Vol. 152, No. 1, 1988
April 15, 1988

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
Pages 351-358

CONFORMATION AND SANDWICHING OF BASES BY AZIDO GROUPS IN THE CRYSTAL STRUCTURE OF 3'-AZIDO-3'-DEOXY-THYMIDINE (AZT), AN ANTIVIRAL AGENT THAT INHIBITS HIV REVERSE TRANSCRIPTASE

R. Parthasarathy* and Heasook Kim

Center for Crystallographic Research
Roswell Park Memorial Institute
666 Elm Street
Buffalo, NY 14263

Received February 29, 1988

Summary: The crystal structure of 3'-azido-3'-deoxy-thymidine (AZT), an antiviral agent that inhibits HIV reverse transcriptase, has been determined from three-dimensional x-ray diffractometer data. The crystal structure contains two independent molecules of AZT forming a hydrogen bonded dimer but exhibiting different conformations. These conformations are different from those theoretically calculated by molecular mechanics methods. The azido groups associate with each other and interrupt the base stacking, forming a sandwich of two stacked bases. The close conformational similarity of AZT to thymidine explains why AZT is a good substrate for thymidine kinase. The selective inhibition of reverse transcriptase by AZT is not due to any conformational restrictions imposed by the azido group but likely due to their stereoelectronic properties.

Introduction: Initially developed as an anticancer drug in the 1960's (1), 3'-azido-3'-deoxy-thymidine (AZT) has emerged as an important and successful drug with proven benefit in the prolongation of life among patients with acquired immune deficiency syndrome (AIDS) (2-5). AZT inhibits the human immunodeficiency virus (HIV) that has been identified as the principal causative agent for AIDS (6). Once AZT is phosphorylated, it selectively competes for viral reverse transcriptase and inhibits viral replication (7) since the 3'-substitution prevents further 5' to 3' phosphodiester linkages and terminates viral DNA chain synthesis. In order to find agents superior to AZT, a variety of AZT and other nucleoside analogs are currently under investigation (2,8,9). Because the reverse transcriptase has a unique association with retroviruses, it has been considered as an attractive target for drug development. Since different inhibitors develop differential sensitivity to reverse transcriptase and other DNA polymerases, a

* Author for correspondence.

0006-291X/88 $1.50

thorough study of the conformational preferences of AZT and its phosphorylated form, especially as in enzyme-bound state, is relevant and important to the drug design of other possibly more potent reverse transcriptase inhibitors. This communication reports the x-ray analysis of AZT containing two molecules in the crystal structure exhibiting different conformations.

Methods: Crystals of AZT (from Sigma Chemical Co.) obtained from aqueous solutions are monoclinic, $P2_1$, with cell constants at (22 + 3)°C: a = 5.713(1), b = 11.996(1), c = 17.658(2)Å, β = 94.31(1)°, Z = 4, D_{obsd} = 1.49, D_{calc} = 1.47 g/cc, formula of the asymmetric unit consisting of two independent molecules, $(C_{10}H_{13}N_5O_4)_2$. Three dimensional intensity data (2668 reflections to the limit 2θ = 154° for Cukα radiation) were collected using an Enraf-Nonius CAD-4 automatic diffractometer. The structure was solved by direct methods using the computer program SHELX (10) and refined to an R of 0.04 for 2427 reflections > 3σ using full-matrix least-squares procedures. All the 26 hydrogen atoms in the structure were located from electron-density difference maps; their positional and individual isotropic thermal parameters were included in the refinement. The atomic coordinates and thermal parameters are given in Table 1.

