Synthesis and *in Vivo* Evaluation of Prodrugs of 9-[2-(Phosphonomethoxy)ethoxy]adenine

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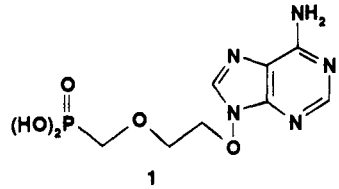
A number of esters and amides of the anti-HIV nucleotide analogue 9-[2-(phosphonomethoxy)-ethoxy]adenine (**1**) have been synthesized as potential prodrugs and evaluated for oral bioavailability in mice. Dialkyl esters **17**–**20** were prepared via a Mitsunobu coupling of alcohols **8**–**11** with 9-hydroxypurine **12** whereas (acyloxy)alkyl esters **25**–**33** and bis-[(alkoxycarbonyl)methyl] and bis(amidomethyl) esters **34**–**39** were obtained by reaction of **1** with a suitable alkylating agent. Phosphonodichloridate chemistry was employed for the preparation of dialkyl and diaryl esters **42**–**65**, and bis(phosphonoamidates) **66** and **67**. Following oral administration to mice, most of the dialkyl esters **17**–**20** were well-absorbed and then converted to the corresponding monoesters, but minimal further metabolism to **1** occurred. Bis[(pivaloyloxy)methyl] ester **25** displayed an oral bioavailabilty of 30% that was 15-fold higher than the bioavailability observed after dosing of **1**. Methyl substitution at the α carbon of the bis[(pivaloyloxy)methyl] ester **25** (**33**) increased the oral bioavailability of **1** to 74%. Some of the diaryl esters also showed improved absorption properties in comparison with that of **1**. In particular, the crystalline hydrochloride salt of diphenyl ester **55** was well-absorbed and efficiently converted to the parent compound with an oral bioavailability of 50%. On the basis of these results as well as the physicochemical properties of the prodrugs and their stability in mouse duodenal contents, the hydrochloride salt of diphenyl ester **55** was identified as the preferred prodrug of **1**.

Introduction

9-[2-(Phosphonomethoxy)ethoxy]adenine (**1**) is a novel acyclic nucleotide analogue[1,2] with potent and selective activity against human immunodeficiency virus-1 (HIV-1) (IC_{50} 0.2 μM) and human immunodeficiency virus-2 (HIV-2) (IC_{50} 0.3 μM) in peripheral blood lymphocytes.[3] Compound **1** also displayed selective *in vitro* activity against simian immunodeficiency virus (IC_{50} 3 μM) and feline immunodeficiency virus (IC_{50} 1.7 μM) and good *in vivo* efficacy against Rauscher murine leukemia virus when administered subcutaneously to mice.[4] Moreover, **1** has been shown to be at least 1500 times less toxic than 3′-azido-3′-deoxythymidine (AZT) to human bone marrow erythroid progenitor cells in tissue culture.[4]



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However, after oral administration of this compound to mice only 2% of the phosphonate **1** was detectable in the blood.[5] The related antiviral nucleotide analogues 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA) and 9-[2-(phosphonomethoxy)propyl]adenine (PMPA) have also been reported to be poorly absorbed after oral administration to rats (7%)[6] and monkeys (<1%).[7]

Phosphonate and phosphate derivatives of nucleoside analogues are charged at physiological pH and often display limited permeability through mucosal and cellular membranes. To facilitate the cellular penetration of these molecules, a variety of protecting groups for the anionic oxygens at the phosphorus have been examined. Recently, the bis[(pivaloyloxy)methyl] esters of PMEA and PMPA were shown to have 100-fold increased cellular uptake and considerably greater antiviral activity *in vitro* in comparison with the parent compounds.[8,9] Similarly, the bis[(pivaloyloxy)methyl] esters of 2′-dideoxyuridine 5′-monophosphate[10] and 5-fluoro-2′-deoxyuridine 5′-monophosphate[11] as well as aryl phosphate derivatives[12] of AZT were found to be good prodrugs for the intracellular delivery of the bioactive phosphates. Interesting results were also obtained from evaluation of 4-(acyloxy)benzyl esters[13,14] of phosphonoacetate and PMEA *in vitro*. Thus, it was demonstrated that the enzymatic hydrolysis of bis- and mono[4-(acyloxy)benzyl] phosphoesters proceeded via cleavage of the acyl group with the formation of highly unstable 4-hydroxybenzyl phosphoester intermediates and that the rate of hydrolysis could be controlled by varying the length and steric bulk of the acyl group.

Despite considerable interest in the antiviral activity of acyclic nucleoside phosphonates[15–20] and promising results from studies on their ester prodrugs *in vitro*, until recently[21,22] only limited data were available on gastrointestinal absorption properties of such prodrugs. During preparation of the manuscript a paper describing oral bioavailability determination of PMEA esters was published.[21] Several prodrugs of PMEA, mainly its dialkyl and bis[(acyloxy)alkyl] esters, were evaluated in rats. The results of those studies together with our findings on prodrugs of **1** considerably enlarge the scant, existing knowledge of phosphonate drug delivery.

In this publication, we report the synthesis of a systematic range of derivatives of **1**, including the bis-

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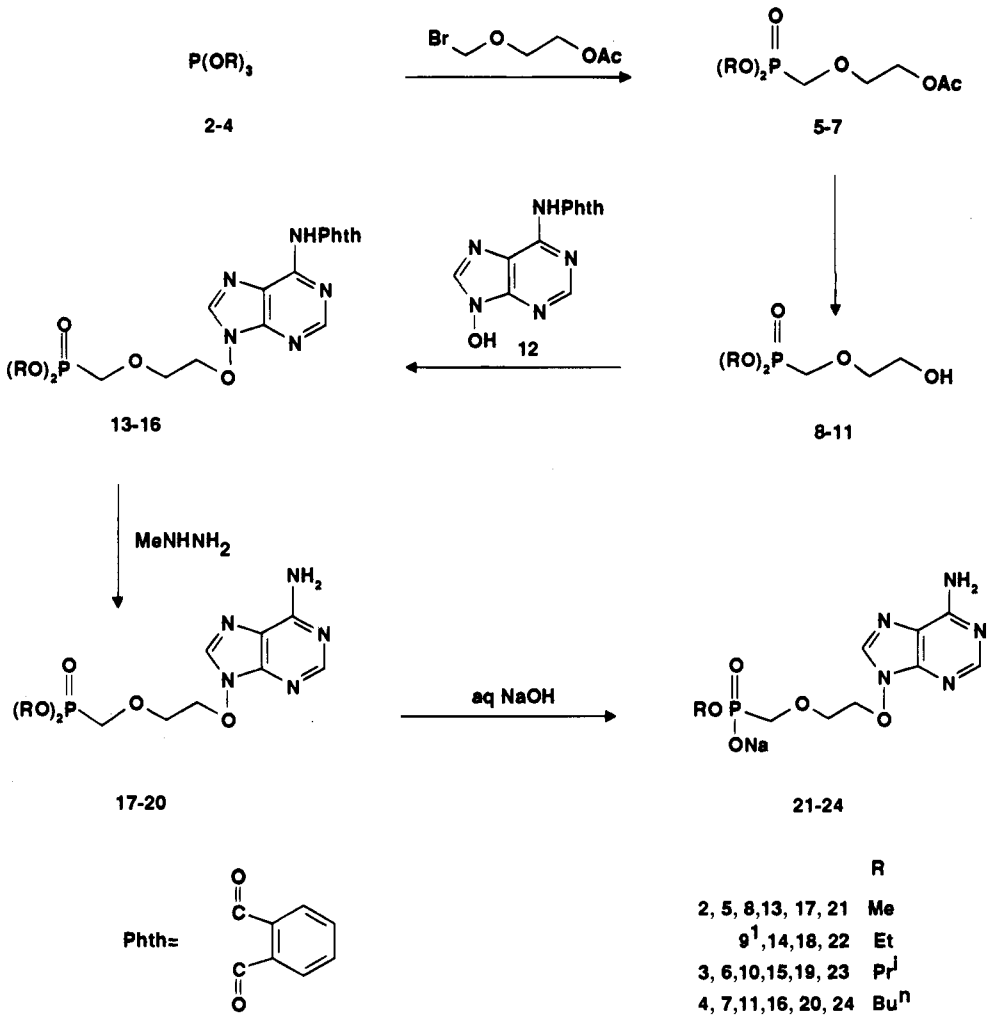
Scheme 1



