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Pyridopyrimidines. 14. Conformational Studies of 5,6,7,8-Tetrahydropyrido[3,2-*d*]pyrimidines. Potential Multisubstrate Analogue Inhibitors of Thymidylate Synthetase

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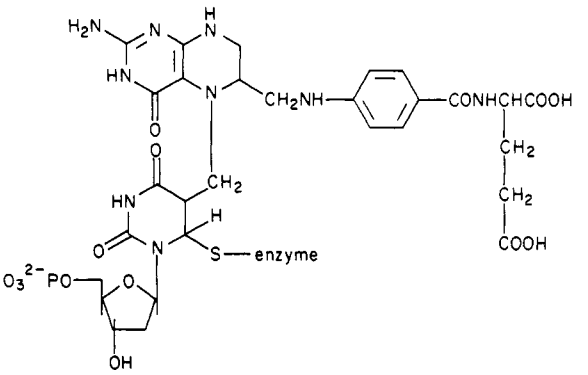
The synthesis of 5-[(2,4-dioxo-6-methyl-5,6,7,8-tetrahydropyrido[3,2-*d*]pyrimidin-5-yl)methyl]-2′-deoxyuridine and a number of related compounds as models of potent inhibitors of thymidylate synthetase has been accomplished. The ^{1}H and ^{13}C NMR spectra of certain of these molecules show unusual and remarkably stable conformational effects. A partial conformational analysis was undertaken in order to determine the origins of these effects. These studies revealed that the presence of a diastereomeric center at C-6 is responsible for the observed phenomena.

A major goal of this laboratory is the synthesis of a series of potential "multisubstrate analogue" inhibitors of thymidylate synthetase, a key enzyme in the biosynthesis of DNA. Such inhibitors (sometimes referred to as "transition state analogues"), provided they possess appropriate functional groups in te correct geometry, are expected to bind many orders of magnitude more tightly than would the substrates.[1]

Thymidylate synthetase has been well studied in this regard; the work of Santi,[2] Ellis and Dunlap,[3] and Kisliuk,[4] in particular, has revealed a great deal. Although many details remain to be explored, it is known that a ternary complex among 2′-deoxyuridylate, a tetrahydrofolate, and thymidylate synthetase is formed, as illustrated by **1**.[2]

An appropriate combination of key elements of a reduced folate and thymidylate ought, then, to lead to very potent inhibitors. In support of this view is the recent report[5] of a very simple tetrahydroquinoxaline analogue, **2**, which turned out to be a reasonably potent inhibitor of thymidylate synthetase (K_i = 0.75 μM). The present report describes the synthesis of a series of model potential inhibitors that represent a major step toward the goal



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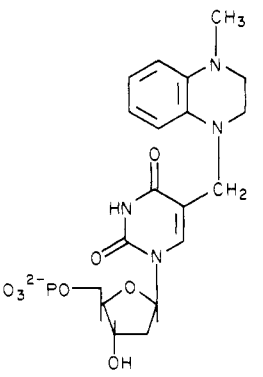

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(1) Lindquist, R. N. "Drug Design"; Ariens, E. J., Ed.; Academic Press: New York, 1975; Vol. 5, p 27–46.

(2) Santi, D. V.; McHenry, C. S.; Sommer, H. *Biochemistry* **1974**, *13*, 471; James, T. L.; Pogolotti, A. L.; Ivanetich, K. M.; Wataya, Y.; Lam, S. S. M.; Santi, D. V. *Biochem. Biophys. Res. Commun.* **1976**, *72*, 404. For a review, see Santi, D. V. *J. Med. Chem.* **1980**, *23*, 103.

(3) Lewis, C. A., Jr.; Ellis, P. D.; Dunlap, R. B., *Biochemistry* **1981**, *20*, 2275.

(4) (a) Beckage, M. J.; Blumenstein, M.; Kisliuk, R. L. *Mol. Cell. Biochem.* **1980**, *32*, 45. (b) Beaudette, N. V.; Langerman, N.; Kisliuk, R. L. *Arch. Biochem. Biophys.* **1980**, *200*, 410.

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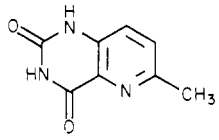
described above. A detailed NMR investigation has afforded substantial insight into the geometry of these model systems.

The key starting materials for this project were 2,4-dioxo-6-methylpyrido[3,2-*d*]pyrimidine[6] (**3**) and the analogous 2-amino-4-oxo compound[7] **4**. It was originally reasoned that alkylation at N-5 of **3** or **4** would provide a quaternary intermediate, the pyridine ring of which could be very readily reduced to give the desired 5,6,7,8-tetrahydro derivative. However, alkylation of either **3** or **4** could



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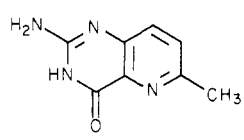
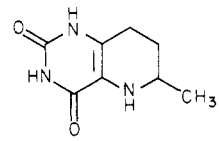


4

not be accomplished; even under forcing conditions (dimethyl sulfate, DMF, steam bath), only starting material was observed. Lack of alkylation on the pyridine nitrogen may be attributed to the severe steric constraints imposed by the peri oxo group at C-4 and the 6-methyl group.

