The Crystal and Molecular Structure of 5-*N*-Methyl-β-D-arabinofurano[1′,2′:4,5]oxazolo-*S*-triazine-4,6-dione: Molecular Modeling Studies on the Pattern of Alkylation of β-D-Arabinofurano[1′,2′:4,5]oxazolo-*S*-triazin-4-one-6-thione

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The involvement of the 5′-hydroxyl group on β-D-arabinofurano[1′,2′:4,5]oxazolo-*s*-triazin-4-one-6-thione (**1b**), to form an intramolecular covalent adduct at C6, is postulated to explain the formation of almost equal amounts of 5-*N*-alkyl-β-D-arabinofurano[1′,2′:4,5]oxazolo-*s*-triazin-4-one-6-thione and 5-*N*-alkyl-β-D-arabinofurano[1′,2′:4,5]oxazolo-*s*-triazine-4,6-dione during alkylation of **1b**. An X-ray crystallographic study was conducted on 5-*N*-methyl-β-D-arabinofurano[1′,2′:4,5]oxazolo-*s*-triazine-4,6-dione (**2a**) and its solid state structure was established. This was compared to the energy minimized structure of the same compound that was generated by the molecular modeling program, MACROMODEL. Force field calculations (Allinger's MM2) on this structure and other intermediates lend support to the concept of formation of the intramolecular covalent adduct.

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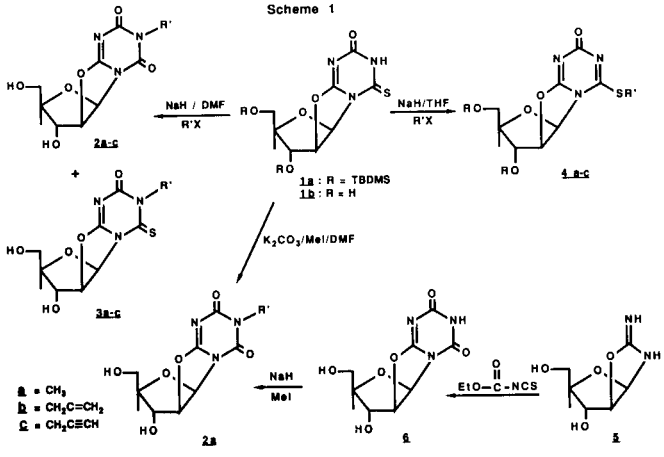
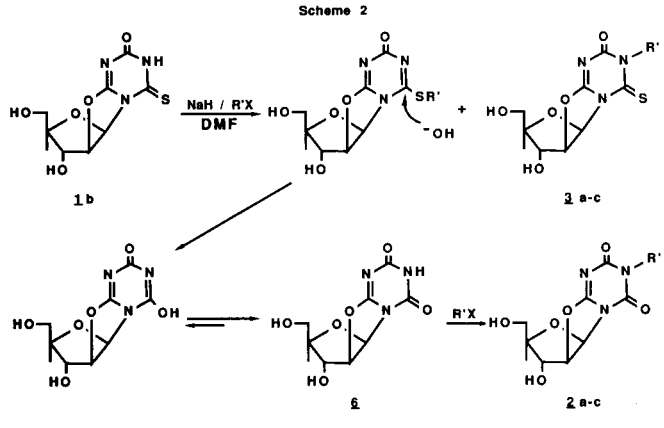
Molecular mechanics is a powerful tool for the calculation of molecular properties [1,2]. Recently, this technique has been effectively used to identify a series of tumor promoters [3,4,5] and to predict the affinity of a drug for a specific receptor [6]. In addition, computer assisted molecular modeling techniques have been used to predict the stereochemistry of mobile systems [7] and the favored geometries of transition states [8,9]. This latter aspect, *i.e.*, predicting transition state geometries, prompted us to use this technique to help explain an unusual pattern of alkylation which we encountered in one of our synthetic programs. We now wish to report the use of computer-generated energy minimized structures as possible support for the mode of formation of 5-*N*-methyl-β-D-arabinofurano[1′,2′:4,5]oxazolo-*s*-triazine-4,6-dione (**2a**) and its C6-thione counterpart (**3a**).

