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On the Existence of the Chair Conformation in Six-Membered Ring Phosphates Bearing an Aryl Group Axially Oriented at the C4 Position: Cyclic Nucleotides As Model Compounds for Cyclic Phosph(on)ate and Phosphoramide Prodrugs

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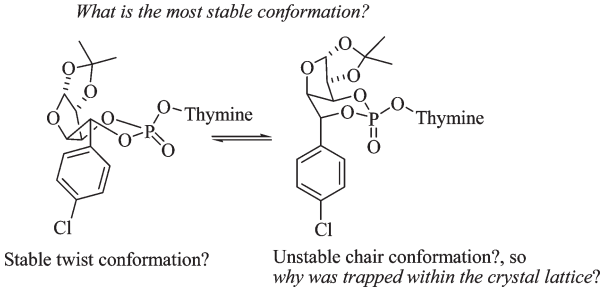

A series of cyclic nucleotide analogues to HepDirect prodrugs were prepared by a three-component reaction of protected thymine, phosphoryl chloride, and 5-aryl-α-D-xylofuranoses derivatives. One of the cyclic nucleotides showed NMR data that suggest a predominant twisted conformation; however, in spite of having an aryl group at the C4 position within the crystal lattice, the cyclic nucleotide had a chair conformation with the aryl group axially oriented. By analyzing the unprecedented X-ray structure, it was observed that the oxygen atom from the phoshoryl group (P═O) is found in close proximity to the *o*-hydrogen atom of the aryl group (2.51 Å), suggesting thus an attractive nonbonding electrostatic interaction, which might be the driving force that overcomes the steric diaxial interactions imposed by the aryl group. Theoretical studies (NBO) for two model compounds showed that there are indeed interactions between filled (donor) Lewis-type NBOs and empty (acceptor) *non*-Lewis NBOs corresponding to the $n\mathrm{O}\rightarrow\sigma^*_{\mathrm{C-H}}$ interaction. Additionally, conversion of a diastereomeric mixture of cyclic nucleotides into the more stable diastereomeric cyclic nucleotide was observed and explained by spontaneous isomerization in the phosphorinane ring. This finding supports the recently established hypothesis for the mode of action of prodrug cleavage, for which the anomeric effect plays an important role.

Introduction

In the search for new antiviral and anticancer candidate drugs, the use of site-selective drug deliveries (prodrugs) has emerged as an excellent strategy for drug administration into the body.[1−9] In this regard, six-membered ring phosph(on)ates represent a new prodrug strategy for treating liver diseases such as hepatitis B and C, and hepatocellular

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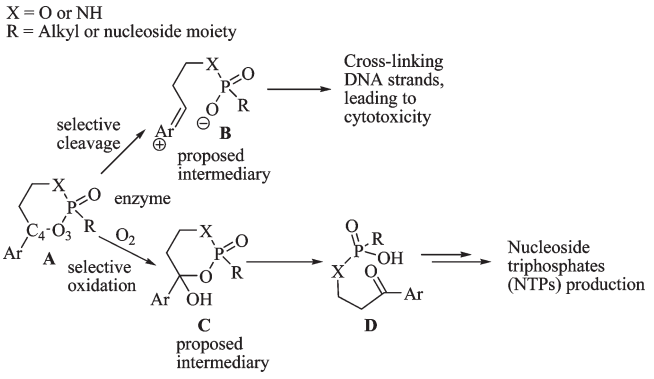
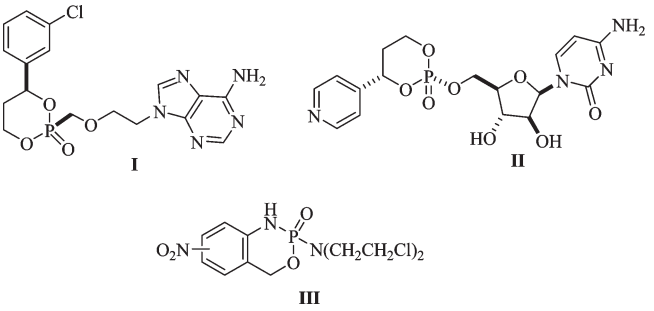
SCHEME 1. Proposed Mechanistic Mode of Action for Six-Membered Ring P-Heterocyclic Prodrugs A



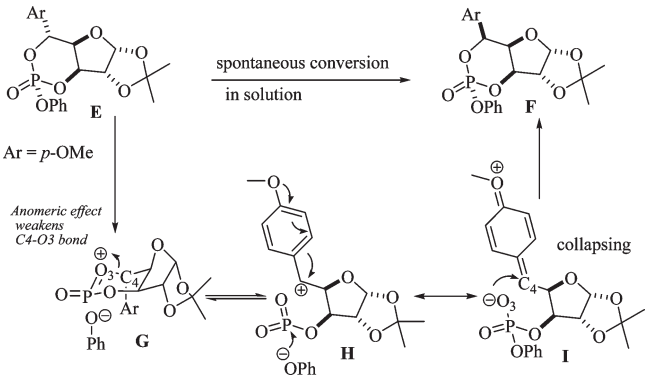
carcinoma.[1,2,8,9] Erion's gruop[10−12] has successfully introduced HepDirect prodrugs (**I** and **II**) for the treatment of the above-mentioned diseases. Similarly, cyclic aryl phosphoramides (**III**)[13,14] represent another class of prodrugs for cancer therapy.



