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5-FLUOROURACIL NUCLEOSIDE ANALOGS HAVING ACYCLIC-SUGAR CHAINS DERIVED FROM THE FOUR D-ALDOPENTOSES*†

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ABSTRACT

Fusion of 5-fluoro-2,4-bis(trimethylsilyloxy)pyrimidine (**1**) with 2,3,4,5-tetra-*O*-acetyl-1-bromo-1-*S*-ethyl-1-thio-D-arabinitol (**2**) gave 59% of crystalline 2,3,4,5-tetra-*O*-acetyl-1-*S*-ethyl-1-(5-fluorouracil-1-yl)-1-thio-D-*gluco*-pentitol [(1*R*)-2,3,4,5-tetra-*O*-acetyl-1-*S*-ethyl-1-(5-fluorouracil-1-yl)-1-thio-D-arabinitol] (**6**), converted by methanolic ammonia into the corresponding, crystalline, free tetrol **10**; X-ray crystallography, chiroptical data, and n.m.r. spectroscopy established the C-1′ stereochemistry of **6** and **10**, and showed that the compounds favor an extended conformation with the ethylthio group antiparallel to the planar, zigzag, carbon chain. The D-*xylo* analog (**3**) of the bromide **2** condensed with **1** to give a mixture from which 35% of the crystalline (1*S*) nucleoside derivative (**7**) was isolated, and converted into the crystalline tetrol; the conformation of **7** in solution was non-extended (sickle). A similar sequence from the D-*lyxo* bromide (**3**) gave a syrupy, (1*R*,1*S*) mixture of protected nucleosides that afforded the corresponding tetrols, also as an amorphous mixture. The D-*ribo* bromide gave a 1:2 (1*R*,1*S*) mixed adduct; de-esterification produced the corresponding mixed tetrols (**13** + **14**) as a glass from which the (1*S*) isomer **13** could be obtained crystalline.

INTRODUCTION

Synthesis of nucleoside analogs in which a purine or pyrimidine base is attached to an acyclic-sugar chain has been developed in detail in this laboratory[2]; the general approach has involved conversion of a suitably protected aldose dithioacetal into its 1-monohalo analog, which is then coupled to an activated derivative of the base. The procedure has been applied with various sugars coupled to natural pyrimidines (uracil[3], cytosine[3], and thymine[4]) and a purine (adenine[5]), as well as with such purine analogs as 6-mercaptopurine[2,6,7] that have antineoplastic activity and whose biological response might be potentiated or modified by attachment of a "fraudulent" sugar component.

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†Portions of this work have been reported in preliminary form; see ref. 1.

Complete, structural characterization of these coupled products is not simple. The point of attachment of the sugar chain to the base needs to be firmly established, and there are significant difficulties in elucidating the stereochemistry at the asymmetrically substituted, C-1 terminus of the acyclic-sugar chain. Furthermore, it is important to determine the favored conformation of the acyclic-sugar chain, as this factor may be important in relation to the topology of the molecule at a potential, biological receptor-site. Earlier work[3-5,7] left undefined the stereochemistry at C-1′ in this class of nucleoside analogs, but more-recent studies[1,2,6] have employed X-ray crystallographic analyses of key examples to provide reference points for broader correlation of the stereochemistry at C-1′ in various series of related derivatives.

As 5-fluorouracil, a pyrimidine analog, is a clinically useful, antileukemic drug[8], it was of interest to synthesize, for comparative purposes, a series of nucleoside analogs having, coupled to N-1 of 5-fluorouracil, acyclic-sugar chains derived from the four D-aldopentoses.

RESULTS AND DISCUSSION

Synthesis. — The procedure used for coupling 5-fluorouracil through N-1 thereof to C-1 of the 5-carbon sugar chain involved the general fusion technique of Nishimura and Iwai[9]; the base was transformed by the action of boiling hexamethyldisilazane into 5-fluoro-2,4-bis(trimethylsilyloxy)pyrimidine[10] (**1**). The acetylated D-aldopentose dithioacetals were converted into the syrupy, unstable bromo derivatives (**2**–**5**) by the action of 1 equiv. of bromine in ether in the cold[6]. In each coupling reaction, equimolar amounts of **1** and the respective bromide (**2**, **3**, **4**, or **5**) were fused for 0.5 h *in vacuo* at 140°, and the resultant mixtures were treated with aqueous methanol to remove the 4-*O*-trimethylsilyl group. The acetylated products (**6**, **7**, **8**, and **9**) were purified to acceptable criteria of compositional purity (elemental analysis) by means of column chromatography on silica gel.

That the coupling would lead to linkage between C-1 and N-1 is presumed on conventional, mechanistic grounds (N-3 would be much less nucleophilic than N-1; conceivable *O*-substitution would have been evident from the properties of the products), and this linkage was directly confirmed (see later) by X-ray, crystal-structure analysis.

The product (**6**) from the D-*arabino* precursor (**2**) crystallized in 59% yield from ethanol; the strongly dextrorotatory ($[\alpha]_D$ +132° in chloroform), sharp-melting, yellow crystals consisted of a single epimer at C-1′ and there was no evidence for formation of an appreciable proportion of the other C-1′ epimer.

The ^{1}H-n.m.r. spectra of all of the coupled products in this series (**6**–**9**) showed the H-6 signal as a distinctive, low-field (δ 8.07 for **6**) doublet ($J_{H,F}$ ~6.5 Hz). Whenever the product was a mixture of 1′-epimers, the spectrum showed two such doublets (having somewhat different chemical shifts); the relative intensities of the doublets gave indication of the approximate proportions of the two C-1′ epimers present, even when chromatography failed to resolve these isomers. Similar differences between the 1′-epimers were observed in the H-1′ signals.

