Secondary arylalkylamines, like 3, did not react in our hands to give formation of enamines.

Pharmacological Methods. Antiinflammatory activity was measured by the method of Winter. Fifteen male Wistar rats weighing 170–220 g were used for each group. Compounds having amino groups in their structures were injected intraperitoneally as their soluble oxalic salts. Insoluble compounds were administered by gavage. Thirty minutes later, carrageenin (0.05 mL, 1%) in physiological saline was injected subcutaneously under the plantar skin of the hind paw. The volume of the injected paw was measured just before (T_0) and 1, 2, and 3 h after the injection of carrageenin for calculation of percent inhibition (Table I).

Nucleosides. 114. 5′-*O*-Glucuronides of 5-Fluorouridine and 5-Fluorocytidine. Masked Precursors of Anticancer Nucleosides[1]

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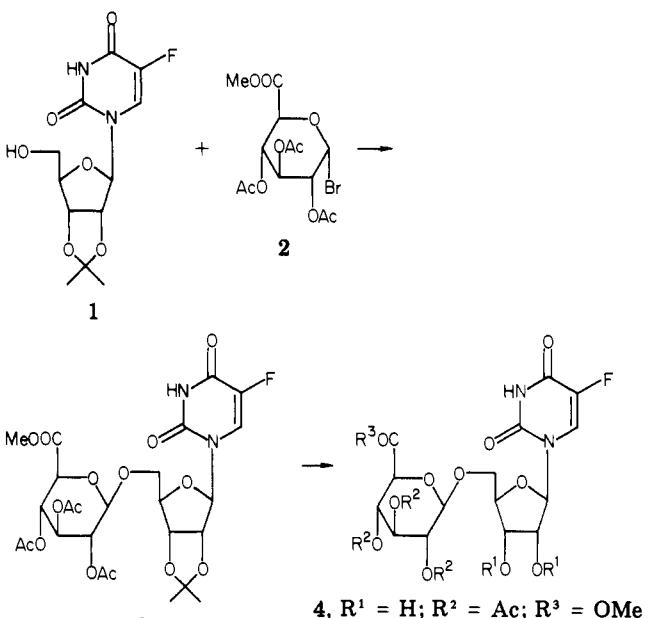
5′-*O*-Glucuronides of anticancer nucleosides, 5-fluorouridine and 5-fluorocytidine, were synthesized by three different methods. The best preparative procedure was the one starting from benzyl 5-*O*-(methyl 2′,3′,4′-tri-*O*-acetyl-β-D-glucopyranosyluronate)-2,3-*O*-isopropylidene-β-D-ribofuranoside (15) that was obtained almost quantitatively by condensation of benzyl 2,3-*O*-isopropylidene-β-D-ribofuranoside (8) with methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate (2). After de-O-isopropylidenation of 15, the crystalline product, benzyl 5-*O*-(methyl 2′,3′,4′-tri-*O*-acetyl-β-D-glucopyranosyluronate)-β-D-ribofuranoside (16), was de-O-benzylated catalytically to 5-*O*-(methyl 2′,3′,4′-tri-*O*-acetyl-β-D-glucopyranosyluronate)-D-ribofuranose (17). Compound 17 was acetylated to crystalline 5-*O*-(methyl 2′,3′,4′-tri-*O*-acetyl-β-D-glucopyranosyluronate)-1,2,3-tri-*O*-acetyl-β-D-ribofuranose (18) and condensed with trimethylsilylated 5-fluorouracil of 5-fluorocytosine in the presence of $SnCl_4$ to afford the corresponding protected nucleosides 5 and 19 in good yields. Saponification of these compounds gave 5′-*O*-β-D-glucuronides of 5-fluorouridine and 5-fluorocytidine (20 and 21) isolated as their crystalline Na salts. These glucuronides were substrates of both bacterial and bovine β-glucuronidase. They were, as expected, much less toxic against several leukemic cell lines in tissue culture.

