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SYNTHESIS OF A CYTOSINE NUCLEOSIDE OF 2-AMINO-2-DEOXY-β-D-XYLOFURANOSE*

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ABSTRACT

1-(2-Amino-2-deoxy-β-D-xylofuranosyl)cytosine (**13**) was synthesized by three routes: (*a*) coupling of 2-deoxy-3,5-di-*O*-*p*-nitrobenzoyl-2-(trifluoroacetamido)-D-xylofuranosyl chloride (**5**) with 2,4-dimethoxypyrimidine and subsequent treatment with methanolic ammonia, (*b*) coupling of **5** with 4-*N*-acetyl-2-*O*,4-*N*-bis(trimethylsilyl)cytosine followed by treatment with methanolic ammonia, and (*c*) thiation of 1-[3,5-di-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-β-D-xylofuranosyl]uracil (**6**) by treatment with phosphorus pentasulfide in pyridine followed by amination of the resulting 4-thionucleoside **12** with methanolic ammonia. The best yield was obtained *via* route (*a*).

INTRODUCTION

Nucleosides of 2-amino-2-deoxy-D-xylofuranose have been of particular interest in this laboratory as possible antitumor agents, because of the biological activity of 9-β-D-xylofuranosyladenine against tumor cells[1]. The syntheses of adenine, thymine, and uracil nucleosides of this sugar have already been reported[2]. An antitumor nucleoside recently[3] described has been shown to be a 9-(2-amino-2-deoxypentofuranosyl)guanine.‡ The present article describes the synthesis of a cytosine nucleoside of 2-amino-2-deoxy-β-D-xylofuranose.

DISCUSSION

The starting material for this synthesis was ethyl 2-deoxy-1-thio-2-(trifluoroacetamido)-α-D-xylofuranoside (**1**), readily obtainable from 2-amino-2-deoxy-D-glucose in five steps[2b]. Compound **1** was treated either with acetic anhydride[2b] or

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‡Note added in proof: The pentose has been shown to have the β-D-*ribo* configuration: T. Nakanishi, T. Iida, F. Tomita, and A. Furuya, *Abstr. Papers*, II, 95th Meeting Pharmaceutical Soc. Japan, Nishinomiya, 1975, p. 275.

with *p*-nitrobenzoyl chloride in pyridine to give the 3,5-diacetate (**2**) or 3,5-di-*p*-nitrobenzoate (**3**), respectively. Treatment of **2** and **3** with chlorine in dichloromethane gave the corresponding glycosyl chlorides (**4** and **5**).

In order to obtain nucleosides in high yield, the conditions for coupling between the glycosyl chlorides and 2,4-bis(trimethylsilyloxy)pyrimidine, as a model reaction, were investigated. The first factor examined was the effect of the acetyl and *p*-nitrobenzoyl groups on the yield. When fused with 2,4-bis(trimethylsilyloxy)-pyrimidine followed by isolation by t.l.c., compound **5** gave the protected nucleoside **7** in 70% yield, whereas **4** under similar conditions gave **6** in 43% yield (Wolfrom and Conigliaro[2b] reported a yield of 48%). The procedural superiority of the *p*-nitrobenzoyl group over the acetyl group was thus demonstrated. In view of the reports[4,5] that pyrimidine nucleosides may be obtained in good yield by reaction at room temperature in inert solvents, the conventional fusion method was compared, as regards yield, with the coupling reaction in dichloromethane and in acetonitrile. The results are shown in Table I. It is evident from Table I that the fusion method gave yields superior to those obtained by coupling in solution.

TABLE I

YIELDS IN THE COUPLING REACTION OF 2-DEOXY-3,5-DI-*O*-*p*-NITROBENZOYL-2-(TRIFLUOROACETAMIDO)-D-XYLOFURANOSYL CHLORIDE WITH VARIOUS PYRIMIDINE DERIVATIVES

Pyrimidine derivative	*Coupling conditions*	*Yield*[a] (%)
2,4-Diethoxy-	2 days at ~25° in CH_2Cl_2[b]	26
	fusion for 10 min at 100–110°	75
2,4-Dimethoxy-	4 days at ~25° in CH_3CN[b]	35
	4 days at ~25° in CH_2Cl_2[b]	52
	fusion for 10 min at 80–90°	79
2,4-Bis(trimethylsilyloxy)-	4 days at ~25° in CH_3CN[b]	49
	fusion for 10 min at 90°	70
	fusion for 5 min at 110–120°	68
4-*N*-Acetyl-2-*O*,4-*N*-bis(trimethylsilyl)cytosine	3 days at ~25° in CH_2Cl_2[b]	44
	fusion for 9 min at 110–120°	57

