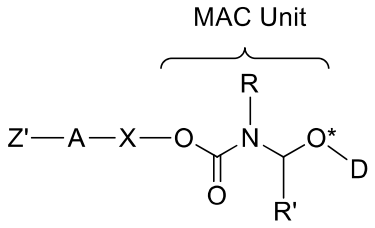
many aliphatic alcohol-containing drugs (see, for example, Toki, et al. *J. Org. Chem.* 2002, 67, 1866-1872). One reason for that may be due to the high pKa of aliphatic alcohol-containing drugs.

[0005] Therefore**,** a need exists for new linker technologies that can be used to attach drugs heretofore believed to be unsuitable for use as ADCs, and Ligand Drug Conjugates (LDCs) in general, including a need for more versatile methods for linking aromatic alcohol- and aliphatic alcohol-containing drugs to other targeting ligands in addition to antibodies. The present invention addresses those and other needs.

BRIEF SUMMARY

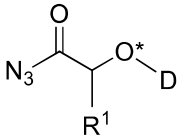
[0006] The disclosure provides *inter alia,* Ligand-Drug Conjugates, wherein the Ligand-Drug Conjugate is comprised of a Self-immolative Assembly Unit having a methylene carbamate unit for conjugation of a drug to a targeting ligand, methods of preparing and using them, and intermediates thereof. The Ligand-Drug Conjugates of the present disclosure are stable in circulation, yet capable of inflicting cell death once free drug is released from a Conjugate in the vicinity or within tumor cells.

[0006a] In one aspect, the present disclosure provides a method of preparing a Drug-Linker Compound, wherein the Drug-Linker compound has the structure of:



said method comprising the step of:

contacting a modified free drug having the structure of:



with an intermediate linker moiety represented by: Z'-A-X'-OH,

under conditions sufficient for providing the indicated MAC unit through Curtius rearrangement,

wherein D is a Drug Unit having a hydroxyl functional group that has been incorporated into the indicated MAC Unit, the oxygen atom from which is designated by O^*;

Z' is a stretcher unit precursor to a Stretcher Unit (Z) in a Ligand-Drug Conjugate and comprises a functional group capable of conjugation to a targeting ligand;

A is an optional Connector Unit;

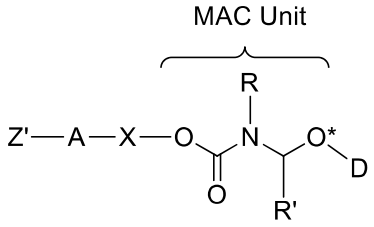
X is an activeatable self-immolative moiety, wherein X is activateable by a protease or glycosidase;

X' is a self-immolative moiety precursor to X, wherein X' has a hydroxyl functional group that participates in the Curtius rearrangement, and X' is otherwise inert to the Curtius rearrangement reaction conditions;

R is hydrogen; and

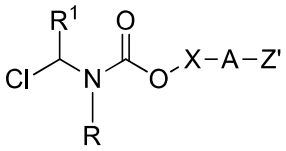
R^1 is hydrogen, or C_1-C_6 alkyl, C_6-C_{14} aryl or C-linked heteroaryl, optionally substituted with suitable protection as required.

[0006b] In another aspect, the present disclosure provides a method of preparing a Drug-Linker compound, wherein the Drug-Linker compound has the structure of:



said method comprising the step of:

contacting a drug having a free hydroxyl functional group with a N-chloromethylamine having the structure of:



under conditions sufficient for substitution of the chlorine atom with the oxygen heteroatom from said free drug functional group,

wherein

Z' is a Stretcher Unit precursor to a Stretcher Unit (Z) of the Drug-Linker Compound and comprises a functional group for attachment of a targeting ligand;

A is an optional Connector Unit;

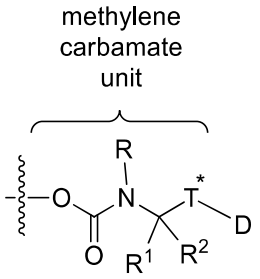
X is an activateable self-immolative moiety, wherein X is inert to the chlorine substitution reaction conditions, and X is activateable by a protease or glycosidase;