[(pivaloyloxy)methyl] ester and other (acyloxy)alkyl esters as well as a number of dialkyl esters, diaryl esters, and diamides. The esters and diamides of **1** were administered orally to mice, and the plasma profile of prodrug, its partially hydrolyzed products, and released **1** was measured. In addition, the stability of selected prodrugs in aqueous solution over a wide pH range and in mouse duodenal contents was also examined. The results of this study may be applicable to other nucleoside analogues with interesting antiviral activity, or indeed to different bioactive phosphonate structures.

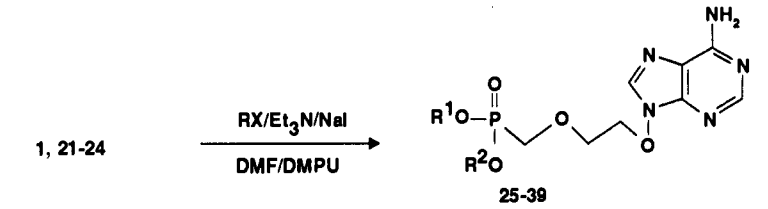
Results and Discussion

Chemistry. The dialkyl phosphonates **17**–**20** were prepared by a route involving the coupling of a suitable alcohol derivative with the protected 9-hydroxypurine **12** under Mitsunobu conditions[1,23] (Scheme 1). Thus, reaction of (2-acetoxyethoxy)methyl bromide with the appropriate alkyl phosphites **2**–**4** followed by acid hydrolysis of the resulting phosphonates **5**–**7** gave phosphonomethoxy alcohols **8**–**11** in good overall yields. Condensation of the alcohols **8**–**11** with 9-hydroxy-6-*N*-phthalimidopurine[24] (**12**) and subsequent cleavage of the resultant *N*-alkoxyphthalimides **13**–**16** with methylhydrazine, afforded the N-deprotected diesters **17**–**20** in 65–98% yield. Hydrolysis of **17**–**20** with aqueous sodium hydroxide provided the corresponding monoalkyl esters **21**–**24** in 6–86% yield.

Initial attempts to prepare the bis[(pivaloyloxy)methyl] ester **25** by alkylation[25–27] of the silver, potassium, or tetrabutylammonium salt of **1** with chloromethyl pivalate or iodomethyl pivalate were unsuccessful. However, when the triethylammonium salt of **1** or its monoalkyl esters **21**–**24** were reacted with chloromethyl pivalate in DMF at 60 °C the diester **25** and the mixed alkyl (pivaloyloxy)methyl esters **26**–**29**, respectively, were obtained in 41–61% yield (Scheme 2). Alkylation of **1** with other chloroalkyl esters, under similar reaction conditions, afforded the bis[(benzoyloxy)methyl] (**30**), bis[[(3-chloropivaloyl)oxy]methyl] (**31**) and bis[1-(acyloxy)ethyl] esters (**32** and **33**) in 6–33% yield, the latter two compounds being isolated as mixtures of diastereomers. As expected, esterification of the triethylammonium salt of **1** with 1-chloroethyl isobutyrate or 1-chloroethyl pivalate[28] was more effective[29] when the reaction was conducted in 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone (DMPU) in the presence of sodium iodide. In this way, the corresponding esters **32** and **33** were obtained in 32% and 53% yield, respectively, also as mixtures of diastereomers.

Alkylation of the triethylammonium salt of **1** with 2-chloro-*N*,*N*-diethylacetamide or 2-bromo-*N*,*N*-dibutylacetamide in DMPU afforded the corresponding esters **38** and **39** in 28% and 29% yield, respectively. A similar reaction of **1** with alkyl bromoacetates in DMF or in

Scheme 2



Acyloxyalkyl esters

	R^1	R^2
25	$Me_3CCO_2CH_2$	$Me_3CCO_2CH_2$
26	$Me_3CCO_2CH_2$	Me
27	$Me_3CCO_2CH_2$	Et
28	$Me_3CCO_2CH_2$	Pr^i
29	$Me_3CCO_2CH_2$	Bu^n
30	$C_6H_5CO_2CH_2$	$C_6H_5CO_2CH_2$
31	$Me_2C(CH_2Cl)CO_2CH_2$	$Me_2C(CH_2Cl)CO_2CH_2$
32	$Me_2CHCO_2CH(Me)$	$Me_2CHCO_2CH(Me)$
33	$Me_3CCO_2CH(Me)$	$Me_3CCO_2CH(Me)$

