Synthesis and Antitumor Activity of 5'-Phosphates and Cyclic 3',5'-Phosphates Derived from Biologically Active Nucleosides†

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Syntheses and biological activities of 12 N^6-substituted adenosine 5'-phosphates and 15 cyclic 3',5'-phosphates are described. Included among these are the cyclic phosphates of the naturally occurring anticodon adjacent modified nucleosides, N^6-(Δ^2-isopentenyl)adenosine and *N*-(purin-6-ylcarbamoyl)-L-threonine ribonucleoside. Also reported in this paper are the 5'-phosphates and cyclic phosphates of the cytokinins, N^6-benzyladenosine, kinetin ribonucleoside, 3-(chloro-*trans*-2-buten-2-yl)adenosine, 6-o-chlorophenylureidopurine ribonucleoside, and 6-allylureidopurine ribonucleoside. The 5'-nucleotides were prepared by direct phosphorylation of the corresponding ribonucleosides with $POCl_3$ and triethyl phosphate. These compounds were converted to the cyclic 3',5'-phosphates by cyclization of the corresponding 5'-nucleotides with dicyclohexylcarbodiimide. Comparison of the cytotoxicity of the ribonucleosides with their 5'-nucleotides and cyclic 3',5'-nucleotides showed that some of the 5'-phosphates and cyclic phosphates were almost as active as the parent nucleosides. The 5'-nucleotides and the cyclic phosphates were more soluble than the parent nucleosides. The cyclic 3',5'-nucleotides were examined as alternate activators of cAMP-dependent protein kinase from beef heart. While all of the analogs studied showed some activity toward this enzyme, several compounds were more effective than cAMP itself. The analogs were also tested as substrates for cyclic 3',5'-nucleotide phosphodiesterase from beef heart. The N^6-alkyl-cAMP analogs were poor substrates for the enzyme, while N^6-carbamoyl-cAMP derivatives were inert toward this enzyme. These compounds did not inhibit the phosphodiesterase. Some of the cyclic phosphates exhibited marginal effect in the inhibition of glycogen synthesis in skin slices.

N^6-(Δ^2-Isopentenyl)adenosine (IPA,‡ Ia) and *N*-(purin-6-ylcarbamoyl)-L-threonine ribonucleoside (PCTR, Ib) are the anticodon adjacent nucleosides in tRNA's which respond to the codons beginning with U and A, respectively.[1] IPA and some of its analogs and the analogs of *N*-(purin-6-ylcarbamoyl)-L-threonine have exhibited cytokinin activity and several of them exerted growth inhibitory effect on cells of leukemic origin grown in culture.[2,3] Among these substances IPA, N^6-benzyladenosine, 6-allylureidopurine ribonucleoside, kinetin ribonucleoside, *N*-(purin-6-ylcarbamoyl)isoamylamine ribonucleoside, and a few others are of particular interest since they showed excellent growth inhibitory activity.[2,3] One of the problems with these compounds has been their poor solubility in water. Recently, LePage et al.[4] have noted that the 5'-nucleotides of arabinosyladenine (AraA) and 6-mercaptopurine (6-MP) ribonucleoside were more soluble than their parent nucleosides and also retained the biological activity of the latter. Thus, we have converted several of the potent antitumor nucleoside analogs to their 5'-phosphates and cyclic 3',5'-phosphates in order to gain two possible advantages: (1) to enhance water solubility;[4] and (2) to see if these 5'-phosphates and cyclic 3',5'-phosphates would serve as sustained release substances for the parent nucleosides.[4]

In the past 3 years there have been several reports suggesting that the cAMP may be involved in controlling the growth and differentiation of mammalian cells. The studies by Hughes and Kimball[5] on AraA cyclic phosphate suggest that part of the inhibitory effect on tumor cells is due to the cyclic phosphates themselves, while some of the effect may be due to the Ara ATP. AraA cyclic phosphate inhibited DNA synthesis to the same degree both in sensitive and 6-methylthioinosine-resistant L1210 cells. Ryan and Heidrick[6] observed that cAMP inhibited a number of tumor cell lines in vitro. Gericke and Chandra[7] have shown that cAMP is effective in inhibition of mouse lymphosarcoma in vivo. Recently, Seller and Benson[8] reported that cAMP, when given with aminophyllin to mice, inhibited growth of Ehrlich carcinoma.

In spite of the general assumption that the cell membrane is impermeable to the charged nucleotides, there is evidence that 5'-phosphates and cyclic 3',5'-phosphates of the nucleosides enter the cell before being cleaved to the nucleosides. Recently, Cohen and his associates[9] have shown that 9-β-D-arabinosyladenine 5'-phosphate can penetrate the mouse fibroblast cells. LePage and Hersh[10] have suggested that the cyclic phosphates of 6-mercaptopurine ribonucleoside and 6-methylmercaptopurine ribonucleoside penetrate intact human lymphocytes and murine tumor cells. Since the analogs of purine and pyrimidine bases generally need to be activated to the 5'-nucleotides in vivo, the use of these preformed 5'-phosphates as well as cyclic phosphates should offer an advantage over the bases and nucleosides particularly in cases of the tissues with low level of kinases. Furthermore, since analogs of cAMP do not serve as substrates for adenosine deaminase,[11] they could be useful in the case of tumors with high levels of this enzyme. Thus, even if the cAMP analogs show poor penetration into the cells, but by virtue of their growth inhibitory activity as well as their water solubiliy, they need to be investigated, particularly in the kinase-deficient tumors.

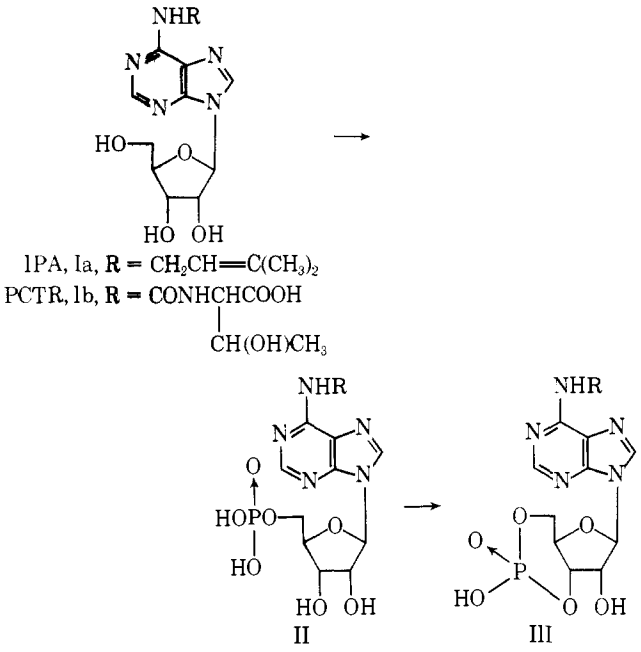
Recently, antitumor and antiviral activities of several cyclic 3',5'-phosphates prepared from the clinically useful as well as other nucleosides have been reported.[12] We have converted several 6-substituted adenosines, which themselves are either of natural origin or have cytokinin or growth inhibitory activities, into the respective 5'-phosphates and cyclic 3',5'-phosphates. This paper describes the synthesis, chemical properties, and biological activities of the 5'-phosphates and cyclic 3',5'-phosphates of several N^6-substituted adenosines.

Chemistry. The N^6-substituted adenosine 5'-phosphates were prepared by direct phosphorylation of the corresponding ribonucleosides with $POCl_3$ and triethyl phosphate.[13,14] The yields varied from 40% to quantitative. For preparation of the cyclic phosphates, the cyclization of the 4-morpholine-*N*,*N*'-dicyclohexylcarboxamidine salts of the 5'-phosphates with DCC in boiling pyridine generally gave good yields of the cyclic 3',5'-phosphates[15] (Scheme I).