Table 1
Positional Parameters and Their Estimated Standard Deviation

Atom	x	y	z	B(A2)
N11	0.4466(4)	0.695	0.3889(1)	3.13(4)
C21	0.5983(4)	0.7262(2)	0.3366(1)	2.87(4)
N31	0.5468(4)	0.6846(2)	0.2646(1)	3.06(4)
C41	0.3677(4)	0.6118(2)	0.2412(1)	2.98(4)
C51	0.2153(4)	0.5804(2)	0.3000(1)	2.96(4)
C61	0.2621(4)	0.6231(2)	0.3695(1)	3.06(4)
O21	0.7676(3)	0.7877(2)	0.3515(1)	3.78(4)
O41	0.3452(4)	0.5799(2)	0.1751(1)	4.12(4)
CM1	0.0169(5)	0.5020(3)	0.2812(2)	3.95(5)
C1'1	0.4816(4)	0.7341(3)	0.4677(1)	3.12(4)
C2'1	0.2825(5)	0.8069(3)	0.4917(2)	3.68(5)
C3'1	0.2767(5)	0.7789(3)	0.5755(1)	3.49(5)
C4'1	0.3526(5)	0.6568(3)	0.5804(1)	3.19(5)
O4'1	0.4900(3)	0.6389(2)	0.51587(9)	3.41(3)
N1AZ1	0.4476(6)	0.8540(3)	0.6179(2)	5.05(6)
N2AZ1	0.4529(4)	0.8453(2)	0.6872(1)	3.77(4)
N3AZ1	0.4694(5)	0.8467(3)	0.7509(1)	4.72(6)
C5'1	0.1530(5)	0.5734(3)	0.5798(2)	4.14(6)
O5'1	-0.0161(4)	0.5949(3)	0.5182(1)	5.06(5)
N12	0.1616(4)	0.8390(2)	0.1143(1)	2.99(4)
C22	0.0259(4)	0.8097(2)	0.1722(1)	2.99(4)
N32	0.0828(4)	0.8606(2)	0.2404(1)	3.27(4)
C42	0.2621(5)	0.9376(3)	0.2572(2)	3.30(5)
C52	0.3962(5)	0.9646(2)	0.1927(2)	3.33(5)
C62	0.3418(4)	0.9142(2)	0.1259(2)	3.16(4)
O22	-0.1348(3)	0.7422(2)	0.1620(1)	3.87(4)
O42	0.2953(4)	0.9749(2)	0.3210(1)	4.65(5)
CM2	0.5923(6)	1.0476(3)	0.2035(2)	4.52(6)
C1'2	0.1118(4)	0.7773(2)	0.0410(1)	2.96(4)
C2'2	-0.1284(5)	0.8072(3)	0.0015(2)	4.09(6)
C3'2	-0.0740(5)	0.8258(3)	-0.0804(2)	3.64(5)
C4'2	0.1781(5)	0.8685(3)	-0.0718(1)	3.23(4)
O4'2	0.2839(3)	0.8060(2)	-0.00845(9)	3.30(3)
N1AZ2	-0.0903(6)	0.7157(3)	-0.1191(2)	4.81(6)
N2AZ2	-0.0647(4)	0.7174(2)	-0.1875(1)	3.77(4)
N3AZ2	-0.0464(6)	0.7081(3)	-0.2499(2)	5.15(6)
C5'2	0.1980(6)	0.9935(3)	-0.0579(2)	3.99(6)
O5'2	0.4329(5)	1.0273(2)	-0.0419(1)	5.13(5)

Anisotropically refined atoms are given in the form of the isotropic equivalent displacement parameter defined as:
(4/3) * [a2*B(1,1) + b2*B(2,2) + c2*B(3,3) + ab(cos gamma)*B(1,2) + ac(cos beta)*B(1,3) + bc(cos alpha)*B(2,3)]

