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(71) Applicants *(for all designated States except US)*: **UNITED THERAPEUTICS CORPORATION** [US/US]; 1040 Spring Street, Silver Spring, MD 20910 (US). **UNIVERSITY OF OXFORD** [GB/GB]; South Parks Road, Oxford OX1 3QU (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **RAMSTEDT, Urban** [SE/US]; C/o United Therapeutics Corporation, 1040 Spring Street, Silver Spring, MD 20910 (US). **KLOSE, Brennan** [US/US]; C/o United Therapeutics Corporation, 1040 Sprng Street, Silver Spring, MD 20910 (US). **ZITZMANN, Nicole** [DE/GB]; 12 Oswestry Road, Oxford OX1 4TL (GB). **DWEK, Raymond, A.** [GB/GB]; Ambleside, Vernon Avenue, Oxford OX2 9AU (GB). **BUTTERS, Terry, D.** [GB/GB]; 1 Pine Close, Garsington, Oxford OX44 9BS (GB).

(74) Agents: **MAEBIUS, Stephen, B.** et al.; Foley & Lardner LLP, 3000 K Street NW, Suite 600, Washington, DC 20007 (US).

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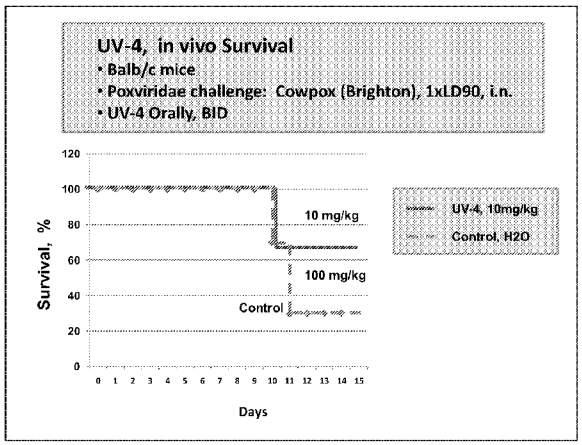
(54) **Title**: METHODS OF TREATING POXVIRAL INFECTIONS

Figure 5



(57) **Abstract**: Provided are methods of treating a disease or condition caused by or associated with a virus belonging to the Poxviridae family using iminosugars, such as DNJ derivatives.

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METHODS OF TREATING POXVIRAL INFECTIONS

RELATED APPLICATIONS

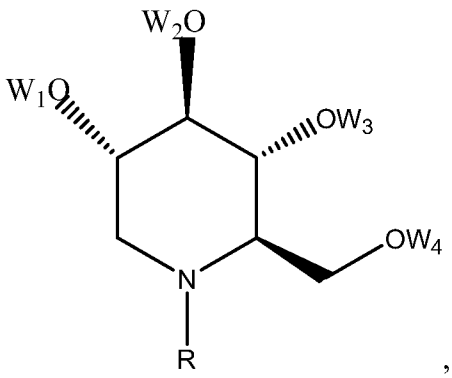
The present application claims priority to U.S. provisional application no. 61/272,252 filed September 4, 2009, which is incorporated herein by reference in its entirety.

FIELD

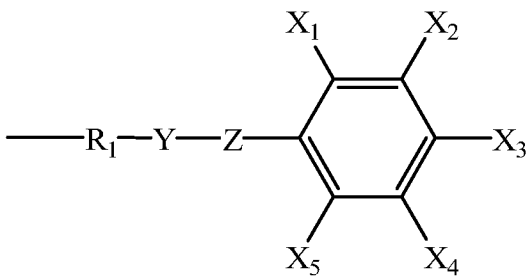
The present application relates to iminosugars and methods of treating viral infections with iminosugars and, in particular, to the use of iminosugars for treatment and/or prevention of viral infections caused by or associated with a virus belonging to the Poxviridae family.

SUMMARY

One embodiment is a method of treating or preventing a disease or condition caused by or associated with a virus belonging to the Poxviridae family, which method comprises administering to a subject in need thereof an effective amount of a compound of the formula,



, or a pharmaceutically acceptable salt thereof, wherein R is either selected from substituted or unsubstituted alkyl groups, substituted or unsubstituted cycloalkyl groups, substituted or unsubstituted aryl groups, or substituted or unsubstituted oxaalkyl groups; or wherein R is



R_1 is a substituted or unsubstituted alkyl group;

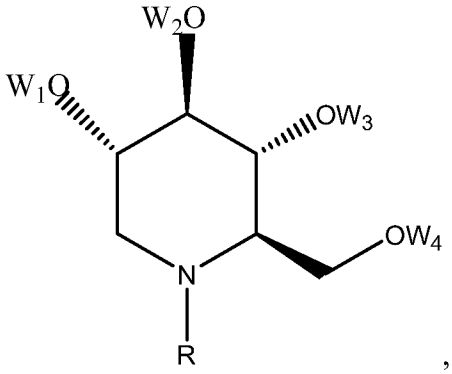
X_{1-5} are independently selected from H, NO_2, N_3, or NH_2;

Y is absent or is a substituted or unsubstituted C_1-alkyl group, other than carbonyl; and

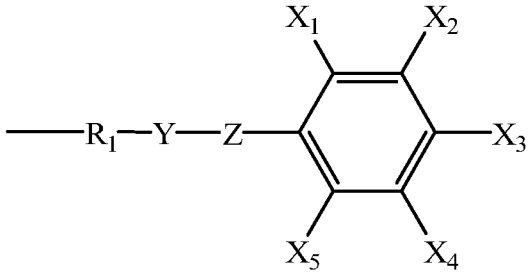
Z is selected from a bond or NH; provided that when Z is a bond, Y is absent, and provided that when Z is NH, Y is a substituted or unsubstituted C_1-alkyl group, other than carbonyl; and

wherein W_{1-4} are independently selected from hydrogen, substituted or unsubstituted alkyl groups, substituted or unsubstituted haloalkyl groups, substituted or unsubstituted alkanoyl groups, substituted or unsubstituted aroyl groups, or substituted or unsubstituted haloalkanoyl groups.

Another embodiment is a method of infectivity of a cell infected with a virus belonging to the Poxviridae family, which method comprises contacting a cell infected with a virus belonging to the Poxviridae family with an effective amount of a compound of the formula,



, or a pharmaceutically acceptable salt thereof, wherein R is either selected from substituted or unsubstituted alkyl groups, substituted or unsubstituted cycloalkyl groups, substituted or unsubstituted aryl groups, or substituted or unsubstituted oxaalkyl groups; or wherein R is



R_1 is a substituted or unsubstituted alkyl group;

X_{1-5} are independently selected from H, NO_2, N_3, or NH_2;

Y is absent or is a substituted or unsubstituted C_1-alkyl group, other than carbonyl; and

Z is selected from a bond or NH; provided that when Z is a bond, Y is absent, and provided that when Z is NH, Y is a substituted or unsubstituted C_1-alkyl group, other than carbonyl; and

wherein W_{1-4} are independently selected from hydrogen, substituted or unsubstituted alkyl groups, substituted or unsubstituted haloalkyl groups, substituted or unsubstituted alkanoyl groups, substituted or unsubstituted aroyl groups, or substituted or unsubstituted haloalkanoyl groups.

DRAWINGS

Figures 1(A)-(E) present chemical formulas of the following iminosugars: A) *N*-Butyl deoxynojirimycin (NB-DNJ, UV-1); B) *N*-Nonyl deoxynojirimycin (NN-DNJ, UV-2); C) *N*-(7-Oxadecyl)deoxynojirimycin (N7-O-DNJ, UV-3); D) *N*-(9-Methoxynonyl) deoxynojirimycin (UV-4); E) *N*-(*N*-{4'-azido-2'-nitrophenyl}-6-aminohexyl)deoxynojirimycin (UV-5).

Figure 2 is a synthesis scheme for NN-DNJ.

Figures 3A-D illustrate synthesis of N7-O-DNJ. In particular, Figure 3A shows a sequence of reactions leading to N7-O-DNJ; Figure 3B illustrates preparation of 6-propyloxy-1-hexanol; Figure 3C illustrates preparation of 6-propyloxy-1-hexanal; Figure 3D illustrates synthesis of N7-O-DNJ.