In the previous paper [10], we described the formation of three unexpected *N*-alkylated anhydronucleoside pairs (**2a-c** and **3a-c**, Scheme 1) during the alkylation of β-D-arabinofurano[1′,2′:4,5]oxazolo-*s*-triazin-4-one-thione (**1b**). Three of these *N*-alkylated derivatives, *i.e.*, **2a-c**, had also experienced a loss of sulfur. The pattern of alkylation as well as the presence of desulfurized products were quite puzzling since alkylation of **1a**, the silyl protected derivative of **1b**, under similar conditions gave as the major or sole product the expected *S*-alkylated derivatives **4a-c** (Scheme 1). At first, we thought that alkylation was occurring on both sulfur and nitrogen (Scheme 2) and that the *S*-alkylated product was immediately attacked by hydroxide ion to generate the dione **6** which in turn was alkylated to furnish **2**. This pathway could account for the formation of **2** and **3**, but not their relative amounts. *S*-Alkylation usually predominates and if this were to happen then **2** would eventually be the major *N*-alkylated species. This was not the case, the ratio of the *N*-alkylated pairs was nearly equal [10]. In addition, if *S*-alkylation occurred then the concentration of alkylating agent present in the reaction would be decreased, thus drastically reduc-

Scheme 1



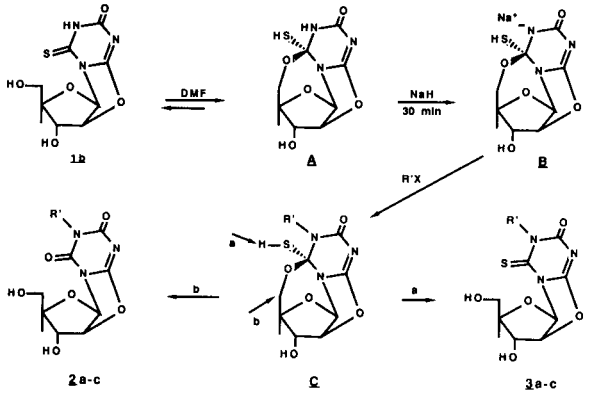
Scheme 2



ing the chance that **6** would be alkylated. Furthermore, we had taken the necessary precautions to keep the reaction anhydrous and the likelihood of water being present in the media was minimal.

Based on our data, we now propose the mechanism depicted in Scheme 3. This proposed pathway can account for the mode of alkylation as well as the observed product ratios. Prior to salt formation, the anhydro nucleoside **1b** is dissolved in dry dimethylformamide (DMF). In solution we believe that **1b** is in equilibrium with the cyclized intermediate **A**, *i.e.*, where the 5′-hydroxyl group is covalently bonded to C6. The formation of such cyclic intermediates and their application in predicting the course of a reaction is well-documented. Piskala and Sorm [11] have implied that crystalline 5-azacytidine (5-AC) actually exists in a 6,5′-cyclic form similar to that proposed for **A**. They suggest that in non-aqueous solutions, the cyclic form of 5-azacytidine is in equilibrium with its anhydro ring opened counterpart. Similarly, Beisler and coworkers [12] observed the formation of a cyclic intermediate with 5,6-dihydro-ara-5-AC. This intermediate was gradually formed by attack of the C2′ hydroxyl group at the C2 position of the *s*-triazine aglycone in deuterium oxide. Use of transient cyclic intermediates is also common in the pyrimidine nucleoside field. For example, 6,5′-anhydro nucleoside intermediates have been proposed to explain rates of reaction [13], product formation [14,15,16] and distribution [17,18]. Therefore, the formation of **A** is conceivable. Once formed and in the presence of sodium hydride, **B** is generated. Salt formation is then followed by alkylation to furnish the *N*-alkylated intermediate **C**. Collapse of this intermediate by either pathway a or b then gives rise to **3a-c** and **2a-c**, respectively.

