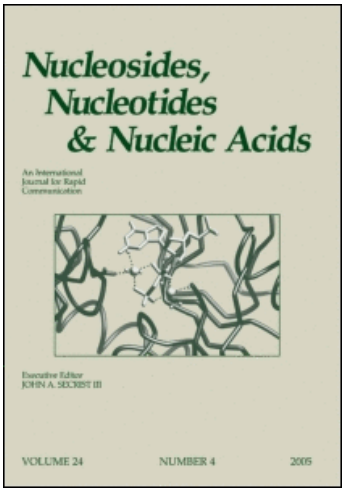

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Crystal Structure of a Tightly Bound Inhibitor of Adenosine Transport N^6-(4-Nitrobenzyl)-β-D-2'-Deoxyadenosine Methanol Solvate, $C_{17}H_{18}N_6O_5.1/2\ CH_3OH$

J. Wilson Quail[a]; Louis T. J. Delbaere[b]; Alan R. P. Paterson[c]
[a] Departments of Chemistry, and Biochemistry, University of Saskatchewan, Saskatoon, Canada [b] Biochemistry, University of Saskatchewan, Saskatoon, Canada [c] Cancer Research Group (McEachern Laboratory), University of Alberta, Edmonton, Alberta, Canada

To cite this Article Quail, J. Wilson , Delbaere, Louis T. J. and Paterson, Alan R. P.(1987) 'Crystal Structure of a Tightly Bound Inhibitor of Adenosine Transport N^6-(4-Nitrobenzyl)-β-D-2'-Deoxyadenosine Methanol Solvate, $C_{17}H_{18}N_6O_5.1/2\ CH_3OH$', Nucleosides, Nucleotides and Nucleic Acids, 6: 5, 877 — 885
To link to this Article: DOI: 10.1080/15257778708073434
URL: http://dx.doi.org/10.1080/15257778708073434

NUCLEOSIDES & NUCLEOTIDES, 6(5), 877-885 (1987)

CRYSTAL STRUCTURE OF A TIGHTLY BOUND INHIBITOR OF ADENOSINE TRANSPORT N^6-(4-NITROBENZYL)-β-D-2′-DEOXYADENOSINE METHANOL SOLVATE, $C_{17}H_{18}N_6O_5 \cdot 1/2\ CH_3OH$

J. Wilson Quail[+], Louis T.J. Delbaere[++] and Alan R.P. Paterson[+++]

[+]Departments of Chemistry, and [++]Biochemistry, University of Saskatchewan, Saskatoon, Canada. S7N 0W0 and [+++]Cancer Research Group (McEachern Laboratory), University of Alberta, Edmonton, Alberta, Canada. T6G 2H7.

Abstract The molecular structure of N^6-(4-nitrobenzyl)-β-D-2′-deoxyadenosine (I) has been determined by single crystal X-ray diffraction. A potent inhibitor of adenosine permeation in cultured S49 mouse lymphoma cells, I binds tightly (K_D 2.4 nM) to high affinity membrane sites present on the nucleoside transporter elements of these cells. Compound I crystallizes in the trigonal space group $P3_221$ with unit cell dimensions $a = b = 8.0009(9)$Å, $c = 49.174(8)$Å, and $Z = 6$. The structure was solved by direct methods and refined by least-squares to a final $R = 0.038$. The mean plane of the 4-nitrobenzyl group, an important substituent for potent nucleoside transport inhibition in a series of S^6-substituted 6-thioinosine derivatives, is inclined at an angle of 120.6° to the plane of the adenine ring. The torsion angles around the methylene carbon atom of this benzyl group are C(6)-N(6)-C(10)-C(11), 96.6° and N(6)-C(10)-C(11)-C(12), 93.6°. The glycosidic torsion angle, χ, is 217.1° which corresponds to the common anti nucleoside conformation. The deoxyribose ring, however, has the unusual C(1′)-exo conformation, with C(1′) displaced 0.608Å from the plane of C(2′), C(3′), C(4′) and O(4′). The conformation about the exocyclic C(4′)-C(5′) bond is gauche[+].

INTRODUCTION

This study concerns the determination of the crystal structure of N^6-(4-nitrobenzyl)-β-D-2′-deoxyadenosine (I), a potent inhibitor of adenosine permeation in cultured S49 mouse lymphoma cells. The inhibition property is due to the binding of I with high affinity to a specific type of binding site located on the nucleoside transporter polypeptides of these cells. The aim of this work is to identify the

structural features (chemical groups and conformations) of I which are responsible for their tight interaction with the nucleoside transport mechanism.