Figures 4A-C relate to synthesis of *N*-(9-Methoxynonyl) deoxynojirimycin. In particular, Figure 4A illustrates preparation of 9-methoxy-1-nonanol; Figure 4B illustrates preparation of 9-methoxy-1-nonanal; Figure 4C illustrates synthesis of *N*-(9-Methoxynonyl) deoxynojirimycin.

Figure 5 presents *in vivo* survival data for mice infected with cowpox virus.

Figure 6 presents *in vivo* safety data for UV-4 and UV-5.

DETAILED DESCRIPTION

Related Applications

The following patent documents, which are all incorporated herein by reference in their entirety, may be useful for understanding the present disclosure:

1) US patent no. 6,545,021;
2) US patent no. 6,809,803;
3) US patent no. 6,689,759;
4) US patent no. 6,465,487;
5) US patent no. 5,622,972;
6) US patent application no. 12/656,992 filed February 22, 2010;
7) US patent application no. 12/656,993 filed February 22, 2010;
8) US patent application no. 12/813,882 filed June 11, 2010;
9) US patent provisional application no. 61/282,507 filed February 22, 2010;
10) US patent provisional application no. 61/272,252 filed September 4, 2009;
11) US provisional application no. 61/272,253 filed September 4, 2009;
12) US provisional application no. 61/272,254 filed September 4, 2009;
13) US provisional application no. 61/282,508 filed February 22, 2010;
14) US provisional application no. 61/353,935 filed June 11, 2010.

Definition of terms

Unless otherwise specified, "a" or "an" means "one or more."

As used herein, the term "viral infection" describes a diseased state, in which a virus invades a healthy cell, uses the cell's reproductive machinery to multiply or replicate and ultimately lyse the cell resulting in cell death, release of viral particles and the infection of other cells by the newly produced progeny viruses. Latent infection by certain viruses is also a possible result of viral infection.

As used herein, the term "treating or preventing viral infection" means to inhibit the replication of the particular virus, to inhibit viral transmission, or to prevent the virus from establishing itself in its host, and to ameliorate or alleviate the symptoms of the disease caused by the viral infection. The treatment is considered therapeutic if there is a reduction in viral load, decrease in mortality and/or morbidity.

IC50 or IC90 (inhibitory concentration 50 or 90) is a concentration of a therapeutic agent, such as an iminosugar, used to achieve 50% or 90% reduction of viral load, respectively.

Disclosure

The present inventors discovered that certain iminosugars, such as deoxynojirimycin derivatives, may be effective against viruses belonging to the Poxviridae family.

In particular, such iminosugars may be useful for treating or preventing a disease or condition caused by or associated with a virus belonging to the Poxviridae family.

The Poxviridae family includes the Chordopoxviridae subfamily and the Entomopoxviridae subfamily. The Chordopoxviridae subfamily includes Orthopox genus, Parapox genus; Aviropox genus; Capripoxvirus genus; Leporipoxvirus genus; Suipoxvirus genus; Molluscipoxvirus genus and Yatapox genus. The Entomopoxviridae subfamily includes Entomopoxviruses A, B and C. Viruses of orthopox, parapox, yatapox and molluscipox genera may infect humans.

Viruses belonging to the Orthopoxvirus genus of the Poxviridae family, i.e., orthopoxviruses, include Buffalopox virus; Camelpox virus; Cowpox virus; Ectromelia virus; Monkeypox virus; Rabbitpox virus; Raccoonpox virus; Sealpox virus; Skunkpox virus; Taterapox virus; Uasin Gishu disease virus; Vaccinia virus; Variola virus; and Volepox virus.

Diseases caused by or associated with orthopoxviruses include Buffalopox; Camelpox; Cowpox; Mousepox (cause by Ectromelia virus); Monkeypox; Rabbitpox, also known as Green Rabbit Syndrome; Raccoonpox; Sealpox; Skunkpox; Taterapox; Uasin Gishu disease; Smallpox; and Volepox.

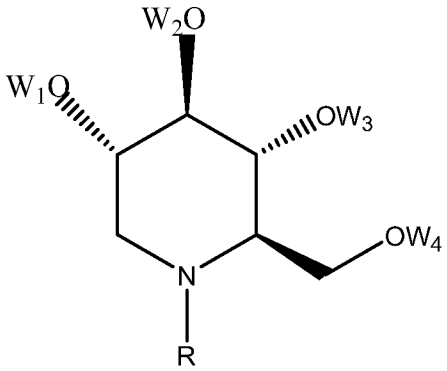
Viruses belonging to the Parapox genus of the Poxviridae family, i.e. parapoxviruses, include orf virus, pseudocowpox and bovine papular stomatitis virus.

Diseases caused by or associated with parapoxviruses include orf, pseudocowpox and bovine papular stomatitis.

Viruses belonging to the Yatapox genus of the Poxviridae family, i.e. yatapoxviruses, include tanapox virus and yaba monkey tumor virus.

Molluscum contagiosum virus is an example of a molluscipox virus, i.e. a virus belonging to the Molluscipox genus of the Poxviridae family.

In many embodiments, the iminosugar may be N-substituted deoxynojirimycin. In some embodiments, as the N-substituted deoxynojirimycin may be a compound of the following formula:



where W_{1-4} are independently selected from hydrogen, substituted or unsubstituted alkyl groups, substituted or unsubstituted haloalkyl groups, substituted or unsubstituted alkanoyl groups, substituted or unsubstituted aroyl groups, or substituted or unsubstituted haloalkanoyl groups.

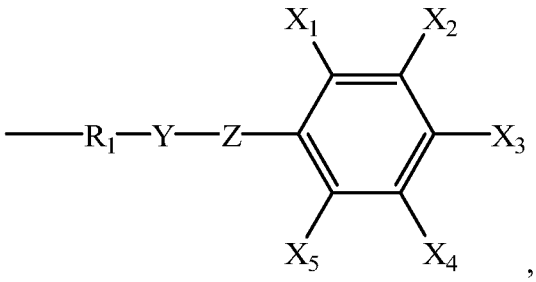
In some embodiments, R may be selected from substituted or unsubstituted alkyl groups, substituted or unsubstituted cycloalkyl groups, substituted or unsubstituted aryl groups, or substituted or unsubstituted oxaalkyl groups.

In some embodiments, R may be substituted or unsubstituted alkyl groups and/or substituted or unsubstituted oxaalkyl groups comprise from 1 to 16 carbon atoms, from 4 to 12 carbon atoms or from 8 to 10 carbon atoms. The term "oxaalkyl" refers to an alkyl derivative, which may contain from 1 to 5 or from 1 to 3 or from 1 to 2 oxygen atoms. The term "oxaalkyl" includes hydroxyterminated and methoxyterminated alkyl derivatives.

In some embodiments, R may be selected from, but is not limited to $-(CH_2)_6OCH_3$, $-(CH_2)_6OCH_2CH_3$, $-(CH_2)_6O(CH_2)_2CH_3$, $-(CH_2)_6O(CH_2)_3CH_3$, $-(CH_2)_2O(CH_2)_5CH_3$, $-(CH_2)_2O(CH_2)_6CH_3$,;$-(CH_2)_2O(CH_2)_7CH_3$; $-(CH_2)_9$-OH; $-(CH_2)_9OCH_3$.

In some embodiments, R may be branched or unbranched, substituted or unsubstituted alkyl group. In certain embodiments, the alkyl group may be a long chain alkyl group, which may be C6-C20 alkyl group; C8-C16 alkyl group; or C8-C10 alkyl group. In some embodiments, R may be a long chain oxaalkyl group, i.e. a long chain alkyl group, which may contain from 1 to 5 or from 1 to 3 or from 1 to 2 oxygen atoms.

In some embodiments, R may have the following formula



, where R_1 is a substituted or unsubstituted alkyl group;

X_{1-5} are independently selected from H, NO_2, N_3, or NH_2;

Y is absent or is a substituted or unsubstituted C_1-alkyl group, other than carbonyl; and

Z is selected from a bond or NH; provided that when Z is a bond, Y is absent, and provided that when Z is NH, Y is a substituted or unsubstituted C_1-alkyl group, other than carbonyl.

In some embodiments, Z is NH and R_1-Y is a substituted or unsubstituted alkyl group, such as C2-C20 alkyl group or C4-C12 alkyl group or C4-C10 alkyl group.

In some embodiments, X_1 is NO_2 and X_3 is N_3. In some embodiments, each of X_2, X_4 and X_5 is hydrogen.

