5'-[4-(Pivaloyloxy)-1,3,2-dioxaphosphorinan-2-yl]-2'-deoxy-5-fluorouridine: A Membrane-Permeating Prodrug of 5-Fluoro-2'-deoxyuridylic Acid (FdUMP)

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5'-[4-(Pivaloyloxy)-1,3,2-dioxaphosphorinan-2-yl]-2'-deoxy-5-fluorouridine (**1c**) was designed as a potential membrane-permeable prodrug of 2'-deoxy-5-fluorouridine 5'-monophosphate (FdUMP), a putative active metabolite of the antitumor drug 5-fluorouracil (FU). It was anticipated that **1c** would be hydrolyzed in vivo by carboxylate esterase (E.C. 3.1.1.1) to the labile 4-hydroxy analogue **2a**, which should penetrate cells by passive diffusion and ring open to the aldehyde **3a**. Spontaneous elimination of acrolein from **3a** would then generate the free nucleotide, FdUMP. **1c** might also penetrate cells directly and undergo the same degradation sequence after hydrolysis by cellular esterases. **1c** was prepared by condensing 2-hydroxy-2-oxo-4-(pivaloyloxy)-1,3,2-dioxaphosphorinane with 2'-deoxy-5-fluorouridine (FUdR) in the presence of triphenylphosphine and diethyl azodicarboxylate. **1c** was moderately stable in aqueous buffers over the pH range 1-7.4 ($t_{1/2}$ > 30 h). In the presence of carboxylate esterase, however, it was degraded, in a concentration-dependent manner, to FdUMP. No intermediates were detected in the incubation mixture. In mouse plasma, **1c** was degraded first to FdUMP and then to FUdR. The latter is presumably formed by dephosphorylation of FdUMP by plasma 5'-nucleotidases or phosphatases. **1c** and FU inhibited the growth of Chinese hamster ovary (CHO) cells in culture at a concentration of 5×10^{-6} M. **1c** was equally potent against a CHO variant that was 20-fold resistant to FU. Administered intraperitoneally for 5 consecutive days, **1c** was as effective as FU at prolonging the life span of mice bearing P-388 leukemia. In the presence of 2-mercaptoehtanesulfonic acid, an acrolein scavenger, **1c** was equally effective against a P-388 mutant cell line that was resistant to FU. Collectively, these data suggest that **1c** acts as a membrane-permeable prodrug of FdUMP. This prodrug strategy may be generally useful for introducing dianionic phosphates and phosphonates into cells.

Introduction

Organophosphate esters, particularly phosphomonoesters and phosphodiesters, play a key role in cell metabolism. They are involved, at one stage or another, in virtually every major metabolic sequence, including the biosynthesis of amino acids, proteins, carbohydrates, lipids, nucleotides, and nucleic acids. The enzymes that mediate intracellular phosphorylation and dephosphorylation reactions afford prime targets for the development of new anticancer and antiviral drugs. The most successful strategy for the design of enzyme inhibitors has been the metabolite-antimetabolite approach, namely, the synthesis of compounds that bear a close structural resemblance to the natural enzyme substrate and bind tightly to the enzyme's active site.

A major problem in applying this strategy to the enzymes involved in nucleic acid biosynthesis is that the majority utilize phosphorylated substrates. Unfortunately, phosphorylated compounds do not readily penetrate into cells.[1,2] The reasons are 2-fold: First, nucleotides are negatively charged at physiologic pH. As a result, they are usually highly hydrophilic and chemically incompatible with lipid cell membranes. Second, most nucleotides are rapidly degraded in the blood and on cell surfaces by nonspecific phosphohydrolases (e.g., acid and alkaline phosphatases, nucleotidases, and phosphodiesterases). Consequently, they have a short biologic half-life.

Many approaches to circumventing these problems have been described.[3-7] The most frequent has been to convert the ionic nucleotides into neutral, lipophilic derivatives that might traverse cell membranes by passive diffusion and then revert, by chemical or enzymatic hydrolyses, to the ionic parent compounds. Although most of these efforts have been unsuccessful, effective methods of introducing nucleotides into cells have been achieved recently with the use of bioreversible phosphate protective groups.[8-15]

In a previously reported[16] attempt to introduce 2'-deoxy-5-fluorouridine 5'-monophosphate (FdUMP) into cells, we synthesized the 5'-(1,3,2-dioxaphosphorinan-2-yl) and 5'-(1,3,2-oxazaphosphorinan-2-yl) derivatives of 2'-deoxy-5-fluorouridine (FUdR), **1a,b** (Scheme 1). We anticipated that these compounds would be oxidatively biotransformed by hepatic P-450-dependent mixed-function oxidases, in a manner similar to cyclophosphamide,[17] to yield the labile hydroxylated intermediates **2a,b**. These intermediates should penetrate cells by passive diffusion and ring open to the aldehyde **3a,b**. Spontaneous E2 elimination of acrolein, **5**, from **3a,b** then generates the corresponding nucleoside 5'-phosphate **4a** and the nucleoside 5'-phosphoramidate **4b**. We expected conversion of **4b** to **4a** to occur by chemical or enzymatic hydrolysis. Unfortunately, **1a,b** were only modestly effective at prolonging the life spans of mice bearing murine leukemia P-388 and inactive against a P-388 variant that was resistant to 5-fluorouracil (FU). Similar derivatives of arabinosyladenine 5'-monophosphate were also modestly active.[18] These nucleoside 5'-

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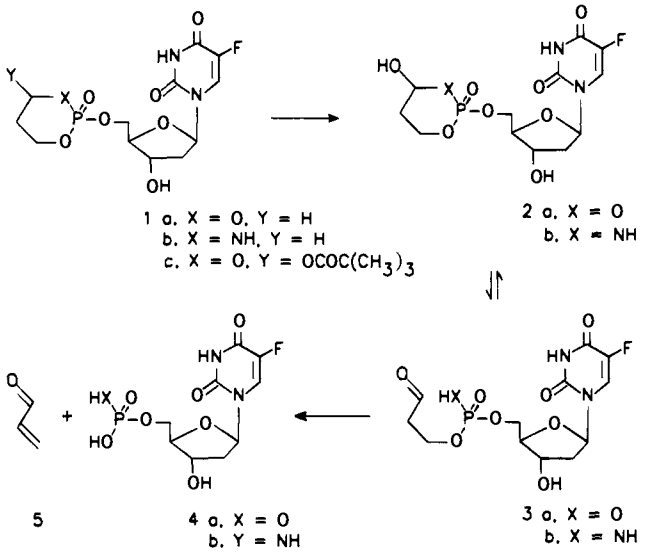
Scheme 1



cyclic compounds, unlike cyclophosphamide, were poor substrates for hepatic P-450 mixed-function oxidases in vitro,[16,18] a finding that most likely accounts for their disappointing antitumor activity in vivo.

