free base was isolated as for 13. The product was crystallized from acetone, and the hydrochloride was prepared: yield 0.98 g (70%); mp 105 °C; UV (H_2O) λ_{max} 331 nm (ϵ 5720); ^1H NMR ($CDCl_3$) δ 8.56 (1 H, s, 1-CH), 8.4–8.2 (4 H, m, 3-CH, 4-CH, 5-CH, 8-CH), 7.85–7.7 (2 H, m, 6-CH, 7-CH), 3.62–3.45 (2 H, q, CH_2), 2.76–2.5 (6 H, m, NCH_2), 1.15–1.10 (6 H, t, CH_3); CIMS (ammonia), m/e 352 (M^+ +2), 351 (M^+ +1). Anal. ($C_{21}H_{22}N_2O_3$) C, H, N, Cl.

Compounds 23 and 24 were prepared in an analogous manner in yields of 69 and 73%, respectively.

23: mp 100–102 °C; UV (H_2O) 331 nm (ϵ 5293); ^1H NMR ($CDCl_3$) δ 8.7 (1 H, s, 1-CH), 8.5–8.38 (4 H, m, 3-CH, 5-CH, 8-CH), 7.96–7.85 (2 H, m, 6-CH, 7-CH), 3.8–3.6 (2 H, q, CH_2), 2.75–2.62 (2 H, t, CH_2), 2.5 (6 H, s, CH_3), 2.45–2.25 (1 H, br s, replaceable with D_2O, NH), 1.95–1.8 (2 H, t, CH_2); MS, m/e 336 (M^+). Anal. ($C_{20}H_{20}N_2O_3$·HCl) C, H, N, Cl.

24: mp 90–93 °C; UV (H_2O) λ_{max} 330 nm (ϵ 5687); ^1H NMR ($CDCl_3$) δ 8.55 (1 H, s, 1-CH) 8.4–8.2 (4 H, m, 3-CH, 4-CH, 5-CH, 8-CH), 7.88–7.74 (2 H, m, 6-CH, 7-CH), 4.32–4.1 (1 H br s, replaceable with D_2O, NH), 2.68–2.4 (7 H, NCH_2 and methine), 1.75–1.6 (4 H, m, CH_2), 1.36–1.28 (3 H, d, CH_3), 1.1–0.92 (6 H, t, CH_3); CIMS (ammonia), m/e 394 (M^+ +2), 393 (M^+ +1). Anal. ($C_{24}H_{28}N_2O_3$·HCl) C, H, N, Cl.

DNA Binding. Solutions were prepared in pH 7.0, 0.05 M NaCl, 0.008 M Tris-Cl buffer and for compounds 18–21 also in pH 5.8, Sorensen's citrate II buffer. Calf thymus DNA (Sigma Type I) in buffer (1 mg mL^{-1}) was assayed using the figure $\epsilon(P)_{260}$ = 6600. All compounds obeyed Beer–Lambert's law over the concentration range used. All glassware was silanized before use.

(a) Determination of Spectral Shifts. Six solutions of drug (2.5×10^{-5} M) and DNA were prepared with DNA/drug ratios of 0, 1, 2, 5, 10, and 15. The spectra for each drug were recorded superimposed.

(b) Effect of pH on the Drug λ_{max} in the Presence and Absence of DNA. Solutions were prepared containing just drug (2.5×10^{-5} M) and drug and DNA (2.5×10^{-5} and 3.75×10^{-4} M, respectively) in pH 7.0 and in pH 9.3 buffer (pH 5.8 and 7.8 for 18–21). The spectra for each compound were recorded superimposed.

(c) Spectrophotometric Titration. Aliquots (4×40, 12×20, and 7×100 μL) of DNA solution (2.5×10^{-3} M) were added sequentially to three samples of drug solution (5×10^{-5} M) in pH 7.0 buffer (3.0 mL) at 25 °C (pH 5.8 buffer for compounds 18–21), and the absorbance at the λ_{max} of the unbound drug was determined after a 5-min equilibration against a blank of buffer treated in an identical manner. The binding parameters K_1 and n_1 were then determined for a one-site binding model and, if necessary, for a two-site binding model by nonlinear regression of absorbance (as the dependent variable) against cumulative volume of DNA added (as independent variable) by the method previously reported.[28]

(d) Spectrofluorimetric Studies. For compounds 13–17, aliquots of DNA solution were added sequentially to three samples of drug solution (2.5×10^{-6} M in pH 7.0 buffer) at 25 °C, and the fluorescence polarization was determined at the λ_{max} of emission for the unbound drug when irradiated with polarized light at the λ_{max} of excitation for the unbound drug. The usual correction was applied to account for spurious polarization due to the instrument. The fluorescence polarization titrations were continued until ratios of DNA to drug were reached at which the drug is fully bound. The change (percent) in fluorescence was also calculated at these DNA/drug ratios. Additionally, polarization values at DNA/drug ratios of 50, 100, and 150 were determined.

(e) Thermal Denaturation. Two solutions with an identical concentration of DNA (about 15×10^{-5} M) were prepared in 0.018 M NaCl, 0.003 M Tris-chloride buffer at pH 7.0, one just containing DNA and the other also containing drug (about 1.5×10^{-5} M) such that the ratio of drug to DNA was precisely 1:10. The absorbance of each solution was measured at 260 nm, as the temperature was raised from 55 to 105 °C (0.25 °C min^{-1}), against a blank containing GMP, the absorbance of which was exactly matched to the starting absorbance of the sample.

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Use of Adenine Nucleotide Derivatives to Assess the Potential of Exo-Active-Site-Directed Reagents as Species- or Isozyme-Specific Enzyme Inactivators. 3.[1] Synthesis of Adenosine 5′-Triphosphate Derivatives with N^6- or 8-Substituents Bearing Iodoacetyl Groups

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Several series of N^6- or 8-substituted derivatives of adenosine 5′-triphosphate (ATP) were synthesized. N^6-(ω-Aminoalkyl) derivatives of adenosine 5′-monophosphate (AMP) were converted into their ω-*N*-carbobenzyloxy derivatives, and these were converted, via the 2′,3′-*O*-carbonyl derivatives of their 5′-phosphorimidazolidates, into the corresponding ATP derivatives. Hydrogenolytic removal of the carbobenzyloxy groups, followed by iodoacetylation of the ω-amino groups with *N*-(iodoacetoxy)succinimide, gave N^6-R-ATP, where R = $(CH_2)_nNHCOCH_2I$ (n = 2–8) or $(CH_2)_nCON(CH_3)(CH_2)_mN(CH_3)CO(CH_2)_nNHCOCH_2I$ ($n = m = 3$; $n = 3$, $m = 4$; $n = 4$, $m = 3$; $n = m = 4$). Condensation of N^6-(ω-aminoalkyl) derivatives of AMP with *N*-hydroxysuccinimide esters of ω-[*N*-(carbobenzyloxy)amino] carboxylic acids gave N^6-$(CH_2)_nNHCO(CH_2)_mNH$-Cbz derivatives of AMP which, upon conversion to the corresponding derivatives of ATP, followed by removal of the carbobenzyloxy group and iodoacetylation, as described above, gave N^6-$(CH_2)_nNHCO(CH_2)_mNHCOCH_2I$-ATP derivatives ($n$ = 3, m = 5 or 6; n = 4, m = 5; n = 6, m = 1–6). The same sequence of reactions starting with N^6-[ω-(methylamino)alkyl] derivatives of N^6-CH_3-AMP gave N^6-CH_3, N^6-$(CH_2)_nN(CH_3)CO(CH_2)_mNHCOCH_2I$ derivatives of ATP (n = 4, m = 3, 5 or 6; n = 6, m = 5 or 6). Reaction of α,ω-diaminoalkanes with 8-Br-ATP gave 8-$NH(CH_2)_nNH_2$ derivatives of ATP, which upon iodoacetylation gave 8-$NH(CH_2)_nNHCOCH_2I$ derivatives of ATP (n = 2, 4, 6, or 8). Substrate and inhibitor properties indicated that the ATP derivatives are potential exo-ATP-site-directed inactivators of hexokinases, adenylate kinases, and pyruvate kinases.