In some embodiments, the iminosugar may be a DNJ derivative disclosed in U.S. Patent application publication no. 2007/0275998, which is incorporated herein by reference.

In some embodiments, the iminosugar may be one of the compounds presented in Figure 1.

Methods of synthesizing deoxynojirimycin derivatives are disclosed, for example, in U.S. Patent Nos. 5,622,972, 5,200,523, 5,043,273, 4,994,572, 4,246,345, 4,266,025, 4,405,714, and 4,806,650 and U.S. Patent application publication no. 2007/0275998, which are all incorporated herein by reference.

In some embodiments, the iminosugar may be in a form of a salt derived from an inorganic or organic acid. Pharmaceutically acceptable salts and methods for preparing salt forms are disclosed, for example, in Berge et al. *(J. Pharm. Sci.* 66:1-18, 1977). Examples of appropriate salts include but are not limited to the following salts: acetate, adipate, alginate, citrate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, camphorate, camphorsulfonate, digluconate, cyclopentanepropionate, dodecylsulfate, ethanesulfonate, glucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, fumarate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, nicotinate, 2-naphthalenesulfonate, oxalate, palmoate, pectinate,

persulfate, 3-phenylpropionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, tosylate, mesylate, and undecanoate.

In some embodiments, the iminosugar may also used in a form of a prodrug. Prodrugs of DNJ derivatives, such as the 6-phosphorylated DNJ derivatives, are disclosed in U.S. Patents nos. 5,043,273 and 5,103,008.

In some embodiments, the iminosugar may be used as a part of a composition, which further comprises a pharmaceutically acceptable carrier and/ or a component useful for delivering the composition to an animal. Numerous pharmaceutically acceptable carriers useful for delivering the compositions to a human and components useful for delivering the composition to other animals such as cattle are known in the art. Addition of such carriers and components to the composition of the invention is well within the level of ordinary skill in the art.

In some embodiments, the pharmaceutical composition may consist essentially of *N*-substituted deoxynojirimycin, which may mean that the *N*-substituted deoxynojirimycin is the only active ingredient in the composition.

Yet in some embodiments, *N*-substituted deoxynojirimycin may be administered with one or more additional antiviral compounds.

In some embodiments, the iminosugar may be used in a liposome composition, such as those disclosed in US publications nos. 2008/0138351 and 2009/0252785 as well as in US application No. 12/732630 filed March 26, 2010.

The iminosugar, such as a DNJ derivative, may be administered to a cell or an animal affected by a virus. The iminosugar may inhibit morphogenesis of the virus, or it may treat the individual. The treatment may reduce, abate, or diminish the virus infection in the animal.

Animals that may be infected with poxviruses include mammals including bovids, such as buffalos, sheep, goats and cattle (cows); camels; rodents, such as mice, voles, and gerbils; leporids, such as rabbits and hares; raccoons; seals; skunks; equines, including horses; primates, including monkeys and humans.

The amount of iminosugar administered to an animal or to an animal cell to the methods of the invention may be an amount effective to inhibit the morphogenesis of a poxvirus from the cell. The term "inhibit" as used herein may refer to the detectable reduction and/or

elimination of a biological activity exhibited in the absence of the iminosugar. The term "effective amount" may refer to that amount of the iminosugar necessary to achieve the indicated effect. The term "treatment" as used herein may refer to reducing or alleviating symptoms in a subject, preventing symptoms from worsening or progressing, inhibition or elimination of the causative agent, or prevention of the infection or disorder related to the poxvirus in a subject who is free therefrom.

Thus, for example, treatment of the disease caused by or associated with a virus may include destruction of the infecting agent, inhibition of or interference with its growth or maturation, and neutralization of its pathological effects. The amount of the iminosugar which may be administered to the cell or animal is preferably an amount that does not induce any toxic effects which outweigh the advantages which accompany its administration.

Actual dosage levels of active ingredients in the pharmaceutical compositions may vary so as to administer an amount of the active compound(s) that is effective to achieve the desired therapeutic response for a particular patient.

The selected dose level may depend on the activity of the iminosugar, the route of administration, the severity of the condition being treated, and the condition and prior medical history of the patient being treated. However, it is within the skill of the art to start doses of the compound(s) at levels lower than required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved. If desired, the effective daily dose may be divided into multiple doses for purposes of administration, for example, two to four doses per day. It will be understood, however, that the specific dose level for any particular patient may depend on a variety of factors, including the body weight, general health, diet, time and route of administration and combination with other therapeutic agents and the severity of the condition or disease being treated. In some embodiments, the adult human daily dosage may range from between about one microgram to about one gram, or from between about 10 mg and 100 mg, of the iminosugar per 10 kilogram body weight. In some embodiments, a total daily dose may be from 0.1 mg/kg body weight to 100 mg/kg body weight or from 1 mg/kg body weight to 60 mg/kg body weight or from 2 mg/kg body weight to 50 mg/kg body weight or from 3 mg/kg body weight to 30 mg/kg body weight. The daily dose may be administered over one or more administering events over day. For example, in some embodiments, the daily dose may be distributed over two (BID)

administering events per day, three administering events per day (TID) or four administering events (QID). In certain embodiments, a single administering event dose ranging from 1 mg/kg body weight to 10 mg/kg body weight may be administered BID or TID to a human making a total daily dose from 2 mg/kg body weight to 20 mg/kg body weight or from 3 mg/kg body weight to 30 mg/kg body weight. Of course, the amount of the iminosugar which should be administered to a cell or an animal may depend upon numerous factors well understood by one of skill in the art, such as the molecular weight of the iminosugar and the route of administration.

Pharmaceutical compositions that are useful in the methods of the invention may be administered systemically in oral solid formulations, ophthalmic, suppository, aerosol, topical or other similar formulations. For example, it may be in the physical form of a powder, tablet, capsule, lozenge, gel, solution, suspension, syrup, or the like. In addition to the iminosugar, such pharmaceutical compositions may contain pharmaceutically-acceptable carriers and other ingredients known to enhance and facilitate drug administration. Other possible formulations, such as nanoparticles, liposomes, resealed erythrocytes, and immunologically based systems may also be used to administer the iminosugar. Such pharmaceutical compositions may be administered by a number of routes. The term "parenteral" used herein includes subcutaneous, intravenous, intraarterial, intrathecal, and injection and infusion techniques, without limitation. By way of example, the pharmaceutical compositions may be administered orally, topically, parenterally, systemically, or by a pulmonary route.

These compositions may be administered in a single dose or in multiple doses which are administered at different times. Because the inhibitory effect of the composition upon a poxvirus may persist, the dosing regimen may be adjusted such that virus propagation is retarded while the host cell is minimally effected. By way of example, an animal may be administered a dose of the composition of the invention once per week, whereby virus propagation is retarded for the entire week, while host cell functions are inhibited only for a short period once per week.

Embodiments described herein are further illustrated by, though in no way limited to, the following working examples.

Working Examples

1. Synthesis of N-Nonyl DNJ

Table 1. Materials for NN-DNJ synthesis

Name	Amount
DNJ	500 mg
Nonanal	530 mg
Ethanol	100 mL
AcOH	0.5 mL
Pd/C	500 mg

Procedure: A 50-mL, one-necked, round-bottom flask equipped with a magnetic stirrer was charged with DNJ (500 mg), ethanol (100 mL), nonanal (530 mg), and acetic acid (0.5 mL) at room temperature. The reaction mixture was heated to 40-45 °C and stirred for 30-40 minutes under nitrogen. The reaction mixture was cooled to ambient temperature and Pd/C was added. The reaction flask was evacuated and replaced by hydrogen gas in a balloon. This process was repeated three times. Finally, the reaction mixture was stirred at ambient temperature overnight. The progress of reaction was monitored by TLC (Note 1). The reaction mixture was filtered through a pad of Celite and washed with ethanol. The filtrate was concentrated *in vacuo* to get the crude product. The crude product was purified by column chromatography (230-400 mesh silica gel). A solvent gradient of methanol in dichloromethane (10-25%) was used to elute the product from the column. All fractions containing the desired product were combined, and concentrated *in vacuo* to give the pure product (420mg). Completion of the reaction was monitored by thin layer chromatography (TLC) using a thin layer silica gel plate; eluent; methanol : dichloromethane = 1:2