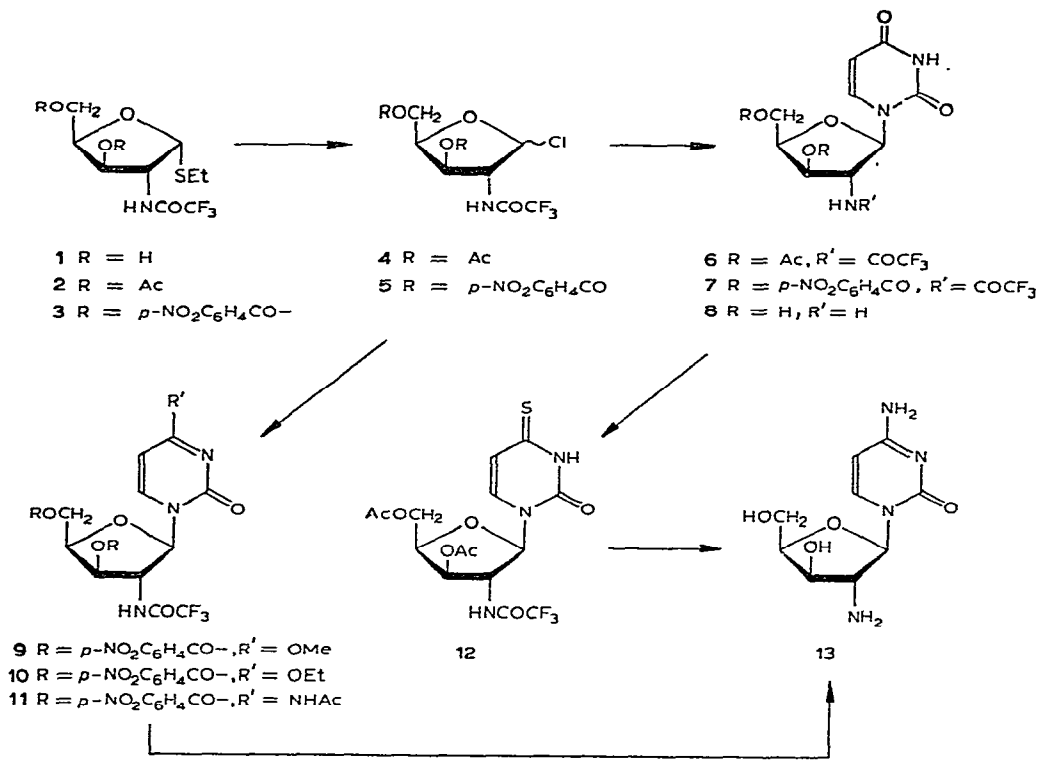
[a]Yield of the fully protected nucleosides (**7**, **9**, **10**, and **11**) from the 1-thioglycoside (**3**). [b]Reaction in the presence of molecular sieve, type 5A (Linde).

The cytosine nucleoside **13** may be prepared by several routes, of which the following three were considered.

(*a*) Coupling of **5** with 2,4-dimethoxypyrimidine, and subsequent treatment with methanolic ammonia to introduce an amino group at C-4 of the pyrimidine base.

(*b*) Coupling of **5** with 4-*N*-acetyl-2-*O*,4-*N*-bis(trimethylsilyl)cytosine, followed by removal of the protective groups.

(*c*) Condensation of the glycosyl chloride **4** with 2,4-bis(trimethylsilyloxy)-pyrimidine, followed by thiation, and subsequent amination at C-4 of the pyrimidine base.



As described here, route (*a*) gave **13** in the highest yield. Thus, the glycosyl chloride **5** was treated with 2,4-dimethoxypyrimidine for 10 min at 80–90° under diminished pressure, and the 4-methoxy nucleoside **9** was isolated after thin-layer chromatography (t.l.c.) on silica gel. The 4-ethoxy nucleoside **10** was also prepared, in similar yield. By heating with methanolic ammonia for 10 h at 90°, compound **9** was converted in 90% yield into the cytosine nucleoside **13**, which was isolated as its crystalline sulfate. The overall yield from the 1-thiofuranoside **3** was 59%.