For cyclic phosphoramides, selective prodrug cleavage is caused by delocalization of the electron density from the aromatic ring toward the P-heterocycle (**A** → **B**), affording thus ionic species for the cross-linkage of DNA strands, thus producing high cytotoxity.[13] On the contrary, Erion's HepDirect prodrugs[10−12] suffer a selective oxidation (**A** → **C**) catalyzed by a specific enzyme, followed by cleavage of the O3−C4 bond (**C** → **D**), thus leading to an increment of the concentration of nucleoside triphosphates (NTPs) within the living cell, which interfere with the viral replication mechanism (Scheme 1).

Apparently, the aryl or pyridyl group at the C4 position is responsible for the selective O3−C4 bond cleavage. Erion observed that the prodrug cleavage is favored not only by the presence of an aryl group at the C4 position, but also by the cis-configuration of the P-heterocycle; however, it was relatively independent of the nucleoside and absolute stereochemistry at C4.[10] This suggests that the stereochemistry and conformation of the P-heterocycle plays a key role in the prodrug cleavage. With this information in mind, it was recently proposed, based on simple model phosphates, that the anomeric effect is involved in the spontaneous cleavage of cyclic phosph(on)ate prodrugs.[15] The spontaneous conversion of unstable cyclic phosphates (**E**) to their most stable cyclic phosphates (**F**) demonstrates that isomerization is favored by natural hyperconjugation between the *n*O *non*-bonding and σ^*-P−X *anti*bonding orbital (anomeric effect), which generates the double-bonded *non*-bonding resonance structure (**G**), thus weakening the O3−C4 bond and favoring the selective and spontaneous cleavage of the phosphate ring (**G** ⇌ **H**); finally, after a rapid collapse of the phosphate group, the more stable cyclic phosphate **F** is formed (Scheme 2). Additionally, it was also postulated that the steric hindrance caused by the 1,3-*syn* diaxial interaction between aryl and phenoxy groups influences the O3−C4 bond cleavage, even though the chair conformation has been found to be less favorable.

SCHEME 2. Anomeric and Steric Effects Involved in the Spontaneous Conversion of Cyclic Phosphates (E → F)



Crucial for the above findings was the previous conformational and configurational analysis of a series of conformationally restricted 4-substituted-1,3,2-dioxaphosphorinanes.[15−18] NMR spectroscopic and X-ray diffraction have shown that the conformational equilibria for the phosphorinane ring depended on both the configuration at the phosphorus atom and the substituent at the C5 atom (or C4 according to the nomenclature for 1,3,2-dioxaphosphorinanes). For cyclic phosphates with R_P configuration and bearing an equatorially oriented aryl group at the C4 position, a chair ⇌ boat equilibrium (**C1** ⇌ **B1**) was observed, whereas a chair ⇌ twist equilibrium (**C1** ⇌ **T**) was observed for the S_P-epimers. Conformers **C2** and **B2** (of which **B2** has been proposed for related cyclic phosphates)[20,21] were not considered in the study: first, because equilibrium **C1** ⇌ **B1** was supported having trapped both conformers in the solid state within the same crystal lattice (for R = Ph, equatorially oriented in **C1**),[18] second, because equilibrium **C1** ⇌ **T** could be established

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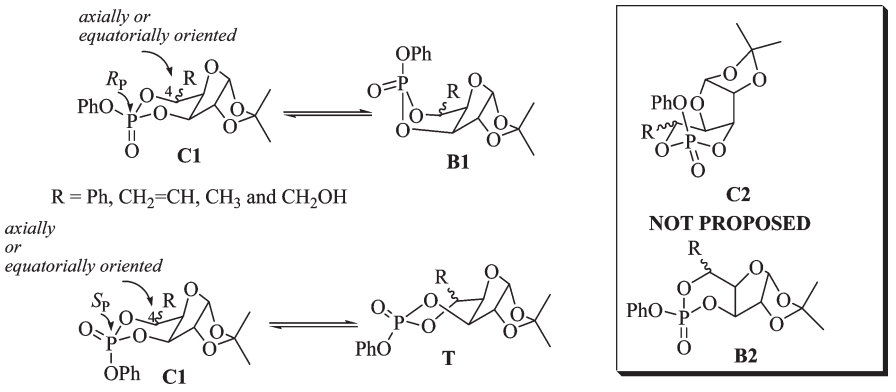
SCHEME 3. Conformational Equilibria for 4-Substituted-1,3,2-dioxaphosphorinanes Containing the 1,2-*O*-Isopropylidene-α-D-xylofuranose Template



based on NMR data, and third because conformation **T** was corroborated by an X-ray crystallographic study for R = *p*-Cl-Ph (Scheme 3).[15,18]

