JOC The Journal of Organic Chemistry

Note

pubs.acs.org/joc

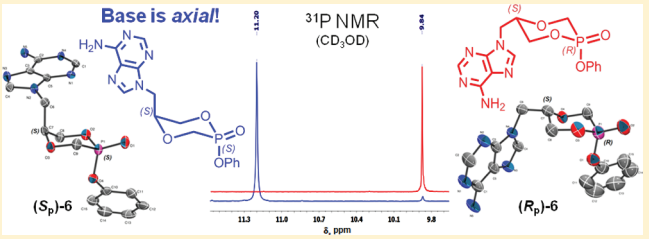
Structure of Cyclic Nucleoside Phosphonate Ester Prodrugs: An Inquiry

Ivan S. Krylov, Valeria M. Zakharova, Michaela Serpi, Ralf Haiges, Boris A. Kashemirov, and Charles E. McKenna*

Department of Chemistry, University of Southern California, Los Angeles, California 90089-0744, United States

Supporting Information

ABSTRACT: The configuration at phosphorus in cyclic (*S*)-HPMPC (**1**, cidofovir) and (*S*)-HPMPA (**2**) phenyl ester (**5** and **6**, respectively) diastereomers ((*R*$_p$)-**5**, (*R*$_p$)-**6**, (*S*$_p$)-**6**) was determined by X-ray crystallography and correlated to their ^{1}H and ^{31}P NMR spectra in solution. (*R*$_p$)-**5** and (*R*$_p$)-**6** have chair conformations with the nucleobase substituent equatorial and the P-OPh axial. Perhaps surprisingly, (*S*$_p$)-**6** is (*a*, *a*) in the crystal and exists largely as an equilibrium of (*a*, *a*)/(*e*, *e*) conformers in chloroform or acetonitrile.



Six-membered phosphonate rings constitute an essential structural feature of cyclic prodrug diesters derived from acyclic nucleoside phosphonates (ANPs),[1−6] which are dNMP analogues that are broadly active against DNA viruses and retroviruses. More generally, six-membered ring phosphates and related compounds have been a durable topic of interest in organic chemistry.[7−15] An example of a clinically important ANP is cidofovir (**1**, (*S*)-HPMPC, Vistide; Figure 1),[16−18]

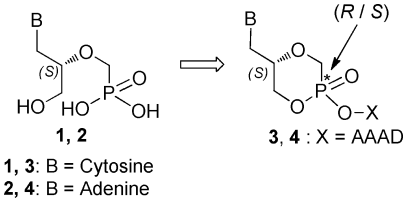


Figure 1. Structures of (*S*)-HPMPC (**1**) and (*S*)-HPMPA (**2**) and corresponding AAAD prodrug forms (**3** and **4**).

which is used for the treatment of CMV retinitis in patients with AIDS.[17] A well-known drawback of ANPs as antiviral drugs is their poor oral bioavailability, owing to the presence of a phosphonic acid group that ionizes at physiological pH. Various prodrug strategies have been devised to circumvent this limitation.[19−22] One currently under development in our laboratory involves esterification of the cyclic form of **1** and **2** (cHPMPC and cHPMPA) with an alcoholic amino acid derivative (AAAD), such as serine, threonine, or tyrosine.[1,3,23−25]

HPMP-based ANPs are chiral compounds, exerting their maximal antiviral effect as a single enantiomer.[18] Esterification of the remaining P-OH group in the cyclic form of **1** and **2** by the promoiety leads to the formation of a new stereocenter at the phosphorus atom, resulting in generation of (*S*$_p$)- and (*R*$_p$)-diastereomers (**3**, **4**; Figure 1). The diastereomers of cyclic **1** aryl esters[6] and also AAAD prodrugs[1,3,24] **3** and **4** have significantly different pharmacokinetic properties. It is clearly important to define the phosphorus configuration and phostonate[26] ring conformations of these prodrugs in order to understand their structure−activity relationships. ^{1}H, ^{13}C, ^{31}P, and 2D NMRs[2,4,6] and dipole moment calculations[3] have been previously applied to predict their absolute configurations (AC). The prodrug diastereomers of **5** (Scheme 1) were distinguished as *axial*/*equatorial*[6] depending on the stipulated position of the exocyclic aryl phosphonate ester group, with the *axial* isomer assigned to the upfield ^{31}P NMR peak (Figure 2). Conversely, phosphonate ester prodrugs of cyclic 9-(*S*)-[3-hydroxy-2-(phosphonomethoxy)propyl]-2,6-diaminopurine (HPMPDAP) and cyclic 1-(*S*)-[3-hydroxy-2-(phosphonomethoxy)-propyl]-5-azacytosine were distinguished as *cis*/*trans*[2,4] with the *cis* isomer assigned to the downfield ^{31}P chemical shift (Figure 2).

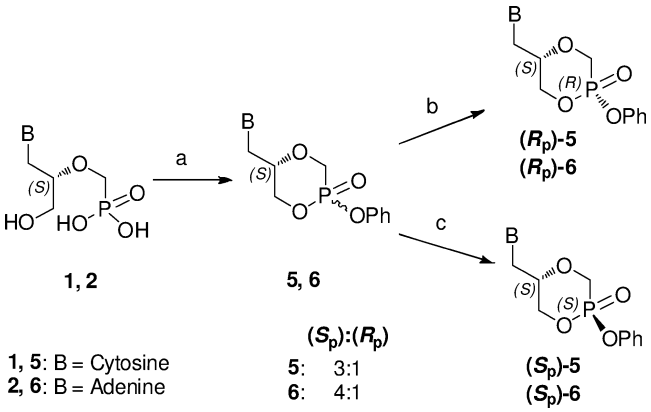
A key assumption underlying the *axial*/*equatorial* descriptives frequently used[6,27−29] to distinguish cyclic (*S*)-HPMPC and (*S*)-HPMPA diastereomeric diesters is that the nucleobase is invariably equatorial. However, *trans*-2-phenyloxy-2-oxo-5-phenyl-1,3,2-dioxaphosphorinane[30] and *trans*-2-methoxy-2-oxo-5-*tert*-butyl-1,3,2-dioxaphosphorinane[31] (*a*, *a*) conformers have been observed in both the solution and solid states, suggesting that in six-membered cyclic *phosphates* the anomeric axial preference of the PhO- or CH_3O-ester groups can compensate for steric repulsions when a 5-phenyl or 5-*tert*-butyl group is axial.

