Pyrido[2,3-*d*]pyrimidines. III. Synthesis of Some 8-(β-D-Ribofuranosyl)pyrido[2,3-*d*]pyrimidines Structurally Related to the Antibiotic Sangivamycin[1]

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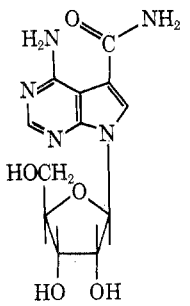
The chemical synthesis of a series of 6-carbethoxy- and 6-carboxamido-5-oxopyrido[2,3-*d*]pyrimidines *via* the requisite diethyl (6-pyrimidinyl)aminomethylenemalonates is described. Certain of these pyrido[2,3-*d*]pyrimidines are converted into 8-(β-D-ribofuranosyl) derivatives, which may be regarded as analogs of the antibiotic sangivamycin. A unique H–N–H geminal coupling in several 4-amino-5-oxo- and 4-amino-6-carboxamido-5-oxopyrido[2,3-*d*]pyrimidine derivatives is described.

The pyrrolo[2,3-*d*]pyrimidine nucleoside antibiotic sangivamycin (**1**) has been found to possess potent antitumor activity.[2,3] The pyrido[2,3-*d*]pyrimidine ring system may be regarded as a simple homolog of the pyrrolo[2,3-*d*]pyrimidine system. This consideration suggested the synthesis of pyrido[2,3-*d*]pyrimidine nucleoside analogs of sangivamycin (for example, **2**).



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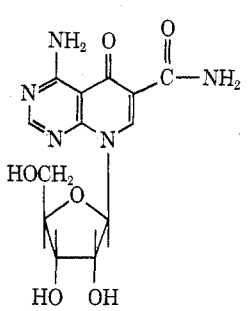

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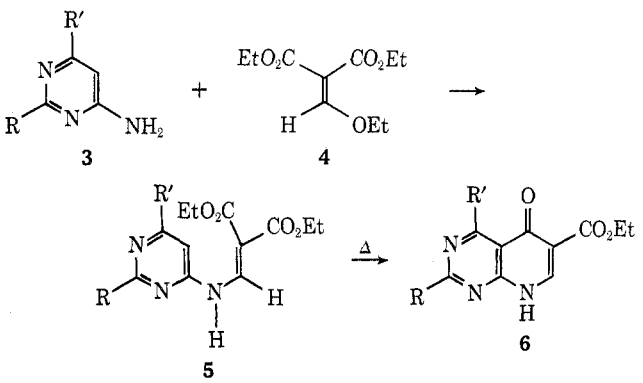
The synthesis and chemistry of the requisite pyrido[2,3-*d*]pyrimidine bases and nucleosides form the basis of this report.

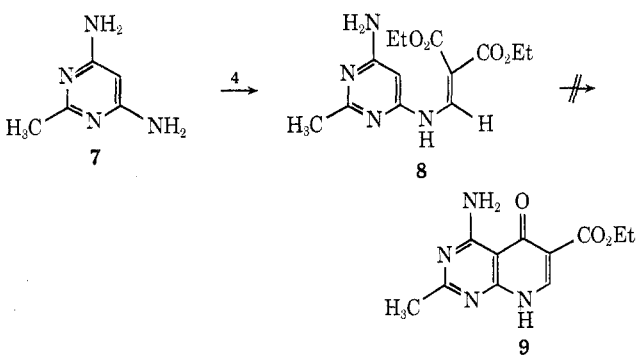
Results and Discussion

Pyrido[2,3-*d*]pyrimidine Bases.—A fundamental structural requirement for the synthesis of pyrido[2,3-*d*]pyrimidine analogs of **1** is the presence of a carboxamide function at position 6. Of the many routes available to the synthesis of the pyrido[2,3-*d*]pyrimidine system,[4] the Gould–Jacobs reaction is the method of choice for introduction of the desired functionality at C-6.[5] This reaction consists of the condensation of an appropriately substituted 6-aminopyrimidine (**3**) with diethyl ethoxymethylenemalonate (**4**) followed by thermal cyclization to the 5-oxo-6-carbethoxypyrido[2,3-*d*]pyrimidine (**6**).[6]

It has been suggested[6b] that an electron-releasing group in the pyrimidine moiety is essential to effect cyclization of the intermediate (6-pyrimidinyl)aminomethylenemalonate diester (**5**). It is, however, noteworthy that, while 4,6-diamino-2-methylpyrimidine (**7**) was condensed readily with **4** to give the intermediate **8**, repeated attempts to cyclize **8** to **9** were unsuccessful.[6]

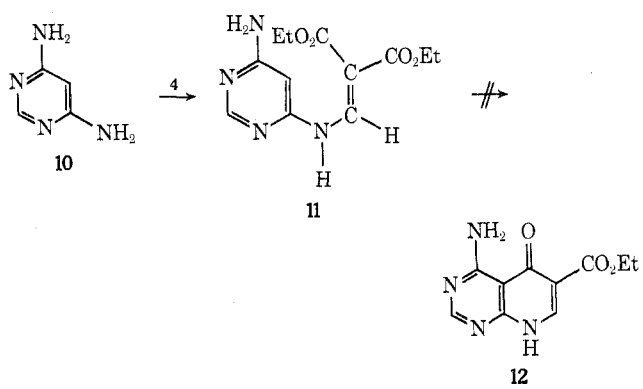


3 **4** **5** **6**



7 **8** **9**

The same situation prevailed in the reaction of 4,6-diaminopyrimidine (**10**) with **4**. The condensation product **11** was readily obtained in good yield and char-



10 **11** **12**

(1) (a) Supported by Research Grant No. CA 12823 from the National Cancer Institute, NIH. (b) Paper II in this series: B. H. Rizkalla, A. D. Broom, M. G. Stout, and R. K. Robins, *J. Org. Chem.*, **37**, 3975 (1972).