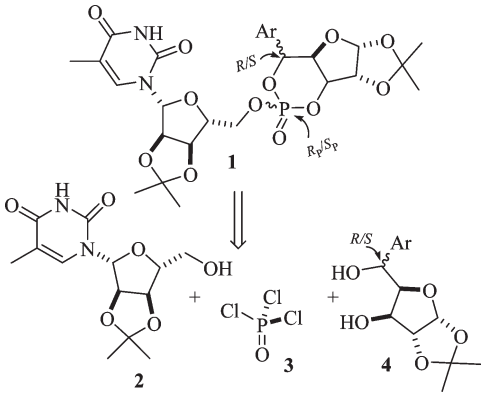
The four above-mentioned conformations were established in solution and corroborated by X-ray crystallographic studies for phosphates with both R_P and S_P configuration, and having an equatorial oriented R group. The only conformation that has not been corroborated by X-ray diffraction analysis is that having an axially oriented aryl group at the C4 position. Many authors have considered that an aryl group at the C4 position can only have an equatorial or *pseudo*-equatorial orientation, and based on the idea of a locked chair conformation they have taken advantage of this anchoring element for stereoselective chemical transformations.[19,22−25]

In the present contribution, the first crystal structure of a six-membered ring phosphate bearing an axially oriented aryl group at the C4 position with a chair conformation is reported. Additionally, with the expectation to provide further experimental and theoretical evidence that supports the above-mentioned proposal related to the selective prodrug-cleavage, the synthesis and the conformational study of cyclic nucleotide analogues to HepDirect prodrugs are also reported.

Results and Discussions

The synthetic strategy for the preparation of the HepDirect analogues was based on a three-component reaction between protected thymine **2**, phosphoryl chloride **3**, and 5-aryl-α-D-xylofuranoses **4a**−**d** (Scheme 4).

SCHEME 4. Synthetic Strategy for the Synthesis of HepDirect Analogues 1



Thus, thymine **5** was selectively protected in one step at the 2′,3′-positions by treatment with an excess of 2,2-dimethoxypropene and catalytic amounts of *p*-TSA at room temperature for 24 h under solvent-free conditions. It is important to note that after 2 h of reaction, the starting material **5** was consumed and two compounds were observed by TLC (presumably the two possible protected isomers); however, after a further 22 h of reaction at room temperature, the presence of **2** as the sole product was observed.

On the other hand, the diasteroisomeric 1,3-diols **4a**−**d** and **4a′**−**d′** were obtained by applying a Sequential Hydrolysis-Oxidation-Grignard Reagent Addition procedure[15,17,18] to diacetone-α-D-glucose **6** (Scheme 5). Then, protected thymine **2** and 1,3-diols **4a**−**d** and **4a′**−**d′**were treated with phosphoryl chloride **3** in the presence of triethylamine (in the case of **4d** and

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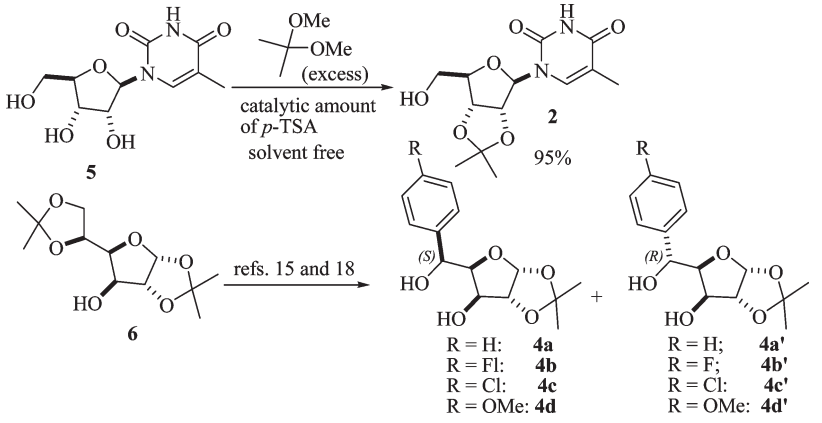
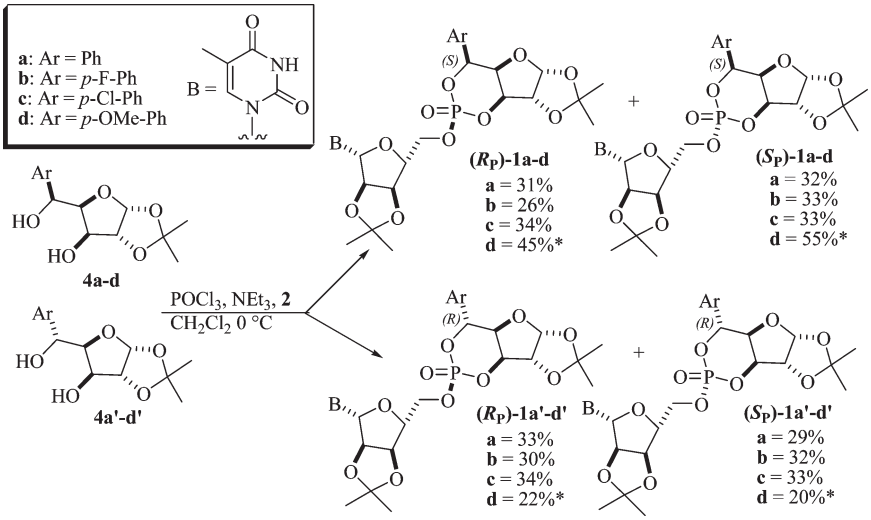
(26) We have assigned, here and previously, the stereochemistry at phosphorus atom by considering the oxygen atom of the phosphoryl group with the highest priority (as a P═O multiple bond, not as a P—O single bond). We understand that, the P═O bond should not be taken like the conventional double bond of carbon to oxygen, the reactivity of both groups is totally different. However, due to the controversy of the role for the phosphorus 3d-orbital into the phosphoryl group, in which, according to Cahn, Ingold, and Prelog assignment rules, contributions by d-orbital to bonds of quadriligants atoms are neglected, hence the P═O bond should be taken as a P—O single bond with the assignment of the least priority (see: Venukadasula, P. K. M.; Chegondi, R.; Maitra, S.; Hanson, P. R. *Org. Lett*. **2010**, 12, 1556−1559.), we decided to take the concept of the phosphoryl multiple bonding only for representative purposes but with the understanding of its real meaning. For a better discussion on this matter, please see: *A Guide to Organophosphorus Chemistry*; Quin, L. D., Ed.; Wiley-Interscience: New York, 2000.

