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Synthesis and Enzymic Studies of 5-Aminoimidazole and N-1- and N^6-Substituted Adenine Ribonucleoside Cyclic 3′,5′-Phosphates Prepared from Adenosine Cyclic 3′,5′-Phosphate

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Alkaline treatment of adenosine cyclic 3′,5′-phosphate 1-oxide gave inosine cyclic 3′,5′-phosphate 1-oxide, 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamidoxime cyclic 3′,5′-phosphate (3), and the Dimroth rearragement product, *N*-hydroxyadenosine cyclic 3′,5′-phosphate. Similar basic treatment of 1-methoxy-, 1-ethoxy-, and 1-benzyloxyadenosine cyclic 3′,5′-phosphates gave the analogous ring-opened *O*-alkyl-5-aminoimidazole-4-carboxamidoxime nucleotides and *N*-alkoxyadenosine cyclic 3′,5′-phosphates. Hydrogenation of 3 or 5-amino-*O*-methyl-1-β-D-ribofuranosylimidazole-4-carboxamidoxime cyclic 3′,5′-phosphate gave 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamidine cyclic 3′,5′-phosphate (9). Basic hydrolysis of 9 gave 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide cyclic 3′,5′-phosphate. Treatment of 5-amino-*O*-methyl-1-β-D-ribofuranosylimidazole-4-carboxamidoxime cyclic 3′,5′-phosphate with pyridine–H_2S gave 5-amino-1-β-D-ribofuranosylthioimidazole-4-carboxamide cyclic 3′,5′-phosphate. A facile synthesis of 9-β-D-ribofuranosylpurine-6(1*H*)-thione cyclic 3′,5′-phosphate from *N*-methoxyadenosine cyclic 3′,5′-phosphate is reported. Alkylation of adenosine cyclic 3′,5′-phosphate (1, cAMP) with α-bromoacetophenone gave 8-phenyl-3-β-D-ribofuranosylimidazo[2,1-*i*]purine cyclic 3′,5′-phosphate (14). Alkylation of 1 with MeI gave 1-methyladenosine cyclic 3′,5′-phosphate. *N*-Alkoxyadenosine cyclic 3′,5′-phosphates were similar in potency to cAMP for activation of bovine brain kinase. With the exception of 14 all the N-1-substituted purine 3′,5′-cyclic nucleotides and 1-β-D-ribofuranosylimidazole cyclic 3′,5′-phosphates were hydrolyzed slower than cAMP by rabbit kidney phosphodiesterase. The N^6 and N-1 derivatives of cAMP were found to be generally better than theophylline as inhibitors of rabbit lung and bovine heart phosphodiesterases.

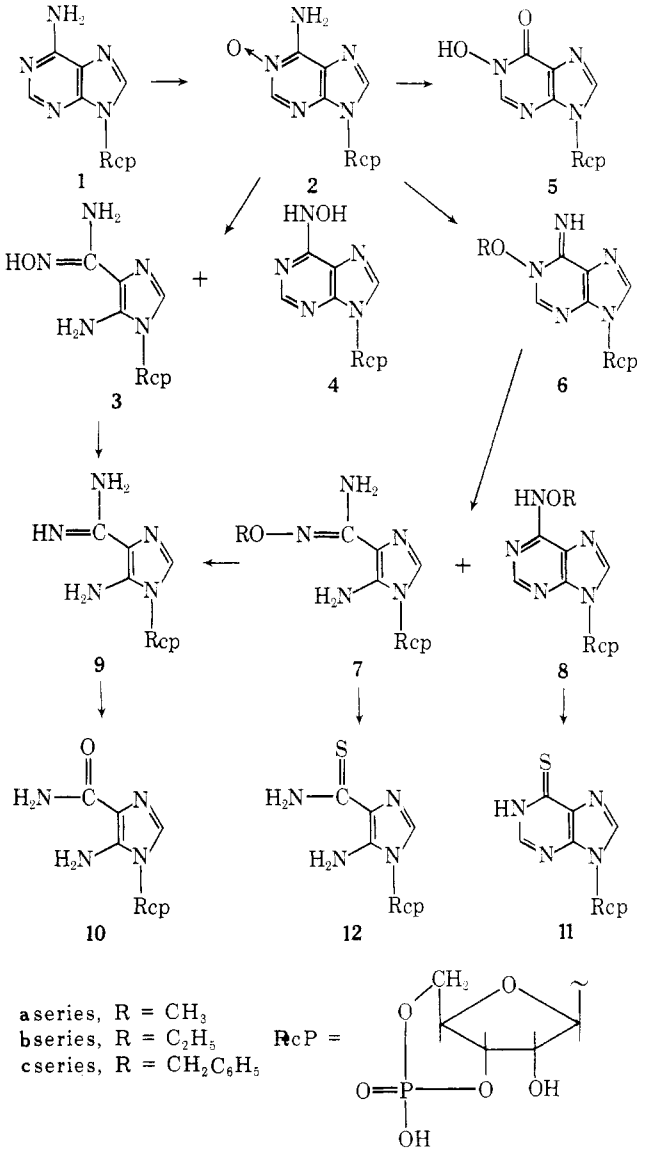
In previous studies from this laboratory we have reported how modification of the adenosine cyclic 3′,5′-phosphate (1, cAMP) molecule affected certain biological activities.[1-4] We have previously studied cAMP derivatives with substituents at the 6 and 8 positions of the purine ring.[1,2,4] As a preliminary measure of the potential biological activity of these compounds, their ability to mimic cAMP in stimulating a purified cAMP-dependent protein kinase isolated from bovine brain was examined. In addition, the ability of these purine nucleoside cyclic 3′,5′-phosphates to inhibit or act as substrates for cyclic nucleotide phosphodiesterase was studied. The present work will describe the synthesis of cAMP derivatives which contain modifications in the pyrimidine portion of the purine ring and the effect that these modifications have on such biological activity.