Elevated β-glucuronidase activity in malignant human tumors was first reported by Fishman et al.[2] Subsequently, many reports up to 1966 were reviewed by Levvy and Conchie[3] showing that the activity of this enzyme in human cancer tissues is extraordinarily high, relative to normal tissues. Sweeney et al.[4] later showed that mycophenolic acid, a compound which inhibits mecca lymphosarcoma and CA-755 mammary carcinoma, is converted into the glucuronide as the only metabolite. They also showed[4] that, in general, the tumors most responsive to mycophenolic acid are those in which β-glucuronidase activity is highest, while it is the lowest in nonresponsive tumors. Conners et al.[5] demonstrated that the *N,N*-bis-(2-chloroethyl)aniline is oxidized in vivo to the corresponding 4-hydroxy derivative, which is then converted into its glucuronide. This aromatic nitrogen mustard is uniquely effective against the mouse ADJ/PCS plasma cell tumor which exhibits particularly high levels of β-glucuronidase activity.[6]

5-Fluorouracil (FU) is a potent agent presently used in the treatment of certain solid tumors.[7] The corresponding ribonucleoside (FUR)[8] and deoxyribonucleoside (FUDR)[9] are also active. These compounds are converted in vivo into FUDR-5′-P, which then interferes with thymidylate

Scheme I



4, R^1 = H; R^2 = Ac; R^3 = OMe
5, R^1 = R^2 = Ac; R^3 = OMe
6, R^1 = R^2 = H; R^3 = NH_2
7, R^1 = R^2 = H; R^3 = OH

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synthetase,[10] or are metabolized to FUR-5′-P, which eventually is incorporated into RNA, producing some abnormally functioning RNA species.[11a,b] Regardless of which mechanism operates, metabolic conversion of these drugs into 5′-nucleotides by cellular enzymes is a prerequisite for a chemotherapeutic effect.

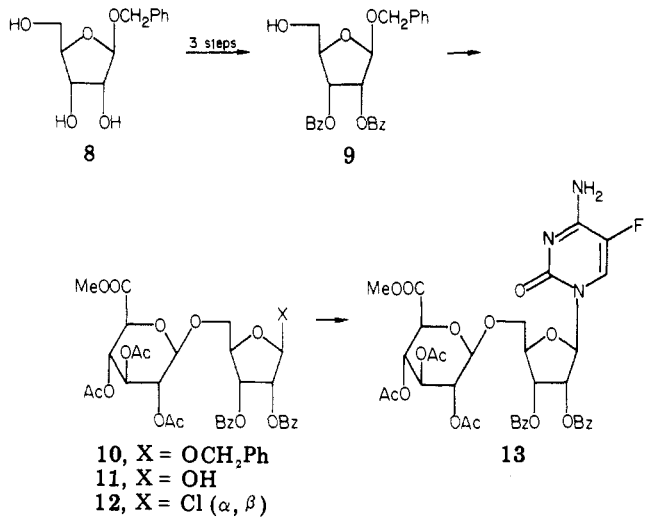
On this basis, one would expect that 5′-glucuronides of such nucleosides would be relatively inactive.[12] Enzymatic hydrolysis of the glucuronide moiety from such nucleoside glucuronides which could enable subsequent entry into nucleotide metabolic pathways may be expected to occur more readily in tumor tissues rich in β-glucuronidase resulting, hopefully, in selective toxicity.

As with other glucuronides, nucleoside 5′-glucuronides should be resistant to *chemical* hydrolysis under physiological conditions. Also, such nucleoside glucuronides would probably not serve as substrates for nucleoside phosphorylase (with the liberation of FU) due to the high degree of structural specificity which such enzymes require. The only metabolic fate of the glucuronides should be cleavage by β-glucuronidase, liberating biologically active nucleosides.

