GEISSMAN, T. A. (1962). *The Chemistry of Flavonoid Compounds*. London: Pergamon Press.
GERMAIN, G., MAIN, P. & WOOLFSON, M. M. (1971). *Acta Cryst.* A**27**, 368–376.
KAUFMANN, F. & LAM, J. (1967). *Acta Chem. Scand.* **21**, 311–313.
MARIEZCURRENA, R. A. & RASMUSSEN, S. E. (1973). *Acta Cryst.* B**29**, 1030–1035.
PHILBIN, E. M. & WHEELER, T. S. (1958). *Proc. Chem. Soc. London*, pp. 167–168.
STEWART, R. F., DAVIDSON, E. R. & SIMPSON, W. T. (1965). *J. Chem. Phys.* **42**, 3175–3187.

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8-[(2-Aminoethyl)amino]adenosine Cyclic 3′,5′-Monophosphate Tetrahydrate

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Abstract. $C_{12}H_{18}N_7O_6P.4H_2O$ (I), orthorhombic, $P2_12_12_1$, $a = 13{\cdot}180$ (2), $b = 22{\cdot}439$ (7), $c = 6{\cdot}661$ (1) Å, $M_r = 459{\cdot}4$, $D_x = 1{\cdot}55$ g cm^{-3}. The adenine base displays a *syn* conformation ($\chi = -107{\cdot}0°$) with respect to the glycosyl bond, the furanose ring a twist ${}_4T^3$ [C(3′)-*endo*–C(4′)-*exo*] conformation relative to the glycosyl N(9). The adenine heterocycles form base stacks of 2_1 symmetry with an interplanar distance of 3·17 Å. Base overlap is limited to only 5·9% of the maximum possible as a result of steric contact, 2·94 Å, between N(6) and O(1′) of the sugar ring of the screw-related molecule.

Introduction. Adenosine cyclic 3′,5′-monophosphate (cAMP) dependent protein kinases occur in a wide variety of animal tissues. Their highly specific recognition of the cAMP, which is important for control and regulatory mechanisms of the human body, suggests that affinity chromatography should provide a convenient means for their purification. In this method a column containing a cAMP derivative bonded to an inert matrix is used to separate chromatographically the coordinate protein from impurities. In (I), which was specifically synthesized for this purpose, the purine base of the nucleotide is connected to the inert matrix *via* the C(8) side chain (Fig. 1). A knowledge of the molecular geometry of (I) and thereby the conformational preference at the glycosyl bond should provide an insight into the spatial characteristics of the specific cAMP binding site of the coordinate protein.

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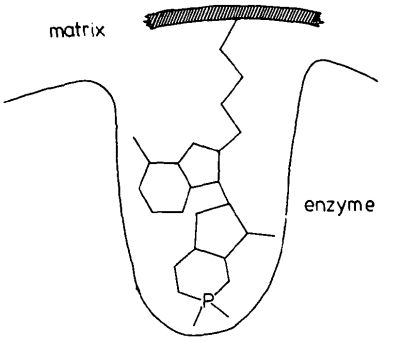

Fig. 1. The principle of affinity chromatography.

1 ml of ethylenediamine (14·8 mmol) was added to a suspension of 200 mg (490 μmol) of 8-bromo-cAMP (Boehringer, Mannheim) in 10 ml of ethanol and heated to 100°C in a bomb for 2 h (Muneyama, Bauer, Shuman & Robins, 1971). After cooling, the reaction mixture was added at a pH of 11·5 to a Dowex 1-X2 column (Serva, Feinbiochemica, Heidelberg) and washed with water to remove the residual ethylenediamine. 8-(2-Aminoethyl)amino-cAMP (I) was eluted with water/0·5 *M* acetic acid (1:1, v/v). The product fractions were reduced to 10 ml and, after dilution with 10 ml of methanol, added to a Sephadex LH 20 column (Pharmacia, Frankfurt/Main) equilibrated with methanol/water (1:1, v/v). (I) was crystallized as long prisms from the methanol/water solution of the appropriate fractions (yield 81%): 100 MHz ^{1}H NMR (D_2O), $\delta = 8{\cdot}06$ [*s*, H(2)], 5·88 [*s*, H(1)′] and 4·23

[poorly resolved *m*, H(2)′–H(5)′], 368 (*t*, α-CH_2), 3·26 (*t*, β-CH_2).