†This work was supported by a grant from the U.S. Public Health Service, CA 14185. Presented in part at the 166th National Meeting of the American Chemical Society, Chicago, Ill., August 1973.

‡Abbreviations used are as follows: IPA, N^6-(Δ^2-isopentenyl)adenosine; PCTR, *N*-(purin-6-ylcarbamoyl)-L-threonine ribonucleoside; cIPAMP, N^6-(Δ^2-isopentenyl)adenosine cyclic 3',5'-phosphate; cBzAMP, N^6-benzyladenosine cyclic 3',5'-phosphate; cPCTRP, *N*-(purin-6-ylcarbamoyl)-L-threonine ribonucleoside cyclic 3',5'-phosphate; DCC, dicyclohexylcarbodiimide.

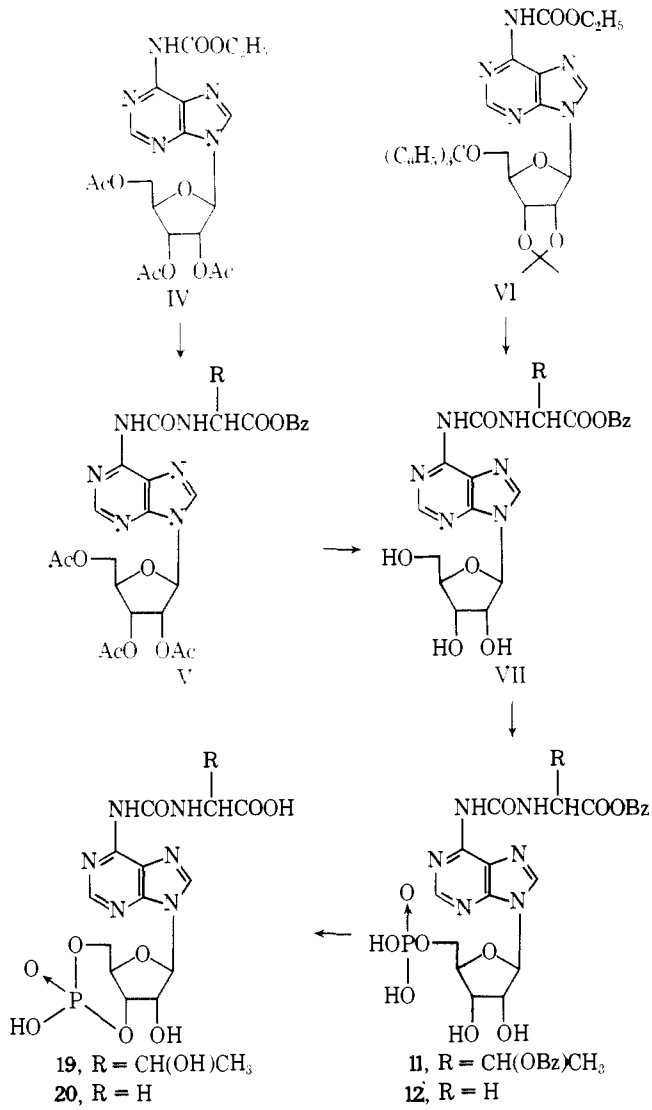
Scheme I



However, in the case of 6-phenylureidopurine[§] and 6-*o*-chlorophenylureidopurine derivatives **26** and **27**, the yields were poor. During the reaction of the 6-phenylureidopurine ribonucleoside 5′-phosphates (**9** and **10**) and DCC, degradation occurred to give large amounts of cAMP (35%). This method, however, failed to give good yields in the case of 6-ureidopurine ribonucleoside 5′-phosphates with an amino acid side chain. For example, the threonine derivative, PCTR (**Ib**), cyclized to give mixture of products, when functional groups of threonine were not protected. Thus in this case, *N*-(purin-6-ylcarbamoyl)-*O*-benzyl-L-threonine benzyl ester ribonucleoside (VII, R = $CH(OCH_2C_6H_5)CH_3$) was prepared by a reaction of the urethane IV[16] and *O*-benzyl-L-threonine benzyl ester followed by selective hydrolysis of the acetyl groups with NH_3–MeOH at 0° for 6 hr (Scheme II, Table I). However, the treatment of glycine compound V (R = H) with NH_3–MeOH at 0° even for 2 hr led to the formation of a transesterified product, the ribonucleoside of the *N*-(purin-6-ylcarbamoyl)glycine methyl ester instead of the desired deacetylated benzyl ester. Thus the preparation of the *N*-(purin-6-ylcarbamoyl)glycine benzyl ester ribonucleoside necessitated the protection of sugar hydroxyls by acid-labile protecting groups. Ethyl 9-(2′,3′-isopropylidene-5′-trityl-β-D-ribofuranosyl)-9*H*-purine-6-carbamate (VI) was synthesized in 55% yield by a reaction of 2′,3′-isopropylidene-5′-trityladenosine with ethyl chloroformate in cold pyridine at −10°. The displacement of the ethoxy group of the urethane VI by refluxing with glycine benzyl ester in pyridine gave the fully protected desired nucleoside. Removal of the isopropylidene and the trityl groups by heating in 80% AcOH gave *N*-(purin-6-ylcarbamoyl)glycine benzyl ester ribonucleoside (VII, R = H). These side-chain protected ribonucleosides VII were then phosphorylated with $POCl_3$ and cyclized with DCC by the usual procedures. Removal of benzyl group by hydrogenation over PdO gave *N*-(purin-6-ylcarbamoyl)-L-threonine ribonucleoside cyclic 3′,5′-phosphate (**19**) and *N*-(purin-6-ylcarbamoyl)glycine ribonucleoside cyclic 3′,5′-phosphate (**20**) in 23% yield (Scheme II). The N^6-substituted adenosine cyclic 3′,5′-phosphates (**13–27**) were purified by cellulose column chromatography using

[§]This compound was prepared in 40% yield by Boswell et al.[19] starting from cAMP.

Scheme II



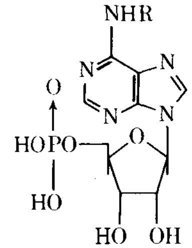
Ac = $COCH_3$; Bz = $CH_2C_6H_5$

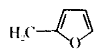
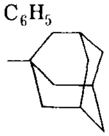
2-propanol–NH_4OH–H_2O (7:1:2) as the eluting solvent. Elemental analyses, comparison of the chromatographic mobilities of the cyclic nucleotide with the corresponding ribonucleoside and 5′-nucleotide, and uv and NMR spectra (in particular, $J_{1',2'}$ = <1 Hz)[17] verified the structures of the cyclic 3′,5′-nucleotides (Table II).

Results and Discussion

Antiproliferative Activity. In order to relate the magnitude of the antiproliferative activity, various compounds were assigned >++, ++, ±, and NA at 10^{-4} *M* as indicated in Table III. The groups of N^6 substituents resulting in the most potent nucleoside, nucleotide, and cyclic phosphate were furfuryl, isopentenyl, benzyl, *n*-octylureido, and 3-chloro-*trans*-buten-2-yl, respectively. Of intermediary activity were the compounds with adamantyl and *o*-chlorophenylureido as N^6 substituents. In general, 5′-phosphates and cyclic 3′,5′-phosphates of the nucleosides led to the increase in the solubility of the parent ribonucleoside with retention of the growth inhibitory activity. For example, N^6-benzyladenosine 5′-phosphate (**2**) and the cyclic 3′,5′-phosphate **14** are 25 times more soluble on a molar basis in saline than the parent nucleoside, N^6-benzyladenosine. In the case of the nucleosides, which were more soluble in water as such, 5′-phosphorylation and cyclic 3′,5′-phosphorylation increased the solubility to a lesser degree (five-

