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SYNTHESIS AND SPECTRAL PROPERTIES OF CYTOSINE NUCLEOSIDES OF 2-AMINO-2-DEOXY-α-D-ARABINO-FURANOSE AND -PYRANOSE*

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ABSTRACT

Ethyl 2-deoxy-3,5-di-*O*-*p*-nitrobenzoyl-1-thio-2-(trifluoroacetamido)-β-D-arabinofuranoside (**3**) was converted into the glycosyl chloride. Condensation of the latter with 2,4-dimethoxypyrimidine, followed by amination, gave 1-(2-amino-2-deoxy-α-D-arabinofuranosyl)cytosine (**6**), which was also obtained from the α-D anomer (**4**) of **3**. Similarly, 1-(2-amino-2-deoxy-α-D-arabinopyranosyl)cytosine (**12**) was synthesized from ethyl 2-deoxy-3,4-di-*O*-*p*-nitrobenzoyl-1-thio-2-(trifluoroacetamido)-α-D-arabinopyranoside (**9**). The p.m.r. spectra of these nucleosides, as well as those of the 1-thioglycosides, are discussed in terms of the conformation of the sugar portion. In particular, a large change of the $J_{1,2}$ coupling constants of the α-D-furanosides, according to the substituents at C-1 and C-2, was interpreted on the basis of conformational mobility.

INTRODUCTION

The synthesis of nucleosides of 2-amino-2-deoxy sugars has been under investigation in this laboratory. In view of the continued interest in the antiviral and antitumor activities of 9-β-D-arabinofuranosyl-adenine and -cytosine[1], investigations on the synthesis and properties of the nucleosides of 2-amino-2-deoxy-D-arabinose are of especial interest. We report here the synthesis and spectral properties of cytosine nucleosides of 2-amino-2-deoxy-α-D-arabinose in the furanose and pyranose forms.

DISCUSSION

Ethyl 2-deoxy-5-*O*-*p*-nitrobenzoyl-1-thio-2-(trifluoroacetamido)-β-D-arabino-furanoside[2] (**1**), prepared from the corresponding dithioacetal by treatment with

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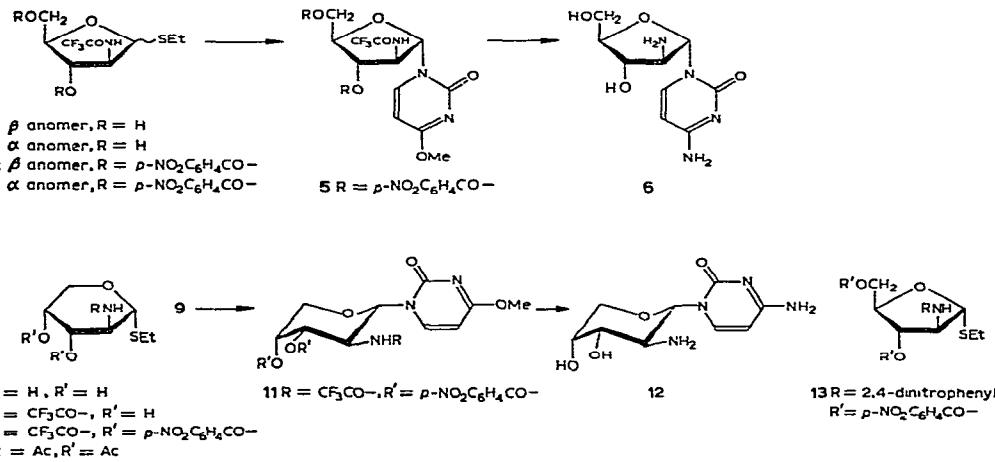
bromine, was *p*-nitrobenzoylated to give the crystalline 3,5-di-*p*-nitrobenzoate (**3**). Treatment of **3** with chlorine in dichloromethane produced the glycosyl chloride, which, without isolation, was immediately brought into reaction with 2,4-dimethoxypyrimidine under diminished pressure for 10 min at 95°. The resulting, fully protected α-D-nucleoside **5** was isolated after purification by thin-layer chromatography (t.l.c.) on silica gel.

When the ethyl 1-thio-α-D-furanoside (**4**) corresponding to **3** was treated similarly with chlorine and then with 2,4-dimethoxypyrimidine, the same α-D-nucleoside (**5**) was obtained. Careful examination of the reaction products by t.l.c. indicated the formation of only one anomer. Amination of nucleoside **5** by treating it with methanolic ammonia for 10 h at 90° gave 1-(2-amino-2-deoxy-α-D-arabinofuranosyl)cytosine (**6**) directly, the *p*-nitrobenzoyl and trifluoroacetyl groups being removed simultaneously. The cytosine nucleoside **6** crystallized as its dihydrochloride, after purification by chromatography on a column of AG-1 X2 (OH^-) resin.



