JOC The Journal of Organic Chemistry

Featured Article

pubs.acs.org/joc

Design and Synthesis of α-Carboxy Nucleoside Phosphonate Analogues and Evaluation as HIV-1 Reverse Transcriptase-Targeting Agents

Sarah J. Keane,[†] Alan Ford,[†] Nicholas D. Mullins,[†] Nuala M. Maguire,[†] Thibaut Legigan,[†] Jan Balzarini,[‡] and Anita R. Maguire*,[§]

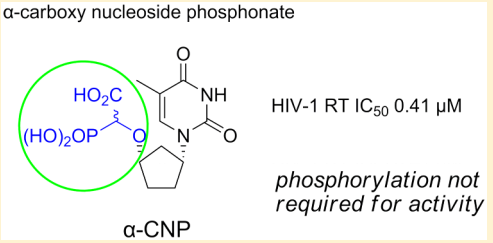
[†]Department of Chemistry, Analytical and Biological Chemistry Research Facility, Synthesis and Solid State Pharmaceutical Centre, University College, Cork, Ireland

[‡]Rega Institute for Medical Research, KU Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium

[§]Department of Chemistry and School of Pharmacy, Analytical and Biological Chemistry Research Facility, Synthesis and Solid State Pharmaceutical Centre, University College, Cork, Ireland

Supporting Information

ABSTRACT: The synthesis of the first series of a new class of nucleoside phosphonate analogues is described. Addition of a carboxyl group at the α position of carbocyclic nucleoside phosphonate analogues leads to a novel class of potent HIV reverse transcriptase (RT) inhibitors, α-carboxy nucleoside phosphonates (α-CNPs). Key steps in the synthesis of the compounds are Rh-catalyzed O−H insertion and Pd-catalyzed allylation reactions. In cell-free assays, the final products are markedly inhibitory against HIV RT and do not require phosphorylation to exhibit anti-RT activity, which indicates that the α-carboxyphosphonate function is efficiently recognized by HIV RT as a triphosphate entity, an unprecedented property of nucleoside monophosph(on)ates.



1. INTRODUCTION

The human immunodeficiency virus (HIV) was first identified as the causative agent of acquired immunodeficiency syndrome (AIDS) in 1983.[1] At the close of 2012, there were an estimated 35 million people living with the retrovirus worldwide, with approximately 2.3 million people newly infected in 2012 alone.[2] The introduction of HAART (highly active antiretroviral therapy) in 1996 has transformed HIV from a lethal infection to a manageable chronic condition with considerable declines in HIV-associated morbidity and mortality.[3−6] However, as a result of the high genetic variability of the retrovirus, resistance to current drug therapies is a major problem, and in addition to HIV there are numerous other chronic viral infections such as hepatitis B and C.[7] Approximately 1 in 12 persons worldwide, or a total of some 500 million people, are living with chronic viral hepatitis.[2] In light of this, a vast amount of time and effort has been invested in the design and synthesis of antiviral agents, most notably nucleoside analogues, and the discovery of new, more efficient antiviral agents is still imperative.

Nucleoside reverse transcriptase inhibitors (NRTIs) were the first class of anti-HIV drugs approved and, despite the discovery of numerous other classes of anti-HIV agents (e.g., nucleotide reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, protease inhibitors, integrase inhibitors, cell entry inhibitors and coreceptor inhibitors), they have continued to play a pivotal role in HIV treatment.[8] NRTIs disrupt viral replication through two distinct modes; competitive inhibition of HIV RT with respect to the dNTP substrate, and DNA chain termination.[9,10] However, in order to do this, these compounds must be first converted via a series of host cell kinases to their active triphosphate form.[10−12] The triphosphorylated drug molecules then compete with the natural nucleotides to be accepted into the growing DNA chain, and upon incorporation, DNA chain elongation is terminated since the NRTIs lack the 3′-OH group of endogenous nucleosides.[10] Poor cell membrane permeability coupled with the labile nature of the phosphate bond precludes the direct delivery of the active triphosphorylated form of the drug into the virus-infected cell.[13] This predicament was partially overcome by the discovery of the phosphonate as a stable isostere for the phosphate bond[14,15] and by the use of phosphoramidate, CycloSal, or alkoxyalkyl prodrug technology.[16−19]

The discovery of (*S*)-HPMPA as a broad spectrum antiviral agent[15] swiftly led to the development of a new class of anti(retro)viral agents: the nucleotide reverse transcriptase inhibitors (NtRTIs). Tenofovir ((*R*)-PMPA)[20] is the only nucleotide reverse transcriptase inhibitor currently approved by the FDA for the treatment of HIV and HBV. It is marketed as the prodrug tenofovir disoproxil fumarate (TDF), which is hydrolyzed in vivo to tenofovir.[8,10] The presence of the phosphonate group enables the compound to bypass the initial phosphorylation step, which is often rate-limiting, and just two

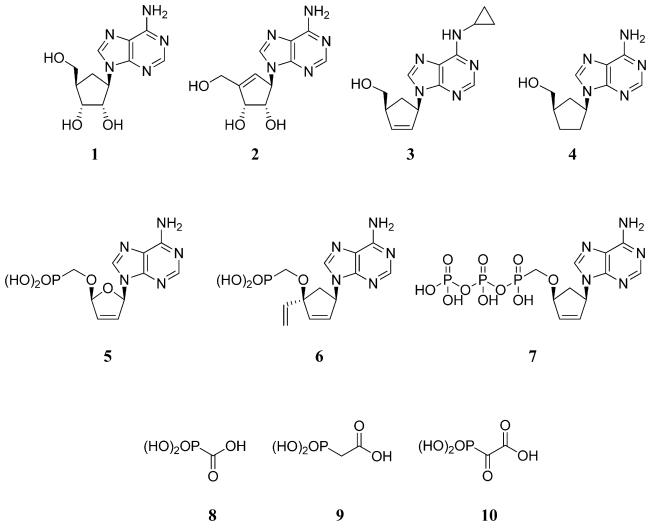
Received: November 7, 2014
Published: December 22, 2014

DOI: 10.1021/jo502549y
J. Org. Chem. 2015, 80, 2479−2493

more phosphorylations are required to furnish the anti(retro)-virally active tenofovir diphosphate.[8]

Beside the N(t)RTIs, two more compound classes have been described as potent inhibitors of HIV-1: non-nucleoside RT inhibitors (NNRTI)[21] and nucleoside-competing reverse transcriptase inhibitors (NcRTI), such as INDOPY-1.[22] Both represent agents with a structure different from nucleosides and do not need metabolic activation (phosphorylation) to interact with their RT target.

Carbocyclic nucleosides are an important subclass of NRTIs where the oxygen of the furanose ring has been replaced by a methylene group.[23−25] This substitution renders these compounds stable to cleavage by intracellular phosphorylases and hydrolases as they lack the labile glycosidic bond of natural nucleosides. Carbocyclic nucleosides also exhibit increased lipophilicity relative to conventional nucleosides leading to increased in vivo half-life, oral uptake efficiency, and cell membrane penetration.[23] Naturally occurring compounds of this type include aristeromycin (**1**) and neplanocin A (**2**),[26] which possess potent antitumor and antiviral activities. Synthetic carbocyclic derivatives include the anti(retro)viral agents abacavir (**3**)[27] and carbocyclic-ddA (**4**).[28]



The phosphononucleoside **5**[29] and the carbocyclic phosphononucleoside **6**[30] possess significant anti-HIV activity, and the diphosphorylated carbocyclic phosphononucleoside derivative **7** strongly inhibits HIV-RT.[14] In addition to this, the anti(retro)viral properties of phosphonoformic acid (PFA, **8**) and phosphonoacetic acid (PAA, **9**) were established almost 3 decades ago.[31] McKenna et al. later synthesized a range of halogen- and methyl-substituted derivatives of PAA, a number of which were found to possess potent anti(retro)viral activity. Interestingly, the carbonyl derivative **10** was significantly more active than **9**.[32] The attachment of PAA and PFA by ester and amide linkages to the 5′-*O* and *N*- positions of 3TC has been reported, but the resulting derivatives were less active against HIV-1 than the parent compound.[33]

In general, phosphononucleoside research involves compounds bearing a simple $CH_2PO(OH)_2$ substituent. However, there have been some reports of derivatives bearing substituents geminal to the phosphonic acid moiety.[34−39] Vederas et al. reported the synthesis of nucleoside dicarboxylates as potential nucleoside diphosphate isosteres.[40] Recently, Janeba has described acyclic nucleoside phosphonates incorporating an additional remote carboxylic acid function, but these compounds did not exhibit any anti(retro)viral activity.[41] Gilbert and co-workers described the synthesis of citrate derivatives of nucleosides as potential mimics of nucleoside triphosphates.[42,43] The compounds were found to be inactive, indicating that the citrate moiety is not a good replacement for the phosphate group(s).

We have previously examined the O−H insertion reaction of diazophosphonoacetates with nucleoside derivatives as a route to obtain functionalized phosphonate analogues.[44,45] The *α*-carboxyphosphonate function was designed as a novel structural motif, which we originally considered as a potential diphosphate (or monophosphate) mimic, to provide a new class of antiviral agents. Although the compounds in the original nucleoside series were found to be biologically inactive, we envisaged that related carbocyclic compounds **11a−f** would be much more sterically similar to the natural nucleoside substrates and therefore more likely to inhibit HIV RT, either in phosphorylated or nonphosphorylated form (Figure 1). We were also interested in compounds such as the unsaturated compound **12** and the diol **13**.

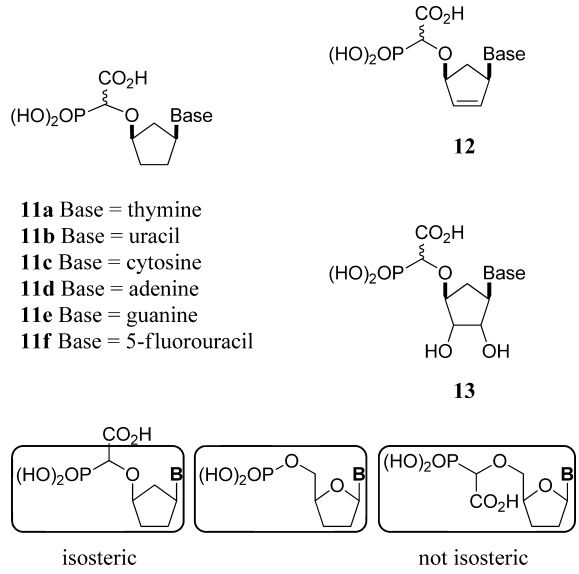


Figure 1. Structural comparison of carbocyclic-CNP, nucleoside monophosphate, and nucleoside carboxymethylphosphonate.

The objective of this study was to synthesize a series of *α*-carboxy nucleoside phosphonates (*α*-CNPs) **11a−f**, **12**, and **13** incorporating a carbocyclic framework for evaluation as potential HIV-1 RT inhibitors.

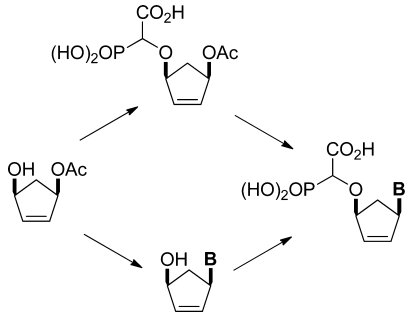
2. RESULTS AND DISCUSSION

Two strategies can be envisaged for the approach to the desired compounds **11**, viz. O−H insertion on the cyclopentanol core prior to introduction of the nucleoside base or alternatively insertion of the base, initially followed by O−H insertion (Scheme 1). For this series of compounds, we elected to use the former approach; while the latter approach is feasible, that route requires extra synthetic steps due to the need for protecting groups to block competing reactions at the nucleoside base, or poisoning of the catalyst by the base.

2.1. O−H Insertion Reactions. We have recently reported that rhodium-catalyzed O−H insertion provides a mild and neutral way of attaching the phosphonate group to suitably protected nucleosides.[44,45] For the preparation of the

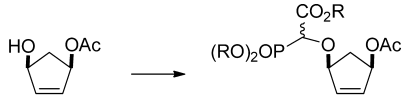
DOI: 10.1021/jo502549y
J. Org. Chem. 2015, 80, 2479−2493

Scheme 1. Two Approaches to Target Compounds



carbocyclic nucleoside phosphonate analogues, the key O−H insertion reactions were carried out using the racemic acetoxy alcohol **14** and trimethyl phosphonodiazoacetate in the presence of rhodium(II) acetate or copper(II) triflate to afford the desired product **15** (Scheme 2). During the early stages of

Scheme 2. O−H Insertion Reaction[a]



14 **15** R = Me 80-90% yield; **16** R = Et 69% yield

[a]Conditions: $(RO)_2OPC(N_2)CO_2R$, $Rh_2(OAc)_4$ or $Cu(OTf)_2$, C_6H_6, 80 °C.

the project, reactions were also carried out with triethyl phosphonodiazoacetate; while these reactions proceeded smoothly to afford **16** in 69% yield, the triethyl derivatives were set aside in favor of the trimethyl derivatives due to ease of deprotection later in the synthetic sequence.

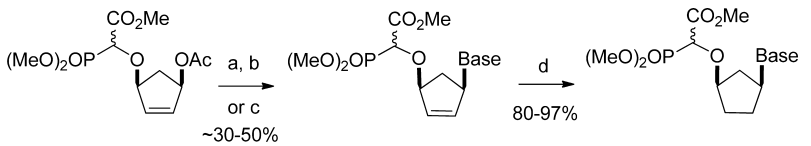
The O−H insertion reactions were essentially complete within 4−5 h at reflux in benzene; however, since it was determined that a longer reaction time did not deleteriously impact on the yield, the reactions were generally heated overnight (17−24 h). The isolated yields were typically high: 70−80% with $Cu(OTf)_2$ and 80−90% with $Rh_2(OAc)_4$. The product **15** was formed as an equimolar mixture of diastereomers, readily identified spectroscopically from the characteristic signals in the ^1H and ^{13}C NMR spectra for the CH adjacent to the phosphorus. Although separation of the diastereomers can be achieved to an extent by chromatography, complete separation of the diastereomers was not attempted.

2.2. Base Insertion Reactions. The introduction of the nucleobases onto the allylic acetate **15** was next undertaken. Tsuji−Trost-type palladium(0)-catalyzed allylic substitution offers a mild method for the attachment of the nucleobases and has the added advantage of regio- and stereoselectivity.[46−49] We investigated the use of different palladium catalysts including $Pd(PPh_3)_4$, $Pd(dba)_2$, or $Pd_2(dba_3)\cdot CHCl_3$ with various phosphines, the use of Cs_2CO_3 and Na_2CO_3 at various concentrations, the use of different solvents at various temperatures and reaction times, and the use of microwave irradiation (Scheme 3). The reactions were found to be variable, sometimes affording 30−60% yields of the desired products **17**, but sometimes failing inexplicably. The most reliable conditions we found involved the use of $Pd(dba)_2$, dppb, and Na_2CO_3 in aqueous acetonitrile at 55 °C overnight or under microwave irradiation for 45−60 min. The reactions carried out in the microwave have the added advantage of short reaction times. We prepared compounds **17a−f**, incorporating thymine, uracil, cytosine, adenine, 2-amino-6-chloropurine, and 5-fluorouracil in this way. The crude adenine and 2-amino-6-chloropurine derivatives were isolated as mixtures of N-7 and N-9 isomers, but after purification the N-9 isomer was obtained in pure form.

2.3. Characterization and Stability of the Phosphononucleosides. Although we did not attempt full separation of the diastereomers of **17**, chromatography of these compounds often led to the isolation of fractions with different diastereomeric ratios, and we observed that the diastereomeric ratio of the bulk crude product of the base insertion reaction was the same as that of the starting allylic acetate. As is the case for the products of the O−H insertion, the majority of ^1H and ^{13}C NMR signals are quite well-distinguished for the two individual diastereomers. The trimethyl derivatives **17a,b** could be kept at room temperature neat or in solution for extended periods without any detectable decomposition, while derivatives **17c−e** were found to be more labile. In particular, the trimethyl cytosine derivative **17c** was found to be labile in solution leading to a complex mixture of unidentifiable products.

2.4. Hydrogenation Reactions. Compounds **17a−f** were hydrogenated at 20−50 psi over palladium on carbon catalyst

Scheme 3. Base Insertion and Hydrogenation Reactions[a]



15

	Base	Yield		Base	Yield
17a	thymine	51%	**18a**	thymine	92%
17b	uracil	38%	**18b**	uracil	86%
17c	cytosine	36%	**18c**	cytosine	80%
17d	adenine	27%	**18d**	adenine	80%
17e	2-amino-6-chloropurine	14%	**18f**	5-fluorouracil	97%
17f	5-fluorouracil	41%			

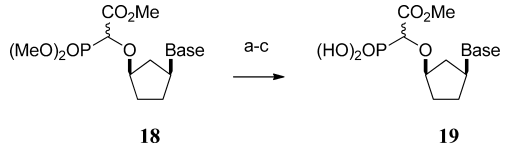
[a]Conditions: (a) base, $Pd(PPh_3)_4$, Na_2CO_3, aq MeCN, 55 °C; (b) base, $Pd(dba)_2$, dppb, Na_2CO_3, aq MeCN, 55 °C; (c) base, $Pd(dba)_2$, dppb, Na_2CO_3, aq MeCN, microwave 55 °C; (d) H_2, Pd/C, MeOH.

DOI: 10.1021/jo502549y
J. Org. Chem. 2015, 80, 2479−2493

(Scheme 3) affording the saturated derivatives **18a−f**. For the thymine and uracil derivatives **17a,b** the reaction was complete within 1.5 h at 20−30 psi, the crude yield was quantitative, and these compounds were isolated in 86−92% yields following chromatography. These reactions could also be carried out over 24−48 h at atmospheric pressure using a hydrogen balloon. The cytosine derivative **17c** required longer reaction times or increased catalyst loading and was isolated in lower yield (80%) than the thymine and uracil derivatives. The adenine derivative **17d** also required more forcing conditions. Complete hydrogenation of the 2-amino-6-chloropurine derivative **17e** was not achieved.

2.5. Deprotection. The derivatives **18a−d** and **18f** were deprotected using essentially the same procedure we applied for the deprotection of the related series of nucleoside derivatives.[44,45] Thus, the trimethyl derivatives were treated with excess TMSBr, followed by addition of water and then treatment with aqueous NaOH (1 M, 10 equiv) at room temperature or at 50 °C. The reaction with TMSBr could also be carried out in the microwave, leading to cleavage of the phosphonate esters after 10−15 min irradiation at 50 °C (Scheme 4). In the case of the adenine derivative, a small amount of the carboxylic methyl ester remained intact (~5%) even after prolonged stirring with aqueous NaOH at 50 °C (3 days). It is possible that heating at a higher temperature could drive the reaction to completion.

