Elementary Patterns in Protein-Nucleic Acid Interactions. II.† Crystal Structure of 3-(Adenin-9-yl)propionamide

Midori TAKIMOTO,* Akio TAKENAKA, and Yoshio SASADA

Laboratory of Chemistry for Natural Products, Tokyo Institute of Technology, Nagatsuta, Midori-ku, Yokohama 227.

(Received November 8, 1980)

In order to study adenine-amide interactions, 3-(adenin-9-yl)propionamide has been synthesized and its crystal structure examined. The crystals are monoclinic, the space group being $P2_1/a$, with unit-cell dimensions of $a=25.668$ (1), $b=8.025$ (1), $c=4.684$ (1) Å, $\beta=103.54$ (1)°, and $Z=4$. The adenine bases are linked along the two-fold screw axis through the hydrogen bonds, N(6)H···N(1) and N(6)H···N(7). The amide group is hydrogen-bonded to N(3) of adjacent adenine moiety, the distance NH···N(3) being 3.055 Å. The model fitting by computer graphics indicates that this binding scheme could be an elementary interaction pattern between the amide group in protein and adenine in the minor groove of double helical DNA.

Studies have been carried out on elementary patterns in protein–nucleic acid interactions using model crystals with the anticipation that their specificity might arise from the interaction between purine-pyrimidine bases and amino acid residues supported sterically and electronically by their three-dimensional structures. Several elementary patterns due to hydrogen bonds have been found in the following combinations: cytosine-carboxyl,[1-4] adenine-indole,[5,6] and adenine-carboxyl group.[7]

Attempts were made to extend the study to the adenine–glutamine or asparagine system. These amino acids contain an amide group in the side chain and significance of their interaction with nucleic acid base was proposed.[8,9] In order to examine the intermolecular hydrogen bonds between adenine and amide moieties, we have synthesized a new compound, 3-(adenin-9-yl)-propionamide and examined its crystal structure.

Experimental and Structure Determination

3-(Adenin-9-yl)propionic acid, synthesized from adenine and β-propiolactone by the method of Kondo *et al.*,[10] was converted into ester in CH_3OH solution saturated with HCl and then into amide in NH_3/CH_3OH. The amide formation was confirmed by elemental analysis and IR spectrum (δ_{N-H} 1600 cm^{-1}, amide group). By slow evaporation, prism crystals were obtained from water–methanol (1 : 1) solution at room temperature. Oscillation and Weissenberg photographs showed the space group to be $P2_1/a$. Density was measured by flotation in a mixture of cyclohexane and carbon tetrachloride. A crystal, $0.3\times0.5\times0.1$ mm in size, was mounted on a Rigaku automated four-circle diffractometer. Nickel-filtered Cu $K\alpha$ radiation ($\lambda=1.54178$ Å) was used. The accurate unit-cell dimensions were determined by least-squares calculation with 2θ values of 40 high-angle reflexions.

TABLE 1. CRYSTAL DATA

3-(Adenin-9-yl)propionamide	
$C_8H_{10}N_6O$	
Space group: $P2_1/a$	
$a=25.668(1)$ Å	$U=938.1(1)$ Å^3
$b=8.025(1)$ Å	$Z=4$
$c=4.684(1)$ Å	$D_x=1.46$ g cm^{-3}
$\beta=103.54(1)°$	$D_m=1.46$ g cm^{-3}

† Part I of this series is Ref. 5.

Crystallographic data are summarized in Table 1.

Intensity data were collected on the diffractometer by means of $\omega/2\theta$ scanning ($8°<2\theta<115°$), the scan speed being 4° (in 2θ) min^{-1} and scan width 0.9° (in ω) plus α_1-α_2 divergence. Five reference reflexions monitored periodically showed no significant intensity deterioration. Corrections were made for Lorentz and polarization factors, but not for absorption effects. A total of 1280 independent reflexions were obtained, 175 of which had no net intensities: the observational threshold value, F_{1im}, was 1.69. The standard deviations were estimated

TABLE 2. FRACTIONAL COORDINATES AND ISOTROPIC TEMPERATURE FACTORS

The B values with ⟨ ⟩ are the equivalent isotropic temperature factors calculated from anisotropic thermal parameters using the equation $B=8\pi^2\Sigma(U_1+U_2+U_3)/3$, where U_1, U_2, and U_3 are the principal components of mean square displacement matrix U. Values in ⟨ ⟩ are anisotropicity defined by $(\Sigma(B-8\pi^2U_i)^2/3)^{1/2}$. The e.s.d.'s in ( ) refer to last decimal places.