(2) J. A. Cavins, *Proc. Amer. Ass. Cancer Res.*, **7**, 12 (1966).

(3) J. A. Cavins, *et al.*, *Cancer Chemother. Rep.*, **51**, 197 (1967).

(4) For a recent review see W. J. Irwin and D. G. Wibberley, *Advan. Heterocycl. Chem.*, **10**, 149 (1969).

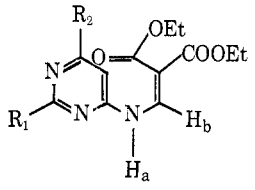
(5) L. A. Paquette, "Principles of Modern Heterocyclic Chemistry," W. A. Benjamin, New York, N. Y., 1968, p 280.

(6) (a) G. Y. Lesher and N. Y. Schodack, U. S. Patent 3,320,257 (1967); (b) S. Nishigaki, K. Ogiwara, K. Senga, S. Fukazawa, K. Aida, Y. Machica, and F. Yoneda, *Chem. Pharm. Bull.*, **18**, 1385 (1970).

acterized by elemental analysis and pmr spectra. The pmr spectrum of **11** revealed two singlets at δ 6.16 and 8.18 (Table I) corresponding to C-5 H and C-2 H, respectively, and doublets at δ 8.95 and 10.62 having the same coupling constant. Upon addition of D_2O to the solution the δ 8.95 doublet collapsed to a sharp singlet and the δ 10.62 doublet disappeared, permitting assignment of the former to the vinyl C–H and the latter to the adjacent N–H and confirming the structure of **11** as shown. Attempts to cyclize **11** to **12** under a variety of conditions failed, apparently because of a competing polymerization reaction.

TABLE I

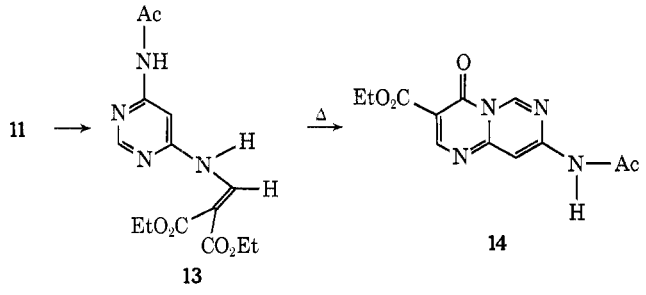
PROTON MAGNETIC RESONANCE FREQUENCIES FOR VARIOUS DIETHYL *N*-(6-PYRIMIDINYL)AMINOMETHYLENEMALONATES[a]



Compd no.	R_1		R_2		C-5		
	SCH_3	H	NH_2	$NHCOCH_3$	H	H_b	H_a
11		8.18 (s)	6.93 (s)		6.16 (s)	8.95 (d)	10.62 (d)
13		8.36 (s)		2.11 (s)	7.66 (s)	8.61 (d)	10.73 (d)
17	2.5 (s)			2.06 (s)	7.48 (s)	8.73 (d)	10.66 (d)
21		8.08 (s)			6.13 (s)	8.66 (d)	10.50 (d)
23	2.53 (s)				6.00 (s)	8.90 (d)	10.46 (d)

[a] All spectra run in DMSO-d_6 using DSS (2,2-dimethyl-2-silapentane sulfonate sodium salt) as internal reference.

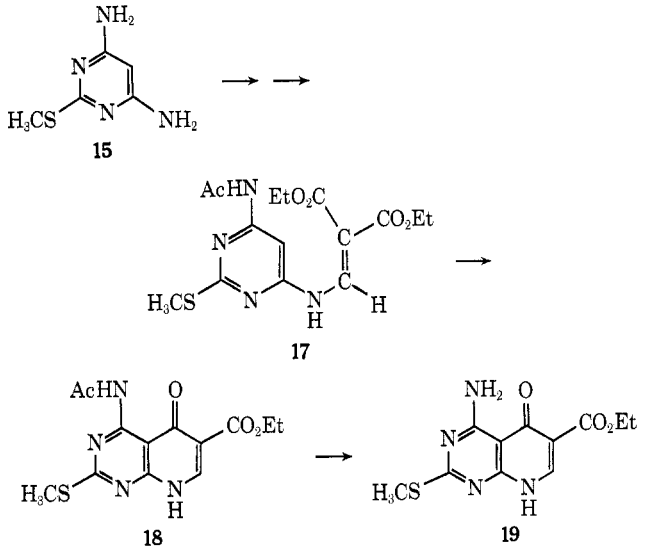
In an attempt to prevent the polymerization, **11** was N-acetylated to give **13**. Thermal cyclization of **13** gave a compound (**14**) which gave the correct elemental analysis for the *N*-acetyl derivative of **12**. The pmr spectrum of **14** revealed, however, that the product was not a pyrido[2,3-*d*]pyrimidine, but that cyclization had occurred at the pyrimidine ring nitrogen to give 8-acetamido-3-carbethoxy-4-oxopyrimido[1,6-*a*]pyrimidine (**14**). The assignment was based on the appearance of three 1-proton singlets in the pmr spectrum of **14** (not exchangeable with D_2O) at δ 8.0, 8.7, and 9.6. Only two such "aromatic" protons would be found in the *N*-acetyl derivative of **12**.



