

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
6 November 2008 (06.11.2008)

PCT

(10) International Publication Number  
WO 2008/133966 A1

(51) International Patent Classification:  
A61K 47/48 (2006.01)

(21) International Application Number:  
PCT/US2008/005322

(22) International Filing Date: 25 April 2008 (25.04.2008)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
60/914,532 27 April 2007 (27.04.2007) US

(71) Applicant (for all designated States except US):  
CHIMERIX, INC. [US/US]; 2505 Meridian Park-  
way, Suite 340, Durham, North Carolina 27713 (US).

(72) Inventor; and

(75) Inventor/Applicant (for US only): PAINTER, George R.  
[US/US]; Chapel Hill, North Carolina (US).

(74) Agent: MYERS BIGEL SIBLEY & SAJOVEC, P.A.;  
P.O. Box 37428, Raleigh, North Carolina 27612 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:  
— with international search report

(54) Title: METHODS OF REDUCING NEPHROTOXICITY IN SUBJECTS ADMINISTERED WITH NUCLEOSIDE

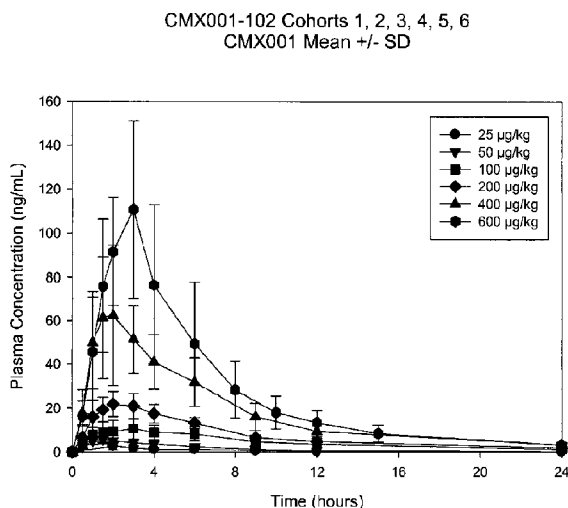
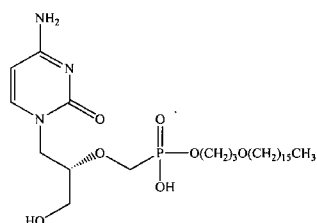


Figure 1. Plasma concentration curves of CMX001 following a single dose administration of CMX001

(57) Abstract: A conjugate compound comprising an acyclic nucleoside phosphonate covalently coupled to a lipid for the therapeutic and/or prophylactic treatment of viral infection in an immunodeficient subject is described, along with compositions and methods of using the same. A preferred conjugate compound is CMX001, having formula (I) or a pharmaceutically acceptable salt thereof.



WO 2008/133966 A1

## METHODS OF REDUCING NEPHROTOXICITY IN SUBJECTS ADMINISTERED NUCLEOSIDE PHOSPHONATES

George R. Painter

### Related Applications

This application claims the benefit of United States provisional patent application Serial No. 60/914,532, filed April 27, 2007, the disclosure of which is incorporated by reference herein in its entirety.

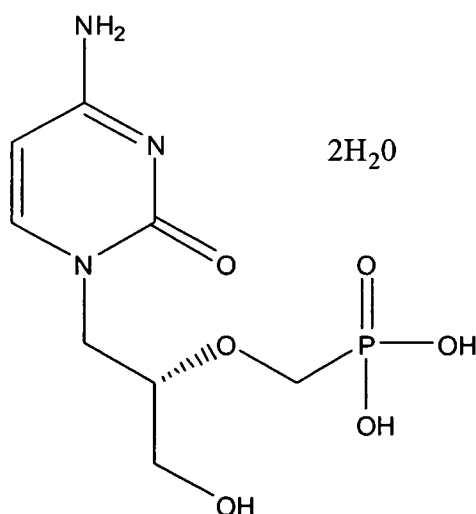
### Field of the Invention

The present invention concerns methods of treatment with nucleoside phosphonates, compositions useful in such methods, and the use of such compounds.

5

### Background of the Invention

Cidofovir (VISTIDE®) is a nucleoside analog approved by the US FDA for the treatment of CMV retinitis in patients with AIDS. It is active against all dsDNA viruses that cause human disease. Cidofovir has the structure:



10 Cidofovir requires intravenous infusion and is dose-limited by its nephrotoxicity. Cases of acute renal failure resulting in dialysis and/or contributing to death have occurred with as few as one or two doses of VISTIDE® Cidofovir. *See, e.g.,* Gilead Letter, Important Drug Warning (Sept. 1996) (available from the US FDA). Hence, prehydration with normal saline

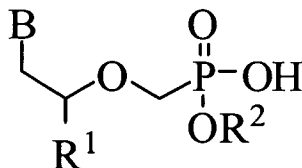
and probenecid co-administration are required with Cidofovir therapy. *See, e.g., S. Lacy, Toxicological Sciences* **44**, 97-106 (1998).

US Patent Nos. 6,716,825; 7,034,014; 7,094,772; and 7,098,197, to Hostetler et al. describe lipid conjugates of phosphonate compounds, including cidofovir, for the treatment  
5 of disease.

### Summary of the Invention

The present invention provides a conjugate compound comprising an acyclic nucleoside phosphonate covalently coupled to a lipid for the therapeutic and/or prophylactic  
10 treatment of viral infection in an immunodeficient subject.

Preferably the conjugate compound comprises a phosphonate of an antiviral compound of the formula:



or an enantiomer, diastereomer, racemate, stereoisomer, tautomer, rotamer or a mixture  
15 thereof, wherein:

R<sup>1</sup> is hydrogen, —CH<sub>3</sub>, —CH<sub>2</sub>OH, —CH<sub>2</sub>F, —CH=CH<sub>2</sub>, or —CH<sub>2</sub>N<sub>3</sub>;

R<sup>2</sup> is hydrogen; and

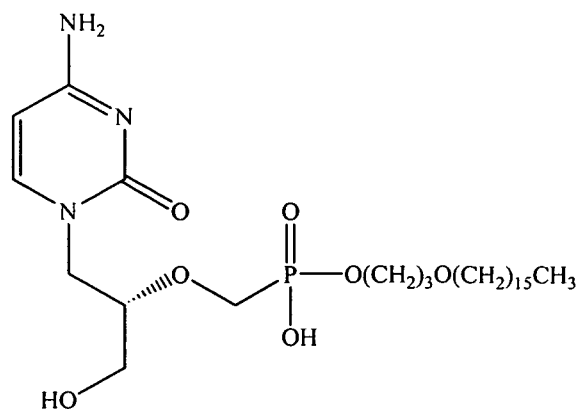
B is a purine or pyrimidine;

covalently linked to an alkylglycerol, alkylpropanediol, 1-S-alkylthioglycerol,  
20 alkoxyalkanol, alkylethanol, hexadecylpropanediol or octadecylpropanediol;  
or a pharmaceutically acceptable salt thereof.

In one embodiment of the invention, the conjugate compound is in the form of an enantiomer, diastereoisomer, racemate or a mixture thereof.

Preferably said acyclic nucleoside phosphonate is selected from the group consisting  
25 of cidofovir, cyclic cidofovir, tenofovir, and adefovir.

In a preferred embodiment of the invention, said conjugate compound is:



or a pharmaceutically acceptable salt thereof.

5 A conjugate compound of the present invention may be used for the therapeutic and/or prophylactic treatment of viral infection in an immunodeficient subject wherein the immunodeficient subject has primary or acquired immunodeficiency.

In one embodiment of the invention, the immunodeficient subject has acquired immunodeficiency as a result of immunosuppressive therapy. Cyclosporine for example is an immunosuppressant drug widely used in post-allogeneic organ transplant to reduce the activity of the patient's immune system and so the risk of organ rejection. In one embodiment of the invention therefore, the immunodeficient subject is a transplant patient. The immunodeficient subject may be a renal transplant patient, a hepatic transplant patient or a bone marrow transplant patient. In an alternative embodiment of the invention, the subject is suffering from chronic fatigue syndrome.

15 In one embodiment of the invention, the viral infection to be treated is resistant to treatment with an unconjugated acyclic nucleoside phosphonate, e.g., cidofovir, cyclic cidofovir, tenofovir, and adefovir, *etc.* Alternatively or additionally, an unconjugated acyclic nucleoside phosphonate exhibits toxic side effects in said immunodeficient subject.

20 Preferably the immunodeficient subject is infected with at least one dsDNA virus. The dsDNA virus may be selected from any of the groups consisting of: human immunodeficiency virus (HIV), influenza, herpes simplex virus (HSV), human herpes virus 6 (HHV-6), cytomegalovirus (CMV), hepatitis B and C virus, Epstein-Barr virus (EBV), varicella zoster virus, variola major and minor, vaccinia, smallpox, cowpox, camelpox, monkeypox, ebola virus, papilloma virus, adenovirus or polyoma viruses including John  
25 Cunningham virus (JCV), BK virus and Simian vacuolating virus 40 or Simian virus 40 (SV40).

In one embodiment of the invention, the immunodeficient subject is infected with a virus or any combination of viruses selected from the groups consisting of: HCMV, BK virus, HHV-6, Adenovirus and EBV.

5 In another embodiment of the invention, the immunodeficient subject is infected with two or more viruses, at least one of which is preferably a dsDNA virus, and the viruses exhibit synergistic action. Preferably the viruses are HCMV and BK.