Atom	x	y	z	B/Å^2
N(1)	0.28388(9)	0.5192(3)	0.7473(5)	3.3⟨9⟩
C(2)	0.3200(1)	0.5599(4)	0.5953(7)	3.7⟨8⟩
N(3)	0.35669(9)	0.4652(3)	0.5157(5)	3.3⟨8⟩
C(4)	0.3535(1)	0.3075(3)	0.6099(6)	2.9⟨6⟩
C(5)	0.3181(1)	0.2456(3)	0.7663(6)	2.8⟨5⟩
C(6)	0.2819(1)	0.3597(3)	0.8375(6)	2.9⟨6⟩
N(6)	0.2453(1)	0.3221(3)	0.9881(6)	3.6⟨18⟩
N(7)	0.32743(9)	0.0772(2)	0.8253(5)	3.3⟨13⟩
C(8)	0.3675(1)	0.0420(4)	0.7041(7)	3.7⟨11⟩
N(9)	0.38475(9)	0.1751(3)	0.5681(5)	3.2⟨9⟩
C(11)	0.4332(1)	0.1864(4)	0.4528(7)	3.5⟨13⟩
C(12)	0.4794(1)	0.2404(6)	0.6945(7)	4.5⟨26⟩
C(13)	0.5297(1)	0.2860(4)	0.5952(6)	3.6⟨11⟩
N(13)	0.5689(1)	0.3501(4)	0.7988(6)	4.4⟨23⟩
O(13)	0.53325(8)	0.2627(3)	0.3422(4)	5.9⟨42⟩
H(2)	0.319(1)	0.681(3)	0.533(5)	1.0(6)
H(6A)	0.224(1)	0.403(4)	1.023(7)	3.1(9)
H(6B)	0.244(1)	0.223(3)	1.068(6)	1.6(8)
H(8)	0.384(1)	−0.062(3)	0.704(5)	0.8(6)
H(11A)	0.424(1)	0.269(3)	0.283(5)	1.0(6)
H(11B)	0.437(1)	0.071(4)	0.372(6)	2.9(8)
H(12A)	0.489(1)	0.150(4)	0.840(8)	5.8(2)
H(12B)	0.470(1)	0.338(4)	0.801(7)	4.9(1)
H(13A)	0.597(1)	0.397(3)	0.751(6)	2.1(8)
H(13B)	0.565(1)	0.365(4)	0.987(6)	3.3(9)

by the equation $\sigma^2(F_o)=\sigma_p^2(F_o)+qF_o^2$, where $\sigma_p(F_o)$ was evaluated by counting statistics and q (6.8×10^{-6}) was derived from the variations of the monitored reflexions.[11]

The structure was solved by the symbolic addition procedure, the parameters being refined by the block-diagonal leasts-quares method. The quantity minimized was $\sum w(|F_o|-|F_o|)^2$, where $w=1/(\sigma^2(F_o))$. All the hydrogen atoms, found on a difference map, were included. The zero-reflexions, for which $|F_c|>F_{llm}$, were included in the least-squares calculation by assuming $F_o=F_{llm}$ and $w=w(F_{llm})$, The final R value was 0.049 for 1170 reflexions with $F_o\geq3\sigma(F_o)$; the maximum shift of parameters in the last cycle was 0.02σ for C, N, and O, and 0.1σ for H. Atomic scattering factors were taken from "International Tables for X-Ray Crystallography".[12] Atomic parameters are given in Table 2, and the observed and calculated structure factors in Table 3.[13]

Results and Discussion

Molecular Structure. Bond distances and angles are shown in Fig. 1. Bond angles involving hydrogen atoms are given in Table 4, and the least-squares planes of adenine and amide moieties in Table 5. Figure 2 shows a stereoscopic view of the 3-(adenin-9-yl)-propionamide molecule. The bond lengths and angles of the adenine ring are in good agreement with those found in 9-methyladenine[14] and other related compounds.[15] The adenine moiety is planar with maximum shift of 0.009 Å for C(5) and C(8) from the least-squares plane.

The dimensions of the amide group is similar to those in L-asparagine monohydrate (neutron study),[16] and L-glutamine (neutron study).[17] Small differences might be related to the strengths of the hydrogen bondings. A comparison of the dimensions of amide group is given in Table 6.