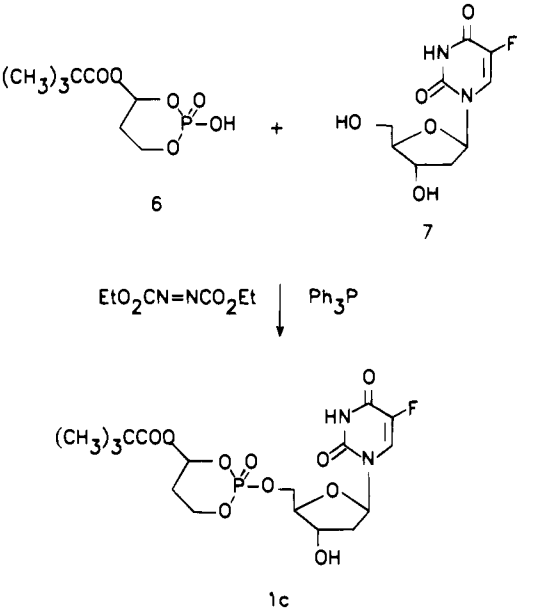
To circumvent the requirement for oxidative biotransformation of **1a**, we have now devised an alternative bioactivation mechanism. In this new approach, the activating hydroxyl group has been introduced into the 4-position of the 1,3,2-dioxaphosphorinane ring in the form of a stable carboxylate ester derivative (**1c**). Since carboxylate esterases are ubiquitous in plasma and other tissues and exhibit low substrate specificity,[19] **1c** should be facilely hydrolyzed in vivo to the putative membrane-transport analogue **2a**. **1c** might also penetrate directly into tumor cells and undergo the same degradation sequence after initial hydrolysis by cellular esterases. This article reports the synthesis of **1c** and documents several of its molecular and biological properties.

Results

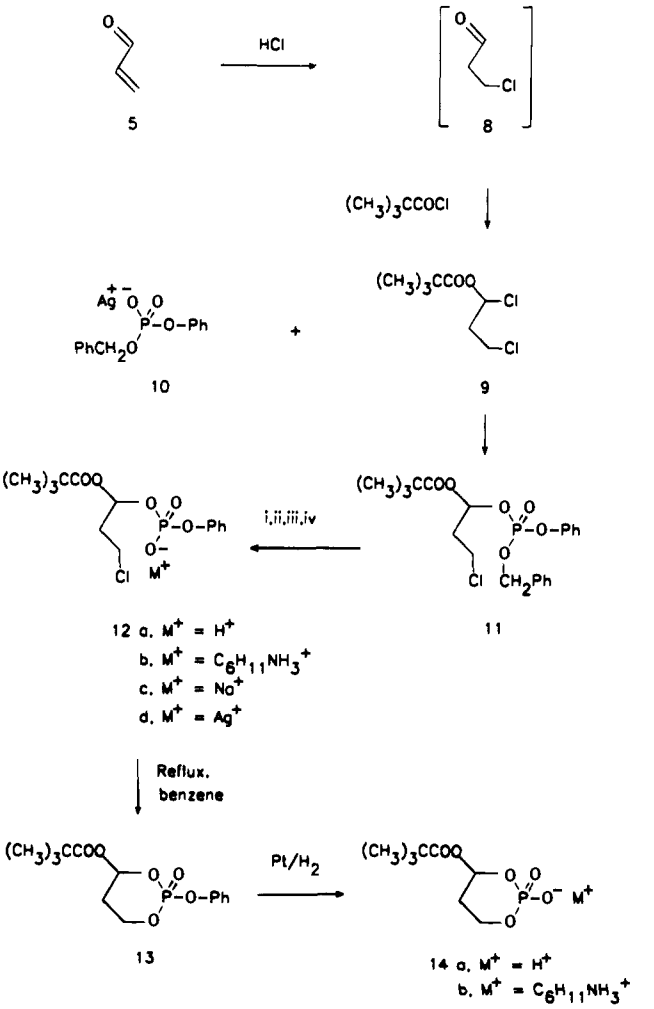
Synthesis. The most convenient synthetic route to **1c** appeared to be by condensation of the cyclic phosphorinane **6** with FUdR, **7**, in the presence of the Mitsunobo reagent[20] as shown in Scheme 2. A major advantage of this approach is that the condensation reaction can be effected under mild, neutral conditions, thus avoiding premature degradation of the (pivaloyloxy)methyl group. A further advantage is that the reaction is selective for the 5′-position of 2′-deoxynucleosides,[20] thus precluding the requirement for protection and subsequent deprotection of the 3′-OH group.

The cyclic phosphodiester **6** has not been reported before. This structure embodies three different functional groups, namely, a carboxylate ester, an acetal, and a phosphodiester. Since both acid- and base-sensitive groups are present in the molecule, neutral conditions had to be employed in the final steps of the synthesis. Of several approaches investigated, the route shown in Scheme 3 was the most successful. Thus, reaction of acrolein with pivaloyl chloride in the presence of hydrogen chloride gave 1-(pivaloyloxy)-1,3-dichloropropane (**9**). Reaction of **9** with a suspension of silver phenyl benzyl phosphate (**10**) in refluxing

Scheme 2

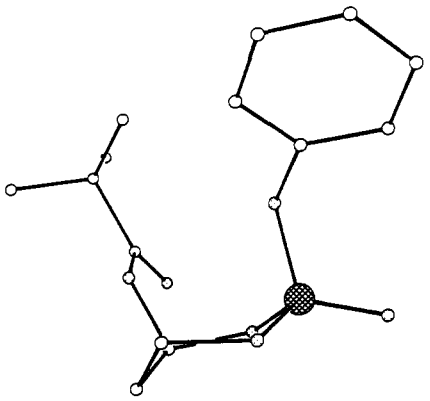


Scheme 3[a]

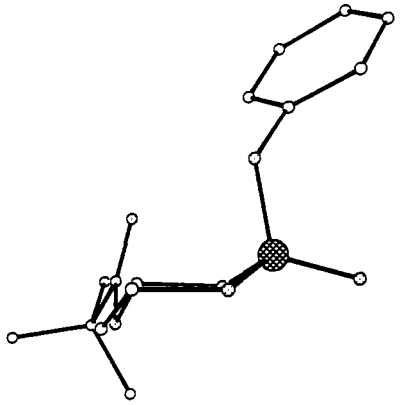


[a] Key: (i) Pd/H_2; (ii) $C_6H_{11}NH_2$; (iii) Na^+ ion-exchange resin; (iv) $AgNO_3$.

benzene for 7 h gave 1-(pivaloyloxy)-3-chloropropan-1-yl phenyl benzyl phosphate (**11**) in 69% yield. The benzyl group was removed by catalytic hydrogenation over 5% Pd/C, and the resulting phosphodiester **12a** was



(2R,4S)-13



(2R,4R)-13

Figure 1. X-ray structures of two diastereomers of 2-phenoxy-2-oxo-4-(pivaloyloxy)-1,3,2-dioxaphosphorinane (**13**). In the crystal state, each of these diastereomers is present as one of a pair of enantiomers (see the Results section).

