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(54) Title: GENETIC SEQUENCE/SMALL MOLECULE-CARBOHYDRATE CONJUGATES FOR ENHANCED LUNG-SPECIFIC TARGETING

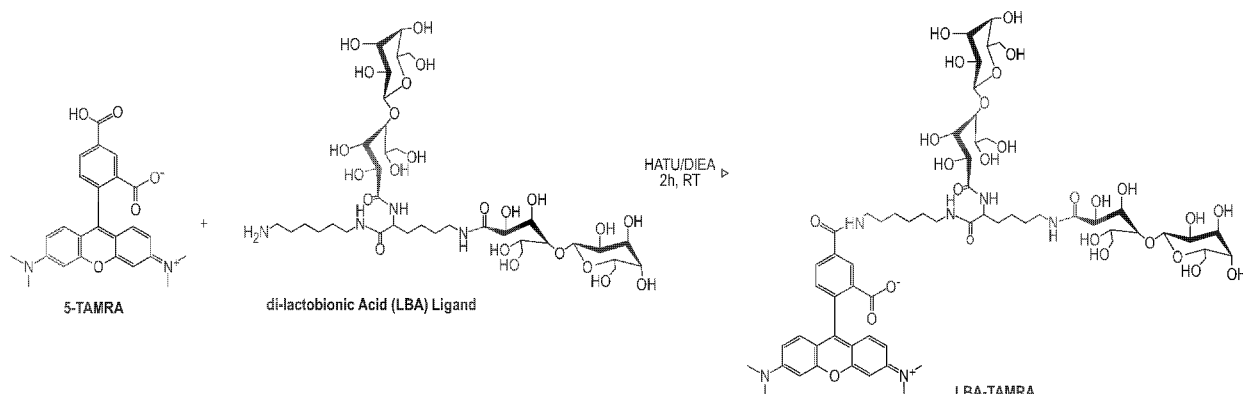
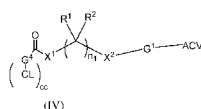


FIG. 1



(57) Abstract: Disclosed herein is a carbohydrate conjugate of formula (IV), an enantiomer, a diastereoisomer, a pharmaceutically acceptable salt, a hydrate, a solvate or a combination thereof: (IV) wherein ACV is a pharmaceutically active agent, R_1 and R_2 are each independently selected from H or a substituted or unsubstituted C_1 to C_6 alkyl, X^1 is selected from the group consisting of O, NR^3 , $C=O$, and $C(R^3)_2$ where R^3 is H, $-C(O)CH_3$, or a substituted or unsubstituted C_1 to C_6 alkyl, X^2 is O, NR^3 , or $C(R^3)_2$ where R^3 is H or a substituted or unsubstituted C_1 to C_6 alkyl; n^1 is 1 to 20, G^1 and G^4 are each a direct bond or a chemical linker; and G^4 -CL is a carbohydrate ligand structure comprising 2 to 16 carbohydrate residues each derived from a monosaccharide, a disaccharide, a trisaccharide, or a tetrasaccharide; where cc is 2 to 16.

Declarations under Rule 4.17:

- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
- *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

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GENETIC SEQUENCE/SMALL MOLECULE-CARBOHYDRATE CONJUGATES FOR ENHANCED LUNG-SPECIFIC TARGETING

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This disclosure claims priority to U.S. Provisional Application No. 63/469,840 filed on May 31, 2023, the entire contents of which are incorporated herein in their entirety.

BACKGROUND

Field of the Disclosure

[0002] This disclosure is directed to compositions and methods that target the lungs via intratracheal administration. More particularly, the present disclosure relates to compositions and methods including genetic sequence/small molecule-carbohydrate conjugates to target the lungs via intratracheal administration for treatment and/or diagnosis of diseases and conditions.

Description of the Related Art

[0003] Glutamic acid-alanine-leucine-alanine (GALA) peptides are often explored for delivering nucleic acids due to their ability to form stable complexes and facilitate cellular uptake. While GALA peptides can facilitate cellular uptake, the efficiency of nucleic acid delivery into the cytoplasm and subsequent release may be suboptimal, affecting the overall efficacy of the therapeutic intervention. In addition, GALA peptides may degrade quickly in biological fluids due to proteolytic enzymes, reducing their effectiveness for in vivo applications. At higher concentrations, GALA peptides may exhibit cytotoxicity, affecting not only the target cells but also surrounding healthy tissues.

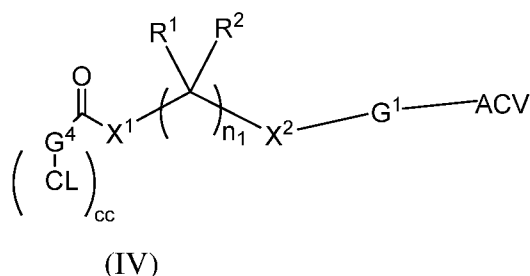
[0004] Modifying lipid nanoparticles (LNPs) with GALA peptides is a strategy to enhance the delivery of nucleic acids to the lungs. This approach combines the beneficial properties of both peptides and lipid nanoparticles, potentially overcoming some of the drawbacks associated with each component individually.

[0005] However, there are disadvantages to the use of lipid nanoparticles as they can cause toxicity and unwanted side effects. The formulation of GALA peptide-modified LNPs can be complex and require precise control over various parameters, making large-scale production challenging. While LNPs can reduce some immunogenicity, the combination with GALA peptides might still elicit an immune response, which needs to be carefully monitored and managed.

[0006] Therefore, there is an urgent need for improved compositions and methods for lung targeting to achieve desired therapeutic outcomes.

SUMMARY

[0007] Disclosed herein is a carbohydrate conjugate of formula (IV), an enantiomer, a diastereoisomer, a pharmaceutically acceptable salt, a hydrate, a solvate or mixtures thereof:



wherein ACV is a pharmaceutically active agent,

R_1 and R_2 are each independently selected from H or a substituted or unsubstituted C_1 to C_6 alkyl,

X^1 is selected from the group consisting of O, NR^3 , $C=O$, and $C(R^3)_2$ where R^3 is H, - $C(O)CH_3$, or a substituted or unsubstituted C_1 to C_6 alkyl,

X^2 is O, NR^3 , or $C(R^3)_2$ where R^3 is H or a substituted or unsubstituted C_1 to C_6 alkyl;

n^1 is 1 to 20,

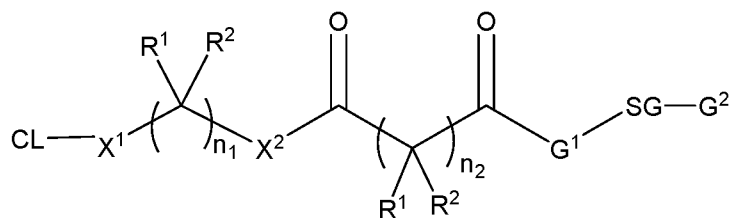
G^1 and G^4 are each a direct bond or a chemical linker; and

G^4 -CL is a carbohydrate ligand structure comprising 2 to 16 carbohydrate residues each derived from a monosaccharide, a disaccharide, a trisaccharide, or a tetrasaccharide; where cc is 2 to 16;

optionally wherein the carbohydrate ligand is fully or partially acylated on a hydroxy or amino group thereof with a C_2 to C_{15} acyl group, preferably wherein the carbohydrate ligand is fully or partially acetylated on a hydroxy or amino group thereof.

[0008] Disclosed herein is a composition for pulmonary delivery, the composition comprising:

a genetic sequence-carbohydrate conjugate of a formula A1, an enantiomer, a diastereoisomer, a pharmaceutically acceptable salt, a hydrate, a solvate or mixtures thereof:



(A1)

wherein

SG is a small molecule (SM), a genetic sequence (GS), or a combination thereof, wherein, SM is a small molecule, preferably a drug or a compound targeting lung, and GS is a genetic sequence, preferably a peptide nucleic acid or an oligonucleotide such as an mRNA sequence, an siRNA sequence, or a DNA sequence, optionally wherein each genetic sequence is natural or modified, for example comprises a gamma-serine modified gamma peptide nucleic acid, an alanine gamma peptide nucleic acid, a clamp G-modified peptide nucleic acid, a locked nucleic acid (LNA), a phosphorothioate (PS), a phosphorodiamidate morpholino (PMO), a 2'-O-methyl (2'-O-Me), 2'-O-methoxyethyl (2'-O-MOE), 2'-fluoro (2'F), a 5'-methylcytosine, or a combination thereof, the genetic sequence having a 3' end and a 5' end,

wherein the small molecule comprises ivacaftor, elexacaftor, tezacaftor, lumacaftor, pirfenidone, N-acetylcysteine, nintedanib, cisplatin, carboplatin, docetaxel, paclitaxel, pemetrexed, or a combination thereof;

R₁ and R₂ are each independently H or a substituted or unsubstituted C₁ to C₆ alkyl, X¹ is O, NR³, C=O, or C(R³)₂ where R³ is H or a substituted or unsubstituted C₁ to C₆ alkyl,

X² is O, NR³, or C(R³)₂ where R³ is H or a substituted or unsubstituted C₁ to C₆ alkyl,

G¹ is a direct bond or a group linking the PNA to the conjugate,

G² is H or a functional moiety,

CL is a carbohydrate ligand comprising 2 to 16 carbohydrate residues derived from a monosaccharide, a disaccharide, a trisaccharide, or a tetrasaccharide, preferably wherein CL comprises a carbohydrate residue derived from a monosaccharide or a disaccharide,

optionally wherein the carbohydrate ligand is fully or partially acylated on a hydroxy or amino group thereof with a C₂ to C₁₅ acyl group, preferably wherein the carbohydrate ligand is fully or partially acetylated on a hydroxy or amino group thereof,

n^1 is 1 to 20, and n^2 is 0 to 20; where the composition is delivered to the lungs and where the genetic sequence/small molecule-carbohydrate conjugate is effective to target a macrophage receptor type 2.

[0009] Disclosed herein too is a method of delivering a pharmaceutically active agent to the lungs of a patient, comprising inhalationally administering to a patient an effective amount of the carbohydrate conjugate or the composition detailed above; where the carbohydrate conjugate or the composition is delivered to the lungs; and where upon delivery, the pulmonary retention of the active agent is increased.

BRIEF DESCRIPTION OF THE FIGURES

[0010] FIG. 1 is a schematic depiction of the synthetic scheme of TAMRA and di-lactobionic acid ligand. Synthesis scheme of amide conjugation of TAMRA and di-lactobionic acid ligand to afford LBA-TAMRA.

[0011] FIGS. 2A and 2B are confocal microscopy images of cellular distribution of TAMRA, LBA-TAMRA and GalNAc-TAMRA in different cell lines. FIG. 2A) Cellular uptake in HepG2 cells. Blue-Nuclei, Red-TAMRA. Fig. 2B) Cellular uptake in A549 cells. Blue-Nuclei, Red-TAMRA. White arrows indicate ligand cellular distribution.

[0012] FIGS. 3A and 3B depict cellular uptake of LBA and GalNAc in different cell lines. FIG. 3A depicts flow cytometry histogram plots depicting cellular uptake of ligands. FIG. 3B depicts percentage cellular uptake of LBA-TAMRA and GalNAc-TAMRA in different cell lines. Data represent mean + standard error mean.

[0013] FIGS. 4A to 4D depict ASGPR gene expression and cellular uptake of LBA and GalNAc ligands following ASGPR silencing. FIG. 4A depicts ASGPR gene expression levels in different cell lines. Data represent mean + standard error mean. FIG. 4B depicts flow cytometry histogram plots depicting reduction in cellular uptake post ASGPR silencing. FIG. 4C and 4D depict percentage cellular uptake of LBA-TAMRA and GalNAc-TAMRA in different cell lines post ASGPR silencing. Data represent mean + standard error mean.

[0014] FIGS. 5A to 5C depict MRC2 gene expression and cellular uptake of LBA ligand following ASGPR & MRC2 silencing in A549 cells. FIG. 5A shows MRC2 gene expression levels in different cell lines. Data represent mean + standard error mean. FIG. 5B depicts flow cytometry histogram plots depicting reduction in cellular uptake post ASGPR & MRC2 silencing. FIG. 5C depicts percentage cellular uptake of LBA-TAMRA in A549 cells post ASGPR & MRC2 silencing. Data represent mean + standard error mean.

[0015] FIGS. 6A and 6B depict *in vivo* biodistribution studies. FIG. 6A is an IVIS image of liver, lungs, heart, kidney, and spleen taken at 4hr, 24hr, 48hr and normalized with PBS control. FIG. 6B depicts lung cross-section images after 4hrs of LBA-TAMRA treatment. Red puncti represent LBA-TAMRA signal.

[0016] FIGS. 7A to 7D depict flow cytometry analysis of 4hr LBA-TAMRA treatment. FIG. 7A is a histogram depicting total lung LBA-TAMRA uptake after 4hrs. FIG. 7B is a histogram depicting macrophage LBA-TAMRA uptake after 4hrs. FIG. 7C is a histogram depicting epithelial cell LBA-TAMRA uptake after 4hrs. FIG. 7D is a histogram depicting endothelial cell LBA-TAMRA uptake after 4hrs.

[0017] FIGS. 8A to 8D depict flow cytometry analysis of 24hr and 48hr LBA-TAMRA treatment. FIG. 8A is a histogram depicting total lung LBA-TAMRA uptake after 24hrs and 48hrs. FIG. 8B is a histogram depicting macrophage LBA-TAMRA uptake after 24hrs and 48hrs. FIG. 8C is a histogram depicting epithelial cell LBA-TAMRA uptake after 24hrs and 48hrs. FIG. 8D is a histogram depicting endothelial cell LBA-TAMRA uptake after 24hrs and 48hrs.

DETAILED DESCRIPTION

Definitions

[0018] Therapeutic cargo refers to any biologically active substance or molecule that is delivered to cells, tissues, or organs for therapeutic purposes. This term encompasses a wide range of agents that can be used to treat diseases, repair tissues, or modulate biological processes. A therapeutic cargo is the payload that is intended to produce a therapeutic effect once delivered to the target site within the body.

[0019] Carbohydrate-based ligands are molecules that contain carbohydrate (sugar) structures and can specifically bind to certain receptors, often on cell surfaces. These ligands play valuable roles in various biological processes, including cell-cell communication, pathogen recognition, and immune responses. They may be exploited in various biomedical applications, including targeted drug delivery, vaccine development, and diagnostic assays. They leverage the specific interactions between carbohydrates and their receptors to achieve precise targeting and therapeutic effects.

[0020] 5-TAMRA is a bright, red-orange fluorescent dye with an excitation wavelength around 555 nm and an emission wavelength around 580 nm. It produces a strong fluorescence signal, making it useful for sensitive detection methods. The dye contains a

carboxyl group (-COOH) that can be activated for covalent attachment to biomolecules, such as proteins, peptides, nucleic acids, and other amine-containing molecules.

[0021] Inhalational mechanism refers to the process by which substances are delivered to and absorbed by the respiratory system through inhalation. This mechanism is commonly used for the administration of medications and delivery of therapeutic agents. It involves the act of drawing air along with any suspended particles or aerosols into the lungs through the nose or mouth.

[0022] A549 cell line is a widely used human cell line in scientific research, particularly in studies related to lung biology and disease.

[0023] MRC2 is a mannose receptor involved in binding and endocytosis of mannose-containing glycoconjugates, expressed in various immune cells and endothelial cells. MRC2, also known as the Endo180 receptor or CD280, is a transmembrane glycoprotein.

[0024] A small molecule is a low molecular weight organic compound, typically having a molecular weight of less than 900 Daltons, preferably 100 to 500 Daltons. These molecules are distinct from large molecules such as proteins, nucleic acids, and polysaccharides.

Detailed Description

[0025] Disclosed herein is an inhalational composition comprising a genetic sequence/small molecule-carbohydrate conjugate that may be used for improved pulmonary targeting along with the ability to optimize delivery of a therapeutic cargo to the target site. The composition is preferably administered by a pulmonary administration. In particular, the composition is preferably administered via an aerosolized composition or via a dry powder to the lung(s) of the patient by inhalation or by other means of local administration to the lung, bronchi and/or airways. It is preferably delivered to the lungs via the nose and/or mouth which involves delivery through the respiratory tract.

[0026] Target sites are typically within the respiratory system and preferably the lung. In an embodiment, the composition may comprise a carbohydrate ligand that is conjugated with a small molecule, a genetic sequence, or a combination of a small molecule and a genetic sequence. The term “genetic sequence/small molecule-carbohydrate conjugate” is used to describe a carbohydrate ligand that is conjugated with a small molecule, a genetic sequence, or a combination of a small molecule and a genetic sequence. In an embodiment, the small molecule is a fluorescent dye that may be used to determine the targeting

capabilities of the genetic sequence/small molecule-carbohydrate conjugate. The genetic sequence/small molecule-carbohydrate conjugate includes carbohydrate-based ligands, such as a lactobionic acid (LBA) ligand, galactose, N-acetylgalactosamine, mannose, and fucose, and can be used for the delivery of nucleic acids with greater pulmonary retention to target various lung diseases such as pulmonary fibrosis, cystic fibrosis, lung cancer, viral respiratory diseases, and asthma.

[0027] In an embodiment, the composition is preferably delivered via an inhalational mechanism to a site within the respiratory system. In another embodiment, the composition is preferably delivered via an inhalational mechanism to the lungs. In another embodiment, the composition may be delivered via other mechanisms such as orally or intravenously to other parts of the body (of a living being) such as the liver and/or the kidney. Inhalational delivery to the lungs is preferred.

[0028] In an embodiment, the method delivering a therapeutic amount of a pharmaceutically active agent to the lungs of a patient, where the pharmaceutically active agent comprises the genetic sequence/small molecule-carbohydrate conjugate detailed herein.

[0029] Currently, GALA peptides and ionizable lipid nanoparticles are utilized for pulmonary delivery of therapeutics. PEGylation of nanoparticles (which refers to the attaching of polyethylene glycol chains to the nanoparticles) has also shown increased biodistribution to the lungs. Although nanoparticle delivery has been an attractive approach to drug delivery to the lungs, longer pulmonary retention and formulation stability has remained a challenge.

[0030] When lipid nanoparticles or derivatives thereof are used to target damaged or diseased lungs, they often cause toxicity and unwanted side effects. Lipid-based formulations, such as liposomes or lipid nanoparticles, might have limited ability to specifically target damaged lung tissue, potentially leading to off-target effects and reduced therapeutic efficacy. The thick mucus layer in the respiratory tract can impede the efficient delivery of lipid-based carriers to the target cells within the lungs. Moreover, nano formulations such as lipid nanoparticles face issues of lower loading of therapeutic cargo, which leads to reduced delivery of cargo to the target sites. Thus, to achieve positive therapeutic outcomes, higher doses of nano formulations are recommended.

[0031] Described herein are genetic sequence/small molecule-carbohydrate conjugates (hereinafter “genetic sequence/small molecule-carbohydrate conjugates” or “the conjugate”) including carbohydrate-based ligands for targeted delivery of the conjugates to

specific organs in mammals, in particular humans, and more in particular the lungs of mammals (such as a human beings).

[0032] In an aspect, the genetic sequence-carbohydrate conjugate is a peptide nucleic acid (PNA)-carbohydrate conjugate (PNAC). In particular, the conjugate is a molecule including a carbohydrate ligand covalently linked to a genetic sequence via a linker backbone covalently bonded to both. In an embodiment, the conjugate is a molecule including a carbohydrate ligand covalently linked to a small molecule via a linker backbone covalently bonded to both. In an embodiment, the small molecule-carbohydrate conjugate is a FDA approved small molecule drug-carbohydrate conjugate. In an embodiment, the conjugate includes a small molecule, a genetic sequence or a combination thereof. The carbohydrate ligand can be selected to target the lungs. Preferably the conjugate selectively binds to specific receptors on cells to deliver the genetic sequence or other therapeutic agent to the cells bearing the receptors.

[0033] Disclosed herein are new formulations and methods including genetic sequence/small molecule-carbohydrate conjugates that include carbohydrate-based ligands, such as a lactobionic acid (LBA) ligand, for delivery of nucleic acids and/or small molecules with greater pulmonary retention to target various lung diseases. Lactobionic acid is also referred to as 4-*O*- β -galactopyranosyl-D-gluconic acid. Advantageously, this formulation results in reduced toxicity and improved pulmonary targeting along with optimal delivery of the therapeutic cargo to the target site.

[0034] Carbohydrate ligands such as lactobionic acid show longer retention in the lungs and require simple conjugation steps for its synthesis, making scale up easier. The *in vivo* biodistribution data confirms the properties of enhanced lung uptake for up to 48 to 72 hours, proving to be a viable alternative to nanoparticle delivery to the lungs. Another advantage is that these carbohydrate-based conjugates can be characterized in a more robust manner than nano-formulations, which help in establishing better safety of the delivered therapeutic modality.

[0035] The carbohydrate-based ligands can be monovalent, divalent, trivalent, tetravalent, or polyvalent. Thus, the genetic sequence/small molecule-carbohydrate conjugates including carbohydrate-based ligands, such as lactobionic acid (LBA) ligand, result in cost effective and efficient pulmonary targeting for safe delivery of therapeutic agents. This leads to numerous applications for treating and diagnosis of pulmonary diseases. The formulations and techniques disclosed may be applied for the delivery of nucleic acids

and/or small molecules with greater pulmonary retention to target various lung diseases such as pulmonary fibrosis, cystic fibrosis, lung cancer, viral respiratory diseases, and asthma.

[0036] The carbohydrate-based ligands disclosed herein, such as LBA, can be conjugated with both nucleic acid and other small molecule therapeutic drugs thus providing a versatile approach for the treatment and diagnosis of pulmonary diseases such as fibrosis, asthma, viral respiratory diseases, lung cancer, cystic fibrosis and other genetic disorders (associated with the lungs). In an embodiment, the carbohydrate-based ligands, such as the LBA ligand, can be used for the functionalization of lipids, biopolymers, and synthetic polymers for enhanced pulmonary delivery of various therapeutic and diagnostic agents and pulmonary vaccine delivery.

[0037] As an alternative to nanoparticle delivery systems, LBA is a safe alternative to deliver drugs to the lungs while precluding toxicity. Additionally, for administration of LBA ligand conjugates (with genetic sequences and/or small therapeutic molecules) no additional polymer or special formulation is needed, which will be a key advantage over nano-formulations. The final product formulation would comprise a liquid or solid drug formulation containing a propellant which may be administered via respiratory tract as liquid aerosolized droplets or solid powder or by any other forms. The final product formulation can be a dry powder inhaler or aerosol. The product can be inhaled as aerosols or dry powder. It has been discovered that the LBA ligand conjugate shows wider distribution throughout the lungs, whereas the dispersion and distribution of nanoparticles that employ LBAs are limited by particle size. In addition, the LBA ligand can be further modified using carbohydrate chemistry for more specific and/or broader distribution and dispersion throughout the lungs. The designed ligand is highly versatile and can undergo conjugation with diverse a variety of functional groups and nucleic acid analogs. Scale-up for nucleic acid conjugation can be done easily with simple chemistry steps.

[0038] The conjugates and/or ligands used herein include a genetic sequence (GS) having a 3' end and a 5' end, which can be a peptide nucleic acid (PNA) or an oligonucleotide such as a messenger ribonucleic acid (mRNA) sequence, a small interfering ribonucleic acid (siRNA) sequence, or a deoxyribonucleic acid (DNA) sequence. Each genetic sequence can be natural or optionally modified, for example in an order of nucleotides or via modifications as a gamma-serine modified gamma peptide nucleic acid, an alanine gamma peptide nucleic acid, a clamp G- modified peptide nucleic acid, a locked nucleic acid (LNA), a phosphorothioates (PS), a phosphorodiamidate morpholino (PMO), a 2'-O-methyl (2'-O-

Me), 2'-O-methoxyethyl (2'-O-MOE), 2'-fluoro (2'F), a 5'-methylcytosine, or a combination thereof. In an aspect, the genetic sequence is a PNA.

[0039] In an aspect, the conjugates bear 1 to 8, or 1 to 5, or 2 to 5, or 2 to 4 carbohydrate residues or ligands. In an embodiment, the carbohydrate-based ligands (also called as carbohydrate ligands) can be a monovalent, a divalent, a trivalent, a tetravalent, or a polyvalent carbohydrate ligands. In an embodiment, the carbohydrate-based ligands can be a monovalent, a divalent, a trivalent, or a tetravalent carbohydrate ligands. In an embodiment, the carbohydrate-based ligands can be a monovalent or a divalent carbohydrate ligands. In an embodiment, the carbohydrate-based ligand is a divalent carbohydrate ligands. The number and type of carbohydrate ligands are selected to target the lungs, preferably to selectively target the lungs. For example, the carbohydrate ligand can be selected to target mannose receptors involved in binding and endocytosis of mannose-containing glycoconjugates, expressed in various immune cells and endothelial cells. In an aspect the conjugates bear 1 to 8, or 1 to 5, or 2 to 5, or 2 to 4 galactose ligands to target the mannose receptor C type 2 (MRC2) on lung cells. MRC2, also known as the Endo180 receptor or CD280, is a transmembrane glycoprotein. It is involved in the recognition and endocytosis of glycoproteins containing mannose, fucose, and N-acetylglucosamine. MRC2 specifically binds to glycoproteins with terminal mannose residues, which distinguishes it from other glycan-binding receptors. MRC2 is expressed in a variety of cell types, including macrophages, dendritic cells, and endothelial cells.

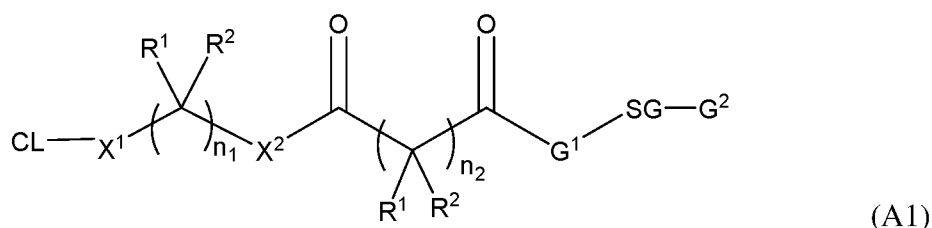
[0040] In an aspect the conjugates bear 1 to 8, or 1 to 5, or 2 to 5, or 2 to 4 galactose amine (GalNAc) ligands to target the MRC2 on lung cells. In another aspect the conjugates bear 1 to 8, or 1 to 5, or 2 to 5, or 2 to 4, or 2 to 3 lactobionic acid ligands to target the MRC2 on lung cells.

[0041] In yet another embodiment, the composition can be effective to target an asialoglycoprotein receptor involved in the clearance of desialylated glycoproteins, predominantly expressed in hepatocytes.

[0042] The carbohydrate ligand(s) of the conjugate can be fully or partially acylated on a hydroxy or amino group thereof with a C2 to C15 acyl group. For example, the carbohydrate ligand can be fully or partially acetylated on a hydroxy or amino group. The acetylation of a carbohydrate ligand can be performed using an acetylating reagent such as acetic anhydride, acetyl chloride, mixed anhydrides, acids with coupling agents such as DCC or like reagents and a base such as triethyl amine, pyridine, DIEA, DMAP or the like, or an organic, inorganic, or polymeric base as used in the art. In an aspect, the carbohydrate ligand

is a GalNAc residue that is fully or partially acylated, preferably acetylated, preferably fully acetylated. Alternatively, the carbohydrate ligand is a lactobionic acid residue that is fully or partially acylated, preferably acetylated, preferably fully acetylated.

[0043] In an aspect, the conjugate includes a genetic sequence/small molecule-carbohydrate conjugate of a formula A1, an enantiomer, a diastereoisomer, a pharmaceutically acceptable salt, a hydrate, a solvate or mixtures thereof:



wherein SG is a small molecule (SM), a genetic sequence (GS), or a combination thereof, wherein, SM is a small molecule, preferably a drug, a dye or a compound targeting lung, and GS is a genetic sequence, preferably a peptide nucleic acid or an oligonucleotide such as an mRNA sequence, an siRNA sequence, or a DNA sequence, optionally wherein each genetic sequence is natural or modified, for example comprises a gamma-serine modified gamma peptide nucleic acid, an alanine gamma peptide nucleic acid, a clamp G-modified peptide nucleic acid, a locked nucleic acid (LNA), a phosphorothioate (PS), a phosphorodiamidate morpholino (PMO), a 2'-O-methyl (2'-O-Me), 2'-O-methoxyethyl (2'-O-MOE), 2'-fluoro (2'F), a 5'-methylcytosine, or a combination thereof, the genetic sequence having a 3' end and a 5' end,

R₁ and R₂ are each independently H or a substituted or unsubstituted C₁ to C₆ alkyl, X¹ is O, NR³, C=O, or C(R³)₂ where R³ is H or a substituted or unsubstituted C₁ to C₆ alkyl,

X² is O, NR³, or C(R³)₂ where R³ is H or a substituted or unsubstituted C₁ to C₆ alkyl,

G¹ is a direct bond or a group linking the PNA to the conjugate,

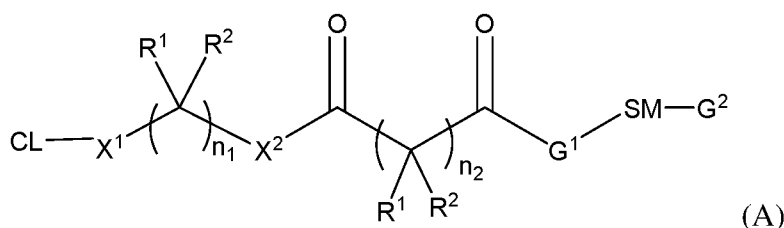
G² is H or a functional moiety,

CL is a carbohydrate ligand comprising 2 to 16 carbohydrate residues derived from a monosaccharide, a disaccharide, a trisaccharide, or a tetrasaccharide, preferably wherein CL comprises a carbohydrate residue derived from a monosaccharide or a disaccharide, optionally wherein the carbohydrate ligand is fully or partially acylated on a hydroxy or amino group thereof with a C₂ to C₁₅ acyl group, preferably wherein the carbohydrate ligand is fully or partially acetylated on a hydroxy or amino group thereof,

n¹ is 1 to 20, and

n^2 is 0 to 20; where the composition is delivered to the lungs and where the genetic sequence and/or small molecule conjugate is effective to target a macrophage receptor type 2. n^2 is 0 to 20; where the composition is delivered to the lungs and where the carbohydrate ligand is effective to target a macrophage receptor type 2. It is to be noted that when the composition is delivered to the lungs, either the carbohydrate ligand, the small molecule, the genetic sequence, or a combination thereof from the genetic sequence/small molecule-carbohydrate ligand is effective to target a macrophage receptor type 2. In an embodiment, the conjugate is effective to target a macrophage receptor type 2. In an embodiment, the carbohydrate ligand is effective to target a macrophage receptor type 2.

[0044] In an aspect, disclosed is a genetic sequence/small molecule-carbohydrate conjugate including carbohydrate-based ligands to target the lungs. In an aspect, the genetic sequence/small molecule-carbohydrate conjugate, is a compound having a formula A, an enantiomer, a diastereoisomer, a pharmaceutically acceptable salt, a hydrate, a solvate or mixtures thereof:

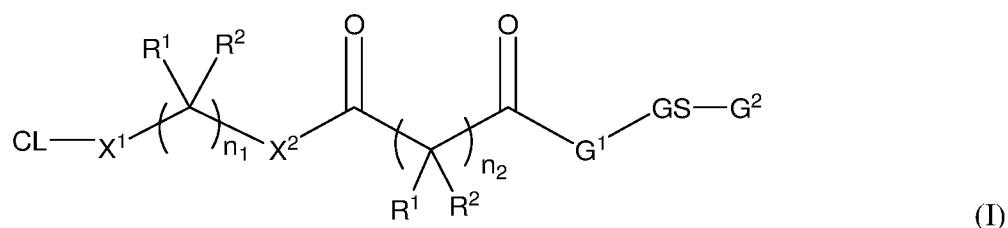


wherein SM is a small molecule, preferably a drug or a compound targeting lung, R_1 and R_2 are each independently H or a substituted or unsubstituted C_1 to C_6 alkyl, X^1 is O, NR^3 , $C=O$, or $C(R^3)_2$ where R^3 is H or a substituted or unsubstituted C_1 to C_6 alkyl, X^2 is O, NR^3 , or $C(R^3)_2$ where R^3 is H or a substituted or unsubstituted C_1 to C_6 alkyl, G^1 is a direct bond or a group linking the small molecule to the conjugate, G^2 is H or a functional moiety, CL is a carbohydrate ligand comprising 2 to 16 carbohydrate residues derived from a monosaccharide, a disaccharide, a trisaccharide, or a tetrasaccharide, preferably wherein CL comprises a carbohydrate residue/ligand derived from a monosaccharide, a disaccharide, or a polysaccharide, optionally wherein the carbohydrate ligand is fully or partially acylated on a hydroxy or amino group thereof with a C_2 to C_{15} acyl group, preferably wherein the carbohydrate ligand is fully or partially acetylated on a hydroxy or amino group thereof, n^1 is 1 to 20, and n^2 is 0 to 20.

[0045] In an embodiment, the genetic sequence/small molecule carbohydrate conjugate is effective to target a macrophage receptor type 2. In an embodiment, the small molecule can be ivacaftor, ellexacaftor, tezacaftor, lumacaftor, pirfenidone, N-acetylcysteine,

nintedanib, cisplatin, carboplatin, docetaxel, paclitaxel, pemetrexed, azathioprine, mycophenolate mofetil, tocilizumab, cyclophosphamide, saracatinib, or a combination thereof.

[0046] In another aspect, disclosed is a genetic sequence/small molecule-carbohydrate conjugate including carbohydrate-based ligands to target the lungs. In an aspect, the genetic sequence/small molecule-carbohydrate conjugate, is a compound having a formula I, an enantiomer, a diastereoisomer, a pharmaceutically acceptable salt, a hydrate, a solvate or mixtures thereof:



wherein GS is a genetic sequence, preferably a peptide nucleic acid or an oligonucleotide such as an mRNA sequence, an siRNA sequence, or a DNA sequence, optionally wherein each genetic sequence is natural or modified, for example comprises a gamma-serine modified gamma peptide nucleic acid, an alanine gamma peptide nucleic acid, a clamp G-modified peptide nucleic acid, a locked nucleic acid (LNA), a phosphorothioate (PS), a phosphorodiamidate morpholino (PMO), a 2'-O-methyl (2'-O-Me), 2'-O-methoxyethyl (2'-O-MOE), 2'-fluoro (2'F), a 5'-methylcytosine, or a combination thereof, the genetic sequence having a 3' end and a 5' end, R₁ and R₂ are each independently H or a substituted or unsubstituted C₁ to C₆ alkyl, X¹ is O, NR³, C=O, or C(R³)₂ where R³ is H or a substituted or unsubstituted C₁ to C₆ alkyl, X² is O, NR³, or C(R³)₂ where R³ is H or a substituted or unsubstituted C₁ to C₆ alkyl, G¹ is a direct bond or a group linking the PNA to the conjugate, G² is H or a functional moiety, CL is a carbohydrate ligand comprising 2 to 16 carbohydrate residues derived from a monosaccharide, a disaccharide, a trisaccharide, or a tetrasaccharide, preferably wherein CL comprises a carbohydrate residue/ligand derived from a monosaccharide, a disaccharide, or a polysaccharide, optionally wherein the carbohydrate ligand is fully or partially acylated on a hydroxy or amino group thereof with a C₂ to C₁₅ acyl group, preferably wherein the carbohydrate ligand is fully or partially acetylated on a hydroxy or amino group thereof, n¹ is 1 to 20, and n² is 0 to 20. In an embodiment, the genetic sequence/small molecule-carbohydrate conjugate is effective to target a macrophage receptor type 2. In an embodiment, the conjugate is effective to target a macrophage receptor

type 2. In an embodiment, the carbohydrate ligand is effective to target a macrophage receptor type 2.

