6-Substituted and 5,6-Disubstituted Derivatives of Uridine: Stereoselective Synthesis, Interaction with Uridine Phosphorylase, and *in Vitro* Antitumor Activity

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Stereoselective procedures are described for the synthesis of 6-alkyluridines by Lewis acid-catalyzed condensation of (a) trimethylsilylated 6-alkyl-4-alkylthiouracils with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranose (ABR) and (b) trimethylsilylated 6-alkyl-3-benzyluracils with ABR. The 4-methylthio group was subsequently removed with the use of 1 N trifluoroacetic acid and the 3-benzyl group by a new modified procedure with the use of the complex BBr_3-THF. Furthermore, 6-(hydroxymethyl)uridine (**39**) and 5-fluoro-6-(hydroxymethyl)uridine (**40**) were obtained by sequential oxidation with SeO_2 and reduction with tetrabutylammonium borohydride of the 6-methyl group of 6-methyluridine (**5**) and 5-fluoro-6-methyluridine (**35**), and their corresponding 6-fluoromethyl congeners **41** and **42** were obtained by DAST treatment of **39** and **40**, respectively. For all the foregoing nucleosides in the fixed *syn* conformation about the glycosyl bond, ^{1}H NMR spectroscopy further demonstrated that the pentose rings exist predominantly in the conformation N (3′-*endo*). Most of the nucleosides were weak substrates of *Escherichia coli* pyrimidine nucleoside phosphorylase. Enhanced susceptibility to phosphorolysis was exhibited by two of them, **39** and **41**, with 6-CH_2OH and 6-CH_2F substituents capable of formation of an additional hydrogen bond with the enzyme. The 5-fluoro-6-substituted uridines were the poorest substrates. Cytotoxicities of the nucleosides were examined vs the human tumor cell lines MOLT-3, U-937, K-562, and IM-9, as well as PHA-stimulated human lymphocytes. Two of the analogues, 5-fluoro-6-(fluoromethyl)uridine (**42**) and 5-fluoro-6-(hydroxymethyl)uridine (**40**), exhibited cytotoxicities comparable to that of 5-fluorouracil.

Introduction

In both aqueous and nonaqueous media most pyrimidine nucleosides exist predominantly in the *anti* conformation about the glycosyl bond, in part due to repulsive interaction between the C(2) carbonyl and the furanose ring. One notable exception is the naturally occurring orotidine (6-carboxyuridine), which is constrained to the *syn* conformation by steric hindrance between the sugar ring and the bulky 6-carboxy substituent.[1] Pyrimidine nucleosides constrained to the *syn* conformation by a C(6)-substituent are of interest (a) as potential antimetabolites, e.g., 6-thiocarboxamide-UMP, a structural analogue of orotidine-5′-phosphate (OMP), is a potent inhibitor of OMP decarboxylase,[2] and (b) as model compounds to determine whether the parent nonsubstituted nucleoside (or nucleotide) is involved in a given enzymatic reaction in the *syn* and/or *anti* conformation; e.g., it was long ago shown that 6-methyluridine is a moderate substrate for uridine phosphorylase from *Salmonella typhimurium*.[3]

Since modification of substrate/inhibitor properties may be due not only to constrainment to the *syn* conformation by a C(6)-substituent but also to steric effects of the latter, it is clearly desirable to examine the biological effects of various C(6)-substituents. We herein describe procedures for the stereospecific synthesis of a number of C(6)-substituted uridines and 5-fluorouridines, their substrate properties with uridine phosphorylase from *Escherichia coli*, and their cytotoxicities toward a variety of human tumor cell lines.

Chemistry

The major obstacle to synthesis of 6-substituted and 5,6-disubstituted pyrimidine nucleosides by condensation methods is the partial, or exclusive, formation of the undesired N(3)-isomers, even under the optimal conditions described by Vorbruggen et al.[4] for maximal yields of the N(1)-isomers. With a view to avoiding formation of the N(3)-glycosides, we have developed condensation methods in which the pyrimidine N(3) is blocked, e.g., N(3)-benzyl or 4-alkylthio derivatives.

Condensation of the TMS derivative **2** of 3-benzyl-6-methyluracil (**1**) with 1-*O*-acetyl-2,3,5-tri-*O*-benzoylribfuranose (ABR), catalyzed by trimethylsilyl triflate (TMSOTfl) in MeCN (Scheme 1), in our hands led to 2′,3′,5′-tri-*O*-benzoyl-3-benzyl-6-methyluridine (**3**) in 76% yield. Earlier attempts to remove the benzyl group from 3-benzyl-6-methyluridine (**4**) by catalytic reduction or with sodium in liquid ammonia were unsuccessful.[5] The use of sodium naphthalene was subsequently found to be effective,[6] and we initially attempted to use it for removal of the benzyl group from 3-benzyl-6-methyluridine (**4**). But, since preparation of the reagent is tedious, requiring the use of oxygen-free medium devoid of CO_2, we made use of the complex of boron tribromide with THF, formed *in situ*. This procedure is simpler

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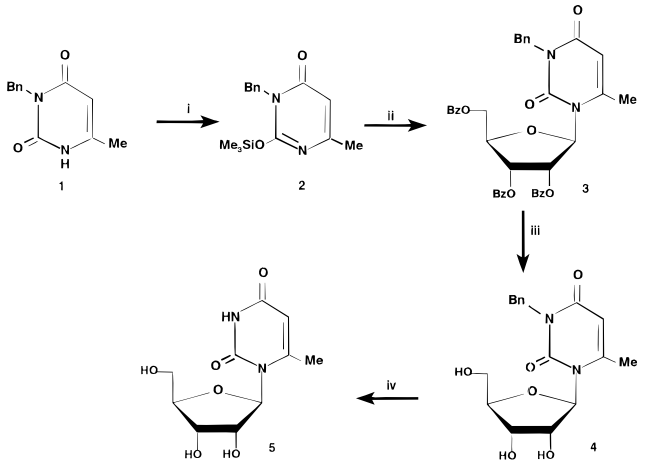
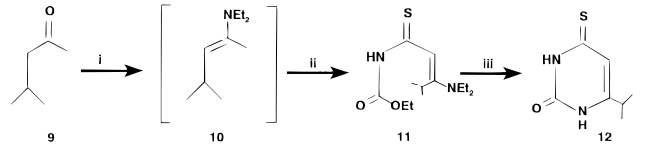
Scheme 1[a]



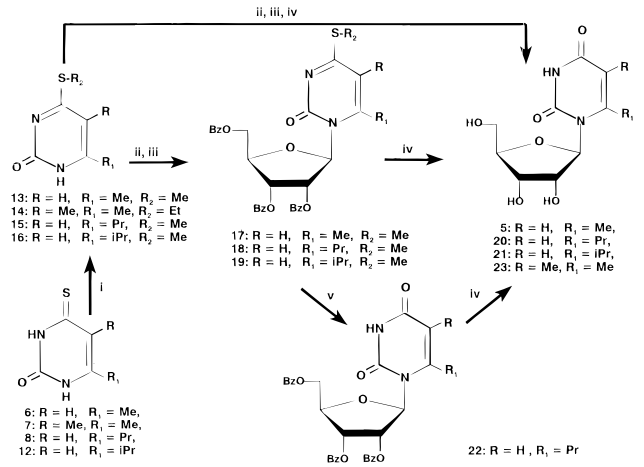
[a] Reagents: (i) HMDS/$(NH_4)_2SO_4$, reflux, 12 h; (ii) ABR, TMSTfl/CH_3CN, 2 h; (iii) NaOMe/MeOH; (iv) BBr_3/THF, room temperature, 2 h.

Scheme 2[a]



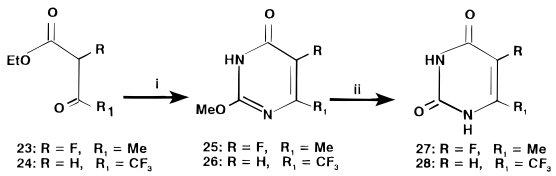
[a] Reagents: (i) Et_2NH, $TiCl_4$/hexane, room temperature, 1 h; (ii) ethoxycarbonyl isothiocyanate, Et_2O, room temperature, 1 h; (iii) NH_3 (aqueous), room temperature, 48 h.

Scheme 3[a]



[a] Reagents: (i) MeI or EtI, NaOH, room temperature; (ii) HMDS/$(NH_4)_2SO_4$, reflux, 12 h; (iii) ABR, TMSTfl/MeCN; (iv) NaOMe/MeOH; (v) 1 N TFA/1,4-dioxane, room temperature.

Scheme 4[a]



[a] Reagents: (i) methylpseudoisourea, $Ca(OH)_2$, H_2O/EtOH, room temperature, 72 h; (ii) 2 N H_2SO_4, 70 °C, 2 h.

and effective; e.g., it permitted conversion of **4** to 6-methyluridine (**5**) in 67% yield.

A 4-methylthio group was employed by Mizuno et al. and Winkley and Robins in the noncatalyzed condensation of 5-(methylthio)-6-azauracil[7a,b] and 4-(methylthio)-6-methyluracil[7c] with 1-halogeno-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose, and the products, without isolation, were subjected to amination to obtain 6-substituted cytidines. Despite the low yield of 6-methylcytidine (8%), this procedure appeared promising if applied in conjunction with catalyzed condensation methods.

