Species- or Isozyme-Specific Enzyme Inhibitors. 7.[1] Selective Effects in Inhibitions of Rat Adenylate Kinase Isozymes by Adenosine 5'-Phosphate Derivatives

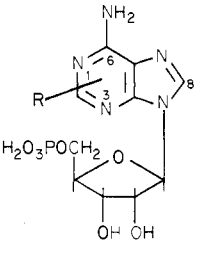
Ton T. Hai, Donald Picker, Masanobu Abo, and Alexander Hampton*

The Institute for Cancer Research, The Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111.
Received February 16, 1982

Monosubstituted derivatives of adenosine 5'-phosphate (AMP) with substituents of 1–3 atoms or group replacements at any of 11 positions have been synthesized and examined as substrates and inhibitors of the rat muscle adenylate kinase isozyme (AK-M), and the rat AK II and III isozymes predominant in poorly differentiated hepatoma tissue and normal liver tissue, respectively. Inhibition indexes of the compounds were expressed as K_M (AMP)/K_i for competitive inhibition or as K_M (AMP)/K_M when only K_M was available. Substituents at N(1), N^6, or C(8) or on ionizable phosphate oxygen reduced inhibition below measurable levels; 2'-deoxy-AMP and adenosine 5'-sulfate had identical inhibition indexes with all three isozymes; compounds with substituents at C(2), O(2'), O(3'), C(4'), C(5'), or O(5') had higher inhibition indexes with AK-M than with AK II or III and the same or similar indexes for AK II and III. The most effective and/or selective inhibitors were 2-NHMe-AMP (index with AK-M, 0.2; index ratio, AK-M/AK III, 9.1), 2'-*O*-Me-AMP (index with AK-M, 0.14; index ratio, AK-M/AK III, 8.2), 2',3'-*O*-CMe_2-AMP (index with AK-M, 0.25; index ratio, AK-M/AK II, 6.6), 4'-allyl-AMP (index with AK-M, 0.97; index ratio, AK-M/AK III, 8.1), and 5'(*S*)-Et-AMP (index with AK-M, 0.64; index ratio, AK-M/AK II, 11.2). The study provides additional evidence that the attachment of simple substituents to various atoms in turn of a substrate is a potentially useful approach in early stages of the attempted design of isozyme-selective inhibitors.

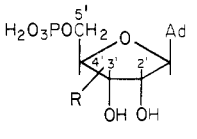
It was reported recently that inhibitors of rat adenylate kinases with isozyme selectivity were produced when substituents were attached to either of two atoms of one of the substrates (ATP).[2] Later, thymidine derivatives bearing a substituent at any of eight positions were studied, and seven of the eight types were found to exhibit isozyme-selective inhibition of rat thymidine kinases.[3] These differential effects were of interest to us in view of evidence, summarized previously,[4] that selective inhibitors of fetal-type isozymes may prove to be a class of compounds useful in the design of new types of antineoplastic agents. The present work further explores the tendency of substrate derivatives monosubstituted at various atoms to exhibit isozyme-selective inhibitions. Derivatives (**1–11**, **13**, **14**, and **16**) resulting from single substitutions or replacements of groups at 11 atoms of adenosine 5'-phosphate (AMP) have been synthesized and studied as substrates and/or inhibitors of the rat muscle isozyme (AK-M) of adenylate kinase, the rat liver isozyme AK III, and the AK II isozyme that predominates in poorly differentiated rat hepatoma tissue.[5,6] The atoms of AMP at which substitutions or replacements were made were N(1) (**1**), C(2) (**2a–f**), N^6 (**3a,b**), C(8) (**4a,b**), O(2') (**5**), C(2') (**16**), O(3') (**6**), C(4') (**8**), C(5') (**9a–c** and **10a–c**), O(5') (**11** and **14**), and ionizable phosphate oxygen (**13**); three doubly substituted AMP derivatives (**7a–c**) were included in the study. In addition, we investigated the effects on inhibition of the AK isozymes of increased rigidity in the phosphate–ribose bridge region of AMP (**12**) or increased flexibility in the ribosyl moiety (**15**).

Syntheses. All AMP derivatives were isolated as disodium salts, and yields in each case refer to these salts. The 2-alkylamino derivatives **2d–f** of AMP were prepared in 68–74% yield by treatment of 2-chloro-AMP[7] with aqueous alkylamine at 100 °C. Compounds **2d–e** had been previously prepared via phosphorylation of 2',3'-*O*-isopropylidene derivatives of the respective 2-(alkylamino)-



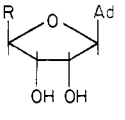
R

1	1-Me
2a	2-Cl
b	2-SMe
c	2-NH_2
d	2-NHMe
e	2-NHEt
f	2-NMe_2
3a	N^6-Me
b	N^6-$(CH_2)_2$NHCOMe
4a	8-Br
b	8-SEt

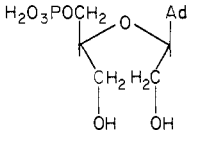


R

5	2'-*O*-Me
6	3'-*O*-Me
7a	2',3'-*O*-CHMe
b	2',3'-*O*-CHEt
c	2',3'-*O*-CMe_2
8	4'-$CH_2CH{=}CH_2$
9a	5'(*R*)-Me
b	5'(*R*)-Et
c	5'(*R*)-*n*-Pr
10a	5'(*S*)-Me
b	5'(*S*)-Et
c	5'(*S*)-*n*-Pr

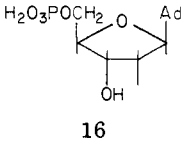


R



15

11	$CH_2CH_2PO_3H_2$
12	trans $CH{=}CHPO_3H_2$
13	$CH_2PO(OH)(OMe)$
14	CH_2OSO_3H



16

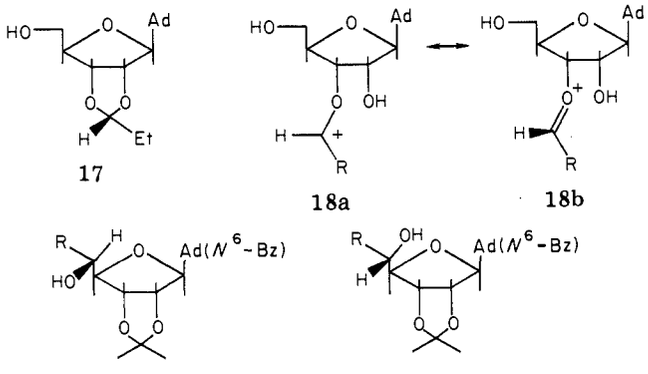
Ad = adenin-9-yl.

(1) For Paper 6 of this series, see Hampton, A.; Hai, T. T.; Kappler, F.; Chawla, R. R. *J. Med. Chem.*, preceding paper in this issue.
(2) Hampton, A.; Picker, D. *J. Med. Chem.* **1979**, *22*, 1529.
(3) Hampton, A.; Chawla, R. R.; Kappler, F. *J. Med. Chem.* **1982**, *25*, 644.
(4) Hampton, A.; Kappler, F.; Maeda, M.; Patel, A. D. *J. Med. Chem.* **1978**, *21*, 1137.
(5) Criss, W. E.; Litwack, G.; Morris, H. P.; Weinhouse, S. *Cancer Res.* **1970**, *30*, 370.
(6) Sapico, V.; Litwack, G.; Criss, W. E. *Biochim. Biophys. Acta* **1972**, *258*, 436.
(7) Gough, G.; Maguire, M. H.; Michal, F. *J. Med. Chem.* **1969**, *12*, 494.

