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(54) Title: NOVEL COMPLEXES OF IMMUNOSTIMULATORY COMPOUNDS, AND USES THEREOF

(57) Abstract: The present invention concerns the preparation of complexes formed between a cationic lipid and at least one of various cyclic dinucleotide (CDN) agonists of STING, and proposes uses of such complexes. Specifically, such complexes can be used to provide enhanced or distinct biological activities in vitro, ex vivo or in vivo, relative to use of the corresponding CDN component alone.

NOVEL COMPLEXES OF IMMUNOSTIMULATORY COMPOUNDS, AND USES THEREOF

FIELD OF THE INVENTION

The present invention is related to the fields of molecular biology, cell biology, immunology and organic chemistry.

BACKGROUND OF THE INVENTION

STING, STING agonists and cytokines

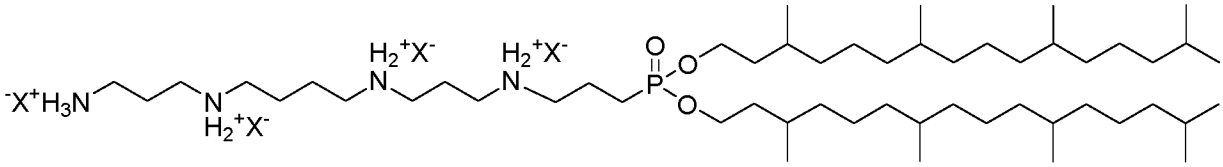
A major player in physiological production of cytokines is *stimulator of interferon genes* (STING; also known as *ERIS*, *MITA, MPYS,* or *TM173*), a transmembrane receptor protein that is paramount in innate immunity. Activation of STING leads to production of Type I interferons (*e.g.* IFN-α and IFN-β), via the IRF3 (interferon regulatory factor 3) pathway; and to production of pro-inflammatory cytokines (IL-1α, IL-1β, IL-2, IL-6, TNF-α, etc.), via the oncogenic transcription factor NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) pathway. STING agonists include naturally occurring and synthetic cyclic dinucleotides (CDNs), which are used in STING-related research and have recently garnered attention for their therapeutic potential.

New reagents for enhanced activity of STING agonists

Given that STING is located inside the cell, targeting of it with small molecules such as CDNs *in vitro* and *in vivo* demands that such molecules efficiently enter the cell. There remains a need to develop new reagents to enhance the cellular activity of CDN STING agonists *in vitro* and *in vivo*, especially reagents that would provide advantages in terms of cytokine induction; immune cell response; therapeutic (*e.g.* anti-tumor or anti-viral) activity; dose and dosing; pharmacokinetics (ADME, etc.); and other research or therapeutic parameters. A single molecule capable of providing such benefits would be especially interesting.

SUMMARY OF THE INVENTION

The present invention proposes use of the cationic lipid complexing agent CL338 (as defined hereafter) to form complexes with cyclic dinucleotides (CDNs), and further proposes uses of such complexes. Specifically, it relates to complexes in which the CDN is a cyclic purine ribodinucleotide or a cyclic purine deoxyribodinucleotide. It further relates to CDNs that are agonists of STING. We show here that the CDN/CL338 complexes of the present invention provide enhanced and/or distinct biologic activities *in vitro, ex vivo* and *in vivo*, including therapeutically beneficial activities, relative to the corresponding CDN alone (*i.e.* in non-complexed form). Surprisingly, we found that, relative to the corresponding CDN alone, a complex comprising that CDN and CL338 can induce stronger and/or distinct STING pathway-dependent biologic activities, including induction of Type I and Type III interferons, *in vitro* in mammalian cells, *ex vivo* in mammalian blood samples and *in vivo* in mammals.



CL338

STING agonists

Human STING is activated three ways: **via** binding of the exogenous (3',3) cyclic dinucleotides (CDNs) c-diGMP, c-diAMP and c-GAMP, which are released by invading bacteria or archaea (see (Gomelsky, 2011) and references therein); **via** binding of the (2',3') CDN cyclic guanosine monophosphate–adenosine monophosphate (2',3')c-GAMP), a recently discovered endogenous cyclic dinucleotide that is produced by the enzyme cyclic GMP-AMP synthase (cGAS; also known as *C6orf150* or *MB21D1*) in the presence of exogenous double-stranded DNA (*e.g.* that released by invading bacteria, viruses or protozoa) or of self-DNA in mammals (see, for example: (Ablasser, Goldeck, *et al.*, 2013) and (Zhang *et al.*, 2013)); or **via** binding of synthetic ligands, such as analogs of the aforementioned naturally-occurring cyclic dinucleotides (see, for example: (Dubensky, Kanne, & Leong, 2013) and (Li *et al.*, 2014)).

CDNs and STING

Cyclic dinucleotides (CDNs) are small nucleic acids that were originally discovered as microbial messenger molecules and later found to be potent immunostimulants in mammals. Bacteria and other microbes produce three different CDNs (c-diGMP, c-diAMP and c-diGAMP), which they use for their own growth and development and which they release into host cells during infection. In contrast, metazoans synthesize a structurally distinct CDN (2'3'-cGAMP), which serves as a messenger molecule in immune signaling pathways triggered by the presence of pathogenic or self DNA in the cytosol. All four of these compounds directly bind to and activate — albeit to varying degrees — stimulator of interferon genes (STING). Accordingly, CDNs have garnered attention for their therapeutic potential to manipulate the STING pathway in areas such as infectious diseases, oncology and autoimmunity. Thus, synthetic CDNs that exhibit favorable drug-like properties and activate all known variants of human STING are currently being sought.

Physiologic transport and uptake of CDNs

Since naturally occurring CDNs are directly released (by pathogens) into or synthesized (by eukaryotic cells) in the cytoplasm, in physiologic contexts they do not have to cross the cell membrane alone from the extracellular space. Interestingly, 2'3'-cGAMP has been reported to be shuttled from the cells in which it is produced, to neighboring cells, via gap junctions (Ablasser, Schmid-Burgk, *et al.*, 2013) and to be transported, inside of viral particles and extracellular vesicles, from virally infected cells into target cells (Gentili *et al.*, 2015). To date, only one example of a surface receptor for CDNs has been identified: Tosolini *et al.* (Tosolini *et al.*, 2015) reported that adenosine-containing CDNs (*e.g.* c-diAMP

and c-GAMP), but not other CDNs (*e.g.* c-diGMP), bind to the G-protein-coupled surface receptor A2a in monocytes. Thus, it is currently not known whether other receptors for surface binding and/or cellular uptake of CDNs exist.

Delivery of CDNs into cells

For practical applications that involve addition of CDNs directly to cell cultures, blood or tissue samples, or administration of them to organisms, the effective delivery of these molecules into cells can pose a challenge, as they are inherently negatively charged (at their phosphodiester or phosphorothioate bonds). Indeed, practical use of charged molecules both *in vitro* and *in vivo* is typically limited by their poor uptake by eukaryotic cells, which are encased in a highly lipophilic cell membrane. Consequently, a CDN that demonstrates strong and specific activity against a particular protein target in a protein-binding assay can later exhibit little or no activity in a whole-cell assay, for the simple reason that it cannot cross the cell membrane. Accordingly, administration of such a molecule *in vivo* to an animal or patient would be of little therapeutic value and could even be toxic, regardless of any desirable qualities that the molecule might purportedly possess. One possible compensatory strategy to offset the poor uptake of certain charged molecules is to administer very high doses of them to cells, animals or human subjects, in the hopes that a small proportion of the administered dose will somehow successfully cross the cell membrane. However, in a therapeutic context, such a strategy can lead to toxicity and other side effects from high levels of metabolites generated after administration. Furthermore, in laboratory as well as clinical settings, deliberately administering a high dose of an active molecule to subsequently obtain a drastically lower effective dose inside cells is not cost effective and can imply wasting precious material. Thus, researchers have sought ways to facilitate delivery of CDNs into cells.

State of the art in delivery of CDNs into cells

Different strategies can be used to make cells more permeable to molecules that are negatively charged at physiological pH, such as CDNs. These include electroporation of cells or use of membrane-permeabilizing agents such as digitonin. For instance, WO/2014/179335 A1, Yi *et al.* (Yi *et al.*, 2013), and Sauer *et al.* (Sauer *et al.*, 2011) each describes whole-cell assays in which human or murine cells are first treated with digitonin and subsequently, with CDN STING agonists.

Another option to ameliorate the cellular uptake — and consequently, the biological activity — of CDNs is by delivering them into cells using some type of neutralizing (cationic) agent, which can be lipophilic (*e.g.* Lipofectamine®) or non-lipophilic (*e.g.* Effectene® and polyethyleneimine [PEI]). These agents are used to shuttle CDNs to the membrane to enable cellular uptake via non-specific endocytosis or other mechanisms. Commonly used materials in this class include commercially available transfection reagents such as Viromer®, Lipofectamine®, Effectene® or SuperFect. For recent examples of the delivery of CDN STING agonists into cells using these reagents, see: for Viromer®, (Paijo *et al.*, 2016); for Lipofectamine®, (Gentili *et al.*, 2015); for Effectene®, WO/2013/185052; and for SuperFect, (Prantner *et al.*, 2012). Interestingly, the group of Nakamura, Miyabe *et al.* has designed a multicomponent PEGylated liposome, YSK05, which they employed to deliver the STING agonist c-

diGMP into cells in diverse *in vitro* and *in vivo* applications (Nakamura *et al.*, 2015) (Miyabe *et al.*, 2014). The related applications US/2014/0341976 A1 and US/2014/0205653 A1 both allow for the use of lipid or liposomal reagents for delivery of CDN STING agonists to cells. However, the aforementioned applications do not explicitly propose any specific reagents or structures, nor do they specify any particular functionality or advantage that could be afforded by the use of such lipid or liposomal reagents.

Other vehicles that have been employed to deliver CDN STING agonists into cells include virus-like particles (US/2016/0074507); PEGylated phosphatidylcholine nanoparticles (Hanson *et al.*, 2015); linear polyethyleneimine/hyaluronic acid (PEI/HA) hydrogels (Lee *et al.*, 2016); and an Arg(9) cell-penetrating peptide (Yildiz *et al.*, 2015). In most of these examples, the delivery vehicle or complexing agent comprises multiple molecules, rather than a single molecule. Alternatively, US/2014/0065223, which relates chiefly to covalent perfluorocarbon (PFC) complexes of nucleic acids and analogs, proposes PFC complexes of c-diGMP. However, the aforementioned application does not mention STING, nor does it discuss any possible differences in cytokine induction between c-diGMP alone and PFC complexes of c-diGMP. Lastly, EP/1959989 (2008), which defines a set of CDNs for use as vaccine adjuvants, proposes vaccines that contain one of these CDNs plus additional components such as glycolipids and antigen-delivery systems (*e.g.* liposomes and virus-like particles). However, EP1959989 does not specify any particular functionality or advantage that could be afforded by the use of such additional components, nor does it mention STING.

Several strategies have been reported for enhancing the activity and properties (*e.g.* lipophilicity, enzymatic stability, cellular uptake or hybridization) of nucleic acids larger than CDNs (*e.g.* DNA, RNA and oligonucleotides [ODNs]) *in vitro* and *in vivo*. These include chemical (covalent) modifications of the molecule itself; use of conjugates and complexes (*e.g.* with liposomes and cationic lipids); use of delivery vehicles such as microparticles and nanoparticles; and/or use of electroporation *in vivo* (*e.g.* for intratumoral administration). The person skilled in the art will be familiar with many of these strategies. For exemplary reviews on this topic, see: (Wang, Miao, Satterlee, & Huang, 2015); (Menzi, Lightfoot, & Hall, 2015); (Silva, Lopes, Sousa Lobo, & Amaral, 2015); (Yin *et al.*, 2014); and (Kanasty, Dorkin, Vegas, & Anderson, 2013). Recently reported examples from this field include a multicomponent lipid vesicle for delivery of nucleic acids, in which structurally and functionally distinct phospholipids are mixed together to form a vesicle that is subsequently added to a therapeutic nucleic acid (US/2015/0272886); a family of rapidly-degradable cationic amphipathic lipids for delivery of nucleic acids or polynucleotides into cells (US/9107931/B2); and a complex that comprises a carrier and a liposome, in which the liposome contains a helper lipid and can contain a nucleic acid (US/2015/0359906). Alternatively, US/2015/0265708 describes a family of cationic lipids for use in multi-component lipid particles that also contain a neutral lipid, a sterol and PEG-DMG, for subsequent use in delivery of therapeutic agents (principally, nucleic acids). As illustrated by these examples, nucleic acids are often delivered using multicomponent systems.

Another approach to enhance the bioactivity of nucleic acids *in vitro* or *in vivo* is to optimize the method or conditions of administration. For example, US 2016/0038612 proposes treatment of a subject by intramuscular or intradermal injection of a chemically modified nucleic acid (*e.g.* mRNA) or complexes thereof, including lipid complexes, followed by electroporation at or near the injection site.

Lipid formulations of therapeutic molecules can exhibit immunogenicity, which can be advantageous (*e.g.* by boosting the immune system) or disadvantageous (*e.g.* by causing unwanted immune responses such as acute inflammation), depending on the context. For example, EP/2125011/B1 relates to the use of cationic lipid formulations of diverse antigens to deliberately generate an immunostimulatory therapeutic response via MAP-kinase signaling, as a strategy for therapeutic modulation of T cells; and WO2008/148057 proposes lipid-based adjuvants for nucleic acid vaccines that encode immunogens, in which the adjuvants enable enhanced *in vivo* immune responses to the immunogens. In contrast, WO/2014/182661 proposes an itinerated dosing approach for administration of lipid-formulated nucleic acids as a strategy to avoid the adverse acute immune responses (known as "infusion-related reactions") that are often observed shortly after treatment.

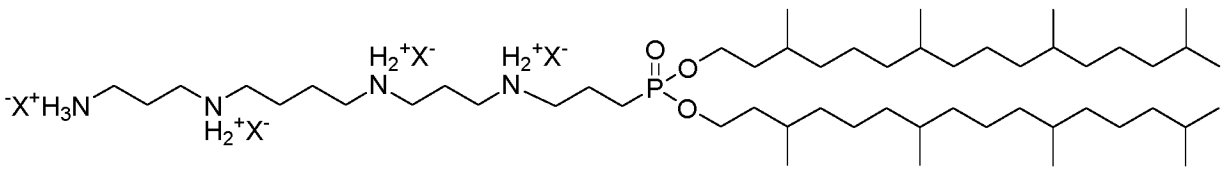
Lastly, some cationic lipids used for delivery of nucleic acids can show toxicity, which inventors have sought to reduce. For instance, US/2016/0009637 relates to the delivery of nucleic acids (siRNAs, aptamers or plasmids) using biodegradable cationic lipids that, relative to other cationic lipids, purportedly offer specific pharmacologic benefits (*e.g.* faster clearance) and consequently, lower toxicity.