isolated as the cyclohexylammonium salt **12b**. The latter was converted to the corresponding sodium salt **12c** by ion-exchange chromatography and then to the silver salt **12d** by treatment of **12c** with silver nitrate in aqueous solution. On heating in anhydrous benzene for 17 h, **12d** was converted to 2-phenoxy-2-oxo-4-(pivaloyloxy)-1,3,2-dioxaphosphorinane (**13**). This product was resolved by chromatography on silica gel into two components (in approximately a 3:7 ratio). X-ray crystallographic analysis of the first component to elute from the column showed that it consisted of a mixture of enantiomers in a slightly twisted chair conformation, with both the 2-phenoxy group and the 4-pivaloyloxy group axial. According to the Cahn–Ingold–Prelog rules,[21] the configurations of these enantiomers are 2*R*,4*S*(phosphorus and carbon, respectively) and 2*S*,4*R*. One of these enantiomers, 2*R*,4*S*-**13**, is shown in Figure 1. The second component to elute also existed in the solid state as a mixture of enantiomers in the chair conformation; the 2-phenoxy group was axial, as before, but now the 4-pivaloyloxy group was equatorial. These enantiomers possess the 2*R*,4*R* and 2*S*,4*S* configurations. One of these enantiomers, 2*R*,4*R*-**13**, is shown in Figure 1. Catalytic hydrogenolysis of **13** (complete racemic mixture) over PtO_2 yielded the phosphodiester acid **14a** which was purified through the intermediacy of its cyclohexylammonium salt **14b**. Condensation of **14a** with FUdR in the presence of diethyl azodicarboxylate and triphenylphosphine afforded the target phosphotriester **1c** as a mixture of stereoisomers, in 55% yield. Since these stereoisomers proved difficult to separate by preparative chromatography, the physicochemical and biological studies of **1c** were conducted on the mixture.

Table 1. Half-Lives[a] of **1c** in Aqueous Media under Different pH Conditions

conditions	half-life (h)
0.20 M HCl/KCl solution, pH 1.0	35.4
0.05 M sodium acetate buffer, pH 4.0	36.5
0.05 M potassium phosphate buffer, pH 7.4	31.4
0.05 M Tris-HCl buffer, pH 9.0	21.5
0.05 M sodium hydroxide, pH 12.6	<0.03

[a] Half-lives represent the average of triplicate determinations.

Table 2. Half-Lives[a] of **1c**[b] in 0.05 M Phosphate Buffer, pH 7.4, at 37 °C in the Absence or Presence of Hog Liver Carboxylate Esterase[c]

conditions	half-life (h)
buffer only	31.4
buffer + 3-unit equiv of enzyme	5.9
buffer + 10-unit equiv of enzyme	4.0
buffer + 25-unit equiv of enzyme	1.5

[a] Half-lives represent the average of triplicate determinations. [b] The initial concentration of **1c** was 10^{-4} M. [c] One unit of the enzyme will convert 1 μmol of ethyl butyrate to butyric acid per minute at 25 °C. The disappearance of **1c** from solution was monitored by HPLC.

Solubility of 1c in Aqueous Buffer. The maximum solubility of **1c** in 0.05 M phosphate buffer, pH 7.4, determined by stirring the compound with the buffer for 2 h at ambient temperature, was 1.29 mg/mL.

Partition Coefficient of 1c. The partition coefficient (*P*) of **1c** between *n*-octanol and 0.05 M potassium phosphate buffer, pH 7.4 (i.e., [*n*-octanol]/[buffer]), was 1.27 (log *P* = 0.10).

Stability Studies of 1c. 1. Aqueous Media. 1c was moderately stable ($t_{1/2}$ > 30 h) when stirred at ambient temperature for 24 h with aqueous buffers over the pH range 1–7.4 (Table 1). In 0.05 N NaOH solution, however, it was rapidly hydrolyzed ($t_{1/2}$ < 2 min).

2. Enzyme Studies. a. Esterases. 1c was progressively degraded when incubated with hog liver carboxylate esterase (Table 2). The rate of hydrolysis was dependent, as expected, on the amount of enzyme used. In the presence of 3-unit equiv of the enzyme (i.e., 3 units of enzyme/μmol of substrate), **1c** was degraded with a half-life of 5.9 h. This decreased to 4.0 h in the presence of 10-unit equiv of the enzyme and to 1.5 h in the presence of 25-fold unit equiv of the enzyme. The only product detected in the incubation mixtures was FdUMP.

b. Phosphohydrolases. 1c was incubated for 2 h at 37 °C with a 10-fold unit excess of alkaline phosphatase (E.C. 3.1.3.1), 5′-nucleotidase (E.C. 3.1.3.5), phosphodiesterase I (E.C. 3.1.4.1), and crude snake venom in 0.1 M Tris buffer, pH 8.0. The rate of degradation of **1c** under these conditions was the same as that in buffer alone, indicating that the compound was not a substrate for these nucleotide-catabolizing enzymes.

c. Mouse Plasma. When incubated with mouse plasma in 0.05 M phosphate buffer, pH 7.4 (1:3), **1c** was degraded to FdUMP with a half-life of 30 min (Figure 2). No reaction intermediates were detected in the

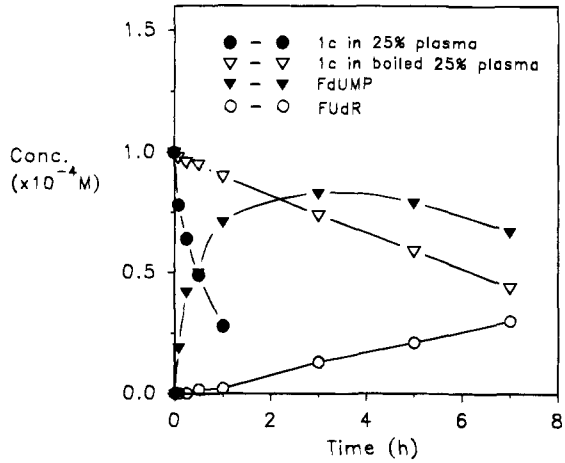


Figure 2. Degradation of 5′-[4-(pivaloyloxy)-1,3,2-dioxaphosphorinan-2-yl]-2′-deoxy-5-fluorouridine (**1c**) in mouse plasma–0.05 M phosphate buffer, pH 7.4 (1:3), at 37 °C.

Table 3. Growth Inhibitory Effects of **1c** on CHO[a] cells in Culture[b]

	IC_{50}[c] (μM)		
cell line	FU	**1c**	acrolein
CHO	5	5	50
CHO/FU	100	5	50

[a] Each value represents the average of three determinations. [b] CHO = Chinese hamster ovary. [c] The minimum drug concentration that gives rise to colonies containing fewer than 50 cells.

incubation mixtures. With continued incubation, FdUMP was progressively converted to FUdR, presumably by the action of plasma 5′-nucleotidases or phosphatases.

Cell Growth Inhibitory Studies. The growth inhibitory activity of **1c** was determined against Chinese hamster ovary (CHO) cells and a subline with 20-fold resistance to FU (CHO/FU) (Table 3). The minimal concentration of **1c** required to reduce cell growth to less than 50 cells per colony was 5×10^{-6} M, the same concentration as required for FU. **1c** was similarly potent against a CHO mutant that was 20-fold resistant to FU. Acrolein was approximately 10-fold less effective than **1c** at inhibiting the growth of both cell lines.

