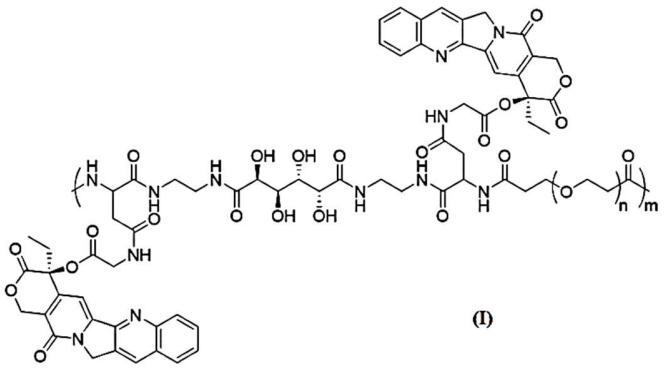
for development of breast, ovarian, prostate, melanoma, and pancreatic cancers. Tumor cells with defective HR repair show increased sensitivity to chemotherapeutic agents, which suggests that HR proficient tumor cells might be sensitized to chemotherapeutics if HR repair could be therapeutically inactivated.

Topoisomerase inhibitors damage DNA by binding to topoisomerase I which prevents the unwinding of DNA required for replication. This results in a stalled replication fork that can be repaired by poly(ADP-ribose) polymerase (PARP). PARP inhibitors block PARP enzyme DNA repair activity and have been shown to induce apoptosis in tumor cells. In addition to direct tumor killing, inhibition of PARP also increases the cytotoxicity of chemotherapeutic drugs, and increases the cytotoxicity of DNA-damaging agents such as topoisomerase inhibitors, camptothecin (CPT) and its derivatives.

Unfortunately, while CPT is known for its anti-tumor activity, including over a wide spectrum of human cancers, it has poor water solubility, low plasma stability, and dose-limiting toxicity.

SUMMARY

The present disclosure provides methods, compositions, and uses of camptothecin (CPT) conjugated nanoparticles. In particular, the present disclosure is directed to compositions, formulations, and methods of use that include formulations of nanoparticles comprising 20(S)-camptothecin covalently attached to a mucic acid-based polymer carrier via a glycyl linkage (MAP-Gly-CPT) comprising Formula (I):



(I)

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where n is the number of ethylene glycol repeating units, which is in the range of 20 to 200 units and m is the number of repeating units of MAP-Gly-CPT, which is in the range of 5 to 200.

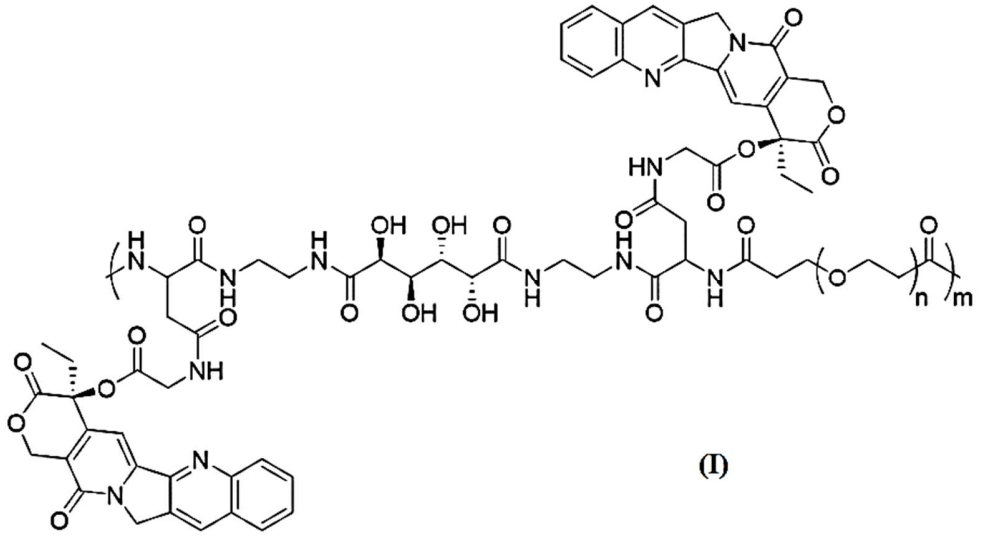
In preferred examples, the MAP-Gly-CPT compound is DAN-222, which has the structure of Formula I, wherein the number of repeating ethylene glycol repeating units (n) averages a number in a range from about 75 to 85. In preferred examples, in the formulations and compositions of the disclosure n averages about 79. In certain examples, the number of repeating units of MAP-Gly-CPT (m) averages 16 ± 4.

