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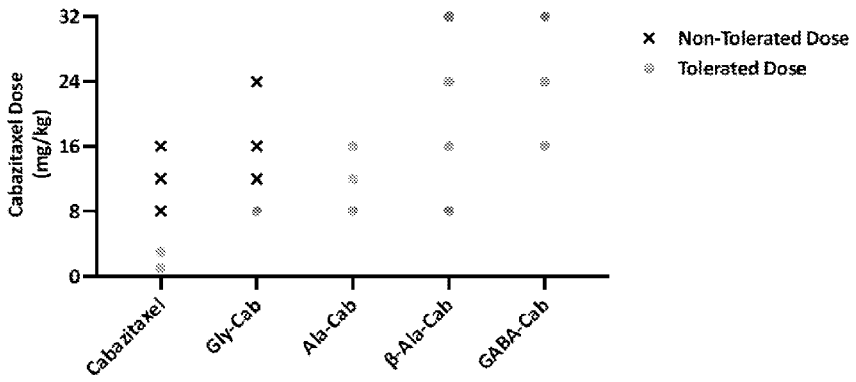
(54) Title: COMPOSITIONS AND METHODS FOR TREATMENT OF PROSTATE CANCER

FIGURE 5



(57) Abstract: Provided herein are compositions comprising cabazitaxel for use in the treatment of prostate cancer; and reactive derivatives of cabazitaxel that are useful in the manufacture of these compositions.

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COMPOSITIONS AND METHODS FOR TREATMENT OF PROSTATE CANCER

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of United States provisional patent application No. 63/386,076; entitled "Compositions and Methods for Treatment of Prostate Cancer;" filed December 5, 2022 and of United States provisional patent application No. 63/512,296; entitled "Compositions and Methods for Treatment of Prostate Cancer;" filed July 7, 2023. The disclosures of both of the foregoing applications, including all text and figures, are incorporated by reference in their entireties, for all purposes.

STATEMENT REGARDING FEDERAL SUPPORT

[0002] Not applicable.

FIELD

[0003] The present disclosure is in the field of chemotherapeutic compositions and vehicles for their delivery.

BACKGROUND

[0004] Certain prostate cancers are treated by castration, either physical or chemical. Physical castration is generally accomplished by removal of one or both testicles (orchiotomy, orchidectomy). Chemical castration is accomplished using, *e.g.*, anti-androgenic drugs that slow or block the production of testosterone and/or dihydrotestosterone.

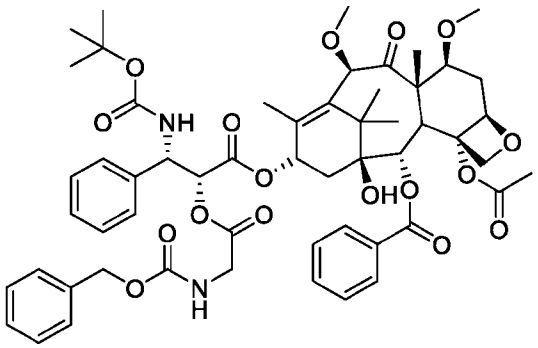
[0005] Prostate cancers that recur after either physical or chemical castration (metastatic castration-resistant prostate cancer, mCRPC) can be treated with docetaxel, but many mCRPCs eventually develop resistance to docetaxel. Cabazitaxel is a tubulin-binding taxane with low affinity for P-glycoprotein, thereby lowering the chances for development of resistance, and is currently the treatment of choice for mCRPC. See, for example, Abidi (2013) *J. Pharmacol. Pharmacother.* **4:**230-237.

[0006] However, cabazitaxel is not well-tolerated. Accordingly, more stable and well-tolerated formulations of cabazitaxel are needed.

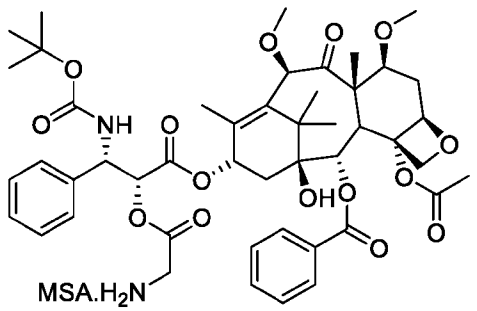
SUMMARY

[0007] The present disclosure provides compositions comprising cabazitaxel for use in the treatment of solid tumors such as prostate cancer. Also provided are reactive derivatives of cabazitaxel that are useful in the manufacture of these compositions. The therapeutic compositions include a polymer, each of whose repeating units contains a polyol (*e.g.*, mucic acid) portion, a polyalkylene glycol (*e.g.*, polyethylene glycol) portion, and one or more reactive groups. Nanoparticles containing the polymers disclosed herein are also provided. Accordingly, some of the embodiments provided herein include:

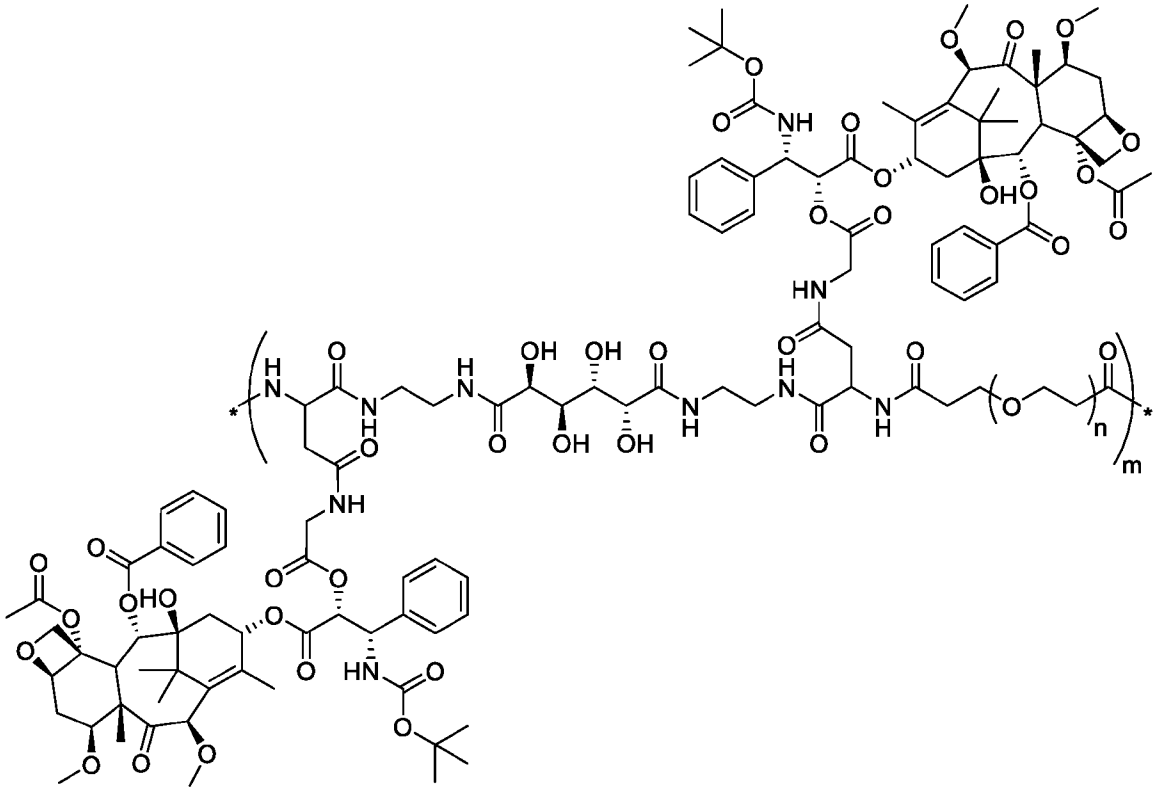
[0008] 1. Cabazitaxel-2'-carboxybenzyl-glycinate having the structure:



[0009] 2. Cabazitaxel-2'-glycinate.methane sulfonic acid having the structure:



[0010] 3. A mucic acid polymer cabazitaxel glycinate conjugate having the structure:



wherein:

m is from 5 to 200 inclusive; and

n is from 20 to 200 inclusive. In certain embodiments, * is -H or -OH or amino acid-CAB, or any combination of the foregoing.

[0011] 4. The conjugate of embodiment 3, further comprising one or more targeting molecules.

[0012] 5. The conjugate of embodiment 4, wherein the targeting molecule is selected from the group consisting of a protein, a peptide, an aptamer, and a ligand for a cellular receptor.

[0013] 6. The conjugate of embodiment 5, wherein the protein is an antibody or an antibody fragment.

[0014] 7. The conjugate of embodiment 6, wherein the antibody or antibody fragment is directed to prostate-specific membrane antigen (PSMA), HER2, or TROP2.

[0015] 8. The conjugate of embodiment 4, wherein the targeting molecule is linked to the conjugate through a polyethylene glycol (PEG) polymer containing one or more nitrophenylboronic acid (NPBA) moieties.

[0016] 9. A nanoparticle comprising the conjugate of any of embodiment 3-8.

[0017] 10. A pharmaceutical composition comprising the nanoparticle of embodiment 9 and a pharmaceutically acceptable carrier or excipient.

[0018] 11. A method for the treatment of a disease or disorder in a subject, the method comprising administering the pharmaceutical composition of embodiment 10 to the subject.

[0019] 12. The method of embodiment 11, wherein the disorder is a malignancy.

[0020] 13. The method of embodiment 12, wherein the malignancy is a solid tumor.

[0021] 14. The method of embodiment 13, wherein the solid tumor is a prostate tumor, a breast tumor, or a lung tumor.

[0022] 15. The method of embodiment 14, wherein the prostate tumor is metastatic castration-resistant prostate cancer (mCRPC).

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] Figure 1 shows kinetics of release of CAB from nanoparticles in mouse plasma *in vitro*. The nanoparticles tested contained different amino acid linkers between the CAB and the MAP components of the nanoparticle, as follows: hexanoic acid (Hex), valine (Val), gamma-amino butyric acid (GABA), beta-alanine (β-Ala), alanine (Ala) and glycine (Gly).

[0024] Figure 2 shows levels of total CAB in male Sprague Dawley rats at 0-120 hours after injection of CAB-containing nanoparticles with different amino acid linkers between the CAB and the MAP, as follows: gamma-amino butyric acid (GABA-CAB, inverted triangles, top line); β-alanine (β-ala-CAB, triangles, second line from top); alanine (Ala-CAB, squares, third line from top) and glycine (Gly-CAB, circles, bottom line).

[0025] Figure 3 shows levels of total CAB in male Sprague Dawley rats at 0-24 hours after injection of CAB-containing nanoparticles with different amino acid linkers between the CAB and the MAP, as follows: gamma-amino butyric acid (GABA-CAB, inverted triangles, top line); β-alanine (β-ala-CAB, triangles, second line from top); alanine (Ala-CAB, squares, third line from top) and glycine (Gly-CAB, circles, bottom line).

[0026] Figure 4 shows levels of free (unconjugated) CAB in male Sprague Dawley rats at 0-120 hours after injection of CAB-containing nanoparticles with different amino acid linkers between the CAB and the MAP, as follows: alanine (Ala-CAB, squares, top line); glycine (Gly-

CAB, circles, second line from top); β-alanine (β-ala-CAB, triangles, third line from top) and gamma-amino butyric acid (GABA-CAB, inverted triangles, bottom line).

[0027] **Figure 5** shows the results of two studies assessing the maximum tolerated dose (MTD) of CAB and CAB-containing nanoparticles in male NSG mice. Mice were dosed with unconjugated cabazitaxel (Cabazitaxel, leftmost set of data points); MAP-Gly-CAB nanoparticles (Gly-Cab, next set of data points to the right); MAP-Ala-CAB nanoparticles (Ala-Cab, next set of data points to the right); MAP-β-Ala-CAB nanoparticles (β-Ala-Cab, next set of data points to the right); and MAP-GABA-CAB nanoparticles (GABA-Cab, rightmost set of data points). Tolerated doses are indicated by circles; non-tolerated doses are indicated by x's.

DETAILED DESCRIPTION

[0028] The present disclosure may be understood more readily by reference to the following description taken in connection with the accompanying Examples, all of which form a part of this disclosure. It is to be understood that this disclosure is not limited to the specific products, methods, conditions or parameters described or shown herein, and that the terminology used herein is for the purpose of describing particular embodiments by way of example only and is not intended to be limiting of any claimed invention. Similarly, unless specifically otherwise stated, any description as to a possible mechanism or mode of action or reason for improvement is meant to be illustrative only, and the disclosure herein is not to be constrained by the correctness or incorrectness of any such suggested mechanism or mode of action or reason for improvement. Throughout this text, it is recognized that the descriptions refer to compositions and methods of making and using said compositions. That is, where the disclosure describes or claims a feature or embodiment associated with a composition or a method of making or using a composition, it is appreciated that such a description or claim is intended to extend these features or embodiment to embodiments in each of these contexts (i.e., compositions, methods of making, and methods of using).

[0029] In the present disclosure the singular forms "a," "an," and "the" include the plural reference, and reference to a particular numerical value includes at least that particular value, unless the context clearly indicates otherwise. Thus, for example, a reference to "a material" is a reference to at least one of such materials and equivalents thereof known to those skilled in the art, and so forth.

[0030] When a value is expressed as an approximation by use of the descriptor "about," it will be understood that the particular value forms another embodiment. In general, use of the term "about" indicates approximations that can vary depending on the desired properties sought to be obtained by the disclosed subject matter and is to be interpreted in the specific context in which it is used, based on its function. The person skilled in the art will be able to interpret this as a matter of routine. In some cases, the number of significant figures used for a particular value may be one non-limiting method of determining the extent of the word "about." In other cases, the gradations used in a series of values may be used to determine the intended range available to the term "about" for each value. Where present, all ranges are inclusive and combinable. That is, references to values stated in ranges include every value within that range.

[0031] In certain embodiments, when the term "about" is used with respect to a value *x*, "about *x*" means within ± 0.5% of *x*. In other embodiments, "about *x*" means within ±1% of *x*. In other embodiments, "about *x*" means within ±2% of *x*. In other embodiments, "about *x*" means within ±3% of *x*. In other embodiments, "about *x*" means within ±4% of *x*. In other embodiments, "about *x*" means within ±5% of *x*. In other embodiments, "about *x*" means within ±6% of *x*. In other embodiments, "about *x*" means within ±7% of *x*. In other embodiments, "about *x*" means within ±8% of *x*. In other embodiments, "about *x*" means within ±9% of *x*. In other embodiments, "about *x*" means within ±10% of *x*. In other embodiments, "about *x*" means within ±15% of *x*. In other embodiments, "about *x*" means within ±20% of *x*. In other embodiments, "about *x*" means within ±25% of *x*. In other embodiments, "about *x*" means within ±50% of *x*.

[0032] It is to be appreciated that certain features of the disclosure which are, for clarity, described herein in the context of separate embodiments, may also be provided in combination in a single embodiment. That is, unless obviously incompatible or specifically excluded, each individual embodiment is deemed to be combinable with any other embodiment(s) and such a combination is considered to be another embodiment. Conversely, various features of the disclosure that are, for brevity, described in the context of a single embodiment, may also be provided separately or in any sub-combination. Finally, while an embodiment may be described as part of a series of steps or part of a more general structure, each said step may also be considered an independent embodiment in itself, combinable with others.

[0033] When a list is presented, unless stated otherwise, it is to be understood that each individual element of that list, and every combination of that list, is a separate embodiment. For example, a list of embodiments presented as "A, B, or C" is to be interpreted as including the embodiments, "A," "B," "C," "A or B," "A or C," "B or C," or "A, B, or C." Likewise, a term such as C1-3 alkyl also includes, as separate embodiments, C1 alkyl, C2 alkyl, C3 alkyl, C1-2 alkyl, and C2-3 alkyl.

