Synthesis and Characterization of a Novel Liver-Targeted Prodrug of Cytosine-1-β-D-arabinofuranoside Monophosphate for the Treatment of Hepatocellular Carcinoma

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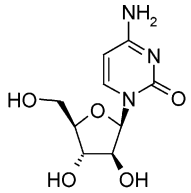
Cytotoxic nucleosides have proven to be ineffective for the treatment of hepatocellular carcinoma (HCC) due, in part, to their inadequate conversion to their active nucleoside triphosphates (NTP) in the liver tumor and high conversion in other tissues. These characteristics lead to poor efficacy, high toxicity, and a drug class associated with an unacceptable therapeutic index. Cyclic 1-aryl-1,3-propanyl phosphate prodrugs selectively release the monophosphate of a nucleoside (NMP) into CYP3A4-expressing cells, such as hepatocytes, while leaving the prodrug intact in plasma and extrahepatic tissues. This prodrug strategy was applied to the monophosphate of the well-known cytotoxic nucleoside cytosine-1-β-D-arabinofuranoside (cytarabine, araC). Compound **19*S*** (MB07133), in mice, achieves good liver targeting compared to araC, generating >19-fold higher cytarabine triphosphate (araCTP) levels in the liver than levels of araC in the plasma and >12-fold higher araCTP levels in the liver than in the bone marrow, representing a >120-fold and >28-fold improvement, respectively, over araC administration.

Introduction

Hepatocellular carcinoma (HCC)[a] remains a poorly treated cancer and, as the fifth most lethal malignancy worldwide, afflicts >500,000 people each year with survival rates of only 23% and <5% at 1 and 5 years, respectively.[1] One reason for the high mortality rate is that treatment options are limited with no chemotherapeutic regimen currently approved for the treatment of unresectable HCC, which represents >80% of cases. The only truly successful therapy is liver transplantation, which is limited due to the paucity of available organs. The difficulties associated with the chemotherapeutic treatment of HCC may stem from the presence of a multitude of drug resistance mechanisms that exist in the liver and correspondingly the primary liver tumor. These include mechanisms for rapid drug metabolism, including both primary and secondary metabolism pathways. Moreover, a vast array of xenobiotics and drug transport systems exist in the liver which are well-known to transport certain drugs out of the tumor (e.g., p-glycoprotein). Last, there exists a high concentration of agents (e.g., glutathione) that inactivate certain oncolytic drugs. These properties limit the uptake and retention of oncolytic drugs in the primary liver tumor and therefore result in the need for very high doses to achieve an antitumor response, which then leads to extrahepatic toxicity.

One class of drugs that typically is ineffective against solid tumors and, more specifically, HCC is the nucleoside oncolytic drug class. The reason for their inability to inhibit cell proliferation may stem from the low levels of specific nucleoside kinases in hepatocytes, whether normal or tumorigenic, which leads to poor conversion of oncolytic nucleosides to biologically active nucleoside triphosphates (NTPs). Accordingly, bypassing the nucleoside kinase may result in high intracellular NTP levels, since NTPs are usually not subject to metabolism (other than dephosphorylation) or export from cells.

Chart 1



Cytosine-1-β-D-arabinofuranoside
(Cytarabine, araC)

Cytosine-1-β-D-arabinofuranoside (cytarabine, araC, Chart 1) is a nucleoside oncolytic currently used clinically for the treatment of acute myelocytic leukemia (AML). Like most nucleosides, araC is inactive and requires phosphorylation to its triphosphate, araCTP, to exert its antineoplastic activity by inhibiting DNA polymerase[2] or by incorporation into elongating DNA strands[3] in a cell cycle dependent manner. However, the use of araC is not suitable to treat other types of cancers, as it is inactive against solid tumors due to poor phosphorylation and conversion into its triphosphate, araCTP.[4] In addition, any attempts to reach therapeutic levels of araCTP in tissues with low kinase activity, such as the liver, by raising the dose are thwarted by dose-limiting myelosuppresion, which arises from rapid phosphorylation and conversion into araCTP in the otherwise healthy bone marrow cells.

Recently we have reported our findings on a new class of prodrugs (**1**, Figure 1), which have proven capable of selectively delivering nucleoside monophosphates (NMPs) to the liver.[5,6] These prodrugs are activated to their NMPs **4**, via hydroxylation at the benzylic carbon by the cytochrome P_{450} isozyme-3A4

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[a] Abbreviations: HCC, hepatocellular carcinoma; cytarabine, araC, cytosine-1-β-D-arabinofuranoside; araCMP, cytosine-1-β-D-arabinofuranoside monophosphate; araCTP, cytosine-1-β-D-arabinofuranoside triphosphate; CYP3A4, cytochrome P_{450}-3A4; adefovir, 9-(2-phosphonylmethoxyethyl)adenine; pradefovir, 9*H*-purin-6-amine-9-[2-[[(2*R*,4*S*)-4-(3-chlorophenyl)-2-oxido-1,3,2-dioxaphosphorinan-2-yl]methoxy]ethyl]; HBV, hepatitis B virus.

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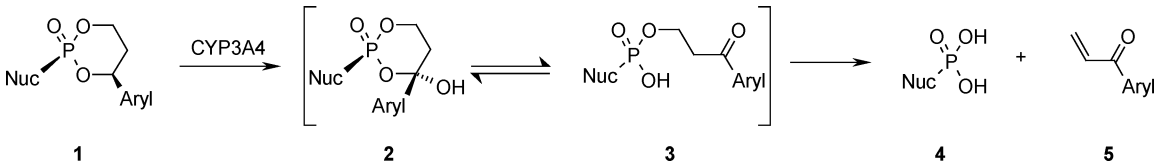
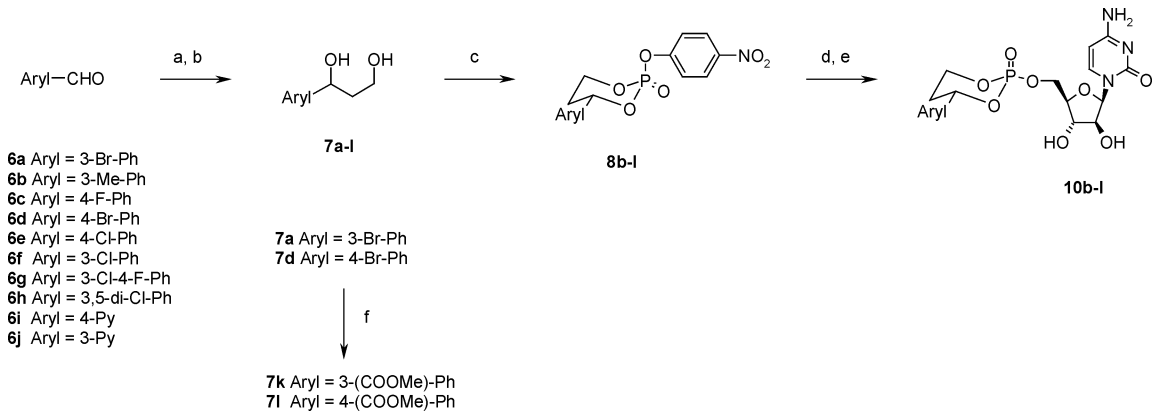


Figure 1. Activation mechanism of cyclic 1-(aryl)-1,3-propanyl prodrugs.

Scheme 1[a]



[a] Reagents: (a) LDA, EtOAc, THF, −78 °C; (b) $LiAlH_4$, ether, 0 °C or $NaBH_4$, EtOH, reflux, 24−83% for 2 steps; (c) (i) $Cl_2P(O)O(4\text{-}NO_2\text{-}Ph)$, Et_3N, THF, rt; (ii) $NaO(4\text{-}NO_2\text{-}Ph)$, rt, 23−92%; (d) *t*-BuMgCl, 2′,3′-di-*O*-TBS-araC (**9**),[5] THF, rt; (e) Et_4NF, THF or TBAF, 13−59% for 2 steps; (f) $Pd(PPh_3)_4$, CO, MeOH, Et_3N, sealed bomb, 85 °C, 66−87%.

(CYP3A4) and subsequent elimination of the β-nucleoside-monophosphate. In this report, the cyclic 1-(3-chlorophenyl)-1,3-propanyl prodrug (**10f**) of cytarabine monophosphate (araCMP) was shown to generate more than 80-fold more araCTP in rat hepatocytes compared to araC.[5] As the successful application of this prodrug strategy to araC for the treatment of HCC is highly dependent on the presence of CYP3A4 in tumor cells, there are numerous reports showing that CYP3A4 is retained in primary HCC tumors.[7] Moreover, delivery of araCTP to the normal, healthy liver, as well as the diseased liver, is not expected to be cytotoxic because normal hepatocytes proliferate at a much slower rate than liver tumor cells and araCTP exerts its efficacy during cell replication.

Intracellular araCTP is relatively short-lived. Consequently, cytarabine is administered to leukemic patients by continuous intravenous (i.v.) infusion, which ensures the presence of araCTP within individual cells during the time that they enter into the proliferative phase.[8] Similarly, we sought an i.v. formulation of our liver-targeted prodrug of araCMP and therefore set out to identify a prodrug with good aqueous solubility and stability suitable for parenteral administration. In this report we describe the identification of the liver-targeted prodrug of araCMP, **19*S*** (MB07133), with good aqueous solution characteristics, high production of araCTP in the liver, and reduced extrahepatic exposure.