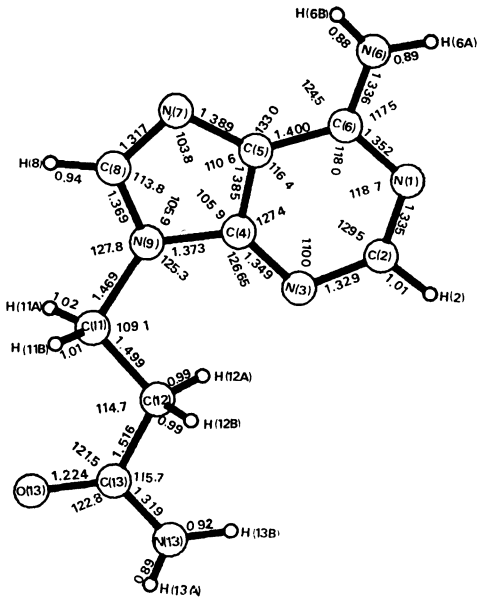

Fig. 1. Atomic numbering of 3-(adenin-9-yl)propionamide with bond lengths (l/Å) and angles (ϕ/°). E.s.d.'s are 0.006 Å and 0.3° for C, N, and O atoms, and 0.04 Å for H atoms. Bond angles involving H atoms are given in Table 4.

TABLE 4. BOND ANGLES (ϕ) INVOLVING HYDROGEN ATOMS OF 3-(ADENIN-9-YL) PROPIONAMIDE

	ϕ/°
N(1)–C(2)–H(2)	115(2)
N(3)–C(2)–H(2)	116(2)
C(6)–N(6)–H(6A)	118(2)
C(6)–N(6)–H(6B)	122(2)
H(6A)–N(6)–H(6B)	120(3)
N(7)–C(8)–H(8)	127(2)
N(9)–C(8)–H(8)	120(2)
N(9)–C(11)–H(11B)	105(2)
N(9)–C(11)–H(11A)	106(2)
N(11A)–C(11)–H(11B)	109(2)
C(12)–C(11)–H(11B)	114(2)
C(12)–C(11)–H(11A)	113(2)
C(11)–C(12)–H(12A)	109(2)
C(11)–C(12)–H(12B)	112(2)
C(13)–C(12)–H(12A)	107(2)
C(13)–C(12)–H(12B)	107(2)
H(12A)–C(12)–H(12B)	107(3)
C(13)–N(13)–H(13A)	121(2)
C(13)–N(13)–H(13B)	121(2)
H(13A)–N(13)–H(13B)	117(3)

TABLE 5. LEAST-SQUARES PLANES AND DEVIATIONS OF ATOMS FROM THE PLANES

X, Y, and Z are in Å along a^*, b, and c, respectively.

Plane 1 (ethyl chain)

$0.264(1)X-0.9538(9)Y+0.143(5)Z=1.31(2)$

Plane 2 (adenine plane)

$0.6557(5)X+0.2236(7)Y+0.7212(5)Z=10.243(2)$

Plane 3 (amide group)

$0.365(2)X-0.9164(8)Y+0.165(2)Z=2.65(3)$

Deviations (l/Å)					
Plane 1		Plane 2		Plane 3	
N(9)*	−0.060	N(1)*	0.005	C(12)*	0.001
C(11)*	0.053	C(2)*	−0.001	C(13)*	−0.003
C(12)*	0.067	N(3)*	0.002	N(13)*	0.001
C(13)*	−0.060	C(4)*	−0.002	O(13)*	0.001
N(13)	−0.190	C(5)*	−0.009	C(11)	−0.163
O(13)	−0.031	C(6)*	−0.0		
		N(7)*	−0.001		
		C(8)*	−0.009		
		N(9)*	−0.001		
		N(6)	0.001		
		C(11)	0.209		

* Atoms included in the calculations of the least-squares plane.

The ethyl chain linking the adenine ring with the amide group is nearly planar within 0.067 Å. The torsion angles of N(9)–C(11)–C(12)–C(13) and C(11)–C(12)–C(13)–N(13) are −170.0(3)° and 173.3(3)°, respectively.