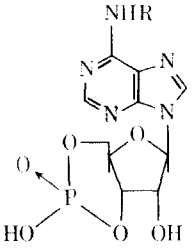
Table I. 5′-Nucleotides

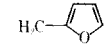
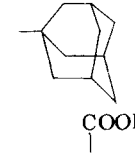
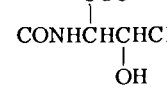


Compd no.	R	Method	Yield, %	Formula[a]	Uv max, nm ($\epsilon \times 10^{-3}$): 0.1 *N* HCl	H_2O	0.1 *N* NaOH	$R_f \times 100$, TLC (solvent A): Cellulose	Silica gel
1[b] (IPAMP)	$CH_2CH{=}C(CH_3)_2$	A	42.6	$C_{15}H_{22}N_5O_7P$	263 (18.9)	266 (17.8)	266 (19.3)	55.6	31.3
2[c] (BzAMP)	$CH_2C_6H_5$	A	45.5	$C_{17}H_{20}N_5O_7P \cdot 0.5H_2O$[d]	264 (21.0)	268 (20.7)	267 (22.3)	46.7	32.2
3	$CH_2CH{=}C(Cl)CH_3$	A	54.1	$C_{14}H_{19}ClN_5O_7P \cdot 2H_2O$	263 (19.6)	266 (19.9)	266 (21.8)	51.1	27.8
4		A	57.9	$C_{15}H_{18}N_5O_8P \cdot 2H_2O$	264 (17.7)	265 (19.0)	265 (19.0)	36.3	21.7
5	C_6H_5	A	59.5	$C_{16}H_{16}N_5O_7P \cdot 2NH_4 \cdot 0.5H_2O$	273 (18.3)	287 (21.7)	287 (23.1)	38.5	21.7
6		A	22.5	$C_{20}H_{26}N_5O_7P \cdot 2NH_4$	267 (19.9)	271 (18.2)	271 (19.2)	69.6	32.2
7	$CONHC(CH_3)_3$	A	45.7	$C_{15}H_{21}N_6O_8P \cdot Ba$	276 (26.7)	269 (25.5), 275 sh[e] (21.6)	269 (23.8), 275 (21.4), 296 (5.3)	54.1	23.5
8	$CONH(CH_2)_7CH_3$	A	97	$C_{19}H_{29}N_6O_8P \cdot 2NH_4 \cdot H_2O$	276 (19.5)	268 (20.0), 275 sh (17.1)	269 (17.0), 276 (15.9), 296 (8.3)	68.9	27.0
9	$CONHC_6H_5$	A	60.2	$C_{17}H_{17}N_6O_8P \cdot 2NH_4$	285 (27.0)	276 (28.5)	306 (29.4)	31.9	19.1
10	$CONHC_6H_4Cl(2)$	A	71.0	$C_{17}H_{16}ClN_6O_8P \cdot 2NH_4 \cdot H_2O$	280 (26.3)	276 (27.5)	305 (31.1)	35.6	25.1
11	$CONHCH(COOCH_2C_6H_5)CH(OCH_2C_6H_5)CH_3$	A	50.2	$C_{29}H_{33}N_6O_{11}P \cdot H_2O$	270 sh (17.1), 275 (18.3)	268 (20.7), 274 sh (17.7)	269 (19.0), 275 (18.3), 295 sh (7.9)	68.9	29.6
12	$CONHCH_2COOCH_2C_6H_5$	A	46.8	$C_{20}H_{23}N_6O_{10}P$	269 sh, 275	268, 274 sh	269, 276, 297		

[a]All compounds were analyzed for C, H, N, and P except **12**. The analytical results were within ±0.4% of the theoretical values. [b]This compound was reported by Grim and Leonard.[28] [c]This compound was reported by Kikugawa et al.[29] [d]N: calcd, 15.69; found, 15.12. [e]sh, shoulder.

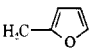
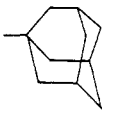
Table II. Cyclic 3′,5′-Nucleotides



Compd no.	R	Method	Yield, %	Formula[a]	Uv max, nm ($\epsilon \times 10^{-3}$)			$R_f \times 100$, TLC (solvent A)	
					0.1 *N* HCl	H_2O	0.1 *N* NaOH	Cellulose	Silica gel
13 (CIPAMP)	$CH_2CH{=}C(CH_3)_2$	B	83.2	$C_{15}H_{19}N_5O_6P \cdot NH_4$	263 (17.9)	265 (16.9)	265 (18.7)	88.1	74.8
14 (cBzAMP)	$CH_2C_6H_5$	B	51	$C_{17}H_{17}N_5O_6P \cdot NH_4 \cdot 1.5H_2O$	263 (20.8)	268 (20.5)	268 (23.2)	90.4	75.6
15	$CH_2CH{=}C(Cl)CH_3$	B	70.2	$C_{14}H_{16}ClN_5O_6P \cdot NH_4$	263 (18.8)	266 (18.5)	265 (20.5)	91.9	75.6
16		B	52	$C_{15}H_{15}N_5O_7P \cdot NH_4 \cdot 3H_2O$[b]	263 (19.3)	265 (20.0)	265 (22.1)	85.2	73.0
17	C_6H_5	B	25.8	$C_{16}H_{15}N_5O_6P \cdot NH_4 H_2O$	272 (18.1)	288 (20.9)	287 (22.0)	86.7	73.9
18		B	31.5	$C_{20}H_{25}N_5O_6P \cdot NH_4 \cdot H_2O$	266 (19.8)	270 (17.8)	270 (19.9)	91.8	78.3
19		C	22.8	$C_{15}H_{17}N_6O_{10}P \cdot Ba \cdot 1.5H_2O$[c]	276 (22.1)	269 (22.7), 275 sh[d] (19.2)	269 (19.2), 276 (18.0), 295 (9.8)	35.5	46.1
20	$CONHCH_2COOH$	C	22.3	$C_{13}H_{14}N_6O_9P \cdot NH_4 \cdot 0.75H_2O$[e]	275 (20.3)	268 (21.0), 274 sh (17.8)	268 (16.0), 275 (15.6), 295 (11.4)	28.1	48.7
21	$CONHCH_2CH{=}CH_2$	B	44.2	$C_{14}H_{16}N_6O_7P \cdot NH_4 \cdot H_2O$	276 (24.9)	268 (22.9), 275 sh (19.3)	269 (17.1), 276 (17.0), 296 (15.1)	81.5	69.6
22	$CONHC(CH_3)_2$	B	43.5	$C_{14}H_{18}N_6O_7P \cdot NH_4 \cdot 0.5H_2O$	276 (24.8)	268 (23.1), 275 sh (19.6)	269 (17.8), 276 (17.1), 296 (13.1)	87.4	70.4
23	$CONHC(CH_3)_3$	B	30.3	$C_{15}H_{20}N_6O_7P \cdot NH_4 \cdot 2H_2O$	276 (25.1)	269 (23.8), 275 sh (20.1)	269 (22.2), 276 (19.6), 296 (6.3)	90.4	73.0
24	$CONHCH_2CH_2(CH_3)_2$	B	39.8	$C_{16}H_{22}N_6O_7P \cdot NH_4 \cdot 2H_2O$	276 (26.9)	268 (25.4), 275 sh (21.8)	269 (21.0), 276 (19.8), 296 (11.9)	94.1	76.5
25	$CONH(CH_2)_7CH_3$	B	11.8	$C_{19}H_{28}N_6O_7P \cdot NH_4 \cdot H_2O$[f]	276 (21.8)	268 (22.5), 275 sh (19.3)	269 (18.7), 275 (17.7), 295 (11.3)	92.6	80.9
26	$CONHC_6H_5$[g]	B	21.1	$C_{17}H_{16}N_6O_7P \cdot NH_4 \cdot H_2O$	285 (26.0)	277 (27.1)	306 (31.2)	77.0	74.8
27	$CONHC_6H_4Cl(2)$	B	18.5	$C_{17}H_{15}ClN_6O_7P \cdot NH_4 \cdot H_2O$	279 (25.2)	276 (26.6)	305 (31.4)	88.1	84.3