The α-D configuration assigned to the nucleosides (**5** and **6**) was based on o.r.d. and c.d. studies. Reports[3,4] have indicated that correlation of the sign of the Cotton effect associated with the B_{2u} electronic transition and the anomeric configuration of pyrimidine nucleosides is applicable to ribosyl- and arabinosyl-cytosines, regardless of the configurations of the carbon atoms of the sugar moiety. A negative Cotton effect of **6** associated with the B_{2u} electronic transition at 266 nm indicated the α-D configuration, although the amplitude (−16,000°) is smaller than that[5] of 1-α-D-ribofuranosylcytosine (−32,200°). β Anomers should show a positive Cotton effect, as 1-β-D-ribofuranosyl[5]- and 1-β-D-arabinofuranosyl[3]-cytosine show positive Cotton effects, with amplitudes of +16,200 and +28,000°, respectively. The 4-methoxypyrimidinone (**5**) showed two negative c.d. maxima, a major one at 280 nm associated with the B_{2u} band, and a minor one at 248 nm that probably arises from an electronic transition of the *p*-nitrobenzoyl groups.

Comparison of the p.m.r. spectra of derivatives of α-D-arabinofuranose provided useful information on the conformation of these furanosyl derivatives. As already noted[2], the coupling constants of ring protons of the α-D anomers changed substantially upon changing substituents, whereas the corresponding β-D anomers exhibited little variation of $J_{1,2}$. Table I summarizes the first-order coupling-constants of four compounds (**4**, **5**, **6**, and **13**) in various solvents. Of these, the nucleoside **5**

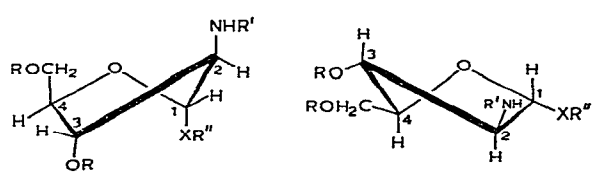
TABLE I

FIRST-ORDER COUPLING-CONSTANTS OF SOME 2-AMINO-2-DEOXY-α-D-ARABINOFURANOSE (**4**, **5**, **6**, AND **13**) AND α-D-ARABINOPYRANOSE DERIVATIVES (**9**, **10**, **11**, AND **12**) IN VARIOUS SOLVENTS

Compound	*Substituent at*		*Solvent*	*Coupling constants (Hz)*		
	C-1	*C-2*		$J_{1,2}$	$J_{2,3}$	$J_{3,4}$
4	SEt	CF_3CONH	acetone-d_6	5.9	5.0	
5	pyrimidine	CF_3CONH	acetone-d_6	6.5	6.5	6.5
5	pyrimidine	CF_3CONH	pyridine-d_5	6.5	6.5	6.5
5	pyrimidine	CF_3CONH	$CDCl_3$	6.9	6.8	6.5
6	cytosine	NH_2	D_2O (DCl)	3.5	4.9	
6	cytosine	NH_2	D_2O (free base)	4.8	3.8	
13	SEt	RNH[b]	pyridine-d_5	1.8		
13	SEt	RNH[b]	$CDCl_3$	1.3	2.0	2.0
9	SEt	CF_3CONH	acetone-d_6	10.0	9.5	
10	SEt	AcNH	$CDCl_3$	9.0	9.0	3.5
11	pyrimidine[a]	CF_3CONH	pyridine-d_5	9.8	9.8	3.0
11	pyrimidine[a]	CF_3CONH	acetone-d_6	10.3	10.3	
11	pyrimidine[a]	CF_3CONH	$CDCl_3$	5.7		
12	cytosine	NH_2	D_2O (sulfate)	9.2		

[a]4-Methoxy-(1*H*)-pyrimidinone. [b]R = 2,4-dinitrophenyl.

showed the largest $J_{1,2}$ value (6.9 Hz), whereas the 2-(2,4-dinitroanilino)-1-thio derivative (**13**) showed the smallest $J_{1,2}$ coupling (1.3 Hz). The 2-(trifluoroacetamido)-1-thio derivative (**4**) and the cytosine nucleoside (**6**) showed intermediate $J_{1,2}$ values. The substantial variation with the α-D anomers indicated a conformational change, rather than major changes in the Karplus constants, as a result of change of substituents.



$^2T_3$ X = O ; S ; N $^3T_2$

On the basis of minimum, non-bonded interactions, Bishop and Cooper[6] proposed two energetically favored conformations ($^3T_2$ and $^2T_3$) for methyl α-D-

arabinofuranoside. In the $^3T_2$ conformation, the out-of-plane atoms (C-2 and C-3) are on the opposite side and on the same side of the substituent at C-4, respectively, and the dihedral angles between H-1 and H-2, H-2 and H-3, and H-3 and H-4 are close to 180°. In the $^2T_3$ form, the dihedral angles between H-1, H-2, H-3, and H-4 are all close to 90°. Therefore, the approximate values of $J_{1,2}$, $J_{2,3}$, and $J_{3,4}$ would be 8–10 Hz in the $^3T_2$, and 0–1 Hz in the $^2T_3$ conformation, if the Karplus equation is employed as an approximate guide. Assuming that there is rapid equilibrium between the $^2T_3$ and $^3T_2$ forms in solution, the variation of the J values among the α-D anomers may reasonably be explained. Thus, when the $J_{1,2}$ value decreased from 6.5 to 1.3 Hz, the $J_{2,3}$ and $J_{3,4}$ values decreased concurrently from 6.5 and 6.5 to 2.0 Hz, corresponding to a partial shift from the $^3T_2$ to the $^2T_3$ conformation. From these data, it is suggested that the sugar portion of the nucleoside **5** exists mainly in the $^3T_2$ form, and that the $^2T_3$ conformation is preponderant in the dinitroanilino derivative **13**. However, this does not preclude other furanose conformations that are energetically similar, and further study would be needed to confirm this suggestion. Stevens and Fletcher[7] discussed the conformation of methyl 2,3,5-tri-*O*-benzoyl-α-D-arabinofuranoside and suggested the $^0T_1$ conformation from the J values ($J_{1,2}$ <0.5, $J_{2,3}$ 1.6, and $J_{3,4}$ 4.9 Hz in acetonitrile). It may be noted that these J values for an *O*-glycoside are somewhat similar to those displayed by **13**.