6-Methyl-4-thiouracil (**6**), 5,6-dimethyl-4-thiouracil (**7**), and 6-propyl-4-thiouracil (**8**) were obtained by thiation of the parent alkyluracils with the Lawesson reagent in 1,4-dioxane.[8] 6-Isopropyl-4-thiouracil (**12**) was prepared by pyrimidine ring closure[9] (Scheme 2), whereby the reaction of methyl isopropyl ketone (**9**) with diethylamine gave initially the enamine **10**. This was treated with ethoxycarbonyl isothiocyanate to give the adduct **11** (75%) which was converted with ammonia to the desired product **12** in 92% yield.

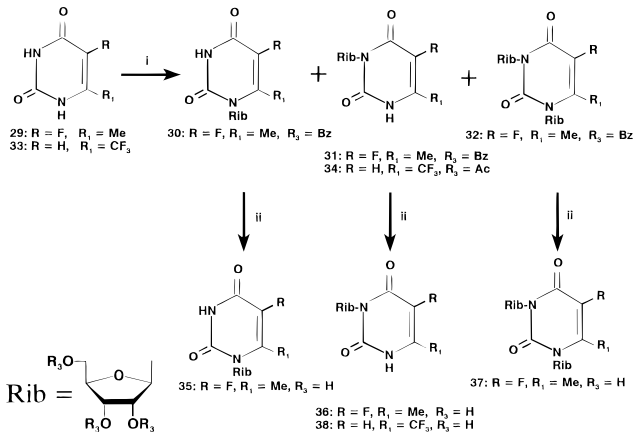
The four (alkylthio)uracils **13**−**16** were obtained by treatment of the corresponding 4-thiouracils with the appropriate alkyl iodide in the presence of base.[7c] As shown in Scheme 3, condensation of the TMS derivatives of 4-(alkylthio)uracils **13**−**16** with ABR was conducted in MeCN. With 0.5 mol equiv of TMSOTfl as catalyst, the course of the reaction was smooth, with good yields (80−90%) and no cleavage of the 4-alkylthio group. The condensation products **17** and **19** were deblocked with methoxide, and the 4-alkylthio group was subjected to hydrolysis with 1 N TFA (80% yields). This procedure led to 6-methyluridine (**5**) and 6-isopropyluridine (**21**). 1-β-D-Ribofuranosyl-6-propyluracil (**20**) was obtained by first removing the 4-methylthio group from the product of condensation (**18**) with the use of 1 N TFA and then crystallization of 1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-6-propyluracil (**22**) in 74% yield followed by methanolysis with sodium methoxide.

The foregoing high condensation yields were profited from to select conditions for direct synthesis of 6-methyluridine (**5**) from 4-(methylthio)-6-methyluracil (**69**) and (ABR), by methanolysis of the product of condensation, followed by hydrolysis of the 4-methylthio group with 1 N TFA, leading to 6-methyluridine (**5**) in 80% yield. The same procedure with 5,6-dimethyl-4-(ethylthio)uracil (**14**) gave 5,6-dimethyluridine (**23**) in 60% yield.

Unfortunately, the foregoing simple and selective procedures proved inapplicable to the synthesis of analogues with electrophilic substituents at C(5) or C(6), since prolongation of the reaction time, or the use of higher concentrations of catalyst, was inconsistent with the lability of 4-alkylthio derivatives. Consequently, for synthesis of 5-fluoro-6-methyluridine (**35**) and 6-(trifluoromethyl)uridine from 5-fluoro-6-methyluracil (**27**) and 6-(trifluoromethyl)uracil (**28**), respectively, use was made of the optimal conditions described by Vorbrüggen et al.[4] for condensation of 6-methyluracil with the ribose derivative ABR.

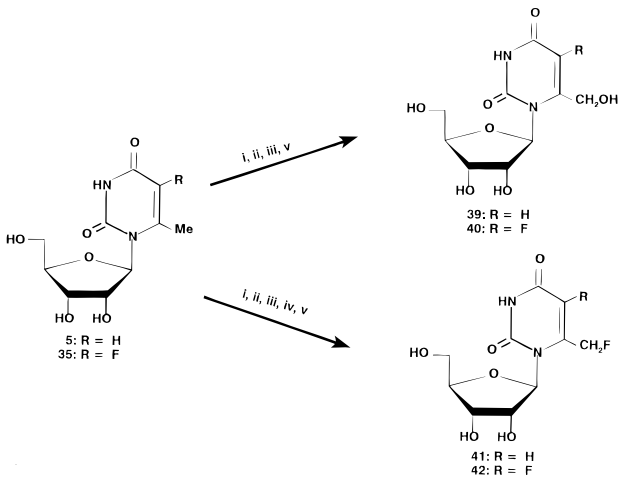
5-Fluoro-6-methyluracil (**27**)[10] and 6-(trifluoromethyl)uracil (**28**)[10,11] were prepared from the appropriate β-keto esters: ethyl α-(fluoroacetyl)acetate (**23**) and ethyl (trifluoroacetyl)acetate (**24**), as shown in Scheme 4, using the procedure hitherto applied for preparation of 5,6-dialkyl- and 6-alkyluracils.[12] Reaction of the appropriate β-keto ester in aqueous alcohol with methylpseudoisourea, catalyzed by $Ca(OH)_2$, gave 5-fluoro-2-methoxy-6-methyluracil (**25**) in 63% yield and 2-meth-

Scheme 5[a]



[a] Reagents: (i) with **29**, HMDS/TCS, reflux, 6 h, ABR, TMSTfl/MeCN, room temperature, 2 h; with **33**, BSTFA/MeCN, 1,2,3,5-tetra-*O*-acetyl-1-*β*-D-ribofuranose, TMSTfl/MeCN, room temperature, 30 min; (ii) NaOMe/MeOH.

Scheme 6[a]



[a] Reagents: (i) Ac_2O, DMAP, room temperature; (ii) SeO_2, 1,4-dioxane/AcOH, reflux, 18 h; (iii) $TBABH_4$/MeOH; (iv) DAST/CH_2Cl_2, room temperature; (v) NH_3/MeOH, 20 °C.

oxy-6-(trifluoromethyl)uracil (**26**) in 66% yield. Acid hydrolysis of **25** and **26** led to 85% yields of **27** and **28**.

Treatment of **27** with HMDS/TCS gave 2,4-bis-*O*-(trimethylsilyl)-5-fluoro-6-methyluracil (**29**), which was condensed with ABR in MeCN (Scheme 5) in the presence of 1.1 equiv of TMSOTfl to give a mixture of blocked isomers consisting of 1-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)-5-fluoro-6-methyluracil (**30**; 45%), the corresponding 3-isomer **31** (21%), and the 1,3-disubstituted analogue **32** (20%). These were fractionated on a column of activated Al_2O_3. Methanolysis catalyzed by MeONa led to the free nucleosides **35** (80%), **36** (85%), and **37** (87%). Use of a stronger Lewis acid, $SnCl_4$, as catalyst resulted in formation of only the N(3)-riboside **31**. The UV spectrum of **35** exhibited an increase in absorbance, relative to that of **5**, characteristic for a 5-fluoronucleoside. Spectrophotometric titration gave a pK_a for **35** of 7.75, as compared to 9.80 for **5**, characteristic for 5-fluorouridine.[13]

In contrast to **29**, condensation of di-*O*-TMS-6-(trifluoromethyl)uracil (**33**) with 1,2,3,5-tetra-*O*-acetyl-1-*β*-D-ribofuranose gave only the N(3)-isomer **34**. The reaction was conducted in MeCN, with formation of **33** *in situ* in the reaction mixture, but with replacement of BSA as the silylating reagent by the more effective BSTFA. The catalyst in the condensation reaction was 1.5 equiv of TMSOTfl in MeCN. Following 0.5 h one product (**34**) appeared (87%) which, following methanolysis catalyzed with MeONa, was identified as 3-*β*-D-ribofuranosyl-6-(trifluoromethyl)uracil (**38**). Replacement of TMSOTfl by $SnCl_4$ and the solvent MeCN by 1,2-dichloroethane did not lead to the desired N(1)-isomer. The UV spectrum of **38** exhibited, characteristic for N(3)-substituted uracils,[14] a strong bathochromic shift for the anionic form relative to the neutral species.

As mentioned above, uracils with an electrophilic substituent at C(6), when used in a condensation reaction with ABR, lead to formation of only the N(3)-ribosides. On the other hand, introduction of a fluorine at C(5) of 6-substituted uridines is technically difficult, involving use of toxic and explosive F_2 or CF_3OF.[15] Hence, to prepare the biologically interesting 5-fluoro-6-(fluoromethyl)uridine (**42**), recourse was made to modification of an analogue with a 5-fluoro substituent. Introduction of a fluoromethyl substituent at C(6) was based on the relatively mild exchange of an alcoholic hydroxyl with fluorine, e.g., with the use of DAST ((diethylamino)sulfur trifluoride). The key step was the use of an analogue with a hydroxymethyl group at C(6). As starting compounds, we employed **5** or 5-fluoro-6-methyluridine (**35**), as shown in Scheme 6. These were acetylated with acetic anhydride in the presence of (*N*,*N*-dimethylamino)pyridine (DMAP) as catalyst and oxidized *in situ* with SeO_2 followed by reduction with tetrabutylammonium borohydride, which is very soluble in organic solvents. The crude products, *O*′-acetyl derivatives of 6-(hydroxymethyl)uridine (**39**) or its 5-fluoro congener **40**, were subjected to fluorination with DAST and, after initial workup, the acetyl groups removed with MeOH-saturated ammonia. The products were isolated by preparative layer chromatography to give 6-(fluoromethyl)uridine (**41**; 45%) and 5-fluoro-6-(fluoromethyl)uridine (**42**; 35%). The crude acetylated derivatives of **39** and **40** were deblocked with methanolic ammonia to obtain free 6-(hydroxymethyl)uridine (**39**; 45%) and 5-fluoro-6-(hydroxymethyl)uridine (**40**; 48%).