Camptothecin (CPT), the active ingredient in DAN-222, is a naturally occurring, pentacyclic quinolone alkaloid isolated from the bark of *Camptotheca acuminata*. CPT is a topoisomerase I inhibitor that acts by forming a ternary complex with topoisomerase I and DNA during replication and transcription in tumor cells, which blocks the re-ligation of DNA, resulting in torsional tension downstream of the transcription fork or replication fork that causes DNA strand breaks.

Unfortunately, while CPT is known for its anti-tumor activity, including over a wide spectrum of human cancers, it has poor water solubility, low plasma stability, and dose-limiting toxicity.

However, as discovered by the present inventors, DAN-222 is more efficacious than other topoisomerase I inhibitors, including free CPT, due to its accumulation in tumors through an enhanced permeability and retention (EPR) effect, which leads to a greater and prolonged topoisomerase I inhibition.

In certain examples, the present disclosure provides aqueous formulations comprising nanoparticles made of a mucic acid polymer (MAP) camptothecin (CPT) conjugate compound of Formula (I):



(I)

,

wherein n is a number in a range from 20 to 200; and

wherein m is a number in a range from 5 to 200.

In certain formulations, n is a number in a range from about 75 to 85. In certain formulations, m is a number in a range from about 12 to 20.

In certain formulations of the disclosure, the compound of Formula (I) has a molecular weight of about 75,000 Da. In preferred examples, nanoparticles of the formulation comprise on average two strands comprising the compound of Formula (I) and have an average molecular weight of about 150,000 Da. The nanoparticles of the formulation may have an average particle size of about 20 nm to 80 nm. Preferably, the nanoparticles have an average particle size of about 30 to 40 nm. In certain examples, the formulation has a nanoparticle concentration of about 17 – 23 mg/mL.

Formulations of the disclosure include those having a total concentration of CPT of between 1.7 and 3.6 mg/mL. In preferred examples, the formulation has a total concentration of CPT of about 2.6 mg/mL. In certain examples, the formulation comprises a concentration of about 20 mg/mL of the compound of Formula (I).

In certain examples, the formulation has a pH between pH 4 and pH 5. In certain examples, the formulation has a pH between pH 4 and pH 4.6. In preferred examples, the formulation has a pH of about pH 4.3.

A formulation of the disclosure may further include at least one buffer selected from, *e.g.*, sodium succinate, sodium citrate, sodium acetate, phosphoric acid, histidine-HCL, and sodium

phosphate. In preferred examples, the buffer is a sodium acetate buffer. The formulation may further include at least one tonicity modifier selected from, *e.g.*, KCl, NaCl, Proline, Arginine-HCl, sucrose, and glycine. Preferably, the at least one tonicity modifier is NaCl.

In preferred examples, the formulation comprises about 20 mg/mL of the compound of Formula (I), about 20 mM sodium acetate, about 200 mM NaCl, and has a pH of 4.3 ± 0.3.

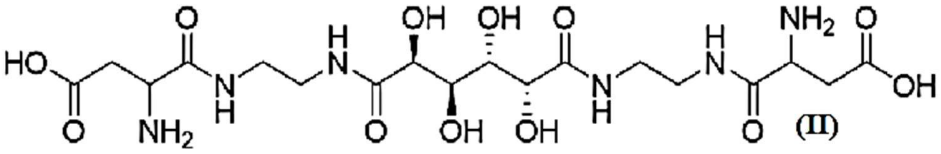
The present disclosure also provides processes for producing a formulation comprising the compound of Formula (I). In certain examples, a process for producing a formulation of a compound of Formula (I) comprises: (i) performing a derivatization of camptothecin (CPT) to yield Gly-CPT as the trifluoracetic acid (TFA) salt; (ii) synthesizing a parent mucic acid polymer (MAP); (iii) covalently attaching the Gly-CPT to the parent MAP to yield solid, amorphous MAP-Gly-CPT; and (iv) preparing an aqueous formulation using the amorphous MAP-Gly-CPT thereby producing an aqueous formulation of nanoparticles comprising the compound of Formula (I).