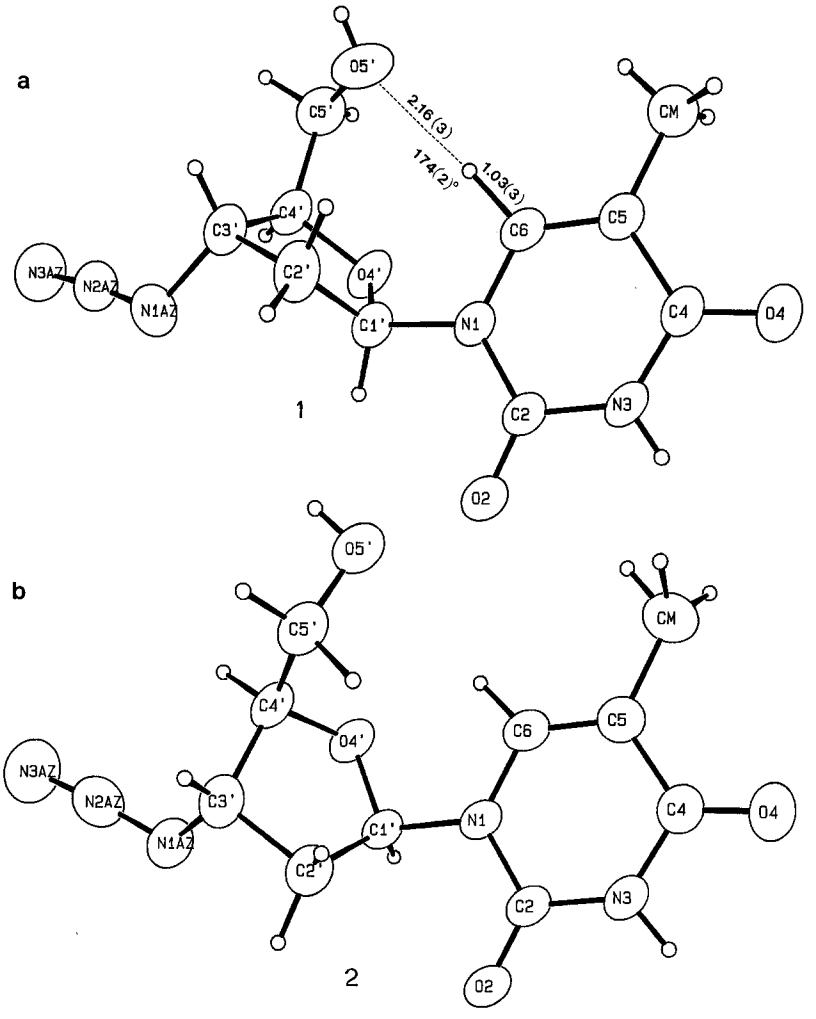

Figure 1. Illustrates the different conformations of molecule 1 (in Figure 1(a)) and molecule 2 (in Figure 1(b)). Note the internal C6-H6---O5' hydrogen bond for molecule 1 in Figure 1(a).

Results and Discussions: Figure 1 illustrates the conformation of the two independent molecules 1 and 2 in the asymmetric unit. Both molecules 1 and 2 exhibit anti conformation across the glycosidic bond but differ considerably in their χ_{CN}(C6-N1-C1'-O4') values of 52.8(3) and 3.0(3)°, respectively. They also differ in their sugar pucker: 1 shows C2'-endo-C3'-exo pucker with g^+-conformation across the C4'-C5' bond while 2 exhibits C4'-endo-C3'-exo pucker with t-conformation across the C4'-C5' bond. The values of θ_m and P, the pseudo rotation parameters (11), and the conformation angle C3'-C4'-C5'-O5' are, respectively, 33.0(2)°, 173.6(3)°, 50.6(4)° for 1 and 36.7(2)°, 212.3(3)°, 173.4(4)° for 2. The conformation of 1 is stabilized by an

