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MONOGRAPH

LANINAMIVIR OCTANOATE

Rec INNM

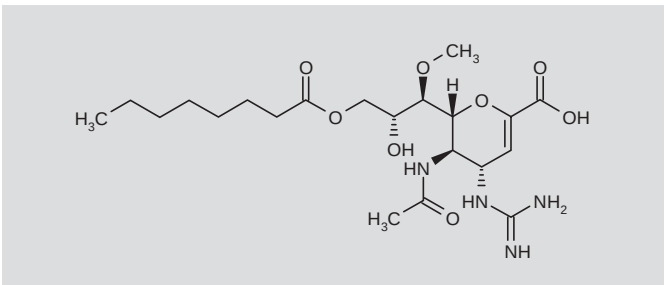
Neuraminidase Inhibitor
Treatment of Influenza

CS-8958
R-118958

5-(Acetamido)-4-(guanidino)-2,6-anhydro-3,4,5-trideoxy-7-*O*-methyl-9-*O*-octanoyl-D-*glycero*-D-*galacto*-non-2-enonic acid

3(*R*)-Acetamido-4(*S*)-guanidino-2-[2(*R*)-hydroxy-1(*R*)-methoxy-3-(octanoyloxy)propyl]-3,4-dihydro-2*H*-pyran-6-carboxylic acid

InChI: 1S/C21H36N4O8/c1-4-5-6-7-8-9-16(28)32-11-14(27)18(31-3)19-17(24-12(2)26)13(25-21(22)23)10-15(33-19)20(29)30/h10,13-14,17-19,27H,4-9,11H2,1-3H3,(H,24,26)(H,29,30)(H4,22,23,25)/t13-,14+,17+,18+,19+/m0/s1



$C_{21}H_{36}N_4O_8$
Mol wt: 472.5325
CAS: 203120-46-1
CAS: 203120-47-2 (monotrifluoroacetate)
EN: 340894

SUMMARY

Influenza is an extremely contagious disease caused by infection with influenza A and B viruses. The available treatments for influenza include two types of drugs: M2 ion channel inhibitors and neuraminidase (NA) inhibitors. Vaccines are also available for prophylaxis. Antiviral resistance and limited antiviral efficacy in severe cases of influenza are the two principal factors that drive the medical need for the development of new and alternative antiviral agents. Multiple potential targets are now being actively explored in the search for new treatments for influenza. In this context, laninamivir and its octanoyl ester prodrug laninamivir octanoate are considered to be a very interesting option. Laninamivir octanoate has shown very weak or no inhibition of viral NA in vitro, but when administrated intranasally in mice it prolonged survival. Laninamivir octanoate is generally well tolerated, and since it remains in the lungs for a long time, it may be ideal for the prophylaxis and treatment of influenza. A single inhalation of laninamivir octanoate may be sufficient to treat influenza and once-weekly inhalation may be sufficient for prevention.

SYNTHESIS*

Laninamivir octanoate can be prepared following several related synthetic strategies:

Condensation of amine (I) with *N,N'*-di-Boc-thiourea (II) using $HgCl_2$ and Et_3N in DMF (1) or with di-Boc-amidinopyrazole (III) in THF gives the protected guanidine derivative (IV), which by deacetylation with methanolic sodium methoxide followed by saponification of the deacylated methyl pyranosoate (V) leads to the carboxylic acid (VI). Alternatively, acid (VI) is prepared by direct hydrolysis of compound (IV) by means of aqueous NaOH or K_2CO_3 in H_2O/MeOH. After conversion of acid (VI) to the corresponding benzhydryl ester (VII) by treatment with diphenyldiazomethane and $BF_3{\cdot}Et_2O$, selective acylation of the primary hydroxyl group with octanoyl chloride (VIII) and Et_3N in CH_2Cl_2 yields the 9-octanoate ester (IX) (1). Finally, compound (IX) is deprotected by removal of *N*-Boc and *O*-benzhydryl protecting groups by treatment with trifluoroacetic acid in CH_2Cl_2, followed by basification of the obtained TFA salt with $NaHCO_3$ (2-8). Scheme 1.

Alternatively, reaction of the 4-amino-5,6-dihydropyran derivative (X) with di-Boc-amidinopyrazole (III) provides the corresponding 4-guanidinodihydropyran, which is hydrolyzed with K_2CO_3 in MeOH/H_2O to give carboxylic acid (VI). In an alternative strategy, carboxylic acid (VI) is prepared by hydrolysis of the 4-amino-5,6-dihydropyrancarboxylate ester (X) with NaOH followed by reaction with the amidinopyrazole derivative (III). Deprotection of di-Boc intermediate (VI) by stirring at 80 °C in MeOH gives laninamivir (XI), which is finally selectively acylated with trimethyl orthooctanoate (XII) in the presence of methanolic HCl. Laninamivir (XI) can also be obtained by hydrolysis of intermediate (X) with NaOH followed by reaction of the resulting free amine (XIII) with pyrazole-1-

