Pyridine Nucleosides Related to 5-Fluorocytosine (1)

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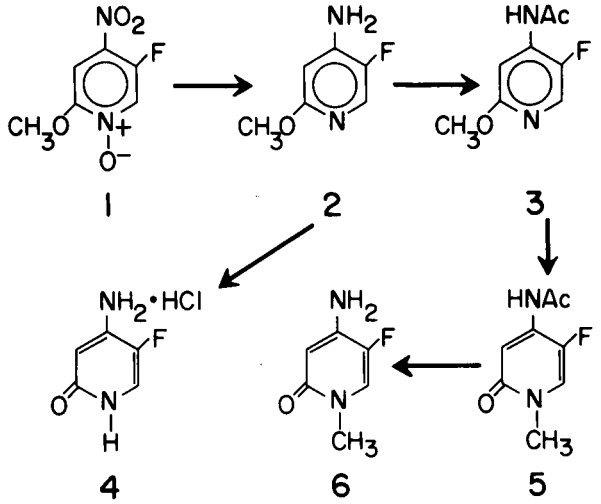
4-Amino-5-fluoro-2-pyridone (**4**) [5-fluoro-3-deazacytosine] was isolated as the hydrochloride salt from the dealkylation of 4-amino-5-fluoro-2-methoxypyridine (**2**), which was obtained from the reduction of 5-fluoro-2-methoxy-4-nitropyridine-*N*-oxide (**1**). Acetylation of **2** gave 4-acetamido-5-fluoro-2-methoxypyridine (**3**), which was condensed with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide to give the blocked nucleoside (**8**). Removal of the protecting groups gave 5-fluoro-3-deazacytidine. Fusion of the trimethylsilyl derivative of **4** (10), with 2-deoxy-3,5-di-*O*-*p*-toluoyl-D-*erythro*pentofuranosyl chloride gave a mixture of the β and α-anomers **12** and **13**, which were separated and deblocked to yield 5-fluoro-2′-deoxy-3-deazacytidine (**14**) and its α-anomer (**15**). Several alkylated and acetylated derivatives of **2** were prepared as model compounds for use in the proof of structure.

In a continuing program to explore the effect of structural modifications on the activity of tumor-inhibitory drugs (3), we undertook to synthesize the 3-deaza derivatives of 5-fluorocytosine nucleosides.

The 3-deazapyrimidine nucleosides of uracil (4), cytosine (4), 5-fluorouracil (5a), thymine (5a,b) and 5-fluoro-2-pyrimidone (3) have been reported. 3-Deazauridine and 3-deazacytidine are cytotoxic both to bacteria and mammalian cells in culture (6) and the former compound has been found to have anti-RNA viral activity (7). 5-Fluorouracil has been proved clinically effective in the treatment of solid tumors (8), while 5-fluoro-2′-deoxycytidine has been shown to be deaminated to 5-fluoro-2′-deoxyuridine, a nucleoside more effective than 5-fluorouracil in inhibiting the growth of tumor cells in culture (9,10).

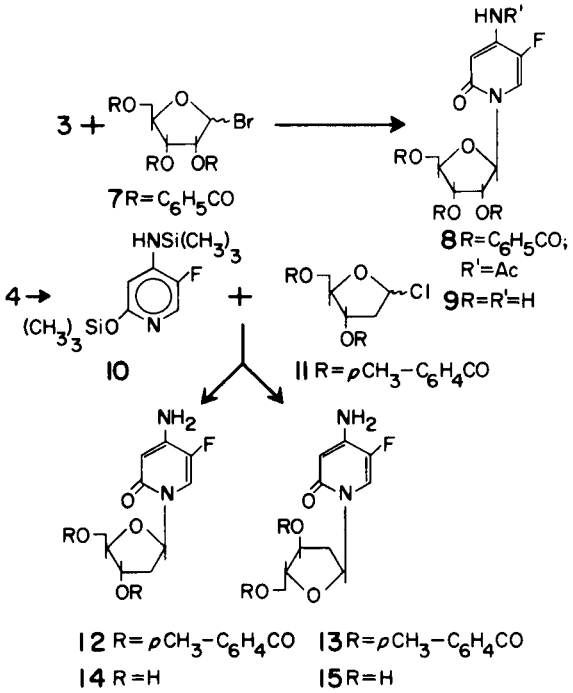
5-Fluoro-2-methoxy-4-nitropyridine-*N*-oxide (**1**) (3) was easily reduced by the action of zinc and hydrochloric acid to 4-amino-5-fluoro-2-methoxypyridine (**2**) in 86% yield (Scheme 1). Acetylation of **2** with acetic anhydride gave the protected base, 4-acetamido-5-fluoro-2-methoxypyridine (**3**). Dealkylation of **2** with 25% hydrochloric acid gave the heterocycle, 4-amino-5-fluoro-2-pyridone [5-fluoro-3-deazacytosine] (**4**) as the hydrochloride. The yield was 88%, in striking contrast to the low yield reported for the dealkylation of 2,4-dialkoxypyridines using the same procedure (5a).