It is now recognized that enzymes which catalyze the same transformation, whether they be derived from different species or are isozymes characteristic of different tissues of the same species, exhibit structural dissimilarities

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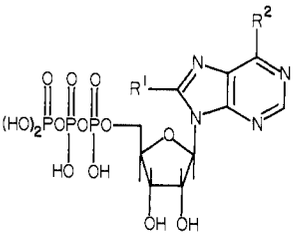
from each other which occur most frequently in regions other than the enzymatic active sites. This led Baker[2-4] to propose that it might be feasible systematically to design species- or isozyme-specific enzyme inactivators through studies of substrate derivatives which are able to bind to a substrate site of the target enzyme and which have the potential subsequently to form a covalent bond with an amino acid residue outside that site by means of a substituent bearing an electrophilic group. Early studies of Baker with this approach led in 1964 to several compounds which selectively inactivated either rabbit muscle or beef heart lactate dehydrogenase, two enzymes in which a species difference is combined with an isozymic difference.[5] Subsequently, however, the approach has produced well-documented examples of only one isoenzyme-specific exo-active-site-directed reagent[1] and of only two species-specific exo-active-site-directed reagents.[6,7] Baker's group reported apparent selective inactivation of dihydrofolate reductase activity in mouse L1210 leukemia cells in comparison with the same activity in a normal mouse tissue,[8] but evidence for the existence of two separate isozymes was lacking, and the nature of the inactivation was not elucidated by kinetic or other studies. In addition, Baker and co-workers reported an instance of apparent species-selective enzyme inactivation involving rat liver and rabbit liver guanine deaminases,[9] but the characteristics of this effect were likewise not delineated.

We have undertaken a series of studies directed at assessing the tendency of exo-active-site-directed reagents of the above type to act as species- or isozyme-specific enzyme inactivators in cell-free systems. The overall approach adopted in this assessment arose from reports that attachment of substituents to N^6 of the adenine nucleotides ADP and ATP did not prevent specific binding from taking place at the ATP sites of certain phosphotransferases, including adenylate kinase, pyruvate kinase, and hexokinase.[10] We decided to synthesize a series of N^6-monosubstituted ATP derivatives bearing an electrophilic group on the N^6-substituent (**1–3**, R′ = H) and to evaluate them as potential irreversible inhibitors (inactivators) of three known rat isozymes of adenylate kinase, four rat isozymes of hexokinase, and three rat isozymes of pyruvate kinase. To these test enzymes we added a bacterial adenylate kinase, a yeast hexokinase, and a bacterial thymidine kinase which we had found could bind N^6-substituted ATP derivatives at its ATP site. We also obtained evidence, reported in this paper, that N^6-alkyl substituents attached to N^6-Me-ATP permit binding to occur at ATP sites of isozymes of adenylate kinase, pyruvate kinase, and hexokinase. Several N^6,N^6-disubstituted compounds of this type (**3**, R′ = CH_3) were, hence, included in the survey. In addition, some 8-substituted ATP derivatives (structure **4**) were synthesized in light of



1, R^1 = H; R^2 = $NH(CH_2)_nNHCOCH_2I$
2, R^1 = H; R^2 = $NH(CH_2)_nCON(CH_3)(CH_2)_mN(CH_3)CO(CH_2)_nNHCOCH_2I$
3, R^1 = H; R_2 = $NR'(CH_2)_nNR'CO(CH_2)_mNHCOCH_2I$ (R′ = H or CH_3)
4, R^1 = $NH(CH_2)_nNHCOCH_2I$; R^2 = NH_2

evidence that 8-substituents do not prevent binding to some adenylate kinase isozymes[11] or to yeast hexokinase, as reported herein. Adenine nucleotide derivatives were employed in the present assessment of exo-active-site-directed reagents as potential species- or isozyme-specific enzyme inactivators primarily because of the practical advantage that each potential enzyme inactivator that was synthesized could be studied with a relatively large number of enzymes.

The previous papers in this series described interactions of several N^6-iodoacetamidoalkyl derivatives of ATP (**1**, n = 5–8) with muscle adenylate kinases of three species[7] and interactions of analogous derivatives of ADP (n = 2–8) with rat pyruvate kinase isozymes.[1] The present report describes an improved route for synthesis of the N^6-substituted ATP derivatives **1** (n = 2–8; 5–11 spacer atoms between N^6 and iodine), together with routes devised for syntheses of ATP derivatives of the structural types **2** and **3** that possess longer N^6 substituents (16–19 and 12–17 spacer atoms, respectively). Syntheses of 8-[(iodoacetamido)alkyl]amino derivatives (**4**) of ATP are also described. Amide linkages were introduced into the substituents of **2** and **3** in order to confer sufficient solubility in water for subsequent studies of the interaction of as much as 5–10 mM levels of these compounds with the test enzymes. Because of the tendency of amide linkages in peptides to form inter- and intramolecular hydrogen bonds with each other and because of the relatively rigid nature of amide linkages under physiological conditions, N-methyl amide linkages were introduced into compounds **2** and a number of the compounds **3** in order to promote substituent flexibility while maintaining sufficient solubility in water. The highly reactive iodoacetyl group was selected as the electrophile in the ATP derivatives **1–4** because the ATP derivatives **1** (n = 5–8) had been found to be adequately stable[7] and because, for the purposes of the present study, it was desirable to maximize the probability of observing enzyme inactivations with the test compounds. Compounds **1–4**, like iodoacetamide, have the potential to alkylate lysine, histidine, cysteine, tyrosine, methionine, glutamate, and aspartate residues in enzymes.

Chemical Syntheses. 6-Chloro-9-β-D-ribofuranosylpurine was synthesized from 2′,3′,5′-tri-*O*-acetylinosine by the method of Zemlicka and Sorm[12] and was treated with phosphorus oxychloride containing 6% water (v/v)[13] to give 6-chloro-9-β-D-ribofuranosylpurine 5′-phosphate (**5**).

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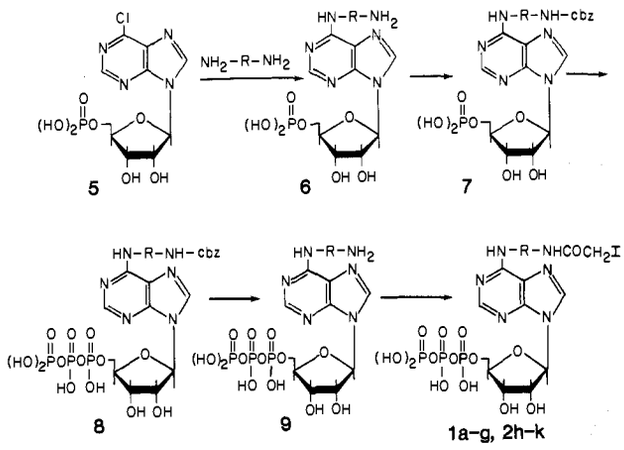
Scheme I