0022-2623/82/1825-0806$01.25/0

adenosines.[8] 3′-*O*-Methyl-AMP (**6**) was obtained in 80% yield following treatment of 3′-*O*-methyladenosine[9,10] with $POCl_3$–H_2O–pyridine in acetonitrile,[11] adsorption of the product on partially inactivated charcoal[12] to separate it from inorganic phosphate, and anion-exchange chromatography.

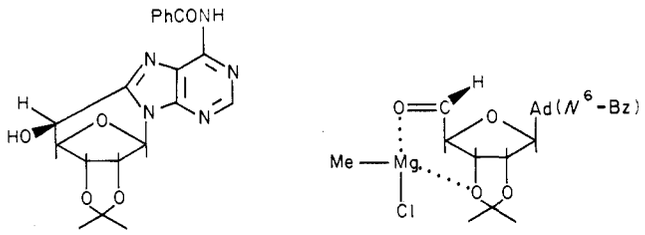
Syntheses of 2′,3′-*O*-isopropylidene-AMP (**7c**)[13] and the endo diastereomer of 2′,3′-*O*-ethylidene-AMP (**7a**)[14] were reported previously; 2′,3′-*O*-*n*-propylidene-AMP (**7b**) was conveniently obtained in 25% yield by HCl-catalyzed reaction of tri-*n*-butylammonium AMP in DMF with 3 equiv each of propionaldehyde and triethyl orthoformate. Treatment of **7b** with alkaline phosphatase gave a nucleoside identical with a crystalline 2′,3′-*O*-*n*-propylideneadenosine (**17**) obtained in 45% yield by



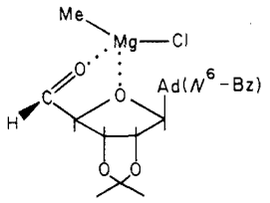
17 **18a** **18b**

19 [C(5′)-*R*] **20** [C(5′)-*S*]

a, R = Me; b, R = Et; c, R = *n*-Pr



21 **22**



23

treatment of adenosine with excess of propionaldehyde in DMF solution in the presence of 10 equiv of toluene-*p*-sulfonic acid. Compound **17** appeared from its ^1H NMR spectrum and from reverse-phase HPLC to consist of only one of the two possible diastereomers epimeric about the chiral acetal function. Acid-catalyzed condensation of aldehydes with ribonucleosides[15–17] and other types of 1,2-diols[18] gives predominantly and sometimes exclusively *endo*-acetals with an *R* configuration, as depicted for **17**. It has been proposed that acetal formation proceeds via an oxocarbonium ion (**18a**) possessing considerable oxonium character and that, by analogy with olefins, the transoid oxonium form **18b** is more stable than the cisoid form and furnishes the kinetically favored endo diastereomer.[18]

Phosphorylation of N^6-benzoyl-2′,3′-*O*-isopropylidene-4′-*C*-prop-2-enyladenosine by the β-cyanoethyl phosphate–dicyclohexylcarbodiimide method,[19] followed by removal of the blocking groups, gave homogeneous disodium 4′-allyl-AMP (**8**) N^6-benzoyl-9-(6-deoxy-2,3-*O*-isopropylidene-β-D-allofuranosyl)adenine [**19a**,[21] 5′(*R*)] and its α-L-talofuranosyl epimer [**20a**,[21] 5′(*S*)] in a 2.5:1 ratio and a combined yield of 24%. A hitherto unreported finding was that this Grignard reaction produced also N^6-benzoyl-2′,3′-*O*-isopropylidene-8,5′(*S*)-cycloadenosine (**21**; yield 4%). The 5′(*S*) configuraion of **21** was established by debenzoylation and acetylation, which yielded a crystalline compound similar in melting point and ^1H NMR characteristics with 5′-*O*-acetyl-2′,3′-*O*-isopropylidene-8,5′(*S*)-cycloadenosine[22] and dissimilar from the corresponding *R* epimer. The *R* epimer of **21** was not formed in detectable amounts in the above Grignard reaction. Stereoselective reactions of Grignard reagents and carbonyl groups in carbohydrates are well known[23] and in some cases have been rationalized in terms of Mg–O coordinated adducts between the reactants. Attack by C-8 of the O(3′)–O(5′) Grignard adduct **22** on C(5′) would selectively produce the 8,5′(*S*) cycloadenosine epimer **21**, while attack by a second molecule of MeMgCl on the least hindered side of C(5′) of adduct **22** would produce the 5′(*R*) epimer **19a**. Formation of the 5′(*S*) epimer **20a** could occur via similar attack by MeMgCl on the O(4′)–O(5′) adduct **23**. This type of adduct has been invoked as an intermediate in the stereospecific conversion of an α-D-xylopentodialdo-1,4-furanose to a β-L-*ido* derivative.[24]

Grignard reactions between the above blocked 5′-aldehyde and ethylmagnesium chloride or *n*-propylmagnesium chloride furnished a mixture of N^6-benzoyl-5′(*R*)-ethyl-2′,3′-*O*-isopropylideneadenosine (**19b**) and its 5′(*S*) epimer **20b** (the ratio of **19b** to **20b** being ca. 4:1) and a mixture of the corresponding 5′-*n*-propyl epimers **19c** and **20c** (in a ratio of ca. 5:1). In the reactions of the alkyl-

(8) Gough, G. R.; Nobbs, D. M.; Middleton, J. C.; Penglis-Caredes, F.; Maguire, H. *J. Med. Chem.* **1978**, *21*, 520.
(9) Robins, M. J.; Naik, S. R.; Lee, A. S. K. *J. Org. Chem.* **1974**, *39*, 1891.
(10) Robins, M. J.; Lee, A. S. K.; Norris, F. A. *Carbohydr. Res.* **1975**, *41*, 304.
(11) Sowa, T.; Ouchi, S. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 2084.
(12) Symons, R. H. *Biochim. Biophys. Acta* **1970**, *209*, 296.
(13) Hampton, A.; Sasaki, T.; Perini, F.; Slotin, L. A.; Kappler, F. *J. Med. Chem.* **1976**, *19*, 1029.
(14) Hampton, A.; Slotin, L. A.; Kappler, F.; Sasaki, T.; Perini, F. *J. Med. Chem.* **1976**, *19*, 1371.
(15) Seela, F.; Ott, J.; Rosemeyer, H. *Z. Naturforsch., C: Biosci.* **1979**, *34C*, 350.
(16) Ott, J.; Seela, F. *Bioorg. Chem.* **1981**, *10*, 82.
(17) Baggett, N.; Foster, A. B.; Webber, J. M.; Lipkin, D.; Phillips, B. E. *Chem. Ind. (London)* **1965**, 136.
(18) Clode, D. M. *Chem. Rev.* **1979**, *79*, 491.
(19) Tener, G. M. *J. Am. Chem. Soc.* **1961**, *83*, 159.
(20) Secrist III, J. A.; Winter, W. J. *J. Am. Chem. Soc.* **1978**, *100*, 2554.
(21) Ranganathan, R. S.; Jones, G. H.; Moffatt, J. G. *J. Org. Chem.* **1974**, *39*, 290.
(22) Matsuda, A.; Tezuka, M.; Ueda, T. *Tetrahedron* **1978**, *34*, 2449.
(23) Inch, T. D. *Adv. Carbohydr. Chem.* **1972**, *27*, 191.
(24) Wolfrom, M. L.; Hanessian, S. *J. Org. Chem.* **1962**, *27*, 1800.