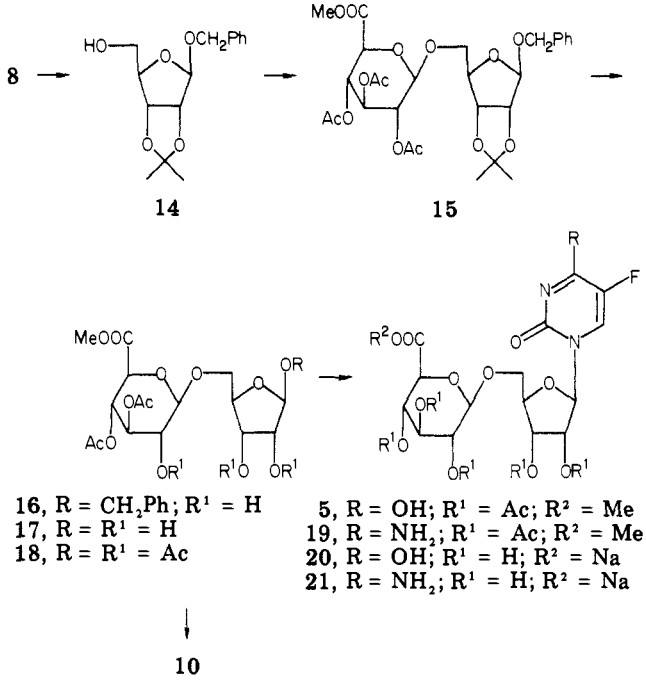
Two basic approaches to the preparation of 5′-glucuronides of nucleosides are possible: 5′-glucuronidation of a preformed nucleoside or condensation of heterocyclic bases with preformed disaccharides containing the glucuronide moiety. Several disaccharide nucleosides have been synthesized both by glycosylation of preformed nucleosides[13-16] and by condensation of heterocyclic bases with preformed disaccharides.[17-20] We studied first the preparation of 5-fluorouridine-5′-*O*-β-glucuronide (**7**, Scheme I) via the Koenigs–Knorr condensation of 2′,3′-*O*-isopropylidene-5-fluorouridine (**1**) with methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate (**2**). In our hands, the condensation occurred most smoothly under the modified conditions of Reynolds and Evans[21] using silver oxide catalyst in the presence of Drierite and iodine in a mixture of benzene and acetonitrile. The protected glucuronide (**3**) was obtained in 65% yield as a syrup. The product contained three acetyl and one each of isopropylidene and ester methyl groups in addition to the 5-fluorouracil base as evidenced by ^{1}H NMR and UV spectral analyses. The β configuration at the glucuronide linkage was assigned later on the basis of the large coupling constant observed between H-1″ and H-2″ in the free glucuronamide (**6**) ($J_{1'',2''}$ = 7.3 Hz). Deketalization of **3** in aqueous acetic acid was accompanied by extensive cleavage of the disaccharide linkage, and the product **4** was obtained in only 38% yield as a powder.

Acetylation of **4** to **5**, followed by ammonolysis, gave the crystalline glucuronamide **6** in good yield. Saponification of **5** with sodium hydroxide, followed by treatment with Dowex 50 (H^+), afforded the free glucuronide (**7**).

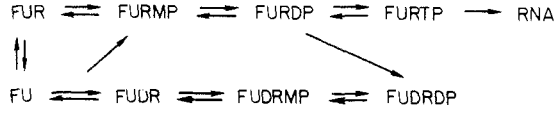
Scheme II



Scheme III



(10) Both FUR and FUDR are directly or indirectly metabolized to FUDR-5′- or FUR-5′-P according to the following scheme:



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The Koenigs–Knorr condensation of 1 to 3 was most erratic and depended heavily on the nature of the silver oxide catalyst. Our attempts to achieve reproducibly good yields in this reaction by variations in the preparation of the catalyst were also unsuccessful. We therefore investigated an alternative approach from benzyl β-D-ribofuranoside (8, Scheme II).