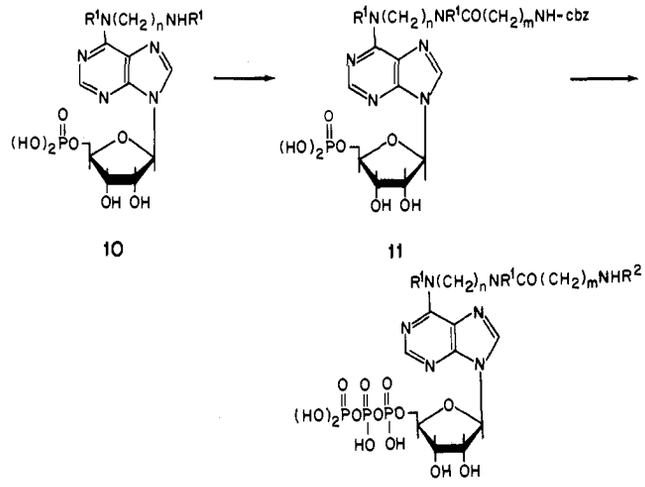


a series, R = $(CH_2)_2$
b series, R = $(CH_2)_3$
c series, R = $(CH_2)_4$
d series, R = $(CH_2)_5$
e series, R = $(CH_2)_6$
f series, R = $(CH_2)_7$
g series, R = $(CH_2)_8$
h series, R = $(CH_2)_3CON(CH_3)(CH_2)_3N(CH_3)CO(CH_2)_3$
i series, R = $(CH_2)_3CON(CH_3)(CH_2)_4N(CH_3)CO(CH_2)_3$
j series, R = $(CH_2)_4CON(CH_3)(CH_2)_3N(CH_3)CO(CH_2)_4$
k series, R = $(CH_2)_4CON(CH_3)(CH_2)_4N(CH_3)CO(CH_2)_4$

The N^6-(ω-aminoalkyl)adenosine 5′-phosphates **6a–k** (Scheme I) and the N^6-methyl-N^6-[ω-(methylamino)alkyl]adenosine 5′-phosphates **10j,q,s** (Scheme II) were obtained by treating **5** for 6 h at 80 °C in aqueous solution (pH 7) with 20 molecular equiv of the appropriate α,ω-diaminoalkane; the conditions used were based on the reported conversion of **5** to N^6-(6-aminohexyl)adenosine 5′-phosphate.[13] The four diamines required for the synthesis of **6h–k** were prepared by condensing 1,3- or 1,4-bis(methylamino)alkanes with 2 equiv of the *N*-hydroxysuccinimide ester of the appropriate *N*-carbobenzyloxy-ω-amino carboxylic acid and subsequently removing the two carbobenzyloxy groups of the product by hydrogenation. Compounds **6a–k**, and **10j,q,s** were isolated in the free acid form in 50–60% overall yields from 6-chloropurine ribonucleoside after elution with acetic acid from a Dowex-1 (acetate) column. Compounds **6a–k** possessed the UV maximum at pH 7 of 267 nm characteristic of N^6-alkyladenosines,[14] and compounds **10j,q, s** had a maximum at 277 nm similar in wavelength and magnitude to that of other N^6,N^6-dialkyladenosines.[15] In further agreement with their assigned structures, **6a–k** and **10j,q,s** migrated as monoanions on paper electrophoretograms run at pH 7.6 and produced in each case a single UV-absorbing component which reacted positively toward ninhydrin spray.

Treatment of **6a–k** in aqueous sodium hydroxide with benzylchloroformate produced the N^6-[ω-[(carbobenzyloxy)amino]alkyl]adenosine 5′-phosphates **7a–k**, which were obtained in 63–82% yields as their homogeneous triethylammonium salts after elution from a column of DEAE-cellulose with aqueous triethylammonium bicarbonate. These products possessed the same UV spectra as **6a–k** and migrated on paper electrophoretograms run at pH 7.6 as ninhydrin-negative dianions, thus confirming attachment of the carbobenzyloxy group to the aliphatic amino group. The triethylammonium salts of **7a–k** were converted, via their phosphorimidazolidates, to the corresponding N^6-substituted derivatives of ATP, **8a–k**,

Scheme II



Series	R^1	n	m	Series	R^1	n	m
a	H	3	5	h	H	6	5
b	H	3	6	i	H	6	6
c	H	4	5	j	CH_3	4	3
d	H	6	1	k	CH_3	4	5
e	H	6	2	p	CH_3	4	6
f	H	6	3	q	CH_3	6	5
g	H	6	4	r	CH_3	6	6
				s	CH_3	3	

utilizing minor modifications of the method of Hoard and Ott[16] for the conversion of 2′-deoxynucleoside 5′-phosphates to 2′-deoxynucleoside 5′-di or -triphosphates. The products arising from the Hoard–Ott procedure were treated at room temperature with triethylamine in aqueous methanol to remove the 2′,3′-*O*-carbonyl groups that are introduced into ribonucleotides under these conditions.[17] The N^6-[ω-[(carbobenzyloxy)amino]alkyl]adenosine 5′-triphosphates **8a-k** were purified by anion-exchange chromatography on DEAE-cellulose and isolated as their tetrasodium salts in 58-75% yields. They were homogeneous as judged by UV extinction coefficient and by paper chromatography and electrophoresis. Compounds **8a–k** were hydrogenated in aqueous methanol in the presence of a palladium catalyst to remove the carbobenzyloxy groups. The reactions, which were conveniently monitored by means of paper electrophoresis at pH 3.5, furnished the corresponding N^6-(ω-aminoalkyl)adenosine 5′-triphosphates **9a–k** as their homogeneous trisodium salts in yields in excess of 90%. We found in earlier work[7] that *N*-(iodoacetoxy)succinimide is a satisfactory reagent for selective iodoacetylation of the aliphatic amino groups of the AMP derivatives **6d–g**. When the ATP derivatives **9a–k** were treated with this reagent at room temperature in aqueous 2-methoxyethanol, they were rapidly converted into the N^6-[ω-(iodoacetamido)alkyl]adenosine 5′-triphosphates **1a-g** and **2h-k**. These were purified by anion-exchange chromatography on DEAE-cellulose and isolated in 68–81% yields as their tetrasodium salts, which were homogeneous as judged by paper chromatography and electrophoresis, by UV extinction coefficient, and by phosphate and elemental analysis (Table I). Compounds

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Table I. Physical Properties of the ADP and ATP Derivatives 1-4, 15, and 17-19