Chemistry. Brown and coworkers have described the preparation and some reactions of the 1-*N*-oxides of adenine,[5,6] adenosine,[5,6] and the 2′-, 3′-, and 5′-adenylic acids,[7] including the cleavage of the pyrimidine ring with alkali to give the corresponding 5-aminoimidazole-4-carboxamidoxime derivatives. More recently, Fujii, Itaya, and coworkers have described the preparation of 1-alkoxy-9-alkyladenines[8-10] by alkylation of 9-alkyladenine 1-oxides. This group has also shown[11,12] that these compounds readily undergo the Dimroth rearrangement to *N*-alkoxy-9-alkyladenines, or that they are cleaved to 1,*O*-dialkyl-5-aminoimidazole-4-carboxamidoximes under alkaline conditions.[12] The latter reaction has also been shown to occur in the case of 1-benzyloxyadenosine.[13,14]

In the present work we desired to apply these reactions to the 3′,5′-cyclic ribonucleotides, as shown in Scheme I. A facile large scale synthesis of adenosine cyclic 3′,5′-phosphate 1-oxide[15] (2) was developed. The action of *m*-chloroperbenzoic acid on cAMP in a buffered two-phase system gave crystalline 2 in 89% yield after neutralization of the reaction mixture. A trace of cAMP was apparent by tlc, but the material was quite suitable for further transformation. Treatment of 2 with refluxing 2 *N* NaOH for 10 min gave 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamidoxime cyclic 3′,5′-phosphate (3) in 43% yield and the rearrangement product, *N*-hydroxyadenosine cyclic 3′,5′-phosphate (4) in 12% yield. This appears to be the first example of the synthesis of a derivative of *N*-hydroxyadenine from a 9-substituted adenine 1-oxide by the Dimroth rearrangement. The products were readily separated by anion-exchange chromatography on Dowex 1-X2 (formate form) using a formic acid gradient as eluent, and could readily be identified by their uv spectra. The spectra of these and other representative nucleotides are given in Table II (see Experimental Section). When a preliminary run of this reaction was worked up by passage through a strong cation exchange resin, the first product, emerging in very low yield, was identified as inosine cyclic 3′,5′-phosphate 1-oxide (5). This product, presumably formed by alkaline deamination of adenosine cyclic 3′,5′-

phosphate 1-oxide (2), was identical with 5 also prepared by nitrous acid deamination of 2.

Scheme I



Alkylation of adenosine cyclic 3′,5′-phosphate 1-oxide (2) with MeI, EtI, or $PhCH_2Br$ gave the corresponding 1-alkoxyadenosine cyclic 3′,5′-phosphates (6). The alkylation was very conveniently performed in DMSO solution using the soluble 1,5-diazabicyclo[5.4.0]-5-undecene (DBU) salt of 2; upon completion of the reaction, the product separated after addition of EtOH.