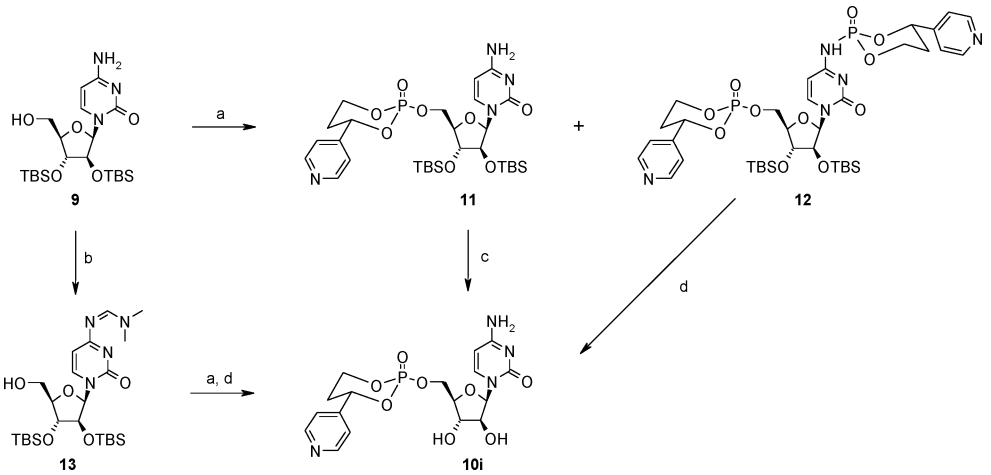
Results

Chemistry. Cyclic *cis*-1-(aryl)-1,3-propanyl prodrugs of cytarabine **10b**−**l** (Scheme 1) were stereospecifically prepared by an S_N2-like reaction between 2′,3′-di-*O*-*tert*-butyldimethylsilyl-cytarabine (**9**, 2′,3′-di-*O*-TBS-araC) and the corresponding *trans*-(4-nitrophenyl)-phosphate reagents **8b**−**l** as previously described.[5,9] 1-Aryl-1,3-propane diols **7a**−**j** were synthesized as racemic mixtures via the two-step sequence aldol condensation/reduction in 24−83% overall yield. Palladium-catalyzed carbonylation of bromophenyl diols **7a**,**d** gave the corresponding methyl benzoate diols **7k**,**l** in 66−87% yield. Reaction of diols **7b**−**l** with 4-nitrophenyl phosphorodichloridate followed by epimerization of the *cis*-phosphate to the thermodynamically more stable *trans*-phosphate[10] with sodium-nitrophenoxide afforded *trans*-phosphate reagents **8b**−**l** in 23−92% yield. Phosphorylation of the 5′-magnesium-alkoxide of protected cytarabine **9**[5] with *trans*-phosphate reagents **8b**−**l**, followed by fluoride or acid deprotection, stereospecifically gave *cis*-prodrugs **10b**−**l** as a mixture of diastereomers at the C4-carbon of the dioxaphosphorinane ring and with minimal epimerization at the phosphorus center (<5%) in 13−59% overall yield.

Prodrug **10i** was selected as the lead for further testing. However, before proceeding toward the evaluation of single stereoisomers, a more efficient synthesis was needed. While prodrug formation with the other phosphorylating reagents was accomplished in good yields, phosphorylation with the 4-pyridyl phosphate reagent **8i** was slow and yields decreased significantly as the scale increased. Addition of excess phosphorylating reagent and further increasing the temperature of the reaction further decreased the yield due to the formation of diphosphorylated cytarabine nucleoside **12** (Scheme 2). The phosphoramidate moiety could be selectively cleaved with 70% aqueous trifluoroacetic acid (TFA) at 60 °C, with concomitant removal of the silyl groups, to produce the desired prodrug **10i** in 50% yield, but this process was judged unsatisfactory. A significant improvement was made by protecting the 4-amino group as a dimethylformamidine (DMF dimethylacetal, 99%), which led to the smooth formation of prodrug **10i**, after removal of all the protecting groups with TFA, in 50−65% overall yield.

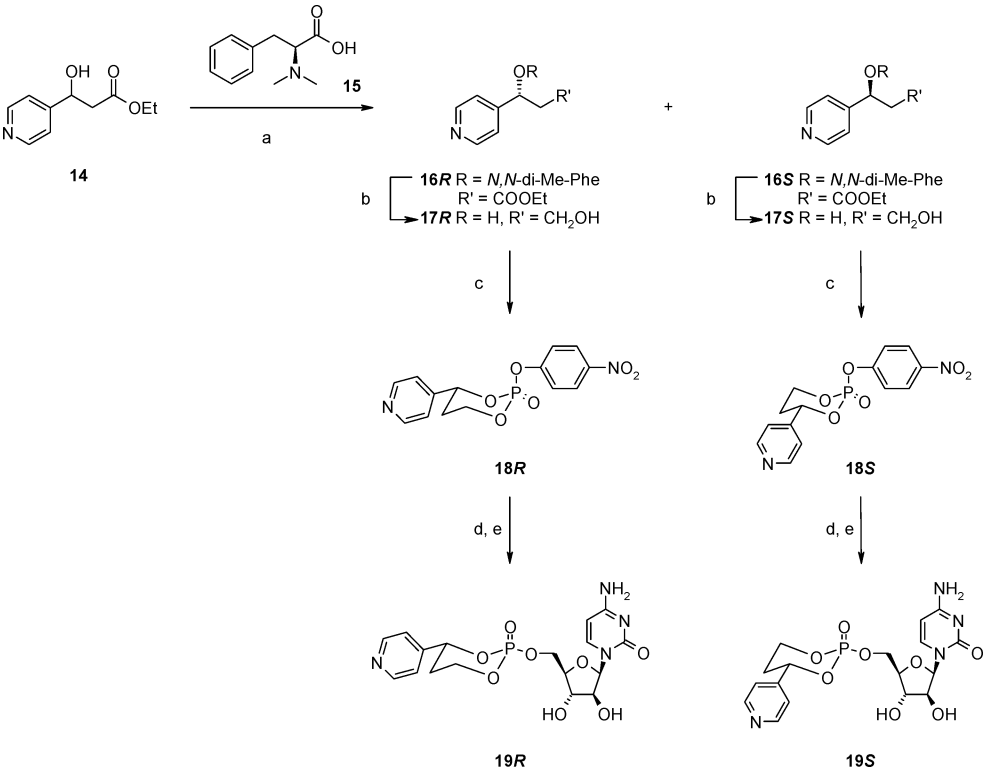
Synthesis of both stereoisomers of prodrug **10i** was accomplished by first resolving racemic diol **7i**. While high optical purity was obtained in the resolution of diol **7f** with (−)-menthone,[5] chromatographic separation of acetals of diol **7i** proved to be very difficult. Alternatively, esterification of racemic β-hydroxy ester **14** with *N*,*N*-dimethyl-phenylalanine (**15**, Scheme 3) led to an easier separation of both diastereomers in high optical purities. Reduction of phenylalanates **16*R*** and **16*S*** gave desired diols **17*R*** and **17*S***, respectively. Stereochemical assignments were initially made on the basis of the comparison of the sign of the optical rotation of **17*S*** with the

Scheme 2[a]



[a] Reagents: (a) *t*-BuMgCl, THF, rt, **11** (13%), **12** (20%); from **13** (70−80%); (b) DMF-dimethyl acetal, pyridine, rt, 99%; (c) Et_4NF, THF, 40%; (d) 70% aq TFA, 60 °C, 80%.

Scheme 3[a]



[a] Reagents: (a) 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide·HCl (EDCI), 4-dimethylaminopyridine (DMAP), CH_2Cl_2, **16*R*** (77%), **16*S*** (83%); (b) $LiAlH_4$, ether, **17*R*** (33%), **17*S*** (66%); (c) (i) $Cl_2P(O)O(4\text{-}NO_2\text{-}Ph)$, pyridine, THF, rt; (ii) $NaO(4\text{-}NO_2\text{-}Ph)$, rt, **18*R*** (60%), **18*S*** (63%); (d) *t*-BuMgCl, **13**, THF, rt; (e) 70% aq TFA, 60 °C, **19*R*** (53%), **19*S*** (65%) for 2 steps.

one of commercially available (−)-(*S*)-1-phenyl-1,3-propanediol. While formation of phosphorylating reagents **18*R*** and **18*S*** using the previously described reaction conditions led to a significant loss of optical purity at the carbon stereocenter, high optical purity was achieved by substituting pyridine for triethylamine. Phosphorylation of protected cytarabine **13** with phosphate **18*R*** or **18*S*** followed by TFA deprotection produced *cis*-prodrugs **19*R*** and **19*S*** in high optical purities. An unambiguous stereochemical assignment was made following single-crystal X-ray structure determination of compound **19*S***. An ORTEP diagram is shown in Figure 2. The absolute stereochemistry of the benzylic carbon on the prodrug moiety was established as *S*, as initially assigned. Most importantly, the X-ray crystal structure clearly establishes the relative stereochemistry between the pyridyl ring and the nucleoside as *cis*.

Biological Results. Cytarabine and prodrugs of cytarabine were evaluated in freshly isolated suspended rat hepatocytes over 4 h for production of araCTP as previously described.[5] Cytarabine produced very little araCTP while prodrugs **10b**−**l** produced substantially higher levels of araCTP with relative amounts depending on the aryl substitution (Table 1). However, no correlation was observed between the electronic effects of the substituents and the amounts of araCTP produced.

The aqueous stability was clearly related to the electronic effects of the substituents. As the electron density of the aryl group decreased, the aqueous stability increased. This effect was

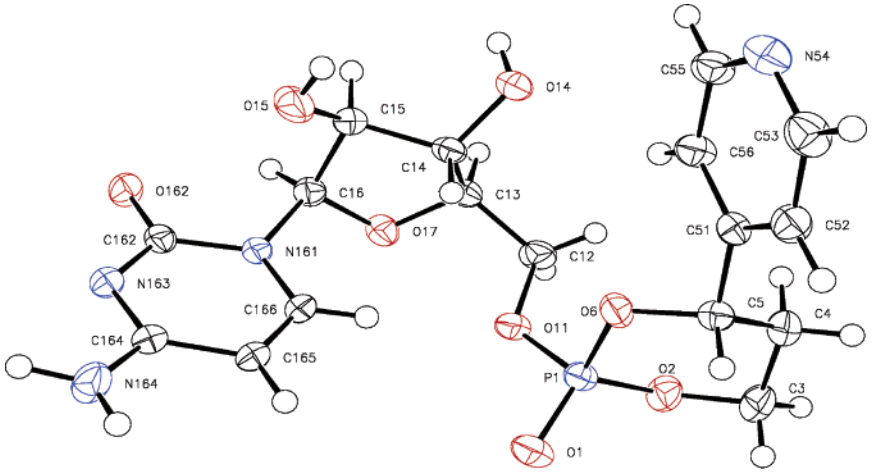


Figure 2. ORTEP Drawing of compound **19*S***. Atoms are represented by 50% probability anisotropic thermal ellipsoids.

