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Absolute Configuration of (+)-Cyclophosphamide. A Crystal and Molecular Structure Determination by X-Ray Diffraction

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Abstract: The crystal and molecular structure of enantiomerically homogeneous cyclophosphamide ($C_7H_{15}N_2O_2PCl_2$) has been determined by x-ray diffraction with the absolute configuration being established by the anomalous dispersion of the Cl and P atoms. It is found that the dextrorotatory enantiomer of cyclophosphamide ($[\alpha]_D^{20}$ 2.3° (*c* 3.0, methanol)) has the *R* configuration at phosphorus. The compound crystallized in the rhombohedral space group *R*3 with the three molecules in the cell related by the threefold axis forming a trimeric unit by NH···O=P hydrogen bonding. Cell parameters are a = 10.520 (5) Å and α = 108.9 (1)°. The conformation of the enantiomerically homogeneous cyclophosphamide as compared to the racemate differs mainly in the orientation of one of the chloroethyl chains.

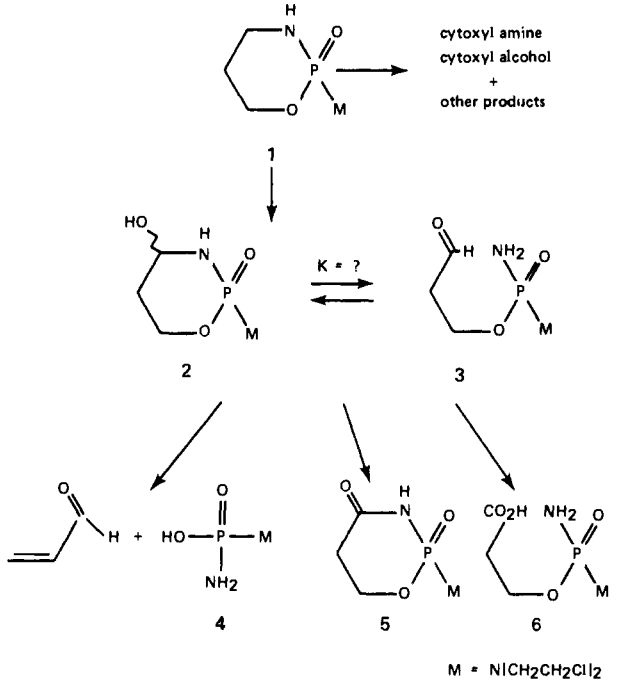
Cyclophosphamide (2-[bis(2-chloroethyl)amino]-2*H*-1,3,2-oxazaphosphorinane 2-oxide, **1**) is a widely used anticancer drug which is prepared synthetically and administered clinically in racemic form (Cytoxan).[2] The broad spectrum of activity[3] exhibited by **1** has led to considerable interest in its metabolism, and a substantial amount of chemical and biochemical data supports the degradative pathway shown in Scheme I. Fragmentation of enzymatically produced 4-hydroxycyclophosphamide (**2**) and/or its putative aldehyde tautomer, aldophosphamide (**3**), affords acrolein and phosphoramide mustard (**4**), generally regarded as the ultimate DNA cross-linking agent. Competing enzymatic conversion of **2** and/or **3** into 4-ketocyclophosphamide (**5**) and carboxyphosphamide (**6**) is associated with drug detoxification.[4] Since biological systems normally exhibit a marked enantiomeric selectivity, it was expected[5] and recently found[6] that the antipodal forms of **1** exhibit significantly different therapeutic indices[7] with (−)-**1** being more effective against PC6 mouse

Table I. Fractional Coordinates and Thermal Parameters[a] for (+)-Cyclophosphamide

Atom	x	y	z	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
Cl(1)	0.7872	0.3609	0.1009	5.97	6.21	6.90	3.07	3.47	2.65
Cl(2)	0.0968	0.2920	0.0039	5.00	9.14	6.74	2.14	2.15	2.23
P	0.6263	0.7198	0.3883	4.32	6.77	4.74	2.33	2.18	1.93
O(1)	0.7020	0.8721	0.3757	6.78	6.13	8.07	3.22	4.00	4.73
O(2)	0.5049	0.7188	0.4381	5.34	8.98	6.51	2.71	3.55	2.12
N(1)	0.5514	0.5706	0.2222	3.25	6.66	5.04	1.76	1.95	1.05
N(2)	0.7764	0.7210	0.5055	5.18	5.98	3.72	3.15	2.11	1.55
C(1)	0.6664	0.4067	0.1764	5.01	9.22	3.36	2.90	3.01	1.49
C(2)	0.6506	0.5390	0.1572	5.51	4.86	4.45	1.65	1.29	1.27
C(3)	0.3784	0.5024	0.1042	15.90	7.30	10.59	6.45	9.44	6.74
C(4)	0.3137	0.3801	0.1294	9.71	8.00	9.89	5.23	7.10	4.19
C(5)	0.8118	1.0130	0.5163	6.73	3.74	8.60	0.91	3.12	0.31
C(6)	0.9479	0.9953	0.5965	7.28	7.30	7.86	3.09	3.56	2.26
C(7)	0.9039	0.8635	0.6351	3.97	5.51	4.85	1.12	0.58	1.33