In an attempt to elucidate this seeming contradiction, we have now synthesized, isolated, and analyzed by X-ray crystallography the individual diastereomers of the phenyl ester prodrugs **5** and **6** ((*R*$_p$)-**5**, (*R*$_p$)-**6** and (*S*$_p$)-**6**),[32] which

Received: August 18, 2011
Published: November 30, 2011

   dx.doi.org/10.1021/jo201735f | *J. Org. Chem.* 2012, 77, 684−689

Scheme 1. Preparation of Individual Cyclic (*S*)-HPMPC and (*S*)-HPMPA Phenyl Ester Diastereomers ((*R*$_p$)-5, -6 and (*S*$_p$)-5, -6)[a]



[a]Reagents and conditions: (a) PhOH, PyBOP, *N*,*N*-diisopropylethylamine, DMF, 40 °C, 2 h; (b) Cs_2CO_3, DMF, 0.1 equiv of PhOH; recrystallization from CH_3OH/acetone (for (*R*$_p$)-5) or *i*-PrOH/EtOAc (for (*R*$_p$)-6); (c) recrystallization from CH_3OH/acetone/hexane (for (*S*$_p$)-5) or CH_3CN (for (*S*$_p$)-6).

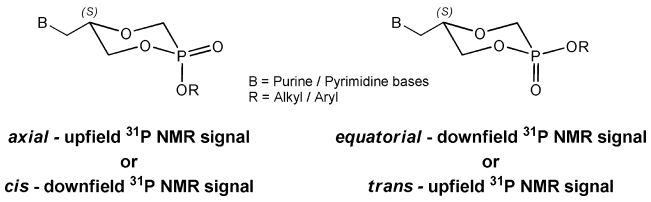


Figure 2. Proposed *cis*/*trans*[2,4] and *axial*/*equatorial*[6] ^{31}P NMR correlations for different cyclic ANP drug diastereomers.

may be regarded as model compounds for **3** or **4** where the AAAD esterifying group is tyrosine.[33] The solution phase conformations of the **5** and **6** diastereomers were studied by correlating the ^{31}P NMR δ values with the X-ray structures and by examining solvent polarity effects on the $^3J_{HCOP}$ and $^3J_{HH}$ coupling constant values.

Esters **5** and **6** were prepared by our previously described procedure for the synthesis of tyrosine side chain ester conjugates of **1** and **2**.[1] After PyBOP-mediated coupling, **5** and **6** were obtained as diastereomeric mixtures enriched with the diastereomer having the more downfield ^{31}P NMR resonance (11.20−11.28 ppm) (Scheme 1). These mixtures could be substantially enriched in the initially minor diastereomer (^{31}P NMR resonance 9.84−10.12 ppm) by treatment with Cs_2CO_3 and 0.1 equiv of phenol in DMF at rt. The pure (*R*$_p$)-**5**, -**6** and (*S*$_p$)-**5**, -**6** diastereomers were obtained by recrystallization and characterized by ^{1}H, ^{13}C, ^{31}P, and 2D HSQC NMR, LC−MS, and by X-ray crystallography.[32]

(*R*$_p$)-**5** crystallizes with one molecule of methanol in the monoclinic space group *P*2(1) with a = 6.9573(7) Å, b = 6.9976(7) Å, c = 34.145(4) Å, and β = 90.050(3)° (Z = 4) (Figure 3). (*R*$_p$)-**6** and (*S*$_p$)-**6** crystallize in the monoclinic space group P2(1) with the unit cell parameters a = 8.2341(9) Å, b = 8.3291(9) Å, c = 12.9089(14) Å, β = 104.660(2)° (Z = 2) (Figure 4) and a = 5.7894(15) Å, b = 43.207(11) Å, c = 6.8100(18) Å, β = 112.184(4)° (Z = 4) (Figure 5), respectively.

In the (*R*$_p$)-diastereomers of **5** and **6**, the PhO-ester group is axial and the nucleobase is equatorial (Figures 3 and 4). However, in the (*S*$_p$)-**6** diastereomer (Figure 5) the nucleobase

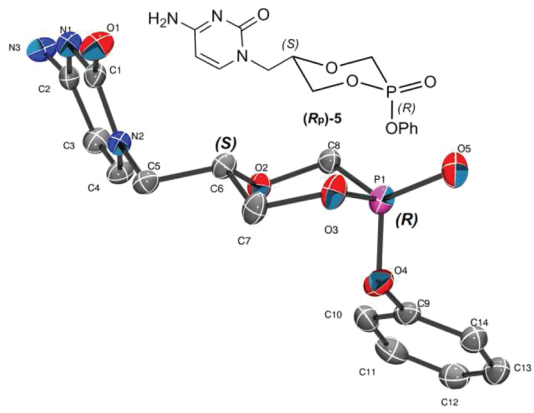


Figure 3. X-ray crystal structure of (*R*$_p$)-**5**. Ellipsoids enclose 50% probability.

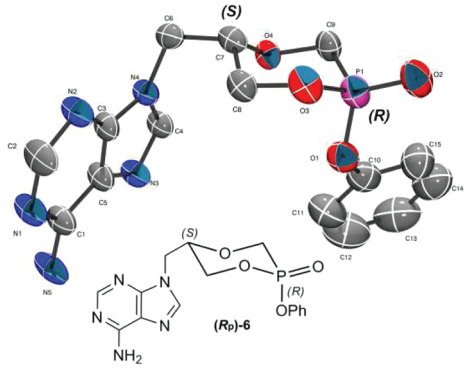


Figure 4. X-ray crystal structure of (*R*$_p$)-**6**. Ellipsoids enclose 50% probability.