Interestingly, we have previously described (US/2013/0336996 and EP/2674170) conjugates used to complex nucleic acids, in which TLR7 or TLR8 agonists are covalently bound to cationic lipids, the latter of which include the cationic lipid listed therein as "Intermediate 11" and denoted herein as "CL338".

DETAILED DESCRIPTION

One object of the invention is a complex formed from the following compounds:

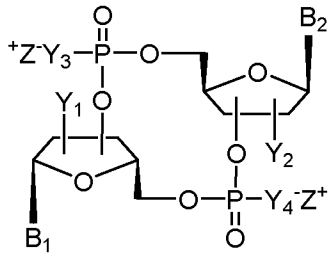
a. a lipid compound of Formula I:



Formula I

wherein X^- is a pharmaceutically acceptable anion ; and

b. a cyclic purine dinucleotide of Formula II:



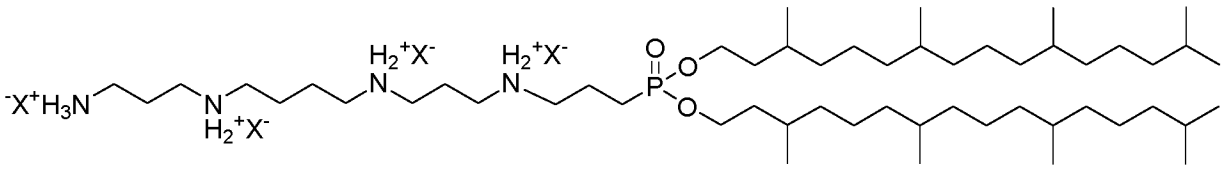
Formula II

wherein:

- B_1 and B_2 are purine bases independently chosen from adenine, guanine or hypoxanthine;
- Y_1 and Y_2 are independently chosen from H, OH or F;
- Y_3 and Y_4 are independently chosen from O or S; and
- Z^+ is a pharmaceutically acceptable cation.

Within the meaning of the present invention, the term “complex” means the chemical entity formed by the association of two or more compounds, in the present case compound of Formula I and compound of Formula II.

The lipid of Formula I is polycationic at physiological pH (i.e. it is positively charged on its amino groups at physiological pH).



Formula I

X^- is a pharmaceutically acceptable anion. It can be a polyatomic anion or a monoatomic anion. Polyatomic anions include for instance acetate, carbonate, nitrate, sulfate, hydrogen phosphate. Monoatomic anions include for instance halides such as chloride, fluoride, bromide, iodide. For example, X^- can be chosen from chloride, acetate, benzenesulfonate, benzoate, bromide, carbonate, citrate, fluoride, formate, fumarate, galacturonate, gluconate, glutarate, lactate, nitrate, succinate, tartrate, maleate, phosphate, pyruvate, sulfate, tosylate, trifluoroacetate or any other pharmaceutically acceptable anion.

The cyclic purine dinucleotide of Formula II (CDN) is dianionic at physiological pH (i.e. it is negatively charged on its phosphate or phosphorothioate groups at physiological pH).

In the present invention, the term “cyclic dinucleotide” (abbreviated as “CDN”) represents a class of cyclic molecules with two phosphodiester linkages, or two phosphorothioate diester linkages, or one phosphodiester linkage and one phosphorothioate diester linkage, between two nucleosides. This includes (3’,5’)-(3’,5’) nucleotide linkages (abbreviated as (3’,3’)); (3’,5’)-(2’,5’) nucleotide linkages (abbreviated as (3’,2’)); (2’,5’)-(3’,5’) nucleotide linkages (abbreviated as (2’,3’)); and (2’,5’)-(2’,5’) nucleotide linkages (abbreviated as (2’,2’)).

The term “nucleoside” refers to a glycosylamine comprising a nitrogenous base and a five-carbon sugar, wherein the nitrogenous base is bound to the five-carbon sugar via a beta-glycosidic linkage.

The term “nucleotide” refers to any nucleoside linked to a phosphate group at position 5’, 3’ or 2’ of the sugar moiety.

A particular class of CDN is CDNs in which at least one of the ribose sugars is substituted with a fluorine atom at the 2’ position.

Z^+ is a pharmaceutically acceptable cation. It can be a polyatomic cation or a monoatomic cation. Polyatomic cations include for instance ammonium, phosphoniums and sulfoniums. Monoatomic cations include for instance cations derived from alkali metals such as potassium and sodium, and alkaline earth metals such as calcium and magnesium.

In one embodiment, the cyclic purine dinucleotide of Formula II is an agonist of stimulator of interferon genes (STING).

In one embodiment, the cyclic purine dinucleotide of Formula II is chosen from the following compounds (as defined in Table 1): CL592, CL603, CL605, CL632, CL657, CL655, CL656, CL614, CL661, CL674, CL695, CL702.

In another embodiment, the cyclic purine dinucleotide of Formula II is chosen from the following compounds (as defined in Table 1), in which the internucleotide linkages in the CDN are two phosphodiester linkages: CL592, CL603, CL605, CL657, CL614, CL674.

In another embodiment, the cyclic purine dinucleotide of Formula II is chosen from the following compounds (as defined in Table 1), in which the internucleotide linkages in the CDN are two phosphorothioate linkages: CL632, CL655, CL656, CL661, CL695, CL702.

In another embodiment, the cyclic purine dinucleotide of Formula II is chosen from the following compounds (as defined in Table 1), in which at least one of the ribose sugars in the CDN is substituted with a fluorine atom at the 2’ position: CL603, CL632, CL614, CL656, CL674, CL702.

Another object of the invention is a pharmaceutical composition comprising the complex of the invention and a pharmaceutically acceptable excipient.

A pharmaceutically acceptable excipient means an excipient or carrier that is useful in preparing a pharmaceutical composition that is safe, non-toxic and neither biologically nor otherwise undesirable.

In one embodiment, the pharmaceutical composition further comprises a surfactant. In the sense of the present invention, the term surfactant means any compound having both a lipophilic portion and a hydrophilic portion in particular a ionic or non-ionic surfactant, which confers to the complex an enhancement of a particular aspect of formulation such as, but not limited to, solubility.

In another embodiment, a complex described in any of the preceding embodiments is added to cell cultures in order to provoke a STING pathway-dependent response such as, but not limited to, production of cytokines (*e.g.* Type I and Type III interferons, TNF-α, IL-1β, IL-6, etc.). Accordingly, another object of the invention is the use of the complex of the invention to induce the production of cytokines in mammalian cell cultures, in particular, by activation of a STING pathway.

In another embodiment, a complex described in any of the preceding embodiments is added to a mammalian blood or tissue sample *ex vivo* in order to provoke a STING pathway-dependent response such as, but not limited to, production of cytokines (*e.g.* Type I and Type III interferons, TNF- α, IL-1β, IL-6, etc.). Accordingly, another object of the invention is the use of a complex of the invention to induce the production of cytokines in mammalian blood or tissue samples *ex vivo,* in particular by activation of a STING pathway.

In another embodiment, a complex described in any of the preceding embodiments is administered to a living mammal in order to provoke a STING pathway-dependent response such as, but not limited to, production of cytokines (*e.g.* Type I and Type III interferons, TNF- α, IL-1β, IL-6, etc.). Accordingly, another object of the invention is the complex of the invention for use to induce the production of cytokines in a mammal, in particular by activation of a STING pathway.

In another embodiment, a complex described in any of the preceding embodiments is administered to a living mammal in order to provoke a STING pathway-dependent therapeutic response such as, but not limited to, an anti-tumor response, or other therapeutic response that is useful for treatment of cancer, such as an anti-metastatic response. Accordingly, another object of the invention is the complex of the invention for use in the treatment of cancer.

As used herein, the term "cancer" refers to the physiological condition in subjects that is characterized by unregulated or dysregulated cell growth or death. The term "cancer" includes solid tumors and blood born tumors, whether malignant or benign.

In one embodiment, the cancer is acinar adenocarcinoma, acinar carcinoma, acral-lentiginous melanoma, actinic keratosis, adenocarcinoma, adenocystic carcinoma, adenosquamous carcinoma, adnexal carcinoma, adrenal rest tumor, adrenocortical carcinoma, aldosterone secreting carcinoma, alveolar soft part sarcoma, amelanotic melanoma, ameloblastic thyroid carcinoma, angiosarcoma, apocrine carcinoma, Askin's tumor, astrocytoma, basal cell carcinoma, basaloid carcinoma, basosquamous cell carcinoma, biliary cancer, bone cancer, bone marrow cancer, botryoid sarcoma, brain cancer, breast cancer, bronchioalveolar carcinoma, bronchogenic adenocarcinoma, bronchogenic carcinoma, carcinoma ex pleomorphic adenoma, cervical cancer, chloroma, cholangiocellular carcinoma, chondrosarcoma, choriocarcinoma, choroid plexus carcinoma, clear cell adenocarcinoma, colon cancer, colorectal cancer, comedocarcinoma, cortisol-producing carcinoma, cylindrical cell carcinoma, dedifferentiated liposarcoma, ductal adenocarcinoma of the prostate, ductal carcinoma, ductal carcinoma in situ, duodenal cancer, eccrine carcinoma, embryonal carcinoma, endometrial carcinoma, endometrial stromal carcinoma, epithelioid sarcoma, esophageal cancer, Ewing's sarcoma, exophytic carcinoma, fibroblastic sarcoma, fibrocarcinoma, fibrolamellar carcinoma, fibrosarcoma, follicular thyroid carcinoma, gallbladder cancer, gastric adenocarcinoma, giant cell carcinoma, giant cell sarcoma, giant cell tumor of bone, glioma, glioblastoma multiforme, granulose cell carcinoma, head & neck cancer, hemangioma, hemangiosarcoma, hepatoblastoma, hepatocellular carcinoma, Hürthle cell carcinoma, ileal cancer, infiltrating lobular carcinoma, inflammatory carcinoma of the breast, intraductal carcinoma, intraepidermal carcinoma, jejuna cancer, Kaposi's sarcoma, Krukenberg's tumor, Kulchitsky cell carcinoma, Kupffer cell sarcoma, large cell carcinoma, larynx cancer, lentigo maligna melanoma, liposarcoma, liver cancer, lobular carcinoma, lobular carcinoma in situ, lung cancer, lymphoepithelioma, lymphoepithelioma, lymphosarcoma, malignant melanoma, medullary carcinoma, medullary thyroid carcinoma, medulloblastoma, meningeal carcinoma, Merkel cell carcinoma, micropapillary carcinoma, mixed cell sarcoma, mucinous carcinoma, mucoepidermoid carcinoma, mucosal melanoma, myxoid liposarcoma, myxosarcoma, nasopharyngeal carcinoma, nephroblastoma, neuroblastoma, nodular melanoma, non-clear cell renal cancer, non-small cell lung cancer, oat cell carcinoma, ocular melanoma, oral cancer, osteoid carcinoma, osteosarcoma, ovarian cancer, Paget's carcinoma, pancreatic cancer, pancreatoblastoma, papillary adenocarcinoma, papillary carcinoma, papillary thyroid carcinoma, pelvic cancer, periampullary carcinoma, phyllodes tumor, pituitary cancer, pleomorphic liposarcoma, pleuropulmonary blastoma, primary intraosseous carcinoma, prostate cancer, rectal cancer, renal cell carcinoma, retinoblastoma, rhabdomyosarcoma, round cell liposarcoma, scar cancer, schistosomal bladder cancer, schneiderian carcinoma, sebaceous carcinoma, signet-ring cell carcinoma, skin cancer, small cell lung cancer, small cell osteosarcoma, soft tissue sarcoma, spindle cell carcinoma, spindle cell sarcoma, squamous cell carcinoma, stomach cancer, superficial spreading melanoma, synovial sarcoma, telangiectatic sarcoma, terminal duct carcinoma, testicular cancer, thyroid cancer, transitional cell carcinoma, tubular carcinoma, tumorigenic melanoma, undifferentiated carcinoma, urachal adenocarcinoma, urinary bladder cancer, uterine cancer, uterine corpus carcinoma, uveal melanoma, vaginal cancer, cerrucous carcinoma, villous carcinoma, well-differentiated liposarcoma, Wilm's tumor or yolk sac tumor.

In another embodiment, a complex described in any of the preceding embodiments is administered to a living organism in order to provoke the STING pathway-dependent therapeutic response such as, but not limited to, an anti-viral or anti-microbial response, or other therapeutic response that is useful for treatment of infectious diseases. Accordingly, another object of the invention is the complex of the invention for use in the treatment of an infectious disease, typically bacterial, viral, fungal or parasitic infectious diseases.

In another embodiment, a complex described in any of the preceding embodiments is administered to a living organism as a vaccine adjuvant to a mammal to provoke a STING pathway-dependent therapeutic response. Accordingly, another object of the invention is the complex of the invention for use as a vaccine adjuvant.

The term “adjuvant” refers to a secondary therapeutic substance that is administered together (either sequentially in any order, or concurrently) with a primary therapeutic substance to achieve some kind of complimentary, synergic or otherwise beneficial effect that could not be achieved through use of the primary therapeutic substance alone. An adjuvant can be used together with a vaccine, chemotherapy, or some other therapeutic substance. Adjuvants can enhance the efficacy of the primary therapeutic substance, reduce the toxicity or side effects of the primary therapeutic substance, or provide some kind of protection to the subject that receives the primary therapeutic substance, such as, but not limited to, improved functioning of the immune system.

The complex or pharmaceutical composition described herein can be administered by any suitable route including, but not limited to the following routes: enteral, gastroenteral, oral, transdermal, epidural (peridural), intracerebral (into the cerebrum), intracerebroventricular (into the cerebral ventricles), epicutaneous (application onto the skin), intradermal, (into the skin itself), subcutaneous (under the skin), nasal administration (through the nose), intravenous (into a vein), intraarterial (into an artery), intramuscular (into a muscle), intracardiac (into the heart), intraosseous infusion (into the bone marrow), intrathecal (into the spinal canal), intraperitoneal, (infusion or injection into the peritoneum), intravesical infusion, intravitreal, (through the eye), intracavemous injection, (into the base of the penis), intravaginal administration, intrauterine, intratumoral, extra-amniotic administration, transdermal (diffusion through the intact skin for systemic distribution), transmucosal (diffusion through a mucous membrane), insufflation (snorting), sublingual, sublabial, enema, eye drops (onto the conjunctiva), or in ear drops. The pharmaceutical compositions can also be administered via topical administration.

