# Synthesis and Biological Activity of 5-Fluoro-4'-thiouridine and Some Related Nucleosides

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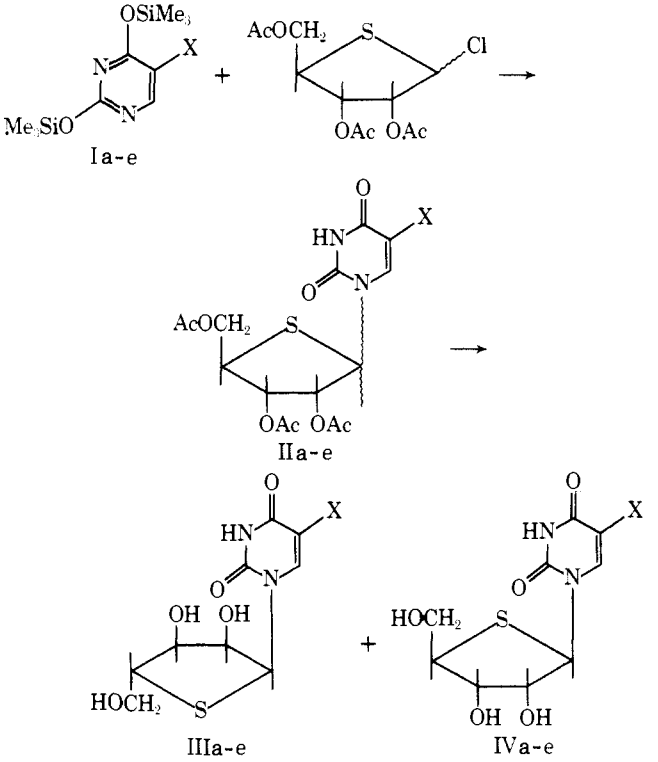
The synthesis of a series of 4'-thio-5-halogenopyrimidine nucleosides, including the 5-fluoro, chloro, bromo and iodo derivatives, has been carried out by condensation of the 2,4-bis-*O*-trimethylsilyl derivatives of the corresponding pyrimidine bases with the protected 4-thio-D-ribofuranosyl chloride. Among these, the $\alpha$ and $\beta$ anomers of 4'-thio-5-fluorouridine inhibited the growth of leukemia L1210 cells at concentrations of $4 \times 10^{-7}$ and $2 \times 10^{-7}$ *M*, respectively, and that of *S. faecium* at $4 \times 10^{-9}$ and $6 \times 10^{-10}$ *M*, respectively. These compounds retained marked activity against strains of *S. faecium* resistant to $10^{-3}$ *M* 5-fluorouracil or 5-fluorouridine. As determined in *S. faecium* cultures, 4'-thio-5-fluorouridine decreased the total protein content of the cells more markedly than it did their RNA or DNA content. X-Ray crystallography showed that substitution of sulfur for the oxygen in the carbohydrate ring markedly changes the conformation of that moiety.

Because of the pronounced biological activity of 5-fluorinated pyrimidines,[1] and the fact that the 4'-thio derivatives of various purine nucleoside analogs have been shown to retain their growth inhibitory activity against cells resistant to the corresponding ribofuranosyl analogs,[2,3] the synthesis of some 4'-thio-5-halogenopyrimidine nucleosides was undertaken. These compounds were tested for the in vitro growth inhibitory activity which they exert in some tumor and bacterial cell systems. The chemical synthesis of the compounds and the results of their biological evaluation are reported in this paper. A preliminary account of these data has been given.[4]

