

METHODS AND COMPOUNDS FOR PREPARING CC-1065 ANALOGS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Patent Application No. 5 60/730,804, filed October 26, 2005, which is incorporated herein by reference in its entirety.

BACKGROUND

CC-1065 is known to be a potent cytotoxin. CC-1065 was first isolated from 10 *Streptomyces zelensis* in 1981 by the Upjohn Company (Hanka *et al.*, *J. Antibiot.* **31**: 1211 (1978); Martin *et al.*, *J. Antibiot.* **33**: 902 (1980); Martin *et al.*, *J. Antibiot.* **34**: 1119 (1981)) and was found to have potent antitumor and antimicrobial activity both *in vitro* and in experimental animals (Li *et al.*, *Cancer Res.* **42**: 999 (1982)). CC-1065 binds to double-stranded B-DNA within the minor groove (Swenson *et al.*, *Cancer Res.* **42**: 2821 (1982)) 15 with the sequence preference of 5'-d(A/GNTTA)-3' and 5'-d(AAAAA)-3' and alkylates the N3 position of the 3'-adenine by its CPI left-hand unit present in the molecule (Hurley *et al.*, *Science* **226**: 843 (1984)). Despite its potent and broad antitumor activity, CC-1065 cannot be used in humans because it causes delayed death in experimental animals.

Many analogs and derivatives of CC-1065 are known in the art. The research into 20 the structure, synthesis and properties of many of the compounds has been reviewed. See, for example, Boger *et al.*, *Angew. Chem. Int. Ed. Engl.* **35**: 1438 (1996); and Boger *et al.*, *Chem. Rev.* **97**: 787 (1997).

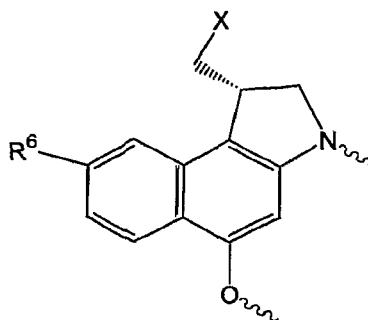
A group at Kyowa Hakko Kogyo Co., Ltd. has prepared a number of CC-1065 derivatives. See, for example, U.S. Pat. No. 5,101,038; 5,641,780; 5,187,186; 5,070,092; 25 5,703,080; 5,070,092; 5,641,780; 5,101,038; and 5,084,468; and published PCT application, WO 96/10405 and published European application 0 537 575 A1.

The Upjohn Company (Pharmacia Upjohn) has also been active in preparing derivatives of CC-1065. See, for example, U.S. Patent No. 5,739,350; 4,978,757, 5,332, 837 and 4,912,227.

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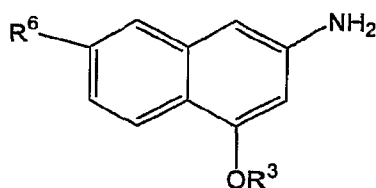
BRIEF SUMMARY

One embodiment is a method of making a compound (I) or a salt thereof,



(I)

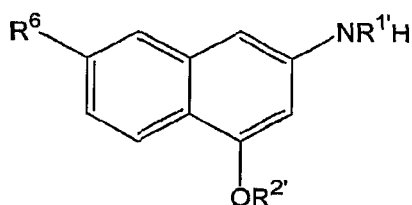
where R^1 and R^2 are each independently H, alkyl, $-C(O)OR'$, $-C(O)NR'R''$, or a protecting group, where R' and R'' are independently selected from the group consisting of H,
 5 substituted alkyl, unsubstituted alkyl, substituted aryl, unsubstituted aryl, substituted heteroaryl, unsubstituted heteroaryl, substituted heterocycloalkyl, and unsubstituted heterocycloalkyl; R^6 is H, substituted or unsubstituted lower alkyl, cyano, or alkoxy; and X is halogen. In this method, protecting groups $R^{1'}$ and $R^{2'}$ are added to a compound (II)



(II)

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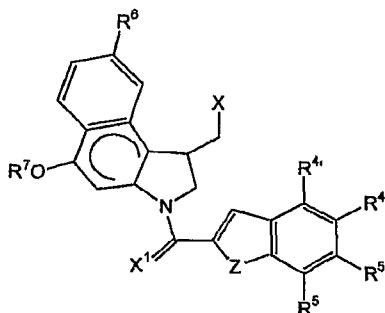
to form a compound (III)



(III)

wherein R^3 is H or alkyl. A five membered ring is generated comprising the amine
 15 nitrogen of compound (III).

Another embodiment is a method of making a CBI CC-1065 analog, or a pharmaceutically acceptable salt thereof, having the following formula:



where X is halo;

X^1 and Z are each independently selected from O, S and NR^8 , where R^8 is a member selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, and acyl;

R^4 , R^{4a} , R^5 and R^{5a} are members independently selected from the group consisting of H, substituted alkyl, unsubstituted alkyl, substituted aryl, unsubstituted aryl, substituted heteroaryl, unsubstituted heteroaryl, substituted heterocycloalkyl, unsubstituted heterocycloalkyl, halogen, NO_2 , NR^9R^{10} , $NC(O)R^9$, $OC(O)NR^9R^{10}$, $OC(O)OR^9$, $C(O)R^9$, SR^9 , OR^9 , $CR^9=NR^{10}$, and $O(CH_2)_nNR^{11}R^{11'}$,

where R^9 and R^{10} are independently selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted heterocycloalkyl, and substituted or unsubstituted peptidyl or where R^9 and R^{10} together with the nitrogen atom to which they are attached are optionally joined to form a substituted or unsubstituted heterocycloalkyl ring system having from 4 to 6 members, optionally containing two or more heteroatoms, and

R^{11} and $R^{11'}$ are each independently H or lower alkyl;

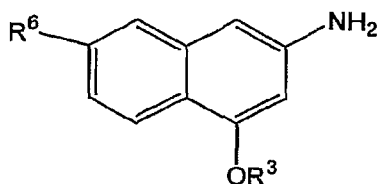
R^6 is H, substituted or unsubstituted lower alkyl, cyano, or alkoxy; and

R^7 is a member selected from the group consisting of H, substituted alkyl, unsubstituted alkyl, substituted heteroalkyl, unsubstituted heteroalkyl, diphosphates, triphosphates, acyl, $C(O)R^{12}R^{13}$, $C(O)OR^{12}$, $C(O)NR^{12}R^{13}$, $P(O)(OR^{12})_2$, $C(O)CHR^{12}R^{13}$, SR^{12} and $SiR^{12}R^{13}R^{14}$,

where R^{12} , R^{13} , and R^{14} are members independently selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl and substituted or unsubstituted aryl, or where R^{12} and R^{13} together with the nitrogen or carbon atom to which they are attached are optionally joined to form a substituted or unsubstituted

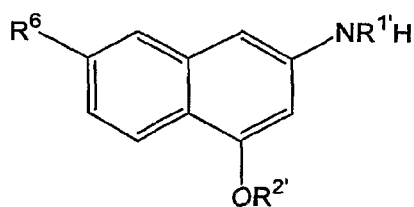
heterocycloalkyl ring system having from 4 to 6 members, optionally containing two or more heteroatoms.

The method includes adding protecting groups $R^{1'}$ and $R^{2'}$ to a compound (II)



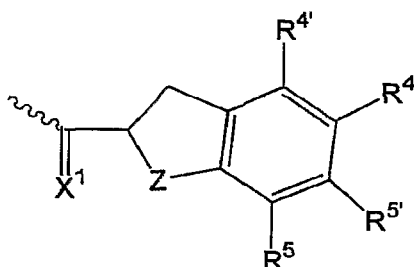
(II)

to form a compound (III)

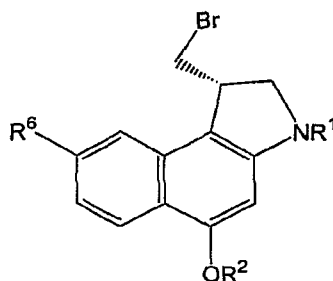


(III)

where R^3 is H or alkyl. A five membered ring is generated comprising the amine nitrogen of compound (III). A binding unit is added to compound (III), the binding unit comprising



Yet another embodiment is a compound of formula (I), or a pharmaceutically acceptable salt thereof:



(I)

where R^1 and R^2 are each independently H, alkyl, $-C(O)OR'$, $-C(O)NR'R''$, or a protecting group, where R' and R'' are independently selected from the group consisting of H,

substituted alkyl, unsubstituted alkyl, substituted aryl, unsubstituted aryl, substituted heteroaryl, unsubstituted heteroaryl, substituted heterocycloalkyl, and unsubstituted heterocycloalkyl.

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BRIEF DESCRIPTION OF THE DRAWINGS

Non-limiting and non-exhaustive embodiments of the present invention are described with reference to the following drawings. In the drawings, like reference numerals refer to like parts throughout the various figures unless otherwise specified.

For a better understanding of the present invention, reference will be made to the following Detailed Description, which is to be read in association with the accompanying drawings, wherein:

FIG. 1 is a synthetic scheme for one embodiment of a method of forming a CBI CC-1065 analog;

FIG. 2 is a synthetic scheme for another embodiment of a method of forming a CBI CC-1065 analog; and

FIG. 3 is a synthetic scheme for a third embodiment of a method of forming a CBI CC-1065 analog.

DETAILED DESCRIPTION

As used herein, "Boc" refers to *t*-butyloxycarbonyl.

"CPI" refers to cyclopropapyrroloindole.

"CBI" refers to cyclopropabenzindole.

"Cbz" is carbobenzoxy.

"DCM," refers to dichloromethane.

"DMF" is N,N-dimethylformamide.

"Fmoc" refers to 9-fluorenylmethyloxycarbonyl.

"TEA" refers to triethylamine.

"THF" refers to tetrahydrofuran.

"EDC" refers to 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride.

Unless defined otherwise, all technical and scientific terms used herein generally have the same meaning as commonly understood by one of ordinary skill in the art to

which this invention belongs. Generally, the nomenclature used herein and the laboratory procedures in cell culture, molecular genetics, organic chemistry and nucleic acid chemistry and hybridization described below are those well known and commonly employed in the art. The techniques and procedures are generally performed according to conventional methods in the art and various general references. The nomenclature used herein and the laboratory procedures in analytical chemistry, and organic synthetic described below are those well known and commonly employed in the art. Standard techniques, or modifications thereof, are used for chemical syntheses and chemical analyses.

The term "therapeutic agent" is intended to mean a compound that, when present in a therapeutically effective amount, produces a desired therapeutic effect on a mammal. For treating carcinomas, it is desirable that the therapeutic agent also be capable of entering the target cell.

The term "cytotoxin" is intended to mean a therapeutic agent having the desired effect of being cytotoxic to cancer cells. Cytotoxic means that the agent arrests the growth of, or kills, the cells.

The terms "prodrug" and "drug conjugate" are used herein interchangeably. Both refer to a compound that is relatively innocuous to cells while still in the conjugated form but which is selectively degraded to a pharmacologically active form by conditions, e.g., enzymes, located within or in the proximity of target cells.

The symbol $\sim$, whether utilized as a bond or displayed perpendicular to a bond indicates the point at which the displayed moiety is attached to the remainder of the molecule, solid support, substituent, etc.

The term "alkyl," by itself or as part of another substituent, means, unless otherwise stated, a straight or branched chain, or cyclic hydrocarbon radical, or combination thereof, which may be fully saturated, mono- or polyunsaturated and can include di- and multivalent radicals, having the number of carbon atoms designated (*i.e.* C₁-C₁₀ means one to ten carbons). Examples of saturated hydrocarbon radicals include, but are not limited to, groups such as methyl, ethyl, n-propyl, isopropyl, n-butyl, t-butyl, isobutyl, sec-butyl, cyclohexyl, (cyclohexyl)methyl, cyclopropylmethyl, homologs and isomers of, for example, n-pentyl, n-hexyl, n-heptyl, n-octyl, and the like. An unsaturated alkyl group is one having one or more double bonds or triple bonds. Examples of unsaturated alkyl groups include, but are not limited to, vinyl, 2-propenyl, crotyl, 2-isopentenyl, 2-

(butadienyl), 2,4-pentadienyl, 3-(1,4-pentadienyl), ethynyl, 1- and 3-propynyl, 3-butyryl, and the higher homologs and isomers. The term "alkyl," unless otherwise noted, is also meant to include those derivatives of alkyl defined in more detail below, such as "heteroalkyl." Alkyl groups, which are limited to hydrocarbon groups are termed

5 "homoalkyl".