[0034] Reference to alcohols, aldehydes, amines, carboxylic acids, ketones, or other similarly reactive functional groups also includes their protected analogs. For example, reference to hydroxy or alcohol also includes those substituents wherein the hydroxy is protected by an acetyl (Ac), benzoyl (Bz), benzyl (Bn, Bnl), β-methoxyethoxymethyl (MEM), dimethoxytrityl (DMT), methoxymethyl (MOM), monomethoxytrityl (MMT), p-methoxybenzyl (PMB), methylthiomethyl, pivaloyl (Piv), tetrahydropyranyl (THP), tetrahydrofuran (THF), trityl (triphenylmethyl, Tr), silyl (*e.g.,* trimethylsilyl (TMS), tert-butyldimethylsilyl (TBDMS), tri-iso-propylsilyloxymethyl (TOM), triisopropylsilyl (TIPS)), or ethoxyethyl (EE) group. Reference to amines also includes those substituents wherein the amine is protected by a tert-butyloxycarbonyl (Boc), carbobenzyloxy (Cbz), p-methoxybenzyl carbonyl (Moz or MeOZ), 9-fluorenylmethyloxycarbonyl (Fmoc), acetyl (Ac), benzoyl (Bz), benzyl (Bn, Bnl), carbamate, p-methoxybenzyl (PMB), 3,4-dimethoxybenzyl (DMPM), p-methoxyphenyl (PMP), tosyl (Ts), or sulfonamide (Nosyl & Nps) group. Reference to substituent containing a carbonyl group also includes those substituents wherein the carbonyl is protected by an acetal or ketal, acylal, or dithiane group. Reference to substituent containing a carboxylic acid or carboxylate group also includes those substituents wherein the carboxylic acid or carboxylate group is protected by its methyl ester, benzyl ester, tert-butyl ester, an ester of 2,6-disubstituted phenol (*e.g.* 2,6-dimethylphenol, 2,6-diisopropylphenol, 2,6-di-tert-butylphenol), a silyl ester, an orthoester, or an oxazoline. Protecting groups for imidazole groups (*e.g.*, histidine) include trityl (Trt); monomethoxytrityl (Mmt); 4-methyltrityl (Mtt); benzyloxymethyl (Bom); and 2,4-dinitrophenyl (Dnp).

Abbreviations

[0035] The following abbreviations and acronyms may be used in this disclosure:

AA: acetic acid

Ab: antibody

ACN: acetonitrile

ARS: alizarin red S

Asp: aspartate, aspartic acid

BBB: blood-brain barrier

Boc: tert-butyloxycarbonyl

Boc-Lys(Boc)-OSu: bis-Boc-L-lysine-N-hydroxysuccinimide ester

Bom: benzyloxymethyl

BTB: blood-tumor barrier

CAB: cabazitaxel

Cbz: carboxybenzyl

CPME: cyclopentyl methyl ether

CPT: camptothecin

CV: column volume

cMAP: cationic mucic acid polymer, cationic mucic acid-containing polymer

cP: cationic polymer

Da: dalton

DCM: dichloromethane, methylene chloride

DF: diafiltration

DIC: N,N'-diisopropylcarbodiimide

DIPEA: N,N'-diisopropylethyamine

diSPA-PEG: di(succinimidyl propionate)-PEG

DLS: dynamic light scattering

DMA: dimethylacetamide

DMAP: 4-dimethylaminopyridine

DMF: N,N-dimethyl-formamide

DMSO: dimethyl sulfoxide

Dnp: 2,4-dinitrophenyl

EDC: 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride

EEDQ: N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline

EGFR: epidermal growth factor receptor

ESI: electrospray ionization

EtOAc: ethyl acetate

FPLC: fast protein liquid chromatography

g: gram

GABA: gamma-amino butyric acid

Gly: glycine, glycyl

GPC: gel permeation chromatography

h: hour

HBSS: Hank's balanced salt solution

HCl: hydrochloric acid

HEPES: N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid

Hex: hexanoic acid, *n*-caproic acid

HIC: hydrophobic interaction chromatography

HOPO: hydroxypyridine-N-oxide

HPLC: high-performance liquid chromatography

HPLC-UV: HPLC with ultraviolet absorbance detection

Im: imidazole, imidazolium

Im-cMAP-Im: cationic mucic acid flanked by imidazole moieties

Im.Na: 4-imidazoleacetic acid, sodium salt hydrate

IPA: isopropyl alcohol

kDa: kilodalton

L: liter

LAR: linker-to-antibody ratio

MAM: polymerizable mucic acid monomer

MAP: mucic acid polymer

MeOH: methanol

MES: 2-(N-morpholino)ethanesulfonic acid, 2-ethanesulfonic acid

mg: milligram

mL: milliliter

mmol: millimole

Mmt: monomethoxytrityl

mol: mole

mPEG: methoxy polyethylene glycol

mRNA: messenger ribonucleic acid

MTBE: methyl tert-butyl ether

Mtt: 4-methyltrityl

MWCO: molecular weight cut-off

N-Boc diamine: N-Boc-ethylenediamine

NHS: N-hydroxy succinimide

NMM: N-methylmorpholine

NP: nanoparticle

NPBA: 3-carboxy-5-nitrophenylboronic acid, nitrophenylboronic acid

PAA: poly(amino acid)

PAMAM: poly(amidoamine)

PBAE: poly(β-amino ester)

PBS: phosphate-buffered saline

Pd/C: palladium on carbon catalyst

PEG: polyethylene glycol

PEGm: methoxy polyethylene glycol

Pent: pentanoic acid; *n*-valeric acid

PEO: polyethylene oxide

PES: polyethersulfone

PEI: polyethyleneimine

PFP: pentaflurorphenyl

$(PFPO)_2O$: bis(pentafluorophenyl) carbonate

PrOAc: isopropyl acetate

PVDF: polyvinylidene fluoride, polyvinylidene difluoride

PyAOP: (7-azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate

RNA: ribonucleic acid

siRNA: short interfering ribonucleic acid, silencing ribonucleic acid

SN38: 7-ethyl-10-hydroxy-camptothecin

SpP: strands per particle

TBDPS: tert-butyldiphenylsilane; tert-butyldiphenylsilyl

TBDPSCl: tert-butlyl(chloro)diphenylsilane

TEA: triethylamine

Tf: holo-transferrin

TfR: transferrin receptor

TFA: trifluoroacetate, trifluoroacetic acid

THF: tetrahydrofuran

TIPS: triisopropyl silane

Tras: trastuzumab (Herceptin)

Trt: trityl

UF/DF: ultrafiltration & diafiltration

μg: microgram

μL: microliter

μmol: micromole

UV: ultraviolet

Val: valine, valyl

Reaction components

[0036] The procedures described herein include standard solvents and catalysts as are known in the art. Although particular compounds (e.g., acids, bases, coupling agents) are recited in the disclosure, it is clear to one of skill in the art that different reagents can be used.

[0037] To that end, exemplary solvents include but are not limited to chlorinated solvents (e.g., dichloromethane, chloroform, 1,2-dichloroethane), ethers (e.g., diethyl ether, tert-butyl methyl ether, cyclopentyl methyl ether, diglyme, 1,4-dioxane, 2-methyltetrahydrofuran), alcohols (e.g., methanol, ethanol, isopropanol, tert-butanol), alkanes (e.g., pentane, hexanes, heptanes), glycols (e.g., ethylene glycol, polyethylene glycol), polar aprotic solvents (e.g., dimethylacetamide, acetonitrile, dimethyl sulfoxide, dimethyl formamide, acetone, N-methyl-2-pyrrolidone,), and polar protic solvents (e.g., water, ethanol, acetic acid, propionic acid).

[0038] Exemplary acids include but are not limited to mineral acids (e.g., hydrochloric acid, nitric acid, phosphoric acid, sulfuric acid) and organic acids (e.g., acetic acid, malonic acid,

methanesulfonic acid, propionic acid, thioacetic acid, p-toluenesulfonic acid, tribromoacetic acid, trichloroacetic acid, trifluoroacetic acid).

[0039] Exemplary bases include but are not limited to amino bases (*e.g.* 1,4-diazabicyclo[2.2.2]octane, diethylamine, triethylamine, N,N-diisopropylethylamine, lithium amide, lithium bis(trimethylsilyl)amide, morpholine, piperidine), alkoxides (*e.g.*, barium tert-butoxide, lithium tert-butoxide, sodium methoxide), hydroxides (*e.g.*, tetrabutylammonium hydroxide, sodium hydroxide, potassium hydroxide), organometallic bases (*e.g.*, n-butyllithium, tert-butyllithium, butyl magnesium chloride), pyridines (*e.g.*, 4-dimethylaminopyridine, 2,6-lutidine, pyridine), carbonates (*e.g.*, lithium carbonate, sodium carbonate, magnesium carbonate, potassium carbonate), and hydrides (*e.g.*, sodium hydride, calcium hydride, potassium hydride).

[0040] Exemplary coupling agents include but are not limited to carbodiimide reagents (*e.g.*, N,N'-diisopropylcarbodiimide, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, N,N'-dicyclopentylcarbodiimide), additives for carbodiimide reagents (*e.g.*, 1-hydroxy-7-azabenzotriazole, 6-chloro-1-hydroxy benzotriazole, N-hydroxysuccinimide, 1-hydroxy-2-pyridinone, 6-chloro-N-hydroxy-2-phenylbenzimidazole, ethyl 2-cyano-2-(hydroxyimino)acetate), anhydride-based or -forming reagents (*e.g.*, ditertbutyl carbonate, acetic anhydride, 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline, ethylchloroformate), acylazoles (*e.g.*, carbonyl diimidazole), acid halide generating reagents (*e.g.*, thionyl chloride, phosgene, cyanuric chloride, benzyltriphenylphosphonium dihydrogen trifluoride), phosphonium salt coupling reagents (*e.g.*, benzotriazol-1-yloxytris(diemthylamino)phosphonium hexafluorophosphate), tetramethyl aminium reagents (*e.g.*, 2-(2-oxo-1(2H)-pyridyl-1,1,3,3-tetramethyluronium tetrafluoroborate), aminium reagents (*e.g.*, 2-chloro-1,3-dimethylimidazolidinium hexafluorophosphate), oxyma salts (*e.g.*, 1-((1-cyano-2-ethoxy-2-oxoethylideneaminooxy)(morpholino)methylene)pyrrolidinium hexafluorophosphate), antimonate uranium salts (*e.g.*, benzotriazol-1-yloxuy-N,N-dimethyl-methaniminium hexachloroantimonate), organophosphorus reagents (*e.g.*, diethylcyanophosphonate, 1-oxo-chlorophospholane, 2-propanephosphonic acid anhydride), triazine based reagents (*e.g.*, 2-chloro-4,6-dimethoxy-1,3,5-triazine), organosulfur reagents (*e.g.*, pentafluorophenyl-4-nitrobenzenesulfonate), pyridinium reagents (*e.g.*, 2-chloro-1-methylpyridinium iodide), polymer bound reagents (*e.g.*, polymer supported N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline).

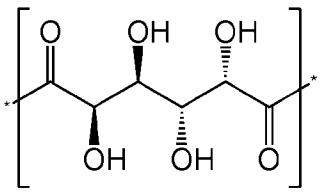
Polymers

[0041] A nanoparticle as described herein contains, as part of its structure, a polymer whose repeat units comprise a polyol containing at least two vicinal diols, a polyalkylene glycol segment, and one or more reactive groups. In certain embodiments, the polyol is mucic acid:

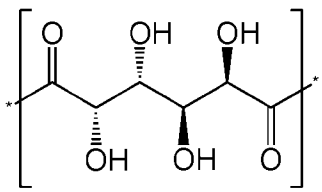
HOOC-CH(OH)-CH(OH)-CH(OH)-CH(OH)-COOH

Thus, certain of the polymers disclosed herein contain -O-C(=O)-CH(OH)-CH(OH)-CH(OH)-CH(OH)-C(=O)-O- as part of a repeating unit.

[0042] In other embodiments, certain of the polymers disclosed herein contain repeating units containing:



or

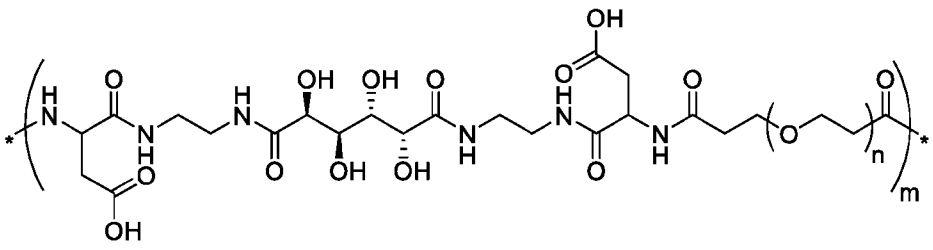


These structures are rotational isomers of each other and for present purposes are functionally equivalent. As used herein, representation of one of these structures connotes either or both of these structures in the context of their use.

[0043] In certain embodiments, the polyalkylene glycol segment is made up of repeating units of polyethylene glycol (PEG). Thus, certain of the polymers disclosed herein contain repeating units of (-O-CH_2-CH_2-) , as part of a repeat unit that also contains mucic acid or another polyol.

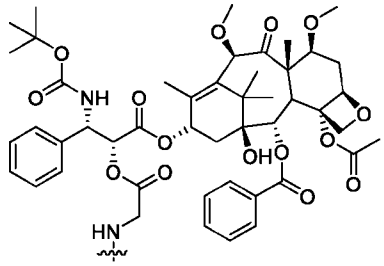
[0044] In certain embodiments, the reactive group is a carbonyl group, a carboxyl group or an amine group.

[0045] In certain embodiments, a mucic acid polymer (MAP) has the following structure:



(20),

wherein the end groups represented by * can be -H, -OH or -R, wherein R is aminoacyl-cabazitaxel such as:



(see, *e.g.*, Example 3).

[0046] The number of polymer repeat units, ***m*** in Compound 20, is between 5 and 200 (or any integral values therebetween; *e.g.*, between 10 and 150, between 20 and 120, between 50 and 100, or between 10 and 25). In certain embodiments, ***m*** is about 16. In other embodiments, ***m*** is 16.

[0047] The number of polyethylene glycol repeat units, ***n*** in Compound **20**, is between 20 and 200 (or any integral value therebetween, *e.g.*, between 25 and 150, between 30 and 120, or between 50 and 100). In certain embodiments, ***n*** is chosen so as to provide a number average molecular weight for the PEG portion of Compound 20 (*i.e.*, -O-CH_2-CH_2-) in a range from about 500 Da to about 50,000 Da. In certain embodiments, ***n*** is about 114 (PEG 5k). In other embodiments, ***n*** is about 80 (PEG 3.5k). In additional embodiments, ***n*** is about 46 (PEG 2k). In certain embodiments, ***n*** is 46, or ***n*** is 80 or ***n*** is 114.

[0048] In certain embodiments, the weight average molecular weight of the MAP is between 5 and 150 kDa or any integral values therebetween, *e.g.*, between 20 and 120 kDa, and the values of *m* and *n* are chosen accordingly.

[0049] In additional embodiments, values of *m* and *n* are chosen such that, after assembly of the MAP, or the CAB-conjugated MAP, into a nanoparticle, the size of the nanoparticle is between 10 and 900 nm or any integral values therebetween, *e.g.*, between 100 and 800 nm, between 200 and 500 nm, between 400 and 700 nm, or between 20 and 100 nm.

[0050] If desired, the length of a polymer can be determined as described in US Patent No. 11,738,092.

[0051] Any and all combinations of two or more of the preceding embodiments are also contemplated as additional embodiments of the compositions disclosed herein.

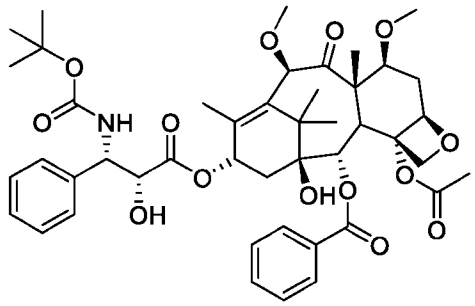
[0052] Certain methods for the synthesis of mucic acid polymers are provided in co-owned US Patent Number 11,738,092; the disclosure of which is incorporated by reference in its

entirety for the purposes of describing synthesis of mucic acid polymers, properties of mucic acid polymers, and uses of mucic acid polymers.

