Solid-State Conformation of Anti-Human Immunodeficiency Virus Type-1 Agents: Crystal Structures of Three 3′-Azido-3′-deoxythymidine Analogues

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Abstract: The crystal structures of three anti-HIV 3′-azido-3′-deoxynucleosides have been determined to gain conformational information for structure–activity studies. The compounds 3′-azido-3′-deoxythymidine (AZT), 3′-azido-2′,3′-dideoxyuridine (CS-87), and 3′-azido-2′,3′-dideoxy-5-ethyluridine (CS-85) are all active inhibitors of HIV-1 replication. X-ray diffraction data for all three compounds were measured at 165 K. AZT: $P2_1$, $a = 17.538$ (1) Å, $b = 12.021$ (1) Å, $c = 5.6435$ (3) Å, $\beta = 95.596$ (8)°, $Z = 4$, $R_{all} = 0.038$. CS-87: $P2_1$, $a = 9.7314$ (8) Å, $b = 6.7517$ (6) Å, $c = 8.3464$ (7) Å, $\beta = 91.854$ (7)°, $Z = 2$, $R_{all} = 0.046$. CS-85: $P2_1$, $a = 5.567$ (5) Å, $b = 22.093$ (9) Å, $c = 21.149$ (4) Å, $\beta = 96.005$ (9)°, $Z = 8$, $R_{all} = 0.036$. Large differences are observed in the conformations of the seven independent observations, including four different conformations for the glycosyl link and three furanose ring geometries. The azido group is nonlinear and has a preferred conformation trans to the C2′–C3′ bond (four molecules). The azido group of one CS-85 molecule is disordered. Two positions, cis to C2′–C3′ and 26° from this position, are fully resolved. The three structures show extensive but distinct hydrogen-bonding patterns that include two different base-pairing geometries. AZT has one dimer formed by two molecules bonded by N3–O2 hydrogen bonds. CS-85 has two dimers, one based on N3–O2 hydrogen bonds and one based on N3–O4 hydrogen bonds. The structure of CS-87 does not show base pairing but rather linear strands of molecules hydrogen bonded through the bases. The 5′-hydroxyl groups form additional strong hydrogen bonds with either the remaining carbonyl oxygens or 4′ ether oxygens. Strong correlations between intramolecular conformational differences and intermolecular interactions are observed. Variations in the positions of the furanose ring substituents correlate not only with differences in the geometry of the ring but also with differences in base-pairing geometries. These correlations would suggest a long-range effect of the furanose substitution on the interactions of the bases.

Analogues of nucleosides that lack the 3′-hydroxyl group are being studied extensively as potential therapeutic agents for the treatment of acquired immunodeficiency syndrome (AIDS). These compounds have been shown to be effective inhibitors of human immunodeficiency virus type-1 (HIV-1), the causative agents of AIDS.[1–4] Recently, 3′-azido-3′-deoxythymidine (AZT) was licensed by the FDA for the treatment of certain HIV infections. Other compounds that have shown promising activity in preliminary trials include 2′,3′-dideoxycytidine and 2′,3′-dideoxyadenosine.[5] 3′-Deoxynucleosides are thought to function by blocking chain elongation, inhibiting the viral enzyme reverse transcriptase. The exact nature of this inhibitory mechanism remains to be determined. Differences in activity and molecular composition of the various analogues are not always consistent with a simple model of incorporation of the 3′-deoxynucleoside in DNA that would block the attachment of additional nucleotides. For example, the importance of the 3′-azido group in the activity of thymidine analogues is not easily explained. Full understanding of the requirements for effectively binding to and inhibition of the enzyme is required before a systematic approach can be taken to develop more effective drugs. This requires structure–activity studies, including correlation of geometric features of the various compounds obtained from conformational studies with activity and toxicity levels observed in vitro and in vivo. In addition, the role of other biological processes such as metabolism will need to be examined.

In this paper we describe the solid-state conformations of AZT and two of its analogues, 3′-azido-2′,3′-dideoxyuridine (CS-87) and 3′-azido-2′,3′-dideoxy-5-ethyluridine (CS-85). These compounds were chosen not only because of their structural similarities to AZT but also for their promising biological activities. CS-87 is a potent anti-HIV-1 agent.[6,7] MIC values for CS-87 and AZT in ATH-8 cells are 0.40 and 0.32 μM, respectively. In peripheral blood mononuclear (PBM) cells, AZT is 50-fold more potent than
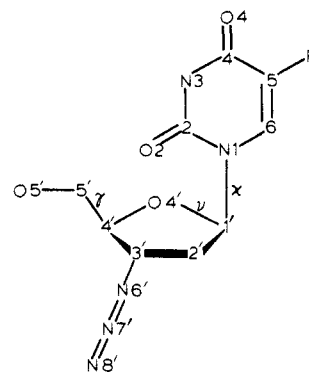
AZT, R = CH₃
CS-87, R = H
CS-85, R = C₂H₅

CS-87. However, the bone marrow toxicity levels of CS-87 are markedly lower than those of AZT.[6] CS-87 is currently a strong candidate for clinical studies. CS-85 is also a selective anti-HIV-1 agent in PBM cells with reduced toxicity.[8]