Compound 8 was converted into the crystalline 2,3-dibenzoate (9) in 37% yield by tritylation and benzoylation, followed by detritylation. Condensation of 9 with 2 under the Helferich conditions[22] afforded the crystalline disaccharide 10 in 37% yield. After hydrogenolysis of the benzyl group of 10, compound 11 was obtained also as colorless crystals. Replacement of the free anomeric hydroxyl group of 11 by a halogen, by treatment either with carbon tetrachloride and triphenylphosphine in acetonitrile[23] or with carbon tetrabromide and triphenylphosphine in pyridine,[24] did not go to completion. Treatment of tris(trimethylsilyl)-5-fluorocytosine with a mixture containing the halide 12 and unreacted 11 generally afforded low yields of the protected disaccharide nucleoside 13 which was obtained as a foam after chromatographic purification. Saponification of 13 afforded the sodium salt of glucuronide (7), which was identical with sodium 5′-O-glucopyranosyl(5-fluorocytidine)uronate (21, Scheme III), prepared by an alternate route (see below).

There are several disadvantages in the above procedure for the preparation of nucleoside glucuronides. The overall yield of the key intermediate 10 is rather poor, and the conversion of 11 into the corresponding halogenose 12 is very slow and also low yielding. A modified approach (Scheme III) from 8 was developed to overcome these disadvantages. Isopropylidenation of 8, followed by condensation of the product 14 with 2, afforded disaccharide 15 in almost quantitative yield. Deketalization of 15 in aqueous acetic acid was accompanied by some cleavage of the disaccharide linkage, but the desired product 16 was obtained in about 70% yield after chromatographic purification and crystallization. Benzoylation of 16 afforded the dibenzoate 10 (Scheme II). Reductive debenzylation of 16 in the presence of palladium on carbon catalyst gave the crystalline glucuronosyl-D-ribofuranose 17, which was acetylated by treatment with acetic anhydride in pyridine. The β anomer of the peracetylated product 18 was obtained in crystalline form in more than 60% yield. A small amount of the α anomer of 18 was obtained from the mother liquor as a syrup. Condensation of 18 with trimethylsilylated 5-fluorouracil or 5-fluorocytosine in the presence of freshly distilled stannic chloride under Niedballa–Vorbrüggen conditions[20] afforded the corresponding protected nucleoside disaccharides 5 and 19, respectively. The ^{1}H NMR spectra of 5 and 19 showed that each contained five acetyl and one ester methyl groups, in addition to a 5-fluoro pyrimidine base (H-6). After saponification of these nucleoside glucuronides in aqueous sodium hydroxide, the corresponding unblocked nucleosides (20 and 21) were obtained as their crystalline sodium salts. The ^{1}H NMR spectra showed, clearly, large couplings between H-1″ and H-2″ (6.7 and 7.9 Hz for 20 and 21, respectively), thus establishing the β configuration of the glucuronide linkage in these molecules.

Preliminary biological studies showed, as expected, that these nucleoside glucuronides are much less cytotoxic relative to their parent nucleosides against P815 cells; the ID_{50} values for the glucuronides of FUR and FCR were both approximately 6 μg/mL, whereas those for the parent nucleosides were found to be in the 0.004–0.006 μg/mL range.[25] These compounds (20 and 21) were substrates for both bacterial and bovine β-glucuronidase. Extensive biological studies with these nucleoside glucuronides are underway at this institute.

Experimental Section

General. Melting points were determined on a Thomas-Hoover apparatus (capillary method) and are uncorrected. The ^{1}H NMR spectra were recorded on a JEOL PFT-100 spectrometer using Me_4Si as the internal standard for organic solvents and $Me_3Si(CH_2)_3SO_3Na$ for D_2O. Chemical shifts are reported in parts per million (δ), and signals are described as s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Values given for coupling constants are first order. TLC was performed on Uniplates (silcia gel coated) purchased from Analtech Co., Newark, DE, and spots were detected with UV light and by spraying with 20% (v/v) H_2SO_4 in EtOH followed by charring. Evaporations were carried out in vacuo with bath temperatures below 45 °C. UV spectra were recorded on a UNICAM-800 spectrometer and IR spectra on a Perkin-Elmer Infracord Model 137B spectrometer. Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, TN, and by Spang Microanalytical Laboratory, Eagle Harbor, MI. All new compounds, except 17, were analyzed correctly.

