location in the parenchyma in an in situ cerebral perfusion assay. In

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vitro BBB assays, such as the model developed by CELLIAL™ Technologies, may be used to identify such vectors.

Assays to determine accumulation in other tissues may be performed as well. Labeled conjugates of a polypeptide can be administered to an animal, and accumulation in different organs can be measured. For example, a polypeptide conjugated to a detectable label (e.g., a near-IR fluorescence spectroscopy label such as Cy5.5) allows live in vivo visualization. Such a polypeptide can be administered to an animal, and the presence of the polypeptide in an organ can be detected, thus allowing determination of the rate and amount of accumulation of the polypeptide in the desired organ. In other embodiments, the polypeptide can be labeled with a radioactive isotope (e.g., ¹²⁵I). The polypeptide is then administered to an animal. After a period of time, the animal is sacrificed, and the animal's organs are extracted. The amount of radioisotope in each organ can then be measured using any means known in the art. By comparing the amount of a labeled candidate polypeptide in a particular organ without amount of labeled control, the ability of the candidate polypeptide the rate or amount of accumulation of a candidate polypeptide in a particular tissue can be ascertained. Appropriate negative controls include any polypeptide known not be transported into a particular cell type.

20

TABLE 10**SEQ ID
NO:**

1	T	F	V	Y	G	G	C	R	A	K	R	N	N	F	K	S	A	E	D
2	T	F	Q	Y	G	G	C	M	G	N	G	N	N	F	V	T	E	K	E
3	P	F	F	Y	G	G	C	G	G	N	R	N	N	F	D	T	E	E	Y
4	S	F	Y	Y	G	G	C	L	G	N	K	N	N	Y	L	R	E	E	E
5	T	F	F	Y	G	G	C	R	A	K	R	N	N	F	K	R	A	K	Y
6	T	F	F	Y	G	G	C	R	G	K	R	N	N	F	K	R	A	K	Y
7	T	F	F	Y	G	G	C	R	A	K	K	N	N	Y	K	R	A	K	Y
8	T	F	F	Y	G	G	C	R	G	K	K	N	N	F	K	R	A	K	Y

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TABLE 10

9	T	F	Q	Y	G	G	C	R	A	K	R	N	N	F	K	R	A	K	Y
10	T	F	Q	Y	G	G	C	R	G	K	K	N	N	F	K	R	A	K	Y
11	T	F	F	Y	G	G	C	L	G	K	R	N	N	F	K	R	A	K	Y
12	T	F	F	Y	G	G	S	L	G	K	R	N	N	F	K	R	A	K	Y
13	P	F	F	Y	G	G	C	G	G	K	K	N	N	F	K	R	A	K	Y
14	T	F	F	Y	G	G	C	R	G	K	G	N	N	Y	K	R	A	K	Y
15	P	F	F	Y	G	G	C	R	G	K	R	N	N	F	L	R	A	K	Y
16	T	F	F	Y	G	G	C	R	G	K	R	N	N	F	K	R	E	K	Y
17	P	F	F	Y	G	G	C	R	A	K	K	N	N	F	K	R	A	K	E
18	T	F	F	Y	G	G	C	R	G	K	R	N	N	F	K	R	A	K	D
19	T	F	F	Y	G	G	C	R	A	K	R	N	N	F	D	R	A	K	Y
20	T	F	F	Y	G	G	C	R	G	K	K	N	N	F	K	R	A	E	Y
21	P	F	F	Y	G	G	C	G	A	N	R	N	N	F	K	R	A	K	Y
22	T	F	F	Y	G	G	C	G	G	K	K	N	N	F	K	T	A	K	Y
23	T	F	F	Y	G	G	C	R	G	N	R	N	N	F	L	R	A	K	Y
24	T	F	F	Y	G	G	C	R	G	N	R	N	N	F	K	T	A	K	Y
25	T	F	F	Y	G	G	S	R	G	N	R	N	N	F	K	T	A	K	Y
26	T	F	F	Y	G	G	C	L	G	N	G	N	N	F	K	R	A	K	Y
27	T	F	F	Y	G	G	C	L	G	N	R	N	N	F	L	R	A	K	Y
28	T	F	F	Y	G	G	C	L	G	N	R	N	N	F	K	T	A	K	Y
29	T	F	F	Y	G	G	C	R	G	N	G	N	N	F	K	S	A	K	Y
30	T	F	F	Y	G	G	C	R	G	K	K	N	N	F	D	R	E	K	Y
31	T	F	F	Y	G	G	C	R	G	K	R	N	N	F	L	R	E	K	E
32	T	F	F	Y	G	G	C	R	G	K	G	N	N	F	D	R	A	K	Y
33	T	F	F	Y	G	G	S	R	G	K	G	N	N	F	D	R	A	K	Y
34	T	F	F	Y	G	G	C	R	G	N	G	N	N	F	V	T	A	K	Y
35	P	F	F	Y	G	G	C	G	G	K	G	N	N	Y	V	T	A	K	Y
36	T	F	F	Y	G	G	C	L	G	K	G	N	N	F	L	T	A	K	Y
37	S	F	F	Y	G	G	C	L	G	N	K	N	N	F	L	T	A	K	Y
38	T	F	F	Y	G	G	C	G	G	N	K	N	N	F	V	R	E	K	Y
39	T	F	F	Y	G	G	C	M	G	N	K	N	N	F	V	R	E	K	Y
40	T	F	F	Y	G	G	S	M	G	N	K	N	N	F	V	R	E	K	Y
41	P	F	F	Y	G	G	C	L	G	N	R	N	N	Y	V	R	E	K	Y

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TABLE 10

42	T	F	F	Y	G	G	C	L	G	N	R	N	N	F	V	R	E	K	Y			
43	T	F	F	Y	G	G	C	L	G	N	K	N	N	Y	V	R	E	K	Y			
44	T	F	F	Y	G	G	C	G	G	N	G	N	N	F	L	T	A	K	Y			
45	T	F	F	Y	G	G	C	R	G	N	R	N	N	F	L	T	A	E	Y			
46	T	F	F	Y	G	G	C	R	G	N	G	N	N	F	K	S	A	E	Y			
47	P	F	F	Y	G	G	C	L	G	N	K	N	N	F	K	T	A	E	Y			
48	T	F	F	Y	G	G	C	R	G	N	R	N	N	F	K	T	E	E	Y			
49	T	F	F	Y	G	G	C	R	G	K	R	N	N	F	K	T	E	E	D			
50	P	F	F	Y	G	G	C	G	G	N	G	N	N	F	V	R	E	K	Y			
51	S	F	F	Y	G	G	C	M	G	N	G	N	N	F	V	R	E	K	Y			
52	P	F	F	Y	G	G	C	G	G	N	G	N	N	F	L	R	E	K	Y			
53	T	F	F	Y	G	G	C	L	G	N	G	N	N	F	V	R	E	K	Y			
54	S	F	F	Y	G	G	C	L	G	N	G	N	N	Y	L	R	E	K	Y			
55	T	F	F	Y	G	G	S	L	G	N	G	N	N	F	V	R	E	K	Y			
56	T	F	F	Y	G	G	C	R	G	N	G	N	N	F	V	T	A	E	Y			
57	T	F	F	Y	G	G	C	L	G	K	G	N	N	F	V	S	A	E	Y			
58	T	F	F	Y	G	G	C	L	G	N	R	N	N	F	D	R	A	E	Y			
59	T	F	F	Y	G	G	C	L	G	N	R	N	N	F	L	R	E	E	Y			
60	T	F	F	Y	G	G	C	L	G	N	K	N	N	Y	L	R	E	E	Y			
61	P	F	F	Y	G	G	C	G	G	N	R	N	N	Y	L	R	E	E	Y			
62	P	F	F	Y	G	G	S	G	G	N	R	N	N	Y	L	R	E	E	Y			
63	M	R	P	D	F	C	L	E	P	P	Y	T	G	P	C	V	A	R	I			
64	A	R	I	I	R	Y	F	Y	N	A	K	A	G	L	C	Q	T	F	V	Y	G	
65	Y	G	G	C	R	A	K	R	N	N	Y	K	S	A	E	D	C	M	R	T	C	G
66	P	D	F	C	L	E	P	P	Y	T	G	P	C	V	A	R	I	I	R	Y	F	Y
67	T	F	F	Y	G	G	C	R	G	K	R	N	N	F	K	T	E	E	Y			
68	K	F	F	Y	G	G	C	R	G	K	R	N	N	F	K	T	E	E	Y			
69	T	F	Y	Y	G	G	C	R	G	K	R	N	N	Y	K	T	E	E	Y			
70	T	F	F	Y	G	G	S	R	G	K	R	N	N	F	K	T	E	E	Y			
71	C	T	F	F	Y	G	C	C	R	G	K	R	N	N	F	K	T	E	E	Y		
72	T	F	F	Y	G	G	C	R	G	K	R	N	N	F	K	T	E	E	Y	C		
73	C	T	F	F	Y	G	S	C	R	G	K	R	N	N	F	K	T	E	E	Y		
74	T	F	F	Y	G	G	S	R	G	K	R	N	N	F	K	T	E	E	Y	C		

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TABLE 10

75	P	F	F	Y	G	G	C	R	G	K	R	N	N	F	K	T	E	E	Y			
76	T	F	F	Y	G	G	C	R	G	K	R	N	N	F	K	T	K	E	Y			
77	T	F	F	Y	G	G	K	R	G	K	R	N	N	F	K	T	E	E	Y			
78	T	F	F	Y	G	G	C	R	G	K	R	N	N	F	K	T	K	R	Y			
79	T	F	F	Y	G	G	K	R	G	K	R	N	N	F	K	T	A	E	Y			
80	T	F	F	Y	G	G	K	R	G	K	R	N	N	F	K	T	A	G	Y			
81	T	F	F	Y	G	G	K	R	G	K	R	N	N	F	K	R	E	K	Y			
82	T	F	F	Y	G	G	K	R	G	K	R	N	N	F	K	R	A	K	Y			
83	T	F	F	Y	G	G	C	L	G	N	R	N	N	F	K	T	E	E	Y			
84	T	F	F	Y	G	C	G	R	G	K	R	N	N	F	K	T	E	E	Y			
85	T	F	F	Y	G	G	R	C	G	K	R	N	N	F	K	T	E	E	Y			
86	T	F	F	Y	G	G	C	L	G	N	G	N	N	F	D	T	E	E	E			
87	T	F	Q	Y	G	G	C	R	G	K	R	N	N	F	K	T	E	E	Y			
88	Y	N	K	E	F	G	T	F	N	T	K	G	C	E	R	G	Y	R	F			
89	R	F	K	Y	G	G	C	L	G	N	M	N	N	F	E	T	L	E	E			
90	R	F	K	Y	G	G	C	L	G	N	K	N	N	F	L	R	L	K	Y			
91	R	F	K	Y	G	G	C	L	G	N	K	N	N	Y	L	R	L	K	Y			
92	K	T	K	R	K	R	K	K	Q	R	V	K	I	A	Y	E	E	I	F	K	N	Y
93	K	T	K	R	K	R	K	K	Q	R	V	K	I	A	Y							
94	R	G	G	R	L	S	Y	S	R	R	F	S	T	S	T	G	R					
95	R	R	L	S	Y	S	R	R	R	F												
96	R	Q	I	K	I	W	F	Q	N	R	R	M	K	W	K	K						
97	T	F	F	Y	G	G	S	R	G	K	R	N	N	F	K	T	E	E	Y			
98	M	R	P	D	F	C	L	E	P	P	Y	T	G	P	C	V	A	R	I			
	I	R	Y	F	Y	N	A	K	A	G	L	C	Q	T	F	V	Y	G	G			
	C	R	A	K	R	N	N	F	K	S	A	E	D	C	M	R	T	C	G	G	A	
99	T	F	F	Y	G	G	C	R	G	K	R	N	N	F	K	T	K	E	Y			
100	R	F	K	Y	G	G	C	L	G	N	K	N	N	Y	L	R	L	K	Y			
101	T	F	F	Y	G	G	C	R	A	K	R	N	N	F	K	R	A	K	Y			
102	N	A	K	A	G	L	C	Q	T	F	V	Y	G	G	C	L	A	K	R	N	N	F
	E	S	A	E	D	C	M	R	T	C	G	G	A									

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TABLE 10

103	Y G G C R A K R N N F K S A E D C M R T C G G A
104	G L C Q T F V Y G G C R A K R N N F K S A E
105	L C Q T F V Y G G C E A K R N N F K S A
107	T F F Y G G S R G K R N N F K T E E Y
108	R F F Y G G S R G K R N N F K T E E Y
109	R F F Y G G S R G K R N N F K T E E Y
110	R F F Y G G S R G K R N N F R T E E Y
111	T F F Y G G S R G K R N N F R T E E Y
112	T F F Y G G S R G R R N N F R T E E Y
113	C T F F Y G G S R G K R N N F K T E E Y
114	T F F Y G G S R G K R N N F K T E E Y C
115	C T F F Y G G S R G R R N N F R T E E Y
116	T F F Y G G S R G R R N N F R T E E Y C

Peptide no. 5 includes the sequence of SEQ ID NO:5 and is amidated at its C-terminus (see for example Fig. 1)

Peptide No. 67 includes the sequence of SEQ ID NO:67 and is amidated at its C-terminus (see for example Fig. 1)

5 Peptide No. 76 includes the sequence of SEQ ID NO:76 and is amidated at its C-terminus (see for example Fig. 1).

Peptide no. 91 includes the sequence of SEQ ID NO:91 and is amidated at its C-terminus (see for example Fig. 1).

Peptide No. 107 includes the sequence of SEQ ID NO:97 and is acetylated at its N-terminus.

10 Peptide No. 109 includes the sequence of SEQ ID NO:109 and is acetylated at its N-terminus.

Peptide No. 110 includes the sequence of SEQ ID NO:110 and is acetylated at its N-terminus.

The amine groups of Angiopep-1 (SEQ ID NO:67) and Angiopep-2 (SEQ ID NO:97) have been used as sites for conjugation of agents. To study the role of amine groups in conjugation and their impact in the overall transport capacity of these vectors, new vectors, based on the Angiopep-1 and Angiopep-2 sequence, were designed with variable reactive amine groups and variable overall charge. These polypeptides are shown in Table 11.

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Table 11: Vectors with variable amine group targets

Polypeptide Name	Polypeptide Sequences	Reactive amines (positions)	Charge	SEQ ID No.
Angiopep-3*	Ac ¹ -TFFYGGSRGKRNNFKTEEY	2 (10,15)	+1	107
Angiopep-4b	RFFYGGSRGKRNNFKTEEY	3 (1,10,15)	+3	108
Angiopep-4a	Ac ¹ -RFFYGGSRGKRNNFKTEEY	2 (10,15)	+2	109
Angiopep-5	Ac ¹ -RFFYGGSRGKRNNFRTEEY	1 (10)	+2	110
Angiopep-6	TFFYGGSRGKRNNFRTEEY	2 (1,10)	+2	111
Angiopep-7	TFFYGGSRGRRNNFRTEEY	1 (1)	+2	112

*Angiopep-3 is an acetylated form of Angiopep-2.

¹Ac represents acetylation.