Antitumor Screening. The antitumor activity of **1c** was determined in mice bearing leukemia P-388 and a subline with acquired resistance to FU (FU/P-388). Administered intraperitoneally for 5 consecutive days at optimal dosage (189 mg/kg), **1c** was as effective as FU at prolonging the life span of BDF_1 mice inoculated with the wild-type tumor (Table 4). However, it was slightly less effective than FUdR. At the two highest dose levels, **1c** was unexpectedly toxic to mice bearing the FU-resistant tumor. Because **1c** is designed to liberate acrolein during activation, it was also screened in combination with 2-mercaptoethanesulfonic acid (MESNA), a nontoxic compound that abrogates the acrolein-induced cystitis occasionally observed in patients receiving high dosages of cyclophosphamide.[22] Against the P-388 tumor, the combination increased the life span 64% over that of saline-treated controls compared to 55% for 5-FU. The combination was similarly effective against the FU-resistant tumor. Animals in the FU/P-388 group that received FU along died before saline-treated controls, presumably because of the combined effects of drug toxicity and lack of drug efficacy. 5-FUdR, administered at an optimal daily dosage of 150 mg/kg, was also ineffective against the resistant tumor.

Discussion

The studies described here are an extension of our previous attempts[16,18] to exploit nucleoside 5′-cyclic phosphates and 5′-cyclic phosphoramidates as membrane-permeable prodrugs of nucleoside 5′-monophosphates. These earlier efforts were unsuccessful, apparently because the cyclic compounds were not metabolically activated in vivo as anticipated. We have now selected 5′-(1,3,2-dioxaphosphorinan-2-yl)-2′-deoxy-5-fluorouridine, **1c**, as a model 5′-cyclic nucleotide and introduced the activating hydroxyl substituent into the molecule as a stable pivaloate ester group. Since carboxylate esterases are present in all tissues[19] and hydrolyze a broad structural variety of esters, we anticipated that the pivaloate **1c** would be readily converted in vivo to the 4-hydroxy intermediate **2a**.

Table 4. Effect of **1c** on the Survival of BDF_1 Mice[a] Implanted Intraperitoneally with P-388 Leukemia[b] Sensitive (P-388/0) or Resistant (P-388/FU) to FU

		P-388/0			P-388/FU		
compd	dose (mg/kg, qd, 1–5)	MST[c] (days)	% ILS[d]	% wt[e] change	MST[c] (days)	% ILS[d]	% wt[e] change
0.9% saline	(0.2 mL)	11	–	18	11	–	13
Emulphor vehicle	(0.2 mL)	11	–	17	11	–	16
FU[f]	40				9	−18	−18
	30	17	55	−15	9	−18	−15
	20	16	45	−5	10	−9	−6
FUdR[f]	150	20	82	−5	13	18	−10
	100	19	73	−4	13	18	−10
	67	16	45	−2	12	9	−6
1c[g]	189	17	55	−5	11	toxic	−10
	126	16	45	−8	11	toxic	−11
	84	16	45	−11	15	36	−4
1c[g+]	189	18	64	−9	17	55	−4
MESNA[h]	126	17	55	−6	16	45	−8
	84	16	45	−2	15	36	−4
MESNA[h]	100	11	0	10	11	0	12

[a] Six mice per group, average weight 22 g. [b] 1×10^{-6} cells were inoculated intraperitoneally on day 0. [c] Median survival time (MST) of 15 mice used as controls was 11 days. [d] Percentage increase in life span of treated animals (T) compared with controls (C) was determined by the formula: $(T/C - 1) \times 100$. [e] The average percentage weight change on day 5 was taken as a measure of drug toxicity. [f] FU and FUdR were administered in 0.9% saline. [g] **1c** was formulated in EtOH–Emulphor 620–0.9% saline (1:1.5:7.5, v/v/v); see the Experimental Section. [h] 2-Mercaptoethanesulfonic acid, sodium salt.

The experimental findings validate this mechanistic approach. The moderate stability of **1c** in aqueous media over the pH range 1.0–7.4 indicates that it is well suited to formulation as a FdUMP prodrug. Moreover, the partition coefficient (1.27) of the compound between *n*-octanol and aqueous buffer, pH 7.4, suggests that it should be able to penetrate membrane barriers by passive diffusion. The finding that **1c** was resistant to degradation by various nucleotide-catabolizing enzymes such as 5′-nucleotidases, phosphatases, and phosphodiesterases indicates that it is unlikely to be directly degraded in vivo by these enzymes before it reaches the target site. As anticipated, **1c** was readily converted to FdUMP in the presence of carboxylate esterases or plasma. No intermediates were detected in these reactions by HPLC analysis, a finding consistent with the anticipated lability of the primary metabolite, **2a**. Once formed, it is expected that **2a** will ring open to the corresponding aldehyde, which should then spontaneously dissociate to form FdUMP and acrolein. The corresponding hydroxy and aldehyde intermediates derived from the bioactivation of cyclophosphamide likewise cannot be detected by direct HPLC analysis of biological samples, despite the fact that 4-hydroxycyclophosphamide is generally believed to be the blood-borne transport form of the drug which delivers the ionic active metabolite phosphorodiamidic mustard into cells.[17]

The finding that **1c** is as effective as FU at inhibiting the growth of CHO cells in culture and at prolonging the life spans of mice bearing P-388 leukemia is consistent with the proposed mechanism of prodrug action. Further evidence in support of this interpretation is that **1c** retains full antiproliferative activity against variants of these cell types that are resistant to FU or FUdR. This eliminates the possibility that the observed activity was due to extracellular catabolism of **1c** to FU or FUdR followed by the uptake of these compounds by cells and conversion to FdUMP. Unfortunately, the mechanisms of resistance to FU and FUdR in the CHO and P-388 cells have not been established. In addition to the reduced capacity of the cells to convert FU to FdUMP, resistance could be due to altered thymidylate synthase or the diminished incorporation of FU into RNA. However, **1c** would be expected to overcome only the first of these possible mechanisms of resistance and have little, if any, effect on the latter two. Collectively, these findings constitute strong circumstantial evidence that **1c** acts, as designed, as an esterase-activated prodrug of FdUMP.

A potential shortcoming of the prodrug strategy is that acrolein, a chemically reactive compound, is generated as a byproduct of drug activation. As already noted, acrolein is also formed in the oxidative bioactivation of cyclophosphamide and ifosfamide, two of the most effective and widely used antitumor drugs in the clinic. At normal drug dosages, the formation of acrolein does not cause toxicologic problems. However, hemorrhagic cystitis has been observed at high drug dosages, particularly in patients receiving ifosfamide, and has been attributed to the formation of acrolein.[22] Fortunately, this toxicity is well controlled by the concurrent administration of MESNA, an ionic sulfonate that localizes in the genitourinary system and reacts with acrolein to form a nontoxic thioether.[23] Mice that received MESNA in conjunction with **1c** generally tolerated the drug better, particularly in the FU-resistant group, at the higher dosages.

Conclusion

We have prepared a neutral derivative of FdUMP that is fairly stable in aqueous media, yet facilely reverts to the parent nucleotide in the presence of carboxylate esterases. The biological data suggest that this derivative acts as a membrane-permeating precursor of FdUMP. This prodrug strategy is not limited to FdUMP, which was selected as a model dianionic 5′-nucleotide, but can be used to facilitate the passage of any dianionic phosphate or phosphonate across membrane barriers. Potential applications of the strategy are to overcome biological resistance to therapeutic nucleosides arising from the deletion of activating kinases and to make available new structural classes of therapeutic organophosphates and organophosphonates.

