Pyridopyrimidines. 9. An Unusual Rearrangement in the 8-Substituted Pyrido[2,3-*d*]pyrimidine Series. Application of the Selective Nuclear Overhauser Effect to Unambiguous Proton Chemical Shift Assignment

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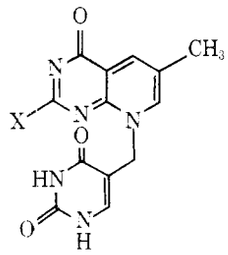
The synthesis of a series of 8-substituted pyrido[2,3-*d*]pyrimidines, the prototype of which was 5-(6-methyl-2-methylthio-4-oxopyrido[2,3-*d*]pyrimidine-8-yl)methyluracil (**6**), is reported. A facile rearrangement of the uracilmethyl moiety from N-8 to N-3 of the pyridopyrimidine was observed. The sites of alkylation on the various pyridopyrimidines were established in part by ^{1}H NMR. Unequivocal assignment of the various proton signals was made by the first application of a new ^{13}C NMR technique, selective nuclear Overhauser effect (SNOE), to so complex a system of spins. The mechanism of the rearrangement was determined to be inter- rather than intramolecular by crossover experiments in which the rearrangement of an 8-substituted pyridopyrimidine in the presence of a different pyridopyrimidine gave a mixture of both 3-substituted pyridopyrimidines. Further details of the mechanism are discussed.

As a part of a program directed toward the synthesis of "transition state" inhibitors of thymidylate synthetase, the synthesis of some model 5-(pyrido[2,3-*d*]pyrimidin-8-yl)-methyluracil derivatives was undertaken. These models (**1**)



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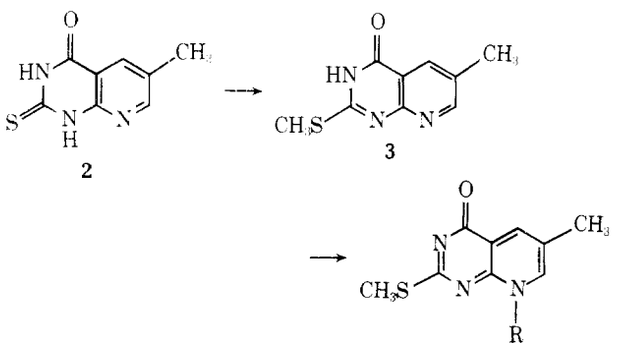
contain certain structural elements which have been implicated[2,3] in the one-carbon transfer from a reduced folate to 2′-deoxyuridylic acid in the synthesis of thymidylic acid.

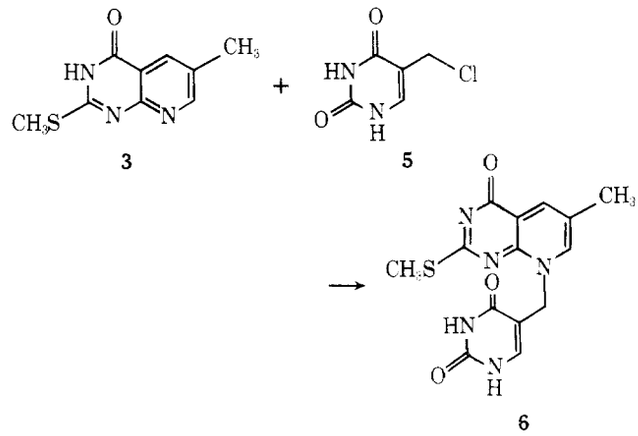
It was established from previous studies that the site most readily alkylated in pyrido[2,3-*d*]pyrimidines containing an aromatic pyridine ring in neutral aprotic solvent was N-8.[4] Alkylation of 6-methyl-2-methylthio-4-oxopyrido[2,3-*d*]-pyrimidine (**3**) (prepared by methylation of the corresponding 2-thione derivative **2**[5]) with alkyl halide (e.g., methyl iodide, 1-bromo-3-methyl-2-butene) in anhydrous dimethylformamide gave the 8-alkylpyrido[2,3-*d*]pyrimidine derivatives **4a** and **4b**. A large bathochromic shift (about 50 nm) in the UV



2 **3**

4a, R = CH_3
b, R = $(CH_3)_2C{=}CHCH_2$-

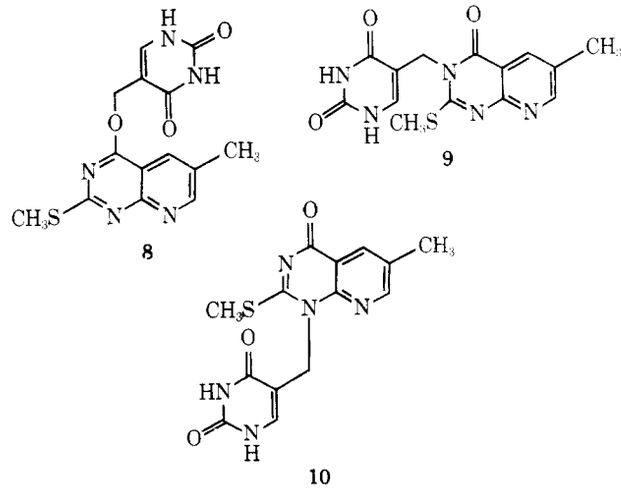
maxima of neutral and anionic species, the similarity (342 nm for **3** and 345 nm for **4**) in acidic solution, and a downfield shift of 0.30 ppm of the pyridine γ proton in the ^{1}H NMR spectrum (vide infra) confirmed the site of alkylation.[4] In a similar reaction, carried out under identical conditions, alkylation of **3** with 5-chloromethyluracil (**5**)[6] gave 5-(6-methyl-2-methylthio-4-oxopyrido[2,3-*d*]pyrimidin-8-yl)methyluracil (**6**). The



3 **5** **6**

site of alkylation was confirmed by the similarity of the UV spectrum and the proton chemical shifts with those of **4**.

An attempted crystallization of **6** from dimethylformamide–water gave a colorless compound whose UV spectrum was completely different from that of **6**. Elemental analysis indicated that the new compound was a structural isomer of **6**. ^{1}H NMR spectral data revealed that the aromatization of the pyridine moiety had occurred, as shown by the similarity of α- and γ-pyridine proton chemical shifts with those of **3**. The above facts suggested that a rearrangement occurred during crystallization. Three possible structures (**8**–**10**) can be written



8 **9**

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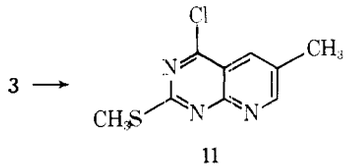
for the new compound. Structure **8** was eliminated from consideration by comparison of the UV spectrum of the product with that of 4-methoxy-2-methylmercapto-6-methylpyrido[2,3-*d*]pyrimidine (**12**), prepared from **3** by



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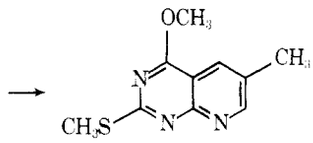

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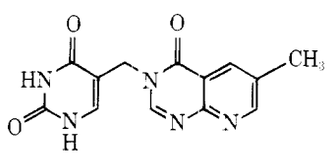
treatment with phosphorus oxychloride followed by a nucleophilic displacement of the 4-chlorine in **11** by methoxide. Though these spectra eliminated structure **8**, it was not possible to differentiate between structures **9** and **10** on the basis of UV spectral evidence.