The coupling reaction between the glycosyl chloride **5** and 4-*N*-acetyl-2-*O*,4-*N*-bis(trimethylsilyl)cytosine for 9 min at 110–120° gave, in 57% yield, the 4-*N*-acetylcytosine nucleoside **11**, which was then deprotected with methanolic ammonia to give **13**. Compound **13** was obtained as its crystalline dihydrochloride after chromatography on a column of AG-1 X2 (OH^-) resin. The overall yield from **3** was 45%. The u.v. and p.m.r. spectra of **13** sulfate obtained *via* route (*a*) were identical with those of **13** dihydrochloride prepared *via* route (*b*), thus supporting formulation as the N-1 glycosylated derivative.

Refluxing of 1-[3,5-di-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-β-D-xylofuranosyl]uracil (**6**), which was obtained by condensation of **4** with 2,4-bis(trimethylsilyloxy)pyrimidine, with phosphorus pentasulfide in pyridine for 4 h gave the 4-thio nucleoside **12** in 24% yield. The latter (**12**) was treated with methanolic ammonia for

10 h at 90°, and the cytosine nucleoside **13** was obtained in 71% yield (overall yield from **2**, 8%).

Assignment of the β configuration to the cytosine nucleoside (**13**) was made by comparing the sign of the Cotton effect associated with the B_{2u} electronic transition[6] with those of appropriate nucleosides of known configuration. The observed positive sign and amplitude of the Cotton effect of **13** at 272 nm were in accord with those found for 1-β-D-pentofuranosylcytosines[7].

It was noted throughout this work that only one anomer (β-D) was isolated from the reaction mixtures under various coupling conditions. No evidence was obtained for formation of the α-D anomer. The coupling of 2,3,5-tri-*O*-acylpentofuranosyl halides with 2,4-bis(trimethylsilyloxy)pyrimidine[7], 4-*N*-acetyl-2-*O*,4-*N*-bis(trimethylsilyl)cytosine[7], or 2,4-dialkoxypyrimidines[8] does not always obey the *trans* rule[9], and gives a mixture of both anomers in many instances. As far as 2-deoxy-2-(trifluoroacetamido) sugars are concerned, there has been no report of the isolation of 1,2-*cis* anomers by coupling of the sugar halides with either 2,4-bis(trimethylsilyloxy)-pyrimidines or 2,4-dialkoxypyrimidines, even though glycosyl halides prepared from the ethyl 1-thio-α- and -β-glycosides were used[10]. This complete conformance with the *trans* rule suggests that the 2-trifluoroacetamido group may be a stronger participating group than the 2-acyloxy group.

EXPERIMENTAL

General methods. — Melting points were determined with a Thomas–Hoover apparatus. Specific rotations were measured in a 2-dm polarimeter tube. I.r. spectra were recorded with a Perkin–Elmer Infracord spectrometer. U.v., o.r.d., and c.d. spectra were recorded with a Jasco ORD/UV-5 spectrometer. P.m.r. spectra were recorded with a Varian A-60A spectrometer; unless otherwise noted, solutions in deuterium oxide or chloroform-*d*, with an internal standard of sodium 4,4-dimethyl-4-silapentane-1-sulfonate or tetramethylsilane, respectively, were used. Microanalytical determinations were made by W. N. Rond. X-Ray powder diffraction data give interplanar spacings, Å, for CuKα radiation. Relative intensities were estimated visually: m, moderate; s, strong; w, weak. The strongest lines are numbered (1, strongest); multiple numbers indicate approximately equal intensities. T.l.c. was performed by the ascending method with Silica Gel G (E. Merck, Darmstadt, Germany) containing a 1:1 mixture of zinc orthosilicate and zinc sulfide (0.5%), with detection by ninhydrin for free amino compounds, by potassium permanganate for thio sugars, and by u.v. light for u.v.-absorbing materials. The indicated amounts of developing solvents are by volume. Unless otherwise noted, evaporations were performed under diminished pressure below 40°.

