Synthesis of New Nucleoside Phosphoraziridines as Potential Site-Directed Antineoplastic Agents

Robert G. Breiner,[†] William C. Rose,[‡] Joseph A. Dunn,[†] Joan E. MacDiarmid,[†] and Thomas J. Bardos*[,†]

Department of Medicinal Chemistry, State University of New York at Buffalo, Amherst, New York 14260, and the Pharmacological Research and Development Division, Bristol-Myers Squibb Company, Wallingford, Connecticut 06492.
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With the aim of increasing the selectivity of the 2,2-dimethylphosphoraziridine type antitumor agents toward the intracellular site of DNA synthesis, a series of new compounds was synthesized in which the reactive bis(2,2-dimethyl-1-aziridinyl)phosphinyl (2,2-DMAP) group was linked through a carbamate or amide linkage to thymidine or cytosine nucleoside moieties. The 3′- and 5′-(2,2-DMAP)carbamates of thymidine (1 and 2) were found to be highly unstable, therefore the corresponding *O*-acetyl derivatives **5** and **6** were prepared by reacting 5′- and 3′-acetylthymidine, respectively, with dichloroisocyanatophosphine oxide followed by the addition of 2,2-dimethylaziridine and triethylamine. The 3′- and 5′-(2,2-DMAP)amides of thymidine **14** and **15** were prepared by reacting the appropriate thymidinylamines with bis(2,2-dimethyl-1-aziridinyl)phosphinyl chloride (**17**). The N^4-(2,2-DMAP)amides of cytidine, 2′-deoxycytidine, and cytosine arabinoside (**18**, **19**, and **20**, respectively) were prepared by reacting the hydrochlorides of the *O*-peracetylated cytosine nucleosides with triethylamine and $POCl_3$ and, subsequently, with 2,2-dimethylaziridine and triethylamine, to give the corresponding N^4-(2,2-DMAP)cytosine nucleoside peracetates **21**, **22**, and **23**, respectively, which were then deacetylated by aminolysis. However, the peracetate intermediates were found to be more stable and, probably for the same reason, also more active against P388 leukemia in mice than the deacetylated products. Particularly, **22** and **23** showed sufficient activity in this in vivo assay system to warrant further evaluation. The relationships between the antitumor activities, the chemical alkylating activities, and the cholinesterase inhibitory activities of these agents are discussed.

Introduction

Previous work in our laboratory resulted in the synthesis of a special class of alkylating agents containing the bis-(2,2-dimethyl-1-aziridinyl)phosphinyl moiety as their reactive function.[1-10] These compounds sharply differed from conventional alkylating agents, including their own ring-C-unsubstituted bis(1-aziridinyl)phosphinyl analogues, in their chemical reaction mechanisms as well as in their biological activity patterns.[11] Two prototypes of this class of compounds, sometimes referred to as 2,2-dimethylphosphoraziridines, ethyl[bis(2,2-dimethyl-1-aziridinyl)-phosphinyl]carbamate (AB-132) and ethyl bis(2,2-dimethyl-1-aziridinyl)phosphinate (AB-163), have shown significant antitumor activities in animals and in human cancer patients, particularly in conjunction with X-irradiation,[10,12] and at the same time exhibited remarkably low hematologic toxicities. Their dose-limiting side effects in the clinical trials were in the area of CNS (central nervous system) toxicity which could be, in part, attributed to their potent cholinesterase inhibitory activities.[13,14] It appeared that the latter were due to the phosphorylating activity of a transient hydrolysis product[14] capable of covalent-bond formation with the serine hydroxyl at the active site of the cholinesterases, while the antitumor effects were directly related to the alkylating activities[10] of these agents, enabling them to react with DNA. These two types of activities were independent of each other and could be separated in the case of a series of bis(2,2-dimethyl-1-aziridinyl)phosphinic amides which showed extreme variations in their antitumor as well as anticholinesterase activities (without any relationship between the two), depending on even minor structural differences in their amide moieties.[8]

It was thought that one could enhance the selectivity of these agents for the DNA template by coupling the bis(2,2-dimethyl-1-aziridinyl)phosphinyl group to a nucleoside "carrier" and thus take advantage of the nucleoside transport mechanisms to deliver the alkylating moiety at the intracellular sites of DNA metabolism. In our first attempt to achieve this, we coupled the phosphoraziridine moiety through an ester linkage to the 5′-position of 3′-acetylthymidine; the resulting compound showed only moderate antitumor effects in vivo against L-1210 and P-388 leukemias in mice, but it was unexpectedly a potent inhibitor of horse serum cholinesterase.[7] This could be attributed to rapid hydrolysis of the phosphor–ester linkage; therefore, it was decided that the use of carbamate and amide linkages would be explored in the syntheses of new nucleoside phosphoraziridines. These are the subjects of the present paper.

While the testing of these compounds was being completed, Lin et al. reported the synthesis of two ring-C-unsubstituted phosphoraziridine nucleosides, i.e., bis(1-aziridinyl)phosphinic *N*-5′- and *N*-3′-thymidinylamides, both of which showed in vitro cytotoxicity against the L1210 leukemia, with the N-3′ derivative being the more active.[15]

Chemistry

Initially, we attempted to synthesize the 3′- and 5′-*O*-

[†] State University of New York at Buffalo.
[‡] Bristol-Myers Squibb Co.

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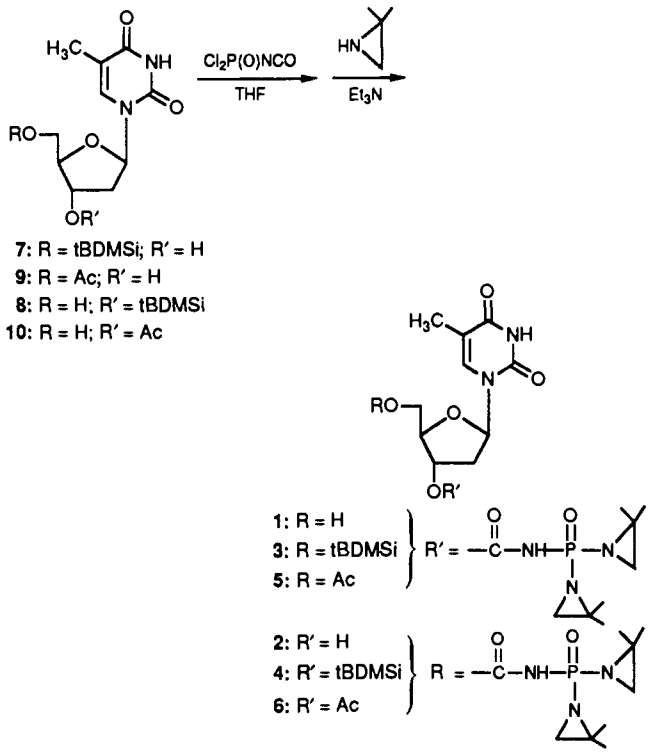
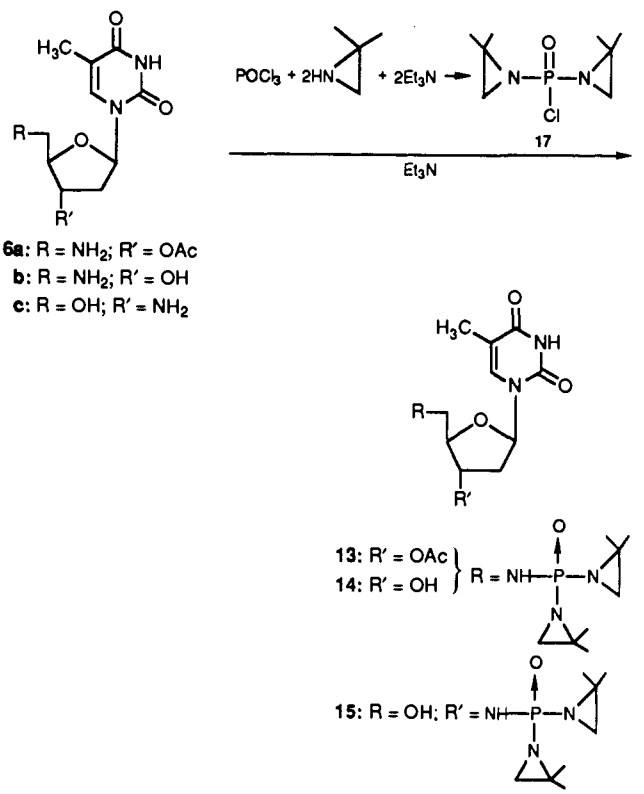
Scheme I



