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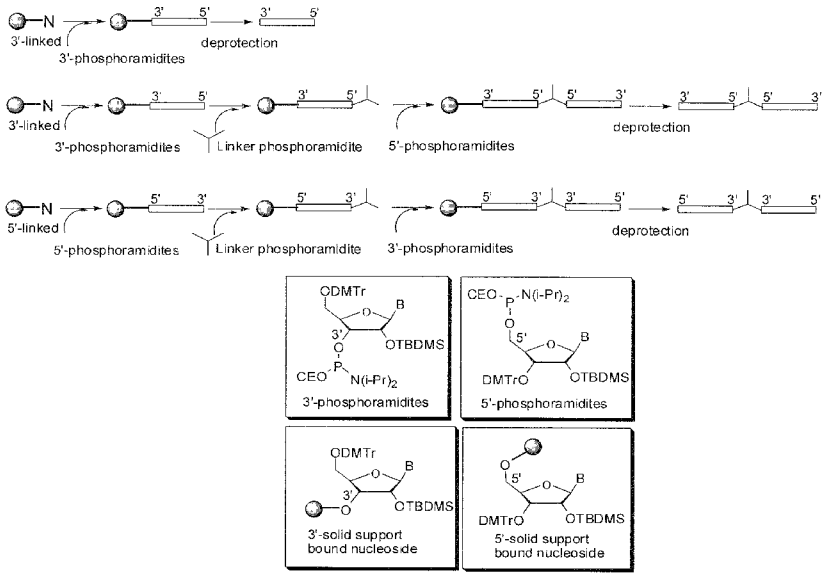
(54) **Title**: NOVEL AGONISTS OF TOLL-LIKE RECEPTOR 3 AND METHODS OF THEIR USE

Figure 1A.



(57) **Abstract**: TLR3 agonist compounds, compositions and methods are provided for stimulating the activity of TLR3. The compositions comprise oligonucleotide-based compounds that bind to and activate TLR3. The compositions may also comprise oligonucleotide-based compounds that bind to and activate TLR3 in combination with other therapeutic and/or prophylactic compounds and/or compositions. Methods of using these compounds and compositions for stimulation of TLR3 activity and for prevention or treatment of diseases wherein modulation of TLR3 activity would be beneficial are provided.

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NOVEL AGONISTS OF TOLL-LIKE RECEPTOR 3 AND METHODS OF THEIR USE

(Atty. Docket No: IDE10639P00281PC)

BACKGROUND OF THE INVENTION

Related Applications

[0001] This application claims the benefit of U.S. Provisional Application Serial No. 61/358,543, filed on June 25, 2010; U.S. Provisional Application Serial No. 61/419,488, filed on December 3, 2010; and U.S. Provisional Application Serial No. 61/435,434, filed on January 24, 2011, the contents of which are incorporated herein by reference in their entirety.

Field of the invention

[0002] The present invention relates to modulating the immune system. In particular, the invention relates to oligonucleotide-based compounds that selectively stimulate an immune response through binding to and activating Toll-Like Receptor 3 (TLR3), and their use, alone or in combination with other agents, for treating or preventing diseases wherein modulation of TLR3 activity would be beneficial.

Summary of the related art

[0003] Toll-like receptors (TLRs) are present on many cells of the immune system and have been shown to be involved in the innate immune response (Hornung, V. et al., (2002) J. Immunol. 168:4531-4537). TLRs are a key means by which mammals recognize and mount an immune response to foreign molecules and also provide a means by which the innate and adaptive immune responses are linked (Akira, S. et al. (2001) Nature Immunol. 2:675-680; Medzhitov, R. (2001) Nature Rev. Immunol. 1:135-145). In vertebrates, this family consists of at least 11 proteins called TLR1 to TLR11, which are known to recognize pathogen associated molecular patterns (PAMP) from bacteria, fungi, parasites, and viruses and induce an immune response mediated by a number of transcription factors.

[0004] Some TLRs are located on the cell surface to detect and initiate a response to extracellular pathogens and other TLRs are located inside the cell to detect and initiate a response to intracellular pathogens. Table 1 provides a representation of TLRs, the known agonists therefore and the cell types known to contain the TLR (Diebold, S.S. et al. (2004)

Science 303:1529-1531; Liew, F. et al. (2005) Nature 5:446-458; Hemmi H et al. (2002) Nat Immunol 3:196-200; Jurk M et al., (2002) Nat Immunol 3:499; Lee J et al. (2003) Proc. Natl. Acad. Sci. USA 100:6646-6651); (Alexopoulou, L. (2001) Nature 413:732-738).

Table 1:

TLR Molecule	Agonist	Cell Types Containing Receptor
Cell Surface TLRs:		
TLR2	bacterial lipopeptides	• Monocytes/macrophages • Myeloid dendritic cells • Mast cells
TLR4	gram negative bacteria	• Monocytes/macrophages • Myeloid dendritic cells • Mast cells • Intestinal epithelium
TLR5	motile bacteria	• Monocyte/macrophages • Dendritic cells • Intestinal epithelium
TLR6	gram positive bacteria	• Monocytes/macrophages • Mast cells • B lymphocytes
Endosomal TLRs:		
TLR3	double stranded RNA viruses	• Dendritic cells • B lymphocytes
TLR7	single stranded RNA viruses; RNA-immunoglobulin complexes	• Monocytes/macrophages • Plasmacytoid dendritic cells • B lymphocytes
TLR8	single stranded RNA viruses; RNA-immunoglobulin complexes	• Monocytes/macrophages • Dendritic cells • Mast cells
TLR9	DNA containing unmethylated "CpG" motifs; DNA-immunoglobulin complexes	• Monocytes/macrophages • Plasmacytoid dendritic cells • B lymphocytes

[0005] The signal transduction pathway mediated by the interaction between a ligand and a TLR is shared among most members of the TLR family and involves a toll/IL-1 receptor (TIR domain), the myeloid differentiation marker 88 (MyD88), IL-1R-associated kinase (IRAK), interferon regulating factor (IRF), TNF-receptor-associated factor (TRAF), TGFβ-activated kinase1, IκB kinases, IκB, and NF-κB (see for example: Akira, S. (2003) J. Biol. Chem. 278:38105 and Geller at al. (2008) Curr. Drug Dev. Tech. 5:29-38). More specifically, for TLRs

1, 2, 4, 5, 6, 7, 8, 9 and 11, this signaling cascade begins with a PAMP ligand interacting with and activating the membrane-bound TLR, which exists as a homo-dimer in the endosomal membrane or the cell surface. Following activation, the receptor undergoes a conformational change to allow recruitment of the TIR domain containing protein MyD88, which is an adapter protein that is common to all TLR signaling pathways except TLR3. MyD88 recruits IRAK4, which phosphorylates and activates IRAK1. The activated IRAK1 binds with TRAF6, which catalyzes the addition of polyubiquitin onto TRAF6. The addition of ubiquitin activates the TAK/TAB complex, which in turn phosphorylates IRFs, resulting in NF-kB release and transport to the nucleus. NF-kB in the nucleus induces the expression of proinflammatory genes (see for example, Trinchieri and Sher (2007) Nat. Rev. Immunol. 7:179-190).

[0006] TLR3 signaling occurs through a MyD88 independent pathway that begins with the TLR3 ligand interacting with and activating TLR3, which exists as a homo-dimer. Following activation, TLR3 undergoes a conformational change, allowing recruitment of a TIR-domain-containing adapter-inducing interferon-β (TRIF), which activates TANK-binding Kinase 1 (TBK1). TBK1 phosphorylates and activates IRF-3, resulting in the activation of interferons α and β and ultimately the generation of an inflammatory immune response (see for example: Miggin and O'Neill (2006) J. Leukoc. Biol. 80:220-226).

[0007] As a result of their involvement in regulating an inflammatory response, TLRs have been shown to play a role in the pathogenesis of many diseases, including autoimmunity, infectious disease and inflammation (Papadimitraki et al. (2007) J. Autoimmun. 29: 310-318; Sun et al. (2007) Inflam. Allergy Drug Targets 6:223-235; Diebold (2008) Adv. Drug Deliv. Rev. 60:813-823; Cook, D.N. et al. (2004) Nature Immunol. 5:975-979; Tse and Horner (2008) Semin. Immunopathol. 30:53-62; Tobias & Curtiss (2008) Semin. Immunopathol. 30:23-27; Ropert et al. (2008) Semin. Immunopathol. 30:41-51; Lee et al. (2008) Semin. Immunopathol. 30:3-9; Gao et al. (2008) Semin. Immunopathol. 30:29-40; Vijay-Kumar et al. (2008) Semin. Immunopathol. 30:11-21).

[0008] The selective localization of TLRs and the signaling generated therefrom, provides some insight into their role in the immune response. The immune response involves both an innate and an adaptive response based upon the subset of cells involved in the response. For example, the T helper (Th) cells involved in classical cell-mediated functions such as delayed-

type hypersensitivity and activation of cytotoxic T lymphocytes (CTLs) are Th1 cells. This response is the body's innate response to antigen (e.g. viral infections, intracellular pathogens, and tumor cells), and results in a secretion of IFN-gamma and a concomitant activation of CTLs.

[0009] TLR3 is known to localize in endosomes inside the cell and recognizes nucleic acids (DNA and RNA) and small molecules such as nucleosides and nucleic acid metabolites. TLR3 has been shown to recognize and respond to double stranded RNA (dsRNA) viruses (Diebold, S.S., et al., (2004) Science 303:1529-1531). In addition, it has been shown that small interfering RNA (siRNA) molecules and non-targeted dsRNA molecules can non-specifically activate TLR3 (Alexopoulou et al. (2008) Nature 413:732-738). However, this non-specific activation of TLR3 was determined to be dependent on a MyD88 pathway, indicating that such dsRNA molecules have the potential to generate immune responses that are not specific to TLR3.

[0010] In addition to naturally existing and synthetic dsRNA ligands for TLR3, other synthetic oligonucleotide analogs have been shown to activate TLR3. The poly-inosinic acid poly-cytidylic acid complex (poly(I:C)), a synthetic double stranded RNA molecule that is designed to mimic viral dsRNA, is composed of a long strand of poly(I) annealed to a long strand of poly(C). Due to the need for long strands, poly(I:C) compounds are routinely synthesized using enzymatic processes. As a result of the enzymatic synthesis, the size of poly(I:C) compounds and preparations is known to vary between 0.2 kb and 8 kb. Poly(I:C) has been shown to induce interferon (Field et al. (1968) Proc. Natl. Acad. Sci.U.S.A. 61:340). Subsequent to this discovery, it was determined that poly(I:C) induces interferon through activation of TLR3 and, as compared to dsRNA molecules, poly(I:C) is preferentially recognized by TLR3 (Alexopoulou et al. (2001) Nature 413:732-738; Okahira et al. (2005) DNA Cell Biol. 24:614-623). The interferon inducing properties of poly(I:C) as well as its preferential binding to TLR3 make poly(I:C) a desirable molecule for use at inducing interferon in vivo. However, poly(I:C) exists as long strands of nucleic acids that have been shown to form undesirable helix-with-loop structures (Ichikawa et al. (1967) Bul. Chem. Soc. Japan 40:2272-2277) and to have toxic properties when administered in vivo (Absher and Stinebring (1969) Nature 223:1023; Lindsay et al. (1969) Nature 223:717; Adamson and Fabro (1969) Nature 223:718; Leonard et al. (1969) Nature 224:1023). Thus, the medical, therapeutic, and prophylactic use of poly(I:C) is limited.

[0011] Attempts have been made to modify the structure of poly(I:C) to retain its immune stimulatory properties while reducing its toxicity (WO2008109083). These compounds insert mismatches into the poly(I:C) strand by replacing cytosine with uracil at defined positions throughout the double stranded molecule. The compounds are referred to as poly(I:C_{12}U). However, these compounds have had limited therapeutic success because their in vivo efficacy has been questioned and they have been rejected by the U.S.A. Food and Drug Administration.

[0012] Thus, it would be desirable to have a selective TLR3 agonist that retains the immune stimulatory activity and therapeutic activity of a poly(I:C) oligonucleotide without the undesired enzymatic synthesis, helix-with-loop structures, toxicity, and lack of efficacy of the currently available poly(I:C), poly(I:C_{12}U), and dsRNA compounds.

BRIEF SUMMARY OF THE INVENTION

[0013] The present invention relates to TLR3 agonist compounds, compositions comprising such compounds, and their use for stimulating a TLR3-mediated immune response.

[0014] In a first aspect, the invention provides novel, synthetic TLR3 agonists compromising a first oligoribonucleotide having a first complementary domain and a poly-inosinic acid domain and a second oligoribonucleotide having a second complementary domain and a poly-cytidylic acid domain, wherein the complementary domain of the first oligoribonucleotide is complementary to the complementary domain of the second oligoribonucleotide and wherein the hybridization of the first and second oligonucleotides to each other is in such a manner that either the complementary domains or the poly-inosinic acid and poly-cytidylic acid domains are free, such that further first oligoribonucleotides and further second oligoribonucleotides can hybridize to the free poly-inosinic acid or free poly-cytidylic acid or free complementary domains.

[0015] In a second aspect, the invention provides a composition comprising a TLR3 agonist according to the invention and a physiologically acceptable carrier.

[0016] In a third aspect, the invention provides a method of stimulating TLR3 activity. In this method, a TLR3 agonist according to the invention is specifically contacted with or bound by TLR3 *in vitro*, *in vivo*, *ex vivo* or in a cell.

[0017] In a fourth aspect, the invention provides methods for stimulating the activity of TLR3 in a mammal, particularly a human, such methods comprising administering to the mammal a TLR3 agonist according to the invention.

[0018] In a fifth aspect, the invention provides a method for stimulating a TLR3-mediated immune response in a mammal, the method comprising administering to the mammal a TLR3 agonist according to the invention in a pharmaceutically effective amount.

[0019] In a sixth aspect, the invention provides a method for therapeutically treating a mammal having a disease treatable by TLR3 activation or TLR3-mediated immune stimulation, such method comprising administering to the mammal, particularly a human, a TLR3 agonist according to the invention, or a composition thereof, in a pharmaceutically effective amount. The invention also relates to the TLR3 agonist and compositions thereof, which are disclosed

herein in methods of treating diseases and illnesses, for use in treating diseases and illnesses and for use as vaccine adjuvants.

[0020] In a seventh aspect, the invention provides methods for preventing a disease or disorder or for use as vaccine adjuvants in a mammal, particularly a human, at risk of contracting or developing a disease or disorder preventable by TLR3 activation or TLR3-mediated stimulation of an immune response. The method according to this aspect of the invention comprises administering to the mammal a TLR3 agonist according to the invention, or a composition thereof, in a prophylactically effective amount.

[0021] In an eighth aspect, the TLR3 agonists and compositions thereof according to the invention are also useful for examining the function of the TLR3 in a cell or in a control mammal or in a mammal afflicted with a disease associated with TLR3 or immune stimulation or immune suppression. The cell or mammal is administered the TLR3 agonist according to the first or second aspects of the invention, and the activity of TLR3 is examined.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] Figure 1A is a synthetic scheme for the linear synthesis of TLR3 agonist of the invention. DMTr = 4,4'-dimethoxytrityl; CE = cyanoethyl.

[0023] Figure 1B is a synthetic scheme for the parallel synthesis of TLR3 agonists of the invention. DMTr = 4,4'-dimethoxytrityl; CE = cyanoethyl.

[0024] Figures 2A and 2B and Table 6 depict the immune stimulatory activity of exemplary TLR3 agonists according to the invention in HEK293 cells expressing human TLR3. Briefly, the HEK293 cells were treated with TLR3 agonists of the invention for 18 hr, and the levels of NF-κB subsequently produced were determined using SEAP (secreted form of human embryonic alkaline phosphatase) assay. The data demonstrate the ability of exemplary TLR3 agonists according to the invention to stimulate TLR3 activity in a dose dependent fashion in HEK293 cells that were cultured and treated according to Example 2. More generally these data demonstrate that TLR3 agonists of the invention can activate TLR3 and generate an immune response.

[0025] Figure 3 and Tables 7, 8, 9, 10, and 11 depict the immune stimulatory activity of an exemplary TLR3 agonist according to the invention in J774 macrophage cells, which naturally contain TLR3. Briefly, the J774 cells were treated with a TLR3 agonist of the invention for 18 hr, and the levels of IL-12 subsequently produced were determined using ELISA. The data demonstrate the ability of exemplary TLR3 agonists according to the invention to stimulate TLR3 activity in a dose dependent fashion in J774 cells that were cultured and treated according to Example 2. More generally these data demonstrate that TLR3 agonists of the invention can activate TLR3 and generate an immune response in immune cells.

[0026] Figure 4 is a graphical representation of the immune stimulatory activity of an exemplary TLR3 agonist according to the invention in J774 macrophage cells, which naturally contain TLR3. Briefly, the J774 cells were treated with a TLR3 agonist of the invention for 18 hr, and the levels of IL-6 subsequently produced were determined using ELISA. The data demonstrate the ability of exemplary TLR3 agonists according to the invention to stimulate TLR3 activity in a dose dependent fashion in J774 cells that were cultured and treated according to Example 2. More generally these data demonstrate that TLR3 agonists of the invention can activate TLR3 and generate an immune response in immune cells.

[0027] Figure 5 is a graphical representation of the immune stimulatory activity of exemplary TLR3 agonists according to the invention in J774 macrophage cells, which naturally contain TLR3. Briefly, the J774 cells were treated with selected TLR3 agonists of the invention for 18 hr, and the levels of IFNβ subsequently produced were determined using ELISA. The data demonstrate the ability of exemplary TLR3 agonists according to the invention to stimulate TLR3 activity in a dose dependent fashion in J774 cells that were cultured and treated according to Example 2. More generally these data demonstrate that TLR3 agonists of the invention can activate TLR3 and generate an immune response in immune cells.

[0028] Figure 6 is a graphical representation of the immune stimulatory activity of exemplary TLR3 agonist according to the invention in J774 macrophage cells, which naturally contain TLR3. Briefly, the J774 cells were treated with TLR3 agonists of the invention for 18 hr, and the levels of IL-6 subsequently produced were determined using ELISA. The data demonstrate the ability of exemplary TLR3 agonists according to the invention to stimulate TLR3 activity in a dose dependent fashion in J774 cells that were cultured and treated according to Example 2. More generally these data demonstrate that TLR3 agonists of the invention can activate TLR3 and generate an immune response in immune cells.

[0029] Figure 7 is a graphical representation of the immune stimulatory activity of exemplary TLR3 agonist according to the invention in J774 macrophage cells, which naturally contain TLR3. Briefly, the J774 cells were treated with TLR3 agonists of the invention for 18 hr, and the levels of IFNβ subsequently produced were determined using ELISA. The data demonstrate the ability of exemplary TLR3 agonists according to the invention to stimulate TLR3 activity in a dose dependent fashion in J774 cells that were cultured and treated according to Example 2. More generally these data demonstrate that TLR3 agonists of the invention can activate TLR3 and generate an immune response in immune cells.

[0030] Figure 8 and Table 14 depict serum cytokine induction in C57BL/6 mice (n=3) 2 hours after they were treated and analyzed according to Example 3. Briefly, the C57BL/6 mice were injected subcutaneously with 0 mg/kg or 25 mg/kg dose of TLR3 agonists, and 2 hours after administration of the agonist, serum was analyzed for immune stimulatory cytokine levels, and IL-12 levels are presented. The data demonstrate that *in vivo* administration of a TLR3 agonist of the invention generates a distinct TLR-mediated *in vivo* cytokine profile.

[0031] Figure 9 is a graphical representation of serum cytokine induction in C57BL/6 mice (n=3) 2 hours after they were treated and analyzed according to Example 3. Briefly, the C57BL/6 mice were injected subcutaneously with 0 mg/kg or 25 mg/kg dose of TLR3 agonists, and 2 hours after administration of the agonist, serum was analyzed for cytokine and chemokine levels, and IL-1b, IL-2, IL-6, IL-10, IL-12, IP-10, KC, MCP-1, MIG, MIP-1α, TNFα levels are presented. The data demonstrate that in vivo administration of a TLR3 agonist of the invention generates a distinct TLR-mediated *in vivo* cytokine and chemokine profile.

[0032] Figure 10 is a graphical representation of immune stimulatory activity of exemplary TLR3 agonists according to the invention in HEK293 cells expressing human TLR7 that were treated and analyzed according to example 2. Briefly, the HEK293 cells were treated with TLR3 agonists of the invention for 18 hr, and the levels of NF-κB subsequently produced were determined using SEAP (secreted form of human embryonic alkaline phosphatase) assay. The data demonstrate the specificity of exemplary TLR3 agonists according to the invention as such compounds did not stimulate TLR7-mediated NF-κB, which is known to be a TLR that responds to single stranded RNA molecules. More generally these data demonstrate that TLR3 agonists of the invention induce a TLR3 specific immune response.

