

reported in Table III. Carbon-13 nmr data were collected using a Varian Associates XL-100-15 spectrometer operating in the Fourier transformer mode at 25.160 MHz.

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A New Purine Ring Closure and the Synthesis of 2-Substituted Derivatives of Adenosine Cyclic 3',5'-Phosphate¹

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Abstract: A new and useful procedure for closure of the purine ring under relatively mild conditions is reported. Treatment of 5-amino-1- β -D-ribofuranosylimidazole-4-carboxamide cyclic 3',5'-phosphate (**2**) with aldehydes under mild oxidative conditions has provided a series of unusual 2-alkyl- and aryladenosine cyclic 3',5'-phosphates. Trifluoroacetamide and **2** gave 2-CF₃-cAMP. Triethyl orthoacetate and orthopropionate and **2** gave 2-methyl- and 2-ethyl-cAMP, respectively. Ring closure of **2** with 1,1'-carbonyldiimidazole gave 2-hydroxy-cAMP, and 2 and 1,1'-thiocarbonyldiimidazole gave 2-thio-cAMP. Methylation of the latter compound gave 2-MeS-cAMP. Treatment of **2** with nitrous acid gave 2-aza-cAMP. The new cAMP derivatives are activators of a cAMP-dependent protein kinase and inhibit the hydrolysis of cAMP by phosphodiesterase.

We wish to report a new procedure for ring closure of an imidazole precursor to provide 2-substituted adenines under conditions which may have implications in the possible biochemical synthesis of such naturally occurring modified nucleic acid constituents as 2-methyladenine.²

The desire for derivatives of adenosine cyclic 3',5'-phosphate (**1**, cAMP) with substituents in the 2 position of the adenine ring prompted a search for new synthetic procedures which would allow the introduction of such a substituent at the final step in the synthetic scheme. Classically, 2-substituted adenine derivatives have been synthesized from the appropriate 2-substituted 4,5,6-triaminopyrimidine or related derivatives by introduction of the C₈ carbon fragment.³⁻⁵ This method is not generally useful in the direct synthesis of adenines with a substituent at the 9 position, a group comprising almost all adenine nucleosides and nucleotides of biological interest. Routes to 2-substituted adenines from 5(4)-aminoimidazole-4(5)-carbonitriles were available⁶ but were of limited scope and required, for our purposes, difficultly obtainable intermediates.

We now wish to report methods which provide a wide variety of 2-substituted derivatives of adenine with a preintroduced substituent on the 9 position. In particular, the key intermediate 5-amino-1- β -D-ribo-

furanosylimidazole-4-carboxamide cyclic 3',5'-phosphate⁷ (**2**) has been treated with various reagents under mild conditions to furnish directly the desired 2-substituted cAMP derivatives. Of particular interest is the reaction of **2** with aldehydes under oxidative conditions. This new procedure, which should be generally applicable to 1-substituted 5-aminoimidazole-4-carboxamides, readily provides a multitude of 2-alkyl and 2-aryl-9-substituted adenines.

The imidazole nucleotide **2** was also converted to 2-thio- and 2-hydroxy-cAMP and to 2-aza-cAMP by modifications of known procedures.

The attractive feature of these methods, for the purpose of this study, is the introduction of the 2 substituent in the final step of the scheme. This provides a direct route to the required cAMP derivative and avoids the more lengthy route of purine \rightarrow nucleoside \rightarrow 5'-nucleotide \rightarrow cyclic 3',5'-nucleotide⁸ for each compound.

The rapid proliferation, in recent years, of literature regarding the biochemical and physiological functions of cAMP has encouraged studies in these laboratories of the effects of modification of the cAMP molecule on these various functions. Indeed, such compounds should provide useful tools for understanding of the biological mechanisms through which cAMP operates. We have previously studied substituents in the 1,⁷ 6,^{7,9,10} and 8^{11,12} positions of the purine ring of cAMP,

(7) R. B. Meyer, D. A. Shuman, R. K. Robins, J. P. Miller, and L. N. Simon, *J. Med. Chem.*, **16**, 1319 (1973).

(8) For typical examples, see (a) M. Smith, G. I. Drummond, and H. G. Khorana, *J. Amer. Chem. Soc.*, **83**, 698 (1961); (b) R. K. Borden and M. Smith, *J. Org. Chem.*, **31**, 3248 (1966); (c) T. Posternak, I. Marcus, A. Baggai, and G. Cehovic, *C. R. Acad. Sci., Ser. D*, **269**, 2409 (1969).

(9) R. B. Meyer, D. A. Shuman, R. K. Robins, R. J. Bauer, M. K. Dimmitt, and L. N. Simon, *Biochemistry*, **11**, 2704 (1972).