It was reasoned that reduction of the pyridine ring should greatly facilitate alkylation at N-5, since the resulting half-chair conformers would impose much less steric restriction. The only reduction of a pyrido[3,2-*d*]pyrimidine in the literature was reported by DeGraw et al.[8] with H_2/PtO_2 on 8-deazafolic acid, but no experimental details were given. It was found that while **3** could be smoothly reduced in either methanol or methanolic HCl, **4** required acidic conditions (trifluoroacetic acid–methanol or 0.1 N HCl) with the PtO_2 catalyst. The structures of products **5** and **6** were confirmed by mass spectra (M^+ 181



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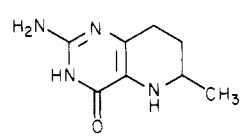


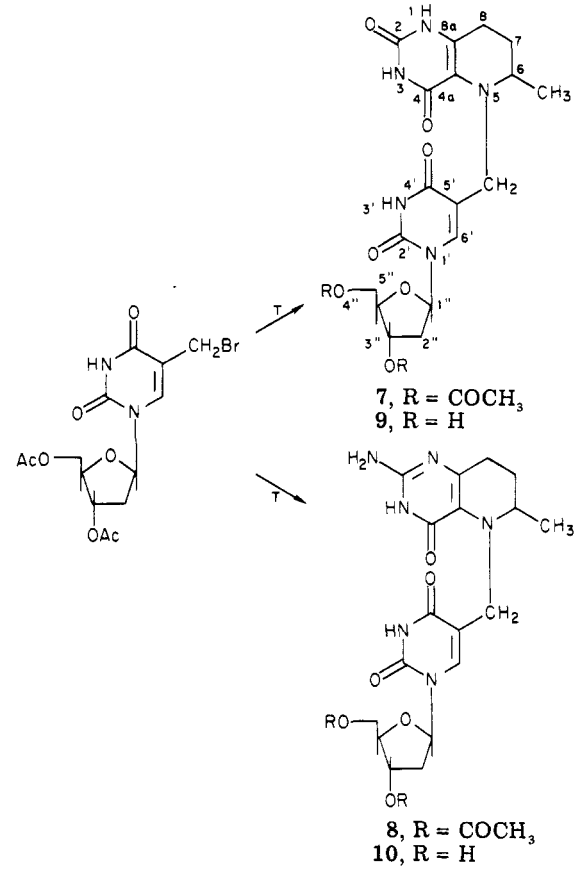
6

and 180 for **5** and **6**, respectively), NMR [the 6-CH_3 groups appeared as a doublet (J = 6.4 Hz) at about δ 1.1] and by a comparison of the UV spectrum of **6** with that of the 6-*n*-amyl derivative synthesized by DeGraw and Brown.[9]

Alkylations were carried out with 5-(bromomethyl)-3′,5′-di-*O*-acetyl-2′-deoxyuridine.[10] Both **5** and **6** reacted smoothly to give **7** and **8**, which were readily deblocked to give the target nucleosides **9** and **10** (Scheme I). The mass spectra of all these nucleosides (trimethysilyl derivatives in the case of **9** and **10**) yielded molecular ions that were predicted from the assigned structures.[11]

The readily accessible dioxo derivative **7** was studied in more detail to establish that the alkylation of **5** did, indeed, lead to N-5 substitution. The assignment proved straightforward; starting material **5** gave rise to three D_2O-exchangeable single-proton resonances at δ 10.28, 10.91 (lactam N-H's), and 3.97 (N-5H). Upon N-alkylation to give **7**, the high-field signal disappeared and was replaced with a new lactam signal at δ 11.44 (uracil N-3 H);

Scheme I



7, R = $COCH_3$
9, R = H

8, R = $COCH_3$
10, R = H

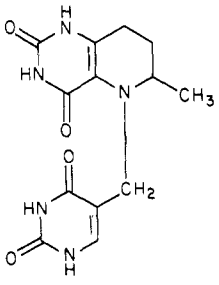
the other lactam signals remained virtually unchanged at δ 10.44 and 10.90.

However, examination of the 90-MHz ^1H NMR spectrum of **7** revealed some unusual features. The signal for the 6-methyl group appeared as a triplet; this enhanced multiplicity was no surprise, since a new pair of diastereoisomers (at C-6) had been generated upon alkylation of N-5 with a chiral reagent. However, it was most surprising to find that both pyrimidine H-6′ and one of the acetyl methyl signals exhibited magnetic nonequivalence and appeared as two peaks of equal intensity.

In order to be certain that this phenomenon was indeed due to the presence of two diastereoisomers in **7**, we prepared two additional compounds. Compound **11**, 2,4-dioxo-6-methyl-5-(uracil-5-ylmethyl)-5,6,7,8-tetrahydropyrido[3,2-*d*]pyrimidine, was prepared by alkylation of **5** with 5-chloromethyluracil,[12] and **12** resulted from the reaction of di-*O*-acetyl-5-(bromomethyl)-2′-deoxyuridine with 2,4-dioxo-5,6,7,8-tetrahydroprido[3,2-*d*]pyrimidine (**13**). Neither of these molecules showed any unusual multiplicity in their ^1H NMR spectra, establishing that 6-*R* and 6-*S* isomers of **7** do, indeed, give rise to different spectra. The same general features were observed in **8** and in **9** and **10**, although the nonequivalence was less pronounced.

