JOC The Journal of Organic Chemistry

ARTICLE

pubs.acs.org/joc

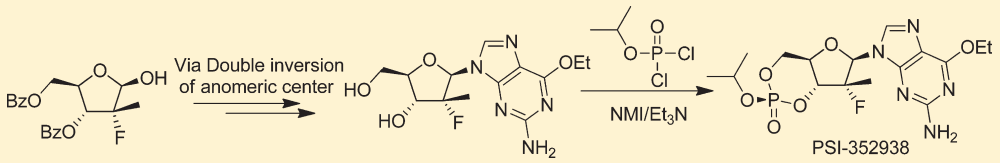
Stereoselective Synthesis of PSI-352938: A β-D-2′-Deoxy-2′-α-fluoro-2′-β-C-methyl-3′,5′-cyclic Phosphate Nucleotide Prodrug for the Treatment of HCV

P. Ganapati Reddy, Byoung-Kwon Chun, Hai-Ren Zhang, Suguna Rachakonda, Bruce S. Ross,* and Michael J. Sofia

Pharmasset, Inc., 303A College Road East, Princeton, New Jersey 08540, United States

Supporting Information

ABSTRACT:



PSI-352938 is a novel 2′-deoxy-2′-α-fluoro-2′-β-C-methyl 3′,5′-cyclic phosphate nucleotide prodrug currently under investigation for the treatment of hepatitis C virus (HCV) infection. PSI-352938 demonstrated superior characteristics *in vitro* that include broad genotype coverage, superior resistance profile, and high levels of active triphosphate *in vivo* in the liver compared to our first and second generation nucleoside inhibitors of this class. Consequently, PSI-352938 was selected for further development and an efficient and scalable synthesis was sought to support clinical development. We report an improved, diastereoselective synthesis of a key 1′-β-nucleoside intermediate **13** via S_N2 displacement of 1-α-bromo ribofuranose sugar **16** with the potassium salt of 6-chloro-2-amino purine and an efficient method to prepare *cis*-Rp cyclic phosphate (PSI-352938) in a highly stereoselective manner without any chromatographic purification. The 1-α-bromo sugar **16** was stereospecifically prepared from the corresponding 1-β-lactol in high yield under mild bromination conditions using CBr_4/PPh_3 (Appel reaction). The desired *cis*-Rp 3′,5′-cyclic phosphate construction was accomplished using isopropyl phosphorodichloridate readily obtained from $POCl_3$ and isopropyl alcohol. The base combination of Et_3N/NMI was identified as a key factor for producing PSI-352938 as the major (>95%) diastereomer (*cis*-Rp) in high yield after the final cyclization step. The current route described in this article was successfully used to produce PSI-352938 on multikilogram scale.

INTRODUCTION

According to the World Health Organization, an estimated 180 million individuals worldwide are infected with the hepatitis C virus (HCV).[1] Currently there is no vaccine to prevent HCV infection, and the current standard of care (SOC), the combination of PEGylated interferon-α and ribavirin, provides less than a 50% response rate among patients infected with the most prevalent genotype 1 virus.[2] There is an urgent medical need for more effective and well-tolerated anti-HCV agents to treat HCV infections across all genotypes.[3] We have shown that nucleoside/tide analogues with 2′-α-*F*-2′-β-*C*-methyl substitution represent an important class of HCV NS5B polymerase inhibitors with broad genotype coverage.[4] This class is represented by the two clinical candidates: the cytidine analogue RG7128 (**1**) and the uridine nucleotide prodrug analogue PSI-7977[5] (**2**) (Figure 1). RG7128 is a 3′,5′-diisobutyrate ester prodrug of our first generation nucleoside PSI-6130 (**3**).[4,6] In a four week combination study with SOC, RG7128 demonstrated efficacy in genotype 1, 2, and 3 patients[7] and was the first direct-acting antiviral to show pan-genotype coverage in the clinic.[8] In a more recent study, following 28 days of treatment in combination with SOC, our second generation uridine nucleotide prodrug PSI-7977 exhibited potent and consistent antiviral activity in HCV genotype 1 infected patients[9] and is currently being studied in HCV genotype 2 and 3 infected patients.

In our continued efforts to identify new and improved agents for HCV therapy, we investigated purine derivatives of the 2′-α-*F*-2′-β-*C*-methyl class of nucleosides. We recently described the discovery of a novel double prodrug of 2′-deoxy-2′-α-fluoro-2′-β-C-methyl guanosine monophosphate, PSI-352938 (**4**) with improved *in vitro* and *in vivo* characteristics.[10] In addition, PSI-352938 demonstrated similar anti-HCV potency in both the wild type and the S282T resistant mutant replicon assays.[11] Recently, PSI-352938 completed phase I multiple ascending dose (MAD) study in treatment-naïve patients with chronic HCV (genotype 1) infection. In this 7 day MAD monotherapy study, when PSI-352938 was administered orally at 200 mg QD, significant antiviral activity was observed with a median HCV RNA change from baseline of 4.64 log IU/mL and 5 out of 8 patients achieving

Received: January 13, 2011
Published: April 06, 2011

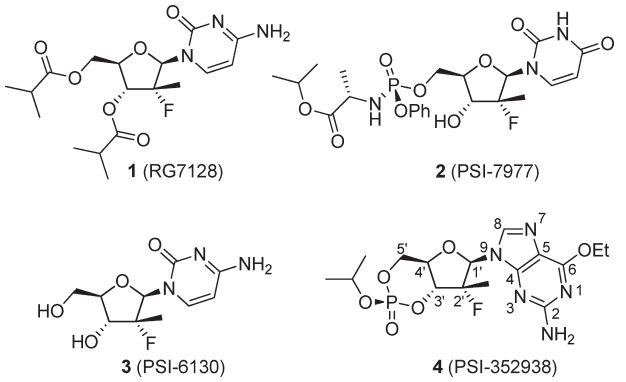


Figure 1. 2′-Deoxy-2′-α-fluoro-2′-*β*-*C*-methyl nucleoside/tide inhibitors of HCV.

HCV RNA levels below the limit of detection.[12] PSI-352938 was shown to be generally safe and well tolerated across all doses studied to date with no serious adverse events and no discontinuations in phase 1 clinical trials.

To support ongoing clinical development, an efficient and scalable synthesis of PSI-352938 was required. PSI-352938 is a novel 3′,5′-cyclic phosphate of a 2′-deoxy-2′-α-*F*-2′-*β*-*C*-methyl purine nucleoside analogue with five chiral centers including the phosphorus center (*cis*-Rp). Two approaches can be considered for the preparation of PSI-352938: linear or convergent. The linear synthesis would require modification of the preexisting nucleoside. In this approach, usually the sugar stereochemistry is established; however, the synthesis could be lengthy if extensive modification of the sugar is required. In addition, reagent compatibility can limit the linear approach as a result of inherent instability of the nucleoside. In the convergent synthesis, the sugar portion is synthesized separately and later coupled to the appropriate base under various conditions. The original discovery synthesis of 2′-deoxy-2′-α-*F*-2′-*β*-*C*-methyl purine nucleosides utilized a linear approach starting from commercially available 2-amino-6-chloro purine riboside **5** as shown in Scheme 1.[4a,13] This route provided direct access to the purine **8**, an advanced intermediate required for making cyclic phosphate **4**, albeit in low yield. Nevertheless, this sequence involved expensive tetraisopropyldisiloxanyl (TIPDS) and atom-inefficient trityl protecting groups. Though attempted optimization of this route with a range of protecting groups and oxidizing agents provided some improvements in the early steps, the late-stage DAST fluorination provided only modest yields. Significant amounts of undesired elimination (2′-exomethylene) and 2′-α-hydroxy byproducts produced in the DAST fluorination reaction necessitated purification by column chromatography, which made scale-up using this route unattractive.

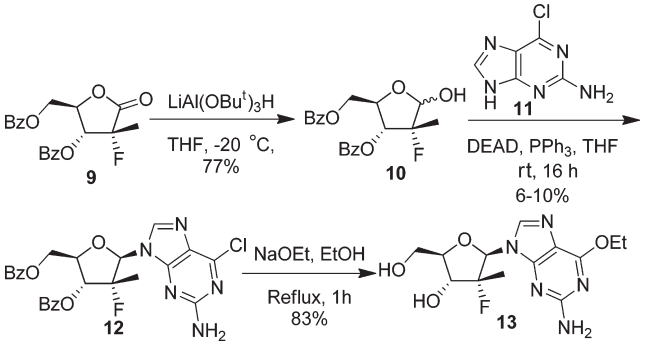
Consequently, we investigated a convergent approach to prepare the nucleoside precursor **8**. Recently, we reported an efficient, convergent and diastereoselective synthesis of **3** that employs a Vorbrüggen-type coupling of the base portion of the molecule and benzoyl protected 2-deoxy-2-fluoro-2-*C*-methyl ribonolactol.[6] Although the sugar subunit in **4** is identical to that found in the pyrimidine analogue **3**, the base-sugar coupling method used to prepare the pyrimidine counterpart **3** suffered from very low yield and poor anomeric stereoselectivity when applied to the purine series.