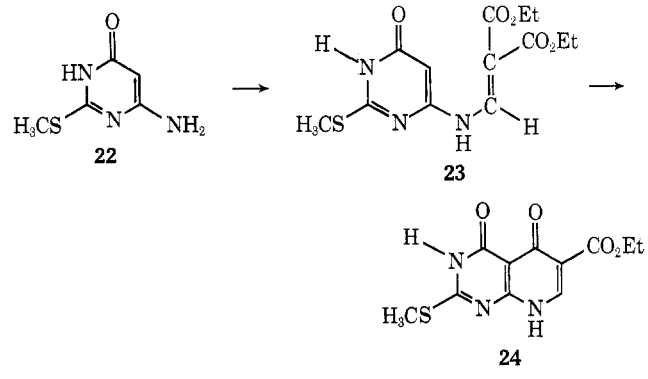
Because N-acylation markedly reduces the electron-donating capability of an amino group, a pyrimidine bearing an additional electron-releasing group, 4,6-diamino-2-methylthiopyrimidine (**15**),[7] was selected.

(7) D. Söll and W. Pfleiderer, *Chem. Ber.*, **96**, 2977 (1963).

Treatment of **15** with diethyl ethoxymethylenemalonate (**4**) gave the expected intermediate **16** which was acylated with acetic anhydride to give diethyl *N*-(4-acetamido-2-methylthio-6-pyrimidinyl)aminoethylenemalonate (**17**). It is noteworthy that, while thermal cyclization of **13** gave only the pyrimido[1,6-*a*]pyrimidine derivative in 70% yield, application of the same conditions to **17** gave only the desired 4-acetamido-6-carbethoxy-2-methylthio-5-oxopyrido[2,3-*d*]pyrimidine (**18**), also in about 70% yield. Alkaline hydrolysis of **18** in ethanolic sodium ethoxide afforded the 4-amino derivative (**19**).



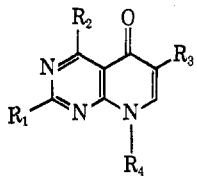
Similar results were obtained in reactions leading to the 4,5-dioxopyrido[2,3-*d*]pyrimidines. Treatment of 4-amino-6-oxopyrimidine (**20**) with **4** gave the intermediate diethyl *N*-(4-oxopyrimidinyl)aminomethylenemalonate (**21**), but no conditions could be found for the cyclization of **21**. Again, however, it was found that 4-amino-2-methylthio-6-oxopyrimidine (**22**) could be readily converted into 6-carbethoxy-4,5-dioxo-2-methylthiopyrido[2,3-*d*]pyrimidine (**24**) *via* the usual intermediate (**23**). In each of these cyclization reactions the assigned structure is supported by the disappearance of the typical pyrimidine C-5 H signal in the pmr spectrum at about δ 6 together with the appearance of a new peak at about δ 8–8.5 characteristic of the α proton of a 4-pyridone type compound (C-7 H).



Pyrido[2,3-*d*]pyrimidine Nucleosides.—When applicable, the direct fusion of heterocyclic bases with fully acylated sugars is the most facile of the many methods

TABLE II

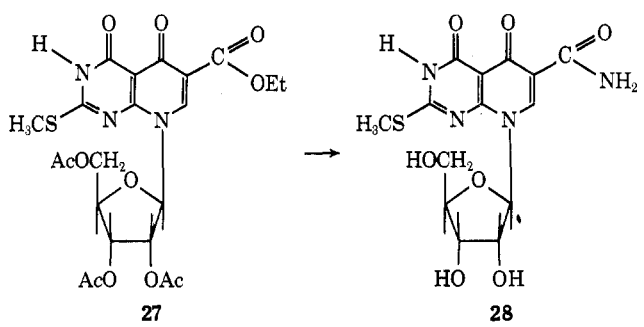
PROTON MAGNETIC RESONANCE FREQUENCIES (δ) FOR VARIOUS 5-OXOPYRIDO[2,3-*d*]PYRIMIDINE DERIVATIVES



Compd no.	R_1: SCH_3	R_1: H	R_2: NH_2	R_2: $NHCCH_3$ (O)	R_3: $COOCH_2CH_3$ CH_2	R_3: $COOCH_2CH_3$ CH_3	R_3: $-CONH_2$	C-7 H	R_4: C-1′ H
2		8.21 (s)	8.83, (d), 9.43 (d) J_{H-N-H} = 4.0 Hz				7.60 (d), 8.50 (d) J_{H-N-H} = 4.4 Hz	8.91 (s)	6.38 (d) $J_{1',2'}$ = 4 Hz
18	2.5 (s)			2.5 (s)	4.15 (q)	1.28 (t)		8.16 (s)	
19	2.46 (s)		8.1 (d), 9.7 (d)		4.15 (q)	1.28 (t)		8.16 (s)	
24	2.53 (s)				4.23 (q)	1.3 (t)		8.33 (s)	
27	2.41 (s)				4.21 (m)	1.23 (t)		8.80 (s)	6.43 (d) $J_{1',2'}$ = 3 Hz
28	2.56 (s)						7.43 (br), 8.7 (br)	8.90 (s)	6.43 (d) $J_{1',2'}$ = 2.5 Hz
29	2.5 (s)			2.5 (s)	4.15 (m)	1.28 (t)		8.58 (s)	6.48 (d) $J_{1',2'}$ = 2.6 Hz
30	2.5 (s)		8.36 (d), 9.5 (d) J_{H-N-H} = 3.4 Hz				7.4 (d), 8.1 (d) J_{H-N-H} = 3.6 Hz	8.85 (s)	6.4 (d) $J_{1',2'}$ = 3 Hz

of nucleoside synthesis.[8] Because of the low solubility and high melting point of 6-carbethoxy-4,5-dioxo-2-methylthiopyrido[2,3-*d*]pyrimidine (**24**), direct fusion coupling with tetra-*O*-acetyl-D-ribofuranose is not practicable. These problems may be readily overcome, however, by trimethylsilylation of the heterocycle.[1b,9,10] Thus **24** was treated with hexamethyldisilazane in anhydrous toluene to give an uncharacterized trimethylsilyloxy derivative **25**. Compound **25** was fused with tetra-*O*-acetyl-D-ribofuranose (**26**) giving 6-carbethoxy-4,5-dioxo-2-methylthio-8-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrido[2,3-*d*]pyrimidine (**27**). When **27** was subjected to the action of liquid ammonia at room temperature, the 6-carboxamido derivative of the free nucleoside (**28**) was obtained.