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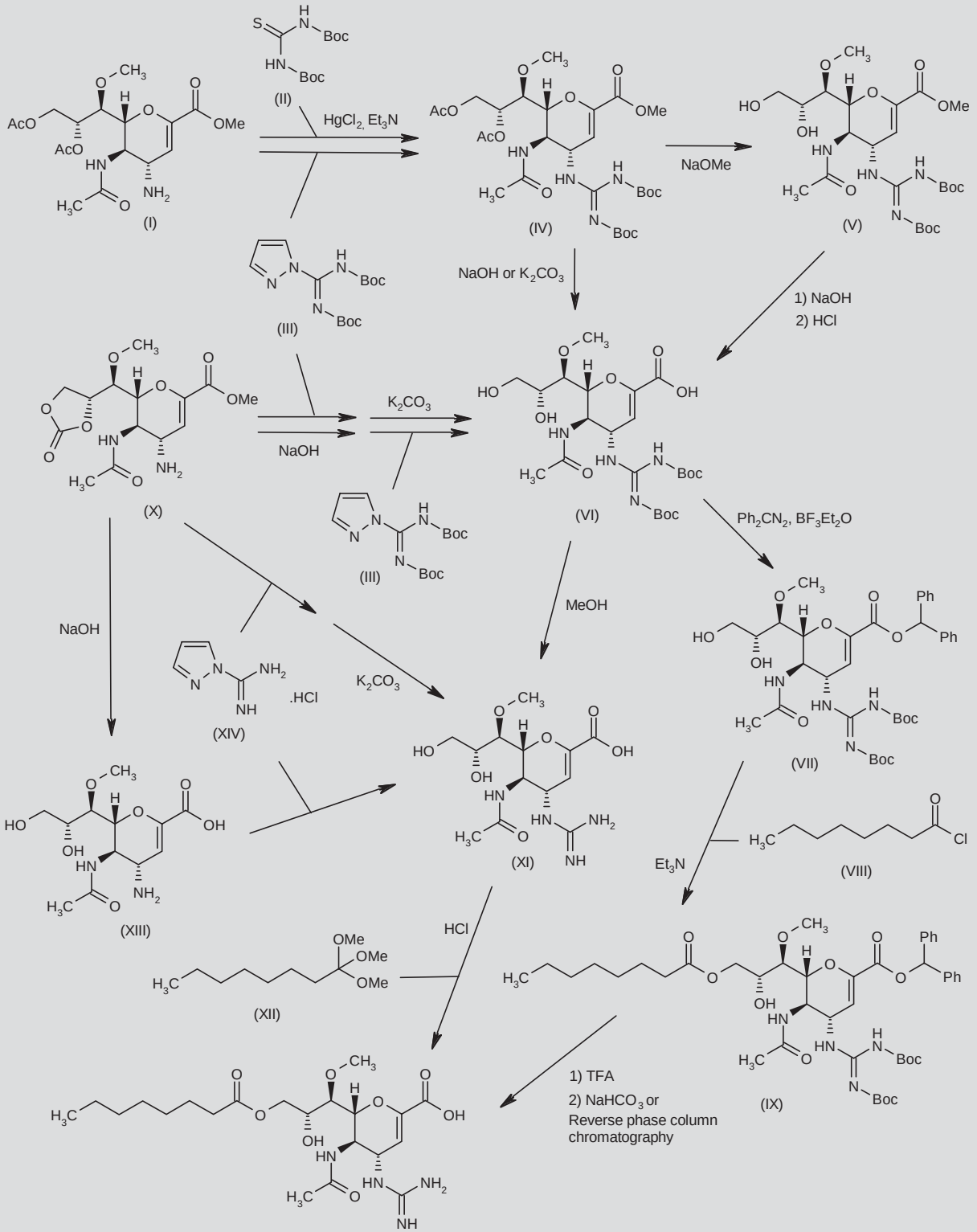
*Synthesis prepared by R. Pandian, R. Castañer. Thomson Reuters, Provença 388, 08025 Barcelona, Spain.