In one embodiment, the complex or pharmaceutical composition described herein is administered in a way which allows them cross the bloodbrain barrier, vascular barrier, or other epithelial barrier.

In one embodiment, the complex or pharmaceutical composition described herein are administered via subcutaneous, intravenous, intraperitoneal, intratumoral, intranasal, oral, intravaginal, transdermal or topical administration.

Another object of the invention is a method for preparing the complex of the invention, said method comprising the step of contacting the compound of Formula I with the compound of Formula II.

The contact between the compound of Formula I and the compound of Formula II can be performed by any means known in the art, e.g., by mixing a solution of the compound of Formula I and a solution of the compound of Formula II.

The concentration of each of the constituent solutions is adjusted prior to mixing such that the desired final compound of Formula I/compound of Formula II ratio and the desired final concentration of compound of Formula II is obtained upon mixing the two solutions.

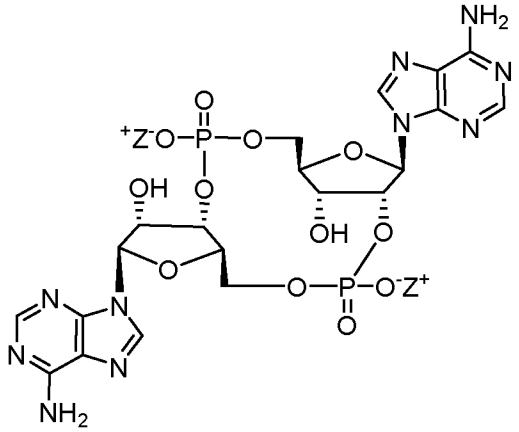
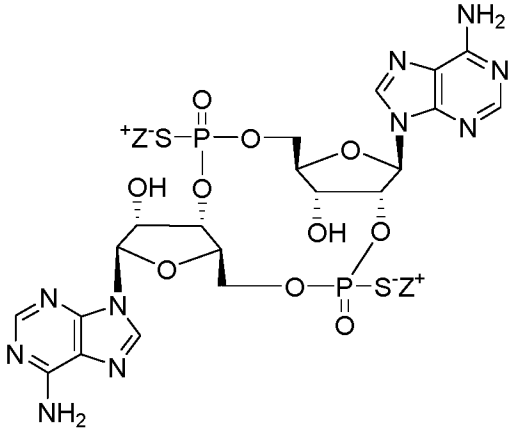
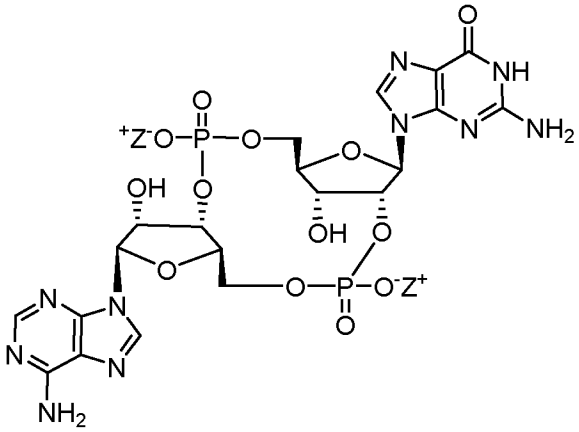
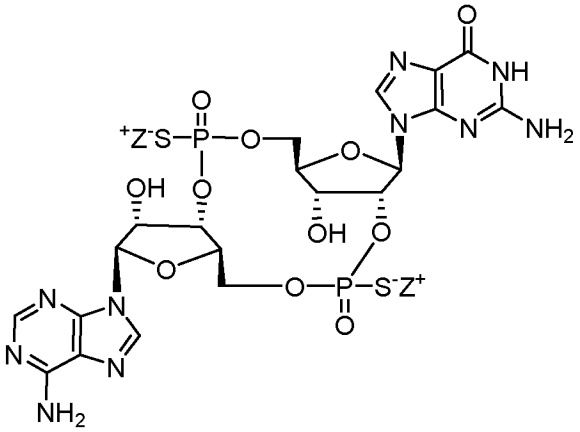
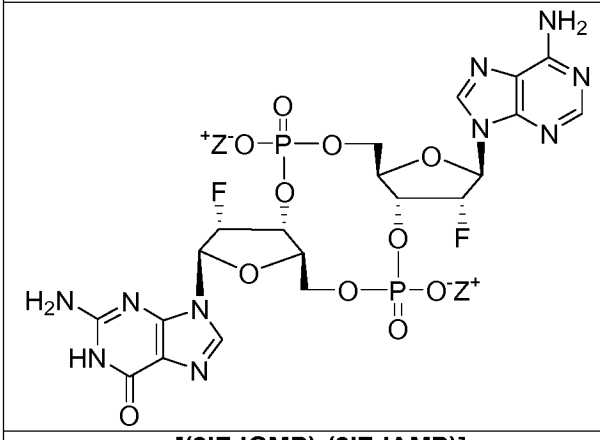
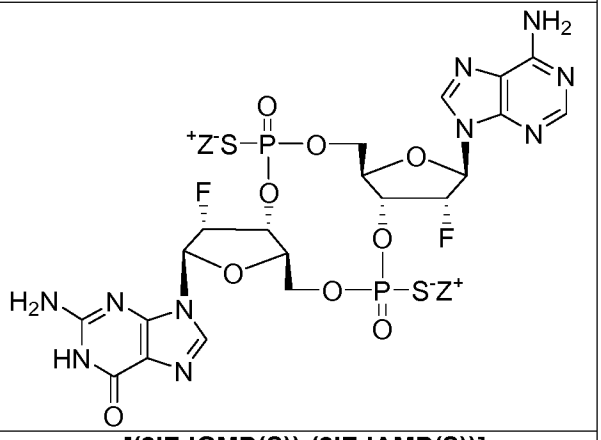
The contact between compound of Formula I and compound of Formula II is preferably performed in an aqueous medium. Typically, an aqueous solution of compound of Formula I and an aqueous solution of compound of Formula II are prepared. For instance, compound of Formula I can be dissolved first in ethanol, then the resulting solution is diluted with H_2O, or an aqueous solution, to a predetermined concentration. Compound of Formula II can be dissolved either in H_2O or an aqueous solution such as aqueous glucose (e.g. 5%). Then, a volume of the solution of compound of Formula I is added dropwise to a volume of the solution of compound of Formula II. The resultant solution is vortexed and then, incubated for at least 20 minutes at room temperature.

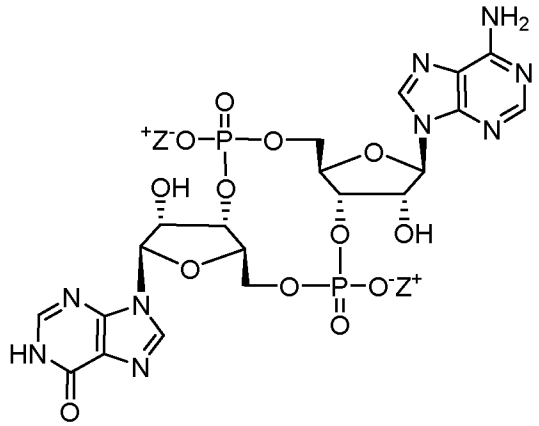
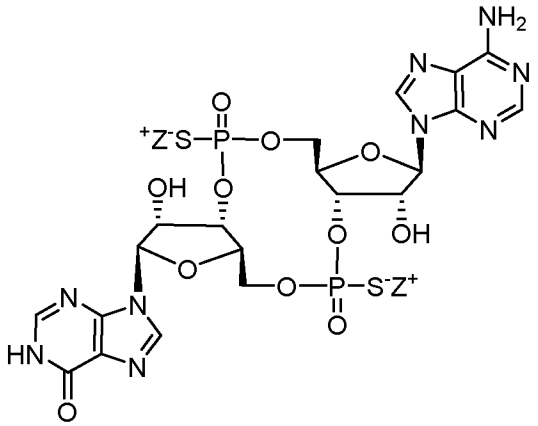
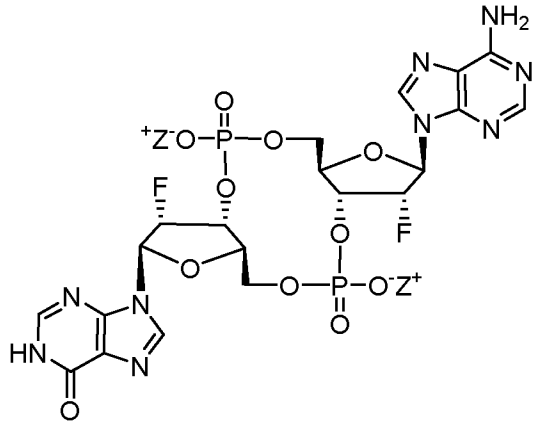
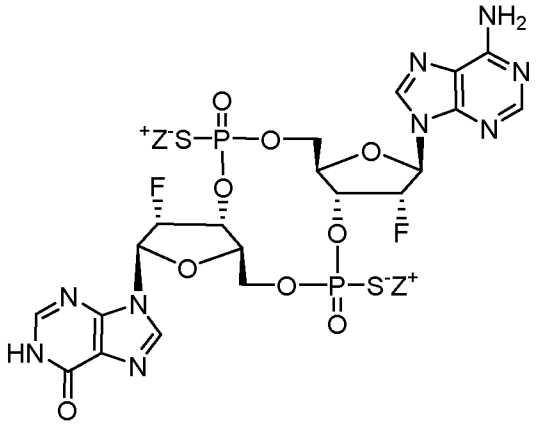
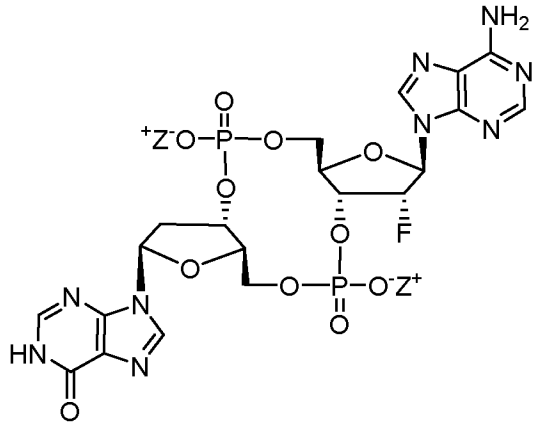
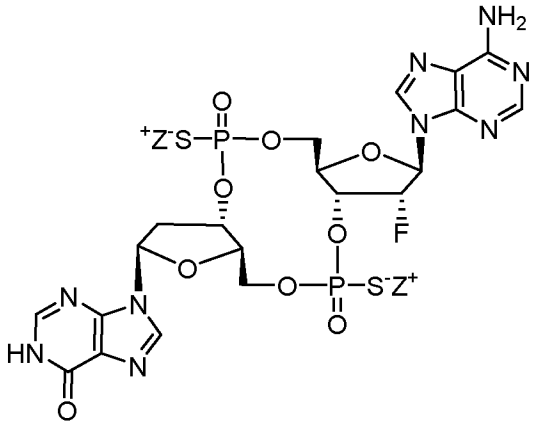
The size of the complex of the invention is typically from 100 nm to 200 nm, as measured by using a Zetasizer Nano ZS (Malvern) at a scattering angle of 90° at 25 °C.

EXAMPLE 1: CHEMISTRY

The CDNs used in the present invention and their corresponding code numbers (format: CL###) are shown below in table **1**.

TABLE 1. The CDNs used in the present invention.

	
(2',3')c-diAMP **CL657**	**(2',3')c-diAM(PS)$_2$** **CL695**
	
(2',3')c-GAMP **CL605**	**(2',3')c-GAM(PS)$_2$** **CL661**
	
c-[(2'FdGMP)-(2'FdAMP)] **CL603**	**c-[(2'FdGMP(S))-(2'FdAMP(S))]** **CL632**

	
cAIMP **CL592**	**cAIM(PS)$_2$** **CL655**
	
c-[(2'FdAMP)-(2'FdIMP)] **CL614**	**c-[(2'FdAMP(S))-(2'FdIMP(S))]** **CL656**
	
c-[(2'FdAMP)-(dIMP)] **CL674**	**c-[(2'FdAMP(S))-(dIMP(S))]** **CL702**

Wherein Z is as defined above.

Example 1.1: Synthesis of CDNs

The CDNs CL592, CL655, CL603, CL632, CL614, CL656, CL674 and CL702 were synthesized in the form of a sodium salt according to a procedure similar to the one described in PCT/EP2015/070635. The remaining CDNs were obtained from InvivoGen:

- CL603 (catalog code: tlrl-nacgaf),
- CL605 (catalog code: tlrl-nacga23),

- CL614 (catalog code: tlr-nacaidf),
- CL656 (catalog code: tlr-nacairs),
- CL657 (catalog code: tlr-nacda-23),
- CL661 (catalog code: tlrl-cga2srs),
- CL674 (catalog code: tlr-nacaimf) and
- CL695 (catalog code: tlr-nacda2r-01).

Note on phosphorothioate CDNs: The phosphodiester linkages in naturally occurring CDNs are both non-chiral and prochiral sites. Substitution of one of the oxygen atoms of the phosphate moiety of a nucleotide with another atom yields an asymmetric center on the phosphorus atom. Since a nucleotide unit already contains a first asymmetrical center within its sugar moiety, further asymmetry at the phosphorus atom of the nucleotide yields a diasymmetric nucleotide. Such a diasymmetric nucleotide is a chiral nucleotide having Sp and Rp diastereomers. Phosphorothioate CDNs can be synthesized using known procedures, which generate racemic mixtures of Rp and Sp diastereomers at the individual phosphorothioate linkages. Thus, theoretically a phosphorothioate CDN can have 2^n possible diastereoisomeric forms, in which *n* is the number of phosphorothioate internucleosidic linkages in the molecule. Accordingly, a CDN with two phosphorothioate linkages can have 2^2 (four) possible stereoisomers.