Raney nickel dethiation of the rearrangement product **8** (or **10**) gave a new compound **13**, with a molecular ion at *m/e* 285. The absence of the 2-CH_3S group was evident in the ^1H NMR spectrum by the disappearance of $-SCH_3$ at δ 2.66 and the appearance of a singlet at δ 8.55. The UV spectrum of this compound (at pH 1, 7, and 11) closely resembled that of 3-methyl-4-oxopyrido[2,3-*d*]pyrimidine (**14**), the structure of which had been established by an unambiguous synthetic procedure.[4] On the basis of these data the structure of **13** was



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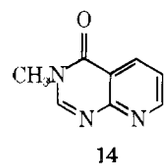

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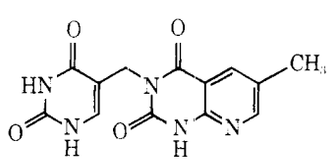
established to be 5-(6-methyl-4-oxopyrido[2,3-*d*]pyrimidin-8-yl)methyluracil. Therefore the structure of the rearrangement product should be **9** rather than **10**.

To confirm the structure **9** the compound was hydrolyzed in 18% HCl to **15**. The UV spectrum closely resembled that of **16**[4] in acidic, neutral, and basic solutions.



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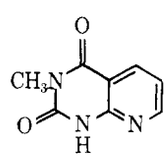

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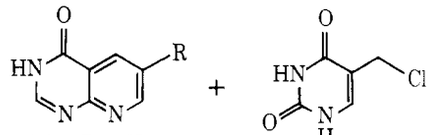
Alkylation of 4-oxopyrido[2,3-*d*]pyrimidine (**17a**) and its 6-methyl derivative **17b** with 5-chloromethyluracil (**5**) gave the 5-(4-oxopyrido[2,3-*d*]pyrimidin-8-yl)methyluracils (**18**).

These compounds also underwent rearrangement to give **19** and **13**; the latter was identical with the one (**13**) obtained by Raney nickel dethiation of **9**. The above rearrangements were readily carried out by refluxing the compounds in dimethylacetamide for 5–10 min, or in the case of **18**, simply by dissolution in Me_2SO. It is noteworthy that the presence of a methylthio group at C-2 of the pyridopyrimidine in **6** afforded some stabilization; it was necessary to heat Me_2SO solutions of **6** for several minutes at ~50 °C to effect complete rearrangement.

In order to assess the importance of an ionizable proton on the uracil moiety in promoting the rearrangement, a 1,3-



17a, R = H
b, R = CH_3

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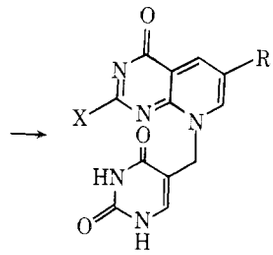
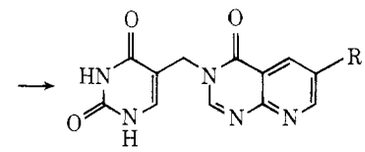
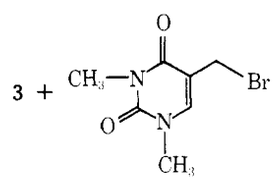


18a, R = H
b, R = CH_3



19, R = H
13, R = CH_3

dimethyluracil derivative was prepared. The alkylating agent selected was 5-bromomethyl-1,3-dimethyluracil (**21**). Methylation of 5-benzyloxymethyluracil[7] gave the 1,3-dimethyl derivative, which was converted to 5-bromomethyl-1,3-dimethyluracil (**21**) by treatment with HBr in dioxane. The reaction of **3** with the 5-bromomethylpyrimidine **21** gave the expected 1,3-dimethyl-5-(6-methyl-2-methylthio-4-oxopyrido[2,3-*d*]pyrimidin-8-yl)methyluracil (**22**). Both **22** and the



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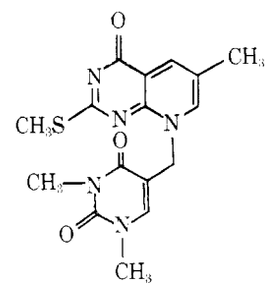

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8-(3-methyl-2-butenyl) derivative **4b** were stable in DMF at reflux (2 days) and could be recrystallized from ethanol.

Nuclear Magnetic Resonance Studies

Proton magnetic resonance spectra have been used in the assignment of the site of alkylation of the pyrido[2,3-*d*]pyrimidine nucleus in the following manner. Alkylation at the pyridine nitrogen of an aromatic pyridine,[8] pyrido[2,3-*d*]-pyrimidine,[4] or other fused-ring system containing a pyridine ring[9] leads to downfield shifts of the pyridine γ proton and either upfield or downfield shifts, generally of smaller magnitude, of the α proton (the "α effect"[10]). Alkylation of a lactam system (for example N-1 or N-3 of **17**), on the other hand, leads to a pronounced downfield shift of an adjacent proton signal with almost no effect on other proton chemical shifts.[4,11] This technique was used in an earlier study in his series to assign the site of methylation and ribosylation of 4-oxo- and 2,4-dioxopyrido[2,3-*d*]pyrimidines.[4] It is obvious that the success of the technique relies on accurate assignments of the pyridine α and γ signals (pyrido[2,3-*d*]pyrimidine H-7 and H-5, respectively). In the previous study,[4] the more downfield of the two proton signals was assigned to H-7 in accord with numerous studies on pyridines and fused pyridines.[12] The

Table I. ^{1}H NMR Chemical Shifts for a Series of Pyrido[2,3-*d*]pyrimidines

Compd	Registry no.	Chemical shift, δ[a]					
		H-2	H-5	H-6	H-7	$-CH_2-$	H-6′ [b]
3	64600-46-0		8.23		8.73		
4b	64600-47-1		8.53		8.68		
6	64600-48-2		8.53		8.67	5.26	7.70
9	64600-49-3		8.28		8.78	4.92	7.12
13	64600-50-6	8.55	8.27		8.80	4.78	7.62
15[c]	64600-51-7		8.57		8.97	5.15	7.97
17a	24410-19-3	8.40	8.53	7.57	8.98		
17b	64600-52-8	8.20	8.25		8.73		
18a[c]	64600-53-9	8.70	9.30	8.03	9.50	5.90	8.33
18b[c]	64600-54-0	8.80	9.37		9.53	5.98	8.50
19	64600-55-1	8.63	8.50	7.53	8.93	4.79	7.63
22	64600-56-2		8.55		8.72	5.40	8.07
23	21038-66-4		8.23	7.22	8.57		
24	49738-87-6		8.07		8.45		

[a] Unless otherwise noted, ^{1}H NMR spectra were recorded at 60 MHz in Me_2SO-d_6 with DSS (sodium 2,2-dimethyl-2-dimethyl-2-silapentanesulfonate) as internal reference. [b] H-6′ refers to the proton at position 6 of the uracil moiety. [c] Trifluoroacetic acid with internal Me_4Si; rapid rearrangement occurred in Me_2So-d_6.