Reference compounds needed for the uv spectral analyses of structure were prepared in the following manner. Reaction of **3** with methyl iodide gave the *N*-methyl isomer, 4-acetamido-5-fluoro-1-methyl-2-pyridone (**5**), and subsequent hydrolysis gave 4-amino-5-fluoro-1-methyl-2-pyridone (**6**).



Condensation of **3** with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide (**7**) in acetonitrile gave the protected ribonucleoside **8**, which upon removal of the blocking groups with sodium methoxide gave 4-amino-5-fluoro-1-(β-D-ribofuranosyl)-2-pyridone [5-fluoro-3-deazacytidine] (**9**) in 33% overall yield (based on **3**) (Scheme 2).

The 2′-deoxyribonucleosides of 4-fluoro-3-deazacytosine were prepared by a fusion reaction with the trimethylsilyl derivative of **4** (10) and 2-deoxy-3,5-di-*O*-*p*-toluoyl-D-



*erythro*pentofuranosyl chloride (**11**) (11) at 120°. The overall yield was 28% (based on **4**) and the ratio of α and β anomers was 1:1. The mixture of anomers was separated by chromatography on silica, and each was deblocked with sodium methoxide to give 4-amino-5-fluoro-1-(2-deoxy-β-D-*erythro*pentofuranosyl)-2-pyridone[5-fluoro-2′-deoxy-3-deazacytidine] (**14**) and the α-anomer (**15**).

The site of glycosylation in compounds **9**, **14** and **15** is assigned to N-1. C-glycosylation can be excluded due to the presence of two aromatic ring protons (by pmr) in each product. The similarity of the uv spectra (Table I) of compounds **9**, **14** and **15** with **6** in both methanol and 1 *N* sodium hydroxide, and the dissimilarity of those spectra with those of **4** or **2** indicate that glycosylation on the 4-amino or 2-oxygen had not occurred.

Ribonucleoside **9** was assigned a β configuration based on the method of synthesis (3,5a), the pmr spectra of the anomeric proton (3,5a) and the circular dichroism spectrum which exhibits a positive Cotton effect centered at 263 nm. Closely related nucleosides such as 5-fluoro-3-deazauridine, 3-deazathymidine (5a), 5-fluoro-4-methoxy-3-deazauridine, 4-deoxy-5-fluoro-3-deazauridine (3), 3-deazauridine, 3-deazacytidine (4) as well as uridine and cytidine (12), all exhibit positive Cotton effects in their circular dichroism spectra.

The deoxyribonucleosides (**14**, **15**) were assigned β and α configurations respectively, based on the pmr spectra of the anomeric protons: **14**, triplet, peak width = 14.6 Hz: **15**, pair of doublets, peak width = 6.9 Hz (13) and the Cotton effects in their circular dichroism spectra.

These compounds are presently being tested for biological activity, and the results will be reported elsewhere.

EXPERIMENTAL

Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Uv spectra were recorded on a Gilford Model 2400S or a Beckman Model 25 spectrophotometer. Pmr spectra were obtained on a Perkin-Elmer R-12 using tetramethylsilane as internal reference. Circular dichroism spectra were obtained in methanol on a Cary spectropolarimeter Model 60. Elemental analyses were performed by Spang Micro-analytical Laboratory, Ann Arbor, Michigan. Analytical tlc was performed on Eastman chromatoplates.

4-Amino-5-fluoro-2-methoxypyridine (**2**).