24 → 25 →



27 28

Proof of structure of **28** was based primarily on pmr spectrometry. It has been previously shown that substitution of an alkyl group for hydrogen on the nitrogen of a potentially tautomeric (lactam–lactim) heteroaromatic system results in a substantial downfield shift of the pmr signal for an adjacent proton.[11] A downfield shift of the C-7 H resonance of 0.57 ppm (Table II) is observed upon ribosylation of **24**, whereas the signal for the methyl protons of the 2-methylthio group remained unchanged; this permits assignment of N-8 as the site of alkylation. The uv spectrum of **28** resembles that of **24** at pH 7 (Table III), supporting the assignment of alkylation as N-8. Assignment of the anomeric configuration as β was based on the small coupling constant for H-1′ ($J_{1',2'}$ = 2.5 Hz).[12]

The synthesis of compound **2** was accomplished starting with the blocked pyrido[2,3-*d*]pyrimidine **18**. Compound **18** possessed melting point and solubility characteristics such that it was suitable for the direct fusion with tetra-*O*-acetyl-D-ribofuranose (**26**). Uncatalyzed fusion of **18** and **26** provided the fully blocked nucleoside **29** in good yield. Both the site of alkylation and the anomeric configuration of **29** were readily assigned by pmr spectroscopy in the same manner as described for nucleoside **28**. Thus alkylation with the sugar resulted in a downfield shift of 0.42 ppm for the C-7 proton resonance of **29** relative to that of the starting material **18** while the methyl proton signals of the 2-methylthio groups remained essentially unchanged. The $H_{1'},H_{2'}$ coupling constant ($J_{1',2'}$ =

(8) For a recent review see L. Goodman in "The Carbohydrates: Chemistry and Biochemistry," Vol. IIA, 2nd ed, W. Pigman and D. Horton, Ed., Academic Press, New York, N. Y., 1970, p 9.

(9) M. G. Stout and R. K. Robins, *J. Org. Chem.*, **33**, 1219 (1968).

(10) L. Birkhofer, A. Ritter, and H. P. Kuehlthan, *Angew. Chem.*, **75**, 209 (1963).

(11) A. D. Broom and R. K. Robins, *J. Org. Chem.*, **34**, 1025 (1969).

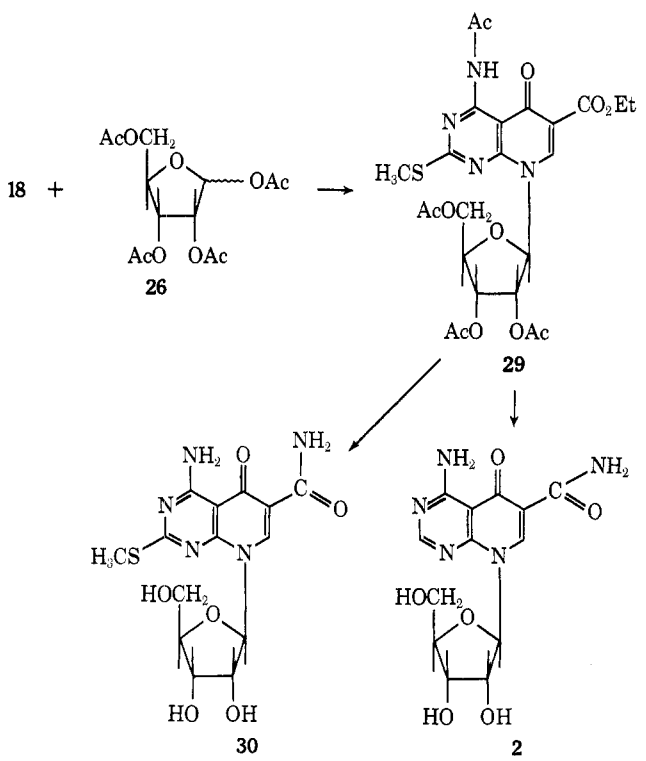
(12) R. U. Lemieux and D. R. Lineback, *Annu. Rev. Biochem.*, **32**, 155 (1963).

TABLE III
ULTRAVIOLET ABSORPTION DATA FOR VARIOUS PYRIDO[2,3-*d*]PYRIMIDINE DERIVATIVES

Compd no.	pH 1 λ_{max}, nm	pH 1 ϵ	pH 7 λ_{max}, nm	pH 7 ϵ	pH 11 λ_{max}, nm	pH 11 ϵ
2	258	27,200	272	21,700	272	21,400
	294	11,250	300	15,800	300	15,900
18	272	43,100	276	40,500	262 (s)	26,700
	310 (s)	14,200	310 (s)	14,200	274 (s)	30,500
					287	33,600
					322 (s)	12,200
19	272	38,500	272	41,500	270	40,200
	294	18,400	297 (s)	14,800	318	17,200
24	267	31,500	267	37,000	267	38,750
	291	20,700	291 (s)	19,000	307	15,400
27	273	3,700	263	3,150	262 (s)	3,700
			273	3,750	272	4,000
28	273	34,400	263 (s)	25,500	263 (s)	34,000
	294	19,200	273	37,200	273	38,000
			294 (s)	17,600	301	13,300
29	276	23,000	276	23,600	275	21,800
	312	7,600	312	7,800	302	7,500
30	276	17,600	275	14,800	275	14,900
	302	39,500	302	40,500	302	42,500