Table II. ^{13}C NMR Chemical Shifts[a] and ^{13}C–Proton Coupling Constants[b] for a Series of Pyrido[2,3-*d*]pyrimidines at 89 °C

Compd	C-2	C-4	C-4a	C-5	C-6	C-7	C-8a
17a	149.4 (204.9, z)	162.5 (6.5, 4.0)	118.9 (7.2)	136.4 (165.5, 6.3, 2.3)	123.2 (166.7, 6.8, 1.6)	156.4 (179.8, 7.9, 3.8)	159.5 (12.0, 6.0)
23	151.2 (z)	163.1 (4.3)	111.0 (7.5, 1.3)	137.2 (166.3, 6.6, 2.2)	119.7 (168.1, 7.5, 1.3)	155.3 (180.6, 5.8, 3.7)	153.4 (9.9, 6.0)
24	151.1 (z)	163.2 (3.8)	110.4 (z)	136.8 (164.5, m)	129.0 (z)	155.7 (178.0, m)	151.4 (o)
3	160.5 (o)	162.8 (3.9)	115.4 (z)	135.7 (164.1, m)	131.7 (o)	156.9 (177.2, m)	159.6 (10.8, 6.0)

[a] Referenced to internal dioxane at 67.4 ppm with an accuracy of ±0.2 ppm. The chemical shift of the 6-methyl carbon in **22** and **3** is 18.0 and 18.3 ppm, respectively. The $-S^{13}CH_3$ chemical shift in **3** is 13.6 ppm. [b] ^{13}C–^{1}H coupling constants (listed in parentheses) are in units of hertz with an accuracy of ±0.5 Hz. The following abbreviations are used: (m) complicated multiplet from long-range coupling to methyl and other ring protons; (o) long-range couplings not analyzable due to overlapping structure; (z) no long-range couplings to within experimental error.

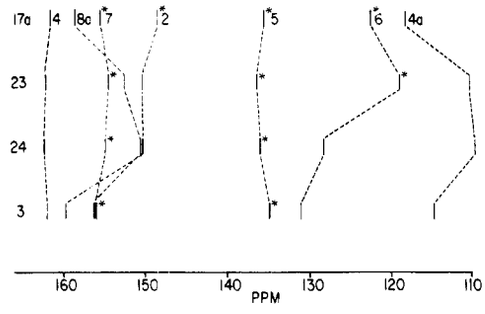
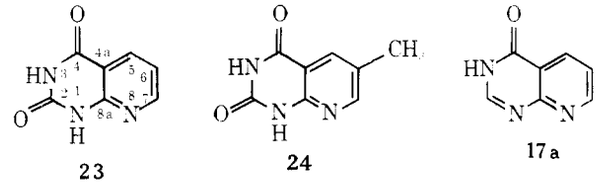


Figure 1. ^{13}C NMR chemical shift correlation diagram. Methine carbon resonances are indicated by asterisks. Chemical shifts are referenced to Me_4Si.

greater downfield shift of the higher field (H-5) proton signal upon alkylation was used to assign the site as N-8.

Subsequently a study on the synthesis, ^{1}H NMR, and ^{13}C NMR of a series of closely related pyrido[2,3-*d*]pyrimidines appeared in which these proton chemical shift assignments were reversed[5] without, however, reference to the earlier publication.[4] This report[5] clearly necessitated a reexamination of the original assignments and a reevaluation of the ^{1}H NMR technique described above for the assignment of the site of alkylation. In order to resolve these questions,[13] the following set of pyridopyrimidines was selected for further study: 4-oxo- (**17a**),[14] 2,4-dioxo- (**23**), 2,4-dioxo-6-methyl- (**24**),[5,15] and 6-methyl-2-methylthio-4-oxopyrido[2,3-*d*]pyrimidine (**3**).



A detailed analysis of the ^{1}H NMR and ^{13}C NMR spectra of compounds **23, 24, 3,** and **17a** was undertaken. The proton spectra (Table I) were unambiguous except for the assignment of H-7 and H-5; the assignments for the other nonexchangeable protons for **17a, 23,** and **24** were reported earlier.[4,5] It remained then to remove the ambiguity in the assignment of H-5 and H-7. The procedure to be followed was to obtain accurate ^{13}C NMR assignments for the compounds under study, then to observe the effect of selective saturation[16] of the ^{13}C satellites in the ^{1}H NMR spectrum on the ^{13}C NMR signals.

The ^{13}C NMR data are presented in Table II, and the correlation diagram for the ^{13}C chemical shifts is shown as Figure 1. The chemical shift assignments were based on the following analysis of spectroscopic data. The fully proton-coupled spectrum of each compound was compared with the proton-decoupled spectrum. Carbon resonances associated with di-

rectly bonded protons were immediately identified by the large (164–205 Hz) splitting patterns. These methine ^{13}C chemical shifts are indicated by an asterisk in Figure 1, and the values of $^1J_{^{13}C-^1H}$ are listed in parentheses in Table II. The long-range $J_{^{13}C-^1H}$ values are also listed, although they were not identified by selective proton-decoupling experiments. Because of the limited digital resolution of the spectrometer and because only simple first-order analysis was applied to interpret the coupled spectra, couplings <1.5 Hz were not reported and the estimated error limits were correspondingly large. However, the effects of long-range coupling, both resolved and unresolved, were used to assign resonances to specific carbons. For example, the two high-field methyl resonances of **3** were differentiated by the sharpness of the 1:3:3:1 quartet centered at 13.6 ppm, which contrasted with the diffuse 1:3:3:1 quartet at 18.0 ppm. Since the methyl group at C-6 can exhibit long-range coupling to H-5 and H-7, while $-S^{13}CH_3$ has no near protons that are not in rapid exchange, the assignment is unquestionably correct, but qualitative with respect to long-range coupling constants.