SCHEME 5. Preparation of Protected Nucleoside 2and 1,3-Diols 4a−d and 4a′−d′



SCHEME 6. Three-Component Reaction for the Synthesis of Cyclic Nucleotides 1a−d and 1a′−d′[a]



[a]Unless otherwise noted, yields were determined after chromatography. An asterisk indicates that yields were determined by ^{1}H and ^{31}P NMR.

4d′, a stronger base such as *t*-BuOK and low temperature were needed), giving an almost equimolar mixture of cyclic nucleotides (R_P)-**1a**−**d**/(S_P)-**1a**−**d** and (R_P)-**1a′**−**d′**/(S_P)-**1a′**−**d′** in moderate to good yields (Scheme 6).

After a careful separation of the diastereoisomeric pairs (R_P)-**1a**−**d**/(S_P)-**1a**−**d** and (R_P)-**1a′**−**d′**/(S_P)-**1a′**−**d′**, determination of the absolute stereochemistry at the phosphorus atom was achieved with the aid of NMR spectroscopy and single-crystal X-ray diffraction analysis.[26] In the case of compounds (R_P)-**1d**/(S_P)-**1d** and (R_P)-**1d′**/(S_P)-**1d′**, NMR data were obtained from the crude reaction mixture because these compounds spontaneously isomerize at room temperature (see Figure 1 and Table 2 in Supporting Information for a description of the rate of isomerization). Following Gorenstein's criteria,[27−29] the cyclic nucleotides giving upfield shifted NMR signals (^{31}P NMR) were assigned the absolute configuration S_P.[30,31] The vicinal coupling constants, such as $^{3}J_{H5-P}$ for nucleotides (R_P)-**1a**−**d** and (S_P)-**1a**−**d**, showed values in the range of 0.5−1.0 Hz, indicating that the aryl group at the C5 position is oriented equatorially or pseudoequatorially (absolute configuration *S*), meanwhile values in the range of 9.2−10.4 Hz for (R_P)-**1a′**−**d′** and (S_P)-**1a′**−**d′** (absolute configuration *R*) suggested that the aryl group was axially or pseudoaxially oriented. These coupling constants are almost identical with those previously observed for 2-phenoxy-1,3,2-dioxaphaphosphorinanes derived from 1,2-*O*-isopropylidene-α-D-xylofuranoses.[15,18] Apparently, no significant conformational changes occur when the OPh group is switched by the nucleosyl group at the P-atom. Therefore, in agreement with the general conformational equilibria shown in Scheme 3, chair−boat equilibria (**C1** ⇌ **B1**) for (R_P)-**1a**−**d** and (R_P)-**1a′**−**d′**, and chair−twist equilibria (**C1** ⇌ **T**) for (S_P)-**1a**−**d**, and (S_P)-**1a′**−**d′** can be proposed.

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(31) For a detailed description of the important NMR data, see Table 1 in the Supporting Information.