[a] Thermal parameters are of the form: $T = \exp[\frac{1}{4}(B_{11}h^2a^{*2} + B_{22}k^2b^{*2} + B_{33}l^2c^{*2} + 2B_{12}hka^*b^* + 2B_{13}hla^*c^* + 2B_{23}klb^*c^*)]$.

Scheme I



tumors than either (+)-**1** or (±)-**1** (the racemic mixture).[6]

The relative anticancer activities of (+)- and (−)-**1** presumably derive from a combination of different rates of, inter alia, enzymatic reaction, binding, and transport involving **1**,[8] and/or subsequent chiral metabolites (**2**, **3**, **5**, and **6**). A knowledge of the absolute stereochemistry of **1** is central to a detailed understanding of the in vivo action of this drug and antecedent to a totally rational approach to the design of improved analogues. We now report the results of the x-ray diffraction determination of the absolute configuration of (+)-**1**.

Results

Anhydrous (+)-**1** ($[\alpha]_D^{20}$ 2.3 ± 0.20° (*c* 3.0 methanol); mp 65–66.5 °C) was prepared in optically active form by catalytic hydrogenolysis of diastereomerically pure *N*-(α-methylbenzyl)cyclophosphamide **7**;[5,9,10] the enantiomeric purity of this sample was unambiguously established by nuclear magnetic resonance methods (observation of **1** in a chiral medium).[11] Material suitable for x-ray diffraction study was obtained by slow recrystallization from 30–60 °C petroleum ether/methylene chloride at 0 °C. The colorless lathlike crystals have the acicular axis parallel to the [110] direction. Many of the crystals form hollow tubes with a diamond cross-section. The shape of the crystal as well as the precession alignment photographs belied the true symmetry and, with the first crystal, data were collected as if for a triclinic cell. Three reflections used as standards during the data collection lost intensity quite anisotropically so that it was impossible to scale the data properly. These data were used to obtain the approximate structure. A second single crystal of dimensions 0.13 × 0.18 × 0.50 mm was employed to collect data for the determination of the absolute configuration.

A four-circle automatic diffractometer was used to measure the intensities with the θ–2θ scan mode, a scan speed of 2°/min, a scan width of $2.0° + 2\theta(\alpha_2) - 2\theta(\alpha_1)$, and a background count of 10 s at either end of the scan. Reflections 330, $\bar{5}50$, and 005 were monitored after every 50 intensity scans. With this crystal, the unique reflections for the rhombohedral space group $R3$ were recorded to $2\theta_{max} = 110°$ (for Cu K_α radiation). Data collection was stopped at this scattering angle since the three reflections monitored as standards had each lost ~15% of their intensity and after this point the three calibration curves for scaling the data had diverged. The data were corrected for Lorentz and polarization factors and rescaled for intensity loss.

The space group is $R3$ with $a = 10.520$ (5) Å, $\alpha = 108.9$ (1)°, $Z = 3$, $V = 914.6$ Å^3, mol wt = 261.1, and $d_{calcd} = 1.42$ g/cm^3. The structure was solved by obtaining phases directly from the magnitudes of the structure factors by means of the symbolic addition procedure.[12] An interesting aspect of the phase determination should be noted. For space group $R3$, $\phi_{hkl} = \phi_{klh} = \phi_{l\bar{h}\bar{k}}$ and $\phi_{hkl} = -\phi_{\bar{h}\bar{k}\bar{l}}$ (where $E_{hkl} = |E_{hkl}|e^{i\phi_{hkl}}$). Reflection $2\bar{2}0$ is very strong and its phase was denoted by the symbol x. The triple $2\bar{2}0 + \bar{2}02 = 0\bar{2}2$ gives rise to the phase relation $2x = -x$. An immediate impulse is to allow $3x = 0$ and $x = 0$; however, this is an incorrect assumption. The correct evaluation is that $3x = 2\pi$ and $x = (2/3)\pi$, an example of the 2π ambiguity.[12] The actual phase of reflection $2\bar{2}0$ is 2.23 radians as compared to 2.09 radians derived from the phase determination.