The methine ^{13}C resonances were assigned by considering both the chemical shifts and the magnitude of $^1J_{^{13}C-^1H}$. It has been shown[17,18] that the carbon α to the nitrogen atom in pyridine-like aromatic systems resonates at lower field than either the β or γ carbons and that $^1J_{^{13}C_\alpha-^1H}$ is approximately 15 Hz larger than $^1J_{^{13}C_\beta-^1H}$ and $^1J_{^{13}C_\gamma-^1H}$.[17] This empirical rule identified C-7 in all four compounds studied here. C-2 in **17a** was identified by the unique magnitude of $^1J_{^{13}C-^1H}$ and the *absence of long-range couplings*. C-6 was identified in **24** and **3** by the characteristic 9–10 ppm downfield shift of one of the two remaining (C-5 and C-6) methine resonances in **23** when the proton was replaced by a methyl group.[19] By elimination, the remaining ^{13}C absorption with a proton directly bonded must have been C-5; in addition, this absorption frequency was relatively constant over this closely related series. Thus, from low to high field, these carbon resonances occurred in the order C-7, C-5, and C-6. It should be noted that the long-range coupling patterns are in total qualitative agreement with these conclusions.

The quaternary carbon assignments are included for completeness; they were based on long-range coupling patterns and conclusions drawn from the chemical shift correlation diagram. C-4a was the highest field quaternary ring carbon resonance, and was thus easily identified in **23** and **17a.** In **24** and **3** the quaternary resonances at 129.0 and 131.7 ppm were previously assigned to C-6 on the basis of the methyl substituent effect;[19] thus, by the process of elimination, the upfield resonances were C-4a. The long-range splitting patterns were entirely consistent if it were assumed that $^2J_{^{13}C-^1H}$ to H-5 was 1.3 Hz or less and $^3J_{^{13}C-^1H}$ to H-6 in **23** and **17a** was about 7.4 Hz. The furthest downfield resonance for each compound was assigned as C-4; this assignment gave a consistent $^3J_{^{13}C-^1H}$ of about 4 Hz with H-5, as well as an additional three-bond coupling constant of 6.5 Hz with H-2 in **71a.** The chemical shifts of C-4 are remarkably constant over this series of compounds. C-2 was identified by its very sharp resonances in **23, 24,** and **17a,** indicating the absence of any nearby nonexchanging protons that could provide fine structure or broadening. In **3** C-2 was assigned by the process of elimination, since it was overlapped with the fine structure of C-7. The long-range couplings of C-8a were used for conclusive identification in **23, 17a,** and **3.** In **17a** the doublet of doublets was quite broad, indicating a third unresolved $^3J_{^{13}C-^1H}$. In **24** all other resonances were identified, so C-8a was assigned by default to 151.4 ppm. The assigned order of carbon signals, from low to high field, for **24** was established as C-4, C-7, C-8a, C-2, C-5, C-6, C-4a, C-methyl. This may be contrasted with the earlier reported order[5] of C-4, C-5, C-2 = C-8a, C-7, C-4a, C-6, C-methyl. Thus, by utilizing the coupled and decoupled

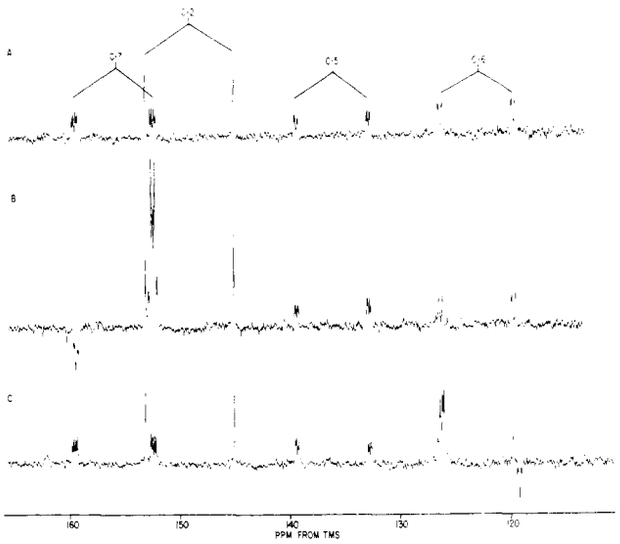


Figure 2. The proton-coupled ^{13}C NMR spectrum of 4-oxopyrido[2,3-*d*]pyrimidine (**17a**). Transients (20 000) of 2 s each and a 90° flip angle were accumulated, weighted, and Fourier transformed to give the above spectra. The quaternary carbons are saturated under these conditions, and hence not visible. (A) Proton decoupler *off* at all times, giving a reference intensity pattern. (B) Same as A except proton decoupler *on* continuously at approximately −18 dBm at 90 Hz downfield from 8.98 ppm. (C) Same as B except decoupler frequency at 83 Hz upfield from 7.57 ppm. Very small decoupling effects in B and C are evident, since it was necessary to saturate a spectral region of about 15 Hz to effect the SNOE.

^{13}C NMR spectra in conjunction with two highly reliable empirical rules, the ^{13}C NMR spectra were completely assigned with high confidence in their accuracy.

Once the carbon assignments were firmly established, it became possible to make unequivocal assignments of the H-5 and H-7 proton signals for each of these compounds. The method used was the recently described[16] selective nuclear Overhauser effect (SNOE); this represents the first practical application of this useful technique for unambiguous proton signal assignments in complex organic molecules.

The $^{13}C-^1H$ one-bond coupling constants were readily available from the fully coupled ^{13}C NMR spectrum. Thus, even though the ^{13}C satellite signals were not detectable in proton spectra under the present conditions of measurement, their absolute positions were known with certainty. The SNOE technque involves moderate power rf irradiation of a narrow spectral band corresponding to a single ^{13}C satellite in the proton spectrum while observing the effect on the ^{13}C NMR spectrum. As may be seen in Figure 2, irradiation of the lowest field ^{13}C satellite proton signal of **17a** (90 Hz downfield from 8.98 ppm) caused a dramatic intensity alteration in the multiplet at lowest field of the four protonated carbon signals, leaving the others essentially unchanged. Since that carbon signal was unequivocally identified as that of C-7, the lowest field proton signal must be attributable to H-7. Similarly, irradiation of the high-field satellite (83 Hz upfield from 7.57 ppm) caused a major alteration in the intensity of the high-field ^{13}C NMR multiplet with only minor effects on the rest of the spectrum. This finding is compatible only with the order (from low to high field) of H-7 > H-5 > H-6 and provides the first unequivocal evidence for that assignment. The SNOE technique was similarly applied to the satellites of H-5 in **17a** and of H-7 and H-5 in **3**; the resulting proton assignments are shown in Table I.

