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The Synthesis of 6-Fluoro-9-methylpurine*

ALDEN G. BEAMAN and ROLAND K. ROBINS

Department of Chemistry, Arizona State University, Tempe, Arizona

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4,6-Dichloro-5-nitropyrimidide (I), upon treatment with silver fluoride, gave 4,6-difluoro-5-nitropyrimidine (II) which was monoaminated and reduced to yield 4,5-diamino-6-fluoropyrimidine (IV). This substance failed to cyclize to 6-fluoropurine by standard cyclization procedures. Reduction of 4,6-difluoro-5-nitropyrimidine (II) yielded 5-amino-4,6-difluoropyrimidine (VII), and VII with methylamine gave 5-amino-6-fluoro-4-methylaminopyrimidine (VIII) which was readily cyclized to 6-fluoro-9-methylpurine (X). The chemistry of X is discussed.

The clinical success of the use of 6-chloropurine in the treatment of chronic granulocytic leukemia[1a] has stimulated the preparation of other 6-halogenated purines. 6-Chloro-9-methylpurine[1b] and other 9-alkyl-6-chloropurines have exhibited significant antitumor activity in experimental mice.[1c] The antitumor action of 6-chloropurine,[1d-g] 6-bromopurine,[1h] and 6-iodopurine[1h] strongly suggests that 6-fluoropurine and derivatives thereof would be excellent candidates for study in various tumor systems. There has been great interest in the synthesis of 6-fluoropurine, and it is noteworthy that the

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hitherto published attempted syntheses of this compound have all started with a purine having some group in the 6-position which it was hoped could be converted to fluorine. Thus, the following methods have been tried without success: hypoxanthine and phosphorus oxyfluoride,[2] adenine, sodium nitrite and fluoroboric acid,[2,3] 6-hydrazinopurine and ferric fluoride,[2,3] and 6-chloropurine (or 6-iodopurine) and a variety of fluorides.[3] In addition, we have found[4] that the Schiemann reaction with adenine-N-oxide failed to give 6-fluoropurine-N-oxide, and that heating of purine-6-sulfonyl fluoride[5] failed to give 6-fluoropurine. It has been suggested[2] that the inability to prepare 6-fluoropurine may be due to its instability. Bendich[6] has predicted that 6-fluoropurine would be unstable to acid; in addition, thermal instability might be mentioned. The latter may explain the failure to obtain 6-fluoropurine when purine-6-sulfonyl fluoride was heated at about 200° in tetralin, under which conditions sulfur dioxide was evolved copiously.

Thus, a completely different approach was sought *via* fluoropyrimidines, which it was hoped would avoid the difficulties inherent in the previous synthetic methods aimed at 6-fluoropurine. In addition, appropriate 4-substituted aminopyrimidines could serve as intermediates for various 9-substituted-6-fluoropurines of interest. Finally, because of the antitumor activity of 5-fluorouracil,[7] it was felt that certain fluoropyrimidines might be of interest in themselves.

Schroeder[8] found that heating 2,4,6-trichloropyrimidine with silver fluoride gave 2,4,6-trifluoropyrimidine. We found that 4,6-dichloro-5-nitropyrimidine (I)[9] and silver fluoride gave the corresponding difluoronitropyrimidine (II) which upon monoamination and reduction gave the important intermediate 4,5-diamino-6-fluoropyrimidine (IV).

The standard reagents for cyclizing 6-chloro-4,5-diaminopyrimidine to 6-chloropurine, *viz.*, ethyl orthoformate–acetic anhydride,[10,11] diethoxymethyl acetate $[(C_2H_5O)_2CHOCOCH_3]$[12] and ethyl orthoformate plus an acidic catalyst,[13] all gave hypoxanthine when applied