Crystal Structure. The crystal structure viewed down the c axis is shown in Fig. 3. Hydrogen bond lengths and angles are given in Table 7. Adenine moieties are arranged so as to form ribbons along the

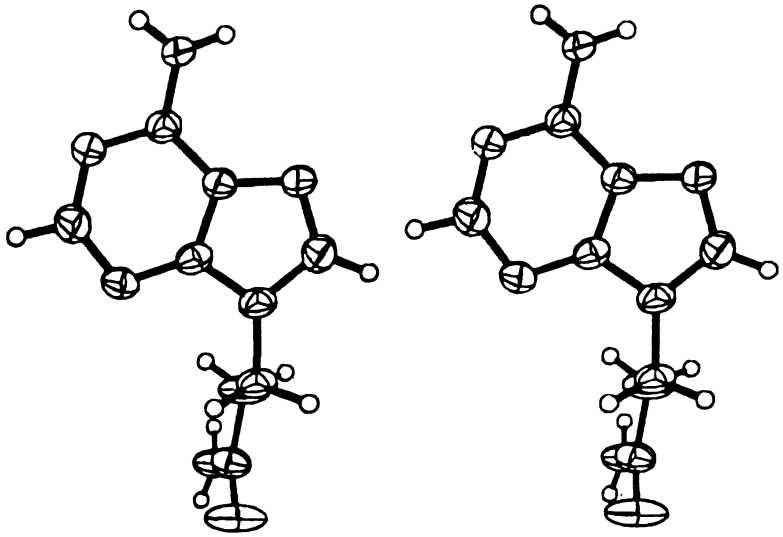

Fig. 2. Stereoview of 3-(adenin-9-yl)propionamide with 50% probability ellipsoids for the non-hydrogen atoms.

Table 6. Dimensions of the amide groups(l/Å)

	3-(Adenin-9-yl) propionamide	L-Asparagine[16] monohydrate	L-Glutamine[17]
C=O	1.224(4)	1.251(8)	1.228(3)
C–N	1.319(4)	1.352(6)	1.332(2)
C–C	1.516(6)	1.513(7)	1.509(2)

two-fold screw axis through the hydrogen bonds, N(6)H···N(1) and N(6)H···N(7). The arrangement is the same as that found in 3-(adenin-9-yl)propionic acid[7] and 5′-*O*-acetyladenosine.[18]

The amino group in amide is hydrogen-bonded to N(3) of the adenine moiety in the molecule related by inversion. The same kind of hydrogen bonding is found in 3-(adenin-9-yl)tryptamide crystal, in which the secondary amide of the peptide bond is a donor of hydrogen bond to N(3) of adenine moiety.

In the present crystal, the N(13)H···N(3) hydrogen bond links adenine ribbons in an anti-parallel mode to each other to form a sheet. The molecular sheet resembles that found in the 3-(adenin-9-yl)propionic acid crystal, though in the latter the linkage is the O–H···N(3) hydrogen bond, the adenine ribbons being parallel to each other.

The remaining hydrogen atom of the amide group participates in the hydrogen bond N(13)H···O(13) between the amide groups to link the molecular sheets (Fig. 4).

Table 7. Hydrogen bond distances and angles of 3-(adenin-9-yl)propionamide
Standard deviations are given in parentheses.

Distances	l/Å		
N(6)···N(7)^{c}	3.033(4)	C(6)–N(1)^{d}···H(6B)	125.4(9)
H(6A)···N(7)^{c}	2.15(3)	C(2)–N(1)^{d}···N(6)	107.7(2)
N(6)···N(1)^{d}	2.905(4)	C(2)–N(1)^{d}···H(6B)	112.5(9)
H(6B)···N(1)^{d}	2.06(3)	N(13)–H(13A)···N(3)^{b}	158(3)
N(13)···N(3)^{b}	3.055(4)	N(13)···N(3)–C(4)^{b}	134.9(2)
H(13A)···N(3)^{b}	2.21(3)	H(13A)···N(3)–C(4)^{b}	137.5(8)
N(13)···O(13)^{a}	2.983(4)	N(13)···N(3)–C(2)^{b}	115.0(2)
H(13B)···O(13)^{a}	2.18(3)	H(13A)···N(3)–C(2)^{b}	112.4(8)
Angles	ϕ/°	N(3)^{b}···N(13)···O(13)^{a}	151.3(1)
N(6)–H(6A)···N(7)	170(3)	O(13)^{a}···N(13)–C(13)	101.0(2)
N(6)···N(7)–C(8)^{c}	125.1(2)	C(13)–N(13)···N(3)^{b}	107.4(2)
H(6A)···N(7)–C(8)^{c}	127.1(9)	C(13)–O(13)^{a}···N(13)	153.8(2)
N(6)···N(7)–C(5)^{c}	128.7(2)	N(13)–H(13B)···O(13)^{a}	146(3)
H(6A)···N(7)–C(5)^{c}	127.5(9)	H(13B)···O(13)–C(13)^{a}	143.9(1)
C(6)–N(6)···N(7)^{c}	123.8(2)	Symmetry codes	
N(7)^{c}···N(6)···N(1)^{d}	101.8(1)	(a) $x, y, 1+z$	
N(1)^{d}···N(6)–C(6)	134.3(2)	(b) $1-x, 1-y, 1-z$	
N(6)–H(6B)···N(1)^{d}	161(3)	(c) $1/2-x, 1/2+y, 2-z$	
C(6)–N(1)^{d}···N(6)	128.8(2)	(d) $1/2-x, -1/2+y, 2-z$	

