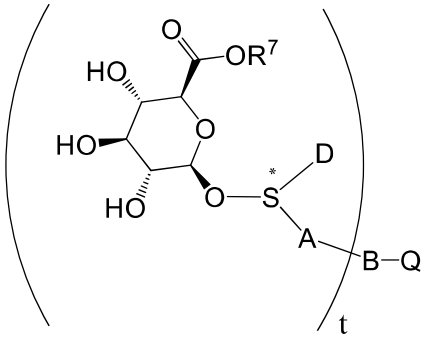
glycosidase recognition site that in some aspects is cleavable by an enzyme having β-glucuronidase activity thereby releasing free drug. Glucuronide-based linkers improve the solubility of LDCs and exhibit sufficient serum stability to provide targeted delivery of a conjugated drug to a targeted cell.

[0003] Literature-reported syntheses of the β-glucuronide-based linkers (Jeffrey, S,C, et al., "Development and properties of β-glucuronide linkers for monoclonal antibody drug conjugates" *Bioconj. Chem.* **17**(3): 831-840) involve generation of a series of intermediates that incorporate a protected β-glucuronide carbohydrate moiety. Global deprotection of that carbohydrate moiety by conventional base hydrolysis methods can generate of up to 20-25% of an impurity that has been identified as a product of β-elimination within the carbohydrate moiety of the Glucuronide Unit. That in turn results in loss of material during, and the impurities generated from that loss can be difficult to remove without further reduction in yield. Therefore, there is a need for improved methods for preparing β-glucuronide-based Drug Linker compounds with higher yields and reduced amounts of contaminating impurities.

BRIEF SUMMARY

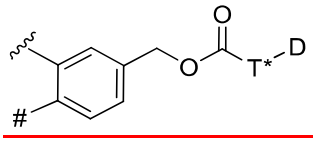
[0004] The disclosure provides *inter alia,* improved processes in producing Drug Linker compounds containing a Glucuronide Unit, as well as intermediates thereof.

[0004a] In one aspect, the present disclosure refers to a method for preparing a compound of Formula **VID**:



(VID)

or a salt thereof, wherein Q is an optionally protected hydroxyl, thiol or amine functional group; A is an optional Connector Unit; B is an optional Branching Unit and is present when subscript t is greater than 1 and is absent when subscript t is 1; S* is a Self-Immolating Unit, wherein the S*-D has the structure of Formula **XIII**:



(**XIII**);

the wavy line indicates the point of attachment to A, and # indicates the point of attachment to the O atom connected to S*;

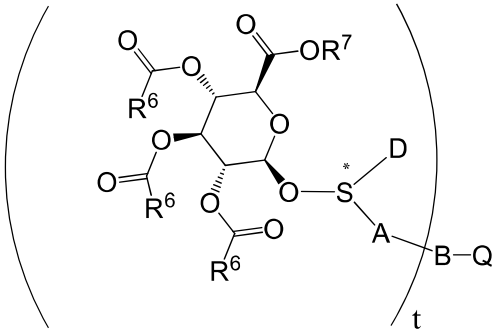
D is a Drug Unit incorporating a DNA minor groove binder, DNA damaging agent or DNA replication inhibitor;

T* is an oxygen, sulfur, or optionally substituted nitrogen atom from a hydroxyl, thiol or amine functional group of the DNA minor groove binder, DNA damaging agent or DNA replication inhibitor;

D' is the remainder of D;

R^7 is C_1-C_8 alkyl or optionally substituted phenyl so that –OR^7 provides for an ester functional group that is a suitable carboxylic acid protecting group; and

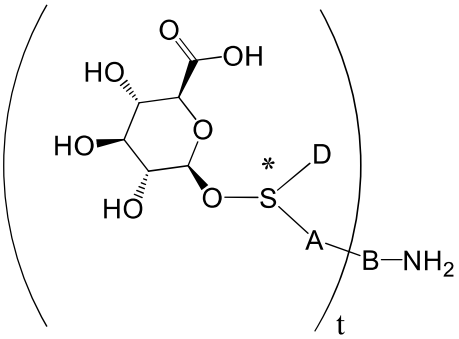
subscript t is 1, 2, 3, or 4, the method comprising the step of: (c) contacting a compound of Formula **VIC** with a Grignard reagent or an alkoxy magnesium halide ~~with either~~ in a suitable alcohol-containing solvent, wherein the Formula **VIC** compound has the structure of:



(**VIC**)

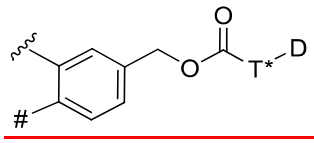
or a salt thereof, wherein each of R^6 is independently C_1-C_8 alkyl or optionally substituted phenyl such that $R^6C(=O)$- provides for an ester functional group that is a suitable hydroxyl protecting group; and the remaining variable groups are as previously defined, wherein said Grignard reagent or alkoxy magnesium halide contacting selectively removes the hydroxyl protecting groups whereby the Formula **VID** compound or its salt is obtained.