The term "alkylene" by itself or as part of another substituent means a divalent radical derived from an alkane, as exemplified, but not limited, by $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-$, and further includes those groups described below as "heteroalkylene." Typically, an alkyl (or alkylene) group will have from 1 to 24 carbon atoms, with those groups having 10 or fewer

10 carbon atoms being preferred in the present invention. A "lower alkyl" or "lower alkylene" is a shorter chain alkyl or alkylene group, generally having eight or fewer carbon atoms.

The term "heteroalkyl," by itself or in combination with another term, means, unless otherwise stated, a stable straight or branched chain, or cyclic hydrocarbon radical, or combinations thereof, consisting of a number of carbon atoms and at least one

15 heteroatom selected from the group consisting of O, N, Si and S, and wherein the nitrogen, carbon and sulfur atoms may optionally be oxidized and the nitrogen heteroatom may optionally be quaternized. The heteroatom(s) O, N, S and Si may be placed at any interior position of the heteroalkyl group or at the position at which the alkyl group is attached to

20 the remainder of the molecule. Examples include, but are not limited to, $-\text{CH}_2\text{-CH}_2\text{-O-CH}_3$, $-\text{CH}_2\text{-CH}_2\text{-NH-CH}_3$, $-\text{CH}_2\text{-CH}_2\text{-N(CH}_3\text{)-CH}_3$, $-\text{CH}_2\text{-S-CH}_2\text{-CH}_3$, $-\text{CH}_2\text{-CH}_2\text{-S(O)-CH}_3$, $-\text{CH}_2\text{-CH}_2\text{-S(O)}_2\text{-CH}_3$, $-\text{CH=CH-O-CH}_3$, $-\text{Si(CH}_3\text{)}_3$, $-\text{CH}_2\text{-CH=N-OCH}_3$, and $-\text{CH=CH-N(CH}_3\text{)-CH}_3$. Up to two heteroatoms may be consecutive, such as, for example, $-\text{CH}_2\text{-NH-OCH}_3$ and $-\text{CH}_2\text{-O-Si(CH}_3\text{)}_3$. Similarly, the term "heteroalkylene" by itself or as

25 part of another substituent means a divalent radical derived from heteroalkyl, as exemplified, but not limited by, $-\text{CH}_2\text{-CH}_2\text{-S-CH}_2\text{-CH}_2-$ and $-\text{CH}_2\text{-S-CH}_2\text{-CH}_2\text{-NH-CH}_2-$. For heteroalkylene groups, heteroatoms can also occupy either or both of the chain termini (e.g., alkyleneoxy, alkylenedioxy, alkyleneamino, alkylenediamino, and the like). The terms "heteroalkyl" and "heteroalkylene" encompass poly(ethylene glycol) and its

30 derivatives (see, for example, Shearwater Polymers Catalog, 2001). Still further, for alkylene and heteroalkylene substituents, no orientation of the substituent is implied by the direction in which the formula of the substituent is written. For example, the formula $-\text{C(O)}_2\text{R}'$ represents both $-\text{C(O)}_2\text{R}'$ and $-\text{R}'\text{C(O)}_2$.

The term "lower" in combination with the terms "alkyl" or "heteroalkyl" refers to a moiety having from 1 to 6 carbon atoms.

The terms "alkoxy," "alkylamino," "alkylsulfonyl," and "alkylthio" (or thioalkoxy) are used in their conventional sense, and refer to those alkyl groups attached to the remainder of the molecule via an oxygen atom, an amino group, an SO₂ group or a sulfur atom, respectively. The term "arylsulfonyl" refers to an aryl group attached to the remainder of the molecule via an SO₂ group, and the term "sulfhydryl" refers to an SH group.

In general, an "acyl" substituent is also selected from the group set forth above. As used herein, the term "acyl" substituent refers to groups attached to, and fulfilling the valence of a carbonyl carbon that is either directly or indirectly attached to the polycyclic nucleus of the compounds of the present invention.

The terms "cycloalkyl" and "heterocycloalkyl", by themselves or in combination with other terms, represent, unless otherwise stated, cyclic versions of substituted or unsubstituted "alkyl" and substituted or unsubstituted "heteroalkyl", respectively. Additionally, for heterocycloalkyl, a heteroatom can occupy the position at which the heterocycle is attached to the remainder of the molecule. Examples of cycloalkyl include, but are not limited to, cyclopentyl, cyclohexyl, 1-cyclohexenyl, 3-cyclohexenyl, cycloheptyl, and the like. Examples of heterocycloalkyl include, but are not limited to, 1-(1,2,5,6-tetrahydropyridyl), 1-piperidinyl, 2-piperidinyl, 3-piperidinyl, 4-morpholinyl, 3-morpholinyl, tetrahydrofuran-2-yl, tetrahydrofuran-3-yl, tetrahydrothien-2-yl, tetrahydrothien-3-yl, 1-piperazinyl, 2-piperazinyl, and the like. The heteroatoms and carbon atoms of the cyclic structures are optionally oxidized.

The terms "halo" or "halogen," by themselves or as part of another substituent, mean, unless otherwise stated, a fluorine, chlorine, bromine, or iodine atom. Additionally, terms such as "haloalkyl," are meant to include monohaloalkyl and polyhaloalkyl. For example, the term "halo(C₁-C₄)alkyl" is meant to include, but not be limited to, trifluoromethyl, 2,2,2-trifluoroethyl, 4-chlorobutyl, 3-bromopropyl, and the like.

The term "aryl" means, unless otherwise stated, a substituted or unsubstituted polyunsaturated, aromatic, hydrocarbon substituent which can be a single ring or multiple rings (preferably from 1 to 3 rings) which are fused together or linked covalently. The term "heteroaryl" refers to aryl groups (or rings) that contain from one to four heteroatoms selected from N, O, and S, wherein the nitrogen, carbon and sulfur atoms are optionally

oxidized, and the nitrogen atom(s) are optionally quaternized. A heteroaryl group can be attached to the remainder of the molecule through a heteroatom. Non-limiting examples of aryl and heteroaryl groups include phenyl, 1-naphthyl, 2-naphthyl, 4-biphenyl, 1-pyrrolyl, 2-pyrrolyl, 3-pyrrolyl, 3-pyrazolyl, 2-imidazolyl, 4-imidazolyl, pyrazinyl, 2-oxazolyl, 4-oxazolyl, 2-phenyl-4-oxazolyl, 5-oxazolyl, 3-isoxazolyl, 4-isoxazolyl, 5-isoxazolyl, 2-thiazolyl, 4-thiazolyl, 5-thiazolyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-pyrimidyl, 4-pyrimidyl, 5-benzothiazolyl, purinyl, 2-benzimidazolyl, 5-indolyl, 1-isoquinolyl, 5-isoquinolyl, 2-quinoxaliny, 5-quinoxaliny, 3-quinolyl, and 6-quinolyl. Substituents for each of the above noted aryl and heteroaryl ring systems are selected from the group of acceptable substituents described below. "Aryl" and "heteroaryl" also encompass ring systems in which one or more non-aromatic ring systems are fused, or otherwise bound, to an aryl or heteroaryl system.

For brevity, the term "aryl" when used in combination with other terms (*e.g.*, aryloxy, arylthioxy, arylalkyl) includes both aryl and heteroaryl rings as defined above. Thus, the term "arylalkyl" is meant to include those radicals in which an aryl group is attached to an alkyl group (*e.g.*, benzyl, phenethyl, pyridylmethyl and the like) including those alkyl groups in which a carbon atom (*e.g.*, a methylene group) has been replaced by, for example, an oxygen atom (*e.g.*, phenoxymethyl, 2-pyridyloxymethyl, 3-(1-naphthyloxy)propyl, and the like).

Each of the above terms (*e.g.*, "alkyl," "heteroalkyl," "aryl" and "heteroaryl") include both substituted and unsubstituted forms of the indicated radical. Preferred substituents for each type of radical are provided below.

Substituents for the alkyl, and heteroalkyl radicals (including those groups often referred to as alkylene, alkenyl, heteroalkylene, heteroalkenyl, alkynyl, cycloalkyl, heterocycloalkyl, cycloalkenyl, and heterocycloalkenyl) are generally referred to as "alkyl substituents" and "heteroalkyl substituents," respectively, and they can be one or more of a variety of groups selected from, but not limited to: -OR', =O, =NR', =N-OR', -NR'R'', -SR', -halogen, -SiR'R''R''', -OC(O)R', -C(O)R', -CO₂R', -CONR'R'', -OC(O)NR'R'', -NR''C(O)R', -NR'-C(O)NR''R''', -NR''C(O)₂R', -NR-C(NR'R''R''')=NR''', -NR-C(NR'R'')=NR''', -S(O)R', -S(O)₂R', -S(O)₂NR'R'', -NRSO₂R', -CN and -NO₂ in a number ranging from zero to (2m'+1), where m' is the total number of carbon atoms in such radical. R', R'', R''' and R'''' each preferably independently refer to hydrogen, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, *e.g.*, aryl

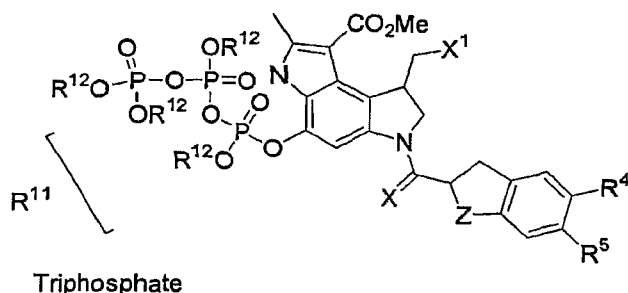
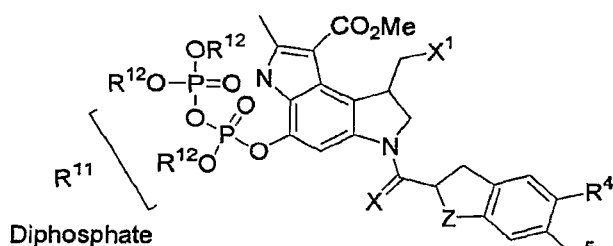
substituted with 1-3 halogens, substituted or unsubstituted alkyl, alkoxy or thioalkoxy groups, or arylalkyl groups. When a compound of the invention includes more than one R group, for example, each of the R groups is independently selected as are each R', R'', R''' and R'''' groups when more than one of these groups is present. When R' and R'' are
 5 attached to the same nitrogen atom, they can be combined with the nitrogen atom to form a 5-, 6-, or 7-membered ring. For example, -NR'R'' is meant to include, but not be limited to, 1-pyrrolidinyl and 4-morpholinyl. From the above discussion of substituents, one of skill in the art will understand that the term "alkyl" is meant to include groups including carbon atoms bound to groups other than hydrogen groups, such as haloalkyl (*e.g.*, -CF₃
 10 and -CH₂CF₃) and acyl (*e.g.*, -C(O)CH₃, -C(O)CF₃, -C(O)CH₂OCH₃, and the like).

Similar to the substituents described for the alkyl radical, the aryl substituents and heteroaryl substituents are generally referred to as "aryl substituents" and "heteroaryl substituents," respectively and are varied and selected from, for example: halogen, -OR', =O, =NR', =N-OR', -NR'R'', -SR', -halogen, -SiR'R''R''', -OC(O)R', -C(O)R', -CO₂R', -
 15 CONR'R'', -OC(O)NR'R'', -NR''C(O)R', -NR'-C(O)NR''R'', -NR''C(O)₂R', -NR-C(NR'R'')=NR''', -S(O)R', -S(O)₂R', -S(O)₂NR'R'', -NRSO₂R', -CN and -NO₂, -R', -N₃, -CH(Ph)₂, fluoro(C₁-C₄)alkoxy, and fluoro(C₁-C₄)alkyl, in a number ranging from zero to the total number of open valences on the aromatic ring system; and where R', R'', R''' and R'''' are preferably independently selected from hydrogen, (C₁-C₈)alkyl and
 20 heteroalkyl, unsubstituted aryl and heteroaryl, (unsubstituted aryl)-(C₁-C₄)alkyl, and (unsubstituted aryl)oxy-(C₁-C₄)alkyl. When a compound of the invention includes more than one R group, for example, each of the R groups is independently selected as are each R', R'', R''' and R'''' groups when more than one of these groups is present.

Two of the aryl substituents on adjacent atoms of the aryl or heteroaryl ring may
 25 optionally be replaced with a substituent of the formula -T-C(O)-(CRR')_q-U-, wherein T and U are independently -NR-, -O-, -CRR'- or a single bond, and q is an integer of from 0 to 3. Alternatively, two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula -A-(CH₂)_r-B-, wherein A and B are independently -CRR'-, -O-, -NR-, -S-, -S(O)-, -S(O)₂-, -S(O)₂NR'- or a single bond,
 30 and r is an integer of from 1 to 4. One of the single bonds of the new ring so formed may optionally be replaced with a double bond. Alternatively, two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula -(CRR')_s-X-(CR''R''')_d-, where s and d are independently integers of from 0

to 3, and X is -O-, -NR'-, -S-, -S(O)-, -S(O)₂-, or -S(O)₂NR'-. The substituents R, R', R'' and R''' are preferably independently selected from hydrogen or substituted or unsubstituted (C₁-C₆) alkyl.