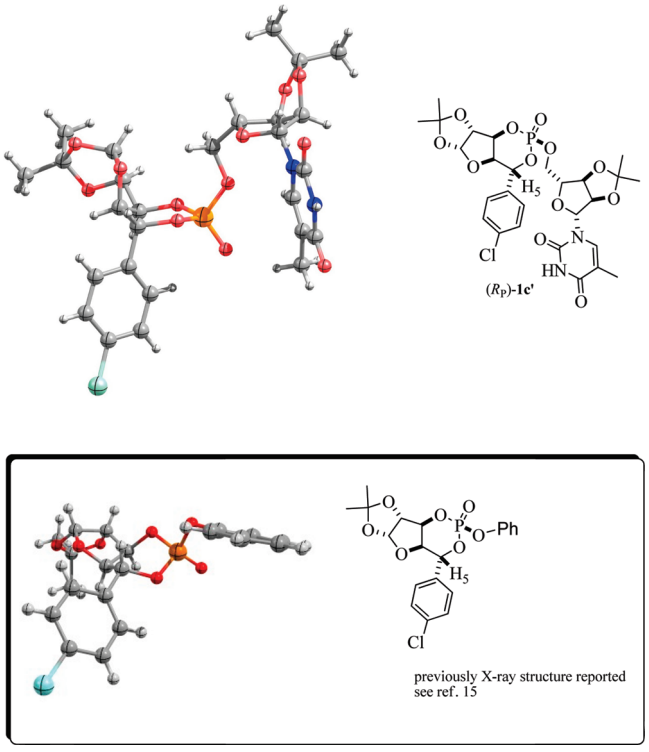

FIGURE 1. Perspective view of the molecular structure of (R_P)-**1c**′ showing that it possesses **C1** conformation and crystal structure previously reported that it possesses twist **T** conformation.

An important contribution of this study is the determination of the molecular structure of (R_P)-**1c**′ by single-crystal X-ray analysis,[32] which not only confirmed the assignment of the absolute configuration at the P-atom for cyclic nucleotides, but also revealed for the first time the existence of a stable chair conformation of a six-membered ring phosphate bearing an aryl group axially oriented at the C4 position (Figure 1).

Moreover, the vicinal H−P coupling constant ($^3J_{H5-P}$ = 10.4 Hz) matches the vicinal H−P coupling constant ($^3J_{H5-P}$ = 10.4 Hz) for the cyclic phosphate with a crystal structure in the twist (**T**) conformation previously reported (Figure 1).[15] The P−O−C4−H dihedral angles for the solid state structures in twist conformation (previously reported) and chair conformation (R_P)-**1c**′ are 52° and 149°, respectively. Indeed, a vicinal H−P coupling constant within the range of 10−14 Hz does not establish a higher population of *non*-chair conformation, but may indicate an equimolar equilibrium of chair and twist conformation in solution.[33−35] Therefore, with this unprecedented X-ray chair conformation **C1** and the previously determined twisted structured **T**, it can be possible now to establish that **C1** ⇌ **T** conformational equilibria are energetically accessible for 4-aryl-1,3,2-dioxaphosphorinanes, and that an equatorially oriented aryl group at C4 is insufficient to freeze the chair conformation.

What force overcomes the strong *syn*-1,3-diaxial steric interactions and thus allowed the revelation of this unprecedented molecular structure? When examining the molecular structure of (R_P)-**1c**′ we found an intramolecular C−H···O hydrogen bonding interaction between the P=O bond and the *o*-hydrogen atom of the aryl group at the C4 atom (C−H, 0.93 Å; H···O, 2.51 Å; C···O, 3.39 Å; C−H···O, 159°, Figure 2). It is important to mention that this interaction is within the previously established limits for such contacts.[36,37]

To investigate this interaction, theoretical calculations for model structure **J** using density functional theory (B3LYP/6-31+G(d,p)) were realized.[38] In model **J**, the nucleoside moiety was replaced by a methyl group. This, besides saving computational time, permitted evaluation of whether the nucleoside moiety imposes any interactions that could facilitate the chair conformation or whether the axial aryl ring contributed to this electrostatic interaction. Although the experimental chair conformation was slightly flattened, the calculated chair conformation in **J** showed a similar value for aryl-H to P=O distance (2.25 Å).

To further demonstrate the above-mentioned interaction, we examined the filled (donor) Lewis-type NBOs and empty (acceptor) *non*-Lewis NBOs of the $nO \rightarrow \sigma^*_{C-H}$ interaction (Figure 3).[39] Additionally, to establish whether the chlorine atom in the para-position influences the formation of the C−H···O hydrogen bonding interaction, the **Jb** structure was also analyzed, in which the chlorine atom was replaced by a hydrogen atom. As for structure **J**, the stereoelectronic $nO \rightarrow \sigma^*_{C-H}$ interaction was observed also in this case, with a similar distance (2.28 Å). These results suggest that the **C1** ⇌ **T** equilibria for 4-aryl-1,3,2-dioxaphosphorinanes do not depend on the presence of the chlorine atom; therefore, the same conformational equilibrium could be readily accessible for other 4-aryl-1,3,2-dioxaphosphorinane compounds, at least for those having 1,3-cis oriented P=O and aryl groups.