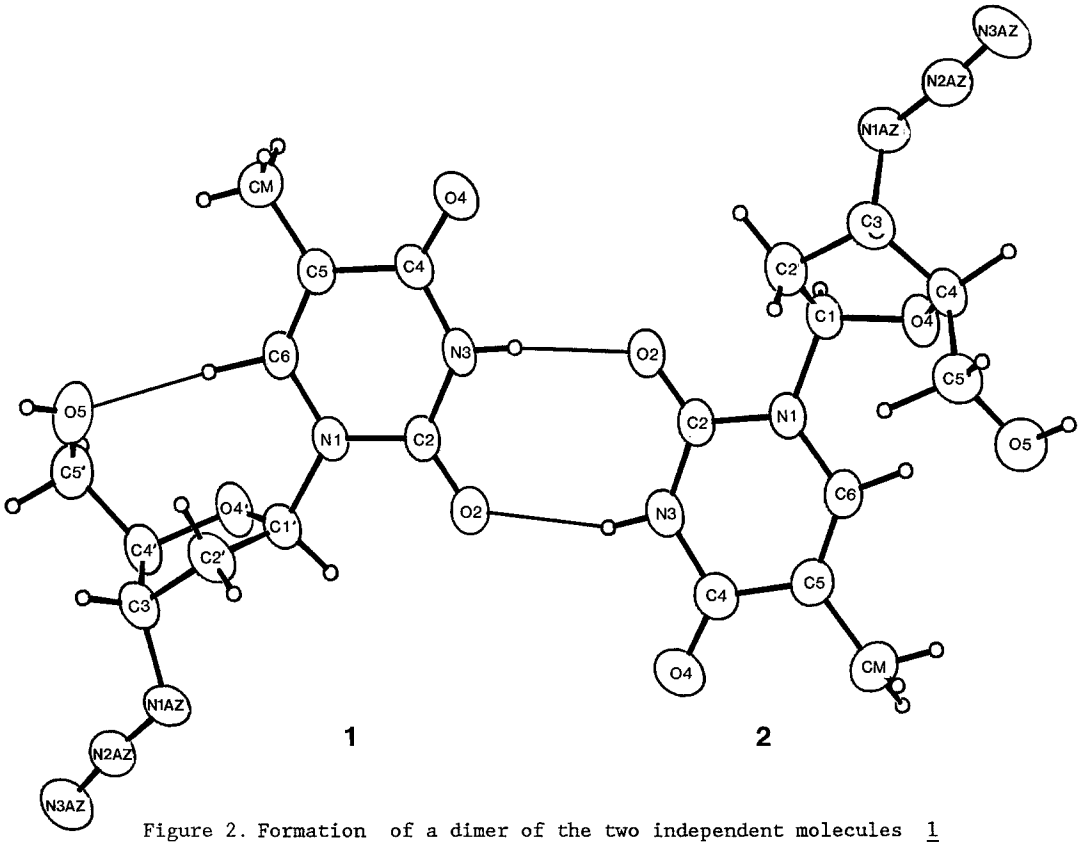

Figure 2. Formation of a dimer of the two independent molecules 1 and 2 of AZT by utilizing two hydrogen bonds.

intramolecular C6-H---O5' bond (C6-H, 1.03(3)Å, H---O5', 2.16(3)Å, C6-H---O5' angle, 174(3)°). Molecules 1 and 2 form a dimer in the crystal (Figure 2), being associated by two hydrogen bonds; N3-H of 1 hydrogen bonds to O2 of 2 and N3-H of 2 hydrogen bonds to O2 of 1 (N31-HN31---022, N31-HN31, 0.82(3)Å, HN31---022, 1.93(3)Å, N31-HN31--022, 176(3)°; N32-HN32---021, N32-HN32, 0.83(4)Å, HN32---021, 2.09(4)Å, N32-HN32---021, 164(4)°). O4' of molecule 1 accepts a hydrogen bond from O5'-H of a neighboring molecule 1 (O5'1-HO5'1---O4'1, O5'1-HO5'1, 0.79(4)Å, HO5'1---O4'1, 2.15(4)Å, O5'1-HO5'1---O4'1, 151(4)°). But the O5'H of 2 hydrogen bonds to O4 of a neighboring molecule 1 (O5'2-HO5'2---041, O5'2-HO5'2, 0.82(3)Å, HO5'2---041, 2.03(3)Å, O5'2-HO5'2---041, 166(3)°). The azido groups are not involved in any hydrogen bonding, but act as terminators of base stacking (Figure 3). There is extensive base stacking between the thymine moiety of 1 and 2, but the base stacking does not extend beyond dimers. The azido groups on either side sandwich the stacked bases (Figure 3).

The molecular dimensions of 1 and 2, in general, agree well with one another and with other nucleosides. The most pronounced difference