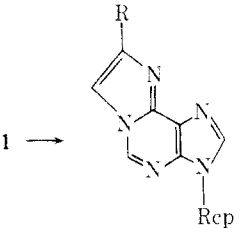
Following the general procedures of Fujii, *et al.*,[11,12] the 1-alkoxy-cAMP derivatives (6) were converted to *O*-alkyl-5-amino-1-β-D-ribofuranosylimidazole-4-carboxamidoxime cyclic 3′,5′-phosphates (7) and *N*-alkoxyadenosine cyclic 3′,5′-phosphates (8). Thus, treatment of **6a** (R = Me) with dilute NaOH at room temperature gave 5-amino-*O*-methyl-1-β-D-ribofuranosylimidazole-4-carboxamidoxime cyclic 3′,5′-phosphate (**7a**) and *N*-methoxyadenosine cyclic 3′,5′-phosphate (**8a**) in 65 and 8% yield, respectively, after anion exchange chromatography as in the synthesis of **3** and **4**. The action of less alkaline media on **6a** (refluxing $NaHCO_3$ solution) affected predominant rearrangement, giving **7a** and **8a** in 18 and 53% respective yields.

Reduction of 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamidoxime cyclic 3′,5′-phosphate (3) or its *O*-methyl derivative (**7a**) with H_2 and sponge nickel catalyst gave 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamidine cyclic 3′,5′-phosphate (**9**), an analog of cAMP lacking carbon in the 2 position. Alkaline hydrolysis of 9 gave 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide cyclic 3′,5′-phosphate (**10**)† in 68% yield.

A recent report[17] on the replacement of H_2NOMe from *N*-methoxyadenosine with liquid H_2S in aqueous pyridine prompted the adaptation of this method to the synthesis of 9-β-D-ribofuranosylpurine-6(1*H*)-thione cyclic 3′,5′-phosphate (**11**).[2,18] Compound **11** has been previously studied[2] as an analog of cAMP and has shown significant activity against L1210 mouse leukemia.[19] *N*-Methoxyadenosine cyclic 3′,5′-phosphate (**8a**) was converted to **11** in 82% yield, and this method represents a more satisfactory route than those previously reported.[2,18]

It was of interest to determine if this same metathesis of H_2NOMe by H_2S could be applied to the synthesis of a thiocarboxamide from an *O*-methylcarboxamidoxime. Such a method would, for the purpose of this work, provide a shorter route to the desired 5-amino-1-β-D-ribofuranosylthioimidazole-4-carboxamide cyclic 3′,5′-phosphate (**12**) than more conventional methods. Accordingly, 5-amino-*O*-methyl-1-β-D-ribofuranosylimidazole-4-carboxamidoxime cyclic 3′,5′-phosphate (**7a**) was treated with liquid H_2S in aqueous pyridine at 60–70°. Although tlc showed several minor impurities, **12** was directly crystallized from an aqueous solution of the reaction mixture in 14% yield.

Additional N-1-substituted derivatives of cAMP (1) were synthesized by treatment of 1 with alkylating reagents. Chloroacetaldehyde and 1 gave 3-β-D-ribofuranosylimidazo[2,1-*i*]purine cyclic 3′,5′-phosphate (**13**);‡ 1 and α-bromoacetophenone gave the corresponding 8-phenyl derivative (**14**). The assignment of the location of the phenyl group of **14** at the 8 position and not the 7 position was based on the expected initial alkylation[21] by α-bromoacetophenone at N-1, followed by cyclization and dehydration.



13, R = H
14, R = C_6H_5

1-Methyladenosine cyclic 3′,5′-phosphate (**15**) was readily synthesized from the DBU salt of 1 and MeI in DMSO.

Biochemical Methods. All procedures have been previously described.[1,4] The ability of the nucleotides to stimulate a purified cAMP-dependent protein kinase from bovine brain was measured by the incorporation of $^{32}PO_4^{3-}$ from [γ-^{32}P]ATP into histone.[1] The activity of the analogs was expressed as the K_a', the ratio of the K_a of cAMP to the K_a of the test compound. The K_a of cAMP in this system was 4×10^{-8} *M*.

The inhibition of 3′,5′-cyclic nucleotide phosphodiesterase from rabbit lung and beef heart by the nucleotides was assessed by determining the concentration that caused 50% inhibition of hydrolysis of cAMP at a substrate concentration of 1.7×10^{-7} *M*.[4] The extent to

† While this work was in progress, the synthesis of **10**, from both 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide 5′-phosphate and inosine cyclic 3′,5′-phosphate, was reported.[16]

‡ While this work was in progress, the synthesis and biological properties of **13** were reported.[20]

which the cyclic nucleotides were substrates for the phosphodiesterase from rabbit kidney was measured as the ratio of the rate of cleavage of the test compound to that of cAMP,[4] both at a concentration of 5×10^{-3} *M*.

The results of the enzyme assays are shown in Table I.