Table I. Chromatographic and Electrophoretic Properties of Adenosine 5′-Phosphate Derivatives

compd	yield, %	electrophoresis,[a] pH 3.6	R_f system A[b]	R_f system B[b]	HPLC t_R,[c] min
AMP		1.00	0.19	0.10	4
2a	69	1.35	0.24	0.17	13.2
2c	60	0.55	0.10	0.05	2.4
2d	70	0.57	0.18	0.10	5.2
2e	68	0.54	0.24	0.15	10
2f	74	0.52	0.30	0.20	15
5	81	1.00	0.30	0.22	7.6
6	80	0.95	0.28	0.17	7.5
7a			0.30	0.26	19.6
7b	25	0.93[d]	0.41	0.34	32
8	50	1.00	0.30	0.24	20.2
9a	68	1.00	0.23	0.11	6
10a	70	1.00	0.26	0.13	10
9b	62	1.00	0.31	0.17	11.6
10b	65	1.00	0.33	0.20	17.5
9c	63	1.00	0.37	0.23	18.8
10c	62	1.00	0.39	0.30	26.8
17	45				21[e]

[a] AMP is assigned a value of 1.0. [b] Compositions given under Experimental Section. [c] Conditions given under Experimental Section. [d] Mobility 0.77 in aqueous 0.05 M $Na_2B_4O_7$. [e] C_{18} μ-Bondapak eluted with MeOH-H_2O (3:7).

magnesium chlorides with the blocked 5′-aldehyde, the predominant 5′-epimer (**19a–c**) in all three cases was also the less polar epimer; hence, these epimers were assigned the 5′(*R*) (β-D-*allo*) configuration established for **19a**.[21] Phosphorylation of **19a–c** and **20a–c** by the β-cyanoethyl phosphate method,[19] followed by basic and acidic treatments to remove the blocking groups, gave the 5′(*R*)-alkyl-AMP derivatives **9a–c** and the 5′(*S*)-alkyl-AMP derivatives **10a–c** in 62–70% yield. In accord with their configurational assignments, **9a–c** and **10a–c** behaved as separate classes of compounds in respect to their substrate and inhibitor properties with the adenylate kinases used in the present studies.

Studies of Interactions of 1–16 with Rat Adenylate Kinases. Table II lists substrate constants with the three AK isozymes determined by an enzymatic assay for the ADP produced by AK-catalyzed transfer of phosphate from ATP to an AMP derivative. For some compounds with no apparent substrate activity, more sensitive tests were carried out using higher levels of enzyme activity, longer reaction times, and HPLC analysis to detect formation of ADP and ADP derivatives. Table II also lists inhibition constants. Substrate and inhibition constants were determined in the presence of a level of MgATP that was 3.5–3.9 times the K_M value of ATP. Because the K_M of AMP with the muscle isozyme AK-M and the AK II and III isozymes is not the same, the inhibitory potency of each compound for a given isozyme is shown in Table II as an inhibition index calculated from K_M (AMP)/K_i for competitive inhibition or from K_M (AMP)/K_M in the few instances where only K_M was available. K_i values for competitive inhibition were in fair agreement with K_M values when comparison was possible, i.e., with AK III and **10a** and AK-M and **2a** and **10a**.

It was possible to determine relative inhibition indexes for the AK isozyme for 7 of the 11 atoms of AMP at which substitutions or replacements were made. Attachment of groups at the other four atoms, i.e., at the purine 1-, N^6-, and 8-positions and at phosphate oxygen (**13**), produced inhibition that was noncompetitive or too weak for ready determination of K_i, together with substrate activity too weak to allow determination of K_M. The nonfuranoid AMP analogue **15** also did not inhibit AK II and III. The weak inhibition by **3a** could be of steric origin inasmuch as X-ray diffraction analysis of pig AK-M[25] indicates that AMP is bound in a relatively small pocket and that the 6-amino group is within hydrogen-bonding distance of an amino acid residue. The weak inhibition by **1** could likewise be of steric origin, but electronic factors could also be important. 8-Bromo-AMP, which also was a weak inhibitor, exists in aqueous solution as syn-type 9-1′ rotamers,[26,27] whereas AMP is in the form of an anti conformer both in solution[28] and when bound to muscle AK.[29]

Selective inhibition of AK-M was produced by substituents or group replacements at C(2) (**2a–f**), O(2′) (**5**), O(3′) (**6**), C(4′) (**8**), C(5′) (**10a–c**), and O(5′) (**11**). The 2′,3′-acetal derivatives of AMP (**7a–c**) also inhibited AK-M more than AK II or III. In all but one instance (**8** with AK-M), attachment of a substituent to AMP produced inhibitors of weak or moderate potency [K_M (AMP)/K_i < 0.65] for the three isozymes. The substituents gave lower inhibition indexes with AK II and III than with AK-M to give rise to the selective inhibitions of AK-M. Adenosine 5′-sulfate (**14**) and 2′-deoxyadenosine 5′-phosphate (**16**) showed no selective inhibitions. The vinyl phosphonate analogue **12** of AMP, which was of interest because of the reduced flexibility in its ribose–phosphate bridge region, inhibited AK-M 5–6 times more strongly than AK II or III. In general, the AMP derivatives inhibited AK II and III to a similar degree; the largest difference occurred with 4′-allyl-AMP (**8**) and was less than 2-fold. The most potent and selective inhibitors of AK-M in the present survey were **2d**, **5**, **6**, **7c**, **8**, **10b**, **10c**, and **12**.