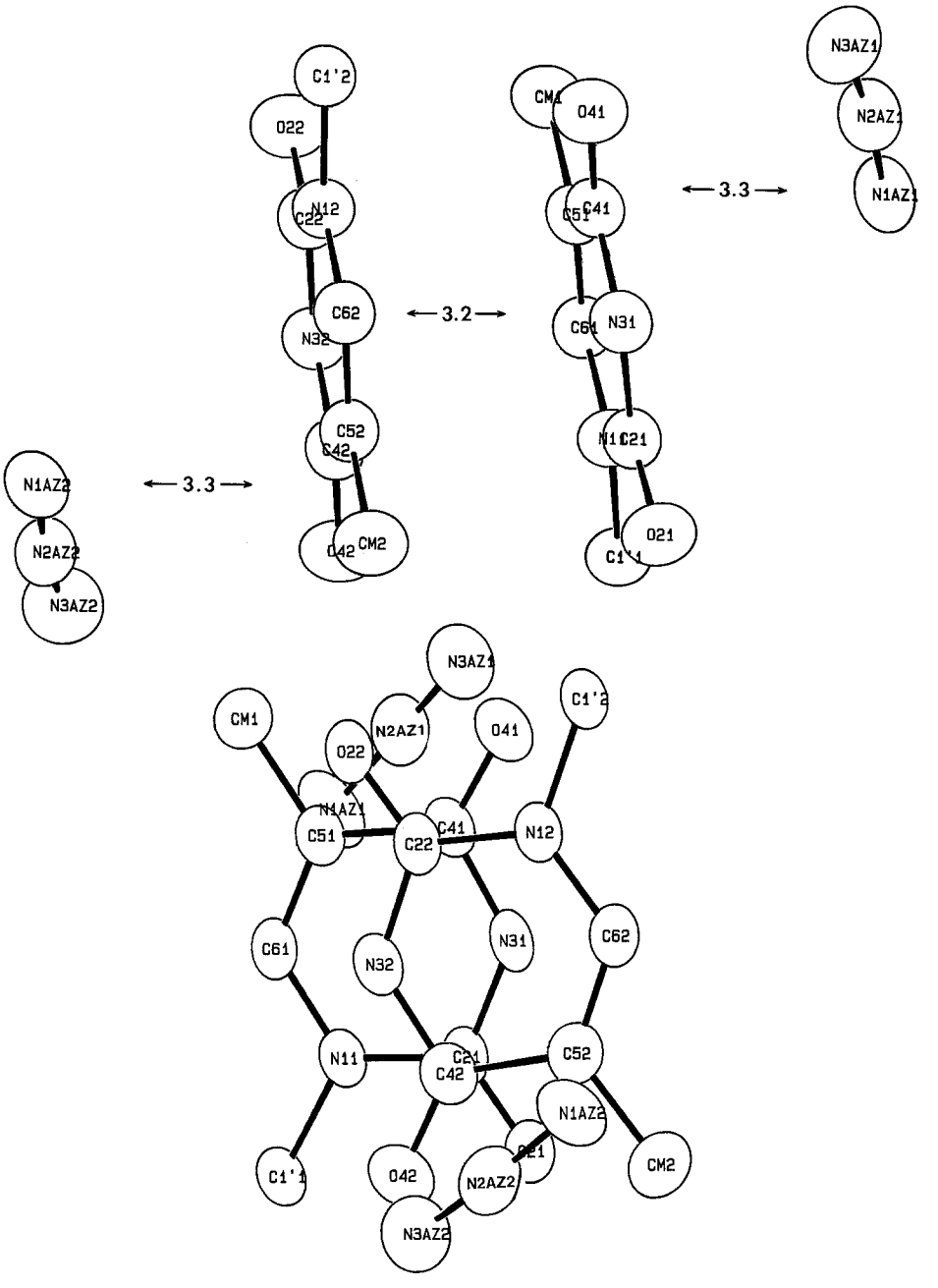

Figure 3. The sandwiching of the two bases by the azido groups on either side. Molecules 1 and 2 are identified by the last numeral in the atom label.

is for N1-C1′ bond; this bond has a length of 1.470(3)Å for 1 and 1.501(3)Å for 2, reflecting a correlation between this bond length and the glycosidic torsion angle (12,13). Molecule 1 does not show as pronounced a bond shortening anomeric effect (14,15) as molecule 2; the C1′-O4′ and C4′-O4′ bond distances for molecules 1 and 2 are,