In addition to the challenging base-sugar coupling, formation of the cyclic phosphate portion of PSI-352938 was low-yielding and required a scale-limiting chromatographic purification. Despite significant efforts to prepare the desired cyclic phosphate using several literature methods,[14,15] the only method that gave reasonable yields of *cis*/*trans* mixture was a two-step protocol that proceeded via a cyclic phosphite intermediate followed by oxidation.[14] However, this sequence used tetrazole in the first cyclization step and *t*BuOOH or *m*CPBA in the final oxidation. Both are undesired because of safety concerns on scale-up. Therefore, it was necessary to find a practical synthetic route for both the purine nucleoside starting material and the *cis*-cyclic phosphate prodrug moiety to support clinical development efforts of PSI-352938 (**4**). Herein, we report an efficient stereoselective synthesis of the 2′-deoxy-2′-α-*F*-2′-*β*-*C*-methyl-6-ethoxy purine nucleoside and the *cis*-cyclic phosphate prodrug **4**.

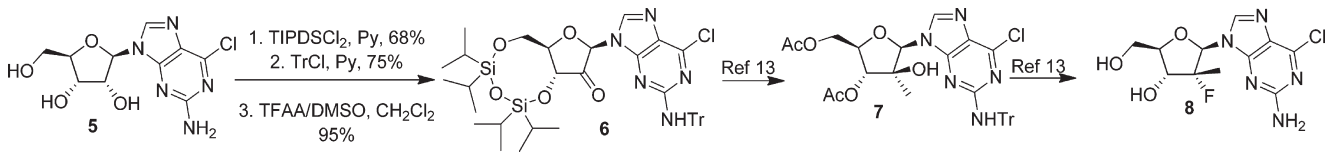
RESULTS AND DISCUSSION

In order to develop a scalable synthesis of **4**, first we needed an efficient synthesis of nucleoside precursor **13** (Scheme 2). Since we had already developed a scalable synthesis of **9**,[6] we explored an alternative convergent approach to nucleoside precursor **13** based on Mitsunobu coupling of lactol **10** with 2-amino-6-chloro purine **11**.[16] Reduction of lactone **9** using hindered reducing agent lithium tri-*tert*-butoxyaluminum hydride gave the lactol **10** in about 2:1 β/α anomeric ratio. Further coupling of **11** under Mitsunobu conditions using DEAD/PPh_3 gave 6-chloropurine analogue **12** as a mixture of α/β anomers in about 1:1 ratio. The mixture was separated by column chromatography to give pure β-isomer in only 6−10% yield. Nucleophilic displacement of the 6-chloro group of **12** followed by debenzoylation using sodium

Scheme 2. Early Synthesis of the Nucleoside Precursor 13

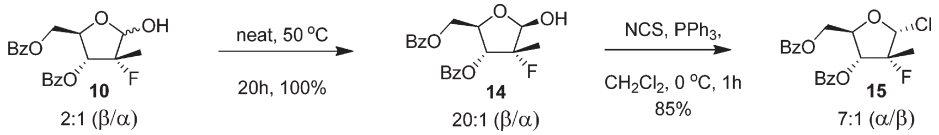


Scheme 1. Early Discovery Synthesis of 2′-Deoxy-2′-α-*F*-2′-*β*-*C*-methyl Purine Nucleosides



dx.doi.org/10.1021/jo200060f |*J. Org. Chem.* 2011, 76, 3782–3790

Scheme 3. Dynamic Crystallization of β-Lactol 14 and Chlorination

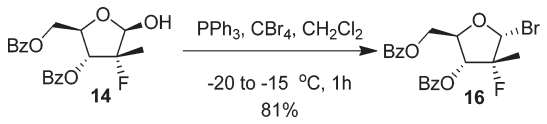


ethoxide in ethanol afforded the nucleoside precursor **13** in 83% yield. In this step, the 3′-hydroxyl group competes with ethanol to displace the 6-chloro substituent of the base, and thus a significant amount of the intermolecular 3′-O−C-6 bridged byproduct[17] was formed that could only be removed by column chromatographic purification.

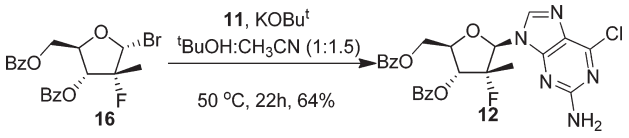
Although the above synthetic route provided enough material for our initial SAR studies, this method is not amenable to large scale synthesis due to the number of expensive chromatographic purifications and the poor yield in the coupling reaction. Alternatively, S_N2 coupling of 1-α-halo sugar or its equivalent with a metal salt of a purine base or a silylated pyrimidine base (Hilbert−Johnson method) could be used to produce the desired β-isomer enriched mixture.[18] However, the success of this approach would rely on development of an effective method for preparation of a 1-α-halo sugar. Typically, the preparation of a 1-α-halo riboside requires treatment of a 1-methoxy or acetoxy sugar with HCl or HBr in acetic acid.[19] However, in our case these methods resulted in an unfavorable anomeric mixture of products. Treatment of the lactol **10** with phosphorus halides generated unpredictable anomeric mixtures due to their acidic nature. Therefore, mild conditions for the stereoselective production of the 1-α-halo sugar were explored.

In an attempt to make the 1-chloro sugar using the Appel reaction[20] (*N*-chlorosuccinimide (NCS)/PPh_3), we found that the 1-α-chloro ribofuranose **15** was produced as the major isomer in about 7:1 α/β ratio (Scheme 3). The fact that the Appel reaction is usually stereospecific prompted us to examine the actual ratio of ribonolactol precursor **10** used in this reaction. To our surprise, we found that it was predominately β-lactol **14** (Scheme 3) with only traces of α-anomer as determined by ^1H NMR. The key finding was that the initial lactol **10** was obtained as a 2:1 β/α mixture after the reduction with lithium tri-*tert*-butoxyaluminum hydride, but isomerized to the thermodynamically stable crystalline β-lactol **14** upon standing, presumably by crystallization induced dynamic resolution.[21] This was an important observation as the β-lactol **14** has the potential to control the stereochemical outcome to produce the desired β-nucleoside after double inversion via 1-α-halo sugar. On larger scale, the crystallization induced dynamic resolution of β-lactol **14** took several days even after seeding with pure β-isomer as the seeds could not spread evenly due to the gummy nature of the mixture. Hence, the neat gummy residue was heated to just below the melting point of the β-anomer (50 °C) to give an oil and seeded with pure β-lactol **14** to produce a 20:1 β/α mixture of **14** as a white crystalline solid in 20 h. With the 1-α-chloro ribofuranose **15** in hand, it was disappointing to find that **15** was not reactive enough with the sodium or potassium salt of the 2-amino-6-chloropurine **11** to produce an acceptable yield of the desired β-nucleoside **12**. However, this transformation provided an indication of the 1-α-halo sugar's potential as a precursor for the S_N2-type glycosylation to give the β-nucleoside with high degree of selectivity.

Scheme 4. Preparation of 1-α-Bromo Ribofuranose 16



Scheme 5. S_N2-Type Coupling of 1-α-Bromo Ribofuranose 16 with 2-Amino-6-chloro Purine 11

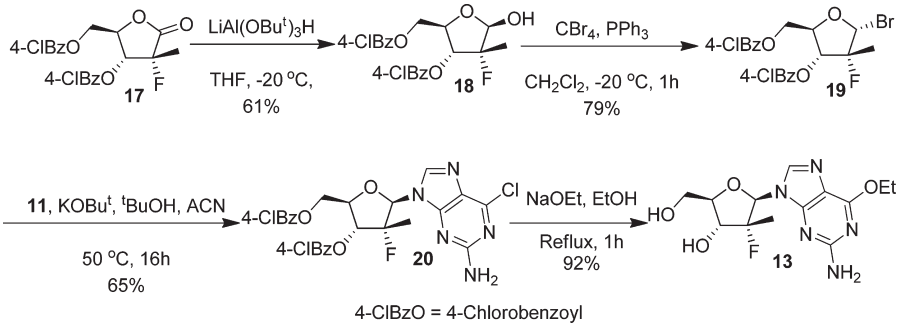


Therefore, preparation of the more reactive 1-bromo or 1-iodo sugar was explored using several halogenating agents. Unlike with NCS, negligible or no stereoselectivity to the desired α-bromo sugar **16** was observed with N-bromosuccinimide in combination with PPh_3 in various solvents. Also, no reaction was observed with lactol **14** using bromine/PPh_3 in the presence of imidazole as a base. When base was excluded, the I_2/PPh_3 reagent system provided no required 1-iodo product and addition of a base compromised the reaction stereoselectivity. In addition, no reaction was observed with CI_4/PPh_3 combination. Interestingly, treatment of the lactol **14** with PPh_3/CBr_4 (Appel reaction) between −20 and −15 °C provided the desired 1-bromo ribofuranose **16** with the best α/β ratio (10:1) (Scheme 4).[18c] However, major isomerization at the anomeric center was observed during the workup at room temperature. Filtration of the cold reaction mixture through a pad of silica gel was found to be key for preventing the isomerization. Further purification of the crude by a quick filtration through silica gel provided the bromo sugar **16** with improved α/β ratio (20:1) in 81% yield.