Scheme 1. Synthesis of Laninamivir Octanoate

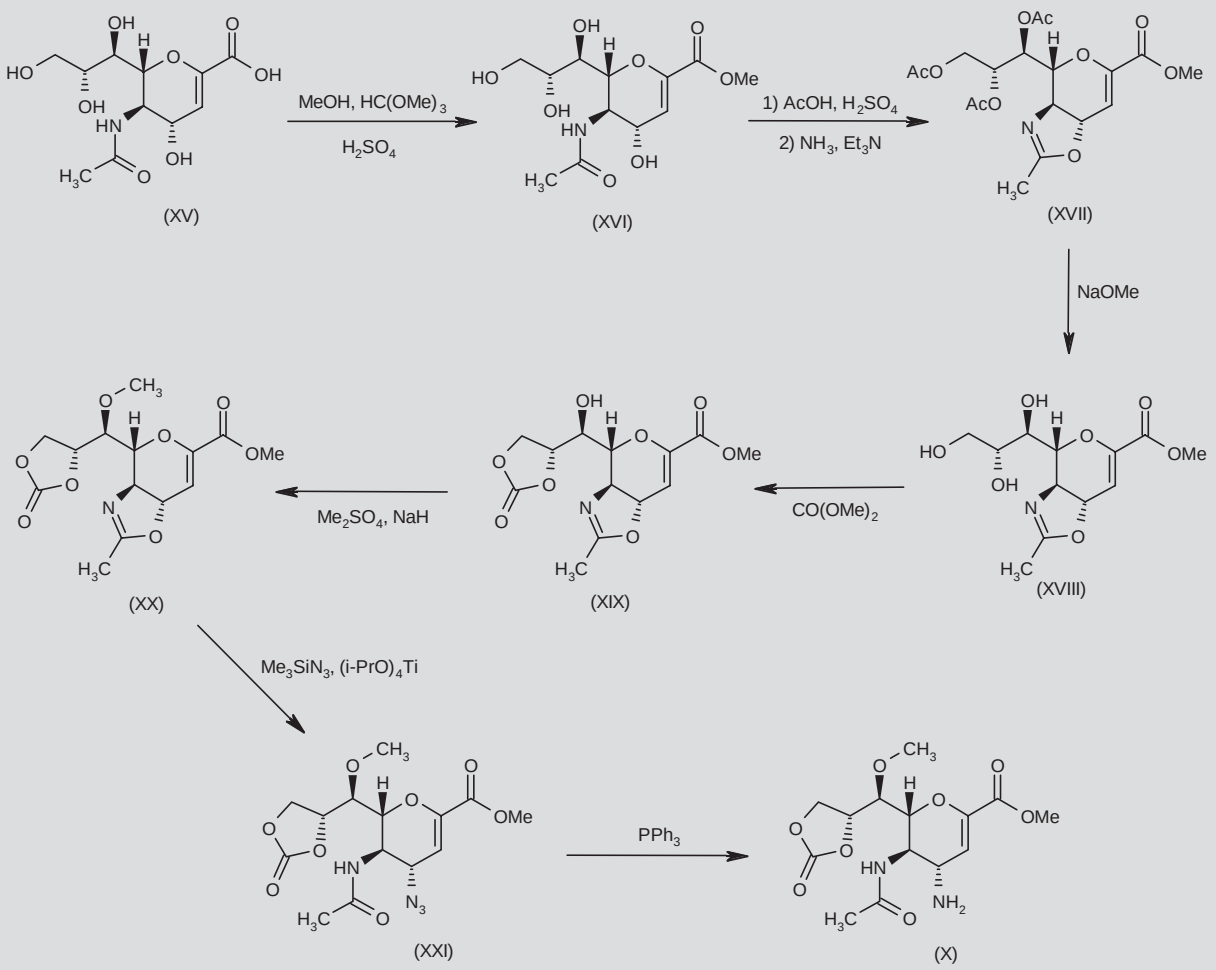


carboxamidine HCl (XIV) or, alternatively, by treatment of 4-amino-5,6-dihydropyran (X) with pyrazole-1-carboxamidine HCl (XIV), and then hydrolysis of the methyl ester with K_2CO_3 in MeOH (3). Scheme 1.