10 Preferably the conjugate compound is used to treat a dsDNA viral infection in an immunodeficient subject wherein said subject is resistant to valganciclovir hydrochloride (or ganciclovir) or wherein said subject exhibits side effects to valganciclovir hydrochloride (or ganciclovir). Alternatively or additionally, the conjugate is used to treat cytomegalovirus (CMV) subsequent to treatment with (val) ganciclovir, preferably wherein the CMV infection is emergent. The patient may be a bone marrow stem cell transplant patient, especially where there is a risk (real or perceived) for bone marrow toxicity from ganciclovir in the patient.

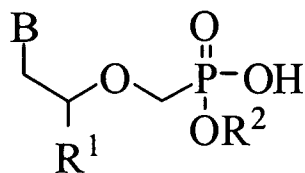
15 In a preferred embodiment of the invention, the immunodeficient subject is a human subject.

20 Preferably the conjugate compound of the invention is administered orally, preferably at a dosage of less than 5 mg/Kg, more preferably at a dosage of less than 1 mg/Kg. More preferably said conjugate compound is administered to said subject at a dosage of 10 or 20, up to 200 or 300 or up to 5000 ug/Kg. The lipid conjugates of the invention can be administered daily, every other day, once a week or once every 2 weeks.

25 The present invention also provides for the use of a conjugate compound comprising an acyclic nucleoside phosphonate covalently coupled to a lipid in the manufacture of a medicament for the therapeutic and/or prophylactic treatment of viral infection in an immunodeficient subject.

In another aspect the invention provides a method for the therapeutic and/or prophylactic treatment of viral infection in an immunodeficient subject, the method comprising administering a conjugate compound to the subject, said conjugate compound comprising an acyclic nucleoside phosphonate covalently coupled to a lipid.

30 Preferably said conjugate compound comprises a phosphonate of an antiviral compound of the formula:



or an enantiomer, diastereomer, racemate, stereoisomer, tautomer, rotamer or a mixture thereof, wherein:

R<sup>1</sup> is hydrogen, —CH<sub>3</sub>, —CH<sub>2</sub>OH, —CH<sub>2</sub>F, —CH=CH<sub>2</sub>, or —CH<sub>2</sub>N<sub>3</sub>;

5 R<sup>2</sup> is hydrogen; and

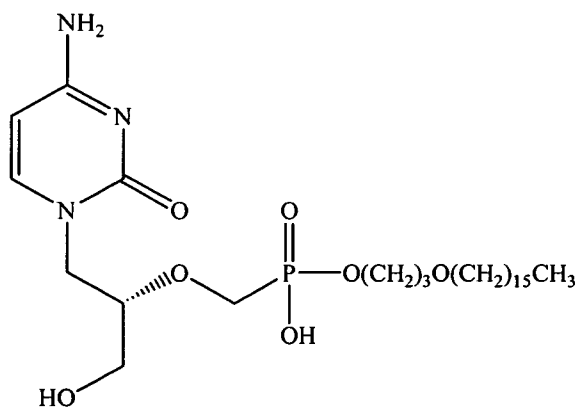
B is a purine or pyrimidine;

covalently linked to an alkylglycerol, alkylpropanediol, 1-S-alkylthioglycerol, alkoxyalkanol, alkylethanol, hexadecylpropanediol or octadecylpropanediol;

or a pharmaceutically acceptable salt thereof.

10 Preferably said compound is in the form of an enantiomer, diastereoisomer, racemate or a mixture thereof. More preferably said acyclic nucleoside phosphonate is selected from the group consisting of cidofovir, cyclic cidofovir, tenofovir, and adefovir.

In a preferred embodiment, the conjugate compound is:



15

or a pharmaceutically acceptable salt thereof.

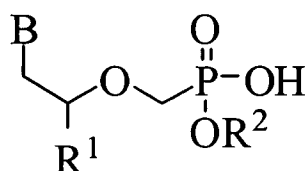
### **Brief Description of the Drawings**

20 **Figure 1** shows plasma concentration curves of CMX001 following a single dose administration; and

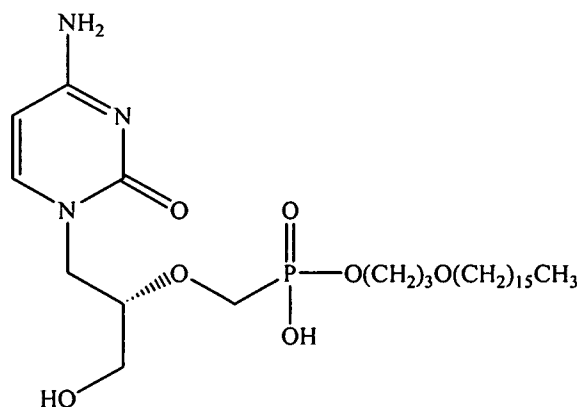
**Figure 2**, shows plasma concentration curves of Cidofovir following a single dose of CMX001.

### Detailed Description of the Invention

As noted above, the present invention provides, among other things, a method of treating a subject (*e.g.*, a human subject) with an acyclic nucleoside phosphonate, which acyclic nucleoside phosphonate (also sometimes referred to as an acyclic phosphonate nucleoside herein) induces nephrotoxicity in said subject, the improvement comprising: administering (*e.g.*, oral administering) said acyclic nucleoside phosphonate as a conjugate compound so that said nephrotoxicity is reduced, said conjugate compound comprising said acyclic nucleoside phosphonate covalently coupled to a lipid. In some embodiments, the conjugate compound selected from the group consisting of a phosphonate of an antiviral compound of the formula:



or an enantiomer, diastereomer, racemate, stereoisomer, tautomer, rotamer or a mixture thereof, wherein: R<sup>1</sup> is hydrogen, —CH<sub>3</sub>, —CH<sub>2</sub>OH, —CH<sub>2</sub>F, —CH=CH<sub>2</sub>, or —CH<sub>2</sub>N<sub>3</sub>; R<sup>2</sup> is hydrogen; and B is a purine or pyrimidine covalently linked to an alkylglycerol, alkylpropanediol, 1-S-alkylthioglycerol, alkoxyalkanol or alkylethanediol; or a pharmaceutically acceptable salt thereof. In some embodiments, the covalent link is to alkylglycerol, alkylpropanediol, 1-S-alkylthioglycerol, alkoxyalkanol, alkylethanediol, octadecylpropanediol, alkylglycerol, hexadecylpropanediol, or octadecylpropanediol. In some embodiments, the compound is:



or a pharmaceutically acceptable salt thereof. In some embodiments, the acyclic nucleoside phosphonate is selected from the group consisting of cidofovir, cyclic cidofovir, tenofovir, and adefovir. In some embodiments, the conjugate compound is administered to said subject at a dosage of less than 1 mg/Kg; in some embodiments the conjugate compound is administered to said subject at a dosage of 10 or 20 up to 200 or 300 µg/Kg. Also provided is the use of an acyclic nucleoside phosphonate or lipid conjugate thereof as described above for the preparation of a medicament for reducing nephrotoxicity in a subject being treated with an acyclic nucleoside phosphonate according to a method as described above.

In some embodiments, the present invention is particularly useful in treating subjects afflicted with at least two different dsDNA which synergistically activate one another (*e.g.*, CMV and HIV virus in combination, CMV and BK virus in combination; etc.) *See, e.g.*, LT Feldman et al., PNAS, Aug 15, 1982, 4952-4956; B. Bielora et al., Bone Marrow Transplant, 2001 Sep; 28(6): 613-4.

The present invention is explained in greater detail below.

15

#### **A. Definitions.**

"Alkyl" as used herein refers to a monovalent straight or branched chain or cyclic radical of from one to twenty-four carbon atoms, including methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, tert-butyl, n-hexyl, and the like.

"Substituted alkyl" as used herein comprises alkyl groups further bearing one or more substituents selected from hydroxy, alkoxy (of a lower alkyl group), mercapto (of a lower alkyl group), cycloalkyl, substituted cycloalkyl, heterocyclic, substituted heterocyclic, aryl, substituted aryl, heteroaryl, substituted heteroaryl, aryloxy, substituted aryloxy, halogen, trifluoromethyl, cyano, nitro, nitrone, amino, amido, -C(O)H, acyl, oxyacyl, carboxyl, carbamate, sulfonyl, sulfonamide, sulfonyl, and the like.

"Alkenyl" as used herein refers to straight or branched chain hydrocarbyl groups having one or more carbon-carbon double bonds, and having in the range of about 2 up to 24 carbon atoms, and "substituted alkenyl" refers to alkenyl groups further bearing one or more substituents as set forth above.

"Aryl" as used herein refers to aromatic groups having in the range of 6 up to 14 carbon atoms and "substituted aryl" refers to aryl groups further bearing one or more substituents as set forth above.

"Heteroaryl" as used herein refers to aromatic groups containing one or more heteroatoms (e.g., N, O, S, or the like) as part of the ring structure, and having in the range of 3 up to 14 carbon atoms and "substituted heteroaryl" refers to heteroaryl groups further bearing one or more substituents as set forth above.

5 "Bond" or "valence bond" as used herein refers to a linkage between atoms consisting of an electron pair.

"Pharmaceutically acceptable salts" as used herein refers to both acid and base addition salts.