[0033] Figure 11 is a graphical representation of the immune stimulatory activity of exemplary TLR3 agonists according to the invention in HEK293 cells expressing human TLR8 that were treated and analyzed according to example 2. Briefly, the HEK293 cells were treated with TLR3 agonists of the invention for 18 hr, and the levels of NF-κB subsequently produced were determined using SEAP (secreted form of human embryonic alkaline phosphatase) assay. The data demonstrate the specificity of exemplary TLR3 agonists according to the invention as such compounds did not stimulate TLR8-mediated NF-κB, which is known to be a TLR that responds to single stranded RNA molecules. More generally these data demonstrate that TLR3 agonists of the invention induce a TLR3 specific immune response.

[0034] Figures 12 and 13 depict serum cytokine induction in C57BL/6 mice (n=2) 2 hours after they were treated and analyzed according to Example 5. Briefly, the C57BL/6 mice were injected subcutaneously with 10 mg/kg dose of TLR3 agonists, and 2 hours after administration of the agonist, serum was analyzed for immune stimulatory cytokine levels, and IL-12 levels are

presented. The data demonstrate that *in vivo* administration of a TLR3 agonist of the invention generates a distinct TLR-mediated *in vivo* cytokine profile.

[0035] Table 3 and Table 12 depict cytokine and chemokine concentrations from human PBMCs that were treated and analyzed according to example 2. Briefly, the PBMCs were isolated from freshly obtained healthy human volunteer's blood and cultured with 250 μg/ml of exemplary TLR3 agonists of the invention for 24 hr, and supernatants were collected and analyzed by Luminex multiplex assay cytokine and chemokine levels. The data demonstrate that administration of a TLR3 agonist of the invention generates a distinct, TLR3-mediated cytokine and chemokine profile in human immune cells.

[0036] Table 4 depicts cytokine and chemokine concentrations from human plasmacytoid dendritic cells (pDCs) that were isolated, treated, and analyzed according to example 2. Briefly, the pDCs were isolated from freshly obtained healthy human volunteer's blood PBMCs and cultured with 250 μg/ml dose of TLR3 agonists of the invention for 24 hr, and supernatants were collected and analyzed by Luminex multiplex assay for cytokine and chemokine levels. The data demonstrate that administration of a TLR3 agonist of the invention generates a distinct, TLR3-mediated cytokine and chemokine profile in human immune cells.

[0037] Tables 5A, 5B, 5C, and 5D depict the immune stimulatory activity of TLR3 agonists that do not have the preferred structure of the TLR3 agonists of the invention and that were isolated, treated, and analyzed according to example 2. Briefly, the HEK293 cells were treated with TLR3 agonists lacking the preferred structure of the TLR3 agonists of the invention for 18 hr, and the levels of NF-κB subsequently produced were determined using SEAP (secreted form of human embryonic alkaline phosphatase) assay. The data demonstrate the compounds lacking the preferred structure of the TLR3 agonists of the invention do not induce a tTLR3-mediated immune response.

[0038] Table 13 depicts cytokine and chemokine concentrations from human myeloid dendritic cells (mDCs) that were isolated, treated, and analyzed according to Example 2. Briefly, the pDCs were isolated from freshly obtained healthy human volunteer's blood PBMCs and cultured with 300 μg/ml dose of TLR3 agonists of the invention for 18 hr, and supernatants were collected and analyzed by Luminex multiplex assay for cytokine and chemokine levels. The data

demonstrate that administration of a TLR3 agonist of the invention generates a distinct, TLR3-mediated cytokine and chemokine profile in human immune cells.

[0039] Table 15 depicts serum cytokine induction in C57BL/6 mice (n=3) 2 hours after they were treated and analyzed according to Example 4. Briefly, the C57BL/6 mice were injected subcutaneously with 0 mg/kg or 10 mg/kg dose of TLR3 agonists, and 2 hours after administration of the agonist, serum was analyzed for immune stimulatory cytokine levels, and IL-12 levels are presented. The data demonstrate that *in vivo* administration of a TLR3 agonist of the invention generates a distinct TLR-mediated *in vivo* cytokine profile.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0040] The invention relates to TLR3 agonist compounds, compositions comprising such compounds, and their use for stimulating a TLR3-mediated immune response. The TLR3 agonists according to the invention are stable, specific, and capable of activating an innate immune response, thereby overcoming the problems of certain previously attempted approaches to create TLR3 agonists. Pharmaceutical and other compositions comprising the compounds according to the invention are also provided. Further provided are methods of stimulating a TLR3-mediated immune response in cells or tissues comprising contacting said cells or tissues with one or more of the TLR3 agonist compounds or compositions thereof alone or in combination with other prophylactic or therapeutic compounds or compositions.

[0041] The invention provides TLR3 agonist compounds that are designed to specifically and potently stimulate TLR3. These TLR3 agonists have unique structures that are preferentially bound by TLR3, resulting in optimal stimulation of a TLR3-mediated immune response.

[0042] The TLR3 agonists according to the invention stimulate an immune response in various *in vitro* and *in vivo* experimental models. As such, the TLR3 agonists or compositions thereof according to the invention are useful as tools to study the immune system, as well as to compare the immune systems of various animal species, such as humans and mice.

[0043] Further provided are methods of treating an animal, particularly a human, having, suspected of having, or being prone to develop a disease or condition that would benefit from TLR3-mediated immune stimulation by administering a therapeutically or prophylactically effective amount of one or more of the TLR3 agonist compounds or compositions of the invention. These can be used for immunotherapy applications such as, but not limited to, treatment of cancer, asthma, allergy, airway inflammation, inflammatory disorders, autoimmune disorders, skin disorders, diseases caused by a pathogen, and infectious diseases and as vaccine adjuvants in adult and pediatric human and veterinary applications.

[0044] In addition, TLR3 agonist oligonucleotides of the invention are useful in the prevention and/or treatment of various diseases, either alone, in combination with or co-administered with other drugs or prophylactic or therapeutic compositions, for example, DNA vaccines, antigens, antibodies, and allergens, TLR antagonist, such as TLR7 and or TLR8 antagonist, and/or other TLR agonists; and in combination with chemotherapeutic agents such as

both traditional chemotherapy and modern targeted therapies for prevention and treatment of diseases.

[0045] The patents and publications cited herein reflect the level of knowledge in the art and are hereby incorporated by reference in their entirety. Any conflict between the teachings of these patents and publications and this specification shall be resolved in favor of the latter.

[0046] The foregoing and other objects of the present invention, the various features thereof, as well as the invention itself may be more fully understood from the following description, when read together with the accompanying drawings in which:

[0047] The term "2'-O-substituted" means substitution of the 2' position of the pentose moiety with an -O- lower alkyl group containing 1-6 saturated or unsaturated carbon atoms (for example, but not limited to, 2'-O-methyl), or with an -O-aryl or allyl group having 2-6 carbon atoms, wherein such alkyl, aryl or allyl group may be unsubstituted or may be substituted, (for example, with 2'-O-ethoxy-methyl, halo, hydroxy, trifluoromethyl, cyano, nitro, acyl, acyloxy, alkoxy, carboxyl, carbalkoxyl, or amino groups); or with a hydroxy, an amino or a halo group, but not with a 2'-H group. In some embodiments the oligonucleotides of the invention include four or five ribonucleotides 2'-O-alkylated at their 5' terminus (i.e., 5' 2-O-alkylated ribonucleotides), and/or four or five ribonucleotides 2'-O-alkylated at their 3' terminus (i.e., 3' 2-O-alkylated ribonucleotides). In exemplar embodiments, the nucleotides of the synthetic oligonucleotides are linked by at least one phosphorothioate internucleotide linkage. The phosphorothioate linkages may be mixed Rp and Sp enantiomers, or they may be stereoregular or substantially stereoregular in either Rp or Sp form (see Iyer et al. (1995) Tetrahedron Asymmetry 6:1051-1054).

[0048] The term " 3' ", when used directionally, generally refers to a region or position in a polynucleotide or oligonucleotide 3' (toward the 3'end of the nucleotide) from another region or position in the same polynucleotide or oligonucleotide.

[0049] The term " 5' ", when used directionally, generally refers to a region or position in a polynucleotide or oligonucleotide 5' (toward the 5'end of the nucleotide) from another region or position in the same polynucleotide or oligonucleotide.

[0050] The term "about" generally means that the exact number is not critical. Thus, oligonucleotides having one or two fewer nucleoside residues, or from one to several additional nucleoside residues are contemplated as equivalents of each of the embodiments described above.

[0051] The term "agonist" generally refers to a substance that binds to a receptor of a cell and induces a response. An agonist often mimics the action of a naturally occurring substance such as a ligand.

[0052] The term "airway inflammation" generally includes, without limitation, inflammation in the respiratory tract caused by allergens, including asthma.

[0053] The term "allergen" generally refers to an antigen or antigenic portion of a molecule, usually a protein, which elicits an allergic response upon exposure to a subject. Typically the subject is allergic to the allergen as indicated, for instance, by the wheal and flare test or any method known in the art. A molecule is said to be an allergen even if only a small subset of subjects exhibit an allergic (e.g., IgE) immune response upon exposure to the molecule.

[0054] The term "allergy" generally includes, without limitation, food allergies, respiratory allergies and skin allergies.

[0055] The term "antigen" generally refers to a substance that is recognized and selectively bound by an antibody or by a T cell antigen receptor. Antigens may include but are not limited to peptides, proteins, nucleosides, nucleotides and combinations thereof. Antigens may be natural or synthetic and generally induce an immune response that is specific for that antigen.

[0056] The term "antagonist" generally refers to a substance that attenuates the effects of an agonist.

[0057] The term "cancer" generally refers to, without limitation, any malignant growth or tumor caused by abnormal or uncontrolled cell proliferation and/or division. Cancers may occur in humans and/or mammals and may arise in any and all tissues. Treating a patient having cancer may include administration of a compound, pharmaceutical formulation or vaccine according to the invention such that the abnormal or uncontrolled cell proliferation and/or division, or metastasis is affected.

[0058] The term "carrier" generally encompasses any excipient, diluent, filler, salt, buffer, stabilizer, solubilizer, oil, lipid, lipid containing vesicle, microspheres, liposomal encapsulation, or other material well known in the art for use in pharmaceutical formulations. It will be understood that the characteristics of the carrier, excipient, or diluent will depend on the route of administration for a particular application. The preparation of pharmaceutically acceptable formulations containing these materials is described in, for example, *Remington's Pharmaceutical Sciences*, 18th Edition, ed. A. Gennaro, Mack Publishing Co., Easton, PA, 1990.

[0059] The term "co-administration" or "co-administered" generally refers to the administration of at least two different substances sufficiently close in time to modulate an immune response. Co-administration refers to simultaneous administration, as well as temporally spaced order of up to several days apart, of at least two different substances in any order, either in a single dose or separate doses.

[0060] The term "in combination with" generally means administering a TLR3 agonist or composition thereof according to the invention and another agent useful for treating the disease or condition that does not abolish the activity of the TLR3 agonist or composition thereof in the course of treating a patient. Such administration may be done in any order, including simultaneous administration, as well as temporally spaced order from a few seconds up to several days apart. Such combination treatment may also include more than a single administration of the TLR3 agonist or composition thereof according to the invention and/or independently the other agent. The administration of the TLR3 agonist or composition thereof according to the invention and the other agent may be by the same or different routes.

[0061] The term "individual" or "subject" or "vertebrate" generally refers to a mammal, such as a human.

[0062] The term "linear synthesis" generally refers to a synthesis that starts at one end of an oligonucleotide and progresses linearly to the other end. Linear synthesis permits incorporation of either identical or non-identical (in terms of length, base composition and/or chemical modifications incorporated) monomeric units into an oligonucleotide.

[0063] The term "mammal" is expressly intended to include warm blooded, vertebrate animals, including, without limitation, humans, non-human primates, rats, mice, cats, dogs, horses, cattle, cows, pigs, sheep and rabbits.

[0064] The term “nucleoside” generally refers to compounds consisting of a sugar, usually ribose or deoxyribose, and a purine or pyrimidine base.

[0065] The term “nucleotide” generally refers to a nucleoside comprising a phosphorous-containing group attached to the sugar.

[0066] The term "modified nucleoside" generally is a nucleoside that includes a modified heterocyclic base, a modified sugar moiety, or any combination thereof. In some embodiments, the modified nucleoside is a non-natural pyrimidine or purine nucleoside, as herein described. For purposes of the invention, a modified nucleoside, a pyrimidine or purine analog or non-naturally occurring pyrimidine or purine can be used interchangeably and refers to a nucleoside that includes a non-naturally occurring base and/or non-naturally occurring sugar moiety. For purposes of the invention, a base is considered to be non-natural if it is not guanine, cytosine, adenine, thymine or uracil and a sugar is considered to be non-natural if it is not β-ribo-furanoside or 2’-deoxyribo-furanoside. For purposes of the invention, a “modified nucleotide” is a modified nucleoside comprising a phosphorous-containing group attached to the sugar.

[0067] The term "modified oligonucleotide" as used herein describes an oligonucleotide in which at least two of its nucleotides are covalently linked via a synthetic linkage, i.e., a linkage other than a phosphodiester linkage between the 5' end of one nucleotide and the 3' end of another nucleotide or the 5’ end of a nucleotide and the 2’ end of another nucleotide in which the 5' nucleotide phosphate has been replaced with any number of chemical groups. The term "modified oligonucleotide" also encompasses oligonucleotides having at least one modified nucleotide.

[0068] The term "nucleic acid" encompasses a genomic region or an RNA molecule transcribed therefrom. In some embodiments, the nucleic acid is mRNA.

[0069] The term “nucleotidic linkage” generally refers to a chemical linkage to join two nucleosides through their sugars (e.g. 3’-3’, 2’-3’, 2’-5’, 3’-5’) consisting of a phosphorous atom and a charged, or neutral group (e.g., phosphodiester, phosphorothioate, phosphorodithioate or methylphosphonate) between adjacent nucleosides.

[0070] For purposes of the invention, a "non-nucleotidic linker" is any moiety that can be linked to the oligonucleotides by way of covalent or non-covalent linkages. Preferably such

linker is from about 2 angstroms to about 200 angstroms in length. Several examples of preferred linkers are set forth below. Non-covalent linkages include, but are not limited to, electrostatic interaction, hydrophobic interactions, π-stacking interactions, and hydrogen bonding. The term "non-nucleotidic linker" is not meant to refer to an internucleoside linkage, as described above, e.g., a phosphodiester, phosphorothioate, or phosphorodithioate functional group, that directly connects the 3'-hydroxyl groups of two nucleosides.

[0071] The term “oligonucleotide” refers to a polynucleoside formed from a plurality of linked nucleoside units. The nucleoside units may be part of viruses, bacteria, cell debris or oligonucleotide-based compositions (for example, siRNA and microRNA). Such oligonucleotides can also be obtained from existing nucleic acid sources, including genomic or cDNA, but are preferably produced by synthetic methods. In certain embodiments each nucleoside unit includes a heterocyclic base and a pentofuranosyl, trehalose, arabinose, 2’-deoxy-2’-substituted nucleoside, 2’-deoxy-2’-substituted arabinose, 2’-O-substitutedarabinose or hexose sugar group. The nucleoside residues can be coupled to each other by any of the numerous known internucleoside linkages. Such internucleoside linkages include, without limitation, phosphodiester, phosphorothioate, phosphorodithioate, methylphosphonate, alkylphosphonate, alkylphosphonothioate, phosphotriester, phosphoramidate, siloxane, carbonate, carboalkoxy, acetamidate, carbamate, morpholino, borano, thioether, bridged phosphoramidate, bridged methylene phosphonate, bridged phosphorothioate, and sulfone internucleoside linkages. The term "oligonucleotide-based compound" also encompasses polynucleosides having one or more stereospecific internucleoside linkage (e.g., (R_P)- or (S_P)-phosphorothioate, alkylphosphonate, or phosphotriester linkages). As used herein, the terms "oligonucleotide" and "dinucleotide" are expressly intended to include polynucleosides and dinucleosides having any such internucleoside linkage, whether or not the linkage comprises a phosphate group. In certain exemplar embodiments, these internucleoside linkages may be phosphodiester, phosphorothioate or phosphorodithioate linkages, or combinations thereof.

[0072] The term "peptide" generally refers to polypeptides that are of sufficient length and composition to affect a biological response, for example, antibody production or cytokine activity whether or not the peptide is a hapten. The term "peptide" may include modified amino

acids (whether or not naturally or non-naturally occurring), where such modifications include, but are not limited to, phosphorylation, glycosylation, pegylation, lipidization and methylation.

[0073] The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of a compound according to the invention or the biological activity of a compound according to the invention.

[0074] The term "physiologically acceptable" refers to a non-toxic material that is compatible with a biological system such as a cell, cell culture, tissue, or organism. Preferably, the biological system is a living organism, such as a mammal, particularly a human.

[0075] The term "prophylactically effective amount" generally refers to an amount sufficient to prevent or reduce the development of an undesired biological effect.

[0076] The term "therapeutically effective amount" or "pharmaceutically effective amount" generally refers to an amount sufficient to affect a desired biological effect, such as a beneficial result, including, without limitation, prevention, diminution, amelioration or elimination of signs or symptoms of a disease or disorder. Thus, the total amount of each active component of the pharmaceutical composition or method is sufficient to show a meaningful patient benefit. Thus, a "pharmaceutically effective amount" will depend upon the context in which it is being administered. A pharmaceutically effective amount may be administered in one or more prophylactic or therapeutic administrations. When applied to an individual active ingredient, administered alone, the term refers to that ingredient alone. When applied to a combination, the term refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

[0077] The term "treatment" generally refers to an approach intended to obtain a beneficial or desired result, which may include alleviation of symptoms, or delaying or ameliorating a disease progression.

[0078] In a first aspect, the invention provides a synthetic TLR3 agonist comprising a first oligoribonucleotide having the structure: 5'-Domain A-Domain B-3' and a second oligoribonucleotide having the structure: 5'-Domain C-Domain D-3', wherein Domain A is a first complementary domain, Domain B is a polyriboininosine domain, Domain C is a second complementary domain and Domain D is a polyribocytidine domain, wherein Domain A and

Domain C are complementary to each other. The first oligoribonucleotide and the second oligoribonucleotide bind to each other through intermolecular hydrogen bonding between either the complementary domains leaving a free polyriboininosine domain and a free polyriboincytidine domain or between the polyriboininosine and polyribocytidine domains leaving a free first complementary domain and a free second complementary domain. Additional first and/or second oligoribonucleotides can bind to the free complementary and/or free polyriboinosine or polyribocytidine domains, thereby creating a chain of oligoribonucleotides.

[0079] The invention further provides a synthetic TLR3 agonist comprising a first oligoribonucleotide having the structure: 5'-Domain B-Domain A-3' and a second oligoribonucleotide having the structure: 5'-Domain D-Domain C-3', wherein Domain A is a first complementary domain, Domain B is a polyriboininosine domain, Domain C is a second complementary domain and Domain D is a polyribocytidine domain, wherein Domain A and Domain C are complementary to each other. The first oligoribonucleotide and the second oligoribonucleotide bind to each other through intermolecular hydrogen bonding between either the complementary domains leaving a free polyriboininosine domain and a free polyriboincytidine domain or between the polyriboininosine and polyribocytidine domains leaving a free first complementary domain and a free second complementary domain. Additional first and/or second oligoribonucleotides can bind to the free complementary and/or polyriboinosine or polyribocytidine domains, thereby creating a chain of oligoribonucleotides.

[0080] The invention further provides a synthetic TLR3 agonist comprising a first oligoribonucleotide having the structure: 5'-Domain A-3'-3'-Domain B-5' and a second oligoribonucleotide having the structure: 5'-Domain C-3'-3'-Domain D-5', wherein Domains A and B and Domains C and D are covalently linked via a direct nucleotide to nucleotide linkage at their 3' ends through the 3' positions of the sugars or through a modified sugar or modified nucleobase, wherein Domain A is a first complementary domain, Domain B is a polyriboininosine domain, Domain C is a second complementary domain and Domain D is a polyribocytidine domain, wherein Domain A and Domain C are complementary to each other. The first oligoribonucleotide and the second oligoribonucleotide bind to each other through intermolecular hydrogen bonding between either the complementary domains leaving a free polyriboininosine domain and a free polyriboincytidine domain or between the polyriboininosine and polyribocytidine domains leaving a free first complementary domain and a free second

complementary domain. Additional first and/or second oligoribonucleotides can bind to the free complementary and/or polyriboinosine or polyribocytidine domains, thereby creating a chain of oligoribonucleotides.

