Pradefovir: A Prodrug That Targets Adefovir to the Liver for the Treatment of Hepatitis B

K. Raja Reddy,*,† Michael C. Matelich,† Bheemarao G. Ugarkar,† Jorge E. Gómez-Galeno,† Jay DaRe,† Kristin Ollis,† Zhili Sun,† William Craigo,† Timothy J. Colby,‡ James M. Fujitaki,‡ Serge H. Boyer,† Paul D. van Poelje,‡ and Mark D. Erion†,‡

Departments of Chemistry and Biochemistry, Metabasis Therapeutics, Inc., 11119 North Torrey Pines Road, La Jolla, California 92037

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Adefovir dipivoxil, a marketed drug for the treatment of hepatitis B, is dosed at submaximally efficacious doses because of renal toxicity. In an effort to improve the therapeutic index of adefovir, 1-aryl-1,3-propanyl prodrugs were synthesized with the rationale that this selectively liver-activated prodrug class would enhance liver levels of the active metabolite adefovir diphosphate (ADV-DP) and/or decrease kidney exposure. The lead prodrug (**14**, MB06866, pradefovir), identified from a variety of in vitro and in vivo assays, exhibited good oral bioavailability ($F = 42\%$, mesylate salt, rat) and rate of prodrug conversion to ADV-DP. Tissue distribution studies in the rat using radiolabeled materials showed that cyclic 1-aryl-1,3-propanyl prodrugs enhance the delivery of adefovir and its metabolites to the liver, with pradefovir exhibiting a 12-fold improvement in the liver/kidney ratio over adefovir dipivoxil.

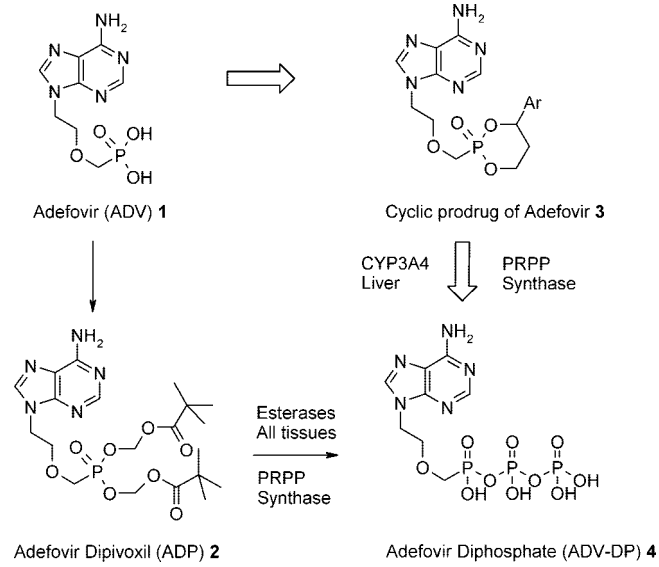
Introduction

More than 2 billion people worldwide are infected with the hepatitis B virus (HBV), resulting in 500 000 to 1.2 million deaths each year (10th leading cause of death worldwide).[1] While several drugs are currently available for the treatment of HBV,[2–5] none are optimal, necessitating the need for new therapies that are safe and well tolerated, show minimal cross-resistance, and are associated with low rates of viral resistance.

Adefovir dipivoxil (ADP[a]) (**2**, Scheme 1), a prodrug of adefovir (ADV, **1**, 9-(2-phosphonomethoxyethyl)adenine; PMEA), is an effective hepatitis B drug associated with a low rate of viral resistance.[4] In HBV-infected patients, ADP lowered serum HBV DNA levels by 3.5 log at a dose of 10 mg/day and 4.7 log at 30 mg/day after 48 weeks of treatment.[4a] Results from the study indicated significant improvements in liver histology scores and enhanced rates of seroconversion compared to placebo-treated patients, with no evidence of viral resistance. Furthermore, ADP was also shown to be effective in lamivudine-resistant HBV-infected individuals.[6] However, the frequency of renal laboratory abnormalities in the group given ADP at 30 mg/day was elevated relative to placebo-treated patients.[7] On the basis of these findings, the less efficacious 10 mg/day dose was approved and is currently the dose marketed for the treatment of HBV-infected patients.

Since the renal toxicity associated with ADP therapy is thought to arise from active transport of ADV into the renal proximal tubules via renal organic anion transporters,[7] we focused on a prodrug strategy that would increase liver exposure to adefovir and/or decrease distribution of adefovir to the kidney. The cyclic 1-aryl-1,3-propanyl prodrug is a new type of prodrug moiety reported to target phosphate- and phosphonate-containing drugs to the liver.[8] This highly plasma-stable class of prodrugs is activated by the liver-specific cytochrome P450 isozyme CYP3A4.[9] In contrast to other prodrug approaches,[10] a cyclic 1-aryl-1,3-propanyl prodrug of ADV (**3**) was envisioned to enhance ADV delivery to the liver while minimizing systemic exposure. Liver targeting arises following oral administration by first pass hepatic extraction. Prodrug that escapes the liver distributes into other tissues, but because it is metabolically stable, the prodrug eventually returns to the circulation and passes through the liver. The prodrug is converted in the liver to adefovir, which then undergoes subsequent phosphorylation to the HBV polymerase inhibitor, adefovir diphosphate (ADV-DP, **4**, Scheme 1).

Scheme 1. ADV and Cyclic 1-Aryl-1,3-propanyl Prodrug of ADV Conversion to ADV-DP (PRPP Synthase: 5-Phospho-D-ribosyl α-1-Pyrophosphate (PRPP) Synthase)



In the work described herein, our primary objective was to discover a cyclic 1-aryl-1,3-propanyl prodrug of ADV that would significantly improve the therapeutic index of ADV relative to ADP. More specifically, we sought to identify a prodrug with improved distribution of ADV to the liver and

* To whom correspondence should be addressed. Phone: 858-622-5530. Fax: 858-622-5573. E-mail: rajar@mbasis.com.
† Department of Chemistry.
‡ Department of Biochemistry.

[a] Abbreviations: ADV, adefovir; ADV-DP, adefovir diphosphate; ADP, adefovir dipivoxil; DCC, *N,N′*-dicyclohexylcarbodiimide; HMDS, hexamethyldisilazane; TMSOTf, trimethylsilyl trifluoromethanesulfonate.

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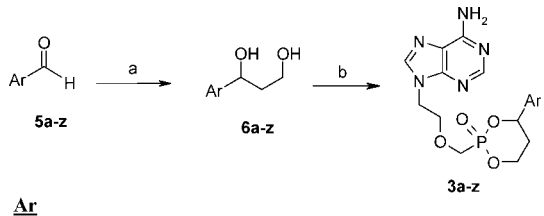
Scheme 2. General Synthesis of Cyclic 1-Aryl-1,3-propanyl Prodrugs of Adefovir [a]



Ar

a: Ph	**j**: 2,3-F_2-Ph	**s**: 3,5-Cl_2-Ph
b: 3,5-Me_2-Ph	**k**: 2,4-F_2-Ph	**t**: 2-Br,5-OMe-Ph
c: 4-*t*-Bu-Ph	**l**: 2,5-F_2-Ph	**u**: 5-Br,2-OMe-Ph
d: 2-F-Ph	**m**: 2,6-F_2-Ph	**v**: 3-Br,4-OMe-Ph
e: 2-Cl-Ph	**n**: 3,5-F_2-Ph	**w**: 4-pyridyl
f: 2-Br-Ph	**o**: 2,3-Cl_2-Ph	**x**: 3-pyridyl
g: 3-F-Ph	**p**: 2,4-Cl_2-Ph	**y**: 3- furanyl
h: 3-Cl-Ph	**q**: 2,6-Cl_2-Ph	**z**: 3- thienyl
i: 3-Br-Ph	**r**: 3,4-Cl_2-Ph	

[a] Conditions: (a) (i) EtOAc, LDA, THF, −78 °C; (ii) LAH, THF or $NaBH_4$, EtOH; (b) ADV (**1**), $SOCl_2$, CH_2Cl_2, reflux or DCC, pyridine, DMF, 100 °C.

lower exposure in the kidney, thereby achieving greater efficacy and safety.

Results

Chemistry. A. General Synthesis of Cyclic 1-Aryl-1,3-propanyl Prodrugs of Adefovir. Cyclic 1-aryl-1,3-propanyl prodrugs of ADV with various aromatic activating groups were synthesized by coupling ADV (**1**)[11] with 1-aryl-1,3-propanediols (**6a**−**z**, Scheme 2). While numerous methods exist for the synthesis of the latter reagents,[12] in the present work the diols were prepared by condensation of substituted aromatic aldehydes (**5a**−**z**) with the lithium enolate of alkyl acetate followed by ester reduction.[12a]

Prodrugs of ADV were initially prepared by making the dichloridate with thionyl chloride followed by coupling[13a] with the corresponding diol in the presence of a non-nucleophilic base such as triethylamine. Because the reaction was low-yielding, attempts were made to optimize the coupling reaction. After exploration of a variety of reaction conditions, heating a mixture of ADV and substituted propane-1,3-diol with dicyclohexylcarbodiimide (DCC) and pyridine in DMF at 100 °C for 12–16 h was found to be optimal in terms of isolated yields of coupled products.[13b] Neither the monoacid nor the diester was observed in the product mixture in significant quantities, indicating that ring closure to form the cyclic ester was a much more favorable process than reaction with a second diol molecule.