In certain methods of the disclosure, step (i) of the process includes dissolving CPT, N-(tert-butoxycarbonyl)-Gly-OH (Boc-Gly), and 4-dimethylaminopyridine (DMAP) in methylene chloride (DCM) to form a reaction mixture; and adding diisopropylcarbodiimide (DIC) to the reaction mixture thereby producing a solution comprising a Boc-Gly-CPT intermediate. In certain examples, the reaction is conducted at 25-30°C for about six hours.

In certain methods of the disclosure, step (i) further includes removing between 90% and 99% of the DCM from the solution comprising the Boc-Gly-CPT intermediate. In preferred methods, the DCM is removed via vacuum distillation. Step (i) may further include precipitating the Boc-Gly-CPT intermediate using methanol. Such methods may also include washing the precipitated Boc-Gly-CPT intermediate using isopropyl alcohol (IPA).

In certain methods of the disclosure, step (i) further comprises treating the Boc-Gly-CPT intermediate with TFA in DCM to yield the TFA salt of Gly-CPT. In certain examples, treatment with TFA is conducted at 25-30°C for about two hours.

In preferred methods for producing the formulation, step (ii) includes: charging a reactor with (succinimidyl propionate)$_2$-PEG$_{3500}$ (diSPA-PEG$_{3500}$) and a mucic acid monomer (MAM) neutral species of Formula (II):



(II)

;

adding dimethyl sulfoxide (DMSO) to the reactor; initiating the reaction by adding TFA to the reactor; and adding DIPEA to the reaction, thereby yielding the parent MAP. In certain examples, the reaction is conducted at 21-23°C for about three hours.

In certain examples, the comonomer ratio of DIPEA:MAM used in step (ii) is at least about 2.5. Preferably, the comonomer ratio is at least 2.6.

In certain methods of the disclosure, step (ii) further includes precipitating the parent MAP from the DMSO via addition of at least one anti-solvent. An exemplary anti-solvent is isopropyl alcohol (IPA). In preferred examples, the precipitating step includes a filtration step under an inert atmosphere, such as, for example, nitrogen or argon.

In certain methods of the disclosure, step (iii) includes: adding the parent MAP and DMSO to a reaction vessel; adding the Gly-CPT (TFA salt) and (7-azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyAOP) to the reaction vessel; and adding DIPEA to the reaction vessel, thereby producing a solution that comprises MAP-Gly-CPT. In certain examples, the reaction is conducted at 19-25°C for 16-22 hours.

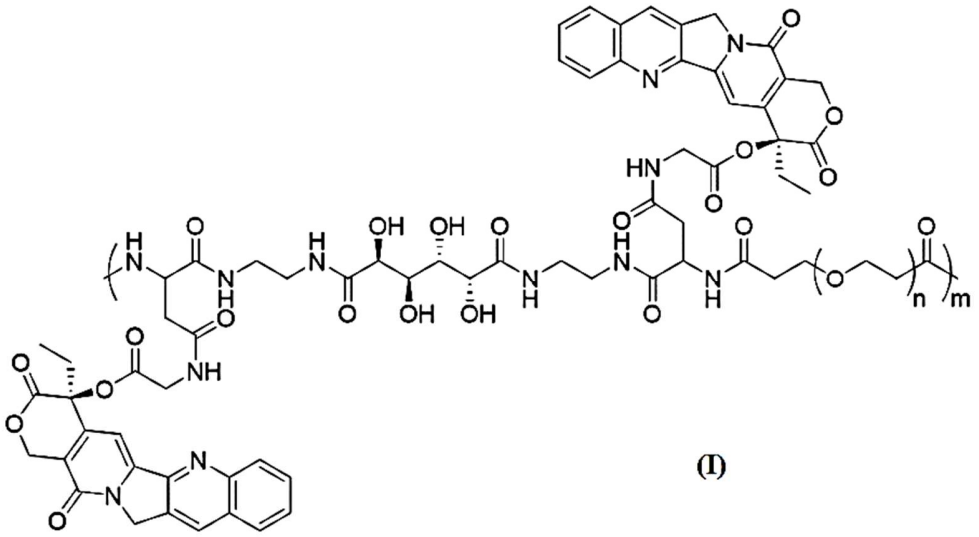
Step (iii) may further include purifying amorphous MAP-Gly-CPT from the solution using tangential flow filtration (TFF). Preferably, prior to the tangential flow filtration, the solution is diluted with pH 3 water and the tangential flow filtration uses polyethersulfone (PESU) TFF filters. In additional examples, membranes made of regenerated cellulose are used for TFF.