10 "Prodrug" as used herein refers to derivatives of pharmaceutically active compounds that have chemically or metabolically cleavable groups and become the pharmaceutically active compound by solvolysis or under in vivo physiological conditions.

"Parenteral" as used herein refers to subcutaneous, intravenous, intra-arterial, intramuscular or intravitreal injection, or infusion techniques.

15 "Topically" as used herein encompasses administration rectally and by inhalation spray, as well as the more common routes of the skin and mucous membranes of the mouth and nose and in toothpaste.

20 "Effective amount" as used herein as applied to the phosphonate prodrugs of the invention is an amount that will prevent or reverse the disorders noted above. Particularly with respect to disorders associated with bone metabolism, an effective amount is an amount that will prevent, attenuate, or reverse abnormal or excessive bone resorption or the bone resorption that occurs in the aged, particularly post-menopausal females or prevent or oppose bone metastasis and visceral metastasis in breast cancer.

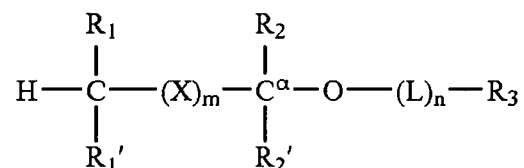
25 "Immunodeficiency" (or "immune deficiency") as used herein refers to a state in which the ability of the immune system to fight infectious disease is compromised or entirely absent. A person who has an immunodeficiency of any kind is said to be immunocompromised. An immunocompromised person may be particularly vulnerable to opportunistic infections, in addition to normal infections.

30 "Treatment" as used herein includes any procedure with a purpose to prevent, pre-empt, treat or cure a disease. Prophylactic treatment may include either *primary* prophylaxis (to prevent the development of a disease) and/or *secondary* prophylaxis (whereby the disease has already developed and the patient is protected against worsening of this process).

## **B. Compounds.**

Compounds, compositions, formulations, and methods of treating subjects that can be used to carry out the present invention include but are not limited to those described in US Patent No. 6,716,825; 7,034,014; 7,094,772; and 7,098,197, the disclosures of which are incorporated by reference herein in their entirety.

5 In some embodiments the phosphonate compounds of the invention have the structure:



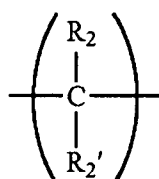
wherein:

10  $\text{R}_1$  and  $\text{R}_1'$  are independently  $-\text{H}$ , optionally substituted  $-\text{O}(\text{C}_1-\text{C}_{24})\text{alkyl}$ ,  $-\text{O}(\text{C}_1-\text{C}_{24})\text{alkenyl}$ ,  $-\text{O}(\text{C}_1-\text{C}_{24})\text{acyl}$ ,  $-\text{S}(\text{C}_1-\text{C}_{24})\text{alkyl}$ ,  $-\text{S}(\text{C}_1-\text{C}_{24})\text{alkenyl}$ , or  $-\text{S}(\text{C}_1-\text{C}_{24})\text{acyl}$ , wherein at least one of  $\text{R}_1$  and  $\text{R}_1'$  are not  $-\text{H}$ , and wherein said alkenyl or acyl moieties optionally have 1 to 6 double bonds,

15  $\text{R}_2$  and  $\text{R}_2'$  are independently  $-\text{H}$ , optionally substituted  $-\text{O}(\text{C}_1-\text{C}_7)\text{alkyl}$ ,  $-\text{O}(\text{C}_1-\text{C}_7)\text{alkenyl}$ ,  $-\text{S}(\text{C}_1-\text{C}_7)\text{alkyl}$ ,  $-\text{S}(\text{C}_1-\text{C}_7)\text{alkenyl}$ ,  $-\text{O}(\text{C}_1-\text{C}_7)\text{acyl}$ ,  $-\text{S}(\text{C}_1-\text{C}_7)\text{acyl}$ ,  $-\text{N}(\text{C}_1-\text{C}_7)\text{acyl}$ ,  $-\text{NH}(\text{C}_1-\text{C}_7)\text{alkyl}$ ,  $-\text{N}((\text{C}_1-\text{C}_7)\text{alkyl})_2$ , oxo, halogen,  $-\text{NH}_2$ ,  $-\text{OH}$ , or  $-\text{SH}$ ;

$\text{R}_3$  is a pharmaceutically active phosphonate, bisphosphonate or a phosphonate derivative of a pharmacologically active compound, linked to a functional group on optional linker L or to an available oxygen atom on  $\text{C}_\alpha$ ;

20 X, when present, is:

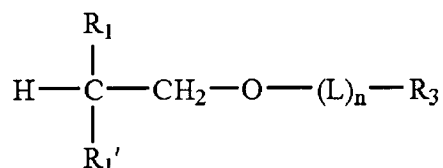


L is a valence bond or a bifunctional linking molecule of the formula  $-J-(CR_2)_t-G-$ , wherein t is an integer from 1 to 24, J and G are independently  $-O-$ ,  $-S-$ ,  $-C(O)O-$ , or  $-NH-$ , and R is  $-H$ , substituted or unsubstituted alkyl, or alkenyl;

m is an integer from 0 to 6; and

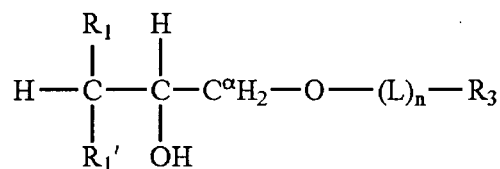
5 n is 0 or 1.

In some embodiments,  $m=0, 1$  or  $2$ . In these embodiments,  $R_2$  and  $R_2'$  are preferably H, and the prodrugs are then ethanediol, propanediol or butanediol derivatives of a therapeutic phosphonate. A preferred ethanediol phosphonate species has the structure:



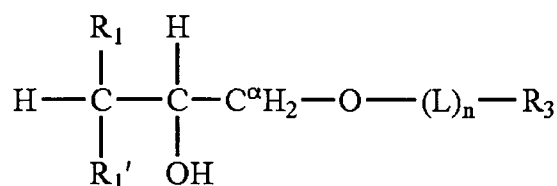
10 wherein  $R_1, R_1', R_3, L,$  and  $n$  are as defined above.

One propanediol species has the structure:



wherein  $m=1$  and  $R_1, R_1', R_3, L$  and  $n$  are as defined above in the general formula.

A glycerol species has the structure:



15 wherein  $m=1, R_2=H, R_2'=OH,$  and  $R_2$  and  $R_2'$  on  $C^\alpha$  are both  $-H$ . Glycerol is an optically active molecule. Using the stereospecific numbering convention for glycerol, the sn-3 position is the position which is phosphorylated by glycerol kinase. In compounds of the invention having a glycerol residue, the  $-(L)_n-$  moiety may be joined at either the sn-3 or  
20 sn-1 position of glycerol.

In all species of the pharmacologically active agents of the invention,  $R_1$  is preferably an alkoxy group having the formula  $-O-(CH_2)_t-CH_3$ , wherein t is 0-24. More preferably t is 11-19. Most preferably t is 15 or 17.

Some examples of antiviral phosphonates derived by substituting  $\text{—CH}_2\text{—PO}_3\text{H}_2$  for the 5'-hydroxyl are: AZT phosphonate, d4T phosphonate, ddC phosphonate, Adefovir, ganciclovir phosphonate, acyclovir phosphonate, ganciclovir cycloic phosphonate, and 3'-thia-2',3'-dideoxycytidine-5'-phosphonic acid

5 Other examples of antiviral nucleotide phosphonates contemplated for use in the practice of the invention are derived similarly from antiviral nucleosides including ddA, ddI, ddG, L-FMAU, DXG, DAPD, L-dA, L-dI, L-(d)T, L-dC, L-dG, FTC, penciclovir, and the like.

10 Additionally, antiviral phosphonates such as cidofovir, cyclic cidofovir, adefovir, tenofovir, and the like, may be used as an  $\text{R}_3$  group in accordance with the present invention.

Certain compounds of the invention possess one or more chiral centers, e.g. in the sugar moieties, and may thus exist in optically active forms. Likewise, when the compounds contain an alkenyl group or an unsaturated alkyl or acyl moiety there exists the possibility of cis- and trans-isomeric forms of the compounds. Additional asymmetric carbon atoms can be present in a substituent group such as an alkyl group. The R- and S-isomers and mixtures thereof, including racemic mixtures as well as mixtures of cis- and trans-isomers are contemplated by this invention. All such isomers as well as mixtures thereof are intended to be included in the invention. If a particular stereoisomer is desired, it can be prepared by methods well known in the art by using stereospecific reactions with starting materials that contain the asymmetric centers and are already resolved or, alternatively, by methods that lead to mixtures of the stereoisomers and resolution by known methods.

