ADMINISTRATION OF TLR7 LIGANDS AND PRODRUGS THEREOF FOR TREATMENT OF INFECTION BY HEPATITIS C VIRUS

[0001] This application claims the benefit of U.S. Provisional Application No. 60/500,339, filed September 5, 2003, U.S. Provisional Application No. 60/518,996, filed November 10, 2003, and U.S. Provisional Application No. 60/518,997, filed November 10, 2003.

1. FIELD OF THE INVENTION

[0002] This invention relates to methods for treating or preventing hepatitis C virus infections in mammals using Toll-Like Receptor (TLR)7 ligands and prodrugs thereof. More particularly, this invention relates to methods of orally administering a therapeutically effective amount of one or more prodrugs of TLR7 ligands for the treatment or prevention of hepatitis C viral infection. Oral administration of these TLR7 immunomodulating ligands and prodrugs thereof to a mammal provides therapeutically effective amounts and reduced undesirable side effects.

2. BACKGROUND OF THE INVENTION

[0003] Immunomodulation by small molecules can be achieved by identifying compounds that bind and activate Toll-Like Receptors (TLRs). TLRs play an important role in innate immune responses in mammals and are often the first line of defense against pathogens such as bacteria and viruses. The various TLRs vary in their abundance in different mammalian cell types and also vary regarding the molecular structures that bind the TLR and activate signaling pathways. These signaling pathways lead to the range of responses associates with innate immunity.

[0004] TLRs detect PAMPs (pathogen-associated molecular patterns) and stimulate immune cells via the MyD88-dependent interleukin 1 receptor (IL-1R)-TLR signaling pathway, which leads to activation of the transcription factor NF-κB2. Ten functional family members of TLRs (TLR1 to TLR10) have been identified in humans. Akira S. *et al.*, *Nature Immunol.*, 2, 675-680 (2001). TLR2, TLR4, and TLR5 are crucial for the recognition of peptidoglycan, lipopolysacharide, and flagellin. Hayashi, F. *et al.*, *Nature*, 410, 1099-1103 (2001). TLR6 associates with TLR2 and recognizes lipoproteins from mycoplasma. Ozinsky, A., *et al.*, *Proc. Natl. Acad. Sci USA.*, 97, 13766-13771 (2000). TLR9 detects bacterial DNA containing unmethylated CpG motifs and TLR3 activates immune cells in response to double-stranded RNA. Hemmi, H. *et al.*, Nature, 408, 740-745 (2000).

[0005] A number of compounds, including guanosine analogs, substituted pyrimidines, and imidazoquinolines have been reported as ligands for TLR7. See, e.g.,

Hemmi *et al.*, *Nature* Immunol., 3, 196-200 (2002) (imiquimod and R-848 (resiquimod)); Jurk *et al.*, *Nat. Immunol.*, 3, 499 (2002) (R-848); and Lee *et al.*, *Proc. Natl. Acad. Sci USA*, 100, 6646-6651 (2003) (wherein guanosine analogs loxoribine, 7-thia-8-oxoguanosine (isatoribine), and 7-deazaguanosine, and the imidazoquinolines imiquimod and R-848 (resiquimod) selectively activate TLR7).

[0006] Prior to being linked as potential TLR7 ligands, guanosine analogs and other D- and L-purine nucleosides have been the subject of considerable research the past two decades. See, e.g., Reitz *et al.*, *J. Med. Chem.*, 37, 3561-78 (1994); Michael *et al.*, *J. Med. Chem.*, 36, 3431-36 (1993) (immunomodulatory guanosine analogs having substituents at the 7-and/or 8-positions); Patent No. 5,821,236 to Krenitsky *et al.* (disclosing 6-alkoxy derivatives of arabinofuranosyl purine derivatives that are useful for tumor therapy); U.S. Patent No. 5,041,426 to Robins *et al.* (certain pyrimido[4,5-d]pyridimine nucleosides are disclosed in as being effective in treatment against L1210 in BDF1 mice); Revankar *et al.*, *J. Med. Chem.*, 27, 1489-96 (1984) (3-Deazaguanine nucleosides and nucleotides demonstrating significant broad spectrum antiviral activity against certain DNA and RNA viruses);

[0007] A number of compounds known to be immunostimulants have recently been identified in the literature as TLR7 ligands, *see, e.g.*, Heil *et al., Eur. J. Immunol.*, 33(11), 2987-97 (2003), Lore *et al., J. Immunol.*, 171(8), 4320-8 (2003), Nagase *et al., J. Immunol.*, 171(8), 3977-82 (2003), Mohty *et al., J. Immunol.*, 171(7), 3385-93 (2003), Pinhal-Enfield, *et al., Am. J. Pathol.*, 163(2), 711-21 (2003), Doxsee *et al, J. Immunol.*, 171(3), 1156-63 (2003), Bottcher *et al., Neurosci. Lett.*, 344(1), 17-20 (2003), Kaisho *et al., Curr. Mol. Med.*, 3(4), 373-85 (2003), Okada *et al., Eur. J. Immunol.*, 33(4), 1012-9 (2003), Edwards *et al., Eur. J. Immunol.*, 33(4), 827-33 (2003), Akira *et al., Immunol. Lett.*, 85(2), 85-95 (2003), Ito *et al., Hum. Immunol.*, 63(12), 1120-5 (2002), Rothenfusser *et al., Hum. Immunol.*, 63(12), 1111-9 (2002), Yamamoto *et al., J. Immunol.*, 169(12), 6668-72 (2002), Gibson *et al., Cell Immunol.*, 218(1-2), 74-86 (2002), Horng *et al., Nature*, 420 (6913), 329-33 (2002), Yamamoto *et al., Nature*, 420(6913), 324-9 (2002), Applequist *et al., Int. Immunol.*, 14(9), 1065-74 (2002), Sato *et al., Int. Immunol.*, 14(7), 783-91 (2002); Jurk *et al., Nat. Immunol.*, 3(6), 499 (2002); Hornung *et al., J. Immunol.*, 168(9), 4531-7 (2002), Hemmi *et al., Nat. Immunol.*, 3(2), 196-200 (2002); Bruno *et al., Eur. J. Immunol.*, 31(11), 3403-12 (2001); Jarrossay *et al., Eur. J. Immunol.*, 31(11), 3388-93 (2001); Miettinen *et al., Genes Immun.*, 2(6), 349-55 (2001), Chuang *et al., Eur. Cytokine Netw.*, 11(3), 372-8 (2000), and Du *et al., Eur. Cytokine Netw.*, 11(3), 362-71 (2000).

[0008] These TLR7 ligands are known to stimulate immune responses *in vitro* and in animal species, and this has led to testing of the uses of these compounds for several therapeutic uses, including antiviral and cancer therapies. These compounds have been characterized as analogs or derivatives of a) guanosine, b) imidazoquinoline, and c) pyrimidine. See Akira, *Current Opinion*, 15, 5-11 (2003). One member (imiquimod) of the imidazoquinoline chemical class has been found effective for treating topical genital infections by papilloma virus. A second member of the imidazoquinoline class, resiquimod, has been tested for the treatment of HCV, but this compound failed to show anti-HCV effect at tolerated oral doses. Pockros *et al.*, *Gastroenterology*, 124 (Suppl 1), A-766 (2003).

[0009] Thus, while there has been some limited use of TLR7 ligands for the treatment of immunological disease and viral infections; see, *e.g.*, U.S. Patent Nos. 5,041,426 and 4,880,784 to Robins *et al.* (3-ß-D-ribofuranosylthiazolo[4,5-d]pyridimines demonstrating significant immunoactivity, including murine spleen cell proliferation and in vivo activity against Semliki Forest virus); United States Patent Application Publication No. US 2003/0199461 and WO 03/045968 to Averett *et al.* (3-ß-D-ribofuranosylthiazolo[4,5-d]pyrimidine nucleosides demonstrating activity against acute and chronic infections of both RNA and DNA viruses); to date ligands have proved ineffective for the treatment or prevention of Hepatitis C virus.

[0010] It is also known that the oral administration of many purine nucleoside analogs are subject to difficulties arising from poor absorption, poor solubility, or degradation in the digestive tract as a result of acidic or alkaline conditions or the action of enzymes, and/or combinations of these phenomena. Thus there remains a need for purine nucleoside analogs with improved oral availability and administration that are used to modulate aspects of the immune system.

[0011] Moreover, immunomodulatory nucleosides have relatively poor oral tolerability when compared to that of the intravenous route. Also, the gastrointestinal tract presents a particular tolerability barrier to immunologic agents by virtue of the large amount of immune tissue associated with the intestinal wall (i.e., gut). Although this is an important biologic mechanism for preventing invasion of the body by gut flora, the immune tissue also may become preferentially affected after oral administration of immunomodulatory compounds because of the high local concentrations of the administered compound in the gut. This leads to undesirable side effects, for example in the case of immune activating agents there is observed gastroenteritis and localized hemorrhagic effects.

[0012] A solution to the problem of effective oral delivery of immunomodulators is not evident in the literature. Available evidence indicates that systemic levels of administered drugs in this class have been limited by gastrointestinal toxicities arising after low oral doses. Therefore there remains a need for immunomodulating TLR7 ligands that have improved oral availability and reduced gastrointestinal irritancy.

3. SUMMARY OF THE INVENTION

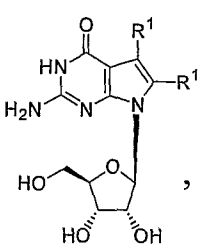
3.1 TLR7 Ligands

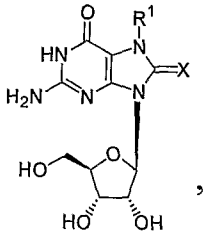
[0013] This invention encompasses novel methods for the treatment or prevention of hepatitis C viral infection, and novel pharmaceutical compositions which utilize TLR7 ligands or pharmaceutically acceptable salts, hydrates, metabolites or stereoisomers thereof.

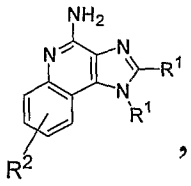
[0014] In one embodiment, the invention encompasses a method of treating or preventing a hepatitis C virus infection in a patient in need thereof comprising administering to the patient a therapeutically or prophylactically effective amount of a TLR7 ligand or a pharmaceutically acceptable salt, hydrate, metabolite or stereoisomer thereof or a pharmaceutically acceptable salt or hydrate of said stereoisomer.

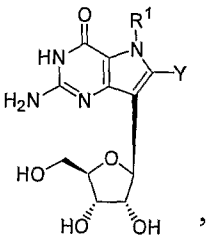
[0015] In another embodiment, the invention encompasses a method of treating or preventing a hepatitis C virus infection in a patient in need thereof comprising administering to the patient a therapeutically or prophylactically effective amount of a TLR7 ligand selected from analogs and derivatives of a) guanosine b) imidazoquinoline c) adenine, and d) pyrimidine.

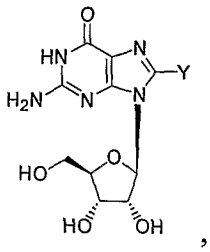
[0016] In another embodiment, the invention encompasses a method of treating or preventing a hepatitis C virus infection in a patient in need thereof comprising administering to the patient a therapeutically or prophylactically effective amount of a TLR7 ligand selected from

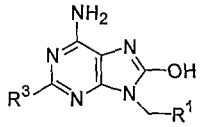

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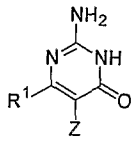

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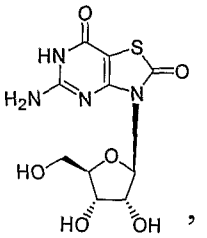

Id


Ie


If


Ig

, and


Ih

wherein:

each R^1 is H, or a substituted or unsubstituted alkyl, alkenyl, or alkynyl, which may be interrupted by one or more O, S, or N heteroatoms, or a substituted or unsubstituted aryl or heteroaryl;

R^2 is H, OH, SH, halo, or a substituted or unsubstituted alkyl, alkenyl, or alkynyl, which may be interrupted by one or more O, S, or N heteroatoms, or a substituted or unsubstituted -O-(alkyl), -O-(aryl), -O-(heteroaryl), -S-(alkyl), -S-(aryl), -S-(heteroaryl), aryl, or heteroaryl;

R^3 is H, OH, or SH, or a substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, heteroaryl, -O-(alkyl), -O-(aryl), -O-(heteroaryl), -S-(alkyl), -S-(aryl), -S-(heteroaryl), -NH(alkyl), -NH(aryl), -NH(heteroaryl), -NH(R^4)(alkyl), -NH(R^4)(aryl), or -NH(R^4)(heteroaryl), wherein R^4 is a substituted or unsubstituted alkyl;

X is O or S;

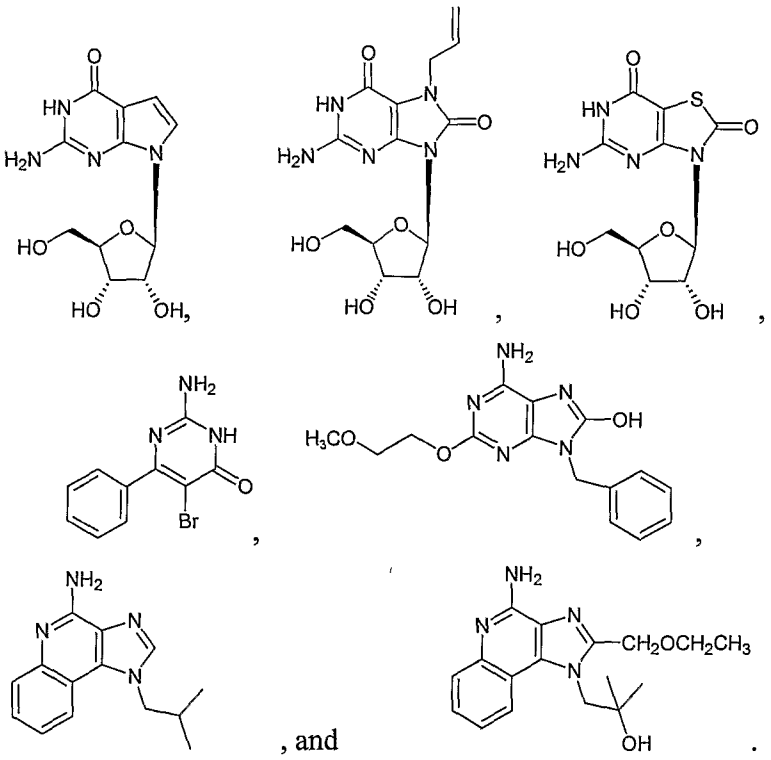
Y is H, halo, OH, OR^4, SH, SR^4, or a substituted or unsubstituted alkyl or aryl;

Z is H, halo, OH, OR^4, SH, or SR^4;

or a pharmaceutically acceptable salt, hydrate, metabolite or stereoisomer thereof or a pharmaceutically acceptable salt or hydrate of said stereoisomer.

[0017] In another embodiment, the invention encompasses a method of treating or preventing a hepatitis C virus infection in a patient in need thereof comprising administering to the patient a therapeutically or prophylactically effective amount of a TLR7 ligand selected from Formula Ia, Ib, Ic, Id, Ie, If, Ig, and Ih, wherein R^1 is H or a substituted or unsubstituted alkyl, alkenyl, or alkynyl; R^2 is H, OH, halo, or a substituted or unsubstituted alkyl, alkenyl, or alkynyl, or $-CH_2$-O-(alkyl); R^3 is H, OH, or SH, or a substituted or unsubstituted -O-(alkyl), -S-(alkyl), or -NH(alkyl); X is O or S; Y is H, halo, OH, OR^4, SH, or SR^4; and Z is H, halo, OH, OR^4, SH, or SR^4.

[0018] In another embodiment, the invention encompasses a method of treating or preventing a hepatitis C virus infection in a patient in need thereof comprising administering to the patient a therapeutically or prophylactically effective amount of a TLR7 ligand selected from



or a pharmaceutically acceptable salt, hydrate, metabolite or stereoisomer thereof.

[0019] In one aspect, the invention encompasses a method for treating or preventing hepatitis C virus infection in a mammal in need thereof, preferably in a human in need thereof.

[0020] In an alternative embodiment, the invention encompasses a method for treating or preventing hepatitis C virus infection in a patient in need thereof, comprising administering to the patient a therapeutically or prophylactically effective amount of a TLR7 ligand and a pharmaceutically acceptable excipient, carrier, or vehicle.

[0021] In an alternative embodiment, the invention encompasses a method for treating or preventing hepatitis C virus infection in a patient in need thereof, comprising administering to the patient a therapeutically or prophylactically effective amount of a TLR7 ligand orally, mucosally, topically or transdermally.

[0022] In a preferred embodiment, the invention encompasses a method for treating or preventing hepatitis C virus infection in a patient in need thereof, comprising administering to the patient a therapeutically or prophylactically effective amount of a TLR7 ligand parenterally.

[0023] In a separate embodiment, the invention encompasses a method for treating or preventing hepatitis C virus infection in a patient in need thereof, comprising administering to the patient a therapeutically or prophylactically effective amount of a TLR7 ligand and an additional therapeutic agent, preferably an additional antiviral or immunomodulatory agent.

[0024] The invention also encompasses pharmaceutical compositions suitable for parenteral administration to a patient comprising a therapeutically or pharmaceutically acceptable amount of a TLR7 ligand of the invention in a sterile form; pharmaceutical compositions suitable for oral administration to a patient comprising a therapeutically or pharmaceutically acceptable amount of a TLR7 ligand of the invention, wherein such compositions are formulated to reduce exposure of the subepithelial immune anatomy to the TLR7 ligand while improving systemic absorption of the TLR7 ligand; pharmaceutical compositions suitable for mucosal administration to a patient comprising a therapeutically or pharmaceutically acceptable amount of a TLR7 ligand of the invention, wherein such compositions are formulated to reduce exposure of the subepithelial immune anatomy to the TLR7 ligand while improving systemic absorption of the TLR7 ligand; and pharmaceutical compositions suitable for topical administration to a patient comprising a therapeutically or pharmaceutically acceptable amount of a TLR7 ligand of the invention, wherein such compositions are formulated to reduce exposure of the subepithelial immune anatomy to the TLR7 ligand while improving systemic absorption of the TLR7 ligand. Depending on the specific tissue to be treated, additional components, such as penetration enhancers, may be used prior to, in conjunction with, or subsequent to treatment with active ingredients of the invention. In a preferred embodiment, each of these compositions is in single unit dosage

form and comprising an amount of active ingredient sufficient to treat or prevent human infection by hepatitis C virus.

[0025] In a specific embodiment, the invention encompasses a pharmaceutical composition comprising a TLR7 ligand selected from analogs and derivatives of a) guanosine, b) imidazoquinoline, c) adenine, and d) pyrimidine.

[0026] In another specific embodiment, the invention encompasses a pharmaceutical composition comprising a TLR7 ligand selected from Formulas Ia, Ib, Ic, Id, Ie, If, Ig, and Ih, or a pharmaceutically acceptable salt, hydrate, metabolite or stereoisomer thereof or a pharmaceutically acceptable salt or hydrate of said stereoisomer.

3.2 TLR7 Ligand Prodrugs

[0027] This invention also encompasses novel methods for the treatment or prevention of hepatitis C viral infection, and novel pharmaceutical compositions which utilize TLR7 ligand prodrugs or pharmaceutically acceptable salts, hydrates, metabolites or stereoisomers thereof.

[0028] This invention also encompasses novel methods of treating diseases responsive to immuno therapy with immunologic agents, comprising orally administering a TLR7 ligand prodrug to a patient in need of immuno therapy, wherein the TLR7 prodrug achieves a therapeutically effective plasma concentration of the TLR7 ligand in the patient.

[0029] In one embodiment, the invention encompasses a method of treating a hepatitis C virus infection in a patient comprising orally administering to the patient a TLR7 ligand prodrug or a pharmaceutically acceptable salt, hydrate, or stereoisomer thereof, wherein the oral administration of the TLR7 ligand prodrug achieves a therapeutically effective plasma concentration of the TLR7 ligand while reducing undesirable side effects associated with TLR7 ligands. In a preferred embodiment, the TLR7 ligand prodrug is a masked TLR7 ligand prodrug.

[0030] In another embodiment, the invention also encompasses a method of treating diseases responsive to immuno therapy while reducing undesirable side effects associated with immunologic agents, comprising orally administering a TLR7 ligand prodrug to a patient in need of immuno therapy, wherein the TLR7 prodrug achieves a therapeutically effective plasma concentration of the TLR7 ligand in the patient. In a preferred embodiment, the TLR7 ligand prodrug is a masked TLR7 ligand prodrug.

[0031] In another embodiment, the oral administration of the TLR7 ligand prodrug improves the *in vivo* bioavailability of the TLR7 ligand. In a preferred embodiment, the oral administration of the TLR7 ligand prodrug achieves an *in vivo* effective plasma concentration of the TLR7 ligand that is 10% to 500% of the effective *in vivo* exposure

obtained upon oral administration of the TLR7 ligand alone. In another preferred embodiment, the oral administration of the masked TLR7 ligand prodrug achieves an *in vivo* effective plasma concentration of the TLR7 ligand that is 50% to 200% of the effective *in vivo* exposure obtained upon oral administration of the TLR7 ligand alone.

[0032] In another embodiment, the oral administration of the TLR7 ligand prodrug reduces adverse side effects. In a preferred embodiment, the side effect comprises gastrointestinal irritancy, wherein gastrointestinal irritancy comprises hemorrhage, lesions, and emesis.

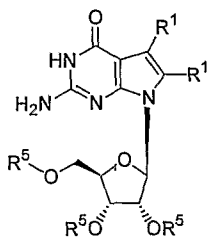
[0033] In another embodiment, the TLR7 ligand prodrug improves oral availability by at least 25% and reduces gastrointestinal irritancy by at least 50% in a patient relative to the oral administration of the TLR7 ligand alone. In another embodiment, the TLR7 ligand prodrug improves oral availability by at least 50% and reduces gastrointestinal irritancy by such that other toxicities become limiting in a patient relative to the oral administration of the TLR7 ligand alone.

[0034] In a preferred embodiment, the TLR7 ligand prodrug achieves a therapeutically effective plasma concentration that is 25% to 200% of the effective *in vivo* concentration of the TLR7 ligand in a patient after oral administration, with minimal gastrointestinal irritancy.

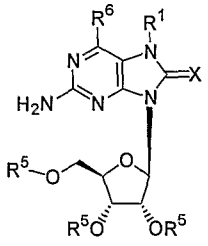
[0035] In one embodiment, the methods of the invention encompass administering to a patient in need thereof a therapeutically or prophylactically effective amount of a prodrug of a TLR7 ligand selected from analogs and derivatives of a) guanosine, b) imidazoquinoline, c) adenine, and d) pyrimidine.

[0036] In another embodiment, the methods of the invention encompass administering to a patient in need thereof a therapeutically or prophylactically effective amount of a prodrug of a TLR7 ligand selected from analogs and derivatives of a) guanosine, b) imidazoquinoline, c) adenine, and d) pyrimidine, wherein the prodrug is an (a) amide, carbamate, or amidine moiety after conversion of a TLR7 ligand amine substituent, (b) ester, carbonate, carbamate, ether, imidate, acetal, aminal, or ketal moiety after conversion of a TLR7 ligand alcohol substituent, (c) acetal or ketal moiety after conversion of a TLR7 ligand keto substituent, (d) imidate moiety after conversion of a TLR7 ligand carbonyl of an amido substituent, (e) deoxygenated moiety after conversion of a TLR7 ligand oxo substituent of pyrimidine or guanosine, or (f) amine.

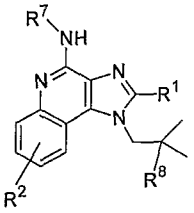
[0037] In another embodiment, the methods of the invention encompass administering to a patient in need thereof a therapeutically or prophylactically effective amount of a prodrug of a TLR7 ligand selected from



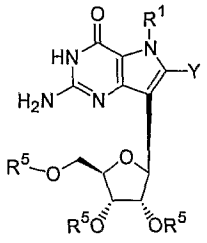
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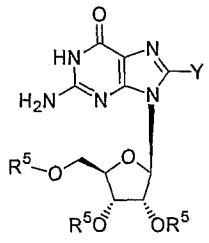
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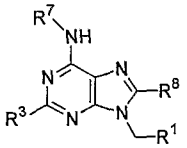
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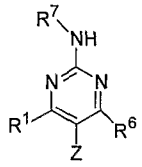
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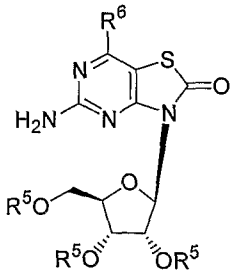
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IIf ,



IIg ; and



IIh ,

wherein:

each R^1 is H, or a substituted or unsubstituted alkyl, alkenyl, or alkynyl, which may be interrupted by one or more O, S, or N heteroatoms, or a substituted or unsubstituted aryl or heteroaryl;

R^2 is H, OH, SH, halo, or a substituted or unsubstituted alkyl, alkenyl, or alkynyl, which may be interrupted by one or more O, S, or N heteroatoms, or a substituted or unsubstituted -O-(alkyl), -O-(aryl), -O-(heteroaryl), -S-(alkyl), -S-(aryl), -S-(heteroaryl), aryl, or heteroaryl;

R^3 is H, OH, or SH, or a substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, heteroaryl, -O-(alkyl), -O-(aryl), -O-(heteroaryl), -S-(alkyl), -S-(aryl), -S-(heteroaryl), -NH(alkyl), -NH(aryl), -NH(heteroaryl), -NH(R^4)(alkyl), -NH(R^4)(aryl), or -NH(R^4)(heteroaryl);

R^4 is a substituted or unsubstituted alkyl;

R^5 is independently H, –C(O)(C_{1-18}alkyl), or a racemic, L-, or D- amino acid group

$-C(O)CHNH_2R^9$;

R^6 is H, OR^{10}, or $N(R^{11})_2$;

R^7 is independently H or a substituted or unsubstituted $-C(O)(C_1\text{-}_{18}alkyl)$ or $-C(O)_2(C_1\text{-}_{18}alkyl)$;

R^8 is H, –OH, -O-(alkyl), $-OCO_2(C_1\text{-}_{18}alkyl)$, $-OC(O)(C_1\text{-}_{18}alkyl)$, or a racemic, L-, or D-amino acid group $-OC(O)CHNH_2R^1$;

R^9 is H, or a substituted or unsubstituted alkyl, $C(O)CH(C_{1\text{-}6}\ alkyl)NH_2$, or $-C(O)CH(CH_2\text{-}aryl)NH_2$;

R^{10} is independently H, $C_{1\text{-}6}$ alkyl, $C_{3\text{-}7}$ alkenyl, $C_{3\text{-}7}$ alkynyl, $-(CR^{12}R^{13})_t(C_6\text{-}C_{10}\ aryl)$, $-(CR^{12}R^{13})_t(C_3\text{-}C_{10}\ cycloalkyl)$, $-(CR^{12}R^{13})_t(C_4\text{-}C_{10}\ heterocyclic)$, $-(CR^{12}R^{13})_{t>1}OH$, $-(CR^{12}R^{13})_{t>0}CO_2C_{1\text{-}18}$ alkyl, and $-(CR^{12}R^{13})_{t>0}N(R^{14})CO_2C_{1\text{-}18}$ alkyl, and $SO_2(aryl)$, wherein t is an integer from 0 to 6 unless otherwise indicated, and wherein the alkyl, alkenyl, alkynyl, aryl, cycloalkyl, and heterocyclic moieties of the foregoing groups are optionally substituted with substituents independently selected from halo, cyano, nitro, trifluoromethyl, trifluoromethoxy, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, hydroxy, C_1-C_6 alkoxy, $-NH_2$, -NH-alkyl, $-N(alkyl)_2$, -NH-aryl, -N(alkyl)(aryl), $-N(aryl)_2$, -NHCHO, -NHC(O)alkyl, -NHC(O)aryl, -N(alkyl)C(O)H, -N(alkyl)C(O)alkyl, -N(aryl)C(O)H, -N(aryl)C(O)alkyl, $-NHCO_2$alkyl, $-N(alkyl)CO_2$alkyl, $-NHC(O)NH_2$, $-N(alkyl)C(O)NH_2$, -NHC(O)NH-alkyl, $-NHC(O)\ N(alkyl)_2$, -N(alkyl)C(O)NH-alkyl, $N(alkyl)C(O)\ N(alkyl)_2$, $-NHSO_2$-alkyl, $-N(alkyl)SO_2$-alkyl, -C(O)alkyl, -C(O)aryl, -OC(O)alkyl, -OC(O)aryl, $-CO_2$-alkyl, $-CO_2$-aryl, $-CO_2H$, $-C(O)NH_2$, -C(O)NH-alkyl, $-C(O)N(alkyl)_2$, -C(O)NH-aryl, $-C(O)N(aryl)_2$, -C(O)N(alkyl)(aryl), -S(O)alkyl, -S(O)aryl, $-SO_2$alkyl, $-SO_2$aryl, $-SO_2NH_2$, $-SO_2$NH-alkyl, and $-SO_2N(alkyl)_2$;

R^{11} is independently H, $C_{1\text{-}6}$ alkyl, C_3-C_{10} cycloalkyl, or together with nitrogen forms a 5- or 6-membered heterocyclic ring;

R^{12} and R^{13} are independently H, $C_{1\text{-}6}$ alkyl, $C_{2\text{-}6}$ alkenyl, or $C_{2\text{-}6}$ alkynyl;

R^{14} is H, $C_{1\text{-}6}$ alkyl, or $-CH_2$-aryl;

X is O or S;

Y is H, halo, OH, OR^4, SH, SR^4, or a substituted or unsubstituted alkyl or aryl; and

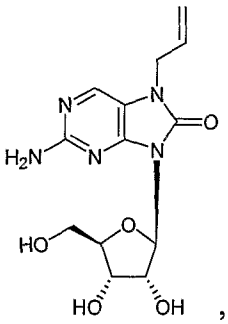
Z is H, halo, OH, OR^4, SH, or SR^4;

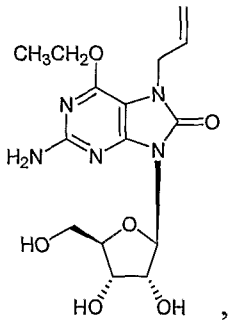
or a pharmaceutically acceptable salt, hydrate, metabolite or stereoisomer thereof or a pharmaceutically acceptable salt or hydrate of said stereoisomer.

[0038] In another embodiment, the invention encompasses a method of treating or preventing a hepatitis C virus infection in a patient in need thereof comprising administering to the patient a therapeutically or prophylactically effective amount of a TLR7 ligand

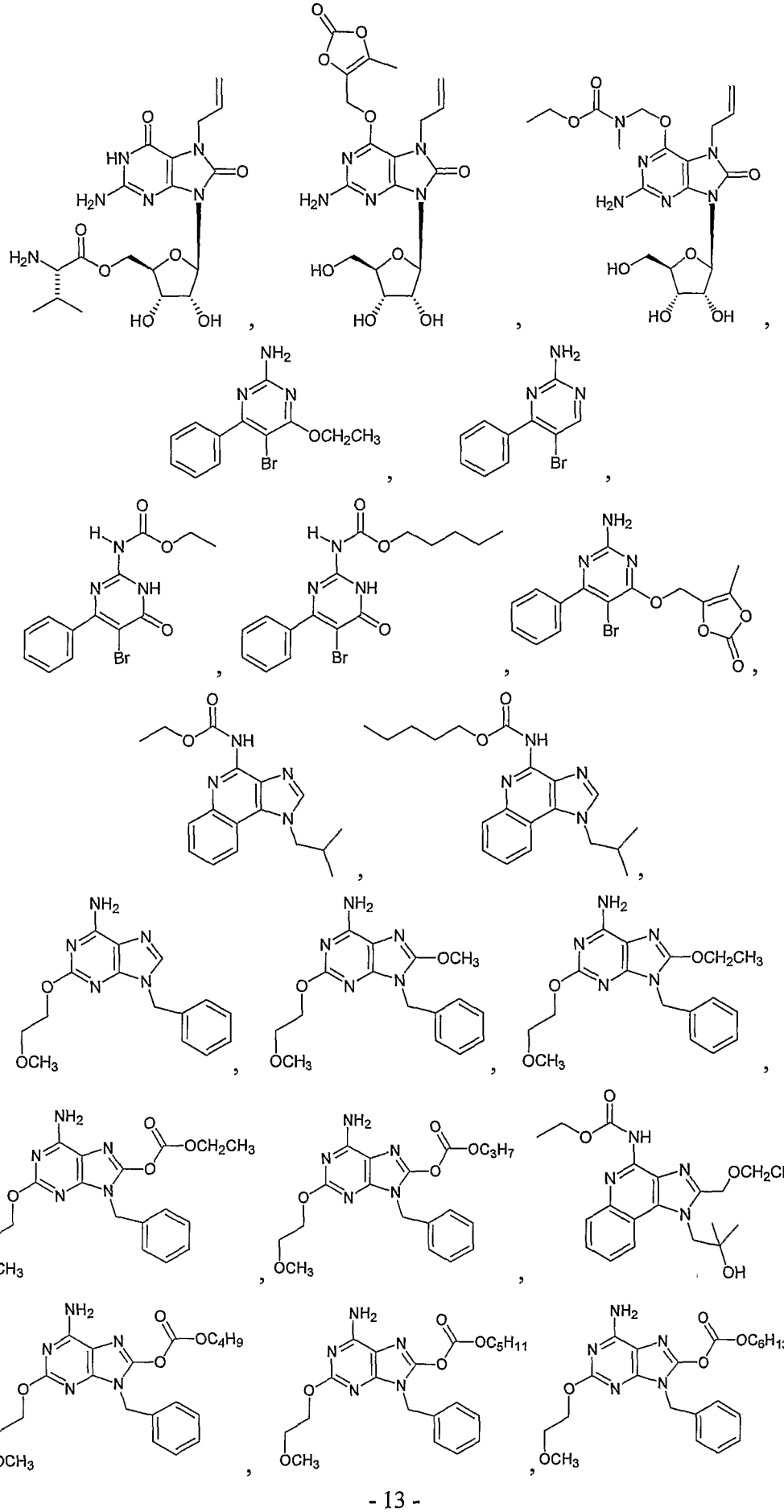
selected from Formula IIa, IIb, IIc, IId, IIe, IIf, IIg, and IIh, wherein R^1 is H or a substituted or unsubstituted alkyl, alkenyl, or alkynyl; R^2 is H, OH, halo, or a substituted or unsubstituted alkyl, alkenyl, or alkynyl, or $-CH_2$-O-(alkyl); R^3 is H, OH, or SH, or a substituted or unsubstituted -O-(alkyl), -S-(alkyl), or -NH(alkyl); R^5 is independently H, $-C(O)(C_{1-18}$alkyl), or a racemic, L-, or D- amino acid group $-C(O)CHNH_2R^9$, wherein R^9 is an unsubstituted alkyl; R^6 is H or OR^{10}, wherein R^{10} is independently C_{1-6} alkyl, C_{3-7} alkenyl, C_{3-7} alkynyl, $-(CR^{12}R^{13})_t(C_6-C_{10}$ aryl), $-(CR^{12}R^{13})_t(C_4-C_{10}$ heterocyclic), and $-(CR^{12}R^{13})_{t>0}N(R^{14})CO_2C_{1-18}$ alkyl, wherein t is an integer from 0 to 4 unless otherwise indicated, and wherein the alkyl, alkenyl, aryl, and heterocyclic moieties of the foregoing groups are optionally substituted with 1 to 3 substituents independently selected from halo, cyano, nitro, trifluoromethyl, trifluoromethoxy, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, hydroxy, C_1-C_6 alkoxy, $-CO_2$-alkyl, $-CO_2$-aryl, -OC(O)alkyl, and -OC(O)aryl, and wherein R^{12} and R^{13} are independently H, C_{1-6} alkyl, or C_{2-6} alkenyl; and R^{14} is H, $-CH_3$, or $-CH_2CH_3$; R^7 is independently H or a substituted or unsubstituted $-C(O)(C_{1-18}$alkyl) or $-C(O)_2(C_{1-18}$alkyl); R^8 is H, –OH, -O-(alkyl), $-OCO_2(C_{1-18}$alkyl), or a racemic, L-, or D- amino acid group $-OC(O)CHNH_2R^1$; X is O or S; Y is H, halo, OH, OR^4, SH, or SR^4; and Z is H, halo, OH, OR^4, SH, or SR^4.

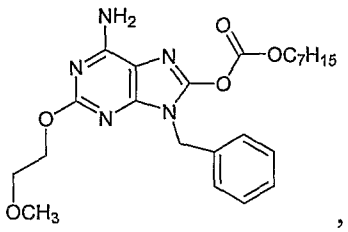
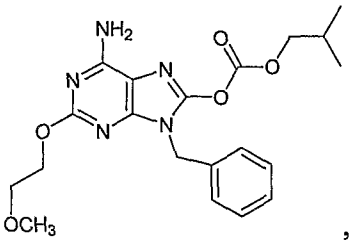
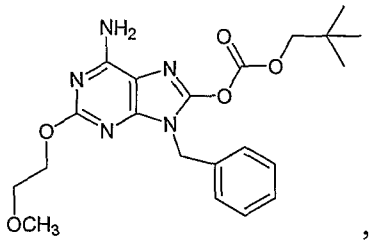
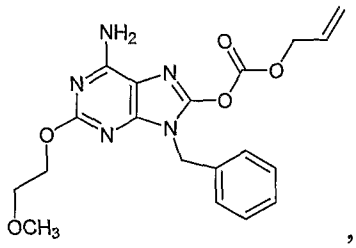
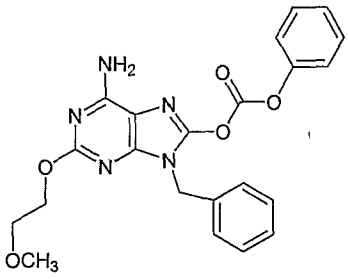
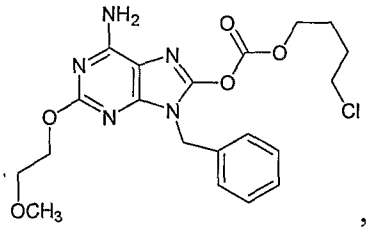
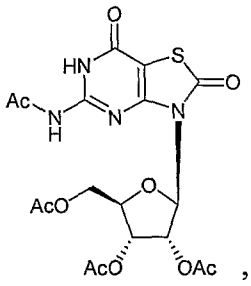
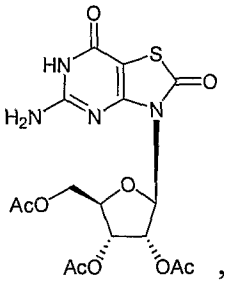
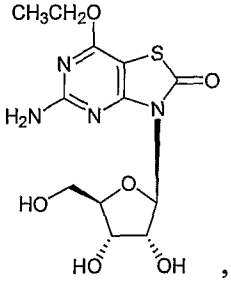
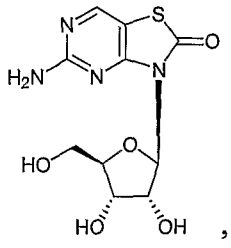
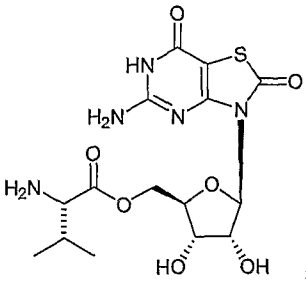
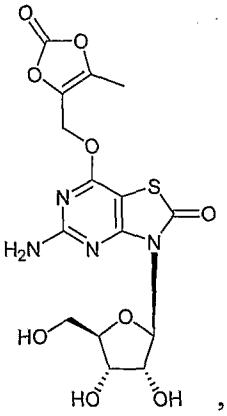
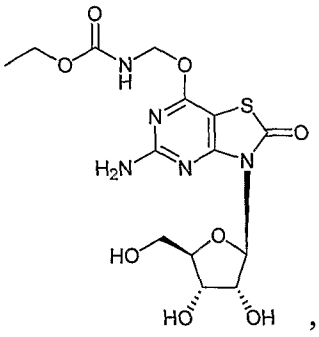
[0039] In specific embodiment, the invention encompasses a method of treating or preventing a hepatitis C virus infection in a patient in need thereof comprising administering to the patient a therapeutically or prophylactically effective amount of a prodrug of a TLR7 ligand selected from

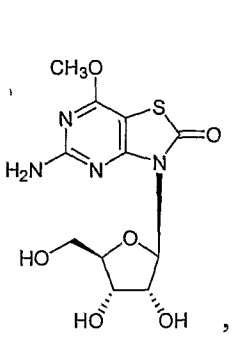
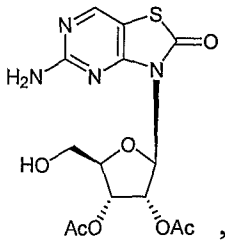
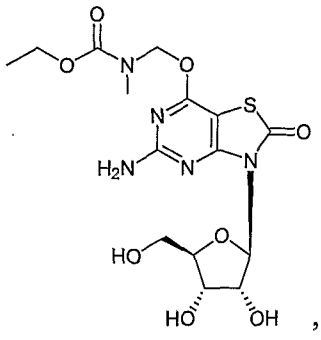


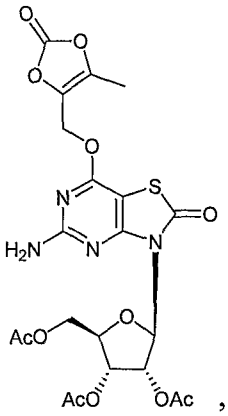
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or a pharmaceutically acceptable salt, hydrate, or stereoisomer thereof or a pharmaceutically acceptable salt or hydrate of said stereoisomer.

[0040] In another preferred embodiment of the invention, the TLR7 ligand prodrug is an amino acid ester prodrug of the TLR7 ligand. In another preferred embodiment, the amino acid ester prodrug of the TLR7 ligand is a valyl ester.

[0041] In one embodiment of the invention, R^5 is not a racemic, L-, or D- amino acid group $-C(O)CHNH_2R^9$. In another embodiment, R^5 is not a racemic, L-, or D- amino acid group $-C(O)CHNH_2R^9$ when the TLR7 ligand prodrug is selected from a compound of Formula IIh.

[0042] In another alternative embodiment, the invention encompasses a method for treating or preventing hepatitis C virus infection in a patient in need thereof, comprising administering to the patient a therapeutically or prophylactically effective amount of a prodrug of a TLR7 ligand and a pharmaceutically acceptable excipient, carrier, or vehicle.

[0043] In a separate embodiment, the invention encompasses a method for treating or preventing hepatitis C virus infection in a patient in need thereof, comprising administering to the patient a therapeutically or prophylactically effective amount of a prodrug of a TLR7 ligand and an additional therapeutic agent, preferably an additional antiviral or immunomodulatory agent.

[0044] The invention also encompasses pharmaceutical compositions suitable for parenteral administration to a patient comprising a therapeutically or pharmaceutically

acceptable amount a prodrug of a TLR7 ligand of the invention in a sterile form; pharmaceutical compositions suitable for parenteral administration to a patient comprising a therapeutically or pharmaceutically acceptable amount of a prodrug of a TLR7 ligand of the invention; pharmaceutical compositions suitable for mucosal administration to a patient comprising a therapeutically or pharmaceutically acceptable amount of a prodrug of a TLR7 ligand of the invention; and pharmaceutical compositions suitable for topical administration to a patient comprising a therapeutically or pharmaceutically acceptable amount of a prodrug of a TLR7 ligand of the invention. Depending on the specific tissue to be treated, additional components, such as penetration enhancers, may be used prior to, in conjunction with, or subsequent to treatment with active ingredients of the invention. In a preferred embodiment, each of these compositions is in single unit dosage form and comprising an amount of active ingredient sufficient to treat or prevent human infection by hepatitis C virus.