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Experimental Section

(a) General Procedures. Procedures for the synthesis of AZT[9] and CS-87[10] have been previously reported. However, the samples of AZT, CS-85, and CS-87 used for this study were prepared by different procedures, which will be reported elsewhere. Crystals were obtained by slow evaporation methods. All three structure determinations were performed under identical conditions unless specified. X-ray diffraction intensities were measured on a Nicolet P3 diffractometer using Ni-filtered Cu $K\alpha$ radiation ($\lambda = 1.5418$ Å). The crystal was cooled with a forced nitrogen stream. The temperature at the nozzle was 142 ± 2 K. Measurement of the temperature at the site of the crystal prior to data collection indicated that the crystal temperature should be considered to be about 165 K. All data between $4 < 2\theta < 115°$ were measured. Six reference reflections were measured after every 120th measurement but showed no significant variations. The data with $F > 3\sigma(F)$ were considered observed, where $\sigma^2(F) = (k/Lp I)[\sigma^2(I) + (0.01I)^2]$. Lorentz and polarization corrections were applied, but no absorption correction. The structures were determined by direct methods and refined by full-matrix least-squares, minimizing $\sum w(F_o - F_c)^2$, where $w = 1/\sigma^2(F)$ for the observed data and $w = 0$ for the unobserved data. Because of the limited number of data and the low thermal motion, only the exocyclic non-hydrogen atoms were refined with anisotropic thermal parameters. Hydrogen atoms were located in difference maps and refined in separate cycles after the refinement of the non-hydrogen atoms had converged. Two or three cycles of hydrogen atom refinement were alternated with two cycles of non-hydrogen atom refinement until the shift/error ratio in the first cycles of each set was less than 0.25. Atomic scattering factors and dispersion corrections were taken from ref.[11] Programs used include the data reduction package DREAM[12] and the direct methods programs MULTAN[13] and SHELX84.[14]

(b) AZT ($C_{10}H_{13}N_5O_4$). The sample was recrystallized from a 1:1 methanol–water mixture. The crystals are monoclinic, space group $P2_1$, $a = 17.538$ (1) Å, $b = 12.021$ (1) Å, $c = 5.6435$ (3) Å, $\beta = 95.596$ (8)°, $V = 1184.15$ Å^3, $Z = 4$, $M_r = 267.25$, $D_{calcd} = 1.499$ g cm^{-1}, $\mu = 0.963$ mm^{-1}. A total of 1701 unique data were measured of which 1698 were considered observed. Final residuals were $R = 0.037$ and $R_w = 0.055$ for the observed data and $R_{all} = 0.038$; the standard deviation of an observation of unit weight was $s = 5.995$. All hydrogen atoms were located and refined.

(c) CS-87 ($C_9H_{11}N_5O_4$). The sample was recrystallized from ethyl acetate. The crystals are monoclinic, space group $P2_1$, $a = 9.7314$ (8) Å, $b = 6.7517$ (6) Å, $c = 8.3464$ (7) Å, $\beta = 91.854$ (7)°, $V = 548.09$ Å^3, $Z = 2$, $M_r = 253.22$, $D_{calcd} = 1.534$ g cm^{-3}, $\mu = 1.01$ mm^{-1}. A total of 827 unique data were measured of which 821 were considered observed. Final residuals were $R = 0.046$, $R_w = 0.065$, and $R_{all} = 0.046$, $s = 6.939$. All hydrogen atoms were located and refined.

(d) CS-85 ($C_{11}H_{15}N_5O_4$). The sample was recrystallized from acetonitrile. The crystals are monoclinic, space group $P2_1$, $a = 5.567$ (5) Å, $b = 22.093$ (9) Å, $c = 21.149$ (4) Å, $\beta = 96.005$ (9)°, $V = 2587.24$ Å^3, $Z = 8$, $M_r = 281.27$, $D_{calcd} = 1.449$ g cm^{-1}, $\mu = 0.908$ mm^{-1}. A total of 3627 unique data were measured of which 3567 were considered observed. Final residuals were $R = 0.035$, $R_w = 0.041$, and $R_{all} = 0.036$, $s = 3.081$. The azido group of molecule D is disordered. Two independent positions for N7'D and N8'D have been observed. Initially, occupancies and isotropic thermal parameters were refined until a suitable fitting of the observed electron density could be obtained. Occupanies of 0.55 and 0.45 were chosen and were held constant during refinement with anistropic thermal parameters. All hydrogens were located in difference maps. Atom H2'B of molecule A became nonpositive definite during refinement. Its B value was reset to 4.00 Å^2 and held fixed.

Results and Discussion

Table I lists the atomic coordinates and isotropic thermal parameters (B_{iso}) for the non-hydrogen atoms. Where appropriate, B_{iso} represents the equivalent B calculated from the anisotropic thermal parameters.[15] The numbering scheme, definitions of

torsion angles and conformational geometries were taken from Saenger[16] and are consistent with the rules of the IUPAC–IUB Commission on Biochemical Nomenclature.[17,18] None of the bond lengths and angles are exceptional. The double bonds in the uridine rings are highly localized. The uridine bond lengths and angles do not appear influenced by substitution at the 5-position. The azido group is not linear. The N6'–N7'–N8' angle ranges from 171.0 (3) to 173.7 (3)° in the AZT, CS-87, and first three CS-85 molecules. The azido group in the fourth CS-85 molecule is somewhat less well determined as the result of the disorder, but all values are within the range observed for other azido groups. The 28 observations contained in the Cambridge Crystallographic Database[19] have N–N–N angles ranging from 168.0 to 176.7° with an average value of 172 (2)°.

The structure of AZT[20] has been determined previously, but coordinates were not available to us at the time of this study and a structure determination under identical conditions as those used for CS-85 and CS-87 was thought to be beneficial for the comparison of structural details.