(10) K. H. Boswell, J. P. Miller, D. A. Shuman, R. W. Sidwell, L. N. Simon, and R. K. Robins, *J. Med. Chem.*, **16**, 1075 (1973).

(1) A portion of this work was presented at the Fourth International Congress of Heterocyclic Chemistry, Salt Lake City, Utah, July, 1973.

(2) (a) H. W. Dion, D. G. Calkins, and J. J. Pffner, *J. Amer. Chem. Soc.*, **76**, 948 (1954); (b) J. W. Littlefield and D. B. Dunn, *Biochem. J.*, **70**, 642 (1958).

(3) E. C. Taylor, O. Vogl, and C. C. Cheng, *J. Amer. Chem. Soc.*, **81**, 2442 (1959).

(4) R. K. Robins in "Heterocyclic Compounds," R. C. Elderfield, Ed., Wiley, New York, N. Y., 1967, p 208.

(5) J. H. Lister, "Fused Pyrimidines," D. J. Brown, Ed., Wiley-Interscience, New York, N. Y., 1971, p 31.

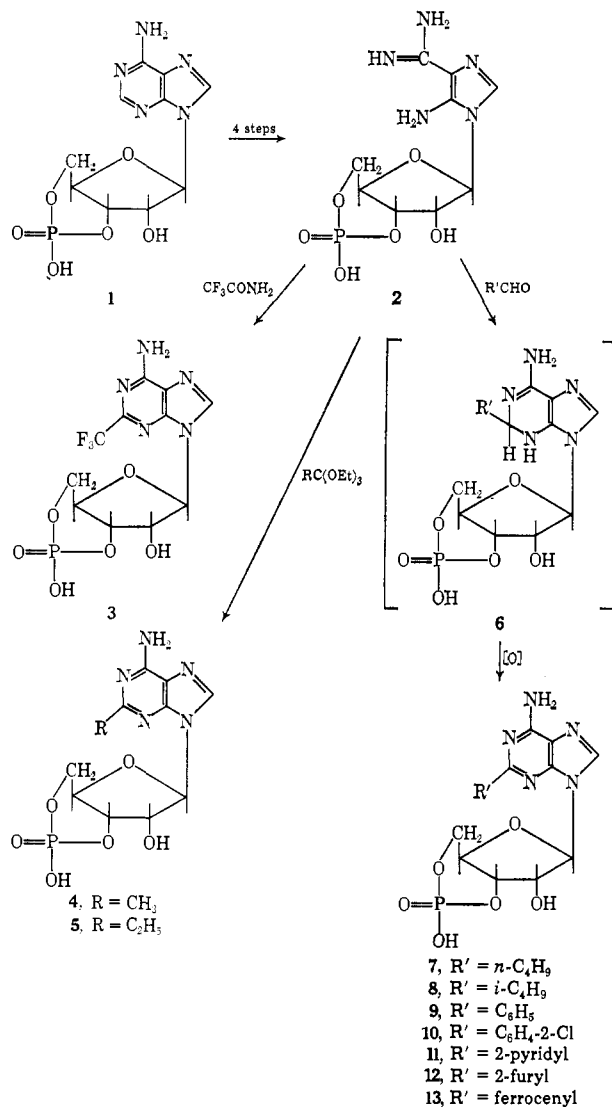
(6) E. C. Taylor and A. L. Borrer, *J. Org. Chem.*, **26**, 4967 (1961).

and, with the methods presented herein, 2-substituted cAMP derivatives have now been prepared for further biochemical study.

2-Alkyl- and 2-Aryl-cAMP (Scheme I)

The only previous report of a 2-alkyladenine from an aminoimidazolecarboxamide directly was the synthesis of 2-trifluoromethyladenine from 5(4)-aminoimidazole-4(5)-carboxamide and refluxing trifluoroacetamide.¹³ 5-Amino-1- β -D-ribofuranosylimidazole-4-carboxamide cyclic 3',5'-phosphate (**2**) was quite insoluble in nonaqueous media as its zwitterion and it was found necessary to form its salt with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) or 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) for the sake of solubility. Thus the DBU salt of **2** and trifluoroacetamide at 135° in *N,N,N',N'*-tetramethylurea solution gave 2-trifluoromethyladenosine cyclic 3',5'-phosphate (**3**).

Scheme I



The latter method is limited, however, to ring closure with strongly electron deficient carboxamides, and a more general method was sought for ring closure of **2**.

(11) K. Muneyama, R. J. Bauer, D. A. Shuman, R. K. Robins, and L. N. Simon, *Biochemistry*, **10**, 2390 (1971).

(12) J. P. Miller, K. H. Boswell, K. Muneyama, L. N. Simon, R. K. Robins, and D. A. Shuman, *Biochemistry*, **12**, 5310 (1973).

(13) A. Ginner-Sorolla and A. Bendich, *J. Amer. Chem. Soc.*, **80**, 5744 (1958).