respectively, for molecule 1 1.423(3), 1.447(3)Å and for molecule 2 1.406(3), 1.440(3)Å. The two azido groups N1-N2-N3 are similar and the values of N1-N2, N2-N3 bond lengths and N1-N2-N3 angle for 1 and 2 are, respectively, 1.225(4), 1.123(3)Å, 173.2(3)° and 1.227(4), 1.121(4)Å, 173.1(3)° corresponding to C-N=$\overset{+}{N}$=$\overset{-}{N}$ configuration for the azido group. A recent publication (16) discusses low energy conformations of AZT using molecular mechanics techniques and also refers in a footnote to an unpublished crystal structure study of AZT. These calculations show that for AZT the major preferred conformations with a statistical weight greater than 0.2 are in the range of anti across glycosidic bond, C3'-endo (with P ~ 22 to 26°) and g^+ across C3'-C4'-C5'-O5'. These results are not in agreement with our structure in the solid state; neither one of the two molecules in the crystal exhibits C3'-endo class of pucker. Also, none of the conformations mentioned in (16) as corresponding to low energy agrees with our x-ray results. The C2'-C3'-N1AZ-N2AZ angle in the two molecules are, respectively, trans, with values of 176.1(4) for molecule 1 and 175.8(4)° for molecule 2.

Since AZT acts as a metabolic analog of thymidine (dThd), a study of the conformational preferences of AZT and dThd will be very important. dThd (17) has anti conformation across the glycosidic bond, C2'-endo-C3'-exo sugar pucker with trans conformation across the C4'-C5' bond; the value of χ_{CN} and the sugar pucker for dThd is in between the values found here for molecules 1 and 2 indicating that the azido substitution did not radically alter the conformational preferences of dThd. Other substituents like 3'-O-acetyl also do not change the sugar pucker from C2'-endo (18) whereas the presence of 2'-OH group or 5'-phosphorylation alters the conformation of the sugar to C3'-endo pucker, as for example, in 5-methyluridine (19) and calcium dThd-5'-P (20). The conformational similarity explains why AZT is a good substrate for cellular kinase and is phosphorylated as efficiently as dThd (7). Why AZT or its triphosphate is a selective inhibitor of replication by HIV is most likely not due to any conformational restrictions of AZT nucleoside but possibly due to the stereoelectronic properties of the azido group itself. When the triphosphate form of AZT is used by reverse transcriptase, the 3'-substitution makes subsequent 5'—> 3' phosphodiester linkage impossible, thereby acting as a chain terminator of DNA synthesis. AZT does not depend either upon a nucleoside transporter or any other carrier system to permeate cell membrane and traverses the membrane by nonfacilitated diffusion quite unlike dThd, probably due to the "lipophilicity conferred by the azido group" (21). AZT is known to inhibit dTMP incorporation catalyzed by HIV reverse transcriptase; the reverse transcriptase was

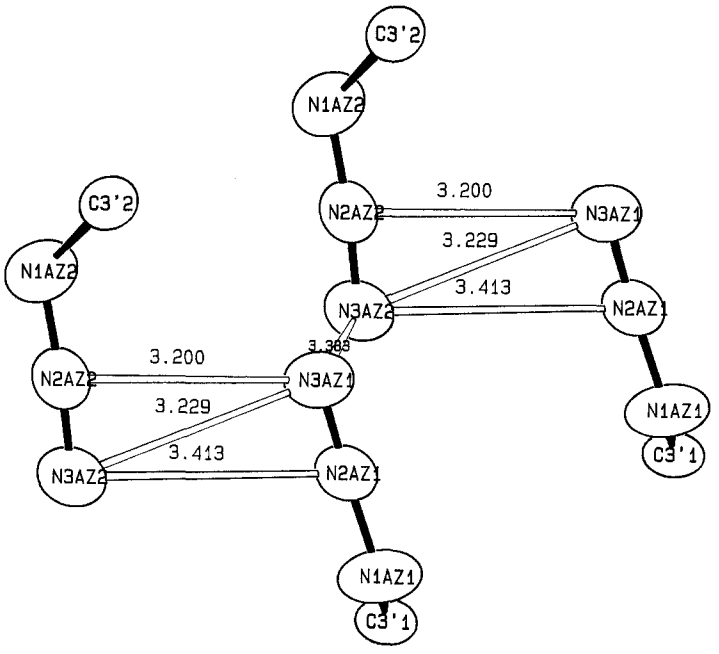

Figure 4. The interactions of the azido groups. The strongest interaction, as judged by the shortest of N---N contact is from N^+ to N^- atom. The azido groups are not involved in any hydrogen bonding.