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(3) A. Giner-Sorolla and A. Bendich, *J. Am. Chem. Soc.*, **80**, 5744 (1958).
(4) Unpublished observations of the authors.
(5) A. G. Beaman and R. K. Robins, *J. Am. Chem. Soc.*, **83**, 4038 (1961).
(6) Ref. 2, p. 16.
(7) See *Cancer Chemotherapy Repts.*, No. 6, p. 94 (1960) for a compilation of the references on 5-fluorouracil.
(8) H. Schroeder, *J. Am. Chem. Soc.*, **82**, 4115 (1960).
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(12) J. A. Montgomery and C. Temple, Jr., *J. Am. Chem. Soc.*, **79**, 5238 (1957).
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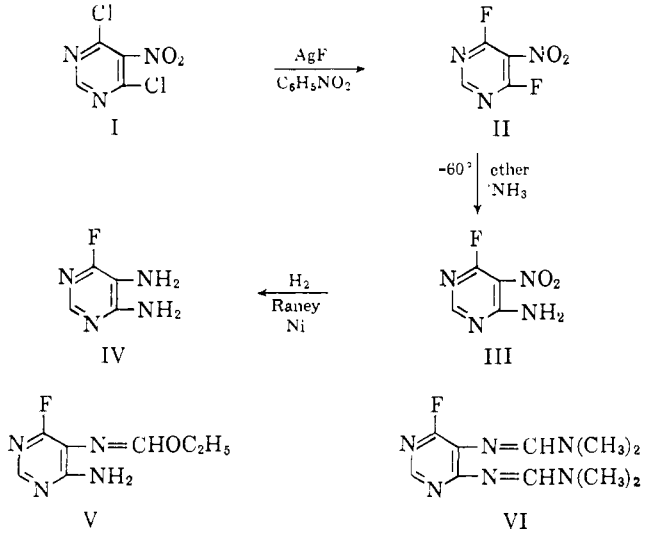


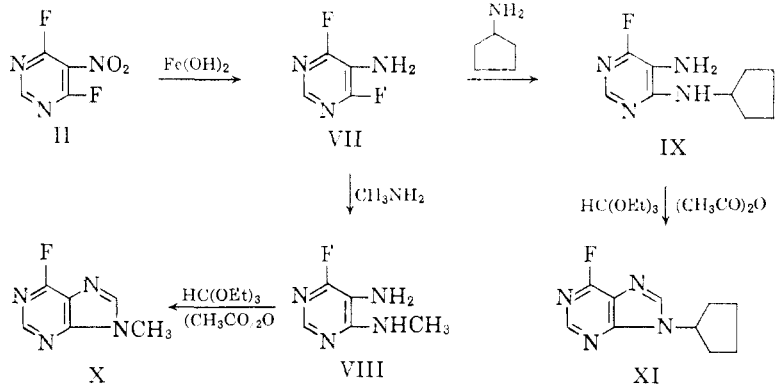
to 4,5-diamino-6-fluoropyrimidine. With ethyl orthoformate alone 4,5-diamino-6-fluoropyrimidine gave 4-amino-5-ethoxymethyleneimino-6-fluoropyrimidine (V), which is analogous to the findings of Montgomery[13] for the corresponding chloro compound. When 4,5-diamino-6-fluoropyrimidine was treated with phosphorus oxychloride and N,N-dimethylformamide according to the method of Clark and Lister,[14] 4,5-bis(dimethylaminomethyleneimino)-6-fluoropyrimidine (VI) was obtained. It was found that this method with 6-chloro-4,5-diaminopyrimidine[15] gave a mixture of 6-chloropurine and 4,5-bis(dimethylaminomethyleneamino)-6-chloropyrimidine.

The very reactive and unstable 4,6-difluoro-5-nitropyrimidine (II) was best reduced with ferrous hydroxide to 5-amino-4,6-difluoropyrimidine (VII) by the method of Brown.[16] This compound reacted readily with methylamine and with cyclopentylamine in refluxing ethanol to give 5-amino-6-fluoro-4-methylaminopyrimidine (VIII) and 5-amino-6-fluoro-4-cyclopentylaminopyrimidine (IX), respectively. Refluxing VIII with ethyl orthoformate-acetic anhydride gave 6-fluoro-9-methylpurine (X). Similarly, IX gave 9-cyclopentyl-6-fluoropurine (XI). 6-Fluoro-9-methylpurine (X) was stable to storage and recrystallization from organic solvents such as benzene. When boiled with concentrated aqueous ammonia, X gave 9-methyl-

(14) J. Clark and J. H. Lister, *J. Chem. Soc.*, 5048 (1961).

(15) R. K. Robins, K. J. Dille, C. H. Willits, and B. E. Christensen, *J. Am. Chem. Soc.*, **75**, 263 (1953).

(16) D. J. Brown, *J. Appl. Chem.*, **4**, 72 (1954).



adenine indicating that the fluorine atom is more readily replaced than the chlorine atom in 6-chloro-9-methylpurine.[1b] 6-Fluoro-9-methylpurine (X) was changed slowly to 9-methylhypoxanthine in aqueous solution over a period of one week. These reactions provide evidence that the fluorine atom is at position 6 and that no unexpected rearrangement has occurred.

6-Fluoro-9-methylpurine (X) was negative when screened against Adenocarcinoma 755 at dosages up to 68 mg./kg. per day. At this dosage no toxicity was evident. Further testing of X and related derivatives against a wider spectrum of tumors is in progress.