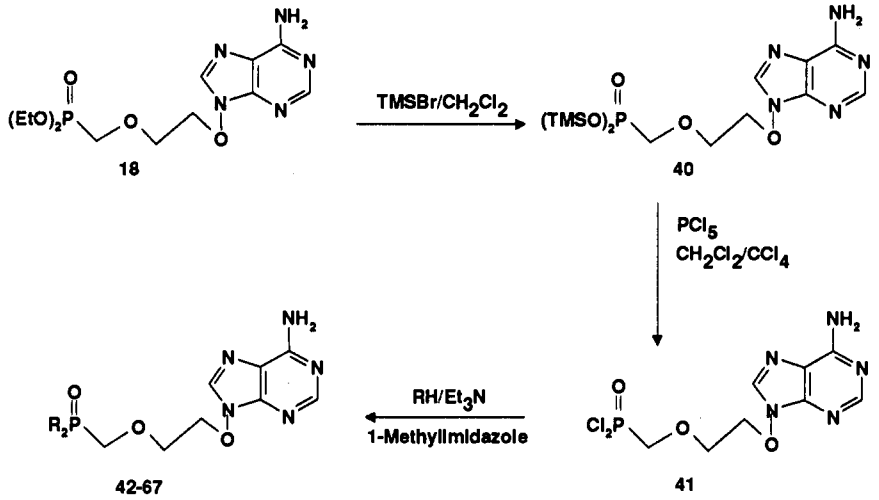
Alkoxycarbonylmethyl esters

	$R^1=R^2$
34	$MeOCOCH_2$
35	$EtOCOCH_2$
36	Pr^iOCOCH_2
37	Bu^tOCOCH_2

Amidomethyl esters

	$R^1=R^2$
38	Et_2NCOCH_2
39	$Bu_2^nNCOCH_2$

Scheme 3



Benzyl esters

	R
42	$C_6H_5CH_2O$
43	$4\text{-}BrC_6H_4CH_2O$
44	$4\text{-}ClC_6H_4CH_2O$
45	$4\text{-}MeC_6H_4CH_2O$
46	$4\text{-}Me_2CHCO_2C_6H_4CH_2O$
47	$4\text{-}AcOC_6H_4CH_2O$

Substituted ethyl and propyl esters

	R
48	$Br(CH_2)_2O$
49	Cl_2CHCH_2O
50	$EtO(CH_2)_2O$
51	$C_6H_5CH_2O(CH_2)_2O$
52	$AcO(CH_2)_2O$
53	$AcOCH_2CH(OAc)CH_2O$
54	$Me_3CCO_2(CH_2)_2O$

Aryl esters

	R
55	C_6H_5O
56	$4\text{-}BrC_6H_4O$
57	$4\text{-}ClC_6H_4O$
58	$2\text{-}MeC_6H_4O$
59	$2,6\text{-}DIMeC_6H_3O$
60	$2\text{-}AcOC_6H_4O$
61	$4\text{-}AcOC_6H_4O$
62	$2\text{-}AcOCH_2C_6H_4O$
63	$4\text{-}AcOCH_2C_6H_4O$
64	$4\text{-}MeOC_6H_4O$
65	$4\text{-}Me_3CCO_2C_6H_4O$

Phosphonoamidates

	R
66	Et_2N
67	MeO_2CCH_2NH

DMPU resulted in formation of the bis[(alkoxycarbonyl)-methyl] esters **34**–**37** in 8–30% yield.

A different approach, utilizing the phosphonodichloridate[30] intermediate **41**, was used for the preparation of diesters **42**–**65** and bis(phosphonoamidates) **66** and **67** (Scheme 3). Initial transesterification of **18** with bromotrimethylsilane followed by chlorination of the resulting silyl ester **40** with phosphorus pentachloride afforded the phosphonodichloridate **41**, which in turn was reacted *in situ* with the appropriate alcohol or amine in the presence of triethylamine and 1-methylimidazole to give the expected diesters **42**–**65** and diamides **66** and **67** in 7–40% overall yield.

This method proved very convenient for the synthesis of a series of esters with diverse structures such as phenyl, and substituted phenyl esters **55**–**65**, benzyl and substituted benzyl esters **42**–**47**, and alkyl esters **48**–**54**. Most of the diesters of **1** were isolated as oils. Since the diphenyl ester **55** displayed good oral bioavailability, it was of particular importance to obtain this compound in a crystalline form. Although attempts at crystallization of **55** from several solvents were

Table 1. Concentrations of 9-[2-(Phosphonomethoxy)ethoxy]adenine **1** and Its Mono- and Dialkyl Esters in the Blood following Oral Administration of Esters to Mice

diester	total AUC 15–180 min (μM) 1	monoester	diester	bioavailability of 1 (%)[a,b]
alkyl				
17	0	9	39	0
18	4	35	9	8
19	5	29	10	10
20	0	69	2	0
(acyloxy)alkyl				
25	15	0	0	30
26	0	9	0	0
27	0	40	0	0
28	0	8	0	0
29	0	11	0	0
31	0	0	0	0
32	12	0	0	24
33	37	0	0	74
benzyl				
42	2	18	0	4
43	1	3	9	2
44	4	12	31	8
46	0.5	0	0	1
47	4	0	0	8
substituted ethyl				
48	4	0	0	8
49	3	13	15	6
50	0	22	13	0
aryl				
55	13	1	0	26
55A	25	0	0	50
56	3	0	0	6
58	11	0	0	22
60	5	16	0	10
61	4	5	0	8
63	0	1	0	0
64	5	14	0	10
65	0	0	0	0

[a] The bioavailability of **1** after oral administration of prodrugs was calculated from the equation: % bioavailability = (AUC 1)/(iv AUC 1) × 100 where iv AUC 1 = 50 μM h. [b] The oral bioavailability of 1 for compounds not shown in Table 1 was 0%.

unsuccessful, its hydrochloride salt (**55A**) was isolated as a stable, crystalline solid (mp 139–143 °C).

Studies on the hydrolysis of the diphenyl ester **55** under acidic, neutral, and basic conditions showed that this compound was quite stable at pH levels between 2.0 and 5.0. Only 2% of the corresponding monoester was present in the reaction mixture at pH 2.0 after 65 h at 23 °C whereas 9% of the monoester was formed at pH 2.0 after 3.5 h at 37 °C. The rate of hydrolysis of **55** was similar between pH 3.0 and 5.0, and only 4–5% of the monoester was formed after 2 h at 37 °C. Increased alkalinity facilitated the hydrolysis of **55** and as a result 11% of the monoester was detected at pH 7.0, 43% at pH 8.0, and 66% at pH 8.5 after 2 h at 37 °C.

Gastrointestinal Absorption and Conversion to 1 in Mice. Esters **17–65** and diamides **66** and **67** were administered orally to mice, and concentrations of **1**, the prodrug, and its partially hydrolyzed products in the blood were determined by HPLC. Oral bioavailability, as measured by area under the curve (AUC), was expressed as percentage of the intravenous bioavailability of the sodium salt of **1**.

Most of the unsubstituted dialkyl esters **17–20** appeared to be well-absorbed after oral administration as assessed by the total concentrations of diester, monoester, and **1** measured in the blood (Table 1). However, minimal oral bioavailability of **1** was observed for these compounds due to the inefficient cleavage of the diesters and the corresponding monoesters to **1**. In particular, the dimethyl ester **17** was highly resistant toward the hydrolysis, and most of this compound remained unchanged in the blood. The diethyl and diisopropyl esters **18** and **19** were only partially converted to the monoesters and then to **1**, providing 8% and 10% bioavailability of **1**, respectively. The more bulky diisobutyl ester **20** was very well-converted to the monoester, but no further metabolism occurred. When the monoesters **21–24** themselves were administerd orally, there was no evidence of any absorption (data not shown). Conversion of the monoesters to the mixed (pivaloyloxy)methyl alkyl esters **26–29** improved their absorption, but the (pivaloyloxy)methyl group was preferentially hydrolyzed, leaving the monoalkyl ester which was resistant to futher metabolism. The diamides **66** and **67** did not afford any detectable metabolites in the blood.