The bond lengths, angles, torsion angles, and deviations from planes that are essential for the description of the molecular conformations of the seven independent observations (two for AZT, one for CS-87, and four for CS-85) are listed in Table II. The molecules are shown in Figure 1. Dimers are shown to allow simultaneous illustration of the molecular conformations and the principal intermolecular interactions: the base pairing. Each pair of molecules represents two crystallographically independent molecules for AZT and CS-85. The dimer for CS-87 shows two symmetry-related molecules. There is no a priori reason to assume that the difference in substitution at the 5-position would significantly alter the molecular conformation. However, large variations in the conformational features are observed. Figure 2 shows all seven molecules superimposed by least-squares fitting of the uridine moiety. Assuming that differences in torsion angles smaller than about 15° are not important for conformational analysis, four different conformations are observed for the glycosyl link, two for the furanose ring, two for the C4'–C5' link, and four for the azido group. Even the 5-ethyl substituent in CS-85 adopts two distinct positions. Only two of the seven molecules (AZTB, CS-85B) have identical conformations. AZTA and CS-85A differ from one another only in the position of the azide group. The glycosyl links (angle χ) are all anti but span a range of 65°. Four furanose rings (angle ν and out-of-plane deviations) are best described as having distorted twist conformations with 2'-endo. The others (AZTB, CS-85B, CS-85D) have distorted 3'-exo envelope conformations. Average torsion angles and deviations of atoms C2' and C3' from the plane of C1', O4', and C4' indicate the large variation in the distortions. Four azido groups are in the trans position to the C2'–C3' bond, one (CS-85C) is trans to the C4'–C3' bond, and one (CS-85A) is 30° out of the C2'–C3' trans position. The last bond (CS-85D) is disordered. Two separate positions of nearly equal occupancy (0.55 and 0.45) have been determined, one cis with the C2'–C3' bond and one 26° removed from this position. The orientation about the C4'–C5' bond (angle γ) is +sc (or gauche–gauche) in five molecules and −sc (or gauche–trans) in the remaining two (AZTB and CS-85B). The uridine ring is not always perfectly planar. In molecules CS-85C, CS-85D, and CS-87 the average torsion angles of the

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Table I. Atomic Coordinates (×10^4) and Isotropic Thermal Parameters (×10^2) for AZT, CS-87, and CS-85^a