5 **Modified polypeptides**

The compositions and methods of the invention may also include a polypeptide having a modification of an amino acid sequence described herein (e.g., polypeptide having a sequence described in any one of SEQ ID NOS:1-105 and 107-116 such as AngioPep-3, -4a, -4b, -5, -6, or -7). In certain embodiments, the modification does not destroy significantly a desired biological activity. In some embodiments, the modification may cause a reduction in biological activity (e.g., by at least 5%, 10%, 20%, 25%, 35%, 50%, 60%, 70%, 75%, 80%, 90%, or 95%). In other embodiments, the modification has no effect on the biological activity or may increase (e.g., by at least 5%, 10%, 25%, 50%, 100%, 200%, 500%, or 1000%) the biological activity of the original polypeptide. The modified polypeptide may have or may optimize one or more of the characteristics of a polypeptide of the invention which, in some instance might be needed or desirable. Such characteristics include in vivo stability, bioavailability, toxicity, immunological activity, or immunological identity.

20 Polypeptides used in the invention may include amino acids or sequences modified either by natural processes, such as posttranslational processing, or by

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chemical modification techniques known in the art. Modifications may occur anywhere in a polypeptide including the polypeptide backbone, the amino acid side-chains and the amino- or carboxy-terminus. The same type of modification may be present in the same or varying degrees at several sites in a given

5 polypeptide, and a polypeptide may contain more than one type of modification. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides may result from posttranslational natural processes or may be made synthetically. Other modifications include pegylation, acetylation, acylation,

10 addition of acetomidomethyl (Acm) group, ADP-ribosylation, alkylation, amidation, biotinylation, carbamoylation, carboxyethylation, esterification, covalent attachment to flavin, covalent attachment to a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of drug, covalent attachment of a marker (e.g., fluorescent or radioactive), covalent

15 attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent crosslinks, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation,

20 proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation and ubiquitination.

A modified polypeptide may further include an amino acid insertion, deletion, or substitution, either conservative or non-conservative (e.g., D-amino

25 acids, desamino acids) in the polypeptide sequence (e.g., where such changes do not substantially alter the biological activity of the polypeptide).

Substitutions may be conservative (i.e., wherein a residue is replaced by another of the same general type or group) or non-conservative (i.e., wherein a

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residue is replaced by an amino acid of another type). In addition, a non-naturally occurring amino acid may substituted for a naturally occurring amino acid (i.e., non-naturally occurring conservative amino acid substitution or a non-naturally occurring non-conservative amino acid substitution).

5 Polypeptides made synthetically may include substitutions of amino acids not naturally encoded by DNA (e.g., non-naturally occurring or unnatural amino acid). Examples of non-naturally occurring amino acids include D-amino acids, an amino acid having an acetylaminoethyl group attached to a sulfur atom of a cysteine, a pegylated amino acid, the omega amino acids of the formula
10 $\text{NH}_2(\text{CH}_2)_n\text{COOH}$ wherein n is 2-6, neutral nonpolar amino acids, such as sarcosine, t-butyl alanine, t-butyl glycine, N-methyl isoleucine, and norleucine. Phenylglycine may substitute for Trp, Tyr, or Phe; citrulline and methionine sulfoxide are neutral nonpolar, cysteic acid is acidic, and ornithine is basic. Proline may be substituted with hydroxyproline and retain the conformation
15 conferring properties.

Analogues may be generated by substitutional mutagenesis and retain the biological activity of the original polypeptide. Examples of substitutions identified as “conservative substitutions” are shown in Table 12. If such substitutions result in a change not desired, then other type of substitutions, denominated “exemplary
20 substitutions” in Table 12, or as further described herein in reference to amino acid classes, are introduced and the products screened.

Substantial modifications in function or immunological identity are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the
25 substitution, for example, as a sheet or helical conformation. (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side chain properties:

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(1) hydrophobic: norleucine, methionine (Met), Alanine (Ala), Valine (Val), Leucine (Leu), Isoleucine (Ile), Histidine (His), Tryptophan (Trp), Tyrosine (Tyr), Phenylalanine (Phe),

(2) neutral hydrophilic: Cysteine (Cys), Serine (Ser), Threonine (Thr)

5 (3) acidic/negatively charged: Aspartic acid (Asp), Glutamic acid (Glu)

(4) basic: Asparagine (Asn), Glutamine (Gln), Histidine (His), Lysine (Lys), Arginine (Arg)

(5) residues that influence chain orientation: Glycine (Gly), Proline (Pro);

(6) aromatic: Tryptophan (Trp), Tyrosine (Tyr), Phenylalanine (Phe),

10 Histidine (His),

(7) polar: Ser, Thr, Asn, Gln

(8) basic positively charged: Arg, Lys, His, and;

(9) charged: Asp, Glu, Arg, Lys, His

Other conservative amino acid substitutions are listed in Table 3.

15 **Table 12: Amino acid substitution**

Original residue	Exemplary substitution	Conservative substitution
Ala (A)	Val, Leu, Ile	Val
Arg (R)	Lys, Gln, Asn	Lys
Asn (N)	Gln, His, Lys, Arg	Gln
Asp (D)	Glu	Glu
Cys (C)	Ser	Ser
Gln (Q)	Asn	Asn
Glu (E)	Asp	Asp
Gly (G)	Pro	Pro
His (H)	Asn, Gln, Lys, Arg	Arg
Ile (I)	Leu, Val, Met, Ala, Phe, norleucine	Leu
Leu (L)	Norleucine, Ile, Val, Met, Ala, Phe	Ile
Lys (K)	Arg, Gln, Asn	Arg
Met (M)	Leu, Phe, Ile	Leu

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Original residue	Exemplary substitution	Conservative substitution
Phe (F)	Leu, Val, Ile, Ala	Leu
Pro (P)	Gly	Gly
Ser (S)	Thr	Thr
Thr (T)	Ser	Ser
Trp (W)	Tyr	Tyr
Tyr (Y)	Trp, Phe, Thr, Ser	Phe
Val (V)	Ile, Leu, Met, Phe, Ala, norleucine	Leu

Additional analogues

The polypeptides and conjugates used in the invention may include polypeptide analogs of aprotinin known in the art. For example, U.S. Patent No. 5,807,980 describes Bovine Pancreatic Trypsin Inhibitor (aprotinin)-derived inhibitors as well as a method for their preparation and therapeutic use, including the polypeptide of SEQ ID NO:102. These polypeptides have been used for the treatment of a condition characterized by an abnormal appearance or amount of tissue factor and/or factor VIIIa such as abnormal thrombosis. U.S. Patent No. 5,780,265 describes serine protease inhibitors capable of inhibiting plasma kallikrein, including SEQ ID NO:103. U.S. Patent No. 5,118,668 describes Bovine Pancreatic Trypsin Inhibitor variants, including SEQ ID NO:105. The aprotinin amino acid sequence (SEQ ID NO:98), the Angiopep-1 amino acid sequence (SEQ ID NO:67), and SEQ ID NO:104, as well as some sequences of biologically active analogs may be found in International Application Publication No. WO 2004/060403.

An exemplary nucleotide sequence encoding an aprotinin analogue is illustrated in SEQ ID NO:106 (atgagaccag atttctgcct cgagccgccg tacactgggc cctgcaaagc tcgtatcatc cggtacttct acaatgcaaa ggcaggcctg tgtcagacct tcgtatacgg cggctgcaga gctaagcgta acaactcaa atccgcggaa gactgcatgc gtacttgccg tggtgcttag;

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Genbank accession No. X04666). This sequence encodes a lysine at position 16 instead of a valine, as found in SEQ ID NO:98. A mutation in the nucleotide sequence of SEQ ID NO:106 may be introduced by methods known in the art to change the produce the polypeptide of SEQ ID NO:98 having a valine in position
5 16. Additional mutations or fragments may be obtained using any technique known in the art.

Other examples of aprotinin analogs may be found by performing a protein BLAST (Genebank: www.ncbi.nlm.nih.gov/BLAST/) using the synthetic aprotinin sequence (or portion thereof) disclosed in International Application No.
10 PCT/CA2004/000011. Exemplary aprotinin analogs are found under accession Nos. CAA37967 (GI:58005) and 1405218C (GI:3604747).

Preparation of polypeptide derivatives and peptidomimetics

In addition to polypeptides consisting only of naturally occurring amino
15 acids, peptidomimetics or polypeptide analogs can also be used in the present invention. Polypeptide analogs are commonly used in the pharmaceutical industry as non-polypeptide drugs with properties analogous to those of the template polypeptide. The non-polypeptide compounds are termed “polypeptide mimetics” or peptidomimetics (Fauchere et al., *Infect. Immun.* 54:283-287, 1986; Evans et al.,
20 *J. Med. Chem.* 30:1229-1239, 1987). Polypeptide mimetics that are structurally related to therapeutically useful polypeptides may be used to produce an equivalent or enhanced therapeutic or prophylactic effect. Generally, peptidomimetics are structurally similar to the paradigm polypeptide (i.e., a polypeptide that has a biological or pharmacological activity) such as naturally-
25 occurring receptor-binding polypeptides, but have one or more peptide linkages optionally replaced by linkages such as $-\text{CH}_2\text{NH}-$, $-\text{CH}_2\text{S}-$, $-\text{CH}_2-\text{CH}_2-$, $-\text{CH}=\text{CH}-$ (cis and trans), $-\text{CH}_2\text{SO}-$, $-\text{CH}(\text{OH})\text{CH}_2-$, $-\text{COCH}_2-$ etc., by methods well known in the art (Spatola, *Peptide Backbone Modifications*, Vega Data,

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1(3):267, 1983); Spatola et al. (*Life Sci.* 38:1243-1249, 1986); Hudson et al. (*Int. J. Pept. Res.* 14:177-185, 1979); and Weinstein. B., 1983, Chemistry and Biochemistry, of Amino Acids, Peptides and Proteins, Weinstein eds, Marcel Dekker, New-York). Such polypeptide mimetics may have significant advantages
5 over naturally-occurring polypeptides including more economical production, greater chemical stability, enhanced pharmacological properties (e.g., half-life, absorption, potency, efficiency), reduced antigenicity and others.

While the polypeptides used in the invention may be effective in entering particular cell types (e.g., those described herein), their effectiveness may be
10 reduced by the presence of proteases. Serum proteases have specific substrate requirements. The substrate must have both L-amino acids and peptide bonds for cleavage. Furthermore, exopeptidases, which represent the most prominent component of the protease activity in serum, usually act on the first peptide bond of the polypeptide and require a free N-terminus (Powell et al., *Pharm. Res.*
15 10:1268-1273, 1993). In light of this, it is often advantageous to use modified versions of polypeptides. The modified polypeptides retain the structural characteristics of the original L-amino acid polypeptides that confer biological activity with regard to IGF-1, but are advantageously not readily susceptible to cleavage by protease and/or exopeptidases.

20 Systematic substitution of one or more amino acids of a consensus sequence with D-amino acid of the same type (e.g., D-lysine in place of L-lysine) may be used to generate more stable polypeptides. Thus, a polypeptide derivative or peptidomimetic used in the present invention may be all L, all D or mixed D, L polypeptide. The presence of an N-terminal or C-terminal D-amino acid increases
25 the *in vivo* stability of a polypeptide because peptidases cannot utilize a D-amino acid as a substrate (Powell et al., *Pharm. Res.* 10:1268-1273, 1993). Reverse-D polypeptides are polypeptides containing D-amino acids, arranged in a reverse sequence relative to a polypeptide containing L-amino acids. Thus, the C-terminal

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residue of an L-amino acid polypeptide becomes N-terminal for the D-amino acid polypeptide, and so forth. Reverse D-polypeptides retain the same tertiary conformation and therefore the same activity, as the L-amino acid polypeptides, but are more stable to enzymatic degradation *in vitro* and *in vivo*, and thus have
5 greater therapeutic efficacy than the original polypeptide (Brady and Dodson, *Nature* 368:692-693, 1994; Jameson et al., *Nature* 368:744-746, 1994). In addition to reverse-D-polypeptides, constrained polypeptides comprising a consensus sequence or a substantially identical consensus sequence variation may be generated by methods well known in the art (Rizo and Gierasch, *Ann. Rev.*
10 *Biochem.* 61:387-418, 1992). For example, constrained polypeptides may be generated by adding cysteine residues capable of forming disulfide bridges and, thereby, resulting in a cyclic polypeptide. Cyclic polypeptides have no free N- or C-termini. Accordingly, they are not susceptible to proteolysis by exopeptidases, although they are, of course, susceptible to endopeptidases, which do not cleave at
15 peptide termini. The amino acid sequences of the polypeptides with N-terminal or C-terminal D-amino acids and of the cyclic polypeptides are usually identical to the sequences of the polypeptides to which they correspond, except for the presence of N-terminal or C-terminal D-amino acid residue, or their circular structure, respectively.

20 A cyclic derivative containing an intramolecular disulfide bond may be prepared by conventional solid phase synthesis while incorporating suitable S-protected cysteine or homocysteine residues at the positions selected for cyclization such as the amino and carboxy termini (Sah et al., *J. Pharm. Pharmacol.* 48:197, 1996). Following completion of the chain assembly,
25 cyclization can be performed either (1) by selective removal of the S-protecting group with a consequent on-support oxidation of the corresponding two free SH-functions, to form a S-S bonds, followed by conventional removal of the product from the support and appropriate purification procedure or (2) by removal of the

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polypeptide from the support along with complete side chain de-protection, followed by oxidation of the free SH-functions in highly dilute aqueous solution.

The cyclic derivative containing an intramolecular amide bond may be prepared by conventional solid phase synthesis while incorporating suitable amino and carboxyl side chain protected amino acid derivatives, at the position selected for cyclization. The cyclic derivatives containing intramolecular -S-alkyl bonds can be prepared by conventional solid phase chemistry while incorporating an amino acid residue with a suitable amino-protected side chain, and a suitable S-protected cysteine or homocysteine residue at the position selected for cyclization.

Another effective approach to confer resistance to peptidases acting on the N-terminal or C-terminal residues of a polypeptide is to add chemical groups at the polypeptide termini, such that the modified polypeptide is no longer a substrate for the peptidase. One such chemical modification is glycosylation of the polypeptides at either or both termini. Certain chemical modifications, in particular N-terminal glycosylation, have been shown to increase the stability of polypeptides in human serum (Powell et al., *Pharm. Res.* 10:1268-1273, 1993). Other chemical modifications which enhance serum stability include, but are not limited to, the addition of an N-terminal alkyl group, consisting of a lower alkyl of from one to twenty carbons, such as an acetyl group, and/or the addition of a C-terminal amide or substituted amide group. In particular, the compositions and methods of the present invention can include modified polypeptides consisting of polypeptides bearing an N-terminal acetyl group and/or a C-terminal amide group.

Also included by the present invention are other types of polypeptide derivatives containing additional chemical moieties not normally part of the polypeptide, provided that the derivative retains the desired functional activity of the polypeptide. Examples of such derivatives include (1) N-acyl derivatives of the amino terminal or of another free amino group, wherein the acyl group may be an alkanoyl group (e.g., acetyl, hexanoyl, octanoyl) an aroyl group (e.g., benzoyl)

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or a blocking group such as F-moc (fluorenylmethyl-O-CO-); (2) esters of the carboxy terminal or of another free carboxy or hydroxyl group; (3) amide of the carboxy-terminal or of another free carboxyl group produced by reaction with ammonia or with a suitable amine; (4) phosphorylated derivatives; (5) derivatives
5 conjugated to an antibody or other biological ligand and other types of derivatives.

