

INHIBITORS OF HIV REVERSE TRANSCRIPTASE

Cross Reference to Related Applications

This application claims the benefit of United States Provisional Application Serial Numbers 60/088,448, filed June 8, 1998, and 60/093,072, filed July 16, 1998, both of which are incorporated by reference in their entirety.

BACKGROUND OF THE INVENTION

Field of the Invention

This invention relates to novel multibinding compounds (agents) that inhibit the enzyme human immunodeficiency virus ("HIV") reverse transcriptase and to pharmaceutical compositions comprising such compounds. Accordingly, the multibinding compounds and pharmaceutical compositions of this invention are useful in to inhibit HIV reverse transcriptase and, accordingly, the replication of HIV *in vivo*.

References

The following publications are cited in this application as superscript numbers:

¹ Christ et al., U.S. Patent 5,874,430, issued February 23, 1999

All of the above publications are herein incorporated by reference in their entirety to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference in its entirety.

State of the Art

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Two distinct retroviruses, human immunodeficiency virus (HIV) type-1 (HIV-1) or type-2 (HIV-2), have been etiologically linked to the immunosuppressive disease, acquired immunodeficiency syndrome (AIDS). HIV seropositive individuals are initially asymptomatic but typically have CD4 count less than normal, and have detectable viremia. They typically develop AIDS related complex (ARC) followed by AIDS. Affected individuals exhibit severe immunosuppression which predisposes them to debilitating and ultimately fatal opportunistic infections.

10 The disease AIDS is the end result of an HIV-1 or HIV-2 virus following its own complex life cycle. The virion life cycle begins with the virion attaching itself to the host human T-4 lymphocyte immune cell through the bonding of a glycoprotein on the surface of the virion's protective coat (gp120) with the CD4 glycoprotein on the lymphocyte cell. Once attached, the virion sheds its
15 glycoprotein coat, penetrates into the membrane of the host cell, and uncoats its RNA. The virion enzyme, reverse transcriptase, directs the process of transcribing the RNA into single-stranded DNA. The viral RNA is degraded and a second DNA strand is created. The now double-stranded DNA is integrated into the human cell's genes and those genes are used for virus reproduction.

20 At this point, RNA polymerase transcribes the integrated DNA into viral RNA. The viral RNA is translated into the precursor gag-pol fusion polyprotein. The polyprotein is then cleaved by the HIV protease enzyme to yield the mature viral proteins. Thus, HIV protease is responsible for regulating a cascade of
25 cleavage events that lead to the virus particle's maturing into a virus that is capable of full infectivity.

 The typical human immune system response, killing the invading virion, is taxed because the virus infects and kills the immune system's T cells. In
30 addition, viral reverse transcriptase, the enzyme used in making a new virion



particle, lacks an error correction system. Thus, errors accumulate and the mutations that occur in the DNA/RNA that codes for the coat proteins tend not to be lethal. This results in continually changed glycoproteins on the surface of the viral protective coat. This lack of constancy decreases the immune system's effectiveness because specific families of antibodies produced against one glycoprotein may be useless against another, hence reducing the number of antibodies available to block viral entry. The virus continues to reproduce while the immune response system continues to weaken. Eventually, the HIV largely holds free reign over the body's immune system, allowing opportunistic infections to set in and without the administration of antiviral agents, immunomodulators, or both, death may result.

There are at least three critical points in the virus's life cycle which have been identified as possible targets for antiviral drugs: (1) the initial attachment of the virion to the T-4 lymphocyte or macrophage site (gp120, CD4, CXCR4, CCR5), (2) the transcription of viral RNA to viral DNA (reverse transcriptase, RT), and (3) the processing of gag-pol protein (HIV protease). Inhibition of the virus at the second critical point, the viral RNA to viral DNA transcription process, has provided a number of the current therapies used in treating AIDS. This transcription must occur for the virion to reproduce because the virion's genes are encoded in RNA and the host cell reads only DNA. By introducing drugs that block the reverse transcriptase from completing the formation of viral DNA, HIV-1 replication can be stopped.

A number of compounds that interfere with viral replication have been developed to treat AIDS. Nucleoside analogs such as, by way of example only, 3'-azido-3'-deoxythymidine (AZT), 2',3'-dideoxycytidine (ddC), 2',3'-dideoxythymidinene (d4T), 2',3'-dideoxyinosine (ddI), and 2',3'-dideoxy-3'-thiacytidine (3TC) have been shown to be relatively effective in halting HIV replication at the reverse transcriptase (RT) stage (also called



nucleoside reverse transcriptase inhibitors, hereinafter "NRTIs" NRTIs generally suffer from specificity-related side effects because they may also be substrates for host RNA and DNA polymerases.

5 Non-nucleoside HIV reverse transcriptase inhibitors (hereinafter "NNRTIs") have also been discovered. As an example, it has been found that certain benzoxazinones, e.g., efavirenz, are useful in the inhibition of HIV reverse transcriptase, the prevention or treatment of infection by HIV and the treatment of AIDS.

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Even with the current success of reverse transcriptase inhibitors, it has been found that HIV patients can become resistant to a single inhibitor.

Accordingly, more potent inhibitors of HIV reverse transcriptase would
15 have significant advantages over currently available inhibitors. It has now been discovered that HIV reverse transcriptase inhibitors prepared by linking at least one nucleoside reverse transcriptase inhibitor (NRTI) and at least one non-nucleoside reverse transcriptase inhibitor (NNRTI) to one or more linkers have an increased potency. Such multibinding compounds provide improved
20 biological and/or therapeutic effects compared to the aggregate of the unlinked ligands due to their multibinding properties.

SUMMARY OF THE INVENTION

This invention is directed to novel multibinding compounds (agents) that
25 inhibit HIV reverse transcriptase. The multibinding compounds of this invention are useful to inhibit HIV reverse transcriptase, and accordingly, the replication of HIV *in vivo*.

Accordingly, in one of its composition aspects, this invention provides a
30 multibinding compound comprising from 2 to 10 ligands covalently attached to



one or more linkers wherein each of said ligands independently comprises at least one nucleoside reverse transcriptase inhibitor and at least one non-nucleoside reverse transcriptase inhibitor; and pharmaceutically-acceptable salts thereof.

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In another of its composition aspects, this invention provides a multibinding compound of formula I:



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wherein each L is independently a ligand comprising at least one nucleoside reverse transcriptase inhibitor and at least one non-nucleoside reverse transcriptase inhibitor; each X is independently a linker; p is an integer of from 2 to 10; and q is an integer of from 1 to 20; and pharmaceutically-acceptable salts thereof.

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Preferably, q is less than p in the multibinding compounds of this invention. More preferably, $p=2$ and $q=1$.

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Preferably, each nucleoside reverse transcriptase inhibitor ligand, L, in the multibinding compound of formula I is independently selected from zidovudine, didanosine, zalcitabine, stavudine, lamivudine, abacavir, adefovir, raluridine, oral PMPA prodrug, azidouridine, IVX-E-59, emitricitabine and lodenosine, and each non-nucleoside reverse transcriptase inhibitor ligand, L, in the multibinding compound of formula I is independently selected from

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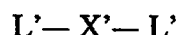
nevirapine, delavirdine, efavirenz, MKC-442, loviride, S-1153, talviraline, calanolide A and tivirapine.

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Preferably, the nucleoside reverse transcriptase inhibitor is emitricitabine and the non-nucleoside reverse transcriptase inhibitor is efavirenz.



In still another of its composition aspects, this invention provides a multibinding compound of formula II:



II

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wherein each L' is independently a ligand comprising at least one nucleoside reverse transcriptase inhibitor and at least one non-nucleoside reverse transcriptase inhibitor and X' is a linker; and pharmaceutically-acceptable salts thereof.

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Preferably, in the multibinding compound of formula II, each nucleoside reverse transcriptase inhibitor ligand, L', is independently selected from the group consisting of zidovudine, didanosine, zalcitabine, stavudine, lamivudine, abacavir, adefovir, raluridine, oral PMPA prodrug, azidouridine, IVX-E-59, emitricitabine and lodenosine, and each non-nucleoside reverse transcriptase inhibitor ligand, L, independently selected from the group consisting of nevirapine, delavirdine, efavirenz, MKC-442, loviride, S-1153, talviraline, calanolide A and tivrapipe.

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Preferably, the nucleoside reverse transcriptase inhibitor is emitricitabine and the non-nucleoside reverse transcriptase inhibitor is efavirenz.

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In yet another of its composition aspects, this invention provides a pharmaceutical composition comprising a pharmaceutically acceptable carrier and an effective amount of a multibinding compound comprising from 2 to 10 ligands covalently attached to one or more linkers wherein each of said ligands independently comprises at least one nucleoside reverse transcriptase inhibitor and at least one non-nucleoside reverse transcriptase inhibitor; and pharmaceutically-acceptable salts thereof.

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This invention is also directed to pharmaceutical compositions comprising a pharmaceutically acceptable carrier and an effective amount of a multibinding compound of formula I or formula II.

5 The multibinding compounds of this invention are effective inhibitors of the enzyme HIV reverse transcriptase, an enzyme involved in the conversion of viral RNA to double stranded DNA. Accordingly, in one of its method aspects, this invention provides a method for inhibiting HIV replication in a patient infected with HIV, the method comprising administering to a patient a
10 pharmaceutical composition comprising a pharmaceutically-acceptable carrier and a therapeutically-effective amount of a multibinding compound comprising from 2 to 10 ligands covalently attached to one or more linkers, wherein each of said ligands independently comprises at least one nucleoside reverse transcriptase inhibitor and at least one non-nucleoside reverse transcriptase inhibitor; and
15 pharmaceutically-acceptable salts thereof.

 This invention is also directed to general synthetic methods for generating large libraries of diverse multimeric compounds which multimeric compounds are candidates for possessing multibinding properties. The diverse multimeric
20 compound libraries provided by this invention are synthesized by combining a linker or linkers with a ligand or ligands to provide for a library of multimeric compounds wherein the linker and ligand each have complementary functional groups permitting covalent linkage. The library of linkers is preferably selected to have diverse properties such as valency, linker length, linker geometry and
25 rigidity, hydrophilicity or hydrophobicity, amphiphilicity, acidity, basicity and polarization. The library of ligands is preferably selected to have diverse attachment points on the same ligand, different functional groups at the same site of otherwise the same ligand, and the like.



This invention is further directed to libraries of diverse multimeric compounds which multimeric compounds are candidates for possessing multibinding properties to HIV reverse transcriptase. These libraries are prepared via the methods described above and permit the rapid and efficient evaluation of what molecular constraints impart multibinding properties to a
5 ligand or a class of ligands targeting an enzyme.

Accordingly, in one of its method aspects, this invention is directed to a method for identifying multimeric ligand compounds possessing multibinding
10 properties to HIV reverse transcriptase which method comprises:

- (a) identifying a ligand or a mixture of ligands wherein each ligand contains at least one reactive functionality;
- (b) identifying a library of linkers wherein each linker in said library comprises at least two functional groups having complementary reactivity to at
15 least one of the reactive functional groups of the ligand;
- (c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the ligand or mixture of ligands identified in (a) with the library of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said
20 linker and at least two of said ligands; and
- (d) assaying the multimeric ligand compounds produced in (c) above to identify multimeric ligand compounds possessing multibinding properties.

In another of its method aspects, this invention is directed to a method for
25 identifying multimeric ligand compounds possessing multibinding properties to HIV reverse transcriptase which method comprises:

- (a) identifying a library of ligands wherein each ligand contains at least one reactive functionality;



- (b) identifying a linker or mixture of linkers wherein each linker comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand;
- (c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the library of ligands identified in (a) with the linker or mixture of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands; and
- (d) assaying the multimeric ligand compounds produced in (c) above to identify multimeric ligand compounds possessing multibinding properties.

The preparation of the multimeric ligand compound library is achieved by either the sequential or concurrent combination of the two or more stoichiometric equivalents of the ligands identified in (a) with the linkers identified in (b).

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The assay protocols recited in (d) can be conducted on the multimeric ligand compound library produced in (c) above, or preferably, each member of the library is isolated by preparative liquid chromatography mass spectrometry (LCMS).

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In one of its composition aspects, this invention is directed to a library of multimeric ligand compounds which may possess multivalent properties to HIV reverse transcriptase which library is prepared by the method comprising:

- (a) identifying a ligand or a mixture of ligands wherein each ligand contains at least one reactive functionality;
- (b) identifying a library of linkers wherein each linker in said library comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand; and
- (c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the ligand or mixture of ligands identified

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in (a) with the library of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands.

5 In another of its composition aspects, this invention is directed to a library of multimeric ligand compounds which may possess multivalent properties to HIV reverse transcriptase which library is prepared by the method comprising:

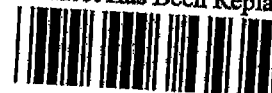
10 (a) identifying a library of ligands wherein each ligand contains at least one reactive functionality;

 (b) identifying a linker or mixture of linkers wherein each linker comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand; and

15 (c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the library of ligands identified in (a) with the linker or mixture of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands.

20 In a preferred embodiment, the library of linkers employed in either the methods or the library aspects of this invention is selected from the group comprising flexible linkers, rigid linkers, hydrophobic linkers, hydrophilic linkers, linkers of different geometry, acidic linkers, basic linkers, linkers of different polarization and/or polarizability and amphiphilic linkers. For
25 example, in one embodiment, each of the linkers in the linker library may comprise linkers of different chain length and/or having different complementary reactive groups. Such linker lengths can preferably range from about 2 to 100Å.

 In another preferred embodiment, the ligand or mixture of ligands is
30 selected to have reactive functionality at different sites on said ligands in order to



provide for a range of orientations of said ligand on said multimeric ligand compounds. Such reactive functionality includes, by way of example, carboxylic acids, carboxylic acid halides, carboxyl esters, amines, halides, pseudohalides, isocyanates, vinyl unsaturation, ketones, aldehydes, thiols, alcohols, anhydrides, boronates, phosphates, phosphonates and precursors thereof. It is understood, of course, that the reactive functionality on the ligand is selected to be complementary to at least one of the reactive groups on the linker so that a covalent linkage can be formed between the linker and the ligand.

10 In addition to the combinatorial methods described herein, this invention provides for an iterative process for rationally evaluating what molecular constraints impart multibinding properties to a class of multimeric compounds or ligands targeting an enzyme. Specifically, this method aspect is directed to a method for identifying multimeric ligand compounds possessing multibinding properties which method comprises:

15 (a) preparing a first collection or iteration of multimeric compounds which is prepared by contacting at least two stoichiometric equivalents of the ligand or mixture of ligands which target an enzyme with a linker or mixture of linkers wherein said ligand or mixture of ligands comprises at least one reactive functionality and said linker or mixture of linkers comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand wherein said contacting is conducted under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands;

20 (b) assaying said first collection or iteration of multimeric compounds to assess which if any of said multimeric compounds possess multibinding properties;

(c) repeating the process of (a) and (b) above until at least one multimeric compound is found to possess multibinding properties;



(d) evaluating what molecular constraints imparted multibinding properties to the multimeric compound or compounds found in the first iteration recited in (a)- (c) above;

(e) creating a second collection or iteration of multimeric compounds
5 which elaborates upon the particular molecular constraints imparting multibinding properties to the multimeric compound or compounds found in said first iteration;

(f) evaluating what molecular constraints imparted enhanced multibinding properties to the multimeric compound or compounds found in the
10 second collection or iteration recited in (e) above;

(g) optionally repeating steps (e) and (f) to further elaborate upon said molecular constraints.

Preferably, steps (e) and (f) are repeated at least two times, more
15 preferably at from 2-50 times, even more preferably from 3 to 50 times, and still more preferably at least 5-50 times.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A shows representative chemical structures of non-nucleoside
20 reverse transcriptase inhibitors and nucleoside reverse transcriptase inhibitors.

Figure 1B shows representative generic structures of non-nucleoside synthons and representative generic chemical structures of nucleoside synthons.

25 Figures 2 through 11 illustrate preparative chemical synthetic procedures.

Figures 12 through 18 illustrate synthetic procedures for representative compounds of this invention.



Figure 19 illustrates examples of multibinding compounds comprising 2 ligands attached in different formats to a linker.

Figure 20 illustrates examples of multibinding compounds comprising 3 ligands attached in different formats to a linker.

Figure 21 illustrates examples of multibinding compounds comprising 4 ligands attached in different formats to a linker.

Figure 22 illustrates examples of multibinding compounds comprising >4 ligands attached in different formats to a linker.

DETAILED DESCRIPTION OF THE INVENTION

This invention is directed to multibinding compounds which inhibit the enzyme HIV reverse transcriptase, pharmaceutical compositions containing such compounds and methods for inhibiting HIV replication in a patient infected with HIV. When discussing such compounds, compositions or methods, the following terms have the following meanings unless otherwise indicated. Any undefined terms have their art recognized meanings.

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The term "alkyl" refers to a monoradical branched or unbranched saturated hydrocarbon chain preferably having from 1 to 40 carbon atoms, more preferably 1 to 10 carbon atoms, and even more preferably 1 to 6 carbon atoms. This term is exemplified by groups such as methyl, ethyl, *n*-propyl, *iso*-propyl, *n*-butyl, *iso*-butyl, *n*-hexyl, *n*-decyl, tetradecyl, and the like.

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The term "substituted alkyl" refers to an alkyl group as defined above, having from 1 to 5 substituents, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy,

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amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy,
5 hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO₂-alkyl, -SO₂-substituted alkyl, -SO₂-aryl and -SO₂-heteroaryl.

The term "alkylene" refers to a diradical of a branched or unbranched
10 saturated hydrocarbon chain, preferably having from 1 to 40 carbon atoms, more preferably 1 to 10 carbon atoms and even more preferably 1 to 6 carbon atoms. This term is exemplified by groups such as methylene (-CH₂-), ethylene (-CH₂CH₂-), the propylene isomers (e.g., -CH₂CH₂CH₂- and -CH(CH₃)CH₂-) and the like.

15 The term "substituted alkylene" refers to an alkylene group, as defined above, having from 1 to 5 substituents, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl,
20 azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO₂-alkyl, -SO₂-substituted alkyl, -SO₂-aryl and
25 -SO₂-heteroaryl. Additionally, such substituted alkylene groups include those where 2 substituents on the alkylene group are fused to form one or more cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, heterocyclic or heteroaryl groups fused to the alkylene group. Preferably such
30 fused groups contain from 1 to 3 fused ring structures.



The term "alkaryl" refers to the groups -alkylene-aryl and -substituted alkylene-aryl where alkylene, substituted alkylene and aryl are defined herein. Such alkaryl groups are exemplified by benzyl, phenethyl and the like.

5 The term "alkoxy" refers to the groups alkyl-O-, alkenyl-O-, cycloalkyl-O-, cycloalkenyl-O-, and alkynyl-O-, where alkyl, alkenyl, cycloalkyl, cycloalkenyl, and alkynyl are as defined herein. Preferred alkoxy groups are alkyl-O- and include, by way of example, methoxy, ethoxy, *n*-propoxy, *iso*-propoxy, *n*-butoxy, *tert*-butoxy, *sec*-butoxy, *n*-pentoxy, *n*-hexoxy, 1,2-
10 dimethylbutoxy, and the like.

The term "substituted alkoxy" refers to the groups substituted alkyl-O-, substituted alkenyl-O-, substituted cycloalkyl-O-, substituted cycloalkenyl-O-, and substituted alkynyl-O- where substituted alkyl, substituted alkenyl,
15 substituted cycloalkyl, substituted cycloalkenyl and substituted alkynyl are as defined herein.

The term "alkylalkoxy" refers to the groups -alkylene-O-alkyl, alkylene-O-substituted alkyl, substituted alkylene-O-alkyl and substituted
20 alkylene-O-substituted alkyl wherein alkyl, substituted alkyl, alkylene and substituted alkylene are as defined herein. Preferred alkylalkoxy groups are alkylene-O-alkyl and include, by way of example, methylenemethoxy (-CH₂OCH₃), ethylenemethoxy (-CH₂CH₂OCH₃), *n*-propylene-*iso*-propoxy (-CH₂CH₂CH₂OCH(CH₃)₂), methylene-*t*-butoxy (-CH₂-O-C(CH₃)₃) and the like.

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The term "alkylthioalkoxy" refers to the group -alkylene-S-alkyl, alkylene-S-substituted alkyl, substituted alkylene-S-alkyl and substituted alkylene-S-substituted alkyl wherein alkyl, substituted alkyl, alkylene and substituted alkylene are as defined herein. Preferred alkylthioalkoxy groups are



alkylene-S-alkyl and include, by way of example, methylenethiomethoxy ($-\text{CH}_2\text{SCH}_3$), ethylenethiomethoxy ($-\text{CH}_2\text{CH}_2\text{SCH}_3$), *n*-propylene-*iso*-thiopropoxy ($-\text{CH}_2\text{CH}_2\text{CH}_2\text{SCH}(\text{CH}_3)_2$), methylene-*t*-thiobutoxy ($-\text{CH}_2\text{SC}(\text{CH}_3)_3$) and the like.

- 5 The term "alkenyl" refers to a monoradical of a branched or unbranched unsaturated hydrocarbon group preferably having from 2 to 40 carbon atoms, more preferably 2 to 10 carbon atoms and even more preferably 2 to 6 carbon atoms and having at least 1 and preferably from 1-6 sites of vinyl unsaturation. Preferred alkenyl groups include ethenyl ($-\text{CH}=\text{CH}_2$), *n*-propenyl
10 ($-\text{CH}_2\text{CH}=\text{CH}_2$), *iso*-propenyl ($-\text{C}(\text{CH}_3)=\text{CH}_2$), and the like.

- The term "substituted alkenyl" refers to an alkenyl group as defined above having from 1 to 5 substituents, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl,
15 substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocycloxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic,
20 heterocycloxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO₂-alkyl, -SO₂-substituted alkyl, -SO₂-aryl and -SO₂-heteroaryl.

- The term "alkenylene" refers to a diradical of a branched or unbranched
25 unsaturated hydrocarbon group preferably having from 2 to 40 carbon atoms, more preferably 2 to 10 carbon atoms and even more preferably 2 to 6 carbon atoms and having at least 1 and preferably from 1-6 sites of vinyl unsaturation. This term is exemplified by groups such as ethenylene ($-\text{CH}=\text{CH}-$), the propenylene isomers (e.g., $-\text{CH}_2\text{CH}=\text{CH}-$ and $-\text{C}(\text{CH}_3)=\text{CH}-$) and the like.



The term "substituted alkenylene" refers to an alkenylene group as defined above having from 1 to 5 substituents, and preferably from 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO₂-alkyl, -SO₂-substituted alkyl, -SO₂-aryl and -SO₂-heteroaryl. Additionally, such substituted alkenylene groups include those where 2 substituents on the alkenylene group are fused to form one or more cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, heterocyclic or heteroaryl groups fused to the alkenylene group.

The term "alkynyl" refers to a monoradical of an unsaturated hydrocarbon preferably having from 2 to 40 carbon atoms, more preferably 2 to 20 carbon atoms and even more preferably 2 to 6 carbon atoms and having at least 1 and preferably from 1-6 sites of acetylene (triple bond) unsaturation. Preferred alkynyl groups include ethynyl ($\text{-C}\equiv\text{CH}$), propargyl ($\text{-CH}_2\text{C}\equiv\text{CH}$) and the like.

The term "substituted alkynyl" refers to an alkynyl group as defined above having from 1 to 5 substituents, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted



thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO₂-alkyl, -SO₂-substituted alkyl, -SO₂-aryl and -SO₂-heteroaryl.

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The term "alkynylene" refers to a diradical of an unsaturated hydrocarbon preferably having from 2 to 40 carbon atoms, more preferably 2 to 10 carbon atoms and even more preferably 2 to 6 carbon atoms and having at least 1 and preferably from 1-6 sites of acetylene (triple bond) unsaturation. Preferred
10 alkynylene groups include ethynylene ($-C\equiv C-$), propargylene ($-CH_2C\equiv C-$) and the like.

The term "substituted alkynylene" refers to an alkynylene group as defined above having from 1 to 5 substituents, and preferably 1 to 3 substituents,
15 selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted
20 thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO₂-alkyl, -SO₂-substituted alkyl, -SO₂-aryl and -SO₂-heteroaryl

25 The term "acyl" refers to the groups $HC(O)-$, $alkyl-C(O)-$, substituted $alkyl-C(O)-$, $cycloalkyl-C(O)-$, substituted $cycloalkyl-C(O)-$, $cycloalkenyl-C(O)-$, substituted $cycloalkenyl-C(O)-$, $aryl-C(O)-$, $heteroaryl-C(O)-$ and heterocyclic- $C(O)-$ where alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, heteroaryl and heterocyclic are as
30 defined herein.



The term "acylamino" or "aminocarbonyl" refers to the group $-C(O)NRR$ where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, heterocyclic or where both R groups are joined to form a heterocyclic group (e.g., morpholino) wherein alkyl, substituted alkyl, aryl, heteroaryl and
5 heterocyclic are as defined herein.

The term "aminoacyl" refers to the group $-NRC(O)R$ where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, or heterocyclic wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined
10 herein.

The term "aminoacyloxy" or "alkoxycarbonylamino" refers to the group $-NRC(O)OR$ where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, or heterocyclic wherein alkyl, substituted alkyl, aryl, heteroaryl
15 and heterocyclic are as defined herein.

The term "acyloxy" refers to the groups $alkyl-C(O)O-$, substituted $alkyl-C(O)O-$, $cycloalkyl-C(O)O-$, substituted $cycloalkyl-C(O)O-$, $aryl-C(O)O-$, heteroaryl- $C(O)O-$, and heterocyclic- $C(O)O-$ wherein alkyl, substituted alkyl,
20 $cycloalkyl$, substituted $cycloalkyl$, aryl, heteroaryl, and heterocyclic are as defined herein.

The term "aryl" refers to an unsaturated aromatic carbocyclic group of from 6 to 20 carbon atoms having a single ring (e.g., phenyl) or multiple
25 condensed (fused) rings (e.g., naphthyl or anthryl). Preferred aryls include phenyl, naphthyl and the like.

Unless otherwise constrained by the definition for the aryl substituent, such aryl groups can optionally be substituted with from 1 to 5 substituents,
30 preferably 1 to 3 substituents, selected from the group consisting of acyloxy,



hydroxy, thiol, acyl, alkyl, alkoxy, alkenyl, alkynyl, cycloalkyl, cycloalkenyl,
substituted alkyl, substituted alkoxy, substituted alkenyl, substituted alkynyl,
substituted cycloalkyl, substituted cycloalkenyl, amino, substituted amino,
aminoacyl, acylamino, alkaryl, aryl, aryloxy, azido, carboxyl, carboxylalkyl, -
5 cyano, halo, nitro, heteroaryl, heteroaryloxy, heterocyclic, heterocycloxy,
aminoacyloxy, oxyacylamino, thioalkoxy, substituted thioalkoxy, thioaryloxy,
thioheteroaryloxy, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -
SO₂-alkyl, -SO₂-substituted alkyl, -SO₂-aryl, -SO₂-heteroaryl and trihalomethyl.
Preferred aryl substituents include alkyl, alkoxy, halo, cyano, nitro,
10 trihalomethyl, and thioalkoxy.

The term "aryloxy" refers to the group aryl-O- wherein the aryl group is
as defined above including optionally substituted aryl groups as also defined
above.

15

The term "arylene" refers to the diradical derived from aryl (including
substituted aryl) as defined above and is exemplified by 1,2-phenylene, 1,3-
phenylene, 1,4-phenylene, 1,2-naphthylene and the like.

20

The term "amino" refers to the group -NH₂.

The term "substituted amino" refers to the group -NRR where each R is
independently selected from the group consisting of hydrogen, alkyl, substituted
alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl,
25 cycloalkenyl, substituted cycloalkenyl, alkynyl, substituted alkynyl, aryl,
heteroaryl and heterocyclic provided that both R's are not hydrogen.

The term "carboxyalkyl" or "alkoxycarbonyl" refers to the groups
"-C(O)O-alkyl", "-C(O)O-substituted alkyl", "-C(O)O-cycloalkyl", "-C(O)O-
30 substituted cycloalkyl", "-C(O)O-alkenyl", "-C(O)O-substituted alkenyl",



"-C(O)O-alkynyl" and "-C(O)O-substituted alkynyl" where alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, alkynyl and substituted alkynyl are as defined herein.

5 The term "cycloalkyl" refers to cyclic alkyl groups of from 3 to 20 carbon atoms having a single cyclic ring or multiple condensed rings. Such cycloalkyl groups include, by way of example, single ring structures such as cyclopropyl, cyclobutyl, cyclopentyl, cyclooctyl, and the like, or multiple ring structures such as adamantanyl, and the like.

10

 The term "substituted cycloalkyl" refers to cycloalkyl groups having from 1 to 5 substituents, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocycloxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocycloxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO₂-alkyl, -SO₂-substituted alkyl, -SO₂-aryl and -SO₂-heteroaryl.