Experimental Section

Nuclear magnetic resonance spectra (^{1}H NMR, ^{13}C NMR, and ^{31}P NMR) were recorded at ambient temperature on an IBM-Bruker Model NR/200 AF spectrometer in the Fourier transform mode, in $CDCl_3$, using tetramethylsilane as an internal standard. Some low-resolution ^{1}H NMR spectra were recorded, as noted, on a Varian Associates T-60 A spectrometer. ^{31}P chemical shifts are reported in parts per million downfield from external 85% H_3PO_4. Mass spectra were obtained on a Finnegan Model 3300 quadrupole spectrometer in the electron impact mode or using methane as the reagent gas in the chemical ionization mode. Elemental analyses wee performed by Galbraith Laboratories, Inc., Knoxville, TN; where indicated only by the symbols of elements, the results were within ±0.4% of the theoretical values. Melting points were determined on a Hoover capillary apparatus and are uncorrected. All reactions were carried out in dry glassware and protected from atmospheric moisture. Solvents were dried over freshly activated (300 °C/4 h) molecular sieves (type 4 Å). Reactions with silver salts were conducted in dry glassware in the dark and protected from atmospheric moisture. The silver salts were dried in vacuo over P_2O_5 before use. The homogeneity of the products was determined by ascending TLC on silica-coated glass plates (silica gel 60 F 254; Merck) using mixtures of $CHCl_3$–MeOH (typically 1–10% MeOH) as the eluting solvent. Chromatograms were visualized under a UV lamp (254 nm) or by placing the air-dried plates in a tank of I_2 vapor. Preparative separations were performed by flash chromatography on silica gel (Merck; 230–400 mesh) using mixtures of EtOAc–hexane or $CHCl_3$–MeOH as eluent. All chemical reagents were purchased from Aldrich Chemical Co., Milwaukee, WI.

1-(Pivaloyloxy)-1,3-dichloropropane (9). Acrolein (25.0 mL, 0.37 mol), contained in a 250-mL round bottom flask, was cooled to 0 °C in an ice bath and then saturated with dry HCl gas. Trimethylacetyl chloride (50 mL, 0.41 mol) was added followed by anhydrous $ZnCl_2$ (0.2 g), and the mixture was allowed to warm to room temperature with stirring. A vigorous exothermic reaction suddenly occurred (CAUTION), and the flask was quickly reimmersed in the ice bath to control the reaction rate. Once the reaction had subsided, the mixture was allowed to stand at room temperature for 30 min and then poured carefully, with stirring, into an ice-cold 5% $NaHCO_3$ solution (500 mL). The mixture was extracted with CH_2Cl_2 (3 × 100 mL), and the extracts were combined, dried (Na_2SO_4), and evaporated. The remaining liquid was fractionated on a Vigreux column under reduced pressure. The main boiling fraction (bp 78 °C/2.4 mmHg) yielded 49.1 g (62%) of **9**. ^{1}H NMR (60 MHz, CCl_4): δ 6.48 (t, 1 H, CHOCO, J = 6 Hz), 3.60 (t, 2 H, CH_2Cl, J = 6 Hz), 2.43 (q, 2 H, C-CH_2-C, J = 6 Hz), 1.17 (s, 9 H, $C(CH_3)_3$). MS: m/z 213/215 (MH^+, Cl^{35}, Cl^{37}). Anal. ($C_8H_{14}Cl_2O_2$) C, H.

Phenyl Benzyl Hydrogen Phosphate, Silver Salt (10). This compound was prepared from phenyl dibenzyl phosphate as described by Mostert et al.[24]

3-Chloro-1-(pivaloyloxy)propyl Phenyl Benzyl Phosphate (11). 1-(Pivaloyloxy)-1,3-dichloropropane (**9**) (23.4 g,

0.11 mol) was added to a suspension of finely divided silver phenyl benzyl phosphate (**10**) (34.0 g, 0.092 mol) in dry benzene (700 mL), and the mixture was heated under reflux for 7 h. The silver salts were removed by filtration, and the solution was evaporated. The residue was taken up in $CHCl_3$ (300 mL), and the solution was washed with a 3% $NaHCO_3$ solution (2 × 300 mL) and H_2O (300 mL), dried ($MgSO_4$), and evaporated. The residue was chromatographed on a column of silica gel (ca. 1.5 kg) using EtOAc–hexane (2 L of 20:80, v/v, solution increasing linearly to 2 L of 50:50, v/v, solution). **11** was isolated as a viscous oil (27.8 g, 69%). It was apparent from the NMR spectrum that the product consisted of a mixture of two stereoisomers in an approximately equal ratio. ^1H NMR (60 MHz, $CDCl_3$): δ 7.0–7.4 (m, 10 H, 2 × C_6H_5), 6.4–6.8 (m, 1 H, CHOCO), 5.15 (d, 2 H, $CH_2C_6H_5$, J = 8.5 Hz), 3.43 and 3.46 (tt, 1 H, 2 H, CH_2Cl), 2.30 and 2.31 (qq, 2 H, C-CH_2-C), 1.15 (s, 9 H, $C(CH_3)_3$). Anal. ($C_{21}H_{26}ClO_6P$) C, H.

3-Chloro-1-(pivaloyloxy)propyl Phenyl Phosphate, Cyclohexylammonium Salt (12b). A solution of **11** (27.0 g, 61.2 mmol) in EtOAc (200 mL) was hydrogenated over 10% Pd/C (2.5 g) at 30 psi for 30 min at ambient temperature. The catalyst was removed by filtration, and cyclohexylamine (ca. 7.0 mL) was added, dropwise, with stirring, until a drop of the solution gave a pH of 7.0 when tested against wet universal litmus paper. The mixture was concentrated to about 50 mL and then diluted to near turbidity with hexane and stored overnight at 4 °C. The white precipitate of cyclohexylammonium salt **12b** that separated was filtered (23.4 g, 85%), mp 141–142 °C. ^1H NMR (60 MHz, $CDCl_3$): δ 7.0–7.3 (m, 5 H, C_7H_5), 6.4–6.8 (m, 1 H, CHOCO), 3.53 (br t, 2 H, CH_2Cl), 2.4–3.2 (m, 1 H, $HCNH_3^+$), 2.0–2.5 (m, 2 H, C-CH_2-C), 1.07 (s, 9 H, $C(CH_3)_3$), 0.8–2.2 (m, 10 H, cyclohexyl-H). Anal. ($C_{20}H_{33}ClNO_6P$) C, H, N.

3-Chloro-1-(pivaloyloxy)propyl Phenyl Phosphate, Silver Salt (12d). **12b** (17.0 g, 37.8 mmol) was dissolved in EtOH (50 mL) and distilled H_2O (500 mL), and the solution was passed through a column (2.5 × 30 cm) of Bio-Rad AG 50W × 12 cation-exchange resin (200–400 mesh, 2.3 mequiv/mL of resin bed) in the Na^+ form. The resin was washed with three column volumes of distilled H_2O, and the effluents were combined, frozen, and lyophilized. The solid residue of **12c** (13.1 g, 93%) was taken up in distilled H_2O (25 mL), and the solution was added, dropwise, to a stirred solution of $AgNO_3$ (6.5 g, 38.3 mmol) in H_2O (12 mL). The mixture was stored overnight at 4–5 °C. The white precipitate of **12d** was filtered, washed with ice-cold H_2O, and dried in vacuo over P_2O_5 for 24 h (14.3 g, 89%). Anal. ($C_{14}H_{19}ClO_6PAg$) C, H, Ag.