With the proton assignments firmly established, it was possible to reexamine the technique described above for establishing the site of N-alkylation. All the ^1H NMR data hitherto reported for *N*-alkylpyrido[2,3-*d*]pyrimidines were.

when correct[4,13b,20] rather than erroneous[5,21] assignments of chemical structure were made, completely consistent with the described technique; i.e., the largest deshielding effect resulting from N-8 alkylation was experienced by the H-5 (pyridine γ) proton, and alkylation of a lactam nitrogen resulted in marked deshielding of an adjacent carbon-bound proton with little effect on other protons in the molecule. The first of these approaches may be illustrated by comparing the data (Table I) for 6-methyl-2-methylthio-4-oxopyrido[2,3-*d*]pyrimidine (**3**) with its 9-(3-methyl-2-butenyl) (**4b**), 8-(uracil-5-methyl) (**6**), and 3-(uracil-5-methyl) (**9**) derivatives. The relevant protons in this case are H-5 (γ) and H-7 (α). In the spectra of both **4b** and **6**, as predicted, the H-5 signal appeared 0.30 ppm downfield from those of starting **3**, while the H-7 protons were shielded by about 0.05 ppm. In the spectrum of the 3-substituted derivative **9**, the chemical shifts closely resembled those of the parent heterocycle. The effect upon a neighboring proton of alkylation at lactam nitrogen is illustrated by comparing the spectral data for **13** and **17**; H-2 was deshielded by 0.35 ppm, whereas the signals for H-5 and H-7 were virtually unaffected.

To summarize, rigorous assignments of the proton chemical shifts for H-5, H-6, and H-7 in the pyridine ring of the pyrido[2,3-*d*]pyrimidine ring system have been carried out by means of the new technique of selective saturation of ^{13}C satellites. These assignments, in turn, confirmed the validity of the proton chemical shift approach to the determination of the site of alkylation in this ring system.

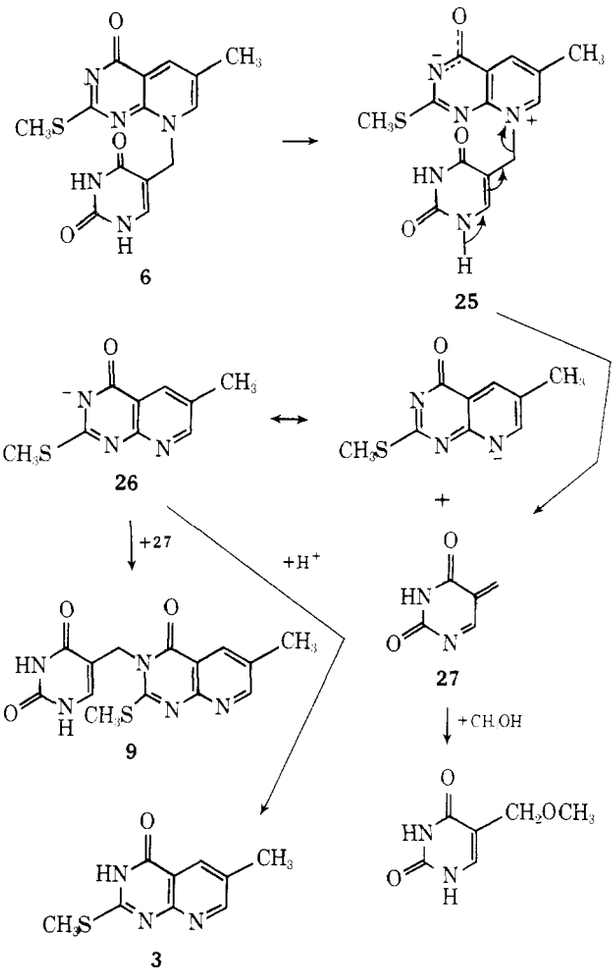
Mechanism

Careful examination of the reaction mixture in the rearrangement of **6** to **9** revealed the presence of a small amount of 6-methyl-2-methylthio-4-oxopyrido[2,3-*d*]pyrimidine. This suggested that the reaction might be intermolecular rather than intramolecular in nature. Confirmation of this hypothesis was provided by three experiments. First a solution of **6** in methanol was heated for a short while at reflux; the only products were 5-methoxymethyluracil[22] and **3**. Second, **6** was rearranged in the presence of 6-methyl-4-oxopyrido[2,3-*d*]-pyrimidine (**17b**). Complete reaction gave rise to 5-(6-methyl-4-oxopyrido[2,3-*d*]pyrimidin-3-yl)methyluracil (**13**) and its 2-methylthio derivative **9** in a 2:1 ratio. Third, the reverse experiment (transalkylation of **3** by **18b**) also gave **13** and **9**; in this case a 4:1 ratio was observed.

A mechanism which is fully consistent with the above observations is presented in Scheme I. The intense fluorescence exhibited by **6** is indicative of substantial zwitterionic character as shown in resonance structure **25**. The pyridopyrimidinyl moiety in such a molecule must be a good leaving group. It would be expected from Santi's excellent study on the methanolysis of 5-(*p*-nitrophenoxy)methyluracil[23] that loss of the proton at the pyrimidine N-1 would greatly facilitate cleavage of the C–N bond between the heterocycles; as noted above, even slightly basic conditions result in extremely rapid rearrangement. Such cleavage would lead to the neutral, electrophilic, highly reactive species **27**[23] and the anion of the pyridopyrimidine **26**. Protonation of the anion and nucleophilic attack of methanol on the exocyclic methylene group of **27** led to the products observed in methanol (vide supra).

The transalkylation reactions between **17b** and **6** and between **3** and **18b** provided unequivocal evidence for the intermolecularity of the rearrangement. Two routes by which a uracilmethyl moiety might be transferred from N-8 of one derivative to N-3 of another required consideration. The first was a rapid acid–base equilibration between, for example, anion **26** and neutral pyridopyrimidine **17b**. The resulting mixture of anions could then undergo attack on N-3 of either

Scheme I



molecule by reactive intermediate **27**. The alternative route would be alkylation at N-8 of **17b** by **6** (or **3** by **18b**) followed by rearrangement of each 8-alkyl derivative as shown in Scheme I. Either of these pathways could account for the observed differences in product ratios; such factors as rate of dissociation of **6** or **17b** to anion and **27**, rate and site of alkylation, rate of proton transfer from neutral species to anions, and steric effects of the methylthio group vs. the proton at C-2 would make a detailed kinetic analysis a formidable problem indeed. However, the differing stabilities toward rearrangement of **6** and **18b** permitted a choice to be made between the two routes by means of a simple ^1H NMR experiment.

A solution of 6-methyl-2-methylthio-4-oxopyrido[2,3-*d*]-pyrimidine (**3**) in Me_2SO-d_6 was treated with 5-(6-methyl-4-oxopyrido[2,3-*d*]pyrimidin-8-yl)methyluracil (**18b**). The ^1H NMR spectrum was recorded immediately after mixing and at 5-min intervals for about 0.5 h. The initial spectrum contained three peaks in the δ 4.5–5.5 region characteristic of the methylene groups. The peak locations and their assignments (made by comparison with the pure compounds **6**, **9**, **18b**, and **13**) were at δ 5.45 (**18b**), 5.33 (**6**), and 4.77 (**13**). No signal attributable to **9** was observed. During the subsequent 0.5 h the signal arising from **18b** gradually disappeared, whereas those attributable to **6** and **13** increased in intensity. Finally, the solution was briefly heated to ~50 °C. The methylene resonance of **6** disappeared and the only remaining signals, at δ 4.75 and 4.88, were those of the two rearranged products **13** and **9** in a ratio of 2.3:1. This experiment provided unequivocal evidence that the second of the two routes described above was operative; namely, that N-8 alkylation is prerequisite to formation of the rearranged product.

