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Synthesis of Diastereomerically Pure Nucleotide Phosphoramidates

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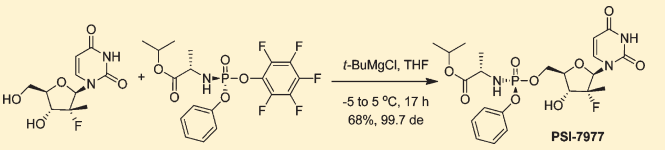
Supporting Information

ABSTRACT: Prodrugs of therapeutic nucleoside monophosphates masked as phosphoramidate derivatives have become an increasingly important class of antiviral drugs in pharmaceutical research for delivering nucleotides in vitro and in vivo. Conventionally, phosphoramidate derivatives are prepared as a mixture of two diastereomers. We report a class of stable phosphoramidating reagents containing an amino acid ester and two phenolic groups, one unsubstituted and the other with electron-withdrawing substituents. The reagents can be isolated as single diastereomers and reacted with the 5′-hydroxyl group of nucleosides through selective nucleophilic displacement of the substituted phenol to prepare single diastereomer phosphoramidate products. This method has been used to prepare the HCV clinical candidate PSI-7977 in high yield and high diastereomeric purity.



INTRODUCTION

Antiviral nucleosides must be converted to their respective 5′-*O*-triphosphate nucleotides through three separate kinases in order to inhibit the viral polymerases. Often, the first kinase for conversion of the nucleoside to its respective 5′-*O*-monophosphate nucleotide is the most discriminating. Nucleotides themselves do not have acceptable pharmacokinetic properties for drug development. Medicinal chemists have long sought to circumvent the first phosphorylation step by creating prodrugs of the monophosphate. In the case of phosphoramidates,[1] the monophosphate is masked by substitution with an aromatic ester and an amine such as an amino acid ester. Phosphoramidate prodrug strategies have been applied widely in the development of treatments for hepatitis C,[2] hepatitis B,[3] and HIV.[4] This prodrug construct provides suitable pharmacokinetic properties for in vivo absorption and in the case of treatments for HCV and HBV provides liver targeting characteristics. Once in the liver, the phosphoramidate is metabolized to the monophosphate nucleotide through a cascade of steps starting with the cleavage of the amino acid ester by esterases.[5] Our second generation nucleoside inhibitor, PSI-7977[6] (**3**, Scheme 1), utilizes this strategy and has demonstrated clinical proof[7] of concept. PSI-7977 is a single *Sp* isomer that is obtained by the first selective crystallization of a nucleotide phosphoramidate from the diastereomeric mixture and is the more potent diastereomer. However, this separation-by-crystallization approach is an inefficient method to use in the last step of a multistep synthesis. Therefore, we undertook an effort to develop a diastereoselective synthesis of PSI-7977.

Unlike the wealth of information available for stereoselective synthesis of carbon centers, the stereoselective synthesis of phosphorus centers[8] and especially phosphoramidates is limited. For a phosphorus(V) electrophilic reagent such as **2**, the phosphorus stereochemistry is already set assuming that the reagent is stable. Nucleophilic substitution on the phosphorus center leads to an inversion of configuration. Using conventional chemistry for our synthesis, the chloro reagent **2** is produced as an even mixture of two diastereomers. Logically, it would be more convergent to develop a synthesis of a single isomer of a phosphoramidating reagent which then could be used to make the desired nucleoside phosphoramidate diastereomer by nucleophilic displacement. If any chiral separation were needed, it could be done on the relatively inexpensive reagent. Unfortunately, reagent **2** was not sufficiently stable. As commonly done for other phosphoramidating reagents,[9] **2** was prepared as a crude mixture just prior to use. It is possible to purify the reagent on silica gel if care is taken to avoid contact with any moisture or alcohols, but it would not be practical to purify it by chiral chromatography. Consequently, a stable phosphoramidate reagent that was sufficiently reactive to allow ester bond formation with a nucleophilic 5′-hydroxyl group while maintaining the desired phenolic phosphate ester intact was required. Upon completion of our work, Meier[10] published a method to prepare single diastereomers of phosphoramidates using a chiral auxiliary on the phosphoramidating reagent. This method was limited by the need to chromatographically purify the reagent, modest yields, and demonstration only on a nucleoside substrate with no competing 2′- or 3′-hydroxyl groups. Here we describe the preparation of a series of stable, crystalline single diastereomer phosphoramidating reagents and a general method for the efficient preparation of diastereomerically pure 5′-nucleotide phosphoramidates.

RESULTS AND DISCUSSION

In our search for a desirable reactive phosphoramidating reagent, we were aware that Hayakawa[11] et al. tested a series of phosphorochloridate diesters and phenolic phosphate triesters as phosphorylation reagents used to make nucleoside phosphate

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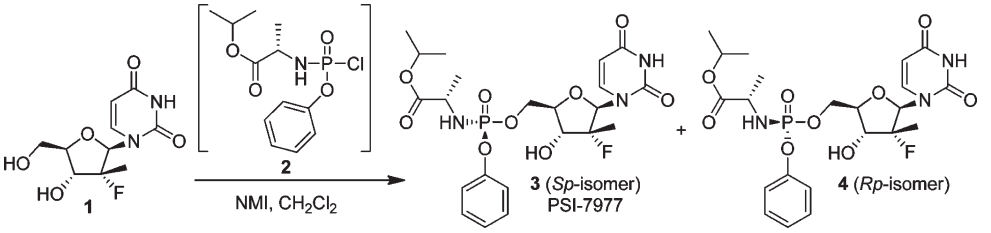
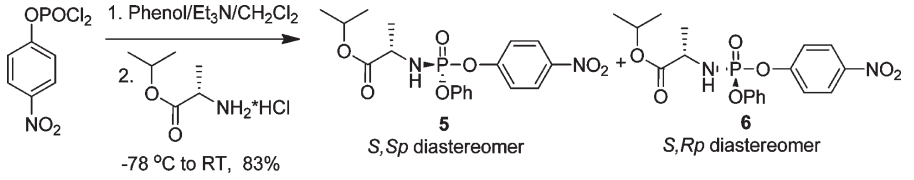
Scheme 1. Conventional Preparation of Phosphoramidates



Scheme 2. Synthesis of 4-Nitrophenyl Reagent 5 and 6



triesters. Interestingly, they reported that the chloride leaving group could be replaced with a *p*-nitrophenyl ester even in the presence of a competing but albeit less reactive *o*-chlorophenyl ester substitution. To promote nucleophilic displacement, a strong base was needed to deprotonate the 5′-hydroxyl group. *tert*-Butylmagnesium chloride had been shown to afford 5′-*O*-nucleoside regioselectivity in the construction of 5′-*O*-phosphotriesters[12] and 5′-*O*-phosphoramidates.[13] Therefore, we decided to test a phosphoramidating reagent containing a *p*-nitrophenyl ester and to use *tert*-butylmagnesium chloride as the base.

The desired nitrophenyl phosphoramidating reagent could be readily prepared by reacting commercial *p*-nitrophenyl dichlorophosphate and isopropyl L-alanate in the presence of triethylamine, followed by phenol to give a 1:1 mixture of diastereomers **5** and **6** as a chromatographically homogeneous oil in 83% yield (Scheme2).

Nucleoside **1** was then reacted with the reagent mixture in the presence of two equivalents of *tert*-butylmagnesium chloride in THF. One equivalent of the Grignard base was consumed by deprotonation of the relatively acidic hydrogen on the uracil base. The resulting precipitated salt of the starting nucleoside redissolved as the reaction progressed. The reaction proceeded to approximately 65% completion after 2 days at ambient temperature despite active reagent remaining. ^{31}P NMR of the crude reaction revealed high regioselectivity for the desired 5′-hydroxyl group with no detectable remaining monosubstitution on the 3′-hydroxyl group, but 5% of 3′,5′-bis-substituted byproduct. More interestingly, ^{31}P NMR of the crude reaction mixture revealed that there was a 3:1 diastereoselectivity for the *Sp* isomer **3** as well as a corresponding 1:3 (*Sp*/*Rp*) ratio of the remaining reagent diastereomers. After chromatographic purification, the recovered yield was 47% of the diastereomeric mixture of **3** and **4**.

