13, 664 (1970); (e) G. W. Moersch, D. F. Morrow, and W. A. Neuklis, *ibid.*, **10**, 154 (1967); (f) H. P. S. Chawla, P. K. Grover, N. Anand, V. P. Kamboj, and A. B. Kar, *ibid.*, **13**, 54 (1970); (g) R. R. Crenshaw, A. T. Jeffries, G. M. Luke, L. C. Cheney, and G. Bialy, *ibid.*, **14**, 1185 (1971); (h) R. R. Crenshaw, G. M. Luke, T. A. Jenks, G. Bialy, and M. E. Bierwagen, *ibid.*, **15**, 1162 (1972).
(5) An account of this area is described in "Contraception", D. Lednicer, Ed., Marcel Dekker, New York, N.Y., 1969.
(6) R. Breslow, T. Eicher, A. Krebs, R. A. Peterson, and J. Posner, *J. Am. Chem. Soc.*, **87**, 1320 (1965).
(7) L. A. Carpino and L. V. McAdams, III, *J. Am. Chem. Soc.*, **87**, 5804 (1965); L. A. Carpino and R. H. Rynbrandt, *ibid.*, **88**, 5682 (1966).
(8) J. Ciabattoni and G. A. Berchtold, *J. Org. Chem.*, **31**, 1336 (1966).
(9) R. West, D. C. Zecher, and W. Goyert, *J. Am. Chem. Soc.*, **92**, 149 (1970); T. Eicher and A. H. Hansen, *Chem. Ber.*, **102**, 319 (1969).
(10) M. H. Rosen and G. Bonet, *J. Org. Chem.*, **39**, 3805 (1974).
(11) Since the end of our investigation, this chemistry has been extensively studied by several authors: (a) M. A. Steinfels and A. S. Dreiding, *Helv. Chim. Acta*, **55**, 702 (1972); (b) V. Bilinski and A. S. Dreiding, *ibid.*, **55**, 1271 (1972); (c) V. Bilinski, M. A. Steinfels, and A. S. Dreiding, *ibid.*, **55**, 1075 (1972); (d) T. Eicher and S. Böhm, *Tetrahedron Lett.*, 2603 (1972); (e) T. Eicher and S. Böhm, *Chem. Ber.*, **107**, 2186 (1974).
(12) Certain structural similarities can be seen with amino ketone **19** and 2,3-diphenylacrylophenones which were reported to have antiimplantation effects: R. N. Iyer, R. Gopalachari, V. P. Kamboj, and A. B. Kar, *Ind. J. Exp. Biol.*, **5**, 169 (1967).
(13) A preliminary account of this chemistry was reported by us: M. H. Rosen, I. Fengler, and G. Bonet, *Tetrahedron Lett.*, 949 (1973).
(14) The reaction of thiirene 1,1-dioxides with enamines has been described by us in ref 10.
(15) T. Giannina, M. Butler, F. Popick, and B. Steinetz, *Contraception*, **3**, 347 (1971).

Synthesis of Some 1,8- and 2,8-Disubstituted Derivatives of Adenosine Cyclic 3',5'-Phosphate and Their Interaction with Some Enzymes of cAMP Metabolism

Hitoshi Uno, Rich B. Meyer, Jr., Dennis A. Shuman, Roland K. Robins, Lionel N. Simon, and Jon P. Miller*

ICN Pharmaceuticals, Inc., Nucleic Acid Research Institute, Irvine, California 92664. *Received August 6, 1975*

1,8-Disubstituted derivatives of adenosine cyclic 3',5'-phosphate (cAMP) were synthesized by N-oxidation or N-methylation of previously reported 8-substituted cAMP derivatives to yield 8-bromoadenosine cyclic 3',5'-phosphate 1-oxide and 8-(benzylthio)-1-methyladenosine cyclic 3',5'-phosphate. Substituents were introduced into the 8 position of 2-methyladenosine cyclic 3',5'-phosphate and 2-butyladenosine cyclic 3',5'-phosphate by bromination, followed by treatment with sodium benzylmercaptide, sodium *p*-chlorothiophenolate, or, in the former case, sodium azide. Each of the 1,8- and 2,8-disubstituted derivatives of cAMP was tested as activators of cAMP-dependent protein kinase and as substrates for and inhibitors of cyclic nucleotide phosphodiesterases. Depending on the substitutions, examples were found where the disubstituted derivatives were either more active, equally as active, or less active than the monosubstituted parent compounds as protein kinase activators. For the compounds reported, 8-substitution completely or substantially eliminated the ability of 1- or 2-substituted derivatives of cAMP to serve as substrates for phosphodiesterase and diminished the ability of these latter derivatives to inhibit cAMP hydrolysis.