The resulting product mixture contained both the racemic cis and trans diastereomers in a ratio of 55:45 to 60:40, favoring the cis isomer. Structures were assigned on the basis of ^{1}H and ^{31}P NMR chemical shifts reported for other cyclic phosph(on)ate esters.[14] Specifically, the relative prodrug stereochemistry (cis and trans) was established by analysis of the chemical shift of the benzylic methine proton in the ^{1}H NMR spectra. The benzylic methine proton for the cis isomer is consistently farther downfield (with a difference of 0.2 ppm in DMSO-d_6) than for the corresponding trans isomer. The magnitude of the shift is enhanced in polar NMR solvents such as DMSO-d_6. The isomers can also be differentiated by their ^{31}P NMR chemical shifts,

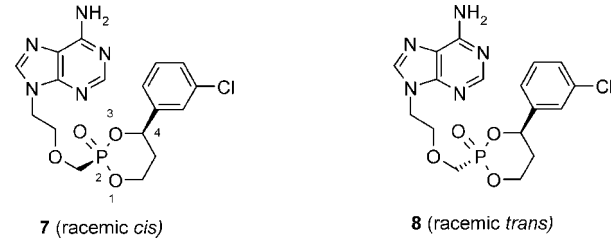


Figure 1. Racemic cis and trans cyclic prodrugs of adefovir.

where the resonance for the cis isomer is also downfield, with a shift of ∼1 ppm, compared to that of the trans isomer.[15]

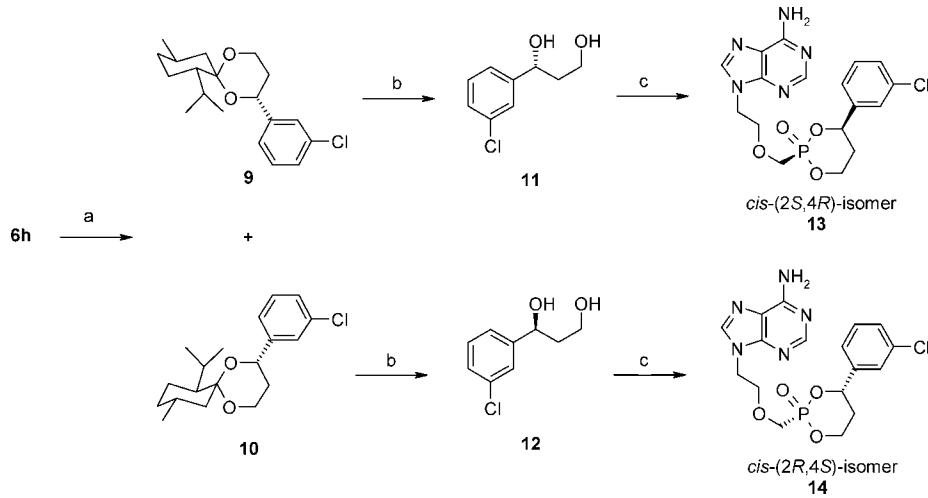
B. Synthesis of Isomers of Cyclic 1-(3-Chlorophenyl)-1,3-propanyl ADV Prodrugs (3h). Prodrug **3h** was initially synthesized as a racemic mixture of two diastereomers. In order to probe the effects of the relative stereochemistry on prodrug activation, the cis (**7**) and trans prodrugs (**8**) (Figure 1) were separated by a succession of column chromatographies and crystallizations.

In an effort to explore the influence of the absolute stereochemistry on prodrug activation, absorption, and pharmacokinetics, the individual enantiomers of racemic mixture **7** were prepared via resolution of the precursor diol (Scheme 3).[16] Diastereomeric menthone adducts **9** and **10** of racemic diol **6h** were prepared via silylation followed by Lewis acid catalyzed ketal formation. Hydrolysis of enantiomerically enriched ketals resulted in 33% and 37% yields of diols **11** and **12**, respectively.[16] Individual coupling of the resulting diols **11** and **12** with ADV, using optimized reaction conditions,[17] gave the single cis enantiomers **13** and **14** (in addition to their trans counterparts).

Biology. A. Activation in Rat Microsomes. Rates of ADV prodrug activation for prodrugs **3a**−**z** were evaluated in male rat liver microsomes by measuring the formation of ADV upon incubation for 5 min with 250 μM prodrug. Unsubstituted phenyl analogue **3a** showed an activation rate of 2.9 nmol min^{-1} mg^{-1} (Table 1), whereas addition of alkyl substituents on the aryl group resulted in a lower rate of activation (e.g., 3,5-dimethyl (**3b**) or 4-*tert*-butyl (**3c**) analogues). Incorporation of halogens at the ortho position of the phenyl group gave a lower rate of activation in the case of fluorine (**3d**) and a higher rate with the corresponding chloro (**3e**), whereas the 2-bromo analogue (**3f**) was found to be similar to the unsubstituted prodrug **3a**. Among the meta-substituted monohalogenated prodrug analogues, the 3-chloro-substituted phenyl prodrug **3h** gave the highest rate of activation (10.9 nmol min^{-1} mg^{-1}) with a clear distinction between the corresponding 3-fluoro (**3g**) and 3-bromo (**3i**) analogues. Evaluation of disubstituted phenyl prodrugs (**3j**–**s**) to further explore the halogen effects resulted in lower activation when compared to the monosubstituted analogue **3h**. While the 2,3-difluoro analogue (**3j**) showed the highest rate of activation among the disubstituted prodrugs, the dichloro analogues (**3o**–**s**) did not show any improvement in rate. Among the other disubstituted prodrugs, the 2-bromo-5-methoxyphenyl prodrug **3t** had a rate of activation of 4 nmol min^{-1} mg^{-1}, whereas two other regioisomers (**3u** and **3v**) had much lower rates. Heteroaryl substitution in place of phenyl with 4-pyridyl (**3w**) and 3-furanyl (**3y**) resulted in rates of 2.9 and 4 nmol min^{-1} mg^{-1}, respectively, whereas the thienyl analogue (**3z**) showed a lower rate of activation.

B. Intracellular ADV-DP Formation in Primary Rat Hepatocytes. Prodrug analogues that showed activation in rat microsomes were evaluated for cellular activation by incubation with primary male rat hepatocytes. Intracellular levels of ADV-

Scheme 3. Synthesis of the Individual Enantiomers of cis Prodrug **7** via Resolution of Racemic 1-(3-Chlorophenyl)-1,3-dihydroxypropane [a]



[a] Conditions: (a) (i) HMDS, cat. TMSOTf, 0 °C; (ii) (−)-menthone, cat. TMSOTf, −40 °C; (b) conc HCl, MeOH (**11**, 33%; **12**, 37%); (c) (i) **1**, $(COCl)_2$, diethylformamide, CH_2Cl_2, reflux, 4.5 h; (ii) diol **11** or **12**, triethylamine, pyridine, −70 °C, 1.5 h; (iii) AcOH, EtOH, reflux, 33% (**13**), 40% (**14**).

Table 1. Activation of ADV Prodrugs **3a**−**z** by Rat Microsomes

compd	Ar	rate of activation in rat liver microsomes (nmol min^{-1} mg^{-1})[a]
3a	phenyl	2.9
3b	3,5-dimethylphenyl	1.6
3c	4-*tert*-butylphenyl	0.5
3d	2-fluorophenyl	<1
3e	2-chlorophenyl	6.2
3f	2-bromophenyl	3.5
3g	3-fluorophenyl	<1
3h	3-chlorophenyl	10.9
3i	3-bromophenyl	1.9
3j	2,3-difluorophenyl	6.3
3k	2,4-difluorophenyl	2.5
3l	2,5-difluorophenyl	2.3
3m	2,6-difluorophenyl	1.5
3n	3,5-difluorophenyl	1.5
3o	2,3-dichlorophenyl	<4.5
3p	2,4-dichlorophenyl	2.5
3q	2,6-dichlorophenyl	<1
3r	3,4-dichlorophenyl	1.5
3s	3,5-dichlorophenyl	1.9
3t	2-bromo-5-methoxyphenyl	4
3u	5-bromo-2-methoxyphenyl	<1
3v	3-bromo-4-methoxyphenyl	1.1
3w	4-pyridyl	4.5
3x	3-pyridyl	2.9
3y	3-furanyl	4
3z	3-thienyl	0.5

[a] A detailed procedure for determination of rate of activation in rat microsomes is described in the Experimental Section.

Table 2. Intracellular ADV-DP Formation of Selected Prodrugs in Primary Rat Hepatocytes

compd	Ar	intracellular ADV-DP (nmol·h/g)[a]
3a	phenyl	149.1
3b	3,5-dimethylphenyl	40.7
3c	4-tert-butylphenyl	0
3f	2-bromophenyl	43.1
3h	3-chlorophenyl	109.3
3i	3-bromophenyl	70.3
3n	3,5-difluorophenyl	61.9
3p	2,4-dichlorophenyl	9.8
3r	3,4-dichlorophenyl	6.2
3s	3,5-dichlorophenyl	64.4
3t	2-bromo-5-methoxyphenyl	<0.5
3v	3-bromo-4-methoxyphenyl	<0.5
3w	4-pyridyl	106.2
3x	3-pyridyl	26.7
3y	3-furanyl	0
3z	3-thienyl	0

[a] A detailed procedure for determination of ADV-DP formation in primary rat hepatocytes is described in the Experimental Section.

Table 3. Liver ADV-DP Levels in Rats Dosed with 30 mg/kg Orally

compd	Ar	liver ADV-DP (nmol/g)[a]
3a	phenyl	0.6
3f	2-bromophenyl	7.1
3h	3-chlorophenyl	6.6
3i	3-bromophenyl	2.2
3n	3,5-difluorophenyl	2.2
3s	3,5-dichlorophenyl	2
3w	4-pyridyl	1.2

[a] A detailed procedure for determination of ADV-DP formation in rat liver is described in the Experimental Section.

DP were measured after 2 h incubation (Table 2). While the 3-chlorophenyl prodrug **3h** had the highest rate of activation in rat microsomes, the phenyl analogue (**3a**) gave the highest intracellular levels of the active metabolite ADV-DP (149.1 nmol/g). Alkyl substituted analogues (**3b**−**c**) gave low levels of ADV-DP, consistent with the low rate of activation observed in microsomes. The 3-chlorophenyl substituted prodrug (**3h**) gave higher levels of ADV-DP (109.3 nmol/g) than any of the other monohalogenated (**3f**, **3i**) or dihalogenated phenyl prodrugs (**3n**−**s**). Both the 2-bromo-5-methoxyphenyl (**3t**) and 3-bromo-4-methoxyphenyl (**3v**) regioisomers showed low levels of ADV-DP. Among the heteroaryl substituted prodrugs, the 4-pyridyl analogue (**3w**) showed higher ADV-DP levels than the 3-pyridyl analogue (**3x**), whereas the 3-furanyl (**3y**) and 3-thienyl (**3z**) prodrugs gave no detectable levels.