Experimental[17]

4,6-Difluoro-5-nitropyrimidine (II).—A mixture of 200 g. of 4,6-dichloro-5-nitropyrimidine,[9] 1000 g. of silver fluoride (Harshaw Chem. Co.), and 200 ml. of nitrobenzene was stirred and refluxed at 170–180° for 45 min. The 4,6-difluoro-5-nitropyrimidine which formed (about 150 ml.) was distilled at atmospheric pressure, b.p. 185–195°. This compound was unstable and was used without purification.

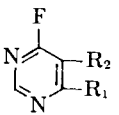
4-Amino-6-fluoro-5-nitropyrimidine (III).—4,6-Difluoro-5-nitropyrimidine (150 ml.) was diluted with 2.5 l. of anhydrous ether, filtered and into the stirred filtrate (at −55 to −65°) was bubbled anhydrous ammonia until a drop of the reaction mixture gave a basic reaction with pH "Hydrion" paper. The yellow solid was filtered, washed with ether, boiled with 4 l. of anhydrous benzene, and filtered. From the filtrate crystallized 38 g. (24%) (including a second crop) of III, m.p. 161–163° (sublimation).

Anal. Calcd. for $C_4H_2FN_4O_2$: C, 30.4; H, 1.9; F, 12.0; N, 35.4. Found: C, 30.2; H, 1.2; F, 12.0; N, 35.6.

4,5-Diamino-6-fluoropyrimidine (IV). Method A.—A solution of 10 g. of 4-amino-6-fluoro-5-nitropyrimidine in methanol was hydrogenated at room temperature in a Parr shaker with 2.46–2.8 kg. of hydrogen per cm.^{2} using Raney

(17) All melting points were taken on a Fisher–Johns melting point apparatus.

TABLE I
ULTRAVIOLET ABSORPTION OF SOME FLUOROPYRIMIDINES



		MeOH		pH 1		pH 11	
R_1	R_2	λ_{max}, mμ	ε	λ_{max}, mμ	ε	λ_{max}, mμ	ε
NH_2	NO_2	324	4,800	278	4,300	278	6,500
				326	7,100	340	11,100
NH_2	NH_2	259	9,350	263	9,100	254	8,350
		273	9,380	292	9,100	268	8,380
NH_2	$N{=}CHOC_2H_5$	255	8,300	265	7,800	254	9,100
		278	6,250	293	7,550	270–275[a]	6,800
$N{=}CHN(CH_3)_2$	$N{=}CHN(CH_3)_2$	252	19,300	223	26,100	247	18,000
		267	18,900	250–260[a]	18,200	257–262[a]	16,700
		308	22,700	300–308[a]	6,000	310	22,000
F	NH_2	242	14,600	239	13,200	238	14,500
		262–274[a]	5,100	260–270[a]	4,800	262–272[a]	5,200
	NH_2	274	12,900	272	11,700	264	13,400
				280–295[a]	11,100		
$NHCH_3$	NH_2	271	12,600	269	11,000	261	12,100
				278–285[a]	10,500		

[a] Shoulder.

nickel catalyst. After 30 min. the nickel was filtered and the filtrate evaporated to give 7.4 g. (90%) of crude IV. This was recrystallized from boiling water (charcoal) to give 6.4 g. of pale-yellow needles, m.p. 218–220° (sublimation).

Anal. Calcd. for $C_4H_5FN_4$: C, 37.5; H, 3.9; F, 14.8; N, 43.7. Found: C, 37.7; H, 4.0; F, 15.0; N, 43.5.

Method B.—5-Amino-4,6-difluoropyrimidine (VII, 0.9 g.) was dissolved in 100 ml. of absolute ethanol. Ammonia gas was bubbled into the solution for 10 min., and the solution was refluxed with ammonia bubbling through it for 4 hr. The solution was evaporated and the solid suspended in a small amount of water to give 0.4 g. of crude product which was sublimed and recrystallized from water to give colorless needles of IV, m.p. 215–219°, undepressed with material prepared by method A. The ultraviolet absorption spectrum of the product was identical with that of the material prepared by reduction of III.

4-Amino-5-ethoxymethyleneamino-6-fluoropyrimidine (V).—Two grams of 4,5-diamino-6-fluoropyrimidine was boiled with 30 ml. of ethyl orthoformate until the temperature reached 145° and for 15 min. thereafter. The ethyl orthoformate was removed under vacuum to give 2.3 g. (80%) of crude V. Two recrystallizations from benzene gave colorless needles, m.p. 122–123°.

Anal. Calcd. for $C_7H_9FN_4O$: C, 45.7; H, 4.9; F, 10.3; N, 30.4. Found: C, 45.7; H, 4.8; F, 10.4; N, 30.3.

