

DESCRIPTION

NOVEL DERIVATIVES OF UNCIALAMYCIN AND METHODS OF SYNTHESIS

This application claims the benefit of U.S. Provisional Application No. 61/865,896, filed on August 14, 2013, U.S. Provisional Application No. 61/868,783, filed on August 22, 2013, and U.S. Provisional Application No. 61/937,003, filed on February 7, 2014, the entirety of each is incorporated herein by reference.

BACKGROUND

1. Field of the Invention

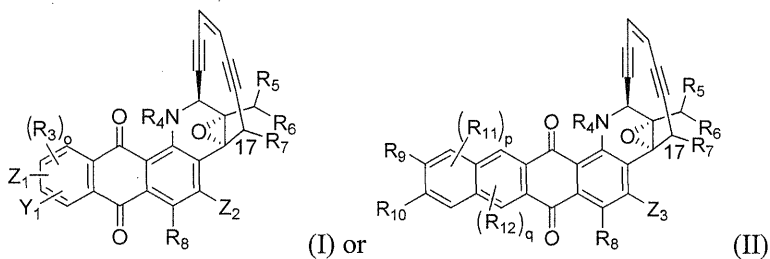
This disclosure relates to the fields of medicine, pharmacology, chemistry and oncology. In particular, compounds, drug conjugates, methods of treatment, and methods of synthesis relating to uncialamycin and analogs thereof are disclosed.

2. Related Art

Uncialamycin is an antibiotic which has shown great promise as a potential anticancer reagent. The use of uncialamycin as an anticancer agent has been described in Nicolaou, *et al.*, 2007 and Nicolaou, *et al.*, 2008, both of which are incorporated herein by reference. The compound has been shown to be a potent antitumor cancer agent with an IC_{50} in the picomolar. Furthermore, the compound has been envisioned as a potential antibody payload. Unfortunately, the current synthetic methods lack the flexibility to produce numerous analogs and generate sufficient quantities of the desired compounds. Given the widespread and world wide impact of cancer, new therapeutic agents are of the commercial importance.

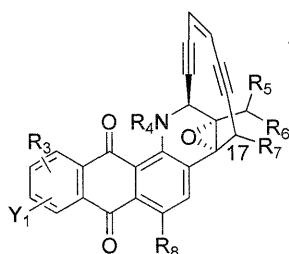
SUMMARY

The present invention refers to subject matter as defined in the attached set of claims. Thus, in accordance with the present disclosure, there is provided-disclosed a compound of the formula:

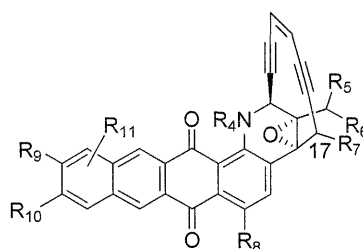


wherein: Y_1 is $-O(CH_2)_mY'$, $-NH(CH_2)_mY'$, $-S(CH_2)_mY'$, or $-(CH_2)_mNR_1R_2$, or is taken together with Z_1 as defined below; wherein: Y' is hydroxy, halo, mercapto, $alkyl_{(C1-12)}$, substituted $alkyl_{(C1-12)}$, $alkenyl_{(C2-12)}$, substituted $alkenyl_{(C2-12)}$, $alkynyl_{(C2-12)}$, substituted $alkynyl_{(C2-12)}$, $acyl_{(C\leq 12)}$, substituted $acyl_{(C1-12)}$, $acyloxy_{(C1-12)}$, substituted $acyloxy_{(C1-12)}$, $alkylamino_{(C1-12)}$, or substituted $alkylamino_{(C1-12)}$; m is 1, 2, 3, 4, 5, or 6; and R_1 and R_2 are each independently selected from hydrogen, hydroxy, $alkyl_{(C1-12)}$, substituted $alkyl_{(C1-12)}$, $alkenyl_{(C2-12)}$.

12), substituted heteroaryl_(C1-12), heterocycloalkyl₍₂₋₁₂₎, substituted heterocycloalkyl_(C2-12), acyl_(C1-12), substituted acyl_(C1-12), or a hydroxy protecting group; X₂ and X₃ are independently selected from hydrogen, alkyl_(C1-12), substituted alkyl_(C1-12), alkenyl_(C2-12), substituted alkenyl_(C2-12), alkynyl_(C2-12), substituted alkynyl_(C2-12), aryl_(C6-12), substituted aryl_(C6-12), aralkyl_(C7-12), substituted aralkyl_(C7-12), heteroaryl_(C1-12), substituted heteroaryl_(C1-12), heterocycloalkyl_(C2-12), substituted heterocycloalkyl_(C2-12), acyl_(C1-12), substituted acyl_(C1-12), a monovalent amine protecting group, or when X₂ and X₃ are taken together form a divalent amine protecting group; X₄ is alkyl_(C1-12), substituted alkyl_(C1-12), alkenyl_(C2-12), substituted alkenyl_(C2-12), alkynyl_(C2-12), substituted alkynyl_(C2-12), aryl_(C6-12), substituted aryl_(C6-12), aralkyl_(C7-12), substituted aralkyl_(C7-12), heteroaryl_(C1-12), substituted heteroaryl_(C1-12), heterocycloalkyl_(C2-12), substituted heterocycloalkyl_(C2-12), acyl_(C1-12), substituted acyl_(C1-12), or a thiol protecting group; Y₂ is alkanediyl_(C1-12) or substituted alkanediyl_(C1-12); and R₁₃ is hydroxy, amino, mercapto, -OX₁, -NX₂X₃, or -SX₄, or heteroaryl_(C1-12), heterocycloalkyl_(C2-12), acyl_(C1-12), alkoxy_(C1-12), acyloxy_(C1-12), alkylamino_(C1-12), dialkylamino_(C2-12), alkylthio_(C1-12), amido_(C1-12), or a substituted version of any of these groups; and p and q are each independently 1 or 2; Z₃ is hydrogen, hydroxy, halo, amino, cyano, nitro, phosphate, or mercapto, or alkyl_(C1-12), alkenyl_(C2-12), alkynyl_(C2-12), aryl_(C6-12), aralkyl_(C7-12), heteroaryl_(C1-12), heterocycloalkyl_(C2-12), acyl_(C1-12), alkoxy_(C1-12), acyloxy_(C1-12), alkylamino_(C1-12), dialkylamino_(C2-12), amido_(C1-12), or a substituted version of any of these groups; provided that Y₁ is not -NHMe or -NHCH₂CH₂NH₂; or a pharmaceutically acceptable salt thereof. In some ~~embodiments~~ examples, the compound is further defined as:

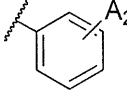


(III) or

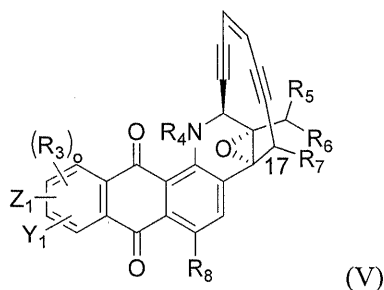


(IV)

wherein: Y₁ is -O(CH₂)_mY', -NH(CH₂)_mY', -S(CH₂)_mY' or -CH₂NR₁R₂, wherein: Y' is alkyl_(C1-12), substituted alkyl_(C1-12), alkenyl_(C2-12), substituted alkenyl_(C2-12), alkynyl_(C2-12), substituted alkynyl_(C2-12), acyl_(C1-12), substituted acyl_(C1-12), acyloxy_(C1-12), substituted acyloxy_(C1-12), alkylamino_(C1-12), or substituted alkylamino_(C1-12); m is 1, 2, 3, 4, 5, or 6; R₁ and R₂ are each independently selected from hydrogen, hydroxy, alkyl_(C1-12), alkenyl_(C1-12), alkynyl_(C1-12), aryl_(C6-12), aralkyl_(C7-12), heteroaryl_(C1-12), heterocycloalkyl_(C2-12), acyl_(C1-12), alkoxy_(C1-12), or a substituted version of any of these groups; R₁ and R₂ are taken together and are a divalent protecting group, alkanediyl₍₁₋₁₂₎, alkoxydiyl_(C1-8); or a substituted version of either of these groups; or -C(O)O(CH₂)_nS-

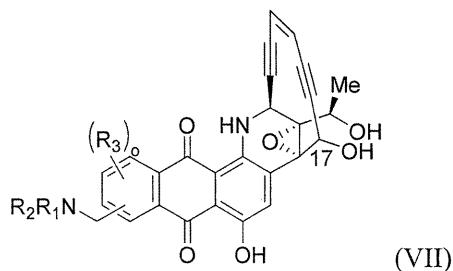
A₁, or -C(O)O(CH₂)_nS(O)₂-A₁, wherein: A₁ is aryl_(C6-12), substituted aryl_(C6-12), or , wherein: A₂ is alkenyl_(C2-12), substituted alkenyl_(C2-12), alkynyl_(C2-12), substituted alkynyl_(C2-12), acyl_(C1-12), substituted acyl_(C1-12), acyloxy_(C1-12), substituted acyloxy_(C1-12), alkoxy_(C1-12), substituted alkoxy_(C1-12), alkylamino_(C1-12), substituted alkylamino_(C1-12), dialkylamino_(C2-12), or substituted dialkylamino_(C2-12); wherein A₂ is not -CO₂H, -CO₂CH₃, -OCH₃, -OCH₂CH₃, -C(O)CH₃, -NHCH₃, -NHCH₂CH₃, -N(CH₃)₂, -C(O)NH₂, and -OC(O)CH₃; n is 1, 2, 3, 4, or 5; R₃ is hydrogen, hydroxy, halo, amino, cyano, nitro, phosphate, or mercapto, or alkyl_(C1-12), alkenyl_(C2-12), alkynyl_(C2-12), aryl_(C6-12), aralkyl_(C7-12), heterocycloalkyl_(C2-12), acyl_(C1-12), alkoxy_(C1-12), acyloxy_(C1-12), alkylamino_(C1-12), dialkylamino_(C2-12), amido_(C1-12), or a substituted version of any of these groups; R₄ is