[0045] In a specific embodiment, the invention encompasses a pharmaceutical composition comprising a prodrug of a TLR7 ligand selected from Formula IIa, IIb, IIc, IId, IIe, IIf, IIg, and IIh, or a pharmaceutically acceptable salt, hydrate or stereoisomer thereof or a pharmaceutically acceptable salt or hydrate of said stereoisomer.

[0046] In another embodiment of the invention, and depending on the specific tissue to be treated, additional components including, but not limited to penetration enhancers, molecules which target the area of the infection and molecules which reduce the *in vivo* toxicity of the prodrug of a TLR7 ligand may be used prior to, in conjunction with, or subsequent to treatment with one or more prodrugs of TLR7 ligands of the invention.

[0047] The TLR7 ligand prodrugs are useful as immune system enhancers and have certain immune system properties including modulation, mitogenicity, augmentation, and/or potentiation or they are intermediates for compounds that have these properties. The compounds are expected to express effects after administration to a mammal on at least one of the cell populations characterized as the natural killer cells, macrophages, dendritic cells, and lymphocyte cells of the immune system of a host. Because of these properties they are useful as an anti-infective including, but not limited to antiviral agents, and as antitumor agents or as intermediates for the same. They can be used to treat an affected host by serving as the active ingredients of suitable pharmaceutical compositions.

[0048] In one aspect of the invention, TLR7 ligand prodrugs are utilized to treat the full range of viral diseases in mammals by administering to the mammal a therapeutically effective amount of the compounds. Viral diseases contemplated to be treated with TLR7 ligand prodrugs include acute and chronic infections caused by both RNA and DNA

viruses. Without limiting in any way the range of viral infections that may be treated, TLR7 ligand prodrugs are particularly useful in the treatment of infections caused by adenovirus, cytomegalovirus, hepatitis A virus (HAV), hepatitis B virus (HBV), flaviviruses including Yellow Fever virus, hepacivirus including hepatitis C virus (HCV), herpes simplex type 1 and 2, herpes zoster, human herpesvirus 6, human immunodeficiency virus (HIV), human papilloma virus (HPV), influenza A virus, influenza B virus, measles, parainfluenza virus, pestivirus, poliovirus, poxvirus (including smallpox and monkeypox virus), rhinovirus, coronovirus, respiratory syncytial virus (RSV), multiple families of viruses that cause hemorrhagic fevers, including the Arenaviruses (LCM, Junin virus, Machupo virus, Guanarito virus, and Lassa Fever), the Bunyaviruses (Hanta viruses and Rift Valley Fever) and Filoviruses (Ebola and Marburg virus), a range of viral encephalitides including West Nile virus, LaCrosse virus, California Encephalitis virus, Venezuelan Equine Encephalitis virus, Eastern Equine Encephalitis virus, Western Equine Encephalitis virus, Japanese Encephalitis virus, Kysanur Forest virus, and tickborne viruses such as Crimean-Congo Hemorrhagic fever virus.

[0049] In another aspect of the invention, TLR7 ligand prodrugs are utilized to treat bacterial, fungal, and protozoal infections in mammals by administering to the mammal a therapeutically effective amount of the prodrugs. The full range of pathogenic microorganisms is contemplated to be treatable by the TLR7 ligand prodrugs of the present invention, including without limitation those organisms that are resistant to antibiotics. The ability of TLR7 ligand prodrugs to activate multiple components of the immune system bypasses resistance mechanisms commonly found to reduce susceptibility to antibiotics, and thus treatment of infections in a mammal caused by such resistant microorganisms by TLR7 ligand prodrugs is a particular utility of the present invention.

[0050] In another aspect of the invention, TLR7 ligand prodrugs are utilized to treat tumors in mammals by administering to the mammal a therapeutically effective amount of the prodrugs. Tumors or cancers contemplated to be treated include both those arising from aberrations in normal cellular processes as well as those caused by virus, and the effect may involve inhibiting the spread of cancerous cells, accelerating the killing of cancerous cells, inhibiting transformation of virus-infected cells to a neoplastic state, inhibiting the spread of viruses from transformed cells to other normal cells, and/or arresting the growth of virus-transformed cells. The prodrugs of TLR7 ligands are expected to be useful against a broad spectrum of tumors including but not limited to carcinomas, sarcomas, and leukemias. Included in such a class are mammary, colon, bladder, lung, prostate, stomach, and pancreas carcinomas and lymphoblastic and myeloid leukemias.

[0051] In another aspect of the invention, a method of treating a mammal comprises administering a therapeutically and/or prophylactically effective amount of a pharmaceutical containing a TLR7 ligand prodrug of the invention. In this aspect the effect may relate to modulation of some portion of the mammal's immune system, especially modulation of cytokine activities of Th1 and Th2, including but not restricted to the interleukin family, e.g., IL-1 through IL-12, and other cytokines such as TNF alpha, and interferons including interferon alpha, interferon beta, and interferon gamma, and their downsteam effectors. Where modulation of Th1 and Th2 cytokines occurs, it is contemplated that the modulation may include stimulation of both Th1 and Th2, suppression of both Th1 and Th2, stimulation of either Th1 or Th2 and suppression of the other, or a bimodal modulation in which one effect on Th1/Th2 levels (such as generalized suppression) occurs at a high concentration, while another effect (such as stimulation of either Th1 or Th2 and suppression of the other) occurs at a lower concentration.

[0052] In another aspect of this invention, pharmaceutical compositions containing a prodrug of a TLR7 ligand are administered in therapeutically effective doses to a mammal that is receiving immunomodulatory drugs not included in this invention. In a preferred aspect, the doses of the immunomodulatory drug are reduced below their customary effective dose, to reduce adverse effects. In a second preferred aspect, the immunomodulatory drug is used at its customary dose, but with an improved therapeutic effect when a prodrug of a TLR7 ligand is also administered.

[0053] In another aspect of the invention, pharmaceutical compositions containing a prodrug of a TLR7 ligand are administered in a therapeutically effective dose to a mammal that is receiving anti-infective drugs not included in this invention. In a preferred aspect of this invention, the pharmaceutical compositions containing a prodrug of a TLR7 ligand are administered in a therapeutically effective dose with anti-infective drug(s) that act directly upon the infectious agent to inhibit the growth of or kill the infectious agent.

4. BRIEF DESCRIPTION OF THE DRAWINGS

[0054] Figure 1 is a graphical depiction of plasma levels of isatoribine and interferon alpha in mice.

[0055] Figure 2 is a graphical depiction of Viral Load Changes in HCV infected Patients receiving isatoribine.

5. DETAILED DESCRIPTION OF THE INVENTION

5.1 DEFINITIONS

[0056] Where the following terms are used in this specification, they are used as defined below:

[0057] The terms "comprising" and "including" are used herein in their open, non-limiting sense.

[0058] The term "nucleoside" refers to a compound composed of any pentose or modified pentose moiety attached to a specific position of a heterocycle or to the natural position of a purine (9-position) or pyrimidine (1-position) or to the equivalent position in an analog.

[0059] The term "purine" refers to nitrogenous bicyclic heterocycles.

[0060] The term "D-nucleosides" refers to the nucleoside compounds that have a D-ribose sugar moiety (e.g., Adenosine).

[0061] The term "L-nucleosides" refers to the nucleoside compounds that have a L-ribose sugar moiety.

[0062] The term "immunomodulator" refers to natural or synthetic products capable of modifying the normal or aberrant immune system through stimulation or suppression..

[0063] The term "NOAEL" is the No Observed Adverse Event Level, which is a toxicology term for the dose of drug that results in no significant toxicity under the specified conditions of dose level, frequency, duration in a selected species.

[0064] "Ligand" means a low molecular weight molecule capable of binding to a biologic receptor. A ligand may be either an agonist or an antagonist, or may have no effect.

[0065] An "agonist" is a ligand that, upon binding, stimulates the receptor to exert a biologic response that is consistent with the normal biologic activity of the receptor.

[0066] An "antagonist" is a ligand that, upon binding, causes the receptor to not exert the normal biologic activity of the receptor.

[0067] The term "mammal" includes both animals and humans.

[0068] The term "preventing" refers to the ability of a compound or composition of the invention to prevent a disease identified herein in mammals diagnosed as having the disease or who are at risk of developing such disease. The term also encompasses preventing further progression of the disease in mammals who are already suffering from or have symptoms of such disease.

[0069] The term "treating" refers to:

(i) preventing a disease, disorder, or condition from occurring in a mammal that may be predisposed to the disease, disorder and/or condition, but has not yet been diagnosed as having it;

(ii) inhibiting the disease, disorder, or condition, i.e., arresting its development; and

(iii) relieving the disease, disorder, or condition, i.e., causing regression of the disease, disorder, and/or condition.

[0070] The terms "α" and "β" indicate the specific stereochemical configuration of a substituent at an asymmetric carbon atom in a chemical structure as drawn.

[0071] The terms "patient" or "subject" mean an animal (e.g., cow, horse, sheep, pig, chicken, turkey, quail, cat, dog, mouse, rat, rabbit, guinea pig, etc.) or a mammal, including chimeric and transgenic animals and mammals. In the treatment or prevention of HCV infection, the term "patient" or "subject" preferably means a monkey or a human, most preferably a human. In a specific embodiment the patient or subject is infected by or exposed to the hepatitis C virus. In certain embodiments, the patient is a human infant (age 0-2), child (age 2-17), adolescent (age 12-17), adult (age 18 and up) or geriatric (age 70 and up) patient. In addition, the patient includes immunocompromised patients such as HIV positive patients, cancer patients, patients undergoing immunotherapy or chemotherapy. In a particular embodiment, the patient is a healthy individual, i.e., not displaying symptoms of other viral infections.

[0072] The term a "therapeutically effective amount" refers to an amount of the TLR7 ligand or prodrug of a TLR7 ligand of the invention sufficient to provide a benefit in the treatment or prevention of viral disease, to delay or minimize symptoms associated with viral infection or viral-induced disease, or to cure or ameliorate the disease or infection or cause thereof. In particular, a therapeutically effective amount means an amount sufficient to provide a therapeutic benefit *in vivo*. Used in connection with an amount of a compound of the invention, the term preferably encompasses a non-toxic amount that improves overall therapy, reduces or avoids symptoms or causes of disease, or enhances the therapeutic efficacy of or synergies with another therapeutic agent.

[0073] The term a "prophylactically effective amount" refers to an amount of a compound of the invention or other active ingredient sufficient to result in the prevention of infection, recurrence or spread of viral infection. A prophylactically effective amount may refer to an amount sufficient to prevent initial infection or the recurrence or spread of the infection or a disease associated with the infection. Used in connection with an amount of a compound of the invention, the term preferably encompasses a non-toxic amount that improves overall prophylaxis or enhances the prophylactic efficacy of or synergizes with another prophylactic or therapeutic agent.

[0074] The term "in combination" refers to the use of more than one prophylactic and/or therapeutic agents simultaneously or sequentially and in a manner that their respective effects are additive or synergistic.

[0075] The term "pharmaceutically acceptable salts" refer to salts prepared from pharmaceutically acceptable non- toxic acids or bases including inorganic acids and bases and organic acids and bases. If the inventive TLR7 ligand prodrug is a base, the desired pharmaceutically acceptable salt may be prepared by any suitable method available in the art, for example, treatment of the free base with an inorganic acid, such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and the like, or with an organic acid, such as acetic acid, maleic acid, succinic acid, mandelic acid, fumaric acid, malonic acid, pyruvic acid, oxalic acid, glycolic acid, salicylic acid, a pyranosidyl acid, such as glucuronic acid or galacturonic acid, an alpha-hydroxy acid, such as citric acid or tartaric acid, an amino acid, such as aspartic acid or glutamic acid, an aromatic acid, such as benzoic acid or cinnamic acid, a sulfonic acid, such as p-toluenesulfonic acid or ethanesulfonic acid, or the like. If the inventive TLR7 ligand prodrug is an acid, the desired pharmaceutically acceptable salt may be prepared by any suitable method, for example, treatment of the free acid with an inorganic or organic base, such as an amine (primary, secondary or tertiary), an alkali metal hydroxide or alkaline earth metal hydroxide, or the like. Illustrative examples of suitable salts include organic salts derived from amino acids, such as glycine and arginine, ammonia, primary, secondary, and tertiary amines, and cyclic amines, such as piperidine, morpholine and piperazine, and inorganic salts derived from sodium, calcium, potassium, magnesium, manganese, iron, copper, zinc, aluminum and lithium.

[0076] The term "prodrug" is intended to mean any chemical entity that after administration is converted via metabolic actions or solvolysis to a different chemical entity that retains biological activity.

[0077] The term "TLR7 ligand prodrug" is intended to mean any chemical entity that after administration is converted via metabolic actions or solvolysis to a different chemical entity that retains biological activity and that is a ligand for TLR7. A TLR7 ligand prodrug may itself be a ligand for TLR7, or it may be "masked" in that it does not function efficiently as a TLR7 ligand.

[0078] The term "masked TLR7 ligand prodrug" is intended to mean any chemical entity that after administration is converted via metabolic actions or solvolysis to a different chemical entity that retains biological activity and that is a ligand for TLR7, and where the

administered chemical entity is a less efficient ligand for TLR7 than the chemical entity arising from metabolic conversion or solvolysis.

[0079] The term "a pharmaceutically active metabolite" is intended to mean a pharmacologically active product produced through metabolism in the body of a specified compound or salt thereof. After entry into the body, most drugs are substrates for chemical reactions that may change their physical properties and biologic effects. These metabolic conversions, which usually affect the polarity of the TLR7 ligand, alter the way in which drugs are distributed in and excreted from the body. However, in some cases, metabolism of a drug is required for therapeutic effect. For example, many anticancer drugs of the anti-metabolite class must be converted to their active forms after they have been transported into a cancer cell.

[0080] As used herein, unless otherwise specified, the term "alkyl" means a saturated straight chain or branched non-cyclic hydrocarbon having from 1 to 20 carbon atoms, preferably 1-10 carbon atoms and most preferably 1-4 carbon atoms. Representative saturated straight chain alkyls include -methyl, -ethyl, -n-propyl, -n-butyl, -n-pentyl, -n-hexyl, -n-heptyl, -n-octyl, -n-nonyl and -n-decyl; while saturated branched alkyls include -isopropyl, -sec-butyl, -isobutyl, -tert-butyl, -isopentyl, 2-methylbutyl, 3-methylbutyl, 2-methylpentyl, 3-methylpentyl, 4-methylpentyl, 2-methylhexyl, 3-methylhexyl, 4-methylhexyl, 5-methylhexyl, 2,3-dimethylbutyl, 2,3-dimethylpentyl, 2,4-dimethylpentyl, 2,3-dimethylhexyl, 2,4-dimethylhexyl, 2,5-dimethylhexyl, 2,2-dimethylpentyl, 2,2-dimethylhexyl, 3,3-dimtheylpentyl, 3,3-dimethylhexyl, 4,4-dimethylhexyl, 2-ethylpentyl, 3-ethylpentyl, 2-ethylhexyl, 3-ethylhexyl, 4-ethylhexyl, 2-methyl-2-ethylpentyl, 2-methyl-3-ethylpentyl, 2-methyl-4-ethylpentyl, 2-methyl-2-ethylhexyl, 2-methyl-3-ethylhexyl, 2-methyl-4-ethylhexyl, 2,2-diethylpentyl, 3,3-diethylhexyl, 2,2-diethylhexyl, 3,3-diethylhexyl and the like. An alkyl group can be unsubstituted or substituted.

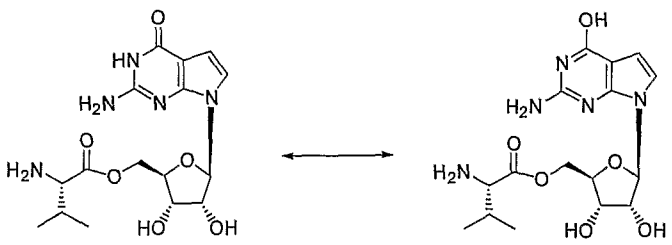
[0081] As used herein, unless otherwise specified the term "aryl" means a carbocyclic aromatic ring containing from 5 to 14 ring atoms. The ring atoms of a carbocyclic aryl group are all carbon atoms. Aryl ring structures include compounds having one or more ring structures such as mono-, bi-, or tricyclic compounds as well as benzo-fused carbocyclic moieties such as 5,6,7,8-tetrahydronaphthyl and the like. Preferably, the aryl group is a monocyclic ring or bicyclic ring. Representative aryl groups include phenyl, tolyl, anthracenyl, fluorenyl, indenyl, azulenyl, phenanthrenyl and naphthyl. A carbocyclic aryl group can be unsubstituted or substituted.

[0082] The term "substituted" means that the specified group or moiety bears one or more substituents. The term "unsubstituted" means that the specified group bears no

substituents. A "substituted alkyl" or "substituted aryl" is substituted by one or more substituents including halogen (F, Cl, Br, or I), lower alkyl (C_{1-6}), -OH, $-NO_2$, -CN, $-CO_2H$, -O-lower alkyl, -aryl, -aryl-lower alkyl, $-CO_2CH_3$, $-CONH_2$, $-OCH_2CONH_2$, $-NH_2$, $-SO_2NH_2$, haloalkyl (e.g., $-CF_3$, $-CH_2CF_3$), -O-haloalkyl (e.g., $-OCF_3$, $-OCHF_2$), and the like.

[0083] As used herein and unless otherwise indicated, the term "optically pure" or "stereomerically pure" means a composition that comprises one stereoisomer of a compound and is substantially free of other stereoisomers of that compound. For example, a stereomerically pure compound having one chiral center will be substantially free of the opposite enantiomer of the compound. A typical stereomerically pure compound comprises greater than about 80% by weight of one stereoisomer of the compound and less than about 20% by weight of other stereoisomers of the compound, more preferably greater than about 90% by weight of one stereoisomer of the compound and less than about 10% by weight of the other stereoisomers of the compound, even more preferably greater than about 95% by weight of one stereoisomer of the compound and less than about 5% by weight of the other stereoisomers of the compound, and most preferably greater than about 97% by weight of one stereoisomer of the compound and less than about 3% by weight of the other stereoisomers of the compound. Since many of the compounds of the invention comprise saccharides which can exist in either the D or L forms, the invention encompasses either or both D and L sugars. As such, for example, a stereomerically pure D sugar will be substantially free of the L form. In an alternative embodiment, the use of L forms of a TLR7 ligand will be substantially free of the D form. Thus, the methods and compositions disclosed herein include in an alternative embodiment the use of such levorotatory sugars or polymers made therefrom.

[0084] The compounds of the invention may exhibit the phenomenon of tautomerism. While Formulas I and II cannot expressly depict all possible tautomeric forms, it is to be understood that Formula I is intended to represent any tautomeric form of the depicted compound and are not to be limited merely to a specific compound form depicted by the formula drawings. For example, it is understood that regardless of whether or not the substituents are shown in their enol or their keto form, they represent the same compound (as shown in the Formula IIa example below).



5.2 Identification of TLR7 Ligands

[0085] Known TLR7 ligands include, but are not limited to (1) guanosine analogs, such as 7-deazaguanosine and related compounds, including but not limited to those described in Townsend, *J. Heterocyclic Chem*, 13, 1363 (1976), and Seela, *et al, Chem. Ber.*, 114(10), 3395-3402 (1981); 7-allyl, 8-oxo-guanosine (loxorabine) and related compounds, including but not limited to those described in Reitz, *et al., J. Med. Chem*, 37, 3561-3578 (1994); 7-methyl, 9-deazaguanosine and related compounds including, but not limited to, those described in Girgis *et al., J. Med. Chem.*, 33, 2750-2755 (1990); 8-bromoguanosine and other 8-halogen substituted purines compounds including, but not limited to, those described in United States Patent No. 4,643,992; 6-amino-9-benzyl-2-butoxy-9*H*-purin-8-ol, and other 2, 6, 8, 9 -substituted purines including, but not limited to, those described in Hirota *et al., J. Med. Chem.*, 45, 5419-5422 (2002), Henry *et al., J. Med. Chem.*,33, 2127-2130 (1990), Michael *et al., J. Med. Chem.*, 36, 3431-3436 (1993), Furneaux *et al., J. Org. Chem.*, 64 (22), 8411-8412 (1999), Barrio *et al; J. Org. Chem.*, 61, 6084-6085 (1996), United States Patent No. 4,539,205, United States Patent No. 5,011,828, United States Patent No. 5,041,426, United States Patent No. 4,880,784, and International Patent Application Publication Nos. WO 94/07904; (2) imidazoquinolines, including but not limited to 1-(4-amino-2-ethoxymethyl-imidazo[4,5-*c*]quinolin-1-yl)-2-methyl-propan-2-ol (imiquimoid), as described in International Patent Application Publication No. WO 94/17043; 1-isobutyl-1*H*-imidazo[4,5-*c*]quinolin-4-ylamine (resiquimoid) as described in International Patent Application Publication No. WO 94/17043 and United States Patent Application Nos. 10/357,777 (United States Patent Application Publication No. US 2003/0195209), 10/357,733 (United States Patent Application Publication No. US 2003/0186949), 10/358,017(United States Patent Application Publication No. US 2003/0176458), 10/357,995(United States Patent Application Publication No. US 2003/0162806), 10/165,222 (United States Patent Application Publication No. US 2003/0100764), 10/011,921(United States Patent Application Publication No. US 2003/0065005) and 10/013,059 (United States Patent Application Publication No. US 2002/0173655); United States Patent No. 5,395,937; International Patent Application Publication No. WO 98/17279; and (3) pyrimidine derivatives, including but not limited to 2-amino-6-bromo-5-phenyl-3*H*-pyrimidin-4-one (bropirimine), and similar substituted pyrimidines including, but not limited to, those described in Wierenga *et al., J. Med. Chem*, 23, 239-240 (1980), Fan *et al., J. Heterocyclic Chem.*, 30, 1273 (1993), Skilnick *et al., J. Med. Chem.*, 29, 1499-1504 (1986), Fried, *et al., J. Med. Chem.*, 23, 237-239 (1980), and Fujiwara *et al., Bioorg. Med. Chem. Lett.*, 10(12) 1317-1320 (2000). The entireties of each

of the patents, patent publications and publications identified herein are incorporated herein by reference.

[0086] In addition to the above TLR7 ligands, additional TLR7 ligands can be readily identified by known screening methods. See, e.g., Hirota *et al.*, *J. Med. Chem.*, 45, 5419-5422 (2002); and Akira S. *et al.*, *Immunology Letters*, 85, 85-95 (2003). Using a variant of one of these known screening methods (as described in Section 6.1), analogs and derivatives of adenine were also identified as TLR7 ligands. Adenine derivatives known in the art are described in European Patent Application Publication Nos. EP 1 035 123, EP 1 043 021, and EP 0 882 727; United States Patent No. 6,376,501; United States Patent No. 6,329,381; United States Patent No.6,028,076, and United States Patent Application Publication No. US 2003/0162806.

[0087] The TLR7 ligands of Formulas Ia-Ih can be synthesized using methods known to one of skill in the art, particularly in light of the references and patents listed above.

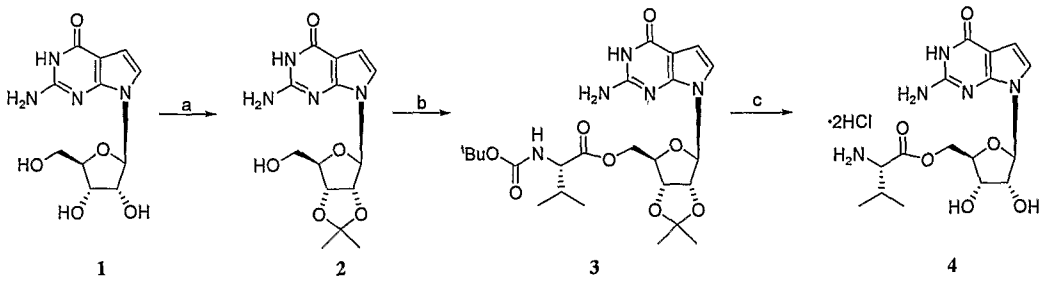
5.3 Preparation of TLR7 Ligand Prodrugs

[0088] The TLR7 ligand prodrugs of the invention are prepared by making an (a) amide, carbamate, or amidine moiety after conversion of a TLR7 ligand amine substituent, (b) ester, carbonate, carbamate, ether, imidate, acetal, or ketal moiety after conversion of a TLR7 ligand alcohol substituent, (c) acetal or ketal moiety after conversion of a TLR7 ligand amine substituent, (d) imidate moiety after conversion of a TLR7 ligand carbonyl of an amido substituent, (e) deoxygenated moiety after conversion of a TLR7 ligand oxo substituent of pyrimidine or guanosine, or (f) amine. For example, TLR7 ligand prodrugs are prepared by either (1) converting an hydroxyl (OH) substituents of the TLR7 ligand into an amino acid ester, or (2) making an amine substituent of the TLR7 ligand into an amide or carbamate. The process for preparing prodrugs is well known in the art and is described by *Burger's Medicinal Chemistry and Drug Chemistry*, 1, 172-178, 949-982 (1995). See also Bertolini *et al.*, *J. Med. Chem.*, 40, 2011-2016 (1997); Shan, *et al.*, *J. Pharm. Sci.*, 86 (7), 765-767; Bagshawe, *Drug Dev. Res.*, 34, 220-230 (1995); Bodor, *Advances in Drug Res.*, 13, 224-331 (1984); Bundgaard, *Design of Prodrugs* (Elsevier Press 1985); Larsen, *Design and Application of Prodrugs*, Drug Design and Development (Krogsgaard-Larsen *et al.*, eds., Harwood Academic Publishers, 1991); Dear *et al.*, *J. Chromatogr. B*, 748, 281-293 (2000); Spraul *et al.*, *J. Pharmaceutical & Biomedical Analysis*, 10, 601-605 (1992); and Prox *et al.*, *Xenobiol.*, 3, 103-112 (1992).

[0089] Schemes 1-18 show a general procedure to prepare representative compounds of Formula II.

[0090] Schemes 1-6 describe how 5'-amino acid esters can be synthesized from analogs and derivatives of guanosine.

Scheme 1

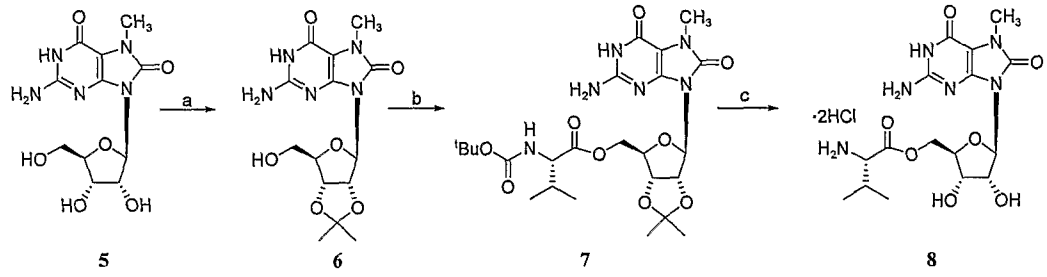


1 2 3 4

Townsend, *JHC*, 13, **1976**, 1363
Seela, et al, *Chem. Ber.*, 114, *10*, **1981**, 3395-3402

a) 2,2-dimethoxypropane, acetone, DMSO, $MeSO_3H$, 0 °C
b) BOC-NHCHR$^1CO_2H$, EDC, DMAP, PhMe, 0 °C - rt
c) anh. HCl, *i*PrOAc, *i*PrOH

Scheme 2

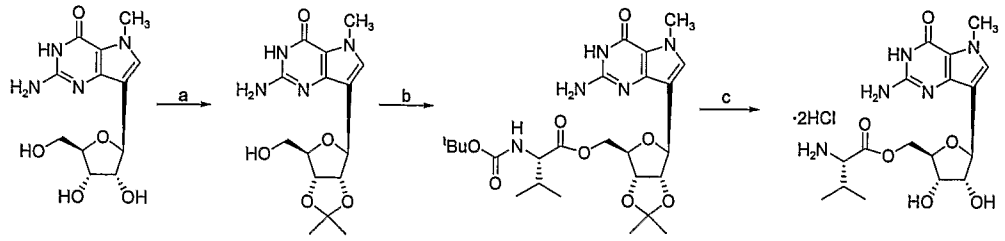


5 6 7 8

Reitz, et al, *JMC*, 37, **1994**, 3561-3578

a) 2,2-dimethoxypropane, acetone, DMSO, $MeSO_3H$, 0 °C
b) BOC-NHCHR$^1CO_2H$, EDC, DMAP, PhMe, 0 °C - rt
c) anh. HCl, *i*PrOAc, *i*PrOH

Scheme 3

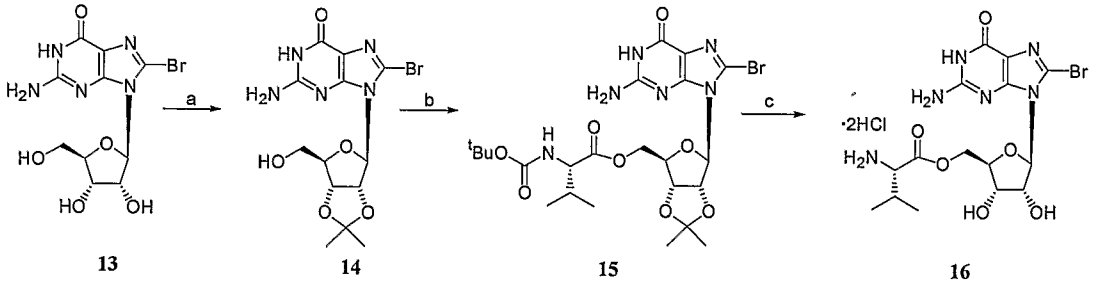


9 10 11 12

Girgis, et al, *JMC*, 33, 1990, 2750-2755

a) 2,2-dimethoxypropane, acetone, DMSO, $MeSO_3H$, 0 °C
b) BOC-NHCHR$^1CO_2H$, EDC, DMAP, PhMe, 0 °C - rt
c) anh. HCl, *i*PrOAc, *i*PrOH

Scheme 4



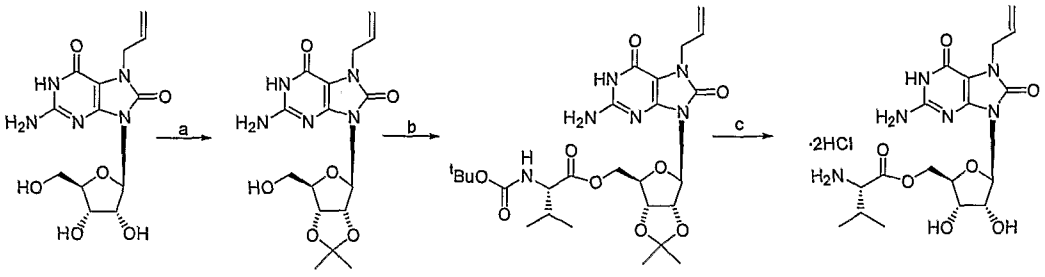
13 14 15 16

8-bromoguanosine [303136-79-0]
commercially available

a) 2,2-dimethoxypropane, acetone, DMSO, $MeSO_3H$, 0 °C
b) BOC-NHCHR$^1CO_2H$, EDC, DMAP, PhMe, 0 °C - rt
c) anh. HCl, *i*PrOAc, *i*PrOH

[0091] In a typical synthetic route, the 2',3'-hydroxyl groups of the β-D-ribose moiety of Formulaa Ia, Ib, Id, Ie, or Ih can first protected, preferably with an acetonide as shown for **2**, **6**, **10**, or **14**. The free 5'-hydroxyl can then be subjected to a variety of esterification methods with a *N*-protected amino acid to form **3**, **7**, **11**, or **15**. The nitrogen of the amino acid ester and the 2',3'-hydroxyls of the ribose unit can then be subjected to various deprotection conditions, preferably concurrently, followed by salt formation of the free amine of the amino acid ester as illustrated for **4**, **8**, **12**, or **16**.

Scheme 5

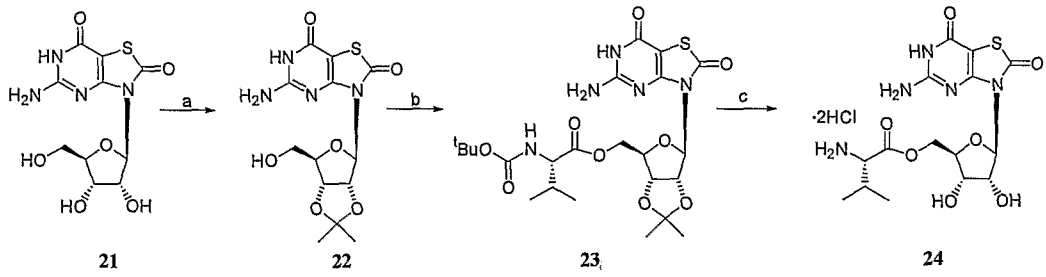


17 18 19 20

Reitz, et al, *JMC*, 37, 1994, 3561-3578

a) 2,2-dimethoxypropane, acetone, DMF, $MeSO_3H$, 0 °C
b) BOC-NHCHR$^1CO_2H$, EDC, DMAP, PhMe, 0 °C - rt
c) AcCl, CH_3OH

Scheme 6



21 22 23 24

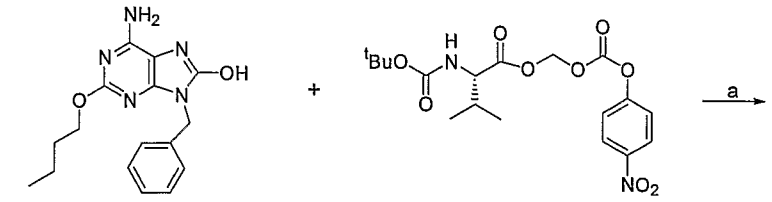
Kini, et al, *JMC*, 34, 1991, 3006-3010

a) 2,2-dimethoxypropane, acetone, DMSO, $MeSO_3H$, 0 °C
b) BOC-NHCHR$^1CO_2H$, EDC, DMAP, PhMe, 0 °C - rt
c) anh. HCl, *i*PrOAc, *i*PrOH

[0092] In the synthetic routes shown in Schemes 5 and 6, the 2',3'-hydroxyl groups of the β-D-ribose moiety of compound **17** and **21** were first protected with an acetonide to form **18** and **22** respectively. The free 5'-hydroxyl was then subjected to esterification with a *N*-tert-butoxycarbonyl valine to form **19** and **23** respectively. The nitrogen of the amino acid ester and the 2',3'-hydroxyls of the ribose were concurrently deprotected forming the hydrochloride salts as illustrated for **20** and **24**.

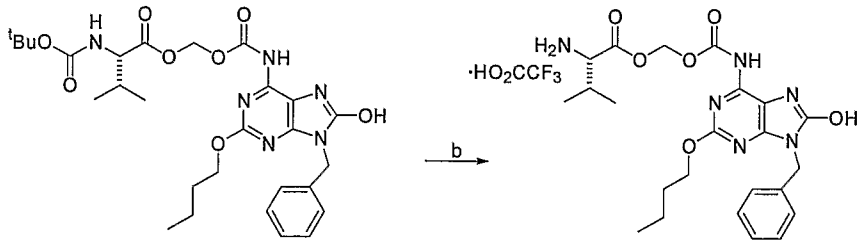
[0093] Schemes 7 and 8 describe how carbamates and carbonates can be synthesized from analogs and derivatives of adenine.

Scheme 7



25 Hirota, et al, *JMC*, 45, **2002**, 5419-5422

26 Gangwar, et al, *JOC*, 62, **1997**, 1356-1362



27

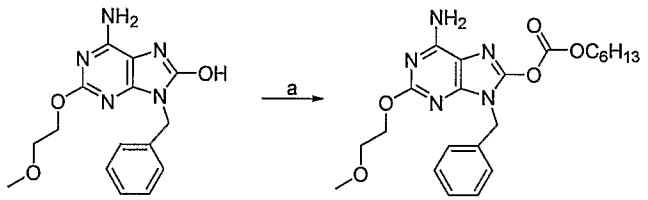
28 (Example of **IIf**)

a) HOBT, DMF,CH_2Cl_2, 0 °C

b) TFA, CH_2Cl_2, 0 °C - rt

[0094] In a typical synthetic route, the amino group of Formula **If** can be subjected to a variety of conditions with carbonates or chloroformates to form carbamates. In the case of **27**, the N-terminal protected amine of the resulting amino acid ester can be subjected to deprotection conditions to form salts such as **28**.

Scheme 8



29

30

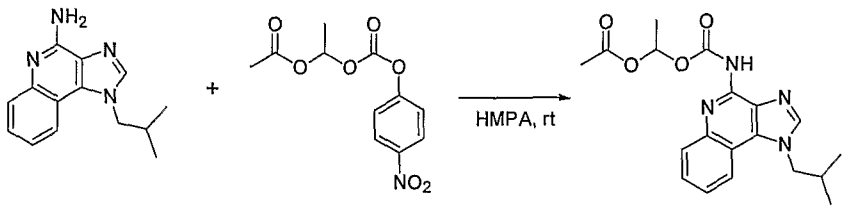
Kurimota, et al, *Bioorg. Med. Chem.*, 12, **2004**, p.1091-1099

a) $C_6H_{13}OC(O)Cl$, $(iPr)_2NEt$, CH_2Cl_2, MeOH, DMAP, 0-35 °C

[0095] In Scheme 8 the hydroxyl group of adenine derivative **29** was esterified with n-hexyl chlorofomate to give carbonate **30**.

[0096] Schemes 9 and 10 describes how carbamates and can be synthesized from imidazoquinoline analogs.

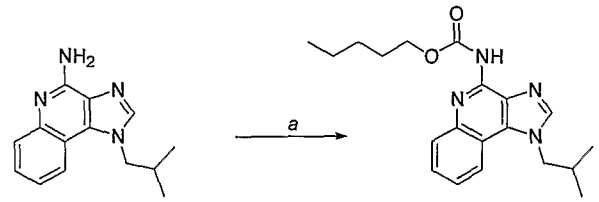
Scheme 9



31 Nicolaides et al, WO 94/17043 **32** Alexander, et al, *JMC*, 31, **1988**, 318-322 **33** (Example of **IIc**)

[0097] In a typical synthetic route, the amino group of analogs of Formula **Ic** can be subjected to a variety of conditions with carbonates, pyrocarbonates or chloroformates to form carbamates.

Scheme 10



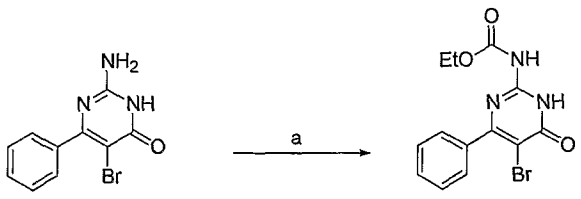
31 Nicolaides et al, WO 94/17043 **34**

a) $[C_5H_{11}OC(O)]_2O$, NEt_3, $CHCl_3$, 40 °C

[0098] In Scheme 10 imidazoquinoline **31** was treated with n-pentyl pyrocarbonate to give pentyl carbamate **34**.

[0099] Schemes 11-12 described how to synthesize carbamates and imidates of pyrimidines of formula **Ig**.

Scheme 11



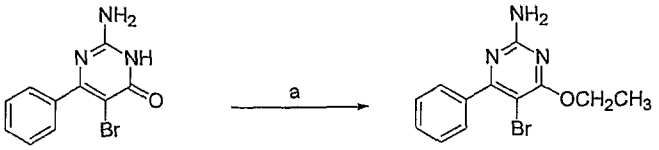
35 **36** (Example of **IIg**)

Wierenga et al, *JMC*, 23, **1980**, 239-240 Fan et al, *JHC*, 30, **1993**, 1273-1276

a) $[EtO(CO)]_2O$, NEt_3, DMF, 65 °C

[00100] In a typical synthesis of carbamates, the amino group of **35** was subjected to ethyl pyrocarbonate under conditions shown above to form carbamate **36**.

Scheme 12



35 37 (Example of IIg)

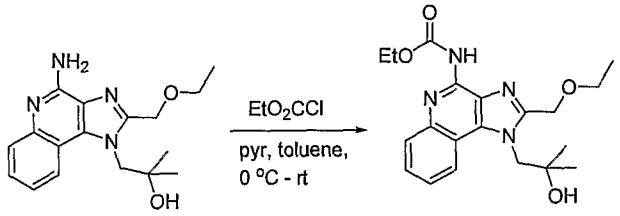
Wierenga et al, *JMC*, 23, **1980**, 239-240

a) polymer supported PPh_3, EtOH, DEAD, THF, rt

[00101] In a typical synthesis of imidates, the amino group of **35** was subjected to ethyl alcohol under Mitsunobu type conditions shown above to form ethoxy derivative **37**.

[00102] Scheme 13 describes how carbamates and can be synthesized from imidazoquinoline analogs.

Scheme 13

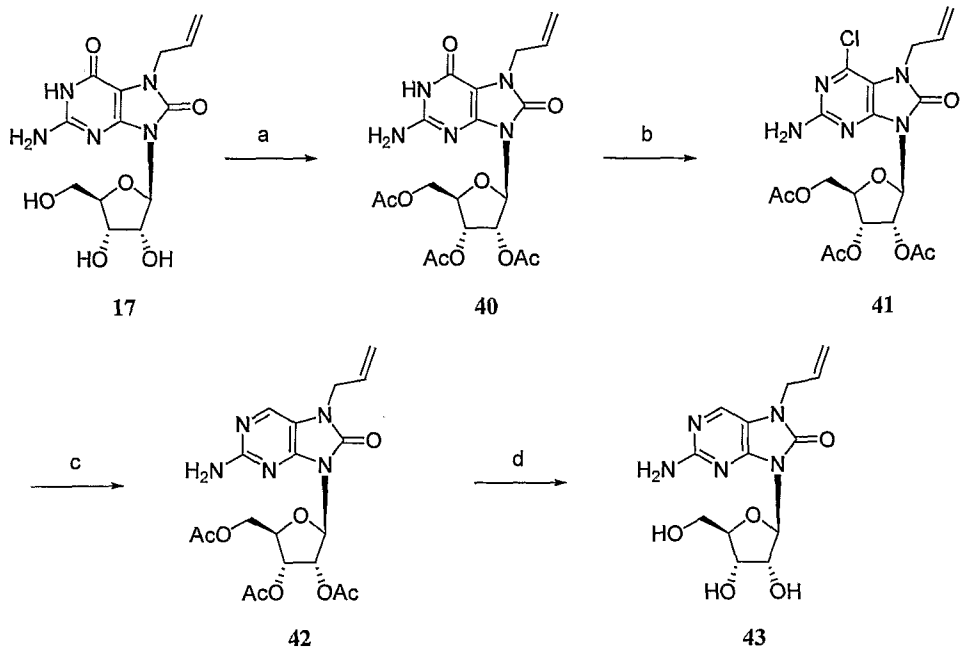


38 Nicolaides, et al, WO 94/17043 39 (Example of IIc)

[00103] In a typical synthetic route, the amino group of a derivative of Formula **Ic** can be subjected to a variety of conditions with carbonates, pyrocarbonates or chloroformates to form carbamates.

[00104] Scheme 14 shows a general procedure for preparing 7-allyl-2-Amino-9-β-D-ribofuranosyl-7,9-dihydro-purin-8-one.