Longer polypeptide sequences which result from the addition of additional amino acid residues to the polypeptides used in the invention are also encompassed. Such longer polypeptide sequences would be expected to have the same biological activity (e.g., entering particular cell types) as the polypeptides
10 described above. While polypeptides having a substantial number of additional amino acids are not excluded, it is recognized that some large polypeptides may assume a configuration that masks the effective sequence, thereby preventing binding to a target (e.g., a member of the LRP receptor family such as LRP or LRP2). These derivatives could act as competitive antagonists. Thus, while the
15 present invention encompasses polypeptides or derivatives of the polypeptides described herein having an extension, desirably the extension does not destroy the cell targeting activity of the polypeptide or derivative.

Other derivatives that can be used in present invention are dual polypeptides consisting of two of the same, or two different polypeptides described
20 herein covalently linked to one another either directly or through a spacer, such as by a short stretch of alanine residues or by a putative site for proteolysis (e.g., by cathepsin, see e.g., U.S. Patent No. 5,126,249 and European Patent No. 495 049). Multimers of the polypeptides used in the present invention consist of polymer of molecules formed from the same or different polypeptides or derivatives thereof.

25 The present invention also encompasses polypeptide derivatives that are chimeric or fusion proteins containing a polypeptide described herein, or fragment thereof, linked at its amino- or carboxy-terminal end, or both, to an amino acid sequence of a different protein. Such a chimeric or fusion protein may be produced

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by recombinant expression of a nucleic acid encoding the protein. For example, a chimeric or fusion protein may contain at least 6 amino acids of a polypeptide used in the present invention and desirably has a functional activity equivalent or greater than a polypeptide used in the invention.

5 Polypeptide derivatives used in the present invention can be made by altering the amino acid sequences by substitution, addition, or deletion or an amino acid residue to provide a functionally equivalent molecule, or functionally enhanced or diminished molecule, as desired. The derivatives used in the present invention include, but are not limited to, those containing, as primary amino acid
10 sequence, all or part of the amino acid sequence of the polypeptides described herein (e.g., any one of SEQ ID NOS:1-105 and 107-116) including altered sequences containing substitutions of functionally equivalent amino acid residues. For example, one or more amino acid residues within the sequence can be substituted by another amino acid of a similar polarity which acts as a functional
15 equivalent, resulting in a silent alteration. Substitution for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the positively charged (basic) amino acids include, arginine, lysine and histidine. The nonpolar (hydrophobic) amino acids include, leucine, isoleucine, alanine, phenylalanine, valine, proline, tryptophan and
20 methionine. The uncharged polar amino acids include serine, threonine, cysteine, tyrosine, asparagine and glutamine. The negatively charged (acid) amino acids include glutamic acid and aspartic acid. The amino acid glycine may be included in either the nonpolar amino acid family or the uncharged (neutral) polar amino acid family. Substitutions made within a family of amino acids are generally
25 understood to be conservative substitutions.

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Assays to identify peptidomimetics

As described above, non-peptidyl compounds generated to replicate the backbone geometry and pharmacophore display (peptidomimetics) of the polypeptides identified by the methods can possess attributes of greater metabolic
5 stability, higher potency, longer duration of action and better bioavailability.

The peptidomimetics compounds used in the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring
10 deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to polypeptide libraries, while the other four approaches are applicable to polypeptide, non-peptide oligomer or small molecule libraries of compounds (Lam, *Anticancer Drug Des.* 12:145, 1997). Examples of methods for the
15 synthesis of molecular libraries can be found in the art, for example, in: DeWitt et al. (*Proc. Natl. Acad. Sci. USA* 90:6909, 1993); Erb et al. (*Proc. Natl. Acad. Sci. USA* 91:11422, 1994); Zuckermann et al., *J. Med. Chem.* 37:2678, 1994); Cho et al. (*Science* 261:1303, 1993); Carell et al. (*Angew. Chem, Int. Ed. Engl.* 33:2059, 1994 and *ibid* 2061); and in Gallop et al. (*Med. Chem.* 37:1233, 1994). Libraries
20 of compounds may be presented in solution (e.g., Houghten, *Biotechniques* 13:412-421, 1992) or on beads (Lam, *Nature* 354:82-84, 1991), chips (Fodor, *Nature* 364:555-556, 1993), bacteria or spores (U.S. Patent No. 5,223,409), plasmids (Cull et al., *Proc. Natl. Acad. Sci. USA* 89:1865-1869, 1992) or on phage (Scott and Smith, *Science* 249:386-390, 1990), or luciferase, and the enzymatic
25 label detected by determination of conversion of an appropriate substrate to product.

Once a polypeptide that can be used the present invention is identified, it may be isolated and purified by any number of standard methods including, but not

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limited to, differential solubility (e.g., precipitation), centrifugation, chromatography (e.g., affinity, ion exchange, size exclusion, and the like) or by any other standard techniques used for the purification of polypeptides, peptidomimetics or proteins. The functional properties of an identified
5 polypeptide of interest may be evaluated using any functional assay known in the art. Desirably, assays for evaluating downstream receptor function in intracellular signaling are used (e.g., cell proliferation).

For example, the peptidomimetics compounds used in the present invention may be obtained using the following three-phase process: (1) scanning the
10 polypeptides used in the present invention to identify regions of secondary structure necessary for targeting the particular cell types described herein; (2) using conformationally constrained dipeptide surrogates to refine the backbone geometry and provide organic platforms corresponding to these surrogates; and (3) using the best organic platforms to display organic pharmacophores in libraries of
15 candidates designed to mimic the desired activity of the native polypeptide. In more detail the three phases are as follows. In phase 1, the lead candidate polypeptides are scanned and their structure abridged to identify the requirements for their activity. A series of polypeptide analogs of the original are synthesized. In phase 2, the best polypeptide analogs are investigated using the
20 conformationally constrained dipeptide surrogates. Indolizidin-2-one, indolizidin-9-one and quinolizidinone amino acids (I^2aa , I^9aa and Qaa respectively) are used as platforms for studying backbone geometry of the best polypeptide candidates. These and related platforms (reviewed in Halab et al., *Biopolymers* 55:101-122, 2000; and Hanessian et al. *Tetrahedron* 53:12789-12854, 1997) may be introduced
25 at specific regions of the polypeptide to orient the pharmacophores in different directions. Biological evaluation of these analogs identifies improved lead polypeptides that mimic the geometric requirements for activity. In phase 3, the platforms from the most active lead polypeptides are used to display organic

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surrogates of the pharmacophores responsible for activity of the native polypeptide. The pharmacophores and scaffolds are combined in a parallel synthesis format. Derivation of polypeptides and the above phases can be accomplished by other means using methods known in the art.

5 Structure function relationships determined from the polypeptides, polypeptide derivatives, peptidomimetics, or other small molecules used in the present invention may be used to refine and prepare analogous molecular structures having similar or better properties. Accordingly, the compounds used in the present invention also include molecules that share the structure, polarity,
10 charge characteristics and side chain properties of the polypeptides described herein.

In summary, based on the disclosure herein, those skilled in the art can develop polypeptides and peptidomimetics screening assays which are useful for identifying compounds for targeting an agent to particular cell types (e.g., those
15 described herein). The assays may be developed for low-throughput, high-throughput, or ultra-high throughput screening formats. Assays of the present invention include assays which are amenable to automation.

Conjugates

20 The polypeptides described herein or derivatives thereof may be linked to an agent. For example, the polypeptide (e.g., any described herein) may be attached to a therapeutic agent, a diagnostic agent, or to a label. In certain embodiments, the polypeptide is linked to or labeled with a detectable label, such as a radioimaging agent, for diagnosis of a disease or condition. Examples of these
25 agents include a radioimaging agent-antibody-vector conjugate, where the antibody binds to a disease or condition-specific antigen (e.g., for diagnosis or therapy). Other binding molecules are also contemplated by the invention. In other cases, the polypeptide or derivative is linked to a therapeutic agent, to treat a

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disease or condition, or may be linked to or labeled with mixtures thereof. The disease or condition may be treated by administering a vector-agent conjugate to an individual under conditions which allow transport of the agent across the BBB or into a particular cell type. Each polypeptide may include at least 1, 2, 3, 4, 5, 6,
5 or 7 agents. In other embodiments, each agent has at least 1, 2, 3, 4, 5, 6, 7, 10, 15, 20, or more polypeptides attached thereto. The conjugates of the invention may be able to promote accumulation (e.g., due to increased uptake or reduced removal) of the agent in a particular cell type or tissue such as liver, lung, kidney, spleen or muscle of a subject.

10 The agent may be releasable from the vector after transport into a particular cell type or across the BBB. The agent can be released, for example, by enzymatic cleavage or other breakage of a chemical bond between the vector and the agent. The released agent may then function in its intended capacity in the absence of the vector.

15
Therapeutic agents. A therapeutic agent may be any biologically active agent. For example, a therapeutic may be a drug, a medicine, an agent emitting radiation, a cellular toxin (for example, a chemotherapeutic agent), a biologically active fragment thereof, or a mixture thereof to treat a disease (e.g., to killing
20 cancer cells) or it may be an agent to treat a disease or condition in an individual. A therapeutic agent may be a synthetic product or a product of fungal, bacterial or other microorganism (e.g., mycoplasma or virus), animal, such as reptile, or plant origin. A therapeutic agent and/or biologically active fragment thereof may be an enzymatically active agent and/or fragment thereof, or may act by inhibiting or
25 blocking an important and/or essential cellular pathway or by competing with an important and/or essential naturally occurring cellular component. Other therapeutic agents include antibodies and antibody fragments.

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Anticancer agents. Any anticancer agent known in the art may be part of a conjugate used in the invention. In certain embodiments, the agent is paclitaxel or a paclitaxel analog (e.g., those described herein). Cancers of the brain may be treated with a conjugate containing a vector that is efficiently transported across the BBB (e.g., AngioPep-1, AngioPep-2, AngioPep-3, AngioPep-4a, AngioPep-4b, AngioPep-5, or AngioPep-6). Liver, lung, kidney, or spleen cancers may be treated with an anticancer agent conjugated to a vector that is transported efficiently into the appropriate cell type (e.g., AngioPep-7). Exemplary agents include abarelix, aldesleukin, alemtuzumab, alitretinoin, allopurinol, altretamine, amifostine, anakinra, anastrozole, arsenic trioxide, asparaginase, azacitidine, BCG Live, bevacuzimab, bexarotene, bleomycin, bleomycin, bortezombi, bortezomib, busulfan, busulfan, calusterone, capecitabine, carboplatin, carmustine, celecoxib, cetuximab, chlorambucil, cisplatin, cladribine, clofarabine, cyclophosphamide, cytarabine, dacarbazine, dactinomycin, actinomycin D, dalteparin (e.g., sodium), darbepoetin alfa, dasatinib, daunorubicin, daunomycin, decitabine, denileukin, Denileukin diftitox, dexrazoxane, docetaxel, doxorubicin, dromostanolone propionate, eculizumab, epirubicin (e.g., HCl), epoetin alfa, erlotinib, estramustine, etoposide (e.g., phosphate), exemestane, fentanyl (e.g., citrate), filgrastim, floxuridine, fludarabine, fluorouracil, 5-FU, fulvestrant, gefitinib, gemcitabine (e.g., HCl), gemtuzumab ozogamicin, goserelin (e.g., acetate), histrelin (e.g., acetate), hydroxyurea, ibritumomab tiuxetan, idarubicin, ifosfamide, imatinib (e.g., mesylate), Interferon alfa-2b, irinotecan, lapatinib ditosylate, lenalidomide, letrozole, leucovorin, leuprolide (e.g., acetate), levamisole, lomustine, CCNU, meclorethamine (nitrogen mustard), megestrol, melphalan (L-PAM), mercaptopurine (6-MP), mesna, methotrexate, methoxsalen, mitomycin C, mitotane, mitoxantrone, nandrolone phenpropionate, nelarabine, nofetumomab, oprelvekin, oxaliplatin, paclitaxel, palifermin, pamidronate, panitumumab, pegademase, pegaspargase, pegfilgrastim, peginterferon alfa-2b, pemetrexed (e.g.,

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disodium), pentostatin, pipobroman, plicamycin (mithramycin), porfimer (e.g., sodium), procarbazine, quinacrine, rasburicase, rituximab, sargramostim, sorafenib, streptozocin, sunitinib (e.g., maleate), talc, tamoxifen, temozolomide, teniposide (VM-26), testolactone, thalidomide, thioguanine (6-TG), thiotepa, 5 thiotepa, thiotepa, topotecan (e.g., hcl), toremifene, Tositumomab/I-131 (tositumomab), trastuzumab, trastuzumab, tretinoin (ATRA), uracil mustard, valrubicin, vinblastine, vincristine, vinorelbine, vorinostat, zoledronate, and zoledronic acid.

10 *Detectable labels.* For the purpose of detection or diagnosis, the conjugate used in the invention may be labeled. Detectable labels, or markers, may be a radiolabel, a fluorescent label, a nuclear magnetic resonance active label, a luminescent label, a chromophore label, a positron emitting isotope for PET scanner, chemiluminescence label, or an enzymatic label. Exemplary 15 radioimaging agents emitting radiation (detectable radiolabels) include indium-111, technitium-99, or low dose iodine-131. Gamma and beta emitting radionuclides include ^{67}Cu , ^{67}Ga , ^{90}Y , ^{111}In , $^{99\text{m}}\text{Tc}$, and ^{201}Tl). Positron emitting radionuclides include ^{18}F , ^{55}Co , ^{60}Cu , ^{62}Cu , ^{64}Cu , ^{66}Ga , ^{68}Ga , ^{82}Rb , and ^{86}Y . Fluorescent labels include Cy5.5, Alexa 488, green fluorescent protein (GFP), 20 fluorescein, and rhodamine. Chemiluminescence labels include luciferase and β -galactosidase. Enzymatic labels include peroxidase and phosphatase. A histag may also be a detectable label. For example, conjugates may include a vector moiety and an antibody moiety (antibody or antibody fragment), which may further include a label. In this case, the label may be attached to either the vector 25 or to the antibody.