Results and Discussion

Protein Kinase. The modifications to cAMP incorporated into the compounds in this report are all in the pyrimidine ring portion of the molecule. They may be formulated as modifications at N-1 (group A: **2**, **6**, **15**), N^6 (group B: **4**, **8**), C-2, N-1, and N-3 (group C: all of the imidazole nucleotides), and N-1 and N^6 (group D: **13**, **14**). Additionally, compounds **6** and **15** probably exist in the N^6-imino tautomer as opposed to the N^6-amino tautomer of adenine. This property could alter hydrogen bonding characteristics of the compounds, but, more importantly, suggests that the aglycon is more basic than that of **1** and is protonated under assay conditions (a pK_a of 8.55 has been reported[9] for 1-methoxy-9-methyladenine).

It has been postulated that the mechanism by which cAMP modulates cellular functions is by stimulation of a cAMP-dependent protein kinase.[22] The novel cyclic nucleotides were thus examined for their ability to activate a preparation of that enzyme purified from bovine brain, and their relative potency was expressed as the K_a' (see Biochemical Methods). As can be seen in Table I, the 1-oxide (**2**) of cAMP was 20-fold less potent than cAMP in this system. Compound **2** probably bears the least steric and electronic variance from the parent molecule, the major difference being the loss of the hydrogen bonding site at N-1. The remaining compounds in group A, which also bear N-1 substituents, but are strongly basic, showed a generally greater loss in stimulatory ability.

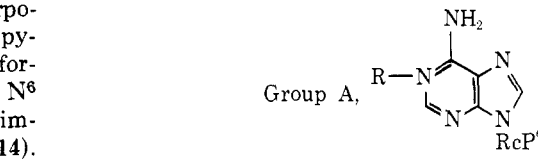
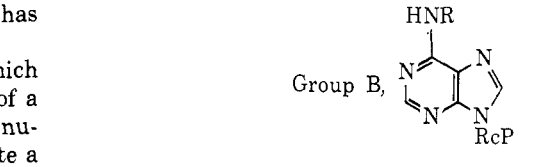
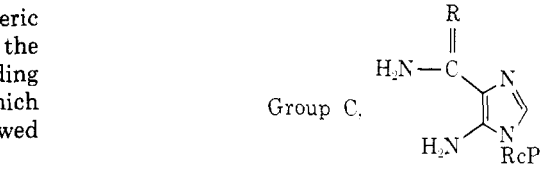
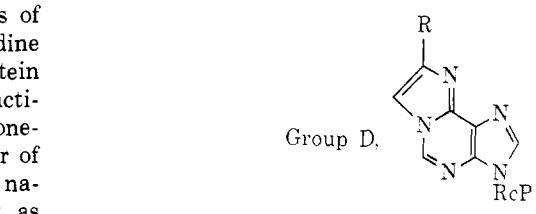
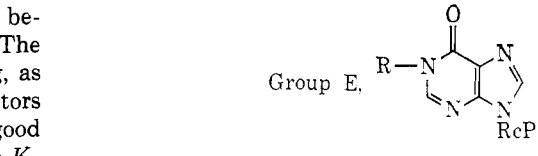
The compounds in group B, the N^6-alkoxy-cAMP derivatives, exhibited activity almost equivalent to **1**, however. N^6-Hydroxy-cAMP (**4**) was able to stimulate the protein kinase as well as **1**, and compounds **8a**, **8b**, and **8c** were nearly as good. Thus the previous finding[2,23,24] that N^6-alkyl groups on cAMP do not markedly alter activity in this system can now be extended to the N^6-alkoxy groups.

The K_a' values of the imidazole cyclic nucleotides of group C in Table I demonstrate that the intact pyrimidine ring is important for the interaction of cAMP with protein kinase. All of the nucleotides in this group were poor activators. *O*-Benzylcarboxamidoxime **7c**, which gave one-tenth the stimulation of cAMP, was the best activator of group C. These K_a' values indicate that the aromatic nature of the ring system is necessary for binding, or that, as suggested above, a hydrogen bond accepting site is needed in the location of N-1 on cAMP.

The compounds of group D, with an ethylene bridge between N-1 and N^6, are, at first glance, anomalous. The substituent at N-1 would seem to deny strong binding, as in group A, but **13** and **14** were among the best activators of the protein kinase in this report; **14** was twice as good as cAMP (compound **13** has been reported [20] to have a K_a for bovine skeletal muscle tenfold higher than that of cAMP). This can possibly be explained by enhanced hydrophobic bonding of the fused imidazole ring; indeed, the addition of a phenyl substituent increases the activity of **14** over **13** by threefold. Another consideration is increased electron delocalization in the aromatic system (**13** and **14** are highly fluorescent[20]), thus possibly increasing π–π interactions between the compound and the active site.

