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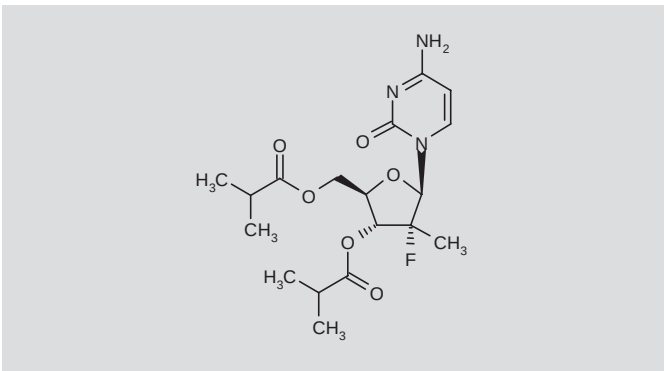
R-7128

RNA-Directed RNA Polymerase (NS5B) Inhibitor
Treatment of Hepatitis C Virus Infection

RO-5024048

2'-Deoxy-2'(*R*)-fluoro-2'-methyl-3',5'-di-*O*-isobutyrylcytidine

InChI=1/C18H26FN3O6/c1-9(2)14(23)26-8-11-13(28-15(24)10(3)4)18(5,19)16(27-11)22-7-6-12(20)21-17(22)25/h6-7,9-11,13,16H,8H2,1-5H3,(H2,20,21,25)/t11-,13-,16-,18-/m1/s1



$C_{18}H_{26}FN_3O_6$
Mol wt: 399.4139
CAS: 940908-79-2
EN: 454918

ABSTRACT

Hepatitis C virus (HCV) infection is almost certainly more widespread than is commonly thought, and chronic disease can be severely debilitating. The standard treatment, the combination of pegylated interferon alfa and ribavirin, is not effective in all patients, with considerable variation depending on HCV genotype and efficacy particularly limited in genotype 1 patients. The standard of care is also limited by adverse effects. One approach to improving treatment is the use of nucleoside analogues targeting HCV NS5B protein, an RNA-dependent RNA polymerase necessary for viral replication. Several of these agents have reached the stage of clinical investigation, although success has been limited. One agent that showed particular promise in preclinical evaluation was PSI-6130 (R-1656), which demonstrated potent inhibition of HCV replication in vitro. PSI-6130 was then evaluated in a phase I study in which it was generally well tolerated. Its pharmacokinetic characteristics are less than optimal, however, and this led to the preparation of a PSI-6130 prodrug, R-7128. In studies in humans, R-7128 has demonstrated the ability to efficiently deliver PSI-6130 and reduce HCV RNA in HCV-infected patients when given as monotherapy and combined with pegylated interferon and ribavirin, without evidence of the development of resistance. It is now entering phase II clinical evaluation in combination with pegylated interferon and ribavirin.

SYNTHESIS

R-7128 can be synthesized by the following alternative strategies:

Acylation of cytidine (I) with benzoic anhydride in DMF provides N^4-benzoylcytidine (II), which is regioselectively protected at the 3'- and 5'-hydroxyl groups with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane in pyridine, giving the cyclic siloxane derivative (III). Subsequent Swern oxidation of the 2'-alcohol group of compound (III) using DMSO, TFAA and Et_3N followed by addition of methyllithium to the obtained ketone (IV) and desilylation with AcOH leads to the 2'-*C*-methylcytidine derivative (V). After reprotection of the primary and secondary hydroxyl groups of the cytidine (V) with benzoyl chloride in pyridine, the tertiary hydroxyl of compound (VI) is fluorinated using DAST in cold toluene to give the benzoyl-protected 2'-deoxy-2'-fluoro-2'-methylcytidine (VII) (1-3). In a different strategy, the nucleoside derivative (VII) is prepared by coupling of the methyl (VIIIa) (1-3) or acetyl (VIIIb) glycosides (4) or the glycosyl chloride (VIIIc) (5) with silylated N^4-benzoylcytosine (IX) by means of either trimethylsilyl triflate or tin tetrachloride. The tribenzoylated precursor (VII) is then deprotected with methanolic NaOMe (1, 2) or ammonia (3, 4) to obtain PSI-6130 (X), which is finally esterified with isobutyryl chloride (XI) in the presence of DMAP and Et_3N in THF/H_2O (6). Scheme 1.

The methyl ribofuranose derivative (VIIIa) is prepared by addition of methyllithium to the furanone (XII) in ethyl ether to give the 2-*C*-methylarabinofuranose (XIII), which is converted to the 2-deoxy-2-fluoro-2-methylribofuranose derivative (XIV) by treatment with DAST in CH_2Cl_2. After debenzylation of compound (XIV) by transfer hydrogenolysis with Pd/C and cyclohexene, the deprotected compound (XV) is finally esterified with benzoyl chloride and pyridine (1-3). Scheme 2.