Antibodies. Antibodies may also be part of a conjugate used in the invention. The conjugation by accomplished using any means known in the art

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(e.g., using the conjugation strategies described herein). Any diagnostic or therapeutic antibody may be conjugated to one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) vectors of the invention. In addition, antibody fragments (e.g., capable of binding to an antigen) may also be conjugated to the vectors of the invention. Antibody fragments include the Fab and Fc regions, heavy chain, and light chain of an antibody (e.g., of any antibody described herein). Exemplary antibodies for use in diagnosis and therapy of cancer include ABX-EGF (Panitimumab), OvaRex (Oregovemab), Theragyn (pemtumomabytrrium-90), Therex, Bivatuzumab, Panorex (Edrecolomab), ReoPro (Abciximab), Bexxar (Tositumomab), MAb, idiotypic 105AD7, Anti-EpCAM (Catumaxomab), MAb lung cancer (from Cytoclonal), Herceptin (Trastuzumab), Rituxan (Rituximab), Avastin (Bevacizumab), AMD Fab (Ranibizumab), E-26 (2nd gen. IgE) (Omalizumab), Zevalin (Rituxan + yttrium-90) (Ibritumomab tiuxetan), Cetuximab, BEC2 (Mitumomab), IMC-1C11, nuC242-DM1, LymphoCide (Epratuzumab), LymphoCide Y-90, CEA-Cide (Labetuzumab), CEA-Cide Y-90, CEA-Scan (Tc-99m-labeled arcitumomab), LeukoScan (Tc-99m-labeled sulesomab), LymphoScan (Tc-99m-labeled bectumomab), AFP-Scan (Tc-99m-labeled), HumaRAD-HN (+ yttrium-90), HumaSPECT (Votumumab), MDX-101 (CTLA-4), MDX-210 (her-2 overexpression), MDX-210/MAK, Vitaxin, MAb 425, IS-IL-2, Campath (alemtuzumab), CD20 streptavidin, Avidicin, (albumin + NRLU13), Oncolym (+ iodine-131) Cotara (+ iodine-131), C215 (+ staphylococcal enterotoxin, MAb lung/kidney cancer (from Pharmacia Corp.), nacolomab tafenatox (C242 staphylococcal enterotoxin), Nuvion (Visilizumab), SMART M195, SMART 1D10, CEAVac, TriGem, TriAb, NovoMAb-G2 radiolabeled, Monopharm C, GlioMAb-H (+ gelonin toxin), Rituxan (Rituximab), and ING-1. Additional therapeutic antibodies include 5G1.1 (Ecluizumab), 5G1.1-SC (Pexelizumab), ABX-CBL (Gavilimomab), ABX-IL8, Antegren (Natalizumab), Anti-CD11a (Efalizumab), Anti-CD18 (from Genetech), Anti-LFA1, Antova, BTI-

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322, CDP571, CDP850, Corsevin M, D2E7 (Adalimumab), Humira
(Adalimumab), Hu23F2G (Rovelizumab), IC14, IDEC-114, IDEC-131, IDEC-
151, IDEC-152, Infliximab (Remicade), LDP-01, LDP-02, MAK-195F
(Afelimomab), MDX-33, MDX-CD4, MEDI-507 (Siplizumab), OKT4A, OKT3
5 (Muromonab- CD3), and ReoPro (Abciximab).

Conjugation linkers

The conjugate (e.g., a polypeptide-agent conjugate) may be obtained using
any cross-linking (conjugation) reagent or protocol known in the art, many of which
are commercially available. Such protocols and reagents include, cross-linkers
10 reactive with amino, carboxyl, sulfhydryl, carbonyl, carbohydrate and/or phenol
groups. The amounts, times, and conditions of such protocols can be varied to
optimize conjugation. Cross-linking reagents contain at least two reactive groups
and are generally divided into homofunctional cross-linkers (containing identical
reactive groups) and heterofunctional cross-linkers (containing non-identical
15 reactive groups). The cross-linkers of the invention may be either
homobifunctional and/or heterobifunctional. Furthermore the cross-linker may
incorporate a 'spacer' between the reactive moieties, or the two reactive moieties
in the cross-linker may be directly linked. Bonds may include ester bonds.

Exemplary linkers include BS³ [Bis(sulfosuccinimidyl)suberate], NHS/EDC
20 (N-hydroxysuccinimide and N-ethyl-(dimethylaminopropyl)carbodiimide, Sulfo-
EMCS ([N-e-Maleimidocaproic acid]hydrazide), SATA (N-succinimidyl-S-
acetylthioacetate), and hydrazide. BS³ is a homobifunctional N-
hydroxysuccinimide ester that targets accessible primary amines. A conjugation
scheme is exemplified in Fig. 2. NHS/EDC allows for the conjugation of primary
25 amine groups with carboxyl groups. Sulfo-EMCS are heterobifunctional reactive
groups (maleimide and NHS-ester) that are reactive toward sulfhydryl and amino
groups. Amine coupling using sulfo-NHS/EDC activation may be used to cross-
link therapeutic antibodies with the polypeptides of the invention, as exemplified

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in Figs. 3 and 4. This is a fast, simple and reproducible coupling technique. The resulting conjugate is stable and retains the biological activity of the antibody. Moreover, it has a high conjugation capacity that can be reliably controlled and a low non-specific interaction during coupling procedures. SATA is reactive
5 towards amines and adds protected sulfhydryls groups. The NHS-ester reacts with primary amines to form stable amide bonds. Sulfhydryl groups may be deprotected using hydroxylamine. This conjugation method is exemplified in Fig. 5. Hydrazide can be used to link carboxyl groups to primary amines, as shown in Fig. 6, and may therefore be useful for linking glycoproteins. Additional
10 exemplary linkers are illustrated in Fig. 7.

Small molecules such as therapeutic agents can be conjugated to polypeptides (e.g., those described herein). The exemplary small molecule, paclitaxel, has two strategic positions (position C2' and C7) useful for conjugation. Conjugation of a vector or vector of the invention to paclitaxel can be performed
15 as follows (Fig. 8). Briefly, paclitaxel is reacted with anhydride succinic pyridine for three hours at room temperature to attach a succinyl group in position 2'. The 2'-succinyl paclitaxel has a cleavable ester bond in position 2' can simply release succinic acid. This cleavable ester bond can be further used for various modifications with linkers, if desired. The resulting 2'-O-succinyl-paclitaxel is
20 then reacted with EDC/NHS in DMSO for nine hours at room temperature, followed by the addition of the vector or vector in Ringer/DMSO for an additional reaction time of four hours at room temperature. The reaction of conjugation depicted in Fig. 8 is monitored by HPLC. Each intermediate, such as paclitaxel, 2'-O-succinyl-paclitaxel and 2'-O-NHS-succinyl-paclitaxel, is purified and
25 validated using different approaches such as HPLC, thin liquid chromatography, NMR (^{13}C or ^1H exchange), melting point, mass spectrometry. The final conjugate is analyzed by mass spectrometry and SDS-polyacrylamide gel electrophoresis.

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This allows determining the number of paclitaxel molecules conjugated on each vector.

Pharmaceutical compositions

5 Because hydrophobic agents often exhibit limited solubility in aqueous solution, pharmaceutical compositions of the inventions may include solubilizing agents. Our exemplary formulations of ANG1005 include DMSO and Solutol HS 15; however, other solubilizing agents, either in place of or in addition to these agents may be useful in the compositions of the invention. The compositions may
10 further include buffering agents, tonicity agents, and lyophilization agents (e.g., bulking or cryoprotectant agents).

Solubilizing agents

The compositions and methods of the invention may include any
15 solubilizing agent known in the art. Such agents may make up at least 1%, 2%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 60%, or 70% of the mass of the composition. Exemplary solubilizers include water-soluble organic solvents (e.g., polyethylene glycol 300, polyethylene glycol 400, ethanol, propylene glycol, glycerin, *N*-methyl-2-pyrrolidone, dimethylacetamide, and dimethylsulfoxide),
20 non-ionic surfactants (e.g., Cremophor EL, Cremophor RH 40, Cremophor RH 60, *d*- α -tocopherol polyethylene glycol 1000 succinate, polysorbate 20, polysorbate 80, Solutol HS 15 (Macrogol 15 Hydroxystearate), sorbitan monooleate, poloxamer 407, Labrafil M-1944CS, Labrafil M-2125CS, Labrasol, Gellucire 44/14, Softigen 767, and mono- and di-fatty acid esters of PEG 300, 400, or 1750),
25 water-insoluble lipids (e.g., castor oil, corn oil, cottonseed oil, olive oil, peanut oil, peppermint oil, safflower oil, sesame oil, soybean oil, hydrogenated vegetable oils, hydrogenated soybean oil, and medium-chain triglycerides of coconut oil and palm seed oil), organic liquids/semi-solids (beeswax, *d*- α -tocopherol, oleic acid,

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medium-chain mono- and diglycerides), cyclodextrins (e.g., α -cyclodextrin, β -cyclodextrin, hydroxypropyl- β -cyclodextrin, and sulfobutylether- β -cyclodextrin), and phospholipids (e.g., hydrogenated soy phosphatidylcholine, distearoylphosphatidylglycerol, 1- α -dimyristoylphosphatidylcholine, 1- α -
 5 dimyristoylphosphatidylglycerol).

Buffering agents

The compositions and methods of the invention may also include one or more buffering agents. Depending on the hydrophobic agent, it may be desirable
 10 to maintain the pH or tonicity of the pharmaceutical composition (e.g., to minimize degradation of the active agent or to maximize safety or efficacy of the agent when used in treatment). Buffering to any particular pH or range of pH may be accomplished using the appropriate buffer (e.g., to pH 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5, 11.0, 11.5, 12.0, 12.5, 13.0,
 15 or any range between these values). The buffer may present at any strength necessary to achieve the desired buffering effect (e.g., 1 mM, 10 mM, 20 mM, 50 mM, 100 mM, 200 mM, 500 mM, 1.0 M, 1.5 M, or any range between these values). Exemplary buffering agents include citric acid/phosphate, acetate, barbital, borate, Britton-Robinson, cacodylate, citrate, collidine, formate, maleate,
 20 McIlvaine, phosphate, Prideaux-Ward, succinate, citrate-phosphate-borate (Teorell-Stanhagen), veronal acetate, MES (2-(N-morpholino) ethanesulfonic acid), BIS-TRIS (bis(2-hydroxyethyl) iminotris-(hydroxymethyl) methane), ADA (N-(2-acetamido)-2-iminodiacetic acid), ACES (N-(carbamoylmethyl)-2-aminoethanesulfonic acid), PIPES (piperazine-N,N'-bis(2-ethanesulfonic acid)),
 25 MOPSO (3-(N-morpholino)-2-hydroxypropanesulfonic acid), BIS-TRIS PROPANE (1,3-bis(tris (hydroxy-methyl) methylamino) propane), BES (N, N-bis(2-hydroxyethyl)-2-amino-ethanesulfonic acid), MOPS (3-(N-morpholino) propanesulfonic acid), TES (N-tris (hydroxymethyl) methyl-2-

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aminoethanesulfonic acid), HEPES (N-(2-hydroxyethyl) piperazine-N'-(2-ethanesulfonic acid), DIPSO (3-(N,N-bis(2-hydroxyethyl) amino)-2-hydroxypropanesulfonic acid), MOBS (4-(N-morpholino) butanesulfonic acid), TAPSO (3-(N-tris (hydroxymethyl) methyl-amino)-2-hydroxypropanesulfonic acid), TRIZMA (tris (hydroxymethyl-aminomethane), HEPPSO (N-(2-hydroxyethyl) piperazine-N'-(2-hydroxy-propanesulfonic acid), POPSO (piperazine-N,N'-bis(2-hydroxypropane-sulfonic acid)), TEA (triethanolamine), EPPS (N-(2-hydroxyethyl)-piperazine-N'-(3-propanesulfonic acid), TRICINE (N-tris (hydroxy-methyl) methylglycine), GLY-GLY (glycylglycine), BICINE (N,N-bis(2-hydroxyethyl) glycine), HEPBS (N-(2-hydroxyethyl)piperazine-N'-(4-butanesulfonic acid)), TAPS (N-tris (hydroxymethyl) methyl-3-amino-propanesulfonic acid), AMPD (2-amino-2-methyl-1,3-propanediol), and/or any other buffer known in the art.

Tonicity may, in addition to or in place of a buffering agent, be maintained using any pharmaceutically acceptable salt known in the art. Exemplary salts include sodium acetate, sodium lactate, sodium chloride, potassium chloride, and calcium chloride. Such salts, either along or in combination with the buffering agents, may be present in amount sufficient to maintain the desired tonicity (e.g., 1 mM, 10 mM, 20 mM, 50 mM, 100 mM, 200 mM, 500 mM, 1.0 M, 1.5 M, or any range between these values).

Other excipients

In certain embodiments, the compositions and methods of the invention include other excipient such as a bulking agent or cryoprotectant). Bulking agents are particularly desirable where the pharmaceutical composition is provided in a dehydrated (e.g., lyophilized) form. Lyophilized compositions may contain less than 10% (e.g., less than 8%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%) water or other solvent by weight. Because dehydrated compositions administered by

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parenteral routes are typically dissolved in an aqueous solution prior to administration to a patient, it can be important that the dehydration process proceed in a manner allowing for resolubilization. Bulking agents can be added to ensure that the lyophilized product can be resolubilized more readily. Such agents
5 are known in the art and include polyethylene glycol, polyvinyl alcohol, polyvinyl pyrrolidone, dextran; sugars such as dextrose, mannitol, sucrose, lactose, trehalose, and sorbitol; amino acids such as glycine, arginine, aspartic acid; and soluble proteins such as collagen, gelatin, or serum albumin.

The compositions may further comprise preservatives (e.g., thimerosal,
10 benzyl alcohol, parabens), covalent attachment of polymers such as polyethylene glycol to the protein, complexation with metal ions, or incorporation of the material into or onto particulate preparations of polymeric compounds such as polylactic acid, polyglycolic acid, hydrogels, etc, or onto liposomes, microemulsions, micelles, unilamellar or multilamellar vesicles, erythrocyte
15 ghosts, or spheroplasts. Such compositions will influence the physical state, solubility, stability, rate of in vivo release, and rate of in vivo clearance. Controlled or sustained release compositions include formulation in lipophilic depots (e.g., fatty acids, waxes, oils). Also comprehended by the invention are particulate compositions coated with polymers (e.g., poloxamers or poloxamines).
20 Other embodiments of the compositions of the invention incorporate particulate forms protective coatings, protease inhibitors or permeation enhancers for various routes of administration, including parenteral, pulmonary, nasal, oral, vaginal, rectal routes. In one embodiment the pharmaceutical composition is administered parenterally, paracancerally, transmucosally, transdermally, intramuscularly,
25 intravenously, intradermally, subcutaneously, intraperitoneally, intraventricularly, intracranially, and intratumorally.

Solid dosage forms for oral use

Formulations for oral use include tablets containing the active ingredient(s) in a mixture with non-toxic pharmaceutically acceptable excipients, and such formulations are known to the skilled artisan (e.g., U.S.P.N.: 5,817,307, 5,824,300, 5,830,456, 5,846,526, 5,882,640, 5,910,304, 6,036,949, 6,036,949, and 6,372,218.

These excipients may be, for example, inert diluents or fillers (e.g., sucrose, sorbitol, sugar, mannitol, microcrystalline cellulose, starches including potato starch, calcium carbonate, sodium chloride, lactose, calcium phosphate, calcium sulfate, or sodium phosphate); granulating and disintegrating agents (e.g., cellulose derivatives including microcrystalline cellulose, starches including potato starch, croscarmellose sodium, alginates, or alginic acid); binding agents (e.g., sucrose, glucose, sorbitol, acacia, alginic acid, sodium alginate, gelatin, starch, pregelatinized starch, microcrystalline cellulose, magnesium aluminum silicate, carboxymethylcellulose sodium, methylcellulose, hydroxypropyl methylcellulose, ethylcellulose, polyvinylpyrrolidone, or polyethylene glycol); and lubricating agents, glidants, and anti-adhesives (e.g., magnesium stearate, zinc stearate, stearic acid, silicas, hydrogenated vegetable oils, or talc). Other pharmaceutically acceptable excipients can be colorants, flavoring agents, plasticizers, humectants, buffering agents, and the like.