Ethyl 2-deoxy-3,5-di-O-p-*nitrobenzoyl-1-thio-2-(trifluoroacetamido)*-α-D-*xylofuranoside* (**3**). — A solution of ethyl 2-deoxy-1-thio-2-(trifluoroacetamido)-α-D-xylofuranoside[2b] (**1**, 923 mg) in pyridine (15 ml) was treated with *p*-nitrobenzoyl chloride (1.8 g) for 2 days at room temperature. Benzene was added, the resulting

precipitate was filtered off, and the filtrate was evaporated to dryness. The residue was extracted with a mixture of ethyl acetate and benzene, and the extract was successively washed with saturated, aqueous sodium hydrogen carbonate and water, dried (Drierite), and evaporated to a syrup that crystallized from benzene; yield, 1.78 g (95%). Recrystallization from benzene–cyclohexane gave an analytical sample of **3**, m.p. 115–118°, $[\alpha]_D^{21}$ +119° (*c* 1.02, chloroform), λ_{max}^{Nujol} 3.02, 3.27 (NH), 5.78 (CO–O), and 5.83 μm (CO–NH); p.m.r. data ($CDCl_3$): δ 8.37 (aromatic C*H*), 5.98 (H-3), 5.97 (H-1), 2.84 (CH_2–CH_3), 1.38 (CH_2–CH_3), $J_{1,2}$ 6.7, $J_{2,3}$ 6.4, and $J_{3,4}$ 6.4 Hz; (pyridine-d_5): δ 6.59 (H-3), 6.56 (H-1), $J_{1,2}$ 7.0, $J_{2,3}$ 5.0, and $J_{3,4}$ 5.0 Hz; X-ray powder diffraction data: 10.34 s (3), 8.18 m, 6.39 w, 5.68 m, 4.69 s (2), 4.07 s (1), and 3.59 m.

Anal. Calc. for $C_{23}H_{20}F_3N_3O_{10}S$: C, 47.02; H, 3.43; N, 7.15; S, 5.46. Found: C, 46.68; H, 3.47; N, 6.91; S, 5.41.

*1-[2-Deoxy-3,5-di-*O*-p-nitrobenzoyl-2-(trifluoroacetamido)-β-*D*-xylofuranosyl]-uracil* (**7**). — Dry chlorine gas was passed for 10 min into a solution of **3** (118 mg) in dichloromethane (10 ml), and then dry nitrogen gas was passed in for 10 min to remove the excess of chlorine. Evaporation of the solvent gave a clear syrup that was redissolved in dichloromethane (5 ml). To this solution was added 2,4-bis(trimethylsilyloxy)pyrimidine (300 mg), the solvent was removed by evaporation, and the residue was heated for 10 min at 90° under diminished pressure (water aspirator), and then kept overnight at room temperature. The mixture was extracted with hot acetone containing a small proportion of methanol, the extract was evaporated to dryness, and the crude product (279 mg) was purified by t.l.c. on a plate of silica gel, with 9:1 chloroform–*tert*-butyl alcohol as the developer. Compound **7** was obtained as an amorphous powder (88.5 mg, 70%)*; m.p. 105–110° (dec.), $[\alpha]_D^{21}$ +110° (*c* 1.49, acetone); λ_{max}^{Nujol} 3.10, 3.28 (NH), 5.78, and 5.89 μm (uracil, CO–O, CO–NH); λ_{max}^{MeOH} 257 nm (ε 35,000); c.d. data (MeOH): 267 nm ([θ] +30,000); p.m.r. data (acetone-d_6): δ 8.50, 8.45 (aromatic C*H*), 8.19 (H-6), 6.57 (H-1′), 6.32 (H-3′), 5.98 (H-5), $J_{1',2'}$ 5.6, and $J_{5,6}$ 8.2 Hz; (pyridine-d_5): δ 8.31 (aromatic C*H*), 8.27 (H-6), 6.97 (H-1′), 6.58 (H-3′), 6.08 (H-5), $J_{1',2'}$ 5.8, $J_{2',3'}$ 4.4, $J_{3',4'}$ 4.4, and $J_{5,6}$ 8.2 Hz.

Anal. Calc. for $C_{25}H_{18}F_3N_5O_{12}$: C, 47.10; H, 2.85; N, 10.99. Found: C, 47.65; H, 3.11; N, 11.20.

Compound **7** (128 mg) was converted into 1-(2-amino-2-deoxy-β-D-xylofuranosyl)uracil (**8**) in 80% yield by treatment overnight at room temperature with methanol (10 ml) saturated with ammonia. The crystalline dihydrochloride that was obtained after addition of hydrochloric acid had m.p. 249–250° (dec.), $[\alpha]_D^{21}$ +38° (*c* 0.70, water), and gave i.r. and o.r.d. spectra, and X-ray powder diffraction pattern identical to those of an authentic sample[2b].