2-Phenoxy-2-oxo-4-(pivaloyloxy)-1,3,2-dioxaphosphorinane (13). A suspension of finely divided **12d** (12.0 g, 26.2 mmol) in dry benzene (250 mL) was heated under reflux for 17 h. The silver salts were removed by filtration, and the solution was evaporated to dryness. The residual solid was taken up in chloroform, and the solution was washed with 3% $NaHCO_3$ solution (2 × 100 mL) and H_2O (2 × 100 mL). The organic layer was dried ($MgSO_4$) and evaporated. The clear viscous residue was chromatographed on silica gel (700 g) using EtOAc–hexane as mobile phase (1.5 L of 20:80, v/v, solution linearly increasing to 1.5 L of 50:50, v/v, solution). Two racemic forms of the product were obtained. The first to elute from the column, 2*RS*,4*SR*-**13** [i.e., a mixture of 2*R*,4*S*-**13** and 2*S*,4*R*-**13**], was obtained as a crystalline solid (2.1 g, 26%), mp 125–126 °C. TLC: EtOAc–hexane, 1:1, R_f = 0.64. HPLC: μC-18 column (Phenomenex, Torrance, CA; 150 mm × 3.9 mm), CH_3CN–0.01 M phosphate buffer, pH 7.4 (40:60), flow rate 1.5 mL/min, retention time 8.08 min. ^1H NMR (Bruker 200 MHz, $CDCl_3$): δ 7.2–7.4 (m, 5 H, C_6H_5), 6.56–6.63 (m, 1 H, H-4), 4.5–4.7 (m, 2 H, H-6), 2.1–2.4 (m, 2 H, H-5), 1.28 (s, 9 H, $C(CH_3)_3$). ^{13}C NMR (50 MHz, $CDCl_3$): δ 176.76 (O*C*OC(CH_3)$_3$), 150.13 (C-1′), 129.87 (C-3′,5′), 125.39 (C-4′), 119.99 (C-2′,6′, J_{CP} = 4.5 Hz), 92.99 (C-4, J_{CP} = 5.5 Hz), 64.74 (C-6, J_{CP} = 6.0 Hz), 38.78 (OCO*C*(CH_3)$_3$), 30.06 (C-5, J_{CP} = 6.0 Hz), 26.77 (OCOC(*C*H_3)$_3$). ^{31}P NMR ($CDCl_3$): δ −14.33. MS (CI, CH_4): m/z 315 (MH^+). Anal. ($C_{14}H_{19}O_6P$) C, H.

The second racemate to eluate, 2*RS*,4*RS*-**13** [i.e., a mixture of 2*R*,4*R*-**13** and 2*S*,4*S*-**13**], was also obtained as a crystalline solid (4.1 g, 50%), mp 99–100 °C. TLC: EtOAc–hexane, 1:1, R_f = 0.41. HPLC: μC-18 column (Phenomenex, Torrance, CA; 150 mm × 3.9 mm), CH_3CN–0.01 M phosphate buffer, pH 7.4 (40:60), flow rate 1.5 mL/min, retention time 6.89 min. ^1H NMR (Bruker 200 MHz, $CDCl_3$): δ 7.1–7.4 (s, 5 H, C_6H_5), 6.5–6.7 (m, 1 H, H-4), 4.3–4.9 (m, 2 H, H-6), 1.9–2.7 (m, 2 H, H-5), 1.16 (s, 9 H, $C(CH_3)_3$). ^{13}C NMR (50 MHz, $CDCl_3$): δ 175.62 (O*C*OC(CH_3)$_3$), 150.47 (C-1′), 129.85 (C-3′,5′), 125.09 (C-4′), 119.59 (C-2′,6′, J_{CP} = 6.0 Hz), 93.14 (C-4, J_{CP} = 10.0 Hz), 64.52 (C-6, J_{CP} = 7.0 Hz), 38.90 (OCO*C*(CH_3)$_3$), 29.72 (C-5, J_{CP} = 8.5 Hz), 26.76 (OCOC(*C*H_3)$_3$). ^{31}P NMR ($CDCl_3$): δ −15.54. MS (CI, CH_4): m/z 315 (MH^+). Anal. ($C_{14}H_{19}O_6P$) C, H.

2-Hydroxy-2-oxo-4-(pivaloyloxy)-1,3,2-dioxaphosphorinane (14a). A solution of **13** (complete racemic mixture) (6.0 g, 0.019 mol) in EtOAc (70 mL) was shaken with PtO_2 (0.5 g) under a hydrogen atmosphere at 35 psi for 40 min at ambient temperature. The catalyst was removed by filtration, and cyclohexylamine (approximately 2.2 mL) was added, dropwise, with stirring, until the mixture gave a pH of 7.0 when tested against wet universal litmus paper. The solution was concentrated to about 20 mL, cyclohexane was added to near turbidity, and the solution was left overnight at 4–5 °C. The white precipitate of **14b** was filtered (5.1 g, 79%), mp 162–163 °C. ^1H NMR (60 MHz, $CDCl_3$): δ 6.2–6.5 (m, 1 H, H-4), 3.9–4.3 (m, 2 H, H-6), 2.6–3.3 (m, 1 H, $HCNH_3^+$), 1.8–2.5 (m, 2 H, H-5), 1.23 (s, 9 H, $C(CH_3)_3$), 0.8–2.2 (m, 10 H, cyclohexyl-H). Anal. ($C_{14}H_{28}NO_6P$) C, H, N.

14b (2.75 g, 8.15 mmol) was taken up in the minimum amount of distilled H_2O, and the solution was transferred to a column (2.5 × 10 cm) of Bio-Rad AG 50W × 12 cation-exchange resin (200–400 mesh, 2.3 mequiv/mL of resin bed) in the H^+ form. The resin was washed with three column volumes of distilled H_2O, and the effluents were immediately frozen and lyophilized. **14a** was obtained as a viscous oil (1.8 g, 93%). Because of the unsuitability of this compound for long-term storage, it was used immediately in the subsequent condensation reaction. ^1H NMR ($CDCl_3$): δ 6.23–6.63 (m, 1 H, H-4), 4.0–4.9 (m, 2 H, H-6), 1.73–2.93 (m, 2 H, H-5), 1.25 (s, 9 H, $C(CH_3)_3$). In later studies, the free acid obtained by catalytic hydrogenolysis of **13** was used directly in the condensation reaction.