Previous studies showed that a large proportion of monosubstituted thymidine derivatives bearing substituents at 6–8 different positions exhibited species- and/or isozyme-selective inhibition of bacterial and rat thymidine kinases.[3,30] In addition, ATP derivatives monosubstituted at two positions that were studied were found to exhibit species- and isozyme-selective inhibition of bacterial and rat adenylate kinases.[2] The present work extends this type of finding to AMP derivatives as inhibitors of rat adenylate kinases and provides a further example of the potential usefulness of the approach in early stages of the attempted design of potent species- or isozyme-selective enzyme inhibitors. A problem in this approach is the tendency of substrate substituents to produce inhibitors of low or moderate potency. Recent findings have shown, however, that isoenzyme-selective inhibitors with enhanced potency can sometimes by obtained by suitable modification of the substituent[3] or by attaching a substituent to a relatively potent inhibitor of a type that binds simultaneously to two adjoining enzymic substrate sites.[31]

Experimental Section

Chemical Synthesis. General. Paper chromatography was carried out on Whatman No. 1 paper in solvent systems A (2-propanol–0.25 M aqueous NH_4HCO_3, 65:35) and B (2-

(25) Pai, E. F.; Sachsenheimer, W.; Schirmer, R. H.; Schulz, G. E. *J. Mol. Biol.* **1977**, *114*, 37.
(26) Sarma, R. H.; Lee, C.-H.; Evans, F. E.; Yathindra, N.; Sandaralingam, M. *J. Am. Chem. Soc.* **1974**, *96*, 7337.
(27) Uesugi, S.; Ikehara, M. *J. Am. Chem. Soc.* **1977**, *99*, 3250.
(28) Davies, D. B. *Prog. Nucl. Magn. Reson. Spectrosc.* **1978**, *12*, 135.
(29) Hampton, A.; Harper, P. J.; Sasaki, T. *Biochemistry* **1972**, *11*, 4965.
(30) Hampton, A.; Kappler, F.; Chawla, R. R. *J. Med. Chem.* **1979**, *22*, 1524.
(31) Hampton, A.; Kappler, F.; Picker, D. *J. Med. Chem.* **1982**, *25*, 638.

Table II. Substrate and Inhibitor Constants of AMP Derivatives with Rat Adenylate Kinase (AK) Isozymes

	AK II			AK III			AK-M			inhibn index [K_M (AMP)/K_i or K_M][a]			rel inhibn indexes	
compd	K_i, mM (type of inhibn)[b]	K_M,[c] mM	rel V_{max}	K_i, mM (type of inhibn)	K_M, mM	rel V_{max}	K_i, mM (type of inhibn)	K_M, mM	rel V_{max}	AK II	AK III	AK-M	AK-M/ AK II	AK-M/ AK III
AMP		0.08	100		0.08	100		0.58	100					
1	[d]		0	[d]		0	[d]		0					
2a		1.6	5.8		1.6	7.5	2.3 (C)	2.7	0.13	0.050	0.050	0.21	4.2	4.2
2b			>0[e]	3.0 (NC)		>0[e]	3.2 (NC)		>0[e]					
2c		1.6	18		1.9	24		3.5	7.1	0.050	0.042	0.17	3.4	4.0
2d		3.0	9.7		3.7	10		2.9	0.7	0.027	0.022	0.20	7.4	9.1
2e					5.4	2.7		4.2	0.4		0.015	0.14		9.3
2f	5.8 (C)		0[f]	4.7 (C)		0[f]	3.7 (NC)	7.5	0.04	0.014	0.017	0.077	5.5	4.5
3a	4.2 (NC)		0	[g]		0	[g]		0					
3b	11.4 (NC)		0	12.4 (NC)		0	3.1 (NC)		0					
4a	23 (NC)		0	45 (NC)		0	37 (NC)		0					
4b	21 (NC)		0	24 (NC)		0	15 (NC)		0					
5	3.6 (C)		>0[h]	4.8 (C)		>0[h]	4.1 (C)		>0[h]	0.022	0.017	0.14	6.4	8.2
6	0.94 (C)		>0[h]	0.63 (C)		>0[h]	1.7 (C)		>0[h]	0.085	0.13	0.34	4.0	2.6
7a	2.7 (C)		0	2.9 (C)		0	4.5 (C)		0	0.030	0.028	0.13	4.3	4.6
7b	2.7 (C)		0	2.5 (C)		0	6.5 (C)		0	0.030	0.032	0.089	3.0	2.8
7c	2.1 (C)		0	1.8 (C)		0	2.3 (C)		0	0.038	0.044	0.25	6.6	5.7
8	0.38 (C)		>0[h]	0.67 (C)		>0[h]	0.60 (C)		>0[h]	0.21	0.12	0.97	4.6	8.1
9a	9.8 (C)		>0[e]	10.0 (C)		>0[e]	16 (NC)		0[i]	0.0082	0.008			
9b	5.4 (C)		0[f]	5.4 (C)		0[f]	11 (NC)		0[f]	0.015	0.015			
9c	3.2 (C)		0[f]	3.8 (C)		0[f]	4.5 (NC)		0[f]	0.025	0.021			
10a		1.3	7	0.80 (C)	1.1	8	2.1 (C)	2.0	1.0	0.062	0.10	0.28	4.7	2.8
10b	2.4 (C-NC)	1.4	2.4	2.6 (C-NC)		>0[j]	0.91 (C)		>0[e]	0.057		0.64	11.2	
10c	2.1 (NC)	1.1	1.3	1.8 (NC)	0.83	0.82	1.40 (C)		>0[e]	0.073	0.096	0.41	5.6	4.3
11		2.5	11.2		2.2	21		4.2	2.2	0.032	0.036	0.14	4.4	3.9
12		1.6	12		1.4	13		2.0	2.1	0.050	0.057	0.29	5.8	5.1
13	[k]		0	[k]		0	52 (NC)		0					
14			0	0.73 (C)		0	5.7 (C)		0		0.11	0.12		1.1
15	[m]		0	[m]		0			0					
16		0.91	29		0.79	21		5.5	5.6	0.088	0.10	0.11	1.2	1.1

[a] In cases where values for both K_i for competitive inhibition and K_M were available, relative affinity was calculated from K_i. [b] C = competitive and NC = noncompetitive inhibition with respect to AMP. [c] K_M = concentration of substrate for half-maximal velocity (V_{max}). [d] No inhibition at 5 mM in the presence of 0.13 mM AMP. [e] AK-catalyzed formation of the nucleoside 5′-di- and triphosphates was observed with HPLC analysis. Substrate activity was not detected with the coupled enzyme assay. [f] No substrate activity was observed by HPLC analysis; for conditions see Experimental Section. [g] 1.3 mM 3a produced 15% inhibition in the presence of 0.65 mM AMP. [h] The initial velocity with 1 mM of the AMP derivative was 0.08–0.26% that with 1 mM AMP. [i] Substrate activity not detected by HPLC analysis using 6-fold more AK activity than in HPLC-analyzed substrate tests with AK II and III. [j] HPLC analysis indicated conversion of 10b to the corresponding 5′-diphosphate. [k] No inhibition by 6.2 mM 13 in the presence of 0.13 mM AMP. [m] No inhibition by 8.9 mM 15 in the presence of 0.13 mM AMP.

propanol–NH_4OH–H_2O, 7:1:2). Electrophoresis was carried out on Whatman No. 1 paper at pH 3.5 (0.05 M citrate). ^1H NMR spectra were obtained on a Perkin-Elmer 24B spectrometer, and chemical shifts are given as parts per million downfield from Me_4Si. Melting points are uncorrected. UV spectra were obtained on a Cary Model 15 spectrophotometer. Elemental analyses were performed by Galbraith Laboratories Inc., Knoxville, TN. Where analyses are indicated by the symbols of the elements, analytical results were within ±0.4% of the theoretical values. Prior to analysis, nucleotides were dried at 22 °C and nucleosides at 78 °C. High-pressure liquid chromatography was performed on a Waters Model 204 chromatograph equipped with a dual solvent-delivery system (Model M-6000 A) and a Model 660 programmer. Compounds were analyzed on a Waters RCM-100 unit containing a μ-Bondapak C_{18} column eluted at 3.3 mL/min with 0.1 M KH_2PO_4–0.025 M Bu_4NHSO_4 (pH 5) with a linear gradient of 10–30% MeOH over 20 min.