The tablets may be uncoated or they may be coated by known techniques, optionally to delay disintegration and absorption in the gastrointestinal tract and thereby providing a sustained action over a longer period. The coating may be adapted to release the agent in a predetermined pattern (e.g., in order to achieve a controlled release formulation) or it may be adapted not to release the agent(s) until after passage of the stomach (enteric coating). The coating may be a sugar coating, a film coating (e.g., based on hydroxypropyl methylcellulose, methylcellulose, methyl hydroxyethylcellulose, hydroxypropylcellulose, carboxymethylcellulose, acrylate copolymers, polyethylene glycols, and/or

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polyvinylpyrrolidone), or an enteric coating (e.g., based on methacrylic acid copolymer, cellulose acetate phthalate, hydroxypropyl methylcellulose phthalate, hydroxypropyl methylcellulose acetate succinate, polyvinyl acetate phthalate, shellac, and/or ethylcellulose). Furthermore, a time delay material such as, e.g.,
5 glyceryl monostearate or glyceryl distearate, may be employed.

The solid tablet compositions may include a coating adapted to protect the composition from unwanted chemical changes, (e.g., chemical degradation prior to the release of the active substances). The coating may be applied on the solid dosage form in a similar manner as that described in *Encyclopedia of*
10 *Pharmaceutical Technology*, supra.

Formulations for oral use may also be presented as chewable tablets, or as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent (e.g., potato starch, lactose, microcrystalline cellulose, calcium carbonate, calcium phosphate, or kaolin), or as soft gelatin capsules wherein the active
15 ingredient is mixed with water or an oil medium, for example, peanut oil, liquid paraffin, or olive oil. Powders and granulates may be prepared using the ingredients mentioned above under tablets and capsules in a conventional manner using, e.g., a mixer, a fluid bed apparatus, or spray drying equipment.

20 **Methods of treatment**

The invention also features methods of treatment using the agents described herein. The anticancer agents and conjugates described herein (e.g., ANG1005) can be used to treat any cancer known in the art. Conjugates of the invention including the peptides described herein may be capable of crossing the BBB (e.g.,
25 AngioPep-1 through AngioPep-6) and thus may be used to treat any brain or central nervous system disease (e.g., a brain cancer such as glioblastoma, astrocytoma, glioma, meduloblastoma, and oligodendroma, neuroglioma, ependymoma, and meningioma). These conjugates may also be efficiently

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transported to the liver, lung, kidney, spleen or muscle (e.g., AngioPep-1 through AngioPep-7) and therefore may also be used, in conjunction with an appropriate therapeutic agent, to treat a disease associated with these tissues (e.g., a cancer such as hepatocellular carcinoma, liver cancer, small cell carcinoma (e.g., oat cell cancer), mixed small cell/large cell carcinoma, combined small cell carcinoma, and metastatic tumors. Metastatic tumors can originate from cancer of any tissue, including breast cancer, colon cancer, prostate cancer, sarcoma, bladder cancer, neuroblastoma, Wilm's tumor, lymphoma, non-Hodgkin's lymphoma, and certain T-cell lymphomas). Additional exemplary cancers that may be treated using a composition of the invention include hepatocellular carcinoma, breast cancer, cancers of the head and neck including various lymphomas such as mantle cell lymphoma, non-Hodgkins lymphoma, adenoma, squamous cell carcinoma, laryngeal carcinoma, cancers of the retina, cancers of the esophagus, multiple myeloma, ovarian cancer, uterine cancer, melanoma, colorectal cancer, bladder cancer, prostate cancer, lung cancer (including non-small cell lung carcinoma), pancreatic cancer, cervical cancer, head and neck cancer, skin cancers, nasopharyngeal carcinoma, liposarcoma, epithelial carcinoma, renal cell carcinoma, gallbladder adenocarcinoma, parotid adenocarcinoma, endometrial sarcoma, multidrug resistant cancers; and proliferative diseases and conditions, such as neovascularization associated with tumor angiogenesis, macular degeneration (e.g., wet/dry AMD), comeal neovascularization, diabetic retinopathy, neovascular glaucoma, myopic degeneration and other proliferative diseases and conditions such as restenosis and polycystic kidney disease. Brain cancers that may be treated with vector that is transported efficiently across the BBB include astrocytoma, pilocytic astrocytoma, dysembryoplastic neuroepithelial tumor, oligodendrogliomas, ependymoma, glioblastoma multiforme, mixed gliomas, oligoastrocytomas, medulloblastoma, retinoblastoma, neuroblastoma, germinoma, and teratoma.

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A conjugate or composition of the invention may be administered by any means known in the art; e.g., orally, intraarterially, intranasally, intraperitoneally, intravenously, intramuscularly, subcutaneously, transdermally or *per os* to the subject. The agent may be, for example, an anti-angiogenic compound.

5

Dosages

The dosage of any conjugate or composition described herein or identified using the methods described herein depends on several factors, including: the administration method, the disease (e.g., cancer) to be treated, the severity of the disease, whether the cancer is to be treated or prevented, and the age, weight, and health of the subject to be treated.

With respect to the treatment methods of the invention, it is not intended that the administration of a vector, conjugate, or composition to a subject be limited to a particular mode of administration, dosage, or frequency of dosing; the invention contemplates all modes of administration. The conjugate, or composition may be administered to the subject in a single dose or in multiple doses. For example, a compound described herein or identified using screening methods of the invention may conjugate be administered once a week for, e.g., 2, 3, 4, 5, 6, 7, 8, 10, 15, 20, or more weeks. It is to be understood that, for any particular subject, specific dosage regimes should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the composition. For example, the dosage of a composition can be increased if the lower dose does not provide sufficient activity in the treatment of a disease or condition described herein (e.g., cancer). Conversely, the dosage of the composition can be decreased if the disease (e.g., cancer) is reduced or eliminated.

While the attending physician ultimately will decide the appropriate amount and dosage regimen, a therapeutically effective amount of a vector, conjugate, or

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composition described herein, may be, for example, in the range of 0.0035 μg to 20 $\mu\text{g}/\text{kg}$ body weight/day or 0.010 μg to 140 $\mu\text{g}/\text{kg}$ body weight/week. Desirably a therapeutically effective amount is in the range of 0.025 μg to 10 $\mu\text{g}/\text{kg}$, for example, at least 0.025, 0.035, 0.05, 0.075, 0.1, 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 5 3.5, 4.0, 5.0, 6.0, 7.0, 8.0, or 9.0 $\mu\text{g}/\text{kg}$ body weight administered daily, every other day, or twice a week. In addition, a therapeutically effective amount may be in the range of 0.05 μg to 20 $\mu\text{g}/\text{kg}$, for example, at least 0.05, 0.7, 0.15, 0.2, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 10.0, 12.0, 14.0, 16.0, or 18.0 $\mu\text{g}/\text{kg}$ body weight administered weekly, every other week, every three weeks or once a month.

10 Furthermore, a therapeutically effective amount of a compound may be, for example, in the range of 0.1 mg/m^2 to 2,000 mg/m^2 administered every other day, once weekly, every other week or every three weeks. For example ANG1005, may be administered at 50, 100, 200, 300, 400, 420, 500, 600, 700, 800, or 1,000 mg/m^2 every one, two, three, four weeks, or every month or every other month. In

15 one particular example, ANG1005 is administered at 300 mg/m^2 or 420 mg/m^2 every three weeks. In another embodiment, the therapeutically effective amount is in the range of 1000 $\mu\text{g}/\text{m}^2$ to 20,000 $\mu\text{g}/\text{m}^2$, for example, at least 1000, 1500, 4000, or 14,000 $\mu\text{g}/\text{m}^2$ of the compound administered daily, every other day, twice weekly, weekly, or every other week.

20

The following examples are intended to illustrate rather than limit the invention.

Example 1

Solubility of ANG1005

25

The solubility of ANG1005 was tested in a number of solvents and surfactants. The results from single agents are shown in Table 13 below.

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Table 13: ANG1005 solubilization List of solvents/surfactants tested as single agents		
Solvents/surfactants	Solubility	Concentration
Acetonitrile (100%)	No	
EtOH dehydrated	No	
Methyl-tert-butyl-ether	No	
Acetone	No	
Ethyl acetate	No	
Tert-butyl alcohol	No	
N,N-Dimethylacetamide	Yes	25 mg/ml
DMSO	Yes	120 mg/ml
Polysorbate 80 (Tween 80)	No	
Cremophor EL	No	
Cremophor ELP (BASF)	No	
PEG 300	No	
PEG	No	
PEG	No	
Polyvinylpyrrolidone (Kollidon 17)	No	
Polyvinylpyrrolidone (Kollidon 19)	No	
Cyclodextrin	No	
Labrafil	No	

Solubility of ANG1005 was also tested in solvent/surfactant combinations.

These results are shown in Table 14.

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4	5.25	4.0 (± 0.5)	No adjustment	4,8 (± 0.2)	42°C
5	4.50	4.0 (± 0.5)	No adjustment	4,0 (± 0.2)	46°C
6	4.75	4.0 (± 0.5)	No adjustment	4,3 (± 0.2)	46°C
7	5.00	4.0 (± 0.5)	No adjustment	4.5 (± 0.2)	46°C
8	5.25	4.0 (± 0.5)	No adjustment	4,8 (± 0.2)	46°C
9	4.50	4.0 (± 0.5)	No adjustment	4,0 (± 0.2)	50°C
10	4.75	4.0 (± 0.5)	No adjustment	4,3 (± 0.2)	50°C
11	5.00	4.0 (± 0.5)	No adjustment	4.5 (± 0.2)	50°C
12	5.25	4.0 (± 0.5)	No adjustment	4,8 (± 0.2)	50°C
Pilot	Target final pH	DMSO pH (5%)	Solutol/buffer: 80/20 (25%)	Buffer pH (70%)	Heating temperature
13	4.50	4.0 (± 0.5)	pH=5.0 (± 0.5)	5.0 (± 0.2)	50°C
14	4.75	4.0 (± 0.5)	pH=5.5 (± 0.5)	5.5 (± 0.2)	50°C
15	5.00	4.0 (± 0.5)	pH=6.0 (± 0.5)	5.5 (± 0.2)	50°C
16	5.25	4.0 (± 0.5)	pH=6.5 (± 0.5)	6.0 (± 0.2)	50°C
Pilot	Target final pH	DMSO pH (5%)	Solutol/water-HCl 80/20 (25%)	Buffer pH (70%)	Heating temperature
17	5.00	4.0 (± 0.5)	pH=6.0 (± 0.5)	5.5 (± 0.2)	50°C

Results of these experiments are shown in Table 16 below.

Table 16

Pilot	Target pH	Purity (%)	DMSO (ppm)*	Comments
1	4.50	95.9	5855	fast, clear reconst. then turbid
2	4.75	94.0	6488	fast, clear reconst. OK
3	5.00	95.0	6256	fast, clear reconst. OK
4	5.25	95.4	6382	fast, clear reconst. OK
5	4.50	96.0	6818	fast, clear reconst then turbid
6	4.75	94.4	6330	fast, clear reconst. OK
7	5.00	94.6	6806	fast, clear reconst. OK
8	5.25	94.0	6930	fast, clear reconst. OK
9	4.50	95.2	6235	fast, clear reconst. OK
10	4.75	93.8	6932	fast, clear reconst. OK
11	5.00	95.1	6302	fast, clear reconst. OK
12 (ref.)	5.25	93.9	7846	fast, clear reconst. OK
13	4.50	97.6	7035	turbid reconst. (++)
14	4.75	97.4	7071	fast, clear rec. then turbid and precipitate
15	5.00	95,4-94,3	6818	fast, clear reconst. OK

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Table 14: ANG1005 solubilization List of solvents/surfactants tested as combination		
Solvents/surfactants	Solubility	Concentration
EtOH/Tween 80	No	
EtOH/Cremophor EL	No	
EtOH/Cremophor ELP	No	
EtOH/PEG	No	
EtOH/Polyvinylpyrrolidone	No	
Solutol HS15/Buffer (with microwave heating)	Yes	6 mg/ml
EtOH/Solutol HS15/Buffer (75°C)	Yes	6 mg/ml
DMSO/Solutol HS15/Buffer (50°C)	Yes	6 mg/ml
DMSO/Tween80/Buffer (65°C)	Yes	6 mg/ml
DMSO/Cremophor/Buffer (65°C)	Yes	6 mg/ml

Example 2

ANG1005 Solubilization conditions

5 ANG1005 was subjected to several solubilization conditions in preparation for lyophilization. A summary of these results is shown below. As described above, the ANG1005 was dissolved first in DMSO. To this mixture, the heated Solutol or Solutol buffer combination was added. Finally, the glycine buffer was added to the ANG1005/DMSO/Solutol mixture. The solubilization conditions in
 10 Table 15 were thus tested.

Table 15: Solubilization of ANG1005

Pilot	Target final pH	DMSO pH (5%)	Solutol (20%)	Buffer pH (75%)	Heating temperature
1	4.50	4.0 (± 0.5)	No adjustment	4.0 (± 0.2)	42°C
2	4.75	4.0 (± 0.5)	No adjustment	4,3 (± 0.2)	42°C
3	5.00	4.0 (± 0.5)	No adjustment	4.5 (± 0.2)	42°C

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16	5.25	95,9-96.2	7155	fast, clear reconst. OK
17	5.00	96.5	6163	turbid reconst. (++)

On this basis of these results, we have determined that the formulation can be processed at temperatures between 40 and 50 °C. The pH of the solution should be above 4.5 to enable the formation of micelles by the Solutol HS 15, as pH 4.5
5 reconstitution can result in turbid solutions. Acidification of the Solutol HS15 prior to adding ANG1005 minimizes its degradation.

Example 3

Lyophilization Conditions

10 Following dissolution, the ANG1005 mixture was diluted in aqueous buffer (e.g., glycine buffer, pH adjusted to 5.0 with HCl, mannitol, and sodium chloride), frozen and lyophilized. Exemplary conditions are described in Table 4 above.

Load temperatures from -70 °C to 25 °C were tested for segment one. The ramp time for segment 2 was varied according to the difference between the
15 temperatures in segments 1 and 3, and may be up to six hours. We determined that segment 3 must be performed for at least 12 hours, as shorter timeframes resulted in a collapse of the lyophilized cake. Segments 8 and 9 can be adjusted within the temperatures shows above to ensure product temperature is between 18°C to 21°C during the secondary drying. The product should remain under 25 °C to avoid
20 melting. Using the solubilization/lyophilization protocols described herein, we were able, in some cases, to generate a product with greater than 96% purity with less than 1% residual DMSO, as shown in Table 17.

Table 17

Table 17: Key results for the GMP batch of ANG1005 for Injection			
	C0807121	C0907124	C1007135

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Purity	93.3%	90.9%	96.5%
2:1 conjugate	4.6%	6.4%	2.2%
Assay	93.8%	94.2%	100.4%
Water	0.2%	0.1%	0.05%
DMSO	8.2%	2.4%	0.6%

Further characterization of the 1007135 batch and other batches is shown in the Table 18 below.