no.	yield, %	UV λ_{max} (H_2O),[a] nm ($\epsilon \times 10^{-3}$)	electrophoresis:[b] pH 3.5	paper chromatography[c] R_f (A)	R_f (B)	R_f (C)	R_f (D)	R_f (E)	phosphate/base ratio[d]	formula	anal.
1a	68	268 (17.2)	1.94	0.32					3.02	$C_{14}H_{18}IN_6O_{14}P_3Na_4 \cdot 1.5CH_3OH$	C, H
1b	75	268 (17.6)	1.92	0.38					2.96	$C_{15}H_{20}IN_6O_{14}P_3Na_4 \cdot 3.5H_2O$	C, H
1c	69	268 (17.2)	1.92	0.43					3.01	$C_{16}H_{22}IN_6O_{14}P_3Na_4 \cdot 1.75H_2O$	C, H
1d[e]	81	268 (17.2)	1.90	0.50					3.05	$C_{17}H_{24}IN_6O_{14}P_3Na_4 \cdot 2.5H_2O$	C, H, N, P; I[f]
1e[e]	80	268 (17.3)	1.90	0.56							
1f[e]	73	268 (17.3)	1.88	0.62					3.08		
1g[e]	75	268 (17.1)	1.88	0.67							
2h	71	268 (17.3)[g]	1.65	0.33	0.25						
2i	68	268 (17.1)	1.63	0.36	0.33				3.09	$C_{26}H_{40}IN_8O_{16}P_3Na_4 \cdot H_2O$	C, H, N, P, I
2j	79	268 (17.2)[g]	1.62	0.38	0.37						
2k	77	268 (17.2)[g]	1.61	0.41	0.40						
3a	76	268 (17.1)	1.69	0.15		0.53				$C_{21}H_{31}IN_7O_{15}P_3Na_4$	C, H, N, P, I
3b	68	268 (17.0)	1.60	0.18		0.62					
3c	89	268 (17.6)	1.51	0.17	0.52				2.98	$C_{22}H_{33}IN_7O_{15}P_3Na_4 \cdot H_2O$	C, H, N, P, I
3d	60	268 (17.6)	1.59	0.14		0.63					
3e	82	268 (17.4)	1.55	0.15		0.69				$C_{21}H_{31}IN_7O_{15}P_3Na_4$	C, H, N, P, I
3f	75	268 (17.3)	1.46	0.22		0.76				$C_{22}H_{33}IN_7O_{15}P_3Na_4$	C, H, N, P, I
3g	65	268 (17.2)	1.37	0.26		0.84					
3h	68	268 (17.5)	1.33	0.31		0.91					
3i	70	268 (17.5)	1.25	0.38	0.53	0.98			2.94	$C_{25}H_{39}IN_7O_{15}P_3Na_4$	C, H, N, P, I
3j	82	276 (19.8)	1.74	0.51						$C_{22}H_{33}IN_7O_{15}P_3Na_4 \cdot 3H_2O$	C, H, N, P, I
3k	67	277 (20.2)[g]	1.72	0.20	0.53				3.04		
3p	78	277 (20.2)[h]	1.62	0.21	0.52				3.02		
3q	89	277 (20.5)[i]	1.52	0.26	0.51				3.07		
3r	85	277 (20.4)	1.49	0.28	0.52				3.03	$C_{27}H_{43}IN_7O_{15}P_3Na_4 \cdot 3H_2O$	C, H, N, P, I
4a	45	279 (16.8)	2.17				0.33	0.42		$C_{14}H_{19}IN_7O_{14}P_3Na_4 \cdot H_2O$	C, H, N, P, I
4b	53	280 (16.9)	1.82				0.40	0.44		$C_{16}H_{23}IN_7O_{14}P_3Na_4 \cdot 2H_2O$	C, H, N, P, I
4c	55	280 (18.0)	1.69				0.49	0.49		$C_{18}H_{27}IN_7O_{14}P_3Na_4 \cdot 2H_2O$	C, H, N, P, I
4d	48	280 (17.5)	1.57				0.58	0.53		$C_{20}H_{31}IN_7O_{14}P_3Na_4 \cdot 2H_2O$	C, H, N, P, I
15a	41	279 (17.0)	1.64				0.17	0.38	2.96	$C_{12}H_{19}N_7O_{13}P_3Na_3 \cdot 2.5H_2O$	C, H, P; N[j]
15b	56	280 (16.8)	1.25				0.20	0.32	3.02	$C_{14}H_{23}N_7O_{13}P_3Na_3 \cdot 5H_2O$	C, H, N, P
15c	47	280 (17.0)	1.09				0.30	0.25	3.04	$C_{16}H_{27}N_7O_{13}P_3Na_3 \cdot 2H_2O$	C, H, N, P
15d	60	280 (17.2)	0.98				0.38	0.34	2.95	$C_{18}H_{31}N_7O_{13}P_3Na_3 \cdot 2H_2O$	C, H, N; P[k]
17	68	277 (20.1)	1.80	0.20	0.30					$C_{18}H_{27}N_6O_{14}P_3Na_4 \cdot 1.5H_2O$	C, H, N, P
18	79	277 (20.3)	1.65	0.25	0.41					$C_{18}H_{27}N_6O_{11}P_2Na_3 \cdot 0.5(CH_3)_2CO$	C, H, N, P
19	85	279 (18.0)	1.52	0.54					2.02		

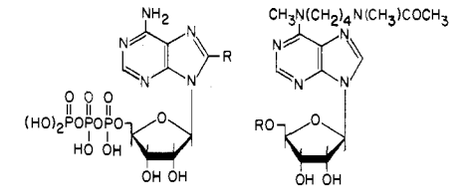
[a] Values of ϵ are calculated for anhydrous salts unless noted otherwise. [b] Mobilities are relative to that of adenosine 5'-phosphate (assigned a value of 1.0). [c] Solvent systems in parentheses. [d] The phosphate measured was that liberated by the action of alkaline phosphatase. [e] Prepared previously by another route; see text. [f] I: calcd, 14.22; found, 12.66. [g] Calculated for a monohydrate. [h] Calculated for a tetrahydrate. [i] Calculated for a dihydrate. [j] N: calcd, 14.50; found, 13.96. [k] P: calcd, 12.35; found, 12.85.

1d–g had previously been prepared by application of the Hoard–Ott procedure to the ω-*N*-iodoacetyl derivatives of **6d–g**.[7] This reaction was found upon further study to give variable and sometimes low yields due to formation of a byproduct of lower electrophoretic mobility at pH 3.6. Imidazole is produced during the Hoard–Ott procedure and it was found that the ω-*N*-iodoacetyl-ATP derivatives **1** react with imidazole under conditions similar to those of the Hoard–Ott procedure to give material of the same electrophoretic mobility at pH 3.6 as the above byproduct.

The route utilized for the preparation of the N^6-substituted ATP derivatives **3a–r** (Scheme II) involved, firstly, introduction of an ω-[(carbobenzyloxy)amino]acyl group at the aliphatic amino groups of the AMP derivatives **10a,c,d,j,q** to give **11a–r**. Compounds **11j–r** were obtained in 43–67% yields as their purified triethylammonium salts by treatment of **10j** and **10q** in aqueous 2-methoxyethanol with a mixed carboxylic–ethyl carbonic anhydride generated in situ from 2 equiv each of the appropriate *N*-carbobenzyloxy-ω-amino carboxylic acid and 1-(ethoxycarbonyl)-2-ethoxy-1,2-dihydroquinoline.[18] The moderate yields of **11j–r** that were obtained were due to the formation of 10–25% as much of a second UV-absorbing product, which was readily separated from **11j–r** by anion-exchange chromatography on DEAE-cellulose. In each case the byproducts eluted earlier than the desired product from the DEAE-cellulose column and migrated more rapidly on paper electrophoretograms. Further characterization of these byproducts was not attempted. In earlier studies, however, larger amounts of a similar byproduct were found to be produced during the *N*-benzoylation of an aliphatic amino group of another nucleotide derivative by the mixed anhydride of benzoic and ethyl carbonic acids,[19] suggesting the possibility that the byproducts obtained in the present studies may be the ω-*N*-carbethoxy derivatives of **10j** and **10q**, respectively. Compound **11j** was obtained in homogeneous form in higher yield (78%) by an alternative procedure involving acylation of **10j** with 3 equiv of the *N*-hydroxysuccinimide ester of 4-[(carbobenzyloxy)amino]butyric acid. Compounds **11a–i** were also obtained in good yields (74–88%) by similar treatment of **10a**, **10c**, or **10d** with the *N*-hydroxysuccinimide esters of the appropriate *N*-carbobenzyloxy-ω-amino carboxylic acids. Conversion of **11a–r** to the corresponding 5′-triphosphates **12a–r**, removal of the carbobenzyloxy groups to give **13a–r**, and iodoacetylation of the ω-amino groups to give **3a–r** were carried out by the procedures described above for the preparation of **8a–k**, **9a–k**, and **1a–g**; compounds **12**, **13**, and **3** were obtained in yields similar to those of **8**, **9**, and **1**, respectively (Tables I and II).