[a] All compounds were analyzed for C, H, N, and P. The analytical results were within ±0.4% of the theoretical values. [b] C: calcd, 37.50; found, 36.39. [c] N: calcd, 13.20; found, 12.32. [d] sh, shoulder. [e] H: calcd, 4.28; found, 4.86. [f] N: calcd, 18.87; found, 18.06. [g] This compound was reported by Boswell et al.[19]

Table III. Inhibitory Effects of Ribonucleosides, Their 5′-Phosphates, and Cyclic 3′,5′-Phosphates on the Viability of Mammalian Cells in Culture

Compound[a]	R (side chain)	Biological activity at 1×10^{-4} *M* (72 hr)[b]		
		Nc-37	RPMI 6410	L1210
IPA		++ (+)[e]	>++ (+)	>++
1 (5′-P)[c]	$CH_2CH{=}C(CH_3)_2$	>++ (+)	>++ (+)	>++
13 (cyclic P)[d]		++ (NA)	++	++
BzAR		++ (+)	>++ (+)	++
2 (5′-P)	$CH_2C_6H_5$	>++	>++	>++
14 (cyclic P)		+	>++	++
ClIPA		+	++	++
3 (5′-P)	$CH_2CH{=}C(Cl)CH_3$	++	++	++
15 (cyclic P)		++	++	++
FurAR		>++ (+)	>++ (++)	>++ (++)
4 (5′-P)		>++ (+)	>++ (+)	>++ (++)
16 (cyclic P)		++ (NA)	>++ (NA)	>++ (NA)
PheAR		NA	NA	NA
5 (5′-P)	C_6H_5	NA	NA	NA
17 (cyclic P)		±	±	±
AdaAR		++	++	++
6 (5′-P)		++ (+)	+ (NA)	+ (NA)
18 (cyclic P)		±	NA	±
PCAlR		±	NA	+
PCAlRP[f] (5′-P)	$CONHCH_2CH{=}CH_2$	NA	NA	++
21 (cyclic P)		NA	NA	NA
PCisoPR		±	±	±
PCisoPRP[f] (5′-P)	$CONHCH(CH_3)_2$	±	±	+
22 (cyclic P)		±	NA	++
PCtBuR		NA	++	++
7 (5′-P)	$CONHC(CH_3)_3$	NA	NA	+
23 (cyclic P)		NA	NA	NA
PCisoAR		±	NA	+
PCisoARP[f] (5′-P)	$CONHCH_2CH_2CH(CH_3)_2$	NA	±	++
24 (cyclic P)		NA	NA	++
PCOctR		++ (NA)	++	>++ (±)
8 (5′-P)	$CONH(CH_2)_7CH_3$	+ (NA)	>++	>++ (±)
25 (cyclic P)		± (NA)	++	++ (NA)
PCAnR		+	+	++
9 (5′-P)	$CONHC_6H_5$	+	+	++
26 (cyclic P)		±	NA	++
PCAnCl(o)R				
10 (5′-P)	$CONHC_6H_4Cl(2)$	+ (NA)	>++ (+)	>++ (+)
27 (cyclic P)		+ (NA)	>++ (NA)	>++ (NA)

[a]IPA = N^6-(Δ^2-isopentenyl)adenosine; BzAR = N^6-benzyladenosine; ClIPA = N^6-(3-chloro-*trans*-buten-2-yl)adenosine; FurAR = N^6-furfuryladenosine; PheAR = N^6-phenyladenosine; AdaAR = N^6-adamantyladenosine; PCAlR = *N*-(purin-6-ylcarbamoyl)allylamine ribonucleoside; PCisoPR = *N*-(purin-6-ylcarbamoyl)isopropylamine ribonucleoside; PCtBuR = *N*-(purin-6-ylcarbamoyl)-*tert*-butylamine ribonucleoside; PCisoAR = *N*-(purin-6-ylcarbamoyl)isoamylamine ribonucleoside; PCOctR = *N*-(purin-6-ylcarbamoyl)-*n*-octylamine ribonucleoside; PCAnR = *N*-(purin-6-ylcarbamoyl)aniline ribonucleoside; PCAnCl(o)R = *N*-(purin-6-ylcarbamoyl)-o-chloroaniline ribonucleoside. [b]The notation represents the viable cell number relative to the controls after 72 hr of incubation: >++, 0–30%; ++, 30–60%; +, 60–80%; ±, 80–90%; NA, 90–110%. [c]5′-P = 5′-monophosphate. [d]Cyclic P = cyclic 3′,5′-monophosphate. [e]Result at 1×10^{-5} *M*. [f]The compounds were reported by Hong and Chheda.[14]

fold). The activities of the nucleosides and nucleotides were fairly similar to each other but were different from the cyclic phosphates as compared to the parent nucleosides. The nucleoside is more active and the cyclic phosphate less active in the potent series of *n*-octylureido and adamantyl derivatives toward Nc-37 cells. In several instances in L1210 cells, the antiproliferative activity increased from the nucleoside to the cyclic phosphate. These compounds are extremely poor substrates of cAMP phosphodiesterase (from beef heart).

There is a decrease in the potency of the most active compounds with dilution to 10^{-5} *M* concentration in each cell line. Thus, examples can be found in the table where these decreases were from >++ to ++, +, ±, and NA.

It appears that increasing the aqueous solubility of a nucleoside by conversion into a nucleotide or cyclic phosphate sufficiently changes the attributes of the compound (uptake, intracellular distribution as well as intrinsic biological activity) that a new compound has been created rather than an analog of merely an altered magnitude of biological activity.

In several instances (*tert*-butylureido, isopropylureido, isoamylureido at N^6) greater antiproliferative activity was observed toward cells of malignant origin than cells derived from a normal individual. The three cell lines used vary considerably in their growth rates, L1210 dividing every 12

Table IV. Protein Kinase and Phosphodiesterase Activities by the Cyclic Nucleotides

Compound	Protein kinase,[a] α[b]					Phosphodiesterase[d] substrate		
	10^{-4} M	10^{-5} M	10^{-6} M	10^{-7} M	10^{-8} M	K_m (10^{-3} M)	V max[e]	% rate of cAMP[f]
cAMP	1.0 (12.0)[c]	1.0 (12.2)	1.0 (12.2)	1.0 (11.9)	1.0 (8.5)	0.87	0.481	100
13 (cIPAMP)	1.15	1.18	0.97	1.10	1.07	0.83	0.083	12
14 (cBzAMP)	1.08	1.39	0.97	0.95	0.95	0.26	0.085	20
15	1.0	1.08	0.91	1.0	0.97	0.33	0.046	9
16	1.09	1.0	0.84	1.05	1.01			1.5
17	1.02	1.20	1.01	1.08	1.03	0.19	0.033	8
18	1.03	1.07	1.01	1.02	1.01	0.67	0.016	2
19 (cPCTRP)	0.99	1.02	1.06	0.93	1.13			
20 (cPCGRP)	1.05	1.18	0.93	0.86	1.10			
21	1.08	1.0	0.95	1.0	1.23			
22	1.08	1.11	1.0	1.02	1.09			
23	0.99	0.96	1.01	1.13	1.02			
24	1.05	1.16	0.94	0.86	1.05			
25	0.95	1.08	1.05	1.81	1.22			
26	1.10	1.04	0.94	1.22	1.07			
27	1.10	1.06	0.98	1.25	1.07			

[a] cAMP-dependent protein kinase from beef heart. [b] α = ratio of pmol incorporated by test compound/pmol incorporated by cAMP. [c] pmol of ^{32}P incorporated into histone/μg of protein. [d] Cyclic 3′,5′-nucleotide phosphodiesterase from beef heart. [e] μmol of Pi/mg of protein/min. [f] The rates of hydrolysis relative to that of cAMP (concentration, 1×10^{-3} M). The actual rate of cAMP hydrolysis was 50 nmol of 5′-nucleotide formed per minute. The N^6-carbamoyl derivatives 19–27 were very poor substrates (<1%).

hr and Nc-37 cells every 24 hr with RPMI 6410 between these two.