Cell dimensions were obtained by least squares from the settings for 15 reflexions (±*hkl*) on a Syntex $P2_1$ diffractometer (Mo *K*, λ = 0·71069 Å). Intensity measurements were carried out in the θ–2θ mode (3·0 $\leq 2\theta \leq$ 60·0°) with graphite-monochromated Mo $K\alpha$ radiation. No absorption correction was applied [μ(Mo $K\alpha$) = 1·6 cm^{-1}]. After application of the rejection criterion $I \leq 2{\cdot}0\sigma(I)$. 4355 unique reflexions (one quadrant of reciprocal space) were considered to be observed. The best *E* map obtained by weighted multi-solution tangent refinement (E_{min} = 1·4, 411 reflexions) revealed 20 of the 26 heavy atoms in the nucleotide (*SHELX*, G. M. Sheldrick). The positions of the other six heavy atoms, four O atoms belonging to water of crystallization, and the H atoms were located from difference syntheses. Anisotropic temperature factors were introduced for the heavy atoms, individual isotropic temperature factors for H. It was necessary to fix the positional parameters of the H atoms of O*W*(2). The terminal value of *R*′ was 0·057 with *R* 0·050. The weights were given by $w = k[\sigma^2(F_o) + gF_o^2]^{-1}$, where *k* and *g* refined to 0·9206 and 0·004159 respectively. Complex neutral-atom scattering factors were employed (Cromer & Waber, 1965; Cromer & Liberman, 1970). Positional parameters, bond lengths and bond angles are given in Tables 1–3.* Fig. 2 shows the numbering system in (I), Fig. 3 is a perspective diagram.

Table 1. *Positional parameters* (×10^4)

	x	*y*	*z*
P	1906 (0)	−2728 (0)	−2968 (1)
N(1)	−1112 (1)	−5613 (1)	−2146 (4)
C(2)	−455 (2)	−5157 (1)	−2093 (5)
N(3)	−628 (1)	−4577 (1)	−2093 (4)
C(4)	−1629 (2)	−4470 (1)	−2162 (4)
C(5)	−2404 (2)	−4876 (1)	−2168 (4)
C(6)	−2120 (2)	−5479 (1)	−2172 (4)
N(6)	−2786 (2)	−5930 (1)	−2252 (5)
N(7)	−3350 (1)	−4595 (1)	−2265 (3)
C(8)	−3123 (2)	−4023 (1)	−2365 (3)
N(9)	−2091 (1)	−3912 (1)	−2278 (4)
N(81)	−3797 (1)	−3566 (1)	−2478 (4)
C(82)	−4871 (2)	−3681 (1)	−2808 (5)
C(83)	−5437 (2)	−3871 (1)	−933 (5)
N(84)	−5186 (2)	−3493 (1)	823 (4)
O(6′)	2469 (1)	−2188 (1)	−2327 (4)
O(7′)	2456 (1)	−3303 (1)	−3211 (4)
O(5′)	1348 (1)	−2571 (1)	−5052 (3)
O(3′)	977 (1)	−2797 (1)	−1407 (3)
C(1′)	−1576 (2)	−3346 (1)	−2425 (4)
C(2′)	−735 (2)	−3227 (1)	−820 (4)
O(2′)	−899 (1)	−2669 (1)	129 (3)
C(3′)	196 (1)	−3186 (1)	−2144 (4)
C(4′)	−238 (2)	−2951 (1)	−4082 (4)
O(1′)	−1091 (1)	−3326 (1)	−4368 (3)
C(5′)	548 (2)	−2973 (1)	−5742 (5)
O*W*(1)	−30 (2)	−4558 (1)	−6728 (4)
O*W*(2)	−1742 (2)	−1215 (1)	−3304 (5)
O*W*(3)	1852 (2)	−4542 (1)	1358 (6)
O*W*(4)	1830 (3)	−4496 (1)	−2820 (7)

Discussion. (I) occurs as a zwitterion, N(84) of the C(8) side chain being protonated by a phosphate proton. The bond distances and angles in the nucleobase are similar to those in other non-protonated adenine heterocycles (Voet & Rich, 1970). Likewise, the distances and angles in the furanose and phosphate ring systems are similar to those in the cyclic nucleotide uridine 3′,5′-monophosphate (cUMP) (Coulter, 1969). A direct comparison with the conformation of the natural metabolite cAMP is not possible, because, as

* Lists of structure factors and anisotropic thermal parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 33427 (28 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

Table 2. *Bond lengths* (Å)