5-Fluoro-2-methoxy-4-nitropyridine *N*-oxide (**1**) (3) [2.00 g., 0.011 mole] was dissolved in 50 ml. of methanol and 60 ml. of 19% hydrochloric acid, whereupon 28 g. of zinc dust was added in small portions. The reaction mixture was filtered, the filtrate evaporated to remove methanol and the *p*H adjusted to 7 with sodium hydroxide. The neutralized solution was extracted exhaustively with chloroform and the combined extracts dried over anhydrous sodium sulfate and evaporated. Recrystallization from chloroform-Skellysolve B (7:3) gave 1.30 g. (86%) of **2** as colorless crystals, m.p. 96-98°; uv: λ max (methanol): 275 nm (ε, 4,110), pmr (deuteriochloroform): τ 2.33 (d, 1, $J_{6\text{-}F}$ = 2.9 Hz, H-6), 4.05 (d, 1, $J_{3\text{-}F}$ = 6.4 Hz, H-3), 5.70 (br.s, 2, $-NH_2$), 6.26 (s, 3, OCH_3).

Anal. Calcd. for $C_6H_7FN_2O$: C, 50.70; H, 4.93; N, 19.72; F, 13.38. Found: C, 50.78; H, 4.88; N, 19.77; F, 13.44.

Table I

Compound	Maximum uv absorption (nm) in 0.1*N* HCl	in MeOH	in 1*N* NaOH
4-Amino-5-fluoro-2-methoxypyridine (**2**)	257	275	272
4-Acetamido-5-fluoro-2-methoxypyridine (**3**)	258	262; 280 (sh)	272
4-Acetamido-5-fluoro-1-methyl-2-pyridone (**5**)	258	256	276; 284 (sh)
4-Amino-5-fluoro-1-methyl-2-pyridone (**6**)	260	263; 286 (sh)	260; 285 (sh)
5-Fluoro-3-deazacytosine (**4**)	260	258; 275 (sh)	288
5-Fluoro-3-deazacytidine (**9**)	264	264; 288 (sh)	262; 288 (sh)
5-Fluoro-2′-deoxy-3-deazacytidine (β-anomer) (**14**)	264	264; 288 (sh)	262; 288 (sh)
5-Fluoro-2′-deoxy-3-deazacytidine (α-anomer) (**15**)	264	264; 288 (sh)	262; 288 (sh)

4-Acetamido-5-fluoro-2-methoxypyridine (**3**).

A mixture of **2** (1.20 g., 0.0845 mole) and 10 ml. of acetic anhydride was refluxed for 30 min., evaporated to a solid, and recrystallized from water to yield 1.18 g. (76%) of **3** as colorless needles, m.p. 145-146°; uv: λ max (methanol): 262 nm, (ε, 6,500), 286 nm (sh) (ε, 4,540); pmr (deuteriochloroform): τ 2.02 (d, 1, $J_{6\text{-}F}$ = 2.8 Hz, H-6), 2.19 (d, 1, $J_{3\text{-}F}$ = 4.4 Hz, H-3), 6.12 (s, 3, OCH_3), 7.75 (s, 3, C-CH_3).

Anal. Calcd. for $C_8H_9FN_2O_2$: C, 52.17; H, 4.89; N, 15.22; F, 10.33. Found: C, 52.13; H, 4.86; N, 15.15; F, 10.36.

4-Amino-5-fluoro-2-pyridone Hydrochloride (**4**).

After heating a solution of **2** (1.26 g., 0.0887 mole) with 5 ml. of 25% hydrochloric acid in a sealed tube at 145° for 5 hours, the reaction mixture was evaporated to dryness. Recrystallization from methanol-ether (6:3) gave 1.29 g. (88%) of **4** as a colorless powder, m.p. 201-205°; uv: λ max (*p*H 1.0): 260 nm (ε, 15,476); pmr (DMSO-d_6): τ 1.95 (d, 1, $J_{6\text{-}F}$ = 5.8 Hz, H-6), 3.46 (d, 1, $J_{3\text{-}F}$ = 7.5 Hz, H-3).

Anal. Calcd. for $C_5H_5FN_2O$·HCl: C, 36.48; H, 3.65; N, 17.03; F, 11.55; Cl, 21.56. Found: C, 36.49; H, 3.66; N, 16.97; F, 11.54; Cl, 21.60.