Scheme 4. General Procedure for the Deprotection of Phosphononucleosides 18a-d[a]



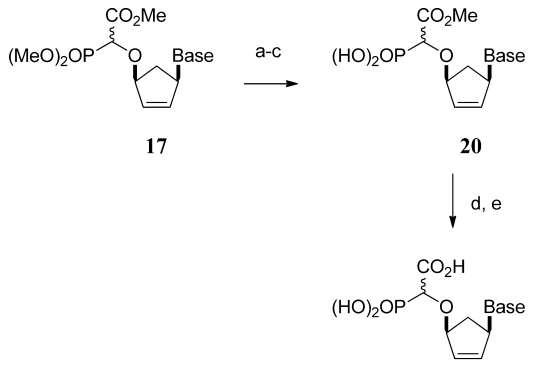
11a thymine	45%
11b uracil	58%
11c cytosine	66%
11d adenine	71%
11f 5-fluorouracil	52%

yield over two steps
isolated as ammonium salts

[a]Conditions: (a) TMSBr, CH_2Cl_2, rt 18 h or MeCN, microwave 50 °C, 15 min; (b) aq MeOH, or H_2O, 30 min; (c) concentration in vacuo; (d) aq NaOH, rt or 50 °C; (e) charcoal chromatography.[44,45]

Exposure of the unsaturated trimethyl ester **17a** to TMSBr resulted in complete degradation of the starting material. Fortunately, when the reaction of **17a** or **17f** with TMSBr was carried out in the presence of 2,6-lutidine,[50] the intermediate phosphonic acid−carboxylic ester **20** could be isolated with no evidence of decomposition. Saponification of the carboxylic ester was then straightforward, giving the fully deprotected unsaturated thymine and 5-fluorouracil derivatives **12a** and **12f** (Scheme 5).

Scheme 5. Deprotection of Unsaturated Derivatives 17a and 17f[a]



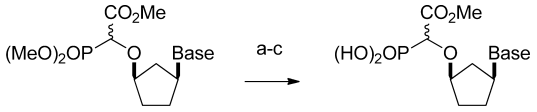
12a thymine	61%
12f 5-fluorouracil	58%

yield over two steps
isolated as ammonium salts

[a]Conditions: (a) TMSBr, lutidine, MeCN microwave 50 °C; (b) H_2O, 30 min; (c) concentration in vacuo; (d) aq. NaOH, 50 °C; (e) charcoal chromatography.[44,45]

The intermediate partially deprotected compounds **19a−d** could be isolated following treatment with TMSBr (5 equiv) and were found to be stable for extended periods of time when stored at neutral pH or as their ammonium salts. To prevent the HBr generated in the reaction from cleaving the carboxylic ester, it was important to adjust the pH of the reaction mixture to 7 with 10% sodium hydroxide before the water was removed at a temperature below 30 °C through coevaporation with acetonitrile. Concentration in vacuo at a higher temperature resulted in partial hydrolysis of the carboxylic ester (Scheme 6).

Scheme 6. Partial Deprotection of 18a−d[a]



19a thymine	82%
19b uracil	96%
19c cytosine	99%
19d adenine	93%

[a]Conditions: (a) TMSBr, CH_2Cl_2, rt 18 h; (b) H_2O, 30 min; (c) NaOH to pH 7.

The novel phosphononucleosides **11a−d** and **11f** were purified using charcoal chromatography.[44,45] The fully deprotected compounds were not stable in acidic solutions; therefore, in each case the crude material was adjusted to pH 1−2.5 immediately prior to adsorption onto the charcoal column. After elution with ammonia, the ammonium salts of **11a−d** and **11f** were isolated in 57−71% yield as clear or pale pink gums. Following lyophilization, these salts were isolated as fine white solids that can be stored for over a year at room temperature without noticeable decomposition. The partially hydrolyzed derivatives **19a−d**, with the intact carboxylic ester, could also be purified by charcoal column chromatography, eluting with 10:10:3 ethanol/water/ammonium hydroxide.

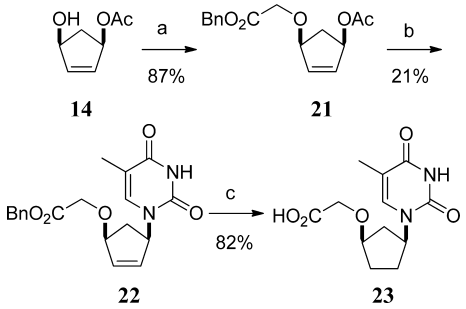
2.6. Synthesis of the Enantioenriched Series of Phosphononucleosides. Having prepared the racemic

DOI: 10.1021/jo502549y
J. Org. Chem. 2015, 80, 2479−2493

phosphononucleosides **11a–d**, the synthesis of these derivatives in enantiopure form ("natural" and "unnatural" enantiomers) was next undertaken. The phosphononucleosides (+)-**11a–d** and (−)-**11a–d** were isolated in each synthetic step in yields similar to those described for the corresponding racemic derivatives **11a–d**. Wherever possible, specific rotations were recorded at each step, and development of HPLC conditions on a chiral stationary phase was undertaken for the unsaturated and saturated phosphononucleosides **17a–d** and **18a–d**. The enantiomers of the intermediates **17b** and **18b** were easily separated by using a Chiralcel OJ-H column, with all four peaks clearly resolved. Separation of the enantiomers of the saturated thymine derivative **18a** was successful to a degree using a Chiralpak AS-H column, although complete resolution of all four peaks was not achieved. Tracking of the enantiopurities of a number of intermediates in the synthesis of the enantioenriched thymine and uracil derivatives **11a** and **11b**, by chiral HPLC and specific rotation, shows that the enantiopurities of the saturated derivatives **18a** and **18b** were the same as those of the starting acetoxy alcohol **14** in each case, thereby confirming that the stereochemical integrity of the precursor **14** is retained throughout the synthetic sequence. While the D-series was prepared starting from (+)-**14** with ≥98% enantiopurity in all cases, we prepared the L-series using several batches of (−)-**14** with varying degrees of enantiopurity (ee 30–99%).

2.7. Structural Modifications. *Nonphosphonate Derivative.* In order to determine whether the phosphonate group is necessary for biological activity we prepared the carboxymethyl derivative **23** (Scheme 7). Rhodium-catalyzed O–H insertion

Scheme 7. Synthesis of Nonphosphonate Derivative 23[a]

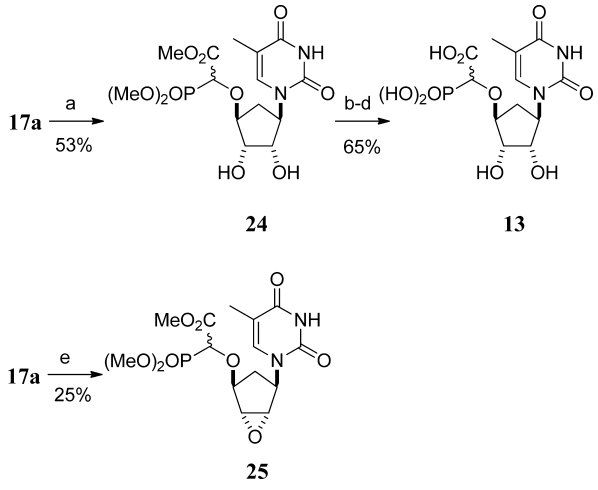


[a]Conditions: (a) N_2CHCO_2Bn, $Rh_2(OAc)_4$, C_6H_6 80 °C; (b) thymine, $Pd(PPh_3)_4$, Na_2CO_3, aq. MeCN 50 °C MW; (c) H_2, Pd/C 1 atm, MeOH.

of the acetoxy alcohol **14** with benzyl diazoacetate afforded the necessary allylic acetate **21** which was converted into the thymine derivative **22** by reaction with thymine in the presence of $Pd(dba)_2$/dppb and sodium carbonate in aqueous acetonitrile. Hydrogenation of **22** resulted in simultaneous cleavage of the benzyl ester and reduction of the double bond to give the desired nonphosphonate free acid derivative **23**. The yields for this sequence were not optimized.

Oxygenated Derivatives. Attempts to use the Upjohn conditions to dihydroxylate the unsaturated derivative **17a** led to low conversion or no reaction. Stoichiometric reaction with OsO_4 did result in the formation of the desired dihydroxylated material **24** (Scheme 8). We assume that the dihydroxylation had taken place on the face of the double-bond opposite to the base and carboxyphosphonate functions. The full deprotection

Scheme 8. Synthesis of Oxygenated Derivatives[a]



[a]Conditions: (a) aq OsO_4, THF, 24 h rt; (b) TMSBr, MeCN, 50 °C MW, 10 min; (c) aq LiOH, 60 °C 3.5 h; (d) charcoal chromatography;[44,45] (e) 30% aq H_2O_2, PhCN, K_2CO_3, MeOH.

to afford the diol-substituted compound **13** was achieved using the normal procedure, using TMSBr followed by saponification.

While attempted epoxidation of **17a** with *m*-CPBA resulted only in recovery of the unsaturated substrate, reaction with H_2O_2 in the presence of benzonitrile[51] did proceed to afford the epoxide **25** (Scheme 8), but a very large excess of the reagent (25 equiv) was required and monitoring of the reaction was complicated by the fact that the starting material and product coeluted on TLC. The coelution also made separation difficult on a preparative scale, but the desired material **25** was isolated cleanly, albeit in low yield (25%). Deprotection of **25** was not achieved, due to limitations of scale.

2.8. Biochemical and Biological Evaluation. The novel compounds **11a–d**, **11f**, (+)-**11a–d**, (−)-**11a–d**, **12a**, **12f**, **13**, **18a**, (+)-**18a**, (−)-**18a**, and **23** were evaluated for their inhibitory activity against a broad range of DNA and RNA viruses and were found to be inactive at ∼250 μM. The following viruses were included in this study: herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2), vaccinia virus, respiratory syncytial virus (RSV), vesicular stomatitis virus (VSV), coxsackie virus B4, parainfluenza virus 3, influenza virus A, influenza virus B, reovirus-1, Sindbis virus, Reovirus-1, Punta Toro virus, human immunodeficiency virus type 1 (HIV-1) and human immunodeficiency virus type 2 (HIV-2). They were not cytostatic/cytotoxic at the highest concentration used (∼250 μM). In the case of the deprotected compounds, these results might be explained by the charged nature of the phosphonic acid/carboxylic acid moiety at physiological pH and, as a result, poor cell permeability.[52,53]

Therefore, the compounds were further investigated using a cell-free HIV-1-RT assay. A number of the compounds were found to strongly inhibit HIV-1 RT (Table 1). Most notably, when examined as potential inhibitors of HIV-1 RT-catalyzed incorporation of [^{3}H]dTTP in a poly rA/oligo dT template/primer, the (−) enantiomers of **11a** and **11b**, corresponding to the "unnatural" L-thymine and L-uracil nucleosides, displayed potent inhibitory activity, and were considerably more active than their (+)-**11a** and (+)-**11b** counterparts. More selective antiviral activity of the L-enantiomer in nucleoside derivatives has been described previously; examples include 3TC and FTC.[54] The thymine derivative **11a** and uracil derivative **11b**

DOI: 10.1021/jo502549y
J. Org. Chem. 2015, 80, 2479–2493

Table 1. Inhibitory Activity of the Compounds against HIV-1 Reverse Transcriptase Using Different Template/Primers and Natural dNTP Substrates

	IC_{50}[a] (μM)		
compd	[^{3}H]dTTP/poly rA·dT	[^{3}H]dCTP/poly rI·dC	[^{3}H]dATP/poly rU·dA
11a	0.41 ± 0.08	>500	293 ± 22
(+)-**11a**	35 ± 5	>500	96 ± 63
(−)-**11a**	0.41 ± 0.00	>500	158 ± 150
11b	3.7 ± 2	>500	139 ± 156
(+)-**11b**	113 ± 105	>500	159 ± 60
(−)-**11b**	3.1 ± 1.7	>500	222 ± 77
11c	219 ± 123	4.5 ± 1.1	102 ± 5.7
(+)-**11c**	22.2 ± 17.7	382 ± 122	51 ± 3
(−)-**11c**	171 ± 22	4.2 ± 0.3	48 ± 0
11d	≥500	>500	0.26 ± 0.05
(+)-**11d**	464 ± 3	>500	32 ± 8
(−)-**11d**	>500	>500	0.19 ± 0.11
11f	3.8 ± 0.0	–[b]	–
12a	1.9 ± 1.1	–	–
12f	2.4 ± 0.8	–	–
13	468	–	–
18a	>500	146 ± 20	>500
(+)-**18a**	>500	>500	>500
(−)-**18a**	>500	>500	>500
23	>700	–	–
AZT-TP	0.069 ± 0.032	–	–
ddCTP	–	11.1 ± 0.9	–
ddATP	–	–	1.16 ± 0.19

[a]Fifty percent inhibitory concentration or compound concentration required to inhibit HIV-RT-catalyzed incorporation of [^{3}H]dNTP in the homopolymeric template/primer. Data are the mean ± SD of at least three to four independent experiments. [b]"–": not performed.

showed no marked, if any, inhibition of the incorporation of [^{3}H]dCTP in poly rI/oligo dC and of [^{3}H]dATP in poly rU/oligo dA at 500 μM, pointing to a specific competition with [^{3}H]dTTP but not with [^{3}H]dCTP or [^{3}H]dATP. Likewise, the cytosine derivative (−)-**11c** displayed strong inhibition in the [^{3}H]dCTP-poly rI/dC system but not in the other systems. Finally, the adenine derivative **11d** proved to be a potent inhibitor of HIV-1 RT in the [^{3}H]dATP-poly rU/dA system but not in the other systems. In all cases, the (−)-enantiomer was by far superior to the (+)-enantiomer, pointing to a high degree of enantiospecificity of these compounds for HIV-1 RT inhibition. The inactivity of compound **23** indicates that both the carboxyl and phosphonate functions are required for HIV-1 RT inhibition.

McClure et al. compared the inhibitory activity of six licensed NRTIs using a cell-free HIV-RT assay.[55] Although not a direct comparison, the results reported by McClure indicate that the most active phosphononucleoside derivatives (−)-**11a** (IC_{50} = 0.41 μM) and (−)-**11d** (IC_{50} = 0.19 μM), possess greater anti-HIV RT activity than all of the NRTIs (IC_{50} = 0.316−10 μM) tested in their study, with the exception of AZT (IC_{50} = 0.1 μM) that showed comparable activity. Our data obtained for AZT-TP, ddCTP and ddATP are in agreement with these findings (Table 1). These data illustrate the remarkable potency of the novel phosphononucleoside derivatives **11a−d**. The most interesting property of the α-carboxy nucleoside phosphonate derivatives is the fact that they are directly inhibitory against HIV-1 RT without the need for prior metabolic conversion to a higher (mono- or di-) phosphate derivative. Indeed, whereas AZT and tenofovir need to be activated (phosphorylated) by cellular nucleoside- and nucleotide kinases to their respective 5′-tri- and diphosphate derivatives in the virus-infected cells, the α-CNPs do not require such metabolic activation steps. In fact, the thymine α-CNP (−)-**11a** was found to lack substrate activity for thymidine kinase and NDP kinase and (−)-**11b** and (−)-**11c** lacked substrate activity for UMP/CMP kinase and NDP kinase (data not shown). Thus, in this respect, the novel agents represent a conceptually and structurally different class of compounds compared with the metabolism-dependent AZT and tenofovir derivatives. The data obtained with the different homopolymeric template/primers and corresponding natural dNTP substrates also revealed that the compounds are base-specific competitive inhibitors of HIV-1 RT, like AZT-TP and tenofovir diphosphate. Also, like AZT-TP and tenofovir diphosphate the α-CNPs inhibit the closely related HIV-2 RT within the same order of magnitude as HIV-1 RT in a biochemical assay.

3. CONCLUSION

In conclusion, we designed and developed synthetic methodology for the first examples of a series of conceptually entirely novel phosphonate derivatives of carbocyclic nucleosides through the incorporation of a carboxylic acid moiety adjacent to the phosphonic acid. We prepared saturated, unsaturated, and oxygenated cyclopentane derivatives featuring this stuctural motif. A number of these new compounds have displayed pronounced inhibitory activity against HIV-1-RT, providing new lead compounds in the nucleoside phosphonate field. Both the phosphonate and carboxyl moieties are required for activity, and the activity resides in the L-enantiomer of the compounds. The new principle of the reported triphosphate mimics may open entirely novel avenues of biological applications given the role of nucleotide triphosphates as important and ubiquitous molecules in living systems. Further studies are underway to prepare additional α-CNP derivatives and to reveal the molecular mechanism of anti-HIV-1 RT activity. In addition, lipophilic prodrug derivatives for testing in cellular assays will be synthesized with initial preference for bis(POM), bis(POC), and phosphoramidate derivatives since such nucleoside phosphonate prodrug entities have proven clinical efficacy.

4. EXPERIMENTAL SECTION

Solvents were distilled prior to use as follows: dichloromethane was distilled from phosphorus pentoxide; ethyl acetate was distilled from potassium carbonate; tetrahydrofuran was distilled from sodium benzophenone; ethanol and methanol were distilled from the corresponding magnesium alkoxide. Benzene was dried before use with activated 4 Å molecular sieves. Organic phases were dried using anhydrous magnesium sulfate. All commercial reagents were used without further purification. Microwave reactions were carried out using a CEM Discover in conjunction with Synergy software, and reaction temperatures were measured with an IR sensor. ^{1}H, ^{13}C, ^{31}P, and ^{19}F NMR spectra were recorded at 20 °C on 300, 400, or 600 MHz spectrometers. Chemical shifts are given in ppm relative to tetramethylsilane (TMS) as an internal standard. ^{31}P chemical shifts are referenced to H_3PO_4 (external standard), and ^{19}F chemical shifts are referenced to C_6F_6. Coupling constants (J) are given in hertz (Hz). Infrared spectra were recorded as potassium bromide (KBr) discs for solids or thin films on sodium chloride plates for oils. Melting points were measured using a capillary melting point apparatus and are not corrected. Optical rotations were measured at 20 °C at 589 nm in a 10 cm cell; concentrations (c) are expressed in g/100 mL, and [α] is expressed in units of 10^{-1} deg cm^2 g^{-1}. Low-resolution mass spectra

DOI: 10.1021/jo502549y
J. Org. Chem. 2015, 80, 2479−2493

were recorded in electrospray ionization (ESI) mode, high resolution mass spectra (HRMS) were recorded on a Time of Flight spectrometer in electrospray ionization (ESI) mode. Column chromatography was performed using silica gel 60. Thin-layer chromatography (TLC) was carried out on precoated silica gel plates (60 PF254). Visualization was achieved by UV (254 nm) detection and/or staining with vanillin, phosphomolybdic acid, or permanganate. The acetoxy alcohol **14**[56] and trimethyl-[57] and triethyl phosphonodiazoacetates[58] were prepared as described in the literature.

