COMPOSITIONS AND METHODS OF MAKING A PHOTOACTIVE AGENT

CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Patent Application No. 60/817,769 filed June 30, 2006, which provisional application is incorporated herein by reference in its entirety.

BACKGROUND

Technical Field

This disclosure generally relates to the field of bio-affecting and body treating compositions and, more particularly, to photo-active compounds, composition, and methods useful in the detection, prevention, and/or treatment of, for example, cancerous tissues.

Description of the Related Art

Mono-L-aspartyl chlorin e_6 tetra sodium salt is commonly known as Talaporfin Sodium, and is known to be a photo-active chemical or drug. For example, U.S. Patent No. RE 37,180 relates to methods of using mono-L-aspartyl chlorin e_6 that broadly include intravenous administration of the drug to a patient and applying light of an appropriate wave length to tissue containing the drug, to photo-activate the drug. Once activated, the drug occludes new blood vessels. Accordingly, the drug is useful in the treatment of diseases where blood vessel occlusion is expected to have a positive effect.

Talaporfin Sodium is typically produced from plant raw material. During the process, which includes both extraction and reaction steps, byproducts are produced. One of these byproducts is di-L-aspartyl chlorin e_6. While diaspartyl chlorin e_6 occurs as a reaction byproduct in relatively low quantity, typically about 5% in well controlled processes, it requires stringent processing for removal to purify the Talaporfin Sodium product to acceptable standards. For example, purifying a crude Talaporfin Sodium reaction product

and/or removing various impurities (*e.g.*, diaspartyl chlorin e_6) usually requires subjecting a reaction product to multiple HPLC (high performance liquid chromatography) steps to achieve an acceptable Talaporfin Sodium purity level. This purification procedure is expensive and time consuming, and may also lead to a substantial loss, up to about 50%, of the Talaporfin Sodium along with the removed impurities.

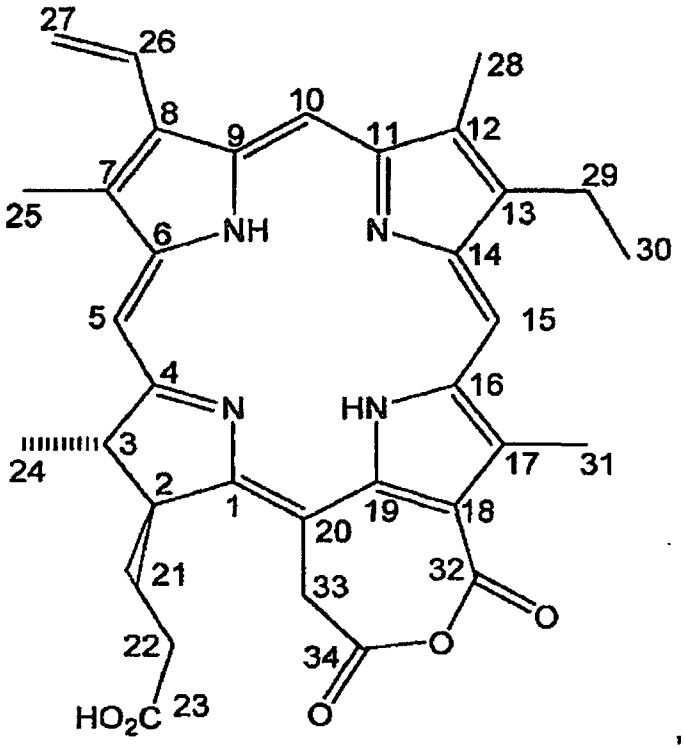
Accordingly, it is desirable to develop a process that produces a Talaporfin Sodium product without the expensive repetitive purification steps or with fewer processing steps. In addition, it is desirable to purify the reaction product without loss of a large proportion of the Talaporfin Sodium, and/or to maintain or increase the yield of Talaporfin Sodium product per mass of raw material input.

Commercial acceptance of Talaporfin Sodium is dependent on a variety of factors, such as cost to manufacture, shelf life, stability during storage, ease-of manufacture, efficacy, bioavailability, pharmacokinetic properties, etc. Therefore, it is desirable to have novel approaches of making Talaporfin Sodium that significantly reduce the manufacturing cost. It is also desirable to have novel compositions that result in purer forms of Talaporfin Sodium.

The present disclosure is directed to overcoming one or more of the shortcomings set forth above, and providing further related advantages.

BRIEF SUMMARY

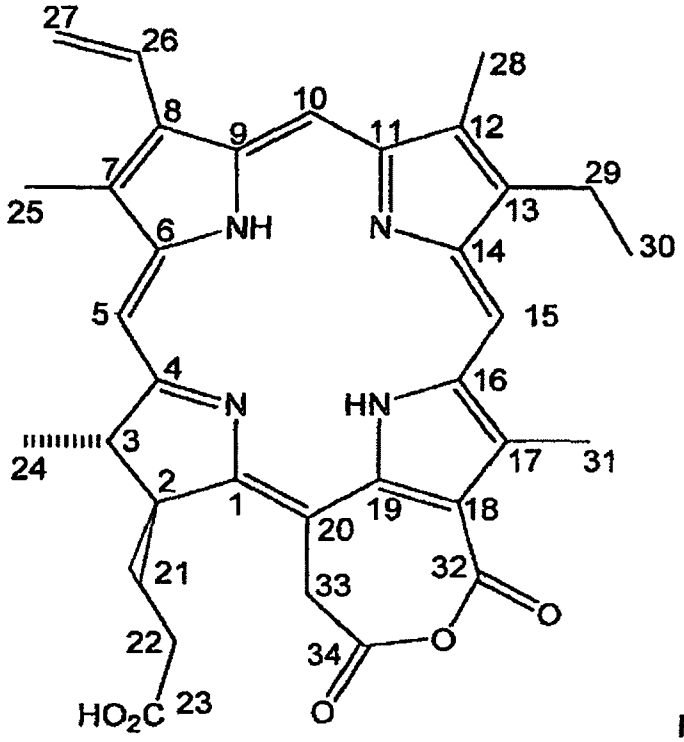
In one aspect, the present disclosure is directed to a chemical reaction product of chlorin e_6 and a carbodiimide. In some embodiments, the chemical reaction product comprises a chlorin e_6 anhydride of the form:



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precursors of diaspartyl chlorin e_6. In some embodiments, the precursors of diaspartyl chlorin e_6 comprise less than about 5 mol% based on the total chlorin species present within the chemical reaction product.

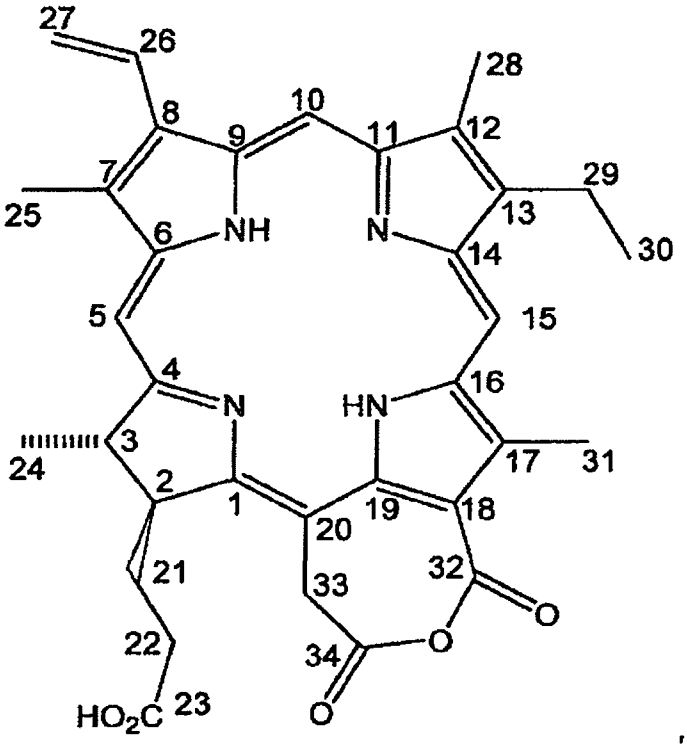
In another aspect, the present disclosure is directed to a composition, comprising a compound of Formula I



Formula I,

or a pharmaceutically acceptable salt thereof, in a substantially pure form.

In another aspect, the present disclosure is directed to a process for preparing a chlorin e_6 anhydride, or a pharmaceutically acceptable salt thereof. The process includes combining chlorin e_6 with a carboxyl activating agent to obtain a mixture comprising a chlorin e_6 anhydride having the following formula



, or a salt thereof.

The process may further include purifying the chlorin e_6 anhydride, or salt thereof.

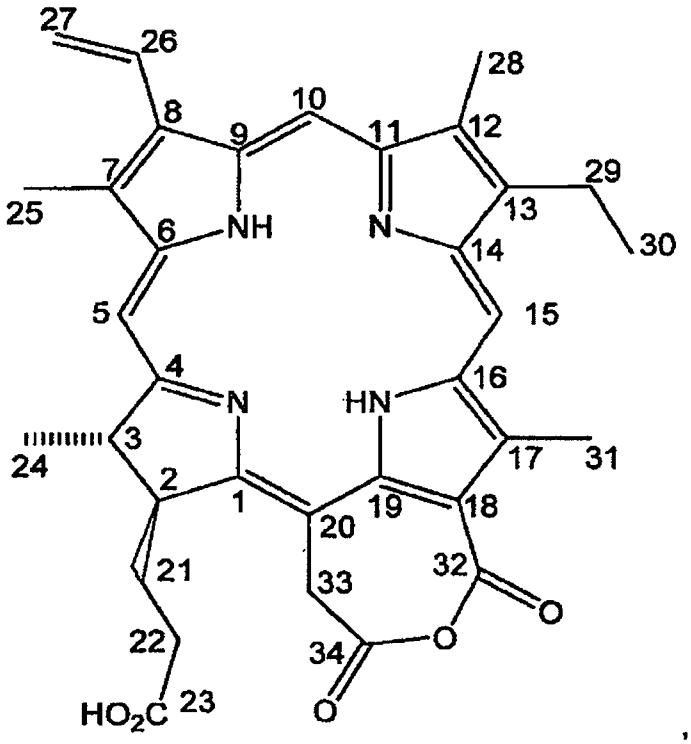
In another aspect, the present disclosure is directed to a method for preparing an intermediate. The method includes activating chlorin e_6 with a carboxyl activating agent to obtain a mixture including an intermediate, the intermediate exhibiting a spectrum comprising chemical shifts in ppm at about 1.63 (t, 3H), 1.72/2.05 (m, 2H), 1.78 (d, 3H), 2.50/2.65 (m, 2H), 3.14 (s, 3H), 3.42 (s, 3H), 3.68 (br. q, 2H), 3.69 (s, 3H), 4.63 (br. q, 1H), 4.67 (br. d, 1H), 5.59/5.56 (d, 2H), 6.37/6.16 (d, 1H), 8.07 (dd, 1H), 8.86 (s, 1H), 9.35 (s, 1H), and 9.67 (s, 1H) when analyzed using proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy, at 500 MHz, using a solvent comprising d_6-acetone and d_6-dimethyl sulfoxide. The method further includes isolating the intermediate.

In another aspect, the present disclosure is directed to a method for preparing Talaporfin Sodium, or a pharmaceutically acceptable salt thereof. The method includes combining an aspartate salt composition having a pH ranging from about 10 to about 12 with a chemical reaction product according to claim 1 in the presence of an organic solvent to form a reaction mixture. The

method may further include rendering the reaction mixture basic. The method may further include precipitating the Talaporfin, or a pharmaceutically acceptable salt thereof, from the basic reaction mixture.