Treatment of **2** with trialkyl orthoesters was investigated as a route to 2-alkyl-cAMP derivatives. In fact, trimethyl orthoacetate and **2** (as the DBU salt in Me₂SO) readily gave 2-methyladenosine cyclic 3',5'-phosphate (**4**); triethyl orthopropionate and **2** gave 2-ethyladenosine cyclic 3',5'-phosphate (**5**). This method was found to be limited to the lower alkyl orthoesters; trimethyl orthobenzoate and **2** failed to give an acceptable amount of the 2-phenyl derivative.

Condensation of **2** with aldehydes under oxidative conditions provided, however, a novel procedure of closure of a purine ring which was found to be remarkably general; the imidazole nucleotide **2** will condense with a wide variety of aldehydes under very mild conditions to give the intermediate 2,3-dihydropurine (**6**). Evidence for this intermediate was provided by addition of CH₃CHO to a solution of **2** in NaOD. The pmr of this solution showed a shift of 0.08 ppm downfield in the imidazole proton resonance and the appearance of a poorly defined pair of doublets centered at δ 1.51 for the CH₃ protons of the stereoisomeric pair of 2,3-dihydro-2-methyl-cAMP's. The $\lambda_{\max}^{\text{pH } 7}$ of this solution was 289 nm, compared to 280 nm for **2**.

It was found necessary to deprotonate the amidine moiety of **2** by addition of 1 equiv of NaOH before the reaction with the requisite aldehyde would occur. The amidine, however, was unstable at elevated temperature as the Na salt and some hydrolysis to the corresponding carboxamide (5-amino-1- β -D-ribofuranosylimidazole-4-carboxamide cyclic 3',5'-phosphate, AICAR cyclic 3',5'-phosphate⁷) was unavoidable. The dehydrogenation of the dihydropurine intermediate *in situ* could be accomplished by several routes. Refluxing aqueous MeOH and Pd/C was efficient in the dehydrogenation, as was chloranil in aqueous DMF and atmospheric oxygen in aqueous alcohol. When the sample of **2** and CH₃CHO in D₂O/NaOH was treated with Pd/C and refluxing MeOH-*d*₄ or with chloranil, the peaks of the dihydropurine were replaced by the expected C₈-H and C₂-CH₃ resonances of 2-methyladenosine cyclic 3',5'-phosphate.

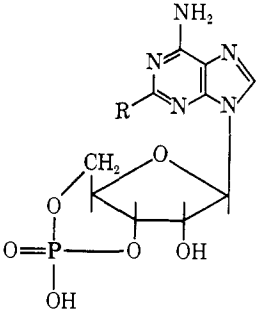
By these methods, 2-*n*-butyl (**7**), 2-isobutyl (**8**), 2-phenyl (**9**), 2-*o*-chlorophenyl (**10**), 2-(2-pyridyl) (**11**), 2-(2-furyl) (**12**), and 2-ferrocenyladenosine cyclic 3',5'-phosphate (**13**) were prepared. The physical constants of these and other compounds prepared from **2** are given in Table I. The 2-aryladenine derivatives are highly fluorescent, a property previously reported³ for this class of compounds.

The condensation of aldehydes with the 5-aminoimidazole-4-carboxamide moiety under oxidative conditions appears to be quite general and should prove useful in the synthesis of many adenines substituted at the 2 position with carbon-linked substituents.

2-Hydroxy- and 2-Thio-cAMP

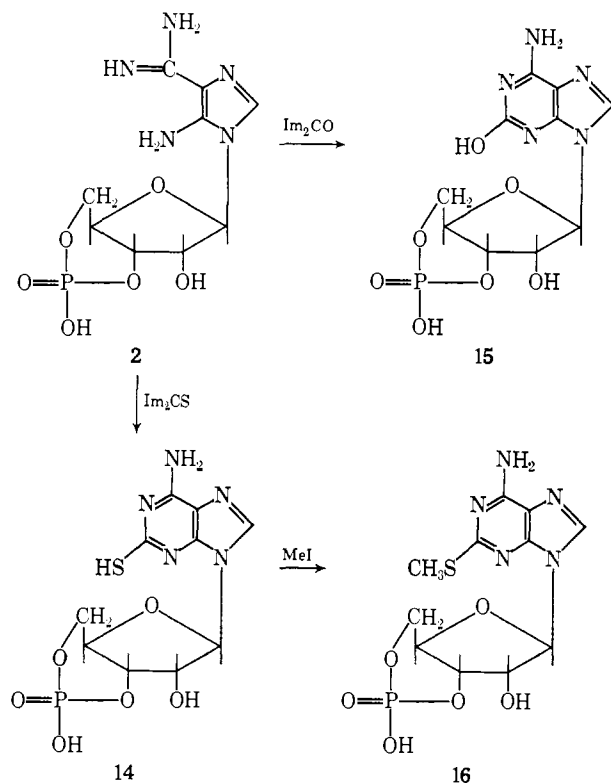
Cyclization of **2** with thiophosgene or CS₂¹⁴ to give 2-thioadenosine cyclic 3',5'-phosphate (**14**) gave dark mixtures which were difficult to purify. The use of 1,1'-thiocarbonyldiimidazole in Me₂SO solution, however, as shown in Scheme II, furnished a facile route to **14**. It was again found most convenient to use the DBU or

(14) R. M. Cresswell and G. B. Brown, *J. Org. Chem.*, **28**, 2560 (1963).