Conformational Aspects

The conformational parameters of the foregoing nucleosides in neutral aqueous medium (D_2O), derived from analyses of the vicinal $^1H,^1H$ coupling constants (see Experimental Section) with the aid of the modified Karplus relationship,[16a] are listed in Table 1. It will be noted that, for all the 6-substituted analogues, irrespective of the presence of a C(5)-fluoro substituent, the pentose ring conformation is predominantly N, i.e., C(3′)-*endo* as compared to 50% for the parent uridine and 60% for 5-fluorouridine. The rotamers Ng^+ and Nt have comparable energies, as in the case of orotidine and cyanuric acid riboside.[1] The 6-substituent on the uracil ring destabilizes the rotamer Sg^+ and stabilizes the Nt rotamer, a characteristic feature associated with the conformation *syn*.[16b,c] This suggests that repulsive interaction occurs between the pentofuranose substituents and C(2) oxygen of the uracil moiety. These results are in accord with previous reports that, for 6-methylribo- and -2′-deoxyribonucleosides in solution, there is a trend in favor of the sugar ring conformation N (C(3′)-*endo*).[16d] Furthermore, bearing in mind that

Table 1. Solution Conformation of 6-Substituted and 5,6-Disubstituted Pyrimidine Nucleosides Calculated from 500-MHz ^{1}H NMR Spectra[a]

nucleoside	conformations								
	S	g^+	t	g^-	Ng^+	Nt	Sg^+	St	Sg^-
Urd	0.46	0.61	0.32	0.08	0.41	0.19	0.20	0.13	0.08
5-F-Urd	0.40	0.67	0.29	0.04	0.42	0.17	0.24	0.12	0.04
6-Me-Urd (**5**)	0.17	0.42	0.52	0.06	0.39	0.43	0.02	0.09	0.06
6-Pr-Urd (**20**)	0.14	0.41	0.52	0.06	0.41	0.45	0.01	0.07	0.06
6-*i*-Pr-Urd (**21**)	0.13	0.41	0.53	0.05	0.40	0.46	0.01	0.07	0.05
5,6-diMe-Urd (**23**)	0.17	0.43	0.51	0.06	0.40	0.42	0.03	0.09	0.06
6-CH_2F-Urd (**39**)	0.21	0.42	0.52	0.06	0.37	0.41	0.04	0.11	0.06
6-CH_2OH-Urd (**41**)	0.17	0.42	0.52	0.06	0.40	0.43	0.02	0.09	0.06
5-F-6-Me-Urd (**35**)	0.15	0.43	0.53	0.03	0.39	0.45	0.04	0.08	0.03
5-F-6-CH_2OH-Urd (**40**)	0.20	0.42	0.52	0.07	0.39	0.41	0.03	0.10	0.07
5-F-6-CH_2F-Urd (**42**)	0.17	0.42	0.52	0.06	0.40	0.43	0.02	0.09	0.06

[a] Spectra obtained in D_2O.

the 5-fluoro-substituted analogues are partially in the monoanionic form at neutral pH (pK_a ~7.9), it appears also that the state of ionization of the pyrimidine ring has little effect on the pentose ring conformation.

It may be concluded that the predominant N conformation of the sugar ring in aqueous medium is imposed by the constrained *syn* conformation due to the steric effect of the 6-substituents. It is therefore of interest that, in the solid state, 6-methyluridine[17a] and 6-methyl-2′-deoxyuridine[17b] both exhibit the pentose ring in the S conformation, i.e., C(2′)-*endo* conformation. In 6-methyl-2′-deoxyuridine, with the exocyclic 5′-CH_2OH in the g^+ rotameric form, this conformation appears at first sight to be stabilized by an intramolecular C(5′)−OH···O(2′) hydrogen bond. However 6-methyluridine exhibits two independent molecules in the asymmetric unit, both with the pentose ring in the S conformation, but only one of them possesses the g^+ rotamer of the exocyclic CH_2OH group, which is hydrogen bonded to the O(2) of the pyrimidine ring. In the other molecule the exocyclic group is in the gt rotameric form, and there is no bond to O(2). It follows that the S conformation in the solid state is not necessarily dependent on intramolecular hydrogen bonding.

An interesting feature displayed by 5-fluoro-6-(hydroxymethyl)uridine (**40**) with respect to **39**, and possibly relevant to its biological activity (see below), is the reduced half-width of the H signal of the 6-CH_2OH group, indicative of a lower rate of proton exchange. Furthermore, the 0.35 ppm upfield shift of this proton, relative to that of **39**, is suggestive of electrostatic interaction between the C(5)-F and 6-CH_2OH, resulting in hindered rotation about the C(6)−CH_2 bond, and reflected by the coalescence of the methylene group signals. In the parent 6-(hydroxymethyl)uridine (**39**), the half-width of the methylene group signals is 2 Hz, consistent with much more rapid, albeit hindered, rotation of the C(6)-CH_2OH group, and the OH signal is broadened by rapid exchange with H_2O.

Biological Results

Interaction with *E. coli* Uridine Phosphorylase. Table 2 exhibits the kinetic constants for phosphorolysis of the C(6)-substituted uridines and 5-fluorouridines, relative to those for the parent uridine and 5-fluorouridine, respectively.

To ensure that the data refer to the behavior of the neutral forms of both series of analogues, those for the 5-fluorouridines (for which the pK_a values are in the range 7.3−7.9) were measured at pH 6. As regards the parent uridine (for which pK_a = 9.3), it will be noted that the kinetic constants at pH 6.0 are slightly lower than those at pH 7.5. By contrast, 5-fluorouridine (pK_a = 7.75) is an appreciably poorer substrate at pH 7.5, where it consists of a mixture of the neutral and monoanionic forms, suggesting that the neutral form is the substrate for phosphorolysis.

Table 2. Kinetic Constants for Phosphorolysis of the Neutral Forms of 6-Substituted and 5-Fluoro-6-substituted Uridines

nucleoside	pK_a	app K_m (μM)	V_{max} (μmol/min × mg of prot)	efficacy (V_{max}/K_m)
		pH 7.5		
Urd	9.23	122 ± 11	340 ± 12	2.8[a]
6-Me-Urd (**5**)	9.50	1030 ± 53	150 ± 5	0.15
6-Et-Urd (**43**)	9.50	1200 ± 90	175 ± 10	0.15
6-Pr-Urd (**20**)	9.50	1150 ± 150	40 ± 4	0.03
6-CH_2F-Urd (**39**)	9.80	476 ± 90	254 ± 27	0.53
6-CH_2OH-Urd (**41**)	9.80	300 ± 27	297 ± 14	0.99
		pH 6.0		
5-F-Urd	7.75	152 ± 20	323 ± 22	2.1[b]
5-F-6-Me-Urd (**35**)	7.70	1355 ± 30	67 ± 1	0.05
5-F-6-CH_2F-Urd (**40**)	7.90	4000	370	0.09
5-F-6-CH_2OH-Urd (**42**)	7.30	979 ± 50	57 ± 4	0.06

[a] At pH 6.0, K_m = 182 ± 25 mM, V_{max} = 312 ± 12, and V_{max}/K_m = 1.7. [b] At pH 7.5, K_m = 110 ± 4.6 mM, V_{max} = 43 ± 1, and V_{max}/K_m = 0.4.

It will be seen from Table 2 that 6-methyluridine (**5**) is a substrate, albeit a weak one, which is consistent with the earlier finding that it is a much better substrate for the analogous enzyme from *S. typhimurium*.[3] As expected, 6-methyluridine (**5**) proved to be a competitive inhibitor with respect to uridine (with the aid of Dixon plots, not shown), with a K_i ~ 2 mM, comparable with the K_m for phosphorolysis, 1.03 mM.

6-Ethyluridine (**43**) is not a better substrate, whereas 6-propyluridine (**20**) is a poorer one, presumably because of enhanced steric effects. In general the foregoing results seem to be consistent with the postulate that the actual substrate in the case of uridine is the *syn* conformer, imposed on binding to the enzyme. This conclusion is supported by the substrate properties of 6-(hydroxymethyl)uridine (**39**) and 6-(fluoromethyl)-uridine (**41**).

Quite unexpected was the finding that, whereas 5-fluorouridine is as good a substrate as uridine, the 5-fluoro-6-substituted congeners are very poor substrates. This is most striking for compounds **40** and **42,** which are the 5-fluoro analogues of the good substrates **39** and **41,** respectively. Although not readily interpretable, this may be relevant to the potential chemotherapeutic activities *in vivo* of these compounds.