O(6′)–P	1·484 (2)	O(7′)–P	1·489 (2)
O(5′)–P	1·610 (2)	O(3′)–P	1·614 (2)
C(2)–N(1)	1·341 (4)	C(6)–N(1)	1·362 (4)
N(3)–C(2)	1·321 (4)	C(4)–N(3)	1·341 (4)
C(5)–C(4)	1·370 (4)	N(9)–C(4)	1·396 (4)
C(6)–C(5)	1·404 (4)	N(7)–C(5)	1·399 (4)
N(6)–C(6)	1·341 (4)	C(8)–N(7)	1·320 (4)
N(9)–C(8)	1·383 (3)	N(81)–C(8)	1·359 (4)
C(1′)–N(9)	1·443 (4)	C(82)–N(81)	1·456 (4)
C(83)–C(82)	1·516 (5)	N(84)–C(83)	1·483 (5)
C(5′)–O(5′)	1·461 (4)	C(3′)–O(3′)	1·435 (3)
C(2′)–C(1′)	1·563 (4)	O(1′)–C(1′)	1·444 (4)
O(2′)–C(2′)	1·420 (4)	C(3′)–C(2′)	1·514 (4)
C(4′)–C(3′)	1·507 (4)	O(1′)–C(4′)	1·417 (4)
C(5′)–C(4′)	1·516 (5)		

Table 3. *Bond angles* (°)

O(7′)–P–O(6′)	119·7 (1)	O(5′)–P–O(6′)	107·4 (1)
O(5′)–P–O(7′)	108·5 (1)	O(3′)–P–O(6′)	105·8 (1)
O(3′)–P–O(7′)	110·9 (1)	O(3′)–P–O(5′)	103·3 (1)
C(6)–N(1)–C(2)	117·5 (2)	N(3)–C(2)–N(1)	129·8 (3)
C(4)–N(3)–C(2)	110·3 (2)	C(5)–C(4)–N(3)	127·9 (3)
N(9)–C(4)–N(3)	126·4 (2)	N(9)–C(4)–C(5)	105·8 (2)
C(6)–C(5)–C(4)	116·3 (3)	N(7)–C(5)–C(4)	111·4 (2)
N(7)–C(5)–C(6)	132·3 (3)	C(5)–C(6)–N(1)	118·2 (2)
N(6)–C(6)–N(1)	118·2 (2)	N(6)–C(6)–C(5)	123·5 (3)
C(8)–N(7)–C(5)	103·8 (2)	N(9)–C(8)–N(7)	113·4 (2)
N(81)–C(8)–N(7)	126·0 (3)	N(81)–C(8)–N(9)	120·5 (2)
C(8)–N(9)–C(4)	105·6 (2)	C(1′)–N(9)–C(4)	126·0 (2)
C(1′)–N(9)–C(8)	128·2 (2)	C(82)–N(81)–C(8)	120·7 (2)
C(83)–C(82)–N(81)	113·8 (3)	N(84)–C(83)–C(82)	112·3 (3)
C(5′)–O(5′)–P	117·8 (2)	C(3′)–O(3′)–P	112·5 (2)
C(2′)–C(1′)–N(9)	115·9 (2)	O(1′)–C(1′)–N(9)	107·3 (2)
O(1′)–C(1′)–C(2′)	107·1 (2)	O(2′)–C(2′)–C(1′)	110·3 (2)
C(3′)–C(2′)–C(1′)	100·8 (2)	C(3′)–C(2′)–O(2′)	109·2 (2)
C(2′)–C(3′)–O(3′)	114·8 (2)	C(4′)–C(3′)–O(3′)	110·6 (2)
C(4′)–C(3′)–C(2′)	102·3 (2)	O(1′)–C(4′)–C(3′)	102·1 (2)
C(5′)–C(4′)–C(3′)	110·7 (2)	C(5′)–C(4′)–O(1′)	115·2 (2)
C(4′)–O(1′)–C(1′)	104·4 (2)	C(4′)–C(5′)–O(5′)	104·1 (3)

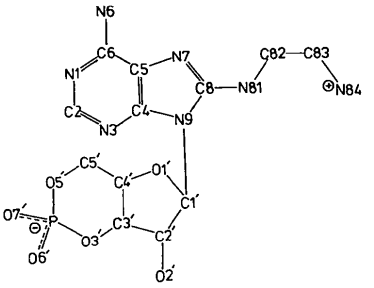

Fig. 2. The numbering system in (I).