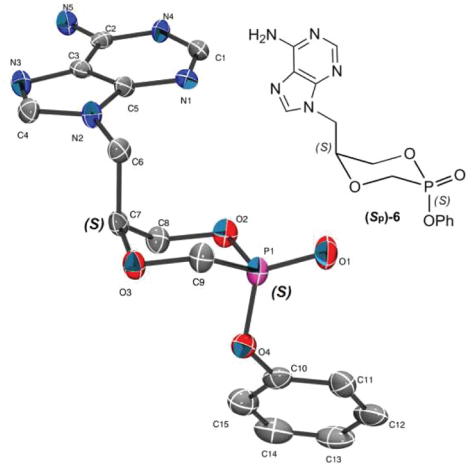


Figure 5. X-ray crystal structure of (*S*$_p$)-**6**. Ellipsoids enclose 50% probability.

is *axial* not *equatorial* as was previously postulated for its (*S*)-HPMPC analogue (*S*$_p$)-**5**.[6]

With the solid-phase structures of the (*R*$_p$)-diastereomer of **5** and the (*R*$_p$)- and (*S*$_p$)-diastereomers of **6** in hand, we then turned to analysis[34−37] of their behavior in solution. Assignment of the signals in their ^{1}H and ^{13}C NMR spectra was based in part on 2D HSQC NMR experiments. Karplus relationships between the HCOP dihedral angle and the $^3J_{HCOP}$ coupling constants for a variety of six-membered phosphorus heterocycles, e.g. 1,3,2-dioxaphosphorinanes, have been previously

dx.doi.org/10.1021/jo201735f | J. Org. Chem. 2012, 77, 684−689

Table 1. ^{1}H NMR Parameters for (R_p)- and (S_p)-Diastereomers of 5 and 6 Recorded at 25 °C and 500 MHz

compd	solvent	δ (A)	δ (B)	δ (C)	δ (D)	δ (X)	δ (^{31}P)	J_{AP}	J_{BP}	J_{CP}	J_{DP}	J_{AX}	J_{BX}
(R_p)-5	CD_3CN	4.47	4.43	4.24	4.02	4.15	9.14	16.6	2.3	10.8	1.3	2.7	10.3
	$CDCl_3$	4.44	4.32	4.17	3.92	4.08	8.01	17.6	1.4	11.0	1.1	2.3	10.5
(S_p)-5	CD_3CN	4.62	4.41	4.36	4.12	4.16	10.49	11.2	8.6	5.7	5.2	2.9	6.7
	$CDCl_3$	4.46	4.40	4.21	3.96	4.16	9.13	14.3	5.6	8.8	2.2	2.9	8.5
(R_p)-6	CD_3CN	4.52	4.41	4.26	4.02	4.29	8.86	17.2	1.6	10.9	1.4	2.1	9.3
	$CDCl_3$	4.50	4.33	4.28	4.00	4.20	7.60	17.4	1.6	10.8	1.2	2.1	10.5
(S_p)-6	CD_3CN	4.71	4.47	4.43	4.09	4.34	10.32	10.0	9.9	4.6	6.1	2.9	5.9
	$CDCl_3$	4.54	4.43	4.31	3.97	4.19	9.03	12.7	7.1	7.2	3.8	2.7	7.7

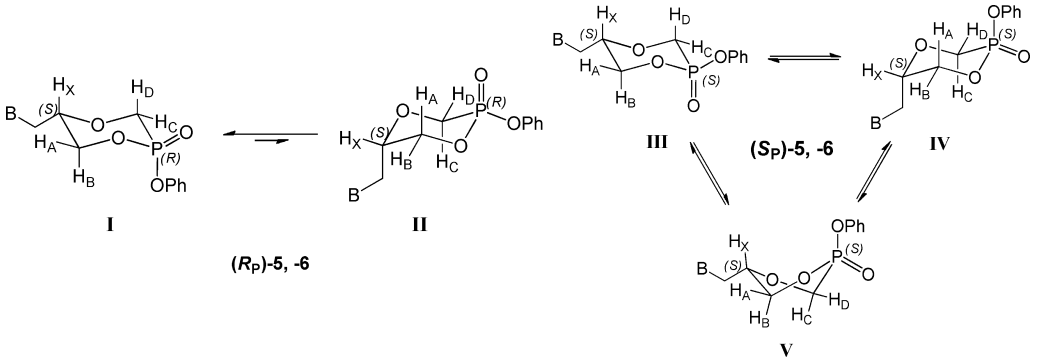


Figure 6. Possible conformer equilibria of (R_p)-**5**, **-6** (**I**-**II**) (left) and (S_p)-**5**, **-6** (**III**–**V**) (right) in solution based on analysis of solvent effect on ^{1}H NMR coupling constant values.

Table 2. Estimated Population of Conformation III for (S_p)-5 and (S_p)-6 Diastereomers

		assumed J, Hz						
compd	solvent	J_{AP}(III)	J_{BP}(III)	J_{AP}(IV)	J_{BP}(IV)	% of III based on J_{AP}(obsd)	% of III based on J_{BP}(obsd)	avg % of III
(S_p)-5	CD_3CN	17.6	1.4	1.4	17.6	60	56	58
	$CDCl_3$					80	74	77
(S_p)-6	CD_3CN	17.6	1.4	1.4	17.6	53	47	50
	$CDCl_3$					70	65	68

defined experimentally.[30,36,38] The $^3J_{HH}$, $^3J_{HCOP}$, and $^2J_{HP}$ values for the individual **5** and **6** diastereomers were measured in two solvents of differing polarities, $CDCl_3$ (ε = 4.81) and CD_3CN (ε = 37.5). The NMR data are summarized in Table 1.