**Chemical.** The method used for the synthesis of the 4'-thiopyrimidine nucleosides was a modified Hilbert–Johnson reaction, 2,4-bis(*O*-trimethylsilyl) derivatives[5] of pyrimidine bases being used in place of 2,4-dialkoxypyrimidines. Condensation of 2,3,5-tri-*O*-acetyl-4-thio-D-ribofuranosyl chloride with the 2,4-bis(trimethylsilyl ethers) of uracil (Ia) or its 5-halogen derivatives (Ib–e, Scheme I) in dry toluene, at 95–100°, in the presence of mercuric acetate gave mixtures of $\alpha$ and $\beta$ anomers of the corresponding protected nucleosides IIa–e. The anomeric mixtures of the blocked nucleosides could not be separated by chromatography on silica gel. However, following deblocking by treatment with methanolic sodium methoxide at room temperature, separation of the anomers was achieved by fractional crystallization from ethanol and by chromatography on a dry column of silica gel, using a methanol–chloroform mixture as the eluent. The ratio of $\alpha$ to $\beta$ anomers obtained by this synthetic procedure was approximately 1:6. The site of glycosidation of the nucleosides (IIIa–e and IVa–e) was assigned at N-1 on the basis of their uv spectra (Table I). The uv absorption spectra of IIIa–e and IVa–e closely resemble those of uridine and its 5-halogen derivatives,[6] and they show little change when compared at pH 7.0 or 12.0. In this they differ markedly from $N^3$-substituted uracil derivatives, which at pH 12.0 demonstrate a strong bathochromic shift of the maxima and an increase in the extinction coefficient.[7,8] This property is also displayed by compound VIb, whose structure was unequivocally established by X-ray crystallography (Figure 1) demonstrating that the sulfur in the ribosyl moiety exerts little influence on the uv spectra. Thus, the assignment of N-1 as the site of attachment appears to be well supported. The anomeric configuration of IVb was determined as $\beta$ by X-ray crystallography (Figure 1). The anomeric configuration of the remaining compounds (IIIa,c–e, IVa,c–e) has not been definitively established, although a tentative assignment on the basis of their optical rotations can be made, the positive rotation designating the $\alpha$ anomer, the negative rotation the $\beta$ anomer.