4-Acetamido-5-fluoro-1-methyl-2-pyridone (**5**).

4-Acetamido-5-fluoro-2-methoxypyridine (**3**) [500 mg., 2.72 mmoles] was heated at 80° for 12 hours in a sealed tube with 5 ml. of methyl iodide. After evaporation *in vacuo*, the residue was triturated with cold chloroform and recrystallized with methanol to yield 355 mg. (71%) of **5** as colorless plates, m.p. 299-300°; uv: λ max (methanol): 256 nm (ε, 15,530); pmr (trifluoroacetic acid): τ 1.34 (d, 1, $J_{6\text{-}F}$ = 6.4 Hz, H-6), 1.87 (d, 1, $J_{3\text{-}F}$ = 4.9 Hz, H-3), 6.02 (s, 3, N-CH_3), 7.50 (s, 3, C-CH_3).

Anal. Calcd. for $C_8H_9FN_2O_2$: C, 51.27; H, 4.89; N, , 5.22; F, 10.35. Found: C, 52.21; H, 4.91; N, 15.23; F, 10.33.

4-Amino-5-fluoro-1-methyl-2-pyridone (**6**).

A mixture of **4** (79 mg., 0.43 mmoles) and 20 ml. of 2 *N* sodium hydroxide was refluxed for 4 hours. Exhaustive extraction with chloroform and recrystallization from methanol-chloroform-Skellysolve B (1:3:7) afforded 48 mg. (79%) of **6** as a colorless powder, m.p. 216-218°; uv: λ max (methanol): 264 nm (ε, 8,690) 288 nm (sh) (ε, 5,630); pmr (deuteriomethanol): τ 2.62 (d, 1, $J_{6\text{-}F}$ = 6.5 Hz, H-6), 4.41 (d, 1, $J_{3\text{-}F}$ = 8.5 Hz, H-3), 6.70 (s, 3, N-CH_3).

Anal. Calcd. for $C_6H_7FN_2O$: C, 50.70; H, 4.93; N, 19.72; F, 13.38. Found: C, 50.62; H, 5.31; N, 19.61; F, 13.25.

4-Acetamido-5-fluoro-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2-pyridone (**8**).

A mixture of **3** (1.104 g., 6 mmoles), **7** [prepared from 3.32 g. (6.6 mmoles) of 1-*O*-acetyl-2,3,5-tri-*O*-benzoylribofuranose], 50 ml. of dry acetonitrile and 1 g. of Linde molecular sieves 3A was sealed under nitrogen and stirred 4 days at room temperature. The reaction mixture was filtered through Celite, the filtrate evaporated to an oil and recrystallized from ethanol-chloroform to give 1.39 g. (38%) of **8** as colorless stout needles m.p. 132-135°.

Anal. Calcd. for $C_{33}H_{27}FN_2O_9$: C, 64.50; H, 4.40; N, 4.56; F, 3.09. Found: C, 64.53; H, 4.22; N, 4.54; F, 3.00.

4-Amino-5-fluoro-1-(β-D-ribofuranosyl)-2-pyridone (**9**).

To a solution of sodium (260 mg., 11.3 g.-atoms) in 50 ml. of dry methanol was added **8** (1.39 g., 2.26 mmoles) and the mixture stirred at room temperature for 3 days. The *p*H was adjusted to **5** with glacial acetic acid and the reaction mixture evaporated to an oil. The oil was partitioned between water and ether and extracted with ether (3 x 50 ml.). The aqueous layer was reduced in volume and placed on a BioRad-70 (H^+-form) column. Fractions were monitored at 264 nm and those containing the product were pooled, evaporated and recrystallized from methyl acetate-methanol (1:1) to yield 519 mg. (88%) of **9** as microcrystals, m.p. 92-94°; uv: λ max 264 nm (ε, 11,450), 287 nm (sh) (ε, 6,300); pmr (DMSO-d_6): τ 2.14 (d, 1, $J_{6\text{-}F}$ = 7.8 Hz, H-6), 3.95 (s, 1, H-1^1), 4.34 (d, 1, $J_{3\text{-}F}$ = 8.0 Hz, H-3); [θ] 28° 263 nm + 19,870.