Table 3. Inhibition of Cell Growth by 6-Substituted and 5,6-Disubstituted Uridines

compound	IC_{50}[a] (μg/mL) MOLT-3[b]	U-937	K-562	IM-9	PHA-Ly
5-F-Ura	1.2	0.5	4.3	0.34	1.4
5-F-Urd	<0.01	<0.01	0.026	<0.01	<0.1
5-F-6-Me-Urd (**35**)	>10.0	>10.0	>10.0	>10.0	>10.0
6-CH_2OH-Urd (**39**)	>10.0	>10.0	>10.0	>10.0	98
5-F-6-CH_2OH-Urd (**40**)	1.2	3.7	4.9	3.0	2.8
6-CH_2F-Urd (**41**)	>10.0	>10.0	>10.0	9.9	>10.0
5-F-6-CH_2F-Urd (**42**)	3.5	3.7	>10.0	>10.0	4.5

[a] IC_{50} is the concentration (μg/mL) of compound which caused a 50% decrease in [^{14}C]leucine incorporation by the cells in culture. Values were calculated from dose−response curves done in triplicate for each analogue. [b] MOLT-3, acute T cell leukemia; U-937, histiocytic lymphoma; K-562, chronic myelogeneous leukemia; IM-9, myeloma; PHA-Ly, phytohemagglutinin-stimulated peripheral blood lymphocytes.

***In Vitro* Antitumor Activity**. The cytotoxicities of the foregoing 6-substituted and 5,6-disubstituted uridine analogues vs four human leukemic cell lines, MOLT-3, U-937, IM-9, and K-562, were determined and compared with those vs phytohemagglutinin-stimulated human lymphocytes (PHA-Ly) with results shown in Table 3.

Two of the analogues, 5-fluoro-6-(hydroxymethyl)-uridine (**40**) and 5-fluoro-6-(fluoromethyl)uridine (**42**), exhibited potent antileukemic activities, comparable to that of the standard drug 5-fluorouracil (FUra). Furthermore both compounds were less toxic to human lymphocytes and, in the case of MOLT-3 and K-562 cells, more selective than FUra.

Conclusions

A number of 6-substituted uridines, all in the nontypical *syn* conformation, are reasonable substrates of *E. coli* uridine phosphorylase, two of them comparable to the parent uridine, and do not exhibit cytotoxicity vs leukemic cells *in vitro.* The 5-fluoro derivatives of these 6-substituted uridines are poorer substrates of the enzyme, and two of them, **40** and **42**, exhibit cytotoxicities comparable to that of 5-fluorouracil vs hematopoietic human leukemic cell lines. These uridine phosphorylase-resistant compounds are reasonable candidates as *in vivo* antitumor agents.

Experimental Section

General Methods. Melting points (uncorrected) were measured on a Boetius microscopic hot stage; UV spectra were recorded on a Cary 3 instrument, using 10-mm path length cuvettes, acetate buffers in the pH range 3−4, and phosphate buffers in the range 6−8.4. Extremes of pH made use of standard solutions of HCl and NaOH. A Cole-Parmer instrument with combination electrode was employed for pH measurements. High-resolution EI mass spectra for pyrimidines were carried out on a Finigan MAT spectrometer and liquid matrix secondary ion mass spectra (LSIMS) for nucleosides with an AMD-604 spectrometer. High-resolution ^{1}H NMR spectra were recorded on a Bruker 500-MHz spectrometer in D_2O with DSS as internal standard, unless otherwise indicated. All evaporations were under vacuum at 35 °C. Thin-layer chromatography (TLC) was run on Merck silica gel F_{254} glass plates (DC, 20 × 20 cm, 0.25 mm, no. 5715), Merck aluminum oxide F_{254} glass plates (DC, 20 × 20 cm, 0.1 mm, no. 5713), and Merck cellulose F glass plates (DC, 20 × 20 cm, 0.1 mm, no. 5718). The following solvents (v/v) were used (A) benzene−EtOAc, 70:30; (B) $CHCl_3$−MeOH, 80:20; (C) $CHCl_3$−MeOH, 90:10; (D) $CHCl_3$−EtOAc, 80:20; (E) benzene−EtOAc, 90:10; (F) benzene−EtOAc, 100:10; (G) $CHCl_3$−Me_2CO, 90:10; (H) benzene−EtOAc, 80:20; (I) $CHCl_3$−MeOH, 85:15; (J) EtOAc−*n*-PrOH−H_2O, 75:16:9; (K) benzene−EtOAc, 60:40; (L) $CHCl_3$−Me_2CO, 85:15; and (M) $CHCl_3$−MeOH, 100:10.

Biology: (a) Enzyme Preparation. Uridine phosphorylase from *E. coli* was purified to homogeneity according to Vita and Magni.[18]

(b) Enzyme Assays. All assays were performed in 50 mM phosphate buffer at 37 °C. For each potential substrate, the pH was selected so that the neutral form predominated. Hence all analogues with a 5-fluoro substituent were assayed at pH 6 and the others at pH 7.5. Phosphorolysis was monitored spectrophotometrically by the decrease in absorbance at 285 nm ($\Delta\epsilon = 3.2 \times 10^3$) for the 5-fluoro analogues and at 280 nm ($\Delta\epsilon = 2.1 \times 10^3$) for the remaining compounds, using a Uvicon 860 instrument fitted with a thermostated cuvette compartment and equipped with a software package for kinetic analysis and statistical treatment of the data. Initial reaction rates were employed for determination of *v* (20 s). Apparent K_m and V_{max} values were evaluated using a computer program that uses the Wilkinson procedure.[19] The program was kindly written by Dr. Z. Kamiński.

For 5-fluoro-6-(hydroxymethyl)uridine (**40**), with a very high K_m (see Table 2), the reaction was conducted for 1 min and terminated by addition of 30 μL of 30% NaOH. A control contained each component, except enzyme, which was added after alkalization. The difference in absorbance was measured at 300 nm ($\Delta\epsilon = 3.5 \times 10^3$). Under these conditions, the enzyme activity was linear with time and enzyme concentration.

Protein was determined by the method of Bradford[20] with bovine serum albumin as standard.

(c) Cytotoxicity Studies. Cytoxicity (IC_{50}) of the compounds was determined by their effects on protein ([^{14}C]-L-leucine incorporation) synthesis in the cell lines (Table 3) obtained from the American Type Culture Collection. Test compounds were added to triplicate cultures in 96-well microplates containing 2×10^4 cells/200 μL well or 10^5 peripheral blood lymphocytes, stimulated with phytohemaglutinin. Cells were cultured in RPMI 1640 medium containing fetal calf serum (10%), in a humidified atmosphere containing 5% CO_2 at 37 °C. [^{14}C]-L-Leucine (specific activity 1.3 mCi/mmol and 0.5 μCi/mL) was added to the cultures for the final 24 h of the 3-day culture period (4 days with PHA-Ly). After incubation, the proteins were precipitated with 0.2 N perchloric acid and collected on glass fiber filters using a multiple cell harvester (Wallac, Finland). The radioactivity incorporated into proteins was measured in a scintillation counter (LKB-Wallac, 81.000; Turku, Finland). The incorporation of [^{14}C]leucine per culture remained constant during the final 24 h of culture. A good correlation between cell number and [^{14}C]leucine incorporation has been demonstrated.[21,22]

Chemistry. 1-β-D-Ribofuranosyl-3-benzyl-6-methyluracil (4). A suspension of 2.04 g (10 mmol) of 3-benzyl-6-methyluracil[5] (**1**) in 25 mL of HMDS with 10 mg of $(NH_4)_2SO_4$ was heated under reflux for 12 h. HMDS was removed under reduced pressure, 50 mL of anhydrous xylene added, and the whole evaporated to an oil (**2**), to which was added 5.04 g (10 mmol) of anhydrous (70 °C, P_2O_5, 0.1 mmHg) ABR in 20 mL of anhydrous MeCN. The mixture was cooled in iced H_2O, 1.11 g (5 mmol) of TMSOTfl added, and the whole left at room temperature for 2 h, the course of the reaction being monitored by TLC on silica gel with solvent A. The reaction was terminated by adding the mixture to 100 mL of vigorously stirred, cooled, saturated $NaHCO_3$. After several minutes the product was extracted with 3 × 50 mL of $CHCl_3$. The combined extracts were brought to dryness under reduced pressure, and the product **3** was isolated by preparative TLC on silica gel with solvent A and dissolved in 100 mL of hot MeOH. To the cooled solution was added 5 mL of 1 N NaOMe in MeOH, and the solution was stirred overnight at room temperature, brought to neutrality with Dowex 50W(H^+), and evaporated to an oil and the product isolated by preparative TLC on silica gel with solvent B, to give **4** (2.26 g) in the form of an oil, chromatographically homogeneous, which was dried over P_2O_5 *in vacuo*: UV λ_{max} (pH 7) 265 nm (ϵ 8.6 × 10^3);

TLC (silica gel) R_f (B) 0.71; MS m/z for $C_{17}H_{20}N_2O_6$ calcd 384.132 136, found 384.132 145.[5]

6-Isopropyl-4-thiouracil (12). To a mixture of 10 mL of anhydrous hexane and 6.2 mL (60 mmol) of diethylamine (distilled over NaH), cooled in ice, was added, portionwise, a solution of 986 μL (9 mmol) of $TiCl_4$ in 4 mL of anhydrous hexane followed by slow addition of 1.064 mL (10 mmol) of methylisopropyl ketone (**9**) and stirring for 1 h at room temperature. The dark brown mixture was diluted with hexane and, following addition of 2 mL of MeOH, filtered through Celite. The pale yellow filtrate was concentrated to an oil (**10**; l.3 g), which was dissolved in 20 mL of Et_2O followed by slow addition, with cooling, of 4 mL of a solution of 940 μL (7.8 mmol) of ethoxycarbonyl isothiocyanate in Et_2O. The mixture was stirred for 1 h at room temperature and 10 mL of hexane added, leading to formation of crystals of **11**. The mixture was stored in the deep freeze for 12 h, and the resulting deep red crystalline adduct **11** was collected by filtration (2.0 g, 75%): mp 75−77 °C. Anal. ($C_{13}H_{23}N_2O_2S$) C, H, N.