hydrogen, alkyl_(C1-12), a monovalent amine protecting group, or substituted alkyl_(C1-12); R₅, R₆, and R₇ are each independently hydrogen, hydroxy, amino, mercapto, -OX₁, -NX₂X₃, or -SX₄; or alkyl_(C1-12), alkoxy_(C1-12), acyloxy_(C1-12), alkylamino_(C1-12), dialkylamino_(C2-12), alkylthio_(C1-12), amido_(C1-12), or a substituted version of any of these groups; wherein: X₁ is a hydroxy protecting group; X₂ and X₃ are independently selected from hydrogen, a monovalent amine protecting group, or when X₂ and X₃ are taken together form a divalent amine protecting group; X₄ is a thiol protecting group; R₈ is hydroxy, amino, or mercapto; or alkoxy_(C1-12), acyloxy_(C1-12), alkylamino_(C1-12), dialkylamino_(C2-12), alkylthio_(C1-12), amido_(C1-12), or a substituted version of any of these groups; R₉, R₁₀, and R₁₁ are each independently selected from hydrogen, hydroxy, amino, mercapto, -OX₁, -NX₂X₃, or -SX₄, or alkyl_(C1-12), alkenyl_(C2-12), alkynyl_(C2-12), aryl_(C6-12), aralkyl_(C7-12), heteroaryl_(C1-12), heterocycloalkyl_(C2-12), acyl_(C1-12), alkoxy_(C1-12), acyloxy_(C1-12), alkylamino_(C1-12), dialkylamino_(C2-12), alkylthio_(C1-12), amido_(C1-12), or a substituted version of any of these groups; or Y₂-R₁₂; wherein: X₁ is alkyl_(C1-12), substituted alkyl_(C1-12), alkenyl_(C2-12), substituted alkenyl_(C2-12), alkynyl_(C2-12), substituted alkynyl_(C2-12), aryl_(C6-12), substituted aryl_(C6-12), aralkyl_(C7-12), substituted aralkyl_(C7-12), heteroaryl_(C1-12), substituted heteroaryl_(C1-12), heterocycloalkyl_(C2-12), substituted heterocycloalkyl_(C2-12), acyl_(C1-12), substituted acyl_(C1-12), or a hydroxy protecting group; X₂ and X₃ are independently selected from hydrogen, alkyl_(C1-12), substituted alkyl_(C1-12), alkenyl_(C2-12), substituted alkenyl_(C2-12), alkynyl_(C2-12), substituted alkynyl_(C2-12), aryl_(C6-12), substituted aryl_(C6-12), aralkyl_(C7-12), substituted aralkyl_(C7-12), heteroaryl_(C1-12), substituted heteroaryl_(C1-12), heterocycloalkyl_(C2-12), substituted heterocycloalkyl_(C2-12), acyl_(C1-12), substituted acyl_(C1-12), a monovalent amine protecting group, or when X₂ and X₃ are taken together form a divalent amine protecting group; X₄ is alkyl_(C1-12), substituted alkyl_(C1-12), alkenyl_(C2-12), substituted alkenyl_(C2-12), alkynyl_(C2-12), substituted alkynyl_(C2-12), aryl_(C6-12), substituted aryl_(C6-12), aralkyl_(C7-12), substituted aralkyl_(C7-12), heteroaryl_(C1-12), substituted heteroaryl_(C1-12), heterocycloalkyl_(C2-12), substituted heterocycloalkyl_(C2-12), acyl_(C1-12), substituted acyl_(C1-12), or a thiol protecting group; Y₂ is alkanediyl_(C1-12) or substituted alkanediyl_(C1-12); or R₁₂ is hydroxy, amino, mercapto, -OX₁, -NX₂X₃, or -SX₄, or heteroaryl_(C1-12), heterocycloalkyl_(C2-12), acyl_(C1-12), alkoxy_(C1-12), acyloxy_(C1-12), alkylamino_(C1-12), dialkylamino_(C2-12), alkylthio_(C1-12), amido_(C1-12), or a substituted version of any of these groups; or provided that Y₁ is not -NHMe, -NHC(O)C₆H₄NH₂, -NHC(O)CH₂NH₂, -NHC(O)CH(CH₂OH)NH₂, -NHC(O)CH(NH₂)CH₂CH₂CH₂CH₂NH₂, or -NHCH₂CH₂NH₂; or a pharmaceutically acceptable salt thereof. In some embodiment examples, the formula is further defined as:

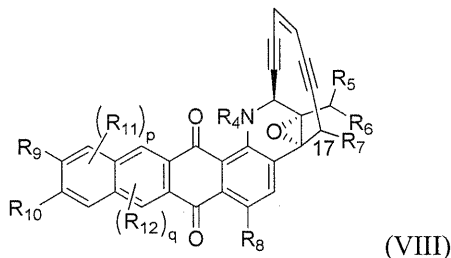


wherein: Y₁ is -O(CH₂)_mY', -NH(CH₂)_mY', -S(CH₂)_mY', or -(CH₂)_mNR₁R₂, or taken together with Z₁ as described below; wherein: Y' is hydroxy, halo, mercapto, alkenyl_(C2-12), substituted alkenyl_(C2-12), alkynyl_(C2-12), substituted alkynyl_(C2-12), acyl_(C1-12), substituted acyl_(C1-12), acyloxy_(C1-12), substituted acyloxy_(C1-12), alkylamino_(C1-12), or substituted alkylamino_(C1-12); m is 1, 2, 3, 4, 5, or 6; and R₁ and R₂ are each independently selected from hydrogen, hydroxy, alkyl_(C1-12), substituted alkyl_(C1-12), alkenyl_(C2-12), substituted alkenyl_(C2-12),

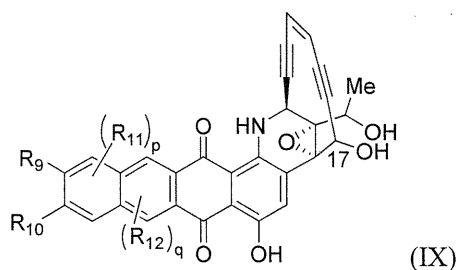
together form a divalent amine protecting group; and X_4 is a thiol protecting group; and R_8 is hydroxy, amino, or mercapto; or a pharmaceutically acceptable salt thereof. In some ~~embodiments~~examples, the formula is further defined as:



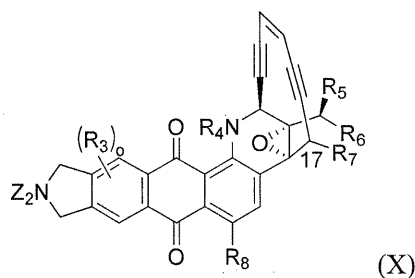
5 wherein: R_1 and R_2 are each independently selected from hydrogen, hydroxy, alkyl_(C1-12), substituted alkyl_(C1-12), aryl₍₆₋₁₂₎, substituted aryl_(C6-12), aralkyl_(C7-12), substituted aralkyl_(C7-12), acyl_(C1-12), substituted acyl_(C1-12), a monovalent amine protecting group, $-C(O)O(CH_2)_nS-C_6H_5$, $-C(O)O(CH_2)_nS(O)-C_6H_5$, or $-C(O)O(CH_2)_nS(O)_2-C_6H_5$, a monovalent amine protecting group, or R_1 and R_2 are taken together and form a divalent amine protecting group, or alkyl_(C1-12), aryl_(C6-12), aralkyl_(C7-12), acyl_(C1-12), or a substituted version of
 10 any of these groups; R_3 is hydrogen, hydroxy, halo, or alkoxy_(C1-12) or substituted alkoxy_(C1-12); and o is 1, 2, or 3; or a pharmaceutically acceptable salt thereof. In some ~~embodiments~~examples, the formula is further defined as:



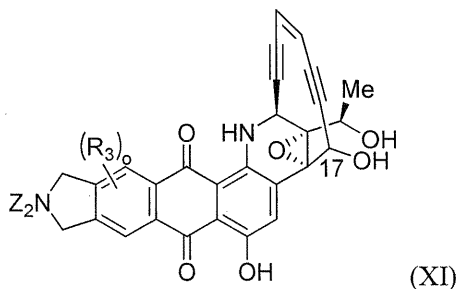
15 wherein: R_4 is hydrogen, alkyl_(C1-12), a monovalent amine protecting group, or substituted alkyl_(C1-12); R_5 , R_6 , and R_7 are each independently hydrogen, hydroxy, amino, mercapto, $-OX_1$, $-NX_2X_3$, or $-SX_4$; or alkyl_(C1-12), alkoxy_(C1-12), acyloxy_(C1-12), alkylamino_(C1-12), dialkylamino_(C2-12), alkylthio_(C1-12), amido_(C1-12), or a substituted version of any of these groups; wherein: X_1 is a hydroxy protecting group; X_2 and X_3 are independently selected from hydrogen, a monovalent amine protecting group, or when X_2 and X_3 are taken together form a divalent amine protecting group; X_4 is a thiol protecting group; R_8 is hydroxy, amino, or mercapto; or
 20 alkoxy_(C1-12), acyloxy_(C1-12), alkylamino_(C1-12), dialkylamino_(C2-12), alkylthio_(C1-12), amido_(C1-12), or a substituted version of any of these groups; R_9 , R_{10} , R_{11} , and R_{12} are each independently selected from hydrogen, hydroxy, amino, $-NX_2X_3$, or heteroaryl_(C1-12), heterocycloalkyl_(C2-12), acyl_(C1-12), alkoxy_(C1-12), acyloxy_(C1-12), alkylamino_(C1-12), dialkylamino_(C2-12), amido_(C2-12), or a substituted version of any of these groups; or Y_2-R_{13} ; wherein: X_2 and X_3 are independently selected from hydrogen, a monovalent amine protecting group, or when
 25 X_2 and X_3 are taken together form a divalent amine protecting group; Y_2 is alkanediyl_(C1-12) or substituted alkanediyl_(C1-12); or R_{13} is hydroxy, amino, or $-NX_2X_3$, or alkylamino_(C1-12), dialkylamino_(C2-12), amido_(C1-12), or a substituted version of any of these groups; or p and q are each independently 1 or 2; a pharmaceutically acceptable salt thereof. In some ~~embodiments~~examples, the formula is further defined as:



wherein: R_9 , R_{10} , R_{11} , and R_{12} are each independently selected from hydrogen, hydroxy, amino, $-NX_2X_3$, or alkoxy_(C1-12), alkylamino_(C1-12), dialkylamino_(C2-12), amido_(C1-12), or a substituted version of any of these groups; or Y_2-R_{13} ; wherein: X_2 and X_3 are independently selected from hydrogen, a monovalent amine protecting group, or when X_2 and X_3 are taken together form a divalent amine protecting group; Y_2 is alkanediyl_(C1-12) or substituted alkanediyl_(C1-12); or R_{13} is hydroxy, amino, or $-NX_2X_3$, or alkylamino_(C1-12), dialkylamino_(C2-12), amido_(C1-12), or a substituted version of any of these groups; and p and q are each independently 1 or 2; or a pharmaceutically acceptable salt thereof. In some embodimentsexamples, the compound is further defined as:

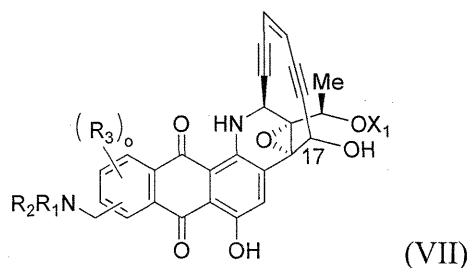


wherein: Z_2 is hydrogen, an amine protecting group, acyl_(C1-12), substituted acyl_(C1-12), $-C(O)O(CH_2)_nS-A_3$, $-C(O)O(CH_2)_nS(O)-A_3$, or $-C(O)O(CH_2)_nS(O)_2-A_3$, wherein: A_3 is aryl_(C6-12) or substituted aryl_(C6-12); R_3 is hydrogen, hydroxy, halo, amino, cyano, nitro, phosphate, or mercapto, or alkyl_(C1-12), alkenyl_(C2-12), alkynyl_(C2-12), aryl_(C6-12), aralkyl_(C7-12), heteroaryl_(C1-12), heterocycloalkyl_(C2-12), acyl_(C1-12), alkoxy_(C1-12), acyloxy_(C1-12), alkylamino_(C1-12), dialkylamino_(C2-12), amido_(C1-12), or a substituted version of any of these groups; o is 1 or 2; R_4 is hydrogen, a monovalent amine protecting group, alkyl_(C1-12), or substituted alkyl_(C1-12); R_5 , R_6 , and R_7 are each independently hydrogen, hydroxy, amino, mercapto, $-OX_1$, $-NX_2X_3$, or $-SX_4$; or alkyl_(C1-12) or substituted alkyl_(C1-12); wherein: X_1 is a hydroxy protecting group; X_2 and X_3 are independently selected from hydrogen, a monovalent amine protecting group, or when X_2 and X_3 are taken together form a divalent amine protecting group; X_4 is a thiol protecting group; R_8 is hydroxy, amino, or mercapto; or a pharmaceutically acceptable salt thereof. In some embodimentsexamples, the compound is further defined as:

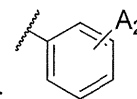


wherein: Z_2 is hydrogen, an amine protecting group, acyl_(C6-12), substituted acyl_(C6-12), $-C(O)O(CH_2)_nS-A_3$, $-C(O)O(CH_2)_nS(O)-A_3$, or $-C(O)O(CH_2)_nS(O)_2-A_3$, wherein: A_3 is aryl_(C6-12) or substituted aryl_(C6-12); R_3 is

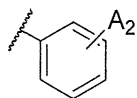
hydrogen, hydroxy, halo, amino, cyano, nitro, phosphate, or mercapto, or alkyl_(C1-12), alkenyl_(C2-12), alkynyl_(C2-12), aryl_(C6-12), aralkyl_(C7-12), heteroaryl_(C1-12), heterocycloalkyl_(C2-12), acyl_(C1-12), alkoxy_(C1-12), acyloxy_(C1-12), alkylamino_(C1-12), dialkylamino_(C2-12), amido_(C1-12), or a substituted version of any of these groups; o is 1 or 2; or a pharmaceutically acceptable salt thereof. In some embodimentsexamples, the formula is further defined as:

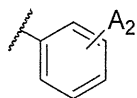


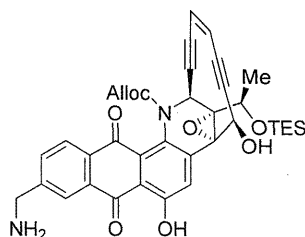
wherein: X₁ is a hydroxy protecting group; R₁ and R₂ are each independently selected from hydrogen, alkyl_(C1-12), or substituted alkyl_(C1-12); and R₃ is hydrogen, alkoxy_(C1-12), or substituted alkoxy_(C1-12); and o is 2; or a pharmaceutically acceptable salt thereof. In some embodimentsexamples, the formula is formula I. In some embodimentsexamples, the formula is formula II. In some embodimentsexamples, Y₁ is taken together with Z₁ and is -alkanediyl_(C≤6)-NZ₂-alkanediyl_(C≤6). In some embodimentsexamples, Y₁ is taken together with Z₁ and is -CH₂CH₂-NZ₂-CH₂CH₂-. In some embodimentsexamples, Z₂ is hydrogen. In other embodimentsexamples, Z₂ is an amine protecting group. In some embodimentsexamples, the amine protecting group is 2-(trimethylsilyl)ethoxycarbonyl or t-butoxycarbonyl. In some embodimentsexamples, Z₂ is -C(O)O(CH₂)_nS-C₆H₅. In some embodimentsexamples, Z₂ is -C(O)O(CH₂)_nS(O)₂-C₆H₅. In some embodimentsexamples, Z₁ is hydrogen. In some embodimentsexamples, Y₁ is -O(CH₂)_mY', -S(CH₂)_mY', or -(CH₂)_mNR₁R₂. In some embodimentsexamples, Y₁ is -(CH₂)_mNR₁R₂. In some embodimentsexamples, m is 1, 2, or 3. In some embodimentsexamples, m is 1 or 2. In some embodimentsexamples, R₁ is -C(O)O(CH₂)_nS-



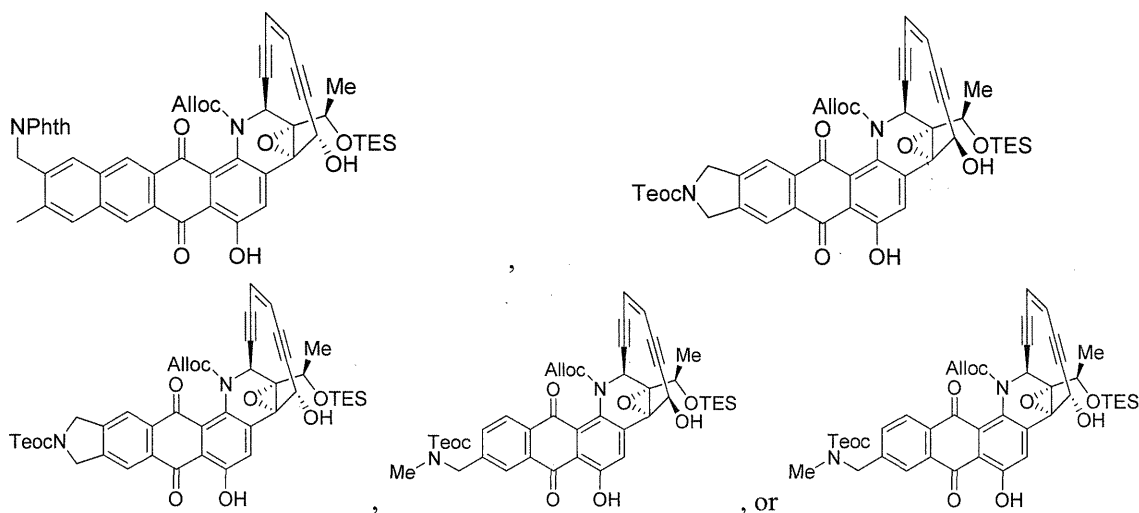
A₁ or -C(O)O(CH₂)_nS(O)₂-A₁, wherein: A₁ is aryl_(C≤12), substituted aryl_(C≤12), or acyloxy_(C≤12), substituted acyloxy_(C≤12), alkoxy_(C≤12), substituted alkoxy_(C≤12), alkylamino_(C≤12), substituted alkylamino_(C≤12), dialkylamino_(C≤12), or substituted dialkylamino_(C≤12), wherein A₂ is not -CO₂H, -CO₂CH₃, -OCH₃, -OCH₂CH₃, -C(O)CH₃, -NHCH₃, -NHCH₂CH₃, -N(CH₃)₂, -C(O)NH₂, and -OC(O)CH₃; and n is 1, 2, 3, 4, or 5. In some embodimentsexamples, R₁ is hydrogen. In other embodimentsexamples, R₁ is alkyl_(C≤12) or substituted alkyl_(C≤12). In some embodimentsexamples, R₁ is alkyl_(C≤12). In some embodimentsexamples, R₁ is methyl. In other embodimentsexamples, R₁ is a monovalent amine protecting group. In some embodimentsexamples, R₁ is 2-(trimethylsilyl)ethoxycarbonyl or t-butoxycarbonyl. In other embodimentsexamples, R₁ is -C(O)O(CH₂)₂SC₆H₅. In other embodimentsexamples, R₁ is -C(O)O(CH₂)₂S(O)₂C₆H₅. In other embodimentsexamples, R₁ is taken together with R₂ and is a divalent protecting group. In some embodimentsexamples, R₁ and R₂ is phthalimide or substituted phthalimide. In some embodimentsexamples, R₂ is hydrogen. In other embodimentsexamples, R₂ is alkyl_(C≤12) or substituted alkyl_(C≤12). In other embodimentsexamples, R₂ is alkyl_(C≤12). In some embodimentsexamples, R₂ is methyl. In other embodimentsexamples, R₂ is a monovalent amine protecting group. In some embodimentsexamples, R₂ is 2-(trimethylsilyl)ethoxycarbonyl or t-butoxycarbonyl. In other embodimentsexamples, R₂ is -C(O)O(CH₂)_nS-A₁ or -C(O)O(CH₂)_nS(O)₂-A₁, wherein: A₁ is