[0047] The carbohydrate ligands can be covalently attached to the small molecule or the genetic sequence by a backbone linker as shown in Formula A and Formula I. A variety of backbones can be used, but in general contain at least two functional groups, for example at least two amino groups, one or more for reaction with the carbohydrate ligand(s) and one or more for reaction with the genetic sequence. The amino groups can be selectively protected as known in the art and as described in the Examples. The backbone can include moieties to modify properties such as solubility. For example, lysine and arginine residues can be present in a backbone. In an embodiment, the carbohydrate ligands can be a monovalent, a divalent, a trivalent, a tetravalent, or a polyvalent carbohydrate ligands.

[0048] In an aspect as shown in Formula A and I, a group G^1 or G^2 can be optionally present. G^1 can be a linker from the backbone to the genetic sequence, for example a linker having 1 to 20 carbon atoms, and optionally one or more reactive groups such as hydroxy, carboxy, thio, or amino. In another aspect, G^1 or G^2 can be a functional moiety. The functional moiety G^1 , G^2 can provide a structural feature to the conjugates that can impart a desired function such as steric separation from a binding ligand, enhancing hydrophilicity or hydrophobicity, facilitating absorption, of the conjugates, facilitating distribution of the conjugate in the body, or other functions advantageous in medicinal chemistry and drug design. The functional moiety can be linked between the backbone and the genetic sequence or at a terminal end of the genetic sequence, or both. In an aspect, a functional moiety G^1 , G^2 is, for example, a residue of a polyethylene glycol, a polypropylene glycol, or a polyethylene-propylene glycol. In an aspect, G^1 or G^2 , or both can be polyethylene glycol (PEG) group. The PEG group can contain 1 to 25 ethylene glycol residues ($-OCH_2CH_2O-$) that can terminate in a free hydroxy, amino, ether, or like functional moiety. The which is optionally bonded to a ligand, a backbone or structure of a conjugate.

[0049] In another aspect, the functional moiety can include a therapeutic agent. Examples include beta-2 agonists (e.g., albuterol (Salbutamol), salmeterol, formoterol), anticholinergics (ipratropium bromide, tiotropium, methylxanthines, theophylline) anti-inflammatory agents (e.g., corticosteroids, beclomethasone, budesonide, fluticasone), leukotriene modifiers (e.g., montelukast), mast cell stabilizers (e.g., cromolyn sodium, nedocromil), antibiotics and antimicrobials (e.g., tobramycin, aztreonam, colistin (Polymyxin E), amphotericin B, voriconazole); antiviral agents (e.g., zanamivir, oseltamivir, ribavirin); mucolytics (e.g., N-acetylcysteine, pulmozyme); immunomodulators; monoclonal antibodies

(e.g., dupilumab (for asthma) omalizumab (for allergic asthma), cyclosporine (investigational for lung transplant patients)); gene therapy and nucleic acid-based therapies (e.g., adenoviral vectors, lentiviral vectors); siRNA and antisense oligonucleotides; pulmonary surfactants (e.g., beractant, poractant alfa); analgesics (e.g., fentanyl); anticancer agents (chemotherapeutic agents such as cisplatin, paclitaxel in nano-formulation form for inhalational delivery); antioxidants and anti-fibrotic agents (e.g., pirfenidone, nintedanib); or a combination thereof.

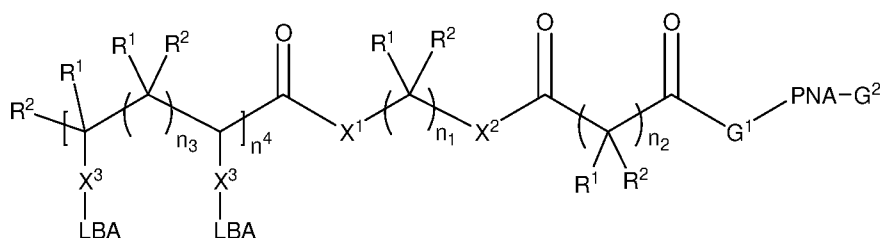
[0050] Other examples of G¹ or G² include kielin, tolvaptan, nintedanib, paclitaxel, bleomycin, cyclosporin, cisplatin, romidepsin, doxorubicin, docetaxel, danunorubicin, vincristine, methotrexate, cyclophosphamide, venetoclax, hydroxyurea, mercaptopurine, prednisolone, cytarabine, deoxyribonuclease I (rhDNase) (dornase alfa), catalase, superoxide dismutase (SOD), glucocerebrosidase, alpha-1-antitrypsin, lipase, hyaluronidase, alpha-galactosidase or pirfenidone.

[0051] Examples of other functional moieties that can be used as a therapeutic agent include vinca alkaloids (e.g., vinblastine, vincristine, and vinorelbine), paclitaxel, epididodophyllotoxins (e.g., etoposide, teniposide), antibiotics (e.g., dactinomycin, actinomycin D, daunorubicin, doxorubicin, penicillin V, penicillin G, ampicillin, amoxicillin, cephalosporin, tetracycline, doxycycline, minocycline, demeclocycline, erythromycin, aminoglycoside antibiotics, polypeptide antibiotics, nystatin, griseofulvin, and idarubicin), anthracyclines, mitoxantrone, bleomycins, plicamycin, mithramycin and mitomycin, enzymes (L-asparaginase, which systemically metabolizes L-asparagine and deprives cells which do not have the capacity to synthesize their own asparagine), antiplatelet agents such as G(GP) IIb/IIIa inhibitors and vitronectin receptor antagonists, anti-proliferative/antimitotic alkylating agents such as nitrogen mustards (e.g., mechlorethamine, cyclophosphamide and analogs, melphalan, chlorambucil), ethylenimines and methylmelamines (e.g., hexamethylmelamine and thiotepa), alkyl sulfonates- busulfan, nitrosoureas (e.g., carmustine (BCNU) and analogs, streptozocin), trazenes--dacarbazine (DTIC), anti-proliferative/antimitotic antimetabolites such as folic acid analogs (e.g., methotrexate), pyrimidine analogs (e.g., fluorouracil, floxuridine, cytarabine), purine analogs and related inhibitors (e.g., mercaptopurine, thioguanine, pentostatin and 2- chlorodeoxyadenosine {cladribine}), platinum coordination complexes (e.g., cisplatin, carboplatin), procarbazine, hydroxyurea, mitotane, aminoglutethimide, hormones (e.g., estrogen), anti-coagulants (e.g., heparin, synthetic heparin salts and other inhibitors of thrombin), fibrinolytic agents (e.g., tissue plasminogen activator, streptokinase and urokinase), aspirin, dipyridamole, ticlopidine,

clopidogrel, abciximab, antimigratory, antisecretory (e.g., brexveldin), anti-inflammatory: such as adrenocortical steroids (e.g., cortisol, cortisone, fludrocortisone, prednisone, prednisolone, 6 α -methylprednisolone, triamcinolone, betamethasone, and dexamethasone), non-steroidal agents (e.g., salicylic acid derivatives such as aspirin, para-aminophenol derivatives such as acetaminophen, indole and indene acetic acids (e.g., indomethacin, sulindac, etodolac), heteroaryl acetic acids (e.g., tolmetin, diclofenac, ketorolac), arylpropionic acids (e.g., ibuprofen and derivatives), anthranilic acids (e.g., mefenamic acid, meclofenamic acid), enolic acids (e.g., piroxicam, tenoxicam, phenylbutazone, oxyphenthatrazone), nabumetone, gold compounds (e.g., auranofin, aurothioglucose, gold sodium thiomalate), immunosuppressives (e.g., cyclosporine, tacrolimus (FK-506), sirolimus (e.g., rapamycin, azathioprine, mycophenolate mofetil), angiogenic agents such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), angiotensin receptor blockers, nitric oxide donors, anti-sense oligonucleotides and combinations thereof, cell cycle inhibitors, mTOR inhibitors, and growth factor receptor signal transduction kinase inhibitors, retinoids, cyclin/CDK inhibitors, HMG co-enzyme reductase inhibitors (statins) or protease inhibitors.

[0052] Other therapeutic agents can be found in the Merck Index published by the Royal Society of Chemistry published in print and online at <https://www.rsc.org/merck-index>. For example, G¹ can be a linker between the backbone and the genetic sequence, and include a therapeutic agent covalently bound thereto. Alternatively, or in addition, the group G² can be a therapeutic agent covalently bound to the genetic sequence either directly or by a linker. Although not shown in Formula A and I, it is also possible for a functional moiety such as a therapeutic agent to be linked to the backbone using a linkage similar to that linking the carbohydrate residue.

[0053] In an aspect, the conjugate is a genetic sequence-lactobionic acid conjugate. Lactobionic acid (LBA) is a disaccharide formed from gluconic acid and galactose. In some embodiments, lactobionic acid is derivatized as part of a conjugate. PNA-lactobionic acid conjugate of Formula Ia, an enantiomer, a diastereoisomer, a pharmaceutically acceptable salt, a hydrate, a solvate or mixtures thereof:

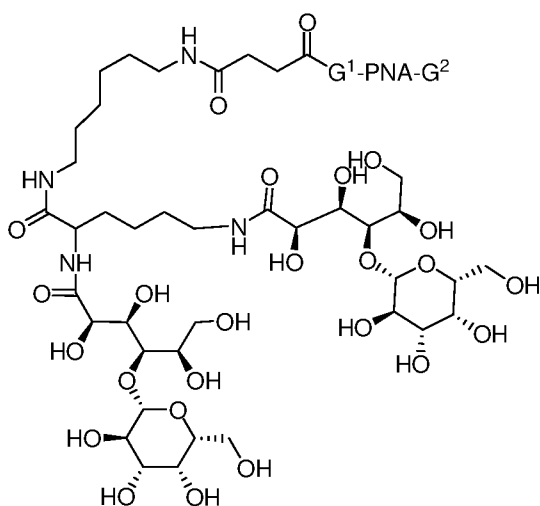


(Ia)

wherein LBA is a lactobionic acid residue, X^1 is NR^3 , O, or $C(R^3)_2$ where R^3 is H or a substituted or unsubstituted C_1 to C_6 alkyl, each X^3 is independently O, NR^3 , or $C(R^3)_2$ where R^3 is H or a substituted or unsubstituted C_1 to C_6 alkyl, n_3 is 0 to 20, and n_4 is 1 to 8.

Preferably, G^1 is a group linking the PNA to the conjugate, R^1 and R^2 are each H, X^1 , X^2 , and X^3 are each NH, and $n_1=6$, $n_2=2$, and $n_3=4$. In an aspect, R^1 and R^2 are each H, $n_3=4$, and the PNA is linked at the 5' end to the conjugate.

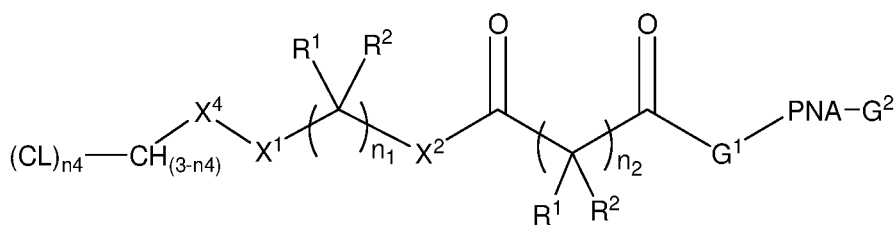
[0054] For example, the PNA-lactobionic acid conjugate can be of formula Ia-1, an enantiomer, a diastereoisomer, a pharmaceutically acceptable salt, a hydrate, a solvate or mixtures thereof.



(Ia-1)

wherein G^1 and G^2 are as defined above, preferably wherein G^1 is a functional moiety linking the PNA to the conjugate and G^2 is a functional moiety. Optionally in any of the Formulas Ia and Ia-1, the hydroxyl groups can be fully or partially acylated with an acyl group having from 2 to 15 carbon atoms or 2 to 8 carbon atoms, preferably acetylated, more preferably fully acetylated as described above.

[0055] In another aspect, the conjugate can be of Formula Ib, an enantiomer, a diastereoisomer, a pharmaceutically acceptable salt, a hydrate, a solvate or mixtures thereof:



(Ib)

wherein X^1 is $C=O$ or $C(R^3)_2$ where R^3 is H or a substituted or unsubstituted C_1 to C_6 alkyl, X^4 is O, NR^3 , or $C(R^3)_2$ where R^3 is H or a substituted or unsubstituted C_1 to C_6 alkyl, CL is a carbohydrate residue linked to CH by a 1 to 30 atom linker chain comprising a substituted or

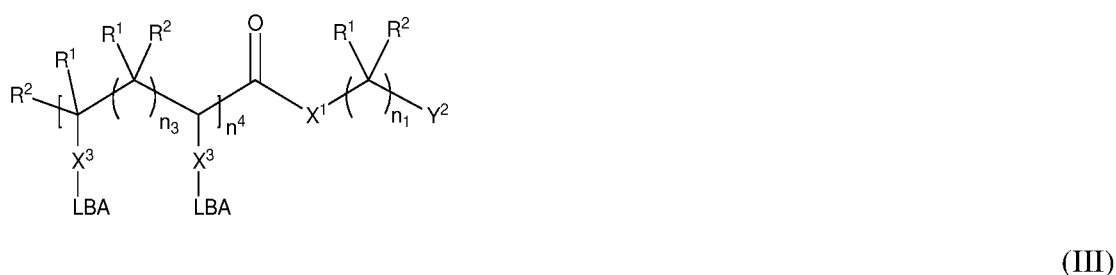
unsubstituted C₁ to C₁₂ alkyl or a C₆ to C₁₂ aryl comprising an amide, ester, or ether group, and n₄ is 2 or 3. The carbohydrate residue in Formula Ib can be derived from N-acetylgalactosamine, and can be a fully or partially acylated carbohydrate residue wherein the acyl groups have 2 to 15 carbon atoms or 2 to 8 carbon atoms, for example a fully or partially acetylated carbohydrate residue, such as fully acetylated.

[0056] A method of conjugating a genetic sequence to a carbohydrate ligand to provide the genetic sequence-carbohydrate conjugate is described. The method includes functionalizing the genetic sequence to provide free -COOH functionality; and forming a bond between the free -COOH functionality of modified genetic sequence and Y² of a compound of a formula II



wherein Y² is -NHR³ or -OH. The method can be performed by solution-phase or solid-phase synthesis or a combination thereof. The genetic sequence, for example a PNA, can be obtained by solution- or solid-phase synthesis as is known in the art, or a combination thereof. It can be modified as described below. In addition, the method can further include modifying the genetic sequence with a precursor of G¹, G², or a combination thereof, before functionalizing the genetic sequence.

[0057] In an aspect, a method of conjugating a genetic sequence to a lactobionic acid-backbone ligand to provide a genetic sequence-lactobionic acid conjugate includes functionalizing the genetic sequence to provide free -COOH functionality; and forming a bond between the free -COOH functionality of modified genetic sequence and Y² of a formula III



wherein Y² is -NHR³ or -OH. Again, the genetic sequence is preferably a PNA. In an aspect, the method can further comprise reacting lactobionic acid with a backbone of a formula IV

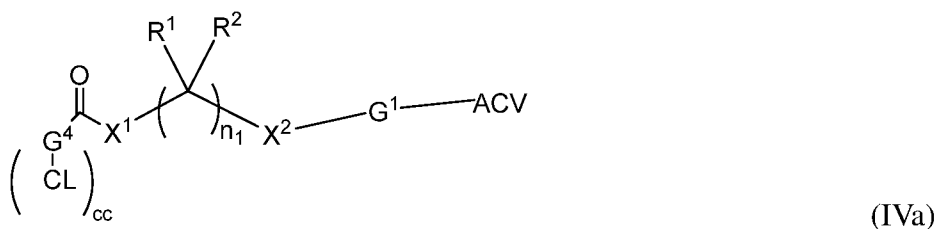


wherein X^3 is an $-OH$ or NHR^3 , and X^2 is a protected O or protected NHR^3 .

[0058] In an aspect, a lactobionic acid residue can be coupled to a backbone comprising a lysine residue by its alpha and epsilon amino groups. The lysine carboxyl group is in turn coupled to an amino group on an alkyl diamine, and the other amino group is coupled to a succinyl COOH group linked to a peptide nucleic acid. In other aspects, the alkyl diamine can be substituted by an alkane diol to form a backbone with ester linkages. Alternatively, the succinic acid at the 5' end can be replaced by a substituted or unsubstituted C to C₂₀ dicarboxylic acid. A PNA is modified with a functional moiety for example a trioxo-miniPEG spacer and succinic acid at the 5' end to provide a free $-COOH$ functionality after cleavage. Some PNAs so modified are commercially available. The free COOH group can then be reacted with an amino group, hydroxy group, alkyl halide, or other suitable functional group on a ligand backbone, for example lactobionic acid or GalNAc.

[0059] Particularly when GalNAc is used, the GalNAcs can be linked to the backbone by groups bearing an alkyl ether, amide, ester residues to provide the carbohydrate ligand. Some of these ligands are available commercially or can be synthesized using chemical synthesis methods familiar to one of ordinary skill in the art. General methods for chemical synthesis may be found in, among other sources, "Comprehensive Organic Transformations: A Guide to Functional Group Preparations," Richard C. Larock, Wiley-VCH: 1999 and in "March's Advanced Organic Chemistry: Reactions, Mechanisms and Structure", Jerry March & Michael Smith, John Wiley & Sons Inc.: 2001. Of course, other carbohydrate residues can be similarly linked to the backbone by a group, e.g., a chain, bearing alkyl ether, amide, or ester residues to form the ligand.

[0060] In an aspect, the genetic sequence/small molecule-carbohydrate conjugate of formula A1 can be reduced to a genetic sequence-carbohydrate conjugate of formula IVa, an enantiomer, a diastereoisomer, a pharmaceutically acceptable salt, a hydrate, a solvate or mixtures thereof, that can target the lungs:



wherein

ACV is a pharmaceutically active agent, and where the pharmaceutically active agent can be a genetic sequence (GS), a small molecule (SM) or a combination of a genetic sequence and a small molecule;

R^1 and R^2 are each independently selected from H or a substituted or unsubstituted C_1 to C_6 alkyl,

X^1 is selected from the group consisting of O, NR^3 , $C=O$, and $C(R^3)_2$ where R^3 is H, - $C(O)CH_3$, or a substituted or unsubstituted C_1 to C_6 alkyl,

X^2 is O, NR^3 , or $C(R^3)_2$ where R^3 is H or a substituted or unsubstituted C_1 to C_6 alkyl;

n_1 is 1 to 20,

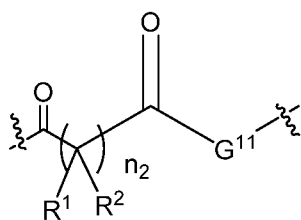
G^1 and G^4 are each a direct bond or a chemical linker; and

G^4 -CL is a carbohydrate ligand structure comprising 2 to 16 carbohydrate residues each derived from a monosaccharide, a disaccharide, a trisaccharide, or a tetrasaccharide; where cc is 2 to 16;

optionally wherein the carbohydrate ligand is fully or partially acylated on a hydroxy or amino group thereof with a C_2 to C_{15} acyl group, preferably wherein the carbohydrate ligand is fully or partially acetylated on a hydroxy or amino group thereof,

n_1 is 1 to 20, and where the carbohydrate conjugate is delivered to the lungs and where upon delivery, the pulmonary retention of the active agent is increased.

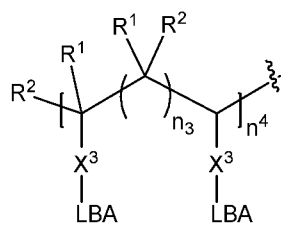
[0061] In an embodiment, G^1 is a 1 to 20 alkylene chain where 1, 2, 3 or 4 carbons on the alkylene chain are substituted with $C=O$ or NH . In another embodiment, G^1 in formula (IVa) is



where n_2 is an integer of 0 to 20 (e.g., n_2 is 4), or 1 to 20;

where R^1 and R^2 are defined above (in formula (IVa)) and G^{11} is a bond or linker moiety. In an embodiment, G^1 is functional moiety linking a nucleic acid to the conjugate or wherein G^1 is a residue of a polyethylene glycol, a polypropylene glycol, or a polyethylene-propylene glycol such a residue of a trioxo-mini polyethylene glycol (PEG) chain.

[0062] In an embodiment, G^4 in formula (IVa) is represented by



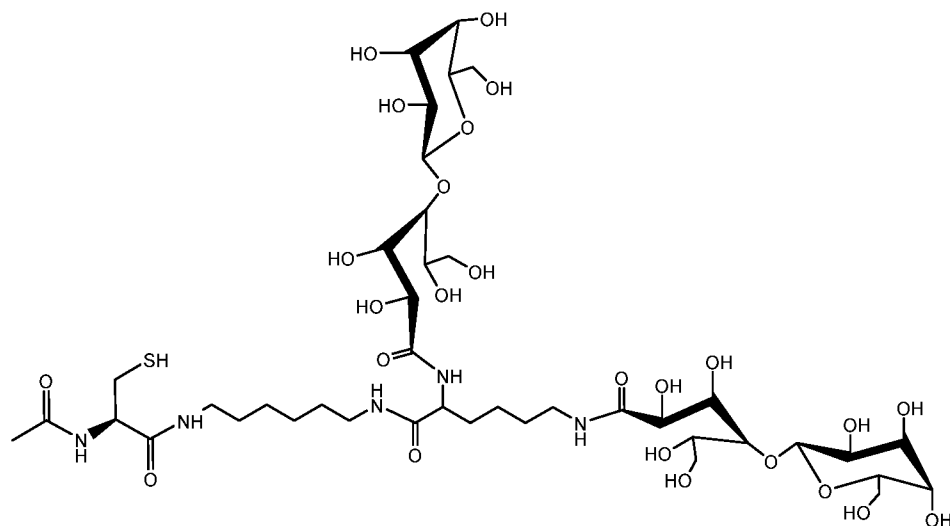
, e.g., where LBA is a lactobionic acid ligand, each X^3 is independently O, NR^3 , or $C(R^3)_2$ where R^3 is H or a substituted or unsubstituted C_1 to C_6 alkyl, n_3 is 0 to 20, and n_4 is 1 to 8.

[0063] In an embodiment in formula (IVa), ACV is selected from the group consisting of a small molecule (SM), a genetic sequence (GS), or a combination thereof, wherein, SM is a small molecule, preferably a drug or a compound targeting the respiratory system (e.g., the lungs), GS is a genetic sequence, preferably a peptide nucleic acid or an oligonucleotide such as an mRNA sequence, an siRNA sequence, or a DNA sequence, optionally wherein each genetic sequence is natural or modified.

[0064] In an embodiment, the genetic sequence GS comprises for example a gamma-serine modified gamma peptide nucleic acid, an alanine gamma peptide nucleic acid, a clamp G-modified peptide nucleic acid, a locked nucleic acid (LNA), a phosphorothioate (PS), a phosphorodiamidate morpholino (PMO), a 2'-O-methyl (2'-O-Me), 2'-O-methoxyethyl (2'-O-MOE), 2'-fluoro (2'F), a 5'-methylcytosine, or a combination thereof, the genetic sequence having a 3' end and a 5' end. In an embodiment, the composition is delivered to the lungs and the genetic sequence/small molecule-carbohydrate conjugate is effective to target a macrophage receptor type 2 (MRC2). In another embodiment, the composition is delivered to the lungs or to the kidney for modulating a target gene, a target mRNA, a microRNA, or a non-coding RNA. Modulating a gene involves controlling its expression or activity, which can have profound effects on cellular function, organismal development, and disease processes.

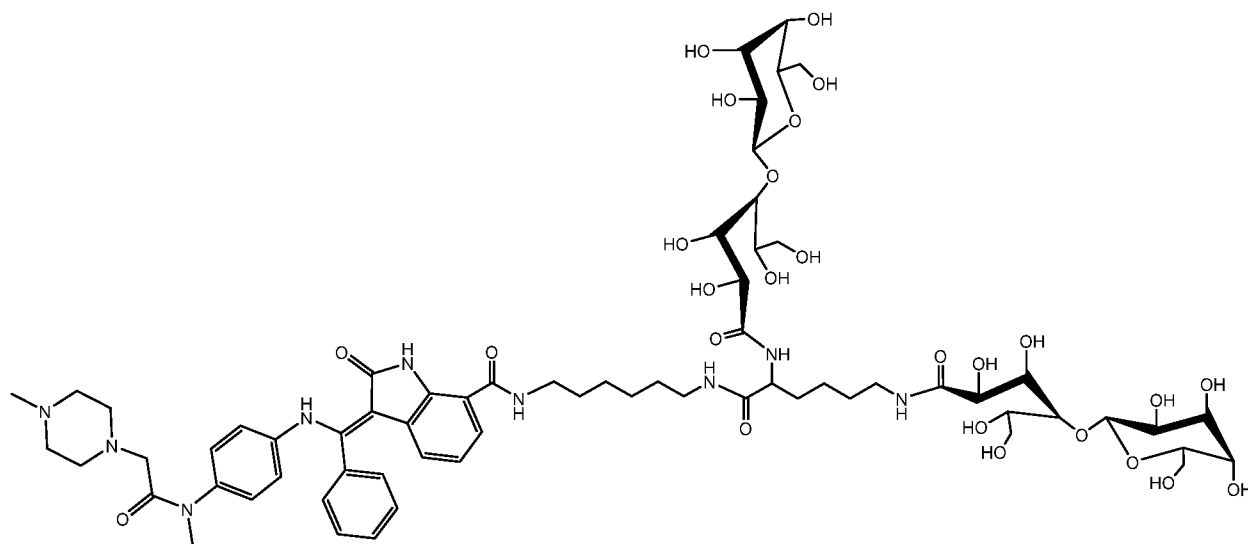
[0065] In an embodiment, the small molecule (SM) can facilitate inhalational therapy of the lungs and comprises antioxidants and mucolytes (e.g., N-acetylcysteine), tyrosine kinase inhibitors (e.g., nintedanib), cystic fibrosis modulators (elexacaftor, tezacaftor, lumacaftor and ivacaftor), antifolate antineoplastic agents (e.g., pemetrexed), microtubule stabilizers (e.g., paclitaxel), chemotherapy drugs (e.g., doxorubicin, cisplatin and carboplatin), antimetabolites (e.g., methotrexate), immunosuppressants (e.g., tacrolimus, mycophenolate mofetil), NSAIDs (e.g., piroxicam), corticosteroids (e.g., prednisone), or a combination thereof.

[0066] Examples of small molecules that can be conjugated with the carbohydrate ligand include N-acetyl cysteine, the carbohydrate conjugate of which (e.g., LBA - N-acetyl cysteine) is shown below in formula (V),



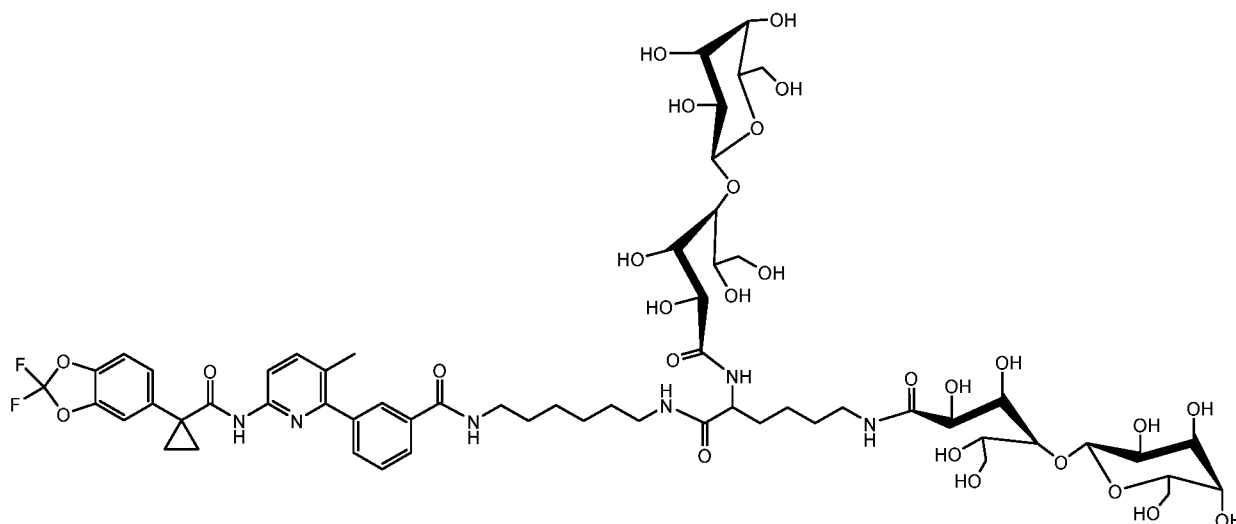
(V),

nintedanib, the carbohydrate conjugate of which (e.g., LBA - nintedanib) is shown below in formula (VI)



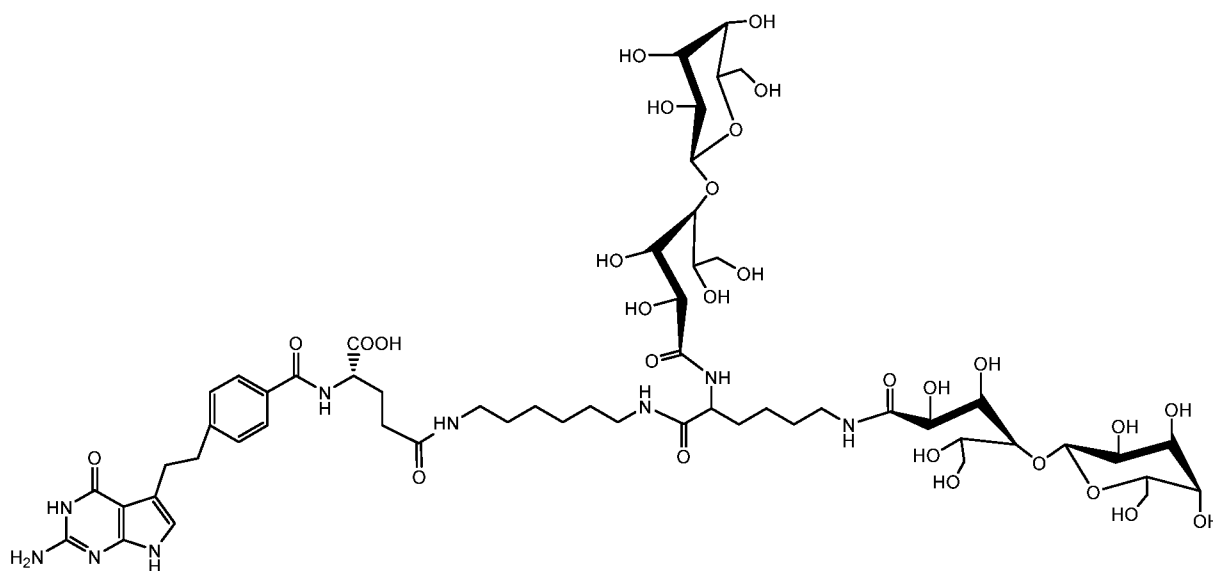
(VI),

lumacaftor, the carbohydrate conjugate of which (e.g., LBA - lumacaftor) is shown below in formula (VII)



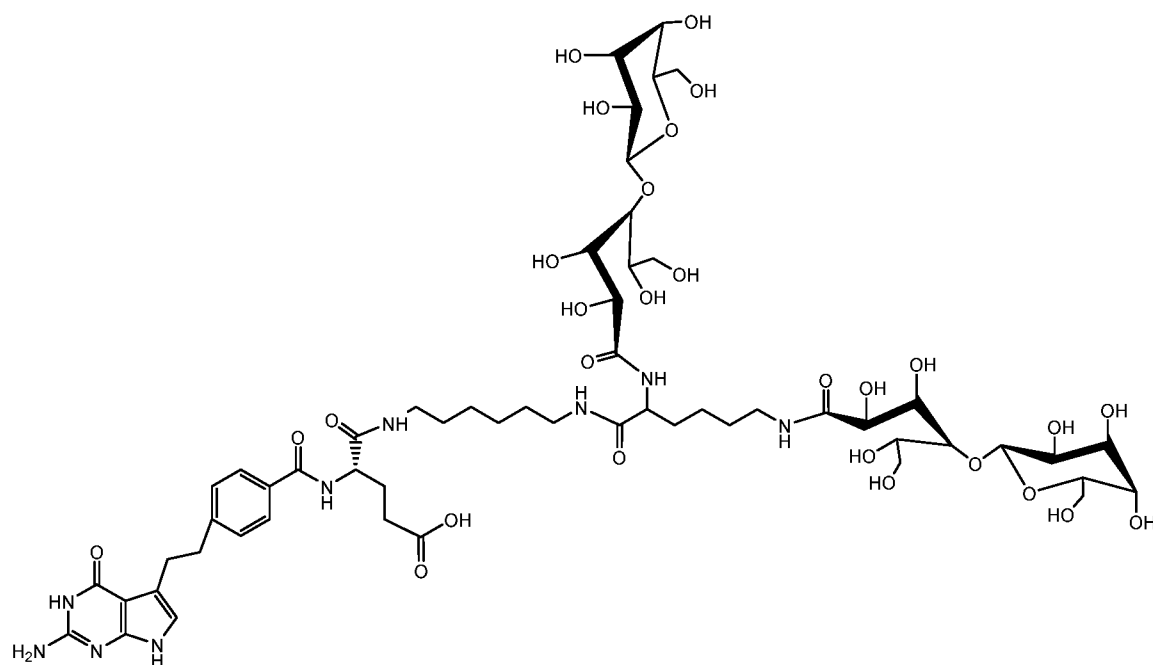
(VII),

pemetrexed-1, the carbohydrate conjugate of which (e.g., LBA – pemetrexed-1) is shown below in formula (VIII)



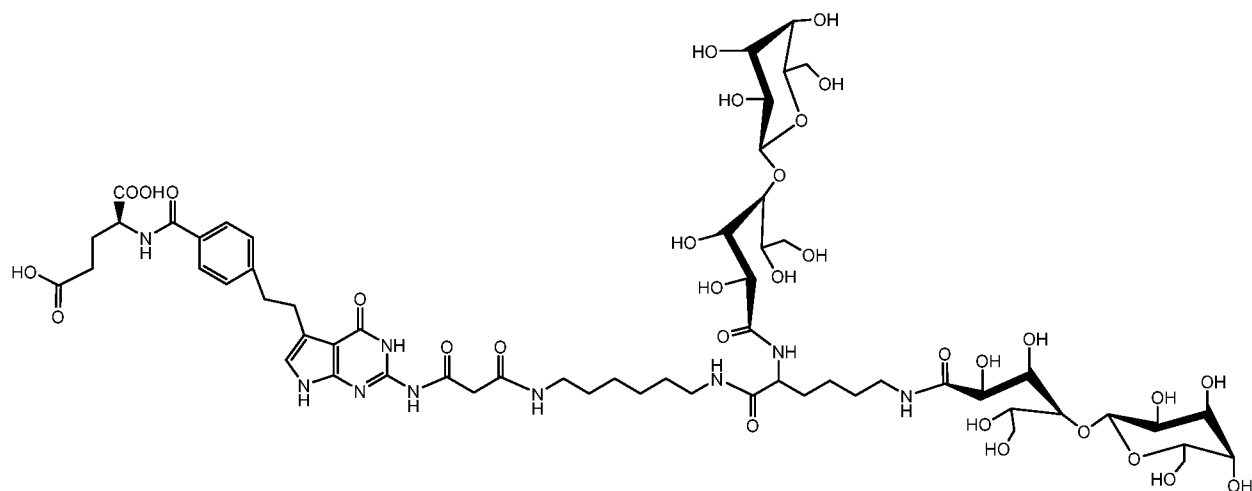
(VIII),

pemetrexed-2, the carbohydrate conjugate of which (e.g., LBA – pemetrexed-2) is shown below in formula (IX)