As used herein, the term "diphosphate" includes but is not limited to an ester of phosphoric acid containing two phosphate groups. The term "triphosphate" includes but is not limited to an ester of phosphoric acid containing three phosphate groups. For example, particular drugs having a diphosphate or a triphosphate include:



As used herein, the term "heteroatom" includes oxygen (O), nitrogen (N), sulfur (S) and silicon (Si).

The symbol "R" is a general abbreviation that represents a substituent group that is selected from substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, and substituted or unsubstituted heterocyclyl groups.

Regarding the term "protecting group," those of skill in the art will understand how to protect a particular functional group from interfering with a chosen set of reaction conditions. For examples of useful protecting groups, *See Greene et al.*, PROTECTIVE GROUPS IN ORGANIC SYNTHESIS, John Wiley & Sons, New York, 1991. Examples of suitable protecting groups include, but are not limited to, BOC, FMOC, 2-

trimethylsilylethoxycarbonyl, allyloxycarbonyl, 4-methyl-1-piperazinecarbonyl, 1-methyl-1-(4-biphenyl)ethoxycarbonyl, diphenyloxycarbonyl, benzyl, t-butyl, tetrahydropyran, trimethylsilyl, t-butyldimethylsilyl, triisopropylsilyl, t-butyldiphenylsilyl, 2,2,2-trichloroethyl oxycarbonyl, diisopropylmethyl oxycarbonyl, vinyl oxycarbonyl, methoxy
5 benzyl oxycarbonyl, nitrobenzyl oxycarbonyl, cyclohexyl oxycarbonyl, cyclopentyl oxycarbonyl, benzyloxycarbonyl, formyl, acetyl, trihaloacetyl, benzoyl, nitrophenylacetyl, 2-nitrobenzenesulfonyl, phthalimido, and dithiasuccinoyl.

The term "pharmaceutically acceptable carrier", as used herein means a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid
10 filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting a chemical agent. Pharmaceutically acceptable carriers include pharmaceutically acceptable salts, where the term "pharmaceutically acceptable salts" includes salts of the active compounds which are prepared with relatively nontoxic acids or bases, depending on the particular substituents found on the compounds described herein.
15 When compounds of the present invention contain relatively acidic functionalities, base addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired base, either neat or in a suitable inert solvent. Examples of pharmaceutically acceptable base addition salts include sodium, potassium, calcium, ammonium, organic amino, or magnesium salt, or a similar salt. When compounds of the
20 present invention contain relatively basic functionalities, acid addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired acid, either neat or in a suitable inert solvent. Examples of pharmaceutically acceptable acid addition salts include those derived from inorganic acids like hydrochloric, hydrobromic, nitric, carbonic, monohydrogencarbonic, phosphoric, monohydrogenphosphoric, dihydrogenphosphoric, sulfuric, monohydrogensulfuric,
25 hydriodic, or phosphorous acids and the like, as well as the salts derived from relatively nontoxic organic acids like acetic, propionic, isobutyric, maleic, malonic, benzoic, succinic, suberic, fumaric, lactic, mandelic, phthalic, benzenesulfonic, p-tolylsulfonic, citric, tartaric, methanesulfonic, and the like. Also included are salts of amino acids such
30 as arginate and the like, and salts of organic acids like glucuronic or galactunoric acids and the like (*see*, for example, Berge *et al.*, "Pharmaceutical Salts", *Journal of Pharmaceutical Science*, 1977, 66, 1-19). Certain specific compounds of the present invention contain both

basic and acidic functionalities that allow the compounds to be converted into either base or acid addition salts.

The neutral forms of the compounds are preferably regenerated by contacting the salt with a base or acid and isolating the parent compound in the conventional manner.

- 5 The parent form of the compound differs from the various salt forms in certain physical properties, such as solubility in polar solvents, but otherwise the salts are equivalent to the parent form of the compound for the purposes of the present invention.

- 10 In addition to salt forms, the present invention provides compounds, which are in a prodrug form. Prodrugs of the compounds described herein are those compounds that readily undergo chemical changes under physiological conditions to provide the compounds of the present invention. Additionally, prodrugs can be converted to the compounds of the present invention by chemical or biochemical methods in an *ex vivo* environment. For example, prodrugs can be slowly converted to the compounds of the present invention when placed in a transdermal patch reservoir with a suitable enzyme or chemical reagent.

- 20 Certain compounds of the present invention can exist in unsolvated forms as well as solvated forms, including hydrated forms. In general, the solvated forms are equivalent to unsolvated forms and are encompassed within the scope of the present invention. Certain compounds of the present invention may exist in multiple crystalline or amorphous forms. In general, all physical forms are equivalent for the uses contemplated by the present invention and are intended to be within the scope of the present invention.

Certain compounds of the present invention possess asymmetric carbon atoms (optical centers) or double bonds; the racemates, diastereomers, geometric isomers and individual isomers are encompassed within the scope of the present invention.

- 25 The compounds of the present invention may also contain unnatural proportions of atomic isotopes at one or more of the atoms that constitute such compounds. For example, the compounds may be radiolabeled with radioactive isotopes, such as for example tritium (^3H), iodine-125 (^{125}I) or carbon-14 (^{14}C). All isotopic variations of the compounds of the present invention, whether radioactive or not, are intended to be encompassed within the scope of the present invention.

30 The terms "polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding

naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymers. These terms also encompass the term "antibody."

The term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, *e.g.*, hydroxyproline, γ -carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, *i.e.*, an α carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, *e.g.*, homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (*e.g.*, norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. One amino acid that may be used in particular is citrulline, which is a precursor to arginine and is involved in the formation of urea in the liver. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but functions in a manner similar to a naturally occurring amino acid. The term "unnatural amino acid" is intended to represent the "D" stereochemical form of the twenty naturally occurring amino acids described above. It is further understood that the term unnatural amino acid includes homologues of the natural amino acids, and synthetically modified forms of the natural amino acids. The synthetically modified forms include, but are not limited to, amino acids having alkylene chains shortened or lengthened by up to two carbon atoms, amino acids comprising optionally substituted aryl groups, and amino acids comprised halogenated groups, preferably halogenated alkyl and aryl groups. When attached to a linker or conjugate of the invention, the amino acid is in the form of an "amino acid side chain", where the carboxylic acid group of the amino acid has been replaced with a keto (C(O)) group. Thus, for example, an alanine side chain is -C(O)-CH(NH₂)-CH₃, and so forth.

Amino acids and peptides may be protected by blocking groups. A blocking group is an atom or a chemical moiety that protects the N-terminus of an amino acid or a peptide from undesired reactions and can be used during the synthesis of a drug-cleavable substrate conjugate. It should remain attached to the N-terminus throughout the synthesis, and may be removed after completion of synthesis of the drug conjugate by chemical or other

conditions that selectively achieve its removal. The blocking groups suitable for N-terminus protection are well known in the art of peptide chemistry. Exemplary blocking groups include, but are not limited to, hydrogen, D-amino acid, and carbobenzoxy (Cbz) chloride.

5 The term "antibody" as referred to herein includes whole antibodies and any antigen binding fragment (*i.e.*, "antigen-binding portion") or single chains thereof. An "antibody" refers to a glycoprotein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, or an antigen binding portion thereof. Each heavy chain is comprised of a heavy chain variable region (V_H) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, C_{H1} , C_{H2} and C_{H3} ,
10 and may be of the mu, delta, gamma, alpha or epsilon isotype. Each light chain is comprised of a light chain variable region (V_L) and a light chain constant region. The light chain constant region is comprised of one domain, C_L , which may be of the kappa or lambda isotype. The V_H and V_L regions can be further subdivided into regions of
15 hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an
20 antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (C1q) of the classical complement system.

 The terms "antibody fragment" or "antigen-binding portion" of an antibody (or simply "antibody portion"), as used herein, refers to one or more fragments of an antibody
25 that retain the ability to specifically bind to an antigen. It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antibody fragment" or "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the V_L , V_H , C_L and C_{H1} domains; (ii) a $F(ab')_2$ fragment, a bivalent
30 fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the V_H and C_{H1} domains; (iv) a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody, (v) a dAb fragment (Ward *et al.*, (1989) *Nature* 341:544-546), which consists of a V_H domain; and (vi) an isolated

complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, V_L and V_H, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules (known as single chain Fv (scFv); see e.g., Bird *et al.* (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies.

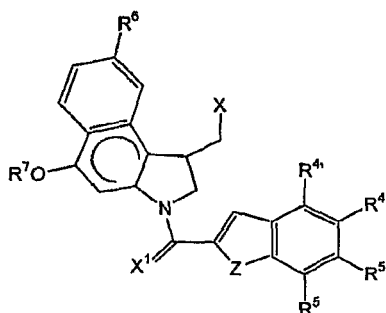
The terms "monoclonal antibody" as used herein refers to a preparation of antibody molecules of single molecular composition. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope.

"Solid support," as used herein refers to a material that is substantially insoluble in a selected solvent system, or which can be readily separated (e.g., by precipitation) from a selected solvent system in which it is soluble. Solid supports useful in practicing the present invention can include groups that are activated or capable of activation to allow selected species to be bound to the solid support. A solid support can also be a substrate, for example, a chip, wafer or well, onto which an individual, or more than one compound, of the invention is bound.

The compounds of the invention are prepared as a single isomer (e.g., enantiomer, cis-trans, positional, diastereomer) or as a mixture of isomers. In a preferred embodiment, the compounds are prepared as substantially a single isomer. Methods of preparing substantially isomerically pure compounds are known in the art. For example, enantiomerically enriched mixtures and pure enantiomeric compounds can be prepared by using synthetic intermediates that are enantiomerically pure in combination with reactions that either leave the stereochemistry at a chiral center unchanged or result in its complete inversion. Alternatively, the final product or intermediates along the synthetic route can be resolved into a single stereoisomer. Techniques for inverting or leaving unchanged a particular stereocenter, and those for resolving mixtures of stereoisomers are well known in the art and it is well within the ability of one of skill in the art to choose and appropriate method for a particular situation. See, generally, Furniss *et al.* (eds.), VOGEL'S

ENCYCLOPEDIA OF PRACTICAL ORGANIC CHEMISTRY 5TH ED., Longman Scientific and Technical Ltd., Essex, 1991, pp. 809-816; and Heller, *Acc. Chem. Res.* **23**: 128 (1990).

Cytotoxic analogs of CC-1065 can be formed using a cyclopropabenzindole (CBI) moiety as an alkylating unit instead of the cyclopropapyrroloindole (CPI) moiety of CC-1065. As one example, CC-1065 CBI analogs include, but are not limited to compounds having the formula (or a pharmaceutically acceptable salt thereof):



where X is halo. Preferably, X is Cl or Br and, more preferably, X is Br.

X¹ and Z are each independently selected from O, S and NR⁸ where R⁸ is a member selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, and acyl.

R⁴, R^{4'}, R⁵ and R^{5'} are members independently selected from the group consisting of H, substituted alkyl, unsubstituted alkyl, substituted aryl, unsubstituted aryl, substituted heteroaryl, unsubstituted heteroaryl, substituted heterocycloalkyl, unsubstituted heterocycloalkyl, halogen, NO₂, NR⁹R¹⁰, NC(O)R⁹, OC(O)NR⁹R¹⁰, OC(O)OR⁹, C(O)R⁹, SR⁹, OR⁹, CR⁹=NR¹⁰, and O(CH₂)_nNR¹¹R^{11'},

where R⁹ and R¹⁰ are independently selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted heterocycloalkyl, and substituted or unsubstituted peptidyl, or where R⁹ and R¹⁰ together with the nitrogen atom to which they are attached are optionally joined to form a substituted or unsubstituted heterocycloalkyl ring system having from 4 to 6 members, optionally containing two or more heteroatoms, and

R¹¹ and R^{11'} are each independently H or lower alkyl.

R⁶ is H, substituted or unsubstituted lower alkyl, cyano, or alkoxy. Preferably R⁶ is methyl, cyano or H. More preferably, R⁶ is H.