Scheme 3



To demonstrate the possibility of the formation of **A**, it's structure was generated using MACROMODEL and subjected to force field calculations (Allinger's MM2). Prior to this, the validity of such calculations had to be established. The solid state structure of 5-*N*-methyl-β-D-

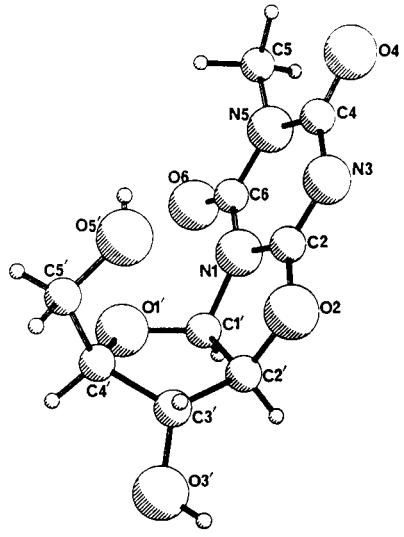


Figure 1 : X-Ray Structure of 2a

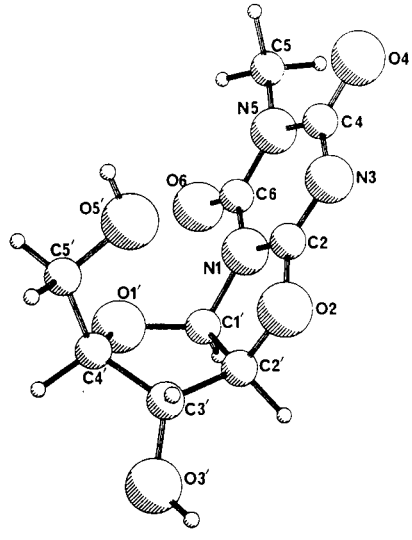


Figure 2 : Energy Minimized Structure of 2a

arabinofurano[1′,2′:4,5]oxazolo-*s*-triazine-4,6-dione (**2a**) was determined by an X-ray crystallographic study (Figure 1) and used as a model. To the best of our knowledge this is the first reported X-ray crystallographic study on a 2,2′-anhydro-*s*-triazine *N*-nucleoside [19]. The anhydro triazine nucleoside has geometries comparable to those of other reported anhydronucleosides (Tables 3,4,5). The C2-O2′ bond is shorter than the normal C-O single bond, whereas, the C2′-O2′ is somewhat longer. Also anhydro ring formation gives rise to considerable distortion in the angles around N1-C1′-C2′.