The synthesis and biological evaluation of analogs of adenosine cyclic 3',5'-phosphate (cAMP) have for the most part been directed toward singly modified derivatives.[1,2] To date, virtually all accessible positions of the cAMP molecule have been modified (the 1, 2, 6, 8, 2', 3', 4', and 5' positions and the phosphorus atom). We have now undertaken a systematic investigation of the effects of multiple substitutions of cAMP on its biological activity. We have previously reported on the synthesis and enzymic activity of 5',6- and 2',8-disubstituted derivatives of cAMP and 2,6-disubstituted derivatives of 9-β-D-ribofuranosylpurine cyclic 3',5'-phosphate (cNMP)[3-5] and on the synthesis of N^6,8-disubstituted derivatives of cAMP and 6,8-disubstituted derivatives of cNMP.[6]

As cAMP-dependent protein kinase activators, the 5',6-, 2',8-, and 2,6-disubstituted analogs were found to be less active than either of the singly modified parent compounds from which they were derived,[3-5] while the 6,8-disubstituted derivatives demonstrated activities intermediate between those of the singly modified parent compounds.[7] As substrates for cyclic nucleotide phosphodiesterase, the 5',6-, 2',8-, and 6,8-disubstituted analogs were resistant to hydrolysis,[3,4,7] while the 2,6-disubstituted derivatives were hydrolyzed at rates which approached those of the singly modified parent compounds.[5]

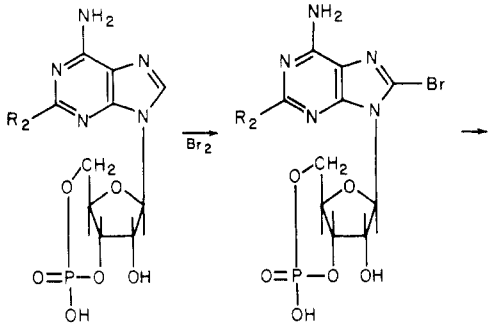
As a logical next step from our reports on the synthesis and enzymic activities of 1-,[8] 2-,[9,10] and 8-monosubstituted[11-13] cAMP derivatives, we here report the synthesis and enzymic properties of some representative 1,8- and 2,8-disubstituted cAMP derivatives and compare them to the singly modified parent compounds.

Synthesis. 2-Methyl- (**1**) and 2-butyladenosine cyclic 3',5'-phosphate[9] (**2**) were treated with aqueous sodium acetate buffer (pH 4) saturated with bromine, in a manner analogous to the preparation of 8Br-cAMP from cAMP,[11] to give 8-bromo-2-methyladenosine cyclic 3',5'-phosphate (**3**) and 8-bromo-2-butyladenosine cyclic 3',5'-phosphate (**4**), respectively. These 8-bromo nucleotides provided convenient intermediates for the preparation of the additional 2,8-disubstituted derivatives by treatment with nucleophiles in the manner previously described.[11] Thus, treatment of **3** with sodium benzylmercaptide, sodium *p*-chlorothiophenolate, and sodium azide gave, respectively, 8-(benzylthio)-2-methyladenosine cyclic 3',5'-phosphate (**5**), 8-(*p*-chlorophenylthio)-2-methyladenosine cyclic 3',5'-phosphate (**6**), and 8-azido-2-methyladenosine cyclic 3',5'-phosphate (**7**). Likewise, 8-(benzylthio)-2-butyladenosine cyclic 3',5'-phosphate (**8**) and 2-butyl-8-(*p*-chlorophenylthio)adenosine cyclic 3',5'-phosphate (**9**) were prepared from **4**.