R^7 is a member selected from the group consisting of H, substituted alkyl, unsubstituted alkyl, substituted heteroalkyl, unsubstituted heteroalkyl, diphosphates, triphosphates, acyl, $C(O)R^{12}R^{13}$, $C(O)OR^{12}$, $C(O)NR^{12}R^{13}$, $P(O)(OR^{12})_2$, $C(O)CHR^{12}R^{13}$, SR^{12} and $SiR^{12}R^{13}R^{14}$,

5 in which R^{12} , R^{13} , and R^{14} are members independently selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl and substituted or unsubstituted aryl, or where R^{12} and R^{13} together with the nitrogen or carbon atom to which they are attached are optionally joined to form a substituted or unsubstituted heterocycloalkyl ring system having from 4 to 6 members, optionally containing two or
10 more heteroatoms.

Examples of CBI CC-1065 analogs are described in co-owned U.S. Patent Applications Serial Nos. 10/160,972; 10/161,233; 10/161,234, 11/134,685, and 11/134,826; all of which are incorporated herein by reference. These references also describe examples of synthesis and uses for these compounds. These compounds can be
15 used as therapeutic agents (e.g., drugs) and as prodrugs. In at least some embodiments, the CBI CC-1065 analogs can be conjugated to targeting agents, such as an antibody, receptor, peptide, lectin, saccharide, nucleic acid or a combination thereof, for use in pharmaceutical compositions that selectively deliver the cytotoxic CBI CC-1065 analogs to desired target cells, such as carcinoma cells.

20 Representative examples of precancerous conditions that may be targeted by these compounds, include, but are not limited to: metaplasia, hyperplasia, dysplasia, colorectal polyps, actinic ketatosis, actinic cheilitis, human papillomaviruses, leukoplakia, lichen planus and Bowen's disease.

Representative examples of cancers or tumors that may be targeted by these
25 compounds include, but are not limited to: lung cancer, colon cancer, prostate cancer, lymphoma, melanoma, breast cancer, ovarian cancer, testicular cancer, CNS cancer, renal cancer, kidney cancer, pancreatic cancer, stomach cancer, oral cancer, nasal cancer, cervical cancer and leukemias. It will be readily apparent to the ordinarily skilled artisan that the particular targeting agent can be chosen such that it targets the drug to the tumor
30 tissue to be treated with the drug (i.e., a targeting agent specific for a tumor-specific antigen is chosen). Examples of such targeting agents are well known in the art, non-limiting examples of which include anti-Her2 for treatment of breast cancer, anti-CD20 for

treatment of lymphoma, anti-PSMA for treatment of prostate cancer and anti-CD30 for treatment of lymphomas, including non-Hodgkin's lymphoma.

These compounds provide a method of killing a cell. The method includes administering to the cell an amount of a compound of the invention sufficient to kill said cell. In an exemplary embodiment, the compound is administered to a subject bearing the cell. In a further exemplary embodiment, the administration serves to retard or stop the growth of a tumor that includes the cell (*e.g.*, the cell can be a tumor cell). For the administration to retard the growth, the rate of growth of the cell should be at least 10% less than the rate of growth before administration. Preferably, the rate of growth will be retarded at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or completely stopped.

Pharmaceutical compositions include compositions wherein the active ingredient is contained in a therapeutically effective amount, *i.e.*, in an amount effective to achieve its intended purpose. The actual amount effective for a particular application will depend, *inter alia*, on the condition being treated. Determination of an effective amount is well within the capabilities of those skilled in the art, especially in light of the detailed disclosure herein.

For any compound described herein, the therapeutically effective amount can be initially determined from cell culture assays. Target plasma concentrations will be those concentrations of active compound(s) that are capable of inhibition cell growth or division. In preferred embodiments, the cellular activity is at least 25% inhibited. Target plasma concentrations of active compound(s) that are capable of inducing at least about 50%, 75%, or even 90% or higher inhibition of cellular activity are presently preferred. The percentage of inhibition of cellular activity in the patient can be monitored to assess the appropriateness of the plasma drug concentration achieved, and the dosage can be adjusted upwards or downwards to achieve the desired percentage of inhibition.

As is well known in the art, therapeutically effective amounts for use in humans can also be determined from animal models. For example, a dose for humans can be formulated to achieve a circulating concentration that has been found to be effective in animals. The dosage in humans can be adjusted by monitoring cellular inhibition and adjusting the dosage upwards or downwards, as described above.

A therapeutically effective dose can also be determined from human data for compounds which are known to exhibit similar pharmacological activities. The applied

dose can be adjusted based on the relative bioavailability and potency of the administered compound as compared with the known compound.

Adjusting the dose to achieve maximal efficacy in humans based on the methods described above and other methods as are well-known in the art is well within the capabilities of the ordinarily skilled artisan.

In the case of local administration, the systemic circulating concentration of administered compound will not be of particular importance. In such instances, the compound is administered so as to achieve a concentration at the local area effective to achieve the intended result.

For use in the prophylaxis and/or treatment of diseases related to abnormal cellular proliferation, a circulating concentration of administered compound of about 0.001 μM to 20 μM is preferred, with about 0.01 μM to 5 μM being preferred.

Patient doses for oral administration of the compounds described herein, typically range from about 1 mg/day to about 10,000 mg/day, more typically from about 10 mg/day to about 1,000 mg/day, and most typically from about 50 mg/day to about 500 mg/day. Stated in terms of patient body weight, typical dosages range from about 0.01 to about 150 mg/kg/day, more typically from about 0.1 to about 15 mg/kg/day, and most typically from about 1 to about 10 mg/kg/day, for example 5 mg/kg/day or 3 mg/kg/day.

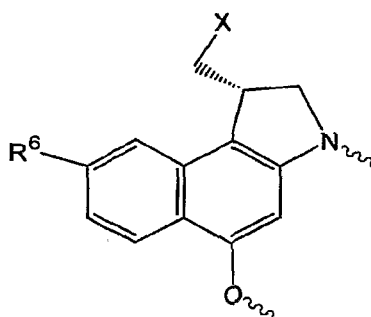
In at least some embodiments, patient doses that retard or inhibit tumor growth can be 1 $\mu\text{mol/kg/day}$ or less. For example, the patient doses can be 0.9, 0.6, 0.5, 0.45, 0.3, 0.2, 0.15, or 0.1 $\mu\text{mol/kg/day}$ or less (referring to moles of the drug) of the drug or a drug conjugate, such as an antibody-drug conjugate. Preferably, the drug or drug conjugate growth of the tumor when administered in the daily dosage amount over a period of at least five days. In at least some embodiments, the tumor is a human-type tumor in a SCID mouse. As an example, the SCID mouse can be a CB17.SCID mouse (available from Taconic, Germantown, NY).

For other modes of administration, dosage amount and interval can be adjusted individually to provide plasma levels of the administered compound effective for the particular clinical indication being treated. For example, in one embodiment, a compound according to the invention can be administered in relatively high concentrations multiple times per day. Alternatively, it may be more desirable to administer a compound of the invention at minimal effective concentrations and to use a less frequent administration

regimen. This will provide a therapeutic regimen that is commensurate with the severity of the individual's disease.

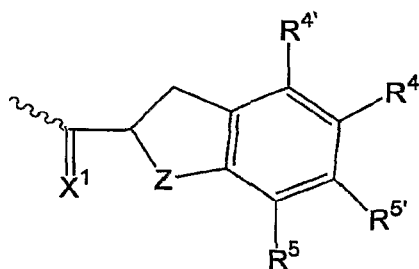
Utilizing the teachings provided herein, an effective therapeutic treatment regimen can be planned which does not cause substantial toxicity and yet is entirely effective to
 5 treat the clinical symptoms demonstrated by the particular patient. This planning should involve the careful choice of active compound by considering factors such as compound potency, relative bioavailability, patient body weight, presence and severity of adverse side effects, preferred mode of administration and the toxicity profile of the selected agent.

Generally, the CBI moiety has the formula:



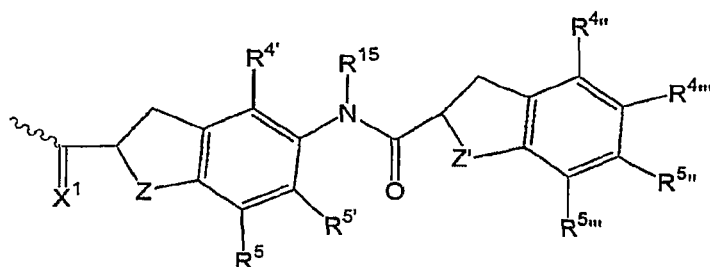
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where substituents can be attached to the oxygen and nitrogen atoms, X is halo, and R^6 is H, substituted or unsubstituted lower alkyl, cyano, or alkoxy. Preferably, R^6 is H, methyl, or cyano. More preferably, R^6 is H. In addition, X is preferably Cl or Br and, more preferably, X is Br. Generally, a binding unit can be attached to the amine substituent of
 15 the CBI moiety. Examples of suitable binding units include, but are not limited to,



where X^1 , Z, R^4 , $R^{4'}$, R^5 , and $R^{5'}$ are as defined above.

Examples of suitable binding units are illustrated and described in U.S. Patent Applications Serial Nos. 10/160,972; 10/161,233; 10/161,234, 11/134,685, and
 20 11/134,826; as well as in U.S. Patent No. 6,534,660, incorporated herein by reference. Suitable binding units within this formula also include binding units with multiple fused rings such as:



where Z' is independently selected from O, S and NR⁸ where R⁸ is a member selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, and acyl.

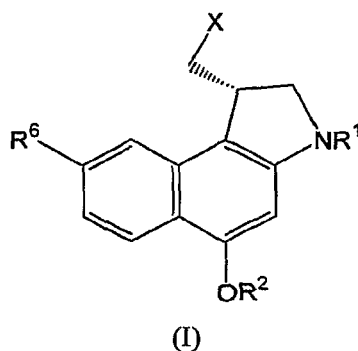
R^{4''}, R^{4'''}, R^{5''}, and R^{5'''} are members independently selected from the group
 5 consisting of H, substituted alkyl, unsubstituted alkyl, substituted aryl, unsubstituted aryl, substituted heteroaryl, unsubstituted heteroaryl, substituted heterocycloalkyl, unsubstituted heterocycloalkyl, halogen, NO₂, NR⁹R¹⁰, NC(O)R⁹, OC(O)NR⁹R¹⁰, OC(O)OR⁹, C(O)R⁹, SR⁹, OR⁹, CR⁹=NR¹⁰, and O(CH₂)_nNR¹¹R^{11'}.

where R⁹ and R¹⁰ are independently selected from H, substituted or
 10 unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted heterocycloalkyl, and substituted or unsubstituted peptidyl, or where R⁹ and R¹⁰ together with the nitrogen atom to which they are attached are optionally joined to form a substituted or unsubstituted heterocycloalkyl ring system having from 4 to 6 members, optionally containing two or
 15 more heteroatoms, and

R¹¹ and R^{11'} are each independently H or lower alkyl.

R¹⁵ can be H, substituted or unsubstituted alkyl, or R¹⁵ and R^{4'} or R^{5'} can be combined to form a ring (e.g., a five- or six-membered ring.)

One intermediate compound useful in the formation of CC-1065 CBI analogs has
 20 the formula (I):

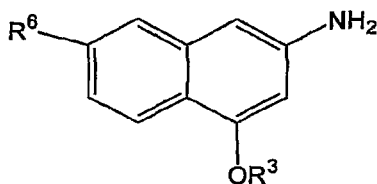


where R^1 and R^2 are each independently H, alkyl, $-C(O)OR'$, $-C(O)NR'R''$, or a protecting group, where R' and R'' are independently selected from the group consisting of H, substituted alkyl, unsubstituted alkyl, substituted aryl, unsubstituted aryl, substituted heteroaryl, unsubstituted heteroaryl, substituted heterocycloalkyl, and unsubstituted heterocycloalkyl, and X is halogen.

In one conventional synthetic method for compound (I), the starting material is 1,3-dihydroxynaphthalene which is then reacted with ammonia in a pressurized chamber (e.g., a bomb) to replace the hydroxy group at the 3-position of the naphthalene with an amine. Examples of this synthetic method can be found in U.S. Patent No. 6,534,660 and D.L. Boger et al., J. Org. Chem. 57, 2873-2876 (1992), both of which are incorporated by reference. The amination reaction is followed by the addition of protecting groups to both the hydroxy and amine moieties.

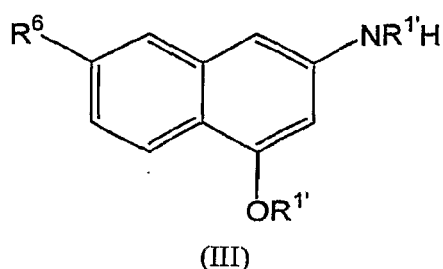
Although the amination reaction may have acceptable yield on a small scale, it can be difficult to scale-up the reaction because of the use of a bomb to contain this pressurized reaction which typically occurs at a pressure substantially greater than 1 atmosphere (about 1.01×10^5 Pa) and generally at a pressure of at least 1.5 atmospheres (1.52×10^5 Pa). This synthetic method has been found to result in a substantially lower yield when scaled-up.