Table 1. Generation of AraCTP in Freshly Isolated Rat Hepatocytes and Aqueous Prodrug Stability and Solubility

		hepatocyte activation	stability, % remaining[c]		water solubility (mg/mL)	
cmpd	aryl[a]	AUC_{0-4h} araCTP (nmol·h/g) [b]	at 24 h	at 72 h	pH 4[e]	pH 7.4[f]
araC		5				
10b	3-Me-Ph	44				
10c	4-F-Ph	44				
10d	4-Br-Ph	159	9	4		
10e	4-Cl-Ph	197	5	4		
10f[d]	3-Cl-Ph	398	63	21	3.7	1.7
10g	3-Cl-4-F-Ph	434	74	52	6.7	3.2
10h	3,5-di-Cl-Ph	558	82	76	1.1	0.5
10i	4-pyridyl	95	92	90	>200	>200
10j	3-pyridyl	84	99	83	172	176
10k	3-Ph-COOMe	170	60	23	10.3	7.2
10l	4-Ph-COOMe	33	84	61	7.4	5.7
19*R*	(*R*)-4-pyridyl	86	>99	>97	37.9	16.7
19*S*	(*S*)-4-pyridyl	99	>99	>95	23.9	7.1

[a] Diols **10b**−**l** are racemic. [b] Activation of 100 μM araCMP prodrugs or araC to araCTP in primary rat hepatocytes: area under the curve (AUC) 0−4 h. [c] Stability of a 100 μM solution of araCMP prodrugs in aqueous buffer at pH 7.4 at 37 °C. [d] See ref 5. [e] 100 mM citrate buffer pH 4. [f] 100 mM phosphate buffer pH 7.4.

Table 2. Comparative Tissue Distribution in Mice after i.p. Administration of 100 mg/kg araC Equivalents

	liver araCTP		plasma araC		bone marrow	
cmpd	peak (nmol/g)	AUC_{0-4h} (nmol/g·h)	peak (μM)	AUC_{0-4h} (μM·h)	peak (μM)	AUC_{0-4h} araCTP (nmol/g)[c]
19*R*	27	68.0	1.6	3.5	<3[b]	<12[b]
19*S*	70	148.8	3	7.6	<3[b]	<12[b]
araC[a]	7.6	<19.2[b]	150	121	19	46

[a] See ref 6. [b] Lower limit of quantitation (LOQ). [c] Area under the curve (AUC) 0−4 h.

further compounded by the position of the substituents on the ring. Compounds with electron-donating groups at the *meta*-position (e.g., **10f**, 63% prodrug remaining at 24 h) had greater stability than prodrugs with similar substitutions at the *para*-position (e.g., **10e**, 5% at 24 h), while compounds with electron-withdrawing groups at the *para*-position had improved stability compared to that of prodrugs with similar substitutions at the *meta*-position (e.g., **10l**, 84% at 24 h vs **10k**, 60%).

The aqueous solubility of relatively stable prodrugs was low (Table 1), and lowering the pH of the buffer solution to 4, to take advantage of the basic character of the cytosine base of cytarabine, showed only a small improvement. Addition of another chloro substituent to prodrug **10f** further decreased the solubility (**10h**) while addition of a fluoro substituent increased the solubility (**10f** vs **10g**). The pyridyl prodrugs **10i** and **10j**, on the other hand, showed very high solubility at both pH 4 and pH 7.4, presumably due to their greater hydrophilicity. Interestingly, the solubilities of the individual isomers **19*R*** and **19*S*** were significantly reduced relative to that observed with **10i.** This is not unduly surprising, since **10i** is a mixture of diastereomers (and likely amorphous), whereas **19*R*** and **19*S*** are isomerically pure (and likely crystalline). Most importantly, both **19*R*** and **19*S*** display solubility sufficient for intravenous administration.

Both diastereomeric *cis*-prodrugs **19*R*** and **19*S*** were tested *in vivo* in mice to compare their tissue distribution and assess their liver targeting indices relative to those of cytarabine. Intraperitoneal (i.p.) administration of 100 mg/kg araC equivalents of prodrugs **19*R*** and **19*S***, respectively, produced 68 and 149 nmol·h/g of araCTP in the liver, over 4 h, while administration of araC produced only 19.2 nmol·h/g[6] (Figure 3; Table 2). Plasma araC exposure was 3.5 μM·h for **19*R***, 7.6 μM·h for **19*S***, and 121 μM·h over 4 h following araC administration.[6] AraCTP levels in the bone marrow, the primary organ of araC toxicity, were below the limit of quantitation (<3 nmol·h/g) at all time points following prodrug administration and 46 nmol·h/g for araC.[6]

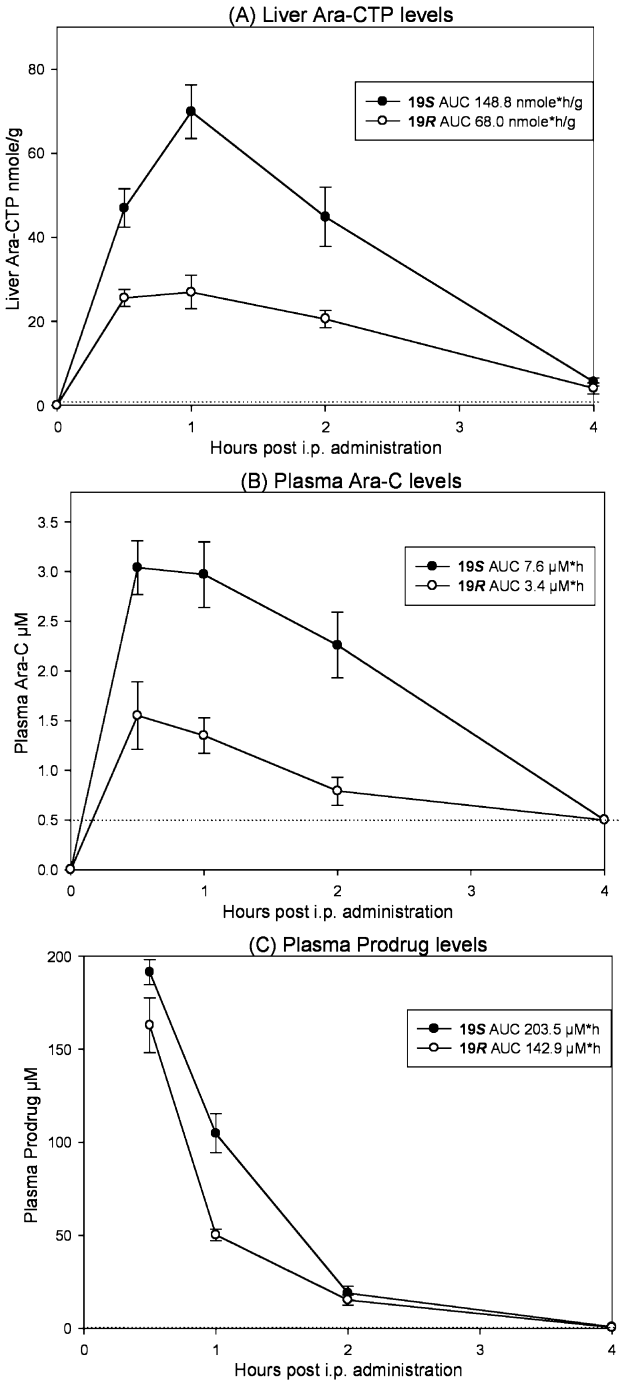


Figure 3. Tissue distribution studies in mice for compounds **19*R*** and **19*S***. (A) AraCTP levels were measured in liver, (B) araC and (C) prodrug levels were measured in plasma at times up to 4 h after i.p. administration of 100 mg/kg of araC equivalents.

Discussion

Cyclic 1-(aryl)-1,3-propanyl prodrugs of NMPs are used to increase NTP levels in the liver while decreasing NTP levels in extrahepatic tissues. Liver targeting has been demonstrated with a variety of structurally different compounds, including both nucleotide and nucleoside analogues. Pradefovir, a prodrug of the antihepatitis B virus (HBV) drug adefovir, is currently in Phase 2 clinical trials. Pradefovir showed excellent liver targeting in rats and monkeys[11] and, most recently, in humans[12] with chronic HBV infections. In this work, we have applied this prodrug strategy to araCMP in order to bypass the nucleoside kinase that is expressed at low levels in the liver and primary liver tumor while simultaneously limiting araC exposure to the bone marrow and gut epithelial cells.

Table 3. Liver Targeting Indexes

cmpd	AUC liver araCTP / AUC plasma araC	AUC liver araCTP / AUC bone marrow araCTP
araC	<0.16[a]	<0.42[a]
19*R*	19.4	>7[a]
19*S*	19.6	>12[a]

[a] Ratio estimated on the basis of the LOQ.

Efficient cleavage of cyclic 1-(aryl)-1,3-propanyl prodrugs of cytarabine was observed in freshly isolated rat hepatocytes. No correlation existed between the different rates of activation and the electronic properties of the activating aryl group. In contrast, a good correlation was observed between the electronic properties and the stability of the prodrug in aqueous media. Prodrugs bearing electron-withdrawing groups were more stable than prodrugs with electron-donating substituents. Furthermore, moving the electron-donating substituent from the *para*-position to the *meta*-position further improved the stability while the opposite effect was observed with electron-withdrawing groups. This is consistent with the S_N1 solvolysis of the benzylic phosphate bond being the primary source of prodrug instability in aqueous medium.[13] Aqueous solubility was poor for all substituted phenyl prodrugs. However, the pyridyl prodrugs **10i** and **10j** showed very good solubility, making them suitable for parenteral administration. On the basis of the superior stability of prodrug **10i** compared with **10j**, the diastereomeric mixture of 4-pyridyl prodrugs **10i** was selected for further characterization of its individual diastereomers.

Contrary to our previous experience with other prodrug single isomers of araC,[5] the two diastereomeric prodrugs **19*R*** and **19*S*** showed no major differences in their hepatocyte activation, aqueous stability, and aqueous solubility. However, a distinct advantage was observed for the *S*-isomer in liver araCTP levels *in vivo*. Liver araCTP exposure after administration of prodrug **19*S*** was twice the exposure generated by prodrug **19*R***. Both prodrugs generated small amounts of araC in the plasma, likely due to dephosphorylation of araCMP in the liver and leakage into the bloodstream. No detectable amount of araCTP was observed in the bone marrow upon administration of either prodrug while large amounts were found upon administration of araC.[6] Comparison of the liver-targeting indices, relative to historical araC levels,[6] clearly demonstrated the *in vivo* advantage of both prodrugs compared with araC in terms of both hepatic araCTP production and reduction of extrahepatic exposure to araC (Table 3).

Due to the higher levels of araCTP produced in the liver, prodrug **19*S*** was selected over prodrug **19*R*** as a lead compound for further evaluation of its potential as a parentally administered nucleoside oncolytic prodrug for the treatment of HCC.