At 270 MHz the spectrum of **7** assumed somewhat more detail. The 6-methyl signal appeared as two doublets centered at δ 0.83 and 0.89 with coupling constants of 6.7 Hz. The axial and equatorial protons attached to C-7 gave rise to complex multiplets at δ 1.49 and 1.77. Both the acetyl methyl signals appeared as doublets and C-6 H gave rise to broad multiplets centered at δ 2.94 and 3.06; C*H*-1″ appeared as two sets of triplets centered at δ 6.20 and 6.23 with coupling constants of 5.8 Hz. A similar pattern was

(6) Irwin, W. J.; Wibberley, D. G. *J. Chem. Soc. C.* **1969**, 1945.
(7) Preparation of **4** was also reported by Kelley, J. L.; McLean, E. W. *J. Heterocycl. Chem.* **1981**, *18*, 671, and Temple, C. L.; Smithers, D. L.; Kussner, C. L.; Bennett, L. L.; Montgomery, J. A. *J. Med. Chem.* **1981**, *24*, 1254.
(8) DeGraw, J. I.; Kisliuk, R. L.; Gaumant, V.; Baugh, C. M. *J. Med. Chem.* **1974**, *17*, 470.
(9) DeGraw, J. I.; Brown, W. H. *J. Heterocycl. Chem.* **1976**, *3*, 349.
(10) Barwolff, D.; Langen, P. "Nucleic Acid Chemistry"; Townsend, L. B.; Tipson, R. S., Ed.; Wiley, New York; 1978, p 359.
(11) Refer to structure **7** (or **9**) for numbering system used.
(12) Giner-Sorolla, A.; Medreck, L. *J. Med. Chem.* **1966**, *9*, 77.



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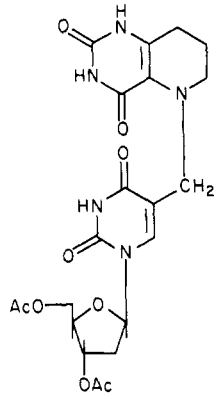

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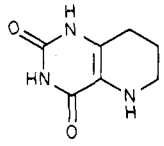

13

observed for 8. The ^1H NMR data for 5–11 are presented in Table I.

Molecular asymmetry is well-known to lead to significant proton and carbon chemical-shift differences. Even in atoms as many as eight bonds removed from the asymmetric center, small chemical-sift differences have been observed and attributed to "through space" (i.e., conformationally dependent) effects.[13] The present examples are remarkable, however, in that the upfield acetyl methyl resonance chemical shifts differ by 0.032 ppm (9.6 Hz), although the protons are 12 or 13 bonds removed from the newly created center of asymmetry (assignment of specific acetyl methyl signals was not undertaken). These data suggest that a substantial degree of conformational rigidity exists in these molecules. Since an increased rate of conformer interconversion relative to the NMR time scale, and subsequent averaging of resonance lines, can often be achieved by heating the sample, a solution of 7 in $(CD_3)_2SO$ was heated to 180 °C. At no stage in this process did coalescence of all lines occur, although chemical-shift differences did decrease, attesting again to the unusual conformational stability of these molecules.

In order to assess which of the possible conformatons predominates, a series of decoupling experiments was performed at 200 MHz. Each diastereoisomer could have C-6 H either equatorially or axially oriented. Since an axial orientation would lead to one large (9–12 Hz, axial–axial) and one small (<4 Hz, axial–equatorial) coupling, and an equatorial proton would give rise to two small couplings (a,e and e,e), it should be possible to decide whether one conformation is preferred. Irradiation of the 6-CH_3 resonances caused a dramatic sharpening and narrowing of the two C6-H signals, resulting in a line width at half-peak height of 5–6 Hz. Although exact coupling constants could not be measured, they are certainly small and, hence, compatible only with an equatorial disposition of C-6 H. Confirmation was obtained by irradiating the C-8 H region (δ 2.40) and noting collapse of the C-7 H resonances into doublets containing a geminal coupling (−13 Hz) and, again, 5–6 Hz line widths containing the small couplings to C-6 H. The signals corresponding to each diastereoisomer behaved identically in all cases. For both isomers the only detectable conformer has the 6-methyl group

(13) Schwarz, M.; Bradley, R. B.; McIntyre, H. M.; Becker, E. D. *Org. Magn. Reson.* **1974**, *6*, 625.

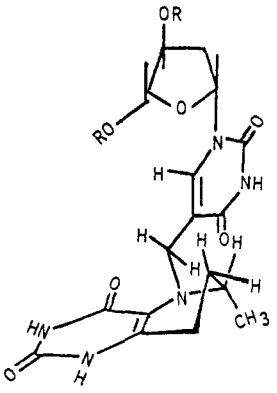

Figure 1. Stereochemical representaton of 7 showing 6-methyl and 5-thymidinyl groups in the trans-diaxial configuraiton.

axially disposed, in sharp contrast to the situation with 6-methyltetrahydropterin in which N-5 bears only a proton,[14] but consistent with the finding by Poe[15] that N-methylation of tetrahydrofolate constrains the side chain to an axial orientation.

The remaining important question, more difficult to answer with certainty, concerns the orientation of the N-5 substituent. Poe et al.[15] suggested, on the basis of space-filling models, that the methyl substituent of 5-methyl-5,6,7,8-tetrahydrofolate was probably axially oriented. CPK models of 7 and 8 are consistent with less steric crowding in the 5,6-diaxial arrangement. Further evidence arises from the NMR spectral data described above. The significant chemical-shift differences noted in the thymidine moiety H-6 and acetyl methyl signals are best accounted for by differential shielding in the two diastereoisomers resulting from a "folded" arrangement whereby the unsaturated heterocyclic rings are stacked in such a way that the pyrimidine H-6 and the acetyl groups, in particular, can experience shielding effects from the pyridopyrimidine. This arrangement is only feasible (as judged by CPK models) when the 6-methyl and 5-thymidinyl groups are trans-diaxial (Figure 1).

It was of interest to determine whether this apparent demonstration of conformational rigidity was supported by ^{13}C NMR spectra. The ^{13}C NMR data presented in Table II were obtained at 67.8 MHz. Again, the detailed discussion refers to dioxo derivative 7 and its precursors, but generally applies also to the 2-amino derivatives.