Except for the compounds **13–15** and **17**, the cyclic nucleotides were not substrates for cAMP phosphodiesterase (Table IV). It would thus seem reasonable to suggest that the activities of cyclic nucleotides in tissue culture are not due to the 5′-phosphate or nucleoside formation.

Protein Kinase. The cyclic 3′,5′-nucleotides were tested for their ability to stimulate a cAMP-dependent protein kinase from beef heart. (The results are shown in Table IV.) The compounds were tested over a concentration range of 10^{-4}–10^{-8} M and their relative effectiveness was compared to that of cAMP. A value for the ratio of activity of the test compound relative to cAMP was calculated at each concentration. The N^6-substitution of cAMP with isopentenyl (**13**), benzyl (**14**), *trans*-chlorobutenyl (**15**), furfuryl (**16**), phenyl (**17**), and adamantyl (**18**) groups led to more active compounds than cAMP in activating protein kinase. Thus these bulky groups or simple alkyls like methyl and ethyl[18] did not possess dramatically high activity. N^6-Carbamoyl derivatives of cAMP did not show much enhancement of the activity as compared to cAMP. However, at the lower concentration (10^{-7} M) the carbamoyl compounds **25–27** possessing a bulky or an aromatic group showed enhanced activity. Similar observation has been made by Boswell et al.[19]

Cyclic 3′,5′-Nucleotide Phosphodiesterase. All the compounds listed in Table II were tested as substrates as well as inhibitors of the phosphodiesterase from beef heart. The relative rates of hydrolysis of the test compounds to that of cAMP at a concentration of 1×10^{-3} M are shown in Table IV. The actual rate of cAMP hydrolysis was 50 nmol of 5′-nucleotide formed per minute. Compounds **13–15** and **17** were hydrolyzed at a rate of 10–20% relative to cAMP, while compounds **16**, **18**, and all the N^6-carbamoyl-cAMP analogs (**19–27**) were found to be very poor substrates for the enzyme. Michaelis constant (K_m) and V max values were also determined from Lineweaver–Burk plots (Table IV). The K_m value for cAMP was 8.7×10^{-4} M and the V max was 0.481 μmol of Pi released per milligram of protein per minute at pH 7.5 with Tris–HCl buffer. The affinity of some of the N^6-substituted cAMP analogs (**13–15**, **17**, and **18**) to the enzyme was as good as that of cAMP. However, the velocity of hydrolysis was slower than that of cAMP. Kinetic studies with the N^6-carbamoyl-cAMP analogs could not be performed because of inertness of these compounds to the enzyme. As reported earlier,[18–21] N^6 substitution of cAMP resulted in resistance of the compound to phosphodiesterase hydrolysis. As inhibitors of the phosphodiesterase, the compounds in Table II were generally inactive.

Glycogen Synthesis. The cyclic 3′,5′-nucleotides were tested for their inhibition against glycogen synthesis in human skin slices. The benzyladenosine analog **14** and *trans*-chlorobutenyl derivative **15** showed 40% inhibition at the concentration of 1×10^{-4} M for a 2-hr assay period and the isopentenyl analog **13** and the allylureido compound **21** showed somewhat a weaker inhibitory activity (10–15%). At the concentration of 1×10^{-6} M, the activity of compounds **14** and **15** dropped to 20% and that of the compound **13** was increased to 23%, while cAMP exerted 90% inhibition. The 5′-nucleotide **1** (IPAMP) also showed 42% inhibition at 1×10^{-4} M.

Experimental Section

Syntheses. Melting points were determined in a capillary tube on a Mel-Temp apparatus and are corrected. Ir, uv, and NMR (Me_4Si as an internal reference) spectra were recorded as reported previously.[3] Evaporations were performed in vacuo at <30°. Whatman cellulose powder (CC31) was used for column chromatography. The eluates from column chromatography were monitored at 254 nm. TLC was carried out on a Brinkman precoated cellulose TLC plate (CEL300-10UV 254) and on Bakerflex silica gel 1B-F using the following solvent systems: (A) *i*-PrOH–H_2O–concentrated NH_4OH (7:2:1); (B) EtOAc–2-ethoxyethanol–16% HCOOH (4:1:2); (c) EtOAc–*n*-PrOH–H_2O (4:1:2). Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn., Schwarzkopf Microanalytical Laboratories, Woodside, N.Y., and Heterocyclic Chemical Co., Harrisonville, Mo. Where analyses are indicated

only by symbols of the elements, the analytical results obtained for these elements were within 0.4% of the theoretical values.

N^6-Alkyl-, N^6-Aralkyl-, and N^6-Aryladenosines. N^6-(Δ^2-Isopentenyl)adenosine,[22] N^6-benzyladenosine, N^6-furfuryladenosine, and N^6-phenyladenosine[2] were prepared according to the reported procedures.

N^6-Adamantyladenosine was prepared by refluxing a mixture of 6-chloropurine ribonucleoside, adamantanamine hydrochloride, and NaOEt in EtOH for 3 days: yield 90%; mp 140–145° effervescent. Anal. ($C_{20}H_{27}N_5O_4$) C, H, N.

6-Ureidopurine Ribonucleosides. These compounds were prepared by our previously published procedures.[3] 6-Octylureidopurine ribonucleoside was prepared by heating a mixture of tri-*O*-acetyladenosine and octyl isocyanate in pyridine at 90° for 7 hr followed by deblocking with NH_3–MeOH. The material was isolated as reported for other compounds: yield 69%; mp 96–98°. Anal. ($C_{19}H_{30}N_6O_5 \cdot 1.5H_2O$) C, H, N.

N^6-Benzyladenosine 5'-Phosphate (2). Method A. To a cooled mixture (at 0°) of 6 ml (ca. 65 mmol) of $POCl_3$ and 150 ml of $(EtO)_3PO$ was added 10.7 g (30 mmol) of N^6-benzyladenosine. The mixture was stirred at 0° for 18 hr and then diluted with ice water (500 ml). The aqueous solution was adjusted to pH 2 with 6 *N* NaOH and then absorbed on a charcoal–Celite& (100 g each) column. The column was washed with H_2O (2000 ml) and then eluted with 2500 ml of EtOH–concentrated NH_4OH–H_2O (10:1:9). The eluate was evaporated to a small volume (200 ml) and the aqueous solution was applied to an AG1-X8 (formate) column (30 g, 2 × 15 cm) prepacked in H_2O. The column was eluted with H_2O (1500 ml) and 0.5 *N* HCO_2H (2000 ml). The 0.5 *N* HCO_2H eluate (2000 ml) was evaporated to dryness and the residue was treated with acetone. The resulting white solid was filtered and washed with acetone: yield 5.96 g (45.5%); ir max 1680, 1620 (C=O, C=C, C=N), 1215 (P=O), and 1020 cm^{-1} (POC); NMR δ 4.53–4.00 (m, 4, 3'-H, 4'-H, 5'-H), 5.08–4.53 (m, 3, CH_2, 2'-H), 6.06 (d, 1, J = 5.5 Hz, 1'-H), 7.42 (s, 5, C_6H_5), 8.38 (s, 1, 2-H), and 8.53 ppm (s, 1, 8-H).

Table I lists the compounds prepared by this procedure. In the case of the 6-ureidopurine derivatives, compounds **7** and **12** were eluted with 2 *N* HCO_2H instead of 0.5 *N* HCO_2H and compound 11 was eluted with 2 *N* HCO_2H in 50% EtOH. The compounds **8**, **9**, and **10** precipitated out when the reaction mixtures were diluted with ice H_2O.