[0053] Additional polymers to which cabazitaxel can be conjugated using the methods and compositions described herein include neutral and cationic polymers such as those described in US Patent No. 10,342,879; US Patent No.9,446,149; US Patent No.11,285,212; US Patent No.10,182,986; US Patent No. 11,041,050; US Patent No. 11,738,092 and U.S. provisional patent application No. 63/588,162 (filed October 5, 2023). The disclosures of all of the foregoing U.S. patents, and of the foregoing patent application, are incorporated by reference in their entireties, for the purposes of describing polymers containing vicinal diols, their synthesis, their properties and their uses.

Reactive derivatives of cabazitaxel

[0054] Cabazitaxel has the structure:



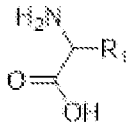
(Compound **1**).

[0055] In preparation for its conjugation to a polymer, cabazitaxel is reacted with a carboxybenzyl-amino acid (*e.g.*, Cbz-Gly, Cbz-Ala, Cbz-β-Ala, Cbz-Val, Cbz-(GABA), or Cbz-Hex) to form a CAB-2'-amino acid-Cbz derivative (Example 1). The CAB-2'-amino acid-Cbz derivative is reacted with methanesulfonic acid to form the CAB-2'-amino acid-methanesulfonic acid salt (Example 2).

Amino acid spacers

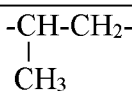
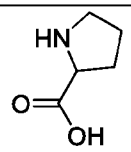
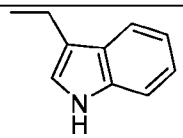
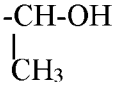
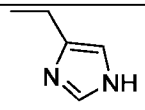
[0056] As noted above, reactive CAB derivatives contain an amino acid spacer. Thus, in certain embodiments, an amino acid, or portion thereof, is used as a spacer molecule in the drug-polymer conjugates disclosed herein. For example, an amino acid can be used as a spacer between a cabazitaxel molecule and a mucic acid polymer.

[0057] The twenty essential amino acids have the general structure:



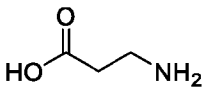
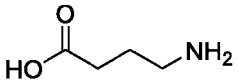
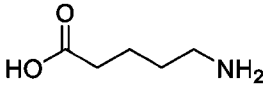
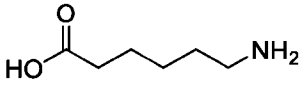
in which R_1 is a functional group that is specific to each particular amino acid. Functional groups for the twenty essential amino acids are given in Table 1. Chemical structures for a number of non-essential amino acids are given in Table 2.

Table 1: Essential amino acid functional groups

Amino acid	Functional group (R_1)
Glycine (Gly) (G)	-H
Alanine (Ala) (A)	$-CH_3$
Valine (Val) (V)	$-CH-(CH_3)_2$
Leucine (Leu) (L)	$-CH_2-CH-(CH_3)_2$
Isoleucine (Ile) (I)	
Methionine (Met) (M)	$-CH_2-CH_2-S-CH_3$
Proline (Pro) (P)*	
Phenylalanine (Phe) (F)	$-CH_2-C_6H_5$
Tryptophan (Trp) (W)	
Serine (Ser) (S)	$-CH_2OH$
Threonine (Thr) (T)	
Asparagine (Asn) (N)	$-CH_2-C(=O)-NH_2$
Glutamine (Gln) (Q)	$-CH_2-CH_2-C(=O)-NH_2$
Tyrosine (Tyr) (Y)	$-CH_2-C_6H_4-OH$
Cysteine (Cys) (C)	$-CH_2-SH$
Lysine (Lys) (K)	$-CH_2-CH_2-CH_2-CH_2-NH_3^+$
Arginine (Arg) (R)	$-CH_2-CH_2-CH_2-NH-C(=NH_2^+)-NH_2$
Histidine (His) (H)	
Aspartic acid (Asp) (D)	$-CH_2-C(=O)O^-$
Glutamic acid (Glu) (E)	$-CH_2-CH_2-C(=O)O^-$

Legend: The left column provides the name of the amino acid and its three-letter and one-letter abbreviations. The right column shows the chemical structure of the functional group (R_1) for each amino acid, except for proline.
* For proline, the structure of the entire amino acid is shown, since R_1 cannot be represented independently.

Table 2: Some Non-essential amino acids and their chemical structures

Amino acid	Structure
β-Alanine (β-Ala)	
-γ-amino butyric acid (GABA)	
5-amino-pentanoic acid	
6-amino-hexanoic acid	

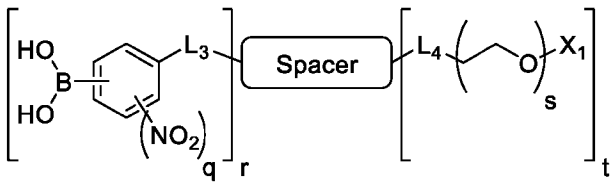
Synthesis of MAP-CAB conjugates

[0058] To form a CAB-polymer conjugate, a CAB-2'-amino acid-methanesulfonic acid salt is combined with a polyol/alkylene glycol polymer (*e.g.*, a mucic acid polymer such as Compound 20) in the presence of dimethyl sulfoxide, PyAOP and DIPEA. The amino group of the MSA salt reacts with one or more carboxyl groups of the polymer, forming an amide bond (Example 3). The product is purified, and appropriate size fractions are obtained, e.g., by dialysis. Percent CAB in the MAP-CAB conjugate is determined as described in Example 4. In certain embodiments, a MAP-CAB conjugate contains at least 5% CAB by weight, at least 10% CAB by weight, at least 15% CAB by weight, at least 20% CAB by weight, at least 25% CAB by weight, at least 30% CAB by weight, at least 35% CAB by weight, at least 40% CAB by weight, at least 45% CAB by weight, at least 50% CAB by weight, at least 55% CAB by weight, or at least 60% CAB by weight,. In additional embodiments, a MAP-CAB conjugate contains between 5-10% CAB by weight, between 10-15% CAB by weight, between 15-20% CAB by weight, between 20-25% CAB by weight, between 25-30% CAB by weight, between 30-35% CAB by weight, between 35-40% CAB by weight, between 40-45% CAB by weight, between 45-50% CAB by weight, between 50-55% CAB by weight, or between 55-60% CAB by weight. Combined and/or overlapping combinations of the above concentrations and ranges are also provided.

Conjugates of CAB-containing polymers with nitrophenylboronic acid-containing polymers

[0059] Polymers containing conjugated cabazitaxel can also have conjugated to them one or more boronic acid-containing polymers. These can include boronic acid-containing polymers, phenyl boronic acid-containing polymers; nitrophenyl boronic acid-containing polymers; and polymers containing one or a plurality of phenyl-boronic acid- or nitrophenyl boronic acid molecules. Boronic acid-containing polymers (including nitrophenylboronic acid-containing polymers, are described, for example, in US Patent No. 10,342,879; US Patent No. 9,446,149; US Patent No. 11,285,212; US Patent No. 10,182,986; US Patent No. 11,041,050; US Patent No. 11,738,092 and U.S. provisional patent application No. 63/588,185; the disclosures of all of which are incorporated by reference in their entireties for the purposes of describing the synthesis, properties and uses of boronic acid-containing polymers, nitrophenyl boronic acid-containing polymers; di-boronic acid-containing polymers and di-nitrophenyl boronic acid-containing polymers.

[0060] In certain embodiments, cabazitaxel-containing polymers, as described above, are conjugated to polyethylene glycol (PEG) polymers containing one or more nitrophenylboronic acid (NPBA) moieties as described, for example, in co-owned U.S. provisional patent application No. 63/588,185. For example, a NPBA-PEG polymer can have a structure as shown in the formulabelowI, in which the NPBA portion of the polymer is to the left of the spacer in the formula, and the PEG portion of the polymer is to the right of the spacer:



wherein:

L_3 is a linking group, or linking groups, between the one or more boronic acid moieties and the spacer;

L_4 is a linking group, or linking groups, between the spacer and the one or more polyethylene oxide linkages;

Spacer is a moiety containing at least one terminal amine and/or at least one terminal carboxylic acid;

q is independently at each occurrence 0, 1, 2, 3, or 4;

r is 1, 2, or 3;

s is independently at each occurrence 20-1200;

t is 1, 2, or 3; and

$\mathbf{X_1}$ is a terminal functional group.

[0061] In certain embodiments, $\mathbf{X_1}$ is either of:

(a) -C_{1-6} alkyl, optionally substituted with -OH, -COOH, -C(=O)O(alkyl), -C(=O)O(aryl), -NH_2, -NH(alkyl), or N(alkyl)$_2$; or

(b) a salt or protected analog of any of the groups of (a); or

$\mathbf{X_1}$ comprises a targeting molecule or a therapeutic molecule. In certain embodiments, a targeting molecule (as described elsewhere herein) is a covalent conjugate of one of the functional groups of (a) or (b) above, such that $\mathbf{X_1}$ has the structure -C-C(=O)-NH-targeting molecule. In additional embodiments, a therapeutic molecule (as described elsewhere herein) is a covalent conjugate of one of the functional groups of (a) or (b) above, such that $\mathbf{X_1}$ has the structure -C-C(=O)-NH-therapeutic molecule.

[0062] In certain embodiments, the spacer is an amino acid. In a preferred embodiment, **q** is 1, **r** is 2, **s** is 50-350, **t** is 1, $\mathbf{L_3}$ is -C(=O)-NH-, $\mathbf{L_4}$ is -C(=O)-NH-, $\mathbf{X_1}$ is -CH_3, and the spacer is a lysyl group. In an additional embodiment, **q** is 1, **r** is 2, **s** is 50-350, **t** is 1, $\mathbf{L_3}$ is -C(=O)-NH-, $\mathbf{L_4}$ is -C(=O)-NH-, $\mathbf{X_1}$ is -C-C(=O)-OH, and the spacer is a lysyl group.

[0063] A NPBA-PEG polymer can be synthesized, for example, by combining an amine-functionalized PEG (*e.g.*, PEG2k, PEG3.5k, PEG5k, PEG10k) with a carboxy NPBA to form a NPBA-PEG conjugate joined by an amide linkage. See, for example, U.S. Patent No. 11,738,092 and U.S. provisional patent application No. 63/588,162; the disclosures of which are incorporated by reference for the purposes of describing synthesis, properties and uses of NPBA-PEG polymers.

Targeting molecules

[0064] In certain embodiments, CAB-containing nanoparticles contain a targeting molecule, or targeting agent, which facilitates homing of a nanoparticle to a target (*e.g.*, a tumor cell) after systemic delivery. Targeting molecules are known in the art and include, for example, small molecules (*e.g.*, vitamins, *e.g.*, folate), saccharides (*e.g.*, mannose, allose, altrose, glucose,

gulose, idose, galactose, talose, disaccharides, trisaccharides, oligosaccharides), peptides (*e.g.*, RGD), polypeptides (*e.g.*, antibodies, proteins that bind cell-surface receptors such as transferrin), nucleic acids (*e.g.*, aptamers), peptoids and peptide nucleic acids (PNAs).

[0065] In additional embodiments, a targeting molecule is a protein, a peptide, an aptamer, or a ligand for a cellular receptor. A targeting molecule that is a protein can be, for example, an antibody or fragment thereof.

[0066] In certain embodiments, the target is the prostate gland, and the targeting agent binds to a molecule present on the surface of a prostate cancer cell, such as prostate-specific antigen (PSA) or prostate-specific membrane antigen (PSMA). In certain embodiments, a targeting molecule is an antibody (or fragment thereof) directed to PSA, or an antibody (or fragment thereof) directed to PSMA, or an antibody (or fragment thereof) directed to B7-H3, or an antibody (or fragment thereof) directed to B7-H4, or an antibody (or fragment thereof) directed to STEAP1, or an antibody (or fragment thereof) directed to HER2, or an antibody (or fragment thereof) directed to HER3, or an antibody (or fragment thereof) directed to TROP2.

[0067] In further embodiments, for targeting to a tumor cell, one or more small molecule targeting molecules can be used. These include, but are not limited to, carbohydrates (such as, for example, fructose, glucose, mannose, levan; or a derivative thereof), vitamins (such as, for example, riboflavin, biotin, thiamine, vitamin B12, and folic acid), and amino acids (such as, for example, glycine, alanine, arginine lysine and tryptophan). Additional small molecule targeting agents include adenosine, anisamide, anacardic acid, phenylboronic acid and methotrexate. *See*, for example, Kaur *et al.* (2023) *J. Controlled Release* **355:**417-433.

[0068] Additional targeting molecules are described in co-owned U.S. provisional patent application No. 63/588,162; the disclosure of which is incorporated by reference for the purposes of describing targeting molecules and methods for their inclusion in therapeutic nanoparticles.

[0069] In certain embodiments, a targeting molecule is covalently coupled to a boronic acid containing polymer, such as, for example a NPBA-PEG polymer as described in co-owned United States provisional patent application No. 63/588,162 filed October 5, 2023; the disclosure of which is hereby incorporated by reference in its entirety for the purposes of describing NPBA-PEG polymers, their structures, their synthesis and their uses.. For example, a targeting molecule can be part of the $\mathbf{X_1}$ moiety of the NPBA-PEG polymer, *e.g.*, the targeting molecule is

a covalent conjugate of one of the functional groups that are specified United States provisional patent application No. 63/588,162 filed October 5, 2023.

[0070] In certain embodiments, a targeting molecule is attached to a NPBA-PEG polymer by way of reaction with a pentafluorophenyl (PFP)-activated ester of the NPBA-PEG polymer, as described, for example, in U.S. Patent No. 11,738,092 and in co-owned U.S. provisional patent application No. 63/588,162; the disclosures of which are incorporated by reference for the purpose of describing methods for attaching polypeptides to NPBA-PEG polymers. Inasmuch as primary amine groups in proteins react with the PFP-activated ester, attachment of a polypeptide (such as, for example, an antibody) to the NPBA-PEG-pentafluorophenyl ester is achieved by combining the ester with the polypeptide. Progress of the reaction can be monitored by high-performance liquid chromatography (HPLC), and the NPBA-PEG-polypeptide conjugate can be purified by hydrophobic interaction chromatography (HIC).

[0071] The targeting agent is attached to the CAB-MAP conjugate, prior to formation of the nanoparticle, by methods described in, for example, U.S. Patents 9,466,149; 10,182,986; 10,342,879; 11,041,050; 11,285,212 and 11,738,092. The disclosures of all of the aforementioned patents are hereby incorporated by reference in their entireties for the purposes of describing targeting molecules and methods for their attachment to MAP-containing polymers and to polyol/PEG-containing polymers.

Therapeutic molecules

[0072] In certain embodiments, cabazitaxel-containing polymers, as described herein, comprise a therapeutic molecule (*e.g.*, a large molecule therapeutic). For example, a therapeutic molecule can be conjugated to a NPBA-PEG polymer in the manner described above for conjugation of a targeting molecule to a NPBA-PEG polymer; i.e., by reaction between the therapeutic molecule and a pentafluorophenyl (PFP)-activated ester of the NPBA-PEG polymer. Thus, in certain embodiments, a therapeutic molecule is part of the $\mathbf{X}_1$ moiety of the NPBA-PEG polymer described in co-owned U.S. provisional patent applications 63/588,162 and 63/588,185. Large molecule therapeutics are known in the art and include, for example, therapeutic polypeptides (*e.g.*, antibodies, enzymes, and bioactive proteins) and nucleic acids.