Scheme 14

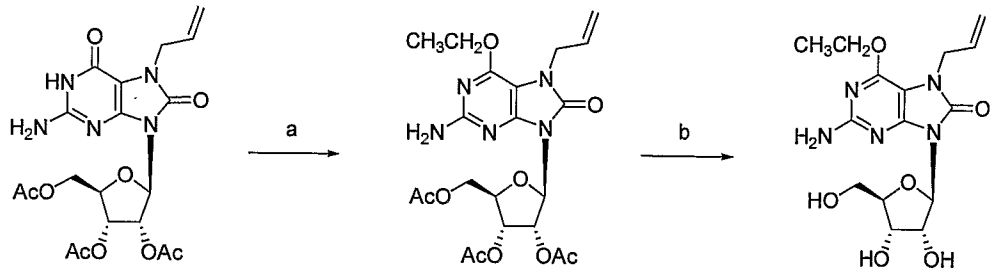


a) Ac_2O, DMAP, NEt_3, CH_3CN
b) $POCl_3$, 75 °C
c) Zn-Cu, AcOH, 70 °C
d) K_2CO_3, CH_3OH, rt

[00105] In a typical synthetic routes, 7-allyl-2-amino-9-β-D-ribofuranosyl-7,9-dihydro-1*H*-purine-6,8-dione **17**, was protected at the 2',3',5'-hydroxyl groups of the β-D-ribose, preferably with acyl groups as shown for **40**, can be subjected to a variety of conditions to convert the carbonyl at the C-6 position to various groups, including but not limited to halogen, as shown for **41**, that are susceptible to reduction. Following reduction under hetero- or homogeneous reaction conditions, the 2',3',5'-hydroxyls of the ribose unit are then subjected to appropriate deprotection conditions, to produce **43**. Compound **43** can further be appropriately modified if so desired.

[00106] Scheme 15 shows a general procedure for preparing 7-allyl-2-Amino-6-ethoxy-9-β-D-ribofuranosyl-7,9-dihydro-purin-8-one.

Scheme 15



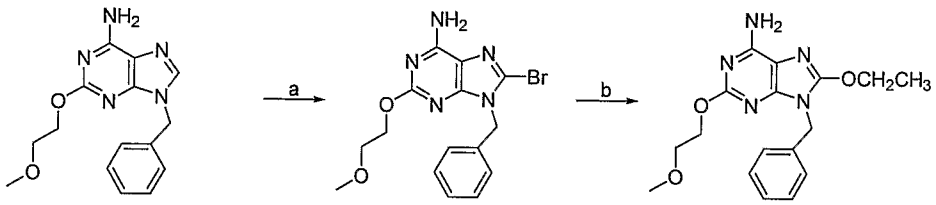
40 44 45

a) polymer supported PPh_3, EtOH, DEAD, THF, rt
b) K_2CO_3, CH_3OH, rt

[00107] In a typical synthetic route, **40** can be subjected to a variety of conditions to convert the carbonyl at the C-6 position to various imido-ethers, including but not limited to ethyl, as shown for **44**. The 2',3',5'-hydroxyls of the ribose unit are then subjected to appropriate deprotection conditions, to produce **45**. Compound **45** can further be appropriately modified if so desired.

[00108] Scheme 16 describes how ethers can be synthesized from analogs and derivatives of adenine.

Scheme 16



62 63 64

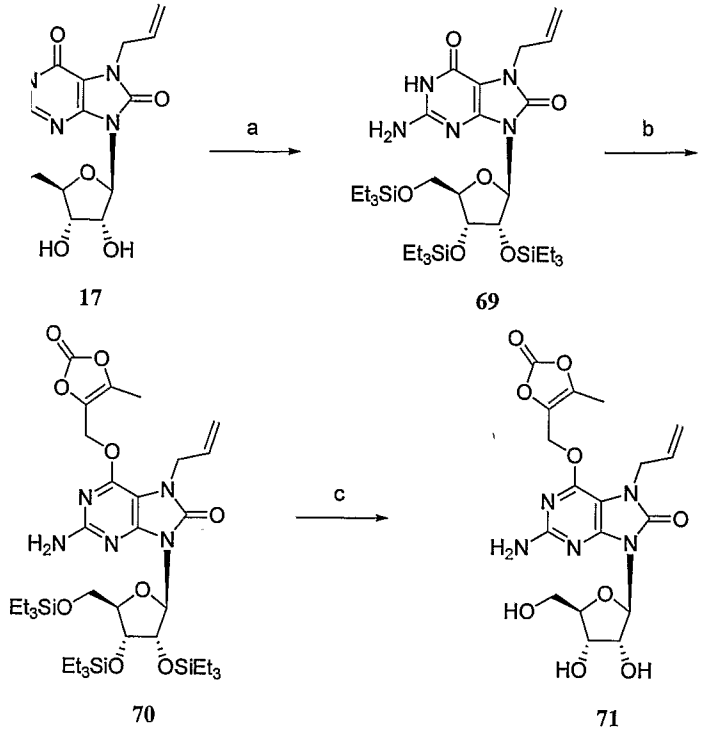
Kurimota, et al, *Bioorg. Med. Chem.*, 12, **2004**, p.1091-1099

a) Br_2, CH_2Cl_2
b) NaOEt, EtOH

[00109] In a typical synthetic route, the adenine derivative can be halogenated at C-8. The halogen can then be displaced with an appropriate alkoxide to form derivatives such as **64**.

[00110] Scheme 17 shows a general procedure for preparing 7-allyl-2-Amino-6-substituted alkoxy-9-β-D-ribofuranosyl-7,9-dihydro-purin-8-ones.

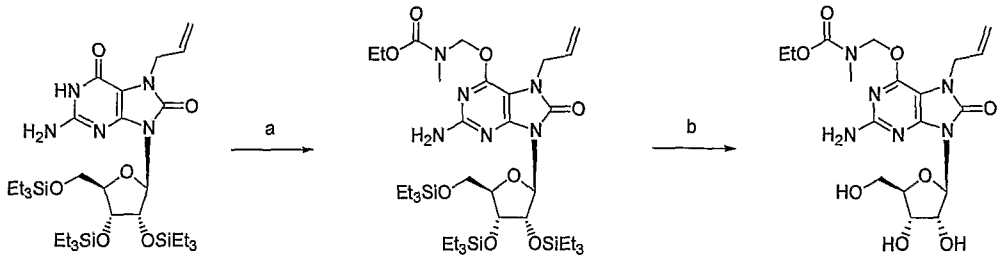
Scheme 17



[00111] In a typical synthetic route, the hydroxyl groups on ribose of **17** can be protected as silyl ethers. The carbonyl at the C-6 position of **69** can be subjected to a variety of conditions to convert the carbonyl to various imido-ethers, including but not limited to the ether of 4-hydroxymethyl-5-methyl-[1,3]dioxol-2-one, as shown for **70**. The 2',3',5'-hydroxyls of the ribose unit are then subjected to appropriate deprotection conditions, to produce **71**.

[00112] Scheme 18 shows a general procedure for preparing 7-allyl-2-Amino-6-substituted alkoxy-9-β-D-ribofuranosyl-7,9-dihydro-purin-8-ones.

Scheme 18



69 72 73

a) $HOCH_2N(CH_3)CO_2Et$, polymer supported PPh_3, DEAD, THF, rt
b) $HF{\cdot}NEt_3$, CH_3OH, rt

[00113] The carbonyl at the C-6 position of **69** can be subjected to a variety of conditions to convert the carbonyl to various imido-ethers, including but not limited to the ether of *N*-methyl-N-(hydroxymethyl)urethane, as shown for **72**. The 2',3',5'-hydroxyls of the ribose unit are then subjected to appropriate deprotection conditions, to produce **73**. Compound **73** can further be appropriately modified if so desired.

5.4 METHODS OF TREATMENT AND PREVENTION OF HEPATITIS C VIRAL INFECTIONS

[00114] The present invention provides methods for treating or preventing a hepatitis C virus infection in a patient in need thereof.

[00115] The present invention further provides methods for introducing a therapeutically effective amount of a TLR7 ligand or a prodrug thereof, or combination of such ligands and prodrugs into the blood stream of a patient in the treatment and/or prevention of hepatitis C viral infections.

[00116] The magnitude of a prophylactic or therapeutic dose of a TLR7 ligand or TLR7 ligand prodrug of the invention or a pharmaceutically acceptable salt, solvate, hydrate, or stereoisomer thereof in the acute or chronic treatment or prevention of an infection will vary, however, with the nature and severity of the infection, and the route by which the active ingredient is administered. The dose, and in some cases the dose frequency, will also vary according to the infection to be treated, the age, body weight, and response of the individual patient. Suitable dosing regimens can be readily selected by those skilled in the art with due consideration of such factors.

[00117] The methods of the present invention are particularly well suited for human patients. In particular, the methods and doses of the present invention can be useful for immunocompromised patients including, but not limited to cancer patients, HIV infected patients, and patients with an immunodegenerative disease. Furthermore, the methods can be useful for immunocompromised patients currently in a state of remission. The methods and doses of the present invention are also useful for patients undergoing other antiviral

treatments. The prevention methods of the present invention are particularly useful for patients at risk of viral infection. These patients include, but are not limited to health care workers, e.g., doctors, nurses, hospice care givers; military personnel; teachers; childcare workers; patients traveling to, or living in, foreign locales, in particular third world locales including social aid workers, missionaries, and foreign diplomats. Finally, the methods and compositions include the treatment of refractory patients or patients resistant to treatment such as resistance to viral polymerase inhibitors, protease inhibitors, etc.

Doses

[00118] Toxicity and efficacy of the compounds of the invention can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}.

[00119] The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage of the compounds for use in humans. The dosage of such compounds lie preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} (*i.e.*, the concentration of the test compound that achieves a half-maximal inhibition of symptoms) as determined in cell culture; alternatively, the dose of the TLR7 ligand prodrug may be formulated in animal models to achieve a circulating plasma concentration range of the TLR7 ligand that corresponds to the concentration required to achieve a fixed magnitude of response. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

[00120] The protocols and compositions of the invention are preferably tested *in vitro*, and then *in vivo*, for the desired therapeutic or prophylactic activity, prior to use in humans. For example, *in vitro* assays which can be used to determine whether administration of a specific therapeutic protocol is indicated, include *in vitro* cell culture assays in which cells that are responsive to the effects of the TLR7 ligands are exposed to the ligand and the magnitude of response is measured by an appropriate technique. The assessment of the TLR7 ligand potency is then evaluated with respect to the TLR7 ligand prodrug potency, and the degree of conversion of the TLR7 ligand prodrug. Compounds for

use in methods of the invention can be tested in suitable animal model systems prior to testing in humans, including but not limited to in rats, mice, chicken, cows, monkeys, rabbits, hamsters, etc. The compounds can then be used in the appropriate clinical trials.

[00121] The magnitude of a prophylactic or therapeutic dose of a prodrug of a TLR7 ligand of the invention or a pharmaceutically acceptable salt, solvate, hydrate, or stereoisomer thereof in the acute or chronic treatment or prevention of an infection or condition will vary with the nature and severity of the infection, and the route by which the active ingredient is administered. The dose, and perhaps the dose frequency, will also vary according to the infection to be treated, the age, body weight, and response of the individual patient. Suitable dosing regimens can be readily selected by those skilled in the art with due consideration of such factors. In one embodiment, the dose administered depends upon the specific compound to be used, and the weight and condition of the patient. Also, the dose may differ for various particular TLR7 ligands prodrugs; suitable doses can be predicted on the basis of the aforementioned in vitro measurements, in particular by use of such measurements of the TLR7 ligand to which the TLR7 ligand prodrug is related, and on the basis of animal studies, such that smaller doses will be suitable for those TLR7 ligand prodrugs that show effectiveness at lower concentrations than other TLR7 ligand prodrugs when measured in the systems described or referenced herein. In general, the dose per day is in the range of from about 0.001 to 100 mg/kg, preferably about 1 to 25 mg/kg, more preferably about 5 to 15 mg/kg. For treatment of humans infected by hepatitis C viruses, about 0.1 mg to about 15 g per day is administered in about one to four divisions a day, preferably 100 mg to 12 g per day, more preferably from 100 mg to 8000 mg per day. In a preferred embodiment for compounds such as prodrugs of 3-β-D-ribofuranosylthiazolo[4,5-d]pyrimidines from 200 mg to 8000 mg per day is administered in about one to four divisions a day. Additionally, the recommended daily dose ran can be administered in cycles as single agents or in combination with other therapeutic agents. In one embodiment, the daily dose is administered in a single dose or in equally divided doses. In a related embodiment, the recommended daily dose can be administered once time per week, two times per week, three times per week, four times per week or five times per week.

[00122] In a preferred embodiment, the compounds of the invention are administered to provide systemic distribution of the compound within the patient. In a related embodiment, the compounds of the invention are administered to produce a systemic effect in the body.

[00123] In another embodiment the compounds of the invention are administered via oral, mucosal (including sublingual, buccal, rectal, nasal, or vaginal), parenteral (including

subcutaneous, intramuscular, bolus injection, intraarterial, or intravenous), transdermal, or topical administration. In a specific embodiment the compounds of the invention are administered via mucosal (including sublingual, buccal, rectal, nasal, or vaginal), parenteral (including subcutaneous, intramuscular, bolus injection, intraarterial, or intravenous), transdermal, or topical administration. In a further specific embodiment, the compounds of the invention are administered via oral administration. In a further specific embodiment, the compounds of the invention are not administered via oral administration.

[00124] Different therapeutically effective amounts may be applicable for different infections, as will be readily known by those of ordinary skill in the art. Similarly, amounts sufficient to treat or prevent such infections, but insufficient to cause, or sufficient to reduce, adverse effects associated with conventional therapies are also encompassed by the above described dosage amounts and dose frequency schedules.

Combination Therapy

[00125] Specific methods of the invention further comprise the administration of an additional therapeutic agent (*i.e.*, a therapeutic agent other than a compound of the invention). In certain embodiments of the present invention, the compounds of the invention can be used in combination with at least one other therapeutic agent. Therapeutic agents include, but are not limited to antibiotics, antiemetic agents, antidepressants, and antifungal agents, anti-inflammatory agents, antiviral agents, anticancer agents, immunomodulatory agents, β-interferons, alkylating agents, hormones or cytokines. In a preferred embodiment the invention encompasses the administration of an additional therapeutic agent that is HCV specific or demonstrates anti-HCV activity.

[00126] The TLR7 ligands prodrugs of the invention can be administered or formulated in combination with antibiotics. For example, they can be formulated with a macrolide (*e.g.*, tobramycin (Tobi®)), a cephalosporin (*e.g.*, cephalexin (Keflex®), cephradine (Velosef®), cefuroxime (Ceftin®), cefprozil (Cefzil®), cefaclor (Ceclor®), cefixime (Suprax®) or cefadroxil (Duricef®)), a clarithromycin (*e.g.*, clarithromycin (Biaxin®)), an erythromycin (*e.g.*, erythromycin (EMycin®)), a penicillin (*e.g.*, penicillin V (V-Cillin K® or Pen Vee K®)) or a quinolone (*e.g.*, ofloxacin (Floxin®), ciprofloxacin (Cipro®) or norfloxacin (Noroxin®)),aminoglycoside antibiotics (*e.g.*, apramycin, arbekacin, bambermycins, butirosin, dibekacin, neomycin, neomycin, undecylenate, netilmicin, paromomycin, ribostamycin, sisomicin, and spectinomycin), amphenicol antibiotics (*e.g.*, azidamfenicol, chloramphenicol, florfenicol, and thiamphenicol), ansamycin antibiotics (*e.g.*, rifamide and rifampin), carbacephems (*e.g.*, loracarbef), carbapenems (*e.g.*, biapenem and imipenem), cephalosporins (*e.g.*, cefaclor, cefadroxil,

cefamandole, cefatrizine, cefazedone, cefozopran, cefpimizole, cefpiramide, and cefpirome), cephamycins (*e.g.*, cefbuperazone, cefmetazole, and cefminox), monobactams (*e.g.*, aztreonam, carumonam, and tigemonam), oxacephems (*e.g.*, flomoxef, and moxalactam), penicillins (*e.g.*, amdinocillin, amdinocillin pivoxil, amoxicillin, bacampicillin, benzylpenicillinic acid, benzylpenicillin sodium, epicillin, fenbenicillin, floxacillin, penamccillin, penethamate hydriodide, penicillin o-benethamine, penicillin 0, penicillin V, penicillin V benzathine, penicillin V hydrabamine, penimepicycline, and phencihicillin potassium), lincosamides (*e.g.*, clindamycin, and lincomycin), amphomycin, bacitracin, capreomycin, colistin, enduracidin, enviomycin, tetracyclines (*e.g.*, apicycline, chlortetracycline, clomocycline, and demeclocycline), 2,4-diaminopyrimidines (*e.g.*, brodimoprim), nitrofurans (*e.g.*, furaltadone, and furazolium chloride), quinolones and analogs thereof (*e.g.*, cinoxacin,, clinafloxacin, flumequine, and grepagloxacin), sulfonamides (*e.g.*, acetyl sulfamethoxypyrazine, benzylsulfamide, noprylsulfamide, phthalylsulfacetamide, sulfachrysoidine, and sulfacytine), sulfones (*e.g.*, diathymosulfone, glucosulfone sodium, and solasulfone), cycloserine, mupirocin and tuberin.

[00127] The TLR7 ligand prodrugs of the invention can also be administered or formulated in combination with an antiemetic agent. Suitable antiemetic agents include, but are not limited to, metoclopromide, domperidone, prochlorperazine, promethazine, chlorpromazine, trimethobenzamide, ondansetron, granisetron, hydroxyzine, acethylleucine monoethanolamine, alizapride, azasetron, benzquinamide, bietanautine, bromopride, buclizine, clebopride, cyclizine, dimenhydrinate, diphenidol, dolasetron, meclizine, methallatal, metopimazine, nabilone, oxyperndyl, pipamazine, scopolamine, sulpiride, tetrahydrocannabinols, thiethylperazine, thioproperazine, tropisetron, and mixtures thereof.

[00128] The TLR7 ligand prodrugs of the invention can be administered or formulated in combination with an antidepressant. Suitable antidepressants include, but are not limited to, binedaline, caroxazone, citalopram, dimethazan, fencamine, indalpine, indeloxazine hydrocholoride, nefopam, nomifensine, oxitriptan, oxypertine, paroxetine, sertraline, thiazesim, trazodone, benmoxine, iproclozide, iproniazid, isocarboxazid, nialamide, octamoxin, phenelzine, cotinine, rolicyprine, rolipram, maprotiline, metralindole, mianserin, mirtazepine, adinazolam, amitriptyline, amitriptylinoxide, amoxapine, butriptyline, clomipramine, demexiptiline, desipramine, dibenzepin, dimetacrine, dothiepin, doxepin, fluacizine, imipramine, imipramine N-oxide, iprindole, lofepramine, melitracen, metapramine, nortriptyline, noxiptilin, opipramol, pizotyline, propizepine, protriptyline, quinupramine, tianeptine, trimipramine, adrafinil, benactyzine, bupropion, butacetin, dioxadrol, duloxetine, etoperidone, febarbamate, femoxetine, fenpentadiol, fluoxetine,

fluvoxamine, hematoporphyrin, hypericin, levophacetoperane, medifoxamine, milnacipran, minaprine, moclobemide, nefazodone, oxaflozane, piberaline, prolintane, pyrisuccideanol, ritanserin, roxindole, rubidium chloride, sulpiride, tandospirone, thozalinone, tofenacin, toloxatone, tranylcypromine, L-tryptophan, venlafaxine, viloxazine, and zimeldine.

[00129] The TLR7 ligands or TLR7 ligand prodrugs of the invention can be administered or formulated in combination with an antifungal agent. Suitable antifungal agents include but are not limited to amphotericin B, itraconazole, ketoconazole, fluconazole, intrathecal, flucytosine, miconazole, butoconazole, clotrimazole, nystatin, terconazole, tioconazole, ciclopirox, econazole, haloprogrin, naftifine, terbinafine, undecylenate, and griseofuldin.

[00130] The TLR7 ligands or TLR7 ligand prodrugs of the invention can be administered or formulated in combination with an anti-inflammatory agent. Useful anti-inflammatory agents include, but are not limited to, non-steroidal anti-inflammatory drugs such as salicylic acid, acetylsalicylic acid, methyl salicylate, diflunisal, salsalate, olsalazine, sulfasalazine, acetaminophen, indomethacin, sulindac, etodolac, mefenamic acid, meclofenamate sodium, tolmetin, ketorolac, dichlofenac, ibuprofen, naproxen, naproxen sodium, fenoprofen, ketoprofen, flurbinprofen, oxaprozin, piroxicam, meloxicam, ampiroxicam, droxicam, pivoxicam, tenoxicam, nabumetome, phenylbutazone, oxyphenbutazone, antipyrine, aminopyrine, apazone and nimesulide; leukotriene antagonists including, but not limited to, zileuton, aurothioglucose, gold sodium thiomalate and auranofin; steroids including, but not limited to, alclometasone diproprionate, amcinonide, beclomethasone dipropionate, betametasone, betamethasone benzoate, betamethasone diproprionate, betamethasone sodium phosphate, betamethasone valerate, clobetasol proprionate, clocortolone pivalate, hydrocortisone, hydrocortisone derivatives, desonide, desoximatasone, dexamethasone, flunisolide, flucoxinolide, flurandrenolide, halcinocide, medrysone, methylprednisolone, methprednisolone acetate, methylprednisolone sodium succinate, mometasone furoate, paramethasone acetate, prednisolone, prednisolone acetate, prednisolone sodium phosphate, prednisolone tebuatate, prednisone, triamcinolone, triamcinolone acetonide, triamcinolone diacetate, and triamcinolone hexacetonide; and other anti-inflammatory agents including, but not limited to, methotrexate, colchicine, allopurinol, probenecid, sulfinpyrazone and benzbromarone.

[00131] The TLR7 ligands or TLR7 ligand prodrugs of the invention can be administered or formulated in combination with another antiviral agent. Useful antiviral agents include, but are not limited to, protease inhibitors, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors and nucleoside analogs. The

antiviral agents include but are not limited to zidovudine, acyclovir, gangcyclovir, vidarabine, idoxuridine, trifluridine, levovirin, viramidine and ribavirin, as well as foscarnet, amantadine, rimantadine, saquinavir, indinavir, amprenavir, lopinavir, ritonavir, the alpha-interferons, beta-interferons, gamma-interferons, adefovir, clevudine, entecavir, and pleconaril.

[00132] The TLR7 ligands or TLR7 ligand prodrugs of the invention can be administered or formulated in combination with an immunomodulatory agent. Immunomodulatory agents include, but are not limited to, methothrexate, leflunomide, cyclophosphamide, cyclosporine A, mycophenolate mofetil, rapamycin (sirolimus), mizoribine, deoxyspergualin, brequinar, malononitriloamindes (e.g., leflunamide), T cell receptor modulators, and cytokine receptor modulators, peptide mimetics, and antibodies (e.g., human, humanized, chimeric, monoclonal, polyclonal, Fvs, ScFvs, Fab or F(ab)2 fragments or epitope binding fragments), nucleic acid molecules (e.g., antisense nucleic acid molecules and triple helices), small molecules, organic compounds, and inorganic compounds. Examples of T cell receptor modulators include, but are not limited to, anti-T cell receptor antibodies (e.g., anti-CD4 antibodies (e.g., cM-T412 (Boeringer), IDEC-CE9.1® (IDEC and SKB), mAB 4162W94, Orthoclone and OKTcdr4a (Janssen-Cilag)), anti-CD3 antibodies (e.g., Nuvion (Product Design Labs), OKT3 (Johnson & Johnson), or Rituxan (IDEC)), anti-CD5 antibodies (e.g., an anti-CD5 ricin-linked immunoconjugate), anti-CD7 antibodies (e.g., CHH-380 (Novartis)), anti-CD8 antibodies, anti-CD40 ligand monoclonal antibodies (e.g., IDEC-131 (IDEC)), anti-CD52 antibodies (e.g., CAMPATH 1H (Ilex)), anti-CD2 antibodies, anti-CD11a antibodies (e.g., Xanelim (Genentech)), and anti-B7 antibodies (e.g., IDEC-114 (IDEC)) and CTLA4-immunoglobulin. Examples of cytokine receptor modulators include, but are not limited to, soluble cytokine receptors (e.g., the extracellular domain of a TNF-α receptor or a fragment thereof, the extracellular domain of an IL-1β receptor or a fragment thereof, and the extracellular domain of an IL-6 receptor or a fragment thereof), cytokines or fragments thereof (e.g., interleukin (IL)-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-15, TNF-α, interferon (IFN)-α, IFN-β, IFN-γ, and GM-CSF), anti-cytokine receptor antibodies (e.g., anti-IFN receptor antibodies, anti-IL-2 receptor antibodies (e.g., Zenapax (Protein Design Labs)), anti-IL-4 receptor antibodies, anti-IL-6 receptor antibodies, anti-IL-10 receptor antibodies, and anti-IL-12 receptor antibodies), anti-cytokine antibodies (e.g., anti-IFN antibodies, anti-TNF-α antibodies, anti-IL-1β antibodies, anti-IL-6 antibodies, anti-IL-8 antibodies (e.g., ABX-IL-8 (Abgenix)), and anti-IL-12 antibodies).

[00133] The TLR7 ligands or TLR ligand prodrugs of the invention can be administered or formulated in combination with an agent which inhibits viral enzymes, including but not limited to inhibitors of HCV protease, such as BILN 2061 and inhibitors of NS5b polymerase such as NM107 and its prodrug NM283 (Idenix Pharmaceuticals, Inc., Cambridge, MA).

[00134] The TLR7 ligands or TLR7 ligand prodrugs of the invention can be administered or formulated in combination with an agent which inhibits HCV polymerase such as those described in Wu, Curr Drug Targets Infect Disord. 2003;3(3):207-19 or in combination with compounds that inhibit the helicase function of the virus such as those described in Bretner M, et al Nucleosides Nucleotides Nucleic Acids. 2003;22(5-8):1531, or with inhibitors of other HCV specific targets such as those described in Zhang X. IDrugs. 2002;5(2):154-8.

[00135] The TLR7 ligands or TLR7 ligand prodrugs of the invention can be administered or formulated in combination with an agent that inhibits viral replication.

[00136] The TLR7 ligands or TLR ligand prodrugs of the invention can be administered or formulated in combination with cytokines. Examples of cytokines include, but are not limited to, interleukin-2 (IL-2), interleukin-3 (IL-3), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-7 (IL-7), interleukin-9 (IL-9), interleukin-10 (IL-10), interleukin-12 (IL-12), interleukin 15 (IL-15), interleukin 18 (IL-18), platelet derived growth factor (PDGF), erythropoietin (Epo), epidermal growth factor (EGF), fibroblast growth factor (FGF), granulocyte macrophage stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), macrophage colony stimulating factor (M-CSF), prolactin, and interferon (IFN), e.g., IFN-alpha, and IFN-gamma).

[00137] The TLR7 ligands or TLR7 ligand prodrugs of the invention can be administered or formulated in combination with hormones. Examples of hormones include, but are not limited to, luteinizing hormone releasing hormone (LHRH), growth hormone (GH), growth hormone releasing hormone, ACTH, somatostatin, somatotropin, somatomedin, parathyroid hormone, hypothalamic releasing factors, insulin, glucagon, enkephalins, vasopressin, calcitonin, heparin, low molecular weight heparins, heparinoids, synthetic and natural opioids, insulin thyroid stimulating hormones, and endorphins.

[00138] The TLR7 ligands or TLR7 ligand prodrugs of the invention can be administered or formulated in combination with β-interferons which include, but are not limited to, interferon beta-1a, interferon beta-1b.

[00139] The TLR7 ligands or TLR7 ligand prodrugs of the invention can be administered or formulated in combination with α-interferons which include, but are not

limited to, interferon alpha-1, interferon alpha-2a (roferon), interferon alpha-2b, intron, Peg-Intron, Pegasys, consensus interferon (infergen) and albuferon.

[00140] The TLR7 ligands or TLR7 ligand prodrugs of the invention can be administered or formulated in combination with an absorption enhancer, particularly those which target the lymphatic system, including, but not limited to sodium glycocholate; sodium caprate; N-lauryl-γ-D-maltopyranoside; EDTA; mixed micelle; and those reported in Muranishi Crit. Rev. Ther. Drug Carrier Syst., 7-1-33, which is hereby incorporated by reference in its entirety. Other known absorption enhancers can also be used. Thus, the invention also encompasses a pharmaceutical composition comprising one or more TLR7 ligand prodrugs of the invention and one or more absorption enhancers.

[00141] The TLR7 ligands or TLR7 ligand prodrugs of the invention can be administered or formulated in combination with an alkylating agent. Examples of alkylating agents include, but are not limited to nitrogen mustards, ethylenimines, methylmelamines, alkyl sulfonates, nitrosoureas, triazenes, mechlorethamine, cyclophosphamide, ifosfamide, melphalan, chlorambucil, hexamethylmelaine, thiotepa, busulfan, carmustine, streptozocin, dacarbazine and temozolomide.

[00142] The compounds of the invention and the other therapeutics agent can act additively or, more preferably, synergistically. In a preferred embodiment, a composition comprising a compound of the invention is administered concurrently with the administration of another therapeutic agent, which can be part of the same composition or in a different composition from that comprising the compounds of the invention. In another embodiment, a compound of the invention is administered prior to or subsequent to administration of another therapeutic agent. In a separate embodiment, a compound of the invention is administered to a patient who has not previously undergone or is not currently undergoing treatment with another therapeutic agent, particularly an antiviral agent.

[00143] In one embodiment, the methods of the invention comprise the administration of one or more TLR7 ligands or TLR7 ligand prodrugs of the invention without an additional therapeutic agent.

PHARMACEUTICAL COMPOSITIONS AND DOSAGE FORMS

[00144] Pharmaceutical compositions and single unit dosage forms comprising a TLR7 ligand or prodrug of the invention, or a pharmaceutically acceptable salt, hydrate or stereoisomer thereof, are also encompassed by the invention. Individual dosage forms of the invention may be suitable for oral, mucosal (including sublingual, buccal, rectal, nasal, or vaginal), parenteral (including subcutaneous, intramuscular, bolus injection, intraarterial, or intravenous), transdermal, or topical administration. Pharmaceutical compositions and dosage forms of the invention typically also comprise one or more pharmaceutically acceptable excipients. Sterile dosage forms are also contemplated.

[00145] In an alternative embodiment,a pharmaceutical composition encompassed by this embodiment includes a TLR7 ligand or prodrug of the invention, or a pharmaceutically acceptable salt, hydrate or stereoisomer thereof, and at least one additional therapeutic agent. Examples of additional therapeutic agents include, but are not limited to, those listed above in section 5.2.2.

[00146] The composition, shape, and type of dosage forms of the invention will typically vary depending on their use. For example, a dosage form used in the acute treatment of a disease or a related disease may contain larger amounts of one or more of the active ingredients it comprises than a dosage form used in the chronic treatment of the same disease. Similarly, a parenteral dosage form may contain smaller amounts of one or more of the active ingredients it comprises than an oral dosage form used to treat the same disease or disorder.

[00147] These and other ways in which specific dosage forms encompassed by this invention will vary from one another will be readily apparent to those skilled in the art. See, e.g., Remington's Pharmaceutical Sciences, 18th ed., Mack Publishing, Easton PA (1990). Examples of dosage forms include, but are not limited to: tablets; caplets; capsules, such as soft elastic gelatin capsules; cachets; troches; lozenges; dispersions; suppositories; ointments; cataplasms (poultices); pastes; powders; dressings; creams; plasters; solutions; patches; aerosols (e.g., nasal sprays or inhalers); gels; liquid dosage forms suitable for oral or mucosal administration to a patient, including suspensions (e.g., aqueous or non-aqueous liquid suspensions, oil-in-water emulsions, or a water-in-oil liquid emulsions), solutions, and elixirs; liquid dosage forms suitable for parenteral administration to a patient; and sterile solids (e.g., crystalline or amorphous solids) that can be reconstituted to provide liquid dosage forms suitable for parenteral administration to a patient.

[00148] Typical pharmaceutical compositions and dosage forms comprise one or more carriers, excipients or diluents. Suitable excipients are well known to those skilled in the art of pharmacy, and non-limiting examples of suitable excipients are provided herein. Whether a particular excipient is suitable for incorporation into a pharmaceutical composition or dosage form depends on a variety of factors well known in the art including, but not limited to, the way in which the dosage form will be administered to a patient. For example, oral dosage forms such as tablets may contain excipients not suited for use in parenteral dosage forms. The suitability of a particular excipient may also depend on the specific active ingredients in the dosage form.

[00149] This invention further encompasses anhydrous pharmaceutical compositions and dosage forms comprising active ingredients, since water can facilitate the degradation of some compounds. For example, the addition of water (e.g., 5%) is widely accepted in the pharmaceutical arts as a means of simulating long-term storage in order to determine characteristics such as shelf-life or the stability of formulations over time. See, e.g., Jens T. Carstensen, Drug Stability: Principles & Practice, 2d. Ed., Marcel Dekker, NY, NY, 1995, pp. 379-80. In effect, water and heat accelerate the decomposition of some compounds. Thus, the effect of water on a formulation can be of great significance since moisture and/or humidity are commonly encountered during manufacture, handling, packaging, storage, shipment, and use of formulations.

[00150] Anhydrous pharmaceutical compositions and dosage forms of the invention can be prepared using anhydrous or low moisture containing ingredients and low moisture or low humidity conditions.

[00151] An anhydrous pharmaceutical composition should be prepared and stored such that its anhydrous nature is maintained. Accordingly, anhydrous compositions are preferably packaged using materials known to prevent exposure to water such that they can be included in suitable formulary kits. Examples of suitable packaging include, but are not limited to, hermetically sealed foils, plastics, unit dose containers (e.g., vials), blister packs, and strip packs.

[00152] The invention further encompasses pharmaceutical compositions and dosage forms that comprise one or more compounds that reduce the rate by which an active ingredient will decompose. Such compounds, which are referred to herein as "stabilizers," include, but are not limited to, antioxidants such as ascorbic acid, pH buffers, or salt buffers.

[00153] Like the amounts and types of excipients, the amounts and specific types of active ingredients in a dosage form may differ depending on factors such as, but not limited to, the route by which it is to be administered to patients. However, typical dosage forms of

the invention comprise TLR7 ligand prodrugs of the invention, or a pharmaceutically acceptable salt, hydrate, or stereoisomers thereof comprise 0.1 mg to 1500 mg per unit to provide doses of about 0.01 to 200 mg/kg per day.

Oral Dosage Forms

[00154] Pharmaceutical compositions of the invention that are suitable for oral administration can be presented as discrete dosage forms, such as, but are not limited to, tablets (e.g., chewable tablets), caplets, capsules, and liquids (e.g., flavored syrups). Such dosage forms contain predetermined amounts of active ingredients, and may be prepared by methods of pharmacy well known to those skilled in the art. See generally, Remington's Pharmaceutical Sciences, 18th ed., Mack Publishing, Easton PA (1990).

[00155] Typical oral dosage forms of the invention are prepared by combining the active ingredient(s) in an intimate admixture with at least one excipient according to conventional pharmaceutical compounding techniques. Excipients can take a wide variety of forms depending on the form of preparation desired for administration. For example, excipients suitable for use in oral liquid or aerosol dosage forms include, but are not limited to, water, glycols, oils, alcohols, flavoring agents, preservatives, and coloring agents. Examples of excipients suitable for use in solid oral dosage forms (e.g., powders, tablets, capsules, and caplets) include, but are not limited to, starches, sugars, micro-crystalline cellulose, diluents, granulating agents, lubricants, binders, and disintegrating agents.

[00156] Because of their ease of administration, tablets and capsules represent the most advantageous oral dosage unit forms, in which case solid excipients are employed. If desired, tablets can be coated by standard aqueous or nonaqueous techniques. Such dosage forms can be prepared by any of the methods of pharmacy. In general, pharmaceutical compositions and dosage forms are prepared by uniformly and intimately admixing the active ingredients with liquid carriers, finely divided solid carriers, or both, and then shaping the product into the desired presentation if necessary.

[00157] For example, a tablet can be prepared by compression or molding. Compressed tablets can be prepared by compressing in a suitable machine the active ingredients in a free-flowing form such as powder or granules, optionally mixed with an excipient. Molded tablets can be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

[00158] Examples of excipients that can be used in oral dosage forms of the invention include, but are not limited to, binders, fillers, disintegrants, and lubricants. Binders suitable for use in pharmaceutical compositions and dosage forms include, but are not limited to, corn starch, potato starch, or other starches, gelatin, natural and synthetic gums

such as acacia, sodium alginate, alginic acid, other alginates, powdered tragacanth, guar gum, cellulose and its derivatives (e.g., ethyl cellulose, cellulose acetate, carboxymethyl cellulose calcium, sodium carboxymethyl cellulose), polyvinyl pyrrolidone, methyl cellulose, pre-gelatinized starch, hydroxypropyl methyl cellulose, (e.g., Nos. 2208, 2906, 2910), microcrystalline cellulose, and mixtures thereof.

[00159] Examples of fillers suitable for use in the pharmaceutical compositions and dosage forms disclosed herein include, but are not limited to, talc, calcium carbonate (e.g., granules or powder), microcrystalline cellulose, powdered cellulose, dextrates, kaolin, mannitol, silicic acid, sorbitol, starch, pre-gelatinized starch, and mixtures thereof. The binder or filler in pharmaceutical compositions of the invention is typically present in from about 50 to about 99 weight percent of the pharmaceutical composition or dosage form.

[00160] Suitable forms of microcrystalline cellulose include, but are not limited to, the materials sold as AVICEL-PH-101, AVICEL-PH-103 AVICEL RC-581, AVICEL-PH-105 (available from FMC Corporation, American Viscose Division, Avicel Sales, Marcus Hook, PA), and mixtures thereof. A specific binder is a mixture of microcrystalline cellulose and sodium carboxymethyl cellulose sold as AVICEL RC-581. Suitable anhydrous or low moisture excipients or additives include AVICEL-PH-103™ and Starch 1500 LM.

[00161] Disintegrants are used in the compositions of the invention to provide tablets that disintegrate when exposed to an aqueous environment. Tablets that contain too much disintegrant may disintegrate in storage, while those that contain too little may not disintegrate at a desired rate or under the desired conditions. Thus, a sufficient amount of disintegrant that is neither too much nor too little to detrimentally alter the release of the active ingredients should be used to form solid oral dosage forms of the invention. The amount of disintegrant used varies based upon the type of formulation, and is readily discernible to those of ordinary skill in the art. Typical pharmaceutical compositions comprise from about 0.5 to about 15 weight percent of disintegrant, specifically from about 1 to about 5 weight percent of disintegrant.

[00162] Disintegrants that can be used in pharmaceutical compositions and dosage forms of the invention include, but are not limited to, agar-agar, alginic acid, calcium carbonate, microcrystalline cellulose, croscarmellose sodium, crospovidone, polacrilin potassium, sodium starch glycolate, potato or tapioca starch, pre-gelatinized starch, other starches, clays, other algins, other celluloses, gums, and mixtures thereof.

[00163] Lubricants that can be used in pharmaceutical compositions and dosage forms of the invention include, but are not limited to, calcium stearate, magnesium stearate,

mineral oil, light mineral oil, glycerin, sorbitol, mannitol, polyethylene glycol, other glycols, stearic acid, sodium lauryl sulfate, talc, hydrogenated vegetable oil (e.g., peanut oil, cottonseed oil, sunflower oil, sesame oil, olive oil, corn oil, and soybean oil), zinc stearate, ethyl oleate, ethyl laureate, agar, and mixtures thereof. Additional lubricants include, for example, a syloid silica gel (AEROSIL 200, manufactured by W.R. Grace Co. of Baltimore, MD), a coagulated aerosol of synthetic silica (marketed by Degussa Co. of Plano, TX), CAB-O-SIL (a pyrogenic silicon dioxide product sold by Cabot Co. of Boston, MA), and mixtures thereof. If used at all, lubricants are typically used in an amount of less than about 1 weight percent of the pharmaceutical compositions or dosage forms into which they are incorporated.

Delayed Release Dosage Forms

[00164] Active ingredients of the invention can be administered by controlled release means or by delivery devices that are well known to those of ordinary skill in the art. Examples include, but are not limited to, those described in U.S. Patent Nos.: 3,845,770; 3,916,899; 3,536,809; 3,598,123; and 4,008,719, 5,674,533, 5,059,595, 5,591,767, 5,120,548, 5,073,543, 5,639,476, 5,354,556, and 5,733,566, each of which is incorporated herein by reference. Such dosage forms can be used to provide slow or controlled-release of one or more active ingredients using, for example, hydropropylmethyl cellulose, other polymer matrices, gels, permeable membranes, osmotic systems, multilayer coatings, microparticles, liposomes, microspheres, or a combination thereof to provide the desired release profile in varying proportions. Suitable controlled-release formulations known to those of ordinary skill in the art, including those described herein, can be readily selected for use with the active ingredients of the invention. The invention thus encompasses single unit dosage forms suitable for oral administration such as, but not limited to, tablets, capsules, gelcaps, and caplets that are adapted for controlled-release.

[00165] All controlled-release pharmaceutical products have a common goal of improving drug therapy over that achieved by their non-controlled counterparts. Ideally, the use of an optimally designed controlled-release preparation in medical treatment is characterized by a minimum of drug substance being employed to cure or control the condition in a minimum amount of time. Advantages of controlled-release formulations include extended activity of the drug, reduced dosage frequency, and increased patient compliance. In addition, controlled-release formulations can be used to affect the time of onset of action or other characteristics, such as blood levels of the drug, and can thus affect the occurrence of side (e.g., adverse) effects.

[00166] Most controlled-release formulations are designed to initially release an amount of drug (active ingredient) that promptly produces the desired therapeutic effect, and gradually and continually release other amounts of drug to maintain this level of therapeutic or prophylactic effect over an extended period of time. In order to maintain this constant level of drug in the body, the drug must be released from the dosage form at a rate that will replace the amount of drug being metabolized and excreted from the body. Controlled-release of an active ingredient can be stimulated by various conditions including, but not limited to, pH, temperature, enzymes, water, or other physiological conditions or compounds.

Parenteral Dosage Forms

[00167] Parenteral dosage forms can be administered to patients by various routes including, but not limited to, subcutaneous, intravenous (including bolus injection), intramuscular, and intraarterial. Because their administration typically bypasses patients' natural defenses against contaminants, parenteral dosage forms are preferably sterile or capable of being sterilized prior to administration to a patient. Examples of parenteral dosage forms include, but are not limited to, solutions ready for injection, dry and/or lyophylized products ready to be dissolved or suspended in a pharmaceutically acceptable vehicle for injection (reconstitutable powders), suspensions ready for injection, and emulsions.

[00168] Suitable vehicles that can be used to provide parenteral dosage forms of the invention are well known to those skilled in the art. Examples include, but are not limited to: Water for Injection USP; aqueous vehicles such as, but not limited to, Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, and Lactated Ringer's Injection; water-miscible vehicles such as, but not limited to, ethyl alcohol, polyethylene glycol, and polypropylene glycol; and non-aqueous vehicles such as, but not limited to, corn oil, cottonseed oil, peanut oil, sesame oil, ethyl oleate, isopropyl myristate, and benzyl benzoate.

[00169] Compounds that increase the solubility of one or more of the active ingredients disclosed herein can also be incorporated into the parenteral dosage forms of the invention.

Transdermal Dosage Forms

[00170] Transdermal dosage forms include "reservoir type" or "matrix type" patches, which can be applied to the skin and worn for a specific period of time to permit the penetration of a desired amount of active ingredients.