2′,3′-O-Isopropylidene-5-fluorouridine (1). A mixture of 5-fluorouridine[8] (5.24 g, 0.02 mol), 2,2-dimethoxypropane (1 mL), and *p*-toluenesulfonic acid (500 mg) in acetone (150 mL) was stirred overnight at room temperature. Solid $NaHCO_3$ was added, and the mixture vigorously stirred for 2 h; insoluble inorganic salts were removed by filtration. The filtrate was concentrated in vacuo, and the residue crystallized from EtOH to give 5.4 g (90%) of 1, mp 197–199 °C (lit. 195–195.5[26] and 198.5 °C[27]).

2′,3′-O-Isopropylidene-5′-O-(methyl 2″,3″,4″-tri-O-acetyl-β-D-glucopyranosyluronate)-5-fluorouridine (3). A mixture of Ag_2O (2.4 g, 0.005 mol), 1 (1.60 g, 0.005 mol), finely pulverized Drierite (40 g, dried at 165 °C for 20 h), and I_2 (100 mg) in dry benzene (100 mL) was boiled gently, and approximately 25 mL of benzene was removed by distillation. A solution of 2[28] (4.0 g, 0.01 mol) in MeCN (100 mL) was added to the hot mixture, and approximately 50 mL of solvent was removed by distillation. The mixture was then refluxed overnight, cooled to room temperature, and filtered from insoluble materials. The filtrate was concentrated in vacuo, and the residue was dissolved in $CHCl_3$ (100 mL), washed with $Na_2S_2O_3$ (0.1 M solution, 100 mL), water (100 mL × 2), dried (Na_2SO_4), and evaporated to a syrup (~5.2 g). The syrup was dissolved in a mixture of EtOAc and C_6H_6 (1:9, 100 mL) and chromatographed over a column of silica gel G (200 g) using EtOAc–C_6H_6 (1:9) as the eluent. Appropriate fractions were combined and evaporated in vacuo to a syrup which could not be crystallized. The yield was 2.02 g (65%): ^{1}H NMR ($CDCl_3$) δ 1.34 (3 H, s, issopropylidene Me), 1.58 (3 H, s, isopropylidene Me), 2.02 (6 H, s, 2 Ac), 3.68 (3 H, s, ester Me), 5.84 (1 H, s, H-1′), 7.73 (1 H, d, H-6, $J_{6,F}$ = 7.0 Hz).

5′-O-(Methyl 2″,3″,4″-tri-O-acetyl-β-D-glucopyranosyluronate)-5-fluorouridine (4). Compound 3 (2.0 g, 0.034 mol) was dissolved in 80% aqueous AcOH (70 mL) and the mixture heated at reflux for 30 min. The solvent was removed by evaporation in vacuo, and the residue was chromatographed over a column of silica gel G (50 g) using MeOH–$CHCl_3$ (1:9) as the eluent. Appropriate fractions were combined and evaporated in vacuo to give 4 as a foam: yield 709 mg (38%); ^{1}H NMR ($CDCl_3$) δ 2.04 (6 H, s, 2 Ac), 2.08 (3 H, s, Ac), 3.78 (3 H, s, ester Me), 5.89 (1 H, narrow m, H-1′), 7.97 (1 H, d, H-6, $J_{6,F}$ = 7.0 Hz); $[\alpha]^{23}_D$ 6.4° (*c* 1.0, $CHCl_3$).