The ^{1}H NMR coupling constant values for (R_p)-diastereomers of **5** and **6** were insensitive to the change in solvent polarity, suggesting that their dominant conformation in solution is **I**, the solid state structure found by X-ray crystallography, which is favored by both steric and electronic factors (Figure 6 (left), Table 1). The vicinal P–O–C–H coupling constant values (J_{AP} = 16.6–17.4 Hz, J_{BP} = 1.4–2.3 Hz) are consistent with an antiperiplanar orientation of the H_A and P atoms and a synclinal relation of the H_B and P atoms confirming a chair conformation.[35] The H_B–C–C–H_X dihedral angle is close to 180° (J_{BX} = 9.3–10.5 Hz, Table 1) corresponding to a sterically favorable equatorial position of the nucleobase.[39] The P-OPh group is axial in this structure, as predicted[10] by the anomeric effect.

Antithetically, the $^3J_{HCOP}$ and $^3J_{HH}$ values of **5** and **6** and their solvent polarity dependence in the (S_p)-diastereomers are consistent with a system of equilibrating conformers as shown in Figure 6 (right).[40] In these stereoisomers, the equatorial preference of the nucleobase (due to a steric effect[39]) and the axial preference of the OPh group (due to the anomeric effect) are opposed, thus stabilizing both chair conformations **III** and **IV** (Figure 6 (right)). Alternatively, these effects could mutually reinforce stabilization of twist conformations **V**, where the OPh remains axial and the nucleobase is pseudoequatorial.

The mole fractions (N) of chair conformations **III** and **IV** in $CDCl_3$ or CD_3CN were estimated by measuring the time averaged coupling constants at 25 °C.[11] Equilibrium of **III** and **IV** interchanges the (S_p)-**5** and (S_p)-**6** H_A and H_B protons, decreasing J_{AP} and increasing J_{BP}, while the sum J_{AP} + J_{BP} remains nearly equal to the sum of the corresponding constants observed for the **5** and **6** (R_p)-diastereomers (Figure 6). For (S_p)-**6** in $CDCl_3$, J_{AP} (12.7 Hz) > J_{BP} (7.1 Hz). This difference becomes smaller in the more polar solvent, CD_3CN, indicating that the conformer ratio approaches 1. The same trend is observed for (S_p)-**5**. In contrast, the (R_p)-diastereomers of **5** and **6** show no solvent-dependent differences in their coupling constants. On the reasonable assumption that the (R_p)-diastereomers essentially retain one conformation, namely that shown in the X-ray structure, we can make the following approximations: J_{AP} (**I**) = J_{AP} (**III**); J_{BP} (**I**) = J_{AP}(**IV**). Using these values and eqs 1–4,[36,41] the ratio of the conformers **III** and **IV** can be estimated (Table 2). The calculations reveal that for (S_p)-**5** and (S_p)-**6** in CD_3CN 50–58% of **III** is present, whereas in $CDCl_3$, the proportion of **III** increases to 68–77%. The assignment of structure **III** rather than **IV** to the major conformer is based upon larger value (5.9 or 6.7 Hz) observed for J_{BX}, relative to the 2–4 Hz value expected for **IV**.

dx.doi.org/10.1021/jo201735f | J. Org. Chem. 2012, 77, 684–689

$$N(\mathbf{III})J_{AP}(\mathbf{III}) + N(\mathbf{IV})J_{AP}(\mathbf{IV}) = J_{AP}(\text{obsd}) \quad (1)$$

$$N(\mathbf{IV}) = 1 - N(\mathbf{III}) \quad (2)$$

therefore

$$N(\mathbf{III}) = [J_{AP}(\text{obsd}) - J_{AP}(\mathbf{IV})]/[J_{AP}(\mathbf{III}) - J_{AP}(\mathbf{IV})] \quad (3)$$

Similarly, for J_{BP}

$$N(\mathbf{III}) = [J_{BP}(\text{obsd}) - J_{BP}(\mathbf{IV})]/[J_{BP}(\mathbf{III}) - J_{BP}(\mathbf{IV})] \quad (4)$$

Taking into consideration earlier work describing the conformations of 2-oxo-1,3,2-oxazaphosphorinane,[34,35] we conclude that the contribution of twist conformations such as **V** (with the nucleobase equatorial to minimize steric repulsion and the OPh pseudoaxial to maximize the anomeric effect) to the equilibrium is negligible, given that the sums of the corresponding spin−spin couplings ($J_{AP} + J_{BP}$) are nearly equal throughout the series of diastereomers. If twist conformations were substantially present, the sums of these J values should decrease by 2−3 Hz because protons H_A and H_B are not interchanged by interconverting the twist and chair conformations.[41]

In summary, the structures of three of the four individual diastereomers of cyclic (*S*)-HPMPC and (*S*)-HPMPA phenyl esters **5** and **6** have been defined by X-ray crystallography. The (S_p)-**6** stereoisomer crystallized with an (*a*, *a*) arrangement of the C-5 nucleobase and phenyl phosphonate ester groups, contradicting the assumption that the nucleobase is exclusively equatorial and indicating that the anomeric effect of the axial OPh group can outweigh steric repulsion of the axial nucleobase. The dependence of the $^3J_{HCOP}$ and $^3J_{HH}$ values of (S_p)-**5** and (S_p)-**6** on solvent polarity suggests that they significantly exist as two conformers at equilibrium in solution; thus, the *axial*/*equatorial* terminology often used to differentiate cyclic (*S*)-HPMPC and (*S*)-HPMPA prodrug diastereomers, and similar compounds, could be misleading. Assignment of the more upfield ^{31}P NMR chemical shifts[6] to axial P-OPh is supported by our work; thus, for the cyclic (*S*)-HPMPC and (*S*)-HPMPA phenyl ester prodrugs, the more downfield ^{31}P NMR signal corresponds to the (S_p) configuration at phosphorus.