**Scheme I**




I-IVa, X = H
b, X = F
c, X = Cl
d, X = Br
e, X = I

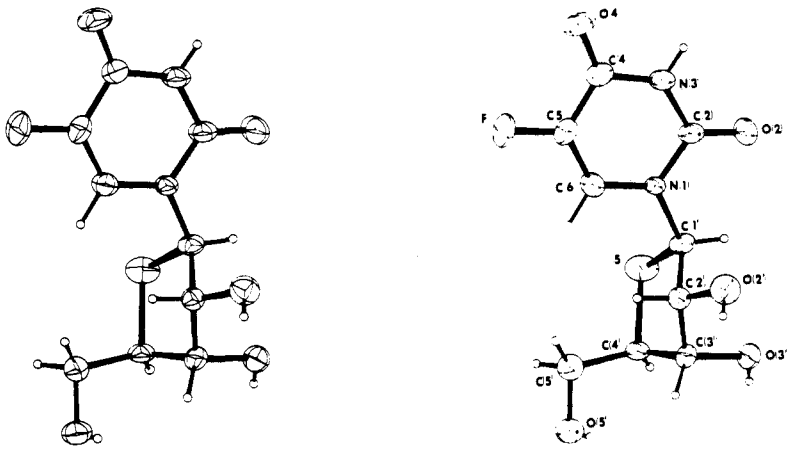



**Figure 1.** Structure of 4'-thio-5-fluorouridine as determined by X-ray crystallography (stereopair).

**Table I.** Physical-Chemical Characteristics of Some 4'-Thio-5-halogenopyrimidine Nucleosides

| Compd | Yield,[a] % | Mp, °C | Solvents used for recrysn at room temp | Uv spectra: 95% EtOH $\lambda_{max}$, nm | Uv spectra: 95% EtOH $\epsilon \times 10^3$ | Uv spectra: pH 12 $\lambda_{max}$, nm | Uv spectra: pH 12 $\epsilon \times 10^3$ | $[\alpha]^{24}D$ |
|---|---|---|---|---|---|---|---|---|
| IIIa | 4 | 244–246[b] | EtOH | 264 | 10.40 | 265 | 6.70 | *c* |
| | | | | 209 | 10.37 | | | |
| IVa | 26 | 190–191[d] | 95% EtOH | 265 | 9.88 | 265.5 | 6.77 | −43.6[d] |
| | | | | 209 | 6.91 | | | |
| IIIb | 5 | 218–219 | EtOH | 271 | 9.73 | 272 | 7.57 | +25.6 |
| | | | | 211 | 11.26 | | | |
| IVb | 28 | 200–201 | 95% EtOH | 271 | 13.51 | 272 | 7.57 | −21.0 |
| | | | | 211 | 11.81 | | | |
| IIIc | 4 | 221–223 | EtOH | 277 | 18.52 | 278 | 7.45 | +63.0 |
| | | | | 214 | 13.23 | | | |
| IVc | 21 | 197–198 | EtOH | 278 | 10.29 | 278 | 7.36 | −55.0 |
| | | | | 214 | 11.76 | | | |
| IIId | 10 | 228–230 | $H_2O$ | 280 | 11.86 | 281 | 6.74 | +63.3 |
| | | | | 214 | 9.49 | | | |
| IVd | 35 | 225–226 | 80% EtOH | 282 | 10.84 | 281.5 | 6.67 | −55.3 |
| | | | | 213 | 13.22 | | | |
| IIIe | 2 | 208–209 | $H_2O$ | 289 | 10.42 | 288 | 6.73 | *c* |
| | | | | 217 | 14.28 | | | |
| IVe | 18 | 225–227 | 90% EtOH | 290 | 8.10 | 288.5 | 6.43 | −71.6 |
| | | | | 217 | 12.73 | | | |

[a] Based on 2,3,5-tri-*O*-acetyl-4-thio-D-ribofuranosyl chloride. [b] Lit.[15] mp 246–248°. [c] Insufficient amount was obtained to measure the optical rotation. [d] Lit.[15] mp 191–192°; $(\alpha)^{25}$D −22.9° (*c* 2.1, water).

**Crystallography.** 4'-Thio-5-fluorouridine was crystallized from aqueous ethanol. The crystals are monoclinic and belong to space group $P2_1$ with unit cell constants $a$ = 6.781 (5) Å, $b$ = 6.240 (5) Å, $c$ = 12.948 (7) Å, $\beta$ = 90.8 (2)°, $Z$ = 2. Three-dimensional intensity data were collected to the limit $2\theta$ −135° for the Cu K$\alpha$ radiation ($\lambda$ K$\alpha_1$ = 1.54051 Å) using a GE XRD 5 diffractometer. The structure was solved by the heavy-atom method and refined by the least-squares method to an $R$ of 0.045. The three-dimensional structure of the molecule is illustrated in Figure 1, showing that the configuration at the anomeric center is $\beta$. The molecule exhibits the anti conformation with the glycosyl torsion angle $\chi_{CN}$ of 59°. The orientation of O(5') with respect to S and C(3') is trans,gauche; the S–C(4')–C(5')–O(5') and C(3')–C(4')–C(5')–O(5') torsion angles are 180 and −61°, respectively. The presence of sulfur in place of the ring oxygen of the ribose moiety gives rise to several changes in the geometry of the five-membered ring. The ring angle C(4')–S–C(1') in the thio sugar is 93.9° as compared to about 110° for C(4')–O–C(1') in the ribofuranose moiety, reflecting the longer bond distances of S–C(1') and S–C(4') as compared to the O–C(1') and O–C(4'). The other bond distances and angles in the two sugars do not differ markedly.