Table I. Enzymic Properties of 1,8- and 2,8-Disubstituted Derivatives of cAMP[a]

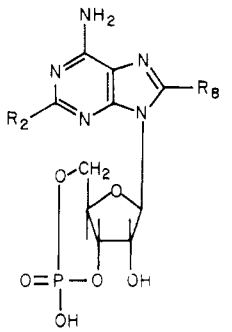
Compd	Substitutions N_1	Substitutions C_2	Substitutions C_8	Protein kinase,[b] K_a'	Hydrolysis by PDE,[c] rel rate	Inhibn of PDE[d] Rabbit lung I_{50}, μM	Inhibn of PDE[d] Beef heart I_{50}, μM
cAMP		-H	-H	1.0	1.0		
1-O-cAMP[e]	-O	-H	-H	0.45	0.33	35	50
1-Me-cAMP[e]	-Me	-H	-H	0.012	0.16	65	80
1[f]		-Me	-H	0.16	0.83	1.6	1.1
2[f]		-*n*-Bu	-H	0.46	0.64	3.0	10
10[g]		-H	-Br	2.9	<0.05	40	7.7
11	-O	-H	-Br	0.15	<0.05	400	59
3		-Me	-Br	3.4	0.15	25	30
4		-*n*-Bu	-Br	0.66	<0.05	17	11
12[g]		-H	$-SCH_2Ph$	2.1	<0.05	53	17
13	-Me	-H	$-SCH_2Ph$	1.3	*h*	61	67
5		-Me	$-SCH_2Ph$	0.95	0.05	9.5	4.3
8		-*n*-Bu	$-SCH_2Ph$	1.1	<0.05	9.5	7.1
8-*p*-ClPhS-cAMP[g]		-H	-SPh-*p*-Cl	18	<0.05	200	22
6		-Me	-SPh-*p*-Cl	2.3	0.05	33	5.0
9		-*n*-Bu	-SPh-*p*-Cl	2.2	<0.05	11	5.0
8-N_3-cAMP[g]		-H	$-N_3$	0.68	<0.05	80	43
7		-Me	$-N_3$	0.45	0.16	67	50

[a] All data represent the results of duplicate determinations which agreed within 15% of each other. [b] K_a' = (K_a for cAMP)/(K_a for the test compound). K_a for cAMP was 82 nM. [c] The ability to serve as a substrate for rabbit kidney cyclic nucleotide phosphodiesterase at a substrate concentration of 5 mM, expressed as the relative rate = (rate of hydrolysis of the analog)/(rate of hydrolysis of cAMP). The rate of hydrolysis of cAMP was 18 nmol of 5'-AMP formed per minute per 310 μg of protein. [d] I_{50} = concentration of the test compound that results in 50% inhibition in the rate of hydrolysis of cAMP (concentration of cAMP = 0.17 μM). [e] Synthesis and enzymic data previously reported[8] wherein the K_a' for 1-O-cAMP was erroneously reported to be 0.045. [f] Synthesis and enzymic data previously reported.[9,10] [g] Synthesis and enzymic data previously reported.[11,12] [h] Not soluble at 5 mM required for assay.