Compound **6** was readily deacetylated by the action of methanolic ammonia, to afford in 88% yield, from acetone, the crystalline, sharp-melting, and strongly dextrorotatory ($[\alpha]_D$ +80° in methanol), free, nucleoside analog **10**. Both **6** and **10** showed typical u.v. absorption near 270 nm (methanol, log ε ~4) for the 1-substituted, 5-fluorouracil chromophore. The X-ray data established[1a,11], for both **6** and **10**, the relative stereochemistry at C-1′ that permits firm attribution of both as the (1*R*) isomers.

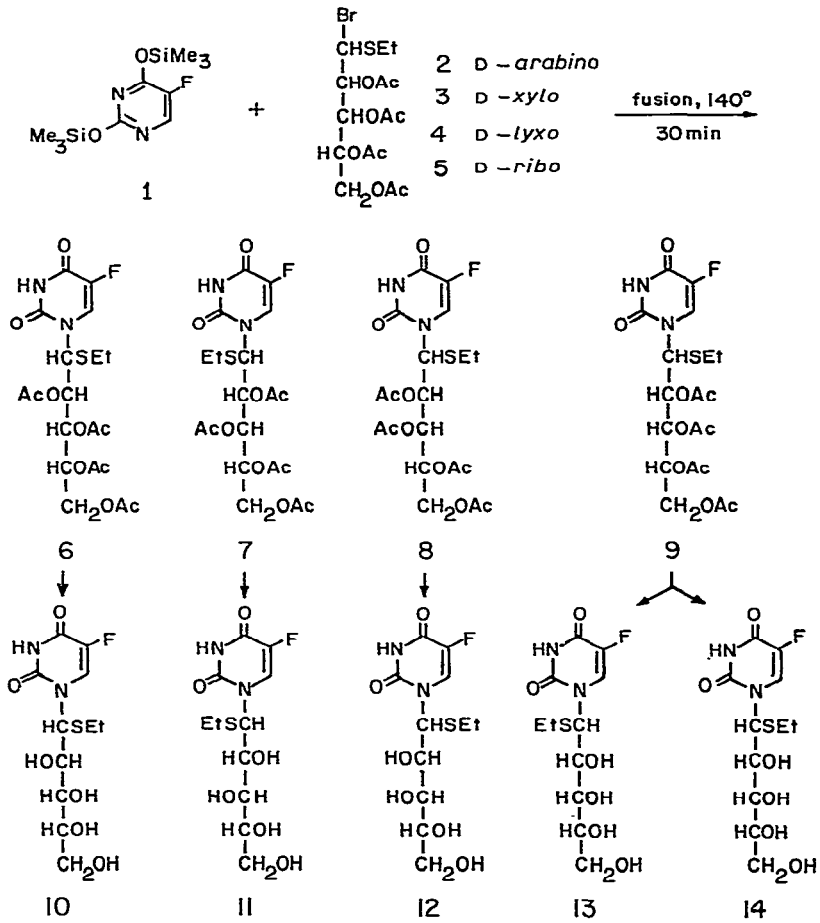
Similar coupling of **1** with the D-xylose derivative **3** gave, after column-chromatographic purification, 35% of crystalline material (m.p. 44–45°) that proved to be an ethanol solvate; heating at 78° converted it into the crystalline, non-solvated, strongly levorotatory product (**7**), m.p. 120–121°, $[\alpha]_D$ −121° in chloroform. ^{1}H-N.m.r. spectroscopy showed that the compound was a single epimer, and from its rotatory behavior (see later) it was assigned as the (1*S*) isomer **7**; undoubtedly, a significant proportion of the (1*R*) form was also produced in the initial mixture, but it was not isolated from other column fractions.

Deacetylation of **7** with methanolic ammonia gave the crystalline (1*S*) tetrol **11**, $[\alpha]_D$ −93° in methanol.

When the coupling reaction was performed between **1** and the D-lyxose derivative **4**, the purified product (**8**) isolated was a yellow glass, having a low value of $[\alpha]_D$ (+9° in chloroform), whose ^{1}H-n.m.r. spectrum indicated that it was an ~1:1 mixture of the (1*R*) and (1*S*) isomers, as evidenced by the presence of two H-6 doublets (at δ 7.84 and 7.75) of approximately equal intensity; two distinct signals for H-1′, corresponding to the two isomers, were also observed. Separation of the C-1′ epimers was not achieved, and they were deacetylated in admixture, to give the amorphous, mixed (1*R*,1*S*) tetrols **12**, $[\alpha]_D$ +4.5°.

Condensation of **1** with the D-ribose derivative **5** afforded 64% of the coupled product **9** as an analytically pure glass, $[\alpha]_D$ −25°, whose ^{1}H-n.m.r. spectrum indicated separate signals for H-6 and H-1′ of the (1*R*) and (1*S*) isomers in ~1:2 ratio; neither separation nor crystallization of these epimers was achieved. Saponification of **9** gave the corresponding 1:2 mixture of the (1*R*) and (1*S*) tetrols (**14** and **13**, respectively) as an amorphous glass. Although these isomers were not resolved in t.l.c., partial crystallization occurred when the product-mixture was triturated with acetone, and the pure, crystalline (m.p. 176–177°), strongly levorotatory ($[\alpha]_D$ −115° in methanol) isomer was obtained; on the basis of its chiroptical behavior, it was identified as the (1*S*) isomer **13**.