 The term "cycloalkenyl" refers to cyclic alkenyl groups of from 4 to 20 carbon atoms having a single cyclic ring and at least one point of internal unsaturation. Examples of suitable cycloalkenyl groups include, for instance, cyclobut-2-enyl, cyclopent-3-enyl, cyclooct-3-enyl and the like.

 The term "substituted cycloalkenyl" refers to cycloalkenyl groups having from 1 to 5 substituents, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted

30



cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, 5 aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO₂-alkyl, -SO₂-substituted alkyl, -SO₂-aryl and -SO₂-heteroaryl.

10 The term "halo" or "halogen" refers to fluoro, chloro, bromo and iodo.

The term "heteroaryl" refers to an aromatic group of from 1 to 15 carbon atoms and 1 to 4 heteroatoms selected from oxygen, nitrogen and sulfur within at least one ring (if there is more than one ring).

15

Unless otherwise constrained by the definition for the heteroaryl substituent, such heteroaryl groups can be optionally substituted with 1 to 5 substituents, preferably 1 to 3 substituents, selected from the group consisting of acyloxy, hydroxy, thiol, acyl, alkyl, alkoxy, alkenyl, alkynyl, cycloalkyl, 20 cycloalkenyl, substituted alkyl, substituted alkoxy, substituted alkenyl, substituted alkynyl, substituted cycloalkyl, substituted cycloalkenyl, amino, substituted amino, aminoacyl, acylamino, alkaryl, aryl, aryloxy, azido, carboxyl, carboxylalkyl, cyano, halo, nitro, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, aminoacyloxy, oxyacylamino, thioalkoxy, substituted 25 thioalkoxy, thioaryloxy, thioheteroaryloxy, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO₂-alkyl, -SO₂-substituted alkyl, -SO₂-aryl, -SO₂-heteroaryl and trihalomethyl. Preferred aryl substituents include alkyl, alkoxy, halo, cyano, nitro, trihalomethyl, and thioalkoxy. Such heteroaryl groups can have a single ring (e.g., pyridyl or furyl) or multiple condensed rings (e.g.,



indolizinyl or benzothienyl). Preferred heteroaryls include pyridyl, pyrrolyl and furyl.

The term "heteroaryloxy" refers to the group heteroaryl-O-.

5

The term "heteroarylene" refers to the diradical group derived from heteroaryl (including substituted heteroaryl), as defined above, and is exemplified by the groups 2,6-pyridylene, 2,4-pyridylene, 1,2-quinolinyne, 1,8-quinolinyne, 1,4-benzofuranylene, 2,5-pyridylene, 2,5-indolenyl and the like.

10

The term "heterocycle" or "heterocyclic" refers to a monoradical saturated unsaturated group having a single ring or multiple condensed rings, from 1 to 40 carbon atoms and from 1 to 10 hetero atoms, preferably 1 to 4 heteroatoms, selected from nitrogen, sulfur, phosphorus, and/or oxygen within the ring.

15

Unless otherwise constrained by the definition for the heterocyclic substituent, such heterocyclic groups can be optionally substituted with 1 to 5, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocycloxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocycloxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO₂-alkyl, -SO₂-substituted alkyl, -SO₂-aryl and -SO₂-heteroaryl. Such heterocyclic groups can have a single ring or multiple condensed rings. Preferred heterocyclics include morpholino, piperidinyl, and the like.

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Examples of nitrogen heterocycles and heteroaryls include, but are not limited to, pyrrole, imidazole, pyrazole, pyridine, pyrazine, pyrimidine, pyridazine, indolizine, isoindole, indole, indazole, purine, quinolizine, isoquinoline, quinoline, phthalazine, naphthylpyridine, quinoxaline, quinazoline, cinnoline, pteridine, carbazole, carboline, phenanthridine, acridine, phenanthroline, isothiazole, phenazine, isoxazole, phenoxazine, phenothiazine, imidazolidine, imidazoline, piperidine, piperazine, indoline, morpholino, piperidinyl, tetrahydrofuranyl, and the like as well as N-alkoxy-nitrogen containing heterocycles.

10

The term "heterocyclooxy" refers to the group heterocyclic-O-.

The term "thioheterocyclooxy" refers to the group heterocyclic-S-.

15

The term "heterocyclene" refers to the diradical group formed from a heterocycle, as defined herein, and is exemplified by the groups 2,6-morpholino, 2,5-morpholino and the like.

20

The term "oxyacylamino" or "aminocarbonyloxy" refers to the group -OC(O)NRR where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, or heterocyclic wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

25

The term "spiro-attached cycloalkyl group" refers to a cycloalkyl group attached to another ring via one carbon atom common to both rings.

The term "thiol" refers to the group -SH.

The term "thioalkoxy" refers to the group -S-alkyl.

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The term "substituted thioalkoxy" refers to the group -S-substituted alkyl.

5 The term "thioaryloxy" refers to the group aryl-S- wherein the aryl group is as defined above including optionally substituted aryl groups also defined above.

10 The term "thioheteroaryloxy" refers to the group heteroaryl-S- wherein the heteroaryl group is as defined above including optionally substituted aryl groups as also defined above.

As to any of the above groups which contain one or more substituents, it is understood, of course, that such groups do not contain any substitution or substitution patterns which are sterically impractical and/or synthetically non-feasible. In addition, the compounds of this invention include all stereochemical isomers arising from the substitution of these compounds.

15

The term "pharmaceutically-acceptable salt" refers to salts which retain the biological effectiveness and properties of the multibinding compounds of this invention and which are not biologically or otherwise undesirable. In many cases, the multibinding compounds of this invention are capable of forming acid and/or base salts by virtue of the presence of amino and/or carboxyl groups or groups similar thereto.

20

Pharmaceutically-acceptable base addition salts can be prepared from inorganic and organic bases. Salts derived from inorganic bases, include by way of example only, sodium, potassium, lithium, ammonium, calcium and magnesium salts. Salts derived from organic bases include, but are not limited to, salts of primary, secondary and tertiary amines, such as alkyl amines, dialkyl amines, trialkyl amines, substituted alkyl amines, di(substituted alkyl) amines, tri(substituted alkyl) amines, alkenyl amines, dialkenyl amines, trialkenyl

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amines, substituted alkenyl amines, di(substituted alkenyl) amines, tri(substituted alkenyl) amines, cycloalkyl amines, di(cycloalkyl) amines, tri(cycloalkyl) amines, substituted cycloalkyl amines, disubstituted cycloalkyl amine, trisubstituted cycloalkyl amines, cycloalkenyl amines, di(cycloalkenyl) amines, tri(cycloalkenyl) amines, substituted cycloalkenyl amines, disubstituted cycloalkenyl amine, trisubstituted cycloalkenyl amines, aryl amines, diaryl amines, triaryl amines, heteroaryl amines, diheteroaryl amines, triheteroaryl amines, heterocyclic amines, diheterocyclic amines, triheterocyclic amines, mixed di- and tri-amines where at least two of the substituents on the amine are different and are selected from the group consisting of alkyl, substituted alkyl, alkenyl, substituted alkenyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, heteroaryl, heterocyclic, and the like. Also included are amines where the two or three substituents, together with the amino nitrogen, form a heterocyclic or heteroaryl group.

15

Examples of suitable amines include, by way of example only, isopropylamine, trimethyl amine, diethyl amine, tri(*iso*-propyl) amine, tri(*n*-propyl) amine, ethanolamine, 2-dimethylaminoethanol, tromethamine, lysine, arginine, histidine, caffeine, procaine, hydrabamine, choline, betaine, ethylenediamine, glucosamine, N-alkylglucamines, theobromine, purines, piperazine, piperidine, morpholine, N-ethylpiperidine, and the like. It should also be understood that other carboxylic acid derivatives would be useful in the practice of this invention, for example, carboxylic acid amides, including carboxamides, lower alkyl carboxamides, dialkyl carboxamides, and the like.

25

Pharmaceutically acceptable acid addition salts may be prepared from inorganic and organic acids. Salts derived from inorganic acids include hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like. Salts derived from organic acids include acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, malic acid, malonic acid, succinic

30



acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, *p*-toluenesulfonic acid, salicylic acid, and the like.

- 5 The term "pharmaceutically-acceptable cation" refers to the cation of a pharmaceutically-acceptable salt.

10 The term "protecting group" or "blocking group" refers to any group which when bound to one or more hydroxyl, thiol, amino or carboxyl groups of the compounds (including intermediates thereof) prevents reactions from occurring at these groups and which protecting group can be removed by conventional chemical or enzymatic steps to reestablish the hydroxyl, thiol, amino or carboxyl group. The particular removable blocking group employed is not critical and preferred removable hydroxyl blocking groups include
15 conventional substituents such as allyl, benzyl, acetyl, chloroacetyl, thiobenzyl, benzylidene, phenacyl, *t*-butyl-diphenylsilyl and any other group that can be introduced chemically onto a hydroxyl functionality and later selectively removed either by chemical or enzymatic methods in mild conditions compatible with the nature of the product.

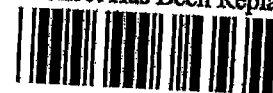
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Preferred removable thiol blocking groups include disulfide groups, acyl groups, benzyl groups, and the like.

25 Preferred removable amino blocking groups include conventional substituents such as *t*-butoxycarbonyl (*t*-BOC), benzyloxycarbonyl (CBZ), fluorenylmethoxycarbonyl (Fmoc), allyloxycarbonyl (ALOC), and the like which can be removed by conventional conditions compatible with the nature of the product.

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Preferred carboxyl protecting groups include esters such as methyl, ethyl, propyl, *t*-butyl etc. which can be removed by mild conditions compatible with the nature of the product.

5 The term "optional" or "optionally" means that the subsequently described event, circumstance or substituent may or may not occur, and that the description includes instances where said event or circumstance occurs and instances where it does not.

10 The term "library" refers to at least 3, preferably from 10^2 to 10^9 and more preferably from 10^2 to 10^4 multimeric compounds. Preferably, these compounds are prepared as a multiplicity of compounds in a single solution or reaction mixture which permits facile synthesis thereof. In one embodiment, the library of multimeric compounds can be directly assayed for multibinding
15 properties. In another embodiment, each member of the library of multimeric compounds is first isolated and, optionally, characterized. This member is then assayed for multibinding properties.

 The term "collection" refers to a set of multimeric compounds which are
20 prepared either sequentially or concurrently (e.g., combinatorially). The collection comprises at least 2 members; preferably from 2 to 10^9 members and still more preferably from 10 to 10^4 members.

 The term "multimeric compound" refers to compounds comprising from
25 2 to 10 ligands covalently connected through at least one linker which compounds may or may not possess multibinding properties (as defined herein).

 The term "pseudohalide" refers to functional groups which react in displacement reactions in a manner similar to a halogen. Such functional groups
30 include, by way of example, mesyl, tosyl, azido and cyano groups.

The term "human immunodeficiency virus reverse transcriptase," (also abbreviated HIV reverse transcriptase, reverse transcriptase, or HIV RT throughout) refers to the HIV enzyme which catalyzes the conversion of viral RNA to single stranded DNA. It may also carry out DNA dependent DNA
5 polymerase activity and ribonuclease activity.

The term "ligand" as used herein denotes a compound that is an inhibitor of the enzyme HIV reverse transcriptase. These inhibitors are further classified as nucleoside reverse transcriptase inhibitors (NRTIs) or non-nucleoside reverse
10 transcriptase inhibitors (NNRTIs). As far as is known, NRTIs are all DNA chain terminators. They are phosphorylated intracellularly to the triphosphate, then incorporated into the growing cDNA chain. As they lack a 3' - OH (hydroxy) group, further extension of the growing cDNA chain is not possible. Resistance to NRTI occurs by one of at least two mechanisms: decreased affinity
15 of reverse transcriptase for the inhibitor, and decreased rate of incorporation of the inhibitor into the growing cDNA chain. Examples of NRTIs include, but are not limited to zidovudine (Retrovir™, AZT, Glaxo Wellcome), didanosine (Videx™, ddI, Bristol-Myers Squibb), zalcitabine (Hivid™, ddC, NIH), stavudine (Zerit™, d4T, Bristol-Myers Squibb), lamivudine (Epivir™, 3TC, Glaxo
20 Wellcome), abacavir (Ziagen™, Glaxo Wellcome), adefovir (Gilead Sciences), raluridine (Burroughs Wellcome), oral PMPA prodrug (Gilead Sciences), azidouridine (AZDU, Schering), IVX-E-59 (Scriptene™, IVX BioScience), emitricitabine (FTC, Triangle Pharmaceuticals) and lodenosine (NIH).

25 Without being limited to any theory, it is believed that the mechanism of action of NNRTIs may be the slight allosteric distortion of one or more of the three catalytic Asp residues in the active site of HIV reverse transcriptase. These Asp residues position Mg^{2+} for catalysis. The mechanism of action can be a decrease in affinity for template or dNTP substrate (rare), or a decreased
30 capacity to catalyze nucleotidyl transfer to growing chain (common). Resistance



mutations are located close to the bound inhibitor, and generally decrease the affinity for the inhibitor. Examples of NNRTIs include, but are not limited to, nevirapine (Viramune™, Boehringer), delavirdine (Rescriptor™, Pharmacia), efavirenz (Sustiva™, DuPont Pharmaceuticals), MKC-442 (Mitsubishi Chemical),
5 loviride (Janssen Pharmaceutica), S-1153 (Shionogi), talviraline (Hoechst-Roussel Pharmaceuticals), calanolide A (NIH) and tivrapipe (Janssen Pharmaceutica).

The specific region or regions of the ligand that is (are) recognized by the
10 enzyme is designated as the "ligand domain". A ligand may be either capable of binding to an enzyme by itself, or may require the presence of one or more non-ligand components for binding (e.g., Ca^{+2} , Mg^{+2} or a water molecule is required for the binding of a ligand to various ligand binding sites).

15 Examples of ligands useful in this invention are described herein. Those skilled in the art will appreciate that portions of the ligand structure that are not essential for specific molecular recognition and binding activity may be varied substantially, replaced or substituted with unrelated structures (for example, with ancillary groups as defined below) and, in some cases, omitted entirely without
20 affecting the binding interaction. The primary requirement for a ligand is that it has a ligand domain as defined above. It is understood that the term ligand is not intended to be limited to compounds known to be useful in binding to HIV reverse transcriptase (e.g., known drugs). Those skilled in the art will understand that the term ligand can equally apply to a molecule that is not
25 normally associated with enzyme binding properties. In addition, it should be noted that ligands that exhibit marginal activity or lack useful activity as monomers can be highly active as multivalent compounds because of the benefits conferred by multivalency.

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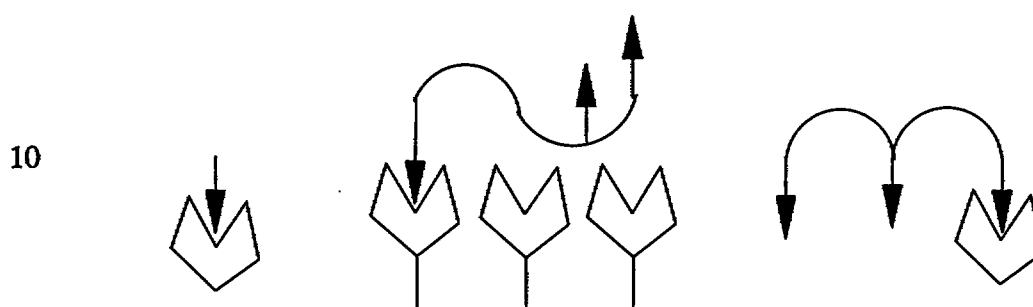
The term "multibinding compound or agent" refers to a compound that is capable of multivalency, as defined below, and which has 2-10 ligands covalently bound to one or more linkers which may be the same or different. Multibinding compounds provide a biological and/or therapeutic effect greater than the aggregate of unlinked ligands equivalent thereto which are made available for binding. That is to say that the biological and/or therapeutic effect of the ligands attached to the multibinding compound is greater than that achieved by the same amount of unlinked ligands made available for binding to the ligand binding sites. Multimeric compounds which are multimers connected through at least one linker may or may not possess multibinding properties.

The phrase "increased biological or therapeutic effect" includes, for example: increased affinity, increased selectivity for target, increased specificity for target, increased potency, increased efficacy, decreased toxicity, improved duration of activity or action, decreased side effects, increased therapeutic index, improved bioavailability, improved pharmacokinetics, improved activity spectrum, and the like. The multibinding compounds of this invention will exhibit at least one and preferably more than one of the above-mentioned affects.

The term "potency" refers to the minimum concentration at which a ligand is able to achieve a desirable biological or therapeutic effect. The potency of a ligand is typically proportional to its affinity for its ligand binding site. In some cases, the potency may be non-linearly correlated with its affinity. In comparing the potency of two drugs, e.g., a multibinding agent and the aggregate of its unlinked ligand, the dose-response curve of each is determined under identical test conditions (e.g., in an *in vitro* or *in vivo* assay, in an appropriate animal model). The finding that the multibinding agent produces an equivalent biological or therapeutic effect at a lower concentration than the aggregate unlinked ligand is indicative of enhanced potency.



The term "univalency" as used herein refers to a single binding interaction between one ligand as defined herein with one ligand binding site as defined herein. It should be noted that a compound having multiple copies of a ligand (or ligands) exhibits univalency when only one ligand is interacting with a
5 ligand binding site. Examples of univalent interactions are depicted below.



15

The term "multivalency" as used herein refers to the concurrent binding of from 2 to 10 linked ligands (which may be the same or different) and two or more corresponding receptors (ligand binding sites) on one or more enzymes which may be the same or different.

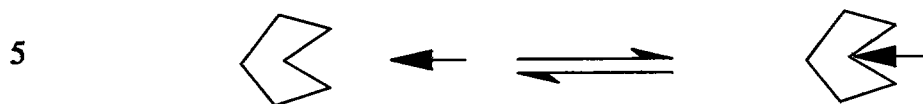
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For example, two ligands connected through a linker that bind concurrently to two ligand binding sites would be considered as bivalency; three ligands thus connected would be an example of trivalency. An example of

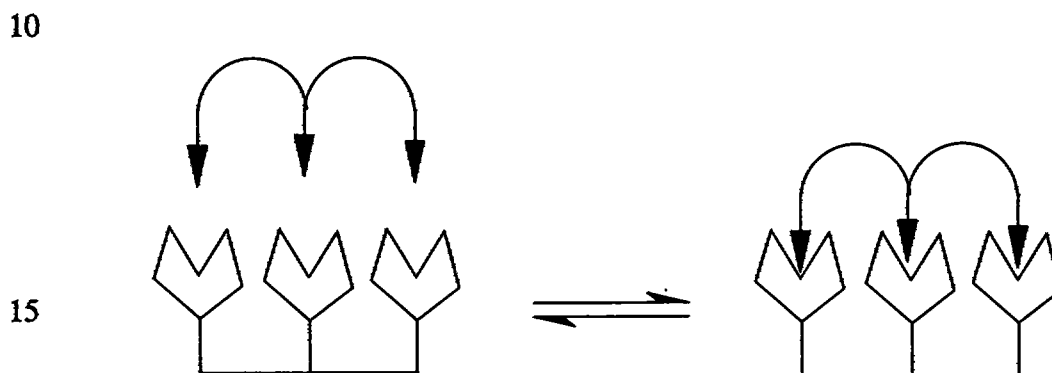
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trivalent binding, illustrating a multibinding compound bearing three ligands versus a monovalent binding interaction, is shown below:



Univalent Interaction



Trivalent Interaction

20 It should be understood that all compounds that contain multiple copies of
a ligand attached to a linker or to linkers do not necessarily exhibit the
phenomena of multivalency, i.e., that the biological and/or therapeutic effect of
the multibinding agent is greater than the sum of the aggregate of unlinked
ligands made available for binding to the ligand binding site (receptor). For
25 multivalency to occur, the ligands that are connected by a linker or linkers have
to be presented to their ligand binding sites by the linker(s) in a specific manner
in order to bring about the desired ligand-orienting result, and thus produce a
multibinding event.

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The term "selectivity" or "specificity" is a measure of the binding preferences of a ligand for different ligand binding sites (receptors). The selectivity of a ligand with respect to its target ligand binding site relative to another ligand binding site is given by the ratio of the respective values of K_d (i.e., the dissociation constants for each ligand-receptor complex) or, in cases
5 where a biological effect is observed below the K_d , the ratio of the respective EC_{50} 's (i.e., the concentrations that produce 50% of the maximum response for the ligand interacting with the two distinct ligand binding sites (receptors)).

10 The term "ligand binding site" denotes the site on the HIV reverse transcriptase enzyme that recognizes a ligand domain and provides a binding partner for the ligand. The ligand binding site may be defined by monomeric or multimeric structures. This interaction may be capable of producing a unique biological effect, for example, agonism, antagonism, modulatory effects, may
15 maintain an ongoing biological event, and the like.

The terms "agonism" and "antagonism" are well known in the art. The term "modulatory effect" refers to the ability of the ligand to change the activity of an agonist or antagonist through binding to a ligand binding site.
20

It should be recognized that the ligand binding sites of the enzyme that participate in biological multivalent binding interactions are constrained to varying degrees by their intra- and inter-molecular associations (e.g., such macromolecular structures may be covalently joined to a single structure,
25 noncovalently associated in a multimeric structure, embedded in a membrane or polymeric matrix, and so on) and therefore have less translational and rotational freedom than if the same structures were present as monomers in solution.

The term "inert organic solvent" or "inert organic solvent" means a
30 solvent which is inert under the conditions of the reaction being described in





conjunction therewith including, by way of example only, benzene, toluene, acetonitrile, tetrahydrofuran, dimethylformamide, chloroform, methylene chloride, diethyl ether, ethyl acetate, acetone, methylethyl ketone, methanol, ethanol, propanol, isopropanol, *t*-butanol, dioxane, pyridine, and the like.

- 5 Unless specified to the contrary, the solvents used in the reactions described herein are inert solvents.

The term "treatment" refers to any treatment of a pathologic condition in a mammal, particularly a human, and includes:

- 10 (i) preventing the pathologic condition from occurring in a subject which may be predisposed to the condition but has not yet been diagnosed with the condition and, accordingly, the treatment constitutes prophylactic treatment for the disease condition;
- (ii) inhibiting the pathologic condition, i.e., arresting its development;
- 15 (iii) relieving the pathologic condition, i.e., causing regression of the pathologic condition; or
- (iv) relieving the conditions mediated by the pathologic condition.

The term "pathologic condition which is modulated by treatment with a ligand" covers all disease states (i.e., pathologic conditions) which are generally
20 acknowledged in the art to be usefully treated with a ligand for the enzyme HIV reverse transcriptase in general, and those disease states which have been found to be usefully treated by a specific multibinding compound of this invention. Such disease states include, by way of example, AIDS.

25

The term "therapeutically effective amount" refers to that amount of multibinding compound which is sufficient to effect treatment, as defined above, when administered to a mammal in need of such treatment. The therapeutically effective amount will vary depending upon the subject and disease condition
30 being treated, the weight and age of the subject, the severity of the disease

condition, the manner of administration and the like, which can readily be determined by one of ordinary skill in the art.

5 The term "linker", identified where appropriate by the symbol X or X', refers to a group or groups that covalently links from 2 to 10 ligands (as identified above) in a manner that provides for a compound capable of multivalency. Among other features, the linker is a ligand-orienting entity that permits attachment of multiple copies of a ligand (which may be the same or different) thereto. It can be chiral, prochiral or achiral. In some cases, the
10 linker may itself be biologically active. The term "linker" does not, however, extend to cover solid inert supports such as beads, glass particles, fibers, and the like. But it is understood that the multibinding compounds of this invention can be attached to a solid support if desired. For example, such attachment to solid supports can be made for use in separation and purification processes and similar
15 applications.

 The extent to which multivalent binding is realized depends upon the efficiency with which the linker or linkers that joins the ligands presents these ligands to the array of available ligand binding sites. Beyond presenting these
20 ligands for multivalent interactions with ligand binding sites, the linker or linkers spatially constrains these interactions to occur within dimensions defined by the linker or linkers. Thus, the structural features of the linker (valency, geometry, orientation, size, flexibility, chemical composition, etc.) are features of multibinding agents that play an important role in determining their activities.

25

 The linkers used in this invention are selected to allow multivalent binding of ligands to the ligand binding sites of HIV reverse transcriptase, whether such sites are located interiorly, both interiorly and on the periphery of the enzyme structure, or at any intermediate position thereof.

30



The ligands are covalently attached to the linker or linkers using conventional chemical techniques providing for covalent linkage of the ligand to the linker or linkers. Reaction chemistries resulting in such linkages are well known in the art and involve the use of complementary functional groups on the linker and ligand. Preferably, the complementary functional groups on the linker are selected relative to the functional groups available on the ligand for bonding or which can be introduced onto the ligand for bonding. Again, such complementary functional groups are well known in the art. For example, reaction between a carboxylic acid of either the linker or the ligand and a primary or secondary amine of the ligand or the linker in the presence of suitable, well-known activating agents results in formation of an amide bond covalently linking the ligand to the linker; reaction between an amine group of either the linker or the ligand and a sulfonyl halide of the ligand or the linker results in formation of a sulfonamide bond covalently linking the ligand to the linker; and reaction between an alcohol or phenol group of either the linker or the ligand and an alkyl or aryl halide of the ligand or the linker results in formation of an ether bond covalently linking the ligand to the linker.

Table I below illustrates numerous complementary reactive groups and the resulting bonds formed by reaction there between.

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Table I

Representative Complementary Binding Chemistries

	<u>First Reactive Group</u>	<u>Second Reactive Group</u>	<u>Linkage</u>
	hydroxyl	isocyanate	urethane
5	amine	epoxide	β -hydroxyamine
	sulfonyl halide	amine	sulfonamide
	carboxyl	amine	amide
	hydroxyl	alkyl/aryl halide	ether
10	The linker is attached to the ligand at a position that retains ligand domain-ligand binding site interaction and specifically which permits the ligand domain of the ligand to orient itself to bind to the ligand binding site. Such positions and synthetic protocols for linkage are well known in the art. The term linker embraces everything that is not considered to be part of the ligand.		
15	The relative orientation in which the ligand domains are displayed derives from the particular point or points of attachment of the ligands to the linker, and on the framework geometry. The determination of where acceptable substitutions can be made on a ligand is typically based on prior knowledge of		
20	structure-activity relationships (SAR) of the ligand and/or congeners and/or structural information about ligand-receptor complexes (e.g., X-ray crystallography, NMR, and the like). Such positions and the synthetic methods for covalent attachment are well known in the art. Following attachment to the selected linker (or attachment to a significant portion of the linker, for example		
25	2-10 atoms of the linker), the univalent linker-ligand conjugate may be tested for retention of activity in the relevant assay.		

Suitable linkers are discussed more fully below.

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At present, it is preferred that the multibinding agent is a bivalent compound, e.g., two ligands which are covalently linked to linker X.

Methodology

5 The linker, when covalently attached to multiple copies of the ligands, provides a biocompatible, substantially non-immunogenic multibinding compound. The biological activity of the multibinding compound is highly sensitive to the valency, geometry, composition, size, flexibility or rigidity, etc. of the linker and, in turn, on the overall structure of the multibinding compound, 10 as well as the presence or absence of anionic or cationic charge, the relative hydrophobicity/hydrophilicity of the linker, and the like on the linker. Accordingly, the linker is preferably chosen to maximize the biological activity of the multibinding compound. The linker may be chosen to enhance the biological activity of the molecule. In general, the linker may be chosen from 15 any organic molecule construct that orients two or more ligands to their ligand binding sites to permit multivalency. In this regard, the linker can be considered as a "framework" on which the ligands are arranged in order to bring about the desired ligand-orienting result, and thus produce a multibinding compound.

20 For example, different orientations can be achieved by including in the framework groups containing mono- or polycyclic groups, including aryl and/or heteroaryl groups, or structures incorporating one or more carbon-carbon multiple bonds (alkenyl, alkenylene, alkynyl or alkynylene groups). Other groups can also include oligomers and polymers which are branched- or straight- 25 chain species. In preferred embodiments, rigidity is imparted by the presence of cyclic groups (e.g., aryl, heteroaryl, cycloalkyl, heterocyclic, etc.). In other preferred embodiments, the ring is a six or ten member ring. In still further preferred embodiments, the ring is an aromatic ring such as, for example, phenyl or naphthyl.