R is hydrogen, or C_1-C_6 alkyl, C_6-C_{14} aryl or C-linked heteroaryl, optionally substituted with suitable protection; and

R^1 is hydrogen, or C_1-C_6 alkyl, C_6-C_{14} aryl or C-linked heteroaryl, optionally substituted with suitable protection as required,

or R and R^1 together with the nitrogen and carbon atoms to which they are attached comprise a pyrrolodinyl or piperidinyl moiety.

[0007] In one principle example, a Ligand Drug Conjugate (LDC), or composition thereof, is comprised of a Ligand Unit, a Drug Unit and a Linker Unit that connects the Ligand Unit to the Drug Unit, wherein the Linker Unit is comprised of a Self-immolative Assembly Unit having a methylene carbamate unit and an activateable self-immolative moiety, wherein activation of the activateable self-immolative moiety results in release of free drug from the LDC from self-immolation, and wherein the methylene carbamate unit is covalently attached to the Drug Unit and the activateable self-immolative moiety, wherein the methylene carbamate unit covalently attached to the Drug Unit is represented by the structure of formula I:



(I)

or a pharmaceutically acceptable salt thereof, wherein

the wavy line indicates covalent attachment of the methylene carbamate unit to an activateable self-immolative moiety (X);

D is a Drug Unit having a functional group (e.g., hydroxyl, thiol, amide or amine functional group) that has been incorporated into the methylene carbamate unit,

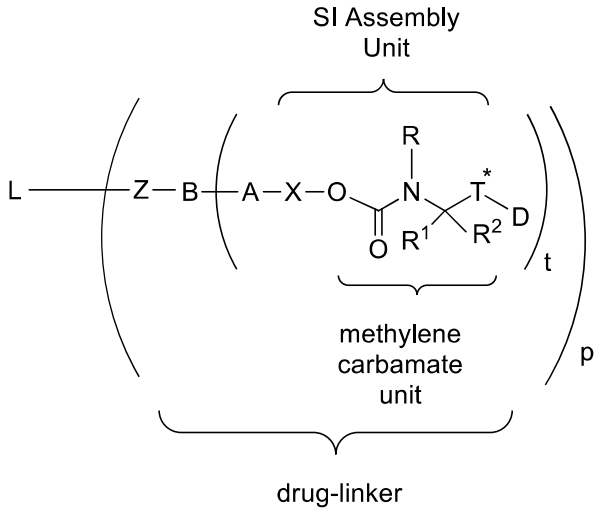
T* is a heteroatom from said functional group (e.g., oxygen, sulfur, optionally substituted nitrogen) that becomes incorporated into the methylene carbamate unit;

X is an activateable self-immolative moiety;

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

or R 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.

[0008] In some of those examples, a Ligand Drug Conjugate, or composition thereof, having the methylene carbamate unit of formula I is represented by the structure of formula II:



(II)

or a pharmaceutically acceptable salt thereof; wherein

L is a Ligand Unit;

Z is a Stretcher Unit;

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

A is an optional Connector Unit;

X is an activateable self-immolative moiety;

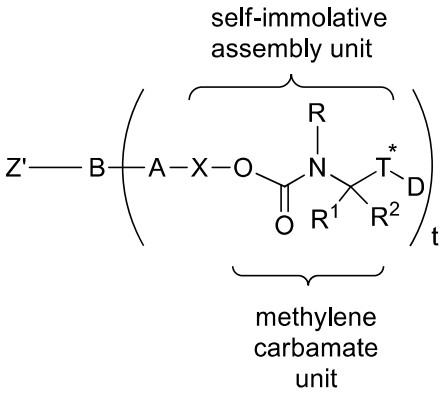
the subscript t ranges from 1 to 4;

the subscript p is an integer (for an individual LDC) or a number (for a population of LDCs in a LDC composition) ranging from 1 to 16; and

and D, T*, R, R^1 and R^2 are as defined in formula I.