For analyses, the compounds (200–250 mg) were passed through a cellulose column (30 g, 2.5 × 23 cm) and then eluted with solvent A as described in method C. The compounds were analyzed as NH_4 salts or Ba salts. The Ba salts were obtained by addition of aqueous BaI_2 to the aqueous solution of the NH_4 salts. Some of the samples were converted back to the free acids by passing the NH_4 salts through an Amberlite CG-50 column and then analyzed as free nucleotides.

N^6-Benzyladenosine Cyclic 3',5'-Phosphate (14). Method B. N^6-Benzyladenosine 5'-phosphate (**2**, 2.19 g, 5 mmol) and 1.47 g (5 mmol) of 4-morpholine-*N*,*N*'-dicyclohexylcarboxamidine were dissolved in 25 ml of H_2O and 125 ml of pyridine. The solution was evaporated and azeotroped with pyridine twice. The residue was dissolved in 200 ml of anhydrous pyridine and the solution was added dropwise to a refluxing solution of 2.06 g (10 mmol) of DCC in 500 ml of anhydrous pyridine for a period of 2 hr. Reflux was continued for an additional 1 hr and then the mixture was evaporated to dryness. The residue was triturated with 200 ml of H_2O and then filtered to remove dicyclohexylurea. The filtrate was passed through AG1-X8 (formate) column (15 g, 2 × 8 cm) prepacked in H_2O. The column was first eluted with H_2O (1500 ml) and then with 1.0 *N* HCO_2H (2500 ml). The formic acid eluate was evaporated to dryness and the residue was treated with acetone. The resulting white solid was filtered and washed with acetone: yield 1.07 g (51%). The analytical sample was prepared (as an NH_4 salt) by passing the product (200 mg) through a cellulose column (30 g, 2.5 × 23 cm) with solvent A as described in method C: ir max 1630 (C=O, C=C, C=N), 1230 (P=O), and 1070 cm^{-1} (POC); NMR δ 4.50–4.08 (m, 3, 4'-H, 5'-H), 5.15–4.65 (m, 4, CH_2, 2'-H, 3'-H), 6.10 (s, 1, 1'-H), 7.34 (s, 5, C_6H_5), 8.37 (s, 1, 2-H), and 8.51 ppm (s, 1, 8-H).

Table II lists the compounds prepared by this procedure. In the case of isolation of the 6-ureidopurine derivatives, compounds **21–24** were eluted with 2 *N* HCO_2H and compounds **25–27** were eluted with 2 *N* HCO_2H in 50% EtOH. The latter three compounds (**25–27**) were also extracted with 50% EtOH from the residue after evaporating the reaction mixture.

N-[9-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-9*H*-purin-6-ylcarbamoyl]glycine Benzyl Ester (V, R = H). The urethane IV[16] (4.65 g, 10 mmol) was refluxed for 7 hr with 5.06 g (15 mmol) of glycine benzyl ester *p*-tosylate in 50 ml of anhydrous pyridine. The reaction mixture was evaporated to dryness and the residue was azeotroped with toluene. The residue was dissolved in 50 ml of $CHCl_3$ and mixed with 60 g of silica gel (100–200 mesh). After drying the mixture, this was applied at the top of a silica gel column (dry packed, 5 × 75 cm) and the column was eluted with EtOAc. The fractions between 2500 and 9100 ml were combined and evaporated to dryness. The residue was crystallized from MeOH: yield 1.99 g (34.1%); mp 140–141°; uv λ max (50% EtOH) 267 nm (ε 21,500), 273 (sh, 17,820); λ max (0.1 *N* HCl) 267 nm (ε 21,200), 273 (sh, 18,670); λ max (0.1 *N* NaOH) 270 nm (ε 14,200), 277 (14,100), and 298 (13,860); ir max 1740 (ester, C=O), 1690 (ureido C=O), and 1210 cm^{-1} (ester, COC); NMR δ 2.03 (s, 3, CH_3), 2.07 (s, 3, CH_3), 2.15 (s, 3, CH_3), 4.22 (d, 2, J = 5.5 Hz, NCH_2), 4.40 (m, 3, 4'-H, 5'-H), 5.20 (s, 2, CH_2), 5.78–5.60 (m, 1, 3'-H), 6.07 (t, 1, J = 6 Hz, 2'-H), 6.37 (d, 1, J = 5 Hz, 1'-H), 7.38 (s, 5, C_6H_5), 8.62 (s, 1, 2-H), 8.70 (s, 1, 8-H), 9.77 (t, 1, J = 6 Hz, CONH), and 9.98 ppm (s, 1, NHCO). Anal. ($C_{26}H_{28}N_6O_{10}$) C, H, N.

N-[9-(β-D-Ribofuranosyl)-9*H*-purin-6-ylcarbamoyl]-*O*-benzyl-L-threonine Benzyl Ester [VII, R = $CH(OCH_2C_6H_5)CH_3$]. The fully protected PCTR benzyl ester V [R = $CH(OCH_2C_6H_5)CH_3$] prepared from 8.4 g (18 mmol) of the urethane IV and 11.7 g (30 mmol) of *O*-benzyl-L-threonine benzyl ester oxalate (1:1) was stirred in 700 ml of NH_3–MeOH (saturated at 0°) for 6 hr. After evaporating the mixture to dryness at 25°, the oily residue was dissolved in 100 ml of EtOH and the ethanolic solution was left at room temperature overnight. The white solid was filtered and washed with cold CH_3CN (1.54 g). The filtrate was evaporated to dryness and the residue was azeotroped with toluene. The residue was weighed 4.7 g [total yield 6.24 g (58.8%)] and was generally used for the phosphorylation without further purification.

The analytical sample was recrystallized twice from boiling CH_3CN: mp 90–100° (slow melting); uv λ max (10% EtOH) 268 nm (ε 22,200), 274 (sh, 18,800); λ max (0.1 *N* HCl) 269 nm (ε 21,660), 275 (21,960); λ max (0.1 *N* NaOH) 270 nm (ε 18,350), 277 (17,930), and 295 (10,230); ir max 1740 (ester, C=O), 1690 (ureido C=O), and 1250 cm^{-1} (ester, COC); NMR δ 1.28 (d, 3, J = 6 Hz, CH_3), 3.85–3.50 (m, 2, 5'-H), 4.38–3.50 (m, 2, 3'-H, 4'-H), 4.75–4.38 (m, 5, NCH, CH, OCH_2, 2'-H), 5.20 (s, 2, OCH_2), 6.08 (d, 1, J = 5.5 Hz, 1'-H), 7.34 (s, 10, C_6H_5), 8.40 (s, 1, 2-H), 8.75 (s, 1, 8-H), and 9.98–10.15 ppm (m, 2, NH). Anal. ($C_{29}H_{32}N_6O_8 \cdot 0.5H_2O$) C, H, N.