The adduct **11** (500 mg, 1.84 mmol) was suspended in 10 mL of vigorously stirred concentrated ammonia, partially dissolved by warming, and left at room temperature for 48 h. Crystallization from aqueous EtOH gave 287 mg (92%) of long needles of **12**: mp 172−174 °C; UV λ_{max} (pH 2) 333 (ϵ 18.9 × 10^3), (pH 7) 330 (ϵ 18.9 × 10^3), (pH 12) 332 nm (ϵ 20.3 × 10^3); TLC (silica gel) R_f (C) 0.72; MS m/z for $C_7H_{10}N_2OS$ calcd 170.051 407, found 170.051 38. Anal. ($C_7H_{10}N_2OS$) C, H, N.

4-(Methylthio)-6-methyluracil (13). This was obtained as described by Winkley and Robins[7c] and recrystallized from H_2O: mp 152−156 °C (lit.[7c] mp 150−157 °C); UV λ_{max} (pH 2) 325 (ϵ 14.5 × 10^3), 316 (ϵ 13.8 × 10^3), 267 (ϵ 5.3 × 10^3), (pH 7) 300 (ϵ 13.9 × 10^3), 270 (ϵ 8.0 × 10^3), (pH 12) 299 (ϵ 11.4 × 10^3), 223 nm (ϵ 12.0 × 10^3); TLC (silica gel) R_f (C) 0.53.

5,6-Dimethyl-4-(ethylthio)uracil (14): prepared as described for **13**, above, with EtI instead of MeI; crystallization from EtOH gave the product in 93% yield; mp 195−197 °C; UV λ_{max} (pH 2) 325 (ϵ 14.5 × 10^3), 316 (ϵ 13.8 × 10^3), 267 (ϵ 5.3 × 10^3), (pH 7) 300 (ϵ 13.9 × 10^3), 270 (ϵ 8.0 × 10^3), (pH 12) 299 (ϵ 11.4 × 10^3), 223 nm (ϵ 12.0 × 10^3); TLC (silica gel) R_f (D) 0.05; MS m/z for $C_8H_{12}N_2OS$ calcd184.067 06, found 184.066 912.

4-(Methylthio)-6-propyluracil (15): prepared as described for **13** (above); rRecrystallization from aqueous EtOH yielded the product in 65% yield; mp 115−117 °C; UV λ_{max} (pH 2) 325 (ϵ 14.5 × 10^3), 316 (ϵ 13.8 × 10^3), 267 (ϵ 5.3 × 10^3), (pH 7) 300 (ϵ 13.9 × 10^3), 270 (ϵ 8.0 × 10^3), (pH 12) 299 (ϵ 11.4 × 10^3), 223 nm (ϵ 12.0 × 10^3); TLC (silica gel) R_f (C) 0.63; MS m/z for $C_8H_{12}N_2OS$ calcd 184.067 06, found 184.066 91.

6-Isopropyl-4-(methylthio)uracil (16): prepared as described for **13** (above); recrystallization from aqueous EtOH gave 90% yield; mp 164−166 °C. UV λ_{max} (pH 2) 325 (ϵ 14.5 × 10^3), 316 (ϵ 13.8 × 10^3), 267 (ϵ 5.3 × 10^3), (pH 7) 300 (ϵ 13.9 × 10^3), 270 (ϵ 8.0 × 10^3), (pH 12) 299 (ϵ 11.4 × 10^3), 223 nm (ϵ 12.0 × 10^3); TLC (silica gel) R_f (C) 0.64; MS m/z for $C_8H_{12}N_2OS$ calcd 184.067 06, found 184.067 27.

1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-4-(methylthio)-6-methyluracil (17). A suspension of 1.56 g (10 mmol) of 4-(methylthio)-6-methyluracil (**13)** in 15 mL of HMDS with 10 mg of $(NH_4)_2SO_4$ was heated under reflux for 12 h. HMDS was removed under reduced pressure, 100 mL of xylene added, and the mixture concentrated to an oil, to which was added 5.04 g (10 mmol) of carefully dried ABR in 20 mL of MeCN. The mixture was cooled on ice, 1.11 g (5 mmol) of TMSOTfl added, and the whole left at room temperature for 30 min, with monitoring of the course of the reaction by TLC on Al_2O_3 with solvent E. The reaction was terminated by pouring the mixture into 100 mL of cooled and vigorously stirred saturated $NaHCO_3$. After several minutes the product was extracted with 3 × 50 mL of $CHCl_3$. The pooled extracts were brought to dryness and fractionated with a mixture of *i*-PrOH and cyclohexane to release a chromatographically homogeneous oily product, which was dried over P_2O_5 under vacuum (5.13 g, 85%): mp 91−92 °C; TLC (Al_2O_3) R_f (F) 0.24. Anal. ($C_{32}H_{28}N_2O_8S\cdot{}^1/_2H_2O$) C, H, N.

1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-4-(methylthio)-6-propyluracil (18). This was obtained from 1.84 g (10 mmol) of 4-(methylthio)-6-propyluracil (**15)** as described above for **17** but with prolongation of the reaction time to 90 min. The product was crystallized from 50 mL of *i*-PrOH/MeOH to yield 2.57 g (90%) of large colorless crystals, mp 105−106 °C; TLC (Al_2O_3) R_f (F) 0.27. Anal. ($C_{34}H_{32}N_2O_8S$) C, H, N.

1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-6-propyluracil (22). A solution of 200 mg (0.32 mmol) of **15** in 10 mL of a 3:7 (v/v) mixture of 1,4-dioxane and 1 N TFA was kept at room temperature until disappearance of **15**, the reaction being monitored by TLC on Al_2O_3 with solvent G. Solvent was removed under reduced pressure and the residue crystallized from EtOH to yield 141 mg (74%) of **22** as thin needles: mp 85−87 °C; TLC (silica gel) R_f (H) 0.16. Anal.($C_{33}H_{30}N_2O_8\cdot 2H_2O$) C, H, N.

1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-6-isopropyl-4-(methylthio)uracil (19). This was prepared as described for **17** with slight modifications: 348 mg (1.90 mmol) of **16** in MeCN was converted to the trimethylsilyl derivative (0.5 mL of BSTFA, 80 °C, 30 min). To the cooled solution was added 200 μL (1.025 mmol) of TMSOTfl with stirring for 30 min at room temperature and monitoring the course of the reaction by TLC on Al_2O_3 with solvent E. Following termination of the reaction and preliminary workup, the product was isolated by preparative TLC on Al_2O_3 (two 20 × 40 cm plates) with solvent E. Crystallization from *i*-PrOH/MeOH gave 1.0 g (88%): mp 79−81 °C; TLC (Al_2O_3) R_f (F) 0.28. Anal. ($C_{31}H_{32}N_2O_8S$) C, H, N.

1-β-D-Ribofuranosyl-6-methyluracil (5). Method A: A solution of 100 mg (0.29 mmol) of 3-benzyl-6-methyl-1-β-D-ribofuranosyluracil (**4**) in 5 mL of anhydrous THF was cooled on an ice bath and 25 μL (0.264 mmol) of BBr_3. Stirring for 2 h at room temperature was followed by addition of 200 μL of MeOH and 100 μL of 25% aqueous ammonia. The solution was brought to dryness under reduced pressure and the product isolated by preparative TLC on silica gel with solvent B. Crystallization from MeOH yielded 50 mg (67%) of **5**.

Method B: A suspension of 1.56 g (10 mmol) of 4-(methylthio)-6-methyluracil (**13**) in 20 mL of HMDS, with a catalytic amount of $(NH_4)_2SO_4$, was heated under reflux for 12 h. HMDS was removed under reduced pressure, 50 mL of xylene added, and the mixture concentrated to an oily residue, to which was added 5.04 g (10 mmol) of ABR in 80 mL of anhydrous MeCN. The mixture was cooled on an ice bath and 1.11 g (5 mmol) of TMSOTfl added. After 2 h the mixture was diluted with 300 mL of $CHCl_3$ and extracted with 2 × 100 mL of saturated $NaHCO_3$ and then with 3 × 200 mL of H_2O. The organic phase was dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was dissolved in 100 mL of 1 N NaOMe and, after 12 h, brought to neutrality with Dowex 50W(H^+), concentrated under vacuum to a small volume, dissolved in 30 mL of H_2O, and extracted with 3 × 25 mL of $CHCl_3$. The aqueous layer was brought to dryness, and the residue was dissolved in 15 mL of 1 N TFA. The mixture was stirred for 12 h at room temperature, brought to dryness, and reevaporated from 30 mL of EtOH, and the residue was crystallized from EtOH/EtOAc to yield 2.06 g (80%) of **5**: mp 174−176 °C (lit.[7c] mp 177−178 °C); TLC (silica gel) R_f (B) 0.34; ^{1}H NMR (D_2O) δ 5.77 (1H, s, 5-H), 5.66 (1H, d, 1′-H, $J_{1'2'}$ = 3.35 Hz), 4.39 (1H, t, 3′-H, $J_{2'3'}$ = 6.39 Hz), 3.97 (1H, m, 4′-H, $J_{3'4'}$ = 7.22 Hz), 3.89 (1H, dd, 5′-H, $J_{4'5'}$ = 3.01 Hz), 3.75 (1H, dd, 5″-H, $J_{4'5''}$ = 6.26 Hz, $J_{5'5''}$ = −12.34 Hz), 2.40 (3H, s, 6-CH_3).