25 Nucleosides useful for treating viral infections may also be converted to their corresponding 5'-phosphonates for use as an  $\text{R}_3$  group. Such phosphonate analogs typically contain either a phosphonate ( $\text{—PO}_3\text{H}_2$ ) or a methylene phosphonate ( $\text{—CH}_2\text{—PO}_3\text{H}_2$ ) group substituted for the 5'-hydroxyl of an antiviral nucleoside. Some examples of antiviral phosphonates derived by substituting  $\text{—PO}_3\text{H}_2$  for the 5'-hydroxyl are:

30 Many phosphonate compounds exist that can be derivatized according to the invention to improve their pharmacologic activity, or to increase their oral absorption, such as, for example, the compounds disclosed in the following patents, each of which are hereby incorporated by reference in their entirety: U.S. Pat. No. 5,043,437 (Phosphonates of azidodideoxynucleosides), U.S. Pat. No. 5,047,533 (Acyclic purine phosphonate nucleotide analogs), U.S. Pat. No. 5,142,051 (N-Phosphonylmethoxyalkyl derivatives of pyrimidine and purine bases), U.S. Pat. No. 5,247,085 (Antiviral purine compounds), U.S. Pat. No. 5,395,826

(Guanidinealkyl 1-1,1-bisphosphonic acid derivatives), U.S. Pat. No. 5,656,745 (Nucleotide analogs), U.S. Pat. No. 5,672,697 (Nucleoside-5'-methylene phosphonates), U.S. Pat. No. 5,717,095 (Nucleotide analogs), U.S. Pat. No. 5,760,013 (Thymidylate analogs), U.S. Pat. No. 5,798,340 (Nucleotide analogs), U.S. Pat. No. 5,840,716 (Phosphonate nucleotide compounds), U.S. Pat. No. 5,856,314 (Thio-substituted, nitrogen-containing, heterocyclic phosphonate compounds), U.S. Pat. No. 5,885,973 (olpadronate), U.S. Pat. No. 5,886,179 (Nucleotide analogs), U.S. Pat. No. 5,877,166 (Enantiomerically pure 2-aminopurine phosphonate nucleotide analogs), U.S. Pat. No. 5,922,695 (Antiviral phosphonmethoxy nucleotide analogs), U.S. Pat. No. 5,922,696 (Ethylenic and allenic phosphonate derivatives of purines), U.S. Pat. No. 5,977,089 (Antiviral phosphonmethoxy nucleotide analogs), U.S. Pat. No. 6,043,230 (Antiviral phosphonmethoxy nucleotide analogs), U.S. Pat. No. 6,069,249 (Antiviral phosphonmethoxy nucleotide analogs); Belgium Patent No. 672205 (Clodronate); European Patent No. 753523 (Amino-substituted bisphosphonic acids); European Patent Application 186405 (geminal diphosphonates); and the like.

Phosphonate analogs, comprising therapeutically effective phosphonates (or phosphonate derivatives of therapeutically effective compounds) covalently linked by a hydroxyl group to a 1-O-alkylglycerol, 3-O-alkylglycerol, 1-S-alkylthioglycerol, or alkoxy-alkanol, may be absorbed more efficiently in the gastrointestinal tract than are the parent compounds. An orally administered dose of the analog is taken up intact from the gastrointestinal tract of a mammal and the active drug is released in vivo by the action of endogenous enzymes. Phosphonate analogs of the invention may also have a higher degree of bioactivity than the corresponding underivatized compounds.

The compounds of the present invention are an improvement over alkylglycerol phosphate prodrugs described in the prior art because the phosphonate-containing moiety is linked directly to the alkyl-glycerol or the alkoxy-alkanol moiety and because the presence of the phosphonate bond prevents enzymatic conversion to the free drug. Other linkers between these groups can be present in the improved analogs. For example, bifunctional linkers having the formula  $-O-(CH_2)_n-C(O)O-$  wherein  $n$  is 1 to 24, can connect the phosphonate to the hydroxyl group of the alkoxy-alkanol or alkylglycerol moiety.

The foregoing allows the phosphonate of the invention to achieve a higher degree of oral absorption. Furthermore, cellular enzymes, but not plasma or digestive tract enzymes, will convert the conjugate to a free phosphonate. A further advantage of the alkoxy-alkanol phosphonates is that the tendency of co-administered food to reduce or abolish phosphonate

absorption is greatly reduced or eliminated, resulting in higher plasma levels and better compliance by patients.

Compounds (or "prodrugs") useful in the invention can be prepared in a variety of ways, as generally depicted in **Schemes I –VI** of US Patent No. 6,716,825. The general phosphonate esterification methods described below are provided for illustrative purposes only and are not to be construed as limiting this invention in any manner. Indeed, several methods have been developed for direct condensation of phosphonic acids with alcohols (*see*, for example, R. C. Larock, *Comprehensive Organic Transformations*, VCH, New York, 1989, p. 966 and references cited therein). Isolation and purification of the compounds and intermediates described in the examples can be effected, if desired, by any suitable separation or purification procedure such as, for example, filtration, extraction, crystallization, flash column chromatography, thin-layer chromatography, distillation or a combination of these procedures. Specific illustrations of suitable separation and isolation procedures are in the examples below. Other equivalent separation and isolation procedures can of course, also be used.

**Scheme I** of US Patent No. 6,716,825. outlines a synthesis of bisphosphonate prodrugs that contain a primary amino group, such as pamidronate or alendronate. Example 1 therein provides conditions for a synthesis of 1-O-hexadecyloxypropyl-alendronate (HDP-alendronate) or 1-O-hexadecyloxypropyl-pamidronate (HDP-pamidronate). In this process, a mixture of dimethyl 4-phthalimidobutanoyl phosphonate (1b, prepared as described in U.S. Pat. No. 5,039,819) and hexadecyloxypropyl methyl phosphite (2) in pyridine solution is treated with triethylamine to yield bisphosphonate tetraester 3b which is purified by silica gel chromatography. Intermediate 2 is obtained by transesterification of diphenyl phosphite as described in Kers, A., Kers, I., Stawinski, J., Sobkowski, M., Kraszewski, A. *Synthesis*, April 1995, 427-430. Thus, diphenyl phosphite in pyridine solution is first treated with hexadecyloxypropan-1-ol, then with methanol to provide compound 2.

An important aspect of the process is that other long chain alcohols may be used in place of hexadecyloxypropan-1-ol to generate the various compounds of this invention. Treatment of intermediate 3b with bromotrimethylsilane in acetonitrile cleaves the methyl esters selectively to yield monoester 4b. Treatment of 4b with hydrazine in a mixed solvent system (20% methanol/80% 1,4-dioxane) results in removal of the phthalimido protecting group as shown. The desired alendronate prodrug is collected by filtration and converted to the triammonium salt by treatment with methanolic ammonia.

**Scheme II** of US Patent No. 6,716,825. illustrates a synthesis of analogs of bisphosphonates lacking a primary amino group, in this case the process steps are similar to those of Scheme 1 except that protection with a phthalimido group and subsequent deprotection by hydrazinolysis are unnecessary.

5 Bisphosphonates having 1-amino groups, such as amino-olpadronate, maybe converted to analogs according to the invention prodrugs using a slightly modified process shown in **Scheme III** of US Patent No. 6,716,825. Treatment of a mixture of compound 2 and 3-(dimethylamino)propionitrile with dry HCl followed by addition of dimethyl phosphite affords tetraester 3 which, after demethylation with bromotrimethylsilane, yields  
10 hexadecyloxypropyl-amino-olpadronate.

**Scheme IV** of US Patent No. 6,716,825 illustrates synthesis of a bisphosphonate analog where the lipid group is attached to a primary amino group of the parent compound rather than as a phosphonate ester.

**Scheme V** of US Patent No. 6,716,825. illustrates a general synthesis of alkylglycerol  
15 or alkylpropanediol analogs of cidofovir, cyclic cidofovir, and other phosphonates. Treatment of 2,3-isopropylidene glycerol, 1, with NaH in dimethylformamide followed by reaction with an alkyl methanesulfonate yields the alkyl ether, 2. Removal of the isopropylidene group by treatment with acetic acid followed by reaction with trityl chloride in pyridine yields the intermediate 3. Alkylation of intermediate 3 with an alkyl halide results in compound 4.  
20 Removal of the trityl group with 80% aqueous acetic acid affords the O,O-dialkyl glycerol, 5. Bromination of compound 5 followed by reaction with the sodium salt of cyclic cidofovir or other phosphonate-containing nucleotide yields the desired phosphonate adduct, 7. Ring-opening of the cyclic adduct is accomplished by reaction with aqueous sodium hydroxide. The preferred propanediol species may be synthesized by substituting 1-O-alkylpropane-3-ol  
25 for compound 5 in Scheme V. The tenofovir and adefovir analogs may be synthesized by substituting these nucleotide phosphonates for cCDV in reaction (f) of Scheme V. Similarly, other nucleotide phosphonates of the invention may be formed in this manner.

**Scheme VI** of US Patent No. 6,716,825. illustrates a general method for the synthesis of nucleotide phosphonates of the invention using 1-O-hexadecyloxypropyl-adefovir as the  
30 example. The nucleotide phosphonate (5 mmol) is suspended in dry pyridine and an alkoxyalkanol or alkylglycerol derivative (6 mmol) and 1,3-dicyclohexylcarbodiimide (DCC, 10 mmol) are added. The mixture is heated to reflux and stirred vigorously until the condensation reaction is complete as monitored by thin-layer chromatography. The mixture is

then cooled and filtered. The filtrate is concentrated under reduced pressure and the residues adsorbed on silica gel and purified by flash column chromatography (elution with approx. 9:1 dichloromethane/methanol) to yield the corresponding phosphonate monoester.