In order to synthesize a 2-amino-2-deoxy-D-arabinopyranosyl nucleoside, ethyl 2-amino-2-deoxy-1-thio-α-D-arabinopyranoside[2] was treated with *S*-ethyl trifluorothioacetate in ethanol to yield the *N*-trifluoroacetyl derivative (**8**), which was then *O*-*p*-nitrobenzoylated. Treatment with chlorine of the 3,4-di-*p*-nitrobenzoate (**9**) thus obtained, followed by condensation with 2,4-dimethoxypyrimidine for 10 min at 110°, gave the fully protected α-D-nucleoside (**11**). Again, no β-D-nucleoside was obtained. By heating for 10 h at 90° in methanolic ammonia, **11** was converted into 1-(2-amino-2-deoxy-α-D-arabinopyranosyl)cytosine (**12**), which crystallized as its sulfate.

The α configuration of **12** was established by p.m.r. spectroscopy, which showed $J_{1,2}$ 9.2 Hz in deuterium oxide. Of the two chair conformations possible for each anomer of 2-amino-2-deoxy-D-arabinopyranose, namely, four conformers, only the α-D anomer in the $^1C_4$(D) conformation gives a large $J_{1,2}$ value (9–10 Hz), arising from the *trans*-diaxial disposition of H-1 and H-2. Large $J_{1,2}$ values were also shown by **11** in acetone-d_6 (10.3 Hz) or pyridine-d_5 (9.8 Hz); moreover, the large $J_{2,3}$ and small $J_{3,4}$ values observed also accord with the $^1C_4$(D) conformation. However, when **11** was examined in chloroform-*d*, the $J_{1,2}$ value was only 5.7 Hz, and broadening of the signals of H-2, H-3, and H-4 was observed. Although the $J_{2,3}$, $J_{3,4}$, and other J values were not obtained on a first-order basis, the small $J_{1,2}$ value suggests some conformational mobility of the pyranoid ring, that is to say, a partial shift from the $^1C_4$ to the $^4C_1$ conformation. For comparison, the J values of ethyl 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-1-thio-α-D-arabinopyranoside (**10**) were measured (see Table I), and this compound was found to be present mainly in the $^1C_4$ conformation in chloroform-*d*.

The o.r.d. and c.d. curves of **15** and, in particular, **14** showed a strongly negative

Cotton effect associated with the B_{2u} band. However, the sign of the Cotton effect of the pyranosyl nucleosides is much less reliable for the determination of the anomeric configuration than that of the furanosyl nucleosides, because there has been no systematic study of the relationship between the sign of the Cotton effect and the anomeric configuration in pyranosyl nucleosides. Although the regularity observed for the furanosyl nucleosides holds for many of the pyranosyl analogs[8], some exceptions have been reported[3,8]. In this connection, it is noteworthy that the u.v. spectra of pyranosyl nucleosides are considerably different from those of the furanosyl nucleosides. Thus, the B_{2u} band of the former (**12**) is red-shifted by about 4 nm in the latter (**6**), and a minor maximum near 230 nm is blue-shifted by about 3–6 nm. This difference, which was first noted by Fox and Shugar[9], indicates that the electronic transition of the chromophore is affected by the ring form of the sugar portion. Any interpretation of the o.r.d. and c.d. curves of the pyranosyl nucleosides must take this perturbation into account.

EXPERIMENTAL

General methods. — Melting points were determined with a Thomas–Hoover apparatus. Specific rotations were determined in a 2-dm polarimeter tube. I.r. spectra were recorded with a Perkin–Elmer Infracord spectrometer. O.r.d., c.d., and u.v. spectra were recorded with a Jasco ORD/UV-5 spectrometer. N.m.r. spectra were recorded with a Varian A-60A spectrometer, with an internal standard of sodium 4,4-dimethyl-4-silapentane-1-sulfonate (for deuterium oxide and for 1:1 pyridine-d_5–deuterium oxide) or tetramethylsilane (for organic solvents). 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 by using Silica Gel G (E. Merck, Darmstadt, Germany) admixed with a 1:1 mixture of zinc orthosilicate and zinc sulfide (0.5%). Detection was by ninhydrin for free amino sugars, by potassium permanganate for thio sugars, and by u.v. light for u.v.-absorbing materials. The amounts of developing solvents indicated 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)-β-*D*-arabino-furanoside* (**3**). — To a solution of ethyl 2-deoxy-5-*O*-*p*-nitrobenzoyl-1-thio-2-(trifluoroacetamido)-β-D-arabinofuranoside[2] (**1**, 70 mg) in pyridine (4 ml) was added *p*-nitrobenzoyl chloride (73 mg), and the mixture was refrigerated overnight. Benzene and cyclohexane were added, and the resulting precipitate was filtered off. The filtrate was evaporated to dryness, and the residue extracted with a mixture of benzene and water. The benzene layer was successively washed with saturated, aqueous sodium hydrogen carbonate and water, and evaporated to give the crude product (119 mg). This was purified by t.l.c. on a plate of silica gel, developed first with