	x/a	y/b	z/c	B$_{iso}$		x/a	y/b	z/c	B$_{iso}$
					AZT				
C(2A)	1647 (2)	9068 (4)	4033 (6)	184 (6)	C(2B)	3285 (2)	8271 (4)	-307 (6)	196 (6)
C(4A)	2599 (2)	10258 (3)	6331 (6)	175 (5)	C(4B)	2425 (2)	6962 (4)	-2654 (6)	198 (6)
C(5A)	2026 (2)	10545 (4)	7867 (6)	195 (6)	C(5B)	3058 (2)	6706 (4)	-4026 (6)	213 (6)
C(6A)	1328 (2)	10095 (4)	7436 (5)	186 (6)	C(6B)	3733 (2)	7232 (4)	-3476 (6)	195 (6)
C(7A)	2211 (2)	11340 (4)	9871 (6)	243 (9)*	C(7B)	2943 (2)	5870 (4)	-5989 (6)	287 (9)*
C(1'A)	338 (2)	8960 (4)	5226 (6)	195 (6)	C(1'B)	4596 (2)	8615 (4)	-1176 (6)	178 (5)
C(2'A)	103 (2)	8254 (4)	7286 (6)	231 (6)	C(2'B)	5000 (2)	8340 (4)	1296 (6)	230 (6)
C(3'A)	-734 (2)	8527 (4)	7314 (6)	219 (6)	C(3'B)	5817 (2)	8125 (4)	779 (6)	223 (6)
C(4'A)	-793 (2)	9746 (3)	6483 (6)	195 (6)	C(4'B)	5720 (2)	7686 (4)	-1771 (6)	189 (6)
C(5'A)	-772 (2)	10596 (4)	8485 (6)	240 (8)*	C(5'B)	5557 (2)	6437 (4)	-1914 (6)	229 (8)*
N(1A)	1123 (1)	9373	5577 (5)	186 (5)	N(1B)	3854 (1)	7995 (3)	-1668 (5)	186 (5)
N(3A)	2362 (1)	9514 (3)	4520 (5)	195 (5)	N(3B)	2596 (1)	7753 (3)	-857 (5)	191 (5)
N(6'A)	-1178 (2)	7779 (4)	5594 (6)	290 (8)*	N(6'B)	6216 (2)	9224 (3)	910 (6)	270 (8)*
N(7'A)	-1879 (2)	7888 (3)	5498 (4)	224 (8)*	N(7'B)	6914 (2)	9186 (3)	653 (5)	240 (8)*
N(8'A)	-2521 (2)	7903 (4)	5283 (5)	283 (8)*	N(8'B)	7546 (2)	9257 (4)	492 (6)	301 (9)*
O(2A)	1494 (1)	8444 (3)	2319 (4)	237 (6)*	O(2B)	3383 (1)	8968 (3)	1320 (4)	230 (6)*
O(4A)	3268 (1)	10612 (3)	6512 (4)	239 (6)*	O(4B)	1783 (1)	6573 (3)	-2966 (4)	276 (6)*
O(4'A)	-159 (1)	9908 (3)	5081 (4)	220 (4)	O(4'B)	5085 (1)	8324 (3)	-2879 (4)	194 (4)
O(5'A)	-138 (1)	10410 (3)	10202 (4)	287 (6)*	O(5'B)	5386 (1)	6087 (3)	-4298 (5)	299 (6)*
					CS-87				
C(2)	12029 (3)	3633 (7)	3188 (4)	180 (6)	N(1)	11352 (3)	5282	2608 (4)	178 (6)
C(4)	13928 (3)	5782 (7)	4108 (4)	193 (7)	N(3)	13306 (3)	3959 (6)	3873 (3)	179 (6)
C(5)	13172 (3)	7431 (7)	3464 (4)	221 (7)	N(6')	6972 (3)	4189 (7)	1443 (5)	308 (9)*
C(6)	11931 (4)	7117 (7)	2755 (4)	211 (7)	N(7')	5869 (3)	4554 (8)	789 (5)	323 (9)*
C(1')	9934 (3)	5021 (7)	1956 (4)	170 (6)	N(8')	4833 (4)	4711 (9)	134 (7)	553 (14)*
C(2')	8865 (4)	4998 (7)	3263 (4)	190 (7)	O(2)	11525 (2)	1947 (6)	3088 (3)	214 (6)*
C(3')	7615 (3)	5833 (7)	2369 (4)	192 (7)	O(4)	15061 (3)	5849 (6)	4815 (4)	261 (7)*
C(4')	8249 (3)	7373 (7)	1255 (4)	190 (6)	O(4')	9611 (2)	6642 (6)	950 (3)	196 (5)
C(5')	8301 (3)	9475 (7)	1919 (5)	232 (8)*	O(5')	9047 (2)	9593 (7)	3435 (3)	250 (6)*
					CS-85				
C(2A)	8736 (6)	1741 (2)	2447 (2)	179 (6)	C(4C)	-168 (7)	6236 (2)	2118 (2)	219 (6)
C(4A)	10174 (6)	1373 (2)	1456 (2)	191 (6)	C(5C)	-2024 (7)	6362 (2)	1608 (2)	233 (7)
C(5A)	11941 (6)	1855 (2)	1498 (2)	191 (6)	C(6C)	-3370 (7)	5898 (2)	1370 (2)	223 (6)
C(6A)	11984 (6)	2240 (2)	1996 (2)	178 (6)	C(7C)	-2273 (8)	7006 (2)	1366 (2)	324 (11)*
C(7A)	13562 (7)	1892 (2)	977 (2)	245 (9)*	C(8C)	-4303 (8)	7102 (2)	852 (2)	326 (11)*
C(8A)	15253 (7)	2431 (2)	1013 (2)	277 (10)*	C(1'C)	-4456 (6)	4823 (2)	1261 (2)	190 (6)
C(1'A)	10513 (6)	2636 (2)	2972 (2)	196 (6)	C(2'C)	-7178 (6)	4905 (2)	1129 (2)	203 (6)
C(2'A)	12753 (7)	2624 (2)	3442 (2)	254 (7)	C(3'C)	-7740 (7)	4578 (2)	496 (2)	239 (7)
C(3'A)	12920 (7)	3272 (2)	3667 (2)	277 (7)	C(4'C)	-5494 (7)	4685 (2)	165 (2)	238 (7)
C(4'A)	12085 (7)	3630 (2)	3064 (2)	214 (6)	C(5'C)	-5681 (8)	5213 (2)	-298 (2)	311 (10)*
C(5'A)	14069 (7)	3850 (2)	2693 (2)	242 (9)*	N(1C)	-3191 (5)	5321 (2)	1619 (1)	209 (5)
N(1A)	10429 (5)	2187	2460 (1)	196 (5)	N(3C)	-90 (5)	5652 (2)	2348 (1)	202 (5)
N(3A)	8705 (5)	1348 (2)	1946 (1)	184 (5)	N(6'C)	-7920 (7)	3914 (2)	583 (2)	343 (10)*
N(6'A)	11092 (8)	3332 (2)	4125 (2)	471 (12)*	N(7'C)	-9564 (7)	3754 (2)	904 (2)	326 (10)*
N(7'A)	11469 (6)	3696 (2)	4559 (1)	279 (9)*	N(8'C)	-10993 (8)	3555 (2)	1188 (2)	450 (12)*
N(8'A)	11554 (7)	4001 (2)	4986 (2)	388 (10)*	O(2C)	-1267 (5)	4673 (2)	2354 (1)	255 (6)*
O(2A)	7326 (4)	1682 (2)	2859 (1)	229 (6)*	O(4C)	1286 (5)	6619 (2)	2352 (1)	289 (7)*
O(4A)	9912 (5)	995 (2)	1026 (1)	246 (6)*	O(4'C)	-3552 (4)	4798 (2)	658 (1)	220 (4)
O(4'A)	10525 (4)	3222 (2)	2682 (1)	196 (4)	O(5'C)	-6593 (5)	5734 (2)	-7 (1)	316 (7)*
O(5'A)	15549 (5)	3361 (2)	2552 (1)	352 (8)*	C(2D)	7195 (6)	6791 (2)	3326 (2)	215 (6)
C(2B)	3566 (6)	423 (2)	2301 (1)	160 (6)	C(4D)	5598 (7)	5760 (2)	3380 (2)	218 (6)
C(4B)	2178 (6)	748 (2)	3313 (2)	193 (6)	C(5D)	7680 (7)	5607 (2)	3811 (2)	228 (7)
C(5B)	389 (6)	273 (2)	3244 (2)	191 (6)	C(6D)	9334 (6)	6032 (2)	3967 (2)	213 (6)
C(6B)	306 (6)	-86 (2)	2731 (2)	186 (6)	C(7D)	7916 (8)	4979 (2)	4086 (2)	335 (11)*
C(7B)	-1292 (7)	212 (2)	3756 (2)	240 (9)*	C(8D)	6198 (9)	4862 (3)	4574 (3)	569 (16)*
C(8B)	-3033 (7)	-315 (2)	3667 (2)	254 (9)*	C(1'D)	10999 (7)	7085 (2)	3923 (2)	231 (6)
C(1'B)	1683 (6)	-409 (2)	1679 (1)	168 (6)	C(2'D)	10082 (7)	7571 (2)	4349 (2)	256 (7)
C(2'B)	3831 (6)	-831 (2)	1669 (2)	220 (6)	C(3'D)	12148 (7)	7647 (2)	4882 (2)	260 (7)
C(3'B)	2687 (7)	-1425 (2)	1426 (2)	228 (6)	C(4'D)	13362 (6)	7034 (2)	4908 (2)	232 (7)
C(4'B)	232 (6)	-1405 (2)	1689 (2)	198 (6)	C(5'D)	12395 (7)	6601 (2)	5373 (2)	269 (10)*
C(5'B)	329 (7)	-1660 (2)	2354 (2)	262 (10)*	N(1D)	9136 (5)	6618 (2)	3739 (1)	216 (5)
N(1B)	1845 (5)	-18 (2)	2264 (1)	173 (5)	N(3D)	5570 (5)	6346 (2)	3148 (1)	211 (5)
N(3B)	3629 (5)	796 (2)	2820 (1)	185 (5)	N(6'D)	13916 (7)	8126 (2)	4768 (2)	393 (11)*
N(6'B)	2441 (6)	-1398 (2)	721 (1)	287 (9)*	N(7'D)	14136 (24)	8247 (7)	4254 (10)	358 (36)*
N(7'B)	1358 (6)	-1838 (2)	458 (1)	253 (8)*	N(7''D)	13371 (29)	8438 (7)	4276 (8)	287 (39)*
N(8'B)	440 (7)	-2215 (2)	164 (2)	316 (9)*	N(8'D)	14378 (17)	8429 (4)	3745 (4)	446 (24)*
O(2B)	4981 (4)	480 (2)	1896 (1)	214 (6)*	N(8''D)	12859 (24)	8797 (7)	3910 (7)	702 (42)*
O(4B)	2501 (4)	1101 (2)	3763 (1)	239 (6)*	O(2D)	6952 (5)	7312 (2)	3126 (1)	296 (7)*
O(4'B)	-410 (4)	-772 (2)	1669 (1)	192 (4)	O(4D)	3946 (5)	5409 (2)	3206 (1)	301 (7)*
O(5'B)	-1964 (6)	-1578 (2)	2584 (1)	394 (8)*	O(4'D)	13019 (4)	6811 (2)	4260 (1)	221 (4)
C(2C)	-1506 (6)	5177 (2)	2126 (2)	192 (6)	O(5'D)	9839 (5)	6586 (2)	5273 (1)	286 (7)*