### 5 **C. Compositions.**

Compounds of the invention can be administered orally in the form of tablets, capsules, solutions, emulsions or suspensions, inhaled liquid or solid particles, microencapsulated particles, as a spray, through the skin by an appliance such as a transdermal patch, or rectally, for example, in the form of suppositories. The lipophilic  
10 prodrug derivatives of the invention are particularly well suited for transdermal absorption administration and delivery systems and may also be used in toothpaste. Administration can also take place parenterally in the form of injectable solutions.

The compositions may be prepared in conventional forms, for example, capsules, tablets, aerosols, solutions, suspensions, or together with carriers for topical applications.  
15 Pharmaceutical formulations containing compounds of this invention can be prepared by conventional techniques, e.g., as described in Remington's Pharmaceutical Sciences, 1985.

The pharmaceutical carrier or diluent employed may be a conventional solid or liquid carrier. Examples of solid carriers are lactose, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, stearic acid, or lower alkyl ethers of cellulose. Examples of liquid  
20 carriers are syrup, peanut oil, olive oil, phospholipids, fatty acids, fatty acid amines, polyoxyethylene or water. The carrier or diluent may include any sustained release material known in the art, such as glyceryl monostearate or distearate, alone or mixed with a wax.

If a solid carrier is used for oral administration, the preparation may be tableted or placed in a hard gelatin capsule in powder or pellet form. The amount of solid carrier will  
25 vary widely, but will usually be from about 25 mg to about 1 gm. If a liquid carrier is used, the preparation may be in the form of a syrup, emulsion, soft gelatin capsule, or sterile injectable liquid such as an aqueous or non-aqueous liquid suspension or solution.

Tablets are prepared by mixing the active ingredient (that is, one or more compounds of the invention), with pharmaceutically inert, inorganic or organic carrier, diluents, and/or  
30 excipients. Examples of such excipients which can be used for tablets are lactose, maize starch or derivatives thereof, talc, stearic acid or salts thereof. Examples of suitable excipients for gelatin capsules are vegetable oils, waxes, fats, semisolid, and liquid polyols. The bisphosphonate prodrugs can also be made in microencapsulated form.

For nasal administration, the preparation may contain a compound of the invention dissolved or suspended in a liquid carrier, in particular, an aqueous carrier, for aerosol application. The carrier may contain solubilizing agents such as propylene glycol, surfactants, absorption enhancers such as lecithin or cyclodextrin, or preservatives.

5           Pharmaceutical compositions of this invention for parenteral injection comprise pharmaceutically acceptable sterile aqueous or non-aqueous liquids, dispersions, suspensions or emulsions as well as sterile powders for reconstitution into sterile injectable solutions or dispersions just prior to use.

10           Suitable excipients for the preparation of solutions and syrups are water, polyols, sucrose, invert sugar, glucose, and the like. Suitable excipients for the preparation of injectable solutions are water, alcohols, polyols, glycerol, vegetable oils, and the like.

15           The pharmaceutical products can additionally contain any of a variety of added components, such as, for example, preservatives, solubilizers, stabilizers, wetting agents, emulsifiers, sweeteners, colorants, flavorings, buffers, coating agents, antioxidants, diluents, and the like.

20           Optionally, the pharmaceutical compositions of the invention may comprise a compound according to the general formula combined with one or more compounds exhibiting a different activity, for example, an antibiotic or other pharmacologically active material. Such combinations are within the scope of the invention.

#### **D. Subjects and methods.**

25           A number of rare diseases feature a heightened susceptibility to infections from childhood onward. Many of these disorders are hereditary and are autosomal recessive or X-linked. There are over 80 recognised primary immunodeficiency syndromes; they are generally grouped by the part of the immune system that is malfunctioning, such as lymphocytes or granulocytes. The treatment of primary immunodeficiencies depends on the nature of the defect, and may involve antibody infusions, long-term antibiotics and (in certain cases) stem cell transplantation.

30           Immune deficiency may also be the result of particular external processes or diseases; the resultant state is called "secondary" or "acquired" immunodeficiency. Common causes for secondary immunodeficiency are malnutrition, aging and particular medications (e.g. chemotherapy, disease-modifying antirheumatic drugs, immunosuppressive drugs after organ transplants, glucocorticoids).

Many specific diseases directly or indirectly impair the immune system. This include many types of cancer, particularly those of the bone marrow and blood cells (leukemia, lymphoma, multiple myeloma), and certain chronic infections. Immunodeficiency is also the hallmark of acquired immunodeficiency syndrome (AIDS), caused by the human immunodeficiency virus (HIV). HIV directly attacks the immune system.

Human cytomegalovirus (HCMV) is a member of the herpes virus family. These dsDNA viruses typically cause mild or subclinical disease, but can cause severe systemic or localised disease in immunocompromised individuals. All herpes viruses share a characteristic ability to remain latent within the body over long periods. Although primary CMV infection in an immunocompromised patient can cause serious disease, the more common problem is the reactivation of the latent virus.

Immunocompromised patients include organ transplant recipients, patients undergoing hemodialysis, patients with cancer, patients receiving immunosuppressive drugs, and HIV-infected patients. Exposure of immunosuppressed patients to outside sources of CMV should be minimized. Whenever possible, patients without CMV infection should be given organs and/or blood products that are free of the virus.

Patients without CMV infection who are given organ transplants from CMV-infected donors should be given prophylactic treatment with valganciclovir (ideally) or ganciclovir and require regular serological monitoring to detect a rising CMV titre, which should be treated early to prevent a potentially life-threatening infection becoming established.

However, despite prophylaxis, often continued for 100 days, disease prevalence at six months is estimated to be from 12 to 22 percent. Safe and efficacious prophylaxis of CMV infection in transplant patients is not possible with current treatments.

CMX001 has a decided advantage over current treatments for prophylaxis of CMV infection in transplant recipients and cancer patients receiving myelosuppressive chemotherapy or radiation therapy, based on the demonstrated lack of nephrotoxicity at potentially effective concentrations. Large patient populations with needs that could previously not be met with existing therapies can now be treated. The surprisingly low levels of nephrotoxicity associated with the compounds of the present invention means that the application of these compounds in treatment of immunocompromised individuals is now possible.

Furthermore, although anti-viral therapies have advanced substantially in recent years, resistance to current agents and significant drug side effects remain an issue for many patients. The conjugate compounds of the present invention demonstrate high oral bioavailability at lower doses than conventional drugs and this has important implications for disease resistance.

More generally, this invention provides methods of treating mammalian disorders related to bone metabolism, viral infections, inappropriate cell proliferation, and the like. The methods particularly comprise administering to a human or other mammal in need thereof a therapeutically effective amount of the prodrugs of this invention. Indications appropriate to such treatment include senile, post-menopausal or steroid-induced osteoporosis, Paget's disease, metastatic bone cancers, hyperparathyroidism, rheumatoid arthritis, algodystrophy, sterno-costoclavicular hyperostosis, Gaucher's disease, Engleman's disease, certain non-skeletal disorders and periodontal disease, human immunodeficiency virus (HIV), influenza, herpes simplex virus (HSV), human herpes virus 6, cytomegalovirus (CMV), hepatitis B virus, Epstein-Barr virus (EBV), varicella zoster virus, lymphomas, hematological disorders such as leukemia, and the like.

In accordance with yet another aspect of the invention, there are provided methods for treating disorders caused by viral infections. Indications appropriate to such treatment include susceptible viruses such as human immunodeficiency virus (HIV), influenza, herpes simplex virus (HSV), human herpes virus 6, cytomegalovirus (CMV), hepatitis B and C virus, Epstein-Barr virus (EBV), varicella zoster virus, and diseases caused by orthopox viruses (e.g., variola major and minor, vaccinia, smallpox, cowpox, camelpox, monkeypox, and the like), ebola virus, papilloma virus, and the like.

The prodrugs of the invention can be administered orally, parenterally, topically, rectally, and through other routes, with appropriate dosage units, as desired.

With respect to disorders associated with viral infections, the "effective amount" is determined with reference to the recommended dosages of the antiviral compound. The selected dosage will vary depending on the activity of the selected compound, the route of administration, the severity of the condition being treated, and the condition and prior medical history of the patient being treated. However, it is within the skill of the art to start doses of the compound(s) at levels lower than required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved. If desired, the effective daily dose may be divided into multiple doses for purposes of administration, for example, two to four doses per day. It will be understood, however, that the specific dose level for any particular patient will depend on a variety of factors, including the body weight, general health, diet, time, and route of administration and combination with other drugs, and the severity of the disease being treated.

Generally, the compounds of the present invention are dispensed in unit dosage form comprising 1% to 100% of active ingredient. The range of therapeutic dosage is from about 0.01 to about 1,000 mg/kg/day with from about 0.10 mg/kg/day to 100 mg/kg/day being preferred, when administered to patients, e.g., humans, as a drug. Actual dosage levels of active ingredients in the pharmaceutical compositions of this invention may be varied so as to administer an amount of the active compound(s) that is effective to achieve the desired therapeutic response for a particular patient.

The present invention is explained in greater detail in the following non-limiting Examples.

### EXAMPLE 1

#### Preclinical Studies of CMX001

As summarized in Tables 1-2 below, pre-clinical studies of CMX001 indicate that it is essentially completely protective against lethal Orthopoxvirus infections in mice and rabbits. The effective dose in these animal models ranges from 1-2 mg/kg daily for 5 days in low titer inoculums, while late stage requires 20-30 mg/kg as a single dose.