2′-Deoxy-5′-[2″-oxo-4″-(pivaloyloxy)-1″,3″,2″-dioxaphosphorinan-2″-yl]-5-fluorouridine (1c). FUdR (1.81 g, 7.35 mmol), **14a** (1.75 g, 7.35 mmol), and triphenylphosphine (2.02 g, 7.70 mmol) were dissolved in dimethylacetamide (15 mL) in a 50-mL round bottom flask, and a solution of diethyl azodicarboxylate (1.21 mL, 7.70 mmol) in dimethylacetamide (5 mL) was added with stirring. The flask was immersed in an oil bath at 60 °C for 5 days under a N_2 atmosphere. The solution was evaporated under reduced pressure, and toluene (25 mL) was added to the residue. The solution was again evaporated; this procedure was twice repeated. The remaining oil was triturated several times with 10-mL portions of *n*-hexane–EtOAc (3:1), and the supernatants were decanted. The residue was dissolved in MeOH (50 mL), silica (10 g) was added, and the solution was evaporated to dryness. After further evaporations from MeOH (2 × 30 mL) and EtOAc (2 × 30 mL), a free-flowing powder was obtained, which was transferred to a column (3.0 × 60 cm) of silica gel previously made up in EtOAc. The product was eluted with EtOAc–MeOH [1 L of EtOAc linearly increasing to 1 L of EtOAc–MeOH (98:2)]. Fractions containing **1c** were combined, evaporated, and dried under vacuum over P_2O_5 for 24 h. A clear viscous oil remained (1.9 g, 55% based on FUdR). HPLC analysis (see Stability Studies) indicated that it consisted of two components (stereoisomers) in the ratio 3:7. UV λ_{max} (H_2O): 268 (ϵ 7962). ^1H NMR ($CDCl_3$): δ 7.8 (d, 1 H, H-6, J = 6 Hz), 6.2–6.8 m (t, 2 H, H-1′, H-4″), 4.1–5.1 (m, 5 H, H-3′, H-4′, H-5′, H-6″), 2.0–2.9 (m, 4 H, H-2′, H-5″), 1.23 (s, 9 H, $C(CH_3)_3$). Anal. ($C_{17}H_{24}N_2O_{13}0FP$) C, H, N.

Single-Crystal X-ray Analysis. (a) 2*RS*,4*SR*-13. Crystals of 2*RS*,4*SR*-**13** were grown from ethyl acetate–hexane solution. A colorless fragment having approximate dimensions 0.30 × 0.30 × 0.55 mm^3 was cut from a long square column and mounted in a random orientation on a Nicolet R3m/V automatic diffractometer. The sample was placed in a stream of dry nitrogen gas at −50 °C. The radiation used was Mo

Kα monochromatized by a highly ordered graphite crystal. Intensities were measured using the omega scan technique, with the scan rate depending on the count obtained in rapid prescans of each reflection. Two standard reflections were monitored after every 2 h or every 100 data collected, and these showed no significant change. During data reduction, Lorentz and polarization corrections were applied. However, no correction for absorption was made due to the small absorption coefficient. The structure was solved by the SHELXTL direct methods program, which revealed the positions of all of the atoms in the molecule. The usual sequence of isotropic and anisotropic refinement was followed, after which all hydrogens were entered in ideal calculated positions and constrained to riding motion, with a single-variable isotropic temperature factor for the methyl hydrogens and a separate variable for the non-methyl hydrogens. After all shift/ESD ratios were less than 0.1, convergence was reached at the agreement factors below. No unusually high correlations were noted between any of the variables in the last cycle of full-matrix least squares refinement. The final difference density map showed a maximum peak of about 0.25 e/A^3. All calculations were made using Nicolet's SHELXTL PLUS (1987) series of crystallographic programs. Final cell constants and other information pertinent to data collection and refinement are as follows: Laue symmetry = mmm; space group = $P2bca$ (orthorhombic); unit cell dimensions, a = 11.174(2) Å, b = 11.300(2) Å, c = 25.633(4) Å; V = 3237(4) Å^3; formula units per cell (Z) = 8; density (ϱ, calcd) = 1.29 g cm^{-3}; absorption coefficient (μ) = 1.85; temperature (T) = −50 °C; radiation λ(Mo Kα) = 0.71073 Å; collection range 4° ≤ 2Θ ≤ 50°; scan width (ΔΘ) = 1.25 + (Kα$_2$ − Kα$_1$)°; scan speed range = 1.5−15° min^{-1}; total data collected = 3232; independent data ($I > 3\sigma$) = 1890; total variables = 192; final R value = 0.041; molecular formula = $C_{14}H_{19}O_6P$; formula weight = 314.3.

(b) 2*RS*,4*RS*-13. Crystals of 2*RS*,4*RS*-**13** were grown from ethyl acetate−hexane solution. A colorless fragment having approximate dimensions 0.40 × 0.45 × 0.50 mm^3 was cut from a large block and mounted in a random orientation on a Nicolet R3m/V automatic diffractometer. The data collection methods and processing parameters were the same as that described for 2*RS*,4*SR*-**13**. Final cell constants and other pertinent data are as follows: Laue symmetry = 2/m; space group = $P2_1/c$ (monoclinic); unit cell dimensions, a = 26.265(6) Å, b = 10.227(2) Å, c = 11.380(2) Å; V = 2990 Å^3; formula units per cell (Z) = 8; density (ϱ, calcd) = 1.40 g cm^{-3}; absorption coefficient (μ) = 2.00; temperature (T) = −50 °C; radiation λ(Mo Kα) = 0.71073 Å; collection range 4° ≤ 2Θ ≤ 45°; scan width (ΔΘ) = 1.30 + (Kα$_2$ − Kα$_1$)°; scan speed range = 1.5−15° min^{-1}; total data collected = 4220; independent data ($I > 3\sigma$) = 3049; total variables = 380; final R value = 0.050; molecular formula = $C_{14}H_{19}O_6P$; formula weight = 314.3.

Solubility of 1c in Aqueous Buffer. Potassium phosphate buffer (0.05 M) pH 7.4 (3 mL), was added to **1c** (15.2 mg) contained in a round bottom flask, and the mixture was stirred at room temperature for 2 h. The contents of the flask were transferred to a 10-mL centrifuge tube and centrifuged at 2000 rpm for 10 min. The supernatant was decanted and passed through a 0.22 μm Millipore filter to remove any remaining particulate matter. The concentration of **1c** in solution was determined by UV absorption using the measured value of 7962 for the molar extinction coefficient (ϵ) at a wavelength of 268 nm.

Partition Coefficient. n-Octanol (3 mL; previously equilibrated with 0.05 M potassium phosphate buffer, pH 7.4) was added to a solution of **1c** (6.0 mg) in 0.05 M potassium phosphate buffer, pH 7.4 (3 mL; previously equilibrated with n-octanol), contained in a 15-mL capped centrifuge tube. The mixture was agitated for 5 min on a Vortex shaker and then centrifuged at 2000 rpm for 5 min. The octanol and aqueous layers were separated, and the concentration of **1c** in each was determined by UV spectrometry as describe above.

Stability Studies. 1. Aqueous Media. Aliquots of a stock solution of **1c** in EtOH (10^{-2} M) were diluted with various buffers to a final concentration of 10^{-4} M. These solutions were stirred at room temperature, and at selected time intervals (typically 2, 4, 8, 12, 24, 30, 50, and 100 h), 20-μL aliquots were removed. These were analyzed immediately for parent drug by HPLC on a μC-18 reversed phase column (Phenomenex, Torrance, CA; 150 mm × 3.9 mm) using MeOH−0.01 M potassium phosphate, pH 5.5 (35:65), as mobile phase at a flow rate of 1.5 mL/min. Eluted compounds were monitored with a variable wavelength UV detector set at 273 nm and 0.01 AUFS sensitivity. Peak areas were quantitated electronically as a function of time using a Hewlett Packard Model 3390 integrator. The retention times of **1c** (which consisted of a mixture of stereoisomers in a 3:7 ratio) were 2.99 and 6.00 min. FdUMP was analyzed on a SAX ion-exchange column (Whatman) using 0.02 M ammonium phosphate buffer, pH 3.5, as mobile phase; the retention time was 5.5 min.