Experimental Section

^{1}H NMR spectra were obtained on a JEOL-60H or Varian-EM-360 spectrometer. All spectra were taken at ambient temperature using 5-mm tubes. UV spectra were run on a Cary-15 spectrophotometer. Melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected. Analyses were performed by Het-Chem-Co, Harrisonville, Mo.

^{13}C NMR data were determined on an XL-100-15 spectrometer operating in the Fourier transform mode. Chemical shifts were measured over 5000-Hz spectral width with an 8K data table (1.25 Hz/real point); coupled spectra were obtained over narrower spectral widths to increase digital resolution. The proton spin decoupler was gated *off* during data acquisition and *on* during the pulse delay to enhance the intensity of the coupled spectra. Also, 48 dB/octave audio low-pass filtering prevented aliasing of interfering signals into the coupled spectral region of interest.

All spectra were taken at 89 °C in 12-mm o.d. tubes. The concentrations varied greatly according to solubilities in Me_2SO-d_6. No special precautions were taken to dry the solvent or compounds, hence exchangeable protons did not exhibit couplings in the ^{13}C NMR spectra.

6-Methyl-2-methylthio-4-oxopyrido[2,3-*d*]pyrimidine (3). To a solution of 10.2 g (52.8 mmol) of 6-methyl-4-oxo-2-thioxopyrido[2,3-*d*]pyrimidine[5] in 110 mL of 1 N sodium hydroxide was added 5 mL (6.66 g, 52.8 mmol) of dimethyl sulfate. The solution was stirred at room temperature for 6 h. The precipitated solid was filtered and air dried. The solid was dissolved in water and made slightly acidic (pH 5–6) with 6 N acetic acid. The precipitate was filtered and dried. The original filtrate was acidified with 6 N acetic acid (pH 5–6). The precipitated solid was filtered and dried.

The combined solids were crystallized from dimethylformamide to give 7.1 g (52%) of **3**. An analytical sample was prepared by crystallization from methanol: mp 255–257 °C; MS *m/e* 207 (M^+), 192 (M − 15); UV λ_{max} (ϵ_{max}) (pH 1) 276 (17 900), 290 (15 600), 342 (14 850); (pH 7) 259 (15 500), 274 (16 700), 312.5 (70); (pH 14) 256 (25 950), 275 (11 650), 328 nm (7600). Anal ($C_9H_9N_3OS$·0.5H_2O): C, H, N.

6-Methyl-4-oxopyrido[2,3-*d*]pyrimidine (17b). To a refluxing solution of 4.32 g (20 mmol) of **3** in 100 mL of dimethylformamide was added 15 g (wet weight) of Raney Ni and the suspension was refluxed for 10 h. The mixture was filtered through Celite and the filtrate was evaporated in vacuo. The solid was crystallized from water to give 2.2 g (65%) of **17b:** mp >270 °C; MS *m/e* 161 (M^+); UV λ_{max} (ϵ_{max}) (pH 1) 266 (5800), 320 (9400); (pH 7) 261 (7500), 305 (18 200), 316 (6170); (pH 11) 275 (5000), 318 nm (8200). Anal. ($C_8H_7N_3O$·0.5H_2O): C, H, N.

6,8-Dimethyl-2-methylthio-4-oxopyrido[2,3-*d*]pyrimidine (4a). To a suspension of 1.08 g (5 mmol) of **3** in 20 mL of anhydrous dimethylformamide was added 1 mL of methyl iodide. A clear solution was obtained. The solution was stirred at room temperature for 6 h. The precipitated solid was filtered and dried. The solid was dissolved in water then made alkaline with dilute ammonium hydroxide. The precipitated solid was filtered and dried. Crystallization from dimethylformamide gave 0.8 g (70%) of **4a:** mp 270 °C; MS *m/e* 221 (M^+), 206 (M − CH_3); UV λ_{max} (ϵ_{max}) (pH 1) 278 (15 650), 298 (14 100), 345 (15 200); (pH 7) 274 (25 500), 376 (12 850); (pH 11) 274 (25 500), 376 nm (12 850). Anal. ($C_{10}H_{11}N_3OS$·0.5H_2O): C, H, N.

6-Methyl-2-methylthio-8-(3-methyl-2-butenyl)-4-oxopyrido[2,3-*d*]pyrimidine (4b). To a suspension of 0.43 g (2 mmol) of **3** in 8 mL of anhydrous dimethylformamide was added 0.3 g (2 mmol) of 1-bromo-3-methyl-2-butene. After 8 h of stirring at room temperature, the precipitated solid was filtered, dried, and dissolved in water. The solution was made alkaline with 5% bicarbonate solution. The bright yellow solid was filtered, dried, and crystallized from ethanol to give 0.36 g (67%) of 4b: mp 223–224 °C; MS *m/e* 275 (M^+); UV λ_{max} (ϵ_{max}) (pH 1) 276 (14 550), 284 (1900), 294 (13 250), 347 (1550); (pH 7) 276 (24 000), 368 (12 650); (pH 11) 276 (24 000), 368 nm (12 650). Anal. ($C_{14}H_{17}N_3OS$·0.5H_2O): C, H, N.

5-(6-Methyl-2-methylthio-4-oxopyrido[2,3-d]pyrimidin-8-yl)methyluracil (6). To a suspension of 2.16 g (10 mmol) of **3** in 50 mL of anhydrous dimethylformamide, 1.61 g (10 mmol) of 5-chloromethyluracil[6] (**5**) was added and the mixture was stirred at room temperature. A clear solution was obtained. After stirring for 6 h at room temperature, the precipitated solid was filtered, dissolved in water, and made alkaline with 5% bicarbonate solution. The bright yellow solid was filtered, washed and a small quantity of ethanol, and dried over P_2O_5 at 20 mm to give 2.54 g (71%) of **6:** mp >300 °C; MS (CI) *m/e* 332 (MH^+); UV λ_{max} (ϵ_{max}) (pH 1) 269 (19 300), 295 (13 000), 351 (1850); (pH 7) 274 (26 700), 371 (13 150); (pH 11) 275 (30 700), 370 nm (13 350). Anal. ($C_{14}H_{13}N_5O_3S$·1.5H_2O): C, H, N.

5-(4-Oxopyrido[2,3-*d*]pyrimidin-8-yl)methyluracil (18a). To a suspension of 1.47 g (10 mmol) of **17a**[14] in 50 mL of anhydrous dimethylformamide was added 1.61 g (10 mmol) of 5-chloromethyluracil (**5**) and the reaction was carried out exactly as described above for **6**. The yield of **18a** was 1.87 g (69%): mp >300 °C; MS *m/e* 271 (M^+); UV λ_{max} (ϵ_{max}) (pH 1) 261 (10 250), 327 (10 700); (pH 7) 246 (13 950), 261 (8800), 361 (9200); (pH 11) 284 (9950), 359 nm (10 000). Anal. ($C_{12}H_9N_5O_3$): C, H, N.