In another aspect, the present disclosure is directed to a method of preparing Talaporfin Sodium. The method includes utilizing a purified intermediate reaction mixture in a reaction to prepare Talaporfin Sodium, the purified intermediate reaction mixture having been obtained from a crude reaction mixture comprising chlorin e_6, a chlorin e_6 anhydride, and precursors of diaspartyl chlorin e_6 from which a significant proportion of the precursors of diaspartyl chlorin e_6 has been removed.

In another aspect, the present disclosure is directed to a reaction product. comprising a product of a coupling reaction between a first reaction volume and a second reaction volume, the first reaction volume comprising a chlorin e_6 anhydride of the formula

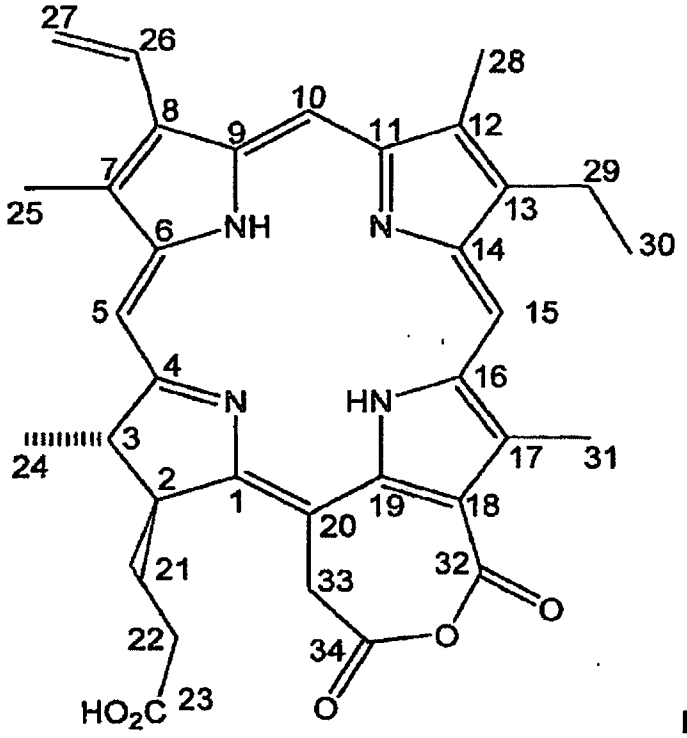


, and

less than about 5 mol% precursors of diaspartyl chlorin e_6 based on total chlorin species within the reaction product; and the second reaction volume

comprising L-aspartic acid or ester thereof. In some embodiments, the reaction product comprises mono-L-aspartyl chlorin e_6, or a pharmaceutically acceptable salt thereof, and diaspartyl chlorin e_6. Examples of chlorin species include chlorin e_6, a chlorin e_6 anhydride, and precursors of diaspartyl chlorin e_6, and the like, as well as compounds comprising a chlorin chromophore.

In another aspect, the present disclosure is directed to a process for preparing mono-L-aspartyl chlorin e_6, or a pharmaceutically acceptable salt thereof. The process includes combining chlorin e_6 with a carboxyl activating agent to obtain a mixture comprising a Formula I intermediate

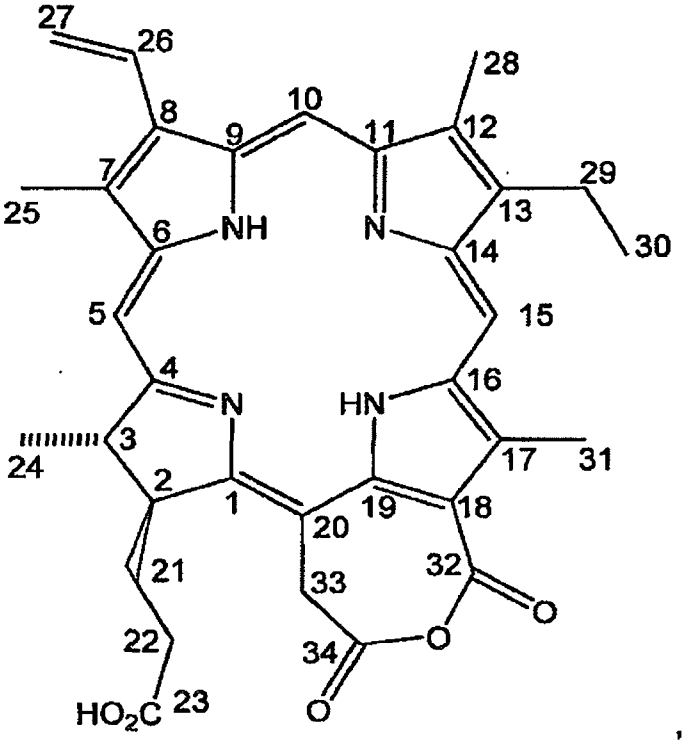


Formula I,

or a salt thereof. The process further includes isolating the Formula I intermediate, or salt thereof. In some embodiments, the process further includes combining the isolated Formula I intermediate, or salt thereof, with an amine-containing reagent to form Talaporfin Sodium, or a pharmaceutically acceptable salt thereof.

In another aspect, the present disclosure is directed to a process for preparing a photoactive agent, or a pharmaceutically acceptable salt

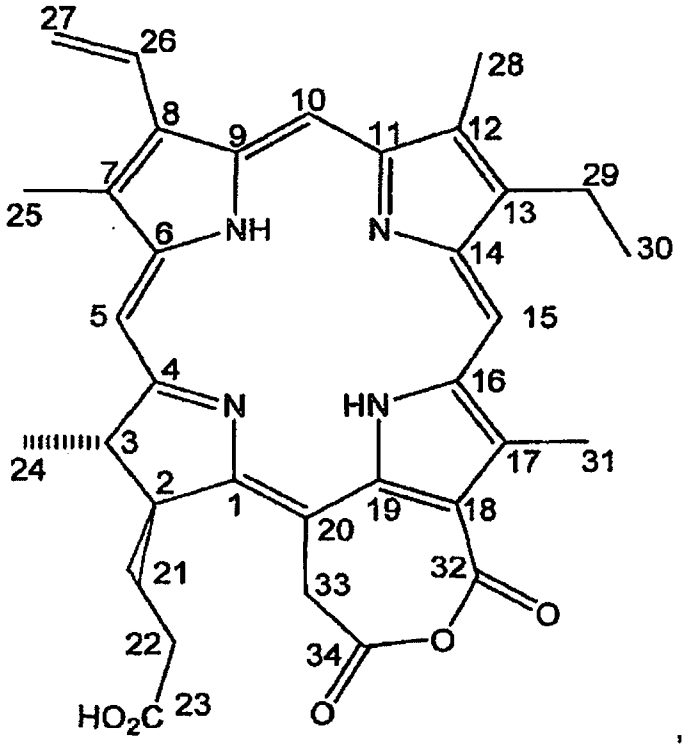
thereof. The process includes combining a composition comprising a compound of the formula



, or

a pharmaceutically acceptable salt thereof, an organic solvent, and one or more of diaspartyl chlorin e_6 precursors, with a basic aqueous sodium aspartate composition to form a crude mono-L-aspartyl chlorin e_6 reaction mixture. In some embodiments, the crude reaction mixture comprises less than about 2 mol% diaspartyl chlorin e_6 based on the total chlorin species present within the crude reaction mixture. The process further includes making the reaction mixture basic. In some embodiments, the process further includes precipitating a substantial amount of the mono-L-aspartyl chlorin e_6 from the reaction mixture.

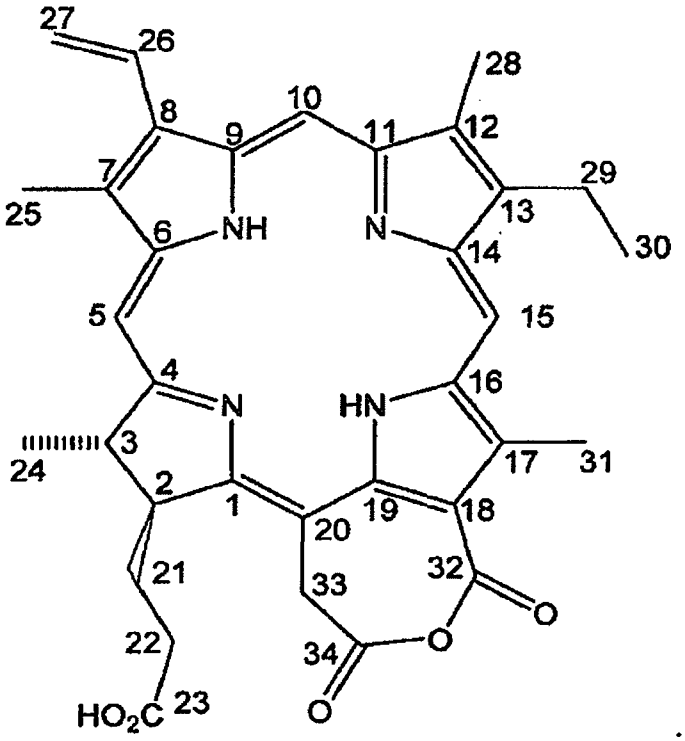
In another aspect, the present disclosure is directed to a chemical reaction product of chlorin e_6 and a dehydration agent, the chemical reaction product comprising a chlorin e_6 anhydride of the form:



, and

precursors of diaspartyl chlorin e_6. In some embodiments, the precursors of diaspartyl chlorin e_6 comprise less than about 5 mol% based on the total chlorin species present within the chemical reaction product.

In another aspect, the present disclosure is directed to a mixture that includes a reaction product of chlorin e_6. The mixture includes the previously unknown chlorin e_6 anhydride, and/or derivatives thereof. The chlorin e_6 anhydride is of the formula:



The mixture has a significantly reduced concentration of precursors of diaspartyl chlorin e_6 and other unwanted species, typically less than 5% based on total chlorin species present.

The mixture may be a purified reaction product of an activation reaction of chlorin e_6 with a carbodiimide. The carbodiimide may be N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) or N,N'-dicyclohexyl-carbodiimide (DCC).

In some embodiments, the chlorin e_6 anhydride of the above formula may be in a mixture that has up to about 5% of precursors of diaspartyl chlorin e_6 based on the total chlorin species in the reaction mixture. In another, the chlorin e_6 anhydride might be in a mixture that contains up to 3% diaspartyl chlorin e_6 precursors based on the total chlorin species in the mixture.

In another aspect, the present disclosure is directed to a method of preparing Talaporfin Sodium that includes the steps of: preparing an intermediate reaction mixture including chlorin e_6, the anhydride thereof, and precursors of diaspartyl chlorin e_6; purifying the mixture by removing a

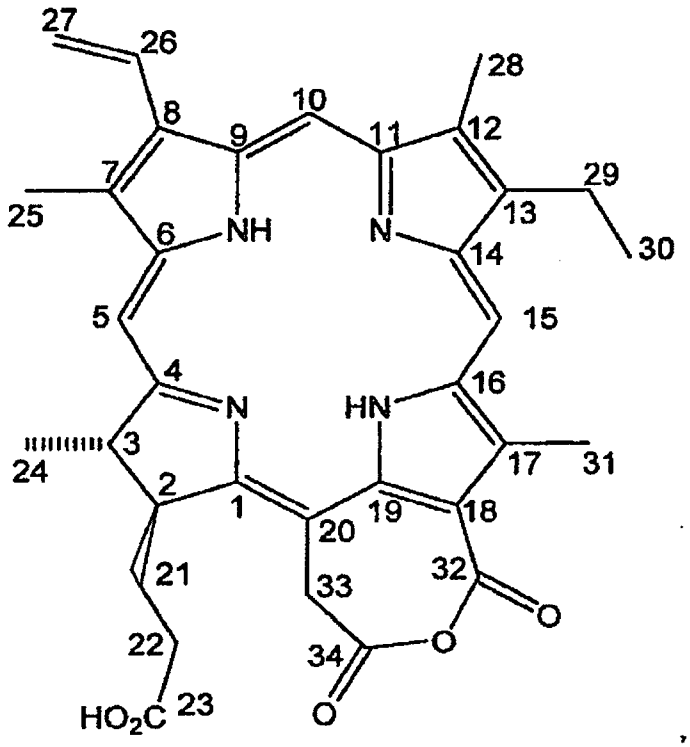
significant proportion of the precursors of diaspartyl chlorin e_6 from the mixture; and utilizing the purified mixture in a reaction to prepare Talaporfin Sodium.