Example 1.2: Characterization of CDNs by HPLC/MS, ^{1}H-NMR and ^{31}P-NMR

Abbreviations used in this section: A: adenosine; d: doublet; dA: deoxyadenosine; dd: doublet of doublets; dI: deoxyinosine; D_2O: deuterium oxide; ES: electrospray ionization; ^{1}H: proton; Hz: hertz; I: inosine; LC: liquid chromatography; m: multiplet; m/z: mass-to-charge ratio; MHz: megahertz; MS: mass spectrometry; NMR: nuclear magnetic resonance; s: singlet; t: triplet; δ: chemical shift.

LC/MS: Analytical LC/ES-MS was performed on an Agilent 1290 Infinity UHPLC system coupled to a diode array detector (DAD; Agilent 1260 Infinity), and on an Agilent 6130 Quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source, controlled by ChemStation (Agilent) software. The LC system was equipped with an Aquity CSH C18 50×2.1 mm 1.7 µm column using gradients of 10 mM ammonium formate and acetonitrile at a flow-rate of 300 µL/min. The UV detection wavelength was 254 nm. The mass spectrometer was operated in positive and negative ESI modes.

NMR: ^{1}H-NMR spectra were acquired on a Bruker 300 MHz (Fourier 300) at room temperature and are reported in ppm downfield from the reference peak of the indicated solvent. ^{31}P-NMR spectra were acquired on a Bruker 500 MHz at room temperature and reported in ppm downfield from the reference peak of the indicated solvent.

The physicochemical properties of the CDNs of the present invention, including their LC/MS retention times and characteristic NMR peaks, are summarized below in table **2**.

TABLE 2. Physicochemical properties of the CDNs of the present invention.

CDN	Chemical formula	LC retention time (min)	MS $[M+1]^+$ $[M-1]^-$	^{1}H-NMR (D_2O, 300 MHz) δ (ppm)	^{31}P-NMR (D_2O, 200 MHz) δ (ppm)
CL657	$C_{20}H_{24}N_{10}O_{12}P_2$	0.575	659 657	8.43 (s, 1H), 8.21 (s, 1H), 8.08 (s, 1H), 8.00 (s, 1H), 6.19 (d, 1H), 6.07 (s, 1H), 5.29 (m, 1H), 4.92 (m, 1H), 4.54 (m, 1H), 4.42 (m, 2H), 4.17-4.08 (m, 4H)	-1.95 -1.94
CL695	$C_{20}H_{24}N_{10}O_{10}P_2S_2$		689 691	8.35 (s, 1H), 8.14-8.11 (m, 2H), 7.94-7.80 (m, 3H), 6.21 (m, 2H), 5.64-5.40 (m, 2H), 5.00 (m, 3H), 4.45 (m, 2H), 4.21 (m, 2H) 4.03-3.92 (m, 6H),	55.52 55.22 51.62 51.52
CL605	$C_{20}H_{24}N_{10}O_{13}P_2$	0.507	675 673	8.26 (s, 1H), 8.22 (s, 1H), 7.81 (s, 1H), 6.13 (s, 1H), 5.91 (d, 1H), 5.61 (m, 1H), 4.99 (m, 1H), 4.54 (m, 1H), 4.42 (m, 2H), 4.17-4.08 (m, 4H)	-1.52 -1.94
CL661	$C_{20}H_{24}N_{10}O_{11}P_2S_2$	1.911 2.306	707 705	8.57 (s, 1H), 8.27 (s, 1H), 7.88 (s, 1H), 6.17 (s, 1H), 5.93 (t, 1H), 5.85-5.50 (m, 1H), 5.35-5.05 (m, 2H), 4.55-4.35 (m, 5H), 4.32-3.99 (m, 3H)	55.85 55.52 51.43 51.39
CL603	$C_{20}H_{22}F_2N_{10}O_{11}P_2$	2.481	679 677	8.17 (s, 1H), 7.83 (s, 1H), 7.63 (s, 1H), 6.09 (d, 1H), 5.94 (d, 1H), 5.62 (m, 1H), 5.50 (m, 1H), 5.15 (m, 2H), 4.42 (m, 4H), 4.03 (m, 2H)	-1.74 -1.99
CL632	$C_{20}H_{22}F2N_{10}O_9P_2S_2$	1.800 2.022	711 709	8.58 (s, 1H), 8.35 (s, 1H), 7.97 (s, 1H), 6.65 (d, 1H), 6.19 (d, 1H), 4.94 (d, 1H), 4.62-4.50 (m, 4H), 4.42 (m, 4H), 4.03 (m, 2H)	54.90 54.78 54.60 54.56
CL592	$C_{20}H_{23}N_9O_{13}P_2$	2.725	660 658	8.34 (s, 1H), 8.27 (s, 1H), 8.10 (s, 1H), 7.86 (s, 1H), 5.94 (s, 2H), 5.06-4.80 (m, 4H), 4.42 (m, 4H), 4.03 (m, 2H)	-1.48 -1.95
CL655	$C_{20}H_{23}N_9O_{11}P_2S_2$	3.452	692 690	8.54 (s, 1H), 8.37 (s, 1H), 8.22 (s, 1H), 7.97 (s, 1H), 6.65 (dd, 1H), 6.15 (dd, 1H), 4.60-4.50 (m, 4H),4.45 (m, 4H), 4.05 (m, 2H)	54.60 54.49 54.10 54.07
CL614	$C_{20}H_{21}F_2N_9O_{11}P_2$	2.786	664 662	8.37 (s, 1H), 8.35 (s, 1H), 8.14 (s, 1H), 7.95 (s, 1H), 6.23 (m, 2H), 5.45 (m, 2H), 5.39 (m, 1H), 4.95 (m, 2H), 4.50 (m, 2H), 4.06 (m, 2H)	-1.88 -1.92
CL656	$C_{20}H_{21}F_2N_9O_9P_2S_2$	3.412	696	8.55 (s, 1H), 8.38 (s, 1H),	54.83

			694	8.20 (s, 1H), 7.99 (s, 1H), 6.63 (dd, 1H), 6.15 (dd, 1H), 5.16-4.95 (m, 4H),4.52 (m, 4H), 4.07 (m, 2H)	54.45 54.37 54.15 54.08
CL674	$C_{20}H_{22}FN_9O_{11}P_2$		646 644	7.99 (s, 1H), 7.88 (s, 1H), 7.79 (s, 1H), 7.72 (s, 1H), 6.12 (m, 1H), 5.61-5.43 (m, 1H), 4.43 (m, 1H), 4.23 (m, 1H), 4.07 (m, 2H), 3.97 (m, 2H)	-1.05 -1.68
CL702	$C_{20}H_{22}FN_9O_9P_2S_2$		678 676	8.48 (s, 1H), 8.24-8.21 (m, 2H), 8.04-7.90 (m, 3H), 6.31 (m, 2H), 5.74-5.50 (m, 2H), 5.02 (m, 3H), 4.45-3.92 (m, 10H),	55.95 55.71 54.76 54.29

Example 1.3: Synthesis of the cationic lipid complexing agent CL338

CL338 (CAS# 1510712-65-8) was synthesized as described in patent n° EP 2 674 170.

Example 1.4: Standard procedure for preparation of CDN/CL338 complexes

The following procedure was used to form complexes between the complexing agent CL338 and a CDN (CL592, CL603, CL605, CL614, CL632, CL655, CL657, CL661, CL656, CL674, CL695 or CL702).

In a typical experiment, CL338 was dissolved (50 mg/mL) in 100% EtOH and then, the resulting solution was diluted in H_2O to a final concentration of 1 mg/mL. The CDN was dissolved (400 µg/mL) in either H_2O or 5% aqueous glucose in a glass container. An aliquot of the CL338 solution was added dropwise to an aliquot (of equal volume) of the CDN solution. The resultant solution was vortexed, and then incubated for at least 20 minutes at room temperature.

Example 1.5: Characterization of complexes for particle size, polydispersity and net charge

Test samples of CDN/CL338 complexes were diluted in H_2O. The mean particle size (Z; in nm), particle-size distribution (polydispersity index [PDI]; unit-less) and net charge (zeta potential [ζ]; in mV) were measured using a Zetasizer Nano ZS (Malvern). The particle size was measured at a scattering angle of 90° at 25 °C. The values represent the mean of at least three measurements.

The mean Z and PDI values for the CDN/CL338 complexes of the present invention are shown in **Figure 1**, which reveal that the complexes range in diameter from *ca.* 120 nm to *ca.* 180 nm (left side of figure) and are relatively homogeneous in terms of PDI (values of *ca.* 0.1 to 0.2; right side of figure). The corresponding mean ζ values are shown in **Figure 2**, which illustrates that all of the CDN/CL338 complexes are stable (*i.e.* not prone to aggregation in solution), as the magnitude of each value exceeds the commonly accepted cutoff value of 30 mV (see, for example: Duffy *et al.*, 2011).

EXAMPLE 2: BIOLOGICAL ASSAYS

Reporter cell lines

All reporter cell lines used in the present invention were obtained directly from InvivoGen (listed here with the corresponding InvivoGen catalog code) or derived from InvivoGen cell lines.

HEK-Blue™ IFN-α/β-KO-STING: These cells, in which the STING gene has been inactivated, are derived from HEK293 cell line known as HEK-Blue™ IFN-α/β (InvivoGen catalog code: hkb-ifnab). HEK-Blue™ IFN-α/β cells enable detection of bioactive human type I IFNs through monitoring of activation of the ISG3 pathway. These cells were generated by stable transfection of HEK293 cells with the human STAT2 and IRF9 genes to obtain a fully active Type-I IFN signaling pathway. The other genes of the pathway (IFNAR1, IFNAR2, JAK1, TyK2 and STAT1) are naturally expressed in sufficient amounts. The cells were further transfected with a SEAP reporter gene under control of an IFN-α/β-inducible ISG54 promoter. This promoter comprises five IFN-stimulated response elements (ISREs) fused to a minimal promoter of the human ISG54 gene, which is unresponsive to activators of the NF-κB or AP-1 pathways. Stimulation of HEK-Blue™ IFN-α/β cells with human IFN-α or IFN-β activates the JAK/STAT/ISGF3 pathway and subsequently induces production of SEAP. Production of Type I interferons in these cells is measured using QUANTI-Blue™.

HEK-Blue™ IL-1R (InvivoGen catalog code: hkb-il1r): The HEK293 cell line known as HEK-Blue™ IL-1R was designed to detect bioactive human and murine IL-1 through monitoring of activation of the NF-κB and AP-1 pathways. Additionally, these cells detect bioactive IL-1 from cynomolgus monkeys, dogs, hamsters and rats. In fact, HEK-Blue™ IL-1R cells can detect IL-1α and IL-1β, as these cytokines bind to the same receptor, IL-1R. These cells derive from HEK-Blue™ IL-1β cells (InvivoGen catalog code: hkb-il1b), in which the TNF-α response is blocked. Therefore, HEK-Blue™ IL-1R cells respond specifically to IL-1. These cells endogenously express the human IL-1 receptor and were stably transfected with the murine IL-1 receptor, rendering them sensitive to both human and murine IL-1β. HEK-Blue™ IL-1R cells express a SEAP reporter gene under control of an IFN-β minimal promoter fused to five NF-κB and five AP-1 binding sites. Binding of IL-1β to IL-1R on the surface of HEK-Blue™ IL-1R cells triggers a signaling cascade that leads to the activation of NF-κB and subsequent production of SEAP. Production of IL-1β in these cells is measured using QUANTI Blue™.

HEK-Blue™ IL-28: This HEK293 cell line is derived from HEK-Blue™ ISG cells (InvivoGen catalog code: hkb-isg). It enables detection of bioactive Type III IFNs (IL-28A [IFN-λ2], IL-28B [IFN-λ3] and IL-29 [IFN-λ1]) through monitoring of activation of the ISG54 pathway. HEK-Blue™ IL-28 cells were generated by inactivation of the IFNAR2 and IFNGR1 genes, to abolish (*i.e.* reduce to undetectable levels) the Type I and Type II IFN response, followed by stable transfection with the human IFNLR1 and IL10R genes, to obtain a strong Type III IFN response. The other genes of the (shared Type I/Type III) IFN pathway (*IFNAR1, JAK1, TyK2* and *STAT1*) are naturally expressed in sufficient amounts. The resultant cells were then transfected with a SEAP reporter gene under control of a

promoter that comprises five IFN-stimulated response elements (ISREs) fused to a minimal promoter of the human ISG54 gene, which is unresponsive to activators of the NF-κB or AP-1 pathways. Stimulation of HEK-Blue™ IL-28 cells with Type III IFNs activates the JAK/STAT/ISGF3 pathway and subsequently induces production of SEAP, which is measured using QUANTI-Blue™.

HEK-Blue™ TNF-α (InvivoGen catalog code: hkb-tnfdmyd): HEK-Blue™ TNF-α cells are a HEK293 cell line that enables detection of bioactive human and murine TNF-α through monitoring of activation of the NF-κB pathway. These cells were generated by stable transfection of HEK293 cells with a SEAP reporter gene under control of an IFN-β minimal promoter fused to five NF-κB and five AP-1 binding sites. They were further rendered unresponsive to IL-1β by knocking out the MyD88 gene. Stimulation of HEK-Blue™ TNF-α cells with TNF-α triggers activation of the NF-κB-inducible promoter and production of SEAP. Production of TNF-α in these cells is measured using QUANTI-Blue™.

ISG Cell lines: The following three cell lines express a secreted luciferase (Lucia) reporter gene under control of an IRF-inducible promoter. This composite promoter comprises five IFN-stimulated response elements (ISREs) fused to a minimal promoter of the human ISG54 gene, which is unresponsive to activators of the NF-κB or AP-1 pathways. Hence, these cells enable monitoring of the IRF pathway based on luciferase (Lucia) or SEAP activity. In the present invention, monitoring of the IRF pathway is used to measure STING activity:

RAW-Lucia™ ISG (InvivoGen catalog code: rawl-isg): These cells were generated from the RAW 264.7 murine macrophage cell line (ATCC® TIB-71™).

HEK293-T-ISG: These cells were generated from the HEK-293T human embryonic kidney cell line (ATCC® CRL-3216™).

HEK293-PEAKrapid-ISG: These cells were generated from the HEK-293 PEAKrapid human embryonic kidney cell line (ATCC® CRL-2828™).

THP1-Dual™ (InvivoGen catalog code: thpd-nfis): These cells were derived from the human monocytic cell line THP-1 by stable integration of two inducible reporter constructs. They enable simultaneous study of the two main signaling pathways for STING: the NF-κB pathway, by monitoring the activity of secreted embryonic alkaline phosphatase (SEAP); and the IRF pathway, by assessing the activity of a secreted luciferase (Lucia).