Group E is presented to show additional evidence of the importance of N-1. Inosine cyclic 3′,5′-phosphate (**17**) has been shown[2] to have a K_a' of 0.59 in this system. Al-

Table I. Protein Kinase and Phosphodiesterase Activity of the Cyclic Nucleotides[a]

No.	R	Protein kinase K_a'[b]	Phosphodiesterase: Substrate,[c] % rate of **1**	Phosphodiesterase: Inhibitor,[d] I_{50}, μM Lung	Phosphodiesterase: Inhibitor,[d] I_{50}, μM Heart
	Group A,  RcP[e]				
2	O	0.045	39	35	50
6a	OCH_3	0.008	36	150	90
6b	OC_2H_5	0.014	77	45	120
6c	$OCH_2C_6H_5$	0.051	53	35	80
15	CH_3	0.012	16	65	80
	Group B,  RcP				
4	OH	1.0	44	13	150
8a	OCH_3	0.65	42	100	80
8b	OC_2H_5	0.44	36	42	68
8c	$OCH_2C_6H_5$	1.0	45	190	240
	Group C,  RcP				
9	NH	0.004	18	300	130
3	NOH	0.002	6	>1000	>1000
7a	$NOCH_3$	0.007	27	550	600
7b	NOC_2H_5	0.034	14	430	300
7c	$NOCH_2C_6H_5$	0.093	57	220	220
10	O	0.05	22	100	170
12	S	0.023	57	80	60
	Group D,  RcP				
13	H	0.65	10	280	590
14	C_6H_5	2.0	20	160	73
	Group E,  RcP				
17	H[f]	0.59	46	80	3.8
5	OH	0.021	20	330	830

[a] The technical assistance of Mary K. Dimmitt and Mieka B. Scholten is acknowledged. [b] $K_a' = K_a$ (cAMP)/K_a (test compound). [c] The ability to serve as a substrate of rabbit kidney phosphodiesterase, expressed as a percentage of the rate of cleavage of **1**. [d] The concentration of test compound for 50% inhibition of the cleavage of **1** (concentration of **1** = 1.7×10^{-7} *M*). The I_{50} of theophylline is 130 μM against bovine heart phosphodiesterase and 250 μM against rabbit lung phosphodiesterase. [e] RcP = β-D-ribofuranosyl cyclic 3,5-phosphate. [f] Data from ref 2.

Table II. Ultraviolet Spectra of the Cyclic Nucleotides

No.	λ_{max}, nm ($\epsilon \times 10^{-3}$) pH 1	pH 7	pH 11
3	276 (8.9)	263 (8.0)	252 (9.6)
4	263 (17.1)	265 (13.6)	270 (9.6), 291 (sh) (8.1)
6a	257 (12.4)		257 (17.5), 285 (sh) (3.5)
7a	281 (9.6)	258 (9.5)	257 (10.2)
8a	264 (15.0)	266 (13.9)	268 (11.8)
9	280 (11.1)	281 (11.1)	280 (10.8)
10	245 (9.2)	263 (12.5)	
	265 (10.4)		
12	275 (10.5)	268 (11.2)	
	323 (17.0)	325 (17.7)	
14	250 (34.2)		251 (42.1)
	256 (sh) (31.0)		261 (sh) (29.6)
	278 (15.2)		280 (sh) (10.5)
			300 (sh) (4.5)

though hypoxanthine derivatives are usually formulated as purin-6(1*H*)-ones, **17** still activated the protein kinase fairly well. Introduction of the 1-oxide function, however, dramatically (30-fold) reduced the binding, as was the case when cAMP was converted to **2**.

Interestingly, the three series of alkoxy compounds **6**, **7**, and **8** exhibited very similar increases in K_a' as the side chain was increased from Me to Et to Bzl. There may, therefore, be hydrophobic interactions of these groups with an area adjacent to the active site of the enzyme.

Phosphodiesterase. All compounds were tested as substrates and inhibitors of cyclic nucleotide phosphodiesterase. Table I shows the results of these studies. Although none of the compounds tested was cleaved by the enzyme as rapidly as **1**, all the compounds were within a 14-fold range of susceptibility. Only 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamidoxime cyclic 3′,5′-phosphate (**3**) was cleaved at less than 10% the rate of **1**. Since addition of substituents in the 8 position of cAMP gave derivatives which were much more stable to phosphodiesterase,[1a] it can be concluded that modification in the pyrimidine ring portion of the molecule of the type described here has much less effect on the interaction of the cyclic nucleotides with the enzyme. Even the addition of the N^6-alkoxy substituents (group B) did not reduce susceptibility to hydrolysis to the degree of that caused by N^6-alkyl substituents.[2,4,25]

As inhibitors of phosphodiesterase, the compounds in groups A and B with an intact purine ring were generally better than theophylline and the nucleotides in group C. The imidazole nucleotides (group C), the N^1,N^6-ethenonucleotides (group D), and the inosine nucleotides (group E) were more variable as inhibitors of phosphodiesterase. Further kinetic studies are required to ascertain the mechanism of inhibition of phosphodiesterase by these compounds.