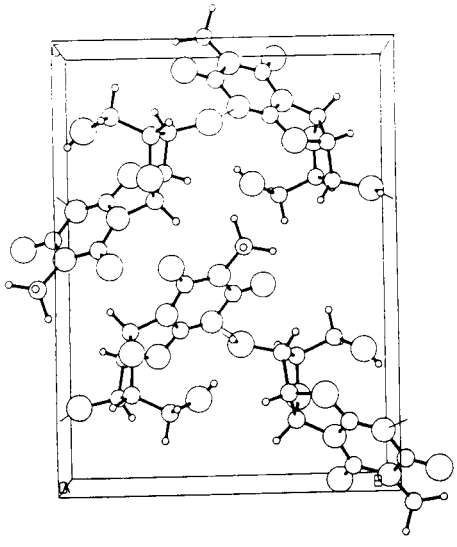


Figure 3 : Packing Diagram of 2a as Viewed Along A-Axis

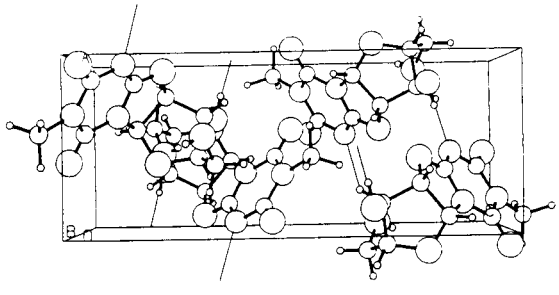


Figure 4 : Packing Diagram of 2a as Viewed Along B-Axis

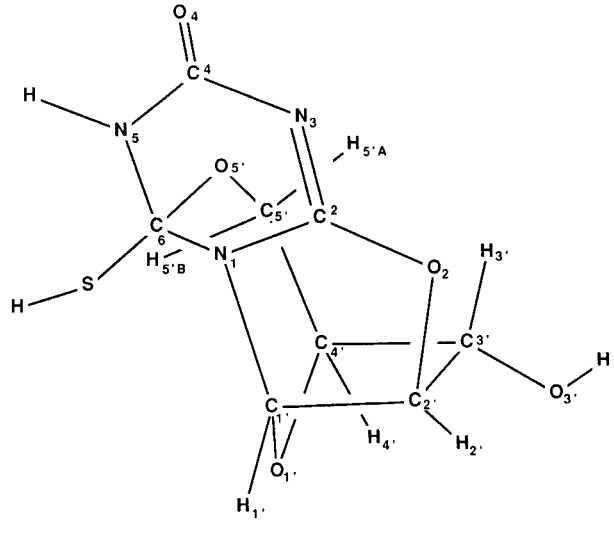


Figure 5 : Energy minimized structure of intermediate A

The atomic coordinates of **2a** were entered in the molecular modeling program, MACROMODEL,

Table 1 : Positional Parameters of 5-N-methyl-β-D-arabinofurano [1',2' : 4,5] oxazolo-s-triazine-4-6-dione

	Atom	X	Y	Z
1.	C 5'	1.1036	0.1735	0.8303
2.	H 5'A	1.0973	0.1901	0.8956
3.	H 5'B	1.2528	0.1486	0.8117
4.	C 4'	1.0622	0.2908	0.7894
5.	H 2'	1.1711	0.3477	0.8116
6.	C 1'	0.9073	0.2962	0.6479
7.	H 1'	0.9130	0.3570	0.6205
8.	C 6	0.9303	0.1253	0.5404
9.	C 5	0.9302	-0.0565	0.4561
10.	H 5A	0.8806	-0.0351	0.3993
11.	H 5B	0.8542	-0.1312	0.4444
12.	H 5C	1.0640	-0.0353	0.4430
13.	C 4	0.6439	-0.0206	0.5657
14.	C 2	0.6536	0.1438	0.6496
15.	C 2'	0.7228	0.3204	0.7130
16.	H 2'	0.6407	0.3903	0.6973
17.	C 3'	0.8318	0.3373	0.8018
18.	H 3'	0.7619	0.2958	0.8434
19.	N 1	0.8360	0.1835	0.6111
20.	N 5	0.8272	0.0209	0.5218
21.	N 3	0.5560	0.0474	0.6322
22.	O 5'	0.9504	0.0875	0.8045
23.	H' O5	0.9895	0.0418	0.7661
24.	O 1'	1.0987	0.2865	0.6950
25.	O 6	1.0565	0.1628	0.5024
26.	O 4	0.5653	-0.1146	0.5448
27.	O 2	0.5853	0.2166	0.7119
28.	O 3'	0.8493	0.4591	0.8221
29.	H'O3	0.7183	0.4823	0.8360

Table 2 : Comparision of Bond Lengths and Bond Angles of X-ray vs MM2 structure of **2a**

Bond Lengths (Å)			Bond Angles		
Atoms	X-ray	MM2	Atoms	X-ray	MM2
C4'-C5'	1.497	1.533	O5'-C5'-C4'	112.9	111.9
C3'-C4'	1.531	1.528	C3'-C4'-C5'	114.7	115.9
O1'-C4'	1.449	1.418	O1'-C4'-C5'	110.6	110.8
C2'-C1'	1.534	1.523	O1'-C4'-C3'	106.1	107.2
N1-C1'	1.469	1.472	N1-C1'-C2'	100.3	98.2
O1'-C1'	1.387	1.427	O5-C1'-C2'	108.5	108.1
N1-C6	1.388	1.324	O1'-C1'-N1	112.5	118.0
N5-C6	1.380	1.334	N5-C6-N1	112.1	116.4
O6-C6	1.203	1.227	O6-C6-N5	125.4	123.2
N5-C5	1.476	1.487	O4-C4-N5	120.3	121.0
N5-C4	1.397	1.337	O4-C4-N3	121.3	118.2
N3-C4	1.383	1.331	N3-C2-N1	126.3	123.5
O4-C4	1.216	1.227	O2-C2-N1	111.4	110.2
N1-C2	1.348	1.308	O2-C2-N3	122.2	126.3
N3-C2	1.282	1.255	N3-C4-N5	118.4	120.8
O2-C2	1.326	1.356	C3'-C2'-C1'	105.3	105.2
C3'-C2'	1.518	1.520	O2-C2'-C1'	106.2	107.9
O2-C2'	1.457	1.418	O2-C2'-C3'	111.9	110.6
O3'-C3'	1.427	1.416	C2'-C3'-C4'	105.1	103.9
			O3'-C3'-C4'	107.1	110.0
			O3'-C3'-C2'	110.4	109.5
			C6-N1-C1'	125.8	124.3
			C2-N1-C1'	112.3	114.1
			C5-N5-C6	117.1	120.5