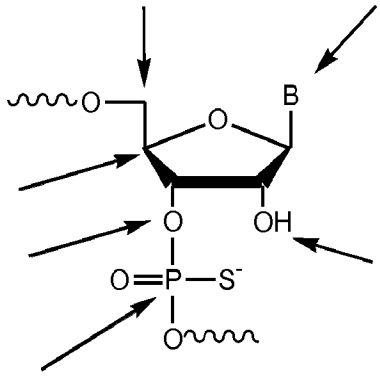
[0081] In some embodiments, the TLR3 agonist comprises at least two first oligoribonucleotides having the structure: 5'-Domain A-Domain B-3' covalently linked via a direct nucleotide to nucleotide linkage at their 3' ends through the 3' positions of the sugars or through a modified sugar or modified nucleobase or via a non-nucleotide linker at their 3' ends through the 3' positions of the sugars or through a modified sugar or modified nucleobase and a second oligoribonucleotide having the structure: 5'-Domain C-Domain D-3', wherein Domain A is a first complementary domain, Domain B is a polyriboininosine domain, Domain C is a second complementary domain and Domain D is a polyribocytidine domain, wherein Domain A and Domain C are complementary to each other. In a further embodiment, the at least two first oligoribonucleotides can have the structure 5'-Domain B-Domain A-3'and the second oligoribonucleotide can have the structure 5'-Domain D-Domain C-3'.

[0082] In some embodiments, the TLR3 agonist comprises a first oligoribonucleotide having the structure: 5'-Domain A-Domain B-3' and at least two second oligoribonucleotides having the structure: 5'-Domain C-Domain D-3'covalently linked via a direct nucleotide to nucleotide linkage at their 3' ends through the 3' positions of the sugars or through a modified sugar or modified nucleobase or via a non-nucleotide linker at their 3' ends through the 3' positions of the sugars or through a modified sugar or modified nucleobase, wherein Domain A is a first complementary domain, Domain B is a polyriboininosine domain, Domain C is a second complementary domain and Domain D is a polyribocytidine domain, wherein Domain A and Domain C are complementary to each other. In a further embodiment, the first oligoribonucleotide can have the structure 5'-Domain B-Domain A-3'and the at least two second oligoribonucleotides can have the structure 5'-Domain D-Domain C-3'.

[0083] As a non-limiting example, the linker may be attached to the 3'-hydroxyl. In such embodiments, the linker comprises a functional group, which is attached to the 3'-hydroxyl by means of a phosphate-based linkage like, for example, phosphodiester, phosphorothioate, phosphorodithioate, methylphosphonate, or by non-phosphate-based linkages. Possible sites of

conjugation for the ribonucleotide are indicated in Structure A, below, wherein B represents a heterocyclic base and wherein the arrow pointing to P indicates any attachment to phosphorous.

Structure A



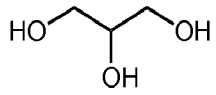
[0084] In some embodiments, the non-nucleotide linker is a small molecule, macromolecule or biomolecule, including, without limitation, polypeptides, antibodies, lipids, antigens, allergens, and oligosaccharides. In some other embodiments, the non-nucleotidic linker is a small molecule. For purposes of the invention, a small molecule is an organic moiety having a molecular weight of less than 1,000 Da. In some embodiments, the small molecule has a molecular weight of less than 750 Da.

[0085] In some embodiments, the small molecule is an aliphatic or aromatic hydrocarbon, either of which optionally can include, either in the linear chain connecting the oligoribonucleotides or appended to it, one or more functional groups including, but not limited to, hydroxy, amino, thiol, thioether, ether, amide, thioamide, ester, urea, or thiourea. The small molecule can be cyclic or acyclic. Examples of small molecule linkers include, but are not limited to, amino acids, carbohydrates, cyclodextrins, adamantane, cholesterol, haptens and antibiotics. However, for purposes of describing the non-nucleotidic linker, the term "small molecule" is not intended to include a nucleoside.

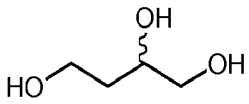
[0086] In some embodiments, the non-nucleotidic linker is an alkyl linker or amino linker. The alkyl linker may be branched or unbranched, cyclic or acyclic, substituted or unsubstituted, saturated or unsaturated, chiral, achiral or racemic mixture. The alkyl linkers can have from about 2 to about 18 carbon atoms. In some embodiments such alkyl linkers have from about 3 to about 9 carbon atoms. Some alkyl linkers include one or more functional groups including, but not limited to, hydroxy, amino, thiol, thioether, ether, amide, thioamide, ester, urea, and thioether. Such alkyl linkers can include, but are not limited to, 1,2 propanediol, 1,2,3

propanetriol, 1,3 propanediol, triethylene glycol hexaethylene glycol, polyethylene glycollinkers (e.g. [-O-CH2-CH2-]$_n$ (n= 1-9)),methyl linkers, ethyl linkers, propyl linkers, butyl linkers, or hexyl linkers. In some embodiments, such alkyl linkers may include peptides or amino acids.

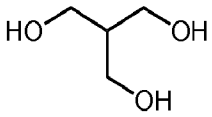
[0087] In some embodiments, the non-nucleotide linker may include, but is not limited to, the following:



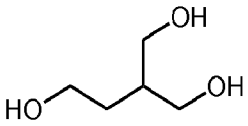
Glycerol (1,2,3-Propanetriol)



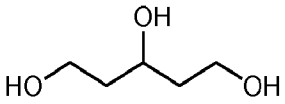
1,2,4-Butanetriol



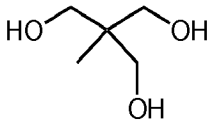
2-(hydroxymethyl)-1,3-propanediol



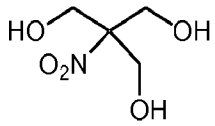
2-(hydroxymethyl)1,4-butanediol



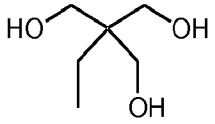
1,3,5-Pentanetriol



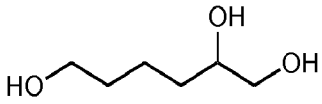
1,1,1-Tris(hydroxymethyl)ethane



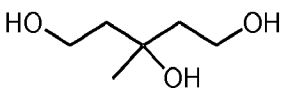
1,1,1-Tris(hydroxymethyl)nitromethane



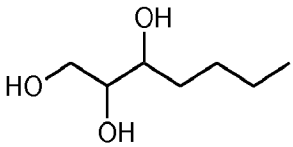
1,1,1-Tris(hydroxymethyl)propane



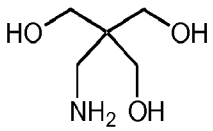
1,2,6-Hexanetriol



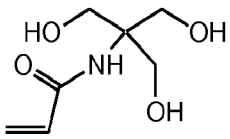
3-Methyl-1,3,5-pentanetriol



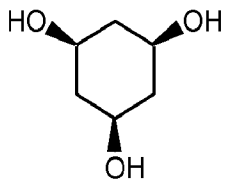
1,2,3-Heptanetriol



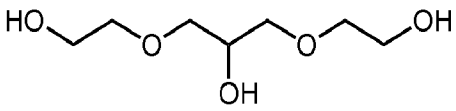
2-Amino-2-(hydroxymethyl)-1,3-propanediol



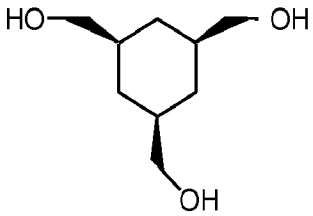
N-[Tris(hydroxymethyl)methyl]acrylamide



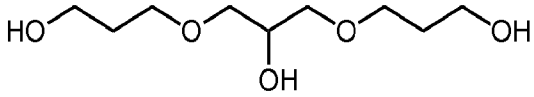
cis-1,3,5-Cyclohexanetriol



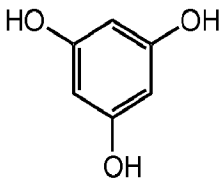
1,3-Di(hydroxyethoxy)-2-hydroxyl-propane



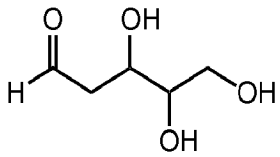
cis-1,3,5-Tri(hydroxymethyl)cyclohexane



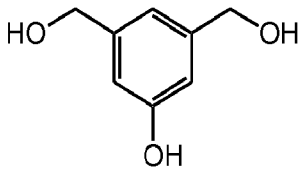
1,3-Di(hydroxypropoxy)-2-hydroxyl-propane



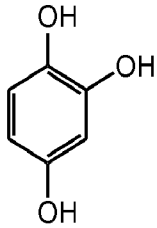
1,3,5,-Trihydroxyl-benzene



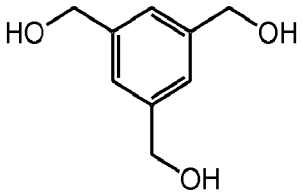
2-Deoxy-D-ribose



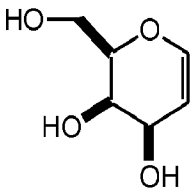
3,5,-Di(hydroxymethyl)phenol



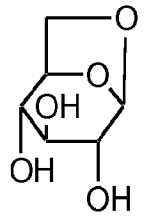
1,2,4,-Trihydroxyl-benzene



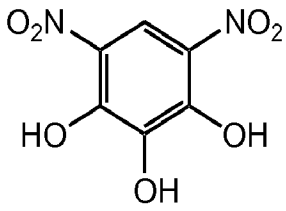
1,3,5,-Tri(hydroxymethyl)benzene



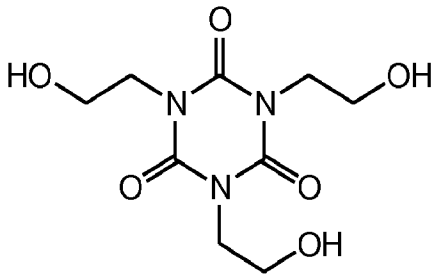
D-Galactoal



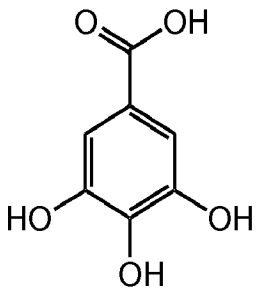
1,6-anhydro-β-D-Glucose



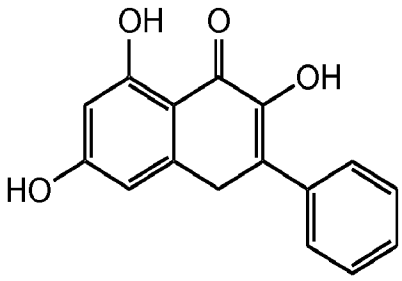
4,6-Nitropyrogallol



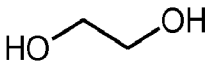
1,3,5-Tris(2-hydroxyethyl)-Cyanuric acid



Gallic acid

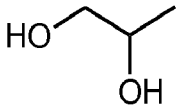


3,5,7-Trihydroxyflavone

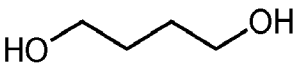


Ethylene glycol

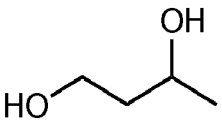
1,3-Propanediol



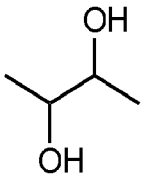
1,2-Propanediol



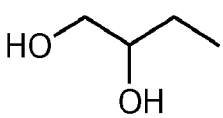
1,4-Butanediol



1,3-Butanediol

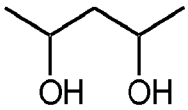


2,3-Butanediol

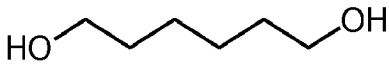


1,4-Butanediol

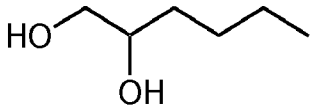
1,5-Pentanediol



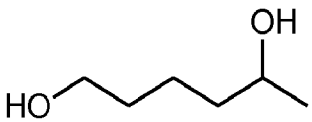
2,4-Pentanediol



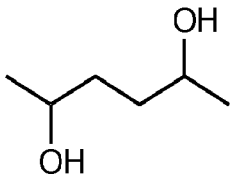
1,6-Hexanediol



1,2-Hexanediol

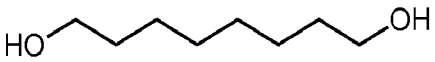


1,5-Hexanediol

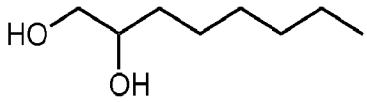


2,5-Hexanediol

1,7-Heptanediol

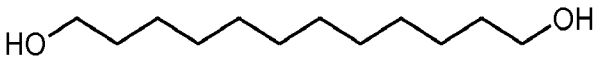


1,8-Octanediol

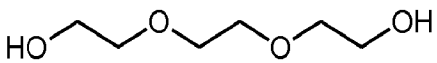


1,2-Octanediol

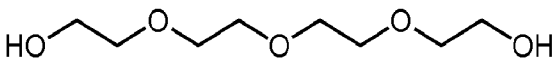
1,9-Nanediol



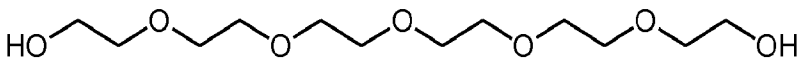
1,12-Dodecanediol



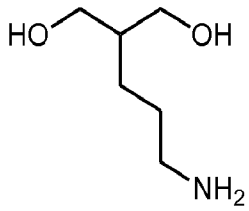
Triethylene glycol



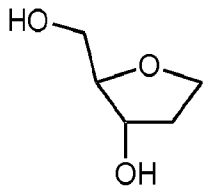
Tetraethylene glycol



Hexaethylene glycol



2-(1-Aminopropyl)-1,3-propanediol



1,2-Dideoxyribose

[0088] In some embodiments, the small molecule linker is glycerol or a glycerol homolog of the formula HO-$(CH_2)_o$-CH(OH)-$(CH_2)_p$-OH, wherein *o* and *p* independently are integers from 1 to about 6, from 1 to about 4, or from 1 to about 3. In some other embodiments, the small molecule linker is a derivative of 1,3-diamino-2-hydroxypropane. Some such derivatives have the formula HO-$(CH_2)_m$-C(O)NH-CH_2-CH(OH)-CH_2-NHC(O)-$(CH_2)_m$-OH, wherein *m* is an integer from 0 to about 10, from 0 to about 6, from 2 to about 6, or from 2 to about 4.

[0089] Some non-nucleotide linkers according to the invention permit attachment of more than two oligoribonucleotides. For example, the small molecule linker glycerol has three hydroxyl groups to which oligoribonucleotides may be covalently attached. Some TLR3 agonist according to the invention, therefore, comprise two or more oligoribonucleotides linked to a nucleotide or a non-nucleotide linker. Such TLR3 agonist are referred to as being "branched".

[0090] Without wishing to be bound to any particular theory, the formation of a chain of first and second oligoribonucleotides of the invention results in a hybrid poly(I:C) that is a specific agonist of TLR3. Specifically, the hybrid poly(I:C) TLR3 agonist of the invention can exists as long strands of nucleic acid but that have reduced ability to form undesirable helix-with-loop structures and that do not have toxic properties or the lack of efficacy when administered *in vivo*.

[0091] As used herein, the term "complementary" means having the ability to hybridize to a nucleic acid. Such hybridization is ordinarily the result of hydrogen bonding between complementary strands, preferably to form Watson-Crick or Hoogsteen base pairs. Intermolecular hydrogen bonding results in the formation of a double-stranded nucleic acid molecule.

[0092] In embodiments of this aspect of the invention, the first complementary domain, as used herein, refers to a domain having a base sequence which, upon suitable alignment with the second complementary domain, may form intermolecular basepairing between G-C, A-T, A-U and/or G-U wobble pairs. Thus, where a plurality of first and second oligoribonucleotides are used together, the complementary domains of the plurality of first oligoribonucleotides and the complementary domains of the plurality of second oligoribonucleotides are capable of hybridizing together through intermolecular hydrogen bonding under high stringency conditions. For example, in some embodiments, the degree of complementarity is at least 93 percent, at least 95 percent, at least 98 percent, or even 100 percent. In preferred embodiments, the degree of complementarity is 100%. Additionally, where a plurality of first and second oligoribonucleotides are used together, the polyriboinosine domains of the plurality of first oligoribonucleotides and the polyribocytidine domains of the plurality of second oligoribonucleotides are capable of hybridizing together.

[0093] "Stringency conditions" for hybridizations is a term of art referring to the conditions (e.g., temperature and buffer concentration) that permit hybridization of a particular nucleic acid

to another nucleic acid in which the first nucleic acid may be perfectly complementary to the second, or the first and second may share some degree of complementarity that is less than perfect. "High stringency conditions" and "moderate stringency conditions" for nucleic acid hybridizations are explained on pages 2.10.1-2.10.16 (see particularly 2.10.8-11) and pages 6.3.1-6 in Current Protocols in Molecular Biology (Ausubel, F. M. et al., eds., Vol. 1, containing supplements up through Supplement 29, 1995), the teachings of which are hereby incorporated by reference. Hybridization requires that the two nucleic acids contain substantially complementary sequences; depending on the stringency of hybridization, however, mismatches may be tolerated. The appropriate stringency for hybridizing nucleic acids depends on the length of the nucleic acids and the degree of complementarily, variables well known in the art.

[0094] In some embodiments, although the first and second oligoribonucleotides are the same number of nucleotides in length, the complementary domains do not necessarily have the same number of nucleotides as the polyriboinosine and polyribocytidine domains. The only requirement is that the first complementary domain and the second complementary domain are the same length and that the polyriboinosine and polyribocytidine domains are the same length. For example, the first and second complementary domains are from about 10 to about 20 nucleotides in length and the polyriboinosine and polyribocytidine domains are from about 30 to about 40 nucleotides in length. In certain embodiments the first and second complementary domains are from about 15 to about 20 nucleotides in length and the polyriboinosine and polyribocytidine domains are from about 30 to about 35 nucleotides in length. In some embodiments, the first and second complementary domains are 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 nucleotides in length and the polyriboinosine and polyribocytidine domains are 30, 31, 32, 33, 34, 35, 36, 37, 38, 39 or 40 nucleotides in length. In some embodiments, the first and second complementary domains are 20 nucleotides in length and the polyriboinosine and polyribocytidine domains are 30 nucleotides in length. In some embodiments, the first and second complementary domains are 15 nucleotides in length and the polyriboinosine and polyribocytidine domains are 35 nucleotides in length. One skilled in the art would understand that the different Domains of the first and second oligoribonucleotides may be shorter or longer as long as the compound retains it TLR3 stimulatory activity without introducing the undesired helix-with-loop structures and toxic properties.

[0095] In embodiments of this aspect of the invention, the first and second oligoribonucleotides can have the following exemplary structures:

Formula I 5'-□-polyI-3'

Formula II 5'-□-polyC-3'

Formula III 5'-polyI-□-3'

Formula IV 5'-polyC-□-3'

Formula V 5'-□-3'-3'-polyI-5'

Formula VI 5-□-3'-3'-polyC-5'

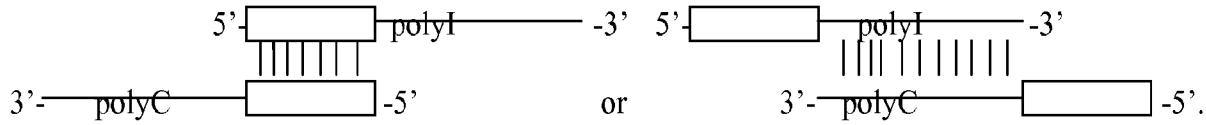
Formula VII 5'-□-3'-X-3'-polyI-5'

Formula VIII 5-□-3'-X-3'-polyC-5'

wherein the □ represents the complementary domains .

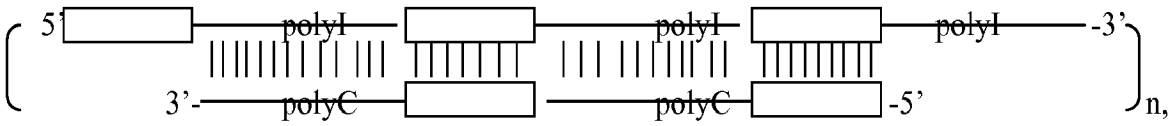
As would be recognized by one skilled in the art, the complementary sequences of the first and second complementary domains and/or the complementary nature of the polyriboinosine and polyribocytidine domains allows for intermolecular hydrogen bonding between the first and second oligoribonucleotides which can have the following exemplary double stranded structures:

Formula IX (e.g., Formulas I and II)



Additional first and second oligoribonucleotides can bind together thereby creating a chain of oligoribonucleotides according to the invention which can have the following exemplary structure:

Formula X (e.g., chain of Formulas I and II)



wherein n is any number.

[0096] As would be recognized by one skilled in the art, double strand structures and/or chains of first and second oligoribonucleotides can also be prepared with Formulas III and IV, Formulas V and VI and Formulas VII and VIII.

[0097] In some embodiments, the TLR3 agonist according to the invention can comprise one or more force binding sites. A force binding site is achieved by the substitution of one or more guanosine(s) for inosine in the polyriboiniosine domain. Such a force binding site can improve the alignment of the polyriboiniosine and polyribocytidine domains and/or increase the strength of the bond between the polyriboiniosine and polyribocytidine domains.