These results provide structural and theoretical evidence that a C4 aryl group can assume an axial orientation in six-membered ring phosph(on)ates, either in the HepDirect or cyclic phosphoramide prodrugs. Additionally, these results also support the recently proposed hypothesis for the factors contributing to phosphorinane ring C4 epimerization where the stereochemistry and conformation are involved (i.e., the anomeric effect). Thereby, the "unstable" chair conformation

(32) The crystallographic coordinates have been deposited with the Cambridge Crystallographic Data Centre; deposition no. 737132. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre, 12 Union Rd., Cambridge CB2 1EZ, UK or via www.ccdc.cam.ac.uk/conts/retrieving.html.

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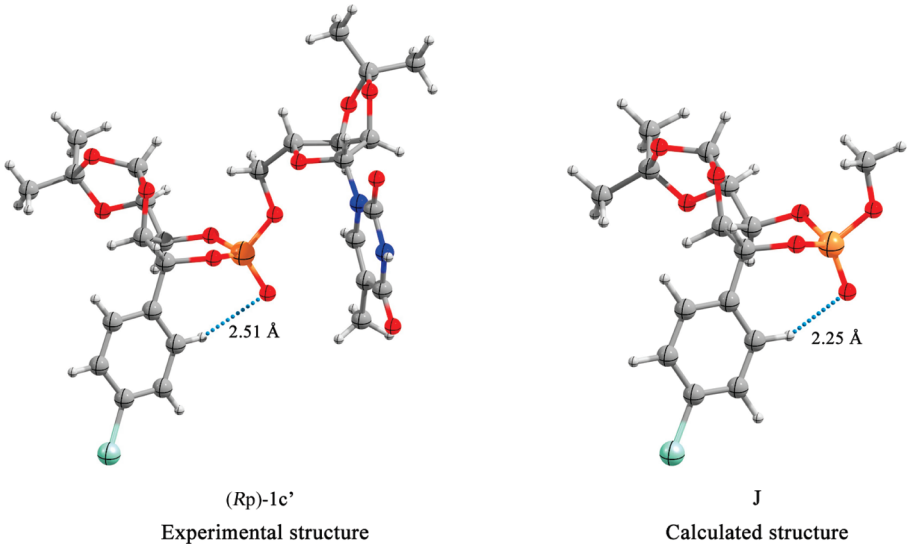


FIGURE 2. Experimental (R_P)-**1c′** and theoretical structure **J** (calculated at the B3LYP/6-31+G(d,p) level of theory) showing the existence of an intramolecular C−H···O hydrogen bonding interaction that stabilizes the chair conformation of the 1,3,2-dioxaphosphorinane.

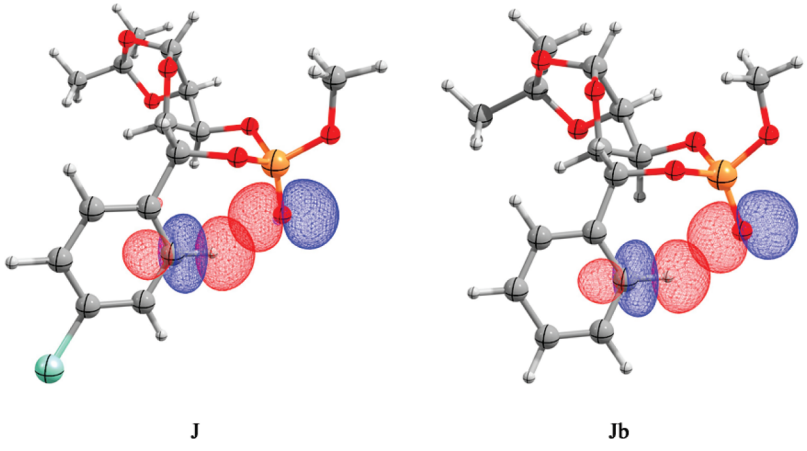


FIGURE 3. NBO analysis for structures **J** and **Jb** showing the $nO \rightarrow \sigma^*_{C-H}$ interaction.

with an axially oriented C4 aryl group was postulated as a key intermediary for the phosphate ring cleavage.[15]

To examine the prodrug isomerization for the series of compounds studied herein, we carried out the phosphorylation reaction of diol **4d′** at −78 °C in the presence of *t*-BuOK as base, and cyclic nucleotides (R_P)-**1′d** and (S_P)-**1′d** were momentarily observed by ^{31}P NMR (−8.9 and −7.8 ppm, respectively);[31] however, after monitoring the crude reaction mixture until reaching room temperature, it could be determined that both nucleotides are selectively transformed to the most stable diastereomeric cyclic nucleotide (S_P)-**1d** (−8.8 ppm) in approximately 4 and 6 h, respectively (Scheme 7).[40]