The carbon spectrum of 7 (Figure 2) is fairly complex. It is readily apparent that nearly all the ^{13}C signals, even those of the di-*O*-acetyl sugar moiety, appear as doublets because of magnetic nonequivalence, lending further support to the apparent rigidity and nonequivalence of the two diastereosomers. Because of the complexity of the spectrum of 7, it was necessary to turn to simpler structures for peak assignment. Assignment of di-*O*-acetylthymidine (see Table I for peak position and also see Figure 1) was based upon the assignments of thymidine.[16] Most of the peak positions in the spectrum of 5 were readily assigned. The two furthest downfield signals (160.26 and 149.24 ppm) correspond to C-4 and C-2, respectively. The signal at 46.08 ppm corresponds to ternary C-6, and the highest filed signal corresponds to the 6-methyl group. (These assignments were confirmed by the doublet and quartet, respectively, observed in the gated

(14) Moad, G.; Luthy, C. L.; Benkovic, P. A.; Benkovic, S. J. *J. Am. Chem. Soc.* **1979**, *101*, 6068.

(15) Poe, M.; Hensens, O. D.; Hoogsteen, K. *J. Biol. Chem.* **1979**, *254*, 1081.

(16) Jones, A. J.; Grant, D. M.; Winkley, M. W.; Robins, R. K. *J. Am. Chem. Soc.* **1970**, *92*, 4079.

Table I. Proton Chemical Shifts of Pyridopyrimidine Derivatives

position	5	7	9	11	6	8	10
CO-NH	10.91 (s, 1), 10.28 (s, 1)	10.90 (s, 1), 10.44 (s, 1)	10.87 (br, 1), 10.44 (br, 1)	10.91 (s, 1), 10.43 (s, 1)			
6-CH	3.04 (br, 1)	2.94 & 3.06 (m each, 1)	3.01 (m, 1)	3.01 (m, 1)	3.08 (m, 1)	2.92 & 3.06 (m each, 1)	3.01 (m, 1)
6-CH_3	1.10 (d, 3), (J = 6.4 Hz)	0.83 & 0.89 (d each, J = 6.7 Hz, 3)	0.89 (d, 3, J = 6.6 Hz)	0.87 (d, 3, J = 6.4 Hz)	1.11 (d, 3, J = 6.1 Hz)	0.83 & 0.89 (d each, 3, J = 6.6 Hz, 3)	0.89 (d, 3, J = 5.6 Hz)
7-CH_2	1.43 & 1.81 (m each, 2)	1.49 & 1.77 (m each, 2)	1.43 & 1.63 (m each, 2)	1.49 & 1.68 (m each, 2)	1.46 & 1.81 (m each, 2)	1.48 & 1.78 (m each, 2)	1.50 & 1.75 (m each, 2)
8-CH_2	2.30 & 2.44 (m each, 2)	2.33 (m, 4, along with 2″-CH_2)	2.00 & 2.15 (m each, 2)	2.25 (m, 2)	2.35 & 2.46 (m each, 2)	2.33 (m, 4, along with 2″-CH_2)	1.96 & 2.08 (m each, 2)
CO-NH		11.44 (s, 1)	11.25 (br, 1)	11.06 (s, 1), 10.75 (d, 1)		11.43 (s, 1)	11.09 (br, 1)
6′-CH		7.76 & 7.84 (s each, 1)	7.85 & 7.87 (s each, 1)	7.44 (d, 1)		7.82 & 7.89 (s each, 1)	7.90 & 7.96 (s each, 1)
1″-CH		6.20 & 6.23 (t each, 1, J = 5.8 Hz)	6.19 (t, 1, J = 6.6 Hz)			6.21 & 6.24 (t each, 1, J = 5.6 Hz)	6.20 (t, 1, J = 6.4 Hz)
2″-CH_2		2.33 (m, 2, along with 8-CH_2)	2.24 (m, 2)			2.33 (m, 2, along with 8-CH_2)	2.25 (m, 2)
3″-CH		5.17 (m, 1)	4.19 (m, 1)			5.17 (m, 1)	4.19 (m, 1)
4″-CH		4.17 (m, 1)	3.53 (m, 3, along with 5″-CH_2)			4.18 (m, 3, along with 5″-CH_2)	3.54 (m, 3, along with 5″-CH_2)
5″-CH_2		4.18 (s, 2)	3.53 (m, 3, along with 4″-CH)			4.18 (m, 3, along with 4″-CH)	3.54 (m, 3, along with 4″-CH)
5-CH_2		3.64 (m, 2)	3.48 (m, 2)	3.53 and 3.66 (d each, 2)		3.62 (m, 2)	3.48 (m, 2)
other		2.07 and 2.08 (s each, 3, $COCH_3$), 1.98 and 2.01 (s each, 3, $COCH_3$)	5.28 (d, 1, 4″-OH), 4.81 (t, 1, 5″-OH)		6.29 (br), (2.2-NH_2)	2.07 and 2.08 (s each, 3, $COCH_3$), 1.93 and 1.98 (s each, 3, $COCH_3$), 6.06 (br, 2,2-NH_2)	5.28 (d, 1, 4″-OH), 4.86 (t, 1, 5″-OH), 6.01 (br, 2,2-NH)