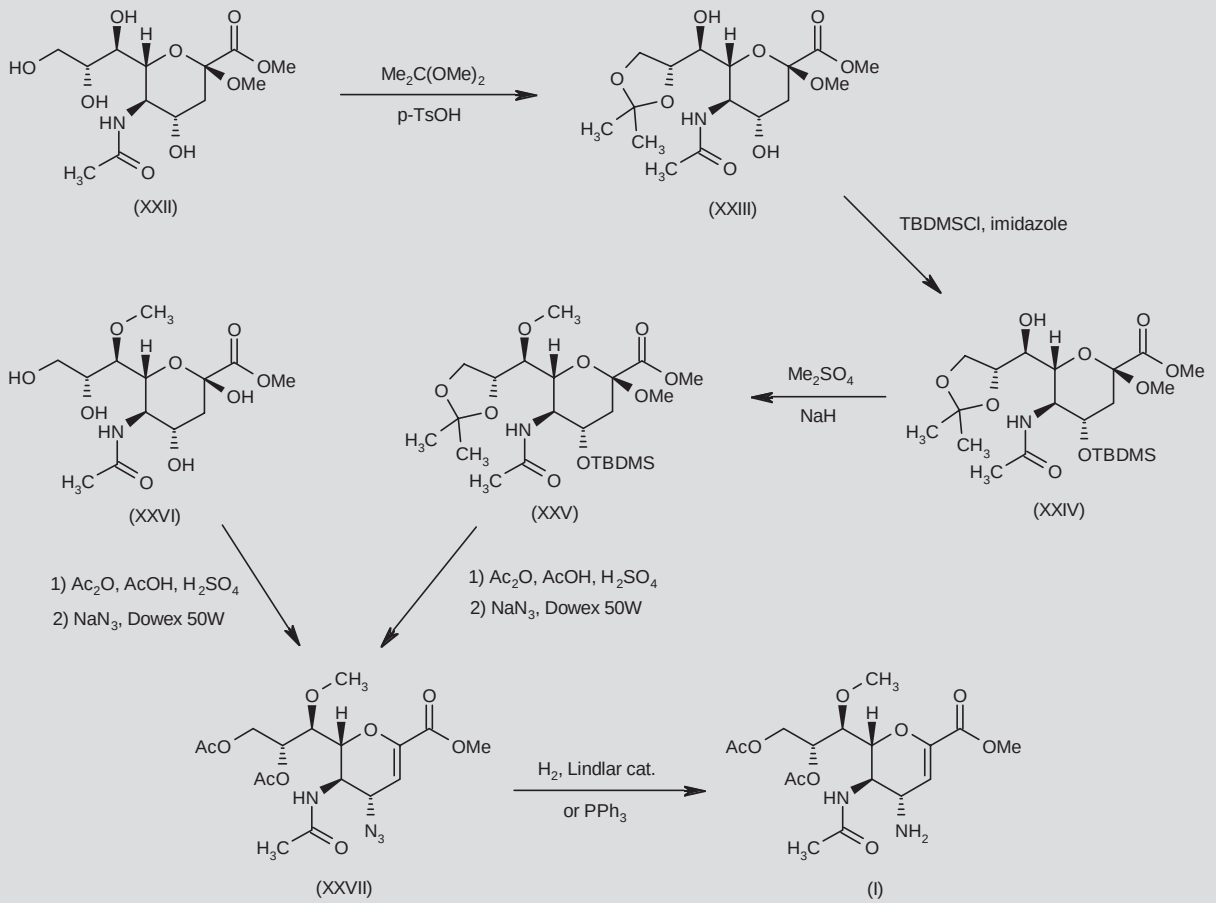
The synthetic precursor (X) is prepared by esterification of *N*-acetyl-2,3-didehydroneuraminic acid (XV) with MeOH in the presence of $HC(OMe)_3$ and H_2SO_4 at 40 °C to yield the methyl ester (XVI), which by *O*-acylation with AcOH by means of H_2SO_4 in heptane at 40 °C followed by cyclization in the presence of NH_3 and Et_3N in toluene/H_2O furnishes the pyrano[3,4-*d*]oxazole derivative (XVII). Hydrolysis of the triacetate (XVII) by means of NaOMe in MeOH gives the corresponding triol (XVIII), which is protected again at the vicinal 8,9-hydroxyl groups as the cyclic carbonate (XIX) by means of $CO(OMe)_2$ in MeOH. Then, methylation of the free C-7 hydroxyl group with Me_2SO_4 in the presence of NaH in THF/DMA gives the corresponding methyl ether (XX), which is submitted to oxazoline ring opening with Me_3SiN_3 in the presence of $(i\text{-PrO})_4Ti$ in *t*-BuOH to furnish the acetamido azide (XXI). Finally, azide (XXI) is reduced by means of PPh_3 in THF or EtOAc (3). Scheme 2.

The synthetic precursor (I) is obtained by ketalization of the α-methyl glycoside of *N*-acetylneuraminic acid methyl ester (XXII) with $Me_2C(OMe)_2$ in the presence of catalytic *p*-TsOH·H_2O in acetone, followed by *O*-protection of the obtained 8,9-*O*-isopropylidene derivative (XXIII) with TBDMSCl by means of imidazole in DMF to yield the protected ether (XXIV) (3). Then, methylation of the remaining free C-7 alcohol group with Me_2SO_4 in the presence of NaH in DMF affords the corresponding methyl ether (XXV) (9). Treatment of either protected (XXV) (9) or unprotected (XXVI) acetamido triol (10) with Ac_2O, AcOH and H_2SO_4 followed by ring opening of the resulting oxazoline with NaN_3 in the presence of Dowex 50W provides azide (XXVII) (9, 10), which is then reduced to amine (I) by means of PPh_3 (3) or H_2 over Lindlar catalyst in EtOH (9, 10). Scheme 3.

Scheme 2. Synthesis of Precursor (X)



Scheme 3. Synthesis of Precursor (I)



The azido acetamide intermediate (XXVII) can be prepared by several alternative methods. Alkylation of the benzyl glycoside of *N*-acetyl-3,6-di-*O*-benzyl-α-D-glucosamine (XXVIII) with iodomethane and NaH in DMF followed by catalytic benzyl group hydrogenolysis of the resulting 4-methoxy derivative (XXIX) with H_2 over Pd/C yields *N*-acetyl-4-*O*-methylglucosamine (XXX), which by subsequent incubation with *N*-acetylneuraminic acid aldolase (*N*-Ac-NAA) in the presence of sodium pyruvate (XXXI) and NaN_3 at pH 10-11 gives the nonuropyranosoic acid derivative (XXXII). Acid (XXXII) is esterified with MeOH in the presence of acidic cation exchange resin (Dowex 50x8) to give methyl ester (XXXIII), which by acetylation with Ac_2O in pyridine and successive treatments with HCl and DBU leads to the non-2-enopyranosonate (XXXIV). Finally, compound (XXXIV) is converted to the azido acetamide intermediate (XXVII) by treatment with boron trifluoride etherate and one equivalent of MeOH in CH_2Cl followed by NaN_3 and Dowex 50x8 resin (1, 7, 8). Scheme 4.