chloroform and then with 1:1 chloroform–benzene. Compound **3** was obtained from the main band of the chromatogram, and was crystallized from dichloromethane–cyclohexane; yield, 82 mg (95%), m.p. 98–99° (sintering at 57–58°), $[\alpha]_D^{22}$ −44° (*c* 0.56, chloroform); λ_{max}^{Nujol} 3.06, 3.24 (NH), 5.76 (CO–O), and 5.84 μm (CO–NH); p.m.r. data (acetone-d_6 containing one drop of deuterium oxide): δ 8.41 (aromatic C*H*), 5.97 (H-3), 5.91 (H-1), 5.18 (H-2), ~4.75 (H-5), ~4.6 (H-4), 2.77 (CH_2–CH_3), 1.27 (CH_2–CH_3), $J_{1,2}$ 6.5, $J_{2,3}$ 6.6, and $J_{3,4}$ 6.5 Hz; ($CDCl_3$): δ 5.74 (H-1), 5.69 (H-3), 5.07 (H-2), $J_{1,2}$ 6.2 Hz; X-ray powder diffraction data: 12.90 m, 11.47 s, 7.66 m, 6.23 w, 5.80 w, 5.01 s (2), 4.36 s (1), 4.14 s (2), and 3.32 m.

Anal. Calc. for $C_{23}H_{20}F_3N_3O_{10}S$: C, 47.02; H, 3.43; N, 7.15. Found: C, 47.15; H, 3.62; N, 7.10.

The low m.p. suggested the possibility of solvation, but no evidence of solvation was obtained by p.m.r. spectroscopy.

*Ethyl 2-deoxy-3,5-di-*O-p-*nitrobenzoyl-1-thio-2-(trifluoroacetamido)-*α-D-*arabino-furanoside* (**4**). — Compound **4** was prepared from ethyl 2-deoxy-5-*O-p*-nitrobenzoyl-1-thio-2-(trifluoroacetamido)-α-D-arabinofuranoside (**2**) by a procedure essentially identical to that used for preparation of the β-D anomer **3**. The product was crystallized from benzene; m.p. 169–169.5°, $[\alpha]_D^{21}$ +91° (*c* 0.59, chloroform); λ_{max}^{Nujol} 3.06, 3.24 (NH), 5.74, 5.80 (CO–O), and 5.85 μm (CO–NH); p.m.r. data (acetone-d_6): δ 8.36 (aromatic C*H*), 5.80 (H-3), 5.66 (H-1), ~4.85 (H-4, H-5), 4.71 (H-2), 2.79 (CH_2–CH_3), 1.31 (CH_2–CH_3), $J_{1,2}$ 5.9, and $J_{2,3}$ 5.0 Hz; X-ray powder diffraction data: 12.02 w, 9.82 s (3), 8.46 m, 4.94 m, 4.70 s (1), 4.48 s (1), 3.57 s, and 3.12 m.

Anal. Calc. for $C_{23}H_{20}F_3N_3O_{10}S$: C, 47.02; H, 3.43; N, 7.15. Found: C, 47.12; H, 3.49; N, 6.89.

*1-[2-Deoxy-3,5-di-*O-p-*nitrobenzoyl-2-(trifluoroacetamido)-*α-D-*arabinofuranosyl]-4-methoxy-2(1*H*)-pyrimidinone* (**5**). — *Method A.* Dry chlorine was passed into a solution of **3** (119 mg) in dried (molecular sieve, type 5A) dichloromethane (20 ml) for 7 min at room temperature, and then dry nitrogen was bubbled through for 10 min to remove the excess of chlorine. 2,4-Dimethoxypyrimidine (800 mg) was then added, and the solvent was immediately evaporated off to yield a syrup, which was heated for 10 min at 95° under diminished pressure (water aspirator), and then kept overnight at room temperature, Benzene was added, and the crystals (33 mg) of compound **15** deposited were filtered off. The filtrate was evaporated to a syrup, which was resolved on a plate (200 × 200 × 1.5 mm) of silica gel developed with 19:1 chloroform–*tert*-butyl alcohol. The band containing the nucleoside was extracted with acetone, and the extract was evaporated to a syrup (116 mg). Repeated t.l.c. of this syrup with the same solvent-system gave compound **5** as an amorphous powder (90 mg, 68%); $[\alpha]_D^{22}$ −41.5° (*c* 0.94, chloroform); λ_{max}^{Nujol} 3.10 (NH), 5.76 (CO–O, CO–NH), 6.02, and 6.09 μm (C=C–C=N); λ_{max}^{MeCN} 260 nm (ε 28,000); c.d. data (acetonitrile): 280 nm ([θ] −30,00) and 248 (−7,500); p.m.r. data (acetone-d_6 containing 1 drop of deuterium oxide: δ 8.37 (aromatic C*H*), 6.48 (H-1), 6.20 (H-3′), 6.14 (H-5), 5.48 (H-2′), 5.29 (H-4′), 4.86 (H-5′), 3.96 (OCH_3), $J_{1',2'}$ 6.5, $J_{2',3'}$ 6.5, $J_{3',4'}$ 6.5, $J_{4',5'}$ 4.0, and $J_{5,6}$ 7.5 Hz; (pyridine-d_5 containing 1 drop of deuterium oxide): δ 8.55 (H-6), 7.07