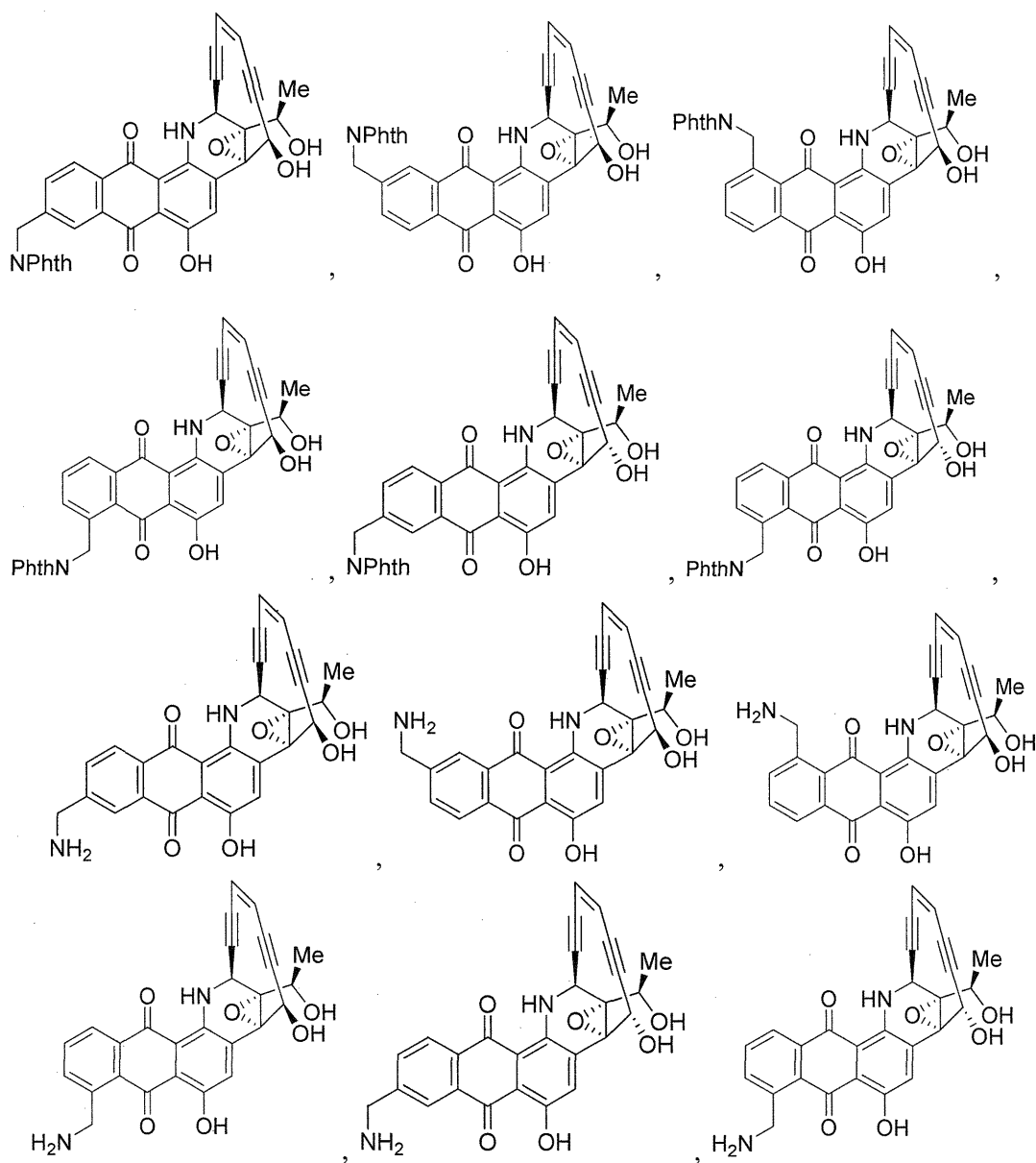
aryl_(C≤12), substituted aryl_(C≤12), or , wherein: A₂ is acyloxy_(C≤12), substituted acyloxy_(C≤12), alkoxy_(C≤12), substituted alkoxy_(C≤12), alkylamino_(C≤12), substituted alkylamino_(C≤12), dialkylamino_(C≤12), or substituted dialkylamino_(C≤12), wherein A₂ is not -CO₂H, -CO₂CH₃, -OCH₃, -OCH₂CH₃, -C(O)CH₃, -NHCH₃, -NHCH₂CH₃, -N(CH₃)₂, -C(O)NH₂, and -OC(O)CH₃; and n is 1, 2, 3, 4, or 5. In other embodimentsexamples, R₂ is -C(O)O(CH₂)₂SC₆H₅. In other embodimentsexamples, R₂ is -C(O)O(CH₂)₂S(O)₂C₆H₅. In other embodimentsexamples, R₂ is taken together with R₁ and is a divalent protecting group. In some embodimentsexamples, R₁ and R₂ are phthalimide or substituted phthalimide. In some embodimentsexamples, R₃ is hydrogen. In other embodimentsexamples, R₃ is alkoxy_(C≤12). In some embodimentsexamples, R₃ is methoxy. In some embodimentsexamples, R₄ is hydrogen. In other embodimentsexamples, R₄ is a monovalent amine protecting group. In some embodimentsexamples, R₄ is -C(O)CH₂CH=CH₂. In some embodimentsexamples, R₅ is alkyl_(C≤12). In some embodimentsexamples, R₅ is methyl. In some embodimentsexamples, R₆ is hydroxy. In some embodimentsexamples, R₇ is hydroxy. In some embodimentsexamples, R₈ is hydroxy. In some embodimentsexamples, R₉ is amino. In other embodimentsexamples, R₉ is alkyl_(C≤12). In some embodimentsexamples, R₉ is methyl. In other embodimentsexamples, R₉ is Y₂-R₁₂. In some embodimentsexamples, Y₂ is alkanediyl_(C≤12). In some embodimentsexamples, Y₂ is -CH₂-. In some embodimentsexamples, R₁₂ is amino. In other embodimentsexamples, R₁₂ is -NX₂X₃. In some embodimentsexamples, X₂ and X₃ are taken together and form a phthalimide. In other embodimentsexamples, X₂ and X₃ are taken together and form a substituted phthalimide. In other embodimentsexamples, X₂ is hydrogen. In some embodimentsexamples, X₃ is t-butoxycarbonyl. In some embodimentsexamples, R₁₀ is amino. In other embodimentsexamples, R₁₀ is alkyl_(C≤12). In some embodimentsexamples, R₁₀ is methyl. In other embodimentsexamples, R₁₀ is Y₂-R₁₂. In some embodimentsexamples, Y₂ is alkanediyl_(C≤12). In some embodimentsexamples, Y₂ is -CH₂-. In some embodimentsexamples, R₁₂ is amino. In other embodimentsexamples, R₁₂ is -NX₂X₃. In some embodimentsexamples, X₂ and X₃ are taken together and form a phthalimide. In other embodimentsexamples, X₂ and X₃ are taken together and form a substituted phthalimide. In other embodimentsexamples, X₂ is hydrogen. In other embodimentsexamples, X₃ is t-butoxycarbonyl. In some embodimentsexamples, R₁₁ is hydrogen. In some embodimentsexamples, carbon atom 17 is in the R configuration. In some embodimentsexamples, carbon atom 17 is in the S configuration. In some embodimentsexamples, the compound is further defined as:

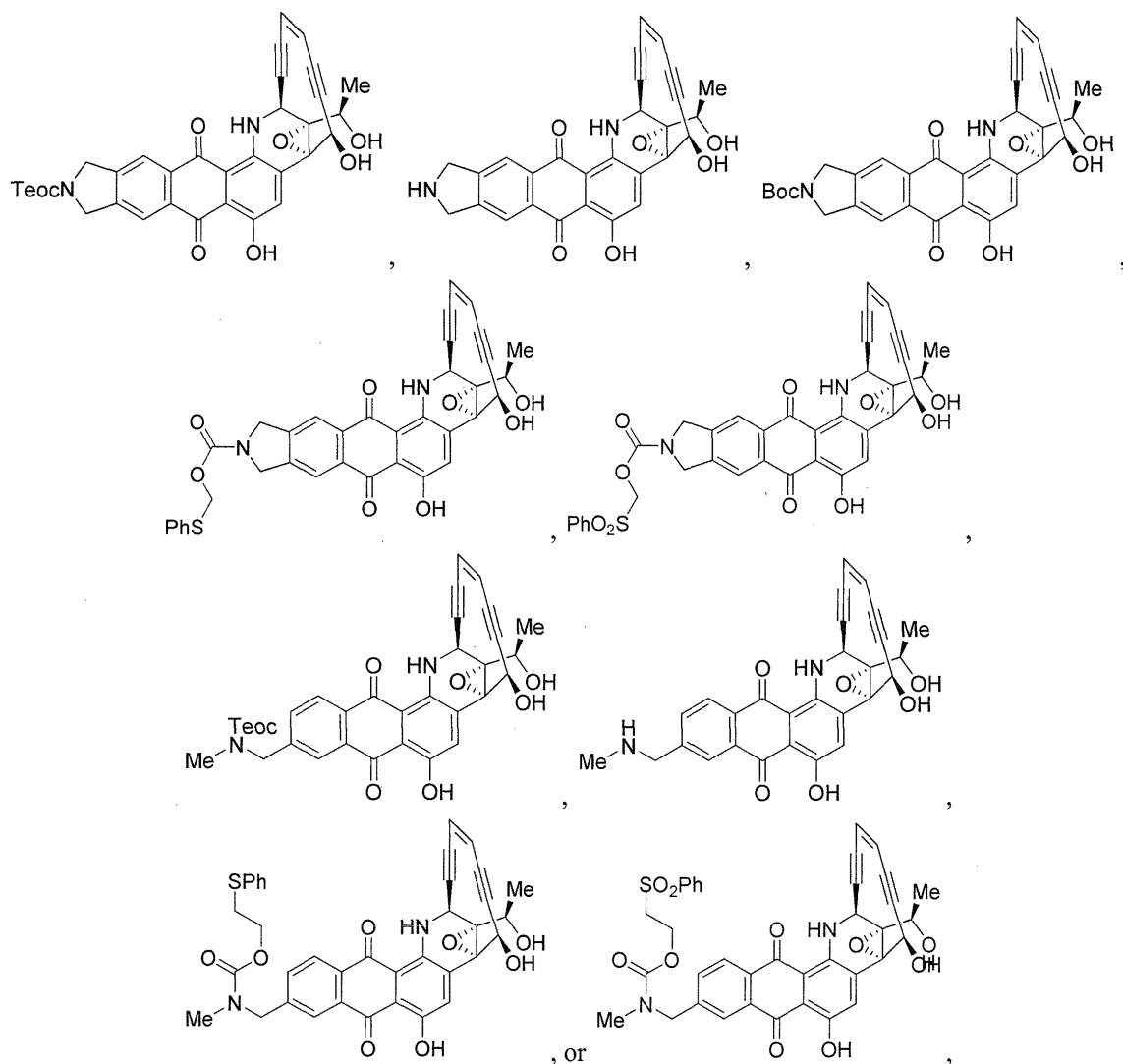


or a pharmaceutically acceptable salt thereof. In some embodimentsexamples, the compound is further defined as:



or a pharmaceutically acceptable salt thereof. In some ~~embodiments~~ examples, the compound is further defined as:



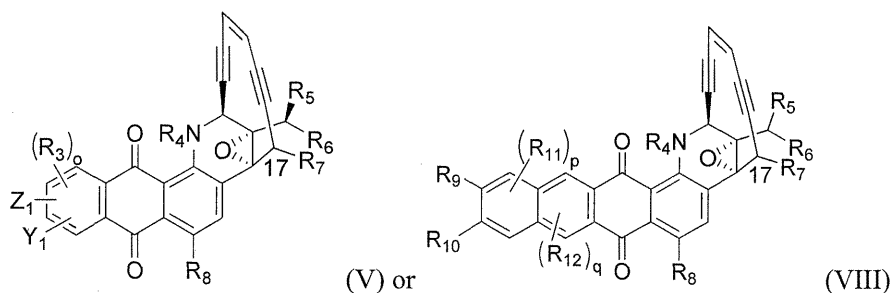


5 or a pharmaceutically acceptable salt thereof.

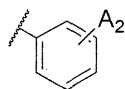
10 In another ~~aspect~~example, the present disclosure ~~provides~~discloses a pharmaceutical composition comprising a compound and an excipient. In some ~~embodiments~~examples, the composition is formulated for oral, intraadiposal, intraarterial, intraarticular, intracranial, intradermal, intralesional, intramuscular, intranasal, intraocular, intrapericardial, intraperitoneal, intrapleural, intraprostatic, intrarectal, intrathecal, intratracheal, intratumoral, intraumbilical, intravaginal, intravenous, intravesicular, intravitreal, liposomal, local, mucosal, parenteral, rectal, subconjunctival, subcutaneous, sublingual, topical, transbuccal, transdermal, vaginal, via a catheter, via a lavage, via continuous infusion, via infusion, via inhalation, via injection, via local delivery, or via localized perfusion administration.