Conclusions

A series of cyclic 1-(aryl)-1,3-propanyl prodrugs of cytarabine was synthesized and optimized for activation in freshly isolated, suspended rat hepatocytes and for aqueous stability and solubility. Both *cis*-isomeric prodrugs contained in the diastereomeric mixture of *cis*-prodrugs **10i** were synthesized in high optical purity and characterized *in vivo* for their ability to produce araCTP in the liver and minimize extrahepatic exposure to araC. Prodrug **19*S*** generated hepatic araCTP levels >12-fold higher than the araCTP levels generated in the bone marrow and >19-fold higher than the levels of araC in the plasma, representing a >28-fold and >120-fold improvement, respectively, over araC

administration, and was selected for further characterization as a potential drug candidate for the treatment of HCC.

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 F_{254} 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 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. C, H, N microanalyses were performed by Robertson Microlit Laboratories, Inc., Madison, NJ, or NuMega Resonance Labs, Inc., San Diego, CA. Single-crystal structure analysis for compound **19*S*** was performed by SSCI Inc., West Lafayette, IN. 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 1-Aryl Substituted Propane-1,3-diols 7a−j. (A) 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 reaction 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 stirring 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 reaction 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 by either chromatography or distillation.

(B) Step B: Reduction of β-Hydroxy Esters. The reduction of the β-hydroxy esters was achieved using either $LiAlH_4$ or $NaBH_4$.

(1) Using $LiAlH_4$. 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 reaction was stirred, allowing the cooling bath to melt and the reaction to reach room temperature. Once the reaction was 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 either chromatography or distillation.

(2) Using $NaBH_4$. $NaBH_4$ was added portionwise to a solution of hydroxy ester 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.

(a) 1-(3-Bromophenyl)-1,3-propanediol (7a). $NaBH_4$ reduction gave an oil (45.1 g, 72% for 2 steps): ^{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).

(b) 1-(3-Methylphenyl)-1,3-propanediol (7b). $LiAlH_4$ reduction gave an oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.30−7.02 (m, 4H), 4.95 (dd, J = 6.0, 4.0 Hz, 1H), 3.85 (t, J = 6.0 Hz, 2H), 2.32 (s, 3H), 2.10−1.85 (m, 2H).

(c) 1-(4-Fluorophenyl)-1,3-propanediol (7c). $LiAlH_4$ reduction gave an oil (5.28 g, 77% for 2 steps): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40−7.34 (m, 2H), 7.07 (t, J = 8.9 Hz, 2H), 4.98 (dd, J = 8.4, 4.2 Hz, 1H), 3.89 (t, J = 5.9 Hz, 2H), 2.10−1.85 (m, 2H).

(d) 1-(4-Bromophenyl)-1,3-propanediol (7d). $LiAlH_4$ reduction gave an oil (3.3 g, 24% for 2 steps): ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.55 (m, 2H), 7.3 (m, 2H), 4.99 (m, 1H), 3.85 (m, 2H), 2.64 (bs, 2H, exchangeable with D_2O), 1.98 (m, 2H).

(e) 1-(4-Chlorophenyl)-1,3-propanediol (7e). $LiAlH_4$ reduction gave an oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.40−7.15 (m, 4H), 4.95−4.85 (dd, J = 6.8, 5.0 Hz, 1H), 3.82 (t, J = 5.0 Hz, 2H), 2.32 (s, 3H), 2.00−1.75 (m, 2H).

(f) 1-(3-Chlorophenyl)-1,3-propanediol (7f). $LiAlH_4$ reduction gave an oil (42.07 g, 63% for 2 steps): ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.18−7.40 (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).

(g) 1-(3-Chloro-4-fluorophenyl)-1,3-propanediol (7g). $NaBH_4$ reduction gave an oil (18 g, 55% for 2 steps): ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.53−7.48 (m, 1H), 7.40−7.35 (m, 3H), 5.33 (d, J = 4.8 Hz, 1H exchangeable with D_2O), 4.75−4.65 (m, 1H), 4.46 (t, J = 5.1 Hz, 1H exchangeable with D_2O), 3.60−3.35 (m, 2H), 1.83−1.65 (m, 2H).

(h) 1-(3,5-Dichlorophenyl)-1,3-propanediol (7h). $NaBH_4$ reduction gave an oil (47 g, 83% for 2 steps): bp 135−137 °C/0.75 mmHg; ^{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), 4.75−4.60 (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).

(i) 1-(Pyridin-4-yl)-1,3-propanediol (7i). $LiAlH_4$ reduction gave a tan solid (45 g, 61%): 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).

(j) 1-(Pyridin-3-yl)-1,3-propanediol (7j). $NaBH_4$ reduction gave an oil (11 g, 77% for 2 steps): ^{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).

(k) 1-(3-Methoxycarbonylphenyl)-1,3-propanediol (7k). A pressure vessel was charged with 1-(3-bromophenyl)-1,3-propane diol (**7a**, 2 g, 8.6 mmol), methanol (30 mL), triethylamine (5 mL), and bis(triphenylphosphine)palladium dichloride (0.36 g, 0.5 mmol). The sealed vessel was pressurize with carbon monoxide at 55 psi and heated at 85 °C for 24 h. The cooled vessel was opened, and the reaction mixture was filtered through Celite and rinsed with methanol. The combined filtrates were concentrated under reduced pressure, and the residue was purified by column chromatography (silica gel, hexanes/ethyl acetate 1/1) to provide diol **7k** (1.2 g, 66%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.02 (s, 1H), 7.95 (d, J = 7.6 Hz, 1H), 7.70−7.35 (m, 2H), 5.07−4.97 (m, 1H), 3.91 (s, 3H), 3.90−3.80 (m, 2H), 2.05−1.90 (m, 2H).

(l) 1-(4-Methoxycarbonylphenyl)-1,3-propanediol (7l). Palladium catalyzed carbonylation of diol **7d** using the procedure described for the synthesis of diol **7k** gave diol **7l** (1.58 g, 87%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.02 (dd, J = 8.4, 1.8 Hz, 2H), 7.44 (d, J = 8.0 Hz, 2H), 5.05 (t, J = 6.3 Hz, 1H), 3.91 (s, 3H), 3.90−3.80 (m, 2H), 2.05−1.90 (m, 2H).

General Procedure for the Synthesis of *trans*-4-(Aryl)-2-(4-nitrophenoxy)-2-oxido-1,3,2-dioxaphosphorinanes. A solution of 1-aryl-1,3-propane diol (**7b−l**, 2.5 g, 13.4 mmol) and triethylamine (6.25 mL, 44.2 mmol) in THF was added to a solution of 4-nitrophenyl-phosphorodichloridate (3.77 g, 14.7 mmol) in THF at room temperature, and the resulting solution was heated at reflux. After 2 h, TLC indicated complete consumption of the starting diol and formation of the *cis* and *trans* isomers in a 50/50 to 60/40 ratio (HPLC). The clear yellow solution was cooled to 30 °C, sodium 4-nitrophenoxide (5.6 g, 40.2 mmol) was added, and the reaction mixture was heated at reflux. After 90 min the reddish reaction mixture was cooled to room temperature, quenched with

a saturated solution of NH_4Cl, and diluted with ethyl acetate. The layers were separated, and the organics were washed four times with a 0.3 N solution of sodium hydroxide, followed by a brine solution, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column chromatography.

(a) *trans*-4-(3-Methylphenyl)-2-(4-nitrophenoxy)-2-oxido-1,3,2-dioxaphosphorinane (8b). Isomerization was conducted with 4-nitrophenol (3 equiv) and DBU (4 equiv) to give phosphate **8b**: ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.26 (d, J = 9.0 Hz, 2H), 7.55 (d, J = 9.0 Hz, 2H), 7.35−7.10 (m, 4H), 5.58 (d, J = 11.7 Hz, 1H), 4.70−4.45 (m, 2H), 2.60−2.30 (m, 1H), 2.38 (s, 3H), 2.18−2.00 (m, 1H). TLC: hexanes/ethyl acetate 6/4; R_f: *cis*-phosphate = 0.2; *trans*-phosphate = 0.5.

(b) *trans*-4-(4-Fluorophenyl)-2-(4-nitrophenoxy)-2-oxido-1,3,2-dioxaphosphorinane (8c). White solid (1.8 g, 43%): mp 123−125 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.24 (d, J = 9.0 Hz, 2H), 7.50−7.20 (m, 4H), 7.10 (t, J = 8.8 Hz, 2H), 5.57 (d, J = 11.4 Hz, 1H), 4.75−4.45 (m, 2H), 2.60−2.35 (m, 1H), 2.20−2.00 (m, 1H).

(c) *trans*-4-(4-Bromophenyl)-2-(4-nitrophenoxy)-2-oxido-1,3,2-dioxaphosphorinane (8d). White solid (2.15 g, 60%): mp 123−125 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.26 (d, J = 9.0 Hz, 2H), 7.55 (d, J = 9.0 Hz, 2H), 7.45 (d, J = 9.0 Hz, 2H), 7.27 (d, J = 9.0 Hz, 2H), 5.58 (d, J = 11.7 Hz, 1H), 4.80−4.50 (m, 2H), 2.55−2.25 (m, 1H), 2.20−2.00 (m, 1H).

(d) *trans*-4-(4-Chlorophenyl)-2-(4-nitrophenoxy)-2-oxido-1,3,2-dioxaphosphorinane (8e). Off-white powder (4.0 g, 51%): mp 102−105 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.30 (d, J = 9.0 Hz, 2H), 7.55−7.25 (m, 6H), 5.60 (d, J = 11.7 Hz, 1H), 4.80−4.50 (m, 2H), 2.55−2.25 (m, 1H), 2.20−2.00 (m, 1H).

(e) *trans*-4-(3-Chlorophenyl)-2-(4-nitrophenoxy)-2-oxido-1,3,2-dioxaphosphorinane (8f). White solid (4.5 g, 91%): mp 115−116 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.26 (d, J = 9.0 Hz, 2H), 7.50−7.20 (m, 6H), 5.56 (d, J = 11.7 Hz, 1H), 4.70−4.40 (m, 2H), 2.60−2.20 (m, 1H), 2.20−2.00 (m, 1H).

(f) *trans*-4-(3-Chloro-4-fluorophenyl)-2-(4-nitrophenoxy)-2-oxido-1,3,2-dioxaphosphorinane (8g). White solid (4.16 g, 73%): mp 115−116 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.28 (d, J = 9.0 Hz, 2H), 7.55−7.45 (m, 3H), 7.35−7.28 (m, 1H), 7.23 (t, J = 8.4 Hz, 1H), 5.60 (dd, J = 11.7, 2.1 Hz, 1H), 4.70−4.55 (m, 2H), 2.55−2.37 (m, 1H), 2.20−2.10 (m, 1H).