Further investigation of the *p*-nitrophenyl phosphoramidate reagent preparation led to identifying conditions that allowed for crystallization of a single diastereomer in 96% diastereomeric excess in 44% yield of theory for one isomer without chromatography. Single crystal X-ray analysis of the crystalline diastereomer **5** revealed it to have the *Sp* configuration. The *Rp* nitrophenol reagent **6** was readily isolated from the mother liquor through supercritical fluid chromatography (SFC) using a chiral stationary phase to afford an oil that slowly solidified upon standing. Considering the change in stereochemical nomenclature priorities, it is the reagent having the *Sp* configuration that should lead, after inversion through a nucleophilic displacement reaction with **1**, to the desired *Sp* product **3**, PSI-7977. This is in fact what we observed. The transference of diastereomeric excess of reagent **5** to the product was complicated by the diastereoselectivity for *Sp* product **3** and a slight racemization of the phosphorus center of the reagent that was dependent on the level of water contamination in the reaction mixture. For product **3**, trace levels of the *Rp* diastereomer **4** could easily be reduced by crystallization. The *Rp* reagent **6** gave the *Rp* phosphoramidate product **4**, in a similar yield.

Even with diastereomerically pure reagent **5**, the reaction on the nucleoside **1** still did not go beyond 80% completion (Scheme 3). A procedure was developed to isolate the product only by crystallization to give 99.7% pure **3** in 40% yield, which was a significant improvement over the conventional synthesis[6] crystallized yield of 15%. Attempts to push the reaction to completion by heating or adding more base or reagent were not productive. A survey of alternative bases failed to find one superior to *tert*-butylmagnesium chloride. Isopropylmagnesium chloride provided similar results, but methylmagnesium bromide gave a complex mixture. DBU and DMAP led first to reaction on the 3′ hydroxyl and racemization of the reagent phosphorus center. Potassium *tert*-butoxide racemized the reagent and failed to react. Sodium hydride did not give any product. Sodium hexamethyldisilazane gave a low yield of product as well as the 3′-regiosiomer and 3′,5′-bis byproducts. Adding cosolvents such as acetonitrile led to lower yields.

Nucleoside substrates without a competing free hydroxyl would be expected to give higher yields. For example, protection of the 3′-hydroxyl group on **1** with *tert*-butyldimethylsilyl did allow the standard reaction to proceed faster and upon heating to 45 °C allowed the reaction to reach completion, affording the 3′-protected product in 70% isolated yield. From an efficiency point of view, with the additional steps for selective 3′-hydroxyl protection and deprotection, the overall yield was not improved.

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Scheme 3. Synthesis of PSI-7977 (3) using *Sp* Nitrophenyl Reagent (5)

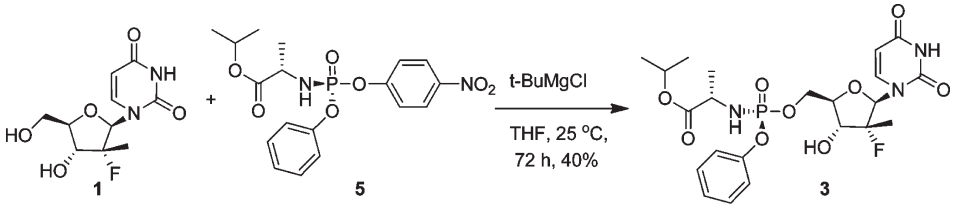
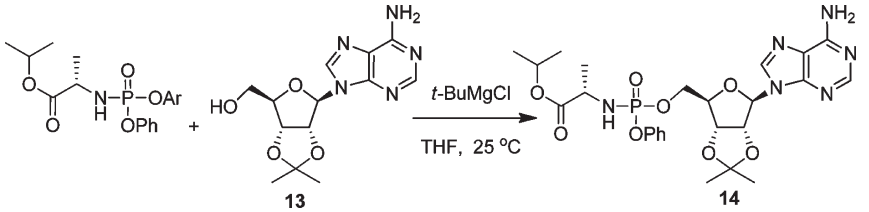


Table 1. Model Reaction To Compare Relative Reactivity of Reagents



Ar-OH	pK_a	reactivity rank	isomer
2,4-dinitrophenol	4.1	1	**7**, *Sp*
pentafluorophenol	5.5	2	**8**, *Sp*
2-chloro-4-nitrophenol	5.4	3	**9**, *Sp*
2-nitrophenol	7.2	4	**11**, *Sp*/*Rp* mix
4-nitrophenol	7.1	5	**5**, *Sp*
2,4-dichlorophenol	7.8	6	**12**, *Sp*/*Rp* mix

Clean desilylation in the presence of the phosphoramidate also proved challenging.

In our desire to increase the rate and extent of reaction, we considered more reactive reagents. Increasing the electrophilicity of the phosphorus center could be accomplished by adding electronegative substituents to the phenolic leaving group. One relative measure of this electronic effect is the pK_a of the phenol derivatives in water. The pK_a of phenol itself is 10.0, whereas 4-nitrophenol has a pK_a of 7.1. We prepared a series of analogous reagents with other acidic phenolic leaving groups in a similar series of reactions as shown in Scheme 2, except that we started with commercial phenyl dichlorophosphate, followed by addition of amino acid ester and then the test phenol. Single diastereomers were isolated by crystallization for **7**, **8**, and **9**. The *Rp* isomer of the reagent from 2-chloro-4-nitrophenol, **10**, could also be isolated through fractional crystallization. The stereochemistry of **7** and **9** was assigned on the basis of the ability to produce **3**. Reagent **8** stereochemistry was assigned on the basis of single crystal X-ray analysis. In order to test the reagents for their relative reactivity, we chose the 2′,3′-*O*-isopropylidene derivative of adenosine (**13**) as a model substrate as it did not have the complications of a competing hydroxyl group or an ionizable hydrogen that could form a salt with reduced solubility. We did observe a positive general correlation between greater acidity of the phenolic leaving group and reactivity (Table 1, Figure 1). The 2-nitrophenyl reagent (**11**) was more active than 4-nitrophenyl reagent (**4**) despite similar pK_a values of the leaving phenol. 2,4-Dinitrophenol was the most acidic, and its reagent derivative **7** was most reactive with **13**. However, when tested with nucleoside **1**, there was less discrimination between the 5′- and 3′-hydroxyl groups, leading to a higher proportion of 3′,5′-bis substitution. The pentafluorophenyl reagent (**8**) was the optimal reagent identified in this series. A separate reaction with reagent **8** and the adenosine derivative **13** was performed and resulted in a 96% isolated yield of **14**. The reaction of reagent **8** with **1** could be driven to completion in a few hours at ambient temperature with good selectivity for the desired 5′-hydroxyl group. Reagent **8** could also be prepared in a higher yield and crystallized readily as a single *Sp* diastereomer with a diastereomeric excess greater than 99.0%. Therefore we selected reagent **8** for further reaction optimization with **1** to produce **3**.

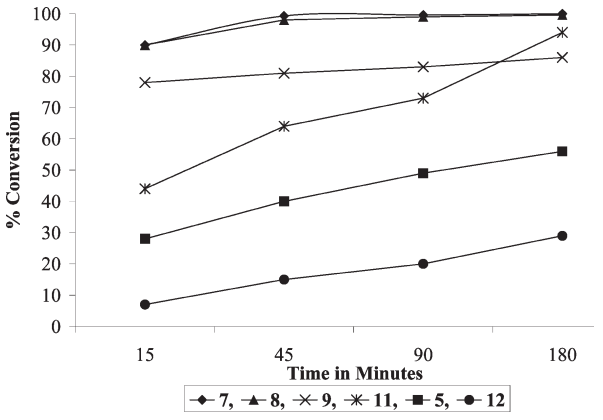


Figure 1. Time course of model reactions to compare relative reactivity of reagents.