O−H Insertion Reactions. *cis-1-[(Methoxycarbonyl)dimethylphosphonomethoxy]-4-acetoxycyclopent-2-ene (**15**).* Rhodium(II) acetate (8 mg, 0.018 mmol, 0.1 mol %) was added to a degassed solution of acetoxy alcohol **14** (2.309g, 16.24 mmol) and trimethyl diazophosphonoacetate (3.712 g, 17.85 mmol) in benzene (35 mL). The reaction mixture was stirred while being heated under reflux for 5 h under a nitrogen atmosphere. The mixture was concentrated and the residue purified by flash chromatography (5% MeOH/CH_2Cl_2) to give **15** as a pale yellow oil (3.873 g, 78% yield, dr 1:1): IR ν_{max}/cm^{-1} (film) 2960 (CH), 1733 (C═O), 1438, 1373, 1244 (P═O), 1111, 1031 (C−O); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.75 (dt, J = 14.7, 3.9, 0.5H), 1.86 (dt, J = 15.0, 3.9, 0.5H), 2.05 (s, 1.5H), 2.06 (s, 1.5H), 2.71−2.84 (m, 1H), 3.81−3.89 (m, 6H), 3.87−3.91 (m, 3H), 4.49 (d, J_{PH} = 20.4, 0.5H), 4.52 (d, J_{PH} = 19.8, 0.5H), 4.60−4.67 (m, 0.5H), 4.69−4.76 (m, 0.5H), 5.45−5.52 (m, 1H), 6.04−6.16 (m, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 21.05, 21.07, 36.7, 36.8, 52.9, 54.2−54.4 (m), 73.3 (d, J_{PC} = 159.5), 73.9 (d, J_{PC} = 159.8), 76.2, 76.3, 84.00 (br d, $J_{PC} \approx$ 11.3), 84.04 (br d, $J_{PC} \approx$ 12.0), 134.37, 134.42, 135.18, 135.23, 168.1 (d, J_{PC} = 2.6), 168.2 (d, J_{PC} = 2.8), 170.6 170.7; ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 16.5, 16.8; HRMS (ES+): calcd for $C_{12}H_{20}O_8P$ (M + H)$^+$ 323.0896, found 323.0909; MS (ES+) m/z 345.0 (M + Na)$^+$.

*(−)-(1S,4R)-1-[(Methoxycarbonyl)dimethylphosphonomethoxy]-4-acetoxycyclopent-2-ene ((−)-(1S,4R)-**15**).* This was synthesized using the procedure described above for **15** using acetoxy alcohol (+)-(1*R*,4*S*)-**14** (1.491 g, 10.49 mmol) and trimethyl diazophosphonoacetate (2.209 g, 10.62 mmol) in benzene (35 mL) and a spatula tip of rhodium(II) acetate. The reaction was stirred while being heated under reflux for 17 h. Purification by flash chromatography (5% MeOH/CH_2Cl_2) afforded (−)-(1*S*,4*R*)-**15** as a pale yellow oil (2.82 g, 88% yield, 98% ee, dr 1:1): $[\alpha]_D^{20}$ −10.58 (*c* 0.95, CH_2Cl_2). The enantiopurity of (−)-(1*S*, 4*R*)-**15** was assigned on the basis of the enantiopurities of the acetoxy alcohol (+)-(1*R*, 4*S*)-**14** and of the base insertion product (+)-(1*R*,4*S*)-**17b**.

*(+)-(1R,4S)-1-[(Methoxycarbonyl)dimethylphosphonomethoxy]-4-acetoxycyclopent-2-ene ((+)-(1R,4S)-**15**).* This was synthesized using the procedure described above for **15** from the acetoxy alcohol (−)-(1*S*,4*R*)-**14** (262 mg, 1.84 mmol, 30% ee) and trimethyl diazophosphonoacetate (415 mg, 2.00 mmol) and a spatula tip of rhodium(II) acetate in benzene (20 mL). The reaction mixture was stirred while being heated under reflux for 6 h. Following purification by flash chromatography (5% MeOH/CH_2Cl_2) the product (+)-(1*R*, 4*S*)-**15** was isolated as a clear oil (545 mg, 92% yield, 30% ee, dr 1:1); $[\alpha]_D^{20}$ + 3.50 (*c* 0.3, CH_2Cl_2). The enantiopurity of (+)-(1*R*,4*S*)-**15** was assigned on the basis of the enantiopurity of the acetoxy alcohol (−)-(1*S*,4*R*)-**14** and of the base insertion product (−)-(1*S*,4*R*)-**17b**.

*cis-1-[(Ethoxycarbonyl)diethylphosphonomethoxy]-4-acetoxycyclopent-2-ene (**16**).* A solution of acetoxy alcohol **14** (259 mg, 1.82 mmol) and triethyl diazophosphonoacetate (500 mg, 2.0 mmol) in benzene (20 mL) was added to a flame-dried 50 mL round-bottomed flask containing activated 3 Å molecular sieve powder (336 mg). The solution was degassed prior to the addition of copper(II) trifluoromethanesulfonate (28 mg, 0.08 mmol, 4 mol %) and heated in a pre-equilibrated oil bath at 92 °C for 19 h. The mixture cooled, filtered, and concentrated in vacuo. Purification by flash chromatography (SiO_2, neat Et_2O) afforded **16** as a colorless oil (459 mg, 69%, dr 1.2:1): IR ν_{max}/cm^{-1} (film) 3459, 2986 (CH), 2939 (CH), 1737 (C═O), 1645 (C═C), 1442, 1371, 1248 (P═O), 1113 (C−N), 1024 (C−O); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.10−1.42 (m, 9H) 1.71−1.80 (dt, J = 14.4, 4.2, 0.4H) 1.82−1.91 (dt, J = 15.0, 3.6, 0.6 H), 2.04 (s, 1.2H), 2.05 (s, 1.8H), 2.77 (dt, J = 15.0, 7.2, 1H), 4.16−4.35 (m, 6H), 4.42 (d, J_{PH} = 19.2, 0.4H), 4.45 (d, J_{PH} = 18.0, 0.6 H), 4.60−4.65 (m, 0.4H), 4.69−4.74 (m, 0.6H), 5.45−5.50 (m, 1H), 6.03−6.08 (m, 1H), 6.10−6.15 (m, 1H); ^{13}C NMR (150.9 MHz, $CDCl_3$) δ 14.1, 16.39, 16.43, 21.06, 21.09, 36.8, 36.9, 61.88, 61.93, 63.7, 63.8, 73.9 (d, J_{PC} = 158.6), 74.4 (d, J_{PC} = 158.9), 76.3, 76.4, 83.90 (d, J_{PC} = 11.6), 83.91 (d, J_{PC} = 12.4), 134.1, 134.7, 134.9, 135.5, 167.8 (br d, $J_{PC} \approx$ 2.0), 167.9 (br d, $J_{PC} \approx$ 2.3), 170.68, 170.70; ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 14.2, 16.5; HRMS (ES+): calcd for $C_{15}H_{26}O_8P$ (M + H)$^+$ 365.1365, found 365.1372; MS (ES+) m/z 387.1 (M + Na)$^+$, 365.1 (M + H)$^+$.

Base Insertion Reactions. *General Procedure for Base Insertion Reactions.* A mixture of 2 M Na_2CO_3 (∼1.2 equiv), nucleobase (∼1.5 equiv), and allylic acetate **15** (1 equiv) in MeCN was thoroughly degassed prior to the addition of a solution of the palladium catalyst (5−10 mol %). The reaction mixture was stirred at the specified temperature for the specified amount of time, allowed to cool, diluted with CH_2Cl_2, and filtered by gravity. The solvents were removed under reduced pressure, and the residue was purified by flash chromatography (SiO_2, 5% MeOH/CH_2Cl_2).

*cis-1-[[4-(Methoxycarbonyl)dimethylphosphonomethoxy]-cyclopent-2-en-1-yl]thymine (**17a**).* This was prepared following the general procedure starting from Na_2CO_3 (2 M, 0.35 mL, ∼0.7 mmol), thymine (112 mg, 0.89 mmol), allylic acetate **15** (186 mg, 0.58 mmol), and $Pd(PPh_3)_4$ (54 mg, 0.047 mmol) in acetonitrile (15 mL). The reaction mixture was stirred at 66 °C for 24 h. Purification by flash chromatography gave **17a** as a cream solid (115 mg, 51%, dr 1.2:1): mp 134−138 °C; IR ν_{max}/cm^{-1} (KBr) 3176 (NH), 3042, 2959 (CH), 1754 (C═O), 1689 (C═O), 1663 (C═O), 1640 (C═C), 1470 (CH), 1264 (P═O), 1102 (C−N), 1029 (C−O); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.73−1.85 (m, 1H), 1.94 (d, J = 1.2, 3H), 2.73−2.87 (m, 1H), 3.81−3.90 (m, 9H), 4.50 (d, J_{PH} = 19.8, 0.55H), 4.53 (d, J_{PH} = 19.2, 0.45H), 4.58−4.64 (m, 0.55H), 4.64−4.71 (m, 0.45H), 5.62−5.73 (m, 1H), 5.91−6.00 (m, 1H), 6.25−6.35 (m, 1H), 7.27 (br q, $J \approx$ 1.2, 0.45H), 7.32 (br q, $J \approx$ 1.2, 0.55H), 9.13 (br s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 12.3, 36.9, 37.1, 53.0, 53.9−54.3 (m), 57.8, 57.9, 74.86 (d, J_{PC} = 159.6), 74.93 (d, J_{PC} = 159.5), 84.7 (d, J_{PC} = 10.0), 84.8 (d, J_{PC} = 11.6), 111.5, 111.6, 134.7, 134.9, 135.4, 135.9, 137.1, 137.2, 151.1, 163.9, 167.6 (br d, $J_{PC} \sim$ 2.5), 167.9 (br d, $J_{PC} \sim$ 2.3); ^{31}P NMR (121 MHz, $CDCl_3$) δ 16.3, 16.5; HRMS (ES+) calcd for $C_{15}H_{22}N_2O_8P$ (M + H)$^+$ 389.1114, found 389.1086; MS (ES+) m/z 389.0 (M + H)$^+$. Anal. Calcd for $C_{15}H_{21}N_2O_8P$: C, 46.40; H, 5.45; N, 7.21. Found: C, 46.27; H, 5.45; N, 6.86.

*(+)-(1R,4S)-1-[4-[(Methoxycarbonyl)dimethylphosphonomethoxy]cyclopent-2-en-1-yl]thymine ((+)-(1R,4S)-**17a**).* This was prepared following the general procedure starting from Na_2CO_3 (2 M, 2.25 mL, ∼4.50 mmol), thymine (714 mg, 5.66 mmol), allylic acetate (−)-(1*S*,4*R*)-**15** (98% ee) (1.25 g, 3.88 mmol), $Pd(dba)_2$ (112 mg, 0.21 mmol, 6 mol %), and dppb (182 mg, 0.427 mmol, 11 mol %) in acetonitrile (50 mL). The reaction mixture was stirred for 5.5 h at 50 °C. Purification by flash chromatography yielded (+)-(1*R*,4*S*)-**17a** as a cream solid (831 mg, 55%, 98% ee, dr 1.2:1): mp 134−135 °C; $[\alpha]_D^{20}$ + 43.70 (*c* 1.00, CH_2Cl_2). The enantiopurity of (+)-(1*R*,4*S*)-**17a** was assigned on the basis of the enantiopurities of the acetoxy alcohol (+)-(1*R*,4*S*)-**14** and of the saturated product (−)-(1*S*,4*R*)-**18a**.

*(−)-(1S,4R)-1-[4-[(Methoxycarbonyl)dimethylphosphonomethoxy]cyclopent-2-en-1-yl]thymine ((−)-(1S,4R)-**17a**).* This was prepared following the general procedure starting from Na_2CO_3 (2 M, 0.7 mL, ∼1.40 mmol), thymine (234 mg, 1.85 mmol), allylic acetate (+)-(1*R*, 4*S*)-**15** (70% ee) (395 mg, 1.23 mmol), $Pd(dba)_2$ (54 mg, 0.10 mmol, 8 mol %), and dppb (60 mg, 0.141 mmol, 11 mol %) in acetonitrile (20 mL). The reaction mixture was stirred for 5.5 h at 50 °C. Purification by flash chromatography yielded (−)-(1*S*,4*R*)-**17a** as a cream solid (261 mg, 55%, 70% ee, dr 1.2:1): mp 136−137 °C; $[\alpha]_D^{20}$ − 33.22 (*c* 0.90, CH_2Cl_2). The enantiopurity of (−)-(1*S*,4*R*)-**17a** was assigned on the basis of the enantiopurities of the acetoxy alcohol (−)-(1*S*,4*R*)-**14** and of the saturated product (+)-(1*R*,4*S*)-**18a**.

*cis-1-[4-[(Methoxycarbonyl)dimethylphosphonomethoxy]cyclopent-2-en-1-yl]uracil (**17b**).* This was prepared following the general procedure starting from Na_2CO_3 (2 M, 0.6 mL, ∼1.2 mmol), uracil (171 mg, 1.52 mmol), allylic acetate **15** (324 mg, 1.01 mmol), and $Pd(PPh_3)_4$ (58 mg, 0.05 mmol, 5 mol %) in acetonitrile (30 mL). The

DOI: 10.1021/jo502549y
J. Org. Chem. 2015, 80, 2479−2493

reaction mixture was stirred at 66 °C for 24 h. Purification via flash chromatography afforded the pure product **17b** as a cream gum (148 mg, 38%, dr 1.2:1): IR ν_{max}/cm^{-1} (film) 3480, 3177 (NH), 3057 (CH), 2961 (CH), 1750 (C═O), 1689, 1625, 1462, 1380, 1260 (P═O), 1105 (C−N), 1032 (C−O); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.74−1.86 (2 × overlapping apparent dt, J = 10.8, 2.7, 8.1, 3.0, 1H), 2.74−2.87 (apparent dt, J = 15.3, 7.5, 1H), 3.80−3.90 (m, 9H), 4.50 (d, J_{PH} = 19.8, 0.5H), 4.53 (d, J_{PH} = 19.2, 0.5H), 4.59−4.65 (m, 0.5H), 4.66−4.72 (m, 0.5H), 5.64−5.72 (m, 1H), 5.74 (br d, $J \sim$ 8.1, 1H), 5.93−6.01 (m, 1H), 6.28−6.37 (m, 1H), 7.49 (d, J = 8.1, 0.5H), 7.51 (d, J = 8.1, 0.5H), 9.46 (br s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 36.9, 37.3, 53.0, 54.1 (2 × overlapping d, $J_{PC} \approx$ 6.6, 6.6), 54.2 (d, J_{PC} = 6.3), 54.3 (d, J_{PC} = 6.2), 58.08, 58.11, 74.78 (d, J_{PC} = 159.8), 74.81 (d, J_{PC} = 160.1), 84.5 (d, J_{PC} = 9.6), 84.6 (d, J_{PC} = 11.7), 102.9, 134.2, 134.6, 135.8, 136.4, 141.6, 151.1, 163.4, 167.5 (br d, $J_{PC} \approx$ 2.5), 167.8 (br d, $J_{PC} \approx$ 2.5); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 16.3, 16.5; HRMS (ES+) calcd for $C_{14}H_{20}N_2O_8P$ $(M + H)^+$ 375.0957, found 375.0952; MS (ES+) m/z 375.0 $(M + H)^+$.

*(+)-(1R,4S)-1-[4-[(Methoxycarbonyl)dimethylphosphonomethoxy]cyclopent-2-en-1-yl]uracil ((+)-(1R,4S)-**17b**).* This was prepared following the general procedure starting from Na_2CO_3 (2 M, 0.7 mL, ∼1.40 mmol), uracil (208 mg, 1.86 mmol), allylic acetate (−)-(1*S*,4*R*)-**15** (98% ee) (395 mg, 1.23 mmol), $Pd(dba)_2$ (38 mg, 0.07 mmol, 6 mol %), and dppb (52 mg, 0.12 mmol, 10 mol %) in acetonitrile. The reaction mixture was stirred for 5.5 h at 50 °C Purification by flash chromatography afforded (+)-(1*R*,4*S*)-**17b** as a cream hygroscopic gum (294 mg, 64%, 98% ee, dr 1.2:1): $[\alpha]_D^{20}$ + 19.56 (*c* 2.30, CH_2Cl_2). HPLC conditions: CHIRALCEL OJ-H column, 30:70 2-propanol/hexane, 0.7 mL/min. 49.2 min, 59.3 min, 83.6 min, 115.0 min.

*(−)-(1S,4R)-1-[4-[(Methoxycarbonyl)dimethylphosphonomethoxy]cyclopent-2-en-1-yl]uracil ((−)-(1S,4R)-**17b**).* This was prepared following the general procedure starting from Na_2CO_3 (2 M, 1.4 mL, ∼2.80 mmol), uracil (422 mg, 3.77 mmol), allylic acetate (+)-(1*R*,4*S*)-**15** (30% ee) (801 mg, 2.5 mmol), $Pd(dba)_2$ (94 mg, 0.18 mmol, 7 mol %), and dppb (107 mg, 0.25 mmol, 10 mol %) in acetonitrile (30 mL). The reaction mixture was stirred for 2.5 h at 55 °C. Purification by flash chromatography afforded the product (−)-(1*S*,4*R*)-**17b** as a cream hygroscopic gum (511 mg, 55%, 30% ee dr 1:1). $[\alpha]_D^{20}$ − 5.87 (*c* 2.23, CH_2Cl_2). HPLC conditions: CHIRALCEL OJ-H column, 30:70 2-propanol/hexane, 0.7 mL/min. 49.2 min, 59.3 min, 83.6 min, 115.0 min.