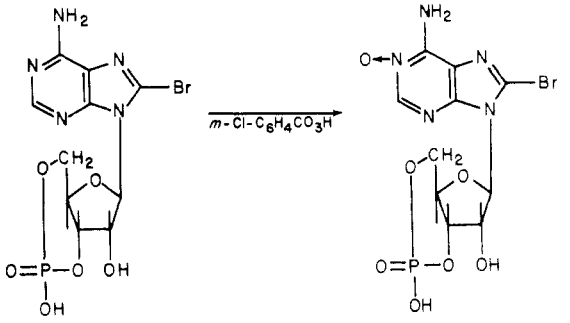
1, $R_2 = CH_3$
2, $R_2 = C_4H_9$

3, $R_2 = CH_3$
4, $R_2 = C_4H_9$

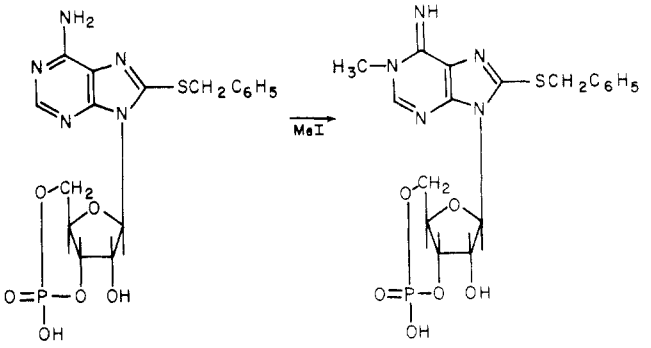


5, $R_2 = CH_3$; $R_8 = SCH_2C_6H_5$
6, $R_2 = CH_3$; $R_8 = SC_6H_4$-*p*-Cl
7, $R_2 = CH_3$; $R_8 = N_3$
8, $R_2 = C_4H_9$; $R_8 = SCH_2C_6H_5$
9, $R_2 = C_4H_9$; $R_8 = SC_6H_4$-*p*-Cl

Oxidation of 8-bromoadenosine cyclic 3',5'-phosphate[11] (**10**) with *m*-chloroperbenzoic acid in a buffered two-phase system as previously described for the N-oxidation of cAMP[8] gave 8-bromoadenosine cyclic 3',5'-phosphate 1-oxide (**11**). The 1-methyl group was introduced by methylation of 8-(benzylthio)adenosine cyclic 3',5'-phosphate[11] (**12**) in Me_2SO with methyl iodide[8] to give 8-(benzylthio)-1-methyladenosine cyclic 3',5'-phosphate (**13**). This procedure was analogous to the methylation of adenosine in *N*,*N*-dimethylacetamide, where 1-methyladenosine was isolated as the sole product.[14]



10 **11**



12 **13**

The integrity of the cyclic phosphate ring of all compounds was verified by examination of the ^1H NMR spectra, which showed an apparent singlet ($J < 1$ Hz) for the anomeric proton.[15]

Results and Discussion

Activation of Bovine Brain cAMP-Dependent Protein Kinase. Each of the 1,8- and 2,8-disubstituted derivatives of cAMP was examined for its ability to activate protein kinase. The results, expressed relative to cAMP (K_a'), for the new derivatives and for the singly modified parent compounds are summarized in Table I.

The effect of substituting cAMP in both the 1 and 8 positions varies with the type of substituent. 8-Substitution of 1-O-cAMP was detrimental to its activity. 8-Br-1-O-cAMP (11) was less active than either 8-Br-cAMP (10) or 1-O-cAMP. In contrast, 8-substitution of 1-Me-cAMP greatly improved its activity: 8-$PhCH_2S$-1-Me-cAMP (13) was almost equal in activity to 8-$PhCH_2S$-cAMP (12), even though 1-Me-cAMP was only one-hundredth as active as either 8-$PhCH_2S$-1-Me-cAMP (13) or 8-$PhCH_2S$-cAMP (12). 1-O-cAMP and 1-Me-cAMP are significantly different chemically. 1-O-cAMP probably is quite similar both sterically and electronically to cAMP, while 1-Me-cAMP probably exists in the N^6-imino tautomer and is protonated at the pH of the assay. We have previously implicated this fact to explain the loss of the protein kinase activating ability of 1-Me-cAMP.[8] It is not clear how 8-substitution of 1-O-cAMP, which is almost as good as cAMP as a kinase activator, can be detrimental to its activity; while 8-substitution of 1-Me-cAMP, which is a very poor activator, can significantly increase its activity.

With only one exception, the K_a' values of the 2,8-disubstituted derivatives were intermediate between the K_a' values of the parent monosubstituted compounds. The exception is 8-Br-2-Me-cAMP (3) which is even slightly more active than the more active of the two parent compounds, 8-Br-cAMP (10). Previous results have shown that 8-substitution of 2' derivatives does not overcome the detrimental effect of the 2' modification.[4,16] In contrast, addition of an 8-substituent to N^6-substituted derivatives of cAMP or to 6-substituted derivatives of cNMP resulted in 6,8-disubstituted derivatives intermediate in activity between the activities of the parent compounds.[7]