The main challenges in reaction optimization were solubility of the nucleoside magnesium salt and balancing reaction

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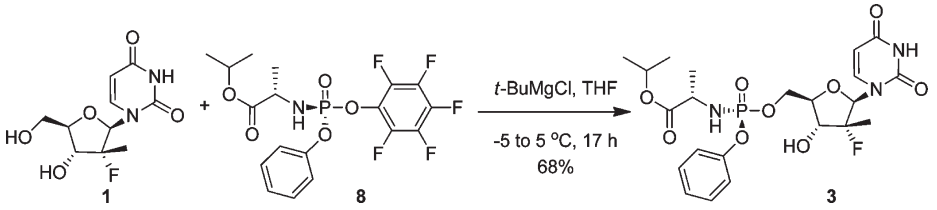
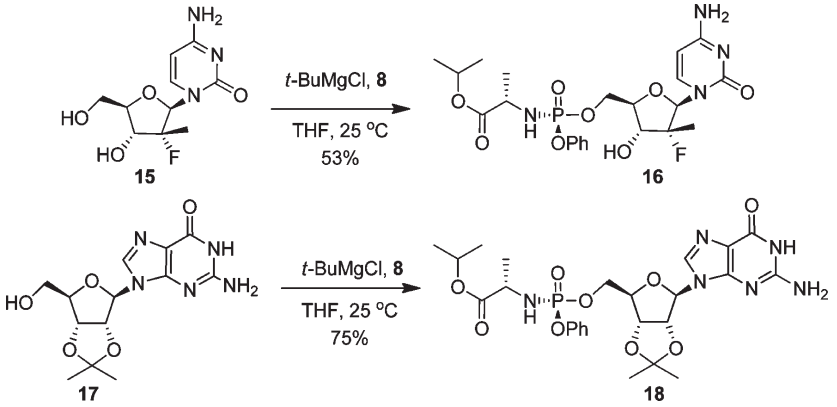
Scheme 4. Synthesis of 3 (PSI-7977) Using Pentafluorophenyl Reagent 8



Scheme 5. Synthesis of Diastereomerically Pure Cytidine and Guanosine Phosphoramidate Derivatives



completion versus minimizing the 3′,5′-bisphosphoramidate byproduct. The magnesium salt of product **3** is more soluble in the reaction solvent than the salt of nucleoside **1**, leading to a higher effective concentration of **3** and more exposure of its 3′-hydroxyl group to the reagent. Cooling the reaction to −5 °C and slow addition of the reagent limited both the remaining unreacted starting material **1** and the 3′,5′-bis byproduct to 5−8% as monitored by HPLC. The *Rp* product (**4**) was present at less than 0.5%. Neither 3′-monophosphoramidate nor the pentafluorophenyl analogue of **4** was observed when compared to prepared standards by HPLC. Lack of the latter side product indicated a very high selectivity for displacing pentafluorophenol instead of phenol in the reagent.

A multigram scale demonstration synthesis of **3** (Scheme 4) started with the addition of 2.1 equiv of *tert*-butylmagnesium chloride in THF at −5 °C to **1** followed by the addition of 1.2 equiv of reagent **8** and warming the reaction to 5 °C for 18 h. This provided **3** as 85% of the total nucleosidic material present in the reaction mixture by HPLC. Aqueous workup followed by two crystallizations of the product produced a 68% isolated yield of the desired **3** as a 99.8% pure crystalline material and with a diastereomeric excess of 99.7%.

This phosphoramidate methodology has the potential to be broadly applicable for a variety of reagents and substrates. Those reagents that do not crystallize as a single isomer can be separated by chiral chromatography. Even when used as a mixture, this method is still superior to existing phosphoramidate methods in regioselectivity, yield, and with the convenience of a shelf-stable reagent. Cytidine and guanosine nucleosides can also be used. For example, as shown in Scheme 5, reaction with reagent **8** and the cytidine analogue **15** (PSI-6130)[14] led to an isolated yield of 53% of product **16**. Although **15** had no ionizable base hydrogens, it had poor solubility in THF, which led to a slower reaction and a greater amount of the 3′,5′-bis substitution byproduct. The purine analogue 2′,3′-*O*-isopropylidine guanosine, **17**, had no competing hydroxyl groups on the sugar but did contain an ionizable hydrogen on the base unit. As for **1**, an extra equivalent of *t*-BuMgCl was added to allow the reaction to reach completion, producing an isolated yield of 75% of **18**.

CONCLUSION

In summary, we have developed a novel synthetic method for the preparation of diastereomerically pure nucleotide phosphoramidates and have demonstrated its use on representative derivatives of all four classes of nucleosides. This method was applied to the synthesis of the anti-HCV clinical agent PSI-7977, **3**. Along with our earlier published work on the synthesis of 2′-fluoro-2′-C-methyl nucleosides, this method now allows for the efficient synthesis of PSI-7977 without the use of any chiral catalysts or chiral auxiliaries and without any chromatography. This method can be applied to the preparation of a variety of stable phosphoramidation reagents and may find great utility in medicinal chemistry exploration of nucleotide phosphoramidates. Optimization and scope of the chiral phosphoramidate reaction is being explored and will be reported in the future.

EXPERIMENTAL SECTION

General Analytical Methods. Reactions were monitored by thin layer chromatography with 250 μm silica gel plates and visualized by UV light or by charring in 5% sulfuric acid in methanol. NMR spectra were recorded in $CDCl_3$, DMSO-d_6, or CD_3OD as noted on a 400 MHz spectrometer with a multinuclear probe. Optical rotations were measured using a polarimeter at ambient temperature and 589 nm. Melting points are uncorrected. HPLC purity was determined on a C18 reverse phase column with a gradient of methanol in 10 mM ammonium acetate

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aqueous buffer at pH 4.0. All solvents, reagents, and the starting nucleosides **13** and **17** were used as received from commercial vendors.

(*S*)-2-[(4-Nitro-phenoxy)-phenoxy-phosphorylamino] Propionic Acid Isopropyl Ester Diastereomeric Mixture (5 and 6). To a stirred solution of 4-nitrophenyl dichlorophosphate (12.8 g, 50 mmol) in dichloromethane (100 mL) was added a solution of phenol (4.71 g, 50 mmol) and triethylamine (Lot 1, 7.7 mL, 55 mmol) in dichloromethane (100 mL) at −78 °C over a period of 20 min. The mixture was stirred at this temperature for 30 min and then transferred to another round-bottom flask containing L-alanine isopropyl ester hydrochloride (8.38 g, 50 mmol) in dichloromethane (100 mL) at 0 °C. To the mixture was added a second lot of triethylamine (Lot 2, 14.6 mL, 105 mmol) over a period of 15 min. The mixture was stirred at 0 °C for 1 h, and then the solvent was evaporated. The residue was triturated with ethyl acetate (150 mL), and the white solid was filtered off. The filtrate was concentrated under reduced pressure to give pale yellow oil. The crude compound was chromatographed using 0−20% ethyl acetate/hexanes gradient to give product (17 g, 83%) as a mixture of diastereomers **5** and **6** in about 1:1 ratio. ^{31}P NMR (162 MHz, $CDCl_3$) δ −2.05, −2.10; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (d, J = 9.2 Hz, 2H), 7.41−7.33(m, 4H), 7.26−7.18(m, 3H), 5.05−4.96(m, 1H), 4.14−4.05(m, 1H), 3.93−3.88(m, 1H), 1.38(d, J = 6.8 Hz, 3H), 1.22 (dd, J = 6.2 and 3.0 Hz, 6H); MS (ESI) m/z 407 $(M - 1)^+$.