*cis-1-[4-[(Methoxycarbonyl)dimethylphosphonomethoxy]cyclopent-2-en-1-yl]cytosine (**17c**).* This was prepared following the general procedure starting from Na_2CO_3 (2 M, 0.45 mL, ∼0.90 mmol), cytosine (128 mg, 1.17 mmol), allylic acetate **15** (255 mg, 0.79 mmol), $Pd(dba)_2$ (25 mg, 0.05 mmol, 6 mol %), and dppb (36 mg, 0.08 mmol, 10 mol %) in acetonitrile (10 mL). The reaction mixture was irradiated (50 W, 55 °C) for 1 h. Purification by flash chromatography afforded **17c** as a cream hygroscopic gum (105 mg, 36%, dr 1:1): IR ν_{max}/cm^{-1} (film) 3423 (NH), 3194 (CH), 2957 (CH), 2853 (CH), 1736 (C═O), 1723 (C═O), 1649, 1233 (P═O), 1101 (C−N), 1051 (C−O); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.67−1.80 (2 overlapping dt, J = 10.2, 2.7, 10.2, 3.0, 1H), 2.76−2.90 (m, 1H), 3.79−3.99 (m, 9H), 4.47 (d, J_{PH} = 19.8, 0.5H), 4.50 (d, J_{PH} = 19.5 0.5H), 4.56−4.62 (m, 0.5H), 4.65−4.71 (m, 0.5H), 5.10−7.00 (br s, 2H), 5.76−5.88 (m and d at 5.83, $J \approx$ 7.2, 2H), 5.94−6.02 (m, 1H), 6.23−6.30 (m, 1H), 7.52 (d, J = 7.2, 0.5H), 7.54 (d, J = 7.2, 0.5H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 37.5, 37.8, 53.0, 54.1−54.4, 58.77, 58.83, 74.65 (d, J_{PC} = 160.0), 74.72 (d, J_{PC} = 159.8, PCH), 85.0 CH, d, $J \approx$ 9.7), 86.0 (br d, $J \approx$ 12.5), 95.2, 134.8, 135.2, 135.5, 135.8, 142.7, 156.3, 165.2, 167.7 (br d, $J_{PC} \approx$ 2.7), 167.9 (br d, $J_{PC} \approx$ 2.3); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 16.4, 16.8; HRMS (ES+) calcd for $C_{14}H_{21}N_3O_7P$ $(M + H)^+$ 374.1117, found 374.1114; MS (ES+) m/z 374.1 $(M + H)^+$. Compound **17c** is not stable in solution.

*(+)-(1R,4S)-1-[4-[(Methoxycarbonyl)dimethylphosphonomethoxy]cyclopent-2-en-1-yl]cytosine ((+)-(1R,4S)-**17c**).* This was prepared following the general procedure starting from Na_2CO_3 (2 M, 0.66 mL, ∼1.32 mmol), cytosine (202 mg, 1.82 mmol), allylic acetate (−)-(1*S*,4*R*)-**15** (98% ee) (390 mg, 1.21 mmol), $Pd(dba)_2$ (35 mg, 0.07 mmol, 5 mol %), and dppb (52 mg, 0.12 mmol, 10 mol %) in acetonitrile (25 mL). The mixture was stirred for 3 h at 55 °C. Purification by flash chromatography afforded (+)-(1*R*,4*S*)-**17c** as a light brown gum (178 mg, 39% yield, 98% ee, dr 1.1:1): $[\alpha]_D^{20}$ + 24.50 (*c* 0.1, CH_2Cl_2). The enantiopurity of (+)-(1*R*,4*S*)-**17c** was assigned on the basis of the enantiopurity of the acetoxy alcohol (+)-(1*R*,4*S*)-**14**.

*(−)-(1S,4R)-1-[4-[(Methoxycarbonyl)dimethylphosphonomethoxy]cyclopent-2-en-1-yl]cytosine ((−)-(1S,4R)-**17c**).* This was prepared following the general procedure starting from Na_2CO_3 (2 M, 1.5 mL ∼3.0 mmol), cytosine (441 mg, 3.97 mmol), allylic acetate (+)-(1*R*,4*S*)-**15** (70% ee) (848 mg, 2.63 mmol), $Pd(dba)_2$ (104 mg, 0.2 mmol, 7.5 mol %), and dppb (114 mg, 0.27 mmol, 10 mol %) in acetonitrile (45 mL). The reaction mixture was stirred for 6.5 h at 55 °C. Purification by flash chromatography afforded (−)-(1*S*,4*R*)-**17c** as a pale brown gum (356 mg, 36%, 70% ee, dr 1.2:1): $[\alpha]_D^{20}$ − 18.25 (*c* 0.2, CH_2Cl_2). The enantiopurity of (−)-(1*S*,4*R*)-**17c** was assigned on the basis of the enantiopurity of the acetoxy alcohol (−)-(1*S*,4*R*)-**14**.

*cis-9-[4-[(Methoxycarbonyl)dimethylphosphonomethoxy]cyclopent-2-en-1-yl]adenine (**17d**).* This was prepared following the general procedure starting from Cs_2CO_3 (2 M, 0.7 mL, ∼1.43 mmol), adenine (316 mg, 2.34 mmol), the allylic acetate **15** (458 mg, 1.42 mmol), $Pd_2(dba_3).CHCl_3$ (80 mg, 0.09 mmol, 6 mol %), and dppb (67 mg, 0.16 mmol, 11 mol %) in acetonitrile (25 mL). The reaction mixture was stirred for 5.5 h at 50 °C. Purification by flash chromatography yielded **17d** as a cream hygroscopic gum (153 mg, 27% dr 1:1): IR ν_{max}/cm^{-1} (film) 3392 (NH), 3185, 2958 (CH), 1749 (C═O), 1655, 1599 (C═C), 1235 (P═O), 1104 (C−N), 1035 (C−O); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.01−2.17 (2 dt, J = 9.6, 3.0, 9.3, 3.0, 1H), 2.84−3.00 (m, 1H), 3.78−3.92 (m, 9H), 4.55 (d, J_{PH} = 20.1, 0.5H), 4.65 (d, J_{PH} = 19.5, 0.5H), 4.70−4.77 (m, 0.5H), 4.79−4.86 (m, 0.5H), 5.61−5.70 (m, 1H), 6.10−6.31 (m, 3H), 6.34−6.41 (m, 1H), 8.10 (s, 0.5H), 8.11 (s, 0.5H), 8.35 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 38.2, 38.6, 53.06, 53.08, 54.0−54.5 (4 overlapping d, $J \approx$ 6.7, 6.8, 6.7, 6.6), 56.5, 56.6, 74.5 (d, J_{PC} = 159.6), 74.6 (d, J_{PC} = 159.8), 84.5 (d, J_{PC} = 10.5), 84.8 (d, J_{PC} = 11.8), 119.46, 119.48, 134.5, 134.8, 135.2, 135.6, 139.59, 139.64, 149.52, 149.54, 152.9, 155.59, 155.61, 167.7 (d, J_{PC} = 2.2), 167.9 [d, J_{PC} = 2.5); ^{31}P NMR (121 MHz, $CDCl_3$) δ 16.3, 16.6; HRMS (ES+) calcd for $C_{15}H_{21}N_5O_6P$ $(M + H)^+$ 398.1229, found 398.1215; MS (ES+) m/z 398.2 $(M + H)^+$.

*(+)-(1R,4S)-9-[4-[(Methoxycarbonyl)dimethylphosphonomethoxy]cyclopent-2-ene-1-yl]adenine ((+)-(1R,4S)-**17d**).* This was prepared following the general procedure starting from Cs_2CO_3 (2 M, 1.35 mL, ∼2.7 mmol), adenine (521 mg, 3.86 mmol), allylic acetate (−)-(1*S*,4*R*)-**15** (98% ee) (701 mg, 2.18 mmol), $Pd(dba)_2$ (75 mg, 0.14 mmol, 7 mol %), and dppb (123 mg, 0.29 mmol, 13 mol %) in acetonitrile (70 mL). The reaction mixture was then stirred for 5 h at 50 °C. Purification by flash chromatography afforded (+)-(1*R*,4*S*)-**17d** as a cream hygroscopic gum (279 mg, 32%, 98% ee, dr 1.1:1). The enantiopurity of (+)-(1*R*,4*S*)-**17d** was assigned on the basis of the enantiopurity of the starting acetoxy alcohol (+)-(1*R*,4*S*)-**14**.

*(−)-(1S,4R)-9-[4-[(Methoxycarbonyl)diethylphosphonomethoxy]-cyclopent-2-en-1-yl]adenine ((−)-(1S,4R)-**17d**).* This was prepared following the general procedure starting from Cs_2CO_3 (2 M, 1.0 mL, ∼2.0 mmol), adenine (352 mg, 2.56 mmol), allylic acetate (+)-(1*R*,4*S*)-**15** (70% ee) (551 mg, 1.70 mmol), $Pd(dba)_2$ (62 mg, 0.12 mmol, 6 mol %), and dppb (80 mg, 0.19 mmol, 11 mol %) in acetonitrile (25 mL). The reaction mixture was stirred for 2.5 h at 55 °C. Purification by flash chromatography afforded (−)-(1*S*,4*R*)-**17d** as a cream hygroscopic gum (268 mg, 40%, 70% ee, dr 1.1:1). The enantiopurity of (−)-(1*S*,4*R*)-**17d** was assigned on the basis of the enantiopurity of the starting acetoxy alcohol (−)-(1*S*,4*R*)-**14**.

*cis-2-Amino-9-[[4-(methoxycarbonyl)dimethylphosphonomethoxy]cyclopent-2-en-1-yl]-6-chloropurine (N-9-**17e**) and cis-2-Amino-7-[[4-(methoxycarbonyl)dimethylphosphonomethoxy]-cyclopent-2-en-1-yl]-6-chloropurine (N-7-**17e**).* This was prepared following the general procedure starting from Cs_2CO_3 (2 M, 0.95 mL, ∼1.89 mmol), 2-amino-6-chloropurine (432 mg, 2.55 mmol), allylic acetate **15** (550 mg, 1.70 mmol), $Pd(dba)_3$ (50 mg, 0.15 mmol, 5 mol %), and dppb (73 mg, 0.17 mmol, 10 mol %) in acetonitrile (25 mL). The reaction mixture stirred for 6.5 h at 45 °C. Purification by flash

DOI: 10.1021/jo502549y
J. Org. Chem. 2015, 80, 2479−2493

chromatography afforded *N*-9-**17e** (101 mg, 14% dr 1.2:1) as a pale yellow oil and *N*-7-**17e** (253 mg, 35% dr 1.2:1) as a yellow oil.

N-9-**17e**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.09 (dt, J = 15.0, 3.3, 0.45H), 2.32 (dt, J = 15.0, 4.5, 0.55H), 2.83−3.02 (m, 1H), 3.79−3.89 (m, 9H), 4.78 (d, J_{PC} = 20.4, 0.45H), 4.79−4.86 (m, 0.45H), 4.99−5.06 (m, 0.55H), 5.25−5.39 (m, 2.1H), 5.41−5.48 (m, 1H,), 5.54 [br s, 1H), 6.04−6.12 (m, 1H), 6.22−6.28 (m, 0.55H), 6.34−6.39 (m, 0.45H), 7.88 (br s, 0.55H), 7.97 (br s, 0.45H).

N-7-**17e**: IR ν_{max}/cm^{-1} (KBr) 3440, 3401, 3327 (NH), 3209 (CH), 2958 (CH), 1749 (C═O), 1626, 1544, 1496 (CH), 1378, 1257, 1226 (P═O), 1107 (C−N), 1028 (C−O); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.98−2.14 (2 dt, J = 14.7, 2.7, 15.0, 3.0, 1H), 2.87−3.03 (m, 1H), 3.72−3.90 (m, 9H), 4.48 (d, J_{PC} = 19.8, 0.55H), 4.52 (d, J_{PC} = 19.2, 0.45H), 4.69−4.75 (m, 0.55H), 4.77−4.83 (m, 0.45H), 5.33 (br s, 2H), 5.76−5.86 (m, 1H), 6.21−6.29 (m, 1H,), 6.43−6.51 (m, 1H,), 8.19 (br s, 0.45H), 8.20 (br s, 0.55H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 39.4, 39.8, 53.08, 53.10, 54.1−54.5 (m), 59.7, 59.8, 74.89 (d, J_{PC} = 159.6), 74.94 (d, J_{PC} = 159.8), 84.5 (d, J_{PC} = 10.7), 84.7 (d, J_{PC} = 12.3), 132.7, 133.3, 134.0, 136.4, 137.1, 143.1, 146.9, 159.3, 164.4, 167.6 (d, J = 2.2), 167.8 (d, J = 2.5); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 16.0, 16.2. Peaks due to unknown impurity (∼10%) visible in ^{13}C NMR spectrum at 141.8, 159.1 ppm and in the ^{1}H NMR at 5.51−5.53 (0.2H, m) and 8.10 (0.2H, s).

*Methyl 2-(Dimethoxyphosphoryl)-2-((4-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)cyclopent-2-en-1-yl)oxy)acetate (**17f**).* This was prepared following the general procedure starting from Na_2CO_3 (2 M, 0.35 mL, 0.7 mmol), 5-fluorouracil (113 mg, 0.87 mmol), allylic acetate **15** (186 mg, 0.58 mmol), and $Pd(PPh_3)_4$ (54 mg, 0,046 mmol) in acetonitrile (15 mL). The reaction mixture was stirred at 60 °C for 2.5 h. Purification via flash chromatography (3% MeOH/CH_2Cl_2) afforded **17f** as a beige solid (94 mg, 41%, dr 1:1): mp 141 °C; IR ν_{max}/cm^{-1} (film) 3486, 3171 (NH), 3066 (CH), 2961 (CH), 1750, 1713 (C═O), 1665, 1467, 1438, 1389, 1260 (P═O), 1104 (C−N), 1033 (C−O); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.80−1.87 (m, 1H), 2.67−2.80 (m, 1H), 3.81−3.87 (m, 9H), 4.51 (d, J = 20.0, 0.5H), 4.53 (d, J = 19.3, 0.5H), 4.58−4.60 (m, 0.5H), 4.66−4.67 (m, 0.5H), 5.65−5.71 (m, 1H), 5.94−6.00 (m, 1H), 6.30−6.37 (m, 1H), 7.64 (d, J = 6.8z, 0.5H), 7.66 (d, J = 6.6 Hz, 0.5H), 9.93 (br s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 36.6, 36.9, 53.2, 54.18, 54.26, 54.31, 54.35, 58.69, 58.74, 74.8 (d, J = 159.7), 84.3 (d, J = 9.6), 84.6 (d, J = 11.5), 125.9 (d, J = 33.5), 126.0 (d, J = 33.4), 134.4, 134.7, 136.2, 136.7, 140.9 (d, J = 237.7), 149.8, 157.1 (d, J = 26.5), 167.6 (d, J = 2.5), 167.8 (d, J = 2.4 Hz); ^{13}P NMR (121.5 MHz, $CDCl_3$) δ 16.26, 16.45; ^{19}F NMR (282.4 MHz, $CDCl_3$) δ − 164.23, − 164.16; HRMS (ES+) calcd for $C_{14}H_{19}FN_2O_8P$ $(M + H)^+$ 393.0863, found 393.0858. MS (ES^-) m/z 391.2 $(M - H)^-$.

Hydrogenation Reactions. *cis-1-[4-[(Methoxycarbonyl)dimethylphosphonomethoxy]cyclopentan-1-yl]thymine (**18a**).* A mixture of alkene **17a** (108 mg, 0.28 mmol) and 10% palladium on carbon (54 mg) in methanol (10 mL) was pressurized with hydrogen to 30 psi and shaken for 2.5 h. The mixture was filtered on Celite, and the cake was rinsed with methanol. The filtrate was concentrated, and the residue was purified by flash chromatography (5% MeOH in CH_2Cl_2) to give **18a** as a cream solid (100 mg, 92%, dr 1.1:1): mp 130−133 °C; IR ν_{max}/cm^{-1}(KBr) 3183 (NH), 3053, 2960 (CH), 1748 (C═O), 1689 (C═O), 1662 (C═O), 1644 (C═C), 1469 (CH), 1261 (P═O), 1113 (C−N), 1023 (C−O); ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.55−1.66 (m, 1H), 1.76−1.90 (m, 2H), 1.98 (s, 1.5H), 2.00 (s, 1.5H), 2.02−2.07 (br dd, $J \approx$ 6.6, 7.2, 0.5H), 2.08−2.14 (br, dd, $J \approx$ 6.6, 7.2, 0.5H), 2.16−2.25 (m, 1H), 2.35−2.43 (m, 1H), 3.83−3.90 (m 9H), 4.15−4.19 (m, 0.5H), 4.20−4.24 (m, 0.5H), 4.40 (d, J_{PH} = 18.6, 0.5H), 4.46 (d, J_{PH} = 19.8, 0.5H), 5.22−5.31 (m, 1H), 7.66 (s, 0.5H), 7.80 (s, 0.5H), 8.75 (br s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 12.25, 12.27, 30.0, 30.1, 30.6, 31.4, 38.4, 38.7, 52.97, 53.00, 53.02, 53.2, 53.8−54.4 (m), 73.3 (d, J_{PC} = 160.8), 74.1 (d, J_{PC} = 160.1), 82.0 (d, J_{PC} = 11.3), 82.8 (d, J_{PC} = 9.1), 111.67, 111.8, 138.0, 138.1, 151.45, 151.48, 163.9, 167.7 (d, J = 2.3), 167.9 (d, J = 2.3); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 16.8, 17.0; HRMS (ES+) calcd for $C_{15}H_{24}N_2O_8P$ $(M + H)^+$ 391.1270, found 391.1263; MS (ES+) m/z 391 $(M + H)^+$. Anal. Calcd for $C_{15}H_{23}N_2O_8P$: C, 46.16; H, 5.94; N, 7.18. Found: C, 45.91; H, 5.84; N, 6.90.

*(−)-(1S,4R)-1-[4-[(Methoxycarbonyl)dimethylphosphonomethoxy]cyclopentan-1-yl]thymine ((−)-(1S,4R)-**18a**).* Following the procedure described for **18a** starting from (+)-(1*R*,4*S*)-**17a** (98% ee) (636 mg, 1.64 mmol) and 5% Pd/C (217 mg) in methanol (25 mL) afforded (−)-(1*S*,4*R*)-**18a** as a white solid (584 mg, 91%, 98% ee, dr 1.1:1): mp 133−135 °C; $[\alpha]_D^{20}$ − 8.48 (c 0.67, CH_2Cl_2). HPLC conditions: CHIRALPAK AS-H column 25:75 IPA/hexane, flow 0.8 mL/min. Retention times: 48.5 min (not resolved), 84.1 min, 102.7 min.