(IX),

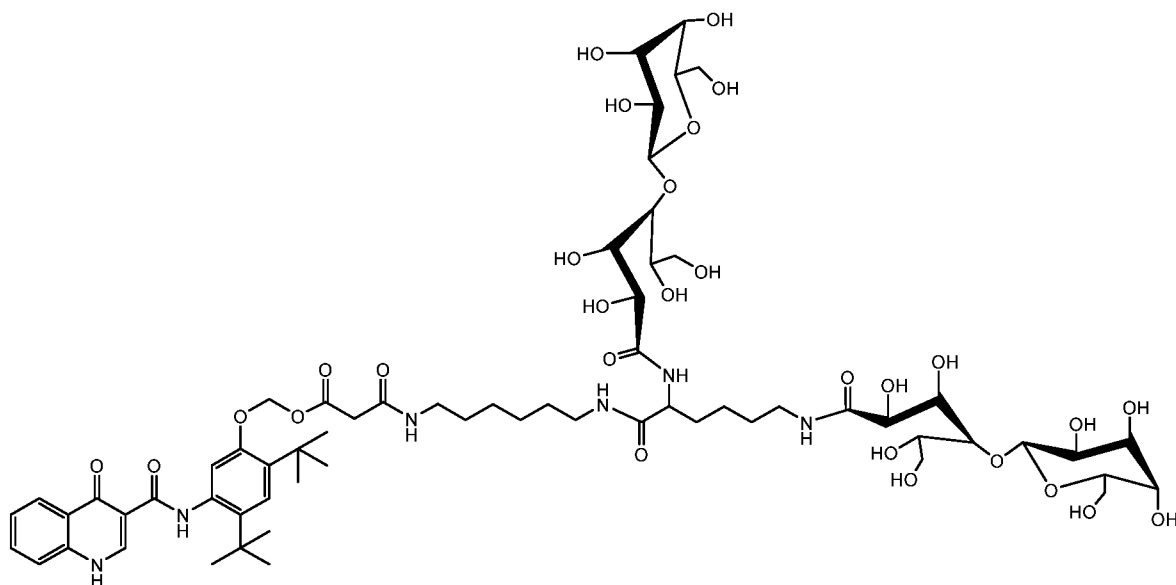
pemetrexed-3, the carbohydrate conjugate of which (e.g., LBA – pemetrexed-3) is shown below in formula (X)



(X),

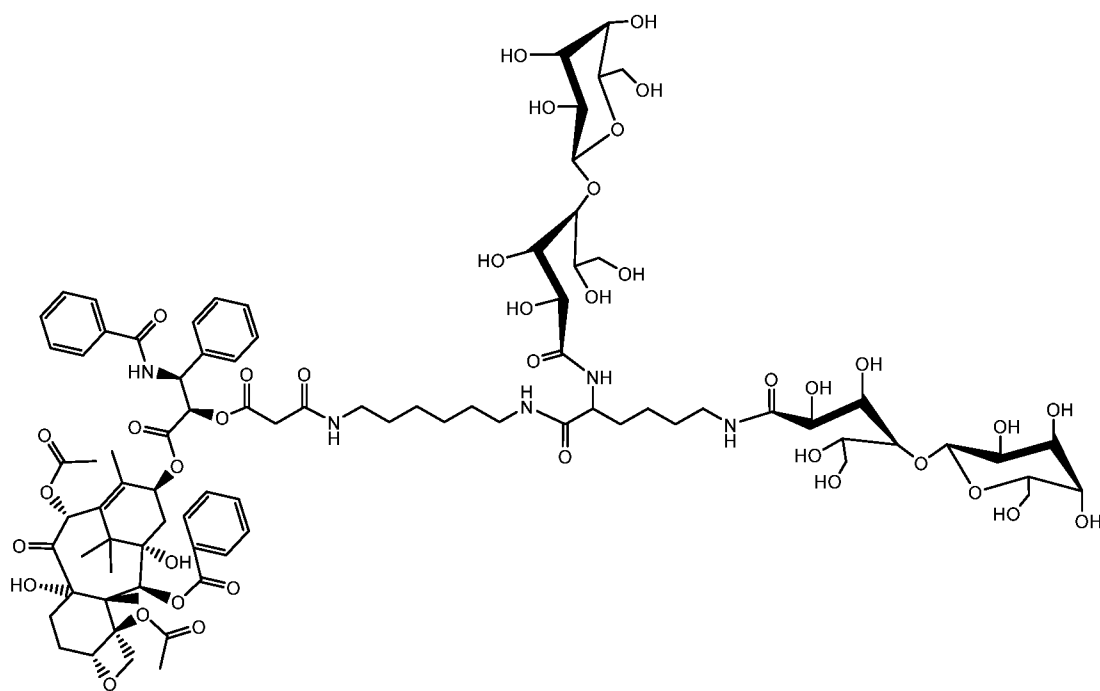
ivacaftor, the carbohydrate conjugate of which (e.g., LBA - ivacaftor) is shown below in formula

(XI)



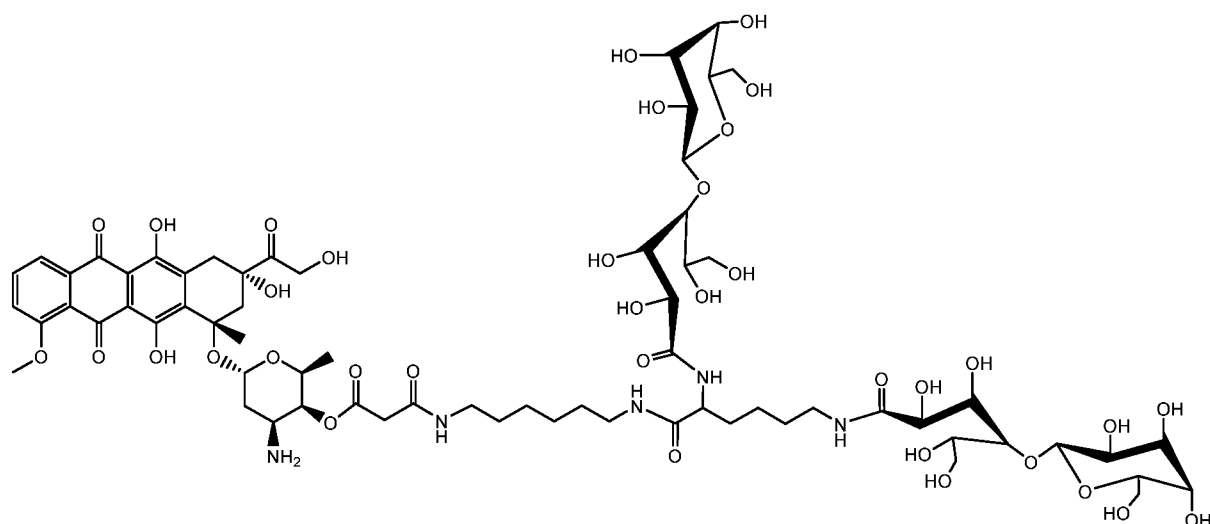
(XI),

paclitaxel-1, the carbohydrate conjugate of which (e.g., LBA – paclitaxel-1) is shown below in formula (XII)



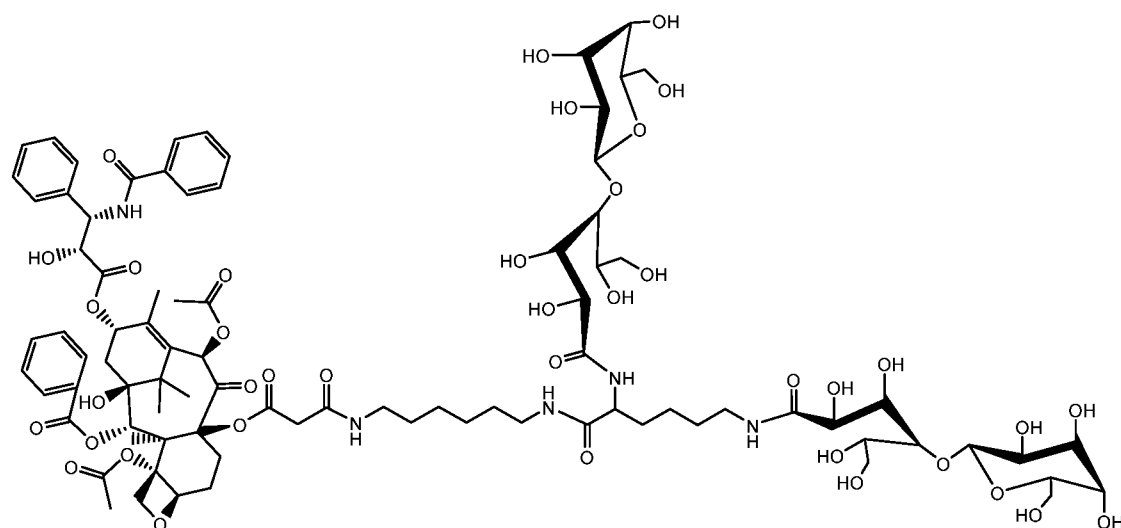
(XII),

doxorubicin-1, the carbohydrate conjugate of which (e.g., LBA – doxorubicin-1) is shown below in formula (XIII)



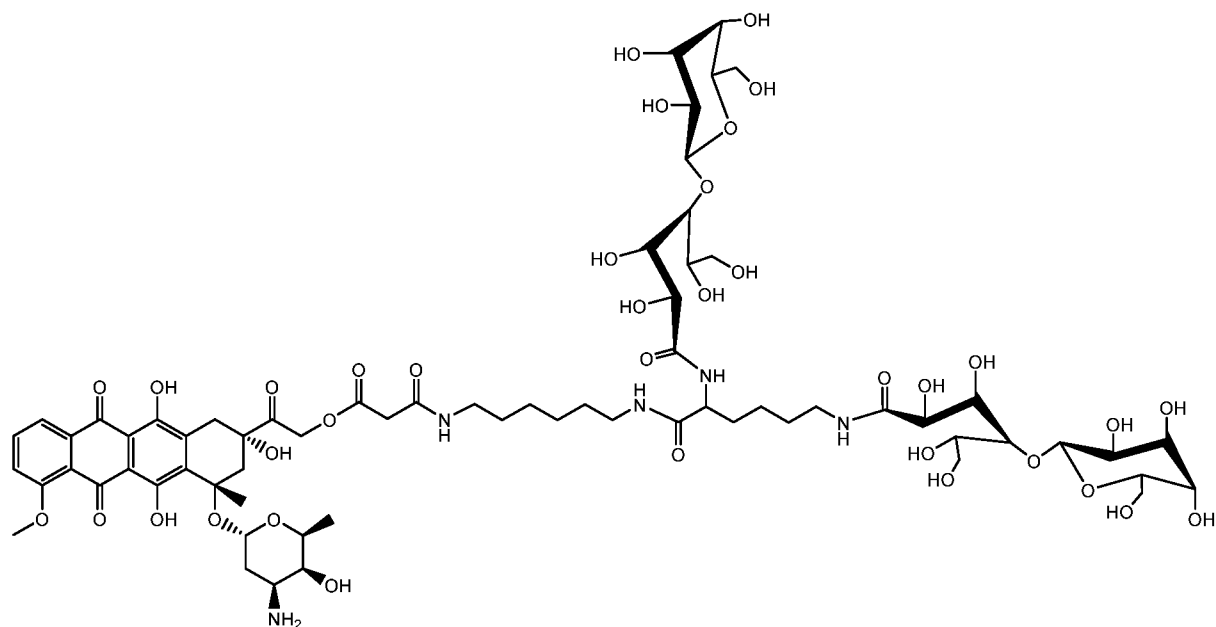
(XIII),

paclitaxel-2, the carbohydrate conjugate of which (e.g., LBA – paclitaxel-2) is shown below in formula (XIV)



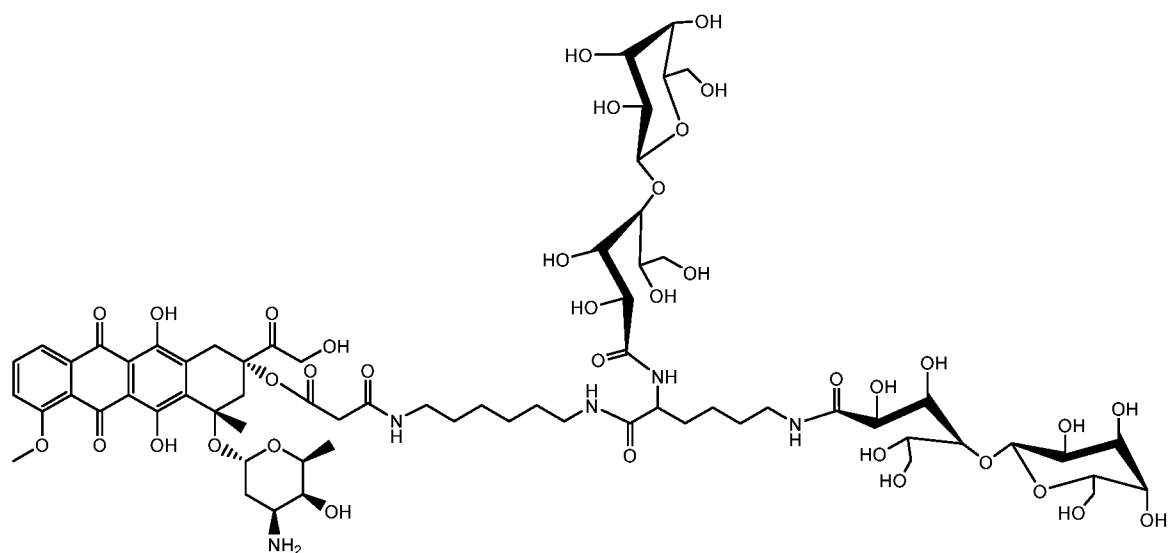
(XIV),

doxorubicin-2, the carbohydrate conjugate of which (e.g., LBA – doxorubicin-2) is shown below in formula (XV)



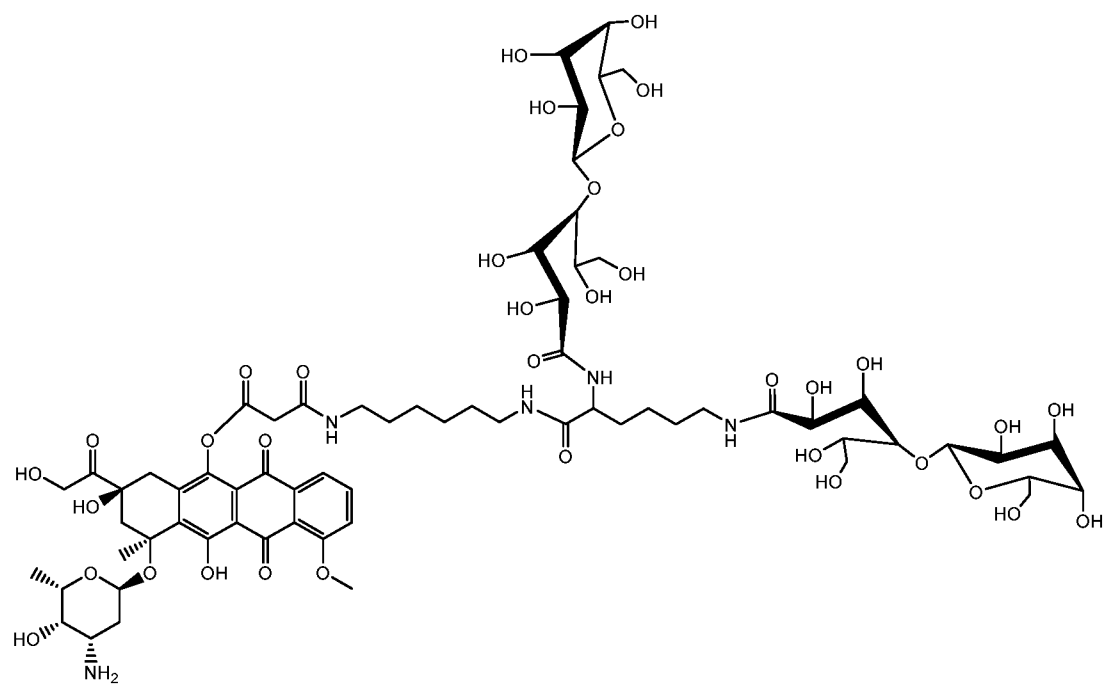
(XV),

doxorubicin-3, the carbohydrate conjugate of which (e.g., LBA – doxorubicin-3) is shown below in formula (XVI)



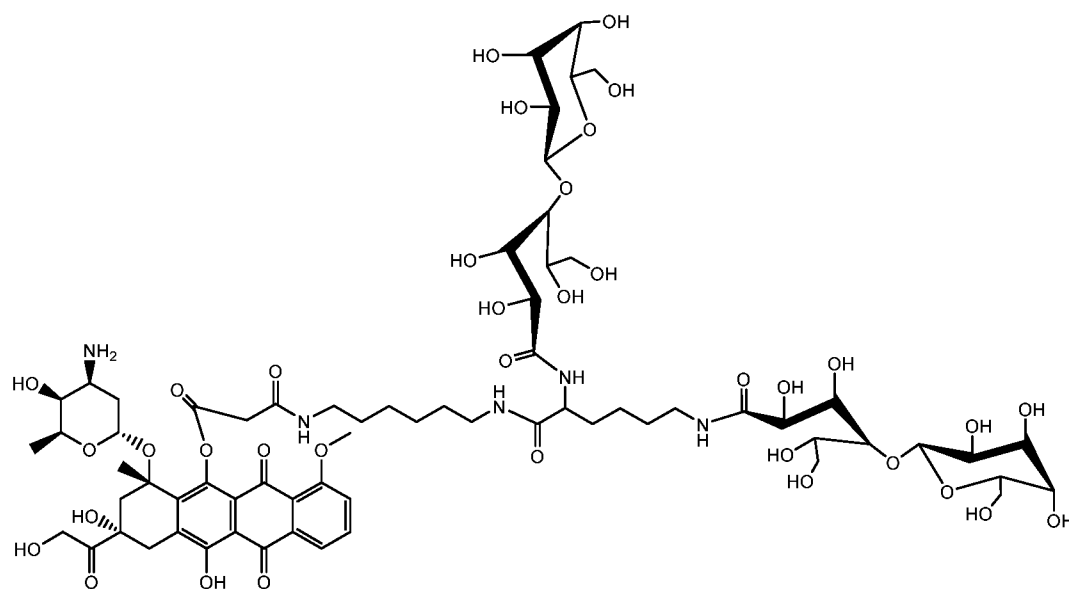
(XVI),

doxorubicin-4, the carbohydrate conjugate of which (e.g., LBA – doxorubicin-4) is shown below in formula (XV)



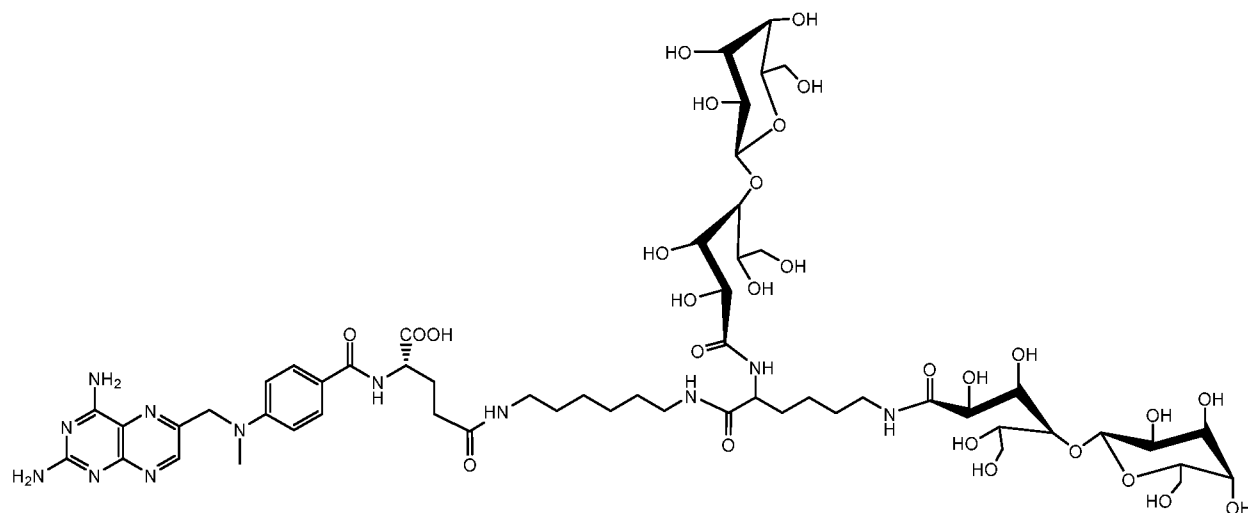
(XV),

doxorubicin-5, the carbohydrate conjugate of which (e.g., LBA – doxorubicin-5) is shown below in formula (XVI)



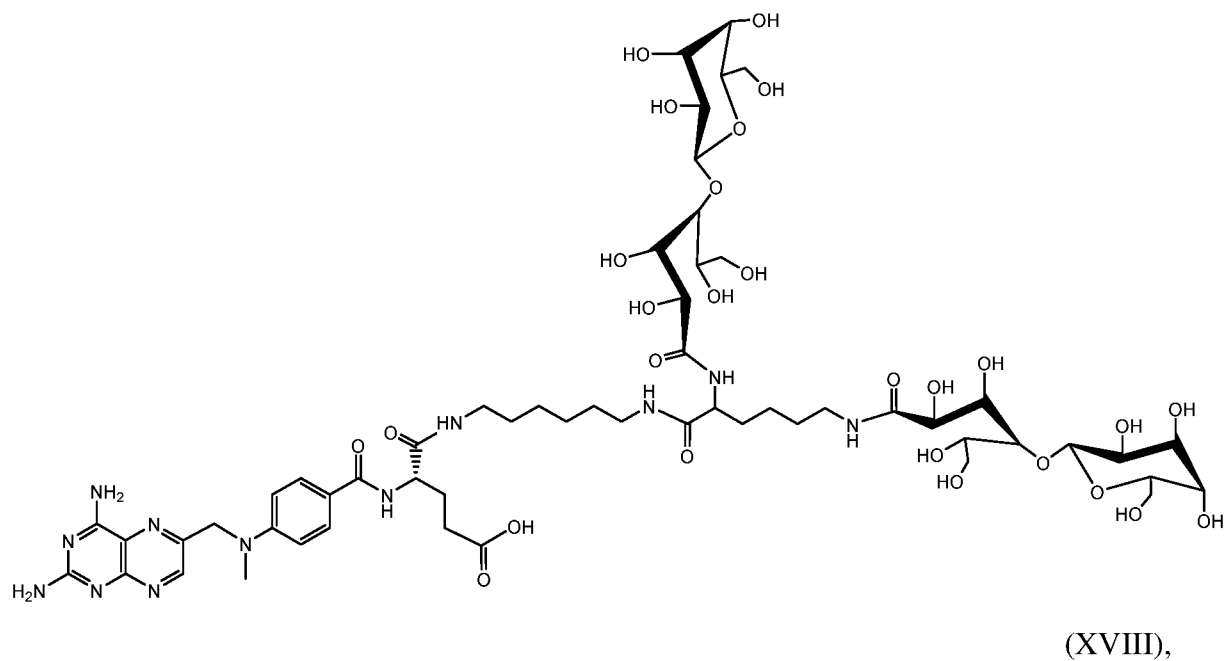
(XVI),

methotrexate-1, the carbohydrate conjugate of which (e.g., LBA – methotrexate-1) is shown below in formula (XVII)

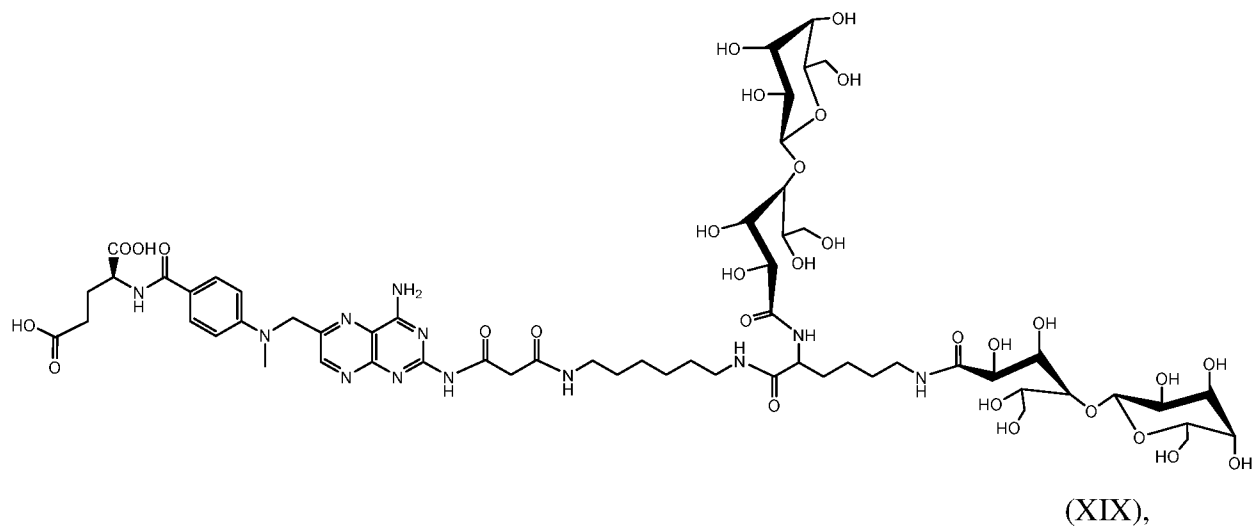


(XVII),

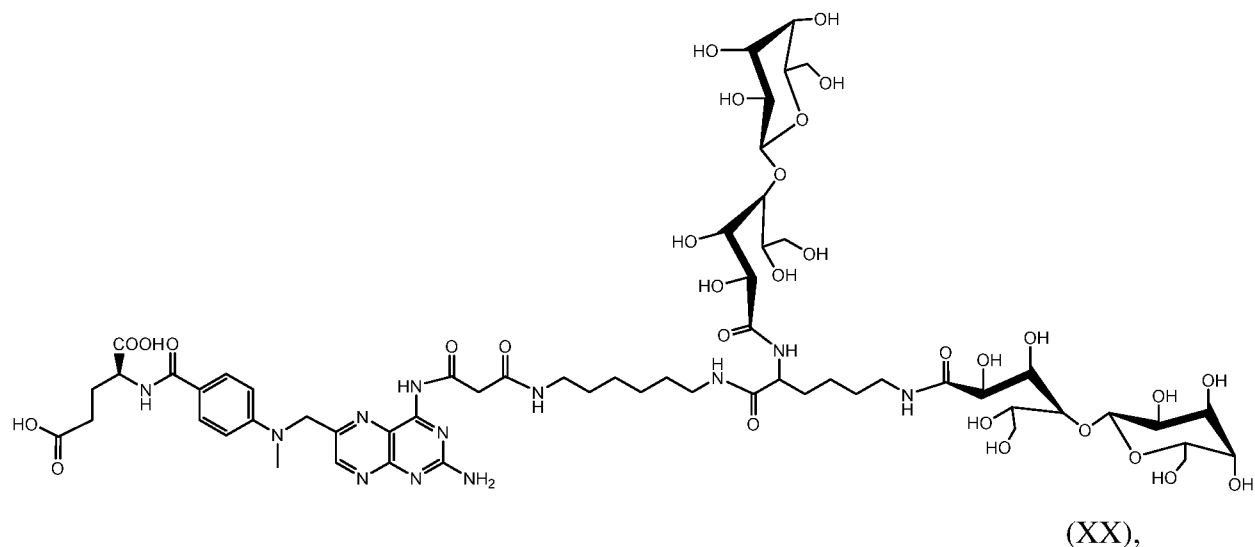
methotrexate-2, the carbohydrate conjugate of which (e.g., LBA – methotrexate-2) is shown below in formula (XVIII)



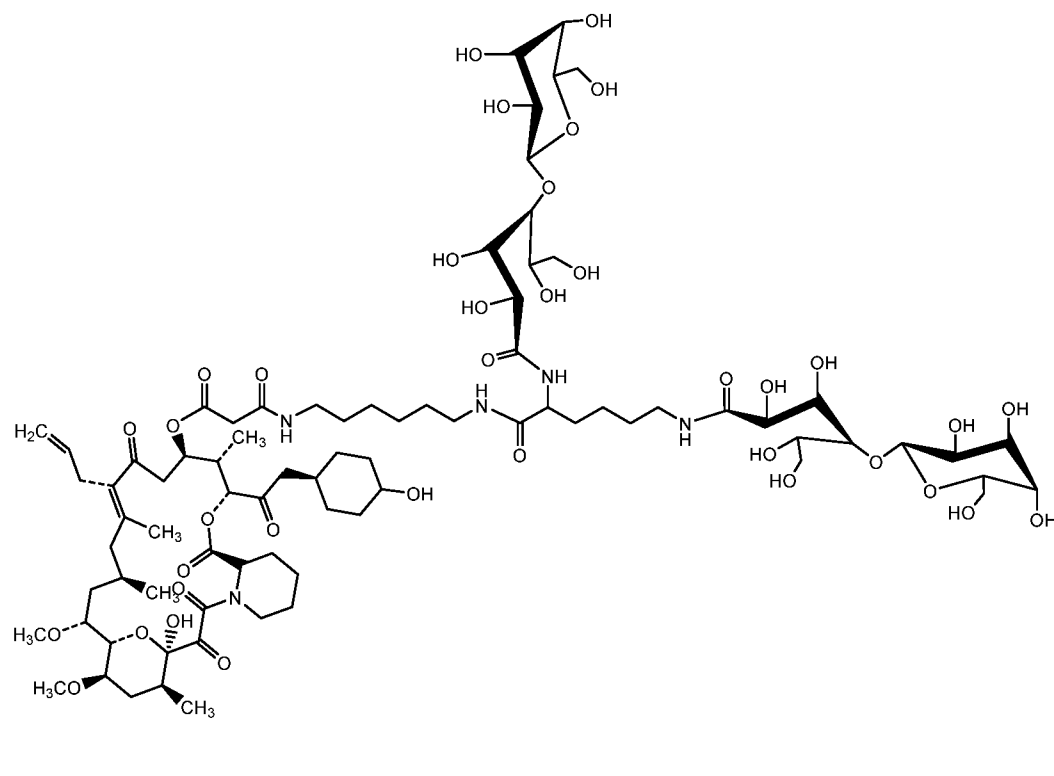
methotrexate-3, the carbohydrate conjugate of which (e.g., LBA – methotrexate-3) is shown below in formula (XIX)



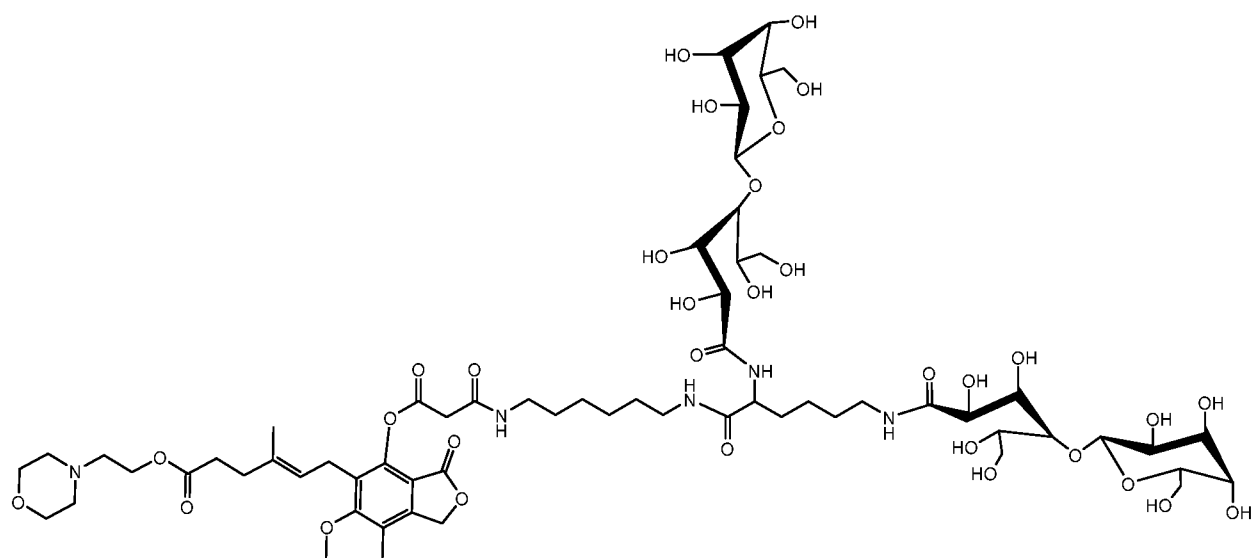
methotrexate-4, the carbohydrate conjugate of which (e.g., LBA – methotrexate-4) is shown below in formula (XX)



tacrolimus, the carbohydrate conjugate of which (e.g., LBA – tacrolimus) is shown below in formula (XXI)

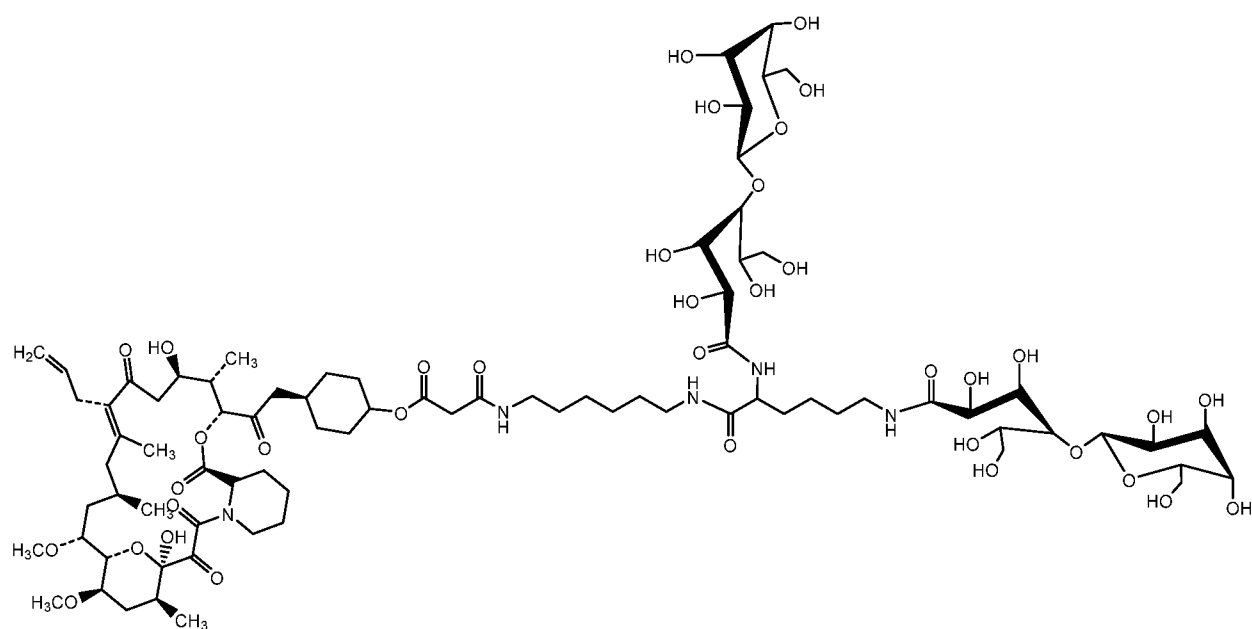


mycophenolate mofetil, the carbohydrate conjugate of which (e.g., LBA – mycophenolate mofetil) is shown below in formula (XXII)