In certain methods of the disclosure, step (iv) includes: adding water with a pH of around pH 4 to the amorphous MAP-Gly-CPT to produce a nanoparticle (NP) solution; and adding a formulation buffer to the NP solution.

In certain examples, the formulation buffer comprises sodium acetate. Preferably, the formulation buffer further comprises NaCl and has a pH of around pH 4.16.

In certain methods, after the step of adding the water with a pH of around pH 4, the NP solution is filtered. In certain examples, after the step of adding the formulation buffer to the NP solution, the NP solution is concentrated by ultrafiltration.

In one aspect, the present disclosure provides a process for producing a formulation of a compound of Formula (I):



(I)

,

wherein n is a number in a range from 20 to 200; and

wherein m is a number in a range from 5 to 200,

the process comprising:

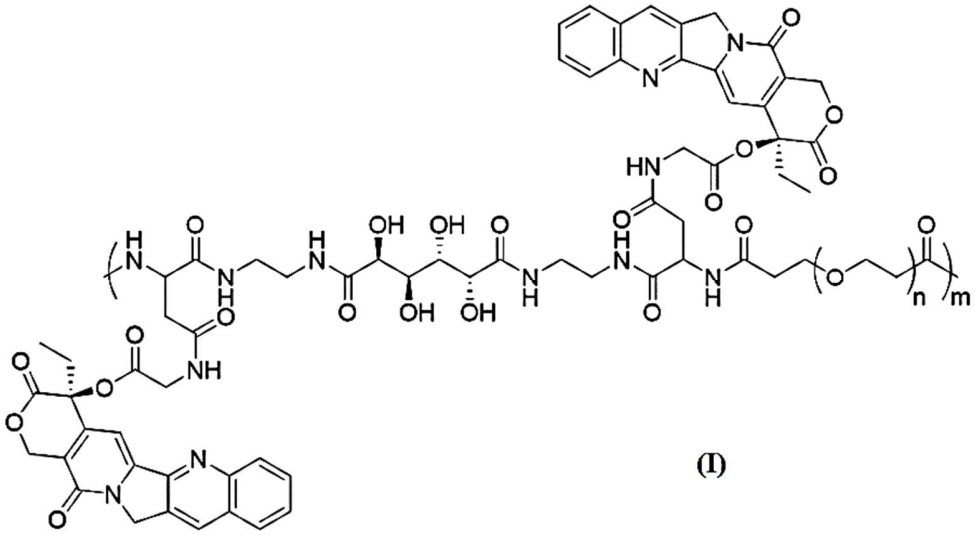
(i) performing a derivatization of camptothecin (CPT) to yield Gly-CPT as the trifluoracetic acid (TFA) salt;

(ii) synthesizing a parent mucic acid polymer (MAP);

(iii) covalently attaching the Gly-CPT to the parent MAP to yield solid, amorphous MAP-Gly-CPT; and

(iv) preparing an aqueous formulation of the amorphous MAP-Gly-CPT, thereby producing an aqueous formulation of nanoparticles comprising the compound of Formula (I).