In contrast to the conventional methods, the starting material can be compound (II), as illustrated in the synthetic schemes of Figures 1 and 2:

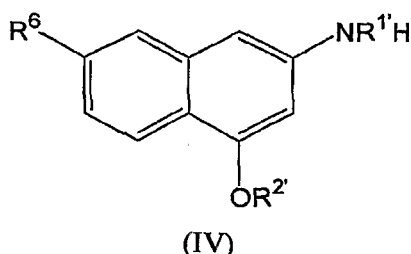


(II)

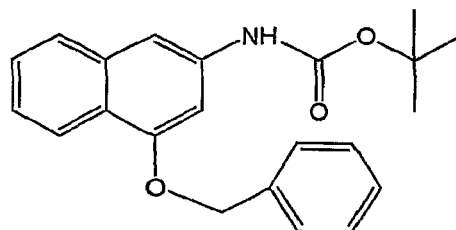
where R^3 is H or alkyl. Preferably, R^3 is C1-5 alkyl and, more preferably, methyl. For example, 4-methoxy-2-naphthylamine is available commercially from Aldrich Chemical Company, Inc., Milwaukee, WI. It is relatively easy to hydrolyze the compound, if R^3 is alkyl, and add protecting groups, $R^{1'}$, to both the hydroxy and amine moieties to form compound (III)



In some embodiments, as illustrated in Figures 1 and 2, it is desirable to provide different protecting groups on the amine and hydroxy functionalities. Accordingly, the initial protecting group, R^{1'}, can be removed from one of these moieties (e.g., from the hydroxy moiety) and replaced with a second, different protecting group, R^{2'}, to provide compound (IV)



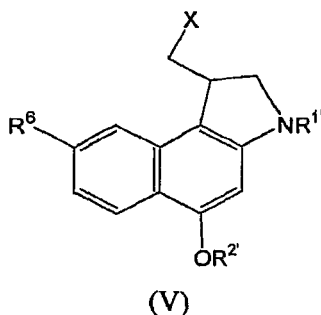
One specific example of compound (IV) has the formula:



Having different protecting groups on the hydroxyl and amine substituents can facilitate later reaction steps where one of the protecting groups can be selectively removed while leaving the other protecting group. Alternatively, different protecting groups can be initially added to the hydroxyl and amine substituents.

Schemes 1 and 2 (Figs. 1 and 2) illustrate one embodiment of the remaining steps in forming compound (I) from compound (IV). These steps can include, for example, forming a ring using the nitrogen of the amine group. This can be accomplished, for example, by alkylation of the aryl ring adjacent the nitrogen followed by a ring closure step. In one embodiment, iodination of compound (IV) by N-iodosuccinimide produces a compound which can then be alkylated using 1,3-dibromopropene or 1,3-dichloropropene.

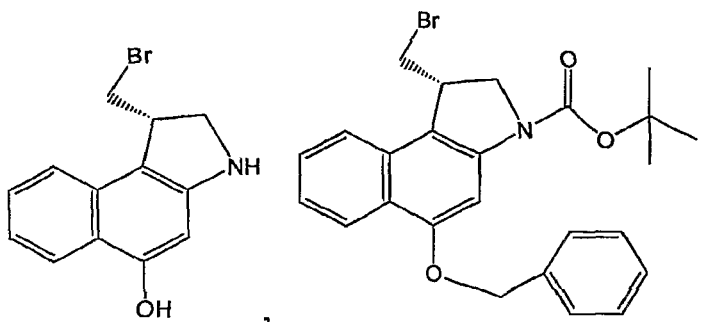
Ring closure can then be performed using tributyltin hydride in the presence of 2,2'-azobisisobutyronitrile (AIBN) to give the racemic CBI-derivative, compound (V):

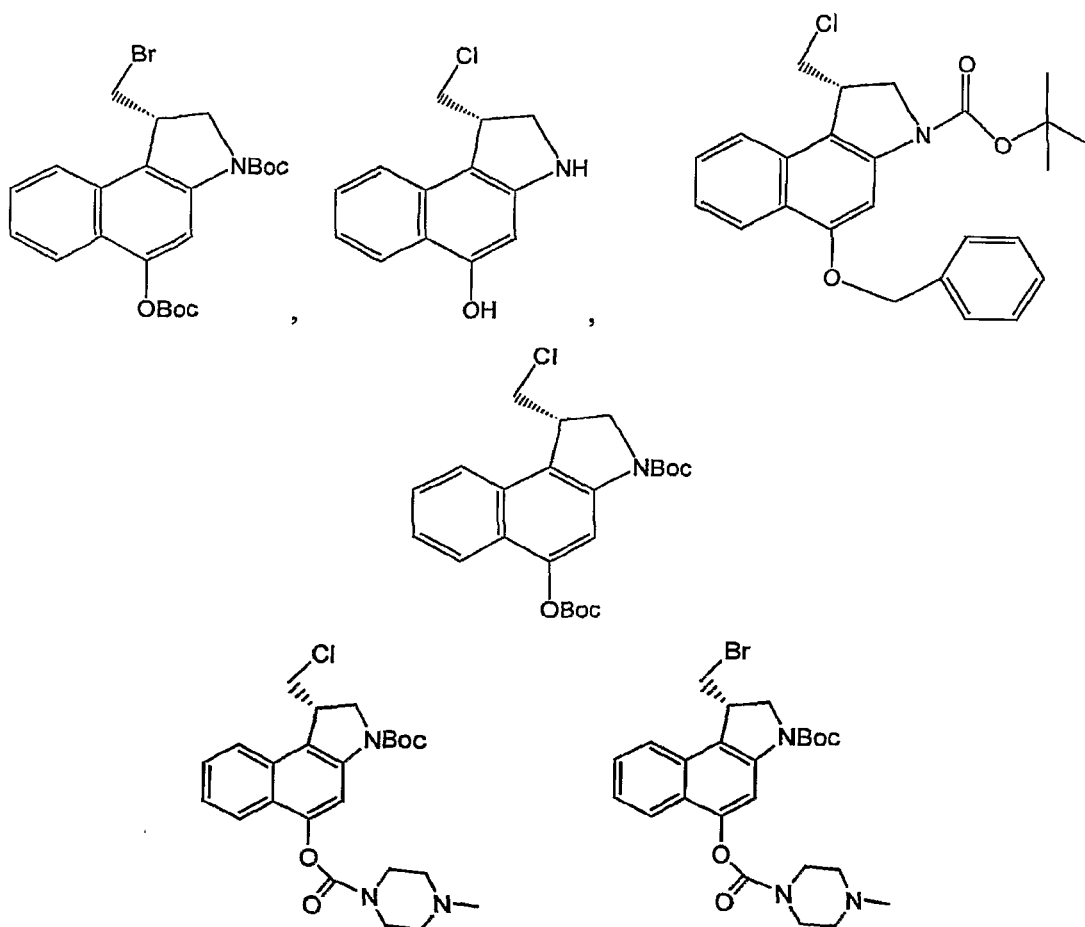


- 5 If desired, the protecting groups can be removed to form CBI. It will be understood that different reactants and catalysts can be used in these reaction steps. Examples can be found in Boger, Chemical Reviews, 97, 787-828 (1997,) incorporated herein by reference.

The racemic CBI-derivative can be separated using known techniques of the separation of enantiomers including the use of chromatographic methods. One particularly
 10 useful technique is high pressure liquid chromatography (HPLC) using a chiral column. For example, separation of such enantiomers has been performed using a HPLC Chiralcel column and hexane/isopropanol (99:1) eluent to give compound (I).

Specific examples of Compound (I) include, but are not limited to,





Compound (I) can be used to form CBI CC-1065 analogs as described, for example, in co-
 5 owned U.S. Patent Applications Serial Nos. 10/160,972; 10/161,233; 10/161,234,
 11/134,685, and 11/134,826, all of which are incorporated herein by reference. For
 example, a binding unit can be added to Compound (I) by deprotecting the amine
 substituent and reacting a compound comprising the binding unit with the deprotected
 amine. Additional substituents can be added to the oxygen atom of the CBI compound by
 10 deprotecting the oxygen and reacting it with appropriate reactant(s).

EXAMPLES

Without further elaboration, it is believed that one skilled in the art can, using the
 15 preceding description, utilize the present invention to its fullest extent. The following
 preferred specific embodiments are, therefore, to be construed as merely illustrative, and
 not limitative of the remainder of the disclosure in any way whatsoever.

In the foregoing and in the following examples, all temperatures are set forth uncorrected in degrees Celsius and, all parts and percentages are by weight, unless otherwise indicated.

5

EXAMPLE 1 - Scheme 1 (Fig. 1)

Synthesis of N-(tert-butyloxycarbonyl)-4-O-(tert-butyloxycarbonyl)-2-naphthylamine (2)

10 A solution of 4-methoxy-2-naphthylamine (230 mg, 1.33 mmol) in glacial acetic acid (9.6 mL) and hydrobromic acid in water (16 mL, 48%) was refluxed under N₂ for 4 h. A small amount of sample (0.1 mL) was diluted with ethyl acetate (0.5 mL), and then water (0.5 mL) and TEA (0.1 mL) were added. TLC (20:1 DCM/methanol) of the organic layer showed no starting material and a new much lower spot (R_f=0.1). The solvent was
15 removed under reduced pressure and the product was dried under vacuum to yield the intermediate 4-hydroxy-2-naphthylamine which was used for the next step without any purification. To a solution of 4-hydroxy-2-naphthylamine in dioxane (10 mL) was added TEA (1 mL) and di-*tert*-butyl dicarbonate (1.149 g, 5.27 mmol). The reaction mixture was refluxed under N₂ for 4 h. TLC (4:1 hexane/ethyl acetate) showed no starting material and
20 a new higher spot (R_f=0.55). The reaction mixture was diluted with ethyl acetate (50 mL) and washed with water. The aqueous layer was extracted with ethyl acetate (2 x 50 mL) and the organics were combined and washed with brine. The organics were dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure to yield N-(tert-butyloxycarbonyl)-4-O-(tert-butyloxycarbonyl)-2-naphthylamine (2, 80% yield) as oil.

25

Synthesis of compound 3

To a solution of compound 2 in acetone (10 mL) was added NaOH solution in water (10 mL, 1 M). The reaction mixture was stirred at room temperature for overnight. TLC (4:1 hexane/ethyl acetate) showed no starting material and a new lower spot. The
30 reaction mixture was extracted with ethyl acetate (50 mL) and washed with water. The aqueous layer was extracted with ethyl acetate (2 x 50 mL) and the organics were washed with brine, dried over anhydrous Na₂SO₄ and concentrated. The residue was purified on 10 g silica gel column with 10-20% ethyl acetate in hexane to yield compound 3 (181 mg, 53%) as an oil.

Synthesis of compound 4:

A solution of compound 3 (5g, 19.3 mmol) in anhydrous DMF (50 ml) under nitrogen atmosphere was treated with benzyl bromide (4 g, 23.1 moles), potassium carbonate (3.7 g, 27 moles) and tetrabutylammonium iodide (70 mg, 0.01 mmols). The reaction mixture was stirred at room temperature for 8 h. The reaction mixture was concentrated under reduced pressure. Chromatographic (4x 10 cm SiO₂, 10-20% EtOAc-hexane gradient elution) separation provided pure compound 4 (5.48 g, 83%) as a cream powder. ¹H NMR (CDCl₃, 400 MHz, ppm) 8.22 (d, 1H J=8.1 Hz, C5-H), 7.68 (d, 1H, J=8.2 Hz, C8-H), 7.3-7.5 (m, 8H, C1-H, C6-H, C7-H, CH₂C₆H₅), 7.06 (d, 1H, J=1.1 Hz, C3-H), 6.62 (br s, 1H, NH), 5.23 (s, 2H, OCH₂(C₆H₅), 1.55 (s, 9H, OC(CH₃)₃).