Alternatively, solvolysis of the cyclic carbonate (XX) with methanolic NaOMe yields diol (XXXV), which is then acetylated with AcOH by means of Et_3N and DMAP in EtOAc to give diacetate (XXXVI). Ring opening of oxazoline (XXXVI) with Me_3SiN_3 in the presence of $(i\text{-}PrO)_4Ti$ in *t*-BuOH furnishes the 4-azido-5,6-dihydropyran derivative (XXVII) (3). Scheme 4.

Intermediates (III) and (XII) are prepared as follows:

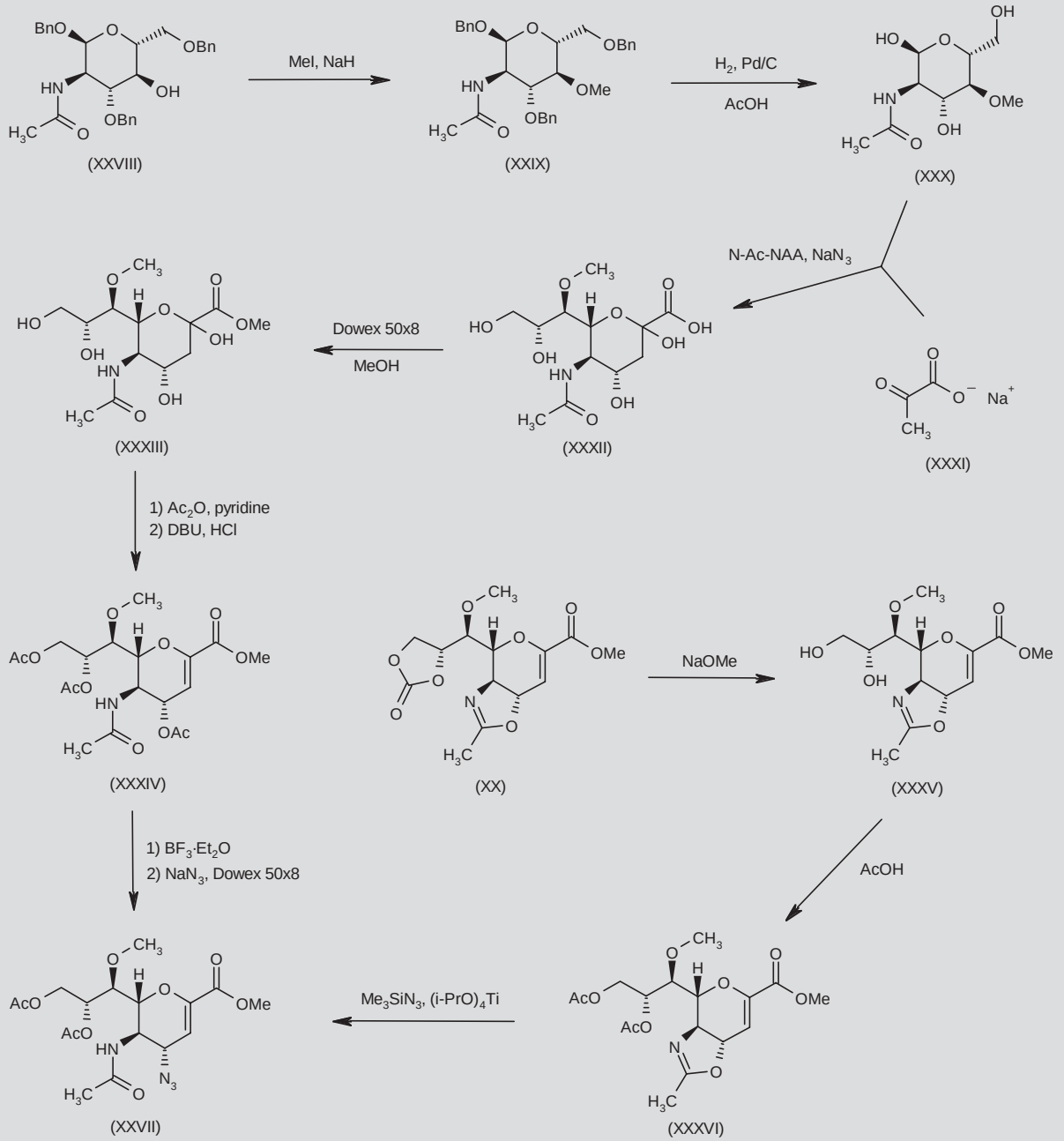
Trimethyl orthooctanoate (XII) is prepared by Pinner reaction of octanonitrile (XXXVII) with MeOH in the presence of HCl in MeOAc followed by treatment of the resulting methyl octanimidate hydrochloride (XXXVIII) with MeOH in methylcyclohexane (3). Scheme 5.