C. Liver ADV-DP Levels in Rats upon Oral Administration. Adefovir prodrugs that showed good activation in rat microsomes and >40 nmol·h/g levels of ADV-DP in primary rat hepatocytes were further evaluated in vivo. Liver levels of ADV-DP were monitored over 8 h after oral administration to rats of 30 mg/kg of prodrug as a PEG-400 solution (Table 3). Phenyl analogue **3a**, which showed a high rate of activation in the microsomal activation assay and formed high levels of ADV-DP in primary rat hepatocytes gave low liver levels of ADV-DP in vivo. The 2-bromophenyl (**3f**) and 3-chlorophenyl (**3h**)

Table 4. Effects of the Stereochemistry of Different Isomers of Prodrug **3h** on Rat Microsomal Activation

compd	P2,C4 relative stereochemistry (P2 stereochemistry)	C4 stereochemistry	rate of activation in rat microsomes (pmol $min^{-1}mg^{-1}$)[a]
7	*cis*-(*R*/*S*)	*R*/*S*	421 ± 77
8	*trans*-(*R*/*S*)	*R*/*S*	<10
13	*cis*-(*S*)	*R*	193 ± 11
14	*cis*-(*R*)	*S*	510 ± 6

[a] A detailed procedure for determination of rate of activation in rat microsomes is described in the Experimental Section.

analogues were found to give the highest liver levels of ADV-DP upon oral administration, 7.1 and 6.6 nmol/g, respectively. Other substituted phenyl analogues including the 3-bromophenyl (**3i**), 3,5-difluorophenyl (**3n**), and 3,5-dichlorophenyl (**3s**) analogues showed moderate levels of the active metabolite. The heteroaryl 4-pyridyl prodrug **3w** generated low ADV-DP levels when administered orally despite producing high levels in hepatocytes.

D. Effects of the Relative and Absolute Stereochemistry of Prodrug Isomers. Prodrugs **3f** and **3h** resulted in 7.1 and 6.6 nmol/g liver levels of ADV-DP in vivo in rats when administered orally. Of these two, the 3-chlorophenyl prodrug **3h** was consistently the better activated prodrug in the microsomal and hepatocyte activation assays. Hence, prodrug **3h**, a mixture of four stereoisomers, was chosen for further exploration of the effects of the stereochemistry of the prodrug isomers on the activation and absorption parameters. The original synthetic protocol (DCC coupling) resulted in a mixture of four diastereomers comprising racemic cis and trans isomers **7** and **8**. The racemic mixture of cis prodrugs (**7**) was found to give a high rate of activation in human microsomes (421 ± 77 pmol min^{-1} mg^{-1}), whereas the racemic mixture of trans diastereomers (**8**) was not activated under these conditions. With this result in hand, the individual enantiomers of **7** were prepared. Among these, the *cis*-(2*R*,4*S*) isomer (**14**), made from *S*-diol, was shown to have a higher rate of activation (510 ± 6 pmol min^{-1} mg^{-1}) than its antipode **13** (193 ± 11 pmol min^{-1} mg^{-1}) (Table 4).

The individual cis enantiomers **13** and **14** were further evaluated in vivo to explore the effects of the absolute stereochemistry on activation and oral absorption. Prodrugs **13** and **14** were administered iv and po to rats (30 mg/kg), followed by quantitation of liver ADV-DP levels. Of the two enantiomers, the *cis*-(2*R*,4*S*) isomer (**14**) resulted in higher ADV-DP levels in the liver upon iv administration compared with the *cis*-(2*S*,4*R*) isomer **13** (Figure 2). Similarly higher liver levels of ADV-DP were formed with prodrug **14** upon oral administration (Figure 3).

E. Tissue Distribution and Pharmacokinetic Parameters for Prodrug 7 in Rats. Tissue distributions for prodrug **7** and adefovir dipivoxil were determined for evidence of enhanced liver production of ADV-DP with prodrug **7**. Radiolabeled compounds were administered at 30 mg/kg ADV equivalents (^{3}H-ADV) to fasted rats by oral gavage. Total ADV equivalents (ADV, adefovir monophosphate (ADV-MP), ADV-DP) over a 24 h period were quantified in tissue extracts of liver, kidney, and small intestine following administration of radiolabeled **7** or adefovir dipivoxil. Liver selectivity was assessed by comparison of the temporal profiles of total radioactivity counts in liver versus kidney and small intestine. Urinary excretion of ADV was also quantified.

Treatment with prodrug **7** resulted in higher ADV exposure to the liver (~3-fold) and reduced ADV distribution to the kidneys and the small intestine vs ADP as shown in Figure 4

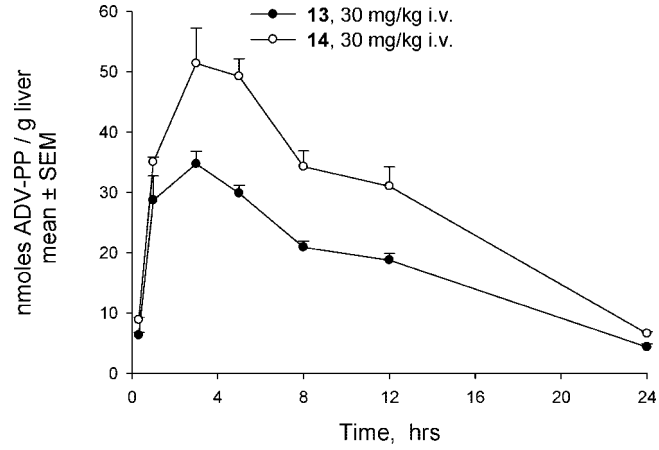


Figure 2. Rat liver ADV-DP levels after 30 mg/kg iv administration of **13** or **14**.

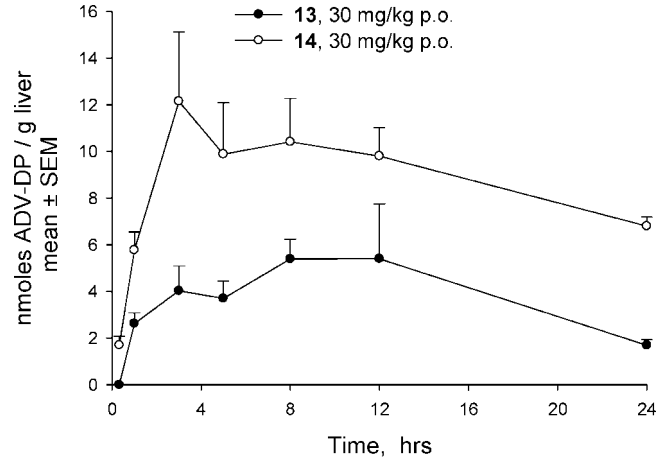


Figure 3. Rat liver ADV-DP levels after 30 mg/kg oral administration of **13** or **14**.

and Table 5.[8b] The reduced ADV exposure in kidneys (~3.5-fold) observed with **7** compared to ADP was mirrored by a similar reduction in the amount of ADV excreted into urine (4-fold). The ratio of adefovir to ADV-MP and to ADV-DP was similar for both prodrugs in the tissues examined (data not shown). Comparison of the liver/kidney ratios showed a 12-fold increase in liver selectivity for **7** vs ADP.

Key pharmacokinetic parameters in liver were determined for lead prodrug **14** and ADV in Sprague-Dawley rats (Table 6). Oral bioavailability was determined by comparing liver levels of ADV-DP following oral vs iv administration of **14** and was found to be 31%. By use of the same protocol, the oral bioavailability of the mesylate salt of **14** in the rat was determined to be 42%. The oral bioavailability of **7** in the beagle dog based on urinary recovery of intact prodrug and metabolites po/iv was 82%.

Discussion

Adefovir dipivoxil, a prodrug of the nucleotide analogue adefovir, is an approved treatment for hepatitis B. However, its efficacy in humans is limited by renal toxicity associated with high systemic adefovir exposure. Cyclic 1-aryl-1,3-propanyl prodrugs were designed to enhance the therapeutic index of phosphate and phosphonate-based drugs by targeting their production to the liver and as a consequence reducing systemic exposure.

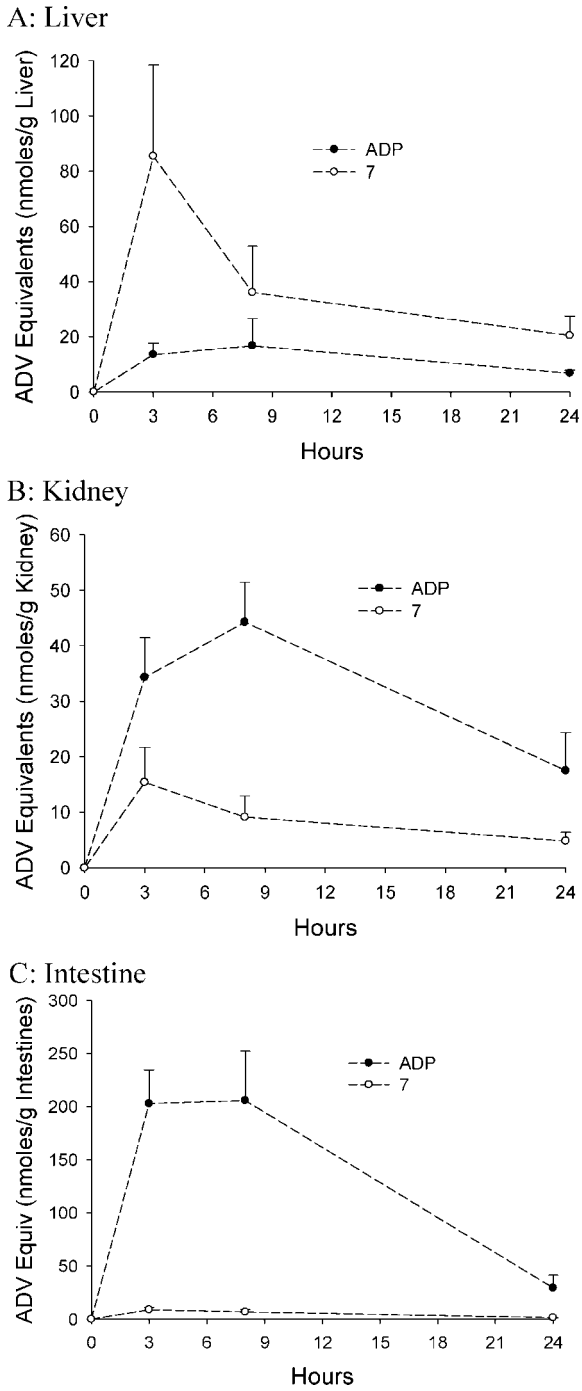


Figure 4. Rat tissue distribution of **7** and ADP in (A) liver, (B) kidney, and (C) intestines.