[0073] Exemplary therapeutic polypeptides include, but are not limited to, antibodies such as trastuzumab (Herceptin, an anti-Her2 antibody), anti-Her3, anti-Trop2, anti-PSMA, anti-

LIV-1, anti-FOLR1, anti-DLL3, anti-PDGF, anti-FRalpha, anti-PTK7, anti-mesothelin, anti-c-MET, anti-MUC1, anti-CD70, anti-CD74, anti-CD30, anti-CD33, anti-FLT3, anti-CD22, anti-CD20, and anti-CD19, anti-CD4, anti-CD8, and antibodies directed against B7-H3, B7-H4, Nectin-4, Claudin 18.2, ROR1, ROR2, BCMA, EpCAM, Claudin 6, CDH6, CEACAM5, and Integrin beta-6.

[0074] Certain polypeptide therapeutics, such as antibodies directed to tumor markers like those exemplified in the previous paragraph, can also be used as targeting molecules.

Biological Agents

[0075] In certain embodiments, nanoparticles as disclosed herein comprise a cabazitaxel-containing polymer and a biological agent. Biological agents include amino acids, peptides, polypeptides, nucleotides, and nucleic acids, such as oligonucleotides and polynucleotides. Exemplary nucleic acids include DNAs such as genomic DNA, cDNA, plasmids and antisense oligonucleotides. Additional exemplary nucleic acids include RNAs, such as, for example, mRNA, siRNA, circular RNA, shRNA, mi RNA, antisense RNA, guide RNA, and tracr RNA. Any of the biological agents mentioned above can be linear, circular or branched (*e.g.*, cyclic peptides, or circular nucleic acids, such as circular RNA).

[0076] Exemplary biological agents for use in RNA interference methods are siRNAs targeted to certain cancer-related genes (*e.g.*, oncogenes) such as, for example, c-myc, c-myb, mdm2, PKA-I, Abl-1, Bcl2, Ras, c-Raf kinase, CDC25 phosphatases, cyclins, cyclin dependent kinases, telomerase, PDGF/sis, erb-B, fos, jun, mos, src, the Bcr/Abl fusion gene, EGFR, BRAF, RRM2, HER2, VEGF, FGFR2, K-RAS, Claudin 18.2, EpCAM, STAT3, mesothelin/MSLN and BCRP.

[0077] In additional embodiments, the biological agent can be negatively charged, such as a nucleic acid. In further embodiments, the nucleic acid can be DNA, RNA or a peptide nucleic acid (PNA). Biological agents that are RNA can be low molecular weight RNAs such as antisense RNA, miRNA and siRNA, which can be introduced into a target cell to regulate (*e.g.*, repress) expression of an endogenous target gene; or high molecular weight RNAs such as mRNAs, which can be used to encode an exogenous protein in a target cell. In addition, a biological agent can be any of a guide RNA and/or a tracr RNA, and/or a RNA encoding the Cas9 nuclease, for use in gene editing using the CRISPR-Cas9 system. Biological agents can also be DNA molecules encoding any of the RNAs described above.

[0078] In further embodiments, a biological agent is a small molecule therapeutic, such as a chemotherapeutic. Exemplary chemotherapeutics include camptothecin and its derivatives and metabolites (such as SN38); taxanes (*e.g.*, taxol, docetaxel) and epithilones.

Nanoparticles

[0079] Nanoparticles are formed by diluting CAB-MAP conjugates (or conjugates of CAB with other polyol/polyalkylene glycol polymers) in water or dilute saline. The CAB-MAP conjugate (or CAB-polyol/polyalkylene glycol conjugate) may optionally also comprise one or more of a targeting molecule, a therapeutic molecule and/or a biological agent, wherein the targeting molecule, therapeutic molecule and/or biological agent is optionally joined to the MAP polymer *via* a NPBA-PEG polymer to which the targeting molecule, therapeutic molecule and/or biological agent is covalently joined. The ionic strength of the nanoparticle solution is adjusted if desired, *e.g.*, by the addition of saline (Example 6).

[0080] The size of the nanoparticles (*i.e.*, cross-sectional diameter) ranges from about 10 to about 60 nm in diameter. Exemplary nanoparticle diameters are 15 nm, 16 nm, 17 nm, 18 nm, 19, nm, 20 nm, 21 nm, 22 nm, 23 nm, 24 nm, 25 nm, 26 nm, 27 nm, 28 nm, 29 nm, 30 nm, 31 nm, 32 nm, 33 nm, 34 nm, 35 nm, 36 nm, 37 nm, 38 nm, 39 nm, 40 nm, 45 nm, 50 nm, 55 nm, 60 nm or larger. Exemplary nanoparticle diameters are 20 nm, 30 nm, 40 nm, 50 nm, 60 nm, or any value within the range of 20-60 nm. Specific embodiments can also describe these nanoparticles as having diameters in a range of from about 20 nm to about 30 nm, from about 30 nm to about 40 nm, from about 40 nm to about 50 nm, from about 50 nm to about 60 nm, or a combination of two or more of these ranges. In certain embodiments, the nanoparticles are spherical and have a diameter of 20-40 nm as determined by DLS and Cryo-transmission electron microscopy.

[0081] In some embodiments, the total size of the nanoparticle is from about 20 nm to about 60 nm. In some embodiments the size of the nanoparticle is from about 20 nm to about 30 nm. In some embodiments the size of the nanoparticle is from about 20 nm to about 40 nm. In some embodiments the size of the nanoparticle is from about 20 nm to about 50 nm. In some embodiments the size of the nanoparticle is from about 20 nm to about 60 nm. In some embodiments the size of the nanoparticle is from about 30 nm to about 40 nm. In some embodiments the size of the nanoparticle is from about 30 nm to about 50 nm. In some

embodiments the size of the nanoparticle is from about 30 nm to about 60 nm. In some embodiments the size of the nanoparticle is from about 40 nm to about 50 nm. In some embodiments the size of the nanoparticle is from about 40 nm to about 60 nm.

Treatment methods

[0082] Also provided are methods for treating malignancies with the nanoparticles disclosed herein. The malignancy to be treated can include any solid tumor for which cabazitaxel is an effective treatment. Examples include, but are not limited to, prostate cancer, metastatic castration-resistant prostate cancer (mCRPC), breast cancer, lung cancer, uterine cancer, cervical cancer, bladder cancer, head and neck cancer, and sarcomas, including Kaposi's sarcoma. In one embodiment, nanoparticles comprising cabazitaxel, and further comprising a targeting agent directed to PSMA, can be used for the treatment of prostate malignancies such as, for example, prostate cancer and mCRPC.

Formulations, kits, and routes of administration

[0083] Therapeutic compositions comprising nanoparticles as disclosed herein are also provided. Such compositions typically comprise the nanoparticles and a pharmaceutically acceptable carrier or excipient. Supplementary active compounds, such as targeting molecules, therapeutic molecules, and/or biological agents, can also be incorporated into nanoparticle compositions.

[0084] The therapeutic compositions disclosed herein are useful for, *inter alia*, treating cancer, cancer metastases and other disorders of the brain and central nervous system. Accordingly, a "therapeutically effective amount" of a composition comprising nanoparticles is any amount that reduces symptoms or, *e.g.*, stimulates tumor regression. For example, dosage amounts of nanoparticles can vary from about 0.1-1.0 mg/kg body weight, or from about 0.5 to 2.0 mg/kg body weight or from about 1-5 mg/kg body weight or from about 1 mg/kg body weight to about 10 mg/kg body weight or more (or any integral value therebetween); with a frequency of administration of, *e.g.*, hourly, twice per day, once per day, twice per week, once per week, twice per month, once per month, depending upon, *e.g.*, body weight, route of administration, severity of disease, *etc*. Thus, a therapeutically effective amount can comprise a plurality of administrations of the same amount, or different amounts, of nanoparticles. In

certain embodiments, a single administration of nanoparticles is a therapeutically effective amount.

[0085] In certain embodiments, nanoparticles are administered at a dosage of 1-20 mg nanoparticle (or any integral or decimal value therebetween) per square meter of the surface area of the body of the subject, as is typical for dosages of chemotherapeutics. For example, a dosage can be any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 122, 13, 14, 15, 16, 17, 18, 19, or 20 mg/m^2; or from 1-5, 2-7, 5-10, 8-12, 10-15, 13-17, or 15-20 mg/m^2.

[0086] In certain embodiments, dosage amounts and schedules are determined empirically and may be different from those listed above.

[0087] Various pharmaceutical compositions and techniques for their preparation and use are known to those of skill in the art in light of the present disclosure. For a detailed listing of suitable pharmacological compositions and techniques for their administration one may refer to texts such as Remington's Pharmaceutical Sciences, 17th ed. 1985; Brunton et al., "Goodman and Gilman's The Pharmacological Basis of Therapeutics," McGraw-Hill, 2005; University of the Sciences in Philadelphia (eds.), "Remington: The Science and Practice of Pharmacy," Lippincott Williams & Wilkins, 2005; and University of the Sciences in Philadelphia (eds.), "Remington: The Principles of Pharmacy Practice," Lippincott Williams & Wilkins, 2008.

[0088] The nanoparticles described herein can be suspended in a physiologically compatible carrier for administration. As used herein, the term "physiologically compatible carrier" refers to a carrier that is compatible with the nanoparticles and with any other ingredients of the formulation, and is not deleterious to the recipient thereof. Those of skill in the art are familiar with physiologically compatible carriers. Examples of suitable carriers include water (*e.g.*, pH 4 water), phosphate-buffered saline, Hank's balanced salt solution+/- glucose (HBSS), and multiple electrolyte solutions such as, *e.g.*, Plasma-LyteTM A (Baxter).

[0089] The volume of a nanoparticle suspension administered to a subject will vary depending on the site of administration, treatment goal and number of nanoparticles in solution. Typically the amount of nanoparticles administered will be a therapeutically effective amount. As used herein, a "therapeutically effective amount" or "effective amount" refers to the number of administered nanoparticles which are required to effect treatment of the particular disorder; *i.e.*, to produce a reduction in the amount and/or severity of the symptoms associated with that disorder. For example, in the case of mCRPC, administration of a therapeutically effective

amount of nanoparticles results in regression of the cancer. Therapeutically effective amounts vary with the type and extent of malignancy, and can also vary depending on the overall condition of the subject; and can be determined by one of skill in the art using established methods.

[0090] The disclosed therapeutic compositions can also include pharmaceutically acceptable materials, compositions or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, *i.e.*, carriers. These carriers can, for example, stabilize the nanoparticles and/or facilitate the retention of the nanoparticles in the body. Each carrier is "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the subject. Some examples of materials which can serve as pharmaceutically-acceptable carriers include: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol and polyethylene glycol; polyols, such as glycerin, sorbitol and mannitol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; phosphate buffer solutions; and other non-toxic compatible substances employed in pharmaceutical formulations. Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

[0091] Nanoparticles can be administered to a subject by any suitable route, including, but not limited to, inhalation, topically, nasally, orally, parenterally (*e.g.*, intravenously, intraperitoneally, intravesically or intrathecally) or rectally in a vehicle comprising one or more pharmaceutically acceptable carriers, the proportion of which is determined by the solubility and chemical nature of the compound, chosen route of administration and standard practice. Administration of the compositionds described herein can be carried out using any method known in the art. For example, administration may be transdermal, parenteral, intravenous, intra-arterial, subcutaneous, intramuscular, intracranial, intraorbital, ophthalmic, intraventricular, intracapsular, intraspinal, intracisternal, intraperitoneal, intracerebroventricular, intrathecal,

intranasal, aerosol, by suppositories, or by oral administration. A pharmaceutical composition of the nanoparticles described herein can be for intravenous administration or for administration by injection, or for oral, pulmonary, nasal, transdermal, or ocular administration.

[0092] Exemplary formulations include, but are not limited to, those suitable for parenteral administration, *e.g.*, intrapulmonary, intravenous, intra-arterial, intra-ocular, intra-cranial, sub-meningeal, or subcutaneous administration, including formulations encapsulated in micelles, liposomes or drug-release capsules (active agents incorporated within a biocompatible matrix designed for slow-release); ingestible formulations; formulations for topical use, such as eye drops, creams, ointments and gels; and other formulations such as inhalants, aerosols and sprays. The dosage of the compositions of the disclosure will vary according to the extent and severity of the need for treatment, the activity of the administered composition, the general health of the subject, and other considerations well known to the skilled artisan.

[0093] In additional embodiments, the compositions described herein are delivered intracranially at or near a site of brain injury or metastasis. Such localized delivery allows for the delivery of the composition non-systemically, thereby reducing the body burden of the composition as compared to systemic delivery. Local delivery can be achieved, for example, by intra-cranial injection, or through the use of various medically implanted devices including, but not limited to, stents and catheters, or can be achieved by inhalation, phlebotomy, or surgery. Methods for coating, implanting, embedding, and otherwise attaching desired agents to medical devices such as stents and catheters are established in the art and contemplated herein.

[0094] Another aspect of the present disclosure relates to kits for carrying out the administration of nanoparticles, optionally in combination with another therapeutic agent, to a subject. In one embodiment, a kit comprises a composition of nanoparticles formulated in a pharmaceutical carrier, suitable for administration, *e.g.*, by injection.

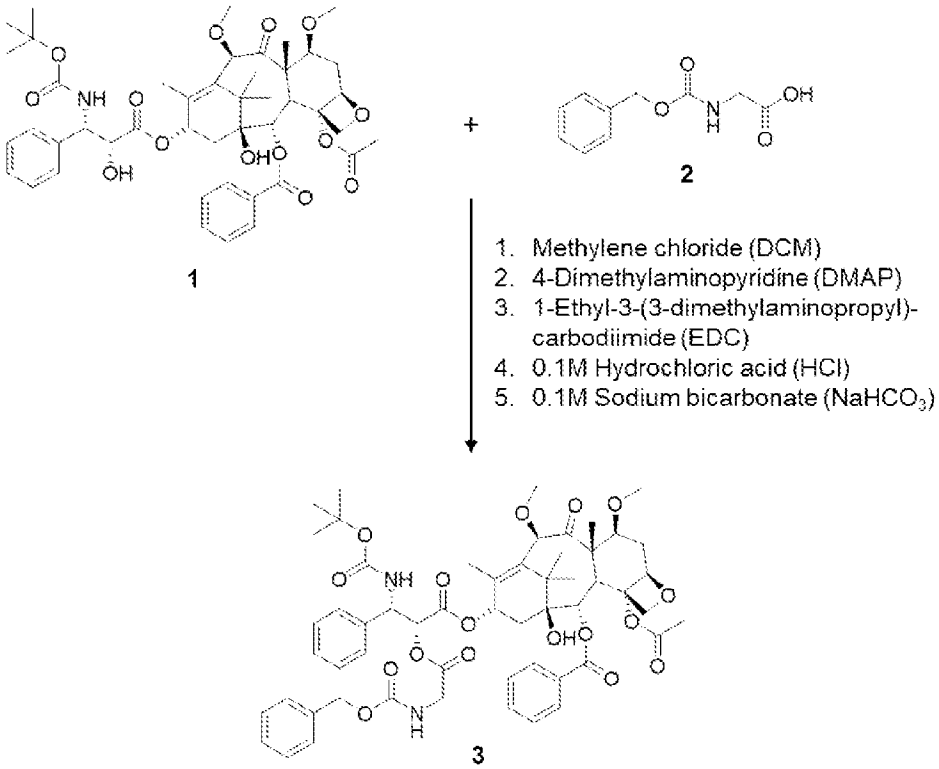
EXAMPLES

Example 1: Synthesis of cabazitaxel-2'-carboxybenzyl-glycinate (CAB-2'-Gly-Cbz) (Compound 3) and related carboxybenzyl-protected cabazitaxel amino acid derivatives (Compounds 9-13)

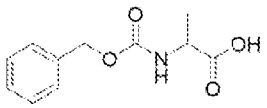
[0095] 0.108 g (0.51 mmol) of Cbz-Gly-OH (Compound **2**), 0.120 g (0.98 mmol) of DMAP, 0.282 g (1.47 mmol) of EDC, and 0.411 g (0.49 mmol) of cabazitaxel (CAB, Compound

1) were measured into an 8 mL vial with a magnetic stir bar and septum. The headspace of the vessel was purged with positive argon pressure. To above mixture of solids was added anhydrous DCM to generate a solution. The reaction was allowed to stir at room temperature overnight. HPLC indicated completion of the reaction. The reaction solution was washed with 0.1 M HCl (3 × 3 mL) to remove the EDC and DMAP, with 0.1 M $NaHCO_3$ (3 × 3 mL) to remove the excess Cbz-Gly-OH, and then with brine (1 × 3 mL). The residue was dried over Na_2SO_4, filtered, and dried under a steam of argon. The material was dried further under high vacuum.