THP1-Dual™-KO-STING (InvivoGen catalog code: thpd-kostg): These cells were generated from the human monocyte THP-1-Dual™, through stable homozygote knockout of the STING gene. Biallelic STING knockout was verified by functional assays, PCR and DNA sequencing.

Cytokine quantification

The aforementioned reporter proteins (SEAP and Lucia luciferase) are readily measurable in the cell culture supernatant when using QUANTI-Blue™ (InvivoGen catalog code: rep-qb1), a SEAP detection reagent that turns purple/blue in the presence of SEAP (quantified by measuring the optical density from 620 nm to 655 nm), or QUANTI-Luc™ (InvivoGen; catalog code: rep-qlc1), a luminometric enzyme assay that measures luciferase expression to report on ISG54 expression (as an indicator of IRF pathway induction and thus, IFN-α/β production).

Example 2.1: Comparison of CDN/CL338 complexes to the corresponding CDN alone, for STING pathway-dependent cytokine induction in cell cultures

- **Cytokine reporter cell lines used**: HEK293-PEAKrapid-ISG, THP1-Dual™ , RAW-Lucia™ ISG, THP1-Dual™-KO-STING
- **Complexes tested:** CL592/CL338, CL603/CL338, CL605/CL338, CL614/CL338, CL632/CL338, CL655/CL338, CL656/CL338, CL657/CL338, CL661/CL338, CL674/CL338, CL695/CL338 and CL702/CL338
- **Reference CDNs:** CL338, CL592, CL603, CL605, CL614, CL632, CL655, CL656, CL657, CL661, CL674, CL695 and CL702
- **Activities evaluated**: Type I IFN induction and NF-κB pathway induction.

Complexes of each CDN with CL338 were prepared according to the general procedure, at a ratio of 20 μg CDN to 50 μg CL338 in 100 μL sterile H_2O (final CDN concentration: 200 μg/mL).

To each well of a flat-bottom 96-well plate were added 10 μL of a solution of either a CDN alone (concentration: 200 μg/mL in sterile water) or a CDN/CL338 complex (see above), followed by 90 μL of a suspension of a cell line (*ca.* 100,000 cells per well). The plate was incubated for 24 h at 37 °C in 5% CO_2. Type I IFN induction was indirectly quantified using QUANTI-Luc™ (for the THP1-Dual™ and RAW-Lucia™ ISG) or QUANTI-Blue™ (for the HEK293-PEAKrapid-ISG), which were prepared and used according to the manufacturer's instructions. NF-κB pathway induction was indirectly quantified using QUANTI-Blue™, which was prepared and used according to the manufacturer's instructions.

The results from this experiment are shown in **Tables 3 to 5**, which reveal three important findings: firstly, that all of the tested CDNs and CDN/CL338 complexes induce Type I interferons in all three cell lines (**Table 3** [top]: THP1-Dual™; **Table 4**: RAW-Lucia™ ISG; and **Table 5**: HEK293-PEAKrapid-ISG); secondly, that all of them induce the NF-κB pathway in the cell line tested for this activity (**Table 3** (bottom): THP1-Dual™); and thirdly, that for both of these activities and in all cell lines tested, each CDN/CL338 complex was more active than was its corresponding CDN alone. Moreover, none of the CDNs or CDN/CL338 complexes induces cytokines in cells that lack STING (data not shown).

TABLE 3. Comparison of CDNs alone vs. their corresponding CDN/CL338 complexes for *in vitro* cytokine induction in THP-1 Dual™ cells: ISG-induced IRF pathway, to report on Type I IFNs (top); and NF-κB pathway, to report on pro-inflammatory cytokines (bottom). NC: not calculable.

EC_{50} for ISG-induced IRF pathway response			
CDN	CDN alone (µg/mL)	CDN/CL338 (µg/mL)	Fold increase
CL592	> 20	5.580	NC
CL655	> 20	0.595	NC
CL614	9.711	1.317	7
CL656	2.801	0.148	19
CL674	> 20	3.394	NC
CL702	> 20	0.396	NC
CL603	4.745	1.222	4
CL632	1.549	0.200	8
CL605	> 20	2.977	NC
CL661	20.750	2.568	8
CL657	> 20	14.610	NC
CL695	16.580	0.409	41

EC_{50} for NF-κB response			
CDN	CDN alone (µg/mL)	CDN/CL338 (µg/mL)	Fold increase
CL592	> 20	> 20	NC
CL655	> 20	0.809	NC
CL614	> 20	4.126	NC
CL656	> 20	0.404	NC
CL674	> 20	15.270	NC
CL702	> 20	0.666	NC
CL603	> 20	4.794	NC
CL632	> 20	0.525	NC
CL605	> 20	17.080	NC
CL661	> 20	8.677	NC
CL657	> 20	> 20	NC
CL695	> 20	0.717	NC

TABLE 4. Comparison of CDNs alone vs. their corresponding CDN/CL338 complexes for *in vitro* cytokine induction (ISG-induced IRF pathway, to report on Type I IFNs) in RAW-Lucia™ ISG cells. NC: not calculable.

EC_{50} for ISG-induced IRF pathway response			
CDN	CDN alone (µg/mL)	CDN/CL338 (µg/mL)	Fold increase
CL592	7.110	0.193	36.8
CL655	3.368	0.011	308
CL614	3.739	0.046	81
CL656	0.866	0.005	188
CL674	10.540	0.251	42
CL702	4.079	0.063	65
CL603	3.906	0.079	49

CL632	0.380	0.017	22
CL605	18.210	0.384	47
CL661	3.889	0.336	12
CL657	> 20	1.110	NC
CL695	2.912	0.039	74

TABLE 5. Comparison of CDNs alone vs. their corresponding CDN/CL338 complexes for *in vitro* cytokine induction (ISG-induced IRF pathway, to report on Type I IFNs) in HEK293-PEAKrapid-ISG cells. NC: not calculable.

EC_{50} for ISG-induced IRF pathway response			
CDN	CDN alone (µg/mL)	CDN/CL338 (µg/mL)	Fold increase
CL592	NC	NC	NC
CL655	> 20	0.98	NC
CL614	1.338	0.32	4.2
CL656	0.2613	0.027	9.6
CL674	> 20	5.5	NC
CL702	3.986	0.78	5.1
CL603	0.97	0.33	2.9
CL632	1.886	0.26	7.25
CL605	13.96	0.59	23.66
CL661	5.7	1.55	3.7
CL657	NC	NC	NC
CL695	NC	0.73	NC

Example 2.2: Comparison of CL338 with commercially available transfection reagents, for delivery of CDN STING agonists into cell cultures

In this experiment, the delivery of two representative CDN STING agonists into cells using CL338 was compared to that using different commercially available lipid or cationic transfection reagents. Each CDN was separately mixed with CL338 or one of the commercially available reagents, and then cells were added. STING pathway-dependent cytokine induction in the cells was used to measure the ability of each reagent to deliver the CDNs into cells.

- **Cytokine reporter cell lines used**: THP1-Dual™
- **Transfection reagents tested:** LyoVec™ (InvivoGen; catalog code: lyec-12); N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium methyl-sulfate (DOTAP; Roche; catalog code: 11202375001); Lipofectamine LTX® (ThermoFisher; catalog code: 15338500); FuGENE® HD (ProMega; catalog code: E2311); jetPEI® (Polyplus Transfection; catalog code: 101-01N); calcium chloride (Sigma-Aldrich; catalog code: C1016; tested only for CL702)
- **Reference transfection reagent:** CL338
- **CDNs tested:** CL592, CL605, CL632 and CL702
- **Activities evaluated**: Type I IFN induction and NF-κB pathway induction.

Each CDN was separately complexed with either CL338 or one of the commercially available transfection reagent (according to the manufacturer's instructions).

The ratios of CDN to transfection reagent used are listed below in **Table 6**.

TABLE 6. Composition of CDN (CL632 or CL702) complexes containing either CL338 or a commercially available transfection reagent. Note: the value for the parameter "N/P", which is specific to jetPEI®, was adapted from the manufacturer's instructions for DNA transfection.

Transfection reagent	Ratio of CDN to transfection reagent
LyoVec™	2:5 (w/w)
DOTAP	2:5 (w/w)
CL338	2:5 (w/w)
Lipofectamine LTX®	1:9 (w/v)
FuGENE® HD	2:5 (w/v)
jetPEI®	N/P = 5

Complexes using transfection reagents: Master solutions of each CDN alone, or of each CDN complex, were separately prepared in water at a CDN concentration of 2 µg/mL, and then serially diluted by a factor of three to provide a final range of six concentrations: 0.0082 µg/mL, 0.025 µg/mL, 0.0074 µg/mL, 0.22 µg/mL, 0.67 µg/mL and 2.0 µg/mL. A 20-µL aliquot of a single solution was added to a single well of a 96-well plate, such that each well corresponded to either a CDN alone at a single concentration, or to a CDN complex at a single concentration. As a control, the wells in one column of each plate were filled with an equivalent volume of water (*i.e.* test concentration = 0 µg/mL). To each well of each plate were then added 200,000 cells, and the plates were incubated for 24 hours at 37 ºC in 5% CO_2. The level of ISG54 activity (as an indicator of Type I IFN induction) was indirectly quantified using QUANTI-Luc™, which was prepared and used according to the manufacturer's instructions. The level of NF-κb pathway activation was measured using QUANTI-Blue™, which was prepared and used according to the manufacturer's instructions.

Complex of CL702 using $CaCl_2$: The CL702/$CaCl_2$ complex was formed as follows: 20 µg CL702 was mixed with 12.4 µL $CaCl_2$ (aq. 2M), and the resulting mixture was brought to a total volume of 100 µL and then, carefully added dropwise to 100 µL 2x HBS (HEPES buffered saline). After 30 minutes at room temperature, the resulting precipitate was serially diluted to provide a final range of six concentrations: 0.0082 µg/mL, 0.025 µg/mL, 0.0074 µg/mL, 0.22 µg/mL, 0.67 µg/mL and 2.0 µg/mL. The rest of the experiment was performed as described above for the transfection reagents, except that the cells were added to each well before the CL702/$CaCl_2$ complex was added.

The results for two of the four CDNs tested in this experiment are shown in **Figure 3A** (CL632: ISG-induced IRF pathway response, to report on Type I IFNs), **Figure 3B** (CL702: ISG-induced IRF pathway response, to report on Type I IFNs), **Figure 4A** (CL632: NF-κB pathway response, to report

on pro-inflammatory cytokines) and **Figure 4B** (CL702: NF-κB pathway response, to report on pro-inflammatory cytokines). The figures show that: firstly, most of the CDN/CL338 complex provides greater induction of ISG-induced IRF pathway (Type IFNs) and of the NF-κB pathway (pro-inflammatory cytokines) than does the corresponding CDN alone; and secondly, among the various complexes, those using CL338 gave the strongest response for both activities.

Example 2.3: Comparison of CDN/CL338 complexes with the corresponding CDN alone for STING pathway-dependent cytokine induction *ex vivo* in human whole-blood samples treated

- **Complexes tested:** CL592/CL338; CL603/CL338; CL605/CL338; CL614/CL338; CL632/CL338; CL655/CL338; CL656/CL338; CL657/CL338; CL661/CL338; CL674/CL338; CL695/CL338; and CL702/CL338
- **Reference compounds:** CL338; CL592; CL603; CL605; CL614; CL632; CL655; CL656; CL657; CL661; CL674; CL695; and CL702
- **Cytokines evaluated:** IFN-α/β (using HEK293-PEAKrapid-ISG cells), IL-1 (using HEK-Blue™ IL-1R cells), TNF-α (using HEK-Blue™ TNF-α cells) and IFN-λ (using HEK-Blue™ IL-28 cells)

Acquisition and handling of human blood samples

Human blood samples were acquired from healthy donors at the *Etablissement Français du Sang* (EFS Pyrénées Méditerranée, Toulouse, France; per agreement # 21/PLER/TOU/CAYLA01/2013-0071). Briefly, the samples were collected by venipuncture into sodium heparin tubes at the time of donation. The samples were analyzed for rhesus (Rh), blood group, hematocrit and serological status (AgHBS, HIV, HCV, HTLV, HBC, CMV, Syph). The tubes were picked up on the day of collection and subsequently tested (blood analysis, and treatment with test items) on the same day.

Treatment of human blood samples

Each blood sample was diluted (1:2 [v/v]) in RPMI medium and aliquoted into 96-well plates (180-µL wells) containing either a CDN at one of seven different concentrations (20 µg/mL, 6.7 µg/mL, 2.2 µg/mL, 0.7 µg/mL, 0.2 µg/mL, 0.08 µg/mL or 0.03 µg/mL), or a CDN/CL338 complex (2:5 [w/w]) at one of seven different (CDN) concentrations (20 µg/mL, 2 µg/mL, 200 ng/mL, 20 ng/mL, 2ng/mL, 200 pg/mL or 20 pg/mL). The plates were incubated at 37 °C in a CO_2 incubator for 18 to 20 hours. Then, the supernatants were collected, transferred into the corresponding wells of round-bottom 96-well plates, and either stored at -80 °C, or immediately tested in the reporter cell line.

Testing of human blood samples

A new 96-well plate was prepared for each of the four reporter cell lines tested, as follows: 10 µL of supernatant from the previous plate (containing the incubated CDNs and plasma) were added to the corresponding well in the new reporter cell plate. Then, a 180-µL aliquot of cells of the desired reporter cell line, previously harvested in medium containing heat-inactivated serum and counted, was added to each well (approximately 50,000 cells/well), and the plate was incubated for approximately 20

hours. The desired cytokine induction activity was determined using the QUANTI-Blue™ assay, as previously described. Briefly, 30 µL of supernatant from the previously incubated plate was transferred to the corresponding well of a new 96-well plate in which 170 µL of QUANTI-Blue™ reagent had previously been added.