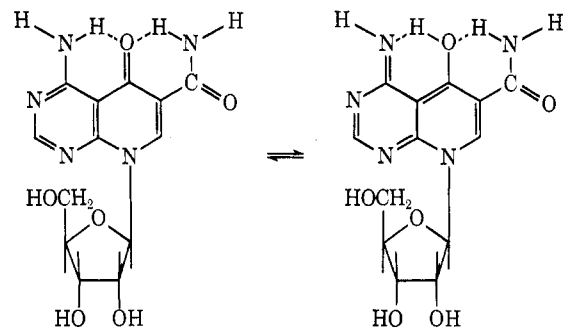
2.6 Hz) permits assignment of the β configuration to the anomeric carbon.[12]

Treatment of **29** with liquid ammonia at room temperature gave 4-amino-6-carboxamido-2-methylthio-5-oxo-8-(β-D-ribofuranosyl)pyrido[2,3-*d*]pyrimidine (**30**).



The blocked nucleoside **29** was also treated with Raney nickel followed by treatment of the product *in situ* with liquid ammonia to give the sangivamycin analog, 4-amino-6-carboxamido-5-oxo-8-(β-D-ribofuranosyl)pyrido[2,3-*d*]pyrimidine (**2**). The pmr spectrum of **2** revealed the loss of the signal due to the protons of the 2-methylthio function and the presence of two 1-proton singlets corresponding to C-2 H (δ 8.21) and C-7 H (δ 8.91).

Pmr Spectral Considerations.—The 4-amino-5-oxopyrido[2,3-*d*]pyrimidines and their ribonucleosides described above gave rise to unique and very interesting pmr spectra in DMSO-d_6 solution. The nucleoside **2** will be used for illustration. The amino group in the 4 position of **2** gave rise to two broad doublets at δ 8.83 and 9.43 (J_{H-N-H} = 4.0 Hz). The nonequivalence of the two N–H protons is not surprising; as clearly seen in structure **31a**, one of the protons may hydrogen bond



31a **31b**

intramolecularly (the chemical shifts remain unchanged upon dilution) to the 5-oxo group. Rotation about the C–N bond is slow on the pmr time scale; the downfield signal (δ 9.43) may thus be assigned to the proton which is hydrogen bonded to the 5-oxo group. Of two possible tautomers **31a** and **31b**, the former is established as the predominant form because of the observed H–N–H geminal coupling. It has been firmly established for a variety of ketamine systems involving a monoalkyl amine that H–N–C–H coupling only occurs when the hydrogen is bound to nitrogen and hydrogen bonded to oxygen as is the case with **31a**.[13] That such a geminal coupling is observed at all appears to be unique; similar amino–oxo hydrogen-bonded systems have been described,[14,15] but no geminal coupling was observed because of ^{14}N quadrupole broadening of the resonance signals of the amino protons. H–N–H geminal coupling has only been observed by application of double resonance techniques; ^{14}N heteronuclear decoupling in amides has demonstrated geminal coupling constants of about 2.4 Hz.[16] Examination of the N–H signals arising from the 6-carboxamide group in **2** revealed the same pattern of two doublets at δ 7.60 and 8.50 (J_{H-N-H} = 4.4 Hz); clearly, then, **2** exists primarily in the conformation shown in **31a**. It is presumed that geminal coupling is observed because of an increase in sp^2 character of the amino groups resulting from H-bond formation.[16]

Experimental Section[17]

Diethyl *N*-(4-Amino-6-pyrimidinyl)aminomethylenemalonate (11).—4,6-Diaminopyrimidine (1 g, 9 mmol) was heated with 1.8 g of diethyl ethoxymethylenemalonate (9 mmol) at an oil bath temperature of 165° for 20 min. The melt was cooled and dissolved in 120 ml of ethyl acetate and 20 ml of methanol by boil-

(13) R. N. Schut, W. G. Strycker, and T. M. H. Lill, *J. Org. Chem.*, **38**, 3046 (1963).
(14) G. O. Dudek and G. P. Volpp, *ibid.*, **30**, 50 (1965).
(15) R. Wasyleshen and T. Schaefer, *Can. J. Chem.*, **49**, 3575 (1971).
(16) H. Kamei, *Bull. Chem. Soc. Jap.*, **38**, 1212 (1965).
(17) Elemental analyses were performed by Heterocyclic Chemical Co., Harrisonville, Mo. Pmr spectra were run on a Jeolco C60H spectrometer at ambient temperature. Uv spectra were obtained using a Cary Model 15 spectrophotometer. Melting points were taken on a Thomas-Hoover apparatus and are uncorrected.

ing. The solution was filtered; the filtrate was concentrated to 60 ml and kept at 5° overnight. The precipitate was filtered, washed with a little methanol and ethyl acetate, and dissolved in 60 ml of boiling methanol by adding 5 ml of ethyl acetate. The solution was treated with charcoal and filtered through a Celite pad. The filtrate was concentrated to 30 ml and kept at 5° overnight. The precipitate was filtered, washed with methanol, and dried *in vacuo* over refluxing toluene in the presence of phosphorus pentoxide to give 0.80 g (33%), mp 196–197°.

Anal. Calcd for $C_{12}H_{16}N_4O_4$: C, 51.70; H, 5.20; N, 20.10. Found: C, 51.50; H, 5.41; N, 20.11.