[0004b] In another aspect, the present disclosure refers to method for preparing a compound of Formula **VIIE**:



(VIIE)

or a salt thereof, wherein A is an optional Connector Unit; B is an optional Branching Unit and is present when subscript t is greater than 1 and is absent when subscript t is 1; S* is a Self-Immolating Unit, wherein the S*-D has the structure of Formula **XIII**:



(XIII);

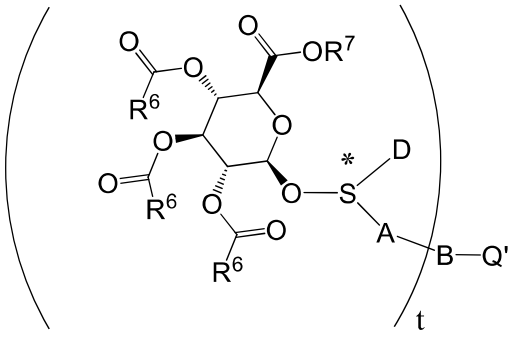
the wavy line indicates the point of attachment to A, and # indicates the point of attachment to the O atom connected to S*;

D is a Drug Unit incorporating a DNA minor groove binder, DNA damaging agent or DNA replication inhibitor;

T* is an oxygen, sulfur, or optionally substituted nitrogen atom from a hydroxyl, thiol or amine functional group of the DNA minor groove binder, DNA damaging agent or DNA replication inhibitor;

the method comprising the steps of:

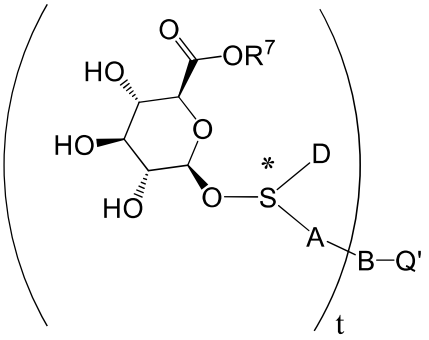
(c) contacting a compound of Formula **VIIC** with a Grignard reagent or an alkoxy magnesium halide ~~with either~~ in a suitable an alcohol-containing solvent, wherein the



Formula **VIIC** compound has the structure of:

(VIIC) or a salt thereof, wherein S*, D, A, B and subscript t are as defined for **VIC** and **VID** ~~of claim 1~~as described herein; Q' is a suitably protected amino group; each of R^6 and R^7 is independently C_1-C_8 alkyl or optionally substituted phenyl such that $R^6C(=O)$- provides for an ester functional group that is a suitable hydroxyl protecting group and $-OR^7$ provides for

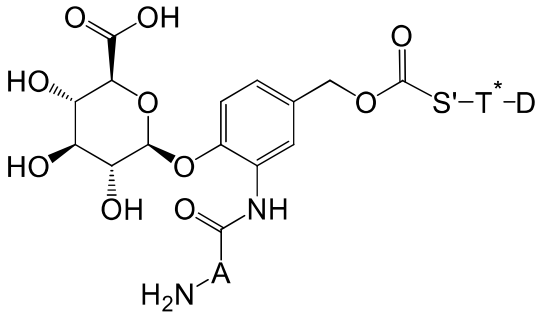
an ester functional group that is a suitable carboxylic acid protecting group; and the remaining variable groups are as previously defined for Formula **VIIE**, wherein said Grignard reagent or alkoxy magnesium halide contacting selectively removes the hydroxyl protecting groups to provide a compound of Formula **VIID**:



(VIID)

or a salt thereof, wherein the variable groups are as previously defined by Formula **VIIIC**; and (d) contacting the Formula **VIID** compound with a first deprotecting agent, wherein said first deprotecting agent contacting removes the amine and carboxylic acid protecting groups whereby the Formula **VIIE** compound or its salt is obtained.

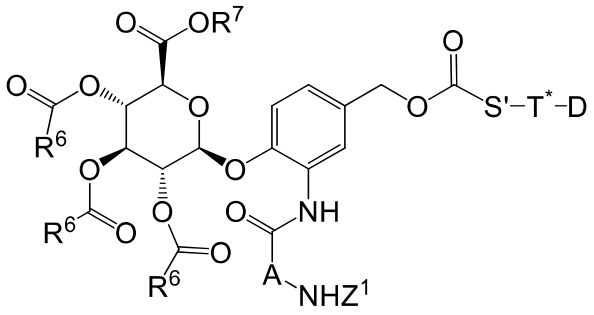
[0004c] In yet another aspect, the present disclosure refers to method for preparing a compound of Formula **IE**:



(IE)