The glycosyl intermediates (VIIIb) and (VIIIc) can be prepared by several related methods. Wittig condensation of 2,3-*O*-isopropylidene-

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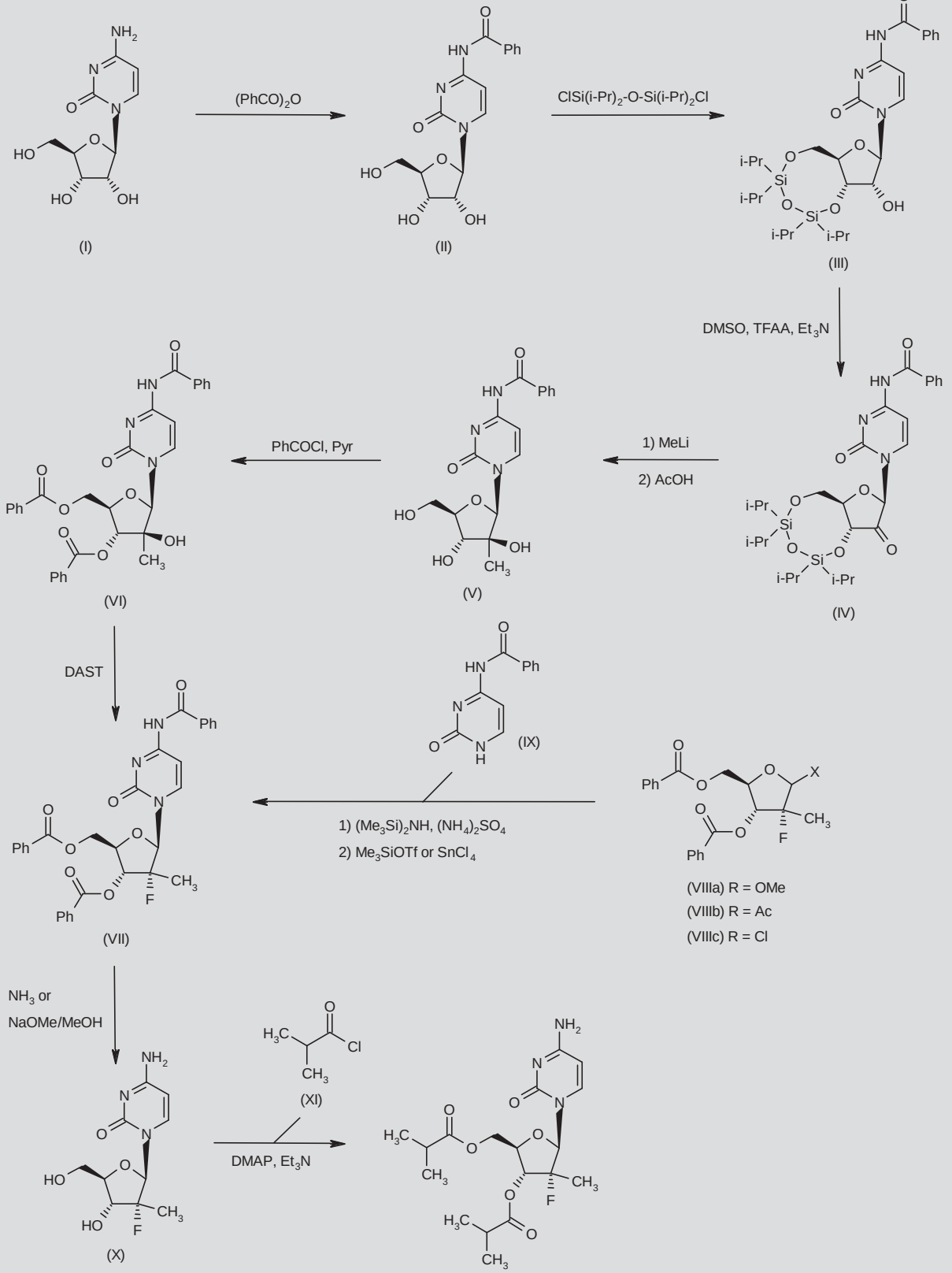
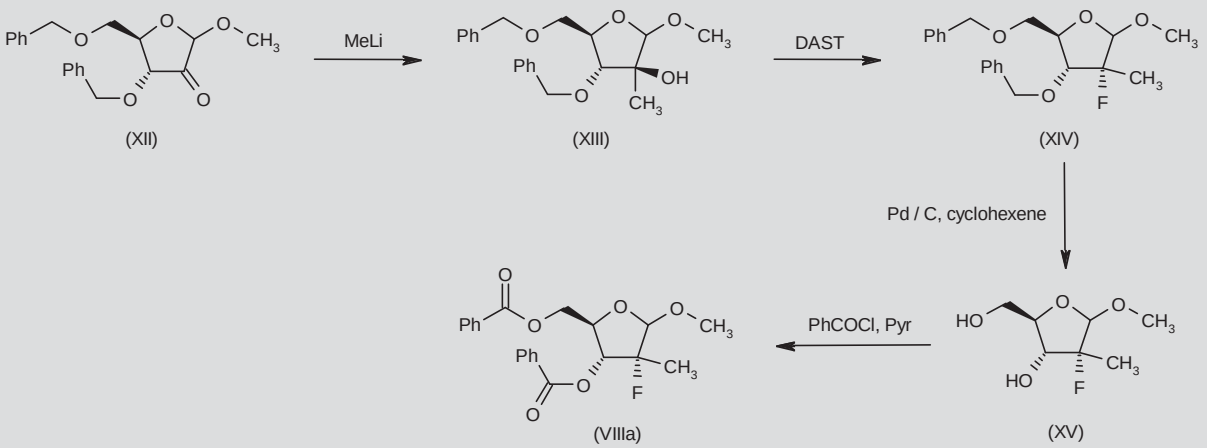
Scheme 1. Synthesis of R-7128