*(+)-(1R,4S)-1-[4-[(Methoxycarbonyl)dimethylphosphonomethoxy]cyclopentan-1-yl]thymine ((+)-(1R,4S)-**18a**).* Following the procedure described for **18a** starting from the alkene (−)-(1*S*,4*R*)-**17a** (50% ee) (167 mg, 0.43 mmol) and 5% Pd/C (76 mg) in methanol (15 mL) afforded (+)-(1*R*,4*S*)-**18a** as a cream solid (143 mg, 85%, 50% ee, dr 1:1): mp 129−130 °C; $[\alpha]_D^{20}$ + 6.11 (c 1.24, CH_2Cl_2). HPLC conditions: CHIRALPAK AS-H column 25:75 IPA/hexane, flow 0.8 mL/min. Retention times: 48.5 min (not resolved), 84.1 min, 102.7 min.

*cis-1-[4-[(Methoxycarbonyl)dimethylphosphonomethoxy]cyclopentan-1-yl]uracil (**18b**).* Following the procedure described for **18a** starting from **17b** (75 mg, 0.19 mmol) and 5% Pd/C (50 mg) in methanol (10 mL) and shaking for 1.5 h afforded the saturated product **18b** as a white hygroscopic gum (69 mg, 86%, dr 1.1:1): IR ν_{max}/cm^{-1} (film) 3425 (NH), 3198, 2959, 2921 (CH), 1744 (C═O), 1688, 1255 (P═O), 1111 (C−N), 1034 (C−O); ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.57−1.67 (m, 1H), 1.78−1.90 (m, 2H), 2.04−2.14 [2 dd, $J \approx$ 6.6, 6.6, 6.6, 6.6, 1H), 2.19−2.29 (m, 1H), 2.34−2.43 (m, 1H), 3.83−3.89 (m, 9H), 4.17−4.22 (m, 1H), 4.39 (d, J_{PH} = 19.2, 0.55H), 4.44 (d, J_{PH} = 19.8, 0.45H), 5.21−5.30 (m, 1H), 5.75 (br d, $J \approx$ 7.8, 0.45H), 5.80 (br d, $J \approx$ 7.8, 0.55H), 7.89 (br d, $J \approx$ 8.4, 0.45H), 7.98 (br d, $J \approx$ 7.8, 0.55H), 8.87 (br s, 1H,); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 30.2, 30.6, 31.6, 38.3, 38.9, 53.01, 53.02, 53.4, 53.5, 53.9 (d, J_{PC} = 6.6), 54.0 (d, J_{PC} = 6.8), 54.1 (d, J_{PC} = 6.6), 54.4 (d, J_{PC} = 6.6), 73.4 (J_{PC} = 160.8), 74.1 (d, J_{PC} = 160.0), 82.0 (d, J_{PC} = 11.2, 82.7 (d, J_{PC} = 9.3), 102.9, 103.1, 142.4, 142.5, 151.38, 151.41, 163.3, 167.6 (br d, $J_{PC} \approx$ 2.0), 167.9 (br d, $J_{PC} \approx$ 2.6); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 16.8, 17.0; HRMS (ES+) calcd for $C_{14}H_{22}N_2O_8P$ $(M + H)^+$ 377.1114, found 377.1114; MS (ES+) m/z 377.1 $(M + H)^+$.

*(−)-(1S,4R)-1-[4-[(Methoxycarbonyl)dimethylphosphonomethoxy]cyclopentan-1-yl]uracil ((−)-(1S,4R)-**18b**).* Following the procedure described for **18a** starting from (+)-(1*R*,4*S*)-**17b** (98% ee) (231 mg, 0.62 mmol) and 5% Pd/C (181 mg) in methanol (35 mL) and shaking for 1.5 h afforded (−)-(1*S*,4*R*)-**18b** as a cream gum (209 mg, 90%, 98% ee, dr 1.1:1): $[\alpha]_D^{20}$ − 4.55 (c 0.62, CH_2Cl_2). HPLC Conditions: CHIRALCEL OJ-H column 30:70 IPA/hexane, flow 0.7 mL/min. Retention times: 42.2 min, 47.5 min, 60.6 min, 68.5 min.

*(+)-(1R,4S)-1-[4-[(Methoxycarbonyl)dimethylphosphonomethoxy]cyclopentan-1-yl]uracil ((+)-(1R,4S)-**18b**).* Following the procedure described for **18a**, starting from (−)-(1*S*,4*R*)-**17b** (30% ee) (351 mg, 0.94 mmol) and 5% Pd/C (120 mg) in methanol (30 mL) and shaking for 1.5 h afforded the (+)-(1*R*,4*S*)-**18b** as a white hygroscopic gum (331 mg, 94%, 30% ee, dr 1.1:1): $[\alpha]_D^{20}$ + 1.46 (c 1.13, CH_2Cl_2). HPLC conditions: CHIRALCEL OJ-H column 30:70 IPA/hexane, flow 0.7 mL/min. Retention times: 42.2 min, 47.5 min, 60.6 min, 68.5 min.

*cis-1-[4-[(Methoxycarbonyl)dimethylphosphonomethoxy]-cyclopentan-1-yl]cytosine **18c**.* Following the procedure described for **18a**, starting from **17c** (97 mg, 0.26 mmol) and 5% Pd/C (60 mg) in methanol (10 mL) and shaking for 15 h at 25 psi afforded **18c** as a white hygroscopic gum (78 mg, 80%, dr 1.1:1): IR ν_{max}/cm^{-1} (film) 3421 (NH), 3198 (CH), 2958 (CH), 1744 (C═O), 1718, 1651, 1531, 1491 (CH), 1260, 1233 (P═O), 1109 (C−N), 1050 (C−O), 1030; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.58−1.90 (m, 3H), 1.97−2.12 (m, 1H), 2.15−2.30 (m, 1H), 2.34−2.48 (m, 1H), 3.81−3.90 (m, 9H), 4.12−4.20 (m, 1H), 4.40 (d, J_{PH} = 19.2, 0.5H), 4.44 (d, J_{PH} = 19.8, 0.5H), 5.27−5.44 (m, 1H), 5.50−7.31 (br s, 2H), 5.90 (d, J = 7.5, 0.5H), 5.93 (d, J = 7.5, 0.5H), 7.86 (d, J = 7.5, 0.5H), 7.94 (d, J = 7.5, 0.5H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 30.6, 30.7, 31.7, 38.5, 39.0,

DOI: 10.1021/jo502549y
J. Org. Chem. 2015, 80, 2479−2493

53.01, 53.04, 53.9−54.3 (m), 73.5 (d, J_{PC} = 160.7), 74.1 (d, J_{PC} = 160.0), 82.3 (d, J_{PC} = 11.2, 82.9 (d, J_{PC} = 9.5), 95.2, 95.4, 143.4, 143.6, 156.9, 164.9, 167.8 (d, J_{PC} = 2.3), 168.0 (d, J_{PC} = 2.7); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 16.9, 17.0; HRMS (ES+) calcd for $C_{14}H_{23}N_3O_7P$ $(M + H)^+$ 376.1274, found 376.1263; MS (ES−) m/z $(M - H)^-$. Compound **18c** is not stable in solution.

*(−)-(1S,4R)-1-[4-[(Methoxycarbonyl)dimethylphosphonomethoxy]cyclopentan-1-yl]cytosine ((−)-(1S,4R)-**18c**).* Following the procedure described for **18a**, starting from (+)-(1*R*,4*S*)-**17c** (98% ee) (167 mg, 0.447 mmol) and 10% Pd/C (83 mg) in methanol (25 mL) and shaking for 15 h at 25 psi afforded (−)-(1*S*,4*R*)-**19c** as a colorless hygroscopic gum (98 mg, 58%, 98% ee, dr 1.2:1). The optical rotation could not be measured due to instability. The enantiopurity of (−)-(1*S*,4*R*)-**18c** was assigned on the basis of the enantiopurity of the acetoxy alcohol (+)-(1*R*,4*S*)-**14**.

*(+)-(1R,4S)-1-[4-[(Methoxycarbonyl)dimethylphosphonomethoxy]cyclopentan-1-yl]cytosine ((+)-(1R,4S)-**18c**).* Following the procedure described for **18a**, starting from (−)-(1*R*,4*S*)-**17c** (70% ee, 171 mg, 0.46 mmol) and 10% Pd/C (86 mg) in methanol (35 mL) and shaking for 15 h at 25 psi afforded the (+)-(1*R*,4*S*)-**18c** as a colorless gum (106 mg, 62%, 70% ee, dr 1.2:1). Optical rotation could not be measured due to instability. The enantiopurity of (+)-(1*R*,4*S*)-**18c** was assigned on the basis of the enantiopurity of the acetoxy alcohol (−)-(1*S*,4*R*)-**14**.

*cis-9-[4-[(Methoxycarbonyl)dimethylphosphonomethoxy]cyclopentan-1-yl]adenine (**18d**).* Following the procedure described for **18a**, starting from *N*-9-**17d** (210 mg, 0.53 mmol) and 5% Pd/C (75 mg) in methanol (15 mL) and shaking for 19 h afforded **18d** as a white hygroscopic gum (169 mg, 80%, dr 1:1): IR ν_{max}/cm^{-1} (film) 3362, 3280 (NH), 3108, 2953, 2922 (CH), 1738 (C═O), 1672, 1601 (C═C), 1230 (P═O), 1108 (C−N), 1073, 1048 (C−O); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.78−1.94 (m, 1H), 2.06−2.27 (m, 3H), 2.33−2.48 (m, 1H), 2.49−2.64 (m, 1H), 3.82−3.92 (m, 9H), 4.26−4.35 (m, 1H,), 4.40 (d, J_{PH} = 19.2, 0.5H), 4.49 (d, J_{PH} = 19.8, 0.5H), 5.11−5.24 (m, 1H), 6.02 (br s, 1H), 6.05 (br s, 1H), 8.35 (s, 1.5 H), 8.46 (s, 0.5H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 30.7, 31.8, 32.0, 32.1, 39.7, 40.3, 52.4, 52.5, 53.0, 54.1 (d, J_{PC} = 6.6), 54.3 (d, J_{PC} = 6.6), 54.5 (d, J_{PC} = 6.6), 73.8 (d, J_{PC} = 160.4), 74.5 (d, J_{PC} = 159.7), 82.0, 82.8, 119.3, 139.8, 139.9, 149.9, 152.8, 155.76, 155.78, 167.7 (d, J_{PC} = 2.0), 168.0 (d, J_{PC} = 2.3); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 16.6, 16.8; HRMS (ES+): calcd for $C_{15}H_{23}N_5O_6P$ $(M + H)^+$ 400.1386, found 400.1379; MS (ES+) m/z 400.1 $(M + H)^+$.

*(−)-(1S,4R)-9-[4-[(Methoxycarbonyl)dimethylphosphonomethoxy]cyclopentan-1-yl]adenine ((−)-(1S,4R)-**18d**).* Following the procedure described for **18a**, starting from (+)-(1*R*,4*S*)-**17d** (98% ee) (158 mg, 0.40 mmol) and 5% Pd/C (75 mg) in methanol (25 mL) and shaking for 19 h afforded (−)-(1*S*,4*R*)-**18d** as a pale yellow hygroscopic gum (118 mg, 74%, 98% ee, dr 1:1): $[\alpha]_D^{20}$ − 4.33 (*c* 0.15, CH_2Cl_2). The enantiopurity of (−)-(1*S*,4*R*)-**18d** was assigned on the basis of the enantiopurity of the acetoxy alcohol (+)-(1*R*,4*S*)-**14**.

*(+)-(1R,4S)-9-[4-[(Methoxycarbonyl)diethylphosphonomethoxy]cyclopentan-1-yl]adenine ((+)-(1R,4S)-**18d**).* Following the procedure described for **18a**, starting from (−)-(1*S*,4*R*)-**17d** (70% ee) (253 mg, 0.64 mmol) and 10% Pd/C (100 mg) in methanol (20 mL) and shaking for 19 h afforded (+)-(1*R*,4*S*)-**18d** as a pale yellow hygroscopic gum (189 mg, 74%, 70% ee, dr 1.1:1): $[\alpha]_D^{20}$ + 10.00 (*c* 0.2, CH_2Cl_2). The enantiopurity of (+)-(1*R*,4*S*)-**18d** was assigned on the basis of the enantiopurity of the acetoxy alcohol (−)-(1*S*,4*R*)-**14**.

*cis-2-Amino-7-[4-[(methoxycarbonyl)dimethylphosphonomethoxy]cyclopentan-1-yl]-6-chloropurine (**18e**).* Following the procedure described for **18a**, starting from *N*-7-**17e** (161 mg, 0.28 mmol) and 10% Pd/C (54 mg) in methanol (10 mL), and shaking for 26 h at 50 psi afforded the saturated compound *N*-7-**18e** as a cream solid (21 mg, 17%, dr 1:1): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.80−2.61 (m, 6H), 3.81−3.90 (m, 9H), 4.26−4.38 (m, 1H,), 4.40 (d, J_{PC} = 19.2, 0.5H), 4.45 (d, J_{PC} = 20.1, 0.5H), 5.13 (br s,1H), 5.14 (br s, 1H), 5.31−5.44 (m, 1H), 8.57 (br s, 0.5H), 8.64 (br s, 0.5H); signals for unreacted *N*-7-**17e** were seen at 5.78−5.89 (m, 0.1H), 6.20−6.27 (m, 0.1H), 6.44−6.50 (m, 0.1H), 8.19 (s, 0.04H) and 8.20 (s, 0.06H). The product is unstable in solution and no further analysis could be obtained.

*Methyl 2-(Dimethoxyphosphoryl)-2-((3-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)cyclopentyl)oxy)acetate (**18f**).* A mixture of **17f** (61 mg, 0.155 mmol) and 5% Pd/C (30 mg) in methanol (5 mL) was stirred for 16 h under a balloon of hydrogen. The mixture was filtrated over Celite, and the cake was rinsed with MeOH. The filtrate was concentrated and the residue was purified by flash chromatography (3% MeOH/CH_2Cl_2) to afford **18f** as a white gum (59 mg, 97% dr 1:1). ν_{max}/cm^{-1} (film): 3490, 3174 (NH), 3067, 2961, 2857, 2825 (CH), 1749, 1700 (C═O), 1469, 1438, 1394, 1359, 1319 (C−H), 1265, 1109, 1032 (C−O, C−N, P−O); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.47−2.42 (m, 6H), 3.82−3.87 (m, 9H), 4.15−4.17 (m, 0.5H), 4.21−4.23 (m, 0.5H), 4.39 (d, *J* = 19.0, 0.5H), 4.45 (d, *J* = 20.3, 0.5H), 5.24−5.33 (m, 1H), 8.06 (d, *J* = 6.7, 0.5H), 8.18 (d, *J* = 6.7, 0.5H), 9.34 (m, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 30.1, 30.2, 30.5, 31.4, 38.7, 39.0, 53.1, 53.4, 53.9, 54.0, 54.2, 54.3, 73.1 (d, *J* = 160.5), 74.1 (d, *J* = 159.3), 81.9 (d, *J* = 11.4), 83.0 (d, *J* = 8.6), 126.8 (d, *J* = 34.3), 140.9 (d, *J* = 236.2), 141.0 (d, *J* = 236.1), 150.1, 156.9 (d, *J* = 26.6), 167.5 (d, *J* = 2.0), 167.9 (d, J_{PC} = 2.9); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 16.64, 16.92; ^{19}F NMR (282.4 MHz, $CDCl_3$) δ − 164.08, − 164.06; HRMS (ES+) calcd for $C_{14}H_{21}FN_2O_8P$ $(M + H)^+$ 395.1020, found 395.1013; MS (ES−) m/z 393.3 $(M - H)^-$.

Deprotection Reactions. General Procedure for Partial Deprotection. A solution of the protected compound **18** (1 equiv) in CH_2Cl_2 or acetonitrile was treated with TMSBr (5 equiv). The mixture was flushed with nitrogen, sealed, and stirred at ambient temperature overnight, or irradiated (50 °C, 50 W) for 15 min, after which water (1 mL) was added, and stirring was continued for 30 min. The mixture adjusted to pH 7 with 10% NaOH, the solvents were removed under reduced pressure (bath temp <30 °C), and the residue was purified by charcoal chromatography, eluting with 10:10:3 EtOH/H_2O/concd NH_4OH.

General Procedure for Full Deprotection. A solution of the protected compound **18** (1 equiv) in CH_2Cl_2 or acetonitrile was treated with TMSBr (5 equiv). The mixture was flushed with nitrogen, stirred at ambient temperature overnight, or sealed and irradiated (50 °C, 50 W) for 15 min, after which water (1 mL) was added and stirring was continued for 30 min. The volatiles were removed, and 1 M NaOH (10 equiv) was added. The resulting solution was stirred overnight at room temperature, then concentrated, and the residue was purified by charcoal chromatography, eluting with 9:1 to 4:1 H_2O/conc. NH_4OH.

*cis-1-[4-[Methoxycarbonyl(phosphono)methoxy]cyclopentan-1-yl]thymine (**19a**).* This was prepared following the general procedure for partial deprotection, starting from **18a** (93 mg, 0.25 mmol) and TMSBr (0.17 mL, 191 mg, 1.25 mmol) CH_2Cl_2, and the reaction mixture was stirred overnight. Purification by charcoal chromatography afforded **19a** as the ammonium salt (77 mg, 82%, dr 1:1): ^{1}H NMR (300 MHz, D_2O) δ 1.51−2.04 (m, 5H,), 1.785 (s, 1.5H), 1.790 (s, 1.5H), 2.26−2.36 (m, 1H,), 3.66 (s, 1.5H), 3.67 (s, 1.5H), 3.99−4.10 (m, 1H), 4.24 (d, J_{PH} = 18.6, 0.5H), 4.27 (d, J_{PH} = 18.9, 0.5H), 4.78−4.92 (m, 1H), 7.73 (br q, *J* = 0.9, 0.5H), 7.82 (br q, *J* = 0.9, 0.5H); ^{13}C NMR (100 MHz, D_2O) δ 11.50, 11.52, 28.7, 28.9, 29.8, 30.2, 37.0, 37.1, 52.4, 54.7, 54.8, 80.6 (d, *J* = 8.4), 81.0 (d, *J* = 6.1), 111.2, 140.2, 140.4, 152.5, 166.5, 168.2, 174.3, 174.5; ^{31}P NMR (121 MHz, D_2O) δ 9.1, 9.5; HRMS (ES+): calcd for $C_{13}H_{20}N_2O_8P$ $(M + H)^+$ 363.0957, found 363.0947; MS (ES+) m/z 363.1 $(M + H)^+$.