In some embodiments, the intermediate reaction mixture may include dimethyl sulfoxide. In some embodiments, the intermediate reaction mixture may include (EDC) and dimethyl formamide (DMF). The purifying step may include filtering through a bed of activated silica.

The purifying step may also include adding agents designed to suppress the formation of precursors to di-L-aspartyl chlorin e_6 and other unwanted species. The purifying step may include removal of diaspartyl chlorin e_6 precursors to a concentration of less than about 5% or less than about 3% based on the total chlorin species present in the intermediate reaction mixture.

The utilizing step may include coupling at high pH in dimethyl sulfoxide with L-aspartic acid or a derivative or ester thereof. The utilizing may also include coupling with L-aspartic acid or a derivative or ester thereof in an aqueous alkaline solution.

In another aspect, the present disclosure is directed to a reaction product that includes: the product of a coupling reaction between a first reaction volume comprising less than about 5% (based on total chlorin species present) of precursors of diaspartyl chlorin e6, the first reaction volume comprising a chlorin e_6 anhydride of the formula:



and a second reactant comprising L-aspartic acid, or a derivative or ester thereof.

The reaction product may include up to about 1% diaspartyl chlorin e_6 based on total chlorin species present. The first reaction volume may include dimethyl sulfoxide. The first reaction volume may be purified to remove precursors of diaspartyl chlorin e_6 and other unwanted reaction products by a suitable separation process, such as filtration through a bed of activated silica, and the like.

In yet another aspect, the present disclosure is directed uses of a purified chlorin e_6 anhydride as an intermediate in the preparation of compounds comprising chlorin e_6 linked via a peptide bond to other chemical moieties.

BRIEF DESCRIPTION OF THE DRAWINGS

A more complete understanding of the present disclosure may be obtained by referring to the detailed description and claims when considered in

conjunction with the following Figures, wherein like reference numbers refer to similar elements throughout the Figures.

Figure 1 is schematic depiction of a two stage process (activation followed by coupling) for synthesizing Talaporfin Sodium from chlorin e_6 according to one illustrative embodiment.

Figure 2A is a relative intensity versus mass-to-charge ratio (m/z) mass spectrometry analysis plot of a purified chlorin e_6 anhydride composition according to one illustrative embodiment.

Figure 2B is an absorbance (AU) at 410 nm versus time (min) HPLC (High Pressure Liquid Chromatography) plot of a chlorin e_6 anhydride composition according to one illustrative embodiment. The peaks labeled as 596.8 and 578.8 are chlorin e_6 and chlorin e_6 anhydride respectively.

Figure 3 is a schematic depiction of a structural formula of one isomer of di-L-aspartyl chlorin e_6 according to one illustrative embodiment.

Figure 4 is a schematic depiction of isolated chlorin e_6 anhydride conversion to Talaporfin Sodium according to one illustrative embodiment.

Figure 5 is a relative intensity versus mass-to-charge ratio (m/z) mass spectrometry analysis plot of a reaction mixture resulting from an activation reaction, after treatment with activated silica, illustrating that the predominant species in the mixture is the chlorin e_6 anhydride according to one illustrative embodiment.

Figure 6 is an absorbance (AU) at 410 nm versus time (min) HPLC plot using UV detection indicating the relative percentage of chlorin species present in a purified reaction product according to one illustrative embodiment.

Figure 7 is a proton nuclear magnetic resonance (^{1}H-NMR) spectrum of chlorin e_6 anhydride in d_6-acetone/d_6-dimethyl sulfoxide, taken at 500 MHz according to one illustrative embodiment.

Figure 8 is comparison of a select region of an ^{1}H-NMR spectrum of chlorin e_6 anhydride in d_6-acetone/d_6-dimethyl sulfoxide, and a select region

of an ^{1}H-NMR spectrum of chlorin e_6 in d_6-acetone/d_6-dimethyl sulfoxide, taken at 500 MHz according to one illustrative embodiment.

DETAILED DESCRIPTION

In the following description, certain specific details are included to provide a thorough understanding of various disclosed embodiments. One skilled in the relevant art, however, will recognize that embodiments may be practiced without one or more of these specific details, or with other methods, components, materials, etc. In other instances, well-known structures associated with extraction, separation, chromatography, and/or purification systems including but not limited to separation columns, filtration beds, and the like, have not been shown or described in detail to avoid unnecessarily obscuring descriptions of the embodiments.

Unless the context requires otherwise, throughout the specification and claims which follow, the word "comprise" and variations thereof, such as, "comprises" and "comprising" are to be construed in an open, inclusive sense, that is as "including, but not limited to."

Reference throughout this specification to "one embodiment," or "an embodiment," or "in another embodiment," or "in some embodiments" means that a particular referent feature, structure, or characteristic described in connection with the embodiment is included in at least one embodiment. Thus, the appearance of the phrases "in one embodiment," or "in an embodiment," or "in another embodiment," or "in some embodiments" in various places throughout this specification are not necessarily all referring to the same embodiment. Furthermore, the particular features, structures, or characteristics may be combined in any suitable manner in one or more embodiments.

It should be noted that, as used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to a chemical reaction of a chlorin e_6 anhydride intermediate with an amine-containing reagent includes a single amine-containing reagent, or two or more

amine-containing reagents. It should also be noted that the term "or" is generally employed in its sense including "and/or" unless the content clearly dictates otherwise.

The following detailed description is merely illustrative in nature and is not intended to limit the disclosure or the application and uses of the disclosure. Furthermore, there is no intention to be bound by any expressed or implied theory presented in the preceding technical field, background, brief summary or the following detailed description.

As used herein and in the claims, the term "dehydration agent" or "carboxyl activating agent" generally refers to a compound, molcule, or substance, capable of activating carboxylic acids with respect to nucleophilic attack. In some embodiments, the dehydration agents or carboxyl activating agents are capable of activating carboxylic acids where the attacking nucleophile is an amine or alcohol, resulting in amide or ester formation.

Non-limiting examples of such dehydration agents include carbodiimides (*e.g.*, N,N'-dicyclohexylcarbodiimide (DCC), N,N'-diisopropylcarbodiimide, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride, and the like), cyanamides.

Further examples of dehydrating reagents include alkyl chloroformates (see *e.g.*, *Kim, Lee*, Kim J. Org. Chem. 1985, 50, 560), which are generally used with a tertiary amine like triethyl amine, diethyl azodicarboxylate (DEAD) with triphenylphosphine (see *e.g.*,*Camp; Jenkins* J. Org. Chem. 1989, 54, 3045, 3049 (the Mitsunobu Reaction)), various chlorosilanes (see *e.g.*, Nakao; Oka; Fukumoto Bull. Chem. Soc. Jpn. 1981, 54, 1267; see als *e.g.*, Brook; Chan, Synthesis 1983, 201), chlorosulfonyl isocyanate (see *e.g.*, Dhar, Murthy Synthesis 1988, 437-450), and N, N'-carbonyldiimidazole (see *e.g.*, Morton, Mangroo, Gerber Can. J. Chem. 1988, 66, 1701). In some embodiments, the dehydration agent is a carbodiimide. In some embodiments, the carbodiimide is N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide hydrochloride or N,N'-dicyclohexyl-carbodiimide.

In some embodiments, dehydration of the reactants may comprise a reaction of carboxylic acids with amines, whether or not they are part of amino acids. In some embodiments, more general ways of dehydrating the reactants in order to obtain the desired coupling reaction may be employed. (see *e.g.*, Beckwith's monograph, pp. 73-185, in The Chemistry of Amides). For example, one general method is the reaction of an amine with an acid chloride. In some embodiments, the reactants are treated with chloroformates with primary amines, because this can be reversed later if the amine is needed for some purpose (see *e.g.*, Zabicky, A.A., Ed.; Wiley: New York, 1970; Raucher; Jones Synth. Commun. 1985, 15, 1025.). As used herein and in the claims, the term "amine-containing agent" generally refers to a compound, molecule, or substance that contains a primary or secondary amine group, or ammonia. Non-limiting examples of such amine-containing agents include amino acids (*e.g.*, L-aspartic acid), aspartic acid, and the like, or esters or derivatives thereof.

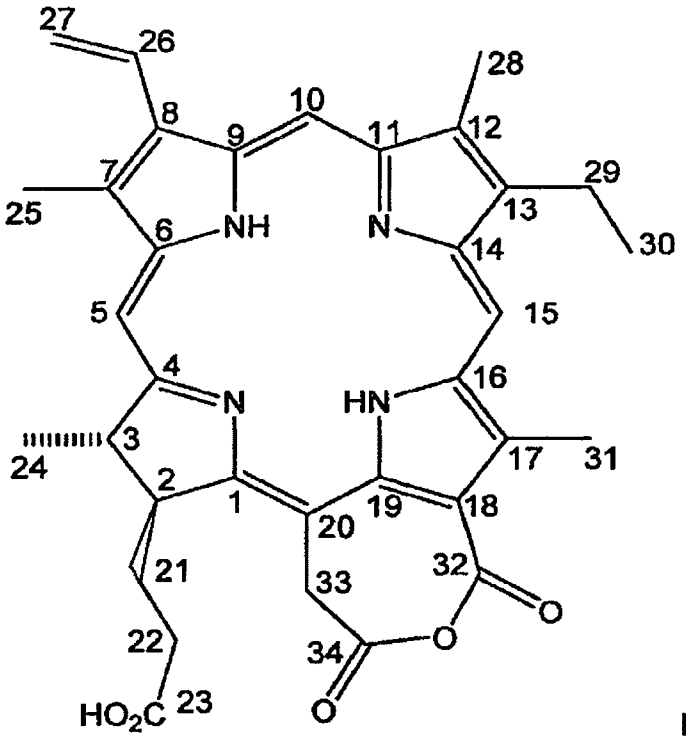
Talaporfin Sodium is commonly known as Mono-L-aspartyl-chlorin e_6, NPe_6, MACE, Taporfin, ME2906, Ace_6, AC_6 and LS11. Its Chemical Abstract Services Registry Number is 220201-34-3, and its chemical name is (+)-Tetrasodium (2*S*,3*S*)-18-carboxylato-20-[N-(*S*)-1,2-dicarboxylatoethyl]-carbamoylmethyl-13-ethyl-3,7,12,17-tetramethyl-8-vinylchlorin-2-propanoate.

Talaporfin Sodium is a photosensitizing agent typically used in light activated drug therapy. After light activation, Talaporfin Sodium forms an extended high energy electronic state that facilitates the formation of singlet oxygen, resulting in singlet oxygen-mediated cell death. In some embodiments, when interacting with cancer cells and exposed to light, the Talaporfin Sodium may kill or destroy the cancer cells.

As shown in Figure 1, producing Talaporfin, or a pharmaceutically acceptable salt thereof, from chlorin e_6 generally requires a two stage process. In some embodiments, in a first stage, chlorin e_6 is activated by dehydration agent, and in a second stage, an activation mixture reacts with an amine-containing agent to form Talaporfin Sodium.

Previously the reaction products of the activation step were thought to be unstable, high-energy, short-lived, species, and hence cannot be isolated or purified. Applicants have identified, however, a significant component of this reaction as the chlorin e_6 anhydride and unexpectedly found that this intermediate is a stable species. In some embodiments, when isolated or purified, the intermediate reacts with L-aspartate to form Talaporfin Sodium that is purer than the case if the isolation or purification of the chlorin e_6 anhydride had not taken place.