15 In yet another ~~aspect~~example, the present disclosure ~~provides~~discloses a method of preparing a compound of the formula:

alkenyl_(C2-12), alkynyl_(C2-12), aryl_(C6-12), aralkyl_(C7-12), heteroaryl_(C1-12), heterocycloalkyl_(C2-12), acyl_(C1-12), alkoxy_(C1-12), acyloxy_(C1-12), alkylamino_(C1-12), dialkylamino_(C2-12), alkylthio_(C1-12), amido_(C1-12), or a substituted version of any of these groups; or Y₂-R₁₃; wherein: X₁ is a hydroxy protecting group; X₂ and X₃ are independently selected from hydrogen, a monovalent amine protecting group, or when X₂ and X₃ are taken together form a divalent amine protecting group; X₄ is a thiol protecting group; Y₂ is alkanediyl_(C1-12) or substituted alkanediyl_(C1-12); or R₁₃ is hydroxy, amino, mercapto, -OX₁, -NX₂X₃, or -SX₄, or heteroaryl_(C6-12), heterocycloalkyl_(C2-12), acyl_(C1-12), alkoxy_(C1-12), acyloxy_(C1-12), alkylamino_(C1-12), dialkylamino_(C2-12), alkylthio_(C1-12), amido_(C1-12), or a substituted version of any of these groups; and p and q are each independently 1 or 2; in the presence of a strong non-nucleophilic base in a solvent under conditions sufficient to cause a reaction. In some embodimentsexamples, Y₁ is not -NH₂, -NHMe, or -NHCH₂CH₂NH₂. In some embodiment, the base is lithium bis(trimethylsilyl)amide, sodium bis(trimethylsilyl)amide, potassium bis(trimethylsilyl)amide, lithium diisopropyl amide, sodium diisopropyl amide, potassium diisopropyl amide, lithium tetramethylpiperidide, lithium t-butoxide, sodium t-butoxide, or potassium t-butoxide, lithium hydride, sodium hydride, or potassium hydride. In some embodimentsexamples, the base is lithium bis(trimethylsilyl)amide. In some embodimentsexamples, the solvent is tetrahydrofuran. In some embodimentsexamples, the method further comprises admixing the compound of formula IV or formula V with the base before contacting the compound of formula III with the compound of formula IV or formula V. In some embodimentsexamples, the compound of formula III is dissolved in THF and added via cannula. In some embodimentsexamples, the method further comprising dissolving the compound of formula I or formula II in an organic solvent and reacting the compound of the formula:



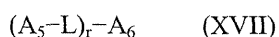
wherein: Y₁ is -O(CH₂)_mY', -NH(CH₂)_mY', -S(CH₂)_mY', or -(CH₂)_mNR₁R₂, or is taken together with Z₁ as described below; wherein: Y' is hydroxy, halo, mercapto, alkyl_(C1-12), substituted alkyl_(C1-12), alkenyl_(C2-12), substituted alkenyl_(C2-12), alkynyl_(C2-12), substituted alkynyl_(C2-12), acyl_(C1-12), substituted acyl_(C1-12), acyloxy_(C1-12), substituted acyloxy_(C1-12), alkylamino_(C1-12), or substituted alkylamino_(C1-12); m is 1, 2, 3, 4, 5, or 6; and R₁ and R₂ are each independently selected from hydrogen, hydroxy, alkyl_(C1-12), substituted alkyl_(C1-12), alkenyl_(C2-12), substituted alkenyl_(C2-12), alkynyl_(C2-12), substituted alkynyl_(C2-12), aryl_(C6-12), substituted aryl_(C6-12), aralkyl_(C7-12), substituted aralkyl_(C7-12), heteroaryl_(C1-12), substituted heteroaryl_(C1-12), heterocycloalkyl_(C2-12), substituted heterocycloalkyl_(C2-12), acyl_(C1-12), substituted acyl_(C1-12), acyloxy_(C1-12), substituted acyloxy_(C1-12), alkylamino_(C1-12), substituted alkylamino_(C1-12); a monovalent amine protecting group, -C(O)O(CH₂)_nS-A₁, -C(O)O(CH₂)_nS(O)-A₁, or -C(O)O(CH₂)_nS(O)₂-A₁, wherein: A₁ is aryl_(C6-12), substituted aryl_(C6-12), or



, wherein: A₂ is alkenyl_(C2-12), substituted alkenyl_(C2-12), alkynyl_(C2-12), substituted alkynyl_(C2-12),

$-\text{OCH}_3$, $-\text{OCH}_2\text{CH}_3$, $-\text{C}(\text{O})\text{CH}_3$, $-\text{NHCH}_3$, $-\text{NHCH}_2\text{CH}_3$, $-\text{N}(\text{CH}_3)_2$, $-\text{C}(\text{O})\text{NH}_2$, and $-\text{OC}(\text{O})\text{CH}_3$; Z_1 is absent, hydrogen or taken together with Y_1 as defined above; R_3 is hydrogen, hydroxy, halo, amino, cyano, nitro, phosphate, or mercapto, or $\text{alkyl}_{(\text{C}1-12)}$, $\text{alkenyl}_{(\text{C}2-12)}$, $\text{alkynyl}_{(\text{C}2-12)}$, $\text{aryl}_{(\text{C}6-12)}$, $\text{aralkyl}_{(\text{C}7-12)}$, $\text{heteroaryl}_{(\text{C}1-12)}$, $\text{heterocycloalkyl}_{(\text{C}2-12)}$, $\text{acyl}_{(\text{C}1-12)}$, $\text{alkoxy}_{(\text{C}1-12)}$, $\text{acyloxy}_{(\text{C}1-12)}$, $\text{alkylamino}_{(\text{C}1-12)}$, $\text{dialkylamino}_{(\text{C}2-12)}$, $\text{amido}_{(\text{C}1-12)}$, or a substituted version of any of these groups; o is 1 or 2; R_5 , R_6 , and R_7 are each independently hydrogen, hydroxy, amino, mercapto, $-\text{OX}_1$, $-\text{NX}_2\text{X}_3$, or $-\text{SX}_4$; or $\text{alkyl}_{(\text{C}1-12)}$, $\text{alkoxy}_{(\text{C}1-12)}$, $\text{acyloxy}_{(\text{C}1-12)}$, $\text{alkylamino}_{(\text{C}1-12)}$, $\text{dialkylamino}_{(\text{C}2-12)}$, $\text{alkylthio}_{(\text{C}1-12)}$, $\text{amido}_{(\text{C}1-12)}$, or a substituted version of any of these groups; wherein: X_1 is a hydroxy protecting group; X_2 and X_3 are independently selected from hydrogen, a monovalent amine protecting group, or when X_2 and X_3 are taken together form a divalent amine protecting group; X_4 is a thiol protecting group; R_8 is hydroxy, amino, or mercapto; or $\text{alkoxy}_{(\text{C}1-12)}$, $\text{acyloxy}_{(\text{C}1-12)}$, $\text{alkylamino}_{(\text{C}1-12)}$, $\text{dialkylamino}_{(\text{C}2-12)}$, $\text{alkylthio}_{(\text{C}1-12)}$, $\text{amido}_{(\text{C}1-12)}$, or a substituted version of any of these groups; and R_9 , R_{10} , R_{11} , and R_{12} are each independently selected from hydrogen, hydroxy, amino, mercapto, $-\text{OX}_1$, $-\text{NX}_2\text{X}_3$, or $-\text{SX}_4$, or $\text{alkyl}_{(\text{C}1-12)}$, $\text{alkenyl}_{(\text{C}2-12)}$, $\text{alkynyl}_{(\text{C}2-12)}$, $\text{aryl}_{(\text{C}6-12)}$, $\text{aralkyl}_{(\text{C}7-12)}$, $\text{heteroaryl}_{(\text{C}1-12)}$, $\text{heterocycloalkyl}_{(\text{C}2-12)}$, $\text{acyl}_{(\text{C}1-12)}$, $\text{alkoxy}_{(\text{C}1-12)}$, $\text{acyloxy}_{(\text{C}1-12)}$, $\text{alkylamino}_{(\text{C}1-12)}$, $\text{dialkylamino}_{(\text{C}2-12)}$, $\text{alkylthio}_{(\text{C}1-12)}$, $\text{amido}_{(\text{C}1-12)}$, or a substituted version of any of these groups; or Y_2-R_{13} ; wherein: X_1 is $\text{alkyl}_{(\text{C}1-12)}$, substituted $\text{alkyl}_{(\text{C}1-12)}$, $\text{alkenyl}_{(\text{C}2-12)}$, substituted $\text{alkenyl}_{(\text{C}2-12)}$, $\text{alkynyl}_{(\text{C}2-12)}$, substituted $\text{alkynyl}_{(\text{C}2-12)}$, $\text{aryl}_{(\text{C}6-12)}$, substituted $\text{aryl}_{(\text{C}6-12)}$, $\text{aralkyl}_{(\text{C}7-12)}$, substituted $\text{aralkyl}_{(\text{C}7-12)}$, $\text{heteroaryl}_{(\text{C}1-12)}$, substituted $\text{heteroaryl}_{(\text{C}1-12)}$, $\text{heterocycloalkyl}_{(\text{C}2-12)}$, substituted $\text{heterocycloalkyl}_{(\text{C}2-12)}$, $\text{acyl}_{(\text{C}1-12)}$, substituted $\text{acyl}_{(\text{C}1-12)}$, or a hydroxy protecting group; X_2 and X_3 are independently selected from hydrogen, $\text{alkyl}_{(\text{C}1-12)}$, substituted $\text{alkyl}_{(\text{C}1-12)}$, $\text{alkenyl}_{(\text{C}2-12)}$, substituted $\text{alkenyl}_{(\text{C}2-12)}$, $\text{alkynyl}_{(\text{C}2-12)}$, substituted $\text{alkynyl}_{(\text{C}2-12)}$, $\text{aryl}_{(\text{C}6-12)}$, substituted $\text{aryl}_{(\text{C}6-12)}$, $\text{aralkyl}_{(\text{C}7-12)}$, substituted $\text{aralkyl}_{(\text{C}7-12)}$, $\text{heteroaryl}_{(\text{C}1-12)}$, substituted $\text{heteroaryl}_{(\text{C}1-12)}$, $\text{heterocycloalkyl}_{(\text{C}2-12)}$, substituted $\text{heterocycloalkyl}_{(\text{C}2-12)}$, $\text{acyl}_{(\text{C}1-12)}$, substituted $\text{acyl}_{(\text{C}1-12)}$, a monovalent amine protecting group, or when X_2 and X_3 are taken together form a divalent amine protecting group; X_4 is $\text{alkyl}_{(\text{C}1-12)}$, substituted $\text{alkyl}_{(\text{C}1-12)}$, $\text{alkenyl}_{(\text{C}2-12)}$, substituted $\text{alkenyl}_{(\text{C}2-12)}$, $\text{alkynyl}_{(\text{C}2-12)}$, substituted $\text{alkynyl}_{(\text{C}2-12)}$, $\text{aryl}_{(\text{C}6-12)}$, substituted $\text{aryl}_{(\text{C}6-12)}$, $\text{aralkyl}_{(\text{C}7-12)}$, substituted $\text{aralkyl}_{(\text{C}7-12)}$, $\text{heteroaryl}_{(\text{C}1-12)}$, substituted $\text{heteroaryl}_{(\text{C}1-12)}$, $\text{heterocycloalkyl}_{(\text{C}2-12)}$, substituted $\text{heterocycloalkyl}_{(\text{C}2-12)}$, $\text{acyl}_{(\text{C}1-12)}$, substituted $\text{acyl}_{(\text{C}1-12)}$, or a thiol protecting group; Y_2 is $\text{alkanediyl}_{(\text{C}1-12)}$ or substituted $\text{alkanediyl}_{(\text{C}1-12)}$; and R_{13} is hydroxy, amino, mercapto, $-\text{OX}_1$, $-\text{NX}_2\text{X}_3$, or $-\text{SX}_4$, or $\text{heteroaryl}_{(\text{C}1-12)}$, $\text{heterocycloalkyl}_{(\text{C}2-12)}$, $\text{acyl}_{(\text{C}1-12)}$, $\text{alkoxy}_{(\text{C}1-12)}$, $\text{acyloxy}_{(\text{C}1-12)}$, $\text{alkylamino}_{(\text{C}1-12)}$, $\text{dialkylamino}_{(\text{C}2-12)}$, $\text{alkylthio}_{(\text{C}1-12)}$, $\text{amido}_{(\text{C}1-12)}$, or a substituted version of any of these groups; and p and q are each independently 1 or 2. In some embodimentsexamples, the palladium salt further comprises a palladium phosphine complex or a palladium salt in the presence of a ligand or other metal complexes or salts. In some embodimentsexamples, the palladium salt is $\text{Pd}(\text{PPh}_3)_4$. In some embodimentsexamples, the base is triethylamine, diisopropylethylamine, ethylenediamine, 1,8-diazabicyclo[5.4.0]undec-7-ene, or morpholine. In some embodimentsexamples, the base is morpholine. In some embodimentsexamples, the solvent is tetrahydrofuran. In some embodimentsexamples, the method further comprises removing one or more protecting groups.