Scheme 2. Synthesis of Intermediate (VIIIa)



D-glyceraldehyde (XVI) with (1-carbethoxyethylidene)triphenylphosphorane (XVII) in dry CH_2Cl_2 provides the unsaturated ester (XVIII) (7). Diastereoselective dihydroxylation of (XVIII) to give diol (XIX) is accomplished using a variety of reagents, including Sharpless oxidation with AD-mix-beta/methanesulfonamide (7), NMMO or *t*-BuOOH and catalytic OsO_4, $K_3Fe(CN)_6$ and catalytic $K_2OsO_2(OH)_4$, tetradecyltrimethylammonium permanganate (TDTAP) and $KMnO_4$, optionally in the presence of crown ether or $RuCl_3/CeCl_3/NaIO_4$ (4) and $NaMnO_4/NaHCO_3$ (5). After conversion of dihydroxy ester (XIX) to the cyclic sulfite (XX) by means of $SOCl_2$ and Et_3N, oxidation employing TEMPO/NaOCl or $RuCl_3/NaIO_4$ provides the corresponding sulfate ester (XXI). Ring opening of the cyclic sulfate (XXI) with HF-triethylamine complex or with tetrabutyl- or tetraethylammonium fluoride produces the respective ammonium fluorosulfates (XXIIa-c), which undergo cyclization to fluorolactone (XXXIII) under acidic conditions. Subsequent acylation of the dihydroxy ribonolactone (XXIII) with benzoyl chloride and DMAP followed by reduction of the resulting dibenzoyloxy lactone (XXIV) with either Red-Al or $LiAlH(O\text{-}t\text{-}Bu)_3$ yields lactol (XXV) (4, 5), which is either acetylated to (VIIIb) using Ac_2O and DMAP in THF (4) or chlorinated to (VIIIc) with sulfuryl chloride and TBAB (5). Alternatively, cyclization and deprotection of dihydroxy ester (XIX) with ethanolic HCl leads to 2-methyl-D-arabino-γ-lactone (XXVI), which, after protection as the dibenzoate ester (XXVII) with benzoyl chloride in dry pyridine, is fluorinated to (XXIV) using either diethylaminosulfur trifluoride (DAST) or bis(2-methoxyethyl)aminosulfur trifluoride (Deoxofluor) in THF (7). In a further method, dihydroxy ester (XIX) is selectively acylated at the secondary hydroxyl group with benzoyl chloride in pyridine to produce compound (XXVIII), which is fluorinated to (XXIX) by treatment with DAST or Deoxofluor in THF. Deprotection and cyclization of compound (XXIX) with TFA in hot CH_3CN/H_2O gives 3-*O*-benzoyl-2-methyl-2-deoxy-2-fluoro-D-ribono-γ-lactone (XXX), which is further benzoylated to (XXIV) by means of benzoyl chloride in the presence of DMAP and pyridine in AcOEt (7). Scheme 3.