[0098] In some embodiments of this aspect of the invention, certain hydrogen atoms in the first and/or second oligoribonucleotide are replaced by a deuterium atom through hydrogen deuterium exchange (also called H-D or H/D exchange). By replacing a hydrogen atom with a deuterium atom, the stability of the TLR3 agonist is improved. Additionally, such an exchange increases the TLR3 agonists resistance to oxidation and/or degradation.

[0099] In other embodiments, the TLR3 agonist can comprise a 5' and/or 3' cap, wherein the 5' and/or 3' end of the TLR3 agonist is attached to another molecule (e.g. a non-nucleotidic linker) or to itself such that the 5' and/or 3' end is not accessible exonuclease degradation or for hybridization to another TLR3 agonist of the invention. Such capping acts to further stabilize the TLR3 agonist and/or to regulate the number of first and second oligoribonucleotides that can bind together and, thereby, allows for a TLR3 agonist having a particular size or length.

[00100] In further embodiments, the TLR3 agonist according to the first aspect of the invention can comprise one or more deuterium atom exchanges. Such deuterium exchanges would be recognized to provide increased resistance to nuclease degradation and/or to increase the stability of hybridization between the first and second oligoribonucleotides and/or to enhance the stability of binding by TLR3. Additionally, such deuterated molecules may comprise a 5' and/or 3' cap.

[00101] In further embodiments, the invention provides a composition comprising one or more of the TLR3 agonists according to the invention and any other therapeutic or prophylactic agent including, but not limited to, one or more vaccines, antigens, antibodies, cytotoxic agents, allergens, antibiotics, antisense oligonucleotides, TLR agonist, TLR antagonist, siRNA, miRNA, peptides, proteins, gene therapy vectors, DNA vaccines, adjuvants or kinase inhibitors to

enhance the specificity or magnitude of the immune response, or co-stimulatory molecules such as cytokines, chemokines, protein ligands, trans-activating factors, peptides and peptides comprising modified amino acids.

[00102] In a second aspect, the invention provides a composition comprising a TLR3 agonist according to the first aspect of the invention and a physiologically acceptable carrier.

[00103] In certain embodiments, the TLR3 agonist is included in the pharmaceutically acceptable carrier in an amount sufficient to deliver to a mammal a pharmaceutically effective amount without causing serious toxic effects. The effective dosage range of the pharmaceutically acceptable derivatives can be calculated based on the weight of the parent compound to be delivered, or by other means known to those skilled in the art.

[00104] In further embodiments, the composition comprising one or more of the TLR3 agonists according to the invention and a physiologically acceptable carrier, further comprises any other therapeutic or prophylactic agent including, but not limited to, one or more vaccines, antigens, antibodies, cytotoxic agents, allergens, antibiotics, antisense oligonucleotides, TLR agonist, TLR antagonist, for example TLR7 and/or TLR8 antagonist, siRNA, miRNA, peptides, proteins, gene therapy vectors, DNA vaccines, adjuvants or kinase inhibitors to enhance the specificity or magnitude of the immune response, or co-stimulatory molecules such as cytokines, chemokines, protein ligands, trans-activating factors, peptides and peptides comprising modified amino acids. In a preferred embodiment, the composition comprising one or more of the TLR3 agonists according to the invention and a physiologically acceptable carrier, further comprises one or more antigens.

[00105] In a third aspect, the invention provides a method for generating a TLR3-mediated immune response in mammals. In this method, a TLR3 agonist according to the first or second aspect of the invention is contacted with or bound by TLR3 *in vitro*, *in vivo*, *ex vivo* or in a cell. For purposes of this invention, the term "mammal" is expressly intended to include humans and animals. In preferred embodiments, the compound, composition or vaccine is administered to a vertebrate in need of immune stimulation.

[00106] In a further embodiment, the invention provides a vaccine. Vaccines according to this aspect comprise a composition according to the invention, and further comprise an antigen. An antigen is a molecule that elicits a specific immune response. Such antigens include, without

limitation, proteins, peptides, nucleic acids, carbohydrates and complexes or combinations of any of the same. Antigens may be natural or synthetic and generally induce an immune response that is specific for that antigen. Any such antigen may optionally be linked to an immunogenic protein, such as keyhole limpet hemocyanin (KLH), cholera toxin B subunit, or any other immunogenic carrier protein.

[00107] Vaccines according to the invention may further include any of the plethora of known adjuvants, including, without limitation, Freund's complete adjuvant, KLH, monophosphoryl lipid A (MPL), alum, Merck alum adjuvant (MAA) and saponins, including QS-21, imiquimod, R848, or combinations thereof.

[00108] In a fourth aspect, the invention provides a method for stimulating TLR3 activity in a mammal such method comprising administering to the mammal a TLR3 agonist according to the first or second aspect of the invention. In some embodiments the mammal is a human. In preferred embodiments, the TLR3 agonist according to the first or second aspect of the invention is administered to a mammal in need of immune stimulation.

[00109] In a fifth aspect, the invention provides a method for stimulating TLR3-mediated immune response in a mammal, such method comprising administering to the mammal a TLR3 agonist according to the first or second aspect of the invention. In some embodiments the mammal is a human. In preferred embodiments, the TLR3 agonist according to the first or second aspect of the invention is administered to a mammal in need of immune stimulation.

[00110] In a sixth aspect, the invention provides a method for treating a mammal having a disease or disorder treatable by TLR3 activation or TLR3-mediated immune stimulation, such method comprising administering to the mammal a TLR3 agonist according to the first or second aspect of the invention in a pharmaceutically effective amount. In some embodiments the mammal is a human. The invention also relates to the TLR3 agonist and compositions thereof, which are disclosed herein in methods of treating diseases and illnesses, for use in treating diseases and illnesses and for use as vaccine adjuvants.

[00111] In a seventh aspect, the invention provides methods for preventing a disease or disorder or for use as vaccine adjuvants in a mammal, particularly a human, at risk of contracting or developing a disease or disorder preventable by TLR3 activation or TLR3-mediated stimulation of an immune response. The method according to this aspect comprises

administering to the mammal a prophylactically effective amount of a TLR3 agonist according to the first or second aspect of the invention.

[00112] In an eighth aspect, the TLR3 agonists and compositions thereof according to the invention are also useful for examining the function of the TLR3 gene in a cell or in a control mammal or in a mammal afflicted with a disease associated with TLR3 or immune stimulation through TLR3. In embodiments of this aspect, the cell or mammal is administered the TLR3 agonist according to the first or second aspects of the invention, and the activity of TLR3 is examined.

[00113] A non-limiting list of TLR3 agonists according to the invention are shown in Table 2 below. In Table 2, the oligonucleotide-based TLR3 agonist compounds have all phosphodiester (PO) linkages, except where indicated as a phosphorothioate (PS) linkage. Those skilled in the art will recognize, however, that a mixture of PS and PO linkages can be used. A list of inactive, control oligonucleotides are shown as compounds nos. 8, 20-24, 64-67, 119-121, 124 and 125 in Table 2 below. In Table 2, the inactive, control oligonucleotides have all phosphodiester (PO) linkages, except where indicated as a phosphorothioate (PS) linkage.

Table 2.

SEQ ID NO.	Compound No. Length		Sequence	Length
1	25	a	5'-GCAGUUGACACCCCCCCCCCCCCCCCCCCCCCCCCCCC-3'	40
2		b	3'-IIIIIIIIIIIIIIIIIIIIIIIIIIICGUCAACUGU-5'	40
3	27	a	5'-CACUGGCAGUUGACACCCCCCCCCCCCCCCCCCCCCCCCCCC CCCCCCCCC-3'	50
4		b	3'-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCAACUGU-5'	50
5	28	a	5'-CACUGGCAGUUGACACAGGUCCCCCCCCCCCCCCCCCCCCCC CCCCCCCC-3'	50
6		b	3'-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCAACUGUGUCCA-5'	50
7	29	a	5'-CACUGGCAGUUGACACCCCCCCCCCCCCCCCCCCCCCCCCCC CCCCCCCC-3'	50
8		b	5'-UGUCAACUGCCAGUGIIIIIIIIIGIIIIIIIIIGIIIIIIIIIGIIII-3'	50
9	30	a	5'-**CACUGGCAGUUGACA**CCCCCCCCCCCCCCCCCCCCCCCCCC CCCCCCCCCC-3'	50
10		b	3'-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCAACUGU-5'	50
11	31	a	5'-**CACUGGCAGUUGACA**CCCCCCCCCCCCCCCCCCCCCCCCCC CCCCCCCCCC-3'	50
12		b	3'-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCAACUGU-5'	50

13 14 	32	a b	5'-CACUGGCAGUUGACACCCCCCCCCCCCCCCCCCCCCCCCC CCCCCCCCCC-X-CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC CCCCACAGUUGACGGUCAC-5' 3'-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCAACUGU-5'	100 50
15 16 	33	a b	5'- CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCACUGG CAGUUGACA-X-ACAGUUGACGGUCACCCCCCCCCCCCCCCCCCCC CCCCCCCCCCCCCCCCCCC-5' 3'-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCAACUGU-5'	100 50
17 18	34	a b	5'-CACUGGCAGUUGACACCCCCCCCCCCCCCCCCCCCCCCCCCC CCCCCCCC-3' 5'-UGUCAACUGCCAGUGIIIIGIIIIIGIIIIGIIIIGIIIIGIIIIGIIII-3'	50 50
19 20	35	a b	5'-CACUGGCAGUUGACACCCCCCCCCCCCCCCCCCCCCCCCCCC CCCCCCCC-3' 5'-UGUCAACUGCCAGUGIIGIIGIIIIGIIGIIGIIIGIIGIIGIIIGIIII-3'	50 50
21 22	1	a b	5'-CCUCCAGCCUUACAGCCAAGUAUGAGAGCU-3' 3'-GGAGGUCGGAAUGUCGGUUCAUACUCUCGA-5'	30 30
23 24	2	a b	5'-GGGAGACAGGCCUGUUCCAUGGCCAACACGUUUGUCUCCC-3' 3'-CCCUCUGUCCGGACAAGGUACCGGUUGUGCAAACAGAGGG-5'	40 40
25 26	3	a b	5'-CUGAACAUCUGCGGACGGACCUAGAUACGGAACCUUUGUU-3' 3'-GACUUGUAGACGCCUGCCUGGAUCUAUGCCUUGGAAACAA-5'	40 40
27 28	4	a b	5'-ACAUCUGCGGACGGACCUAGAUACGGAACCUUUGUUGUUG-3' 3'-UGUAGACGCCUGCCUGGAUCUAUGCCUUGGAAACAACAAC-5'	40 40
29 30	5	a b	5'-**XCCCCCCCCCCCCCCCCCCCCCCX**-3' 3'-**XIIIIIIIIIIIIIIIIIIIIIIX**-5'	24 24
31 32	6	a b	5'-CCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3' 3'-IIIIIIIIIIIIIIIIIIIIIIIIIIIIII-5'	30 30
33 34	7	a b	5'-CCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3' 3'-IIIIIIIIIIIIIIIIIIIIIIIIIIIIII-5'	30 30
35 36	8	a b	5'-CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC CCC-3' 3'-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII-5'	45 45
37 38	9	a b	5'-CCUCCAGCCUUACAGCCAAGUAUGAYYYYYCCUCCAGCCU UACAGCCAAGUAUGA-3' 3'-GGAGGUCGGAAUGUCGGUUCAUACU-5'	55 25
39 40	10	a b	5'-CCUCCAGCCUUACAGCCAAGUAUGAYYYYYYYYYYCCUCCA GCCUUACAGCCAAGUAUGA-3' 3'-GGAGGUCGGAAUGUCGGUUCAUACU-5'	60 25
41 42	11	a b	5'-CCUCCAGCCUUACAGCCAAGUAUGAYYYYYYYYYYYYYYYC CUCCAGCCUUACAGCCAAGUAUGA-3' 3'-GGAGGUCGGAAUGUCGGUUCAUACU-5'	65 25
43 44	12	a b	5'-GGGAGACAAACGUGUUGGCCAUGGAACAGGCCUGUCUCCC -X-CCCUCUGUCCGGACAAGGUACCGGUUGUGCAAACAGAGGG-5' 3'-CCCUCUGUUUGCACAACCGGUACCUUGUCCGGACAGAGGG-5'	81 40
45 46	13	a b	3'-CCCUCUGUCCGGACAAGGUACCGGUUGUGCAAACAGAGGG-X- GGGAGACAAACGUGUUGGCCAUGGAACAGGCCUGUCUCCC-3' 5'-GGGAGACAGGCCUGUUCCAUGGCCAACACGUUUGUCUCCC-3'	81 40
47	14		5'-**CCCIIICCCII-X-IICCCIIICCC**-5'	23
48	15		5'-**CCIICCIICCC-X-CCCIICCIICC**-5'	23
49	16		5'-**CCIICCIICCLCCIICCIICC**-3'	23
50 51	17	a b	5'-**XCCCCCCCCCCC-X-CCCCCCCCCCCX**-3' 5'-**XIIIIIIIIIIIIIIIIIIIIIIX**-3'	25 24

52	18		5'-**IIIIIIIIIIII-X-CCCCCCCCCCCCC**-5'	27
53	19		5'-**ICICICICICICICI-X-ICICICICICICICI**-5'	31
54	20	a	5'-CCCACACCC-3'	9
55		b	3'-IIIIIIUGU-5'	9
56	21	a	5'-CCCCCCACACCCCCC-3'	15
57		b	3'-IIIIIIIIIIIUGU-5'	15
58	22	a	5'-IIIIIIIIIIIIIC$_3$GUGC-3'	20
59		b	3'-GC$_3$AC$_3$GCCCCCCCCCCCCCCC-5'	20
60	23	a	5'-CCCCCCCCCCACACCCCCCCCC-3'	23
61		b	3'-IIIIIIIIIIIIIIIIIUGU-5'	23
62	24	a	5'-GACACCCCCCCCCCCCCCCCCCCCCCCCCC-3'	34
63		b	3'-IIIIIIIIIIIIIIIIIIIIIIIIIICUGU-5'	34
64	26	a	5'-CACUGGCAGUUGACACAGGUUCCUCACUUCACAAAUCGUUC CCCCCCCCC-3'	50
65		b	3'-IIIIIIIIIGUGACCGUCAACUGUGUCCAAGGAGUGAAGUGUUU AGCAA-5'	50
66	36	a	5'-CACUGGCAGUUGACACAGGUUCCUCACUUCACAAAUCGUUCA UCGCCCCC-3'	50
67		b	3'-IIIIGUGACCGUCAACUGUGUCCAAGGAGUGAAGUGUUUAGCA AGUAGC-5'	50
68	37	a	5'-CACUGGCAGUUGACACAGGUUCCUCACUUCCCCCCCCCCCCCC CCCCCCC-3'	50
69		b	3'-IIIIIIIIIIIIIIIIIGUGACCGUCAACUGUGUCCAAGGAGUGAAG-5'	50
70	38	a	5'-CACUGCUCAUUCACACCCCCCCCCCCCCCCCCCCCCCCCCCCC CCCCCC-3'	50
71		b	3'-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACGAGUAAGUGU-5'	50
72	39	a	5'-GUCACAGUCAAGUUCCCCCCCCCCCCCCCCCCCCCCCCCCCCC CCCCCC-3'	50
73		b	3'-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIICAGUGUCAGUUCAAG-5'	50
74	40	a	5'-CGUGAACUGACACUGCCCCCCCCCCCCCCCCCCCCCCCCCCCCC CCCCCC-3'	50
75		b	3'-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGCACUUGACUGUGAC-5'	50
76	41	a	5'- CACUGGCAGUUGACACAGGUCCCCCCCCCCCCCCCCCCCCCCCC CCCCCCCCCCCCCCCCC-3'	60
77		b	3'-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCAACUGUGUCCA-5'	60
78	42	a	5'-CACUGGCAGUUGACACCCCCCCCCCCCCCCCCCCCCCCCCCCCC CCCCCCCCCCCCCCCCC-3'	60
79		b	3'-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCAACUGU-5'	60
80	43	a	5'-CACUGGCAGUUGACACCCCCCCCCCCCCCCCCCCCCCCCCCCCC CCCCCCC-3'	50
81		b	3'-IIIIGIIIIGIIIIGIIIIGIIIIGIIIIGIIIIIGUGACCGUCAACUGU-5'	50
82	44	a	5'-CACUGGCAGUUGACACCCCCCCCCCCCCCCCCCCCCCCCCCCCC CCCCCCC-3'	50
83		b	3'-IIGIIGIIGIIGIIGIIGIIGIIGIIGIIGIIGIIGUGACCGUCAACUGU-5'	50
84	45	a	5'-CACUGGCAGUUGACACCCCUCCCCCCCCCCUCCCCCCCCCCUCCC CCCCCCC-3'	50
85		b	3'-IIIAIIIIIIIIAIIIIIIIIAIIIIIIIIIGUGACCGUCAACUGU-5'	50
86	46	a	5'-CACUGGCAGUUGACACCCCCCCCCCCCCCCCCCCCCCCCCCCCC CCCCCCC-3'	50
87		b	3'-IIIIG$_1$IIIIIIIIG$_1$IIIIIIIIG$_1$IIIIIIIIIGUGACCGUCAACUGU-5'	50

88	47	a	5’-CACUGGCAGUUGACACCCCC$_1$CCCCCCCCCC$_1$CCCCCCCCCC$_1$CCCCCCCC-3’	50
89		b	3’-IIIIGIIIIIIIIIGIIIIIIIIIGIIIIIIIIIGUGACCGUCAACUGU-5’	50
90	48	a	5’-CACUGGCAGUUGACACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3’	50
91		b	3’-IIIIG$_2$IIIIIIIIIG$_2$IIIIIIIIIG$_2$IIIIIIIIIGUGACCGUCAACUGU-5’	50
92	49	a	5’-CACUGGCAGUUGACACCCCC$_1$CCCCCCCCCC$_1$CCCCCCCCCC$_1$CCCCCCCC-3’	50
93		b	3’-IIIIG$_2$IIIIIIIIIG$_2$IIIIIIIIIG$_2$IIIIIIIIIGUGACCGUCAACUGU-5’	50
94	50	a	5’-CACUGGCAGUUGACACCCCC$_2$CCCCCCCCCC$_2$CCCCCCCCCC$_2$CCCCCCCC-3’	50
95		b	3’-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCAACUGU-5’	50
96	51	a	5’-<u>CACUGGCAGUUGACA</u>CCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3’	50
97		b	3’-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCAACUGU-5’	50
98	52	a	5’-<u>CACUGGCAGUUGACACCCCC</u>CCCCCCCCCCCCCCCCCCCCCCCCCC-3’	50
99		b	3’-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCAACUGU-5’	50
100	53	a	5’-CACUGGCAGUUGACA<u>CCCCCCCCCC</u>CCCCCCCCCCCCCCCCCCCCCC-3’	50
101		b	3’-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCAACUGU-5’	50
102	54	a	5’-CACUGGCAGUUGACA<u>CCCCCCCCCCCCCCCCCCCC</u>CCCCCCCCCC-3’	50
103		b	3’-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCAACUGU-5’	50
104	55	a	5’-CACUGGCAGUUGACA<u>CCCCCCCCCCCCCCCCCCCCCCCCCCCCCC</u>-3’	50
105		b	3’-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCAACUGU-5’	50
106	56	a	5’-CACUGGCAGUUGACACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3’	50
107		b	3’-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCAACUGU-5’	50
108	57	a	5’-<u>CACUGGCAGUUGACA</u>CCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3’	50
109		b	3’-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCAACUGU-5’	50
110	58	a	5’-**CACUGGCAGUUGACACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC**-3’	50
111		b	3’-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCAACUGU-5’	50
112	59	a	5’-CACUGGCAGUUGACACCCCUCCCCCCCCCCUCCCCCCCCCCUCCCCCCC-3’	50
113		b	3’-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCAACUGU-5’	50
114	60	a	5’-CACUGGCAGUUGACACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3’	50
115		b	3’-IIIAIIIIIIIIAIIIIIIIIAIIIIIIIIIGUGACCGUCAACUGU-5’	50
116	61	a	5’-CACUGGCAGUUGACA-3’-3’-CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-5’	50
117		b	5’-UGUCAACUGCCAGUG-3’-3’-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIII-5’	50
118	62	a	5’-CACUGGCAGUUGACA-3’-3’-CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-5’	50
119		b	3’-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCAACUGU-5’	50
120	63	a	5’-CACUGGCAGUUGACACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3’	50
121		b	5’-UGUCAACUGCCAGUG-3’-3’-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIII-5’	50
122	64	a	5’-CACUGGCAGUUGACA -3’	15
123		b	3’-GUGACCGUCAACUGU-5’	15