[[bis(2,2-dimethyl-1-aziridinyl)phosphinyl]carbamoyl]-thymidines (**1** and **2**) via the corresponding 5′- and 3′-*O*-(*tert*-butyldimethylsilyl) (tBDMSi) derivatives (**3** and **4**), which in turn could be obtained as uncrystallizable pure white foams by reacting the known[16a,b] 5′- and 3′-*O*-(tBDMSi)thymidines **7** and **8** (**8** being prepared by a method more satisfactory than those[16a,b] previously published), with dichloroisocyanatophosphine oxide (in THF)[17] followed by the addition of 2 equiv each of 2,2-dimethylaziridine and triethylamine.[17] However, upon removal of the tBDMSi blocking groups with tetrabutylammonium fluoride (TBAF) in THF, the resulting products rapidly polymerized.

Since the originally desired unprotected carbamates **1** and **2** proved to be too unstable, the corresponding *O*-acetyl derivatives **5** and **6** were synthesized instead, using 5′-*O*-acetylthymidine **9** and 3′-*O*-acetylthymidine **10**, respectively, as the starting materials for the reaction sequence shown in Scheme I. Although both acetylthymidines are well known and several methods of preparation for each have been reported in the literature,[18-20] substantial improvements in yield have been obtained in the present work simply by using the *p*-methoxytrityl protecting group (instead of the trityl) in the synthesis of **10**[18] and by employing an alternative route for the synthesis of **9**. The latter, although it is a multistep procedure, could be accomplished rapidly and with 70% overall yield based on thymidine as the starting material. Since 5′-*O*-acetylthymidine (**9**) is a generally useful intermediate in nucleoside chemistry, this improved procedure for its preparation is described in the Experimental Section.

Scheme II



To replace the carbamate ester bridge between the phosphoraziridine and thymidine moieties of **1** and **2** (or **5** and **6**) with a simple and presumably more stable amide linkage, the *P*,*P*-bis(2,2-dimethyl-1-aziridinyl)phosphinic *N*-5′- and *N*-3′-thymidinylamides (**14** and **15**, respectively) were synthesized in a manner essentially analogous to that reported by Lin et al. for their ring-C-unsubstituted aziridinyl analogues.[15] The known[21-23] aminonucleosides 5′- and 3′-amino-, 5′- and 3′-deoxythymidines (**16b** and **16c**, respectively) were used as the starting materials. Attempts to protect their hydroxyl groups via acylation of the known precursor 5′- and 3′-azidothymidines[22-26] gave unsatisfactory results, because, upon reduction of the azide, the acyl group migrated from the hydroxyl to the amine, and the desired *O*-acetyl aminonucleosides always contained some of the corresponding *N*-acetyl derivative. Therefore, only a very small amount (6% yield) of the 3′-*O*-acetyl derivative **13** of one of the target compounds could be obtained, by reacting freshly prepared 3′-*O*-acetyl-5′-amino-5′-deoxythymidine[25] (**16a**) with freshly prepared bis(2,2-dimethyl-1-aziridinyl)phosphinyl chloride (**17**). However, **14** and **15** could be obtained directly by the selective reaction of the phosphinyl monochloride (**17**) with the corresponding unprotected aminonucleosides **16b** and **16c** (see Scheme II).

Phosphinic amides **14** and **15**, each having an unprotected hydroxyl group, were obtained as uncrystallizable, white, foamy residues which appeared to be pure by TLC and NMR immediately after isolation but showed partial decomposition after a few days of storage in a desiccator

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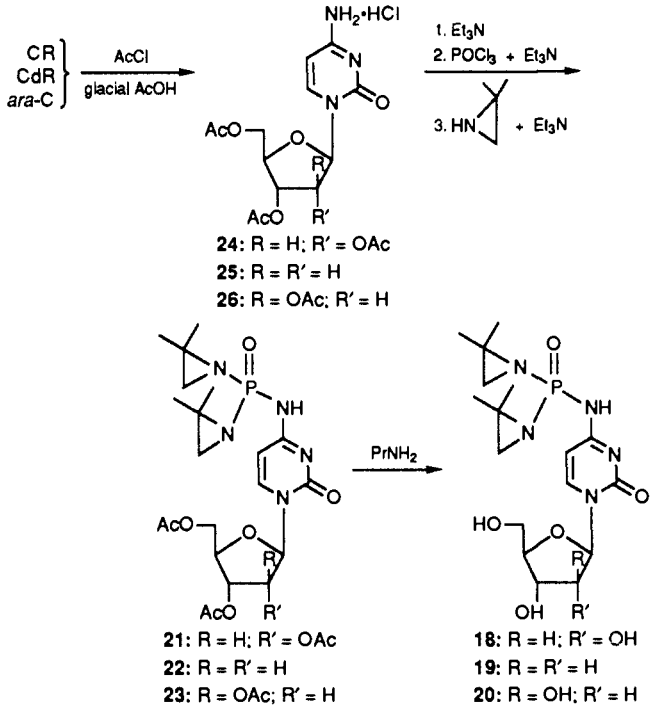
Scheme III



at 4 °C. Therefore, the identification of these compounds was based on the well-resolved peaks of their NMR spectra, which integrated satisfactorily, rather than on their elemental analyses.