To accomplish the S_N2 coupling between **16** and **11**, we investigated the coupling reaction using various metal salts of 2-amino-6-chloropurine **11**. Treatment of the 2-amino-6-chloropurine **11** with LHMDS in tetrahydrofuran or acetonitrile gave the lithium salt and further reaction of the lithium salt with 1-α-bromo ribofuranose **16** at room temperature was found to be very sluggish resulting in very low conversion after several days. Higher temperature provided significant undesired N7 regioisomer. Similar results were observed when NaH was used as the base in DMF or acetonitrile. After further investigation, treatment of the 1-α-bromo ribofuranose **16** with the potassium salt of 2-amino-6-chloro purine **11** (generated in situ using potassium tert-butoxide in 1:1.5 mixture of tert-butanol and acetonitrile) gave the required 6-chloro purine analogue **12** in 64% yield (Scheme 5) analogous to purine coupling reported by

dx.doi.org/10.1021/jo200060f |*J. Org. Chem.* 2011, 76, 3782–3790

Scheme 6. Non-chromatographic Scalable Synthesis of Nucleoside 13



Bauta et al.[18e] At room temperature the reaction was very slow (5days), however, the optimum reaction temperature for this transformation was found to be 50 °C and required only 22 h to complete. The solvent combination of *t*BuOH−CH_3CN was also found to be the key for delivering high yields and minimal byproducts in the coupling reaction.

The next step in the sequence was ethoxy substitution at the C-6 position of the base. This was accomplished by converting the 6-chloropurine **12** to the required 6-ethoxy derivative **13** as shown in Scheme 2. The formation of the intermolecular 3′−O-C-6 bridged byproduct[17] was minimized by use of excess sodium ethoxide as it increased the ethoxide to 3′-alkoxide ratio. On larger scale we could eliminate the chromatographic purification of **13** with a simple acid−base extraction followed by crystallization. After completion of the reaction, ethanol was evaporated and the residue was partitioned between ethyl acetate and 1N aqueous HCl. The required product **13** was extracted into the aqueous layer as a hydrochloride salt leaving the ethyl benzoate byproduct in the organic layer. Basification of the aqueous layer with aqueous Na_2CO_3 followed by extraction with ethyl acetate and crystallization of the crude product from acetone gave pure **13** as a crystalline solid in 79% yield.

Despite several process improvements, the above sequence still required some chromatographic purification at all stages from lactol **14** to 6-chloro purine **13**. Hence, we continued to explore a more facile route for multikilogram scale production of 6-ethoxy purine **13**. Interestingly, we were able to overcome this issue by using the 4-chlorobenzoyl protecting group in place of the simple benzoyl protecting groups at 3′- and 5′-hydroxyl groups (Scheme 6). The lactone **17** was prepared following the same method described for making the benzoyl lactone **9**.[6] Reduction of the lactone **17** with lithium tri-*tert*-butoxyaluminum hydride followed by crystallization of the crude product from methanol/water mixture gave 1-β-lactol **18** (>95% pure) in 61% yield without any chromatographic purification. Under similar bromination conditions, lactol **18** gave bromo ribofuranose **19**. Simple filtration of the cold reaction mixture through a pad of silica gel followed by crystallization from a mixture of ether/hexanes gave 1-α-bromo ribofuranose **19** in high purity (>96%), which was partially contaminated with 2−5% of triphenylphosphine oxide. Treatment of **19** with the potassium salt of 2-amino-6-chloropurine **11** furnished 6-chloro purine **20** in 65% yield after crystallization from ethanol. 6-Ethoxy purine **13** was prepared in 92% yield following similar reaction conditions used for the benzoyl protected nucleoside **12**.

Table 1. Cyclization Using Different Activators

activator	conditions	% yield[a] (**4**)
tetrazole	CH_3CN, 40 °C, 3 h	23
5-(ethylthio)-tetrazole	CH_3CN, 0 °C, 3 h	7
4,5-dicyanoimidazole	CH_3CN, 50 °C, 6 h	41
Py.CF_3SO_3H	CH_3CN, 50 °C, 6 h	21
imidazolium triflate	CH_3CN, 45 °C, 6 h	8
benzimidazolium triflate	CH_3CN, 45 °C, 6 h	ND[b]

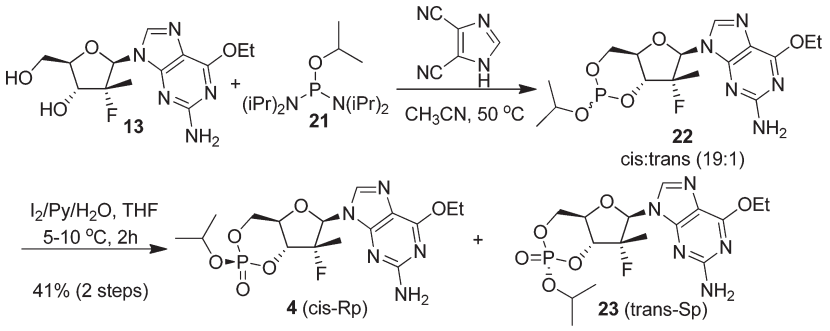
[a] Isolated yields of **4** after oxidation with *t*BuOOH. [b] Less than 5% conversion by TLC.

The final step in the sequence was formation of the 3′,5′-cyclic phosphate moiety. For our initial SAR studies, we followed a two-step literature procedure that proceeded via a cyclic phosphite intermediate followed by oxidation to give the desired cyclic phosphate as a *cis*/*trans* mixture (Scheme 7).[14] Thus cyclization of 6-ethoxy purine **13** with *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite **21** using 1*H*-tetrazole as an activator gave cyclic phosphite intermediate **22** as a mixture of *cis*/*trans* isomers. The cyclic phosphite mixture was further oxidized without isolation using *t*BuOOH to give a *cis*/*trans* mixture of cyclic phosphates **4** and **23**. The mixture was separated by column chromatography providing pure *cis* and *trans* cyclic phosphates (**4** and **23**) in 9% and 10% yield, respectively. Of the two diastereomers, the *cis*-isomer of the cyclic phosphite **22** was found to be the thermodynamically more stable isomer, similar to an analogous compound reported by Broeders.[14a] The initial cyclic phosphite mixture could be equilibrated to *cis*-**22** by heating the reaction mixture.[10] Indeed, after treatment of 6-ethoxy purine **13** with *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite **21** in presence of 1*H*-tetrazole followed by heating the reaction mixture at 40 °C for 6 h, the *cis*/*trans* ratio of the cyclic phosphite **22** was found to be 95:5. Further oxidation and column chromatography of the mixture provided desired *cis*-cyclic phosphate **4** in 23% yield. Due to the synthetic accessibility of a single diastereomer, *cis*-cyclic phosphate **4** was selected for further development.

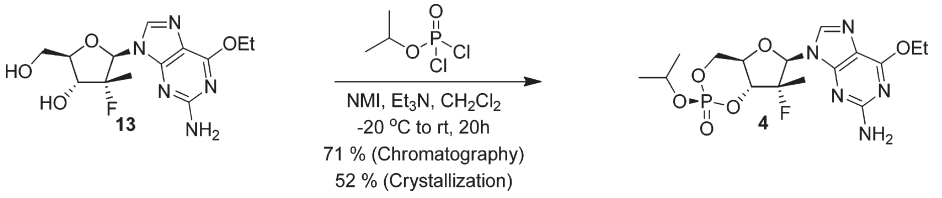
Since the target compound **4** has the *cis*-Rp cyclic phosphate configuration as determined by single crystal X-ray analysis,[10] optimization to produce exclusively the desired cyclic phosphate configuration was pursued. Since the use of tetrazole on large scale is undesirable due to safety concerns, we investigated several other activators for the cyclization step as listed in Table 1. Both 5-(ethylthio)-tetrazole and imidazolium triflate gave unacceptable yields of the cyclic phosphate **4**. Very low conversion was found

dx.doi.org/10.1021/jo200060f |*J. Org. Chem.* 2011, 76, 3782–3790

Scheme 7. Early Synthesis of *cis*-Cyclic Phosphate 4



Scheme 8. Improved Synthesis of *cis*-Cyclic Phosphate 4



with benzimidazolium triflate. Pyridinium triflate gave similar yield to the tetrazole reaction. Intriguingly, 4,5-dicyanoimidazole (DCI) provided an improved *cis*/*trans* ratio (5.6:1) of cyclic phosphites, which upon heating at 50 °C for 6 h followed by oxidation gave 19:1 mixture of cyclic phosphates **4** and **23**, respectively. Further chromatographic purification of the mixture afforded 41% yield of the required *cis* cyclic phosphate **4**. Although DCI is known as an activator in oligonucleotide synthesis,[22] this was the first example where DCI was demonstrated as an activator in cyclic phosphate synthesis. In the final oxidation, we could successfully replace the peroxides with the safer $Py/I_2/H_2O$ reagent system routinely used in the oligonucleotide synthesis.[23]