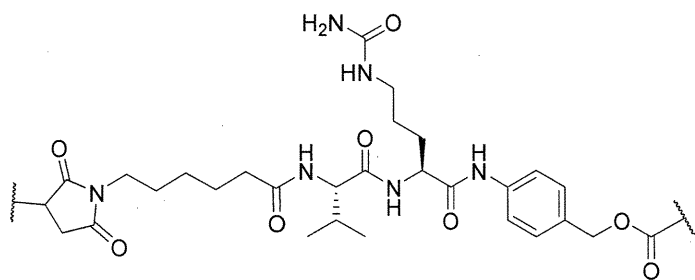
In yet another aspeetexample, the present disclosure provides-discloses a conjugate of the formula:



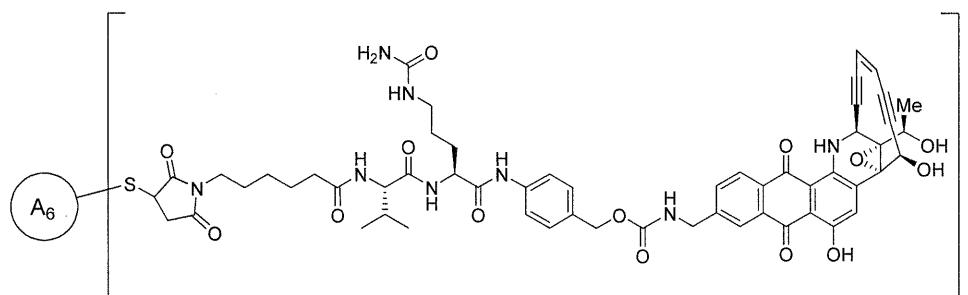
wherein: A_5 is a compound of claim 1; L is a linker; r is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12; and A_6 is a cell targeting moiety. In some embodimentsexamples, L comprises a polypeptide cleavable by an intracellular enzyme.

In

some ~~embodiment~~examples, the enzyme is cathepsin B. In some ~~embodiment~~examples, L comprises a self-immolating group. In some ~~embodiment~~examples, L is:



In some ~~embodiment~~examples, A₆ is an antibody whose antigen is a tumor associated antigen. In some ~~embodiment~~examples, the antigen is mesothelin, glypican-3, or CD70. In some ~~embodiment~~examples, A₅ is a compound according to formula (I). In some ~~embodiment~~examples, the conjugate further comprising a structure of the formula:



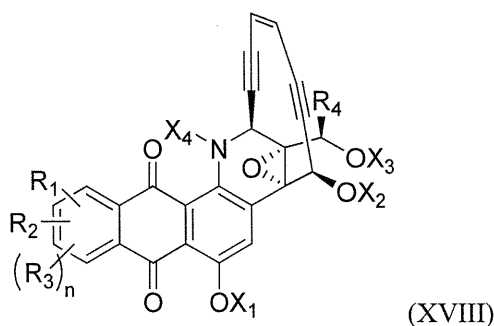
wherein: A₆ is an antibody and r is 1, 2, 3, or 4. In some ~~embodiment~~examples, the antibody is an anti-mesothelin, anti-glypican-3, or anti-CD70 antibody.

In still another ~~example~~aspect, the present disclosure ~~provides~~describes a pharmaceutical composition comprising a conjugate of the present disclosure and an excipient. In some ~~embodiment~~examples, the compound is formulated for oral, intraadiposal, intraarterial, intraarticular, intracranial, intradermal, intralesional, intramuscular, intranasal, intraocular, intrapericardial, intraperitoneal, intrapleural, intraprostatic, intrarectal, intrathecal, intratracheal, intratumoral, intraumbilical, intravaginal, intravenous, intravesicularl, intravitreal, liposomal, local, mucosal, parenteral, rectal, subconjunctival, subcutaneous, sublingual, topical, transbuccal, transdermal, vaginal, via a catheter, via a lavage, via continuous infusion, via infusion, via inhalation, via injection, via local delivery, or via localized perfusion administration.

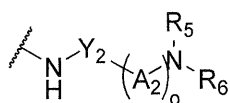
In another ~~example~~aspect, the present disclosure ~~provides~~describes a method of treating a disease or disorder comprising administering to a patient in need thereof a pharmaceutically effective amount of a compound, conjugate, or composition of the present disclosure. In some ~~embodiment~~examples, the disease or disorder is cancer. In some ~~embodiment~~examples, the cancer is of the bladder, blood, bone, brain, breast, central nervous system, cervix, colon, endometrium, esophagus, gall bladder, genitalia, genitourinary tract, head, kidney, larynx, liver, lung, muscle tissue, neck, oral or nasal mucosa, ovary, pancreas, prostate, skin, spleen, small intestine, large intestine, stomach, testicle, or thyroid. In other ~~embodiment~~examples, the cancer is a carcinoma, sarcoma, leukemia, melanoma, mesothelioma, multiple myeloma, or seminoma. In some ~~embodiment~~examples, the cancer is lung cancer, gastric cancer, ovarian cancer, liver cancer, renal cancer, or breast cancer. In some ~~embodiment~~examples, the cancer is H226 lung cancer, N87 gastric cancer, OVCAR3 ovarian cancer, Hep3B liver cancer, HepG2 liver cancer,

786-O renal cancer, or ADR multidrug resistant breast cancer. In some ~~embodiment~~examples, the cancer exhibit one or more tumor markers. In some ~~embodiment~~examples, the tumor marker is mesothelin, prostate specific membrane antigen, CD19, CD22, CD30, CD70, B7H4, protein tyrosine kinase 7, glypican-3, RG1, CTLA-4, or CD44. In some ~~embodiment~~examples, the tumor marker is mesothelin, glypican-3, or CD70. In some ~~embodiment~~examples, the method comprises administering a compound or a composition of a compound of the present disclosure. In some ~~embodiment~~examples, the method comprises administering a conjugate or a composition of the present disclosure. In some ~~embodiment~~examples, the compound comprises an antibody. In some ~~embodiment~~examples, the antibody binds to an antigen on the surface of the cancer cell. In some ~~embodiment~~examples, the binding of the antibody to the antigen on the cell surface triggers the internalization of the compound. In some ~~embodiment~~examples, the internalization of the compound triggers cleavage of a therapeutic compound from the antibody. In some ~~embodiment~~examples, the method further comprises administering a second therapeutic agent. In some ~~embodiment~~examples, the second therapeutic agent is a second chemotherapeutic agent, surgery, a radiotherapy, a genetic therapy, or an immunotherapy. In some ~~embodiment~~examples, the method comprises administering the compound once. In other ~~embodiment~~examples, the method comprises administering the compound two or more times. In some ~~embodiment~~examples, the disease or disorder is a bacterial infection. In some ~~embodiment~~examples, the bacterial infection is an infection of *Staphylococcus aureus*, *Escherichia coli*, or *Burkholderia cepacia*. In some ~~embodiment~~examples, the bacterial infection is an infection of *Burkholderia cepacia*.

In still another example~~aspect~~, the present disclosure ~~provides~~describes a method of preparing a compound of the formula:

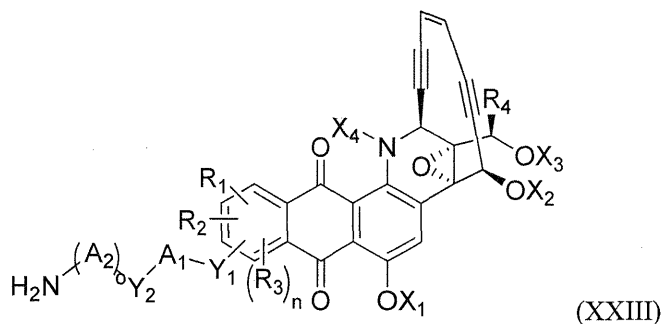


wherein: X_1 , X_2 , X_3 are hydrogen or a hydroxy protecting group; X_4 is hydrogen or an amino protecting group; R_1 and R_2 are each independently absent, hydrogen, or R_1 and R_2 are taken together and are alkylaminodiy_(C1-12), substituted alkylaminodiy_(C1-12); alkenediy_(C2-12), or substituted alkenediy_(C2-12); R_3 is hydrogen, amino, carboxy, cyano, halo, hydroxy, or alkyl_(C1-12), acyl_(C1-12), amido_(C1-12), alkoxy_(C1-12), alkylamino_(C1-12), dialkylamino_(C2-12), or a substituted version of any of these groups; or $-Y_1-A_1$; wherein: Y_1 is alkanediy_(C1-8) or substituted alkanediy_(C1-8); A_1 is a linker wherein the linker has the formula:

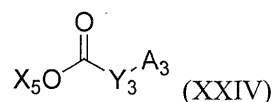


wherein: R_5 is hydrogen, alkyl_(C1-6), or substituted alkyl_(C1-6); R_6 is $-C(O)-Y_3-A_3$, wherein: Y_3 is alkanediy_(C1-12) or substituted alkanediy_(C1-12); and A_3 is a thiol reactive group; Y_2 is $-C(O)-$, $-C(O)-$ alkanediy_(C1-12); substituted $-C(O)-$ alkanediy_(C1-12), or a self immolating group; A_2 is a covalent bond, an amino acid residue,