*cis-1-[4-[Carboxy(phosphono)methoxy]cyclopentan-1-yl]thymine (**11a**).* This was prepared following the general procedure for full deprotection, starting from **18a** (133 mg, 0.34 mmol) and TMSBr (260 mg, 0.22 mL, 1.7 mmol) in CH_2Cl_2 (20 mL) at 0 °C, stirred overnight at room temperature, followed by addition of water (1 mL) and NaOH (1 M, 3.5 mL, ∼3.5 mmol, 10 equiv). Purification by charcoal chromatography gave the fully deprotected phosphonate **11a** as the ammonium salt (56 mg, 45%, dr 1:1): mp 228−230 °C; IR ν_{max}/cm^{-1} (KBr) 3152 (NH), 3025 (CH), 1691 (C═O), 1405, 1273 (P═O), 1058; ^{1}H NMR (600 MHz, D_2O) δ 1.53−1.62 (m, 0.5H), 1.62−1.75 (m, 2.5H), 1.76 (s, 3H), 1.80−1.98 (m, 2H), 2.24−2.31 (m, 1H), 3.92 (d, PCH, J_{PC} = 18.6, 0.5H), 3.95−4.02 (m, 1.5H), 4.73−4.84 (m, 1H), 7.70 (s, 0.5H), 7.72 (s, 0.5H); ^{13}C NMR (150.9 MHz, D_2O) δ 11.4, 11.5, 29.1, 29.2, 29.5, 30.6, 36.6, 37.5, 54.6, 54.8, 77.5 (d,

DOI: 10.1021/jo502549y
J. Org. Chem. 2015, 80, 2479−2493

J_{PC} = 143.5), 78.2 (d, J_{PC} = 143.4), 79.9 (d, J_{PC} = 11.2), 80.6 (d, J_{PC} = 10.9), 111.3, 111.4, 140.3, 140.5, 152.57, 152.59, 166.6, 176.3, 176.5; ^{31}P NMR (121.5 MHz, D_2O) δ 12.4, 12.6; HRMS (ES+) calcd for $C_{12}H_{18}N_2O_8P$ $(M + H)^+$ 349.0801, found 349.0804; MS (ES+) *m/z* 349 $(M + H)^+$.

*(+)-(1S,4R)-1-[4-[Carboxy(phosphono)methoxy]cyclopentan-1-yl]thymine ((+)-(1S,4R)-**11a**).* This was prepared following the general procedure for full deprotection, starting from (−)-(1*S*,4*R*)-**18a** (98% ee) (187 mg, 0.48 mmol) and TMSBr (371 mg, 0.32 mL, 2.42 mmol), in CH_2Cl_2 (20 mL), stirred overnight followed by addition of (0.3 mL) and NaOH (1 M, 5 mL, ∼5.0 mmol, 10 equiv) overnight at 50 °C. Purification by charcoal chromatography gave (+)-(1*S*,4*R*)-**11** as the ammonium salt (81 mg, 46%, 98% ee, dr 1:1): mp 225−227 °C; IR ν_{max}/cm^{-1} (KBr) 3204 (NH), 3025 (CH), 1691 (C═O), 1588 (C═C), 1433 (CH), 1273 (P═O), 1157 (C−N), 1057 (C−O); ^{1}H NMR (300 MHz, D_2O) δ 1.65−2.17 (m, 5H), 1.93 (s, 3H), 2.36−2.50 (m, 1H), 4.04−4.21 (m, 2H), 4.87−5.05 (m, 1H), 7.89 (br s, 1H). The enantiopurity of (+)-(1*S*,4*R*)-**11a** was assigned on the basis of the enantiopurities of the acetoxy alcohol (+)-(1*R*,4*S*)-**14** and of the saturated product (−)-(1*S*,4*R*)-**18a**.

*(−)-(1R,4S)-1-[4-[Carboxy(phosphono)methoxy]cyclopentan-1-yl]thymine ((−)-(1R,4S)-**11a**).* This was prepared following the general procedure for full deprotection, starting from (+)-(1*R*,4*S*)-**18a** (99% ee) (322 mg, 0.83 mmol) and TMSBr (632 mg, 0.55 mL, 4.13 mmol), in CH_2Cl_2 (55 mL) followed by addition of (0.5 mL) and NaOH (1 M, 8.3 mL, ∼8.3 mmol, 10 equiv). Purification by charcoal column chromatography gave (−)-(1*R*,4*S*)-**11a** as the ammonium salt (151 mg, 50%, 99% ee, dr 1:1): mp 229−230 °C; $[\alpha]_D^{20}$ − 2.67 (*c* = 0.52, H_2O). The enantiopurity of (−)-(1*R*,4*S*)-**11a** was assigned on the basis of the enantiopurity of the acetoxy alcohol (−)-(1*S*,4*R*)-**14**.

*cis-1-[4-[Carboxy(phosphono)methoxy]cyclopentan-1-yl]uracil (**11b**).* This was prepared following the general procedure for full deprotection, starting from **18b** (103 mg, 0.27 mmol) and TMSBr (209 mg, 0.18 mL, 1.37 mmol) in CH_2Cl_2 (20 mL) at 0 °C, and stirred overnight at room temperature, followed by addition of (0.1 mL) and NaOH (1 M, 5 mL, ∼5.0 mmol, 18 equiv) and stirred at 50 °C. The crude residue was purified by charcoal chromatography to give **11b** as the ammonium salt (55 mg, 58%, dr 1.1:1): IR ν_{max}/cm^{-1} (KBr) 3201 (OH), 3052 (CH), 1686 (C═O), 1273 (P═O), 1152 (C−O), 1062 (C−N); ^{1}H NMR (600 MHz, D_2O) δ 1.51−1.60 (m, 0.5H), 1.60−177 (m, 2.5H), 1.82−1.93 (m, 1H), 1.94−2.02 (m, 1H), 2.21−2.31 (m, 1H), 3.90 (d, J_{PC} = 18.6, 0.5H), 3.91−4.03 (m, 1.5H), 4.80−4.88 (m, 1H), 5.74 (br d, $J \approx$ 7.8, 0.5H), 5.75 (br d, $J \approx$ 7.2, 0.5H), 7.99 (br d, $J \approx$ 7.8, 1H); ^{13}C NMR (150.9 MHz, D_2O) δ 29.5, 29.7, 31.1, 36.5, 37.7, 54.9, 55.1, 77.5 (br d, $J_{PC} \approx$ 144.0)*, 78.1 (br d, $J_{PC} \approx$ 143.5)*, 80.0 (d, J_{PC} = 11.3, 80.7 (d, J_{PC} = 11.5), 102.1, 102.2, 145.35, 145.38, 152.6, 152.7, 166.5, 176.4, 176.6; ^{31}P NMR (161.9 MHz, D_2O) δ 12.17, 12.23; HRMS (ES+) calcd for $C_{11}H_{16}N_2O_8P$ $(M + H)^+$ 335.0644, found 335.0628; MS (ES+) *m/z* 333 $(M + H)^+$.

*(1S,4R)-1-[4-[Methoxycarbonyl(phosphono)methoxy]cyclopentan-1-yl]uracil ((1S,4R)-**19b**).* This was prepared following the general procedure for partial deprotection, starting from (−)-(1*S*,4*R*)-**18b** (98% ee) (147 mg, 0.39 mmol) and TMSBr (302 mg, 0.26 mL, 1.97 mmol) in CH_2Cl_2 (20 mL) overnight at room temperature, followed by addition of (0.2 mL), to give (1*S*,4*R*)-**19b** (131 mg, 96% yield, 98% ee, dr 1:1): ^{1}H NMR (300 MHz, D_2O) δ 1.49−2.11 (m, 5H), 2.22−2.38 (m, 1H), 3.66 (s, 1.5H), 3.69 (s, 1.5H), 4.03−4.13 (m, 1H), 4.36 (d, J_{PC} = 19.5, 0.5H), 4.40 (d, J_{PC} = 19.5, 0.5H), 4.80−4.96 (m, 1H [partially obscured by water]), 5.76 (d, *J* = 7.8, 1H), 7.90 (br d, J ∼ 8.1, 0.5H), 7.95 (br d, J ∼ 8.1, 0.5H); ^{13}C NMR (75.5 MHz, D_2O) δ 29.1, 29.3, 29.6, 30.8, 36.8, 37.5, 48.7, 54.8, 73.4 (J_{PC} = 151.0), 75.4 (d, J_{PC} = 151.4), 81.7 (d, J_{PC} = 10.5), 82.4 (d, J_{PC} = 10.1), 101.8, 144.65, 144.7, 152.3, 166.2, 170.9 (br d, $J_{PC} \approx$ 2.2), 171.0 (br d, $J_{PC} \approx$ 2.2); ^{31}P NMR (121.5 MHz, D_2O) δ 11.78, 11.96; HRMS (ES+) calcd for $C_{12}H_{18}N_2O_8P$ $(M + H)^+$ 349.0801, found 349.0790; MS (ES+) *m/z* 349 $(M + H)^+$.

*(+)-(1S,4R)-1-[4-[Carboxy(phosphono)methoxy]cyclopentan-1-yl]uracil ((+)-(1S,4R)-**11b**).* This was prepared from crude (+)-(1*S*,4*R*)-**19b** (98% ee) (131 mg, 0.38 mmol, isolated from the experiment above) in water (10 mL) and NaOH (1 M, 5 mL, ∼5 mmol, 18 equiv) and stirring at 50 °C overnight. The crude residue was purified by charcoal chromatography to afford (+)-(1*S*,4*R*)-**11b** as the ammonium salt (63 mg, 48%, 98% ee, dr 1:1): $[\alpha]_D^{20}$ + 8.30 (*c* 0.24, H_2O). The enantiopurity of (+)-(1*S*,4*R*)-**11b** was assigned on the basis of the enantiopurities of the acetoxy alcohol (+)-(1*R*,4*S*)-**14**, the phosphonucleoside (+)-(1*R*,4*S*)-**17b**, and the saturated product (−)-(1*S*,4*R*)-**18b**.

*(−)-(1R,4S)-1-[4-[Carboxy(phosphono)methoxy]cyclopentan-1-yl]uracil ((−)-(1R,4S)-**11b**).* This was prepared following the general procedure for full deprotection, starting from (+)-(1*R*,4*S*)-**18b** (30% ee) (183 mg, 0.49 mmol) and TMSBr (348 mg, 0.30 mL, 2.27 mmol) in CH_2Cl_2 (20 mL) overnight at room temperature, followed by addition of (0.2 mL) and NaOH (1 M, 4.9 mL, ∼4.90 mmol, 10 equiv) at 50 °C overnight. The crude material was purified by charcoal chromatography to afford (−)-(1*R*,4*S*)-**11b** as the ammonium salt (81 mg, 45%, 30% ee, dr 1.1:1): $[\alpha]_D^{20}$ − 1.23 (*c* 0.29, H_2O). The enantiopurity of (−)-(1*R*,4*S*)-**11b** was assigned on the basis of the enantiopurities of the acetoxy alcohol (−)-(1*S*,4*R*)-**14**, the phosphononucleoside (−)-(1*S*,4*R*)-**17b**, and of the saturated product (+)-(1*R*,4*S*)-**18b**.

*cis-1-[4-[Methoxycarbonyl(phosphono)methoxy]cyclopentan-1-yl]cytosine (**19c**).* This was prepared following the general procedure for partial deprotection, starting from **18c** (95 mg, 0.25 mmol) and TMSBr (0.23 mL, 1.70 mmol, 7 equiv) in CH_2Cl_2 (10 mL) at 40 °C for 8 h, followed by addition of (0.1 mL), to give **19c** (88 mg, 99%, dr 1:1): ^{1}H NMR (500 MHz, D_2O) δ 1.59−2.18 (m, 5H), 2.32−2.48 (m, 1H), 3.72 (s, 3H), 4.09−4.16 (m, 1H), 4.27−4.48 (m, 1H,), 4.98−5.07 (m, 1H), 6.14−6.16 (d, *J* = 7.5, 1H), 8.23 (d, *J* = 8.0, 0.5H), 8.29 (d, *J* = 7.5, 0.5H); ^{13}C NMR (125.7 MHz, D_2O) δ 29.6, 29.7, 29.95, 31.0, 37.2, 38.1, 52.9, 56.1, 56.3, 74.8 (d, J_{PC} = 166.0), 75.9 (d, J_{PC} = 166.0), 81.7 (d, J_{PC} = 10.1), 81.4 (d, J_{PC} = 10.1), 94.9, 95.0, 147.8, 147.9, 149.5, 158.7, 171.9, 172.2; ^{31}P NMR (121.5 MHz, D_2O) δ 9.82, 10.13; HRMS (ES+) calcd for $C_{12}H_{19}N_3O_7P$ $(M + H)^+$ 348.0961, found 348.0953; MS (ES+) *m/z* 348 $(M + H)^+$.

*cis-1-[4-[Carboxy(phosphono)methoxy]cyclopentan-1-yl]-cytosine (**11c**).* This was prepared from crude **19c** (88 mg, 0.25 mmol isolated from the experiment above) in water (10 mL) and NaOH (1 M, 2.5 mL, ∼2.5 mmol, 10 equiv), stirring at 40 °C for 28 h. The crude material was purified by charcoal chromatography to afford the fully deprotected phosphonate **11c** (58 mg, 66%, dr 1:1): mp >250 °C; IR ν_{max}/cm^{-1} (KBr) 3432 (br, NH), 2966 (CH), 1723 (C═O), 1650, 1595 (C═C), 1490, 1399, 1286 (P═O), 1172 (C−N), 1087 (C−O); ^{1}H NMR (600 MHz, D_2O) δ 1.52−1.60 (m, 0.5H), 1.60−1.72 (m, 2.5H), 1.81−1.92 (m, 1H), 1.93−2.01 (m, 1H), 2.19−2.31 (m, 1H), 3.88 (d, J_{PH} = 18.0, 0.5H), 3.91−3.95 (m, 0.5H), 3.92 (d, J_{PH} = 18.6, 0.5H), 3.96−4.00 (m, 0.5H), 4.82−4.90 (m, 1H), 5.915 (d, *J* = 7.2, 0.5H), 5.923 (d, *J* = 7.8, 0.5H), 7.96 (d, *J* = 7.2, 0.5H), 7.97 (d, *J* = 7.2, 0.5H); ^{13}C NMR (150 MHz, D_2O) δ 29.4, 30.0, 30.3, 31.1, 36.6, 37.9, 55.3, 55.6, 77.7 (d, J_{PC} = 142.1,), 78.4 (d, J_{PC} = 141.4, 79.8 (d, J_{PC} = 11.5), 80.5 (d, J_{PC} = 11.8), 96.30, 96.34, 144.7, 144.8, 158.42, 158.45, 165.35, 165.37, 176.8, 177.1; ^{31}P NMR (121.5 MHz, D_2O) δ 11.3, 11.5; HRMS (ES+) calcd for $C_{11}H_{17}N_3O_7P$ $(M + H)^+$ 334.0804, found 334.0802; MS (ES−) *m/z* 332.0 $(M - H)^-$.

*(+)-(1S,4R)-1-[4-[Carboxy(phosphono)methoxy]cyclopentan-1-yl]cytosine ((+)-(1S,4R)-**11c**).* This was prepared following the general procedure for full deprotection, starting from (−)-(1*S*,4*R*)-**18c** (98% ee) (98 mg, 0.26 mmol) and TMSBr (0.18 mL, 1.4 mmol, 5 equiv), in CH_2Cl_2 (15 mL), followed by addition of NaOH (1 M, 2.6 mL, ∼2.6 mmol, 10 equiv) at 50 °C for 18 h. The crude material was purified by charcoal chromatography and lyophilized to give (+)-(1*S*,4*R*)-**11c** as a fine cream solid (51 mg, 56% yield, 98% ee, dr 1.1:1): mp >250 °C.

*(−)-(1R,4S)-1-[4-[Carboxy(phosphono)methoxy]cyclopentan-1-yl]cytosine ((−)-(1R,4S)-**11c**).* This was prepared following the general procedure for full deprotection, starting from (+)-(1*R*,4*S*)-**18c** (70% ee) (67 mg, 0.18 mmol) and TMSBr (0.12 mL, 0.9 mmol, 5 equiv) in CH_2Cl_2 (15 mL), followed by addition of NaOH (1 M, 1.8 mL, ∼1.8 mmol, 10 equiv) at 50 °C for 18 h. The crude material was purified by charcoal chromatography afford (−)-(1*R*,4*S*)-**11c** as a fine cream solid (26 mg, 41%, 70% ee, dr 1.1:1): mp >250 °C. The enantiopurity of

DOI: 10.1021/jo502549y
J. Org. Chem. 2015, 80, 2479−2493

(−)-(1*R*,4*S*)-**11c** was assigned on the basis of the enantiopurity of the acetoxy alcohol (−)-(1*S*,4*R*)-**14**.

*cis-9-[4-[Methoxycarbonyl(phosphono)methoxy]cyclopentan-1-yl]adenine (**19d**).* This was prepared following the general procedure for partial deprotection, starting from **18d** (45 mg, 0.11 mmol) and TMSBr (0.08 mL, 0.60 mmol) in CH_2Cl_2 (15 mL) at 40 °C for 9 h, followed by addition of (0.1 mL), to give **19d** (38 mg, 93%, dr 1:1). ^{1}H NMR (300 MHz, D_2O) δ 1.77−2.26 (m, 4H), 2.20−2.37 (m, 1H), 2.41−2.55 (m, 1H), 3.67 (s, 1.5H), 3.70 (s, 1.5H), 4.16−4.27 (m, 1H), 4.37 (d, J_{PC} = 18.9, 0.5H), 4.42 (d, J_{PC} = 19.2, 0.5H), 4.94−5.08 (m, 1H), 8.32 (s, 1H), 8.63 (s, 0.5H), 8.71 (s, 0.4H); ^{13}C NMR (125 MHz, CD_3OD) δ 30.4, 31.0, 31.3, 31.4, 39.4, 39.5, 51.7, 51.8, 54.2, 74.9 (d, J_{PC} = 156.0), 75.2 (d, J_{PC} = 155.1), 82.3 (d, J_{PC} = 11.4), 82.9 (d, J_{PC} = 8.7), 117.5, 117.6, 143.6, 143.7, 143.8, 148.7, 150.0, 169.2, 169.5; ^{31}P NMR (162 MHz, CD_3OD) δ 12.5, 12.9; HRMS (ES+) calcd for $C_{13}H_{18}N_5O_6P$ $(M + H)^+$ 372.1073, found 372.1063; MS (ES+) m/z 372.1 $[M + H]^+$.