[0096] 0.450 g CAB-2'-Gly-Cbz (**3**) was obtained as a white solid (0.438 mmol, 89% yield). ^{1}H NMR (600 MHz, DMSO-d_6): 7.98 (d, 2H), 7.91 (d, 1H). 7.78 (t, 1H), 7.73 (d, 1H), 7.68 (t, 2H), 7.42 (t, 2H), 7.38 (m, 6H), 7.32 (m, 1H), 7.19 (t, 1H). 5.81 (t, 1H), 5.39 (d, 1H), 5.12 (d, 1H), 5.10 (m, 1H), 5.07 (dd, 2H,), 4.96 (d, 1H), 4.70 (s, 1H), 4.50 (s, 1H), 4.01 (dd, 2H), 3.91 (m, 2H), 3.74 (dd, 1H), 3.58 (d, 1H), 3.27 (s, 3H), 3.20 (s, 3H), 2.55 (m, 1H), 2.23 (s, 3H), 1.78 (m, 4H), 1.51 (m, 5H), 1.39 (s, 9H), 0.97 (d, 6H). ESI/MS: 1027.4466. [M+H$^+$].



[0097] Cbz-Gly-OH can be substituted with other Cbz-protected amino acids in the above procedure. By substituting Cbz-Ala-OH (Compound **4**), Cbz-β-Ala-OH (Compound **5**), Cbz-Val-OH (Compound **6**), Cbz-(GABA)-OH (Compound 7), and Cbz-Hex-OH (Compound **8**), the same procedure yields CAB-2'-Ala-Cbz (Compound **9**), CAB-2'-β-Ala-Cbz (Compound **10**), CAB-2'-Val-Cbz (Compound **11**), CAB-2'-GABA-Cbz (Compound **12**), and CAB-2'-Hex-Cbz (Compound **13**), respectively.



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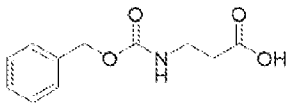

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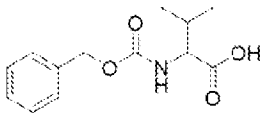

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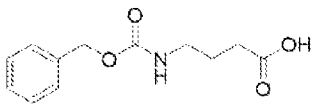

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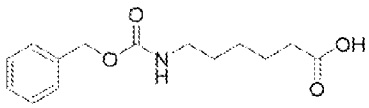

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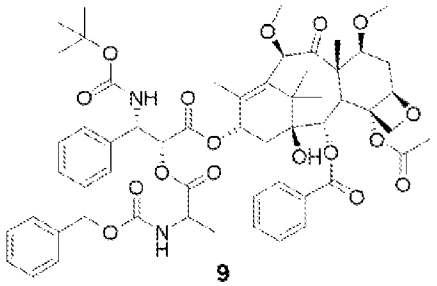

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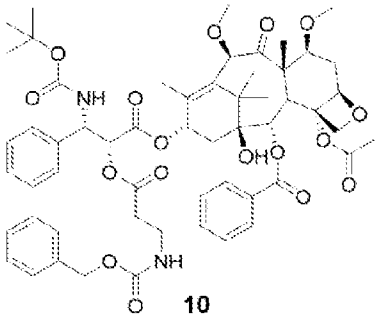

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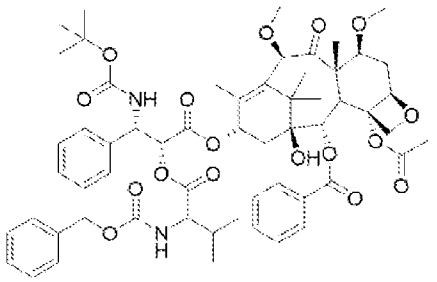

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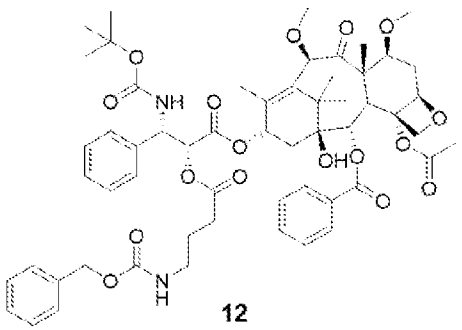

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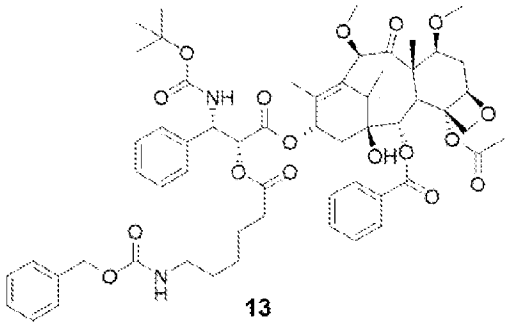

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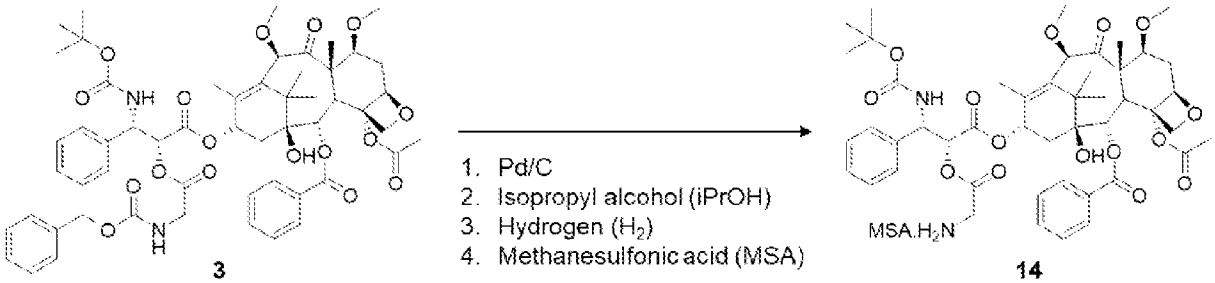
[0098] It will be apparent that additional Cbz-protected amino acids can be used (see, *e.g.*, Tables 1 and 2), in the procedure described in this example, to provide additional Cbz-protected CAB-amino acid derivatives.

Example 2: Synthesis of cabazitaxel-2'-glycinate.methane sulfonic acid (CAB-2'-Gly.MSA) (Compound 14) and related methane sulfonic acid salts of cabazitaxel (Compounds 15-19)

[0099] 0.066 mg Pd/C (10%) and 0.319 g (0.331 mmol) of CAB-2'-Gly-Cbz (**3**) were weighed into an 8 mL vial with a septum and a magnetic stir bar. Air was evacuated under vacuum through a needle. Isopropyl alcohol (IPA, 4mL) was added, and a H_2 balloon with a long needle was inserted into the reaction mixture, allowing H_2 to bubble through the reaction mixture with a vent (short needle) through the septum. Immediately upon insertion of the H_2 balloon, 1 mL of a solution of 0.030 g (0.31 mmol) of methanesulfonic acid in IPA was added via syringe. After stirring for 5 mins, the venting needle was removed, and the reaction mixture was stirred vigorously for 2 hrs. HPLC indicated completion of the reaction. 0.6 g Celite was added to the reaction mixture. The resulting mixture was stirred for 2 mins, then filtered through a Celite plug. The filtrate was washed with IPA (3 × 0.5 mL). The combined filtrates were dried using a steam of argon, and dried further under high vacuum.

[0100] 0.277 g CAB-2'-Gly.MSA (**14**) was obtained as a white solid (0.28 mmol, 90% yield). ^{1}H NMR (600 MHz, DMSO-d_6): 8.01 (d, 2H), 7.91 (d, 1H), 7.73 (t, 1H), 7.63 (t, 2H), 7.44 (t, 2H), 7.38 (d, 1H, 2H), d, 1H)7.21 (t, 1H), 5.87 (t, 1H), 5.39 (d, 1H), 5.18 ((d, 1H), 5.13 (t, 1H), 4.45 (d, 1H), 4.70 (s, 1H), 4.54 (s, 1H), 4.02 (dd, 2H), 3.87 (dd, 2H), 3.74 (s, 3H)m, 1H), 3..59 (d, 1H), 3.29 (s, 3H), 3.20 (s. 3H), 2.65 (m, 1H), 2.29 (s, 3H), 2.27 (s, 3H), 1.85 (m, 1H), 1.83 (s, 3H), 1.64 (m, 1H), 1.51 (s, 3H), 1.48 (m, 1H), 1.34 (s, 9H), 0.95 (d, 6H). ESI/MS: 893.4103 [M+H$^+$].



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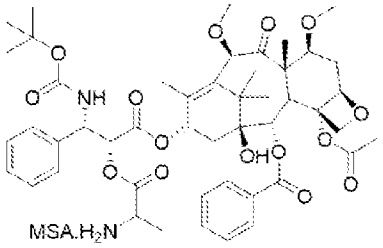
[0101] Substitution, in the method described above, of CAB-2'-Gly-Cbz with CAB-2'-Ala-Cbz (**9**), CAB-2'-β-Ala-Cbz (**10**), CAB-2'-Val-Cbz (**11**), CAB-2'-GABA-Cbz (**12**), and CAB-2'-Hex-Cbz (**13**) yields CAB-2'-Ala.MSA (Compound **15**), CAB-2'-β-Ala.MSA (Compound **16**), CAB-2'-Val.MSA (Compound **17**), CAB-2'-GABA.MSA (Compound **18**), and CAB-2'-Hex.MSA (Compound **19**), respectively.



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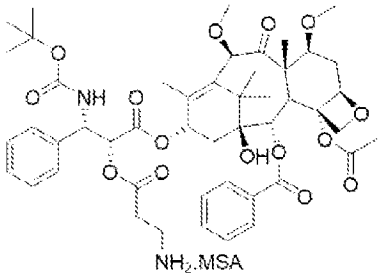

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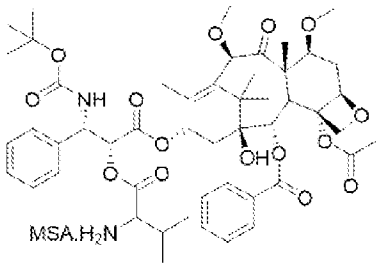

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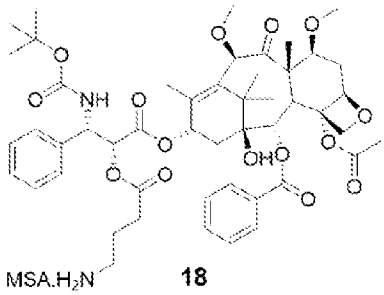

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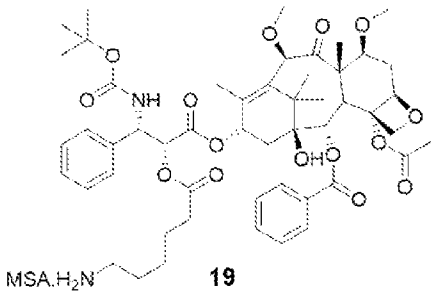

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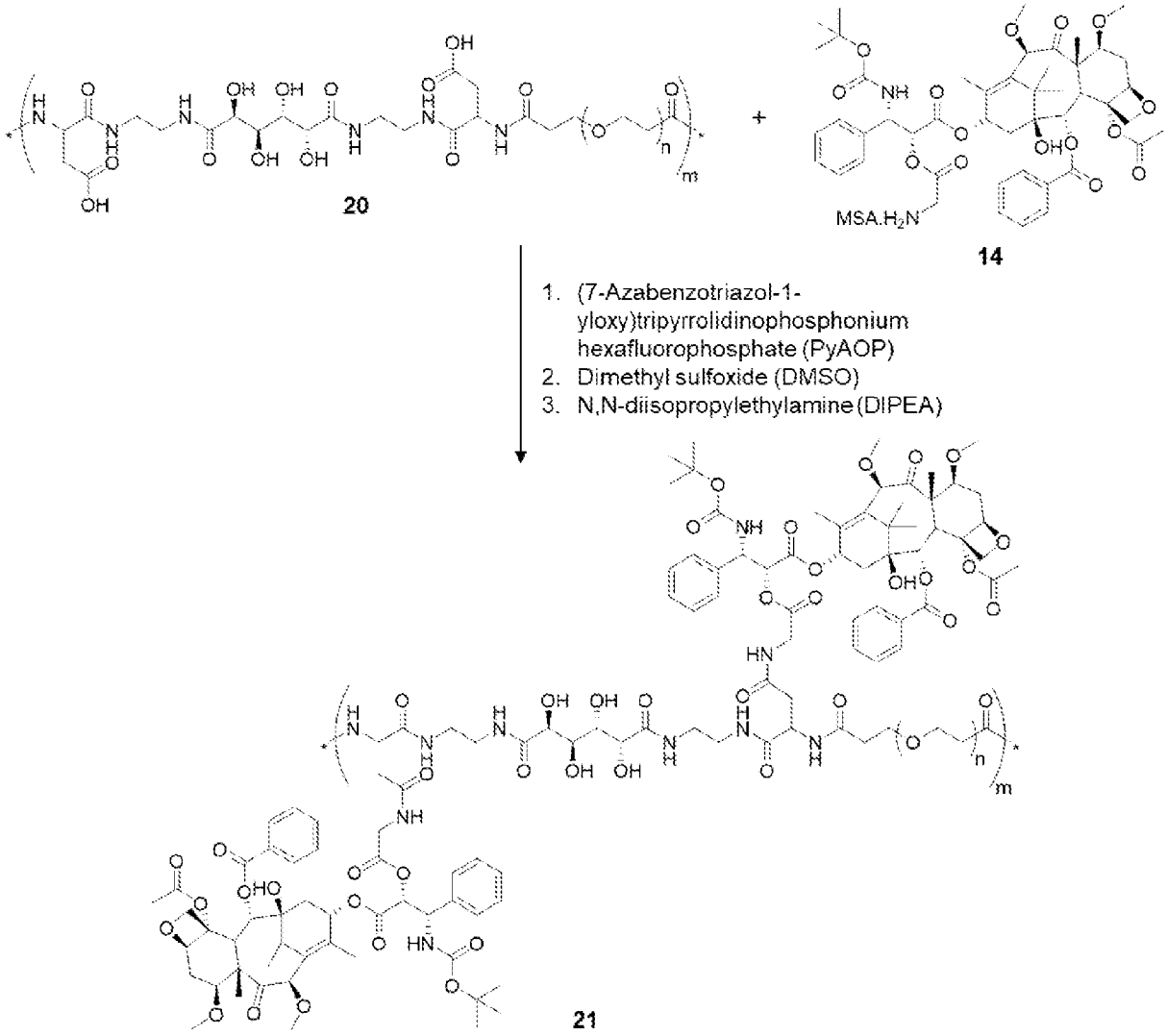
[0102] It will be apparent that additional Cbz-protected CAB-amino acid derivatives can be used (see, *e.g.*, Tables 1 and 2), in the procedure described in this example, to provide additional MSA salts of CAB.