^{a}The esd's are given in parentheses. An asterisk after the thermal parameter indicates that the atom was refined using anisotropic thermal parameters and the B value listed is B$_{eq}$.

Table II. Conformational Parameters: Bond Lengths, Angles, Torsion Angles and Distances between Atoms from Planes That Represent Important Conformational Features

molecule	AZTA	AZTB	CS-87	CS-85A	CS-85B	CS-85C	CS-85D
				Bond Lengths (Å) and Angles (deg)			
N1–C1′	1.460 (4)	1.502 (4)	1.477 (4)	1.467 (4)	1.504 (5)	1.473 (5)	1.486 (5)
C3′–N6′	1.487 (5)	1.494 (6)	1.480 (6)	1.483 (6)	1.485 (5)	1.484 (7)	1.482 (6)
N6′–N7′	1.232 (4)	1.248 (4)	1.214 (4)	1.220 (5)	1.244 (5)	1.246 (6)	1.14 (2)
							1.26 (2)
N7′–N8′	1.122 (4)	1.124 (4)	1.137 (6)	1.124 (5)	1.130 (5)	1.134 (6)	1.17 (2)
							1.12 (2)
C3′–N6′–N7′	115.0 (3)	115.0 (3)	115.8 (3)	118.3 (3)	114.0 (3)	114.3 (3)	117.6 (7)
							114.8 (7)
N6′–N7′–N8′	173.7 (3)	173.2 (3)	173.5 (4)	171.0 (3)	173.1 (3)	173.6 (3)	174 (1)
							168 (1)
				Torsion Angles (deg)			
C2–N1–C1′–O4′ (χ)	−124.4 (3)	−173.6 (3)	−159.8 (3)	−129.9 (3)	−170.8 (3)	−108.8 (4)	−170.6 (3)
C2–N1–C1′–C2′	118.4 (3)	66.7 (4)	82.3 (4)	113.5 (4)	70.4 (4)	133.5 (3)	70.0 (4)
N1–C1′–O4′–C4′ (ν)	−135.4 (3)	−111.4 (3)	−135.6 (3)	−142.1 (3)	−118.8 (3)	−139.4 (3)	−115.9 (3)
O4′–C4′–C5′–O5′	−67.9 (4)	57.1 (4)	−62.1 (4)	−64.2 (4)	58.3 (4)	−70.8 (4)	−69.2 (4)
C3′–C4′–C5′–O5′ (γ)	50.9 (4)	173.4 (3)	57.3 (4)	54.0 (4)	175.1 (3)	48.6 (5)	49.6 (4)
C2′–C3′–N6′–N7′	177.5 (3)	176.2 (3)	172.4 (4)	150.0 (4)	−174.8 (3)	61.7 (5)	−26 (1)
							6 (1)
C4–C5–C7–C8				175.4 (3)	−177.2 (3)	−177.9 (4)	−72.1 (5)
				Average Torsion Angle (deg)			
uridine ring	1.1 (5)	0.4 (6)	2.1 (6)	0.9 (5)	1.4 (6)	2.8 (6)	2.8 (6)
furanose ring	21.4 (4)	23.7 (4)	23.9 (4)	24.3 (3)	22.5 (4)	21.8 (5)	20.5 (4)
				Distance from Plane C1′,C4′,O4′ (Å)			
C2′	0.332	0.272	0.353	0.464	0.079	0.413	0.157
C3′	−0.195	0.732	−0.242	−0.131	0.600	−0.129	0.594
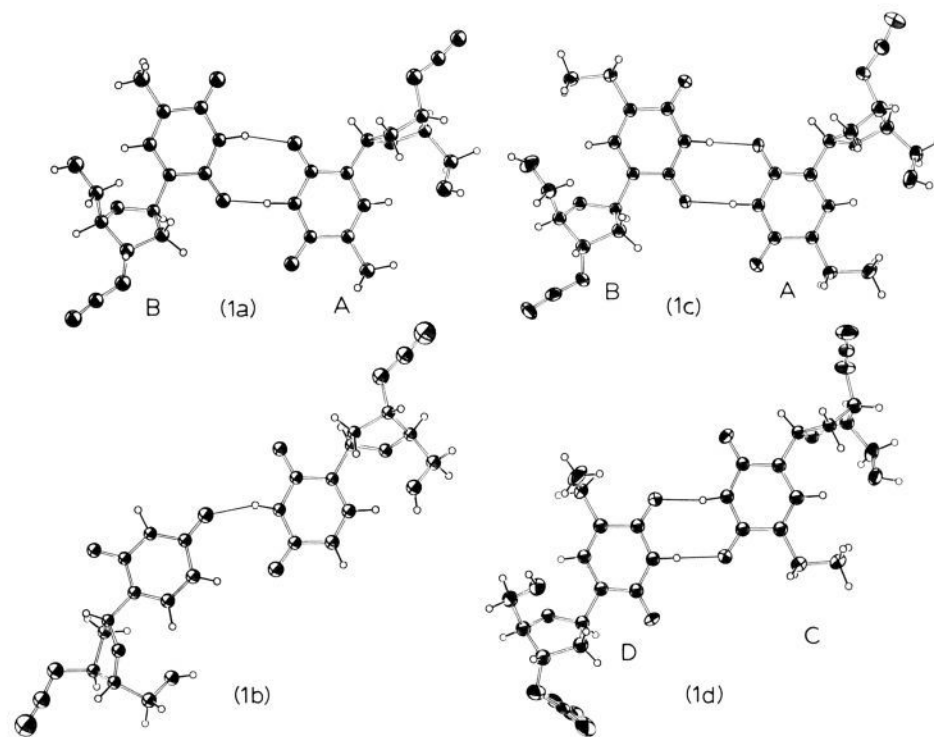
Figure 1. ORTEP[21] drawing showing molecular conformation and base-pairing hydrogen bonding in the crystal structures of AZT, CS-87, and CS-85. Key: (a) AZT dimer A–B; (b) CS-87, molecules related by twofold screw axis; (c) CS-85 dimer A–B; (d) CS-85 dimer C–D. Note the similarity in base pairing and molecular conformations in AZT and CS-85 dimer A–B.