The absorption of substituted dialkyl and dibenzyl esters varied from very poor to moderate, although the bioavailability from these compounds was always less than 10%. The bis[(alkoxycarbonyl)methyl] esters **34–37** and bis(amidomethyl) esters **38** and **39**, as well as some of the protected 2-hydroxyethyl and 2,3-dihydroxypropyl esters **50–54**, did not provide detectable concentrations of **1** in the blood (data shown only for **50**), but 2-halo-substituted ethyl esters **48** and **49** were absorbed and cleaved with different degrees of efficiency to **1**. The oral bioavalability and extent of metabolism of substituted benzyl esters was dependent on the aryl ring substitution, and this was well-demonstrated with bis(4-alkoxybenzyl) esters **46** and **47**. The 4-alkoxybenzyl moiety has been predicted to be an effective phosphonate prodrug moiety through esterase bioactivation[13] followed by chemical breakdown.[13,31] Our studies showed that the bis(4-acetoxybenzyl) ester **47** gave only 8% bioavailability of **1** with no monoester or diester detected in the blood. Replacement of the 4-acetoxy group with a more bulky isobutyryloxy substituent reduced significantly the bioavailability of **1** to just of 1%.

The bis[(pivaloyloxy)methyl] ester **25** was absorbed and readily metabolized to the parent compound with an oral bioavailabilty of 30%, providing maximum concentrations of **1** (8 μM) 15 min after administration. No di- or monoester was detected in the blood samples. Further studies in mouse duodenal contents demonstrated that **25** was rapidly metabolized to the corresponding monoester with no diester remaining after 1 min followed by much slower hydrolysis of the monoester to **1** over the next 40 min. Methyl substitution at the α carbon of the bis[(pivaloyloxy)methyl] ester **25** increased the oral bioavailability of **1** to 74%. The maximum concentration of **1** was 17 μM at the 60 min time point, and no di- or monoester was detected in the blood. These results suggest that the absorption of **33** was slower than that of the bis[(pivaloyloxy)methyl] ester **25**. As expected, the bis[(1-pivaloyloxy)ethyl] ester **33** proved more stable in mouse duodenal contents than the bis[(pivaloyloxy)methyl] ester **25**. After a 2 h incubation at 37 °C only 50% of diester **33** remained with 50% conversion to **1**, and no corresponding monoester was detected. Despite the excellent pharmacokinetic profile obtained from oral administration of **33**,

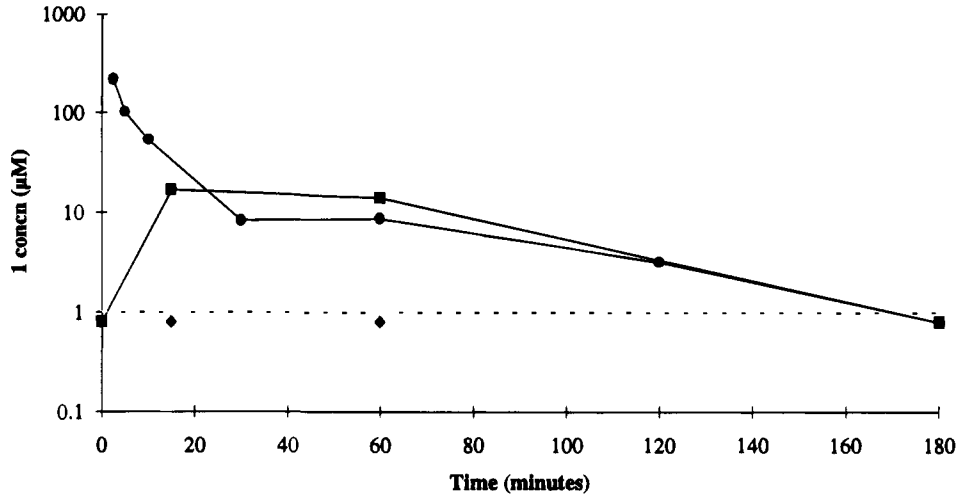


Figure 1. Concentrations of 1 in the blood following intravenous (●) or oral (◆) administration of 1 or oral administration of **55A** (■) to mice.

it was considered that the presence of diastereomers and the physical properties of the compound made it unsuitable for further evaluation as a prodrug.

The overall absorption and the oral bioavailability of **1** for the aryl esters **55**–**65** varied with the aryl substitution, although no clear correlation was observed with the nature of the substituents. In no case was the diaryl ester detected in the blood, metabolism having occurred through to the monoestr and/or **1** itself. The oral bioavailability of **1** ranged from 0 to 26% in this series. Interestingly, the bis(2-methylphenyl) ester **58** was efficiently metabolized to **1** with an oral bioavailability of 22%, but the even more sterically crowded bis-(2,6-dimethylphenyl) ester **59** failed to give any absorption. The unsubstituted diphenyl ester **55** afforded the most promising profile of absorption with 26% bioavailability of **1** and minimal levels of the monoester detected in the blood. The concentrations of **1** ranged from 13 to 20 μM at 15 min after dosing and between 2 and 7 μM at 60 min after dosing.

The crystalline hydrochloride (**55A**) of diphenyl ester **55** gave higher concentrations of **1** (approximately 13 μM) 60 min after dosing (Figure 1), and as a result the oral bioavailability of **1** was increased to 50%. Further studies demonstrated that the diphenyl ester of **1** was relatively stable in mouse duodenal contents with only 10% conversion to **1** after 2 h incubation at 37 °C.

The good oral bioavailability of **1** obtained from **55A** along with the suitable stability profile in aqueous solution and duodenal contents led to the selection of **55A** as the preferred oral prodrug of **1**. Subsequently, oral therapy with **55A** has been shown to inhibit effectively splenomegaly and viraemia in mice infected with Rauscher murine leukemia virus.[5]

Experimental Section

Melting points were determined on a Reichert Kofler apparatus and are uncorrected. ^{1}H NMR spectra were recorded with a JEOL GX-270 270-MHz or a Varian EM-390 90-MHz. Infrared spectra were recorded with a Perkin-Elmer 580 spectrometer, and mass spectra were recorded on a JEOL JMS-SX102 spectrometer. Microanalyses were performed on a Carlo Erba model 1106 analyzer and, where only the symbols for the elements are recorded, were within ±0.4% of the calculated values. Column chromatography was carried out on Merck 7736 silica gel. All compounds were homogenous by TLC on silica gel $60F_{254}$-coated glass plates. Determination of hydrolysis products concentrations in aqueous solutions was by HPLC using Waters 6000A/660 equipment and Spherisorb ODS column. For the analyses the buffer used was 0.1 M NH_4-OAc pH 7.5 and CH_3CN. Dry toluene, methylene chloride, and carbon tetrachloride were obtained by distillation from CaH_2 prior to use.