Although the above modified method was successfully used in a kilo-scale synthesis of **4**, it was less amenable to much large scale synthesis because of the chromatographic purification used in the final step. Consequently, alternative approaches were explored to prepare cyclic phosphate **4** based on P(V) chemistry. A search of the literature revealed no relevant precedent for direct 3′,5′-cyclicphosphate ester synthesis using alkyl- or aryldichlorophosphates. Nevertheless, when the nucleoside **13** was treated with isopropyldichlorophosphate in presence of *N*-methylimidazole (NMI) in dichloromethane at 0 °C, a mixture of cyclic phosphates **4** and **23** was obtained in approximately 85:15 ratio, albeit in low yield (12%). The isopropyldichlorophosphate was readily prepared from $POCl_3$ and isopropyl alcohol. Further optimization of the cyclization reaction with various organic bases did not improve the yield. Surprisingly, there was no reaction when triethylamine or diisopropylethylamine alone was used as a base presumably due to poor solubility of the starting nucleoside under the reaction conditions. Intriguingly, when NMI was added to the above reactions the desired *cis* cyclic phosphate **4** was formed as a major product with less than 5% of the *trans*-isomer **23** (Scheme 8).[24] Further optimization of the reaction temperature and stoichiometry of isopropyldichlorophosphate and Et_3N/NMI gave cyclic phosphate **4** in 71% yield after column chromatographic purification or in 52% yield by direct crystallization of the crude product from ethyl acetate or acetone. The crystallized material was sufficiently pure (>99.5%) with less than 0.2% of the *trans*-cyclic phosphate **23**. The new process has been used successfully on a multikilogram scale to support ongoing clinical development of **4** (PSI-352938).

CONCLUSION

We have demonstrated an efficient, diastereoselective and scalable synthesis of the cyclic phosphate nucleotide prodrug PSI-352938 (**4**). The anomeric carbon stereochemistry was first set by thermal equilibration of the lactol mixture followed by dynamic crystallization of the 1-β-lactol **14**. Double inversion of the anomeric center of the 1-β-lactol **14** provided the desired β-nucleoside with high degree of selectivity. This was accomplished via the 1-α-bromo ribofuranose **16**, which was stereospecifically prepared from the 1-β-lactol **14** in high yield using CBr_4/PPh_3 and successfully used for the glycosylation via S_N2-type coupling to provide the key intermediate **12**. An efficient and non-chromatographic synthesis of the nucleoside **13** was accomplished using 4-chlorobenzoyl protecting groups on the 3′- and 5′-hydroxyl groups. DCI was used as an activator for the first time in the synthesis of a 3′,5′-nucleoside cyclic phosphate to provide gram scale preclinical material of **4**. A highly stereoselective non-chromatographic method for the synthesis of *cis*-Rp cyclic phosphate **4** was developed using inexpensive isopropyldichlorophosphate. This improved process is currently being used to produce PSI-352938 (**4**) clinical material on multikilogram scale.

EXPERIMENTAL SECTION

General Analytical Methods. Reactions were monitored by thin layer chromatography and visualized by UV light or by charring in 5% sulfuric acid in methanol. All solvents and reagents were used as received. NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 as noted. Optical

dx.doi.org/10.1021/jo200060f |*J. Org. Chem.* 2011, 76, 3782–3790

rotations were measured at ambient temperature and 589 nm. High resolution mass spectra were performed using chemical ionization. Melting points are uncorrected.

((2*R*,3*R*,4*R*,5*R*)-3-(Benzoyloxy)-4-fluoro-5-hydroxy-4-methyltetrahydrofuran-2-yl)methyl Benzoate (14). To a dry 5 L three neck round-bottom flask equipped with a mechanical stirrer, addition funnel and thermometer were added the lactone **9** (379 g, 1.018 mol) and anhydrous THF (1.75 L). The solution was cooled to −30 °C under a nitrogen atmosphere, and then was added a solution of lithium tri-*tert*-butoxyaluminohydride (1.0 M in THF, 1.527 L, 1.527 mol), with stirring, over a period of 1 h. After completion of the addition, the reaction mixture was allowed slowly to warm to −10 °C over 1 h 15 min. The reaction mixture was quenched with ethyl acetate (900 mL) followed by saturated aq NH_4Cl (40 mL) below 0 °C. The cloudy supernant liquid was decanted, and the residue was washed with ethyl acetate (2 × 200 mL) and decanted. The combined decants were concentrated under reduced pressure to give an oily residue. The oil was dissolved in ethyl acetate (2 L) and washed with 3 N HCl (600 mL). The aqueous layer was back extracted with ethyl acetate (3 × 400 mL). The combined organic layer was washed with water (3 × 800 mL), saturated aq $NaHCO_3$ (400 mL) and brine (400 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure to give a light brown syrup. The residue was purified by column chromatography (2.2 KG silica gel) using 5−30% ethyl acetate/hexanes gradient to give lactol **10** as colorless syrup. The syrup was heated at 50 °C with seeds of crystalline β-lactol for 20 h to give pure β-lactol **14** as a white solid (293.8 g, 77%, β/α ratio 20:1). For **14**: $[\alpha]^{20}_D$ = +21 (*c* 1.0, MeOH); mp 79−80 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.99 (m, 2 H), 7.93 (m, 2 H), 7.70 (m, 1 H), 7.61 (m, 1 H), 7.55 (m, 2 H), 7.42 (m, 2 H), 7.32 (d, *J* = 5.2 Hz, 1H), 5.54 (dd, *J* = 23.6, 7.2 Hz, 1H), 5.20 (dd, *J* = 10.8, 5.2 Hz, 1H, D_2O exchangeable), 4.55−4.50 (m, 1H), 4.46−4.40 (m, 2H), 1.42 (d, *J* = 22.4 Hz, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 166.4, 166.2, 134.9, 134.3, 130.5, 130.3, 130.2, 129.8, 129.6, 129.6, 101.2 (d, J_{C-F} = 32 Hz), 101.1 (d, J_{C-F} = 180 Hz), 77.1, 75.7 (d, J_{C-F} = 15 Hz), 65.9, 17.6 (d, J_{C-F} = 24 Hz). HRMS-ESI (*m*/*z*): calcd for $C_{20}H_{19}FO_6$ $[M + H]^+$ 375.1238, found 375.1243.

((2*R*,3*R*,4*R*,5*R*)-3-((4-Chlorobenzoyl)oxy)-4-fluoro-5-hydroxy-4-methyltetrahydrofuran-2-yl)methyl 4-Chlorobenzoate (18). Under similar reaction conditions reduction of the lactone **17** (50 g, 113 mmol) followed by crystallization of the crude product from the mixture of methanol/water (4.5:1) gave lactol **18** as a white solid (30.5 g, 61% yield, β/α ratio 35:1). For **18**: $[\alpha]^{20}_D$ = +53 (*c* 1.0, MeOH); mp 101−104 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.96−7.93 (m, 2H), 7.92−7.88 (m, 2H), 7.61−7.58 (m, 2H), 7.52−7.48 (m, 2H), 7.31 (dd, *J* = 5.2, 0.8 Hz, 1H), 5.50 (dd, *J* = 24, 7.2 Hz, 1H), 5.19 (dd, *J* = 10.8, 5.6 Hz, 1H), 4.58−4.54 (m, 1H), 4.45−4.40 (m, 2H), 1.42 (d, *J* = 22.8 Hz, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 165.5, 165.3, 139.8, 139.3, 132.25, 131.9, 129.9, 129.7, 129.1, 128.4, 101.1 (d, *J* = 31.8 Hz), 101.0 (d, *J* = 179.8 Hz), 76.9, 75.9 (d, *J* = 15.1 Hz), 65.9, 17.6 (d, *J* = 23.5 Hz). HRMS-ESI (*m*/*z*): calcd for $C_{20}H_{17}Cl_2FO_6$ $[M + Na]^+$ 465.0278, found 465.0293.