(g) *trans*-4-(3,5-Dichlorophenyl)-2-(4-nitrophenoxy)-2-oxido-1,3,2-dioxaphosphorinane (8h). White solid (5.4 g, 42%): mp 124−126 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.28 (d, J = 9.0 Hz, 2H), 7.44 (d, J = 9.0 Hz, 2H), 7.38 (s, 1H), 7.31 (s, 1H), 5.56 (dd, J = 11.7, 2.1 Hz, 1H), 4.70−4.55 (m, 2H), 2.55−2.37 (m, 1H), 2.20−2.10 (m, 1H).

(h) *trans*-2-(4-Nitrophenoxy)-2-oxido-4-(pyridin-4-yl)-1,3,2-dioxaphosphorinane (8i). White solid (8.9 g, 92%): mp 140−142 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.61 (d, J = 4.8 Hz, 2H), 8.33 (d, J = 9.6 Hz, 2H), 7.57 (d, J = 9.6 Hz, 2H), 7.42 (d, J = 4.8 Hz, 2H), 6.00−5.85 (m, 1H), 4.80−4.50 (m, 2H), 2.6−2.1 (m, 2H). TLC conditions: 3/2 acetone/hexanes; diol: R_f = 0.2; *trans*-phosphate: R_f = 0.6; *cis*-phosphate: R_f = 0.5.

(i) *trans*-2-(4-Nitrophenoxy)-2-oxido-4-(pyridin-3-yl)-1,3,2-dioxaphosphorinane (8j). White solid: mp 165−167 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.70−8.60 (m, 2H), 8.28 (d, J = 9.0 Hz, 2H), 7.90−7.80 (m, 1H), 7.44 (d, J = 9.0 Hz, 2H), 7.45−7.38 (m, 1H), 5.65 (d, J = 11.0 Hz, 1H), 4.80−4.50 (m, 2H), 2.60−2.37 (m, 1H), 2.22−2.07 (m, 1H).

(j) *trans*-4-(3-Methoxycarbonylphenyl)-2-(4-nitrophenoxy)-2-oxido-1,3,2-dioxaphosphorinane (8k). White solid (1.2 g, 54%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.26 (dd, J = 9.2, 2.2 Hz, 2H), 7.80−8.00 (m, 1H), 7.63 (d, J = 7.6 Hz, 1H), 7.60−7.40 (m, 4H), 5.66 (dd, J = 11.6, 2.2 Hz, 1H), 4.80−4.50 (m, 2H), 3.93 (s, 3H), 2.60−2.30 (m, 1H), 2.20−2.05 (m, 1H).

(k) *trans*-4-(4-Methoxycarbonylphenyl)-2-(4-nitrophenoxy)-2-oxido-1,3,2-dioxaphosphorinane (8l). White solid (0.7 g, 23%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.24 (dd, J = 9.2, 2.2 Hz, 2H), 8.09 (dd, J = 8.4, 2.0 Hz, 2H), 7.55−7.37 (m, 4H), 5.66 (dd, J = 11.6, 2.2 Hz, 1H), 4.75−4.50 (m, 2H), 3.93 (s, 3H), 2.55−2.25 (m, 1H), 2.20−2.05 (m, 1H).

General Procedure for the Synthesis of araC Prodrugs. (A) Step A: Phosphorylation Reaction. A solution of *t*-BuMgCl (1.55 mL, 1.55 mmol) in THF was added to a solution of 2′,3′-di-*O*-*tert*-butyldimethylsilyl-cytosine-β-D-arabinofuranoside[5] (**9**, 0.5 g, 1.03 mmol) in THF (30 mL) at room temperature. The tan solution was stirred at room temperature for 30 min, and the respective *trans*-phosphate reagent **8b**−**l** (1.3 equiv) was added in one portion. After stirring at room temperature for 18 h, the tan reaction mixture was quenched with a saturated solution of NH_4Cl (20 mL) and extracted twice with ethyl acetate. The combined organic extracts were washed three times with a 1 N solution of sodium hydroxide and twice with a brine solution, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by flash column chromatography to give the protected prodrug

(B) Step B: Deprotection of the Protected Prodrug. Tetraethylammonium fluoride hydrate (TEAF, 272 mg, 1.83 mmol) or a 1 M solution of tetrabutylammonium fluoride (TBAF, 1.83 mL, 1.83 mmol) was added to a solution of the protected prodrug (428 mg, 0.61 mmol) in THF (6 mL) at room temperature. After stirring at room temperature for 18 h, the reaction mixture was concentrated under reduced pressure and the residue was purified by column chromatography to provide prodrugs **10b**−**l**.

(a) 5′-*O*-*cis*-[4-(3-Methylphenyl)-2-oxido-1,3,2-dioxaphosphorinan-2-yl]-cytosine-β-D-arabinofuranoside (10b). Phosphorylation step (150 mg, 52%). Deprotection with TEAF provided prodrug **10b** as a white solid, (40 mg, 40%): mp 189−192 °C; ^{1}H NMR (200 MHz, CD_3OD) δ 7.80−7.65 (m, 1H), 7.35−7.10 (m, 4H), 6.21 (d, J = 5.5 Hz, 1H), 5.80−5.50 (m, 2H), 4.80−4.00 (m, 76H), 2.50−2.10 (m, 2H), 2.33 (s, 3H). Anal. ($C_{19}H_{24}N_3O_7P\cdot 0.5H_2O$) C, H, N.

(b) 5′-*O*-*cis*-[4-(4-Fluorophenyl)-2-oxido-1,3,2-dioxaphosphorinan-2-yl]-cytosine-β-D-arabinofuranoside (10c). Phosphorylation step (170 mg, 57%). Deprotection with TBAF provided prodrug **10c** as a white solid (86 mg, 86%): mp 190 °C, dec; ^{1}H NMR (200 MHz, DMSO-d_6) δ 7.60−7.40 (m, 7H), 7.30−6.95 (m, 4H, 2H exchangeable with D_2O), 6.08 (d, J = 3.4 Hz, 1H), 5.80−5.45 (m, 4H, 2H exchangeable with D_2O), 4.60−4.10 (m, 4H), 4.00−3.80 (m, 3H), 2.35−2.00 (m, 2H). Anal. ($C_{18}H_{21}FN_3O_8P\cdot H_2O$) C, H, N.

(c) 5′-*O*-*cis*-[4-(4-Bromophenyl)-2-oxido-1,3,2-dioxaphosphorinan-2-yl]-cytosine-β-D-arabinofuranoside (10d). Phosphorylation step (200 mg, 51%). Deprotection with TBAF provided prodrug **10d** as a white solid (105 mg, 87%): mp 196 °C, dec; ^{1}H NMR (200 MHz, DMSO-d_6) δ 7.65−7.30 (m, 5H), 7.20−6.95 (m, 2H, exchangeable with D_2O), 6.08 (d, J = 3.4 Hz, 1H), 5.75−5.45 (m, 4H, 2H exchangeable with D_2O), 4.60−4.15 (m, 4H), 4.00−3.80 (m, 3H), 2.30−2.05 (m, 2H). Anal. ($C_{18}H_{21}BrN_3O_8P\cdot 0.8H_2O$) C, H, N.

(d) 5′-*O*-*cis*-[4-(4-Chlorophenyl)-2-oxido-1,3,2-dioxaphosphorinan-2-yl]-cytosine-β-D-arabinofuranoside (10e). Phosphorylation step (390 mg, 65%). Deprotection with TBAF provided prodrug **10e** as a white solid (130 mg, 64%): mp 190−192 °C; ^{1}H NMR (200 MHz, CD_3OD) δ 7.80−7.65 (m, 1H), 7.50−7.30 (m, 4H), 6.30−6.17 (m, 1H), 5.80−5.60 (m, 2H), 4.75−4.00 (m, 7H), 2.50−2.10 (m, 2H). Anal. ($C_{18}H_{21}ClN_3O_8P\cdot 1.2H_2O$) C, H, N.

(e) 5′-*O*-*cis*-[4-(3-Chlorophenyl)-2-oxido-1,3,2-dioxaphosphorinan-2-yl]-cytosine-β-D-arabinofuranoside (10f). Phosphorylation step (4.48 g, 62%). Deprotection with TEAF provided prodrug **10f** as a white powder (1.62 g, 65%): mp >200 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 7.60−7.37 (m, 5H), 7.07 (br s, 2H, exchangeable with D_2O), 6.10 (d, J = 3.3 Hz, 1H), 5.78−5.65 (m, 1H), 5.65−5.50 (m, 3H, 2H exchangeable with D_2O), 4.60−4.20 (m, 4H), 4.00−3.85 (m, 3H), 2.30−2.10 (m, 2H). Anal. ($C_{18}H_{21}N_3O_8ClP\cdot H_2O$) C, H, N.

(f) 5′-*O*-*cis*-[4-(3-Chloro-4-fluorophenyl)-2-oxido-1,3,2-dioxaphosphorinan-2-yl]-cytosine-β-D-arabinofuranoside (10g). Phosphorylation step (180 mg, 58%). Deprotection with TBAF provided prodrug **10g** as a white solid (101 mg, 83%): mp 128−131 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 7.70−7.40 (m, 3H), 7.2−6.95 (m,

2H, exchangeable with D_2O), 6.08 (d, $J = 3.4$ Hz, 1H), 5.80−5.50 (m, 4H, 2H exchangeable with D_2O), 4.60−4.20 (m, 4H), 4.00−3.80 (m, 2H), 2.30−2.10 (m, 2H). Anal. ($C_{18}H_{21}ClFN_3O_8P\cdot 0.9H_2O$) C, H, N.

(g) 5′-*O*-*cis*-[4-(3,5-Dichlorophenyl)-2-oxido-1,3,2-dioxaphosphorinan-2-yl]-cytosine-β-D-arabinofuranoside (10h). Phosphorylation step (440 mg, 63%). Deprotection with TBAF provided prodrug **10h** as a white solid (240 mg, 81%): mp 195−197 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 7.70−7.43 (m, 4H), 7.15−6.95 (m, 2H, exchangeable with D_2O), 6.09 (d, $J = 3.0$ Hz, 1H), 5.80−5.65 (m, 1H), 5.65−5.50 (m, 3H, 2H exchangeable with D_2O), 4.80−4.20 (m, 4H), 4.00−3.80 (m, 3H), 2.30−2.10 (m, 2H). Anal. ($C_{18}H_{20}Cl_2N_3O_8P\cdot 1.2H_2O$) C, H, N.