For synthesis of the 8-substituted ATP derivatives **4**, sodium 8-Br-ATP (**14**, Chart I) was obtained by bromination of ATP under conditions described for bromination of AMP[20] and was then treated without purification with a concentrated aqueous solution of the appropriate α,ω-diaminoalkane. The triethylammonium salts of the resulting 8-[(ω-aminoalkyl)amino]adenosine 5′-triphosphates, **15a–d**, were isolated in 41–60% yields following anion-exchange chromatography on DEAE-cellulose. These compounds possessed the UV absorption maximum at 280 nm reported for 8-(alkylamino)adenosine nucleotides[20] and were homogeneous as judged by paper chromatography

Chart I

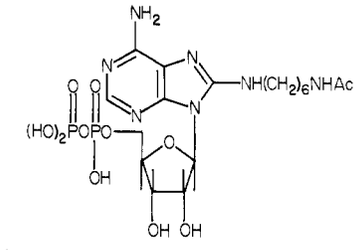


14, R = Br
15, R = $NH(CH_2)_n NHR'$
4, R = $NH(CH_2)_n NHCOCH_2I$
a series, n = 2, R′ = H
b series, n = 4, R′ = H
c series, n = 6, R′ = H
d series, n = 8 R′ = H
e series n = 6, R′ = Ac

16, R = PO_3H_2
17, R = $P_3O_9H_4$
18, R = $P_2O_6H_3$

and electrophoresis, by phosphate/nucleoside ratio, and by UV extinction coefficient; in addition, the trisodium salts of **15a–d** prepared from the triethylammonium salts gave elemental analyses in accord with the structural assignments (Table I). The triethylammonium salts of **15a–d** were treated with 1.5 equiv of *N*-(iodoacetoxy)succinimide in aqueous 2-methoxyethanol, and the 8-[[ω-(iodoacetamido)alkyl]amino]adenosine 5′-triphosphates **4a-d** were purified by anion-exchange chromatography and isolated in 45–55% yield as their homogeneous tetrasodium salts (Table I). Paper electrophoretic analysis showed that the sodium salts of **4a–d** were not stable at room temperature for longer than 4 days. The sodium salts of the N^6-substituted ATP derivatives **1–3** were stable for several weeks. All of the present series of iodoacetamido-substituted ATP derivatives could be stored as their tetrasodium salts at −150 °C for more than 2 years without detectable decomposition. Solutions (10–20 mM) of the ATP derivatives of types **1–3** were stable at −15 °C for several months in 0.05 M Tris-HCl buffer, pH 7.6.

One objective of the present studies was to obtain evidence regarding the effect of an 8-substituent on the affinity of ADP for its substrate site on pyruvate kinases. Since the 8-substituted ATP derivatives **4** proved to be relatively unstable and since nucleoside 5′-diphosphates tend in general to be less stable than the corresponding nucleoside 5′-triphosphates, we decided to prepare an ADP derivative bearing an acetyl group rather than an iodoacetyl group in the 8-substituent. For this purpose, the ω-amino group of **15c** was acetylated with *N*-acetoxysuccinimide under the conditions used to convert **15a–d** to **4a–d**. The product, **15e**, after isolation as its homogeneous tetrasodium salt, was readily converted into the corresponding ADP derivative **19** by the action of yeast



19

hexokinase in the presence of glucose, a method previously found useful in the synthesis of other ADP derivatives.[1] 8-[(6-Acetamidohexyl)amino]adenosine 5′-diphosphate (**19**) was isolated in 85% yield as its trisodium salt, which was homogeneous as indicated by its ultraviolet extinction coefficient, phosphate/nucleoside ratio, and paper electrophoresis and chromatography.

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(19) Hampton, A.; Slotin, L. A.; Kappler, F.; Sasaki, T.; Perini, F. *J. Med. Chem.* **1976**, *19*, 1371.
(20) Lee, C.-Y.; Kaplan, N. O. *Arch. Biochem. Biophys.* **1975**, *168*, 665.

Table II. Physical Properties of Intermediates Used in the Synthesis of the Compounds of Table I[a]

no.[b]	yield, %	UV λ_{max} (H_2O),[c] nm ($\epsilon \times 10^{-3}$)	electrophoresis[d]		paper chromatography[e]				
			pH 3.5	pH 7.6	R_f (A)	R_f (B)	R_f (C)	R_f (F)	R_f (G)
6a	63	268		0.46	0.38				
6c	60	268		0.45	0.39				
6h	62	268 (17.0)[f]		0.41					0.20
6j	61	268		0.40					0.24
7a	71	268		0.88	0.61				
7c	68	268		0.81	0.66				
7e	70	268		0.78	0.72				
7g	63	268		0.75	0.76				
7h	82	268		0.65	0.73				
7j	72	268		0.64	0.76				
8a	73	269 (17.1)	1.85		0.52				
8c	71	269 (17.0)	1.76		0.58				
8e	75	269 (17.3)	1.71		0.65				
8g	66	269 (17.2)	1.66		0.71				
8h	68	268 (17.1)	1.50		0.60				
8i	70	268 (17.5)	1.47		0.62				
8j	71	268 (17.4)	1.45		0.65				
8k	67	268 (17.3)	1.44		0.66				
9a	95	268	1.35		0.16				
9c	93	268	1.26		0.20				
9e	90	268	1.10		0.26				
9g	91	268	0.99		0.30				
9h	93	268	0.98		0.24	0.16			
9j	90	268	0.97		0.35	0.17			
10j	64	277		0.53	0.38	0.64		0.58	
10q	54	277		0.52	0.50	0.69		0.62	
10s	61	277 (19.5)[g]		0.58	0.35			0.57	
11b	85	268		0.59	0.61				
11d	80	268		0.63	0.60				
11f	75	268		0.57	0.65				
11h	88	268		0.53	0.67				
11p	52	277		0.62	0.75	0.78		0.69	
11r	58	277		0.57	0.75	0.77		0.71	
12b	64	268	1.59		0.30		0.78		
12d	65	268	1.64		0.28		0.87		
12h	68	268	1.50		0.50		0.98		
12p	62	277	1.57		0.38	0.53			
12r	58	277	1.43		0.49	0.53			
13c	79	268	1.28		0.08	0.37			
13i	92	268	1.12		0.12				
13j	90	277	1.05		0.19				
13p	77	277	1.03		0.14	0.45			
13r	92	277	1.02		0.18	0.52			
15e	82	279 (18.2)	1.70		0.42				
16	90	277 (20.2)[h]		0.82			0.67		0.57
20[i]	84	268 (17.4)[j]	1.30		0.75				

[a] Among the several series of homologous compounds, physical properties are given for representative members only. [b] Compounds 6d-g have been described previously; see text. [c] Unless specified otherwise, ϵ values are calculated for anhydrous tetrasodium salts. In 0.1 N HCl, the N^6-alkyladenine nucleotides had λ_{max} 266 nm, and the N^6,N^6-dialkyladenine nucleotides had λ_{max} 269 nm. [d] AMP = 1.00. [e] Solvent system in parentheses. [f] Calculated for the anhydrous free acid form. [g] Calculated as a dihydrate. Anal. ($C_{15}H_{25}N_6O_7P \cdot 2H_2O$) C, H, N, P. [h] Calculated as an anhydrous bis(triethylammonium) salt. [i] Compound 20 is the ADP derivative corresponding to 8j. The phosphate/nucleoside ratio was 2.03. [j] Calculated as a trihydrated trisodium salt.

N^6-Methyl-N^6-[4-(N-methyl-N-acetylamino)butyl]-adenosine 5′-triphosphate (**17**, Scheme III) was prepared as a relatively simple, nonalkylating ATP derivative with which to obtain preliminary information on the influence of N^6-methyl-N^6-alkyl substituents on the affinity of ATP for substrate sites. Compound **17** was conveniently synthesized by acetylation of N^6-methyl-N^6-[4-(methylamino)butyl]adenosine 5′-phosphate (**10j**) with N-acetoxysuccinimide and subsequent application to the resulting AMP derivative **16** of the modified Hoard–Ott procedure employed in the syntheses of **8a–k**. Treatment of **17** with hexokinase under the conditions used to prepare **19** furnished in good yield the corresponding derivative (**18**) of ADP (Table I), which was desired for studies of the ADP site of pyruvate kinase.