5-(6-Methyl-4-oxopyrido[2,3-*d*]pyrimidin-8-yl)methyluracil (18b). To a suspension of 0.85 g (5 mmol) of **17b** in 15 mL of anhydrous dimethyl sulfoxide (distilled from calcium hydride) was added 0.81 g (5 mmol) of 5-chloromethyluracil (**5**) and the mixture was stirred for 8 h at room temperature. The yellow solution was poured with stirring into 100 mL of methylene chloride and the precipitate was filtered. The highly hygroscopic solid was quickly dissolved in water. The solution was made alkaline with 5% bicarbonate solution. The precipitated solid was filtered, washed with water and a small amount of ethanol, and dried over P_2O_5 at 20 mm to give 0.84 g (57%) of **18b:** mp >300 °C; MS (CI) *m/e* 286 (MH^+); UV λ_{max} (ϵ_{max}) (pH 1) 263 (1800), 331 (9700); (pH 7) 251 (13 200), 263 (10 300), 365 (7250); (pH 11) 245 (10 300), 284 (1200), 364 nm (8700). Anal. ($C_{13}H_{11}N_5O_3$·0.5H_2O): C, H, N.

5-(6-Methyl-2-methylthio-4-oxopyrido[2,3-*d*]pyrimidin-3-yl)methyluracil (9). A suspension of 3.59 g (10 mmol) of **6** in 40 mL of dimethylacetamide was refluxed for 5 min. Charcoal was added to the hot solution and was filtered through Celite. The filtrate was cooled to room temperature and water was added to the cloud point. After standing for 2 h at room temperature the solid was filtered, washed with water, and dried to give 2.62 g (77%) of **9**. An analytical sample was prepared by crystallization from methanol: mp 258–259 °C; MS *m/e* 331; UV λ_{max} (ϵ_{max}) (pH 1) 266 (19 750), 285 (16 500), 295 (1850), 345 (13 100); (pH 7) 276 (21 550), 320 (6350); (pH 11) 283 (22 650), 321.5 nm (6050). Anal. ($C_{14}H_{13}N_5O_3S$·0.5H_2O): C, H, N.

5-(6-Methyl-4-oxopyrido]2,3-*d*]pyrimidin-3-yl)methyluracil (13). Method A. A suspension of 0.59 g (2 mmol) of **18b** in 8 mL of dimethylacetamide was heated to reflux for 5 min. A white solid precipitated even before complete dissolution of **18b** occurred. The mixture was refluxed for 2–3 min more and allowed to cool to room temperature. The precipitated solid was filtered, dried, and crystallized from dimethylformamide to give 0.40 g (71%) of **13:** mp >270 °C; MS *m/e* 285 (M^+); UV λ_{max} (ϵ_{max}) (pH 1) 264 (12 000), 327 (8600); (pH 7) 265 (15 150), 306 (5850), 317 (4200); (pH 11) 275 (13 100), 287.5 (12 800), 317 nm (4450). Anal. ($C_{13}H_{11}N_5O_3$): C, H, N.

Method B. A solution of 0.68 g (2 mmol) of **9** in 25 mL of dimethylformamide was stirred at 80 °C for 4 h with 4 g (wet weight) of Raney Ni. The solution was filtered hot through Celite and washed with hot dimethylformamide. The combined filtrate was evaporated to a small volume and cooled to give 0.17 g (31%) of **13**, mp >270 °C. This compound is identical with the one (TLC with $CHCl_3$/MeOH 85:15, CH_3CH/H_2O 80:20, MS, and ^{1}H NMR) prepared from **18b**.

5-(4-Oxopyrido[2,3-*d*]pyrimidin-3-y)methyluracil (19). A suspension of 1.35 g (5 mmol) of **18a** in 20 mL of dimethylacetamide was refluxed for 5 min. A white solid precipitated even before complete dissolution of **19** occurred. Refluxing was continued for 2–3 min more and cooled to room temperature. The precipitated solid was filtered, dried, and crystallized from dimethylformamide to give 1.06 g (76%) of **19:** mp >300 °C; MS *m/e* 271; UV λ_{max} (ϵ_{max}) (pH 1) 264 (11 550), 318 (7700); (pH 7) 264 (1150), 299 (5900), 310 (4300); (pH 11) 288 (13 400), 275 (12 250), 310 nm (5100). Anal. ($C_{12}H_9N_5O_3$): C, H, N.

5-(2,4-Dioxo-6-methyl-4-oxopyrido[2,3-*d*]pyrimidin-3-yl)-methyluracil (15). A solution of 0.68 g (2 mmol) of **9** in 20 mL of 18% HCl was refluxed for 8 h. The solution was evaporated to dryness. The white solid was repeatedly evaporated with water to remove traces of acid. The residue was recrystallized from dimethylformamide to give 0.68 g (63%) of **15:** mp >300 °C; MS *m/e* 301 (M^+); UV λ_{max} (ϵ_{max}) (pH 1) 247 (11 800), 262.5 (8000), 315 (6550); (pH 7) 247 (11 650), 262.5 (8000), 315 (6150); (pH 11) 271 (1400), 345 nm (4400). Anal. ($C_{13}H_{11}N_5O_4$·0.5H_2O): C, H, N.

Methanolysis of 5-(6-methyl-2-methylthio-4-oxopyridopyrimidin-8-yl)methyluracil (6). A suspension of 0.17 g (0.5 mmol) of **6** in 20 mL of methanol was refluxed for 6 h. Thin layer chromatography ($CHCl_3$/MeOH 90:10) indicated the presence of two compounds. The mixture was separated on a silica gel column using $CHCl_3$/MeOH (90:10) mixture as the eluent to give 80 mg of 6-methyl-2-methylthio-4-oxopyrido[2,3-*d*]pyrimidine (**3**) and 65 mg of 5-methoxymethyluracil.[22] The above compounds were identified by comparing their ^{1}H NMR, MS, and TLC with those of the authentic material.

Transalkylation of 6-Methyl-4-oxopyrido[2,3-*d*]pyrimidine

17b with 5-(6-Methyl-2-methylthiopyrido[2,3-*d*]pyrimidin-8-yl)methyluracil (6). A mixture of 73.5 mg (0.5 mmol) of **17b** and 170 mg of **6** were heated in 3 mL of dimethylacetamide. A clear solution was obtained. The solution was boiled for 2–3 min. The solid obtained on cooling (70 mg) was filtered and dried. The compound **13** was identical with the one obtained by the rearrangement of **18b** in dimethylacetamide and Raney Ni dethiation of **9** (based on TLC, ^{1}H NMR, and MS). The filtrate was found to contain a small amount of **13** along with 6-methyl-4-oxopyrido[2,3-*d*]pyrimidine (**17b**), 6-methyl-2-methylthio-4-oxopyrido[2,3-*d*]pyrimidine (**3**), and 5-(6-methyl-2-methylthio-4-oxopyrido[2,3-*d*]pyrimidin-3-yl)methyluracil (**9**) based on thin layer chromatography ($CHCl_3$/MeOH 90:10).