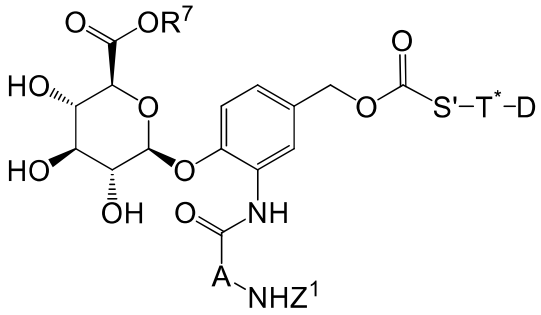
or a salt thereof, wherein A is a an optional Connector Unit; S' is absent or–NR^N-$C(R^1)(R^2)$-, wherein R^N, R^1 and R^2 independently are hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_6-C_{14} aryl, or optionally substituted C-linked C_3-C_8 heteroaryl, or R^N and R^1 together with the nitrogen and carbon atoms to which they are attached comprise an azetidinyl, pyrrolodinyl, piperidinyl or homopiperidinyl moiety, and R^2 is hydrogen; D is a Drug Unit incorporating a DNA minor groove binder, DNA damaging agent or DNA replication inhibitor having a hydroxyl, thiol, or amine functional group, wherein the functional group forms a covalent bond with S'; T* is the oxygen, sulfur, or optionally substituted nitrogen from said functional group; Z^1 is a first suitable amine protecting group; and R^7 is C_1-C_8 alkyl or optionally substituted phenyl such that –OR^7 provides for an ester functional group that is a suitable carboxylic acid protecting group,

the method comprising the steps of: (c) contacting a compound of Formula **IC** with a Grignard reagent or an alkoxy magnesium halide ~~with either~~ in a suitable an alcohol-containing solvent, wherein the Formula **IC** compound has the structure of:



(**IC**)

or a salt thereof, wherein Z^1 is a first suitable amine protecting group; and each of R^6 and R^7 is independently C_1-C_8 alkyl or optionally substituted phenyl such that $R^6C(=O)$- provides for an ester functional group that is a suitable hydroxyl protecting group and $–OR^7$ provides for an ester functional group that is a suitable carboxylic acid protecting group; and the remaining variable groups are as previously defined by Formula **IE**, wherein said Grignard reagent or alkoxy magnesium halide contacting selectively removes the hydroxyl protecting groups to provide a compound of Formula **ID**:



(**ID**)

or a salt thereof, wherein the variable groups are as previously defined by Formula **IC**; and (d) contacting the Formula **ID** compound with a first deprotecting agent, wherein said deprotecting agent contacting removes the amine and carboxylic acid protecting groups whereby the Formula **IE** compound or its salt is obtained.

DESCRIPTION OF THE INVENTION

General

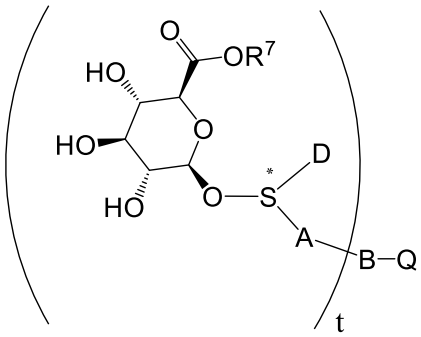
[0005] The present invention is based, in part, on the surprising discovery that the method of deprotection of a Glucuronide Unit in the synthesis of certain Drug Linker

compounds can have a profound effect on the purity and yield of the desired product. Specifically, the present inventors have discovered that using an alkoxymagnesium halide in a solvent comprising an alcohol for removal of acyl protecting groups in the carbohydrate moeity of the Glucuronide Unit, instead of conventionally used reagents such as LiOH, leads to significant reduction of in an undesirable β-eliminated impurity (to below about 5% from about 20%). In some aspects, the alkoxymagnesium halide reagent is prepared *in situ* by contacting a Gringard reagent with the alcohol-containing solvent. Thus, the present invention provides improved processes for preparing certain Drug Linkers comprising a Glucuronide Unit.

Definitions

WHAT IS CLAIMED IS:

1. A method for preparing a compound of Formula **VID**:



(**VID**)

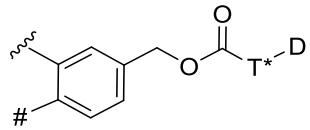
or a salt thereof, wherein

Q is an optionally protected hydroxyl, thiol or amine functional group;

A is an optional Connector Unit;

B is an optional Branching Unit and is present when subscript t is greater than 1 and is absent when subscript t is 1;

S* is a Self-Immolating Unit, wherein the S*-D has the structure of Formula **XIII**:



(**XIII**);

the wavy line indicates the point of attachment to A, and # indicates the point of attachment to the O atom connected to S*;

D is a Drug Unit incorporating a DNA minor groove binder, DNA damaging agent or DNA replication inhibitor;

T* is an oxygen, sulfur, or optionally substituted nitrogen atom from a hydroxyl, thiol or amine functional group of the DNA minor groove binder, DNA damaging agent or DNA replication inhibitor;

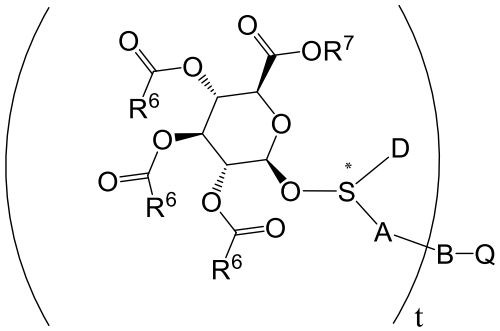
D' is the remainder of D;