Table 18

Lot Number	C1007135	C0108002	C0308011	C0608030	C1108062
Purity	96.5%	96.9%	95.5%	95.6%	96.9%
2:1 conjugate	2.2%	1.4%	2.0%	2.7%	2.3%
Assay	100.4%	97.2%	100.7%	102.6%	105.7%
Total Related Substances	3.5%	3.1%	5.0%	4.4%	3.1%
Unconjugated Angiopep-2	ND	ND	ND	ND	ND
Unconjugated Paclitaxel	0.9%	0.6%	1.0%	0.7%	0.5%
1:1 Conjugate	ND	ND	ND	ND	ND
Unknown	0.5%	1.0%	1.0%	1.0%	0.4%
Water Content	0.05%	0.04%	0.05%	0.12%	0.03%
DMSO	0.65%	0.54%	0.37%	0.54%	0.64%

5 ND = not detected

Example 4

Resuspension of Lyophilized ANG1005

The follow procedure was developed to dissolve and suspend the ANG1005 lyophilized formulation in aqueous solution. The procedure outlined is appropriate
 10 for a single vial containing 120 mg ANG1005.

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The ANG1005 vial was equilibrated at room temperature. The vial was then vented. With a 20 cc syringe fitted with an 18G 1 1/2" needle, 4 ml of anhydrous ethanol was slowly (i.e., over 30 seconds) injected down the side of the vial. The vial was then placed on a nutating mixer for 10 minutes, resulting in the ethanol slowly moistening the cake, thus providing a milky suspension.

The vial was then removed from the mixer and, with a 20-cc plastic syringe fitted to an 18G 1.5" needle, 12 ml of lactated Ringer's with 5% dextrose was injected down the side of the vial. The vial was then placed on the nutating mixer for 5 minutes. The vial was then turned vial 180 degrees and then keep mixing on the nutating mixer for another 5 minutes. At this point, the suspension was clear with minimal foaming. The vial was then allowed to stand on the bench for five minutes before proceeding to the next step (e.g., dilution for injection, analysis).

Alternate diluents were also tested (Table 19). While use of these diluents resulted in a clear solution with complete dissolution, they resulted in greater ANG1005 degradation than the mixture of lactated Ringer with 5% dextrose and ethanol at room temperature.

Table 19: Alternative diluents for Resuspension:

	Quantity	Conditions
Water for Injection	16 ml	Warm to 40-50°C
Water for Injection/Ethanol	12 ml + 4 ml	RT
D5W	16 ml	Warm to 40-50°C
D5W/Ethanol	12 ml + 4 ml	RT
Lactated Ringer-D5W	16 ml	Warm to 40-50°C

Example 5

Testing of additional buffers and bulking agents

Further efforts were undertaken with the objectives of reducing the residual DMSO (0.5%) and shortening the lyophilization cycle (5 days).

We believe that the various excipients of the formulation (especially the glycine and the sodium chloride) resulted in reduced efficiency of DMSO removal

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during the secondary drying of the cycle. Formulations made without NaCl, glycine, mannitol, or water resulted in much lower DMSO content (on the order of 0.01%). Without mannitol however, the cake was waxy-like (mainly consisting of Solutol). These low DMSO formulations all failed re-constitution using ethanol and D5W/lactated ringer. In addition, when the glycine was not present, the pH was not controlled. This resulted in degradation of the ANG1005.

Thus, in a further test, mannitol was kept as a bulking agent, glycine was replaced by buffers including citric acid and lactic acid, and the sodium chloride was removed. These formulations, using a shorter lyophilization cycle, still resulted in cakes with residual DMSO at 0.05%. At this level of DMSO, the cake was not soluble. In an additional formulation, soy lecithin was used in place of mannitol. This resulted in a residual DMSO of 0.2%. At 0.2% residual DMSO, the cake was soluble in Ethanol and D5W/LR. Thus, we believe that a minimum of 0.2-0.4% DMSO may be necessary for the reconstitution of the vials and the further dilution into the infusion bag. The lyophilization time can be adjusted accordingly to allow for DMSO concentrations in this range.

The compositions used in these tests are detailed as follows (Tables 20-22).

Table 20: Pre-lyophilization composition

% w/w	Supplier	F-37	F-38	F-39	F-40
ANG1005		0.72	0.72	0.72	0.72
Solutol HS15		24.92	24.88	24.83	24.88
DMSO, USP	Gaylord	13.39	13.36	13.32	13.36
1N HCl		0.311	0.311	0.306	0.311
Citric acid		0.04			
Lactic acid**			0.21	0.42	0.21
Mannitol		1.54	1.53	1.53	
Soy lecithin	PL90G				1.53
Water for injection		59.08	59.00	58.86	59.00
Total		100.00	100.00	100.00	100.00

** 85% in water

The Solutol/API ratio is same as in the ANG batch record

The DMSO/HCl ratio is same as in the ANG batch record

Citric acid conc is selected to provide pH 5 (based F-34)

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Lactic acid conc is calculated to provide pH 5 (F38) and lower (F39)
Mannitol conc in water is the same as in the ANG batch record
Soy lecithin is a "solublizing" bulking agent to replace mannitol

5

Table 21: Compounding table

Mg/tube	Grade & Lot	F-37	F-38	F-39	F-40
ANG1005		184	184	184	184
Solutol HS15		6328	6328	6328	6328
DMSO, USP		3399	3399	3395	3399
1N HCl		79	79	78	79
Citric acid		10			
Lactic acid**			54	108	54
Mannitol		390	390	390	
Soy lecithin					390
Water for injection		15000	15000	15000	15000
Total		25390	25434	25483	25434
Total Dry wt		6991	7035	7088	7035

** 85% in water

Table 22: Post lyophilization composition table

% w/w	Supplier	F-37	F-38	F-39	F-40
ANG1005		2.64	2.62	2.60	2.62
Solutol HS15		90.65	89.95	89.28	89.95
DMSO, USP	Gaylord				
1N HCl		1.13	1.12	1.10	1.12
Citric acid		0.14			
Lactic acid**			0.77	1.52	0.77
Mannitol		5.59	5.54	5.50	
Soy lecithin					5.54
Water for injection					
Total		100.0	100.0	100.0	100.0

10 ** 85% in water

The compositions were prepared as follows: The DMSO/HCl stock was prepared by weighing out 0.5 g 1N HCl standard solution into a 50 ml Falcon tube. 21.5 g DMSO was added and mixed well to obtain the DMSO+1N HCl stock.

15 For each composition mannitol, Solutol, citric or lactic acid, and WFI were weighed out and placed into a 50 ml Falcon tube. The contents were mixed well to dissolve. The tubes were capped and heated to 51°C ("buffer mixture"). Another 50 ml was used to weigh out ANG1005. The DMSO/HCl stock was then added

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and mixed well by vortex until the solution became clear. The heated buffer mixture was slowly added to the DMSO/ANG1005 mixture while vortexing. The mixtures was then cooled to RT. 1700-1730 mg of the solution was then placed into each vial. The solution was lyophilized as described herein. The compound
 5 was stored at -20°C .

The solutions were then test for their ability to reconstituted. To reconstitute the solutions, ethanol was added and mixed. Ringer's lactate solution was then added. Amounts are shown in Table 23.

Table 23: Reconstitution Volume

wt per vial	Ref Product	F-37 to F-40
API (mg)	125	12.5
Ethanol (mg)	3234	323.4
Ringer's lactate with D5W (mg)	12186	1218.6

10

If reconstitution is successful, then the sample appearance was to be recorded (color, crystal or solid PPT under microscope etc.). An aliquot would then be analyzed by HPLC (e.g., assay and purity). pH would also be measured.

15

Example 6

Stability testing of the lyophilized ANG1005 product

Stability testing over time of the ANG1005 product is being tested. The lyophilized product, which is being store at about -15°C , was monitored for activity, purity, appearance, pH, and degradation. The results of these tests are
 20 shown in Table 24 below.

Table 24: Stability Results for ANG1005 for Injection, Lot Number C1007135 Stored at $-15 \pm 5^{\circ}\text{C}$								
ANG1005 for Injection. Lot Number C1007135					Strength: 120 mg/vial of ANG1005			
Date of Manufacture: October 2007					Stability Study Start Date: November 2007			
Package: 60 ml vial, with a 15 mm Daikyo stopper and 15 mm flip-off seal					Length of Study: 24 months			
Tests	Initial	1 Month	2 Months	3 Months	6 Months	9 Months	12 Months	16 months
Appearance	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms

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Clarity and completeness of Solution	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms
pH	5.2	5.9	5.5	5.5	5.5	5.8	5.8	5.7
Water Content	0.1%	0.1%	0.1%	0.1%	0.1%	ND	ND	0.1%
Assay (wt/wt)	100.4%	97.6%	98.4%	97.5%	93.7%	103.3%	103.5%	101.3%
Purity	96.5%	97.4%	97.2%	96.7%	96.2%	97.3%	97.4%	97.2%
2:1 Conjugate	2.2%	1.4%	1.5%	1.5%	1.4%	1.3%	1.2%	1.4%
Paclitaxel	0.9%	0.6%	0.7%	0.6%	1.1%	0.5%	0.5%	0.6%
Unknown Rel	0.5%	0.4%	0.4%	1.2%	1.3%	0.8%	0.8%	0.8%
Substances	ND	ND	ND	ND	ND	ND	ND	ND
Imp RRT	ND	ND	ND	ND	ND	ND	ND	ND
0.60	ND	ND	ND	ND	ND	ND	ND	ND
Imp RRT	ND	ND	ND	ND	ND	ND	ND	ND
0.61	ND	ND	ND	ND	ND	ND	ND	ND
Imp RRT	ND	ND	ND	ND	ND	ND	ND	ND
0.62	0.1%	ND	ND	ND	0.1%	ND	ND	ND
Imp RRT	ND	ND	ND	ND	ND	ND	ND	ND
0.64	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%
Imp RRT	ND	ND	ND	0.1%	ND	ND	0.1%	0.1%
0.66	ND	ND	ND	0.3%	0.2%	ND	ND	0.2%
Imp RRT	ND	0.2%	0.2%	0.2%	0.1%	ND	ND	ND
0.79	0.2%	ND	ND	ND	0.2%	ND	0.2%	0.1%
Imp RRT	0.1%	0.1%	0.1%	0.2%	0.2%	0.5%	0.2%	0.1%
0.83	0.1%	0.1%	ND	0.2%	ND	ND	ND	ND
Imp RRT								
0.91								
Imp RRT								
0.93								
Imp RRT								
0.95								
Imp RRT								
0.98								
Imp RRT								
1.03								
Imp RRT								
1.04								
Imp RRT								
1.06								
Imp RRT								
1.07								
Sterility	Sterile	N/Ap	N/Ap	N/Ap	N/Ap	N/Ap	Sterile	N/Ap
Particulate Matter	Conforms	N/Ap	N/Ap	N/Ap	N/Ap	N/Ap	Conforms	N/Ap

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Example 7

Stability of the ANG1005 product following reconstitution

Several experiments have been performed to evaluate the stability of ANG1005 following reconstitution into solution. These experiments are described
 5 below.

Experiment 1

Product from lot number C0108002 of ANG1005 for Injection was reconstituted as described herein to a concentration range of 1.0 to 2.0 mg/ml. The
 10 concentration of 2.0 mg/ml of ANG1005 was previously determined to be the highest feasible dose for clinical use as previously presented in the IND. These preliminary experiments were conducted in small volumes in glass vials. Samples were kept at room temperature and visually inspected at the various time-points. Selected samples were filtered prior to HPLC analysis.

15 Table 25 shows the visual clarity of the solutions across the concentrations tested over time. The appearance of cloudiness appears to correlate with both increasing concentration and time. HPLC analysis of selected samples revealed a single ANG1005 peak that did not significantly change in purity over the various time-points. No changes in the profile of the related substances were observed
 20 (Table 26). Two related substance peaks are noted and identified as the 2:1 conjugate (RRT 0.88) and unconjugated paclitaxel (RRT 0.95). A peak was observed with a RRT of 1.15 (8.1 minutes); this peak is an impurity from the HPLC column, as it is also present in the blank chromatograms.

Table 25: Visual appearance of reconstituted ANG1005 diluted in D5W

	0h	1h	2h	3h	4h	6h
1.0 mg/ml	Clear	Clear	Clear	Clear	Clear	X
1.5 mg/ml	Clear	Clear	X	X	XX	XXX
2.0 mg/ml	Clear	X	XX	XX	XXX	XXX

25 X: Slightly cloudy
 XX: Cloudy
 XXX: Very cloudy

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Table 26: Purity of reconstituted ANG1005 for Injection diluted in D5W

	0h	1.5h	3.5h	6h
1.0 mg/ml	97.3% Related Substances: 1.3% (0.88) 1.4% (0.95)	NT	97.1% Related Substances: 1.4% (0.88) 1.5% (0.95)	NT
1.5 mg/ml	97.3% Related Substances: 1.3% (0.88) 1.4% (0.95)	97.1% Related Substances: 1.4% (0.88) 1.5% (0.95)	NT	96.8% Related Substances: 1.5% (0.88) 1.7% (0.95)
2.0 mg/ml	97.2% Related Substances: 1.3% (0.88) 1.5% (0.95)	97.1% Related Substances: 1.4% (0.88) 1.5% (0.95)	NT	NT

NT = Not tested

Related substances: report single peak greater than 0.5% with relative retention time in parentheses

Related substance at RRT 0.88 represents the 2:1 conjugate

Related substance at RRT 0.95 represents the unconjugated paclitaxel

Note: A peak is observed with a relative retention time of 1.15 (8.1 min); this peak is an impurity from the HPLC column as it is

also present in blank chromatogram.

10 **Experiment 2**

In order to verify the stability results at 1.0 mg/ml that was obtained in Experiment 1, an additional study was conducted under the conditions of clinical use. ANG1005 for Injection, lot number C0108002, was reconstituted as described to prepare a final concentration of 1.0 mg/ml in the 500 ml D5W infusion bag. The solution remained visually clear over the 6-hour observation period at room temperature with no significant changes in purity or related substance profiles (see Table 27 and Figure 2).

Table 27: Purity of reconstituted ANG1005 for Injection diluted with D5W to 1.0 mg/ml under conditions of clinical use

Time	0h	2h	4h	6h
Purity	97.4% Related Substances: 1.2% (0.88) 1.4% (0.95)	97.3% Related Substances: 1.3% (0.88) 1.4% (0.95)	97.0% Related Substances: 1.5% (0.88) 1.5% (0.95)	97.2% Related Substances: 1.4% (0.88) 1.4% (0.95)

20 Related substances: report single peak greater than 0.5% with relative retention time in parentheses.

Related substance at RRT 0.88 represents the 2:1 conjugate

Related substance at RRT 0.95 represents the unconjugated paclitaxel

Note: A peak is observed with a relative retention time of 1.15 (8.1 min); this peak is an impurity from the HPLC column as it is

25 also present in blank chromatogram.

