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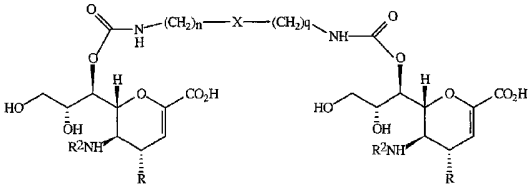
(54) Title: DIMERIC COMPOUNDS AND THEIR USE AS ANTI-VIRAL AGENTS



(I)

(57) Abstract: The invention relates to compounds of general formula (I), in which: R is an amino or guanidino group; R^2 is acetyl or trifluoroacetyl; n and q are either the same or different and selected from 0, 1 or 2; and X is an optionally substituted phenyl, optionally substituted naphthyl or optionally substituted phenyl-Y-optionally substituted phenyl in which Y is selected from a covalent bond, CH_2, CH_2CH_2, O or SO_2, or a pharmaceutically acceptable derivative thereof, with the proviso that when X is phenyl or naphthyl, n and q are both 2 and when X is phenyl-Y-phenyl in which Y is a covalent bond, then n and q are not both 0, methods for their preparation, pharmaceutical formulations containing them or their use in the prevention or treatment of a viral infection.

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DIMERIC COMPOUNDS AND THEIR USE AS ANTI-VIRAL AGENTS

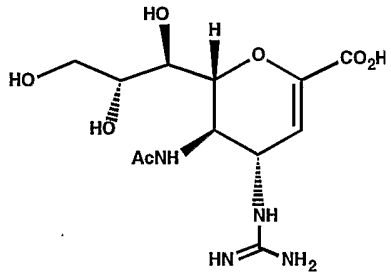
This invention relates to new chemical compounds and their use in medicine. In particular the invention concerns novel dimeric compounds, methods for their preparation, pharmaceutical formulations thereof and their use as anti-viral agents.

BACKGROUND OF THE INVENTION

Enzymes with the ability to cleave N-acetyl neuraminic acid (NANA), also known as sialic acid, from other carbohydrates are present in many microorganisms. These include bacteria such as *Vibrio cholerae*, *Clostridium perfringens*, *Streptococcus pneumoniae* and *Arthrobacter sialophilus*, and viruses such as influenza virus, parainfluenza virus, mumps virus, Newcastle disease virus and Sendai virus. Most of these viruses are of the orthomyxovirus or paramyxovirus groups, and carry a neuraminidase activity on the surface of the virus particles. Many of these neuraminidase-possessing organisms are major pathogens of man and/or animals, and some, such as influenza virus and Newcastle disease virus, cause diseases of enormous importance.

It has long been thought that inhibitors of neuraminidase might prevent infection by neuraminidase-bearing viruses. Most of the known neuraminidase inhibitors are analogues of neuraminic acid, such as 2-deoxy-2,3-dehydro-N-acetylneuraminic acid (DANA) and some of its derivatives (Meindl *et al*, Virology, 1974 58 457). Our International Patent Publication No. WO 91/16320 describes a number of analogues of DANA which are active against viral neuraminidase, and it has been shown in particular that 4-guanidino-2-deoxy-2,3-dehydro-N-acetylneuraminic acid (Compound (A), code number GG167) is useful in the treatment of influenza A and B (N. Engl. J. Med., 1997 337 874-880). Other patent applications describe various closely-related sialic acid derivatives (*eg.* PCT Publications No. WO 95/18800, No. WO 95/20583 and No. WO 98/06712), and anti-viral macromolecular conjugates

of GG167 have also been described (International Patent Application No. PCT/AU97/00771).



Compound (A)

Ac represents acetyl

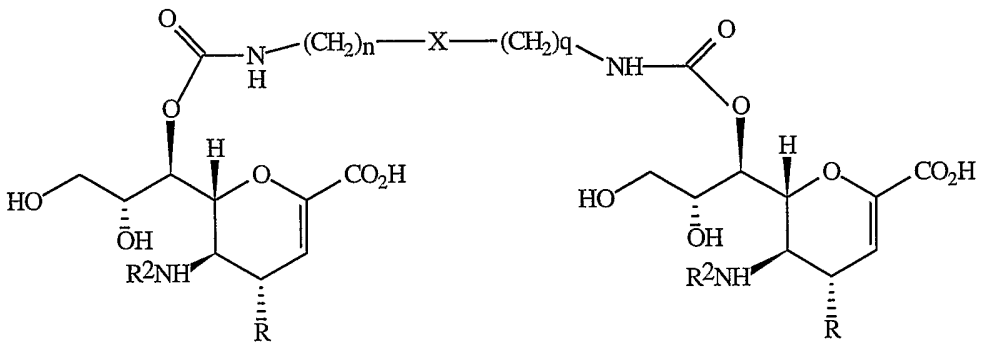
International Patent Publication No. WO 00/55149, describes dimeric compounds which comprise two neuraminidase binding molecules, such as compound (A), attached to a common spacer or linking group of up to 100 atoms in length.

We have now discovered a novel class of compounds which fall within the generic scope of International Patent Publication No. WO 00/55149, but which are not specifically disclosed therein, and exhibit a surprisingly advantageous anti-influenza activity profile which includes a long lung residency time and high potency.

Without wishing to be bound by theory, the basis for the long residency time in the lungs is thought to be due to the size and molecular weight of the compounds preventing entry through tight junctions in the respiratory epithelium and the polarity of the compounds being such that passage through the cell membranes occurs very inefficiently. An alternative theory is that the compounds themselves interact with the phospholipids in the cell membrane or other components of the respiratory epithelium and increase the residency time in the lungs.

SUMMARY OF THE INVENTION

A compound of general formula (I):



(I)

in which

R is an amino or guanidino group;

R^2 is acetyl or trifluoroacetyl;

n and q are either the same or different and selected from 0, 1 or 2; and

X is an optionally substituted phenyl, optionally substituted naphthyl or optionally substituted phenyl-Y-optionally substituted phenyl in which Y is selected from a covalent bond, CH_2, CH_2CH_2, O or SO_2,

or a pharmaceutically acceptable derivative thereof,

with the proviso that when X is phenyl or naphthyl, n and q are both 2 and when X is phenyl-Y-phenyl in which Y is a covalent bond, then n and q are not both 0.

Preferably R is a guanidino group.

Preferably R^2 is an acetyl group.

Preferably n and q are the same.

Preferably X is phenyl which may be ortho, meta or para substituted, naphthyl or phenyl-Y-phenyl in which each phenyl ring is optionally substituted with alkoxy.

The term "optionally substituted" means that a group may or may not be further substituted with one or more groups selected from alkyl, alkenyl, alkynyl, aryl, halo, haloalkyl, haloalkenyl, haloalkynyl, haloaryl, hydroxy, alkoxy, alkenyloxy, aryloxy, carboxy, benzyloxy, haloalkoxy, haloalkenyloxy, haloaryloxy, nitro, nitroalkyl, nitroalkenyl, nitroalkynyl, nitroaryl, nitroheterocyclyl,

azido, nitroso, amino, alkylamino, alkenylamino, alkynylamino, arylamino, benzylamino, acylamino, acyl, alkenylacyl, alkynylacyl, arylacyl, acylamino, acyloxy, aldehydo, alkylsulphonyl, arylsulphonyl, sulphonylamino, alkylsulphonylamino, arylsulphonylamino, alkylsulphonyloxy, arylsulphonyloxy, heterocyclyl, heterocycloxy, heterocyclylamino, haloheterocyclyl, alkylsulphenyl, arylsulphenyl, carboalkoxy, carboaryloxy, mercapto, sulfonic acid, alkylthio, arylthio and acylthio.

Preferably, the alkyl, alkenyl, alkynyl and alkoxy substituents contain up to 6 carbon atoms.

It will be appreciated by those skilled in the art that the compounds of formula (I) may be modified to provide pharmaceutically acceptable derivatives thereof at any one or more of the functional groups in the compounds of formula (I). Of particular interest as such derivatives are compounds modified at the carboxyl function, hydroxyl functions or at amino groups. Thus compounds of interest include alkyl esters, such as methyl, ethyl, propyl or isopropyl esters, aryl esters, such as phenyl, benzoyl esters, and acetyl esters of the compounds of formula (I).

The term "pharmaceutically acceptable derivative" means any pharmaceutically acceptable salt, ether, ester or salt of such ester of a compound of formula (I) or any other compound which, upon administration to the recipient, is capable of providing a compound of formula (I) or an anti-virally active metabolite or residue thereof. Of particular interest as derivatives are compounds modified at the sialic acid carboxy or glycerol hydroxy groups, or at amino and guanidine groups.

Pharmaceutically acceptable salts of the compounds of formula (I) include those derived from pharmaceutically acceptable inorganic and organic acids and bases. Examples of suitable acids include hydrochloric, hydrobromic, sulphuric, nitric, perchloric, fumaric, maleic, phosphoric, glycollic, lactic, salicylic, succinic, toluene-p-sulphonic, tartaric, acetic, citric, methanesulphonic, formic, benzoic, malonic, naphthalene-2-sulphonic and benzenesulphonic acids. Other acids such as oxalic acid,

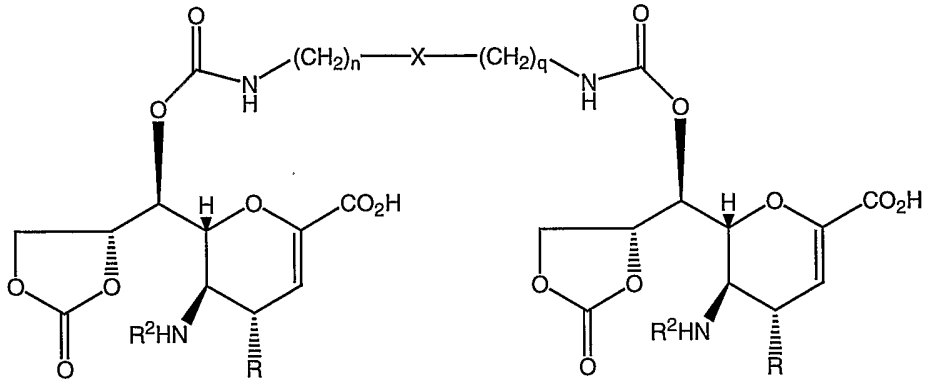
while not in themselves pharmaceutically acceptable, may be useful in the preparation of salts useful as intermediates in obtaining compounds of the invention and their pharmaceutically acceptable acid addition salts.

Salts derived from appropriate bases include alkali metal (*eg.* sodium), alkaline earth metal (*eg.* magnesium), ammonium, and NR_4^+ (where R is C_{1-4}alkyl) salts.