(h) 5′-*O*-*cis*-[2-Oxido-4-(pyridin-4-yl)-1,3,2-dioxaphosphorinan-2-yl]-cytosine-β-D-arabinofuranoside (10i). Phosphorylation step (1.44 g, 52%). Deprotection with TEAF provided prodrug **10i** as a white solid (391 mg, 40%): mp 150 °C, dec; ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.65−8.50 (m, 2H), 7.60−7.25 (m, 3H), 7.20−6.90 (m, 2H, exchangeable with D_2O), 6.15−6.00 (m, 1H), 5.80−5.65 (m, 1H), 5.65−5.50 (m, 3H, 2H exchangeable with D_2O), 4.65−4.10 (m, 4H), 4.05−3.80 (m, 3H), 2.35−2.00 (m, 2H). Anal. ($C_{17}H_{21}N_4O_8P\cdot 0.5H_2O$) C, H, N.

(i) 5′-*O*-*cis*-[2-Oxido-3-(pyridin-3-yl)-1,3,2-dioxaphosphorinan-2-yl]-cytosine-β-D-arabinofuranoside (10j). Phosphorylation step (240 mg, 34%). Deprotection with TEAF provided prodrug **10j** as a white solid (60 mg, 38%): mp 184−188 °C; ^{1}H NMR (200 MHz, CD_3OD) δ 8.65 (s, 1H), 8.60−8.50 (m, 1H), 7.95 (d, $J = 5.7$ Hz, 1H), 7.75−7.65 (m, 1H), 7.55−7.40 (m, 1H), 6.25−618 (m, 1H), 5.90−5.60 (m, 2H), 4.80−4.25 (m, 4H), 4.25−4.00 (m, 3H), 2.60−2.10 (m, 2H). Anal. ($C_{17}H_{21}N_4O_8P\cdot 1.4H_2O$) C, H, N.

(j) 5′-*O*-*cis*-[4-(3-Methoxycarbonylphenyl)-2-oxido-1,3,2-dioxaphosphorinan-2-yl]-cytosine-β-D-arabinofuranoside (10k). Phosphorylation step (220 mg, 72%). Phosphorylation step (120 mg, 80%). Deprotection with TBAF provided prodrug **10k** as a white solid (120 mg, 80%;): mp 130−132 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.00 (s, 1H), 7.93 (d, $J = 7.8$ Hz, 1H), 7.70 (d, $J = 7.6$ Hz, 1H), 7.60−7.45 (m, 2H), 7.05 (d, $J = 9.2$ Hz, 2H, exchangeable with D_2O), 6.08 (d, $J = 3.4$ Hz, 1H), 5.90−5.70 (m, 1H), 7.65−5.50 (m, 3H, 2H exchangeable with D_2O), 4.65−4.20 (m, 4H), 4.00−3.80 (m, 3H), 3.84 (s, 3H), 2.30−2.10 (m, 2H). Anal. ($C_{20}H_{24}N_3O_{10}P\cdot 0.9\ H_2O$) C, H, N.

(k) 5′-*O*-*cis*-[4-(4-Methoxycarbonylphenyl)-2-oxido-1,3,2-dioxaphosphorinan-2-yl]-cytosine-β-D-arabinofuranoside (10l). Phosphorylation step (190 mg, 62%). Deprotection with TBAF provided prodrug **10l** as a white solid (105 mg, 95%): mp 137−140 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 7.97 (d, $J = 8.4$ Hz, 2H), 7.60−7.45 (m, 2H), 7.15−6.95 (m, 2H, exchangeable with D_2O), 6.08 (d, $J = 3.4$ Hz, 1H), 5.85−5.70 (m, 1H), 5.70−5.45 (m, 3H, 2H exchangeable with D_2O), 4.65−4.20 (m, 4H), 4.00−3.80 (m, 3H), 3.83 (s, 3H), 2.25−2.15 (m, 2H). Anal. ($C_{20}H_{24}N_3O_{10}P\cdot 0.7H_2O$) C, H, N.

(l) 2′,3′-di-*O*-*tert*-Butyldimethylsilyl-4-*N*-(*N*,*N*-dimethylformamidine)-cytosine-β-D-arabinofuranoside (13). DMF-dimethyl acetal (3.4 mL, 25.7 mmol) was added dropwise to a stirred solution of 2′,3′-di-*O*-TBS-ara-C (**9**, 9.7 g, 20.56 mmol) in pyridine (100 mL) at room temperature. After stirring the clear solution at room temperature for 16 h, the volatiles were removed under reduced pressure. The crude product was purified by column chromatography on silica gel, eluting with 2−5% ethanol in dichloromethane to afford the desired nitrogen-protected intermediate **13** as a white foam (9.75 g, 90% yield): ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.80 (s, 1H), 7.78 (d, $J = 7.2$ Hz, 1H), 6.25 (d, $J = 2.5$ Hz, 1H), 6.02 (d, $J = 7.2$ Hz, 1H), 4.30−4.22 (m, 1H), 4.10−4.02 (m, 2H), 3.87−3.75 (m, 2H), 3.12 (s, 3H), 3.10 (s, 3H), 0.87 (s, 9H), 0.77 (s, 9H), 0.10 (s, 3H), 0.08 (s, 3H), 0.02 (s, 3H), −0.18 (s, 3H).

(m) Ethyl 3-Hydroxy-3-(pyridin-4-yl)-propanoate (14). Oil (87 g, 97%): ^{1}H NMR ($CDCl_3$) δ 8.53 (d, $J = 6.6$ Hz, 2H), 7.31 (d, $J = 6.6$ Hz, 2H), 5.19−5.11 (m, 1H), 4.18 (q, $J = 7.0$ Hz, 2H), 2.80−2.65 (m, 2H), 1.22 (s, 3H).

Resolution of Diols 17*R* and 17*S*. A solution of methyl 3-hydroxy-3-(pyridin-4-yl)-propanoate (**14**, 23.9 g, 122 mmol) in CH_2Cl_2 (200 mL) was added to a stirring solution of EDCI (35 g, 183 mmol), DMAP (3.7 g, 30.5 mmol), and L-*N*,*N*-dimethylphenylalanine (26 g, 135 mmol) in CH_2Cl_2 (800 mL) at room temperature. After stirring for 16 h at room temperature, water was added and the layers were separated. The aqueous phase was back-extracted with CH_2Cl_2, and the combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Chromatography on silica gel with hexane/acetone 80/20 to 50/50 removed the unreacted starting material. The diastereomers were then separated by a succession of chromatographies with hexane/ethyl acetate (1%TEA) 60/40 to 30/70 to get pure fractions.

(a) Ethyl 3-(L-2-*N*,*N*-Dimethylamino-3-phenyl-propanoyloxy)-3-(pyridin-4-yl)-propanoate (16*S*). Faster moving product (18.78 g. 83%): ^{1}H NMR (200 MHz, CD_3OD) δ 8.27 (d, $J = 6.4$ Hz, 2H), 7.20−6.95 (m, 5H), 6.84 (d, $J = 6.4$ Hz, 2H), 6.03 (t, $J = 6.8$ Hz, 1H), 4.07 (q, $J = 7.3$ Hz, 2H), 3.52 (t, $J = 7.9$ Hz, 1H), 2.90 (d, $J = 7.8$ Hz, 2H), 2.77 (d, $J = 7.6$ Hz, 2H), 2.33 (s, 6H), 1.15 (t, $J = 7.3$ Hz, 3H).

(b) Ethyl 3-(L-2-*N*,*N*-Dimethylamino-3-phenyl-propanoyloxy)-3-(pyridin-4-yl)-propanoate (16*R*). Slower moving product (17.5 g, 77%): ^{1}H NMR (200 MHz, CD_3OD) δ 8.43 (d, $J = 6.4$ Hz, 2H), 7.29 (d, $J = 6.4$ Hz, 2H), 7.23−7.07 (m, 5H), 5.98 (t, $J = 6.8$ Hz, 1H), 3.99 (q, $J = 7.3$ Hz, 2H), 3.52 (t, $J = 7.9$ Hz, 1H), 2.96 (d, $J = 7.8$ Hz, 2H), 2.80−2.55 (m, 2H), 2.27 (s, 6H), 1.10 (t, $J = 7.3$ Hz, 3H).

(c) (−)-(*S*)-1-(Pyridin-4-yl)-1,3-propanediol (17*S*). A three-neck flask was flame dried and charged with $LiAlH_4$ (10.4 g, 170.6 mmol). Ether (750 mL) was added slowly. The slurry was cooled to −20 °C, and a solution of ester **16*S*** (16.97 g, 45.8 mmol) in ether (250 mL) was added dropwise via addition funnel. The reaction progressed for 1 h and then was quenched with EtOAc (150 mL) followed by a saturated solution of Na_2SO_4 (50 mL) and MeOH (100 mL). This mixture was stirred for 1 h and filtered, and the solid was rinsed with MeOH. The filtrate was concentrated under reduced pressure and purified by chromatography (95/5 CH_2Cl_2/MeOH + 1% Et_3N) to give a brown oil that crystallized upon standing. Recrystallization from ethyl acetate gave tan crystals (4.64 g, 66%): mp 98−100 °C. ee = 96% *S*-isomer determined by HPLC: Chiralpak AD, 4.6 × 250 mm^2; 0:90, ethanol/hexane, isocratic; 1.5 mL/min; detection at 254 nm; room temperature: *R*-diol = 12.7 min; *S*-diol = 14 min. $[\alpha]_D^{25} = -50.48°$ (*c* 1.00, MeOH).

(d) (+)-(*R*)-1-(Pyridin-4-yl)-1,3-propanediol (17*R*). Reduction of ester **16*R*** (17.5 g) provided diol **17*R*** (2.4 g, 33%): mp 98−100 °C, ee = 98% *R*-isomer (determined by HPLC).