In some embodiments, the process for preparing Talaporfin, or a pharmaceutically acceptable salt thereof, may include combining chlorin e_6 with a carboxyl activating agent to obtain an activation reaction mixture comprising a Formula I intermediate



Formula I, or a salt thereof.

The process may further include isolating the Formula I intermediate, or salt thereof, from a reaction mixture using one or more separation. Suitable techniques and/or methods for extracting, isolating, separating, and/or purifying the Formula I intermediate, or salt thereof, from the

mixture include extraction, separation, chromatography, and/or purification techniques such as liquid chromatography (*e.g.*, HPLC), column chromatography, thin layer chromatography, planar chromatography, and the like. In some embodiments, the mixture may be passed through an activated silica bed, and the resultant silica-treated reaction product analyzed by mass spectrometry. *Figure 5 shows a mass spectrum for silica-treated reaction* product following an activation reaction of chlorin e_6 by a dehydration agent. The spectrum was recorded on an Agilent 1100 series LC/MSD instrument using electrospray ionization and with polarity setting on positive.

As shown in Figure 5, the peak for the chlorin e_6 anhydride is now proportionately much higher than those for the reaction byproducts. The most intense peak is that of the chlorin e_6 anhydride.

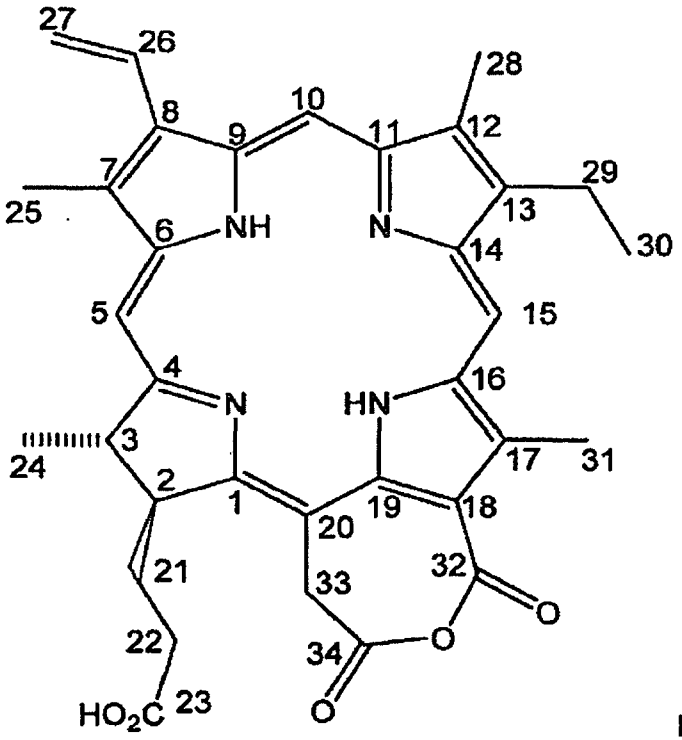
When the silica-treated or otherwise purified activation reaction product, now containing a higher chlorin e_6 anhydride concentration, was allowed to react with L-aspartic acid in aqueous alkaline (sodium hydroxide was used to create alkaline conditions) solution a much purer Talaporfin Sodium-containing reaction product was produced, as compared to the prior art. The HPLC analysis of the coupling reaction product is shown in Figure 6. It shows that about 95% of the coupling reaction product (based on total chlorin species present), is Talaporfin Sodium with only about 5% other reaction products. This coupling reaction product is much simpler and less costly to purify further to 96% Talaporfin Sodium than the reaction product of the prior art that had a much lower proportion of Talaporfin Sodium. In some embodiments, the resulting Talaporfin Sodium comprises a purity greater than from about 90% to about 99% or greater.

The purification of the coupling reaction product requires a series of treatments with HPLC to remove impurities. Consequently, in some embodiments, starting the purification process with a reaction product that has a higher Talaporfin Sodium concentration (and reduced concentrations of other (unwanted) activation reaction products, such as precursors of diaspartyl chlorin e_6) also offers the potential for eliminating the need for HPLC purification

processes. Accordingly, some embodiments offer the potential for higher Talaporfin Sodium yields, as well as higher Talaporfin Sodium purity.

In some embodiments, the activation reaction mixture produced from the activation reaction is first purified to produce a purified activation reaction mixture that has an enhanced concentration of the chlorin e_6 anhydride, and reduced concentrations of other (unwanted) activation reaction products, such as precursors of diaspartyl chlorin e_6 (a drawing of one isomer of diaspartyl chlorin e_6 is shown in Figure 3). Di-L-aspartyl chlorin e_6 typically comprises 3-4% of the total product mixture. Generally, diaspartyl chlorin e_6 is removed by multiple stages in series of reverse phase HPLC steps. The HPLC process employed, however, is both expensive and time consuming. Moreover, multiple stage HPLC separation results in loss of Talaporfin Sodium product, thereby reducing product yield by as much as 50%.

It has now been found that there is an "activated intermediate" heretofore unsuspected in the activation mixture, having the following Formula I structure:



Formula I.

This Formula I chlorin e_6 anhydride may be referred to as "chlorin e_6 cyclic anhydride," or "anhydride." When this chlorin e_6 anhydride, purified from the activation mixture, is allowed to react in the coupling reaction with L-aspartyl chlorin e_6, as shown in Figure 4, the production of diaspartyl chlorin e_6 is dramatically reduced.

In some embodiments, the process may further include combining the isolated Formula I intermediate, or salt thereof, with an amine-containing reagent to form Talaporfin Sodium, or a pharmaceutically acceptable salt thereof. In some embodiments, combining chlorin e_6 with the carboxyl activating agent comprises reacting chlorin e_6 with a carbodiimide. In some embodiments, the carbodiimide is DCC or EDC.

In some embodiments, combining chlorin e_6 with the carboxyl activating agent comprises combining the chlorin e_6 with a carbodiimide in the presence of dimethyl sulfoxide or dimethyl formamide. In some embodiments, combining the chlorin e_6 with the carboxyl activating agent comprises combining the chlorin e_6 with EDC or DCC.

In some embodiments, isolating the Formula I intermediate, or salt thereof, comprises separating the Formula I intermediate, or salt thereof, from the mixture by using activated silica to yield a substantially pure Formula I intermediate, or salt thereof.

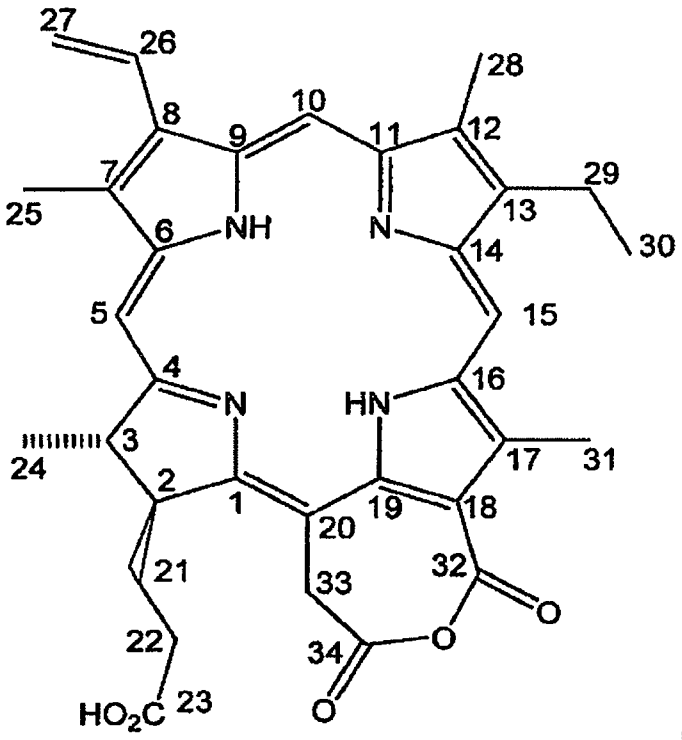
In some embodiments, combining the isolated Formula I intermediate, or salt thereof, with an amine-containing reagent comprises combining the isolated Formula I intermediate, or salt thereof, with sodium aspartate to form Talaporfin Sodium.

In some embodiments, combining the isolated Formula I intermediate, or salt thereof, with an amine-containing reagent comprises combining the isolated Formula I intermediate, or salt thereof, in the presence of dimethyl sulfoxide, with L-aspartic acid or ester thereof to form Talaporfin Sodium.

In some embodiments, combining the isolated Formula I intermediate, or salt thereof, with an amine-containing reagent comprises

coupling the chlorin e_6 anhydride to L-aspartic acid or ester thereof in an aqueous alkaline solution to form a tetra-sodium salt of mono-L-aspartyl chlorin e_6.

Certain embodiments of the present disclosure are directed to a process for preparing a chlorin e_6 anhydride, or a pharmaceutically acceptable salt thereof. The process includes combining chlorin e_6 with a carboxyl activating agent to obtain a mixture comprising a chlorin e_6 anhydride having the following formula



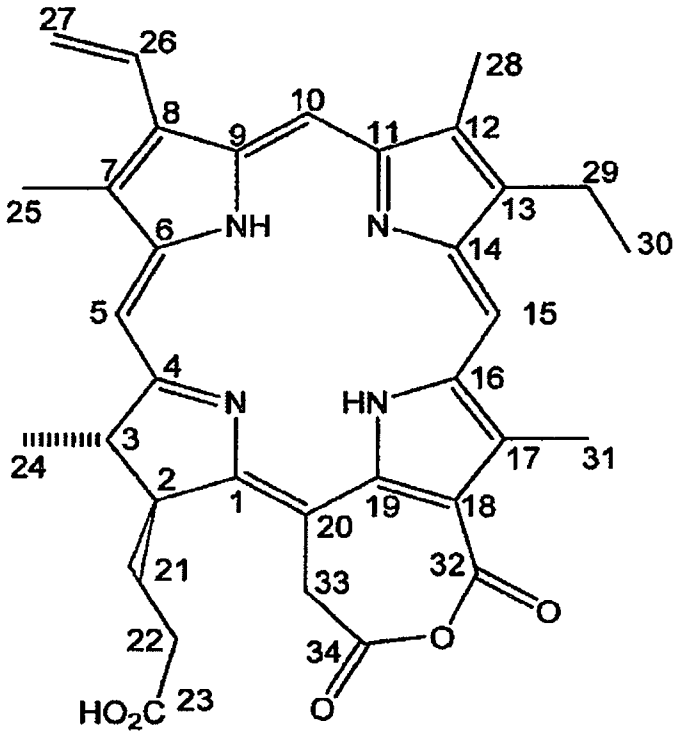
, or a salt thereof.

In some embodiments, the carboxyl activating agent is DCC or EDC.

The process may further includes purifying the chlorin e_6 anhydride, or salt thereof. In some embodiments, purifying the chlorin e_6 anhydride, or salt thereof, comprises isolating the chlorin e_6 anhydride, or salt thereof, from the mixture using one or more extraction, separation, chromatography, and/or purification techniques.