The nonfuranoid AMP analogue **15** was prepared from AMP as described[32] and purified on paper chromatograms run in *i*-PrOH–concentrated NH_4OH–H_2O (6:1:3); **15** had the same UV absorption properties as AMP and was homogeneous (mobility 0.77; AMP = 1.0) on paper electrophoretograms run in aqueous sodium tetraborate of pH 9.0. Adenosine 5′-sulfate and N^6-methyl-AMP were obtained from Sigma Chemical Co. Compound **3b** was obtained by acetylation of N^6-(2-aminoethyl)-AMP.[33] 8-Bromo-AMP was obtained by bromination of AMP[34] and, after purification by elution from Dowex 1 (formate) with 0–0.3 M formic acid, was free of AMP and other UV-absorbing impurities, as judged by HPLC. The phosphonates **11**[35] and **12**[36] were prepared as described. 8-(Ethylthio)-AMP (**4b**) was prepared from 8-bromo-AMP by the method used to convert 8-bromo-ATP to 8-(ethylthio)-ATP;[2] the disodium salt of **4b** had λ_{max} 282 nm (H_2O), ε 17 300 (calculated for a dihydrate). 2-Thioadenosine was prepared from adenosine N^1-oxide by a modification of the procedure of Kikugawa[37] in which cyclization with CS_2 was carried out in aqueous 86% MeOH instead of pyridine–MeOH. S-Methylation of 2-thioadenosine, followed by introduction of a 2′,3′-*O*-isopropylidene group, phosphorylation with β-cyanoethyl phosphate,[19] and deblocking gave the known 2-(methylthio)-AMP (**2b**).[38] 2-Chloro-AMP was prepared from 2-chloroadenosine as described;[7] the latter was obtained by conversion of 2,6-dichloropurine[39] to the corresponding ribonucleoside,[40] followed by amination.[41] 2-Amino-AMP (**2c**)[42] was obtained by phosphorylation[11] of 2-aminoadenosine and was purified by method B below. 2′-*O*-Methyl-AMP (**5**)[43] was obtained by the same procedures from 2′-*O*-methyladenosine.[9,10] **1** and **16** were obtained from P-L Biochemicals Inc. **13** was prepared as described.[44]

General Method for the Synthesis of 2-(Alkylamino)-adenosine 5′-Phosphates (2d–f). A solution of 2-chloroadenosine 5′-phosphate[7] (**2a**; 0.3 mmol) in an aqueous 40% solution of alkylamine (3.5 mL) was diluted with MeOH–water (1:1, 14 mL) and heated for 16 h in a stainless-steel bomb at 100 °C. HPLC showed that conversion of **2a** was complete. Volatiles were removed, and ethanol was evaporated from the residue to remove residual alkylamine. The product was chromatographed on a DEAE-cellulose column (4 × 20 cm). Elution with a linear gradient of 0–0.15 M $Et_3NH{\cdot}HCO_3$ (4 L) gave a triethylammonium salt of the product, which was dissolved in MeOH and treated with NaI, followed by acetone to precipitate the disodium salt.[45] The products were homogeneous in the systems of Table I. The UV spectral properties and R_f values in solvent A of **2d–e** agreed with those reported.[8] 2-(Dimethylamino)adenosine 5′-phosphate (**2f**): UV λ_{max} at pH 1, 261 nm (ε 16 300), 305 (8400); at pH 6–13, 262 nm (ε 12 800), 294 (8400). Anal. ($C_{12}H_{17}N_6O_7PNa_2{\cdot}0.5H_2O{\cdot}0.5MeOH$) C, H, N, P.

Conversion of Nucleosides to Nucleoside 5′-Phosphates. Method I. The 2′,3′-*O*-isopropylideneadenosine derivative (1 mmol) was phosphorylated by the method of Tener.[19] After addition of water to the reaction mixture, the filtered solution was evaporated to dryness, and the residue was treated with water (30 mL) and concentrated NH_4OH (22 mL) at 70 °C for 2 h. After filtration and evaporation of solvent, the residue was treated with trifluoroacetic acid–water (9:1, 15 mL) at 22 °C for 10 min. Solvent was evaporated under reduced pressure, and toluene–methanol (2:1, 2 × 30 mL) was evaporated from the residue. The residue was dissolved in cold MeOH–H_2O (1:1, 30 mL) and neutralized with Et_3N. Removal of solvent gave a product, which was purified by one of the two procedures given below.

Method II. The adenosine derivative (1 mmol) was phosphorylated as described,[11] and the mixture was poured into ice–water. The solution was kept at 5 °C for 1 h and then neutralized with 1 M $Et_3NH{\cdot}HCO_3$ at 5 °C, after which solvent was evaporated. The product usually contained relatively small amounts of byproducts as indicated by HPLC. It was purified by procedure A or B below.

Purification of Nucleoside 5′-Phosphates. Method A. The crude product was chromatographed in solvent B on Whatman No. 17 paper (four sheets, width 46 cm). Inorganic phosphate remained on the origin. The band containing the product was eluted with water, and the eluate was applied to a column of DEAE-cellulose (4 × 20 cm). The column was eluted with a linear gradient of 0.0–0.15 M $Et_3NH{\cdot}HCO_3$ (2 L + 2 L), and appropriate fractions were pooled and evaporated in vacuo. The residue was evaporated several times with ethanol. Addition of 1 M NaI–MeOH to a solution in MeOH of the residual triethylamine salt, followed by addition of acetone, precipitated the sodium salt of the product.

Method B. The crude product was dissolved in cold water (5 °C, 200 mL), and glacial acetic acid was added to bring the pH to 3.5. Partially inactivated charcoal (Sigma Chemical Co., HCl washed)[12] (3 g/mmol of nucleotide) and Celite (3 g) were added, and the mixture was stirred at 5 °C until 98% of the nucleotide had been adsorbed (1 h). The charcoal was collected by filtration and washed with water (500 mL). The nucleotide was desorbed (86–90% recovery) with aqueous 50% ethanol containing 3 mL of concentrated NH_4OH/L (300 mL). The extract was concentrated and chromatographed on DEAE-cellulose as described for procedure A.

3′-*O*-Methyladenosine 5′-Phosphate (6). 3′-*O*-Methyladenosine[9,10] was phosphorylated by method II and purified by procedure B to give **6** as its sodium salt, which was homogeneous by HPLC and paper chromatography: NMR (D_2O, 60 MHz) δ 3.45 (3, OCH_3-3′), 3.9–4.9 (m, 5, H-5′, H-4′, H-3′, H-2′), 6.0 (d, 1, J = 5.5 Hz, H-1′), 8.15 and 8.4 (s, 1, H-2 and H-8); UV λ_{max} (H_2O) 260 nm (ε 14 700). Anal. ($C_{11}H_{14}N_5O_7PNa_2{\cdot}2H_2O$) C, H, N, P.