The compounds of the invention may be prepared by methods described herein. It will be apparent to those skilled in the art, that it is necessary to use protecting groups to protect one or more functional groups of the neuraminidase binding molecule during the process of attaching the monomers to the aryl spacer group. See for example "Protective Groups in Organic Synthesis" by T.W. Green and P.G.M. Nuts (John Wiley & Sons, 1991). Pharmaceutically acceptable salts of the compounds of formula (I) may be prepared according to known procedures.

For ease of preparation and processing, it is preferable that the compounds of formula (I) are in crystalline form.

Accordingly, the present invention also provides a method for the preparation of the compound of formula (I) as defined above, which comprises the step of deprotecting a compound of formula (II)



(II)

in which R, R^2, n, q and x are as defined above.

The compounds of formula (I) possess antiviral activity. In particular these compounds are inhibitors of viral neuraminidase of orthomyxoviruses and

paramyxoviruses, for example the viral neuraminidase of influenza A and B, parainfluenza, mumps and Newcastle disease.

Thus in a second aspect the invention provides a compound of formula (I) or a pharmaceutically acceptable derivative thereof, for use as an active therapeutic agent in the treatment of a viral infection, for example orthomyxovirus and paramyxovirus infections.

In a third aspect the invention provides a method for the prevention or treatment of a viral infection comprising the step of administration to a subject in need thereof of an effective amount of a compound of formula (I), or a pharmaceutically acceptable salt or derivative thereof.

Preferably, the viral infection is an orthomyxovirus or paramyxovirus infection. More preferably the viral infection is an influenza A or B infection.

Preferably the subject is an animal such as a mammal, more preferably a human, or a member of the genus Equus, for example a horse, donkey or mule. Most preferably the mammal is a human.

In a fourth aspect the invention provides use of a compound of the invention for the manufacture of a medicament for the treatment of a viral infection.

It will be appreciated by those skilled in the art that reference herein to treatment extends to prophylaxis against infection as well as to the treatment of established infections or symptoms.

The compounds of the invention may also be used in diagnostic methods, in particular methods for the detection of influenza virus. For use in such methods it may be advantageous to link a compound of the invention to a label, such as a radioactive, fluorescent or chemiluminescent label.

Methods of diagnosis for which the compounds of the invention are suitable are described, for example, in our earlier applications PCT/AU97/00109 and PCT/AU97/00771.

In a fifth aspect the invention provides a method for the detection of a viral infection which comprises the step

of contacting the compound of the invention with a sample suspected of containing the virus.

It will be further appreciated that the amount of a compound of the invention required for use in treatment will vary not only with the particular compound selected but also with the route of administration, the nature of the condition being treated, and the age and condition of the patient, and will ultimately be at the discretion of the attendant physician or veterinarian. In general however, a suitable dose will be in the range of from about 0.001 to 100 mg/kg of bodyweight per day, preferably in the range of 0.01 to 10 mg/kg/day, most preferably in the range of 0.1 to 1 mg/kg/day.

Treatment is preferably commenced before or at the time of infection and continued until virus is no longer present in the respiratory tract. However the compounds are also effective when given post-infection, for example after the appearance of established symptoms.

Suitably treatment is given on one or two occasions, preferably only once only for treatment, and preferably once per week for prophylaxis.

The compound is conveniently administered in unit dosage form, for example containing 1 to 100 mg, more conveniently 1 to 20 mg of active ingredient per unit dosage form.

While it is possible that, for use in therapy, a compound of the invention may be administered as the raw chemical, it is preferable to present the active ingredient as a pharmaceutical formulation.

Thus in a sixth aspect the invention provides a pharmaceutical formulation comprising a compound of formula (I) or a pharmaceutically acceptable salt or derivative thereof, together with one or more pharmaceutically acceptable carriers therefor and, optionally, other therapeutic and/or prophylactic ingredients. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not being deleterious to the recipient thereof.

The compounds of the invention may also be used in combination with other therapeutic and/or prophylactic agents, for example other anti-infective agents. In particular the compounds of the invention may be employed with other antiviral agents. The invention thus provides in a seventh aspect a combination comprising a compound of formula (I) or a pharmaceutically acceptable salt or derivative thereof together with another therapeutically and/or prophylactically active agent, in particular an antiviral agent.

The combinations referred to above may conveniently be presented for use in the form of a pharmaceutical formulation and thus such formulations comprising a combination as defined above together with a pharmaceutically acceptable carrier therefor comprise a further aspect of the invention.

Suitable therapeutic and/or prophylactic agents for use in such combinations include other anti-infective agents, in particular anti-bacterial and anti-viral agents such as those used to treat respiratory infections. For example, other compounds or vaccines effective against influenza viruses, such as the sialic acid analogues referred to above, e.g. zanamivir, oseltamivir, amantadine, rimantadine and ribavirin and FluVax, may be included in such combinations.

The individual components of such combinations may be administered either separately, sequentially or simultaneously in separate or combined pharmaceutical formulations.

When the compounds of the invention are used with a second therapeutic and/or prophylactic agent active against the same virus, the dose of each compound may either be the same as or different from that employed when each compound is used alone. Appropriate doses will be readily appreciated by those skilled in the art.

Pharmaceutical formulations include those suitable for oral, rectal, nasal, topical (including buccal and sub-lingual), vaginal or parenteral (including intramuscular, sub-cutaneous and intravenous) administration, or those in

a form suitable for administration to the respiratory tract (including the nasal passages) for example by inhalation or insufflation. The formulations may, where appropriate, be conveniently presented in discrete dosage units, and may be prepared by any of the methods well known in the art of pharmacy. These methods include the step of bringing into association the active compound with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product into the desired formulation.

Pharmaceutical formulations suitable for oral administration may conveniently be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution, a suspension or as an emulsion. The active ingredient may also be presented as a bolus, electuary or paste. Tablets and capsules for oral administration may contain conventional excipients such as binding agents, fillers, lubricants, disintegrants, or wetting agents. The tablets may be coated according to methods well known in the art. Oral liquid preparations may for example be in the form of aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, emulsifying agents, non-aqueous vehicles, which may include edible oils, or preservatives.

The compounds according to the invention may also be formulated for parenteral administration by injection, for example bolus injection, or continuous infusion, and may be presented in unit dose form in ampoules, pre-filled syringes, small volume infusion or in multi-dose containers with an added preservative. The compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulating agents such as suspending, stabilising and/or dispersing agents. Alternatively, the active ingredient may be in powder form, obtained by aseptic isolation of sterile solid or by

lyophilisation from solution, for constitution with a suitable vehicle, *eg.* sterile, pyrogen-free water, before use.

For topical administration to the epidermis the compounds according to the invention may be formulated as ointments, creams or lotions, or as a transdermal patch. Ointments and creams may, for example, be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agents. Lotions may be formulated with an aqueous or oily base, and will in general also contain one or more emulsifying agents, stabilising agents, dispersing agents, suspending agents, thickening agents, or colouring agents.

Formulations suitable for topical administration in the mouth include lozenges comprising active ingredient in a flavoured base, usually sucrose and gum acacia or gum tragacanth; pastilles comprising the active ingredient in an inert base such as gelatin or sucrose and gum acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

Pharmaceutical formulations suitable for rectal administration wherein the carrier is a solid are most preferably presented as unit dose suppositories. Suitable carriers include cocoa butter and other materials commonly used in the art, and the suppositories may be conveniently formed by admixture of the active compound with the softened or melted carrier(s) followed by chilling and shaping moulds.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or sprays containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

For administration to the respiratory tract, including intranasal administration, the neuraminidase inhibitors may be administered by any of the methods and formulations employed in the art for administration to the respiratory tract.

Thus in general the compounds may be administered in the form of a solution or a suspension or as a dry powder.

Solutions and suspensions will generally be aqueous, for example prepared from water alone (for example sterile or pyrogen-free water) or water and a physiologically acceptable co-solvent (for example ethanol, propylene glycol or polyethylene glycols such as PEG 400).

Such solutions or suspensions may additionally contain other excipients for example preservatives (such as benzalkonium chloride), solubilising agents/surfactants such as polysorbates (*eg.* Tween 80, Span 80, benzalkonium chloride), buffering agents, isotonicity-adjusting agents (for example sodium chloride), absorption enhancers and viscosity enhancers. Suspensions may additionally contain suspending agents (for example microcrystalline cellulose, carboxymethyl cellulose sodium).

Solutions or suspensions are applied directly to the nasal cavity by conventional means, for example with a dropper, pipette or spray. The formulations may be provided in single or multidose form. In the latter case a means of dose metering is desirably provided. In the case of a dropper or pipette this may be achieved by the patient administering an appropriate, predetermined volume of the solution or suspension. In the case of a spray this may be achieved for example by means of a metering atomising spray pump.

Administration to the respiratory tract may also be achieved by means of an aerosol formulation in which the compound is provided in a pressurised pack with a suitable propellant, such as a chlorofluorocarbon (CFC), for example dichlorodifluoromethane, trichlorofluoromethane or dichlorotetrafluoroethane, carbon dioxide or other suitable gas. The aerosol may conveniently also contain a surfactant such as lecithin. The dose of drug may be controlled by provision of a metered valve.

Alternatively the compounds may be provided in the form of a dry powder, for example a powder mix of the compound in a suitable powder base such as lactose, starch, starch derivatives such as hydroxypropylmethyl cellulose

and polyvinylpyrrolidine (PVP). Conveniently the powder carrier will form a gel in the nasal cavity. The powder composition may be presented in unit dose form, for example in capsules or cartridges of *eg.* gelatin, or blister packs from which the powder may be administered by means of an inhaler.

In formulations intended for administration to the respiratory tract, including intranasal formulations, the compound will generally have a small particle size, for example of the order of 5 microns or less. Such a particle size may be obtained by means known in the art, for example by micronisation.

When desired, formulations adapted to give sustained release of the active ingredient may be employed.

Preferably the compounds of the invention are administered to the respiratory tract by inhalation, insufflation or intranasal administration, or a combination thereof.

"Relenza" is administered by oral inhalation as a free-flow powder via a "Diskhaler" (trade marks of the GlaxoSmithKline group of companies). A similar formulation would be suitable for the present invention.

Thus, according to an eighth aspect of the present invention there is provided an inhaler which contains a formulation as defined above.

It will be appreciated that the inhaler may also be in the form of a meter dose aerosol inhaler.

For the purposes of this specification it will be clearly understood that the word "comprising" means "including but not limited to", and that the word "comprises" has a corresponding meaning.

All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

DETAILED DESCRIPTION OF THE INVENTION

The invention will now be described in detail by way of reference only to the following non-limiting examples.