[00171] Suitable excipients (e.g., carriers and diluents) and other materials that can be used to provide transdermal and topical dosage forms encompassed by this invention are well known to those skilled in the pharmaceutical arts, and depend on the particular tissue to which a given pharmaceutical composition or dosage form will be applied. With that fact in mind, typical excipients include, but are not limited to, water, acetone, ethanol, ethylene glycol, propylene glycol, butane-1,3-diol, isopropyl myristate, isopropyl palmitate, mineral oil, and mixtures thereof.

[00172] Depending on the specific tissue to be treated, additional components may be used prior to, in conjunction with, or subsequent to treatment with active ingredients of the invention. For example, penetration enhancers can be used to assist in delivering the active ingredients to the tissue. Suitable penetration enhancers include, but are not limited to: acetone; various alcohols such as ethanol, oleyl, and tetrahydrofuryl; alkyl sulfoxides such as dimethyl sulfoxide; dimethyl acetamide; dimethyl formamide; polyethylene glycol; pyrrolidones such as polyvinylpyrrolidone; Kollidon grades (Povidone, Polyvidone); urea; and various water-soluble or insoluble sugar esters such as Tween 80 (polysorbate 80) and Span 60 (sorbitan monostearate).

[00173] The pH of a pharmaceutical composition or dosage form, or of the tissue to which the pharmaceutical composition or dosage form is applied, may also be adjusted to improve delivery of one or more active ingredients. Similarly, the polarity of a solvent carrier, its ionic strength, or tonicity can be adjusted to improve delivery. Compounds such as stearates can also be added to pharmaceutical compositions or dosage forms to advantageously alter the hydrophilicity or lipophilicity of one or more active ingredients so as to improve delivery. In this regard, stearates can serve as a lipid vehicle for the formulation, as an emulsifying agent or surfactant, and as a delivery-enhancing or penetration-enhancing agent. Different salts, hydrates or solvates of the active ingredients can be used to further adjust the properties of the resulting composition.

Topical Dosage Forms

[00174] Topical dosage forms of the invention include, but are not limited to, creams, lotions, ointments, gels, solutions, emulsions, suspensions, or other forms known to one of skill in the art. See, e.g., Remington's Pharmaceutical Sciences, 18th eds., Mack

Publishing, Easton PA (1990); and Introduction to Pharmaceutical Dosage Forms, 4th ed., Lea & Febiger, Philadelphia (1985).

[00175] Suitable excipients (e.g., carriers and diluents) and other materials that can be used to provide transdermal and topical dosage forms encompassed by this invention are well known to those skilled in the pharmaceutical arts, and depend on the particular tissue to which a given pharmaceutical composition or dosage form will be applied. With that fact in mind, typical excipients include, but are not limited to, water, acetone, ethanol, ethylene glycol, propylene glycol, butane-1,3-diol, isopropyl myristate, isopropyl palmitate, mineral oil, and mixtures thereof.

[00176] Depending on the specific tissue to be treated, additional components may be used prior to, in conjunction with, or subsequent to treatment with active ingredients of the invention. For example, penetration enhancers can be used to assist in delivering the active ingredients to the tissue. Suitable penetration enhancers include, but are not limited to: acetone; various alcohols such as ethanol, oleyl, and tetrahydrofuryl; alkyl sulfoxides such as dimethyl sulfoxide; dimethyl acetamide; dimethyl formamide; polyethylene glycol; pyrrolidones such as polyvinylpyrrolidone; Kollidon grades (Povidone, Polyvidone); urea; and various water-soluble or insoluble sugar esters such as Tween 80 (polysorbate 80) and Span 60 (sorbitan monostearate).

Mucosal Dosage Forms

[00177] Mucosal dosage forms of the invention include, but are not limited to, ophthalmic solutions, sprays and aerosols, or other forms known to one of skill in the art. See, e.g., Remington's Pharmaceutical Sciences, 18th eds., Mack Publishing, Easton PA (1990); and Introduction to Pharmaceutical Dosage Forms, 4th ed., Lea & Febiger, Philadelphia (1985). Dosage forms suitable for treating mucosal tissues within the oral cavity can be formulated as mouthwashes or as oral gels. In one embodiment, the aerosol comprises a carrier. In another embodiment, the aerosol is carrier free.

[00178] The TLR7 ligands or TLR7 ligand prodrugs of the invention may also be administered directly to the lung by inhalation. For administration by inhalation, a TLR7 ligand can be conveniently delivered to the lung by a number of different devices. For example, a Metered Dose Inhaler ("MDI") which utilizes canisters that contain a suitable low boiling propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas can be used to deliver a TLR7 ligand directly to the lung. MDI devices are available from a number of suppliers such as 3M Corporation, Aventis, Boehringer Ingleheim, Forest Laboratories, Glaxo-Wellcome, Schering Plough and Vectura.

[00179] Alternatively, a Dry Powder Inhaler (DPI) device can be used to administer a TLR7 ligand to the lung (see, e.g., Raleigh *et al.*, Proc. Amer. Assoc. Cancer Research Annual Meeting, 1999, 40, 397, which is herein incorporated by reference). DPI devices typically use a mechanism such as a burst of gas to create a cloud of dry powder inside a container, which can then be inhaled by the patient. DPI devices are also well known in the art and can be purchased from a number of vendors which include, for example, Fisons, Glaxo-Wellcome, Inhale Therapeutic Systems, ML Laboratories, Qdose and Vectura. A popular variation is the multiple dose DPI ("MDDPI") system, which allows for the delivery of more than one therapeutic dose. MDDPI devices are available from companies such as AstraZeneca, GlaxoWellcome, IVAX, Schering Plough, SkyePharma and Vectura. For example, capsules and cartridges of gelatin for use in an inhaler or insufflator can be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch for these systems.

[00180] Another type of device that can be used to deliver a TLR7 ligand or TLR7 ligand prodrug to the lung is a liquid spray device supplied, for example, by Aradigm Corporation. Liquid spray systems use extremely small nozzle holes to aerosolize liquid drug formulations that can then be directly inhaled into the lung.

[00181] In a preferred embodiment, a nebulizer device is used to deliver TLR7 ligands or TLR ligand prodrugs to the lung. Nebulizers create aerosols from liquid drug formulations by using, for example, ultrasonic energy to form fine particles that can be readily inhaled (See e.g., Verschoyle *et al.*, British J. Cancer, 1999, 80, Suppl 2, 96, which is herein incorporated by reference). Examples of nebulizers include devices supplied by Sheffield/Systemic Pulmonary Delivery Ltd, Aventis, and Batelle Pulmonary Therapeutics. See U.S. Pat. Nos. 5,954,047; 5,950,619; 5,970,974, which are herein incorporated by reference.

[00182] In a particularly preferred embodiment, an electrohydrodynamic ("EHD") aerosol device is used to deliver TLR7 ligands and TLR7 ligand prodrugs to the lung. EHD aerosol devices use electrical energy to aerosolize liquid drug solutions or suspensions (see, e.g., Noakes *et al.*, U.S. Pat. No. 4,765,539; Coffee, U.S. Pat. No., 4,962,885; Coffee, PCT Application, WO 94/12285; Coffee, PCT Application, WO 94/14543; Coffee, PCT Application, WO 95/26234, Coffee, PCT Application, WO 95/26235, Coffee, PCT Application, WO 95/32807, which are herein incorporated by reference). The electrochemical properties of the TLR7 ligands and TLR7 ligand prodrugs formulation may be important parameters to optimize when delivering this drug to the lung with an EHD aerosol device and such optimization is routinely performed by one of skill in the art. EHD

aerosol devices may more efficiently delivery drugs to the lung than existing pulmonary delivery technologies. Other methods of intra-pulmonary delivery of TLR7 ligand and TLR7 ligand prodrugs will be known to the skilled artisan and are within the scope of the invention.

[00183] Liquid drug formulations suitable for use with nebulizers and liquid spray devices and EHD aerosol devices will typically include a TLR7 ligand or TLR7 ligand prodrug with a pharmaceutically acceptable carrier. Preferably, the pharmaceutically acceptable carrier is a liquid such as alcohol, water, polyethylene glycol or a perfluorocarbon. Optionally, another material may be added to alter the aerosol properties of the solution or suspension of the TLR7 ligand or prodrug of a TLR7 ligand. Preferably, this material is liquid such as an alcohol, glycol, polyglycol or a fatty acid. Other methods of formulating liquid drug solutions or suspension suitable for use in aerosol devices are known to those of skill in the art (see, e.g., Biesalski, U.S. Pat. Nos. 5,112,598; Biesalski, 5,556,611, which are herein incorporated by reference) A TLR7 ligand or prodrug of a TLR7 ligand can also be formulated in rectal or vaginal compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases such as cocoa butter or other glycerides.

[00184] In addition to the formulations described previously, a TLR7 ligand or TLR7 ligand prodrug can also be formulated as a depot preparation. Such long acting formulations can be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds can be formulated with suitable polymeric or hydrophobic materials (for example, as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

[00185] Alternatively, other pharmaceutical delivery systems can be employed. Liposomes and emulsions are well known examples of delivery vehicles that can be used to deliver TLR7 ligands and TLR7 ligand prodrugs. Certain organic solvents such as dimethylsulfoxide can also be employed, although usually at the cost of greater toxicity. A TLR7 ligand or prodrug of a TLR7 ligand can also be delivered in a controlled release system. In one embodiment, a pump can be used (Sefton, CRC Crit. Ref Biomed Eng., 1987, 14, 201; Buchwald *et al.*, Surgery, 1980, 88, 507; Saudek *et al.*, N. Engl. J. Med., 1989, 321, 574). In another embodiment, polymeric materials can be used (see Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Fla. (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J. Macromol. Sci. Rev.

Macromol. Chem., 1983, 23, 61; see also Levy *et al.*, Science, 1985, 228, 190; During *et al.*, Ann. Neurol., 1989,25,351; Howard *et al.*, 1989, J. Neurosurg. 71, 105). In yet another embodiment, a controlled-release system can be placed in proximity of the target of the compounds of the invention, e.g., the lung, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115 (1984)). Other controlled-release system can be used (see, e.g. Langer, Science, 1990, 249, 1527).

[00186] Suitable excipients (e.g., carriers and diluents) and other materials that can be used to provide mucosal dosage forms encompassed by this invention are well known to those skilled in the pharmaceutical arts, and depend on the particular site or method which a given pharmaceutical composition or dosage form will be administered. With that fact in mind, typical excipients include, but are not limited to, water, ethanol, ethylene glycol, propylene glycol, butane-1,3-diol, isopropyl myristate, isopropyl palmitate, mineral oil, and mixtures thereof, which are non-toxic and pharmaceutically acceptable. Examples of such additional ingredients are well known in the art. See, e.g., Remington's Pharmaceutical Sciences, 18th eds., Mack Publishing, Easton PA (1990).

[00187] The pH of a pharmaceutical composition or dosage form, or of the tissue to which the pharmaceutical composition or dosage form is applied, can also be adjusted to improve delivery of one or more active ingredients. Similarly, the polarity of a solvent carrier, its ionic strength, or tonicity can be adjusted to improve delivery. Compounds such as stearates can also be added to pharmaceutical compositions or dosage forms to advantageously alter the hydrophilicity or lipophilicity of one or more active ingredients so as to improve delivery. In this regard, stearates can serve as a lipid vehicle for the formulation, as an emulsifying agent or surfactant, and as a delivery-enhancing or penetration-enhancing agent. Different salts, hydrates or solvates of the active ingredients can be used to further adjust the properties of the resulting composition.

KITS

[00188] The invention provides a pharmaceutical pack or kit comprising one or more containers comprising a TLR7 ligand prodrug useful for the treatment or prevention of a Hepatitis C virus infection. In other embodiments, the invention provides a pharmaceutical pack or kit comprising one or more containers comprising a TLR7 ligand prodrug useful for the treatment or prevention of a Hepatitis C virus infection and one or more containers comprising an additional therapeutic agent, including but not limited to those listed in section 5.2.2 above, in particular an antiviral agent, an interferon, an agent which inhibits

viral enzymes, or an agent which inhibits viral replication, preferably the additional therapeutic agent is HCV specific or demonstrates anti-HCV activity.

[00189] The invention also provides a pharmaceutical pack or kit comprising one or more containers comprising one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

ASSAYS

[00190] The TLR7 ligands, TLR ligand prodrugs, compositions and dosage forms of the invention can be tested *in vitro* or *in vivo* by a variety of methods known in the art to test activity. See, for example, the methods discussed below and used throughout the examples.

[00191] A range of assays for the purpose of evaluating TLR7 activity are available, and are described in the following publications, each of which is incorporated-by-reference: Hirota *et al.*, *J. Med. Chem.*, 45, 5419-5422 (2002); and Akira S. *et al.*, *Immunology Letters*, 85, 85-95 (2003). For example, one system useful for the assay of TLR7 ligands is where the gene for TLR7 is cloned by methods known to those of skill in the art and transfected into an appropriate cell type such that TLR7 is expressed and coupled to a NFkB-luciferase reporter plasmid. In this cell system, exposure to TLR7 ligand prodrugs in cell culture results in a measurable luminescence signal. See, e.g., Lee *et al.*, *Proc. Nat. Acad. USA*, 100, 6646-6651 (2003); Hemmi *et al.*, *Nat. Immunol.*, 3, 196-200 (2002); and Jurk *et al.*, *Nat. Immunol.*, 3, 499 (2002) (wherein the Lee *et al.*, Hemmi *et al.*, and Jurk *et al.*, references are all incorporated herein by reference).

[00192] Another example of an in vitro method is to expose human peripheral blood mononuclear cells (PBMC) to the candidate TLR7 ligand prodrug for a predetermined interval (e.g., 2 hours to 24 hours), followed by measurement of immunologic activity. Such immunologic activity may include induction of the synthesis of cytokines, which can be measured in the culture supernate by ELISA assay of the cytokine protein, such as a Type 1 interferon (interferon alpha, interferon beta) or Type 2 interferon (interferon gamma). Alternatively, the PBMC can be harvested after incubation with the candidate TLR7 ligand prodrug, the PBMC RNA extracted, and the level of induction of immune system genes determined by RNAse protection assays of the extracted RNA. Genes typically induced include 2'5'-OAS, or interferon gamma, but a range of cytokines can be measured. See, e.g., Hirota *et al.*, J. Med. Chem., 45, 5419-5422 (2002).

[00193] RNAse protection assays (RPA) are a method known in the art wherein RNA analytes are quantitated by first hybridizing them to a radioalabeled RNA sequence specific for the analyte RNA, followed by digestion with enzymes that selectively degrade single stranded RNA. The sample is then submitted to gel electrophoresis under conditions that resolve the hybridized, protected double stranded RNA. The gel is then autoradiographed to reveal the location and intensity of the bands. These can be quantitated by methods well known in the art. Multiple analyte RNA species can be simultaneously assayed, if the protected fragments are sufficiently different in size to allow their separation by gel electrophoresis. Comparison of the levels of an analyte RNA to control RNA species that is expressed at constant levels in cells provides an internal control that enable changes in levels of the analyte RNA species to be monitored even if the total amount of RNA varies. Such RNAse protection assays can be performed as follows:

[00194] RNA is purified from PMBC pellets using the "RNAeasy" kit (Qiagen), according to the manufacturer's instructions. Template sets may be obtained from PharMingen (BD Biosciences); a useful set that is commercially available from this supplier contains materials allowing the assay of TNF-a, IL12 p35, IP10, IL-1a, IL-1b, IL-6, Interferon gamma, and the control RNA species L32 and GAPDH. This template set contains DNA that is appropriate for synthesis of the proper RNA probe for each of the listed genes.

[00195] Probe synthesis is performed using the PharMingen in vitro transcription kit provided in the kit. The reaction contains RNase inhibitor; transcription buffer; 50 ng of the tempate set; 0.1375 mM of each of rGTP, rCTP, rATP; 0.003 mM rUTP;10 mM DTT, 0.010 mCi of [alpha 32P] UTP, and 20 Units of T7 RNA polymerase in a volume of 20 microliters. The reaction mixture is incubated for one hour at 37 °C, and then stopped by addition of 2 units of RNAse free DNAse, with an additional incubation of 30 minutes at 37 °C. The RNA probes synthesized in this incubation are extracted once with 5.2 mM EDTA, 25 µl of Tris Saturated phenol, 25 µL of chloroform, and 4 µg of yeast tRNA, and then extracted with 50 µL of chloroform. The RNA is precipitated by addition of 50 µL of 4M ammonium acetate and 250 µL of ice-cold 100% ethanol, and after incubation at –80 °C for 30 minutes the preparation is centrifuged at high speed for 30 minutes. The pellet is washed in 100 % ethanol, and after removal of the ethanol the probe is resuspended and used in the RNAse protection incubation.

[00196] The RNAse protection assay uses the probe material prepared above and RNA extracted from PBMC. The PBMC RNA is washed in 100% ethanol, quantitated by absorbance at 260 nm. The RPA is performed as described in the protocol provided in the

PharMingen RiboQuant kit. Eight μL of the PBMC RNA samples are mixed with the 2 μL of the probe set in a thin-walled PCR tube, mixed well, briefly centrifuged and then overlaid with mineral oil. The tube is then placed in a 90 °C PCR block, cooled to 56 °C, and incubated for 16 hours. The samples are then cooled to 37 °C and RNAse A and RNAse T1 are added. The mixture is incubated for 45 minutes at 30 °C, and the reaction stopped with a mix of protease K and yeast tRNA. The RNA is extracted with phenol-chloroform, and then precipitated from the phenol-chloroform with ammonium acetate-ethanol. The pellet is washed with ethanol and resuspended in buffer for electrophoresis. The samples are analysed by gel electrophoresis by methods well known in molecular biology.

[00197] A number of assays may be employed in accordance with the present invention in order to determine the degree of anti-viral activity of a compound of the invention such as cell culture, animal models, and administration to human subjects. The assays described herein may be used to assay viral growth over time to determine the growth characteristics of a virus in the presence of a compound of the invention.

[00198] In another embodiment, a virus and a compound of the invention are administered to animal subjects susceptible to infection with the virus. The incidence, severity, length, virus load, mortality rate of infection, etc. can be compared to the incidence, severity, length, virus load, mortality rate of infection, etc. observed when subjects are administered the virus alone (in the absence of a compound of the invention). Anti-virus activity of the compound of the invention is demonstrated by a decrease in incidence, severity, length, virus load, mortality rate of infection, etc. in the presence of the compound of the invention. In a specific embodiment, the virus and the compound of the invention are administered to the animal subject at the same time. In another specific embodiment, the virus is administered to the animal subject before the compound of the invention. In another specific embodiment, the compound of the invention is administered to the animal subject before the virus.

[00199] In another embodiment, the growth rate of the virus can be tested by sampling biological fluids/clinical samples (e.g., nasal aspirate, throat swab, sputum, broncho-alveolar lavage, urine, saliva, blood, or serum) from human or animal subjects at multiple time points post-infection either in the presence or absence of a compound of the invention and measuring levels of virus. In specific embodiments, the growth rate of a virus is assayed by assessing the presence of virus in a sample after growth in cell culture, growth on a permissible growth medium, or growth in subject using any method well-known in the art, for example, but not limited to, immunoassay (*e.g.*, ELISA; for discussion regarding ELISAs *see, e.g.*, Ausubel *et al.*, eds, 1994, Current Protocols in Molecular Biology, Vol. I,

John Wiley & Sons, Inc., New York at 11.2.1), immunofluorescent staining, or immunoblot analysis using an antibody which immunospecifically recognizes the virus to be assayed or detection of a virus-specific nucleic acid (*e.g.*, by Southern blot or RT-PCR analysis, etc.).

[00200] In a specific embodiment, viral titers can be determined by obtaining biological fluids/clinical samples from infected cells or an infected subject, preparing a serial dilution of the sample and infecting a monolayer of cells that are susceptible to infection with the virus (e.g. primary cells, transformed cell lines, patient tissue samples, etc) at a dilution of the virus that allows for the emergence of single plaques. The plaques can then be counted and the viral titer expressed as plaque forming units per milliliter of sample.

[00201] In one specific embodiment, the growth rate of a virus in a subject can be estimated by the titer of antibodies against the virus in the subject. Antibody serum titer can be determined by any method well-known in the art, for example, but not limited to, the amount of antibody or antibody fragment in serum samples can be quantitated by, e.g., ELISA.

[00202] Additionally, *in vivo* activity of a TLR7 ligand or prodrug of a TLR7 ligand can be determined by directly administering the compound to a test animal, collecting biological fluids (e.g., nasal aspirate, throat swab, sputum, broncho-alveolar lavage, urine, saliva, blood, or serum) and testing the fluid for anti-virus activity.

[00203] In embodiments where samples to be assayed for virus levels are biological fluids/clinical samples (e.g., nasal aspirate, throat swab, sputum, broncho-alveolar lavage, urine, saliva, blood, or serum), the samples may or may not contain in tact cells. Samples from subjects containing intact cells can be directly processed, whereas isolates without intact cells may or may not be first cultured on a permissive cell line (e.g. primary cells, transformed cell lines, patient tissue samples, etc) or growth medium (e.g., LB broth/agar, YT broth/agar, blood agar, etc.). Cell suspensions can be cleared by centrifugation at, e.g., 300xg for 5 minutes at room temperature, followed by a PBS, pH 7.4 (Ca++ and Mg++ free) wash under the same conditions. Cell pellets can be resuspended in a small volume of PBS for analysis. Primary clinical isolates containing intact cells can be mixed with PBS and centrifuged at 300xg for 5 minutes at room temperature. Mucus is removed from the interface with a sterile pipette tip and cell pellets can be washed once more with PBS under the same conditions. Pellets can then be resuspended in a small volume of PBS for analysis.

[00204] In another embodiment, a compound of the invention is administered to a human subject infected with a virus. The incidence, severity, length, viral load, mortality rate of infection, etc. can be compared to the incidence, severity, length, viral load,

mortality rate of infection, etc. observed in human subjects infected with a virus in the absence of a compound of the invention or in the presence of a placebo. Anti-viral activity of the compound of the invention is demonstrated by a decrease in incidence, severity, length, viral load, mortality rate of infection, etc. in the presence of the compound of the invention. Any method known in the art can be used to determine anti-viral activity in a subject such as those described previously.

[00205] Additionally, *in vivo* activity of a TLR7 ligand or prodrug of TLR7 ligand can be determined by directly administering the compound to an animal or human subject, collecting biological fluids/clinical samples (e.g., nasal aspirate, throat swab, sputum, broncho-alveolar lavage, urine, saliva, blood, or serum) and testing the biological fluids/clinical samples for anti-viral activity (e.g., by addition to cells in culture in the presence of the virus).

[00206] The foregoing has demonstrated the pertinent and important features of the present invention. One of skill in the art will be appreciate that numerous modifications and embodiments may be devised. Therefore, it is intended that the appended claims cover all such modifications and embodiments.

6. EXAMPLES

[00207] The following examples are for the purpose of illustration only and are not intended as limiting the scope of the invention.

6.1 TLR7 Ligand Identification

[00208] There are three known chemical classes of TLR7 ligands: guanosines, imidazoquinolines, and pyrimidines (see Section 5.2). As described above, additional TLR7 ligands are readily identified by known screening methods. For example, adenine analogs and derivatives were identified as TLR ligands by using the following screening procedure. See Tables 1 and 2.

A stable HEK293-hTLR7 cell line was obtained from Invivogen (San Diego, California), transfected with pNiFty2-Luc, an NF-kB inducible luciferase reporter plasmid (Invivogen) and (dual) stable transfectants selected. The resultant dual (hTLR7/pNiFty2-Luc) cell lines were functionally tested by response to loxoribine and isatoribine as measured by fold luciferase induction relative to a no drug control. The C23 line was chosen due to its satisfactory response and sensitivity profile with these (and other) TLR7 agonists. The biological rationale which connects TLR7 engagement and NF-kB activation has met with widespread acceptance (for a review, see Akira S. *et al.*, *Immunol. Lett.*, 85, 85-95 (2003))

and as a consequence the HEK293-TLR-NF-kB inducible reporter system is accepted as a standard assay which has been consistently used to assay TLR(7) agonists, in either transient or stable system format. See, e.g., Hemmi H. *et al.*, *Nat. Immunol.*, 3, 196-200 (2002); Jurk M. *et al.*, *Nat. Immunol.*, 3, 499 (2002); and Lee J. *et al.*, *Proc. Natl. Acad. Sci. USA* , 100, 6646-51 (2003).

[00209] For a typical C23 assay, cells are seeded at $6x10^4$ cells/well in 96 well plates and 4-24 hours later are treated with various concentrations of compound. After 2-48 hours exposure the cell monolayers are lyzed with passive lysis buffer (Promega) and the firefly luciferase assay carried out using the luciferase assay reagent (Promega) as specified by the manufacturer. Relative luciferase activities are expressed as folds of induction compared to the no drug control. A two-fold induction over background is considered a *bona fide* TLR7 agonist, dependent on this being a statistically significant increase.

Table 1: Isatoribine Activates Human TLR7 in HEK293 Assay

Compound No.	Compound concentration, μM			
	50	100	250	500
21	3.1 (± 0.2)	3.4 (± 0.1)	6.2 (± 0.5)	10.9 (± 0.7)

Table 2: Adenine Derivative Activates Human TLR7 in HEK293 Assay

Compound No.	Compound concentration, μM			
	1.0	3.2	10	32
29	20.8 (± 1.1)	37.3 (± 0.5)	47.5 (± 0.2)	48.4 (± 0.4)

[00210] In Table 1, isatoribine, was added to C23 cells for forty-eight hours, and the cells were then harvested and assayed for luciferase activity. Each time-point was assayed in triplicate. The data displayed is the mean fold induction compared to the no drug control, together with the standard deviation in parentheses.

[00211] In Table 2, an adenine derivative **29** was added to C23 cells for twenty-four hours, and the cells were then harvested and assayed for luciferase activity. Each time-point

was assayed in triplicate. The data displayed is the mean fold induction compared to the no drug control, together with the standard deviation in parentheses.

6.2 TLR7 Ligands Tested as anti-HCV Agents

HCV Viral Load Reduction

[00212] Isatoribine investigational drug product was supplied as a 1 mg/mL solution in sterile normal saline contained in a 50 mL vial. Isatoribine was administered in humans by intravenous infusion once daily for 7 days, at 200, 400, 600 or 800 mg per dose. All doses were administered by constant rate infusion over a 60-minute period, except the 800 mg dose was administered over an 80-minute period. The flow rate for each dose was as follows: 3.33 mL/min for the 200 mg dose; 6.67 mL/min for the 400 mg dose; 8.33 mL/min for the 500 mg dose; or 10.0 mL/min for the 600 mg and 800 mg dose.

[00213] Four to twelve patients were enrolled in each of the dose groups (200 mg, 400 mg, 600 mg and 800 mg per dose) and received once daily intravenous infusions for 7 days. Prior to dosing, a blood sample was drawn from each patient for assessment of the genotype of the HCV virus.

[00214] Plasma HCV RNA was determined at baseline (an average of 2 pre-treatment measurements taken on Day –1 or pre-treatment and on Day 1) and once daily prior to the start of the first daily isatoribine intravenous infusion on Days 2 through 7 for these daily (x7 days) dosing groups. See Figure 2. The viral load was measured by the branched DNA method (Versant™ v3.0 bDNA assay, Bayer Diagnostics). For plasma HCV RNA, the maximum change from the pre-treatment baseline was estimated using log-transformed values.

[00215] Plasma HCV RNA decreased over the course of isatoribine treatment, with the larger changes generally occurring in patients who received the higher daily doses (Figure 2). Eight of 12 patients who received isatoribine 800 mg QD x 7 days showed a plasma viral load decrease of more than 0.5 log10 units, with a mean change in these 12 patients of -0.76 log10 units and range of –2.85 to +0.21 log10 units. This decrease in viral load was statistically significant for the 800 mg QD dose group ($p=0.008$). Plasma viral load declines generally reversed upon cessation of treatment.

HCV Replicon-Based Viral Bioassay

[00216] It has been demonstrated that HCV replicons are highly sensitive to the inhibitory effects of interferon-α and interferon-γ. Therefore the HCV replicon becomes a very useful system for measuring the amount of biologically active interferons in supernatants from human PBMCs stimulated with a TLR7 agonist. A quantitative assay was developed which is based on measuring the activity of the luciferase reporter gene that

was integrated into an HCV replicon. By using this system, interferons from TLR7 agonist-treated PBMCs were measured and their inhibitory activity was assessed using the luciferase reporter replicon.

[00217] Human PBMCs isolated from a healthy donor were placed in replicate cell culture wells (5×10^6 cells per well). The PBMCs are incubated in the absence of test compounds at 37 °C in a humidified atmosphere containing 5% CO_2 for 24 hours to allow stabilization to the culture conditions, and then the TLR7 ligand or a drug free control is added to replicate wells containing PBMCs from the same donor. The concentrations of TLR7 ligand may be varied to suit the particular experiment, and the PBMC cultures are then incubated at 37 °C in a humidified atmosphere containing 5% CO_2 for eight hours. Samples of cell culture supernatant are taken at the eight hour time point (or twenty four hour time point in case of Loxoribine and its prodrugs) from TLR7 ligand treated and control wells and are assayed for interferon-alpha production by ELISA. Supernatants from compound-treated cells and a no-drug control were diluted at 1:10, 1:100, and 1:1000 in RPMI media and applied to a 96-well plate of Huh7 hepatocyte cells containing the luciferase reporter replicon. Cells were incubated for 48 hours at 37 °C in a tissue culture incubator.

[00218] After the incubation period, the 96-well plates are washed 2X with PBS and are lysed with passive lysis buffer (Promega). Plates are shaken at room temperature for 20 minutes and standard luciferase assay reagent (Promega) is added to each well by injection and the plate is read on an Lmax luminometer (Molecular Devices). The raw relative light units are converted to a percent inhibition that is compared to the no-drug control wells to determine the level of inhibition observed in the replicon assay. The estimated maximal concentration of interferons required to inhibit HCV replicon replication was determined to be at a 1:10 dilution of supernatant of PBMC-stimulated cells which fell within our test range of the dilution series. For all TLR7 agonists tested, 100% inhibition was observed on the luciferase reporter replicon system at the 1:10 dilution.

[00219] The data presented in Tables 3-8 represents the inhibition of the HCV replicon system by the supernatant collected after exposure of PBMC cells to the compound at an initial concentration for the incubation time indicated and diluted as specified in the first column ("PBMC exposed to compound"). A supernatant collected from PBMC cells non-exposed to the compound and diluted as specified in the first column was used as a control ("Blank supernatant"). The PBMC cells were isolated from a single blood donor as specified.

Table 3: Antiviral Effect of Isatoribine in the *in vitro* HCV Replicon Bioassay

No. 1
Incubation time: 8 hours
Initial concentration: 100 μM
Blood donor number: FL72035

Dilution of supernatant	HCV replicon inhibition, %	
	Blank PBMC	PBMC exposed to compound
1:100	0	100
1:1000	0	94

No. 2
Incubation time: 8 hours
Initial concentration: 100 μM
Blood donor number: FL75287

Dilution of supernatant	HCV replicon inhibition, %	
	Blank PBMC	PBMC exposed to compound
1:10	0	100
1:100	0	87
1:1000	0	6

No. 3
Incubation time: 24 hours
Initial concentration: 100 μM
Blood donor number: FL75864

Dilution of supernatant	HCV replicon inhibition, %	
	Blank PBMC	PBMC exposed to compound
1:10	0	100
1:100	23	99
1:1000	0	64

Table 4: Antiviral Effect of Loxoribine in the *in vitro* HCV Replicon Bioassay

No. 1
Incubation time : 24 hours
Initial concentration: 100 μM
Blood donor number: FL75864

Dilution of supernatant	HCV replicon inhibition, %	
	Blank PBMC	PBMC exposed to compound
1:10	0	100
1:100	23	74
1:1000	0	17

Table 5: Antiviral Effect of Imiquimod in the *in vitro* HCV Replicon Bioassay

No. 1
Incubation time: 8 hours
Initial concentration: 3.2 μM
Blood donor number: FL75287

Dilution of supernatant	HCV replicon inhibition, %	
	Blank PBMC	PBMC exposed to compound
1:10	0	100

1:100	0	100
1:1000	0	89

No. 2
Incubation time: 8 hours
Initial concentration: 3.2 μM
Blood donor number: FL75287

Dilution of supernatant	HCV replicon inhibition, %	
	Blank PBMC	PBMC exposed to compound
1:10	3	100
1:100	13	75
1:1000	3	1

Table 6: Antiviral Effect of Resiquimod in the *in vitro* HCV Replicon Bioassay

No. 1
Incubation time: 8 hours
Initial concentration: 10 μM
Blood donor number: FL75287

Dilution of supernatant	HCV replicon inhibition, %	
	Blank PBMC	PBMC exposed to compound
1:10	0	100
1:100	0	98
1:1000	0	21

No. 2
Incubation time: 8 hours
Initial concentration: 10 μM
Blood donor number: FL75287

Dilution of supernatant	HCV replicon inhibition, %	
	Blank PBMC	PBMC exposed to compound
1:10	3	100
1:100	13	88
1:1000	3	6

Table 7: Antiviral Effect of Bropirimine in the *in vitro* HCV Replicon Bioassay

No. 1
Incubation time: 8 hours
Initial concentration: 100 μM
Blood donor number: FL72035

Dilution of supernatant	HCV replicon inhibition, %	
	Blank PBMC	PBMC exposed to compound
1:10	0	100
1:100	0	95
1:1000	0	0

No. 2
Incubation time: 8 hours
Initial concentration: 100 μM

Blood donor number: FL72036

Dilution of supernatant	HCV replicon inhibition, %	
	Blank PBMC	PBMC exposed to compound
1:10	0	100
1:100	8	95
1:1000	0	33

Table 8: Antiviral Effect of Adenine Derivative in the *in vitro* HCV Replicon Bioassay

No. 1
Incubation time: 8 hours
Initial concentration: 0.1 μM
Blood donor number: FL76418
Form of the compound: a TFA salt

Dilution of supernatant	HCV replicon inhibition, %	
	Blank PBMC	PBMC exposed to compound
1:10	0	100
1:100	3	73
1:1000	3	0

No. 2
Incubation time: 8 hours
Initial concentration: 0.1 μM
Blood donor number: FL76418
Form of the compound: a TFA salt

Dilution of supernatant	HCV replicon inhibition, %	
	Blank PBMC	PBMC exposed to compound
1:10	0	100
1:100	3	64
1:1000	3	9

6.3 Preparation of TLR7 Ligand Prodrugs

[00220] Compounds of the invention can be synthesized using the methodology described in Schemes 1-18 above. Unless otherwise indicated all temperatures are set forth in degrees Celsius and all parts and percentages are by weight. Reagents are purchased from commercial suppliers such as Aldrich Chemical Company or Lancaster Synthesis Ltd. and are used without further purification unless otherwise indicated. Tetrahydrofuran (THF) and N,N-dimethylforamide (DMF) are purchased from Aldrich in Sure Seal bottles and used as received. Unless otherwise indicated, the following solvents and reagents are distilled under a blanket of dry nitrogen. THF and Et_2O are distilled from Na-benzophenone ketyl; CH_2Cl_2 (DCM), diisopropylamine, pyridine and Et_3N are distilled from CaH_2; MeCN is distilled first from P_2O_5, then from CaH_2; MeOH is distilled from Mg; PhMe, EtOAc and i-PrOAc are distilled from CaH_2; TFAA was purified via simple atmospheric distillation under dry argon.

The reactions set forth are done generally under a positive pressure of argon at an ambient temperature (unless otherwise stated) in anhydrous solvents, and the reaction flasks are fitted with rubber septa for the introduction of substrates and reagents via syringe. Glassware is oven dried and/or heat dried. The reactions are assayed by TLC and terminated as judged by the consumption of starting material. Analytical thin layer chromatography (TLC) is performed on aluminum-backed silica gel 60 F254 0.2 mm plates (EM Science), and visualized with UV light (254 nm) followed by heating with commercial ethanolic phosphomolybdic acid. Preparative thin layer chromatography (TLC) is performed on aluminum-backed silica gel 60 F254 1.0 mm plates (EM Science) and visualized with UV light (254 nm). HPLC is performed on a Waters Micromass ZQ system consisting of a model 2525 binary gradient pump with an Alltech model 800 ELSD detector and a Waters model 996 photodiode array detector.

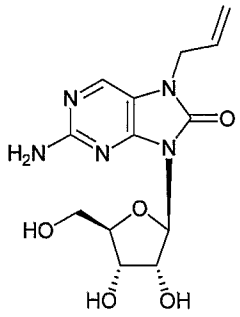
[00221] Work-ups are typically done by doubling the reaction volume with the reaction solvent or extraction solvent and then washing with the indicated aqueous solutions using 25% by volume of the extraction volume unless otherwise indicated. Product solutions are dried over anhydrous Na_2SO_4 and/or Mg_2SO_4 prior to filtration and evaporation of the solvents under reduced pressure on a rotary evaporator and noted as solvents removed in vacuo. Column chromatography is completed under positive pressure using 230-400 mesh silica gel or 50-200 mesh neutral alumina. Hydrogenolysis is done at the pressure indicated in the examples or at ambient pressure.

[00222] ^{1}H-NMR spectra is recorded on a Varian Mercury-VX400 instrument operating at 400 MHz and ^{13}C-NMR spectra are recorded operating at 75 MHz. NMR spectra are obtained as $CDCl_3$ solutions (reported in ppm), using chloroform as the reference standard (7.27 ppm and 77.00 ppm), CD_3OD (3.4 and 4.8 ppm and 49.3 ppm), DMSO-d_6, or internally tetramethylsilane (0.00 ppm) when appropriate. Other NMR solvents are used as needed. When peak multiplicities are reported, the following abbreviations are used: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broadened), dd (doublet of doublets), dt (doublet of triplets). Coupling constants, when given, are reported in Hertz (Hz).

[00223] Infrared (IR) spectra are recorded on a Thermo Nicolet Avatar 370 FT-IR as neat oils or solids, and when given are reported in wave numbers (cm^{-1}). Mass spectra reported (+)-ES Thermo Finnegan LCQ LC/MS conducted by the Analytical Chemistry Department at Anadys Pharmaceuticals, Inc. Elemental analyses are conducted by the Atlantic Microlab, Inc. in Norcross, GA or by NuMega, in San Diego, CA. Melting points (mp) are determined on an open capillary apparatus, and are uncorrected.

[00224] The described synthetic pathways and experimental procedures utilize many common chemical abbreviations, THF (tetrahydrofuran), DMF (N,N-dimethylformamide), EtOAc (ethyl acetate), DMSO (dimethyl sulfoxide), DMAP (4-dimethylaminopyridine), DBU (1,8-diazacyclo[5.4.0]undec-7-ene), DCM (4-(dicyanomethylene)-2-methyl-6-(4-dimethylamino-styryl)-4-H–pyran), MCPBA (3-chloroperoxybenzoic acid), EDC (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride), HATU (O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate), HOBT (1-hydroxybenzotriazole hydrate), TFAA (trifluoroacetic anhydride), pyBOP (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate), DIEA (diisopropylethylamine), and the like.

Example 1: **7-Allyl-2-amino-9-β-D-ribofuranosyl-7,9-dihydro-purin-8-one (43)**



Step 1: Preparation of 7-Allyl-2-amino-9-(2',3',5'-tri-O-acetyl-β-D-ribofuranosyl)-7,9-dihydro-1H-purine-6,8-dione (**40**)

[00225] A heterogeneous mixture of 7-allyl-2-amino-9-β-D-ribofuranosyl-7,9-dihydro-1*H*-purine-6,8-dione **17** (1.00 g, 2.95 mmol, prepared according to Reitz *et al.*, *JMC*, 37, 3561-3578 (1994)), DMAP (0.036 mg, 0.29 mmol) and NEt_3 (2.05 mL, 14.74 mmol) was stirred in dry acetonitrile (25 mL). Acetic anhydride (0.862 mL, 9.13 mmol) was added slowly to the suspension and the reaction mixture was stirred at ambient temperature for 16 h. The solvent was removed under vacuum and the residue dissolved in dichloromethane (DCM). The organic phase was then washed with saturated aqueous sodium bicarbonate ($NaHCO_3$), brine and thereafter dried with anhydrous magnesium sulfate ($MgSO_4$). The solvent was concentrated under vacuum and dried at room temperature under high vacuum to give 1.33 g of **40** (97%) as a pale yellow solid: ^1H NMR (400 MHz, $CDCl_3$) δ 6.12 (t, J = 6.0 Hz, 1H), 6.01 (d, J = 3.6 Hz, 1H), 5.89 (m, 1H), 5.82 (t, J = 6.0 Hz, 1H), 5.39 (br s, 2H), 5.21 (m, 2H), 4.58 (br s, 2H), 4.51 (m, 1H), 4.32 (m, 2H), 2.16 (s, 3H), 2.15 (s, 3H), 2.10 (s, 3H); MS (+)-ES $[M+H]^+$ 466.2 *m/z*.

Step 2: Preparation of 7-Allyl-2-amino-6-chloro-9-(2',3',5'-tri-O-acetyl-β-D-ribofuranosyl)-7,9-dihydro-purin-8-one (**41**)

[00226] Compound **40** (0.65 g, 1.39 mmol) was dissolved in phosphorus oxychloride (10 mL) and heated to 75 °C for 16 h. The reaction mixture was concentrated under vacuum and the crude product dissolved in DCM. The mixture was then washed with $NaHCO_3$ solution, brine, dried (MgSO4) and filtered. The filtrate was concentrated under vacuum and purified by flash chromatography using 10 to 50% gradient of ethyl acetate in hexanes. Removal of the solvent afforded 280 mg (41%) of the desired product **41**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.04 (d, J = 4.0 Hz, 1H), 6.03 (t, J = 5.6 Hz, 1H), 5.87 (m, 1H), 5.86 (t, J = 5.6 Hz, 1H), 5.18 (m, 4H), 4.59 (d, J = 8.0 Hz, 2H), 4.45 (d, J = 7.6 Hz, 1H), 4.31 (m, 2H), 2.10 (s, 3H), 2.08 (s, 3H), 2.04 (s, 3H); MS (+)-ES $[M+H]^+$ 484.2 *m/z*.

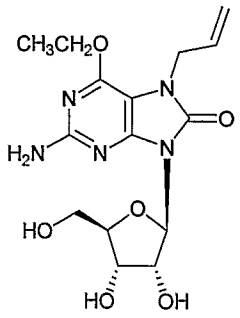
Step 3: Preparation of 7-Allyl-2-amino-9-(2',3',5'-tri-O-acetyl-β-D-ribofuranosyl)-7,9-dihydro-purin-8-one (**42**)

[00227] Compound **41** (0.27 g, 0.56 mmol) was dissolved in acetic acid and Zn-Cu couple was added to the solution. The mixture was heated at 70 °C for 18 h. The suspended particles were filtered off and the filtrate concentrated under vacuum. The residue was purified by flash chromatography using 10% to 100% gradient of ethyl acetate in hexanes. The solvent was removed to give 150 mg (60 %) of **42** as off-white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.05 (t, J = 4.0 Hz, 1H), 6.03 (d, J = 4.0 Hz, 1H), 5.87 (t, J = 6.0 Hz, 1H), 5.83 (m, 1H), 5.48 (br s, 2H), 5.33 (s, 1H), 5.29 (d, J = 5.6 Hz, 1H), 4.49 (d, J = 3.2 Hz, 1H), 4.46 (d, J = 3.2 Hz, 1H), 4.41 (d, J = 5.6 Hz, 2H), 4.27 (m, 2H), 2.12 (s, 3H), 2.10 (s, 3H), 2.05 (s, 3H); MS (+)-ES $[M+H]^+$ 450.0 *m/z*.