4,5-Bis(dimethylaminomethyleneamino)-6-fluoropyrimidine (VI).—4,5-Diamino-6-fluoropyrimidine (0.20 g.) was dissolved in 1 ml. of N,N-dimethylformamide at room temperature. Phosphorus oxychloride (0.25 ml.) was added dropwise over a period of 10 min. at 25–35°. After 1 hr. at room temperature the reaction mixture was poured on ice and the pH adjusted to 10 with ammonia and then to 6.5 with acetic acid. The solution was extracted with ethyl acetate to give 0.19 g. of crystalline solid. Recrystallization from benzene–petroleum ether gave needles, m.p. 116–118°.

Anal. Calcd. for $C_{10}H_{15}FN_6$: C, 50.4; H, 6.3; F, 8.0; N, 35.3. Found: C, 50.3; H, 5.2; F, 8.2; N, 35.9.

Reaction of 6-Chloro-4,5-diaminopyrimidine with Phosphorus Oxychloride and N,N-Dimethylformamide.—6-Chloro-4,5-diaminopyrimidine[15] (5.0 g.) was dissolved in 50 ml. of N,N-dimethylformamide at room temperature, and 10 ml. of phosphorus oxychloride was added slowly so that a reaction temperature of 85–90° was maintained. The mixture was allowed to stand for 1 hr.; the excess solvents were removed under vacuum and the syrup was decomposed with ice. The pH was adjusted to 10 with concd. potassium hydroxide solution and the solid collected and recrystallized from acetone to give 3.1 g. of 6-chloro-4,5-bis(dimethylaminomethyleneamino)pyrimidine, m.p. 95–100°.

Anal. Calcd. for $C_{10}H_{15}ClN_6$: C, 47.1; H, 5.9; Cl, 13.9; N, 33.0. Found: C, 47.2; H, 6.2; Cl, 13.7; N, 32.7.

The aqueous filtrate from the crude 6-chloro-4,5-bis(dimethylaminomethyleneamino)pyrimidine was adjusted to pH 3 with hydrochloric acid and the solid which formed recrystallized from acetone to give 0.7 g. of 6-chloropurine identified by its characteristic ultraviolet absorption spectrum.[14]

5-Amino-4,6-difluoropyrimidine (VII).—4,6-Difluoro-5-nitropyrimidine (about 55 ml.) from 67 g. of 4,6-dichloro-5-nitropyrimidine[9] and tetralin, in lieu of nitrobenzene as the solvent, was reduced with hot aqueous ferrous hydroxide, using the method described by Brown[16] for the reduction of 4,6-dichloro-5-nitropyrimidine, to give 11.5 g. (25%) of crude product (extracted with ethyl acetate).

This was recrystallized from absolute ethanol to give VII as flakelets, m.p. 157–159°.

Anal. Calcd. for $C_4H_3F_2N_3$: C, 36.7; H, 2.3; F, 29.0; N, 32.1. Found: C, 37.0; H, 2.0; F, 28.8; N, 32.3.

5-Amino-4-cyclopentylamino-6-fluoropyrimidine (IX).—5-Amino-4,6-difluoropyrimidine (0.55 g.), ethanol (25 ml.), and cyclopentylamine (2 ml.) (Aldrich Chemical Co.) were refluxed 2 hr. and evaporated to a gum which was triturated with water. A yellow solid formed, wt. 0.70 g. (85%). Recrystallization from water gave flat needles, m.p. 125–127°.

Anal. Calcd. for $C_9H_{13}FN_4$: C, 55.1; H, 6.7; F, 9.7; N, 28.5. Found: C, 55.2; H, 6.5; F, 10.0; N, 28.4.

9-Cyclopentyl-6-fluoropurine (XI).—A solution of 0.47 g. of 5-amino-4-cyclopentylamino-6-fluoropyrimidine (IX) in 7 ml. of 2:1 (v./v.) ethyl orthoformate:acetic anhydride (aged one year) was boiled in an open flask for 20 min. The excess solvent was removed *in vacuo*, and the residue was dissolved in benzene–heptane and allowed to evaporate to give a low-melting solid which was recrystallized from low-boiling petroleum ether to give 150 mg. of a crystalline solid, m.p. 55°. This material was further purified by dissolving it in benzene and passing it over a small column of Florisil to give a crystalline product, m.p. 55–58°. A qualitative test for fluorine was positive.

Anal. Calcd. for $C_{10}H_{11}FN_4$: C, 58.2; H, 5.4. Found: C, 57.6; H, 6.1. Ultraviolet absorption in methanol: λ_{max} 243, 275 (shoulder) mμ, ϵ 16,480, 3,090.