((2*R*,3*R*,4*R*,5*S*)-3-(Benzoyloxy)-5-chloro-4-fluoro-4-methyltetrahydrofuran-2-yl)methyl Benzoate (15). To a solution of the lactol **14** (1.0 g, 2.67 mmol) and PPh_3 (1.4 g, 5.34 mmol) in CH_2Cl_2 (15 mL) was added *N*-chlorosuccimide (1.07 g, 8.01 mmol) portion-wise at 0 °C. Then the resulting mixture was stirred at rt for 1 h and poured into a silica gel column and eluted with a mixture of EtOAc/hexanes (1:4). The collected fractions were combined, concentrated under reduced pressure, coevaporated two times with CH_2Cl_2 and used in the next step without further purification (1.0 g, 95%, α/β ratio 7:1). For major α-isomer **15**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12−8.10 (m, 4H, aromatic), 8.04 (m, 4H, aromatic), 7.63−7.56 (m, 2H, aromatic), 7.50−7.42 (m, 4H, aromatic), 6.00 (broad s, 1H, H-1), 5.27 (dd, 1H, *J* = 3.6, 5.6 Hz, H-3), 4.87 (m, 1H, H-4), 4.76 (dd, 1H, *J* = 3.2, 12.4 Hz, H-5), 4.61 (dd, 1H, *J* = 4.8, 12.4 Hz, H-5′), 1.72 (d, 3H, *J* = 21.6 Hz, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.0, 165.8, 133.7, 133.4, 130.0, 129.7, 129.3, 128.8, 128.6, 128.5, 97.1 (d, *J* = 22.0 Hz), 94.8 (d, *J* = 209.3 Hz), 81.2, 73.4 (d, *J* = 15.9 Hz), 62.7, 22.8 (d, *J* = 25.8 Hz).

((2*R*,3*R*,4*R*,5*R*)-3-(Benzoyloxy)-5-bromo-4-fluoro-4-methyltetrahydrofuran-2-yl)methyl Benzoate (16). To a stirred solution of PPh_3 (205.4 g, 0.783 mol) in dichloromethane (5.6 L) was added the β-lactol **14** (209.4 g, 0.559 mol) below −20 °C under N_2 atmosphere. After the mixture stirred for 15 min, CBr_4 (278.2 g, 0.839 mol) was added portion-wise while maintaining the reaction temperature between −25 and −20 °C under N_2 flow. After completion of the addition, the reaction mixture was stirred below −17 °C for 20 min. Silica gel (230 g) was added to the mixture, filtered through a pad of silica gel (680 g) and washed with dichloromethane. The combined filtrates were concentrated under reduced pressure at room temperature to give colorless oil. ^{1}H NMR of a crude sample at this stage indicated 10:1 α/β mixture. The residue was purified by column chromatography (2.1 kg silica gel) using 0−25% EtOAc/hexanes gradient to give **16** as colorless oil which solidified upon standing to give waxy solid. (197 g, 81%, α/β ratio 20:1). For **16**: $[\alpha]^{20}_D$ = +136 (*c* 1.0, $CHCl_3$); mp 59−61 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.13 (d, *J* = 7.2 Hz, 2H), 8.02 (d, *J* = 7.6 Hz, 2H), 7.63−7.56 (m, 2H), 7.50−7.42 (m, 4H), 6.34 (s, 1H), 5.29 (dd, *J* = 5.3, 2.8 Hz, 1H), 4.89−4.86 (m, 1H), 4.80−4.76 (m, 1H), 4.65−4.61 (m, 1H), 1.72 (d, *J* = 21.2 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 165.9, 165.7, 133.7, 133.4, 130.0, 129.6, 129.2, 128.8, 128.5, 128.5, 94.6 (d, *J* = 210 Hz), 92.0 (d, *J* = 23 Hz), 81.9, 73.3 (d, *J* = 15 Hz), 62.4, 22.8 (d, *J* = 27 Hz). HRMS-ESI (*m*/*z*): calcd for $C_{20}H_{18}BrFO_5$ $[M + H]^+$ 437.0394, found 437.0393.

(2*R*,3*R*,4*R*,5*S*)-5-Bromo-2-(((4-chlorobenzoyl)oxy)methyl)-4-fluoro-4-methyltetrahydrofuran-3-yl 4-Chlorobenzoate (19). Under similar reaction conditions lactol **18** (23.5 g, 53 mmol) gave bromide **19** (21.23 g, 79% yield, α/β ratio 65:1, contaminated with 2% of triphenylphosphine) as a white solid after crystallization of the crude product from the mixture of ether/hexanes (3.4:1). For **19**: $[\alpha]^{20}_D$ = +133 (*c* 1.0, $CHCl_3$); mp 122−124 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.06−8.03 (m, 2H), 7.96−7.93 (m, 2H), 7.46−7.40 (m, 4H), 6.33 (s, 1H), 5.22 (dd, *J* = 5.6, 1H), 4.86−4.83 (m, 1H), 4.78−4.74 (m, 1H), 4.64−4.60 (m, 1H), 1.69 (d, *J* = 21.6 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 165.1, 164.8, 140.3, 140.0, 131.4, 131.0, 127.6, 127.1, 94.5 (d, *J* = 209.4 Hz), 91.8 (d, *J* = 23.5 Hz), 81.7, 73.5 (d, *J* = 15.2 Hz), 62.5, 22.8 (d, *J* = 26.6 Hz). HRMS-ESI (*m*/*z*): calcd for $C_{20}H_{16}BrCl_2FO_5$ $[M + Na]^+$ 528.9412, found 528.9431.

(2*R*,3*R*,4*R*,5*R*)-5-(2-Amino-6-chloro-9*H*-purin-9-yl)-2-(benzoyloxymethyl)-4-fluoro-4-methyltetrahydrofuran-3-yl Benzoate (12). A 12 L three-neck round-bottomed flask was charged with 6-chloro-2-aminopurine (225.4 g, 1.329 mol) followed by anhydrous *t*BuOH (4.5 L) with stirring with a mechanical stirrer. To the above stirred solution was added potassium *tert*-butoxide (151.6 g, 1.35 mol) portion-wise at room temperature under N_2 flow. After 30 min, a solution of 1-α-bromo ribofuranose **16** (197 g, 0.451 mol) in anhydrous acetonitrile (4 L) was added in one lot over a period of 5 min at room temperature. The mixture was slowly heated to 50 °C with a heating mantle at which temperature it was stirred for 22 h. To the reaction mixture was added solid NH_4Cl (75 g) followed by water (200 mL), and the mixture was filtered through a short pad of Celite and washed with toluene (3 × 200 mL). The filtrate was neutralized with 6 N HCl and concentrated under reduced pressure to a volume of 800 mL. The mixture was filtered through Celite to remove additional salts that were precipitated during the concentration, washed with toluene (200 mL) and concentrated. The residue was purified by column chromatography (1.6 kg silica gel) using 40−45% EtOAc/hexanes gradient to give 6-chloropurine **12** as a white foamy solid (150.7 g, 64% yield, β/α ratio 14:1). A portion of the solid was crystallized from methanol to give pure

12. For **12**: $[\alpha]^{20}_{D} = -21$ (*c* 1.0, $CHCl_3$); mp 160−162 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.03 (m, 2H), 7.93 (m, 2H), 7.88 (s, 1H), 7.60 (m, 1H), 7.50 (m, 1H), 7.44 (m, 2H), 7.33 (m, 2H), 6.43 (dd, *J* = 22.8, 9.2 Hz, 1H), 6.12 (d, *J* = 18 Hz, 1H), 5.41 (s, 2H, NH_2), 5.00 (dd, *J* = 12, 4.4 Hz, 1H), 4.75 (m, 1H), 4.59 (dd, *J* = 12, 5.6 Hz, 1H), 1.33 (d, *J* = 22.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.2, 165.5, 159.1, 152.6, 152.1, 140.7, 133.8, 133.3, 130.0, 129.5, 129.2, 128.5, 128.4, 128.4, 125.8, 100.3 (d, *J* = 185 Hz), 90.3 (d, *J* = 39 Hz), 77.7, 73.4 (d, *J* = 16 Hz), 62.9, 17.3 (d, *J* = 24 Hz). HRMS-ESI (*m*/*z*): calcd for $C_{25}H_{21}ClFN_5O_5$ $[M + H]^+$ 526.1288, found 526.1289.