developed by W. C. Still (Columbia University). A model of the structure of **2a** was then generated with these expertimentally determined coordinates. The aim of this study was to determine the lowest energy conformation of this rigid molecule. The computer model of **2a** was constructed from uridine-5'-monophosphate in the nucleic acid menu, subjected to energy minimization (global), and further refined using the Full Matrix Newton Rhapson technique. Comparison of the energy minimized structure (Figure 2) with the structure generated from the X-ray coordinates showed that they were nearly identical (root mean square deviation (0.049 Å)) on superimposition (Table 2). Using the cartesian coordinates of the energy minimized struc-

Table 3 : Comparison of Certain Bond Lengths of **2a** with Other Anhydronucleosides, Å

Atoms	**2a**	5-S-(Me)2-2,2'-CC[a]	5-Cl-2,2'-CC[a]	6,2'-CC[a]	2-S-2,2'-CU[b]	2,2'-CU[a]	2,2'-α-CXyloU[c]
N1-C2	1.348	1.339	1.345	1.382	1.371	1.335	1.342
C2-N3	1.282	1.299	1.287	1.342	1.297	1.298	1.293
N3-C4	1.383	1.382	1.372	1.332	1.381	1.396	1.386
N5-C6	1.380	1.421	1.407	1.351	1.342	1.342	1.344
C4-N5(C5)	1.397	1.416	1.403	1.411	1.458	1.445	1.445
C6-N1	1.388	1.399	1.388	1.351	1.371	1.370	1.382
N1-C1'	1.469	1.483	1.490	1.436	1.482	1.472	1.465
C2-O2(S2)	1.325	1.327	1.329	1.399	1.731	1.342	1.328
C2'-O2(S2)	1.457	1.469	1.451	1.458	1.809	1.461	1.466

5-S-(Me)2-2,2'-CC : 2,2'-anhydro-1-β-D-arabinofuranosyl-5-dimethylsulfonio-6-oxocytosine chloride
5-Cl-2,2'-CC : 2,2'-anhydro-1-[3',5'-di-O-acetyl-β-D-arabinofuranosyl]-5-chloro-6-oxocytosine
6,2'-CC : 6,2'-anhydro-1-β-D-arabinofuranosyl-6-hydroxycytosine
2-S-2,2'-CU : 2,2'-anhydro-1-β-D-arabinifuranosyl-2-thiouracil
2,2'-CU : 2,2'-cyclouridine
2,2'- -CXyloU : 2,2'-anhydro-1-α-xylofuranosyluracil
a : Reference [21]; b : Reference [22]; c : Reference [23].

Table 4 : Comparison of Certain Bond Angles of **2a** with Other Anhydronucleosides [a]

Atoms	**2a**	5-S-(Me)2-2,2'-CC	5-Cl-2,2'-CC	6,2'-CC	2-S-2,2'-CU	2,2'-CU	2,2'-α-CXyloU
N1-C1'-C2'	100.4	100.3	99.5	101.9	106.7	100.6	100.6
N1-C2-N3	126.2	128.2	126.4	118.3	125.1	127.3	127.1
C2-N3-C4	116.8	114.5	115.4	119.0	118.9	115.7	116.7
C5-C6-N1	112.2	111.9	113.4	121.9	120.0	118.3	118.0
N1-C2-O2	111.5	112.5	110.8	118.0	112.4	111.8	111.8
C2-N1-C1'	112.2	111.6	112.8	126.2	118.1	112.3	112.4
C6-N1-C1'	125.9	126.5	125.1	111.9	123.3	128.1	128.5
C1'-C2'-O2	106.1	106.2	105.9	104.4	109.1	106.0	105.6

[a] See Table 3 for abbreviations and names of compounds.