Entry of the physiological nucleosides into animal cells is mediated by nucleoside-specific transport elements of the plasma membrane[1,2]. In S49 cells and various other cell types, nucleoside transport is blocked by nanomolar concentrations of NBMPR (S^6-(4-nitrobenzyl)-β-D-thioinosine) and by related compounds[3,4]. It must be noted that nucleoside transport mechanisms with low NBMPR sensitivity also occur in various cell types; these are significantly inhibited only at millimolar NBMPR concentrations[3,4]. In cells with nucleoside transporters having high NBMPR sensitivity, NBMPR is bound tightly (K_D 0.1-1 nM), but reversibly, to plasma membrane sites which appear to be on nucleoside transporter proteins[5]. Occupancy of these sites by NBMPR correlates directly with inhibition of nucleoside transport[6].

An important group which affects the interaction of NBMPR with the nucleoside transport mechanism is the 4-nitrobenzyl substituent[7]. This group evidently contributes to the interaction of a series of N^6-substituted adenine nucleosides with the nucleoside transport mechanism[3]. Compound I, a member of this series, is a potent inhibitor of adenosine transport and is bound tightly (K_D 2.4 nM) at the high affinity, nucleoside transporter sites of S49 mouse lymphoma cells (at which the K_D for bound NBMPR is about 0.1 nM)[3]. Potent inhibitors of nucleoside transport such as NBMPR and (I) may have applications as modifiers of the *in vivo* disposition of nucleoside analogs with therapeutic uses as antineoplastic and antiviral agents[2,3,8].

RESULTS AND DISCUSSION

Selected bond distances and angles of I are given in TABLES 1 (a) and (b), respectively. Selected torsion angles are listed in TABLE 2. A view of the molecule is given in FIG. 1. The bond distances and angles in the 4-nitrophenyl and the adenine rings of (I) are similar to those found for these groups in other structure determinations.

The deoxyribose moiety bond distances and angles are as expected with C(1′)-O(4′), 1.414(5)Å, being significantly smaller than C(4′)-O(4′), 1.442(4) Å.

TABLE 1 (a). Selected bond distances (Å) for I.

N(1)-C(2)	1.332(5)*	C(10)-C(11)	1.504(8)
N(1)-C(6)	1.344(4)	C(11)-C(12)	1.389(5)
N(3)-C(2)	1.326(5)	C(11)-C(16)	1.384(7)
N(3)-C(4)	1.353(4)	C(12)-C(13)	1.381(8)
N(7)-C(5)	1.393(4)	C(13)-C(14)	1.371(7)
N(7)-C(8)	1.317(5)	C(14)-C(15)	1.370(6)
N(9)-C(1')	1.456(5)	C(15)-C(16)	1.375(8)
N(9)-C(4)	1.366(4)	C(1')-O(4')	1.414(5)
N(9)-C(8)	1.371(5)	C(1')-C(2')	1.528(9)
N(6)-C(6)	1.342(5)	C(2')-C(3')	1.525(7)
N(6)-C(10)	1.461(5)	C(3')-O(3')	1.419(6)
N(14)-C(14)	1.479(7)	C(3')-C(4')	1.535(7)
N(14)-O(14A)	1.224(5)	C(4')-O(4')	1.442(4)
N(14)-O(14B)	1.223(6)	C(4')-C(5')	1.514(5)
C(4)-C(5)	1.380(5)	C(5')-O(5')	1.412(8)
C(5)-C(6)	1.402(5)		

TABLE 1 (b). Selected bond angles (deg.)