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Experiment 3

ANG1005 for Injection, lot number C0108002, was reconstituted and diluted in D5W to a final concentration of 2.0 mg/ml in a glass vial. The sample was kept at room temperature for ~6h. The solution became cloudy and was
5 centrifuged. The resulting sediment was collected by decanting the supernatant, was solubilized in DMSO, and was analyzed by HPLC. The main peak of the re-solubilized sediment was identified as ANG1005 with a purity of 97.2%. No change in the profile of related substances was observed and only the 2 expected additional peaks were present (1.3% at RRT 0.88 and 1.5% at RRT 0.95). The
10 HPLC chromatogram of this sample is shown in Figure 3. A peak is observed with a RRT of 1.15 (8.1 minutes); this peak is an impurity from the HPLC column and is also present in the blank chromatograms.

The collective data indicate that the turbidity/cloudiness observed is a result of intact ANG1005 going out of solution without any degradation, likely due to an
15 interaction between the components of the drug product and D5W. This phenomenon appears to be concentration and time-dependent.

To reduce the turbidity, a reduction of the final concentration to ≤ 1 mg/ml for all patients receiving doses of ANG1005 > 300 mg/m² was suggested.

There is some data to suggest that this finding is mostly due to the reduction
20 of the amount of residual DMSO in the drug product. The first batch of drug product, lot number C0807121, had a residual DMSO content of 8.2%, whereas a more recent lot, lot number C0108002, has a residual DMSO content of 0.54%. It appears that this change may have affected the solubility of the substance.

Additional experiments are underway to modify the dilution procedure to
25 increase the stability of the reconstituted drug. Solutions prepared with Lactated Ringer's instead of 5% Dextrose Injection as the diluent in the last step of the reconstitution process are being tested: each vial of ANG1005 for Injection will be first reconstituted with 4 ml of anhydrous ethanol and 12 ml of Lactated

Ringer's/5% Dextrose Injection as before to achieve a concentration of 6 mg/ml, and then further diluted with Lactated Ringer's Injection. Preliminary data, shown in Table 28, suggest the replacement of D5W by Lactated Ringer's at the same concentration range (up to 2.0 mg/ml) can prevent the observed cloudiness of the infusion solutions. All solutions remained clear throughout the observation period without affecting the purity of ANG 1005.

Table 28: Purity of reconstituted ANG1005 for Injection diluted with Lactated Ringer's Injection

	0h	1h	2h	4h
0.5 mg/ml	97.2% Related Substances 1.4% (0.88) 1.4% (0.95)	96.9% Related Substances 1.5% (0.88) 1.6% (0.95)	96.7% Related Substances 1.7% (0.88) 1.6% (0.95)	96.3% Related Substances 2.0% (0.88) 1.7% (0.95)
1.0 mg/ml	96.8% Related Substances 1.6% (0.88) 1.6% (0.95)	96.7% Related Substances 1.7% (0.88) 1.6% (0.95)	96.6% Related Substances 1.8% (0.88) 1.6% (0.95)	96.6% Related Substances 1.8% (0.88) 1.6% (0.95)
1.5 mg/ml	97.0% Related Substances 1.5% (0.88) 1.5% (0.95)	96.9% Related Substances 1.6% (0.88) 1.5% (0.95)	96.6% Related Substances 1.8% (0.88) 1.6% (0.95)	96.6% Related Substances 1.8% (0.88) 1.6% (0.95)
2.0 mg/ml	97.1% Related Substances 1.4% (0.88) 1.5% (0.95)	96.8% Related Substances 1.6% (0.88) 1.6% (0.95)	96.6% Related Substances 1.8% (0.88) 1.6% (0.95)	96.6% Related Substances 1.8% (0.88) 1.6% (0.95)

Related substances report single peak greater than 0.5% with relative retention time in parentheses

Related substance at RRT 0.88 represents the 2:1 conjugate

Related substance at RRT 0.95 represents the unconjugated paclitaxel

10

CLAIMS

1. A composition comprising:
 - (a) a conjugate comprising:
 - (i) a polypeptide comprising an amino acid sequence having at least 70% identity to AngioPep-1 as set forth in SEQ ID NO:67; AngioPep-2 as set forth in SEQ ID NO:97, or AngioPep-7 as set forth in SEQ ID NO:112; and
 - (ii) a therapeutic agent which is paclitaxel or a paclitaxel analog, wherein said therapeutic agent is conjugated to the polypeptide;
 - (b) an optional tonicity agent;
 - (c) a buffering agent, wherein said buffering agent maintains a pH of 4.5-6;
 - (d) a bulking agent;
 - (e) a solubilizing agent that is not ethoxylated castor oil; and
 - (f) 0.2 to 10% DMSO by non-water weight,
 wherein said paclitaxel analog is ((azidophenyl)ureido)taxoid, (2 α ,5 α ,7 β ,9 α ,10 β ,13 α)-5,10,13,20-tetraacetoxytax-11-ene-2,7,9-triol, (2 α ,5 α ,9 α ,10 β)-2,9,10-triacetoxy-5-((β -D-glucopyranosyl)oxy)-3, 11-cyclotax-11-en-13-one, 1 β -hydroxybaccatin I, 1,7-dihydroxytaxinine, 1-acety-5,7,10-deacetyl-baccatin I, 1-dehydroxybaccatin VI, 1-hydroxy-2-deacetoxy-5-decinnamoyltaxinine j, 1-hydroxy-7,9-dideacetyl baccatin I, 1-hydroxybaccatin I, 10-acetyl-4-deacetyl taxotere, 10-deacetoxypaclitaxel, 10-Deacetyl baccatin III dimethyl sulfoxide disolvate, 10-deacetyl-10-(3-aminobenzoyl)paclitaxel, 10-deacetyl-10-(7-(diethylamino)coumarin-3-carbonyl)paclitaxel, 10-deacetyl-9-dihydrotaxol, 10-deacetyl baccatine III, 10-deacetylpaclitaxel, 10-deacetyltaxinine, 10-deacetyltaxol, 10-deoxy-10-C-morpholinoethyl docetaxel, 10-O-acetyl-2-O-(cyclohexylcarbonyl)-2-debenzoyltaxotere, 10-O-sec-aminoethyl docetaxel, 11-desmethyllaulimalide, 13-deoxo-13-acetyloxy-7,9-diacetyl-1,2-dideoxytaxine, 13-deoxybaccatin III, 14-hydroxy-10-deacetyl-2-O-debenzoylbaccatin III, 14-hydroxy-10-deacetyl baccatin III, 14 β -benzoyloxy-13-deacetyl baccatin IV, 14 β -benzoyloxy-2-deacetyl baccatin VI, 14 β -benzoyloxybaccatin IV, 19-hydroxybaccatin III, 2',2''-methylenedocetaxel, 2',2''-methylenepaclitaxel, 2'-(valyl-leucyl-lysyl-PABC)paclitaxel, 2'-acetyltaxol, 2'-O-acetyl-7-O-(N-(4'-fluoresceincarbonyl)alanyl)taxol, 2,10,13-triacetoxy-taxa-4(20), 11-diene-5,7,9-triol, 2,20-O-diacetyltaxumairol N, 2-(4-azidobenzoyl)taxol, 2-deacetoxytaxinine J, 2-debenzoyl-2-methoxybenzoyl-7-triethylsilyl-13-oxo-14-hydroxybaccatin III 1, 14-carbonate, 2-O-(cyclohexylcarbonyl)-2-debenzoylbaccatin III 13-O-(N-(cyclohexylcarbonyl)-3-cyclohexylisoserinate), 2 α , 7 β ,9 α ,10 β ,13 α -pentaacetoxyltaxa-4 (20), 11-dien-5-ol, 2 α ,5 α ,7 β ,9 α ,13 α -pentahydroxy-10 β -acetoxytaxa-4(20), 11-diene, 2 α ,7 β ,9 α ,10 β ,13-pentaacetoxyl-11 β -hydroxy-5 α -(3'-N,N-dimethylamino-

3'-phenyl)-propionyloxytaxa-4(20), 12-diene, 2 α ,7 β -diacetoxy-5 α , 10 β , 13 β - trihydroxy-2(3-20)abeotaxa-4(20), 11-dien-9-one, 2 α ,9 α -dihydroxy-10 β ,13 α -diacetoxy-5 α -(3'-methylamino-3'-phenyl)-propionyloxytaxa-4(20), 11-diene, 2 α -hydroxy-7 β ,9 α ,10 β , 13 α tetraacetoxy-5 α -(2'-hydroxy-3'-N,N-dimethylamino-3' -phenyl)-propionyloxytaxa-4(20), 11- diene, 3'-(4-azidobenzamido)taxol, 3'-N-(4-benzoyldihydrocinnamoyl)-3'-N-debenzoyl paclitaxel, 3' -N-m-aminobenzamido-3'-debenzamidopaclitaxel, 3'-p-hydroxypaclitaxel, 3,11-cyclotaxinine NN-2, 4-deacetyltaxol, 5,13 -diacetoxy-taxa-4(20), 11- diene-9,10-diol, 5-O-benzoylated taxinine K, 5-O-phenylpropionyloxytaxinine A, 5 α ,13 α -diacetoxy-10 β -cinnamoyloxy-4(20), 11-taxadien-9 α -ol, 6,3'-p-dihydroxypaclitaxel, 6- α -hydroxy-7 -deoxy-10-deacetylbaaccatin-III, 6-fluoro-10-acetyldocetaxel, 6-hydroxytaxol, 7,13- diacetoxy-5-cinnamyloxy-2(3-20)-abeo-taxa-4(20), 11-diene-2, 10-diol, 7,9-dideacetylbaaccatin VI, 7-(5'-Biotinylamidopropanoyl)paclitaxel, 7-acetyltaxol, 7-deoxy-10-deacetylbaaccatin-III, 7- deoxy-9-dihdropaclitaxel, 7-epipaclitaxel, 7-methylthiomethylpaclitaxel, 7-O-(4- benzoyldihydrocinnamoyl)paclitaxel, 7-O-(N-(4'-fluoresceincarboxyl)alanyl)taxol, 7-xylosyl- 10-deacetyltaxol, 8,9-single-epoxy brevifolin, 9-dihydrobaaccatin III, 9-dihydrotaxol, 9 α -hydroxy-2 α ,10 β ,13 α -triacetoxy-5 α -(3'-N,N-dimethylamino-3'-phenyl)-propionyloxytaxa-4(20), 11-diene, baaccatin III, baaccatin III 13-O-(N-benzoyl-3-cyclohexylisoserinate), BAY59, benzoyltaxol, BMS 181339, BMS 185660, BMS 188797, brevifoliol, butitaxel, cephalomannine, dantaxusin A, dantaxusin B, dantaxusin C, dantaxusin D, dibromo-10-deacetylcephalomannine, DJ927, docetaxel, Flutax 2, glutarylpaclitaxel 6-aminohexanol glucuronide, IDN 5109, IDN 5111, IDN 5127, IDN 5390, isolaulimalide, laulimalide, MST 997, N-(paclitaxel-2'-O-(2-amino)phenylpropionate)-O-(β -glucuronyl)carbamate, N-(paclitaxel-2'- O-3,3-dimethyl butanoate)-O-(β -glucuronyl)carbamate, N-debenzoyl-N-(3- (dimethylamino)benzoyl)paclitaxel, nonataxel, octreotide-conjugated paclitaxel, paclitaxel-transferrin, PNU 166945, poly(ethylene glycol)-conjugated paclitaxel-2'-glycinate, polyglutamic acid-paclitaxel, protax, protaxel, RPR 109881A, SB T-101187, SB T-1102, SB T-1213, SB T-1214, SB T-1250, SB T-12843, tasumatrol E, tasumatrol F, tasumatrol G, taxa-4(20), 11(12)-dien-5-yl acetate, taxa-4(20), 11(12)-diene-5-ol, taxane, taxchinin N, taxcultine, taxezopidine M, taxezopidine N, taxine, taxinine, taxinine A, taxinine M, taxinine NN-1, taxinine NN-7, taxol C-7-xylose, taxol-sialyl conjugate, taxumairol A, taxumairol B, taxumairol G, taxumairol H, taxumairol I, taxumairol K, taxumairol M, taxumairol N, taxumairol O, taxumairol U, taxumairol V, taxumairol W, taxumairol-X, taxumairol-Y, taxumairol-Z, taxusin, taxuspinnanane A, taxuspinnanane B, taxuspine C, taxuspine D, taxuspine F, taxuyunnanine C, taxuyunnanine S, taxuyunnanine T, taxuyunnanine U, taxuyunnanine V, tRA-96023, wallifoliol, 1-deoxypaclitaxel, 10-deacetoxy-7-deoxypaclitaxel, 10-O-deacetylpaclitaxel 10-monosuccinyl ester, 10-succinyl paclitaxel, 12b-acetyloxy- 2 α ,3,4,4 α ,5,6,9,10,11,12,12 α ,12 β -dodecahydro-4, 11-dihydroxy-12-(2,5-dimethoxybenzyloxy)-4 α ,8,13,13-tetramethyl-5-oxo-7, 11-

methano-1H-cyclodeca(3,4)benz(1,2-b)oxet-9-yl 3-(tert-butyloxycarbonyl)amino-2-hydroxy-5-methyl-4-hexanoate, 130-nm albumin-bound paclitaxel, 2'-paclitaxel methyl 2-glucopyranosyl succinate, 3'-(4-azidophenyl)-3'-dephenylpaclitaxel, 4-fluoropaclitaxel, 6,6,8-trimethyl-4,4a,5,6,7,7a,8,9-octahydrocyclopenta(4,5)cyclohepta(1,2-c)-furan-4,8-diol 4-(N-acetyl-3-phenylisoserinate), 6,6,8-trimethyl-4,4a,5,6,7,7a,8,9-octahydrocyclopenta(4,5)cyclohepta(1,2-c)-furan-4,8-diol 4-(N-tert-butoxycarbonyl-3-phenylisoserinate), 7-(3-methyl-3-nitrosothiobutyl)paclitaxel, 7-deoxypaclitaxel, 7-succinylpaclitaxel, A-Z-CINN 310, AI-850, albumin-bound paclitaxel, AZ 10992, isotaxel, MAC321, MBT-0206, NK105, Pacliex, paclitaxel poliglumex, paclitaxel-EC-1 conjugate, polilactofate, or TXD 258.

2. The composition of claim 1, wherein said therapeutic agent is paclitaxel.
3. The composition of claim 2, wherein said conjugate is ANG1005.
4. The composition of any one of claims 1-3, wherein said tonicity agent is sodium chloride.
5. The composition of any one of claims 1-4, wherein said buffering agent is glycine, citric acid, or lactic acid, or said bulking agent is mannitol or sorbitol.
6. The composition of any one of claims 1-5, wherein said solubilizing agent is a polyoxyethylene ester of a fatty acid.
7. The composition of claim 6, wherein said solubilizing agent is 12-Hydroxystearic acid-polyethylene glycol copolymer.
8. The composition of any one of claims 1-7, wherein said composition is substantially free from ethoxylated castor oil.
9. The composition of any one of claims 1-7, wherein said composition is free of ethoxylated castor oil.