[0009] Other principle examples are Drug-Linker Compounds useful as intermediates for preparing Ligand-Drug Conjugates, wherein the Drug-Linker Compound is comprised of a Drug Unit and a Linker Unit, wherein the Linker Unit is comprised of a Stretcher Unit precursor (Z') capable of forming a covalent bond to a targeting ligand that provides for a Ligand Unit, a Self-immolative Assembly Unit having a methylene carbamate unit and an activateable self-immolative moiety, wherein activation of the activateable self-immolative moiety in a LDC in which the Drug-Linker Compound is incorporated results in release of free drug from the LDC by self-immolation, and wherein the methylene carbamate unit is covalently attached to the Drug Unit and the activateable self-immolative moiety.

[0010] In some of those examples the Drug-Linker Compound having the methylene carbamate unit of formula I has the structure of formula V:



(V)

or a pharmaceutically acceptable salt thereof, wherein

Z' is a Stretcher Unit precursor to a Stretcher Unit (Z) and is comprised of a functional group that provides for covalent attachment of a Ligand Unit to Z;

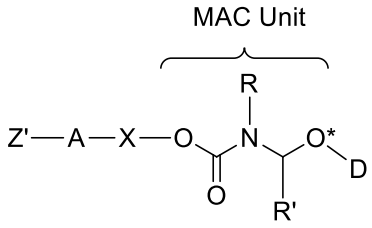
and B, A, X, R, R^1, R^2, T*, D, and the subscript t are as defined for Formula (II).

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] Figure 1 demonstrates the stability *ex vivo* in rat and mouse plasma of an ADC composition having an average drug loading of 4 in which the ADCs of the composition are comprised of a methylene alkoxy carbamate unit (MAC unit) that incorporates the oxygen heteroatom from the hydroxyl functional group of Auristatin E.

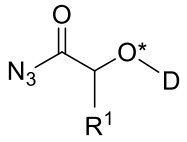
WHAT IS CLAIMED IS:

1. A method of preparing a Drug-Linker Compound, wherein the Drug-Linker compound has the structure of:



said method comprising the step of:

contacting a modified free drug having the structure of:



with an intermediate linker moiety represented by: Z'-A-X'-OH,

under conditions sufficient for providing the indicated MAC unit through Curtius rearrangement,

wherein D is a Drug Unit having a hydroxyl functional group that has been incorporated into the indicated MAC Unit, the oxygen atom from which is designated by O^*;

Z' is a stretcher unit precursor to a Stretcher Unit (Z) in a Ligand-Drug Conjugate and comprises a functional group capable of conjugation to a targeting ligand;

A is an optional Connector Unit;

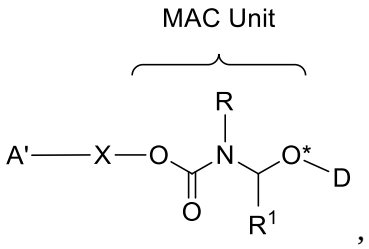
X is an activeatable self-immolative moiety, wherein X is activateable by a protease or glycosidase;

X' is a self-immolative moiety precursor to X, wherein X' has a hydroxyl functional group that participates in the Curtius rearrangement, and X' is otherwise inert to the Curtius rearrangement reaction conditions;

R is hydrogen; and

R^1 is hydrogen, or C_1-C_6 alkyl, C_6-C_{14} aryl or C-linked heteroaryl, optionally substituted with suitable protection as required.