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2',3'-Di-*O*-acetyl-5'-*O*-(methyl 2'',3'',4''-tri-*O*-acetyl-β-D-glucopyranosyluronate)-5-fluorouridine (5). Compound 4 (550 mg, 0.95 mmol) was acetylated with Ac_2O (1 mL) in pyridine (2 mL) at room temperature. The reaction mixture was poured onto iced water (20 mL) and extracted with CH_2Cl_2 (20 mL × 3). The combined extracts were washed with water (50 mL), dried (Na_2SO_4), and evaporated to dryness to a syrup, which was purified by column chromatography (silica gel G, 50 g) with C_6H_6–EtOH (9:1) as the eluent. Fractions containing UV-absorbing material were combined and evaporated to a slightly yellowish foam: yield 570 mg (97%); $[\alpha]^{23}_D$ −8.2° (*c* 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.05 (3 H, s, Ac), 2.07 (3 H, s, Ac), 2.10 (6 H, s, 2 Ac), 2.12 (3 H, s, Ac), 3.75 (3 H, s, ester Me), 5.04 (1 H, d, H-5'', $J_{4'',5''}$ = 6.8 Hz), 6.29 (1 H, dd, H-1', $J_{1',2'}$ = 7.1, $J_{1',F}$ = 0.5 Hz), 7.87 (1 H, d, H-6, $J_{6,F}$ = 6.3 Hz).

5'-*O*-(β-D-Glucopuranosyluronamide)-5-fluorouridine (6). Compound 5 (190 mg) was dissolved in $CHCl_3$ (2 mL). To the solution was added MeOH/NH_3 (3 mL, saturated at 0 °C), and the mixture was kept overnight at room temperature. The crystals which separated were collected by filtration and washed with EtOH (3 mL) and Et_2O (5 mL) to give 92 mg (83%) of 6, which did not show a definite melting point but slowly effervesced above 107 °C: ^1H NMR (Me_2SO-d_6) δ 4.35 (1 H, d, H-1'', $J_{1'',2''}$ = 7.3 Hz), 5.77 (1 H, br s, H-1'), 7.35 (2 H, br s, NH_2), 7.96 (1 H, d, H-6, $J_{6,F}$ = 7.0 Hz).

5'-*O*-(β-D-Glucopyranosyluronic acid)-5-fluorouridine (7). Compound 5 (190 mg) was dissolved in MeOH (2 mL). The solution was diluted with 0.1 N NaOH (2 mL), and the mixture was stirred for 2 h and then passed through a column of Dowex 50 (H^+, 2 mL). The column was washed with water until no more UV-absorbing material was eluted. The UV-absorbing fractions were combined and evaporated in vacuo to a powder, which was crystallized from MeOH to give 7: yield 19 mg; mp 216–220 °C dec.

Benzyl 2,3-Di-*O*-benzoyl-β-D-ribofuranoside (9). A mixture of benzyl β-D-ribofuranoside (8;[29] 24 g, 0.1 mol) and TrCl (31 g, 0.11 mol) in pyridine (50 mL) was shaken at room temperature for 16 h and then cooled in an ice bath. Benozyl chloride (10 g, 0.07 mol) was added to the cooled mixture with stirring. The thick reaction mixture was diluted with pyridine (25 mL) and benzoyl chloride (19 g, 0.13 mol) was added. After 4 h at room temperature, the mixture was poured into an ice–water mixture (500 mL). The supernatant was removed by decantation, and the residual syrup was dissolved in CH_2Cl_2 (400 mL), which was successively washed with water (200 mL), saturated $NaHCO_3$ solution (400 mL), and water (400 mL) and then dried (Na_2SO_4). The solvent was removed by evaporation in vacuo, and the residue was triturated with EtOH (500 mL). The EtOH supernatant was removed by decantation, and the residue was dissolved in CH_2Cl_2 (250 mL) and then treated with 88% HCO_2H (200 mL). After 35 min, water was added. The organic layer was separated and washed with water (200 mL × 2), saturated $NaHCO_3$ solution (200 mL × 2), and water (200 mL). EtOH (200 mL) was added to the separated organic phase, the mixture was concentrated in vacuo to ca. 200 mL and cooled in an ice bath, and the crystalline precipitate (TrOH) was removed by filtration. The filtrate was treated with concentrated NH_4OH (2 mL) for 10 min to hydrolyze the 5-formate ester. The mixture was concentrated in vacuo to a syrup, which was chromatographed over a column of silica gel (500 g) using EtOH–$CHCl_3$ (1:19) as the eluent. Appropriate fractions were combined and evaporated in vacuo, and the residue was crystallized from EtOH to give 22.6 g (50.4%) of 9, mp 83–85 °C.