2. Synthesis of N-7-Oxadecyl DNJ

2a. Synthesis of 6-propyloxy-1-hexanol

Table 2. Materials for synthesis of 6-propyloxy-1-hexanol

Name	Amount
1,6-hexanediol	6.00 g
1-Iodopropane	8.63 g
Potassium tert-butoxide	5.413 mg
THF	140 mL

Procedure: a 500-mL, one-necked, round-bottom flask equipped with a magnetic stirrer was charged with 1,6-hexanediol (6.00 g), potassium tert-butoxide (5.413 g) at room temperature. The reaction mixture was stirred for one hour, and then 1-iodopropane (8.63 g) was added. The reaction mixture was heated to 70-80 °C and stirred overnight. The progress of reaction was monitored by TLC (Note 1). After completion of the reaction, water was added to the reaction mixture, and extracted with ethyl acetate (2 x 100 mL). The combined organic layers were concentrated *in vacuo* to get the crude product. The crude product was dissolved in dichloromethane and washed with water, and then brine, dried over sodium sulfate. The organic layer was concentrated *in vacuo* to get the crude product. The crude product was purified by column chromatography using 230-400 mesh silica gel. A solvent gradient of ethyl acetate in hexanes (10-45%) was used to elute the product from the column. All fractions containing the desired pure product were combined and concentrated *in vacuo* to give pure 6-propyloxy-1-hexanol (lot D-1029-048, 1.9 g, 25%) Completion of the reaction was monitored by thin layer chromatography (TLC); (eluent: 60% ethyl acetate in hexanes).

2b. Preparation of 6-propyloxy-1-hexanal

Table 3. Materials for preparation of 6-propyloxy-1-hexanal

Name	Amount
6-Propyloxy-1-hexanol	1.00 g
PDC	4.70 g
Celite	1.00 g
NaOAc	100 mg
CH_2Cl_2	10 mL

Procedure: a 50-mL, one-necked, round-bottom flask equipped with a magnetic stirrer was charged with 6-propyloxy-1-hexanol (1.0 g), PDC (4.7 g), dichloromethane (10 mL), Celite (1.0 g), and sodium acetate (100 mg). The reaction mixture was stirred at room temperature under nitrogen for 5 minutes. PDC (4.70 g) was added to the reaction mixture, and stirred overnight. The progress of reaction was monitored by TLC (Note 1). After completion of the reaction, the reaction mixture was directly loaded on the column (230-400 mesh silica gel). A solvent gradient of dichloromethane in ethyl acetate (10-20%) was used to elute the product from the column. All fractions containing the desired pure product were combined and concentrated *in vacuo* to give pure 6-propyloxy-1-hexanal (lot D-1029-050, 710 mg, 71%). Completion of the reaction was monitored by thin layer chromatography (TLC); (eluent: 60% ethyl acetate in hexanes).

2c Synthesis of N-7-Oxadecyl-DNJ

Table 4. Materials for Synthesis of N-7-Oxadecyl-DNJ

Name	Amount
DNJ	500 mg
6-Propyloxy-1-hexanal	585 mg
Pd/C	125 mg
Ethanol	15 mL
Acetic acid	mL

Procedure: a 50-mL, one-necked, round-bottom flask equipped with a magnetic stirrer was charged with DNJ (500 mg), ethanol (15 mL), 6-propyloxy-1-hexanal (585 mg), and acetic acid (0.1mL) t room temperature. The reaction mixture was heated to 40-45 °C and stirred for 30-40 minutes under nitrogen. The reaction mixture was cooled to ambient temperature and Pd/C was added. The reaction flask was evacuated and replaced by hydrogen gas in a balloon. This process was repeated three times. Finally, the reaction mixture was stirred at ambient temperature overnight. The progress of reaction was monitored by TLC (Note 1). The reaction mixture was filtered through a pad of Celite and washed with ethanol. The filtrate was concentrated *in vacuo* to get the crude product. The crude product was purified by column chromatography (230-400 mesh silica gel). A solvent gradient of methanol in

dichloromethane (10-40%) was used to elute the product from the column. All fractions containing the desired product were combined, and concentrated *in vacuo* to give the pure product. (Lot: D-1029-052 (840 mg). Completion of the reaction was monitored by thin layer chromatography (TLC); (eluent: 50% methanol in dichloromethane).

3. Synthesis of N-(9-methoxy)-nonyl DNJ

3a Preparation of 9-methoxy-1-nonanol

Table 5. Materials for preparation of 9-methoxy-1-nonanol

Name	Amount
1,9-nonanediol	10.0 g
Dimethyl sulfate	41.39 g
Sodium hydroxide	5.0g
DMSO	100 mL

Procedure: a 500-mL, one-necked, round-bottom flask equipped with a magnetic stirrer and stir bar was charged with 1,9-nonanediol (10.00 g, 62.3 mmol) in dimethyl sulfoxide (100 mL) and H_2O (100 mL). To this was added slowly a solution of sodium hydroxide (5.0 g, 125.0 mmol) in H_2O (10 mL) at room temperature. During addition of sodium hydroxide the reaction mixture generated heat and the temperature rose to ~40 °C. The mixture was stirred for one hour, and then dimethyl sulfate (16.52 g, 131 mmol) was added in four portions while maintaining the temperature of the reaction mixture at ~ 40^0C. The reaction mixture was stirred at room temperature overnight. Progress of the reaction was monitored by TLC (Note 1). TLC monitoring indicated that the reaction was 25 % conversion. At this stage additional dimethyl sulfate (24.78g, 196.44 mmol) was added and the resulting mixture was stirred at room temperature for an additional 24 h. After completion of the reaction, sodium hydroxide (10% solution in water) was added to the reaction mixture to adjust the pH of the solution to 11-13. The mixture was stirred at room temperature for 2 h and extracted with dichloromethane (3 x 100 mL). The combined organic layers were washed with H_2O (200 mL), brine (150 mL), dried over anhydrous sodium sulfate (20 g), filtered and concentrated *in*

vacuo to obtain a crude product (14 g). The crude product was purified by column chromatography using 250-400 mesh silica gel. A solvent gradient of ethyl acetate in hexanes (10-50%) was used to elute the product from the column. All fractions containing the desired pure product were combined and concentrated *in vacuo* to give pure 9-methoxy-1-nonanol (lot D-1027-155, 2.38 g, 21.9 %). Completion of the reaction was monitored by thin layer chromatography (TLC) using a thin layer silica gel plate; eluent: 60% ethyl acetate in hexanes.

3b Preparation of 9-methoxy-1-nonanal

Table 6. Materials for preparation of 9-methoxy-1-nonanal

Name	Amount
9-methoxy-1-nonanol	1.0 g
PDC	4.7 g
Molecular sieves, 3A	1.0 g
NaOAc	0.1g
CH_2Cl_2	10 mL

Procedure: a 50-mL, one-necked, round-bottom flask equipped with a magnetic stirrer and stir bar was charged with 9-methoxy-nonanol (1.0 g, 5.9 mmol), dichloromethane (10 mL), molecular sieves (1.0 g, 3A), sodium acetate (0.1 g) at room temperature. The reaction mixture was stirred at room temperature under nitrogen for 5 minutes. The reaction mixture was charged with pyridinium dichromate (4.7 g, 12.5 mmol) and stirred overnight. The progress of reaction was monitored by TLC (Note 1). After completion of the reaction, the reaction mixture was filtered through a bed of silica gel (~15 g). The filtrate was evaporated in *vacuo* to obtain a crude compound. This was purified by column chromatography using silica gel column (250-400 mesh, 40 g). A solvent gradient of ethyl acetate in hexane (10-50%) was used to elute the product from the column. All fractions containing the desired pure product were combined and concentrated *in vacuo* to give pure 9-methoxy-nonanal (lot D-1027-156, 553 mg, 54.4%). Completion of the reaction was monitored by thin layer chromatography (TLC) using a thin layer silica gel plate; eluent: 60% ethyl acetate in hexanes.