Dimethyl [(2-Acetoxyethoxy)methyl]phosphonate (5). To stirred trimethyl phosphite **2** (6.84 mL, 0.058 mmol) at 80 °C was added dropwise (2-acetoxyethoxy)methyl bromide[1] (10.0 g, 0.05 mmol). The temperature was then increased to 140 °C, and the reaction mixture was stirred at this temperature for 5 h. After the solution was cooled to room temperature, an excess of trimethyl phosphite was removed and the residue was distilled (120 °C/0.5 mmHg) to give **5** as an oil; (6.81 g, 60%): ^{1}H NMR ($CDCl_3$) δ 2.01 (3 H, s, CH_3CO), 3.70 (10 H, m, CH_2CH_2O and 2 × CH_3OP), 4.20 (2H, m, CH_2P). Anal. ($C_7H_{15}O_6P$·0.5H_2O) C, H, N.

Diisopropyl [(2-Acetoxyethoxy)methyl]phosphonate (6) and Dibutyl [(2-Acetoxyethoxy)methyl]phosphonate (7). Compounds **6** and **7** were obtained from the corresponding phosphites **3** and **4** according to the procedure described previously for **5**. Reaction time was 2 h for **6** and 5 h for **7**. The products **6** and **7** were used in the next step without purification.

Preparation of Dialkyl [(2-Hydroxyethoxy)methyl]-phosphonates (8, 10, and 11). To a solution of **5**–**7** (25 mmol) in methanol or 2-propanol (50–70 mL) was added aqueous 2 M HCl (25 mmol), and the resulting mixture was stirred under reflux for 1.5–3 h. The solvents were removed, and the residue was purified by column chromatography on silica gel eluting with dichloromethane–methanol or ethyl acetate–hexane mixtures.

Dimethyl 2-[(Hydroxyethoxy)methyl]phosphonate (8): yield 77%; colorless oil; ^{1}H NMR (Me_2SO-d_6) δ 3.53 (4 H, m, CH_2CH_2O), 3.68 (6 H, d, J = 10.7 Hz, 2 × CH_3OP), 3.86 (2 H, d, J = 8.2 Hz, CH_2P), 4.65 (1 H, bs, D_2O exchangeable, OH). Anal. ($C_5H_{13}O_5P$·0.5H_2O) C, H.

Preparation of 9-*N*-Alkoxy-6-*N*-phthalimidopurines, Compounds 13–16. To a solution of dialkyl ((2-hydroxyethoxy)methyl)phosphonate **8**–11 (15.5 mmol), triphenylphosphine (23.5 mmol), and 9-hydroxy-6-*N*-phthalimidopurine **12** (15.5 mmol) in THF (45 mL) at 5 °C was added diethyl azodicarboxylate (23.3 mmol). The resulting solution was stirred at room temperature for 1.5–15 h. The solvent was removed and the residue was purified by column chromatography on silica gel eluting with ethyl acetate–methanol (9:1) or hexane-acetone (3:1) to give the products.

9-[2-((Diethoxyphosphoryl)methoxy)ethoxy]-6-*N*-phthalimidopurine (14): yield 68%; gum; ^{1}H NMR (Me_2SO-d_6) δ 1.24 (6 H, t, J = 7.1 Hz, 2 × CH_3), 3.93 (4 H, m, CH_2P and CH_2O), 4.05 (4 H, m, 2 × CH_2OP), 4.68 (2 H, m, CH_2ON), 8.06 (4 H, m, aromatic protons), 8.98 (1 H, s, H-2 or H-8), 9.10 (1

H, s, H-2 or H-8); FABMS (positive ion, thioglycerol) 476 (MH^+). Anal. ($C_{20}H_{22}N_5O_7P$) H; C: calcd, 50.53; found, 51.08; N: calcd, 14,73; found 13.76.

Preparation of Dialkyl Esters 17–20. To a solution of the appropriate 6-*N*-phthalimidopurine **13–16** (7.8 mmol) in dichloromethane (15 mL) was added methylhydrazine (0.5 mL, 9.4 mmol). The resulting mixture was stirred at room temperature for 0.75–1 h. The solvent was removed, and the residue was purified by column chromatography on silica gel eluting with dichloromethane–methanol mixtures.

9-[2-((Diethoxyphosphoryl)methoxy)ethoxy]purine (18): yield 83%; mp 74–76 °C; (lit.[2] mp 74–76 °C); ^1H NMR (Me_2SO-d_6) δ 1.25 (6 H, t, J = 7.1 Hz, 2 × CH_3), 3.86 (2 H, m, CH_2O), 3.90 (2 H, d, J = 8.2 Hz, CH_2P), 4.06 (4 H, m, 2 × CH_2OP), 4.50 (2 H, m, CH_2ON), 7.37 (2 H, s, D_2O exchangeable, NH_2), 8.15 (1 H, s, H-2 or H-8), 8.35 (1 H, s, H-2 or H-8); FABMS (positive ion, thioglycerol) 346 (MH^+). Anal. ($C_{12}H_{20}N_5O_5P$) C, H, N.

Preparation of Monoalkyl Esters of 1, Compounds 21–24. To a solution of dialkyl ester **13–16** (1 mmol) in dioxane (1.6–7 mL) was added aqueous 1 M NaOH (1.6–7 mL). The resulting mixture was stirred at room temperature for 1–48 h, and then it was neutralized with Dowex 50W-X8 (H). The resin was filtered off and washed with water, and the combined filtrates were evaporated to dryness. The residue was purified on a reverse-phase C_{18} column, eluting with water, water–methanol (1:1), or methanol. Fractions containing the product were combined and evaporated to dryness.

9-[2-((Ethoxyphosphoryl)methoxy)ethoxy]purine, sodium salt (22): yield 57%; colorless gum; ^1H NMR (Me_2SO-d_6) δ 1.20 (3 H, t, J = 7.1 Hz, CH_3), 3.71 (2 H, d, J = 8.0 Hz, CH_2P), 3.83 (2 H, m, CH_2O), 3.96 (2 H, m, CH_2OP), 4.48 (2 H, m, CH_2ON), 7.39 (2 H, s, D_2O exchangeable, NH_2), 8.15 (1 H, s, H-2 or H-8), 8.38 (1 H, s, H-2 or H-8); FABMS (positive ion, thioglycerol) 340 (MH^+). Anal. ($C_{10}H_{15}N_5O_5PNa{\cdot}H_2O$) H, N; C: calcd, 33.62; found, 34.38.