*It was found that the protected nucleoside **7**, and compounds **9** and **11**, were partially methanolyzed under neutral conditions when the methanolic solutions were kept overnight at room temperature. From the reaction mixture was isolated methyl *p*-nitrobenzoate, and the formation of partially deprotected nucleosides was shown by t.l.c. on silica gel.

1-[3,5-Di-O-acetyl-2-deoxy-2-(trifluoroacetamido)-β-D-xylofuranosyl]-4-thiouracil (**12**). — A solution of 1-[3,5-di-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-β-D-xylofuranosyl]uracil[2b] (**6**) (0.8 g), phosphorus pentasulfide (1.6 g), and pyridine (15 ml) was refluxed for 4 h with stirring. The mixture, which turned dark, was poured into ice–water, and extracted with chloroform. The extract was evaporated to give a dark glass that was resolved on 12 thin-layer plates (40 × 40 cm) of silica gel with 2:1 chloroform–acetone as the developer. Extraction of the yellow band with acetone, and evaporation of the extract, gave the thio nucleoside (**12**), which crystallized from chloroform–methanol; yield, 200 mg (24%), m.p. 115–120° (dec., with sintering at 85°), $[\alpha]_D^{22}$ +64° (*c* 0.11, acetone); λ_{max}^{EtOH} 331 (ε 18,600) and 252 (sh) nm (5,700); c.d. data (acetone): 425 ([θ] +690) and 381 nm (−218); X-ray powder diffraction data: 11.62 s (1), 8.88 w, 7.96 w, 6.39 w, 5.88 m, 5.05 m (3), 4.73 m, 3.96 m (2), and 1.99 m.

Anal. Calc. for $C_{15}H_{16}F_3N_3O_7S$: C, 41.00; H, 3.67; N, 9.56. Found: C, 41.36; H, 3.87; N, 9.88.

1-[2-Deoxy-3,5-di-O-p-nitrobenzoyl-2-(trifluoroacetamido)-β-D-xylofuranosyl]-4-methoxy-(1H)-pyrimidin-2-one (**9**). — To a solution of 2-deoxy-3,5-di-*O*-*p*-nitrobenzoyl-2-(trifluoroacetamido)-D-xylofuranosyl chloride [**5**, prepared from the 1-thioglycoside **3** (114 mg) by the general procedure described for the preparation of **7**] in dichloromethane was added 2,4-dimethoxypyrimidine (189 mg), and the solvent was immediately removed by evaporation. The residue was heated for 10 min at 80–90° under diminished pressure, and kept overnight at room temperature, during which time crystals of the nucleoside were deposited. They were filtered off and washed with benzene. The crude, crystalline product and the mother liquor were separately chromatographed on plates of silica gel by developing with 9:1 chloroform–methanol. The nucleoside fractions were combined, and extracted with acetone. Evaporation of the extract yielded 112 mg (79%) of **9**, which was recrystallized from acetone; m.p. 227–228.5°, $[\alpha]_D^{21}$ +111° (*c* 0.88, acetone); λ_{max}^{Nujol} 3.13, 3.32 (NH), 5.80 (CO–O, CO–NH), 6.05, and 6.13 μm (pyrimidinone); λ_{max}^{MeOH} 260 nm (ε 29,000); c.d. data (MeOH): 277 ([θ] +56,000) and 240 (sh) nm; p.m.r. data (pyridine-d_5): δ 8.59 (H-6), 8.37 (aromatic C*H*), 7.08 (H-1′), 6.64 (H-3′), 6.23 (H-5), 3.91 (OCH_3), $J_{1',2'}$ 5.0, $J_{2',3'}$ 3.9, $J_{3',4'}$ 3.9, and $J_{5,6}$ 7.5 Hz; X-ray powder diffraction data: 12.90 m, 9.87 s (3), 6.73 w, 5.66 m, 4.94 m, 4.39 s (2), and 3.81 s (1).

Anal. Calc. for $C_{26}H_{20}F_3N_5O_{12}$: C, 47.93; H, 3.09; N, 10.75. Found: C, 47.45; H, 3.17; N, 10.50.