3c Synthesis of N-(9-methoxy)-nonyl DNJ

Table 7. Materials for synthesis of N-(9-methoxy)-nonyl DNJ

Name	Amount
DNJ	300 mg
9-methoxy-1-nonanal	476 mg
Pd/C	200 mg
Ethanol	20 mL

Procedure: a 50-mL, two-necked, round-bottom flask equipped with magnetic stirrer and a stir bar was charged with DNJ (300 mg, 1.84 mmol), ethanol (20 mL), 9-methoxy-1-nonanal (476 mg, 2.76 mmol) at room temperature. The reaction mixture was stirred for 5-10 minutes under nitrogen and Pd/C was added at room temperature. The reaction mixture was evacuated and was replaced by hydrogen gas using a balloon. This process was repeated three times and then reaction mixture was stirred under atmospheric hydrogen at room temperature. The progress of reaction was monitored by TLC (Note 1). The reaction mixture was filtered through a bed of Celite and was washed with ethanol (20 mL). The filtrate was concentrated *in vacuo* to get a crude product. The crude product was purified by column chromatography using 250-400 mesh silica gel (20 g). A solvent gradient of methanol in ethyl acetate (5-25%) was used to elute the product from the column. All fractions containing the desired pure product were combined, and concentrated *in vacuo* to give an off white solid. The solid was triturated in ethyl acetate (20 mL), filtered and dried in high vacuum to give a white solid [lot: D-1027-158 (165.3 mg, 28.1%). Completion of the reaction was monitored by thin layer chromatography (TLC) using a thin layer silica gel plate; eluent: 50% methanol in dichloromethane.

4. Effects of iminosugars against Vaccinia Virus

Table 7 provides data for inhibition of infectivity of Vaccinia virus for NB-DNJ (UV-1), NN-DNJ (UV-2), N7-O-DNJ (UV-3), N9-DNJ (UV-4) and NAP-DNJ (UV-5).

Table 7.

Compound	IC50, μM
UV-1	90
UV-2	21
UV-3	7
UV-4	59
UV-5	3

Procedure. The compounds were screened for inhibition of generation of infectious virus was conducted on the UV compounds at concentrations from 4 μM up to 250 μM. The orthopoxvirus Vaccinia NYCBOH strain was evaluated for virus inhibition. BSC-40 cells (vervet monkey kidney epithelial cell line) obtained from American Type Culture Collection (ATCC, Manassas, Virginia). Cells were cultured in lx modified Eagle medium (MEM, Gibco), supplemented with 5% fetal bovine serum, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin in cell culture treated 24-well flat bottom plates at 37°C in a 5% CO2 incubator for 24 hr or until 80% confluent prior to assay. Cells were pretreated with compounds in a final concentration of 0.5% DMSO for 1 hr followed addition of virus inoculums in EMEM with 5% FBS. Three days later virus containing supernatants were collected and 10 fold dilutions of virus-containing supernatants was done in a virus plaque assay. To titer, 12-well plates with 80% confluent BSC-40 cells in growth medium were used. Viral supernatant were diluted from 10^{-3} to 10^{-8} and added to the cells and incubated at 37°C for 1 hour with shaking every 5-10 minutes. Viral infection medium were aspirated and replace with 1mL pre-warmed 2% low-melt agarose mixed 1:1 with 2X MEM (5% fetal calf serum final concentration) and incubated at 37°C, 5% CO_2 for 2 days followed by plaque visualization by neutral red staining.

EXAMPLE 5

The study assessed the efficacy of the iminosugar compound, UV-4, in promoting survival of mice challenged with Cowpox Brighton. This compound was previously tested in both in vitro (CC50 of 125 to >2,000uM) and in vivo (no weight loss or adverse effects observed in multiple mouse studies) and shown it possesses low toxicity. In this study, the compound was administered as a free drug dissolved in water. The UV-4 compound was given by the oral route (2x per day intragastric via oral gavage - IG) for a total number of 10 days after the

start of the compound dosing. Study animals were infected intranasally with cowpox brighton with ~1 LD90 (1.00e6 pfu/mouse) 1 hour before the first UV-4 dose.

Methods:

Infection: 4-6 week old female BALB/C mice were anesthetized with isofluorene prior to intranasal inoculation with 100uL Cowpox Brighton (Where did you obtain this strain? Is it publically available?) at a concentration of 1xLD90.
Dosing: 2X per day mice (n=10) were orally gavaged with 100ul of the compound dilution (prepared in H2O). Treatments lasted for 10 days.

Results:

Table 8.

Days post infection.	Control + H_2O, %	UV-4 0.2mg, %
0	100	100
10	70	100
11	30	70

Figure 5 shows survival data for mice that were infected with a 1xLD90 dose of cowpox brighton and dosed 3x per day for 10 days with either water (control group) or UV-4 (treated group). Table 8 shows a percentage of surviving mice in a) the control group treated with water and b) the group treated with UV-4 on days indicated in the left column. Each of the control and treated groups included 10 mice.
Kaplan-Meier analysis of the control and UV-4 treated groups. Log-rank (Mantel Cox) Analysis indicating p values between the groups. A p value of <0.05 indicates significance. Mice P-value for UV-4 0.2 mg is 0.046.

EXAMPLE 6

Iminosugar Safety Study

Methods and Discussion: BALB/c and C57/Bl/6 mice were given oral suspensions of UV-1, UV-4, UV-5, twice a day for seven days, in 100ul per mouse at 100 and 10 mg/kg (2mg and 0.2 mg/mouse, respectively) 8 hours apart for 7 days, and then monitored for weight loss and general health. After seven days of treatment, the mice did not show any significant signs of weight loss compared to the "vehicle only" control. The results of these experiments are in Figure 6.

When the BALB/c mice were treated with UV-5 at the highest concentration, they displayed signs of diarrhea, red urine, and a ruffled appearance although they did not show signs of weight loss. The C57/Bl/6 mice displayed these same symptoms but without the ruffled look. These symptoms promptly ceased when treatment was done, and by day 11 (day 4 post compound treatment) the BALB/c mice in these groups looked very healthy.

Conclusions: These compounds have shown to be relatively non-toxic in this mouse model and these concentrations of compound are deemed safe.

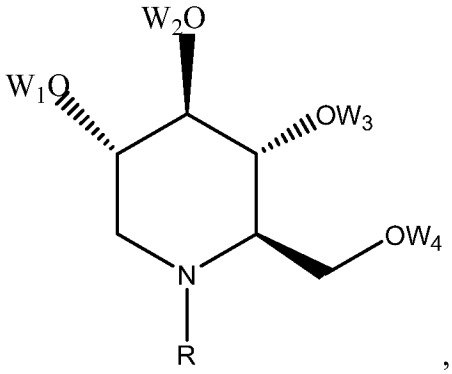
* * *

Although the foregoing refers to particular preferred embodiments, it will be understood that the present invention is not so limited. It will occur to those of ordinary skill in the art that various modifications may be made to the disclosed embodiments and that such modifications are intended to be within the scope of the present invention.

All of the publications, patent applications and patents cited in this specification are incorporated herein by reference in their entirety.

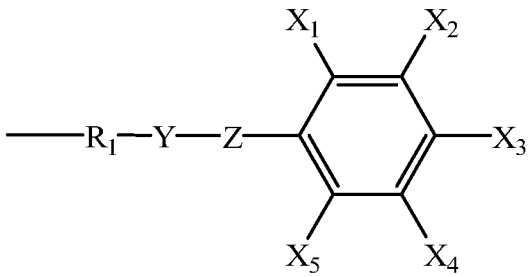
WHAT IS CLAIMED IS:

1. A method of treating or preventing a disease or condition caused by or associated with a virus belonging to the Poxviridae family, the method comprising administering to a subject in need thereof an effective amount of a compound of the formula,



, or a pharmaceutically acceptable salt thereof,

wherein R is either selected from substituted or unsubstituted alkyl groups, substituted or unsubstituted cycloalkyl groups, substituted or unsubstituted aryl groups, or substituted or unsubstituted oxaalkyl groups; or wherein R is



R_1 is a substituted or unsubstituted alkyl group;

X_{1-5} are independently selected from H, NO_2, N_3, or NH_2;

Y is absent or is a substituted or unsubstituted C_1-alkyl group, other than carbonyl; and

Z is selected from a bond or NH; provided that when Z is a bond, Y is absent, and provided that when Z is NH, Y is a substituted or unsubstituted C_1-alkyl group, other than carbonyl; and

wherein W_{1-4} are independently selected from hydrogen, substituted or unsubstituted alkyl groups, substituted or unsubstituted haloalkyl groups, substituted or unsubstituted alkanoyl groups, substituted or unsubstituted aroyl groups, or substituted or unsubstituted haloalkanoyl groups.