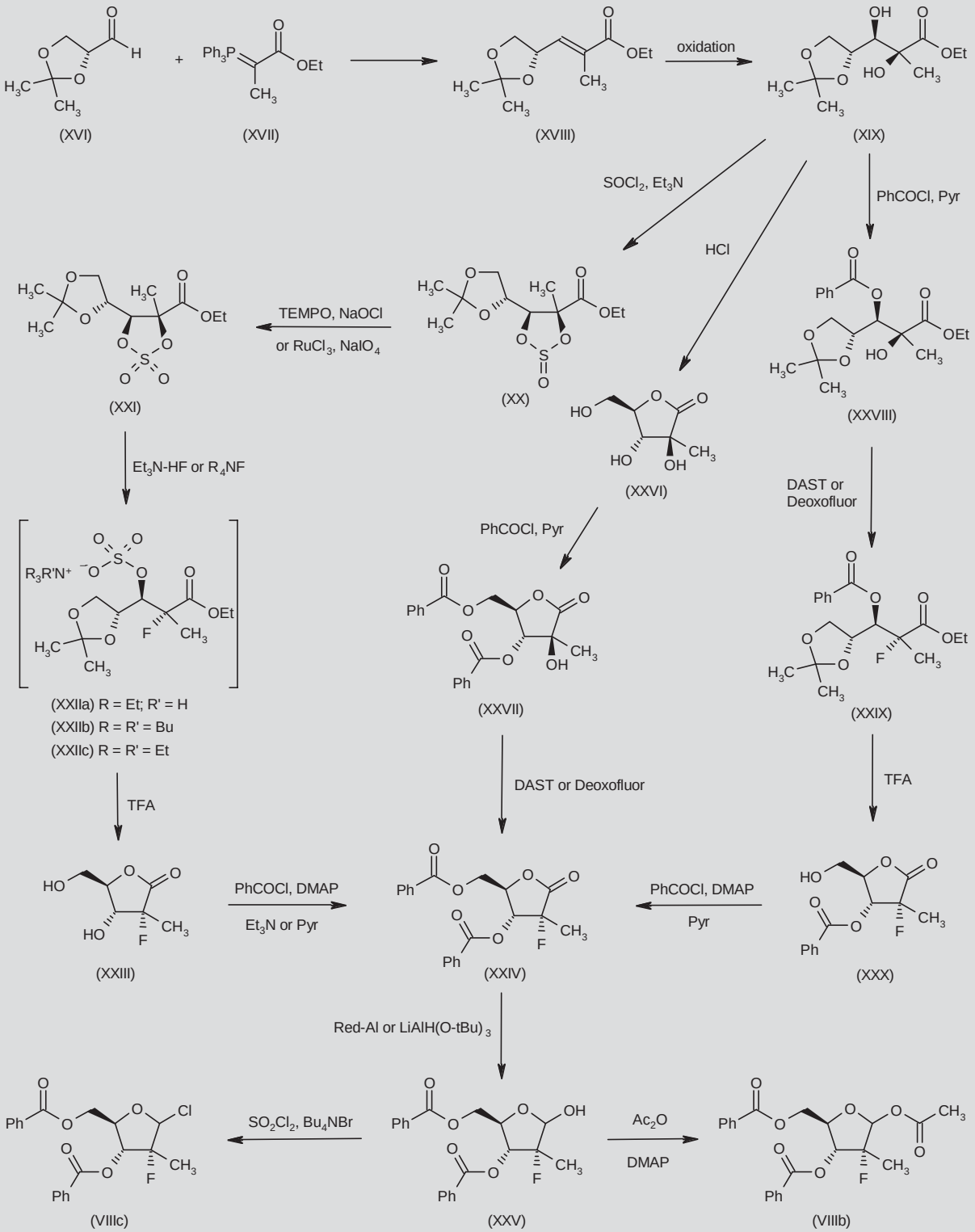
The synthetic precursor (XXIV) can be prepared by a number of alternative methods.

Condensation of 2,3-*O*-isopropylidene-D-glyceraldehyde (XVI) with ethyl 2-fluoropropionate (XXXI) by means of LDA in cold THF gives a mixture of diastereomeric fluoro-hydroxy esters (XXXIIa) and (XXXIIb) along with minor amounts of isomer (XXXIIc). After removal of the undesired (2*S*,3*R*)-isomer (XXXIIb) by selective enzymatic hydrolysis with *Candida antarctica* lipase form B (CALB), the remaining mixture of fluoro-hydroxy esters (XXXIIa) and (XXXIIc) undergoes cyclization to the respective lactones (XXIII) and (XXXIII) upon heating with aqueous H_2SO_4 (8). Esterification of the mixture of diols (XXIII) and (XXXIII) with benzoyl chloride and DMAP in Et_3N/CH_3CN followed by recrystallization of the resulting dibenzoates from isopropanol provides compound (XXIV) (8, 9). Scheme 4.