By replacing thymidine with a cytosine nucleoside as the "carrier" of the phosphoraziridine moiety, it was possible to link the latter through a phosphinic amide bond to the pyrimidine base rather than to the sugar moiety of the nucleoside. Thus, the N^4-bis(2,2-dimethyl-1-aziridinyl)-phosphinyl derivative (**18**, **19**, and **20**) of cytidine (CR), 2′-deoxycytidine (CdR), and 1′-β-arabinosylcytosine (*ara*-C) were obtained by aminolysis (with propylamine) of the corresponding *O*-peracetyl derivatives (**21**, **22**, and **23**) which, in turn, were synthesized from the corresponding selectively *O*-acetylated[27] nucleoside hydrochlorides (**24**, **25**, and **26**) by addition of triethylamine (in THF) to remove the HCl, followed by coupling of the free 4-amino group with phosphorus oxychloride and subsequent reaction of the dichloride intermediate with 2,2-dimethylaziridine, both stages being carried out in the presence of an equivalent amount of triethylamine in an aprotic solvent (see Scheme III).

All three per-*O*-acetyl nucleoside phosporaziridines **21–23** were obtained in virtually quantitative yields, after filtration and evaporation of the reaction mixtures, as white, foamy residues which were homogeneous by TLC and gave the expected NMR spectra and correct elemental analyses without any purification. They were stable on prolonged storage at 4 °C under dry conditions. Deacetylation markedly decreased their stability; two of the three deblocked target nucleosides (**18** and **19**) continued to give inconsistent and unsatisfactory elemental analyses after repeated chromatography. At the same time, all three target compounds **18–20** were homogeneous by TLC and gave self-consistent NMR spectra which integrated satisfactorily.

Alkylating Activities. The comparative chemical alkylating activities of the new phosphoraziridine nucleosides are graphically represented in Figures 1 and 2, which show their rates of reaction with the model nucleophile 4-(*p*-nitrobenzyl)pyridine (NBP) under carefully standardized conditions.[11] All of these rate curves show the characteristic pattern of the 2,2-dimethylphosphoraziridines, i.e., high initial alkylation rate tapering off after 10–20 min to zero (plateau). The two thymidine [bis(2,2-dimethylaziridinyl)phosphinyl]carbamates **5** and **6**, as well as the analogous amide **14**, are compared with the corresponding ethyl carbamate standard, AB-132, in Figure 1; the 3′-carbamate **5** has approximately the same alkylating activity as AB-132, while the 5′-carbamate **6** is somewhat less reactive and the 5′-amide **14** shows virtually no alkylating activity. It should be noted, however, that

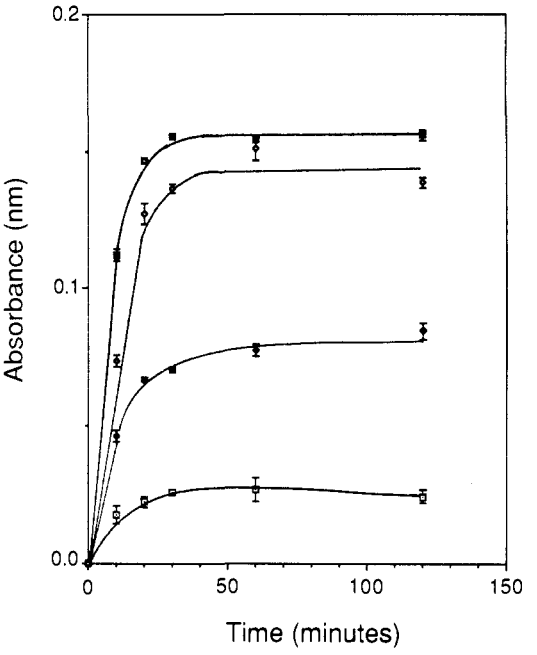


Figure 1. Comparative rates of alkylation of 4-(*p*-nitrobenzyl)pyridine (NBP) with thymidine 3′- and/or 5′-[[bis(2,2-dimethylaziridinyl)phosphinyl]carbamates] (**5** and **6**) and amide (**14**): **5**, ■; **6**, ♦; **14**, ⊡; and AB-132 (standard), ◇.

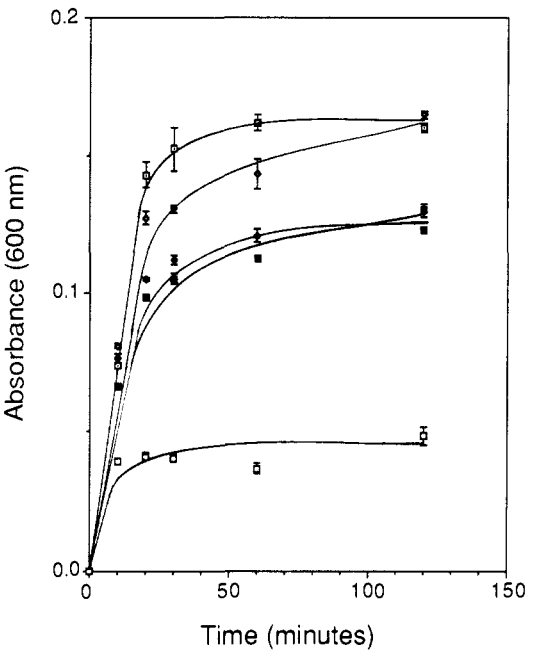


Figure 2. Comparative rates of alkylation of NBP with N^4-[bis(2,2-dimethylaziridinyl)phosphinyl]cytosine nucleoside *O*-peracetates: **23** (*ara*-C), ◇; **22** (CdR), ■; **21** (CR), ◆; AB-163 (standard), ⊡; **27** (reference compound), □.

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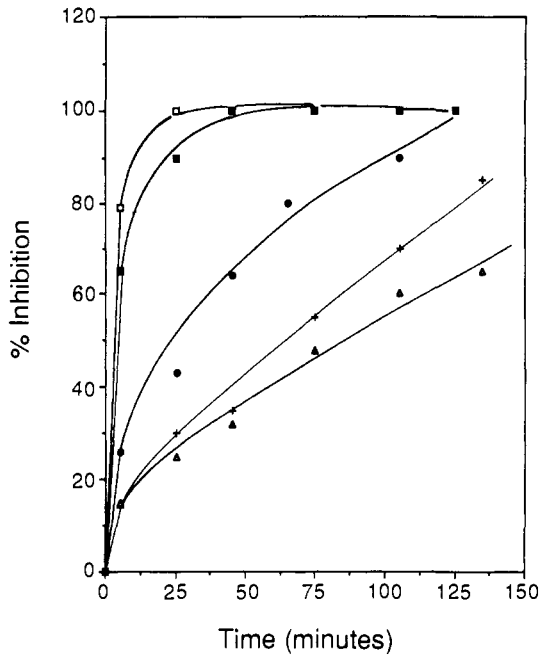

Figure 3. Inhibition of horse serum cholinesterase by bis(2,2-dimethylaziridinyl)phosphinyl nucleosides: **5**, ■; **6**, □; **14**, +; **15**, △; AB-132 (standard), ●.

in both **5** and **6** the "second" sugar hydroxyl (5′ or 3′, respectively) is blocked by acetyl, while the 5′-amide **14** has a free 3′-hydroxyl group which may be responsible for its decomposition (or polymerization) under the relatively drastic conditions of the NBP reaction at 80 °C. The three *O*-acetylated N^4-[bis(2,2-dimethyl-1-aziridinyl)phosphinyl]cytosine nucleosides (**21–23**, Figure 2) show good alkylating activities, comparable to those of the prototype 2,2-dimethylphosphoraziridines (AB-163, AB-132),[11] with the following decreasing order of reactivity: **23** (*ara*-C), **22** (CdR), **21** (CR). Reference compound **27**, in which the cytosine nucleoside is replaced by an anilide moiety,[8] showed much less alkylating activity. The three deacetylated cytosine nucleoside analogues **18–20** were too unstable and apparently decomposed under these reaction conditions.