Table 5. Comparison of the Tissue Distribution and Liver Selectivity of Radiolabeled ADP and **7** in Rats

	ADV equivalents $AUC_{0\text{-}24h}$ (nmol·h/g)[a]				liver selectivity index	
prodrug	liver	kidney	small intestine	plasma	liver/ kidney	liver/ intestine
ADP	284	742	3206	34	0.4	0.08
7	884	196	118	55	4.5	7.5
7/ADP	3	0.3	0.04	1.6	11.8	84.4

[a] A detailed procedure for determination of ADV-equivalents in tissues is described in the Experimental Section.

The unsubstituted phenyl prodrug of ADV (**3a**) was found to be well activated in both microsomal and cellular activation assays. The addition of alkyl substituents to the phenyl group lowered the rate of activation as well as intracellular ADV-DP formation irrespective of the position or size of the substituents. In contrast, halogen substituents were associated with significant improvements in the in vitro activation of these prodrugs with the chloro substituent providing the highest activation rates and in general better stability[18] (observed during synthesis and isolation of the prodrugs). As observed previously,[18] the results did not correlate with the number of substituents or their position on the aryl ring, suggesting that prodrug activation is governed by factors beyond simple lipophilicity or electronic effects.

Table 6. Comparison of Key Pharmacokinetic Parameters, Based on ADV-DP Liver Levels, in Rat after Oral Administration of 30 mg/kg (ADV Equivalents) of 3-Chlorophenyl Prodrugs[a]

compd	F (%)	liver $AUC_{0\text{-}48h}$ (nmol·h/L)	liver C_{max} (nmol/g)
7		64	8.0
13	21	93	5.4
14	31	213	12.2
14-mesylate	42	288	19.6

[a] A detailed procedure for determination of ADV-DP formation in liver is described in the Experimental Section.

Most prodrugs showing good ADV-DP formation in hepatocytes showed good liver ADV-DP levels in vivo, with the halogenated prodrugs consistently exhibiting the better profile. While the nonsubstituted phenyl analogue **3a** exhibited a high rate of activation and high levels of ADV-DP in hepatocytes relative to most analogues, low ADV-DP formation was observed in vivo, which was attributed to the unstable nature of this particular prodrug.[18] In contrast, the low in vivo liver levels upon oral administration of the 4-pyridyl prodrug **3w**, despite a better in vitro profile, may arise from poor oral bioavailability due to its low log *P*. Among all the evaluated ADV prodrugs, the 3-chlorophenyl mixture of prodrugs **3h** consistently exhibited a high rate of activation in microsomes, in primary hepatocytes, and in rats following oral administration. Evaluation of its individual isomers led to the selection of single prodrug isomer *cis*-(2*R*,4*S*) (**14**) as the lead compound. Prodrug **14** had good oral bioavailability in rats and dogs, which was further increased by using the mesylate salt to increase water solubility.[17]

Comparative tissue distribution of ADV-equivalent levels in liver, kidneys, and small intestine after oral administration of radiolabeled cyclic 1-aryl-1,3-propanyl prodrug **7** and ADP demonstrated evidence of liver targeting with a 12-fold improvement of the liver/kidney exposure over ADP. This concomitant increase in liver exposure and decrease in kidney exposure are the result of the liver-selective activation of this class of prodrug coupled with its stability in extrahepatic tissues and likely increase in biliary excretion. The higher degree of liver targeting of ADV demonstrated in our animal studies with prodrug 14-mesylate (MB06866, pradefovir) translated well into the recently completed phase II clinical trials and resulted in significant antiviral activity in a 48-week study.[19]

Conclusion

Cyclic 1-aryl-1,3-propanyl prodrugs of adefovir were synthesized and evaluated for in vitro microsomal activation, ADV-DP formation in rat hepatocytes, and liver levels of ADV-DP in rats after oral administration. These screens identified the 3-chlorophenyl prodrug **3h** (a mixture of four diastereomers) as the lead compound. Synthesis and evaluation of each diastereomer demonstrated selective activation of the cis isomers and a 2.5-fold greater activation of the (2*R*,4*S*)-isomer (**14**) compared to the (2*S*,4*R*)-isomer (**13**). Oral administration of prodrug **7** to rats led to a 12-fold improvement in the liver/

kidney exposure ratio relative to ADP. The mesylate salt of prodrug **14**, which was selected as the development candidate based on its improved oral bioavailability in the rat, is currently under clinical evaluation in hepatitis B patients.[19]

Experimental Section

General Information. Glassware for moisture-sensitive reactions was flame-dried and cooled to room temperature in a desiccator, and all reactions were carried out under an atmosphere of nitrogen. Anhydrous solvents were purchased from Aldrich or Acros. Thin layer chromatography was performed on EM Science silica gel 60 F254 plates and was visualized with a UV lamp (254 nm) or cerium stain. Column chromatography was performed on 230–400 mesh EM Science silica gel 60. Melting points were recorded on a Thomas-Hoover capillary melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were obtained on a Varian Gemini-200 operating at 200 and 50 MHz, respectively, or a Varian Mercury-300 operating at 300 and 75 MHz, respectively. ^{1}H and ^{13}C NMR spectra were recorded in δ units using the solvent's chemical shift as the reference line. ^{1}H NMR data for only characteristic signals are provided where a mixture of prodrug isomers was characterized, whereas complete characterization is presented in the case of single isomer prodrugs. C, H, N microanalyses were performed by Robertson Microlit Laboratories, Inc., Madison, NJ, or by NuMega Resonance Laboratories, Inc., San Diego, CA. All protocols involving animal experimentation were reviewed and approved by the Metabasis Therapeutics IACUC (Institution Animal Care and Use Committee) and follow the guidelines established by the NRC "Guide for the Care and Use of Laboratory Animals".

General Procedure for Preparation of Racemic 1-Aryl Substituted Propane-1,3-diols 6a–z. Step A. To a solution of diisopropylamine (2 mmol) in THF (0.7 mL/mmol diisopropylamine) at −78 °C was slowly added a solution of *n*-BuLi (2 mmol, 2.5 M in hexanes). The mixture was then stirred for 15 min at −78 °C before a solution of ethyl acetate (2 mmol) in THF (0.14 mL/mmol ethyl acetate) was slowly introduced. After the mixture was stirred for an additional 30 min at −78 °C, a THF solution containing the aryl aldehyde (1.0 mmol in 0.28 mL of THF) was added. The mixture was then stirred at −78 °C for 30 min, warmed to room temperature, and stirred an additional 2 h. After aqueous workup (0.5 M HCl), the organic layer was concentrated to a crude oil and purified either by chromatography or by distillation.

Step B. $LiAlH_4$ Reduction of β-Hydroxy Esters. A solution of hydroxyester (1 mmol) in ether (1 M) was added to a suspension of $LiAlH_4$ (3 mmol) in ether (1 M) at 0 °C. The mixture was stirred, allowing the cooling bath to melt and the mixture to reach room temperature. Once the reaction was judged complete (TLC), the reaction mixture was cooled back to 0 °C and quenched with ethyl acetate. Aqueous workup (0.5 M HCl) afforded the crude diol, which was purified by chromatography or distillation.

$NaBH_4$ Reduction of β-Hydroxy Esters. $NaBH_4$ was added portionwise to a solution of hydroxyester in EtOH at room temperature. After 20 min the heterogeneous white reaction mixture was heated to reflux until all starting material was consumed (3–5 h, TLC). The cooled mixture was concentrated under reduced pressure and partitioned between water and EtOAc. The layers were separated, and the aqueous layer was back-extracted with EtOAc. The combined extracts were dried (Na_2SO_4), concentrated under reduced pressure, and purified by chromatography on silica gel to give the corresponding diol. Typical overall yields of diols for two steps were 40−80%.

1-(3,5-Dimethylphenyl)-1,3-propanediol (6b). ^{1}H NMR (200 MHz, $CDCl_3$): δ 6.98 (s, 2H), 6.93 (s, 1H), 4.89 (dd, J = 8.2, 4.8 Hz, 1H), 3.85 (dd, J = 6.8, 6.1 Hz, 2H), 2.49 (br s, 2H), 2.32 (s, 6H), 2.10–1.81 (m, 1H).

1-(4-*tert*-Butylphenyl)-1,3-propanediol (6c). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.41–7.28 (m, 4H), 4.95 (dt, J = 9.5, 3.4 Hz, 1H), 3.87 (q, J = 6.1 Hz, 2H), 2.56 (d, J = 3.4 Hz, 1H), 2.31 (t, J = 6.1 Hz, 1H), 2.10–1.85 (m, 1H).

1-(2-Fluorophenyl)-1,3-propanediol (6d). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.52 (td, J = 6.2, 0.8 Hz, 1H) 7.3–6.9 (m, 3H), 5.25 (t, J = 6.2 Hz, 1H), 3.84 (t, J = 5.1 Hz, 2H), 3.5 (br s, 1H), 2.8 (br s, 1H), 1.97 (dd, J = 6.2, 5.1 Hz, 2H).

1-(2-Chlorophenyl)-1,3-propanediol (6e). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.63 (d, J = 7.0 Hz, 1H) 7.39–7.15 (m, 3H), 5.35 (dd, J = 8.4, 2.7 Hz, 1H), 3.90 (br s, 1H), 3.22 (br s, 1H), 2.42 (br s, 1H), 2.15–1.80 (m, 2H).

1-(2-Bromophenyl)-1,3-propanediol (6f). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.60–7.53 (m, 2H) 7.43–7.37 (m, 1H), 7.21–7.16 (m, 1H), 5.36–5.35 (m, 1H), 4.95–4.91 (m, 1H), 4.49–4.46 (m, 1H), 3.61–3.35 (m, 2H), 1.79–1.58 (m, 2H).

1-(3-Fluorophenyl)-1,3-propanediol (6g). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.22 (dd, J = 12.0, 8.0 Hz, 1H), 7.09–6.98 (m, 2H), 6.89 (td, J = 12.0, 2.2 Hz, 1H), 4.84 (br d, J = 4.4 Hz, 1H), 4.30 (br s, 1H), 3.73 (br d, J = 4.4 Hz, 2H), 3.40 (br s, 1H), 1.84 (dd, J = 6.4, 5.0 Hz, 2H).