In another aspect, the present disclosure provides a formulation comprising nanoparticles, said nanoparticles comprising a mucic acid polymer (MAP) camptothecin (CPT) conjugate compound of Formula (I):



(I)

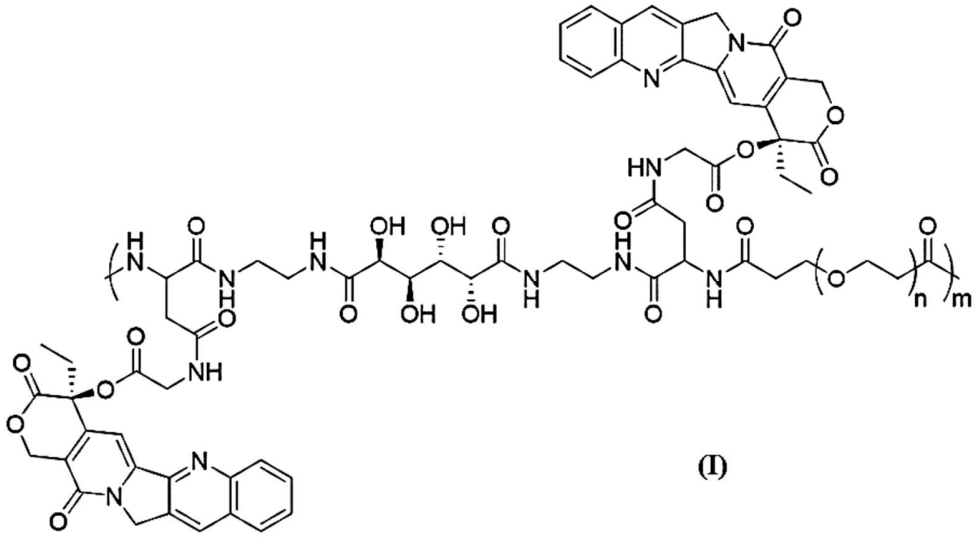
,

wherein n is a number in a range from 20 to 200; and

wherein m is a number in a range from 5 to 200,

formulated as an aqueous formulation.

The present disclosure also provides methods for treating cancer in a subject using DAN-222 formulations as described herein. An exemplary method includes providing to a subject having cancer at least one dose of a composition comprising a compound of Formula (I):



(I)

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wherein n is a number in a range from 20 to 200; and wherein m is a number in a range from 5 to 200. In preferred methods: n is a number in a range from about 75 to 85; m is a number in a range from about 12 to 20; and the composition has a pH between pH 4 and pH 4.6.

Preferably, the composition is provided in solution for infusion.

In preferred methods, the composition comprises between 2 mg/mL and 3 mg/mL of camptothecin (CPT). In certain examples, the dose of the composition comprises between 2 mg/m^2 and 16 mg/m^2 of the compound of Formula (I). An exemplary dose of the composition comprises about 2 mg/m^2, about 4 mg/m^2, about 6 mg/m^2, about 8 mg/m^2, about 10 mg/m^2, about 12 mg/m^2, about 14 mg/m^2, or about 20 mg/m^2 of the compound of Formula (I).

In certain methods, a plurality of doses is provided to a subject during a treatment cycle. Preferably, the doses are provided as weekly doses, i.e., one dose provided every seven days.

Exemplary methods of treatment further include providing at least one dose of a poly(ADP-ribose) polymerase (PARP) inhibitor to the subject. The PARP inhibitor may include, for example, one of olaparib, rucaparib, niraparib, and talazoparib. Preferably, the PARP inhibitor is niraparib. Preferably, the subject receives a dose of niraparib daily during the treatment cycle. In exemplary methods, each dose of niraparib is a 100 mg dose.

In exemplary methods of the disclosure, the cancer is one or more cancers of the breast, ovary, brain, lung, testicle, head, neck, esophagus, lymphoma, central nervous system, peripheral nervous system, bladder, stomach, pancreas, liver, oral mucosa, colorectal, anus, kidney, bladder, uroepithelium, prostate, endometrium, uterus, fallopian tube, mesothelioma, melanoma, myeloma, leukemia, and Kaposi's sarcoma.

In preferred methods, the cancer is a breast cancer. The breast cancer may include, for example, a breast cancer that is a homologous recombination repair deficiency (HRD)-positive or HRD-negative breast cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the formation of a DAN-222 engineered nanoparticle.