Full matrix least-squares refinements on F values, using anomalous dispersion factors for the Cl and P atoms,[13] with anisotropic thermal factors for the heavy atoms and constant parameters for the hydrogen atoms (calculated positions), resulted in unweighted agreement factors:

$$R = \frac{\sum||F_o| - |F_c||}{\sum|F_o|}$$

of 6.2% for the absolute configuration shown in this paper and

Table II. Nearest Intermolecular Contacts

	Separation, Å	Location of primed atom		
$Cl_1 \cdots C_1'$	3.70	$1+z$	x	y
$Cl_1 \cdots C_6'$	3.73	z	$-1+x$	$-1+y$
$Cl_2 \cdots C_5'$	3.70	$-1+y$	z	$-1+x$
$Cl_2 \cdots C_4'$	3.78	z	x	y
$Cl_2 \cdots C_6'$	3.80	$-1+x$	$-1+y$	$-1+z$
$O_1 \cdots C_5'$	3.40	z	x	$-1+y$
$O_2 \cdots C_1'$	3.20	z	x	y
$C_3 \cdots C_3'$	3.67	z	x	$-1+y$
$Cl_1 \cdots Cl_1'$	3.90	y	z	$-1+x$
$Cl_1 \cdots Cl_1''$	3.90	$1+z$	x	y
$Cl_1 \cdots Cl_2'$	3.92	$1+x$	y	z

7.2% for its optical antipode. The difference Fourier maps were essentially featureless after the refinements. The anomalous dispersion had a relatively small effect on the intensities of individual pairs of *hkl* and $\overline{hkl}$ reflections. Only 13 pairs had intensity ratios deviating from 1.0 by 3 to 9%. Of these 13 pairs, 12 were consistent with the configuration shown in this paper. Moreover, the significance test[14] on the ratio of the two R factors and the 536 independent reflections used in the least-squares refinement indicates a confidence level of better than 99.5%. The R configuration at phosphorus is thus established for **1**.[15]

Fractional coordinates and thermal parameters are listed in Table I, bond lengths, bond angles, and torsional angles are shown in Figures 1a, b, and c, respectively. The molecule of (+)-cyclophosphamide in its absolute configuration, R, is depicted in the stereodiagram in Figure 2.

Discussion

(+)-Cyclophosphamide crystallizes without water of hydration, in contrast to racemic cyclophosphamide[16,17] which contains one water molecule per asymmetric unit. Also in contrast to racemic **1** which is quite stable, (+)-**1** exhibits a moderate instability which is accelerated by exposure to x rays. Other cyclophosphamide analogues, such as the peroxy dimer,[18] decompose readily in the x-ray beam. Although the thermal parameters for all the atoms in (+)-**1** are somewhat high, those for C_3 and C_4 are particularly large (see Figure 2 and Table I), indicating considerable motion in these atoms that are possibly in the process of rearrangement to form a new compound. The three nearest bonding neighbors to the exocyclic N atom do not form a coplanar array with the N, although the uncertainty in the coordinates of C_3 may account for this apparent deviation from planarity. It is interesting to note that in the peroxy dimer[18] these same atoms have very large thermal parameters and exhibit disorder. Thermal parameters and coordinates have not been published for the crystal structures of most of the other cyclophosphamide analogues and derivatives[19-23] for comparison. In (+)-**1**, the C_4-Cl_2 distance appears to be 1.92 Å, at least 0.14 Å larger than a normal C-Cl bond length. In the first crystal of (+)-**1** which was analyzed, the C_4-Cl_2 distance also had a similarly large value. The large distance suggests that the Cl_2 atom is dissociating from the molecule.

The conformation of (+)-**1** and that found in the crystal of the racemate[16,17] can be compared in the stereodiagrams in Figures 2 and 3 and with the torsional angles shown in Figure 1c. In both molecules, the ring assumes the chair conformation, the phosphoryl oxygen is axial to the ring, the NCCCl chains are in the trans conformation, and C_2 is trans to O_2. The main difference between the molecules is in the torsional angle defined by $C_1C_2N_1P$, which changes from +105 to −102° and causes C_1 and C_4 to be syn in the (+)-**1** crystal and anti in the (±)-**1** crystal.