Conversion of (R_P)-**1d′** to (S_P)-**1d** occurs faster than conversion of (S_P)-**1d′** to (S_P)-**1d** even though this conversion requires the inversion of two stereogenic centers (at C4 and the P-atom), meanwhile only one stereogenic center is inverted in the case of (S_P)-**1d′**. These transformations are readily explained by the σ^* and the π^* route. The naming of the routes indicates which orbital is involved in the O3−C4 bond that leads to the transformations. The isomerization of (R_P)-**1d′** to (S_P)-**1d** is possible by both routes. In the σ^* route, conformation **B1**enables $nO \rightarrow \sigma^*_{P-ONu}$ hyperconjugation (**K**) that weakens the O3−C4 bond to give zwitterion **L**, which after recombination affords (S_P)-**1d**. Following the π^* route, conformation **C1** enables now $nO \rightarrow \pi^*_{P=O}$ hyperconjugation[15,41,42] (**M**) to give zwitterion **N**, which scrambles before collapsing to give the isomerizated product (S_P)-**1d**. On the other hand, the situation with (S_P)-**1d′** is somewhat less complicated because the conversion only requires inversion of the C4 atom and must, therefore, follow the σ^* route, in which starting from conformation **C1** zwitterion **P** is formed that rapidly collapses to afford (S_P)-**1d** (Scheme 7).

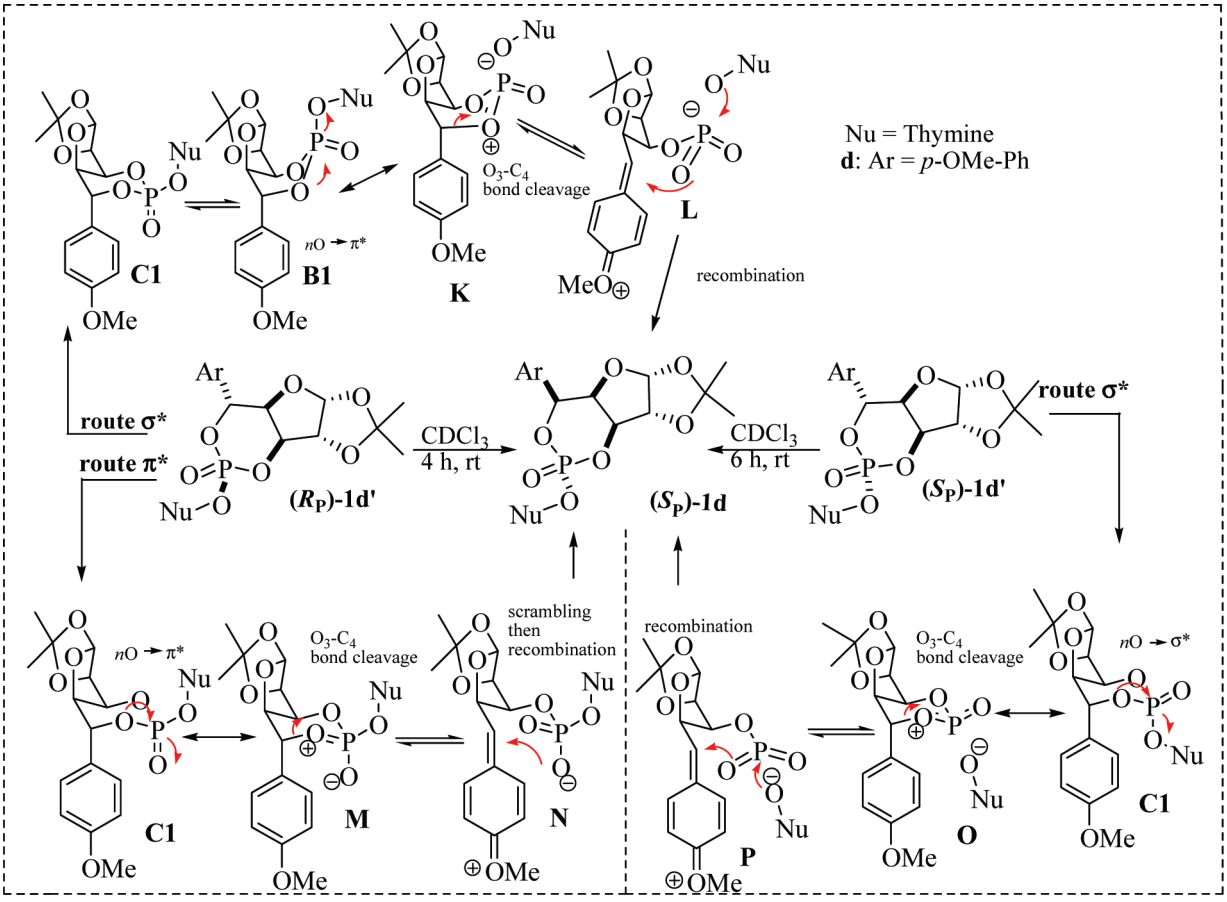
Conclusions

The X-ray crystallographic and theoretical studies reported herein demonstrated the existence of a stable chair

(40) Please see the experimental procedure of ref 15 for details in the determination of the conversion time.