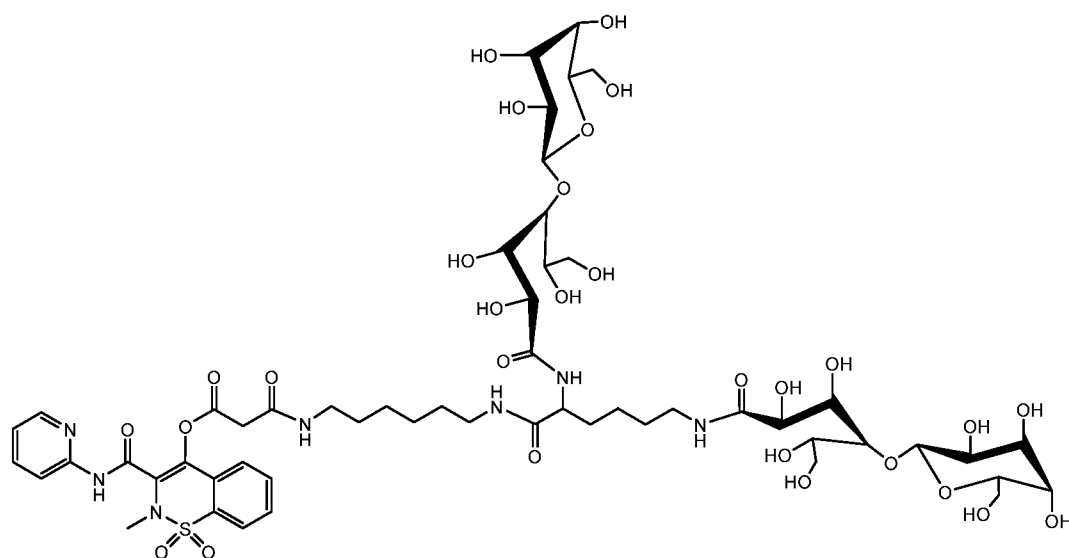
(XXII),

tacrolimus-2, the carbohydrate conjugate of which (e.g., LBA – tacrolimus-2) is shown below in formula (XXIII)



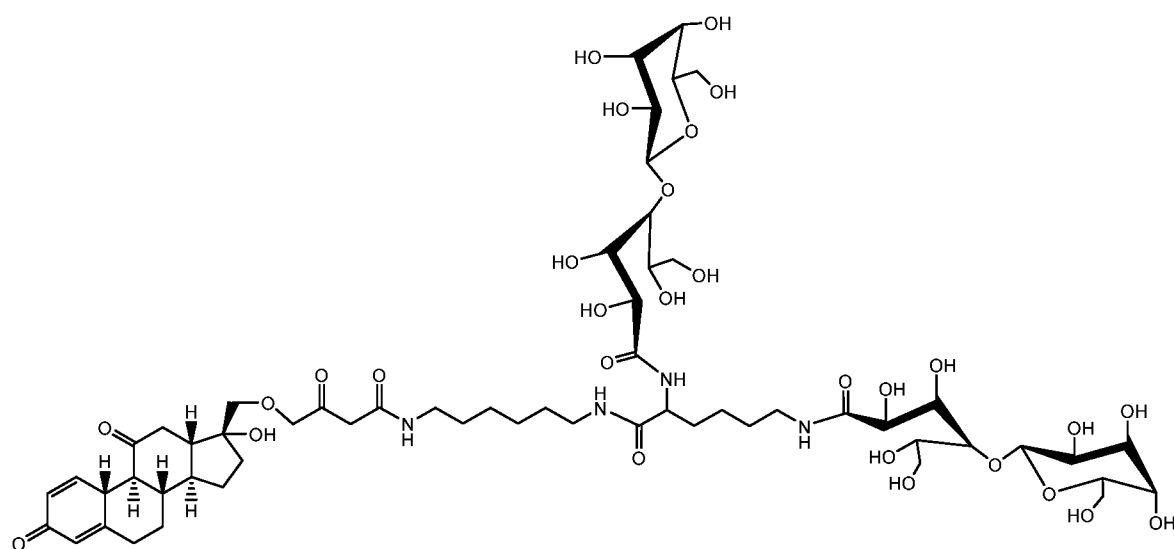
(XXIII),

piroxicam, the carbohydrate conjugate of which (e.g., LBA – piroxicam) is shown below in formula (XXIV)



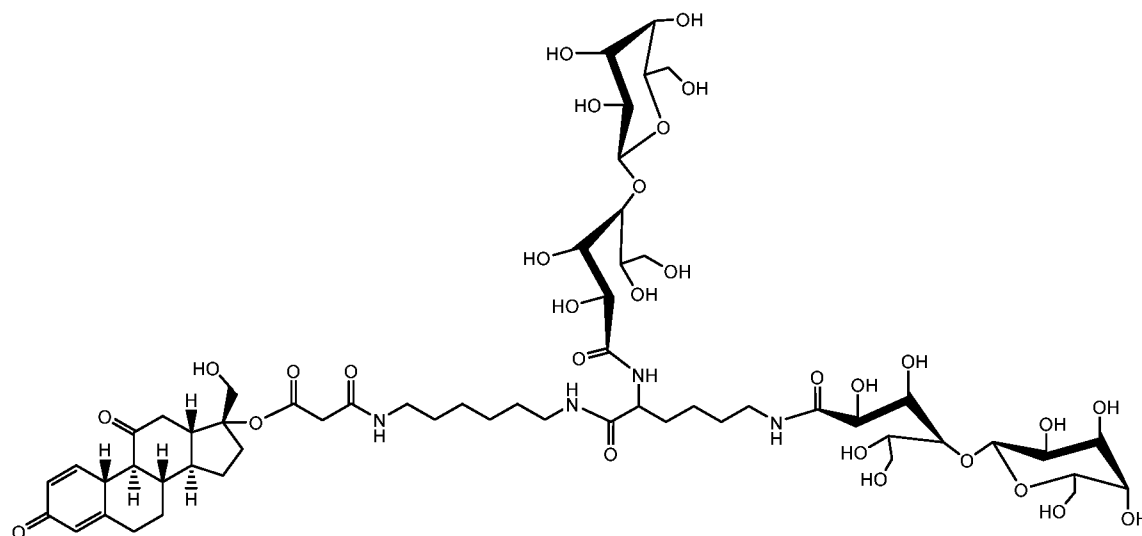
(XXIV),

prednisone-1, the carbohydrate conjugate of which (e.g., LBA – prednisone-1) is shown below in formula (XXV)



(XXV),

prednisone-2, the carbohydrate conjugate of which (e.g., LBA – prednisone-2) is shown below in formula (XXVI)



(XXVI);

an enantiomer, a diastereoisomer, a pharmaceutically acceptable salt, a hydrate, a solvate or mixtures thereof.

[0067] In an aspect, disclosed are methods for the use of the genetic sequence/small molecule-carbohydrate conjugate, to target the lungs. In an embodiment, the lungs are targeted using a pulmonary delivery including the disclosed genetic sequence/small molecule-carbohydrate conjugate and/or the carbohydrate-based ligands. In an embodiment, the conjugate, is the genetic sequence (for example, PNA)-carbohydrate conjugate. In an embodiment, the conjugate, is the small molecule (for example, an FDA approved small molecule drug)-carbohydrate conjugate. For example, the conjugates can be used to treat cancers in the lung. In an embodiment, the conjugates can be used to treat non-small cell lung cancer. In an embodiment, the conjugates can be used to treat small-cell carcinoma. In an embodiment, the conjugate can be used to treat large cell carcinoma. In an embodiment, the conjugate can be used to treat lung adenocarcinoma. In an embodiment, the conjugate can be used to treat lung mesothelioma. In an embodiment, the conjugate can be used to treat squamous cell carcinoma. The formulations can be administered directly to a subject for in vivo gene therapy.

[0068] The conjugates, in particular the peptide nucleic acid-carbohydrate conjugates (PNACs), can be used as an RNA therapeutic agent. In particular, the conjugates in particular the PNACs, can target microRNA (miRNA) sequences. The conjugates can be used to control gene expression at the post-transcription level. miRNAs play key roles in maintaining physiological processes by controlling gene expression through regulating messenger RNA (mRNA) stability and translation. Use of the conjugates to target an RNA in a cell, such as an mRNA or miRNA, can inhibit expression of the RNA at the translational

stage in the case of mRNA, and/or affect gene expression by downregulation or upregulating expression of the miRNA and its downstream effects on its target genes. The conjugates can be used to control aberrant expression of miRNAs causing several devastating diseases. The conjugates can be used to treat cancers wherein, atypical miRNA levels lead to altered processes, including differentiation, proliferation, and apoptosis. In a preferred embodiment, the conjugates are used to treat cancers in the lungs. In an aspect, the conjugate can be used to treat a lung disease.

[0069] Accordingly, in an aspect, a method for reducing expression of a targeted RNA involved in a health disorder in a subject comprises providing to a cell of the subject *in vivo* or *ex vivo* the genetic sequence/small molecule-lactobionic acid conjugate as described herein, wherein the binding of the PNA of the conjugate to the targeted RNA reduces expression of the targeted RNA, in particular, the targeted RNA is a microRNA. In an aspect, the RNA therapeutics are used in targeting lung cells, or a combination thereof to regulate expression of cellular nucleic acid function of a subject in need thereof, in particular lung cancer cells. In an aspect, the PNA comprises a lung-specific microRNA, still more specifically miR-21. The condition (need) for treatment can be lung cancer or any other lung disease.

[0070] The genetic sequence/small molecule-carbohydrate conjugate can be used for treatment of a subject in need thereof *ex vivo* or *in vivo*. The methods typically include contacting a cell *ex vivo* or *in vivo* with an effective amount of a conjugate, optionally in combination with a potentiating agent, to deliver a therapeutic agent, for example to modify the expression of an RNA. In an aspect, the method includes contacting a population of target cells with an effective amount of the conjugate, to modify the expression of RNA to achieve a therapeutic result.

[0071] In another aspect, a method for targeting DNA and gene editing in a health disorder in a subject comprises: providing to a cell of the subject *in vivo* or *ex vivo* a genetic sequence-carbohydrate conjugate according to any one of claims 1 to 20, wherein the DNA of the conjugate targeted to the cell modulates expression of a gene.

[0072] The genetic sequence/small molecule-carbohydrate conjugate is generally provided as a formulation including include an effective amount of a conjugate and a polymer, lipid, protein, or other pharmaceutical excipient for the lung-specific delivery. Pharmaceutically acceptable carrier (also referred to as an excipient in the art), where the formulation is selected to suit the mode of administration. Pharmaceutically acceptable carriers are determined in part by the particular conjugate being administered, as well as by

the particular method used to administer the conjugate. For example, the formulations may be for administration topically, locally, or systemically in a suitable pharmaceutical carrier. Accordingly, there is a wide variety of suitable formulations for the conjugates. Remington's Pharmaceutical Sciences, 15th Edition by E. W. Martin (Mark Publishing Company, 1975), discloses typical carriers and methods of preparation. For example, the formulations can include pharmaceutically acceptable carriers such as salts, carriers, buffering agents, emulsifiers, diluents, excipients, chelating agents, fillers, drying agents, antioxidants, antimicrobials, preservatives, binding agents, bulking agents, silicas, solubilizers, or stabilizers. The conjugates can also be encapsulated in suitable biocompatible microcapsules, microparticles, nanoparticles, or microspheres formed of biodegradable or non-biodegradable polymers or proteins or liposomes for targeting to lung cells. The particles can be capable of controlled release of the active agent. The particles can be microparticle(s) and/or nanoparticle(s). The particles can include one or more polymers. One or more of the polymers can be a synthetic polymer. The particle or particles can be formed by, for example, single emulsion technique or double emulsion technique or nanoprecipitation. Such systems are well known to those skilled in the art and may be optimized for use with the appropriate nucleic acid.

[0073] In an embodiment, the small molecule or nucleic acid (that is to be conjugated with the carbohydrate) can be a liquid solution or dry powder which can be formulated as an aerosol product or dry powder inhaler to deliver ligand conjugates to the lungs. This can be administered via a spray nozzle or in a barometric chamber.

[0074] In an embodiment, the composition may be aerosolized for inhalational delivery into the respiratory system of a living being. Aerosols for delivery to the respiratory system are created through various methods that generate fine particles or droplets capable of being inhaled into the lungs. These methods involve specialized devices and formulations designed to produce aerosols with the appropriate particle size and characteristics for effective respiratory delivery.

[0075] In an embodiment, the composition may be aerosolized for inhalational therapy using compressed air or oxygen to convert the composition into a mist. In an embodiment, a high-velocity airstream passes through a narrow opening, creating a low-pressure area that draws up the liquid composition (the genetic sequence/small molecule-carbohydrate conjugate) and breaks it into fine droplets. In another manner, ultrasonic nebulization may be used to provide a therapeutic dose to a patient via the respiratory system. High-frequency ultrasonic waves may be used to create vibrations in the liquid composition,

generating a mist of fine droplets. In yet another embodiment, a mesh nebulizer with a vibrating mesh having microscopic holes may be used to produce a fine mist from the liquid medication. These are compact, efficient, and produce consistent particle sizes.

[0076] The composition disclosed herein containing the genetic sequence/small molecule-carbohydrate conjugate can be delivered to the respiratory system via inhalation and is capable of greater pulmonary retention to target various lung diseases. In addition, the genetic sequence/small molecule-carbohydrate conjugate described herein is less toxic and shows less unwanted side effects compared to the lipid nanoparticles used for the same purpose.

[0077] The composition disclosed herein is exemplified by the following non-limiting examples.

EXAMPLES

[0078] This example is conducted to demonstrate that the genetic sequence-carbohydrate conjugate is preferentially retained in the lung. This disclosure is illustrated by the following Examples, which are not intended to limit the claims.

Synthesis of TAMRA and di-lactobionic acid ligand

[0079] TAMRA (tetramethylrhodamine - a fluorescent dye) and di-lactobionic acid ligand were synthesized as shown in FIG. 1. TAMRA is the small molecule in this particular example and is used to provide imaging analysis of the uptake of the lactobionic acid ligand. TAMRA was conjugated with di-lactobionic acid ligand using HATU (1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate) and DIEA (N,N-Diisopropylethylamine) coupling reagents which yielded LBA-TAMRA.

In vitro cellular uptake

[0080] The cellular uptake of diLBA and GalNAc conjugated to TAMRA was evaluated in different cell lines namely, HepG2 and A549 at a dose of 4 micromolar (μM) for 6 hours. Based on our early confocal microscopy results, LBA-TAMRA showcased lower cytoplasmic distribution than GalNAc-TAMRA in HepG2 (See FIG. 2A). However, for A549 cell lines, LBA-TAMRA exhibited higher cytoplasmic distribution (See FIG. 2B). This observation led to an investigation of the route of receptor mediated cellular uptake in these different cell lines. Since galactose-based ligands show cellular uptake through

asialoglycoprotein receptors (ASGP-R) in hepatocytes, gene expression ASGP-R was first checked in liver cancer cell lines HepG2, Hep3B and SNU-398 along with lung adenocarcinoma cell line A549. Gene expression results showed highest ASGP-R levels in HepG2, followed by Hep3B (See FIG. 4A). A549 and SNU-398 showed negligible levels of ASGP-R expression.

[0081] Cellular uptake of LBA-TAMRA and GalNAc-TAMRA was evaluated in these cell lines at 4 μ M dose for 6 hours. Flow cytometry analysis of cellular uptake indicated the highest cellular uptake for GalNAc-TAMRA as compared to LBA-TAMRA in HepG2 and Hep3B cells which showcased high ASGP-R receptors (See FIGS. 3A & 3B). LBA-TAMRA on the other hand, displayed higher cellular uptake as compared to GalNAc-TAMRA in A549 and SNU-398 cells which have low ASGP-R levels. The distinct higher uptake of LBA-TAMRA in specific cells led to an investigation of the effect of ASGP-R silencing on cellular uptake. Following ASGP-R silencing, significant reduction in uptake was observed for GalNAc ligand for HepG2 (~43.4%), A549 (~29.55%), Hep3B (~29.83%) and SNU-398 (~18.77%) cells suggesting that the GalNAc ligand follows ASGP-R mediated cellular uptake (See FIGS. 4B and 4D). However, for LBA ligand, lesser reduction in cellular uptake was seen for HepG2 (~22.9%) and A549 (~17.7%) cells (See FIGS. 4B and 4C).

[0082] Interestingly, uptake of LBA ligand in SNU-398 did not show any effect following ASGP-R knockdown, suggesting different receptor mediated uptake. Since A549 cells do not express ASGP-R and show better uptake of LBA over GalNAc, it was decided to evaluate other receptors associated with galactose ligands present in lung. The gene expression of mannose receptor C type 2 (MRC2) was evaluated in A549 cells and compared with its expression levels against liver cancer cell lines of HepG2, Hep3B and SNU-398 (See FIG. 5A). SNU-398 and A549 showed high levels of MRC2 receptors followed by Hep3B and HepG2. Cellular uptake of LBA in A549 cells was investigated with ASGPR and MRC2 knockdown and observed 21% and 26% reduction in cellular uptake (See FIGS. 5B and 5C).

In vivo biodistribution studies

[0083] The biodistribution of LBA in C57BL6/J mice was determined through intratracheal administration with a 100 μ M/50 μ L dose (0.325mg/kg). The retention duration was evaluated using a time dependent study model where the mice were treated for 4, 24, 48, 72, and 96hrs and compared the uptake with PBS treated mice. The IVIS images of the organs at each time point show significant uptake in the lung with retention of up to 48hrs

(See FIG. 6A). The lung tissue was sectioned and imaged to confirm total lung uptake after 4 hours (See FIG. 6B). Retention of the LBA ligand compared with PBS and TAMRA dye was confirmed. Flow cytometry analysis was performed to quantify the uptake in different cell types including macrophages, epithelial cells, and endothelial cells at 4, 24, and 48 hours after administration. In 4 hours, the lung retains the LBA ligand and is distributed throughout the macrophages, epithelial cells and endothelial cells (See FIG. 7). Flow cytometry shows uptake in total lung, macrophages, and epithelial cells for up to 48 hours (See FIG. 8).

[0084] The following terms are used to describe the invention of the present disclosure. In instances where a term is not specifically defined herein, that term is given an art-recognized meaning by those of ordinary skill applying that term in context to its use in describing the present disclosure.

[0085] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art. For example, any nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics, and protein and nucleic acid chemistry and hybridization described herein are well known and commonly used in the art. In case of conflict, the present disclosure, including definitions, will control. Exemplary methods and materials are described below, although methods and materials similar or equivalent to those described herein can be used in practice or testing of the embodiments and aspects described herein.

[0086] As used herein, the terms “amino acid,” “nucleotide,” “polynucleotide,” and “protein” have their common meanings as would be understood by a biochemist of ordinary skill in the art. Standard single letter nucleotides (A, C, G, T, U) and standard single letter amino acids (A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y) are used herein.

[0087] Compounds and materials are described using standard nomenclature. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which this invention belongs. The following terms are used to describe the invention of the present disclosure. In instances where a term is not specifically defined herein, that term is given an art-recognized meaning by those of ordinary skill applying that term in context to its use in describing the present disclosure.

[0088] As used herein, the term “hydrocarbyl” and “hydrocarbon” refer to a substituent comprising carbon and hydrogen, optionally with 1 to 3 heteroatoms, for example, oxygen, nitrogen, halogen, silicon, sulfur, or a combination thereof; “alkyl” refers to a

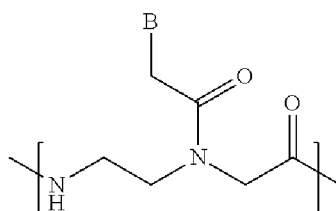
straight or branched chain, saturated monovalent hydrocarbon group; “alkylene” refers to a straight or branched chain, saturated, divalent hydrocarbon group; “alkylidene” refers to a straight or branched chain, saturated divalent hydrocarbon group, with both valences on a single common carbon atom; “alkenyl” refers to a straight or branched chain monovalent hydrocarbon group having at least two carbons joined by a carbon-carbon double bond; “cycloalkyl” refers to a non-aromatic monovalent monocyclic or multicyclic hydrocarbon group having at least three carbon atoms, “cycloalkenyl” refers to a non-aromatic cyclic divalent hydrocarbon group having at least three carbon atoms, with at least one degree of unsaturation; “aryl” refers to an aromatic monovalent group containing only carbon in the aromatic ring or rings; “arylene” refers to an aromatic divalent group containing only carbon in the aromatic ring or rings; “alkylaryl” refers to an aryl group that has been substituted with an alkyl group as defined above, with 4-methylphenyl being an exemplary alkylaryl group; “arylalkyl” refers to an alkyl group that has been substituted with an aryl group as defined above, with benzyl being an exemplary arylalkyl group; “acyl” refers to an alkyl group as defined above with the indicated number of carbon atoms attached through a carbonyl carbon bridge ($-C(=O)-$); “alkoxy” refers to an alkyl group as defined above with the indicated number of carbon atoms attached through an oxygen bridge ($-O-$); and “aryloxy” refers to an aryl group as defined above with the indicated number of carbon atoms attached through an oxygen bridge ($-O-$).

[0089] Unless otherwise indicated, each of the foregoing groups can be unsubstituted or substituted, provided that the substitution does not significantly adversely affect synthesis, stability, or use of the compound. The term “substituted” as used herein means that at least one hydrogen on the designated atom or group is replaced with another group, provided that the designated atom’s normal valence is not exceeded. When the substituent is oxo (i.e., $=O$), then two hydrogens on the atom are replaced. Combinations of substituents or variables are permissible provided that the substitutions do not significantly adversely affect synthesis or use of the compound. Exemplary groups that can be present on a “substituted” position include, but are not limited to, cyano; hydroxyl; nitro; azido; alkanoyl (such as a C_{2-6} alkanoyl group such as acyl); carboxamido; C_{1-6} or C_{1-3} alkyl, cycloalkyl, alkenyl, and alkynyl (including groups having at least one unsaturated linkages and from 2 to 8, or 2 to 6 carbon atoms); C_{1-6} or C_{1-3} alkoxy; C_{6-10} aryloxy such as phenoxy; C_{1-6} alkylthio; C_{1-6} or C_{1-3} alkylsulfinyl; C_{1-6} or C_{1-3} alkylsulfonyl; aminodi(C_{1-6} or C_{1-3})alkyl; C_{6-12} aryl having at least one aromatic rings (e.g., phenyl, biphenyl, naphthyl, or the like, each ring either substituted or unsubstituted aromatic); C_{7-19} arylalkyl having 1 to 3 separate or fused rings and from 6 to

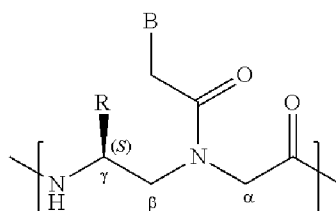
18 ring carbon atoms; or arylalkoxy having 1 to 3 separate or fused rings and from 6 to 18 ring carbon atoms, with benzyloxy being an exemplary arylalkoxy. The indicated number of carbon atoms of a group do not include any substituents.

[0090] As used herein, a peptide nucleic acid (PNA) is an artificially synthesized polymer with a backbone comprising repeating N-(2-aminoethyl)-glycine units linked by peptide bonds. The various purine and pyrimidine bases are linked to the backbone by a methylene bridge (-CH₂-) and a carbonyl group (-C(=O)-) to a nitrogen on the backbone as shown in Figure 1. By convention, PNA is represented with the N-terminus upward (or side to the left) and the C-terminus downward (or side to the right) as in peptides. A PNA is not a peptide or a nucleic acid in the formal sense, but rather a hybrid of the two.

[0091] In some aspects, the PNA monomers forming a PNA oligomer are modified at the gamma position in the polyamide backbone (γ PNAs) as illustrated below (wherein “B” is a nucleobase and “R” is a substitution at the gamma position).



PNA

Chiral γ PNA

[0092] Substitution at the gamma position creates chirality and provides helical pre-organization to the PNA oligomer, yielding substantially increased binding affinity to the target RNA. Other advantageous properties can be conferred depending on the chemical nature of the specific substitution at the gamma position (the “R” group in the chiral γ PNA above). The synthesis of γ PNAs is described in U.S. Patent No. 10,221,216, incorporated herein by reference for the disclosure of γ PNA and methods of synthesis of γ PNA.

[0093] Examples of γ substitution with other side chains include that of alanine, serine, threonine, cysteine, valine, leucine, isoleucine, methionine, proline, phenylalanine, tyrosine, aspartic acid, glutamic acid, asparagine, glutamine, histidine, lysine, arginine, and the derivatives thereof. The “derivatives thereof” herein are defined as those chemical

moieties that are covalently attached to these amino acid side chains, for instance, to that of serine, cysteine, threonine, tyrosine, aspartic acid, glutamic acid, asparagine, glutamine, lysine, and arginine.

[0094] In an aspect, the PNA oligomer forming a PNA/RNA/PNA triplex is a γ PNA with a tail clamp, or a γ tcPNA.

[0095] Chemical modifications of the basic PNA structure are known and can be used. For example, fluorine-modified, cyclopentyl-modified, mini-peg-modified, guanidinium-modified, pyrrolidinyl-modified, and 2-aminopyridien-modified PNAs are known in the art and can be chosen for preparation of the PNA oligomer to improve cell permeability or increase RNA binding affinity. Mini-PEG-containing γ -PNAs and their methods of synthesis are described in US. Patent No. 10,793,605, the entire contents of which are hereby incorporated by reference.

[0096] The PNA oligomers can also include other positively charged moieties to increase the solubility of the PNA, for increased cell permeability, and/or to increase the affinity of the PNA for the target RNA. Commonly used positively charged moieties include the amino acids lysine and arginine, although other positively charged moieties may also be useful. Lysine and arginine residues can be added to a tcPNA linker or can be added to the carboxy or the N-terminus of a PNA oligomer strand.

[0097] Exemplary modifications to PNA include, but are not limited to, incorporation of charged amino acid residues, such as lysine at the termini or in the interior part of the oligomer; inclusion of polar groups in the backbone, carboxymethylene bridge, and in the nucleobases; chiral PNAs bearing substituents on the original N-(2-aminoethyl)glycine backbone; replacement of the original aminoethylglycyl backbone skeleton with a negatively-charged scaffold; conjugation of high molecular weight polyethylene glycol (PEG) to one of the termini; fusion of PNA to RNA to generate a chimeric oligomer, redesign of the backbone architecture, conjugation of PNA to DNA or RNA. These modifications improve solubility but often result in reduced binding affinity and/or sequence specificity.

[0098] Gamma-PNA modifications include serine modified, lysine modified, glutamic acid modified, or alanine modified. Particularly when the PNAs are serine gamma modified, the $\square$ PNAs target RNA more efficiently compared to the conventional full length PNAs based on their binding affinity.

[0099] Phosphorothioate analogues of DNA, RNA and OMe-RNA have sulfur in place of oxygen as one of the non-bridging ligands bonded to phosphorus.

[0100] A morpholino, also known as a morpholino oligomer and as a phosphorodiamidate morpholino oligomer (PMO), is a type of oligomer used in molecular biology to modify gene expression. Its molecular structure contains DNA bases attached to a backbone of methylenemorpholine rings linked through phosphorodiamidate groups. Morpholinos block access of other molecules to small (~25 base) specific sequences of the base-pairing surfaces of ribonucleic acid (RNA). Morpholinos are used as research tools for reverse genetics by knocking down gene function.

[0101] Locked nucleic acid is an RNA derivative in which the ribose ring is constrained by a methylene linkage between the 2'-oxygen and the 4'-carbon. This conformation restriction increases binding affinity for complementarity sequences and provides a chemical approach for the control of gene expression and optimization of microarrays.

[0102] 2'-O-methylation is a nucleoside modification of RNA, where a methyl group is added to the 2' hydroxyl of the ribose moiety of a nucleoside, producing a methoxy group. 2'-O-methylated nucleosides are mostly found in ribosomal RNA and small nuclear RNA and occur in the functionally essential regions of the ribosome and spliceosome.

[0103] Like the 2'-OMe nucleoside modification of RNA, the 2'-O-methoxyethyl-RNA (2'-MOE) backbone provides enhanced duplex stability, significant nuclease resistance.

[0104] 2'-Fluoro (2'-F) is a potent RNA analogue that possesses high RNA binding affinity and resistance to nuclease degradation.

[0105] 5'-Methylcytosine is a methylated form of the DNA base cytosine (C) that regulates gene transcription and takes several other biological roles. When cytosine is methylated, the DNA maintains the same sequence, but the expression of methylated genes can be altered

[0106] The G-clamp heterocycle modification, a cytosine analog that clamps on to guanine by forming an additional hydrogen bond, was rationally designed to enhance oligonucleotide/RNA hybrid affinity. PNAs containing internally-linked guanidinium moieties (GPNAs) are readily taken-up by mammalian cells, and bind to DNA and RNA with high affinity and sequence specificity.

[0107] A protecting group is a functional group that transforms a reactive functional group in an organic molecule so that it does not undergo a reaction meant for another functional group in the structure. Protecting groups are widely used in various forms in organic synthesis. The need for protection and deprotection, and the selection of appropriate protecting groups can be readily determined by one skilled in the art. The chemistry of

protecting groups can be found, for example, in Greene, et al., *Protective Groups in Organic Synthesis*, 4d. Ed., Wiley & Sons, 2007. Adjustments to the protecting groups and formation and cleavage methods described herein may be adjusted as necessary in light of the various substituents.

[0108] A residue is used to describe any of the parts that integrate to make up a larger molecule such as a conjugate. For example, a lysine residue refers to a lysine amino acid structure integral to a conjugate covalently bonded to an alkyl diamine via the lysine carboxyl group (by an amide function) and covalently bonded by its alpha and epsilon amino groups to lactobionic acid molecules (by amide bonds) as shown in the drawings herein. A residue may also be referred to as a moiety.

[0109] RNA as used herein includes different types of RNA that serve different functions including messenger RNA, transfer RNA, ribosomal RNA, and microRNA. Micro RNA (miRNA) is involved in gene expression. miRNA is a non-coding region of mRNA that is believed to be important in the either promotion or inhibition of gene expression. These may involve small sequences of about 25 nucleotides.

[0110] A sequence of bases is a succession of bases signified by a series of a set of five different letters that indicate the order of nucleotides forming alleles within a DNA (using GACT) or RNA (GACU) molecule. By convention, sequences are usually presented from the 5' end to the 3' end. For DNA, the sense strand is used. Because nucleic acids are normally linear (unbranched) polymers, specifying the sequence is equivalent to defining the covalent structure of the entire molecule.

[0111] Gene expression is the process by which a genes coded information is converted into the structures present and operating in the cell. Expressed genes include those that are transcribed into mRNA and then translated into protein and those that are transcribed into RNA but not translated into protein (for example, transfer and ribosomal RNAs). miRNA is a non-coding region of mRNA that is believed to be important in the either promotion or inhibition of gene expression.

[0112] Below are the names or brief descriptions of some gene names and micro RNAs and genes known in the art. The nonlimiting examples of the microRNAs include miR-33, miR-145, miR-143, miR-21, miR-182, or a combination thereof. The nonlimiting examples of the genes include c-myc, PTEN, PI3K, KRAS, TP53, EGFR, MET, LKB1, PRAF, PIK3CA, ALK, RET, ROS1, CFTR, PDGFR, TGF- β , or a combination thereof. Other information can be found on the website <https://www.genecards.org/> which is incorporated herein by reference.

[0113] The use of the terms “a” and “an” and “the” and similar referents (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. By way of example, “an element” means one element or more than one element.

[0114] As used herein, the term “substantially” means to a great or significant extent, but not completely.

[0115] It should also be understood that, in certain methods described herein that include more than one step or act, the order of the steps or acts of the method is not necessarily limited to the order in which the steps or acts of the method are recited unless the context indicates otherwise. Furthermore, the terms first, second, etc., as used herein are not meant to denote any particular ordering, but simply for convenience to denote a plurality of, for example, layers.

[0116] The terms “comprising”, “having”, “including”, and “containing” are to be construed as open-ended terms (i.e., meaning “including, but not limited to”) unless otherwise noted.

[0117] The terms “about” or “approximately,” as used herein, is inclusive of the stated value and means within an acceptable range of deviation for the particular value as determined by one of ordinary skill in the art, considering the measurement in question and the error associated with measurement of the particular quantity (i.e., the limitations of the measurement system). For example, “about” can mean within one or more standard deviations, or within $\pm 10\%$ or 5% of the stated value. Recitation of ranges of values are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All ranges disclosed herein include both end points as discrete values as well as all integers and fractions specified within the range. For example, a range of 0.1-2.0 includes 0.1, 0.2, 0.3, 0.4 . . . 2.0. All methods described herein can be performed in a suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., “such as”), is intended merely to better illustrate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention as used herein.

[0118] The phrase “and/or,” as used herein in the specification and in the claims, should be understood to mean “either or both” of the elements so conjoined, i.e., elements

that are conjunctively present in some cases and disjunctively present in other cases. Multiple elements listed with "and/or" should be construed in the same fashion, i.e., "one or more" of the elements so conjoined. Other elements may optionally be present other than the elements specifically identified by the "and/or" clause, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, a reference to "A and/or B", when used in conjunction with open-ended language such as "comprising" can refer, in one embodiment, to A only (optionally including elements other than B); in another embodiment, to B only (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); etc.

[0119] As used herein in the specification and in the claims, "or" should be understood to have the same meaning as "and/or" as defined above. For example, when separating items in a list, "or" or "and/or" shall be interpreted as being inclusive, i.e., the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally, additional unlisted items. Only terms clearly indicated to the contrary, such as "only one of" or "exactly one of," or, when used in the claims, "consisting of," will refer to the inclusion of exactly one element of a number or list of elements. In general, the term "or" as used herein shall only be interpreted as indicating exclusive alternatives (i.e., "one or the other but not both") when preceded by terms of exclusivity, such as "either," "one of," "only one of," or "exactly one of."

[0120] As used herein in the specification and in the claims, the phrase "at least one," in reference to a list of one or more elements, should be understood to mean at least one element selected from anyone or more of the elements in the list of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements and not excluding any combinations of elements in the list of elements. This definition also allows that elements may optionally be present other than the elements specifically identified within the list of elements to which the phrase "at least one" refers, whether related or unrelated to those elements specifically identified. Thus, as a nonlimiting example, "at least one of A and B" (or, equivalently, "at least one of A or B," or, equivalently "at least one of A and/or B") can refer, in one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another embodiment, to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another embodiment, to at least one, optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); etc.

[0121] The phrase "one or more," as used herein, means at least one, and thus includes individual components as well as mixtures/combinations of the listed components in any combination.