(H-1′), 6.64 (H-3′), 6.14 (H-5), 6.02 (H-2′), 5.64 (H-4′), 5.16 (H-5′), $J_{1',2'}$ 6.5, $J_{2',3'}$ 6.5, $J_{3',4'}$ 6.5, $J_{4',5'}$ 3.0, and $J_{5,6}$ 7.6 Hz; ($CDCl_3$ containing 1 drop of deuterium oxide): δ 7.84 (H-6), 6.55 (H-1′), 6.09 (H-5), 5.97 (H-3′), 5.16 (H-2′), ~4.8 (H-4′, H-5′), $J_{1',2'}$ 6.9, $J_{2',3'}$ 6.8, $J_{3',4'}$ 6.5, and $J_{5,6}$ 7.5 Hz.

Anal. Calc. for $C_{26}H_{20}F_3N_5O_{12}$: C, 47.93; H, 3.09; N, 10.75. Found: C, 47.69; H, 3.14; N, 10.84.

Examination of other t.l.c. bands gave no evidence for formation of the β-D-nucleoside. From the bands migrating more slowly than **5**, there were isolated two crystalline products, **14** and **15**. Compound **14** was identified as 1,2-dihydro-4-methoxy-1-methyl-2-pyrimidinone, m.p. 149–150° (recrystallized from benzene) (lit.[10] m.p. 149–150°), $[\alpha]_D^{21}$ 0° (*c* 0.44, acetonitrile); λ_{max}^{Nujol} 6.00 and 6.10 μm (pyrimidinone), λ_{max}^{MeCN} 279 nm (ε 5,300); p.m.r. data ($CDCl_3$): δ 7.55 (H-6), 5.93 (H-5), 3.98 (OCH_3), 3.52 (NCH_3), $J_{5,6}$ 7.3 Hz; X-ray powder diffraction data: 8.08 m, 6.02 s (2), 5.09 m (3), 4.48 m, 4.03 m, 3.55 w, 3.26 s (1), 2.90 m, 2.77 w, and 2.57 m.

Anal. Calc. for $C_6H_8N_2O_2$: C, 51.42; H, 5.75; N, 19.99. Found: C, 51.57; H, 5.51; N, 19.82.

Compound **15** was tentatively identified as 1,2-dihydro-4-methoxy-2-pyrimidinone, m.p. 202–205° (recrystallized from acetone) (lit.[11] m.p. 206–208°), $[\alpha]_D^{21}$ 0° (*c* 0.34, acetonitrile); λ_{max}^{Nujol} 3.21 (NH) and 6.10 μm (pyrimidinone); λ_{max}^{MeCN} 273 nm (ε 4,000); p.m.r. data (methyl sulfoxide-d_6): δ 7.75 (H-6), 5.91 (H-5), 3.85 (OCH_3), $J_{5,6}$ 7.1 Hz; X-ray powder diffraction data: 7.16 w, 6.21 m, 5.40 s (2), 4.56 w, 3.82 m, 3.59 m (3), 3.24 s (1), 3.06 w, and 2.96 w.

Anal. Calc. for $C_5H_6N_2O_2$: C, 47.62; H, 4.80; N, 22.21. Found: C, 47.69; H, 4.74; N, 22.09.

Method B. Compound **4** (52 mg) was treated with chlorine, and the resultant glycosyl chloride was condensed with 2,4-dimethoxypyrimidine (400 mg) under the same conditions as those described for the β-D anomer **3**. The yield of nucleoside **5** was 34 mg (59%). Again, formation of the β-D-nucleoside was not observed.

1-(2-Amino-2-deoxy-α-D-arabinofuranosyl)cytosine (**6**). — A solution of the 4-methoxy derivative **5** (83 mg) in methanol (15 ml) presaturated 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, insoluble materials were removed by filtration, and the filtrate was washed with chloroform. The aqueous layer was separated and evaporated to a syrup, which was dissolved in a small volume of water, and the solution made neutral with 0.5M sulfuric acid. The sulfate thus obtained failed to crystallize from water–ethanol. A solution of the sulfate in water was chromatographed on a column of AG-1 X2 (OH^-) resin. The column was washed with water, and the nucleoside **6** was eluted with 1:2 methanol–water. The basic effluents were made neutral with M hydrochloric acid and evaporated to give the syrupy dihydrochloride, which crystallized from methanol; yield, 30 mg (75%), m.p. 177–178° (dec., with partial sublimation), $[\alpha]_D^{21}$ −18° (*c* 0.22, water); λ_{max}^{Nujol} 3.04, 3.22 (OH, NH), 5.75, and 5.88 μm (C=C–C=N); $\lambda_{max}^{H_2O}$ 270 (ε 8,000) and 230 (sh) nm (6,500); $\lambda_{max}^{0.05M\ HCl}$ 278 (ε 12,500) and