The results from this experiment are summarized in **Table 7**, which illustrate that at all concentrations tested, the CDN/CL338 complexes provide, compared to the corresponding CDN alone: firstly, a stronger Type I IFN response (although for CL661, the difference was minimal); secondly, a stronger IL-1 response (this trend could not be confirmed for CL-592, CL-605, CL657 or CL695); thirdly, a similar TNF-α response; and lastly, and rather surprisingly, a stronger IFN-λ response. Also surprisingly, the stronger IFN-λ (also known collectively as "Type III interferons (IFNs)"; and individually, as IL-28A [IFN-λ2], IL-28B [IFN-λ3] and IL-29 [IFN-λ1]) response observed for the CDN/CL338 complexes relative to the corresponding CDNs alone was especially marked for the phosphorothioate CDNs (compare the EC_{50} values in the following pairs: CL656/CL338 vs. CL614/CL338; CL702/CL338 vs. CL674/CL338; CL632/CL338 vs. CL603/CL338; and CL655/CL338 vs. CL592/CL338). These trends are plotted for the representative CDN CL614, in **Figure 5A** (Type I IFNs), **Figure 5B** (TNF-α) and **Figure 5C** (IL-1); and for the representative CDN CL656, in **Figure 5D** (IFN-λ). Together, these findings demonstrate that each CDN/CL338 complex provides a distinct cytokine response profile relative to its corresponding CDN alone. Accordingly, these results suggest that a given CDN/CL338 complex could be used to obtain a particular cytokine response that could not be obtained by using that CDN alone. One possible scenario in which such a complex could prove especially utile is in a therapeutic context that is based on modulation of the STING pathway and in which a Type I and/or Type III IFN response is desirable but a pro-inflammatory cytokine (*e.g.* IL-1 and TNF-α) response is not. Lastly, we would like to highlight here that, to the best of our knowledge, the present invention represents the first report of *ex vivo* induction of IFN-λ by CDN STING agonists in human blood, and the first report that such activity could differ greatly between phosphodiester CDNs and their corresponding phosphorothioate CDNs.

TABLE 7. Comparison of CDNs alone vs. their corresponding CDN/CL338 complexes for *ex vivo* cytokine induction in human blood. From top to bottom: Type I IFNs, IL-1, TNF-α and IFN-λ. NC: not calculable.

EC_{50} for Type I IFNs			
CDN	CDN alone (µg/mL)	CDN/CL338 (ng/mL)	Fold increase
CL592	1.8	22.4	80
CL655	3.5	4.4	795
CL614	0.9	0.6	1413
CL656	0.7	0.2	4209
CL674	2.3	7.9	286
CL702	1.8	13.2	140
CL603	0.7	0.1	6759
CL632	0.3	1.9	159

CL605	> 20	9.4 (µg/mL)	NC
CL661	2.9	1.8 (µg/mL)	1.6
CL657	7.3	24.2	304
CL695	0.9	5.1	187
EC_{50} for IL-1			
CDN	CDN alone (µg/mL)	CDN/CL338 (µg/mL)	Fold increase
CL592	> 20	> 20	NC
CL655	6.7	3.6	2
CL614	0.8	0.1	14
CL656	0.7	7.1 (ng/mL)	95
CL674	7.9	0.8	10
CL702	3.6	0.5	7
CL603	0.6	46.6 (ng/mL)	12
CL632	0.2	0.1	3
CL605	> 20	> 20	NC
CL661	2.4	1.4	2
CL657	> 20	> 20	NC
CL695	> 20	> 20	NC
EC_{50} for TNF-α			
CDN	CDN alone (µg/mL)	CDN/CL338 (µg/mL)	
CL592	0.2	0.2	
CL655	0.3	6.8	
CL614	5.7	12.7	
CL656	> 20	> 20	
CL674	> 20	> 20	
CL702	> 20	> 20	
CL603	> 20	> 20	
CL632	> 20	> 20	
CL605	1.2	> 20	
CL661	1.8	2.8	
CL657	> 20	> 20	
CL695	> 20	> 20	
EC_{50} for IFN-λ			
CDN	CDN alone (µg/mL)	CDN/CL338 (µg/mL)	Fold increase
CL592	> 20	0.1	NC
CL655	> 20	0.04	NC
CL614	> 20	> 20	NC
CL656	5.4	7 (ng/mL)	767
CL674	> 20	8.9	NC
CL702	> 20	1.2	NC
CL603	> 20	> 20	NC
CL632	3.3	0.7	5

Example 2.4: Comparison of CDNs alone with their corresponding CDN/CL338 complexes for *in vitro* antiviral activity

In this experiment, CDNs and their corresponding CDN/CL338 complexes were tested for their possible effects on infection of human retinal cells by human cytomegalovirus (CMV). The anti-CMV drug ganciclovir, which is a standard of care for retinal CMV infection (reference: http://www.merckmanuals.com/professional/infectious-diseases/herpesviruses/cytomegalovirus-%28cmv%29-infection), was used as positive control.

ARPE19 human retinal pigment epithelial (RPE) cells (reference: ATCC® CRL-2302™) were seeded in 96-well plates (5,000 cells/well) and treated with the indicated CDN (10 µg/mL), CDN/CL338 complex (10 µg/mL CDN conc.; 2:5 [w/w]), saline or ganciclovir (3 µg/mL [12.5 µM]). Immediately after treatment, the cells were infected with the autofluorescent ANCHOR™ strain of human CMV (see Gros *et al.*, *ACS Infect Dis*, 2015) at a multiplicity-of-infection (MOI) of 1. Ten days post-infection, the cells were fixed in formalin for 20 min at room temperature, and then incubated in PBS containing Hoetsch 33342 (1 µg/mL). Micrographs of the cells were acquired on a Thermo Cellomics Arrayscan microscope. Viral DNA in the nucleus of the cells was quantified based on green fluorescent protein (GFP) signal intensity (expressed as fluorescence units [FU]), and then normalized using the compartmental analysis algorithm. In the cells whose GFP intensity was greater than the background GFP intensity, the total GFP fluorescence intensity in the nucleus was scored automatically for at least 10,000 cells/well. The results were normalized according to the control (untreated) cells. The indicated values are the mean +/-SD of duplicate wells.

The results of this experiment are shown in **Figure 6**, which reveals that, rather surprisingly, the CL338 complexes provide an anti-viral activity similar to that of ganciclovir.

Example 2.5: Comparison of CL592 alone with its corresponding complex CL592/CL338, for *in vivo* anti-tumor activity in a hamster model of pancreatic cancer

On Day 1, 18 hamsters (Golden Syrian) received an orthotopic injection of PC-1.0 tumor cells (1×10^6) in the tail of their pancreas. The hamsters were then divided into three groups of six animals. Each group received a different treatment by intraperitoneal (i.p.) injection starting on day 8 post-inoculation: either saline, CL592 alone (at 2.5 mg/Kg) or CL592/CL338 complex (CL592 concentration: 0.5 mg/Kg). Treatments were administered once daily on days 8 to 13, days 16 and 17, and days 21 to 23 post-inoculation. On day 24, necropsy was performed and tumor volumes were evaluated. Values are the mean +/-SD per group.

The results of this experiment are shown in **Figure 7**, which reveals that, at five times lower concentration, the CL592/CL338 complex provides similar anti-tumor activity to CL592.

FIGURE LEGENDS

Figure 1. Particle size (Z; in nm) and polydispersity index (PDI; unit-less) for the CDN/CL338 complexes of the present invention.

Figure 2. Zeta potential (ζ; in mV) for the CDN/CL338 complexes of the present invention.

Figure 3. Comparison of transfection agents for ISG-induced IRF activation by complexes containing either CL632 (Fig. 3A) or CL702 (Fig. 3B), in THP1-Dual™ cells.

Figure 4. Comparison of transfection agents for NF-κB pathway activation by complexes containing either CL632 (Fig. 4A) or CL702 (Fig. 4B), in THP1-Dual™ cells.

Figure 5. Comparison of CDNs alone with their corresponding CDN/CL338 complexes for *ex vivo* cytokine induction in human blood: **(A)** CL614 vs. CL614/CL338 for induction of Type I IFNs; **(B)** CL614 vs. CL614/CL338 for induction of TNF-α (CL614); **(C)** CL614 vs. CL614/CL338 for induction of IL-1; and **(D)** f CL656 vs. CL656/CL338 or induction of IFN-λ.

Figure 6. Comparison of CDNs alone with their corresponding CDN/CL338 complexes for *in vitro* anti-viral (anti-hCMV) activity in human retinal cells.

Figure 7. Comparison of CL592 alone (2.5 mg/Kg) with its corresponding complex CL592/CL338 at five times lower concentration (0.5 mg CL592/Kg) for *in vivo* anti-tumor activity in a hamster model of pancreatic cancer (orthotopic PC1.0 tumors). The results are shown relative to the control (saline) group.

PRIOR ART

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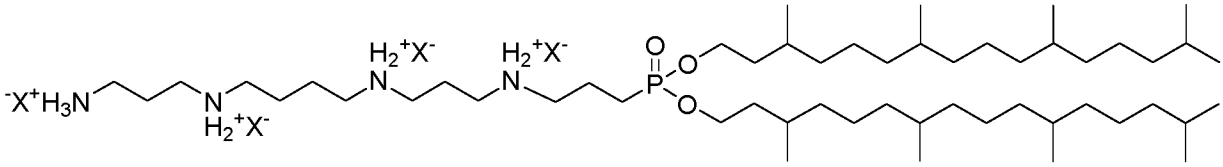
CLAIMS

1. A complex formed from the following compounds:

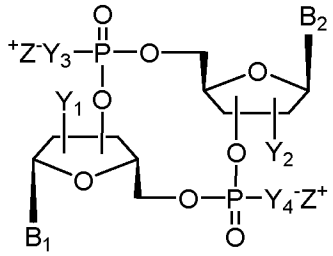
a. a lipid compound of Formula I:



Formula I

wherein X^- is a pharmaceutically acceptable anion ; and

b. a cyclic purine dinucleotide of Formula II:



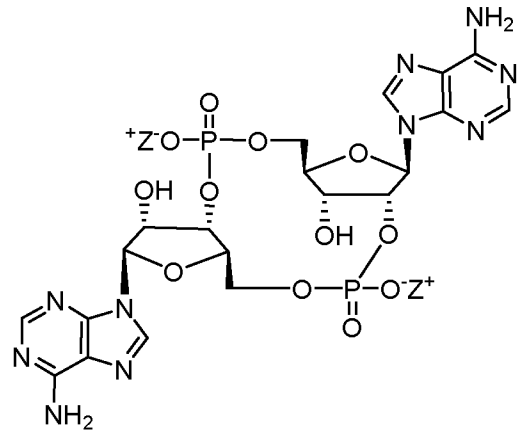
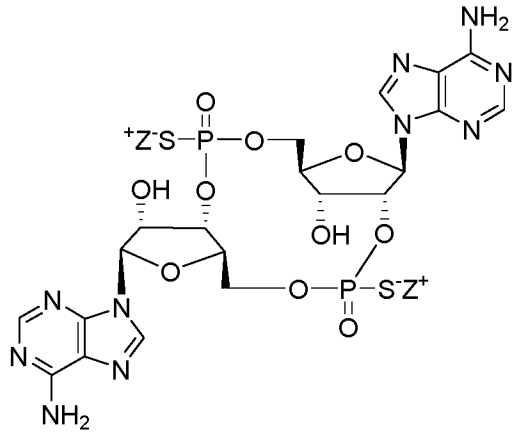
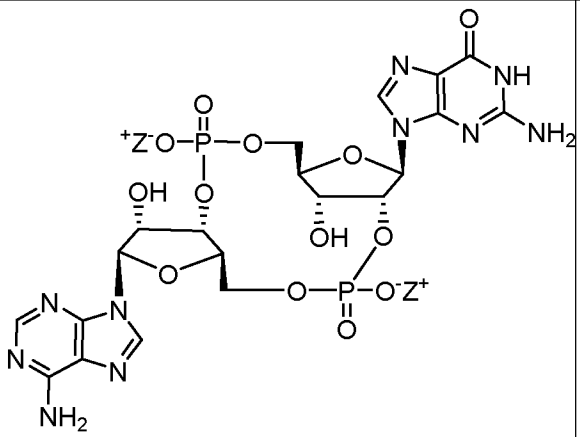
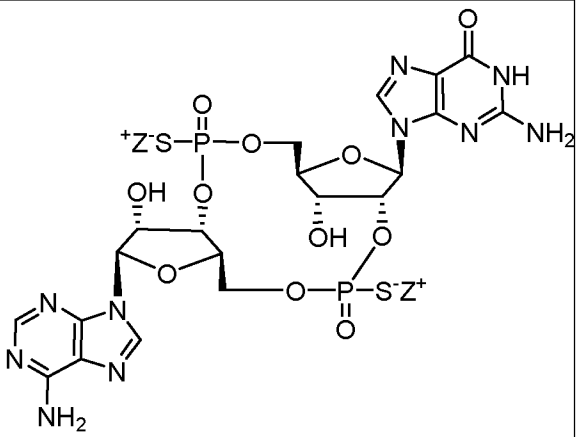
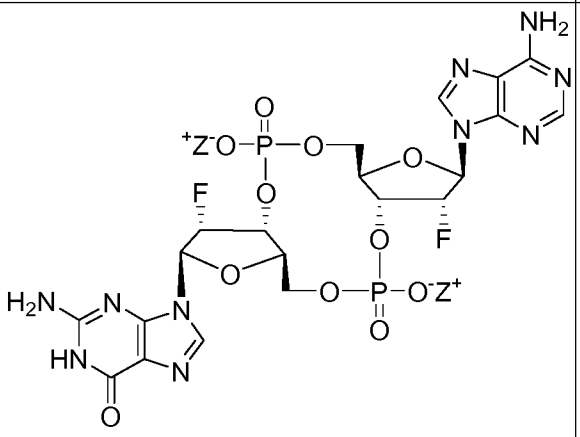
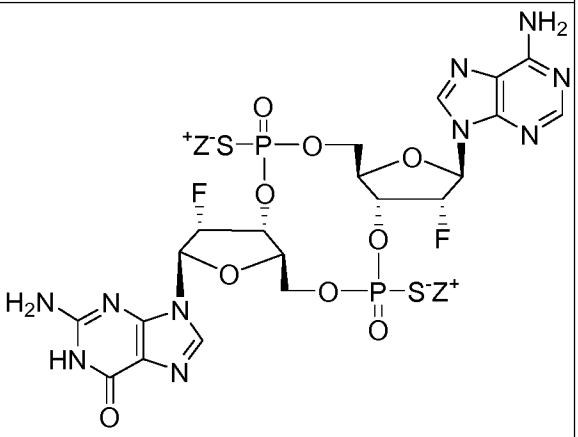
Formula II