Diethyl *N*-(4-Acetamido-6-pyrimidinyl)aminomethylenemalonate (13).—A 1-g sample of **11** (3.6 mmol) was refluxed with 50 ml of acetic anhydride overnight. The acetic anhydride was removed *in vacuo*. The residue was dissolved in 60 ml of boiling ethanol, treated with charcoal, and filtered. The filtrate was concentrated to 50 ml and kept at 5° overnight. The yellowish white precipitate was filtered, washed with ethanol, and air-dried to give 0.68 g (60%), mp 204–205°.

Anal. Calcd for $C_{14}H_{18}N_4O_5$: C, 52.20; H, 5.60; N, 17.40. Found: C, 52.18; H, 5.56; N, 17.42.

8-Acetamido-3-carbethoxy-4-oxopyrimido[1,6-*a*]pyrimidine (14).—Compound **13** (1 g, 3.1 mmol) was heated in diphenyl ether at 205–210° internal temperature for 1 hr with stirring. The solution was cooled, and the brown precipitate was filtered. The precipitate was dissolved in 50 ml of a boiling chloroform–methanol mixture, treated with charcoal, and filtered. The filtrate was concentrated to 30 ml and kept at 5° overnight. The precipitate was filtered, washed with chloroform, and air-dried to give 0.60 g (70%), mp 263–264°. For analysis a sample was dried *in vacuo* over refluxing toluene in presence of phosphorus pentoxide.

Anal. Calcd for $C_{12}H_{12}N_4O_4$: C, 52.12; H, 4.35; N, 20.35. Found: C, 52.35; H, 4.25; N, 20.36.

Diethyl *N*-(2-Methylthio-4-amino-6-pyrimidinyl)aminomethylenemalonate (16).—A 6.30-g sample of 2-methylthio-4,6-diaminopyrimidine (20.0 mmol) was heated for 40 min with 4.00 g of diethyl ethoxymethylenemalonate (20 mmol) at 165°. The temperature was reduced to 135°, and heating was continued for another 60 min. The melt was cooled and dissolved in 150 ml of boiling ethanol, treated with charcoal, filtered, and concentrated to 80 ml. The solution was heated to reflux and water was added until the cloud point was reached. The solution was kept at 5° overnight. The yellowish precipitate was filtered, washed with ethanol, and air-dried to give 4.90 g (70%), mp 174–176°.

Anal. Calcd for $C_{13}H_{18}N_4O_4S$: C, 47.70; H, 5.20; N, 12.81; S, 9.8. Found: C, 47.44; H, 5.28; N, 12.43; S, 9.64.

Diethyl *N*-(4-Acetamido-2-methylthio-6-pyrimidinyl)aminomethylenemalonate (17). A 2.0-g sample of **16** (6.0 mmol) was refluxed overnight in 50 ml of acetic anhydride. The excess acetic anhydride was removed by distillation under reduced pressure. The residue was crystallized from 80 ml of an ethyl acetate–ethanol mixture (10:90). The precipitate was filtered, washed with cold ethanol, and dried over refluxing toluene in the presence of phosphorus pentoxide to give 1.5 g (68%), mp 164–164.5°.

Anal. Calcd for $C_{15}H_{20}N_4O_5S$: C, 48.90; H, 5.48; N, 15.22. Found: C, 48.81; H, 5.39; N, 15.31.

4-Acetamido-6-carbethoxy-2-methylthio-4-oxopyrido[2,3-*d*]pyrimidine (18).—A 1.3-g sample of **17** (3.70 mmol) was refluxed in 30 ml of Dowtherm A for 15 min. The solution was cooled to room temperature and diluted with 70 ml of diethyl ether. The precipitate was filtered, washed with diethyl ether, and air-dried. The residue was dissolved in 200 ml of 1,2-dimethoxyethane, treated with charcoal, and filtered. The filtrate was concentrated to 20 ml and kept at 5° overnight. The white precipitate was filtered, washed with cold 1,2-dimethoxyethane, and air-dried to yield 0.80 g (71.5%), mp 227–228°. For analysis a sample was dried over refluxing toluene in the presence of phosphorus pentoxide.

Anal. Calcd for $C_{13}H_{14}N_4O_4S$: C, 48.5; H, 4.35; N, 17.39. Found: C, 48.15; H, 4.36; N, 17.47.

4-Amino-6-carbethoxy-2-methylthio-5-oxopyrido[2,3-*d*]pyrimidine (19).—Compound **18** (1 g, 3.3 mmol) was dissolved in 150 ml of absolute ethanol. A solution of 0.2 g of metallic sodium (freshly cut) in 20 ml of absolute ethanol was added. The solution was refluxed for 20 min. The mixture was cooled and neutralized with acetic acid to pH 7.0. The solution was concentrated to about 30 ml, and the precipitate which formed was filtered, washed with ethanol, and dissolved in a hot mixture of 10 ml of water and 40 ml of ethanol. The solution was filtered hot and the filtrate left at room temperature. The precipitate was filtered, washed with ethanol, and air-dried to yield 0.80 g (86.5%), mp 274–275°. For analysis a sample was dried *in vacuo* over refluxing toluene in the presence of phosphorus pentoxide.

Anal. Calcd for $C_{11}H_{12}N_4O_3S \cdot H_2O$: C, 44.20; H, 4.70; N, 18.95. Found: C, 44.27; H, 4.66; N, 18.65.

Diethyl *N*-(4-Oxo-6-pyrimidinyl)aminomethylenemalonate (21).—Amino-6-oxopyrimidine (1.1 g, 10 mmol) and 1.97 g of diethyl ethoxymethylenemalonate (10 mmol) were refluxed in 15 ml of dimethylformamide for 9 hr. The reaction mixture was filtered. The filtrate was added to 180 ml of diethyl ether; the suspension was stirred for 1 min and filtered. The filtrate was kept at 5° overnight, and the bright yellowish orange precipitate was filtered and washed with diethyl ether to yield 0.85 g (30.5%), mp 190–191°.