uridine ring are 2.8 (6), 2.8 (6), and 2.1 (6)° with individual angles exceeding 5.0°.

Comparison of these results with the data on 138 uridine analogues present in the Cambridge Crystallographic Database[19] shows some interesting features. Figure 3 shows histograms of the observed values for the torsion angles χ (C2–N1–C1′–O4′), ν (N1–C1′–O4′–C4′), and γ (C3′–C4′–C5′–O5′). The values of the torsion angle χ show a bimodal distribution. A total of 124 structures have χ values between −176 and −82° (anti) with a

mean value of −135° and a standard deviation of 22°. The remaining 14 previous observations have χ values between 60 and 89° (cis) with a mean of 69° and a standard deviation of 8°. All molecules in this study have χ values that coincide with the largest of these two groups, but three of them (AZTB, CS-85B, CS85D) are at the extreme end of the range. The angle ν appears to have a rather broad distribution between −170 and −100°, with two preferred values. Two-thirds of the observations have ν values of about −140° with the remaining third distributed about −115°.

3'-Azido-3'-deoxythymidine Analogue Structures

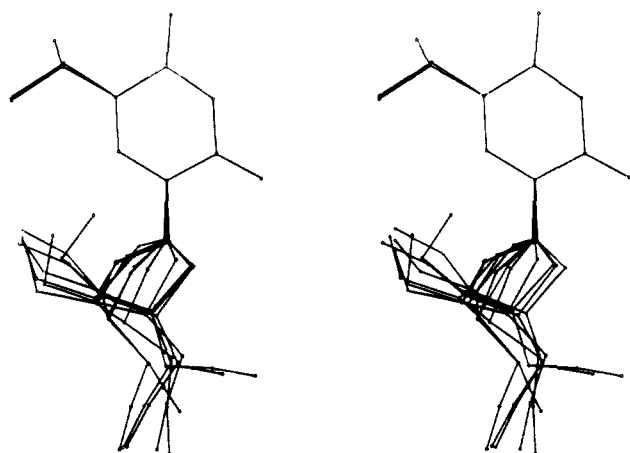J. Am. Chem. Soc., Vol. 110, No. 7, 1988   2281

Figure 2. ORTEP[21] stereodrawing of all seven independent molecules superimposed by least-squares fitting of the uridine ring (atoms N1, C2, O2, N3, C4, O4, C5, C6).