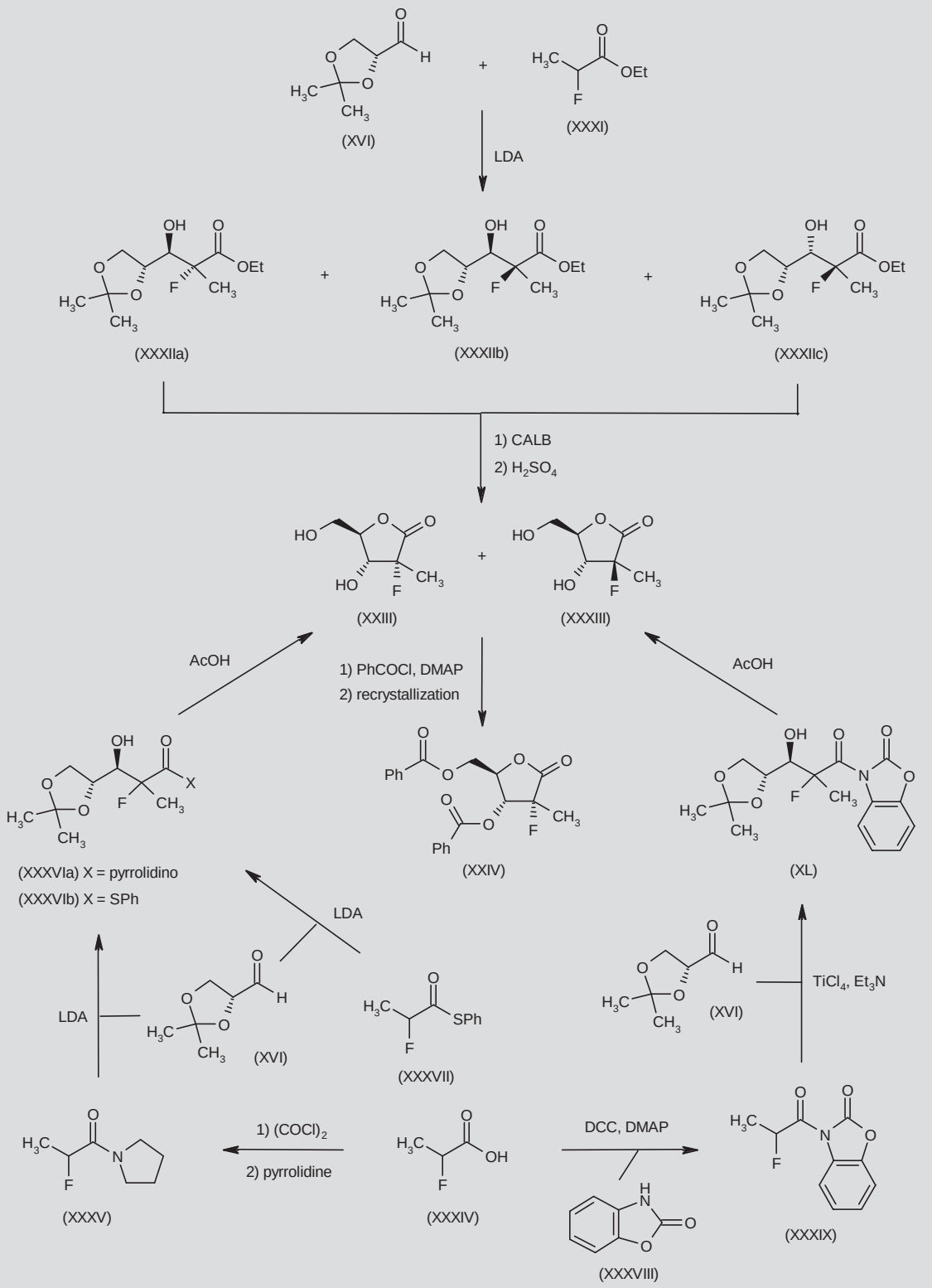
In a related method, chlorination of 2-fluoropropionic acid (XXXIV) with oxalyl chloride and catalytic DMF in toluene followed by reaction with pyrrolidine leads to amide (XXXV). Subsequent condensation of fluoro-amide (XXXV) with glyceraldehyde acetonide (XVI) by means of LDA in THF produces fluoro-hydroxyamide (XXXVIa), which upon heating with aqueous AcOH generates lactone (XXIII) along with minor amounts of its diastereoisomer (XXXIII). Similarly, condensation of *S*-phenyl 2-fluoropropanethioate (XXXVII) with aldehyde (XVI) yields hydroxy thioester (XXXVIb), which undergoes cyclization to lactones (XXIII) and (XXXIII) under acidic conditions (9). Scheme 4.

In a further related method, acyl benzoxazolone (XXXIX) —prepared by coupling 2-fluoropropionic acid (XXXIV) with benzoxazolone (XXXVIII) in the presence of DCC and DMAP— is condensed with glyceraldehyde acetonide (XVI) by means of $TiCl_4$ and Et_3N in CH_2Cl_2 to furnish adduct (XL). Subsequent hydrolysis and cyclization of (XL) in $AcOH/H_2O$ at 90 °C produces the mixture of lactones (XXIII) and (XXXIII) (9). Scheme 4.

Scheme 3. Synthesis of Intermediates (VIIIb) and (VIIIc)



Scheme 4. Synthesis of Precursor (XXIV)



BACKGROUND

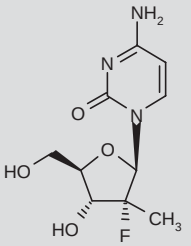
Hepatitis C virus (HCV) infection is exceedingly common, affecting millions of people around the world and as many as one in five people in Egypt, the country with the highest incidence of infection (10). Infected individuals may appear perfectly healthy for many years but are most likely to progress to chronic hepatitis, as 75-85% of acutely infected individuals do so (11). At this stage, symptoms may become apparent, including jaundice, fatigue, abdominal pain, dark urine, anorexia, intermittent nausea and vomiting. Such patients are also at an increased risk of developing liver cancer (12), may develop serious liver damage, may have to undergo liver transplantation or may die from the disease.

HCV infection is the only curable chronic human viral infection (13). The standard treatment at present is the combination of pegylated interferon alfa and ribavirin, but many patients with HCV genotype 1 – perhaps half – do not achieve a sustained virological response (undetectable HCV RNA 6 months after the end of therapy) with this treatment (13, 14). The distribution of HCV by genotype varies by region and over time, but in some areas, genotype 1 infection is predominant (15). The introduction of the pegylated form of interferon for combination with ribavirin appears to have increased efficacy but at a cost of increased adverse events compared to interferon combined with ribavirin, as shown in a Cochrane systematic review of 18 randomized trials in 4,811 patients. The increased adverse events included neutropenia, thrombocytopenia, arthralgia and injection-site reactions (16).

The management of chronic hepatitis C is complicated still more by coinfection with HIV. The incidence of HIV and HCV coinfection is increasing, primarily in injected drug users, and the U.S. Public Health Service has recognized HCV as an opportunistic infection of HIV, meaning that the disease acts more aggressively in HIV-infected patients (17, 18).