(*S*)-2-[(*S*)-(4-Nitro-phenoxy)-phenoxy-phosphorylamino] Propionic Acid Isopropyl Ester (5). L-Alanine isopropyl ester hydrochloride (330 g, 1.97 mol) was predried by coevaporation with toluene (2 × 400 mL) under reduced pressure and then dried in a vacuum oven (50 °C, 0.2 mmHg, 17 h). To a stirred solution of 4-nitrophenyl dichlorophosphate (500.0 g, 1.95 mol) in anhydrous dichloromethane (3.0 L) was added a solution of phenol (183.8 g, 1.95 mol) and triethylamine (300 mL, 2.15 mol) in dichloromethane (900 mL) at −60 °C internal temperature over a period of 3 h. The mixture was stirred at this temperature for additional 30 min and then allowed to warm to −5 °C over 2.5 h. The predried amino acid ester was added between −5 and 0 °C under an atmosphere of nitrogen over 10 min. The mixture was stirred at 0 °C for 40 min, and additional triethylamine (571 mL, 4.10 mol) was added over a period of 40 min at 0 °C. The mixture was stirred between 0 and 10 °C for 3 h, and then the white solid (triethylamine hydrochloride) was filtered off and rinsed with dichloromethane (3 × 300 mL). The filtrate was concentrated under reduced pressure, and the residue was triturated with TBME (*tert*-butylmethyl ether, 4 L). The additional triethylamine hydrochloride salt was filtered off and washed with TBME (3 × 150 mL). The filtrate was concentrated under reduced pressure to give clear light brown color oil. The residue was coevaporated with hexanes (2 × 140 mL) to remove any residual TBME and further dried under vacuum at 40 °C for 2 h. The dry residue was dissolved in diisopropyl ether (1.1 L) and stirred at 5 °C in an ice−water bath. Small amount of seeds of the desired *Sp* isomer **5** was added to the solution and the mixture was stirred at 5 °C for 22 h to form moderately thick slurry. This was allowed to stand in a freezer (−10 °C) for 44 h The product was collected via filtration and washed with a precooled mixture of diisopropyl ether and hexanes (1:1, 3 × 190 mL). The white solid was dried under vacuum (0.5 mmHg) at ambient temperature until a constant weight was obtained to give 227.2 g (yield: 29%). The ratio of two diastereomers *Sp*:*Rp* was 9.7/1 based on ^{31}P NMR (162 MHz, DMSO-d_6, δ −0.31 (desired *Sp* isomer **5**), −0.47 (*Rp* isomer **6**). The product was recrystallized from diisopropyl ether (840 mL) with seeds of *Sp* isomer **5**. A white solid was formed within 2 h, and the flask was allowed to stand in a freezer (−10 °C) for 16 h. A white and fine crystalline solid obtained was filtered, washed with precooled diisopropyl ether (3 × 50 mL), and dried under vacuum (0.5 mmHg) to give a white fluffy solid (177.7 g, 22% overall yield or 44% overall yield based on theoretical yield of the *Sp* isomer **5**) with diastereomeric ratio of 48/1 based on ^{31}P NMR (96% de). A small sample was recrystallized from isopropyl ether to provide suitable crystals for single crystal X-ray analysis which confirmed the *Sp* stereochemistry. $[\alpha]^{25}_D$ (c 1.00, $CHCl_3$) +1.7; mp 62−66 °C; ^{31}P NMR (162 MHz, DMSO-d_6) δ −0.31; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.30−8.27 (m, 2H), 7.49(d, J = 8.8 Hz, 2H), 7.41−7.37(m, 2H), 7.23−7.19 (m, 3H), 6.66 (dd, J = 13.6, 10.0 Hz, 1H), 4.86−4.78 (m, 1H), 3.97−3.86 (m, 1H), 1.19 (d, J = 7.2 Hz, 3H), 1.10 (d, J = 6.4 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.4 (d, J = 8.4 Hz), 155.5 (d, J = 6.1 Hz), 150.2 (d, J = 6.9 Hz), 144.6, 129.8, 125.6, 125.4, 120.7 (d, J = 5.3 Hz), 120.1 (d, J = 4.5 Hz), 69.5, 50.5, 21.6 (d, J = 7.6 Hz), 20.9 (d, J = 3.8 Hz); HRMS-ESI (m/z): calcd for $C_{18}H_{22}N_2O_7P$ $[M + H]^+$ 409.1165, found 409.1150.

Separation of (*S*)-2-[(*R*)-(4-Nitro-phenoxy)-phenoxy-phosphorylamino] Propionic Acid Isopropyl Ester (6) from 5 and 6 Mixture. A sample of the mixture of diastereomers (4.8 g) enriched with the *Rp* isomer was subjected to SFC using a chiral column (2 × 15 cm) and eluted with 35% isopropanol in carbon dioxide at 100 bar. An injection loading of 4 mL of sample at a concentration of 17 mg/mL of methanol was used. The *Rp* isomer (**6**) eluted first. The appropriate fractions of the multiple runs were combined and concentrated under reduced pressure to give 2.9 g of **6** as a light yellow viscous oil that solidified upon standing over several days, mp 42−43 °C and 1.9 g of the *Sp* isomer **5** as a white solid. Both isomers were >99.9% pure by analytical SFC. Analytical data for **6**: $[\alpha]^{20}_D$ (c 1.0, MeOH) −5.6 ; ^{31}P NMR (162 MHz, DMSO-d_6) δ −0.47; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.30−8.27 (m, 2H), 7.46−7.38 (m, 4H), 7.27−7.20 (m, 3H), 6.68 (dd, J = 13.8, 10.2 Hz, 1H), 4.86−4.77 (m, 1H), 3.97−3.86 (m, 1H), 1.20 (d, J = 7.2 Hz, 3H), 1.10(dd, J = 6.2, 2.2 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.8 (d, J = 6.8 Hz), 155.8 (d, J = 6.8 Hz), 150.5 (d, J = 6.8 Hz), 144.8, 130.1, 125.8, 125.7, 121.1 (d, J = 5.3 Hz), 120.3 (d, J = 5.3 Hz), 69.7, 50.8, 21.9, 21.8, 21.0 (d, J = 5.3 Hz); MS (ESI) m/z 407 $(M - 1)^+$; HRMS-ESI (m/z): calcd for $C_{18}H_{22}N_2O_7P$ $[M + H]^+$ 409.1165, found 409.1167.

(*S*)-2-[(*S*)-(2,4-Dinitro-phenoxy)-phenoxy-phosphorylamino] Propionic Acid Isopropyl Ester (7). Phenyl dichlorophosphate (10.0 g, 47.4 mmol) was dissolved in dry dichloromethane (60 mL), and the solution was cooled to −78 °C. A solution of 2,4-dinitrophenol (8.72 g, 47.4 mmol) and triethylamine (7.27 mL, 52.1 mmol) in dichloromethane (20 mL) was slowly added at −78 °C over a period of 30 min. The reaction mixture was warmed to 0 °C and stirred for 2.5 h at this temperature before L-alanine isopropyl ester (7.95 g, 47.4 mmol) was added in one lot. After stirring for 40 min at 0 °C, triethylamine (13.9 mL, 99.54 mmol) was added and stirred for additional 3 h at 0 °C. The reaction mixture was evaporated under reduced pressure, and the residue was suspended in TBME (100 mL). The triethylamine hydrochloride salt was removed by filtration, and the filtrate was concentrated under reduced pressure to give yellow syrup. ^{31}P NMR of the crude sample indicated mixture of two diastereomers in the ratio of 1:1. A mixture of EtOAc/hexanes (1:1, 50 mL) was added, and the mixture was allowed to stir for 15 h. The white solid thus formed was filtered off and washed with EtOAc/hexanes (1:1, 20 mL) and dried under vacuum to give 6.0 g (28%) of **7**. The phosphorus stereochemistry was assigned as *Sp* on the basis of subsequent reactions to produce **3**, which has a known phosphorus stereochemistry. Based on ^{1}H NMR, the ratio of *Sp*/*Rp* isomers was 15.7:1 (88% de). Mp 104−106 °C; ^{31}P NMR ($CDCl_3$, 162 MHz) δ −1.82; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.82−8.81 (m, 1 H), 8.43−8.40 (m, 1 H), 7.89−7.86 (m, 1 H), 7.36−7.32 (m, 2 H), 7.23−7.19 (m, 3 H), 4.96 (hepta, J = 6.4 Hz, 1 H), 4.19−4.08 (m, 2 H), 1.42 (d, J = 6.4 Hz, 3 H), 1.20 (d, J = 6.4 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.3 (d, J = 8.4 Hz), 149.8 (d, J = 6.8 Hz), 148.4 (d, J = 5.3 Hz), 143.6, 140.9 (d, J = 6.8 Hz), 130.0, 128.8, 125.8, 123.9 (d, J = 2.3 Hz), 121.6, 120.1 (d, J = 5.4 Hz), 69.6, 50.6, 21.6 (d, J = 5.3 Hz), 20.8 (d, J = 4.6 Hz); HRMS-ESI (m/z): calcd for $C_{18}H_{21}N_3O_9P$ $[M + H]^+$ 454.1010, found 454.1003.