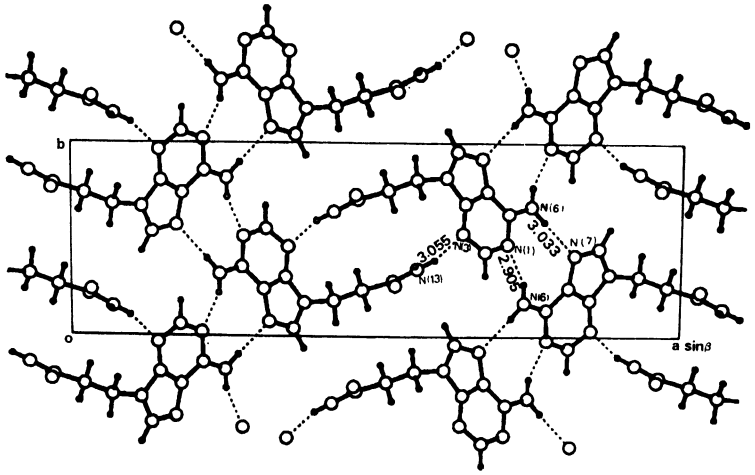

Fig. 3. Crystal structure of 3-(adenin-9-yl)propionamide projected along the c axis.

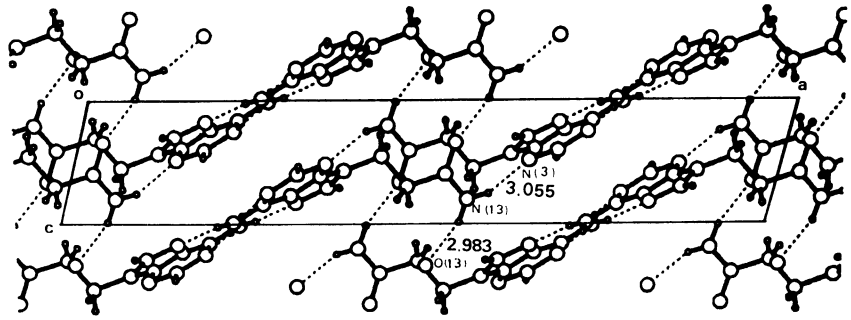

Fig. 4. Crystal structure of 3-(adenin-9-yl)propionamide projected along the b axis.

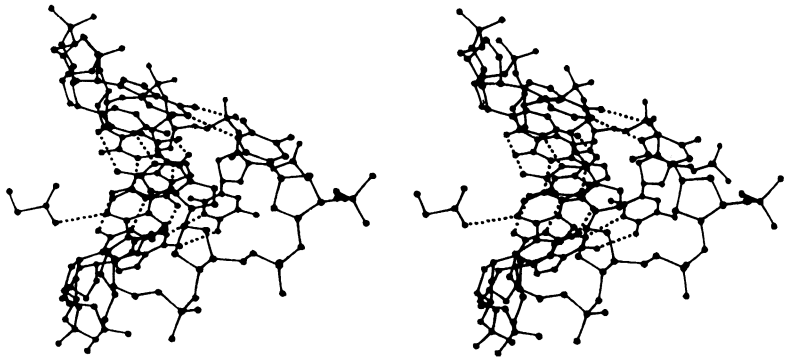

Fig. 5. Fitting model between amide group of the side chain of protein and adenine moiety paired with thymine residue in the minor groove of double helical DNA. (Conformation A)

Elementary Pattern of Interaction between Adenine and Amide Group. In order to see if the binding mode between adenine N(3) and amide group N(13) is an elementary pattern of protein-nucleic acid interactions, we have tried to fit the binding geometry into a minor groove of the double helical DNA by an interactive computer graphics; atomic coordinates of DNA were taken from the work of Arnott and Hukins.[19]

In DNA (A form), the amide side chain of amino acid fitting into adenine N(3) with the observed binding geometry (Table 8)[3] is free from any steric hindrance as shown in Fig. 5. However, the groove is not very large and the rotation of the amide group about the N(13)H···N(3) hydrogen bond (60°) gives rise to a close contact between O(13) and the ribose ring of the neighbouring nucleotide.