Studies with Phosphokinases. At early stages of the work, substrate and inhibitor properties were studied of several N^6- or 8-substituted ATP or ADP derivatives that possessed structures representative of those occurring in compounds **1–4**. These determinations were made in order to confirm and extend previous findings[10] which suggested that the substituents of compounds **1–4** should permit affinity to persist for the ATP or ADP sites of most of the phosphokinases which it was planned to employ in the study. The enzyme kinetic data are presented in Table III.

The N^6-substituted ATP derivatives **1g** and **2j** and the N^6,N^6-disubstituted ATP derivative **3j** were comparable to or better than ATP as substrates of yeast hexokinase, as judged by initial reaction velocities produced by a common substrate concentration (5 mM) that was more than 5 times higher than the saturation level for ATP (K_M = 0.1 mM). These properties accord with high V_{max} values relative to the V_{max} of ATP reported for N^6-t-Bu-ATP[21]

Table III. Substrate and Inhibitor Properties of ATP or ADP Derivatives with a Hexokinase, Pyruvate Kinases, and Adenylate Kinases

enzyme	no.	substrate properties			inhibn constants:[d]
		K_m,[a] mM	V_{max},[b] rel %	velocity rel to ATP[c]	K_i, mM
hexokinase	1g			1.8	
(yeast)	2j			0.73	
	3j			1.2	
	4d			0.54	
pyruvate kinase					
rat liver	18	0.7	21		
rat kidney	18	1.7	18		
rat muscle	18	3.6	20		
rabbit muscle	19		0[e]		
rat muscle	19		0[f]		
rat muscle	20[g]	0.07	1.5		
adenylate kinase					
AK II (rat liver)	17		0		1.1
AK III (rat liver)	17		0		0.9

[a] K_M (Michaelis constant) = substrate concentration for half-maximal velocity. [b] Maximal velocity relative to ATP (100) (for adenylate kinase and hexokinase) or to ADP (100) (pyruvate kinase). [c] The value for ATP is 1.00. The initial concentration of ATP and its derivatives was 5 mM. [d] Inhibitions were competitive with respect to ATP. [e] The level of enzyme activity was 200-fold higher than that normally employed with ADP as substrate. [f] The enzyme activity was 8-fold higher than in the normal assay with ADP as substrate. [g] Compound 20 is the ADP analogue corresponding to 8j.

and N^6-Bz-ATP.[10] High V_{max} values are given also by ATP derivatives in which small groups (bromo, amino) have been introduced at C-8.[21] The present studies revealed (Table III) that good substrate activity with yeast hexokinase is retained when the much larger (iodoacetamidooctyl)amino group of **4d** is present at C-8.

Those derivatives of ADP which correspond to the N^6-substituted ATP derivatives **1a–g** are substrates of the three pyruvate kinase isozymes of muscle, liver, and kidney, respectively.[1] In order to determine if longer and more complex N^6-substituents are compatible with substrate activity, we converted **8j** by the action of yeast hexokinase to the corresponding ADP derivative **20**, and this was isolated as its homogeneous trisodium salt (Table II) by the methods used for the preparation of the ADP derivative **18**. Compound **20** was a substrate of rat muscle pyruvate kinase (Table III); its activity appears to be significantly less than that of the less complex ADP analogues of **1a–g**.[1] Disubstitution on the 6-amino group of ADP also allows formation of pyruvate kinase–ADP complexes, as evidenced by the substrate activity of **18** with pyruvate kinases of rat muscle, liver, and kidney (Table III). This suggests that the more complex but structurally similar type of N^6,N^6-disubstitution present in compounds **3j–r** will also allow formation of complexes between the ADP analogues of these compounds and the ADP site of rat pyruvate kinases. The 8-substituted ADP derivative **19**, on the other hand, showed no detectable substrate activity with rat or rabbit muscle pyruvate kinases under the conditions employed.

N^6-[(Acylamino)alkyl]-ATP derivatives of the structure **1** were reported previously to be moderately good substrates of the adenylate kinases AK II and AK III from rat liver[11] and weak substrates of rabbit, pig, and carp muscle adenylate kinases.[7] In these earlier studies,[11] no substrate activity was detected with rat muscle adenylate kinase with the compounds (**1d** and **1e**) tested, presumably because only relatively low levels of enzyme activity could be employed. More recently, high-performance liquid chromatographic analysis employing higher levels of enzyme activity has provided evidence that **1d** is a weak substrate of rat muscle adenylate kinase.[22] The N^6,N^6-disubstituted ATP derivative **17** showed no substrate activity with AK II or AK III. However, it behaved toward both these isozymes as an inhibitor that was competitive with respect to ATP with a K_M (ATP)/K_i (inhibitor) ratio in both cases of ca. 0.1 (Table III). Interactions of 8-substituted ATP derivatives with adenylate kinases have been studied previously, and it was reported that 8-SR derivatives of ATP (R = phenyl, *n*-amyl, and smaller groups) were substrates and competitive inhibitors of rat AK II and AK III, whereas with rat muscle AK they lacked detectable substrate activity and behaved as inhibitors that were noncompetitive with respect to ATP.[11]

In summary, the present work has furnished convenient routes for synthesis of four types of N^6- or 8-substituted ATP derivatives (structures **1–4**) and has provided kinetic data which indicate that frequently all four types of derivatives possess affinity for the ATP sites of adenylate kinases, pyruvate kinases, and hexokinases from various rat tissues and from yeast. The ATP derivatives show no decomposition upon storage for several days at room temperature as 10 mM solutions in neutral aqueous buffers, and thus they are sufficiently stable to test as potential ATP-site-directed enzyme-alkylating agents, even in cases when alkylation proceeds slowly. Studies of their interactions with adenylate kinases, pyruvate kinases, and hexokinases from several rat tissues and microorganisms are described in the following two papers in this issue.

Experimental Section

Chemical Synthesis. General. ω-Aminoalkylamines were purchased from Aldrich Chemical Co., *N*,*N*′-dimethyl-ω-aminoalkylamines from Ames Laboratories, and *N*-carbobenzyloxy-ω-amino carboxylic acids from Sigma Chemical Co. *N*,*N*-Dimethylformamide was distilled from calcium hydride and stored over molecular sieves. Tri-*n*-butylammonium pyrophosphate[16] was prepared at room temperature and stored at 5 °C. Paper chromatography was carried out with Whatman no. 1 paper in (A) 1-butanol–acetic acid–water (5:2:3, v/v), (B) 2-propanol–1% ammonium sulfate (2:1), (C) isobutyric acid–1 M NH_4OH (6:4), (D) 1-butanol–acetic acid–water (1:1:1), (E) 2-propanol–water (2:1), (F) 2-propanol–concentrated NH_4OH–water (7:1:2), or (G) 1-butanol–acetic acid–water (4:1:5, upper phase). The procedures for paper electrophoresis, for determination of ultraviolet spectra, and for phosphate analyses of nucleotides have been described.[7]

(21) Gabbei, A.; Posternak, Th. *Helv. Chim. Acta* 1971, *54*, 2141.

(22) Hampton, A.; Kappler, F., unpublished results.

Elemental analyses were performed by Galbraith Laboratories Inc., Knoxville, TN, and Atlantic Microlabs, Atlanta, GA, and unless otherwise indicated are within ±0.4% of the theoretical values. Evaporations were carried out in vacuo at bath temperatures below 30 °C.

N-Hydroxysuccinimide Esters of N-Carbobenzyloxy-ω-amino Carboxylic Acids. These were prepared by activation of the appropriate N-carbobenzyloxy-ω-amino acid with dicyclohexylcarbodiimide for reaction with N-hydroxysuccinimide. The conditions used were similar to those of de Groot et al.[23] The products, after crystallization from ethanol, possessed melting points in agreement with reported values.