213 nm (7,500); $\lambda_{max}^{0.05M\ NaOH}$ 271 (ε 8,500) and 230 nm (7,000); c.d. data (H_2O): 270 nm ([θ] −11,000); (0.05M HCl): 276 nm (−9,500); (0.05M NaOH): 273 nm (−10,000); p.m.r. data (D_2O): δ 8.12 (H-6), 6.38 (H-5), 6.16 (H-1′), ~4.15 (H-3′, H-4′), 4.22 (H-2′), 3.94 (H-5′), $J_{1',2'}$ 3.5, $J_{2',3'}$ 4.9, and $J_{5,6}$ 8.1 Hz; (free base, D_2O): δ 7.81 (H-6), 6.07 (H-5), 5.92 (H-1′), ~4.35 (H-3′, H-4′), 3.91 (H-5′), 3.80 (H-2′), $J_{1',2'}$ 4.8, $J_{2',3'}$ 3.8, and $J_{5,6}$ 7.5 Hz; X-ray powder diffraction data: 14.84 vw, 6.30 s (2), 6.04 s (2), 4.72 m, 3.42 w, 3.25 s (1), 3.00 m, 2.89 w, and 2.80 w.

Anal. Calc. for $C_9H_{16}Cl_2N_4O_4$: C, 34.30; H, 5.12; N, 17.78. Found: C, 34.19; H, 5.19; N, 17.72.

Ethyl 2-deoxy-1-thio-2-(trifluoroacetamido)-α-D-arabinopyranoside (**8**). — A solution of ethyl 2-amino-2-deoxy-1-thio-α-D-arabinopyranoside (**7**, 100 mg) in ethanol (10 ml) was treated with *S*-ethyl trifluorothioacetate (330 mg) for 18 h at room temperature, and then evaporated to dryness. The crystalline residue was recrystallized from ethyl acetate to give **8**; yield, 95 mg (64%), m.p. 236–237° (dec., with partial sublimation above 200°), $[\alpha]_D^{22}$ −20.3° (*c* 0.16, methanol); λ_{max}^{Nujol} 5.90 and 6.40 μm (CO–NH); X-ray powder diffraction data: 13.80 m, 9.99 m, 7.02 s (3), 4.94 s (1), 4.53 m, 4.06 s (2), 3.89 s, 3.19 w, and 2.45 w.

Anal. Calc. for $C_9H_{14}F_3NO_4$: C, 37.37; H, 4.88; N, 4.84. Found: C, 37.51; H, 5.01; N, 5.29.

*Ethyl 2-deoxy-3,4-di-*O-p-*nitrobenzoyl-1-thio-2-(trifluoroacetamido)-α-D-arabinopyranoside* (**9**). — A mixture of the *N*-trifluoroacetyl derivative **8** (129 mg), pyridine (30 ml), and *p*-nitrobenzoyl chloride (223 mg) was kept for 2 days at room temperature, and then evaporated to dryness. The residue was extracted with a mixture of ethyl acetate and benzene, the extract was washed three times with saturated, aqueous sodium hydrogen carbonate and twice with water, and evaporated to give a crude product (273 mg). This was purified by t.l.c. on silica gel developed with chloroform. The main band, containing **9**, was extracted with a mixture of acetone and methanol, and the extract was evaporated to give essentially pure **9**; yield, 184 mg (70%). Recrystallization from benzene gave an analytical sample, m.p. 165–166°, $[\alpha]_D^{22}$ −146° (*c* 1.02, chloroform); λ_{max}^{Nujol} 3.07, 3.27 (NH), 5.78 (CO–O), and 5.87 μm (CO–NH); p.m.r. data (acetone-d_6): δ 7.59, 7.42 (aromatic C*H*), 5.85 (H-4), 5.78 (H-3), 5.14 (H-1), 4.74 (H-2), 4.33 (H-5), 2.93 (CH_2CH_3), 1.35 (CH_2–CH_3), $J_{1,2}$ 10.0, and $J_{2,3}$ 9.5 Hz; X-ray powder diffraction data: 10.21 s (2), 8.11 s, 6.39 w, 5.73 m, 4.69 s (3), 4.10 s (1), and 3.60 w.

Anal. Calc. for $C_{23}H_{20}F_3N_3O_{10}S$: C, 47.02; H, 3.43; N, 7.15. Found: C, 47.26; H, 3.34; N, 7.42.

*Ethyl 2-acetamido-3,4-di-*O-*acetyl-2-deoxy-1-thio-α-D-arabinopyranoside* (**10**). — A solution of ethyl 2-amino-2-deoxy-1-thio-α-D-arabinopyranoside (**7**, 106 mg) in dry pyridine (5 ml) was treated with acetic anhydride (0.9 ml) for 2 days at room temperature, and then evaporated to dryness. The residue was crystallized from acetonitrile and benzene to give compound **10** (120 mg). Further crops (30 mg) were recovered from the mother liquors upon concentration and addition of petroleum ether; total yield, 86%. A sample recrystallized from acetonitrile–benzene showed

m.p. 190–191°, $[\alpha]_D^{21}$ +28° (*c* 0.25, chloroform); λ_{max}^{Nujol} 3.10 (NH), 5.75 (CO–O), and 6.04 μm (CO–NH); p.m.r. data ($CDCl_3$): δ 5.14 (H-4), 5.23 (H-3), 4.67 (H-1), 4.19 (H-2), 4.10 (H-5*e*), 3.69 (H-5*a*), 2.72 (CH_2–CH_3), 2.15, 2.05, 2.00 (CH_3CO), 1.28 (CH_2–CH_3), $J_{1,2}$ 9.0, $J_{2,3}$ 9.0, $J_{3,4}$ 3.5, $J_{4,5e}$ 2.0, and $J_{4,5a}$ 1.0 Hz; X-ray powder diffraction data: 9.99 w, 8.04 s (1), 6.62 m, 5.92 s (3), 5.35 m, 4.36 m, 3.94 w, and 3.70 s (2).