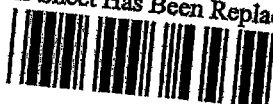
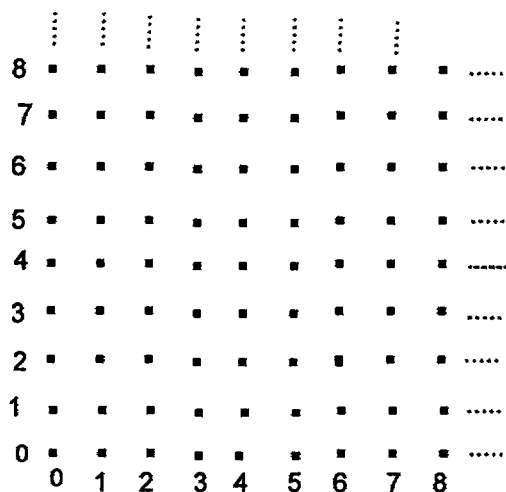
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Different hydrophobic/hydrophilic characteristics of the linker as well as the presence or absence of charged moieties can readily be controlled by the skilled artisan. For example, the hydrophobic nature of a linker derived from hexamethylene diamine ($\text{H}_2\text{N}(\text{CH}_2)_6\text{NH}_2$) or related polyamines can be modified to be substantially more hydrophilic by replacing the alkylene group with a poly(oxyalkylene) group such as found in the commercially available "Jeffamines".

Different frameworks can be designed to provide preferred orientations of the ligands. Such frameworks may be represented by using an array of dots (as shown below) wherein each dot may potentially be an atom, such as C, O, N, S, P, H, F, Cl, Br, and F or the dot may alternatively indicate the absence of an atom at that position. To facilitate the understanding of the framework structure, the framework is illustrated as a two dimensional array in the following diagram, although clearly the framework is a three dimensional array in practice:



5

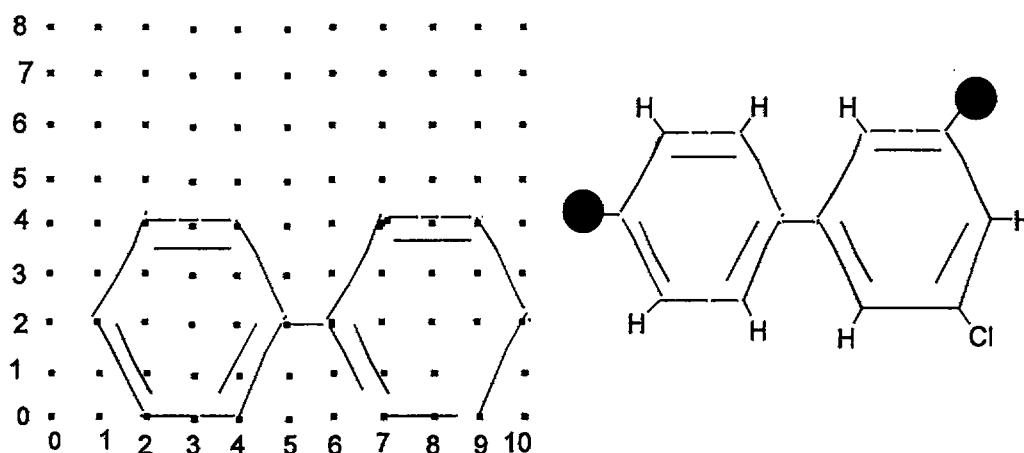
Each dot is either an atom, chosen from carbon, hydrogen, oxygen, selenium, nitrogen, sulfur, phosphorus, or halogen, or the dot represents a point in space (i.e., an absence of an atom). As is apparent to the skilled artisan, only certain atoms on the grid have the ability to act as an attachment point for the
10 ligands, namely, C, O, N, S and P.

Atoms can be connected to each other via bonds (single, double or triple bonds with acceptable resonance and tautomeric forms), with regard to the usual constraints of chemical bonding. Ligands may be attached to the framework via
15 single, double or triple bonds (with chemically acceptable tautomeric and resonance forms). Multiple ligand groups (2 to 10) can be attached to the framework such that the minimal, shortest path distance between adjacent ligand groups does not exceed 100 atoms. Preferably, the linker connections to the
20 ligand is selected such that the maximum spatial distance between two adjacent ligands is no more than 100Å.

An example of a linker as presented by the grid is shown below for a biphenyl construct.

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Nodes (1,2), (2,0), (4,4), (5,2), (4,0), (6,2), (7,4), (9,4), (10,2), (9,0),
 5 (7,0) all represent carbon atoms. Node (10,0) represents a chlorine atom. All
 other nodes (or dots) are points in space (i.e., represent an absence of atoms).

Nodes (1,2) and (9,4) are attachment points. Hydrogen atoms are affixed
 to nodes (2,4), (4,4), (4,0), (2,0), (7,4), (10,2) and (7,0). Nodes (5,2) and (6,2)
 10 are connected by a single bond.

The carbon atoms present are connected by either a single or double
 bonds, taking into consideration the principle of resonance and/or tautomerism.

15 The intersection of the framework (linker) and the ligand group, and
 indeed, the framework (linker) itself can have many different bonding patterns.
 Examples of acceptable patterns of three contiguous atom arrangements are
 shown in the following diagram:



5	CCC	NCC	OCC	SCC	PCC
	CCN	NCN	OCN	SCN	PCN
	CCO	NCO	OCO	SCO	PCO
	CCS	NCS	OCS	SCS	PCS
	CCP	NCP	OCP	SCP	PCP
10	CNC	NNC	ONC	SNC	PNC
	CNN	NNN	ONN	<u>SNN</u>	PNN
	CNO	NNO	<u>ONO</u>	SNO	PNO
	CNS	<u>NNS</u>	ONS	SNS	PNS
	CNP	<u>NNP</u>	ONP	SNP	PNP
15	COC	NOC	<u>OOC</u>	SOC	POC
	CON	<u>NON</u>	<u>ONN</u>	SON	PON
	<u>COO</u>	<u>NOO</u>	<u>OOO</u>	<u>SOO</u>	<u>POO</u>
	COS	<u>NOS</u>	<u>OOS</u>	<u>SOS</u>	<u>POS</u>
	COP	<u>NOP</u>	<u>OOP</u>	<u>SOP</u>	<u>POP</u>
20	CSC	NSC	OSC	SSC	PSC
	CSN	NSN	OSN	SSN	<u>PSN</u>
	CSO	NSO	OSO	<u>SSO</u>	<u>PSO</u>
	CSS	NSS	OSS	<u>SSS</u>	<u>PSS</u>
	CSP	<u>NSP</u>	<u>OSP</u>	<u>SSP</u>	<u>PSP</u>
25	CPC	NPC	OPC	SPC	<u>PPC</u>
	CPN	NPN	OPN	SPN	<u>PPN</u>
	CPO	NPO	OPO	SPO	<u>PPO</u>
	CPS	NPS	OPS	SPS	<u>PPS</u>
	<u>CPP</u>	<u>NPP</u>	<u>OPP</u>	<u>SPP</u>	<u>PPP</u>

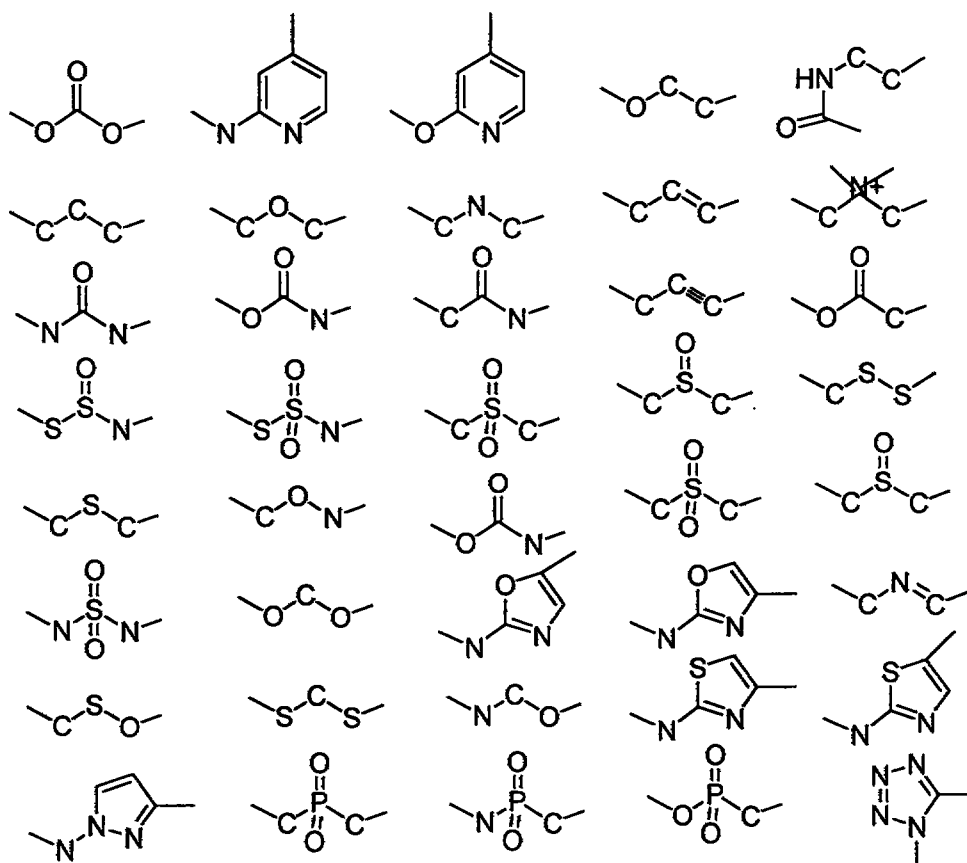
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One skilled in the art would be able to identify bonding patterns that would produce multivalent compounds. Methods for producing these bonding arrangements are described in March, "Advanced Organic Chemistry", 4th Edition, Wiley-Interscience, New York, New York (1992). These arrangements are described in the grid of dots shown in the scheme above. All of the possible arrangements for the five most preferred atoms are shown. Each atom has a variety of acceptable oxidation states. The bonding arrangements underlined are less acceptable and are not preferred.

10 Examples of molecular structures in which the above bonding patterns could be employed as components of the linker are shown below.



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The identification of an appropriate framework geometry and size for ligand domain presentation are important steps in the construction of a multibinding compound with enhanced activity. Systematic spatial searching strategies can be used to aid in the identification of preferred frameworks through an iterative process. Figure 19 illustrates a useful strategy for determining an optimal framework display orientation for ligand domains. Various other strategies are known to those skilled in the art of molecular design and can be used for preparing compounds of this invention.

As shown in Figure 19, display vectors around similar central core structures such as a phenyl structure and a cyclohexane structure can be varied, as can the spacing of the ligand domain from the core structure (i.e., the length of the attaching moiety). It is to be noted that core structures other than those shown here can be used for determining the optimal framework display orientation of the ligands. The process may require the use of multiple copies of the same central core structure or combinations of different types of display cores.

The above-described process can be extended to trimers (Figure 20) and compound of higher valency.

Assays of each of the individual compounds of a collection generated as described above will lead to a subset of compounds with the desired enhanced activities (e.g., potency, selectivity, etc.). The analysis of this subset using a technique such as Ensemble Molecular Dynamics will provide a framework orientation that favors the properties desired. A wide diversity of linkers is commercially available (see, e.g., Available Chemical Directory (ACD)). Many of the linkers that are suitable for use in this invention fall into this category. Other can be readily synthesized by methods well known in the art and/or are described below.



Having selected a preferred framework geometry, the physical properties of the linker can be optimized by varying the chemical composition thereof. The composition of the linker can be varied in numerous ways to achieve the desired physical properties for the multibinding compound.

5

It can therefore be seen that there is a plethora of possibilities for the composition of a linker. Examples of linkers include aliphatic moieties, aromatic moieties, steroidal moieties, peptides, and the like. Specific examples are peptides or polyamides, hydrocarbons, aromatic groups, ethers, lipids, cationic
10 or anionic groups, or a combination thereof.

Examples are given below, but it should be understood that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. For example, properties of the linker
15 can be modified by the addition or insertion of ancillary groups into or onto the linker, for example, to change the solubility of the multibinding compound (in water, fats, lipids, biological fluids, etc.), hydrophobicity, hydrophilicity, linker flexibility, antigenicity, stability, and the like. For example, the introduction of one or more poly(ethylene glycol) (PEG) groups onto or into the linker enhances
20 the hydrophilicity and water solubility of the multibinding compound, increases both molecular weight and molecular size and, depending on the nature of the unPEGylated linker, may increase the *in vivo* retention time. Further PEG may decrease antigenicity and potentially enhances the overall rigidity of the linker.

25 Ancillary groups which enhance the water solubility/hydrophilicity of the linker and, accordingly, the resulting multibinding compounds are useful in practicing this invention. Thus, it is within the scope of the present invention to use ancillary groups such as, for example, small repeating units of ethylene glycols, alcohols, polyols (e.g., glycerin, glycerol propoxylate, saccharides,
30 including mono-, oligosaccharides, etc.), carboxylates (e.g., small repeating



units of glutamic acid, acrylic acid, etc.), amines (e.g., tetraethylenepentamine), and the like) to enhance the water solubility and/or hydrophilicity of the multibinding compounds of this invention. In preferred embodiments, the ancillary group used to improve water solubility/hydrophilicity will be a
5 polyether .

The incorporation of lipophilic ancillary groups within the structure of the linker to enhance the lipophilicity and/or hydrophobicity of the multibinding compounds described herein is also within the scope of this invention.

10 Lipophilic groups useful with the linkers of this invention include, by way of example only, aryl and heteroaryl groups which, as above, may be either unsubstituted or substituted with other groups, but are at least substituted with a group which allows their covalent attachment to the linker. Other lipophilic groups useful with the linkers of this invention include fatty acid derivatives
15 which do not form bilayers in aqueous medium until higher concentrations are reached.

Also within the scope of this invention is the use of ancillary groups which result in the multibinding compound being incorporated or anchored into a
20 vesicle or other membranous structure such as a liposome or a micelle. The term "lipid" refers to any fatty acid derivative that is capable of forming a bilayer or a micelle such that a hydrophobic portion of the lipid material orients toward the bilayer while a hydrophilic portion orients toward the aqueous phase. Hydrophilic characteristics derive from the presence of phosphato, carboxylic, sulfato, amino, sulfhydryl, nitro and other like groups well known in the art.
25 Hydrophobicity could be conferred by the inclusion of groups that include, but are not limited to, long chain saturated and unsaturated aliphatic hydrocarbon groups of up to 20 carbon atoms and such groups substituted by one or more aryl, heteroaryl, cycloalkyl, and/or heterocyclic group(s). Preferred lipids are
30 phosphglycerides and sphingolipids, representative examples of which include



phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine,
phosphatidylinositol, phosphatidic acid, palmitoyleoyl phosphatidylcholine,
lysophosphatidylcholine, lysophosphatidyl-ethanolamine,
dipalmitoylphosphatidylcholine, dioleoylphosphatidylcholine, distearoyl-
5 phosphatidylcholine or dilinoleoylphosphatidylcholine could be used. Other
compounds lacking phosphorus, such as sphingolipid and glycosphingolipid
families are also within the group designated as lipid. Additionally, the
amphipathic lipids described above may be mixed with other lipids including
triglycerides and sterols.

10

The flexibility of the linker can be manipulated by the inclusion of
ancillary groups which are bulky and/or rigid. The presence of bulky or rigid
groups can hinder free rotation about bonds in the linker or bonds between the
linker and the ancillary group(s) or bonds between the linker and the functional
15 groups. Rigid groups can include, for example, those groups whose
conformational lability is restrained by the presence of rings and/or multiple
bonds within the group, for example, aryl, heteroaryl, cycloalkyl, cycloalkenyl,
and heterocyclic groups. Other groups which can impart rigidity include
polypeptide groups such as oligo- or polyproline chains.

20

Rigidity may also be imparted by internal hydrogen bonding or by
hydrophobic collapse.

Bulky groups can include, for example, large atoms, ions (e.g., iodine,
25 sulfur, metal ions, etc.) or groups containing large atoms, polycyclic groups,
including aromatic groups, non-aromatic groups and structures incorporating one
or more carbon-carbon multiple bonds (i.e., alkenes and alkynes). Bulky groups
can also include oligomers and polymers which are branched- or straight-chain
species. Species that are branched are expected to increase the rigidity of the
30 structure more per unit molecular weight gain than are straight-chain species.



In preferred embodiments, rigidity is imparted by the presence of cyclic groups (e.g., aryl, heteroaryl, cycloalkyl, heterocyclic, etc.). In other preferred embodiments, the linker comprises one or more six-membered rings. In still further preferred embodiments, the ring is an aryl group such as, for example,
5 phenyl or naphthyl.

Rigidity can also be imparted electrostatically. Thus, if the ancillary groups are either positively or negatively charged, the similarly charged ancillary groups will force the presenter linker into a configuration affording the
10 maximum distance between each of the like charges. The energetic cost of bringing the like-charged groups closer to each other will tend to hold the linker in a configuration that maintains the separation between the like-charged ancillary groups. Further ancillary groups bearing opposite charges will tend to be attracted to their oppositely charged counterparts and potentially may enter
15 into both inter- and intramolecular ionic bonds. This non-covalent mechanism will tend to hold the linker into a conformation which allows bonding between the oppositely charged groups. The addition of ancillary groups which are charged, or alternatively, bear a latent charge when deprotected, following addition to the linker, include deprotection of a carboxyl, hydroxyl, thiol or
20 amino group by a change in pH, oxidation, reduction or other mechanisms known to those skilled in the art which result in removal of the protecting group, is within the scope of this invention.

In view of the above, it is apparent that the appropriate selection of a
25 linker group providing suitable orientation, restricted/unrestricted rotation, the desired degree of hydrophobicity/hydrophilicity, etc. is well within the skill of the art. Eliminating or reducing antigenicity of the multibinding compounds described herein is also within the scope of this invention. In certain cases, the antigenicity of a multibinding compound may be eliminated or reduced by use of
30 groups such as, for example, poly(ethylene glycol).



As explained above, the multibinding compounds described herein comprise 2-10 ligands attached to a linker that links the ligands in such a manner that they are presented to the enzyme for multivalent interactions with ligand binding sites thereon/therein. The linker spatially constrains these interactions to occur within dimensions defined by the linker. This and other factors increases the biological activity of the multibinding compound as compared to the same number of ligands made available in monobinding form.

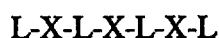
The compounds of this invention are preferably represented by the empirical formula $(L)_p(X)_q$ where L, X, p and q are as defined above. This is intended to include the several ways in which the ligands can be linked together in order to achieve the objective of multivalency, and a more detailed explanation is described below.

As noted previously, the linker may be considered as a framework to which ligands are attached. Thus, it should be recognized that the ligands can be attached at any suitable position on this framework, for example, at the termini of a linear chain or at any intermediate position.

The simplest and most preferred multibinding compound is a bivalent heterodimeric compound which can be represented as L-X-L, where each L is independently nucleoside reverse transcriptase inhibitor (NRTI) ligand and a non-nucleoside reverse transcriptase inhibitor (NNRTI), and each X is independently the linker. Examples of such bivalent compounds are provided in Figure 19 where each shaded circle represents a ligand (with the understanding that the two ligands are at least one NRTI and one NNRTI). A trivalent compound could also be represented in a linear fashion, i.e., as a sequence of repeated units L-X-L-X-L, in which L is a ligand, at least one ligand being a NRTI and at least one ligand being a NNRTI, and X is the same or different at each occurrence. However, a trimer can also be a radial multibinding compound



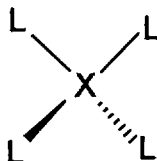
comprising three ligands attached to a central core, and thus represented as $(L)_3X$, where the linker X could include, for example, an aryl or cycloalkyl group, and, as before, at least one L is a NRTI and at least one L is a NNRTI. Illustrations of trivalent and tetravalent compounds of this invention are found in
5 Figures 20 and 21 respectively where, again, the shaded circles represent ligands. Tetravalent compounds can be represented in a linear array, e.g.,



10 in a branched array, e.g.,



(a branched construct analogous to the isomers of butane -- *n*-butyl, *iso*-butyl, *sec*-butyl, and *t*-butyl) or in a tetrahedral array, e.g.,



20 where X and L are as defined herein. Alternatively, it could be represented as an alkyl, aryl or cycloalkyl derivative as above with four (4) ligands attached to the core linker. Again, at least one L is a NRTI and at least one L is a NNRTI.

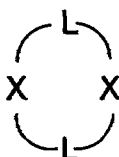
The same considerations apply to higher multibinding compounds of this
25 invention containing 5-10 ligands as illustrated in Figure 22 where, as before, the shaded circles represent ligands. However, for multibinding agents attached to a central linker such as aryl or cycloalkyl, there is a self-evident constraint



that there must be sufficient attachment sites on the linker to accommodate the number of ligands present; for example, a benzene ring could not directly accommodate more than 6 ligands, whereas a multi-ring linker (e.g., biphenyl) could accommodate a larger number of ligands.

5

Ceratin of the above described compounds may alternatively be represented as cyclic chains of the form:



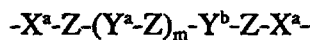
and variants thereof.

10

All of the above variations are intended to be within the scope of the invention defined by the formula $(L)_p(X)_q$.

With the foregoing in mind, a preferred linker may be represented by the following formula:

15



in which:

20

m is an integer of from 0 to 20;

X^a at each separate occurrence is selected from the group consisting of -O-, -S-, -NR-, -C(O)-, -C(O)O-, -C(O)NR-, -C(S), -C(S)O-, -C(S)NR- or a covalent bond where R is as defined below;

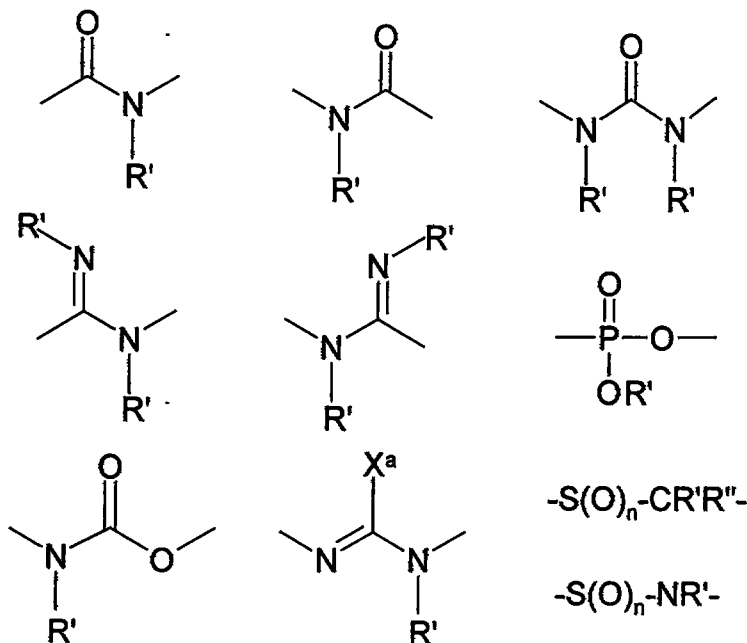
Z is at each separate occurrence is selected from the group consisting of alkylene, substituted alkylene, cycloalkylene, substituted cycloalkylene, alkenylene, substituted alkenylene, alkynylene, substituted alkynylene,

25



cycloalkenylene, substituted cycloalkenylene, arylene, heteroarylene, heterocyclene, or a covalent bond;

Y^a and Y^b at each separate occurrence are selected from the group consisting of:



5

-S-S- or a covalent bond;

in which:

n is 0, 1 or 2; and

10 R , R' and R'' at each separate occurrence are selected from the group consisting of hydrogen, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, cycloalkenyl, substituted cycloalkenyl, alkynyl, substituted alkynyl, aryl, heteroaryl and heterocyclic.

15 Additionally, the linker moiety can be optionally substituted at any atom therein by one or more alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, cycloalkenyl, substituted cycloalkenyl, alkynyl, substituted alkynyl, aryl, heteroaryl and heterocyclic group.



In one embodiment of this invention, the linker (i.e., X or X') is selected those shown in Table II:

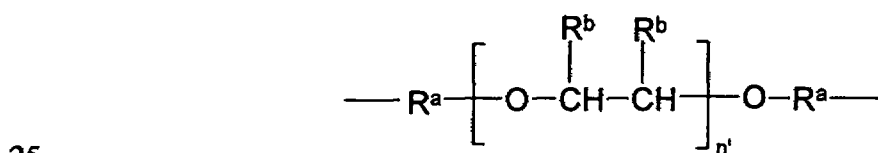
Table II
Representative Linkers

5	Linker
	-HN-(CH ₂) ₂ -NH-C(O)-(CH ₂) ₂ -C(O)-NH-(CH ₂) ₂ -NH-
	-HN-(CH ₂) ₂ -NH-C(O)-(CH ₂) ₂ -C(O)-NH-(CH ₂) ₂ -NH-
10	-HN-(CH ₂) ₂ -NH-C(O)-(CH ₂) ₃ -C(O)-NH-(CH ₂) ₂ -NH-
	-HN-(CH ₂) ₂ -NH-C(O)-(CH ₂) ₄ -C(O)-NH-(CH ₂) ₂ -NH-
	-HN-(CH ₂) ₂ -NH-C(O)-(CH ₂) ₅ -C(O)-NH-(CH ₂) ₂ -NH-
	-HN-(CH ₂) ₂ -NH-C(O)-(CH ₂) ₆ -C(O)-NH-(CH ₂) ₂ -NH-
	-HN-(CH ₂) ₂ -NH-C(O)-(CH ₂) ₇ -C(O)-NH-(CH ₂) ₂ -NH-
15	-HN-(CH ₂) ₂ -NH-C(O)-(CH ₂) ₈ -C(O)-NH-(CH ₂) ₂ -NH-
	-HN-(CH ₂) ₂ -NH-C(O)-(CH ₂) ₉ -C(O)-NH-(CH ₂) ₂ -NH-
	-HN-(CH ₂) ₂ -NH-C(O)-(CH ₂) ₁₀ -C(O)-NH-(CH ₂) ₂ -NH-
	-HN-(CH ₂) ₂ -NH-C(O)-(CH ₂) ₁₁ -C(O)-NH-(CH ₂) ₂ -NH-
	-HN-(CH ₂) ₂ -NH-C(O)-(CH ₂) ₁₂ -C(O)-NH-(CH ₂) ₂ -NH-
20	-HN-(CH ₂) ₂ -NH-C(O)-Z-C(O)-NH-(CH ₂) ₂ -NH- where Z is 1,2-phenyl
	-HN-(CH ₂) ₂ -NH-C(O)-Z-C(O)-NH-(CH ₂) ₂ -NH- where Z is 1,3-phenyl
	-HN-(CH ₂) ₂ -NH-C(O)-Z-C(O)-NH-(CH ₂) ₂ -NH- where Z is 1,4-phenyl
	-HN-(CH ₂) ₂ -NH-C(O)-Z-O-Z-C(O)-NH-(CH ₂) ₂ -NH- where Z is 1,4-phenyl
25	-HN-(CH ₂) ₂ -NH-C(O)-(CH ₂) ₂ -CH(NH-C(O)-(CH ₂) ₈ -CH ₃)-C(O)-NH-(CH ₂) ₂ -NH-
	-HN-(CH ₂) ₂ -NH-C(O)-(CH ₂) ₂ -O-(CH ₂) ₂ -C(O)-NH-(CH ₂) ₂ -NH-
	-HN-(CH ₂) ₂ -NH-C(O)-Z-C(O)-NH-(CH ₂) ₂ -NH- where Z is 5-(n-octadecyloxy)-1,3-phenyl
30	-HN-(CH ₂) ₂ -NH-C(O)-(CH ₂) ₂ -CH(NH-C(O)-Z)-C(O)-NH-(CH ₂) ₂ -NH- where Z is 4-biphenyl



Linker	
	-HN-(CH ₂) ₂ -NH-C(O)-Z-C(O)-NH-(CH ₂) ₂ -NH- where Z is 5-(n-butyloxy)-1,3-phenyl
	-HN-(CH ₂) ₂ -NH-C(O)-(CH ₂) ₈ - <i>trans</i> -(CH=CH)-C(O)-NH-(CH ₂) ₂ -NH-
5	-HN-(CH ₂) ₂ -NH-C(O)-(CH ₂) ₂ -CH(NH-C(O)-(CH ₂) ₁₂ -CH ₃)-C(O)-NH-(CH ₂) ₂ -NH-
	-HN-(CH ₂) ₂ -NH-C(O)-(CH ₂) ₂ -CH(NH-C(O)-Z)-C(O)-NH-(CH ₂) ₂ -NH- where Z is 4-(n-octyl)-phenyl
	-HN-(CH ₂) ₂ -Z-O-(CH ₂) ₆ -O-Z-(CH ₂) ₂ -NH- where Z is 1,4-phenyl
10	-HN-(CH ₂) ₂ -NH-C(O)-(CH ₂) ₂ -NH-C(O)-(CH ₂) ₃ -C(O)-NH-(CH ₂) ₂ -C(O)-NH-(CH ₂) ₂ -NH-
	-HN-(CH ₂) ₂ -NH-C(O)-(CH ₂) ₂ -CH(NH-C(O)-Ph)-C(O)-NH-(CH ₂) ₂ -NH-
	-HN-(CH ₂) ₂ -NH-C(O)-(CH ₂) ₂ -N + ((CH ₂) ₉ -CH ₃)(CH ₂ -C(O)-NH-(CH ₂) ₂ -NH ₂)- (CH ₂) ₂ -C(O)-NH-(CH ₂) ₂ -NH-
	-HN-(CH ₂) ₂ -NH-C(O)-(CH ₂) ₂ -N((CH ₂) ₉ -CH ₃)-(CH ₂) ₂ -C(O)-NH-(CH ₂) ₂ -NH-
15	-HN-(CH ₂) ₂ -NH-C(O)-(CH ₂) ₂ -NH-C(O)-(CH ₂) ₂ -NH-C(O)-(CH ₂) ₃ -C(O)-NH-(CH ₂) ₂ -C(O)-NH-(CH ₂) ₂ -C(O)-NH-(CH ₂) ₂ -NH-
	-HN-(CH ₂) ₂ -NH-C(O)-Z-C(O)-NH-(CH ₂) ₂ -NH- where Z is 5-hydroxy-1,3-phenyl

20 In another embodiment of this invention, the linker (i.e., X or X') has the formula:



wherein

each R^a is independently selected from the group consisting of a covalent bond, alkylene, substituted alkylene and arylene;



each R^b is independently selected from the group consisting of hydrogen, alkyl and substituted alkyl; and

n' is an integer ranging from 1 to about 20.