Ethyl 9-(2',3'-Isopropylidene-5'-trityl-β-D-ribofuranosyl)-9*H*-purine-6-carbamate (VI). To a stirred solution of 27.5 g (0.05 mol) of 2',3'-isopropylidene-5'-trityladenosine at −10° in 700 ml of anhydrous pyridine was added, dropwise, 15 ml (ca. 0.15 mol) of ethyl chloroformate over a period of 30 min. The mixture was then stirred at ambient temperature for 18 hr and evaporated to dryness. The residue was dissolved in 150 ml of $CHCl_3$ and the solution was mixed with silica gel (100 g). After drying the mixture, this was applied at the top of a silica gel column (100–200 mesh, dry packed, 5 × 100 cm) and the column was eluted with EtOAc. The first 1500 ml of the eluate was evaporated to dryness and the resulting product isolated as a glass, 17 g (54.7%). The analytical sample was further purified by silica gel preparative TLC in EtOH–EtOAc (1:49): mp 90–100° (slowly melt); uv λ max (50% EtOH) 267 nm (ε 15,600), 273 (sh, 13,200); λ max (0.1 *N* HCl) 269 nm (ε 14,900), 272 (14,800); λ max (0.1 *N* NaOH) 291 nm (ε 17,500); ir max 1725 (urethane, C=O) and 1210 cm^{-1} (=COC); NMR δ 1.27 (d, 3, J = 6.5 Hz, CH_3), 1.33 (s, 3, CH_3), 1.55 (s, 3, CH_3), 4.22 (q, 2, J = 7 Hz, CH_2), 4.38 (m, 2, 5'-H), 5.67–4.85 (m, 3, 2'-H, 3'-H, 4'-H), 6.36 (d, 1, J = 2.5 Hz, 1'-H), 7.28 (s, 15, C_6H_5), 8.48 (s, 1, 2-H), 8.60 (s, 1, 8-H), and 10.48 ppm (s, 1, NH). Anal. ($C_{35}H_{35}N_5O_6 \cdot 0.5H_2O$) C, H, N.

N-[9-(β-D-Ribofuranosyl)-9*H*-purin-6-ylcarbamoyl]glycine Benzyl Ester (VII, R = H). A mixture of 3.73 g (6 mmol) of urethane VI and 3.04 g (9 mmol) of glycine benzyl ester *p*-tosylate in 200 ml of anhydrous pyridine was refluxed 18 hr and then evaporated to dryness. The residue was dissolved in EtOH and sufficient H_2O was added to get a gummy precipitate. The cloudy water was removed by decantation and the gummy solid was washed with H_2O (100 ml) three times. This was crystallized from hot EtOH (3 g). The fully protected crude product was then heated in 200 ml of 80% AcOH on a steam bath for 1 hr and evaporated to

&The charcoal–Celite column was prepared by first mixing 100 g of each with 100 ml of water and then packing into a precision bore glass column (5 cm in diameter) with a plunger.

dryness. The residue was partitioned between 200 ml of ether and 300 ml of H_2O. The aqueous phase was evaporated to dryness. The residue was dissolved in hot EtOH and petroleum ether (bp 30–60°) was added to turbidity. The resulting white precipitate was filtered and washed with petroleum ether: yield 0.974 g (35.44%). The analytical sample was recrystallized from hot H_2O: mp 128–130°; uv λ max (25% EtOH) 268 nm (ε 23,460), 274 (sh, 19,600); λ max (0.1 *N* HCl) 269 nm (ε 22,760), 275 (23,000); λ max (0.1 *N* NaOH) 269 nm (ε 18,800), 276 (17,860), and 297 (11,400); ir max 1750 (ester, C=O), 1680 (ureido C=O), and 1200 cm^{-1} (ester, =COC); NMR δ 3.75–3.50 (m, 2, 5′-H), 4.10–3.87 (m, 2, 3′-H, 4′-4), 4.18 (d, 2, J = 6 Hz, N-CH_2), 4.68–4.55 (m, 1, 2′-H), 5.20 (s, 2, OCH_2), 6.30 (d, 1, J = 5.5 Hz, 1′-H), 7.39 (s, 5, C_6H_5), 8.57 (s, 1, 2-H), 8.71 (s, 1, 8-H), and 9.92–9.67 ppm (m, 2, NH). Anal. ($C_{20}H_{22}N_6O_7$) C, H, N.

***N*-(Purin-6-ylcarbamoyl)-L-threonine Ribonucleoside Cyclic 3′,5′-Phosphate (19). Method C.** A solution of 673 mg (1 mmol) of compound 11 in 10 ml of water was added to a solution of 4-morpholine-*N*,*N*′-dicyclohexylcarboxamidine in 50 ml of pyridine. The solution was evaporated to dryness and then azeotroped with pyridine twice. The residue dissolved in 100 ml of anhydrous pyridine was added, dropwise, to a refluxing solution of 412 mg (2 mmol) of DCC in 100 ml of anhydrous pyridine for a period of 2 hr. Reflux was continued for an additional 1 hr and then evaporated to dryness. The residue was triturated with 50% EtOH (100 ml) and filtered to remove dicyclohexylurea. The ethanolic solution was hydrogenated over PdO (200 mg, Matheson Coleman and Bell) at atmospheric pressure for 48 hr. Fresh PdO (100 mg) was added and hydrogenation was continued an additional 24 hr. After removing the catalyst, the filtrate was evaporated to dryness and the residue was treated with H_2O to remove dicyclohexylurea. The filtrate was evaporated to dryness and the residue was dissolved in 10 ml of solvent A and purified on the cellulose column (30 g, 2.5 × 23 cm) prepacked in solvent A. A few drops of Thymol Blue solution were placed on the column before applying the sample on the column and the fractions were collected when the blue indicator had reached to the bottom of the column. The fractions between 75 and 120 ml were pooled and evaporated to dryness. The residue was triturated with acetone and the resultant white solid was collected on a filter: yield 112 mg (22.8%); ir max 1680 (ureido C=O), 1220 (P=O), and 1070 cm^{-1} (POC); NMR δ 1.02 (d, 3, J = 6 Hz, CH_3), 4.45–3.58 (m, 4, 3′-H, 4′-H, 5′-H), 4.82–4.45 (m, 3, 2′-H, =CH, NCH), 6.05 (s, 1, 1′-H), 8.58 (s, 1, 2-H), 8.65 (s, 1, 8-H), and 9.62–9.72 ppm (m, 2, NH).

***N*-[9-(β-D-Ribofuranosyl)-9*H*-purin-6-ylcarbamoyl]glycine Methyl Ester.** Compound V (R = H) (100 mg) was stirred in 25 ml of NH_3–MeOH (saturated at 0°) at 0° for 2 hr and evaporated to dryness. The residue was crystallized from EtOH twice: yield quantitative; mp 97–100° (softens) and 188–190° (melts); uv λ max (10% EtOH) 268 nm (ε 20,700), 274 (17,400); λ max (0.1 *N* HCl) 269 nm (sh, ε 19,600), 275 (21,000); λ max (0.1 *N* NaOH) 269 nm (ε 15,800), 276 (15,600), and 298 (12,800); ir max 1730 (ester, C=O), 1700, 1680 (ureido C=O), and 1220 cm^{-1} (ester, =COC); NMR δ 3.52 (m, 2, 5′-H), 3.72 (s, 3, CH_3), 4.40–3.92 (m, 4, NCH_2, 3′-H, 4′-H), 4.83–4.50 (m, 1, 2′-H), 6.11 (d, J = 5 Hz, 1′-H), 8.65 (s, 1, 2-H), 8.77 (s, 1, 8-H), 9.83 (s, 1, NH), and 9.95 ppm (s, 1, NH). Anal. ($C_{13}H_{18}N_6O_7$) C, H, N.

Biochemical. Growth Inhibition. Cultured cells derived from the buffy coat of a normal individual (Nc-37), patients with myeloblastic leukemia (RPMI 6410), and mouse leukemia (L1210) were used for determining the growth inhibitory properties. The compounds were dissolved in 0.5% DMSO in growth medium (RPMI 1640 + 10% fetal calf serum) to achieve concentrations of 10^{-4} *M*. The results are expressed as percent of viable cell number relative to control containing 0.5% DMSO, after 72 hr of incubation. Controls with compared DMSO and NH_4^+ (the ammonium salt) concentrations did not affect growth. Table III shows the results.

Enzyme Assays. cAMP-dependent protein kinase from beef heart, cyclic 3′,5′-nucleotide phosphodiesterase (beef heart), and 5′-nucleotidase were purchased from Sigma Chemical Co. [γ-^{32}P]-ATP (6.80 Ci/mmol) was purchased from New England Nuclear Corp. and [8-^{3}H]-cAMP (28 Ci/mmol) was purchased from Schwarz/Mann.