2. Enzyme Studies. a. Esterases. Porcine liver carboxylate esterase (E.C. 3.1.1.1) was obtained from Sigma Chemical Co., St. Louis, MO, and used as received. The specific activity of the preparation was 200 units/mg of protein, where 1 unit is defined as the amount that will hydrolyze 1.0 μmol of ethyl butyrate to butyric acid and EtOH per minute at pH 8.0 and 25 °C. **1c** was dissolved in 0.05 M phosphate buffer, pH 7.4, at a concentration of 10^{-4} M. One-milliliter aliquots of this solution contained in a 5.0-mL screw-capped glass vial were incubated at 37 °C in the absence or presence of the enzyme. At selected time intervals (typically 2, 4, 8, 12, 24, 30, 50, 100 and 250 h in the absence of enzyme), aliquots (40 μL) were removed and analyzed immediately for parent drug by HPLC as described above. For the enzyme studies, 3, 10, and 25 units of esterase/μmol of substrate were used. A control reaction was run using 25 units of enzyme that had been boiled for 10 min. To begin the reaction, 20 μL of the enzyme was added to 0.198 mL of the drug solution contained in 1.5-mL microcentrifuge tubes that were preequilibrated at 37 °C. At intervals of 0.25, 0.5, 1, 3, 5, 7, and 24 h, the vials were removed from the water bath, and three volumes (0.6 mL) of cold MeOH were added to deactivate the enzyme. The vials were agitated on a Vortex shaker for 20 s and then centrifuged for 4 min at 1000 rpm. Aliquots (40 μL) of the clear supernatant were analyzed by HPLC for parent compound and metabolites as described above. The half-lives were determined by linear least squares regression analysis of the pseudo-first-order reactions.

b. Phosphohydrolases. **1c** (0.7 μmol) in H_2O (393 μL) was added to the following enzyme solutions maintained at 37 °C in a shaking water bath, and the mixtures were incubated for 2 h: (a) 5′-nucleotidase (*Crotalus adamanteus*) (20 μL, 2.25 units/mL) in 0.1 M Tris-HCl buffer−0.01 mM $MgCl_2$, pH 8.0 (100 μL); (b) alkaline phosphatase (*Escherichia coli*) (20 μL, 2.18 units/mL) in 0.1 M Tris-HCl buffer, pH 8 (100 μL); (c) phosphodiesterase I (*C. adamanteus*) (20 μL, 1.69 units/mL) in 0.1 M Tris-HCl buffer−0.01 mM $MgCl_2$, pH 8.0 (100 μL); and (d) snake venom (*C. adamanteus*) (20 μL, 1.25 mg/mL) in 0.1 M Tris-HCl buffer−0.01 mM $MgCl_2$, pH 8.0 (200 μL). Immediately after the addition of **1c**, and at 0.5, 1.0, and 2.0 h, thereafter, aliquots (50 μL) of the reaction mixture were withdrawn and added to 100 μL of MeOH in a centrifuge tube. The mixtures were agitated on Vortex mixer for 20 s and then centrifuged at 2000 rpm for 5 min. The supernatants were analyzed by HPLC as described above.

c. Mouse Plasma. Blood was obtained from female BDF_1 mice (Timco Co., Houston, TX). The samples were centrifuged at 2000g for 10 min at 4 °C, and the plasma supernatants were removed by aspiration. The plasma was diluted with three volumes of 0.05 M phosphate buffer, pH 7.4. The reaction was started by adding 20 μL of a stock solution (2 × 10^{-2} M) of **1c** in EtOH to plasma (1.98 mL) contained in a 5-mL vial, such that the final concentration was 2 × 10^{-4} M. The sample was agitated for 15 s on a Vortex shaker to ensure thorough mixing and then immersed in a water bath at 37 °C. Samples (100 μL) were withdrawn at 0, 5, 15, 30, 60, 180, 360, and 1440 min and diluted with four volumes of MeOH. The mixture was agitated on a Vortex shaker for 1 min and then centrifuged at 10 000 rpm for 10 min to sediment precipitated protein. The supernatants were analyzed for **1c** and FdUMP as described above. FUdR was determined by HPLC on a μC-18 reversed phase column (Phenomenex, Torrance, CA; 150 mm × 3.9 mm) using MeOH−0.01 M potassium phosphate, pH 5.5 (5:95), as

mobile phase at a flow rate of 1.5 mL/min; the retention time was 5.9 min. A control experiment was conducted using mouse plasma that had been boiled for 10 min and then homogenized.

Cytotoxicity Studies. Chinese hamster ovary (CHO) cells were dispersed into a series of 35-mm plastic dishes (approximately 200 cells/dish) and allowed to form clones in the presence of drug at the following concentrations: 0, 0.5, 1.5, 5, 10, 50, and 100 μM. After incubation for 7 days in humidified CO_2 at 37 °C, the media were decanted and the clones were fixed with 10% formaldehyde for 10 min and stained with 0.1% crystal violet. The minimum inhibitory drug concentration was the lowest concentration tested that resulted in the formation of colonies containing fewer than 50 cells.

Antitumor Screening. Mice weighing 20–23 g were obtained from Jackson Laboratories, Madison, WI. Murine leukemia P-388 either sensitive or resistant to FU (P-388/0 and P-388/FU) were obtained from the National Cancer Institute Tumor Repository, Frederick, MD. The tumors were maintained by weekly passage in female DBA/2 mice. For antitumor screening, 1×10^6 cells were inoculated intraperitoneally into male BDF_1 mice. FU and FUdR were formulated in 0.9% saline solution. However, **1c** was insufficiently soluble in saline at the dosages administered and so was formulated with the aid of Emulphor 620, a polyoxyethylated vegetable oil (GAF Corp., NY). This was accomplished by first preparing a stock solution of **1c** in EtOH. Emulphor 620 (1.5 vol) was then added, and the solution was thoroughly mixed on a Vortex shaker. Finally, a 0.9% saline solution (7.5 vol) was added, with stirring, so that each individual dose was contained in 0.2 mL of vehicle. The drugs were administered ip daily for 5 consecutive days beginning 24 h after tumor implantation. Animals were observed until the time of death. Antitumor activity was determined by comparing the median survival time of the treated animals (T) with that of saline-treated controls (C) and expressed as the percentage increase in life span (% ILS), where % ILS = $(T/C - 1) \times 100$.

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Supplementary Material Available: X-ray crystallography data on 2-phenoxy-2-oxo-4-(pivaloyloxy)-1,3,2-dioxaphosphorinane (racemates 2*RS*,4*SR*-**13** and 2*RS*,4*RS*-**13**) (23 pages). Ordering information is given on any current masthead page.

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