Table II. ^{13}C NMR Chemical Shifts of Pyridopyrimidine Derivatives

position	5	7	9	11	12	13	6	8	10	diacetylthymidine[d]
2-C[a]	149.24	149.70	149.63	149.72	149.23	149.74	159.39	160.52, 160.39	160.51	
4-C=O	160.26	162.05, 161.96	162.01	161.98	160.34	161.49	148.34	149.72	149.77	
5-CH_2		47.85, 47.08	47.53, 46.97	46.92		46.87		47.76, 47.35	47.27, 46.96	
6-C[b]	46.08	50.93, 49.93	50.17, 49.84	49.75	40.57	46.87	46.28	50.85, 50.66	50.50, 60.19	
6-CH_3	21.08	17.90, 17.41	17.83	17.78			21.06	18.32, 18.12	18.20	
7-CH_2	28.69	20.94, 20.78	21.02	21.01	20.92	16.59	29.95	25.21, 25.05	25.06	
8-CH_2	22.79	20.06, 19.85	19.98	19.96	22.95	23.96	25.95	23.01	22.50	
4a-C	118.51	117.85	117.75	117.75	118.81	119.77	120.96	121.61	121.12	
8a-C	126.73	137.13, 136.87	136.73	136.92	126.72	137.86	136.83	148.75, 148.61	148.25	
2′-C=O		150.07	150.09	151.08		150.09		150.06	150.12	151.41
4′-C=O		163.14, 163.01	163.13	164.39		163.23		163.09, 163.02	163.22	164.61
5′-C		112.32, 112.29	111.65	110.17		111.90		112.97, 112.80	112.21, 112.04	110.90
6′-CH		137.88, 137.25	138.35	140.01		137.95		138.18, 137.92	138.68, 138.46	136.66
1″-CH		81.15, 81.04	83.70			81.34		8.130, 8.121	83.77	82.14
2″-CH_2		36.11	*c*			36.13		36.19	*c*	36.57
3″-CH		74.10, 73.90	70.58			74.20		73.97, 73.89	70.60	74.96
4″-CH		84.13, 83.89	87.12			84.45		84.54, 84.38	87.24	85.12
5″-CH_2		63.81, 63.48	61.71			63.62		63.58, 63.45	61.70	64.61
$COCH_3$		170.10, 169.95, 169.92				170.13, 169.96		169.86, 169.69		171.09, 171.00
$COCH_3$		20.66, 20.33, 20.19				20.68, 20.17		20.47, 20.19, 20.10		21.65, 21.47

[a] Refers to 2 C-NH_2 in the case of *6*, *8* and *10*. [b] Refers to 6 CH_2 in the case of *12* and *13*. [c] 5″-CH_2 is present along with solvent peak. [d] Position of 5′ CH_3 is 13.75 ppm.

off-resonance decoupled spectrum.) This leaves two pairs of lines requiring assignment, a downfield pair corresponding to quaternary carbons C-4a and C-8a and a high-field pair arising from methylene carbon-7 and carbon-8. Of the downfield pair, the signal at 118.51 ppm was tentatively assigned to C-4a and that at 126.73 to C-8a by analogy with 6-methyl-5-(methylamino)uracil.[17] The assignments in 6-methyl-5-(methylamino)uracil were based on the coupled spectrum combined with selective heteronuclear decoupling (data not shown).

Initially, an intuitive assignment was made that allylic C-8 would resonate at lower field than aliphatic C-7. However, selective irradiation of the allylic protons at δ 2.38 led to a collapse of the signal at 22.79 ppm to a singlet, while irradiaiton at δ 1.62 similarly affected the downfield signal at 28.69 ppm, thereby establishing the former as C-8 and the latter as C-7.

The next step was to couple deazapteridine to pyrimidine in such a way that no new center of asymmetry was introduced. The ^{13}C NMR spectrum of **11** should yield significant conformational information without the complexities observed in the spectrum of **7**. In this case, the chemical shifts were virtually identical for comparable carbons in **11** and the nucleosides **7** and **9**. This finding, coupled with the ^1H NMR data described above, supports the view that 5,6-disubstitution, presumably of the kind required at some point in the thymidylate synthetase reaction, results in a relatively rigid, 5,6-diaxial array of substituents.

Because these compounds are models for more complex structures to be prepared, it was of interest to determine the effects of N and C substitution on the carbon chemical shifts in these somewhat unusual and highly hindered systems. The direction and magnitude of chemical-shift differences on saturated carbons were predictable and consistent with those observed in piperidine[18,19] and cyclohexane.[20] For example, if one compares the chemical shifts of C-6 in **5** and **12**, one finds the expected downfield shift of 5.5 ppm upon replacing proton with methyl. The adjacent carbon-7 signal similarly undergoes a downfield shift of nearly 8 ppm, while C-8 remains unaffected. N-Substitution leads to the effects in the saturated portion of the molecule as reported for piperidine.[18] However, the effect of N-alkylaton on C-4a and C-8a was significant. In every case studied, N-alkylation made essentially no change in the chemical shift of carbon-4a but caused a substantial downfield shift (10–12 ppm) in the chemical shift of the C-8a resonance. The large deshielding of C-8a upon N-alkylation should prove very useful in determining the site of N-alkylation of complex deazafolates.

The data described above provided ample basis for straightforward assignment of the signals in the complex spectra of **7** and **9**. A similar approach enabled the assignments to be made for 2-amino derivatives **6**, **8**, and **10**. It should be noted that the carbon spectra, unlike the proton spectra, do not provide useful information concerning half-chair conformations of the tetrahydropyridine moiety but do provide information that should uniquely permit assignment of the alkylation site.

Experimental Section

^1H and ^{13}C NMR data were obtained on a JEOL FX-270 NMR spectrometer at 269.65 and 67.8 MHz, respectively, in the Fourier Transform mode in $(CD_3)_2SO$ with tetramethylsilane as the internal standard. Proton spectra, 200 MHz, were obtained on a Varian XL-200 NMR spectrometer. UV spectra were run on a Cary 15 or Beckman DU-8 spectrophotometer. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are not corrected. Mass spectra were obtained on a Varian 112S or LKB-GCMS 9000 S spectrometer. Elemental analyses were performed by Heterocyclic Chemical Corp., Harrisonville, MO, and Galbraith Laboratories, Knoxville, TN.