Step 4: Preparation of 7-Allyl-2-amino-9-β-D-ribofuranosyl-7,9-dihydro-purin-8-one (**43**)

[00228] To a solution of **42** (0.13 g, 0.29 mmol) in methanol (4 mL) was added solid K_2CO_3 (0.024g, 0.17 mmol) and the reaction stirred at ambient temperature for 18 h. To the cloudy mixture was added Amberlite CG-50 (0.5 g) and stirred till neutral and filtered. The filtrate was concentrated to give an off-white solid, which was washed with water and dried under high vacuum to give 93.5 mg of pure **43** in quantitative yield as an off white solid: ^{1}H NMR (400 MHz, d_6-DMSO) δ 7.88 (s, 1H), 6.33 (br s, 2H), 5.85 (m, 1H), 5.66 (d, J = 6.0 Hz, 1H), 5.30 (d, J = 5.6 Hz, 1H), 5.20 (s, 1H), 5.16 (d, J = 8.4 Hz, 1H), 5.01 (d, J = 4.8 Hz, 1H), 4.89 (q, J = 5.6 Hz, 1H), 4.75 (br s, 1H), 4.35 (d, J = 5.2 Hz, 2H), 4.10 (t, J = 8.4 Hz, 1) 3.80 (q, J = 3.6 Hz, 1H), 3.57 (m, 1H), 3.44 (m, 1H). MS (+)-ES $[M+H]^+$ 324.1 *m/z*.

Example 2: **7-Allyl-2-amino-6-ethoxy-9-β-D-ribofuranosyl-7,9-dihydro-purin-8-one (45)**



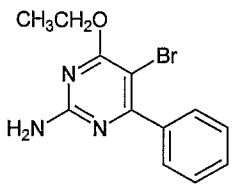
Step 1: Preparation of 7-Allyl-2-amino-6-ethoxy-9-(2',3',5'-tri-O-acetyl-β-D-ribofuranosyl)-7,9-dihydro-purin-8-one (**44**)

[00229] To a solution of **40** (0.30 g, 0.64 mmol) in dry THF (15 mL) was added polymer-supported triphenylphosphine (0.89 g, 1.93 mmol) and EtOH (0.11 mL, 1.93 mmol) at room temperature. To the stirring mixture was added diethyl azodicarboxylate (0.12 mL, 0.77 mmol) and stirring continued for 18 h. The spent polymer support was filtered off and the solvent removed under vacuum. The residue was then purified by flash chromatography using 10 to 50% gradient of ethyl acetate in hexanes. Removal of the solvent afforded 85 mg (26%) of the desired product **6** as a clear oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.07 (d, J = 4.0 Hz, 1H), 6.06 (d, J = 4.0 Hz, 1H), 6.01 (d, J = 3.6 Hz, 1H), 5.96 (t, J = 6.0 Hz, 1H), 5.87 (m, 1H), 5.14 (d, J = 2.2 Hz, 1H), 5.15 (m, 1H), 4.80 (br s, 2H), 4.46 (m, 4H), 4.37 (q, J = 7.2 Hz, 2H), 4.29 (m, 2H), 2.09 (s, 3H), 2.08 (s, 3H), 2.04 (s, 3H), 1.35 (t, J = 7.6 Hz, 3H); MS (+)-ES $[M+H]^+$ 494.1 *m/z*.

Step 2: Preparation of 7-Allyl-2-amino-6-ethoxy-9-β-D-ribofuranosyl-7,9-dihydro-purin-8-one (**45**)

[00230] To a solution of **44** (0.084 g, 0.17 mmol) in methanol (4 mL) was added solid K_2CO_3 (0.014g, 0.17 mmol) and the reaction stirred at ambient temperature for 1 h. To the cloudy mixture was added Amberlite CG-50 (0.5 g) and stirred till neutral and filtered. The filtrate was concentrated and purified by flash chromatography using 100% DCM to 10 % of methanol in DCM. Removal of the solvent afforded 62 mg of 7 (99%) as a clear oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.97 (d, J = 8.0 Hz, 1H), 5.93 (m, 1H), 5.25 (d, J = 32.4, Hz, 1H), 5.21 (s, 1H), 5.02 (t, J = 8.0 Hz, 1H), 4.62 (br s, 2H), 4.47 (d, J = 5.6 Hz, 2H), 4.25-4.45 (m, 3H), 4.21 (q, J = 6.8 Hz, 2H), 3.77 (ABq, $\Delta\upsilon_{AB}$ = 0.14, J_{AB} = 12.4 Hz, 2H), 1.37 (t, J = 6.8 Hz, 3H), 1.27 (t, 7.6, 2H); MS (+)-ES $[M+H]^+$ 368.0 *m/z*.

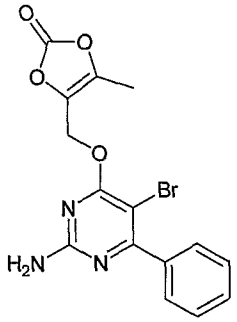
Example 3: **5-Bromo-4-ethoxy-6-phenyl-pyrimidin-2-ylamine (37)**



Step 1: Preparation of 5-Bromo-4-ethoxy-6-phenyl-pyrimidin-2-ylamine (**37**)

[00231] In a manner similar to step 2 of Example **2** was prepared the title compound as a white solid from 2-amino-5-bromo-6-phenyl-3H-pyrimidin-4-one **35** (Wierenga, *et al.*, *JMC*, 23, 239-240 (1980)) in 13% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (m, 2H0, 7.42 (m, 3H), 5.15 (br s, 2H), 4.23 (q, J = 7.2 Hz, 2H), 1.44 (t, 6.8 Hz, 3H); MS (+)-ES $[M]^+$ 294.1 $[M+2]^+$ 296.0 *m/z*. Elemental analysis for $C_{12}H_{12}BrN_3O$: calc'd: C, 49.00; H, 4.11; N, 14.29; found: C, 48.94; H, 4.18; N, 14.01.

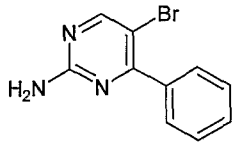
Example 4: **4-(2-Amino-5-bromo-6-phenyl-pyrimidin-4-yloxymethyl)-5-methyl-[1,3]dioxol-2-one (46)**



Step 1: Preparation of 4-(2-Amino-5-bromo-6-phenyl-pyrimidin-4-yloxymethyl)-5-methyl-[1,3]dioxol-2-one (**46**)

[00232] In a manner similar to step 2 of Example **2** was prepared the title compound as a white solid from 2-Amino-5-bromo-6-phenyl-3H-pyrimidin-4-one **35** in 4% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (m, 2H), 7.45 (m, 3H), 5.18 (s, 2H), 5.07 (s, 2H), 2.26 (s, H); MS (+)-ES $[M]^+$378.2 $[M+2]^+$ 380.1 *m/z*. Elemental analysis for $C_{15}H_{12}BrN_3O_4$: calc'd: C, 47.64; H, 3.20; N, 11.11; found: C, 46.98; H, 3.23; N, 10.70.

Example 5: **5-Bromo-4-phenyl-pyrimidin-2-ylamine (48)**



Step 1: Preparation of 4-Phenyl-pyrimidin-2-ylamine (47)

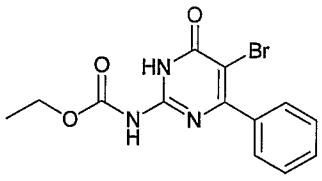
[00233] To a solution of bromobenzene (4.43 mL, 42.06 mmol) in dry THF (100 mL) at -78 °C was added BuLi (394 mL, 63.08 mmol) and the mixture left to stir at –78 °C for 2 h. To this was added 2-aminopyrimidine (2.0 g, 21.03 mmol) in hot toluene (80 mL) over a 15 minutes period. The mixture was refluxed for 16 h and allowed to cool to room temperature and carefully quenched with aqueous $NaHCO_3$. The mixture was filtered and the filtrate concentrated under vacuum. The residue was then dissolved in DCM and washed with aqueous $NaHCO_3$, brine and dried (MgSO4). The solvent was removed to afford 350

mg of **47** (10%) as a pale yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (d, J = 4.8 Hz, 1H), 7.97 (m, 2H), 7.45 (m, 3H), 7.02 (J = 4.8 Hz, 1H). 5.27 (br s, 2H); MS (+)-ES $[M+H]^+$ 172.2 *m/z*.

Step 2: Preparation of 5-Bromo-4-phenyl-pyrimidin-2-ylamine (**48**)

[00234] Compound **47** (0.30 g, 1.75 mmol) was dissolved in glacial acetic acid (15 ml) and warmed to 45 °C. Br_2 (0.09 mL, 1.75 mmol) was added slowly. The resulting mixture was then allowed to stir at room temperature for 3 h. The solvent was removed under vacuum to a solid residue. This was then transferred onto a filter funnel and washed with DCM, followed by water. The remaining solid was then dried under high vacuum for 16 h to give 197 mg of **13** (45%) as a pale yellow solid: ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.40 (s, 1H), 7.61 (m, 2H), 7.45 (m, 3H), 6.96 (s, 2H); MS (+)-ES $[M]^+$ 250.0 $[M+2]^+$ 252.0 *m/z*. Elemental analysis for $C_{10}H_8BrN_3$: calc'd: C, 48.02; H, 3.22; Br, 31.95; N, 16.80; found: C, 47.91; H, 3.28; Br, 32.15; N, 16.80.

Example 6: **(5-Bromo-6-oxo-4-phenyl-1,6-dihydro-pyrimidin-2-yl)-carbamic acid ethyl ester (36)**

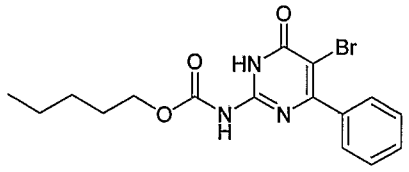


Step 1: Preparation of (5-Bromo-6-oxo-4-phenyl-1,6-dihydro-pyrimidin-2-yl)-carbamic acid ethyl ester (**36**)

To a solution of **35** (0.25g, 0.94 mmol) in DMF (8 mL) was added NEt_3 (0.14 mL, 0.99 mmol) and diethyl pyrocarbonate (0.27 mL, 1.89 mmol). The reaction mixture was maintained at 65 °C for 20 h. The solvent was removed and the residue treated with DCM. The resulting mixture was filtered to remove the remaining starting material **35** and the filtrate washed with aqueous $NaHCO_3$, brine and dried ($MgSO_4$). The filtrate was concentrated and purified by HPLC (Thomson ODS-A 100A 5μ 150 x 21.2 mm column; flow rate = 30 mL/min; CH_3CN with 0.05 % TFA (A), Water with 0.05 % TFA (B); Make up pump flow = 0.9 mL/min; Make up pump mobile phase; MeOH with 0.05% TFA using a gradient system as follows: t = 0; 15 % A, 85 % B; t = 3.0 min; 15 % A, 85 % B; t = 9.5 min; 70 % A, 30 % B; t = 10.0 min; 100 % A, 0 % B; t = 12.0 min; 100 % A, 0 % B; t = 12.5 min; 15 % A, 85 % B; t = 15.0 min; 15 % A, 85 % B.) to afford 54 mgs of **36** (17%) as a clear oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (m, 1H), 7.44 (m, 3H), 4.26 (q, J = 7.6 Hz, 2H), 1.32 t, J = 6.8 Hz, 3H); MS (+)-ES $[M]^+$ 338.1 $[M+2]^+$ 340.0 *m/z*. Elemental analysis

for $C_{13}H_{12}BrN_3O_3$: calc'd: C, 46.17; H, 3.58; N, 12.43; found: C, 46.43; H, 3.74; N, 11.95.

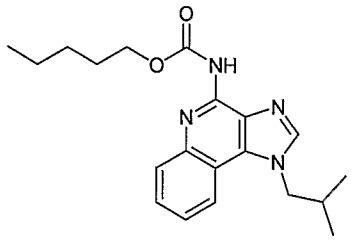
Example 7: **(5-Bromo-6-oxo-4-phenyl-1,6-dihydro-pyrimidin-2-yl)-carbamic acid pentyl ester (49)**



Step 1: Preparation of (5-Bromo-6-oxo-4-phenyl-1,6-dihydro-pyrimidin-2-yl)-carbamic acid pentyl ester (**49**)

[00235] In a manner similar to step 1 of Example 6 the title compound was prepared from **35** and dipentyl pyrocarbonate as a clear oil in 9% yield after HPLC purification (Thomson ODS-A 100A 5μ 150 x 21.2 mm column; flow rate = 30 mL/min; CH_3CN with 0.05 % TFA (A), Water with 0.05 % TFA (B); Make up pump flow = 0.9 mL/min; Make up pump mobile phase; MeOH with 0.05% TFA using a gradient system as follows: t = 0; 35 % A, 65 % B; t = 3.0 min; 35 % A, 65 % B; t = 10 min; 100 % A, 0 % B; t = 12.0 min; 100 % A, 0 % B; t = 12.5 min; 35 % A, 65 % B; t = 15.0 min; 35 % A, 65 % B.). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (br s, 1H), 7.67 (m, 2H), 7.43 (d, J = 2.0 Hz, 3H), 4.17 (t, J = 7.2 Hz, 2H), 1.64 (t, J = 6.8 Hz, 2H), 1.34 (m, 4H), 0.92 (t, J = 6.4 Hz, 3H); MS (+)-ES $[M]^+$ 380.1 $[M+2]^+$ 382.1 *m/z*.

Example 8: **(1-Isobutyl-1H-imidazo[4,5-*c*]quinolin-4-yl)-carbamic acid pentyl ester (34)**

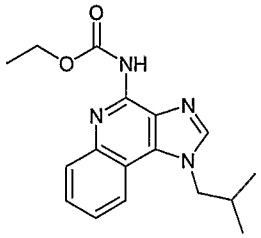


Step 1: Preparation of (1-Isobutyl-1H-imidazo[4,5-c]quinolin-4-yl)-carbamic acid pentyl ester (**34**)

[00236] To a suspension of 1-Isobutyl-1H-imidazo[4,5-c]quinolin-4-ylamine **31** (0.15 g, 0.62 mmol, prepared according to the procedure given in WO94/17043) in $CHCl_3$ (5 mL) was added NEt_3 (0.09 mL, 0.65 mmol) and dipentyl pyrocarbonate (0.231 g, 0.94 mmol). The mixture was stirred at 40 °C for 60 h. The reaction mixture was washed with aqueous $NaHCO_3$, brine and dried over $MgSO_4$. The filtrate was concentrated and purified by flash chromatography using a 10% to 70% gradient of ethyl acetate in hexanes to give 50.5 mg of **34** (23%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (br s, 1H), 8.15 (t, J = 8.0 Hz, 2H), 7.85 (t, J = 7.2 Hz, 1H), 7.77 (t, J = 8.0 Hz, 1H), 4.43 (d, J = 7.6 Hz, 2H), 4.36 (t, J

= 7.2 Hz, 2H), 2.31 (m, 1H), 1.75 (t, J = 6.8 Hz, 2H), 1.36 (m, 4H), 1.06 (d, J = 6.4 Hz, 6H), 0.89 (t, J = 6.8 Hz, 2H); MS (+)-ES $[M+H]^+$ 355.3 *m/z*.

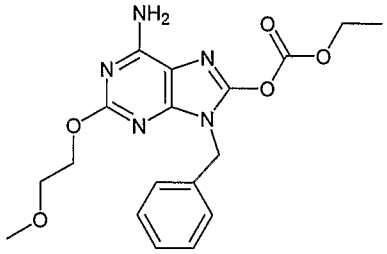
Example 9: **(1-Isobutyl-1H-imidazo[4,5-*c*]quinolin-4-yl)-carbamic acid ethyl ester (50)**



Step 1: Preparation of (1-Isobutyl-1H-imidazo[4,5-c]quinolin-4-yl)-carbamic acid ethyl ester (**50**)

[00237] In a manner similar to step 1 of Example 8 was prepared the title compound as a white solid from **31** and diethyl pyrocarbonate in 67% yield as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.32 (br s, 2H), 8.2 (d, J = 8.0 Hz, 2H) 8.12 (d, J = 8.0 Hz, 1H), 7.83 (t, J = 7.2 Hz, 1H), 7.74 (t, J = 8.0 Hz,1H) 4.43 (m, 4H), 2.35 (m, 1H), 1.39 (t, J = 7.2 Hz, 3H), 1.08 (d, J = 6.4 Hz, 6H); MS (+)-ES $[M+H]^+$ 313.2 *m/z*.

Example 10: **Carbonic acid 6-amino-9-benzyl-2-(2-methoxy-ethoxy)-9*H*-purin-8-yl ester ethyl ester (51)**



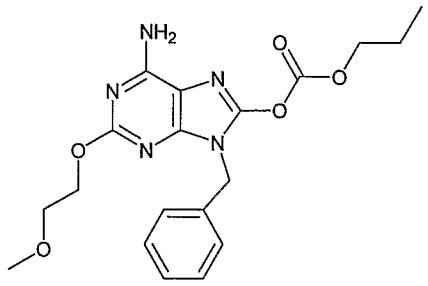
Step 1: Preparation of Carbonic acid 6-amino-9-benzyl-2-(2-methoxy-ethoxy)-9H-purin-8-yl ester ethyl ester (**51**)

[00238] 6-Amino-9-benzyl-2-(2-methoxy-ethoxy)-9*H*-purin-8-ol, **29** (11.75 mg, 0.027 mmol, prepared according to the procedure described by Kurimota, *et al., Bioorg. Med. Chem.*, 12, 1091-109 (2004) was suspended in CH_2Cl_2 (0.6 mL) and cooled to 0 °C. DIEA (11.96 μL, 0.068mmol) and ethyl chloroformate (3.86 mg, 0.036 mmol, added as a 10% by volume solution in dichloromethane) were then added to the suspension. The reaction mixture was stirred at 0 °C for 10 minutes then allowed to warm to room temperature for 15 minutes. TLC of the reaction mixture shows that starting material remained. The reaction mixture was heated to 35 °C, DMAP (cat.), methanol (60 μL portions) was added to dissolve **29**, and additional aliquots of ethyl chloroformate (3.86 mg, 0.036 mmol, added as a 10% by volume solution in dichloromethane) were added until the reactions was complete. The crude mixture was purified by flash chromatography using a 0

to 100% gradient of ethyl acetate in hexane. The desired peaks were collected and concentrated in-vacuo to yield 8.5 mg (80%) of compound 51 as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (d, J = Hz, 2H), 7.27 (m, 3H), 4.98 (s, 2H), 4.46 (m, 4H), 3.74 (m, 2H), 3.42 (s, 2H), 1.46 (t, 3H); MS [M+H]+ m/z 388.3.

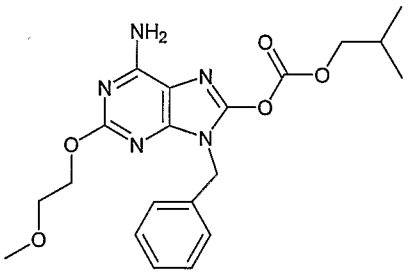
[00239] Examples 11-20 were prepared from 6-amino-9-benzyl-2-(2-methoxy-ethoxy)-9H-purin-8-ol, **29** and the appropriate chloroformate according to the procedure described in Example 10.

Example 11: **Carbonic acid 6-amino-9-benzyl-2-(2-methoxy-ethoxy)-9*H*-purin-8-yl ester propyl ester (52)**



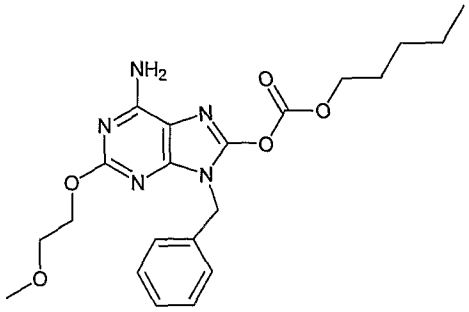
86% yield as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (d, J = 6.4 Hz, 2H), 7.27 (m, 3H), 4.98 (s, 2H), 4.46 (m, 4H), 3.74 (t, J = 5.2 Hz, 2H), 3.42 (s, 3H), 1.46 (t, J = 7.6 Hz, 3H); MS $[M+H]^+$ *m/z* 402.2.

Example 12: **Carbonic acid 6-amino-9-benzyl-2-(2-methoxy-ethoxy)-9*H*-purin-8-yl ester isobutyl ester (53)**



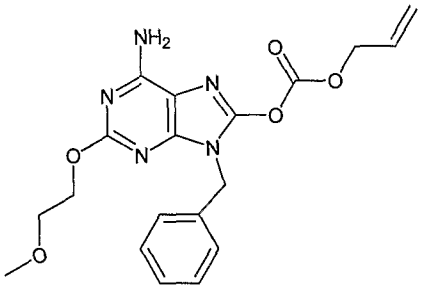
[00240] 92% yield as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (d, J = 6.4 Hz 2H), 7.27 (m, 3H), 4.99 (s, 2H), 4.45 (m, 2H), 4.17 (d, J =7.4 Hz, 2H), 3.73 (t, J = 4.8 Hz, 2H), 3.4 (s, 3H), 2.15 (m, 1H), 1.06 (d, J = 6.8 Hz, 6H); MS [M+H]+ m/z 416.3.

Example 13: **Carbonic acid 6-amino-9-benzyl-2-(2-methoxy-ethoxy)-9*H*-purin-8-yl ester pentyl ester (54)**



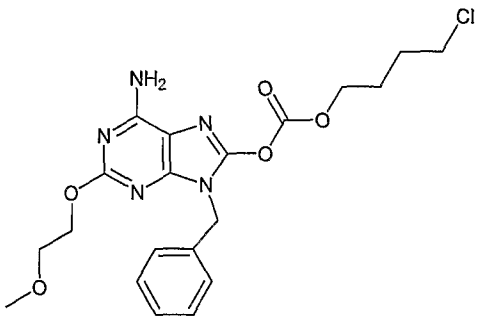
[00241] 92% yield as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (d, J = 6.4 Hz, 2H), 7.27 (m, 3H), 4.99 (s, 2H), 4.45 (t, J = 4.8 Hz, 2H), 4.39 (t, J = 7.2 Hz, 2H), 3.73 (t, J = 5.2 Hz, 2H), 3.4 (s, 3H), 2.15 (m, 1H), 1.82 (m, 2H), 1.40 (m, 4H), 0.93 (t, J = 6.8 Hz, 3H); MS [M+H]+ m/z 430.2.

Example 14: **Carbonic acid allyl ester 6-amino-9-benzyl-2-(2-methoxy-ethoxy)-9*H*-purin-8-yl ester (55)**



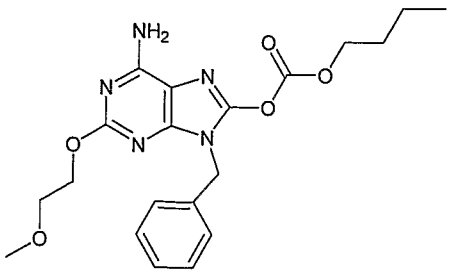
[00242] 93% yield as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (d, J = 6.0 Hz, 2H), 7.25 (m, 3H), 6.0 (m, 1H), 5.5 (m, 1H), 5.35 (m, 1H), 4.99 (s, 2H), 4.89 (d, J = 2.4 Hz, 2H), 4.46 (t, J = 4.8 Hz, 2H), 3.74 (d, J = 5.2 Hz, 2H), 3.42 (s, 3H); MS [M+H]+ m/z 400.2.

Example 15: **Carbonic acid 6-amino-9-benzyl-2-(2-methoxy-ethoxy)-9*H*-purin-8-yl ester 4-chloro-butyl ester (56)**



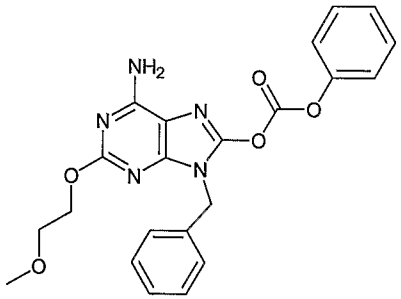
[00243] 98% yield as a white solid: 1H ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (d, J = 6.0 Hz, 2H), 7.25 (m, 3H), 4.98 (s, 2H), 4.45 (m, 4H), 3.63 (t, J = 5.2 Hz, 2H), 3.42 (s, 3H), 1.99 (m, 4H); MS [M+H]+ m/z 450.2.

Example 16: **Carbonic acid 6-amino-9-benzyl-2-(2-methoxy-ethoxy)-9H-purin-8-yl ester butyl ester (57)**



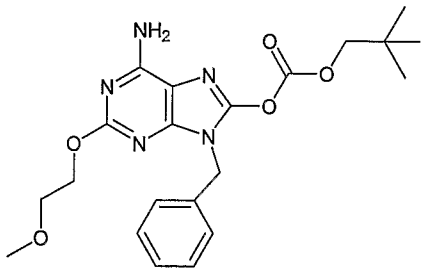
[00244] 100% yield as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (d, J = 6.0 Hz, 2H), 7.27 (m, 3H), 4.99 (s, 2H), 4.46 (m, 2H), 4.41 (t, J = 4.4 Hz, 2H), 3.73 (t, J = 7.2 Hz, 2H), 3.42 (s, 3H), 1.79 (m, 2H), 1.48 (m, 2H), 0.96 (t, J = 7.6 Hz, 3H); MS [M+H]+ m/z 416.2.

Example 17: **Carbonic acid 6-amino-9-benzyl-2-(2-methoxy-ethoxy)-9*H*-purin-8-yl ester phenyl ester (58)**



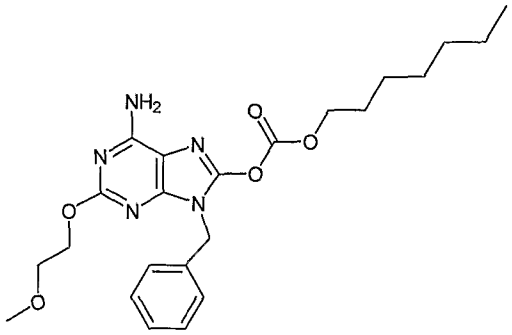
[00245] 100% yield as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (d, J = 6.8 Hz, 2H), 7.41 (m, 2H), 7.30 (m, 3H), 7.25 (m, 3H), 4.99 (s, 2H), 4.47 (t, J = 4.8 Hz, 2H), 3.75 (t, J = 4.8 Hz, 2H), 3.43 (s, 3H); MS [M+H]+ m/z 436.2.

Example 18: **Carbonic acid 6-amino-9-benzyl-2-(2-methoxy-ethoxy)-9*H*-purin-8-yl ester 2,2-dimethyl-propyl ester (59)**



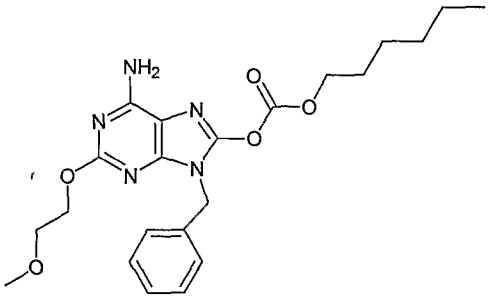
[00246] 100% yield as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (d, 2H), 7.25 (m, 3H), 4.99 (s, 2H), 4.47 (t, 2H), 4.08 (s, 2H), 3.75 (t, 2H), 3.42 (s, 3H), 1.07 (s, 9H); MS [M+H]+ m/z 430.2.

Example 19: **Carbonic acid 6-amino-9-benzyl-2-(2-methoxy-ethoxy)-9H-purin-8-yl ester heptyl ester (60)**



[00247] 100% yield as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (d, J = 6.0 Hz, 2H), 7.27 (m, 3H), 4.99 (s, 2H), 4.46 (t, J = 5.2 Hz, 2H), 4.39 (t, J = 7.2 Hz, 2H), 3.74 (t, J = 5.2 Hz, 2H), 3.42 (s, 3H), 1.8 (m, 2H), 1.4 (m, 2H), 1.3 (m, 6H) 0.87 (t, J = 7.2 Hz, 3H); MS [M+H]+ m/z 458.3.

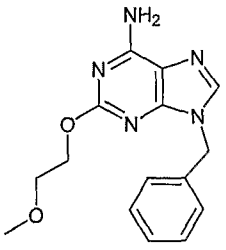
Example 20: **Carbonic acid 6-amino-9-benzyl-2-(2-methoxy-ethoxy)-9H-purin-8-yl ester hexyl ester (30)**



[00248] 74% yield as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (d, J = 5.6 Hz, 2H), 7.27 (m, 3H), 4.98 (s, 2H), 4.45 (t, J = 4.8 Hz, 2H), 4.41 (t, J = 6.8 Hz, 2H), 3.73 (t, J = 4.4 Hz, 2H), 3.42 (s, 3H), 1.81 (m, 2H), 1.34 (m, 2H), 1.31 (m, 2H), 1.26 (m, 2H), 0.89 (t, J = 2 Hz, 3H); MS [M+H]+ m/z 444.4.

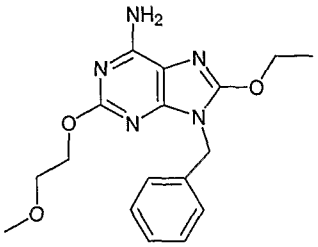
[00249] Examples 22 and 23 were prepared from 9-benzyl-2-(2-methoxy-ethoxy)-9H-purin-6-ylamine, 62, via 9-benzyl-8-bromo-2-(2-methoxy-ethoxy)-9H-purin-6-ylamine, 63 and sodium ethoxide or methoxide respectively according to the procedures of Kurimota *et al., Bioorg. Med. Chem.*, 12, 1091-1099 (2004).

Example 21: **9-Benzyl-2-(2-methoxy-ethoxy)-9H-purin-6-ylamine (62)**



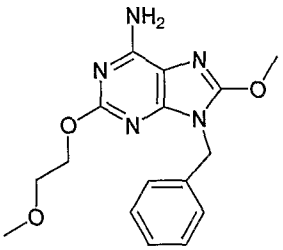
[00250] 100% yield as a brown solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (s, 1H), 7.27 (m, 5H), 5.83 (s, 2H), 5.26 (s, 2H), 4.49 (t, J = 4.8 Hz, 2H), 3.75 (t, J = 5.2 Hz, 2H), 3.43 (s, 3H); MS $[M+H]^+$ *m/z* 300.2.

Example 22: **9-Benzyl-8-ethoxy-2-(2-methoxy-ethoxy)-9*H*-purin-6-ylamine (64)**



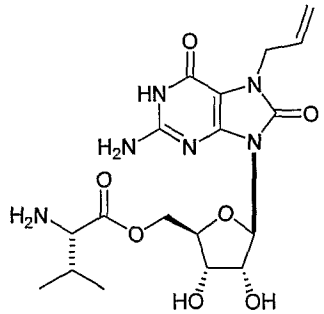
[00251] 91% yield as a brown solid: ^{1}H NMR (400 MHz, d_6-DMSO) δ 7.24 (m, 2H), 7.24 (m, 3H), 5.00 (s, 2H), 4.42 (m, 2H), 4.27 (t, J = 4.8 Hz, 2H), 3.58 (t, J = 4.8 Hz, 2H), 3.26 (s, 3H), 1.32 (t, J = 7.2 Hz, 3H); MS $[M+H]^+$ *m/z* 344.1.

Example 23: **9-Benzyl-8-methoxy-2-(2-methoxy-ethoxy)-9*H*-purin-6-ylamine (65)**



[00252] 91% yield as a brown solid: ^{1}H NMR (400 MHz, d_6-DMSO) δ 7.28 (m, 2H), 7.22 (m, 3H), 6.86 (s, 2H), 5.01 (s, 2H), 4.26 (t, J = 4.4 Hz, 2H), 4.02 (s, 3H), 3.58 (t, J = 4.8 Hz, 2H), 3.25 (s, 3H); MS $[M+H]^+$ *m/z* 330.2.

Example 24: **7-Allyl-2-Amino-9-(5'-*O*-L-valinyl-β-D-ribofuranosyl)-7,9-dihydro-1*H*-purine-6,8-dione (68)**



Step 1: Preparation of 7-Allyl-2-amino-9-(2',3'-O-isopropylidene-β-D-ribofuranosyl)-7,9-dihydro-1H-purine-6,8-dione (**66**)

[00253] Compound **17** (0.17g, 0.49 mmol) was dissolved in DMF (4.0 mL) and acetone (3.0 mL) was added to the solution. To the mixture was added 2,2-dimethoxypropane (0.18 mL, 1.47 mmol) and $MeSO_3H$ (0.02 mL, 0.05mmol). The reaction

mixture was stirred at ambient temperature for 20 min and quenched with saturated aqueous $NaHCO_3$. The aqueous phase was then extracted (4x) with CH_2Cl_2. The combined organic phase was dried ($MgSO_4$), filtered and concentrated under vacuum to afford 130 mg of **66** in 70% yield as a white solid: ^{1}H NMR (400 MHz, CD_3OD) δ 5.97 (d, J = 2.4 Hz, 1H), 5.93 (m, 1H), 5.34 (dd, J = 4.4, 2.0 Hz, 1H), 5.15 (m, 1H), 5.12 (dd, J = 7.6, 1.2 Hz, 1H), 4.98 (m, 1H), 4.52 (d, J = 5.6, 2H), 4.16 (m, 1H), 3.71 (m, 2H), 1.56 (s, 3H), 1.36 (s, 3H); MS (+)-ES $[M+H]^+$ 380.0 *m/z*.

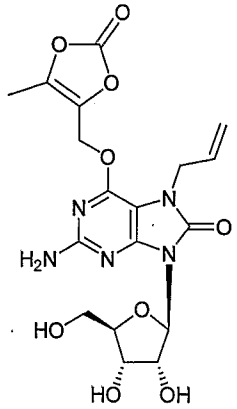
Step 2: Preparation of 7-Allyl-2-amino-9-(2',3'-O-isopropylidene-5'-N-tert-butoxycarbonyl-L-valinyl)-β-D-ribofuranosyl)-7,9-dihydro-1H-purine-6,8-dione (**67**)

[00254] A mixture of **66** (0.13 g, 0.34 mmol), BOC-Valine (0.08 g, 0.36 mmol), EDC (0.07g, 0.38 mmol), DMAP (0.05g, 0.38 mmol) in THF (8.0 mL) and pyridine (0.8 mL) under N_2 atmosphere was stirred at ambient temperature for 16 h. The solvents were removed under vacuum and the residue dissolved in EtOAc. The organic phase was washed with saturated aqueous $NaHCO_3$, brine, dried ($MgSO_4$) and filtered. The filtrate was concentrated under vacuum and the purified by flash chromatography using a 2% to 5% gradient of MeOH in CH_2Cl_2 to give 180 mg of **67** (91%) as pale yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.07 (s, 1H), 5.80 (m, 1H), 5.56 (br s, 2H), 5.41 (d, J = 5.6 Hz, 1H), 5.15 (m, 3H), 4.97 (br s, 1H), 4.53 (br s, 2H), 4.46 (m, 1H), 4.32 (m, 2H), 4.20 (m, 1H), 2.11 (m, 1H), 1.56 (s, 3H), 1.44 (s, 9H), 1.36 (s, 3H), 0.93 (d, J = 6.8 Hz, 3H), 0.84 (d, J = 6.8 Hz, 3H); MS (+)-ES $[M]^+$ 578.9 *m/z*.

Step 3: 7-Allyl-2-Amino-9-(5'-O-L-valinyl-β-D-ribofuranosyl)-7,9-dihydro-1H-purine-6,8-dione (**68**)

[00255] To a solution of **67** (0.18 g, 0.311 mmols) in MeOH (10 mL) was added AcCl (0.86 mL, 12.07 mmol) under N_2 atmosphere. The reaction mixture was left to stir at ambient temperature for 18 h and thereafter carefully neutralized with saturated aqueous $NaHCO_3$. To the mixture was added silica gel and concentrated under vacuum. The residue was purified by flash chromatography using a 10% to 20% gradient of MeOH in CH_2Cl_2 to give 80 mg of **68** (59%) as a white solid: ^{1}H NMR (400 MHz, d_6-DMSO) δ 6.72 (br s, 2H), 5.84 (m, 1H), 5.59 (d, J = 4.8 Hz, 1H), 5.43 (d, J = 5.6 Hz, 1H), 5.15 (br s, 1H), 5.07 (d, J = 12 Hz, 1H), 5.0 (d, J = 18.8 Hz, 1H), 4.77 (q, J = 4.8 Hz, 1H), 4.36 (m, 3H), 4.26 (t, J = 4.4 Hz, 1H), 4.20 (m, 1H), 3.93 (m, 1H), 3.57 (br s, 1H), 2.01 (m, 1H), 0.86 (d, J = 4.4 Hz, 3H), 0.85 (d, J = 5.2 Hz, 3H); MS (+)-ES $[M+H]^+$ 439.1 *m/z*.

Example 25: **7-Allyl-2-amino-9-β-D-ribofuranosyl-6-(5-methyl-2-oxo-[1,3]dioxol-4-ylmethoxy)-7,9-dihydro-purin-8-one (71)**



Step 1: Preparation of 7-Allyl-2-amino-9-(2',3',5'-tris-O-triethylsilanyl-β-D-ribofuranosyl)-7,9-dihydro-1H-purine-6,8-dione (**69**)

[00256] To a solution of **17** (0.46 g, 1.36 mmol) and imidazole (0.93 g, 13.64 mol) in DMF (13 mL) was added chlorotriethylsilane (0.92 mL, 5.46 mmol) dropwise and stirred at ambient temperature for 2.5 h. The reaction mixture was treated with saturated aqueous $NaHCO_3$ and the resulting two phases separated. The aqueous phase was washed with diethyl ether (2x). The organic layers were combined and washed with water, dried ($MgSO_4$) and concentrated after filtration. The residue was purified by flash chromatography using a 2% to 10% gradient of MeOH in CH_2Cl_2 to give 830 mg of **69** (89%) as pale yellow oil: ^1H NMR (400 MHz, $CDCl_3$) δ 5.92 (m, 1H), 5.85 (d, J = 6.8 Hz, 1H), 5.3 (m, 1H), 5.15 - 5.23 (m, 2H), 5.09 (br s, 2H), 4.54 (d, J = 4.4 Hz, 2H), 4.33 (m, 1H), 3.98 (m, 1H), 3.67 – 3.80 (m, 2H), 0.85 – 1.02 (m, 26H), 0.48 – 0.71 (m, 19H): MS (+)-ES $[M]^+$ 682.6 *m/z*.

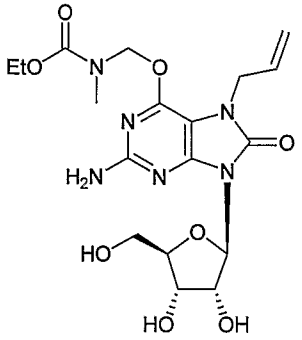
Step 2: Preparation of 7-Allyl-2-amino-9-(2',3',5'-tris-O-triethylsilanyl-β-D-ribofuranosyl)-6-(5-methyl-2-oxo-[1,3]dioxol-4-ylmethoxy)-7,9-dihydro-purin-8-one (**70**)

[00257] In a manner similar to Step 1 of Example **2**, compound **70** was prepared from compound **69** and 4-hydroxymethyl-5-methyl-[1,3]dioxol-2-one (prepared according to the procedure of Alepegiani, *Syn. Comm.*, 22(9), 1277-82 (1992)) in 5% yield as a white solid after HPLC purification (Thomson ODS-A 100A 5u 50 x 21.2 mm column; flow rate = 30 mL/min; CH_3CN with 0.05 % TFA (A), Water with 0.05 % TFA (B); Make up pump flow = 1.0 mL/min; Make up pump mobile phase; MeOH with 0.05% TFA using a gradient system as follows: t = 0; 50 % A, 50 % B; t = 2.0 min; 50 % A, 50 % B; t = 5.0 min; 100 % A, 0 % B; t = 9.5 min; 100 % A, 0 % B; t = 10.0 min; 50 % A, 50 % B; t = 13.0 min; 50 % A, 50 % B.); MS (+)-ES $[M]^+$ 794.1 *m/z*.

Step 3: Preparation of 7-Allyl-2-amino-9-β-D-ribofuranosyl-6-(5-methyl-2-oxo-[1,3]dioxol-4-ylmethoxy)-7,9-dihydro-purin-8-one (**71**)

[00258] To a solution of **70** (9.0 mg, 0.012 mmol) in MeOH (1.5 mL) was added $3HF{\cdot}NEt_3$ (0.01 mL, 0.07 mmol) and stirred at ambient temperature for 16 h. The solvent was removed under vacuum and the residue purified by flash chromatography. The desired product was eluted with 2% to 5% gradient of MeOH in CH_2Cl_2 to afford 3.26 mg of **71** (64%) as white solid: ^{1}H NMR (400 MHz, $CDCl_3$) 5.96 (d, J = 7.6 Hz, 1H), 5.85 (m, 1H), 5.17 (m, 4H), 4.96 (t, J = 7.2 Hz, 1H), 4.47 (d, J = 6.0 Hz, 2H), 4.25 (J = 5.2 Hz, 1H), 4.24 (s, 1H), 3.81 (ABq, $\Delta\upsilon_{AB}$ = 0.17, J_{AB} = 11.6 Hz, 2H), 2.22 (s, 3H): MS (+)-ES $[M+H]^+$ 452.4 *m/z*.

Example 26: **(7-Allyl-2-amino-9-β-D-ribofuranosyl-8-oxo-8,9-dihydro-7*H*-purin-6-yloxymethyl)-methyl-carbamic acid ethyl ester (73)**



Step 1: Preparation of (7-Allyl-2-amino-9-*(2'3',5'-tris-O-triethylsilanyl-β-D-ribofuranosyl)*-8-oxo-8,9-dihydro-7*H*-purin-6-yloxymethyl)-methyl-carbamic acid ethyl ester (**72**)

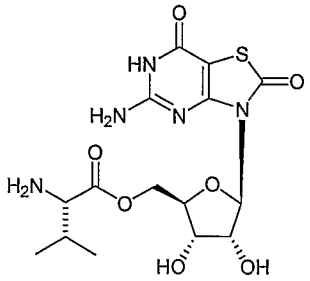
[00259] In a manner similar to Step 1 of Example **2** compound **72** was prepared in 4% yield from compound **69** and *N-methyl-N-(hydroxymethyl)urethane* (Kelper, *JOC*, 52, **1987**, p.453-455) as a white solid after HPLC purification: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.94 (m, 1H), 5.82 (d, J = 6.4 Hz, 1H), 5.53 (br s, 1H), 5.23 – 5.29 (m, 2H), 5.15 (t, J = 9.6 Hz, 1H), 4.57 (d, J = 5.2 Hz, 2H), 4.35 (br s, 1H), 4.21 (m, 2H), 3.96 (br s, 1H), 3.73 (m, 2H), 3.06 (s, 3H), 1.30 (m, 4H), 0.87 – 1.01 (m, 24H), 0.57 – 0.68 (m, 19H): MS (+)-ES $[M+H]^+$ 797.7 *m/z*.