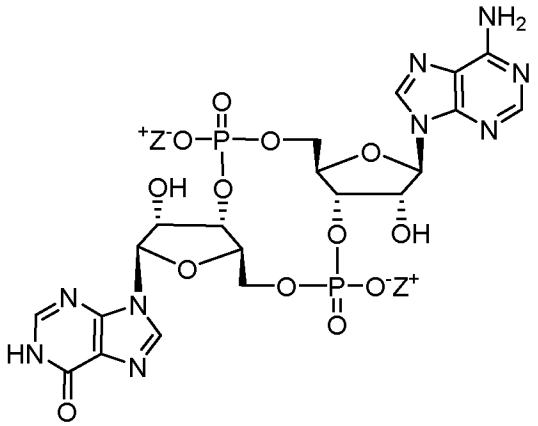
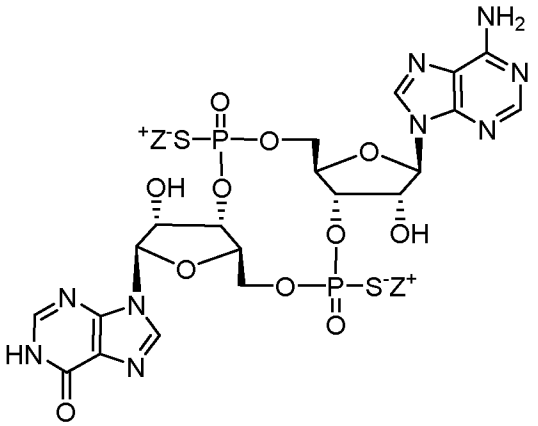
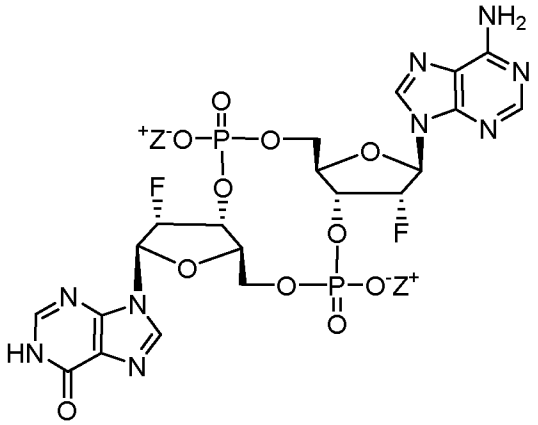
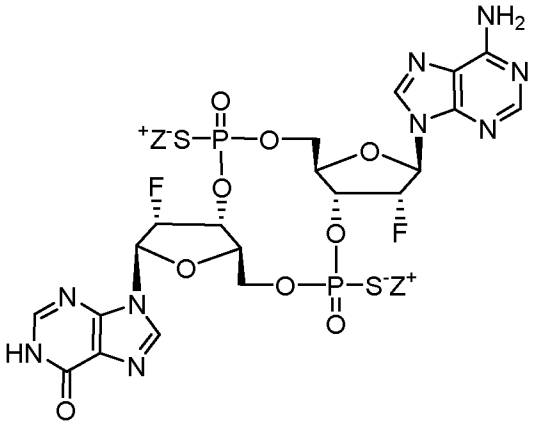
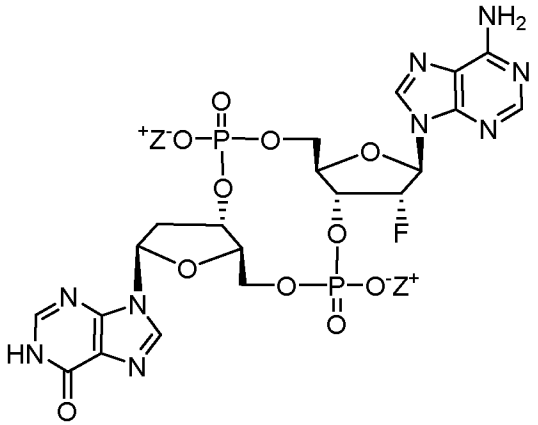
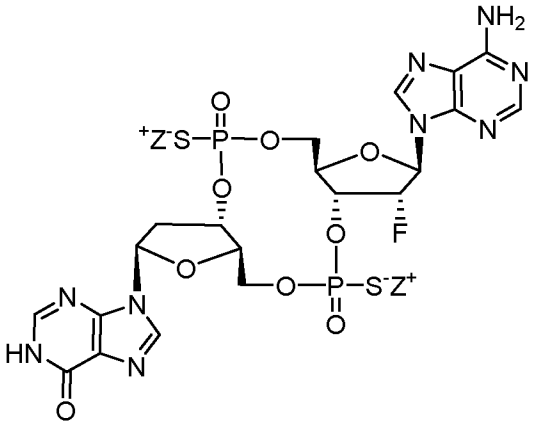
wherein:

- B_1 and B_2 are purine bases independently chosen from adenine, guanine or hypoxanthine;
- Y_1 and Y_2 are independently chosen from H, OH or F;
- Y_3 and Y_4 are independently chosen from O or S;
- Z^+ is a pharmaceutically acceptable cation.

2. The complex according to claim 1, wherein the cyclic purine dinucleotide of Formula II activates the stimulator of interferon genes (STING) pathway.

3. The complex according to claim 1 or claim 2, wherein the cyclic purine dinucleotide of Formula II is chosen from the following CDNs:

	
(2',3')c-diAMP CL657	**(2',3')c-diAM(PS)$_2$ CL695**
	
(2',3')c-GAMP CL605	**(2',3')c-GAM(PS)$_2$ CL661**
	
c-[(2'FdGMP)-(2'FdAMP)] CL603	**c-[(2'FdGMP(S))-(2'FdAMP(S))] CL632**

	
cAIMP **CL592**	**cAIM(PS)$_2$** **CL655**
	
c-[(2'FdAMP)-(2'FdIMP)] **CL614**	**c-[(2'FdAMP(S))-(2'FdIMP(S))]** **CL656**
	
c-[(2'FdAMP)-(dIMP)] **CL674**	**c-[(2'FdAMP(S))-(dIMP(S))]** **CL702**

4. A pharmaceutical composition comprising the complex according to any one of claims 1 to 3, and a pharmaceutically acceptable excipient.

5. The pharmaceutical composition of claim 4, further comprising a surfactant.

6. Use of the complex according to any one of claims 1 to 3 or of the pharmaceutical composition according to claim 4 or claim 5 to induce the production of cytokines in mammalian cell cultures by activation of the STING pathway.

7. Use of a complex according to any one of claims 1 to 3 or of the pharmaceutical composition according to claim 4 or claim 5 to induce the production of cytokines in mammalian blood or tissue samples *ex vivo* by activation of the STING pathway.

8. The complex according to any one of claims 1 to 3 or the pharmaceutical composition according to claim 4 or claim 5 for use in a method for treatment of a mammal.

9. The complex according to any one of claims 1 to 3 or the pharmaceutical composition according to claim 4 or claim 5 for use to induce the production of cytokines in a mammal by activation of the STING pathway.

10. The complex according to any one of claims 1 to 3 or the pharmaceutical composition according to claim 4 or claim 5 for use in the treatment of cancer.

11. The complex according to any one of claims 1 to 3 or the pharmaceutical composition according to claim 4 or claim 5 for use in the treatment of an infectious disease.

12. The complex according to any one of claims 1 to 3 or the pharmaceutical composition according to claim 4 or claim 5 for use as a vaccine adjuvant.

13. The complex for use according to any one of claims 8 to 12 or the pharmaceutical composition for use according to any one of claims 8 to 12, wherein said complex or said pharmaceutical composition is administered via subcutaneous, intravenous, intraperitoneal, intratumoral, intranasal, oral, intravaginal, transdermal or topical administration.

14. A method for preparing the complex according to any one of claims 1 to 3, said method comprising the step of contacting the compound of Formula I with the compound of Formula II in an aqueous solution.