^{13}C chemical shifts were measured over 13000-Hz spectral width with a 16K data table. Methine, methylene, and methyl carbons were identified by the Ernest–Doddrell method[21] with the program supplied by JEOL. Alternatively, assignment of methylene carbons utilized heteronuclear decoupling based on proton chemical-shift assignments.

2-Amino-6-methyl-4-oxopyrido[3,2-*d*]pyrimidine (4). 2,4-Diamino-6-methylpyrido[3,2-*d*]pyrimidine[22] (0.87 g, 5 mmol) in 25 mL of 1 N NaOH was refluxed for 8–10 h. The hot solution was filtered and neutralized with 2 N acetic acid. The precipitated solid was collected by filtration and washed with water to give 0.7 g (79%) of **4**: mp λ >300 °C; MS, *m/e* 176 (M^+); UV λ_{max} 247 nm (ϵ_{max} 12800), 285 (3500), 315 (5100), 321 (4700); λ_{max} (pH 7) 261 nm (ϵ_{max} 10600), 270 (9000), 320 (5000), 325 (4800); UV (pH 13) 234 nm (ϵ_{max} 20500), 268 (8900), 334 (5000); ^1H NMR δ 2.48 (s, 3, 6-CH_3), 6.46 (br, 2, 2-NH_2), 7.41 and 7.48 (d each, J = 8.6 Hz, 1, 2, C-7 H and C-8 H) and 11.13 (br, 1, 3-NH).

Anal. Calcd for $C_8H_8N_4O$: C, 54.54; H, 4.57; N, 31.80. Found: C, 54.37; H, 4.70; N, 31.59.

2,4-Dioxo-6-methyl-5,6,7,8-tetrahydropyrido[3,2-*d*]pyrimidine (5). A solution of 1.77 g (10 mmol) of **3**[6] in 100 mL of methanol was hydrogenated at 1 atm in the presence of 100 mg of Adams catalyst in a Parr apparatus. The reaction was followed by TLC (silica gel; Merck F-254; $CHCl_3/CH_3OH$, 85:15) and was generally complete within 3 h. The catalyst was removed by filtration through Celite, and the filtrate was evaporated to give 1.5 g (82%) of **5**. An analytical sample was prepared by crystallization from ethanol: mp 273–274 °C; MS, *m/e* 181 (M^+); UV λ_{max} (pH 1) 259 nm (ϵ_{max} 14000; λ_{max} (pH 7) 300nm (ϵ_{max} 9000); UV (pH 13) 300 nm (ϵ_{max} 9600).

Anal. Calcd for $C_8H_{11}N_3O_2 \cdot 0.5H_2O$:C, 50.52; H, 6.35; N, 22.09. Found: C, 50.89; H, 6.63; N, 22.49.

2-Amino-6-methyl-4-oxo-5,6,7,8-tetrahydropyrido[3,2-*d*]pyrimidine (6). A suspension of 0.88 g (5 mmol) of **4** in 100 mL of methanol containing 5 mL of trifluoroacetic acid was hydrogenated in the presence of 50 mg of Adams catalyst at 42 psi as described in **5**. The catalyst was removed by filtraton through Celite, and the filtrate was evaporated to dryness. The oily residue was dissolved in methanol and evaporated to dryness. This process was repeated until a dry solid was obtained. The solid was dissolved in warm water and was neutralized with 5% $NaHCO_3$ solution. The precipitated solid was filtered and washed with water to give ϵ_{max} 0.75 g (83%) of **6**: mp >300 °C; MS, *m/e* 180 (M^+); UV λ_{max} (pH 1) 259 nm (ϵ_{max} 5000); UV λ_{max} (pH 7) 249 nm (ϵ_{max} 8000), 295 (5400); UV λ_{max} (pH 13) 247 nm (ϵ_{max} (8400), 300 (6100).

Anal. Calcd for $C_8H_{12}N_4O$: C, 53.31; H, 6.73; N, 31.08. Found: C, 53.11; H, 6.89; N, 31.16.

2,4-Dioxo-5,6,7,8-tetrahydropyrido[3,2-*d*]pyrimidine (13). A solution of 2,4-dioxopyrido[3,2-*d*]pyrimidine[23] (0.82 g, 5 mmol) in 75 mL of 0.1 N HCl was hydrogenated in the presence of 50 mg of Adams catalyst as described for **5**. The catalyst was removed by filtration through Celite, and the filtrate was evaporated to dryness. The residue was dissolved in water, neutralized with 5% $NaHCO_3$ solution, and kept in the refrigerator. The precipitated solid was filtered, washed with cold water, and dried over P_2O_5 to give 0.6 g (70%) of **12**: mp >280 °C; MS, *m/e* 167

(17) ^{13}C NMR chemical shifts for 6-methyl-5-(methylamino)uracil are: 162.57 (C-4), 149.77 (C-2), 120.80 (C-5), 139.07 (C-6), 35.45 (N-CH_3), 14.43 (6-CH_3) ppm. 6-Methyl-5-(methylamino)uracil was prepared according to the procedure of Wheeler, H. L.; Jamieson, G. S. *Am. Chem. J.* **1904**, *82*, 1955.