[0122] Other than in the operating examples, or where otherwise indicated, all numbers expressing quantities of ingredients and/or reaction conditions are to be understood as being modified in all instances by the term "about," meaning within 10% of the indicated number (e.g., "about 10%" means 9%-11% and "about 2%" means 1.8%-2.2%).

[0123] All percentages and ratios are calculated by weight unless otherwise indicated. All percentages are calculated based on the total composition unless otherwise indicated. Generally, unless otherwise expressly stated herein, "weight" or "amount" as used herein with respect to the percent amount of an ingredient refers to the amount of the raw material comprising the ingredient, wherein the raw material may be described herein to comprise less than and up to 100% activity of the ingredient. Therefore, weight percent of an active in a composition is represented as the amount of raw material containing the active that is used and may or may not reflect the final percentage of the active, wherein the final percentage of the active is dependent on the weight percent of active in the raw material.

[0124] All ranges and amounts given herein are intended to include subranges and amounts using any disclosed point as an end point. Thus, a range of "1% to 10%, such as 2% to 8%, such as 3% to 5%," is intended to encompass ranges of "1% to 8%," "1% to 5%," "2% to 10%," and so on. All numbers, amounts, ranges, etc., are intended to be modified by the term "about," whether or not so expressly stated. Similarly, a range given of "about 1% to 10%" is intended to have the term "about" modifying both the 1% and the 10% endpoints. Further, it is understood that when an amount of a component is given, it is intended to signify the amount of the active material unless otherwise specifically stated.

[0125] As used herein, the term "administering" means the actual physical introduction of a composition into or onto (as appropriate) a subject, a host, or cell. Any and all methods of introducing the composition into the subject, host or cell are contemplated according to the invention; the method is not dependent on any particular means of introduction and is not to be so construed. Means of introduction are well-known to those skilled in the art, and also are exemplified herein. "Providing" means giving, administering, selling, distributing, transferring (for profit or not), manufacturing, compounding, or dispensing.

[0126] As used herein, “optional” or “optionally” means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where said event or circumstance occurs and instances where it does not.

[0127] As used herein, the term “pharmaceutically acceptable” refers to compositions that are physiologically tolerable and do not typically produce an allergic or similar untoward reaction when administered to a subject, preferably a human or a non-human subject. Preferably, as used herein, the term “pharmaceutically acceptable” means approved by a regulatory agency of a federal or state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans.

[0128] As used herein, the terms “treat,” “treating,” and “treatment” include inhibiting the pathological condition, disorder, or disease, *e.g.*, arresting or reducing the development of the pathological condition, disorder, or disease or its clinical symptoms; or relieving the pathological condition, disorder, or disease, *e.g.*, causing regression of the pathological condition, disorder, or disease or its clinical symptoms. Treatment means any way the symptoms of a pathological condition, disorder, or disease are ameliorated or otherwise beneficially altered. Preferably, the subject in need of such treatment is a mammal, preferably a human. Treatment also means providing an active compound to a patient in an amount sufficient to measurably reduce any cancer symptom, slow cancer progression or cause cancer regression. These terms also encompass therapy and cure. In certain embodiments treatment of the cancer may be commenced before the patient presents symptoms of the disease.

[0129] As used herein, the term “effective amount” or “therapeutically effective amount” refers to the amount of a therapy, which is sufficient to reduce or ameliorate the severity and/or duration of a disorder or one or more symptoms thereof, inhibit or prevent the advancement of a disorder, cause regression of a disorder, inhibit or prevent the recurrence, development, onset or progression of one or more symptoms associated with a disorder, detect a disorder, or enhance or improve the prophylactic or therapeutic effect(s) of another therapy (*e.g.*, prophylactic or therapeutic agent). An effective amount can require more than one dose. As used herein, the term “prophylaxis” refers to preventing or reducing the progression of a disorder, either to a statistically significant degree or to a degree detectable by a person of ordinary skill in the art.

[0130] As used herein, the term “dose” denotes any form of an active ingredient formulation or composition, including cells, that contains an amount sufficient to initiate or produce a therapeutic effect with at least one or more administrations. “Formulation” and

“composition” are used interchangeably herein. As used herein, the terms “control,” or “reference” are used herein interchangeably. A “reference” or “control” level may be a predetermined value or range, which is employed as a baseline or benchmark against which to assess a measured result. “Control” also refers to control experiments or control cells.

[0131] Effective amounts may vary depending upon the biological effect desired in the individual, condition to be treated, and/or the specific characteristics of the composition according to the present invention and the individual. In this respect, any suitable dose of the composition can be administered to the patient (*e.g.*, human), according to the type of disease to be treated. Various general considerations taken into account in determining the “effective amount” are known to those of skill in the art and are described, *e.g.*, in Gilman et al., eds., Goodman And Gilman’s: *The Pharmacological Bases of Therapeutics*, 8th ed., Pergamon Press, 1990; and Remington’s *Pharmaceutical Sciences*, 17th Ed., Mack Publishing Co., Easton, Pa., 1990, each of which is herein incorporated by reference.

[0132] The term “subject” or “patient” is used herein to refer to an animal, such as a mammal, including a primate (such as a human, a non-human primate, *e.g.*, a monkey, and a chimpanzee), a non-primate (such as a cow, a pig, a camel, a llama, a horse, a goat, a rabbit, a sheep, a hamster, a guinea pig, a cat, a dog, a rat, a mouse, and a whale), a bird (*e.g.*, a duck or a goose), and a shark. In an embodiment, the subject or patient is a human subject or a human patient, such as a human being treated or assessed for a disease, disorder or condition, a human at risk for a disease, disorder or condition, a human having a disease, disorder or condition, and/or human being treated for a disease, disorder or condition as described herein. In one embodiment, the subject is about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 years of age. In another embodiment, the subject is about 5-10, 10-15, 15-20, 20-25, 25-30, 30-35, 35-40, 40-45, 45-50, 50-55, 55-60, 60-65, 65-70, 70-75, 75-80, 80-85, 85-90, 90-95, 95-100 years of age. Values and ranges intermediate to the above recited ranges are also intended to be part of this invention. In addition, ranges of values using a combination of any of the above-recited values as upper and/or lower limits are intended to be included. As used herein, a subject is “in need of treatment” if such subject would benefit biologically, medically, or in quality of life from such treatment. A subject in need of treatment does not necessarily present symptoms, particular in the case of preventative or prophylaxis treatments.

[0133] All methods described herein can be performed in a suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (*e.g.*, “such as”), is intended merely to better illustrate the invention and does not pose a limitation on the scope of the invention unless otherwise

claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention as used herein. Unless defined otherwise, technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art of this disclosure.

[0134] Furthermore, the disclosure encompasses all variations, combinations, and permutations in which one or more limitations, elements, clauses, and descriptive terms from one or more of the listed claims are introduced into another claim. For example, any claim that is dependent on another claim can be modified to include one or more limitations found in any other claim that is dependent on the same base claim. Where elements are presented as lists, *e.g.*, in Markush group format, each subgroup of the elements is also disclosed, and any element(s) can be removed from the group.

[0135] All compounds are understood to include all possible isotopes of atoms occurring in the compounds. Isotopes include those atoms having the same atomic number but different mass numbers and encompass heavy isotopes and radioactive isotopes. By way of general example, and without limitation, isotopes of hydrogen include tritium and deuterium, and isotopes of carbon include ^{11}C , ^{13}C , and ^{14}C . Accordingly, the compounds disclosed herein may include heavy or radioactive isotopes in the structure of the compounds or as substituents attached thereto. Examples of useful heavy or radioactive isotopes include ^{18}F , ^{15}N , ^{18}O , ^{76}Br , ^{125}I and ^{131}I .

[0136] “Pharmaceutical compositions” means compositions comprising at least one active agent, such as a compound or salt of Formula (I), and at least one other substance, such as a carrier. Pharmaceutical compositions meet the U.S. FDA’s GMP (good manufacturing practice) standards for human or non-human drugs.

[0137] “Carrier” means a diluent, excipient, or vehicle with which an active compound is administered. A “pharmaceutically acceptable carrier” means a substance, *e.g.*, excipient, diluent, or vehicle, that is useful in preparing a pharmaceutical composition that is generally safe, non-toxic and neither biologically nor otherwise undesirable, and includes a carrier that is acceptable for veterinary use as well as human pharmaceutical use. A “pharmaceutically acceptable carrier” includes both one and more than one such carrier.

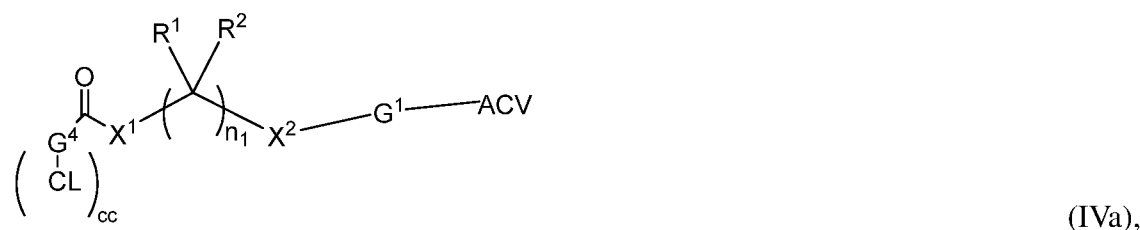
[0138] A significant change is any detectable change that is statistically significant in a standard parametric test of statistical significance such as Student’s T-test, where $p < 0.05$.

[0139] While the invention has been described with reference to some embodiments, it will be understood by those skilled in the art that various changes may be made and equivalents may be substituted for elements thereof without departing from the scope of the

invention. In addition, many modifications may be made to adapt a particular situation or material to the teachings of the invention without departing from essential scope thereof. Therefore, it is intended that the invention not be limited to the particular embodiments disclosed as the best mode contemplated for carrying out this invention, but that the invention will include all embodiments falling within the scope of the appended claims.

What is claimed is:

1. A carbohydrate conjugate of formula (IVa), an enantiomer, a diastereoisomer, a pharmaceutically acceptable salt, a hydrate, a solvate or a combination thereof:



wherein ACV is a pharmaceutically active agent,

R_1 and R_2 are each independently selected from H or a substituted or unsubstituted C_1 to C_6 alkyl,

X^1 is selected from the group consisting of O, NR^3 , $C=O$, and $C(R^3)_2$ where R^3 is H, - $C(O)CH_3$, or a substituted or unsubstituted C_1 to C_6 alkyl,

X^2 is O, NR^3 , or $C(R^3)_2$ where R^3 is H or a substituted or unsubstituted C_1 to C_6 alkyl;

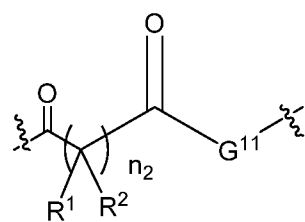
n^1 is 1 to 20,

G^1 and G^4 are each a direct bond or a chemical linker; and

G^4 -CL is a carbohydrate ligand structure comprising 2 to 16 carbohydrate residues each derived from a monosaccharide, a disaccharide, a trisaccharide, or a tetrasaccharide; where cc is 2 to 16;

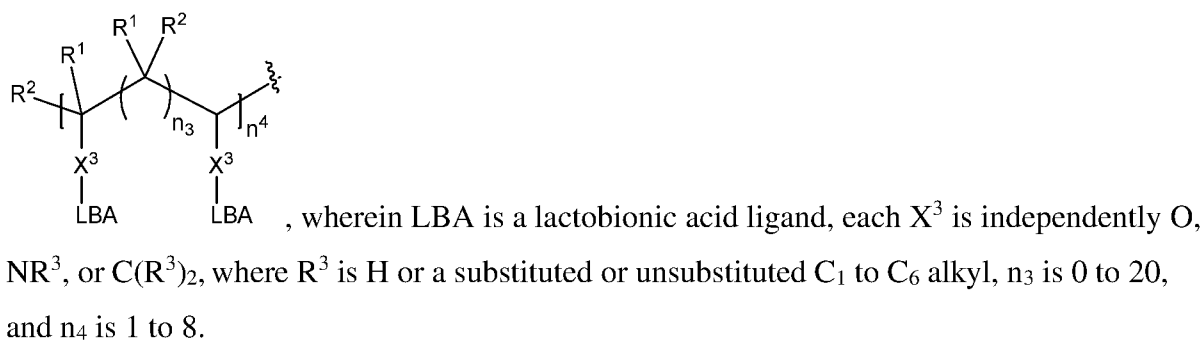
optionally wherein the carbohydrate ligand is fully or partially acylated on a hydroxy or amino group thereof with a C_2 to C_{15} acyl group, preferably wherein the carbohydrate ligand is fully or partially acetylated on a hydroxy or amino group thereof.

2. The carbohydrate conjugate of Claim 1, wherein G^1 comprises:



where n_2 is 1-20; and G^{11} is a bond or linker; wherein G^1 has 1 to 20 carbon atoms, or wherein G^1 is functional moiety linking the PNA to the conjugate or wherein G^1 is a residue of a polyethylene glycol, a polypropylene glycol, or a polyethylene-propylene glycol such a residue of a trioxo-mini polyethylene glycol (PEG) chain.

3. The carbohydrate conjugate of Claims 1 or 2, wherein G^4 comprises:



4. The carbohydrate conjugate of any one of Claims 1 to 3, wherein the ACV is selected from the group consisting of a small molecule (SM), a genetic sequence (GS), or a combination thereof, wherein, SM is a small molecule, preferably a drug or a compound targeting lung, GS is a genetic sequence, preferably a peptide nucleic acid or an antisense oligonucleotide such as a mRNA sequence, a siRNA sequence, or a DNA sequence, optionally wherein each genetic sequence is natural or modified, for example comprises a gamma-serine modified gamma peptide nucleic acid, an alanine gamma peptide nucleic acid, a clamp G-modified peptide nucleic acid, a locked nucleic acid (LNA), a phosphorothioate (PS), a phosphorodiamidate morpholino (PMO), a 2'-O-methyl (2'-O-Me), 2'-O-methoxyethyl (2'-O-MOE), 2'-fluoro (2'F), a 5'-methylcytosine, or a combination thereof, the genetic sequence having a 3' end and a 5' end.

5. The carbohydrate conjugate of any one of Claims 1 through 4, wherein the carbohydrate conjugate is delivered to a lung and where the pharmaceutically active agent is effective to target a macrophage receptor type 2 and preferably wherein CL comprises a carbohydrate residue derived from a monosaccharide or a disaccharide.

6. The carbohydrate conjugate of any one of Claims 1 through 5, wherein the genetic sequence comprises a PNA, mRNA, siRNA or antisense oligonucleotide that comprises a chemically modified nucleotide, for example a locked nucleic acid (LNA), phosphorothioate (PS), phosphorodiamidate morpholino (PMO), 2'-O-methyl (2'-O-Me), G-clamp, 2'-O-methoxyethyl (2'-O-MOE), siRNA, 2'-fluoro (2'F), 5'-methylcytosine, or a combination thereof.

7. The carbohydrate conjugate of Claim 6, wherein the genetic sequence is effective to target a MRC2 receptor or wherein the genetic sequence is effective to modulating a target gene, a target mRNA, a microRNA, or a non-coding RNA or genomic DNA.

8. The carbohydrate conjugate of any one of Claims 1 through 7, wherein G^1 is a 1 to 20 alkylene chain and where 1, 2, 3 or 4 carbons on the alkylene chain are substituted with C=O or NH.

10. The carbohydrate conjugate of Claim 4, wherein the small molecule comprises ivacaftor, elxacaftor, tezacaftor, lumacaftor, pirfenidone, N-acetylcysteine, nintedanib, cisplatin, carboplatin, docetaxel, paclitaxel, pemetrexed, prednisone, or a combination thereof.

11. The carbohydrate conjugate of any one of Claims 1 through 10, wherein the carbohydrate ligand further comprises a linker for attachment to X^1 .

12. The carbohydrate conjugate of any one of Claims 1 through 11, wherein the carbohydrate residue is a fully or partially acylated carbohydrate residue wherein the acyl groups have 2 to 15 carbon atoms.

13. The carbohydrate conjugate of any one of Claims 1 through 12, wherein a functional moiety on the conjugate further comprises a linker for attachment to kielin, tolvaptan, nintedanib, paclitaxel, bleomycin, cyclosporin, cisplatin, romidepsin, doxorubicin, docetaxel, danunorubicin, vincristine, methotrexate, cyclophosphamide, venetoclax, hydroxyurea, mercaptopurine, prednisolone, cytarabine, or pirfenidone.

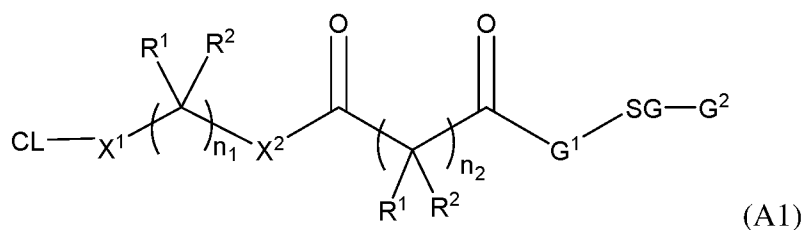
14. The carbohydrate conjugate of any one of Claims 1 through 13, wherein the carbohydrate residue is derived from N-acetylgalactosamine (GalNAc), galactose, lactobionic acid or an acetylated ester thereof, preferably wherein the carbohydrate residue is a fully or partially acetylated product of N-acetylgalactosamine (GalNAc), galactose, or lactobionic acid.

15. A method of delivering a pharmaceutically active agent to the lungs of a patient, comprising inhalationally administering to a patient an effective amount of the carbohydrate conjugate of any one of Claims 1 through 14;

where the carbohydrate conjugate is delivered to the lungs; and

where upon delivery, the pulmonary retention of the active agent is increased.

16. A composition for pulmonary delivery, the composition comprising:
a genetic sequence/small molecule-carbohydrate conjugate of a formula A1, an enantiomer, a diastereoisomer, a pharmaceutically acceptable salt, a hydrate, a solvate or a combination thereof:



wherein

SG is a small molecule (SM), a genetic sequence (GS), or a combination thereof, wherein, SM is a small molecule, preferably a drug or a compound targeting lung, and GS is a genetic sequence, preferably a peptide nucleic acid or an oligonucleotide such as an mRNA sequence, an siRNA sequence, or a DNA sequence, optionally wherein each genetic sequence is natural or modified, for example comprises a gamma-serine modified gamma peptide nucleic acid, an alanine gamma peptide nucleic acid, a clamp G-modified peptide nucleic acid, a locked nucleic acid (LNA), a phosphorothioate (PS), a phosphorodiamidate morpholino (PMO), a 2'-O-methyl (2'-O-Me), 2'-O-methoxyethyl (2'-O-MOE), 2'-fluoro (2'F), a 5'-methylcytosine, or a combination thereof, the genetic sequence having a 3' end and a 5' end,

wherein the small molecule comprises ivacaftor, elxacaftor, tezacaftor, lumacaftor, pirfenidone, N-acetylcysteine, nintedanib, cisplatin, carboplatin, docetaxel, paclitaxel, pemetrexed, or a combination thereof;

R₁ and R₂ are each independently H or a substituted or unsubstituted C₁ to C₆ alkyl, X¹ is O, NR³, C=O, or C(R³)₂ where R³ is H or a substituted or unsubstituted C₁ to C₆ alkyl,

X² is O, NR³, or C(R³)₂ where R³ is H or a substituted or unsubstituted C₁ to C₆ alkyl,

G¹ is a direct bond or a group linking the PNA to the conjugate,

G^2 is H or a functional moiety,

CL is a carbohydrate ligand comprising 2 to 16 carbohydrate residues derived from a monosaccharide, a disaccharide, a trisaccharide, or a tetrasaccharide, preferably wherein CL comprises a carbohydrate residue derived from a monosaccharide or a disaccharide,

optionally wherein the carbohydrate ligand is fully or partially acylated on a hydroxy or amino group thereof with a C_2 to C_{15} acyl group, preferably wherein the carbohydrate ligand is fully or partially acetylated on a hydroxy or amino group thereof,

n^1 is 1 to 20, and

n^2 is 0 to 20; where the composition is delivered to the lungs.

17. The composition of claim 16, wherein the genetic sequence comprises a PNA, mRNA, or siRNA that comprises a chemically modified nucleotide, for example a locked nucleic acid (LNA), phosphorothioate (PS), phosphorodiamidate morpholino (PMO), 2'-O-methyl (2'-O-Me), G-clamp, 2'-O-methoxyethyl (2'-O-MOE), siRNA, 2'-fluoro (2'F), 5'-methylcytosine, or a combination thereof.

18. The composition of any one of Claims 1 to 14 and 16 to 17, wherein the carbohydrate ligand or the carbohydrate conjugate is effective to target a MRC2 receptor.

19. The composition any one of Claims 16 through 18, for modulating a target gene, a target mRNA, a microRNA, or a non-coding RNA.

20. The composition of any one of Claims 16 through 19, wherein G^1 has 1 to 20 carbon atoms, or wherein G^1 is functional moiety linking the PNA to the conjugate or wherein G^1 is a residue of a polyethylene glycol, a polypropylene glycol, or a polyethylene-propylene glycol such a residue of a trioxo-mini polyethylene glycol (PEG)chain.

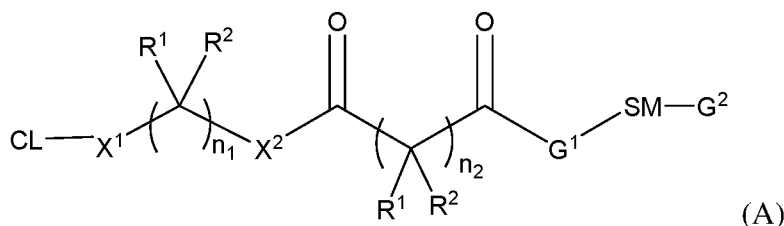
21. The composition of any one of Claims 16 through 20, wherein the carbohydrate ligand further comprises a linker for attachment to X^1 .

22. The composition of any one of Claims 16 through 21, wherein the carbohydrate residue is a fully or partially acylated carbohydrate residue wherein the acyl groups have 2 to 15 carbon atoms.

23. The composition of any one of Claims 16 through 22, wherein a functional moiety G^2 on the conjugate further comprises a linker for attachment to kielin, tolvaftan, nintedanib, paclitaxel, bleomycin, cyclosporin, cisplatin, romidepsin, doxorubicin, docetaxel, danunorubicin, vincristine, methotrexate, cyclophosphamide, venetoclax, hydroxyurea, mercaptopurine, prednisolone, cytarabine, deoxyribonuclease I (rhDNase) (dornase alfa), catalase, superoxide dismutase (SOD), glucocerebrosidase, alpha-1-antitrypsin, lipase, hyaluronidase, alpha-galactosidase or pirfenidone.

24. The composition of any one of Claims 16 through 23, wherein the carbohydrate residue is derived from N-acetylgalactosamine (GalNAc), galactose, lactobionic acid or an acetylated ester thereof, preferably wherein the carbohydrate residue is a fully or partially acetylated product of N-acetylgalactosamine (GalNAc), galactose, or lactobionic acid.

25. The composition of any one of Claims 16 through 24, of a formula A, an enantiomer, a diastereoisomer, a pharmaceutically acceptable salt, a hydrate, a solvate or a combination thereof:

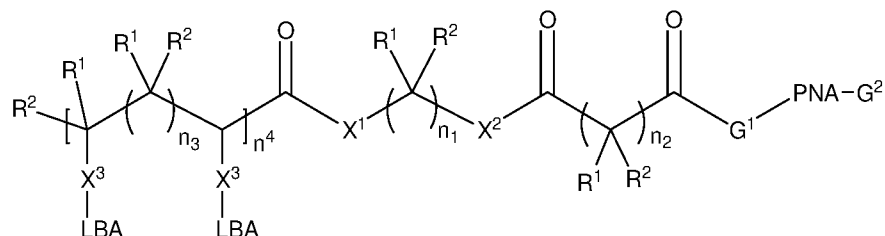


wherein SM is a small molecule, preferably a drug or a compound targeting lung, R_1 and R_2 are each independently H or a substituted or unsubstituted C_1 to C_6 alkyl, X^1 is O, NR^3 , $C=O$, or $C(R^3)_2$ where R^3 is H or a substituted or unsubstituted C_1 to C_6 alkyl, X^2 is O, NR^3 , or $C(R^3)_2$ where R^3 is H or a substituted or unsubstituted C_1 to C_6 alkyl, G^1 is a direct bond or a group linking the small molecule to the conjugate, G^2 is H or a functional moiety, CL is a carbohydrate ligand comprising 2 to 16 carbohydrate residues derived from a monosaccharide, a disaccharide, a trisaccharide, or a tetrasaccharide, preferably wherein CL comprises a carbohydrate residue/ligand derived from a monosaccharide, a disaccharide, or a polysaccharide, optionally wherein the carbohydrate ligand is fully or partially acylated on a hydroxy or amino group thereof with a C_2 to C_{15} acyl group, preferably wherein the carbohydrate ligand is fully or partially acetylated on a hydroxy or amino group thereof, n^1 is 1 to 20, and n^2 is 0 to 20.

26. The composition of Claim 25, wherein the small molecule comprises ivacaftor, ellexaftor, tezacaftor, lumacaftor, pirfenidone, N-acetylcysteine, nintedanib, cisplatin, carboplatin, docetaxel, paclitaxel, pemetrexed, prednisone, or a combination thereof.

27. The composition of any one of Claims 16 through 26, where the genetic sequence/small molecule-carbohydrate conjugate is effective to target a macrophage receptor type 2.

28. The composition of any one of Claims 17 through 27, of a formula



wherein

LBA is a lactobionic acid residue,

X^1 is NR^3 , O, or $C(R^3)_2$ where R^3 is H or a substituted or unsubstituted C_1 to C_6 alkyl,

each X^3 is independently O, NR^3 , or $C(R^3)_2$ where R^3 is H or a substituted or unsubstituted C_1 to C_6 alkyl,

n_3 is 0 to 20, and

n_4 is 1 to 8.

29. The composition of claim 27, wherein

G^1 is a group linking the PNA to the conjugate,

R^1 and R^2 are each H,

X^1 , X^2 , and X^3 are each NH, and

$n_1=6$, $n_2=2$, and $n_3=4$.

30. The composition of claim 28, wherein

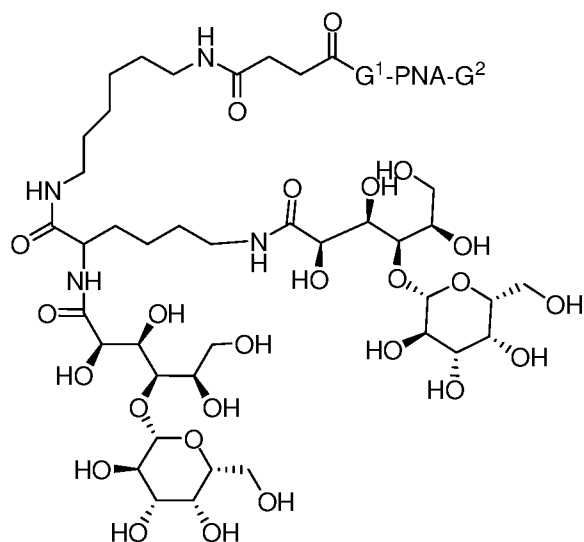
R^1 and R^2 are each H,

$n_3=4$, and

the PNA is linked at the 5' end to the conjugate.

31. The composition of any one of Claims 16 through 30, where the composition is aerosolized for inhalational delivery or delivered via local administration to the lung, bronchi and/or airways.

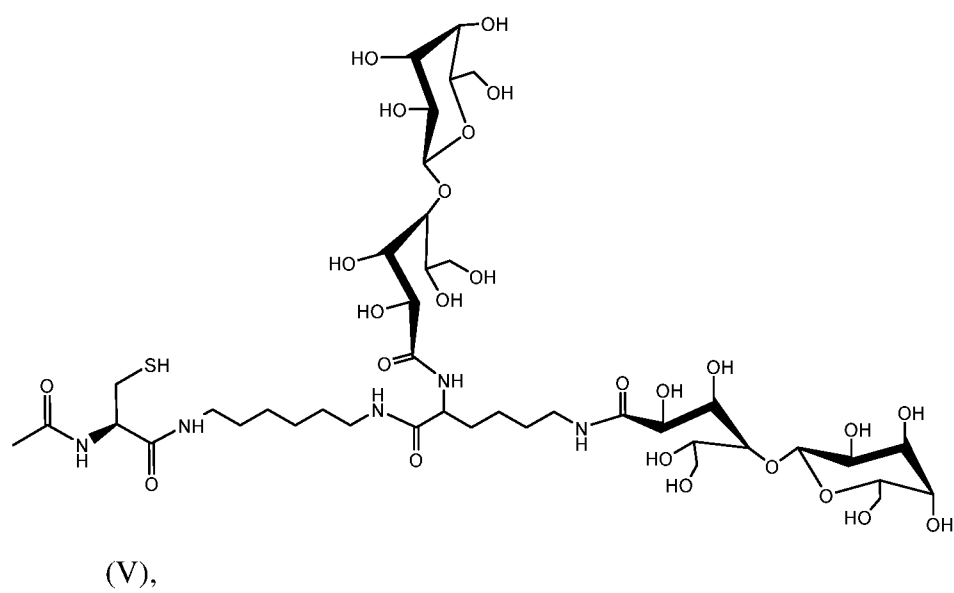
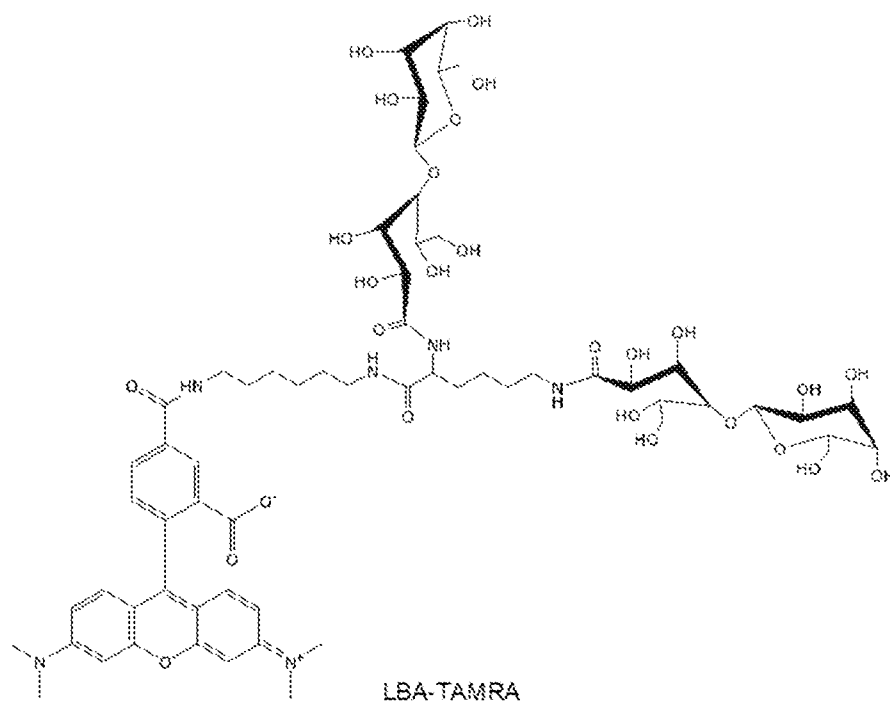
32. The composition of any one of Claims 27 through 30, where the composition has the structure:

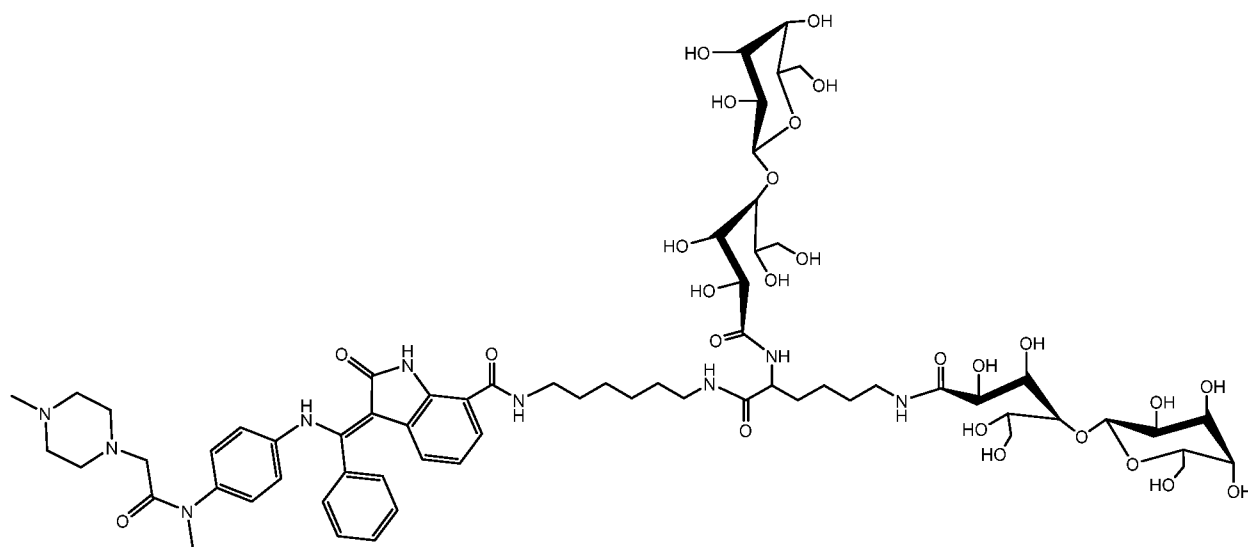


, an enantiomer, a diastereoisomer, a pharmaceutically acceptable salt, a hydrate, a solvate or a combination thereof, wherein G^1 is a functional moiety linking the PNA to the conjugate and G^2 is a functional moiety.

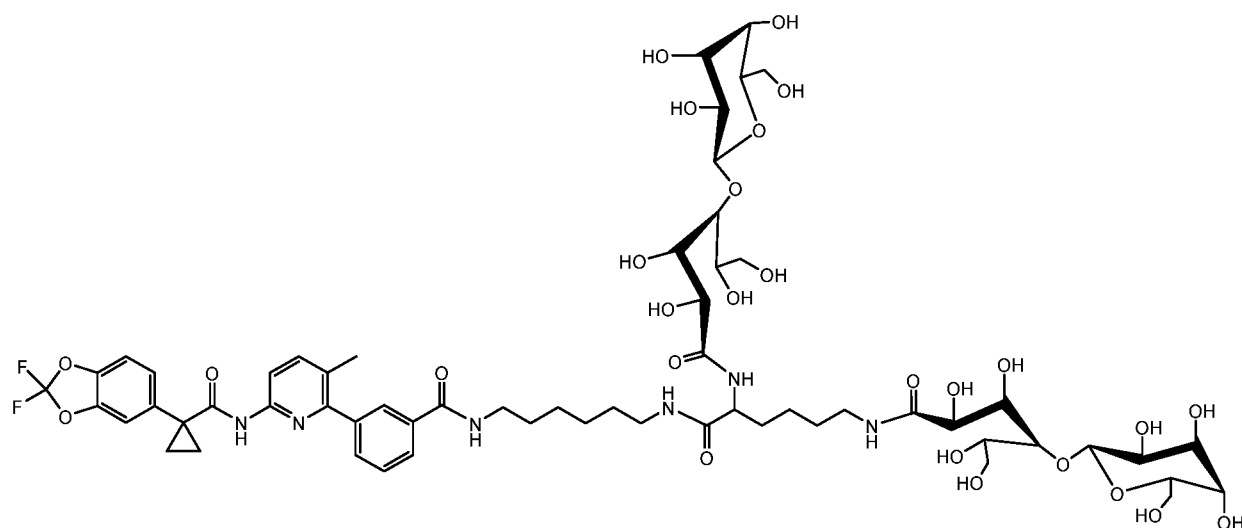
33. The composition of any one of Claims 16 through 32, wherein the lactobionic acid residue is fully or partially acylated with an acyl group having from 2 to 15 carbon atoms, preferably wherein the lactobionic acid residue is fully or partially acetylated.