<b>Virus</b>	<b>Cell Line</b>	<b>Cidofovir EC50 (μM)</b>	<b>CMX001 EC50 (μM)</b>	<b>Enhanced Activity</b>
Variola major	Vero 76	27.3	0.1	271
Vaccinia Virus	HFF	46	0.8	57
HCMV(AD169)	MRC-5	0.38	0.0009	422
BK Virus	WI-38	115.1	0.13	885
HSV-1	MRC-5	15	0.06	250
HHV-6	HSB-2	0.2	0.004	50
Adenovirus	HFF	1.3	0.02	65
HPV 18	HeLa	516	0.42	1229
HPV 11	A431	716	17	42
EBV	Dardi	>170	0.04	>4250

**Table 2: CMX001 is protective against lethal orthopoxvirus infections in mice and rabbits.**

	<b>Viral Inoculum (PFU)</b>	<b>100% Protective Dose of CMX001*</b>
<b>Mice Infected with Ectromelia</b>	1.2	1 mg/kg/day
	27	4 mg/kg/day
	270	4 mg/kg/day
	9200	8 mg/kg/day
<b>Rabbits Infected with Rabbitpox</b>	100	2 mg/kg/day
	500	10 mg/kg/day
	1000	20 mg/kg/day

\*Dose was orally administered for five consecutive days

In addition, over twenty-one toxicology studies have been conducted in mice, rats, rabbits and monkeys with CMX001 being delivered by the oral route. In none of these studies (as opposed to the delivery of efficacious doses of cidofovir by i.v.), has there been any indication of nephrotoxicity (*see, e.g.*, Example 2 below).

## **EXAMPLE 2**

### **Clinical Studies**

An initial study was conducted to evaluate the safety and pharmacokinetics of CMX001 in healthy volunteers. The study consisted of a single dose arm (SD) and a multiple dose arm (MD). In the single dose arm 7 cohorts of 6 subjects were treated (4 subjects received active drug and 2 placebo). Enrollment was staggered as 2 subjects (one active, one placebo) followed by 4 subjects (Groups A and B). The estimated single doses for the two highest doses treated for a 75 kg subject were 40 mg (0.6mg/kg cohort 6) and 70 mg (1mg/kg cohort 7). In the multiple dose arm, cohort 6MD received 0.1 mg/kg on Day 0, 6 and 12; Cohort 7MD received 0.2 mg/kg on Day 0, 6 and 12. Levels of cidofovir, CMX001 and CMX064 (major metabolite) were measured in blood and urine of subjects during the course of the study. Gastrointestinal (GI) monitoring of the subjects included (a) monitoring for clinical signs of GI adverse events, (b) monitoring for clinical symptoms using a visual Analog Scale, (c) monitoring for appetite loss/anorexia, nausea, vomiting, diarrhea, constipation and intestinal gas/bloating, (d) laboratory tests for fecal occult blood; serum

electrolytes, urine specific gravity, BUN/creatinine ratio; serum albumin, and lipids, and (e) diagnostic studies (the Wireless capsule endoscopy (PillCam®, Given Imaging)).

Upon the completion of the study of cohort 6 (600 $\mu$ g/kg) (while still blinded) it was observed as follows:

5 No post-dose clinically significant gastrointestinal capsule endoscopy findings attributable to drug.

No drug associated clinically significant changes to clinical laboratory values, including those indicative of kidney dysfunction.

10 No serious adverse events (SAEs), no significant adverse events (AEs) (i.e.  $\geq$  Grade 2), no AEs directly attributable to drug.

Plasma concentration curves of CMX001 following a single dose administration are shown in **Figure 1**, and plasma concentration curves of Cidofovir following a single dose of CMX001 are shown in **Figure 2**.

15 **Table 3** illustrates the PK comparison of CMX 001 with CMX 021 and CMX 064 for mouse, rabbit and human.

**TABLE 3**

Species	Dose (mg/kg)	CMX001		CMX021		CMX064	
		Cmax (ng/mL)	AUCO $\rightarrow$ (ng*h/mL)	Cmax (ng/mL)	AUCO $\rightarrow$ (ng*h/mL)	Cmax (ng/mL)	AUCO $\rightarrow$ (ng*h/mL)
Mouse-Rabbit	2	7.9-18.0	83.14-102.4	BQL-5.44	ND-50.1	-	-
Human	0.025	2.36	18.51	BQL	ND	1.69	11.83
	0.050	5.63	36.32	1.51	33.28	4.63	38.95
	0.100	10.62	133.47	3.44	125.14	2.85	34.02
	0.200	24.48	225.49	5.41	189.92	4.55	39.73
	0.400	68.13	526.37	10.44	444.76	23.03	202.99
	0.600	114.73	728.8	12.19	519.0	24.86	187.0

Calculated based on mouse doses of 2 and 10 mg/kg, rabbit doses of 5 and 10 mg/kg

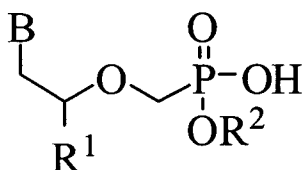
ND Not Determined, BQL Below Quantitation Limit

20 The foregoing is illustrative of the present invention, and is not to be construed as limiting thereof. The invention is defined by the following claims, with equivalents of the claims to be included therein.

**That which is claimed is:**

1. A conjugate compound comprising an acyclic nucleoside phosphonate covalently coupled to a lipid for the therapeutic and/or prophylactic treatment of viral infection in an immunodeficient subject.

2. A conjugate compound according to claim 1, comprising a phosphonate of an antiviral compound of the formula:



or an enantiomer, diastereomer, racemate, stereoisomer, tautomer, rotamer or a mixture thereof, wherein:

R<sup>1</sup> is hydrogen, —CH<sub>3</sub>, —CH<sub>2</sub>OH, —CH<sub>2</sub>F, —CH=CH<sub>2</sub>, or —CH<sub>2</sub>N<sub>3</sub>;

R<sup>2</sup> is hydrogen; and

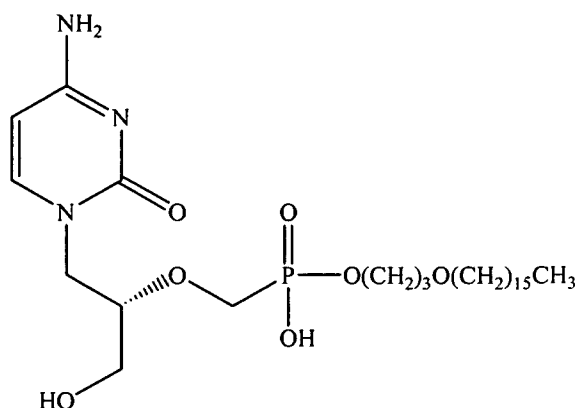
B is a purine or pyrimidine;

covalently linked to an alkylglycerol, alkylpropanediol, 1-S-alkylthioglycerol, alkoxyalkanol, alkylethanol, hexadecylpropanediol or octadecylpropanediol; or a pharmaceutically acceptable salt thereof.

3. A conjugate compound according to claim 1 or claim 2, wherein said conjugate compound is in the form of an enantiomer, diastereoisomer, racemate or a mixture thereof.

4. A conjugate compound according to any of claims 1 to 3, wherein said acyclic nucleoside phosphonate is selected from the group consisting of cidofovir, cyclic cidofovir, tenofovir, and adefovir.

5. A conjugate compound according to claim 4, wherein said conjugate compound is:



or a pharmaceutically acceptable salt thereof.

6. A conjugate compound according to any of the preceding claims, wherein said immunodeficient subject has primary or acquired immunodeficiency.

7. A conjugate compound according to claim 6, wherein said immunodeficient subject has acquired immunodeficiency as a result of immunosuppressive therapy, particularly by cyclosporine.

8. A conjugate compound according to any of the preceding claims, wherein said immunodeficient subject is a transplant patient.

9. A conjugate compound according to claim 8, wherein said subject is a renal transplant patient, a hepatic transplant patient or a bone marrow transplant patient.

10. A conjugate compound according to any of the preceding claims, wherein said subject is suffering from chronic fatigue syndrome.

11. A conjugate compound according to any of the preceding claims, wherein the viral infection is resistant to treatment with an unconjugated acyclic nucleoside phosphonate.

12. A conjugate compound according to any of the preceding claims, wherein an unconjugated acyclic nucleoside phosphonate exhibits toxic side effects in said immunodeficient subject.

13. A conjugate compound according to any of the preceding claims, wherein said immunodeficient subject is infected with at least one dsDNA virus.

14. A conjugate compound according to claim 13, wherein said dsDNA virus is selected from any of the groups consisting of: human immunodeficiency virus (HIV), influenza, herpes simplex virus (HSV), human herpes virus 6 (HHV-6), cytomegalovirus (CMV), hepatitis B and C virus, Epstein-Barr virus (EBV), varicella zoster virus, variola major and minor, vaccinia, smallpox, cowpox, camelpox, monkeypox, ebola virus, papilloma virus, adenovirus or polyoma virus including JC virus, BK virus and SV40.

15. A conjugate compound according to claim 13 or claim 14 wherein said immunodeficient subject is infected with a virus or any combination of viruses selected from the groups consisting of: HCMV, BK virus, HHV-6, Adenovirus and EBV.

16. A conjugate compound according to any of claims 13 to 15, wherein said immunodeficient subject is infected with two or more viruses and said two or more viruses exhibit synergistic action.