Anal. Calc. for $C_{13}H_{21}NO_6S$: C, 48.89; H, 6.63; N, 4.39. Found: C, 48.95; H, 6.55; N, 4.40.

*1-[2-Deoxy-3,4-di-*O*-p-nitrobenzoyl-2-(trifluoroacetamido)-α-*D*-arabinopyranosyl]-4-methoxy-2(1H)-pyrimidinone* (**11**). — Into a solution of compound **9** (116 mg) in dichloromethane (15 ml) was passed dry chlorine for 7 min; then, dry nitrogen was passed in for 10 min to expel the excess of chlorine. Evaporation of the solvent gave a clear syrup, which was redissolved in dichloromethane. After addition of 2,4-dimethoxypyrimidine (800 mg), the solvent was immediately evaporated off, and the residue was heated for 10 min at 100–110° under diminished pressure, and cooled to room temperature; benzene was added, and insoluble materials were filtered off and washed with benzene. The filtrate and washings were combined and evaporated to dryness. The crude product was resolved on a plate (200 × 200 × 1.5 mm) of silica gel, with 19:1 chloroform–*tert*-butyl alcohol as the developer, and detection by u.v. light. The major zone was excised, and extracted with acetone. Evaporation of the solvent left a clear glass, repeated t.l.c. of which on a plate of silica gel gave compound **11** (99 mg, 77%). Compound **11** crystallized slowly from chloroform–benzene, and was recrystallized from dichloromethane; m.p. 284–285°, $[\alpha]_D^{22}$ −220° (*c* 0.64, chloroform); λ_{max}^{Nujol} 3.03, 3.24 (NH), 5.72, 5.74 (CO–O, CO–NH), 5.92, and 6.08 μm (C=C–C=N); λ_{max}^{MeCN} 260 nm (ε 31,000); c.d. data (acetonitrile): 280 nm ([θ] +95,000) and 245 (+22,000); p.m.r. data (acetone-d_6 containing 1 drop of D_2O): δ 8.47, 8.25 (aromatic C*H*), 8.22 (H-6), 6.37 (H-1′), 6.20 (H-5), ~6.0 (H-3′, H-4′), 5.09 (H-2′), 4.54 (H-5′), 3.94 (OCH_3), $J_{1',2'}$ 10.3, $J_{2',3'}$ 10.3, $J_{5,6}$ 7.5 Hz; ($CDCl_3$ containing 1 drop of D_2O): δ 8.28 (H-6), 6.40 (H-1′), 6.16 (H-5), ~5.85 (H-3′, H-4′), ~5.0 (H-2′), 4.39 (H-5′), $J_{1',2'}$ 5.7, and $J_{5,6}$ 7.5 Hz; (pyridine-d_5 containing 1 drop of D_2O): δ 8.67 (H-6), 6.93 (H-1′), 6.50 (H-3′), 6.33 (H-4′), 6.19 (H-5), 5.67 (H-2′), 4.60 (H-5′), $J_{1',2'}$ 9.8, $J_{2',3'}$ 9.8, $J_{3',4'}$ 3.0, $J_{4',5'}$ 5.5, and $J_{5,6}$ 7.5 Hz; X-ray powder diffraction data: 11.18 s (3), 7.08 s, 5.68 m, 4.86 s (1), 4.48 w, 4.23 w, 3.62 s (2), 3.23 w, and 3.04 m.

Anal. Calc. for $C_{26}H_{20}F_3N_5O_{12}$: C, 47.93; H, 3.09; N, 10.75. Found: C, 48.20; H, 2.90; N, 10.97.

*1-(2-Amino-2-deoxy-α-*D*-arabinopyranosyl)cytosine* (**12**). — A solution of the 4-methoxy derivative **11** (79 mg) in methanol (15 ml) saturated with ammonia at 4° was heated in a sealed tube for 7 h at 80–85°, and then evaporated to dryness. The residue was dissolved in water, and the solution washed with chloroform. Evaporation of the aqueous layer gave a pale-yellow powder, which was redissolved in a small volume of water. Insoluble materials were removed by filtration, and the filtrate was made neutral with 0.5M sulfuric acid. Addition of ethanol caused crystallization of the

sulfate. Recrystallization from water–methanol gave compound **12** as the sulfate (30 mg, 73%); m.p. 245–248° (dec.), $[\alpha]_D^{21}$ −96° (*c* 0.36, water)*; λ_{max}^{Nujol} 2.94, 3.22 (OH, NH), and 5.77 μm (C=C–C=N); $\lambda_{max}^{H_2O}$ 266 (ε 9,300), 235 nm (8,300); $\lambda_{max}^{0.05M\ HCl}$ 274 (ε 13,400), and 210 (sh) nm (9,000), $\lambda_{max}^{0.05M\ NaOH}$ 268 (ε 9,000) and 233 nm (8,000); c.d. data (H_2O): 266 nm ([θ] −14,000); (0.05M HCl): 277 nm ([θ] −9,500); (0.05M NaOH): 263 nm ([θ] −9,000); p.m.r. data (D_2O): δ 8.09 (H-6), 6.43 (H-5), 5.92 (H-1′), $J_{1',2'}$ 9.2, and $J_{5,6}$ 8.0 Hz; (D_2O, free base): δ 7.75 (H-6), 6.09 (H-5), 5.69 (H-1′), $J_{1',2'}$ 9.4, and $J_{5,6}$ 7.5 Hz; X-ray powder diffraction data: 8.07 vw, 6.08 m, 5.75 s (2), 5.08 m, 4.46 w, 4.07 s (1), 3.89 w, 3.59 m, and 3.42 s (3).