Hydrolysis by Rabbit Kidney Cyclic Nucleotide Phosphodiesterase. Each of the analogs was tested for its ability to be hydrolyzed by phosphodiesterase (PDE). Addition of an 8-Br substituent to 1-O-cAMP resulted in a compound, 8-Br-1-O-cAMP (11), which was resistant to enzymic hydrolysis. A similar result was observed when 8-substituents were added to 2' derivatives of cAMP,[4] to N^6-substituted derivatives of cAMP,[7] or to 6-substituted derivatives of cNMP.[7] Without the 8-substituent these compounds were hydrolyzed by the PDE, but addition of an 8-substituent resulted in a 2',8- or 6,8-disubstituted derivative which was resistant to enzymic hydrolysis. The results with the 2,8 derivatives were apparently mixed. Addition of an 8-Br-, 8-$PhCH_2S$-, 8-*p*-ClPhS-, or 8-N_3-group to 2-Me-cAMP (1) decreased the rate at which the resulting 2,8 derivative [8-Br-2-Me-cAMP (3), 8-$PhCH_2S$-2-Me-cAMP (5), 8-*p*-ClPhS-2-Me-cAMP (6), or 8-N_3-2-Me-cAMP (7), respectively] was hydrolyzed, but the rate of hydrolysis was still measurable. In contrast, addition of an 8-Br-, 8-*p*-ClPhS-, or 8-$PhCH_2S$- group to 2-*n*-Bu-cAMP (2) resulted in 2,8 derivatives [8-Br-2-*n*-Bu-cAMP (4), 8-*p*-ClPhS-2-*n*-Bu-cAMP (9), or 8-$PhCH_2S$-2-*n*-Bu-cAMP (8), respectively] which were either not hydrolyzed or were hydrolyzed so slowly that the rate could not be determined by the method used.

Inhibition of cAMP Hydrolysis. The inhibition of cAMP hydrolysis by the 1,8- and 2,8-disubstituted derivatives of cAMP is summarized in Table I. 8-Br-1-O-cAMP (11) had an I_{50} tenfold greater than either 8-Br-cAMP (10) or 1-O-cAMP with the lung enzyme, while the I_{50} values of the latter three compounds with the heart enzyme were essentially the same. In contrast, no such drastic difference was seen between 8-$PhCH_2S$-1-Me-cAMP (13), 8-$PhCH_2S$-cAMP (12), and 1-Me-cAMP with either enzyme. With the 2,8-disubstituted analogs, the results were mixed. Addition of an 8-Br or 8-N_3 substituent to 2-Me-cAMP (1) or to 2-*n*-Bu-cAMP (2) resulted in 2,8-disubstituted derivatives with I_{50} values that were significantly greater than those of the 2-substituted parent compound and which were very close to the 8-substituted parent compounds. In contrast, addition of an 8-$PhCH_2S$- or 8-*p*-ClPhS substituent to 2-Me-cAMP (1) or to 2-*n*-Bu-cAMP (2) resulted in 2,8-disubstituted derivatives with I_{50} values intermediate between the 2-substituted and 8-substituted parent compounds.

2-Substituted derivatives of cAMP have been shown to be quite good as substrates for and inhibitors of phosphodiesterase,[5,10] while 8-substituted derivatives have been shown to be essentially resistant to enzymic hydrolysis and poor inhibitors of phosphodiesterase.[11-13] Within the scope of the compounds reported herein, 8-substitutents counteract the beneficial effect of 2-substituents on the ability of the analogs to either be hydrolyzed by phosphodiesterase or to inhibit the hydrolysis of cAMP by this enzyme.

Experimental Section

Uv spectra were taken on a Cary 15. Analytical analyses were performed by Galbraith Laboratories, Knoxville, Tenn. TLC was run on EM-cellulose 300-F (Brinkman), developed in solvent system A (*i*-PrOH–concentrated NH_4OH–H_2O, 17:1:2) or B (MeCN–0.1 N NH_4Cl, 4:1). Samples were dried under vacuum at 80°.

8-Bromo-2-methyladenosine Cyclic 3',5'-Phosphate (3). To a solution of 2-methyladenosine cyclic 3',5'-phosphate[9] (1, 144 mg, 0.38 mmol) in 0.4 ml of 1 N NaOH was added 2.5 ml of 1 M sodium acetate buffer (pH 3.9), followed by 3.5 ml of 1 M sodium acetate buffer (pH 3.9) saturated with bromine. The mixture was stirred overnight at room temperature and the resulting precipitate was filtered, washed with H_2O, and recrystallized from H_2O to give 40 mg (25%) of 3: $\lambda_{max}^{pH\ 1}$ 262 nm (ϵ 18000); $\lambda_{max}^{pH\ 11}$ 267 nm (ϵ 16800); R_f 0.16 (A), 0.30 (B). Anal. ($C_{11}H_{13}BrN_5O_6P \cdot 1.5H_2O$) C, H, N, Br.