Table III. Intermolecular Hydrogen Bonding

	distance, Å		angle, deg
bond: donor···acceptor	D···A	H(D)···A	D–H···A
AZT			
N3A···O2B (x, y, z)	2.744 (4)	1.69 (5)	173 (4)
O5'A···O4'A $(x, y, 1 + z)$	2.823 (3)	2.15 (4)	154 (3)
N3B···O2A (x, y, z)	2.883 (4)	1.92 (4)	162 (2)
O5'B···O4A $(1 - x, -^1/_2 + y, -z)$	2.834 (4)	2.00 (4)	169 (3)
CS-87			
N3···O4 $(3 - x, -^1/_2 + y, 1 - z)$	2.832 (5)	1.99 (8)	136 (5)
O5'···O2 $(x, 1 + y, z)$	2.911 (4)	2.13 (9)	151 (7)
CS-85			
N3A···O2B (x, y, z)	2.817 (5)	1.84 (4)	179 (3)
O5'B···O2D $(-1 + x, -1 + y, z)$	2.798 (5)	1.92 (6)	169 (4)
N3C···O4D (x, y, z)	2.789 (4)	1.85 (3)	170 (3)
O5'A···O4'A $(1 + x, y, z)$	2.773 (4)	2.03 (6)	146 (4)
N3B···O2A (x, y, z)	2.835 (5)	1.87 (5)	170 (3)
O5'C···O4A $(-x, ^1/_2 + y, -z)$	2.749 (3)	1.86 (4)	162 (3)
N3D···O4C (x, y, z)	2.837 (4)	1.90 (4)	179 (3)
O5'D···O4B $(1 - x, ^1/_2 + y, 1 - z)$	2.749 (4)	1.97 (4)	166 (3)

Three of the seven molecules observed in this study belong to the smaller population. The γ angle has a trimodal distribution. One group consisting of 90 observations centered about 60° and two smaller ones at 180° (22 observations) and –60° (26 observations). The distribution in this study appears to be consistent, with five observations in the range of the largest population and the other two in the smallest. Review of the database shows a large variety of conformations for the five-membered ring. Saenger[22] observed correlations between the χ angle on the one hand and the furanose ring conformation and the N1–C1' bond length on the other. χ angles between –180 and –138° were correlated with C3'-endo geometry and 1.52-Å bond lengths, χ values between –115 and –144° were correlated with C2'-endo conformations and 1.48-Å bond lengths. The conformations observed in these structures are essentially consistent with these observations.

Table III lists the intermolecular hydrogen bonds. Very strong intermolecular hydrogen bonding is observed, but the patterns are very different in the three structures. The primary interaction between nucleoside molecules is expected to take place between the bases. Stable base pairing requires two hydrogen bonds between bases. In this case two symmetric (N3A···O2B, N3B···O2A; N3A···O4B, N3B···O4A) patterns and one asymmetric (N3A···O2B, N3B···O4A) pattern are possible. AZT adopts the symmetric pattern using O2. CS-85 has one dimer (A···B) that has the O2 symmetric pattern and one dimer (C···D) that has the O4 symmetric pattern. CS-87 does not form standard base pairing

(22) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984; pp 70–74.
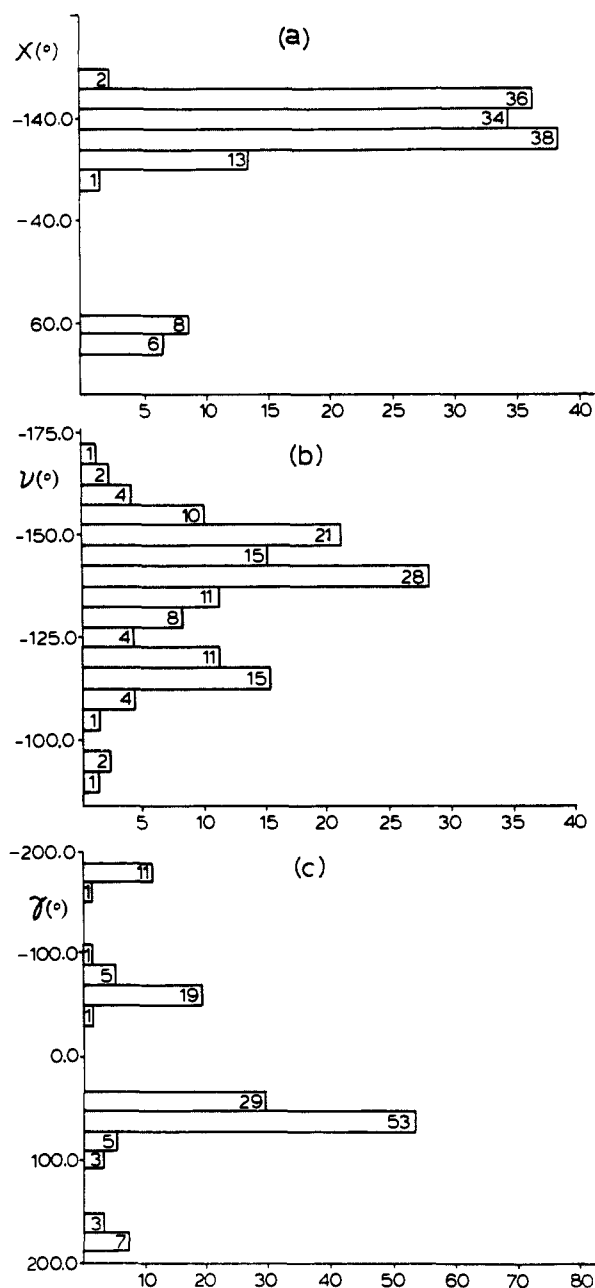

Figure 3. Histograms showing the distributions of previously observed values contained in the Cambridge Crystallographic Database for the torsion angles (a) χ, (b) ν, (c) γ.