Machine Methods

Method A(LC/MS)

Micromass Platform II mass spectrometer operating in positive ion electrospray mode, mass range 100-1000 amu.
Column : 3.3cm x 4.6mm ID, 3µm ABZ+PLUS
Flow Rate : 3ml/min
Injection Volume : 5µl
Solvent A : 95% acetonitrile + 0.05% formic acid
Solvent B : 0.1% formic acid + 10mMolar ammonium acetate
Gradient : 0% A/0.7min, 0-100% A/3.5min, 100% A/1.1min, 100-0% A/0.2min

Method B (LC/MS)

Waters ZQ mass spectrometer operating in positive ion electrospray mode, mass range 100-1000 amu.
Column : 3.3cm x 4.6mm ID, 3µm ABZ+PLUS
Flow Rate : 3ml/min
Injection Volume : 5µl
Solvent A : 95% acetonitrile + 0.05% formic acid
Solvent B : 0.1% formic acid + 10mMolar ammonium acetate
Gradient : 0% A/0.7min, 0-100% A/3.5min, 100% A/1.1min, 100-0% A/0.2min

Method C (Autoprep HPLC)

The prep column used was a Supelcosil ABZplus (10cm x 2.12cm).
UV wavelength : 230nm
Injection Volume: 2ml
Flow : 4ml/min
Solvent A : acetonitrile + 0.05% TFA
Solvent B : water + 0.1% TFA

Gradient : 5-40% A/20min, 40% A/20 min, 40-100% A/0.3 min, 100% A/15 min, 100-5% A/3min

Method D (Mass directed autoprep HPLC)

The prep column used was a Supelcosil ABZplus (10cm x 2.12cm)
UV wavelength : 200-320nM
Flow : 20ml/min
Injection volume: 1ml
Solvent A : 0.1% formic acid
Solvent B : 95% acetonitrile + 5% formic acid
Gradient : 100% A/1min, 100-80% A/9min, 80-1% A/3.5min, 1% A/1.4min, 1-100%A/0.1min

Method E (Prep HPLC)

The prep column used was a Dynamax 60Å C18 (25cm x 4.14cm)
UV wavelength : 230nM
Flow : 40ml/min
Solvent A : acetonitrile + 0.05% TFA
Solvent B : water + 0.1% TFA
Gradient : 0-50% A/25 min, 50-100% A/0.3 min, 100% A/15 min, 100-0% A/3min

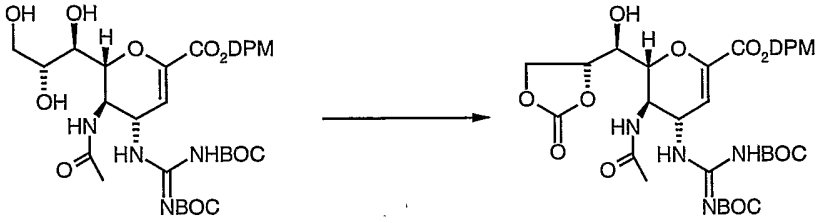
Method F (Prep HPLC)

The prep column used was a Kromasil C18 (20cmx5cm)
UV wavelength : 230nM
Flow : 80ml/min
Solvent A : 1% TFA
Solvent B : 80% acetonitrile + 1% TFA
Gradient : 0-100%B/70 min
Following HPLC, appropriate fractions were combined and volatile components removed by evaporation under reduced pressure. The aqueous was applied to a column of Amberchrom CG-161 resin (10x2.5cm) which was eluted with water (500ml), then a 2:2:1 mixture of acetonitrile : MeOH : water (500ml).

Abbreviations

TFA	trifluoroacetic acid
DMAP	4-dimethylaminopyridine
DCM	dichloromethane
EtOAc	ethyl acetate
Et_2O	diethyl ether
MeOH	methanol
HPLC	high pressure liquid chromatography
DPM	diphenylmethyl
SPE	solid phase extraction
NMR	nuclear magnetic resonance
LC/MS	Liquid chromatography/mass spectroscopy

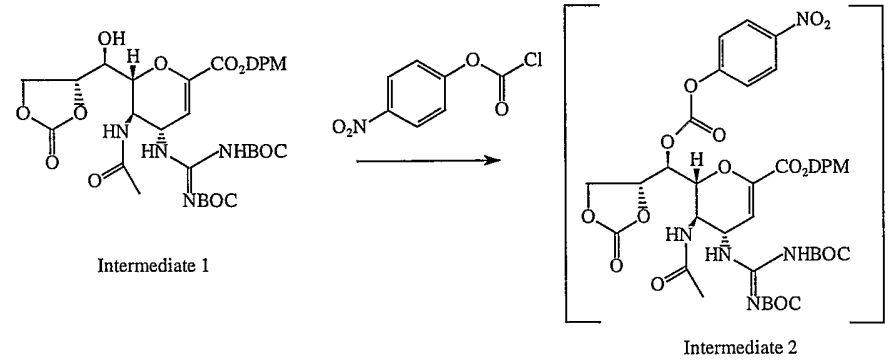
Intermediate 1



Intermediate 1

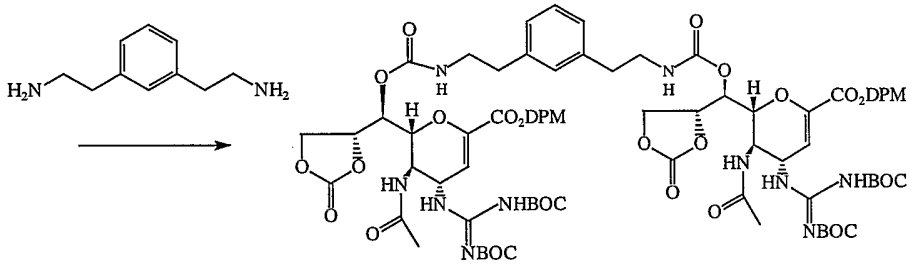
Benzhydryl (2*R*,3*R*,4*S*)-3-(acetylamino)-4-({(*E*)-[(*tert*-butoxycarbonyl)amino][(*tert*-butoxycarbonyl)imino]methyl}amino)-2-[(1*R*,2*R*)-1,2,3-trihydroxypropyl]-3,4-dihydro-2*H*-pyran-6-carboxylate (see *J. Med. Chem.* 1998, *41*, 787-797) (12.38g; 17.7mmoles) was dissolved in dry acetonitrile (130ml) under nitrogen at room temperature. The solution was stirred and 1,1'-carbonyldiimidazole (2.87g; 17.7mmoles) was added. After 16 hours LC/MS showed the presence of starting triol so further 1,1'-carbonyldiimidazole (total of 0.493g; 3mmoles) was added. After a few hours LC/MS showed no triol present. The solvent was evaporated and the residue purified by flash column chromatography on silica, eluting with 1:1 ethyl acetate/40-60 petroleum ether. Fractions containing the product were evaporated then taken up in dichloromethane, dried with sodium sulphate, filtered and evaporated to give Intermediate 1 (benzhydryl (2R,3R,4S)-3-(acetylamino)-4-({[(tert-butoxycarbonyl)amino][(tert-butoxycarbonyl)imino]methyl}amino)-2-{(S)-hydroxy[(4R)-2-oxo-1,3-dioxolan-4-yl]methyl}-3,4-dihydro-2H-pyran-6-carboxylate) as an off white solid (11.05g; 86%).

Intermediate 3



Intermediate 1

Intermediate 2



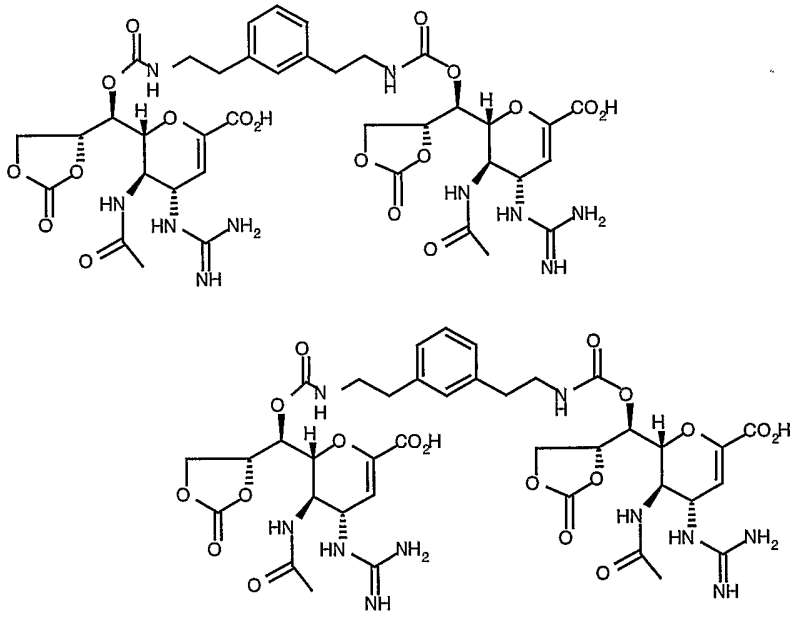
Intermediate 3

Intermediate 1 (2.0g, 2.76mmol) was dried by azeotroping with anhydrous toluene (3x20ml), then dissolved in anhydrous pyridine (8ml). To this was added DMAP (1.01g, 8.29mmol) and p-nitrophenyl chloroformate (0.67g, 3.3mmol) and the mixture was stirred at room temperature overnight. A further portion of p-nitrophenyl chloroformate (0.28g, 1.38mmol) was added and stirring continued for 2h. LCMS (Method B) showed MH^+ = 890; T_{RET} = 4.19 min corresponding to Intermediate 2.

A portion of the mixture (1.6ml) was transferred to another reaction vessel and treated with DMAP (0.20g, 1.66mmol), triethylamine (0.08ml, 0.55mol), then 1,3-benzenediethanamine dihydrochloride (65mg, 0.27mmol) [for preparation see Chem. Ber., 1984, 117(4), 1487-1496] and the mixture was stirred for 70h then concentrated *in vacuo* and partitioned between DCM (10ml) and water (5ml). Isolation of the organic layer was carried out using a

hydrophobic frit cartridge. The DCM layer was concentrated by blow down under nitrogen, then applied to a 5g silica SPE cartridge and eluted first with cyclohexane : Et_2O (1:1), then with Et_2O, then Et_2O : EtOAc (9:1), then Et_2O : EtOAc (5:1), then Et_2O : EtOAc (3:1), then Et_2O : EtOAc (1:1) and finally with EtOAc to afford Intermediate 3 as a white solid (0.29g; 63% yield). LC/MS (Method A) showed $(M+2H^+)/2$ = 834; T_{RET} = 4.56 min.