8-Bromo-2-butyladenosine cyclic 3',5'-phosphate (4) was prepared from 2-butyladenosine cyclic 3',5'-phosphate[9] (2) in the same manner as 3 in 33% yield: $\lambda_{max}^{pH\ 1}$ 263 nm (ϵ 18800); $\lambda_{max}^{pH\ 7}$ 267 nm (ϵ 17400); $\lambda_{max}^{pH\ 11}$ 267 nm (ϵ 16900); R_f 0.50 (A), 0.81 (B). Anal. ($C_{14}H_{19}BrN_5O_6P \cdot H_2O$) C, H, N, Br.

8-(Benzylthio)-2-methyladenosine Cyclic 3',5'-Phosphate Sodium Salt (5). Benzylmercaptan (200 mg, 1.6 mmol) and sodium methoxide (162 mg, 3.0 mmol) were dissolved in 10 ml of MeOH. The solution was stirred for 30 min at room temperature. After addition of 3 (300 mg, 0.7 mmol), the mixture was refluxed for 7 h and then allowed to stand overnight at room temperature. The resulting precipitate was filtered, washed with MeOH and Et_2O, and dried to give 220 mg (57%) of 5: $\lambda_{max}^{pH\ 1}$ 283 nm (ϵ 18700); $\lambda_{max}^{pH\ 7}$ 283 nm (ϵ 16800); $\lambda_{max}^{pH\ 11}$ 282 nm (ϵ 16300); R_f 0.40 (A), 0.92 (B). Anal. ($C_{18}H_{19}N_5NaO_6PS \cdot 3.5H_2O$) C, H, N, S.

8-(*p*-Chlorophenylthio)-2-methyladenosine Cyclic 3',5'-Phosphate (6). *p*-Chlorothiophenol (360 mg, 2.5 mmol) and sodium methoxide (300 mg, 4.5 mmol) were dissolved in 20 ml of MeOH. The solution was stirred at room temperature for 30 min. To the solution was added 3 (500 mg, 1.1 mmol) and the mixture was refluxed for 5 h. H_2O (50 ml) was added to the mixture and insoluble substances were filtered off and washed well with H_2O. The filtrate and washings were acidified with concentrated HCl and resulting precipitate was collected and washed with EtOH and then Et_2O. The crude product was dissolved in 100 ml of 2 N NH_4OH, filtered, warmed to 50°, and then acidified with concentrated HCl. The solid which separated was filtered, washed with EtOH and then Et_2O, and dried to give 400 mg (76%) of 6: $\lambda_{max}^{pH\ 1}$ 278 nm (ϵ 19700); $\lambda_{max}^{pH\ 7}$ 283 nm (ϵ 17600); $\lambda_{max}^{pH\ 11}$ 283 nm (ϵ 17600); R_f 0.50 (A), 0.93 (B). Anal. ($C_{17}H_{17}ClN_5O_6PS \cdot H_2O$) C, H, N, S.

8-Azido-2-methyladenosine Cyclic 3',5'-Phosphate (7). A solution of 3 (450 mg, 1 mmol) and sodium azide (130 mg, 2 mmol) in 40 ml of *N*,*N*-dimethylformamide was stirred at 70–80° overnight. The solvent was evaporated to dryness. The residue

was taken up in a small volume of H_2O and passed onto a column of 30 ml of Dowex 50 X8 (H^+, 100–200 mesh) resin. The column was eluted with H_2O and the uv-absorbing fractions were pooled. After evaporation of the solvent, the residue was triturated with EtOH to give 220 mg (62%) of **7**: $\lambda_{max}^{pH\ 1}$ 276 nm (ϵ 14950); $\lambda_{max}^{pH\ 7}$ 275 nm (ϵ 11600); $\lambda_{max}^{pH\ 11}$ 275 nm (ϵ 11500); R_f 0.18 (A), 0.31 (B). Anal. ($C_{11}H_{13}N_8O_6P \cdot H_2O$) C, H, N.