2. The method of claim 1, wherein each of W_1, W_2, W_3 and W_4 is hydrogen.

3. The method of claim 1, wherein R is selected from substituted or unsubstituted alkyl groups, substituted or unsubstituted cycloalkyl groups, substituted or unsubstituted aryl groups, or substituted or unsubstituted oxaalkyl groups.

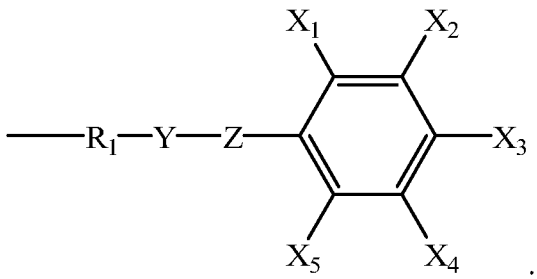
4. The method of claim 1, wherein R is C6-C12 alkyl or oxaalkyl group.

5. The method of claim 1, wherein R is C8-C10 alkyl or oxaalkyl group.

6. The method of claim 1, wherein said administering comprises administering N-nonyl deoxynojirimycin or a pharmaceutically acceptable salt thereof.

7. The method of claim 1, wherein said administering comprises administering N-(7-oxadecyl)deoxynojirimycin or a pharmaceutically acceptable salt thereof.

8. The method of claim 1, wherein said administering comprises administering N-(9-Methoxynonyl)deoxynojirimycin or a pharmaceutically acceptable salt thereof.

9. The method of claim 1, wherein R is 

.

10. The method of claim 9, wherein X_1 is NO_2 and X_3 is N_3.

11. The method of claim 9, wherein each of X_2, X_4 and X_5 is hydrogen.

12. The method of claim 1, wherein said administering comprises administering is *N*-(*N*-{4'-azido-2'-nitrophenyl}-6-aminohexyl)deoxynojirimycin or a pharmaceutically acceptable salt thereof.

13. The method of claim 1, wherein the subject is a mammal.

14. The method of claim 1, wherein the subject is a human being.

15. The method of claim 1, wherein the virus belongs is the Orthopoxvirus family.

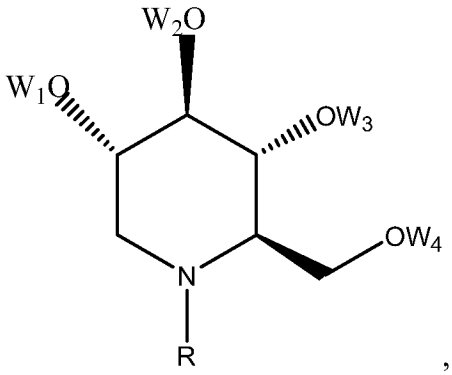
16. The method of claim 15, wherein the virus is Vaccinia virus.

17. The method of claim 15, wherein the virus is a cowpox virus.

18. The method of claim 17, wherein said administering comprises administering N-(9-Methoxynonyl)deoxynojirimycin or a pharmaceutically acceptable salt thereof.

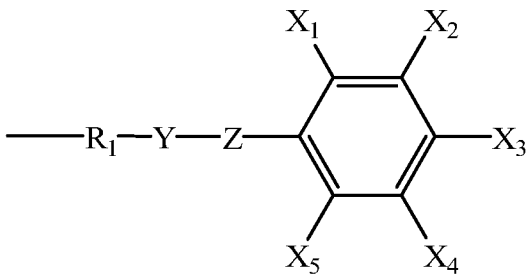
19. A method of infectivity of a cell infected with a virus belonging to the Poxviridae family, the method comprising

contacting a cell infected with a virus belonging to the Poxviridae family with an effective amount of a compound of the formula,



, or a pharmaceutically acceptable salt thereof,

wherein R is either selected from substituted or unsubstituted alkyl groups, substituted or unsubstituted cycloalkyl groups, substituted or unsubstituted aryl groups, or substituted or unsubstituted oxaalkyl groups; or wherein R is



R_1 is a substituted or unsubstituted alkyl group;

X_{1-5} are independently selected from H, NO_2, N_3, or NH_2;

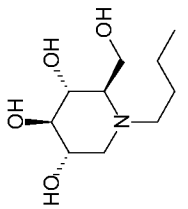
Y is absent or is a substituted or unsubstituted C_1-alkyl group, other than carbonyl; and

Z is selected from a bond or NH; provided that when Z is a bond, Y is absent, and provided

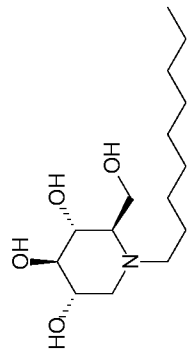
that when Z is NH, Y is a substituted or unsubstituted C_1-alkyl group, other than carbonyl; and

wherein W_{1-4} are independently selected from hydrogen, substituted or unsubstituted alkyl groups, substituted or unsubstituted haloalkyl groups, substituted or unsubstituted alkanoyl groups, substituted or unsubstituted aroyl groups, or substituted or unsubstituted haloalkanoyl groups.

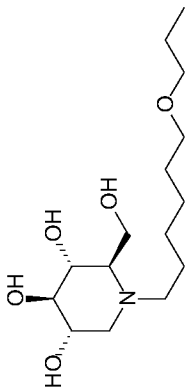
FIGURES 1(A)-(E)