Experimental Section

All compounds were chromatographed on either Merck silica gel F-254 or Baker-Flex cellulose F plates in MeCN–0.1 *N* NH_4Cl either 2:1, 7:3, or 4:1. Uv spectra were determined on a Cary 15. All compounds were dried at 80° under high vacuum. Combustion analyses were performed by either Galbraith Laboratories, Knoxville, Tenn., or by Heterocyclic Chemical Co., Harrisonville, Mo. Pertinent uv spectra are recorded in Table II.

Adenosine Cyclic 3′,5′-Phosphate 1-Oxide[15] (2). A biphasic mixture of 100 g of **1** (0.304 mol), 800 ml of 1 *N* NaOAc, 800 ml of 1 *N* AcOH, 1.5 l. of EtOAc, and 180 g (1.10 mol) of *m*-chloroperbenzoic acid was stirred 24 hr at ambient temperature. The aqueous phase was separated, and 500 ml of $CHCl_3$ and 800 ml of 1 *N* HCl were added with stirring. After 2 hr additional stirring, the crystallized product was collected on a filter and washed with H_2O, *i*-PrOH, and Et_2O; yield 103 g (89%). *Anal.* ($C_{10}H_{12}N_5O_7P \cdot 2H_2O$) C, H, N. This material contained a trace of **1** as determined by tlc, but was suitable for further transformations. For enzymic evaluation, a portion of the Na salt of **2** was precipitated from H_2O with EtOH to give a chromatographically pure sample of **2**.

5-Amino-1-β-D-ribofuranosylimidazole-4-carboxamidoxime Cyclic 3′,5′-Phosphate (3) and *N*-Hydroxyadenosine Cyclic 3′,5′-Phosphate (4). A solution of 70 g of **2** (0.184 mol) in 90 ml of 2 *N* NaOH was poured into a refluxing solution of 700 ml of 2 *N* NaOH. After 10 min additional refluxing, ice was added to bring the temperature to 25°, and the pH was adjusted to 11 with Dowex 50-X8 (H^+). The resin was filtered and washed, and the filtrate and washings were passed through a 7 × 42 cm column of Dowex 1-X2 ($HCOO^-$ form, 100–200 mesh). After washing with H_2O, the column was eluted with a gradient using 4 l. of H_2O in the mixing chamber and 4 l. of 0.5 *N* HCOOH in the reservoir. Fractions containing **3**, which appeared after *ca.* 5 l. of eluate and began crystalling in the tubes, were evaporated to a small volume, diluted with EtOH, and chilled. The pure **3** was filtered and washed with EtOH; yield 28.0 g (43%). *Anal.* ($C_9H_{14}N_5O_7P \cdot H_2O$) C, H, N. After elution of **3**, the mixing chamber was filled with 4 l. of 1 *N* HCOOH and the reservoir with 4 l. of 2 *N* HCOOH, and elution was continued. Appropriate fractions containing **4** were pooled and evaporated to a small volume. Dilution with EtOH and chilling gave **4** (8.0 g, 12%). *Anal.* ($C_{10}H_{12}N_5O_7P \cdot H_2O$) C, H, N. Compounds **3** and **4** are most easily detected by tlc on cellulose plates (developed in MeCN–0.2 *N* NH_4Cl 3:2) which are sprayed with 1% $FeCl_3$ in dilute HOAc. With this spray, **2** gave an orange spot, **3** purple, and **4** a blue spot.

Inosine Cyclic 3′,5′-Phosphate 1-Oxide (5). A solution of 5.9 g of **2** (15.5 mmol), 7.0 g of $NaNO_2$ (101 mmol), 50 ml of H_2O, and 10 ml of AcOH was stirred for 2 hr at 0°, then 16 hr at ambient temperature. An additional 5 g of $NaNO_2$ and 5 ml of AcOH were added, and stirring was continued 24 hr. The solution was evaporated and AcOH was added, then evaporated. The solution was dissolved in 50 ml of H_2O and passed through a column of 500 ml of Dowex 50-X8 (H^+, 100–200 mesh). The product (0.70 g, 13%) crystallized directly from the fraction tubes. *Anal.* ($C_{10}H_{11}N_4O_8P$) C, H, N.