Figure 7 shown an ^1H NMR spectrum of chlorin e_6 anhydride in acetone d_6 according to one illustrative embodiment. Referring to TABLE 1, the

position assignments are numbered according to the corresponding atoms as indicated by the following Formula I chlorin e_6 anhydride



Formula I

Table 1: ^{1}H NMR Peak Assignments

Position Assignment	δ (ppm) Chemical Shift (ppm)	M Multiplicity	J (Hz) J (Hz)
2	4.67	br d	10.7/2.0
3	4.63	br q	7.3
5	9.35	s	
10	8.86	s	
15	9.67	s	
21	1.72/2.05	m	
22	2.50/2.65	m	
24	1.78	d	7.3
25	3.42	s	

Position Assignment	δ (ppm) Chemical Shift (ppm)	M Multiplicity	J (Hz) J (Hz)
26	8.07	dd	17.9/11.5
27	6.37/6.16	d	17.9/11.5
28	3.14	s	
29	3.68	br q	7.7
30	1.63	t	7.7
31	3.69	s	
33	5.59/5.56	d	13.7

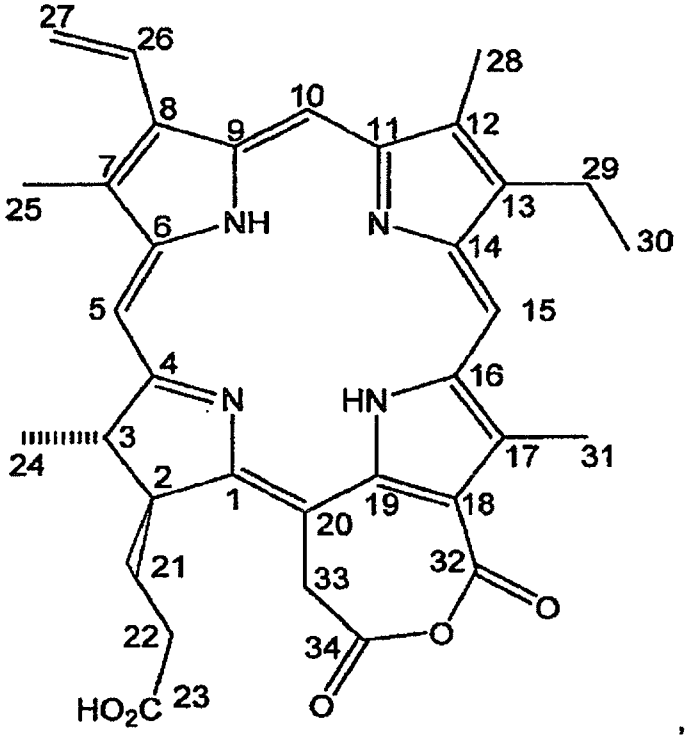
Figure 8 shows a 4-6 ppm expanded portion of the NMR spectrum in Figure 7, as well as a 4-6 ppm expanded portion of an NMR spectrum of chlorin e_6 taken under similar conditions. The plots evidence some of the structural difference between chlorin e_6 and the anhydride.

Certain embodiments of the present disclosure are directed to a method for preparing an intermediate. The method includes activating chlorin e_6 with a carboxyl activating agent to obtain a mixture including an intermediate, the intermediate exhibiting a spectrum comprising chemical shifts in ppm at about 1.63 (t, 3H), 1.72/2.05 (m, 2H), 1.78 (d, 3H), 2.50/2.65 (m, 2H), 3.14 (s, 3H), 3.42 (s, 3H), 3.68 (br. q, 2H), 3.69 (s, 3H), 4.63 (br. q, 1H), 4.67 (br. d, 1H), 5.59/5.56 (d, 2H), 6.37/6.16 (d, 1H), 8.07 (dd, 1H), 8.86 (s, 1H), 9.35 (s, 1H), and 9.67 (s, 1H) when analyzed using proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy, at 500 MHz, using d_6-acetone/d_6-dimethyl sulfoxide as a solvent.

The method further includes isolating the intermediate. In some embodiments, isolating the intermediate comprises separating the intermediate from the mixture using one or more extraction, separation, chromatography, and/or purification techniques. In some embodiments, the one or more extraction, separation, chromatography, and/or purification techniques includes contacting the mixture with activated silica.

The method may further include drying the isolated intermediate in the presence of a vacuum.

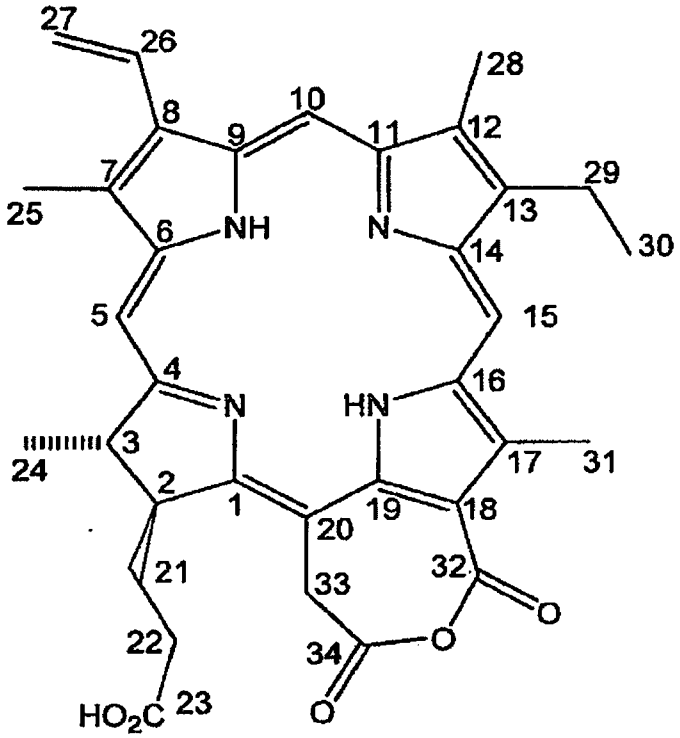
Certain embodiments of the present disclosure are directed to a method for preparing Talaporfin, or a salt thereof. The method includes combining an intermediate (prepared by combining chlorin e_6 with a carboxyl activating agent to obtain a mixture comprising a chlorin e_6 anhydride having the following formula



, or a salt thereof,

and purifying the chlorin e_6 anhydride, or salt thereof) with an amine-containing reagent to form a mono amino acid chlorin e_6, or a pharmaceutically acceptable salt thereof. In some embodiments, the amine-containing reagent is L-aspartic acid or an ester thereof.

Certain embodiments of the present disclosure are directed to a reaction product. The reaction comprises a product of a coupling reaction between a first reaction volume and a second reaction volume, the first reaction volume comprising a chlorin e_6 anhydride of the formula



, and

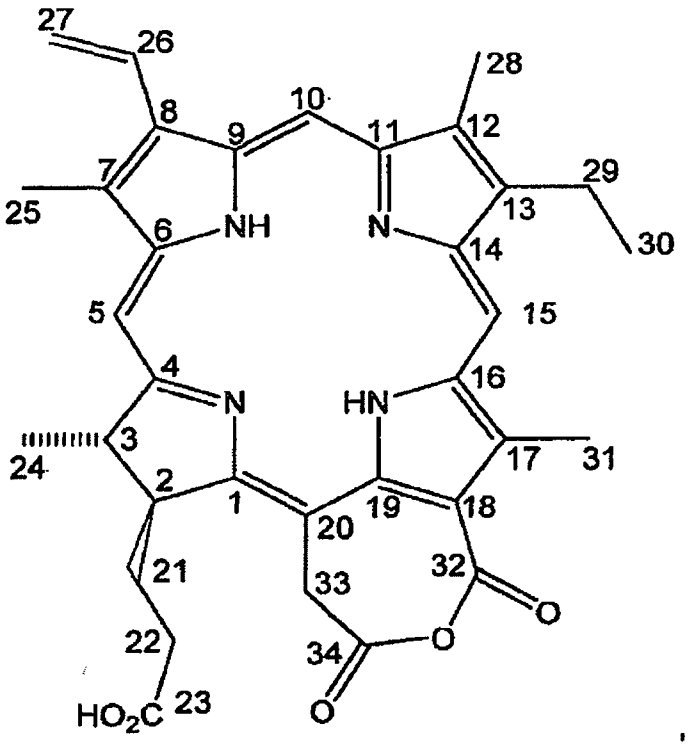
less than about 5 mol% precursors of diaspartyl chlorin e_6 based on total chlorin species present in the first reaction volume; and the second reaction volume comprising L-aspartic acid or ester thereof. In some embodiments, the units mol% and %AUC (area under the curve) are used interchangeably. %AUC is defined as percent area under curve and is determined by HPLC-UV absorbance analysis using a detection signal having wavelength of about 400 nm or about 410 nm. %AUC is calculated by dividing area under curve of the peak of interest by area under curve of all peaks in the chromatogram. In some embodiments, %AUC is expected to be proportional to the concentration of the chlorin species in the mixture as they share the same chromophore. Accordingly, by measuring the %AUC of the separated species in an HPLC chromatogram, it is possible to determine the relative concentration and/or mol% of species present in a reaction mixture. In some embodiments, the relative AUC for the respective HPLC plot peaks may be use to determine the relative concentration and/or mol% of species present in a reaction mixture.

In some embodiments, the reaction product comprises mono-L-aspartyl chlorin e_6, or a pharmaceutically acceptable salt thereof, and diaspartyl

chlorin e_6. In some embodiments, the reaction product comprises less than about 2 mol% diaspartyl chlorin e_6 based on total chlorin species present in the reaction product. In some embodiments, the reaction product comprises less than about 1 mol% diaspartyl chlorin e_6 based on total chlorin species present in the reaction product. In some embodiments, the first reaction volume further comprises dimethyl sulfoxide or dimethyl formamide.

The first reaction volume can be purified to remove precursors of diaspartyl chlorin e_6 by a separation process. In some embodiments, the separation process is activated silica chromatography. In some embodiments, the resulting activation mixture is combined with L-aspartic acid (as shown in Figure 4) in an alkaline aqueous solution to yield Talaporfin Sodium among other byproducts.

Certain embodiments of the present disclosure are directed to a chemical reaction product of chlorin e_6 and a carbodiimide, the chemical reaction product comprising a chlorin e_6 anhydride of the form:

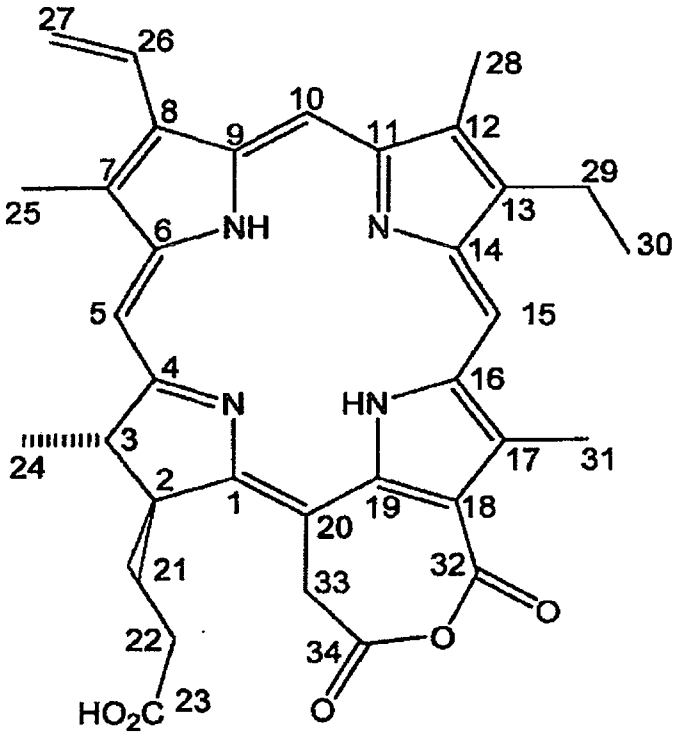


,and precursors of diaspartyl chlorin e_6.

In some embodiments, the precursors of diaspartyl chlorin e_6 comprise less than about 5 mol% of a total chlorin species present within the chemical reaction product. In some further embodiments, the precursors of diaspartyl chlorin e_6 comprise less than about 3 mol% of total chlorin species present within the chemical reaction product. In some embodiments, the chlorin e_6 anhydride comprises at least about 85 mol% of the total chlorin species present within the chemical reaction product.