Cholinesterase Inhibition. The in vitro anticholinesterase activities of the thymidine-linked phosphoraziridines are shown in Figure 3. The two carbamates, **5** and **6**, are seen to be extremely potent inhibitors of horse serum cholinesterase, causing 70–80% inactivation of the enzyme in 5-min contact time and complete inactivation in 10–20 min. Thus, they are faster inactivators than the prototype 2,2-dimethylphosphoraziridines (AB-163, AB-132) and almost as fast as the earlier reported thymidine 5′-ester analogue.[7] The two thymidinylamides **14** and **15** are, on the other hand, much slower acting inhibitors, significantly less potent than AB-163, and are about in the middle of the broad range of anticholinesterase activities reported earlier for a series of *N*-alkylbis(2,2-dimethylaziridinyl)phosphinic amides.[8] The cytosine nucleoside derivatives **21–23** (or **18–20**) could not be tested in this system because their UV absorbances overlapped with those of the procaine substrate used in the enzyme assay.

In Vivo Antitumor Activity. The new phosphoraziridine nucleosides were screened against P388 leukemia in mice. The results are given in Table I. Among the thymidine derivatives, carbamates **5** and **6** showed only borderline activities at the highest dose tested (which was 256 mg/kg for all the new compounds in this experiment), while 5′-amide **14** showed modest but significant activity at all dose levels between 32 and 256 mg/kg. The isomeric 3′-amide **15** could not be tested due to insufficient material. The N^4-amide derivatives of 2′-deoxycytidine and *ara*-C, **19** and **20**, respectively, were significantly active at the highest dose tested while the corresponding cytidine derivative **18** showed only borderline activity. Most active among this new series of compounds at all dose levels tested were **22** and **23**, which are the *O*-acetyl derivatives of **19** and **20**, respectively; the corresponding cytidine analogue **21** was also more active than its deacetylated counterpart **18**. It should be noted that all nucleoside derivatives have molecular weights about twice those of the standard phosphoraziridines; thus, their molar concentrations at the various dose levels are only half of those of the standards at the same doses. In addition, their lack of toxicity at 256 mg/kg would allow further testing at higher doses to achieve presumably higher activities.

Table I. Screening of 2,2-Dimethylphosphoraziridine Nucleosides against P388 Leukemia in Mice

compd	dose,[a] mg/kg	MST % T/C	AWC,[b] g (day 6)
5	256[c]	125	−0.5
6	256[c]	113	−1.3
14	256[c]	156	−0.7
	128	150	−0.8
	64	150	−0.4
	32	131	−0.7
18	256[c]	122	−1.1
19	256[c]	150	−1.6
	128[c]	128	−0.7
20	256[c]	150	−1.7
	128	122	−1.0
21	256[c]	150	−1.6
	128	125	−0.7
22	256[c]	188	−1.8
	128	163	−1.1
	64	150	−1.3
	32	150	−0.8
23	256[c]	206	−1.0
	128	169	−0.4
	64	150	−0.8
	32	150	−0.8
AB-132[d]	400	244	−1.3
	200	231	−0.2
	100	181	−0.4
	50	163	−0.7
AB-163[d]	200	433	−5.2
	100	289	−2.6
27[e]	256	169	−0.8
	128	138	−0.7
	64	144	−1.3

[a] Injected ip on day 1 following ip implant of 10^6 P388 cells in six CDF_1 mice per dosage tested. [b] Average weight change of mice. [c] Highest dose level evaluated. [d] Prototype 2,2-dimethylphosphoraziridines. [e] Phosphinic anilide derivative[8] used as a reference for N^4-amides.

Discussion

Previous studies of the mechanism of action of 2,2-dimethylphosphoraziridines have led to the conclusion that the interesting biological activities of these agents depend on protonation followed by stepwise opening of the two aziridine rings, giving rise to the formation of tertiary carbonium ion intermediates which are capable of very rapid alkylation reactions via an S_N1 mechanism[1] and, alternatively, of recyclization to oxazaphospholidine intermediates having no alkylating but potent phosphorylating activities.[13,14] We estimated the former by determining the comparative rates of reaction of the various compounds with the model nucleophile 4-(*p*-nitrobenzyl)pyridine (NBP) and the latter by measuring their inhibitory effects on horse serum cholinesterase.

The two thymidine carbamates **5** and **6** are anomalous in that their relatively high chemical alkylating activities

in the NBP assay are not matched by their rather poor showing in the in vivo P388 test. This anomaly may be due to instability in biological media of the nucleoside carbamate linkage which, if it hydrolyzes at the phosphorus, would release the phosphoraziridine moiety in the form of a phosphinate anion. This would accelerate recyclization of the carbonium ion to form the nonalkylating oxazaphospholidine moiety (see above). Indeed the very potent anticholinesterase activities of both **5** and **6** indicate that this may be the case. These compounds are similar to their previously reported phosphinate ester analogue.[7]

The phosphinic amide linkage present in all the other nucleoside phosphoraziridines reported in this paper appears to be relatively stable to hydrolysis. However, in those compounds in which the sugar hydroxyls are unprotected, i.e., **14**, **15**, and **18–20**, the free hydroxyl group(s) are susceptible to phosphorylation and thus either intramolecular cyclization or polymerization may occur, resulting in loss of activity. This would be accelerated at higher temperature as indicated in the case of **14**, which showed no activity in the NBP assay while demonstrating moderate but significant in vivo activity against P388. On the other hand, the chemical alkylating activities of the *O*-acetyl-protected nucleoside analogues **21–23** showed excellent correlation with their in vivo activities. Moreover, compounds **22** and **23** demonstrated significant in vivo activities against P388 leukemia even at remarkably low doses (particularly on a mmol/kg basis) without apparent toxicity at the highest dose tested; therefore, these compounds merit future evaluation in other tumor systems.

Despite the promising activities of **22** and **23**, the present results are still within the broad range of antitumor activities of our previously reported series of *N*-alkyl-substituted bis(2,2-dimethylaziridinyl)phosphinic amides[8] and, therefore, they do not provide evidence for a "carrier" effect of the nucleoside moiety. However, the nucleoside transport mechanism may be more operative in some other tumor systems than in P388 leukemia, and in such systems a "carrier" effect may be demonstrable in future studies of these compounds.