124	65	a	5’-CACUGGCAGUUGACACACUGGCAGUUGACACACUGGCAGUUGACA -3’	45
125		b	3’-GUGACCGUCAACUGUGUGACCGUCAACUGUGUGACCGUCAACUGU-5’	45
126	66	a	5’-CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCACUGGCAGUUGACA -3’	50
127		b	3’-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCAACUGU-5’	50
128	67	a	5’-UGUCAACUGCCAGUGCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC -3’	50
129		b	3’-CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCACAGUUGACGGUCAC-5’	50
130	68	a	5’-CACUGGCAGUUGACAIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII-3’	50
131		b	3’-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCAACUGU-5’	50
132	69	a	5’-CAAGGCAAGCAUUCGCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3’	50
133		b	3’-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIII GUUCCGUUCGUAAGC-5’	50
134	70	a	5’-GCUACUGUUCGUCGUCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3’	50
135		b	5‘-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIII CGAUGACAAGCAGCA-3‘	50
136	71	a	5’-GAAGUCAGUAGUCUCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3’	50
137		b	3’- IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIICUUCAGUCAUCAGAG-5‘	50
138	72	a	5’-CACUGAGACUGAUGCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3’	50
139		b	3’- IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACUCUGACUACG-5‘	50
140	73	a	5’-UACAGCAGUCAGUCUCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3’	50
141		b	3’-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIAUGUCGUCAGUCAGA-5‘	50
142	74	a	5’-CGAUGACUGACUACGCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3’	50
143		b	5‘-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGCUACUGACUGAUGC-3‘	50
144	75	a	5’-CCCCGGCCGCCGCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3’	50
145		b	5’-IGICIICIGCCIGIGIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII-3’	50
146	76	a	5’-GCCCCGCCCCGCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3’	50
147		b	5’-IIGICGIGICGIGICIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII-3’	50
148	77	a	5’-CACUGCUCAUUCACACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3’	50
149		b	3’- IIIIIIIIG$_1$IIIIIIIIG$_1$IIIIIIIIG$_1$IIIIGUGACGAGUAAGUGU-5’	50
150	78	a.	5’-UACAGCAGUCAGUCUCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3’	50
151		b.	5’-IIIIIG$_1$IIIIIIIIG$_1$IIIIIIIIG$_1$IIIIIIIIAUGUCGUCAGUCAGA-3’	50
152	79	a.	5’-CGAUGACUGACUACGCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3’	50
153		b.	5’-IIIIIIIG$_1$IIIIIIIG$_1$IIIIIIIG$_1$IIIIIIIGCU ACUGACUGAUGC-3’	50
154	80	a.	5’-CACUGAGACUGAUGCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3’	50
155		b.	5’-IIIIIG$_1$IIIIIIIIG$_1$IIIIIIIIG$_1$IIIIIIIIGUGACUCUGACUACG-3’	50
156	81	a.	5’-CAAGGCAAGCAUUCGCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3’	50
157		b.	5’-IIIIIIIG$_1$IIIIIIIG$_1$IIIIIIIG$_1$IIIIIIIGUUCCGUUCGUAAGC-3’	50
158	82	a	5’-CACUGCUCAUUCACACCCCCCCCCC$_3$CCCCCCCCCC$_3$CCCCCCCCC$_3$CCCCC-3’	50
159		b	3’-IIIIIIIIGIIIIIIIIGIIIIIIIIGIIIIGUGACGAGUAAGUGU-5’	50

160	83	a	5'-UACAGCAGUCAGUCUCCCCCC$_3$CCCCCCCCCC$_3$CCCCCCCCCC$_3$CCCCCCCC-3'	50
161		b	3'-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIAUGUCGUCAGUCAGA-5'	50
162	84	a	5'-CGAUGACUGACUACGCCCCCCCCCC$_3$CCCCCCCCC$_3$CCCCCCCCC$_3$CCCCCCCC-3'	50
163		b	5'-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGCUACUGACUGAUGC-3'	50
164	85	a	5'-CACUGAGACUGAUGCCCCCCC$_3$CCCCCCCCCC$_3$CCCCCCCCCC$_3$CCCCCCCC-3'	50
165		b	3'-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACUCUGACUACG-5'	50
166	86	a	5'-CAAGGCAAGCAUUCGCCCCCCCCCC$_3$CCCCCCCCC$_3$CCCCCCCCC$_3$CCCCCCCC-3'	50
167		b	3'-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUUCCGUUCGUAAGC-5'	50
168	87	a	5'-CACUGGCAGUUGACACCCCCC$_3$CCCCCC$_3$CCCCCC$_3$CCCCCC$_3$CCCCCC$_3$CCCCC-3'	50
169		b	3'-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII GUGACCGUCAACUGU-5'	50
170	88	a	5'-CACUGGCAGUUGACACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3'	50
171		b	3'-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII GUGACCGUCAACUGU-5'	50
172	89	a	5'-CACUGGCAGUUGACACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3'	50
173		b	3'-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII GUGACCGUCAACUGU-5'	50
174	90	a	5'-GUCCUCAGCGAUAGCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3'	50
175		b	3'- IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII CAGGAGUCGCUAUCG-5'	50
176	91	a	5'-CAUCGCUCCUCUCCACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3'	50
177		b	5'-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII GUAGCGAGGAGAGGU-3'	50
178	92	a	5'-CUCUACCGUUCGCUCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3'	50
179		b	5'-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGAGAUGGCAAGCGAG-3'	50
180	93	a	5'-CACUGGCAGUUGACA-HEG-CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3'	50
181		b	5'- IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII-HEG-GUGACCGUCAACUGU-3'	50
182	94	a	5'-CACUGGCAGUUGACA-HEG-CCCCCCCCCC-HEG-CCCCCCCCCC-HEG-CCCCCCCCCCCCCCCC-3'	50
183		b	5'-IIIIIIIII-HEG-IIIIIIIII-HEG-IIIIIIIIIIIIIIIIGUGACCGUCAACUGU-3'	50
184	95	a	5'-CACUGGCAGUUGACACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3'	50
185		b	3'-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCAACUGU-5'	50
186	96	a	5'-CACUGGCAGUUGACACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3'	50
187		b	3'-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCAACUGU-5'	50
188	97	a	5'-CACUGGCAGUUGACACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3'	50
189		b	3'-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCAACUGU-5'	50
190	98	a	5'-CACUGGCAGUUGACACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3'	50
191		b	3'-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCAACUGU-5'	50
192	99	a	5'-CACUGGCAGUUGACACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3'	50
193		b	3'-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCAACUGU-5'	50
194	100	a	5'-CACUGGCAGUUGACACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3'	50
195		b	3'-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCAACUGU-5'	50

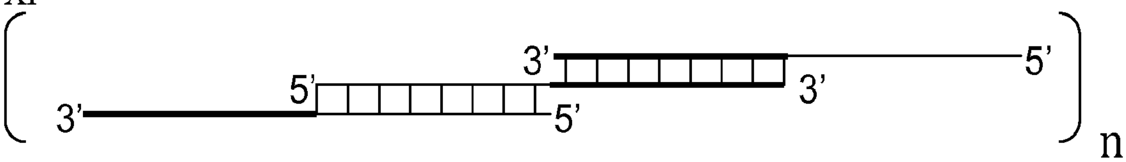
196	101	a	5’-CACUGGCAGUUGACACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3’	50
197		b	3’-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCAACUGU-5’	50
198	102	a	5’-CACUGGCAGUUGACACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3’	50
199		b	3’-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCAACUGU-5’	50
200	103	a	5’-CACUGGCAGUUGACACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3’	50
201		b	3’-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCAACUGU-5’	50
202	104	a	5’-CACUGGCAGUUGACACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3’	50
203		b	3’-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCAACUGU-5’	50
204	105	a	5’-CACUGGCAGUUGACACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3’	50
205		b	3’-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCAACUGU-5’	50
206	106	a	5’-**CAC**UGGCAGUUGACACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3’	50
207		b	3’-**III**IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCAAC**UGU**-5’	50
208	107	a	5’-**CAC**UGGCAGUUGACACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3’	50
209		b	3’-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCAACUGU-5’	50
210	108	a	5’-CACUGGCAGUUGACACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3’	50
211		b	3’-**III**IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCAAC**UGU**-5’	50
212	109	a	5’-**CACU**GGCAGUUGACACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3’	50
213		b	3’-**IIII**IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCAA**CUGU**-5’	50
214	110	a	5’-**CACU**GGCAGUUGACACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3’	50
215		b	3’-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCAACUGU-5’	50
216	111	a	5’-CACUGGCAGUUGACACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3’	50
217		b	3’-**IIII**IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCAA**CUGU**-5’	50
218	112	a	5’-CACUGGCAGUUGACACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3’	50
219		b	3’-**IIIII**IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCAACUGU-5’	50
220	113	a	5’-CACUGGCAGUUGACACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3’	50
221		b	3’-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCAACUGU-5’	50
222	114	a	5’-CACUGGCAGUUGACACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3’	50
223		b	3’-**IIIII**IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCAACUGU-5’	50
224	115	a	5’-CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCACUGGC AGUUGACA -3’	50
225		b	5’-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIUGUCAACUGCCAGUG-3’	50
226	116	a	3’-CCCCCCCCCCCCCCCCCCCCACAGUUGACGGUCACCCCCCCCCCCC CCCCCCC-5’	50
227		b	3’-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCAACUGU-5’	50
228	117	a	5’-CACUGGCAGUUGACAUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUU UUUUUUUUU-3’	50
229		b	3’-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCAACUGU-5’	50
230	118	a	5’-CACUGGCAGUUGACAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAA-3’	50
231		b	3’-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCAACUGU-5’	50

232	119	a	5'-ACACCCCCCC-3'	10
233		b	3'-IIIIIIIUGU-5'	10
234	120	a	5'-ACACCCCCCCCCCCCCCCCC-3'	20
235		b	3'-IIIIIIIIIIIIIIIIIUGU-5'	20
236	121	a.	5'-CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3'	50
237		b.	3'-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII-5'	50
238	122	a	5'-CACUGCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3'	50
239		b	3'-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGAC-5'	50
240	123	a	5'-CACUGGCAGUCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3'	50
241		b	3'-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCA-5'	50
242	124	a	5'-CACUGGCAGUUGACACAGGUUCCUCACUUCACAAAUCGUUCAUCGUUCAC-3'	50
243		b	3'-GUGACCGUCAACUGUGUCCAAGGAGUGAAGUGUUUAGCAAGUAGCAAGUG-5'	50
244	125	a	5'-CAAUGGCACUUAACACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3'	50
245		b	3'-IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGUGACCGUCAACUGU-5'	50

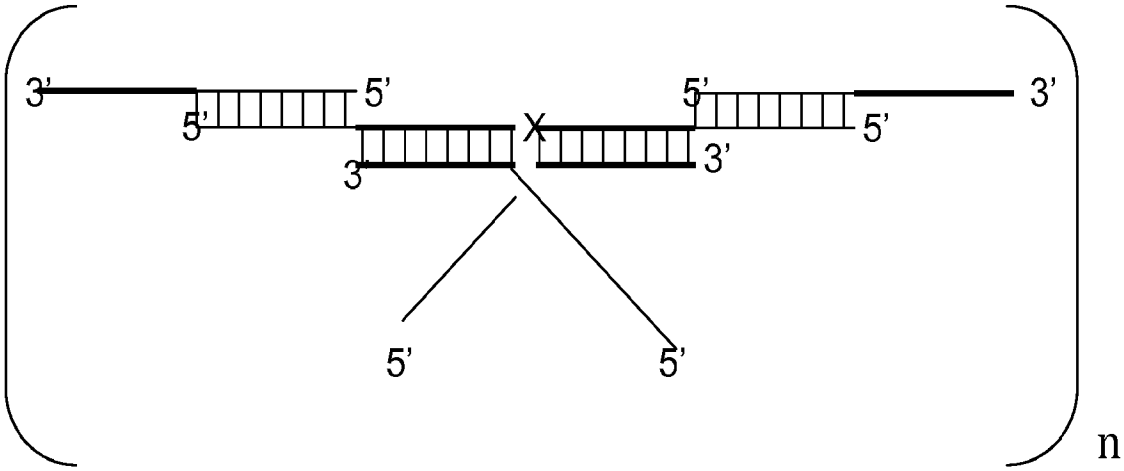
I = inosine; X = glycerol; Y =1,3-propanediol; C_1 = ara-C; C_2 = 5-Me-C; C_3 = 5-methyl-cytidine; G_1 = 7-deaza-G; G_2 = ara-G; **bold** = phosphorothioate linkage; underscore = 2'-methoxy-nucleoside ; HEG = hexaethylene glycol.

[00114] Additional structures that may be formed by the TLR3 agonists of the invention include Formulas XI, XII and XIII.

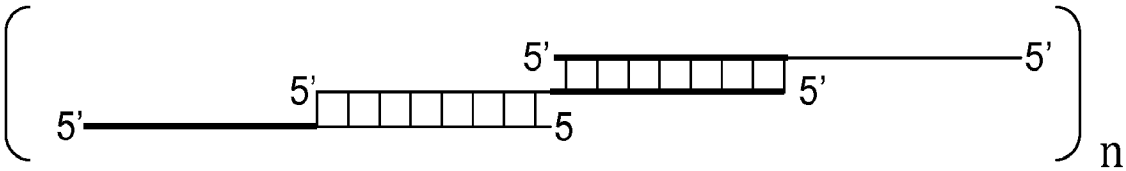
Formula XI



Formula
XII



Formula
XIII



[00115] In any of the methods according to the invention, a therapeutically or prophylactically effective amount of a TLR3 agonist of the invention and effective in stimulating TLR3 activity is administered to a cell. This cell may be part of a cell culture, a neovascularized tissue culture, or may be part or the whole body of a mammal such as a human or other mammal. Administration of the therapeutic compositions of TLR3 agonist can be carried out using known procedures at dosages and for periods of time effective to reduce symptoms or surrogate markers of the disease, depending on the condition and response, as determined by those with skill in the art. It may be desirable to administer simultaneously, or sequentially a therapeutically effective amount of one or more of the therapeutic TLR3 agonists of the invention to an individual as a single treatment episode. In some exemplary embodiments of the methods of the invention described above, the TLR3 agonist is administered locally and/or systemically. The term "administered locally" refers to delivery to a defined area or region of the body, while the term “systemic administration” is meant to encompass delivery to the whole organism.

[00116] In any of the methods according to the invention, one or more of the TLR3 agonists or composition thereof can be administered alone or in combination with any other agent useful

for treating the disease or condition that does not diminish the immunostimulatory effect of the TLR3 agonists. In any of the methods according to the invention, the agent useful for treating the disease or condition includes, but is not limited to, one or more vaccines, antigens, antibodies, cytotoxic agents, allergens, antibiotics, antisense oligonucleotides, TLR agonist, TLR antagonist, siRNA, miRNA, peptides, proteins, gene therapy vectors, DNA vaccines, adjuvants or kinase inhibitors to enhance the specificity or magnitude of the immune response, or co-stimulatory molecules such as cytokines, chemokines, protein ligands, trans-activating factors, peptides and peptides comprising modified amino acids. For example, in the treatment of cancer, it is contemplated that the TLR3 agonist or composition thereof according to the invention may be administered in combination with one or more targeted therapeutic agents and/or monoclonal antibodies. Alternatively, the agent can include DNA vectors encoding for antigen or allergen. In these embodiments, the TLR3 agonist of the invention can produce direct immunostimulatory effects. When co-administered with one or more other therapies, the TLR3 agonist of the invention may be administered either simultaneously with the other treatment(s), or sequentially.

[00117] In the various methods according to the invention the route of administration may be by any suitable route including, without limitation, parenteral, mucosal delivery, oral, sublingual, transdermal, topical, inhalation, intranasal, aerosol, intraocular, intratracheal, intrarectal, vaginal, by gene gun, dermal patch or in eye drop or mouthwash form.

[00118] When a therapeutically effective amount of TLR3 agonist of the invention is administered orally, the TLR3 agonist will be in the form of a tablet, capsule, powder, solution or elixir. When administered in tablet form, the pharmaceutical composition of the invention may additionally contain a solid carrier such as a gelatin or an adjuvant. The tablet, capsule, and powder contain from about 5 to 95% synthetic oligonucleotide and preferably from about 25 to 90% synthetic oligonucleotide. When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, sesame oil, or synthetic oils may be added. The liquid form of the pharmaceutical composition may further contain physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol. When administered in liquid form, the pharmaceutical composition contains from about 0.5 to 90% by weight of the synthetic oligonucleotide or from about 1 to 50% synthetic oligonucleotide.

[00119] When a therapeutically effective amount of TLR3 agonist of the invention is administered by parenteral, mucosal delivery, oral, sublingual, transdermal, topical, inhalation, intranasal, aerosol, intraocular, intratracheal, intrarectal, vaginal, by gene gun, dermal patch or in eye drop or mouthwash form, the TLR3 agonist will be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A pharmaceutical composition for parenteral, mucosal delivery, oral, sublingual, transdermal, topical, inhalation, intranasal, aerosol, intraocular, intratracheal, intrarectal, vaginal, by gene gun, dermal patch or in eye drop or mouthwash form should contain, in addition to the TLR3 agonist, an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection or other vehicle as known in the art. The pharmaceutical composition of the present invention may also contain stabilizers, preservatives, buffers, antioxidants or other additives known to those of skill in the art.

[00120] When administered parenteral, mucosal delivery, oral, sublingual, transdermal, topical, inhalation, intranasal, aerosol, intraocular, intratracheal, intrarectal, vaginal, by gene gun, dermal patch or in eye drop or mouthwash form, doses ranging from 0.01% to 10% (weight/volume) may be used. When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, sesame oil or synthetic oils may be added. Topical administration may be by liposome or transdermal time-release patch.

[00121] The amount of TLR3 agonist in the pharmaceutical composition of the present invention will depend upon the nature and severity of the condition being treated, and on the nature of prior treatments that the patent has undergone. It is contemplated that the various pharmaceutical compositions used to practice the method of the present invention should contain about 10 micrograms to about 20 mg of synthetic oligonucleotide per kg body or organ weight.

[00122] The duration of intravenous therapy using the pharmaceutical composition of the present invention will vary, depending on the severity of the disease being treated and the condition and potential idiosyncratic response of each individual patient.

[00123] Some diseases lend themselves to acute treatment while others require longer-term therapy. Both acute and long-term intervention in diseases are worthy goals. Injections of TLR3

agonists can be an effective means of inhibiting certain diseases in an acute situation. However for long-term therapy over a period of weeks, months or years, systemic delivery (intraperitoneal, intramuscular, subcutaneous, intravenous) either with carriers such as saline, slow release polymers or liposomes are likely to be considered.

[00124] In some chronic diseases, systemic administration of TLR3 agonists of the invention may be preferable. The frequency of injections is from continuous infusion to once a month, several times per month or less frequently will be determined based on the disease process and the biological half-life of the TLR3 agonist.

[00125] The TLR3 agonists and methods of the invention are also useful for examining the function of TLR3 in a cell or in a control mammal or in a mammal afflicted with a disease associated with TLR3 or immune stimulation through TLR3. In such use, the cell or mammal is administered the TLR3 agonists, and the activity of TLR3 is examined.

[00126] Without being limited to any theory or mechanism, it is generally believed that the activity of TLR3 agonists according to the invention depends on the binding of the TLR3 agonist to TLR3, thus stimulating the activity of TLR3. Such stimulation under physiological conditions is measured as a practical matter by observing the down-stream activity of TLR3. Thus, an exemplary TLR3 agonist used in accordance with the invention is capable of forming a stable bond with TLR3; activating TLR3 and initiating a cascade of activity through various signaling molecules.

[00127] The following examples illustrate the exemplary modes of making and practicing the present invention, but are not meant to limit the scope of the invention since alternative methods may be utilized to obtain similar results.

Example 1

Synthesis of TLR3-Agonists

[00128] The immune modulatory oligoribonucleotides were chemically synthesized using phosphoramidite chemistry on automated DNA/RNA synthesizer. TAC protected (Except U) 2'-O-TBDMS RNA monomers, A, G, C and U, were purchased from Sigma-Aldrich. 7-deaza-G, inosine, and loxoribine monomers were purchased from ChemGenes Corporation. 0.25M 5-ethylthio-1H-tetrazole, PAC- anhydride Cap A and Cap B were purchased from Glen Research.

3% trichloroacetic acid (TCA) in dichloromethane (DCM) and 5% 3H-1,2-Benzodithiole-3-one-1,1-dioxide (Beaucage reagent) were made in house.

[00129] Immune modulatory oligoribonucleotides were synthesized at 1-2 μM scale using a standard RNA synthesis protocol.

Cleavage and base deprotection

[00130] Immune modulatory oligoribonucleotides were cleaved from solid support and the solution was further heated at 65 °C to removing protecting groups of exo cyclic-amines. The resulting solution was dried completely in a SpeedVac.

IE HPLC Purification

[00131] Immune modulatory oligoribonucleotides were purified by ion exchange HPLC.