but forms a single strand of molecules using hydrogen bonds in which O4 accepts a hydrogen from N3 of a symmetry-related molecule and N3 donates to O4 of a similarly related molecule translated by one cell dimension along b. Nonplanarity of the uridine ring is largest in those molecules for which O4 is used in base pairing. In each molecule, O5' forms intermolecular hydrogen bonds to connect the dimers in AZT and CS-85 and the linear strands in CS-87, into a three-dimensional lattice. The O5'A hydroxyl groups of AZT and CS-85 donate their hydrogen to ether oxygens O4'A of translationally related molecules. This leaves one carbonyl oxygen unused for hydrogen bondng in each structure. The O5'···O4' hydrogen bonds are expectedly somewhat weaker than those involving carbonyl acceptors, as can be seen from the angle at the hydrogen atom. But their occurrence appears correlated with the molecular geometry (χ angle and N1–C1' bond length). The unused carbonyl groups (O4B in AZT, O2C in CS-85) do not appear to be less accessible than the others. Other intermolecular interactions are limited to a partial stacking of parallel pyridimine rings in AZT and CS-85. In CS-85, A···B dimers stack by positioning N3B $(1 + x, y, z)$ over the uridine

ring of molecule A and N3A over uridine ring B (−1 + x, y, z). Atom C2B is stacked approximately right above C4A and C4B above C2A. The C···D dimer stacks atoms O4, C4, N3, and C2 of molecule C approximately right above atoms C2, N3, C4, and O4 of molecule D (−1 + x, y, z). The pattern in AZT is intermediate between these two but involves molecules A and B (x, y, 1 + z). In all three cases the distance between the planes is about 3.2 Å. No short intermolecular contacts other than hydrogen bonds are observed in the structure of CS-87.

Conclusions

Low-temperature data collection has allowed accurate determination of conformational parameters, including complete resolution of a disordered azido substituent. Relatively large variations are observed in the conformations of molecules that have only very small differences in intramolecular steric interactions. However, correlations can be observed between the most important intermolecular contacts (hydrogen bonding between bases and with the 5'-hydroxyl group) and the variations in molecular conformations. Pairs of molecules with identical base pairing (AZT, the A–B dimer of CS-85) have identical conformations in the glycosyl link and the furanose ring geometry. The alternate base pairing observed in the second dimer of CS-85 and the chain hydrogen bonding in CS-87 is correlated with differences in internal conformational features. The latter molecules also have the least planar uridine rings. The preferred conformation of the azido group is trans to the C2'–C3' bond. However, deviation from this position is correlated with an increased C2'-endo conformation of the furanose ring. On the other hand, tendency toward C3'-envelope conformations appears to be correlated with a χ angle close to 180° and a trans conformation for the γ angle.

The large variation in packing arrangements appears to be the result of several different factors, including hydrogen bonding, base stacking, and effects of molecular conformation. One would assume that strongest type interaction possible in these structures would be the base pairing involving two hydrogen bonds and stacking of the uridine rings. Both interactions are observed in the structures of AZT and CS-85 but not in the structure of CS-87, which lacks the substitution at the 5 position. The geometries of the uridine rings are essentially identical. The only difference, a small deviation from planarity appears to be correlated with the use of O4 in hydrogen bonding and not with the substitution at C5. Therefore, this substitution does not greatly alter the electron density distribution in the uridine ring but may well enhance dipole–dipole interactions that promote base stacking and base pairing. These intermolecular interactions are strong enough to compensate for the destabilizing effect of the use of ether oxyens as acceptors for hydrogen bonds while carbonyl oxygen atoms remain unused. Crystallization of all three compounds have been attempted from a large range of solvents and at different temperatures. Crystals have been obtained from several of these conditions, but all have been isomorphous with the structures reported here. This indicates that the observed structures are very stable low-energy structures achieved by a combination of low-energy molecular conformations and favorable intermolecular interactions.

Registry No. AZT, 30516-87-1; CS-87, 84472-85-5; CS-85, 105380-83-4.

Supplementary Material Available: Tables of anisotropic thermal parameters, hydrogen atom coordinates and thermal parameters and bond distances and angles (9 pages); listing of observed and calculated structure factor amplitudes (43 pages). Ordering information is given on any current masthead page.

Antibody Catalysis Approaching the Activity of Enzymes[†]

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Abstract: Antibodies were raised to phosphonate esters that model a carboxyl esterolytic transition state. Twenty monoclonal antibodies were screened by a direct assay for hydrolysis of the carboxylic ester deduced from the structure of the transition-state analogue. Five of these were found to be esterases. The transition-state analogue is a specific inhibitor of the activity. One antibody accelerates the hydrolysis of a related substrate with k_{cat} of 20 s⁻¹ and K_m of 1.5 mM at pH 8.0. This represents an acceleration of several million times above the spontaneous rate of hydrolysis. The kinetic constants for two substrates and the inhibition constant suggest the better binding of transition states than ground states to the antibody. The pH dependence of the activity can be explained by the titration of an amino acid side chain with a pK_a of about 8.9. Activity is abolished by protein nitration that is specific for tyrosyl groups, and the nitration of two or more groups is prevented by the presence of the phosphonate ligand. Thus, the residues that constitute the combining site of the antibody may act analogously to residues in the active site of enzymes. These results demonstrate that an esterolytic antibody can be relatively efficient as compared with similar enzymes.

The binding of ligands to antibodies is mediated by the same fundamental interactions used by enzymes to bind substrates. This analogy gives cause to speculate that the immune system might be manipulated to yield antibodies with catalytic activity. Recent reports have provided the first experimental evidence for this possibility. We have obtained esterolytic antibodies with a predicted specificity by using a presumed transition-state mimic as part of an immunogen.[1,2] A subsequent report has shown that antibodies elicited to a similar transition-state analogue may also catalyze carbonate hydrolysis.[3] These findings begin to define the properties of a new class of catalyst that has many of the advantages and characteristics of enzymes. For example, lactonization of a δ-hydroxy ester is catalyzed by an antibody with

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(3) Jacobs, J.; Schultz, P. G.; Sugasawara, R.; Powell, M. *J. Am. Chem. Soc.* **1987**, *109*, 2174.