Table I. Physical Constants of the 2-Substituted Adenosine Cyclic 3',5'-Phosphates


No.	R	R_f^a		λ_{max} , nm ($\epsilon \times 10^{-3}$) ^b		
		A	B	pH 1	pH 7	pH 11
3	CF ₃	0.60	0.80	258 (12.9)		258 (13.3)
4	CH ₃	0.15	0.46	256 (13.5)		261 (14.4)
5	C ₂ H ₅	0.24	0.56	256 (13.9)		261 (15.0)
7	<i>n</i> -C ₄ H ₉	0.43	0.71	257 (14.5)		261 (15.6)
8	<i>i</i> -C ₄ H ₉	0.45	0.74	257 (14.9)		261 (15.1)
9	C ₆ H ₅	0.47	0.63	270 (16.5), 287 sh (13.7)		238 (24.3), 268 sh (14.4)
10	C ₆ H ₄ -2-Cl	0.55	0.74	263 (17.6), 283 sh (10.3)		262 (15.9)
11	2-C ₅ H ₄ N	0.17	0.46	232 (16.7), 263 (12.4), 329 (8.6)		231 (22.4), 261 (13.6), 294 (10.4)
12	2-C ₄ H ₃ O	0.28	0.50	279 (15.1), 318 (20.8)		253 sh (18.4), 258 (19.3), 287 sh (18.0), 299 (20.0)
13	C ₁₀ H ₉ Fe	0.63	0.79	265 (13.4), 302 (14.2)		231 (26.9), 259 (14.3), 292 (14.2)
14	SH	0.05	0.11	231 (15.1), 288 (21.6)	229 (16.4), 286 (20.6)	241 (21.4), 283 (16.7)
15	OH	0.04	0.12	234 (6.7), 280 (12.7)	247 (9.6), 292 (11.9)	252 (7.3), 283 (10.9)
16	SCH ₃	0.23	0.50	268 (16.7)		234 (21.8), 272 (14.7)
17	2-Aza	0.147	0.33	252 (7.1), 281 (3.4)		255 (7.1), 296 (5.2)

^a R_f on E. Merck 254-F Cellulose plates in solvent system A (MeCN/0.1 N NH₄Cl, 3:1) or B (*i*-PrOH/concd NH₄OH/H₂O, 7:1:2). In these systems, **1** has $R_f(A) = 0.12$ and $R_f(B) = 0.40$ and **2** has $R_f(A) = 0.08$ and $R_f(B) = 0.19$. ^b λ_{max} of **2** is 280 nm (ϵ 11,100) at pH 1, 7, and 11. pH 7 values are the same as pH 11 unless otherwise indicated. sh refers to a shoulder.

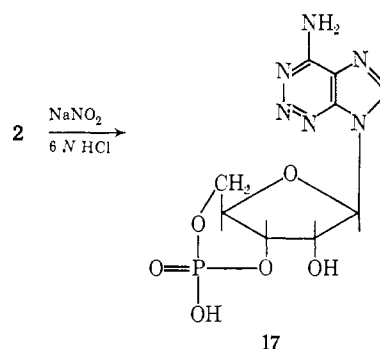
Scheme II

DBN salts of **2** for purposes of solubility. 1,1'-Carbonyldiimidazole and **2** under the same conditions gave 2-hydroxyadenosine cyclic 3',5'-phosphate (**15**, isoguanosine cyclic 3',5'-phosphate).

Methylation of **14** was readily accomplished with methyl iodide in aqueous alkali, giving 2-(methylthio)adenosine cyclic 3',5'-phosphate (**16**).

2-Aza-cAMP

Finally, the 2-aza analog of cAMP was prepared (Scheme III). The closure of the ribonucleoside

Scheme III

analog of **2**, 5-amino-1- β -D-ribofuranosylimidazole-4-carboxamide, to 2-azaadenosine has been reported by addition of aqueous NaNO₂ to the HCl salt of imidazole nucleoside.^{15,16} We employed the conditions of Kawana, *et al.*¹⁷ (cold 6 N HCl and NaNO₂), and obtained 2-aza-cAMP (**17**, 4-amino-7- β -D-ribofuranosyl-

(15) J. A. Montgomery and H. J. Thomas, *Chem. Commun.*, 458 (1969).