On the other hand, in DNA (B form) with a much narrower minor groove the amide side chain fitting into adenine N(3) gives rise to an abnormal contact between N(13) and oxygen atom of the ribose ring of the neighbouring nucleotide. Even after considerable adjustments of torsional angles and bond angles a close contact of 2.5 Å still remains. Conformational change of DNA should be introduced.

Table 8. Binding parameters between adenine and amide group in Fig. 5

Interaction bond distance			
l_{kl}/Å		3.055	
Bond angle			
θ_{jkl}/°		115	
θ_{klm}/°		107	
Torsion angle[a)]			
ϕ_{ijkl}/°		176	
ϕ_{jklm}/°		275	
ϕ_{klmn}/°		203	
Atoms in adenine		Atoms in amide group	
i	N(1)	l	N(13)
j	C(2)	m	C(13)
k	N(3)	n	C(12)

a) Values for the molecules related by inversion.

Figures 1, 3, 4, and 5 were drawn by TSD : XTAL and TSD: MODL,[20] computer-graphics interactive modelling programmes for NOVA 3 computer. The present work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture.

References

1) M. Ohki, A. Takenaka, H. Shimanouchi, and Y. Sasada, *Bull. Chem. Soc. Jpn.*, **48**, 848 (1975).
2) C. Tamura, T. Hata, S. Sato, and N. Sakurai, *Bull. Chem. Soc. Jpn.*, **45**, 3254 (1972).
3) A. Takenaka, M. Ohki, and Y. Sasada, *Bull. Chem. Soc. Jpn.*, **53**, 2724 (1980).
4) T. Hata, M. Yoshioka, S. Sato, and C. Tamura, *Acta Crystallogr., Sect. B*, **31**, 312 (1975).
5) M. Ohki, A. Takenaka, H. Shimanouchi, and Y. Sasada, *Bull. Chem. Soc. Jpn.*, **50**, 2573 (1977).
6) M. Ohki, A. Takenaka, H. Shimanouchi, and Y. Sasada, *Acta Crystallogr., Sect. B*, **33**, 2954 (1977).
7) A. Takenaka and Y. Sasada, 38th National Meeting of the Chemical Society of Japan, Nagoya, October 1978, Abstr., p. 1004.
8) N. C. Seeman, J. M. Rosenberg, F. L. Suddath, J. J. P. Kim, and A. Rich, *J. Mol. Biol.*, **104**, 109 (1976).
9) G. Lancelot and C. Héléne, *Nucleic Acids Res.*, **16**, 1063 (1979).
10) K. Kondo, M. Miyata, and K. Takemoto, *Bull. Chem. Soc. Jpn.*, **44**, 2554 (1971).
11) L. E. McCandlish and G. H. Staut, *Acta Crystallogr., Sect. A*, **31**, 245 (1975).
12) "International Tables for X-Ray Crystallography," Kynoch Press, Birmingham (1974), Vol. IV, p. 71.
13) Table 3 is deposited as Document No. 8130, at the Office of the Bulletin of the Chemical Society of Japan.
14) T. J. Kistenmacher and M. Rossi, *Acta Crystallogr., Sect. B*, **33**, 253 (1977).
15) D. Voet and A. Rich, *Prog. Nucl. Acids. Res. Molec. Biol.*, **10**, 183 (1970).
16) J. J. Verbist, M. S. Lehmann, T. F. Koetzle, and W. C. Hamilton, *Acta Crystallogr., Sect. B*, **28**, 3006 (1972).
17) T. F. Koetzle, M. N. Frey, M. S. Lehmann, and W. C. Hamilton, *Acta Crystallogr., Sect. B*, **28**, 1571 (1973).
18) T. Koizumi, Y. Ohashi, and Y. Sasada, Annual Meeting of the Crystallographic Society of Japan, Nagoya, November, 1979, Abstr., p. 1B-13.
19) S. Arnott and D. W. L. Hukins, *Biochem. Biophys. Res. Commun.*, **47**, 1504 (1972).
20) A. Takenaka and Y. Sasada, *J. Cryst. Soc. Jpn.*, **22**, 214 (1980).