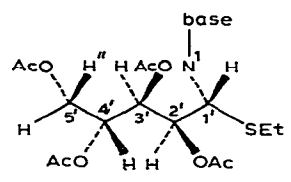
Electron-impact, mass spectra of the acetylated nucleosides **6–9** showed prominent molecule-ions (*m/e* 492) in all instances, together with important fragment-ions corresponding to $M^{+\cdot}$ − ·SEt (*m/e* 431), C-1–C-2 cleavage to give base-$\overset{+}{C}$HSEt (*m/e* 203), the protonated base (*m/e* 130 and 131), and the families of daughter-ions expected from conventional loss of ketene (42 m.u.), acetyl (43 m.u.), acetic acid (60 m.u.), and the elements of acetic anhydride (102 m.u.); fuller details are recorded in ref. 1c. The unsubstituted nucleosides **10–14** were extensively decomposed under electron



impact, but chemical-ionization, mass spectrometry of **10** with ammonia as the reagent gas showed[1c] a moderate $M \cdot NH_4^+$ capture-ion (*m/e* 342), accompanied by intense ions resulting from C-1–base cleavage *m/e* 342 with retention of the reagent gas in the sugar fragment (giving *m/e* 212), or the base fragment (leading to *m/e* 148).

Chirality at C-1' and conformation of the acyclic-sugar nucleosides. — The key point of reference in this series was the D-arabinose-derived, protected nucleoside **6** (and its deprotected analog **10**). From all of the comparative, conformational studies on acyclic-sugar systems performed in this laboratory[12], it could safely be anticipated that a chain having the D-*arabino* configuration at C-3–C-5 would favor planar, extended geometry, and that nonhydrogen substituents at C-1 would be so oriented that no such group would lie in parallel disposition with the 3-substituent on the chain. The ^{1}H-n.m.r. spectrum of **6** at 100 MHz in acetone-d_6 was essentially first-order[1c] (see Experimental section for data), and the large (7.7 Hz) value of $J_{1',2'}$ indicated that the favored conformation of **6** has H-1 and H-2 of the sugar chain in antiparallel disposition. The single-crystal structures of **6** and **10** were determined

by Drs. A. Ducruix and C. Pascard-Billy as part of a collaborative study, and the results, as already noted in a preliminary report[1c], showed that there is very close correspondence between the conformational behavior of **6** in solution and in the crystalline state; the results firmly establish the relative, spatial distribution of groups about C-1′, permitting the (1*R*) chirality to be assigned unambiguously to **6** (and also to **10**). The detailed crystallographic data[11], obtained with CuKα radiation, showed that the monoethanol solvate of **6** forms monoclinic crystals of space group $P2_1$ and cell parameters $a = 851.2$, $b = 1695.1$, and $c = 983.2$ pm, $\beta = 108.12°$, and $Z = 2$; the final index of refinement was $R = 0.06$. The positions of the hydrogen atoms on the chain were localized, thus permitting proton–proton, dihedral angles to be estimated for the molecule in the solid state, and allowing direct comparison between these angles and the corresponding angles estimated from proton–proton spin-couplings determined for the molecule in solution. The following formula gives a schematic representation of the favored conformation of **6**, together with the corresponding ^{1}H-n.m.r. spin-couplings observed and the proton–proton, dihedral angles estimated[11] from the crystal data.



Planar, extended conformation of **6**

Spin couplings (Hz)		Dihedral angles in crystal (degrees)	
$J_{1',2'}$	7.7	H-1′–H-2′	+170
$J_{2',3'}$	2.8	H-2′–H-3′	+53
$J_{3',4'}$	7.5	H-3′–H-4′	−171
$J_{4',5'}$	3.4	H-4′–H-5′	+68
$J_{4',5''}$	5.8	H-4′–H-5″	−53

It may be seen that there is very close correspondence between the two sets of data if the reliable, qualitative principle is invoked that large (8–9 Hz) spin-couplings denote nominally antiparallel (~180°) protons, and small (~3 Hz) couplings correspond to a nominal gauche (~60°) disposition. It may be noted that the 5-acetoxyl group occupies a gauche, rather than an extended, orientation in the crystal, which may result from the operation of intermolecular packing-forces; the n.m.r. data indicate, in solution, substantial population also of the C-4–C-5 rotamer having the 5-acetoxyl group extended.

From the observed disposition of the groups about C-1, it is evident that the ethylthio group is sterically more demanding than the 5-fluorouracil base, and so the latter is forced into gauche orientation; the (1*R*) chirality at C-1 of the sugar chain is definitively established, as is the point of attachment of the sugar chain to N-1 of the heterocycle*.

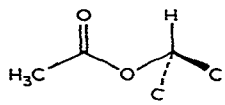
The deacetylated nucleoside **10** was obtained from acetone as anhydrous, hexagonal crystals. The crystal-structure analysis[1a,11] showed the space group to be $P6_3$, $a = b = 2.1150$ nm, and $c = 600.6$ pm; $\alpha = \beta = 90°$, and $\gamma = 120°$ for 1141

*In ref. 11, the nitrogen atom to which the sugar chain is attached is erroneously numbered throughout as N-3, but the structures depicted correctly display the point of attachment as N-1 in conventional nucleoside ring-numbering.

reflections; and the R index, 0.09. The hydrogen atoms were not located, but the overall, conformation features of the molecule are essentially identical to those of **6**, except that O-5′ occupies the extended, rather than the gauche disposition.