R^7 is C_1-C_8 alkyl or optionally substituted phenyl so that $–OR^7$ provides for an ester functional group that is a suitable carboxylic acid protecting group; and

subscript t is 1, 2, 3, or 4,

the method comprising the step of:

(c) contacting a compound of Formula **VIC** with a Grignard reagent or an alkoxy magnesium halide ~~with either~~ in a suitable alcohol-containing solvent, wherein the Formula **VIC** compound has the structure of:



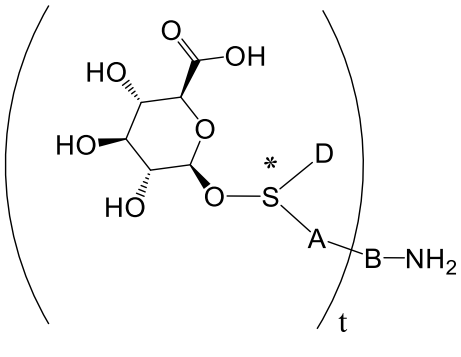
(**VIC**)

or a salt thereof, wherein

each of R^6 is independently C_1-C_8 alkyl or optionally substituted phenyl such that $R^6C(=O)$- provides for an ester functional group that is a suitable hydroxyl protecting group; and the remaining variable groups are as previously defined,

wherein said Grignard reagent or alkoxy magnesium halide contacting selectively removes the hydroxyl protecting groups whereby the Formula **VID** compound or its salt is obtained.

2. A method for preparing a compound of Formula **VIIE**:



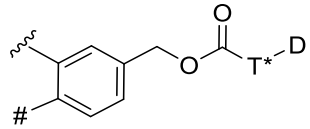
(**VIIE**)

or a salt thereof, wherein

A is an optional Connector Unit;

B is an optional Branching Unit and is present when subscript t is greater than 1 and is absent when subscript t is 1;

S* is a Self-Immolating Unit, wherein the S*-D has the structure of Formula **XIII**:



(**XIII**);

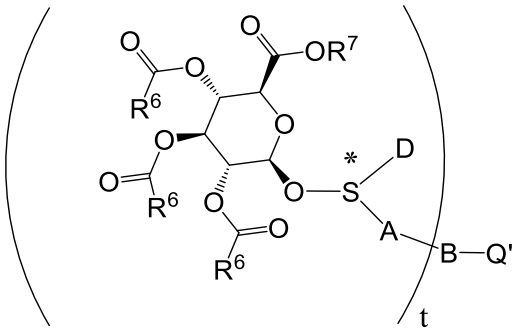
the wavy line indicates the point of attachment to A, and # indicates the point of attachment to the O atom connected to S*;

D is a Drug Unit incorporating a DNA minor groove binder, DNA damaging agent or DNA replication inhibitor;

T* is an oxygen, sulfur, or optionally substituted nitrogen atom from a hydroxyl, thiol or amine functional group of the DNA minor groove binder, DNA damaging agent or DNA replication inhibitor;

the method comprising the steps of:

(c) contacting a compound of Formula **VIIC** with a Grignard reagent or an alkoxy magnesium halide ~~with either~~ in a suitable an alcohol-containing solvent, wherein the Formula **VIIC** compound has the structure of:



(**VIIC**)

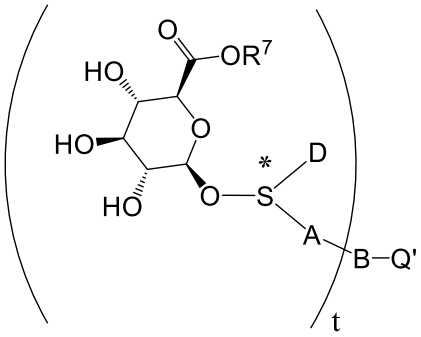
or a salt thereof, wherein

S*, D, A, B and subscript t are as defined for **VIC** and **VID** of claim 1;

Q' is a suitably protected amino group;

each of R^6 and R^7 is independently C_1-C_8 alkyl or optionally substituted phenyl such that $R^6C(=O)$- provides for an ester functional group that is a suitable hydroxyl protecting group and $-OR^7$ provides for an ester functional group that is a suitable carboxylic acid protecting group; and the remaining variable groups are as previously defined for Formula **VIIE**,

wherein said Grignard reagent or alkoxy magnesium halide contacting selectively removes the hydroxyl protecting groups to provide a compound of Formula **VIID**:

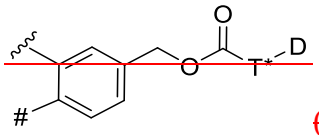


(**VIID**)

or a salt thereof, wherein the variable groups are as previously defined by Formula **VIIIC**; and

(d) contacting the Formula **VIID** compound with a first deprotecting agent, wherein said first deprotecting agent contacting removes the amine and carboxylic acid protecting groups whereby the Formula **VIIE** compound or its salt is obtained.