Synthesis of compound 5:

To a 1000 ml round bottom flask equipped with a stir bar and a rubber septum was combined compound 4 (13 g, 0.0372 moles) and THF (300 ml). The clear yellow solution was cooled to -20 °C with a dry ice bath under a nitrogen atmosphere. *p*-Toluenesulfonic acid (0.10 g, 0.0005 moles) was added to the reaction and the solution was stirred for 10 minutes. *N*-Iodosuccinimide (10 g, 0.0446 moles) was dissolved in THF (50 ml) and added to the reaction by cannula (approximately 1 hr). The solution was stirred in the ice bath for 2 hr and turned brownish. The solution was then removed from the ice bath and let warm to room temperature under nitrogen for 1.5 hr. TLC (2:1 hexane/DCM) showed no starting material and a new higher spot. The reaction was quenched with saturated NaHCO₃ (200 ml) and a white solid formed. After stirring the solution for 10 minutes, added EtOAc (200 ml) and water (100 ml) to the reaction. The aqueous layer was extracted with EtOAc (2 x 100 ml) and the organics were combined and extracted with brine (100 ml). The organics were dried over MgSO₄, filtered, and concentrated under vacuum to a dark red-brown solid. The solid was purified by column chromatography using 2:1 hexane/DCM as eluant to yield compound 5 (14 g, 79%) as a brown solid.

Synthesis of compound 6:

To a 500 ml round bottom flask equipped with stir bar and nitrogen inlet was combined compound 5 (22.5 g, 0.0473 moles) and anhydrous DMF (250 ml). The yellow-orange solution was cooled to 0 °C with an ice/salt bath under a nitrogen atmosphere. NaH (60%,

5.6 g, 0.146 moles) was added to the reaction in one portion. The solution turned cloudy and a gas was formed. The reaction was stirred in the ice bath for 15 minutes and then the ice bath was removed and the solution was stirred for another 15 minutes. *cis/trans*-1,3-Dibromopropene (14 ml, 0.14 moles) was added to the reaction in portions by syringe.

- 5 The reaction was stirred under nitrogen at room temperature for 1 hr and turned a cloudy brown. The temperature rose to 40 °C. The reactions was allowed to cool to room temperature. TLC (4:1 hexane/EtOAc) showed no starting material and a new lower spot. The reaction was quenched with water (500 ml). The aqueous layer was extracted with EtOAc (4 x 100 ml) and the organics were washed with brine (2 x 75 ml). The organics
10 were dried over MgSO₄, filtered and concentrated under vacuum to a brown oil. The product was purified by column chromatography using 1:1 DCM/hexane as eluant to yield compound 6 (25 g, 89%) as a brown oil.

Synthesis of compound 7:

- 15 To a 1000 ml three necked round bottom flask equipped with stir bar, temperature probe, reflux condenser and nitrogen inlet was combined compound 6 (25 g, 0.0421 moles), toluene (500 ml), 2,2'-azobis(2-methylpropionitrile) (0.15 g, 0.0009 moles) and tributyltin hydride (3.4 ml, 0.0126 moles) [by syringe]. Nitrogen was bubbled through the solution for 15 minutes and then the reaction was heated to 80 °C under nitrogen. After
20 heating at 80 °C for 15 minutes, tributyltin hydride (3.4 ml, 0.0126 moles) was added to the reaction by syringe. After heating for another 15 minute tributyltin hydride (3.4 ml, 0.0126 moles) was added to the reaction by syringe. After a further 15 minutes, tributyltin hydride (3.4 ml, 0.0126 moles) was added to the reaction by syringe. The total amount of tributyltin hydride added was 13.6 ml, 0.0505 moles. The reaction was heated at 80 °C for
25 30 minutes and then allowed to cool to room temperature. TLC (10% EtOAc/hexane) showed starting material and a new higher spot. The solution was concentrated under vacuum to a yellow solid. The solid was purified by column chromatography using as eluant 1:1 dicloromethane/hexane to give a yellow solid. The solid was recrystallized from hexane (200 ml, 45 C for 30 min, cooled in fridge for 2 hr, collected by filtration, dried
30 under vacuum) to yield compound 7 (11.70 g, 59% yield) as a pale yellow solid.
- NMR(1H, CDCl₃, 400 MHz): δ 1.61 (9H, s, C-(CH₃)₃); 3.30 (1H, t, J = 26 Hz, CH-CH₂-N); 3.82 (1H, d, J = 26 Hz, Br-CH₂-CH); 4.04 (1H, d, J = 19 Hz, Br-CH₂-CH) 4.14 (1H, t, J = 26 Hz, CH-CH₂-N); 4.21 (1H, m, CH₂-CH-CH₂); 5.26 (2H, s, O-CH₂-C₆H₅); 7.3-7.55 (8H, m, O-CH₂-C₆H₅, C₁₀H₅); 7.63 (1H, d, J = 21 Hz, C₁₀H₅); 8.3 (1H, d, J = 21 Hz, C₁₀H₅)

Resolution of compound 7:

The racemic compound 7 was dissolved in DCM (50mg, 1 ml). The solution was then diluted with hexane (9 ml). The solution was then loaded onto a Chiralcel OD prep column (10 micron, 20 x 250 mm) and separated using hexane/isopropanol (99:1, 15 ml/min). The first enantiomer (7a) elutes from 10 to 15 min and the second enantiomers (7b) elutes from 17.5 to 25 min. The analytical column (Chiralcel OD, 0.46 x 25 cm, 20 micrometers) gives a retention time of 7.71 min for 7a and 12.9 min for 7b (99:1 hexane/IPA, 1 ml/min, 15 minute run). NMR(1H, CDCl₃, 400 MHz): δ 1.61 (9H, s, C-(CH₃)₃); 3.30 (1H, t, J = 26 Hz, CH-CH₂-N); 3.82 (1H, d, J = 26 Hz, Br-CH₂-CH); 4.04 (1H, d, J = 19 Hz, Br-CH₂-CH) 4.14 (1H, t, J = 26 Hz, CH-CH₂-N); 4.21 (1H, m, CH₂-CH-CH₂); 5.26 (2H, s, O-CH₂-C₆H₅); 7.3-7.55 (8H, m, O-CH₂-C₆H₅, C₁₀H₅); 7.63 (1H, d, J = 21 Hz, C₁₀H₅); 8.3 (1H, d, J = 21 Hz, C₁₀H₅).

EXAMPLE 2 - Scheme 2 (Fig. 2)

The synthetic method procedure is as described above in Example 1 through the synthesis of compound 5.

Synthesis of compound 8:

To a 250 ml round bottom flask equipped with stir bar and nitrogen inlet was combined compound 5 (8.4 g, 0.0177 moles) and anhydrous DMF (125 ml). The yellow-orange solution was cooled to 0 °C with an ice/salt bath under a nitrogen atmosphere. NaH (60%, 2.22 g, 0.0554 moles) was added to the reaction in one portion. The solution turned cloudy and a gas was formed. The reaction was stirred in the ice bath for 15 minutes and then the ice bath was removed and the solution was stirred for another 15 minutes. *cis/trans*-1,3-Dichloropropene (5.3 ml, 0.0571 moles) was added to the reaction in portions by syringe. The reaction was stirred under nitrogen at room temperature for 3 hr and turned a cloudy brown. TLC (4:1 hexane/EtOAc) showed no starting material and a new lower spot. The reaction was quenched with water (250 ml). The aqueous layer was extracted with EtOAc (3 x 100 ml) and the organics were washed with brine (2 x 50 ml). The organics were dried over MgSO₄, filtered and concentrated under vacuum to a brown oil. The product was purified by column chromatography using 1:1 DCM/hexane as eluant to yield (*E/Z*)-*tert*-butyl 4-(benzyloxy)-1-iodonaphthalene-2-yl(3-chloroallyl)carbamate (8) (9 g, 93%) as a yellow oil.

Synthesis of compound 9:

To a 500 ml three necked round bottom flask equipped with stir bar, temperature probe, reflux condenser and nitrogen inlet was combined compound 8 (9 g, 0.0164 moles), toluene (200 ml), 2,2'-azobis(2-methylpropionitrile) (0.15 g, 0.0009 moles) and tributyltin hydride (1.5 ml, 0.0056 moles) [by syringe]. Nitrogen was bubbled through the solution for 15 minutes and then the reaction was heated to 80 °C under nitrogen. After heating at 80 °C for 15 minutes, tributyltin hydride (1.5 ml, 0.0056 moles) was added to the reaction by syringe. After heating for another 15 minute tributyltin hydride (1.5 ml, 0.0056 moles) was added to the reaction by syringe. After a further 15 minutes, tributyltin hydride (1.0 ml, 0.0037 moles) was added to the reaction by syringe. The total amount of tributyltin hydride added was 5.5 ml, 0.0204 moles. The reaction was heated at 80 °C for 30 minutes and then allowed to cool to room temperature. TLC (10% EtOAc/hexane) showed no starting material and a new higher spot. The solution was concentrated under vacuum to a yellow oil. The oil was purified by column chromatography using as eluant 100% Hexane to 5% EtOAc/Hexane to 10% EtOAc/Hexane to give a pale yellow solid. The solid was recrystallized from hexane (100 ml, 45 C for 30 min, cooled in fridge for 2 hr, collected by filtration, dried under vacuum) to yield compound 9 (4.16 g, 60% yield) as a white solid.

Resolution of compound 9:

The racemic compound 9 was dissolved in DCM (50mg, 1 ml). The solution was then diluted with hexane (9 ml). The solution was then loaded onto a Chiralcel OD prep column (10 micron, 20 x 250 mm) and separated using hexane/isopropanol (99:1, 15 ml/min). The first enantiomer (9a) elutes from 11.5 to 15 min and the second enantiomers (9b) elutes from 17.5 to 25 min. The analytical column (Chiralcel OD, 0.46 x 25 cm, 20 microns) gives a retention time of 6.5 min for 9a and 10.6 min for 9b (99:1 hexane/IPA, 1 ml/min, 15 minute run). NMR(1H, CDCl₃, 400 MHz): d 1.61 (9H, s, C-(CH₃)₃); 3.44 (1H, t, J = 25 Hz, CH-CH₂-N); 3.9-4.0 (2H, m, Cl-CH₂-CH); 4.12 (1H, t, J = 26 Hz, CH-CH₂-N); 4.25 (1H, m, CH₂-CH-CH₂); 5.26 (2H, s, O-CH₂-C₆H₅); 7.2-7.5 (8H, m, O-CH₂-C₆H₅, C₁₀H₅); 7.63 (1H, d, J = 21 Hz, C₁₀H₅); 8.3 (1H, d, J = 21 Hz, C₁₀H₅).

EXAMPLE 3 - Scheme 3 (Fig. 3)

The synthetic method procedure is as described above in Example 1 through the synthesis of compound 3.

Synthesis of compound 10:

A solution of *tert*-butyl-4-hydroxynaphthalen-2-ylcarbamate (**3**) (500 mg, 2.89 mmol), 4-methyl-1-piperazinecarbonyl chloride hydrochloride (858 mg, 4.34 mmol),
5 anhydrous pyridine (4.98 ml, 57.8 mmol), and allyl alcohol (4.98 ml, 73.2 mmol) in anhydrous DCM (20 ml) was stirred at room temperature for overnight. TLC (9:1 DCM/MeOH) showed no starting material and a much lower spot. The reaction mixture was quenched with water. The aqueous layer was extracted with EtOAc (3 x 50 ml) and the organics were washed with brine (2x 50 ml). The organics were dried over Na₂SO₄,
10 filtered and concentrated under vacuum to brown oil. The crude product was purified by column chromatography with 1-5% methanol in DCM to yield **10** (602 mg, 82%) as yellow solid.

¹HNMR (DMSO-d₆) δ 9.68 (s, 1H), 7.91 (s, 1H), 7.80 (d, 1H), 7.69 (s, 1H), 7.47 (t, 1 H), 7.42 (s, 1H), 7.39 (t, 1H), 3.78 (s, 2H), 3.42 (s, 2H), 2.44 (s, 2H), 2.39 (s, 2H), 2.21
15 (s, 3H), 1.50 (s, 9H).

Synthesis of compound 11:

Compound **10** (82 mg, 0.21 mmol), *p*-Toluenesulfonic acid (10 mg, 0.05 mmol), and *N*-iodosuccinimide (96 mg, 0.43 mmol) in anhydrous THF (5 ml) was stirred at room
20 temperature overnight. TLC (9:1 DCM/MeOH) showed a small amount of starting material and a higher spot. The reaction mixture was quenched with saturated NaHCO₃ (10 ml). After stirring at room temperature for 10 min, the reaction mixture was extracted with EtOAc (3 x 20 ml) and the organics were washed with brine (2x 20 ml). The organics were dried over Na₂SO₄, filtered and concentrated under vacuum to brown oil. The crude
25 product was purified by column chromatography with 1-5% methanol in DCM to yield **11** (52 mg, 48%) as yellow oil.