C(2)-N(1)-C(6)	117.4(3)	C(10)-C(11)-C(12)	120.0(4)
C(2)-N(3)-C(4)	110.5(3)	C(10)-C(11)-C(16)	121.3(4)
C(5)-N(7)-C(8)	103.9(3)	C(12)-C(11)-C(16)	118.7(5)
C(4)-N(9)-C(8)	105.9(3)	C(11)-C(12)-C(13)	121.0(5)
C(6)-N(6)-C(10)	123.4(3)	C(12)-C(13)-C(14)	118.2(4)
C(14)-N(14)-O(14A)	118.2(4)	N(14)-C(14)-C(13)	118.4(4)
C(14)-N(14)-O(14B)	117.9(4)	N(14)-C(14)-C(15)	119.1(4)
O(14A)-N(14)-O(14B)	123.9(5)	C(13)-C(14)-C(15)	122.5(5)
N(1)-C(2)-N(3)	130.6(3)	C(14)-C(15)-C(16)	118.6(5)
N(3)-C(4)-C(5)	125.7(3)	C(11)-C(16)-C(15)	121.0(4)
N(3)-C(4)-N(9)	127.8(3)	N(9)-C(1')-C(2')	115.6(5)
N(9)-C(4)-C(5)	106.5(3)	N(9)-C(1')-O(4')	108.2(3)
N(7)-C(5)-C(4)	110.2(3)	C(2')-C(1')-O(4')	104.1(3)
N(7)-C(5)-C(6)	132.3(3)	C(1')-C(2')-C(3')	101.3(5)
C(4)-C(5)-C(6)	117.4(3)	C(2')-C(3')-C(4')	104.2(4)
N(1)-C(6)-N(6)	120.3(3)	C(2')-C(3')-O(3')	112.0(3)
N(1)-C(6)-C(5)	118.4(3)	C(3')-C(4')-C(5')	115.0(4)
N(6)-C(6)-C(5)	121.3(3)	C(3')-C(4')-O(4')	106.3(3)
N(7)-C(8)-N(9)	113.5(3)	C(4')-C(5')-O(5')	111.7(5)
N(6)-C(10)-C(11)	114.5(4)	C(1')-O(4')-C(4')	105.0(3)

* Estimated standard deviation of the last digit is given in parentheses.

TABLE 2. Selected torsion angles (deg).

C(1')-C(2')-C(3')-C(4')	-20.4	O(5')-C(5')-C(4')-O(4')	-71.0
C(2')-C(3')-C(4')-O(4')	4.6	O(5')-C(5')-C(4')-C(3')	49.2
C(3')-C(4')-O(4')-C(1')	30.6	O(4')-C(1')-N(9)-C(4)	217.1
C(4')-O(4')-C(1')-C(2')	-44.2	C(6)-N(6)-C(10)-C(11)	96.6
O(4')-C(1')-C(2')-C(3')	39.8	N(6)-C(10)-C(11)-C(12)	93.6

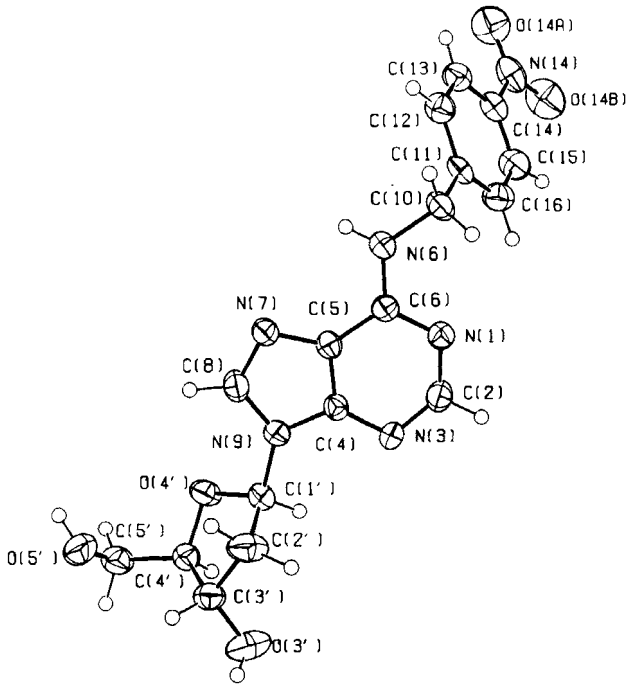

FIG. 1. A perspective view of N^6-(4-nitrobenzyl)-β-D-2′-deoxyadenosine, I, as found in the crystal structure.

The nucleoside (I) has the usual anti conformation with the glycosidic torsion angle, C(4)-N(9)-C(1′)-O(4′), χ = 217.1°. This may be compared to that occurring for adenosine[9] where, χ = 189.9° and deoxyadenosine[10] where, χ = 186.7°.

Deoxyribose ring.