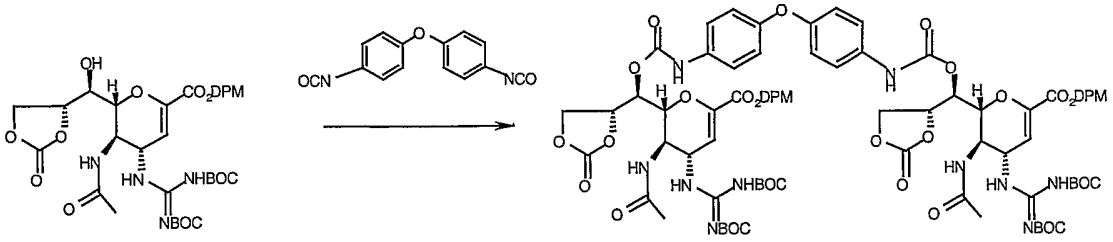
Intermediate 4



Intermediate 4

Intermediate 3 (0.29g, 0.2mmol) was dissolved in a 10:1 mixture of DCM : anisole (0.80ml) and treated with TFA (0.73ml). The resulting solution was stirred at room temperature for 2h then evaporated to dryness by blow down under nitrogen. Trituration of the residue with diethyl ether afforded the bis-TFA salt of Intermediate 4 as a white solid (0.142g, 76% yield). LC/MS (Method B) showed $(M+2H^+)/2$ = 467; T_{RET} = 2.05 min.

Intermediate 5

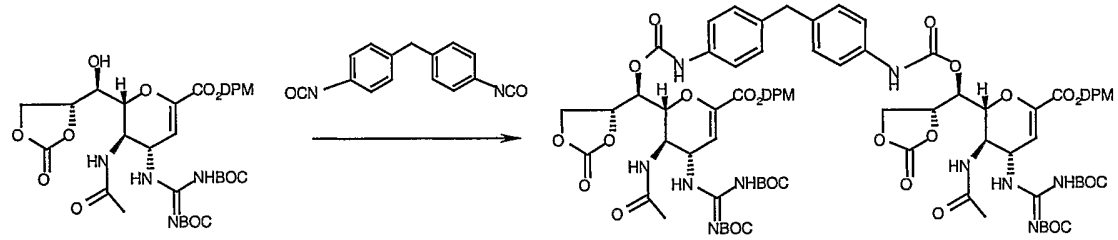


Intermediate 1

Intermediate 5

Intermediate 1 (0.10g, 0.14mmol) was dried by azeotroping three times with anhydrous toluene, then dissolved in anhydrous DCM (0.5ml). To the resultant solution was added DMAP (0.005g, cat.) followed by 1,1'-oxybis[4-isocyanatobenzene] (0.012g, 0.046mmol) and a few 3 Å molecular sieve pellets. The mixture was refluxed overnight then allowed to cool and applied directly to a 5g silica SPE cartridge. This was eluted first with Et_2O (10x20ml), then with EtOAc (5x20ml) to afford Intermediate 5 as a colourless glass (0.025g, 33% yield). LC/MS (Method A) showed $(M+2H^+)/2 = 852$; $T_{RET} = 4.55$ min.

Intermediate 6



Intermediate 1

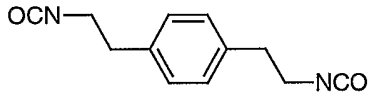
Intermediate 6

Intermediate 1 (4.0 g, 5.6mmol) was dried by azeotroping with anhydrous toluene then dissolved in anhydrous DCM (5ml). To the resultant solution stirring under nitrogen was added 4,4'-methylenebis[phenylisocyanate] (0.48g, 1.9mmol), a few 3Å molecular sieve pellets and DMAP (0.2g, cat.), then the mixture was refluxed for 20h. After cooling the mixture was concentrated *in vacuo* and purified

by flash column chromatography on silica. Elution was carried out first with DCM, then with Et_2O, then sequentially with Et_2O : EtOAc (95:5), Et_2O : EtOAc (90:10) and Et_2O : EtOAc (80:20) to afford Intermediate 6 as a white solid (2.60g, 80% yield).

LCMS (Method B) showed $(M+2H^+)/2$ = 850; T_{RET} = 4.57 min.

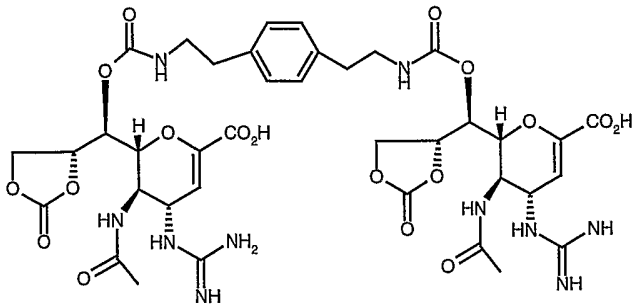
Intermediate 7



1,4-Phenylenedipropionic acid (0.50g, 2.25mmol) was azeotroped with toluene then suspended in dioxane (5ml) over a few 3Å molecular sieve pellets and stirred under nitrogen for 10 min. Triethylamine (0.68ml, 4.90mmol) was added, followed by diphenylphosphoryl azide (0.96ml, 4.50mmol) and the mixture was stirred at room temperature for 2h. The temperature was then raised to 80°C and the mixture stirred for 45min before cooling and filtering to remove insoluble material. The solid was washed with petroleum ether (40-60°C) and the combined filtrates were concentrated *in vacuo*. The resultant oil was extracted with petroleum ether (40-60°C) to afford Intermediate 7 as a colourless oil (0.12g, 25% yield). ^1H NMR (400MHz, $CDCl_3$) δ (ppm)

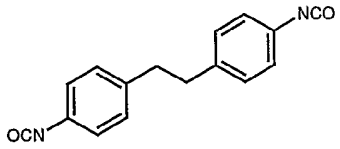
7.12 (s, 4H), 3.45 (t, 4H), 2.83 (t, 4H).

Intermediate 8



Fully protected Intermediate 8 was prepared from Intermediates 7 and 1 following the same procedure as for Intermediates 5 and 6 and was then deprotected as follows: (0.09g, 0.05mmol) was dissolved in a mixture of DCM (0.37ml) and anisole (0.037ml) and cooled in an ice bath. The mixture was treated with TFA (0.37ml) and the resulting solution allowed to warm to room temperature, then stirred for 2.5h before concentration *in vacuo*. The mixture was triturated with Et_2O to afford the bis-TFA salt of Intermediate 8 as a white solid (0.05g; 93% yield). LC/MS (Method B) showed $(M+2H^+)/2$ = 467; T_{RET} = 2.01min.

Intermediate 9

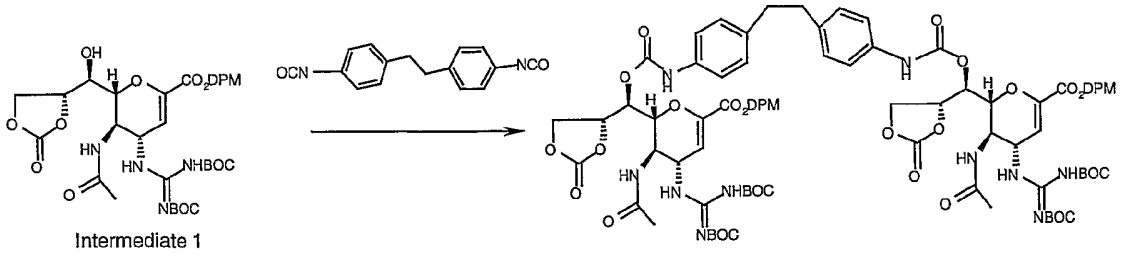


Intermediate 9

A solution of 4,4'-ethylene dianiline (0.50g, 2.36mmol) in anhydrous toluene (100ml) was treated with triphosgene (1.40g, 4.70mmol) and the mixture heated at reflux (120°C) for 4h. The mixture was allowed to cool and filtered under gravity to remove insoluble residues. The filtrate was concentrated *in vacuo* to afford Intermediate 9 as a yellow solid (0.54g, 86% yield).

^1H NMR (400MHz, $CDCl_3$) δ(ppm) 6.98-7.05 (8H, ABq) and 2.86 (4H, s)

Intermediate 10

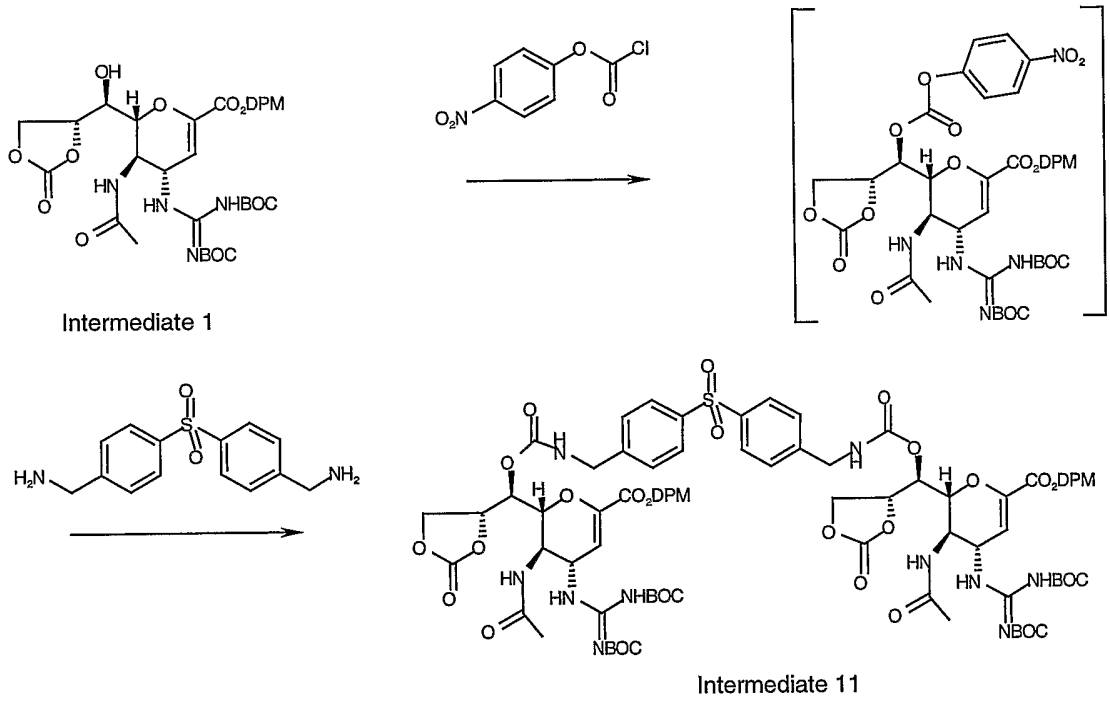


Intermediate 1

Intermediate 10

Intermediate 1 (0.40g, 0.56mmol) was azeotroped twice from anhydrous toluene then dissolved in anhydrous DCM (0.4ml). To the resultant solution was added DMAP (0.02g, cat.) followed by Intermediate 9 (0.05g, 0.19mmol) and a few 3Å molecular sieve pellets. The mixture was refluxed for 18h then applied directly to a 40g silica Biotage cartridge. This was eluted with Et_2O : EtOAc (6:1) to afford Intermediate 10 as a white solid (0.10g, 31% yield). LC/MS (Method B) showed $(M+2H^+)/2$ = 858; T_{RET} = 4.57 min.