1-[2-Deoxy-3,5-di-O-p-nitrobenzoyl-2-(trifluoroacetamido)-β-D-xylofuranosyl]-4-ethoxy-(1H)-pyrimidin-2-one (**10**). — Compound **10** was prepared in 75% yield by fusion of 2,4-diethoxypyrimidine with the glycosyl chloride **5** under the same conditions as those described for the preparation of the methoxy analog (**9**); m.p. 226–227°, $[\alpha]_D^{21}$ +107° (*c* 0.60, acetone); p.m.r. data (pyridine-d_5): δ 8.59 (H-6), 8.36 (aromatic C*H*), 7.07 (H-1′), 6.63 (H-3′), 6.23 (H-5), 4.43 (CH_2–CH$_3$), 1.26 (CH$_2$–CH_3), $J_{1',2'}$ 5.0, $J_{2',3'}$ 3.9, $J_{3',4'}$ 3.9, and $J_{5,6}$ 7.6 Hz; X-ray powder diffraction data: 13.48 m, 10.27 s (2), 6.78 w, 5.94 s (2), 4.72 s, 4.33 w, 3.97 m, 3.62 s (1), and 3.36 s.

Anal. Calc. for $C_{27}H_{22}F_3N_5O_{12}$: C, 48.73; H, 3.33; N, 10.52. Found: C, 48.93; H, 3.53; N, 11.03, 9.90.

*4-Acetamido-1-[2-deoxy-3,5-di-*O*-p-nitrobenzoyl-2-(trifluoroacetamido)-β-D-xylofuranosyl]-(1H)-pyrimidin-2-one* (**11**). — To a solution of 2-deoxy-3,5-di-*O*-*p*-nitrobenzoyl-2-(trifluoroacetamido)-D-xylofuranosyl chloride (**5**), prepared from **3** (129 mg) by the same procedure as that described for preparation of **7**, was added 4-*N*-acetyl-2-*O*,4-*N*-bis(trimethylsilyl)cytosine (250 mg). The mixture was quickly evaporated, heated for 5 min at 110–120°, and cooled. Methanol was added, and the *N*-acetylcytosine that was deposited was filtered off. The filtrate was evaporated to dryness, and the residue chromatographed on a plate of silica gel, by developing twice with 10:1 chloroform–methanol. The nucleoside band was extracted with acetone, and evaporation of the extract afforded **11** as a clear syrup that crystallized from dichloromethane–benzene; yield, 84 mg (57%), m.p. 207.5–210.5°, $[\alpha]_D^{21}$ +83° (*c* 1.01, acetone); λ_{max}^{Nujol} 3.07, 3.27 (NH), 5.78, and 5.87 μm; λ_{max}^{MeOH} 296 (sh) (ε 10,000) and 250 nm (38,000); c.d. data (MeOH): 296 ([θ] +49,000) and 246 nm (−27,000); p.m.r. data (acetone-d_6): δ 8.65 (H-6), 8.40 (aromatic C*H*), 7.63 (H-5), 6.59 (H-1′), 6.19 (H-3′), 2.28 (COCH_3), $J_{1',2'}$ 4.0, $J_{2',3'}$ 3.6, $J_{3',4'}$ 3.6, and $J_{5,6}$ 7.8 Hz; (pyridine-d_5): δ 8.88 (H-6), 8.43 (aromatic C*H*), 7.99 (H-5), 7.18 (H-1′), 6.72 (H-3′), 2.43 (COCH_3), $J_{1',2'}$ 4.0, and $J_{5,6}$ 7.5 Hz; X-ray powder diffraction data: 12.90 w, 11.33 m, 8.31 m, 6.19 w, 5.71 s (2), 4.96 s (1), 4.35 s (3), 4.11 m, 3.92 m, and 3.65 m.

Anal. Calc. for $C_{27}H_{21}F_3N_6O_{12}$: C, 47.80; H, 3.12; N, 12.39. Found: C, 48.15; H, 3.06; N, 12.32.