1-Methoxyadenosine Cyclic 3′,5′-Phosphate (6a). A solution of **2** (76.0 g, 0.200 mol) and 31 g (0.204 mol) of 1,5-diazabicyclo-[5.4.0]-5-undecene (DBU, Aldrich Chemical Co.) in 400 ml of DMSO was cooled to 15°, and 40 ml of MeI was added with vigorous stirring. After 30 min, the mixture had jelled; 2.5 l. of EtOH was added and the mixture was homogenized. The filtered product was resuspended in 2 l. of EtOH, filtered, and washed with EtOH, giving 71.0 g (96%) of chromatographically homogeneous **6a**, suitable for further transformations. A sample of **6a** was precipitated from aqueous MeOH with Et_2O for analysis. *Anal.* ($C_{11}H_{14}N_5O_7P \cdot 0.5\ H_2O$) C, H, N.

1-Ethoxyadenosine cyclic 3′,5′-phosphate (6b) was synthesized in 83% yield from **2** and EtI by the method described for **6a**. *Anal.* ($C_{12}H_{16}N_5O_7P \cdot H_2O$) C, H, N.

1-Benzyloxyadenosine Cyclic 3′,5′-Phosphate (6c). To a solution of 3.81 g of **2** (10 mmol), 1.52 g (10 mmol) of DBU, and 50 ml of DMSO was added 2.4 ml of $PhCH_2Br$. After 2-days stirring at ambient temperature the solution was diluted with 800 ml of Me_2CO. The filtered solid was recrystallized from aqueous EtOH: yield 2.11 g (48%). *Anal.* ($C_{17}H_{18}N_5O_7P \cdot 0.25H_2O$) C, H, N.

5-Amino-*O*-methyl-1-β-D-ribofuranosylimidazole-4-carboxamidoxime Cyclic 3′,5′-Phosphate (7a) and *N*-Methoxyadenosine Cyclic 3′, 5′-Phosphate (8a). Method A. A solution of 74 g of **6a** (206 mmol), 45 g of $NaHCO_3$ (536 mmol), and 300 ml of H_2O was refluxed for 45 min. The pH was adjusted to 2.5 with Dowex 50-X8 (H^+), and a water pump vacuum was applied to the solution while warm until CO_2 evolution ceased. The pH was readjusted to 10 with NaOH and the resin was filtered and washed. The combined filtrate and washings were subjected to anion exchange chromatography as in the preparation of **3** and **4**, giving 12.85 g (18%) of **7a** (recrystallized from aqueous EtOH) and 39.0 g (53%) of **8a** (precipitated from aqueous MeOH with Et_2O).

Method B. Compound **6a** (20 g, 54 mmol) was stirred 24 hr in 450 ml of 0.67 *N* NaOH, then worked up as in the synthesis of **3** and **4**; yield 12.20 g of **7a** (65%) and 1.70 g of **8a** (8.5%). *Anal.* **7a** ($C_{10}H_{16}N_5O_7P$) C, H, N; **8a** ($C_{11}H_{14}N_5O_7P \cdot 0.5H_2O$) C, H, N.

5-Amino-*O*-ethyl-1-β-D-ribofuranosylimidazole-4-carboxamidoxime cyclic 3′,5′-phosphate (7b) and *N*-ethoxyadenosine cyclic 3′,5′-phosphate (8b) were prepared from **6b** by method B in 56 and 5% yields, respectively. *Anal.* **7b** ($C_{11}H_{18}N_5O_7P \cdot 0.5\ H_2O$) C, H, N; **8b** ($C_{12}H_{16}N_5O_7P \cdot 0.5\ H_2O$) C, H, N.

5-Amino-*O*-benzyl-1-β-D-ribofuranosylimidazole-4-carboxamidoxime cyclic 3′,5′-phosphate (7c) and *N*-benzyloxyadenosine cyclic 3′,5′-phosphate (8c) were prepared from **6c** using method A, except that H_2O was replaced by 50% aqueous EtOH in the gradient chambers. The yield of **7c** and **8c** was 21 and 10%, respectively. *Anal.* **7c** ($C_{16}H_{20}N_5O_7P$) C, H, N; **8c** ($C_{17}H_{18}N_5O_7P \cdot H_2O$) C, H, N.