¹HNMR (DMSO-d₆) δ 8.81 (s, 1H), 8.14 (d, 1H), 7.79 (d, 1H), 7.65 (t, 1 H), 7.58 (t, 1H), 7.45 (t, 1H), 3.78 (s, 2H), 3.42 (s, 2H), 2.44 (s, 2H), 2.39 (s, 2H), 2.21 (s, 3H), 1.50 (s, 9H).
30

Synthesis of compound 12:

A solution of compound **11** (102 mg, 0.2 mmol) in anhydrous DMF (5 ml) was cooled in an ice bath. Sodium hydride (60% in mineral oil, 32 mg, 0.8 mmol) was added

to the reaction. The reaction mixture was stirred at 0 °C for 15 min and at room temperature for 15 min. *cis/trans*-1,3-Dichloroprepene (83.36 µl, 0.9 mmol) was added to the reaction. The reaction mixture was stirred at room temperature for 1 hr. TLC (9:1 DCM/MeOH) showed no starting material. The reaction mixture was quenched with

5 water. The aqueous layer was extracted with EtOAc (3 x 10 ml) and the organics were washed with brine (2x 10 ml). The organics were dried over Na₂SO₄, filtered and concentrated under vacuum to brown oil. The crude product was purified by column chromatography with 1-5% methanol in DCM to yield **12** (82 mg, 70%) as yellow solid.

¹HNMR (DMSO-d₆) δ 8.20 (d, 1H), 7.82 (d, 1 H), 7.62 (m, 2H), 7.38 (d, 1H), 6.38 (m, 1H), 6.18 (m, 1H), 3.98-4.46 (dd, 2H), 3.78 (s, 2H), 3.42 (s, 2H), 2.44 (s, 2H), 2.39 (s, 2H), 2.21 (s, 3H), 1.50 (s, 9H).

10

Synthesis of compound 13:

To a solution of **12** (82mg, 0.14 mmol) in anhydrous toluene (3 ml), dry nitrogen

15 was bubbled for 15 min. Tributyltin hydride (47.1 µl, 0.18 mmol) and 2,2'-azobisisobutyronitrile (10 mg, 0.06mmol) were added to the reaction. The reaction mixture was heated at 80 °C for 15 min under nitrogen. TLC (9:1 DCM/MeOH) showed new blue spot and no starting material. The reaction mixture was concentrated under vacuum to yellow oil. The crude product was purified by column chromatography with 1-

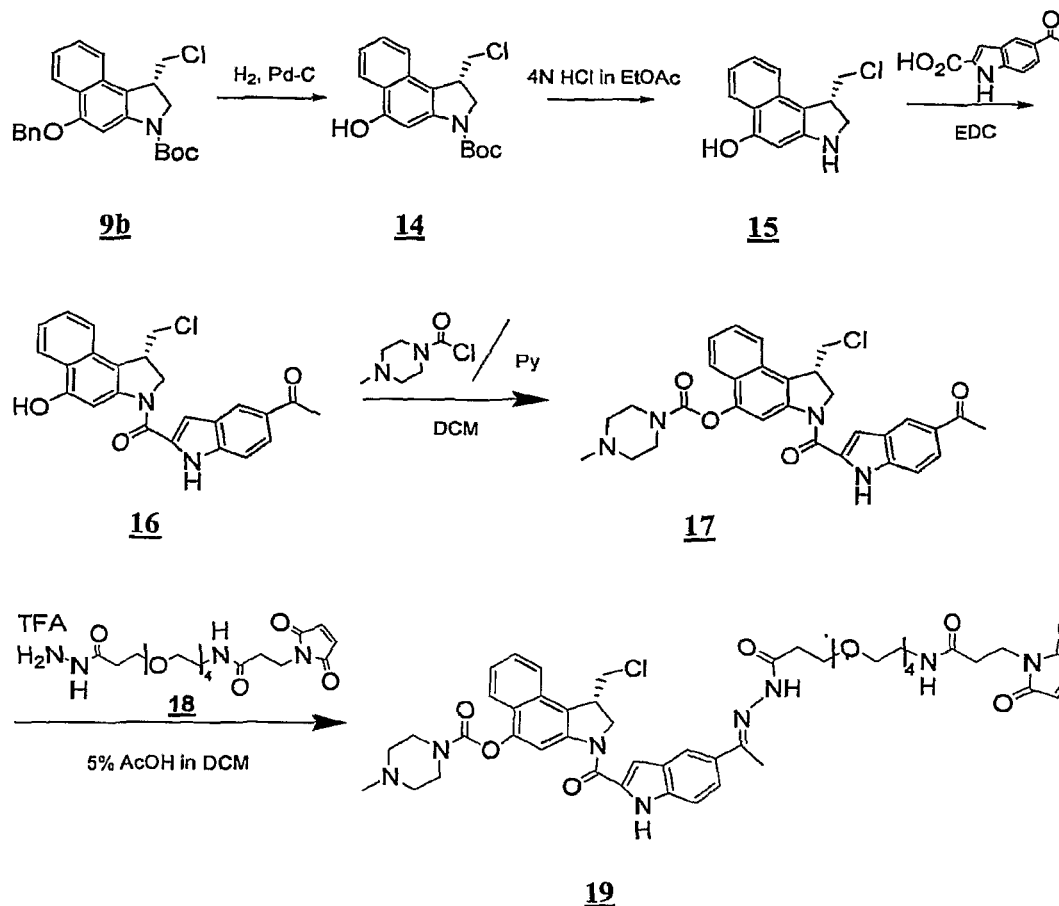
20 5% methanol in DCM to yield **13** (52 mg, 82%) as white solid.

¹HNMR (DMSO-d₆) δ 7.92 (d, 1H), 7.83 (m, 1H), 7.78 (d, 1 H), 7.58 (t, 1H), 7.42 (t, 1H), 4.20 (m, 2H), 4.04 (m, 2H), 3.92 (m, 1H), 3.78 (s, 2H), 3.42 (s, 2H), 2.44 (s, 2H), 2.39 (s, 2H), 2.21 (s, 3H), 1.50 (s, 9H).

25 Resolution of compound 13:

The racemic compound **13** is dissolved in methanol. The solution is then loaded onto a CHIRALPAK AD prep column (20 micron, 20 x 250 mm) and separated using methanol (15 ml/min). The first enantiomer (**13a**) elutes from 5.1 min and the second enantiomers (**13b**) elutes from 7.1 min. ¹HNMR (DMSO-d₆) δ 7.92 (d, 1H), 7.83 (m, 1H), 7.78 (d, 1 H), 7.58 (t, 1H), 7.42 (t, 1H), 4.20 (m, 2H), 4.04 (m, 2H), 3.92 (m, 1H), 3.78 (s, 2H), 3.42 (s, 2H), 2.44 (s, 2H), 2.39 (s, 2H), 2.21 (s, 3H), 1.50 (s, 9H).

30

Example 4 - Synthesis of CBI CC-1065 Analog

- Synthesis of Compound (14).** A solution of **9b** (100 mg, 0.24 mmol) and 10% Pd-C (35 mg) in MeOH/CH₂Cl₂ (1/2, 10 ml) was degassed in vacuo for 40 s. The resulting mixture was placed under an atmosphere of hydrogen and stirred at 25 °C for 7 h. The reaction mixture was filtered through Celite (CH₂Cl₂ wash). The solvent was removed in vacuo. Chromatography on silica gel eluted with EtOAc/Hex (2/8) afforded **14** (77 mg, 98%).
- ¹NMR DMSO-d₆) δ 10.36 (s, 1H), 8.04 (d, 1H, J=8.2 Hz), 7.72 (d, 1H, J=8.2 Hz), 7.61 (br s, 1H), 7.45 (t, 1H, J=8.4 Hz), 7.261 (t, 1H, J=8.4 Hz), 4.06 (m, 4H), 3.73 (m, 1H), 1.52 (s, 9H).

- Synthesis of Compound (16).** A solution of **14** (35 mg, 0.1 mmol) in 4 M HCl-EtOAc (5 ml) was stirred at 25 °C under Ar for 30 min. The solvent was removed in vacuo. To the residue was added 5-acetylidone-2-carboxylic acid (24.4 mg, 0.12 mmol). A solution of EDC (22.9 mg, 0.12 mmol) in DMF (3 ml) was added and the reaction mixture was stirred

at 25 °C for 5 h. The solvent was removed. The crude product was chromatographed on silica gel eluted with 10% MeOH in CH₂Cl₂ to give **16** (40.7mg, 93%). ¹HNMR DMSO-d₆) δ 12.13 (s, 1H), 10.47 (s, 1H), 8.45 (s, 1H), 8.10 (d, 1H, J=8.4 Hz), 7.96 (br s, 1H), 7.85 (d, 2H, J=8.4 Hz), 7.54 (d, 1H, J=8.4 Hz), 7.51 (t, 1H, J=8.2 Hz), 7.36 (t, 1H, J=7.6), 7.35 (s, 1H), 4.81 (t, 1H, 11.2 Hz), 4.54 (dd, 1H, 8.8 Hz), 4.23 (m, 1H), 4.01 (dd, 1H, J=10.2 Hz), 3.86 (dd, 1H, J=10.7 Hz), 2.61 (s, 3H).

Synthesis of Compound (17). 4-Methyl-1-piperazinecarbonyl chloride hydrochloride (19.9 mg, 0.1 mmol) was added to a solution of **16** (20 mg, 0.05 mmol) and anhydrous pyridine (25 µl, 0.3 mmol) in 3% allyl alcohol in dry methylene chloride (4 ml) and the mixture was stirred for 16 h. Purification of the crude product on silica gel yielded **17** (23.6 mg, 91%). ¹NMR DMSO-d₆) δ 12.03 (s, 1H), 8.41 (s, 1H), 8.21 (s, 1H), 8.01 (d, 1H, J=8.4 Hz), 7.88 (d, 1H, J=8.4 Hz), 7.82 (dd, 1H, J=8.4 Hz), 7.58 (t, 1H, J=8.1 Hz), 7.51 (d, 1H, J=8.4 Hz), 7.46 (t, 1H, J=7.6 Hz), 7.37 (s, 1H), 4.86 (t, 1H, J=10.8 Hz), 4.57 (dd, 1H, J=10.8 Hz), 4.38 (m, 1H), 4.06 (dd, 1H, J=10.8 Hz), 3.86 (dd, 1H, J=11 Hz), 3.41 (br, 4H), 3.29 (br, 4H), 2.82 (s, 3H), 2.57 (s, 3H).

Synthesis of Compound (19). A solution of **17** (13 mg, 24 µmol) and linker **18** (16.9 mg, 31 µmol) in 5% acetic acid in dry methylene chloride (1 ml) was stirred for 30 min at 25 °C. The solvent was completely removed in vacuo and purified by HPLC (SymmetryPrep C₁₈, 7µm, 19 x 150 mm column) to give **19** (18.5 mg, 81%). MS: calcd for C₄₈H₅₇ClN₈O₁₁ (M+H) m/z 958.38, found 958.10.

EXAMPLE 5: Proliferation Assays

The biological activity of the cytotoxic compounds of the invention can be assayed using the well established ³H-thymidine proliferation assay. This is a convenient method for quantitating cellular proliferation, as it evaluates DNA synthesis by measuring the incorporation of exogenous radiolabeled ³H-thymidine. This assay is highly reproducible and can accommodate large numbers of compounds.

To carry out the assay, promyelocytic leukemia cells, HL-60, are cultured in RPMI media containing 10 % heat inactivated fetal calf serum (FCS). On the day of the study, the cells are collected, washed and resuspended at a concentration of 0.5 x 10⁶ cells/ml in RPMI containing 10% FCS. 100 µl of cell suspension is added to 96 well plates. Serial

dilutions (3-fold increments) of doxorubicin (as a positive control) or test compounds are made and 100 μ l of compounds are added per well. Finally 10 μ l of a 100 μ Ci/ml 3 H-thymidine is added per well and the plates are incubated for 24 hours. The plates are harvested using a 96 well Harvester (Packard Instruments) and counted on a Packard Top
5 Count counter. Four parameter logistic curves are fitted to the 3 H-thymidine incorporation as a function of drug molarity using Prism software to determine IC₅₀ values.

The CBI CC-1065 analogs (e.g., compound 19 of Example 4) generally have an IC₅₀ value in the above assay of from about 1 pM to about 100 nM, preferably from about 10 pM to about 10 nM.

10

EXAMPLE 6: Conjugation of Drug Molecules to Antibodies

This example describes reaction conditions and methodologies for conjugating a drug molecule of the invention (optionally including other groups, such as spacers, reactive functional groups and the like) to an antibody as a targeting agent. The conditions and
15 methodologies are intended to be exemplary only and non-limiting. Other approaches for conjugating drug molecules to antibodies are known in the art.