In both **6** and **10**, the backbone chain is approximately planar, small deviations from complete planarity being attributable to steric effects of the base's causing some displacement of C-1′; these deviations are reinforced by hydrogen-bonding effects in the case of **10**.

The disposition of the acetic ester groups in **6** is such that each carbonyl group is essentially eclipsed with the carbon–hydrogen bond of the alcohol to which it is



attached. This behavior is in accord with that noted in earlier work from this laboratory[13] with acetylated, cyclic sugars, and appears to be a general phenomenon for esters, at least of sugars.

Compounds **6** and **10**, having (1*R*) chirality, are strongly dextrorotatory (+132° in chloroform, and +80° in methanol, respectively) at the sodium D line, thus according with predictions from the generalized, rotation rule of El Khadem[14]; these values increase with decreasing wavelength to a positive peak (near 290 nm for **6**, and 350 nm for **10**), and then descend to a trough (near 276 nm for **6**, and 280 nm for **10**), before once more increasing to a strong, positive maximum at short wavelength (~245 nm). These complex, o.r.d. curves thus show an initial, *positive* Cotton-effect, with an inflection close to the principal, u.v. absorption (~270 nm), upon which is superimposed the effect of a strong, optically active band at short (<220 nm) wavelength.

The opposite effect is observed with the 1′-epimerically pure, D-*xylo* derivative **7**; its high, negative levorotation (−121° in chloroform) becomes progressively more negative with decreasing wavelength, before reaching a trough at 288 nm, and then rising to a peak at 266 nm. This first, *negative* Cotton-effect with inflection near the principal u.v. absorption at 270 nm is then followed at shorter wavelength by another negative extremum (near 240 nm) that reflects a short-wavelength (<220 nm), optically active band.

As the effect of atomic asymmetry involving strongly polarizable groups at C-1′ is expected to provide the overwhelmingly large, quantitative contribution to the total rotation of these molecules, the fact that **6** and **7** have large D-line rotations of *opposite* sign, together with initial Cotton-effects of *opposite* sign, leads to the conclusion that the two molecules have opposite chirality at C-1′, and thus **7** (and consequently **11**) may be assigned the (1*S*) configuration.

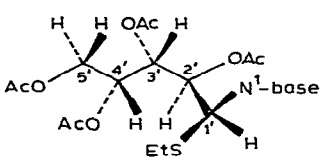
The epimerically pure, crystalline, D-*ribo* derivative (**13**) displays strong levorotation (−115° in methanol) at the D-line, descending to a trough near 290° before rising to a peak at 270°; at lower wavelength, a negative extremum is reached near 244 nm. Again, the strong, D-line levorotation and *negative*, initial Cotton-effect

(associated with the 270-nm absorption) lead to assignment of (*S*) chirality at C-1′ in this product.

The quantitatively small, D-line rotation of the 1:1, C-1′ epimerically mixed products **8** and **12** reinforces the conclusion that the C-1′ center is quantitatively dominant in determining the sign and value of the rotation, and the fact that **9**, an ~1:2 (1*R*,1*S*) mixture, is moderately levorotatory (—25° in chloroform) bears this out.

The c.d. spectra of these pyrimidine derivatives show at least two, optically active absorptions, but these show no simple, direct correlation with chirality at C-1′; the correlations based on the sign of large, D-line rotations and the longest-wavelength Cotton-effect appear to be the most reliable indications of stereochemistry at this center. These polarimetric and Cotton-effect correlations parallel those recently[6] made with purine analogs.

The ^{1}H-n.m.r. spectrum of 2,3,4,5-tetra-*O*-acetyl-1-*S*-ethyl-1-(5-fluorouracil-1-yl)-1-thio-D-*gulo*-pentitol [(1*S*)-2,3,4,5-tetra-*O*-acetyl-1-*S*-ethyl-1-(5-fluorouracil-1-yl)-1-thio-D-xylitol] (**7**) in chloroform-*d* at 250 MHz was entirely first-order, and the magnitudes of the vicinal, proton–proton couplings observed were, as expected, inconsistent with an extended conformation (such an arrangement would generate a parallel O-2–O-4 interaction). The data accord most closely with the $_2G^-$ conformation of the sugar chain, as depicted, which brings H-2 and H-3 into antiparallel disposition; the rotameric state about C-1–C-2 would be one of the two forms not having H-1 and H-2 antiparallel (the data do not allow firm selection of one of these



$_2G^-$ Conformation of **7**

Observed couplings (Hz)	
$J_{1',2'}$	4.4
$J_{2',3'}$	6.8
$J_{3',4'}$	4.0
$J_{4',5'}$	4.8
$J_{4',5''}$	6.3
$J_{1',F}$	1.3

two). The fact that the spin-couplings are not "extreme" values suggests the participation of other, minor contributors to the conformational equilibrium. Significant (1.3 Hz) ^6J coupling exists between H-1′ and F-5 (of the base); similar, long-range coupling has previously[15] been noted for certain 1-aldofuranosyl-5-fluorouracils.

Detailed, conformational interpretations concerning **8**, **9**, and **13** require better-resolved spectra, or separation of the 1′-epimers of **8** and **9**, or both.

Preliminary *in vivo*, and *in vitro*, antitumor assays indicated that 5-fluorouracil itself is more active than the compounds having an acyclic-sugar chain attached.

EXPERIMENTAL

General methods. — These were essentially as given in the preceding paper[2]. Molecular rotations refer to ($[\alpha]_D$ × mol. wt.)/100. Fluorine analyses were performed

by Galbraith Analytical Laboratories, Knoxville, TN. Chemical-ionization, mass spectra were recorded by Dr. Rodger Foltz of Battelle Columbus Laboratories with a Finnigan Model 3200 quadrupole spectrometer. The chemical-ionization source was maintained at 150°, and the pressure of the reagent gas was ~0.5 torr.