(16) J. A. Montgomery and H. J. Thomas, *J. Med. Chem.*, **15**, 182 (1972).

(17) M. Kawana, G. A. Ivanovics, R. J. Rousseau, and R. K. Robins, *J. Med. Chem.*, **15**, 841 (1972).

imidazo[4,5-*d*]-*v*-triazine cyclic 3',5'-phosphate) in 76% yield from **2**.

Biochemical

All the new 2-substituted cAMP derivatives were examined for their ability to activate a cAMP-dependent protein kinase from bovine brain, as measured by incorporation of $^{32}\text{PO}_4^{3-}$ from ATP- γ - ^{32}P into histone as previously described.¹¹ The best activity of the compounds reported here was found with 2-*n*-butyl-cAMP (**7**), which was half as effective as cAMP itself. 2-Methyl-cAMP (**4**) and 2-ethyl-cAMP (**5**) were found to be potent inhibitors of cAMP phosphodiesterase from rabbit lung. 2-Methyl-cAMP caused 50% inhibition of the hydrolysis of cAMP at a concentration of 1.5×10^{-6} M when assayed at a substrate concentration of 1.7×10^{-7} M as previously described.¹⁸ A detailed account of the biochemistry of these and related compounds will be the subject of a subsequent communication.

Experimental Section

UV spectra were recorded on a Cary 15. Nmr spectra were recorded on a Hitachi Perkin-Elmer R-20A. Elemental analyses were performed by Heterocyclic Chemical Corp., Harrisonville, Mo., or Galbraith Laboratories, Inc., Knoxville, Tenn. Nmr and ir spectra and paper electrophoresis were consistent with the reported structures. The chloranil in these procedures was freshly recrystallized. Thin-layer chromatograms were run on E. Merck Cellulose-254F plates, developed with either solvent system A (MeCN/0.1 N NH₄Cl, 3:1) or B (*i*-PrOH/concd NH₄OH/H₂O, 7:1:2).

In all cases the anomeric proton appeared as an apparent singlet in the pmr, which is indicative of the presence of the cyclic 3',5'-phosphate.^{9,19}

2-Trifluoromethyladenosine Cyclic 3',5'-Phosphate (3). A mixture of 1.0 g (3.14 mmol) of **2**, 0.4 g (3.22 mmol) of 1,5-diazabicyclo[4.3.0]non-5-ene (DBN), and 3.0 g (26.6 mmol) of trifluoroacetamide was surrounded by an oil bath at 135° and stirred for 30 min. After addition of 5 ml of *N,N,N',N'*-tetramethylurea the mixture was stirred at 135° an additional 4 hr and then poured into 100 ml of Et₂O. The liquid was decanted, and the residue was taken up in 50 ml of H₂O and percolated through a 2.6 × 20 cm column of Dowex 50-X8 (H⁺, 100–200 mesh) and washed with H₂O. The first 500 ml of eluate was evaporated to dryness, and the residue was taken up in 20 ml of EtOH. The solution was diluted with 40 ml of EtOAc and filtered. On standing, 0.59 g (47%) of product was deposited.

Anal. Calcd for C₁₁H₁₀F₃N₅O₆P: C, 33.34; H, 2.54; F, 14.39; N, 17.68. Found: C, 33.31; H, 2.71; F, 14.67; N, 17.45.

2-Methyladenosine Cyclic 3',5'-Phosphate (4). A mixture of 3.0 g (9.4 mmol) of **2**, 2.0 g (13 mmol) of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), and 20 ml of Me₂SO was warmed to solution and 3 ml (16.5 mmol) of triethyl orthoacetate was added. The solution was surrounded by an oil bath at 150° and stirred for 45 min. The hot solution was poured into 100 ml of H₂O and 1 ml of formic acid, and this solution was passed through a 2.6 × 20 cm column of Dowex 1-X2 (formate form, 100–200 mesh). After washing with H₂O, the column was eluted with a gradient of 2 l. of H₂O in the mixing chamber and 2 l. of 5 N formic acid in the reservoir. Evaporation of the appropriate fractions gave 2.54 g (75%) of product.

Anal. Calcd for C₁₁H₁₃N₅O₆P·H₂O: C, 36.57; H, 4.46; N, 19.39. Found: C, 36.90; H, 4.54; N, 19.69.

2-Ethyladenosine Cyclic 3',5'-Phosphate (5). Treatment of 0.50 g (1.57 mmol) of **2** with triethyl orthopropionate as described for the synthesis of **4** gave, after the ion-exchange chromatography, 0.35 g (66%) of **8**.

Anal. Calcd for C₁₂H₁₅N₅O₆P·H₂O: C, 38.40; H, 4.84; N, 18.66. Found: C, 38.57; H, 4.73; N, 18.77.