5-Amino-1-β-D-ribofuranosylimidazole-4-carboxamidine Cyclic 3′,5′-Phosphate (9). A solution of 5.0 g of **7a** (14.3 mmol) in 200 ml of H_2O, preheated to 60° and containing *ca.* 5 g of moist sponge nickel catalyst, was shaken with 2–3 atm of H_2 at 60° for 2 hr. The filtered solution was evaporated to dryness to give 3.75 g of **9** (82%). A sample was recrystallized from H_2O for analysis. *Anal.* ($C_9H_{14}N_5O_6P$) C, H, N.

5-Amino-1-β-D-ribofuranosylimidazole-4-carboxamide Cyclic 3′,5′-Phosphate[16] (10). A mixture of 4.0 g of **9** (12.5 mmol) and 100 ml of concentrated NH_4OH was heated in a bomb at 100° for 16 hr, then cooled and evaporated *in vacuo*. The residue was taken up in 100 ml of H_2O and applied to a 2.5 × 20 cm column of Dowex 1-X2 ($HCOO^-$ form, 100–200 mesh). After the column was washed with H_2O, it was eluted with a gradient of 1 l. of H_2O in the mixing chamber and 1 l. of 3 *N* HCOOH in the reservoir. Fractions containing the product, appearing near the end of the elution, were evaporated. Trituration of the residue with EtOH gave 2.90 g (68%). *Anal.* ($C_9H_{13}N_4O_7P \cdot H_2O$) C, H, N.

5-Amino-1-β-D-ribofuranosylthioimidazole-4-carboxamide Cyclic 3′,5′-Phosphate (12). To a frozen solution of 10 g of **7a** (28.7 mmol) in 25 ml of H_2O in a bomb was added a solution of 50 ml of liquid H_2S in 25 ml of pyridine. The sealed bomb was placed in an oil bath maintained at 60–70° for 5 hr, then cooled and opened. The solution, after being allowed to warm to room temperature, was diluted with 100 ml of H_2O, filtered, and evaporated to dryness. The residue was taken up in 200 ml of H_2O and filtered, and the pH was adjusted to 2.0 with HCl. The resulting precipitate was purified by dissolution in H_2O with sufficient NH_4OH added to bring the pH to 7, then acidification to pH 2.0. The filtered and dried product weighed 1.48 g (14.5%). *Anal.* ($C_9H_{13}N_4O_6PS \cdot H_2O$) C, H, N.

8-Phenyl-3-β-D-ribofuranosylimidazo[2,1-*i*]purine Cyclic 3′,5′-Phosphate (14). A suspension which contained **1** (4.0 g, 12 mmol), α-bromoacetophenone (6.0 g, 28 mmol), EtOH (20 ml), and 2 *M* NaOAc (20 ml) was stirred at 65° for 64 hr. The solution was diluted to 300 ml with H_2O and extracted with $CHCl_3$. The aqueous layer was passed through a Dowex 50 (H^+, 3 × 25 cm) column. The column was washed with H_2O until no uv absorption was detected in the eluate. Further elution with 50% aqueous EtOH removed the product **14**. The appropriate fractions were evaporated to a solid; yield 0.67 g (13%). *Anal.* ($C_{18}H_{15}N_5O_6P \cdot 1.5H_2O$) C, H, N.

1-Methyladenosine Cyclic 3′,5′-Phosphate (15). To a solution of 3.29 g of **1** (10 mmol) and 1.6 g of DBU (10.4 mmol) in 30 ml of DMSO was added 7 ml of MeI. After 2 hr stirring the solution was diluted with 200 ml of EtOH. The filtered product was recrystallized from aqueous EtOH, giving 0.48 g (13%). An additional 0.67 g (total yield 31%) was obtained by dilution of the initial mother liquors with Et_2O, followed by recrystallization. *Anal.* ($C_{11}H_{14}N_5O_6P \cdot 1.5H_2O$) C, H, N.

9-β-D-Ribofuranosylpurine-6(1*H*)-thione Cyclic 3′,5′-Phosphate[2,18] (11). To a frozen solution of 12.0 g of **8a** in 25 ml of H_2O and 5 ml of pyridine in a bomb was added a solution of 50 ml of liquid H_2S and 25 ml of pyridine. The sealed bomb was kept at 60° for 48 hr, then cooled, opened, and allowed to warm to room temperature. The resulting mixture was diluted with 100 ml of H_2O, filtered, and evaporated. The residue was taken up in 100 ml of H_2O, filtered, and applied to a column of 1000 ml of Dowex 50-X8 (H^+, 100–200 mesh), and the column was eluted with water. The product (6.3 g) crystallized in the fraction tubes; additional product (3.45 g) was obtained upon evaporation of the appropriate fractions; total yield 9.75 g (82%). This product was identical in its uv and pmr spectra and tlc mobility to that previously prepared.[2]

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