5 In view of the above description of the linker, it is understood that the term "linker" when used in combination with the term "multibinding compound" includes both a covalently contiguous single linker (e.g., L-X-L) and multiple covalently non-contiguous linkers (L-X-L-X-L) within the multibinding compound.

10

Preparation of Multibinding Compounds

 The multibinding compounds of this invention can be prepared from readily available starting materials using the following general methods and procedures. It will be appreciated that where typical or preferred process
15 conditions (i.e., reaction temperatures, times, mole ratios of reactants, solvents, pressures, etc.) are given, other process conditions can also be used unless otherwise stated. Optimum reaction conditions may vary with the particular reactants or solvent used, but such conditions can be determined by one skilled in the art by routine optimization procedures.

20

 Additionally, as will be apparent to those skilled in the art, conventional protecting groups may be necessary to prevent certain functional groups from undergoing undesired reactions. The choice of a suitable protecting group for a particular functional group as well as suitable conditions for protection and
25 deprotection are well known in the art. For example, numerous protecting groups, and their introduction and removal, are described in T. W. Greene and G. M. Wuts, *Protecting Groups in Organic Synthesis*, Second Edition, Wiley, New York, 1991, and references cited therein.



Any nucleoside reverse transcriptase inhibitor or non-nucleoside reverse transcriptase inhibitor compound which inhibits HIV reverse transcriptase can be used as a ligand in this invention. At least one ligand is a nucleoside reverse transcriptase inhibitor (NRTI) and at least one other ligand is a non-nucleoside reverse transcriptase inhibitor (NNRTI), as described above. As discussed in further detail below, numerous such NRTIs and NNRTIs are known in the art and any of these known compounds or derivatives thereof may be employed as ligands in this invention. Typically, a compound selected for use as a ligand will have at least one functional group, such as an amino, hydroxyl, thiol or carboxyl group and the like, which allows the compound to be readily coupled to the linker via a covalent bond. Compounds having such functionality are either known in the art or can be prepared by routine modification of known compounds using conventional reagents and procedures.

The NRTI and the NNRTI ligand can be covalently attached to the linker through any available position on the ligand, provided that when attached to the linker, the ligand retains its ability to inhibit HIV reverse transcriptase. Preferably, the linker is attached to a site on the ligand where structure-activity studies show that substituents are tolerated without total loss of inhibition activity. For example, many known NRTIs and NNRTIs contain, among other structural features, amino, hydroxyl, carboxyl, vinyl, acetylenic and other groups which permit ready attachment to a complementary reactive group on the linker. In addition, such groups can be modified in order to facilitate coupling to the linker. For example, a carboxyl group can be modified via conventional techniques well known in the art to the corresponding carboxyl halide, activated ester or anhydride to facilitate coupling reactions with, for example, an amine to form an amide linkage.

The ligand, however, is preferably not attached to the linker through any functional group which would result in abrogation of activity.

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A first group of preferred ligands for use in this invention are set forth as compounds 1-5 of FIG. 1A and 11-13 of FIG. 1B, and suitable attachment points for these ligands to linkers is illustrated in compounds 6-10 and 14-15 of FIG. 1B. Such ligands (and the precursors thereof) are well-known in the art and/or can be
5 readily prepared using art-recognized starting materials, reagents and reaction conditions as illustrated in the figures and exemplified in the Examples below.

Reaction schemes for the synthesis of such preferred ligands are illustrated in FIG.s 2 through 11. Specifically, preparation 1 of FIG. 2 illustrates the
10 addition of alkynyl lithium compound 17 to ketone 16 under conventional coupling conditions resulting in intermediate 18, which is then cyclized in the presence of a cyclization agent such as phosgene or phosgene equivalent per conventional procedures well known in the art to provide for compound 19. Bromination of compound 19 to provide for bromo derivative 20 is accomplished
15 again via conventional techniques using for example, carbon tetrabromide/triphenyl phosphine as the brominating agent.

Preparations 2 and 3 of FIG. 2 parallel the synthesis described above for preparation 1 with the exception that the bromo analog prepared is further
20 derivatized to amino or thiol derivatives using conventional techniques.

Preparations 4 and 5 of FIG. 3 illustrate conventional alkylation reactions of aniline. Subsequent to N-alkylation, addition of an alkynyl lithium compound to the ketone functionality followed by cyclization yields compounds 30 and 34.
25 Each of these steps is conducted in the manner described above.

Preparations 6 through 9 of FIGs. 3 and 4 illustrate displacement of an aromatic halide via a primary amine followed by cyclization via displacement of an aromatic methyl ether by the secondary amine using conventional conditions
30 well known in the art. Functional groups introduced with the primary amine can



then be further derivatized via conventional techniques. For example,
conversion of the primary alcohol 38 to the corresponding bromo derivative
follows the procedures set forth above. Similarly, the hydroxyl group can be
converted to a tosyl derivative which permits formation of a thiol functional as
5 shown in compounds 43 and 44.

Preparation 10 of FIG. 4 illustrates conventional N-alkylation of an amide
via an α -bromo- ω -carboxyl ester, compound 48, to provide for compound 49.
Conventional deesterification yields compound 50.

10

Preparation 11 of FIG. 5 is similar to the synthesis described above for
preparations 6 through 9.

Preparations 12 through 15 and 24 through 25 illustrate bromination or
15 alkylation of a primary alcohol again using well known conditions.

Preparation 16 illustrates conventional protection of the primary alcohol
of compound 66 by an alkyldiarylsilyl halide to provide for compound 67
followed by triflation of the uracil group to provide for compound 68 again
20 employing conventional conditions. Triflate displacement by 1,3-propylene
diamine provides for compound 69.

Preparation 17 parallels the synthesis set forth above for preparation 16
with the exception that coupling of fluorouracil to the silylated acetate 70 occurs
25 by displacement of the acetate functional group to provide for compound 71.

Preparations 18 and 19 merely illustrate conversion of the uracil to a
chloro derivative again using conventional techniques. Conversion of the chloro
group in compound 73 by, for example, a lithium acetylide provides for



compounds 76 and 78 which can subsequently be converted to tosyl and carboxyl derivatives 77 and 79 as illustrated in preparation 20.

Preparation 21 parallels the synthesis of preparation 20.

5

Alternatively, chlorouracil compounds 73 and 74 can be employed in an amino displacement reaction to provide for compounds 82 and 83 as illustrated in Preparations 22 and 23. Such amino displacement reactions are well known in the art.

10

In yet another alternative embodiment, chlorouracil compound 87 can be employed in etheration or thioetheration reaction to provide for compounds 88 and 89 as illustrated in Preparation 26. Such reactions are well known in the art.

15

Preparation 27, as illustrated in FIG. 11, depicts conventional displacement of a halo group of haloalkyl compound 90 to provide for compound 92.

20

As will be readily apparent to those of ordinary skill in the art, the synthetic procedures described herein or those known in the art may be readily modified to afford a wide variety of compounds within the scope of this invention.

Combinatorial Libraries

25

The methods described above lend themselves to combinatorial approaches for identifying multimeric compounds which possess multibinding properties that inhibit HIV reverse transcriptase.

30

Specifically, factors such as the proper juxtaposition of the individual ligands of a multibinding compound with respect to the relevant array of binding



sites on a target or targets is important in optimizing the interaction of the multibinding compound with its target(s) and to maximize the biological advantage through multivalency. One approach is to identify a library of candidate multibinding compounds with properties spanning the multibinding parameters that are relevant for a particular target. These parameters include:

5 (1) the identity of ligand(s), (2) the orientation of ligands, (3) the valency of the construct, (4) linker length, (5) linker geometry, (6) linker physical properties, and (7) linker chemical functional groups.

10 Libraries of multimeric compounds potentially possessing multibinding properties (i.e., candidate multibinding compounds) and comprising a multiplicity of such variables are prepared and these libraries are then evaluated via conventional assays corresponding to the ligand selected and the multibinding parameters desired. Considerations relevant to each of these variables are set

15 forth below:

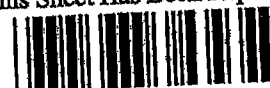
Selection of ligand(s)

A single ligand or set of ligands is (are) selected for incorporation into the libraries of candidate multibinding compounds which library is directed

20 against a particular biological target or targets. The only requirement for the ligands chosen is that they are capable of interacting with the selected target(s). Thus, ligands may be known drugs, modified forms of known drugs, substructures of known drugs or substrates of modified forms of known drugs (which are competent to interact with the target), or other compounds. Ligands

25 are preferably chosen based on known favorable properties that may be projected to be carried over to or amplified in multibinding forms. Favorable properties include demonstrated safety and efficacy in human patients, appropriate PK/ADME profiles, synthetic accessibility, and desirable physical properties such as solubility, logP, etc. However, it is crucial to note that ligands which

30 display an unfavorable property from among the previous list may obtain a more



favorable property through the process of multibinding compound formation; i.e., ligands should not necessarily be excluded on such a basis. For example, a ligand that is not sufficiently potent at a particular target so as to be efficacious in a human patient may become highly potent and efficacious when presented in multibinding form. A ligand that is potent and efficacious but not of utility because of a non-mechanism-related toxic side effect may have increased therapeutic index (increased potency relative to toxicity) as a multibinding compound. Compounds that exhibit short *in vivo* half-lives may have extended half-lives as multibinding compounds. Physical properties of ligands that limit their usefulness (e.g. poor bioavailability due to low solubility, hydrophobicity, hydrophilicity) may be rationally modulated in multibinding forms, providing compounds with physical properties consistent with the desired utility.

Orientation: selection of ligand attachment points and linking chemistry

Several points are chosen on each ligand at which to attach the ligand to the linker. The selected points on the ligand/linker for attachment are functionalized to contain complementary reactive functional groups. This permits probing the effects of presenting the ligands to their receptor(s) in multiple relative orientations, an important multibinding design parameter. The only requirement for choosing attachment points is that attaching to at least one of these points does not abrogate activity of the ligand. Such points for attachment can be identified by structural information when available. For example, inspection of a co-crystal structure of a protease inhibitor bound to its target allows one to identify one or more sites where linker attachment will not preclude the enzyme:inhibitor interaction. Alternatively, evaluation of ligand/target binding by nuclear magnetic resonance will permit the identification of sites non-essential for ligand/target binding. See, for example, Fesik, et al., U.S. Patent No. 5,891,643. When such structural information is not available, utilization of structure-activity relationships (SAR) for ligands will suggest positions where substantial structural variations are and are not allowed. In the



absence of both structural and SAR information, a library is merely selected with multiple points of attachment to allow presentation of the ligand in multiple distinct orientations. Subsequent evaluation of this library will indicate what positions are suitable for attachment.

5

It is important to emphasize that positions of attachment that do abrogate the activity of the monomeric ligand may also be advantageously included in candidate multibinding compounds in the library provided that such compounds bear at least one ligand attached in a manner which does not abrogate intrinsic activity. This selection derives from, for example, heterobivalent interactions within the context of a single target molecule. For example, consider a receptor antagonist ligand bound to its target receptor, and then consider modifying this ligand by attaching to it a second copy of the same ligand with a linker which allows the second ligand to interact with the same receptor molecule at sites proximal to the antagonist binding site, which include elements of the receptor that are not part of the formal antagonist binding site and/or elements of the matrix surrounding the receptor such as the membrane. Here, the most favorable orientation for interaction of the second ligand molecule with the receptor/matrix may be achieved by attaching it to the linker at a position which abrogates activity of the ligand at the formal antagonist binding site. Another way to consider this is that the SAR of individual ligands within the context of a multibinding structure is often different from the SAR of those same ligands in monomeric form.

25

The foregoing discussion focused on bivalent interactions of dimeric compounds bearing two copies of the same ligand joined to a single linker through different attachment points, one of which may abrogate the binding/activity of the monomeric ligand. It should also be understood that bivalent advantage may also be attained with heterodimeric constructs bearing two different ligands that bind to common or different targets. For example, a

30



5HT₄ receptor antagonist and a bladder-selective muscarinic M₃ antagonist may be joined to a linker through attachment points which do not abrogate the binding affinity of the monomeric ligands for their respective receptor sites. The dimeric compound may achieve enhanced affinity for both receptors due to favorable interactions between the 5HT₄ ligand and elements of the M₃ receptor proximal to the formal M₃ antagonist binding site and between the M₃ ligand and elements of the 5HT₄ receptor proximal to the formal 5HT₄ antagonist binding site. Thus, the dimeric compound may be more potent and selective antagonist of overactive bladder and a superior therapy for urinary urge incontinence.

10

Once the ligand attachment points have been chosen, one identifies the types of chemical linkages that are possible at those points. The most preferred types of chemical linkages are those that are compatible with the overall structure of the ligand (or protected forms of the ligand) readily and generally formed, stable and intrinsically innocuous under typical chemical and physiological conditions, and compatible with a large number of available linkers. Amide bonds, ethers, amines, carbamates, ureas, and sulfonamides are but a few examples of preferred linkages.

15

20 Linkers: spanning relevant multibinding parameters through selection of valency, linker length, linker geometry, rigidity, physical properties, and chemical functional groups

In the library of linkers employed to generate the library of candidate multibinding compounds, the selection of linkers employed in this library of linkers takes into consideration the following factors:

25

Valency. In most instances the library of linkers is initiated with divalent linkers. The choice of ligands and proper juxtaposition of two ligands relative to their binding sites permits such molecules to exhibit target binding affinities and specificities more than sufficient to confer biological advantage. Furthermore,

30



divalent linkers or constructs are also typically of modest size such that they retain the desirable biodistribution properties of small molecules.

Linker length. Linkers are chosen in a range of lengths to allow the
5 spanning of a range of inter-ligand distances that encompass the distance
preferable for a given divalent interaction. In some instances the preferred
distance can be estimated rather precisely from high-resolution structural
information of targets, typically enzymes and soluble receptor targets. In other
instances where high-resolution structural information is not available (such as
10 7TM G-protein coupled receptors), one can make use of simple models to
estimate the maximum distance between binding sites either on adjacent receptors
or at different locations on the same receptor. In situations where two binding
sites are present on the same target (or target subunit for multisubunit targets),
preferred linker distances are 2-20 Å, with more preferred linker distances of 3-
15 12 Å. In situations where two binding sites reside on separate (e.g., protein)
target sites, preferred linker distances are 20-100 Å, with more preferred
distances of 30-70 Å.

Linker geometry and rigidity. The combination of ligand attachment site,
20 linker length, linker geometry, and linker rigidity determine the possible ways in
which the ligands of candidate multibinding compounds may be displayed in
three dimensions and thereby presented to their binding sites. Linker geometry
and rigidity are nominally determined by chemical composition and bonding
pattern, which may be controlled and are systematically varied as another
25 spanning function in a multibinding array. For example, linker geometry is
varied by attaching two ligands to the ortho, meta, and para positions of a
benzene ring, or in *cis*- or *trans*-arrangements at the 1,1- vs. 1,2- vs. 1,3- vs.
1,4- positions around a cyclohexane core or in *cis*- or *trans*-arrangements at a
point of ethylene unsaturation. Linker rigidity is varied by controlling the
30 number and relative energies of different conformational states possible for the



linker. For example, a divalent compound bearing two ligands joined by 1,8-octyl linker has many more degrees of freedom, and is therefore less rigid than a compound in which the two ligands are attached to the 4,4' positions of a biphenyl linker.

5

Linker physical properties. The physical properties of linkers are nominally determined by the chemical constitution and bonding patterns of the linker, and linker physical properties impact the overall physical properties of the candidate multibinding compounds in which they are included. A range of linker compositions is typically selected to provide a range of physical properties (hydrophobicity, hydrophilicity, amphiphilicity, polarization, acidity, and basicity) in the candidate multibinding compounds. The particular choice of linker physical properties is made within the context of the physical properties of the ligands they join and preferably the goal is to generate molecules with favorable PK/ADME properties. For example, linkers can be selected to avoid those that are too hydrophilic or too hydrophobic to be readily absorbed and/or distributed *in vivo*.

Linker chemical functional groups. Linker chemical functional groups are selected to be compatible with the chemistry chosen to connect linkers to the ligands and to impart the range of physical properties sufficient to span initial examination of this parameter.

Combinatorial synthesis

25 Having chosen a set of n ligands (n being determined by the sum of the number of different attachment points for each ligand chosen) and m linkers by the process outlined above, a library of $(n!)m$ candidate divalent multibinding compounds is prepared which spans the relevant multibinding design parameters for a particular target. For example, an array generated from two ligands, one which has two attachment points (A1, A2) and one which has three attachment

30



points (B1, B2, B3) joined in all possible combinations provide for at least 15 possible combinations of multibinding compounds:

	A1-A1	A1-A2	A1-B1	A1-B2	A1-B3	A2-A2	A2-B1	A2-B2
5	A2-B3	B1-B1	B1-B2	B1-B3	B2-B2	B2-B3	B3-B3	

When each of these combinations is joined by 10 different linkers, a library of 150 candidate multibinding compounds results.

10 Given the combinatorial nature of the library, common chemistries are preferably used to join the reactive functionalities on the ligands with complementary reactive functionalities on the linkers. The library therefore lends itself to efficient parallel synthetic methods. The combinatorial library can employ solid phase chemistries well known in the art wherein the ligand and/or

15 linker is attached to a solid support. Alternatively and preferably, the combinatorial library is prepared in the solution phase. After synthesis, candidate multibinding compounds are optionally purified before assaying for activity by, for example, chromatographic methods (e.g., HPLC).

20 Analysis of array by biochemical, analytical, pharmacological, and computational methods

 Various methods are used to characterize the properties and activities of the candidate multibinding compounds in the library to determine which compounds possess multibinding properties. Physical constants such as

25 solubility under various solvent conditions and logD/clogD values are determined. A combination of NMR spectroscopy and computational methods is used to determine low-energy conformations of the candidate multibinding compounds in fluid media. The ability of the members of the library to bind to the desired target and other targets is determined by various standard methods,

30 which include radioligand displacement assays for receptor and ion channel



targets, and kinetic inhibition analysis for many enzyme targets. *In vitro* efficacy, such as for receptor agonists and antagonists, ion channel blockers, and antimicrobial activity, are determined. Pharmacological data, including oral absorption, everted gut penetration, other pharmacokinetic parameters and
5 efficacy data are determined in appropriate models. In this way, key structure-activity relationships are obtained for multibinding design parameters which are then used to direct future work.

The members of the library which exhibit multibinding properties, as
10 defined herein, can be readily determined by conventional methods. First those members which exhibit multibinding properties are identified by conventional methods as described above including conventional assays (both *in vitro* and *in vivo*).

15 Second, ascertaining the structure of those compounds which exhibit multibinding properties can be accomplished via art recognized procedures. For example, each member of the library can be encrypted or tagged with appropriate information allowing determination of the structure of relevant members at a later time. See, for example, Dower, et al., International Patent
20 Application Publication No. WO 93/06121; Brenner, et al., Proc. Natl. Acad. Sci., USA, 89:5181 (1992); Gallop, et al., U.S. Patent No. 5,846,839; each of which are incorporated herein by reference in its entirety. Alternatively, the structure of relevant multivalent compounds can also be determined from soluble and untagged libraries of candidate multivalent compounds by methods known in
25 the art such as those described by Hindsgaul, et al., Canadian Patent Application No. 2,240,325 which was published on July 11, 1998. Such methods couple frontal affinity chromatography with mass spectroscopy to determine both the structure and relative binding affinities of candidate multibinding compounds to receptors.

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The process set forth above for dimeric candidate multibinding compounds can, of course, be extended to trimeric candidate compounds and higher analogs thereof.

5 Follow-up synthesis and analysis of additional array(s)

Based on the information obtained through analysis of the initial library, an optional component of the process is to ascertain one or more promising multibinding "lead" compounds as defined by particular relative ligand orientations, linker lengths, linker geometries, etc. Additional libraries can then
10 be generated around these leads to provide for further information regarding structure to activity relationships. These arrays typically bear more focused variations in linker structure in an effort to further optimize target affinity and/or activity at the target (antagonism, partial agonism, etc.), and/or alter physical properties. By iterative redesign/analysis using the novel principles of
15 multibinding design along with classical medicinal chemistry, biochemistry, and pharmacology approaches, one is able to prepare and identify optimal multibinding compounds that exhibit biological advantage towards their targets and as therapeutic agents.

20 To further elaborate upon this procedure, suitable divalent linkers include, by way of example only, those derived from dicarboxylic acids, disulfonylhalides, dialdehydes, diketones, dihalides, diisocyanates, diamines, diols, mixtures of carboxylic acids, sulfonylhalides, aldehydes, ketones, halides, isocyanates, amines and diols. In each case, the carboxylic acid, sulfonylhalide,
25 aldehyde, ketone, halide, isocyanate, amine and diol functional group is reacted with a complementary functionality on the ligand to form a covalent linkage. Such complementary functionality is well known in the art as illustrated in the following table:

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COMPLEMENTARY BINDING CHEMISTRIES

	<u>First Reactive Group</u>	<u>Second Reactive Group</u>	<u>Linkage</u>
	hydroxyl	isocyanate	urethane
5	amine	epoxide	β -hydroxyamine
	sulfonyl halide	amine	sulfonamide
	carboxyl acid	amine	amide
	hydroxyl	alkyl/aryl halide	ether
	aldehyde	amine/ NaCNBH_4	amine
10	ketone	amine/ NaCNBH_4	amine
	amine	isocyanate	urea

Exemplary linkers include the following linkers identified as X-1 through X-418 as set forth below:

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Diacids					
X-1	X-2	X-3	X-4	X-5	X-6
X-7	X-8	X-9	X-10	X-11	X-12
X-13	X-14	X-15	X-16	X-17	X-18
X-19	X-20	X-21	X-22	X-23	X-24
X-25	X-26	X-27	X-28	X-29	X-30
X-31	X-32	X-33	X-34	X-35	X-36
X-37	X-38	X-39	X-40	X-41	X-42
X-43	X-44	X-45	X-46	X-47	X-48
X-49	X-50	X-51	X-52	X-53	X-54
X-55	X-56	X-57	X-58	X-59	X-60
X-61	X-62	X-63	X-64	X-65	X-66
X-67	X-68	X-69	X-70	X-71	X-72
X-73	X-74	X-75	X-76	X-77	X-78
X-79	X-80	X-81	X-82	X-83	X-84



X-85	X-86	X-87	X-88	X-89	X-90
X-91	X-92	X-93	X-94	X-95	X-96
X-97	X-98	X-99	X-100	X-101	X-102
X-103	X-104	X-105	X-106	X-107	X-108
X-109	X-110	X-111	X-112	X-113	X-114
X-115	X-116	X-117	X-118	X-119	X-120
X-121	X-122	X-123	X-124	X-125	X-126
X-127	X-128	X-129	X-130	X-131	X-132
Disulfonyl Halides					
X-133	X-134	X-135	X-136	X-137	X-138
X-139	X-140	X-141	X-142	X-143	X-144
X-145	X-146	X-147	X-148	X-149	X-150
X-151	X-152				
Dialdehydes					
X-153	X-154	X-155	X-156	X-157	X-158

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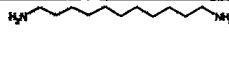
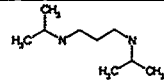
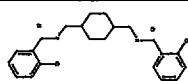
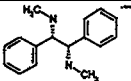
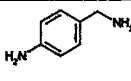
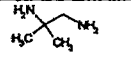
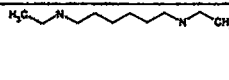
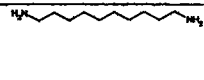
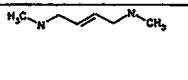
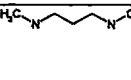
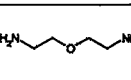
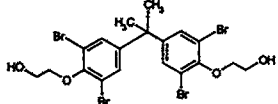
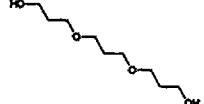
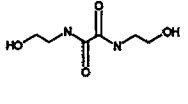
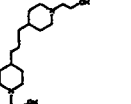
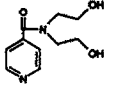
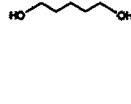
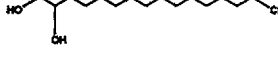
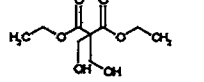
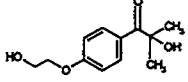
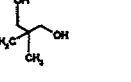
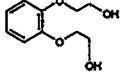
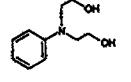
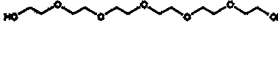
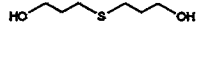
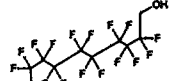
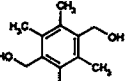
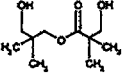
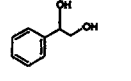
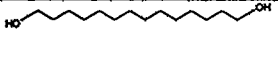
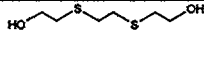
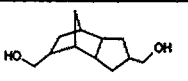
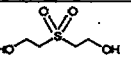
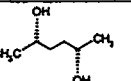
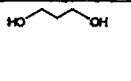
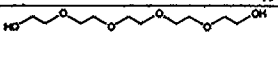
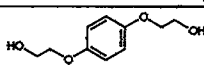
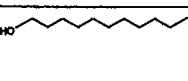
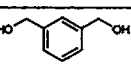
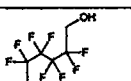
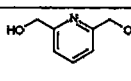
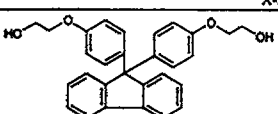
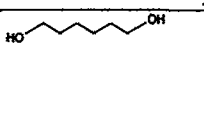
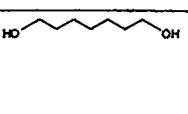
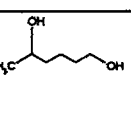
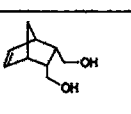
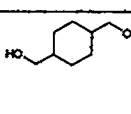
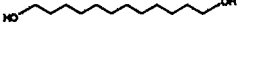
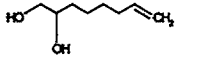
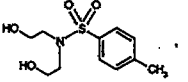
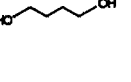
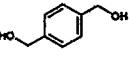
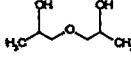
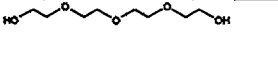
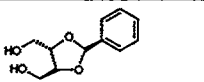
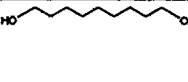
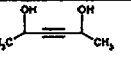
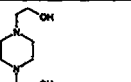
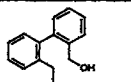
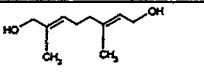
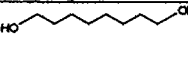
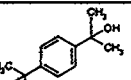
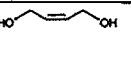
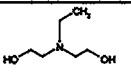
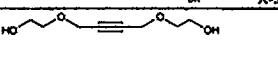
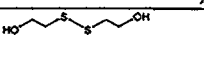
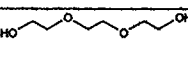
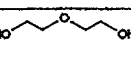
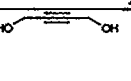
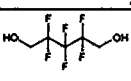
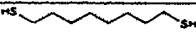
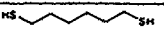


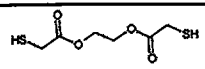
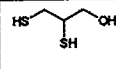
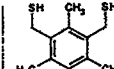
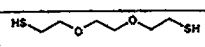
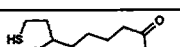
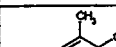
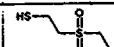
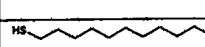
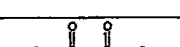
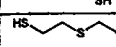
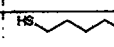
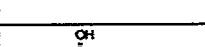
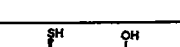
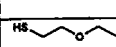
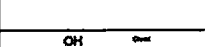
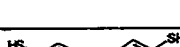
X-159	X-160	X-161	X-162	X-163	X-164
X-165	X-166	X-167	X-168	X-169	X-170
X-171	X-172	X-173	X-174		
Dihalides					
X-175	X-176	X-177	X-178	X-179	X-180
X-181	X-182	X-183	X-184	X-185	X-186
X-187	X-188	X-189	X-190	X-191	X-192
X-193	X-194	X-195	X-196	X-197	X-198
X-199	X-200	X-201	X-202	X-203	X-204
X-205	X-206	X-207	X-208	X-209	X-210
X-211	X-212	X-213	X-214		
Diisocyanates					
X-215	X-216	X-217	X-218	X-219	X-220
X-221	X-222	X-223	X-224	X-225	X-226
X-227	X-228	X-229	X-230	X-231	X-232



X-233	X-234	X-235	X-236	X-237	X-238
X-239	X-240	X-241	X-242	X-243	
X-244	X-245	X-246	X-247	X-248	
Diamines					
X-249	X-250	X-251	X-252	X-253	X-254
X-255	X-256	X-257	X-258	X-259	X-260
X-261	X-262	X-263	X-264	X-265	X-266
X-267	X-268	X-269	X-270	X-271	X-272
X-273	X-274	X-275	X-276	X-277	X-278
X-279	X-280	X-281	X-282	X-283	X-284
X-285	X-286	X-287	X-288	X-289	X-290
X-291	X-292	X-293	X-294	X-295	X-296
X-297	X-298	X-299	X-300	X-301	X-302
X-303	X-304	X-305	X-306	X-307	X-308
X-309	X-310	X-311	X-312	X-313	X-314

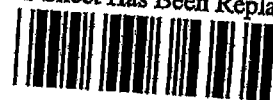


					
X-3151	X-3161	X-3171	X-3181	X-3191	X-3201
					
X-3211	X-3221	X-3231	X-3241	X-3251	
Diols					
					
3261	X-3271	X-3281	X-3291	X-3301	X-3311
					
X-3321	X-3331	X-3341	X-3351	X-3361	X-3371
					
X-3381	X-3391	X-3401	X-3411	X-3421	X-3431
					
X-3441	X-3451	X-3461	X-3471	X-3481	X-3491
					
X-3501	X-3511	X-3521	X-3531	X-3541	X-3551
					
3561	X-3571	X-3581	X-3591	X-3601	X-3611
					
X-3621	X-3631	X-3641	X-3651	X-3661	X-3671
					
X-3681	X-3691	X-3701	X-3711	X-3721	X-3731
					
X-3741	X-3751	X-3761	X-3771	X-3781	X-3791
					
X-3801	X-3811	X-3821	X-3831	X-3841	X-3851
Dithiols					
					
X-3861	X-3871	X-3881	X-3891	X-3901	X-3911

					
X-392	X-393	X-394	X-395	X-396	X-397
					
X-398	X-399	X-400	X-401	X-402	X-403
					
X-404	X-405	X-406	X-407	X-408	X-409
					
X-410	X-411	X-412	X-413	X-414	X-415
					
X-416	X-417	X-418			

This Sheet Has Been Replaced





Representative nucleoside reverse transcriptase inhibitors (NRTI) ligands (L_1) for use in this invention include, by way of example, L_1 -1 through L_1 -13: zidovudine (L_1 -1), didanosine (L_1 -2), zalcitabine (L_1 -3), stavudine (L_1 -4),
 5 lamivudine (L_1 -5), abacavir (L_1 -6), adefovir (L_1 -7), raluridine (L_1 -8), oral PMPA prodrug (L_1 -9), azidouridine (L_1 -10), IVX-E-59 (L_1 -11), emitricitabine (L_1 -12) and lodenosine (L_1 -13).