(e) (−)-(4*S*)-*trans*-2-(4-Nitrophenoxy)-2-oxido-4-(pyridin-4-yl)-1,3,2-dioxaphosphorinane (18*S*). To a stirred solution of 4-nitrophenyl phosphorodichloridate (7.35 g, 28.7 mmol) in THF (80 mL) at 0 °C was slowly added pyridine (6.3 mL, 78.3 mmol) over 1 h. The reaction mixture was stirred for 5 min at 0 °C and slowly added to a solution of (−)-(*S*)-1-(pyridin-4-yl)-1,3-propanediol (**17*S***, 97% ee, 4.0 g, 26.1 mmol) in THF (240 mL) at 0 °C over 1 h. The reaction mixture was allowed to warm to room temperature and was stirred for 2.5 h. Sodium 4-nitrophenoxide (10.91 g, 78.3 mmol) was added, and the heterogeneous orange reaction mixture was heated at 40 °C for 4 h. The reaction was cooled to 0 °C, quenched with a saturated solution of NH_4Cl (250 mL), and extracted with ethyl acetate (100 mL × 2). The combined organic extracts were washed with a 0.3 N solution of sodium hydroxide (100 mL × 4) and dried ($MgSO_4$). The filtered solution was concentrated under reduced pressure to give an oil that crystallized upon standing. The yellow solid was recrystallized from 100 mL of 2-propanol to afford *trans*-phosphate **18*S*** as a white solid (4.74 g, ee 99.4%). A second crop was obtained by concentrating the mother liquor (780 mg, 98% ee) to bring the total yield to 5.52 g, 63%: mp 140−142 °C. $[\alpha]_D^{20}$ −80.9 (*c* 1.0, MeOH). de = 99% *trans* by HPLC: Zorbax Rx-C18 (4.6 × 250 mm^2); 35% acetonitrile/ 65% 20 mM phosphate buffer pH 7.95; 0.5 mL/min; detection at 250 nm; retention times: *cis* isomer = 9.39 min, *trans* isomer = 10.11 min; ee = 99.4% by

HPLC: Chiral Pak AD; 1:1 2-propanol/hexanes; 1.0 mL/min; detection at 254 nm; retention times in min: *trans*-phosphate = 7.02.

(f) (+)-(4*R*)-*trans*-2-(4-Nitrophenoxy)-2-oxido-(4-pyridinyl)-1,3,2-dioxaphosphorinane (18*R*). Starting from 5.0 g of (+)-(*R*)-1-(pyridin-4-yl)-1,3-propanediol (**17*R***, 96% ee) gave an oil that crystallized upon standing. The yellow solid was recrystallized from 100 mL of 2-propanol to afford *trans*-phosphate **18*R*** as a white solid (6.67 g, 60%, 95% ee, $[\alpha]_D^{20}$ + 74.2° (*c* 1.0, MeOH)).

(g) (4*S*)-5′-*O*-*cis*-[2-Oxido-4-(pyridin-4-yl)-1,3,2-dioxaphosphorinan-2-yl]-cytosine-β-D-arabinofuranoside (19*S*). To a stirred solution of 2′,3′-di-*O*-TBS-4-*N*-(*N*,*N*-dimethylformamidine)-cytidine-β-D-arabinofuranoside (**13**, 1.8 g, 3.42 mmol) in THF (40 mL) at room temperature was slowly added a solution of *t*-BuMgCl (1 M in THF, 4.44 mL, 4.44 mmol). After 30 min, *trans*-phosphate **18*S*** (1.94 g, 5.77 mmol) was added and the reaction mixture was stirred at room temperature for 16 h. The reaction was cooled to 0 °C, quenched with a saturated aqueous solution of ammonium chloride, and extracted with ethyl acetate (50 mL × 2). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel, eluting with acetone to afford the desired protected prodrug as an off-white solid (1.92 g, 78%): mp 162−164 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.80 (s, 1H), 8.64 (dd, *J* = 4.4, 1.4 Hz, 2H), 7.78 (d, *J* = 7.2 Hz, 1H), 7.26 (dd, *J* = 4.4, 1.4 Hz, 2H), 6.34 (d, *J* = 2.8 Hz, 1H), 6.03 (d, *J* = 7.2 Hz, 1H), 5.70−5.60 (m, 1H), 4.80−4.15 (m, 6H), 4.04 (s, 1H), 3.14 (s, 3H), 3.12 (s, 3H), 2.40−2.05 (m, 2H), 0.89 (s, 9H), 0.77 (s, 9H), 0.12 (s, 3H), 0.10 (s, 3H), 0.01 (s, 3H), −0.19 (s, 3H).

A stirred solution of the protected prodrug (4.96 g, 2.64 mmol) in 70% aqueous TFA (50 mL) was heated at 60 °C for 16 h, and the solvent was removed under reduced pressure. To the residue was added methanol (20 mL), and the mixture was made slightly basic with sodium bicarbonate. The cloudy solution was dried ($MgSO_4$), filtered, and concentrated under reduced pressure, and the crude product was purified by column chromatography on silica gel, eluting with methanol/acetone (1:1) to afford **19*S*** as an off-white solid (2.55 g, 83%): mp 153−155 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.58 (dd, *J* = 4.7, 1.8 Hz, 2H), 7.51 (d, *J* = 7.6 Hz, 1H), 7.41 (dd, *J* = 4.7, 1.8 Hz, 2H), 7.13 (s, 1H, exchangeable with D_2O), 7.05 (s, 1H, exchangeable with D_2O), 6.09 (d, *J* = 3.6 Hz, 1H), 5.80−5.70 (m, 1H), 5.63 (d, *J* = 2.7 Hz, 1H exchangeable with D_2O), 5.62 (d, 1H exchangeable with D_2O), 5.57 (d, *J* = 7.6 Hz, 1H), 4.60−4.20 (m, 4H), 4.00−3.85 (m, 3H), 2.25−2.15 (m, 2H). ^{13}C NMR (50 MHz, DMSO-d_6) δ 164.9, 154.4, 149.3, 147.3 (d, *J* = 8.0 Hz), 142.2, 119.6, 91.9, 85.7, 81.7 (d, *J* = 6.8 Hz), 77.7 (d, *J* = 5.0 Hz), 75.6, 73.5, 67.1 (d, *J* = 6.5 Hz), 66.6 (d, *J* = 5.7 Hz), 31.5 (d, *J* = 6.8 Hz). Anal. ($C_{17}H_{21}N_4O_8P$·0.1 H_2O) C, H, N.

(h) (4*R*)-5′-*O*-*cis*-[2-Oxido-4-(pyridin-4-yl)-1,3,2-dioxaphosphorinan-2-yl]-cytosine-β-D-arabinofuranoside (19*R*). Phosphorylation of 2′,3′-di-*O*-*tert*-butyldimethylsilyl-4-*N*-(*N*,*N*-dimethylformamidine)-cytidine-β-D-arabinofuranoside (**13**, 2.4 g, 4.55 mmol) with *trans*-phosphate **18*R*** (1.84 g, 5.50 mmol) afforded the protected prodrug as an off-white solid (2.19 g, 66%, mp 142−145 °C); ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.78 (s, 1H), 8.63 (dd, *J* = 4.4, 1.4 Hz, 2H), 7.73 (d, *J* = 7.2 Hz, 1H), 7.26 (dd, *J* = 4.4, 1.4 Hz, 2H), 6.33 (d, *J* = 2.8 Hz, 1H), 5.99 (d, *J* = 7.2 Hz, 1H), 5.70−5.55 (m, 1H), 4.80−4.1 (m, 6H), 4.04 (s, 1H), 3.14 (s, 3H), 3.11 (s, 3H), 2.30−2.10 (m, 2H), 0.90 (s, 9H), 0.77 (s, 9H), 0.12 (s, 3H), 0.10 (s, 3H), 0.01 (s, 3H), −0.19 (s, 3H).

Deprotection as for **19*S*** provided prodrug **19*R*** as an off-white solid (1.06 g, 80%): mp 178−180 °C; ^{1}H NMR (DMSO-d_6) δ 8.61 (d, *J* = 6.0 Hz, 2H), 7.52 (d, *J* = 7.4 Hz, 1H), 7.44 (d, *J* = 5.8 Hz, 2H), 7.15−7.00 (m, 2H, exchangeable with D_2O), 6.11 (d, *J* = 3.6 Hz, 1H), 5.80−5.70 (m, 1H), 5.70−5.65 (m, 3H, 2H exchangeable with D_2O), 4.65−4.20 (m, 4H), 4.00−3.85 (m, 3H), 2.25−2.15 (m, 2H). Anal. ($C_{17}H_{21}N_4O_8P$·0.7 H_2O) C, H, N.

Biological Methods. (A) Hepatocyte Activation Assay. Rat hepatocytes were isolated using standard procedures described previously.[9] Compounds were incubated at 100 μM in suspended rat hepatocytes, shaken for 4 h under oxygen at 37 °C, and then extracted by brief centrifugation (10 000 rpm in microfuge) through a mineral/silicon (4:1) oil layer into 10% perchloric acid (PCA). The perchloric acid layer was centrifuged for 10 min (14 000 rpm). The resulting supernatant was neutralized with 0.3 volumes of 3 M KOH/3 M $KHCO_3$. Neutralized hepatocyte extracts were treated with sodium periodate to remove interfering ribonucleoside triphosphates and analyzed by ion exchange chromatography for araCTP levels on a Whatman Partisil SAX column (4.6 × 250 mm^2) eluted with a linear gradient of buffer A (10 mM ammonium phosphate pH 3.5 and 6% v/v ethanol) and buffer B (1 M ammonium phosphate pH 3.5 and 6% v/v ethanol) [30−80% buffer B over 0−25 min] at a flow rate of 1.25 mL/min and with detection at 280 nm. AraCTP eluted at approximatively 12 min and was quantified relative to a known araCTP standard spiked into neutralized extracts.

(B) Stability Assay. Prodrug stability studies were performed using 100 μM concentrations of prodrugs in 10 mM phosphate buffer, pH 7.4 at 37 °C. Intact prodrug was quantified by HPLC.

(C) Aqueous Solubility. Solubility measurements of prodrugs were conducted by dissolving 10 mg/mL of prodrug in 100 mM K_i, pH 7.4, or 100 mM citrate buffer, pH 4.0 (prodrugs **10i**, **10j**, **19*R***, and **19*S*** were dissolved at 200 mg/mL). Samples were then sonicated for 20 min in a sonication bath and allowed to stand at room temperature for ∼10 min before being filtered through a 0.45 μm, nylon membrane, 4 mm syringe filter. Samples were then diluted 1:100 or 1:10000 in DMSO before being analyzed by HPLC.

(D) HPLC Analysis. Samples were analyzed on a HP1100 (Hewlett-Packard) using a C-18 Phenomenex Luna column, 150 mm × 4.6 mm, catalog # 00F-4252-E0, with a C-18 Alltech Econosphere guard column, 7.5 mm × 4.6 mm, catalog # 96121. Fifty microliters of filtrate was injected onto the column with mobile phase buffer, 20 mM potassium phosphate buffer, pH 6.2. Samples were eluted with an acetonitrile gradient: 10−80% over 10 min. The column was allowed to equilibrate for 5 min before the next injection. The flow rate was 1 mL/min with a column temperature of 40 °C. Prodrugs eluted around 3−7 min and were monitored at 270 nm.