Intermediate 11



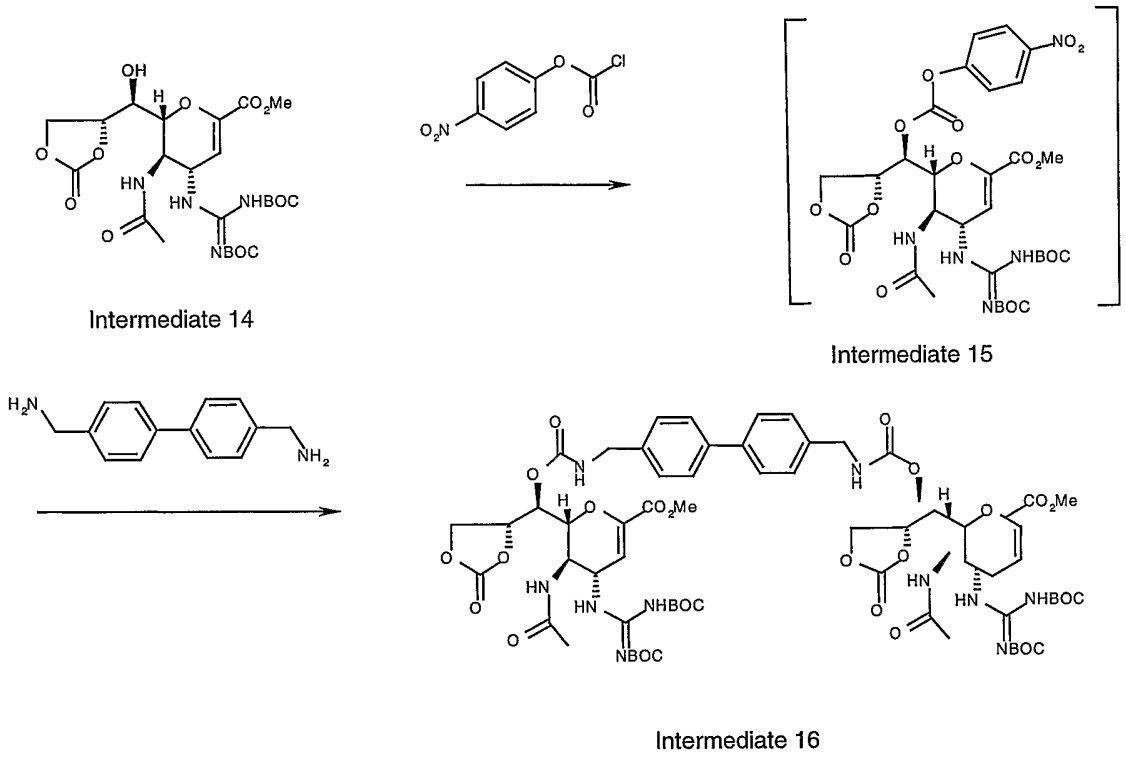
Intermediate 1 (2.0g, 2.76mmol) was dried by azeotroping three times from anhydrous toluene, then dissolved in anhydrous pyridine (8ml). To this was added DMAP (1.01g, 8.29mmol) and p-nitrophenyl chloroformate (0.67g, 3.30mmol) and the mixture was stirred at room temperature for 18h. A further portion of p-nitrophenyl chloroformate (0.28g, 1.38mmol) was added and stirring continued for 2h. LC/MS (Method B) showed MH^+ = 890; T_{RET} = 4.19 min corresponding to Intermediate 2.

A portion of the mixture (1.6ml) was transferred to another reaction vessel and treated with DMAP (0.20g, 1.66mmol), triethylamine (0.08ml, 0.55mmol), then 4,4'-sulfonylbis-benzylamine dihydrochloride (0.10g, 0.28mmol) [for preparation see *J. Chem. Soc.*, 1946, 466] and the mixture stirred for 70h. The mixture was concentrated *in vacuo* and partitioned between DCM (10ml) and water (5ml). Separation of the two phases was carried out using a 50ml hydrophobic frit cartridge. The DCM layer was concentrated by blow down under nitrogen, then applied to a 5g silica SPE cartridge and eluted first with cyclohexane : Et_2O (1:1) then with Et_2O, followed by Et_2O : EtOAc (9:1), then Et_2O : EtOAc (5:1), then Et_2O : EtOAc (3:1) then Et_2O : EtOAc (1:1) and finally with EtOAc to afford Intermediate 11 as an off-white solid (0.15g; 30% yield). LCMS (Method A) showed $(M+2H^+)/2$ = 890; T_{RET} = 4.47 min.

Similarly prepared were the following:

X	Starting amine	Product	LC/MS Method	$(M+2H^+)$ /2	T_{RET}(min)
O	4,4'-oxybis-benzylamine dihydrochloride (*Chem. Comm.*, 1998, 2297-2298)	**Intermediate 12**	A	866	4.48
CH_2	4,4'-methylenebis-benzylamine dihydrochloride (*J. Med. Chem.*, 1998, 41, 2-5)	**Intermediate 13**	A	865	4.51

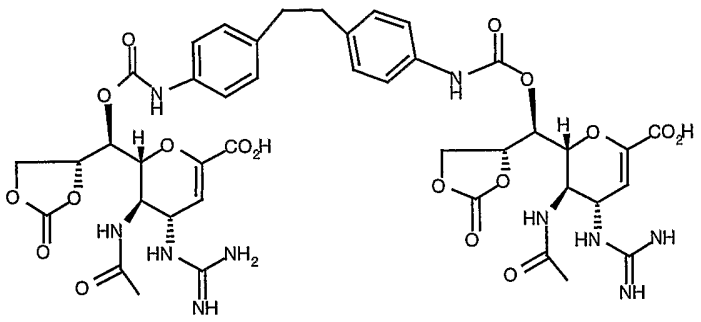
Intermediate 16



Intermediate 14 (2.74g, 4.79mmol) was dried by azeotroping four times from anhydrous toluene, then dissolved in anhydrous pyridine (13.75ml). To this was added DMAP (1.46g, 11.98mmol) and p-nitrophenyl chloroformate (1.06g, 5.27mmol) and the mixture was stirred at room temperature for 3h. LCMS (Method B) showed MH^+ = 738; T_{RET} = 3.87 min corresponding to Intermediate 15.

To the mixture was then added more pyridine (8.25ml), followed by [1,1'-Biphenyl]-4,4'-dimethanamine (0.51g, 2.4mmol) (prepared according to *J. Med. Chem.*, 2000, 43, 420-431) and stirring was continued for a further 16h. The mixture was concentrated *in vacuo* and applied as a solution in DCM to a 90g, silica Biotage cartridge. This was eluted with diethyl ether, followed by Et_2O : EtOAc (1:1), then Et_2O : EtOAc (1:2) and finally EtOAc to afford Intermediate 16 as a white solid (1.92g, 57% yield). LC/MS (Method A) showed $(M+2H^+)/2$ = 705; T_{RET} = 3.96 min.

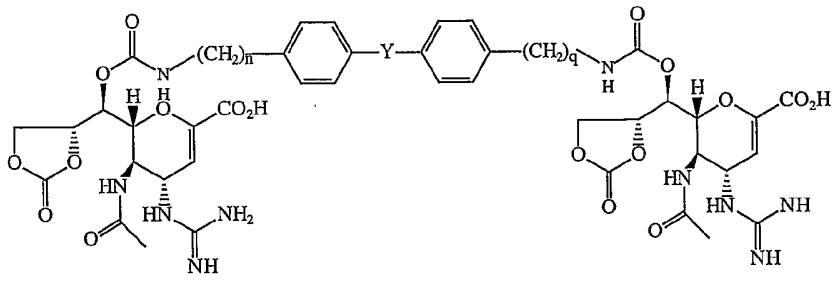
Intermediate 17



Intermediate 17

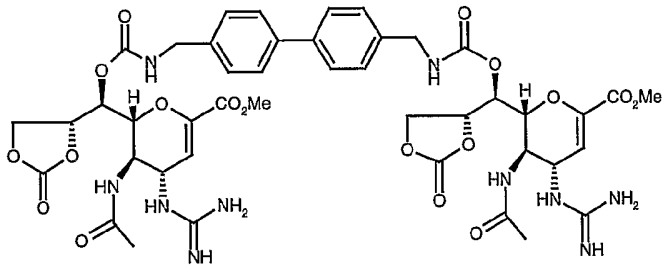
Intermediate 10 (0.1g, 0.06mmol) was dissolved in a 10:1 mixture of DCM : anisole (0.44ml) in a glass vial and treated with TFA (0.04ml). The resulting solution was stirred at room temperature for 2h then concentrated *in vacuo*. Trituration of the residue with diethyl ether afforded the bis-TFA salt of Intermediate 17 as a white solid (0.06g, 87% yield). LC/MS (Method A) showed $(M+2H^+)/2$ = 491; T_{RET} = 2.38 min.

Similarly prepared were the following:



Y	n	q	Starting material	Product	LC/MS Method	$(M+2H^+)/2$	T_{RET}(min)
O	0	0	Intermediate 5	Intermediate 18	A	485	2.25
CH_2	0	0	Intermediate 6	Intermediate 19	B	484	2.26
SO_2	1	1	Intermediate 11	Intermediate 20	A	523	2.08
O	1	1	Intermediate 12	Intermediate 21	A	499	2.21
CH_2	1	1	Intermediate 13	Intermediate 22	A	498	2.25

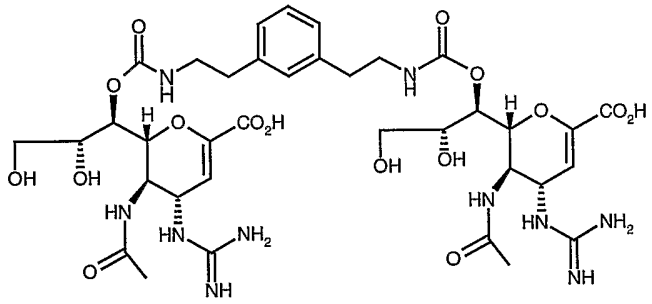
Intermediate 23



Intermediate 16 (1.92g, 1.36mmol) was dissolved in a 10:1 mixture of DCM: anisole (27.5ml) and treated with TFA (25ml). The resulting solution was stirred at room temperature for 2h then concentrated *in vacuo*. Trituration of the residue with diethyl ether afforded the bis-TFA salt of Intermediate 23 as a white solid (1.59g, 94% yield). LCMS (Method A) showed $(M+2H^+)/2$ = 505; T_{RET} = 2.27 min.