Protein kinase activity was assayed by measuring the incorporation of [γ-^{32}P]phosphate into histone from γ-^{32}P-labeled ATP. The modified literature procedures were used.[23,24] The incubation mixture contained (final volume of 0.2 ml) sodium glyerol buffer (10 μmol, pH 6.5), histone (0.5 mg), [γ-^{32}P]-ATP (1 μCi, 0.5 nmol), $MgAc_2$ (2 μmol), NaF (2 μmol), theophylline (0.4 μmol), ethylene glycol bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid (0.06 μmol), and cAMP or analogs as indicated and protein kinase (25 μg). The mixture was incubated at 30° for 5 min and then an aliquot (0.1 ml) of the incubation mixture was transferred to a Whatman No. 3MM filter paper piece (2.5 × 2.5 cm). The paper pieces were air-dried and washed with 6% trichloroacetic acid for 15 min at room temperature three times, with EtOH–ether (1:1), and finally with ether. The air-dried papers were transferred to liquid scintillation vials and radioactivity was determined.

When testing cAMP analogs as substrates for cyclic 3′,5′-nucleotide phosphodiesterase, the release of inorganic phosphate was utilized as a measure of phosphodiesterase activity. The method was patterned after the literature procedure.[25,26] The incubation mixture contained (in a volume of 0.9 ml) Tris–HCl (40 μmol, pH 7.5), $MgCl_2$ (1 μmol), various concentrations of cAMP or analogs, and the phosphodiesterase (0.2 mg). The mixture was incubated at 30° for 20 min and the reaction was terminated by heating at 100° for 3 min. The mixture was then incubated with 0.1 mg of 5′-nucleotidase in 0.1 ml of 10 m*M* Tris–HCl (pH 7.5) at 30° for 10 min and the reaction was terminated by addition of 0.1 ml of cold 55% trichloroacetic acid. After centrifugation, a 0.5-ml portion of the supernatant solution was analyzed for inorganic phosphate by the reported procedure.[27]

The assay for inhibition of the phosphodiesterase was carried out according to the procedure of Miller et al.[20] The mixture contained (in 1.0 ml) Tris–HCl (50 μmol, pH 7.5), $MgCl_2$ (10 μmol), the phosphodiesterase (0.2 mg), [8-^{3}H]-cAMP (100 nmol), and varying concentrations of the analog being tested as an inhibitor. The incubation and the termination of reaction was followed as described above. The intact nucleoside cyclic phosphate was absorbed onto AG1-X8 (formate) and the radioactivity of the nucleoside fraction was determined.

Glycogen Assay in the Skin Slices. Human skin was cut in a sheet at a depth of 0.3 mm using an electric keratone. The pieces of skin were floated on Ringer's solution containing 5 m*M* glucose and maintained a pH 6.7–7.0 during the 2-hr incubation. For each time period (15, 30, 60, and 120 min) and for each concentration of compound (10^{-4}–10^{-6} *M*), three pieces were removed, put in a boiling water bath for 5 min, lyophilized, and then homogenized in Tris–HCl (50 m*M*, pH 4.8). The glycogen was assayed by adding amyloglucosidase (Boehringer Mannheim) to break the glycogen down to glucose. The glucose was assayed before and after the glycogen was broken down by coupling it to hexokinase and glucose-6-phosphate dehydrogenase. The NADPH formed was monitored fluorometrically.

Acknowledgments. For part of this project, we wish to acknowledge financial support from Westwood Pharmaceuticals, Inc., Buffalo, N.Y. The authors thank Dr. G. P. Murphy and Dr. E. D. Holyoke for their continued keen interest in this work. N^6-(3-Chloro-*trans*-buten-2-yl)adenosine was a gift from Dr. H. F. Vorbrüggen, Schering, A.G., West Berlin, Germany.

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Synthesis and Biological Activity of 4-(β-D-Ribofuranosyl)-1,3-dihydroxybenzene ("1,3-Dideazauridine")

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In view of the marked antitumor activity of 3-deazauridine, the synthesis of 4-(β-D-ribofuranosyl)-1,3-dihydroxybenzene (1,3-dideazauridine) and its dibenzyl derivative was carried out. 4-Bromo-1,3-dihydroxybenzene was converted to its dibenzyl derivative, which, upon reaction with *n*-butyllithium followed by treatment with anhydrous cadmium chloride, gave bis(1,3-dibenzyloxyphenyl-4)cadmium. Condensation of this intermediate with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride in refluxing toluene, and subsequent removal of the protecting benzoyl groups, afforded 4-(β-D-ribofuranosyl)-1,3-dibenzyloxybenzene which, upon catalytic hydrogenation over Pd/C, furnished the desired 4-(β-D-ribofuranosyl)-1,3-dihydroxybenzene. The β configuration at the anomeric center was established by NMR and hydrogen bonding studies. 4-(β-D-Ribofuranosyl)-1,3-dibenzyloxybenzene inhibited the growth of leukemia L1210 cells by 50% at 7×10^{-6} *M*, and that of mammary carcinoma TA_3 cells at 5×10^{-5} *M*. Dideazauridine itself was less active, inhibiting the leukemia L1210 but not the TA_3 cells at 1×10^{-4} *M*, but the compound was significantly active against herpes simplex (type I) virus in vitro.

The replacement of nitrogen 3 by carbon in the heterocyclic moiety of uridine and cytidine has provided the analogs 3-deazauridine and 3-deazacytidine which have demonstrated marked antitumor activity in vitro and in vivo.[1-6] Because of this activity, we considered it worthwhile to synthesize 4-(β-D-ribofuranosyl)-1,3-dihydroxybenzene (1,3-dideazauridine, **8**). The present communication reports the synthesis and some biological effects of the compound and its 1,3-dibenzyl derivative. Part of this work has been presented in a preliminary communication.[7]

Chemical. The synthesis of **8** was approached by the procedure employed for the synthesis of pseudouridine[8] (Scheme I). 4-Bromo-1,3-dihydroxybenzene (**1**) was converted to 4-bromo-1,3-dibenzyloxybenzene (**2**) by refluxing with benzyl chloride in dry acetone in the presence of anhydrous potassium carbonate. Treatment of **2** with *n*-butyllithium in absolute ether afforded 1,3-dibenzyloxyphenyl-4-lithium (**3**), which was condensed under nitrogen with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride in dry toluene. Thin-layer chromatography of the reaction mixture revealed numerous products, and no attempt was made to separate them.

This result was not completely unexpected, since numerous products and poor yields had also been obtained when 2,4-dimethoxypyrimidine-5-lithium[8] and 2,6-dibenzyloxypyridyl-3-lithium[9] were condensed with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride. We, therefore, considered the preparation of 1,3-dibenzyloxyphenylcadmium (**4**) for condensation with the chloro sugar **5**, since, as has been demonstrated,[10] diphenylcadmium does not react with the acyl-protecting groups, thus offering greater selectivity in reactions with halo sugars. By use of this method, 1-deazauridine[9] and more recently β-D-ribofuranosylbenzene have been synthesized.[11] The cadmium derivative **4** was prepared by refluxing **3** with anhydrous cadmium chloride in absolute ether (Scheme I) under nitrogen atmosphere.

Addition of the chloro sugar **5** to the cadmium compound **4** in refluxing toluene, followed by removal of the protecting benzoyl groups with methanolic sodium methoxide, furnished a mixture which, as shown by tlc, contained three products. The fastest moving product with R_f 0.95 was readily isolated and characterized as 1,3-dibenzyloxybenzene. In fact, this was the major product of the reaction, isolated in 50% yield. Although we have not fully characterized the second product which has an R_f of 0.67, preliminary evidence obtained from mass spectrometry indicates that it is presumably the sugar ketal formed by the reaction of cadmium compound **4** with the 2-*O*-benzoyl group in the