Representative non-nucleoside reverse transcriptase inhibitors (NNRTIs)
 10 ligands L_2 for use in this invention include, by way of example, L_2 -1 through L_2 -9: nevirapine (L_2 -1), delavirdine (L_2 -2), efavirenz (L_2 -3), MKC-442 (L_2 -4), loviride (L_2 -5), S-1153 (L_2 -6), talviraline (L_2 -7), calanolide A (L_2 -8) and tivirapine (L_2 -9).

15 Combinations of ligands (L_1 and L_2) and linkers (X) per this invention include, by way example only, heterodimers wherein a first ligand, L_1 , selected from L_1 -1 through L_1 -13 above, and a second ligand, L_2 , and a linker, X, are selected from the following:

20	L_2 -1/X-1-	L_2 -1/X-2-	L_2 -1/X-3-	L_2 -1/X-4-	L_2 -1/X-5-	L_2 -1/X-6-
	L_2 -1/X-7-	L_2 -1/X-8-	L_2 -1/X-9-	L_2 -1/X-10-	L_2 -1/X-11-	L_2 -1/X-12-
	L_2 -1/X-13-	L_2 -1/X-14-	L_2 -1/X-15-	L_2 -1/X-16-	L_2 -1/X-17-	L_2 -1/X-18-
	L_2 -1/X-19-	L_2 -1/X-20-	L_2 -1/X-21-	L_2 -1/X-22-	L_2 -1/X-23-	L_2 -1/X-24-
	L_2 -1/X-25-	L_2 -1/X-26-	L_2 -1/X-27-	L_2 -1/X-28-	L_2 -1/X-29-	L_2 -1/X-30-
25	L_2 -1/X-31-	L_2 -1/X-32-	L_2 -1/X-33-	L_2 -1/X-34-	L_2 -1/X-35-	L_2 -1/X-36-
	L_2 -1/X-37-	L_2 -1/X-38-	L_2 -1/X-39-	L_2 -1/X-40-	L_2 -1/X-41-	L_2 -1/X-42-
	L_2 -1/X-43-	L_2 -1/X-44-	L_2 -1/X-45-	L_2 -1/X-46-	L_2 -1/X-47-	L_2 -1/X-48-
	L_2 -1/X-49-	L_2 -1/X-50-	L_2 -1/X-51-	L_2 -1/X-52-	L_2 -1/X-53-	L_2 -1/X-54-
	L_2 -1/X-55-	L_2 -1/X-56-	L_2 -1/X-57-	L_2 -1/X-58-	L_2 -1/X-59-	L_2 -1/X-60-
30	L_2 -1/X-61-	L_2 -1/X-62-	L_2 -1/X-63-	L_2 -1/X-64-	L_2 -1/X-65-	L_2 -1/X-66-

This Sheet Has Been Replaced



--78--

	L ₂ -1/X-67-	L ₂ -1/X-68-	L ₂ -1/X-69-	L ₂ -1/X-70-	L ₂ -1/X-71-	L ₂ -1/X-72-
	L ₂ -1/X-73-	L ₂ -1/X-74-	L ₂ -1/X-75-	L ₂ -1/X-76-	L ₂ -1/X-77-	L ₂ -1/X-78-
	L ₂ -1/X-79-	L ₂ -1/X-80-	L ₂ -1/X-81-	L ₂ -1/X-82-	L ₂ -1/X-83-	L ₂ -1/X-84-
	L ₂ -1/X-85-	L ₂ -1/X-86-	L ₂ -1/X-87-	L ₂ -1/X-88-	L ₂ -1/X-89-	L ₂ -1/X-90-
5	L ₂ -1/X-91-	L ₂ -1/X-92-	L ₂ -1/X-93-	L ₂ -1/X-94-	L ₂ -1/X-95-	L ₂ -1/X-96-
	L ₂ -1/X-97-	L ₂ -1/X-98-	L ₂ -1/X-99-	L ₂ -1/X-100-	L ₂ -1/X-101-	L ₂ -1/X-102-
	L ₂ -1/X-103-	L ₂ -1/X-104-	L ₂ -1/X-105-	L ₂ -1/X-106-	L ₂ -1/X-107-	L ₂ -1/X-108-
	L ₂ -1/X-109-	L ₂ -1/X-110-	L ₂ -1/X-111-	L ₂ -1/X-112-	L ₂ -1/X-113-	L ₂ -1/X-114-
	L ₂ -1/X-115-	L ₂ -1/X-116-	L ₂ -1/X-117-	L ₂ -1/X-118-	L ₂ -1/X-119-	L ₂ -1/X-120-
10	L ₂ -1/X-121-	L ₂ -1/X-122-	L ₂ -1/X-123-	L ₂ -1/X-124-	L ₂ -1/X-125-	L ₂ -1/X-126-
	L ₂ -1/X-127-	L ₂ -1/X-128-	L ₂ -1/X-129-	L ₂ -1/X-130-	L ₂ -1/X-131-	L ₂ -1/X-132-
	L ₂ -1/X-133-	L ₂ -1/X-134-	L ₂ -1/X-135-	L ₂ -1/X-136-	L ₂ -1/X-137-	L ₂ -1/X-138-
	L ₂ -1/X-139-	L ₂ -1/X-140-	L ₂ -1/X-141-	L ₂ -1/X-142-	L ₂ -1/X-143-	L ₂ -1/X-144-
	L ₂ -1/X-145-	L ₂ -1/X-146-	L ₂ -1/X-147-	L ₂ -1/X-148-	L ₂ -1/X-149-	L ₂ -1/X-150-
15	L ₂ -1/X-151-	L ₂ -1/X-152-	L ₂ -1/X-153-	L ₂ -1/X-154-	L ₂ -1/X-155-	L ₂ -1/X-156-
	L ₂ -1/X-157-	L ₂ -1/X-158-	L ₂ -1/X-159-	L ₂ -1/X-160-	L ₂ -1/X-161-	L ₂ -1/X-162-
	L ₂ -1/X-163-	L ₂ -1/X-164-	L ₂ -1/X-165-	L ₂ -1/X-166-	L ₂ -1/X-167-	L ₂ -1/X-168-
	L ₂ -1/X-169-	L ₂ -1/X-170-	L ₂ -1/X-171-	L ₂ -1/X-172-		
	L ₂ -1/X-173-	L ₂ -1/X-174-	L ₂ -1/X-175-	L ₂ -1/X-176-	L ₂ -1/X-177-	L ₂ -1/X-178-
20	L ₂ -1/X-179-	L ₂ -1/X-180-	L ₂ -1/X-181-	L ₂ -1/X-182-	L ₂ -1/X-183-	L ₂ -1/X-184-
	L ₂ -1/X-185-	L ₂ -1/X-186-	L ₂ -1/X-187-	L ₂ -1/X-188-	L ₂ -1/X-189-	L ₂ -1/X-190-
	L ₂ -1/X-191-	L ₂ -1/X-192-	L ₂ -1/X-193-	L ₂ -1/X-194-	L ₂ -1/X-195-	L ₂ -1/X-196-
	L ₂ -1/X-197-	L ₂ -1/X-198-	L ₂ -1/X-199-	L ₂ -1/X-200-	L ₂ -1/X-201-	L ₂ -1/X-202-
	L ₂ -1/X-203-	L ₂ -1/X-204-	L ₂ -1/X-205-	L ₂ -1/X-206-	L ₂ -1/X-207-	L ₂ -1/X-208-
25	L ₂ -1/X-209-	L ₂ -1/X-210-	L ₂ -1/X-211-	L ₂ -1/X-212-	L ₂ -1/X-213-	L ₂ -1/X-214-
	L ₂ -1/X-215-	L ₂ -1/X-216-	L ₂ -1/X-217-	L ₂ -1/X-218-	L ₂ -1/X-219-	L ₂ -1/X-220-
	L ₂ -1/X-221-	L ₂ -1/X-222-	L ₂ -1/X-223-	L ₂ -1/X-224-	L ₂ -1/X-225-	L ₂ -1/X-226-
	L ₂ -1/X-227-	L ₂ -1/X-228-	L ₂ -1/X-229-	L ₂ -1/X-230-	L ₂ -1/X-231-	L ₂ -1/X-232-
	L ₂ -1/X-233-	L ₂ -1/X-234-	L ₂ -1/X-235-	L ₂ -1/X-236-	L ₂ -1/X-237-	L ₂ -1/X-238-
30	L ₂ -1/X-239-	L ₂ -1/X-240-	L ₂ -1/X-241-	L ₂ -1/X-242-	L ₂ -1/X-243-	L ₂ -1/X-244-

	L ₂ -1/X-245-	L ₂ -1/X-246-	L ₂ -1/X-247-	L ₂ -1/X-248-	L ₂ -1/X-249-	L ₂ -1/X-250-
	L ₂ -1/X-251-	L ₂ -1/X-252-	L ₂ -1/X-253-	L ₂ -1/X-254-	L ₂ -1/X-255-	L ₂ -1/X-256-
	L ₂ -1/X-257-	L ₂ -1/X-258-	L ₂ -1/X-259-	L ₂ -1/X-260-	L ₂ -1/X-261-	L ₂ -1/X-262-
	L ₂ -1/X-263-	L ₂ -1/X-264-	L ₂ -1/X-265-	L ₂ -1/X-266-	L ₂ -1/X-267-	L ₂ -1/X-268-
5	L ₂ -1/X-269-	L ₂ -1/X-270-	L ₂ -1/X-271-	L ₂ -1/X-272-	L ₂ -1/X-273-	L ₂ -1/X-274-
	L ₂ -1/X-275-	L ₂ -1/X-276-	L ₂ -1/X-277-	L ₂ -1/X-278-	L ₂ -1/X-279-	L ₂ -1/X-280-
	L ₂ -1/X-281-	L ₂ -1/X-282-	L ₂ -1/X-283-	L ₂ -1/X-284-	L ₂ -1/X-285-	L ₂ -1/X-286-
	L ₂ -1/X-287-	L ₂ -1/X-288-	L ₂ -1/X-289-	L ₂ -1/X-290-	L ₂ -1/X-291-	L ₂ -1/X-292-
	L ₂ -1/X-293-	L ₂ -1/X-294-	L ₂ -1/X-295-	L ₂ -1/X-296-	L ₂ -1/X-297-	L ₂ -1/X-298-
10	L ₂ -1/X-299-	L ₂ -1/X-300-	L ₂ -1/X-301-	L ₂ -1/X-302-	L ₂ -1/X-303-	L ₂ -1/X-304-
	L ₂ -1/X-305-	L ₂ -1/X-306-	L ₂ -1/X-307-	L ₂ -1/X-308-	L ₂ -1/X-309-	L ₂ -1/X-310-
	L ₂ -1/X-311-	L ₂ -1/X-312-	L ₂ -1/X-313-	L ₂ -1/X-314-	L ₂ -1/X-315-	L ₂ -1/X-316-
	L ₂ -1/X-317-	L ₂ -1/X-318-	L ₂ -1/X-319-	L ₂ -1/X-320-	L ₂ -1/X-321-	L ₂ -1/X-322-
	L ₂ -1/X-323-	L ₂ -1/X-324-	L ₂ -1/X-325-	L ₂ -1/X-326-	L ₂ -1/X-327-	L ₂ -1/X-328-
15	L ₂ -1/X-329-	L ₂ -1/X-330-	L ₂ -1/X-331-	L ₂ -1/X-332-	L ₂ -1/X-333-	L ₂ -1/X-334-
	L ₂ -1/X-335-	L ₂ -1/X-336-	L ₂ -1/X-337-	L ₂ -1/X-338-	L ₂ -1/X-339-	L ₂ -1/X-340-
	L ₂ -1/X-341-	L ₂ -1/X-342-	L ₂ -1/X-343-	L ₂ -1/X-344-	L ₂ -1/X-345-	L ₂ -1/X-346-
	L ₂ -1/X-347-	L ₂ -1/X-348-	L ₂ -1/X-349-	L ₂ -1/X-350-	L ₂ -1/X-351-	L ₂ -1/X-352-
	L ₂ -1/X-353-	L ₂ -1/X-354-	L ₂ -1/X-355-	L ₂ -1/X-356-	L ₂ -1/X-357-	L ₂ -1/X-358-
20	L ₂ -1/X-359-	L ₂ -1/X-360-	L ₂ -1/X-361-	L ₂ -1/X-362-	L ₂ -1/X-363-	L ₂ -1/X-364-
	L ₂ -1/X-365-	L ₂ -1/X-366-	L ₂ -1/X-367-	L ₂ -1/X-368-	L ₂ -1/X-369-	L ₂ -1/X-370-
	L ₂ -1/X-371-	L ₂ -1/X-372-	L ₂ -1/X-373-	L ₂ -1/X-374-	L ₂ -1/X-375-	L ₂ -1/X-376-
	L ₂ -1/X-377-	L ₂ -1/X-378-	L ₂ -1/X-379-	L ₂ -1/X-380-	L ₂ -1/X-381-	L ₂ -1/X-382-
	L ₂ -1/X-383-	L ₂ -1/X-384-	L ₂ -1/X-385-	L ₂ -1/X-386-	L ₂ -1/X-387-	L ₂ -1/X-388-
25	L ₂ -1/X-389-	L ₂ -1/X-390-	L ₂ -1/X-391-	L ₂ -1/X-392-	L ₂ -1/X-393-	L ₂ -1/X-394-
	L ₂ -1/X-395-	L ₂ -1/X-396-	L ₂ -1/X-397-	L ₂ -1/X-398-	L ₂ -1/X-399-	L ₂ -1/X-400-
	L ₂ -1/X-401-	L ₂ -1/X-402-	L ₂ -1/X-403-	L ₂ -1/X-404-	L ₂ -1/X-405-	L ₂ -1/X-406-
	L ₂ -1/X-407-	L ₂ -1/X-408-	L ₂ -1/X-409-	L ₂ -1/X-410-	L ₂ -1/X-411-	L ₂ -1/X-412-
	L ₂ -1/X-413-	L ₂ -1/X-414-	L ₂ -1/X-415-	L ₂ -1/X-416-	L ₂ -1/X-417-	L ₂ -1/X-418-



	L ₂ -2/X-1-	L ₂ -2/X-2-	L ₂ -2/X-3-	L ₂ -2/X-4-	L ₂ -2/X-5-	L ₂ -2/X-6-
	L ₂ -2/X-7-	L ₂ -2/X-8-	L ₂ -2/X-9-	L ₂ -2/X-10-	L ₂ -2/X-11-	L ₂ -2/X-12-
	L ₂ -2/X-13-	L ₂ -2/X-14-	L ₂ -2/X-15-	L ₂ -2/X-16-	L ₂ -2/X-17-	L ₂ -2/X-18-
	L ₂ -2/X-19-	L ₂ -2/X-20-	L ₂ -2/X-21-	L ₂ -2/X-22-	L ₂ -2/X-23-	L ₂ -2/X-24-
5	L ₂ -2/X-25-	L ₂ -2/X-26-	L ₂ -2/X-27-	L ₂ -2/X-28-	L ₂ -2/X-29-	L ₂ -2/X-30-
	L ₂ -2/X-31-	L ₂ -2/X-32-	L ₂ -2/X-33-	L ₂ -2/X-34-	L ₂ -2/X-35-	L ₂ -2/X-36-
	L ₂ -2/X-37-	L ₂ -2/X-38-	L ₂ -2/X-39-	L ₂ -2/X-40-	L ₂ -2/X-41-	L ₂ -2/X-42-
	L ₂ -2/X-43-	L ₂ -2/X-44-	L ₂ -2/X-45-	L ₂ -2/X-46-	L ₂ -2/X-47-	L ₂ -2/X-48-
	L ₂ -2/X-49-	L ₂ -2/X-50-	L ₂ -2/X-51-	L ₂ -2/X-52-	L ₂ -2/X-53-	L ₂ -2/X-54-
10	L ₂ -2/X-55-	L ₂ -2/X-56-	L ₂ -2/X-57-	L ₂ -2/X-58-	L ₂ -2/X-59-	L ₂ -2/X-60-
	L ₂ -2/X-61-	L ₂ -2/X-62-	L ₂ -2/X-63-	L ₂ -2/X-64-	L ₂ -2/X-65-	L ₂ -2/X-66-
	L ₂ -2/X-67-	L ₂ -2/X-68-	L ₂ -2/X-69-	L ₂ -2/X-70-	L ₂ -2/X-71-	L ₂ -2/X-72-
	L ₂ -2/X-73-	L ₂ -2/X-74-	L ₂ -2/X-75-	L ₂ -2/X-76-	L ₂ -2/X-77-	L ₂ -2/X-78-
	L ₂ -2/X-79-	L ₂ -2/X-80-	L ₂ -2/X-81-	L ₂ -2/X-82-	L ₂ -2/X-83-	L ₂ -2/X-84-
15	L ₂ -2/X-85-	L ₂ -2/X-86-	L ₂ -2/X-87-	L ₂ -2/X-88-	L ₂ -2/X-89-	L ₂ -2/X-90-
	L ₂ -2/X-91-	L ₂ -2/X-92-	L ₂ -2/X-93-	L ₂ -2/X-94-	L ₂ -2/X-95-	L ₂ -2/X-96-
	L ₂ -2/X-97-	L ₂ -2/X-98-	L ₂ -2/X-99-	L ₂ -2/X-100-	L ₂ -2/X-101-	L ₂ -2/X-102-
	L ₂ -2/X-103-	L ₂ -2/X-104-	L ₂ -2/X-105-	L ₂ -2/X-106-	L ₂ -2/X-107-	L ₂ -2/X-108-
	L ₂ -2/X-109-	L ₂ -2/X-110-	L ₂ -2/X-111-	L ₂ -2/X-112-	L ₂ -2/X-113-	L ₂ -2/X-114-
20	L ₂ -2/X-115-	L ₂ -2/X-116-	L ₂ -2/X-117-	L ₂ -2/X-118-	L ₂ -2/X-119-	L ₂ -2/X-120-
	L ₂ -2/X-121-	L ₂ -2/X-122-	L ₂ -2/X-123-	L ₂ -2/X-124-	L ₂ -2/X-125-	L ₂ -2/X-126-
	L ₂ -2/X-127-	L ₂ -2/X-128-	L ₂ -2/X-129-	L ₂ -2/X-130-	L ₂ -2/X-131-	L ₂ -2/X-132-
	L ₂ -2/X-133-	L ₂ -2/X-134-	L ₂ -2/X-135-	L ₂ -2/X-136-	L ₂ -2/X-137-	L ₂ -2/X-138-
	L ₂ -2/X-139-	L ₂ -2/X-140-	L ₂ -2/X-141-	L ₂ -2/X-142-	L ₂ -2/X-143-	L ₂ -2/X-144-
25	L ₂ -2/X-145-	L ₂ -2/X-146-	L ₂ -2/X-147-	L ₂ -2/X-148-	L ₂ -2/X-149-	L ₂ -2/X-150-
	L ₂ -2/X-151-	L ₂ -2/X-152-	L ₂ -2/X-153-	L ₂ -2/X-154-	L ₂ -2/X-155-	L ₂ -2/X-156-
	L ₂ -2/X-157-	L ₂ -2/X-158-	L ₂ -2/X-159-	L ₂ -2/X-160-	L ₂ -2/X-161-	L ₂ -2/X-162-
	L ₂ -2/X-163-	L ₂ -2/X-164-	L ₂ -2/X-165-	L ₂ -2/X-166-	L ₂ -2/X-167-	L ₂ -2/X-168-
	L ₂ -2/X-169-	L ₂ -2/X-170-	L ₂ -2/X-171-	L ₂ -2/X-172-		
30	L ₂ -2/X-173-	L ₂ -2/X-174-	L ₂ -2/X-175-	L ₂ -2/X-176-	L ₂ -2/X-177-	L ₂ -2/X-178-



	L ₂ -2/X-179-	L ₂ -2/X-180-	L ₂ -2/X-181-	L ₂ -2/X-182-	L ₂ -2/X-183-	L ₂ -2/X-184-
	L ₂ -2/X-185-	L ₂ -2/X-186-	L ₂ -2/X-187-	L ₂ -2/X-188-	L ₂ -2/X-189-	L ₂ -2/X-190-
	L ₂ -2/X-191-	L ₂ -2/X-192-	L ₂ -2/X-193-	L ₂ -2/X-194-	L ₂ -2/X-195-	L ₂ -2/X-196-
	L ₂ -2/X-197-	L ₂ -2/X-198-	L ₂ -2/X-199-	L ₂ -2/X-200-	L ₂ -2/X-201-	L ₂ -2/X-202-
5	L ₂ -2/X-203-	L ₂ -2/X-204-	L ₂ -2/X-205-	L ₂ -2/X-206-	L ₂ -2/X-207-	L ₂ -2/X-208-
	L ₂ -2/X-209-	L ₂ -2/X-210-	L ₂ -2/X-211-	L ₂ -2/X-212-	L ₂ -2/X-213-	L ₂ -2/X-214-
	L ₂ -2/X-215-	L ₂ -2/X-216-	L ₂ -2/X-217-	L ₂ -2/X-218-	L ₂ -2/X-219-	L ₂ -2/X-220-
	L ₂ -2/X-221-	L ₂ -2/X-222-	L ₂ -2/X-223-	L ₂ -2/X-224-	L ₂ -2/X-225-	L ₂ -2/X-226-
	L ₂ -2/X-227-	L ₂ -2/X-228-	L ₂ -2/X-229-	L ₂ -2/X-230-	L ₂ -2/X-231-	L ₂ -2/X-232-
10	L ₂ -2/X-233-	L ₂ -2/X-234-	L ₂ -2/X-235-	L ₂ -2/X-236-	L ₂ -2/X-237-	L ₂ -2/X-238-
	L ₂ -2/X-239-	L ₂ -2/X-240-	L ₂ -2/X-241-	L ₂ -2/X-242-	L ₂ -2/X-243-	L ₂ -2/X-244-
	L ₂ -2/X-245-	L ₂ -2/X-246-	L ₂ -2/X-247-	L ₂ -2/X-248-	L ₂ -2/X-249-	L ₂ -2/X-250-
	L ₂ -2/X-251-	L ₂ -2/X-252-	L ₂ -2/X-253-	L ₂ -2/X-254-	L ₂ -2/X-255-	L ₂ -2/X-256-
	L ₂ -2/X-257-	L ₂ -2/X-258-	L ₂ -2/X-259-	L ₂ -2/X-260-	L ₂ -2/X-261-	L ₂ -2/X-262-
15	L ₂ -2/X-263-	L ₂ -2/X-264-	L ₂ -2/X-265-	L ₂ -2/X-266-	L ₂ -2/X-267-	L ₂ -2/X-268-
	L ₂ -2/X-269-	L ₂ -2/X-270-	L ₂ -2/X-271-	L ₂ -2/X-272-	L ₂ -2/X-273-	L ₂ -2/X-274-
	L ₂ -2/X-275-	L ₂ -2/X-276-	L ₂ -2/X-277-	L ₂ -2/X-278-	L ₂ -2/X-279-	L ₂ -2/X-280-
	L ₂ -2/X-281-	L ₂ -2/X-282-	L ₂ -2/X-283-	L ₂ -2/X-284-	L ₂ -2/X-285-	L ₂ -2/X-286-
	L ₂ -2/X-287-	L ₂ -2/X-288-	L ₂ -2/X-289-	L ₂ -2/X-290-	L ₂ -2/X-291-	L ₂ -2/X-292-
20	L ₂ -2/X-293-	L ₂ -2/X-294-	L ₂ -2/X-295-	L ₂ -2/X-296-	L ₂ -2/X-297-	L ₂ -2/X-298-
	L ₂ -2/X-299-	L ₂ -2/X-300-	L ₂ -2/X-301-	L ₂ -2/X-302-	L ₂ -2/X-303-	L ₂ -2/X-304-
	L ₂ -2/X-305-	L ₂ -2/X-306-	L ₂ -2/X-307-	L ₂ -2/X-308-	L ₂ -2/X-309-	L ₂ -2/X-310-
	L ₂ -2/X-311-	L ₂ -2/X-312-	L ₂ -2/X-313-	L ₂ -2/X-314-	L ₂ -2/X-315-	L ₂ -2/X-316-
	L ₂ -2/X-317-	L ₂ -2/X-318-	L ₂ -2/X-319-	L ₂ -2/X-320-	L ₂ -2/X-321-	L ₂ -2/X-322-
25	L ₂ -2/X-323-	L ₂ -2/X-324-	L ₂ -2/X-325-	L ₂ -2/X-326-	L ₂ -2/X-327-	L ₂ -2/X-328-
	L ₂ -2/X-329-	L ₂ -2/X-330-	L ₂ -2/X-331-	L ₂ -2/X-332-	L ₂ -2/X-333-	L ₂ -2/X-334-
	L ₂ -2/X-335-	L ₂ -2/X-336-	L ₂ -2/X-337-	L ₂ -2/X-338-	L ₂ -2/X-339-	L ₂ -2/X-340-
	L ₂ -2/X-341-	L ₂ -2/X-342-	L ₂ -2/X-343-	L ₂ -2/X-344-	L ₂ -2/X-345-	L ₂ -2/X-346-
	L ₂ -2/X-347-	L ₂ -2/X-348-	L ₂ -2/X-349-	L ₂ -2/X-350-	L ₂ -2/X-351-	L ₂ -2/X-352-
30	L ₂ -2/X-353-	L ₂ -2/X-354-	L ₂ -2/X-355-	L ₂ -2/X-356-	L ₂ -2/X-357-	L ₂ -2/X-358-

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	L ₂ -2/X-359-	L ₂ -2/X-360-	L ₂ -2/X-361-	L ₂ -2/X-362-	L ₂ -2/X-363-	L ₂ -2/X-364-
	L ₂ -2/X-365-	L ₂ -2/X-366-	L ₂ -2/X-367-	L ₂ -2/X-368-	L ₂ -2/X-369-	L ₂ -2/X-370-
	L ₂ -2/X-371-	L ₂ -2/X-372-	L ₂ -2/X-373-	L ₂ -2/X-374-	L ₂ -2/X-375-	L ₂ -2/X-376-
	L ₂ -2/X-377-	L ₂ -2/X-378-	L ₂ -2/X-379-	L ₂ -2/X-380-	L ₂ -2/X-381-	L ₂ -2/X-382-
5	L ₂ -2/X-383-	L ₂ -2/X-384-	L ₂ -2/X-385-	L ₂ -2/X-386-	L ₂ -2/X-387-	L ₂ -2/X-388-
	L ₂ -2/X-389-	L ₂ -2/X-390-	L ₂ -2/X-391-	L ₂ -2/X-392-	L ₂ -2/X-393-	L ₂ -2/X-394-
	L ₂ -2/X-395-	L ₂ -2/X-396-	L ₂ -2/X-397-	L ₂ -2/X-398-	L ₂ -2/X-399-	L ₂ -2/X-400-
	L ₂ -2/X-401-	L ₂ -2/X-402-	L ₂ -2/X-403-	L ₂ -2/X-404-	L ₂ -2/X-405-	L ₂ -2/X-406-
	L ₂ -2/X-407-	L ₂ -2/X-408-	L ₂ -2/X-409-	L ₂ -2/X-410-	L ₂ -2/X-411-	L ₂ -2/X-412-
10	L ₂ -2/X-413-	L ₂ -2/X-414-	L ₂ -2/X-415-	L ₂ -2/X-416-	L ₂ -2/X-417-	L ₂ -2/X-418-

and so on, substituting L₂-3, L₂-4, L₂-5, L₂-6, L₂-7, L₂-8, and L₂-9 in turn for L₂-1 and L₂-2 exemplified above.