Example 1

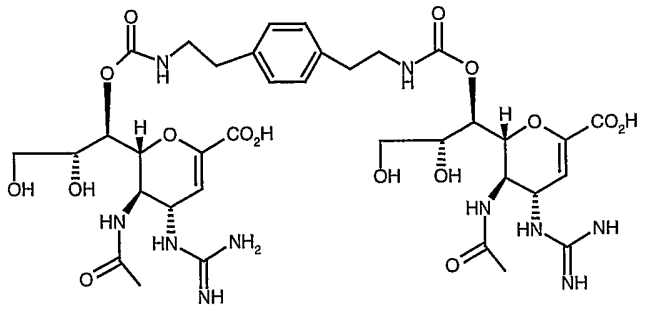
(2R,3R,4S)-3-(acetylamino)-2-{(1R,2R)-1-[({[2-(3-{2-[({[(1R,2R)-1-((2R,3R,4S)-3-(acetylamino)-4-{[amino(imino)methyl]amino}-6-carboxy-3,4-dihydro-2H-pyran-2-yl)-2,3-dihydroxypropyl]oxy}carbonyl)amino]ethyl}phenyl)ethyl]amino }carbonyl)oxy]-2,3-dihydroxypropyl}-4-{[amino(imino)methyl]amino}-3,4-dihydro-2H-pyran-6-carboxylic acid



Intermediate 4 (0.14g, 0.15mmol) was dissolved in a mixture of water (1.20ml) and methanol (1.20ml). To this was added triethylamine (0.30ml) and the solution was shaken for 2 hours then concentrated *in vacuo*. Reverse phase preparative HPLC (Method D) gave Example 1 (0.048g; 44% yield). LC/MS (Method A) showed $(M+2H^+)/2$ = 441; T_{RET} = 1.83 min.

Example 2

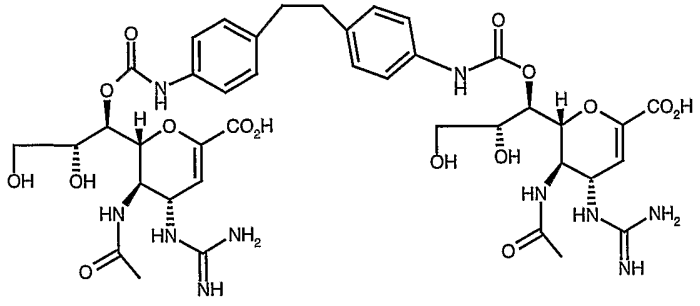
(2R,3R,4S)-3-(acetylamino)-2-{(1R,2R)-1-[({[2-(4-{2-[({[(1R,2R)-1-((2R,3R,4S)-3-(acetylamino)-4-{[amino(imino)methyl]amino}-6-carboxy-3,4-dihydro-2H-pyran-2-yl)-2,3-dihydroxypropyl]oxy}carbonyl)amino]ethyl}phenyl)ethyl]amino } carbonyl)oxy]-2,3-dihydroxypropyl}-4-{[amino(imino)methyl]amino}-3,4-dihydro-2H-pyran-6-carboxylic acid



Intermediate 8 (0.03g; 0.06mmol) was dissolved in a mixture of water (1ml) and methanol (1ml). To this was added triethylamine (0.25ml) and the solution was stirred for 1h then concentrated *in vacuo*. The residue was applied as an aqueous solution to a C18 SPE cartridge (pre-conditioned with methanol). The column was eluted with acetonitrile : water (5:95) (3x5ml), then acetonitrile : water (7.5:93.5) (3x5ml) and finally acetonitrile : water (15:85) (3x5ml) to afford Example 2 as a white solid (0.005g; 9% yield). LC/MS (Method B) showed $(M+2H^+)/2$ = 441; T_{RET} = 1.79 min.

Example 3

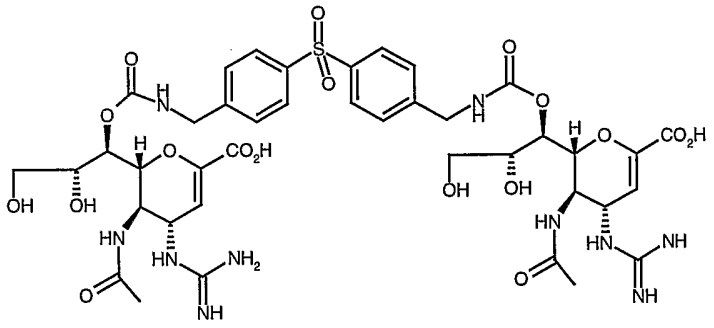
(2R,3R,4S)-3-(acetylamino)-2-{(1R,2R)-1-[({[4-(2-{4-[({[(1R,2R)-1-((2R,3R,4S)-3-(acetylamino)-4-{[amino(imino)methyl]amino}-6-carboxy-3,4-dihydro-2H-pyran-2-yl)-2,3-dihydroxypropyl]oxy}carbonyl)amino]phenyl}ethyl)phenyl]amino}carbonyl)oxy]-2,3-dihydroxypropyl}-4-{[amino(imino)methyl]amino}-3,4-dihydro-2H-pyran-6-carboxylic acid bis TFA salt



A solution of Intermediate 17 (0.06g, 0.06mmol) in a 2:1 mixture of dioxane : water (3ml) was treated with triethylamine (1ml) and the mixture stirred at room temperature for 18h. Purification by reverse phase HPLC (Method C) afforded Example 3 as a white solid (0.01g, 22% yield). LC/MS (Method A) showed $(M+2H^+)/2$ = 465; T_{RET} = 2.16 min.

Example 4

(2R,3R,4S)-3-(acetylamino)-2-[(1R,2R)-1-({[({4-[(4-{[({[(1R,2R)-1((2R,3R,4S)-3-(acetylamino)-4-{[amino(imino)methyl]amino}-6-carboxy-3,4-dihydro-2H-pyran-2-yl)-2,3-dihydroxypropyl]oxy}-carbonyl)amino]methyl}phenyl)sulfonyl]phenyl}methyl)amino]carbonyl}oxy)-2,3-dihydroxypropyl]-4-{[amino(imino)methyl] amino}-3,4-dihydro-2H-pyran-6-carboxylic acid



Intermediate 20 (0.09g, 0.07mmol) was dissolved in a mixture of water (0.70ml) and methanol (0.70ml). To this was added triethylamine (0.18ml) and the solution was shaken for 2 hours then concentrated *in vacuo*. Reverse phase preparative HPLC (Method D) gave Example 4 as the bis-TFA salt (0.014g, 20% yield). LC/MS (Method B) showed $(M+2H^+)/2$ = 497; T_{RET} = 1.93 min.

Similarly prepared were the following:

Y	Starting material	Product	LC/MS Method	$(M+2H^+)$ /2	T_{RET}(min)
O	Intermediate 21	**Example 5**	A	473	2.07
CH_2	Intermediate 22	**Example 6**	A	472	2.11

Example 5

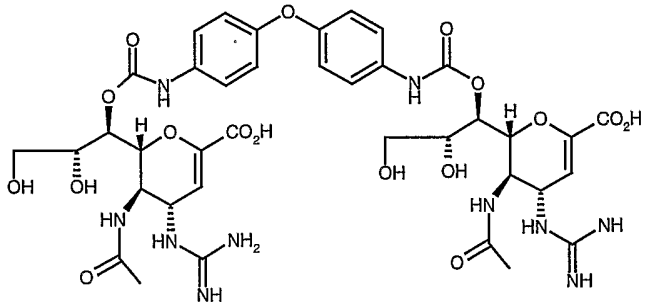
(2R,3R,4S)-3-(acetylamino)-2-[(1R,2R)-1-({[({4-[(4-{[({[(1R,2R)-1-((2R,3R,4S)-3-(acetylamino)-4-{[amino(imino)methyl]amino}-6-carboxy-3,4-dihydro-2H-pyran-2-yl)-2,3-dihydroxypropyl]oxy}carbonyl)amino]methyl}phenyl)oxy]phenyl}methyl) amino] carbonyl}oxy)-2,3-dihydroxypropyl]-4-{[amino(imino)methyl]amino}-3,4-dihydro-2H-pyran-6-carboxylic acid

Example 6

(2R,3R,4S)-3-(acetylamino)-2-[(1R,2R)-1-({[({4-[(4-{[({[(1R,2R)-1((2R,3R,4S)-3-(acetylamino)-4-{[amino(imino)methyl]amino}-6-carboxy-3,4-dihydro-2H-pyran-2-yl)-2,3-dihydroxypropyl]oxy}carbonyl)amino]methyl}phenyl)methyl]phenyl} methyl) amino]carbonyl}oxy)-2,3-dihydroxypropyl]-4-{[amino(imino)methyl] amino}-3,4-dihydro-2H-pyran-6-carboxylic acid

Example 7

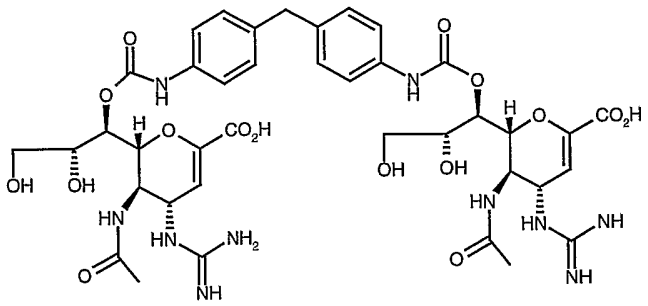
(2R,3R,4S)-3-(acetylamino)-2-{(1R,2R)-1-[({[4-({4-[({[(1R,2R)-1-((2R,3R,4S)-3-(acetylamino)-4-{[amino(imino)methyl]amino}-6-carboxy-3,4-dihydro-2H-pyran-2-yl)-2,3-dihydroxypropyl]oxy}carbonyl)amino]phenyl}oxy)phenyl]amino}carbonyl)oxy]-2,3-dihydroxypropyl}-4-{[amino(imino)methyl]amino}-3,4-dihydro-2H-pyran-6-carboxylic acid bis TFA salt



Intermediate 18 (0.005g, 0.004mmol) was dissolved in water (1ml) and heated at 40°C for 8 hours. The mixture was allowed to cool and applied directly to a 500mg C18 SPE cartridge (pre-conditioned with methanol). The column was eluted with water (5ml), then acetonitrile : water (15:85) (2x5ml). The acetonitrile containing fractions contained impure product and so were combined and concentrated *in vacuo*. The residue was re-dissolved in water (1ml) containing a drop of TFA to aid solubility and re-applied to a 500mg C18 SPE cartridge (pre-conditioned with methanol). The column was eluted with acetonitrile : water (2:98) (2x5ml), then acetonitrile : water (4:96) (2x5ml), then acetonitrile : water (6:94) (2x5ml), then acetonitrile : water (8:92) (2x5ml) and finally acetonitrile : water (10:90) (2x5ml) to afford Example 7 as a white solid (0.002g, 47% yield). LC/MS (Method A) showed $(M+2H^+)/2$ = 459; T_{RET} = 2.01min.