The substitution of S for O in the ring markedly affects the conformation of the five-membered ring and the orientation of O(5') with respect to S and C(4'). The "best" four-atom least-squares plane is through S, C(1'), C(2'), and C(4'); C(3') and C(5') are displaced from this plane by −0.63 and +1.30 Å, respectively. The next best four-atom least-squares plane is through S, C(1'), C(3'), and C(4'); C(2') is displaced by +0.59 Å, C(5') by +1.49 Å. Consequently, the conformation of this sugar is C(3')-exo–C(2')-endo. The commonly encountered conformation of the sugar moiety of various pyrimidine nucleosides is C(2')-endo or C(3')-endo.[9] The trans,gauche (tg) orientation

Table II. Effect of α- and β-4′-Thio-5-fluorouridine on the in Vitro Growth of Various Cell Systems[a]

| Compd | Molar concn for 50% growth inhibition of | | | |
|---|---|---|---|---|
| | Leukemia L1210 | Mammary carcinoma TA-3 | *S. faecium* | *E. coli* B |
| 4′-Thio-α-5-fluorouridine | $4 \times 10^{-7}$ | $>10^{-4}$ | $4 \times 10^{-9}$ | $6 \times 10^{-7}$ |
| 4′-Thio-β-5-fluorouridine | $2 \times 10^{-7}$ | $3 \times 10^{-7}$ | $6 \times 10^{-10}$ | $4 \times 10^{-5}$ |
| 5-Fluorouridine | $5 \times 10^{-8}$ | | $6 \times 10^{-11}$ | $7 \times 10^{-10}$ |

[a] The α and β anomers of 5-iodo-, 5-chloro-, and 5-bromo-4′-thiouridine were inactive at $1 \times 10^{-4}$ *M* concentrations.

Table III. Inhibition of the Growth of 5-Fluoropyrimidine-Resistant Strains of *S. faecium* by α- and β-4′-Thio-5-fluorouridine

| Compd | Molar concn for 50% growth inhibition | |
|---|---|---|
| | Sensitive | Resistant |
| 5-Fluorouracil | $7 \times 10^{-12}$ | $>10^{-3}$ |
| 5-Fluorouridine | $6 \times 10^{-11}$ | $>10^{-3}$ |
| 5-Fluoro-2′-deoxyuridine | $4 \times 10^{-11}$ | $>10^{-3}$ |
| β-4′-Thio-5-fluorouridine | $6 \times 10^{-10}$ | $2 \times 10^{-7}$ |
| α-4′-Thio-5-fluorouridine | $4 \times 10^{-9}$ | $4 \times 10^{-5}$ |

Table IV. Reversal by Pyrimidines of the Inhibition of *S. faecium* Growth Caused by 4′-Thio-5-fluorouridine and 5-Fluorouridine

| Pyrimidine | Inhibition index ($\times 10^3$)[a] | |
|---|---|---|
| | 4′-Thio-5-fluoro-uridine | 5-Fluoro-uridine |
| 2′-Deoxyuridine | 18 | 8 |
| Thymidine | 50 | 40 |
| Uridine | 0.7 | 0.3 |
| 2′-Deoxycytidine | 0.6 | 0.09 |
| Cytidine | 0.008 | 0.01 |

[a] Inhibition index = [I]/[S] for 50% growth inhibition.

found for O(5′) is also encountered infrequently in nucleosides.[10-14] The most favored conformation is the gauche,-gauche (gg).[9]

**Biological.** The effect which the α and β anomers of 4′-thio-5-fluorouridine exert on the growth of various cell systems is shown in Table II. Of primary interest is the observation that both the α and β anomers of 5-fluoro-4′-thiouridine are active against leukemia L1210 cells, whereas only the β anomer is inhibitory to mammary carcinoma TA-3 cells, the α anomer being inactive. Neither the α nor the β anomers of the iodo, chloro, or bromo analogs demonstrated any inhibitory activity at the highest concentration ($10^{-4}$ *M*) tested. Because the 4′-thio derivatives of antimetabolites such as 6-thioinosine or toyocamycin were active against cells which were resistant to the antimetabolites,[2,3] it was of interest to determine whether, by analogy, 4′-thio-5-fluorouracil would retain activity against cells resistant to 5-fluorouridine or 5-fluoro-2′-deoxyuridine. As shown in Table III, this is indeed the case. The growth of strains of *Streptococcus faecium* resistant to $10^{-3}$ *M* concentrations of various 5-fluoropyrimidines was still sensitive to inhibition by both the α and β anomers of 4′-thio-5-fluorouridine, although this sensitivity was somewhat less than that encountered in the parent strain.