The figure of 170 million people affected worldwide repeatedly draws the eye during a perusal of HCV literature (19), and this figure represents 3% of the world's population. There are thus many people who need more effective and tolerable treatment. This explains why there are presently 79 drugs in active clinical development for HCV and 52 in preclinical development. For such an important and pervasive illness, it may be surprising that the first drug marketed for HCV (interferon beta; Serono/Toray) was launched in 1985. The most recent drug to reach the market (interferon alfa, Multiferon; Viragen) did so in 2004.

Some of the efforts aimed at meeting the need for improved HCV treatments target the HCV NS5B protein, an RNA-dependent RNA polymerase required for viral replication, with nucleoside analogues (2, 20). Drugs of this type that have reached clinical development include PSI-6130 and its prodrug R-7128, valopicitabine, MK-0608 and R-1626, a prodrug of R-1479. Clinical investigation of R-1626 was terminated after high rates of grade 4 neutropenia and HCV infection relapse were seen in a phase IIA study (21). Valopicitabine also reached phase II development, but further investigation was put on hold based on the overall risk/benefit ratio seen in clinical testing. MK-0608 entered phase I investigation but did not feature in Merck & Co.'s 2009 pipeline (22).



PSI-6130 (R-1656, **1**)

PSI-6130 (R-1656, **1**) was developed through a partnership formed by Roche and Pharmasset to synthesize nucleoside polymerase inhibitors for HCV treatment. The compound potently inhibited HCV replication in subgenomic HCV replicon cells (EC_{90} = 4.6 μM) (23). In a phase I study in 24 healthy male volunteers, single oral doses of PSI-6130 were generally well tolerated and no serious adverse events were seen with doses up to 3000 mg (24). Pharmacokinetic studies in rhesus monkeys, however, revealed slow and incomplete absorption and an oral bioavailability of 24.0%; total bioavailability, including the parent drug and the deaminated metabolite PSI-6206, was 64%. An attempt at the design of a prodrug yielded PSI-6419, which did not produce high levels of PSI-6130 in serum. Further efforts to improve absorption and drug exposure and to limit or eliminate the formation of the uridine metabolite led to the identification of another PSI-6130 prodrug, R-7128 (25).

R-7128 received fast track designation from the FDA in 2007, and a phase II study of combination therapy with pegylated interferon and ribavirin in treatment-naïve, genotype 1 or genotype 4 HCV-infected patients is scheduled to begin soon (26).

PRECLINICAL PHARMACOLOGY

R-7128 was designed to improve the delivery of the oral cytidine nucleoside analogue inhibitor of NS5B polymerase PSI-6130 (R-1656), which showed an EC_{90} of 4.6 μM in the subgenomic HCV replicon assay. PSI-6130 was not active against bovine diarrhea virus and had little activity against other flaviviruses, indicating that it is a specific inhibitor of HCV. PSI-6130 also showed little or no cytotoxicity against a variety of cell types and no mitochondrial toxicity. When toxicity was evaluated in mice, the no-effect dose was found to be 100 mg/kg/day i.p. or more (27). In vitro experiments including an assay of inhibition of wild-type and S282T mutant replicons indicated that PSI-6130 was associated with a high genetic barrier to resistance (28). Enhanced antiviral activity was seen when PSI-6130 or the active moiety of another nucleoside inhibitor, R-1626, was combined with ITMN-191, an inhibitor of HCV NS3/4A protease activity, in in vitro experiments. The combinations were also associated with suppression of ITMN-191-resistant variants (29).

PHARMACOKINETICS AND METABOLISM

The pharmacokinetics of PSI-6130 after administration of single oral doses of R-7128 were examined in a study in healthy volunteers (30). Plasma exposure to R-7128 was negligible and that to PSI-6130, PSI-6206 and the uridine metabolite of PSI-6130 increased with

increasing dose. At doses of 500, 1500, 4500, 6000 and 9000 mg the C_{max} values for PSI-6130 were 4.5, 7.5, 10.9, 18.1 and 25.8 µg/mL, respectively, median t_{max} values were 0.9, 2.0, 2.0, 2.5 and 4.0 h, respectively, $t_{1/2}$ was 4.84, 5.57, 5.59, 5.88 and 6.5 h, respectively, and $AUC_{(0\text{-}inf)}$ values were 22.7, 58.6, 103, 152 and 280 µg.h/mL, respectively. In subjects administered a dose of 1500 mg with food the respective C_{max}, median t_{max}, $t_{1/2}$ and $AUC_{(0\text{-}inf)}$ values were 8.9 µg/mL, 3.0 h, 5.16 h and 71.3 µg.h/mL.

Pharmacokinetic data from a study in patients with genotype 1 HCV infection failing prior interferon therapy administered multiple doses of R-7128 (750 or 1500 mg once or twice daily for 14 days) showed dose-dependent but not dose-proportional exposure to PSI-6130, while plasma concentrations of R-7128 were below the limit of detection. The $t_{1/2}$ for PSI-6130 was approximately 5 h and 20 h for PSI-6206 (31, 32).