*cis-9-[4-[Carboxy(phosphono)methoxy]cyclopentan-1-yl]-adenine (**11d**).* This was prepared following the general procedure for full deprotection, starting from **18d** (125 mg, 0.31 mmol) and TMSBr (240 mg, 0.21 mL, 1.57 mmol) in CH_2Cl_2 (15 mL) under reflux for 7 h, followed by addition of (0.1 mL) and NaOH (1 M, 3.1 mL, 3.10 mmol, 10 equiv) at 50 °C overnight. Purification by charcoal chromatography gave **11d** as the ammonium salt (86 mg, 71%, dr 1.2:1): mp 236−240 °C; ν_{max}/cm^{-1} (KBr) 3342 (NH), 3198, 2961 (CH), 1603 (C═O), 1396, 1176 (C−N), 1071 (C−O); ^{1}H NMR (600 MHz, D_2O) δ 1.86−2.23 (m, 5H), 2.55−2.64 (m, 1H), 3.91 (d, J_{PC} = 16.8, 0.55H), 3.96 (d, J_{PC} = 16.8, 0.45H), 4.09−4.17 (m, 1H), 4.75−4.83 (m [partially obscured by water], 1H), 8.11 (s, 0.45H,), 8.12 (s, 0.55H), 8.45 (s, 0.45H), 8.50 (s, 0.55H); ^{13}C NMR (150 MHz, D_2O) δ 29.3, 30.5, 30.6, 30.7, 37.5, 38.6, 53.6, 53.8, 77.4 (d, J_{PC} = 145.9), 78.1 (d, J_{PC} = 148.8), 79.9 (d, J_{PC} = 10.3), 80.5 (d, J_{PC} = 10.7), 117.8, 117.9, 141.11, 141.14, 148.2, 151.2, 154.5, 154.6, 176.6, 176.8; ^{31}P NMR (121.5 MHz, D_2O) δ 11.7, 11.8; HRMS (ES+) calcd for $C_{12}H_{17}N_5O_6P$ $(M + H)^+$ 358.0916, found 358.0898; MS (ES−) m/z 356.1 $(M - H)^-$. A sample of **11d** checked after 1 year showed ∼10% degradation.

*(+)-(1S,4R)-9-[4-[Carboxy(phosphono)methoxy]cyclopentan-1-yl]adenine ((+)-(1S,4R)-**11d**).* This was prepared following the general procedure for full deprotection, starting from (−)-(1*S*,4*R*)-**18d** (98% ee) (114 mg, 0.29 mmol) and TMSBr (218 mg, 0.19 mL, 1.43 mmol) in CH_2Cl_2 (15 mL), followed by addition of (0.1 mL) and NaOH (1 M, 2.9 mL, ∼2.90 mmol, 10 equiv) at 50 °C. The crude material was purified by charcoal chromatography to afford (+)-(1*S*,4*R*)-**11d** as the ammonium salt (88 mg, 78%, 98% ee, dr 1.1:1): mp 234−239 °C; $[\alpha]_D^{20}$ + 13.50 (*c* 0.2, CH_2Cl_2) The enantiopurity of (+)-(1*S*,4*R*)-**11d** was assigned on the basis of the enantiopurities of the acetoxy alcohol (+)-(1*R*,4*S*)-**14**.

*(−)-(1R,4S)-9-[4-[Carboxy(phosphono)methoxy]cyclopentan-1-yl]adenine ((−)-(1R,4S)-**11d**).* This was prepared following the general procedure for full deprotection, starting from (−)-(1*R*,4*S*)-**18d** (70% ee) (122 mg, 0.31 mmol) and TMSBr (239 mg, 0.21 mL, 1.55 mmol) in CH_2Cl_2 (15 mL) at 40 °C for 9 h, followed by addition of (0.1 mL) and NaOH (1 M, 3.0 mL, ∼3.0 mmol, 10 equiv) overnight at 50 °C. The crude material was purified by charcoal chromatography to afford (−)-(1*R*,4*S*)-**11d** as the ammonium salt (59 mg, 59%, 70% ee, dr 1.1:1): mp 237−241 °C; $[\alpha]_D^{20}$ − 6.70 (*c* 1.00, CH_2Cl_2). The enantiopurity of (−)-(1*R*,4*S*)-**11d** was assigned on the basis of the enantiopurity of the acetoxy alcohol (−)-(1*S*,4*R*)-**14**.

*2-((3-(5-Fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)cyclopentyl)oxy)-2-phosphonoacetic Acid (**11f**).* This was prepared following the general procedure for full deprotection, starting from **18f** (51 mg, 0.129 mmol) and TMSBr (79 mg, 67 μL, 0.517 mmol) in acetonitrile (3 mL) and irradiated (50 W, 50 °C) for 10 min, followed by 50% aq MeOH (0.2 mL) and LiOH (31 mg, 1.29 mmol) in water (3 mL) for 2 h at 50 °C. The crude material was purified by charcoal chromatography to afford **11f** as the ammonium salt (25 mg, 52%): mp 243 °C; ^{1}H NMR (300 MHz, D_2O) δ 1.69−2.17 (m, 5H), 2.40−2.51 (m, 1H), 3.99−4.14 (m, 2H), 4.93−5.01 (m, 1H), 8.33 (d, *J* = 8.4, 0.5H), 8.35 (d, *J* = 8.8, 0.5H); ^{13}C NMR (150 MHz, D_2O) δ 29.3, 29.35, 29.4, 36.6, 37.5, 55.4, 55.6, 78.4 (d, *J* = 139.8), 78.9 (d, *J* = 139.3), 79.7 (d, *J* = 10.6), 80.3 (d, *J* = 10.2), 128.7, 128.9, 140.2, 141.7, 152.0, 160.5, 160.7, 177.6, 178.0; ^{31}P NMR (121.5 MHz, D_2O) δ 12.09, 12.32; ^{19}F NMR (282.4 MHz, D_2O) δ − 165.89, − 165.86; HRMS (ES+) calcd for $C_{11}H_{15}FN_2O_8P$ $(M + H)^+$ 353.0550, found 353.0535; calcd for $C_{11}H_{14}DFN_2O_8P$ $(M + H)^+$ 354.0613, found 354.0597; MS (ES−) m/z 351.2 $(M - H)^-$. Some indication of D-exchange visible in ^{1}H and ^{19}F NMR spectra.

Unsaturated Derivatives. *cis-1-[4-[(Methoxycarbonyl)-phosphonomethoxy]cyclopent-2-en-1-yl]thymine (**20a**).* A solution of **17a** (0.23 g, 0.6 mmol) and 2,6-lutidine (284 μL, 0.26 g, 2.4 mmol) in acetonitrile (3 mL) was treated with TMSBr (325 μL, 0.38 g, 2.4 mmol), and the resulting mixture was irradiated (50 W, 50 °C) for 10 min. The reaction was quenched by the addition of MeOH/H_2O (95:5) and the mixture concentrated under reduced pressure. The residue was purified by charcoal chromatography to provide an amber glass (0.21 g) which was crystallized from methanol/ether to afford **20a** as a cream solid (0.17 g, 78%): mp 198−205 °C; ^{1}H NMR (400 MHz, D_2O) δ 1.51−1.64 (m, 1H), 1.76 (s, 3H), 2.69−2.86 (m, 1H), 3.64 (s, 1.5H), 3.68 (s, 1.5H), 4.28 (d, J_{PH} = 18.5, 0.5H), 4.29 (d, J_{PH} = 17.9, 0.5H), 4.52−4.59 (m, 0.5H), 4.59−4.66 (m, 0.5H), 5.28−5.40 (m, 1H), 5.79−5.89 (m, 1H), 6.13−6.30 (m, 1H,), 7.39 (s, 0.5H), 7.44 (s, 0.5H); ^{13}C NMR (150 MHz, CD_3OD) δ 12.4, 37.8, 37.9, 52.5, 59.6, 59.8, 78.7 (d, J_{PC} = 140.3), 79.0 (d, J_{PC} = 141.8), 85.2 (d, J_{PC} = 10.6), 85.4 (d, J_{PC} = 9.0), 111.9, 112.0, 134.1, 134.4, 138.0, 139.9, 140.0, 152.9, 153.0, 166.6, 166.7, 172.8, 173.0; ^{31}P NMR (202 MHz, CD_3OD) δ 8.6, br; HRMS (ES+) calcd for $C_{13}H_{18}N_2O_8P$ $(M + H)^+$ 361.0801, found 361.0795; MS (ES+) m/z 383.3 $(M + Na)^+$

*cis-1-[4-(Carboxyphosphonomethoxy)cyclopent-2-en-1-yl]-thymine (**12a**).* A solution of **20a** (113 mg, 0.3 mmol) and LiOH (63 mg, 2.6 mmol) in water (2.5 mL was stirred at 60 °C for 4.25 h. The mixture was concentrated and purified by charcoal chromatography to afford **12a** as a white solid (84 mg, 78%): mp 236−239 °C; ^{1}H NMR (400 MHz, D_2O) δ 1.50−1.68 (m, 1H), 1.76 (s, 3H), 2.67−2.90 (m, 1H), 3.78−4.10 (m, 1H), 4.44−4.66 (m, 1H), 5.27−5.38 (m, 1H), 5.72−5.82 (m, 1H), 6.14−6.33 (m, 1H), 7.44 (s, 0.5H), 7.46 (s, 0.5H); ^{13}C NMR (150 MHz, D_2O) δ 11.3, 36.6, 36.8, 58.9, 59.0, 78.4 (br), 83.4, 111.3, 132.1, 132.2, 136.7, 137.2, 139.8, 139.9, 152.3, 166.8, 177.1; ^{31}P NMR (202 MHz, D_2O) δ 12.3 br; HRMS (ES+) calcd for $C_{12}H_{16}N_2O_8P$ $(M + H)^+$ 347.0644, found 347.0653; MS (ES+) m/z 347.3 $(M + H)^+$.

*2-((4-(5-Fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-cyclopent-2-en-1-yl)oxy)-2-phosphonoacetic Acid (**12f**).* A solution of **17f** (94 mg, 0.240 mmol) and 2,6-lutidine (103 mg, 112 μL, 0.960 mmol) in acetonitrile (3 mL) was treated with TMSBr (147 mg, 124 μL, 0.960 mmol). The resulting solution was irradiated (50 W, 50 °C) for 10 min. Water (0.3 mL) and methanol (0.3 mL) were added, and the mixture was stirred for 20 min at room temperature. The mixture was concentrated, and the residue was stirred with lithium hydroxide (57 mg, 2.40 mmol) in water (5 mL) for 1 h at 50 °C and then concentrated again. The crude material was purified by charcoal chromatography to afford **12f** as the ammonium salt (71 mg, 0.193 mmol, 81%, 1:1 dr): ^{1}H NMR (300 MHz, D_2O) δ 1.74−1.82 (m, 1H), 2.87−2.97 (m, 1H), 4.14 (d, *J* = 18.5, 0.50H), 4.17 (d, *J* = 17.9, 0.5H), 4.66−4.78 (m, 1H), 5.49−5.55 (m, 1H), 5.96−6.00 (m, 1H), 6.40 (d, *J* = 5.6, 0.5H), 6.46 (d, *J* = 5.5, 0.5H), 7.98 (d, *J* = 6.5, 0.5H), 7.99 (d, *J* = 6.4,0.5H); ^{13}C NMR (75.5 MHz, D_2O) δ 36.2, 36.5, 59.76, 59.85, 78.6 (d, *J* = 133.6), 83.2 (d, *J* = 13.2), 128.1 (d, *J* = 33.5), 128.2 (d, *J* = 33.4), 132.0, 132.1, 137.3, 137.8, 140.9 (d, *J* = 232.2), 150.9, 159.8 (d, *J* = 25.4), 176.9; ^{31}P NMR (121.5 MHz, D_2O) δ 12.08, 12.31; ^{19}F NMR (282.4 MHz, D_2O) δ − 166.20, − 166.16; HRMS (ES+) calcd for $C_{11}H_{13}FN_2O_8P$ $(M + H)^+$ 351.0394, found 351.0407; MS (ES−) m/z 349.2 $(M - H)$.

Nonphosphonate Compounds. *Benzyl 2-((4-Acetoxycyclopent-2-en-1-yl)oxy)acetate (**21**).* The allylic acetate **14** (350 mg, 2.46 mmol) was dissolved in benzene (20 mL) and purged with nitrogen. Rhodium acetate (20 mg) was added, and the mixture stirred for 5 min at room temperature. Benzyl diazoacetate (800 mg, 4.54 mmol) was added, and the reaction was refluxed overnight. The reaction mixture cooled to room temperature and filtered and the

DOI: 10.1021/jo502549y
J. Org. Chem. 2015, 80, 2479−2493

solvent removed under vacuum to afford a green residue which was purified by chromatography (SiO_2, 25% ethyl acetate/hexane) to give the desired product **21** as a clear oil (0.14 g, 19%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.71−1.79 (m, 1H), 2.00 (s, 3H), 2.71−2.81 (m, 1H), 4.14 (s,2H), 4.52−4.56 (m, 1H), 5.19 (s, 2H), 5.49−5.56 (m, 1H), 5.99−6.08 (m, 1H), 6.12−6.17 (m, 1H), 7.31−7.39 (m, 5H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 21.1, 37.0, 66.0, 66.6, 76.5, 82.6, 128.4, 128.5, 128.6, 133.7, 135.4, 135.5, 170.3, 170.8; HRMS (ES+): calcd for $C_{16}H_{18}NaO_5$ $(M + Na)^+$ 313.1052, found 313.1047; MS (ES+) m/z 313 $(M + Na)^+$.

*Benzyl 2-((4-(5-Methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-cyclopent-2-en-1-yl)oxy)acetate (**22**).* A microwave vial containing a degassed suspension of thymine (63 mg, 0.50 mmol) and sodium carbonate (38 mg, 0.35 mmol) in water (1 mL) and acetonitrile (1 mL) was heated under microwave conditions (50 °C, 200 W) for 30 min. A degassed solution of **21** (140 mg, 0.48 mmol) in acetonitrile (1 mL), $Pd(dba)_2$ (10 mg, 4 mol %), and 1,4-bis(diphenylphosphino)-butane (dppb) (14 mg, 7 mol %) was added to the vial. The resulting solution was irradiated (50 °C, 200 W) for 30 min whereupon a second portion of $Pd(dba)_2$ (10 mg) and dppb (14 mg) was added followed by irradiation (50 °C, 200 W) for a further 30 min. The reaction mixture was cooled to room temperature, gravity filtered, and concentrated under vacuum to give a purple residue which was purified by chromatography (SiO_2, 5% methanol/dichloromethane) to afford compound **22** as an oil (36 mg, 21%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.66−1.73 (m, 1H), 1.88 (s, 3H), 2.76−2.86 (m, 1H), 4.21 (s,2H), 4.52−4.55 (m, 1H), 5.21 (s, 2H), 5.61−5.66 (m, 1H), 5.86−5.89 (m, 1H), 6.28−6.31 (m, 1H), 7.21 (s, 1H), 7.33−7.38 (m, 5H), 9.01 (bs, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 12.4. 37.3, 57.9, 66.8, 67.1, 83.2, 111.5, 128.5, 128.6, 128.7, 133.5, 135.2, 136.5, 137.1, 151.1, 163.9, 170.0; HRMS (ES+) calcd for $C_{19}H_{21}N_2O_5$ $(M + H)^+$ 357.1450, found 357.1436; MS (ES+) m/z 357 $(M + H)^+$.

*2-((3-(5-Methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)cyclopentyl)oxy)acetic Acid (**23**).* The base insertion product **22** (36 mg, 0.10 mmol) was dissolved in methanol (2 mL) and flushed with nitrogen. Palladium on carbon 10% (10 mg) was added and the suspension stirred under a hydrogen filled balloon at room temperature for 18 h. The reaction mixture was filtered through a pad of Celite and concentrated under vacuum to give the title product **23** as a colorless solid (22 mg, 82%): ^{1}H NMR (300 MHz, CD_3OD) δ 1.62−1.75 (m, 1H), 1.79−1.89 (m, 2H), 1.91 (s, 3H), 2.06−2.21 (m, 2H), 2.31−2.41 (m, 1H), 4.04−4.14 (m, 3H), 5.09−5.20 (m, 1H), 7.89 (s, 1H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 10.9, 29.5, 30.5, 37.4, 53.8, 65.3, 80.2, 110.6, 139.0,151.8, 165.0, 172.7; HRMS (ES+) calcd for $C_{12}H_{17}N_2O_5$ $(M + H)^+$ 269.1137, found 269.1135; MS (ES+) m/z 269 $(M + H)^+$.

Oxygenated Derivatives. *cis-1-[4-[Dimethyl(methoxycarbonyl)-phosphonomethoxy]-2,3-dihydroxycyclopentan-1-yl]thymine (**24**).* A suspension of **17a** (45 mg, 0.12 mmol) in THF (3 mL) was treated with 4% aq osmium tetraoxide (810 μL, 810 mg, 0.13 mmol). The resulting solution was stirred for 24 h, quenched with 5% $Na_2S_2O_5$, concentrated under reduced pressure, and purified by flash chromatography (10% $MeOH/CH_2Cl_2$) to afford the desired product **24** as a white solid (26 mg, 53%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.67−1.82 (m, 1H), 1.90 (s, 1.5H), 1.92 (s, 1.5H), 2.53−2.82 (m, 1H), 3.76−3.92 (m, 9H), 3.94−4.09 (m, 1H), 4.12−4.27 (m, 1H), 4.29−4.41 (m, 1H), 4.43−4.76 (m, 1H), 4.62 (d, J_{PH} = 19.1, 0.5H), 4.72 (d, J_{PH} = 20.0, 0.5H), 4.86−5.12 (m, 2H), 7.46 (s, 0.5H), 7.53 (s, 0.5H), 10.33 (br s, 0.5H), 10.36 (br s, 0.5H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 12.2, 12.3, 33.2, 33.5, 53.1, 54.1−54.6 (m), 59.2, 59.3, 74.1, 74.4, 74.5 (d, J_{PC} = 159.8), 74.6 (d, J_{PC} = 158.6), 76.5, 76.6, 84.1−84.4 (m), 111.7, 111.9, 138.1, 138.2, 152.2, 152.3, 164.4, 167.6 (d, J = 1.6), 167.7 (d, J = 2.0); ^{31}P NMR (202 MHz, $CDCl_3$) δ 16.82, 16.84; HRMS (ES+) calcd for $C_{15}H_{24}N_2O_{10}P$ $(M + H)^+$ 423.1169, found 423.1165; MS (ES+) m/z 423.3 $(M + H)^+$.