The reason for the retention of this partial sensitivity is at present unclear. It remains to be demonstrated whether the 4′-thio nucleosides require activation by phosphorylation. In any event, the 4′-thio analogs, like the 5-fluoropyrimidines, appear to exert their inhibitory effects along the pyrimidine path, as indicated by the fact that their inhibition of cell growth is prevented, competitively, by the natural pyrimidines. Purines do not affect the inhibition. An inhibition analysis carried out with both 4′-thio-5-fluorouridine and 5-fluorouridine shows a similar reversal pattern (Table IV). That does not mean, however, that the two compounds act entirely at identical sites. Indeed, as shown in Figure 2, 5-fluorouridine appears to be a more potent inhibitor of DNA synthesis than is 4′-thio-5-fluorouridine, the latter appearing to act more effectively on RNA synthesis. In both instances, protein synthesis appears ultimately to be inhibited most markedly. In this cell system, the initial pronounced inhibition of DNA synthesis by FUR is replaced, after approximately 1 hr of incubation, by the more marked inhibition of protein synthesis. This shift might result from the incorporation of the analog into RNA,[1] which may have a more permanent effect on cell growth than does its inhibition of the thymidylate synthetase.[1]

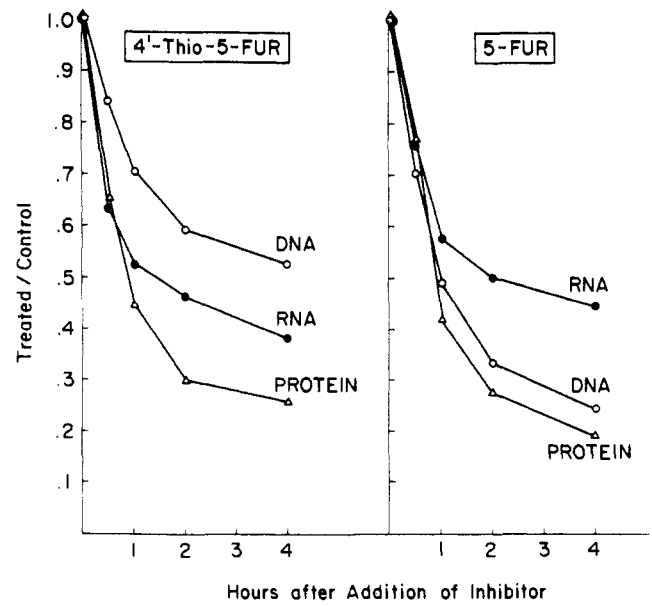



Figure 2. Comparative effect of 4′-thio-5-fluorouridine and 5-fluorouridine on total RNA, DNA, and protein content of *S. faecium*.

## Experimental Section

Melting points were determined on a Fisher-Johns apparatus; uv spectra were recorded on a Cary Model 14 spectrophotometer. Optical rotations are equilibrium values and were determined on a Perkin-Elmer Model 141 polarimeter at 0.5 *M* concentration in methanol. Solvent concentration was conducted under reduced pressure in a rotary evaporator. Satisfactory analytical results (C, H, N, and S within 0.4% of the theoretical values) were obtained from Robertson Laboratory, Florham Park, N.J.