Example 3: Synthesis of MAP cabazitaxel glycinate conjugate (MAP-Gly-CAB) (Compound 21) and related MAP-cabazitaxel (MAP-CAB) conjugates (Compounds 22-26)

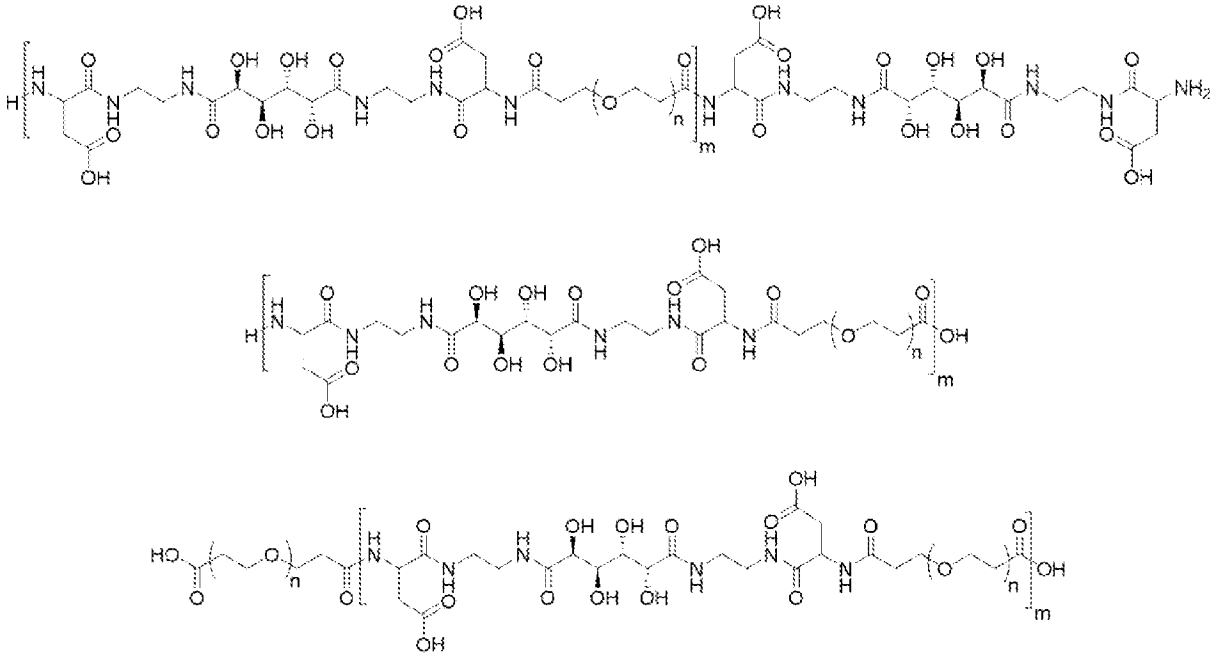
[0103] CAB-2'-Gly.MSA (**14**) was conjugated to a mucic acid polymer (MAP, as described, for example, in US Patent No. 11,738,092) as follows. 40 mg (0.019 mmol COOH) of MAP (compound **20**, identified as compound **6** in US 11,738,092) and 16.2 mg (0.016 mmol) of CAB-2'-Gly.MSA (**14**) were combined in an 8 mL reaction vial with a septum and a magnetic stirring bar. The vial was charged with 3mL anhydrous DMSO. In a separate vial, 30.1 mg

(0.058 mmol) PyAOP and 1 mL anhydrous DMSO were combined to generate a solution. The PyAOP solution was added to the reaction vial. Then, 12 mL (0.069 mmol) DIPEA was added, with stirring. The reaction mixture was stirred at ambient temperature overnight; then dialyzed against DMF (4 × 900 mL) and water (4 × 900 mL) using a 10 kDa membrane. The last water dialysis product was frozen, and lyophilized to afford 46.7 mg MAP-Gly-CAB (**21**).

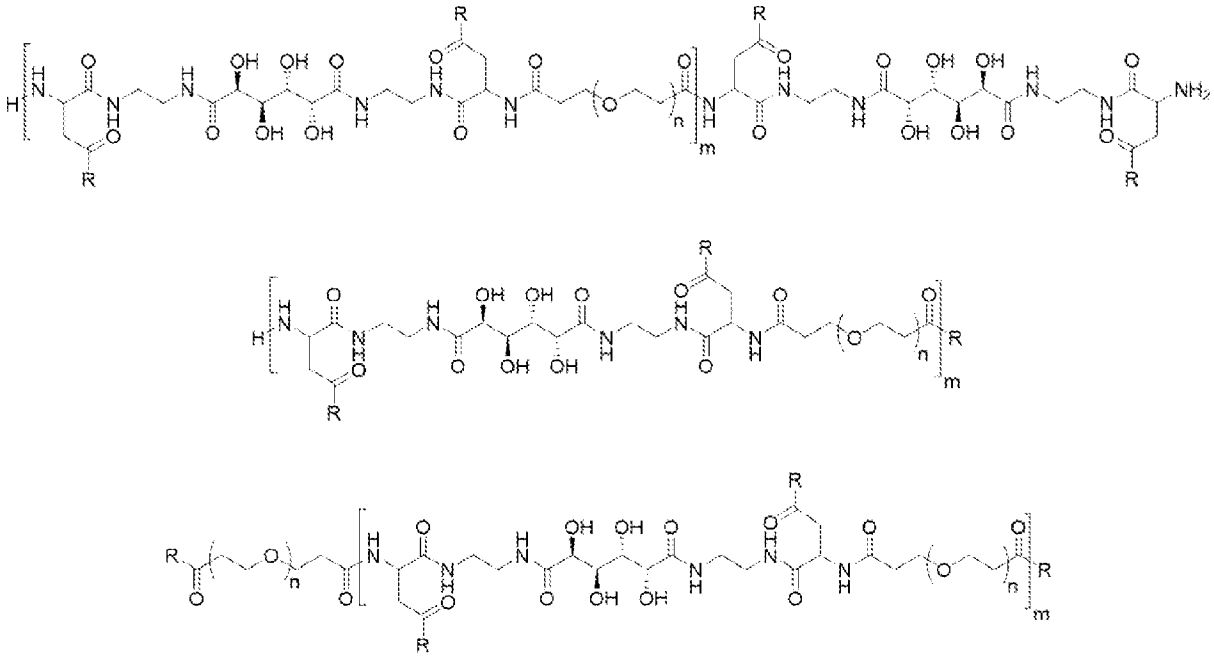
[0104] ^{1}H NMR (600 MHz, DMSO-d_6): 8.33 (s, br, 1H), 8.02 (d, , 1H), 7.98 (d, 2H), 7.89 (s, br, 1H), 7.78 (s, br, 1H), 7.72 (t, 2H), 7.65 (t, 3H), 7.41 (t, 2H), 7.34 (d, 2H), 7.18 (t, 1H), 5.81 (t, 1H), 5.36 (d, 1H), 5.12 (t, 1H), 5.08 (dd, 2H), 4.92 (d, 1H), 4.69 (s, 1H), 4.52 (dd, 1H), 4.47 (s, 1H), 4.39 (s, br, 1H), 4.12 (d, 1H), 4.0 (m, 4H), 3.81 (s, br, 1H), 3.73 (t, 1H), 3.29 (s, 3H), 3.21 (s, 3H), 2.63 (m, 2H), 2.37 (m, 2H), 2.23 (s, 3H), 1.80 (m, 1H), 1.79 (s, 3H), 1.51 (m, 2H), 1.50 (s, 3H), 1.37 (s, 9H), 0.95 (d, 6H).



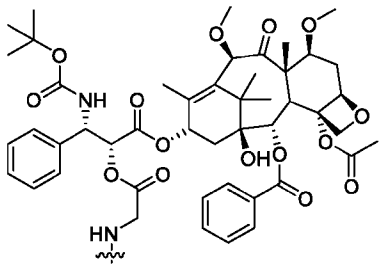
[0105] Before conjugation of CAB, MAP polymers can have a number of terminal structures, as shown below. The polymer can contain amine groups at both ends (top); an amine group at one end and a carboxylic acid group at the other end (middle); or carboxylic acid groups at both ends (bottom).



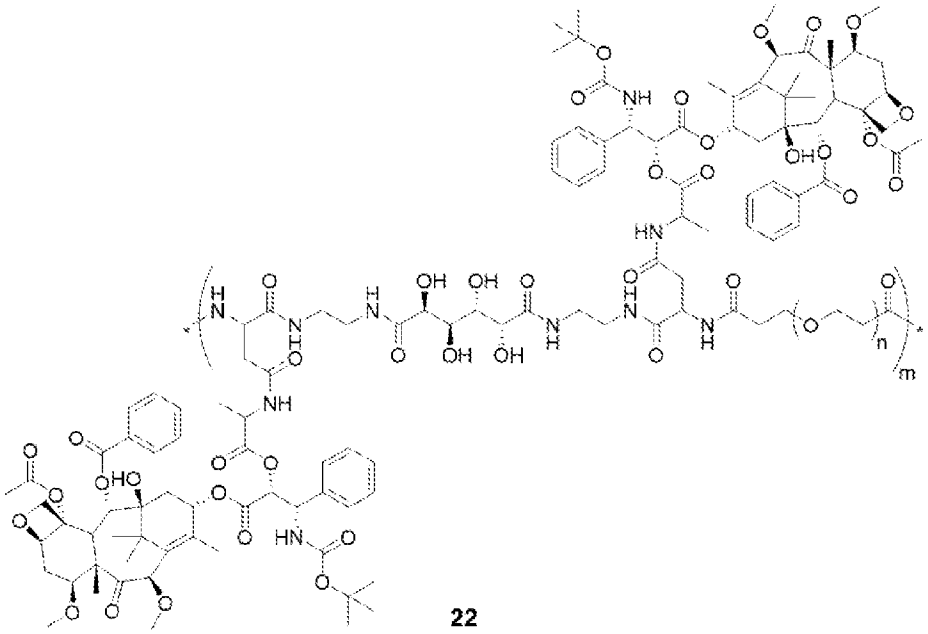
[0106] After conjugation of CAB, carboxylic acid end groups of the MAP polymers, as well as the carboxylic acid groups within the polymer repeat unit, may be derivatized in the resulting MAP-CAB conjugates, as described by the structures below:



wherein **R** is:



[0107] Additional MAP-amino acid-CAB conjugates are produced by the same method using CAB-2'-Ala.MSA (**15**), CAB-2'-β-Ala.MSA (**16**), CAB-2'-Val.MSA (**17**), CAB-2'-GABA.MSA (**18**), and CAB-2'-Hex.MSA (**19**) as starting material, instead of CAB-2'-Gly.MSA, to generate MAP-Ala-CAB (Compound **22**), MAP-β-Ala-CAB (Compound **23**), MAP-Val-CAB (Compound **24**), MAP-GABA-CAB (Compound **25**), and MAP-Hex-CAB (Compound **26**), respectively.



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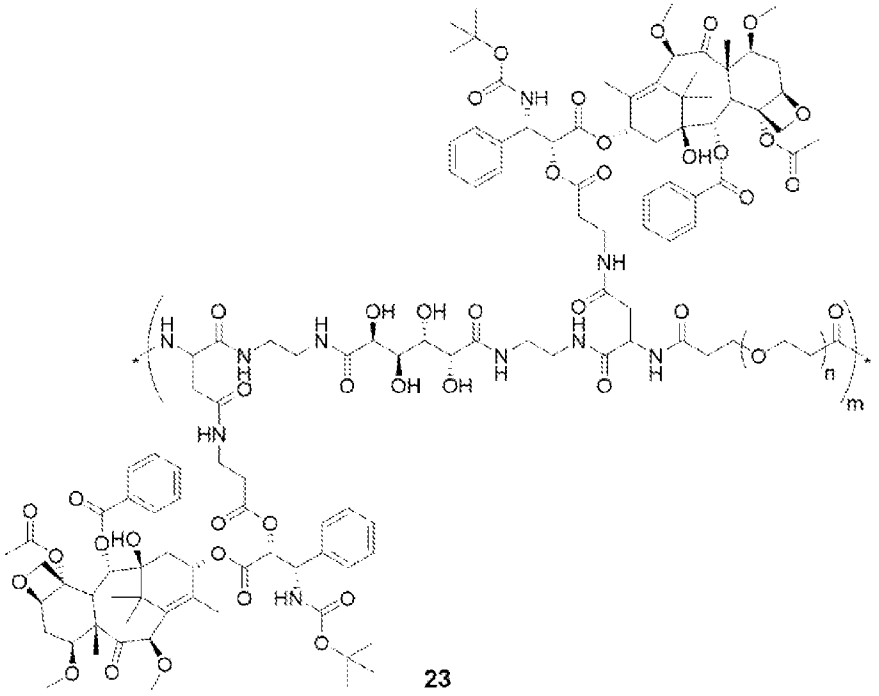

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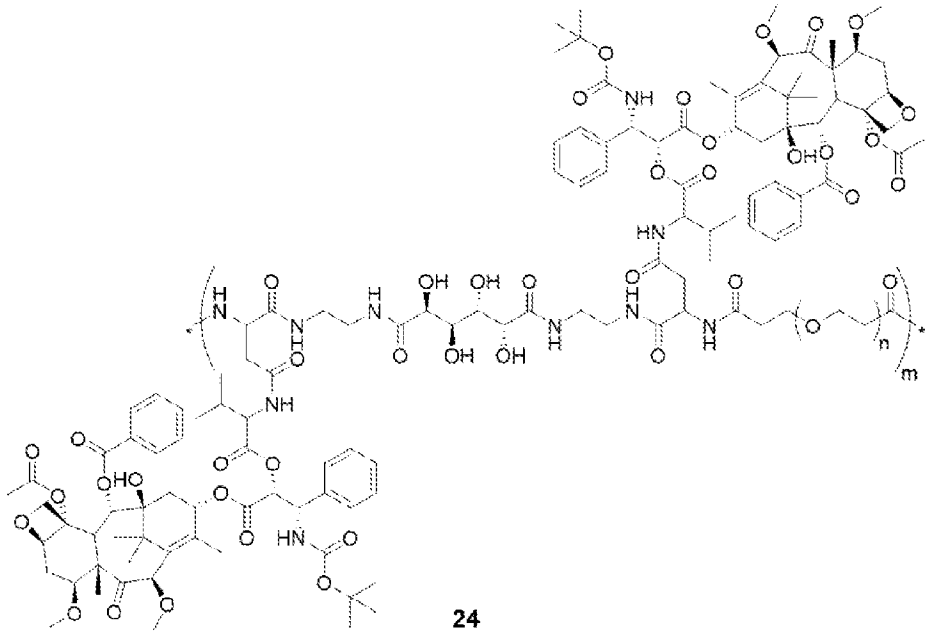

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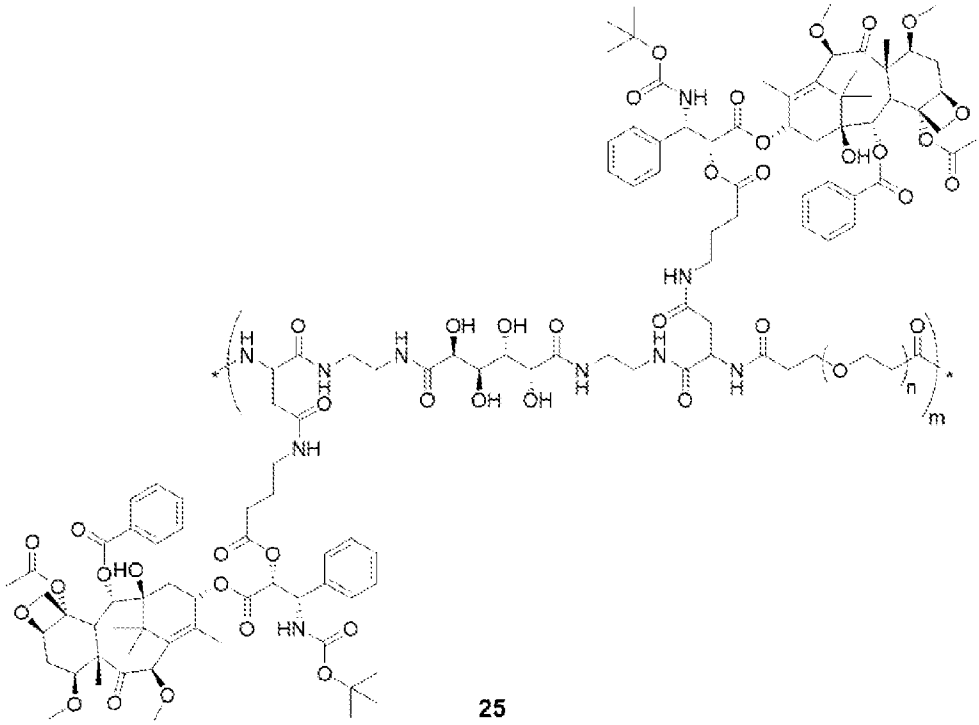