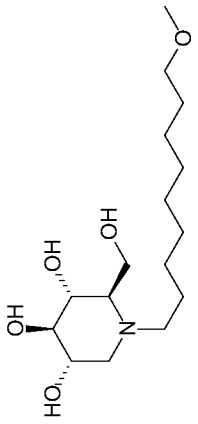
A



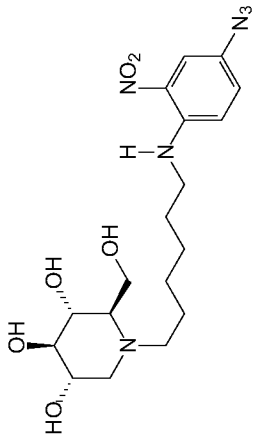
B



C



D



E

FIGURE 2

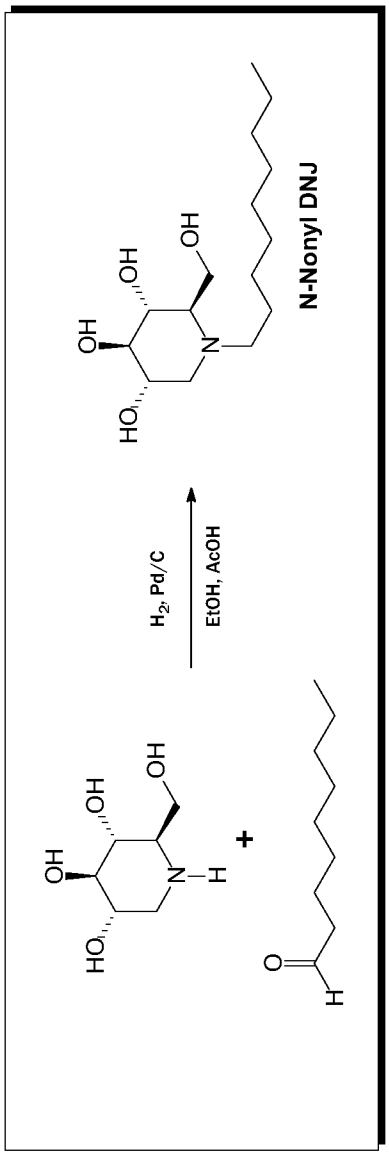
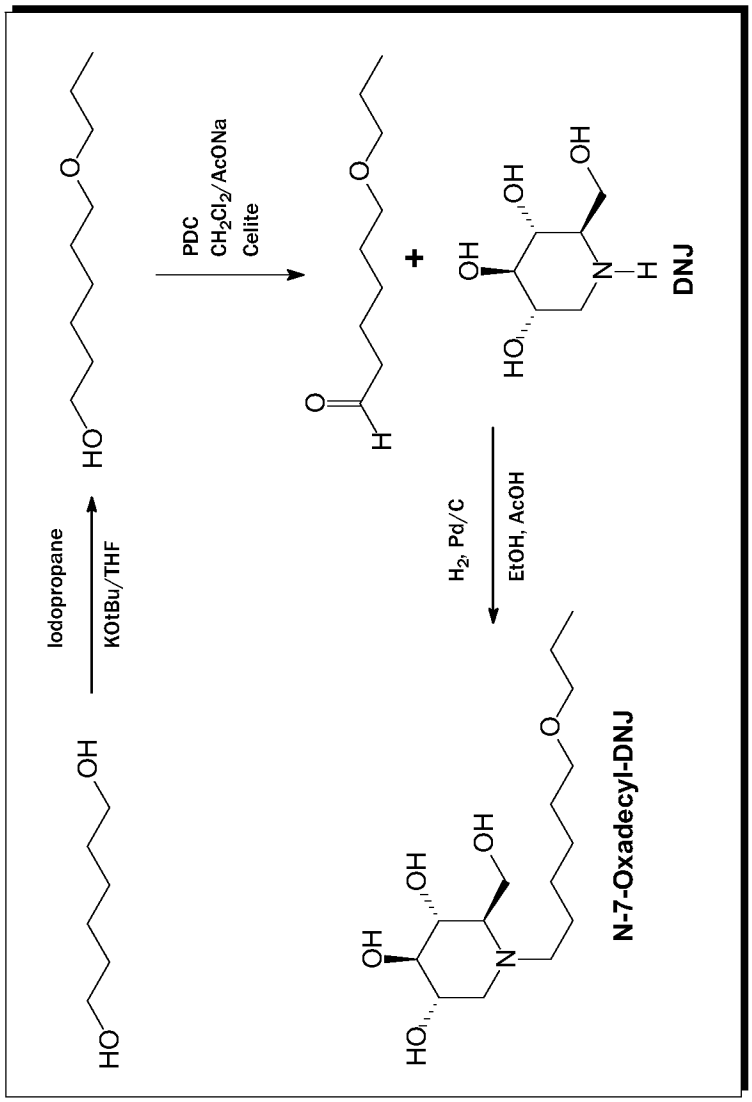
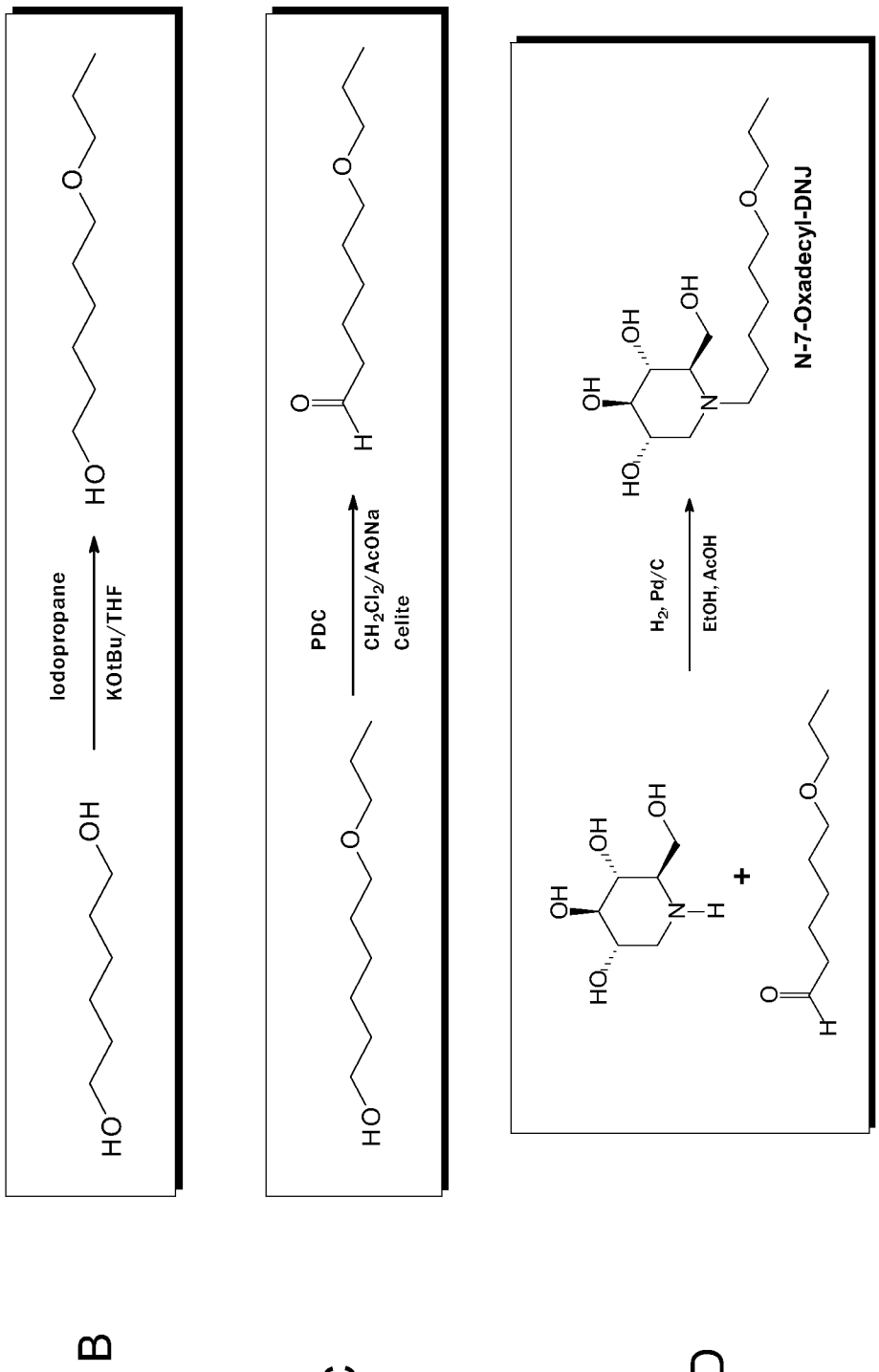


FIGURE 3A


Iodopropane
KOtBu/THF
PDC
CH_2Cl_2/AcONa
Celite
H_2, Pd/C
EtOH, AcOH
DNJ
N-7-Oxadecyl-DNJ

A

FIGURES 3B-D


B
Iodopropane
KOtBu/THF
HO
OH
C
PDC
CH_2Cl_2/AcONa
Celite
D
H_2, Pd/C
EtOH, AcOH
N-7-Oxadecyl-DNJ