Preparation of Bis((acyloxy)alkyl) Esters 25–33 and Bis((alkoxycarbonyl)methyl) Esters 35 and 37. A mixture of 1 or **21–24** (1 mmol) and triethylamine (2.2 mmol for 1 and 1.1 mmol for **21–24**) in DMF (10 mL) was stirred at room temperature for 5 min. Then, the appropriate alkylating agent (chloromethyl pivalate, chloromethyl benzoylate, chloromethyl 3-chloropivalate, 1-chloromethyl isobutyrate,[28] 1-chloroethyl pivalate,[28] ethyl bromoacetate or *tert*-butyl bromoacetate) (2–8 mmol) was added, and the resulting solution was stirred at 60–80 °C for 2–6 h. The solvent was removed and the residue was dissolved in chloroform (100 mL). The chloroform solution was washed with saturated aqueous $NaHCO_3$ (2 × 20 mL) and water (1 × 20 mL) and dried ($MgSO_4$). The solvent was removed and the residue was purified by column chromatography on silica gel, eluting with chloroform–ethanol mixtures.

9-[2-[[Bis((pivaloyloxy)methoxy)phosphoryl]methoxy]ethoxy]adenine (25): yield 41%; colorless oil; ^1H NMR (Me_2SO-d_6) δ 1.16 (18 H, s, 2 × $(CH_3)_3C$), 3.86 (2 H, s, CH_2O), 4.04 (2 H, d, J = 7.7 Hz, CH_2P), 4.51 (2 H, m, CH_2ON), 5.66 (4 H, d, J = 12.6 Hz, 2 × CH_2OP), 7.38 (2 H, s, D_2O exchangeable, NH_2), 8.14 (1 H, s, H-2 or H-8), 8.33 (1 H, s, H-2 or H-8); HRMS calcd for $C_{20}H_{32}N_5O_9P$ 518.2017, found 518.2020. Anal. ($C_{20}H_{32}N_5O_9P{\cdot}0.3H_2O$) C, H, N.

9-[2-[Ethoxy((pivaloyloxy)methoxy)phosphoryl]methoxy]ethoxy]adenine (27): yield 61%; colorless oil; ^1H NMR (Me_2SO-d_6) δ 1.17 (9 H, s, $(CH_3)_3C$), 1.26 (3 H, t, J = 7.1 Hz, CH_3), 3.86 (2 H, m, CH_2O), 3.97 (2 H, d, J = 8.0 Hz, CH_2P), 4.05 (2H, m, CH_2OP), 4.51 (2H, m, CH_2ON) 5.62 (2H, d, J = 12.9 Hz, CH_2OP), 7.38 (2 H, s, D_2O exchangeable, NH_2), 8.14 (1 H, s, H-2 or H-8), 8.34 (1 H, s, H-2 or H-8); HMRS calcd for $C_{16}H_{26}N_5O_7P$ 431.1572, found 431.1572. Anal. ($C_{16}H_{26}N_5O_7P{\cdot}H_2O$) C, H, N.

9-[2-[[Bis(1-(isobutyryloxy)ethoxy)phosphoryl]methoxy]ethoxy]adenine (32): yield 6%; yellow oil (mixture of diastereomers); ^1H NMR (Me_2SO-d_6) δ 1.09 (12 H, m, 2 × $(CH_3)_2C$), 1.48 (6 H, m, 2 × CH_3C), 2.54 (2 H, m, 2 × CHCO), 3.85 (2 H, m, CH_2O), 3.97 (2 H, m, CH_2P), 4.49 (2 H, m, CH_2ON), 6.50 (2 H, m, 2 × CHOP), 7.38 (2 H, s, D_2O exchangeable, NH_2), 8.15 (1H, s, H-2 or H-8), 8.33 (1H, s, H-2 or H-8); HRMS calcd for $C_{20}H_{32}N_5O_9P$ 517.1938, found 517.1938.

9-[2-[[Bis(1-(pivaloyloxy)ethoxy)phosphoryl]methoxy]ethoxy]adenine (33): yield 18%; yellow oil (mixture of diastereomers); ^1H NMR (Me_2SO-d_6) δ 1.14 (18 H, s, 2 × $(CH_3)_3C$), 1.48 (6 H, m, 2 × CH_3), 3.83 (2 H, m, CH_2O), 3.96 (2 H, m, CH_2P), 4.48(2 H, m, CH_2ON), 6.40 (2 H, m, 2 × CHOP), 7.37 (2 H, s, D_2O exchangeable, NH_2), 8.14 (1 H, s, H-2 or H-8), 8.31 (1 H, s, H-2 or H-8); HRMS calcd for $C_{22}H_{36}O_9N_5P$ 545.2251, found 545.2251. Anal. ($C_{22}H_{36}N_5O_9P{\cdot}H_2O$) C, H, N.

9-[2-[[Bis(1-(isobutyryloxy)ethoxy)phosphoryl]methoxy]ethoxy]adenine (32), Alkylation in DMPU. A mixture of 1 (0.42 g, 1.45 mmol) and triethylamine (0.48 mL, 3.42 mmol) in DMPU (5 mL) was stirred at room temperature for 10 min. Then, 1-chloroethyl butyrate[28] (0.5 mL) was added followed by sodium iodide (0.25 g, 1.67 mmol). The resulting reaction mixture was stirred at 60 °C for 5 h. A further quantity of sodium iodide (1.67 mmol, 0.25 g) was added, and the stirring was continued for another 1 h. The precipitate was filtered off and washed with dichloromethane (2 × 20 mL). The filtrate and washings were combined and concentrated. The residue was precipitated into hexane (300 mL) at 0 °C, and the hexane solution was removed by decantation. The resulting oil was dissolved in chloroform (150 mL), and the solution was washed with saturated aqueous $NaHCO_3$ (30 mL) and water (30 mL) and dried ($MgSO_4$). The solvent was evaporated, and the residue was purified by column chromatography on silica gel, eluting with chloroform–ethanol (95:5) to afford **32** as a yellow oil (mixture of diastereomers): yield 0.23 g, 32%; ^1H NMR and HRMS as for **32** prepared in DMF. Anal. ($C_{20}H_{32}N_5O_9P{\cdot}0.25CHCl_3$) C, H, N.

9-[2-[[Bis(1-(pivaloyloxy)ethoxy)phosphoryl]methoxy]ethoxy]adenine (33), Alkylation in DMPU. The compound was obtained by alkylation of 1 with 1-chloroethyl pivalate[28] according to the method described previously for **32**, but no further amount of sodium iodide was added after 5 h of stirring at 60 °C: yield 53%; (mixture of diastereomers); ^1H NMR, C, H, N, and HRMS as for **33** prepared in DMF.