(*S*)-2-[-(*S*)-(2,3,4,5,6-Pentafluoro-phenoxy)-phenoxy-phosphorylamino] Propionic Acid Isopropyl Ester (8). To a stirred solution of L-alanine isopropyl ester hydrochloride (8.0 g 47.7 mmol) in

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anhydrous dichloromethane (50 mL) was added triethylamine (10.0 g, 13.8 mL, 99.0 mmol) at −70 °C over 15 min dropwise. To this mixture was added a solution of phenyl dichlorophosphate (10.0 g, 47.3 mmol) in anhydrous dichloromethane (50 mL) over 1 h. The reaction mixture was stirred at this temperature for additional 30 min and then allowed to warm to 0 °C over 2 h and stirred for 1 h. To this mixture was added a solution of 2,3,4,5,6-pentafluoro phenol (8.72 g, 47.3 mmol) and triethylamine (5.27 g, 7.26 mL, 52.1 mmol) in dichloromethane (30 mL) over 20 min. The crude mixture was allowed to stir at 0 °C for 4 h, and the white solid (triethylamine hydrochloride) was filtered off and washed with dichloromethane (1 × 25 mL). The filtrate was concentrated under reduced pressure, the residue was triturated with TBME (150 mL), and the triethylamine hydrochloride salt was removed by filtration. The cake was washed with TBME (2 × 25 mL), and the combined filtrate was concentrated under reduced pressure to give 22 g of crude solid containing an even mixture of diastereomers. The mixture was triturated with 20% EtOAc in hexanes (100 mL) and collected by filtration to give 7.4 g (34%) of **8** as a white solid (>98% de as determined by NMR). A small portion was crystallized from the mixture of 2-propanol and TBME to give suitable crystals for single crystal X-ray analysis, which confirmed the *Sp* stereochemistry. $[\alpha]^{25}_{D}$ (*c* 1.00, $CHCl_3$) +3.8; mp 130−134 °C; ^{31}P NMR ($CDCl_3$, 162 MHz) δ −0.50; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.38−7.34 (m, 2 H), 7.27−7.24 (m, 2 H), 7.23−7.19 (m, 1 H), 5.04 (m, J = 6.4 Hz, 1 H), 4.18−4.09 (m, 1 H), 4.00−3.95 (m, 1 H), 1.45 (d, J = 7.2 Hz, 3 H), 1.25 (d, J = 6 Hz, 3 H), 1.24 (d, J = 6 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.4 (d, J = 9.1 Hz), 150.1 (d, J = 6.8 Hz), 142.7−142.5 (m), 140.2−140.0 (m), 139.3−139.0 (m), 137.8−137.4 (m), 136.8−136.5 (m), 129.8, 125.6, 120.0 (d, J = 4.5 Hz), 69.6, 50.6, 21.6 (d, J = 2.2 Hz), 20.9 (d, J = 4.6 Hz); HRMS-ESI (*m*/*z*): calcd for $C_{18}H_{18}F_5NO_5P$ $[M + H]^+$ 454.0837, found 454.0829.

(*S*)-2-[(*S*)-(2-Chloro-4-nitro-phenoxy)-phenoxy-phosphorylamino] Propionic Acid Isopropyl Ester (9) and (*S*)-2-[(*R*)-(2-Chloro-4-nitro-phenoxy)-phenoxy-phosphorylamino] Propionic Acid Isopropyl Ester (10). Phenyl dichlorophosphate (10.0 g, 47.3 mmol) was dissolved in 50 mL of dry dichloromethane and cooled to 0 °C. After addition of solid L-alanine isopropyl ester HCl salt (7.94 g, 47.3 mmol), the reaction mixture was cooled to −70 °C and then treated with triethylamine (13.8 mL, 94.6 mmol) dissolved in 50 mL of dry dichloromethane. The resulting mixture was stirred for 30 min at this temperature and then allowed to warm to 0 °C. A solution of 2-chloro-4-nitrophenol (8.2 g, 47.3 mmol) and triethylamine (6.6 mL, 47.3 mmol) in 20 mL of dry dichloromethane was added over 5−10 min and stirred for additional 2 h. The solution was filtered, and the filtrate was concentrated under reduced pressure. The resulting residue was suspended in 50 mL of TBME and stirred for 10 min at room temperature. The white solid was removed by filtration, and the filtrate was concentrated under reduce pressure. Column chromatography of the residue using dichloromethane as an eluent gave the desired product (12.2 g, *Sp*/*Rp* ratio of 2:1 as determined by ^{31}P NMR) as a pale yellow solid. The product was recrystallized twice from EtOAc/hexane (2:3) to give pure **9** (5.2 g, 25% yield, >98% de). Stereochemistry at phosphorus center was assigned as *Sp* on the basis of the ability of the product to make **3**. Upon cooling the mother liquor to −5 °C, the *Rp* isomer **10** was crystallized out as a white solid that was collected by filtration (1.5 g, 7% yield, >98% de). *Sp* isomer **9**: $[\alpha]^{25}_{D}$ (*c* 1.00, $CHCl_3$) −16.1; mp 70−72 °C; ^{31}P NMR ($CDCl_3$, 162 MHz) δ −1.97; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.33 (m, 1 H), 8.13−8.10 (m, 1 H), 7.73−7.71 (m, 1 H), 7.36−7.33 (m, 2 H), 7.25−7.18 (m, 3 H), 5.00 (m, 1 H), 4.19−4.10 (m, 1 H), 4.02−3.97 (m, 1 H), 1.43 (d, 3 H), 1.23−1.21 (m, 6 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.3 (d, J = 8.3 Hz), 151.9 (d, J = 5.3 Hz), 150.0 (d, J = 7.6 Hz), 144.4, 129.9, 126.4 (d, J = 8.4 Hz), 126.1, 125.7, 123.4, 121.5 (d, J = 2.3 Hz), 120.2 (d, J = 4.6 Hz), 69.6, 50.6, 21.6 (d, J = 7.6 Hz), 21.0 (d, J = 4.5 Hz); HRMS-ESI (*m*/*z*): calcd for $C_{18}H_{21}ClN_2O_7P$ $[M + H]^+$ 443.0769, found 443.0759. *Rp* isomer **10**: $[\alpha]^{25}_{D}$ (*c* 1.00, $CHCl_3$) +26.6; mp 77−80 °C; ^{31}P NMR ($CDCl_3$, 162 MHz) δ −2.02; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.32−8.31 (m, 1H), 8.13−8.10 (m, 1 H), 7.73−7.71 (m, 1 H), 7.38−7.34 (m, 2 H), 7.28−7.19 (m, 3 H), 5.02 (m, 1 H), 4.21−4.11 (m, 1 H), 4.01−3.95 (m, 1 H), 1.40 (d, 3 H), 1.25−1.22 (m, 6 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.4 (d, J = 9.1 Hz), 150.1 (d, J = 6.8 Hz), 142.7−142.5 (m), 140.2−140.0 (m), 139.3−139.0 (m), 137.8−137.4 (m), 136.8−136.5 (m), 129.8, 125.6, 120.0 (d, J = 4.5 Hz), 69.6, 50.6, 21.6 (d, J = 2.2 Hz), 20.9 (d, J = 4.6 Hz); HRMS-ESI (*m*/*z*): calcd for $C_{18}H_{21}ClN_2O_7P$ $[M + H]^+$ 443.0769, found 443.0760.

(2*S*)-Isopropyl 2-(((2-Nitrophenoxy)(phenoxy)phosphoryl) amino)propanoate (11). To a stirred solution of phenyl dichlorophosphate (30 g, 142.2 mmol) in dichloromethane (150 mL) was added a solution of *o*-nitrophenol (19.76 g, 142.2 mmol) and triethylamine (19.8 mL, 142.2 mmol) in dichloromethane (150 mL) over a period of 1 h at −70 °C. The mixture slowly warmed to 0 °C over 2 h. To the mixture was added L-alanine isopropyl ester hydrochloride salt (26.2 g, 156.3 mmol) in one lot followed by a solution of triethylamine (43.7 mL, 313.4 mmol) in dichloromethane (150 mL) over 20 min, and the reaction mixture was stirred for additional 1 h. The solid was filtered, and the filtrate was concentrated. The residue was purified by column chromatography (20% EtOAc/hexanes) on a silica gel to give **11** as a mixture of diastereomers (14.4 g, 25% yield). ^{31}P NMR ($CDCl_3$, 162 MHz) δ −1.55 (isomer I), −1.76 (isomer II); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.94−7.90 (m, 1 H), 7.67−7.63 (m, 1 H), 7.57−7.54 (m, 1 H), 7.33−7.26 (m, 3 H), 7.23−7.14 (m, 3 H), 5.04−4.89 (m, 1 H), 4.21−4.04 (m, 2 H), 1.38 (d, 3 H, isomer I), 1.33 (d, 3 H, isomer II), 1.23−1.17 (m, 6 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.6 (s), 172.5 (s), 150.3−150.2 (m), 143.7−143.6 (m), 141.4 (s), 134.5−134.4 (m), 129.8 (d), 125.7−125.2 (m), 123.2−123.0 (m), 120.3−120.2 (m), 69.4−69.3 (m), 68.3 (s), 50.5−50.3 (m), 22.9 (s), 21.6−21.5 (m), 21.0−20.9 (m); HRMS-ESI (*m*/*z*): calcd for $C_{18}H_{22}N_2O_7P$ $[M + H]^+$ 409.1165. Observed: 409.1154.