10. The composition of any one of claims 1-9, wherein said composition is dissolved in water.

11. The composition of any one of claims 1-10, wherein said polypeptide comprises the amino acid sequence of AngioPep-1 as set forth in SEQ ID NO: 67; AngioPep-2 as set forth in SEQ ID NO: 97 or AngioPep-7 as set forth in SEQ ID NO: 112.

12. A composition comprising:

- i) 0.1-5% by non-water weight of ANG1005;
- ii) 1-15% by non-water weight of a tonicity agent;
- iii) 1-10% by non-water weight of a buffering agent;
- iv) 0-15% by non-water weight of a bulking agent;
- v) 45-75% by non-water weight of 12-Hydroxystearic acid-polyethylene glycol copolymer as a solubilizing agent; and
- vi) 0.2-10% by non-water weight of DMSO.

13. The composition of any one of claims 1-12, wherein said tonicity agent is sodium chloride, said buffering agent is glycine, and said bulking agent is mannitol.

14. The composition of claim 12 comprising:

- i) 1.8-2.3% by non-water weight of ANG1 005;
- ii) 9-11 % by non-water weight of a tonicity agent;
- iii) 4.5-6% by non-water weight of a buffering agent;
- iv) 8-10% by non-water weight of a bulking agent;
- v) 69-75% by non-water weight of 12-Hydroxystearic acid-polyethylene glycol copolymer as a solubilizing agent; and
- vi) 0.2-2% by non-water weight of DMSO.

15. A composition comprising:

- i) 1.8-4% by non-water weight of ANG1005;
- ii) 0.1-6% by non-water weight of a buffering agent;
- iii) 2-10% by non-water weight of a bulking agent;
- iv) 80-95% by non-water weight of 12-Hydroxystearic acid-polyethylene glycol copolymer as a solubilizing agent; and
- v) 0.2-1% by non-water weight of DMSO.

16. The composition of any one of claims 1-12, 14, and 15, wherein said buffering agent is lactic acid or citric acid and said bulking agent is mannitol.

17. The composition of claim 15, comprising the following:

- i) 2-3% by non-water weight of ANG1 005;
- ii) 0.5-6% by non-water weight of a buffering agent;
- iii) 2-7% by non-water weight of a bulking agent;
- iv) 85-95% by non-water weight of 12-Hydroxystearic acid-polyethylene glycol copolymer; and
- v) 0.2-0.6% by non-water weight of DMSO.

18. Use of a composition as defined in any one of claims 1-17 for the treatment of cancer.

19. A sealed container containing a composition as defined in any one of claims 1-17.

20. A method for preparing a composition as defined in any one of claims 1-11, said method comprising:

- (a) dissolving said conjugate in DMSO to form a mixture;
- (b) adding said solubilizing agent to the mixture of step (a);
- (c) adding water and said buffering agent to said mixture; and
- (d) lyophilizing the mixture of step (c); wherein said lyophilization results in a reduction of the amount of said DMSO that results in a final concentration of 0.2-10% DMSO, but does not substantially reduce the amount of said solubilizing agent, thereby preparing said composition.

21. The method of claim 20, wherein said conjugate is ANG1005.

22. The method of claim 20 or 21, wherein said step (d) lyophilizing comprises:

- (i) freezing said mixture to obtain a frozen product;
- (ii) drying said frozen product at a first temperature and pressure sufficient to remove at least a portion of said water; and
- (iii) drying said product at a second temperature and pressure sufficient to remove at least a portion of said DMSO.

23. The method of any one of claims 20-22, wherein the mixture of step (c) is filtered prior to step (d) lyophilizing or wherein said mixture is placed into a vial prior to step (d) lyophilizing.

24. A method for producing a composition as defined in claim 1, said method comprising the steps:

(a) dissolving in DMSO said conjugate, wherein the therapeutic agent of said conjugate is paclitaxel or docetaxel, thereby forming a mixture;

(b) adding 12-Hydroxystearic acid-polyethylene glycol copolymer to said mixture;

(c) adding water, said buffering agent, said bulking agent, and optionally said tonicity agent to said mixture; and

(d) lyophilizing said mixture under conditions which remove said water and said DMSO from said mixture, wherein said DMSO remains at a final concentration of 0.2-10%, thereby producing said composition.

25. The method of claim 24, wherein said 12-Hydroxystearic acid-polyethylene glycol copolymer is mixed with water, said buffering agent, said bulking agent, and optionally said tonicity agent prior to adding to said mixture, wherein said water, buffering agent, bulking agent, and optional tonicity agent are added in an amount which maintains solubility of said conjugate in said mixture.

26. The method of claim 24 or 25, wherein said DMSO is acidified between pH 3.5 and 4.5 prior to said step (a) dissolving.

27. The method of any one of claims 24-26, wherein said conjugate comprises AngioPep-2 as set forth in SEQ ID NO: 97.

28. The method of claim 27, wherein said conjugate is ANG1005.

Flow Chart of the Drug Product Manufacturing Process

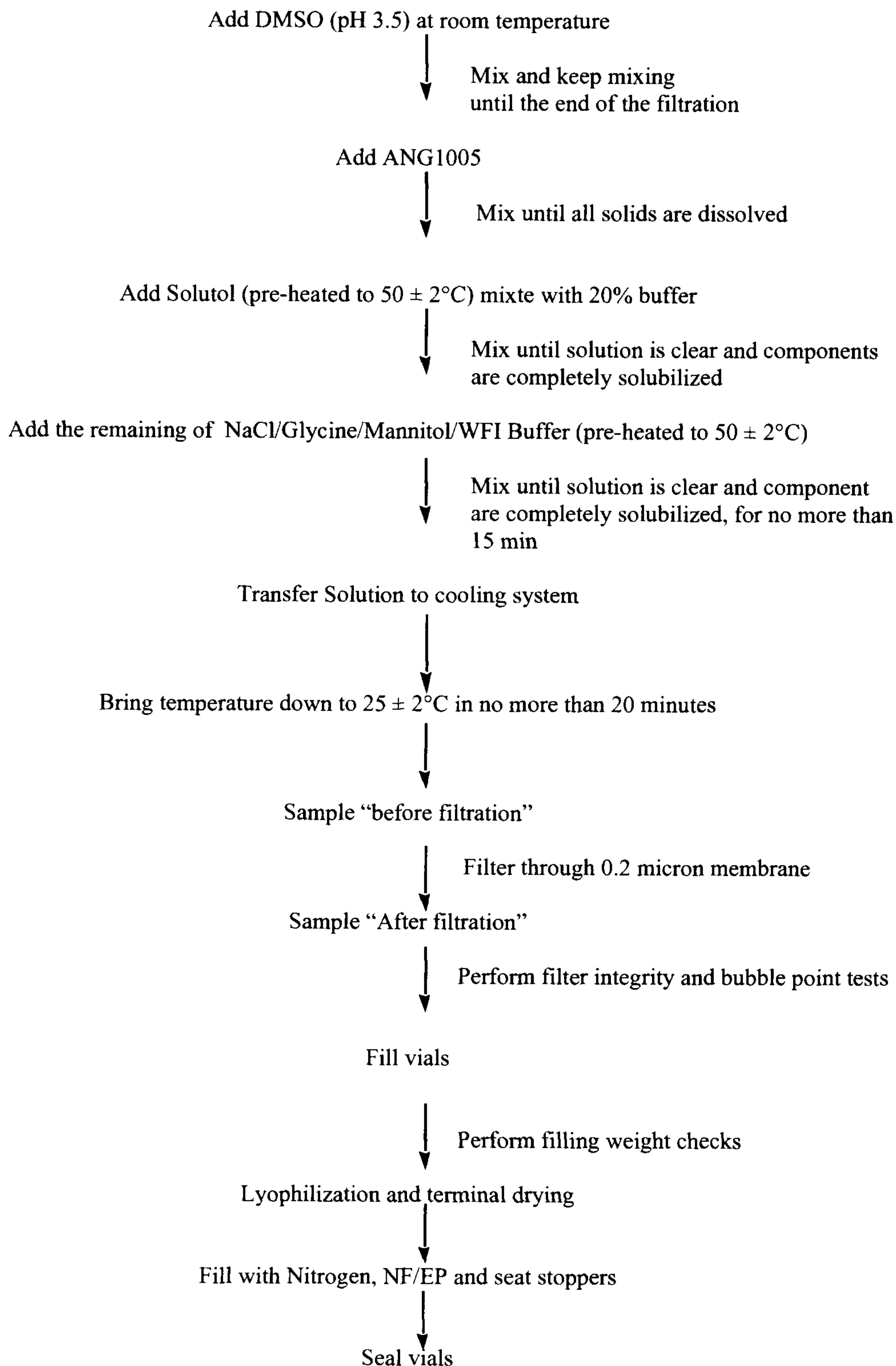
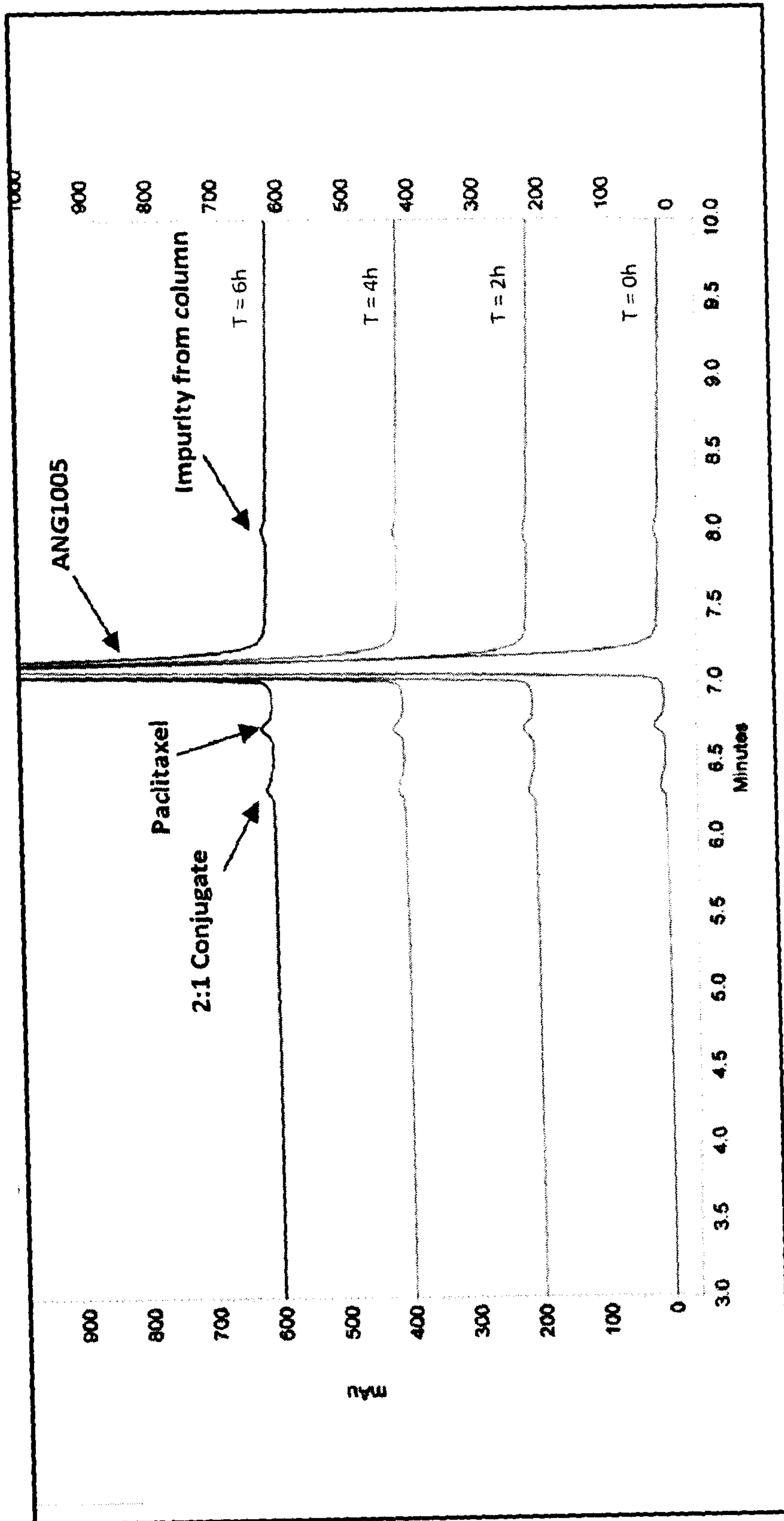


Figure 1

Figure 2



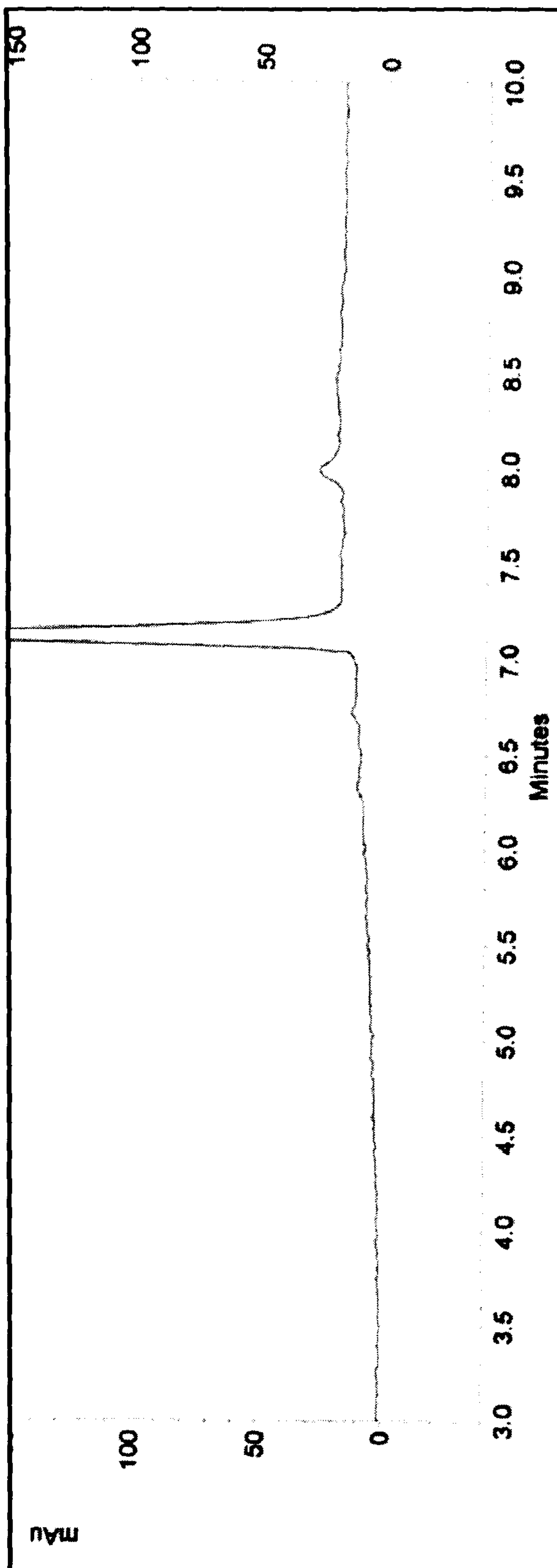


Figure 3

Flow Chart of the Drug Product Manufacturing Process