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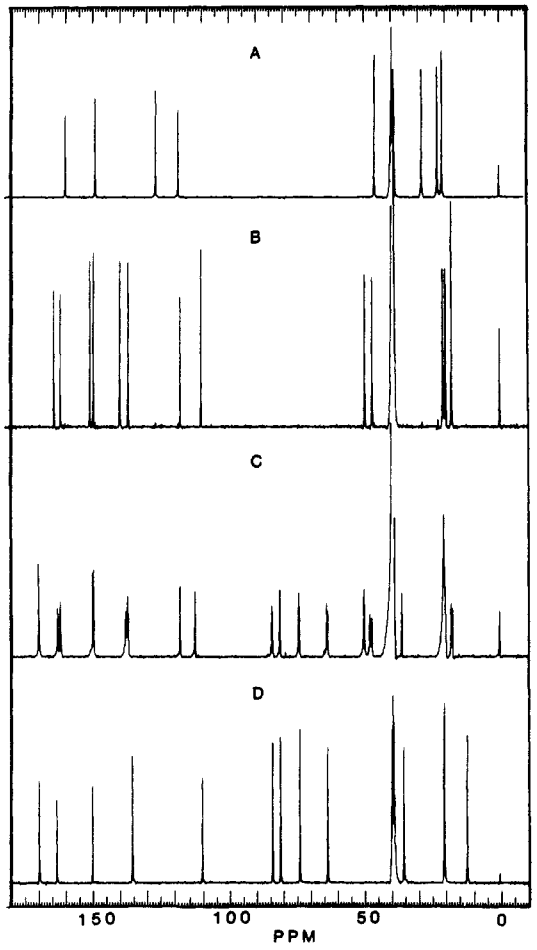

Figure 2. 67.8-MHz C-13 NMR spectra of **5** (A), **11** (B), **7** (C), and diacetylthymidine (D) in Me_2SO-d_6.

(M⁺); UV λ_{max} (pH 1) 260 nm (ϵ_{max} 14 000); UV λ_{max} (pH 7) 303 nm (ϵ_{max} 8100); UV λ_{max} (pH 13) 300 nm (9000); ^{1}H NMR δ 1.76 (m, 2, 7-CH_2), 2.33 (t, 2, 8-CH_2), 2.98 (t, 2, 6- CH_2), 4.11 (br, 1, 5-NH), 10.25 (s, 1, CONH), 10.93 (s, 1, CONH).

Anal. Calcd for $C_7H_9N_3O_2$: C, 50.29; H, 5.42, N, 25.13. Found: C, 50.39; H, 5.45; N, 25.17.

2,4-Dioxo-6-methyl-5-(uracil-5-ylmethyl)-5,6,7,8-tetrahydropyrido[3,2-*d*]pyrimidine (11). A solution of 0.41 g (2.5 mmol) of **5** in 20 mL of anhydrous dimethylformamide was stirred with 5-(chloromethyl)uracil (0.40 g, 2.5 mmol) for 8 h. The solution was evaporated to dryness in vacuo, dissolved in 15–20 mL of water, neutralized with 5% $NaHCO_3$ solution, and allowed to stand at room temperature. A yellow solid crystallized after several hours to give 0.52 g (71%) of **11**: mp >280 °C; MS, *m/e* 305 (M⁺); UV λ_{max} (pH 1) 263 nm (ϵ_{max} 13 000); UV λ_{max} (pH 7) 265 nm (ϵ_{max} 7000), 306 (5000); UV λ_{max} (pH 13) 290 nm (ϵ_{max} 10 000).

Anal. Calcd for $C_{13}H_{15}N_5O_4$·0.5H_2O: C, 49.67; H, 5.13; N, 22.28. Found: C, 49.64; H, 5.10; N, 22.24.

General Procedure for the Alkylation of 5, 6, and 13 with 5-(Bromomethyl)-3′,5′-di-*O*-acetyl-2′-deoxyuridine. A solution of tetrahydropyridopyrimidine derivative in anhydrous dimethylformamide (distilled over P_2O_5 and stored over molecular sieves) (10 mL of DMF/mmol of base) was stirred with 1.1 molar equiv of 5-(bromomethyl)-3′,5′-di-*O*-acetyl-2′-deoxyuridine at room temperature in the presence of an equimolar amount of solid $NaHCO_3$ (dried over refluxing toluene in vacuo over P_2O_5.) The reaction was followed by thin-layer chromatography (silica gel; Merck F-254; $CHCl_3$/CH_3OH, 85.15). The solvent was removed in vacuo when chromatography indicated the absence of starting tetrahydropyridopyrimidine derivative (which took approximately 3–5 h). The residue was suspended in water, extracted with chloroform, dried over anhydrous Na_2SO_4, and concentrated to a small volume. The solution was poured onto a silica gel column (60–400 mesh), and eluted with $CHCl_3$/CH_3OH (98:2) to remove the unreacted bromo derivative. Final elution with $CHCl_3$/CH_3OH (97:3) in the case of **5** and **12** and $CHCl_3$/CH_3OH (94:6) in the case of **6** gave the protected nucleosides.

3′,5′-Di-*O*-acetyl-5-[(2,4-dioxo-6-methyl-5,6,7,8-tetrahydropyrido[3,2-*d*]pyrimidin-5-yl)methyl]-2′-deoxyuridine (7): yield 66%; mp 158–160 °C; MS, *m/e* 505 (M⁺); UV λ_{max} (pH 1) 265 nm (ϵ_{max} 16 000); UV λ_{max} (pH 7) 264 nm (ϵ_{max} 8800), 300 (3700); UV λ_{max} (pH 11) 265 nm (ϵ_{max} 9000), 300 (5000).

Anal. Calcd for $C_{22}H_{27}N_5O_9$·0.5H_2O: C, 51.36; H, 5.48; N, 13.61. Found: C, 51.50; H, 5.66; N, 13.37.