(2*S*)-Isopropyl 2-(((2,4-Dichlorophenoxy)(phenoxy)phosphoryl)amino)propanoate (12). To a stirred solution of phenyl dichlorophosphate (10.0 g, 47.4 mmol) in dry dichloromethane (60 mL) was added a solution of 2,4-dichlorophenol (7.73 g, 47.4 mmol) and triethylamine (7.27 mL, 52.1 mmol) in dichloromethane (20 mL) at −78 °C over 30 min. The reaction mixture was allowed to warm to 0 °C and stirred for additional 2.5 h. To the above mixture was added L-alanine isopropyl ester hydrochloride (7.95 g, 47.4 mmol) in one lot followed by triethylamine (13.9 mL, 99.54 mmol). The mixture was stirred for an additional 3 h at 0 °C, and the solvent was evaporated under reduced pressure. The residue was chromatographed using ethyl acetate/hexanes gradient to give **12** as colorless viscous oil (13.6 g, 66% yield, 1:1 mixture of diastereomers). ^{31}P NMR ($CDCl_3$, 162 MHz) δ −1.52 (isomer I), −1.54 (isomer II); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.47−7.44 (m, 1 H), 7.42−7.39 (m, 1 H), 7.35−7.30 (m, 2 H), 7.24−7.15 (m, 3 H), 5.05−4.94 (m, 1 H), 4.19−4.08 (m, 1 H), 3.96−3.89 (m, 1 H), 1.41−1.35 (m, 1 H), 1.24−1.19 (m, 6 H); ^{13}C NMR ($CDCl_3$, 100 MHz) 172.5−172.4 (m), 150.4−150.2 (m), 145.6−145.4 (m), 130.4−129.6 (m), 127.9 (d), 126.2 (d), 125.2 (s), 122.5−122.4 (m), 120.3−120.1 (m), 69.3 (d), 50.5−50.4 (m), 21.6 (s), 21.5−20.9 (m). HRMS-ESI (*m*/*z*): calcd for $C_{18}H_{21}Cl_2NO_5P$ 1:1 $[M + H]^+$ 432.0529, found 432.0521.

(*S*)-Isopropyl (((2*R*,3*R*,4*R*,5*R*)-5-(2,4-Dioxo-3,4-dihydropyrimidin-(2*H*)-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy)phosphorylamino)propanoate Diastereomeric Mixture (3 and 4). To a stirred solution of 1-((2*R*,3*R*,4*R*,5*R*)-3-fluoro-4-hydroxy-5-hydroxymethyl-3-methyl-tetrahydro-furan-2-yl)-1*H*-pyrimidine-2,4-dione (130 mg, 0.5 mmol) in dry THF (1.5 mL) was added a 1.0 M solution of *tert*-butylmagnesium chloride (1.05 mL, 1.05 mmol, 2.1 equiv) at room temperature over a

period of 5 min. After 30 min, a solution of (*S*)-2-[(4-nitro-phenoxy)-phenoxy-phosphorylamino] propionic acid isopropyl ester (1:1 mixture of isomers **5** and **6**, 408 mg, 1.0 mmol) in THF (1.5 mL) was added dropwise over a period of 5 min. The mixture was allowed to stir at room temperature for 48 h and then quenched with saturated aqueous NH_4Cl (20 mL). The mixture was partitioned between ethyl acetate (50 mL) and water (20 mL). The combined organic extract was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to give a pale yellow residue. Column chromatography of the residue using 0−5% MeOH/dichloromethane gradient gave a white foamy solid (125 mg, 47% yield, mixture of *Sp*/*Rp* diastereomers **3** and **4** in about 3.05:1.0 ratio, respectively). Analytical data of the product was consistent with the original mixture of diastereomers.[6]

(*S*)-Isopropyl 2-((*R*)-(((2*R*,3*R*,4*R*,5*R*)-5-(2,4-Dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy)phosphorylamino)-propanoate (4). To a stirred solution of 1-((2*R*,3*R*,4*R*,5*R*)-3-fluoro-4-hydroxy-5-hydroxymethyl-3-methyl-tetrahydro-furan-2-yl)-1*H*-pyrimidine-2,4-dione (**1**, 260 mg, 1 mmol) in dry THF (6 mL) was added a 1.7 M solution of *tert*-butylmagnesium chloride (1.23 mL, 2.1 mmol, 2.1 equiv) at room temperature over a period of 5 min. After 30 min, a solution of (*S*)-2-[(*R*)-(4-nitro-phenoxy)-phenoxy-phosphorylamino] propionic acid isopropyl ester (**6**, *Rp* isomer) in THF (3 mL) was added dropwise over a period of 3 min. The mixture was allowed to stir at room temperature for 96 h and then quenched with saturated aqueous NH_4Cl (10 mL). The mixture was partitioned between ethyl acetate (50 mL) and water (2 × 20 mL). The combined organic extract was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to give a pale yellow residue (490 mg). The residue was chromatographed using 0−5% MeOH/dichloromethane gradient to give product **4** as a white solid (160 mg, 30% yield). Analytical data of the product was consistent with standard sample of **4**.[6]

(*S*)-Isopropyl 2-((*S*)-(((2*R*,3*R*,4*R*,5*R*)-5-(2,4-Dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy)phosphorylamino)propanoate (3) Using Reagent 5. 1-((2*R*,3*R*,4*R*,5*R*)-3-Fluoro-4-hydroxy-5-hydroxymethyl-3-methyl-tetrahydro-furan-2-yl)-1*H*-pyrimidine-2,4-dione (**1**, 10.0 g, 38.5 mmol) was dried under vacuum at 50 °C for 20 h and dissolved in dry THF (200 mL) in a two neck 1 L round-bottom flask equipped with an addition funnel, nitrogen inlet, and magnetic stir bar. The flask was immersed in a tap water bath at ambient temperature, and then was added a 1.7 M solution of *tert*-butylmagnesium chloride (47.5 mL, 80.7 mmol, 2.1 equiv) over a period of 30 min (slightly exothermic). After an additional 30 min, a solution of (*S*)-2-[(*S*)-(4-nitro-phenoxy)-phenoxyphosphorylamino] propionic acid isopropyl ester (**5**) in THF (20 mL) was added in over a period of 15 min. The mixture was allowed to stir at room temperature for 60 h. TLC of the reaction mixture indicated ∼20% of unreacted starting material. The reaction mixture was quenched with saturated aq ammonium chloride (5 mL). The solvent was evaporated under reduced pressure, and the residue was dried under high vacuum to give yellow foam. The residue was suspended in ethyl acetate (400 mL) and then washed with water (2 × 50 mL). The organic layer was washed with 5% aqueous sodium bicarbonate (6 × 50 mL), until TLC of the organic layer indicated the absence of nitrophenol. The organic layer was washed with water (3 × 50 mL) and brine (50 mL), dried over anhydrous sodium sulfate, and concentrated to give a light brown solid, 18.2 g. Dichloromethane (45 mL) was added to the solid and heated at reflux for 3 h, then allowed to cool to ambient temperature, and stirred for 65 h. The white solid was filtered, washed with 1:1 (v/v) isopropyl ether/dichloromethane (20 mL), and dried under high vacuum to give white solid, 9.2 g. The solid was redissolved in dichloromethane (275 mL), heated to reflux, and filtered hot through filter paper. The dichloromethane was concentrated at atmospheric pressure to a volume of 200 mL. The solution was cooled to ambient temperature and allowed to stand for 17 h. The white needles obtained were filtered and dried under high vacuum for 17 h to give **3** (8.1 g, 40% yield, 99.7% HPLC purity). Analytical data of the product was consistent with the standard sample of **3**.[6]