EXPERIMENTAL SECTION

General Experimental Methods. ^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded on 400, 500, 600 MHz spectrometers. Chemical shifts (δ) are reported in parts per million (ppm) relative to internal CH_3OH (^{1}H NMR, δ = 3.34; ^{13}C NMR, δ = 49.86); $CHCl_3$ (^{1}H NMR, δ = 7.26); CH_3CN (^{1}H NMR, δ = 1.96); or external 85% H_3PO_4 (^{31}P NMR, δ = 0.00). ^{31}P NMR spectra were proton-decoupled, and ^{1}H and ^{13}C coupling constants (J values) are given in Hz. The following NMR abbreviations are used: s (singlet), d (doublet), m (unresolved multiplet), dd (doublet of doublets), ddd (doublet of doublet of doublet), br (broad signal). LC−MS analysis of compounds (S_p)-**5**, (R_p)-**5**, (S_p)-**6** and (R_p)-**6** was performed on a mass spectrometer in positive ion mode (ESI), equipped with a PDA UV detector and HPLC solvent delivery system. HPLC separations were performed on a C18 HPLC column (5 μm, 250 mm × 4.6 mm) with a 0−30% CH_3CN gradient in 60 mM ammonium acetate buffer, pH 5.5, at a flow rate of 1.0 mL/min. MS parameters were optimized as follows: sheath gas (N2) flow rate 20 arb, I spray voltage 5 kV, capillary temperature 275 °C, capillary voltage 35 V, tube lens offset 55 V. Full scan mass spectra were recorded over a range of m/z 200−600. The UV detector was operated at 274 or 260 nm for (*S*)-HPMPC or (*S*)-HPMPA derivatives, respectively. (*S*)-HPMPC and (*S*)-HPMPA were purchased from a commercial supplier. All other reagents were purchased from commercial sources and used as obtained, unless specified otherwise.

General Procedure for Synthesis of Cyclic 1 and 2 Phenyl Esters. To a suspension of (*S*)-HPMPC (**1**) or (*S*)-HPMPA (**2**) (0.42 mmol) in dry DMF (5 mL) were added dry *N*,*N*-diisopropylethylamine (DIEA) (10 mmol, 1.8 mL), PhOH (60 mg, 0.63 mmol), and (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP) (1.05 mmol, 0.546 g). The reaction mixture was stirred under N_2 at 40 °C for 2 h. The reaction was monitored by ^{31}P NMR, and additional portions of PyBOP were added as necessary. After reaction completion, DMF and DIEA were removed under vacuum. The residue was washed with diethyl ether and purified using silica gel column chromatography [CH_2Cl_2, then CH_2Cl_2/acetone (2:1), then CH_2Cl_2/acetone/CH_3OH (6:3:1)]. Solvents were removed under vacuum, yielding the product as a mixture of (S_p)- and (R_p)-diastereomers in a ratio of 3:1 (assignment based on integration of the corresponding signals in ^{31}P NMR and the X-ray crystallographic structures) for cHPMPC-Ph (**5**) (83% yield) and 4:1 for cHPMPA-Ph (**6**) (71% yield). Diastereomeric mixtures of **5** or **6** enriched in the (S_p)-diastereomers were recrystallized as described in the following text to furnish the pure (S_p)-diastereomers used for X-ray crystallography and NMR experiments.

General Procedure for Isomerization. Molecular sieves (0.4 nm) were added to a solution of PhOH (0.02 mmol) and a mixture of cHPMPC-Ph or cHPMPA-Ph diastereomers (0.2 mmol) enriched with the (S_p)-diastereomer in absolute DMF (5 mL). Cs_2CO_3 (0.4 mmol, 130 mg) was added after 30 min in N_2 atmosphere. The reaction mixture was stirred for 24 h at room temperature and monitored by ^{31}P NMR until the ratio of (S_p):(R_p) diastereomers was ∼1:9. In case of cHPMPC-Ph, the reaction mixture was additionally heated for 2 h at 40 °C, affording a ∼1:13 ratio of (S_p):(R_p) diastereomers. Molecular sieves and Cs_2CO_3 were removed by filtration, DMF was evaporated under vacuum, and the residue was purified utilizing the same procedure as described above, yielding enriched products (cHPMPC-Ph (**5**), 74% yield; cHPMPA-Ph (**6**), 84% yield), which were recrystallized as described below to furnish the pure (R_p)-diastereomers used for X-ray crystallography and NMR experiments.

4-Amino-1-[[(5*S*)-2-oxido-2-phenoxy-1,4,2-dioxaphosphinan-5-yl]methyl]pyrimidin-2(1*H*)-one (5). The (R_p)-**5 diastereomer** was obtained by recrystallization from CH_3OH/acetone. ^{1}H NMR (400 MHz, CD_3OD): δ = 7.50 (d, J = 7.4 Hz, 1H, 6-H), 7.41−7.37 (m, 2H, 2 arom *m*-CH), 7.26−7.21 (m, 3H, 3 arom CH), 5.83 (d, J = 7.6 Hz, 1H, 5-H), 4.52 (ddd, $^3J_{PH}$ = 16.9 Hz, J_{gem} = 12.0 Hz, J_{vic} = 3.0 Hz, 1H, CH_AH$_B$O), 4.44 (ddd, J_{gem} = 12.1 Hz, J_{vic} = 10.1 Hz, $^3J_{PH}$ = 1.8 Hz, 1H, CH$_A$H$_B$O), 4.32 (dd, J_{gem} = 14.9 Hz, $^2J_{HP}$ = 10.6 Hz, 1H, CH_CH$_D$P), 4.20−4.15 (m, 1H, CH_XO), 4.07 (dd, J_{gem} = 14.9 Hz, $^2J_{PH}$ = 1.2 Hz, 1H, CH$_C$H$_D$P), 4.01 (dd, J_{gem} = 14.5 Hz, J_{vic} = 3.9 Hz, 1H, C*H*HN), 3.77 ppm (dd, J_{gem} = 14.3 Hz, J_{vic} = 6.8 Hz, 1H, CH*H*N); ^{13}C NMR (126 MHz, CD_3OD) δ 166.7 (CNH_2), 157.6 (CO), 149.6 (d, $^2J_{CP}$ = 8.4 Hz, arom *ipso*-C), 147.0 (C-6), 129.8 (2 arom *m*-CH), 125.4 (arom *p*-CH), 120.0 (d, $^3J_{CP}$ = 4.3 Hz, 2 arom. *o*-CH), 94.1 (C-5), 74.3 (d, $^3J_{CP}$ = 5.5 Hz, CHO), 73.2 (d, $^2J_{CP}$ = 8.6 Hz, CH_2OP), 62.1 (d, $^1J_{CP}$ = 144.0 Hz, CH_2P), 48.5 ppm (CH_2N); ^{31}P NMR (162 MHz, CD_3OD): δ = 10.12 ppm.