3′,5′-Di-*O*-acetyl-5-[(2-amino-6-methyl-4-oxo-5,6,7,8-tetrahydropyrido[3,2-*d*]pyrimidin-5-yl)methyl]-2′-deoxyuridine (8): yield 63%; mp 164–167 °C; MS, *m/e* 504 (M⁺); UV λ_{max} (pH 1) 265 nm (ϵ_{max} 14 500); UV λ_{max} (pH 7) 260 nm (ϵ_{max} 12 500), 305 (4500); UV λ_{max} (pH 13) 2 45 nm (ϵ_{max} 11 000), 305 (4600).

Anal. Calcd for $C_{22}H_{28}N_6O_8$·0.5H_2O: C, 51.45; H, 5.69, N, 16.36. Found: C, 51.24; H, 5.95; N, 16.10.

3′,5′-Di-*O*-acetyl-5-[(2,4-dioxo-5,6,7,8-tetrahydropyrido-[3,2-*d*]pyrimidin-5-yl)methyl]-2′-deoxyuridine (12): yield 64%; mp 142–144 °C; MS, *m/e* 491 (M⁺); UV λ_{max} (pH 1) 264 nm (ϵ_{max} 17 000); UV λ_{max} (pH 7) 264 nm (ϵ_{max} 9000), 301 (4000); UV λ_{max} (pH 13) 265 nm (ϵ_{max} 9000), 300 (5000); ^{1}H NMR δ 11.43 (s, 1, CONH), 10.89 (s, 1, CONH), 10.45 (s, 1, CONH), 7.75 (s, 1, C-6′ H), 6.19 (t, J = 6.2 Hz, 1, 1″-H), 5.18 (m, 1, 3″-H), 4.20 (m, 3, 4″-H and 5″-OCH_2), 3.68 (s, 2, 5′-CH_2), 2.78 (m, 2, 6-CH_2), 2.32 (m, 4, 2″-CH_2 and 8-CH_2), 2.07 (s, 3, $COCH_3$), 2.00 (s, 3, $COCH_3$), 1.64 (m, 2, 7-CH_2).

Anal. Calcd for $C_{21}H_{25}N_5O_9$·1.0H_2O: C, 49.52; H, 5.34; N, 13.71. Found: C, 49.86, H, 5.34; N, 13.26.

General Procedure of Deacetylation of 7 and 8. One millimole of the protected nucleoside (**7** and **8**) in 50 mL of ethanolic ammonia (saturated at 0 °C) was stirred at room temperature for 24 h. The solvent was removed in vauco. The residue was redissolved in ethanol and was evaporated to dryness. This process was repeated until the presence of ammonia was no longer evident. The residue was stirred with warm acetone to remove the acetamide and filtered. The solid was washed with acetone and dried over P_2O_5.

5-[(2,4-Dioxo-6-methyl-5,6,7,8-tetrahydropyrido[3,2-*d*]pyrimidin-5-yl)methyl]-2′-deoxyuridine (9): yield 71%; MS, *m/e* 781 [(Me_4Si)$_5$ derivative] (M⁺); UV λ_{max} (pH 1) 265 nm (ϵ_{max} 16 000); UV λ_{max} (pH 7) 267 nm (ϵ_{max} 13 500), 303 (5500); UV λ_{max} (pH 13) 267 nm (ϵ_{max} 13 000), 303 (5500).

For analytical purposes, an ethanolic solution of the nucleoside was treated with etheral hydrogen chloride to give the hydrochloride salt, mp 207–211 °C.

Anal. Calcd for $C_{18}H_{23}N_5O_7$·1.0HCl·2.0H_2O: C, 43.23; H, 5.92; N, 13.97. Found: C, 43.77; H, 5.51; N, 14.17.

The proton and carbon NMR reported in Tables I and II, respectively, were recorded on the free nucleoside.

5-[(2-Amino-6-methyl-4-oxo-5,6,7,8-tetrahydropyrido[3,2-*d*]pyrimidin-5-yl)methyl]-2′-deoxyuridine (10): yield 60%; mp 186–188 °C; MS, *m/e* 780 [(Me_4Si)$_5$ derivative) (M⁺); UV λ_{max} (pH 1) 267 nm (ϵ_{max} 15 000); UV λ_{max} (pH 7) 260 nm (ϵ_{max} 13 500), 305 (4500); UV λ_{max} (pH 13) 255 nm (ϵ_{max} 11 000), 305 (4500).

Anal. Calcd for $C_{18}H_{24}N_6O_6$·1.0H_2O: C, 49.31; H, 5.97; N, 19.16. Found: C, 49.50; H, 6.18; N, 18.98.

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Registry No. **3**, 2499-96-9; **4**, 78711-30-5; **5**, 82933-82-2; **6**, 78711-33-8; **7**, 82933-83-3; **8**, 82855-83-2; **9**, 82933-84-4; **10**, 82855-84-3; **11**, 82933-85-5; **12**, 82951-12-0; **13**, 82933-86-6; 2,4-diamino-6-methylpyrido[3,2-*d*]pyrimidine, 82933-87-7; 2,4-dioxopyrido[3,2-*d*]-pyrimidine, 37538-68-4; 5-(chloromethyl)uracil, 3590-48-5; 5-(bromomethyl)-3′,5′-di-*O*-acetyl-2′-deoxyuridine, 58589-18-7; thymidylate synthetase, 9031-61-2.

Supplementary Material Available: ^{1}H NMR spectra of compounds **7**, **8**, **11**, and **12** and ^{13}C NMR spectra of **11** and **12** (6 pages). Ordering information is given on any current masthead page.