(*S*)-Isopropyl 2-((*S*)-(((2*R*,3*R*,4*R*,5*R*)-5-(2,4-Dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy)phosphorylamino) propanoate (3) Using Reagent 8. To a stirred suspension of 1-((2*R*,3*R*,4*R*,5*R*)-3-fluoro-4-hydroxy-5-(hydroxymethyl)-3-methyl-tetrahydrofuran-2-yl)pyrimidine-2,4(1*H*,3*H*)-dione (**1**, 5.0 g, 19.1 mmol, dried under vacuum at 50 °C for 20 h) in dry THF (75 mL) was added a 1.7 M solution of *tert*-butylmagnesium chloride in THF (23.7 mL, 40.35 mmol) using an addition funnel over a period of 30 min at −5 °C. The white suspension was stirred at this temperature for 30 min and then warmed to ambient temperature (20 °C) at which temperature it was stirred for additional 30 min. The reaction mixture was cooled to 5 °C, and then was added a solution of (*S*)-2-[(*S*)-(2,3,4,5,6-pentafluorophenoxy)-phenoxyphosphorylamino] propionic acid isopropyl ester (**8**, 10.45 g, 23.06 mmol) in THF (50 mL) over a period of 30 min. The mixture was stirred at 5 °C for 18 h, cooled to −5 °C, and then quenched with 2 N HCl (25 mL). Toluene (100 mL) was added to the mixture and warmed to room temperature. After 20 min the layers were separated. The organic layer was washed with 1 N HCl (2 × 10 mL), water (10 mL), 5% aqueous Na_2CO_3 (4 × 10 mL), water (2 × 10 mL), and brine (10 mL). All of the aqueous layers were re-extracted with toluene (20 mL) and washed with 5% aqueous Na_2CO_3 (2 × 5 mL), water (10 mL), and brine (5 mL). The combined organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated to an approximate volume of 20 mL. Dichloromethane (20 mL) was added to the solution, and the mixture was stirred at room temperature for 18 h. The solid was filtered, washed with 1:1 TBME/dichloromethane mixture (2 × 10 mL), and dried under high vacuum to give white solid (7.7 g). HPLC of the solid indicated 98.21% of **3**, 0.18% of unreacted nucleoside **1** and 0.67% of 3′,5′-bis-phosphoramidate impurity. The above solid was redissolved in dichloromethane (77 mL, heated in a pressure vessel at 55 °C) and allowed to stand at room temperature for 20 h. The crystalline solid was filtered washed with cold dichloromethane (5 mL, 0 °C) and dried under high vacuum to give pure product as a white solid (6.9 g, 68% yield) matching a standard sample of **3**.[6] HPLC analysis showed a total purity of 99.79% with impurities composed of 0.07% unknown and 0.14% of the *Rp* isomer, resulting in a diastereomeric excess of 99.72%.

(*S*)-Isopropyl 2-(((*S*)-(((3a*R*,4*R*,6*R*,6a*R*)-6-(6-Amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro-[3,4-*d*][1,3]dioxol-4-yl)methoxy)(phenoxy)phosphoryl)amino)propanoate (14). To a stirred solution of ((3a*R*,4*R*,6*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methanol (**13**, 307 mg, 1 mmol) in dry THF (3 mL) was added a 1.7 M solution of *tert*-butylmagnesium chloride in THF (0.706 mL, 1.2 mmol) over a period of 3 min at room temperature. The white suspension was stirred at this temperature for 30 min, and then was added a solution of (*S*)-2-[(*S*)-(2,3,4,5,6-pentafluorophenoxy)-phenoxyphosphorylamino] propionic acid isopropyl ester (**8**, 544 mg, 1.2 mmol) in THF (3 mL) over a period of 3 min. The mixture was stirred at this temperature for 18 h. The reaction mixture was quenched with methanol (1 mL), solvent was evaporated, and the residue was chromatographed using 0−10% methanol/dichloromethane gradient to give pure **14** as a white amorphous solid, mp 43−65 °C (552 mg, 96% yield). There was no other isomer detectable by ^{31}P or ^{1}H NMR. Stereochemistry was assigned as *Sp* on the basis of the known stereochemistry of the regent. $[\alpha]^{20}_{D}$ (*c* 1.0, MeOH) −25.7; ^{31}P NMR (162 MHz, $CDCl_3$) δ 2.81; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (s, 1H), 7.94 (s, 1H), 7.29−7.25 (m, 2H), 7.17−7.10 (m, 3H), 6.10 (d, *J* = 2.0 Hz, 1H), 5.92 (bs, 2H), 5.38(dd, *J* = 6.4, 2.0 Hz, 1H), 5.07 (dd, *J* = 6.0, 3.2 Hz, 1H), 4.94 (m, *J* = 6.4 Hz, 1H), 4.51−4.47 (m, 1H), 4.37−4.32 (m, 1H), 4.28−4.22 (m, 1H), 4.02 (t, *J* = 10.4 Hz, 1H), 3.94−3.84 (m, 1H), 2.10 (bs, 1H), 1.61 (s, 3H),

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1.38 (s, 3H), 1.23 (d, J = 6.8 Hz, 3H), 1.18 (dd, J = 5.6, 1.6 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): 173.4 (d, J = 7.6 Hz), 156.1, 153.5, 150.8 (d, J = 6.8 Hz), 149.4, 140.0, 129.9, 125.2, 120.4 (d, J = 4.6 Hz), 120.3, 114.8, 91.2, 85.8 (d, J = 8.6 Hz), 84.5, 81.8, 69.5, 66.5(d, J = 5.5 Hz), 50.5, 27.4, 25.6, 21.9 (d, J = 5.3 Hz), 21.2 (d, J = 5.3 Hz). HRMS-ESI (m/z): calcd for $C_{25}H_{34}N_6O_8P$ $[M + H]^+$ 577.2170, found 577.2163.

(*S*)-Isopropyl 2-(((*S*)-(((2*R*,3*R*,4*R*,5*R*)-5-(4-Amino-2-oxopyrimidin-1(2*H*)-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)amino)propanoate (16). To a stirred suspension of 4-amino-1-((2*R*,3*R*,4*R*,5*R*)-3-fluoro-4-hydroxy-5-(hydroxymethyl)-3-methyltetrahydrofuran-2-yl)pyrimidin-2(1*H*)-one (**15**,[14] 259 mg, 1 mmol) in dry THF (3 mL) was added a 1.7 M solution of *tert*-butylmagnesium chloride in THF (0.71 mL, 1.2 mmol) over a period of 3 min at room temperature. The white suspension was stirred at this temperature for 30 min, and then was added a solution of (*S*)-2-[(*S*)-(2,3,4,5,6-pentafluorophenoxy)-phenoxyphosphorylamino] propionic acid isopropyl ester (**8**, 544 mg, 1.2 mmol) in THF (3 mL) over a period of 3 min. The mixture was stirred at this temperature for 18 h. The reaction mixture was quenched with methanol (1 mL), solvent was evaporated, and the residue was chromatographed using 0−15% methanol/dichloromethane gradient to give pure **16** as a white amorphous solid, mp 67−95 °C (445 mg, 53% yield). There was no other isomer detectable by ^{31}P or ^{1}H NMR. Stereochemistry was assigned as *Sp* on the basis of the known stereochemistry of the regent. $[\alpha]^{20}_D$ (c 1.0, MeOH) +57.8 ; ^{31}P NMR (162 MHz, CD_3OD) δ 4.57; ^{1}H NMR (400 MHz, CD_3OD) δ 7.63 (d, J = 7.2 Hz, 1H), 7.39−7.36 (m, 2H), 7.28−7.26 (m, 2H), 7.22−7.19 (m, 1H), 6.24 (bd, J = 12.4 Hz, 1H), 5.84 (d, J = 7.6 Hz, 1H), 4.95 (m, J = 6.2 Hz, 1H), 4.53 (br d dd, J = 11.2, 5.2 Hz, 1H), 4.38 (ddd, J = 12.0, 5.8, 3.6 Hz, 1H), 4.11−4.08 (m, 1H), 3.95−3.87 (m, 2H), 1.34 (dd, J = 7.2 Hz, 3H), 1.29 (d, J = 22.4 Hz, 3H), 1.20 (d, J = 6.0 Hz, 6H); ^{13}C NMR (100 MHz, CD_3OD) δ 173.2 (d, J = 5.3 Hz), 166.3, 156.9, 150.9 (d, J = 6.0 Hz), 140.4, 129.7, 125.1, 120.2, (d, J = 5.3 Hz), 101.3, 99.5, 95.5, 89.8, 79.5, 71.9 (d, J = 17.4 Hz), 69.0, 64.6, 50.5, 20.8, 20.7, 19.5 (d, J = 6.1 Hz), 15.8 (d, J = 25.1 Hz). HRMS-ESI (m/z): calcd for $C_{22}H_{31}FN_4O_8P$ $[M + H]^+$ 529.1865, found 529.1849.