Anal. Calc. for $C_9H_{16}N_4O_8S$: C, 31.76; H, 4.74; N, 16.46. Found: C, 31.84; H, 4.38; N, 16.52.

*Ethyl 2-deoxy-2-(2,4-dinitroanilino)-3,5-di-*O*-p-nitrobenzoyl-1-thio-α-*D*-arabino-furanoside* (**13**). — Syrupy ethyl 2-amino-2-deoxy-1-thio-α-D-arabinofuranoside[2] (268 mg) was dissolved in a mixture of methanol (14 ml) and water (7 ml). Sodium hydrogen carbonate (160 mg) and fluoro-2,4-dinitrobenzene (370 mg) were added with stirring. The mixture was stirred for 18 h at room temperature and then evaporated to dryness. The residue was extracted with a mixture of isopropyl alcohol and ethyl acetate, and the extract was evaporated to give the syrupy 2,4-dinitroanilino derivative (667 mg). This was dissolved in dry pyridine, and *p*-nitrobenzoyl chloride (570 mg) was added. The solution was kept for 18 h at room temperature, and then evaporated to dryness. The residue was dissolved in chloroform, and the solution was washed successively with saturated, aqueous sodium hydrogen carbonate and water, and then evaporated to give crude **13** (1.23 g).

Purification of this product was performed by t.l.c. on silica gel, by developing three times with chloroform and then once with benzene. Extraction of the main band with acetone, and evaporation of the solvent, gave **13** (570 mg, 67%), which crystallized from benzene; m.p. 113–115°, $[\alpha]_D^{22}$ −26° (*c* 0.65, chloroform); λ_{max}^{Nujol} 3.04, 3.27 (NH), and 5.78 μm (CO–O); p.m.r. data ($CDCl_3$): δ 9.05 (H-3 of 2,4-dinitrophenyl), 8.98 (H-6 of 2,4-dinitrophenyl), 8.38 (H-5 of 2,4-dinitrophenyl), 8.36, 8.26 (C*H* of *p*-nitrobenzoyl), 5.69 (H-1), 5.58 (H-3), 4.51 (H-2), 2.90 (CH_2–CH_3), 1.43 (CH_2–CH_3), $J_{1,2}$ 1.3, $J_{2,3}$ 2.0, and $J_{3,4}$ 2.0 Hz; (pyridine-d_5): δ 6.11 (H-1), 6.11 (H-3), 4.23 (H-2), $J_{1,2}$ 1.8 Hz; X-ray powder diffraction data: 15.63 w, 13.70 m, 11.70 s (1), 7.05 w, 5.73 m, 4.94 s (3), 4.06 m, 3.54 s (2), and 3.33 s (3).

Anal. Calc. for $C_{27}H_{23}N_5O_{13}S$: C, 49.32; H, 3.53; N, 10.65. Found: C, 49.77; H, 3.48; N, 10.93.

REFERENCES

1 S. COHEN, *Progr. Nucleic Acid Res. Mol. Biol.*, 5 (1966) 1–88; A. BLOCH, in E. ARIENS (Ed.), *Drug Design*, Academic Press, New York, 1973, pp. 285–378.
2 M. L. WOLFROM AND S. INOUYE, *Carbohydr. Res.*, 41 (1975) 117–133.
3 T. R. EMERSON, R. J. SWAN, AND T. L. V. ULBRICHT, *Biochemistry*, 6 (1967) 843–850.
4 J. A. WRIGHT, D. P. WILSON, AND J. J. FOX, *J. Med. Chem.*, 13 (1970) 269–272.

*The specific optical rotation reported[12] for 1-α-D-arabinopyranosylcytosine is $[\alpha]_D^{26}$ −101° (water).

5 B. SHIMIZU, *Ann. Sankyo Res. Lab.*, 19 (1967) 1–64.
6 C. T. BISHOP AND F. P. COOPER, *Can. J. Chem.*, 41 (1963) 2743–2758; J. W. GREEN, *Advan. Carbohydr. Chem.*, 21 (1966) 95–142.
7 J. D. STEVENS AND H. G. FLETCHER, JR., *J. Org. Chem.*, 33 (1968) 1799–1805.
8 I. FRIČ, J. ŠMEJKAL, AND J. FARKAŠ, *Tetrahedron Lett.*, (1966) 75–79.
9 J. J. FOX AND D. SHUGAR, *Biochim. Biophys. Acta*, 9 (1952) 369–384.
10 G. E. HILBERT AND T. B. JOHNSON, *J. Amer. Chem. Soc.*, 52 (1930) 2001–2007.
11 C. W. NOELL AND C. C. CHENG, *J. Heterocycl. Chem.*, 5 (1968) 25–28.
12 J. J. FOX AND I. GOODMAN, *J. Amer. Chem. Soc.*, 73 (1951) 3256–3258.