Experimental Section

Melting points were obtained with a MelTemp apparatus and are uncorrected. NMR spectra were recorded on a Varian T-60A spectrophotometer and are reported in ppm (δ) downfield from TMS. IR spectra were obtained with a Perkin-Elmer 197 spectrophotometer calibrated with polystyrene or a Nicolet 7199 FT spectrophotometer. Thin-layer chromatographic analyses were performed with Analtech silica gel GF or GHLF Uniplates, and components were visualized with UV light and/or iodine vapor. Elemental analyses were provided by Atlantic Microlabs, Inc., Atlanta, GA, or by Galbraith Laboratories, Inc., Knoxville, TN.

All organic solvents were distilled by using conventional procedures to ensure dryness. The 2,2-dimethylaziridine was purchased from Polysciences, Inc., stored over pelleted KOH, and used without further purification.

Improved Synthesis of 3′-*O*-(*tert*-Butyldimethylsilyl)thymidine (8). To a solution of *tert*-butyldimethylsilyl chloride (1.65 g, 11 mmol) and imidazole (1.50 g, 22 mmol) in DMF (10 mL) was added crude 5′-(*p*-methoxytrityl)thymidine[28] (from 2.42 g, 10 mmol thymidine) in 3 mL of DMF. The mixture was stirred at room temperature for 3 h and was then poured into 500 mL of rapidly stirred ice/water. The resulting precipitate was collected and dissolved in 20 mL of 80% acetic acid. After 3 h at room temperature, the solution was concentrated to an oil. Column chromatography (silica gel, elution with 0–5% MeOH gradient in CH_2Cl_2) yielded **8** as a white, solid foam. Crystallization from ethanol/water afforded 2.9 g (81%) of **8** as white needles: mp 118–120 °C (lit.[16a] mp 83–84 °C); ^{1}H NMR ($CDCl_3$) δ 0.09 (s, 1 H, $SiCH_3$), 0.89 (s, 9 H, $C(CH_3)_3$), 1.92 (s, 3 H, C^5-CH_3), 2.39 (t, 2 H, 2′-H), 2.96 (br s, 1 H, 4′-H), 3.92 (br s, 3 H, 5′- and 3′-H), 4.50 (br s, 1 H, 5′-OH), 6.18 (t, 1 H, 1′-H), 7.43 (s, 1 H, C^6-H), 9.32 (br s, 1 H, N^3-H). Anal. ($C_{16}H_{28}N_2O_5Si\cdot 0.3H_2O$) C, H, N.

3′-*O*-(*tert*-Butyldimethylsilyl)-5′-*O*-[[bis(2,2-dimethyl-1-aziridinyl)phosphinyl]carbamoyl]thymidine (4). To a stirred and cooled solution (0.5 °C, ice bath) of dichloroisocyanatophosphine oxide[17] (0.138 mL, 1.4 mmol) in 5 mL of THF was added, dropwise, 3′-*O*-(*tert*-butyldimethylsilyl)thymidine (500 mg, 1.4 mmol) in 5 mL of THF. After 4 h at this temperature, TLC (10% MeOH/CH_2Cl_2) indicated reaction completion, with only one apparent product. The resulting solution of the dichloridate 3′-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-[(dichlorophosphinyl)carbamoyl]thymidine was transferred to a dropping funnel and added slowly to a stirred and cooled (0–5 °C) solution of 2,2-dimethylaziridine (0.253 mL, 2.8 mmol) and triethylamine (0.391 mL, 2.8 mmol) in 5 mL of THF. Following addition, the mixture was stirred at 4 °C overnight (22 h) and then filtered to remove the Et_3N·HCl. The filtrate was evaporated to dryness and then placed under high vacuum to give **4** as a white solid foam: ^{1}H NMR ($CDCl_3$) δ 0.16 (s, 6 H, CH_3), 0.94 (s, 9 H, CH_3), 1.45 (s, 12 H, aziridine CH_3), 2.1 (s, 3 H, C^5-CH_3), ca. 2.25 (m, 2 H, 2′-H), 2.40 (d, 4 H, aziridine CH_2, J_{PH} = 14 Hz), 3.95 (s, 3 H, 5′-H and 4′-H), 4.10 (s, 1 H, 3′-H), 6.01 (d, 1 H, carbamate N-H), 7.65 (s, 1 H, C^6-H), 9.85 (br, 1 H, N^3-H).

5′-*O*-(*tert*-Butyldimethylsilyl)-3′-*O*-[[bis(2,2-dimethyl-1-aziridinyl)phosphinyl]carbamoyl]thymidine (3). The synthesis and physicochemical properties (including NMR spectra) of the compound were analogous to those of its isomer **4**.

Improved Synthesis of 3′-*O*-(*p*-Methoxytrityl)thymidine (12). A stirred suspension of *tert*-butyldimethylsilyl chloride (6.85 g, 45.4 mmol), imidazole (6.18 g, 91.0 mmol), and thymidine (10.0 g, 41.3 mmol) in 10 mL of anhydrous DMF was stirred for 5 h and then concentrated to a viscous oil. The oil was dissolved in 25 mL of anhydrous pyridine, treated with *p*-methoxytrityl chloride (14.0 g, 45.4 mmol), and stirred on a steam bath for 2 h. The solution was stirred and slowly cooled to room temperature over an additional 1 h and then poured into 1.5 L of rapidly stirred ice/water. The resulting precipitate (**11**) was collected by filtration, washed with water, dried under vacuum, and then added to 100 mL of THF and tetrabutylammonium fluoride trihydrate (26.0 g, 82.56 mmol). This solution was stirred at room temperature for 1 h. The solvent was evaporated and the resulting oil was partitioned between ether and water. The ether layer was isolated, washed with water, and dried ($MgSO_4$), and the product was crystallized by the addition of hexane, yielding 19.0 g (81%) of **12**: mp 125–127 °C (lit.[29] mp 126–128 °C).

Improved Synthesis of 5′-*O*-Acetylthymidine (9). Acetic anhydride (13.8 mL, 146 mmol) was added, dropwise, to a cold (0–5 °C) solution of 3′-(methoxytrityl)thymidine (**12**; 15.0 g, 29.1 mmol) in 25 mL of anhydrous pyridine. The solution was stirred for 4 h at room temperature and then poured into 1 L of rapidly stirred ice/water. The precipitate was collected by filtration, washed with water, dissolved in 30 mL of 80% acetic acid, and stirred at room temperature for 1.5 h. The mixture was poured into 1 L rapidly stirred ice/water and filtered through Celite, and the filtrate was evaporated to give an oil consisting of primarily one component (**9**) with two minor impurities. Precipitation of a solid with ether and recrystallization from acetone/petroleum ether yielded 7.0 g (84%) of **9** as white needles: mp 152–152.5 °C (lit.[30] mp 153–154 °C); ^{1}H NMR (DMSO-d_6) δ 1.8 (s, 3 H, C^5-CH_3), 2.07 (s, 3 H, acetyl CH_3), 2.18 (obscured t, 2 H, 2′-H), 3.42 (s, 2 H, 5′-H), 3.95 (m, 1 H, 4′-H), 4.27 (br, 3 H, 3′-H; 3′-OH; N^3H), 6.18 (t, 1 H, 1′-H), 7.45 (s, 1 H, C^6-H).