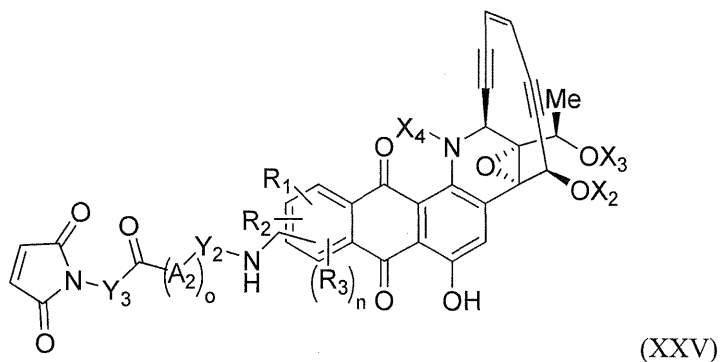
wherein: R_1 , R_2 , R_3 , R_4 , X_1 , X_2 , X_3 , X_4 , n , o , Y_1 , Y_2 , A_1 , A_2 , and R_5 are as defined above; c) deprotecting one or more functional groups on the compound of formula XXII comprising reacting the compound with a reagent which removes the protecting group under conditions sufficient to cause a reaction to form a compound of the formula:



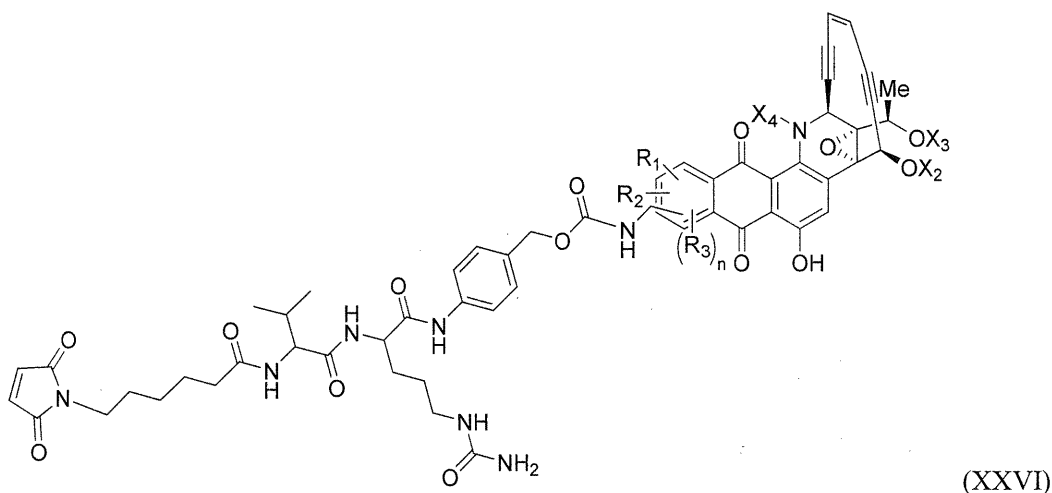
wherein: R_1 , R_2 , R_3 , R_4 , X_1 , X_2 , X_3 , X_4 , n , o , Y_1 , Y_2 , A_1 , and A_2 are as defined above; d) reacting the compound of formula XXIII with a compound of the formula:



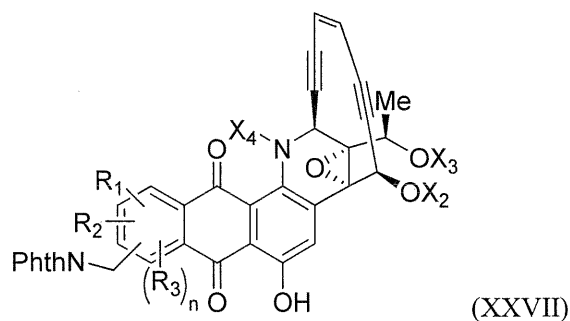
wherein: X_5 is hydrogen or an activating agent; Y_3 is alkanediyl_(C1-12) or substituted alkanediyl_(C1-12); and A_3 is a thiol reactive group; in the presence of a base in solvent under conditions sufficient to cause a reaction to form a compound of formula XVIII. In some embodiments, the compound of the formula is further defined as:



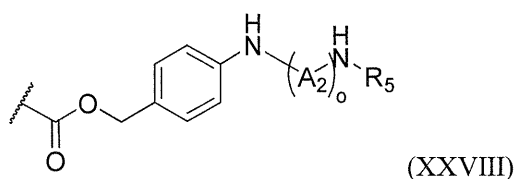
wherein: X_2 and X_3 are hydrogen or a hydroxy protecting group; X_4 is hydrogen or an amino protecting group; R_1 and R_2 are each independently absent, hydrogen, or R_1 and R_2 are taken together and are alkylaminodiyl_(C1-8); substituted alkylaminodiyl_(C1-8); alkenediyl_(C2-8), or substituted alkenediyl_(C2-8); R_3 is hydrogen, amino, carboxy, cyano, halo, hydroxy, or alkoxy_(C1-12) or substituted alkoxy_(C1-12); or Y_2 is $-C(O)-$, $-C(O)-$ alkanediyl_(C1-12), substituted $-C(O)-$ alkanediyl_(C1-12), or a self immolating group; A_2 is a covalent bond, an amino acid residue, or a polypeptide; and o is 1, 2, 3, 4, 5, 6, 7, or 8; n is 1, 2, or 3; and Y_3 is a covalent bond, alkanediyl_(C1-12), or substituted alkanediyl_(C1-12). In some embodiments, the compound of formula XXV is further defined as:



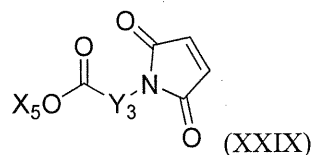
wherein: X_2 and X_3 are hydrogen or a hydroxy protecting group; X_4 is hydrogen or an amino protecting group; R_1 and R_2 are each independently absent, hydrogen, or R_1 and R_2 are taken together and are alkylaminodiyl_(C1-8); substituted alkylaminodiyl_(C1-8); alkenediyl_(C2-8), or substituted alkenediyl_(C2-8); R_3 is hydrogen, amino, carboxy, cyano, halo, hydroxy, or alkoxy_(C1-12) or substituted alkoxy_(C1-12); and n is 1, 2, or 3. In some embodimentsexamples, the compound of formula XIX is further defined as:



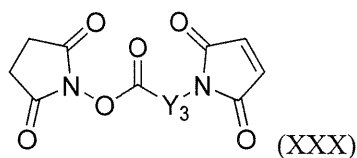
wherein: R_1 , R_2 , R_3 , n , X_2 , X_3 , and X_4 are as defined above. In some embodimentsexamples, the compound of formula XXI is further defined as:



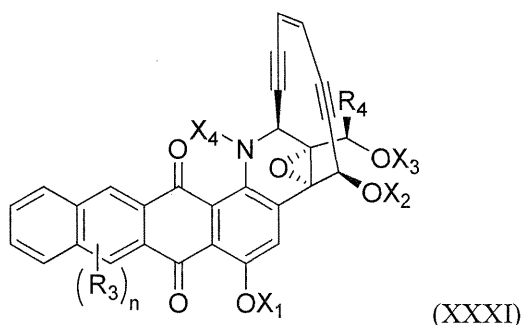
wherein: R_5 is an amine protecting group; A_2 is a covalent bond, an amino acid residue, or a polypeptide; and o is 1, 2, 3, 4, 5, 6, 7, or 8. In some embodimentsexamples, the compound of formula XXIV is further defined as:



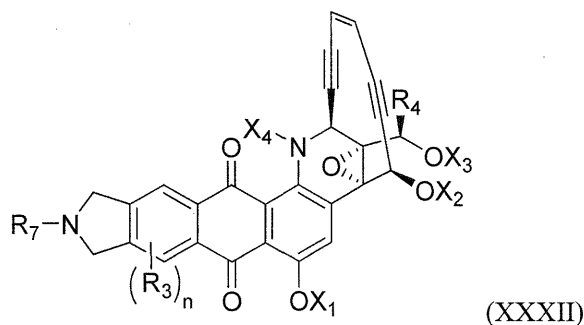
wherein: X_5 is hydrogen or an activating agent; and Y_3 is alkanediyl_(C1-12) or substituted alkanediyl_(C1-12). In some embodimentsexamples, the compound of formula XXIV is further defined as:



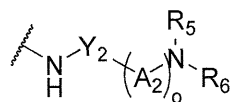
wherein: Y_3 is alkanediyl_(C1-12) or substituted alkanediyl_(C1-12). In some embodimentsexamples, R_1 is hydrogen. In other embodimentsexamples, R_1 is alkoxy_(C1-12). In some embodimentsexamples, R_1 is methoxy. In other embodimentsexamples, R_1 and R_2 are taken together and are alkenediyl_(C2-12) or substituted alkenediyl_(C2-12). In some embodimentsexamples, the formula is further defined as:



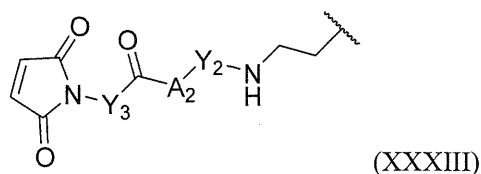
wherein: R_3 , R_4 , n , X_1 , X_2 , X_3 , and X_4 are as defined above. In other embodimentsexamples, R_1 and R_2 are taken together and are alkylaminodiyl_(C1-12) or substituted alkylaminodiyl_(C1-12). In some embodimentsexamples, the formula is further defined as:



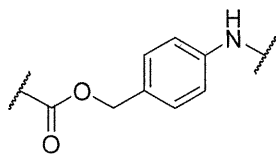
wherein: R_3 , R_4 , n , X_1 , X_2 , X_3 , and X_4 are as defined above; and R_7 is hydrogen or an amino protecting group. In some embodimentsexamples, R_3 is $-Y_1-A_1$; wherein: Y_1 is alkanediyl_(C1-8) or substituted alkanediyl_(C1-8); A_1 is a linker wherein the linker has the formula:



wherein: R_5 is hydrogen, alkyl_(C1-6), or substituted alkyl_(C1-6); R_6 is $-C(O)-Y_3-A_3$, wherein: Y_3 is alkanediyl_(C1-12) or substituted alkanediyl_(C1-12); and A_3 is a thiol reactive group; Y_2 is $-C(O)-$, $-C(O)-$ alkanediyl_(C1-12), substituted $-C(O)-$ alkanediyl_(C1-12), or a self immolating group; A_2 is a covalent bond, an amino acid residue, or a polypeptide; and o is 1, 2, 3, 4, 5, 6, 7, or 8. In some embodimentsexamples, R_3 is



wherein Y₂, A₂, and Y₃ are as defined above. In some embodimentsexamples, A₂ is –HN–Val–Cit–C(O)O–. In some embodimentsexamples, Y₂ is a self-immolating group. In some embodimentsexamples, Y₂ is



In some embodimentsexamples, R₄ is methyl. In some embodimentsexamples, X₁, X₂, or X₃ are hydrogen. In some embodimentsexamples, X₃ is a hydroxy protecting group. In some embodimentsexamples, X₄ is hydrogen. In some embodimentsexamples, X₄ is an amine protecting group. In some embodimentsexamples, the alkylamine_(C1-12) or substituted alkylamine_(C1-12) of step a) is methylamine. In some embodimentsexamples, the base of step b) is a nitrogenous base and the base is diisopropylethylamine. In some embodimentsexamples, the reagent which removes the protecting group of step c) is a base and the base is piperidine. In some embodimentsexamples, the base of step d) is a nitrogenous base and the base is diisopropylethylamine. In some embodimentsexamples, the method further comprises one or more deprotection steps. In other embodimentsexamples, these compounds of the present disclosure are envisioned to be joined to an antibody or other cell targeting moiety as disclosed in US Patent 8,798,431, US Patent Application No. 2013/0209494 and PCT Patent Application WO 2013/122823, all of which are incorporated herein by reference.