Step 2: Preparation of (7-Allyl-2-amino-9-β-D-ribofuranosyl-8-oxo-8,9-dihydro-7H-purin-6- yloxymethyl)-methyl-carbamic acid ethyl ester (**73**)

[00260] In a manner similar to Step 3 of Example **25** was prepared the title compound **73** in 64% as a white solid after HPLC purification (Thomson ODS-A 100A 5μ 50 x 21.2 mm column; flow rate = 30 mL/min; CH_3CN with 0.05 % TFA (A), Water with 0.05 % TFA (B); Make up pump flow = 1.0 mL/min; Make up pump mobile phase; MeOH with 0.05% TFA using a gradient system as follows: t = 0; 50 % A, 50 % B; t = 2.0 min; 50 % A, 50 % B; t = 5.0 min; 100 % A, 0 % B; t = 9.5 min; 100 % A, 0 % B; t = 10.0 min; 50 % A, 50 % B; t = 13.0 min; 50 % A, 50 % B.). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.92 (m, 1H),

5.92 (d, J = 7.6 Hz, 1H), 5.51 (m, 2H), 5.22 (d, J = 15.6 Hz, 1H), 5.16 (d, J = 9.2 Hz, 1H), 4.92 (t, J = 7.2 Hz, 1H), 4.57 (d, J = 6.0, 2H), 4.40 (d, J = 6.0, 1H), 4.21 (m, 3H), 3.79 (ABq, $\Delta\upsilon_{AB}$ = 0.178, J_{AB} = 14.0 Hz, 2H), 3.06 (s, 3H), 1.31 (t, J = 7.2 Hz, 3H): MS (+)-ES $[M]^+$ 455.4 *m/z*.

Example 27: **5-Amino-3-(5'-O-L-valinyl-β-D-ribofuranosyl)thiazolo[4,5-d]pyrimidine-2,7-dione Dihydrochloride (24)**



Step 1: *Preparation of 5-Amino-3-(2',3'-O-isopropylidene-β-D-ribofuranosyl) thiazolo[4,5-d]pyrimidine-2,7-dione* (**22**)

[00261] To a heterogeneous mixture of **21** (5.37 g, 17.0 mmol, prepared according to the procedure given in U.S. Patent No. 5,041,426 (Example 2), which is incorporated by reference in its entirety) in acetone (40 mL) contained in a 250 mL Morton flask was added successively 2,2-DMP (6.26 mL, 50.9 mmol), DMSO (6.6 mL), and $MeSO_3H$ (220 μL, 3.39 mmol) at room temperature. The reaction mixture was stirred vigorously, becoming homogeneous and golden yellow as the diol was consumed. TLC analysis (SiO_2, 10% MeOH-$CHCl_3$) indicated reaction completion after 6 h. Undissolved solids were removed via gravity filtration using fluted Whatman type 1 filter paper. This was followed by pouring of the filtrate into 10 volumes of ice water (~400 mL), resulting in immediate precipitation of a white solid. After a brief period of stirring, $NaHCO_3$ (285 mg, 3.39 mmol) dissolved in water (10 mL) was added to neutralize the $MeSO_3H$. Vigorous stirring in the Morton reactor was continued for 15 min, whereupon the mixture was filtered through a coarse scintered glass funnel. The solid material was washed with ice water (100 mL), air dried, then dried further under high vacuum at 65 °C, affording 5.36 g (88%) of the acetonide **22** as a white solid: mp 280-81 °C; ^1H (DMSO-d_6) δ 1.28 (s, 3H), 1.47 (s, 3H), 3.43-3.55 (m, 2H), 3.95-3.99 (m, 1H), 4.77-4.80 (m, 1H), 4.88-4.91 (m, 1H), 5.24-5.26 (m, 1H), 5.99 (s, 1H), 6.97 (br s, 2H), 11.25 (s, 1H).

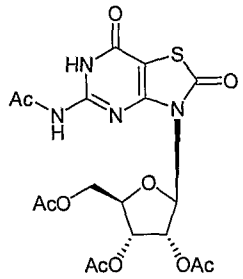
Step 2: Preparation of 5-Amino-3-(2',3'-O-isopropylidene –5'-N-tert-butoxycarbonyl-L-valinyl)-β-D-ribofuranosyl)-thiazolo[4,5-d]pyrimidine-2,7-dione (**23**)

[00262] To a solution of *N*-butoxycarbonyl-(L)-valine (671 mg, 2.81 mmol) in THF (9 mL) at 0 °C was added EDC (588 mg, 3.07 mmol). The resultant homogeneous mixture was stirred 45 min at 0 °C, at which point it had become heterogeneous, and solid acetonide **2** from Step 1 above (1.00 g, 2.81 mmol) was added as one portion. Subsequently added was solid DMAP (522 mg, 4.27 mmol). The reaction mixture was permitted to reach room temperature, and stirred an additional 5 h, whereupon it was concentrated at 25 °C via rotary evaporation to a yellow syrup. The residue was dissolved in EtOAc (50 mL), partitioned with 1 N HCl (10 mL) followed by neutralization of acid with saturated aqueous $NaHCO_3$ (10 mL). The acidic aqueous phase was further extracted with EtOAc (2 x 50 mL), and then partitioned with the basic aqueous phase. The combined organic phases were dried over Na_2SO_4, filtered through a short pad of SiO_2, and concentrated, affording 1.480 g (96%) of Boc-protected amino acid ester **23** as a foam: mp 158 °C (dec); ^1H ($CDCl_3$) δ 0.86 (d, J = 7.0, 3H), 0.95 (d, J = 7.0, 3H), 1.35 (s, 3H), 1.44 (s, 9H), 1.56 (s, 3H), 1.75 (br s, 1H), 2.08-2.19 (m, 1H), 4.20-4.24 (m, 2H), 4.30-4.37 (m, 1H), 4.56 (dd, J = 11.0, 5.9, 1H), 4.96 (dd, J = 6.2, 3.7, 1H), 5.11 (br d, J = 8.8, 1H), 5.29 (br d, J = 6.6, 1H), 5.88 (br s, 2H), 6.23 (s, 1H).

Step 3: Preparation of 5-Amino-3-(5'-O-L-valinyl-β-D-ribofuranosyl)thiazolo[4,5-d]pyrimidine-2,7-dione Dihydrochloride (**24**)

[00263] A stream of HCl gas was passed through a bubbler of concentrated H_2SO_4, and subsequently directed (via fritted dispersion tube) into a 250 mL 3-neck Morton flask containing dry isopropyl acetate (80 mL) at 0 °C until a saturated solution was obtained. To this was added a solution of the Boc-amino acid ester from Step 2 above (5.53 g, 9.95 mmol) in isopropyl acetate (30 mL), resulting in the formation of a white solid precipitate within 5 min. To this was added 10% (v/v) IPA (11 mL). The reaction mixture was warmed to room temperature, then stirred 12 h. The heterogeneous reaction mixture was diluted with dry toluene (100 mL). Filtration using a medium pore scintered glass funnel under N_2 provided an off-white, amorphous solid. Trituration of the solid in dry THF was followed by filtration and vacuum drying at 65 °C, affording 3.677 g (81%) of the title compound **24** as a white solid: mp 166-68 °C (dec); ^1H (DMSO-d_6) δ 0.90 (d, J = 7.0, 3H), 0.94 (d, J = 7.0, 3H), 2.14-2.18 (m, 1H), 3.83-3.85 (m, 1H), 3.96-4.00 (m, 1H), 4.23-4.28 (m, 2H), 4.42 (dd, J = 11.7, 3.4, 1H), 4.75 (dd, J = 10.3, 5.5, 1H), 5.81 (d, J = 4.4, 1H), 6.46 (br s, 3H), 7.23 (br s, 2H), 8.47 (s, 3H), 11.5 (br s, 1H). Elemental analysis for $C_{15}H_{21}N_5O_7S \cdot 2HCl$: calc'd: C, 36.89; H, 4.75; Cl, 14.52; N, 14.34; S, 6.57; found: C, 37.03: H, 4.74; Cl, 14.26; N, 14.24; S, 6.42.

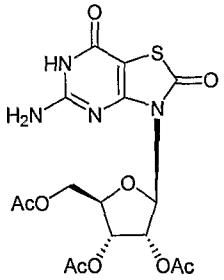
Example 28: **5-Acetylamino-3-(2',3',5'-tri-O-acetyl-β-D-ribofuranosyl)thiazolo[4,5-*d*]pyrimidine-2,7(6*H*)-dione (74)**



Step 1: Preparation of 5-Acetylamino-3-(2',3',5'-tri-O-acetyl-β-D-ribofuranosyl)thiazolo[4,5-d]pyrimidine-2,7(6H)-dione (74)

[00264] Anhydrous **21** (8.0 g, 39.5 mmol) was dissolved in dry pyridine (65 mL). DMAP (3.1g, 25.3 mmol) and acetic anhydride (19.1 mL 202.4 mmol) were added sequentially. The reaction was allowed to progress for 2 h at room temperature, whereupon it was quenched with saturated $NaHCO_3$ (100 mL) and extracted with DCM (3 x 200 mL). The organic phase was concentrated, and then triturated with ether. This provided 12.5 g (103%) of slightly impure 5-acetylamino-3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)thiazolo-[4,5-*d*]pyrimidin-2,7(6H)-dione as a white solid **74**: mp 246.7-248.1 °C; R_f= 0.20 (SiO_2, 50% EtOAc-$CHCl_3$); ^{1}H NMR (400MHz, d_6-DMSO) δ 12.23 (s, 1H), 11.85 (s, 1H), 5.97 (m, 2H), 5.48 (t, J = 6, 1H), 4.35-4.40 (m, 1H), 4.25-4.31 (m, 1H), 4.08-4.18 (m, 1H), 2.49 (s, 3H), 2.07 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H).

Example 29: **5-Amino-3-(2',3',5'-tri-O-acetyl-β-D-ribofuranosyl)thiazolo[4,5-*d*]pyrimidine-2,7(6*H*)-dione (75)**

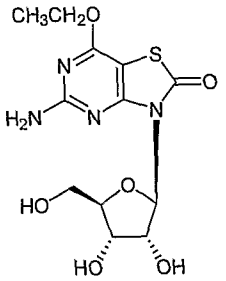


Step 1: Preparation of 5-Amino-3-(2',3',5'-tri-O-acetyl-β-D-ribofuranosyl)thiazolo[4,5-d]pyrimidine-2,7(6H)-dione (75)

[00265] To a suspension of **21** (5.00 g, 15.8 mmol) in acetonitrile (160 mL) at 0 °C was added successively Et_3N (11.0 mL, 79.0 mmol), DMAP (195 mg, 1.59 mmol), and Ac_2O (4.47 mL, 47.4 mmol). The reaction mixture was stirred at room temperature for 2 h, whereupon it was concentrated to a brown syrup. The residue was purified by flash column chromatography (silica, MeOH/$CHCl_3$ = 1-10%) to afford 6.22 g (89%) of triacetate **75** as a white solid: mp 198-199 °C; ^{1}H (400 MHz, d_6-DMSO) δ 11.34 (s, 1H), 7.02 (br s, 2H), 5.90

(m, 2H), 5.51 (t, J = 6.0 Hz, 1H), 4.36 (dd, J = 12.4, 3.2 Hz, 1H), 4.21 (m, 1H), 4.08 (q, J = 6.0 Hz, 1H), 2.06 (s, 3H), 2.06 (s, 3H), 2.00 (s, 3H); MS (+)-ES $[M+H]^+$ *m/z* 443.3.

Example 30: **5-Amino-7-ethoxy-3-β-D-ribofuranosyl-thiazolo[4,5-*d*]pyrimidin-2-one (77)**



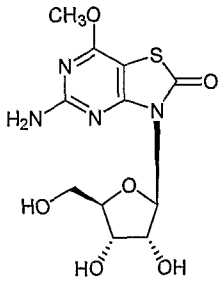
Step 1: Preparation of 5-Acetylamino-7-ethoxy-3-(2',3',5'-tri-O-acetyl-β-D-ribofuranosyl)-thiazolo[4,5-d]pyrimidin-2-one (**76**)

[00266] In a manner similar to Example 2, step 1, **76** was prepared from **74** and ethanol in 72% yield as a white foam: MS (+)-ES $[M+H]^+$ *m/z* 513. R_f = 0.45 (75% Ethyl acetate-$CHCl_3$).

Step 2: Preparation of 5-Amino-7-ethoxy-3-β-D-ribofuranosyl-thiazolo[4,5-d]pyrimidine-2-one (**77**)

[00267] In a manner similar to Example 2, step 2, the title compound was prepared from **76** in 65% yield as a white solid: ^{1}H NMR (400 MHz, d_6-DMSO) δ 6.87 (s, 2H), 5.85 (d, J = 4.8 Hz, 1H), 5.27 (d, J = 5.6 Hz, 1H), 4.96 (d, J = 5.2 Hz, 1H), 4.78 (m, 1H), 4.66 (m, 1H), 4.36 (m, 2H), 4.09 (m, 1H), 3.74 (m, 1H), 3.58 (m,1H), 3.40 (m, 1H), 1.29 (m, 3H); MS (+)-ES $[M+H]^+$ *m/z* 445, $[2M+H]^+$ *m/z* 689. R_f = 0.2 (50% THF-$CHCl_3$). Elemental Analysis for $C_{12}H_{16}N_4O_6S \cdot 0.25\ H_2O$: calc'd: C, 41.31; H, 4.77; N, 16.06; S, 9.19. Found: C, 41.24; H, 4.71; N, 15.89; S, 9.06.

Example 31: **5-Amino-7-methoxy-3-β-D-ribofuranosyl-thiazolo[4,5-*d*]pyrimidin-2-one (79)**



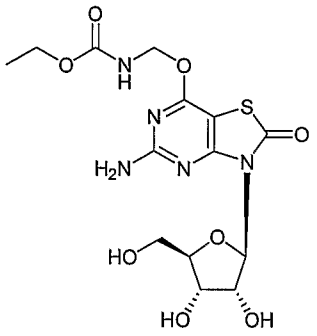
Step 1: Preparation of 5-Acetylamino-7-methoxy-3-(2',3',5'-tri-O-acetyl-β-D-ribofuranosyl)-thiazolo[4,5-d]pyrimidin-2-one (**78**)

[00268] In a manner similar to Example 2, step 1, **77** was prepared from **74** and methanol in 65% yield as a white foam: MS (+)-ES $[M+H]^+$ 499. R_f= 0.5 (75% Ethyl acetate-$CHCl_3$).

Step 2: Preparation of 5-Amino-7-methoxy-3-β-D-ribofuranosyl-thiazolo[4,5-d]pyrimidin-2-one (**79**)

[00269] In a manner similar to Example 2, step 2, the title compound was prepared from **78** in 78% yield as a white solid: ^{1}H NMR (400 MHz, d_6-DMSO) δ 6.91 (s, 2H), 5.86 (d, J= 5.2 Hz, 1H), 5.28 (d, J= 5.2 Hz, 1H), 4.96 (d, J= 5.2 Hz, 1H), 4.77 (m, 1H), 4.66 (m, 1H), 4.09 (m, 1H), 3.90 (s, 3H), 3.75 (m, 1H), 3.56 (m, 1H), 3.43 (m, 1H); MS (+)-ES $[M+H]^+$ 331. R_f= 0.2 (50% THF-$CHCl_3$). Elemental Analysis for $C_{11}H_{14}N_4O_6S \cdot 0.25\ H_2O$: calc'd: C, 39.46; H, 4.37; N, 16.73; S, 9.58. Found: C, 39.59; H, 4.17; N, 16.55; S, 9.52.

Example 32: **(5-Amino-2-oxo-3-β-D-ribofuranosyl-2,3-dihydro-thiazolo[4,5-*d*]pyrimidin-7-yloxymethyl)-carbamic acid ethyl ester (82)**



Step 1: Preparation of 5-Amino-3-(2',3',5'-tris-O-triethylsilanyl-β-D-ribofuranosyl)-thiazolo[4,5-d]pyrimidin-2,7-dione (**80**)

[00270] To a suspension of **21** (1.00 g, 3.16 mmol) in DMF (20 mL) at room temperature was added successively imidazole (753 mg, 11.06 mmol), DMAP (39 mg, 0.32 mmol), and chlorotriethylsilane (1.64 mL, 9.80 mmol). The reaction mixture was stirred at room temperature for 2 h, whereupon it was quenched by saturated $NaHCO_3$ solution (20 mL). The mixture was extracted with $CHCl_3$ (3 x 20 mL), dried over $MgSO_4$ and concentrated. The residue was purified by flash column chromatography (silica, MeOH/$CHCl_3$ = 1-5%) to afford 1.91 g (92%) of compound **80** as a white solid: ^{1}H (400 MHz, d_6-DMSO) δ 5.99 (s, 1H), 5.62 (br s, 2H), 5.19 (dd, J= 4.4, 6.0 Hz, 1H), 4.35 (dd, J= 2.8, 4.4 Hz, 1H), 3.99 (m, 1H), 3.77 (dd, J= 7.6, 10.8 Hz, 1H), 3.68 (dd, J= 4.8, 10.4 Hz, 1H), 1.10 (t, J= 7.1 Hz, 3H), 0.96 (t, J= 7.1 Hz, 3H), 0.89 (t, J= 7.1 Hz, 3H), 0.68 (q, J= 7.1 Hz, 2H), 0.61 (q, J= 7.1 Hz, 2H), 0.54 (m, 2H); MS (+)-ES $[M+H]^+$ *m/z* 660.0.

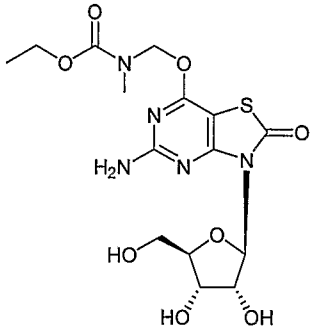
Step 2: Preparation of 5-Amino-3-(2',3',5'-tris-O-triethylsilanyl-β-D-ribofuranosyl)- 2,3-dihydro-thiazolo[4,5-d]pyrimidin-7-yloxymethyl)-carbamic acid ethyl ester (**81**)

[00271] In a manner similar to Step 1 of Example 2, compound **81** was prepared from **80** and *N*-ethylurethane as a white solid in 31% yield: $[M+H]^+$ 760.5; ^1H NMR (400 MHz, $CDCl_3$) δ 6.43 (br s, 2H), 6.09 (t, *J* = 7.6 Hz, 1H), 5.94 (d, *J* = 6.0 Hz, 1H), 5.31 (d, *J* = 4.8 Hz, 2H), 5.19 (dd, *J* = 6.0, 4.8 Hz, 1H), 4.35 (dd, *J* = 4.8, 2.8 Hz, 1H), 4.19 (q, *J* = 6.4 Hz, 2H), 3.98 (m, 1H), 3.76 (dd, *J* = 10.8, 7.6 Hz, 1H), 3.68 (dd, *J* = 10.4, 4.8 Hz, 1H), 1.29 (t, *J* = 6.8 Hz, 3H), 1.02 (t, *J* = 8.0 Hz, 3H), 0.96 (t, *J* = 7.6 Hz, 3H), 0.90 (t, *J* = 8.0 Hz, 3H), 0.69 (q, *J* = 8.0 Hz, 2H), 0.61 (q, *J* = 8.0 Hz, 2H), 0.55 (m, 2H); $[M+H]^+$ 760.5.

Step 3: Preparation of (5-Amino-2-oxo-3-β-D-ribofuranosyl-2,3-dihydro-thiazolo[4,5-d]pyrimidin-7-yloxymethyl)-carbamic acid ethyl ester (**82**)

[00272] A solution of **81** (244 mg, 321 μmol), 5M HF in pyridine (321 μL, 1.60 mmol) and THF (3.20 mL) were stirred at room temperature for 5 h. Removal of the solvents under vacuum left a residue that was purified by flash chromatography (SiO_2, 10% MeOH-$CHCl_3$) to afford **82** (119 mg, 90%) as a white solid: ^1H NMR (400 MHz, d_6-DMSO) δ 8.43 (br s, 1H), 7.76 (br s, 2H), 5.82 (d, *J* = 5.2 Hz, 1H), 5.78 (s, 2H), 5.32 (d, *J* = 5.6 Hz, 1H), 5.24 (dd, *J* = 6.0, 4.8 Hz, 1H), 5.00 (d, *J* = 5.6 Hz, 1H), 4.82 (q, *J* = 5.6 Hz, 1H), 4.68 (t, *J* = 6.0, 1H), 4.11 (q, *J* = 5.2 Hz, 1H), 4.09 (q, *J* = 7.2 Hz, 2H), 3.78 (q, *J* = 5.6 Hz, 1H), 3.60 (m, 1H), 3.46 (m, 1H), 1.21 (t, *J* = 7.2 Hz, 3H); $[M+H]^+$ 418.2.

Example 33: **(5-Amino-2-oxo-3-β-D-ribofuranosyl-2,3-dihydro-thiazolo[4,5-*d*]pyrimidin-7-yloxymethyl)-methyl-carbamic acid ethyl ester (84)**



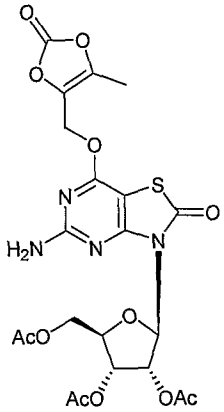
Step 1: Preparation of (5-Amino-2-oxo-3-(2',3',5'-tri-O-acetyl-β-D-ribofuranosyl)-2,3-dihydro-thiazolo[4,5-d]pyrimidin-7-yloxymethyl)-methyl-carbamic acid ethyl ester (**83**)

[00273] In a manner similar to Example 2, step 1, compound **83** was prepared from **75** and *N*-methyl-*N*-(hydroxymethyl)urethane as a white solid in 24% yield: R_f = 0.4 (33% EtOAc-$CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 11.49 (br s, 1H), 6.08 (d, *J* = 4.0 Hz, 1H), 5.75 (t, *J* = 6.0 Hz, 1H), 5.53 (s, 2H), 4.49 (dd, *J* = 13.5, 8.4 Hz, 1H), 4.30 (m, 5H), 3.62 (q, *J* = 7.2 Hz, 2H), 2.30 (s, 3H), 2.12 (s, 3H), 2.09 (s, 3H), 2.08 (s, 3H), 1.36 (t, *J* = 6.8 Hz, 3H), 1.20 (t, *J* = 6.8 Hz, 3H); $[M+H]^+$ 614.2.

Step 2: Preparation of (5-Amino-2-oxo-3-β-D-ribofuranosyl-2,3-dihydro-thiazolo[4,5-d]pyrimidin-7-yloxymethyl)-methyl-carbamic acid ethyl ester (**84**)

[00274] In a manner similar to Example 1, step 4, the title compound was prepared from **83** as a white solid in 20% yield: ^{1}H NMR (400 MHz, d_6-DMSO) δ 7.86 (br s, 2H), 5.82 (d, J = 4.8 Hz, 1H), 5.47 (s, 2H), 5.31 (d, J = 5.2 Hz, 1H), 5.00 (d, J = 5.6 Hz, 1H), 4.82 (q, J = 5.2 Hz, 1H), 4.67 (q, J = 5.6 Hz, 1H), 4.18 (q, J = 6.4 Hz, 2H), 4.12 (m, 1H), 3.78 (q, J = 6.0 Hz, 1H), 3.60 (m, 1H), 3.47 (m, 1H), 3.30 (s, 3H), 1.27 (t, J = 6.8 Hz, 3H); $[M+H]^+$ 432.3.

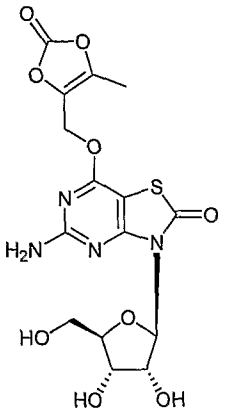
Example 34: **5-Amino-7-(5-methyl-2-oxo-[1,3]dioxol-4-ylmethoxy)-3-(2',3',5'-tri-*O*-acetyl-β-D-ribofuranosyl)-thiazolo[4,5-*d*]pyrimidin-2-one (85)**



Step 1: Preparation of 5-Amino-7-(5-methyl-2-oxo-[1,3]dioxol-4-ylmethoxy)-3-(2',3',5'-tri-O-acetyl-β-D-ribofuranosyl)-thiazolo[4,5-d]pyrimidin-2-one (**85**)

[00275] To a solution of triacetate **75** (1.55 g, 3.50 mmol) in THF (50 mL) at 0 °C was added polymer supported-triphenylphosphine (4.95 g, 10.50 mmol, Argonaut). To this mixture was added 4-hydroxymethyl-5-methyl-[1,3]dioxol-2-one (0.91 g, 7.00 mmol), prepared according to the procedure of Alepegiani, *Syn. Comm.*, 22(9), 1277-82 (1992) Diethyl azodicarboxylate (0.73 ml, 4.60 mmol) was then added dropwise. The resulting mixture was stirred at room temperature for 48 h, filtered and washed with MeOH and $CHCl_3$. The filtrate was concentrated and purified by flash column chromatography (silica, acetone/$CHCl_3$ = 10-20%) to afford dioxolone derivative **85** (1.38 g, 71%) as white solid: ^{1}H (400 MHz, d_6-DMSO); δ 7.06 (s, 2H), 6.00 (d, J = 4.0 Hz, 1H), 5.92 (dd, J = 6.6, 4.4 Hz, 1H), 5.56 (t, J = 6.4 Hz, 1H), 5.30 (s, 2H), 4.38 (dd, J = 11.6, 3.6 Hz, 1H), 4.25 (t, J = 3.6 Hz, 1H), 4.10 (q, J = 6.0 Hz, 1H), 2.23 (s, 3H), 2.08 (s, 3H), 2.07 (s, 3H), 2.00 (s, 3H); MS (+)-ES $[M+H]^+$ *m/z* 555.3. Elemental Analysis calc'd for $C_{21}H_{22}N_4O_{12}S \cdot Me_2CO$: C, 47.06; H, 4.61; N, 9.15; S, 5.23. Found: C, 47.25; H, 4.37; N, 9.53; S, 5.52.

Example 35: **5-Amino-7-(5-methyl-2-oxo-[1,3]dioxol-4-ylmethoxy)-3-β-D-ribofuranosyl-thiazolo[4,5-*d*]pyrimidin-2-one (87)**



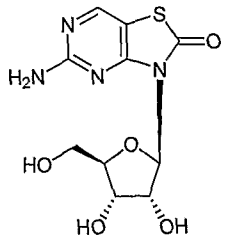
Step 1: Preparation of 5-Amino-7-(5-methyl-2-oxo-[1,3]dioxol-4-ylmethoxy)-3-(2',3',5'-tris-O-triethylsilanyl-β-D-ribofuranosyl)-thiazolo[4,5-d]pyrimidin-2-one (**86**)

[00276] In a manner similar to Example 34, compound **86** was prepared from **80** and 4-hydroxymethyl-5-methyl-[1,3]dioxol-2-one as a white solid in 45% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.06 (d, J = 6.0 Hz, 1H), 5.21 (dd, J = 6.0, 4.8 Hz, 1H), 5.18 (d, J = 3.2 Hz, 2H), 4.94 (br s, 2H), 4.38 (dd, J = 4.8, 2.8 Hz, 1H), 4.00 (m, 1H), 3.79 (dd, J = 11.2, 8.0 Hz, 1H), 3.69 (dd, J = 10.8, 5.2 Hz, 1H), 2.23 (s, 3H), 1.02 (t, J = 8.0 Hz, 3H), 0.96 (t, J = 7.6 Hz, 3H), 0.89 (t, J = 8.4 Hz, 3H), 0.70 (q, J = 7.6 Hz, 2H), 0.61 (q, J = 8.0 Hz, 2H), 0.53 (m, 2H); $[M+H]^+$ 771.5.

Step 2: Preparation of 5-Amino-7-(5-methyl-2-oxo-[1,3]dioxol-4-ylmethoxy)-3-β-D-ribofuranosyl-thiazolo[4,5-d]pyrimidin-2-one (**87**)

[00277] In a manner similar to Steps 3 of Example 32, the title compound was prepared from **86** as a white solid in 89% yield: ^{1}H NMR (400 MHz, d_6-DMSO) δ 7.03 (br s, 2H), 5.90 (d, J = 5.2 Hz, 1H), 5.33 (s, 2H), 5.02 (d, J = 4.8 Hz, 1H), 4.83 (q, J = 5.6 Hz, 1H), 4.71 (t, J = 6.0 Hz, 1H), 4.14 (q, J = 5.2 Hz, 1H), 3.80 (q, J = 4.8 Hz, 1H), 3.62 (m, 1H), 3.47 (m, 1H), 2.27 (s, 3H); $[M+H]^+$ 429.2.

Example 36: **5-Amino-3-β-D-ribofuranosyl-3*H*-thiazolo-[4,5-*d*]pyrimidin-2-one (90)**



Step 1: Preparation of 5-Amino-7-thioxo-3-(2',3',5'-tri-O-acetyl-β-D-ribofuranosyl)-thiazolo[4,5-d]pyrimidin-2-one (**88**)

[00278] To a solution of **75** (1 g, 2.26 mmol) in pyridine (50 mL) was added at room temperature P_2S_5 (2.13 g, 4.79 mmol). The solution was refluxed gently (bath temperature 130 – 140 °C) for 29 h. The reaction mixture was evaporated to dryness in vacuo. The

excess P_2S_5 was decomposed by the addition of H_2O (40 mL) at 60 °C. The mixture was stirred for 1 h at 60 °C and then cooled to room temperature. The mixture was extracted with $CHCl_3$ (3 x 40 mL). The dried ($MgSO_4$) organic layer was evaporated to yield a syrup, which was purified by flash column chromatography (silica, acetone/$CHCl_3$ = 15%) to afford 0.93 g (90%) of **88** as a yellow solid: ^1H (400 MHz, d_6-DMSO) δ 12.50 (s, 1H), 7.35 (br s, 2H), 5.89 (m, 2H), 5.51 (t, J = 6.4 Hz, 1H), 4.36 (dd, J = 12.0, 4.0 Hz, 1H), 4.24 (m, 1H), 4.10 (q, J = 6.0 Hz, 1H), 2.07 (s, 3H), 2.06 (s, 3H), 2.01 (s, 3H); MS (+)-ES $[M+H]^+$ *m/z* 459.3.

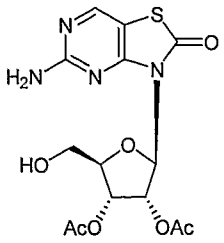
Step 2: Preparation of 5-Amino-3-(2',3',5'-tri-O-acetyl-β-D-ribofuranosyl)-3H-thiazolo[4,5-d]pyrimidin-2-one (**89**)

[00279] A suspension of Raney® 2800 nickel (3 big spatula, pre-washed with H_2O, MeOH and acetone) in acetone (50 mL) was stirred at refluxing for 1 h. Triacetate **88** (0.93 g, 2.03 mmol) was subsequently added into the above suspension at reflux. The mixture was stirred for 5 min, cooled to room temperature over 30 min. The reaction was quenched by bubbling H_2S (g) into the mixture for 2 h. The resulting mixture was filtered through a short pad of Celite® and washed with EtOH. The filtrate was concentrated and purified by flash column chromatography (silica, MeOH/$CHCl_3$ = 1-2%) to afford 0.52 g (60%) of **89** as a white solid: mp 121-123 °C; ^1H (400 MHz, d_6-DMSO) δ 8.38 (s, 1H), 6.93 (s, 2H), 6.03 (d, J = 3.6 Hz, 1H), 5.93 (dd, J = 6.4, 3.6 Hz, 1H), 5.58 (t, J = 6.0 Hz, 1H), 4.38 (dd, J = 11.6, 3.6 Hz, 1H), 4.26 (m, 1H), 4.11 (q, J = 6.0 Hz, 1H), 2.08 (s, 3H), 2.07 (s, 3H), 2.00 (s, 3H); MS (+)-ES $[M+H]^+$ *m/z* 427.2. Elemental Analysis calc'd for $C_{16}H_{18}N_4O_8S \cdot 0.5\ CH_3OH \cdot 0.25\ H_2O$: C, 44.34; H, 4.62; N, 12.54; S 7.17. Found: C, 44.54; H, 4.88; N, 12.16; S, 7.17.

Step 3: Preparation of 5-Amino-3-β-D-ribofuranosyl-3H-thiazolo[4,5-d]pyrimidin-2-one (**90**)

[00280] To a solution of **89** (0.52 g, 1.22 mmol) in MeOH (20 mL) was added K_2CO_3 (25 mg, 0.18 mmol). The reaction was stirred at room temperature overnight, then neutralized with AcOH (21 μL, 0.36 mmol). The resulting mixture was stirred at room temperature for additional 30 min, concentrated, and triturated with H_2O (2 ml) to afford 0.33 g of compound **90** (89%) as a white solid: mp 220 °C (Dec); ^1H (400 MHz, d_6-DMSO) δ 8.34 (s, 1H), 6.85 (s, 2H), 5.90 (d, J = 4.8 Hz, 1H), 5.31 (d, J = 5.6 Hz, 1H), 4.98 (d, J = 5.6 Hz, 1H), 4.81 (q, J = 5.2 Hz, 1H), 4.67 (t, J = 6.0 Hz, 1H), 4.11 (q, J = 5.2 Hz, 1H), 3.77 (dd, J = 10.8, 4.8 Hz, 1H), 3.58 (m, 1H), 3.44 (m, 1H); MS (+)-ES $[M+H]^+$ *m/z* 301.1. Elemental Analysis calc'd for $C_{10}H_{12}N_4O_5S \cdot 0.3\ H_2O$: C, 39.29; H, 4.15; N, 18.33; S 10.49. Found: C, 39.51; H, 4.18; N, 17.95; S, 10.27.

Example 37: **5-Amino-3-(2',3'-di-*O*-acetyl-β-D-ribofuranosyl)-3*H*-thiazolo[4,5-*d*]pyrimidin-2-one (93)**



Step 1: Preparation of 5-Amino-3-(5'-O-tert-butyl-dimethylsilanyl-β-D-ribofuranosyl)-3H-thiazolo[4,5-d]pyrimidin-2-one (**91**)

[00281] To a solution of **90** (0.68 g, 2.28 mmol) in DMF (10 mL) was added imidazole (0.54 g, 7.93 mmol) and tert-butyldimethylsilyl chloride (0.68 g, 4.56 mmol) sequentially. The reaction mixture was stirred at room temperature for 2 h, at which point it was concentrated and purified by flash column chromatography (silica, MeOH/$CHCl_3$; gradient = 5-20%) to afford 0.49 g (52%) **91** as a white solid: ^{1}H (400 MHz, d_6-DMSO) δ 8.33 (s, 1H), 6.87 (s, 2H), 5.90 (d, J = 4.0 Hz, 1H), 5.33 (d, J = 5.6 Hz, 1H), 5.00 (d, J = 5.2 Hz, 1H), 4.79 (q, J = 5.2 Hz, 1H), 4.16 (q, J = 5.2 Hz, 1H), 3.77 (m, 2H), 3.64 (dd, J = 12.0, 7.2 Hz, 1H), 0.84 (s, 9H), 0.00 (s, 6H); MS (+)-ES $[M+H]^+$ *m/z* 415.4.

Step 2: Preparation of 5-Amino-3-(2',3'-di-O-acetyl, 5'-O-tert-butyl-dimethylsilanyl-β-D-ribofuranosyl)-3H-thiazolo[4,5-d]pyrimidin-2-one (**92**)

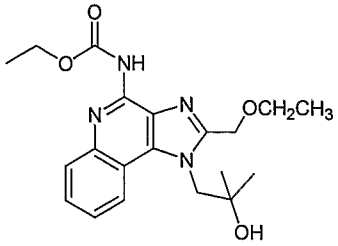
[00282] To a solution of **91** (0.20 g, 0.48 mmol) in acetonitrile (5 mL) at 0 °C was added successively Et_3N (0.26 mL, 1.86 mmol) and Ac_2O (91 μL, 0.96 mmol). The reaction mixture was stirred at room temperature for 24 h, whereupon it was concentrated and purified by flash column chromatography (silica, acetone/$CHCl_3$: gradient = 5-10%) to afford 0.22 g (92%) of **92** as a white solid: ^{1}H (400 MHz, d_6-DMSO) δ 8.36 (s, 1H), 6.90 (s, 2H), 6.00 (m, 2H), 5.57 (t, J = 6.0 Hz, 1H), 4.07 (q, J = 5.2 Hz, 1H), 3.77 (m, 2H), 2.07 (s, 3H), 2.06 (s, 3H), 0.83 (s, 9H), 0.00 (d, J = 2.4 Hz, 6H); MS (+)-ES $[M+H]^+$ *m/z* 499.5.

Step 3: Preparation of 5-Amino-3-(2',3'-di-O-acetyl-β-D-ribofuranosyl)-3H-thiazolo[4,5-d]pyrimidin-2-one (**93**)

[00283] To a solution of **92** (0.22 g, 0.44 mmol) in THF (5 mL) in a plastic vial was added HF/pyridine (0.70 mL). The reaction was stirred for 2h, concentrated and purified by flash column chromatography (silica, MeOH/$CHCl_3$: gradient = 5-10%) to afford 0.17 g (100%) of the title compound as a white solid: mp 109-111 °C; ^{1}H (400 MHz, d_6-DMSO) δ 8.37 (s, 1H), 6.91 (s, 2H), 6.00 (m, 2H), 5.48 (t, J = 6.0 Hz, 1H), 4.91 (t, J = 6.0 Hz, 1H), 4.04 (dd, J = 10.4, 6.0 Hz, 1H), 3.64 (m, 1H), 3.52 (m, 1H), 2.08 (s, 3H), 2.05 (s, 3H); MS

(+)-ES $[M+H]^+$ *m/z* 385.3. Elemental Analysis calc'd for $C_{14}H_{16}N_4O_7S \cdot 0.5\ CH_3OH \cdot 0.2\ CHCl_3$: C, 41.61; H, 4.32; N, 13.21; S 7.56: Found: C, 41.73; H, 4.29; N, 12.86; S, 7.33.

Example 38: **[2-Ethoxymethyl-1-(2-hydroxy-2-methyl-propyl)-1*H*-imidazo[4,5-c]quinolin-4-yl]-carbamic acid ethyl ester (39)**



Step 1: Preparation of [2-Ethoxymethyl-1-(2-hydroxy-2-methyl-propyl)-1H-imidazo[4,5-

[00284] In a manner similar to step 1 of Example 8 except, substituting MeOH as the solvent, was prepared the title compound from 1-(4-amino-2-ethoxymethyl-imidazo[4,5-*c*]quinolin-1-yl)-2-methyl-propan-2-ol (**38**) (prepared according to the procedure given in International Publication No. WO 94/17043) and diethyl pyrocarbonate as an oil in 39% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (d, J = 8.0 Hz, 1H), 8.05 (d, J = 8.0 Hz, 1H), 7.70 (t, J = 7.2 Hz, 1H), 7.61 (t, J = 8.0 Hz, 1H), 4.96 (br s, 2H), 4.80 (s, 2H), 4.39 (q, J = 7.2 Hz, 2H), 3.62 (q, J = 7.2 Hz, 2H), 1.40 (t, J = 7.2 Hz, 3H), 1.36 (br s, 6H), 1.24 (t, J = 6.8 Hz, 3H); MS (+)-ES $[M+H]^+$ 387.4 *m/z*.

6.4 Masking Effect of TLR7 Ligand Prodrugs

[00285] A typical experiment would use human peripheral blood mononuclear cells (PBMC) isolated from a healthy donor and placed in replicate cell culture wells; typically, $2x10^6$ to $5x10^6$ cells are placed in each well. The PBMC are incubated in the absence of test compounds at 37 °C in a humidified atmosphere containing 5% CO_2 for 24 hours to allow stabilization to the culture conditions, and then 100 micromolar isatoribine, the TLR7 ligand and a corresponding TLR7 ligand prodrug are added to separate wells containing PBMC from the same donor; untreated controls are included. The concentrations of TLR7 ligand and TLR7 ligand prodrug may be varied to suit the particular experiment, and the PBMC cultures are then incubated at 37 °C in a humidified atmosphere containing 5% CO_2 for a period of time ranging from two hours to 48 hours. Samples of cell culture supernate media are taken during the incubation. These are assayed for cytokine production by ELISA. Additionally the amount of TLR7 ligand and TLR7 ligand prodrug remaining at the end of the incubation may be assayed by LC-MS. Cytokine production is calculated relative to production in the isatoribine control, following subtraction of the cytokine production in untreated controls. The cytokine results are compared to determine the extent that the TLR7 ligand is more active than the corresponding TLR7 ligand prodrug.

[00286] Thus, if the TLR7 ligand generates more interferon alpha (a conveniently measured cytokine) than does the corresponding TLR7 ligand prodrug after a similar duration of exposure and concentration, the TLR7 ligand prodrug may be deemed a "masked" TLR7 ligand prodrug. The magnitude of reduction in cytokine production that constitutes "masking" may be as little as a 25% reduction relative to the parent TLR, since this would afford a corresponding increase in administered dose for a given level of tolerability.

[00287] Tables 9 through 14 provide data illustrating that TLR7 ligands of multiple chemical classes can be masked. The examples shown demonstrate substantial masking relative to the parent TLR7 ligand. The chemical substitutions shown are exemplary, and in no way restrictive of the invention, since additional chemical substitutions may also exhibit masking and are contemplated in the invention. Masking can be achieved by introduction of substitutions at a range of locations on any TLR7 ligand, and as shown can incorporate a variety of chemical linkages. It will be appreciated that the preferred substitution and linkage may vary for different parent TLR7 ligands.

Table 9: Masking of Isatoribine Prodrugs

Parent molecule and its prodrugs	Compound No.	Amount of INFa relative to that induced by isatoribine at 100 μM, %
Parent molecule: Isatoribine	**21**	100
Prodrug: Amino acid ester	**24**	1
Prodrug: Deoxy	**93**	0
Prodrug: 6-Ethoxy	**77**	0
Prodrug: 6-Methoxy	**79**	0
Prodrug: Aminal	**84**	0
Prodrug: Aminal	**82**	0
Prodrug: Dioxolenone	**85**	0

[00288] The masked property of isatoribine prodrugs can be demonstrated in a PBMC assay. The results of the PBMC assay (Table 9) show the amount of INFa released after exposure of the parent compound and its prodrugs for either 8 hours (val-isatoribine, **24**) or 24 hours (other prodrugs) at the initial concentration of 100 μM. The amount of the released INFa was normalized to that induced by 100 μM of isotoribine at 100 μM in the same blood donor with the same exposure time.

Table 10: Masking of Loxoribine Prodrugs

Parent molecule and its prodrugs	Compound No.	Amount of INFa relative to that induced by isatoribine at 100 μM, %
Parent molecule: Loxoribine	**17**	50
Prodrug: 6-Ethoxy	**45**	0
Prodrug: Deoxy	**43**	0
Prodrug: Valyl ester	**68**	0

[00289] The masked property of loxoribine prodrugs can be demonstrated in a PBMC assay. The results of the PBMC assay (Table 10) show the amount of INFa released after exposure of the parent compound and its prodrugs for 24 hours at the initial concentration 100 μM. The amount of the released INFa was normalized to that induced by 100 μM of isotoribine at 100 μM in the same blood donor with the same exposure time.

Table 11: Masking of Imiquimod Prodrugs

Parent molecule and its prodrugs	Compound No.	Amount of INFa relative to that induced by isatoribine at 100 μM, %
Parent molecule: Imiquimod	**31**	60-76*
Prodrug: Pentyl carbamate	**34**	0
Prodrug: Ethyl carbamate	**50**	0

Results of two experiments with three different donors

[00290] The masked property of imiquimod prodrugs can be demonstrated in a PBMC assay. The results of the PBMC assay (Table 11) show the amount of INFa released after exposure of the parent compound and its prodrugs for 24 hours at the initial concentration 100 μM. The amount of the released INFa was normalized to that induced by 100 μM of isatoribine at 100 μM in the same blood donor with the same exposure time.

Table 12: Masking of Resiquimod Prodrugs

Parent molecule and its prodrugs	Compound No.	Amount of INFa relative to that induced by isatoribine at 100 μM, %
Parent molecule: Resiquimod	**39**	95 @ 1 μM
Prodrug: Ethyl Carbamate	**38**	9 @ 100 μM

[00291] The masked property of resiquimod prodrugs can be demonstrated in a PBMC assay. The results of the PBMC assay (Table 12) show the amount of INFa released after exposure of the parent compound and its prodrugs for 24 hours at the initial concentrations of either 1 or 100 μM. The amount of the released INFa was normalized to that induced by 100 μM of isatoribine at 100 μM in the same blood donor with the same exposure time.