5′-*O*-Acetyl-3′-*O*-[[bis(2,2-dimethyl-1-aziridinyl)phosphinyl]carbamoyl]thymidine (5). A solution of 5′-*O*-acetylthymidine (500 mg, 1.76 mmol) in 4 mL of THF was added to a stirred solution of dichloroisocyanatophosphine oxide[17] (0.174 mL, 1.76 mmol) in 5 mL of THF at 0–5 °C, and the resulting solution was stirred for 1 h at 0–5 °C. TLC (10% MeOH/CH_2Cl_2)

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showed reaction completion and only one apparent product. This dichloridate solution was transferred to a dropping funnel and added dropwise to a solution of 2,2-dimethylaziridine (0.318 mL, 3.52 mmol) and triethylamine (0.49 mL, 3.52 mmol) in 5 mL of THF at 0–5 °C. To this was added, dropwise, the freshly prepared dichloridate with stirring. Following addition the resulting mixture was stirred in a cold room (4 °C) overnight (17 h) and then filtered to remove Et_3N·HCl. The filtrate was evaporated to a white foam and chromatographed on a silica gel column pretreated with 1% Et_3N in methylene chloride, eluting with a gradient of 0–10% methanol in methylene chloride to afford 0.80 g (89%) of **5** as a white foam: mp 78–81 °C; ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 12 H, aziridine CH_3), 1.92 (s, 3 H, C^5-CH_3), 2.13 (s, 3 H, acetyl), 2.35 (d, 4 H, aziridine CH_2, J_{PH} = 14 Hz), ca. 2.2 (m, 2 H, 2′-H), 4.35 (br, 3 H, 5′-H, and 4′-H), 5.27 (m, 1 H, 3′-H), 7.3 (s, 1 H, C^6-H). Anal. ($C_{21}H_{32}N_5O_8P$·$^2/_3H_2O$) C, H, N.

3′-*O*-Acetyl-5′-*O*-[[bis(2,2-dimethyl-1-aziridinyl)phosphinyl]carbamoyl]thymidine (6). The preparation of this compound from 3′-*O*-acetylthymidine[18] was analogous to that of its isomer **5**, except that the final product was crystallized from chloroform/hexane to give 0.380 g (70%) of **6** as white plates: mp 141–143 °C; ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 12 H, aziridine CH_3), 1.82 (s, 3 H, C^5-CH_3), 2.1 (s, 3 H, acetyl), 2.30 (d, 4 H, aziridine CH_2, J_{PH} = 14 Hz), 2.2 (m, 2 H, 2′-H), 4.23 (br s, 1 H, 3′-H), 4.38 (br s, 2 H, 5′-H), 5.33 (br, 1 H, 4′-H), 6.43 (t, 1 H, 1′-H), 7.43 (s, 1 H, C^6-H), 8.45 (br d, 1 H, carbamate N-H), 10.58 (br, 1 H, N^3-H). Anal. ($C_{21}H_{32}N_5O_8P$·H_2O) C, H, N.

Bis(2,2-dimethyl-1-aziridinyl)phosphinyl Chloride (17). A solution of triethylamine (0.197 mL, 1.41 mmol) and 2,2-dimethylaziridine (0.128 mL, 1.41 mmol) in 3 mL of dry THF was added over 10 min to phosphorus oxychloride (0.066 mL, 0.75 mmol) in 2 mL of dry THF, stirred at 0–5 °C. After an additional 10 min, the reaction was filtered to remove the Et_3N·HCl. The filtrates were used immediately in the ensuing reactions. (Although tetrahydrofuran was the solvent used for the preparation of **17** in most cases, 1,2-dimethoxyethane was also used occasionally as a reaction solvent, with equivalent results.)

3′-*O*-Acetyl-5′-[[bis(2,2-dimethyl-1-aziridinyl)phosphinyl]amino]-5′-deoxythymidine (13). A solution of **17** (2.82 mmol) in 7 mL of THF was added dropwise over 15 min to 800 mg (2.82 mmol) of 3′-*O*-acetyl-5′-amino-5′-deoxythymidine[25] (**16a**; which was found to contain some of the isomeric 5′-acetylamino-5′-deoxythymidine; see the text) and triethylamine (0.392 mL, 2.82 mol) in 5 mL of dry DMF, stirred at 0–5 °C. The resulting mixture was stirred at 4 °C overnight (14 h) and then concentrated with a rotary evaporator. The resulting oil was dissolved in THF, the precipitate was filtered and washed with THF, and the filtrate was reduced in volume to ca. 1 mL. This was then chromatographed on preparative silica gel plates with 20% methanol/methylene chloride; elution of the major band yielded 0.080 g (6%) of **13** as a chromatographically pure glass: ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 12 H, aziridine CH_3), 1.92 (s, 3 H, C^5-CH_3), 2.1 (s, 3 H, acetyl), 2.17 (d, 4 H, aziridine CH_2, J_{PH} = 14 Hz), ca. 3.4 (br, 4 H, 5′-H, 5′-NH, N^3-H), 4.05 (br, 1 H, 4′-H), 5.27 (br, 1 H, 3′-H), 6.27 (t, 1 H, 1′-H), 7.3 (s, 1 H, C^6-H). Anal. ($C_{20}H_{32}N_5O_6P$·CH_3OH) C, H, N.

5′-[[Bis(2,2-dimethyl-1-aziridinyl)phosphinyl]amino]-5′-deoxythymidine (14). A freshly prepared solution of **17** (4.15 mmol) in 10 mL of dry THF was added dropwise over 10 min to 5′-aminothymidine[22] (1.0 g, 4.15 mmol) and triethylamine (0.636 mL, 4.6 mmol) in 10 mL of dry DMF stirred at 0–5 °C. The reaction was stirred at 4 °C for 10 h and at room temperature for an additional 5 h and then filtered to remove the Et_3N·HCl. The filtrate was evaporated to a viscous oil which was chromatographed on preparative layer silica gel plates with 20% MeOH/CH_2Cl_2. Elution of the major band afforded 0.83 g (47%) of **14** as a white foam, chromatographically homogeneous by TLC (20% MeOH/CH_2Cl_2): ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 12 H, aziridine CH_3), 1.9 (s, 3 H, C^5-CH_3), 2.13 (d, 4 H, aziridine CH_2, J_{PH} = 14 Hz), ca. 2.2 (m, 2 H, 2′-H), 3.33 (br, 3 H, 5′-H and 4′-H), 3.8 (br, 1 H, 3′-H), 4.4 (br, 1 H, 3′-OH), 6.23 (t, 1 H, 1′-H), 7.23 (s, 1 H, C^6-H), 10.17 (br, 1 H, N^3-H).