In some embodiments, the carbodiimide is EDC or DCC.

Certain embodiments of the present disclosure are directed to a composition comprising a compound of Formula I



(Formula I),

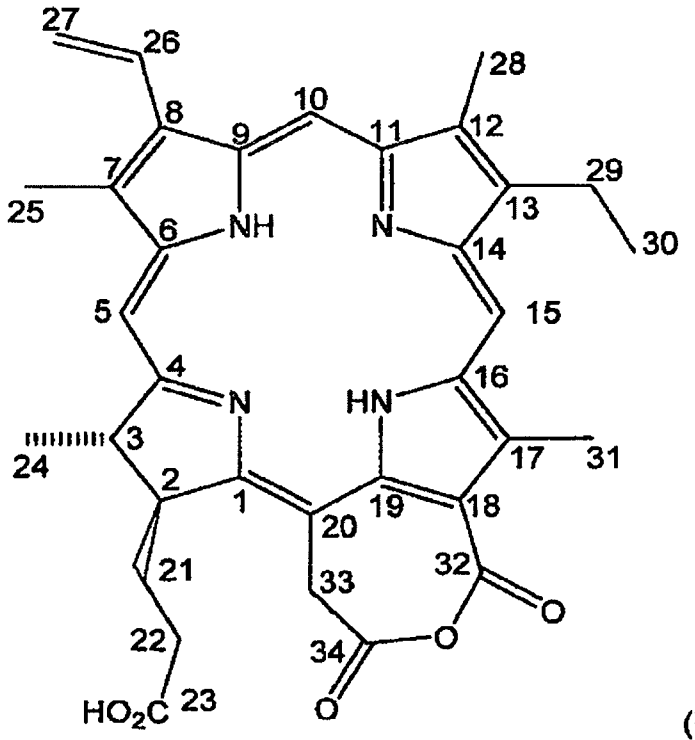
or a pharmaceutically acceptable salt thereof, in a substantially pure form.

In some embodiments, the total content of the Formula I compound, or a pharmaceutically acceptable salt thereof, is greater than about 85 mol% of total chlorin species within the composition. In some embodiments, the total content of the Formula I compound, or a pharmaceutically acceptable salt thereof, is greater than about 90 mol% of total chlorin species within the

composition. In some embodiments, the total content of the Formula I compound, or a pharmaceutically acceptable salt thereof, is greater than about 95 mol% of total chlorin species within the composition.

In some embodiments, the total content of the Formula I compound, or a pharmaceutically acceptable salt thereof, is greater than about 85 wt% of the composition. In some embodiments, the total content of the Formula I compound, or a pharmaceutically acceptable salt thereof, is greater than about 90 wt% of the composition. In some embodiments, the total content of the Formula I compound, or a pharmaceutically acceptable salt thereof, is greater than about 95 wt% of the composition.

Certain embodiments of the present disclosure are directed to a chemical reaction product comprising a compound of Formula I



(Formula I),

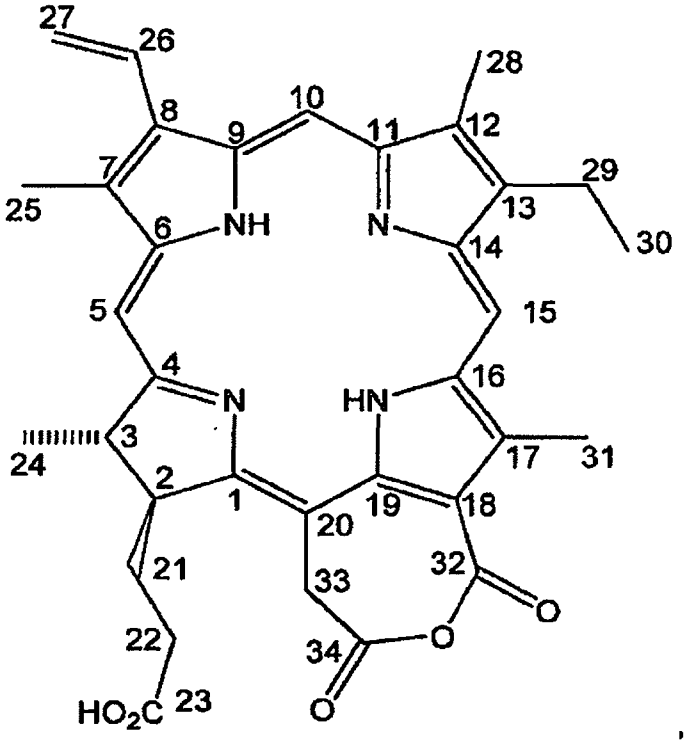
or a pharmaceutically acceptable salt thereof, in a substantially pure form.

In some embodiments, the total content of the Formula I compound is greater than about 85 mol% based on the total amount of chlorin species present within the chemical reaction product. In some embodiments,

the total content of the Formula I compound is greater than about 90 mol% based on the total amount of chlorin species present within the chemical reaction product. In some embodiments, the total content of the Formula I compound is greater than about 95 mol% based on the total amount of chlorin species present within the chemical reaction product.

Certain embodiments of the present disclosure are directed to a method for preparing Talaporfin Sodium, or a pharmaceutically acceptable salt thereof. The method includes combining an aspartate salt composition having a pH ranging from about 8 to about 12 with a chemical reaction product of chlorin e_6 and a carbodiimide the presence of an organic solvent to form a reaction mixture. In some embodiments, the organic solvent is selected from dimethyl formamide, dimethyl sulfoxide, tetrahydrofuran, ethyl acetate, methylene chloride, or mixtures thereof.

In some embodiments, the chemical reaction product comprises a chlorin e_6 anhydride of the form:



, and

precursors of diaspartyl chlorin e_6. In some embodiments, the precursors of diaspartyl chlorin e_6 comprise less than about 5 mol% based on the total amount of chlorin species present within the chemical reaction product.

The method may further include rendering the reaction mixture basic. In some embodiments, rendering the reaction mixture basic comprises adding hydroxide to maintain a pH of the reaction mixture at about 8 to about 12.

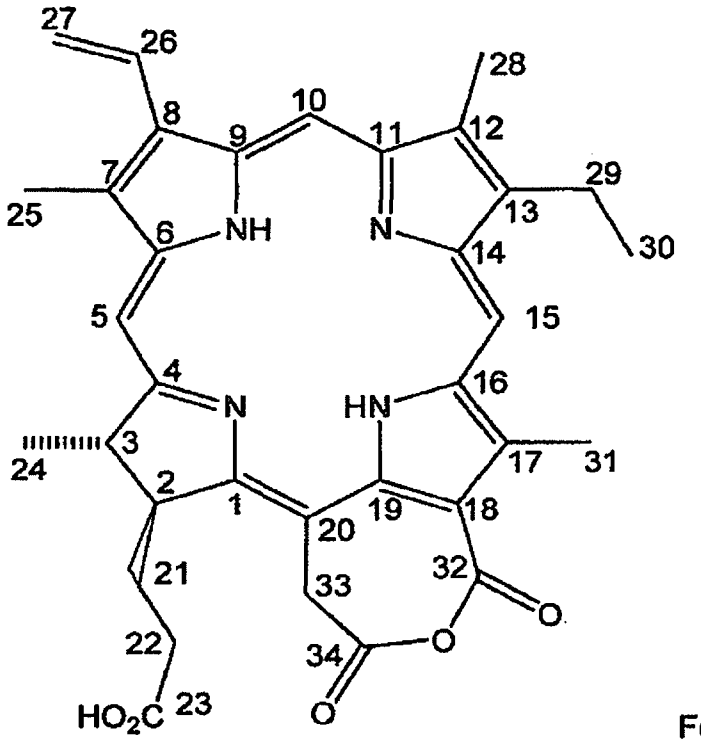
In some embodiments, the method may further include precipitating the Talaporfin, or a pharmaceutically acceptable salt thereof, from the basic reaction mixture. In some embodiments, precipitating the Talaporfin, or a pharmaceutically acceptable salt thereof, comprises adding a suitable precipitation solvent to the basic reaction mixture.

The method may further include separating the Talaporfin (or a pharmaceutically acceptable salt thereof) precipitate from the basic reaction mixture by filtration, centrifugation, and/or chromatography.

Certain embodiments of the present disclosure are directed to a method of preparing Talaporfin Sodium. The method includes utilizing a purified intermediate reaction mixture in a reaction to prepare Talaporfin Sodium, the purified intermediate reaction mixture having been obtained from a crude reaction mixture comprising chlorin e_6, a chlorin e_6 anhydride, and precursors of diaspartyl chlorin e_6 from which a significant proportion of the precursors of diaspartyl chlorin e_6 has been removed.

In some embodiments, the purified intermediate reaction mixture comprises less than about 5% precursors of diaspartyl chlorin e_6 based on total chlorin species. In some embodiments, the purified intermediate reaction mixture comprises less than about 3 mol% precursors of diaspartyl chlorin e_6 based on total chlorin species.

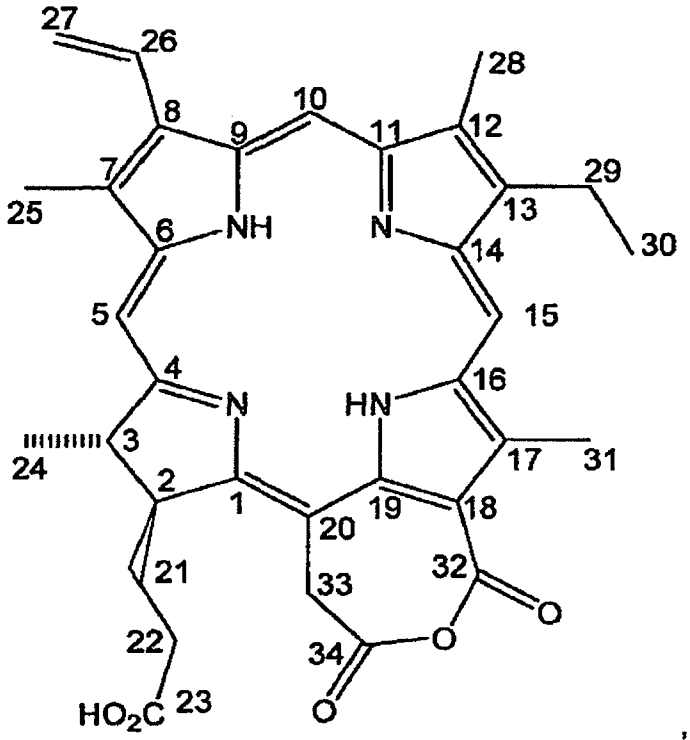
In some embodiments, the chlorin e_6 cyclic anhydride is a Formula I chlorin e_6 cyclic anhydride



Formula I, or salt thereof.

In some embodiments, the purified intermediate reaction mixture further comprises dimethyl sulfoxide. In some embodiments, the purified intermediate reaction mixture further comprises EDC or DCC and dimethyl formamide. In some embodiments, a significant proportion of the precursors of diaspartyl chlorin e_6 has been removed by using activated silica.