2′,3′-*O*-*n*-Propylideneadenosine 5′-Monophosphate (7c). AMP (free acid, 2.5 mmol) was dissolved in a mixture of pyridine (100 mL), water (16 mL), and tri-*n*-butylamine (1.19 mL, 5 mmol). Solvents were evaporated in vacuo, and the residue was rendered

(32) (a) Schwartz, D. E.; Gilham. P. T. *J. Am. Chem. Soc.* **1972**, *94*, 8921. (b) Haar, F.; Schlimme, E.; Gomez-Guillen, M.; Cramer, F. *Eur. J. Biochem.* **1971**, *24*, 296.
(33) Hampton, A.; Patel, A. D.; Maeda, M.; Hai, T. T.; Chang, C.-D.; Kang, J. B.; Kappler, F.; Abo, M.; Preston, R. K. *J. Med. Chem.* **1982**, *25*, 373.
(34) Lee, C.-Y.; Kaplan, N. O. *Arch. Biochem. Biophys.* **1975**, *168*, 665.
(35) Jones, G. H.; Moffatt, J. G. *J. Am. Chem. Soc.* **1968**, *90*, 5337.
(36) Hampton, A.; Kappler, F.; Perini, F. *Bioorg. Chem.* **1976**, *5*, 31.
(37) Kikugawa, K.; Suehero, H. *J. Carbohydr. Nucleosides Nucleotides* **1975**, *2*, 159.
(38) Michal, F.; Maguire, M. H.; Gough, G. *Nature (London)* **1969**, *222*, 1073.
(39) Elion, G. B.; Hitchings, G. H. *J. Am. Chem. Soc.* **1956**, *78*, 3508.
(40) Jimoku, S.; Maruyama, A.; Sato, T. Japanese Patent 70 11 702 (1970); *Chem. Abstr.* **1970**, *73*, 35708*w*.
(41) Montgomery, J. A.; Hewson, K. *J. Heterocycl. Chem.* **1964**, *1*, 213.
(42) Kikugawa, K.; Iizuka, K.; Higuchi, Y.; Hirayama, H.; Ichino, M. *J. Med. Chem.* **1972**, *15*, 387.
(43) Tazawa, I.; Tazawa, S.; Alderfer, J. L.; Ts'o, P. O. P. *Biochemistry* **1972**, *26*, 4931.
(44) Moffatt, J. G.; Khorana, H. G. *J. Am. Chem. Soc.* **1961**, *83*, 649.
(45) Hoard, D. E.; Ott, D. G. *J. Am. Chem. Soc.* **1965**, *87*, 1785.
(46) Zemlicka, J. In "Synthetic Procedures in Nucleic Acid Chemistry"; Zorbach, W. W.; Tipson, R. S., Eds.; Wiley: New York, 1968; Vol. 1, p 202.

anhydrous by repeated evaporations of pyridine and dry DMF. To a suspension of this material in DMF (8 mL) was added triethyl orthoformate (1.247 mL, 7.5 mmol), propionaldehyde (0.54 mL, 7.5 mmol), and DMF–HCl[46] (5.08 M, 2.47 mL). The mixture was stirred at 22 °C, and the reaction was followed by cellulose TLC (solvent B) or electrophoresis in 0.05 M $Na_2B_4O_7$ buffer (pH 9). After 24 h, volatiles were removed in vacuo. The residue was dissolved in 2 N ethanolic ammonia (60 mL), and solvent was evaporated. The residue was applied to paper chromatograms (Whatman No. 17, 46 × 57 cm, 5 sheets) for development in solvent B. The product was eluted with water and applied to DEAE-cellulose (3 × 20 cm). Elution with a linear gradient of 0.0–0.15 M $Et_3NH{\cdot}HCO_3$ gave the triethylammonium salt of **7c** (0.63 mmol, 25%), which was converted to its sodium salt with NaI–MeOH: UV λ_{max} (H_2O) 260 nm (ϵ 14 900); NMR (D_2O) δ 1.0 (t, 3, J = 7 Hz, β-CH_3), 1.8 (m, 2, α-CH_2), 2.1 (acetone), 4.05 (broad, 2, H-5′), 4.7 (m, 1, masked by H_2O, H-4′), 5.2 (m, 3, H-2′, H-3′, H-acetal), 6.1 (d, 1, J = 3 Hz, H-1′), 8.0 and 8.3 (s, 1, H-2 and H-8). Anal. ($C_{13}H_{16}N_5O_7PNa_2{\cdot}3H_2O{\cdot}^1/_3Me_2CO$) C, H, N, P.

A solution of **7c** (20 mg) in Tris buffer (1 mL, pH 10) was treated with alkaline phosphatase (Sigma, type VII, 40 μL) overnight. The precipitate was collected by centrifugation, washed with water, and crystallized from methanol to give a product identical with **17** by HPLC, mp, and TLC on silica gel in $CHCl_3$–MeOH (10:1).

2′,3′-*O*-*n*-Propylideneadenosine (17). A suspension of adenosine (4 g, 15 mmol) in DMF (60 mL) and propionaldehyde (75 mL) was cooled in an ice bath, and to this was slowly added *p*-toluenesulfonic acid (28.5 g, 150 mmol). After dissolution was complete, the solution was stored at 22 °C for 7 h and then added to a solution of 2 N ethanolic ammonia (200 mL), and the mixture evaporated to dryness. A solution of the residue in $CHCl_3$–MeOH (8:2, 1 L) was washed with water (2 × 300 mL) and then dried (Na_2SO_4) and evaporated. The residue was chromatographed on a column (Merck silica gel 60, particle size 0.063–0.200 mm, 300 g); elution with a linear gradient of 0–5% MeOH in $CHCl_3$ (1 L + 1 L) gave material which was crystallized from MeOH to give 2 g of **17**: mp 198–199 °C; homogeneous on silica gel TLC in $CHCl_3$–MeOH (10:1.5), R_f 0.46; UV λ_{max} (MeOH) 260 nm; NMR (Me_2SO-d_6) δ 0.9 (t, 3, J = 7 Hz, β-CH_3), 1.70 (m, 2, α-CH_2), 3.35 (m, 2, H-5′; became d, $J_{5',4'}$ = 4.5 Hz at 3.55 when exchanged with D_2O), 3.55 (broad, 1, OH-5′, exchanged with D_2O), 4.25 (m, 1, H-4′), 4.85 (dd, 1, $J_{2',3'}$ = 6 Hz, $J_{3',4'}$ = 2 Hz, H-3′), 5.0 (t, 1, J = 5 Hz, acetal H), 5.25 (dd, 1, $J_{1',2'}$ = 6 Hz, $J_{2',3'}$ = 6 Hz, H-2′), 6.05 (d, 1, $J_{1',2'}$ = 3 Hz, H-1′), 7.3 (s, 2, exchanges with D_2O, NH_2), 8.15 and 8.30 (s, 1, H-2 and H-8). Anal. ($C_{13}H_{17}N_5O_7$) C, H, N.