3-[[Bis(2,2-dimethyl-1-aziridinyl)phosphinyl]amino]-3′-deoxythymidine (15). The compound was prepared from 3′-aminothymidine[21] in a manner analogous to that described above for **14**. Preparative silica gel thin-layer chromatography using 20% MeOH/CH_2Cl_2 yielded **15** in 50% yield as a white foam which was chromatographically homogeneous: ^{1}H NMR ($CDCl_3$) δ 1.3 (s, 12 H, aziridine CH_3), 1.87 (s, 3 H, C^5-CH_3), 2.05 (d, 4 H, aziridine CH_2, J_{PH} = 14 Hz), ca. 2.2 (m, 2 H, 2′-H), 3.28 (m, 2 H, 5′-H), 3.87 (m, 1 H, 4′-H), 4.4 (br, 1 H, 3′-H), 5.2 (br, 1 H, 5′-OH), 6.2 (t, 1 H, 1′-H), 7.4 (s, 1 H, C^6-H), 11.15 (br, 1 H, N^3-H). Anal. ($C_{18}H_{30}N_5O_5P$·$1.5H_2O$) C, H, N.

***N*⁴-[Bis(2,2-dimethyl-1-aziridinyl)phosphinyl]-2′,3′,5′-triacetylcytidine (21).** To a suspension of 2′,3′,5′-tri-*O*-acetylcytidine hydrochloride[27] (**24**; 1.0 g, 2.46 mmol) in 10 mL of dry THF was added triethylamine (0.755 mL, 5.42 mmol) and the mixture was stirred for 30 min and then filtered, and the residue was washed with 2 mL of dry THF to remove the Et_3N·HCl. To the filtrate was added Et_3N (8.350 mL, 2.46 mmol) and this solution was added dropwise, over 5 min, to phosphorus oxychloride (0.241 mL, 2.59 mmol) in 5 mL of dry THF at 0–5 °C. The mixture was stirred for 30 min and then filtered to remove the Et_3N·HCl. The filtrate containing the phosphorodichloridate of the cytidine triacetate was transferred to a dropping funnel and added to a stirred solution of 2,2-dimethylaziridine (0.468 mL, 5.18 mmol) and triethylamine (0.722 mL, 5.18 mmol) in 5 mL of dry THF at 0–5 °C. The mixture was stirred for 14 h at 4 °C and filtered and the Et_3N·HCl was washed with 5 mL of THF. The filtrate was evaporated under reduced pressure to a chromatographically homogeneous (R_f 0.61, 10% MeOH/CH_2Cl_2) white foam (1.4 g (100%) of **21** after a 12-h evacuation at 30 °C): ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 12 H, aziridine-CH_3), 2.1 (s, 9 H, acetyl), 2.22 (d, 4 H, aziridine CH_2, J_{PH} = 14 Hz), 4.37 (s, 3 H, 5′- and 4′-H), 5.38 (m, 2 H, 3′- and 4′-H), 6.03 (d, 1 H, 1′-H), 6.27 (d, 1 H, C^5-H), 7.43 (d, 1 H, C^6-H). Anal. ($C_{23}H_{34}N_5O_9P$) C, H, N.

***N*⁴-[Bis(2,2-dimethyl-1-aziridinyl)phosphinyl]cytidine (18).** A solution of N^4-[bis(2,2-dimethyl-1-aziridinyl)phosphinyl]cytidine 2′,3′,5′-triacetate (**21**; 500 mg, 0.9 mmol) in 10 mL of absolute methanol at 0–5 °C was treated with *N*-propylamine (2 mL, 24.33 mmol) and stirred for 14 h at 4 °C. This mixture was evaporated under reduced pressure to give an oil, which was chromatographically purified on a silica gel column pretreated with 1% Et_3N/CH_2Cl_2, using a 5–25% MeOH/CH_2Cl_2 gradient. The appropriate fractions were concentrated to give a chromatographically homogeneous (R_f 0.49, 20% MeOH/CH_2Cl_2) white foam (310 mg (80%) of **18** after overnight evacuation at 30 °C under high vacuum: ^{1}H NMR ($CDCl_3$/5% DMSO-d_6) δ 1.42 (s, 12 H, aziridine CH_3), 2.18 (d, 4 H, aziridine CH_2, J_{PH} = 14 Hz), 3.83 (br s, 2 H, 5′-H), 3.97–4.33 (br m, 3 H, 3′- and 4′-H), 5.88 (br s, 1 H, 1′-H), 6.27 (d, 1 H, C^5-OH), 8.03 (d, 1 H, C^6-H).

3′,5′-*O*-Diacetyl-2′-deoxycytidine Hydrochloride (25). A solution of 2′-deoxycytidine (2.0 g, 8.8 mmol) and acetyl chloride (2.5 mL, 35.2 mmol) in glacial acetic acid (12.6 mL) was stirred at room temperature for 10 min, then 10 mL of dry chloroform was added and the stirring was continued for 21 h. The solution was evaporated under reduced pressure, reevaporated from 20 mL of ethanol, and evacuated at room temperature for 12 h. Recrystallization from ethanol yielded 2.75 g (90%) of **25** as white needles: mp 187–189 °C dec; ^{1}H NMR (DMSO-d_6) δ 2.08 (s, 6 H, acetyl), 2.5 (t, 2 H, 2′-H), 4.3 (s, 3 H, 5′- and 4′-H), 5.23 (m, 1 H, 3′-H), 6.12 (t, 1 H, 1′-H), 6.33 (d, 1 H, C^5-H), 6.33 (d, 1 H, C^5-H), 8.03 (d, 1 H, C^6-H).

3′,5′-Diacetyl-*N*⁴-[bis(2,2-dimethyl-1-aziridinyl)phosphinyl]-2′-deoxycytidine (22). A suspension of 3′,5′-di-*O*-acetyl-2′-deoxycytidine hydrochloride (**25**; 2.5 g, 7.19 mmol) and triethylamine (1.0 mL, 7.19 mmol) in 15 mL of dry THF (at 0–5 °C) was stirred for 30 min and filtered, and the residue was washed with THF. The filtrate was treated with more triethylamine (1.0 mL, 7.19 mmol) and this solution was added dropwise over 10 min to phosphorus oxychloride (0.67 mL, 7.19 mmol) in 10 mL of THF at 0–5 °C. After an additional 40 min, Et_3N·HCl was removed by filtration and washed with THF. The filtrate was transferred to a dropping funnel and added over 25 min to a stirred solution of 2,2-dimethylaziridine (1.3 mL, 14.38 mmol) and triethylamine (2 mL, 14.38 mmol) in 15 mL of dry THF at 0–5 °C. The mixture was stirred for 17 h at 4 °C and then filtered, and the precipitated Et_3N·HCl was washed with 10 mL of THF. The filtrate was evaporated under reduced pressure to a chromatographically homogeneous (R_f 0.57, 10% MeOH/CH_2Cl_2) white foam (3.45 g (97%) of **22** after evacuation at 30 °C for 11 h): ^{1}H

NMR ($CDCl_3$) δ 1.45 (s, 12 H, aziridine CH_3), 2.1 (s, 6 H, acetyl), 2.23 (d, 4 H, aziridine-CH_2, J_{PH} = 15 Hz), 2.4 (m, 2 H, 2'-H), 4.33 (s, 3 H, 5'- and 4'-H), 5.23 (br m, 1 H, 3'-H), 6.28 (partially obscured t, 1 H, 1'-H), 6.32 (d, 1 H, C^5-H), 7.52 (d, 1 H, C^6-H). Anal. ($C_{21}H_{32}N_5O_7P \cdot 0.5H_2O$) C, H, N.