The (S_p)-**5 diastereomer** was obtained by recrystallization from CH_3OH/acetone/hexane: ^{1}H NMR (500 MHz, CD_3OD): δ = 7.53 (d, J = 7.4 Hz, 1H, 6-H), 7.40−7.36 (m, 2H, 2 arom *m*-CH), 7.25−7.18 (m, 3H, 3 arom CH), 5.82 (d, J = 7.6 Hz, 1H, 5-H), 4.52 (ddd, $^3J_{PH}$ = 12.1 Hz, J_{gem} = 12.0 Hz, J_{vic} = 2.7 Hz, 1H, CH_AH$_B$O), 4.48−4.42 (m, 1H, CH$_A$H$_B$O), 4.32 (dd, J_{gem} = 14.4 Hz, $^2J_{HP}$ = 7.4 Hz, 1H, CH_CH$_D$P), 4.19−4.15 (m, 1H, CH_XO), 4.17 (dd, J_{gem} = 15.0 Hz, $^2J_{PH}$ = 3.0 Hz, 1H, CH$_C$H$_D$P), 4.01 (dd, J_{gem} = 14.2 Hz, J_{vic} = 3.3 Hz, 1H, C*H*HN), 3.91 ppm (dd, J_{gem} = 14.2 Hz, J_{vic} = 8.0 Hz, 1H, CH*H*N); ^{13}C NMR (126 MHz, CD_3OD) δ = 166.7 (CNH_2), 157.5 (CO), 149.5

dx.doi.org/10.1021/jo201735f | J. Org. Chem. 2012, 77, 684−689

($^2J_{CP}$ = 8.7 Hz, arom *ipso*-C), 146.7 (C-6), 129.7 (d, $^4J_{CP}$ = 1.0 Hz, 2 arom *m*-CH), 125.5 (arom *p*-CH), 120.1 (d, $^3J_{CP}$ = 4.1 Hz, 2 arom *o*-CH), 94.3 (C-5), 73.3 (d, $^3J_{CP}$ = 5.2 Hz, CHO), 71.6 (d, $^2J_{CP}$ = 7.5 Hz, CH_2OP), 62.1 (d, $^1J_{CP}$ = 146.2 Hz, CH_2P), 48.2 ppm (CH_2N); ^{31}P NMR (162 MHz, CD_3OD) δ = 11.28 ppm.

9-[[(5*S*)-2-Oxido-2-phenoxy-1,4,2-dioxaphosphinan-5-yl]-methyl]-9*H*-purin-6-amine (6). The **(R_p)-6 diastereomer** was obtained by recrystallization from *i*-PrOH/EtOAc: ^1H NMR (600 MHz, CD_3OD) δ = 8.24 (s, 1H, 2-H), 8.13 (s, 1H, 8-H), δ 7.41−7.38 (m, 2H, 2 arom *m*-CH), 7.26−7.24 (m, 1H, arom *p*-CH), 7.19−7.17 (m, 2H, 2 arom *o*-CH), 4.61 (ddd, $^3J_{HP}$ = 17.6 Hz, J_{gem} = 11.6 Hz, *J* = 2.0 Hz, 1H, CH_AH_BO), 4.46 (dd, *J* = 14.4, 2.9 Hz, 1H, C*H*HN), 4.42 (ddd, J_{gem} = 11.6, $^3J_{HP}$ = 1.2 Hz, CH_AH_BO), 4.39−4.33 (m, 3H, CH*H*N, CH_CH_DP, CH_XO), 4.10 ppm (dd, J_{gem} = 15.3 Hz, $^2J_{HP}$ = 1.4 Hz, 1H, CH_CH_DP); ^{13}C NMR (126 MHz, CD_3OD) 156.05 ($C\text{-}NH_2$), 152.51 (C-2), 149.56 (d, $^2J_{CP}$ = 8.3 Hz, arom *ipso*-C), 149.42 (NCC=**C**NN), 142.05 (C-8), 129.73 (d, $^4J_{CP}$ = 0.7 Hz, 2 arom *m*-CH), 125.34 (arom *p*-CH), 119.90 (d, $^3J_{CP}$ = 4.3 Hz, 2 arom *o*-CH), 118.33 (NC**C**=CNN), 73.90 (d, $^3J_{CP}$ = 5.5 Hz, CHO), 72.96 (d, $^2J_{CP}$ = 9.1 Hz, CH_2OP), 62.08 (d, $^1J_{CP}$ = 144.1 Hz, CH_2P), 42.55 ppm (CH_2N); ^{31}P NMR (162 MHz, CD_3OD) δ = 9.84 ppm.