The deoxyribose ring has the unusual C(1′)-exo envelope conformation with C(1′) displaced by 0.61Å from the plane of C(2′), C(3′), C(4′) and O(4′) (planar within 0.03Å). This may be compared with the envelope conformations and displacements which occur for adenosine[9] (C(3′)-endo, 0.55Å from the C(4′), O(4′), C(1′), C(2′) mean plane) and deoxyadenosine[10] (C(3′)-exo, 0.55Å from the C(4′), O(4′), C(1′), C(2′) mean plane). More crystal structures of related adenosine analogues will have to be carried out to see if this C(1′)-exo conformation is specific for these potent nucleoside transport inhibitors. The geometry about the exocyclic C(4′)-C(5′) bond is g^+ (i.e. O(5′) is gauche to

C(3'), ϕ_{OC} = 49.2° and gauche to O(4') ϕ_{OO} = -71.0°). The C(4')-C(5') bond is t for adenosine (ϕ_{OC} = 176.9°, ϕ_{OO} = 60.1°) and is also t for deoxyadenosine (ϕ_{OC} = -173.1°, ϕ_{OO} = 68.1°). Care must be taken in calculating the conformation angles for deoxyadenosine because the structure reported in the literature[10] was solved and mistakenly reported for the L-form of the molecule. The values calculated for the two pseudorotational parameters[11] for compound I are P = 119.7° and m = 41.1°, which corresponds to the $_1E$ conformation.

The 4-nitrobenzyl group is important for the potent nucleoside transport inhibition[7]. The ten nonhydrogen atoms of the adenine base are planar within 0.041Å. The group of six phenyl carbon atoms plus N(6) and C(10) is planar within 0.009Å. The 4-nitrobenzyl group is distal to the imidazole ring of the adenine base (see FIG. 1). The plane of the nitrobenzyl group is inclined at 120.6° to the plane of the adenine ring. The preferred conformations for the NH-CH_2-R moiety have been reported[12]. These torsion angles C(6)-N(6)-C(10)-C(11), 96.6°, and N(6)-C(10)-C(11)-C(12), 93.6°, fall within this preferred range of values. The corresponding torsion angles in N^6-(Δ^2-isopentenyl)-2-methylthioadenine are 103.6° and 143.6°, respectively[13].

Molecular packing.

The crystal structure is stabilized by three hydrogen bonds. A bond N...H-O [2.770(4)Å] is formed between N(3) of one molecule and O(5') of another related by the symmetry operation (x + 1, y + 1, z). Molecules related by (y, x + 1, -z + 1) also form an O-H...O hydrogen bond between O(3') and O(5') [2.721(6)Å]. A third hydrogen bond, (N-H)...N [2.972(5)Å] is found between N(6) of one molecule and N(7) of another related by the symmetry operation (-x, y - x, -z + 2/3).

CONCLUSIONS

The glycosidic torsion angle of I, χ = 217.1°, represents the anti conformation. The deoxyribose ring of I has the unusual C(1')-exo envelope conformation with C(1') displaced 0.61Å from the mean plane of C(2'), C(3'), C(4') and O(4'). This deoxyribose ring conformation may not contribute to the interaction of I with the high affinity transporter sites on S49 cells because the arabinofuranosyl and ribofuranosyl homologs of I have binding affinities at those sites similar to that of I (K_D, 4.7 and 8.7 nM, respectively).

The 4-nitrobenzyl group is required for nucleoside transport inhibition[7]. The mean plane of this moiety is inclined at an angle of 120.6° to the plane of the adenine ring. The torsion angles involving the methylene carbon atom of the 4-nitrobenzyl group are C(6)-N(6)-C(10)-C(11), 96.6° and N(6)-C(10)-C(11)-C(12), 93.6°. These conformations may be important for the interaction of I with the nucleoside transport proteins which are intrinsic to the plasma membrane.

EXPERIMENTAL

The molecule (I) was synthesized and kindly supplied by Dr. M.J. Robins of the University of Alberta. Thin hexagonal plate-like crystals were grown by the vapor diffusion of diethyl ether into a solution of the compound in methanol. Unit-cell parameters were refined from accurately measured 2θ-values of 25 high-angle reflections. A CAD4F diffractometer was used to collect three dimensional Cu K_α intensity data on 2105 reflections ($\sin\theta/\lambda < 0.609 Å^{-1}$) by the ω -2θ scan mode. Lorentz and polarization corrections were applied; no significant change occurred in the intensities of three standard reflections which were monitored throughout the data collection.