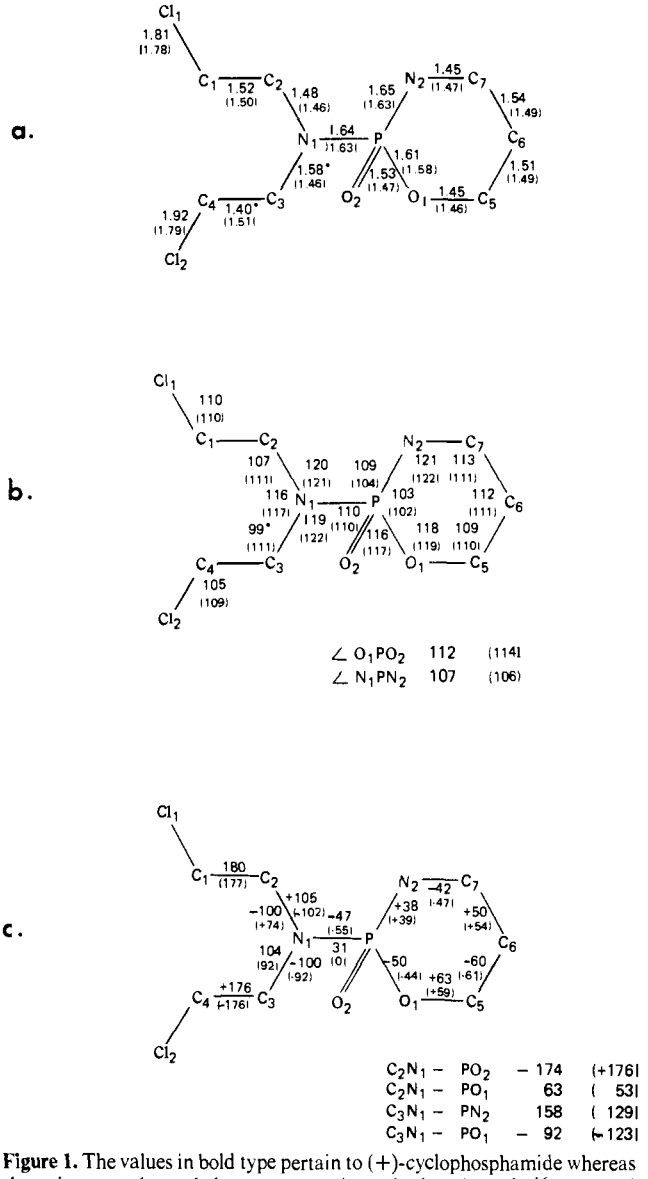
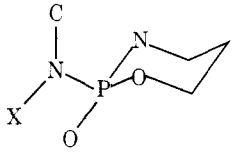


Figure 1. The values in bold type pertain to (+)-cyclophosphamide whereas those in parentheses belong to racemic cyclophosphamide:[16] (a) bond lengths—the standard deviations for (+)-cyclophosphamide are of the order of 0.02-0.03 Å except for C_3* which has very large thermal factors and is not located very well; (b) bond angles—standard deviations are of the order of 1.5-2.0° except for angles containing C_3*; (c) torsional angles—in the figure, the four atoms involved in a particular A_1A_2-A_3A_4 torsional angle surround the value of that angle. A value of 0° corresponds to a cis conformation, whereas 180° corresponds to a trans conformation; the torsion angle is considered positive for a right-handed rotation and when looking along the A_2-A_3 bond the far bond rotates relative to the near bond.

The moiety shown below



where X = H or C··, has essentially the same conformation in (+)-cyclophosphamide, racemic cyclophosphamide,[16,17] 4-peroxy dimer,[18] 4-hydroperoxycyclophosphamide,[19] *cis*-4-hydroperoxyisophosphamide,[19] isophosphamide,[22] and trophosphamide,[19,20] as well as in 4-ketocyclophosphamide[23] where the ring is somewhat flattened in the region of the keto group.[24] The common features of this moiety are a ring in the chair conformation, an axial P=O bond, geometries about exo-