*2,3,4,5-Tetra-*O*-acetyl-1-*S*-ethyl-1-(5-fluorouracil-1-yl)-1-thio-*D*-gluco-pentitol* [*(1*R*)-2,3,4,5-tetra-*O*-acetyl-1-*S*-ethyl-1-(5-fluorouracil-1-yl)-1-thio-*D*-arabinitol*] (**6**). — Syrupy, freshly prepared, 2,3,4,5-tetra-*O*-acetyl-1-bromo-1-deoxy-1-*S*-ethyl-1-thio-D-arabinitol[6] (**2**; 1.67 g, 3.76 mmol) was mixed with 5-fluoro-2,4-bis(trimethylsilyloxy)pyrimidine[10] (**1**; 0.5 g, 3.84 mmol, prepared by boiling 5-fluorouracil in hexamethyldisilazane for 4 h at 140°, and evaporating off the excess of hexamethyldisilazane at ~15 torr) in a 25-mL flask, which was then evacuated to ~15 torr by means of a vacuum pump. The flask was closed, and the mixture heated slowly to fusion on an oil bath, and kept for 30 min at 140°. During this period, the vacuum line was opened briefly to the pump to decrease the buildup of pressure within the flask. After 30 min, the flask was removed from the oil bath, and allowed to cool under vacuum. The dark residue was triturated with 4:1 methanol–water (25 mL), and the resultant mixture filtered. The filtrate was evaporated to dryness, the residue extracted with hot chloroform (50 mL), the extract filtered, and the filtrate washed with water, dried (sodium sulfate), and evaporated. The dark, syrupy residue was taken up in the minimal volume of benzene, and applied to a column (64 × 3.5 cm) of silica gel. Initial elution with benzene was followed by gradient elution with 1:1 ethyl acetate–benzene, to afford crude compound **6**. The material obtained gave an elongated, single spot (R_F 0.65, 1:1 benzene–ethyl acetate) in t.l.c. Concentration of the product-containing fractions afforded a dark, yellow-brown syrup that failed to crystallize from various solvents. Careful chromatography on a column (205 × 2.0 cm) of silica gel with 1:1 benzene–ethyl acetate yielded purer material, giving a sharp, single spot, that was initially isolated as a yellow oil and which subsequently crystallized from abs. ethanol; yield 1.1 g (59%), m.p. 88–90°, $[\alpha]_D^{25}$ +132° (*c* 1.0, chloroform); o.r.d. $[M]_{550}$ +41°, $[M]_{290}$ +370°, $[M]_{276}$ 0°, $[M]_{248}$ +2591°, $[M]_{228}$ +1234°; c.d. $[\theta]_{290}$ −82°, $[\theta]_{264}$ −1349°, $[\theta]_{250}$ −33°; R_F 0.65 (1:1 benzene–ethyl acetate); λ_{max}^{MeOH} 270 nm (log ε 3.9); ν_{max}^{KBr} 3390 (NH), 1750 (C=O of acetate), 1470, 1390, and 1235–1200 cm^{-1} (ester); n.m.r. (100 MHz, acetone-d_6): δ 10.6 (bs, NH), 8.07 (d, $J_{6,F}$ 6.4 Hz, H-6), 5.91 (d, $J_{1',2'}$ 7.7 Hz, H-1′), 5.59 (dd, $J_{2',3'}$ 2.8 Hz, H-2′), 5.34 (dd, $J_{3',4'}$ 7.5 Hz, H-3′), 5.13 (o, H-4′), 4.26 (q, $J_{4',5'}$ 3.4, $J_{5',5''}$ 12.3 Hz, H-5′), 4.04 (q, $J_{4',5''}$ 5.8 Hz, H-5″), 2.62 (q, SCH_2CH_3), 2.10 s, 2.01 s, 1.97 s (4 OAc), and 1.25 (t, SCH_2CH_3); X-ray powder diffraction data: 8.00 vs (1), 7.22 s (3), 6.70 m, 5.5 w, 5.30 w, 4.47 vs (2), 3.89 m, 3.26 m, 3.09 m, and 2.54 w.

Anal. Calc. for $C_{19}H_{25}FN_2O_{10}S$ (492.476): C, 46.34; H, 5.08; N, 5.69; S, 6.50. Found: C, 46.52; H, 5.40; N, 5.83; S, 6.44.

*1-*S*-Ethyl-1-(5-fluorouracil-1-yl)-1-thio-*D*-gluco-pentitol* [*(1*R*)-1-*S*-ethyl-1-(5-fluorouracil-1-yl)-1-thio-*D*-arabinitol*] (**10**). — A solution of the foregoing acetylated derivative **6** (1.0 g, 2.24 mmol) in methanol (25 mL) was cooled to 0°, and dry ammonia was then bubbled through the solution for 30 min. After 18 h at ~25°,