Intermediate (III) can be prepared by consecutive *N*-protection of the amidine nitrogens in pyrazole-1-carboxamidine (XIV), first by treatment with Boc_2O by means of DIEA in DMF, and then reaction of the resulting monoprotected intermediate (XXXIX) with Boc_2O in the presence of NaH in THF (3). Scheme 5.

BACKGROUND

Influenza is a serious and contagious respiratory illness caused by influenza A and B viruses. Influenza A and B viruses infect the respi-

Scheme 4. Synthesis of Intermediate (XXVII)

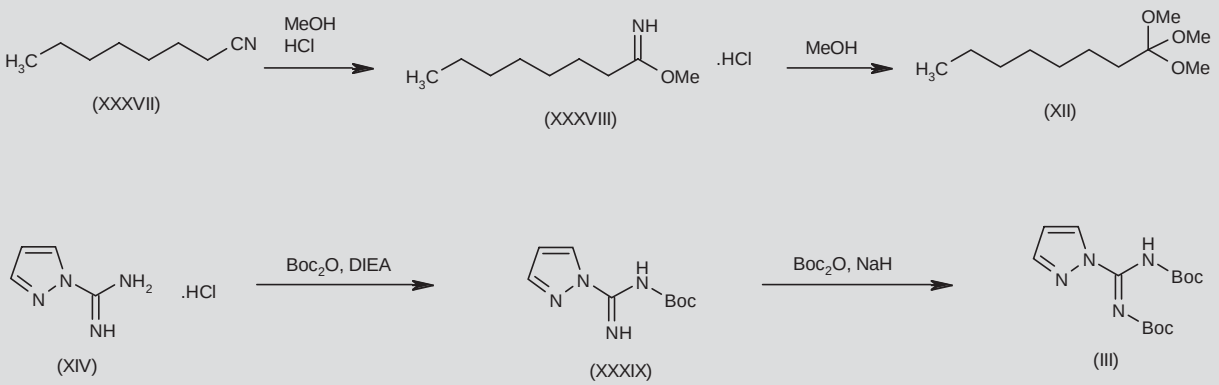


ratory tract and are highly pathogenic for humans. The illness can cause complications, including pneumonia, leading to hospitalization and ultimately death in groups such as young children, the elderly and immunocompromised patients (11).

Influenza A viruses are classified into subtypes based on the antigenicity of hemagglutinin (HA) and neuraminidase (NA) molecules. Sixteen HA subtypes (H1 to H16) and 9 NA subtypes (N1 to N9) have been reported to date, but only a few subtypes have circulated in humans (12).

Vaccination and treatment with antivirals are both available to control human influenza, and although vaccination plays a critical role in influenza prophylaxis, it is not enough, especially against a pandemic virus. Two types of anti-influenza drugs are currently available for the treatment of this disease: M2 ion channel inhibitors (amanta-

Scheme 5. Synthesis of Intermediates (III) and (XII)



dine and rimantadine) and NA inhibitors. The therapeutic use of M2 channel blocker-type drugs is limited, and in some countries, such as the U.S., they are not even recommended (13, 14) because of their side effects, the emergence of antiviral resistance and the lack of activity against influenza B virus replication (15). NA inhibitors bind to the NA surface glycoprotein of newly formed virus particles and prevent their efficient release from the host cell. Two NA inhibitors are licensed for use –zanamivir (Relenza®; inhaled drug, 10 mg/dose) and oseltamivir (Tamiflu®; oral drug, 75 mg/dose)–, both requiring twice-daily administration for effective treatment. Oseltamivir is the predominant choice and is used worldwide for the treatment of influenza. However, the generation and circulation of oseltamivir-resistant seasonal influenza viruses have recently increased (16, 17). This, together with the sporadic appearance of oseltamivir-resistant pandemic 2009 H1N1 viruses (18), as well as H5N1 avian influenza (19), has demonstrated the need for the development of alternative agents.

Laninamivir (also known as R-125489) is a new potent NA inhibitor that is active against various influenza A and B viruses, including oseltamivir-resistant viruses (20-22). Laninamivir octanoate (CS-8958, R-118958) is the octanoyl ester prodrug of laninamivir. Laninamivir octanoate has been reported to have potential advantages compared to the pharmacologically active form and long-lasting antiviral activity (4, 20, 21). In fact, it has been suggested that a single inhaled dose of laninamivir octanoate might be sufficient to treat influenza and a single weekly inhalation might be sufficient for prevention (4, 20-22). Prophylactic and therapeutic efficacy has been observed even against highly pathogenic H5N1 influenza viruses (23).