The **(S_p)-6 diastereomer** was obtained by recrystallization from CH_3CN: ^1H NMR (600 MHz, CD_3OD) δ = 8.20 (s, 1H, 2-H), 8.11 (s, 1H, 8-H), δ 7.38−7.35 (m, 2H, 2 arom *m*-CH), 7.23−7.21 (m, 1H, arom *p*-CH), 7.19−7.17 (m, 2H, 2 arom *o*-CH), 4.71 (ddd, *J* = 12.1 Hz, *J* = 2.8 Hz, 1H, CH_AH_BO), 4.54 (dd, *J* = 15.1, 8.8 Hz, 1H, CH_AH_BN), 4.50 (m, 1H, CH_AH_BO), 4.46 (dd, *J* = 15.0, 7.0, 1H, CH_CH_DP), 4.44 (dd, *J* = 14.7, 3.5 Hz, 1H, CH*H*N), 4.32 (m, CH_XO), 4.15 ppm (dd, *J* = 14.8, 4.0 Hz, CH_CH_DP); ^{13}C NMR (126 MHz, CD_3OD) δ = 156.01 ($C\text{-}NH_2$), 152.53 (C-2), 149.51 (d, $^2J_{CP}$ = 8.6 Hz, arom *ipso*-C), 149.41 (NCC=**C**NN) 141.74 (C-8), 129.70 (2 arom *m*-CH), 125.48 (arom *p*-CH), 120.11 (d, $^3J_{CP}$ = 4.0 Hz, 2 arom *o*-CH), 118.37 (NCC=CNN), 72.97 (d, $^3J_{CP}$ = 5.3 Hz, CHO), 71.42 (d, $^2J_{CP}$ = 7.4 Hz, CH_2OP), 61.47 (d, $^1J_{CP}$ = 145.8 Hz, CH_2P), 42.16 ppm (CH_2N); ^{31}P NMR (202 MHz, CD_3OD) δ = 11.20 ppm.

X-ray Crystallography. Crystals were grown from CH_3OH/acetone ((R_p)-**5**), *i*-PrOH/EtOAc ((R_p)-**6**) or neat acetonitrile ((S_p)-**6**).

The single-crystal X-ray diffraction data were collected on a three-circle platform diffractometer equipped with a CCD detector with the χ-axis fixed at 54.74° and using Mo Kα radiation (λ = 0.71073 Å) from a fine-focus tube. This diffractometer was equipped with an apparatus for low temperature data collection using controlled liquid nitrogen boil off. A complete hemisphere of data was scanned on ω (0.3°) with a run time of 10 s per frame at a detector resolution of 512 × 512 pixels. The structure was solved by the direct method using the SHELX-90 program and refined by the least-squares method on F^2 using SHELXL-97.[42] All non-hydrogen atoms were refined anisotropically.

Crystallographic data have been deposited with the Cambridge Crystallographic Center, CCDC Nos. 795825, 795826, 795827. Copies of the data can be obtained, free of charge, from CCDC, 12 Union Road, Cambridge, CB21EZ, UK (fax: +44−1233−336033; e-mail: deposit@ccdc.cam.ac.uk; Internet: http://www.ccdc.cam.ac.uk).

■ ASSOCIATED CONTENT

Supporting Information

Characterization (NMR, LC-MS, X-ray crystallographic data) for cyclic (*S*)-HPMPC and (*S*)-HPMPA phenyl diesters **5** and **6**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +1 213 740 7007. Fax: +1 213 740 0930. E-mail: mckenna@usc.edu.

■ ACKNOWLEDGMENTS

This work was supported by Grant Nos. AI061457, AI056864, and AI091216 from the National Institutes of Health.