the solvent was removed *in vacuo*. The dark syrup resulting was heated (oil bath) at 50°, with the flask attached to a vacuum pump, and evacuation was maintained overnight. The resulting dark, viscous mass was taken up in the minimal volume of methanol, and the solution placed on a column (105 × 2.0 cm) of silica gel. Elution was initially performed with chloroform, and then methanol was gradually incorporated, the concentration being increased until the desired material was eluted from the column (with 3:17 methanol–chloroform). The same column separation was performed twice more to give material, homogeneous by t.l.c., as a pale-yellow syrup. The syrup was dissolved in dry acetone, and petroleum ether (b.p. 30–60°) was added to incipient turbidity. After 24 h at ~0°, crystal growth was observed. Two more days of refrigeration permitted maximal crystallization, giving the desired nucleoside **10**; yield 0.63 g (88%), m.p. 124–126°, $[\alpha]_D^{25}$ +80° (*c* 0.6, methanol); o.r.d. $[M]_{600}$ +350°, $[M]_{350}$ +979°, $[M]_{304}$ 0°, $[M]_{280}$ −731°, $[M]_{274}$ 0°, $[M]_{246}$ +2104°, $[M]_{230}$ +1140°; c.d. $[\theta]_{310}$ 0°, $[\theta]_{270}$ −30,170°, $[\theta]_{246}$ 0°; R_F 0.45 (3:17 methanol–chloroform); λ_{max}^{MeOH} 272 nm (log ε 4.1); ν_{max}^{KBr} 3280–3125 (OH), 1490, 1390, 1260, and 1085 cm^{-1} (uracil); X-ray powder diffraction data: 10.24 vs (1), 6.83 w, 5.71 m, 5.03 s (2), 4.53 s (3), 4.11 m, 3.86 s, 3.29 s, 3.13 w, 2.99 m, 2.69 m, 2.60 w, 2.53 w, and 2.39 w.

Anal. Calc. for $C_{11}H_{17}FN_2O_6S$ (324.336): C, 40.74; H, 5.25; F, 5.86; N, 8.64; S, 9.87. Found: C, 40.57; H, 5.25; F, 6.13; N, 8.39; S, 9.62.

2,3,4,5-Tetra-O-*acetyl-1*-S-*ethyl-1*-(*5-fluorouracil-1-yl*)-*1-thio*-D-gulo-*pentitol* [(*1S*)-*2,3,4,5-Tetra*-O-*acetyl-1*-S-*ethyl-1*-(*5-fluorouracil-1-yl*)-*1-thio*-D-*xylitol*] (**7**). — Syrupy 2,3,4,5-tetra-*O*-acetyl-1-bromo-1-deoxy-1-*S*-ethyl-1-thio-D-xylitol[6] (**3**; 7.0 g, 16.5 mmol) was fused with **1** (7.94 g, 15.4 mmol), and the product, isolated in a manner analogous to that used for the arabinose derivative **6**, was obtained as granular crystals from abs. ethanol; yield 2.6 g (35%), m.p. 120–121° (after drying at 78°), $[\alpha]_D^{25}$ −121° (*c* 1.76, chloroform); o.r.d. $[M]_{300}$ −1292°, $[M]_{288}$ −2058°, $[M]_{272}$ 0°, $[M]_{266}$ +526°, $[M]_{258}$ 0°, $[M]_{240}$ −2919°, $[M]_{226}$ −1914°; c.d. $[\theta]_{300}$ 0°, $[\theta]_{274}$ −992°, $[\theta]_{272}$ −1006°, $[\theta]_{268}$ −992°, $[\theta]_{250}$ 0°; R_F 0.75 (2:1 ethyl acetate–benzene); λ_{max}^{MeOH} 270 nm (log ε 3.9); ν_{max}^{KBr} 3320 (NH), 1755 (C=O of acetate), 1370, 1250, 1220, and 1090 cm^{-1} (uracil); n.m.r. (250 MHz, $CDCl_3$): δ 10.0 (bs, NH), 7.92 (d, $J_{6,F}$ 6.5 Hz, H-6), 5.72 (dd, $J_{1',2'}$ 4.4, $J_{1',F}$ 1.3 Hz, H-1'), 5.53 (dd, $J_{2',3'}$ 6.8 Hz, H-2'), 5.41 (dd, $J_{3',4'}$ 4.0 Hz, H-3'), 5.50 (m, H-4'), 4.31 (q, $J_{4',5'}$ 4.8, $J_{5',5''}$ 11.8 Hz, H-5'), 4.00 (q, $J_{4',5''}$ 6.3 Hz, H-5''), 2.58 (q, SCH_2CH_3), 2.20 s, 2.08 s, 2.06 s (4 OAc), and 1.30 (t, SCH_2CH_3); X-ray powder diffraction data: 11.18 w, 9.11 w, 7.75 vs (1), 6.53 m (2), 5.43 w, 4.91 w, 4.31 w, 3.52 m (3), and 3.11 w.

Anal. Calc. for $C_{19}H_{25}FN_2O_{10}S$: C, 46.34; H, 5.08; N, 5.69; S, 6.50. Found: C, 46.28; H, 5.22; N, 5.53; S, 6.46.

The crystalline product as initially isolated had m.p. 44–45°, and its n.m.r. spectrum indicated it to be an ethanol solvate. The higher-melting form was non-solvated.

The H-1'–F coupling was verified by ^{19}F-n.m.r. spectroscopy.

1-S-*Ethyl-1*-(*5-fluorouracil-1-yl*)-*1-thio*-D-gulo-*pentitol* [(*1S*)-*1*-S-*ethyl-1*-(*5*-

fluorouracil-1-yl)-1-thio-D-*xylitol*] (**11**). — The tetraacetate **7** (0.91 g, 1.82 mmol) was treated with methanolic ammonia, and the product isolated as described for the *arabino* derivative **10**. Rapid cooling of a solution in 3:17 methanol–chloroform afforded crystalline **11**; yield 0.32 g (54%), m.p. 176–177°, $[\alpha]_D^{25}$ −93° (*c* 1.4, methanol); R_F 0.42 (3:17 methanol–chloroform); λ_{max}^{MeOH} 271 nm (log ε 4.0); ν_{max}^{KBr} 3450 (OH), 1390, 1260, and 1090 cm^{-1} (uracil); X-ray powder diffraction data: 8.29 vs (1), 5.03 m (2), 4.47 m (3), 3.83 w, and 1.94 w.