15

EXAMPLES

In the examples below, the following abbreviations have the following meanings. If an abbreviation is not defined, it has its generally accepted meaning.

	Å	=	Angstroms
20	cm	=	centimeter
	DCC	=	dicyclohexyl carbodiimide
	DMF	=	<i>N,N</i> -dimethylformamide
	DMSO	=	dimethylsulfoxide
	EDTA	=	ethylenediaminetetraacetic acid
25	g	=	gram
	HPLC	=	high performance liquid chromatography
	MEM	=	minimal essential medium
	mg	=	milligram
	MIC	=	minimum inhibitory concentration
30	min	=	minute
	mL	=	milliliter
	mm	=	millimeter
	mmol	=	millimol
	N	=	normal
35	THF	=	tetrahydrofuran

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μL = microliters
 μm = microns

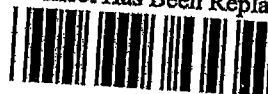
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Preparation 1

1,4-dihydro-6-chloro-4-[2-(bromomethyl)cycloprop-1-ylethynyl]- 4-trifluoromethyl-2H-3,1-benzoxazin-2-one (20)

- 10 A. 2-Ethynylcyclopropylmethanol (17, prepared as described in Tetrahedron Letters, 1992, 33, 4905) (50 mmol) is dissolved in dry THF (25 mL) at 0°. A solution of n-BuLi in hexane (100 mmol) is added. After 10 minutes, a solution of 4-chloro-2-trifluoroacetylaniline (16, prepared as described in WO 8904535) (100 mmol) is added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to sat NaHCO₃, and extracted with EtOAc. The
15 extract is dried and evaporated, and the residue is purified by chromatography to afford the intermediate carbinol 18.
- B. Compound 18 (50 mmol) is dissolved in dry THF (250 mL) and carbonyldiimidazole (100 mmol) is added. The progress of the reaction is monitored
20 by tlc. When it is complete, the mixture is added to dilute HCl, and extracted with EtOAc. The extract is dried and evaporated, and the residue is chromatographed to afford 6-chloro-1,4-dihydro-4-[2-(hydroxymethyl)cycloprop-1-ylethynyl]-4-trifluoromethyl-2H-3,1-benzoxazin-2-one, 19.
- 25 C. The compound 19 (50 mmol) is dissolved in CH₂Cl₂ (150 mL) and CBr₄ (50 mmol) and PPh₃ (50 mmol) are added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water. The organic phase is dried and evaporated, and the residue is chromatographed to afford 4-[2-(bromomethyl)cycloprop-1-ylethynyl]-6-chloro-1,4-dihydro-4-trifluoromethyl-2H-
30 3,1-benzoxazin-2-one, 20.

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D. Using the above procedures, but employing different hydroxyl-substituted terminal acetylenes for 2-ethynylcyclopropylmethanol, there are obtained the corresponding compounds **6** in which X is Br.

- 5 E. In place of a hydroxyl-substituted acetylene, there may be employed in Step A the corresponding trisubstituted silyloxy terminal acetylenes, prepared as described in Preparation 16A with the acetylenic alcohol replacing **66**. The silyloxy group is removed using the procedure of Example 1B, prior to the conversion of the hydroxyl group to the corresponding bromo group.

10

Preparation 2
**4-[2-(6-aminooct-1-ynyl)- 6-chloro-1,4-dihydro-4-trifluoromethyl-
2H-3,1-benzoxazin-2-one (23)**

- 15 A. Using the procedure of Preparation 1, but employing 8-hydroxy-1-octyne in place of 2-ethynylcyclopropylmethanol, there is obtained 4-[2-(8-bromooct-1-ynyl)-6-chloro-1,4-dihydro-4-trifluoromethyl-2H-3,1-benzoxazin-2-one, **22**.

- 20 B. The above compound (100 mmol) is dissolved in THF (200 mL) and the solution is added to concentrated NH_4OH (50 mL) with vigorous stirring. The progress of the reaction is monitored by tlc. When it is complete, the mixture is extracted with EtOAc. The extract is dried and evaporated, and the residue is chromatographed to afford the compound **23**.

25

Preparation 3
**6-chloro-1,4-dihydro-4-[2-(5-mercapto-4-methylpent-1-ynyl)-
4-trifluoromethyl-2H-3,1-benzoxazin-2-one (26)**

- 30 A. Using the procedures of Preparation 1, but employing 4-methyl-5-hydroxypent-1-yne in place of 2-ethynylcyclopropylmethanol, there is obtained the compound 4-[2-(5-bromo-6-chloro-1,4-dihydro-4-methylpent-1-ynyl)-4-trifluoromethyl-2H-3,1-benzoxazin-2-one, **25**.



B. The above compound (50 mmol) is dissolved in EtOH (100 mL) and thiourea (100 mmol) is added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water, and extracted with EtOAc. The extract is dried and evaporated, and the residue redissolved in EtOH (200 mL).

- 5 Tetramethylene pentamine (200 mmol) is added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water, and extracted with EtOAc. The extract is dried and evaporated, and the residue is chromatographed to afford the compound 26.

10

Preparation 4

1-(3-bromopropyl)-6-chloro-1,4-dihydro-4-(cyclopropylethynyl)- 4-trifluoromethyl-2H-3,1-benzoxazin-2-one (30)

- 15 A. 4-Chloro-2-trifluoroacetylaniline, 16, (100 mmol) and 3-bromopropanol (50 mmol) are heated at reflux in EtOH (100 mL). The progress of the reaction is monitored by tlc. When it is complete, the mixture is cooled and added to water. The aqueous solution is extracted with EtOAc, and the residue is dried and evaporated. The residue is chromatographed to afford N-(3-hydroxypropyl)-4-chloro-2-trifluoroacetylaniline, 28.

20

- B. Using the procedures of Preparation 1, but employing cyclopropylacetylene in place of 2-ethynylcyclopropylmethanol in Part A, there is obtained the compound 30.

25

Preparation 5

1-(4-aminobutyl)-6-chloro-1,4-dihydro-4-(cyclopropylethynyl)- 4-trifluoromethyl-2H-3,1-benzoxazin-2-one (34)

- 30 A. Using the conditions of Preparation 4A, 4-chloro-2-trifluoroacetylaniline, 16 is reacted with 4-bromobutylphthalimide 31, to afford N-(4-phthalimidobutyl)-4-chloro-2-trifluoroacetylaniline, 32.

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B. Using the conditions of Preparation 1A and 1B, employing cyclopropylacetylene in place of 2-ethynylcyclopropylmethanol in Part A, using 1.2 equivalents of nBuLi instead of 2 equivalents, there is obtained the compound 6-chloro-4-(cyclopropylethynyl)-1,4-dihydro-1-(4-phthalimidobutyl)-4-trifluoromethyl -
5 2H-3,1-benzoxazin-2-one, 33.

C. The above compound 33 (50 mmol) is dissolved in EtOH (100 mL) and 85% hydrazine hydrate (200 mmol) is added. The mixture is heated at reflux while the progress of the reaction is monitored by tlc. When it is complete, the mixture is
10 cooled and it is then poured into water. The aqueous solution is extracted with CH₂Cl₂. The extract is dried and evaporated. The residue is chromatographed to afford the compound 34.

15 **Preparation 6**
11-[2-(bromomethyl)cyclopropyl]-5,11-dihydro-4-methyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one (39)

A. 2-Chloro-N-(2-methoxy-4-methyl-3-pyridinyl)-3-pyridinecarboxamide, 35, prepared as described in J. Org. Chem., 1995, 60, 1875, (5 mmol) and 2-
20 (hydroxymethyl)cyclopropylamine, 36 (10 mmol), prepared as described in Tetrahedron, 1995, 51, 7194, (10 mmol) are heated at 110° in a sealed tube for 16 hours. The excess amine is removed under vacuum and the residue is chromatographed to afford 2-[2-(hydroxymethyl)cyclopropylamino]-N-(2-methoxy-4-methyl-3-pyridinyl)-3-pyridinecarboxamide, 37.

25

B. The above compound 37, (1 mmol) is dissolved in pyridine (3 mL) and a 1M solution of sodium hexamethyldisilazide (3 mmol) is added. The mixture is heated to 60°; the progress of the reaction is monitored by tlc. When it is complete, the mixture is cooled and it is then poured into water. The aqueous solution is extracted
30 with CH₂Cl₂. The extract is dried and evaporated. The residue is chromatographed to



afford the compound 11-[2-(hydroxymethyl)cyclopropyl]-5,11-dihydro-4-methyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one, 38.

5 C. Using the conditions of Preparation 1C, the compound 38 is converted into the compound 39.

10 D. Using the above procedure, but employing in place of 2-(hydroxymethyl)cyclopropylamine, different hydroxyalkylamines, there is obtained the corresponding bromoalkyl compound 8 in which X is Br.

Preparation 7
5,11-dihydro-4-methyl-11-[4-(p-toluenesulfonyloxy)butyl]-
6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one (41)

15 A. Using the procedures of Preparations 6A and 6B, 2-chloro-N-(2-methoxy-4-methyl-3-pyridinyl)-3-pyridinecarboxamide, 35, and 4-aminobutanol 40, are reacted together and the product is cyclized.

20 B. The compound so obtained (5 mmol) is dissolved in pyridine (50 mL) and p-toluenesulfonyl chloride (6 mmol) is added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is poured into water. The aqueous solution is extracted with CH₂Cl₂. The extract is washed with water, then dried and evaporated. The residue is chromatographed to afford the compound 41.

Preparation 8
5,11-dihydro-11-(5-mercaptopent-2-yl)-4-methyl-
6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one (44)

25 A. Using the procedures of Preparations 6A, 6B and 7B, and employing 2-chloro-N-(2-methoxy-4-methyl-3-pyridinyl)-3-pyridinecarboxamide, 35, and 4-aminopentanol 42 as starting materials, there is obtained the compound 5,11-

30



dihydro-4-methyl-11-[5-(p-toluenesulfonyloxy)pent-2-yl]-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one, 43.

- B. Using the procedure of Preparation 3B, the above compound is converted into the thiol compound 44.

Preparation 9

11-(4-aminobutyl)-5,11-dihydro-4-methyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one (47)

10

- A. Using the procedures of Preparation 6A and 6B, and employing 2-chloro-N-(2-methoxy-4-methyl-3-pyridinyl)-3-pyridinecarboxamide, 35, and 1,4-diaminobutane as starting materials, there is obtained the compound 47.
- 15 B. Using the above procedure, but employing in place of 1,4-diaminobutane, different diamino compounds, there are obtained the corresponding compounds 8 in which X is NH₂.

Preparation 10

5-(5-carboxypentyl)-11-cyclopropyl-5,11-dihydro-4-methyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one (50)

20

- A. Using a procedure similar to that described in J. Med. Chem., 1995, 38, 4830, 11-cyclopropyl-5,11-dihydro-4-methyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one, 2, (Nevirapine) (5 mmol) is dissolved in DMSO (10 mL) and KOBu^t (6 mmol) is added. After 5 minutes, methyl 6-bromohexanoate (48) (10 mmol) is added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is poured into water. The aqueous solution is extracted with CH₂Cl₂. The extract is washed with water, then dried and evaporated. The residue is chromatographed to afford 5-(5-carbomethoxypentyl)-11-cyclopropyl-5,11-dihydro-4-methyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one, 49.

25

30



B. The above compound (1 mmol) is dissolved in THF (5 mL) and a solution of LiOH, H₂O (1.5 mmol) in water (5 mL) is added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is poured into dilute HCl. The aqueous solution is extracted with CH₂Cl₂. The extract is washed with water, then
5 dried and evaporated. The residue is chromatographed to afford the compound 50.

C. Using the above procedure, but different methyl bromoalkanoates for methyl 6-bromohexanoate, there are obtained the corresponding compounds 10 in which X is COOH.

10

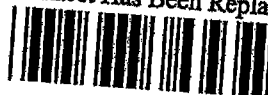
Preparation 11

11-Cyclopropyl-5,11-dihydro-4-(p-toluenesulfonylmethyl)-6H-dipyrido
[3,2-b:2',3'-e][1,4]diazepin-6-one (57) and
4-(4'-Aminophenoxy)-11-cyclopropyl-5,11-dihydro
15 -6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one (59)

A. Using the procedures of Preparation 6A and 6B, 3-amino-4-benzyloxymethyl-2-methoxypyridine 51 and 2-chloro-3-carbonyl chloride, 52, prepared as described in J. Med. Chem., 1997, 40, 2674, or J. Chem. Eng. Data,
20 1976, 21, 246, are reacted together to form the amide 53, which is then cyclized via a two step protocol to afford 4-benzyloxymethyl-11-cyclopropyl-5,11-dihydro-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one, 55.

B. 2 mmol of 55 is dissolved in DMF (15 mL) and Pd/C (0.1 mmol) is added.
25 The mixture is hydrogenated at room temperature. The progress of the reaction is monitored by tlc. When it is complete, the mixture is filtered, diluted with water, and extracted with EtOAc. The extract is dried and evaporated, and the residue is chromatographed to afford 11-cyclopropyl-5,11-dihydro-4-hydroxymethyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one, 56.

30



C. The steps of Preparation 20C (below) are follow using the above compound 56 to synthesize compound 57.

D. 57 (2 mmol) is dissolved in acetonitrile (20 ml). To the solution, 4-(dimethylethoxycarbonyl)-aminophenol (2 mmol) and K_2CO_3 (2 mmol) are added. After 24 hours, the mixture is partitioned between water and ether. The ether phase is separated, evaporated, and the residue is purified by chromatography to give 58.

E. The above compound 58 (1 mmol) is dissolved in TFA. After 30 minutes, the mixture is concentrated. The residue is partitioned between 15% Na_2CO_3 and ether. The ether phase is separated, evaporated and the residue is purified by chromatography to give 59.

Preparation 12

5'-bromo-3'-thia-2',3'-dideoxycytidine, 60.

Using the procedure of Preparation 1C, 3'-thia-2',3'-dideoxycytidine (3TC), 3, is converted into the compound 60.

Preparation 13

5'-bromo-3'-thia-2',3'-dideoxy-5-fluorocytidine, 61.

Using the procedure of Preparation 1C, 3'-thia-2',3'-dideoxy-5-fluorocytidine (FTC), 4, is converted into the compound 61.

Preparation 14

5'-carboxymethyl-3'-thia-2',3'-dideoxycytidine, 63.

A. 3'-Thia-2',3'-dideoxycytidine, 3 (5 mmol) is dissolved in DMF (30 mL) and methyl bromoacetate (5 mmol), K_2CO_3 (0.5g) and KI (50 mg) are added. The mixture is heated to 50° , and the progress of the reaction is monitored by tlc. When it is complete, the mixture is poured into water. The aqueous solution is extracted



with CH_2Cl_2 . The extract is washed with water, then dried and evaporated. The residue is chromatographed to afford 5'-(carbomethoxymethyl)-3'-thia-2',3'-dideoxycytidine **62**.

- 5 B. Using the procedure of Preparation 10B, the above ester **62** is converted into the carboxylic acid **63**.

Preparation 15
5'-carboxymethyl-3'-thia-2',3'-dideoxy-5-fluorocytidine, **65**.

10

Using the procedures of Preparation 14, 3'-thia-2',3'-dideoxy-5-fluorocytidine (FTC), **4**, is converted, via the ester **64**, into the carboxylic acid **65**.

Preparation 16
5'-tert-butyldiphenylsilyloxy-3'-thia-2',3'-dideoxy-4-(3-aminopropyl)cytidine (**69**)

15

- A. 3-Thia-2,3-dideoxyuridine **66**, prepared as described in US Patent 5,700,937 (5 mmol) is dissolved in DMF (100 mL) and imidazole (10 mmol) and tert-butyldiphenylsilyl chloride (6 mmol) are added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is poured into water. The aqueous solution is extracted with CH_2Cl_2 . The extract is washed with water, then dried and evaporated. The residue is chromatographed to afford 5'-tert-butyldiphenylsilyloxy-3'-thia-2',3'-dideoxyuridine, **67**.
- 20
- 25 B The above compound **67** (1 mmol) is dissolved in CH_2Cl_2 (10 mL) and pyridine (2 mL). The solution is cooled to 0° and trifluoromethanesulfonic anhydride (1 mmol) is added. The mixture is left for 1 hour, and then the solvents are removed under vacuum to afford the triflate ester **68**.
- 30 C. The above compound **68** is dissolved in dry THF (5 mL) and the solution is added, with vigorous stirring, to 1,3-diaminopropane (2 mL). After 24 hours, the



mixture was added to water and extracted with CH_2Cl_2 ; the extract was washed with water, then dried and evaporated. The residue was chromatographed to afford the compound 69.

- 5 D. Using the above procedure, but employing in Step C different diamines in place of 1,3-diaminopropane, there are obtained the corresponding compounds 14 in which X is NH and Y is NH_2 .

Preparation 17

10 **4-(3-aminopropyl)-5'-tert-butyldiphenylsilyloxy-3'-thia-2',3'-dideoxy-5-fluorocytidine (72)**

- A. Using a procedure similar to that described in US Patent 5,700,937, 2-(tert-butyldiphenylsilyloxy)methyl-5-acetoxy-1,3-oxathiolane, 70, prepared as described in
15 the aforementioned patent, (2 mmol) is dissolved in CH_2Cl_2 (50 mL) and a premixed solution of silylated 5-fluorouracil, prepared as described in US Patent 5,700,937 (2 mmol) and 1M SnCl_4 in CH_2Cl_2 (4 mL, 4 mmol) is added over 30 minutes. After 6 hours, pyridine (3 mL) is added, and the solvents are removed under vacuum. The residue is dissolved in ethanol, and is then evaporated to low volume. The residue is
20 chromatographed to afford 5'-tert-butyldiphenylsilyloxy-3'-thia-2',3'-dideoxy-5-fluorouridine, 71.

- B. Using the procedures of Preparation 16B and 16C, and employing 1,2-diaminoethane in place of 1,3-diaminopropane in the second step, the compound 71
25 is converted into the compound 72.

Preparation 18

Conversion of 5'-tert-butyldiphenylsilyloxy-3'-thia-2',3'-dideoxyuridine (67) into the iminochloride (73)

30

The compound 67 (5 mmol) is dissolved in CH_2Cl_2 (50 mL) and thionyl chloride (50 mmol) and DMF (0.1 mL) are added. The solution is heated under



reflux for 2 hour, then is cooled. The volatile components are removed under vacuum to afford the compound 73.

Preparation 19

5 **Conversion of 5'-tert-butylidiphenylsilyloxy-3'-thia-2',3'-dideoxy-5-fluorouridine (71) into the iminochloride (75)**

Using the procedure of Preparation 18, the compound 71 is converted into the compound 75.

10

Preparation 20

Coupling reactions of the chloroimine (73) with substituted acetylenes to afford the p-toluenesulfonate (77) and the carboxylic acid (79)

15 A. Using a procedure similar to that described in J. Het. Chem., 1994, 31, 989, (Ph₃P)₂PdCl₂ (0.3g), and CuI (75mg) are added to dry THF (100 mL) under an inert atmosphere. Et₃N (3 mL) and 5-trimethylsilyloxypent-1-yne (20 mmol) are then added. After 10 minutes, a solution of 73 (10 mmol) in THF (25 mL) is added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is
20 diluted with CH₂Cl₂ (100 mL). The solution is washed with dilute HCl, then dried and evaporated. The residue is chromatographed to afford the coupled product 76.

25 B. Using the above procedure, but employing methyl hex-5-ynoate in place of 5-trimethylsilyloxypent-1-yne, there is obtained the coupled compound 78.

C. Using the procedure of Preparation 7B, the compound 76 is converted into the p-toluenesulfonate ester 77.

30 D. Using the procedure of Preparation 10B, the methyl ester 78 is converted into the corresponding acid 79.



E. Using the above procedure, but employing in Steps A and B different trimethylsilyloxy acetylenes or methyl alkynoates, in place of 5-trimethylsilyloxypent-1-yne and methyl hex-5-ynoate, there are obtained the corresponding compounds 15 in which Y is OTs or COOH.

5

Preparation 21
Coupling reactions of the chloroimine (75) with substituted acetylenes to afford the p-toluenesulfonate (80) and the carboxylic acid (81)

10 Using the procedures of Preparation 20, the 5-fluoro chloroimine 75 is converted into the compounds 80 and 81.

Preparation 22
Displacement reaction of the chloroimine (73) with 2-(4-hydroxyphenyl)ethylamine, to afford the phenol (82)

15

2-(4-Hydroxyphenyl)ethylamine (25 mmol) is dissolved in EtOH (50 mL) and the chloroimine 73 (5 mmol) is added. The mixture is heated at reflux while the progress of the reaction is monitored by tlc. When it is complete, the mixture is cooled and then added to water. The aqueous solution is extracted with CH₂Cl₂, and the extract is dried and evaporated. The residue is chromatographed to afford the compound 82.

Preparation 23
Displacement reaction of the 5-fluoro chloroimine (74) with hexane-1,6-diamine to afford the amine (83)

25

A. Using the procedure of Preparation 22, but employing hexane-1,6-diamine in place of 2-(4-hydroxyphenyl)ethylamine, there is obtained the compound 83.

30

B. Using the procedures of Preparations 22 and 23, but employing in place of hexane-1,6-diamine or 2-(4-hydroxyphenyl)ethylamine, different amino, hydroxy or



substituted-phenyl substituted alkylamines, there are obtained the corresponding compounds 14 in which X is NH.

Preparation 24

5 **5'-bromo-2',3'-didehydro-3'-deoxythymidine (84)**

Using the procedure of Preparation 1C, the compound 5 is converted into the compound 84.

10 **Preparation 25**

5'-carboxymethyl-2',3'-didehydro-3'-deoxythymidine (85)

Using the procedure of Preparation 14, the compound 5 is converted into the compound 85.

15

Preparation 26

**Displacement reactions on the chloroimine (87)
derived from D4T, to afford ether and thioether amines (88) and (89)**

20 A. Using the procedure of Preparation 16A, D4T (5) is converted into the 5'-tert-butyldiphenylsilyloxy-2',3'-didehydro-3'-deoxythymidine, 86.

B. Using the procedure of Preparation 18, the compound 86 is converted into the chloroimine compound 86.

25

C. Using the procedure of Preparation 22, but employing 2-mercaptoethylamine in place of 2-(4-hydroxyphenyl)ethylamine, there is obtained the thioether 88.

D. Using the above procedure, but employing different aminothiols in place of 2-mercaptoethylamine, there are obtained the corresponding compounds 14 in which X is S and Y is NH₂.

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- E. Sodium hydride (2 mmol) is added to a solution of 3-aminopropanol (2 mmol) in DMF (15 mL). When hydrogen evolution has stopped, a solution of the chlorimine **87** (1 mmol) in DMF (5 mL) is added. The progress of the reaction is monitored by tlc.
- 5 When it is complete, the mixture is added to water. The aqueous solution is extracted with CH_2Cl_2 , and the extract is dried and evaporated. The residue is chromatographed to afford the compound **89**.
- F. Using the above procedure, but employing different aminoalcohols in place of 3-aminopropanol, there are obtained the corresponding compounds **14** in which X is O and Y is NH_2 .
- 10

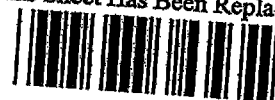
Preparation 27

- 15 Displacement reaction of (**90**), the chloromethyl derivative of D4T, to afford the hexyloxy compound (**92**) in which X is O and Link is $(\text{CH}_2)_5$.

- A. Sodium hydride (1 mmol) is added to a solution of methyl 6-hydroxyhexanoate **91** (1 mmol) in DMF (20 mL). When hydrogen evolution has ceased, a solution of the chloromethyl compound **90**, prepared as described in Antiviral Agents and Chemotherapy, 9, 205, (1 mmol) in DMF (5 mL) is added.
- 20 The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water. The aqueous solution is extracted with CH_2Cl_2 , and the extract is dried and evaporated. The residue is chromatographed to afford the methyl ester of the acid **92** in which X is O and Link is $(\text{CH}_2)_5$.

25

- B. Using the procedure of Preparation 10B, the product of A above is converted into the carboxylic acid **92** in which X is O and Link is $(\text{CH}_2)_5$.
- C. Using the above procedure, but employing different methyl hydroxyalkanoates in place of methyl 6-hydroxyhexanoate, there are obtained compounds **92**.
- 30



Example 1

**Alkylation of an amino derivative of 3TC with
a bromomethyl analog of Efavirenz, to afford an amine-linked dimer (94)**

- 5 A. 4-[2-(Bromomethyl)cycloprop-1-ylethynyl]-6-chloro-1,4-dihydro-4-
trifluoromethyl-2H-3,1-benzoxazin-2-one **20** (1 mmol) and the amine **69** (1 mmol)
are dissolved in DMF (15 mL) containing K_2CO_3 (250 mg) and KI (50 mg). The
progress of the reaction is monitored by tlc. When it is complete, the mixture is
added to water. The aqueous solution is extracted with CH_2Cl_2 , and the extract is
10 dried and evaporated. The residue is chromatographed to afford the silylated dimer
93.
- B. The above product **93** (1 mmol) is dissolved in THF (10 mL) and a solution
of Bu_4NF (2 mmol) in THF (2 mL) is added. The progress of the reaction is
15 monitored by tlc. When it is complete, the mixture is added to water. The aqueous
solution is extracted with CH_2Cl_2 , and the extract is dried and evaporated. The
residue is chromatographed to afford the compound **94**.
- C. Using the above procedure, different bromo-substituted compounds **6** are
20 reacted with different amino-substituted nucleosides **14**, in which Y is NH_2 , to afford
the corresponding dimeric compounds.

Example 2

25 **Alkylation of the chloroimine (75), derived from FTC with (23),
the amine derivative of Efavirenz to afford the amine-linked dimer (96)**

- A. Using the procedure of Preparation 22, equimolar quantities of 4-[2-(6-
amino-oct-1-ynyl)-6-chloro-1,4-dihydro-4-trifluoromethyl-2H-3,1-benzoxazin-2-one,
23 and the chloroimine **75** are reacted together to afford the compound **95**.
30
- B. Using the procedure of Example 1B, the silyl protecting group in compound
95 is removed to afford the compound **96**.



Example 3

Displacement reaction of the p-toluenesulfonate (77) related to 3TC with the thiol (26) derived from Efavirenz, to afford the thioether-linked dimer (98)

- 5 A. 6-Chloro-1,4-dihydro-4-[2-(5-mercapto-4-methylpent-1-ynyl)-4-trifluoromethyl-2H-3,1-benzoxazin-2-one **26** (1 mmol) is dissolved in DMSO (10 mL) and the p-toluenesulfonate **77** (1 mmol) and diisopropylethylamine (5 mmol) are added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water. The aqueous solution is extracted with CH_2Cl_2 , and the
- 10 extract is dried and evaporated. The residue is chromatographed to afford the silylated intermediate compound **97**.
- B. Using the procedure of Example 1B, the compound **97** is converted into the desilylated compound **98**.
- 15 C. Using the above procedure, different compounds **6** in which X is SH, are reacted with different compounds.