Table 5 : Comparision of Certain Torsion Angles of **2a** with Other Anhydronucleosides [a]

Atoms	**2a**	5-S-(Me)2-2,2'-CC	5-Cl-2,2'-CC	6,2'-CC	2-S-2,2'-CU
O1'-C1'-N1-C6	-72.57	302.7	291.1	111.4	295.7
C4'-O1'-C1'-C2'	5.59	19.5	10.0	-31.4	19.2
O1'-C1'-C2'-C3'	6.04	-9.5	1.3	6.2	1.7
C1'-C2'-C3'-C4'	-14.34	-3.1	-11.1	19.7	-20.4
C2'-C3'-C4'-O1'	17.71	14.2	17.3	-38.9	31.8
C3'-C4'-O1'-C1'	-14.80	-21.4	-17.7	44.3	-32.6
O1'-C4'-C5'-O5'	-67.71	-77.4	-72.7	59.4	67.3
C3'-C4'-C5'-O5'	52.28	43.5	50.2	56.8	-173.2

[a] See Table 3 for abbreviations and names of compounds.

ture of **2a** a CPK space-filling model was constructed. Examination of this structure of **2a** showed that 5'-OH was in close proximity (3.78 Å as compared to 4.99 Å in uridine) to the C-6 of the *s*-triazine ring. The close proximity of these two positions lends support to the hypothesized nucleophilic attack on the electron deficient C-6 position of the heterocyclic ring by the 5'-OH of the sugar. Next, the structure of the intermediate **A** was generated and subjected to energy minimization. This energy minimized (-6.12 Kjoule, 1.46 Kcal) structure (Figure 5) had normal bond lengths and bond angles

Table 6 : Bond Lengths and Bond Angles of Intermediate **A** as Generated by MM2

Atoms	Bond Lengths (Å)	Atoms	Bond Angles
C6-O5'	1.456	C4'-C5'-O5'	116.3
C5'-O5'	1.427	C3'-C4'-C5'	116.7
C5'-C4'	1.539	C5'-O5'-C6	116.0
C1'-N1	1.478	C2-O2-C2'	107.0
O2-C2'	1.411	C5'-C4'-O1'	109.6
O2-C2	1.356	N1-C1'-O1'	118.4
O1'-C4'	1.412	C2-N1-C1'	110.6
O1'-C1'	1.416	N1-C2-O2	110.4
N5-C6	1.469	O2-C2'-C1'	105.6
N1-C6	1.467	O2-C2'-C3'	111.1

(Table 6), thus providing additional support for the possible formation of **A**.

In conclusion, the results of this detailed molecular modeling study lend strong support to the probable formation of **A** and the proposed mechanism. The pathway illustrated in Scheme 3 now adequately explains the observed alkylation pattern of **1b** and the observed distribution of *N*-alkylated products.

EXPERIMENTAL

A colorless crystalline plate of **2a** with dimensions 0.14 x 0.50 x 0.68 mm was mounted on a Nicolet R3M/E auto diffractometer. The crystal was orthorhombic. The cell constants based on 25 centered reflections with $15° < 2\theta < 35°$ were calculated to be a = 6.1814 (9), b = 11.4058 (15), c = 15.1634 (16) Å, $\alpha = \beta = \gamma = 90.0°$. Diffraction data were collected between 5.0 and 50.0° 2θ at 20°C. The data were corrected for Lorentz-polarization effects, and an empirical (laminar) absorption correction was applied. 1144 reflections were collected of which 971 were unique and were used in refinement. The structure was solved by direct methods and refined by block-diagonal least-squares technique. All hydrogen atoms were located on difference maps. Non-hydrogen atoms were refined anisotropically and hydrogen atoms were refined isotropically based on F. One of the H atoms attached to C-5 refined to an improbable position, and so this carbon and its attached H atoms were refined as a rigid group. Refinement converged to R = 0.0348 and WR = 0.0483 where $W = 0.3182/[\sigma^2(F) + 0.002464\ F^2]$ and $\sigma(F)^2$ is derived from counting statistics. In the final least-squares cycle, no parameter moved by more than 0.00722 Å which was taken as convergence. The maximum shift/ESD in the final cycle was -0.485. The highest peak in the difference map was 0.165, and the deepest hole was -0.170e⁻/Å³. Final atomic coordinates are given in Table 1. Anisotropic thermal parameters of non-hydrogen atoms, isotropic thermal parameters for hydrogen atoms, and a list of observed and calculated structure factors are available as supplementary material from the authors.