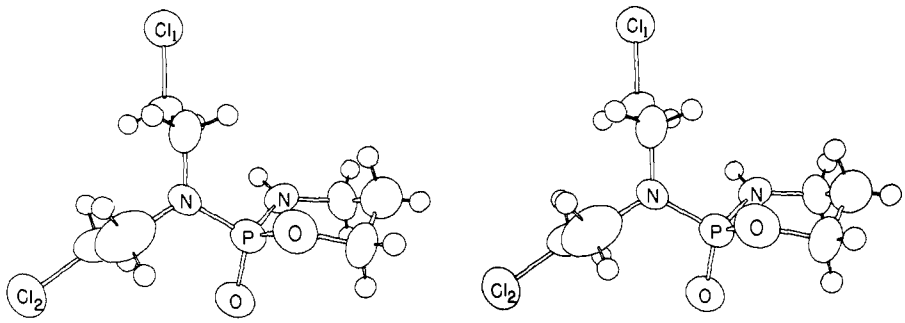


Figure 2. Stereodiagram of the absolute configuration of (+)-cyclophosphamide. The ellipsoids representing the thermal parameters are at a 50% probability level. Hydrogen atoms are represented by small spheres.

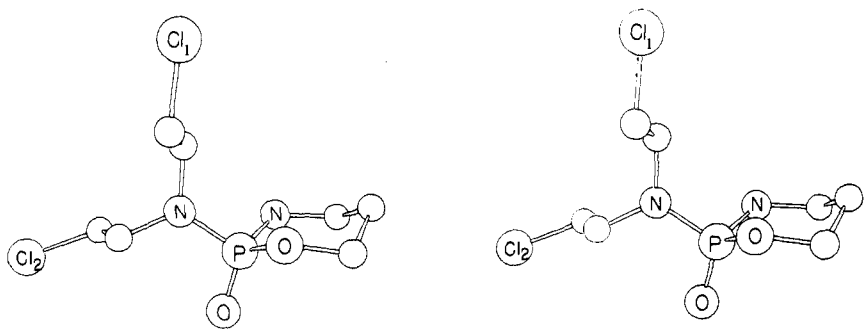


Figure 3. Stereodiagram redrawn from the coordinates of the racemate.[16] The spheres are ranged according to the weight of the atoms and are not related to the thermal parameters.

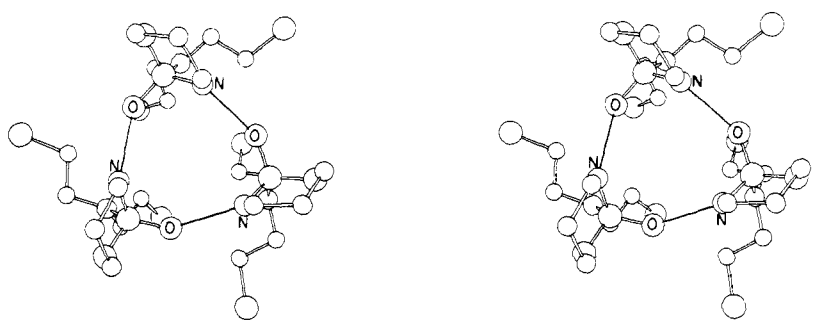


Figure 4. A view down the threefold axis of the unit cell showing the trimer formed by P=O···HN hydrogen bonds.

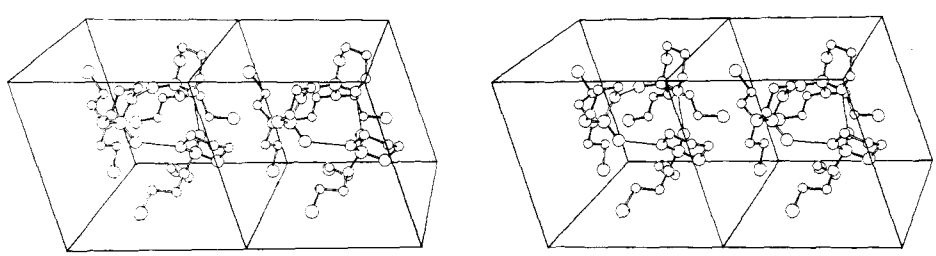


Figure 5. Two unit cells of (+)-cyclophosphamide showing the molecular packing. The axial directions are $a \rightarrow$, $b \uparrow$, and c directed up from the page; $a = b = c$ and $\alpha = \beta = \gamma$.

and endocyclic N atoms that are nearly planar, and a C atom on the exocyclic N that is trans to the phosphoryl oxygen. On the other hand, the chloroethyl chains assume varied conformations.