Certain embodiments of the present disclosure are directed to a process for preparing a photoactive agent, or a pharmaceutically acceptable salt thereof. The process include combining a composition comprising a compound of the formula



, or a pharmaceutically acceptable salt

thereof, an organic solvent, and one or more of diaspartyl chlorin e_6 precursors, with a basic aqueous sodium aspartate composition to form a crude mono-L-aspartyl chlorin e_6 reaction mixture, the crude reaction mixture comprising less than about 2 mol% diaspartyl chlorin e_6 based on total chlorin species present within the crude reaction mixture

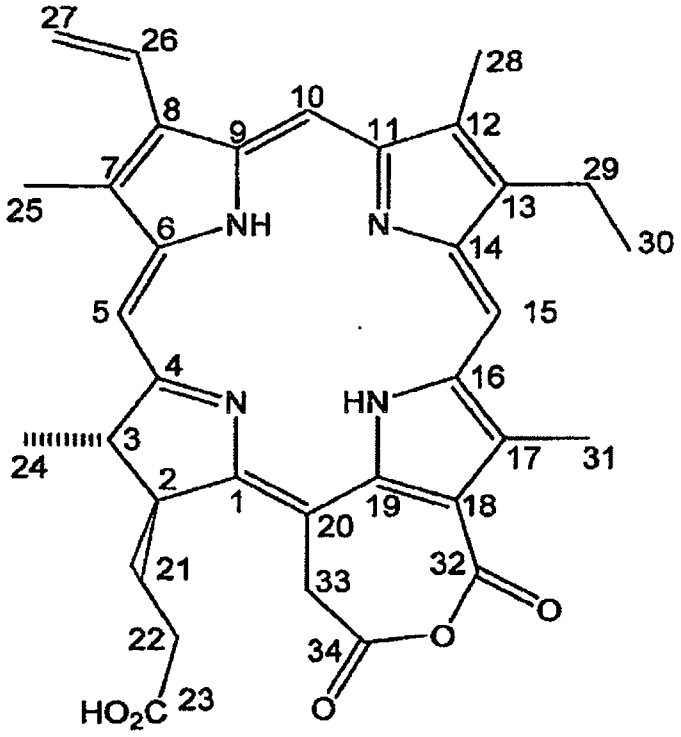
The process may further include making the reaction mixture basic. The process may further include precipitating a substantial amount of the mono-L-aspartyl chlorin e_6 from the reaction mixture. In some embodiments, precipitating a substantial amount of the mono-L-aspartyl chlorin e_6 from the reaction mixture comprises providing a sufficient amount of an organic solvent to cause the precipitation of more than about 50 mol% of the mono-L-aspartyl chlorin e_6 present from the reaction mixture.

In some embodiments, making the reaction mixture basic comprises providing a sufficient amount of an alkaline agent to the reaction mixture to maintain a pH range from about 8 to about 12. In some embodiments, making the reaction mixture basic comprises providing a

sufficient amount of an alkaline agent to the reaction mixture to maintain a pH range from about 10 to about 12.

In some embodiments, the at least one diaspartyl chlorin e_6 precursor comprises from less than about 1 mol% to about 2 mol% of the total chlorin species present in the reaction mixture.

Certain embodiments of the present disclosure are directed to a reaction product comprising a chlorin e_6 anhydride of the form:



and precursors of diaspartyl chlorin e_6; wherein the precursors of diaspartyl chlorin e_6 comprise less than about 1 to 2 mol% of the total chlorin species present.

EXAMPLE 1

CHLORIN E_6 ANHYDRIDE SYNTHESIS

Chlorin e_6 (2.0 g, 3.3 mmol) (Frontier Scientific) was dissolved in 10 mL DMF. EDC (0.61 g, 3.2 mmol) was added to the mixture and allowed to react for 1.5 h. The reaction mixture was loaded onto a bed of silica gel

(Sigma-Aldrich) and eluted with acetone. The colored portion was collected and concentrated *in vacuo*. The anhydride was further dried in the presence of a vacuum.

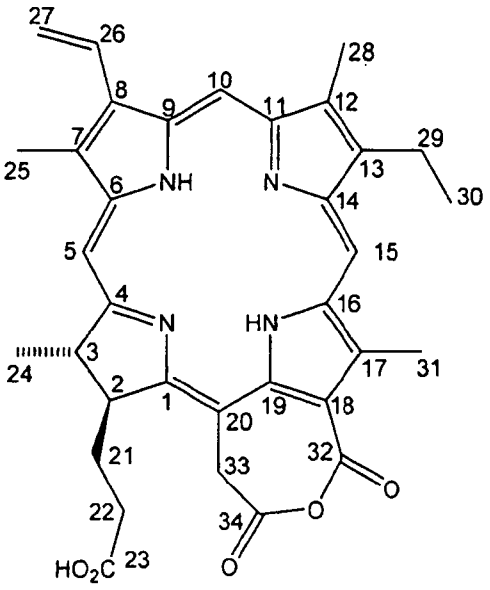
EXAMPLE 2
TALAPORFIN SODIUM SYNTHESIS

A sodium aspartate solution was prepared by adding 10 M NaOH to aspartic acid (~1.3 g, 10 mmol) in 10 mL water until pH 11 was attained. Chlorin e_6 anhydride from EXAMPLE 1 was dissolved in DMF and added slowly to the sodium aspartate solution. The mixture was made basic with 10M NaOH. Excess DMF was added to the dark suspension to precipitate the product. The product was collected by filtration and washed with acetone. The material was re-suspended in acetone and collected by filtration. The resultant crude Talaporfin Sodium was dried in the presence of a vacuum.

While at least one example embodiment has been presented in the foregoing detailed description, it should be appreciated that a vast number of variations exist. It should also be appreciated that the example embodiment or embodiments described herein are not intended to limit the scope, applicability, or configuration of the disclosure in any way. Rather, the foregoing detailed description will provide those skilled in the art with a convenient road map for implementing the described embodiment or embodiments. It should be understood that various changes can be made in the function and arrangement of elements without departing from the scope of the disclosure as set forth in the appended claims and the legal equivalents thereof.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A chemical reaction product of chlorin e_6 and a dehydration agent, the chemical reaction product comprising a chlorin e_6 anhydride of the form:



and precursors of diaspartyl chlorin e_6; wherein the precursors of diaspartyl chlorin e_6 comprise less than about 5 mol% based on the total amount of chlorin species present within the chemical reaction product.

2. The chemical reaction product of claim 1 wherein

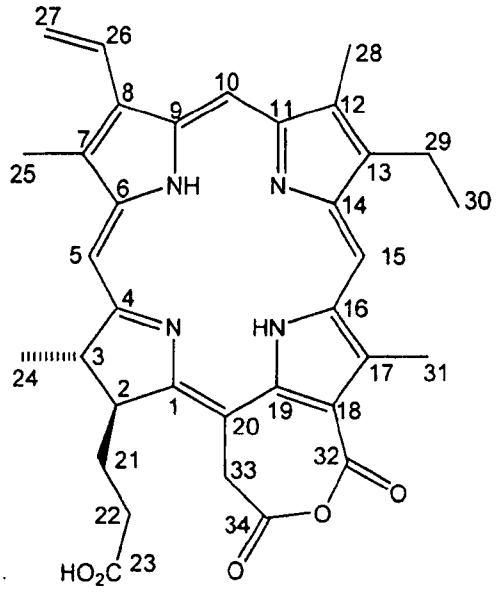
(1) the dehydration agent is a carbodiimide;

(2) the dehydration agent is a carbodiimide and the precursors of diaspartyl chlorin e_6 comprise less than about 3 mol% based on the total amount of chlorin species present within the chemical reaction product;

(3) the dehydration agent is a carbodiimide, and the chlorin e_6 anhydride comprises at least about 85 mol% of the chemical reaction product; or

(4) the dehydration agent is N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide hydrochloride or N,N'-dicyclohexyl-carbodiimide.

3. A composition, comprising a compound of Formula I



Formula I,

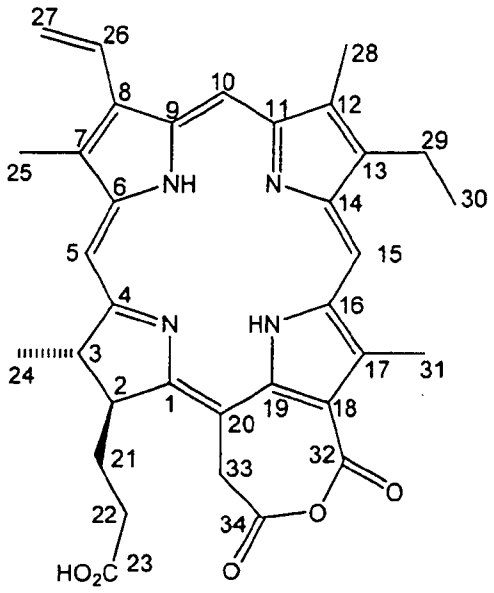
or a pharmaceutically acceptable salt thereof, in a substantially pure form.

4. The composition of claim 3 wherein

(1) a total content of the Formula I compound, or a pharmaceutically acceptable salt thereof, is greater than about 85 wt%, greater than about 90 wt%, or greater than about 95 wt% of the composition; or

(2) a total content of the Formula I compound is greater than about 85 mol%, greater than about 90 mol%, or greater than about 95 mole % based on the total amount of chlorin species present within the composition.

5. A process for preparing a chlorin e_6 anhydride, or a pharmaceutically acceptable salt thereof, comprising combining chlorin e_6 with a dehydration agent to obtain a mixture comprising a chlorin e_6 anhydride having the following formula (I)



(I)

or pharmaceutically acceptable salt thereof; and purifying the chlorin e_6 anhydride or pharmaceutically acceptable salt thereof.

6. The process of claim 5 wherein

(1) the dehydration agent is N,N'-dicyclohexyl-carbodiimide or N-ethyl-N'-(3 dimethylaminopropyl) carbodiimide hydrochloride; or

(2) purifying the chlorin e_6 anhydride, or salt thereof, comprises isolating the chlorin e_6 anhydride, or salt thereof, from the mixture using one or more extraction, separation, chromatography, and/or purification techniques.

7. A method for preparing an intermediate, comprising activating chlorin e_6 with a carboxyl activating agent to obtain a mixture including an intermediate, the intermediate exhibiting a spectrum comprising chemical shifts in ppm at about 1.63 (t, 3H), 1.72/2.05 (m, 2H), 1.78 (d, 3H), 2.50/2.65 (m, 2H), 3.14 (s, 3H), 3.42 (s, 3H), 3.68 (br. q, 2H), 3.69 (s, 3H), 4.63 (br. q, 1H), 4.67 (br. d, 1H), 5.59/5.56 (d, 2H), 6.37/6.16 (d, 1H), 8.07 (dd, 1H), 8.86 (s, 1H), 9.35 (s, 1H), and 9.67 (s, 1H) when analyzed using proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy, at 500 MHz, using d_6-acetone/d_6-dimethyl sulfoxide as a solvent; and isolating the intermediate.

8. The method of claim 7

(1) wherein isolating the intermediate comprises separating the intermediate from the mixture using one or more extraction, separation, chromatography, and/or purification techniques;

(2) wherein isolating the intermediate comprises separating the intermediate from the mixture using one or more extraction, separation, chromatography, and/or purification techniques, the one or more extraction, separation, chromatography, and/or purification techniques including contacting the mixture with activated silica; or

(3) further comprising drying the isolated intermediate in the presence of a vacuum.

9. A method for preparing mono-L-aspartyl chlorin e_6, the method comprising combining an intermediate prepared according to claim 7 with an amine-containing reagent, the amine-containing agent optionally being L-aspartic acid or an ester thereof, to form a mono amino acid chlorin e_6, or a pharmaceutically acceptable salt thereof.

10. A method for preparing mono-L-aspartyl chlorin e_6, or a pharmaceutically acceptable salt thereof, the method comprising:

combining an aspartate salt composition having a pH ranging from about 10 to about 12 with a chemical reaction product according to claim 1 in the presence of an organic solvent to form a reaction mixture;

making the reaction mixture basic; and

precipitating the mono-L-aspartyl chlorin e_6, or a pharmaceutically acceptable salt thereof, from the basic reaction mixture.