1-β-D-Ribofuranosyl-6-ethyluracil (43). This was prepared as described by Holy:[23] mp 115−118 °C (lit.[23] mp 119−121 °C); TLC (silica gel) R_f (B) 0.44; UV spectra as for **5**.

1-β-D-Ribofuranosyl-6-propyluracil (20). 1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-6-propyluracil (**22**) (100 mg, 0.167 mmol) was dissolved in 100 mL of hot EtOH. To the cooled solution was added 5 mL of 1 N NaOMe in MeOH, and the mixture was stirred overnight at room temperature, brought to neutrality with Dowex 50W(H^+), and evaporated to dryness. The product was isolated by preparative TLC on silica gel with solvent B to yield 39 mg (82%) as an amorphous powder: mp 173−176 °C (lit.[24] mp 177−178 °C); TLC (silica gel) R_f (B) 0.53;

^{1}H NMR (D_2O) δ 5.65 (1H, d, 1′-H, $J_{1'2'}$ = 3.34 Hz), 4.39 (1H, t, 3′-H, $J_{2'3'}$ = 6.38 Hz), 3.96 (1H, m, 4′-H, $J_{3'4'}$ = 7.46 Hz), 3.90 (1H, dd, 5′-H, $J_{4'5'}$ = 3.02 Hz), 3.75 (1H, dd, 5″-H, $J_{4'5''}$ = 6.26 Hz, $J_{5'5''}$ = −12.34 Hz), 2.65 (2H, t, 6-CH_2CH$_2$CH$_3$), 1.68 (2H, m, 6-CH$_2$CH_2CH$_3$), 0.99 (3H, t, 6-CH$_2$CH$_2$CH_3).

1-β-D-Ribofuranosyl-6-isopropyluracil (21). 1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-6-isopropyl-4-(methylthio)uracil (**19**) (500 mg, 0.80 mmol) was dissolved in 10 mL of 0.1 N NaOMe in MeOH. The solution stirred for 12 h, brought to neutrality with Dowex 50W(H^+), reduced to a small volume by evaporation, dissolved in 15 mL of H_2O, and extracted with 3 × 15 mL of $CHCl_3$. The aqueous layer was brought to dryness, dissolved in 5 mL of 1 N TFA, stirred for 12 h at room temperature, and brought to dryness; the residue was evaporated from 30 mL of EtOH and crystallized from EtOH/EtOAc to give 189 mg (83%) in an amorphous form: mp 202−204 °C (lit.[24] mp 204−206 °C); TLC (silica gel) R_f (B) 0.52; ^{1}H NMR (D_2O) δ 5.81 (1H, 5-H, s), 5.77 (1H, d, 1′-H, $J_{1'2'}$ = 3.17 Hz), 4.40 (1H, t, 3′-H, $J_{2'3'}$ = 6.64 Hz), 3.95 (1H, m, 4′-H, $J_{3'4'}$ = 7.53 Hz), 3.90 (1H, dd, 5′-H, $J_{4'5'}$ = 2.94 Hz), 3.76 (1H, dd, 5″-H, $J_{4'5''}$ = 6.35 Hz, $J_{5'5''}$ = −12.41 Hz), 3.08 (2H, m, 6-CH_2-(CH$_3$)$_2$), 1.28 (6H, d, 6-CH$_2$(CH_3)$_2$).

1-β-D-Ribofuranosyl-5,6-dimethyluracil (23). This was obtained essentially as described for the 6-methyl analogue **5**, the starting substance being 4-(ethylthio)-6-methyluracil (**14**). The final product was isolated by preparative TLC on silica gel with solvent I and crystallized from *i*-PrOH in 60% yield: mp 180−181 °C (lit.[7c] mp 82 °C); TLC (silica gel) R_f (I) 0.38; ^{1}H NMR (D_2O) δ 5.73 (1H, d, 1′-H, $J_{1',2'}$ = 3.39 Hz), 4.38 (1H, dd, 3′-H, $J_{2',3'}$ = 6.43 Hz, $J_{3',4'}$ = 7.26 Hz), 3.97 (1H, m, 4′-H, $J_{4',5'}$ = 2.97 Hz, $J_{4',5''}$ = 6.16 Hz), 3.90 (1H, dd, 5′-H, $J_{5',5''}$ = −12.38 Hz), 3.75 (1H, dd, 5″-H), 2.41 (3H, s, 5-CH$_3$), 2.20 (3H, s, 6-CH$_3$).

5-Fluoro-2-methoxy-6-methyluracil (**25**). To a solution of 1.72 g (10 mmol) of methylpseudoisourea in 30 mL of H_2O was added 616 mg (11 mmol) of CaO followed by portionwise addition of a solution of 1.46 mL (10 mmol) of ethyl α-(fluoroacetyl)acetate (**23**) in 30 mL of EtOH and stirring for 72 h. The mixture was then acidified to pH 3 with HCOOH and filtered through Celite and the latter washed with EtOH, and the combined filtrates were evaporated to dryness. The residue was crystallized from toluene/*i*-PrOH to yield 995 mg (63%): mp182−185 °C; UV λ_{max} (pH 2) 260 (ϵ 7.7 × 10^3), (pH 7) 265 (ϵ 7.42 × 10^3), (pH 12) 268 nm (ϵ 7.57 × 10^3); TLC (silica gel) R_f (C) 0.64; MS *m*/*z* for $C_6H_7FN_2O_2$ calcd 158.049 15, found 158.049 15.

2-Methoxy-6-(trifluoromethyl)uracil (**26**). To a solution of 1.722 g (10 mmol) of methylpseudoisourea in 30 mL of H_2O was added 616 mg (11 mmol) of CaO followed by portionwise addition of 1.46 mL (10 mmol) of ethyl(trifluoroacetyl)acetate (**24**) in 30 mL of EtOH and then stirring for 72 h, acidification to pH 3 with HCOOH, filtration through Celite, and evaporation to dryness. Crystallization from toluene/*i*-PrOH yielded 1.28 g (66%) of **26**: mp 106−110 °C; UV λ_{max} (pH 2) 272 (ϵ 5.8 × 10^3), (pH 7) 273 (ϵ 5.05 × 10^3), (pH 12) 273 nm (ϵ 5.3 × 10^3); TLC (silica gel) R_f (C) 0.74; MS *m*/*z* for $C_6H_5F_3N_2O_2$ calcd 194.0303 19, found 194.031 17.

5-Fluoro-6-methyluracil (**27**). A suspension of 1 g (6.3 mmol) of 5-fluoro-2-methoxy-6-methyluracil (**25**) in 20 mL of 2 N H_2SO_4 was heated under reflux at 70 °C, and the course of the reaction was monitored by TLC on silica gel with solvent C. After about 2 h, the cooled solution was filtered through Celite, reduced to a small volume, and allowed to crystallize, yielding 792 mg (87%) of **27**: mp 300 °C dec (lit.[10] mp 300 °C dec); UV λ_{max} (pH 2) 270 (ϵ 7.0 × 10^3), (pH 7) 271 (ϵ 6.3 × 10^3), (pH 14) 287 nm (ϵ 6.9 × 10^3); TLC (silica gel) R_f (J) 0.63.

6-(Trifluoromethyl)uracil (**28**). A suspension of 1 g (5.1 mmol) of 2-methoxy-6-(trifluoromethyl)uracil (**26**) in 20 mL of 2 N H_2SO_4 was heated under reflux at 70 °C for 2 h, with monitoring of the course of the reaction on silica gel with solvent C. The solution was cooled, filtered through Celite, and reduced to a small volume for crystallization, to yield 765 mg (83%) of **28**: mp 225−228 °C (lit.[11] mp 220−222 °C); TLC (silica gel) R_f (C) 0.37.