3. The method of claim **1** or **2**, wherein ~~the Self-immolating Unit (S*) has the structure of Formula **XIII:**~~



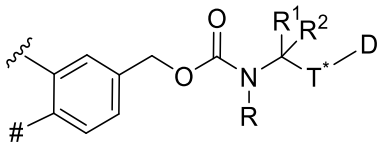
~~(**XIII**)~~

~~wherein~~

T* is an ~~oxygen, sulfur, or~~ optionally substituted nitrogen atom from ~~a hydroxyl, thiol or~~ an amine functional group of the DNA minor groove binder, DNA damaging agent or DNA replication inhibitor that is capable of forming a carbamate functional group in Formula **XIII'**. ~~**XIII**; and~~

~~the wavy line indicates the point of attachment to A, and # indicates the point of attachment to the glycosidic oxygen atom of a carbohydrate moiety (Su).~~

4. The method of claim **1** or **2**, wherein the Self-Immolating Unit has the structure of Formula **XIV**:



(**XIV**),

wherein

T* is an oxygen, sulfur, or optionally substituted nitrogen atom from a hydroxyl, thiol or amine functional group of the DNA minor groove binder, DNA damaging agent or

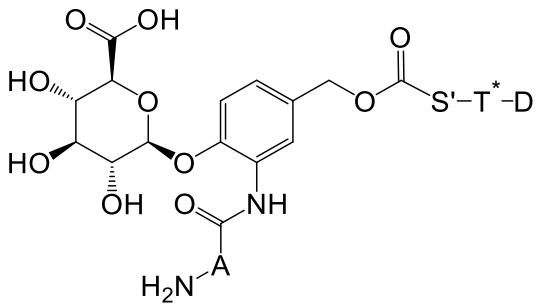
DNA replication inhibitor ~~that is capable of forming a methylene carbamate functional group in Formula **XIV**~~;

R, R^1, and R^2 independently are hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_{6-14} aryl, or optionally substituted C-linked C_3-C_8 heteroaryl, or

both R and R^1 together with the nitrogen and carbon atoms to which they are attached define a pyrrolodinyl or piperidinyl moiety and R^2 is hydrogen; and

the wavy line indicates the point of attachment to A, and # indicates the point of attachment to the ~~glycosidic oxygen atom of a carbohydrate moiety (Su)~~ O atom connected to S*.

5. A method for preparing a compound of Formula **IE**:



(**IE**)

or a salt thereof, wherein

A is a an optional Connector Unit;

S' is absent or $–NR^N$-$C(R^1)(R^2)$-, wherein R^N, R^1 and R^2 independently are hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_6-C_{14} aryl, or optionally substituted C-linked C_3-C_8 heteroaryl, or R^N and R^1 together with the nitrogen and carbon atoms to which they are attached comprise an azetidinyl, pyrrolodinyl, piperidinyl or homopiperidinyl moiety, and R^2 is hydrogen;

D is a Drug Unit incorporating a DNA minor groove binder, DNA damaging agent or DNA replication inhibitor having a hydroxyl, thiol, or amine functional group, wherein the functional group forms a covalent bond with S';

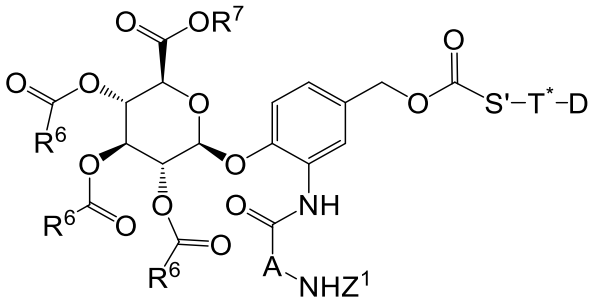
T* is the oxygen, sulfur, or optionally substituted nitrogen from said functional group;

Z^1 is a first suitable amine protecting group; and

R^7 is C_1-C_8 alkyl or optionally substituted phenyl such that $–OR^7$ provides for an ester functional group that is a suitable carboxylic acid protecting group,

the method comprising the steps of:

(c) contacting a compound of Formula **IC** with a Grignard reagent or an alkoxy magnesium halide ~~with either~~ in a suitable an alcohol-containing solvent, wherein the Formula **IC** compound has the structure of:



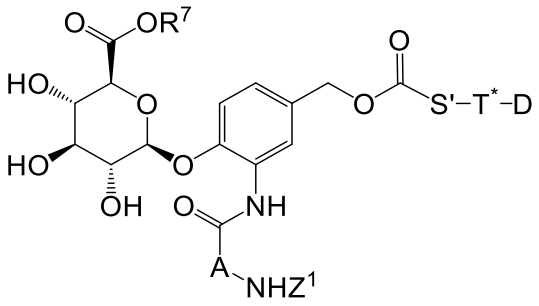
(**IC**)

or a salt thereof, wherein

Z^1 is a first suitable amine protecting group; and

each of R^6 and R^7 is independently C_1-C_8 alkyl or optionally substituted phenyl such that $R^6C(=O)$- provides for an ester functional group that is a suitable hydroxyl protecting group and $–OR^7$ provides for an ester functional group that is a suitable carboxylic acid protecting group; and the remaining variable groups are as previously defined by Formula **IE**,

wherein said Grignard reagent or alkoxy magnesium halide contacting selectively removes the hydroxyl protecting groups to provide a compound of Formula **ID**:



(**ID**)

or a salt thereof, wherein the variable groups are as previously defined by Formula **IC**; and

(d) contacting the Formula **ID** compound with a first deprotecting agent, wherein said deprotecting agent contacting removes the amine and carboxylic acid protecting groups whereby the Formula **IE** compound or its salt is obtained.