FIGURES 4A-C

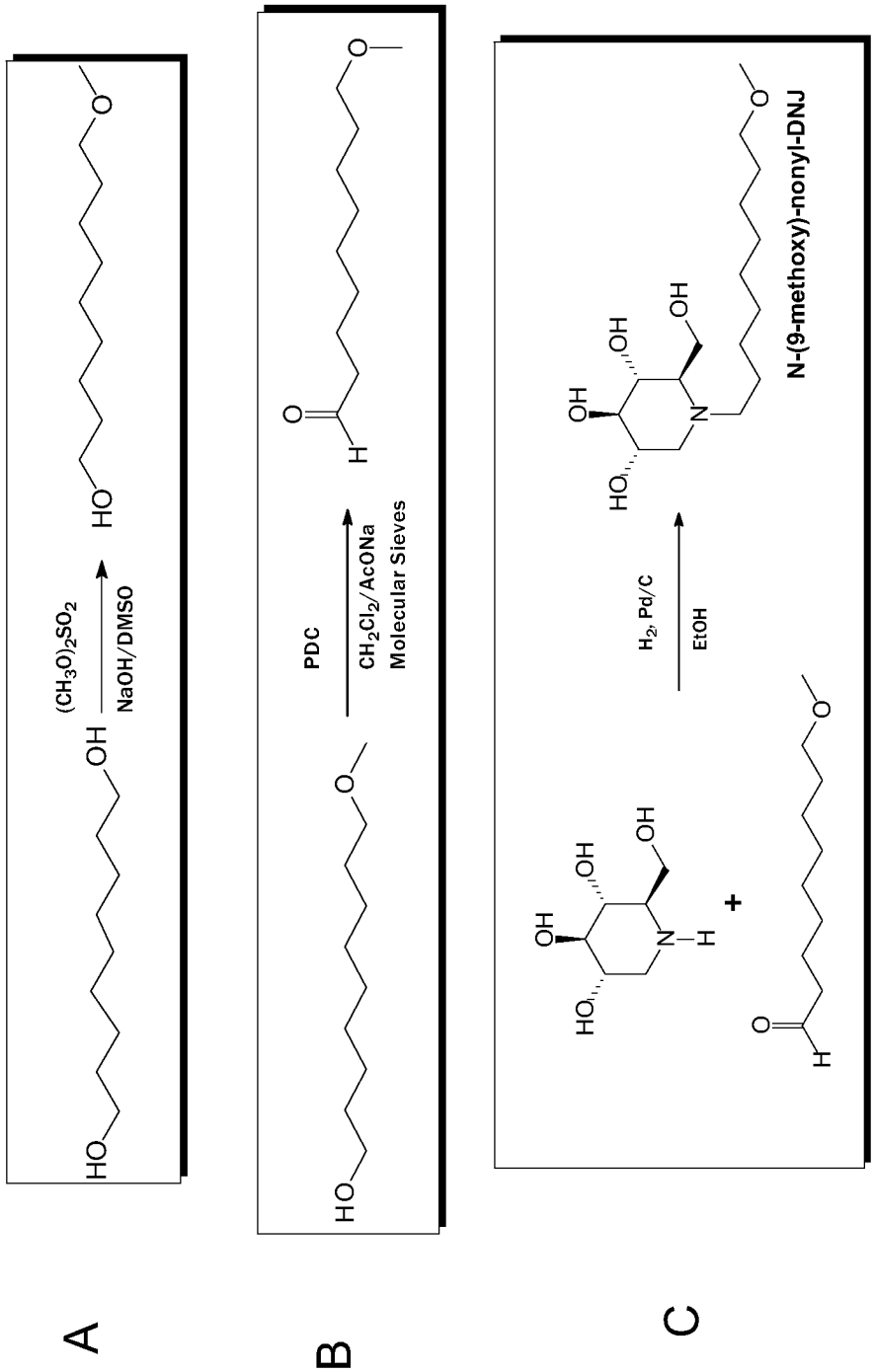

A
B
C
$(CH_3O)_2SO_2$
NaOH/DMSO
PDC
CH_2Cl_2/AcONa
Molecular Sieves
H_2, Pd/C
EtOH
N-(9-methoxy)-nonyl-DNJ

Figure 5

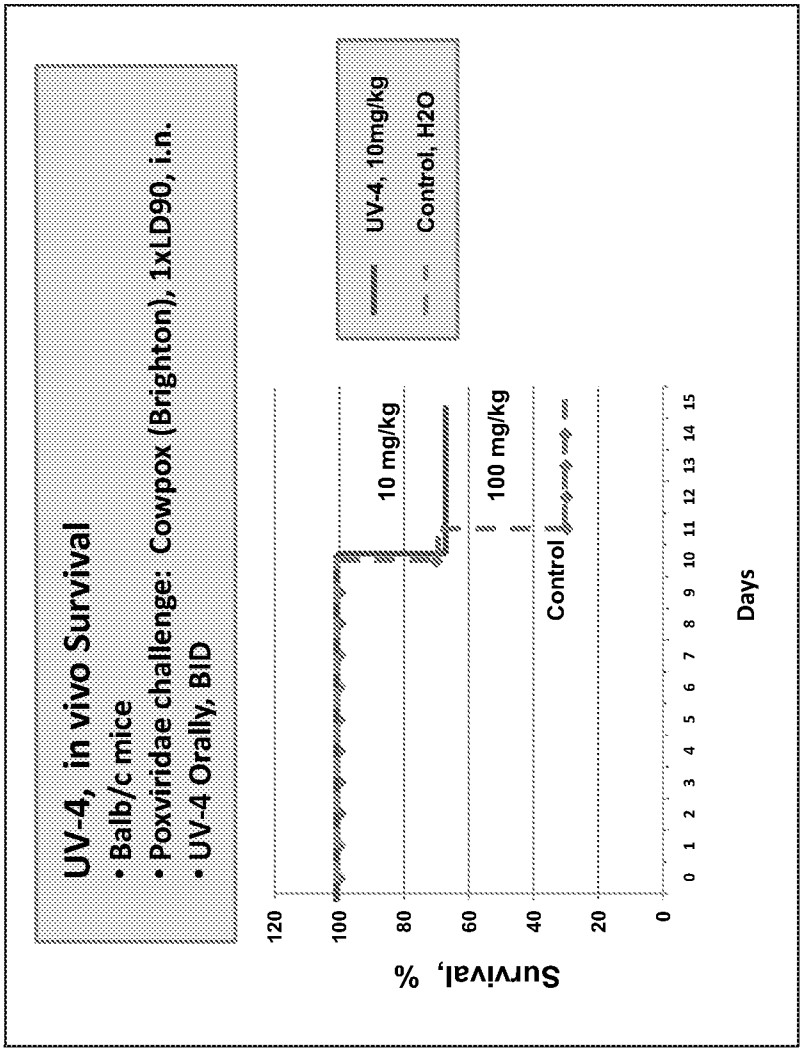
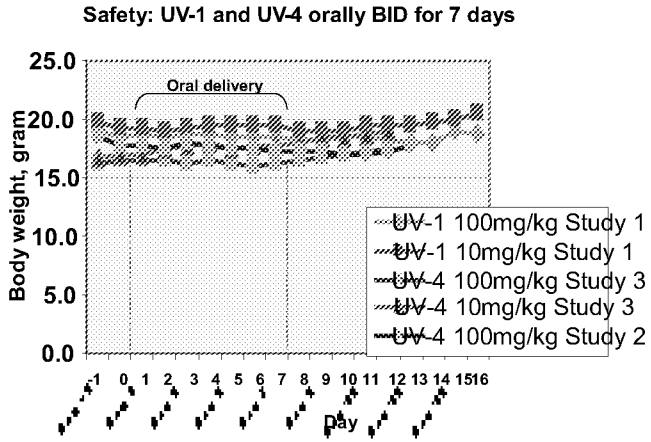

UV-4, in vivo Survival
• Balb/c mice
• Poxviridae challenge: Cowpox (Brighton), 1xLD90, i.n.
• UV-4 Orally, BID
UV-4, 10mg/kg
Control, H2O
10 mg/kg
100 mg/kg
Control
Survival, %
120
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Days

FIGURE 6

UV-4



Methods

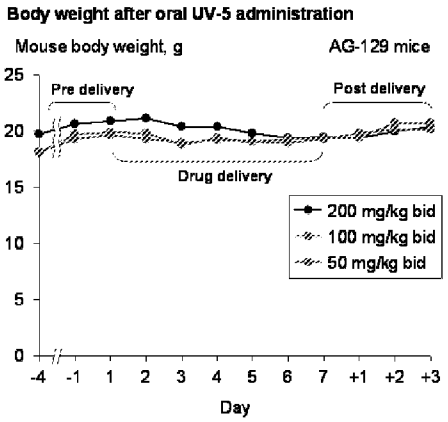
Compound administered orally for 7 days

Results

No significant weight loss

No adverse events

UV-5



Methods

Compound administered orally for 7 days

Results

No significant weight loss

No adverse events

UV compounds are safe *in vivo*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/47498

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C07D 307/48 (2010.01)
USPC - 546/248
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)
USPC - 546/248 (see search terms below)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 546/242; 546/11; 546/184 (see search terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
USPTO-WEST - PGPB,USPT,USOC,EPAB,JPAB keywords: imino sugar, analogs, deoxynojirimycin, arabinitol, alpha-glucosidase, inhibiting, virus, mammal, alpha-glucosidase inhibitors, administering, amount effective, compositions, treatment, inflammatory, diseases, Flaviridae, arenaviridae, Poxviridae, vaccinia viruses, molecular modelling, hydrophobicity.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2007/0275998 A1 (BUTTERS et al.) 29 November 2007 (29.11.2007), para [0031]; [0033]; [0046]; [0048]; [0071] - [0080]; [0126]; [0137].	1-19
Y	WO 2006/124676 A1 (KOHN et al.) 23 November 2006 (23.11.2006), para [0071]; [0090]; [0092]; [0129]; [0188].	1-19
Y	BUTTERS et al., Imino sugar inhibitors for treating the lysosomal glycosphingolipidoses, Glycobiology 15(10), pp 43R-52R, 2005, pg 45R - col 1; pg 46R.	6-8 and 18

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
07 October 2010 (07.10.2010)	22 OCT 2010
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)