Preparation of 9-[2-[[Bis((alkoxycarbonyl)methoxy)phosphoryl]methoxy]ethoxy]adenine (34 and 36) and 9-{2-[[Bis(((*N*,*N*-dialkylamino)carbonyl)methoxy)phosphoryl]methoxy]ethoxy}adenine (38 and 39). A mixture of 1 (1 mmol) and triethylamine (0.31 mL, 2.2 mol) in DMPU (3 mL) was stirred at room temperature for 10 min. The appropriate alkylating agent (methyl bromoacetate, 2-propyl bromoacetate, 2-chloro-*N*,*N*-diethylacetamide or 2-bromo-*N*,*N*-dibutylacetamide) (2 mmol) was then added to it, and the resulting solution was stirred at 60 °C for 45 min (**34** and **36**), 90 min (**38**), and 15 min (**39**). In the case of **34** and **36**, a further quantity of the alkylating agent (2 mmol) was added after 45 min, and the solution was stirred for a further 45 min. The reaction mixture was then allowed to cool to room temperature, and the solution was added dropwise to stirred hexane (150 mL) at 0 °C. After the mixture was stirred at 0 °C for 30 min, the solvents were removed by decantation, and the residue was dissolved in chloroform (70 mL), washed with saturated aqueous $NaHCO_3$ (2 × 20 mL) and water (20 mL), and dried ($MgSO_4$). The solvent was removed, and the residue was purified by column chromatography on silica gel, eluting with chloroform–methanol or chloroform–ethanol (from 98:2 to 84:16) to give the product.

9-[2-[[Bis((methoxycarbonyl)methoxy)phosphoryl]methoxy]ethoxy]adenine (34): yield 21%; tan oil; IR (film) ν_{max} 3320, 3180, 1760, 1640, and 1600 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 3.10 (6 H, s, 2 × CH_3O), 3.88 (2 H, m, CH_2O), 4.08 (2 H, d, J = 8.0 Hz, CH_2P), 4.50 (2 H, m, CH_2ON), 4.74 (4 H, d, J = 11.0 Hz, 2 × CH_2OP), 4.76 (2 H, s, CH_2ON), 7.40 (2 H, s, D_2O exchangeable, NH_2), 8.15 (1 H, s, H-2 or H-8), 8.35 (1 H, s, H-2 or H-8); HRMS calcd for $C_{14}H_{20}N_5O_9P$ 433.0999, found 433.0991. Anal. ($C_{14}H_{20}N_5O_9P{\cdot}0.3CH_3OH$) C, H, N.

9-{2-[[Bis[((*N*,*N*-dibutylamino)carbonyl)methoxy]phosphoryl]methoxy]ethoxy}adenine (39): yield 29%; yellow oil; ^1H NMR (Me_2SO-d_6) δ 0.86 (12 H, t, J = 7.1 Hz, 4 × CH_3), 1.23 (8 H, m, 4 × CH_2), 1.44 (8 H, m, 4 × CH_2), 3.17 (8 H, m, 4 × CH_2), 3.87 (2 H, m, CH_2O), 4.07 (2 H, d, J = 8.2 Hz, CH_2P), 4.49 (2 H, m, CH_2ON), 4.82 (4 H, m, 2 × CH_2OP), 7.36 (2 H, s, D_2O exchangeable, NH_2), 8.15 (1 H, s, H-2 or H-8),

8.39 (1 H, s, H-2 or H-8); FABMS (positive ion, thioglycerol) 628 (MH^+). Anal. ($C_{28}H_{50}N_7O_7P \cdot 0.75H_2O$) C, N; H: calcd, 8.10; found, 7.17.

Preparation of Dibenzyl (42–47), Substituted Alkyl (48–54), and Aryl Esters (55–65) of 1 and Phosphonoamidates 66 and 67. To a solution of **18** (1.45 mmol) in dichloromethane (5 mL) was added bromotrimethylsilane (14.5 mmol). The reaction mixture was stirred at room temperature for 2 h, and the solvents were removed. The residue was coevaporated with toluene (2 × 5 mL), and the resulting glass was dissolved in dichloromethane–carbon tetrachloride solution (3:1, 10 mL). Phosphorus pentachloride (3.0 mmol) was then added, and the reaction mixture was stirred at room temperature for 3 h. The solvents were removed, and the residue was coevaporated with toluene (1 × 10 mL). The resulting phosphonodichloridate intermediate was suspended in dichloromethane (10 mL), the appropriate alcohol or amine (3.0 mmol) was added, and the solution was cooled to 0–3 °C under argon. The mixture was then treated successively with triethylamine (3.6 mmol) and 1-methylimidazole (5.8 mmol) and stirred at 0–3 °C for 10 min and finally at room temperature for 1.5 h. The precipitate was filtered off and washed with dichloromethane (2 × 5 mL), the filtrate and washings were combined, and the solvent was removed. The residue was coevaporated with toluene (2 × 20 mL), dissolved in chloroform (80 mL), and washed with saturated aqueous sodium hydrogen carbonate (2 × 20 mL) and water (2 × 20 mL). The solvent was removed, and the residue was purified by column chromatography on silica gel, eluting with chloroform–ethanol mixtures to give the product.

Most of the alcohols and phenols used in these reactions were commercially available, but some of them such as 2-acetoxy- and 4-acetoxyphenol,[32] 4-(*tert*-butyryloxy)phenol,[32] 4-acetoxybenzyl and 4-(isobutyryloxy)benzyl alcohols,[33] and 2-(acetoxymethyl)- and 4-(acetoxymethyl)phenol[34] were prepared according to the literature procedures.

9-[2-[[Bis(benzyloxy)phosphoryl]methoxy]ethoxy]adenine (42): yield 13%; slightly yellow oil; IR (KBr) ν_{max} 3300, 3180, 1640, 1590, 1240, and 1000 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.84 (2 H, m, CH_2O), 3.85 (2 H, d, J = 8.0 Hz, CH_2P), 4.52 (2 H, m, CH_2ON), 5.10 (4 H, m, 2 × CH_2Ph), 5.80 (2 H, s, D_2O exchangeable, NH_2), 7.35 (10 H, m, aromatic protons), 7.96 (1 H, s, H-2 or H-8), 8.34 (1 H, s, H-2 or H-8); HRMS calcd for $C_{22}H_{24}N_5O_5P$ 469.1515, found 469.1512. Anal. ($C_{22}H_{24}N_5O_5P \cdot 0.5CHCl_3$) C, H, N.

9-[2-[[Bis(2-bromoethoxy)phosphoryl]methoxy]ethoxy]adenine (48): yield 20%; yellow oil; ^1H NMR (Me_2SO-d_6) δ 3.72 (4 H, m, 2 × CH_2Br), 3.86 (2 H, m, CH_2O), 4.04 (2 H, d, J = 8.0 Hz, CH_2P), 4.33 (4 H, m, 2 × CH_2OP), 4.52 (2 H, m, CH_2ON), 7.38 (2 H, s, D_2O exchangeable, NH_2), 8.15 (1 H, s, H-2 or H-8), 8.36 (1 H, s, H-2 or H-8); FABMS (positive ion, thioglycerol) 504 (MH^+). Anal. ($C_{12}H_{18}Br_2N_5O_5P$) C, H, N.