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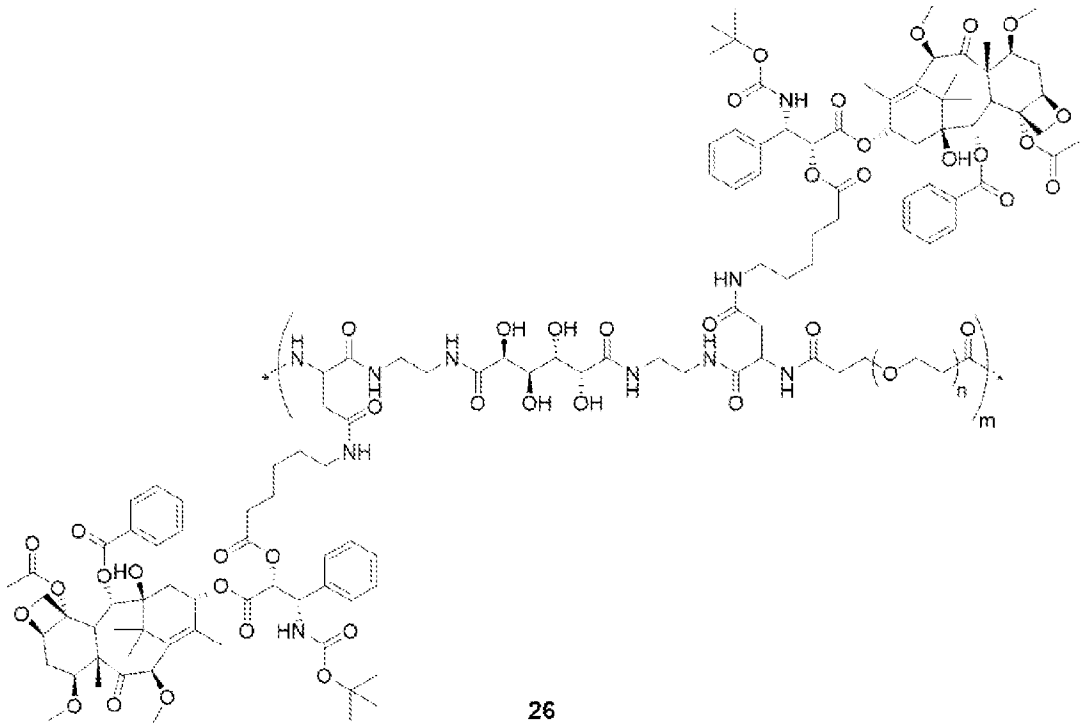

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[0108] It will be apparent that additional MSA salts of CAB-amino acid derivatives (see, *e.g.*, Tables 1 and 2) can be used, in the procedure described in this example, to provide additional MAP-amino acid-CAB conjugates.

[0109] In compounds 20-26, ***n*** can range from 20 to 200 and ***m*** can range from 5 to 200. In certain embodiments, ***n*** is 114 or about 114 (PEG molecular weight about 5 kDa). In other embodiments, ***n*** is 80 or about 80 (PEG molecular weight about 3.5 kDa). In further embodiments, ***n*** is 46 or about 46 (PEG molecular weight about 2 kDa). In certain embodiments, ***m*** is 16 or about 16.

Example 4: CAB content in MAP-CAB conjugates

[0110] Weight percent of CAB in MAP-amino acid-CAB conjugates was determined by high-performance liquid chromatography with ultraviolet absorbance detection (HPLC-UV). MAP-Gly-CAB conjugate was dissolved in dimethyl formamide (DMF) at 1 mg/mL. 10 µL of conjugate solution was added to 6.7 µL 0.5 N NaOH and incubated for 3 h at room temperature. 6.7 µL 0.5 N HCl was subsequently added and incubated for 30 min at room temperature. After incubation with HCl, 23.4 µL of acetonitrile (ACN) was added to complete sample preparation.

[0111] Samples were analyzed using an Agilent 1200 Series HPLC system. Using an isocratic mobile phase of 57:43 Water + 0.01 % trifluoroacetic acid (TFA):ACN + 0.01 % TFA, 20 µL of sample was injected onto a Phenomenex Synergi Hydro-RP (4 µm, 80 Å) reverse-phase column. The amount of CAB in the conjugate solution was quantified by measuring absorbance at 230 nm and comparing to a calibration curve of reference CAB solutions, prepared as described in Example 5.

[0112] Weight percent CAB was then determined using the following formula:

Weight percent CAB = 100% × [(concentration of total CAB)/concentration of MAP-CAB conjugate)].

Example 5: Preparation of reference CAB solutions

[0113] Reference solutions of cabazitaxel (CAB) were prepared by dissolving different concentrations of CAB in dimethyl formamide (DMF). 10 µL of each reference solution was added to 6.7 µL 0.5 N NaOH and the mixture was incubated for 3 h at room temperature. 6.7 µL 0.5 N HCl was subsequently added and the mixture was incubated for 30 min at room temperature. After the incubation with HCl, 23.4 µL of acetonitrile (ACN) was added. The absorbance at 230 nm (A230) was measured for each reference solution and the absorbance values were used to construct a standard curve (calibration curve).

Example 6: Formation of nanoparticles

[0114] 75 mg of MAP-Gly-CAB (**21**) was dissolved in 15 mL of pH 4 ultrapure water. The solution was stirred at room temperature for approximately 3 hours. 1.7 mL of 9 % saline, pH 4, was added and stirring was continued for 1 hour. The nanoparticle solution was concentrated using a 10 kDa membrane, passed through a 0.22 µm filter, and frozen.

[0115] Any of the other MAP-amino acid-CAB conjugates described above (*i.e.*, MAP-Ala-CAB (**22**), MAP-β-Ala-CAB (**23**), MAP-Val-CAB (**24**), MAP-GABA-CAB (**25**), and MAP-Hex-CAB (**26**) are converted to nanoparticles in similar fashion, *i.e.*, by dissolving in water or dilute saline, at pH 4, to a concentration of 1–10 mg/mL.

Example 7. Nanoparticle characterization

[0116] Nanoparticle size (Z_{avg}) and polydispersity index (PDI) were determined using dynamic light scattering (DLS), which was performed on a Malvern Zetasizer Nano ZS instrument. Nanoparticles made from MAP-Gly-CAB were diluted to a concentration of 2.0 mg/mL in pH 4 saline. The results of 3 measurements, 10 runs each, were averaged.

[0117] The surface charge, or zeta potential, of MAP-Gly-CAB nanoparticles was measured using a Malvern Zetasizer Nano ZS instrument. 75 μL of 2.0 mg/mL of MAP-Gly-CAB nanoparticle solution was mixed with 675 μL of 1 mM potassium chloride. The results of 3 measurements, 10 runs each, were averaged.

[0118] Using the methods described above, the Z_{avg} of MAP-Gly-CAB nanoparticles was determined to be 19 nm; the PDI of MAP-Gly-CAB nanoparticles was determined to be 0.21; and the zeta potential of MAP-Gly-CAB nanoparticles was determined to be -0.08 mV.

[0119] Z_{avg}, PDI and zeta potential of nanoparticles made from MAP-Ala-CAB, MAP-β-Ala-CAB, MAP-Val-CAB, MAP-GABA-CAB, and MAP-Hex-CAB are determined in similar fashion.

Example 8: *In vitro* release studies

[0120] Release of CAB from nanoparticles is determined by incubating solutions of nanoparticles in various media and subsequently measuring the amount of unconjugated and total CAB present in solution at different timepoints.

[0121] In a typical experiment, nanoparticles were diluted to a concentration of 1.0 mg/mL in various release media (pH 5.5 phosphate-buffered saline (PBS), pH 7.4 PBS, mouse plasma, rat plasma, or human plasma). This 1.0 mg/mL stock of particles in release media was separated into 14 microcentrifuge tubes with 2 holes poked in the top of each cap. These tubes were subsequently incubated in a Thermo Forma Series II water-jacketed CO_2 incubator at 5 % CO_2 and 37 °C. At 0, 2, 4, 6, 12, 24, 48, 72, 96, and 120 h, two tubes were removed from the incubator, frozen on liquid nitrogen, and stored at -80°C until analysis.

[0122] Frozen samples were thawed and analyzed for total CAB and free (unconjugated) CAB as described in Example 9. Standards were prepared as described in Example 5. Release of CAB from nanoparticles is expressed as the ratio of free CAB to total (*i.e.*, the sum of free and conjugated) CAB. Exemplary results, showing *in vitro* release rates of CAB from nanoparticles

in mouse plasma, are shown in Figure 1. The results indicate that the release rate depends on the amino acid linker present between the CAB and the MAP components of the nanoparticle.

Example 9: *In vivo* rat pharmacokinetics (PK) study

[0123] MAP-Gly-CAB, MAP-Ala-CAB, MAP-β-Ala-CAB, and MAP-GABA-CAB nanoparticles, formulated in 0.9% saline, pH 4, were administered via bolus intravenous injection at 7.5 mg/kg (CAB basis) through a femoral vein canula in male Sprague Dawley rats. At predetermined time points, blood was collected via a jugular vein canula. Samples were kept on ice until centrifugation at 2000 x g for 10 min at 5°C within 1 h of collection. Plasma was directly transferred to cluster tubes and stored at -80°C until analysis.

[0124] Analyses for total CAB and unconjugated CAB were as follows. To determine the amount of unconjugated CAB, 10 µL of sample was mixed with 13.4 µL 0.5 N HCl and incubated for 30 min at room temperature. 23.4 µL ACN was subsequently added and incubated for 3 h at room temperature. After incubation with ACN, the mixture was centrifuged at 14,000 x g for 10 min at 4°C and the supernatant was recovered and filtered through a 0.20 µm filter. To measure the total amount of CAB, 10 µL of sample was mixed with 6.7 µL 0.5 N NaOH and incubated for 3 h. 6.7 µL 0.5 N HCl was subsequently added and incubated for 30 min at room temperature, followed by addition of 23.4 µL ACN and incubation for 3 h at room temperature. After incubation with ACN, the mixture was centrifuged at 14,000 x g for 10 min at 4°C and supernatant was filtered with a 0.20 µm filter. Filtered samples were analyzed using an Agilent 1200 Series HPLC system as described in Example 4 and compared to a calibration curve of reference CAB solutions, prepared as described in Example 5.

[0125] The results, shown in Figures 2-4, indicate that nanoparticles display a biphasic profile with a fast redistribution phase and a long elimination phase. The elimination phase for the nanoparticles was prolonged for increasingly stable linkers, such as GABA and β-ala. The amount of unconjugated (free) CPT was low at all timepoints and for all nanoparticles.

Example 10: *In vivo* mouse maximum tolerated dose (MTD) study: unconjugated CAB and MAP-Gly-CAB nanoparticles

[0126] In a first MTD study, male NSG mice were randomly divided into 7 groups containing 4 mice each. CAB at 8, 12, and 16 mg/kg was administered on days 1, 8 and 15 via

intravenous tail vein injection. MAP-Gly-CAB nanoparticles at 8, 12, 16, and 24 mg/kg (CAB basis) were administered on days 1, 8, and 15 via intravenous tail vein injection. CAB was formulated in 1:1:18 ethanol:polysorbate 80:5% glucose, and nanoparticles were formulated in 0.9% saline, pH 4. Health of the mice was monitored and recorded daily for 4 weeks after the start of treatment. Body weight was monitored and recorded about every second or third day. MTD was defined as the highest dose resulting in less than 20% body weight loss or with less than 20% treatment-related deaths. Animals were euthanized when the criteria for the MTD was exceeded or at the end of the study.

[0127] Administration of CAB at 8, 12, or 16 mg/kg was at or near the MTD. All three weekly doses were completed in these groups. However, there was a 25.0% incidence of deaths in the treatment window in each group. The treatments were associated with weight changes of -15.9%, -13.7% and -15.3%, respectively. There was a trending recovery of the lost body weight in all animals that survived until study end. Administration of MAP-Gly-CAB nanoparticles at 12, 16 or 24 mg/kg (CAB basis) was not tolerated. The incidences of deaths in the treatment window were 25.0%, 25.0% and 75.0%, respectively. The treatments were associated with a -12.2%, -15.5% and -19.9% weight change in the treatment window, respectively. Due to the degree of body weight loss in these groups, the final dose of the scheduled three weekly doses was cancelled. Treatment with MAP-Gly-CAB nanoparticles at 8 mg/kg (CAB basis) was tolerated, resulting in no deaths and a -6.2% weight change in the treatment window. All three weekly doses were completed in this group.

Example 11: *In vivo* mouse MTD study: unconjugated CAB, and nanoparticles containing MAP-Ala-CAB, MAP-β-Ala- CAB and MAP-GABA-CAB

[0128] In a second MTD study, male NSG mice were randomly divided into 12 groups containing 4 mice each. CAB at 1 and 3 mg/kg was administered on days 1, 8 and 15 via intravenous tail vein injection. MAP-Ala-CAB nanoparticles at 8, 12, and 16 mg/kg (CAB basis); MAP-β-Ala-CAB nanoparticles at 8, 16, 24, and 32 mg/kg (CAB basis); and MAP-GABA-CAB nanoparticles at 16, 24, and 32 mg/kg (CAB basis) were administered on days 1, 8, and 15 via intravenous tail vein injection. CAB was formulated in 1:1:18 ethanol:polysorbate 80:5% glucose, and nanoparticles were formulated in 0.9% saline, pH 4. Health of the mice was monitored and recorded daily for 4 weeks after the start of treatment. Body weight was

monitored and recorded about every second or third day. MTD was defined as the highest dose resulting in less than 20% body weight loss or with less than 20% treatment-related deaths. Animals were euthanized when the criteria for the MTD was exceeded or at the end of the study.

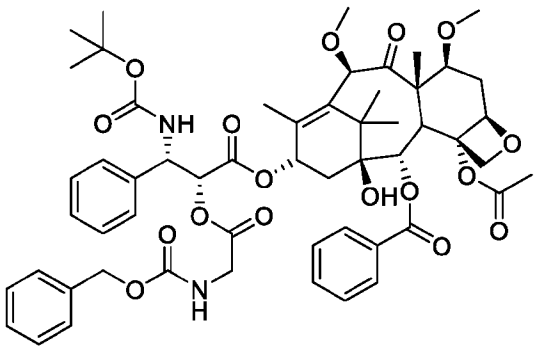
[0129] Administration of CAB at 1 or 3 mg/kg was tolerated, resulting in no deaths and weight change in the treatment windows of -2.9% and -5.8%. Administration of MAP-Ala-CAB nanoparticles at 8, 12 or 16 mg/kg (CAB basis), MAP-β-Ala-CAB nanoparticles at 8, 16, 24, or 32 mg/kg (CAB basis), or MAP-GABA-CAB at 16, 24 or 32 mg/kg (CAB basis) were all tolerated. Weight change in the treatment window across all groups ranged from -6.7% to 3.9%. There were no deaths in the treatment window with the exception of one animal that received MAP-β-Ala-CAB nanoparticles at 24 mg/kg (CAB basis). It should be noted that this single death due to excessive weight loss appears to be an outlier in comparison to the other animals in this group, and the overall tolerance of MAP-β-Ala-CAB nanoparticles is further supported by the test article being tolerated at a higher dose.