1-(3-Chlorophenyl)-1,3-propanediol (6h). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.40–7.18 (m, 4H), 5.00–4.90 (dd, J = 6.8, 6.1 Hz, 1H), 3.85 (t, J = 5.8 Hz, 2H), 2.40 (s, 2H), 2.00–1.90 (m, 2H).

1-(3-Bromophenyl)-1,3-propanediol (6i). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.53 (s, 1H), 7.45–7.40 (m, 1H), 7.35–7.25 (m, 2H), 5.30 (d, J = 4.5 Hz, 1H exchangeable with D_2O), 4.73–4.63 (m, 1H), 4.47 (t, J = 5.1 Hz, 1H exchangeable with D_2O), 3.60–3.35 (m, 2H), 1.85–1.65 (m, 2H).

1-(2,3-Difluorophenyl)-1,3-propanediol (6j). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.30 (m, 1H), 7.15–7.00 (m, 2H), 5.28 (t, J = 5.9 Hz, 1H), 3.87 (t, J = 5.1 Hz, 2H), 3.61 (br s, 1H), 2.63 (br s, 1H), 1.99 (m, 2H).

1-(2,4-Difluorophenyl)-1,3-propanediol (6k). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.45 (t, d, J = 8.6, 6.6 Hz, 1H), 6.92–6.69 (m, 2H), 5.19 (dd, J = 5.8, 4.0 Hz, 1H), 3.83 (br s, 2H), 3.72 (br s, 1H), 2.90 (br s, 1H) 1.93 (q, J = 6.0 Hz, 2H).

1-(2,5-Difluorophenyl)-1,3-propanediol (6l). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.27 (td, J = 5.8, 2.7 Hz, 1H), 7.02–6.82 (m, 2H), 5.24 (dd, J = 7.6, 4.0 Hz, 1H), 3.89 (t, J = 6.6 Hz, 2H), 2.57 (br s, 2H), 2.04–1.91 (m, 2H).

1-(2,6-Difluorophenyl))-1,3-propanediol (6m). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.22 (d, J = 8.4 Hz, 2H), 6.88 (t, J = 8.4 Hz, 1H), 5.32 (dd, J = 9.0, 4.0 Hz, 1H), 3.89 (td, J = 5.9, 5.3 Hz, 2H), 2.36–1.90 (m, 4H).

1-(3,5-Difluorophenyl)-1,3-propanediol (6n). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.24–7.07 (m, 1H), 6.90 (dd, J = 8.4, 1.8 Hz, 1H), 6.70 (tt, J = 8.4, 1.8 Hz, 1H), 4.94 (dd, J = 12.0, 5.8 Hz, 1H), 3.88 (t, J = 5.8 Hz, 2H), 2.36 (br s, 2H), 1.93 (m, 2H).

1-(2,3-Dichlorophenyl))-1,3-propanediol (6o). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.54 (dd, J = 7.4, 1.6 Hz, 1H), 7.35 (dd, J = 7.4, 1.6 Hz, 1H), 7.27 (t, J = 7.4 Hz, 1H), 5.33 (d, J = 8.8 Hz, 1H), 3.88 (br s, 2H), 3.77 (br s, 1H), 2.72 (br s, 1H), 2.15–1.75 (m, 2H).

1-(2,4-Dichlorophenyl)-1,3-propanediol (6p). ^{1}H NMR (200 MHz, C_6D_6): δ 7.60 (d, J = 8.0 Hz, 1H), 7.35–7.27 (m, 2H), 5.32 (dd, J = 8.4, 3.0 Hz, 1H), 3.94 (td, J = 5.6, 2.2 Hz, 2H), 2.30–1.80 (m, 4H).

1-(2,6-Dichlorophenyl)-1,3-propanediol (6q). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.32 (d, J = 13.6 Hz, 2H), 7.14 (t, J = 13.6 Hz, 1H), 5.66 (dd, J = 9.8, 4.0 Hz, 1H), 3.90 (t, J = 6.0 Hz, 2H), 2.55–2.36 (m, 3H), 1.95 (dq, J = 14.6, 4.4 Hz, 1H).

1-(3,4-Dichlorophenyl)-1,3-propanediol (6r). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.47 (d, J = 2.2 Hz, 1H), 7.41 (d, J = 8.4 Hz, 1H), 7.17 (dd, J = 8.4, 2.2 Hz, 1H), 4.92 (dd, J = 6.6, 5.8 Hz, 1H), 3.86 (t, J = 5.0 Hz, 2H), 2.75 (br s, 2H), 1.91 (dd, J = 10.8, 5.6 Hz, 2H).

1-(3,5-Dichlorophenyl)-1,3-propanediol (6s). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.43 (s, 1H), 7.32 (s, 2H), 5.40 (d, J = 4.5 Hz, 1H exchangeable with D_2O), 4.60–4.75 (m, 1H), 4.44 (t, J = 5.1 Hz, 1H exchangeable with D_2O), 3.55–3.31 (m, 2H), 1.77–1.60 (m, 2H).

1-(2-Bromo-5-methoxyphenyl)-1,3-propanediol (6t). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.36 (dd, J = 8.8, 0.8 Hz, 1H), 7.18 (d, J = 3.2 Hz, 1H), 6.68 (ddd, J = 8.8, 3.2, 0.8 Hz, 1H), 5.21 (br d, J =

8.0 Hz, 1H), 3.88 (br s, 2H), 3.79 (s, 3H), 3.55 (br s, 1H), 3.70 (br s, 1H), 3.70 (br s, 1H), 2.10–1.70 (m, 2H).

1-(3-Bromo-6-methoxyphenyl)-1,3-propanediol (6u). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.50 (d, J = 2.2 Hz, 1H), 7.27 (dd, J = 8.4, 2.2 Hz, 1H), 6.67 (d, J = 8.4 Hz, 1H), 5.10 (dt, J = 8.0, 3.6 Hz, 1H), 3.99 (d, J = 4.4 Hz, 1H), 3.75 (br s, 5H), 3.89 (br s, 1H), 1.87 (m, 2H).

1-(3-Bromo-4-methoxyphenyl)-1,3-propanediol (6v). ^{1}H NMR 200 MHz, $CDCl_3$): δ 7.53 (d, J = 2.2 Hz, 1H), 7.32 (dd, J = 8.4, 2.2 Hz, 1H), 6.72 (d, J = 8.4 Hz, 1H), 5.14 (dd, J = 10.2, 5.2 Hz, 1h), 3.80 (br s, 5H), 3.45 (br s, 1H), 2.79 (br s, 1H), 1.93 (dd, J = 10.2, 5.8 Hz, 2H).

1-(Pyrid-4-yl)-1,3-propanediol (6w). Mp 98–100 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.47 (d, J = 6.3 Hz, 2H), 7.30 (d, J = 6.3 Hz, 2H), 5.37 (d, J = 4.5 Hz, 1H exchangeable with D_2O), 4.72–4.62 (m, 1H), 4.48 (t, J = 5.1 Hz, 1H exchangeable with D_2O), 3.60–3.38 (m, 2H), 1.75–1.65 (m, 2H).

1-(Pyrid-3-yl)-1,3-propanediol (6x). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.49 (d, J = 2.1 Hz, 1H), 8.25–8.20 (m, 1H), 7.72–7.65 (m, 1H), 7.45–7.30 (m, 1H), 5.28 (d, J = 4.5 Hz, 1H exchangeable with D_2O), 4.75–4.65 (m, 1H), 4.45 (t, J = 5.1 Hz, 1H exchangeable with D_2O), 3.55–3.33 (m, 2H), 1.85–1.62 (m, 2H).

1-(Furan-3-yl)-1,3-propanediol (6y). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.50–7.42 (m, 1H), 7.28–7.26 (m, 1H), 7.09–7.06 (m, 1H), 5.11 (d, J = 5.5 Hz, 1H), 4.80–4.70 (m, 1H), 4.42 (t, J = 3 Hz, 1H), 3.60–3.40 (m, 2H), 1.90–1.70 (m, 2H).

1-(Thiophen-3-yl)-1,3-propanediol (6z). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.60 (br s, 1H), 7.50 (br s, 1H), 6.50 (br s, 1H), 4.95 (d, J = 5.1 Hz, 1H), 4.55–4.65 (m, 1H), 4.40 (t, J = 3 Hz, 1H), 3.60–3.40 (m, 2H), 1.90–1.70 (m, 1H).

General Procedure for the Synthesis of Prodrugs by Thionyl Chloride Reaction. A suspension of 1 mmol of phosphonic acid in 5 mL of thionyl chloride was heated at reflux temperature for 4 h. The reaction mixture was then cooled and evaporated to dryness. To the resulting residue was added a solution of 1 mmol of alcohol and 2.5 mmol of pyridine in 3 mL of methylene chloride. After being stirred at 25 °C for 4 h, the mixture was subjected to workup and chromatography.

General Procedure for the Synthesis of ADV Cyclic Prodrugs via DCC Coupling. To a solution of ADV (410 mg, 1.5 mmol) in DMF (15 mL) and pyridine (3 mL) was added 1,3-dicyclohexylcarbodiimide (DCC) (925 mg, 4.5 mmol) followed by 1(3-chlorophenyl)propane-1,3-diol (295 mg, 1.57 mmol). The reaction mixture was heated overnight at 100 °C. The mixture was concentrated under reduced pressure and azeotroped with toluene (2 × 10 mL). Crude compound was chromatographed on a silica gel column (3:97 to 10:90 methanol−dichloromethane) to result in pure cyclic prodrug (310 mg).

Typical yield of prodrug formation via DCC method varied between 20% and 70%. ^{1}H NMR data of the prodrug isomers are given for the characteristic C4-methine proton of the prodrug moiety. All the spectra are available in the Supporting Information.

9-{2-[4-(Phenyl)-2-oxo-1,3,2-dioxaphosphorinan-2-yl]methoxyethyl}adenine (3a). White foamy solid, mp 187–188 °C. ^{1}H NMR (300 MHz, CD_3OD): δ 5.65–5.46 (m, 1H), 5.29 (d, J = 11.5 Hz, 1H). Anal. ($C_{17}H_{20}N_5O_4P$) C, H, N.