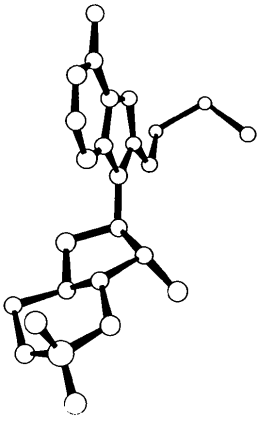

Fig. 3. The molecule of (I) in perspective.

yet, only a preliminary report of its crystal structure has appeared (Watenpaugh, Dow, Jensen & Furberg, 1968). The adenine base displays the *syn* conformation with respect to rotation about the glycosyl C(1′)–N bond, χ (Sundaralingam, 1969) being −107·0°. A *syn* conformation with similar χ values is also observed for other cyclic nucleotides in the crystalline state. χ is −125·8° for 5-methyleneadenosine cyclic 3′,5′-monophosphonate monohydrate (Sundaralingam & Abola, 1972) and −102° for one of the two independent molecules of cAMP in the asymmetric unit of this structure. However, the remaining independent molecule of cAMP occurs in the *anti* conformation (χ = 50°) indicating an intimate relation for the purine nucleotides between their conformation at the C(1′)–N bond and the adoption of a particular crystal-packing scheme. Evidence that the *syn* conformation of (I) is the result of a preference for this conformation by cyclic purine nucleotides rather than a result of the avoidance of potential intramolecular steric contacts between the C(8) side chain and the furanose and phosphate rings, is provided by the fact that the non-cyclic analogue of (I), 8-[(2-aminoethyl)amino]adenosine 3′-monophosphate, displays an *anti* conformation with χ = 48·8° (Sheldrick & Morr, 1978). The *anti* conformation of this non-cyclic 3′-AMP derivative is stabilized by an N(81)–H···O(5′) intramolecular hydrogen bond of length 3·07 Å. The stereochemical rigidity of the cyclic phosphate system obviously leads to the lack of such a hydrogen bond in (I). The *syn* conformation leads to long N(84)···O(6′) and N(84)···O(7′) intramolecular distances of 10·71 and 10·43 Å in (I), which give an indication of the spatial characteristics of the binding site of the coordinate protein. N(6)···O(2′) is 7·89 Å. As was observed for the other cyclic nucleotides, the furanose ring in (I) is in the twist $_4T^3$ [C(3′)-*endo*–C(4′)-*exo*] conformation (Sundaralingam, 1975) relative to the glycosyl N(9) (Table 4). The phosphate ring exhibits a distorted chair conformation with C(5′) −0·735 and the opposite O(3′) 0·654 Å from the least-squares plane of the remaining four ring atoms. The torsion angles φ_{OO} [O(5′)–C(5′)–C(4′)–O(1′)] and φ_{OC} [O(5′)–C(5′)–C(4′)–C(3′)] (Saenger, 1973) are +178·0 and −62·9°, *i.e.* C(5′)–O(5′) is in the *trans–gauche* conformation relative to the sugar ring in this cyclic nucleotide.

The crystal structure (Fig. 4) is dominated by hydrophobic and hydrophilic channels formed by the base stacks of the heterocycles on the one hand and the furanose and phosphate rings hydrogen-bonded to the interspersed four water molecules of crystallization on the other hand. O*W*(1) and O*W*(3) are involved in the maximum of four, O*W*(2) and O*W*(4) in three

Table 4. *Deviations* (Å) *of atoms from least-squares planes*

(*a*) Adenine heterocycle		(*b*) Furanose ring			(*c*) Phosphate ring	
N(1)	−0·032	O(1′)	0·013	0·0	C(3′)	−0·037
C(2)	−0·007	C(1′)	−0·019	0·0	C(4′)	0·040
N(3)	0·021	C(2′)	0·018	0·0	C(5′)	−0·735*
C(4)	0·021	C(3′)	−0·013	−0·084*	O(5′)	−0·038
C(5)	0·034	C(4′)	0·671*	0·611*	P	0·035
C(6)	−0·003	C(5′)	0·542*	0·435*	O(3′)	0·654*
N(7)	0·019	N(9)	−1·183*	−1·129*	C(2′)	0·572*
C(8)	−0·036	O(2′)	1·248*	1·234*	O(1′)	−0·474*
N(9)	−0·017	O(3′)	0·671*	0·558*	O(6′)	1·071*
N(6)	−0·045*				O(7′)	−1·348*

* Distance for atom not included in the least-squares calculation of the plane.