A new formulation of R-7128 (1500 mg b.i.d.) was used in a study in patients with HCV genotype 2 and 3 infection treated with R-7128, peginterferon alfa-2a and ribavirin. This formulation yielded higher plasma exposure ($AUC_{(0\text{-}12)}$ = 83,667.21 ng.h/mL, C_{max} = 14,309.95 ng/mL) compared to the original formulation (respective values of 78,560.57 ng.h/mL and 11,990.86 ng/mL) (33, 34).

SAFETY

The safety and tolerability of R-7128 and its metabolites were assessed in healthy volunteers who received single ascending doses, with the prodrug found to be generally safe and well tolerated at doses up to 9000 mg. In each dose group, 6 subjects received oral R-7128 doses of 500, 1500, 4500, 6000 or 9000 mg and 2 received placebo, with a separate group of 6 subjects given 1500 mg in the fed state. All adverse events were mild to moderate in severity and the most common were headache (3), sunburn (2), nasal congestion (2) and sore throat (2). There were no discontinuations due to adverse events. No laboratory abnormalities were detected and there were no clinically significant changes in vital signs or serial ECGs. Exposure to PSI-6130 was good (30).

These results led to the initiation of a multiple-dose study of the safety and tolerability of R-7128 in HCV genotype 1 patients. Forty patients received oral R-7128 750 mg once daily, 1500 mg once daily, 750 mg b.i.d. or 1500 mg b.i.d. or placebo for 14 days. All patients had failed interferon treatment and were noncirrhotic. Adverse events were more common in placebo-treated patients and no serious adverse events were reported. There were also no adverse events requiring dose modification, dose-related gastrointestinal adverse events or hematological changes. No major organ or other acute toxicities were seen, and the maximum tolerated dose was not identified. The most common adverse events were headache, diarrhea, dry mouth, nausea, fatigue, tiredness and upper respiratory tract infections (31, 32).

Combination therapy with R-7128, peginterferon alfa-2a and ribavirin was evaluated in a randomized, double-blind, placebo-controlled, multicenter trial in treatment-naïve genotype 1 patients. Patients (N = 50) received R-7128 500 or 1500 mg b.i.d. or placebo with peginterferon and ribavirin for 4 weeks followed by peginterferon and ribavirin without R-7128 for a further 4 weeks. The triple combination was safe and well tolerated, with no large differences in tolerability compared to that expected with peginterferon alfa-2a and ribavirin, and no hematological or other toxicity noted. There were no serious adverse events and most events were mild. Among the most common adverse events in the R-7128 groups were headache, fatigue, nausea, diarrhea, injection-site erythema, chills, rash, myalgia, arthralgia and dizziness, each occurring in over 15% of patients in the 500- and 1500-mg b.i.d. groups (33, 34).

When R-7128 was given at a dose of 1500 mg b.i.d. for 28 days to patients with genotype 2 or genotype 3 HCV infection (N = 20) in a placebo-controlled study, the agent was generally well tolerated. The most common adverse events were arthralgia, cough, diarrhea, dyspepsia, fatigue, headache, insomnia, lethargy, myalgia, nausea, pain, pharyngolaryngeal pain, pruritus, pyrexia and vomiting, which occurred in at least two (10%) patients each. No serious adverse events were seen in this study, most were mild and there were no laboratory abnormalities or clinically significant changes in vital signs (34).

CLINICAL STUDIES

Efficient delivery of PSI-6130 has been seen with R-7128 in healthy volunteers and monotherapy with R-7128 was associated with a mean reduction in HCV RNA of 2.7 $\log_{10}$ IU/mL in patients with HCV genotype 1 who previously failed treatment with interferon alfa with or without ribavirin (30, 35). No evidence for the development of viral resistance to R-7128 was seen in patients receiving monotherapy for 2 weeks (28). When combined with pegylated interferon (Pegasys®) and ribavirin (Copegus®), R-7128 1500 mg b.i.d. was associated with an 85% rapid virological response rate (< 15 IU/mL HCV RNA) in treatment-naïve genotype 1 patients (33).

In the multiple-dose study described above in 40 patients with genotype 1 HCV infection, the mean decline in HCV RNA was 2.7 $\log_{10}$ IU/mL and the maximum decline was 4.2 $\log_{10}$ IU/mL after 14 days of therapy. HCV RNA decreases were dose-dependent (see Figs. 1 and 2). In the R-7128 1500 mg b.i.d. group, the range of the decline was 1.2-4.2 $\log_{10}$ IU/mL (35, 36). No signs of development of resistance to R-7128 were observed in this study according to an analysis of 3 patients who had a sustained 0.5 $\log_{10}$ increase in viral load above nadir (nadir = 0.5 $\log_{10}$ decrease from baseline) and in 2 patients with a viral load reduction that remained stable at 1 $\log_{10}$ below baseline during the last 8 and 10 days of treatment. The NS5B polymerase amino acid substitution S282T was previously found to be responsible for a threefold reduction in sensitivity to PSI-6130, and the NS5B replicon phenotypic assay and NS5B population sequencing analysis were used in this study to monitor the development of resistance. In the NS5B replicon phenotypic assay, all NS5B isolates from day 14 were as sensitive to inhibition by PSI-6130 as baseline samples and 2 laboratory reference strains. Population sequence analysis of the entire NS5B coding region revealed no known resistance mutation or any other common amino acid substitutions, and no resistance mutation or amino acid substitution predictive of clinical response or failure was identified in baseline samples of the 40 patients initially treated (36).