1-(2-Amino-2-deoxy-β-D-xylofuranosyl)cytosine (**13**). — *Method A. From the 4-methoxypyrimidinone* **9**. A solution of **9** (56 mg) in methanol (10 ml) saturated with ammonia at 4° was heated in a sealed tube for 10 h at 90°, and then evaporated to dryness. The residue was dissolved in water, and the solution was washed three times with chloroform, and evaporated to a syrup that was mixed with water (2 ml); insoluble material was filtered off, and the filtrate was made neutral with 0.5M sulfuric acid, and concentrated. Addition of ethanol induced crystallization of the sulfate of **13** (21.6 mg, 74%). Recrystallization from water–ethanol gave an analytical sample; m.p. 228–230° (dec.), $[\alpha]_D^{21}$ +44° (*c* 0.71, water) (lit.[11] for 1-β-D-xylofuranosylcytosine +48° in water); λ_{max}^{Nujol} 2.95, 3.25 (OH, NH), 5.74, and 5.87 μm (C=C–C=N); $\lambda_{max}^{H_2O}$ 270 (ε 10,000) and 229 (sh) nm (8,400); $\lambda_{max}^{0.05M\ HCl}$ 278 (ε 14,000) and 212 nm (10,000); $\lambda_{max}^{0.05M\ NaOH}$ 272 (ε 9,500) and 230 (sh) nm (8,000); c.d. data (H_2O): 272 nm ([θ] +10,000); (0.05M HCl): 280 nm ([θ] +9,500); (0.05M NaOH): 272 nm ([θ] +14,000); p.m.r. data (D_2O): δ 8.21 (H-6), 6.34 (H-5), 6.14 (H-1′), 4.09 (H-2′), 4.02 (H-5′), $J_{1',2'}$ 3.6, $J_{2',3'}$ 3.5, $J_{3',4'}$ 3.5, and $J_{5,6}$ 8.0 Hz; (D_2O, free base): δ 7.93 (H-6), 6.06 (H-5), 5.79 (H-1′), 3.99 (H-5′), 3.61 (H-2′), $J_{1',2'}$ 3.0, and $J_{5,6}$ 7.6 Hz; X-ray powder diffraction data: 10.91 s (2), 10.22 s (2), 8.58 w, 6.70 w, 5.66 m, 5.18 s (3), 4.32 s (1), 4.16 w, 3.97 s (1), and 3.03 m.

Anal. Calc. for $C_9H_{16}N_4O_8S$: C, 31.76; H, 4.74; N, 16.46. Found: C, 31.78; H, 4.49; N, 16.27.

Method B. From the 4-acetamidopyrimidinone **11**. A solution of the 4-acetamido

nucleoside **11** (89 mg) in methanol (20 ml) was saturated with ammonia at 4°, and then heated in a sealed tube for 10 h at 80–85°. The solvent was evaporated off, the residue was dissolved in water, and the solution was washed with chloroform, and evaporated to a syrup that was dissolved in water and chromatographed on a column (1.9 × 25 cm) of AG-1 X2 (OH^-) resin, by developing with 1:4 methanol–water. The effluent was monitored by ninhydrin assay and ultraviolet absorptivity, and the fractions positive to both tests were combined, and evaporated to a clear glass that resisted efforts at crystallization. The glass was dissolved in water, and two equivalents of M hydrochloric acid were added. Evaporation of the solvent, and addition of methanol, led to crystallization of the dihydrochloride of **13**; yield, 33 mg (80%). Recrystallization from water–ethanol–isopropyl alcohol gave an analytical sample, m.p. 200–201° (dec.), $[\alpha]_D^{21}$ +42° (*c* 1.04, water); λ_{max}^{Nujol} 3.12, 3.23 (OH, NH), 5.76, and 5.92 μm (cytosine). The R_F values in t.l.c., and the u.v. and p.m.r. spectra of the compound in deuterium oxide, were identical with those of the sulfate of **13**.

Anal. Calc. for $C_9H_{16}Cl_2N_4O_4$: C, 34.30; H, 5.12; N, 17.78. Found: C, 34.62; H, 5.28; N, 17.52.

Method C. From the 4-thiouracil **12**. A solution of the 4-thiouracil **12** (132 mg) in methanol (10 ml) saturated with ammonia at 4° was heated for 10 h at 90°, and then evaporated to dryness. The residue (126 mg) was dissolved in water, and the solution was washed with chloroform, and evaporated to a syrup that was redissolved in a small volume of water. The solution was made neutral with 0.5M sulfuric acid, and the sulfate of **13** was crystallized by addition of methanol and ethanol; yield, 72 mg (71%), m.p. 228–230° (dec.). The i.r. and p.m.r. spectra of this sample were identical with those of the sulfate prepared *via* method A.

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