34. The composition of Claim 1, where the genetic sequence/small molecule-carbohydrate conjugate has the structure:



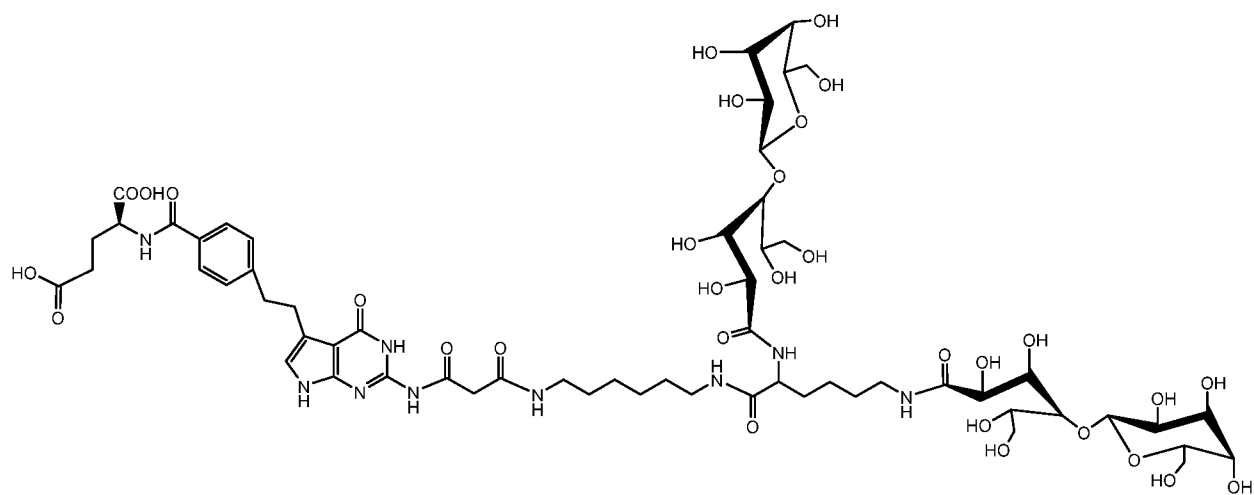


(VI),

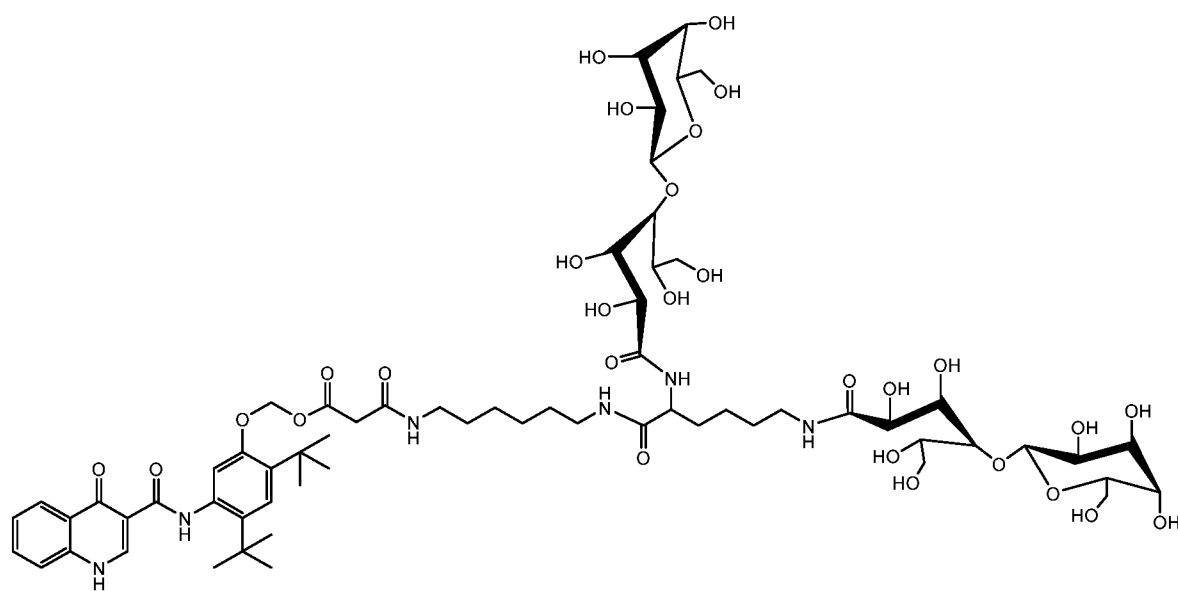


(VII)

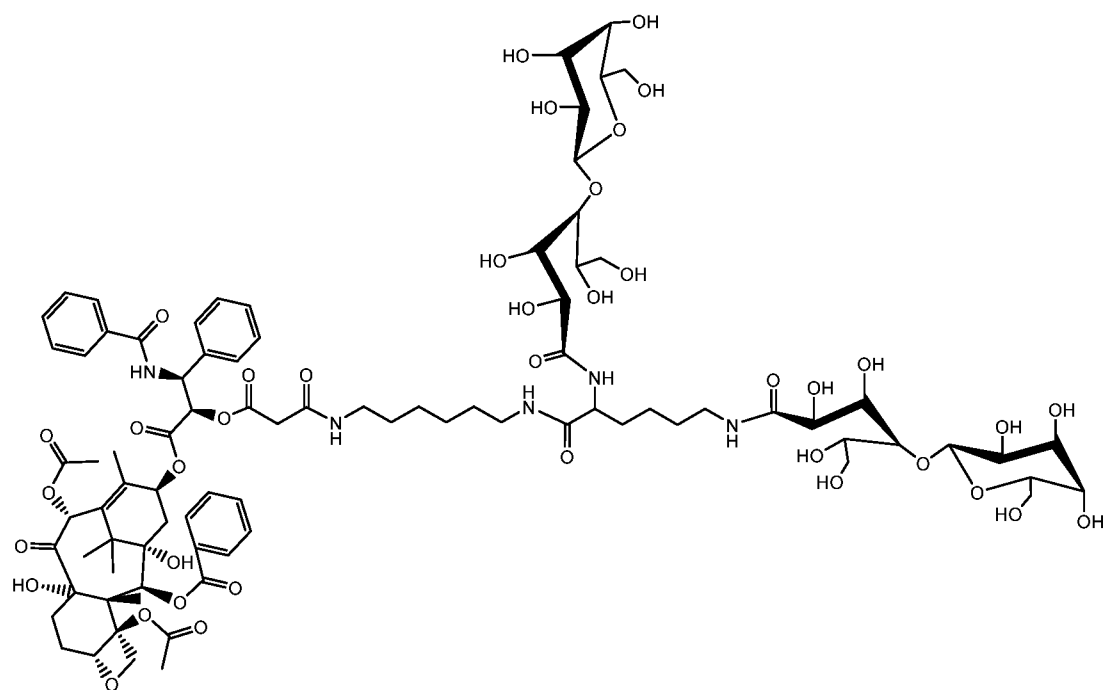




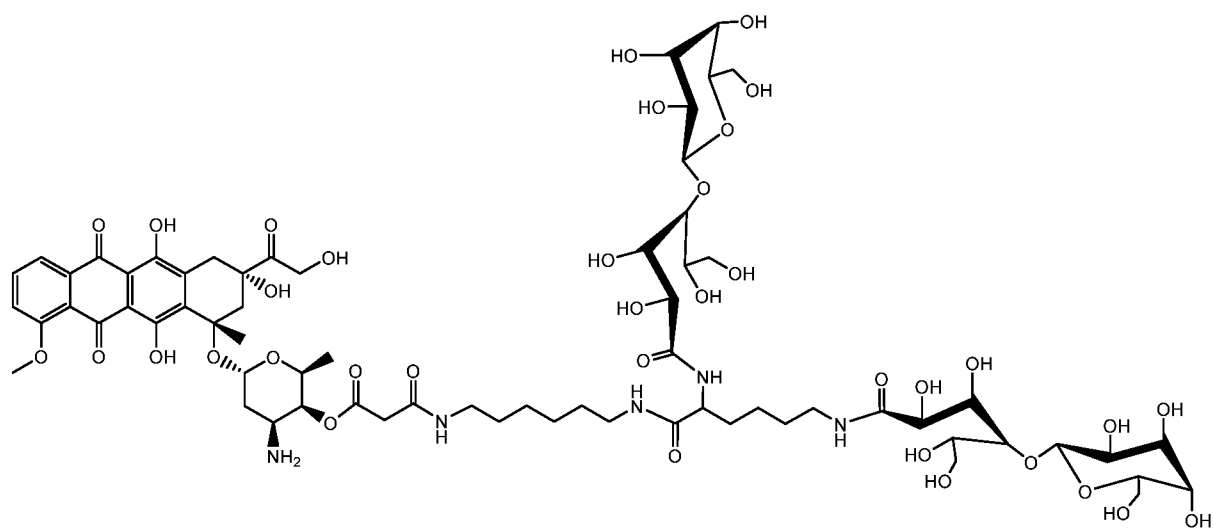
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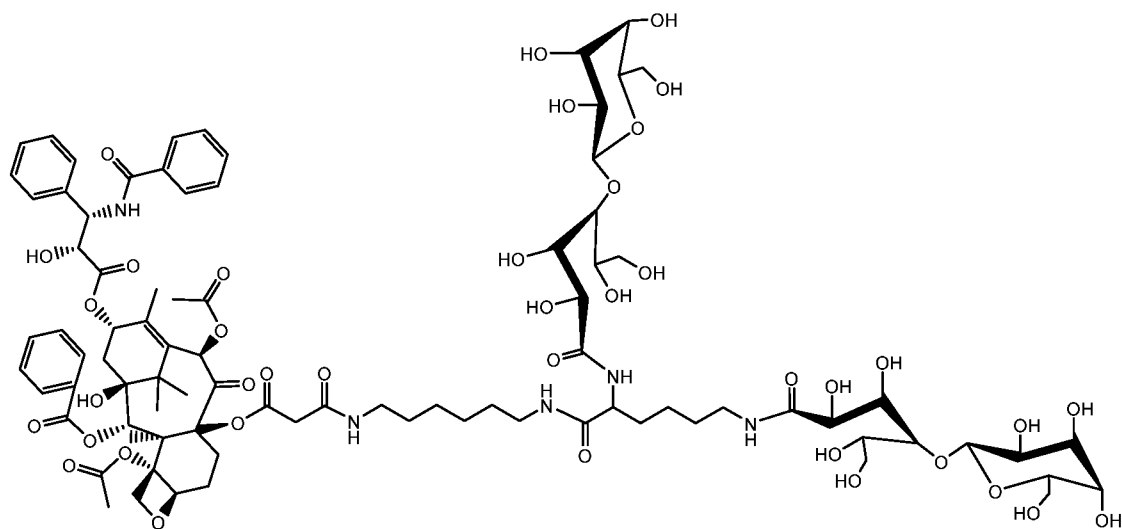
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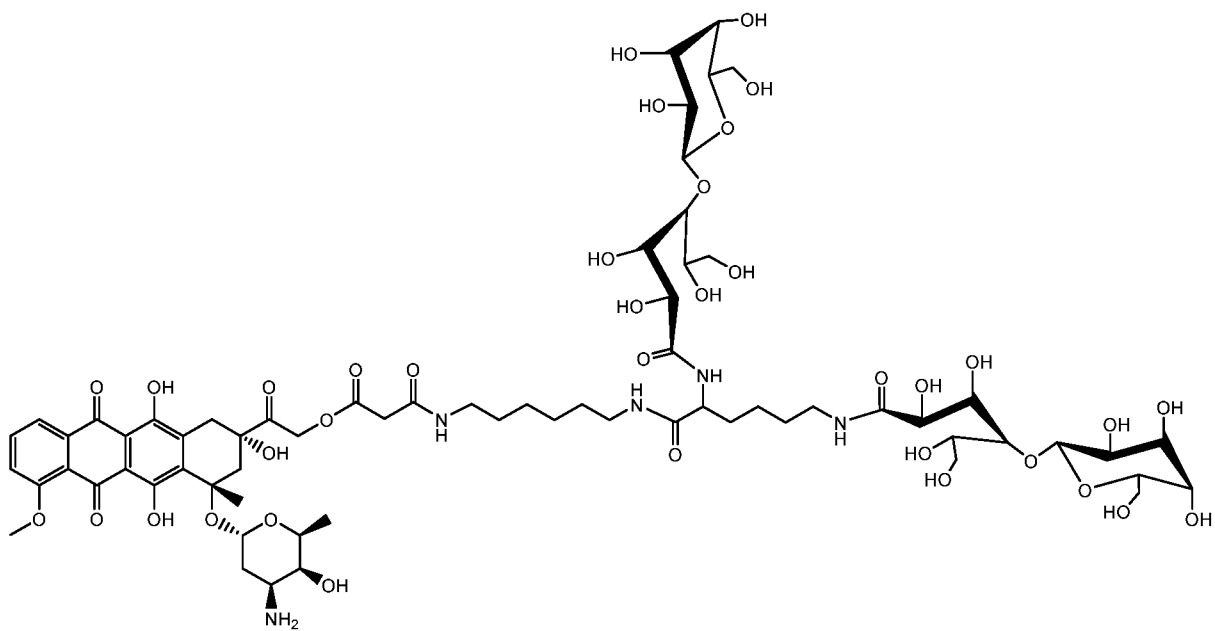
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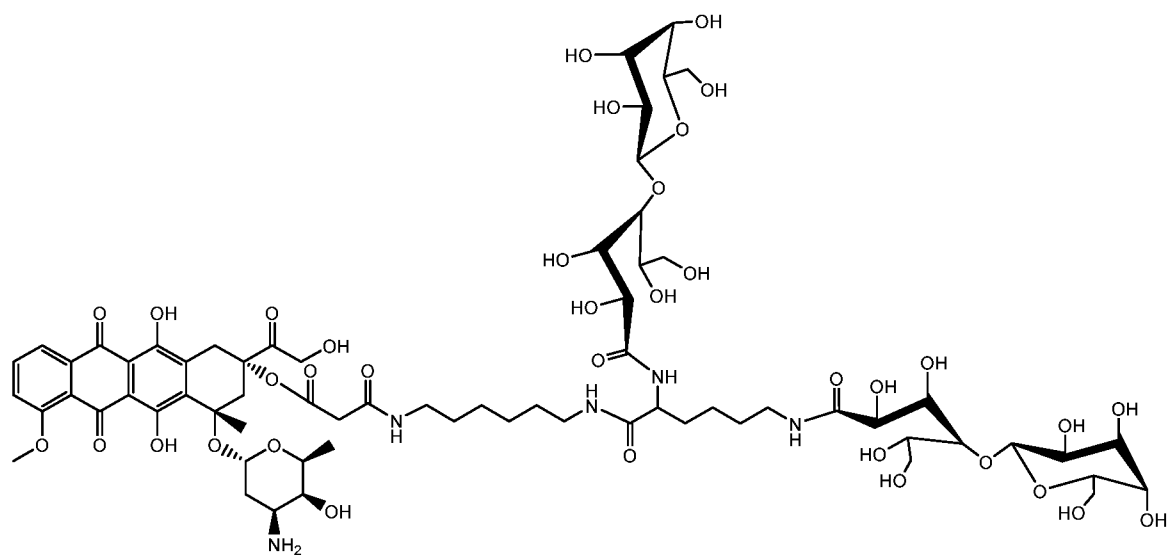
(XIII),



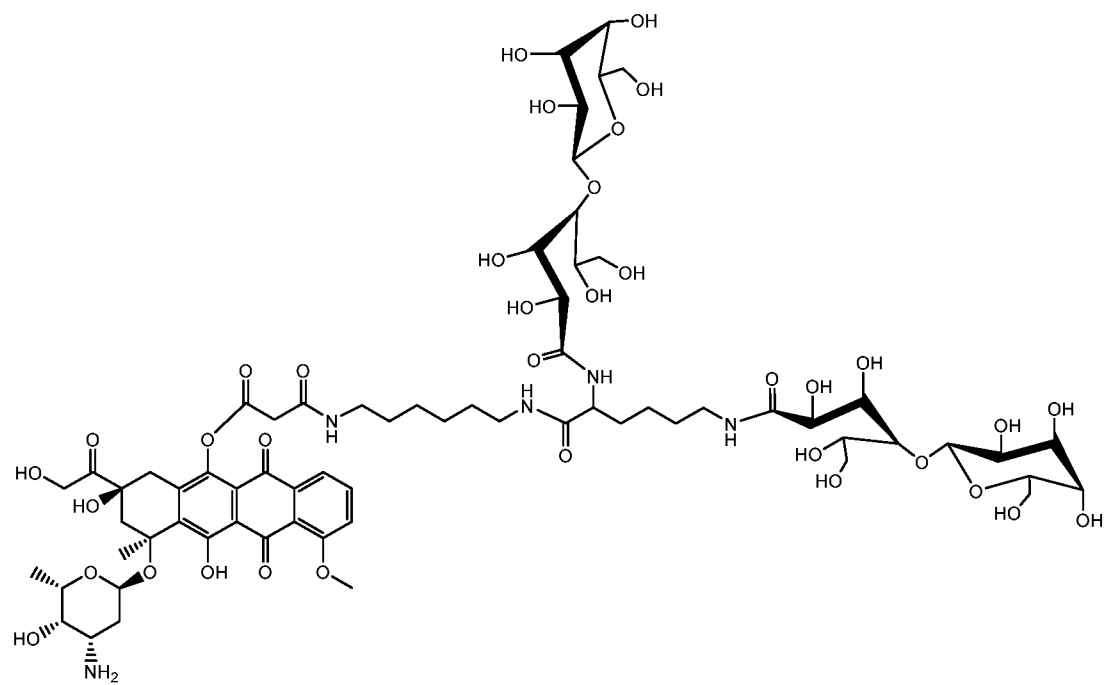
(XIV),



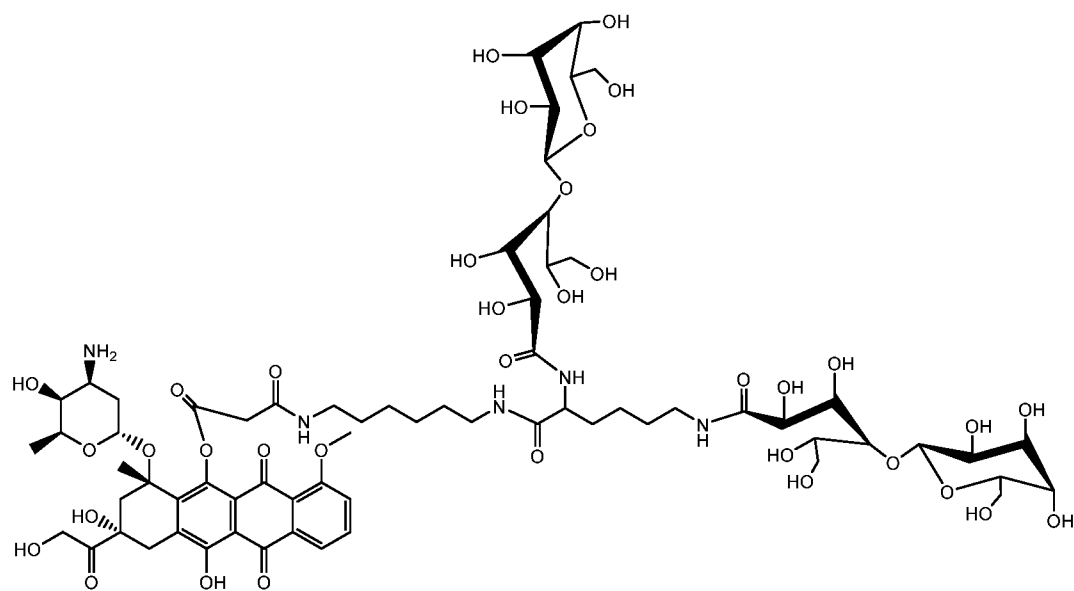
(XV),



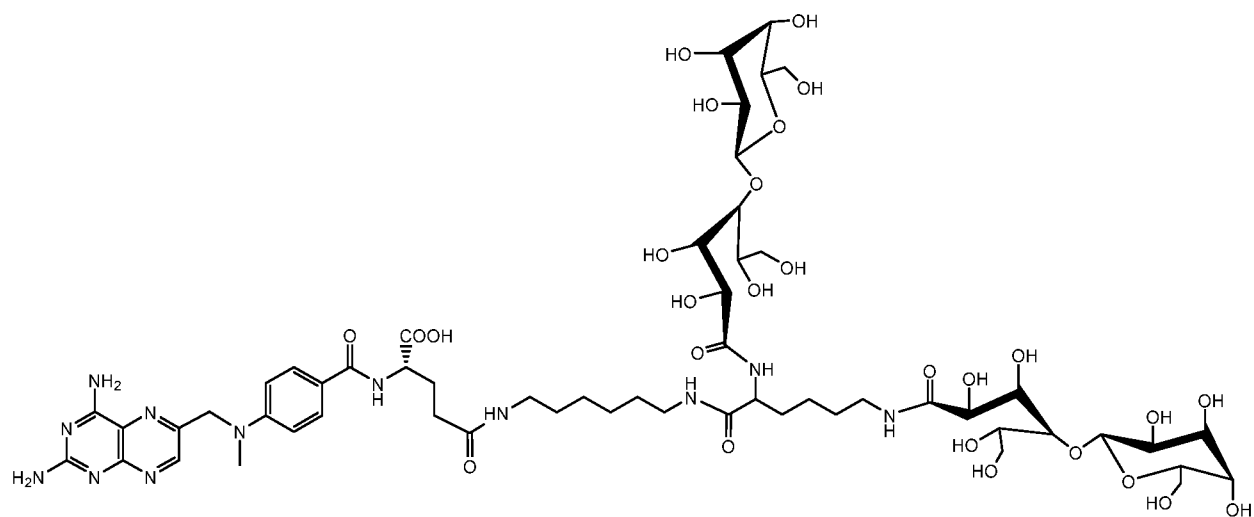
(XVI)



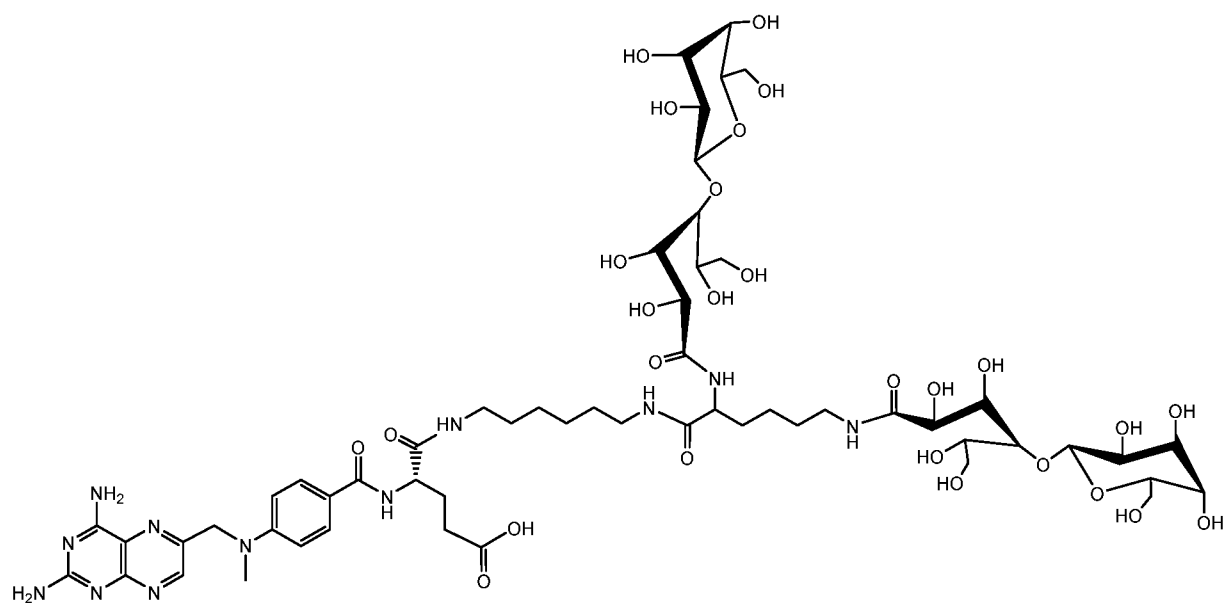
(XV),



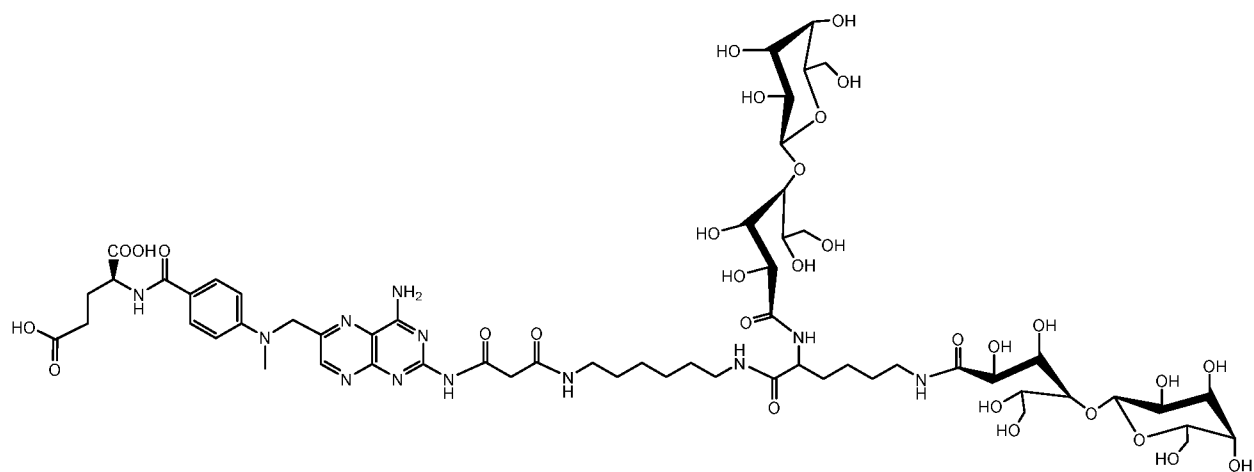
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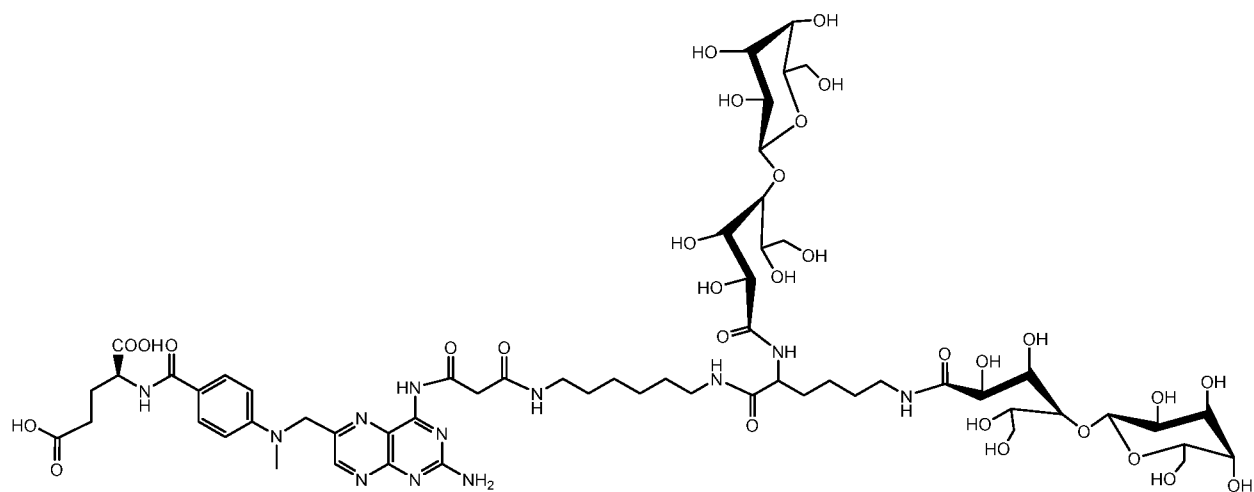
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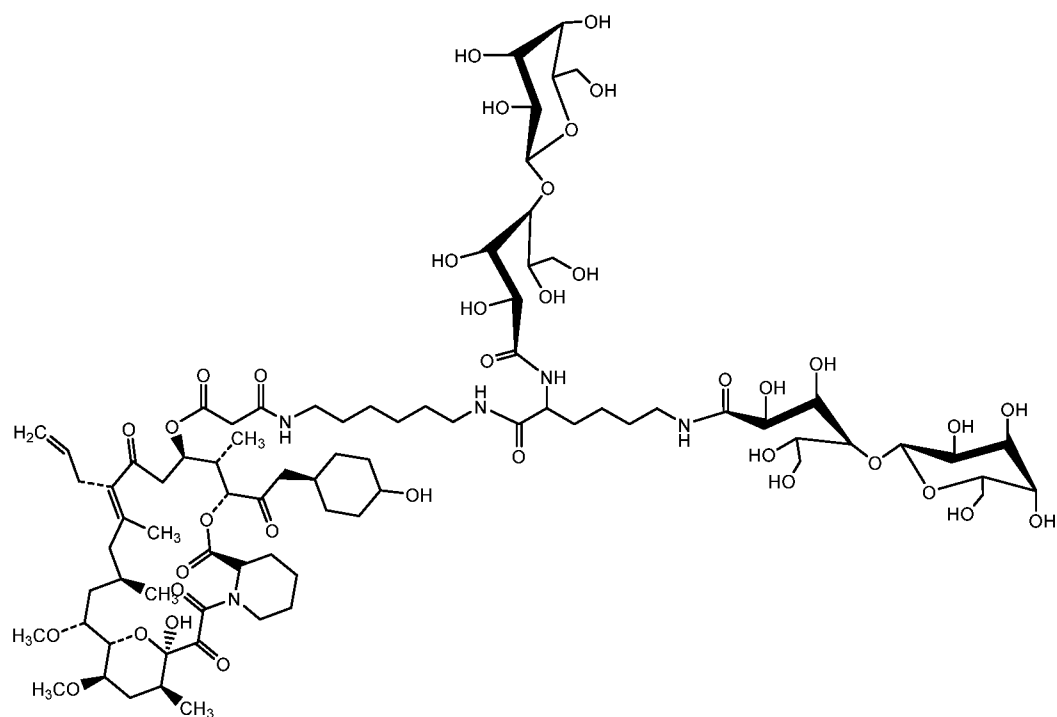
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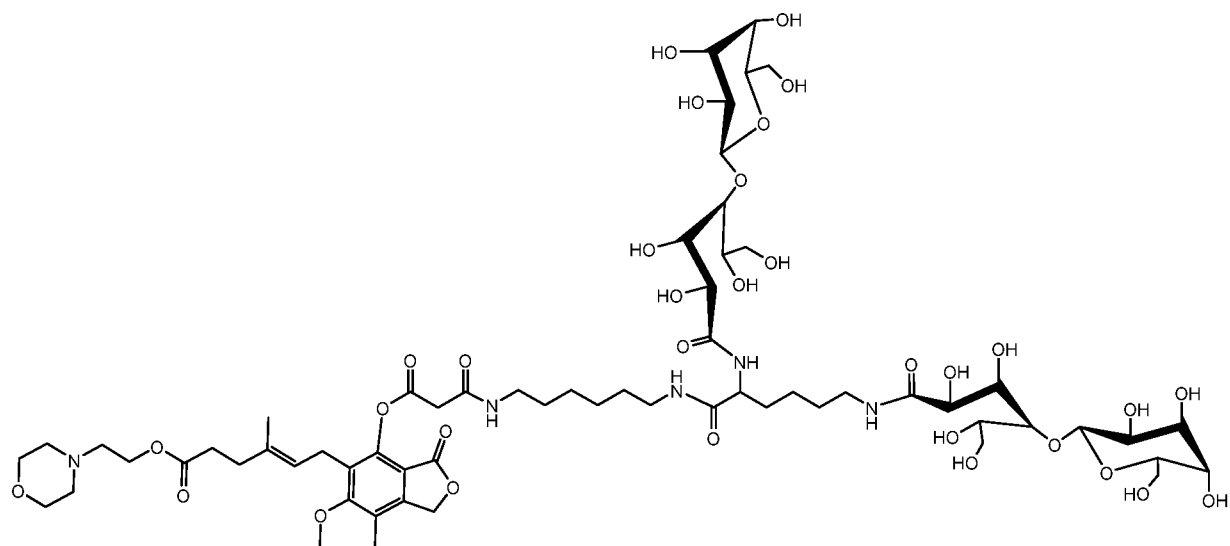
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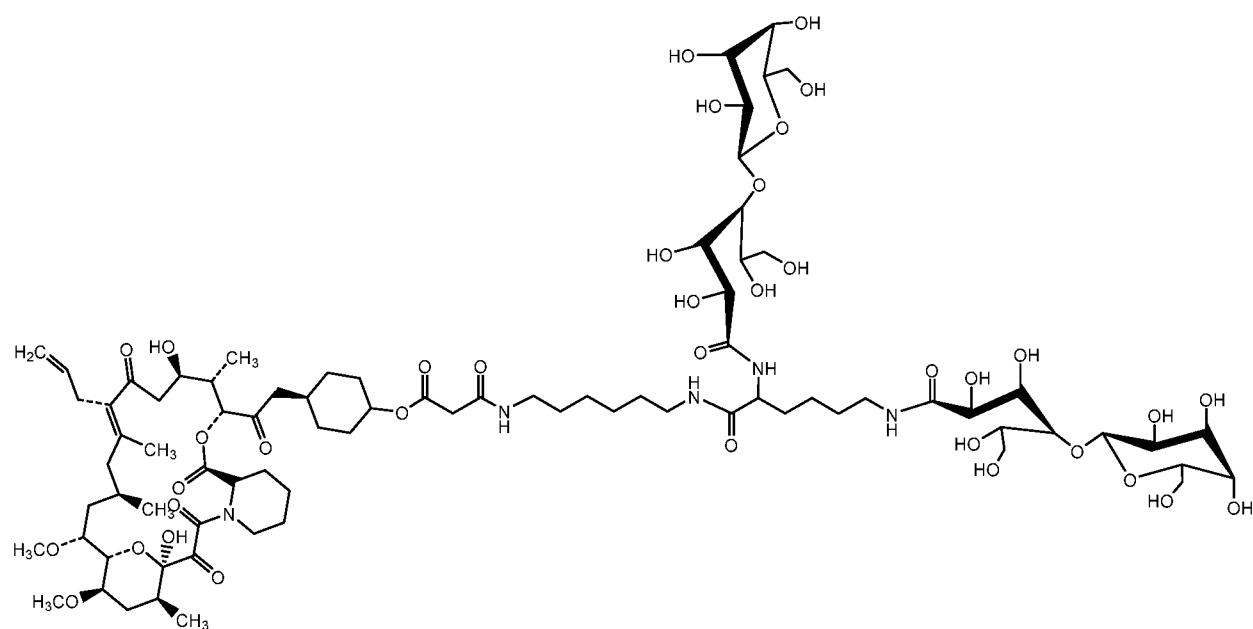
(XX)