(2*R*,3*R*,4*R*,5*R*)-5-(2-Amino-6-chloro-9*H*-purin-9-yl)-2-(4-chlorobenzoyloxymethyl)-4-fluoro-4-methyltetrahydrofuran-3-yl 4-Chlorobenzoate (20). Under similar reaction conditions bromide **19** (2.6 g, 5.14 mmol) gave 6-chloropurine **20** (1.98 g, 65% yield, β/α ratio 26:1) as a white solid after crystallization from 95% ethanol. For **20** $[\alpha]^{20}_{D} = -26$ (*c* 1.0, $CHCl_3$); mp 171−176 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.92−7.89 (m, 2H), 7.85 (s, 1H), 7.85−7.82 (m, 2H), 7.41−7.38 (m, 2H), 7.29−7.26 (m, 2H), 6.47 (dd, *J* = 22.8, 9.2 Hz, 1H), 6.09 (d, *J* = 18 Hz, 1H,), 5.49 (bs, 2H, NH_2), 5.06 (dd, *J* = 11.6, 4.8 Hz, 1H), 4.75−4.70 (m, 1H, 1H), 4.55 (dd, *J* = 12.0, 5.6 Hz, 1H), 1.32 (d, *J* = 22.4 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 165.3, 164.6, 159.1, 152.5, 152.3, 140.7, 140.5, 139.9, 131.3, 130.9, 128.9, 128.7, 127.6, 126.8, 125.9, 100.3 (d, *J* = 184.3 Hz), 90.4 (d, *J* = 39.4 Hz), 77.4, 73.8 (d, *J* = 15.2 Hz), 63.1, 17.2 (d, *J* = 25 Hz). HRMS-ESI (*m*/*z*): calcd for $C_{25}H_{19}Cl_3FN_5O_5$ $[M + H]^+$ 594.0509, found 594.0527.

(2*R*,3*R*,4*R*,5*R*)-5-(2-Amino-6-ethoxy-9*H*-purin-9-yl)-4-fluoro-2-(hydroxymethyl)-4-methyltetrahydrofuran-3-ol (13). From **12**. To a stirred solution of 6-chloropurine **12** (60 g, 114 mmol, coevaporated with absolute ethanol under reduced pressure) in absolute ethanol (236 mL) was added a 21 wt % solution of sodium ethoxide (64 mL, 171 mmol) dropwise at ambient temperature. The cloudy mixture was stirred at room temperature for 1 h and then heated to reflux for 30 min under nitrogen atmosphere. The reaction mixture was cooled to room temperature and neutralized with concentrated HCl (15 mL). The cloudy mixture was concentrated under reduced pressure to give a solid residue. The residue was partitioned between 1 N HCl (198 mL) and dichloromethane (200 mL). The aqueous layer was filtered through a filter paper, and the filtrate was neutralized with of saturated aqueous sodium carbonate to pH 8 and extracted with ethyl acetate (4 × 140 mL). The combined organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure to give white solid. The solid was recrystallized from acetone (90 mL), filtered, washed with acetone (20 mL) to give pure **13** an off-white solid (29.46 g, 79% yield). For **13** $[\alpha]^{20}_{D} = +21$ (*c* 1.0, MeOH); mp 106−110 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.16 (s, 1H), 6.56 (bs, 2H, NH_2), 6.05 (d, *J* = 17.6 Hz, 1H), 5.67 (d, *J* = 7.2 Hz, 1H, D_2O exchangeable), 5.26 (t, *J* = 5 Hz, 1H, D_2O exchangeable), 4.50−4.89 (m, 2H), 4.24−4.13 (m, 1H), 3.92−3.82 (m, 2H), 3.72−3.66 (m, 1H), 1.35 (t, *J* = 7.2 Hz, 3H), 1.06 (d, *J* = 22.4 Hz, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 161.6, 161.0, 154.5, 137.8, 114.6, 102.2 (d, *J* = 180 Hz), 88.6 (d, *J* = 38 Hz), 83.0, 71.5 (d, *J* = 17 Hz), 62.6, 60.2, 17.5 (d, *J* = 25 Hz), 15.5. HRMS-ESI (*m*/*z*): calcd for $C_{13}H_{18}FN_5O_4$ $[M + H]^+$ 328.1416, found 328.1408. From **20**: Under similar reaction conditions 6-chloropurine **20** (313.2 g, 526.5 mol) gave 6-ethoxy purine **13** (151.6 g, 92% yield) after direct crystallization of the crude product from acetone. Analytical data of the product were identical to those of the product obtained from **12**.

Isopropyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (21). Bis(diisopropylamino)chlorophos-phine (250.1 g, 937 mmol) was dissolved in anhydrous ethyl ether (3.6 L), and triethylamine (190 g, 1.87 mol) was introduced. The turbid mixture was cooled to 0 °C, and a solution of 2-propanol (225 g, 287 mL) in ether (200 mL) was added via a funnel. The resulting cloudy mixture was stirred at room temperature for 5.5 h. The reaction was monitored by ^{31}P NMR (δ = 116.10 ppm, S). White solid (triethylamine HCl salt) was removed by filtration. The filtrate was concentrated to furnish **21** as a pale brown liquid (272 g, quantitative) and used for next step without further purification. Note that the P(III) reagent **21** can be purified by vacuum distillation (bp 84−86 °C, 5 mmHg) if desired to furnish a colorless clear oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.91 (m, 1H), 3.51 (m, 4 H), 1.19 (d, 6 H, *J* = 6 Hz), 1.16 (24 H, m); ^{31}P NMR ($CDCl_3$) δ 116.1.

6-Ethoxy-9-((2*R*,4a*R*,6*R*,7*R*,7a*R*)-7-fluoro-2-isopropoxy-7-methyl-2-oxo-tetrahydro-2λ^5-furo[3,2-*d*][1,3,2]dioxaphosphinin-6-yl)-9*H*-purin-2-ylamine (4) and 6-Ethoxy-9-((2*S*,4a*R*,6*R*,7*R*,7a*R*)-7-fluoro-2-isopropoxy-7-methyl-2-oxo-tetrahydro-2λ^5-furo[3,2-*d*][1,3,2]dioxaphosphinin-6-yl)-9*H*-purin-2-ylamine (23). To a stirred suspension of (2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-ethoxy-9*H*-purin-9-yl)-4-fluoro-2-(hydroxymethyl)-4-methyltetrahydrofuran-3-ol (**13**, 65.0 g, 0.199 mol) and 4,5-dicyanoimidazole (59 g, 496 mol) in acetonitrile (1000 mL) at 0−5 °C was added P (III)-reagent **21** (62.35 mL, 0.199 mol), dropwise over a period of 20 min. The solid was dissolved after the completion of the addition of the P (III)-reagent **21**, and a clear solution was observed. After 30 min, the solution was warmed to room temperature and stirred for 3 h. The reaction mixture was then heated at 50 °C (bath) for 6 h (^{31}P NMR of a small aliquot diluted with an equal volume of $CDCl_3$ indicated less than 5% of the minor *trans*-isomer **22** at δ 127.91 ppm). The solvent was evaporated to dryness and the residue was stirred with EtOAc (500 mL) to form a white solid suspension of the DCI salt. The solid was removed by filtration and washed with EtOAc (250 mL). The combined filtrate was concentrated to dryness. To the residue was added a 0.1 M solution of iodine in 70:28:2 (each in v/v %) THF/pyridine/H_2O (2 L) over a period of 30 min at 5−10 °C. After 2 h, more white DCI salt solid was collected by filtration, and the filtrate was concentrated under reduced pressure. The residue was dissolved in ethyl acetate (1 L), washed with a 10% aqueous sodium thiosulfate (200 mL) and then saturated aq$NaHCO_3$ (3 × 250 mL) until DCI was mostly removed as judged by TLC (also note that saturated sodium carbonate solution can remove DCI more efficiently). The organic layer was washed with water (250 mL), dried over anhydrous sodium sulfate, filtered and concentrated to give 86 g of foam. This was combined with similar material from two additional runs totaling 259 mmol of starting nucleoside. The combined crude foams were dissolved in a minimum of dichloromethane and subjected to silica gel chromatography using 3 L of silica gel in a 6 L sintered glass Büchner funnel with a step gradient of 30−75% EtOAc/hexanes to give 83 g of purified product as a foam as the primary fraction and 16 g of a secondary partially purified fraction. The primary fraction was suspended in ethyl ether (250 mL), which immediately gave a fine granular solid. The solid was collected by filtration and dried (40 °C, 0.2 mmHg, 17 h) to 73.5 g of slightly off-white powder containing 20 mol % of ethyl ether. The solid was coevaporated with acetone (200 mL) and redried in a similar manner to 71.5 g of **4** as a white solid with 2 mol % of acetone, and HPLC purity of 98.5%. The secondary contaminated fractions were purified by chromatography to afford an additional 9.0 g of **4** for a total recovery of 80.5 g (41% yield) of pure product. A small portion of the *trans*-isomer **23** (250 mg) was also isolated as an amorphous white foam solid. For *cis*-isomer **4**: $[\alpha]^{20}_{D} = -80$ (*c* 1.0, MeOH); mp 194−197 °C (dec); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (s, 1H), 6.02 (br d, *J* = 19.6 Hz, 1H), 5.46 (br s, 1H), 4.90 (sept, *J* = 6.4 Hz, 1H), 4.84 (br s, 2H, NH_2), 4.69−4.42 (m, 4H), 4.40−4.37 (m, 1H), 1.48−1.33 (m, 9H), 1.35 (d, *J* = 22 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 161.7, 159.3, 152.6, 138.1, 116.8, 97.5 (dd, J_{C-F} = 188, 8.3 Hz), 92.6 (d, *J* = 35 Hz), 79.8 (d, *J* = 16.7 Hz), 73.9 (d, *J* = 5.3 Hz), 70.5, 69.5 (d, *J* = 9.1 Hz), 63.1, 23.9(d, *J* = 5.3 Hz), 23.6 (d *J* = 3.8 Hz), 16.1 (d, *J* = 25 Hz), 14.5; ^{19}F NMR (376.4 MHz, $CDCl_3$) δ −160.41; ^{31}P NMR (162 MHz, $CDCl_3$ with respect to an external