Example 4

20 **Alkylation of the FTC-related amine (72) with the Efavirenz related bromide (30), to afford the amine-linked dimer (100).**

- A. Using the procedure of Example 1A, the amine **72** and the bromo compound **30** are reacted together to afford the silylated dimer **99**.
- 25 B. Using the procedure of Example 1B, the compound **99** is converted into the desilylated dimeric compound **100**
- C. Using the above procedure, different compounds **7**, in which X is Br, are
- 30 reacted with different compounds **14**, in which Y is NH_2 , to afford the corresponding dimeric products.



Example 5

Coupling reaction of the Efavirenz amine ligand (34) with the 3TC derived carboxylic acid (63) to afford an amide-linked dimer (101)

- 5 A. 1-(4-Aminobutyl)-6-chloro-1,4-dihydro-4-(cyclopropylethynyl)-4-trifluoromethyl-2H-3,1-benzoxazin-2-one **34** (2 mmol) and the 3TC-derived carboxylic acid **63** (2 mmol) are dissolved in DMF (20 mL) containing dicyclohexylcarbodiimide (3 mmol)
- The progress of the reaction is monitored by tlc. When it is complete, the mixture is
- 10 added to water. The aqueous solution is extracted with CH_2Cl_2 , and the extract is dried and evaporated. The residue is chromatographed to afford the dimeric compound **101**.
- B. Using the above procedure, different compounds **7** in which X is NH_2 are
- 15 reacted with **63** to afford dimeric amides.

Example 6

Reaction of the phenol (82), derived from 3TC with (39), a bromo derivative of Nevirapine, to afford the phenoxy-linked dimer (103).

- 20 A. Using the procedure of Example 1A, equimolar amounts of 11-[2-(bromomethyl)cyclopropyl]-5,11-dihydro-4-methyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one **39** and the phenol **82** are reacted together to afford the silyl-protected dimeric compound **102**.
- 25 B. Using the procedure of Example 1B, the silyl protecting group is removed from **102** to afford the compound **103**.
- C. Using the above procedure, different compounds **8**, in which X is Br, are
- 30 reacted with different compounds **14**, in which Y is OH or NH_2 , to afford the corresponding dimeric products.



Example 7

Displacement reaction of the p-toluenesulfonate (41) derived from Nevirapine with the amine (89) derived from D4T, to afford the dimeric amine (104)

- 5 A. Using the procedure of Example 1A, followed by the desilylation procedure of Example 1B, equimolar quantities of the amine **89** and 5,11-dihydro-4-methyl-11-[4-(p-toluenesulfonyloxy)butyl]-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one **41** are reacted together to afford the dimeric compound **104**.
- 10 B. Using the above procedure, different compounds **8m** in which X is OTs or Br, are reacted with different compounds **14**, in which Y is NH₂, to afford the corresponding dimeric amines.

Example 8

- 15 **Alkylation of the FTC p-toluenesulfonate (80), with the Nevirapine thiol (44), to afford the thioether-linked dimer (105)**

- A. Using the coupling procedure of Example 3A, and the desilylation procedure of Example 1B, 5,11-dihydro-11-(5-mercaptopent-2-yl)-4-methyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one **44** is reacted with the p-toluenesulfonate **80** to afford the dimeric compound **105**.
- 20
- B. Using the above procedure, different compounds **8**, in which X is SH, are reacted with different compounds **15**, in which Y is OTs, to afford the corresponding
- 25 dimeric thioethers

Example 9

Coupling of the Nevirapine amine (47) with the 3TC carboxylic acid (79) to afford the amide-linked dimer (106)

30

- A. Using the coupling procedure of Example 5, and then the desilylation procedure of Example 1B, 11-(4-aminobutyl)-5,11-dihydro-4-methyl-6H-



dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one, **47** and the carboxylic acid **79** are reacted together to afford the dimeric amide compound **106**.

- 5 B. Using the above procedure, different compounds **8**, in which X is NH₂ are reacted with different compounds **15**, in which Y is COOH, to afford the corresponding dimeric amides.

Example 10

10 **Coupling of the Nevirapine carboxylic acid (50) with the d4T amine (89) to afford the amide-linked dimer (107)**

- 15 A. Using the coupling procedure of Example 5, and then the desilylation procedure of Example 1B, the amine **89** and 5-(5-carboxypentyl)-11-cyclopropyl-5,11-dihydro-4-methyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one **50** are reacted together to afford the dimeric amide compound **107**.

- 20 B. Using the above procedure, different compounds **10**, in which X is COOH, are reacted with different compounds **14**, in which X is NH₂, to afford the corresponding dimeric amides.

Example 11

Alkylation of the d4T amine (88) with the Nevirapine tosylate (57) to afford the amine-linked dimer 108.

- 25 A. Using the alkylation procedure of Example 1A, and the desilylation procedure of Example 1B, the amine **88** and **57** are reacted together to afford the dimeric compound **108**.
- 30 B. Using the above procedure, different compounds **9** in which X is Br are reacted with different compounds **14** in which Y is NH₂, to afford the corresponding dimeric amines.



Example 12

Alkylation reaction of the Nevirapine thiol (44) and the 3TC chloro compound (74) to afford the thioether-linked dimer (109)

5 A. 5,11-Dihydro-11-(5-mercaptopent-2-yl)-4-methyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one, **44** (2 mmol) is dissolved in DMF (10 mL) and 5'-bromo-3'-thia-2',3'-dideoxycytidine, **60** (1 mmol) and diisopropylethylamine (5 mmol) are added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water. The aqueous solution is extracted with CH₂Cl₂, and the
10 extract is dried and evaporated. The residue is chromatographed to afford the dimeric compound **109**.

B. Using the above procedure, different compounds **8** in which X is SH, are reacted with different compounds **11** to afford the corresponding thioether dimers.
15

Example 13

Alkylation of the 3TC chloroimine (74) with the Efavirenz amine (23) to afford the dimeric compound (110)

20 A. Using the procedure of Preparation 22, 6-chloro-4-[2-(6-amino-oct-1-ynyl)-1,4-dihydro-4-trifluoromethyl-2H-3,1-benzoxazin-2-one **23** is reacted with the chloroimine **74** to afford a dimeric product. Using the desilylation procedure of Example 1B, the product is converted into the dimeric compound **110**.

25 B. Using the above procedure, different compounds **6**, in which X is NH₂ are reacted with different compounds **12m** to afford the corresponding dimeric compounds.

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Example 14

Coupling reaction of (59) with (77) to afford the amine-linked dimer (111)

5 A. Using the procedure of Example 11, 59 is reacted with the 77 to afford the corresponding dimeric amine. Using the desilylation procedure of Example 1B, the product is converted into the dimeric compound 111.

10 B. Using the above procedure, different compounds 9 in which X is COOH, are reacted with different compounds 14 in which X is NH₂ to afford the corresponding dimeric amides.

Example 15

Coupling reaction of the Nevirapine amine (47) with the D4T carboxylic acid (92) to afford the amide-linked dimer (112)

15

A. Using the coupling procedure of Example 5, 11-(4-aminobutyl)-5,11-dihydro-4-methyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one, 47, is reacted with the D4T carboxylic acid 92 to afford the dimeric compound 112.

20 B. Using the above procedure, different compounds 8, in which X is NH₂ are reacted with different compounds 92 to afford the corresponding dimeric amides.

Example 16

Alkylation of the D4T chloroimine (85) with the Efavirenz amine (23) to afford the dimer (113)

25

A. Using the procedure of Preparation 22, 6-chloro-4-[2-(6-aminooct-1-ynyl)-1,4-dihydro-4-trifluoromethyl-2H-3,1-benzoxazin-2-one, 23, is reacted with the chloroimine 85 to afford a dimeric amine. Using the desilylation procedure of
30 Example 1B, the product is converted into the compound 113.



B. Using the above procedure, different compounds 6 in which X is NH₂ are reacted with different compounds 12 to afford the corresponding dimeric products.

Example 17

5 **Coupling reaction of the Efavirenz amine (34) with the D4T carboxylic acid (85) to afford the dimeric amide (114)**

A. Using the coupling procedure of Example 5, 1-(4-aminobutyl)-6-chloro-4-(cyclopropylethynyl)-1,4-dihydro-4-trifluoromethyl-2H-3,1-benzoxazin-2-one, 34 is
10 reacted with 5'-carboxymethyl-2',3'-didehydro-3'-deoxythymidine, 85 to afford the dimeric amide compound 114.

B. Using the above procedure, different compounds 7 in which X is NH₂ are reacted with different compounds 13 to afford the corresponding dimeric amide
15 products.

Example 18

Alkylation of the Nevirapine thiol (44) with the D4T bromo compound (84) to afford the thioether linked dimer (115)

20

A. Using the procedure of Example 3A, 5,11-dihydro-11-(5-mercaptopent-2-yl)-4-methyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one, 44 is reacted with 5'-bromo-2',3'-didehydro-3'-deoxythymidine 84 to afford the compound 115.

25 B. Using the above procedure, different compounds 8 in which X is SH or NH₂ are reacted with different compounds 11 to afford the corresponding thioether linked dimeric products.

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Example 19

**Alkylation of the Nevirapine thiol (44) with the 3TC bromo compound (60)
to afford the thioether-linked dimer (116)**

- 5 A. Using the procedure of Example 3A, 5,11-dihydro-11-(5-mercaptopent-2-yl)-4-methyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one, **44** is reacted with 5'-bromo-3'-thia-2',3'-dideoxycytidine, **60** to afford the dimeric compound **116**.

Example 20

- 10 **Alkylation of the FTC bromo compound (61) with the Efavirenz amine (334)
to afford the amine-linked dimer (117)**

- A. Using the procedure of Example 3A, 1-(4-aminobutyl)-6-chloro-4-(cyclopropylethynyl)-1,4-dihydro-4-trifluoromethyl-2H-3,1-benzoxazin-2-one, **34** is
15 reacted with 5'-bromo-3'-thia-2',3'-dideoxy-5-fluorocytidine, **61** to afford the compound **117**.

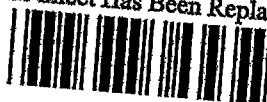
- B. Using the above procedure, different compounds **7** in which X is NH₂ are
reacted with different compounds **11** to afford the corresponding dimeric products.
20

Example 21

**Coupling reaction of the Efavirenz amine (34) with the FTC carboxylic acid (65)
to afford the amide-linked dimer (118)**

- 25 A. Using the coupling procedure of Example 5, 1-(4-aminobutyl)-6-chloro-4-(cyclopropylethynyl)-1,4-dihydro-4-trifluoromethyl-2H-3,1-benzoxazin-2-one, **34** is reacted with 5'-carboxymethyl-3'-thia-2',3'-dideoxy-5-fluorocytidine, **65** to afford the dimeric compound **118**.
- 30 B. Using the above procedure, different amines **7** are reacted with different carboxylic acids **13** to afford the corresponding dimeric amide products.

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Pharmaceutical Formulations

When employed as pharmaceuticals, the compounds of this invention are usually administered in the form of pharmaceutical compositions. These compounds can be administered by a variety of routes including oral, rectal, transdermal,
5 subcutaneous, intravenous, intramuscular, and intranasal. These compounds are effective as both injectable and oral compositions. Such compositions are prepared in a manner well known in the pharmaceutical art and comprise at least one active compound.

10 This invention also includes pharmaceutical compositions which contain, as the active ingredient, one or more of the compounds described herein associated with pharmaceutically acceptable carriers. In making the compositions of this invention, the active ingredient is usually mixed with an excipient, diluted by an excipient or enclosed within such a carrier which can be in the form of a capsule, sachet, paper
15 or other container. When the excipient serves as a diluent, it can be a solid, semi-solid, or liquid material, which acts as a vehicle, carrier or medium for the active ingredient. Thus, the compositions can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols (as a solid or in a liquid medium), ointments containing, for example, up to
20 10% by weight of the active compound, soft and hard gelatin capsules, suppositories, sterile injectable solutions, and sterile packaged powders.

In preparing a formulation, it may be necessary to mill the active compound to provide the appropriate particle size prior to combining with the other
25 ingredients. If the active compound is substantially insoluble, it ordinarily is milled to a particle size of less than 200 mesh. If the active compound is substantially water soluble, the particle size is normally adjusted by milling to provide a substantially uniform distribution in the formulation, e.g. about 40 mesh.

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Some examples of suitable excipients include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, sterile water, syrup, and methyl cellulose. The formulations can additionally
5 include: lubricating agents such as talc, magnesium stearate, and mineral oil; wetting agents; emulsifying and suspending agents; preserving agents such as methyl- and propylhydroxy-benzoates; sweetening agents; and flavoring agents. The compositions of the invention can be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the patient by
10 employing procedures known in the art.

The compositions are preferably formulated in a unit dosage form, each dosage containing from about 0.001 to about 1 g, more usually about 1 to about 30 mg, of the active ingredient. The term "unit dosage forms" refers to physically
15 discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical excipient. Preferably, the compound of formula I above is employed at no more than about 20 weight percent of the pharmaceutical composition, more preferably no
20 more than about 15 weight percent, with the balance being pharmaceutically inert carrier(s).

The active compound is effective over a wide dosage range and is generally administered in a pharmaceutically effective amount. It, will be understood,
25 however, that the amount of the compound actually administered will be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound administered and its relative activity, the age, weight, and response of the individual patient, the severity of the patient's symptoms, and the like.



For preparing solid compositions such as tablets, the principal active ingredient is mixed with a pharmaceutical excipient to form a solid preformulation composition containing a homogeneous mixture of a compound of the present invention. When referring to these preformulation compositions as homogeneous, it is meant that the active ingredient is dispersed evenly throughout the composition so that the composition may be readily subdivided into equally effective unit dosage forms such as tablets, pills and capsules. This solid preformulation is then subdivided into unit dosage forms of the type described above containing from, for example, 0.1 to about 500 mg of the active ingredient of the present invention.

10

The tablets or pills of the present invention may be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can comprise an inner dosage and an outer dosage component, the latter being in the form of an envelope over the former. The two components can be separated by an enteric layer which serves to resist disintegration in the stomach and permit the inner component to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials including a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol, and cellulose acetate.

20

The liquid forms in which the novel compositions of the present invention may be incorporated for administration orally or by injection include aqueous solutions, suitably flavored syrups, aqueous or oil suspensions, and flavored emulsions with edible oils such as corn oil, cottonseed oil, sesame oil, coconut oil, or peanut oil, as well as elixirs and similar pharmaceutical vehicles.

25

Compositions for inhalation or insufflation include solutions and suspensions in pharmaceutically acceptable, aqueous or organic solvents, or mixtures thereof, and powders. The liquid or solid compositions may contain suitable pharmaceutically acceptable excipients as described *supra*. Preferably the compositions are

30



administered by the oral or nasal respiratory route for local or systemic effect. Compositions in preferably pharmaceutically acceptable solvents may be nebulized by use of inert gases. Nebulized solutions may be inhaled directly from the nebulizing device or the nebulizing device may be attached to a face mask tent, or
5 intermittent positive pressure breathing machine. Solution, suspension, or powder compositions may be administered, preferably orally or nasally, from devices which deliver the formulation in an appropriate manner.

The following formulation examples illustrate representative pharmaceutical
10 compositions of the present invention.

Formulation Example 1

Hard gelatin capsules containing the following ingredients are prepared:

15	<u>Ingredient</u>	<u>Quantity</u> <u>(mg/capsule)</u>
	Active Ingredient	30.0
	Starch	305.0
20	Magnesium stearate	5.0

The above ingredients are mixed and filled into hard gelatin capsules in 340 mg quantities.

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Formulation Example 2

A tablet formula is prepared using the ingredients below:

	<u>Ingredient</u>	<u>Quantity</u> <u>(mg/tablet)</u>
5	Active Ingredient	25.0
	Cellulose, microcrystalline	200.0
	Colloidal silicon dioxide	10.0
10	Stearic acid	5.0

The components are blended and compressed to form tablets, each weighing 240 mg.

Formulation Example 3

15 A dry powder inhaler formulation is prepared containing the following components:

	<u>Ingredient</u>	<u>Weight %</u>
	Active Ingredient	5
20	Lactose	95

The active ingredient is mixed with the lactose and the mixture is added to a dry powder inhaling appliance.

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Formulation Example 4

Tablets, each containing 30 mg of active ingredient, are prepared as follows:

	<u>Ingredient</u>	<u>Quantity</u> <u>(mg/tablet)</u>
5	Active Ingredient	30.0 mg
	Starch	45.0 mg
	Microcrystalline cellulose	35.0 mg
	Polyvinylpyrrolidone	
10	(as 10% solution in sterile water)	4.0 mg
	Sodium carboxymethyl starch	4.5 mg
	Magnesium stearate	0.5 mg
	Talc	<u>1.0 mg</u>
15	Total	120 mg

The active ingredient, starch and cellulose are passed through a No. 20 mesh U.S. sieve and mixed thoroughly. The solution of polyvinylpyrrolidone is mixed with the resultant powders, which are then passed through a 16 mesh U.S. sieve.

20 The granules so produced are dried at 50° to 60°C and passed through a 16 mesh U.S. sieve. The sodium carboxymethyl starch, magnesium stearate, and talc, previously passed through a No. 30 mesh U.S. sieve, are then added to the granules which, after mixing, are compressed on a tablet machine to yield tablets each weighing 120 mg.

25

Formulation Example 5

Capsules, each containing 40 mg of medicament are made as follows:

	<u>Ingredient</u>	<u>Quantity</u> <u>(mg/capsule)</u>
30	Active Ingredient	40.0 mg
	Starch	109.0 mg
	Magnesium stearate	<u>1.0 mg</u>
	Total	150.0 mg

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The active ingredient, starch, and magnesium stearate are blended, passed through a No. 20 mesh U.S. sieve, and filled into hard gelatin capsules in 150 mg quantities.

5

Formulation Example 6

Suppositories, each containing 25 mg of active ingredient are made as follows:

	<u>Ingredient</u>	<u>Amount</u>
	Active Ingredient	25 mg
10	Saturated fatty acid glycerides to	2,000 mg

The active ingredient is passed through a No. 60 mesh U.S. sieve and suspended in the saturated fatty acid glycerides previously melted using the minimum heat necessary. The mixture is then poured into a suppository mold of nominal 2.0 g capacity and allowed to cool.

15

Formulation Example 7

Suspensions, each containing 50 mg of medicament per 5.0 mL dose are made as follows:

	<u>Ingredient</u>	<u>Amount</u>
20	Active Ingredient	50.0 mg
	Xanthan gum	4.0 mg
	Sodium carboxymethyl cellulose (11 %)	
	Microcrystalline cellulose (89 %)	50.0 mg
25	Sucrose	1.75 g
	Sodium benzoate	10.0 mg
	Flavor and Color	q.v.
	Purified water to	5.0 mL

The active ingredient, sucrose and xanthan gum are blended, passed through a No. 10 mesh U.S. sieve, and then mixed with a previously made solution of the microcrystalline cellulose and sodium carboxymethyl cellulose in water. The sodium benzoate, flavor, and color are diluted with some of the water and added with stirring. Sufficient water is then added to produce the required volume.

35



Formulation Example 8

A formulation may be prepared as follows:

5	<u>Ingredient</u>	<u>Quantity</u> <u>(mg/capsule)</u>
	Active Ingredient	15.0 mg
	Starch	407.0 mg
	Magnesium stearate	<u>3.0 mg</u>
10	Total	425.0 mg

The active ingredient, starch, and magnesium stearate are blended, passed through a No. 20 mesh U.S. sieve, and filled into hard gelatin capsules in 425.0 mg quantities.

15

Formulation Example 9

A formulation may be prepared as follows:

	<u>Ingredient</u>	<u>Quantity</u>
	Active Ingredient	5.0 mg
20	Corn Oil	1.0 mL

Formulation Example 10

A topical formulation may be prepared as follows:

25	<u>Ingredient</u>	<u>Quantity</u>
	Active Ingredient	1-10 g
	Emulsifying Wax	30 g
	Liquid Paraffin	20 g
	White Soft Paraffin	to 100 g

30

The white soft paraffin is heated until molten. The liquid paraffin and emulsifying wax are incorporated and stirred until dissolved. The active ingredient is added and stirring is continued until dispersed. The mixture is then cooled until solid.

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Another preferred formulation employed in the methods of the present invention employs transdermal delivery devices ("patches"). Such transdermal patches may be used to provide continuous or discontinuous infusion of the compounds of the present invention in controlled amounts. The construction and use of transdermal patches for the delivery of pharmaceutical agents is well known in the art. *See, e.g.*, U.S. Patent 5,023,252, issued June 11, 1991, herein incorporated by reference in its entirety. Such patches may be constructed for continuous, pulsatile, or on demand delivery of pharmaceutical agents.

Other suitable formulations for use in the present invention can be found in *Remington's Pharmaceutical Sciences*, Mace Publishing Company, Philadelphia, PA, 17th ed. (1985).

Utility

The multibinding compounds of this invention inhibit the enzyme HIV reverse transcriptase, an enzyme which catalyzes the conversion of viral RNA to single stranded DNA. Accordingly, the multibinding compounds and pharmaceutical compositions of this invention are useful to inhibit the mechanism of HIV reverse transcriptase *in vivo*, which in turn inhibits the replication of HIV in a patient.

The multibinding compounds of this invention possess reverse transcriptase inhibitory activity, in particular, HIV reverse transcriptase inhibitory efficacy. The compounds of formula I possess HIV reverse transcriptase inhibitory activity and are therefore useful as antiviral agents for the treatment of HIV infection and associated diseases. The multibinding compounds of formula I possess HIV reverse transcriptase inhibitory activity and are effective as inhibitors of HIV growth. The ability of the multibinding compounds of the present invention to inhibit viral growth or infectivity is demonstrated in a standard assay of viral growth or infectivity, for example, using the assay described below.



The multibinding compounds of formula I of the present invention are also useful for the inhibition of HIV in an ex vivo sample containing HIV or expected to be exposed to HIV. Thus, the multibinding compounds may be used to inhibit HIV present in a body fluid sample (for example, a serum or semen sample) which
5 contains or is suspected to contain or be exposed to HIV.

The multibinding compounds are also useful as standard or reference compounds for use in tests or assays for determining the ability of an agent to inhibit viral clone replication and/or HIV reverse transcriptase, for example in a
10 pharmaceutical research program. Thus, the multibinding compounds may be used as a control or reference compound in such assays and as a quality control standard. The multibinding compounds may be provided in a commercial kit or container for use as such standard or reference compound.

15 Since the multibinding compounds exhibit specificity for HIV reverse transcriptase, they may also be useful as diagnostic reagents in diagnostic assays for the detection of HIV reverse transcriptase. Thus, inhibition of the reverse transcriptase activity in an assay (such as the assays described herein) by a multibinding compound would be indicative of the presence of HIV reverse
20 transcriptase and HIV.

When used to treat HIV infection, the multibinding compounds of this invention are typically administered to a patient in need of treatment for HIV infection in a pharmaceutical composition comprising a pharmaceutically acceptable
25 diluent and an effective amount of at least one compound of this invention. The amount of compound administered to the patient will vary depending upon which compound and/or composition is being administered, the purpose of the administration, such as prophylaxis or therapy, the state of the patient, the manner of administration, and the like. In therapeutic applications, compositions are
30 administered to a patient already suffering from HIV infection, for example, in an



amount sufficient to at least partially reduce the rate of HIV replication. Amounts effective for this use will depend on the judgment of the attending clinician depending upon factors such as the degree or severity of the HIV infection in the patient, the age, weight and general condition of the patient, and the like. The
5 pharmaceutical compositions of this invention may contain more than one compound of the present invention.

As noted above, the compounds administered to a patient are in the form of pharmaceutical compositions described above which can be administered by a variety
10 of routes including oral, rectal, transdermal, subcutaneous, intravenous, intramuscular, etc.. These compounds are effective as both injectable and oral deliverable pharmaceutical compositions. Such compositions are prepared in a manner well known in the pharmaceutical art and comprise at least one active compound.

15 The multibinding compounds of this invention can also be administered in the form of pro-drugs, i.e., as derivatives which are converted into a biologically active compound *in vivo*. Such pro-drugs will typically include compounds in which, for example, a carboxylic acid group, a hydroxyl group or a thiol group is converted to
20 a biologically liable group, such as an ester, lactone or thioester group which will hydrolyze *in vivo* to reinstate the respective group.

The following synthetic and biological examples are offered to illustrate this invention and are not to be construed in any way as limiting the scope of this
25 invention. Unless otherwise stated, all temperatures are in degrees Celsius.

BIOLOGICAL EXAMPLES

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Example A
HIV RNA Assay

DNA Plasmids and *in vitro* RNA transcripts

- 5 Plasmid pDAB 72 containing both gag and pol sequences of BH10 (bp
113-1816) cloned into PTZ 19R is prepared according to Erickson-Viitanen et al.
AIDS Research and Human Retroviruses (1989) 5:577. The plasmid is linearized
with Bam HI prior to the generation of *in vitro* RNA transcripts using the Riboprobe
Gemini system II kit (Promega) with T7 RNA polymerase. Synthesized RNA is
10 purified by treatment with RNase free DNase (Promega), phenol-chloroform
extraction, and ethanol precipitation. RNA transcripts are dissolved in water, and
stored at -70 C°. The concentration of RNA is determined from the A₂₆₀.

Probes

- 15 Biotinylated capture probes are purified by HPLC after synthesis on an
Applied Biosystems (Foster City, Calif.) DNA synthesizer by addition of biotin to
the 5' terminal end of the oligonucleotide, using the biotin-phosphoramidite reagent
of Cocuzza, Tet. Lett. (1989) 30:6287. The gag biotinylated capture probe
(5-biotin-CTAGCTCCCTGCTTGCCCATACTA 3') is complementary to nucleotides
20 889-912 of HXB2 and the pol biotinylated capture probe (5'-biotin
-CCCTATCATTTTTGGTTTCCAT 3') is complementary to nucleotides 2374-2395
of HXB2. Alkaline phosphatase conjugated oligonucleotides used as reporter probes
are prepared by Syngene (San Diego, Calif.). The pol reporter probe
(5'CTGTCTTACTTTGATAAAACCTC 3') is complementary to nucleotides
25 2403-2425 of HXB2. The gag reporter probe
(5'CCCAGTATTTGTCTACAGCCTTCT 3') is complementary to nucleotides
950-973 of HXB2. All nucleotide positions are those of the GenBank Genetic
Sequence Data Bank as accessed through the Genetics Computer Group Sequence
Analysis Software Package (Devereau Nucleic Acids Research (1984) 12:387). The
30 reporter probes are prepared as 0.5 µM stocks in 2X SSC (0.3M NaCl, 0.03M



sodium citrate), 0.05M Tris pH 8.8, 1 mg/mL BSA. The biotinylated capture probes are prepared as 100 μ M stocks in water.

Streptavidin coated plates

- 5 Streptavidin coated plates are obtained from Du Pont Biotechnology Systems (Boston, Mass.).

Cells and virus stocks

- MT-2 and MT-4 cells are maintained in RPMI 1640 supplemented with 5% fetal calf serum (FCS) for MT-2 cells or 10% FCS for MT-4 cells, 2 mM L-glutamine and 50 μ g/mL gentamycin, all from Gibco. HIV-1 (RF) is propagated in MT-4 cells in the same medium. Virus stocks were prepared approximately 10 days after acute infection of MT-4 cells and stored as aliquots at -70 C°. Infectious titers of HIV-1(RF) stocks are 1-3 X 10⁷ PFU (plaque forming units)/mL as measured by plaque assay on MT-2 cells (see below). Each aliquot of virus stock used for infection is thawed only once.

- For evaluation of antiviral efficacy, cells to be infected are subcultured one day prior to infection. On the day of infection, cells are resuspended at 5 X 10⁵ cells/mL in RPMI 1640, 5% FCS for bulk infections or at 2 X 10⁶ /mL in Dulbecco's modified Eagles medium with 5% FCS for infection in microtiter plates. Virus is added and culture continued for 3 days at 37 C°.