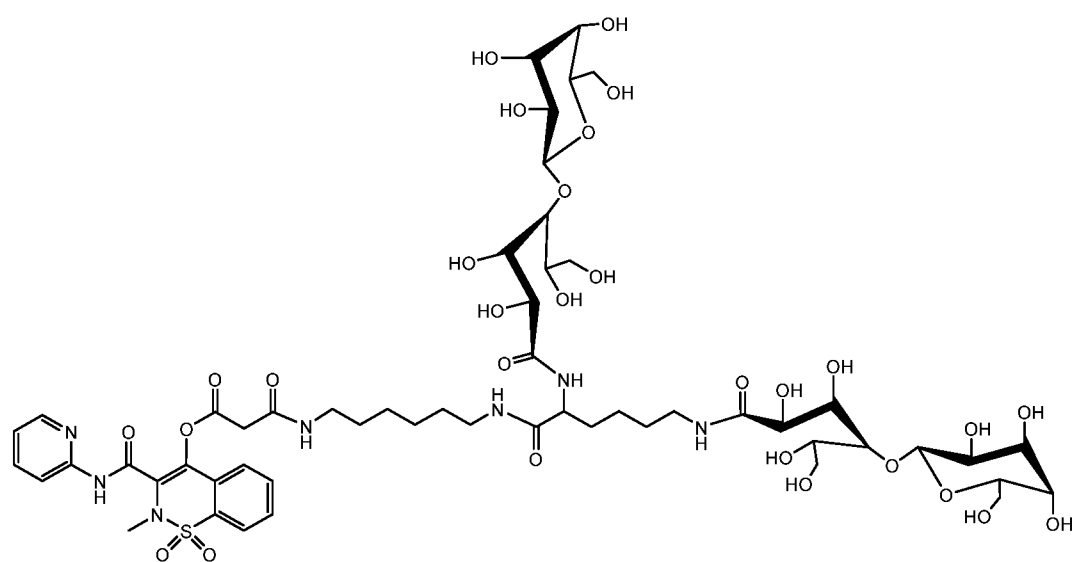
(XXI),



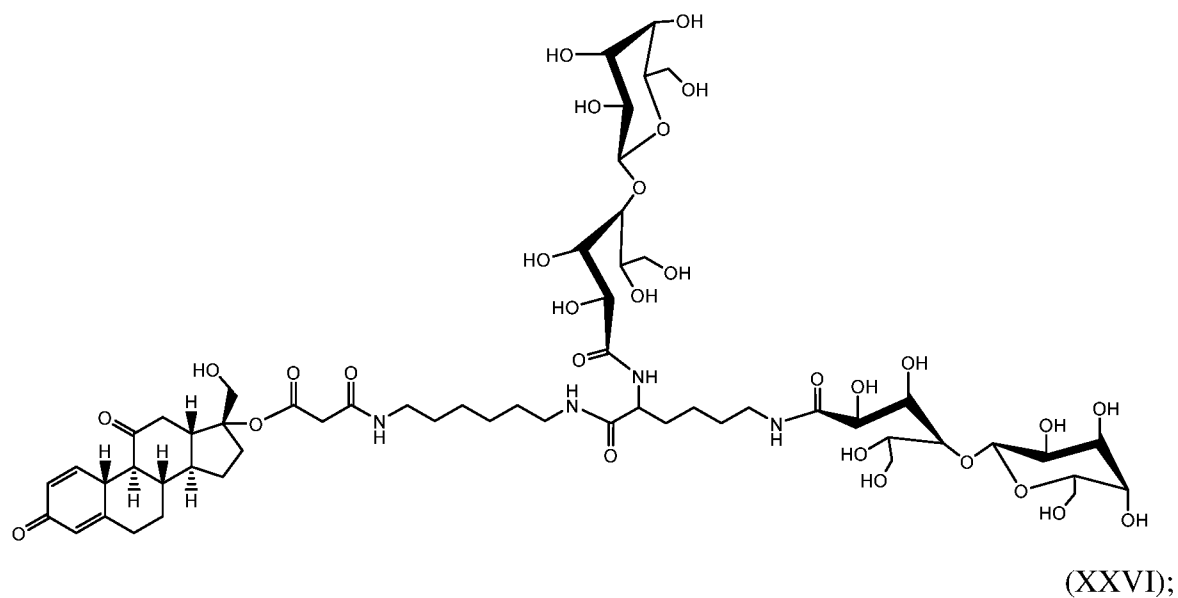
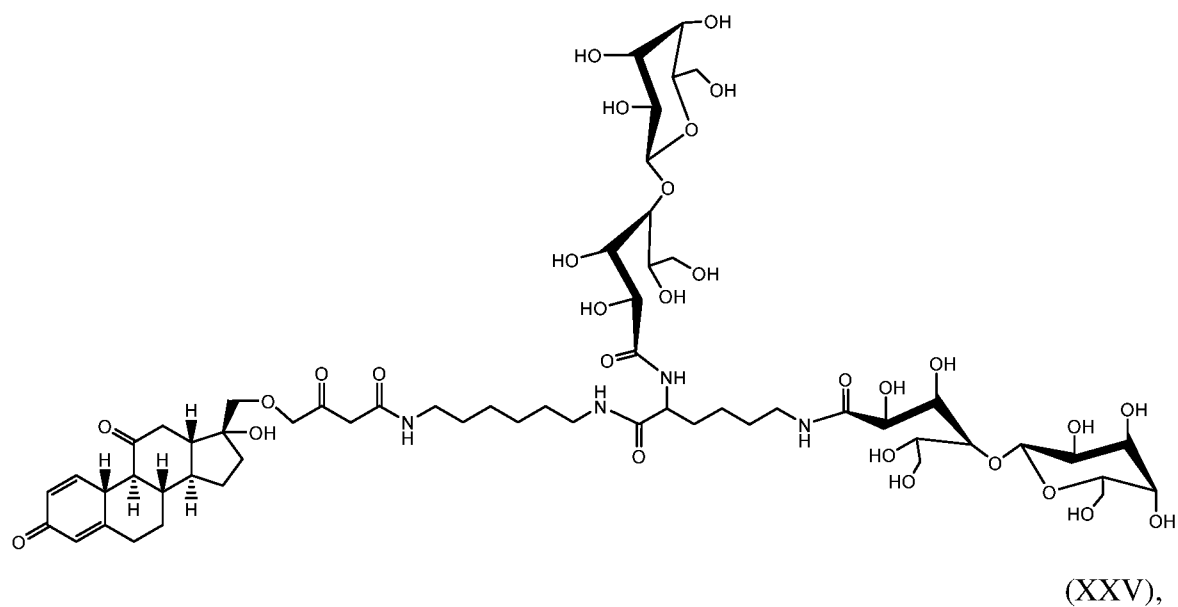
(XXII),



(XXIII),



(XXIV),



an enantiomer, a diastereoisomer, a pharmaceutically acceptable salt, a hydrate, a solvate or a combination thereof.

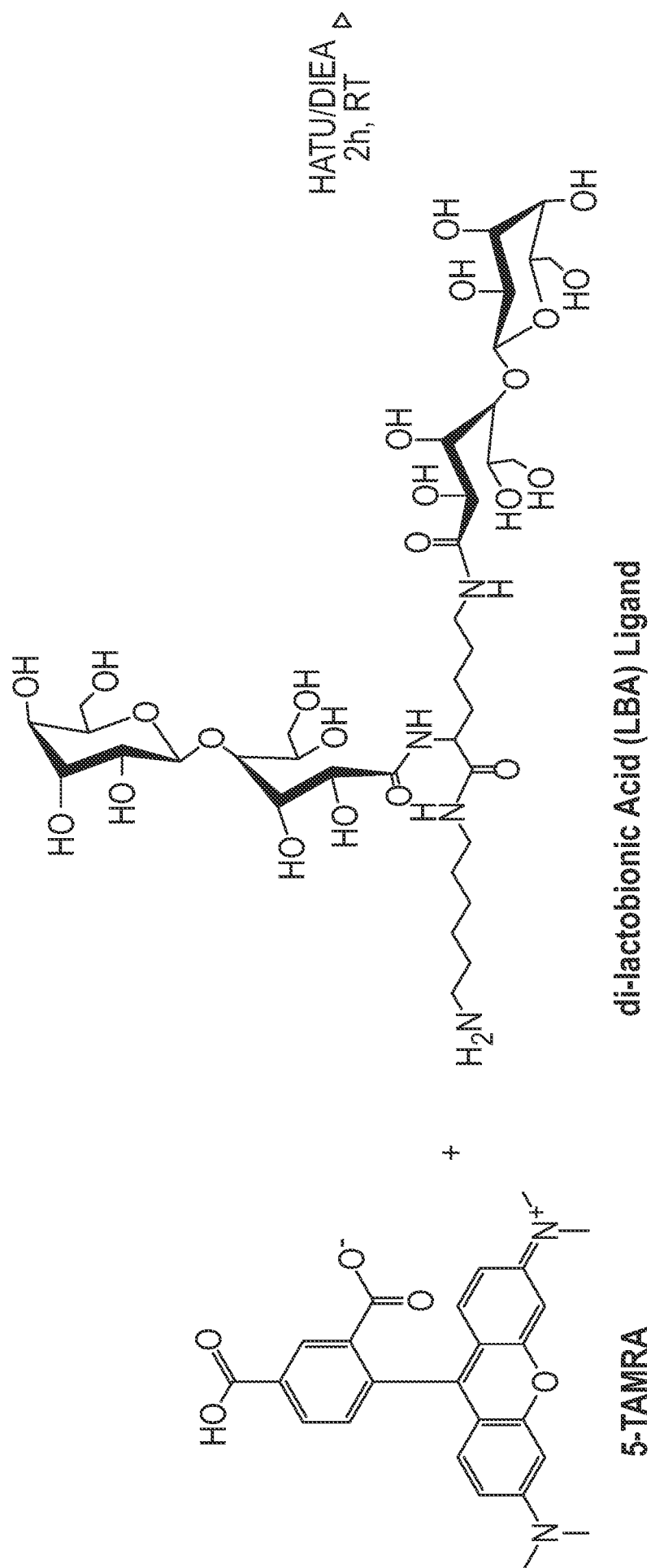


FIG. 1

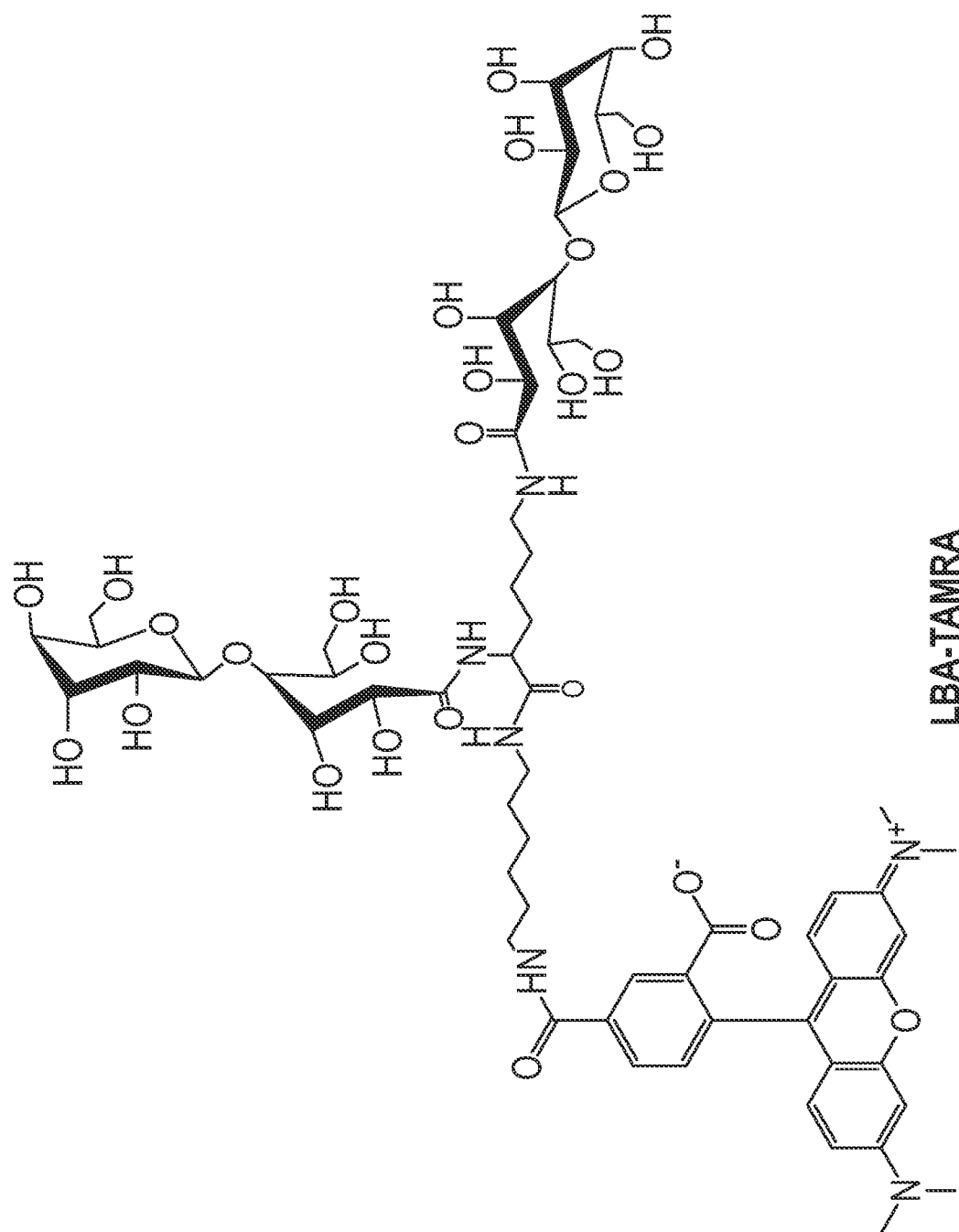


FIG. 1
(Continued)

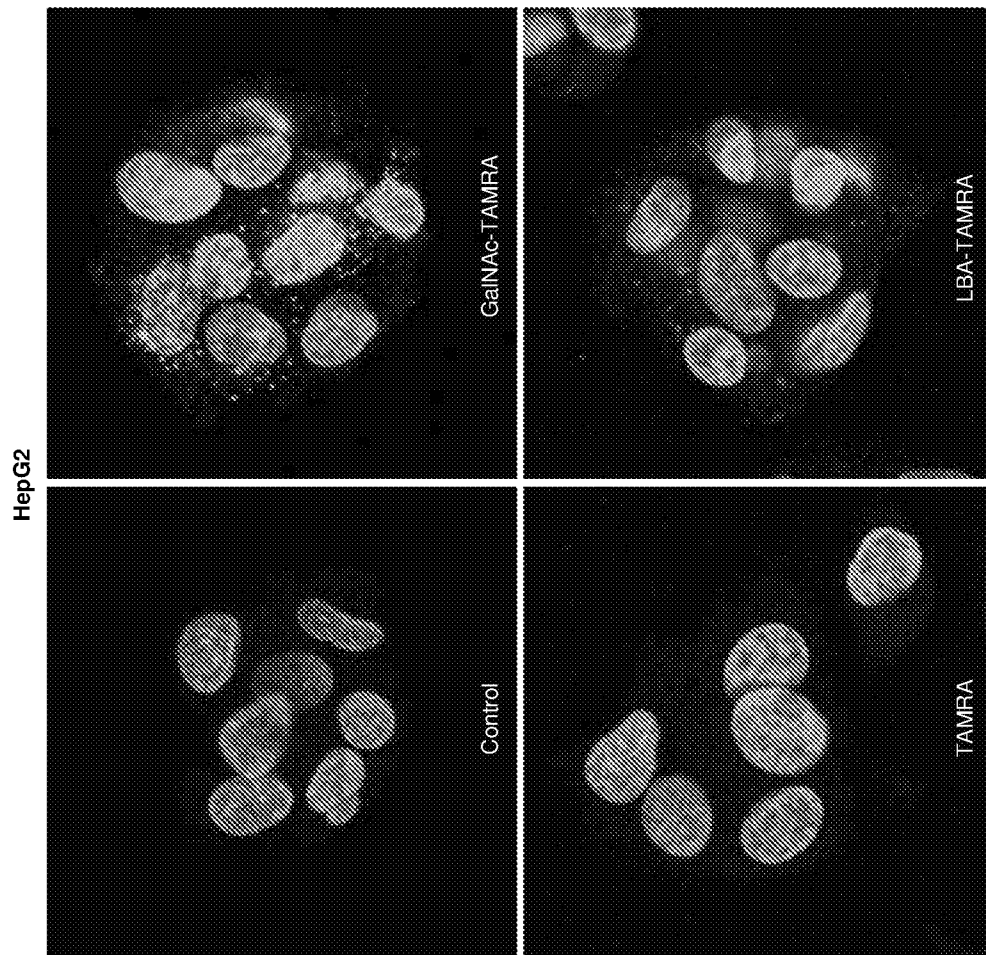


FIG. 2A

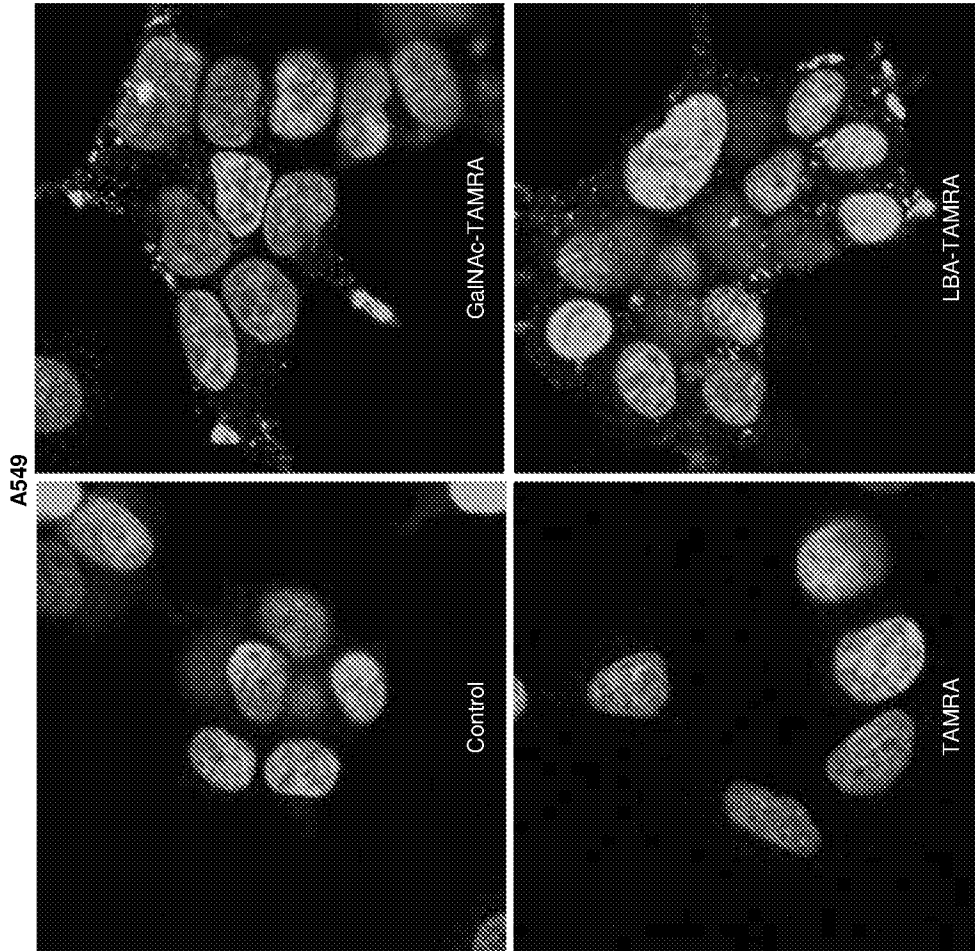
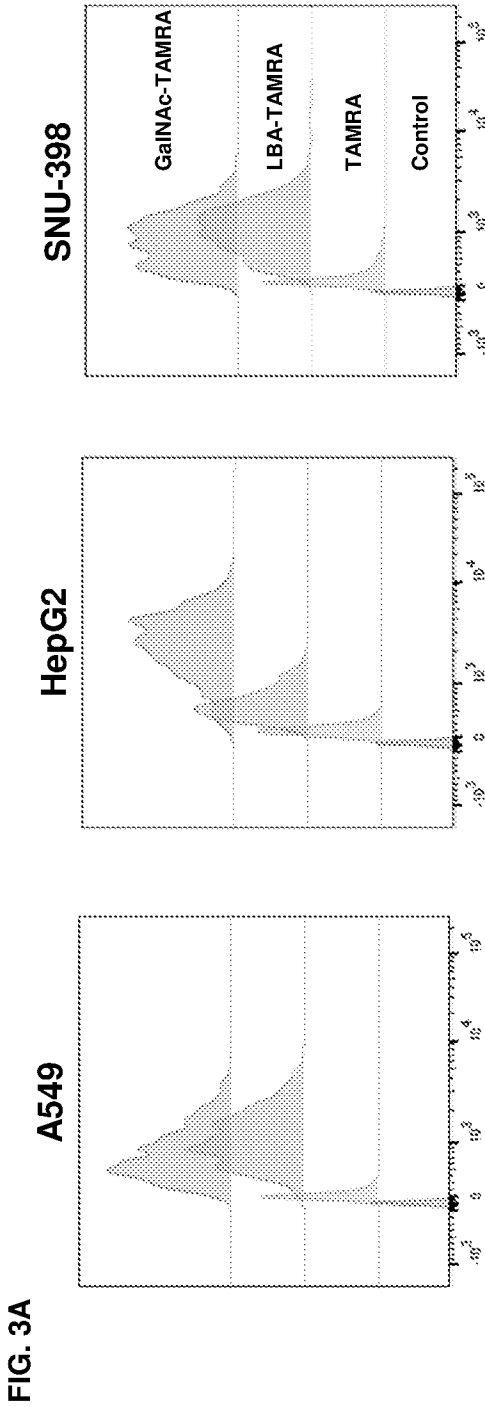


FIG. 2B



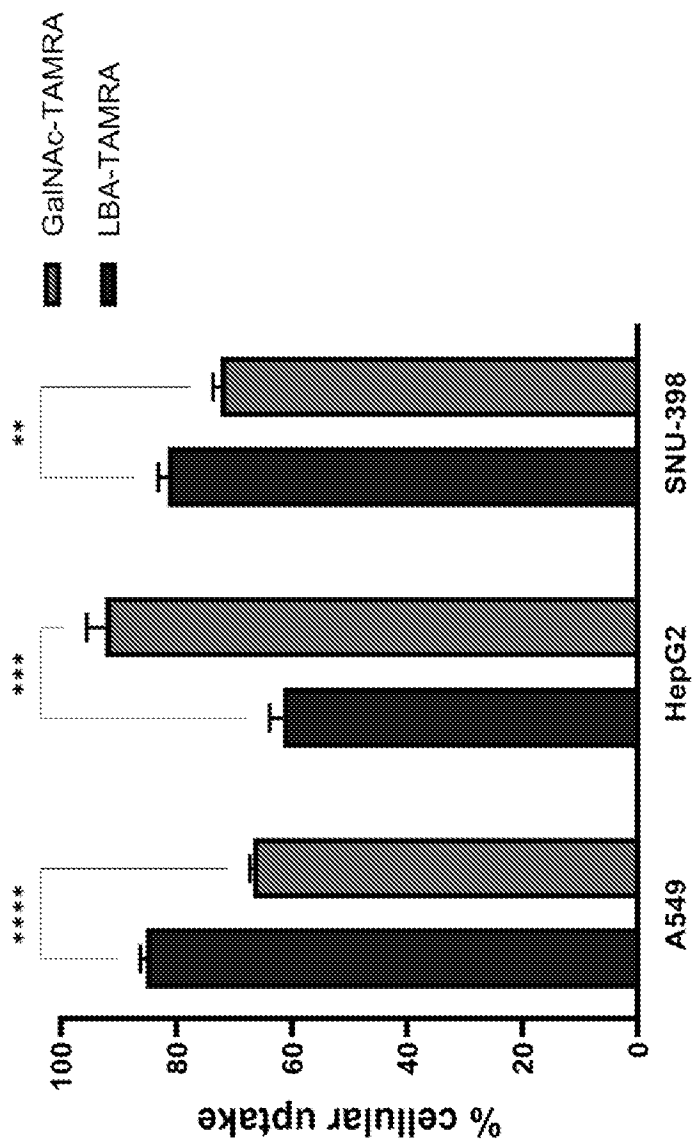


FIG. 3B

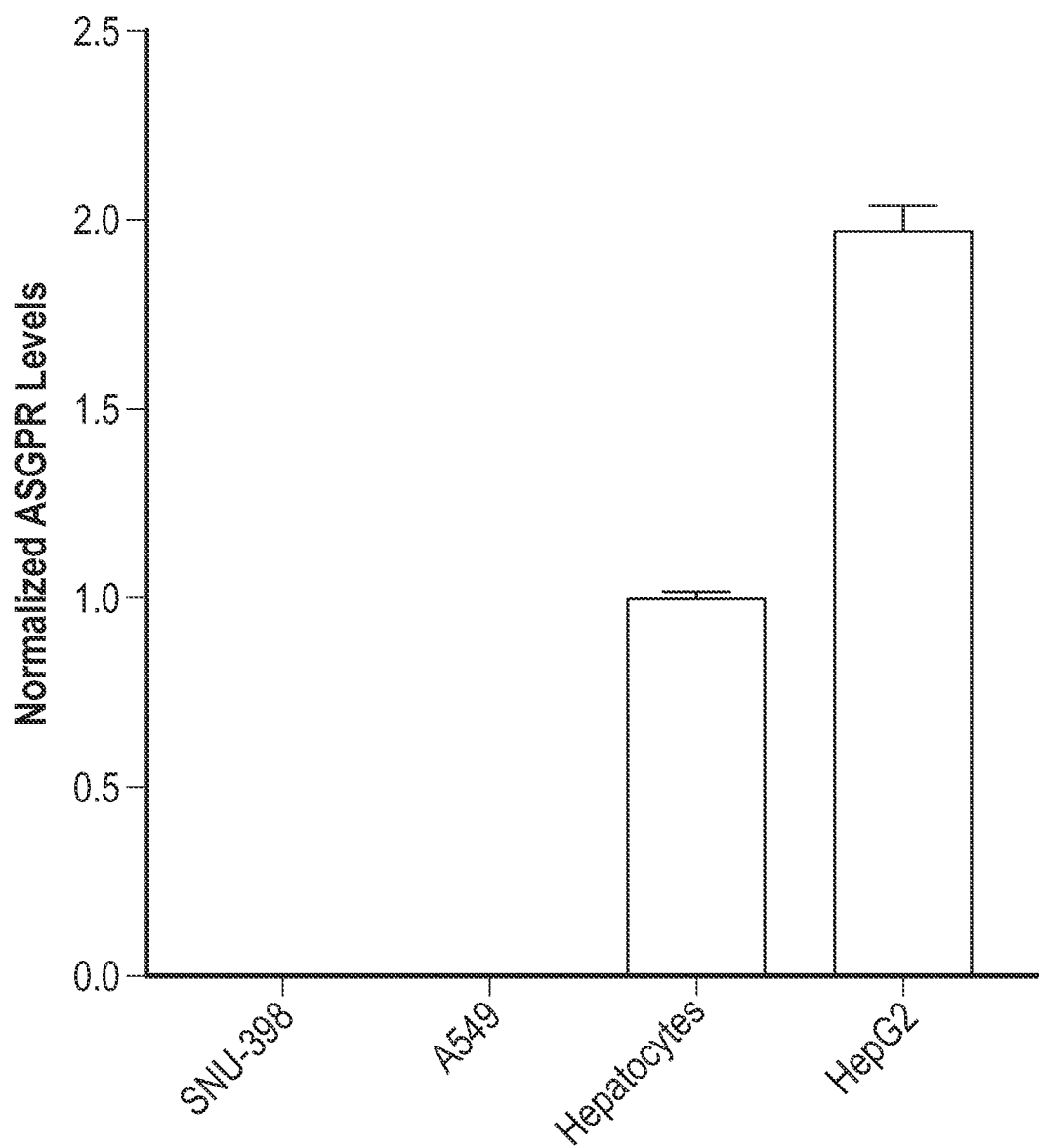


FIG. 4A

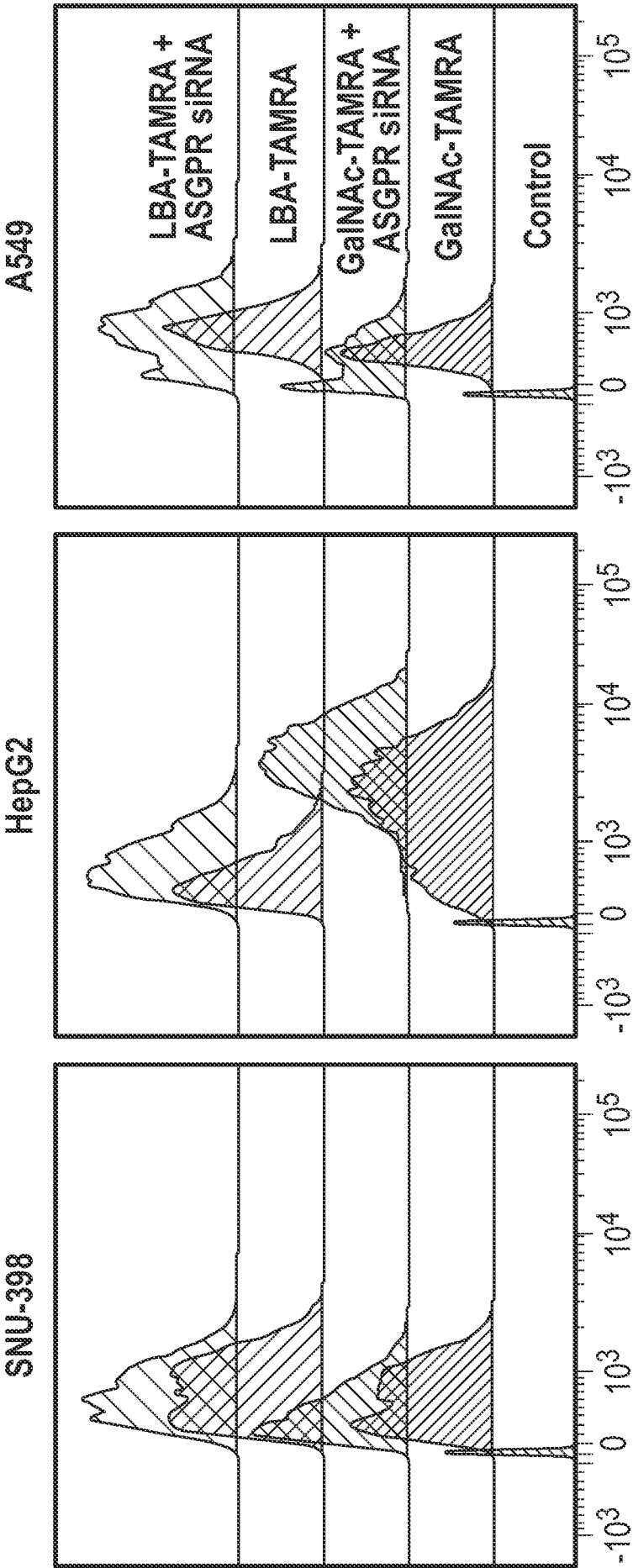


FIG. 4B

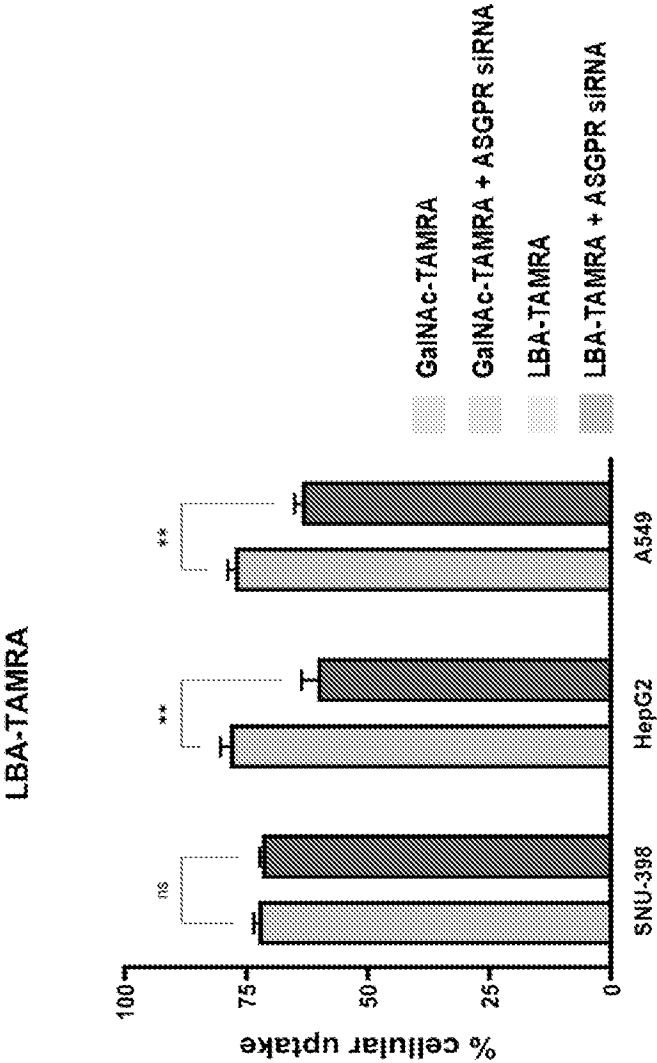
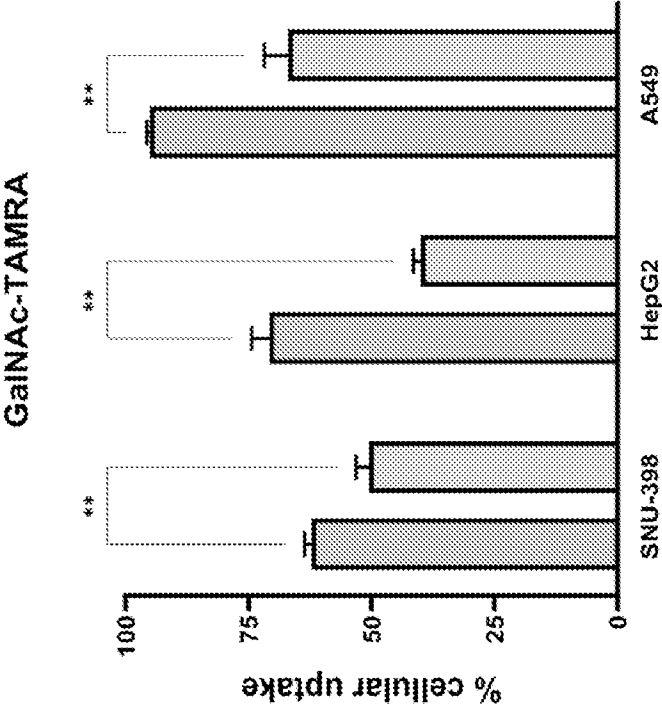
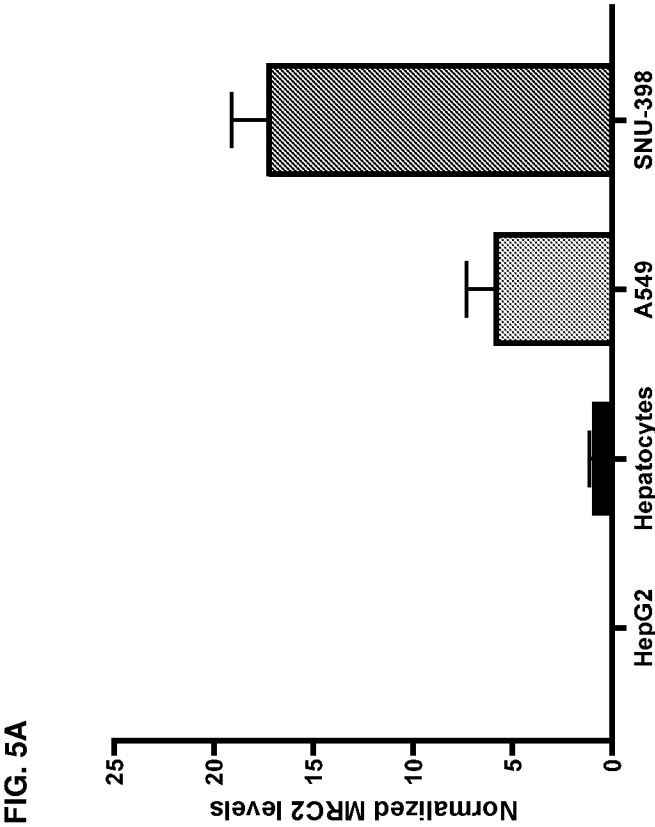