standard of triphenylphosphate in $CDCl_3$ set to −17.80) δ −7.18. LRMS (ESI) $[M + H]^+$ calculated for $C_{16}H_{24}FN_5O_6P$ 432.4, found 432.4. Elemental analysis: Calcd: C, 44.15; H, 5.37; N, 16.24. Found: C, 44.21; H, 5.21; N, 15.90; . For *trans*-isomer **23**: $[\alpha]^{20}_D$ = −79 (*c* 1.0, MeOH); T_g 95−105 °C (glass transition); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (s, 1H), 5.98 (br d, *J* = 20.0 Hz, 1H), 5.78 (br s, 1H), 5.10 (br s, 2H), 4.83 (sept, *J* = 6.4 Hz, 1H), 4.63−4.48 (m, 4H), 4.45−4.38 (m, 1H), 1.47−1.21 (m, 12H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 161.9, 159.8, 152.9, 138.3, 116.7, 98.2 (dd, J_{C-F} = 188, 8.3 Hz), 92.8 (d, *J* = 37.9 Hz), 79.2 (d, *J* = 16.6 Hz), 75.8 (d, *J* = 6.9 Hz), 70.8, 69.2 (d, *J* = 6.8 Hz), 63.1, 23.9 (d, *J* = 2.0 Hz), 23.8 (d, *J* = 3.1 Hz), 16.5 (d, *J* = 24.3 Hz), 14.7. ^{31}P NMR (162 MHz, $CDCl_3$ with respect to an external standard of triphenylphosphate in $CDCl_3$ set to −17.80) δ −3.74 (s). LRMS (ESI) $[M + H]^+$ calculated for $C_{16}H_{24}FN_5O_6P$ 432.4, found 432.4.

Preparation of Isopropyl Phoshorodichloridate. A solution of isopropyl alcohol (38.6 mL, 0.5 mol) and triethylamine (69.83 mL, 0.5 mol) in dichloromethane (250 mL) was added to a stirred solution of $POCl_3$ (50.45 mL, 0.551 mol) in DCM (250 mL), dropwise over a period of 25 min at −5 °C. After the mixture stirred for 1 h, the solvent was evaporated, and the residue was suspended in diethyl ether (400 mL). The triethylamine hydrochloride salt was filtered and washed with ether (100 mL). The filtrate was concentrated, and the residue was distilled under high vacuum (∼10 mm) with a cow-head fraction collector at 85 °C (bath temperature). The required product was collected between 42 and 48 °C (distillation head temperature) as a colorless liquid (82 g, 92.5% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.08−4.96 (m, 1H), 1.48 (dd, *J* = 6.4, 0.8 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 79.4 (d, *J* = 9.1 Hz), 22.9 (d, *J* = 5.3 Hz); ^{31}P NMR (162 MHz, $CDCl_3$ with respect to an external standard of triphenylphosphate in $CDCl_3$ set to −17.80) δ 6.77

Stereoselective Synthesis of (2*R*,4a*R*,6*R*,7*R*,7a*R*)-6-(2-amino-6-ethoxy-9*H*-purin-9-yl)-7-fluoro-2-isopropoxy-7-methyltetrahydro-4*H*-furo[3,2-*d*][1,3,2]dioxaphosphinine 2-Oxide (4). To a stirred suspension of (2*R*,3*R*,4*R*,5*R*)-5-(2-amino-6-ethoxy-9*H*-purin-9-yl)-4-fluoro-2-(hydroxymethyl)-4-methyltetrahydrofuran-3-ol (5.0 g, 15.28 mmol) in dichloromethane (75 mL) was added triethylamine (8.52 mL, 61.1 mmol) at room temperature. The reaction mixture was cooled to −20 °C, and then was added isopropyl phosphorodichloridate (3.51 g, 2.64 mL, 19.9 mmol), dropwise over a period of 10 min. The mixture was stirred at this temperature for 15 min and then was added NMI (2.54 mL, 32.08 mmol), dropwise over a period of 15 min. The mixture was stirred between −20 and −15 °C for 1 h and then slowly warmed to room temperature in 20 h. The solvent was evaporated, and the residue was dried under high vacuum to remove traces of triethyl amine and excess reagent. The residue was suspended in ethyl acetate (150 mL), washed with water (3 × 20 mL) and brine (20 mL), dried over anhydrous sodium sulfate, filtered and concentrated to ∼75 mL of volume. The solution was filtered through a pad of silica gel (15 g) and washed with ethyl acetate two times with 20 mL portions. The filtrate was concentrated to ∼10 mL of volume and heated at reflux for 3 h. The mixture was cooled to room temperature and stirred for 3 h. The white solid was filtered, washed with ethyl acetate (2 mL) and dried to give pure **4** as a white solid (3.52 g, 52% yield). Analytical data of the product obtained by this method was identical to the product from original P(III) method.

■ ASSOCIATED CONTENT

Supporting Information. Copies of ^{1}H and ^{13}C NMR spectra for compounds **4**, **12**−**21**, **23** and isopropyl phosphorodichloridate reagent. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: bruce.ross@pharmasset.com.

■ ACKNOWLEDGMENT

We thank Dr. Wonsuk Chang of Pharmasset for helpful discussions and Dr. Kyungil Kim and Mr. Jae-Hun Jeon, ST Pharm. Co. Ltd. (formerly Samchully Pharm. Co. Ltd.) for providing technical assistance.

■ REFERENCES

(1) Lavanchy, D. The global burden of hepatitis C. *Liver Int.* **2009**, *29* (S1), 74–81.

(2) (a) Zeuzem, S.; Berg, T.; Moeller, B.; Hinrichsen, H.; Mauss, S.; Wedemeyer, H.; Sarrazin, C.; Hueppe, D.; Zehnter, E.; Manns, M. P. C. *J. Viral. Hepat.* **2009**, *16*, 75. (b) Fried, M. W.; Shiffman, M. L.; Reddy, K. R.; Smith, C.; Marinos, G.; Goncales, F. L.; Haussinger, D.; Diango, M.; Carosi, G.; Dhumeaux, D.; Craxi, A.; Lin, A.; Hoffman, J.; Yu, J. *N. Engl. J. Med.* **2002**, *347*, 975.

(3) (a) Burton, J. R.; Everson, G. T. *Clin Liver Dis.* **2009**, *13*, 453–465. (b) Beaulieu, P. L. *Expert Opin. Ther. Pat.* **2009**, *19*, 145.

(4) (a) Clark, J. L.; Hollecker, L.; Mason, J. C.; Stuyver, L. J.; Tharnish, P. M.; Lostia, S.; McBrayer, T. R.; Schinazi, R.; Watanabe, K. A.; Otto, M. J.; Furman, P. A.; Stec, W. J.; Patterson, S. E.; Pankiewicz, K. W. *J. Med. Chem.* **2005**, *48*, 5504. (b) Stuyver, L. J.; McBrayer, T. R.; Tharnish, P. M.; Clark, J. L.; Hollecher, L.; Lostia, S.; Nachman; Grier, J.; Bennett, M. A.; Xie, M.-Y.; Schinazi, R. F.; Morrey, J. D.; Julander, J. L.; Furman, P. A.; Otto, M. J. *Antiviral Chem. Chemother.* **2006**, *17*, 79. (c) Stuyver, L. J.; McBrayer, T. R.; Whitaker, T.; Tharnish, P. M.; Ramesh, M.; Lostia, S.; Cartee, L.; Shi, J.; Hobbs, A.; Schinazi, R. F.; Watanabe, K. A; Otto, M. J. *Antimicrob. Agents Chemother.* **2004**, *48*, 651.

(5) (a) Sofia, M. J.; Bao, D.; Chang, W.; Du, J.; Nagarathnam, D.; Rachakonda, S.; Reddy, P. G.; Ross, B. S.; Wang, P.; Zhang, H.-R.; Bansal, S.; Espiritu, C.; Keilman, M.; Lam, A. M.; Micolochick Steuer, H. M.; Niu, C.; Otto, M. J.; Furman, P. A. *J. Med. Chem.* **2010**, *53*, 7202. (b) Sofia, M. J. ; Du, J. ; Wang, P. ; Nagarathnam, D. Intl. Pat. Appl. WO2008121634, 2008.