Tripeptides of the Structure $H_2N(CH_2)_nCON(CH_3)(CH_2)_mN(CH_3)CO(CH_2)_nNH_2$ ($n = m = 3$; $n = 3$, $m = 4$; $n = 4$, $m = 3$; $n = m = 4$). A solution in acetone (100 mL) of the appropriate N-hydroxysuccinimide ester of the preceding section (50 mmol) was added dropwise to the appropriate N,N'-dimethyl-ω-aminoalkylamine (25 mmol). The mixture was stirred for 1 h, after which water (0.5 L) was added. The pH was adjusted to 9.0 by the addition of $NaHCO_3$. The gummy precipitate was extracted into ethyl acetate (3 × 100 mL). The combined extracts were concentrated to 25 mL and applied to a silica gel column (5 × 54 cm). The column was washed with ethyl acetate (500 mL), after which the N,N'-bis(carbobenzyloxy) derivative of the desired diamine was eluted with ca. 500 mL of 10% methanolic ethyl acetate. Removal of volatiles in vacuo and trituration of the residual gum with light petroleum gave the product as a white powder, which was homogeneous as judged by TLC on silica gel developed with 5% methanolic ethyl acetate. In two instances ($n = 3$, $m = 4$; $n = m = 4$), the N,N'-bis(carbobenzyloxy) derivative was subjected to elemental analysis. Anal. ($C_{30}H_{42}N_4O_6$ and $C_{32}H_{46}N_4O_6$) C, H, N.

The appropriate N,N'-bis(carbobenzyloxy) derivative (20 mmol) was dissolved in methanol (50 mL) containing acetic acid (100 μL) and hydrogenated for 2–3 h at 40 psi in the presence of 10% Pd/C (100 mg). The solution was filtered and evaporated to dryness to give the required diamine in 95–98% yield as a pale yellow oil, which was homogeneous toward TLC on silica gel plates developed with 10% methanol in ethyl acetate.

General Synthesis of N^6-(ω-Aminoalkyl)adenosine 5'-Monophosphates (6a–k) and of N^6-Methyl-N^6-[ω-(methylamino)alkyl]adenosine 5'-Monophosphates (10j,q,s). These compounds were prepared from **5** as described in the text and isolated in the free acid form. Compounds **6a–c**, **6h–k**, and **10j** were precipitated from ethanolic solution by addition of acetone; compounds **6d–g** were crystallized from water; compounds **10q** and **10s** were crystallized from aqueous ethanol. Properties of these nucleotides are given in Table II.

General Synthesis of N^6-[ω-[(Carbobenzyloxy)amino]alkyl]adenosine 5'-Monophosphates (7). To aqueous 0.1 N NaOH (40 mL) at 0 °C was added the appropriate N^6-(aminoalkyl)adenosine 5'-phosphate (**6**; 1.0 mmol), followed by benzyl chloroformate (1.1 mmol). The mixture was stirred at 0 °C for 2 h and then adjusted to pH 8.0 by the addition of solid CO_2. Paper electrophoresis showed a single ninhydrin-negative component of higher mobility than **6**. The solution was applied to a column (2.5 × 30 cm) of DEAE-cellulose (bicarbonate form), and the column was eluted with a linear gradient (0–0.2 M, 2 L) of aqueous $Et_3N{\cdot}HCO_3$. Fractions containing **7** were pooled and evaporated in vacuo, and traces of $Et_3N{\cdot}HCO_3$ were removed from the residual triethylammonium salt of **7** by several evaporations in vacuo with ethanol. The product so obtained was homogeneous upon paper chromatography or electrophoresis (Table II). In several instances, a portion of the product was dissolved in methanol and precipitated as the disodium salt by addition of NaI in acetone.[24] In aqueous solution, the disodium salts exhibited extinction coefficients (calculated for anhydrous material) that were within 3% of the expected value (17 300 at 268 nm). The triethylammonium salts of **7** were used for preparation of the 5'-triphosphates **8** after further drying them by several additions and vacuum evaporations of anhydrous DMF (3 × 25 mL).

General Procedure for Conversion of the Nucleoside 5'-Monophosphates 7 and 11 to the Corresponding 5'-Triphosphates 8 and 12. These conversions, as well as subsequent purification of the products, were carried out by the methods of Hoard and Ott,[16] except that shorter reaction times were employed for conversion of the 5'-monophosphates to the 5'-phosphorimidazolidates (1 h) and of the latter to the 5'-triphosphates (4 h). The crude 5'-triphosphates obtained by evaporation of the reaction solvent were dissolved in a 5% (v/v) solution of Et_3N in aqueous 50% methanol. This solution was kept at 22–24 °C for 1 h to remove 2',3'-O-carbonyl groups,[17] after which volatiles were removed in vacuo and the residue was chromatographed on DEAE-cellulose as described.[16] The products were precipitated as their tetrasodium salts by addition of NaI in acetone to methanolic solutions of the triethylammonium salts.[24] The sodium salts were chromatographically and electrophoretically homogeneous. Properties are given in Table II.

General Synthesis of N^6-(Aminoalkyl)adenosine 5'-Triphosphates (9a–k and 13a–i) and N^6-Methyl-N^6-(aminoalkyl)adenosine 5'-Triphosphates (13j–r). The appropriate N-carbobenzyloxy derivative **8a–k** or **12a–r** (100 μmol) was dissolved in 20 mL of aqueous 50% methanol containing 50 μL of acetic acid and hydrogenated in the presence of 10% Pd/C (35 mg) for 1 h at 30 psi. Electrophoresis at pH 3.5 revealed that the conversion was usually complete after this time. The charcoal was filtered off and washed with 2% NH_4OH in aqueous 50% methanol (50 mL). The combined filtrates were evaporated to dryness, and the residue was dissolved in water (10 mL) and chromatographed on a DEAE-cellulose column (2.5 × 20 cm) with a linear gradient of 1 L each of water and of 0.25 M triethylammonium bicarbonate, pH 7.5. Appropriate fractions were evaporated under vacuum and the product was converted to its trisodium salt in the manner described above. See Table II for properties.

General Synthesis of N^6-[(Iodoacetamido)alkyl]adenosine 5'-Triphosphates (1a–g, 2h–k, and 3a–i) and N^6-Methyl-N^6-[(iodoacetamido)alkyl]adenosine 5'-Triphosphates (3j–r). The appropriate N^6-(aminoalkyl)adenosine 5'-triphosphate (**9a–k** or **13a–r**; 100 μmol) was dissolved in 2-methoxyethanol–water (5:3, 20 mL) in which had been dissolved N-(iodoacetoxy)succinimide[7] (200 μmol) and sodium bicarbonate (200 μmol). The solution was stored at room temperature for 3 h and was then chromatographed at 5 °C on a column (2 × 20 cm) of DEAE-cellulose (HCO_3^- form) with a linear gradient of 1 L each of water and 0.3 M $Et_3N{\cdot}HCO_3$. The products were isolated as their tetrasodium salts in the usual manner. Their properties are listed in Table I.

General Synthesis of N^6-[ω-[N-[ω-[(Carbobenzyloxy)amino]acyl]amino]alkyl]adenosine 5'-Monophosphates (11a–j). The appropriate N^6-(ω-aminoalkyl)adenosine 5'-phosphate (**10a–j**; 1.0 mmol) was dissolved in water (50 mL) at 50 °C. This was cooled to room temperature and to it was added in succession 2-methoxyethanol (50 mL), a solution in 2-methoxyethanol (50 mL) of the N-hydroxysuccinimide ester of the appropriate N-carbobenzyloxy-ω-amino carboxylic acid (3.0 mmol), and a solution of $NaHCO_3$ (4 mmol) in water (30 mL). The mixture was stirred at room temperature for 8 h. Volatiles were removed in vacuo, and the residue was washed with acetone to remove the excess of N-hydroxysuccinimide ester. The insoluble portion was dissolved in water and chromatographed on a column (2.5 × 40 cm) of DEAE-cellulose with a linear gradient of aqueous $Et_3N{\cdot}HCO_3$ (0–0.25 M, 4 L), pH 7.5. The triethylammonium salts of **11a–j** thereby obtained were homogeneous in the electrophoretic and paper chromatographic systems given in Table II and were used for synthesis of **12a–j** after vacuum evaporation from them of anhydrous DMF (3 × 25 mL).