(41) Hernández, J.; Ramos, R.; Sastre, N.; Meza, R.; Hommer, H.; Salas, M.; Gordillo, B. *Tetrahedron* **2004**, *60*, 10927–10941.
(42) Verkade, J. G. *Phosphorus Sulfur* **1976**, *2*, 251–281.

SCHEME 7. Spontaneous and Selective Isomerization of (R_P)-1d′ and (S_P)-1d′ into (S_P)-1d in solution ($CDCl_3$)



conformation for 1,3,2-dioxaphosphorinanes bearing an aryl group axially oriented at the C4 position. This analysis revealed a C−H···O hydrogen bonding interaction between the phosphoryl group (P═O) and the *o*-hydrogen atom of the aryl group, which overcomes the strong steric axial interactions imposed by the aryl group. We have also corroborated that the conversion or isomerization at C4 of cyclic nucleotides, which are analogues of HepDirect prodrugs, occurs through a spontaneous and selective O3−C4 bond cleavage of the phosphorinane ring, which is favored by the conformation and steric anomeric effect. The implications of this finding may not only have relevance on the understanding of the mechanistic course of prodrug cleavage, but also on the re-evaluation of the conformational equilibria of 4-aryl-1,3,2-dioxaphosphorinanes that have been postulated to rarely exhibit a chair conformation. Taking advantage of the above-described findings, along with the introduction of an elegant protocol for the synthesis of novel cyclic nucleotides, we have initiated the synthesis of a series of new cyclic nucleotide analogues to HepDirect that are suitable to undergo oxidative cleavage by a cytochrome P_{450} enzyme (CYP). Results will be published in due course.

Experimental Section

General. The reagents were obtained from commercial sources and used without purification. The solvents were used as technical grade, and freshly distilled prior to use. NMR studies were carried out with 400 and 300 MHz spectrometers. Internal references (TMS) for ^1H and ^{13}C Chemical shifts are stated in parts per million. COSY, HSQC, and NOESY experiments have been carried out in order to assign the ^1H and ^{13}C spectra completely in high resolution mass spectra (FAB^+ ion mode).

2′,3′-*O*-Isopropylidene-5-methyluridine (2).[43] Thymine **5** (2 g, 7.7 mmol) was dissolved in 25 mL of 2,2-dimethoxypropane and catalytic amounts of *p*-toluenesulfonic acid (8 mg, 0.058 mmol) were added. Although the starting material was consumed within 2 h and two products were observed by TLC, the reaction mixture was allowed to react for 24 h at room temperature, whereafter only one product was observed. Excess 2,2-dimethoxypropane was evaporated under reduced pressure and the residue was purified by silica gel column chromatography (*n*-hexane:ethyl acetate = 1:5) to afford **2** (3 g, 95%). White solid; mp 132−134 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 1.36 (s, 3H), 1.57 (s, 3H), 1.90 (d, J = 1.2 Hz, 3H), 3.80 (dd, J = 12.4, 3.6 Hz, 1H), 3.90 (dd, J = 12.4, 2.8 Hz, 1H), 4.26 (q, J = 3.2 Hz, 1H), 4.96 (dd, J = 6.0, 3.2 Hz, 1H), 5.06 (dd, J = 6.4, 2.8 Hz, 1H), 5.55 (d, J = 2.8 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.4, 25.3, 27.3, 62.5, 80.3, 83.4, 86.8, 95.7, 111.0, 114.2, 138.8, 150.4, 163.9.

General Procedure for the Three-Component Reaction of Protected Thymine (2), Phosphoryl Chloride, and 5-Aryl-α-D-xylofuranoses Derivatives. To a solution protected thymine **2** (0.50 mmol) and triethylamine (1.25 mmol) in dry CH_2Cl_2 (30 mL) at 0 °C was added dropwise phosphoryl chloride (0.6 mmol) dissolved in 2 mL of dry CH_2Cl_2. The reaction mixture was allowed to react for 2 h before adding a solution of the corresponding

(43) Chung, R.; Anderson, K. S. *Tetrahedron Lett.* **2006**, *47*, 8361–8363.

1,3-diol **4** (0.50 mmol) and triethylamine (1.25 mmol) in dry CH_2Cl_2 (30 mL) at 0 °C. The reaction mixture was stirred for 3 h and concentrated under reduced pressure; the residue was washed with brine and extracted with ethyl acetate, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (230−400 mesh) with *n*-hexane−EtOAc 2/1 (v/v) to afford the corresponding cyclic nucleotide (R_P)-**1a**−**d** and (S_P)-**1a**−**d**. For the case of 1,3-diol (**4d′**), *t*-BuOK was used as base and ethyl ether as solvent at −78 °C.

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Supporting Information Available: Spectroscopic data, ^1H and ^{13}C NMR spectra for new compounds, Cartesian coordinates for **J** and **Jb** structures, and a CIF file for (R_P)-**1c′**. This material is available free of charge via the Internet at http://pubs.acs.org.