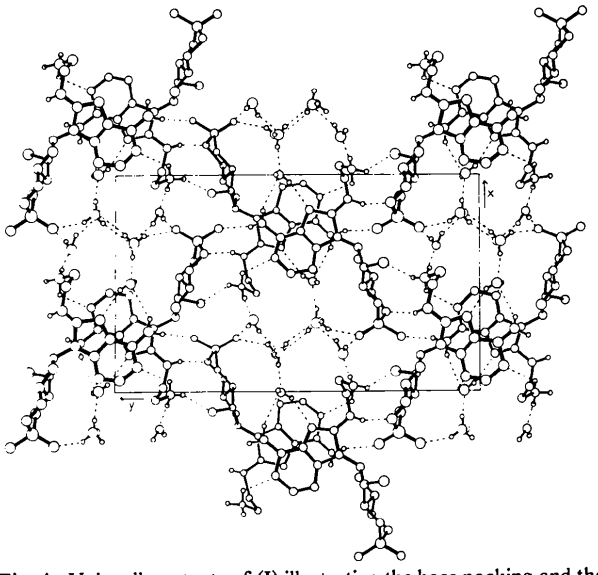

Fig. 4. Unit cell contents of (I) illustrating the base packing and the hydrophilic channels.

hydrogen bonds. The adenine heterocycles are related to one another at an interplanar distance of 3·17 Å by a twofold screw axis perpendicular to the base plane, thereby placing the sugar rings on alternate sides of the base stack (pattern II of Motherwell & Isaacs, 1972). The degree of base overlap is limited to 5·9% of the maximum as a result of (non-hydrogen-bonded) steric contact, 2·94 Å, between N(6) and O(1′) of the sugar ring of the screw-related molecule.

References

COULTER, C. L. (1969). *Acta Cryst.* B**25**, 2055–2065.

CROMER, D. T. & LIBERMAN, D. (1970). *J. Chem. Phys.* **53**, 1891–1898.

CROMER, D. T. & WABER, J. T. (1965). *Acta Cryst.* **18**, 104–109.

MOTHERWELL, W. D. S. & ISAACS, N. W. (1972). *J. Mol. Biol.* **71**, 231–241.

MUNEYAMA, K., BAUER, J. R., SHUMAN, D. A. & ROBINS, R. K. (1971). *Biochemistry*, **10**, 2390–2395.

SAENGER, W. (1973). *Angew. Chem.* **85**, 680–690.

SHELDRICK, W. S. & MORR, M. (1978). Unpublished results.

SUNDARALINGAM, M. (1969). *Biopolymers*, **7**, 821–860.

SUNDARALINGAM, M. (1975). *Ann. NY Acad. Sci.* **255**, 3–42.

SUNDARALINGAM, M. & ABOLA, J. (1972). *J. Am. Chem. Soc.* **94**, 5070–5076.

VOET, D. & RICH, A. (1970). *Prog. Nucleic Acid Res. Mol. Biol.* **10**, 183–265.

WATENPAUGH, K., DOW, J., JENSEN, L. H. & FURBERG, S. (1968). *Science*, **159**, 206–207.

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4-Ethynyl-2,2,6,6-tetramethylpiperidin-4-ol (TMPE)*

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Abstract. $C_{11}H_{19}NO$, monoclinic, $P2_1/n$, a = 6·427 (2), b = 13·203 (4), c = 12·753 (3) Å, β = 91·08 (2)°, Z = 4, V = 1081·97 Å^3, D_x = 1·112 g cm^{-3}, $F(000)$ = 400, μr(Mo $K\alpha$) = 0·02. 1892 independent reflexions were measured on a single-crystal diffractometer. The structure was solved by the symbolic addition method and refined by full-matrix least squares to a final R = 0·0689 for 1010 reflexions with $I > \sigma(I)$. The molecule exists in a chair conformation. The ethynyl group is axial and the hydroxyl group is equatorial. The H bonded to N takes the axial position. There are strong intermolecular O—H···N hydrogen bonds. The H of the ethynyl group forms a hydrogen bond with the O of the hydroxyl group. The correlation between the flatness of the ring and the system of intermolecular hydrogen bonds in related piperidine derivatives is described.

Introduction. Very interesting problems, connected with the conformations of overcrowded systems with strong steric interactions, arise in studies on non-

* Conformation of the Piperidine Ring. I.