inhibited 100 times better than its cellular counterpart, DNA polymerase (7). This high selectivity of the triphosphate of AZT to HIV reverse transcriptase might depend on the stereoelectronic properties of the azido group and its relative orientation to the sugar ring. In this crystal structure, we find that the association of the azido groups between themselves (Figure 4) is strong enough to break the extended base stacking and to facilitate the formation of sandwiching of bases. The high selectivity of the triphosphates of AZT over dThd might result from the extra binding of the azido group in an appropriate pocket in reverse transcriptase containing a positive and negative group in such a way to interact with the azido group in a head to tail fashion as in Figure 4. Whether such an interaction exists or not between AZT and reverse transcriptase will be decided by future x-ray crystallographic studies of reverse transcriptase.

Acknowledgements: This work was supported by a grant GM-24864 from the National Institutes of Health.

References

1. Horwitz, J.P., Chua, J. and Noel, M. (1964) J. Org. Chem. 29, 2076-2078.
2. Clercq, E.C. (1986) J. Med. Chem. 29, 1561-1569.

3. Yarochan, R. and Broder, S. (1987) N. Engl. J. Med. 316, 557-564.
4. Fischl, M.A., Richman, D.D., Grieco, M.H., Gottlieb, M.S., Volberding, P.A., Laskin, O.L., Leedom, J.M., Groopman, J.E., Mildvan, D., Schooley, R.T., Jackson, G.G., Durack, D.T., King, D. and the AZT Collaborative Working Group. N. Engl. J. Med. 317, 185-191.
5. Mitsuya, H. and Broder, S. (1987) Nature 325, 773-778.
6. Gallo, R.C., Salahuddin, S.Z., Popovic, M., Shearer, G.M., Kaplan, M., Haynes, B.F., Palker, T.J., Redfield, R., Oleske, J., Safai, B., White, G., Foster, P. and Markani, P.D. (1984) Science 224, 500-503.
7. Furman, P.A., Fyfe, J.A., St. Clair, M.H., Weinhold, K., Rideout, J.L., Freeman, G.A., Lehrman, S.N., Bolognesi, D.P., Broder, S., Mitsuya, H. and Barry, D.W. (1986) Proc. Natl. Acad. Sci. USA 83, 8333-8337.
8. Lin, T.-S., Chen, M.S., McLaren, C., Gao, Y.-S., Ghazzouli, I., and Prussoff, W.H. (1987) J. Med. Chem. 30, 440-444.
9. Mitsuya, H. and Broder, S. (1986) Proc. Natl. Acad. Sci. USA 83, 1911-1915.
10. Sheldrick, G. (1986) SHELXS-86. Program for Crystal Structure Determinations, University of Gottingen, Federal Republic of Germany.
11. Altona, C. and Sundaralingam, M. (1972) J. Amer. Chem. Soc. 94, 8205-8212.
12. Lo, A., Shefter, E. and Cochran, T.G. (1975) J. Pharm. Sci. 64, 1707-1710.
13. Leeuw, H.P.M., Haasnoot, C.A.G. and Altona, C. (1980) Israel J. Chem. 20, 108-126.
14. Berman, H.M., Chu, S.S.C. and Jeffrey, G.A. (1967) Science 157, 1576-1577.
15. Wolfe, S., Whangbo, H-H. and Mitchell, D.J. (1979) Carbohydrate Res. 69, 1-26.
16. Herzyk, P., Beveridge, A. and Neidle, S. (1987) Biochem. Biophys. Res. Commun. 145, 1356-1361.
17. Young, D.W., Tollin, P. and Wilson, H.R. (1969) Acta Cryst. B25, 1423-1432.
18. Saenger, W. and Suck, D. (1971) Acta Cryst. B27, 2105-2112.
19. Hunt, D.J. and Subramanian, E. (1969) Acta Cryst. B25, 2144-2152.
20. Trueblood, K.N., Horn, P. and Luzzati, V. (1961) Acta Cryst. 14, 965-982.
21. Zimmerman, T.P., Mahony, W.B. and Prus, K.L. (1987) J. Biol. Chem. 262, 5748-5754.