FIG. 2 provides the calculated median tumor volumes (MTV) as a function over time presented by various treatment groups in an HRD-positive (ER-positive, PR-positive, HER2-positive) breast ductal carcinoma (BT-474 model). Treatment groups are:

- vehicle control (circles, top line)
- camptothecin (3 mg/kg) iv once weekly for 3 weeks (diamonds, next lower line)
- irinotecan (100 mg/kg) iv once weekly for 3 weeks (triangles, next lower line)
- DAN-222 (3 mg/kg) iv once weekly for 3 weeks (squares, lowest line).

FIG. 3 provides a Kaplan Meier plot of the time to endpoint (TTE), for animals in each of the treatment groups described in FIG. 2. Endpoint is a tumor volume of 2,000 mm^3. Treatment groups are:

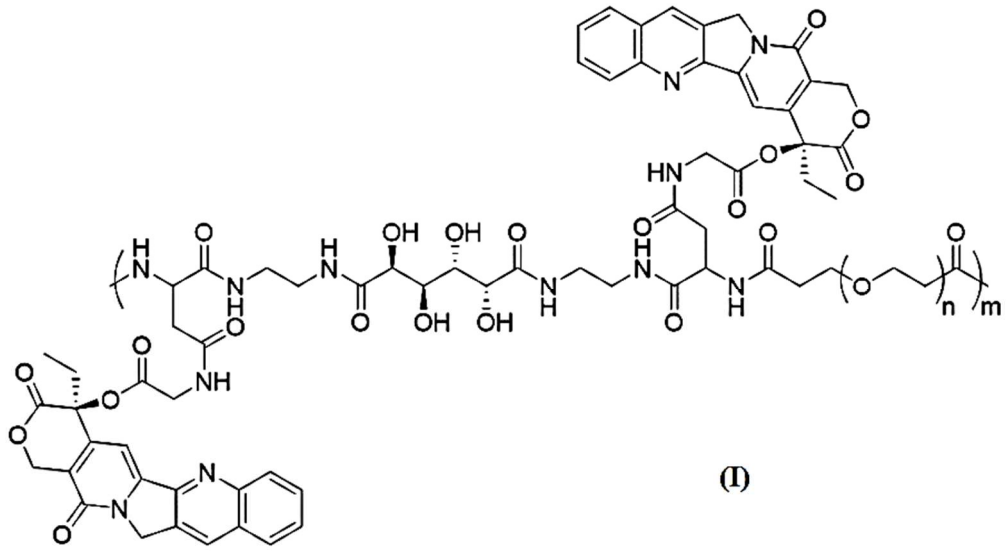
- vehicle control (circles, first line that descends from 100%)
- camptothecin (3 mg/kg) iv once weekly for 3 weeks (diamonds, second line that descends from 100%)
- irinotecan (100 mg/kg) iv once weekly for 3 weeks (triangles, third line that descends from 100%)
- DAN-222 (3 mg/kg) iv once weekly for 3 weeks (square, line that does not descend from 100%).

FIG. 4 shows median tumor volume measurements, over time, for various treatment groups in a HRD-positive, triple negative (ER-negative, PR-negative, HER2-negative) breast

Claims

What is claimed is:

1. A process for producing a formulation of a compound of Formula (I):



(I)

,

wherein n is a number in a range from 20 to 200; and

wherein m is a number in a range from 5 to 200,

the process comprising:

(i) performing a derivatization of camptothecin (CPT) to yield Gly-CPT as the trifluoracetic acid (TFA) salt;

(ii) synthesizing a parent mucic acid polymer (MAP);

(iii) covalently attaching the Gly-CPT to the parent MAP to yield solid, amorphous MAP-Gly-CPT; and

(iv) preparing an aqueous formulation of the amorphous MAP-Gly-CPT, thereby producing an aqueous formulation of nanoparticles comprising the compound of Formula (I).