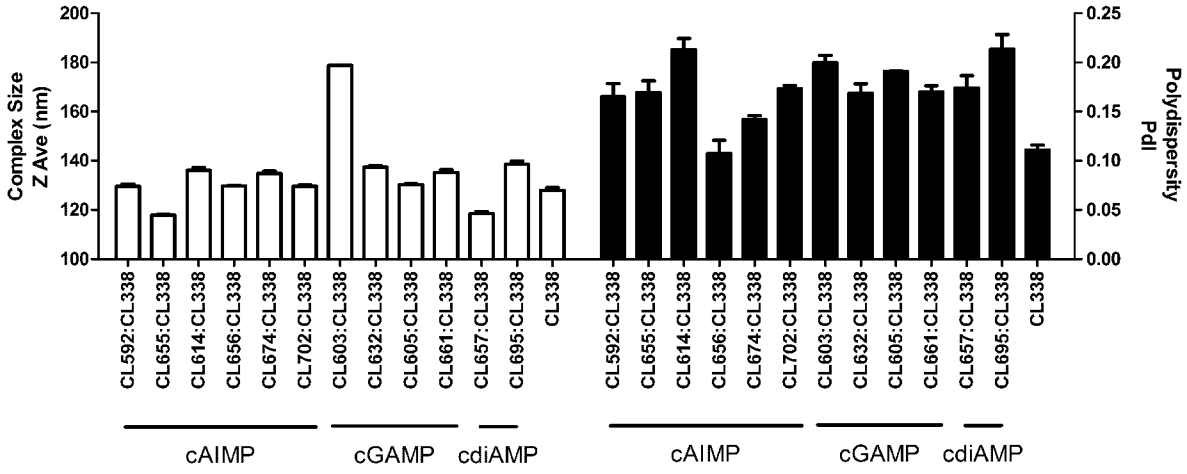


FIGURE 1

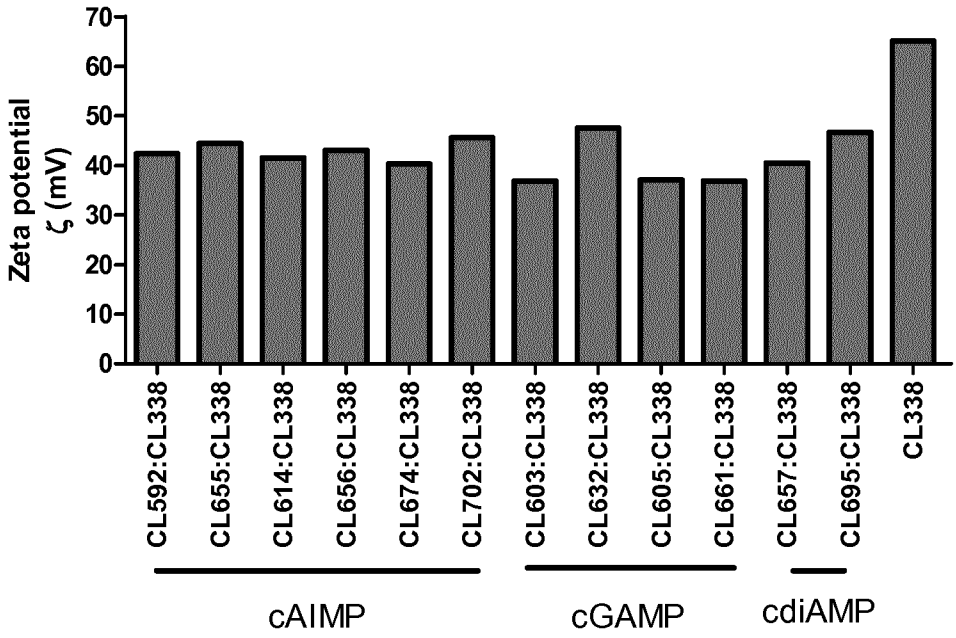


FIGURE 2

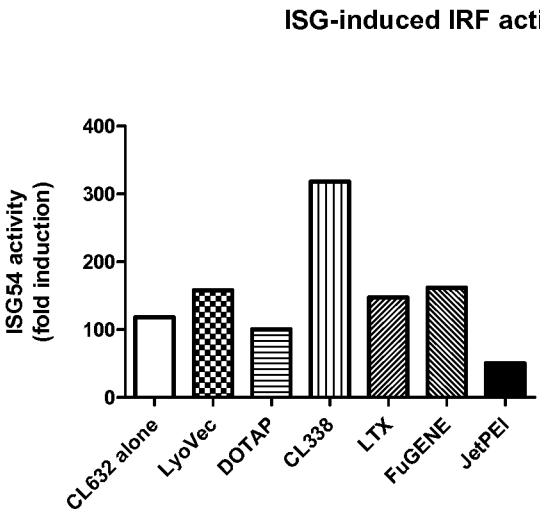


FIGURE 3A

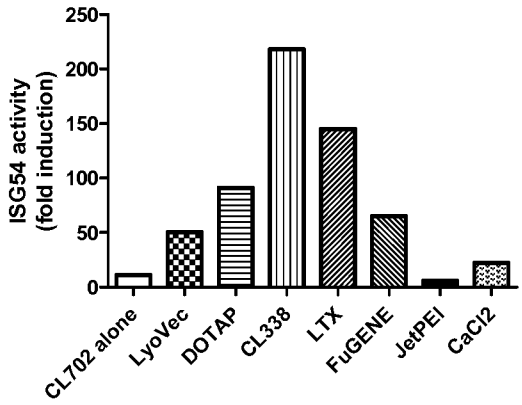


FIGURE 3B

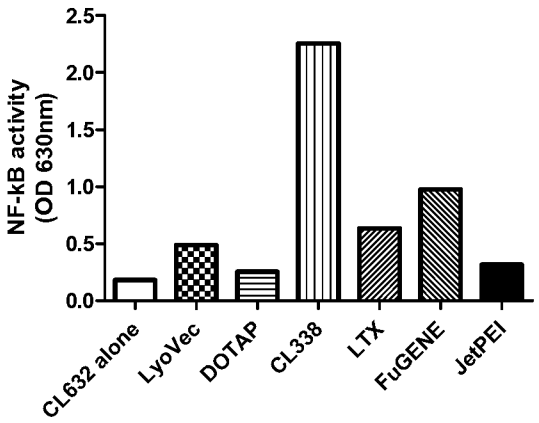


FIGURE 4A

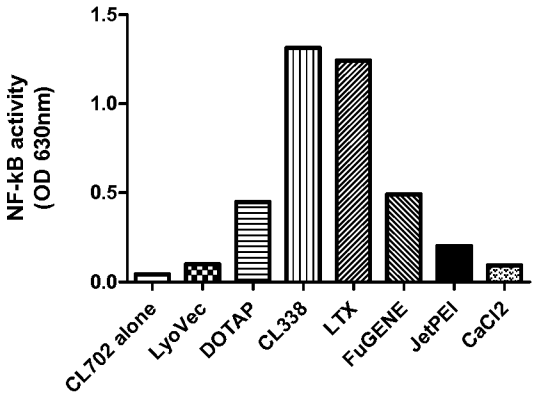


FIGURE 4B

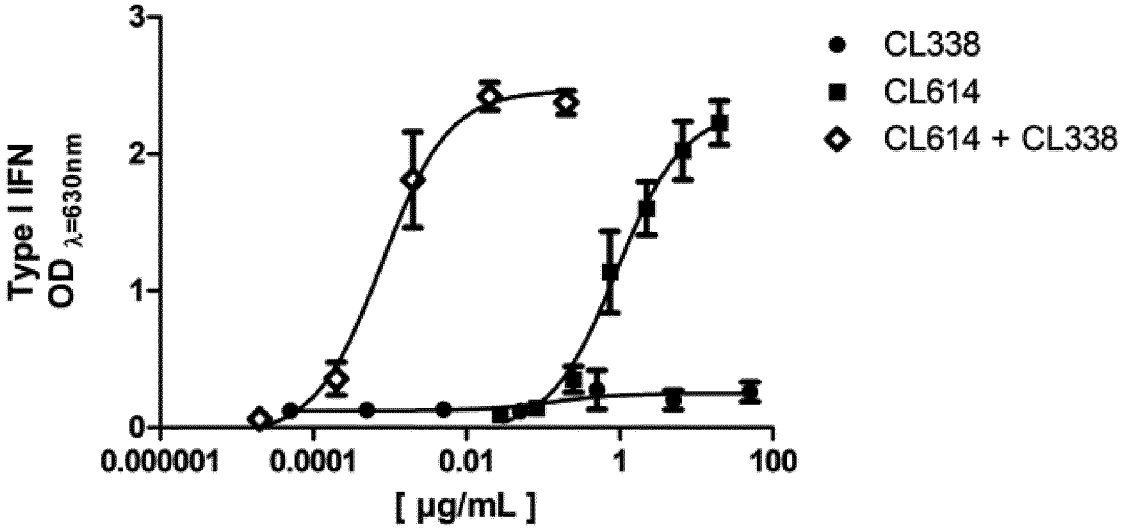


FIGURE 5A

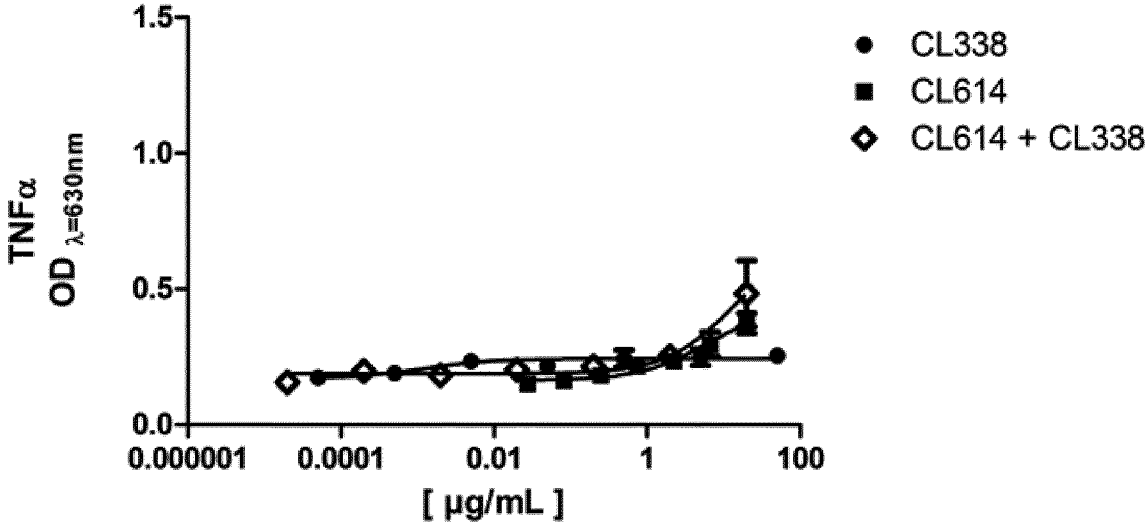


FIGURE 5B

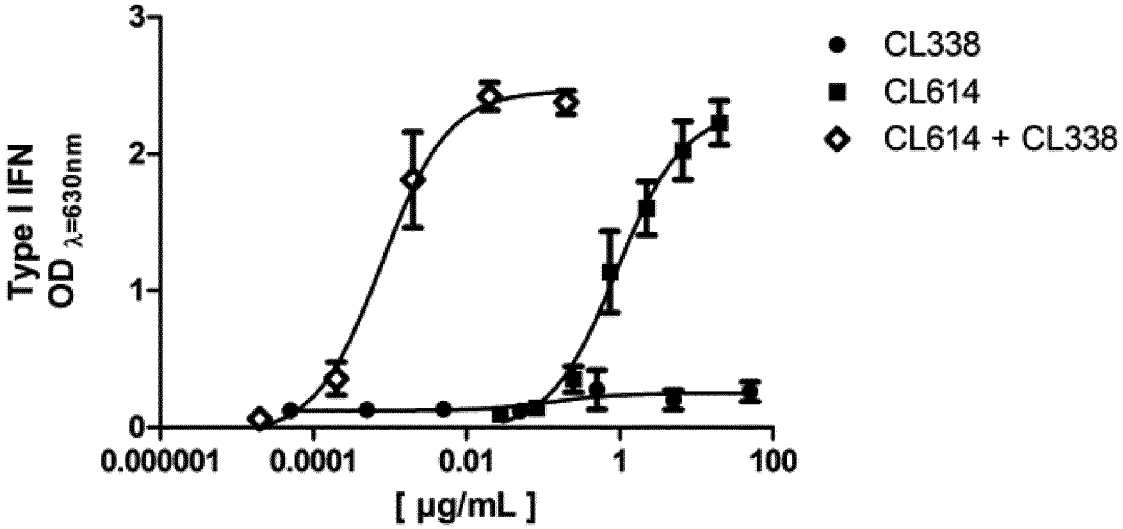


FIGURE 5C

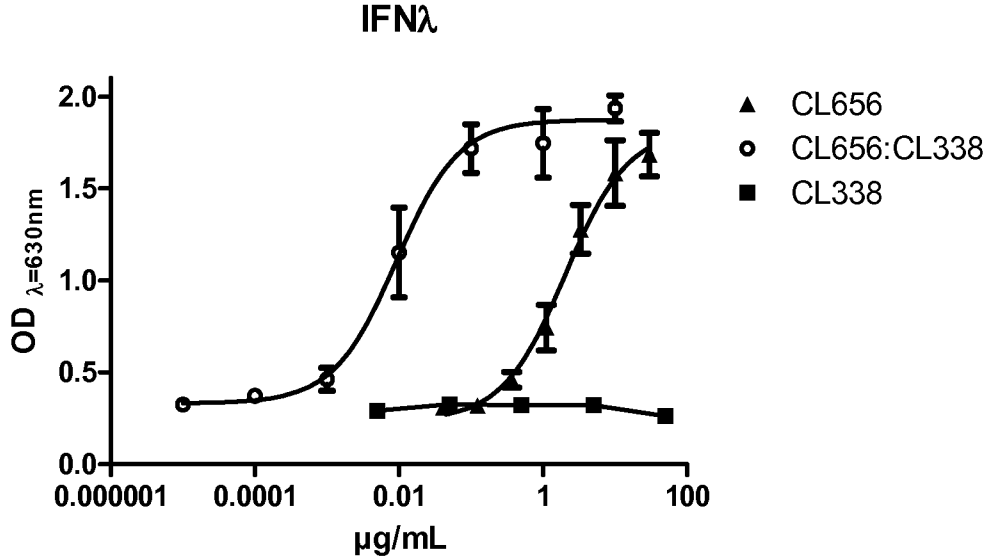


FIGURE 5D

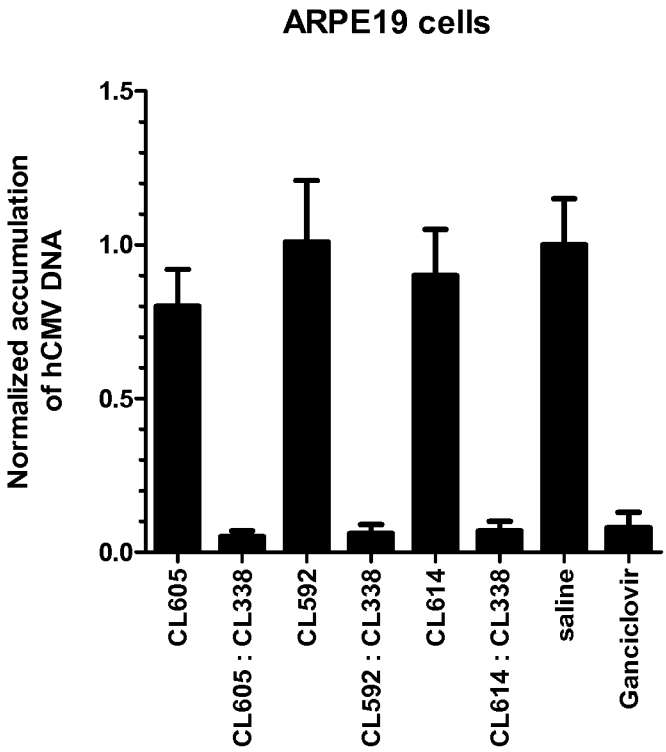

ARPE19 cells
Normalized accumulation of hCMV DNA
1.5
1.0
0.5
0.0
CL605
CL605 : CL338
CL592
CL592 : CL338
CL614
CL614 : CL338
saline
Ganciclovir

FIGURE 6

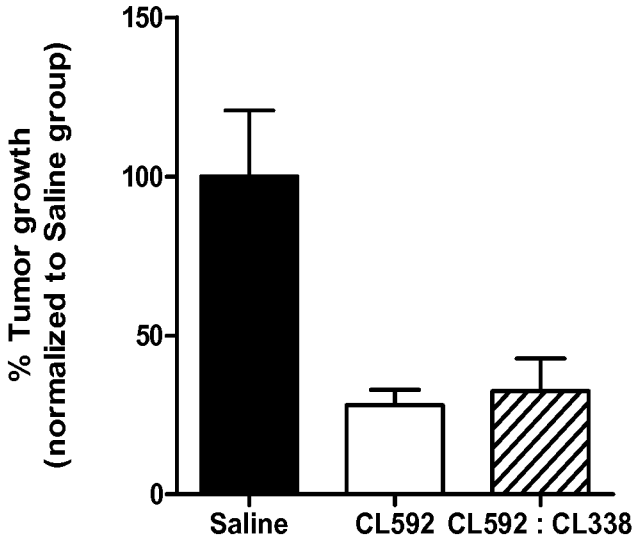

% Tumor growth
(normalized to Saline group)
150
100
50
0
Saline
CL592
CL592 : CL338

FIGURE 7

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/059781

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/7084 A61K31/7076 A61K31/708 A61K33/42 A61K9/00
A61P35/00 A61P31/00
ADD.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, WPI Data, EMBASE, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	NAKAMURA TAKASHI ET AL: "Liposomes loaded with a STING pathway ligand, cyclic di-GMP, enhance cancer immunotherapy against metastatic melanoma", JOURNAL OF CONTROLLED RELEASE, vol. 216, 14 August 2015 (2015-08-14), pages 149-157, XP029276987, ISSN: 0168-3659, DOI: 10.1016/J.JCONREL.2015.08.026 abstract page 149, column 2, paragraph 2 - page 150, column 1 ----- -/--	1-14

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 June 2017	28/06/2017

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Houyvet-Landriscina

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	YUSUKE SATO ET AL: "A pH-sensitive cationic lipid facilitates the delivery of liposomal siRNA and gene silencing activity in vitro and in vivo", JOURNAL OF CONTROLLED RELEASE., vol. 163, no. 3, 1 November 2012 (2012-11-01), pages 267-276, XP055300249, NL ISSN: 0168-3659, DOI: 10.1016/j.jconrel.2012.09.009 abstract figure 1	1-14
A	YILDIZ SONER ET AL: "Enhanced immunostimulatory activity of cyclic dinucleotides on mouse cells when complexed with a cell-penetrating peptide or combined with CpG", EUROPEAN JOURNAL OF IMMUNOLOGY, vol. 45, no. 4, April 2015 (2015-04), pages 1170-1179, XP002761516, page 1170 - page 1171	1-14
A	InvivoGen: "InvivoGen Insight", invivogen.com , February 2014 (2014-02), XP002761517, Retrieved from the Internet: URL:http://www.invivogen.com/docs/Insight_201402.pdf [retrieved on 2016-09-06] pages 1-3	1-14
A	XU ZHANG ET AL: "Cyclic GMP-AMP Containing Mixed Phosphodiester Linkages Is An Endogenous High-Affinity Ligand for STING", MOLECULAR CELL., vol. 51, no. 2, 1 July 2013 (2013-07-01), pages 226-235, XP055300246, US ISSN: 1097-2765, DOI: 10.1016/j.molcel.2013.05.022 abstract; figure 1A	1-14
A	WO 2013/185052 A1 (ADURO BIOTECH [US]; UNIV JOHNS HOPKINS [US]) 12 December 2013 (2013-12-12) cited in the application page 3, paragraph 10-12 page 13, paragraph 56 page 16, paragraph 68 - page 18, paragraph 75	1-14

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/059781

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 2 674 170 A1 (CAYLA [FR]) 18 December 2013 (2013-12-18) cited in the application page 2, paragraphs 1, 3-4 page 4, paragraph 10-11 page 21, paragraphs 73, 77, 78-79 page 35, paragraph 154 -----	1-14
X,P	WO 2016/096174 A1 (INVIVOGEN [FR]) 23 June 2016 (2016-06-23) page 1, lines 3-7 page 3, lines 1-12 page 5, line 18 - page 7, line 35 -----	1-14

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2017/059781

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2013185052 A1	12-12-2013	AU 2013271375 A1	18-12-2014
		CA 2876150 A1	12-12-2013
		CN 104507538 A	08-04-2015
		EP 2858722 A1	15-04-2015
		HK 1204302 A1	13-11-2015
		JP 2015518901 A	06-07-2015
		KR 20150022996 A	04-03-2015
		NZ 702392 A	31-03-2017
		SG 10201610251P A	27-01-2017
		SG 11201407875U A	30-12-2014
		US 2015010613 A1	08-01-2015
		WO 2013185052 A1	12-12-2013
EP 2674170 A1	18-12-2013	EP 2674170 A1	18-12-2013
		US 2013336996 A1	19-12-2013
WO 2016096174 A1	23-06-2016	US 2016362441 A1	15-12-2016
		WO 2016096174 A1	23-06-2016