Example 8

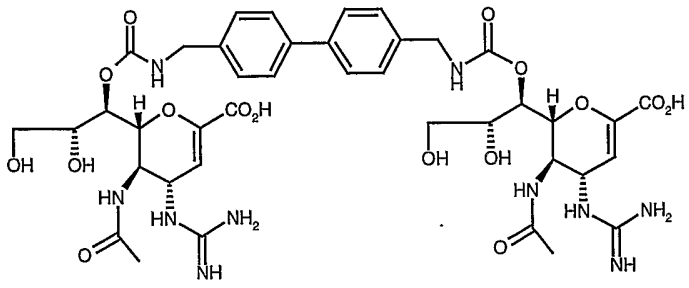
(2R,3R,4S)-3-(acetylamino)-2-{(1R,2R)-1-[({[4-({4-[({[(1R,2R)-1-((2R,3R,4S)-3-(acetylamino)-4-{[amino(imino)methyl]amino}-6-carboxy-3,4-dihydro-2H-pyran-2-yl)-2,3-dihydroxypropyl]oxy}carbonyl)amino]phenyl}methyl)phenyl]amino} carbonyl)oxy]-2,3-dihydroxypropyl}-4-{[amino(imino)methyl]amino}-3,4-dihydro-2H-pyran-6-carboxylic acid bis TFA salt



Intermediate 19 (1.0g, 1.0mmol) was dissolved in a mixture of water (4ml) and methanol (4ml). To this was added triethylamine (1ml) and the solution was stirred for 3.5 hours. Volatile components were removed by evaporation under reduced pressure and the pH of the remaining aqueous solution adjusted to pH3 by addition of TFA. Reverse phase preparative HPLC (Method E) gave the bis-TFA salt of Example 8 as a white solid (0.29g; 24% yield). LCMS (Method B) showed $(M+2H^+)/2$ = 458; T_{RET} = 2.08 min.

Example 9

(2R,3R,4S)-3-(acetylamino)-2-{(1R,2R)-1-[({[(4'-{[({[(1R,2R)-1-((2R,3R,4S)-3-(acetylamino)-4-{[amino(imino)methyl]amino}-6-carboxy-3,4-dihydro-2H-pyran-2-yl)-2,3-dihydroxypropyl]oxy}carbonyl)amino]methyl}-1,1'-biphenyl-4-yl)methyl]amino}carbonyl)oxy]-2,3-dihydroxypropyl}-4-{[amino(imino)methyl]amino}-3,4-dihydro-2H-pyran-6-carboxylic acid bis TFA salt



Intermediate 23 (1.59g; 1.3mmol) was dissolved in a mixture of water (28.5ml) and methanol (28.5ml). To this was added

triethylamine (2.85ml) and the solution stirred for 4 hours. Volatile organic components were removed *in vacuo* and the residual solution adjusted to pH 2 by addition of TFA. Reverse phase preparative HPLC (Method F) gave the zwitterion Example 9 (0.70g; 57% yield). LC/MS (Method A) showed $(M+2H^+)/2$ = 465; T_{RET} = 2.00min.

Example 10: Evaluation of the Compounds of formula (I) - Inhibition of Influenza Virus Replication

Cytopathic effect (CPE) assays were performed essentially as described by Watanabe et al. (J. Virological Methods, 1994 48 257). MDCK cells were infected with a defined inoculum of virus (determined by experimentation to be the minimum sufficient to cause adequate CPE in 72 hours and to be susceptible to control compounds at concentrations considered to be consistent with published norms) in the presence serial dilutions of Compounds of the invention. Cultures were incubated for up to 72 hours at 37°C in a 5% CO_2 atmosphere. The extent of CPE and hence viral replication was determined via metabolism of the viral dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) according to published methods (see for example, Watanabe et al., 1994). The compound concentration that inhibited CPE by 50% (ID_{50}) was calculated using a computer program for curve fitting. Influenza A/Sydney/5/97 and B/Harbin/7/95 viruses were assayed and the results are shown in Table 1. Comparable data for a specifically disclosed compound in WO 00/55149 and for compound A is also shown in Table 1.

Table 1

Description	ID_{50} µg/ml A/Sydney/5/97+	ID_{50} µg/ml B/Harbin/7/95
Compound A	0.023 +/- 0.024	0.013 +/- 0.011
Example 1	0.084	0.0002
Example 4	> 0.100	< 0.00005
Example 8	0.013	< 0.00005
Example 9	> 0.100	0.00008
Compound Number 8 *	0.0007, 0.0005	0.007 +/- 0.01
Compound Number 10 *	0.057	>0.1

* As referenced in WO 00/55149

\+ Data provided in WO 00/55149 related to the virus H3N2 isolate A/Victoria/3/75 rather than A H3N2 isolate A/Sydney/5/97. When comparing such data the person skilled in the art will appreciate that differences in antiviral potency are not uncommon for a given compound when analysed against several different viruses *in vitro*. For example, Woods et al (Antimicrob Agents Chemother 1993 37:1473-9) have reported that Compound A exhibits a wide range of EC50 values (from 0.02 to 0.16 uM) in *in vitro* assays involving recent clinical isolates. Accordingly, compound 8 was found to be more potent in CPE assays involving the recent influenza A H3N2 isolate A/Sydney/5/97 than the earlier H3N2 isolate A/Victoria/3/75.

Data provided in Table 1 demonstrate that the compounds E1 - E5, in addition to being substantially more potent than the highly active compound A, are even more potent against A/Sydney/5/97 and substantially more potent against the recent influenza B isolate B/Harbin/7/95 than compounds 8 and 10 of WO 00/55149.

Example 11: Plaque Reduction Assay

Madin Darby Canine Kidney (MDCK) cells are seeded into six well tissue culture plates and grown to confluency via standard methods. Influenza viruses are diluted in a minimal volume of phosphate buffered saline supplemented with 0.2% bovine serum albumin to yield an estimated titre of 50-100 plaque forming units (pfu) per well. After adsorption to the MDCK cells for one hour at 37°C in a 5% CO_2 atmosphere the viral inocula is aspirated and replaced with viral growth media (minimal Eagle's media supplemented with BSA, trypsin and insulin/transferrin/selenium at optimal concentrations) containing sufficient agar or agarose (generally 1-2%) to cause the media to gel at room temperature and at 37°C in a 5% CO_2 atmosphere until plaques develop (generally 2-4 days). Plaques can be visualised with a suitable stain (e.g. 0.4% crystal violet in formal saline) before counting. Antiviral potency is expressed as the concentration of test article which reduces plaque numbers by 50% of the untreated control value (EC_{50}).

	EC_{50} ng/ml					
	PRA					
Example	**A/WSN***	**A/Vic***	**A/Syd***	**A/New***	**A/Pan***	**A/Bay***
Compound A	56, >100	5.5 +/- 8.2	2.4	0.27, 0.23	2.7, 3	35
Compound 8		0.003	0.19, >1	0.0001		
Compound 9	<0.0001, 0.001	0.000141, 0.038	3.7	0.003	1.8, >10	>1
Amantadine		220		11	157	
Oseltamivir		0.11		0.23	0.3	

***A/WSN/33 BVLV09 (H1N1)**
A/Victoria/3/75 BVLV017 (H3N2)
A/Sydney/5/97 BVLV015 (H3N2)
A/New Caledonia/20/99 BVLV008 (H1N1)
A/Panama/2007/99 BVLV008 (H3N2)
A/Bayern/7/95 BVL006 (H1N1)

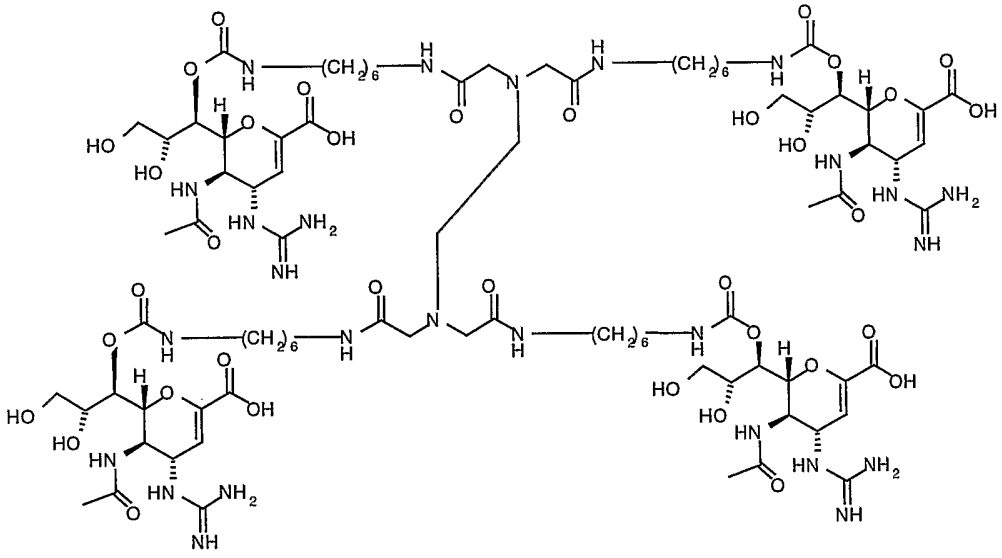
	EC_{50} ng/ml			
	PRA			
Example	**B/Vic***	**B/Harb***	**B/HongK***	**B/Yam***
Compound A	3, 20	0.19	21 +/- 6	0.2, 3.1
Compound 8	0.01, 0.2	<0.0001		0.02
Compound 9			0.23	0.006
Amantadine			>10000	2061
Oseltamivir			32	0.7

***B/Victoria/1/67**
B/Hong Kong/5/72 BVLV012
B/Harbin/7/95 BVLV008
B/Yamanashi/166/98 BVLV007

Example 12: Assessment of long duration of action

Rodents are anaesthetised and dosed with compound of interest by the intra-tracheal route at a dose volume of 0.8 ml/kg. The rodent is then held in the vertical position until full recovery is achieved. At different time points, for example, 2, 8, 24 and 48 hours post-dose, levels of compound in the lung tissue are assessed by analytical methods. Any analytical method suitable for detection of this type of compound may be used. The time at which levels of compound fall below the sensitivity of the analytical techniques identified will determine the residency time of the compound in lung tissue.