General Synthesis of N^6-Methyl-N^6-[ω-[N-methyl-N-[ω-[(carbobenzyloxy)amino]acyl]amino]alkyl]adenosine 5'-Monophosphates (11j–r). A solution of the appropriate N^6-methyl-N^6-[ω-(methylamino)alkyl]adenosine 5'-phosphate (**10j,q**; 0.69 mmol) in water (5 mL) containing Et_3N (3 mmol) was evaporated to dryness in vacuo. The residue was dissolved in a mixture of water (3 mL) and 2-methoxyethanol (14 mL) containing NEt_3 (1.38 mmol). A solution of the appropriate N-carbobenzyloxy-ω-amino acid (1.38 mmol) and 1-ethoxycarbonyl-2-

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ethoxy-1,2-dihydroquinoline (EEDQ; 1.38 mmol) in 2-methoxyethanol (2 mL) was added. Paper electrophoresis showed that the reaction was complete after 4 h at room temperature. Volatiles were removed in vacuo, and the residue was washed with diethyl ether to extract EEDQ. To a solution of the insoluble portion in methanol (1 mL) was added water (10 mL), and the mixture was centrifuged, if necessary, to remove precipitated *N*-Cbz-ω-amino acid. The supernate was chromatographed on DEAE-cellulose as described for the purification of **11a–j**. The triethylammonium salts of **11j–r** so obtained were homogeneous in the systems of Table II and were used for synthesis of **12j–r** after drying by vacuum evaporations from them of anhydrous DMF (3 × 20 mL).

N^6-Methyl-N^6-[4-(*N*-methyl-*N*-acetylamino)butyl]-adenosine 5′-Triphosphate (17). Compound **10j** (0.24 g, 0.5 mmol) was added to a solution of *N*-acetoxysuccinimide (0.75 mmol) and $NaHCO_3$ (1.5 mmol) in aqueous 50% 2-methoxyethanol (50 mL). The mixture was stirred at room temperature for 16 h, after which the solvents were evaporated in vacuo and the residue was dissolved in water and chromatographed on a column (2.5 × 30 cm) of DEAE-cellulose with a linear gradient of water (1 L)–$Et_3N{\cdot}HCO_3$ (0.2 M, 1 L). The triethylammonium salt of the N^6-(acetamidoalkyl)adenosine 5′-phosphate (**16**) so obtained was pure as judged by its extinction coefficient, electrophoresis, and chromatography in two solvent systems (Table II). It was dried by additions and evaporations of anhydrous DMF (3 × 20 mL) and converted to the tetrasodium salt of the corresponding 5′-triphosphate **17** by the procedures used in the synthesis of **8** and **12**. The product was homogeneous in the systems given in Table I.

N^6-Methyl-N^6-[4-(*N*-methyl-*N*-acetylamino)butyl]-adenosine 5′-Diphosphate (18). A solution of the nucleoside 5′-triphosphate **17** (115 mg, 0.15 mmol) was treated for 16 h with yeast hexokinase under conditions used previously for the preparation of N^6-substituted adenosine 5′-diphosphates.[1] The product was chromatographed on a column (2.5 × 20 cm) of DEAE-cellulose using a linear gradient of $Et_3N{\cdot}HCO_3$ (0–0.4 M, 3 L) and isolated as its trisodium salt in the usual manner. The compound was chromatographically and electrophoretically homogeneous in the systems of Table I.

General Synthesis of 8-[(ω-Aminoalkyl)amino]adenosine 5′-Triphosphates (15). Adenosine 5′-triphosphate was converted to sodium 8-bromoadenosine 5′-triphosphate (**14**; 1 mmol) in admixture with sodium acetate using a method[20] for formation of sodium 8-bromoadenosine 5′-phosphate from adenosine 5′-phosphate. To a solution of **14** in water (5 mL) was added the appropriate α,ω-diaminoalkane (2 g) and Na_2CO_3 (1 mmol). After 3 days at 25 °C, the purple mixture was poured into ethanol (50 mL) at 0 °C. The gummy precipitate was collected by centrifugation and precipitated twice more by adding its solution in the minimum volume (ca. 5 mL) of water to ethanol at 0 °C. The product was chromatographed on a column (2.5 × 30 cm) of DEAE-cellulose (bicarbonate form) by elution with ethanol (50 mL), then water (100 mL), and then a linear gradient of $Et_3N{\cdot}HCO_3$ (0–0.5 M, 1.6 L) at a flow rate of 2 mL/min. The triethylammonium salts of compounds **15** were obtained as electrophoretically and chromatographically homogeneous colorless glasses by the usual procedure and were used directly for the preparation of compounds **4**. A portion of each compound **15** was converted to its trisodium salt in the usual manner prior to obtaining elemental analyses and UV extinction coefficients. For properties see Table I.

General Synthesis of 8-[[ω-(Iodoacetamido)alkyl]-amino]adenosine 5′-Triphosphates (4a–d). To a solution of the triethylammonium salt of the appropriate ATP derivative **15** (0.2 mmol) in 2-methoxyethanol–water (1:1, 5 mL) was added *N*-(iodoacetoxy)succinimide (0.3 mmol) and $NaHCO_3$ (0.3 mmol). The suspension was stirred at room temperature for 2 h. The resulting solution was chromatographed at 5 °C on a column (1.8 × 20 cm) of DEAE-cellulose (HCO_3^- form) using a linear gradient of $Et_3N{\cdot}HCO_3$ (0–0.5 M, 1.4 L), pH 7.5. The product **4** was present in fractions containing 0.20–0.26 M $Et_3N{\cdot}HCO_3$. The triethylammonium salts of compounds **4** were isolated immediately in the usual way, except that acetic acid (100 μL) was added together with ethanol in the final four vacuum evaporations in order to prevent partial decomposition of the product by traces of Et_3N. The tetrasodium salts of **15** were obtained by the usual procedure and were found to be homogeneous by paper electrophoresis and paper chromatography (see Table I). They were stored at −150 °C.

Enzyme Kinetic Studies. All assays were carried out at 22–24 °C by measuring the rate of change of optical density at 340 nm 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 systems. Each kinetic study employed five or more concentrations of substrate, and each study of inhibition employed five or more levels of normal substrate for each of two levels of inhibitor. Inhibitor levels were 1–5 times higher than the inhibition constant. Stock solutions of ATP and ATP derivatives used as substrates or inhibitors contained equimolar amounts of $MgCl_2$. Substrate constants were determined from Lineweaver–Burk plots, all of which were linear, and inhibition constants were obtained from replots of inhibitor concentration vs. slopes of the Lineweaver–Burk plots.

The rat liver adenylate kinase isozymes AK II and AK III and rat muscle AK were isolated as described previously,[11] as were the rat muscle, liver, and kidney isozymes of pyruvate kinase.[1] The conditions used for studying substrate and inhibitor properties of ATP derivatives with adenylate kinases were the same as described previously.[11] Substrate properties of ADP derivatives with pyruvate kinases were determined as described previously.[1] Reactions catalyzed by yeast hexokinase were carried out in 1 mL of 0.05 M Tris-HCl (pH 7.6) containing $MgCl_2$ (6.6 mM), α-D-glucose (10 mM), NADP (0.93 mM), and glucose-6-phosphate dehydrogenase (0.35 unit). Yeast hexokinase and glucose-6-phosphate dehydrogenase (grade II) were obtained from Boehringer Mannheim.

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