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REFERENCES AND NOTES

[1] U. Burket and N. L Allinger, "Molecular Mechanics", American Chemical Society, Washington, 1982.

[2] N. L. Allinger, *J. Am. Chem. Soc.*, **99**, 8127 (1977).

[3] A. M. Jeffrey and R. M. J. Liskamp, *Proc. Natl. Acad. Sci., USA*, **83**, 241 (1986).

[4] P. A. Wender, K. F. Koehler, N. A. Sharkey, M. L. Dell'Aquila and P. M. Blumberg, *Proc. Natl. Acad. Sci., USA*, **83**, 4125 (1986).

[5] P. A. Wender, C. M. Cribbs, K. F. Koehler, N. A. Sharkey, C. L. Herald, Y. Kamano, G. R. Pettit and P. M. Blumberg, *Proc. Natl. Acad. Sci., USA*, **85**, 7197 (1988).

[6] A. Thurkauf, P. C. Zenk, R. L. Balster, E. L. May, C. George, F. I. Carroll, S. W. Mascarella, K. C. Rice, A. E. Jacobson, and M. V. Mattson, *J. Med. Chem.*, **31**, 2257 (1988).

[7] W. C. Still and I. Galynker, *Tetrahedron*, **37**, 3981 (1981).

[8] W. C. Still and I. Galynker, *J. Am. Chem. Soc.*, **104**, 1774 (1982).

[9] P. A. Wender and N. C. Ihle, *Tetrahedron Letters*, **28**, 2451 (1987).

[10] S. Purkayastha and R. P. Panzica, *J. Heterocyclic Chem.*, **27**, 743 (1990).

[11] A. Piskala and F. Sorm in "Nucleic Acid Chemistry" Part 1, L. B. Townsend and R. S. Tipson, eds, Wiley-Interscience, New York, 1978, p 455-459.

[12] J. A. Beisler, M. M. Abbasi and J. S. Driscoll, *J. Med. Chem.*, **22**, 1230 (1979).

[13] D. V. Santi and C. F. Brewer, *J. Am. Chem. Soc.*, **90**, 6232 (1968).

[14] Y. Kondo, J.-L. Fourrey and B. Witkop, *J. Am. Chem. Soc.*, **93**, 3527 (1971).

[15] S. A. Salisbury and D. M. Brown, *J. Chem. Soc., Chem Commun.*, 656 (1979).

[16] V. Skaric and J. M. Adamic, *J. Chem. Soc; Perkin Trans. 1*, 779 (1985).

[17] K. Kikugawa and T. Ukita, *Chem. Pharm. Bull.*, **17**, 775 (1969).

[18] A. Matsuda, H. Inoue and T. Ueda, Abstr. 1st Symp. Nucleic Acid Chem., 85 (1973).

[19] Dr. Wendell Wierenga (The Upjohn Company) has informed us that an X-ray crystallographic study was performed on dihydro-5-azathymidine, but is unpublished. R. K. Robins and coworkers [20] determined the structure of 2,4-diamino-6-(β-D-ribofuranosyl)-*s*-triazine by single crystal X-ray diffraction methods, but this compound, *i.e.*, a "C"-nucleoside, can be simply viewed as a trisubstituted *s*-triazine.

[20] T. A. Riley, W. J. Hennen, N. K. Dalley, B. E. Wilson and R. K. Robins, *J. Heterocyclic Chem.*, **23**, 1709 (1986).

[21] Y. Yamagata, M. Koshibi, R. Tokuoka, S. Fujii, T. Fujiwara, T. Kanai and K.-I. Tomita, *Acta Cryst.*, **B 35**, 382 (1979).

[22] Y. Yamagata, J. Yoshimura, S. Fujii, T. Fijiwara, K.-I. Tomita and T. Ueda, *Acta Cryst.*, **B 36**, 343 (1980).

[23] G. I. Birnbaum, J. Giziewicz, C. P. Huber and D. Shugar, *J. Am. Chem. Soc.*, **98**, 4640 (1976).