Reaction of 5-Amino-1-β-D-ribofuranosylimidazole-4-carboxamide Cyclic 3',5'-Phosphate (2) with Acetaldehyde. A solution of **2** (0.16 g, 0.5 mmol) and 0.050 g (0.5 mmol) of 40% NaOD was prepared in 1.0 ml of D₂O. The pmr of this showed a singlet at 7.49 for the C₂ proton relative to internal DSS. When the spectra was recorded immediately after addition of 0.015 g (0.34 mmol) of CH₃CHO to one-half of this sample, this peak was entirely replaced by a new one at δ 7.57, and a new, poorly defined, pair of doublets had appeared centered at δ 1.51. Additionally, a doublet for what was the apparent hydrate of acetaldehyde was seen at δ 1.25. The λ_{max} of this solution at pH 7 was 289 nm.

After either addition of 1 equiv of chloranil in 0.5 ml of Me₂SO-*d*₆ or refluxing 30 min with 0.5 ml of MeOH-*d*₄ and 0.020 g of 10% Pd/C, the peaks at δ 7.57 and 1.51 were entirely replaced by new peaks at ~δ 8.2 and 2.6, the expected C₅-H and C₂-CH₃ resonances of 2-methyladenosine cyclic 3',5'-phosphate.

2-*n*-Butyladenosine Cyclic 3',5'-Phosphate (7). A mixture of 4.0 g (12.5 mmol) of **2**, 2.0 g of DBU (13.0 mmol), 30 ml of H₂O, and 40 ml of EtOH was brought to solution by refluxing 5 min. Following addition of 0.50 g of 10% Pd/C, a solution of 3.0 ml (28.2 mmol) of *n*-valeraldehyde in 25 ml of EtOH was added under reflux. After an additional 1 hr of reflux, the mixture was filtered and the filtrate was evaporated. The residue was taken up in MeOH and filtered and the filtrate was evaporated. The residue was taken up in MeOH, filtered, and again evaporated. The residue was taken up in 200 ml of H₂O and passed through a 16 × 4 cm column of Dowex 1-X2 (formate form, 100–200 mesh). After washing with H₂O the column was eluted with a gradient of 2 l. of H₂O in the mixing chamber and 2 l. of 3 N formic acid in the reservoir: 23 ml fractions were collected. Evaporation of the fractions (47–85) containing the product gave a residue which crystallized upon addition of EtOH; yield, 3.35 g (65%).

Anal. Calcd for C₁₄H₂₀N₅O₆P·1.5H₂O: C, 40.78; H, 5.62; N, 16.99. Found: C, 41.05; H, 5.68; N, 17.08.

2-Isobutyladenosine cyclic 3',5'-phosphate (8) was prepared from isovaleraldehyde and **2** as in the procedure for **7**; yield, 54%.

Anal. Calcd for C₁₄H₂₀N₅O₆P·H₂O: C, 41.69; H, 5.50; N, 17.37. Found: C, 41.68; H, 5.65; N, 17.64.

2-Phenyladenosine Cyclic 3',5'-Phosphate (9). (A) A mixture of 2.0 g (6.3 mmol) of **2**, 0.7 g (7 mmol) of Et₃N, 0.30 g of 10% Pd/C, 0.8 ml (7.9 mmol) of benzaldehyde, and 30 ml of 50% aqueous EtOH was refluxed for 16 hr. The filtered solution was evaporated, and the residue was taken up in 50 ml of water. Adjustment of the pH to 2.0 with HCl caused crystallization of the product (0.55 g, 20%).

Anal. Calcd for C₁₅H₁₅N₅O₆P·H₂O: C, 45.39; H, 4.29; N, 16.55. Found: C, 45.43; H, 4.38; N, 16.57.

(B) A solution of 0.66 g (2 mmol) of **2**, 1 ml of 2 N NaOH, 10 ml of EtOH, and 0.25 ml (2.5 mmol) of benzaldehyde was vigorously stirred 16 hr in an open flask. After evaporation of the solvent, the residue was taken up in 20 ml of H₂O; addition of 1 ml of 2 N HCl deposited 0.49 g (60%) of pure **13**.

2-(2-Chlorophenyl)adenosine Cyclic 3',5'-Phosphate (10). A solution of 1.0 g (3.1 mmol) of **2**, 1.5 ml of 2 N NaOH, 5 ml of H₂O, 15 ml of DMF, and 1 ml (8.9 mmol) of *o*-chlorobenzaldehyde was stirred 3 hr, and then a solution of 1 g (4.1 mmol) of chloranil in 10 ml of DMF was added. After 1 hr of additional stirring, the solution was evaporated and partitioned between 100 ml of EtOAc and 100 ml of H₂O. The aqueous phase was diluted with 100 ml of EtOH and passed through a column of 150 ml of Dowex 50-X2 (100–200 mesh, H⁺ form). The product was eluted with 50% aqueous MeOH. Evaporation of product-containing fractions and precipitation of the residue from aqueous EtOH and Et₂O gave 1.06 g (81%) of product.