The structure was solved by direct methods using MULTAN80[14] to provide the phase angles and XRAY76[15] to calculate the E map. An adenine fragment and a 4-nitrobenzyl fragment were input to the normalization program. The EXFFT program of MULTAN80 could not correctly calculate an E map for the space group being used ($P3_121$). Fourier maps calculated with EXFFT did not have the correct crystallographic symmetry for space group $P3_121$. Consequently, phases from MULTAN80 were input to XRAY76 to calculate an E map. An E map computed with the best set of phases (figure of merit = 3.0, RESID = 14.04) revealed the positions of 24 non-hydrogen atoms. All of the adenine and 4-nitrobenzyl moieties were found as well as a fragment of the deoxyribose. The remaining portion of the deoxyribose was located from difference Fourier maps. At this stage space group $P3_121$ was used. The absolute configuration of the molecule was known from the chemical synthesis and it was apparent that we had the wrong enantiomer. Thus the coordinates were converted to the $P3_221$ equivalent structure for the remainder of this analysis. An unexpected broad peak was found near the

TABLE 3. Positional parameters and average thermal parameters or U_{iso}

ATOM	x/a(x 10^4)	y/b(x 10^4)	z/c(x 10^5)	U_{eq}(Å^2)
N(1)	4967(5)*	8913(5)	37086(6)	0.035
C(2)	5200(6)	8584(6)	39674(8)	0.038
N(3)	4008(5)	7216(5)	41371(6)	0.033
C(4)	2314(6)	6022(5)	40122(7)	0.030
C(5)	1852(6)	6189(6)	37471(7)	0.032
C(6)	3275(6)	7688(5)	35900(7)	0.032
N(7)	-36(5)	4764(5)	36899(6)	0.035
C(8)	-641(6)	3785(6)	39188(8)	0.037
N(9)	722(5)	4472(4)	41209(5)	0.033
N(6)	2970(5)	7910(5)	33274(6)	0.034
C(10)	4303(7)	9575(6)	31644(9)	0.038
C(11)	5683(6)	9258(6)	29944(7)	0.034
C(12)	5225(6)	8648(6)	27264(8)	0.042
C(13)	6468(6)	8345(7)	25665(9)	0.041
C(14)	8178(6)	8681(6)	26786(8)	0.039
C(15)	8692(7)	9310(7)	29407(9)	0.048
C(16)	7426(7)	9578(7)	30985(8)	0.046
N(14)	9533(6)	8398(6)	25065(8)	0.053
O(14A)	9013(5)	7745(5)	22774(7)	0.072
O(14B)	11103(5)	8814(6)	26030(7)	0.078
C(1')	545(6)	3748(6)	43972(8)	0.037
C(2')	-540(9)	4333(7)	45958(9)	0.058
C(3')	-1151(7)	2775(6)	48130(8)	0.041
O(3')	154(6)	3396(5)	50353(6)	0.075
C(4')	-1043(7)	1128(6)	46691(7)	0.036
O(4')	-570(4)	1711(4)	43891(5)	0.040
C(5')	-2865(8)	-824(7)	46853(9)	0.047
O(5')	-4515(5)	-712(5)	46136(6)	0.058
N(100)	-5639(19)	-5618(28)	49051(40)	0.291

ATOM	x/a(x10^3)	y/b(x10^3)	z/c(x10^4)	U_{iso}(Å^2)
H(2)	645(5)	953(5)	4058(7)	0.036
H(8)	-190(5)	256(5)	3934(6)	0.036
H(6)	200(6)	695(6)	3249(7)	0.044
H(101)	351(6)	982(6)	3038(7)	0.047
H(102)	493(6)	1056(5)	3287(7)	0.031
H(12)	403(5)	840(5)	2654(6)	0.020
H(13)	619(5)	798(5)	2394(6)	0.030
H(15)	997(6)	954(6)	3008(7)	0.049
H(16)	770(6)	1000(6)	3285(7)	0.061
H(1')	196(6)	425(6)	4469(8)	0.071
H(21')	-186(6)	389(6)	4493(7)	0.054
H(22')	41(8)	568(7)	4671(9)	0.089
H(3'C)	-249(6)	239(7)	4884(8)	0.069
H(3'O)	-59(8)	374(9)	5139(10)	0.111
H(4')	10(6)	109(6)	4750(7)	0.048
H(51'C)	-283(6)	-176(6)	4559(7)	0.055
H(52'C)	691(5)	865(5)	4876(7)	0.050
H(5'O)	-503(8)	-144(8)	4466(9)	0.099

* Estimated standard deviation of the last digit is given in parentheses; $U_{eq} = 1/3\ (U_{11} + U_{22} + U_{33})$

two-fold axis on the difference Fourier map after the entire molecule had been located. The peak was too distant from any atom in the molecule to be connected by a chemical bond. This peak was interpreted as a disordered methanol solvent molecule centered across the two-fold axis. The scattering factor of this solvent molecule was approximated as a nitrogen atom [N(100)]. This atom refined with very large thermal paremeters, consistent with the large size of the cavity which the methanol molecule apparently occupies. Inclusion of this methanol molecule in the calculation of the contents of the unit cell improved the agreement of the observed (1.46 g cm^{-3}) and calculated (1.47 g cm^{-3}) density of the crystal. After full matrix least-squares refinement on F with anisotropic temperature factors, the positions of all the hydrogens on the molecule were determined by difference Fourier maps.