*cis-1-[4-[(Carboxyl)phosphonomethoxy]-2,3-dihydroxycyclopentan-1-yl]thymine (**13**).* A 10 mL microwave tube was charged with **24** (23 mg, 0.05 mmol), TMSBr (28 μL, 32 mg, 2.3 mmol), and acetonitrile (2 mL), and the mixture was irradiated at 50 °C for 10 min. Thereafter, the reaction was quenched with $MeOH-H_2O$ (95:5) and the mixture concentrated under reduced pressure. The residue was dissolved in water (2 mL), lithium hydroxide (7 mg, 3 mmol) was added, and the solution was stirred at 60 °C for 3.5 h. After concentration under reduced pressure and acidification, the residue was purified by charcoal chromatography to afford the desired product **13** as a white solid (14 mg, 65%): ^{1}H NMR (300 MHz, D_2O) δ 1.67−1.83 (m, 1H), 1.87 (s, 3H), 2.50−2.69 (m, 1H), 3.70−3.93 (m, 1H), 3.93−4.25 (m, 2H), 4.30−4.48 (m, 1H), 4.76−4.95 (m, 1H), 7.70 (s, 0.5H), 7.71 (s, 0.5H); ^{13}C NMR (100 MHz, D_2O) δ 11.6, 31.0, 31.6, 59.5, 60.0, 73.3, 73.4, 73.5, 73.7, 82.2, 82.6, 111.7, 140.0, 140.3, 152.6, 152.7, 166.5, 166.6; ^{31}P NMR (121 MHz, D_2O) δ 11.98; HRMS (ES+) calcd for $C_{12}H_{18}N_2O_{10}P$ $(M + H)^+$ 381.0699, found 381.0692; MS m/z (ES−) 379.2 $(M - H)^-$.

*cis-1-[4-[Dimethyl(methoxycarbonyl)phosphonomethoxy]-2,3-epoxycyclopent-2-en-1-yl]thymine (**25**).* A mixture of **17a** (39 mg, 0.1 mmol), benzonitrile (258 μL, 258 mg, 2.5 mmol), and potassium carbonate (14 mg, 0.1 mmol) in methanol (3 mL) was treated dropwise over 10 min with 30% aq hydrogen peroxide (256 μL, 285 mg, 2.5 mmol). Thereafter, the reaction mixture was stirred for 3 h, quenched with water, and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated, and the residue was purified by flash chromatography (4% MeOH/EtOAc) to afford the desired epoxide **25** as a white solid (10 mg, 25%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45−1.60 (m, 1H), 1.95 (s, 1.5H), 1.96 (s, 1.5H), 2.31−2.42 (m, 1H), 3.60−3.63 (m, 1H), 3.73−3.77 (m, 1H), 3.80−3.93 (m, 9H), 4.17−4.28 (m, 1H), 4.50 (d, J_{PH} = 18.8, 0.5H), 4.59 (d, J_{PH} = 19.2, 0.5H), 4.90−4.98 (m, 1H), 7.49−7.55 (m, 1H), 8.08 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.5, 12.51, 27.8, 27.9, 51.9, 52.0, 53.07, 53.09, 53.9, 54.3−55.2 (m), 74.9 (d, J = 158.8), 75.2 (d, J = 159.2), 78.8 (d, J = 11.2), 79.0 (d, J = 10.7), 111.9, 112.0, 136.69, 136.70, 150.8, 163.4, 167.4, 167.5; ^{31}P NMR (202 MHz, $CDCl_3$) δ 15.7, 15.8; HRMS (ES+) calcd for $C_{15}H_{22}N_2O_9P$ $(M + H)^+$ 405.1063, found 405.1049; MS (ES+) m/z 405.2 $[M + H]^+$.

Antiviral Activity Assays. The compounds were evaluated against the following viruses: herpes simplex virus type 1 (HSV-1) strain KOS, thymidine kinase-deficient (TK^-) HSV-1 strain KOS resistant to ACV (ACV^r), herpes simplex virus type 2 (HSV-2) strain G, vaccinia virus Lederle strain, respiratory syncytial virus (RSV) strain Long, vesicular stomatitis virus (VSV), coxsackie virus B4, parainfluenza virus 3, influenza virus A (subtypes H1N1, H3N2), influenza virus B, Reovirus-1, Sindbis virus, Reovirus-1, Punta Toro virus, human immunodeficiency virus type 1 (HIV-1) strain III_B, and human immunodeficiency virus type 2 (HIV-2) strain ROD. The antiviral, other than anti-HIV, assays were based on inhibition of virus-induced cytopathicity in human embryonic lung fibroblasts (HEL), African green monkey cells (Vero), human epithelial cells (HeLa), or Madin-Darby canine kidney cells (MDCK). Confluent cell cultures in microtiter 96-well plates were inoculated with 100 $CCID_{50}$ of virus (1 $CCID_{50}$ being the virus dose to infect 50% of the cell cultures) in the presence of varying concentrations of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds. Antiviral activity was expressed as the EC_{50} or compound concentration required to reduce virus-induced cytopathicity by 50%.

Anti-HIV Activity Assays. Inhibition of HIV-1(III_B)- and HIV-2(ROD)-induced cytopathicity in CEM cell cultures was measured in microtiter 96-well plates containing ~3 × 10^5 CEM cells/mL infected with 100 CCID50 of HIV per milliliter and containing appropriate dilutions of the test compounds. After 4−5 days of incubation at 37 °C in a CO_2-controlled humidified atmosphere, CEM giant (syncytium) cell formation was examined microscopically. The EC_{50} (50% effective concentration) was defined as the compound concentration required to inhibit HIV-induced giant cell formation by 50%.

HIV-1 RT Assays. To prepare the template/primers for the RT experiments, 0.15 mM poly(U), poly(A), and poly(I) were mixed with an equal volume of 0.0375 mM oligo(dA), oligo(dT), and oligo(dC), respectively. The final concentrations of the templates in the RT reaction mixture were 0.015 mM. The reaction mixture (50 μL) contained 50 mM Tris.HCl (pH 7.8), 5 mM dithiothreitol, 300 mM glutathione, 500 μM EDTA, 150 mM KCl, 5 mM $MgCl_2$, 1.25 μg of

DOI: 10.1021/jo502549y
J. Org. Chem. 2015, 80, 2479−2493

bovine serum albumin, an appropriate concentration of labeled (tritiated) substrate dTTP, dCTP, or dATP (2 μCi/assay), a fixed concentration of the template/primer poly(A)·oligo(dT) (0.015 mM), poly(I).oligo(dC) (0.015 mM), and poly(U)·oligo(dA) (0.015 mM), 0.06% Triton X-100, 10 μL of inhibitor solution (containing various concentrations of the compounds), and 1 μL of the RT preparation. The reaction mixtures were incubated at 37 °C for 30 min, at which time 100 μL of yeast RNA (1 mg/mL) and 1 mL of $Na_4P_2O_7$ (0.02 M) in trichloroacetic acid (5% v/v) were added. The solutions were kept on ice for 30 min, after which the acid-insoluble material was washed and analyzed for radioactivity. The 50% inhibitory concentration (IC_{50}) of the test compounds was determined in the presence of fixed concentrations of 1.25 μM [^{3}H]dTTP, 1.75 μM [^{3}H]dATP, or 2.5 μM [^{3}H]dCTP.

■ ASSOCIATED CONTENT

Supporting Information

Copies of ^{1}H and ^{13}C NMR spectra of all products described in the Experimental Section. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: a.maguire@ucc.ie.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by Science Foundation Ireland (05/PICA/B802) and by the KU Leuven − University of Leuven (GOA 10/14 : PF-10/018). We thank Mrs. Lizette van Berckelaer for excellent technical assistance. We also thank Veronique Chastagner, Richard Donovan, and Stephen J. Plunkett for carrying out experiments during the early part of the project.

■ REFERENCES

(1) Gallo, R. C.; Montagnier, L. *New Engl. J. Med.* **2003**, *349*, 2283−2285.
(2) WHO. *UNAIDS Global Facts & Figures; CDC Worldwide Hepatitis Statistics*; WHO: Geneva, 2013.
(3) Broder, S. *Antiviral Res.* **2010**, *85*, 1−18.
(4) Vandamme, A.-M.; Van Laethem, K.; De Clercq, E. *Drugs* **1999**, *57*, 337−361.
(5) De Clercq, E. *Nat. Rev. Drug Discovery* **2007**, *6*, 1001−1018.
(6) De Clercq, E. *Curr. Opin. Pharmacol.* **2010**, *10*, 507−515.
(7) *Combination Therapy of AIDS*; De Clercq, E., Vandamme, A.-M., Eds.; Birkhauser: Basel, 2004.
(8) De Clercq, E. *Int. J. Antimicrob. Agents* **2009**, *33*, 307−320.
(9) Cihlar, T.; Ray, A. S. *Antiviral Res.* **2010**, *85*, 39−58.
(10) Warnke, D.; Barreto, J.; Temesgen, Z. *J. Clin. Pharmacol.* **2007**, *47*, 1570−1579.
(11) Balzarini, J. *Pharm. World Sci.* **1994**, *16*, 113−126.
(12) Kulik, K.; Radzikowska, E.; Kaczmarek, R.; Baraniak, J.; Stec, W. J.; De, C. E.; Balzarini, J.; Pannecouque, C. *Antiviral Chem. Chemother.* **2011**, *21*, 143−150.
(13) Goldring, A. O.; Gilbert, I. H.; Mahmood, N.; Balzarini, J. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2411−2416.
(14) Coe, D. M.; Roberts, S. M.; Storer, R. *J. Chem. Soc., Perkin Trans. 1* **1992**, 2695−2704.
(15) De Clercq, E.; Holy, A.; Rosenberg, I.; Sakuma, T.; Balzarini, J.; Maudgal, P. C. *Nature (London)* **1986**, *323*, 464−467.
(16) Cahard, D.; McGuigan, C.; Balzarini, J. *Mini Rev. Med. Chem.* **2004**, *4*, 371−381.
(17) Meier, C.; Balzarini, J. *Antiviral Res.* **2006**, *71*, 282−292.
(18) Valiaeva, N.; Beadle, J. R.; Aldern, K. A.; Trahan, J.; Hostetler, K. Y. *Antiviral Res.* **2006**, *72*, 10−19.
(19) Ray, A. S.; Hostetler, K. Y. *Antiviral Res.* **2011**, *92*, 277−291.
(20) Balzarini, J.; Holý, A.; Jindrich, J.; Naesens, L.; Snoeck, R.; Schols, D.; De Clercq, E. *Antimicrob. Agents Chemother.* **1993**, *37*, 332−338.
(21) Zhan, P.; Chen, X.; Li, D.; Fang, Z.; De Clercq, E.; Liu, X. *Med. Res. Rev.* **2013**, *33*, E1−E72.
(22) Jochmans, D.; Deval, J.; Kesteleyn, B.; Van Marck, H.; Bettens, E.; De Baere, I.; Dehertogh, P.; Ivens, T.; Van Ginderen, M.; Van Schoubroeck, B.; Ehteshami, M.; Wigerinck, P.; Götte, M.; Hertogs, K. *J. Virol.* **2006**, *80*, 12283−92.
(23) Casu, F.; Chiacchio, M. A.; Romeo, R.; Gumina, G. *Curr. Org. Chem.* **2007**, *11*, 999−1016.
(24) Jeong, L. S.; Lee, J. A. *Antiviral Chem. Chemother.* **2004**, *15*, 235−250.
(25) Rodriguez, J. B.; Comin, M. J. *Mini Rev. Med. Chem.* **2003**, *3*, 95−114.
(26) Borchardt, R. T.; Keller, B. T.; Patel-Thombre, U. *J. Biol. Chem.* **1984**, *259*, 4353−4358.
(27) Tardibono, L. P., Jr; Miller, M. J.; Balzarini, J. *Tetrahedron* **2011**, *67*, 825−829.
(28) Marce, P.; Diaz, Y.; Matheu, M. I.; Castillon, S. *Org. Lett.* **2008**, *10*, 4735−4738.
(29) Kim, C. U.; Luh, B. Y.; Martin, J. C. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 307−310.
(30) Boojamra, C. G.; Parrish, J. P.; Sperandio, D.; Gao, Y.; Petrakovsky, O. V.; Lee, S. K.; Markevitch, D. Y.; Vela, J. E.; Laflamme, G.; Chen, J. M.; Ray, A. S.; Barron, A. C.; Sparacino, M. L.; Desai, M. C.; Kim, C. U.; Cihlar, T.; Mackman, R. L. *Bioorg. Med. Chem.* **2009**, *17*, 1739−1746.
(31) Mao, J. C.; Otis, E. R.; Von Esch, A. M.; Herrin, T. R.; Fairgrieve, J. S.; Shipkowitz, N. L.; Duff, R. G. *Antimicrob. Agents Chemother.* **1985**, *27*, 197−202.
(32) McKenna, C. E.; Ye, T. G.; Levy, J. N.; Pham, P.; Wen, T.; Bongartz, J. P.; Starnes, M. C.; Cheng, Y. C. *Phosphorus, Sulfur, and Silicon and the Related Elements* **1990**, *49*−*50*, 183−186.
(33) Charvet, A.-S.; Camplo, M. F.. P.; Graciet, J.-P.; Mourier, N.; Chermann, J.-C.; Kraus, J.-L. *J. Med. Chem.* **1994**, *37*, 2216−2223.
(34) Wnuk, S. F.; Robins, M. J. *J. Am. Chem. Soc.* **1996**, *118*, 2519−2520.
(35) Králíková, Š.; Buděšínský, M.; Masojídková, M.; Rosenberg, I. *Tetrahedron Lett.* **2000**, *41*, 955−958.
(36) Romanenko, V. D.; Kukhar, V. P. *Chem. Rev.* **2006**, *106*, 3868−3935.
(37) Chen, W.; Flavin, M. T.; Filler, R.; Xu, Z.-Q. *Tetrahedron Lett.* **1996**, *37*, 8975−8978.
(38) Chen, W.; Flavin, M. T.; Filler, R.; Xu, Z.-Q. *J. Chem. Soc., Perkin Trans. 1* **1998**, 3979−3988.
(39) Chen, X.; Wiemer, A. J.; Hohl, R. J.; Wiemer, D. F. *J. Org. Chem.* **2002**, *67*, 9331−9339.
(40) Boudreau, M. A.; Vederas, J. C. *Org. Biomol. Chem.* **2007**, *5*, 627−635.
(41) Kaiser, M. M.; Jansa, P.; Dracísnky, M.; Janeba, Z. *Tetrahedron* **2012**, *68*, 4003−4012.
(42) Weaver, R.; Gilbert, I. H. *Tetrahedron* **1997**, *53*, 5537−5562.
(43) Weaver, R.; Gilbert, I. H.; Mahmood, N.; Balzarini, J. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2405−2410.
(44) Debarge, S.; Balzarini, J.; Maguire, A. R. *J. Org. Chem.* **2011**, *76*, 105−126.
(45) Hladezuk, I.; Chastagner, V.; Collins, S. G.; Plunkett, S. J.; Ford, A.; Debarge, S.; Maguire, A. R. *Tetrahedron* **2012**, *68*, 1894−1909.
(46) Coe, D. M.; Hilpert, H.; Noble, S. A.; Peel, M. R.; Roberts, S. M.; Storer, R. *J. Chem. Soc., Chem. Commun.* **1991**, 312−314.
(47) Trost, B. M.; Kuo, G. H.; Benneche, T. *J. Am. Chem. Soc.* **1988**, *110*, 621−622.
(48) Merlo, V.; Roberts, S. M.; Storer, R.; Bethell, R. C. *J. Chem. Soc., Perkin Trans. 1* **1994**, 1477−1481.

DOI: 10.1021/jo502549y
J. Org. Chem. 2015, 80, 2479−2493

(49) Amblard, F.; Nolan, S. P.; Agrofoglio, L. A. *Tetrahedron* **2005**, *61*, 7067−7080.
(50) Mackman, R. L.; Lin, K.-Y.; Boojamra, C. G.; Hui, H.; Douglas, J.; Grant, D.; Petrakovsky, O.; Prasad, V.; Ray, A. S.; Cihlar, T. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1116−1119.
(51) Ravn, J.; Thorup, N.; Nielsen, P. *J. Chem. Soc., Perkin Trans. 1* **2001**, 1855−1861.
(52) Gosselin, G. *Antiviral Drug Strategies*; De Clercq, E., Ed.; Wiley-VCH: Weinheim, 2011.
(53) Serrao, E.; Odde, S.; Ramkumar, K.; Neamati, N. *Retrovirology* **2009**, *6*, 25.
(54) (a) Schinazi, R. F.; Chu, C. K.; Peck, A.; McMillan, A.; Mathis, R.; Cannon, D.; Jeong, L. S.; Beach, J. W.; Choi, W. B.; Yeola, S.; Liotta, S. C. *Antimicrob. Agents Chemother.* **1992**, *36*, 672−676. (b) Gosselin, G.; Schinazi, R. F.; Sommadossi, J. P.; Mathé, C.; Bergogne, M. C.; Aubertin, A. M.; Kirn, A.; Imbach, J. L. *Antimicrob. Agents Chemother.* **1994**, *38*, 1292−1297.
(55) Rosenblum, L. L.; Patton, G.; Grigg, A. R.; Frater, A. J.; Cain, D.; Erlwein, O.; Hill, C. L.; Clarke, J. R.; McClure, M. O. *Antiviral Chem. Chemother.* **2001**, *12*, 91−97.
(56) (a) Crandall, J. K.; Banks, D. B.; Collyer, R. A.; Watkins, R. J.; Arrington, J. P. *J. Org. Chem.* **1968**, *33*, 423−425. (c) Deardorff, D. R.; Myles, D. C. *Organic Synthesis*; Wiley: New York, 1993; Collect. Vol. *VIII*, pp 13−15. (b) Korach, M.; Nielson, D. R.; Rideout, W. H. Dihydroxycyclopentene. *Organic Synthesis*; Wiley: New York, 1973; Collect. Vol. *V*, pp 414−418.
(57) Maas, G.; Regitz, M. *Chem. Ber.* **1976**, *109*, 2039−2063.
(58) Moody, C. J.; Sie, E. R. H. B.; Kulagowski, J. J. *Tetrahedron* **1992**, *48*, 3991−4004.

DOI: 10.1021/jo502549y
J. Org. Chem. 2015, 80, 2479−2493