■ REFERENCES

(1) Zakharova, V. M.; Serpi, M.; Krylov, I. S.; Peterson, L. W.; Breitenbach, J. M.; Borysko, K. Z.; Drach, J. C.; Collins, M.; Hilfinger, J. M.; Kashemirov, B. A.; McKenna, C. E. *J. Med. Chem.* **2011**, *54*, 5680−5693.
(2) Krecmerova, M.; Holy, A.; Andrei, G.; Pomeisl, K.; Tichy, T.; Brehova, P.; Masojidkova, M.; Dracinsky, M.; Pohl, R.; Laflamme, G.; Naesens, L.; Hui, H.; Cihlar, T.; Neyts, J.; De, C. E.; Balzarini, J.; Snoeck, R. *J. Med. Chem.* **2010**, *53*, 6825−6837.
(3) Eriksson, U.; Peterson, L. W.; Kashemirov, B. A.; Hilfinger, J. M.; Drach, J. C.; Borysko, K. Z.; Breitenbach, J. M.; Kim, J. S.; Mitchell, S.; Kijek, P.; McKenna, C. E. *Mol. Pharmaceutics* **2008**, *5*, 598−609.
(4) Krecmerova, M.; Holy, A.; Pohl, R.; Masojidkova, M.; Andrei, G.; Naesens, L.; Neyts, J.; Balzarini, J.; De, C. E.; Snoeck, R. *J. Med. Chem.* **2007**, *50*, 5765−5772.
(5) Beadle, J. R.; Hartline, C.; Aldern, K. A.; Rodriguez, N.; Harden, E.; Kern, E. R.; Hostetler, K. Y. *Antimicrob. Agents Chemother.* **2002**, *46*, 2381−2386.
(6) Oliyai, R.; Shaw, J.-P.; Sueoka-Lennen, C. M.; Cundy, K. C.; Arimilli, M. N.; Jones, R. J.; Lee, W. A. *Pharm. Res.* **1999**, *16*, 1687−1693.
(7) Quintero, L.; Sanchez-Vazquez, M.; Cruz-Gregorio, S.; Sartillo-Piscil, F. *J. Org. Chem.* **2010**, *75*, 5852−5859.
(8) Cruz-Gregorio, S.; Rodriguez-Palacios, V.; Hopfl, H.; Quintero, L.; Sartillo-Piscil, F. *J. Org. Chem.* **2009**, *74*, 197−205.
(9) Frank, E.; Wolfling, J. *Curr. Org. Chem.* **2007**, *11*, 1610−1623.
(10) Bentrude, W. G. In *Conformational Behavior of Six-Membered Rings*; Juaristi, E., Ed.; VCH: New York, 1995; pp 245−93.
(11) Bentrude, W. G. In *Phosphorus-31 NMR Spectral Properties in Compound Characterization and Structural Analysis*; Quin, L. D., Verkade, J. D., Eds.; VCH: New York, 1994; pp 41−53.
(12) Gorenstein, D. G. *Chem. Rev.* **1987**, *87*, 1047−77.
(13) Chang, J. W. A.; Gorenstein, D. G. *Tetrahedron* **1987**, *43*, 5187−96.
(14) Gorenstein, D. G. In *Phosphorus-31 NMR: Principles and Applications*; Gorenstein, D. G., Ed.; Academic Press: New York, 1984; pp 37−53.
(15) Maryanoff, B. E.; Hutchins, R. O.; Maryanoff, C. A. *Top. Stereochem.* **1979**, *11*, 187−326.
(16) De Clercq, E. *J. Med. Chem.* **2009**, *53*, 1438−1450.
(17) De Clercq, E.; Andrei, G.; Balzarini, J.; Leyssen, P.; Naesens, L.; Neyts, J.; Pannecouque, C.; Snoeck, R.; Ying, C.; Hockova, D.; Holy, A. *Nucleosides, Nucleotides Nucleic Acids* **2005**, *24*, 331−341.
(18) De Clercq, E.; Sakuma, T.; Baba, M.; Pauwels, R.; Balzarini, J.; Rosenberg, I.; Holy, A. *Antiviral Res.* **1987**, *8*, 261−72.
(19) Peterson, L. W.; McKenna, C. E. *Expert Opin. Drug Delivery* **2009**, *6*, 405−420.
(20) Hostetler, K. Y. *Antiviral Res.* **2009**, *82*, A84−A98.
(21) Hecker, S. J.; Erion, M. D. *J. Med. Chem.* **2008**, *51*, 2328−2345.
(22) He, G.-X.; Krise, J. P.; Oliyai, R. *Biotechnol.: Pharm. Aspects* **2007**, *5*, 223−264.
(23) Peterson, L. W.; Kim, J.-S.; Kijek, P.; Mitchell, S.; Hilfinger, J.; Breitenbach, J.; Borysko, K.; Drach, J. C.; Kashemirov, B. A.; McKenna, C. E. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 4045−4049.
(24) Peterson, L. W.; Sala-Rabanal, M.; Krylov, I. S.; Serpi, M.; Kashemirov, B. A.; McKenna, C. E. *Mol. Pharmaceutics* **2010**, *7*, 2349−2361.
(25) Krylov, I. S.; Peterson, L. W.; Kashemirov, B. A.; Breitenbach, J.; Borysco, K.; Drach, J. C.; Kim, J. S.; Hilfinger, J. M.; McKenna, C. E. *Antiviral Res.* **2009**, *82*, A75−A75.
(26) Eberhard, A.; Westheimer, F. H. *J. Am. Chem. Soc.* **1965**, *87*, 253−60.

(27) Tammewar, A. M.; Cheng, L.; Hostetler, K. Y.; Falkenstein, I.; Beadle, J. R.; Barron, E. C.; Kozak, I.; Freeman, W. R. *J. Ocul. Pharmacol. Ther.* **2007**, *23*, 433−444.
(28) Wan, W. B.; Beadle, J. R.; Hartline, C.; Kern, E. R.; Ciesla, S. L.; Valiaeva, N.; Hostetler, K. Y. *Antimicrob. Agents Chemother.* **2005**, *49*, 656−662.
(29) Kern, E. R.; Hartline, C.; Harden, E.; Keith, K.; Rodriguez, N.; Beadle, J. R.; Hostetler, K. Y. *Antimicrob. Agents Chemother.* **2002**, *46*, 991−995.
(30) Donaldson, B.; Hall, L. D. *Can. J. Chem.* **1972**, *50*, 2111−18.
(31) Warrent, R. W.; Caughlan, C. N.; Hargis, J. H.; Yee, K. C.; Bentrude, W. G. *J. Org. Chem.* **1978**, *43*, 4266−70.
(32) Repeated attempts to grow a crystal of (S_p)-**5** suitable for X-ray crystallographic structure determination were unsuccessful.
(33) The individual prodrug diastereomers reportedly resist crystallization,[4] which was our own experience.
(34) Bentrude, W. G.; Setzer, W. N.; Sopchik, A. E.; Chandrasekaran, S.; Ashby, M. T. *J. Am. Chem. Soc.* **1988**, *110*, 7119−27.
(35) Bentrude, W. G.; Setzer, W. N.; Sopchik, A. E.; Bajwa, G. S.; Burright, D. D.; Hutchinson, J. P. *J. Am. Chem. Soc.* **1986**, *108*, 6669−75.
(36) Holmes, R. R.; Day, R. O.; Setzer, W. N.; Sopchik, A. E.; Bentrude, W. G. *J. Am. Chem. Soc.* **1984**, *106*, 2353−8.
(37) Gorenstein, D. G.; Rowell, R. *J. Am. Chem. Soc.* **1979**, *101*, 4925−8.
(38) Gorenstein, D. G.; Rowell, R.; Findlay, J. *J. Am. Chem. Soc.* **1980**, *102*, 5077−81.
(39) Bushweller, H. In *Conformational Behavior of Six-Membered Rings: Analysis, Dynamics, and Stereochemical Effects*; Juaristi, E., Ed.; VCH: New York, 1995.
(40) Attempts to detect individual conformers in solution at low temperature using ^{1}H and ^{31}P NMR were unsuccessful (acetone, −80 °C; acetonitrile, −40 °C) in part due to limited compound solubility.
(41) Nelson, K. A.; Bentrude, W. G.; Setzer, W. N.; Hutchinson, J. P. *J. Am. Chem. Soc.* **1987**, *109*, 4058−64.
(42) Sheldrick, G. M. *Acta Crystallogr.* **2008**, *A64*, 112−122.