In the double-blind study of combination therapy with R-7128, peginterferon alfa-2a and ribavirin in 50 treatment-naïve genotype 1 patients, a rapid virological response (RVR; HCV RNA < 15 IU/mL) was seen at 4 weeks in 10%, 30% and 85% of patients, respectively, given placebo, R-7128 500 mg b.i.d. and R-7128 1500 mg b.i.d. Changes in HCV RNA in the R-7128 groups were dose-dependent.

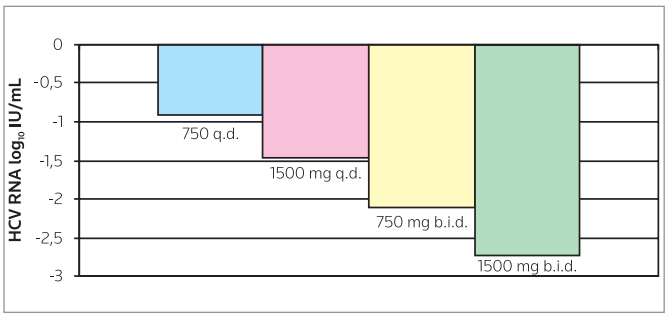


Figure 1. HCV RNA reductions after 14 days of dosing with R-7128 in HCV genotype 1 patients (31).

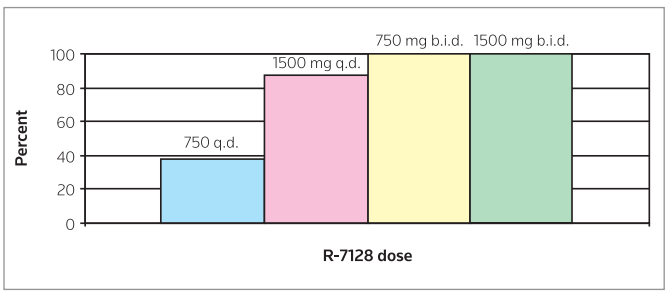


Figure 2. Percent of HCV genotype 1 patients with > 1.0 $\log_{10}$ reduction in HCV RNA after 14 days of dosing with R-7128 (n = 8 in each dose group) (31).

The RVR rate in the R-7128 1500-mg group increased from 10% to 45%, 75% and 85% at weeks 1, 2, 3 and 4, respectively, and the mean decrease in HCV RNA at week 4 was 5.1 $\log_{10}$ IU/mL (33). The combination of R-7128, peginterferon alfa-2a and ribavirin in this study provided significant clinical efficacy regardless of race/ethnicity, as shown in Figure 3. RVR rates for each race/ethnic group increased with the dose of 1500 mg b.i.d. R-7128 compared to 500 mg b.i.d. R-7128. Weight, body mass index and gender were also not determinants of antiviral response (37).

Combination of R-7128 1500 mg b.i.d. with peginterferon and ribavirin was also assessed in patients with genotype 2 (n = 10) and genotype 3 (n = 15) HCV infection who had previously failed interferon therapy. Administration lasted 28 days and 5 of the patients received placebo plus peginterferon and ribavirin. RVR rates were 90% and 60%, respectively, in the R-7128 and placebo groups and plasma HCV RNA declined 5.0 and 4.3 $\log_{10}$ IU/mL from baseline to week 4, respectively, in the R-7128 and placebo groups. Responses were similar in genotype 2 and genotype 3 patients (34).

A phase I study of combination treatment with R-7128 and an HCV protease inhibitor (RO-5190591) in genotype 1 patients is currently recruiting patients (38) and a phase IIb study of R-7128 in treatment-naïve genotype 1 or genotype 4 HCV-infected patients is also expected to begin this year. Approximately 400 patients will be treated with R-7128 500 or 1000 mg b.i.d. plus pegylated interferon and ribavirin (26).

SOURCES

Pharmasset, Inc. (US); being developed in collaboration with Roche.

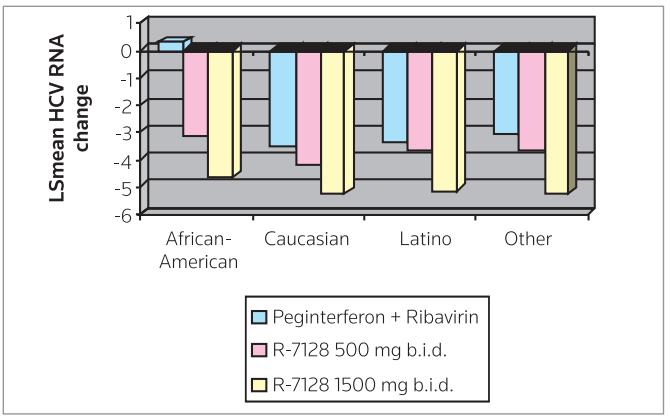


Figure 3. Change in HCV RNA from baseline to week 4 by race/ethnicity (least squares mean change adjusted for baseline) (37).

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