6. The method of claim **1**, **2**, or **5**, wherein the Grignard reagent has the formula of R^gMgX and the alkoxy magnesium halide has the formula of R^gOMgX, wherein R^g is C_1-C_5 alkyl and X is I, Br, or Cl.

7. The method of claim **6**, wherein the Grignard reagent is MeMgI or MeMgCl.

8. The method of claim **6**, wherein the alkoxy magnesium halide is MeOMgI or MeOMgCl.

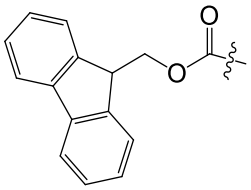
9. The method of claim **1**, **2**, or **5**, wherein the Grignard reagent is MeMgI, the alkoxy magnesium halide is MeOMgI or MeOMgCl and the suitable alcohol-containing solvent comprises a C_1-C_4 alcohol.

10. The method of claim **1**, **2**, or **5**, wherein when the Grignard reagent is in the suitable alcohol-containing solvent, the suitable alcohol-containing solvent further comprises THF.

11. The method of claim **10**, wherein when the Grignard reagent is in the suitable alcohol-containing solvent, the suitable alcohol-containing solvent is a 1:1 (v/v) mixture of methanol and THF.

12. The method claim **5**, wherein the first deprotecting agent for removal of Z^1 is an aqueous-containing solution of LiOH.

13. The method of claim **5**, wherein Z^1 has the formula of:

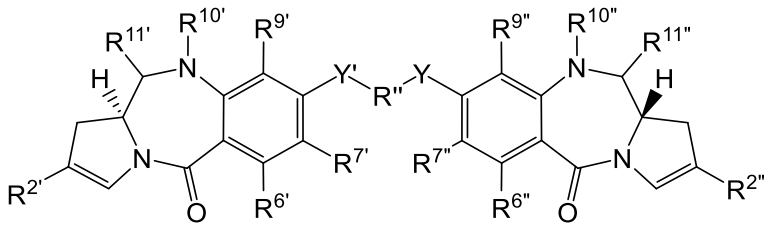


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14. The method of claim **5**, wherein said Grignard reagent or alkoxy magnesium halide contacting and said deprotecting agent contacting to remove Z^1 are done sequentially in one pot.

15. The method of claim **1**, **2**, or **5**, wherein D is a PBD Drug Unit.

16. The method of claim **15**, wherein the PBD Drug Unit has the structure of Formula **X**:



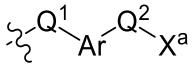
(**X**)

or a salt thereof,

wherein:

the dotted lines represent a tautomeric double bond;

$R^{2"}$ is of formula **XI**:



(**XI**),

wherein the wavy line indicates the site of covalent attachment to the Formula **X** structure;

Ar is an optionally substituted C_{5-7} arylene;

X^a is from a reactive or activateable group for covalent attachment to Q^2, when Q^2 is other than a single bond or to S* or S' when Q^2 is a single bond, wherein X^a is selected from the group comprising: -O-, -S-, -C(O)O-, -C(O)-, -NHC(O)-, and –$N(R^N)$-, wherein R^N is H or C_1-C_4 alkyl, and $(C_2H_4O)_mCH_3$, where subscript m is 1, 2 or 3;

and either:

(i) Q^1 is a single bond; and Q^2 is a single bond or -Z-$(CH_2)_n$-, wherein Z is selected from the group consisting of a single bond, O, S, and NH; and subscript n is 1, 2 or 3, or

(ii) Q^1 is -CH=CH-, and Q^2 is a single bond; and

$R^{2'}$ is a C_{5-10} aryl group, optionally substituted by one or more substituents selected from the group consisting of halo, nitro, cyano, C_{1-7} alkoxy, C_{5-20} aryloxy, C_{3-20} heterocyclyoxy, C_1-C_6 ether, C_1-C_7 alkyl, C_3-C_7 heterocyclyl and bis-oxy-C_1-C_3 alkylene;

$R^{6"}$ and $R^{9"}$ are independently selected from the group consisting of H, R, OH, OR, SH, SR, NH_2, NHR, NRR', nitro, Me_3Sn and halo;

$R^{7"}$ is selected from the group consisting of H, R, OH, OR, SH, SR, NH_2, NHR, NRR', nitro, Me_3Sn and halo; and

R and R' are independently selected from the group consisting of optionally substituted C_1-C_{12} alkyl, optionally substituted C_3-C_{20} heterocyclyl and optionally substituted C_5-C_{20} aryl;

and either:

(a) $R^{10"}$ is H, and $R^{11"}$ is OH or OR^A, wherein R^A is C_1-C_4 alkyl, or