[0130] Figure 5 summarizes the results of both MTD studies, showing that MAP-Gly-CAB nanoparticles are tolerated better than unconjugated cabazitaxel; while nanoparticles containing MAP-Ala-CAB, MAP-β-Ala-CAB and MAP-GABA-CAB are even more well tolerated that MAP-Gly-CAB-containing nanoparticles.

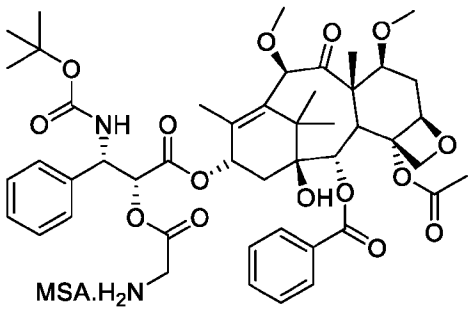
CLAIMS

What is claimed is:

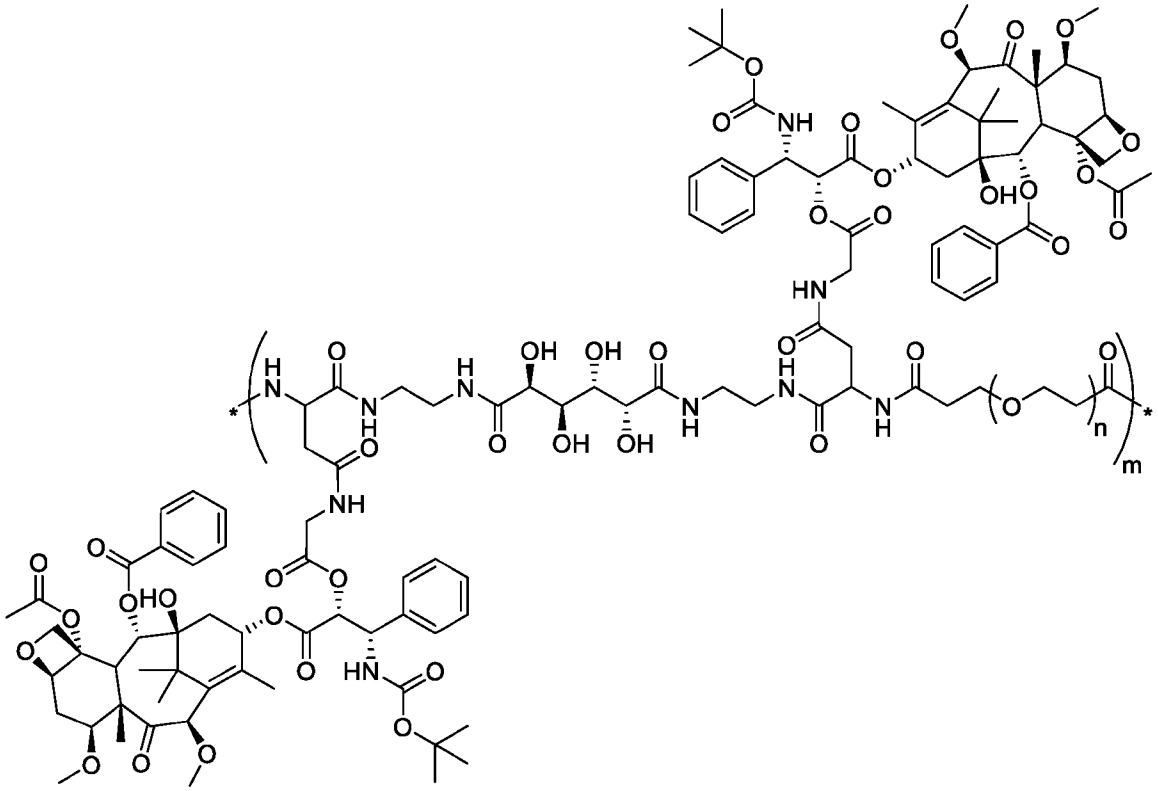
1. Cabazitaxel-2'-carboxybenzyl-glycinate having the structure:



2. Cabazitaxel-2'-glycinate.methane sulfonic acid having the structure:



3. A mucic acid polymer cabazitaxel glycinate conjugate having the structure:



wherein:

m is from 5 to 200 inclusive; and

n is from 20 to 200 inclusive.

4. The conjugate of claim 3, further comprising one or more targeting molecules.

5. The conjugate of claim 4, wherein the targeting molecule is selected from the group consisting of a protein, a peptide, an aptamer, and a ligand for a cellular receptor.

6. The conjugate of claim 5, wherein the protein is an antibody or an antibody fragment.

7. The conjugate of claim 6, wherein the antibody or antibody fragment is directed to prostate-specific membrane antigen (PSMA), HER2, or TROP2.

8. The conjugate of claim 4, wherein the targeting molecule is linked to the conjugate through a polyethylene glycol (PEG) polymer containing one or more nitrophenylboronic acid (NPBA) moieties.

9. A nanoparticle comprising the conjugate of any of claims 3-8.

10. A pharmaceutical composition comprising the nanoparticle of claim 9 and a pharmaceutically acceptable carrier or excipient.

11. A method for the treatment of a disease or disorder in a subject, the method comprising administering the pharmaceutical composition of claim 10 to the subject.

12. The method of claim 11, wherein the disorder is a malignancy.

13. The method of claim 12, wherein the malignancy is a solid tumor.

14. The method of claim 13, wherein the solid tumor is a prostate tumor, a breast tumor, or a lung tumor.

15. The method of claim 14, wherein the prostate tumor is metastatic castration-resistant prostate cancer (mCRPC).

FIGURE 1

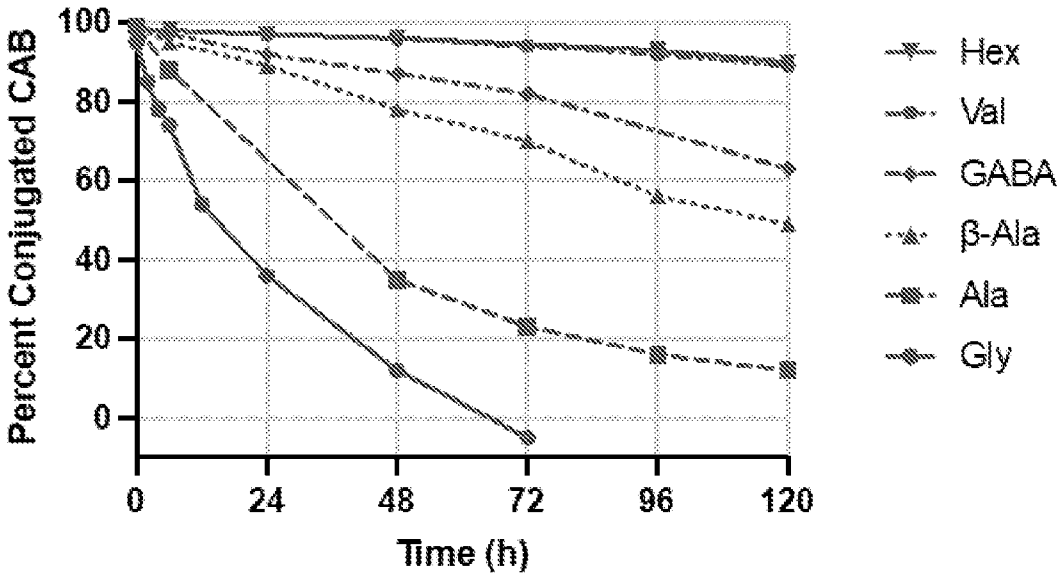

Percent Conjugated CAB
100
80
60
40
20
0
0
24
48
72
96
120
Time (h)
Hex
Val
GABA
β-Ala
Ala
Gly

FIGURE 2

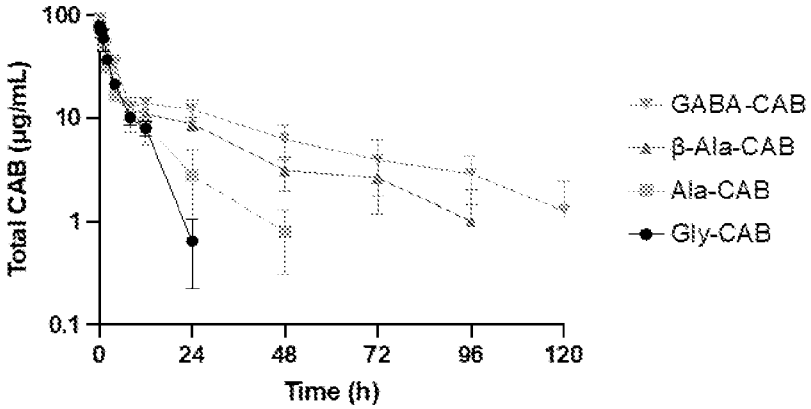


FIGURE 3

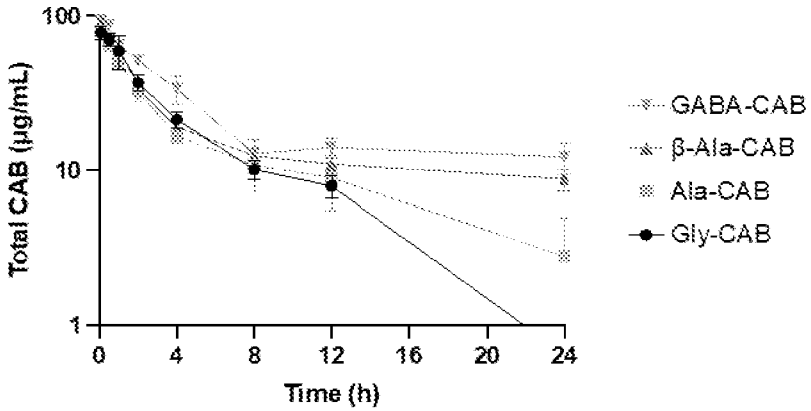


FIGURE 4

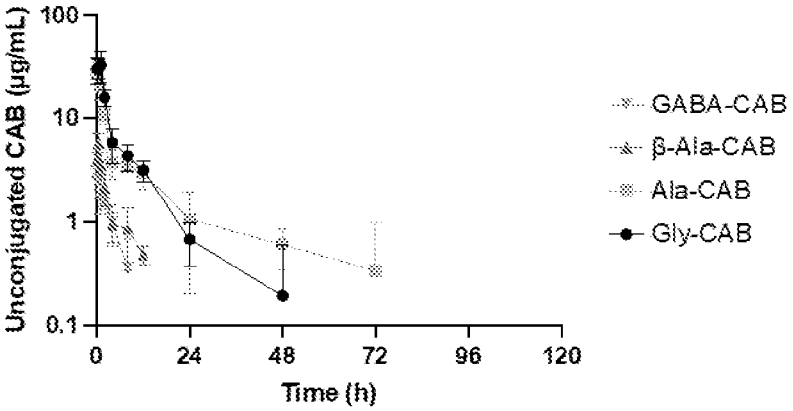
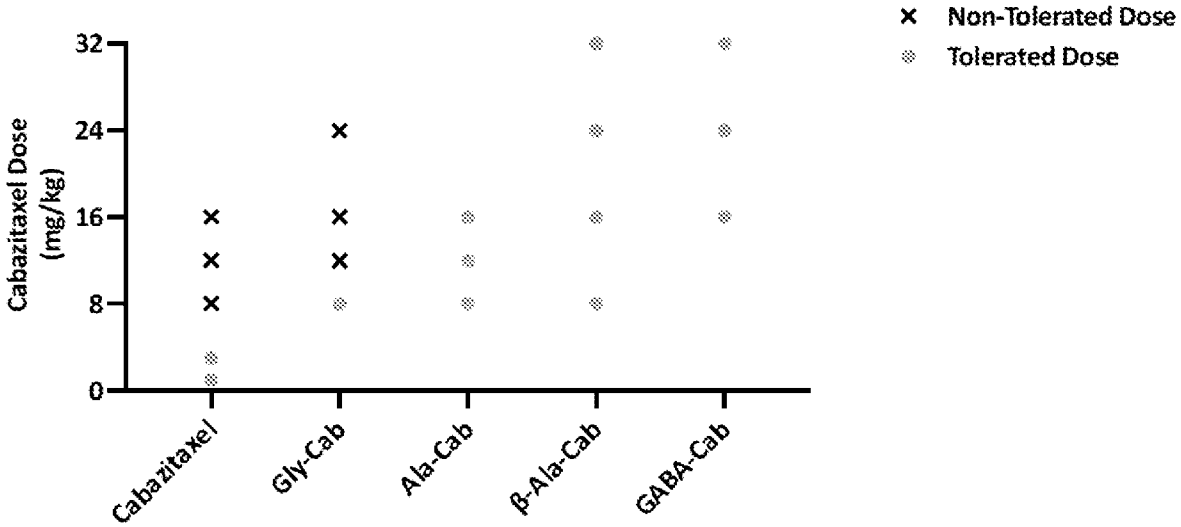


FIGURE 5



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2023/082430

A. CLASSIFICATION OF SUBJECT MATTER

IPC: *A61K 9/14* (2024.01); *A61K 31/337* (2024.01); *A61P 35/00* (2024.01); ***C07D 305/14*** (2024.01)

CPC: ***C07D 305/14;*** *A61K 31/337; A61P 35/00; A61K 9/14*

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2011/0268658 A1 (CRAWFORD et al.) 03 November 2011 (03.11.2011) entire document	1
A	US 2013/0345298 A1 (KOZLOWSKI et al.) 26 December 2013 (26.12.2013) entire document	1
A	US 2022/0378735 A1 (SHENYANG PHARMACEUTICAL UNIVERSITY) 01 December 2022 (01.12.2022) entire document	1
A	US 2014/0024845 A1 (KOZLOWSKI et al.) 23 January 2014 (23.01.2014) entire document	1
A	US 2021/0170049 A1 (DANTARI INC.) 10 June 2021 (10.06.2021) entire document	1
A	US 2011/0105598 A1 (GURJAR et al.) 05 May 2011 (05.05.2011) entire document	1

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"D" document cited by the applicant in the international application

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 February 2024 (29.02.2024)	**22 April 2024 (22.04.2024)**

Name and mailing address of the ISA/US	Authorized officer
Mail Stop PCT, Attn: ISA/US **Commissioner for Patents** **P.O. Box 1450, Alexandria, VA 22313-1450**	**MATOS** **TAINA**
Facsimile No. **571-273-8300**	Telephone No. **571-272-4300**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2023/082430

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I: claim 1 is drawn to Cabazitaxel-2'-carboxybenzyl-glycinate.

Group II: claim 2 is drawn to Cabazitaxel-2'-glycinate.methane sulfonic acid.

Group III: claims 3-15 are drawn to mucic acid polymer cabazitaxel glycinate conjugates, nanoparticles, pharmaceutical compositions, and methods for the treatment of a disease or disorder in a subject.

The inventions listed in Groups I-III do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The special technical features of Group I, Cabazitaxel-2'-carboxybenzyl-glycinate, are not present in Groups II and III; the special technical features of Group II, Cabazitaxel-2'-glycinate.methane sulfonic acid, are not present in Groups I and III; and the special technical features of Group III, mucic acid polymer cabazitaxel glycinate conjugates, nanoparticles, pharmaceutical compositions, and methods for the treatment of a disease or disorder in a subject, are not present in Groups I and II.

Additionally, even if Groups I-III were considered to share the technical features of a Cabazitaxel glycinate 2' derivative, these shared technical features do not represent a contribution over the prior art as disclosed by US 2022/0378735 A1 to Shenyang Pharmaceutical University (hereinafter, "Shenyang").

Shenyang teaches a Cabazitaxel glycinate 2' derivative (Para. [0008], a cabazitaxel weakly-alkaline derivative or a pharmaceutically acceptable salt thereof is provided. The cabazitaxel weakly-alkaline derivative has a structural formula as follows: as shown).

The inventions listed in Groups I-III therefore lack unity under Rule 13 because they do not share a same or corresponding special technical feature.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2023/082430

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☑ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: **1**

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.