Column: Dionex DNAPac 100 column (22X250)
Column Heater: ChromTech TL-105 HPLC column heater, temperature is set to 80 °C.
Buffer A: 20 mM Tris-HCl, pH 7.0, 20% acetinitrile
Buffer B: 3.0 M NaCl, 20 mM Tris-HCl, pH 7.0, 20% acetonitrile
Flow rate: 10ml/min
Gradient:

0-2 min: 0% B
2-11 min: 0% B to 35% B
11-41 min: 35% B to 90% B
41-45 min: 100% B

[00132] Crude immune modulatory oligoribonucleotide solution was injected into HPLC. Above gradient is performed and the fractions were collected. All fractions containing more than 90% desired product were mixed, and then the solution was concentrated to almost dry by RotoVap. RNAse-free water was added to make final volume of 10ml.

C-18 Reversed Phase Desalting

[00133] CC-18 Sep-Pak cartridge purchased from Waters was first conditioned with 10ml of acetonitrile followed by 10 ml of 0.5 M sodium acetate. 10 ml of immune modulatory oligoribonucleotide solution was loaded. 15 ml of water was then used to wash out the salt. The

immune modulatory oligoribonucleotide was finally eluted out by 1 ml of 50% acetonitrile in water.

[00134] The solution is placed in SpeedVac for 30 minutes. The remaining solution was filter through a 0.2 micro filter and then was lyophilized to dryness. The solid was then re-dissolved in water to make the desired concentration. The final solution was stored below 0 °C.

Capillary Electrophoresis

Instrument: Beckman 5010

Capillary: 62cm ssDNA capillary

Sample preparation: 0.2 OD of SIMRA compound was dissolved in 200ul of RNAse-free water.

Injection: electro-kinetic injection at 5KV for 5 seconds.

Running condition: 14KV for 50 minutes at 30 °C.

Ion Exchange HPLC analysis

Column: Dionex DNAPac guard column (22X250)

Column Heater: ChromTech TL-105 HPLC column heater, temperature is set to 80 °C.

Buffer A: 100 mM Tris-HCl, pH 8.0, 20% acetinitrile

Buffer B: 2.0 M LiCl, 100 mM Tris-HCl, pH 8.0, 20% acetonitrile

Flow rate: 2ml/min

Gradient:

0-2 min: 0% B

2-10 min: 0% B to 100% B

10-15 min: 100% B

PAGE analysis

[00135] 0.3 OD of immune modulatory oligoribonucleotide was loaded on 20% polyacrylamide gel and was running at constant power of 4 watts for approximately 5 hours. The gel was viewed under short wavelength UV light.

Example 2:

HEK293 cell cultures:

[00136] HEK293 cells stably expressing human TLR3 and pNifty-2 plasmid containing the SEAP reporter gene were purchased from Invivogen. Cells were maintained in Dulbecco's

modified Eagle's medium with 10% fetal bovine serum (FBS) and 10 μg/ml blasticidin and 100U/ml penicillin and streptomycin.

[00137] For transient transfection assay, cells were trypsinized and plated overnight in DMEM with FBS (no antibiotics) in 48 well plates. Next day, aliquots of 25 μl of the plasmid DNA/lipofectamine2000 mixture containing 100 ng of plasmid DNA and 1 μl of lipofectamine were added to each well of the cell culture plate. TLR3 agonist compounds were added to the cultures, and the cultures were continued for 18 h. At the end of the treatment, 20 μl of culture supernatant was taken from each treatment and used for SEAP assay following manufacturer's protocol (Invivogen).

SEAP Assay:

[00138] SEAP activity was quantified using the Quanti Blue Detection substrate (Invivogen) according to the manufacturer's instructions. To 20 μl of culture supernatant in a 96 well plate, 150 μl of SEAP Detection substrate was added. The samples were assayed in duplicate. The plates were incubated at 37 ^{o}C for 30-40 minutes and read at 620-655nm. The results are expressed as % maximal (agonist) NF-κB activity.

J774 Cell Assay:

[00139] Murine J774 macrophage cells (BIM-67, ATCC) were maintained in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) FBS and antibiotics (100U/ml of penicillin and streptomycin). For the experiments, cells were plated at a density of 7 x10^5 cells/ml in 48-well plates and allowed to attach overnight. Next day the cells were treated with agonist for 18h and then supernatants were collected for measurement of cytokine production by ELISA (IL-6, IL-12, IFNβ), according to manufacturer's instructions (BD Biosciences, PBL respectively).

Human PBMC and Myeloid DC cultures:

[00140] Peripheral blood mononuclear cells (PBMCs) from freshly drawn healthy volunteer blood (Research Blood Components, Brighton, MA) were isolated by Ficoll density gradient centrifugation method (Ficoll-Paque PLUS, GE Health Care).

[00141] Human CD1c (BDCA-1)$^+$ myeloid dendritic cells were isolated from PBMCs by two magnetic separation steps involving depletion of CD19$^+$ B cells and positive selection of CD1c (BDCA-1)$^+$ cells (Miltenyi Biotec, Auburn, CA) according to the manufacturer's instructions.

[00142] The culture medium used for the assay consisted of RPMI 1640 medium supplemented with 1.5 mM glutamine, 1 mM sodium pyruvate, 0.1 mM non-essential amino acids, 50 μM 2-mercaptoethanol, 100 IU/ml penicillin-streptomycin mix and 10% heat-inactivated fetal bovine serum (Hyclone).

Cytokine measurements:

[00143] PBMCs (5 X 10^6 cells/ml) and mDCS (1 X 10^6 cells/ml) were cultured in 96 well flat bottom plates then stimulated with agonist for a period of 24h. Unstimulated cells served as controls.

[00144] At the end of the incubation period supernatants were harvested and stored frozen until the time of assay by Luminex multiplex technology. A 25-plex human cytokine bead kit (Invitrogen) was used according to the manufacturer's instructions. Results from cells treated according to Example 2 are shown in Figures 2A, 2B, 4, 5, 6, 7, 10 or 11, and Tables 3, 4, 5A, 5B, 5C, 5D, 6, 7, 8, 9 10, 11, 12 or 13.

Table 3.

Compound #	Cytokine/Chemokine, pg/ml (+/- SD)				
	IL-1Ra	IL-8	MIP-1β	IP-10	MCP-1
27	667 (220)	1034 (31)	20 (4)	595 (133)	200 (4)
29	269 (46)	113 (7)	5 (6)	466 (81)	39 (7)
30	617 (14)	106 (2)	15 (2)	713 (126)	83 (6)
31	131 (40)	265 (17)	17 (2)	39 (7)	17 (1)
PBS	40 (16)	127 (24)	6 (4)	11 (4)	50 (65)

At 250 μg/ml concentration of compounds.

Table 4.

Compound #	Cytokine/Chemokine, pg/ml (+/-SD)						
	IL-1Ra	IL-6	IL-8	MIP-1α	MIP-1β	IP-10	MCP-1
27	1057 (38)	257 (6)	21907 (526)	299 (8)	955 (7)	2936 (79)	7929 (698)
29	1249 (40)	113 (1)	9618 (35)	129 (4)	521 (0)	4568 (80)	6548 (138)
30	1058 (113)	157 (12)	12007 (388)	123 (9)	489 (14)	2438 (185)	3538 (210)
31	544 (45)	115 (5)	17607 (575)	48 (3)	224 (5)	326 (15)	1189 (38)
PBS	288 (0)	42 (1)	2875 (52)	0 (0)	79 (2)	41 (0)	68 (0)

At 250 μg/ml concentration of compounds.

Table 5A.

Compound	Fold increase in NF-κB activity
Medium	1.0
1	0.8
3	1.3
4	1.0
5	0.9
6	1.0
12	1.7
13	1.0
24	1.0

Concentration of compounds was 250 μg/mL. Data shown are representative of two independent experiments.

Table 5B.

Compound	Fold increase in NF-κB activity
Medium	1.0
8	1.0
14	1.2
18	1.2
20	1.2
21	1.2
22	1.1
23	1.2

Concentration of compounds was 50 μg/mL. Data shown are representative of two independent experiments.

Table 5C.

Compound	Fold increase in NF-κB activity
Medium	1.0
9	1.6
10	1.5
11	1.8

Concentration of compounds was 100 μg/mL. Data shown are representative of two independent experiments.

Table 5D.

Compound	Fold increase in NF-κB activity
M	1.0
16	0.6

Concentration of compounds was 150 μg/mL. Data shown are representative of two independent experiments.

Table 6.

Compound #	Fold increase in NF-κB activity
Medium	1.0
36	0.93
37	1.33
38	5.50
41	3.64
42	5.20

Concentration of compounds was 250 μg/mL. Data shown are representative of two independent experiments.

Table 7.

Compound #	IL-6 (pg/ml)	IL-12 (pg/ml)	IP-10 (pg/ml)	IFN-β (pg/ml)
Medium	0	51.3	0	1.2
36	0	97.3	0	0
37	1004.7	313.1	16583	76.8
38	1114.1	223.5	20361	94.3
41	1271.3	352.9	17900	101.2
42	1359.5	315.4	19493	121.5

Concentration of compounds was 250 μg/mL. Data shown are representative of two independent experiments.

Table 8.

Compound #	IL-6 (pg/ml)	IL-12 (pg/ml)	IP-10 (pg/ml)	IFN-β (pg/ml)
Medium	0	85.1	0	0
43	175.8	287.2	18724	36.5
44	137.0	145.8	13923	0
45	3313.4	3236.6	19175	1416.2
46	5672.4	8599.6	18398	446.5
47	9.44	114.2	9281.5	0
48	2	110.5	8187.5	0
49	11.9	118.8	157.3	0
50	135.4	153.9	14871	68.8

Concentration of compounds was 250 μg/mL. Data shown are representative of two independent experiments.

Table 9.

Compound#	IL-6 (pg/ml)	IL-12 (pg/ml)	IP-10 (pg/ml)	IFN-β (pg/ml)
Medium	11.2	117.2	54.7	0
51	2432.7	1319.4	32923	533.5
52	1296.6	113.3	676833	7.9
53	32.0	133.8	12764	0
54	11.9	114.3	563.4	0
55	11.9	99.0	206.0	0
56	4177.5	1838.0	80034	1037.3
57	27.4	129.0	5424.3	0

Concentration of compounds was 250 μg/mL. Data shown are representative of two independent experiments.

Table 10.

Compound#	IL-6 (pg/ml)	IL-12 (pg/ml)	IP-10 (pg/ml)	IFN-β (pg/ml)
Medium	22.3	149.2	109.4	0
61	22.3	137.8	249.1	3.59
62	966.2	294.4	21736	3.08
63	61.1	141.2	6239.7	0

Concentration of compounds was 250 μg/mL. Data shown are representative of two independent experiments.

Table 11.

Compound#	IL-6 (pg/ml)	IL-12 (pg/ml)	IP-10 (pg/ml)	IFN-β (pg/ml)
Medium	22.3	149.2	109.4	0
64	18.9	119.1	179.8	0
65	32.1	14.5	0	-
66	233.7	168.2	14256.8	0
67	18.9	128.8	244.3	0
68	1379.4	171.4	33076.8	1.43

Concentration of compounds was 250 μg/mL. Data shown are representative of two independent experiments.

Table 12.

Compound#	IL-1Ra (pg/ml)	IL-12 (pg/ml)	IP-10 (pg/ml)	MCP-1 (pg/ml)
Medium	8.0	0.6	3.8	3.2
36	79.6	26.8	16.9	19.8
37	343.0	32.7	51.8	1955.2
38	151.9	34.9	102.4	44.3
41	1440.3	45.1	1526.0	895.0
42	2482.8	125.2	150.6	34655

Concentration of compounds was 300 μg/mL. Data shown are representative of two independent experiments.

Table 13.

Compound#	IL-1Ra (pg/ml)	IL-6 (pg/ml)	IP-10 (pg/ml)	MCP-1 (pg/ml)
Medium	91.5	10.6	0	0
36	58.8	17.3	23.7	27.0
37	344.2	18.7	458.7	321.6
38	1390.7	538.2	1291.6	7251.5
41	962.0	326.1	1001.2	8959.4
42	1237.1	367.3	2257.4	7263.4

Concentration of compounds was 300 μg/mL. Data shown are representative of two independent experiments.

Example 3:

***In vivo* cytokine secretion in mouse model treated with TLR3 agonist compounds**

[00145] C57BL/6 mice, 5-6 weeks old, were obtained from Taconic Farms, Germantown, NY and maintained in accordance with Idera Pharmaceutical's IACUC approved animal protocols. Mice (n=2 or 3) were injected subcutaneously (s.c) with individual TLR3 agonists of the invention at 5 mg/kg, 10 mg/kg or 25 mg/kg (single dose). Naïve animals were not treated with

a TLR3 agonist. Control animals were treated with 25 mg/kg poly(I:C). Serum was collected by retro-orbital bleeding 2 hr after TLR3 agonist administration and cytokine and chemokine levels were determined by ELISA or Luminex multiplex assays. The results are shown in Table 14 and Figures 8 and 9 and demonstrate that *in vivo* administration of TLR3 agonists of the invention generates unique cytokine and chemokine profiles *in vivo*. All reagents, including cytokine and chemokine antibodies and standards were purchased from PharMingen. (San Diego, CA).

Table 14.

Compound#	IL-12 (pg/ml)
Naive	67.1
36	1142.6
37	5093.1
38	4925.0
41	3638.4
42	11902

Mice were dosed with 25 mg/kg of the TLR3 agonist compound. Naïve mice were treated with saline.

Example 4:

***In vivo* cytokine secretion in mouse model treated with TLR3 agonist compounds**

[00146] C57BL/6 mice, 5-6 weeks old, were obtained from Taconic Farms, Germantown, NY and maintained in accordance with Idera Pharmaceutical's IACUC approved animal protocols. Mice (n = 3) were injected subcutaneously (s.c) with individual TLR3 agonists of the invention at 5 mg/kg, 10 mg/kg (single dose). Naïve animals were not treated with a TLR3 agonist. Control animals were treated with 25 mg/kg poly(I:C). Serum was collected by retro-orbital bleeding 2 hr after TLR3 agonist administration and cytokine levels were determined by ELISA assay. The results are shown in Table 15 and demonstrate that *in vivo* administration of TLR3 agonists of the invention generates unique TLR3 stimulation, resulting in induced IL-12 concentrations *in vivo*. All reagents, including cytokine and chemokine antibodies and standards were purchased from PharMingen. (San Diego, CA).

Table 15.

Compound#	IL-12 (pg/ml)
Naive	621.5
39	10078
40	32388
43	35655
44	51066
45	33699
46	24979
47	535.2
48	1311.9
49	181.2
50	41085
51	8470
52	2091
53	416.7
54	329.7
55	331.6
56	10874
57	2948
58	845.9
59	1704
60	928.8
61	535.2
62	41.1
63	221.1

Mice were dosed with 10 mg/kg of the TLR3 agonist compound. Naïve mice were treated with saline.

Example 5:

***In vivo* cytokine secretion in mouse model treated with TLR3 agonist compounds**

[00147] C57BL/6 mice, 5-6 weeks old, were obtained from Taconic Farms, Germantown, NY and maintained in accordance with Idera Pharmaceutical's IACUC approved animal protocols. Mice (n = 2) were injected subcutaneously (s.c) with individual TLR3 agonists of the invention at 10 mg/kg (single dose). Naïve animals were not treated with a TLR3 agonist. Control animals were treated with 25 mg/kg poly(I:C). Serum was collected by retro-orbital bleeding 2 hr after TLR3 agonist administration and cytokine levels were determined by ELISA assay. The results are shown in Figures 12 and 13 and demonstrate that *in vivo* administration of TLR3 agonists of the invention generates unique TLR3 stimulation, resulting in induced IL-12

concentrations *in vivo*. All reagents, including cytokine and chemokine antibodies and standards were purchased from PharMingen. (San Diego, CA).

EQUIVALENTS

[00148] Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific substances and procedures described herein. For example, antisense oligonucleotides that overlap with the oligonucleotides may be used. Such equivalents are considered to be within the scope of this invention, and are covered by the following claims.

What is claimed is:

1. A synthetic TLR3 agonist comprising

i) a first oligoribonucleotide having the structure: 5'-Domain A-Domain B-3'; and

ii) a second oligoribonucleotide having the structure: 5'-Domain C-Domain D-3',

wherein Domain A is a first complementary domain, Domain B is a polyriboininosine domain, Domain C is a second complementary domain and Domain D is a polyribocytidine domain, wherein Domain A and Domain C are complementary to each other, and wherein the first oligoribonucleotide and the second oligoribonucleotide bind to each other through intermolecular hydrogen bonding between (i) the complementary domains leaving a free polyriboininosine domain and a free polyriboincytidine domain or (ii) between the polyriboininosine and polyribocytidine domains leaving a free first complementary domain and a free second complementary domain, and wherein additional first and/or second oligoribonucleotides can bind to the free complementary and/or polyriboinosine or polyribocytidine domains, thereby creating a chain of oligoribonucleotides.

2. A synthetic TLR3 agonist comprising

i) a first oligoribonucleotide having the structure: 5'-Domain B-Domain A-3'; and

ii) a second oligoribonucleotide having the structure: 5'-Domain D-Domain C-3',

wherein Domain A is a first complementary domain, Domain B is a polyriboininosine domain, Domain C is a second complementary domain and Domain D is a polyribocytidine domain, wherein Domain A and Domain C are complementary to each other, and wherein the first oligoribonucleotide and the second oligoribonucleotide bind to each other through intermolecular hydrogen bonding between (i) the complementary domains leaving a free polyriboininosine domain and a free polyriboincytidine domain or (ii) between the polyriboininosine and polyribocytidine domains leaving a free first complementary domain and a free second complementary domain, and wherein additional first and/or second oligoribonucleotides can bind to the free complementary and/or polyriboinosine or polyribocytidine domains, thereby creating a chain of oligoribonucleotides.

3. A composition comprising a TLR3 agonist according to Claim 1 or 2 and a physiologically acceptable carrier.

4. A method for stimulating TLR3 activity comprising contacting TLR3 with a TLR3 agonist according to Claim 1 or 2 or a composition according to Claim 3.

5. A method for stimulating TLR3 activity in a mammal comprising administering to the mammal a TLR3 agonist according to Claim 1 or 2 or a composition according to Claim 3.

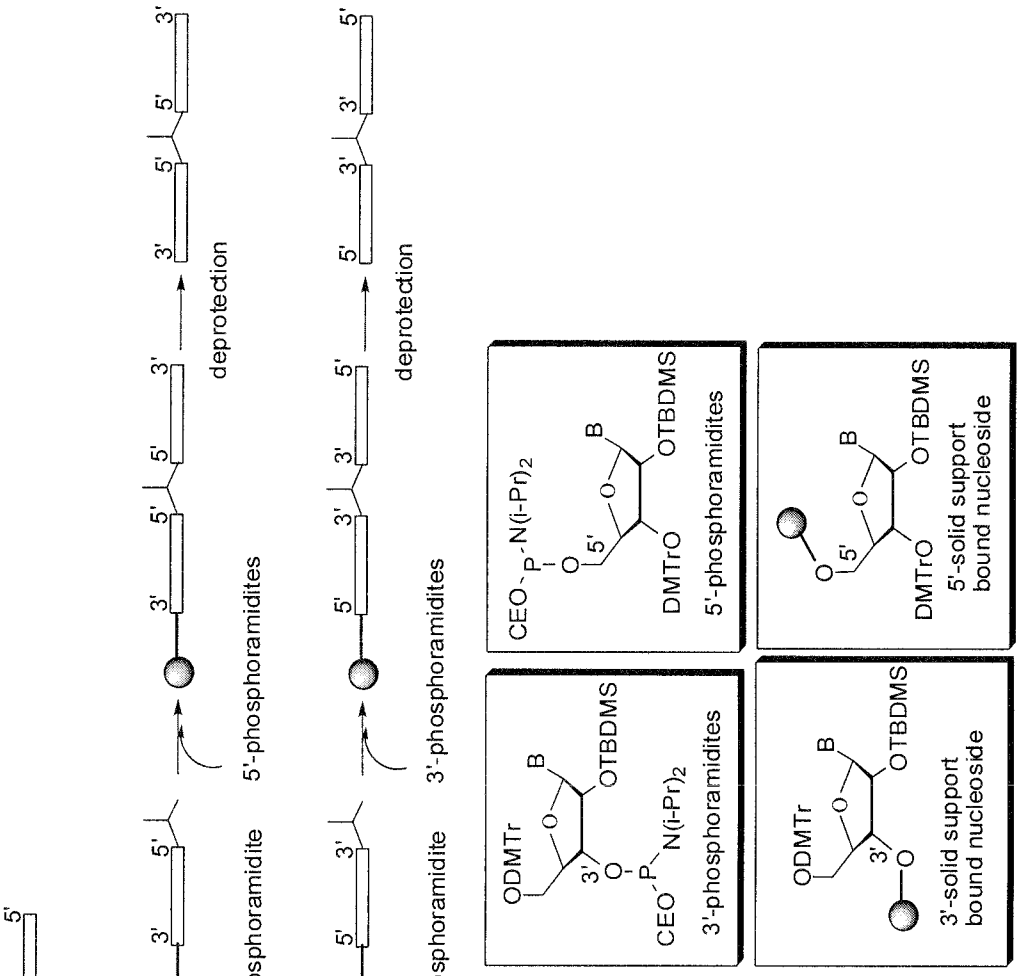
6. A method for stimulating TLR3-mediated immune response in a mammal comprising administering to the mammal a TLR3 agonist according to Claim 1 or 2 or a composition according to Claim 3.