9-{2-[4-(3,5-Dimethylphenyl)-2-oxo-1,3,2-dioxaphosphorinan-2-yl]methoxyethyl}adenine (3b). White solid, mp 155–165 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.45 (d, J = 10.5 Hz, 1H), 5.15 (d, J = 10.5 Hz, 1H). Anal. ($C_{19}H_{24}N_5O_4P\cdot0.5H_2O$) C, H, N.

9-{2-[4-(4-*tert*-Butylphenyl)-2-oxo-1,3,2-dioxaphosphorinan-2-yl]methoxyethyl}adenine (3c). Yellow foam. ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.48 (d, J = 9.1 Hz, 1H), 5.24 (d, J = 11.1 Hz, 1H). Anal.($C_{21}H_{28}N_5O_4P\cdot0.33CH_2Cl_2$) C, H, N.

9-{2-[4-(2-Fluorophenyl)-2-oxo-1,3,2-dioxaphosphorinan-2-yl]methoxyethyl}adenine (3d). White solid, mp 189–191 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.77 (d, J = 11.1 Hz, 1H), 5.73 (d, J = 9.2 Hz, 1H). Anal. ($C_{17}H_{19}FN_5O_4P$) C, H, N.

9-{2-[4-(2-Chlorophenyl)-2-oxo-1,3,2-dioxaphosphorinan-2-yl]methoxyethyl}adenine (3e). White solid, mp 194–195°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.86 (d, J = 11.5 Hz, 1H), 5.79 (d, J = 9.1 Hz, 1H). Anal. ($C_{17}H_{19}ClN_5O_4P\cdot0.3C_3H_6O$) C, H, N.

9-{2-[4-(2-Bromophenyl)-2-oxo-1,3,2-dioxaphosphorinan-2-yl]methoxyethyl}adenine (3f). Tan foam, mp 193–195 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.69 (d, J = 11.4 Hz, 1H). Anal. ($C_{17}H_{19}N_5O_4PBr\cdot1.1H_2O$) C, H, N.

9-{2-[4-(3-fluorophenyl)-2-oxo-1,3,2-dioxaphosphorinan-2-yl]methoxyethyl}adenine (3g). White solid, mp 168–170 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.74–5.61 (m, 1H), 5.33 (d, J = 11.4 Hz, 1H). Anal. ($C_{17}H_{19}FN_5O_4P$) C, H, N.

9-{2-[4-(3-Chlorophenyl)-2-oxo-1,3,2-dioxaphosphorinan-2-yl]methoxyethyl}adenine (3h). Pale-yellow foam, mp 200–202 °C. ^{1}H NMR (200 MHz, CD_3OD): δ 5.62–5.55 (m, 1H), 5.33 (d, J = 11.4 Hz, 1H). Anal. ($C_{17}H_{19}ClN_5O_4P$) C, H, N.

9-{2-[4-(3-Bromophenyl)-2-oxo-1,3,2-dioxaphosphorinan-2-yl]methoxyethyl}adenine (3i). Off-white foam. ^{1}H NMR (200 MHz, CD_3OD): δ 5.62–5.45 (m, 1H), 5.33 (d, J = 11.4 Hz, 1H). Anal. ($C_{17}H_{19}BrN_5O_4P\cdot0.9H_2O$) C, H, N.

9-{2-[4-(2,3-Difluorophenyl)-2-oxo-1,3,2-dioxaphosphorinan-2-yl]methoxyethyl}adenine (3j). White solid, mp 208–210 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.85−5.78 (m, 1H), 5.88 (d, J = 10.4 Hz, 1H). Anal. ($C_{17}H_{18}F_2N_5O_4P$) C, H, N.

9-{2-[4-(2,4-Difluorophenyl)-2-oxo-1,3,2-dioxaphosphorinan-2-yl]methoxyethyl}adenine (3k). White foam. ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.74 (d, J = 11.2 Hz, 1H), 5.66 (d, J = 10.8 Hz, 1H). Anal. ($C_{17}H_{18}F_2N_5O_4P\cdot0.3C_3H_6O$) C, H, N.

9-{2-[4-(2,5-Difluorophenyl)-2-oxo-1,3,2-dioxaphosphorinan-2-yl]methoxyethyl}adenine (3l). White solid, mp 109–110 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 5.70–5.80 (m, 2H). Anal. ($C_{17}H_{18}F_2N_5O_4P\cdot0.2H_2O$) C, H, N.

9-{2-[4-(2,6-Difluorophenyl)-2-oxo-1,3,2-dioxaphosphorinan-2-yl]methoxyethyl}adenine (3m). White foam. ^{1}H NMR (200 MHz, $CDCl_3$): δ 6.00–5.65 (m, 2H). Anal. ($C_{17}H_{18}F_2N_5O_4P\cdot0.4C_3H_6O$) C, H, N.

9-{2-[4-(3,5-Difluorophenyl)-2-oxo-1,3,2-dioxaphosphorinan-2-yl]methoxyethyl}adenine (3n). Off-white foam. ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.56 (t, J = 6.2 Hz, 1H), 5.13 (d, J = 11.0 Hz, 1H). Anal. ($C_{17}H_{18}F_2N_5O_4P\cdot0.4CH_2Cl_2$) C, H, N.

9-{2-[4-(2,3-Dichlorophenyl)-2-oxo-1,3,2-dioxaphosphorinan-2-yl]methoxyethyl}adenine (3o). White foam. ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.74–5.84 (m, 2H). Anal. ($C_{17}H_{18}Cl_2N_5O_4P\cdot0.7H_2O$) C, H, N.

9-{2-[4-(2,4-Dichlorophenyl)-2-oxo-1,3,2-dioxaphosphorinan-2-yl]methoxyethyl}adenine (3p). Brown foam. ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.88–5.80 (m, 2H). Anal. ($C_{17}H_{18}Cl_2N_5O_4P\cdot0.4CH_2Cl_2$) C, H, N.

9-{2-[4-(2,6-Dichlorophenyl)-2-oxo-1,3,2-dioxaphosphorinan-2-yl]methoxyethyl}adenine (3q). White solid, mp 195–197 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.29 (d, J = 10.4 Hz, 1H), 6.17 (d, J = 11.4 Hz, 1H). Anal. ($C_{17}H_{18}Cl_2N_5O_4P\cdot0.3CH_2Cl_2$) C, H, N.

9-{2-[4-(3,4-Dichlorophenyl)-2-oxo-1,3,2-dioxaphosphorinan-2-yl]methoxyethyl}adenine (3r). White foam. ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.67 (d, J = 9.7 Hz, 1H), 5.38 (d, J = 10.3 Hz, 1H). Anal. ($C_{17}H_{18}Cl_2N_5O_4P\cdot0.2CH_2Cl_2$) C, H, N.

9-{2-[4-(3,5-Dichlorophenyl)-2-oxo-1,3,2-dioxaphosphorinan-2-yl]methoxyethyl}adenine (3s). Off-white solid, mp 178–195 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.57 (d, J = 11.5 Hz, 1H), 5.29 (d, J = 9.37 Hz, 1H). Anal. ($C_{17}H_{18}Cl_2N_5O_4P\cdot0.2H_2O$) C, H, N.

9-{2-[4-(2-Bromo-5-methoxybenzyl)-2-oxo-1,3,2-dioxaphosphorinan-2-yl]methoxyethyl}adenine (3t). White solid, mp 104–105 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.76 (d, J = 10.3 Hz, 1H), 5.66 (d, J = 10.2 Hz, 1H). Anal. ($C_{18}H_{21}BrN_5O_5P$) C, H, N.

9-{2-[4-(5-Bromo-2-methoxybenzyl)-2-oxo-1,3,2-dioxaphosphorinan-2-yl]methoxyethyl}adenine (3u). White solid, mp 110–111 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 5.77 (m, 2H). Anal. ($C_{18}H_{21}BrN_5O_5P$) C, H, N.

9-{2-[4-(3-Bromo-4-methoxybenzyl)-2-oxo-1,3,2-dioxaphosphorinan-2-yl]methoxyethyl}adenine (3v). White solid, mp 131–132 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 5.69–5.79 (m, 2H). Anal. ($C_{18}H_{21}BrN_5O_5P$) C, H, N.

9-{2-[4-(4-Pyridyl)-2-oxo-1,3,2-dioxaphosphorinan-2-yl]methoxyethyl}adenine (3w). Yellow sticky solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.62 (d, J = 9.43 Hz, 1H), 5.26 (d, J = 10.8 Hz, 1H). Anal. ($C_{16}H_{19}N_6O_4P\cdot 1.0H_2O$) C, H, N.

9-{2-[4-(3-Pyridyl)-2-oxo-1,3,2-dioxaphosphorinan-2-yl]methoxyethyl}adenine (3x). White powder, mp 117–119 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.74–5.60 (m, 1H), 5.40 (d, J = 10.2 Hz, 1H). Anal. ($C_{16}H_{19}N_6O_4P$) C, H, N.

9-{2-[4-(3-Furanyl)-2-oxo-1,3,2-dioxaphosphorinan-2-yl]methoxyethyl}adenine (3y). White solid, mp 174–177 °C. ^{1}H NMR (200 MHz, CD_3OD): δ 5.62–5.45 (m, 1H), 5.20 (d, J = 11.4 Hz, 1H). Anal. ($C_{15}H_{18}N_5O_5P$) C, H, N.

9-{2-[4-(3-Thienyl)-2-oxo-1,3,2-dioxaphosphorinan-2-yl]methoxyethyl}adenine (3z). White solid, mp 152–155 °C. ^{1}H NMR (200 MHz, CD_3OD): δ 5.62–5.56 (m, 1H), 5.20 (d, J = 10.3 Hz, 1H). Anal. ($C_{15}H_{18}N_5O_4PS\cdot 0.3NH_4Cl\cdot 1.0H_2O$) C, H, N.

9-{2-[2,4-*cis*-4-(3-Chlorophenyl)-2-oxo-1,3,2-dioxaphosphorinan-2-yl]methoxyethyl}adenine (7). Mp 200–204 °C. TLC: R_f = 0.39 (SiO_2, 1:9 MeOH/CH_2Cl_2). ^{1}H NMR (200 Hz, DMSO-d_6): δ 8.09 (s, 1 H), 8.07 (s, 1 H), 7.44–7.34 (m, 2 H), 7.26−7.05 (m, 2 H), 5.64–5.52 (m, 1 H), 4.48–3.80 (m, 8 H), 3.28−3.24 (m, 2 H), 2.04–1.80 (m, 2 H). Anal. Calcd for $C_{17}H_{19}ClN_5O_4P$: C, 48.18; H, 4.52; N, 16.53. Found: C, 48.27; H, 4.37; N, 16.47.