N^4-[Bis(2,2-dimethyl-1-aziridinyl)phosphinyl]-2'-deoxycytidine (19). A solution of N^4-[bis(2,2-dimethyl-1-aziridinyl)-phosphinyl]-2'-deoxycytidine 3',5'-diacetate (**22**; 500 mg, 1.01 mmol) and *n*-propylamine (1.5 mL, 18.25 mmol) in 10 mL of absolute methanol was stirred at 0–5 °C for 15 min and then at room temperature for an additional 3 h. The mixture was concentrated to an oil, which was chromatographed on a pretreated (1% Et_3N/CH_2Cl_2) silica gel column, eluting with a 0–20% $MeOH/CH_2Cl_2$ gradient. The appropriate fractions were concentrated to a chromatographically homogeneous (R_f 0.53, 20% $MeOH/CH_2Cl_2$) white foam (320 mg (77%) of **19** after evacuation (0.1 mm) overnight at 30 °C): ^1H NMR (DMSO-d_6) δ 1.32 (s, 12 H, aziridine CH_3), 2.12 (d, 4 H, aziridine CH_2, J_{PH} = 14 Hz), ca. 2.2 (m, 2 H, 2'-H), 3.58 (m, 2 H, 5'-H), 3.82 (br, 2 H, 3'- and 4'-H), 4.23 (br, 2 H, 3'- and 5'-OH), 6.15 (t, 1 H, 1'-H), 6.45 (d, 1 H, C^5-H), 7.93 (d, 1 H, C^6-H).

N^4-[Bis(2,2-dimethyl-1-aziridinyl)phosphinyl]-1-(2',3',5'-triacetyl-β-D-arabinosyl)cytosine (23). The hydrochloride of (2',3',5'-triacetylarabinosyl)cytosine (**26**) was prepared by the method of Beranek[31] from arabinosylcytosine and acetyl chloride in glacial acetic acid with added chloroform, in a yield of 96% as white needles, mp 170 °C (lit.[31] mp 171–174 °C). A suspension of **26** (2.0 g, 4.9 mmol) in 15 mL of dry THF was treated with triethylamine (0.86 mL, 6.1 mmol) for 25 min and then filtered to remove $Et_3N \cdot HCl$. The filtrate was combined with additional triethylamine (0.86 mL, 6.1 mmol) in an addition funnel and dropped into phosphorus oxychloride (0.67 mL, 4.9 mmol) in 7 mL of THF over 15 min at 0–5 °C. After 45 min of further stirring, the reaction was filtered to remove $Et_3N \cdot HCl$. The filtrate was removed to a dropping funnel and added to a chilled (0–5 °C) solution of 2,2-dimethylaziridine (0.89 mL, 9.9 mmol) and triethylamine (1.37 mL, 9.9 mmol) in 20 mL of dry THF over 30 min. After the solution was stirred at room temperature for 3 h, it was filtered and the filtrate was concentrated to a chromatographically homogeneous (R_f 0.59, 10% $MeOH/CH_2Cl_2$) white, solid foam (2.65 g (97%) of **23** after evacuation for 13 h at 30 °C): ^1H NMR ($CDCl_3$) δ 1.42 (s, 12 H, aziridine CH_3), 1.97 (s, 3 H, acetyl), 2.1 (s, 6 H, acetyl), 2.21 (br d, 4 H, aziridine CH_2, J_{PH} = 14 Hz), 4.2 (br, 3 H, 5'- and 4'-H), 5.07 (m, 1 H, 3'-H), 5.43 (m, 1 H, 2'-H), 6.3 (two overlapping d, 2 H, 1'- and C^5-H), 7.5 (d, 1 H, C^6-H). Anal. ($C_{23}H_{34}N_5O_9P \cdot 0.5H_2O$) C, H, N.

N^4-[Bis(2,2-dimethyl-1-aziridinyl)phosphinyl]-β-D-arabinosylcytosine (20). To a solution of **23** (600 mg, 1.08 mmol) in 10 mL of absolute methanol at 0–5 °C was added *n*-propylamine (1.5 mL, 18.25 mmol), and the mixture was stirred for 15 min at this temperature and then for 5 h at room temperature. The reaction mixture was concentrated to an oil and chromatographed on a pretreated (1% Et_3N/CH_2Cl_2) silica gel column using a 5% $MeOH/CH_2Cl_2$ step gradient. Product-containing fractions were concentrated to give a chromatographically homogeneous (R_f 0.48, 20% $MeOH/CH_2Cl_2$) white, solid foam (360 mg (78%) of **20** after overnight evacuation (0.1 mm) at 30 °C): ^1H NMR ($CDCl_3$)/5% DMSO-d_6) δ 1.42 (s, 12 H, aziridine CH_3), 2.18 (d, 4 H, aziridine CH_2, J_{PH} = 14 Hz), 3.83 (br, 2 H, 5'-H), 4.03 (br, 1 H, 4'-H), 4.18 (br, 2 H, 3'- and 2'-H), 6.18 (br, 2 H, 1'- and C^5-H), 7.83 (d, 1 H, C^6-H). Anal. ($C_{17}H_{28}N_5O_6P \cdot 1.5H_2O$) C, H, N, P.

Alkylation rate studies were conducted according to our general method[32] in its recently described adaptation for phosphoraziridines.[11] Briefly, a chilled solution of 1.0 mL of 5% (w/v) 4-(*p*-nitrobenzyl)pyridine (NBP) in absolute ethanol was combined with 1.0 mL of 50 mM KHP buffer (pH 4.2) and 1.0 mL of the phosphoraziridine (0.2 μmol) in absolute ethanol in a sealed vial for each measurement. After thorough mixing, the vials were incubated at 80 °C (water bath). At given times, three sample tubes and one blank were removed, chilled to 4 °C to terminate the reaction, and treated with 1.0 mL of cold absolute ethanol and 0.6 mL of 0.1 N KOH in 80% ethanol/water to develop the color of the alkylated NBP, which was observed at 600 nm in a colorimeter. The absorbance of the samples minus the absorbance of the blank was charted for each corresponding time point.

Cholinesterase Inhibition Studies. The method of Lalka and Bardos[13] was used to measure the in vitro deactivation of horse serum (butyryl) cholinesterase, specifically as described in a recent publication.[8]

In Vivo Screening against P388 Leukemia. P388 leukemia (maintained in ascitic form in DBA/2 mice) was implanted (10^6 cells ip) into CDF_1 female mice (16–20 g), with 10 mice as a leukemia control group and six mice per dose level tested. For the nucleoside phosphoraziridines and **27**, an arbitrary set of dosages of 256, 128, 64, and 32 mg/kg was selected for evaluation, while other dose regimens were employed in evaluation of AB-132 and AB-163 (see Table I). The drugs, as freshly prepared aqueous solutions, were administered in a single dosage ip 1 day following the implantation of the P388. The mice were observed daily for 30 days postimplant, and antitumor activity was determined on the basis of the median survival time (MST) in the drug-treated vs untreated (tumor-control) groups (% T/C).

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