2. The process of claim 1, wherein step (i) comprises:

dissolving CPT, N-(tert-butoxycarbonyl)Gly-OH (Boc-Gly), and 4-dimethylaminopyridine (DMAP) in methylene chloride (DCM) to form a reaction mixture; and

adding diisopropylcarbodiimide (DIC) to the reaction mixture thereby producing a solution comprising a Boc-Gly-CPT intermediate.

3. The process of claim 2, wherein the step (i) further comprises removing between 90% and 99% of the DCM from the solution comprising the Boc-Gly-CPT intermediate.

4. The process of claim 3, wherein the DCM is removed via vacuum distillation.

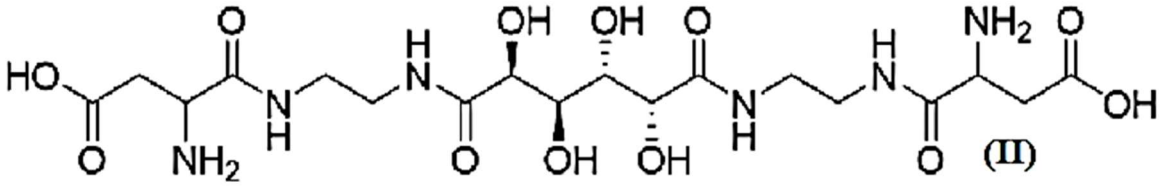
5. The process of claim 3, wherein the step (i) further comprises precipitating the Boc-Gly-CPT intermediate using methanol.

6. The process of claim 5, wherein the step (i) further comprises washing the precipitated Boc-Gly-CPT intermediate using isopropyl alcohol (IPA).

7. The process of claim 6, wherein the step (i) further comprises treating the Boc-Gly-CPT intermediate with TFA in DCM to yield Gly-CPT.

8. The process of claim 1, wherein step (ii) comprises:

charging a reactor with (succinimidyl propionate)$_2$PEG$_{3500}$ (diSPA-PEG$_{3500}$) and a mucic acid monomer (MAM) neutral species of Formula (II):



;

adding dimethyl sulfoxide (DMSO) to the reactor;

initiating the reaction by adding TFA to the reactor; and

adding DIPEA to the reaction,

thereby yielding the parent MAP.

9. The process of claim 8, wherein the comonomer ratio of DIPEA:MAM used in step (ii) is at least about 2.5.

10. The process of claim 9, wherein the comonomer ratio is at least 2.6.

11. The process of claim 8, wherein step (ii) further comprises precipitating the parent MAP from the DMSO via addition of at least one anti-solvent.

12. The process of claim 11, wherein the at least one anti-solvent is IPA.

13. The process of claim 11, wherein the precipitating step is followed by a filtration step under an inert atmosphere.

14. The process of claim 1, wherein step (iii) comprises:

adding the parent MAP and DMSO to a reaction vessel;

adding the Gly-CPT (TFA salt) and 7-azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyAOP) to the reaction vessel; and

adding DIPEA to the reaction vessel, thereby producing a solution that comprises MAP-Gly-CPT.

15. The process of claim 14, wherein step (iii) further comprises purifying amorphous MAP-Gly-CPT from the solution using tangential flow filtration (TFF).

16. The process of claim 15, wherein prior to the tangential flow filtration, the solution is diluted with pH 3 water and wherein the tangential flow filtration uses polyethersulfone (PESU) TFF filters.

17. The process of claim 1, wherein step (iv) comprises:

adding water with a pH of around pH 4 to the amorphous MAP-Gly-CPT to produce a nanoparticle (NP) solution; and

adding a formulation buffer to the NP solution.