9-[2-[(Diphenoxyphosphoryl)methoxy]ethoxy]adenine (55): yield 36%; colorless oil; ^1H NMR (Me_2SO-d_6) δ 3.97 (2 H, m, CH_2O), 4.35 (2 H, d, J = 7.1 Hz, CH_2P), 4.55 (2 H, m, CH_2ON), 7.32 (12 H, m, aromatic protons, D_2O exchangeable, NH_2), 8.15 (1 H, s, H-2 or H-8), 8.31 (1 H, s, H-2 or H-8); HRMS calcd for $C_{20}H_{20}N_5O_5P$ 442.1281, found 442.1271. Anal. ($C_{20}H_{20}N_5O_5P \cdot 0.35CHCl_3$) C, H, N.

9-[2-[[Bis(*N*,*N*-diethylamino)phosphoryl]methoxy]ethoxy]adenine (66): yield 7%; mp 131–135 °C; IR (KBr) ν_{max} 3340, 3180, 1660, 1600, 1520, and 1050 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.12 (12 H, t, J = 7.0 Hz, 4 × CH_3), 3.10 (8 H, m, 4 × CH_2), 3.87 (2 H, d, J = 7.5 Hz, CH_2P), 3.93 (2 H, m, CH_2O), 4.60 (2 H, m, CH_2ON), 6.19 (2 H, s, D_2O exchangeable, NH_2), 8.14 (1 H, s, H-2 or H-8), 8.35 (1 H, s, H-2 or H-8); HRMS calcd for $C_{16}H_{30}N_7O_3P$ 399.2148, found 399.2147.

9-[2-[(Diphenoxyphosphoryl)methoxy]ethoxy]adenine Hydrochloride (55A). To a solution of diphenyl ester **55** (1.1 g) in dichloromethane (10 mL) was added a saturated (0 °C) solution of hydrogen chloride in dichloromethane (30 mL). The reaction mixture was stirred at room temperature for 5 min, and the solvent was removed. The residue was coevaporated with dry dichloromethane (5 × 20 mL) and then with dry toluene (5 × 20 mL) to give a colorless solid: mp 139–143 °C; ^1H NMR (Me_2SO-d_6) δ 4.01 (2 H, m, CH_2O), 4.32 (2 H, d, J = 7.4 Hz, CH_2P), 4.62 (2 H, m, CH_2ON), 7.38 (10 H, m, aromatic protons), 8.49 (1 H, s, H-2 or H-8), 8.69 (1 H, s, H-2 or H-8), 8.7–9.25 (2 H, very broad doublet, NH_2, D_2O exchangeable; HRMS calcd for $C_{20}H_{20}N_5O_5P \cdot HCl$ 442.128, found 442.1280. Anal. ($C_{20}H_{20}N_5O_5P \cdot 1.2HCl \cdot H_2O$) C, H, N.

Hydrolysis of 9-[2-[(Diphenoxyphosphoryl)methoxy]ethoxy]adenine (55). To a sonicated solution at the appropriate pH (0.5 mL) at 23 or 37 °C was added slowly a solution of **55** (1 mg) in dioxane (20 μL). Hydrolysis at pH 6.0 or 7.0 was carried out in a mixture of buffer (0.4 mL) and dioxane (0.1 mL). Determination of **55** and the corresponding monoester in the reaction mixture was by HPLC, and their retention times were found to be 2.7 and 1.5 min, respectively. Spherisorb S5 ODS column was used in conjunction with Waters 600 multisolvent delivery system, Waters 600 E system controller, 745B data module, and 484 tunable absorbance detector. The samples were eluted with 55% acetonitrile in ammonium acetate buffer (pH 7.5, 0.1 M). The mono- and diesters were detected by monitoring the eluate at 254 nm.

Estimates of Compounds' Stability in Mouse Duodenal Contents. From stock solutions (15 mM) of each of the compounds (**25**, **33**, and **55**) prepared in dimethyl sulfoxide, samples (7 μL) were added to the mouse duodenal contents (0.77 g contents suspended in 7.7 mL of PBS, 693 μL) to give a final compound concentration of 150 μL. For controls, each compound was incubated in PBS. Immediately after the compound was thoroughly mixed with the duodenal contents (at 50 s to 1.5 min), an aliquot (100 μL) was taken and added to an equal volume of buffered ethanol (ethanol:500 mM NH_4OAc, pH 5.0 (99:1)), and the mixure was stored at −20 °C. The reminder of the sample was incubated at 37 °C, and the further aliquots were taken after suitable intervals of time. To analyze the samples, they were thawed and centrifuged for 4 min (Eppindorf 5414 microcentrifuge), and the supernatant (100 μL) was removed. The dried supernatants were then taken up in 200 μL of 25 mM NH_4OAc, pH 6.0, and analyzed by HPLC as described for the estimates of oral bioavailability in the mouse.

Estimates of Oral Bioavailability in the Mouse. Compounds were administered by oral gavage to female BALB/c mice as a single 0.2 mmol/kg dose in 0.1 mL of 1% carboxymethyl cellulose plus 1% Tween 80 in water. Food was withheld from the animals for 16 h prior to dosing. Blood was collected from three mice per time point at the indicated times. Equal volumes of blood were pooled and treated with ice-cold ethanol. Following chilling at −20 °C, samples were centrifuged to remove protein. The supernatants were assayed for diesters, diamides, and products of hydrolysis by HPLC using a method similar to that previously described.[35] Briefly, a Nova-PAK C18 column (Waters Associates, Inc., Milford, Mass.) was used in conjunction with two buffers: buffer A consisted of 5 mM potassium dihydrogen phosphate plus 5 mL/L of Q8 (Fisons, Loughborough, UK) in water (the pH of this buffer was adjusted to 2.6 using hydrochloric acid) and buffer B consisted of 80% methanol in water. Each sample was eluted at a flow rate of 0.6 mL/min by using a linear gradient changing from 1% buffer B and 99% buffer A at 1.5 min to 95% buffer B and 5% buffer A at 24 min. The column was equilibrated for 10 min prior to each injection.

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Supplementary Material Available: ^1H-NMR spectra, IR spectra, mass spectra, and yields of compounds **10**, **11**, **13**, **15**, **16**, **17**, **19**, **20**, **21**, **23**, **24**, **26**, **28**, **29**, **30**, **31**, **35**, **36**, **37**, **38**, **43–47**, **49**, **50–54**, **56–65**, and **67** (7 pages). Ordering information is given on any current masthead page.

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