1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-5-fluoro-6-methyluracil (30). A suspension of 1.58 g (11 mmol) of 5-fluoro-6-methyluracil (**27**) in 30 mL of HMDS, to which was added 0.1 mL (0.8 mmol) of TCS, was heated under reflux to obtain a clear solution (about 6 h). The silylating reagents were removed under vacuum, and the residue was codistilled with 3 × 50 mL of anhydrous xylene and taken up in 30 mL of anhydrous MeCN. To the resulting solution of 2,4-bis-*O*-(trimethylsilyl)-5-fluoro-6-methyluracil (**29**), in a round-bottomed flask with protection against moisture, was added 5.04 g (10 mmol) of anhydrous ABR. To this mixture, cooled on an ice bath, was added, dropwise, 20 mL of a solution containing 2.18 mL (12 mmol) of TMSOTfl, with stirring for 2 h. The mixture was brought slowly to room temperature and stirred for 3 h. Following addition of 300 mL of $CHCl_3$, the mixture was extracted with 2 × 100 mL of saturated $NaHCO_3$ and 3 × 200 mL of H_2O. The organic phase was dried over anhydrous Na_2SO_4 and then evaporated to dryness. The residue was deposited on a 2.5 × 25 cm column of neutral Al_2O_3 (Merck; 70−230 mesh, activity according to Brockman III) and eluted with a 9:1 (v/v) mixture of *n*-hexane/EtOAc, to give 1,3-bis(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-5-fluoro-6-methyluracil (**32**). Further elution with a linear gradient (10−100%) of EtOAc in hexane resulted in a component identified as 3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-5-fluoro-6-methyluracil (**31**). Subsequent elution with EtOAc/MeOH (9:l, v/v) yielded 1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-5-fluoro-6-methyluracil (**30**). This third fraction was brought to dryness and crystallized from CH_2Cl_2/Et_2O to give 3 g (45%) of **30**: mp 97−98 °C; TLC (silica gel) R_f (K) 0.45; ^{1}H NMR ($CDCl_3$/Me_4-Si) δ 8.25 (15H, m, aromatic), 6.24−6.14 (2H, m, 2′-H, 3′-H), 5.63 (1H, s, 1′-H), 4.84−4.68 (3H, m, 4′-H, 5′-H, 5″- H), 2.32 (3H, 6-CH$_3$, d, J_{F,CH_3} = 3.5 Hz). Anal. ($C_{31}H_{25}FN_2O_9$) C, H; N: calcd, 4.76; found, 4.34.

3-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-5-fluoro-6-methyluracil (31). The second fraction from the foregoing procedure was brought to dryness and crystallized from EtOH to obtain 1.4 g (21%) of **31**: mp 189−190 °C; TLC (silica gel) R_f (K) 0.38; ^{1}H NMR ($CDCl_3$/Me_4Si) δ 10.45 (1H, br s, 1-NH), 8.30−7.94, 7.75−7.30 (15H, m, aromatic), 6.62 (1H, s, 1′-H), 6.32−6.08 (2H, m, 2′-H, 3′-H), 5.95−5.65 (3H, m, 4′-H, 5′-H, 5″-H), 2.20 (3H, d, 6-CH$_3$, J_{F,CH_3} = 3.5 Hz). Anal. ($C_{31}H_{25}FN_2O_9$) C, H, N.

1,3-Bis(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-5-fluoro-6-methyluracil (32). The first fraction from the foregoing procedure was brought to dryness and crystallized from Et_2O to give 1.33 g (20%) of **32**: mp 133−134 °C; TLC (silica gel) R_f (K) 0.65; ^{1}H NMR ($CDCl_3$/Me_4Si) δ 8.30−7.88, 7.70−7.30 (30H, aromatic), 6.68 (1H, s, 1′-H near N-3), 6.37−6.10 (4H, m, 2′-H, 3′-H), 5.75 (1H, s, 1′-H near N-1), 5.00−4.50 (6H, m, 4′-H, 5′-H, 5″-H), 2.33 (3H, 6-CH$_3$, d, $J_{F,Me}$ = 3.5 Hz). Anal. ($C_{57}H_{45}FN_2O_{16}$) C, H, N.

1-β-D-Ribofuranosyl-5-fluoro-6-methyluracil (35). Compound **30** (2.5 g, 4.3 mmol) was dissolved in 100 mL of hot EtOH. To the cooled solution was added 5 mL of a methanolic solution of 1 N NaOMe; the mixture was stirred overnight at room temperature and then brought to neutrality with Dowex 50W(H^+) and evaporated to dryness. The residue was crystallized from *i*-PrOH/EtOAc and then from EtOH, to yield 938 mg (80%) of **35**: mp 180.5−182 °C; UV λ_{max} (pH 2) 268 (ϵ 9.6 × 10^3), (pH 7) 268 (ϵ 8.6 × 10^3), (pH 12) 269 nm (ϵ 7.15 × 10^3); TLC (cellulose) R_f (J) 0.43; MS *m*/*z* for $C_{10}H_{14}FN_2O_6$ calcd 277.0836, found 277.0840; ^{1}H NMR (D_2O) δ 5.59 (1H, d, 1′-H, $J_{1'2'}$ = 3.51 Hz), 4.35 (1H, t, 3′-H, $J_{2'3'}$ = 6.38 Hz), 3.97 (1H, m, 4′-H, $J_{3'4'}$ = 7.18 Hz), 3.89 (1H, dd, 5′-H, $J_{4'5'}$ = 2.99 Hz), 3.75 (1H, dd, 5″-H, $J_{4'5''}$ = 6.25 Hz, $J_{5'5''}$ = −12.41 Hz), 2.40 (3H, 6-CH$_3$, d, $J_{F,Me}$ = 3.19 Hz). Anal. ($C_{10}H_{14}FN_2O_6$) C, H, N.

3-β-D-Ribofuranosyl-5-fluoro-6-methyluracil (36). This was obtained by deblocking of 1 g of **31**, as described in the previous section for the preparation of **35** from **30**, to give 400 mg (85%) of amorphous **36**: UV λ_{max} (pH 2) 273 (ϵ 8.01 × 10^3), (pH 7) 272 (ϵ 8.26 × 10^3), (pH 12) 300 nm (ϵ 10.4 × 10^3); TLC (cellulose) R_f (J) 0.53; MS *m*/*z* for $C_{10}H_{14}FN_2O_6$ calcd 277.0836, found 277.0842; ^{1}H NMR (D_2O) δ 6.30 (1H, d, 1′-H, $J_{1'2'}$ = 2.85 Hz), 4.50 (1H, t, 3′-H, $J_{2'3'}$ = 6.20 Hz), 4.06 (1H, m, 4′-H, $J_{3'4'}$ = 7.22 Hz), 3.96 (1H, dd, 5′-H, $J_{4'5'}$ = 3.02 Hz), 3.82 (1H, dd, 5″-H, $J_{4'5''}$ = 6.20 Hz, $J_{5'5''}$ = −12.41 Hz), 2.29 (3H, 6-CH$_3$, d, $J_{F,Me}$ = 3.19 Hz). Anal. ($C_{10}H_{13}FN_2O_6 \cdot {}^1/_2H_2O$) C, H, N.

1,3-Di-β-D-ribofuranosyl-5-fluoro-6-methyluracil (37). This was prepared by deblocking 1 g of **32**, as for **35** and **36**, above, to give 355 mg (87%) of amorphous **37**, with no defined melting point:UV λ_{max} (pH 7) 268 nm (ϵ 9.5 × 10^3); TLC (cellulose) R_f (J) 0.21; MS m/z for $C_{15}H_{22}FN_2O_{10}$ calcd 409.125 85, found 409.1262. Anal. ($C_{15}H_{21}FN_2O_{10}\cdot H_2O$) C, H, N.

3-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-6-(trifluoromethyl)uracil (34). To a suspension of 360 mg (2 mmol) of 6-(trifluoromethyl)uracil (**28**) in 10 mL of anhydrous MeCN was added 500 μL (1.88 mmol) of BSTFA and 650 mg (2 mmol) of 1,2,3,5-tetra-*O*-acetyl-1-β-D- ribofuranose, and the mixture was heated under reflux to obtain a clear solution. After cooling to room temperature, 340 mg (11.5 mmol) of TMSOTfl was slowly added, and after 30 min, TLC on silica gel with solvent L demonstrated quantitative conversion to a single product. Following addition of 50 mL of $CHCl_3$, the mixture was extracted with 2 × 10 mL of saturated $NaHCO_3$. The organic phase was dried on anhydrous Na_2SO_4 and evaporated to dryness. The residue was fractionated by preparative TLC (silica gel) with solvent L. Following 2-fold development, the reaction product was eluted with EtOH and brought to dryness, yielding 790 mg (87%) of **34** as a foam, which could not be crystallized: TLC (silica gel) R_f (L) 0.60; MS m/z $(M - CH_3COOH)^+$ for $C_{14}H_{13}F_3N_2O_7$ calcd 378.067 486, found 378.067 526.

3-β-D-Ribofuranosyl-6-(trifluoromethyl)uracil (38). Addition of 454.4 mg (1 mmol) of 3-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)-6-(trifluoromethyl)uracil (**34**) to 20 mL of hot MeOH resulted in a clear solution. After cooling to room temperature, 1.5 mL of 1 N methanolic NaOMe was added and the mixture was stirred overnight, neutralized with Dowex 50W(H^+), and evaporated to dryness to yield 272 mg (87%) of **38** as a foam, which could not be crystallized: mp 114−117 °C; UV λ_{max} (pH 2) 262 (ϵ 5.75 × 10^3), (pH 7) 298 (ϵ 7.65 × 10^3), (pH 12) 298 nm (ϵ 8.15 × 10^3); TLC (silica gel) R_f (C) 0.16; MS m/z for $C_{10}H_{12}F_3N_2O_6$ calcd 313.0648, found 313.064 72; ^{1}H NMR (D_2O) δ 6.27 (1H, d, 1′-H, $J_{1'2'}$ = 3.35 Hz), 6.23 (1H, br s, 5-H), 4.43 (1H, t, 3′-H, $J_{2'3'}$ = 6.56 Hz), 3.99 (1H, m, 4′-H, $J_{3'4'}$ = 7.02 Hz), 3.89 (1H, dd, 5′-H, $J_{4'5'}$ = 3.02 Hz), 3.85 (1H, dd, 5″-H, $J_{4'5''}$ = 6.04 Hz, $J_{5'5''}$ = −12.10 Hz). Anal. ($C_{10}H_{12}F_3N_2O_6\cdot{}^1/_2$MeOH) C, H, N.