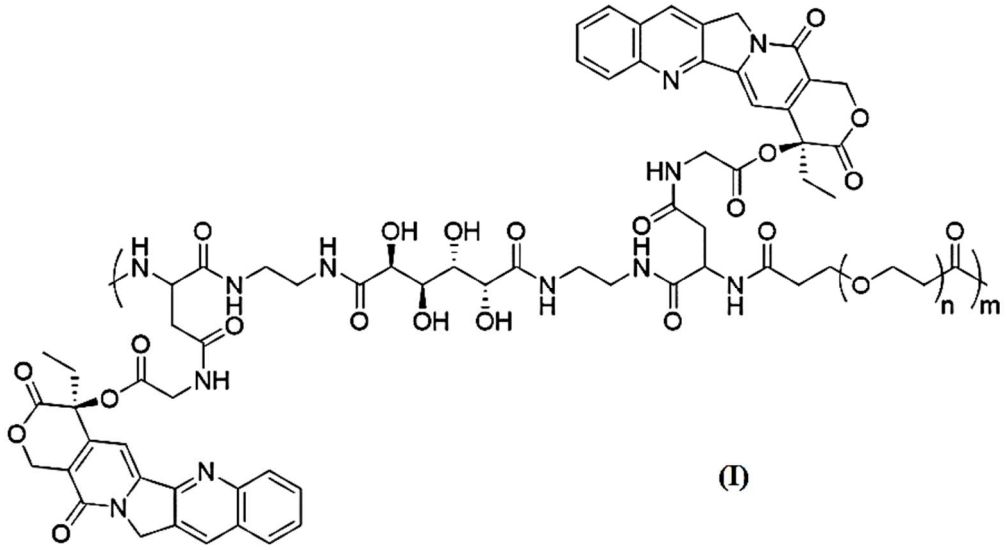
18. The process of claim 17, wherein the formulation buffer comprises sodium acetate.

19. The process of claim 18, wherein the formulation buffer comprises NaCl and has a pH of around pH 4.16.

20. The process of claim 18, wherein after the step of adding the water with a pH of around pH 4, the NP solution is filtered.

21. The process of claim 20, wherein after the step of adding the formulation buffer to the NP solution, the NP solution is concentrated by ultrafiltration.

22. A formulation comprising nanoparticles, said nanoparticles comprising a mucic acid polymer (MAP) camptothecin (CPT) conjugate compound of Formula (I):



(I)

,

wherein n is a number in a range from 20 to 200; and

wherein m is a number in a range from 5 to 200,

formulated as an aqueous formulation.

23. The formulation of claim 22, wherein n is a number in a range from about 75 to 85.

24. The formulation of claim 23, wherein m is a number in a range from about 12 to 20.

25. The formulation of claim 24, wherein the compound of Formula (I) has a molecular weight of about 75,000 Da.

26. The formulation of claim 25, wherein the nanoparticles of the formulation comprise on average two strands comprising the compound of Formula (I) and wherein the nanoparticles have an average molecular weight of about 150,000 Da.

27. The formulation of claim 26, wherein the nanoparticles of the formulation have an average particle size of about 20 nm to 80 nm.

28. The formulation of claim 27, wherein the nanoparticles have an average particle size of about 30 to 40 nm.

29. The formulation of claim 28, wherein the formulation has a nanoparticle concentration of about 17–23 mg/mL.

30. The formulation of claim 24, wherein the formulation has a total concentration of CPT of between 1.7 and 3.6 mg/mL.

31. The formulation of claim 30, wherein the formulation has a total concentration of CPT of about 2.6 mg/mL.

32. The formulation of claim 31, wherein the formulation comprises a concentration of about 20 mg/mL of the compound of Formula (I).

33. The formulation of claim 24, wherein the formulation has a pH between pH 4 and pH 5.

34. The formulation of claim 33, wherein the formulation has a pH between pH 4 and pH 4.6.

35. The formulation of claim 34, wherein the formulation has a pH of about 4.3.

36. The formulation of claim 35, wherein the formulation further comprises at least one buffer selected from sodium succinate, sodium citrate, sodium acetate, phosphoric acid, histidine-HCl, and sodium phosphate.

37. The formulation of claim 36, wherein the at least one buffer is a sodium acetate buffer.

38. The formulation of claim 37, wherein the formulation further comprises at least one tonicity modifier selected from KCl, NaCl, Proline, Arginine-HCl, sucrose, and glycine.

39. The formulation of claim 38, wherein the at least one tonicity modifier is NaCl.

40. The formulation of claim 39, wherein the formulation comprises about 20 mg/mL of the compound of Formula (I).

41. The formulation of claim 40, wherein the formulation comprises about 20 mM sodium acetate, about 200 mM NaCl, and has a pH of 4.3 ± 0.3.