7. A method for treating a mammal having a disease or disorder whose treatment is capable of being mediated by TLR3 comprising administering to the mammal a TLR3 agonist according to Claim 1 or 2 or a composition according to Claim 3.

8. A method of preventing a disease or disorder, whose prevention is capable of being mediated by TLR3, in a mammal at risk of contracting/developing such disease or disorder comprising administering to the mammal a TLR3 agonist according to Claim 1 or 2 or a composition according to Claim 3.

9. A vaccine comprising a composition according to Claim 3, and further comprising an antigen.

Figure 1A.


3'-linked
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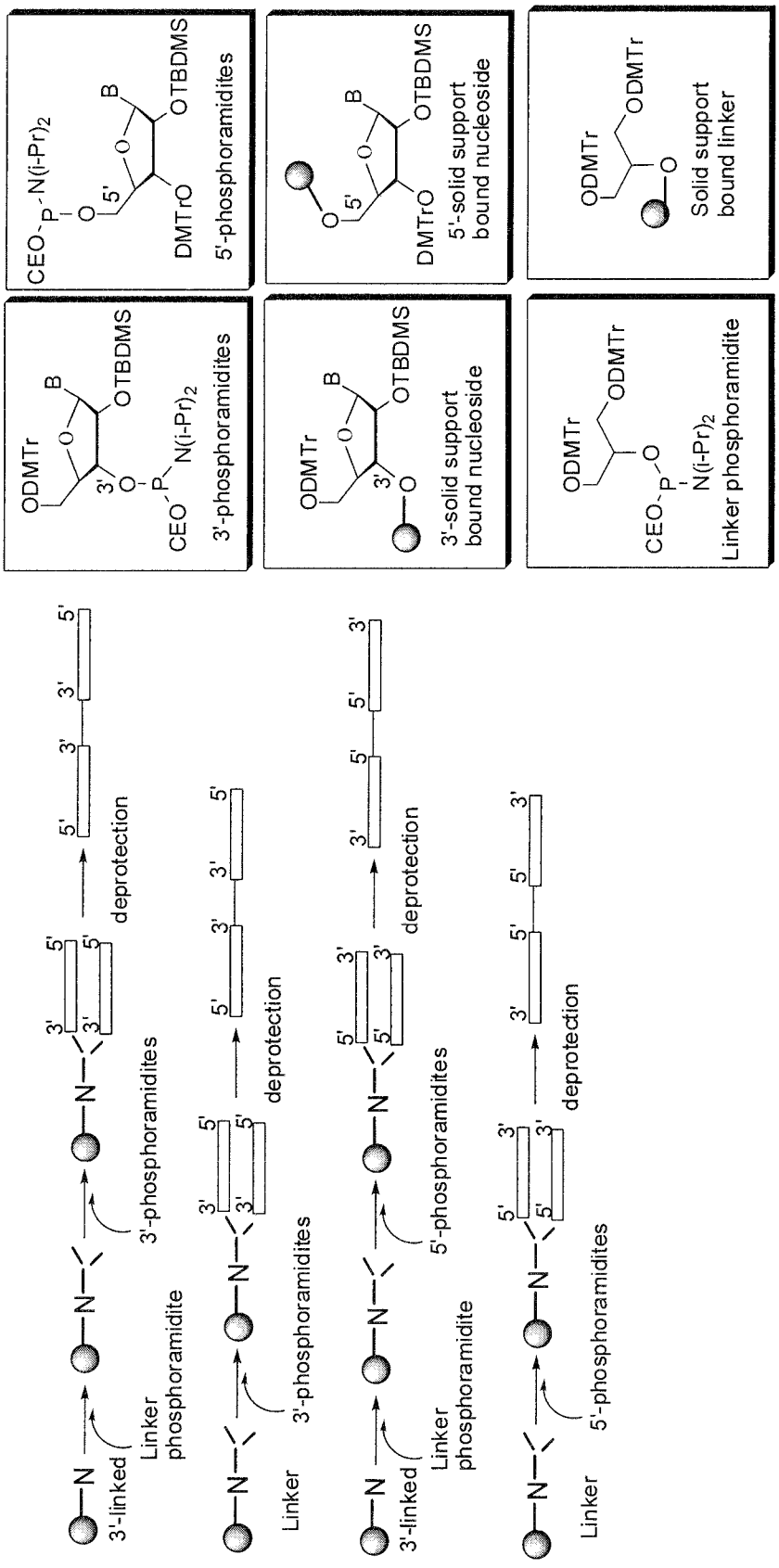
CEO
N(i-Pr)2
3'-phosphoramidites
CEO
N(i-Pr)2
DMTrO
OTBDMS
5'-phosphoramidites
ODMTr
OTBDMS
3'-solid support
bound nucleoside
DMTrO
OTBDMS
5'-solid support
bound nucleoside

Figure 1B.


3'-linked
Linker
phosphoramidite
3'-phosphoramidites
deprotection
Linker
3'-phosphoramidites
deprotection
3'-linked
Linker
phosphoramidite
5'-phosphoramidites
deprotection
Linker
5'-phosphoramidites
deprotection
ODMTr
OTBDMS
CEO
N(i-Pr)2
3'-phosphoramidites
CEO
N(i-Pr)2
DMTrO
OTBDMS
5'-phosphoramidites
ODMTr
OTBDMS
3'-solid support
bound nucleoside
DMTrO
OTBDMS
5'-solid support
bound nucleoside
ODMTr
ODMTr
Solid support
bound linker
ODMTr
ODMTr
CEO
N(i-Pr)2
Linker phosphoramidite

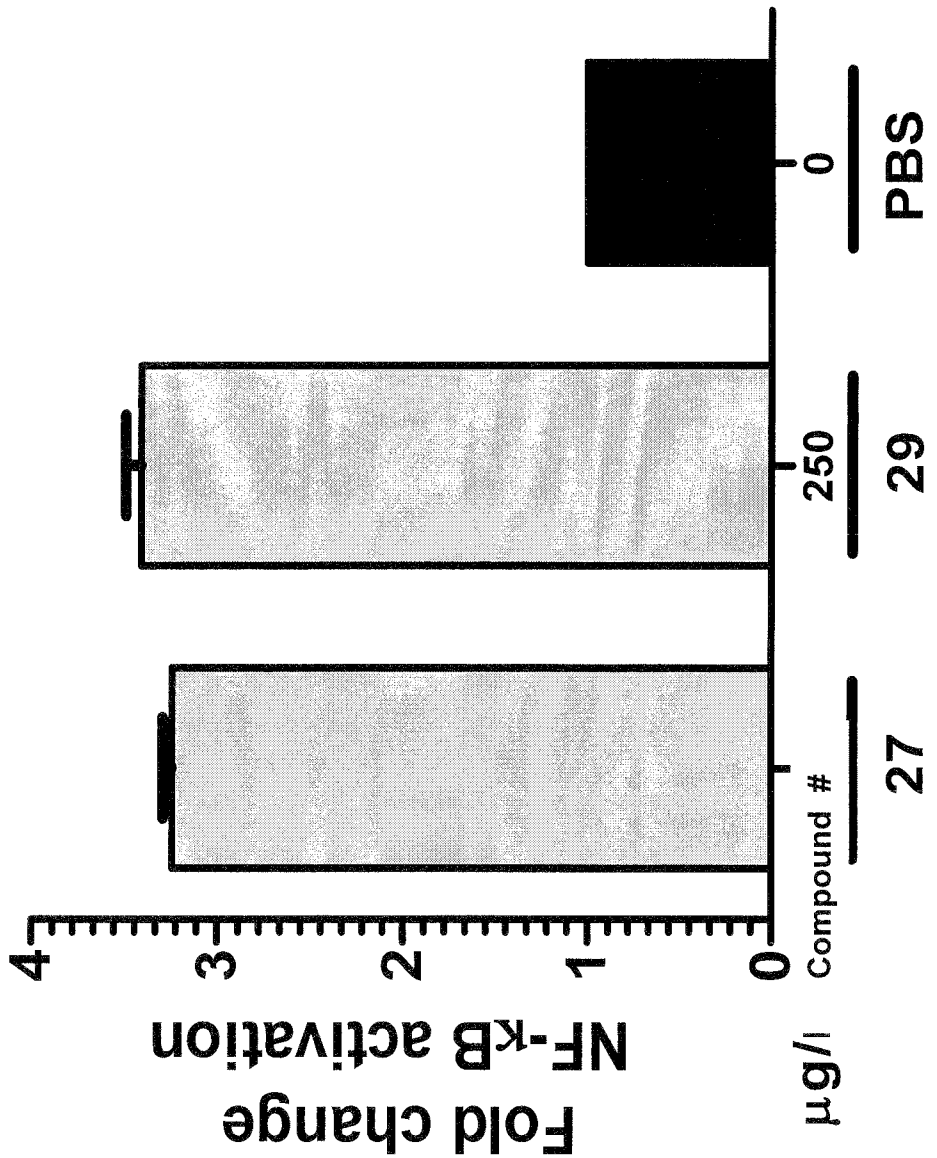
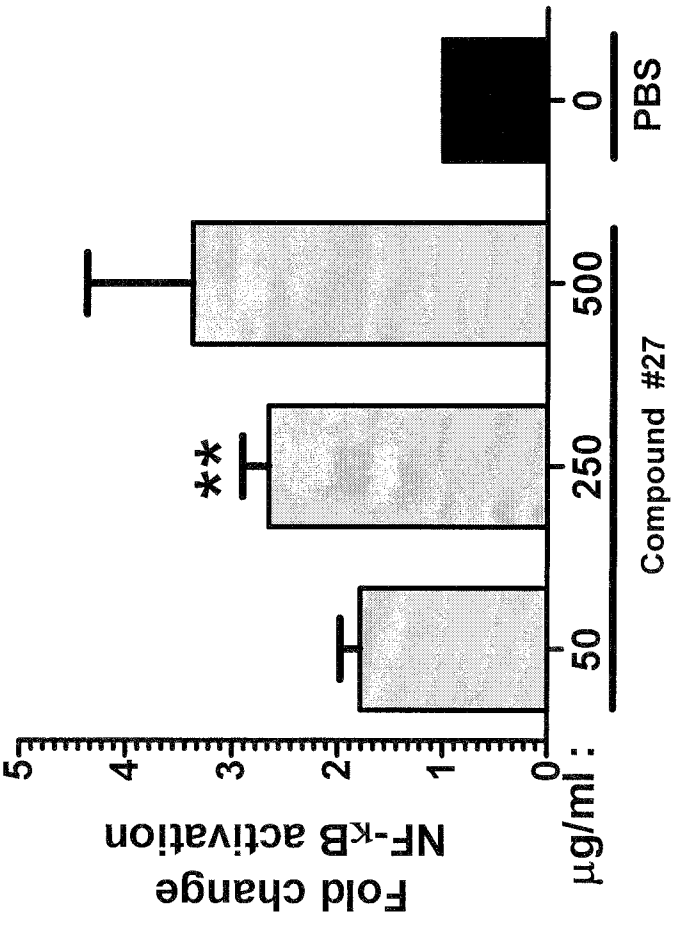

Fold change
NF-κB activation
0
1
2
3
4
μg/l
Compound #
27
29
250
0
PBS

Figure 2A.

Figure 2B.

A



N = 5, ** p = 0.001

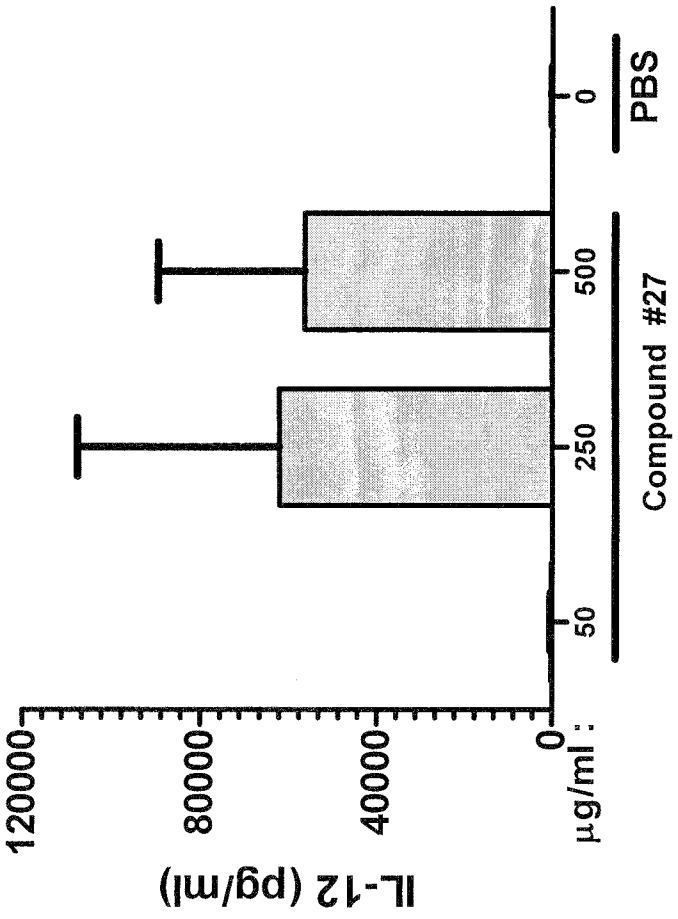

IL-12 (pg/ml)
120000
80000
40000
0
µg/ml :
50
250
500
0
Compound #27
PBS

Figure 3.

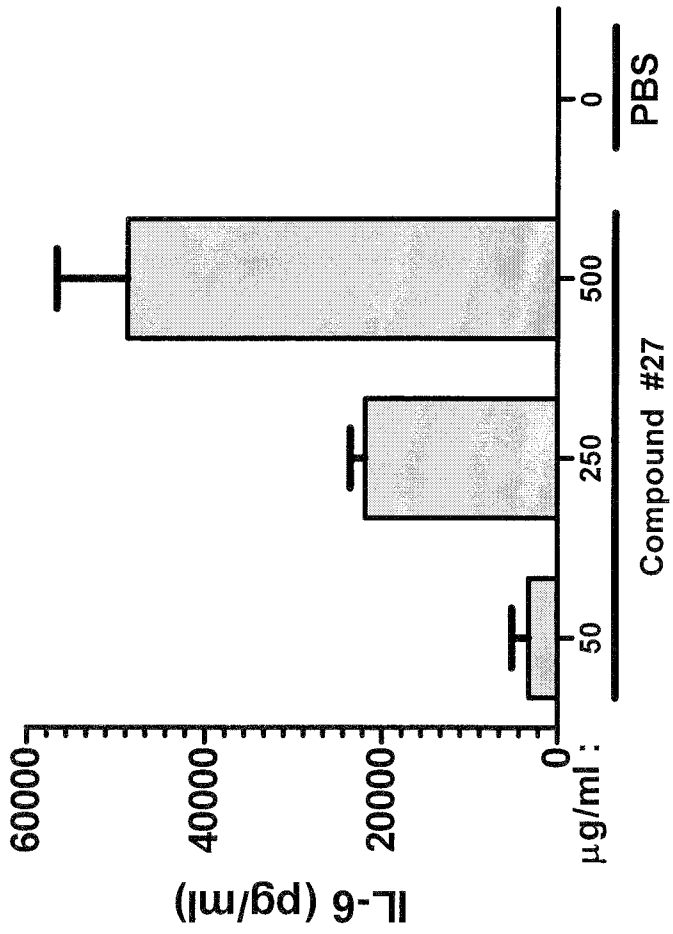


Figure 4.

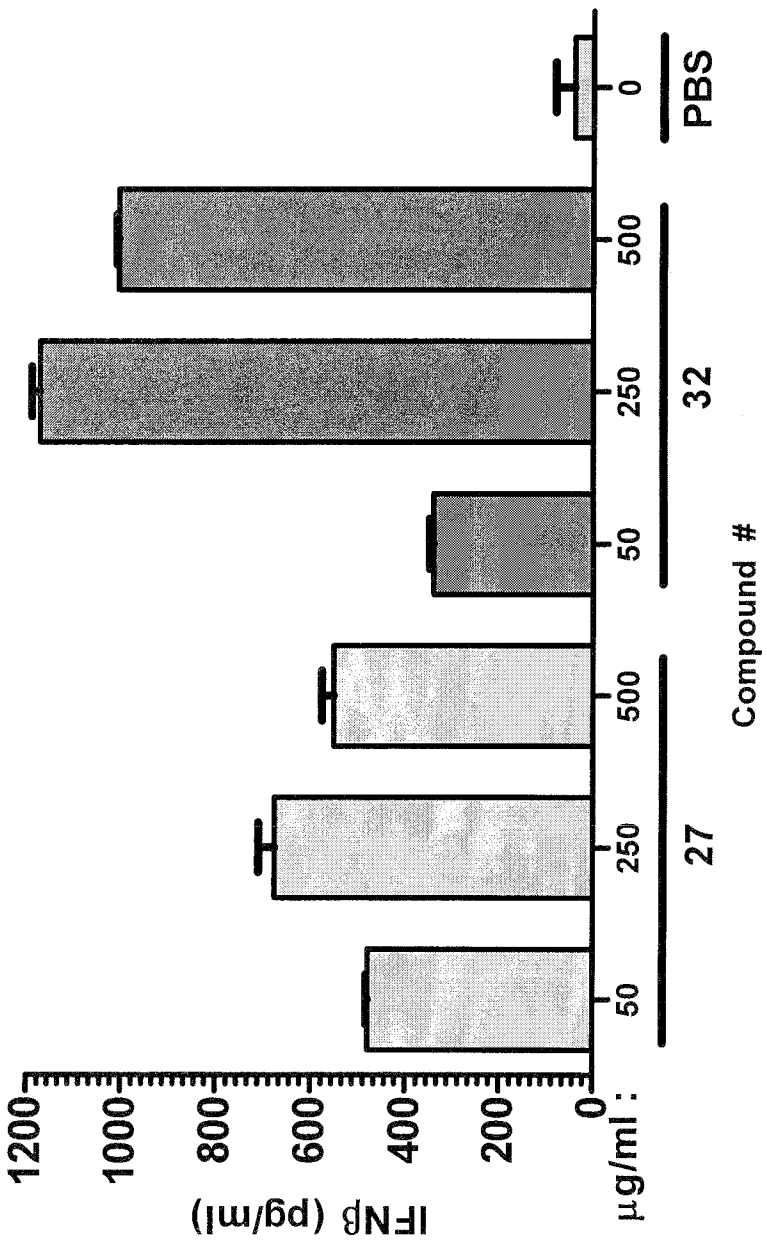

IFNβ (pg/ml)
1200
1000
800
600
400
200
0
μg/ml:
50
250
500
50
250
500
0
27
32
PBS
Compound #

Figure 5.