Anal. Calc. for $C_{11}H_{17}FN_2O_6S$: C, 40.74; H, 5.25; N, 8.64; S, 9.87. Found: C, 40.58; H, 5.29; N, 8.43; S, 9.40.

Preparation of 2,3,4,5-tetra-O-*acetyl*-D-*lyxose diethyl dithioacetal.* — This compound was first prepared by Wolfrom and Moody[16]. The material obtained in the present work solidified when the pyridine–acetic anhydride solution was poured into ice–water. The crude solid was recrystallized from ethanol, to yield material having m.p. 52–53° (lit.[16] m.p. 35°). The product appeared to be a dimorph of the crystals described[16] by Wolfrom and Moody.

This high-melting form showed n.m.r. and i.r. spectra essentially identical with those of the low-melting form, and was used for preparation of bromide **4** (obtained as an unstable syrup) essentially by the procedure used for preparing the arabinose derivative **2**; **4** was used in the subsequent reaction within 1 h to avoid decomposition.

(*1*R,*1*S)-*2,3,4,5-Tetra*-O-*acetyl-1*-S-*ethyl-1-(5-fluorouracil-1-yl)-1-thio*-D-*lyxitol* (**8**). — Syrupy 2,3,4,5-tetra-*O*-acetyl-1-bromo-1-deoxy-1-*S*-ethyl-1-thio-D-lyxitol[6] (**4**; 4.0 g, 9.43 mmol) was fused with 5-fluoro-2,4-bis(trimethylsilyloxy)uracil (**1**; 2.36 g, 9.22 mmol), and the product treated essentially by the procedure used to prepare compound **6**, to yield a thick syrup which was purified on a column (64 × 2.5 cm) of silica gel. Elution with benzene–ethyl acetate gave **8** as a glass; yield 2.1 g (47%), $[\alpha]_D^{25}$ +9.2° (*c* 1.0, chloroform); R_F 0.66 (1:1 benzene–ethyl acetate); λ_{max}^{MeOH} 270 nm (log ε 3.9); ν_{max}^{film} 3510 (NH), 1760 (C=O of acetate), 1470, 1235–1205, and 1065 cm^{-1} (uracil); n.m.r. (100 MHz, $CDCl_3$): δ 7.83 (d, $J_{6,F}$ 6.5 Hz, H-6 of one epimer), 7.75 (d, $J_{6,F}$ 6.2 Hz, H-6 of other epimer), 6.15 (dd, $J_{1',2'}$ 4 Hz, $J_{1',F}$ 1.8 Hz, H-1′ of one epimer), 5.82 (dd, $J_{1',2'}$ 3.8, $J_{1',F}$ 1.8 Hz, H-1′ of other epimer), 5.6 m, 5.3 (m, H-2′,3′,4′), 4.3 m, 3.9 (m, H-5′,5″), 2.55 (m, SCH_2CH_3), 2.23 s, 2.13 s, 2.04 s, 1.99 s (4 OAc), and 1.1 (m, SCH_2CH_3).

Anal. Calc. for $C_{19}H_{25}FN_2O_{10}S$: C, 46.34; H, 5.08; N, 5.69; S, 6.50. Found: C, 46.33; H, 5.12; N, 5.69; S, 6.51.

As judged from the intensities of the H-6 and H-1′ signals, the two epimers were present in ~1:1 ratio.

(*1*R,*1*S)-*1*-S-*Ethyl-1-(5-fluorouracil-1-yl)-1-thio*-D-*lyxitol* (**12**). — The foregoing tetraacetate **8** (0.5 g, 1.54 mmol) was treated with methanolic ammonia, as described for the preparation of the arabinose derivative **10**. Chromatography on a column (205 × 2.0 cm) of silica gel gave amorphous **12**; yield 303 mg (61%), $[\alpha]_D^{25}$ +4.5° (*c* 2.4, methanol); R_F 0.43 (3:17 methanol–chloroform); λ_{max}^{MeOH} 272 nm (log ε 4.0); ν_{max}^{film} 3510 (OH), 1725, 1400, 1265, and 1090 cm^{-1} (uracil).

Anal. Calc. for $C_{11}H_{17}FN_2O_6S$: C, 40.74; H, 5.25; N, 8.64; S, 9.87. Found: C, 40.33; H, 5.16; N, 8.26; S, 9.50.