Anal. Calcd for $C_{12}H_{15}N_3O_5$: C, 51.15; H, 5.35; N, 15.00. Found: C, 51.35; H, 5.37; N, 15.21.

Diethyl *N*-(2-Methylthio-4-oxo-6-pyrimidinyl)aminomethylenemalonate (23).—A 15.7-g sample of **22** (100 mmol) was heated for 1 hr with 19.8 g of diethyl ethoxymethylenemalonate (100 mmol) in a round-bottom flask immersed in an oil bath kept at 175–180°. The solid was dissolved in a boiling chloroform–methanol mixture, treated with charcoal, and filtered. The filtrate was concentrated and kept at 5° overnight. The precipitate was filtered, washed with cold methanol, and air-dried to give 20.6 g (66.7%), mp 232–234°. For analysis a sample was crystallized twice from $CHCl_3$–MeOH and dried *in vacuo*, mp 233–234°.

Anal. Calcd for $C_{13}H_{17}N_3O_5S$: C, 47.70; H, 5.20; N, 12.81; S, 9.80. Found: C, 47.44; H, 5.28; N, 12.43; S, 9.64.

6-Carbethoxy-4,5-dioxo-2-methylthiopyrido[2,3-*d*]pyrimidine (24).—Compound **23** (10 g, 32 mmol) were added to 150 ml of Dowtherm A. The mixture was heated to reflux for 20 min. The solution was cooled and the mixture treated with 300 ml of toluene and filtered. The brown precipitate was washed with toluene, dissolved in a chloroform–methanol mixture, treated with charcoal, and filtered. The solution was concentrated and kept at 5° overnight. The cream-colored precipitate was filtered, washed with methanol, and air-dried to give 6.23 g (73%), mp 269–270°. For analysis a sample was recrystallized twice from $CHCl_3$–MeOH and dried *in vacuo*, mp 270–271°.

Anal. Calcd for $C_{11}H_{11}N_3O_4S$: C, 47.01; H, 3.92; N, 14.96; S, 11.39. Found: C, 46.81; H, 4.06; N, 14.72; S, 11.19.

6-Carbethoxy-4,5-dioxo-2-methylthio-8-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrido[2,3-*d*]pyrimidine (27).—A 2.3-g sample of compound **24** (8.15 mmol) was suspended in 180 ml of toluene and the mixture refluxed for 1 hr; 30 ml of toluene was distilled off. The mixture was cooled, and 4 ml of hexamethyldisilazane was added together with a few crystals of ammonium sulfate. The mixture was refluxed for 4 hr. The clear yellow solution was filtered through a sintered glass funnel. The solvent was removed *in vacuo* at bath temperature of 150°, and oil pump vacuum was applied briefly for 2 min. Five grams of tetra-*O*-acetyl-β-D-ribofuranose (15.7 mmol) was added, and the melt was stirred *in vacuo* at an oil bath temperature of 155–160° for 15 min. The vacuum was broken, and the reaction was protected from moisture with drying tube. Stirring was continued for 10 hr, vacuum being applied for 5 min every hour. The melt was dissolved in methanol, boiled for 10 min, and cooled to room temperature. The precipitate was filtered (starting material), and the filtrate was evaporated to dryness. The viscous residue was dissolved in diethyl ether, filtered, concentrated, and kept at 5° overnight. The precipitate was filtered, washed with cold ether, and air-dried to give 1.75 g, mp 147–148°. A second crop was obtained by further concentration of the filtrate and yielded 0.5 g, mp 146–147°. The total yield was 2.25 g (51%). Two further crystallizations from ether gave a product having mp 148°.

Anal. Calcd for $C_{22}H_{25}N_3O_{11}S$: C, 48.98; H, 4.67; N, 7.80, S, 5.93. Found: C, 48.95; H, 4.67; N, 7.81; S, 5.98.

6-Carboxamido-4,5-dioxo-2-methylthio-8-(β-D-ribofuranosyl)-pyrido[2,3-*d*]pyrimidine (28).—Compound **27** (539 mg, 1.0 mmol) was placed in a glass-lined bomb; 80 ml of liquid ammonia was added, and the bomb was sealed. The solution was left at room temperature for 24 hr. The liquid ammonia was allowed to evaporate. The residue was dissolved in 200 ml of hot methanol. The solution was filtered, and the filtrate was kept at 5° overnight. The white precipitate was filtered and washed with methanol to yield 340 mg (89%), mp 243°.

Anal. Calcd for $C_{14}H_{16}N_4O_7S \cdot 0.5H_2O$: C, 42.90; H, 4.10; N, 14.25. Found: C, 42.81; H, 4.04; N, 14.33.

4-Acetamido-6-carbethoxy-2-methylthio-5-oxo-8-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrido[2,3-*d*]pyrimidine (29).—A 1.0-g sample of compound **18** (2.72 mmol) was mixed with 1.5 g of tetra-*O*-acetyl-β-D-ribofuranose. The mixture was fused at an oil bath temperature of 195–200° for 2.5 hr *in vacuo*. The black melt was dissolved in a minimum volume of chloroform, and the solution was filtered. The filtrate was concentrated to 5.0 ml and placed on a column packed with 50 g of silica gel in chloroform. The column was eluted with chloroform; the first 300 ml of eluent was discarded. The next 800 ml of eluent was evaporated *in vacuo* to dryness. The viscous residue was triturated with 50 ml of ether. On scratching the side wall of the beaker a white precipitate formed. The precipitate was filtered, washed with diethyl ether, and air-dried to give 0.95 g (61%), mp 106–107°. For analysis a sample was dried *in vacuo* over refluxing methanol in presence of phosphorus pentoxide.