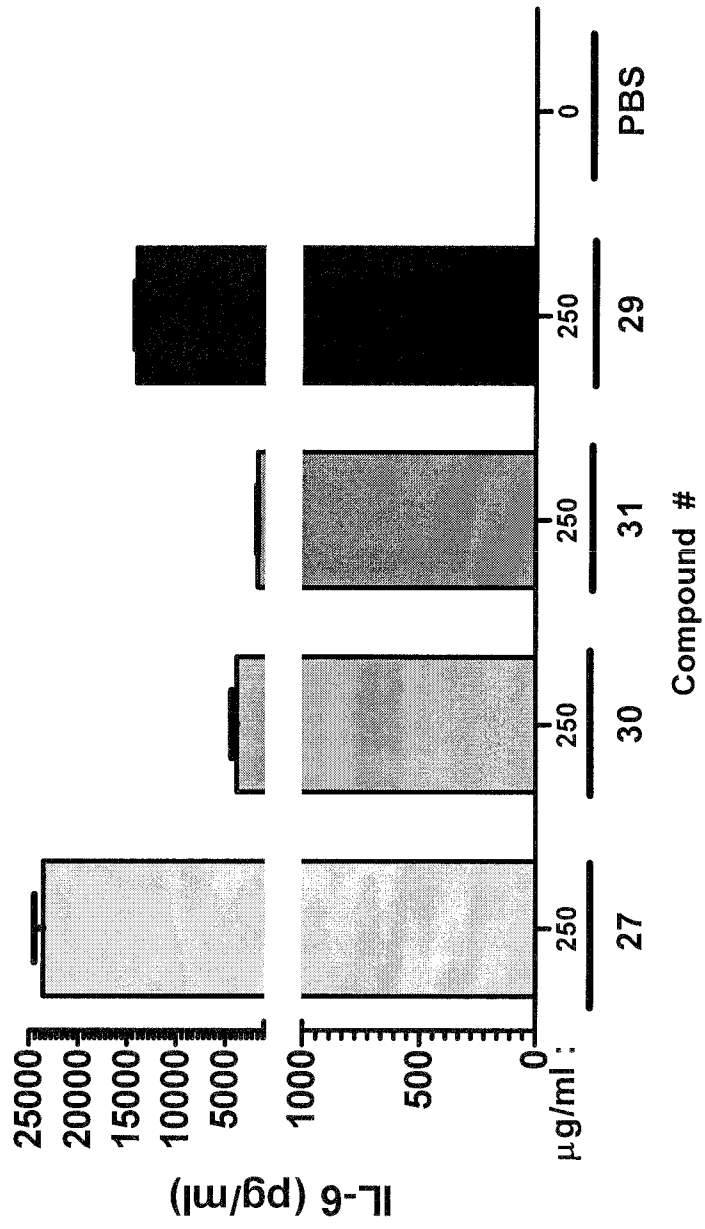
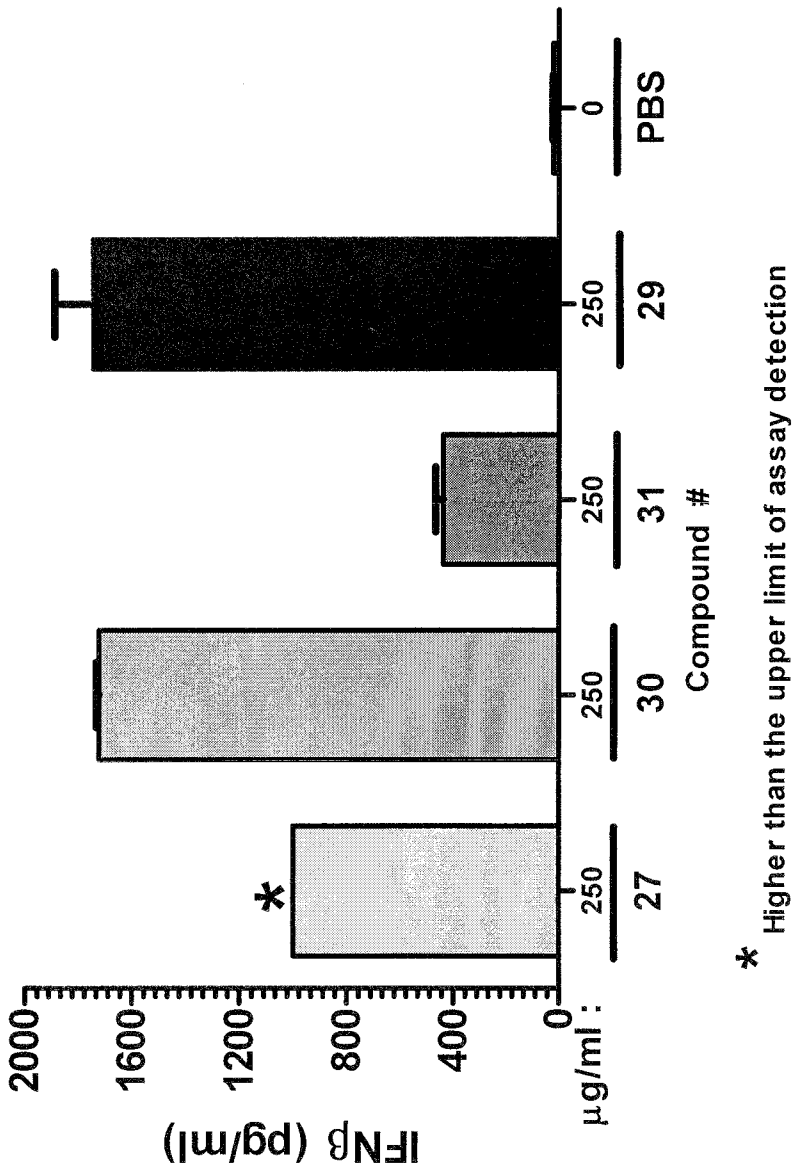

IL-6 (pg/ml)
25000
20000
15000
10000
5000
1000
500
0
µg/ml:
250
250
250
0
27
30
31
29
PBS
Compound #

Figure 6.

Figure 7.


IFNβ (pg/ml)
2000
1600
1200
800
400
0
*
µg/ml :
250
250
250
0
27
30
31
29
PBS
Compound #
* Higher than the upper limit of assay detection

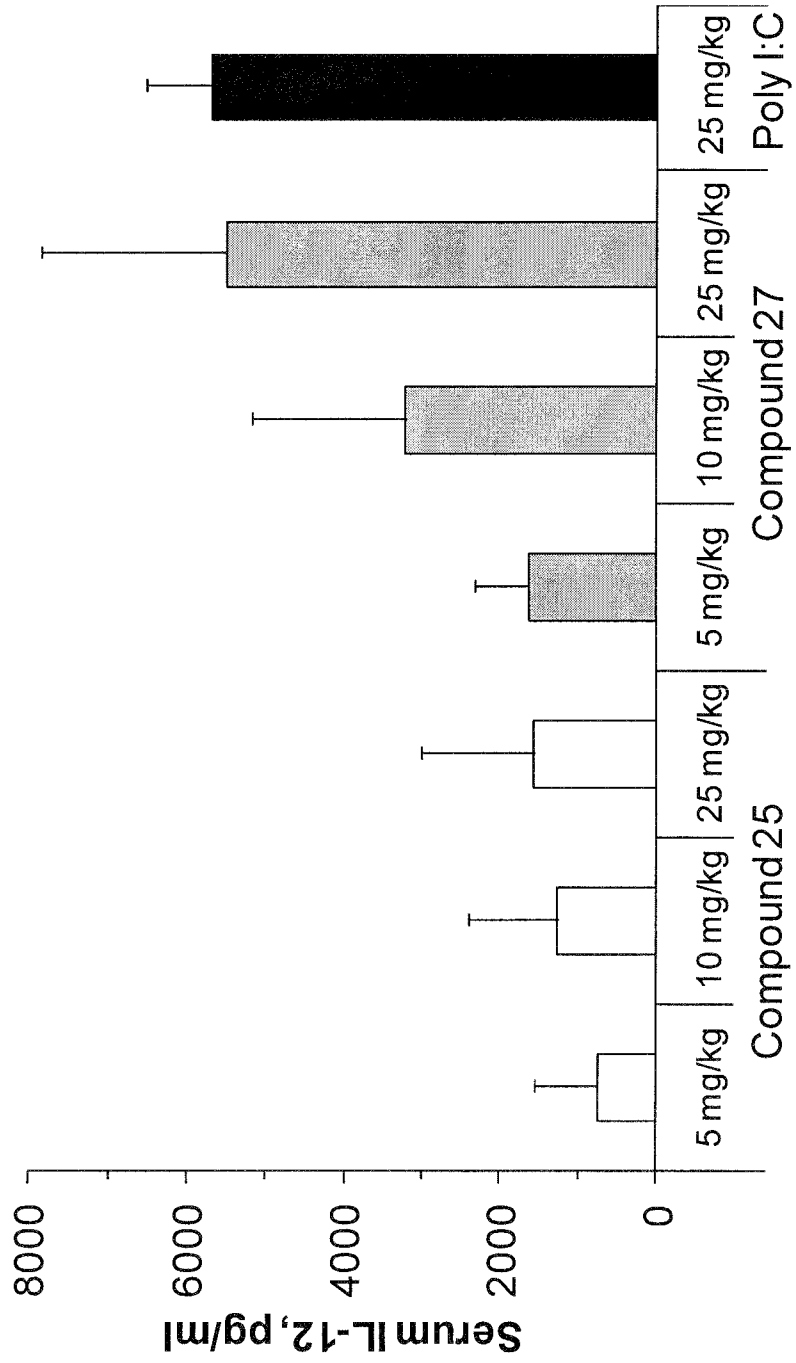

Serum IL-12, pg/ml
8000
6000
4000
2000
0
5 mg/kg
10 mg/kg
25 mg/kg
Compound 25
5 mg/kg
10 mg/kg
25 mg/kg
Compound 27
25 mg/kg
Poly I:C

Figure 8.

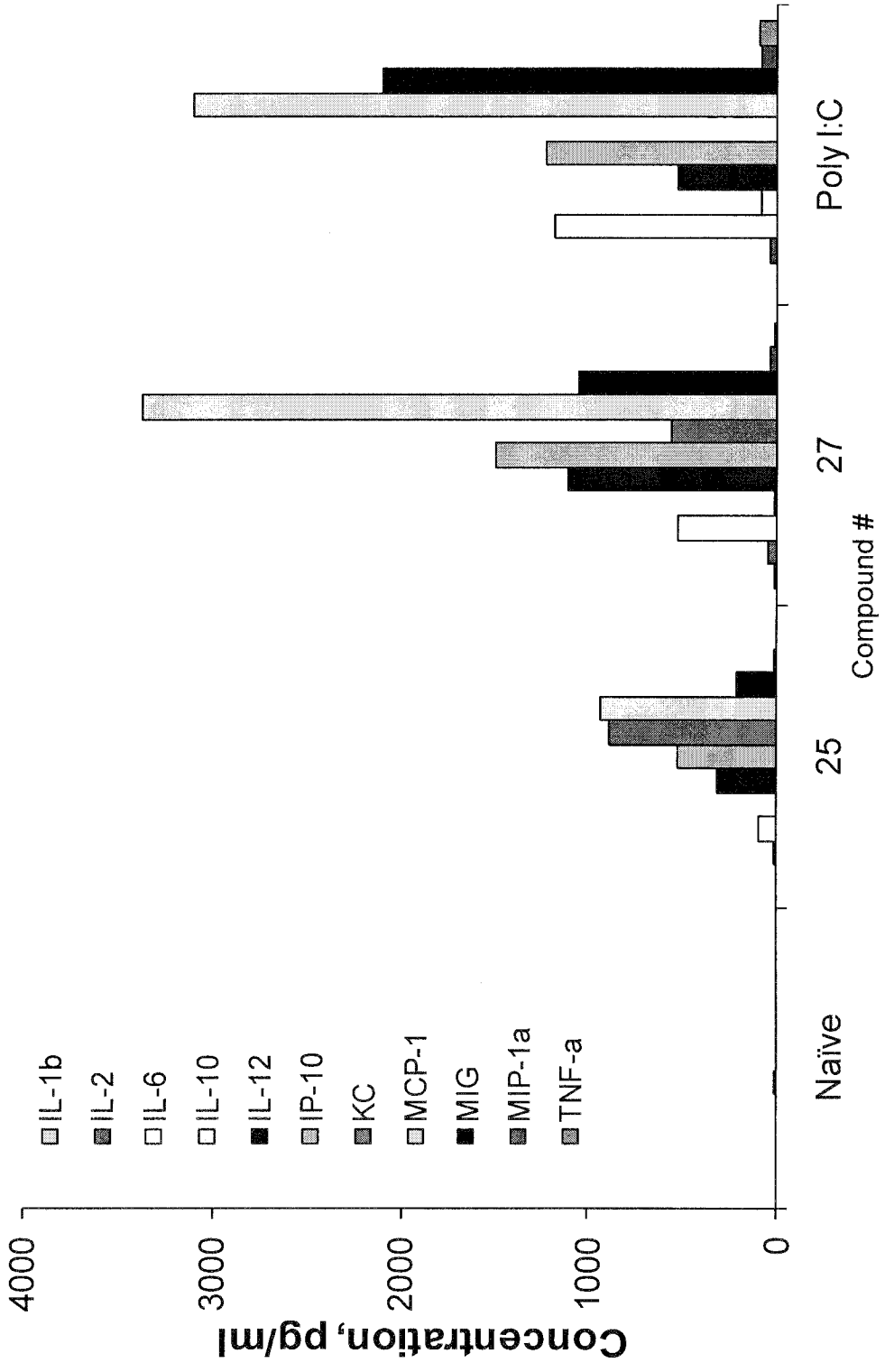

Concentration, pg/ml
4000
3000
2000
1000
0
IL-1b
IL-2
IL-6
IL-10
IL-12
IP-10
KC
MCP-1
MIG
MIP-1a
TNF-a
Naïve
25
27
Poly I:C
Compound #

Figure 9.

Figure 10.

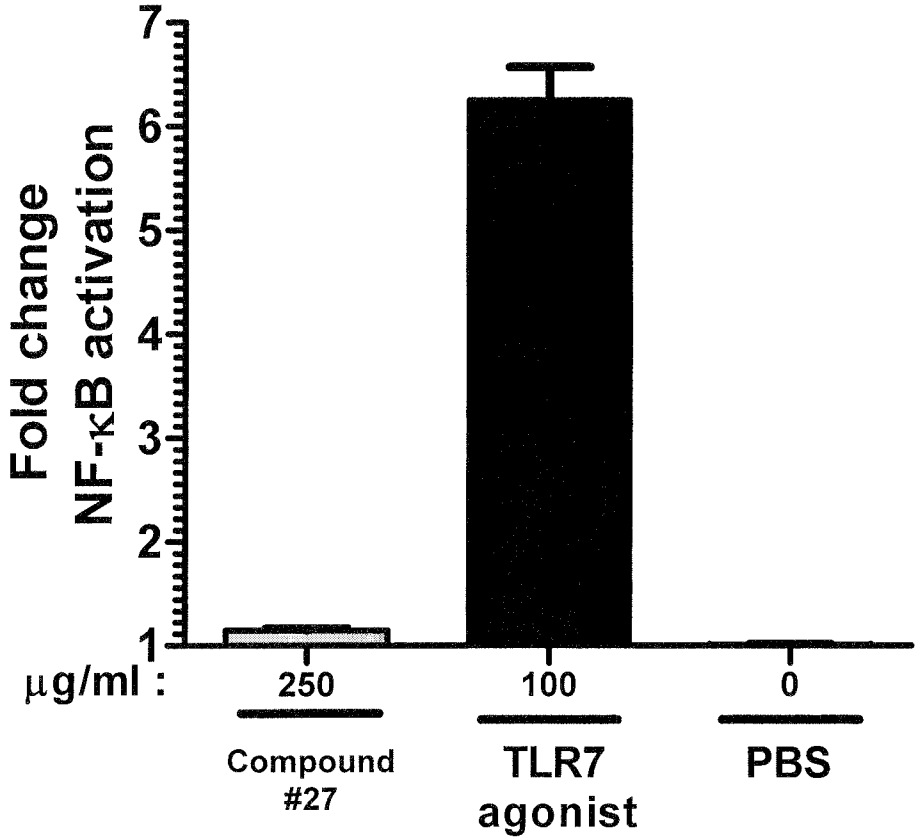

Fold change
NF-κB activation
7
6
5
4
3
2
1
μg/ml :
250
100
0
Compound
#27
TLR7
agonist
PBS

Figure 11.

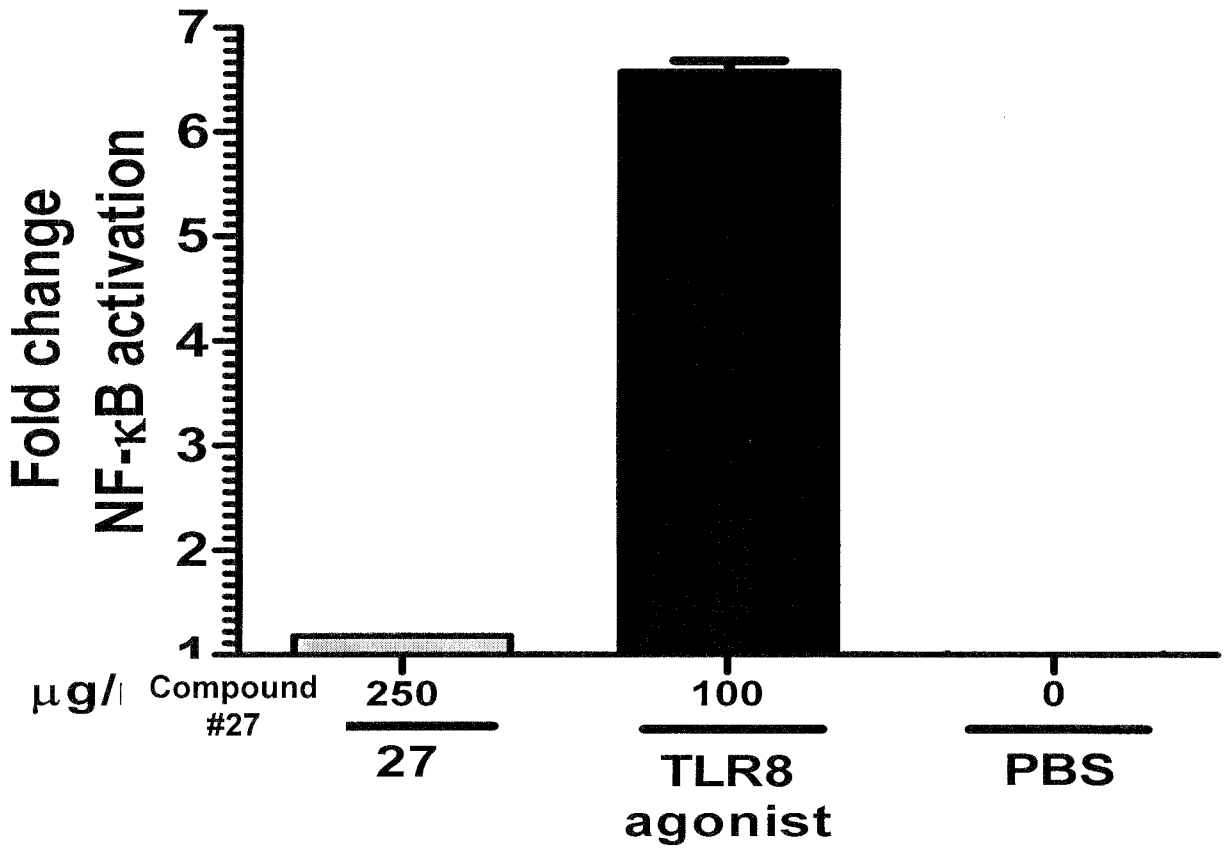

Fold change
NF-κB activation
7
6
5
4
3
2
1
μg/
Compound
#27
250
27
100
TLR8
agonist
0
PBS

Figure 12.

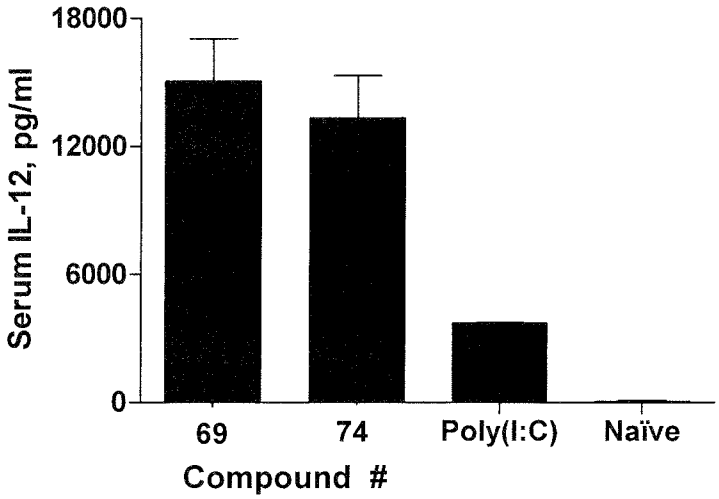
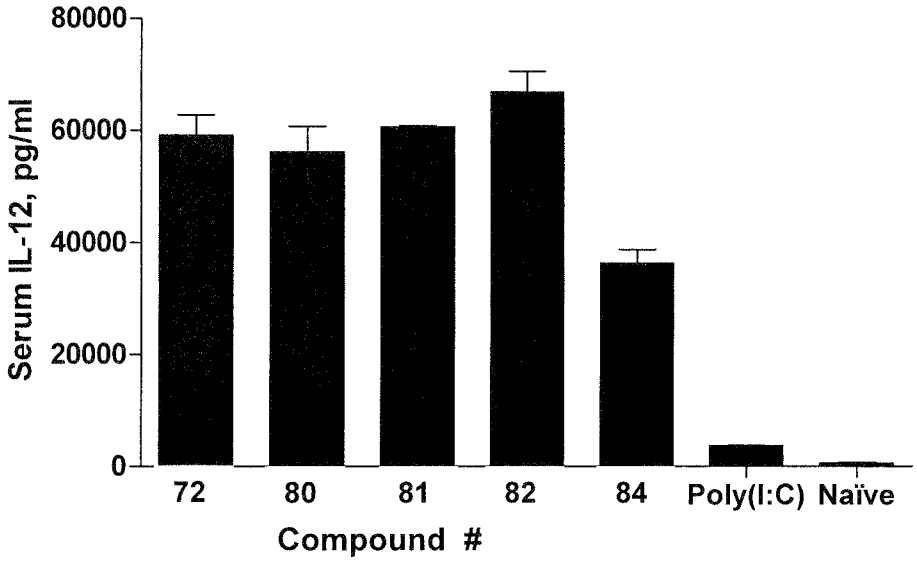

18000
12000
6000
0
Serum IL-12, pg/ml
69
74
Poly(I:C)
Naïve
Compound #

Figure 13.


80000
60000
40000
20000
0
Serum IL-12, pg/ml
72
80
81
82
84
Poly(I:C)
Naïve
Compound #