In the embodimentsexamples of this disclosure, it is contemplated that the conditions sufficient to cause a reaction to occur can encompass a variety of factors which effective the efficacy of the reaction. Modification of such is envisioned within the embodimentsexamples of any of the present methods. Such conditions include the number of equivalents of a compound, the choice of solvent, the choice of temperature, or the reaction time. In some exampleaspects of the present disclosure, the reactions of the present disclosure can be run in any organic solvents. In some embodimentsexamples, the solvent is an amide_(C1-12), an ester_(C1-12), an alcohol_(C1-12), an arene_(C1-12), a substituted arene_(C1-12), an alkane_(C1-12), a substituted alkane_(C1-12), a heterocycloalkane_(C1-12), or an ether_(C1-12). In some embodimentsexamples, the solvent of the present disclosure is diethyl ether, acetonitrile, dimethylformamide, tetrahydrofuran, dimethylsulfoxide, chloroform, dichloromethane, hexanes, methanol, ethanol, pentanes, benzene, toluene, ethyl acetate, or N-methyl-2-pyrrolidone. Furthermore, the conditions can affect the temperature of the reaction. In some embodimentsexamples, the temperature is at room temperature. In some embodimentsexamples, the room temperature or 25 °C can also be performed at a temperature from about 10 °C to about 40 °C or more preferably from about 20 °C to about 30 °C. In some embodimentsexamples, the temperature can be reduced to improve the efficacy of the reaction. In some embodimentsexamples, the reduced temperature is a temperature less than room temperature. In some embodimentsexamples, the temperature is either 0 °C or –78 °C. In some embodimentsexamples, the temperature is within 20 °C of the stated temperature and more preferably 10 °C of the stated temperature. In some embodimentsexamples, the temperature can be raised to speed the reaction. In some embodimentsexamples, the reaction temperature is a temperature is from about 50 °C to about 100 °C. In some embodimentsexamples, the temperature is within 20 °C of the stated temperature and more preferably 10 °C of the stated temperature. Additionally, the reaction time may be varied to increase the efficacy of the reaction. In some embodimentsexamples, the reaction is from about half the exemplified time and to about twice the exemplified time. By way of a non-limiting example, a reaction which has exemplified as reacting for 1 hour, the reaction

time is from about 30 minutes to 2 hours. Finally, the amount of equivalents added can also modify the efficacy of the reaction. In some ~~embodiment~~examples, the addition of a reagent is envisioned from about half the exemplified amount to about 3 times the exemplified amount. By way of a non-limiting example, the addition of the reagent is measured based upon one of the starting material containing a carbon atom which appears in the final product. Additionally, the equivalents of a reagent which is consumed (*i.e.* not catalytic) is envisioned to not be less than 1 equivalent.

It is contemplated that any method or composition described herein can be implemented with respect to any other method or composition described herein.

The use of the word “a” or “an” when used in conjunction with the term “comprising” in the claims and/or the specification may mean “one,” but it is also consistent with the meaning of “one or more,” “at least one,” and “one or more than one.” The word “about” means plus or minus 5% of the stated number.

Other objects, features and advantages of the present disclosure will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating specific embodiments of the disclosure, are given by way of illustration only, since various changes and modifications within the spirit and scope of the disclosure will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE FIGURES

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present disclosure. The disclosure may be better understood by reference to one or more of these drawings in combination with the detailed.

FIG. 1 – Synthesis of uncialamycin analogs

FIG. 2 – Uncialamycin and some examples of aminomethyl uncialamycin and other amine containing uncialamycin analogs.

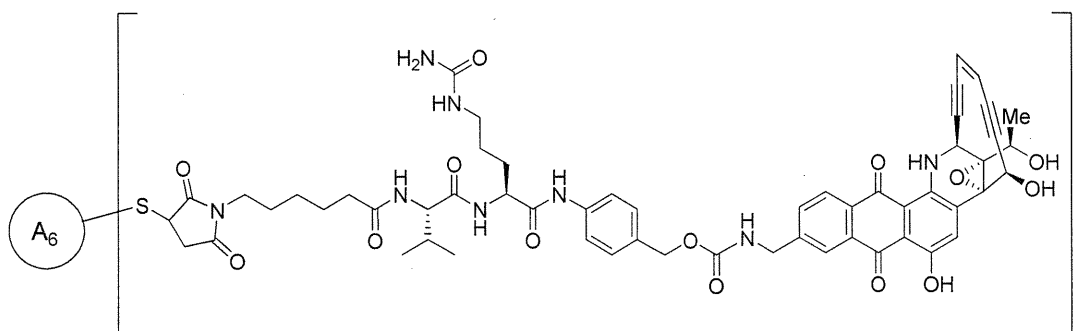
DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

The present disclosure provides new analogs of uncialamycin and provides improved methods of synthesizing uncialamycin and analogs. These compounds have been shown to have picomolar IC₅₀ in cancer cells. Furthermore, these analogs are functionalized with an amine group which can be used to modify cell targeting moieties to generate novel therapeutics such as but not limited to an antibody-drug conjugate.

I. Uncialamycin and Formulations Thereof

The compounds provided by the present disclosure are shown, for example, above in the Summary section and in the claims below. They may be made using the methods outlined in the Examples section. Uncialamycin and its analogs can be synthesized according to the methods described, for example, in the Examples section below. These methods can be further modified and optimized using the principles and techniques of organic chemistry as applied by a person skilled in the art. Such principles and techniques are taught, for example, in *March's Advanced Organic Chemistry: Reactions, Mechanisms, and Structure* (2007), which is incorporated by reference herein.

70. The conjugate of claim 69, wherein the antigen is mesothelin, glypican-3, or CD70.
71. The conjugate of claim 64, wherein A₅ is a compound according to formula I.
72. The conjugate of claim 64, further comprising a structure of the formula:



wherein:

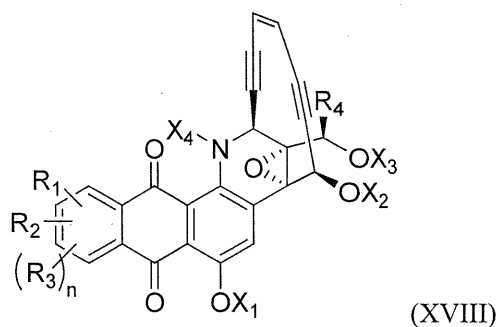
A₆ is an antibody and r is 1, 2, 3, or 4.

73. The conjugate of claim 72, wherein the antibody is an anti-mesothelin, anti-glypican-3, or anti-CD70 antibody.
74. A pharmaceutical composition comprising a conjugate according to any one of claims 64-73 and an excipient.
75. The pharmaceutical composition of claim 74, wherein the compound is formulated for oral, intraadiposal, intraarterial, intraarticular, intracranial, intradermal, intralesional, intramuscular, intranasal, intraocular, intrapericardial, intraperitoneal, intrapleural, intraprostatic, intrarectal, intrathecal, intratracheal, intratumoral, intraumbilical, intravaginal, intravenous, intravesicular, intravitreal, liposomal, local, mucosal, parenteral, rectal, subconjunctival, subcutaneous, sublingual, topical, transbuccal, transdermal, vaginal, via a catheter, via a lavage, via continuous infusion, via infusion, via inhalation, via injection, via local delivery, or via localized perfusion administration.
76. Use of a compound or composition of claims 1-49 or 64-75 in the manufacture of a medicament for A method of treating a disease or disorder, the use comprising a pharmaceutically effective amount of compound or composition of the claims 1-49 or 64-75 to be administered administering to a patient in need thereof a pharmaceutically effective amount of a compound or composition of the claims 1-49 or 64-75.
77. The method use of claim 76, wherein the disease or disorder is cancer.
78. The use method of claim 77, wherein the cancer is lung cancer, gastric cancer, ovarian cancer, liver cancer, renal cancer, or breast cancer.
79. The use method of claim 77, wherein the cancer exhibit one or more tumor markers.
80. The use method of either claim 79, wherein the tumor marker is mesothelin, glypican-3, or CD70.
81. The use method of claim 76, wherein the use method comprises administering a compound of claims 1-49 to be administered.
82. The use method of claim 76, wherein the use method comprises administering a compound of claims 64-75 to be administered.

83. The use method of claim 76, wherein the use method further comprises administering a second therapeutic agent to be administered.
84. The use method of claim 83, wherein the second therapeutic agent is a second chemotherapeutic agent, surgery, a radiotherapy, a genetic therapy, or an immunotherapy.
- 5 | 85. The use method of claim 76, wherein the disease or disorder is a bacterial infection.

86. The ~~use method~~ of claim 85, wherein the bacterial infection is an infection of *Staphylococcus aureus*, *Escherichia coli*, or *Burkholderia cepacia*.

87. A method of preparing a compound of the formula:



wherein:

X_1, X_2, X_3 are hydrogen or a hydroxy protecting group;

X_4 is hydrogen or an amino protecting group;

R_1 and R_2 are each independently absent, hydrogen, or

R_1 and R_2 are taken together and are alkylaminodiyl_(C1-12), substituted alkylaminodiyl_(C1-12); alkenediyl_(C2-12), or substituted alkenediyl_(C2-12);

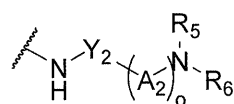
R_3 is hydrogen, amino, carboxy, cyano, halo, hydroxy, or

alkyl_(C1-12), acyl_(C1-12), amido_(C1-12), alkoxy_(C1-12), alkylamino_(C1-12), dialkylamino_(C2-12), or a substituted version of any of these groups; or

$-Y_1-A_1$; wherein:

Y_1 is alkanediyl_(C1-8) or substituted alkanediyl_(C1-8); and

A_1 is a linker wherein the linker has the formula:



wherein:

R_5 is hydrogen, alkyl_(C1-6), or substituted alkyl_(C1-6);

R_6 is $-C(O)-Y_3-A_3$, wherein:

Y_3 is alkanediyl_(C1-12) or substituted alkanediyl_(C1-12); and

A_3 is a thiol reactive group;

Y_2 is $-C(O)-$, $-C(O)-$ alkanediyl_(C1-12); substituted $-C(O)-$ alkanediyl_(C1-12), or a self immolating group;

A_2 is a covalent bond, an amino acid residue, or a polypeptide; and

o is 1, 2, 3, 4, 5, 6, 7, or 8;