5-Amino-6-fluoro-4-methylaminopyrimidine (VIII).—5-Amino-4,6-difluoropyrimidine (5.0 g.) was dissolved in 500 ml. of absolute ethanol, and the solution was refluxed with a stream of anhydrous methylamine bubbling through it for 1.5 hr. The solution was evaporated to dryness and the solid leached with a little water to give 4.5 g. (83%) of crude VIII. Sublimation gave colorless needles, m.p. 142–144°.

Anal. Calcd. for $C_5H_7FN_4$: C, 42.3; H, 5.0; F, 13.4; N, 39.4. Found: C, 42.5; H, 5.4; F, 13.7; N, 39.6.

6-Fluoro-9-methylpurine (X).—A solution of 9.8 g. of 5-amino-6-fluoro-4-methylaminopyrimidine in 125 ml. of 2:1 (v./v.) ethyl orthoformate:acetic anhydride was boiled for 12 min. and the excess solvent removed *in vacuo*. The resulting solid was recrystallized from benzene–heptane to give 7 g. (67%) of colorless needles, m.p. 135–136°.

Anal. Calcd. for $C_6H_5FN_4$: C, 47.4; H, 3.3; F, 12.5; N, 36.8. Found: C, 47.2; H, 3.1; F, 12.8; N, 36.9.

Ultraviolet absorption spectra of 6-fluoro-9-methylpurine: in methanol, λ_{max} 248 mμ, ϵ 8,350; at pH 1, λ_{max} 249 mμ, ϵ 9,150; at pH 11, λ_{max} 259 and 264-267 (shoulder) mμ, ϵ 11,800 and 11,100. The 264 to 267 mμ shoulder at pH 11 appears to be due to a product with base since it increases to a sharp peak on standing at pH 11.

9-Methyladenine.—6-Fluoro-9-methylpurine (0.28 g.) and 10 ml. of aqueous ammonia solution (29% ammonia) were heated in a boiling water bath for 45 min. Every 6–8 min. an additional 5 ml. of concd. aqueous ammonia was added. The hot solution was cooled to give tiny rods, m.p. 310° (preceded by sublimation). A mixture melting point of this product with an authentic sample of 9-methyladenine[1b] showed no depression. The ultraviolet and infrared absorption spectra of the product were identical with those of 9-methyladenine, as were the R_f values in two different solvent systems.

9-Methylhypoxanthine.—6-Fluoro-9-methylpurine (X, 0.15 g.) was dissolved in 5 ml. of distilled water at room temperature. After 7 days crystals formed. The product was identified as 9-methylhypoxanthine by its characteristic ultraviolet spectrum.[1b]

Anal. Calcd. for $C_6H_6N_4O$: C, 48.0; H, 4.0. Found: C, 47.7; H, 4.5.

The Antitumor Activity of 2-Amino-6-alkylthio-9-(β-D-ribofuranosylpurines and related Derivatives of 2-Amino-6-purinethiol (Thioguanine)[1]

C. WAYNE NOELL and ROLAND K. ROBINS

Department of Chemistry, Arizona State University, Tempe, Arizona

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A number of 6-alkylthio-2-aminopurines and their ribosides have been prepared and tested against Adenocarcinoma 755. Alkylation of 2-amino-9-β-D-ribofuranosyl-6-purinethiol with the appropriate alkyl halide gave the desired riboside derivatives. Many of these compounds exhibit excellent activity against Adenocarcinoma 755 and significant activity against Sarcoma 180 and Leukemia 1210.

The antitumor activity of a number of 9-alkyl-2-amino-6-purinethiols[2] and 6-alkylthio-2-aminopurines[3] against Adenocarcinoma 755 suggested the synthesis of the corresponding 6-alkylthio-2-aminopurine-9-ribosides. The possibility of the increased antitumor activity of the 6-alkylthio-2-amino-9-β-D-ribofuranosylpurines was suggested by the fact that 9-β-D-ribofuranosyl-6-purinethiol possesses a therapeutic index[4] of 200 as compared to 30 for 6-mercaptopurine against Adenocarcinoma 755. 6-Mercaptopurine ribonucleoside is also inhibitory at a lower dosage[4] than 6-mercaptopurine in Adenocarcinoma 755. In addition, 2-amino-9-β-D-ribofuranosyl-6-purinethiol (thioguanosine) given orally in the clinic is more active on a

(1) This investigation was supported by Contract No. SA-43-ph-1928 with the Cancer Chemotherapy National Service Center of the National Cancer Institute, National Institutes of Health, U. S. Public Health Service.

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