Table 13: Masking of Bropirimine Prodrugs

Parent molecule and its prodrugs	Compound No.	Amount of INFa relative to that induced by isatoribine at 100 μM, %
Parent molecule: Bropirimine	**35**	22
Prodrug: Deoxy	**48**	0
Prodrug: Ethoxy	**37**	0
Prodrug: Ethyl carbamate	**36**	0

Prodrug: Pentyl carbamate	49	0

[00292] The masked property of bropirimine prodrugs can be demonstrated in a PBMC assay. The results of the PBMC assay (Table 13) show the amount of INFa released after exposure of the parent compound and its prodrugs for 24 hours at the initial concentration of 100 μM. The amount of the released INFa was normalized to that induced by 100 μM of Isatoribine at 100 μM in the same blood donor with the same exposure time.

Table 14: Masking of Adenine Prodrugs

Parent molecule and its prodrugs	Compound No.	Amount of INFa relative to that induced by isatoribine at 100 μM, %
Parent molecule	29	128 @ 0.1μM
Prodrug: Methoxy	65	0 @ 100 μM
Prodrug: Ethoxy	64	0 @ 10 μM
Prodrug: Deoxy	62	0 @ 0.1 μM
Prodrug: Ethyl carbonate	51	18 @ 32 μM
Prodrug: Pentyl carbonate	54	15 @ 10 μM

[00293] The masked property of adenine prodrugs can be demonstrated in a PBMC assay. The results of the PBMC assay (Table 14) show the amount of INFa released after exposure of the parent compound and its prodrugs for 24 hours at different initial concentrations specified in the table. The amount of the released INFa was normalized to that induced by 100 μM of isatoribine at 100 μM in the same blood donor with the same exposure time.

[00294] TLR7 ligand prodrugs can also be assessed *in vitro* for their conversion to the active parent TLR7 ligand. This can be measured by incubation of the prodrug in blood, plasma, or in a cell culture of hepatocytes. At selected time intervals, samples are taken to

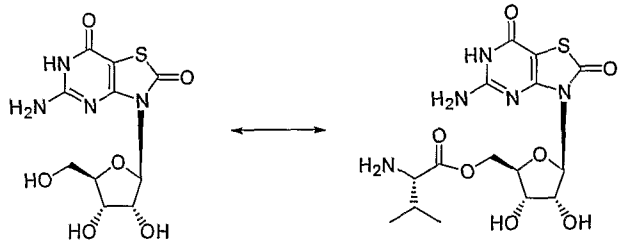
determine the amount of prodrug remaining and the amount of TLR7 ligand produced. Such determinations are readily made by use of analytical tools known in the art such as LC-MS. The determination of the extent of conversion of a masked TLR7 ligand prodrug to the parent TLR7 ligand is useful in interpreting data wherein masking is apparent at shorter times but diminishes upon long incubations in the PBMC assay described herein. The rate of conversion of the prodrug to the TLR7 ligand may be determined to ensure that the cytokine results arise predominately from exposure to prodrug rather than from exposure to TLR7 ligand generated by rapid conversion of the prodrug under the conditions of the experiment.

6.5 Biological Testing of TLR7 Ligand Prodrugs Demonstrating Increased Oral Availability and Decreased Side-Effects

Oral Availability

[00295] The improved bioavailability of TLR7 ligand prodrugs can be assessed by performing studies *in vivo*. In such experiments, the candidate prodrugs are administered by oral administration to mice, rats, monkeys, and/or dogs, and blood samples are taken at selected intervals. The blood samples are analysed for both the prodrug and the desired TLR7 ligand. Additional blood or liver samples may be analyzed for the presence of interferons and other cytokines that indicate functional activation of the TLR7 pathway *in vivo*. Desired candidates will demonstrate a blood exposure to the prodrug and also demonstrate a blood exposure to the desired TLR7 ligand of about 10% to 99% of the applied dose, as measured on a molar basis.

[00296] A representative example is the result obtained with the TLR7 ligand prodrug val-isatoribine (**24**), which as described below in the mouse and dog generated significant amounts of the parent TLR7 ligand isatoribine (**21**) in the blood. See U.S. Patent Application No. 10/305,061 (incorporated herein by reference in its entirety).



21 **24**

Interferon Alpha (Mu-IFN-α) Concentrations in Mice

[00297] The normal mouse provides a useful system for the assessment of the degree to which the inventions described herein provide material improvement in the oral delivery of **21** (isatoribine). Not only can one measure the plasma concentrations of isatoribine

arising from oral administration of the said prodrug(s) but also the extensive immunological research conducted in the mouse has provided reagents suitable for measuring the levels of interferon alpha, a cytokine of interest reflecting one of the desired biologic activities of isatoribine.

[00298] We have used the murine system in a series of experiments that demonstrate that **24**, the 5'-valine ester of **21** (val-isatoribine) elicits an interferon response substantially improved over that resulting from administration of isatoribine itself.

[00299] Table 15 records the results of an assay for murine interferon alpha in the plasma of mice that were dosed two times with isatoribine, formulated in bicarbonate, at a level of 50 mg/kg by the oral route. It is evident that no interferon was measurable even when the dose was repeated after an interval of four hours.

Table 15: Interferon Alpha (Mu-IFN-α) Plasma Concentration (pg/mL) in Mice Following Two Oral 50 mg/kg Doses of Isatoribine 4 Hours Apart

Time, h	Individual Value			Mean	SD
First Dose					
0.00	BQL^{50}	BQL^{125}	BQL^{50}	0.00	0.00
0.03	BQL^{25}	BQL^{250}	BQL^{25}	0.00	0.00
0.08	BQL^{25}	BQL^{25}	BQL^{25}	0.00	0.00
0.25	BQL^{50}	BQL^{25}	BQL^{25}	0.00	0.00
0.50	BQL^{25}	BQL^{25}	BQL^{25}	0.00	0.00
1.00	BQL^{25}	BQL^{25}	BQL^{25}	0.00	0.00
1.50	BQL^{100}	BQL^{25}	BQL^{25}	0.00	0.00
2.00	BQL^{25}	BQL^{75}	BQL^{25}	0.00	0.00
3.00	BQL^{25}	BQL^{25}	BQL^{25}	0.00	0.00
4.00	BQL^{25}	BQL^{25}	BQL^{25}	0.00	0.00
Second Dose					
4.03	BQL^{25}	BQL^{25}	BQL^{25}	0.00	0.00
4.08	BQL^{25}	BQL^{25}	BQL^{25}	0.00	0.00
4.25	BQL^{25}	BQL^{25}	BQL^{25}	0.00	0.00
4.50	BQL^{50}	$BQL^{37.5}$	BQL^{50}	0.00	0.00
5.00	BQL^{50}	BQL^{50}	BQL^{50}	0.00	0.00
5.50	$BQL^{37.5}$	$BQL^{37.5}$	$BQL^{37.5}$	0.00	0.00
6.00	BQL^{50}	$BQL^{41.3}$	$BQL^{37.5}$	0.00	0.00
7.00	BQL^{50}	BQL^{50}	BQL^{50}	0.00	0.00
8.00	BQL^{50}	BQL^{25}	BQL^{50}	0.00	0.00

BQL^{n} - Below Elevated Quantifiable Limit < n pg/mL.

[00300] Table 16 records the results of assays for murine interferon alpha in the plasma of mice that first were dosed with bicarbonate and then four hours later were dosed orally with isatoribine, formulated in bicarbonate, at a level of 50 mg/kg. Interferon was reported in the plasma from four mice, including two that had received the bicarbonate vehicle dose. All the values reported in this experiment were low, and the reported interferon levels were not consistently reported for all three mice assessed at each time point, suggesting that these signals may be artifacts arising from measurement near the lower limits of the assay.

Table 16: Interferon Alpha (Mu-IFN-α) Plasma Concentration (pg/mL) in Mice Following One Vehicle Dose and One 50 mg/kg Doses of Isatoribine 4 Hours Later

Time, h	Individual Value			Mean	SD
First Dose					
0.00	BQL^{50}	BQL^{100}	$BQL^{62.5}$	0.00	0.00
0.03	BQL^{50}	BQL^{50}	$BQL^{37.5}$	0.00	0.00
0.08	BQL^{50}	BQL^{50}	BQL^{50}	0.00	0.00
0.25	BQL^{50}	$BQL^{62.5}$	BQL^{50}	0.00	0.00
0.50	BQL^{50}	BQL^{50}	BQL^{50}	0.00	0.00
1.00	BQL^{50}	BQL^{50}	BQL^{100}	0.00	0.00
1.50	BQL^{50}	BQL^{100}	BQL^{50}	0.00	0.00
2.00	34.9	BQL^{25}	BQL^{25}	11.6	20.15
3.00	BQL^{25}	BQL^{25}	BQL^{25}	0.00	0.00
4.00	BQL^{25}	35.4	BQL^{100}	11.8	20.44
Second Dose					
4.03	BQL^{25}	BQL^{25}	BQL^{25}	0.00	0.00
4.08	BQL^{25}	BQL^{25}	BQL^{25}	0.00	0.00
4.25	BQL^{25}	BQL^{25}	BQL^{25}	0.00	0.00
4.50	BQL^{100}	BQL^{25}	133.2	44.4	76.90
5.00	74.9	BQL^{50}	NR	37.5	52.96
5.50	BQL^{250}	BQL^{75}	BQL^{25}	0.00	0.00
6.00	BQL^{25}	BQL^{75}	BQL^{75}	0.00	0.00
7.00	BQL^{50}	BQL^{50}	BQL^{25}	0.00	0.00
8.00	BQL^{25}	BQL^{25}	BQL^{25}	0.00	0.00

BQL^{n} - Below Elevated Quantifiable Limit < n pg/mL.

NR - Not reportable.

[00301] Table 17 records the results of assays for murine interferon alpha in the plasma of mice that were dosed orally with val-isatoribine, dissolved in bicarbonate, at a dose that is equivalent to 50 mg/kg of isatoribine on a molar basis. It is evident that

interferon was readily measurable at 1.0 hour, 1.5 hours, and 2.0 hours after dosing. Interferon was detected in all mice assayed at a given time point, indicating the reliability of the effect following val-isatoribine administration. Thus a single administration of val-isatoribine was superior to either a single dose or a repeated dose of isatoribine.

Table 17: Plasma Concentration (pg/mL) of Interferon Alpha (Mu-IFN-α) in Mice Following a Single 73.0 mg/kg Dose of Val-Isatoribine

Time, h	Individual Value			Mean	SD
0.00	BQL	BQL^{125}	BQL^{25}	0.00	0.00
0.25	BQL	BQL	BQL	0.00	0.00
0.50	BQL^{25}	BQL^{25}	BQL	0.00	0.00
0.75	BQL	BQL	BQL^{25}	0.00	0.00
1.00	173.2	125.1	89.0	129.1	42.24
1.50	202.9	145.9	294.8	214.5	75.13
2.00	49.2	137.9	138.3	108.5	51.33
3.00	BQL^{25}	NR	NR	0.00	0.00
4.00	BQL^{25}	27.6	BQL	9.20	15.90
5.00	BQL	BQL^{25}	BQL^{25}	0.00	0.00

BQL - Below the Quantifiable Limit < 12.5 pg/mL

BQL^{n} - Below the Elevated Quantifiable Limit < n pg/mL

NR - Not Reportable

[00302] The data tabulated in Tables 15, 16, and 17 may be also considered from the point of view of the incidence of measurable interferon levels. Interferon was detected in the plasma of only 4 of the 114 mice used in the studies of isatoribine, whereas 10 of the 30 mice dosed with val-isatoribine had detectable interferon in their plasma. Thus, the prodrug increased the proportion of mice exhibiting an interferon response from 4% to 30 % and the magnitude of both the average and peak response was increased twofold (100%).

[00303] In other experiments, plasma levels of isatoribine and interferon alpha were measured in mice that were dosed with isatoribine by the intravenous route, and these levels were compared to the levels of isatoribine and interferon alpha arising after oral administration of val-isatoribine. These data are summarized in Figure 1. In this figure it is evident that the levels of interferon alpha induced by oral val-isatoribine ("val-isator") (at 50 mg/kg isatoribine molar equivalent) was similar to that from intravenous isatoribine ("isator") at 25 mg/kg. Thus, oral val-isatoribine provides levels of isatoribine and interferon that are approximately 50% of those observed after intravenous administration of isatoribine itself.

Beagle Dog

[00304] The effect of a prodrug (val-isatoribine, **24**) on the systemic exposure to isatoribine (**21**) after oral administration to beagle dogs was investigated. Isatoribine was prepared in sodium bicarbonate solution. Val-isatoribine and isatoribine were prepared as the following formulations, which were chosen to ensure solubility:

[00305] Formulation 1: Isatoribine in sodium bicarbonate solution, 1 and 4 mg/mL.

[00306] Formulation 2: Val-isatoribine in phosphate buffered saline, 1.62 and 6.48 mg/mL, equivalent to 1 and 4 mg/mL of isatoribine on a molar basis.

[00307] Four male and four female adult beagle dogs weighing between 15 to 27 kg and approximately 1-2 years old were used at the beginning of the study. The animals were divided into 2 groups of 2 males and 2 females each. The test material was administered by gavage on Days 1 and 8, allowing a 7 day washout period between administrations. Blood samples (2 mL) were collected from each animal at predose, 15, 30 minutes, 1, 2, 3, 4, 6, 8 and 10 hours into lithium heparin tubes after each dosing. The plasma was frozen at –70 °C until analysis. The plasma was analyzed for isatoribine by an HPLC-MS/MS assay.

[00308] The pharmacokinetic parameters for isatoribine arising from isatoribine or val-isatoribine in each dog are summarized in Tables 18 and 19. The ratios for the key pharmacokinetic parameters defining the maximum concentration (Cmax) and total exposure as measured by the area under the time-concentration curve (AUC) for the prodrug and the bicarbonate solution at the 50 mg/kg dose are summarized in Table 20. For the prodrug **24**, the Cmax ratio was 2.98 ± 0.695 and the AUC ratio was 2.38 ± 0.485. These results indicate that at 50 mg/kg dose, the prodrug val-isatoribine provided substantially higher Cmax and greater bioavailability than isatoribine in bicarbonate solution.

[00309] The ratios for the Cmax and AUC for the prodrug to the bicarbonate solution for the 10 mg/kg dose are summarized in Table 21. For the prodrug, the Cmax ratio was 2.24 ± 0.249 and the AUC ratio was 1.82 ± 0.529. These results indicate that at 10 mg/kg dose, the prodrug val-isatoribine provided higher Cmax and greater bioavailability than isatoribine in bicarbonate solution.

[00310] Thus, the maximum concentrations of isatoribine achieved after oral dosing are at least doubled, and the systemic exposure to isatoribine is enhanced by approximately 2-fold following oral administration of the prodrug val- isatoribine, compared to isatoribine itself, at both 10 and 50 mg/kg dose.

Table 18: Pharmacokinetic Parameters of Isatoribine in Dogs dosed at 50 mg/kg

Dosing Period	1	2

	Formulation	Isatoribine	Val-isatoribine
Animal Number	Dose, mg/kg molar equivalent isatoribine	50	50
Dog 3517322	Cmax, ng/mL	3038.7	11741.5
	Tmax, h	0.50	0.50
	AUC(0-inf), ng·h/mL	15227.0	33038.1
	T1/2, h	6.4	2.4
Dog 3521451	Cmax, ng/mL	3354.0	10652.1
	Tmax, h	1.00	1.00
	AUC(0-inf), ng·h/mL	9422.2	26552.7
	$T_{1/2}$, h	1.9	1.6
Dog 3528707	Cmax, ng/mL	8915.3	20340.6
	Tmax, h	0.50	0.50
	AUC(0-inf), ng·h/mL	29701.7	53273.0
	$T_{1/2}$, h	2.2	2.3
Dog 3532828	Cmax, ng/mL	6134.7	15987.9
	Tmax, h	0.50	0.50
	AUC(0-inf), ng·h/mL	12069.7	32987.0
	$T_{1/2}$, h	1.4	1.6

Table 19: Pharmacokinetic Parameters of Isatoribine in Dogs Dosed at 10 mg/kg

	Dosing Period	1	2

Animal Number	Formulation	Isatoribine	Val-isatoribine
	Dose, mg/kg molar equivalent isatoribine	10	10
Dog 3524523	Cmax, ng/mL	4091.5	8594.6
	Tmax, h	1.00	0.50
	AUC(0-inf), ng·h/mL	13305.8	17166.2
	$T_{1/2}$, h	2.1	1.7
Dog 3526402	Cmax, ng/mL	1859.5	4047.0
	Tmax, h	1.00	1.00
	AUC(0-inf), ng·h/mL	5774.4	10548.9
	$T_{1/2}$, h	1.6	2.2
Dog 357450	Cmax, ng/mL	1620.3	4228.7
	Tmax, h	0.50	1.00
	AUC(0-inf), ng·h/mL	4387.3	11158.0
	$T_{1/2}$, h	1.5	2.3
Dog 354708	Cmax, ng/mL	2781.2	5784.8
	Tmax, h	0.50	0.50
	AUC(0-inf), ng·h/mL	7522.1	12259.1
	$T_{1/2}$, h	1.6	2.0

Table 20: Ratio of Pharmacokinetic Parameters of Isatoribine in Dogs Dosed at 50 mg/kg

Animal Number	Formulation	Isatoribine	Val-isatoribine
Dog 3517322	Cmax Ratio	1.00	3.86
	AUC Ratio	1.00	2.17

Dog 3521451	Cmax Ratio	1.00	3.18
	AUC Ratio	1.00	2.82
Dog 3528707	Cmax Ratio	1.00	2.28
	AUC Ratio	1.00	1.79
Dog 3532828	Cmax Ratio	1.00	2.61
	AUC Ratio	1.00	2.73
	Mean Cmax Ratio	N/A	2.98
	SD Cmax Ratio	N/A	0.695
	Mean AUC Ratio	N/A	2.38
	SD AUC Ratio	N/A	0.485

Table 21: Ratio of Pharmacokinetic Parameters of Isatoribine in Dogs Dosed at 10 mg/kg

Animal Number	Formulation	Isatoribine	Val-isatoribine
Dog 3524523	Cmax Ratio	1.00	2.10
	AUC Ratio	1.00	1.29
Dog 3526402	Cmax Ratio	1.00	2.18
	AUC Ratio	1.00	2.20
Dog 3527450	Cmax Ratio	1.00	2.61
	AUC Ratio	1.00	2.54
Dog 355708	Cmax Ratio	1.00	2.08
	AUC Ratio	1.00	1.63
	Mean Cmax Ratio	N/A	2.24
	SD Cmax Ratio	N/A	0.249

Mean AUC Ratio	N/A	1.82
SD AUC Ratio	N/A	0.529

[00311] The prodrug val-isatoribine is preferred for several reasons. First, the prodrug is easily formulated to provide a high proportion of active agent. This results in small capsule sizes for a given dose, which is an advantage for an oral product. Second, at the doses tested, val-isatoribine provides plasma levels of isatoribine that are well within the range desirable for biologic effect after oral administration, which is not the case for isatoribine itself.

Cynomolgus Monkey

[00312] From two to four male or female cynomolgus monkeys were used on the animal testing study. The study compound was formulated in a vehicle appropriate for animal oral or intravenous administration. The vehicles used were either as aqueous buffers or a solutions containing Cremophor. Animals were dosed via oral gavage or intravenous bolus injection for each test article. Blood samples (approximately 0.5 mL) were collected at predetermined time points (usually, pre-dose, 15, 30, and 45 minutes and at 1, 1.5, 2, 2.5, 3, 4, 8 and 24 hours post-dose), placed into tubes containing disodium EDTA. The samples were placed on wet ice following collection, and plasma separated as rapidly as possible. The plasma samples were aliquoted into a single vial, and stored frozen at approximately -20°C until shipped on dry ice to the Sponsor. Animals were given food and water approximately 4 hours after the dose.

[00313] The plasma samples were analyzed for a prodrug and a parent compound using well-known LCMS/MS quantitation techniques by triple quadrupole instruments, i.e Sciex API3000. The quantitation results for the parent compound delivered by oral administration of the parent compound itself or by its prodrug administered orally were used to calculate the area-under-the-cureve (AUC) values from time zeto to 24 hours (PO AUC0-24h). The comparison of the AUC values for the parent compound delivered into systemic circulation with that delivered by the prodrug allowed calculating relative oral bioavalibility of the prodrug. See results provided in Tables 22-26. When the AUC data for the parent compound delivered by the parent compound itself after its intravenous administration were available (AUC IV), it allowed calculating the absolute oral bioavailability by deviding the PO AUC (0-24h) for the prodrug by the IV AUC (0-24h) for the parent moleclule.

Table 22: Oral bioavailability of Isatoribine and its Prodrugs in Monkeys

Parent molecule and its prodrugs	Structure	Oral bioavailability in cynomolgus monkey, %
Parent molecule: Isatoribine	**21**	3
Prodrug: Amino acid ester	**24**	7-9*
Prodrug: Deoxy	**93**	80
Prodrug: 6-Ethoxy	**77**	28
Prodrug: 6-Methoxy	**79**	21
Prodrug: Aminal	**84**	14
Prodrug: Aminal	**82**	4
Prodrug: Dioxolenone	**85**	17

* Average of multiple experiments at different doses.

Table 23: Oral bioavailability of Loxoribine and its Prodrugs in Monkeys

Parent molecule and its prodrugs	Compound No.	Oral bioavailability in cynomolgus monkey, %
Parent molecule: Loxoribine	**17**	2
Prodrug: 6-Ethoxy	**45**	9
Prodrug: Deoxy	**43**	13

Table 24: Oral bioavailability of Imiquimod and its Prodrugs in Monkeys

Parent molecule and its prodrugs	Compound No.	Oral bioavailability in cynomolgus monkey, %
Parent molecule: Imiquimod	**31**	100 AUC(0-24h) = 9.0
Prodrug: Pentyl carbamate	**34**	555 AUC(0-24h) = 50
Prodrug: Ethyl carbamate	**50**	234 AUC(0-24h) = 21.1

Table 25: Oral bioavailability of Bropirimine and its Prodrugs in Monkeys

Parent molecule and its prodrugs	Compound No.	Oral bioavailability in cynomolgus monkey, %
Parent molecule: Bropirimine	**35**	100
Prodrug: Deoxy	**48**	137*
Prodrug: Ethoxy	**37**	94
Prodrug: Ethyl carbamate	**36**	33
Prodrug: Pentyl carbamate	**49**	6

* The oral bioavailability exceeding 100% may be associated with gender differences since the parent compound was studied in male monkeys and the prodrug was studied in female monkeys.

Table 26: Oral bioavailability of Adenine Prodrugs in Monkeys

Parent molecule and its prodrugs	Compound No.	Oral bioavailability in cynomolgus monkey, %
Parent molecule:	**29**	46
Prodrug: Methoxy	**65**	1.3
Prodrug: Ethoxy	**64**	4.6
Prodrug: Deoxy	**62**	0.7
Prodrug: Pentyl carbonate	**54**	9.7

Reduction of Gastrointestinal Irritancy

[00314] TLR7 ligand prodrugs of the invention also demonstrate unexpected and greatly reduced toxicology effects, and in particular reduced GI irritancy.

[00315] The gastrointestinal ("GI") tract is lined with substantial immune tissue (e.g., Peyer's patches, etc.). TLR7 ligand prodrugs offer the prospect of masking the active structure as the agent passes through lymphoid tissue lining the gut, which should minimize activation of this tissue and thereby reduce GI irritancy.

[00316] Robins *et al.* have shown that elimination of the 5'-hydroxyl of isatoribine nucleoside eliminates activity. *See* Robins *et al.*, *Adv. Enzyme Regul.*, 29, 97-121 (1989). Without being limited to any particular theory, it was hypothesized that blockade of this hydroxyl site by an ester substitution would similarly eliminate activity but allow transport in the systemic circulation, where the valine ester would be cleaved and result in exposure to isatoribine.

[00317] We have found that the hypothesis was confirmed. Formal toxicology studies of intravenously administered isatoribine and orally administered isatoribine and val- isatoribine were conducted in beagle dogs. The toxicology results for orally administered isatoribine are from a study conducted by ICN/Nucleic Acid Research Institute.

[00318] We compared in the dog the oral toxicology of **21** and **24**, and the intravenous toxicology of **21**. We observed that the oral toxicology of **24** was much more

like intravenous **21** than it was like oral **21**. In particular, the dose limiting toxicology of oral **3** was similar in nature to that of intravenous **21**, and occurred at blood exposures that were similar to those observed after intravenous **21**. In contrast, oral **21** had a different limiting toxicity (gastrointestinal lesions) and this toxicity was observed at a dose lower than the toxic dose of either intravenous **21** or oral **24**. Also, emesis was observed in dogs treated with oral **21** at doses lower than the dose of oral **24** that resulted in emesis. *See* Table 27. Other systems for assessment of emesis also are known, such as in ferrets, allowing comparison of oral and intravenous administration of compounds. *See, e.g.,* Strominger N. *et al.*, *Brain Res. Bull*, 5, 445-451 (2001).

[00319] In each case the compound was administered as a solution, by gavage or by intravenous infusion. Multiple parameters were assessed, as is customary in a toxicology study. In the studies providing higher potential exposure to isatoribine, the plasma concentration of isatoribine was assessed by a LC/MS method. The notable GI findings were graded and are listed in Table 27.

TABLE 27: Effect on GI Tolerance in Dogs after Dosing of Isatoribine (**21**) or Val-Isatoribine (**24**) Ranked by Systemic Exposure (AUC) to Isatoribine in Toxicology Studies.

		Oral Isatoribine		IV Isatoribine		Oral Val-Isatoribine	
Isatoribine equivalent applied dose (mg/kg)	$AUC_{0\text{-}24\ hrs}$ (μg•hr/ml)	Emesis or loose stool	GI lesions or Irritation	Emesis or loose stool	GI lesions or irritation	Emesis or loose stool	GI lesions or irritation
2.5	n.d	Neg.	Neg.				
5	n.d.	+	Neg.				
10	n.d.	++	++				
8.1	11.4					Neg.	Neg.
16	15.6					Neg.	Neg.
12.5	19.5			Neg.	Neg.		
32	31.7					Neg.	Neg.
25	42.8			Neg.	Neg.		
64	71					Neg.	Neg.
130	75.3					+	Neg.
50	87.8			+	Neg.		

		Oral Isatoribine		IV Isatoribine		Oral Val-Isatoribine	
260	127					++	Neg.
390	180					+++	Neg.
100	209			++	Neg.		

[00320] For orally administered isatoribine the principal findings were related to GI tolerability as measured by GI irritancy. The clinical signs noted in Table 27 were emesis and/or loose stools. These clinical signs were more frequent in the 10 mg/kg group, and in one animal at this dose a bloody stool was noted. Gross histopathologic evaluation of the GI tract noted multiple, scattered red lesions on the intestinal mucosa in four of eight dogs at 10 mg/kg, which on microscopic evaluation revealed cellular congestion and hemorrhage, as might be expected for an ongoing localized inflammatory process. The GI effects established the NOAEL as 5 mg/kg.

[00321] Intravenously administered isatoribine resulted in emesis and/or loose stools as a common finding in dogs; this effect occurred at substantially higher applied doses than orally administered isatoribine. No lesions were seen in the GI tract either at necropsy or histopathologic evaluation of tissues. The GI toxicity did not affect the NOAEL, which was established as 12.5 mg/kg on the basis of other findings.

[00322] Orally administered val-isatoribine demonstrated a toxicology profile similar to intravenously administered isatoribine. At higher applied doses, emesis and loose stools were observed. No GI lesions were found, although this was a focus of evaluation in this study. As for intravenously administered isatoribine, the NOAEL was established on the basis of other findings. The correspondence of observed toxicity to systemic exposures of isatoribine is of interest in this study; the threshold of isatoribine AUC for observation of emesis and loose stools is similar for intravenously administered isatoribine and orally administered val-isatoribine (Table 27).

[00323] The data in Table 27 indicate that orally administered val-isatoribine provides an improved toxicity profile over orally administered isatoribine, and is consistent with the hypothesis that chemical masking of the activity of isatoribine is afforded by chemically substituting an ester at the 5'-hydroxyl position of the nucleoside. As illustrated in Tables 9 through 14, it is possible to chemically mask any TLR7 ligand using a variety of substituents. Engineering this substitution to be cleavable upon entry into the body affords systemic exposure to the useful activity of the compound without the limiting GI toxicity arising from the anatomical structure of the GI tract. As illustrated in Tables 22 through 25,

it is possible to design the chemical substitution on a masked TLR7 ligand to be cleavable after administration. Thus, masked TLR7 ligand prodrugs can be generated for any TLR7 ligand. This enables administration of doses that are substantially higher on a molar basis than otherwise would be acceptable, with the result of greater efficacy and reduced side effects when compared to administration of the parental "unmasked" compound alone.

6.6 Oral Composition

[00324] Table 28 illustrates a batch formulation and a single dose unit formulation containing 100 mg of val-isatoribine.

Table 28: Formulation for 100 mg tablet

Material	Percent by Weight	Quantity (mg/tablet)	Quantity (kg/batch)
val-isatoribine	40%	100.00	20.00
Microcrystalline Cellulose, NF	53.5%	133.75	26.75
Pluronic F-68 Surfactant	4.0%	10.00	2.00
Croscarmellose Sodium Type A, NF	2.0%	5.00	1.00
Magnesium Stearate, NF	0.5%	1.25	0.25
Total	100.0%	250.00 mg	50.00 kg

[00325] The microcrystalline cellulose, croscarmellose sodium, and val-isatoribine components are passed through a #30 mesh screen (about 430μ to about 655μ). The Pluronic F-68® (manufactured by JRH Biosciences, Inc. of Lenexa, KS) surfactant is passed through a #20 mesh screen (about 457μ to about 1041μ). The Pluronic F-68® surfactant and 0.5 kgs of croscarmellose sodium are loaded into a 16 qt. twin shell tumble blender and are mixed for about 5 minutes. The mix is then transferred to a 3 cubic foot twin shell tumble blender where the microcrystalline cellulose is added and blended for about 5 minutes. The thalidomide is added and blended for an additional 25 minutes. This pre-blend is passed through a roller compactor with a hammer mill attached at the discharge of the roller compactor and moved back to the tumble blender. The remaining croscarmellose sodium and magnesium stearate is added to the tumble blender and blended for about 3 minutes. The final mixture is compressed on a rotary tablet press with 250 mg per tablet (200,000 tablet batch size).

6.7 Mucosal Composition

[00326] A concentrate is prepared by combining isatoribine, and a 12.6 kg portion of the trichloromonofluoromethane in a sealed stainless steel vessel equipped with a high shear mixer. Mixing is carried out for about 20 minutes. The bulk suspension is then prepared in the sealed vessel by combining the concentrate with the balance of the propellants in a bulk product tank that is temperature controlled to 21° to 27°C. and pressure controlled to 2.8 to 4.0 BAR. 17 ml aerosol containers which have a metered valve which is designed to provide 100 inhalations of the composition of the invention. Each container is provided with the following:

val-isatoribine	0.0120 g
trichloromonofluoromethane	1.6960 g
dichlorodifluoromethane	3.7028g
dichlorotetrafluoroethane	1.5766 g
total	7.0000 g

6.8 Intravenous Composition

[00327] The intravenous formulation is prepared by reconstituting a compound of the invention with an appropriate liquid medium, such as water for injection (WFI) or a 5% dextrose solution. A desired concentration of the intravenous formulation can be obtained by reconstituting an appropriate amount of a compound of the invention with an appropriate volume of liquid medium. A desired concentration of the intravenous formulation provides a therapeutically effective amount of a compound of the invention to the patient, preferably a mammal, more preferably a human, in need of the intravenous pharmaceutical formulation and maintains a therapeutically effective level of a compound of the invention in the patient. The dose which is therapeutically effective will depend on the rate at which the intravenous formulation is delivered to the patient and the concentration of the intravenous formulation.

[00328] For example, one vial containing a composition (e.g., 50 mg of a compound of the invention per vial) are reconstituted with a 5% dextrose solution (14 ml of 5% dextrose solution per vial) yielding a total of 25 mL of solution. The reconstituted solution is incorporated into a dextrose solution in an infusion bag and q.s. to 50 mL, resulting in a solution containing 1 mg/ml of a compound of the invention suitable for intravenous infusion administration. The preferred concentration of a compound of the invention in the

liquid medium, in the infusion bag, is about 0.001 to about 3 mg/ml, preferably about 0.75 to about 1 mg/ml.

[00329] The foregoing has demonstrated the pertinent and important features of the present invention. Many modifications and variations of the present invention can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The specific embodiments described herein are offered by way of example only, and the invention is to be limited only by the terms of the appended claims along with the full scope of equivalents to which such claims are entitled.

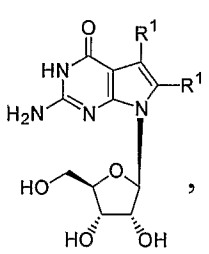
[00330] All references cited herein are incorporated herein by reference in their entirety and for all purposes to the same extent as if each individual publication or patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

What is claimed is:

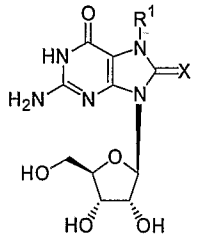
1. A method of treating a hepatitis C virus infection in a patient comprising administering to the patient in need thereof a therapeutically or prophylactically effective amount of a TLR7 ligand or a pharmaceutically acceptable salt, hydrate, or stereoisomer thereof.

2. A method of treating a hepatitis C virus infection in a patient comprising administering to the patient in need thereof a therapeutically or prophylactically effective amount of a TLR7 ligand or a pharmaceutically acceptable salt, hydrate, or stereoisomer thereof, wherein the TLR7 ligand is selected from analogs and derivatives of guanosine, imidazoquinoline, adenine, and pyrimidine.

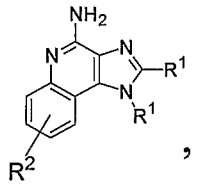
3. The method of claim 2 wherein the TLR7 ligand is selected from



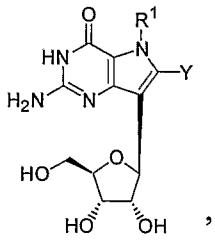
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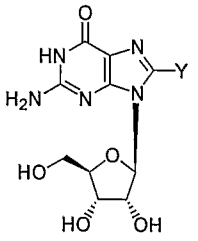
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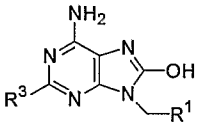
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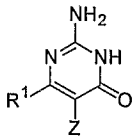
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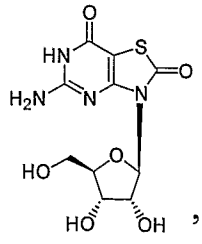


If



Ig

, and



Ih

wherein:

each R^1 is H, or a substituted or unsubstituted alkyl, alkenyl, or alkynyl, which may be interrupted by one or more O, S, or N heteroatoms, or a substituted or unsubstituted aryl or heteroaryl;

R^2 is H, OH, SH, halo, or a substituted or unsubstituted alkyl, alkenyl, or alkynyl, which may be interrupted by one or more O, S, or N heteroatoms, or a substituted or unsubstituted -O-(alkyl), -O-(aryl), -O-(heteroaryl), -S-(alkyl), -S-(aryl), -S-(heteroaryl), aryl, or heteroaryl;

R^3 is H, OH, or SH, or a substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, heteroaryl, -O-(alkyl), -O-(aryl), -O-(heteroaryl), -S-(alkyl), -S-(aryl), -S-(heteroaryl), -NH(alkyl), -NH(aryl), -NH(heteroaryl), -NH(R4)(alkyl), -NH(R^4)(aryl), or -NH(R^4)(heteroaryl), wherein R^4 is a substituted or unsubstituted alkyl;

X is O or S;

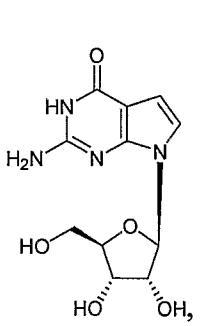
Y is H, halo, OH, OR^4, SH, SR^4, or a substituted or unsubstituted alkyl or aryl;

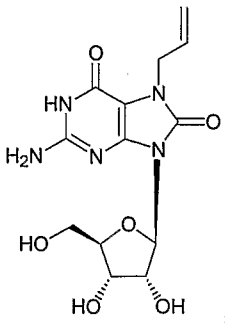
Z is H, halo, OH, OR^4, SH, or SR^4;

or a pharmaceutically acceptable salt, hydrate, metabolite or stereoisomer thereof or a pharmaceutically acceptable salt or hydrate of said stereoisomer.

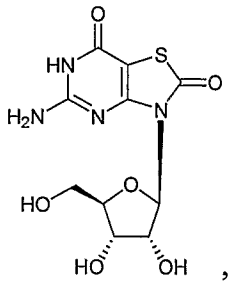
4. The method of claim 2 wherein the TLR7 ligand is selected from Formula Ia, Ib, Ic, Id, Ie, If, Ig, and Ih, wherein R^1 is H or a substituted or unsubstituted alkyl, alkenyl, or alkynyl; R^2 is H, OH, halo, or a substituted or unsubstituted alkyl, alkenyl, or alkynyl, or $-CH_2$-O-(alkyl); R^3 is H, OH, or SH, or a substituted or unsubstituted -O-(alkyl), -S-(alkyl), or -NH(alkyl); X is O or S; Y is H, halo, OH, OR^4, SH, or SR^4; and Z is H, halo, OH, OR^4, SH, or SR^4.

5. The method of claim 2 wherein the TLR7 ligand is selected from

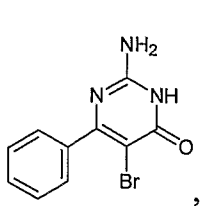




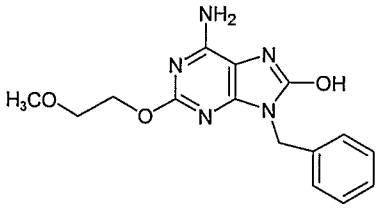
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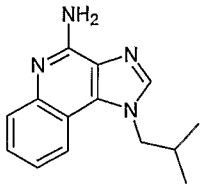
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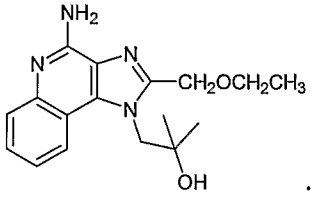
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6. The method of claim 2 wherein the patient is human.

7. The method of claim 2 further comprising administering a pharmaceutically acceptable excipient, carrier, or vehicle.

8. The method of claim 2 further comprising administering an additional therapeutic agent.

9. The method of claim 8 wherein the additional therapeutic agent is an antiviral agent.

10. The method of claim 2 wherein the therapeutically or prophylactically effective dose is 0.001 to 100 mg/kg per day.

11. The method of claim 10 wherein the therapeutically or prophylactically effective dose is about 0.01 to 50 mg/kg per day.

12. The method of claim 11 wherein the therapeutically or prophylactically effective dose is about 0.1 to 20 mg/kg per day.

13. The method of claim 2 wherein the TLR7 ligand is administered parenterally.

14. The method of claim 2 wherein the TLR7 ligand is administered intravenously.

15. The method of claim 2 wherein the TLR7 ligand is administered orally.

16. The method of claim 2 wherein the TLR7 ligand is administered mucosally.

17. A method of treating a hepatitis C virus infection in a patient comprising orally administering to the patient in need thereof a TLR7 ligand prodrug or a pharmaceutically acceptable salt, hydrate, or stereoisomer thereof, wherein the oral administration of the TLR7 ligand prodrug achieves a therapeutically effective plasma concentration of the TLR7 ligand while reducing undesirable side effects associated with oral administration of TLR7 ligands.

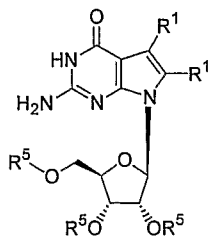
18. A method of treating a hepatitis C virus infection in a patient comprising orally administering to the patient in need thereof a masked TLR7 ligand prodrug or a pharmaceutically acceptable salt, hydrate, or stereoisomer thereof, wherein the oral administration of the masked TLR7 ligand prodrug achieves a therapeutically effective plasma concentration of the TLR7 ligand while reducing undesirable side effects associated with oral administration of TLR7 ligands.

19. The method of claim 18 wherein the masked TLR7 ligand prodrug is selected from analogs and derivatives of guanosine, imidazoquinoline, adenine, and pyrimidine.

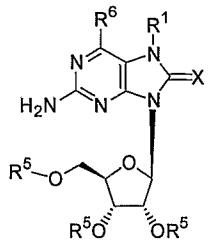
20. The method of claim 19 wherein the prodrug is an (a) amide, carbamate, or amidine moiety after conversion of a TLR7 ligand amine substituent, (b) ester, carbonate, carbamate, ether, imidate, acetal, aminal, or ketal moiety after conversion of a TLR7 ligand alcohol substituent, (c) acetal or ketal moiety after conversion of a TLR7 ligand keto substituent, (d) imidate moiety after conversion of a TLR7 ligand carbonyl of an amido substituent, (e) deoxygenated moiety after conversion of a TLR7 ligand oxo substituent of pyrimidine or guanosine, or (f) amine.

21. The method of claim 20 wherein the prodrug is selected from an (a) carbamate moiety after conversion of a TLR7 ligand amine substituent, (b) ester, ether, or aminal moiety after conversion of a TLR7 ligand alcohol substituent, or (e) deoxygenated moiety after conversion of a TLR7 ligand oxo substituent of pyrimidine or guanosine.