Atomic scattering factors including the anomalous scattering terms f′ and f′′ were obtained from the International Tables for X-ray Crystallography[16]; anisotropic thermal parameters for non-hydrogen atoms and isotropic thermal parameters for hydrogen atoms were included in final cycles and gave a final R = 0.038 (R_w = 0.038) for the 1381 observed reflections. The function minimized was of the form $w(|F_o|-|F_c|)^2$ with $w = (2I)^2 / |F_o|^2 (T + (0.02\ I)^2 + r^2B)$, where I is the net intensity, $|F_o|$ is the observed structure amplitude, T is the total peak count, B is the sum of the background counts and r is the ratio of the peak-scan time to the total background-counting time (r = 2 for this data).

The final atomic coordinates are given in TABLE 3. Supplementary material may be obtained from the publisher[17].

ACKNOWLEDGEMENTS

This work was supported by grants from the Medical Research Council of Canada and the National Cancer Institute of Canada.

REFERENCES

1. P.G.W. Plagemann, and R.M. Wohlheuter. Curr. Top. Membr. Transp. 14, 225 (1980).
2. A.R.P. Paterson, E.S. Jakobs, E.R. Harley N.-W. Fu, M.J. Robins, and C.E. Cass. In "Regulatory Function of Adenosine" (R.M. Berne, T.W. Rall, R. Rubio, eds.) Martinus Nijhoff, The Hague, p 203 (1983).

3. A.R.P. Paterson, E.S. Jakobs, E.R. Harley, C.E. Cass, and M.J. Robins. In "Development of Target-Oriented Anticancer Drugs" (Y.-C, Cheng, B. Goz, and M. Minkoff, eds.) Raven Press, New York, p 41 (1983).
4. J.A. Belt. Mol. Pharmacol. 24, 479 (1983).
5. J.D. Young, S.M. Jarvis, M.J. Robins and A.R.P. Paterson. J. Biol. Chem. 258, 2202 (1983).
6. C.E. Cass, L.A. Gaudette and A.R.P. Paterson. Biochim. Biophys. Acta. 345, 1 (1974).
7. A.R.P. Paterson, S.R. Naik, and C.E. Cass. Mol. Pharmacol. 13, 1014 (1977).
8. N. Kolassa, E.S. Jakobs, G.R. Buzzell and A.R.P. Paterson. Biochem. Pharmacol. 31, 1863 (1982).
9. T.F. Lai and R.E. Marsh. Acta Crystallogr. B28, 1982 (1972).
10. D.G. Watson, D.J. Sutor and P. Tollin. Acta. Crystallogr. 19, 111 (1965).
11. C. Altona and M. Sundaralingham. J. Amer. Chem. Soc. 94, 8205 (1972).
12. M. Soriano-Garcia and R. Parthasarathy. Biochim. Biophys. Res. Commun. 64, 1062 (1975).
13. R.K. McMullan and M. Sundaralingham. J. Amer. Chem. Soc. 93, 7050 (1971).
14. P. Main, S.J. Fiske, S.E. Hull, L. Lessinger, G. Germain, J-P. Declercq and M.M. Woolfson. "MULTAN80, A system of computer programs for the automatic solution of crystal structures from X-ray diffraction data", University of York, England (1980).
15. J.M. Stewart, P.A. Machin, C.W. Dickinson, H.L. Ammon, H. Heck and H. Flack. "XRAY76, The X-ray system of crystallographic computer programs", Computer Science Center, University of Maryland, College Park, MD. (1976).
16. International Tables for X-ray Crystallography, Kynock Press, Birmingham, England, Vol. IV (1974).
17. Anisotropic thermal parameters for non-hydrogen atoms and the observed and calculated structure amplitudes for I can be obtained from the Journal Editorial Department, Marcel Dekker, Inc., 270 Madison Avenue, New York, N.Y., 10016.

Received October 29, 1986.