**Preparation of the Protected Anomeric Nucleosides IIa–e.** Pyrimidine bases were converted to their trimethylsilyl derivatives by treatment with boiling hexamethyldisilazane[5] in the presence of a catalytic amount of trimethylchlorosilane. The excess of hexamethyldisilazane was removed by repeated coevaporation with dry toluene (three times), and the remaining crude trimethylsilyl ethers were used immediately in the next step. A mixture of 0.01 mol of 2,3,5-tri-*O*-acetyl-4-thio-D-ribofuranosyl chloride,[15] 0.011 mol of the 2,4-bis(*O*-trimethylsilyl) derivative of uracil[16] (Ia) or of Ib–e,[17] and 0.01 mol of mercuric acetate in 100 ml of dry toluene was stirred at 95–100° for 2 days. The mixture was cooled to 22° and was concentrated to a syrup, which was dissolved in EtOAc (150 ml). The solution was washed successively with a 20% KI solution (2 × 80 ml) and with $H_2O$ (100 ml), dried ($Na_2SO_4$), and evaporated to a syrup. The syrupy residue was purified by column chromatography on silica gel in a $C_6H_6$–$Me_2CO$ (8:1, v/v) mixture. The anomeric mixtures of the nucleosides IIa–e were obtained after evaporation of the solvent as glassy residues. The yields were as follows: IIa = 42%, IIb = 48%, IIc = 35%, IId = 60%, and IIe = 27%.

**Preparation of the Nucleosides IIIa–e and IVa–e.** The syrupy mixtures of the α and β anomers of the acetylated nucleosides IIa–e (0.005 mol) were dissolved in 50-ml portions of dry MeOH. NaOMe (100 mg) was added, and the solutions were kept at 22° for 15 hr. Dowex-50 ($H^+$) ion exchange resin (3 ml) was added, the mixtures were filtered, and the resin was washed with MeOH (40 ml). Each combined filtrate was concentrated to a syrup, which was dissolved in 95% EtOH (50 ml), and the resulting solution was evaporated to a syrup. The syrupy residue was dissolved in 95% EtOH (5 ml), and the solution kept at 22° for 15 hr. The crystalline compounds IVa–e were collected by filtration, washed with cold EtOH (2 ml), and recrystallized. The mother liquors were combined with the respective washings and concentrated to approximately 2 ml. The solutions were applied to 2 × 60 cm columns of dry silica gel, and these compounds were eluted with $CHCl_3$–MeOH (6:1, v/v). The α anomers eluted from the columns first and they, as well as the β anomers, crystallized after evaporation of the solvent and were recrystallized. The ratio of α:β anomers obtained by this procedure was approximately 1:6. Some of the pertinent physical–chemical characteristics of the newly synthesized compounds are shown in Table I.

**Biological and Biochemical Assay Procedures.** The techniques used for assaying the growth inhibitory activity of the analogs in the bacterial[18] and tumor cell systems,[19] and the procedures employed for isolating the fluoropyrimidine resistant strains,[20] have been published previously. The effect of the agents on RNA, DNA, and protein synthesis in *S. faecium* was determined by procedures also described previously.[18]

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# Synthesis and Pharmacology of 5-Noralkyl-9β-methyl-6,7-benzomorphans and Stereochemistry of Some Intermediates

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2,9β-Dimethyl-2′-hydroxy-6,7-benzomorphan (**18**) has been synthesized from *m*-methoxyphenylacetone (**6a**) or *m*-methoxyphenylacetonitrile (**1**) via bromo-α-tetralone (**10**). Isomeric bromo-α-tetralone **9**, instead of undergoing cyclization to a 6,7-benzomorphan, gave aromatization product **12**. The structures and stereochemical assignments of **9**, **10** (and thus **7** and **8**), and **18** follow from analogy and from NMR data of **9**, **10**, **17**, and **18**. Compound **18** and the deoxy analog **16** are as potent as morphine and codeine, respectively, as analgetics (mice) and are without physical dependence capacity (monkeys).

Recently,[1] we reported that 2,9-dimethyl-6,7-benzomorphan (**16**), not obtainable in the usual way[2] from 3-methylpyridine. could be synthesized in 12 steps from phenylacetonitrile, only the β isomer being formed. By some modifications of this sequence we have now prepared the 2′-hydroxy relative **18**. Described below are the synthesis of **18** and the analgetic and other pharmacologic properties of **16** and **18** and two analogs, **15** and **17**.

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