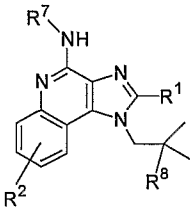
22. The method of claim 18 wherein the prodrug of the masked TLR7 ligand is selected from



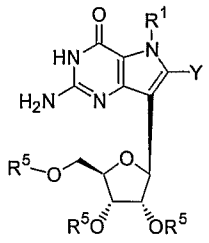
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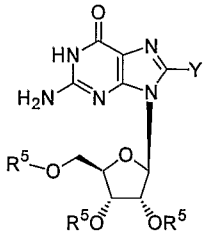
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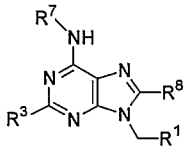
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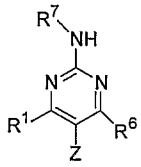
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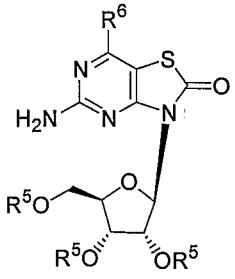
IIe ,



IIf ,



IIg , and



IIh ,

wherein:

each R^1 is H, or a substituted or unsubstituted alkyl, alkenyl, or alkynyl, which may be interrupted by one or more O, S, or N heteroatoms, or a substituted or unsubstituted aryl or heteroaryl;

R^2 is H, OH, SH, halo, or a substituted or unsubstituted alkyl, alkenyl, or alkynyl, which may be interrupted by one or more O, S, or N heteroatoms, or a substituted or unsubstituted -O-(alkyl), -O-(aryl), -O-(heteroaryl), -S-(alkyl), -S-(aryl), -S-(heteroaryl), aryl, or heteroaryl;

R^3 is H, OH, or SH, or a substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, heteroaryl, -O-(alkyl), -O-(aryl), -O-(heteroaryl), -S-(alkyl), -S-(aryl), -S-(heteroaryl), -NH(alkyl), -NH(aryl), -NH(heteroaryl), -NH(R^4)(alkyl), -NH(R^4)(aryl), or -NH(R^4)(heteroaryl);

R^4 is a substituted or unsubstituted alkyl;

R^5 is independently H, –C(O)(C_{1-18}alkyl), or a racemic, L-, or D- amino acid group

$-C(O)CHNH_2R^9$;

R^6 is H, OR^{10}, or $N(R^{11})_2$;

R^7 is independently H or a substituted or unsubstituted $-C(O)(C_{1-18}alkyl)$ or $-C(O)_2(C_{1-18}alkyl)$;

R^8 is H, –OH, -O-(alkyl), $-OCO_2(C_{1-18}alkyl)$, $-OC(O)(C_{1-18}alkyl)$, or a racemic, L-, or D-amino acid group $-OC(O)CHNH_2R^1$;

R^9 is H, or a substituted or unsubstituted alkyl, $C(O)CH(C_{1-6}\ alkyl)NH_2$, or $-C(O)CH(CH_2-aryl)NH_2$;

R^{10} is independently H, C_{1-6} alkyl, C_{3-7} alkenyl, C_{3-7} alkynyl, $-(CR^{12}R^{13})_t(C_6-C_{10}$ aryl), $-(CR^{12}R^{13})_t(C_3-C_{10}$ cycloalkyl), $-(CR^{12}R^{13})_t(C_4-C_{10}$ heterocyclic), $-(CR^{12}R^{13})_{t>1}OH$, $-(CR^{12}R^{13})_{t>0}CO_2C_{1-18}$ alkyl, and $-(CR^{12}R^{13})_{t>0}N(R^{14})CO_2C_{1-18}$ alkyl, and SO_2(aryl), wherein t is an integer from 0 to 6 unless otherwise indicated, and wherein the alkyl, alkenyl, alkynyl, aryl, cycloalkyl, and heterocyclic moieties of the foregoing groups are optionally substituted with substituents independently selected from halo, cyano, nitro, trifluoromethyl, trifluoromethoxy, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, hydroxy, C_1-C_6 alkoxy, $-NH_2$, -NH-alkyl, $-N(alkyl)_2$, -NH-aryl, -N(alkyl)(aryl), $-N(aryl)_2$, -NHCHO, -NHC(O)alkyl, -NHC(O)aryl, -N(alkyl)C(O)H, -N(alkyl)C(O)alkyl, -N(aryl)C(O)H, -N(aryl)C(O)alkyl, $-NHCO_2$alkyl, $-N(alkyl)CO_2$alkyl, $-NHC(O)NH_2$, $-N(alkyl)C(O)NH_2$, -NHC(O)NH-alkyl, $-NHC(O)\ N(alkyl)_2$, -N(alkyl)C(O)NH-alkyl, $N(alkyl)C(O)\ N(alkyl)_2$, $-NHSO_2$-alkyl, $-N(alkyl)SO_2$-alkyl, -C(O)alkyl, -C(O)aryl, -OC(O)alkyl, -OC(O)aryl, $-CO_2$-alkyl, $-CO_2$-aryl, $-CO_2H$, $-C(O)NH_2$, -C(O)NH-alkyl, $-C(O)N(alkyl)_2$, -C(O)NH-aryl, $-C(O)N(aryl)_2$, -C(O)N(alkyl)(aryl), -S(O)alkyl, -S(O)aryl, $-SO_2$alkyl, $-SO_2$aryl, $-SO_2NH_2$, $-SO_2$NH-alkyl, and $-SO_2N(alkyl)_2$;

R^{11} is independently H, C_{1-6} alkyl, C_3-C_{10} cycloalkyl, or together with nitrogen forms a 5- or 6-membered heterocyclic ring;

R^{12} and R^{13} are independently H, C_{1-6} alkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl;

R^{14} is H, C_{1-6} alkyl, or $-CH_2$-aryl;

X is O or S;

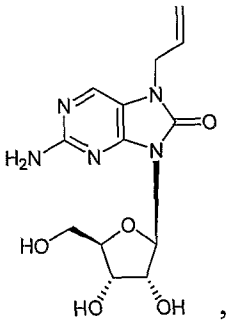
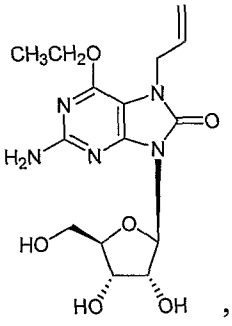
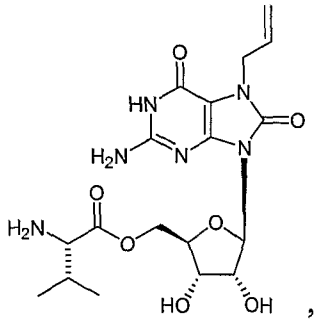
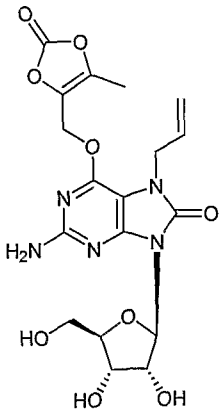
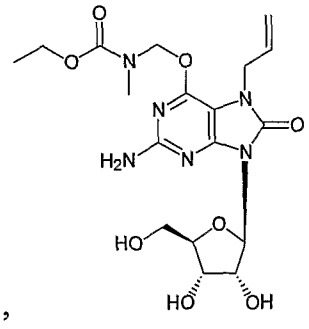
Y is H, halo, OH, OR^4, SH, SR^4, NH_2, NHR^4, $N(R^4)_2$, or a substituted or unsubstituted alkyl or aryl; and

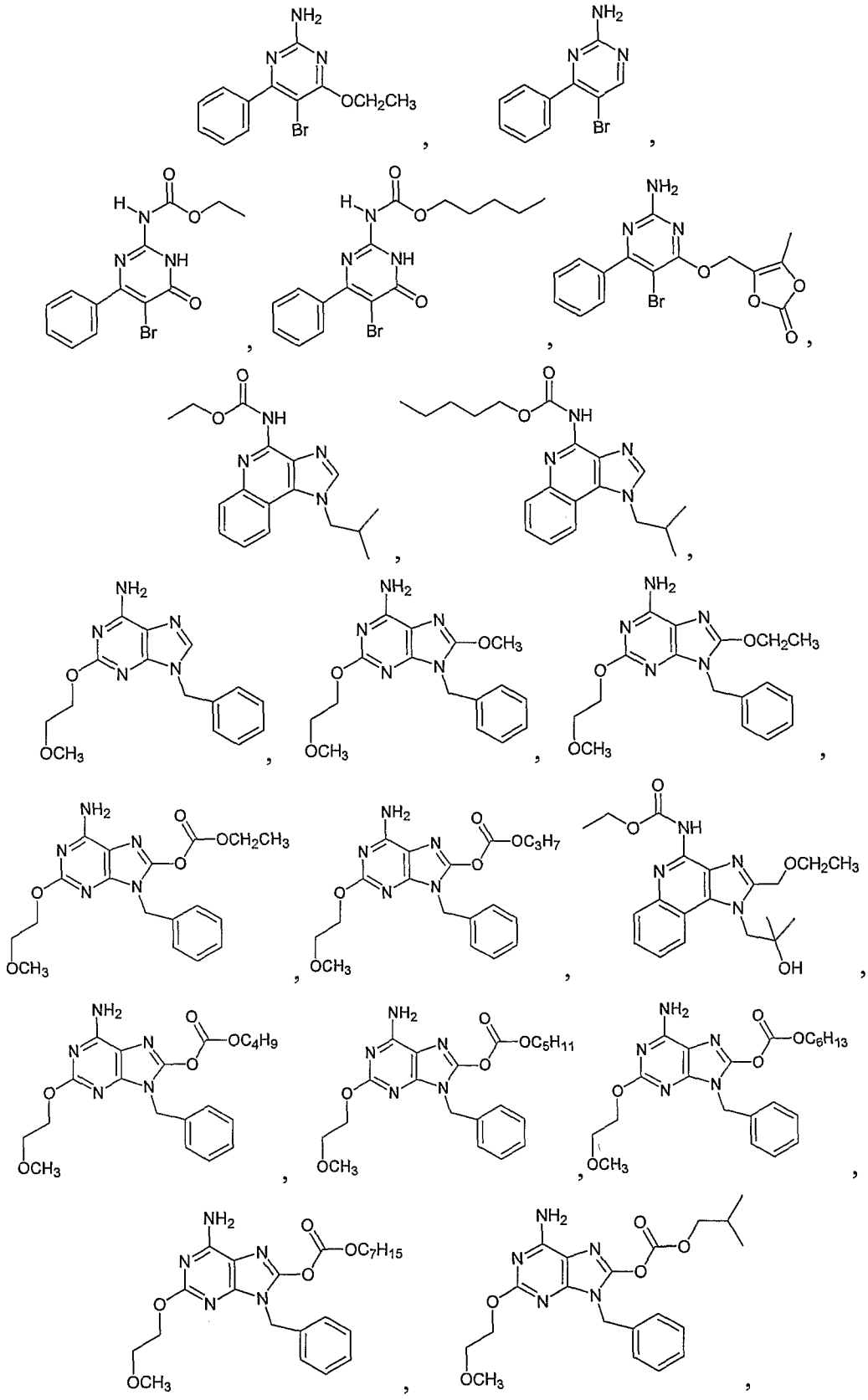
Z is H, halo, OH, OR^4, SH, or SR^4;

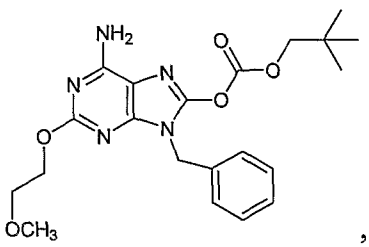
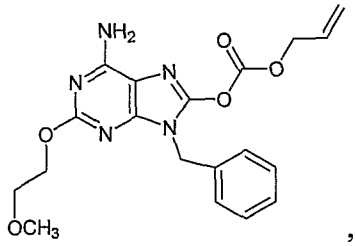
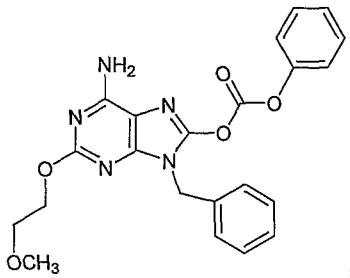
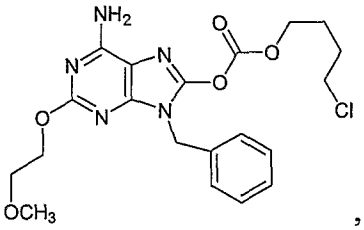
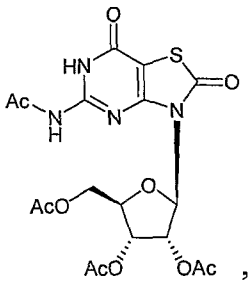
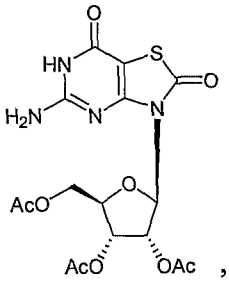
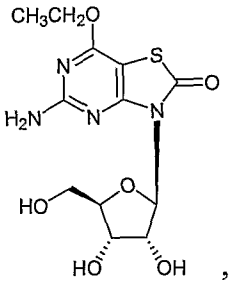
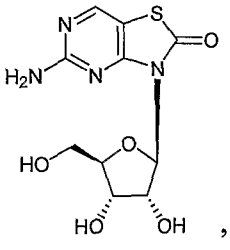
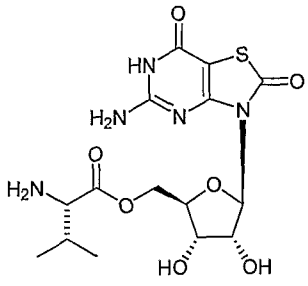
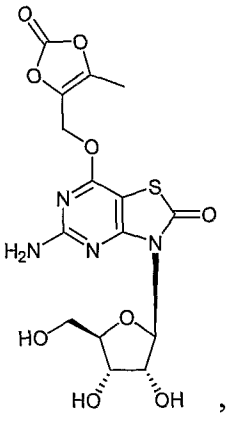
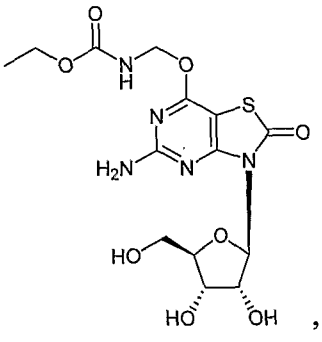
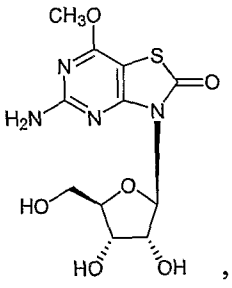
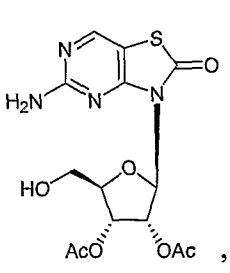
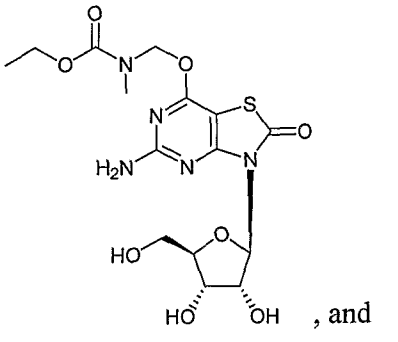
or a pharmaceutically acceptable salt, hydrate, metabolite or stereoisomer thereof or a pharmaceutically acceptable salt or hydrate of said stereoisomer.

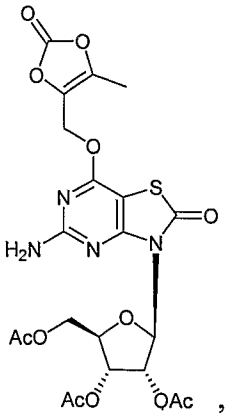
23. The method of claim 22 wherein R^1 is H or a substituted or unsubstituted alkyl, alkenyl, or alkynyl; R^2 is H, OH, halo, or a substituted or unsubstituted alkyl, alkenyl, or alkynyl, or $-CH_2$-O-(alkyl); R^3 is H, OH, or SH, or a substituted or unsubstituted -O-(alkyl), -S-(alkyl), or -NH(alkyl); R^5 is independently H, $-C(O)(C_1$-$_{18}$alkyl), or a racemic, L-, or D- amino acid group $-C(O)CHNH_2R^9$, wherein R^9 is an unsubstituted alkyl; R^6 is H or OR^{10}, wherein R^{10} is independently C_{1-6} alkyl, C_{3-7} alkenyl, C_{3-7} alkynyl, $-(CR^{12}R^{13})_t(C_6$-C_{10} aryl), $-(CR^{12}R^{13})_t(C_4$-C_{10} heterocyclic), and $-(CR^{12}R^{13})_{t>0}N(R^{14})CO_2C_{1-18}$ alkyl, wherein t is an integer from 0 to 4 unless otherwise indicated, and wherein the alkyl, alkenyl, aryl, and heterocyclic moieties of the foregoing groups are optionally substituted with 1 to 3 substituents independently selected from halo, cyano, nitro, trifluoromethyl, trifluoromethoxy, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, hydroxy, C_1-C_6 alkoxy, $-CO_2$-alkyl, $-CO_2$-aryl, -OC(O)alkyl, and -OC(O)aryl, and wherein R^{12} and R^{13} are independently H, C_{1-6} alkyl, or C_{2-6} alkenyl; and R^{14} is H, $-CH_3$, or $-CH_2CH_3$; R^7 is independently H or a substituted or unsubstituted $-C(O)(C_1$-$_{18}$alkyl) or $-C(O)_2(C_1$-$_{18}$alkyl); R^8 is H, –OH, -O-(alkyl), $-OCO_2(C_1$-$_{18}$alkyl), or a racemic, L-, or D- amino acid group $-OC(O)CHNH_2R^1$; X is O or S; Y is H, halo, OH, OR^4, SH, or SR^4; and Z is H, halo, OH, OR^4, SH, or SR^4.

24. The method of claim 18 wherein the masked TLR7 ligand prodrug is selected from


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25. The method of claim 18 wherein the oral administration of the masked TLR7 ligand prodrug achieves an *in vivo* effective plasma concentration of the TLR7 ligand that is 10% to 500% of the effective *in vivo* exposure obtained upon oral administration of the TLR7 ligand alone.

26. The method of claim 18 wherein the oral administration of the masked TLR7 ligand prodrug achieves an *in vivo* effective plasma concentration of the TLR7 ligand that is 50% to 200% of the effective *in vivo* exposure obtained upon oral administration of the TLR7 ligand alone.

27. The method of claim 25 wherein the oral administration of the masked TLR7 ligand prodrug achieves a therapeutically effective plasma concentration of the corresponding TLR7 ligand without causing gastrointestinal irritation.

28. The method of claim 18 wherein the oral administration of the masked TLR7 ligand prodrug reduces undesirable side effects in a patient relative to the side effects upon oral administration of the TLR7 ligand alone.

29. The method of claim 18 wherein the oral administration of the masked TLR7 ligand prodrug reduces undesirable side effects by 50% in a patient relative to the side effects upon oral administration of the TLR7 ligand alone.

30. The method of claim 18 wherein the side effect is gastrointestinal irritancy.

31. The method of claim 30 wherein the irritancy is hemorrhage.

32. The method of claim 30 wherein the irritancy is lesions.

33. The method of claim 30 wherein the irritancy is emesis.

34. The method of claim 18 wherein the patient is human.

35. The method of claim 18 further comprising administering a pharmaceutically acceptable excipient, carrier, or vehicle.

36. The method of claim 18 further comprising administering an additional therapeutic agent.

37. The method of claim 36 wherein the additional therapeutic agent is an antiviral agent.

38. The method of claim 18 wherein the therapeutically or prophylactically effective dose is 0.001 to 100 mg/kg per day.

39. The method of claim 38 wherein the therapeutically or prophylactically effective dose is about 0.1 to 25 mg/kg per day.

40. The method of claim 39 wherein the therapeutically or prophylactically effective dose is about 1 to 20 mg/kg per day.

41. The method of claim 18 wherein the masked TLR7 ligand prodrug is administered parenterally.

42. The method of claim 41 wherein the masked TLR7 ligand prodrug is administered intravenously.

43. The method of claim 18 wherein the masked TLR7 ligand prodrug is administered orally.

44. The method of claim 18 wherein the masked TLR7 ligand prodrug is administered mucosally.

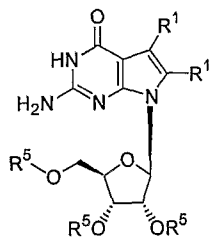
45. A method for treating diseases responsive to immuno therapy while reducing undesirable side effects associated with immunologic agents, comprising orally administering a masked TLR7 ligand prodrug to a patient in need of immuno therapy, wherein the masked TLR7 ligand prodrug achieves a therapeutically effective plasma concentration of a TLR7 ligand in the patient.

46. The method of claim 45 wherein the masked TLR7 ligand prodrug is selected from analogs and derivatives of guanosine, imidazoquinoline, adenine, and pyrimidine.

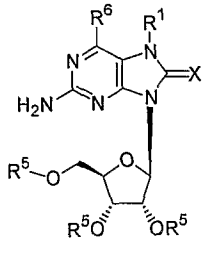
47. The method of claim 45 wherein the prodrug is an (a) amide, carbamate, or amidine moiety after conversion of a TLR7 ligand amine substituent, (b) ester, carbonate, carbamate, ether, imidate, acetal, aminal, or ketal moiety after conversion of a TLR7 ligand alcohol substituent, (c) acetal or ketal moiety after conversion of a TLR7 ligand keto substituent, (d) imidate moiety after conversion of a TLR7 ligand carbonyl of an amido substituent, (e) deoxygenated moiety after conversion of a TLR7 ligand oxo substituent of pyrimidine or guanosine, or (f) amine.

48. The method of claim 47 wherein the prodrug is selected from an (a) carbamate moiety after conversion of a TLR7 ligand amine substituent, (b) ester, ether, or animal moiety after conversion of a TLR7 ligand alcohol substituent, or (e) deoxygenated moiety after conversion of a TLR7 ligand oxo substituent of pyrimidine or guanosine.

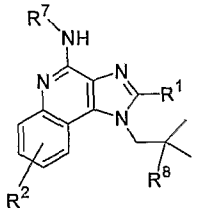
49. The method of claim 45 wherein the masked TLR7 ligand prodrug is selected from



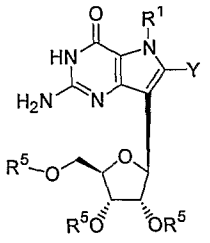
IIa ,



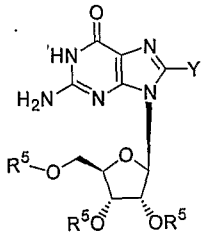
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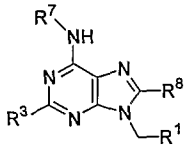
IIc ,



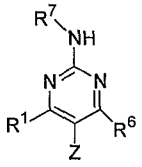
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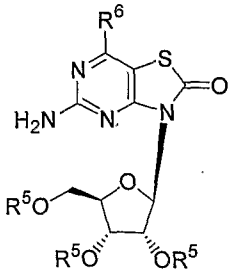
IIe ,



IIf ,



IIg , and



IIh ,

wherein:

each R^1 is H, or a substituted or unsubstituted alkyl, alkenyl, or alkynyl, which may be interrupted by one or more O, S, or N heteroatoms, or a substituted or unsubstituted aryl or heteroaryl;

R^2 is H, OH, SH, halo, or a substituted or unsubstituted alkyl, alkenyl, or alkynyl, which may be interrupted by one or more O, S, or N heteroatoms, or a substituted or unsubstituted -O-(alkyl), -O-(aryl), -O-(heteroaryl), -S-(alkyl), -S-(aryl), -S-(heteroaryl), aryl, or heteroaryl;

R^3 is H, OH, or SH, or a substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, heteroaryl, -O-(alkyl), -O-(aryl), -O-(heteroaryl), -S-(alkyl), -S-(aryl), -S-(heteroaryl), -NH(alkyl), -NH(aryl), -NH(heteroaryl), -NH(R^4)(alkyl), -NH(R^4)(aryl), or -NH(R^4)(heteroaryl);

R^4 is a substituted or unsubstituted alkyl;

R^5 is independently H, –C(O)(C_{1-18}alkyl), or a racemic, L-, or D- amino acid group –C(O)CHNH_2R^9;

R^6 is H, OR^{10}, or N(R^{11})$_2$;

R^7 is independently H or a substituted or unsubstituted –C(O)(C_{1-18}alkyl) or –C(O)$_2$(C_{1-18}alkyl);

R^8 is H, –OH, -O-(alkyl), –OCO$_2$(C_{1-18}alkyl), –OC(O)(C_{1-18}alkyl), or a racemic, L-, or D-amino acid group –OC(O)CHNH_2R^1;

R^9 is H, or a substituted or unsubstituted alkyl, $C(O)CH(C_{1-6}$ alkyl$)NH_2$, or $-C(O)CH(CH_2$-aryl$)NH_2$;

R^{10} is independently H, C_{1-6} alkyl, C_{3-7} alkenyl, C_{3-7} alkynyl, $-(CR^{12}R^{13})_t(C_6$-C_{10} aryl), $-(CR^{12}R^{13})_t(C_3$-C_{10} cycloalkyl), $-(CR^{12}R^{13})_t(C_4$-C_{10} heterocyclic), $-(CR^{12}R^{13})_{t>1}OH$, $-(CR^{12}R^{13})_{t>0}CO_2C_{1-18}$ alkyl, and $-(CR^{12}R^{13})_{t>0}N(R^{14})CO_2C_{1-18}$ alkyl, and SO_2(aryl), wherein t is an integer from 0 to 6 unless otherwise indicated, and wherein the alkyl, alkenyl, alkynyl, aryl, cycloalkyl, and heterocyclic moieties of the foregoing groups are optionally substituted with substituents independently selected from halo, cyano, nitro, trifluoromethyl, trifluoromethoxy, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, hydroxy, C_1-C_6 alkoxy, $-NH_2$, -NH-alkyl, $-N(alkyl)_2$, -NH-aryl, -N(alkyl)(aryl), $-N(aryl)_2$, -NHCHO, -NHC(O)alkyl, -NHC(O)aryl, -N(alkyl)C(O)H, -N(alkyl)C(O)alkyl, -N(aryl)C(O)H, -N(aryl)C(O)alkyl, $-NHCO_2$alkyl, -N(alkyl)CO_2alkyl, $-NHC(O)NH_2$, -N(alkyl)$C(O)NH_2$, -NHC(O)NH-alkyl, -NHC(O) $N(alkyl)_2$, -N(alkyl)C(O)NH-alkyl, N(alkyl)C(O) $N(alkyl)_2$, $-NHSO_2$-alkyl, -N(alkyl)SO_2-alkyl, -C(O)alkyl, -C(O)aryl, -OC(O)alkyl, -OC(O)aryl, $-CO_2$-alkyl, $-CO_2$-aryl, $-CO_2H$, $-C(O)NH_2$, -C(O)NH-alkyl, $-C(O)N(alkyl)_2$, -C(O)NH-aryl, $-C(O)N(aryl)_2$, -C(O)N(alkyl)(aryl), -S(O)alkyl, -S(O)aryl, $-SO_2$alkyl, $-SO_2$aryl, $-SO_2NH_2$, $-SO_2$NH-alkyl, and $-SO_2N(alkyl)_2$;

R^{11} is independently H, C_{1-6} alkyl, C_3-C_{10} cycloalkyl, or together with nitrogen forms a 5- or 6-membered heterocyclic ring;

R^{12} and R^{13} are independently H, C_{1-6} alkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl;

R^{14} is H, C_{1-6} alkyl, or $-CH_2$-aryl;

X is O or S;

Y is H, halo, OH, OR^4, SH, SR^4, NH_2, NHR^4, $N(R^4)_2$, or a substituted or unsubstituted alkyl or aryl; and

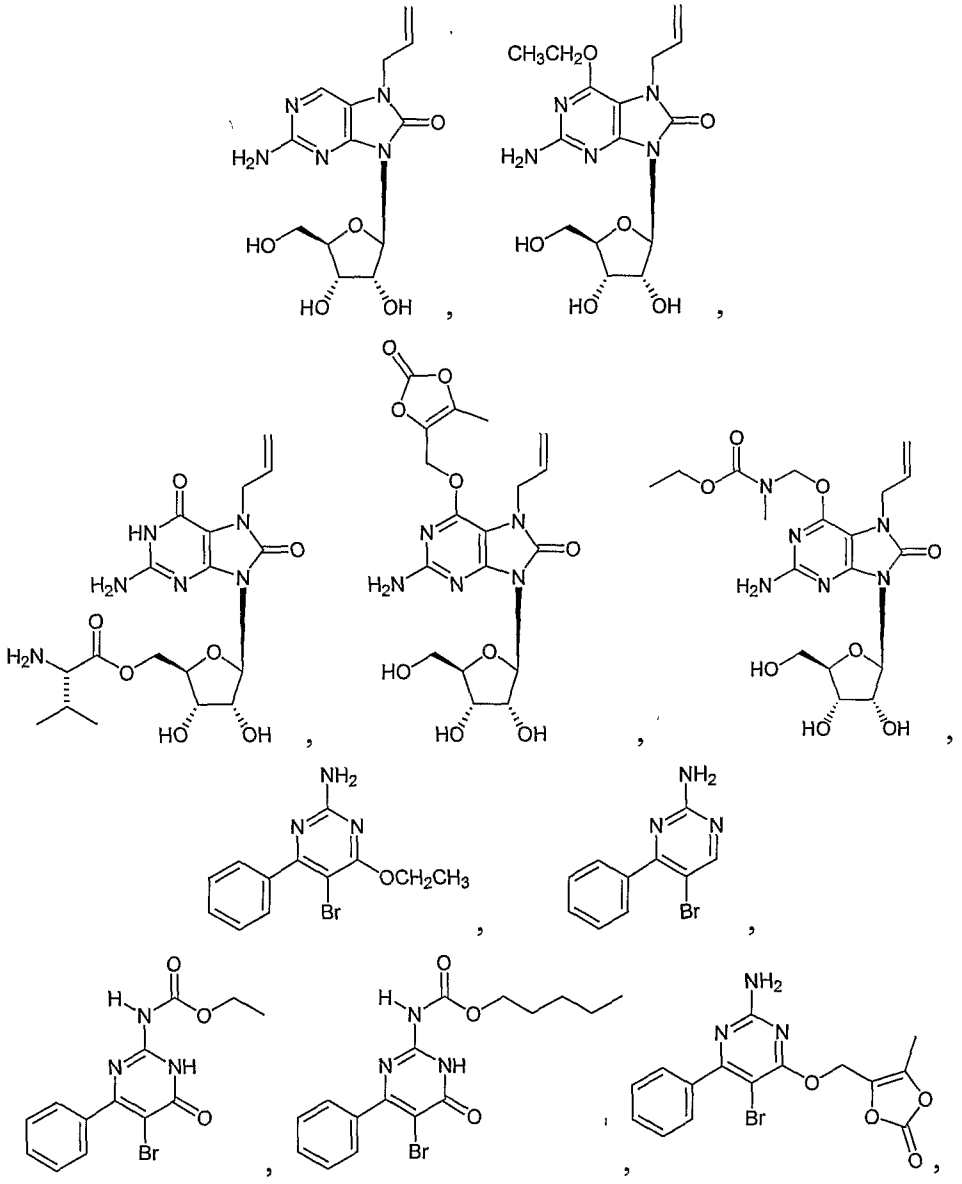
Z is H, halo, OH, OR^4, SH, or SR^4;

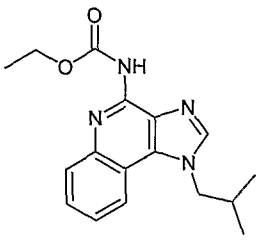
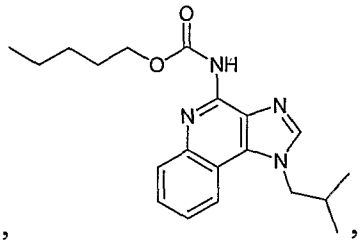
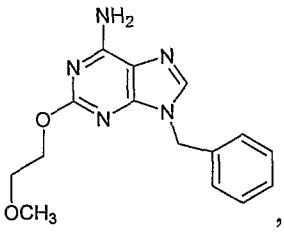
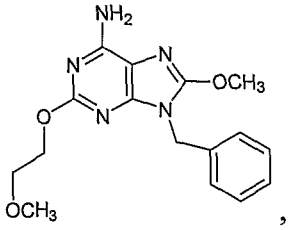
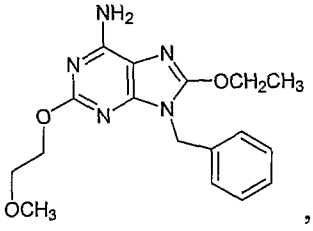
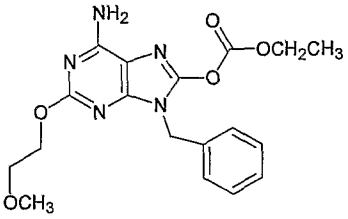
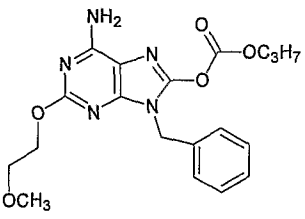
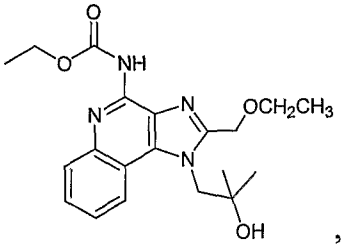
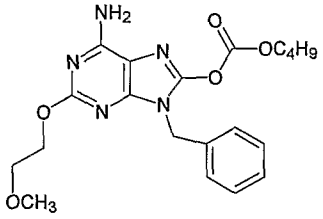
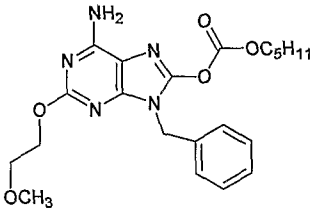
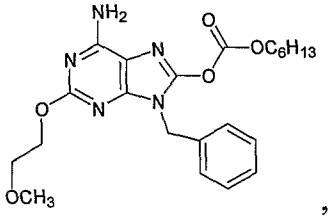
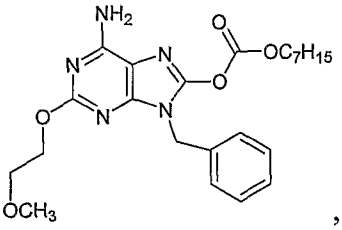
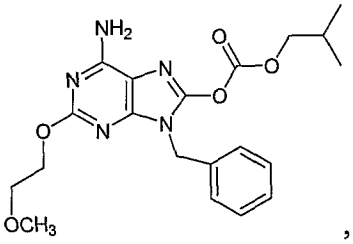
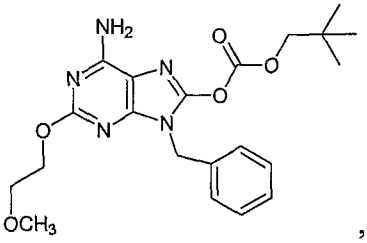
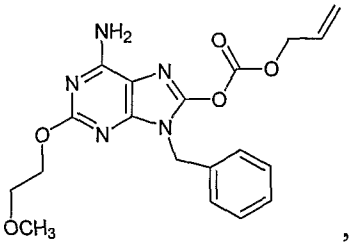
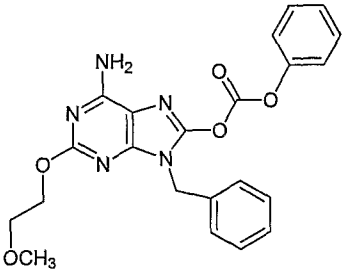
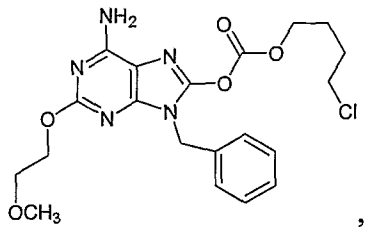
or a pharmaceutically acceptable salt, hydrate, metabolite or stereoisomer thereof or a pharmaceutically acceptable salt or hydrate of said stereoisomer.

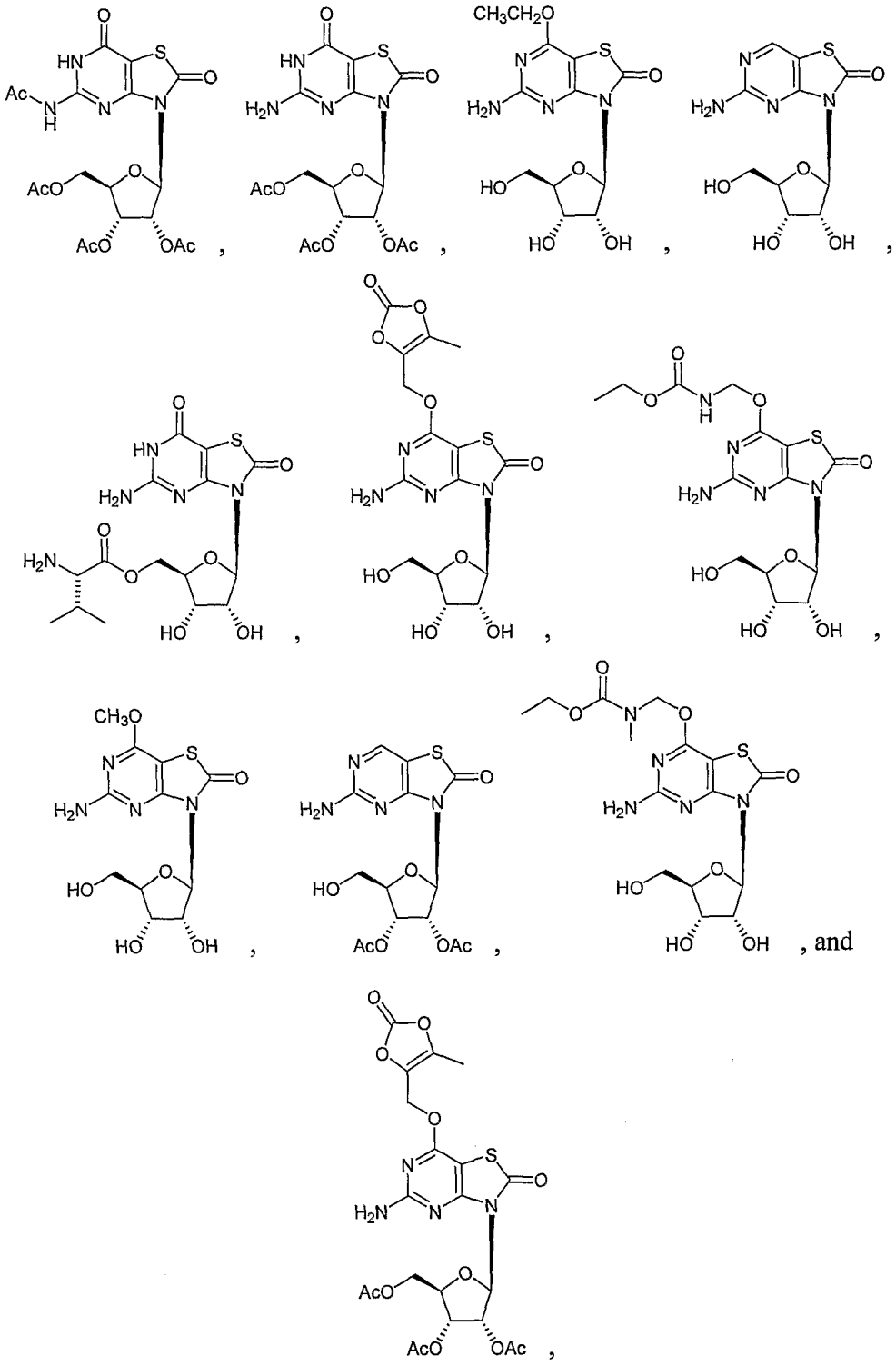
50. The method of claim 49 wherein R^1 is H or a substituted or unsubstituted alkyl, alkenyl, or alkynyl; R^2 is H, OH, halo, or a substituted or unsubstituted alkyl, alkenyl, or alkynyl, or $-CH_2$-O-(alkyl); R^3 is H, OH, or SH, or a substituted or unsubstituted -O-(alkyl), -S-(alkyl), or -NH(alkyl); R^5 is independently H, $-C(O)(C_{1-18}$alkyl), or a racemic, L-, or D- amino acid group $-C(O)CHNH_2R^9$, wherein R^9 is an unsubstituted alkyl; R^6 is H or OR^{10}, wherein R^{10} is independently C_{1-6} alkyl, C_{3-7} alkenyl, C_{3-7} alkynyl, $-(CR^{12}R^{13})_t(C_6$-C_{10} aryl), $-(CR^{12}R^{13})_t(C_4$-C_{10} heterocyclic), and $-(CR^{12}R^{13})_{t>0}N(R^{14})CO_2C_{1-18}$ alkyl, wherein t is an integer from 0 to 4 unless otherwise indicated, and wherein the alkyl, alkenyl, aryl, and

heterocyclic moieties of the foregoing groups are optionally substituted with 1 to 3 substituents independently selected from halo, cyano, nitro, trifluoromethyl, trifluoromethoxy, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, hydroxy, C_1-C_6 alkoxy, -CO_2-alkyl, -CO_2-aryl, -OC(O)alkyl, and -OC(O)aryl, and wherein R^{12} and R^{13} are independently H, C_{1-6} alkyl, or C_{2-6} alkenyl; and R^{14} is H, $-CH_3$, or $-CH_2CH_3$; R^7 is independently H or a substituted or unsubstituted $-C(O)(C_1$-$_{18}$alkyl) or $-C(O)_2(C_1$-$_{18}$alkyl); R^8 is H, –OH, -O-(alkyl), $-OCO_2(C_1$-$_{18}$alkyl), or a racemic, L-, or D- amino acid group $-OC(O)CHNH_2R^1$; X is O or S; Y is H, halo, OH, OR^4, SH, or SR^4; and Z is H, halo, OH, OR^4, SH, or SR^4.

51. The method of claim 45 wherein the masked TLR7 ligand prodrug is selected from




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52. The method of claim 45 wherein the disease is a viral infection.

53. The method of claim 52 wherein the viral infection is selected from an RNA or DNA virus including adenovirus, cytomegalovirus, hepatitis A virus (HAV), hepadnaviruses including hepatitis B virus (HBV), flaviviruses including Yellow Fever

virus, hepaciviruses including hepatitis C virus (HCV), herpes simplex type 1 and 2, herpes zoster, human herpesvirus 6, human immunodeficiency virus (HIV), human papilloma virus (HPV), influenza A virus, influenza B virus, measles, parainfluenza virus, pestivirus, poliovirus, poxvirus (including smallpox and monkeypox virus), rhinovirus, coronovirus, respiratory syncytial virus (RSV), multiple families of viruses that cause hemorrhagic fevers, including the Arenaviruses, the Bunyaviruses and Filoviruses, and a range of viral encephalitides caused by RNA and DNA viruses.

54. The method of claim 45 wherein the disease is a cancer.

55. The method of claim 45 wherein the oral administration of the masked TLR7 ligand prodrug achieves an *in vivo* effective plasma concentration of the TLR7 ligand that is 10% to 500% of the effective *in vivo* exposure obtained upon oral administration of the TLR7 ligand alone.

56. The method of claim 55 wherein the oral administration of the masked TLR7 ligand prodrug achieves an *in vivo* effective plasma concentration of the TLR7 ligand that is 50% to 200% of the effective *in vivo* exposure obtained upon oral administration of the TLR7 ligand alone.

57. The method of claim 56 wherein the oral administration of the masked TLR7 ligand prodrug achieves a therapeutically effective plasma concentration of the corresponding TLR7 ligand without causing gastrointestinal irritation.

58. The method of claim 45 wherein the oral administration of the masked TLR7 ligand prodrug reduces undesirable side effects in a patient relative to the side effects upon oral administration of the TLR7 ligand alone.

59. The method of claim 45 wherein the oral administration of the masked TLR7 ligand prodrug reduces undesirable side effects by 50% in a patient relative to the side effects upon oral administration of the TLR7 ligand alone.

60. The method of claim 45 wherein the side effect is gastrointestinal irritancy.

61. The method of claim 60 wherein the irritancy is hemorrhage.

62. The method of claim 60 wherein the irritancy is lesions.

63. The method of claim 60 wherein the irritancy is emesis.

64. The method of claim 45 wherein the patient is human.

65. The method of claim 45 further comprising administering a pharmaceutically acceptable excipient, carrier, or vehicle.

66. The method of claim 45 further comprising administering an additional therapeutic agent.

67. The method of claim 66 wherein the additional therapeutic agent is an antiviral agent.

68. The method of claim 45 wherein the therapeutically or prophylactically effective dose is 0.001 to 100 mg/kg per day.

69. The method of claim 68 wherein the therapeutically or prophylactically effective dose is about 0.01 to 50 mg/kg per day.

70. The method of claim 69 wherein the therapeutically or prophylactically effective dose is about 0.1 to 20 mg/kg per day.

71. The method of claim 45 wherein the masked TLR7 ligand prodrug is administered parenterally.

72. The method of claim 71 wherein the masked TLR7 ligand prodrug is administered intravenously.

73. The method of claim 45 wherein the masked TLR7 ligand prodrug is administered orally.

74. The method of claim 45 wherein the masked TLR7 ligand prodrug is administered mucosally.