Hydrogen bonds are formed between the P=O and HN of three (+)-cyclophosphamide molecules that are related by the threefold axis in the unit cell and tie these molecules into a trimer. Figure 4 shows a view down the threefold axis. The $O_2 \cdots N_2$ distance is 2.84 Å as compared to 2.92–2.94 Å for the O···N distance in P=O···HN bonds in 4-ketocyclophosphamide[23] (an intermolecular bond across a center of symmetry) and the 4-peroxy dimer[18] (two intramolecular bonds). Molecular packing is illustrated in Figure 5. The nearest intermolecular approaches, listed in Table II, indicate a number of normal van der Waals contacts between the Cl and C atoms. The three closest Cl···Cl contacts, 3.90–3.92 Å, are somewhat larger than the sum of van der Waals radii for Cl (3.6 Å). The calculated density for the $R3$ cell of (+)-**1**, 1.42 g cm^{-3}, is the same as the calculated density for the $P2_1/c$ cell of (±)-**1**·H_2O. Since the $R3$ cell does not contain H_2O molecules, the molecular packing is considerably looser than in the racemate.

The presently reported R configuration for (+)-**1** provides

a convenient and reliable basis for the establishment of the absolute configuration at phosphorus in *all* of the known chiral metabolites of **1** (see Scheme I) which may be synthesized from **1** using reactions that do not involve stereochemical changes at the asymmetric phosphorus center. Our results in this connection will be reported in the future.

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Supplementary Material Available: Tables of observed and calculated structure factors (3 pages). Ordering information is given on any current masthead page.

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(8) In vitro kinetic measurements for (−)-1 → **2/3** using a liver microsomal enzyme preparation gave K_M = 0.57 mM and V_{max} = 27.4 μmol of nor-HN2 equiv g^{-1} h^{-1} whereas (+)-1 gave K_M = 0.48 mM and V_{max} = 22.2 μmol of nor-HN2 equiv g^{-1} h^{-1}.
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8-Azaadenosine. Crystal Structure of Its Monohydrate and Conformational Analysis for Rotation around the Glycosyl Bond

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Abstract: The crystal and molecular structure of the synthetic nucleoside analogue 8-azaadenosine has been determined using three-dimensional x-ray counter data. The nucleoside crystallizes as a monohydrate ($C_9H_{12}N_6O_4 \cdot H_2O$) in the space group $P2_12_12_1$ of the orthorhombic system with four formula units in a cell of dimensions a = 17.125 (2), b = 9.830 (1), and c = 7.428 (1) Å. The crystal structure is isomorphous with that of formycin monohydrate. The structure was refined using 1136 nonzero intensity data to an R factor of 0.033. The conformation of the nucleoside around the glycosyl bond is high-anti with a χ value of 103.7°. The conformation of the C(5′)–O(5′) bond around the C(4′)–C(5′) bond is gauche-trans, and the puckering of the ribose is C(2′)-endo-C(1′)-exo. A CNDO/2 molecular orbital calculation of the energy of the molecule as a function of the torsional angle χ shows that the most stable conformation for the isolated molecule is syn with a χ value of approximately 265°; the solid-state conformation with χ observed in the high-anti range, however, is the next most stable conformation and is only 0.5 kcal mol^{-1} higher in energy than the global minimum. The two energy barriers between the syn and the high-anti conformations are estimated to be 1.25 and 1.5 kcal mol^{-1}, which are considerably lower than the value of approximately 6 kcal mol^{-1} reported for the naturally occurring nucleoside, adenosine. A nonbonded contact search calculation and calculations of atomic charge density by the MO method show that a low-anti conformation with $\chi \lesssim 44°$ is precluded owing to a severe contact between negatively charged atoms N(8) on the base and ring oxygen atom O(4′) on the sugar. The high-anti conformation may be stabilized by an electrostatic attraction between N(8) on the base and C(2′) on the sugar. It is shown that the 8-azapurine nucleosides occurring in the high-anti conformation have their exocyclic bond angle C(4)–N(9)–C(1′) larger than N(8)–N(9)–C(1′) while the naturally occurring purine nucleosides occurring in the high-anti conformation have the reverse relationship.

Ever since the discovery of the antibacterial[1] and the antitumor[2] properties of 8-azaguanine there has been considerable interest in the biochemical[3] and biophysical[4,5] properties of 8-azapurines and their nucleosides (I, II). Our interest in these compounds is primarily structural and is motivated by the belief that the precise structural information provided by x-ray diffraction experiments and theoretical calculations based on the observed geometry would be of value in eventually unra-