The rat lung retention data for selected compounds is shown below. Please note that all experiments included a co-dosed internal standard, namely compound 3 of International Patent Publication No. WO 02/20514, to permit comparison. The data are expressed as a ratio with respect to this compound, the structure of which is shown below.



Compound 3

The data for compound A is included for comparison purposes. The compounds of the invention have significantly greater retention at 7 days than Compound A when expressed as a ratio of compound concentration to standard concentration.

Rat lung retention assay results

time point	Compound	dose	(cmpd)	Mean (cmpd)	(PCT AU01/01128 compound 3)	Mean (PCT AU01/01128 compound 3)	Ratio Mean (lung)
hrs		mg/kg	ng/g	ng/g	ng/g	ng/g	(cmpd)/PCT AU01/01128 compound 3
48	Example 2	0.1	740		1944		
48	Example 2	0.1	667	603	1258	1366	0.44
48	Example 2	0.1	403		894		
168	Example 2	0.1	350		807		
168	Example 2	0.1	172	259	653	755	0.34
168	Example 2	0.1	254		804		
48	Example 9	0.1	570		1346		
48	Example 9	0.1	2389	1405	4101	2710	0.52
48	Example 9	0.1	1255		2684		
168	Example 9	0.1	724		1486		
168	Example 9	0.1	465	835	1253	1849	0.45
168	Example 9	0.1	1317		2806		
48	Compound A (zanamivir)	0.1	421		698		
48	Compound A (zanamivir)	0.1	369	352	1901	1368	0.26
48	Compound A (zanamivir)	0.1	267		1507		
168	Compound A (zanamivir)	0.1	91		815		
168	Compound A (zanamivir)	0.1	47	61	925	750	0.08
168	Compound A (zanamivir)	0.1	45		512		

time point	Compound	dose	(lung)	Mean (lung)	Ratio mean (lung)
hrs		mg/kg	ng/g	ng/g	(cmpd) / PCT AU01/01128 compound 3
48	PCT AU01/01128 compound 3	0.4	-		
48	PCT AU01/01128 compound 3	0.4	3689	3111	-
48	PCT AU01/01128 compound 3	0.4	2534		
168	PCT AU01/01128 compound 3	0.4	1205		
168	PCT AU01/01128 compound 3	0.4	-	1491	-
168	PCT AU01/01128 compound 3	0.4	1777		
48	Example 8	0.4	3795		
48	Example 8	0.4	2704	3253	1.05
48	Example 8	0.4	3259		
168	Example 8	0.4	-		
168	Example 8	0.4	3234	3403	2.28
168	Example 8	0.4	3571		

Example 13: Alternative assessment of long duration of action and efficacy

The protocol for infecting mice has been described previously (1 - 4). Mildly anaesthetised mice are inoculated into the external nares with influenza virus.

Treatment procedure and regimen.

A single dose of compound is administered at a defined time point up to 10 days prior to infection, preferably 4-7 days prior to infection, or following infection, preferably immediately following infection and up to 48 hours post infection. In most experiments, a non-lethal strain of influenza is used, and efficacy is assessed by reductions in lung virus titre. For mice given compound prior to infection, lungs are removed post infection either on a single day, or on days following infection, preferably days 1-4 post infection. Homogenised lung samples are assayed for virus using established methods, and the titres of viral load estimated and compared to titres of virus in lungs of untreated mice.

In those experiments where a mouse-adapted lethal strain of influenza is used, efficacy is assessed by an increase in survival rate and/or numbers of survivors, as compared to untreated mice.

REFERENCES

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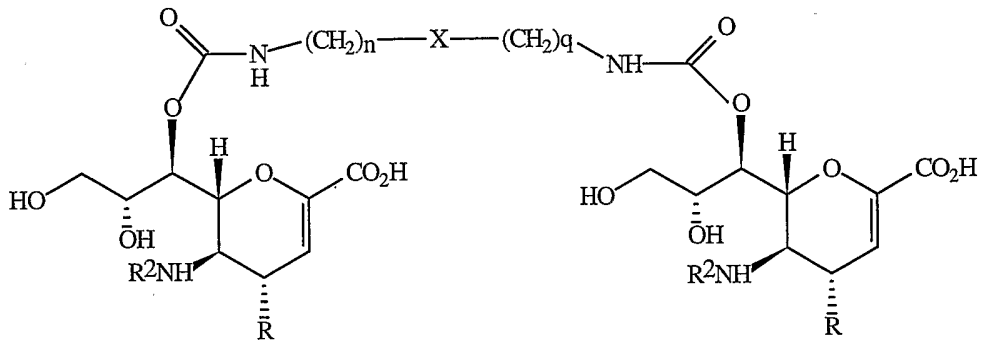
4. Robert J Fenton, Peter J Morley, Ian J Owens, David Gower, Simon Parry, Lee Crossman and Tony Wong (1999). Chemoprophylaxis of influenza A virus infections, with single doses of zanamivir, demonstrates that zanamivir is cleared slowly from the respiratory tract. Antimicrob. Agents and Chemother. 43, 11, 2642-2647.

CLAIMS:

1. A compound of general formula (I):



(I)

in which

R is an amino or guanidino group;

R^2 is acetyl or trifluoroacetyl;

n and q are either the same or different and selected from 0, 1 or 2; and

X is an optionally substituted phenyl, optionally substituted naphthyl or optionally substituted phenyl-Y-optionally substituted phenyl in which Y is selected from a covalent bond, CH_2, CH_2CH_2, O or SO_2,

or a pharmaceutically acceptable derivative thereof,

with the proviso that when X is phenyl or naphthyl, n and q are both 2 and when X is phenyl-Y-phenyl in which Y is a covalent bond, then n and q are not both 0.

2. A compound according to claim 1, in which R is a guanidino group.

3. A compound according to claim 1 or claim 2, in which R^2 is an acetyl group.

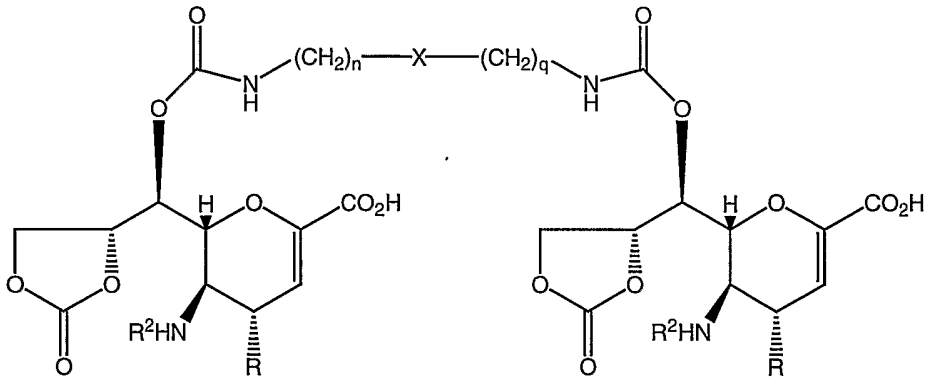
4. A compound according to any one of the preceding claims, in which n and q are the same.

5. A compound according to claim 4, in which the optional substituent on X is alkoxy.

6. A compound according to any one of the preceding claims, which is a derivative modified at one or more of the carboxyl functions, hydroxyl functions, amino groups or guanidine groups.

7. A compound according to any one of the preceding claims, in which the derivative is an alkyl ester, an aryl ester or an acetyl ester.

8. A method for the preparation of the compound of formula (I) according to any one of claims 1 to 7, which comprises the step of deprotecting a compound of formula (II)



(II)

in which R, R^2, n, q and x are as defined in claim 1.

9. A pharmaceutical formulation comprising a compound of formula (I) as defined in any one of claims 1 to 7 or a pharmaceutically acceptable salt or derivative thereof, together with one or more pharmaceutically acceptable carriers.

10. A pharmaceutical formulation according to claim 9, which further comprises one or more other therapeutic and/or prophylactic ingredients.

11. A pharmaceutical formulation according to claim 10, in which the other therapeutic and/or prophylactic ingredient is an anti-infective agent.

12. A pharmaceutical formulation according to claim 11, in which the anti-infective agent is an antiviral or antibacterial agent.

13. A pharmaceutical formulation according to claim 12, in which the anti-bacterial or anti-viral agents are those used to treat respiratory infections.

14. A pharmaceutical formulation according to claim 13, in which the agent is zanamivir, oseltamivir, amantadine, rimantadine, ribavirin and/or FluVax.

15. An inhaler which comprises a compound according to any one of claims 1 to 7 or a formulation according to any one of claims 9 to 14.

16. An inhaler according to claim 15 which is adapted for oral administration as a free-flow powder.

17. An inhaler according to claim 15 which is a metered dose aerosol inhaler.

18. A method for the prevention or treatment of a viral infection comprising the step of administration to a subject in need thereof of an effective amount of a compound of formula (I) as defined in any one of claims 1 to 7.

19. A method according to claim 18, in which the viral infection is an orthomyxovirus or paramyxovirus infection.

20. A method according to claim 18 or claim 19 in which the viral infection is an influenza A or B infection, parainfluenza, mumps or Newcastle disease.

21. A method according to any one of claims 18 to 20 in which the administration is to the respiratory tract by inhalation, insufflation or intranasally or a combination thereof.

22. Use of the compound of formula (I) as defined in any one of claims 1 to 7 for the manufacture of a medicament for the prevention or treatment of a viral infection.

23. Use of the compound of formula (I) as defined in any one of claims 1 to 7 in the prevention or treatment of a viral infection.

24. Use of the compound of formula (I) as defined in any one of claims 1 to 7 as an antiviral agent.

25. A method for the detection of a viral infection which comprises the step of contacting the compound of formula (I) as defined in any one of claims 1 to 7 with a sample suspected of containing the virus.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU02/01526

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: C07D 407/12; A61K 31/351; A61P 31/12, 31/16

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)

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Chem Abs: substructure based on formula (I)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00/55149 A1 (BIOTA SCIENTIFIC MANAGEMENT PTY LTD) 21 September 2000 See whole document and especially page 4 lines 10-29, page 5 lines 1-20, page 14 line 9 to page 15 line 20, and the claims	1-25
P, A	WO 02/20514 A1 (BIOTA SCIENTIFIC MANAGEMENT PTY LTD) 14 March 2002 See whole document	1-25

☐ Further documents are listed in the continuation of Box C ☒ See patent family annex

* Special categories of cited documents:

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"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
16 December 2002	19 DEC 2002
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorised officer G. D. HEARDER Telephone No : (02) 6283 2553

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/AU02/01526

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	200055149	AU	200028966	BR	200008939	CZ	20013274
		EP	1165541	HU	200200190	NO	20014409
		NZ	514041				
WO	200220514	AU	20000010	AU	200185601		

END OF ANNEX