(b) $R^{10"}$ and $R^{11"}$ form a nitrogen-carbon double bond between the nitrogen and carbon atoms to which they are bound, or

(c) $R^{10"}$ is H and $R^{11"}$ is SO_zM, wherein subscript z is 2 or 3; and

R″ is a C_{3-12} alkylene group, the carbon chain of which is optionally interrupted by one or more of O, S or NR^{N2} (where R^{N2} is H or C_1-C_4 alkyl), and/or by one of benzene or pyridine;

Y and Y' are selected from the group consisting of O, S, and NH;

$R^{6'}$, $R^{7'}$, $R^{9'}$ are selected from the same groups as $R^{6"}$, $R^{7"}$ and $R^{9"}$, respectively, and $R^{10'}$ and $R^{11'}$ are the same as $R^{10"}$ and $R^{11"}$, respectively, and if $R^{11"}$ and $R^{11'}$ are SO_zM, each M is either a monovalent pharmaceutically acceptable cation or together represent a divalent pharmaceutically acceptable cation.

17. The method of claim **16**, wherein $R^{7"}$ is selected from the group consisting of H, OH and OR.

18. The method of claim **16**, wherein $R^{7"}$ is a C_{1-4} alkyloxy group.

19. The method of claim **16**, wherein $R^{7"}$ is $–OCH_3$.

20. The method of claim **16**, wherein Y and Y' are O.

21. The method of claim **16**, wherein $R^{9"}$ is H.

22. The method of claim **16**, wherein $R^{6"}$ is selected from the group consisting of H and halo.

23. The method of claim **16**, wherein Ar is phenylene; X^a is selected from the group consisting of -O-, -S- and -NH-; and Q^1 is a single bond.

24. The method of claim **23**, wherein X^a is NH.

25. The method of claim **16**, wherein Q^1 is a single bond and Q^2 is a single bond.

26. The method of claim **16**, wherein $R^{2'}$ is a C_{5-7} aryl group optionally substituted by one or more substituents selected from the group consisting of halo, nitro, cyano, C_{1-7} alkoxy, C_{5-20} aryloxy, C_{3-20} heterocyclyoxy, C_{1-7} alkyl, C_{3-7} heterocyclyl and bis-oxy-C_{1-3} alkylene, wherein the C_{1-7} alkoxy group is optionally substituted by an amino group, and if the C_{3-7} heterocyclyl group is a C_6 nitrogen containing heterocyclyl group, it is optionally substituted by a C_{1-4} alkyl group.

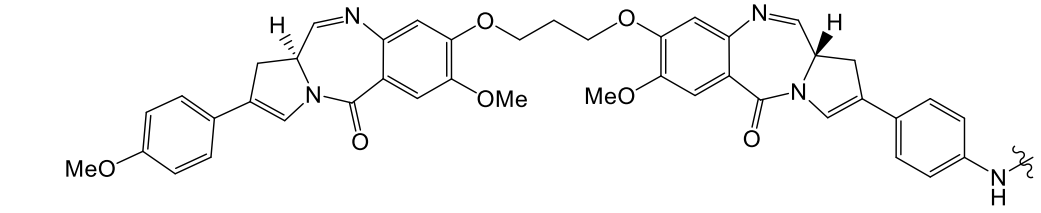
27. The method of claim **26**, wherein $R^{2'}$ is an optionally substituted C_{5-7} aryl group, wherein the optionally substituted C_{5-7} aryl group is optionally substituted phenyl.

28. The method of claim **27**, wherein the optionally substituted C_{5-7} aryl group is phenyl having one to three substituents.

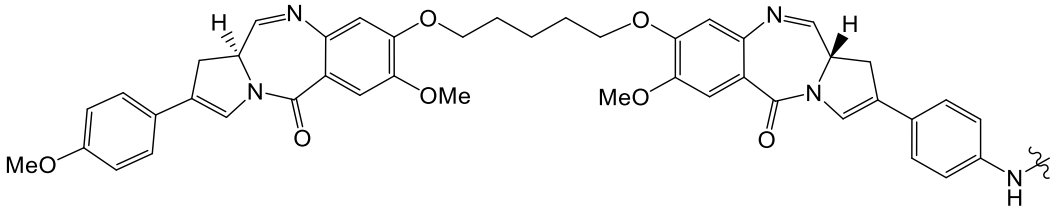
29. The method of claim **16**, wherein $R^{10"}$ and $R^{11"}$ form a nitrogen-carbon double bond.

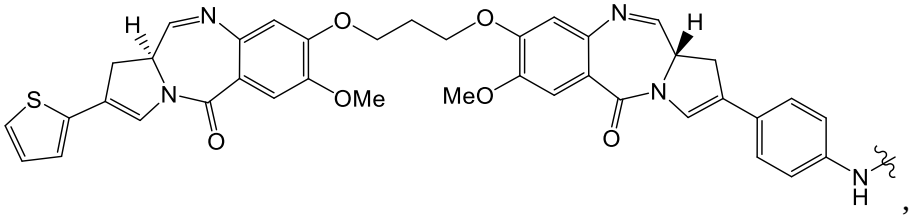
30. The method of claim claim **16**, wherein $R^{6'}$, $R^{7'}$, $R^{9'}$, and Y' are the same as $R^{6"}$, $R^{7"}$, $R^{9"}$, and Y respectively.