(6) Wang, P.; Chun, B.-K.; Rachakonda, S.; Du, J.; Khan, N.; Shi, J.; Stec, W.; Cleary, D.; Ross, B. S.; Sofia, M. J. *J. Org. Chem.* **2009**, *74*, 6819.

(7) (a) Reddy, R.; Rodriguez-Torres, M.; Gane, E.; Robson, R.; Lalezari, J.; Everson, G.; DeJesus, E.; McHutchinson, J.; Vargas, H. 58th Annual Meeting of the American Association of the Study of Liver Disease (AASLD), Boston, November 2007, Abstr. No. LB9. (b) Lalezari, J.; Gane, E.; Rodriguez,-Torres, M.; DeJesus, E.; Nelson, D.; Everson, G.; Jacobson, I.; Reddy, R.; Hill, G. Z.; Beard, A.; Symonds, W. T.; Berrey, M. M.; McHutchison, J. G. 43rd Annual Meeting of the European Association for the Study of the Liver (EASL), Milan, April 2008.

(8) Rodriguez-Torres, M.; Lalezari, J; Gane, E.; DeJesus, E.; Nelson, D.; Everson, G.; Jacobsen, I.; Reddy, R.; McHutchinson, J.; Beard, A.; Walker, S.; Symonds, B.; Berrey, M. 59th Annual Meeting of the American Association for the Study of Liver Disease, San Francisco, November 2008, Abstr. No. 1899.

(9) (a) Lawitz, E.; Lalezari, J.; Rodriguez-Torres, M.; Kowdley, K. V.; Nelson, D. R.; DeJesus, E.; McHutchison, J. G.; Compropst, M. T.; Mader, M.; Albanis, E.; Symonds, W. T.; Berry, M. M. 61st Annual Meeting of the American Association for the Study of Liver Disease, Boston, October 29−November 2, 2010, Abstr. No. 806. (b) Lawitz, E.; Lalezari, J.; Rodriguez-Torres, M.; Kowdley, K. V.; Nelson, D. R.; DeJesus, E.; McHutchison, J. G.; Compropst, M. T.; De La Rosa, A.; Symonds, W. T.; Berry, M. M. 61st Annual Meeting of the American Association for the Study of Liver Disease, Boston, October 29−November 2, 2010, Abstr. No. 1861.

(10) Reddy, P. G.; Bao, D.; Chang, W.; Chun, B.-K.; Du, J.; Nagarathnam, D.; Rachakonda, S.; Ross, B. S.; Zhang, H.-R.; Bansal, S.; Espiritu, C. L.; Keilman, M.; Lam, A. M.; Niu, C.; Micolochick Steuer,

H.; Furman, P. A.; Otto, M. J.; Sofia, M. J. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 7376.

(11) Lam, A. M.; Espiritu, C. L.; Micolochick Steuer, H. M.; Murakami, E.; Niu, C.; Keilman, M.; Frick, D. N.; Heck, J. A.; Sofia, M. J.; Nagarathnam, D.; Chang, W.; Reddy, P. G.; Ross, B. S.; Chun, B.-K.; Wang, P.; Zhang, H.-R.; Rachakonda, S.; Bao, D.; Otto, M. J.; Furman, P. A. 60th Annual Meeting of the American Association for the Study of Liver Disease, Boston, October 30−November 3, 2009, Abstr. No. 1605.

(12) Unpublished results (Pharmasset, Inc. press release, October 28, 2010.)

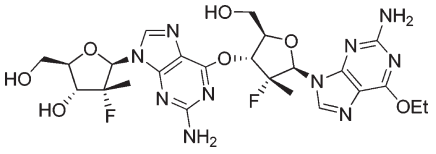
(13) Clark, J. L.; Mason, J. C.; Hollecker, L.; Stuyer, L. J.; Tharnish, P. M.; McBrayer, T. R.; Otto, M. J.; Furman, P. A.; Schinazi, R. F.; Watanabe, K. A. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1712.

(14) (a) Broeders, N. L. H. L.; Van Der Heiden, A. P.; Koole, L. H.; Kanters, J. A.; Schouten, A. *Can. J. Chem.* **1993**, *71*, 855. (b) Broeders, N. L. H. L.; Koole, L. H.; Buck, H. M. *J. Am. Chem. Soc.* **1990**, *112*, 7475.

(15) (a) Cruz-Gregorio, S.; Rodriguez-Palacios, V.; Hopfl, H.; Quintero, L.; Sartillo-Piscil, F. *J. Org. Chem.* **2009**, *74*, 197. (b) Cruz-Gregorio, S.; Sanchez, M.; Clara-Sosa, A.; Bèrnes, S.; Quintero, L.; Sartillo-Piscil, F. *J. Org. Chem.* **2005**, *70*, 7107. (c) Beres, J.; Sagi, G.; Bentrude, W. G.; Balzarini, J.; Clercq, E. D.; Otvos, L. *J. Med. Chem.* **1986**, *29*, 1243. (d) Beres, J.; Sandor, P.; Kalman, A.; Koritsanszky, T.; Otvos, L. *Tetrahedron* **1984**, *40*, 2405. (e) Gunic, E.; Girardet, J.-L.; Ramasamy, K.; Stoisavljevic-Petkov, V.; Chow, S.; Yeh, L.-T.; Hamatake, R. K.; Raney, A.; Hong, Z. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2452.

(16) Eldrup, A. B.; Prhavc, M.; Brooks, J.; Bhat, B.; Prakash, T. P.; Song, Q.; Bera, S.; Bhat, B.; Dande, P.; Cook, P. D.; Bennett, C. F.; Carroll, S. S.; Ball, R. G.; Bosserman, M.; Burlein, C.; Colwell, L. F.; Fay, J. F.; Flores, O. A.; Getty, K.; LaFemina, R. L.; Leone, J.; MacCoss, M.; McMasters, D. R.; Tomassini, J. E.; Langen, D. V.; Wolanski, B.; Olsen, D. B. *J. Med. Chem.* **2004**, *47*, 5284.

(17) Intramolecular 3′-O−C-6 bridged byproduct:



(18) (a) Kazimierczuk, Z.; Cottam, H. B.; Revankar, G. R.; Robins, R. K. *J. Am. Chem. Soc.* **1984**, *106*, 6379. (b) Okabe, M.; Sun, R. -C.; Zenchoff, G. B. *J. Org. Chem.* **1991**, *56*, 4392. (c) Chun, B. K.; Olgen, S.; Hong, J. H.; Newton, M. G.; Chu, C. K. *J. Org. Chem.* **2000**, *65*, 685. (d) Zhong, M.; Nowak, I.; Robins, M. J. *J. Org. Chem.* **2006**, *71*, 7773.(e) Bauta, W.; Schulmeier, B. E.; Cantrell, W. R.: Lovelt, D.; Puente, J. Intl. Pat. Appl. WO 2003/011877.

(19) (a) Hoffer, M. *Chem. Ber* **1960**, *93*, 2777. (b) Howell, H. G.; Brodfuehrer, P. R.; Brundidge, S. P.; Benigni, D. A.; Sapino, C., Jr. *J. Org. Chem.* **1988**, *53*, 85.

(20) Appel, R. *Angew. Chem., Int. Ed.* **1975**, *14*, 801.

(21) ^{1}H NMR studies on pure β-lactol **14** in $CDCl_3$, acetone-d_6, CD_3CN, and CD_3OD indicated anomerization to give β/α mixture in solution. However, there was no isomerization observed in DMSO-d_6. The extent of isomerization is also solvent-dependent, but clearly there was a preference to the β-anomer in all the solvents studied (β/α ratio after 16 h in $CDCl_3$, 2.5:1; acetone-d_6, 6.25:1; CD_3CN, 3.85:1; and CD_3OD, 5.56:1). On the basis of these results, we believe that the anomeric effect favors the β-anomer in the neat liquid mixture of anomers while dynamic crystallization of β-lactol **14** drives the equilibrium to eventually give nearly exclusively the β-anomer solid.

(22) Vargeese, C.; Carter, J.; Yegge, J.; Krivjansky, S.; Settle, A.; Kropp, E.; Peterson, K.; Pieken, W. *Nucleic Acids Res.* **1998**, *26*, 1046.

(23) Beaucage, S. L. In *Current Protocols in Nucleic Acid Chemistry*; Beaucage, S. L., Bergstrom, D. E., Glick, G. D., Jones, R. A., Eds.; John Wiley & Sons: New York, NY, 2001; Chapter 3.

(24) Although the role of NMI is not clear, we believe that NMI serves as a catalyst while triethylamine scavenges the HCl generated in the reaction.