Anal. Calcd for C₁₅H₁₃ClN₅O₆P·H₂O: C, 41.98; H, 3.74; N, 15.30. Found: C, 42.05; H, 3.75; N, 15.48.

2-(2-Pyridyl)adenosine Cyclic 3',5'-Phosphate (11). A mixture of 0.32 g (1 mmol) of **2**, 0.154 g (1 mmol) of DBU, 10 ml of H₂O, 10 ml of MeOH, 0.100 g of 10% Pd/C, and 1.28 g (1.2 mmol) of pyridine-2-carboxaldehyde was refluxed 1 hr, then filtered, evaporated, and taken up in H₂O. The crystals collected after adjustment of the pH of the solution to 2 were recrystallized from H₂O to give 0.270 g (66%) of product.

Anal. Calcd for C₁₅H₁₅N₆O₆P·H₂O: C, 42.46; H, 4.04; N, 19.81. Found: C, 42.93; H, 4.12; N, 19.63.

2-(2-Furyl)adenosine cyclic 3',5'-phosphate (12) was prepared from **2** and furan-2-carboxaldehyde in 73% yield as described in the preparation of **10**.

(18) J. P. Miller, D. A. Shuman, M. B. Scholten, M. K. Dimmitt, C. M. Stewart, T. A. Khwaja, R. K. Robins, and L. N. Simon, *Biochemistry*, **12**, 1010 (1973).

(19) C. D. Jardetzky, *J. Amer. Chem. Soc.*, **84**, 62 (1962).

Anal. Calcd for $C_{14}H_{14}N_5O_7P \cdot H_2O$: C, 40.68; H, 3.90; N, 16.95. Found: C, 40.87; H, 3.85; N, 16.96.

2-Ferrocenyadenosine Cyclic 3',5'-Phosphate (13). An open flask containing a solution of 1.0 g (3.1 mmol) of **2**, 1.5 ml of 2 *N* NaOH, 5 ml of H_2O , 20 ml of MeOH, and 0.70 g (3.3 mmol) of ferrocenecarboxaldehyde was vigorously stirred for 3 days. The evaporated mixture was taken up in 30 ml of 50% aqueous EtOH and warmed to ca. 80°. Addition of 1.5 ml of 2 *N* HCl and cooling gave a solid which was further purified on an Avicel (Brinkman) microcrystalline cellulose column (2.5 × 20 cm), packed in and eluted with *i*-PrOH/concd NH_4OH/H_2O , 8:1:1. Evaporation of the appropriate fractions and acidification of an aqueous solution of the residue gave 0.30 g (19%).

Anal. Calcd for $C_{22}H_{22}FeN_5O_8P$: C, 46.81; H, 3.93; N, 13.65. Found: C, 46.89; H, 4.06; N, 13.49.

2-Thioadenosine Cyclic 3',5'-Phosphate (14). A mixture of 3.5 g (11.3 mmol) of **2**, 1.60 g (10.5 mmol) of DBU, and 50 ml of Me_2SO was brought to solution by heating then was cooled to 0°. With stirring, 2.0 g (11.3 mmol) of 1,1'-thiocarbonyldiimidazole was added. After 10 min of stirring, the solution was stored at -20° for 20 hr, then an additional 1.0 g of 1,1'-thiocarbonyldiimidazole was added. After 30 min of stirring at ambient temperature, the solution was diluted with 100 ml of H_2O and 1 ml of formic acid, then passed through a 2.6 × 20 cm column of Dowex 1-X2 (formate form, 100–200 mesh). The column was washed with H_2O , then eluted with a gradient of 900 ml of 1 *N* formic acid in the mixing chamber and 900 ml of 1 *N* formic acid + 1 *N* ammonium formate in the reservoir. The product began to appear near the end of the gradient, and elution of the product was completed with 1 *N* formic acid + 2 *N* ammonium formate. Fractions containing product were passed through a column containing 1 l. of Dowex 50-X8 (H^+ , 100–200 mesh). Evaporation of the eluate to dryness gave 2.05 g (48%) of product.

Anal. Calcd for $C_{10}H_{12}N_5O_8PS \cdot H_2O$: C, 31.58; H, 3.71; N, 18.42. Found: C, 31.87; H, 3.59; N, 18.58.