Anal. Calcd. for $C_{10}H_{13}FN_2O_5$: C, 46.15; H, 5.00; N, 10.77; F, 7.31. Found: C, 46.07; H, 5.09; N, 10.38; F, 6.98.

4-Amino-5-fluoro-1-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*pentofuranosyl)-2-pyridone (**12**) and 4-Amino-5-fluoro-1-(2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*pentofuranosyl)-2-pyridone (**13**).

A mixture of **4** (493 mg., 3 mmoles), hexamethyldisilazane (5 ml.) and trimethylchlorosilane (0.5 ml.) was refluxed 2 hours and then evaporated *in vacuo* to yield 5-fluoro-4-trimethylsilylamino-2-trimethylsilyloxypyridine (**10**) as a crude oil (pmr exhibited a ratio of aromatic to methyl protons of 1:9). This oil was fused with **11** (1.16 g., 3 mmoles) under vacuum at 120° for 25 minutes. The reaction mixture was then treated with methanol and chromatographed on a silica-gel column (3 cm X 50 cm) using ethyl acetate as elution solvent. The early fractions containing sugar decomposition products were discarded. The β-anomer (**12**) eluted next and was recrystallized from chloroform-heptane to yield 190 mg. (13%), m.p. 188-189°.

Anal. Calcd. for $C_{26}H_{25}FN_2O_6$: C, 65.00; H, 5.21; N, 5.83; F, 3.93. Found: C, 64.99; H, 5.18; N, 5.83; F, 3.90.

The α-anomer (13) eluted soon after the β-anomer and was collected to yield 230 mg. (15%), m.p. 99-191°.

Anal. Found: C, 65.13; H, 5.06; N, 5.84; F, 3.96.

4-Amino-5-fluoro-1-(2-deoxy-β-D-*erythro*pentofuranosyl)-2-pyridone (**14**).

To a solution of sodium (83 mg., 3.6 g.-atoms) in dry methanol (50 ml.) was added **12** (430 mg., 0.90 mmole), and the mixture stirred at room temperature for 2 days. The deblocked nucleoside (**14**) was isolated using the same work-up procedure as described for **9** and 196 mg. (89%); m.p. 194.5-197° was obtained; uv: λ max (methanol): 264 nm (ε, 10,800), 287 nm (sh) (ε, 5,900); pmr (DMSO-d_6): τ 2.22 (d, 1, $J_{6\text{-}F}$ = 7.2 Hz, H-6), 3.65 (t, 1, $J_1{}^1{}_{,2}{}^1$; $_2{}^{11}$ = 7.0 Hz, H-1^1), 4.34 (d, $J_{3\text{-}F}$ = 8.4 Hz, H-3); [θ] 28° 263 nm + 14,930.

Anal. Calcd. for $C_{10}H_{13}FN_3O_4$: C, 49.18; H, 5.53; N, 11.48; F, 7.79. Found: C, 49.18; H, 5.30; N, 11.88; F, 7.80.

4-Amino-5-fluoro-1-(2-deoxy-α-D-*erythro*pentofuranosyl)-2-pyridone (**15**).

To a solution of sodium (115 mg., 5 g.-atoms) in 50 ml. of dry methanol was added **13** (520 mg., 1 mmole) and the mixture stirred at room temperature for 4 days. A yield of 207 mg. (85%) of **15**, m.p. 94-96° was obtained, using an isolation procedure similar to that described for **9**; uv: λ max (methanol): 264 nm (ε, 9,460), 287 nm (sh) (ε, 4,900); pmr (DMSO-d_6): τ 2.50 (d, 1, $J_{6\text{-}F}$ = 8.0 Hz, H-6), 3.71 (d of d, 1, $J_1{}^1{}_{,2}{}^1{}_{,2}{}^{11}$ = 5.5 and 1.0 Hz, H-1^1), 4.73 (d, 1, $J_{3\text{-}F}$ = 8.5 Hz, H-3); [θ] 28° 263 nm -7560.

Anal. Calcd. for $C_{10}H_{13}FN_2O_4$: C, 49.18; H, 5.33; N, 11.48; F, 7.79. Found: C, 49.05; H, 5.51; N, 11.13; F, 7.43.

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