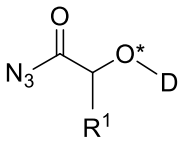
2. A method of preparing an intermediate of a Drug-Linker compound having the structure of claim 1, wherein the intermediate has the structure of:



,

said method comprising the step of:

contacting a modified free drug having the structure of:



with a self-immolative intermediate represented by: A'-X-OH,

under conditions sufficient for providing the indicated MAC Unit through Curtius rearrangement,

wherein D is a Drug Unit having a hydroxyl functional group that has been incorporated into the indicated MAC Unit, the oxygen atom from which is designated by O^*;

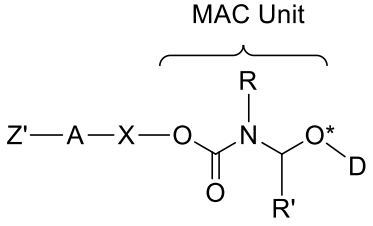
A' is a Connector Unit precursor to a Connector Unit (A) and comprises a functional group for bond formation to the remainder of the Linker Unit of the Drug-Linker compound;

X is an activeatable self-immolative moiety, wherein X is inert to the Curtius rearrangement reaction conditions, and X is activateable by a protease or glycosidase;

R is hydrogen; and

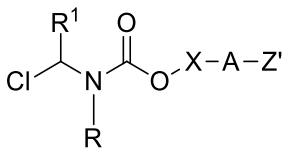
R^1 is hydrogen, or C_1-C_6 alkyl, C_6-C_{14} aryl or C-linked heteroaryl, optionally substituted with suitable protection as required.

3. A method of preparing a Drug-Linker compound, wherein the Drug-Linker compound has the structure of:



said method comprising the step of:

contacting a drug having a free hydroxyl functional group with a N-chloromethylamine having the structure of:



under conditions sufficient for substitution of the chlorine atom with the oxygen heteroatom from said free drug functional group,

wherein

Z' is a Stretcher Unit precursor to a Stretcher Unit (Z) of the Drug-Linker Compound and comprises a functional group for attachment of a targeting ligand;

A is an optional Connector Unit;

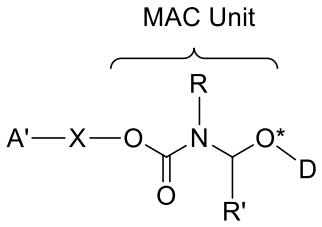
X is an activateable self-immolative moiety, wherein X is inert to the chlorine substitution reaction conditions, and X is activateable by a protease or glycosidase;

R is hydrogen, or C_1-C_6 alkyl, C_6-C_{14} aryl or C-linked heteroaryl, optionally substituted with suitable protection; and

R^1 is hydrogen, or C_1-C_6 alkyl, C_6-C_{14} aryl or C-linked heteroaryl, optionally substituted with suitable protection as required,

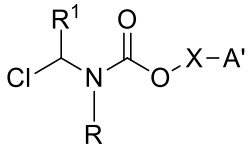
or R and R^1 together with the nitrogen and carbon atoms to which they are attached comprise a pyrrolodinyl or piperidinyl moiety.

4. A method of preparing an intermediate of a Drug-Linker Compound having the structure of claim 1, wherein the intermediate has the structure of:



said method comprising the step of:

contacting a drug having a free hydroxyl functional group with a N-chloromethylamine having the structure of:



under conditions sufficient for substitution of the chlorine atom with the oxygen heteroatom from said free drug functional group,

wherein

Z' is a Stretcher Unit precursor to a Stretcher Unit (Z) of the Drug-Linker Compound and comprises a functional group for attachment of a targeting ligand;

A’ is a Connector Unit precursor to a Connector Unit (A) and comprises a functional group for bond formation to the remainder of the Linker Unit of the Drug-Linker compound;

X is an activatable self-immolative moiety, wherein X is inert to the chlorine substitution reaction conditions, and X is activateable by a protease or glycosidase;

R is hydrogen, or C_1-C_6 alkyl, C_6-C_{14} aryl or C-linked heteroaryl, optionally substituted with suitable protection; and

R^1 is hydrogen, or C_1-C_6 alkyl, C_6-C_{14} aryl or C-linked heteroaryl, optionally substituted with suitable protection as required,

or R and R^1 together with the nitrogen and carbon atoms to which they are attached comprise a pyrrolodinyl or piperidinyl moiety.