17. A conjugate compound according to claim 16 wherein said viruses are HCMV and BK.

18. A conjugate compound according to any of the preceding claims, wherein said conjugate compound is used to treat a dsDNA viral infection in an immunodeficient subject wherein said subject is resistant to valganciclovir hydrochloride (or ganciclovir) or wherein said subject exhibits side effects to valganciclovir hydrochloride (or ganciclovir).

19. A conjugate compound according to any of the preceding claims, wherein said conjugate is used to treat cytomegalovirus (CMV) subsequent to treatment with (val) ganciclovir.

20. A conjugate compound according to any of the preceding claims, wherein said immunodeficient subject is a human subject.

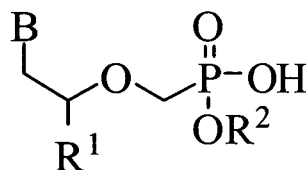
21. A conjugate compound according to any of the preceding claims, wherein conjugate compound is administered to said subject at a dosage of less than 5 mg/Kg.

22. A conjugate compound according to any of the preceding claims, wherein said conjugate compound is administered to said subject at a dosage of 10 or 20 up to 200 or 5000 ug/Kg.

23. The use of a conjugate compound comprising an acyclic nucleoside phosphonate covalently coupled to a lipid in the manufacture of a medicament for the therapeutic and/or prophylactic treatment of viral infection in an immunodeficient subject.

24. A method for the therapeutic and/or prophylactic treatment of viral infection in an immunodeficient subject comprising administering a conjugate compound to the subject, said conjugate compound comprising an acyclic nucleoside phosphonate covalently coupled to a lipid.

25. The method according to claim 24, said conjugate compound comprising a phosphonate of an antiviral compound of the formula:



or an enantiomer, diastereomer, racemate, stereoisomer, tautomer, rotamer or a mixture thereof, wherein:

R<sup>1</sup> is hydrogen, —CH<sub>3</sub>, —CH<sub>2</sub>OH, —CH<sub>2</sub>F, —CH=CH<sub>2</sub>, or —CH<sub>2</sub>N<sub>3</sub>;

R<sup>2</sup> is hydrogen; and

B is a purine or pyrimidine;

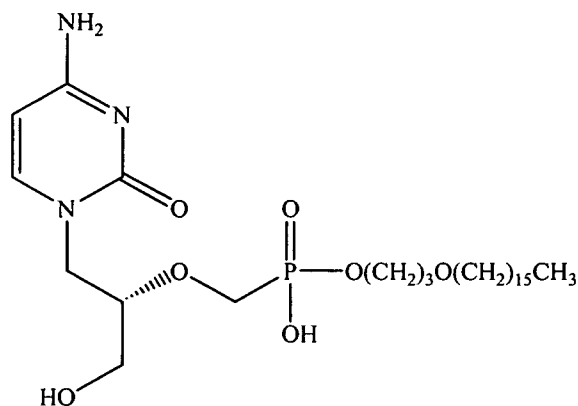
covalently linked to an alkylglycerol, alkylpropanediol, 1-S-alkylthioglycerol, alkoxyalkanol, alkylethanediol, hexadecylpropanediol or octadecylpropanediol;

or a pharmaceutically acceptable salt thereof.

26. The method according to claim 24 or claim 25, wherein said compound is in the form of an enantiomer, diastereoisomer, racemate or a mixture thereof.

27. The method according to any of claims 24 to 26, wherein said acyclic nucleoside phosphonate is selected from the group consisting of cidofovir, cyclic cidofovir, tenofovir, and adefovir.

28. The method according to claim 26, wherein said conjugate compound is:



or a pharmaceutically acceptable salt thereof.

CMX001-102 Cohorts 1, 2, 3, 4, 5, 6  
CMX001 Mean +/- SD

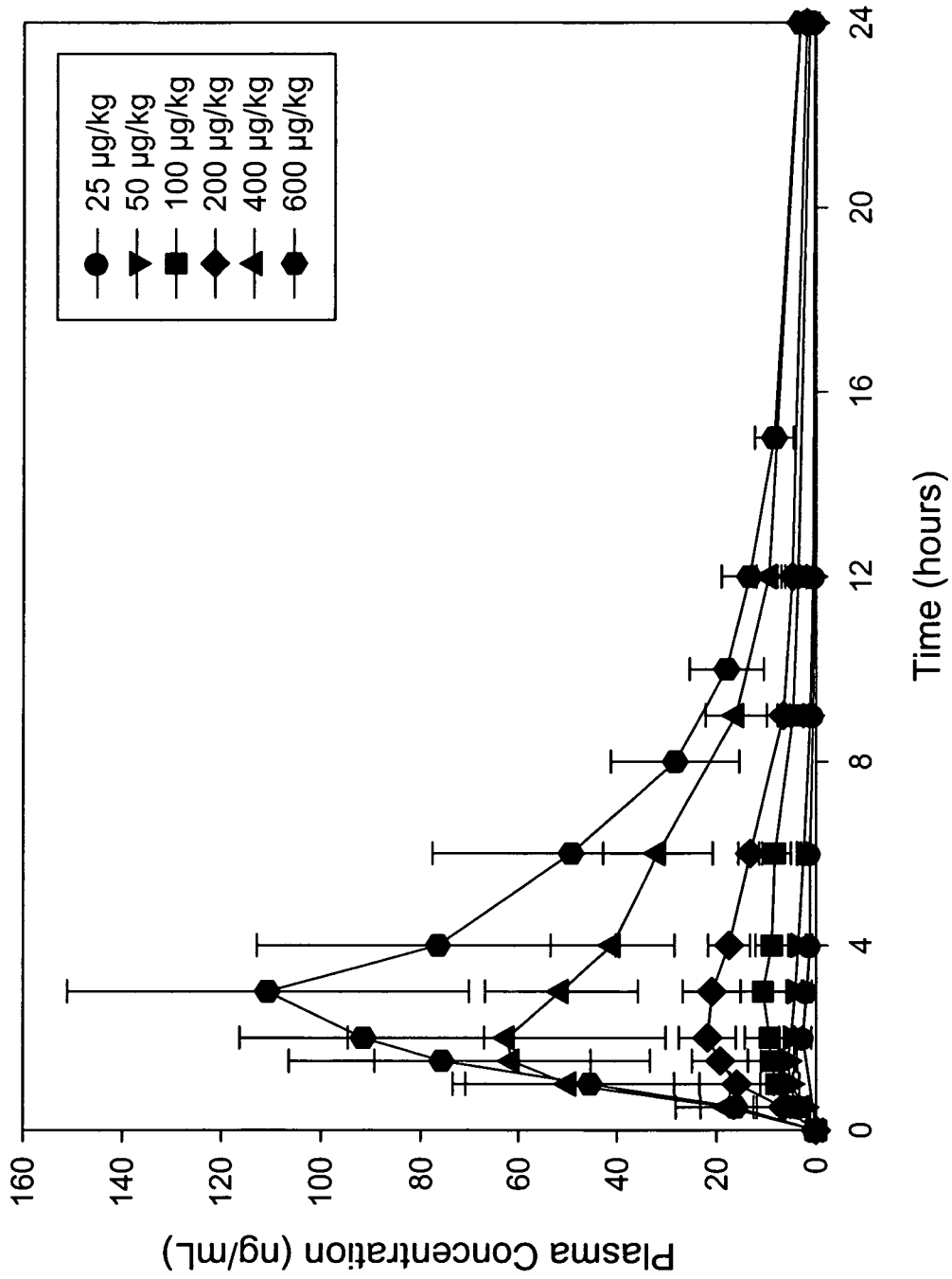


Figure 1. Plasma concentration curves of CMX001 following a single dose administration of CMX001.