11. The method of claim 10,

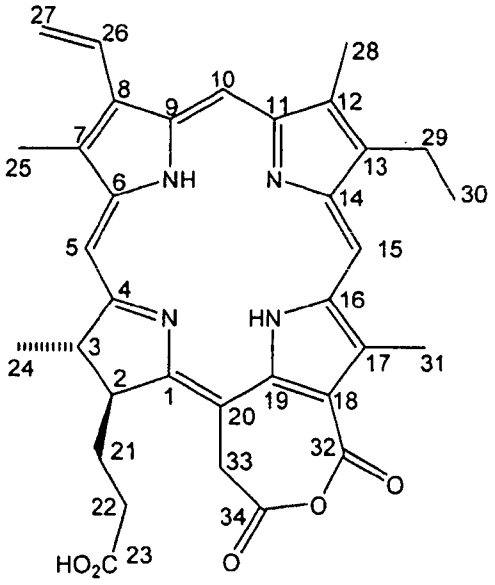
(1) wherein the organic solvent is dimethyl formamide, dimethyl sulfoxide, tetrahydrofuran, ethyl acetate, methylene chloride, or mixtures thereof;

(2) wherein making the reaction mixture basic comprises adding a hydroxide to maintain the pH of the reaction mixture within a range of about 8 to about 12;

(3) wherein precipitating the mono-L-aspartyl chlorin e_6, or a pharmaceutically acceptable salt thereof, comprises adding a suitable precipitation solvent to the basic reaction mixture; or

(4) further comprising separating the mono-L-aspartyl chlorin e_6, or a pharmaceutically acceptable salt thereof, precipitate from the basic reaction mixture by filtration and/or chromatography.

12. A method of preparing Talaporfin Sodium, comprising utilizing a purified intermediate reaction mixture in a reaction to prepare Talaporfin Sodium, the purified intermediate reaction mixture having been obtained from a crude reaction mixture comprising chlorin e_6, chlorin e_6 anhydride of Formula I:



(I)

or a salt thereof, and less than about 5 mol% precursors of diaspartyl chlorin e_6 based on the total amount of chlorin species present within the purified intermediate reaction mixture.

13. The method of claim 12 wherein

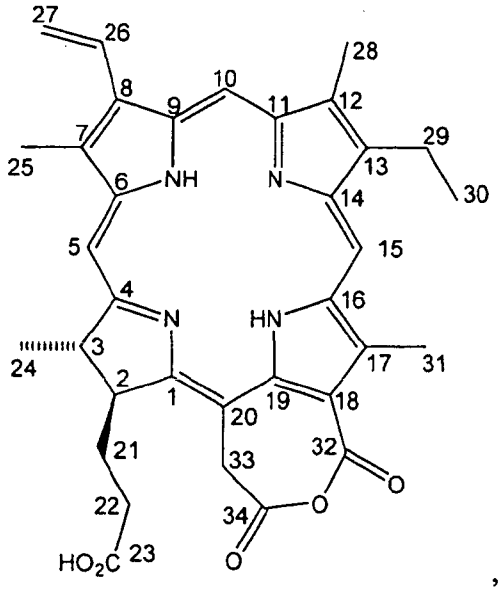
(1) the purified intermediate reaction mixture comprises less than about 3 mol% precursors of diaspartyl chlorin e_6 based on the total chlorin species present within the purified intermediate reaction mixture;

(2) the purified intermediate reaction mixture further comprises dimethyl sulfoxide;

(3) the purified intermediate reaction mixture further comprises N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide hydrochloride, N,N'-dicyclohexyl-carbodiimide, or dimethyl formamide, or combinations thereof; or

(4) the significant proportion of the precursors of diaspartyl chlorin e_6 has been removed by using activated silica.

14. A reaction product, comprising: a product of a coupling reaction between a first reaction volume and a second reaction volume, the first reaction volume comprising a chlorin e_6 anhydride of the formula



and less than about 5 mol% precursors of diaspartyl chlorin e_6 based on total chlorin species present within the first reaction volume; and the second reaction volume comprising L-aspartic acid or ester thereof;

wherein the reaction product comprises mono-L-aspartyl chlorin e_6, or a pharmaceutically acceptable salt thereof, and diaspartyl chlorin e_6.

15. The reaction product of claim 14, wherein

(1) the reaction product comprises less than about 2 mol% diaspartyl chlorin e_6 based on total chlorin species present within the reaction product;

(2) the reaction product comprises less than about 1 mol% diaspartyl chlorin e_6 based on total chlorin species present within the reaction product;

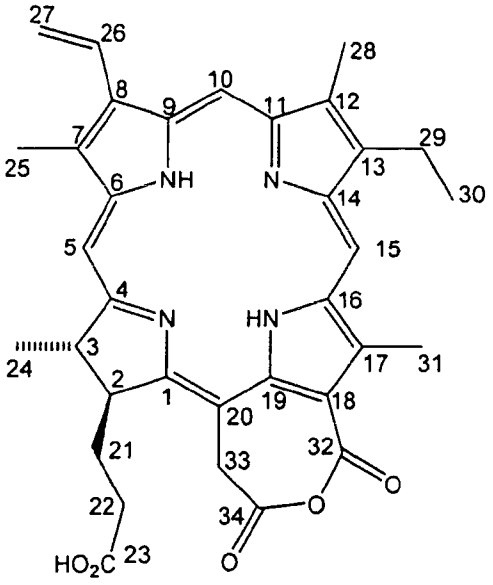
(3) the first reaction volume further comprises dimethyl sulfoxide or dimethyl formamide;

(4) the first reaction volume is purified to remove precursors of diaspartyl chlorin e_6 by a separation process; or

(5) the first reaction volume is purified to remove precursors of diaspartyl chlorin e_6 by activated silica chromatography.

16. A process for preparing mono-L-aspartyl chlorin e_6, or a pharmaceutically acceptable salt thereof, comprising:

combining chlorin e_6 with a dehydration agent to obtain a mixture comprising a Formula I intermediate



Formula I,

or a salt thereof;

isolating the Formula I intermediate, or salt thereof; and

combining the isolated Formula I intermediate, or salt thereof, with an amine-containing reagent to form mono-L-aspartyl chlorin e_6, or a pharmaceutically acceptable salt thereof.

17. The process of claim 16, wherein

(1) combining the chlorin e_6 with the dehydration agent comprises reacting the chlorin e_6 with a carbodiimide;

(2) combining the chlorin e_6 with the dehydration agent comprises reacting the chlorin e_6 with a carbodiimide, the carbodiimide being N,N'-dicyclohexyl-carbodiimide, N-ethyl-N'-(3-dimethylaminopropyl) carbodiimide hydrochloride, or mixtures thereof;

(3) combining the chlorin e_6 with the dehydration agent comprises reacting the chlorin e_6 with a carbodiimide in the presence of dimethyl sulfoxide or dimethyl formamide;

(4) reacting the chlorin e_6 with the dehydration agent comprises reacting the chlorin e_6 with N-ethyl-N'-(3-dimethylaminopropyl) carbodiimide hydrochloride or N,N'-dicyclohexyl-carbodiimide;

(5) isolating the Formula I intermediate, or salt thereof, comprises separating the Formula I intermediate, or salt thereof, from the mixture by using activated silica to yield a substantially pure Formula I intermediate, or salt thereof;

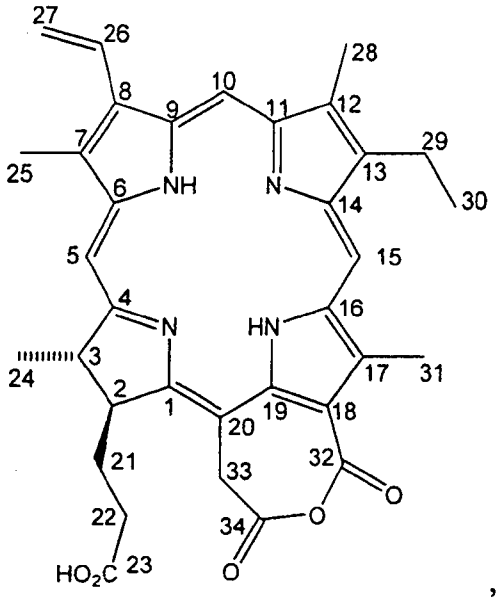
(6) combining the isolated Formula I intermediate, or salt thereof, with an amine-containing reagent comprises reacting the isolated Formula I intermediate, or salt thereof, with sodium aspartate to form Talaporfin Sodium;

(7) combining the isolated Formula I intermediate, or salt thereof, with an amine-containing reagent comprises combining the isolated Formula I intermediate, or salt thereof, in the presence of dimethyl sulfoxide, with L-aspartic acid or ester thereof to form mono-L-aspartyl chlorin e_6 or salt or ester thereof; or

(8) combining the isolated Formula I intermediate, or salt thereof, with an amine-containing reagent comprises coupling the chlorin e_6 anhydride with L-aspartic acid or ester thereof in an aqueous alkaline solution to form a tetra-sodium salt of mono-L-aspartyl chlorin e_6 or ester thereof.

18. A process for preparing mono-L-aspartyl chlorin e_6, or a pharmaceutically acceptable salt thereof, comprising:

combining a composition comprising a compound of the formula (I)



(I)

or a pharmaceutically acceptable salt thereof, an organic solvent, and one or more of diaspartyl chlorin e_6 precursors, with a basic aqueous sodium aspartate composition to form a crude mono-L-aspartyl chlorin e_6 reaction mixture, the crude reaction mixture comprising less than about 2 mol% diaspartyl chlorin e_6 based on the total chlorin species within the composition;

making the reaction mixture basic; and

precipitating a substantial amount of the mono-L-aspartyl chlorin e_6 or a pharmaceutically acceptable salt thereof, from the reaction mixture.

19. The process of claim 18 wherein

(1) the at least one diaspartyl chlorin e_6 precursor comprises less than about 5 mol% based on the total chlorin species within the composition;

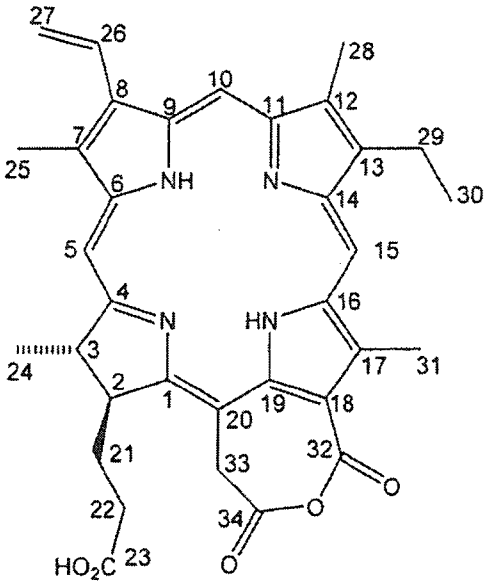
(2) the at least one diaspartyl chlorin e_6 precursor comprises less than about 3 mol% based on the total chlorin species within the composition;

(3) making the reaction mixture basic comprises providing a sufficient amount of an alkaline agent to the reaction mixture to maintain a pH range from about 8 to about 12; or

(4) precipitating a substantial amount of the mono-L-aspartyl chlorin e_6 or a pharmaceutically acceptable salt thereof, from the reaction mixture comprises providing a sufficient amount of an organic solvent to cause the precipitation of more than 50 mol% of

the mono-L-aspartyl chlorin e_6, or a pharmaceutically acceptable salt thereof, present from the reaction mixture.

20. An isolated compound having the following formula (I):



or a pharmaceutically acceptable salt thereof.

21. The compound of claim 20, wherein the compound has a purity of greater than 85 mol%, relative to total chlorin species, as determined by HPLC analysis.

22. The compound of claim 20, wherein the compound has a purity of greater than 90 mol%, relative to total chlorin species, as determined by HPLC analysis.

23. The compound of claim 20, wherein the compound has a purity of greater than 95 mol%, relative to total chlorin species, as determined by HPLC analysis.