FIG. 4C

FIG. 4D





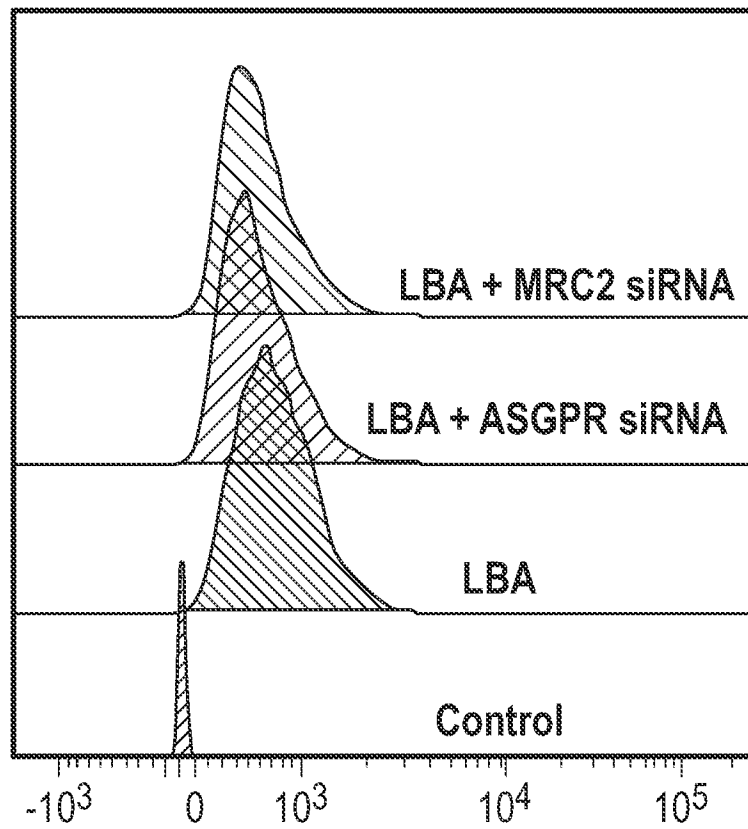
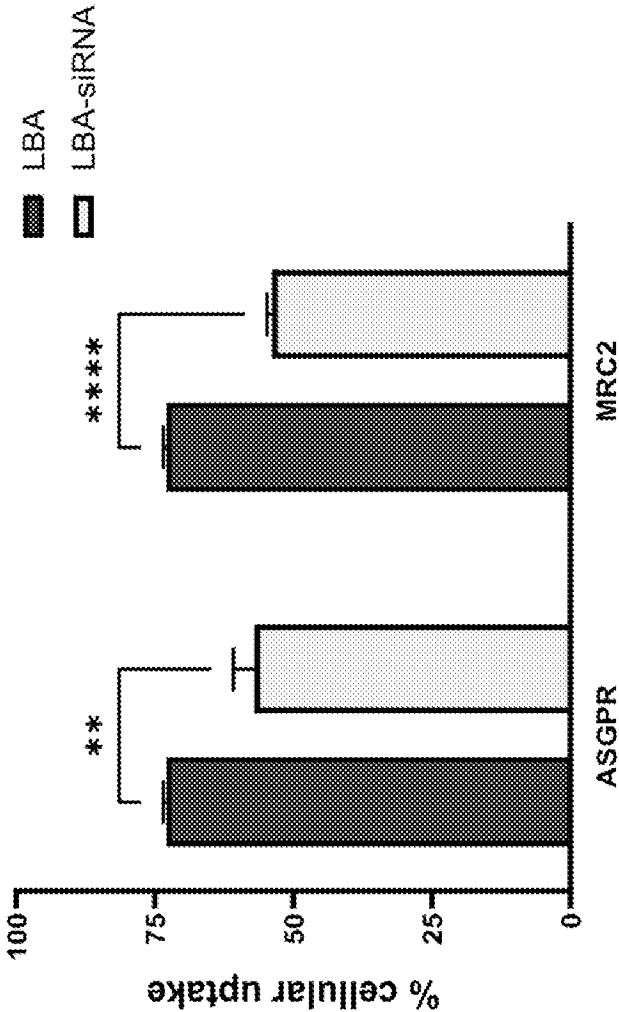


FIG. 5B

FIG. 5C



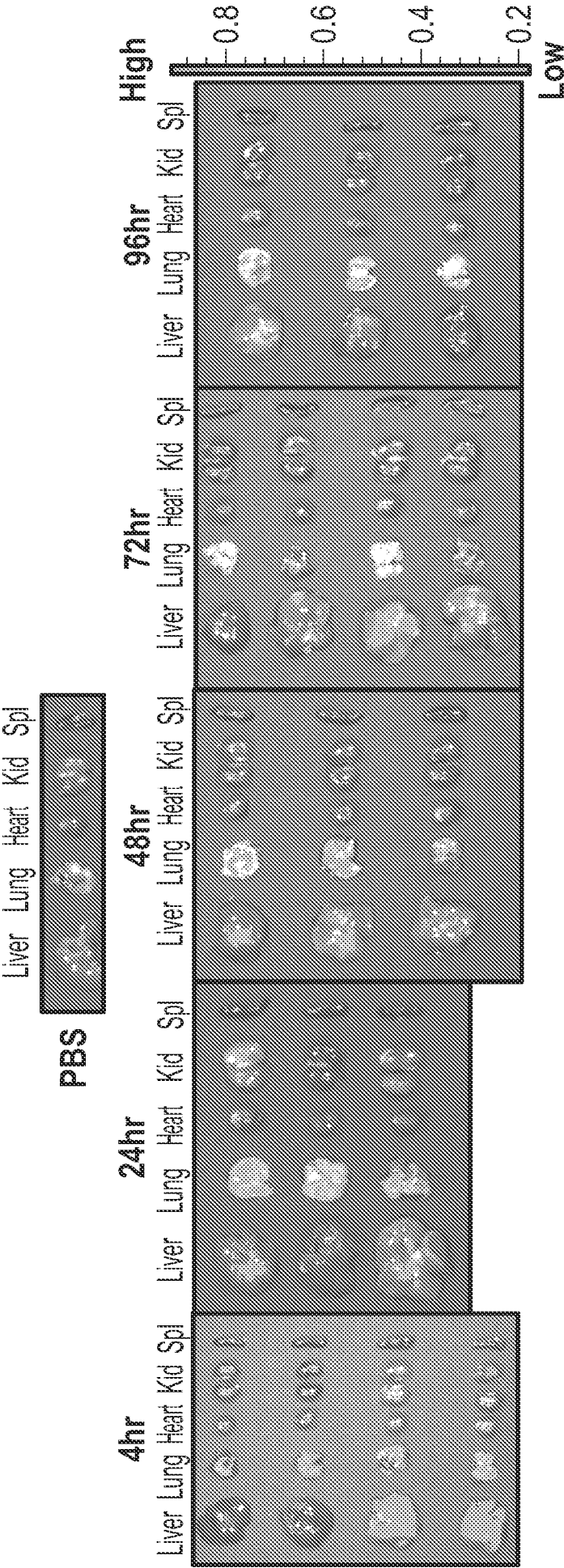


FIG. 6A

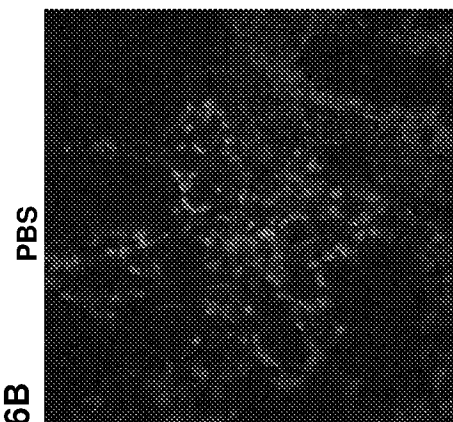
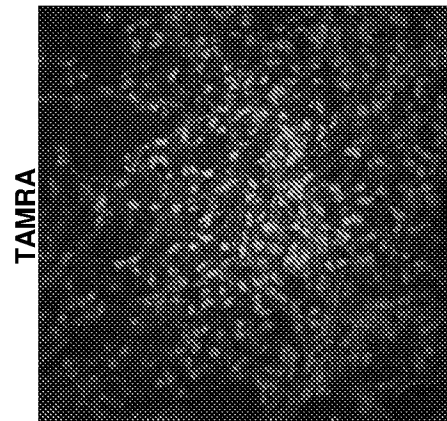
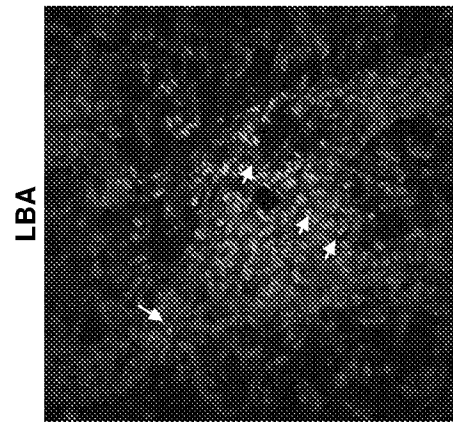
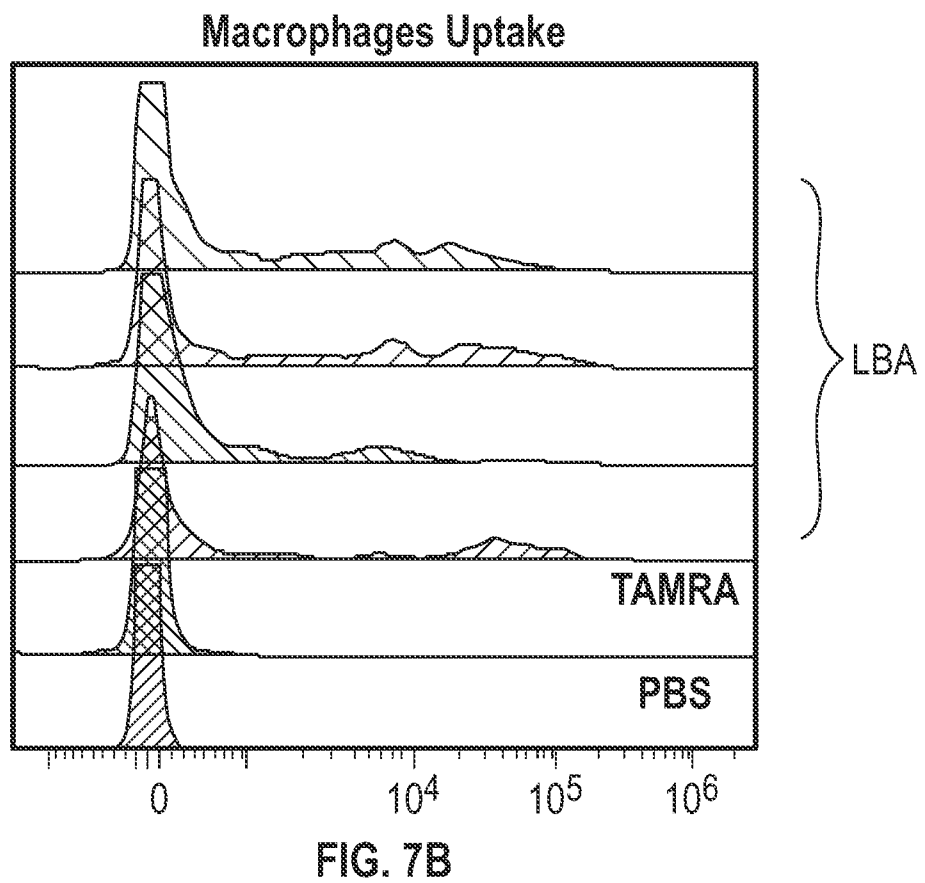
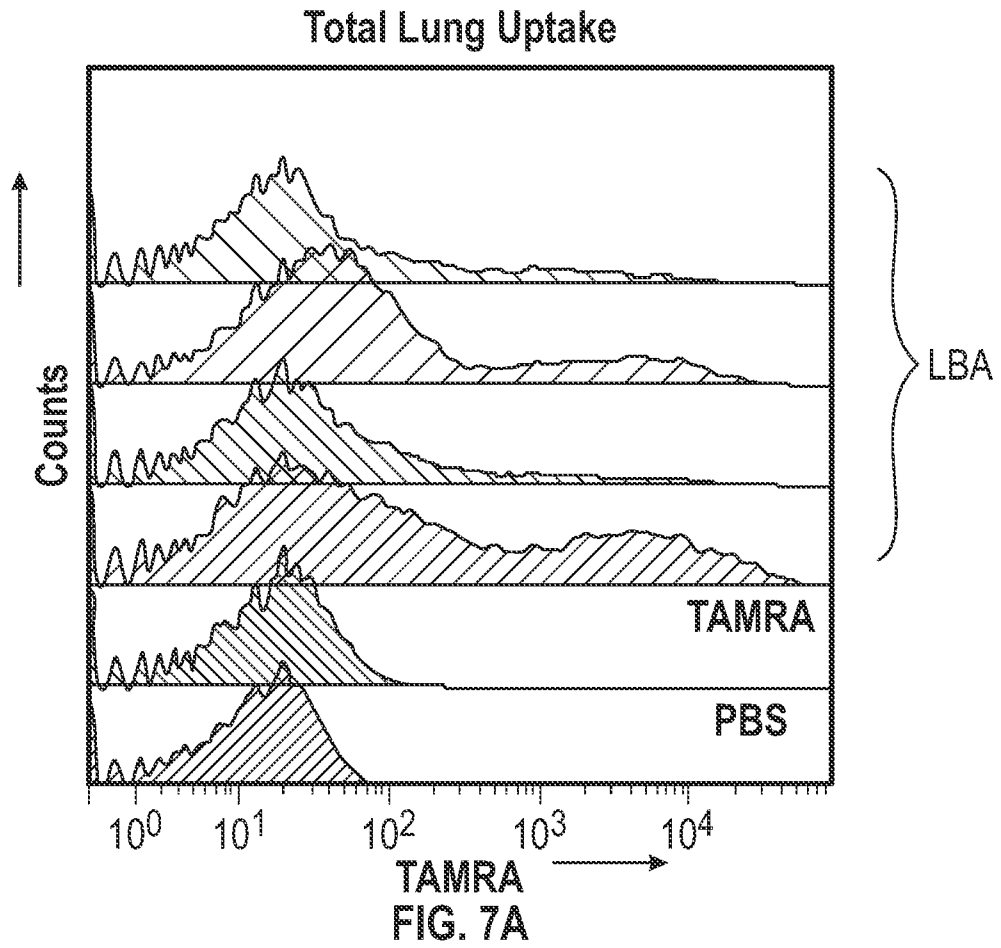


FIG. 6B



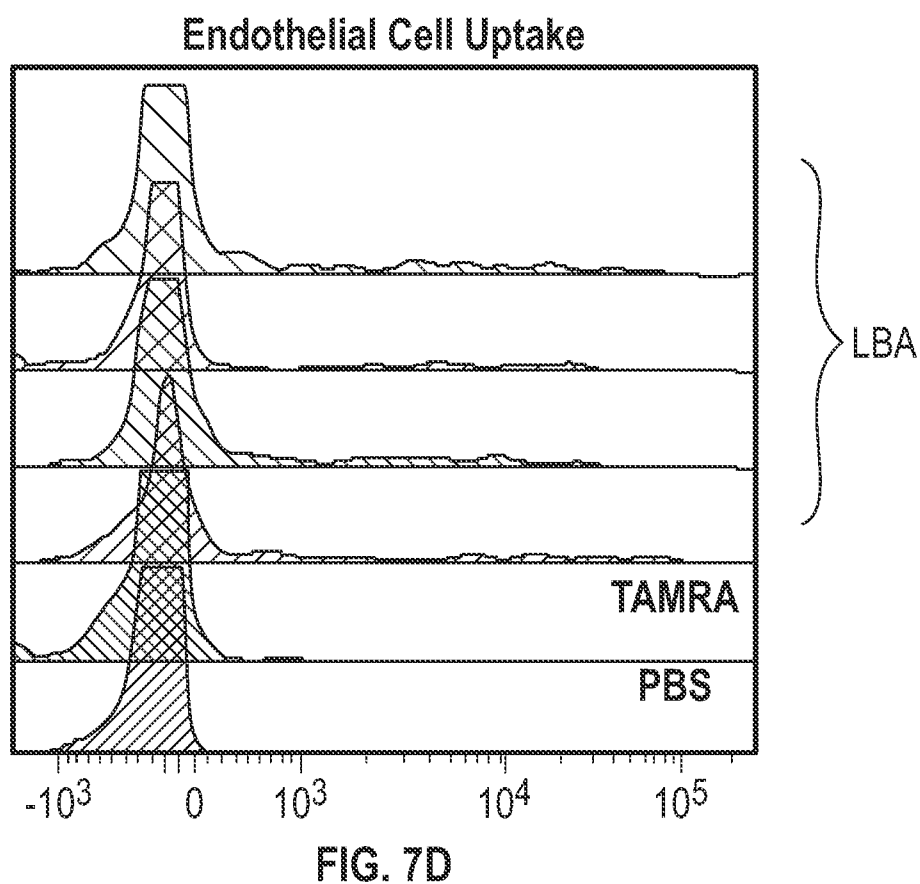
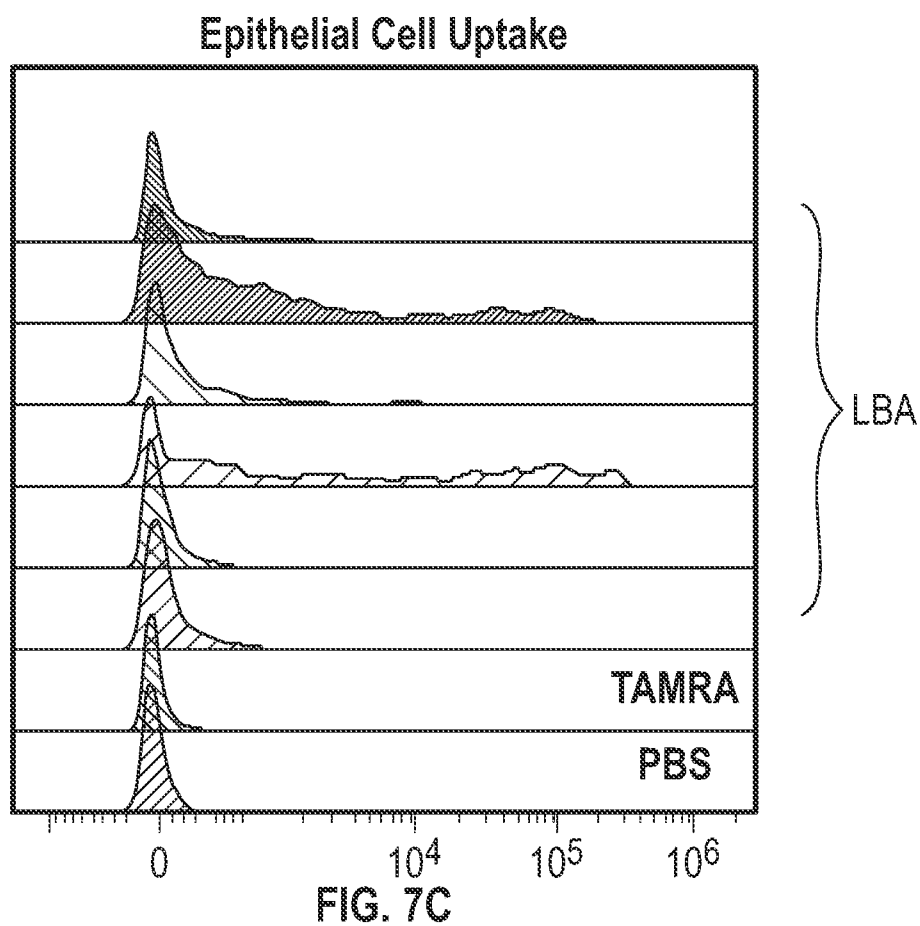
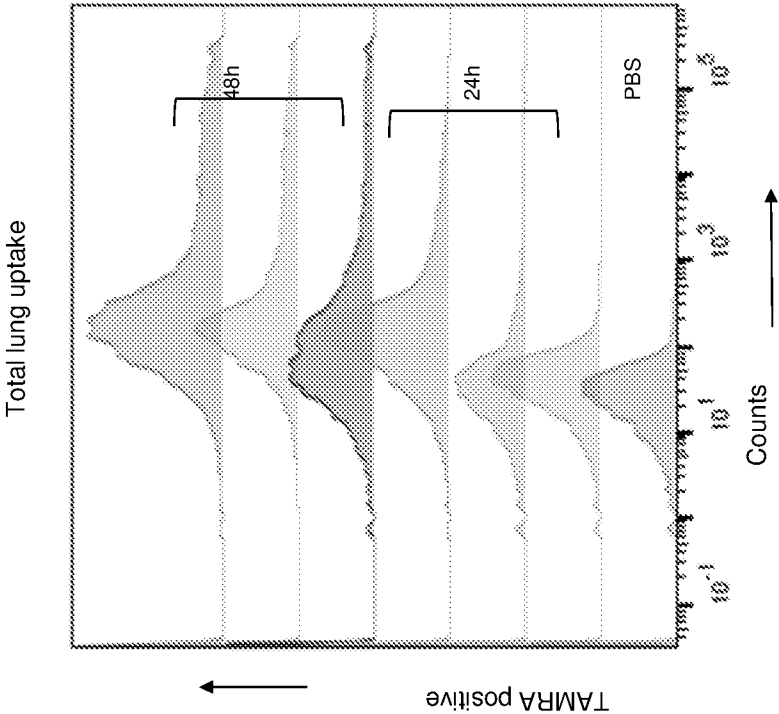


FIG. 8A



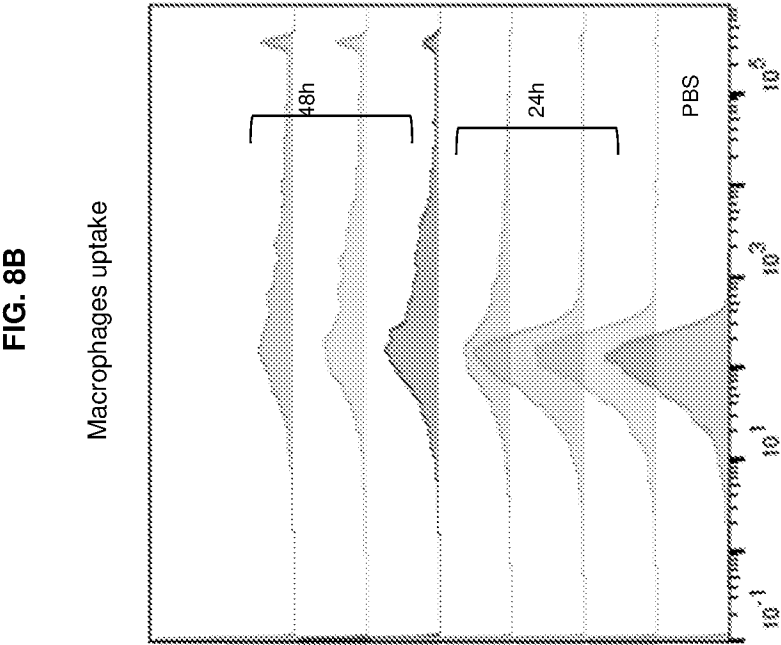


FIG. 8C

Epithelial cell uptake

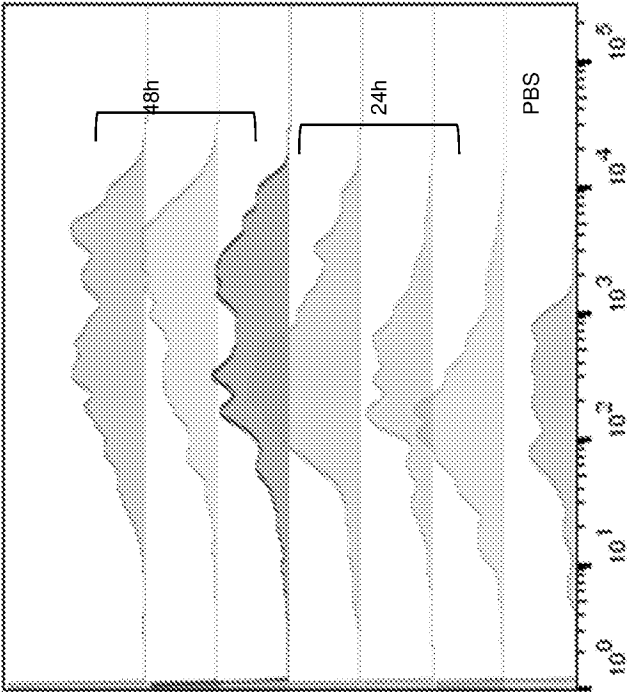
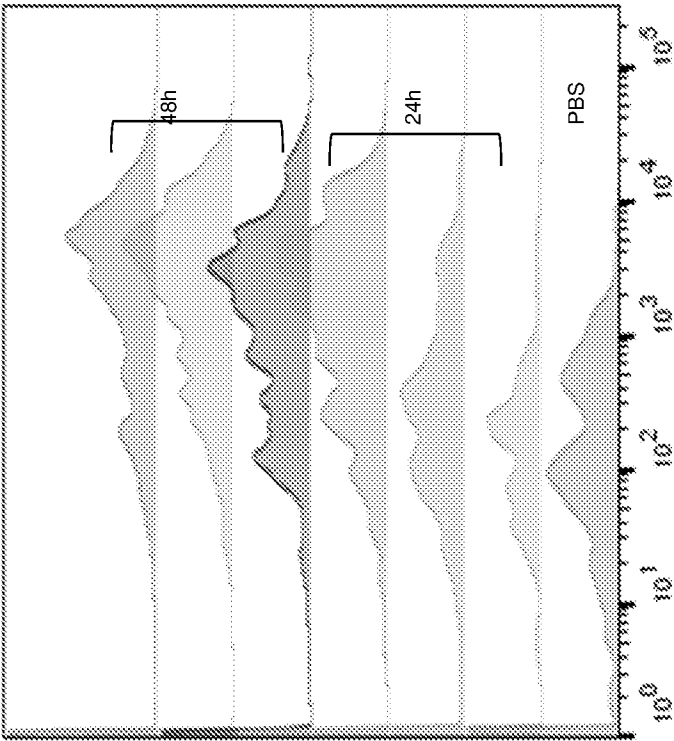


FIG. 8D
Endothelial cell uptake



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/032042

A. CLASSIFICATION OF SUBJECT MATTER

IPC: **C07H 3/04** (2024.01); **C07H 3/06** (2024.01); **A61K 31/7016** (2024.01); **A61K 31/702** (2024.01); **A61K 31/7028** (2024.01)
CPC: **C07H 3/04**; **C07H 3/06**; **A61K 31/7016**; **A61K 31/702**; **A61K 31/7028**

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)

See Search History Document

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

See Search History Document

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

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2012/0136042 A1 (MANOHARAN et al.) 31 May 2012 (31.05.2012) entire document	1
A	US 2015/0368642 A1 (HOFFMANN-LA ROCHE INC. et al.) 24 December 2015 (24.12.2015) entire document	1
A	US 9,173,951 B2 (NEKTAR THERAPEUTICS) 03 November 2015 (03.11.2015) entire document	1
A	US 2023/0010108 A1 (HANGZHOU DAC BIOTECH CO. LTD.) 12 January 2023 (12.01.2023) entire document	1
A	KUMAR et al., Multivalent Lactobionic Acid and N-Acetylgalactosamine-conjugated Peptide Nucleic Acids for Efficient in vivo Targeting of Hepatocytes, Advanced Healthcare Materials, Vol. 12, 20 January 2023 [retrieved on 09 August 2024]. Retrieved from the Internet: <URL: https://onlinelibrary.wiley.com/doi/epdf/10.1002/adhm.202202859 >. Pgs. 1-14	1



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

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

“D” document cited by the applicant in the international application

“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

17 August 2024 (17.08.2024)

Date of mailing of the international search report

15 October 2024 (15.10.2024)

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450, Alexandria, VA 22313-1450

Authorized officer

MATOS
TAINA

Facsimile No. 571-273-8300

Telephone No. 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/032042

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: **4-8, 10-15, 18-33**
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

Claims 1, 9, and 34 are objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because claims 1, 9, and 34 are indefinite for the following reason:

With regards to claim 1, the claim contains exemplary claim language (preferably), which renders the intended scope of the claim unclear. For the purposes of this written opinion, claim 1 is best interpreted without the exemplary claim language.

Claim 9 is not present in the claims.

With regards to claim 34, the claim cites its dependency from claim 1. However, claim 1 is lacking antecedent basis for "the genetic sequence/small molecule-carbohydrate conjugate" recited. For the purposes of this written opinion, claim 34 is best interpreted to be dependent on claim 16, which provides proper antecedent basis for "the genetic sequence/small molecule-carbohydrate conjugate."

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I+: claims 1-3 are drawn to carbohydrate conjugates of formula (IVa), an enantiomer, a diastereoisomer, a pharmaceutically acceptable salt, a hydrate, a solvate or a combination thereof.

Group II+: claims 16, 17, and 34 are drawn to compositions for pulmonary delivery, the composition comprising a genetic sequence/small molecule-carbohydrate conjugate of a formula A1, an enantiomer, a diastereoisomer, a pharmaceutically acceptable salt, a hydrate, a solvate or a combination thereof.

The first invention of Group I+ is restricted to a carbohydrate conjugate of formula (IVa), an enantiomer, a diastereoisomer, a pharmaceutically acceptable salt, a hydrate, a solvate or a combination thereof, wherein ACV is a pharmaceutically active agent, specifically wherein the ACV is a small molecule selected to be ivacaftor, R1 and R2 are each independently H, X1 is O, X2 is O, n1 is 1, G1 and G4 are each a direct bond, and CL is a carbohydrate ligand structure comprising 2 carbohydrate residues each derived from a monosaccharide, specifically N-acetylgalactosamine (GalNAc), where cc is 2. The first named invention has been selected based on the guidance set forth in section 10.54 of the PCT International Search and Preliminary Examination Guidelines. Specifically, the first named invention was selected based on the first listed element for each of

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

the variables presented in the claims (see claims 1, 10, 14). It is believed that claim 1 reads on this first named invention and thus this claim will be searched without fee to the extent that they read on the above embodiment.

An exemplary election of Group II+ is a composition for pulmonary delivery, the composition comprising a genetic sequence/small molecule-carbohydrate conjugate of a formula A1, an enantiomer, a diastereoisomer, a pharmaceutically acceptable salt, a hydrate, a solvate or a combination thereof, where the genetic sequence/small molecule-carbohydrate conjugate has the structure of LBA-TAMRA.

Applicant is invited to elect additional formula(e) for each additional compound to be searched in a specific combination by paying an additional fee for each set of election. Each additional elected formula(e) requires the selection of a single definition for each compound variable. An exemplary election would be a carbohydrate conjugate of formula (IVa), an enantiomer, a diastereoisomer, a pharmaceutically acceptable salt, a hydrate, a solvate or a combination thereof, wherein ACV is a pharmaceutically active agent, specifically wherein the ACV is a small molecule selected to be elxacaftor, R1 and R2 are each independently H, X1 is NR3 wherein R3 is H, X2 is O, n1 is 1, G1 and G4 are each a direct bond, and CL is a carbohydrate ligand structure comprising 2 carbohydrate residues each derived from a monosaccharide, specifically N-acetylgalactosamine (GalNAc), where cc is 2. Additional formula(e) will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined.

The inventions listed in Groups I+ and II+ do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The special technical features of Group I+, carbohydrate conjugates of formula (IVa), an enantiomer, a diastereoisomer, a pharmaceutically acceptable salt, a hydrate, a solvate or a combination thereof, are not present in Group II+; and the special technical features of Group II+, compositions for pulmonary delivery, the composition comprising a genetic sequence/small molecule-carbohydrate conjugate of a formula A1, an enantiomer, a diastereoisomer, a pharmaceutically acceptable salt, a hydrate, a solvate or a combination thereof, are not present in Group I+.

The Groups I+ and II+ formulae do not share a significant structural element requiring the selection of alternatives for the compound variables, ACV, R1, R2, X1, X2, n1, G1, G4, CL, SG, n2, and accordingly these groups lack unity a priori.

Additionally, even if Groups I+ and II+ were considered to share the technical features of a carbohydrate conjugate having the core structure of formula (IVa), an enantiomer, a diastereoisomer, a pharmaceutically acceptable salt, a hydrate, a solvate or a combination thereof; and a composition for pulmonary delivery, the composition comprising a genetic sequence/small molecule-carbohydrate conjugate having the core structure of a formula A1, an enantiomer, a diastereoisomer, a pharmaceutically acceptable salt, a hydrate, a solvate or a combination thereof, these shared technical features do not represent a contribution over the prior art as disclosed by the publication entitled "Multivalent Lactobionic Acid and N-Acetylgalactosamine-conjugated Peptide Nucleic Acids for Efficient in vivo Targeting of Hepatocytes" by Kumar et al. (hereinafter, "Kumar") and US 2012/0136042 A1 to Manoharan et al. (hereinafter, "Manoharan").

Kumar teaches a carbohydrate conjugate having the core structure of formula (IVa), an enantiomer, a diastereoisomer, a pharmaceutically acceptable salt, a hydrate, a solvate or a combination thereof (Pg. 5, Fig. 2A, see compounds (14) and (15)); and a genetic sequence/small molecule-carbohydrate conjugate having the core structure of a formula A1, an enantiomer, a diastereoisomer, a pharmaceutically acceptable salt, a hydrate, a solvate or a combination thereof (Pg. 5, Fig. 2A, see compounds (14) and (15)).

Manoharan teaches a composition for pulmonary delivery, the composition comprising a genetic sequence/small molecule-carbohydrate (Para. [0016], the invention provides an iRNA agent that is conjugated with at least one carbohydrate ligand, e.g., monosaccharide, disaccharide, trisaccharide, tetrasaccharide,

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/032042

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

oligosaccharide, polysaccharide; Para. [0497], microparticles can be formulated ... to be delivered by ... intrapulmonary).

The inventions listed in Groups I+ and II+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical feature.

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

Remark on Protest

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