4′-*C*-Prop-2-enyladenosine 5′-Monophosphate (8). N^6-Benzoyl-2′,3′-*O*-isopropylidene-4′-*C*-prop-2-enyladenosine was phosphorylated by method I and purified according to procedure A to give **8** in 50% yield as a sodium salt, which was homogeneous by HPLC and paper chromatography: UV λ_{max} (H_2O) 260 nm (ϵ 15 400); NMR (D_2O) δ 2.1 (s, acetone), 2.5 (d, 2, H-6′), 3.9 (d, 2, H-5′), 4.3 (d, 1, H-3′), 5.0 and 5.3 (m, 3, H-2′ and H-8′), 5.7 (m, 1, H-7′), 6.05 (d, 1, H-1′), 8.0 and 8.4 (s, 1, H-2 and H-8). Anal. ($C_{13}H_{16}N_5O_7PNa_2{\cdot}0.4H_2O{\cdot}0.3CH_3COCH_3$) C, H, N, P.

N^6-Benzoyl-2′,3′-*O*-isopropylidene-8,5′(*S*)-cycloadenosine (21). N^6-Benzoyl-2′,3′-*O*-isopropylideneadenosine (10.3 g, 25 mmol) was converted to the corresponding 5′-aldehyde with Me_2SO–DCC, and excess DCC was removed by reaction with oxalic acid as described.[21] Dicyclohexylurea was removed by filtration and washed with ethanol. Volatiles were removed in vacuo from the combined filtrate and washings, and the Me_2SO was filtered from dicyclohexylurea and then diluted with chloroform (200 mL). The solution was washed successively with saturated sodium bicarbonate (200 mL) and water (6 × 200 mL) and dried (Na_2SO_4), and the chloroform was evaporated. Last traces of water were removed from the residual 5′-aldehyde by distillation with dry benzene (200 mL), and the aldehyde was brought into reaction with MeMgCl as described.[21] The mixture of products was analyzed by TLC on silica gel in toluene–acetone (2:1) (three developments) and found to contain, among other components, **21** (R_f 0.68, 4% yield), **19a** (R_f 0.57), and **20a** (R_f 0.52) in a combined yield of 24% and a 2.5:1 ratio. The crude product was dissolved in $CHCl_3$–toluene–acetone (55:55:1) (70 mL) and filtered from dicyclohexylurea. The filtrate was applied to a column (Merck silica gel 60 H, 200 g, 5 × 29 cm) that was eluted with $CHCl_3$–toluene–acetone (55:55:20); 21-mL fractions were collected. Fractions 90–110 contained homogeneous **19a** (1.75 g), fractions 111–138 contained a mixture of **19a** and **20a** (0.8 g), and fractions 145–152 contained homogeneous **21** (0.24 g): UV λ_{max} (H_2O) 285 nm; mp 174–175 °C from toluene–acetone; NMR (Me_2SO-d_6) δ 1.22 and 1.42 (s, 3, CMe_2), 4.7 (m, 2, H-5′ and H-4′), 5.1 (m, 2, H-3′ and H-2′), 6.35 (s, 1, H-1′), 6.8 (broad, 1, OH-5′), 7.4–8.2 (m, 5, C_6H_5), 8.7 (s, 1, H-2), 10.5 (broad, 1, H-N^6). Anal. ($C_{20}H_{19}N_5O_5$) C, H, N.

A solution of **21** (300 mg) in MeOH–concentrated NH_4OH (1:1, 100 mL) was stored at 22 °C overnight and evaporated to dryness. The residue was subjected to preparative TLC on silica gel with $CHCl_3$–EtOH (100:15) as solvent. Elution with $CHCl_3$–EtOH (1:1) gave a product (90%) identical with 2′,3′-*O*-isopropylidene-8,5′(*S*)-cycloadenosine[22] by R_f on silica gel TLC in $CHCl_3$–EtOH (10:1), ^1H NMR, and UV λ_{max}. Acetylation gave a product identical with 2′,3′-*O*-isopropylidene-5′-*O*-acetyl-8,5′(*S*)-cycloadenosine by ^1H NMR and with mp 130–131 °C from EtOH–water (lit.[22] 132–133 °C).

9-(6-Deoxy-6-methyl-5-*O*-phosphoryl-β-D-allofuranosyl)adenine (9b) and 9-(6-Deoxy-6-methyl-5-*O*-phosphoryl-α-L-talofuranosyl)adenine (10b). N^6-Benzoyl-2′,3′-*O*-isopropylideneadenosine (5.13 g, 12.5 mmol) was converted to anhydrous 5′-aldehyde as described above, and to a solution of this in anhydrous THF (230 mL) was added EtMgCl in THF (2 M, 120 mL); the reaction was carried out, and the mixture worked up as in the preparation of **19a** and **20a**.[21] The mixture of products was chromatographed on a column (Merck silica gel 60 H, 150 g, 4 × 35 cm) using toluene–acetone (6:1) as eluant; 20-mL fractions were collected. The products **19b** (650 mg) and **20b** (130 mg) were collected in fractions 180–194 and 205–220, respectively, and were obtained as glasses upon evaporation of solvents. Fractions 195–204 contained a mixture of **19b** and **20b** (80 mg). **19b**: UV λ_{max} (MeOH) 280, 230 nm (sh); NMR ($CDCl_3$) δ 1.1 (t, 3, J = 7 Hz, H-7′), 1.5 (m, 2, partially obscured by CMe_2, H-6′), 1.35 and 1.65 (s, 3, CMe_2), 3.8 (m, 1, H-5′), 4.35 (m, 1, H-4′), 5.1 (m, 2, H-3′ and H-2′), 5.7 (broad, 1, OH-5′), 5.95 (d, 1, J = 3.5 Hz, H-1′), 7.5–8.2 (m, 5, C_6H_5), 8.1 and 8.75 (s, 1, H-2 and H-8), 9.3 (broad, 1, H-N^6). **20b**: UV λ_{max} (MeOH) 280 and 230 nm (sh); NMR ($CDCl_3$) δ 1.0 (t, 3, J = 7 Hz, H-7′), 1.5 (m, 2, partially obscured by CMe_2, H-6′), 1.45 and 1.70 (s, 3, CMe_2), 3.75 (m, 1, H-5′), 4.45 (broad, 1, H-4′), 5.2 (m, 2, H-2′ and H-3′), 5.6 (broad, 1, OH-5′), 6.05 (d, 1, J = 3.5 Hz, H-1′), 7.6–8.1 (m, 5, C_6H_5), 8.2 and 8.75 (s, 1, H-2 and H-8), 9.5 (broad, 1, H-N^6).

Phosphorylation of **20b** was carried out by method I, and the product was purified by method A to give **10b** as a disodium salt that was homogeneous by HPLC and paper chromatography: UV λ_{max} (H_2O) 260 nm (ϵ 15 100); NMR (D_2O) δ 1.0 (t, 3, J = 7 Hz, H-7′), 1.8 (m, 2, H-6′), 4.0–4.9 (m, 4, masked by D_2O, H-5′, H-4′, H-3′, H-2′), 6.05 (d, 1, J = 5 Hz), 8.05 and 8.45 (s, 1, H-2 and H-8).

Phosphorylation of **19b** (1 mmol) by method I and purification of the product by method A gave **9b** as a disodium salt that was homogeneous by HPLC and paper chromatography: UV λ_{max} (H_2O) 260 nm (ϵ 14 900); NMR (D_2O) δ 1.0 (t, 3, J = 7 Hz, H-7′), 1.8 (m, 2, H-6′), 4.0–4.9 (m, 4, masked by D_2O, H-5′, H-4′, H-3′, and H-2′), 6.0 (d, 1, J = 5.5 Hz, H-1′), 8.0 and 8.4 (s, 1, H-2 and H-8). Anal. ($C_{12}H_{16}N_5O_7PNa_2{\cdot}2.7H_2O$) C, H, N, P.