PRECLINICAL PHARMACOLOGY

In vitro, laninamivir, the active form, inhibited the NA activity of the influenza viruses tested to a level similar to zanamivir, but did not inhibit NAs from other pathogens such as *Vibrio cholerae*, *Clostridium perfringens* and Newcastle disease virus (24).

Some in vitro studies have determined the activity of NA inhibitors against several human and animal influenza viruses, including oseltamivir-resistant strains isolated from patients (20, 22, 24).

Laninamivir inhibited the replication of a broad range of human and animal influenza viruses in Madin-Darby canine kidney (MDCK) cells (20, 22, 24). However, the IC_{50} range was higher than that exhibited by either zanamivir or oseltamivir (20, 22). Laninamivir octanoate showed either no or weak inhibitory activity. When oseltamivir-resistant influenza viruses were tested, IC_{50} ratios were calculated by comparing the mutant viruses and their corresponding wild types. Zanamivir and laninamivir showed significantly lower IC_{50} ratios than oseltamivir against all type A viruses tested. For type B virus mutants, laninamivir showed inhibitory activity within a similar range as zanamivir and oseltamivir carboxylate. Mutant/wild-type ratios were not calculated in this case (20).

In vivo, the efficacy of laninamivir, zanamivir and laninamivir octanoate was investigated by intranasal administration of 0.2 µmol/kg in A/PR/8/34 virus-infected mice. All control mice died at day 6 postinfection (p.i.), but animals administered zanamivir died at day 10 p.i. Although there was no difference in the survival rate between laninamivir and zanamivir, laninamivir octanoate showed a higher survival rate than zanamivir, as 20% of the mice survived at day 20 p.i. Interestingly, all mice treated with laninamivir octanoate survived after 20 days of inoculation (20). Under similar conditions, laninamivir octanoate was also found to be the most effective compound when compared to other ester prodrugs of the biologically active compound laninamivir (4).

In a similar study, zanamivir and laninamivir octanoate were administered either as a single or twice-daily repeated doses in mice infected with the A/PR/8/34 virus (25). The virus titer reductions observed in the lungs were significantly higher than those reported for the control group. No statistically significant differences were observed between the groups treated with a single dose of laninamivir octanoate and a twice-daily dose. The efficacy of a single administration of laninamivir octanoate was also compared to twice-daily administration of oseltamivir. The reductions in virus titers achieved by laninamivir octanoate were significantly higher compared to with repeated administration of oseltamivir. Similar results were obtained by Kiso et al., where laninamivir octanoate was shown

to confer more potent and long-lasting protection of mice than oseltamivir (22).

Laninamivir octanoate or zanamivir was administered intranasally to mice as a single dose of 0.5 µmol/kg on days 10, 7, 4 and 1 before infection (b.i.) with A/PR/8/34 virus to evaluate the efficacy of prophylactic administration. While only the group given zanamivir 1 day b.i. showed significant benefit, all the groups receiving laninamivir octanoate showed significantly prolonged survival (20, 26). In fact, all the mice treated with laninamivir octanoate at day 4 b.i., as well as 50% of the mice treated at day 7 b.i., survived after 20 days of infection. On the contrary, when zanamivir was administered at day 4 b.i., only 20% of the mice survived. None of the mice receiving zanamivir at day 7 b.i. survived after 20 days of infection (26).

Additionally, single administration of various doses of zanamivir or laninamivir octanoate at day 7 b.i. in mice with A/PR/8/34 at 500 PFU significantly prolonged survival compared to the control group. The doses of 5.2 µmol/kg of zanamivir and 0.78 µmol/kg of laninamivir octanoate resulted in survival of one-third and 83% of the treated mice, respectively (24).

Against H5N1 (HN30408cl7) influenza virus, a single intranasal dose of 750 or 1500 µg/kg of laninamivir octanoate significantly protected mice even when treated 7 days prior to infection (21).

PHARMACOKINETICS AND METABOLISM

The tissue distribution of laninamivir octanoate was studied after a single intranasal administration to mice in order to evaluate its contribution to the increased pharmacological effect. Laninamivir octanoate was metabolized/hydrolyzed to laninamivir and then remained in the respiratory tract, the primary site of influenza virus infection and replication, for a long time (21, 27, 28). In fact, when unlabeled laninamivir octanoate was administered, laninamivir was slowly eliminated from the lung with a half-life ($t_{1/2}$) of 41.4 h after reaching peak plasma concentrations (C_{max}) at 3 h after dose. This suggested that laninamivir octanoate would be deposited not only in the respiratory tract but also in the liver, where it would be converted/hydrolyzed to laninamivir (21, 28).

A similar tissue distribution profile was observed in rats when laninamivir octanoate was administered intratracheally (28, 29). The plasma concentrations reached a maximum at 0.5-0.7 h after dose and disappeared with a $t_{1/2}$ of 0.8-0.9 h, while the C_{max} of laninamivir was reached 1.0-2.0 h after dose and its $t_{1/2}$ was considerably longer than after i.v. administration of laninamivir at the same dose. Oral administration of laninamivir octanoate resulted in very low plasma concentrations and bioavailability of both laninamivir octanoate and laninamivir (28).