The conjugation method described herein is based on introduction of free thiol groups to the antibody through reaction of lysines of the antibody with 2-iminothiolane, followed by reaction of the drug-linker molecule with an active maleimide group. Initially
20 the antibody to be conjugated is buffer exchanged into 0.1M phosphate buffer pH 8.0 containing 50mM NaCl, 2mM DTPA, pH 8.0 and concentrated to 5-10 mg/ml. Thiolation is achieved through addition of 2-iminothiolane to the antibody. The amount of 2-iminothiolane to be added is determined in preliminary experiments and varies from antibody to antibody. In the preliminary experiments, a titration of increasing amounts of
25 2-iminothiolane is added to the antibody, and following incubation with the antibody for one hour at room temperature, the antibody is desalted into 50mM HEPES buffer pH 6.0 using a Sephadex G-25 column and the number of thiol groups introduced determined rapidly by reaction with dithiodipyridine (DTDP). Reaction of thiol groups with DTDP results in liberation of thiopyridine which is monitored at 324nm. Samples at a protein
30 concentration of 0.5-1.0 mg/ml can be used. The absorbance at 280nm is used to accurately determine the concentration of protein in the samples, and then an aliquot of each sample (0.9ml) is incubated with 0.1 ml DTDP (5mM stock solution in ethanol) for 10 minutes at room temperature. Blank samples of buffer alone plus DTDP are also incubated alongside.

After 10 minutes, absorbance at 324nm is measured and the number of thiols present quantitated using an extinction coefficient for thiopyridine of 19800M^{-1} .

Typically a thiolation level of three thiol groups per antibody is desired. For example, with one particular antibody this can be achieved through adding a 15 fold molar excess of 2-iminothiolane followed by incubation at room temperature for 1 hour. Antibody to be conjugated is therefore incubated with 2-iminothiolane at the desired molar ratio and then desalted into conjugation buffer (50mM HEPES buffer pH 6.0 containing 5mM glycine, 3% Glycerol and 2mM DTPA). The thiolated material is maintained on ice whilst the number of thiols introduced is quantitated as described above.

After verification of the number of thiols introduced, the drug molecule containing an active maleimide group (e.g., compound 15 of Example 3) is added at a 3-fold molar excess per thiol. The conjugation reaction is carried out in conjugation buffer also containing a final concentration of 5% ethylene glycol dimethyl ether (or a suitable alternative solvent). Commonly, the drug stock solution is dissolved in 90% ethylene glycol dimethyl ether, 10% dimethyl sulfoxide. For addition to antibody, the stock solution is added directly to the thiolated antibody, which has enough ethylene glycol dimethyl ether added to bring the final concentration to 5%, or pre-diluted in conjugation buffer containing a final concentration of 10% ethylene glycol dimethyl ether, followed by addition to an equal volume of thiolated antibody.

The conjugation reaction is incubated at room temperature for 2 hours with mixing. Following incubation the reaction mix is centrifuged at 14000 RPM for 15 minutes and the pH can be adjusted to 7.2 if purification was not immediate. Purification of conjugate can be achieved through chromatography using a number of methods. Conjugate can be purified using size-exclusion chromatography on a Sephacryl S200 column pre-equilibrated with 50mM HEPES buffer pH 7.2 containing 5mM glycine, 50mM NaCl and 3% glycerol. Chromatography can be carried out at a linear flow rate of 28 cm/h. Fractions containing conjugate can be collected, pooled and concentrated. Alternatively purification can be achieved through ion-exchange chromatography. Conditions vary from antibody to antibody and need to be optimized in each case. For example, antibody-drug conjugate reaction mix can be applied to an SP-Sepharose column pre-equilibrated in 50mM HEPES, 5mM glycine, 3% glycerol, pH 6.0. The antibody conjugate can be eluted using a gradient of 0-1M NaCl in equilibration buffer. Fractions containing the conjugate

can be pooled, the pH can be adjusted to 7.2 and the sample concentrated as required.

5 The entire disclosures of all applications, patents and publications, cited herein are incorporated by reference herein.

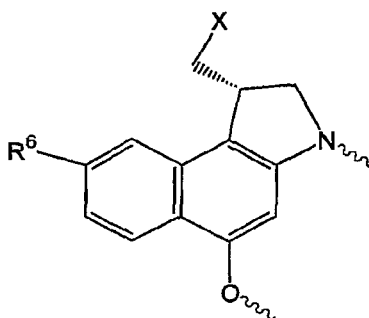
 The preceding examples can be repeated with similar success by substituting the generically or specifically described reactants and/or operating conditions of this invention for those used in the preceding examples.

10 From the foregoing description, one skilled in the art can easily ascertain the essential characteristics of this invention and, without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

CLAIMS

What is claimed as new and desired to be protected by Letters Patent of the United States is:

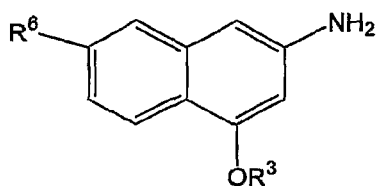
1. A method of making a compound (I) or a salt thereof,



(I)

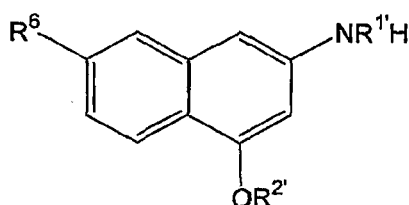
wherein R^1 and R^2 are each independently H, alkyl, $-C(O)OR'$, $-C(O)NR'R''$, or a protecting group, wherein R' and R'' are independently selected from the group consisting of H, substituted alkyl, unsubstituted alkyl, substituted aryl, unsubstituted aryl, substituted heteroaryl, unsubstituted heteroaryl, substituted heterocycloalkyl, and unsubstituted heterocycloalkyl; R^6 is H, substituted or unsubstituted lower alkyl, cyano, or alkoxy; and X is halogen, the method comprising:

adding protecting groups $R^{1'}$ and $R^{2'}$ to a compound (II)



(II)

to form a compound (III)

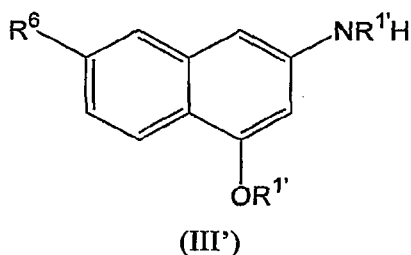


(III)

wherein R^3 is H or alkyl; and

generating a five membered ring comprising the amine nitrogen of compound (III).

2. The method of claim 1, wherein R^3 is methyl.
3. The method of claim 1, wherein X is Cl or Br.
4. The method of claim 3, wherein X is Br.
5. The method of claim 1, wherein $R^{1'}$ and $R^{2'}$ are different protecting groups.
6. The method of claim 5, wherein adding $R^{1'}$ and $R^{2'}$ comprises adding $R^{1'}$ to compound (II) to form compound (III')

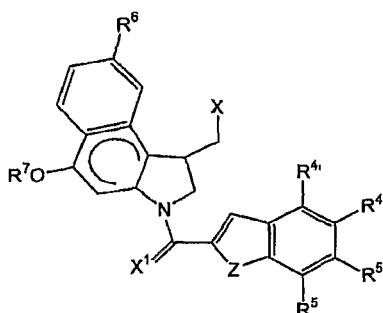


and replacing the protecting group $R^{1'}$ on the hydroxyl substituent with the protecting group $R^{2'}$.

7. The method of claim 5, wherein $R^{1'}$ is tert-butyloxycarbonyl.
8. The method of claim 7, wherein $R^{2'}$ is $-\text{CH}_2\text{Ph}$.
9. The method of claim 1, wherein $R^{1'}$ and $R^{2'}$ are the same.
10. The method of claim 1, further comprising replacing $R^{1'}$ and $R^{2'}$ with hydrogen after generating the five membered ring.
11. The method of claim 1, wherein $R^{1'}$ and R^1 are the same and $R^{2'}$ and R^2 are the same.

12. The method of claim 1, wherein generating the five membered ring comprises iodination of a carbon adjacent the amine substituent of compound (III) followed by alkylation using 1,3-dihalopropene.

13. A method of making a CBI CC-1065 analog, or a pharmaceutically acceptable salt thereof, having the following formula:



wherein X is halo;

X^1 and Z are each independently selected from O, S and NR^8 , wherein R^8 is a member selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, and acyl;

R^4 , $R^{4'}$, R^5 and $R^{5'}$ are members independently selected from the group consisting of H, substituted alkyl, unsubstituted alkyl, substituted aryl, unsubstituted aryl, substituted heteroaryl, unsubstituted heteroaryl, substituted heterocycloalkyl, unsubstituted heterocycloalkyl, halogen, NO_2 , NR^9R^{10} , $NC(O)R^9$, $OC(O)NR^9R^{10}$, $OC(O)OR^9$, $C(O)R^9$, SR^9 , OR^9 , $CR^9=NR^{10}$, and $O(CH_2)_nNR^{11}R^{11'}$,

wherein R^9 and R^{10} are independently selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted heterocycloalkyl, and substituted or unsubstituted peptidyl, or wherein R^9 and R^{10} together with the nitrogen atom to which they are attached are optionally joined to form a substituted or unsubstituted heterocycloalkyl ring system having from 4 to 6 members, optionally containing two or more heteroatoms, and

R^{11} and $R^{11'}$ are each independently H or lower alkyl;

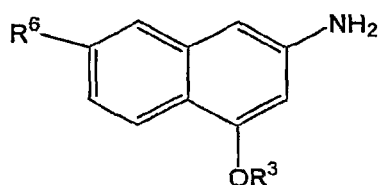
R^6 is H, substituted or unsubstituted lower alkyl, cyano, or alkoxy; and

R^7 is a member selected from the group consisting of H, substituted alkyl, unsubstituted alkyl, substituted heteroalkyl, unsubstituted heteroalkyl, diphosphates,

triphosphates, acyl, $C(O)R^{12}R^{13}$, $C(O)OR^{12}$, $C(O)NR^{12}R^{13}$, $P(O)(OR^{12})_2$, $C(O)CHR^{12}R^{13}$, SR^{12} and $SiR^{12}R^{13}R^{14}$,

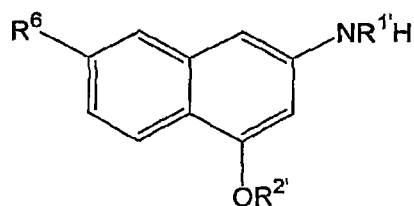
wherein R^{12} , R^{13} , and R^{14} are members independently selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl and substituted or unsubstituted aryl, or wherein R^{12} and R^{13} together with the nitrogen or carbon atom to which they are attached are optionally joined to form a substituted or unsubstituted heterocycloalkyl ring system having from 4 to 6 members, optionally containing two or more heteroatoms; the method comprising:

adding protecting groups $R^{1'}$ and $R^{2'}$ to a compound (II)



(II)

to form a compound (III)



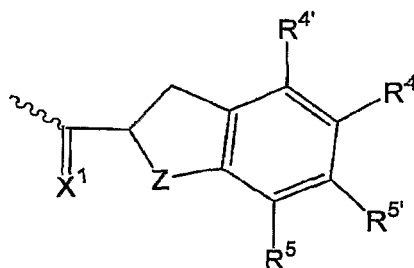
(III)

wherein R^3 is H or alkyl; and

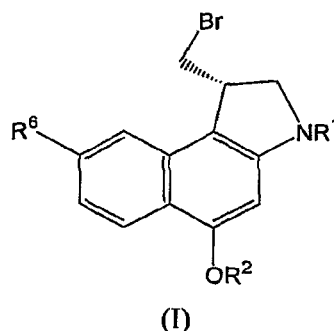
generating a five membered ring comprising the amine nitrogen of compound (III);

and

adding a binding unit to compound (III), the binding unit comprising



14. The method of claim 13, wherein generating the five membered ring comprises iodination of a carbon adjacent the amine substituent of compound (III) followed by alkylation using 1,3-dihalopropene prior to generating the five membered ring.
15. The method of claim 13, wherein adding the binding unit comprises removing the protecting group R^1 .
16. The method of claim 15, wherein adding the binding unit further comprises adding the binding unit to the amine substituent.
17. The method of claim 13, wherein R^6 is H.
18. A compound of formula (I), or a pharmaceutically acceptable salt thereof:



wherein R^1 and R^2 are each independently H, alkyl, $-C(O)OR'$, $-C(O)NR'R''$, or a protecting group, wherein R' and R'' are independently selected from the group consisting of H, substituted alkyl, unsubstituted alkyl, substituted aryl, unsubstituted aryl, substituted heteroaryl, unsubstituted heteroaryl, substituted heterocycloalkyl, and unsubstituted heterocycloalkyl.

19. The compound of claim 18, wherein R^1 and R^2 are different protecting groups.
20. The compound of claim 18, wherein the compound is