9-(6-Deoxy-6-ethyl-5-*O*-phosphoryl-β-D-allofuranosyl)adenine (9c). N^6-Benzoyl-2′,3′-*O*-isopropylideneadenosine (10.3 g, 25 mmol) was oxidized to the crude 5′-aldehyde as given above, and this was treated with *n*-PrMgCl under conditions used for the preparation of **19b** and **20b**. The crude product, which contained **19c** and **20c** in a 5:1 ratio, was dissolved in toluene/acetone (6:1, 70 mL), and the solution was filtered from dicyclohexylurea and applied to a column (Merck silica gel 60 H, 200 g, 5 × 29 cm). This was eluted with toluene–acetone (6:1) and 20-mL fractions were collected; **19c** (0.8 g) contaminated with a small amount of an unknown byproduct was eluted in fractions 151–189. A mixture of **19c** and **20c** (1.2 g) was eluted in fractions 190–260. **19c**: UV λ_{max} (MeOH) 280 nm (ϵ 18 850), 230 sh (12 570); mp 95–96 °C from toluene. Anal. ($C_{23}H_{27}N_5O_5$) C, N, H.

Phosphorylation of **19c** by method I and purification of **9c** by method A gave the disodium salt of **9c**, which was homogeneous by HPLC: UV λ_{max} (H_2O) 260 nm (ϵ 14900). Anal. ($C_{13}H_{18}N_5O_7PNa_2 \cdot 3.5H_2O$) C, H, N, P.

9-(6-Deoxy-6-ethyl-5-*O*-phosphoryl-α-L-talofuranosyl)-adenine (10c). A mixture of **19c** and **20c** (1.4 g, 3.09 mmol) containing principally **19c** was oxidized with Me_2SO–DCC, and the product was immediately reduced with $NaBH_4$ by the procedure described[21] for the preparation of **10a** to give a mixture of **19c** and **20c** in a ratio of ca. 1:2.3 This was applied to a column (Merck silica gel 60 H, 100 g, 4 × 22 cm). Elution with $CHCl_3$–toluene–acetone (10:10:3) gave **19c** (300 mg), **20c** (690 mg), and a mixture of **19c** and **20c** (100 mg). **20c**: UV λ_{max} (MeOH) 280, 230 nm (A_{280}/A_{230} = 1.48); NMR ($CDCl_3$) δ 0.8 (m, 3, H-8′), 1.3 (m, masked by CMe_2, H-7′ and H-6′), 1.25 and 1.5 (s, 3, CMe_2), 3.7 (m, 1, H-5′), 4.25 (broad, 1, H-4′), 5.0 (m, 2, H-3′ and H-2′), 5.9 (d, 1, J = 4 Hz, H-1′), 7.4–7.9 (m, 5, C_6H_5), 8.0 and 8.6 (s, 1, H-2 and H-8), 9.4 (broad, 1, H-N^6).

Compound **20c** was phosphorylated by method I. Purification by method A gave **10c** as a disodium salt, which was homogeneous by HPLC and paper chromatography: UV λ_{max} (H_2O) 260 nm (ϵ 15200). Anal. ($C_{13}H_{18}N_5O_7PNa_2 \cdot 2.8H_2O$) C, H, N, P.

Enzyme Kinetic Studies. Adenosine 5′-monophosphate, adenosine 5′-triphosphate, lactate dehydrogenase (type II, rabbit muscle), and phosphoenolpyruvate were from Sigma Chemical Co., rabbit muscle pyruvate kinase was from Boehringer Mannheim, and NADH was from PL Biochemicals. Preparations of the three rat isozymes of AK were obtained as described previously.[2]

The enzyme-catalyzed reactions were followed at 23 °C by measuring the rate of change of optical density at 340 nm for a period of 5 min in a Cary Model 15 spectrophotometer using 1-cm cells containing a final volume of 1 mL. Initial velocities were linear and proportional to the concentration of primary enzyme and independent of the level of secondary enzymes in the assay system. The assays contained 0.1 M Tris-HCl (pH 7.6), $MgSO_4$ (2 mM), KCl (0.12 M), PEP cyclohexylammonium salt (0.3 mM), NADH (0.38 mM), pyruvate kinase (8.6 units), and lactate dehydrogenase (8.6 units). In studies with AK II and III, 0.35 mM MgATP (3.9 × K_M of ATP) was present, and in studies with AK-M, 2.0 mM MgATP (3.5 × K_M of ATP) was present. Levels of MgATP higher than 2.0 mM were inhibitory to AK-M. The K_M values of AMP, **2a**, **2c**, and **2d** with AK-M were independent of MgATP level in the range 0.5–2.0 mM. Stock solutions of ATP contained an equimolar amount of $MgSO_4$.

Substrate constants were determined from double-reciprocal plots of velocity vs. substrate level, all of which were linear. Five or more levels of substrate were employed. AMP derivatives (1 mM) were tested for possible substrate activity for up to 0.5 h with a level of enzyme activity that was 20-fold higher than in assays in which AMP was the substrate. All compounds for which K_M values are reported produced ADP derivatives during the kinetic determinations which were substrates of the pyruvate kinase present in the assay mixture, and these ADP derivatives contributed equally with the ADP itself to the rate of formation of pyruvic acid that was measured by the lactate dehydrogenase present in the assay mixture. Substrate properties with muscle pyruvate kinase of the 5′-diphosphates arising from **11** and **12** have been published;[36] substrate properties of the remaining ADP derivatives will be reported separately.[47]

Substrate studies by means of HPLC analysis were carried out at 22 °C in mixtures of the AMP derivative (1 mM), 0.1 M Tris-HCl (pH 7.6), $MgSO_4$ (2 mM), and KCl (0.12 M) containing 1 mM MgATP. In the case of **2f**, AK II or III sufficient to produce 20-fold higher activity than in the above spectrophotometric assay was added each day for 4 days, while with **9b–c** enough AK II or III to produce a 10-fold higher activity level was added on 2 successive days (Table II, footnote *f*). With **9a** the AK II or III level was 60 times more than in the normal assay following three additions of enzyme over 26 h. Analyses for the AK-catalyzed formation of ADP derivatives were performed using a μ-Bondapak C_{18} column under conditions given above.

Inhibition studies used five or more levels of AMP in the range of 0.5–4.0 × K_M for each of two inhibitor levels that were in the range 1–4 × K_i. Inhibition constants (K_i values) were obtained from replots of inhibitor concentrations vs. slopes of double-reciprocal plots of velocity vs. substrate level.

Acknowledgment. This work was supported by Public Health Service Research Grant CA-11196 from the National Cancer Institute and by grants to the Institute for Cancer Research (USPHS Grants CA-06927 and RR-05539 and an appropriation from the Commonwealth of Pennsylvania).

(47) Hai, T. T.; Abo, M.; Hampton, A., unpublished results.