(E) Mouse Tissue Distribution. Normal nonfasted male Swiss Webster mice (25−35 g body weight, Harlan Sprague Dawley, Indianapolis, IN) were injected i.p. with **19*R*** or **19*S*** at a molar-equivalent dose of 100 mg/kg of araC. At specified times after injection, mice were anesthetized and blood drawn. The liver was removed, rapidly snap-frozen in liquid nitrogen, and homogenized using a Polytron homogenizer PT 10/35 (Brinkmann Instruments, Westbury, NY) in three volumes of 10% (v/v) PCA. After a 5 min centrifugation at 2500*g*, 1 mL of supernatant was neutralized using 0.3 mL of 3 M KOH/3 M $KHCO_3$ and mixed thoroughly. One hundred microliters of tissue extract was incubated with 4 μL of 0.5 M sodium periodate and 10 μL of 1.8 M methylamine for 30 min at room temperature. The reaction was stopped with the addition of 2 μL of 1 M L-Rhamnose. Resulting samples were analyzed by HPLC as described above. The bone marrow samples were flushed from the marrow cavities of femurs with 1.2 mL of saline into preweighed Eppendorf tubes. After centrifugation for 20−30 s (Eppendorf microfuge, 14 000 rpm) and removal of the supernatant, 12 volumes of 3% PCA (v/v) were added to the bone marrow cell pellets. Samples were then vortexed until the pellet was well dissolved and centrifuged as above for 10 min. Ninety microliters of extracted supernatant was neutralized to pH 7−8 using 30 μL of 1 M KOH/1 M $KHCO_3$ and again centrifuged. Resulting supernatants were periodate-treated, and araCTP levels were determined by HPLC as above.

Blood was transferred to heparinized microcollection tubes and centrifuged in an Eppendorf microfuge at 14 000 rpm for 2 min to collect plasma, which was placed on dry ice and subsequently stored at −20 °C. On the day of analysis, proteins were precipitated by adding 1 mL of acetonitrile to 100 μL of plasma. After a 10 min centrifugation (Eppendorf microfuge, 14 000 rpm), the supernatant was removed and dried in a Savant SpeedVac Plus SC110A.

Samples were reconstituted with 110 μL of mobile phase buffer (20 mM KH_2PO_4, pH 4.5), sonicated for 5 min, and centrifuged for 30 s. Supernatants were analyzed by reverse-phase HPLC: Alltech C18 column (5 μm, 4.6 $\times$ 250 mm^2); acetonitrile gradient 0−10% over 10 min, then to 50% over 15 min. Elution times for araC and prodrugs were around 3 and 19 min, respectively. AraC and prodrugs were detected by absorbance at 280 nm.

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Supporting Information Available: Microanalysis data for all final compounds; crystallographic data for compound **19*S*** (packing diagrams, tables of crystal data and data collection parameters, positional parameters and their estimated standard deviations, anisotropic temperature factor coefficients, bond distances, bond angles, and torsion angles). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Pisani, P.; Parkin, D. M.; Bray, F.; Ferlay, J. Estimates of the worldwide mortality from 25 cancers in 1990. *Int. J. Cancer* **1999**, *83*, 18−29. (b) Bruix, J.; Llovet, J. M. Prognostic prediction and treatment strategy in hepatocellular carcinoma. *Hepatology* **2002**, *35*, 519−24. (c) Yu, A. S.; Keefe, E. B. Management of hepatocellular carcinoma. *Rev. Gastroenterol. Disord.* **2003**, *3*, 8−24.

(2) (a) Furth, J. J.; Cohen, S. S. Inhibition of mammalian DNA polymerase by the 5′-triphosphate of 1-β-D-arabinofuranosylcytosine and the 5′-triphosphate of 9-β-D-arabinofuranosyladenine. *Cancer Res.* **1968**, *28*, 2061−2067. (b) Graham, F. L.; Whitmore, G. F. Studies in mouse L-cells on the incorporation of 1-β-D-arabinofuranosylcytosine into DNA and on inhibition of DNA polymerase by 1-β-D-arabinofuranosylcytosine 5′-triphosphate. *Cancer Res.* **1970**, *30*, 2636−2644.

(3) (a) Major, P. P.; Egan, E. M.; Beardsley, G. P.; Minden, M. D.; Kufe, D. W. Lethality of human myeloblasts correlates with the incorporation of arabinofuranosylcytosine into DNA. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 3235−3239. (b) Kufe, D. W.; Major, P. P.; Egan, E. M.; Beardsley, G. P. Correlation of cytotoxicity with incorporation of ara-C into DNA. *J. Biol. Chem.* **1980**, *255*, 8997−9000.

(4) Verhoef, V.; Sarup, J.; Fridland, A. Identification of the mechanism of activation of 9-β-D-arabinofuranosyladenine in human lymphoid cells using mutants deficient in nucleoside kinases. *Cancer Res.* **1981**, *41*, 4478−4483.

(5) Erion, M. D.; Reddy, K. R.; Boyer, S. H.; Matelich, M. C.; Gómez-Galeno, J.; Lemus, R. H.; Ugarkar, B. G.; Colby, T. J.; Schanzer, J.; van Poelje, P. D. Design, synthesis, and characterization of a series of cytochrome P_{450} 3A-activated prodrugs (HepDirect prodrugs) useful for targeting phosph(on)ate-based drugs to the liver. *J. Am. Chem. Soc.* **2004**, *126*, 5154−5163.

(6) Erion, M. D.; van Poelje, P. D.; MacKenna, D. A.; Colby, T. J.; Montag, A. C.; Fujitaki, J. M.; Linemeyer, D. L.; Bullough, D. A. Liver-targeted drug delivery using HepDirect prodrugs. *J. Pharmacol. Exp. Ther.* **2005**, *312*, 554−560.

(7) (a) Murray, G. I.; Paterson, P. J.; Weaver, R. J.; Ewen, S. W. B.; Melvin, W. T.; Burke, M. D. The expression of cytochrome P-450, epoxide hydrolase, and glutathione S-transferase in hepatocellular carcinoma. *Cancer* **1993**, *71*, 36−43. (b) Philip, P. A.; Kaklamanis, L.; Ryley, N.; Stratford, I.; Wolf, R.; Harris, A.; Carmichael, J. Expression of xenobiotic-metabolizing enzymes by primary and secondary hepatic tumors in man. *Int. J. Radiat. Oncol. Biol. Phys.* **1994**, *29*, 277−283. (c) Zhang, Y. J.; Chen, S.; Tsai, W. Y.; Ahsan, H.; Lunn, R. M.; Wang, L. Y.; Chen, C. J.; Santella, R. M. Expression of cytochrome P450 1A1/2 and 3A4 in liver tissues of hepatocellular carcinoma cases and controls from taiwan and their relationship to hepatitis B birus and aflatoxin B1 and 4-aminobiphenyl DNA adducts. *Biomarkers* **2000**, *5*, 295−306. (d) El, Mouelhi, M.; Didolkar, M. S.; Elias, E. G.; Guengerich, F. P.; Kauffman, F. C. Hepatic drug-metabolizing enzymes in primary and secondary tumors of human liver. *Cancer Res.* **1987**, *47*, 460−466. (e) Kondoh, N.; Wakatsuki, T.; Ryo, A.; Hada, A.; Aihara, T.; Horiuchi, S.; Goseki, N.; Matsubara, O.; Takenaka, K.; Shichita, M.; Tanaka, K.; Shuda, M.; Yamamoto, M. Identification and characterization of genes associated with human hepatocellular carcinogenesis. *Cancer Res.* **1999**, *59*, 4990−4996.

(8) (a) Plunkett, W.; Gandhi V. Pharmacokinetics of arabinosylcytosine. *J. Infusional Chem.* **1992**, *2*, 169−176. (b) Southwest Oncology Group. Cytarabine for acute leukemia in adults: Effect of schedule on therapeutic response. *Arch. Intern. Med.* **1974**, *133* (Feb), 251−259.

(9) Reddy, K. R.; Boyer, S. H.; Erion, M. D. Stereoselective synthesis of nucleoside monophosphate HepDirect prodrugs. *Tetrahedron Lett.* **2005**, *46*, 4321−4324.

(10) Gorenstein, D. G. Stereoelectronic effects in biomolecules. *Chem. Rev.* **1987**, *87*, 1047−1077.

(11) Lin, C.-C.; Yeh, L.-T.; Vitarella, D.; Hong, Z.; Erion, M. D. Remofovir mesylate: A prodrug of PMEA with improved liver-targeting and safety in rats and monkeys. *Antiviral Chem. Chemother.* **2004**, *15*, 307−317.

(12) (a) Lee, K. S.; Lim S. G.; Chuang, W. L.; Hwang, S. G.; Cho, M.; Lai, M. Y.; Chao, Y. C.; Chang, T.-T.; Han, K. H.; Lee, C. M.; Um, S. H.; Yeon, J. E.; Yang, S. S.; Teo, E. K.; Peng, C. Y.; Lin, H. H.; Yang, S. S.; Huo, T. I.; Nguyen, T.; Chen, T. Y.; Hu, K. Q.; Xu, Y.; Sullivan-Bólyai, J. Z. Safety, tolerability, and antiviral activity of pradefovir mesylate in patients with chronic hepatitis B virus infection: 48-Week analysis of a phase 2 study. 41st Annual Meeting of the European Association for the Study of the Liver, April 26−30, 2006, Vienna, Austria. *J. Hepatol.* **2006**, *44*, S274. (b) Lin, C.-C.; Xu, C.; Teng, A.; Yeh, L.-T.; Peterson, J. Pharmacokinetics of pradefovir and PMEA in healthy volunteers after oral dosing of pradefovir. *J. Clin. Pharmacol.* **2005**, *45*, 1250−1258. (c) Erion, M. D.; Bullough, D. A.; Lin, C.-C.; Hong, Z. HepDirect prodrugs for targeting nucleotide-based antiviral drugs to the liver. *Curr. Opin. Invest. Drugs* **2006**, *7*, 109−117.

(13) Kochi, J. K.; Hammond, G. S. Benzyl tosylates. II. The application of the Hammett equation to the rates of their solvolysis. *J. Am. Chem. Soc.* **1953**, *75*, 3445−3451.

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