Anal. Calcd for $C_{24}H_{28}N_4O_{11}S \cdot 0.5H_2O$: C, 49.20; H, 4.95; N, 9.55. Found: 49.09; H, 5.17; N, 9.26.

4-Amino-6-carboxamido-2-methylthio-5-oxo-8-(β-D-ribofuranosyl)pyrido[2,3-*d*]pyrimidine (30).—A 1.16-g sample of **29** (2.0 mmol) was dissolved in 80 ml of liquid ammonia in a glass-lined bomb. The bomb was sealed and left to stand at room temperature for 24 hr. The liquid ammonia was allowed to evaporate. The residue was dissolved in 200 ml of boiling methanol by the addition of water. The solution was filtered, and the filtrate was kept at 5° overnight. The white precipitate was filtered, washed with methanol, and air-dried to give 0.60 g (78%), mp 252–253°. For analysis a sample was dried *in vacuo* over refluxing toluene in the presence of phosphorus pentaoxide.

Anal. Calcd for $C_{14}H_{17}N_5O_8S \cdot H_2O$: C, 41.90; H, 4.73; N, 17.4. Found: C, 42.01; H, 5.03; N, 17.25.

4-Amino-6-carboxamido-5-oxo-8-(β-D-ribofuranosyl)pyrido-[2,3-*d*]pyrimidine (2).—A 0.58-g sample of **29** (1.0 mmol) was dissolved in 30 ml of ethanol; 2.0 g of Raney nickel (weighed wet and prewashed with distilled water followed by ethanol) was added. The mixture was refluxed for 24 hr, and 1 g more of Raney nickel (weighed wet and pretreated as above) was added. Refluxing was continued for another 4 hr. The mixture was filtered hot, and the Raney nickel was washed with 300 ml of boiling ethanol. The filtrate was evaporated to dryness. The residue was transfered to a glass-lined bomb and 80 ml of liquid ammonia was added; the bomb was sealed and left at room temperature for 24 hr. The liquid ammonia was allowed to evaporate. The residue was dissolved in a boiling mixture of 40 ml of methanol and 5 ml of water and kept at 5° overnight. The precipitate was filtered, washed with methanol, and air-dried to give 0.23 g, mp 253–254°, resolidifies (44.5% overall yield). For analysis a sample was dried *in vacuo* over refluxing toluene in the presence of phosphorus pentoxide.

Anal. Calcd for $C_{13}H_{15}N_5O_6$: C, 46.30; H, 4.46; N, 20.74. Found: C, 46.02; H, 4.35; N, 20.61.

Registry No.—**1**, 18417-89-5; **2**, 36707-00-3; **11**, 21025-64-9; **13**, 36707-40-1; **14**, 36707-41-2; **16**, 36707-42-3; **17**, 36707-43-4; **18**, 36707-44-5; **19**, 36707-45-6; **21**, 36707-46-7; **23**, 36707-47-8; **24**, 36707-48-9; **27**, 36707-01-4; **28**, 36707-02-5; **29**, 36707-03-6; **30**, 36707-04-7.

Directed Glycosylation of 8-Bromoadenine. Synthesis and Reactions of 8-Substituted 3-Glycosyladenine Derivatives

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The ratio of 3-glycosyl- *vs.* 9-glycosyladenine nucleosides using several glycosylation procedures was investigated. Treatment of the trimethylsilyl derivative of 8-bromoadenine with glycosyl halides leads to excellent yields of blocked 3-glycosyl-6-amino-8-bromopurine nucleosides. This method has been used to prepare 6-amino-8-bromo-3-β-D-ribofuranosylpurine (**6**), 3-β-D-ribofuranosyladenine (**7**), 6-amino-3-α-D-arabinofuranosyl-8-bromopurine (**10**), 3-α-D-arabinofuranosyladenine (**11**), and 3-β-D-arabinofuranosyladenine (**14**). Deamination of 3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)adenine (**5**) with NOCl–pyridine in DMF and removal of the blocking groups gave improved yields of 3-β-D-ribofuranosylhypoxanthine (**16**). Similar treatment of **11** gave 3-β-D-arabinofuranosylhypoxanthine (**17**). Deamination of 6-amino-3-(2,3,5-tri-*O*-benzoyl)-8-bromopurine (**4**), using NOCl in pyridine, and subsequent debenzoylation gave 3-β-D-ribofuranosyl-8-pyridiniumhypoxanthine betaine (**19**). The reactivity of **4** toward nucleophiles was investigated. Strongly basic nucleophiles such as methoxide, benzyloxide, and hydrazine caused decomposition. Displacement was accomplished with azide ion which gave, after hydrogenation and deblocking, 6,8-diamino-3-β-D-ribofuranosylpurine (**21**).

Interest in 3-substituted purine derivatives has been stimulated by the isolation of 3-β-D-ribofuranosyluric acid and 3-(3-methyl-2-butenyl)adenine from natural sources[1–3] and by the observation of interesting biological properties[4–7] of synthetic 3-β-D-ribofuranosyladenine (**7**). Adenine has been shown to undergo preferential alkylation at the 3 position;[3,8,9] however, direct alkylation of adenine with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide (**1**) afforded 3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)adenine (**5**) in only 26% yield with 18% of the 9 isomer.[10] Glycosylation of 6-benzamidopurine with 2,3,5-tri-*O*-benzyl-α-D-arabinofuranosyl chloride gave some 3-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)adenine (**13**) in addition to the expected 9 isomer.[11] Selective glycosylation at the 3 position has been achieved by utilizing 7-pivaloyloxymethyladenine

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