8-(Benzylthio)-2-butyladenosine cyclic 3',5'-phosphate (8) was prepared from **4** in the same manner as **5** in 38% yield: $\lambda_{max}^{pH\ 1}$ 283 nm (ϵ 18810); $\lambda_{max}^{pH\ 7}$ 285 nm (ϵ 16000); $\lambda_{max}^{pH\ 11}$ 285 nm (ϵ 16000); R_f 0.74 (A), 0.96 (B). Anal. ($C_{21}H_{26}N_5O_6PS \cdot 3H_2O$) C, H, N.

8-(*p*-Chlorophenylthio)-2-butyladenosine cyclic 3',5'-phosphate (9) was prepared from **4** in the same manner as **6** in 70% yield: $\lambda_{max}^{pH\ 1}$ 278 nm (ϵ 20980); $\lambda_{max}^{pH\ 7}$ 282 nm (ϵ 18330); $\lambda_{max}^{pH\ 11}$ 282 nm (ϵ 18330); R_f 0.76 (A), 0.96 (B). Anal. ($C_{20}H_{23}ClN_5O_6PS \cdot 2H_2O$) C, H, N.

8-Bromoadenosine Cyclic 3',5'-Phosphate 1-Oxide (11). A mixture of 7 g of 8-bromoadenosine cyclic 3',5'-phosphate[11] (**10**, 17 mmol), 56 ml of 1 N NaOAc, 56 ml of 1 N HOAc, 105 ml of EtOAc, and 12 g (70 mmol) of *m*-chloroperbenzoic acid was stirred at ambient temperature for 2 days. The aqueous phase was separated, washed with 3 × 100 ml of EtOAc, and passed onto a Dowex 50 X8 (H^+, 4 × 29 cm) column. Elution with water brought off-colored by-product first, and the product appeared after 400 ml of eluate. Product-containing fractions were evaporated to a small volume and diluted with 200 ml of EtOH to yield 3.37 g (48%) of white product: $\lambda_{max}^{pH\ 1}$ 236 nm (ϵ 12500), 262 (13200); $\lambda_{max}^{pH\ 7,11}$ 236 nm (ϵ 35400), 263 (9700), 293 (sh, 2500); R_f 0.06 (A), 0.10 (B). Anal. ($C_{10}H_{11}BrO_7P$) C, H, N.

8-(Benzylthio)-1-methyladenosine Cyclic 3',5'-Phosphate (13). 8-Benzylthioadenosine cyclic 3',5'-phosphate[11] (Na salt) (**12**) (5 g, 10 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (1.61 g, 10.6 mmol) were dissolved in 15 ml of Me_2SO. To this solution was added 3 ml of MeI and the mixture was stirred at room temperature overnight. The solvent was evaporated and the residue was codistilled with H_2O and then EtOH. The residue was triturated with 50 ml of H_2O and resulting white solid was filtered and washed with EtOH and Et_2O to give 2.3 g (48%) of **13**: $\lambda_{max}^{pH\ 1}$ 285 nm (ϵ 18500); $\lambda_{max}^{pH\ 7}$ 285 nm (ϵ 18000); $\lambda_{max}^{pH\ 11}$ 285 nm (ϵ 12500); R_f 0.39 (A), 0.89 (B). Anal. ($C_{18}H_{20}N_5O_6PS \cdot H_2O$) C, H, N, S.