1-β-D-Ribofuranosyl-6-(hydroxymethyl)uracil (39) and 1-β-D-Ribofuranosyl-5-fluoro-6-(hydroxymethyl)uracil (40). To a solution of 774 mg (3 mmol) of 6-methyluridine (**5**) in 10 mL of distilled Ac_2O was added 15 mg (0.12 mmol) of DMAP. The mixture was stirred at room temperature until **5** had disappeared (TLC on silica gel with solvent M). The mixture was evaporated to dryness, 25 mL of EtOH added, and the mixture again brought to dryness. This was repeated with 3 × 30 mL of anhydrous toluene. The residue was then taken up in 9 mL of 1,4-dioxane/AcOH (11:1, v/v), and 1 g (9 mmol) of SeO_2 was added. The mixture was heated to boiling under reflux for 18 h, the reaction course being monitored by TLC (silica gel, solvent M). Following addition of 50 mL of benzene to the cooled solution, it was filtered, the filtrate brought to dryness under reduced pressure, the residue dissolved in 50 mL of $CHCl_3$, and 50 mL of H_2O added. The organic phase was separated and dried over Na_2SO_4. The drying agent was removed by filtration, 25 mL of MeOH added, and 257 mg (1 mmol) of tetrabutylammonium borohydride added in small portions, with the reaction course being monitored by TLC (silica gel with solvent M). The mixture was filtered, brought to a small volume under reduced pressure, and, after addition of 50 mL of $CHCl_3$, washed sequentially with 20 mL of H_2O, 20 mL of 0.1 N HCl, 30 mL of H_2O, and 20 mL of saturated $NaHCO_3$. The organic phase was concentrated under reduced pressure and the residue dissolved in 15 mL of ammonia-saturated MeOH (at 0 °C) and left at room temperature for 5 h. The mixture was brought to dryness, and the product was isolated by preparative TLC on silica gel plates with solvent I, yielding 395 mg (48%) of **39**: mp 173−175 °C (lit.[25] mp 177−178 °C); TLC (silica gel) R_f (B) 0.17; ^{1}H NMR (D_2O) δ 5.99 (1H, d, 5-H), 5.56 (1H, d, 1′-H, $J_{1'2'}$ = 3.38 Hz), 4.85 (1H, dd, 2′-H), 4.40 (1H, t, 3′-H, $J_{2'3'}$ = 6.37 Hz), 3.97 (1H, m, 4′-H, $J_{3'4'}$ = 7.27 Hz), 3.89 (1H, dd, 5′-H, $J_{4'5'}$ = 3.00 Hz), 3.76 (1H, dd, 5″-H, $J_{4'5''}$ = 6.23 Hz, $J_{5'5''}$ = −12.41 Hz).

Compound **40** was prepared in an analogous manner to obtain 422 mg (48%) in an amorphous form, which could not be crystallized: UV λ_{max} (pH 2) 268 (ϵ 9.6 × 10^3), (pH 7) 268 (ϵ 6.9 × 10^3), (pH 12) 269 nm (ϵ 5.7 × 10^3); TLC (silica gel) R_f (I) 0.17; MS m/z for $C_{10}H_{14}O_7N_2F$ calcd 293.0785, found 293.0777; ^{1}H NMR (D_2O) δ 5.66−5.59 (2H, m, 6-CH_2OH), 5.59 (1H, d, 1′-H, $J_{1'2'}$ = 3.44 Hz), 4.80 (1H, dd, 2′-H, $J_{2'3'}$ = 6.52 Hz), 4.41 (1H, dd, 3′-H), 3.98 (1H, m, 4′-H, $J_{3'4'}$ = 7.04 Hz), 3.89 (1H, dd, 5′-H, $J_{4'5'}$ = 3.05 Hz), 3.76 (1H, dd, 5″-H, $J_{4'5''}$ = 6.22 Hz, $J_{5'5''}$ = −12.41 Hz). Anal. ($C_{10}H_{14}N_2O_7F$) C, H, N.

1-β-D-Ribofuranosyl-6-(fluoromethyl)uracil (41) and 1-β-D-Ribofuranosyl-5-fluoro-6-(fluoromethyl)uracil (42). To a solution of 724 mg (3 mmol) of 6-methyluridine (**5**) in 10 mL of distilled Ac_2O was added 15 mg (0.12 mmol) of DMAP, and the mixture was stirred at room temperature until TLC (silica gel, solvent M) demonstrated disappearance of **5**. The mixture was evaporated to dryness and, following addition of 25 mL of EtOH, again brought to dryness. The residue was treated with 30 mL of anhydrous toluene and brought to dryness, and this was repeated twice more. The residue was then dissolved in 9 mL of 1,4-dioxane/AcOH (11:1, v/v) and 1 g of SeO_2 added. The mixture was then brought to boiling under reflux for 18 h, with monitoring of the course of the reaction by TLC (silica gel, solvent M), and then cooled and, following addition of 50 mL of benzene, filtered. The filtrate was brought to dryness under reduced pressure, the residue dissolved in 50 mL of $CHCl_3$, and 50 mL of H_2O added. The organic phase was removed and dried over Na_2SO_4. The drying agent was removed by filtration. To the filtrate was added 25 mL of MeOH followed by portionwise addition of 257 mg (l mmol), tetrabutylammonium borohydride, and the course of the reaction was monitored by TLC (silica gel with solvent M). The reaction mixture was filtered, the filtrate concentrated to a small volume under reduced pressure, 50 mL of $CHCl_3$ added, and the solution washed sequentially with 20 mL of H_2O, 20 mL of 0.1 N HCl, 30 mL of H_2O, and 20 mL of saturated $NaHCO_3$. The organic phase was dried over Na_2SO_4, brought to dryness under reduced pressure, and then dried in an Abderhalden pistol. The residue was dissolved in 20 mL of anhydrous CH_2Cl_2, to which was added 300 μL (2.27 mmol) of DAST in 5 mL of CH_2Cl_2. The solution was stirred at room temperature, and the reaction course was monitored by TLC (silica gel with solvent M). After 13 h, 50 mL of $CHCl_3$ was added, and the mixture was extracted with saturated $NaHCO_3$. The organic phase was washed with 2 × 30 mL of H_2O, dried over Na_2SO_4, and brought to dryness under reduced pressure. The residue was dissolved in 15 mL of ammonia-saturated MeOH (at 0 °C), left at room temperature for 5 h, brought to a small volume, and subjected to preparative TLC on silica gel plates with solvent I to yield 322 mg (37%) of amorphous **41**: TLC (silica gel) R_f (B) 0.33; MS m/z for $C_{10}H_{13}FN_2NaO_6$ calcd 299.065 58, found 299.065 54; ^{1}H NMR δ (D_2O) δ 6.03 (1H, d, 5-H), 5.46 (1H, d, 1′-H, $J_{1'2'}$ = 3.29 Hz), 5.45 (2H, m, 6-CH_2OH), 4.40 (1H, dd, 3′-H, $J_{2'3'}$ = 6.63 Hz), 3.97 (1H, m, 4′-H, $J_{3'4'}$ = 6.97 Hz), 3.89 (1H, dd, 5′-H, $J_{4'5'}$ = 2.97 Hz), 3.76 (1H, dd, 5″-H, $J_{4'5''}$ = 6.28 Hz, $J_{5'5''}$ = −12.41 Hz). Anal. ($C_{10}H_{13}N_2O_6F$) C, H, N.

Compound **42** was synthesized as above (309 mg, 35%) but could not be crystallized: UV λ_{max} (pH 2) 268 (ϵ 9.6 × 10^3), (pH 7) 268 (ϵ 6.9 × 10^3), (pH 12) 269 nm (ϵ 5.7 × 10^3); TLC (silica gel) R_f (J) 0.78; MS m/z for $C_{10}H_{12}F_2N_2O_6$ calcd 295.0742, found 295.0740; ^{1}H NMR (D_2O) δ 5.75−5.40 (2H, m, 6-CH_2F), 5.72 (1H, d, 1′-H, $J_{1'2'}$ = 3.31 Hz), 4.40 (1H, t, 3′-H, $J_{2'3'}$ = 6.51 Hz), 3.96 (1H, m, 4′-H, $J_{3'4'}$ = 7.23 Hz), 3.87 (1H, dd, 5′-H, $J_{4'5'}$ = 3.04 Hz), 3.74 (1H, dd, 5″-H, $J_{4'5''}$ = 6.22 Hz, $J_{5'5''}$ = −12.41 Hz). Anal. ($C_{10}H_{12}F_2N_2O_6\cdot H_2O$) C, H, N.

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