(*1*R)-[*and* (*1*S)]-*2,3,4,5-Tetra*-O-*acetyl-1*-S-*ethyl-1-(5-fluorouracil-1-yl)-1-thio*-D-*ribitol* (**9**). — Syrupy 2,3,4,5-tetra-*O*-acetyl-1-bromo-1-deoxy-1-*S*-ethyl-1-thio-D-ribitol[6] (**5**) (3.5 g, 7.68 mmol) was fused with compound **1** (1.97 g, 7.69 mmol), and the product treated by the general procedure used for compound **6**. The resultant syrup was processed in a manner analogous to that used for compound **6**, and the product was then purified on a column (64 × 3.5 cm) of silica gel, with 9:1 benzene–ethyl acetate as the eluant. The desired product **9** was isolated as a glass; yield 2.4 g (64%), $[\alpha]_D^{24}$ −25° (*c* 4.2, chloroform); R_F 0.69 (1:1 benzene–ethyl acetate); λ_{max}^{MeOH} 268 nm (log ε 4.1); ν_{max}^{film} 3435 (NH), 1740 (C=O of acetate), 1470, 1380, and 1220 cm^{-1} (ester); n.m.r. (100 MHz, $CDCl_3$): δ 10.3 (bs, NH), 7.88 [d, $J_{6,F}$ 6.0 Hz, H-6 of (1*S*) isomer], 7.79 [d, $J_{6,F}$ 6.0 Hz, H-6 of (1*R*) isomer], 6.30 [d, $J_{1',2'}$ 4.0 Hz, H-1′ of (1*S*) isomer], 5.94 [bs, H-1′ of (1*R*) isomer], 5.35 (m, H-2′,3′,4′), 4.25 m, 3.40 (m, H-5′,5″), 2.54 (q, SCH_2CH_3), 2.16, 2.14, 2.09, and 2.03 (4 s, 4 OAc), and 1.30 (m, SCH_2CH_3).

Anal. Calc. for $C_{19}H_{25}FN_2O_{10}S$: C, 46.34; H, 5.08; F, 3.86; N, 5.69; S, 6.50. Found: C, 46.21; H, 4.75; F, 3.73; N, 5.68; S, 6.66.

The intensities of the H-6 and H-1′ signals indicated a ratio of ~1:2 for the (1*R*) and (1*S*) isomers.

(*1*S,*1*R)-*1*-S-*Ethyl-1-(5-fluorouracil-1-yl)-1-thio*-D-*ribitol* (**13** *and* **14**). — The foregoing tetracetate **9** (0.85 g, 1.73 mmol) was treated with methanolic ammonia as described for the preparation of **10**, and the solution was evaporated to a thick syrup that was kept under vacuum for 24 h in an oil bath at 50°. The resultant, dark syrup was chromatographed on a column of silica gel as for the *arabino* analog **10**, to yield an ~2:1 mixture of products **13** and **14** as a glass; yield 0.45 g (88%), $[\alpha]_D^{25}$ −10° (*c* 1.2, methanol); R_F 0.65 (1:1 benzene–ethyl acetate).

Anal. Calc. for $C_{11}H_{17}FN_2O_6S$: C, 40.74; H, 5.25; F, 5.86; N, 8.64; S, 9.87. Found: C, 40.61; H, 5.52; F, 5.84; N, 8.90; S, 9.66.

1-S-*Ethyl-1-(5-fluorouracil-1-yl)-1-thio*-D-allo-*pentitol* [(*1*S)-*1*-S-*ethyl-1-(5-fluorouracil-1-yl)-1-thio*-D-*ribitol*] (**13**). — Trituration of the amorphous mixture of **13** and **14** (1.1 g, 3.39 mmol) with cold, dry acetone led to partial crystallization. The crystals were washed with cold, dry acetone to yield pure **13** (80 mg, 6%), m.p. 176–177°, $[\alpha]_D^{25}$ −115° (*c* 1.6, methanol); o.r.d. $[M]_{290}$ −229°, $[M]_{280}$ 0°, $[M]_{274}$ +45°, $[M]_{270}$ +137°, $[M]_{264}$ −92°, $[M]_{246}$ −1922°, $[M]_{244}$ −2014°, $[M]_{242}$ −1968°, $[M]_{224}$ −732°; c.d. $[\theta]_{310}$ +55°, $[\theta]_{160}$ +897°, $[\theta]_{258}$ +970°, $[\theta]_{256}$ +933°, $[\theta]_{244}$ +18°; R_F 0.65 (1:1 benzene–ethyl acetate); λ_{max}^{MeOH} 272 nm (log ε 4.1); ν_{max}^{KBr} 3330–3175 (OH), 1540, 1470, 1220, 1090, and 1070 cm^{-1} (uracil); n.m.r. (100 MHz, methanol-d_4): δ 8.24 (d, $J_{6,F}$ 7.6 Hz, H-6), 6.20 (bs, H-1′), 3.74 (m, H-2′,3′,4′), 3.31 (m, H-5′,5″), 2.25 (q, SCH_2CH_3), and 1.26 (t, SCH_2CH_3); X-ray powder diffraction data: 8.04 s (2), 6.97 w, 6.14 vs (1), 5.01 s (3), 4.19 w, 3.98 s, 3.45 m, 3.28 w, 3.15 w, 2.94 w, 2.84 2, 2.71 w, and 1.96 w.

Anal. Calc. for $C_{11}H_{17}FN_2O_6S$: C, 40.74; H, 5.25; N, 8.64; S, 9.87. Found: C, 40.55; H, 5.60; N, 8.80; S, 10.21.

Biological testing. — Compounds **6** (NSC 245303), **9** (NSC 245304), and (**13** + **14**) (NSC 245305) were tested *in vivo* in the NCI murine L-1210 leukemia assay. At dose levels up to 200 mg/kg, the compounds were essentially nontoxic and inactive, the highest T/C value (110) was observed with (**13** + **14**) at 50 mg/kg.

In inhibition studies conducted *in vitro* with cultured L5178Y mouse leukemia cells by Dr. Charles Heidelberger of the Department of Oncology, University of Wisconsin, **9** showed >100% inhibition at 100μM, 70% at 10μM, and 0% at 1μM; (**13** + **14**) showed 80% inhibition at 100μM and 0% at 10μM. Compounds **6** and **10** showed only slight inhibition at 100μM; 5-fluorouracil alone gives 100% inhibition at 1μM.

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