The above experiment was repeated and the solvent was removed under vacuum. A ^{1}H NMR analysis of the mixture showed that the ratio of **13** to **9** was 2:1 (based on the ratio of 5-CH_2 protons).

In a similar experiment, when the transalkylation of **3** with **18a** was carried out, the ratio of **13** to **9** was found to be 4:1.

5-Benzyloxymethyl-1,3-dimethyluracil (20). To a solution of 2.32 g (10 mmol) of 5-benzyloxymethyluracil[7] in 20 mL of anhydrous dimethylformamide was added 0.88 g of sodium hydride (55% dispersion in oil). After the hydrogen evolution ceased, 1.5 mL of methyl iodide was added. After stirring for 5–6 h at room temperature, the solution was carefully poured into 50 mL of water and extracted with petroleum ether. The aqueous layer was evaporated to dryness in vacuo. Trituration of the residue with water gave a solid which was filtered, dried, and crystallized from petroleum ether (30–60 °C) to give 1.58 g (61%) of **20:** mp 88–89 °C; ^{1}H NMR ($CDCl_3$) δ 3.31 (s, 3, NCH_3), 3.36 (s, 3, NCH_3), 4.3 (d, J = 2 Hz, 2, CH_2), 4.6 (s, 2, CH_2), 7.21 (d, J = 2 Hz, 1, H-6), 7.33 (s, 5, C_6H_5); MS *m/e* 169 ($M^+ - C_6H_5 - CH_2$). Anal. ($C_{14}H_{16}N_2O_3$): C, H, N.

5-Bromomethyl-1,3-dimethyluracil (21). To 0.78 g (3 mmol) of **20,** 6 mL of 9% HBr in anhydrous dioxane was added. A clear solution was obtained. After 4 h of stirring, the mixture was evaporated to dryness. The residue was triturated with anhydrous ether. The precipitated solid was filtered, washed with a small quantity of ether, and air dried. Crystallization from petroleum ether (30–60 °C) gave 0.54 g (78%) of **21:** mp 165–166 °C; ^{1}H NMR ($CDCl_3$) δ 3.4 (s, 3, NCH_3), 3.46 (s, 3, NCH_3), 4.33 (s, 2, CH_2), 7.47 (s, 1, H-6); MS (CI) *m/e* 233 (MH^+). Anal. ($C_7H_9N_2O_2Br$): C, H, N.

1,3-Dimethyl-5-(6-methyl-2-methylthio-4-oxopyrido[2,3-*d*]-pyrimidin-8-yl)methyluracil (22). To a suspension of 0.43 g (2 mmol) of **3** in 5 mL of anhydrous dimethylformamide was added 0.47 g (2 mmol) of 5-bromomethyl-1,3-dimethyluracil (**21**). A clear solution was obtained in a few mintues. A white solid precipitated after 1 h. The mixture was stirred for 4 h more, filtered, and air dried. The solid was dissolved in water. The solution was made alkaline with 5% bicarbonate solution. The precipitated solid was filtered, washed with water, and dried. Crystallization from ethanol gave bright yellow crystals: mp 246–247.5 °C; MS *m/e* 259 (M^+); UV λ_{max} (ϵ_{max}) (pH 1) 266 (8450), 271 (22 250), 290 (13 300), 350 (15 350); (pH 7) 266 (8450), 274 (29 000), 366 (12 900); (pH 11) 266 (7900), 274 (29 800), 366 (13 400). Anal. ($C_{16}H_{17}N_5O_3S$·0.5H_2O): C, H, N.

4-Chloro-2-methylthio-6-methylpyrido[2,3-*d*]pyrimidine (11). A suspension of 2-methylthio-6-methyl-4-oxopyrido[2,3-*d*]pyrimidine (**3**) (2.16 g, 10 mmol) was refluxed with 25 mL of phosphorus oxychloride for 12 h. The dark brown solution was evaporated under reduced pressure to a small volume. The residue was treated with crushed ice and extracted with methylene chloride. The combined extracts were washed with ice cold water and the organic layer was dried over Na_2SO_4. Evaporation of the solvent gave a brown solid. The solid was refluxed with 600 mL of petroleum ether (30–60 °C), the insoluble portion was removed by filtration, and the filtrate was concentrated to about 100 mL and cooled to give 0.84 g (37%) of **11:** mp 126–127 °C; ^{1}H NMR ($CDCl_3$) δ 2.6 (s, 3, CH_3), 2.73 (s, 3, SCH_3), 8.23 (m, 1, H-5), 9.23 (d, 1, H-7); MS (CI) *m/e* 226 (MH^+); UV λ_{max} (ϵ_{max}) (pH 1) 246 (16 250), 275 (19 400), 375 (8000); (pH 7) 243 (22 300), 271 (20 250), 354 (6350); (pH 11) 235 (15 350), 267 (20 400), 345.5 (8700). Anal. ($C_9H_8N_3ClS$): C, H, N.

4-Methoxy-6-methyl-2-methylthiopyrido[2,3-*d*]pyrimidine (12). To a solution of 70 mg (3 mmol) of Na dissolved in 10 mL of methanol was added 670 mg (3 mmol) of **11** and the solution was stirred at room temperature for 4 h. The solution was evaporated in vacuo and the residue was triturated with water. The precipitated solid was filtered and air dried. The solid was crystallized from petroleum ether (30–60 °C) to give 0.46 g (67%) of **12:** ^{1}H NMR (Me_2SO-d_6) δ 2.5 (s, 3, CH_3), 2.68 (s, 3, SCH_3), 4.1 (s, 3, OCH_3), 8.15 (m, 1, H-5), 8.88 (d, 1, H-7); MS (CI) *m/e* 222 (MH^+); UV λ_{max} (ϵ_{max}) (pH 1) 259 (21 350), 355 (11 650); (pH 7) 241.5 (17 000), 266 (18 200), 329.5 (7500); (pH 11) 241.5 (16 500), 266 (18 000), 329.3 nm (7500). Anal. ($C_{10}H_{11}N_3OS$·0.5H_2O): C, H, N.

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Registry No.—**2,** 49738-95-6; **4a,** 64600-57-3; **5,** 3590-48-5; **11,** 64600-58-4; **2,** 64600-59-5; **20,** 64600-60-8; **21,** 64600-61-9; 1-bromo-3-methyl-2-butene, 870-63-3; 5-benzyloxymethyluracil, 7295-02-5.

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