In humans, the pharmacokinetics of laninamivir octanoate have been studied in both healthy and renally impaired male volunteers.

In a double-blind, placebo-controlled, ascending-single-dose study conducted in healthy male volunteers, laninamivir octanoate was only detected after doses of 5 and 10 mg. However, the concentrations detected were around the limit of quantification up to 3 h after dose. Laninamivir was not detected in plasma, but laninamivir octanoate was detected in urine up to 12 h after doses of 1, 2 and 5 mg, and up to 24 h after a dose of 10 mg (30).

A similar study was carried out where single doses of 5, 10, 20, 40, 80 or 120 mg were administered to the subjects. In this study, laninamivir octanoate appeared rapidly in plasma after inhalation, with median t_{max} values of 0.5-1 h. The plasma concentrations decreased with a $t_{1/2}$ of 1.7-10.7 h, with a median of 1.8 h. The t_{max} of the pharmacologically active form laninamivir was 4.0 h, declining slowly, with $t_{1/2}$ increasing with the dose of laninamivir octanoate administered. The half-life of the urinary excretion rates of laninamivir at high doses was almost comparable to the plasma half-life (31).

A second group of healthy male volunteers was treated with twice-daily doses of 20 and 40 mg for up to 3 days. Similar results as in the previous study were observed for the t_{max} of both laninamivir octanoate and laninamivir. Furthermore, the $t_{1/2}$ was longer with the higher dose and the renal clearance of both compounds was comparable to that observed in the single-dose study (31).

Subjects with normal, mild, moderate and severe renal impairment participated in an open-label, single-dose study, where a single 20-mg dose of laninamivir octanoate was administered. Interestingly, the results obtained for the t_{max} of laninamivir octanoate and the $t_{1/2}$ of laninamivir in the normal subjects were comparable to those obtained in the previous single-dose study, while the $t_{1/2}$ of laninamivir octanoate and the t_{max} of laninamivir were slightly higher. The subjects with mild and/or moderate renal impairment showed similar values for the t_{max} and $t_{1/2}$ of both laninamivir octanoate and laninamivir. In contrast, subjects with severe renal impairment exhibited a greater t_{max} for laninamivir compared to normal subjects, but the $t_{1/2}$ of laninamivir was not affected by renal impairment. The elimination of laninamivir may reflect the slow release of this compound from retaining tissues to plasma, which would not be affected by renal impairment and would result in no change in the terminal half-life of laninamivir among the subjects (32).

SAFETY

No adverse effects attributed to treatment with laninamivir octanoate have been observed in single- and repeated-dose toxicity studies in mice, rats or dogs. Laninamivir octanoate also showed no genotoxic activity (33).

Single and multiple inhaled doses of laninamivir octanoate were generally well tolerated in healthy male volunteers (31, 32). Clinical evaluation and laboratory measurements were studied to assess the safety and tolerability of the prodrug. The adverse events reported were not considered to be related to the study drug (32). No clinically significant changes in laboratory tests were reported (31, 32).

A single dose of laninamivir octanoate was also well tolerated in subjects with mild, moderate and severe renal impairment (33). In this study, three adverse events, i.e., diarrhea and transiently elevated alanine aminotransferase and aspartate aminotransferase, were considered to be related to the study drug. This suggested that increasing renal dysfunction leads to increasing systemic exposure to laninamivir, particularly in severe renal impairment.

No clinically significant changes in electrocardiograms were reported in any participant during the studies (31-33).

In addition, laninamivir octanoate proved to be well tolerated in children with oseltamivir-resistant influenza A (H1N1) virus infection,

although some gastrointestinal events, including diarrhea, nausea and vomiting, occurred. The events were mild to moderate and resolved within a few days. Psychiatric disorders were observed in 3 of 122 patients (approximately 2.4%), but were mild and did not require any treatment (34).

CLINICAL STUDIES

Only one study has been conducted so far on the safety and antiviral activity of laninamivir octanoate in humans, and more interestingly, in children. Influenza virus infection is actually one of the major causes of pediatric hospitalizations in the winter season (35, 36), and schoolchildren and children who attend day care centers are the principal transmitters of influenza in the community (37).

A double-blind, randomized, controlled trial was conducted in children 9 years of age who presented febrile influenza symptoms of no more than 36 h duration. Two groups received laninamivir octanoate (20 or 40 mg) as a single inhaled dose, while the third group was treated with oseltamivir 2 mg/kg orally twice daily for 5 days. Laninamivir octanoate markedly reduced the median time to alleviation of illness in comparison to oseltamivir in patients infected with oseltamivir-resistant influenza A (H1N1). On the other hand, there were no significant differences in the time to alleviation of illness between the laninamivir octanoate groups and the oseltamivir group against influenza A (H3N2) or B infection (34).

SOURCE

Daiichi Sankyo Co., Ltd. (JP).

DISCLOSURES

The author states no conflicts of interest.

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