31. The method of claim **16**, wherein the PBD Drug Unit is:



, or

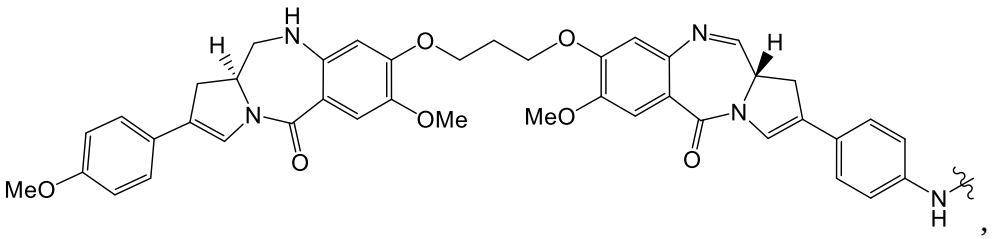


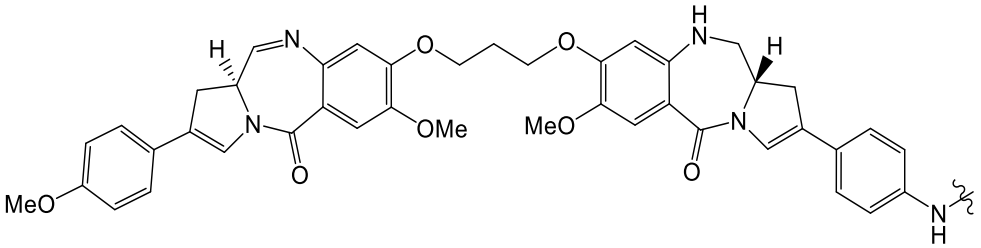


,

or a salt thereof, wherein the wavy line indicates the point of covalent attachment to Q^2, S* or S' in the form of a carbamate functional group.

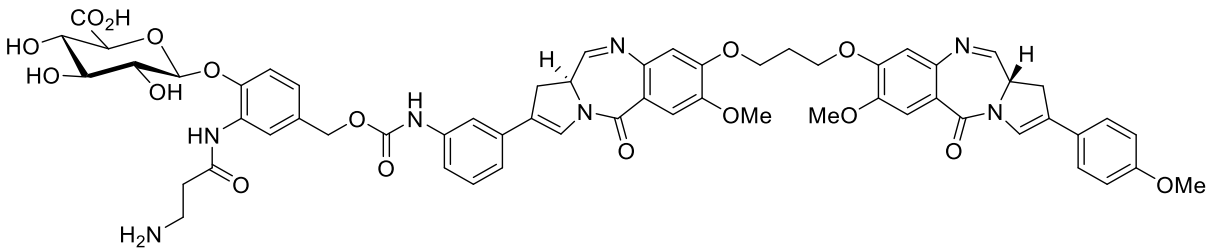
32. The method of claim **25**, wherein D is a PBD Drug Unit, wherein the PBD Drug Unit is:





or a salt thereof, wherein the wavy line indicates the point of covalent attachment to Q^2, S* or S' in the form of a carbamate functional group.

33. The method of claim **5**, wherein D is a PBD Drug Unit, in which the compound of Formula **IE** has the structure of:

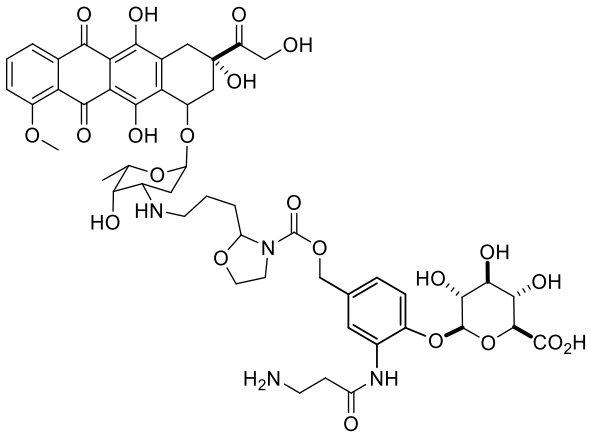


or a salt thereof.

34. The method of claim **1**, **2**, or **5**, wherein D is an anthracyclin Drug Unit.

35. The method of claim **34**, wherein the anthracyclin Drug Unit incorporates the structure of doxorubicin, idarubicin, daunorubicin, doxorubicin propyloxazoline (DPO) or cyanomorpholino-doxorubicin.

36. The method of claim **5**, wherein D is an anthracyclin Drug Unit in which the compound of Formula **IE** has the structure of:



or a salt thereof.

37. The method of claim **1**, **2**, or **5**, wherein D is a camptothecin Drug Unit.

38. The method of claim **1**, **2**, or **5**, wherein D is a duocarmycin Drug Unit.