9-{2-[2,4-*trans*-4-(3-Chlorophenyl)-2-oxo-1,3,2-dioxaphosphorinan-2-yl]methoxyethyl}adenine (8). Mp 197–199 °C. TLC: R_f = 0.35 (SiO_2, 15% MeOH in CH_2Cl_2). ^{1}H NMR (200 Hz, DMSO-d_6): δ 8.22 (s, 1 H), 8.10 (s, 1 H), 7.46–7.14 (m, 6 H), 4.50–3.80 (m, 8 H), 5.18 (d, J = 12 Hz, 1 H) 2.20–1.70 (m, 2 H). Anal. Calcd for $C_{17}H_{19}ClN_5O_4P$: C, 48.18; H, 4.52; N, 16.53. Found: C, 48.19; H, 4.68; N, 16.22.

Synthesis of Chiral Diols via Resolution. (±)-1-(1,3-Bistrimethylsilyloxypropyl)-3-chlorobenzene. To a solution of racemic diol (**6h**) (10 g, 53.7 mmole) in THF (55 mL) was added hexamethyldisilazide (18.2 g, 112.8 mmol) followed by a catalytic amount of trimethylsilyltriflate (2–3 drops). After being stirred at room temperature for 1 h, the mixture was diluted with hexane (200 mL) and washed with ice-cold water (2 × 50 mL). The organic layer was then dried over magnesium sulfate, filtered, and concentrated. The resulting disilylether was purified by chromatography (hexane/ethyl acetate (10:1)) or, if sufficiently pure, used crude in the next reaction. Chromatography gave the pure disilyl ether as a colorless oil (17.7 g, 100%). R_f = 0.85 (hexane/ethyl acetate (4:1)). ^{1}H NMR (200 Hz, $CDCl_3$): δ 7.15–7.35 (m, 4H), 4.80 (dd, J = 8.1, 6.5 Hz, 1H), 3.43–3.77 (m, 2H), 1.74–1.94 (m, 2H), 0.10 (s, 9H), 0.02 (s, 9H).

(2*R*,7*S*,10*R*)-2-(3-Chlorophenyl)-7-isopropyl-10-methyl-1,5-dioxa-spiro[5.5]undecane and (2*S*,7*S*,10*R*)-2-(3-Chlorophenyl)-7-isopropyl-10-methyl-1,5-dioxa-spiro[5.5]undecane. To a solution of disilylether (17.7 g, 53.7 mmol) and (−)-menthone (9.1 g, 59.1 mmol) in dichloromethane (110 mL) at –40 °C was slowly added trimethylsilyltriflate (1.3 g, 5.9 mmol). The light-yellow mixture was then kept at –50 to –60 °C for 48 h, at which time pyridine was added to quench the reaction. After warming to room temperature, the crude mixture was diluted with hexane (200 mL) and washed with saturated aqueous sodium bicarbonate (2 × 75 mL). The two diastereomeric ketals were separated by chromatography on silica gel (hexane/dichloromethane gradient (12:1 to 6:1)). 2-(*R*)-spiroketal: R_f = 0.42 (hexane/dichloromethane (9:1)), waxy solid (7.33 g). ^{1}H NMR (200 Hz, $CDCl_3$): δ 7.20–7.40 (m, 4H), 5.01 (dd, J = 12.2, 3.2 Hz, 1H), 4.01 (td, J = 12.2, 2.6 Hz, 1H), 3.89 (m, 1H), 2.81 (dm, J = 12.2 Hz), 2.59 (sep d, J = 7.7, 1.3 Hz, 1H), 1.28–1.90 (m, 9H), 0.98 (d, J = 7.7 Hz, 3H), 0.97 (d, J = 7.7 Hz, 3H), 0.92 (d, J = 7.7 Hz, 3H). 2-(*S*)-spiroketal: R_f = 0.21 (hexane/dichloromethane (9:1)), oil (7.50 g). ^{1}H NMR (200 Hz, $CDCl_3$): δ 7.20–7.40 (m, 4H), 4.83 (dd, J = 11.6, 3.2 Hz, 1H), 4.23 (td, J = 12.2, 3.2 Hz, 1H), 3.95 (m, 1H), 2.82 (dm, J = 12.9 Hz, 1H), 2.50 (sep d, J = 7.1, 2.3 Hz, 1H), 1.2–2.0 (m, 9H), 0.99 (d, J = 7.1 Hz, 3H), 0.95 (d, J = 7.1 Hz, 3H), 0.90 (d, J = 7.1 Hz, 3H).

(*R*)-(+)-3-(3-Chlorophenyl)-1,3-propanediol (11) and (*S*)-(−)-3-(3-chlorophenyl)-1,3-propanediol (12). The separated ketals (7.33 and 7.50 g, respectively) were each hydrolyzed by adding a catalytic amount of concentrated hydrochloric acid (15 drops) to a methanol (90 mL) solution of each. After the mixture was stirred overnight at room temperature, the methanol was removed under vacuum and the residue was dissolved in water and extracted with ethyl acetate (4 × 40 mL). The resolved diols were further purified by chromatography on silica gel (hexane/ethyl acetate gradient (2:1 to 1:1.5)). (*R*)-(+)-diol: 3.77 g (75.4% from racemic diol based on 50% being theory), oil. $[\alpha]_D$ +52.5° (*c* 0.105, $CHCl_3$). R_f = 0.31 (hexane/ethyl acetate (1:1). ^{1}H NMR (200 Hz, $CDCl_3$): δ 7.18–7.40 (m, 4H), 4.90–5.00 (dd, J = 6.8, 6.1 Hz, 1H), 3.85 (t, J = 5.8 Hz, 2H), 2.40 (s, 2H), 1.90–2.00 (m, 2H). (*S*)-(−)-diol: 3.32 g (66.4% from racemic diol based on 50% being theory), oil. $[\alpha]_D$ −52.8° (*c* 0.105, $CHCl_3$). R_f = 0.31 (hexane/ethyl acetate (1:1). ^{1}H NMR (200 Hz, $CDCl_3$): δ 7.18- 7.40 (m, 4H), 4.90–5.00 (dd, J = 6.8, 6.1 Hz, 1H), 3.85 (t, J = 5.8 Hz, 2H), 2.40 (s, 2H), 1.90–2.00 (m, 2H).

2*R*,4*S*-(+)-9-{2-[4-(3-Chlorophenyl)-2-oxo-1,3,2-dioxaphosphorinan-2-yl]methoxyethyl}adenine (14). A 250 mL flask was charged with ADV (2.50 g, 9.16 mmol), dichloromethane (70 mL), and *N*,*N*-diethylformamide (1.0 g, 9.89 mmol). Oxalyl chloride (2.8 mL) was added slowly to the stirred reaction mixture. After the addition was complete (20 min), the vigorously stirred mixture was heated at reflux for 3 h. The reaction solution was then cooled to room temperature and concentrated in vacuo to a crude yellow foam. The resulting foam was dissolved in dichloromethane (50 mL) and cooled to 0 °C, and pyridine (1.5 mL, 18.32 mmol) was then added slowly to the stirred solution. After the addition was complete (5 min), the resulting pale-orange solution was stored at ice bath temperature until used (30 min). A 500 mL two-neck round-bottom flask was equipped with a thermocouple probe magnetic stirrer and addition funnel (125 mL). The flask was charged with (*S*)-(−)-(3-chlorophenyl)-1,3-propanediol (**12**) (1.7 g, 9.16 mmol), dichloromethane (30 mL), and triethylamine (7.4 mL, 58.13 mmol). The reaction solution was cooled to –78 °C. The above prepared dichloridate solution was charged to the addition funnel, then added slowly with stirring, maintaining the reaction temperature at <−67 °C. After the addition of the dichloridate was complete (45 min), the cooling bath was removed and the stirred mixture was warmed to 0 °C over 30 min. At this point, the crude reaction mixture was washed with water (80 mL). The organic layer was dried over magnesium sulfate, filtered, and concentrated under reduced pressure to provide a brown-orange tar. The crude tar was then dissolved in ethanol (30 mL). Acetic acid (2.3 mL) was added, and the red solution was heated at reflux for 2 h. The reaction mixture was cooled to room temperature and then concentrated in vacuo to a red-orange tar. The crude tar was then dissolved in ethyl acetate (20 mL) and stirred at room temperature for 2.5 h during which time a solid formed. After filtration, the material was split into two streams: solid (55:45, cis/trans) and filtrate (95.5:4.5, cis/trans). The filtrate was concentrated to an oil, dissolved in ethyl acetate (40 mL), and allowed to stand overnight at room temperature without stirring. The solid was suspended in ethyl acetate (20 mL), heated at reflux for 1 h, and then stirred overnight at room temperature. Upon filtration of both streams, the filtrates were combined and concentrated in vacuo. Flash chromatography on silica gel (dichloromethane/methanol (12:1)) gave the title compound as a red-orange oil. An additional chromatography (ethyl acetate/methanol gradient (10:1 to 4:1)) gave the pure prodrug as a light-yellow foam (1.56 g, 40% yield, 97% cis/3% trans). $[\alpha]_D$ + 44.9° (*c* 0.978, MeOH). ^{1}H NMR (200 Hz, DMSO-d_6): δ 8.08 (s, 1H), 8.07 (s, 1H), 7.35–7.43 (m, 3H), 7.19 (br s, 1H), 5.57 (br dd, J = 9.5, 3.4 Hz, 1H), 4.26- 4.55 (m, 3H), 4.05–4.26 (m, 1H), 3.83–4.01 (m, 4H), 1.75–2.04 (m, 2H), Anal. ($C_{17}H_{19}ClN_5O_4P$) C, H, N.