Biochemical Methods. The rabbit lung, beef heart, and rabbit kidney phosphodiesterases were prepared as previously described.[16] The assay for the inhibition of the rabbit lung and beef heart enzymes contained in 0.5 ml: 25 μmol of Tris HCl, pH 7.5; 5 μmol of $MgCl_2$; 20–200 μg of phosphodiesterase protein; 80 pmol of 8-[^{3}H]cAMP (350000 cpm); and varying concentrations of the 3',5'-cyclic nucleotide being tested as an inhibitor. The incubation times were determined from pilot assays to give kinetically valid data. The mixture was heat-inactivated to terminate the reaction and treated with 5'-nucleotidase (crude *Crotalus atrox* venom) to convert the 5'-nucleotide to the corresponding nucleoside. The unhydrolyzed 3',5'-cyclic nucleotide was absorbed onto Dowex-1 and the radioactivity of the nucleoside fraction determined. A control reaction utilizing [^{14}C]-5'-AMP and the highest concentration of cyclic nucleotide derivative was run to determine if the compounds inhibited the action of the nucleotidase. None of the derivatives had any effect on this enzyme. When testing cAMP derivatives as substrates for rabbit kidney phosphodiesterase, the standard reaction mixture contained in 0.60 ml: 3.0 μmol of cyclic nucleotide; 3.0 μmol of Tris HCl, pH 7.5; 6 μmol of $MgCl_2$; and 0.1–0.3 mg of phosphodiesterase protein. After an appropriate incubation period (usually 10–60 min), the reaction was terminated by heating and treated with bacterial alkaline phosphatase, and the phosphate released was assayed colorimetrically. The details of the phosphodiesterase substance and inhibition assays have been previously described.[16] The same rates were observed using higher concentrations of alkaline phosphates or longer periods (times two or three) of incubation with the latter enzyme. In addition, examination of the reaction mixtures by thin-layer chromatography[16] gave the same results.

The bovine brain cAMP-dependent protein kinase was purified through the DEAE-cellulose step as described by Kuo and Greengard.[17] The assay for the stimulation of the cAMP-dependent protein kinase[12] contained in 0.1 ml: 5 μmol of NaOAc, pH 6.0; 1 μmol of $MgCl_2$; 20 μg of histone (Worthington HLY); 0.5 nmol of ATP-γ-^{32}P; protein kinase enzyme (20–200 μg); and various concentrations of the 3',5'-cyclic nucleotide being tested as an activator (10^{-9}–10^{-3} M). After a suitable incubation time to give kinetically valid data, 0.05 ml of each reaction mixture was absorbed onto Whatmann 3MM paper disks, and the disks were dried. The disks were washed sequentially in 10% Cl_3CCO_2H and 5% Cl_3CCO_2H (three times), EtOH, and Et_2O. After thorough drying, the radioactivity in each disk was determined in a liquid scintillation spectrometer.

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References and Notes

(1) L. N. Simon, D. A. Shuman, and R. K. Robins, *Adv. Cyclic Nucleotide Res.*, **3**, 225–353 (1973).
(2) R. B. Meyer, Jr., and J. P. Miller, *Life Sci.*, **14**, 1019 (1974).
(3) D. A. Shuman, J. P. Miller, M. B. Scholten, L. N. Simon, and R. K. Robins, *Biochemistry*, **12**, 2781 (1973).
(4) T. A. Khwaja, K. H. Boswell, R. K. Robins, and J. P. Miller, *Biochemistry*, **14**, 4238 (1975).
(5) R. B. Meyer, Jr., H. Uno, D. A. Shuman, R. K. Robins, L. N. Simon, and J. P. Miller, *J. Cyclic Nucleoside Res.*, **1**, 159 (1975).
(6) K. H. Boswell, L. F. Christensen, D. A. Shuman, and R. K. Robins, *J. Heterocycl. Chem.*, **12**, 1 (1975).
(7) J. P. Miller, K. H. Boswell, L. F. Christensen, R. K. Robins, and R. B. Meyer, Jr., unpublished results.
(8) R. B. Meyer, Jr., D. A. Shuman, R. K. Robins, J. P. Miller, and L. N. Simon, *J. Med. Chem.*, **16**, 1319 (1973).
(9) R. B. Meyer, Jr., D. A. Shuman, and R. K. Robins, *J. Am. Chem. Soc.*, **96**, 4962 (1974).
(10) R. B. Meyer, Jr., H. Uno, R. K. Robins, L. N. Simon, and J. P. Miller, *Biochemistry*, **14**, 3315 (1975).
(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) K. Muneyama, D. A. Shuman, K. H. Boswell, R. K. Robins, L. N. Simon, and J. P. Miller, *J. Carbohydr., Nucleosides, Nucleotides*, **1**, 55 (1974).
(14) J. W. Jones and R. K. Robins, *J. Am. Chem. Soc.*, **85**, 193 (1963).
(15) C. D. Jardetzky, *J. Am. Chem. Soc.*, **84**, 62 (1962).
(16) 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).
(17) J. F. Kuo and P. Greengard, *J. Biol. Chem.*, **245**, 2493 (1970).