(*S*)-Isopropyl 2-(((*S*)-(((3a*R*,4*R*,6*R*,6a*R*)-6-(2-Amino-6-oxo-1*H*-purin-9(6*H*)-yl)-2,2-dimethyl-tetrahydrofuro[3,4-*d*][1,3]-dioxol-4-yl)methoxy)(phenoxy)phosphoryl)amino)propanoate (18). To a stirred solution of 2-amino-9-((3a*R*,4*R*,6*R*,6a*R*)-6-(hydroxymethyl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)-1*H*-purin-6(9*H*)-one (**17**, 323 mg, 1 mmol) in dry THF (3 mL) was added a 1.7 M solution of *tert*-butylmagnesium chloride in THF (1.24 mL, 2.1 mmol) over a period of 3 min at room temperature. The white suspension was stirred at this temperature for 30 min and then was added a solution of (*S*)-2-[(*S*)-(2,3,4,5,6-pentafluorophenoxy)-phenoxyphosphorylamino] propionic acid isopropyl ester (**8**, 544 mg, 1.2 mmol) in THF (3 mL) over a period of 3 min. The mixture was stirred at this temperature for 18 h. The reaction mixture was quenched with methanol (1 mL), solvent was evaporated, and the residue was chromatographed using 0−10% methanol/dichloromethane gradient to give pure **18** as a white amorphous solid, mp 101−136 °C (445 mg, 75% yield). There was no other isomer detectable by ^{31}P or ^{1}H NMR. Stereochemistry was assigned as *Sp* on the basis of the known stereochemistry of the regent. $[\alpha]^{20}_D$ (c 1.0, MeOH) +3.5; ^{31}P NMR (162 MHz, DMSO-d_6) δ 4.67; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.73 (bs, 1H), 7.83 (s, 1H), 7.35−7.31 (m, 2H), 7.18−7.13 (m, 3H), 6.55 (bs, 1H), 6.03−5.98 (m, 2H), 5.16 (dd, J = 6.0, 2.0 Hz, 1H), 5.12 (dd, J = 6.4, 2.8 Hz, 1H), 4.79 (m, J = 6.4 Hz, 1H), 4.31−4.22 (m, 2H), 4.04−3.99 (m, 1H), 3.80−3.69 (m, 1H), 1.49 (s, 3H), 1.29 (s, 3H), 1.15 (d, J = 6.8 Hz, 3H), 1.09 (dd, J = 6.0, 1.5 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.3 (d, J = 4.6 Hz), 157.4, 154.4, 151.3 (d, J = 6.8 Hz), 151.0, 136.9, 130.3, 125.3, 120.8 (d, J = 4.6 Hz), 117.7, 113.9, 89.2, 85.8 (d, J = 8.4 Hz), 84.5, 81.8, 68.6, 66.4, 50.4, 27.7, 25.9, 22.1 (d, J = 3.8 Hz), 20.3 (d, J = 6.1 Hz). HRMS-ESI (m/z): calcd for $C_{25}H_{34}N_6O_9P$ $[M + H]^+$ 593.2127, found 593.2160.

General Experimental for Conversion of 13 to 14 (Model Study). To a stirred solution of nucleoside (1 mmol) in dry THF (3 mL) was added a 1.7 M solution of *tert*-butylmagnesium chloride in THF (0.706 mL, 1.2 mmol) over a period of 3 min at room temperature. The white suspension was stirred at this temperature for 30 min, and then was added a solution of the (*S*)-2-aryloxy-phenoxyphosphorylamino] propionic acid isopropyl ester (1.2 mmol) in THF (3 mL) over a period of 3 min. The progress of the reaction was monitored by HPLC (column: Luna 3 μm C8; 50 mm × 4.6 mm; flow rate: 1.0 mL/min; mobile phase: 2−98% 0.1% TFA in CH_3CN/0.1% TFA in water; UV: 254 nm; retention times: 7.71 min for product and 4.99 min for nucleoside starting material) at 15, 45, 90, and 180 min time points.

■ ASSOCIATED CONTENT

Supporting Information. ^{1}H and ^{13}C NMR spectra for compounds **5**−**12**, **14**, **16**, and **18**; single crystal X-ray data for **5** and **8** in CIF format; and complete refs 2a, 2b, 6, and 7. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ REFERENCES

(1) Siddiqui, A. Q.; Balatore, C.; McGuigan, C.; Pathirana, R. N.; Balzarini, J.; Declercq, E. *J. Med. Chem.* **1999**, *42*, 393.

(2) Selected examples: (a) Gardelli, C.; et al. *J. Med. Chem.* **2009**, *52*, 5394–5407. (b) McGuigan, C.; et al. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4850–4854. (c) Zhou, X.-J.; Pietropaolo, K.; Chen, J.; Khan, S.; Sullivan-Boylai, J.; Mayers, D. *Antimicrob. Agents Chemother.* **2011**, *55*, 76–81. (d) Mehellou, Y.; Balzarini, J.; McGuigan, C. *ChemMedChem* **2009**, *4*, 1779–91. (e) McGuigan, C.; Kelleher, M. R.; Perrone, P.; Mulready, S.; Luoni, G.; Daverio, F.; Rajyaguru, S.; Le Pogam, S.; Najera, I.; Martin, J. A.; Klumpp, K.; Smith, D. B. *Biorg. Med. Chem. Lett.* **2009**, *19* (15), 4250–4254.

(3) Gudmundsson, K. S.; Wang, Z.; Daluge, S. M.; Johnson, L. C.; Hazen, R.; Condreay, L. D.; McGuigan, C. *Nucleosides, Nucleotides Nucleic Acids* **2004**, *23*, 1929–1937.

(4) Venkatachalam, T. K.; Qazi, S.; Uckun, F. M. *Arzneim. Forsch.* **2006**, *56*, 136–151.

(5) Murakami, E.; Tolstykh, T.; Bao, H.; Niu, C.; Micholochick Steuer, H. M.; Bao, D.; Chang, W.; Espiritu, C.; Bansal, S.; Lam, A. M.; Otto, M. J.; Sofia, M. J.; Furman, P. A. *J. Biol. Chem.* **2010**, *285*, 34337–34347.

(6) Sofia, M. J.; et al. *J. Med. Chem.* **2010**, *53*, 7202–7218.

(7) Nelson, D. R. et al. *European Association for the Study of Liver Disease*, Berlin, Germany, March 30−Aprit 3, 2011, abstract 1372.

(8) (a) Nakayama, K.; Thompson, W. J. *J. Am. Chem. Soc.* **1990**, *112*, 6936–6942. (b) Wozniak, L. A; Okruzsek, A. *Chem. Soc. Rev.* **2003**, *32*, 158–169.

(9) Lehsten, D. M.; Baehr, D. N.; Lobl, T. J.; Vaino, A. R. *Org. Process Res. Dev.* **2002**, *6*, 819–822.

(10) Roman, C. A; Balzarini, J.; Meier, C. *J. Med. Chem.* **2010**, *53*, 7675–7681.

(11) Uchiyama, M.; Aso, Y.; Noyori, R.; Hayakawa, Y. *J. Org. Chem.* **1993**, *58*, 373–379.
(12) Howes, P. D.; Slater, M. J.; Wareing, K. *Nucleosides, Nucleotides Nucleic Acids* **2003**, *22* (5−8), 687–689.
(13) Perrone., P.; Luoni, G. M.; Kelleher, M. R.; Daverio, F.; Angell, A.; Mulready, S.; Congiatu, C.; Rajyaguru, S.; Martin, J. A.; Leveque, V.; Le Pogam, S.; Najera, S.; Klumpp, K.; Smith, D. B.; McGuigan, C. *J. Med. Chem.* **2007**, *50*, 1840–1849.
(14) Wang, P.; Chun, B.-K.; Rachakonda, S.; Du, J.; Khan, S.; Shi, J.; Stec, W.; Cleary, D.; Ross, B. S.; Sofia, M. J. *J. Org. Chem.* **2009**, *74*, 6819–6824.