HIV RNA assay

- 25 Cell lysates or purified RNA in 3M or 5M GED are mixed with 5M GED and capture probe to a final guanidinium isothiocyanate concentration of 3M and a final biotin oligonucleotide concentration of 30 nM. Hybridization is carried out in sealed U bottom 96 well tissue culture plates (Nunc or Costar) for 16-20 hours at 37 C°. RNA hybridization reactions are diluted three-fold with deionized water to a final guanidinium isothiocyanate concentration of 1M and aliquots (150 μ L) are



transferred to streptavidin coated microtiter plates wells. Binding of capture probe and capture probe-RNA hybrid to the immobilized streptavidin is allowed to proceed for 2 hours at room temperature, after which the plates are washed 6 times with DuPont ELISA plate wash buffer (phosphate buffered saline(PBS), 0.05% Tween
5 20). A second hybridization of reporter probe to the immobilized complex of capture probe and hybridized target RNA is carried out in the washed streptavidin coated well by addition of 120 μ l of a hybridization cocktail containing 4X SSC, 0.66% TritonX100, 6.66% deionized formamide, 1 mg/mL BSA and 5 nM reporter probe. After hybridization for one hour at 37 C°, the plate is again washed 6 times.
10 Immobilized alkaline phosphatase activity is detected by addition of 100 μ L of 0.2 mM 4-methylumbelliferyl phosphate (MUBP, JBL Scientific) in buffer δ (2.5M diethanolamine pH 8.9 (JBL Scientific), 10 mM MgCl₂, 5 mM zinc acetate dihydrate and 5 mM N-hydroxyethyl-ethylene-diamine-triacetic acid). The plates are incubated at 37 C°. Fluorescence at 450 nM is measured using a microplate fluorometer
15 (Dynateck) exciting at 365 nM.

Microplate based compound evaluation in HIV-1 infected MT-2 cells

Test compounds to be evaluated are dissolved in DMSO and diluted in culture medium to twice the highest concentration to be tested and a maximum DMSO
20 concentration of 2%. Further three-fold serial dilutions of the compound in culture medium are performed directly in U bottom microtiter plates (Nunc). After compound dilution, MT-2 cells (50 μ L) are added to a final concentration of 5X10⁵ per mL (1X10⁵ per well). Cells are incubated with test compounds for 30 minutes at 37 C°. in a CO₂ incubator. For evaluation of antiviral potency, an appropriate
25 dilution of HIV-1 (RF) virus stock (50 μ L) is added to culture wells containing cells and dilutions of the test compounds. The final volume in each well is 200 μ L. Eight wells per plate are left uninfected with 50 μ L of medium added in place of virus, while eight wells are infected in the absence of any antiviral compound. For evaluation of compound toxicity, parallel plates are cultured without virus infection.



After 3 days of culture at 37 C° in a humidified chamber inside a CO₂ incubator, all but 25 µL of medium/well is removed from the HIV infected plates. Thirty seven µL of 5M GED containing biotinylated capture probe is added to the settled cells and remaining medium in each well to a final concentration of 3M GED and 30 nM capture probe. Hybridization of the capture probe to HIV RNA in the cell lysate is carried out in the same microplate well used for virus culture by sealing the plate with a plate sealer (Costar), and incubating for 16-20 hrs in a 37 C° incubator. Distilled water is then added to each well to dilute the hybridization reaction three-fold and 150 µL of this diluted mixture is transferred to a streptavidin coated microtiter plate. HIV RNA is quantitated as described above. A standard curve, prepared by adding known amounts of pDAB 72 in vitro RNA transcript to wells containing lysed uninfected cells, is run on each microtiter plate in order to determine the amount of viral RNA made during the infection.

In order to standardize the virus inoculum used in the evaluation of test compounds for antiviral activity, dilutions of virus are selected which result in an IC₉₀ value (concentration of compound required to reduce the HIV RNA level by 90%) for dideoxycytidine (ddC) of 0.2 µg/mL. IC₉₀ values of other antiviral compounds, both more and less potent than ddC, are reproducible using several stocks of HIV-1 (RF) when this procedure is followed. This concentration of virus corresponds to about 3X10⁵ PFU (measured by plaque assay on MT-2 cells) per assay well and typically produce approximately 75% of the maximum viral RNA level achievable at any virus inoculum. For the HIV RNA assay, IC₉₀ values are determined from the percent reduction of net signal (signal from infected cell samples minus signal from uninfected cell samples) in the RNA assay relative to the net signal from infected, untreated cells on the same culture plate (average of eight wells). Valid performance of individual infection and RNA assay tests is judged according to three criteria. It is required that the virus infection should result in an RNA assay signal equal to or greater than the signal generated from 2 ng of pDAB 72 in vitro RNA transcript. The IC₉₀ for ddC, determined in each assay run, should



be between 0.1 and 0.3 $\mu\text{g/mL}$. Finally, the plateau level of viral RNA produced by an effective reverse transcriptase inhibitor should be less than 10% of the level achieved in an uninhibited infection. A test compound is considered active if its IC_{90} is found to be less than 20 μM .

5

For antiviral potency tests, all manipulations in microtiter plates, following the initial addition of 2X concentrated compound solution to a single row of wells, are performed using a Perkin Elmer/Cetus ProPette.

10 HIV-1 RT Assay Materials and Methods

This assay measures HIV-1 RT RNA dependent DNA polymerase activity by the incorporation of ^3H dTMP onto the template primer Poly (rA) oligo (dT)12-18. The template primer containing the incorporated radioactivity is separated from unincorporated label by one of two methods:

15 Method 1. The template primer is precipitated with TCA, collected on glass fiber filters and counted for radioactivity with a scintillation counter.

Method 2. The template primer is captured on an diethyl amino ethyl (DEAE) ion exchange membrane which is then counted for radioactivity after washing off the free nucleotide.

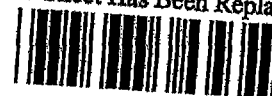
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Materials and Reagents

The template primer Poly (rA) oligo (dT)12-18 and dTTP are purchased from Pharmacia Biotech. The template primer and nucleotide are dissolved in diethyl pyrocarbonate water to a concentration of 1 mg/ml and 5.8 mM respectively. The substrates are aliquoted (template primer at 20 μl /aliquot, dTTP at 9 μl /aliquot) and frozen at -20°C . The ^3H dTTP (2.5 mCi/ml in 10 mM Tricine at pH 7.6; specific activity of 90-120 Ci/mmol) and the recombinant HIV-1 Reverse Transcriptase (HxB2 background; 100 U/10 μl in 100 mM potassium phosphate at pH 7.1, 1 mM dithiothreitol and 50% glycerol) are purchased from DuPont NEN. 1 Unit of enzyme is defined by DuPont NEN as the amount required to incorporate 1 nmol of labelled

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dTTP into acid-insoluble material in 10 minutes at 37 C°. The 3H dTTP is aliquoted at 23.2 µl/microfuge tube (58 µCi) and frozen at -20 C°. The HIV-1 Reverse Transcriptase (RT) was diluted 10 fold with RT buffer (80 mM KCl, 50 mM Tris HCl, 12 mM MgCl₂, 1 mM DTT, 50 µM EGTA, 5 mg/ml BSA, 0.01% Triton-X 100, pH 8.2) and aliquoted at 10 µl/microfuge tube (10 Units/10 µl). One aliquot (enough for 8 assays) is diluted further to 10 Units/100 µl and aliquoted into 8 tubes (1.25 Units/12.5 µl). All aliquots were frozen at -70 C°.

The Millipore Multiscreen DE 96 well filter plates, multiscreen plate adaptors, and microplate press-on adhesive sealing film are purchased from Millipore. The filter plate containing 0.65 µm pore size diethyl amino ethyl cellulose (DEAE) paper disks is pretreated with 0.3M ammonium formate and 10 mM sodium pyrophosphate (2 X 200 µl /well) at pH 8.0 prior to use. A Skatron 96 well cell harvester and glass fiber filter mats are purchased from Skatron Instruments. Microscint 20 scintillation cocktail is purchased from Packard. Beckman Ready Flow III scintillation cocktail is purchased from Beckman.

HIV-1 RT Assay

The enzyme and substrate mixture are freshly prepared from the above stock solutions. 1.25 Units of enzyme is diluted with RT buffer (containing 5 mg/ml BSA) to a concentration of 0.05 Units/10 µl or 0.7 nM. Final enzyme and BSA concentrations in the assay are 0.01 Units or 0.14 nM and 1 mg/ml respectively. The inhibitor and substrate mixture are diluted with RT buffer containing no BSA. All inhibitors are dissolved in dimethyl sulfoxide (DMSO) at a stock concentration of 3 mM and stored at -20 C° after use. A Biomek robot is used to dilute the inhibitors in a 96 well plate. Inhibitors are initially diluted 96 fold from stock and then serially diluted two times (10 fold/dilution) from 31.25 µM to 3125 nM and 312.5 nM. Depending on the potency of the inhibitor, one of the three dilutions is further diluted. Typically the highest concentration (31.25 µM) is serially diluted three times at 5 fold/dilution to 6.25, 1.25, and 0.25 µM. Final inhibitor concentrations in the



assay were 12.5, 2.5, 0.5, and 0.1 μ M. For potent inhibitors of HIV-1 RT, the final inhibitor concentrations used are 0.1 or 0.01 that stated above. The substrate mixture contains 6.25 μ g/ml of Poly (rA) oligo (dT)12-18 and 12.5 μ M of dTTP (58 μ Ci 3H dTTP). The final substrate concentrations are 2.5 μ g/ml and 5 μ M respectively.

5

Using the Beckman Instruments Biomek robot, 10 μ l of HIV-1 RT is combined with 20 μ l of inhibitor in a 96 well U bottom plate. The enzyme and inhibitor are preincubated at ambient temperature for 6 minutes. 20 μ l of the substrate mixture is added to each well to initiate the reaction (total volume is 50 μ l). The reactions are incubated at 37 C° and terminated after 45 minutes.

10

For Method 1, 200 μ l of an ice-cold solution of 13% tritrichloroacetic acid (TCA) and 10 mM sodium pyrophosphate is added to each of the 96 wells. The 96 well plate is then placed in an ice-water bath for 30 minutes. Using A Skatron 96 well cell harvester, the acid precipitable material is collected on a glass fiber filter mat that had been presoaked in 13% TCA and 10 mM sodium pyrophosphate. The filter disks are washed 3 times (2.0 ml/wash) with 1N HCl and 10 mM sodium pyrophosphate. The filter disks are punched out into scintillation vials, 2.0 ml of Beckman Ready Flow III scintillant is added, and the vials are counted for radioactivity for 1 minute.

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For Method 2, the assay is terminated with the addition of 175 μ l/well of 50 mM EDTA at pH 8.0. Then 180 μ l of the mixture is transferred to a pretreated Millipore DE 96 well filter plate. Vacuum is applied to the filter plate to aspirate away the liquid and immobilize the template primer on the DEAE filter disks. Each well is washed 3 times with 200 μ l of 0.3M ammonium formate and 10 mM sodium pyrophosphate at pH 8.0. 50 μ l of microscint 20 scintillation cocktail is added to each well and the plate is counted for radioactivity on a Packard Topcount at 1 minute/well.

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The IC_{50} values are calculated with the equation:

$$IC_{50} = [Inh] / (1 / \text{fractional activity} - 1)$$

- 5 where the fractional activity = RT activity (dpms) in the presence of inhibitor / RT activity (dpms) in the absence of inhibitor. For a given inhibitor, the IC_{50} values are calculated for the inhibitor concentrations that range between 0.1-0.8 fractional activity. The IC_{50} values in this range (generally 2 values) are averaged. A compound is considered active if its IC_{50} is found to be less than 12 μM .

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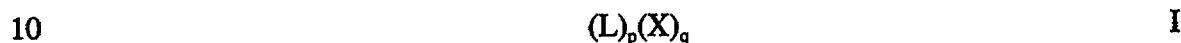




WHAT IS CLAIMED IS:

1. A multibinding compound comprising from 2 to 10 ligands covalently attached to one or more linkers wherein each of said ligands independently comprises at least one nucleoside reverse transcriptase inhibitor and at least one non-nucleoside reverse transcriptase inhibitor; and pharmaceutically-acceptable salts thereof.

2. A multibinding compound of formula I:



wherein each L is independently a ligand comprising at least one nucleoside reverse transcriptase inhibitor and at least one non-nucleoside reverse transcriptase inhibitor; each X is independently a linker; p is an integer of from 2 to 10; and q is an integer of from 1 to 20; and pharmaceutically-acceptable salts thereof.

3. The multibinding compound of Claim 2 wherein q is less than p .

4. The multibinding compound of Claim 3 wherein $p=2$ and $q=1$.

20 5. The multibinding compound of Claim 3 wherein each nucleoside reverse transcriptase ligand is independently selected from zidovudine, didanosine, zalcitabine, stavudine, lamivudine, abacavir, adefovir, raluridine, oral PMPA prodrug, azidouridine, IVX-E-59, emitricitabine and lodenosine, and each non-nucleoside reverse transcriptase ligand is independently selected from nevirapine, delavirdine, efavirenz, MKC-442, loviride, S-1153, talviraline, calanolide A and

25 tivirapine.

30 6. The multibinding compound of Claim 5 wherein the nucleoside reverse transcriptase ligand is emitricitabine.

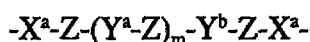




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7. The multibinding compound of Claim 5 wherein the non-nucleoside reverse transcriptase ligand is efavirenz.

8. The multibinding compound of Claim 5 wherein each linker
5 independently has the formula:



wherein

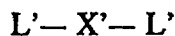
m is an integer of from 0 to 20;

10 X^a at each separate occurrence is selected from the group consisting of -O-, -S-, -NR-, -C(O)-, -C(O)O-, -C(O)NR-, -C(S)-, -C(S)O-, -C(S)NR- or a covalent bond where R is as defined below;

Z is at each separate occurrence is selected from the group consisting of alkylene, substituted alkylene, cycloalkylene, substituted cycloalkylene, alkenylene,
15 substituted alkenylene, alkynylene, substituted alkynylene, cycloalkenylene, substituted cycloalkenylene, arylene, heteroarylene, heterocyclene, or a covalent bond;

Y^a and Y^b at each separate occurrence are selected from the group consisting of -C(O)NR'-, -NR'C(O)-, -NR'C(O)NR'-, -C(=NR')-NR'-,
20 -NR'-C(=NR')-, -NR'-C(O)-O-, -N=C(X^a)-NR'-, -P(O)(OR')-O-, -S(O)_nCR'R"-, -S(O)_n-NR'-, -S-S- and a covalent bond; where n is 0, 1 or 2; and R, R' and R'' at each separate occurrence are selected from the group consisting of hydrogen, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, cycloalkenyl, substituted cycloalkenyl, alkynyl, substituted
25 alkynyl, aryl, heteroaryl and heterocyclic.

9. A multibinding compound of formula II:



II

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wherein each L' is independently a ligand comprising at least one nucleoside reverse transcriptase inhibitor and at least one non-nucleoside reverse transcriptase inhibitor and X' is a linker; and pharmaceutically-acceptable salts thereof.

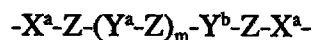
- 5 10. The multibinding compound of Claim 9 wherein each nucleoside reverse transcriptase ligand is independently selected from zidovudine, didanosine, zalcitabine, stavudine, lamivudine, abacavir, adefovir, raluridine, oral PMPA prodrug, azidouridine, IVX-E-59, emitricitabine and lodenosine, and each non-nucleoside reverse transcriptase ligand is independently selected from nevirapine,
10 delavirdine, efavirenz, MKC-442, loviride, S-1153, talviraline, calanolide A and tivrapiine.

11. The multibinding compound of Claim 10 wherein the nucleoside reverse transcriptase ligand is emitricitabine.

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12. The multibinding compound of Claim 10 wherein the non-nucleoside reverse transcriptase ligand is efavirenz.

13. The multibinding compound of Claim 9 wherein X' has the formula:
20



wherein

m is an integer of from 0 to 20;

- X^a* at each separate occurrence is selected from the group consisting of
25 -O-, -S-, -NR-, -C(O)-, -C(O)O-, -C(O)NR-, -C(S), -C(S)O-, -C(S)NR- or a covalent bond where R is as defined below;

Z is at each separate occurrence is selected from the group consisting of alkylene, substituted alkylene, cycloalkylene, substituted cycloalkylene, alkenylene, substituted alkenylene, alkynylene, substituted alkynylene, cycloalkenylene,





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substituted cycloalkenylene, arylene, heteroarylene, heterocyclene, or a covalent bond;

- Y^a and Y^b at each separate occurrence are selected from the group consisting of -C(O)NR'-, -NR'C(O)-, -NR'C(O)NR'-, -C(=NR')-NR'-, -NR'-C(=NR')-, -NR'-C(O)-O-, -N=C(X^a)-NR'-, -P(O)(OR')-O-, -S(O)_nCR'R"-, -S(O)_n-NR'-, -S-S- and a covalent bond; where *n* is 0, 1 or 2; and R, R' and R'' at each separate occurrence are selected from the group consisting of hydrogen, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, cycloalkenyl, substituted cycloalkenyl, alkynyl, substituted alkynyl, aryl, heteroaryl and heterocyclic.

14. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and an effective amount of a multibinding compound comprising from 2 to 10 ligands covalently attached to one or more linkers wherein each of said ligands independently comprises at least one nucleoside reverse transcriptase inhibitor and at least one non-nucleoside reverse transcriptase inhibitor; and pharmaceutically-acceptable salts thereof.

15. A pharmaceutical compositions comprising a pharmaceutically acceptable carrier and an effective amount of a multibinding compound of formula I:



- wherein each L is independently at least one nucleoside reverse transcriptase inhibitor and at least one non-nucleoside reverse transcriptase inhibitor; each X is independently a linker; *p* is an integer of from 2 to 10; and *q* is an integer of from 1 to 20; and pharmaceutically-acceptable salts thereof.

16. The pharmaceutical composition of Claim 15 wherein *q* is less than *p*.

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17. The pharmaceutical composition of Claim 15 wherein $p=2$ and $q=1$.

18. The pharmaceutical composition of Claim 15 wherein each nucleoside reverse transcriptase ligand is independently selected from zidovudine, didanosine, zalcitabine, stavudine, lamivudine, abacavir, adefovir, raluridine, oral PMPA prodrug, azidouridine, IVX-E-59, emitricitabine and lodenosine, and each non-nucleoside reverse transcriptase ligand is independently selected from nevirapine, delavirdine, efavirenz, MKC-442, loviride, S-1153, talviraline, calanolide A and tivrapiine.

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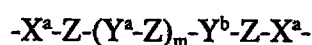
19. The pharmaceutical composition of Claim 18 wherein the nucleoside reverse transcriptase ligand is emitricitabine.

20. The pharmaceutical composition of Claim 18 wherein the non-nucleoside reverse transcriptase ligand is efavirenz.

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21. The pharmaceutical composition of Claim 15 wherein each linker independently has the formula:

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wherein

m is an integer of from 0 to 20;

X^a at each separate occurrence is selected from the group consisting of -O-, -S-, -NR-, -C(O)-, -C(O)O-, -C(O)NR-, -C(S), -C(S)O-, -C(S)NR- or a covalent bond where R is as defined below;

25

Z is at each separate occurrence is selected from the group consisting of alkylene, substituted alkylene, cycloalkylene, substituted cycloalkylene, alkenylene, substituted alkenylene, alkynylene, substituted alkynylene, cycloalkenylene, substituted cycloalkenylene, arylene, heteroarylene, heterocyclene, or a covalent

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bond;

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Y^a and Y^b at each separate occurrence are selected from the group consisting of
-C(O)NR'-, -NR'C(O)-, -NR'C(O)NR'-, -C(=NR')-NR'-,
-NR'-C(=NR')-, -NR'-C(O)-O-, -N=C(X^a)-NR'-, -P(O)(OR')-O-,
-S(O)_nCR'R"-, -S(O)_n-NR'-, -S-S- and a covalent bond; where *n* is 0, 1 or 2; and
5 R, R' and R'' at each separate occurrence are selected from the group consisting of
hydrogen, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl,
substituted alkenyl, cycloalkenyl, substituted cycloalkenyl, alkynyl, substituted
alkynyl, aryl, heteroaryl and heterocyclic.

10 22. A pharmaceutical composition comprising a pharmaceutically
acceptable carrier and an effective amount of a multibinding compound of formula II:



15 wherein each L' is independently a ligand comprising at least one nucleoside
reverse transcriptase inhibitor and at least one non-nucleoside reverse transcriptase
inhibitor and X' is a linker; and pharmaceutically-acceptable salts thereof.

20 23. The pharmaceutical composition of Claim 22 wherein each nucleoside
reverse transcriptase ligand is independently selected from zidovudine, didanosine,
zalcitabine, stavudine, lamivudine, abacavir, adefovir, raluridine, oral PMPA
prodrug, azidouridine, IVX-E-59, emitricitabine and lodenosine, and each non-
nucleoside reverse transcriptase ligand is independently selected from nevirapine,
delavirdine, efavirenz, MKC-442, loviride, S-1153, talviraline, calanolide A and
25 tivrapiine.

24. The pharmaceutical composition of Claim 22 wherein X' has the
formula:





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wherein

m is an integer of from 0 to 20;

X^a at each separate occurrence is selected from the group consisting of -O-, -S-, -NR-, -C(O)-, -C(O)O-, -C(O)NR-, -C(S), -C(S)O-, -C(S)NR- or a covalent bond where R is as defined below;

Z is at each separate occurrence is selected from the group consisting of alkylene, substituted alkylene, cycloalkylene, substituted cycloalkylene, alkenylene, substituted alkenylene, alkynylene, substituted alkynylene, cycloalkenylene, substituted cycloalkenylene, arylene, heteroarylene, heterocyclene, or a covalent bond;

Y^a and Y^b at each separate occurrence are selected from the group consisting of -C(O)NR'-, -NR'C(O)-, -NR'C(O)NR'-, -C(=NR')-NR'-, -NR'-C(=NR')-, -NR'-C(O)-O-, -N=C(X^a)-NR'-, -P(O)(OR')-O-, -S(O)_nCR'R"-, -S(O)_n-NR'-, -S-S- and a covalent bond; where n is 0, 1 or 2; and R, R' and R" at each separate occurrence are selected from the group consisting of hydrogen, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, cycloalkenyl, substituted cycloalkenyl, alkynyl, substituted alkynyl, aryl, heteroaryl and heterocyclic.

25. A method for treating HIV infection, the method comprising administering to a patient having HIV infection a pharmaceutical composition comprising a pharmaceutically-acceptable carrier and a therapeutically-effective amount of a multibinding compound comprising from 2 to 10 ligands covalently attached to one or more linkers wherein each of said ligands independently comprises at least one nucleoside reverse transcriptase inhibitor and at least one non-nucleoside reverse transcriptase inhibitor; and pharmaceutically-acceptable salts thereof.

26. A method for identifying multimeric ligand compounds possessing multibinding properties to HIV reverse transcriptase which method comprises:

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- (a) identifying a ligand or a mixture of ligands wherein each ligand contains at least one reactive functionality;
- (b) identifying a library of linkers wherein each linker in said library comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand;
- (c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the ligand or mixture of ligands identified in (a) with the library of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands; and
- (d) assaying the multimeric ligand compounds produced in the library prepared in (c) above to identify multimeric ligand compounds possessing multibinding properties.
27. A method for identifying multimeric ligand compounds possessing multibinding properties to HIV reverse transcriptase which method comprises:
- (a) identifying a library of ligands wherein each ligand contains at least one reactive functionality;
- (b) identifying a linker or mixture of linkers wherein each linker comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand;
- (c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the library of ligands identified in (a) with the linker or mixture of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands; and
- (d) assaying the multimeric ligand compounds produced in the library prepared in (c) above to identify multimeric ligand compounds possessing multibinding properties.





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28. The method according to Claim 26 or 27 wherein the preparation of the multimeric ligand compound library is achieved by either the sequential or concurrent combination of the two or more stoichiometric equivalents of the ligands identified in (a) with the linkers identified in (b).

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29. The method according to Claim 28 wherein the multimeric ligand compounds comprising the multimeric ligand compound library are dimeric.

30. The method according to Claim 29 wherein the dimeric ligand
10 compounds comprising the dimeric ligand compound library are heterodimeric.

31. The method according to Claim 30 wherein the heterodimeric ligand compound library is prepared by sequential addition of a first and second ligand.

15 32. The method according to Claim 26 or 27 wherein, prior to procedure (d), each member of the multimeric ligand compound library is isolated from the library.

20 33. The method according to Claim 32 wherein each member of the library is isolated by preparative liquid chromatography mass spectrometry (LCMS).

25 34. The method according to Claim 26 or Claim 27 wherein the linker or linkers employed are selected from the group comprising flexible linkers, rigid linkers, hydrophobic linkers, hydrophilic linkers, linkers of different geometry, acidic linkers, basic linkers, linkers of different polarization and/or polarizability and amphiphilic linkers.

30 35. The method according to Claim 34 wherein the linkers comprise linkers of different chain length and/or having different complementary reactive groups.

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36. The method according to Claim 35 wherein the linkers are selected to have different linker lengths ranging from about 2 to 100Å.

37. The method according to Claim 26 or 27 wherein the ligand or mixture
5 of ligands is selected to have reactive functionality at different sites on said ligands.

38. The method according to Claim 37 wherein said reactive functionality is selected from the group consisting of carboxylic acids, carboxylic acid halides, carboxyl esters, amines, halides, pseudohalides, isocyanates, vinyl unsaturation,
10 ketones, aldehydes, thiols, alcohols, anhydrides, boronates, phosphates, phosphonates and precursors thereof wherein the reactive functionality on the ligand is selected to be complementary to at least one of the reactive groups on the linker so that a covalent linkage can be formed between the linker and the ligand.

15 39. The method according to Claim 26 or Claim 27 wherein the multimeric ligand compound library comprises heteromeric ligand compounds.

40. A library of multimeric ligand compounds which may possess multivalent properties to HIV reverse transcriptase which library is prepared by the
20 method comprising:

(a) identifying a ligand or a mixture of ligands wherein each ligand contains at least one reactive functionality;

(b) identifying a library of linkers wherein each linker in said library comprises at least two functional groups having complementary reactivity to at least
25 one of the reactive functional groups of the ligand; and

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the ligand or mixture of ligands identified in (a) with the library of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said
30 linker and at least two of said ligands.

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41. A library of multimeric ligand compounds which may possess multivalent properties to HIV reverse transcriptase which library is prepared by the method comprising:

5 (a) identifying a library of ligands wherein each ligand contains at least one reactive functionality;

(b) identifying a linker or mixture of linkers wherein each linker comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand; and

10 (c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the library of ligands identified in (a) with the linker or mixture of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands.

15 42. The library according to Claim 40 or Claim 41 wherein the linker or linkers employed are selected from the group comprising flexible linkers, rigid linkers, hydrophobic linkers, hydrophilic linkers, linkers of different geometry, acidic linkers, basic linkers, linkers of different polarization and/or polarizability and amphiphilic linkers.

20

43. The library according to Claim 42 wherein the linkers comprise linkers of different chain length and/or having different complementary reactive groups.

25 44. The library according to Claim 43 wherein the linkers are selected to have different linker lengths ranging from about 2 to 100Å.

45. The library according to Claim 40 or 41 wherein the ligand or mixture of ligands is selected to have reactive functionality at different sites on said ligands.





46. The library according to Claim 45 wherein said reactive functionality is selected from the group consisting of carboxylic acids, carboxylic acid halides, carboxyl esters, amines, halides, pseudohalides, isocyanates, vinyl unsaturation, ketones, aldehydes, thiols, alcohols, anhydrides, boronates, phosphates, phosphonates and precursors thereof wherein the reactive functionality on the ligand is selected to be complementary to at least one of the reactive groups on the linker so that a covalent linkage can be formed between the linker and the ligand.

47. The library according to Claim 40 or Claim 41 wherein the multimeric ligand compound library comprises heteromeric ligand compounds.

48. An iterative method for identifying multimeric ligand compounds possessing multibinding properties to HIV reverse transcriptase which method comprises:

(a) preparing a first collection or iteration of multimeric compounds which is prepared by contacting at least two stoichiometric equivalents of the ligand or mixture of ligands which target an enzyme with a linker or mixture of linkers wherein said ligand or mixture of ligands comprises at least one reactive functionality and said linker or mixture of linkers comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand wherein said contacting is conducted under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands;

(b) assaying said first collection or iteration of multimeric compounds to assess which if any of said multimeric compounds possess multibinding properties;

(c) repeating the process of (a) and (b) above until at least one multimeric compound is found to possess multibinding properties;

(d) evaluating what molecular constraints imparted or are consistent with imparting multibinding properties to the multimeric compound or compounds found in the first iteration recited in (a)- (c) above;





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- (e) creating a second collection or iteration of multimeric compounds which elaborates upon the particular molecular constraints imparting multibinding properties to the multimeric compound or compounds found in said first iteration;
- (f) evaluating what molecular constraints imparted or are consistent with
- 5 imparting enhanced multibinding properties to the multimeric compound or compounds found in the second collection or iteration recited in (e) above;
- (g) optionally repeating steps (e) and (f) to further elaborate upon said molecular constraints.

10 49. The method according to Claim 48 wherein steps (e) and (f) are repeated from 2-50 times.

50. The method according to Claim 49 wherein steps (e) and (f) are repeated from 5-50 times.

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