CMX001-102 Cohorts 1, 2, 3, 4, 5, 6  
CMX021 (Cidofovir) Mean +/- SD

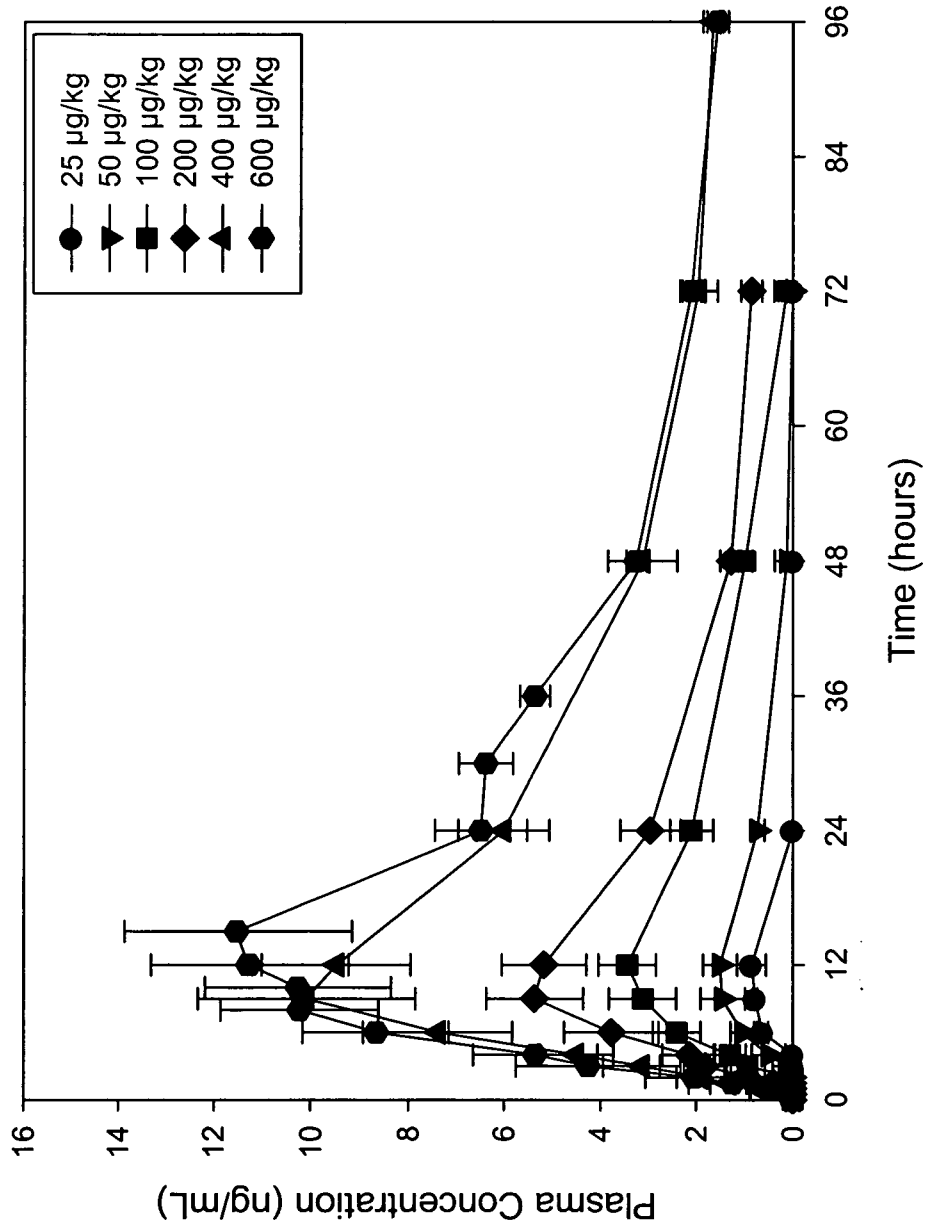


Figure 2. Plasma concentration curves of Cidofovir following a single dose administration of CMX001.

**INTERNATIONAL SEARCH REPORT**

International application No  
PCT/US2008/005322

**A. CLASSIFICATION OF SUBJECT MATTER**  
INV. A61K47/48

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)  
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, EMBASE, BIOSIS, BEILSTEIN Data

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>BIDANSET D. J. ET AL.: "Oral activity of ether lipid ester prodrugs of cidofovir against experimental human cytomegalovirus infection" THE JOURNAL OF INFECTIOUS DISEASES, vol. 190, no. 3, 1 August 2004 (2004-08-01), pages 499-503, XP002485780 abstract page 499, column 1, paragraph 1 page 499, column 1, paragraph 2 - column 2, paragraph 1 page 500, column 2, paragraph 3 page 503, column 1, paragraph 3 - column 2, paragraph 1</p> <p align="center">----- -/--</p>	1-28

Further documents are listed in the continuation of Box C.

See patent family annex.

\* Special categories of cited documents :

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- \* & \* document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

26 June 2008

09/07/2008

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Monami, Amélie

## INTERNATIONAL SEARCH REPORT

International application No  
PCT/US2008/005322

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>PAINTER G R ET AL: "Design and development of oral drugs for the prophylaxis and treatment of smallpox infection" TRENDS IN BIOTECHNOLOGY, ELSEVIER PUBLICATIONS, CAMBRIDGE, GB, vol. 22, no. 8, 1 August 2004 (2004-08-01), pages 423-427, XP004524159 ISSN: 0167-7799 abstract figure 1 tables 2,3 page 423, column 1, line 17 - column 2, line 25 page 425, column 1, line 29 - line 51 page 426, column 1, line 9 - page 427, column 1, line 10 page 425, column 2, line 45 - page 426, column 1, line 8</p>	1-9, 11-14, 20-28
A	<p>BIRON ET AL: "Antiviral drugs for cytomegalovirus diseases" ANTIVIRAL RESEARCH, ELSEVIER SCIENCE BV., AMSTERDAM, NL, vol. 71, no. 2-3, 1 September 2006 (2006-09-01), pages 154-163, XP005605076 ISSN: 0166-3542 page 155, column 2, paragraph 1 - paragraph 3 page 155, column 1, paragraph 2 figure 2(d) page 161, column 1, paragraph 3 - column 2, paragraph 2</p>	
A	<p>DAL POZZO ET AL: "In vitro evaluation of the anti-orf virus activity of alkoxyalkyl esters of CDV, cCDV and (S)-HPMPA" ANTIVIRAL RESEARCH, ELSEVIER SCIENCE BV., AMSTERDAM, NL, vol. 75, no. 1, 17 April 2007 (2007-04-17), pages 52-57, XP022031604 ISSN: 0166-3542 page 55, column 1, paragraph 6 - page 56, column 1, paragraph 2</p>	

-/--

## INTERNATIONAL SEARCH REPORT

International application No  
PCT/US2008/005322

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>CIESLA S.L. ET AL.: "Esterification of cidofovir with alkoxyalkanols increases oral bioavailability and diminishes drug accumulation in kidney" ANTIVIRAL RESEARCH, vol. 59, no. 3, August 2003 (2003-08), pages 163-171, XP002485781 figure 1 abstract page 163, column 2, paragraph 3 - page 164, column 2, paragraph 1 page 168, column 2, paragraph 2 - page 170, column 2, paragraph 2</p>	
A	<p>JOSEPHSON M. A. ET AL.: "Polyomavirus-associated nephropathy : Update on antiviral strategies" TRANSPLANT INFECTIOUS DISEASE, vol. 8, no. 2, June 2006 (2006-06), pages 95-101, XP002485782 page 96, column 1, paragraph 1 page 96, column 2, paragraph 3 - page 97, column 2, paragraph 1</p>	
A	<p>RANDHAWA P. ET AL.: "Ether lipid ester derivatives of cidofovir inhibit polyomavirus BK replication in vitro" ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, vol. 50, no. 4, April 2006 (2006-04), pages 1564-1566, XP002485899 the whole document</p>	
A	<p>US 2004/019232 A1 (HOSTETLER KARL Y [US] ET AL) 29 January 2004 (2004-01-29) cited in the application examples 1-11 examples 17-21</p>	
A	<p>WO 2006/110655 A (CHIMERIX INC [US]; ALMOND MERRICK R [US]; PAINTER GEORGE R [US]) 19 October 2006 (2006-10-19) examples 1-5 page 18, line 10 - line 14</p>	
A	<p>DE CLERCQ ET AL: "The acyclic nucleoside phosphonates from inception to clinical use: Historical perspective" ANTIVIRAL RESEARCH, ELSEVIER SCIENCE BV., AMSTERDAM, NL, vol. 75, no. 1, 17 April 2007 (2007-04-17), pages 1-13, XP022031598 ISSN: 0166-3542</p>	

## INTERNATIONAL SEARCH REPORT

International application No

PCT/US2008/005322

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>KERN ET AL: "Pivotal role of animal models in the development of new therapies for cytomegalovirus infections"  ANTIVIRAL RESEARCH, ELSEVIER SCIENCE BV.,  AMSTERDAM, NL,  vol. 71, no. 2-3,  1 September 2006 (2006-09-01), pages  164-171, XP005605077  ISSN: 0166-3542</p> <p>-----</p>	
P,A	<p>PARKER ET AL: "Efficacy of therapeutic intervention with an oral ether-lipid analogue of cidofovir (CMX001) in a lethal mousepox model"  ANTIVIRAL RESEARCH, ELSEVIER SCIENCE BV.,  AMSTERDAM, NL,  vol. 77, no. 1,  1 January 2008 (2008-01-01), pages 39-49,  XP022405846  ISSN: 0166-3542  abstract  page 39, column 2, paragraph 2 - page 40,  column 1, paragraph 1  page 42, column 1, paragraph 1 - page 43,  column 2, paragraph 1</p> <p>-----</p>	
P,A	<p>PAINTER G. R. ET AL.: "Evaluation of hexadecyloxypropyl-9-R-[2-(phosphonomethoxy)propyl]-adenine, CMX157, as a potential treatment for human immunodeficiency virus type 1 and hepatitis B virus infections"  ANTIMICROBIAL AGENTS AND CHEMOTHERAPY,  vol. 51, no. 10, October 2007 (2007-10),  pages 3505-3509, XP002485783  abstract  page 3505, column 2, paragraph 2 - page  3506, column 1, paragraph 2  compound 3  page 3508, column 1, paragraph 3 - column  2, paragraph 3</p> <p>-----</p>	
A	<p>JACOBSON M. A.: "Treatment of cytomegalovirus retinitis in patients with the acquired immunodeficiency syndrome"  DRUG THERAPY,  vol. 337, no. 2, 1997, pages 105-114,  XP002485817</p> <p>-----</p>	

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US2008/005322

## Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  

Although claims 24-28 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2.  Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3.  Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1.  As all required additional search fees were timely paid by the applicant, this international search report covers allsearchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2008/005322

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2004019232 A1	29-01-2004	US 2004127735 A1	01-07-2004
WO 2006110655 A	19-10-2006	EP 1868628 A2	26-12-2007