(2*S*,4*R*)-(−)-9-{2-[4-(3-Chlorophenyl)-2-oxo-1,3,2-dioxaphosphorinan-2-yl]methoxyethyl}adenine (13). The 4-(*R*) prodrug stereoisomer (**11**) was synthesized on identical scale and in identical

fashion except for the final purification. Here, the crude red-orange tar was also dissolved in ethyl acetate (40 mL), stirred 2.5 h at room temperature, and filtered. However, only the solid was carried forward while the filtrate (cis-prodrug-containing fraction) was set aside and saved. The solid fraction was extracted with ethyl acetate two more times (40 mL each) by first refluxing the suspension for 1 h followed by stirring for 2 h at room temperature and filtering. After the third extraction, the combined filtrates were concentrated in vacuo to a brown foam. The foam was further purified by flash chromatography (dichloromethane/methanol (11:1)), which gave the title compound as a brown foam. An additional chromatography (ethyl acetate/methanol (6:1)) was required to remove the color, and in this way the title compound was obtained as a light-yellow foam (1.28 g, 33% yield, 97% cis/3% trans). $[\alpha]_D$ −43.2° (*c* 0.978, MeOH). ^{1}H NMR (200 Hz, DMSO-d_6): δ 8.08 (s, 1H), 8.07 (s, 1H), 7.35–7.43 (m, 3H), 7.19 (br s, 1H), 5.57 (br dd, J = 9.5, 3.4 Hz, 1H), 4.26–4.55 (m, 3H), 4.05–4.26 (m, 1H), 3.83–4.01 (m, 4H), 1.75–2.04 (m, 2H), Anal. ($C_{17}H_{19}ClN_5O_4P$).

Biological Methods. In Vitro Activation of ADV Prodrug Analogues by Rat Liver Microsomes. Prodrugs (25 and 250 μM) were tested for activation by liver microsomes isolated from rats induced with dexamethasone to enhance CYP3A activity (Human Biologics Inc., Phoenix AZ). Reactions were conducted in 0.1 M KH_2PO_4, pH 7.4, in the presence of 2 mM NADPH and liver microsomes (1 mg/mL). Reaction mixtures were incubated for 5 min in an Eppendorf Thermomixer 5436 (37 °C, speed 6). Reactions were terminated by the addition of 1.5 volumes of methanol. The resulting extracts were clarified by centrifugation at 14 000 rpm in an Eppendorf microfuge (20 min). The supernatants (200 μL) were evaporated under vacuum and heat to dryness before resuspending in 80 μL of buffer A (see below) for ADV analysis. Spiked standards of 9-[2-(phosophonomethoxy)ethyl]adenine (ADV) were prepared in the same reaction mixture and processed in an identical fashion. After resuspension, samples were analyzed by reverse-phase HPLC (Altima C-18 column) with use of an ion-pairing buffer (buffer A) consisting of 10 mM ammonium phosphate and 2.5 mM octyltriethylammonium phosphate, pH 5.5. Samples were loaded in buffer A and eluted with a methanol gradient from 40% to 80% over 20 min. Detection was at 265 nm.

ADV-DP Accumulation in Hepatocytes after Incubation with ADV Prodrug Analogues. Isolated hepatocytes were prepared from fed Sprague-Dawley rats (250–300 g) according to the procedure of Berry and Friend[21] as modified by Groen.[22] Hepatocytes (60 mg/mL wet weight, >85% trypan blue viability) were incubated in 2 mL of Krebs bicarbonate buffer containing 20 mM glucose and 1 mg/mL BSA for 4 h in the presence of 250 μM ADV prodrugs (from 25 mM stock solutions of prodrugs in methanol). At prespecified time points throughout the incubation (0, 1, 2, 4 h), aliquots (400 μL) of the cell suspension were taken and centrifuged through a silicon/mineral oil layer into 10% perchloric acid to extract intracellular nucleotides. The acidic cell extracts were neutralized with 0.3 volumes of ice-cold 3 M KOH/3 M KH_2CO_3 before evaluating ADV-DP (Trilink Technologies, San Diego, CA) levels by anion exchange HPLC (Hewlett-Packard 1050) using a Whatman Partisphere SAX (5 μm, 4.6 × 125 mm) column. Samples were loaded onto the column in 0.3 M ammonium phosphate buffer, pH 3.5, eluted from the column with a gradient to 0.8 M ammonium phosphate, pH 3.5, and monitored by UV absorbance at 265 nm.

ADV-DP Accumulation in Liver Following iv and Oral Administration of ADV Prodrug to Fasted Rats. Following iv bolus or oral administration of 30 mg/kg prodrug (ADV equivalents) to fasted male Sprague-Dawley rats, livers were freeze-clamped at prespecified times (at 8 h postdose for screening purposes and at 20 min and 1, 3, 5, 8, 12, and 24 h postdose for pharmacokinetic evaluations) and homogenized in ice-cold 0.4 N perchloric acid. The acid homogenates were clarified by centrifugation, and the supernatants were neutralized as previously described. Liver concentrations of ADV-DP were determined by HPLC as previously described.

Assessment of the Pharmacokinetics and Oral Bioavailability of ADV Prodrugs in Rat. The relative oral bioavailability of ADV prodrugs was assessed by two methods in rats: (1) comparison of the dose-normalized area-under-the-curve (AUC) values of the liver ADV-DP concentration−time profiles following oral and iv bolus administration of the prodrug and (2) comparison of the percent of dose of ADV prodrug and metabolites excreted in the urine up to 48 h following oral and iv bolus administration of prodrug. The AUC values of liver ADV-DP were determined noncompartmentally by trapezoidal summation of the temporal liver concentration plots from the accumulation studies. The percent of dose of ADV prodrug and metabolites excreted in urine was assessed by a modification of an HPLC assay.[20a] Aliquots (0.25–0.5 mL) of urine samples and spiked standards were incubated with an equal volume of 17% (v/v) of 50% chloroacetaldehyde in 100 mM sodium acetate, pH 4.5, at 50 °C for 4 h. Following centrifugation, the supernatants were evaporated to dryness and reconstituted with mobile phase for chromatographic analysis. The derivatized ADV prodrug and metabolites were analyzed by an HPLC system (Hewlett-Packard 1090) consisting of a Beckman Ultrasphere ODS 4.6 mm × 150 mm (5 μm) column developed with a 20 min gradient of 0–30% acetonitrile in 20 mM potassium phosphate, pH 6.2. Fluorescence was monitored at excitation and emission wavelengths of 240 and 420 nm, respectively. The column temperature was 40 °C, and the flow rate was 1.5 mL/min. The analytes were detected and quantified by comparison to an authentic standard prepared in control rat urine.

The plasma pharmacokinetics of ADP and cyclic 1-aryl-1,3-propanyl prodrugs of ADV were evaluated following oral administration of 30 mg/kg (ADV equivalents) to rats (n = 4). Intact prodrug and the metabolite 9-[2-(phosophonomethoxy)ethyl]adenine (ADV) and the respective monoacids were measured in the plasma at 0 (predose), 0.33, 1, 3, 5, 8, and 12 h postdose. Plasma ADV was determined by a modified HPLC fluorescence assay.[20b] Plasma (100 μL) samples were extracted with 2 volumes of 0.1% TFA in acetonitrile. Following centrifugation to remove the precipitate, the supernatant was evaporated to dryness. The dried plasma was reconstituted with 200 μL of the derivatization cocktail consisting of 0.34% chloroacetaldehyde in 100 mM sodium acetate, pH 4.5, and incubated at 95 °C for 40 min. The samples were evaporated to dryness and reconstituted with mobile phase for analysis by HPLC as previously described. The pharmacokinetic parameters were calculated on the basis of noncompartmental analysis of the concentration−time profile.

Determination of Pharmacokinetics and Oral Bioavailability of 7 in Dog. The pharmacokinetics of prodrug **7** was assessed in dogs. Following an iv bolus administration of 5 mg/kg (ADV equivalents) of prodrug **7** in PEG-400 to male beagle dogs (n = 4), blood (heparin) samples were collected at the following times: 5, 15, 30, and 45 min and 1, 2, 4, 6, 8, 12, and 24 h postdose and then centrifuged to obtain plasma. After a 72 h washout period, the same dogs received an oral dose of 25 mg/kg (ADV equivalents) and blood was sampled as before. In addition, urine was collected over the following intervals: 0–12, 12–24, 24–48, and 48–72 h for each occasion. An API 2000 triple quadrupole mass spectrometer (Applied Biosystems, Inc., Foster City, CA) was used to quantify intact **7** and ADV in plasma and urine samples using a positive multiple reaction monitoring (MRM) mode technique. Plasma and urine samples were extracted with 3 volumes of methanol containing 2% v/v acetic acid. The extracts were clarified by centrifugation, and an amount of 10 μL of supernant was injected in duplicate onto a Phenomenex column (Luna C8, 5 μm, 50 mm × 2.0 mm) for separation. Mobile phase A contained a buffer of 10 mM propionic acid and 5 mM *N*,*N*-dimethylhexylamine in deionized water, and mobile phase B contained 80% methanol in deionized water. A gradient system was used at a constant flow rate of 0.2 mL/min: starting at 0% B for 2.5 min, then increasing to 80% B over 0.5 min and holding for 4 min, then decreasing to 0% B over 0.5 min followed by re-equilibration at 0% B for 2.5 min. Protonated molecular ions were selected for collisional activation, and fragment ions were used for quantitation. Concentrations of **7** (Q1/Q3, 424.04/

150.93) and ADV (274.10/136.00) were determined by comparison to plasma and urine standards. Pharmacokinetic parameters were determined by noncompartmental analysis.

The oral bioavailability of **7** was determined by comparison of the AUC values of the dose-normalized plasma concentration−time profile of ADV and by comparison of the extent of renal excretion of ADV following oral and iv bolus administration of **7**.

Tissue Distribution of ADV Prodrugs and Their Metabolites after Oral Administration of Radiolabeled Compound 7 and ADP. Compound **7** [adenine-2, 8-^{3}H] or ADP [adenine-8-^{3}H] (Moravek Biochemicals, Brea, CA) was administered at 30 mg/kg (ADV equivalents) to fasted rats by oral gavage. Total tritium counts were analyzed in solubilized samples of liver organ, kidney, plasma, urine, red blood cells, small intestine, as well as small intestine contents and feces obtained at various time points over 24 h. Liver specificity was assessed by comparison of the temporal profiles of total tritium in liver versus kidney, small intestine, plasma, and red blood cells. Metabolite profiles in perchloric acid extracts of liver, kidney, small intestine, feces and urine were analyzed by the previously described HPLC assay with monitoring with an online radioactivity detector (Radiomatic, Packard Instruments).

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Supporting Information Available: NMR and elemental analysis data for all final compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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