2-Hydroxyadenosine Cyclic 3',5'-Phosphate (15). A mixture of 2.0 g (6.3 mmol) of **2**, 0.80 g (6.45 mmol) of DBN, and 10 ml of Me_2SO was brought to solution by heating. To this was added, with stirring at 25°, 1.0 g (6.2 mmol) of 1,1'-carbonyldiimidazole. After 30 min of stirring another 1.0 g of 1,1'-carbonyldiimidazole was added, and stirring was continued an additional 30 min. The solution was diluted with 50 ml of H_2O and 1 ml of formic acid and passed through a 2.6 × 10 cm column of Dowex 1-X2 (formate

form, 100–200 mesh). After washing with H_2O , the column was eluted with a gradient of 1 l. of H_2O in the mixing chamber and 1 l. of 5 *N* formic acid in the reservoir. Evaporation of fractions containing product, which appeared between 750 and 1250 ml of eluate, gave 0.79 g (33%).

Anal. Calcd for $C_{10}H_{12}N_5O_7P \cdot 2H_2O$: C, 31.50; H, 4.23; N, 18.37. Found: C, 31.42; H, 4.29; N, 18.44.

2-Methylthioadenosine 3',5'-Cyclic Phosphate (16). A mixture of 1.8 g (4.5 mmol) of **14**, 5 ml of 2 *N* NaOH, 2 ml of MeI (32 mmol), 20 ml of H_2O , and 20 ml of MeOH was stirred 2 hr. The solution was evaporated *in vacuo*, taken up in 100 ml of H_2O , and passed through a column of 50 ml of Dowex 1-X2 (formate, 100–200 mesh). The product appeared as the major component upon elution with a gradient of 1 l. of 1 *N* formic acid in the mixing chamber and 1 l. of 5 *N* formic acid in the reservoir. To remove a trace of impurity, the product, isolated after evaporation of the above fractions, was taken up in water and passed onto a 200 ml column of Dowex 50-X8 (H^+ , 100–200 mesh), which was washed in the 500 ml of H_2O then 1 l. of 0.5 *N* formic acid. Fractions containing product were evaporated, giving 0.64 g (38%).

Anal. Calcd for $C_{11}H_{14}N_5O_8PS \cdot 1.5H_2O$: C, 32.84; H, 4.26; N, 17.41. Found: C, 32.98; H, 4.57; N, 17.49.

4-Amino-7-β-D-ribofuranosylimidazo[4,5-*d*]-*v*-triazine Cyclic 3',5'-Phosphate (17, 2-Azaadenosine Cyclic 3',5'-Phosphate). Compound **2** (1.5 g 4.7 mmol) was dissolved in 92 ml of 6 *N* HCl, pre-cooled to -25°, and a solution of $NaNO_2$ (370 mg, 6.2 mmol) in 14 ml of H_2O was added dropwise with stirring over 25 min. The temperature was maintained at -25°, stirring was continued an additional 40 min, and 30 ml of EtOH was added. With cooling, the solution was neutralized with concentrated NH_4OH . The filtered solution was passed through a 5.5 × 46 cm column of Dowex 50-X8 (H^+ , 100–200 mesh) and the column was eluted with H_2O . The appropriate fractions were combined and evaporated to dryness, giving, after trituration with EtOH, 1.19 g (76%).

Anal. Calcd for $C_9H_{11}N_6O_6P$: C, 32.73; H, 3.35; N, 25.45. Found: C, 32.54; H, 3.47; N, 25.23.

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Polynucleotides. XXIV.¹ Synthesis and Properties of a Dinucleoside Monophosphate Derived from 8,2'-*O*-Cycloadenosine

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Abstract: A dinucleoside monophosphate of *O*-cycloadenosine, 8,2'-anhydro-8-oxy-9-β-D-arabinofuranosyladenine phosphoryl-(3'-5')-8,2'-anhydro-8-oxy-9-β-D-arabinofuranosyladenine (Ib), was synthesized from 5'-monomethoxytrityl-8,2'-anhydro-8-oxy-9-β-D-arabinofuranosyladenine (VI) and *N*⁶-3'-diacetyl-8,2'-anhydro-8-oxy-9-β-D-arabinofuranosyladenine (VIII) using dicyclohexylcarbodiimide as the condensing reagent. From the uv spectra taken under various conditions of temperature and salt concentration, it was deduced that compound I had a well-stacked thermally stable conformation. CD spectra of I, which have curves symmetrically reversed from those of usual ApA, suggest a stacking of two adenine moieties along the left-handed screw axis, as has been previously observed in *S*-cyclonucleoside oligomers. It is suggested that direction of the rotation of the screw axis and the conformational stability of cyclonucleoside oligomers are dependent on the torsion angle of bases in the component nucleosides.

We have synthesized previously a dinucleoside monophosphate^{2,3} and oligomers⁴ of 8,2'-an-

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hydro-8-mercapto-9-β-D-arabinofuranosyladenine (8,2'-*S*-cycloadenosine, A⁸) (Ia) (Chart I). Compared with

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