Benzyl 5-*O*-[Methyl (2',3',4'-tri-*O*-acetyl-β-D-glucopyranosyl)uronate]-2,3-di-*O*-benzoyl-β-D-ribofuranoside (10). A mixture of compound 9 (22.4 g, 0.05 mol) and $Hg(CN)_2$ (25 g, 0.1 mol) in $MeNO_2$ (300 mL) and C_6H_6 (300 mL) was dried by azeotropic distillation of about 100 mL of solvent. A solution of 2 (20 g, 0.005 mol) in CH_2Cl_2 (250 mL) was added, and an additional 250 mL of solvent was removed by distillation. The mixture was heated at reflux for 4 h, and then the mixture was concentrated to dryness in vacuo. The residue was dissolved in $CHCl_3$ (300 mL) and the solution washed with 30% KI solution (100 mL × 2) and water (100 mL) and then was dried (Na_2SO_4). After the solvent was removed by evaporation in vacuo, the residue was chromatographed over a silica gel column (800 g, C_6H_6–EtOAc 19:1 as the eluent). Appropriate fractions were combined and evaporated, and the residue was crystallized from EtOH. Crystals (highly solvated, mp ~40 °C) were collected and dried in vacuo first at room temperature (24 h) and then at 57 °C (24 h) to give 14 g (36.6%) of 10, mp 131–132 °C.

5-*O*-[Methyl (2',3',4'-tri-*O*-acetyl-β-D-glucopyranosyl)-uronate]-2,3-di-*O*-benzoyl-D-ribofuranose (11). Compound 10 (3.1 g, 0.04 mol) was dissolved in glacial AcOH (40 mL). The solution was diluted with water (10 mL) and hydrogenated over Pd/C (10%, 500 mg) in a Parr apparatus with initial pressure of 2.8 kg/cm² for 5 days. The catalyst was removed by filtration and the filtrate evaporated to dryness. The residue was chromatographed on a column of silica gel G (100 g, C_6H_6–EtOAc 4:1). Appropriate fractions were combined and evaporated, and the residue was crystallized from Et_2O to give 2.1 g (76%) of 11: mp 158.5–160 °C; ^1H NMR ($CDCl_3$) δ 2.03 (6 H, s, 2 Ac), 2.14 (3 H, s, Ac), 3.75 (3 H, s, ester Me), 4.80 (1 H, d, H-1', $J_{1',2'}$ = 7.0 Hz), 7.3–8.1 (10–12 H, m, aromatic).

2',3'-Di-*O*-benzoyl-5'-*O*-(methyl 2'',3'',4''-tri-*O*-acetyl-β-D-glucopyranosyluronate)-5-fluorocytidine (13). A mixture of 11 (1.02 g, 1.5 mmol) and PPh_3 (1.75 g, 6.7 mmol) in MeCN (25 mL) was treated with CCl_4 (4 mL); the mixture was heated at reflux for 3 h. After evaporation of the solvent in vacuo, the residue was triturated with Et_2O and filtered, and the filtrate was evaporated to dryness in vacuo. The residue was dissolved in MeCN (15 mL) and added to tris(trimethylsilyl)-5-fluorocytosine (prepared from 1.0 g of 5-fluorocytosine); the mixture was heated at reflux for 24 h. The solvent was removed in vacuo, and the residue was dissolved in CH_2Cl_2 (15 mL). The solution was treated with MeOH (2 mL) and saturated $NaHCO_3$ solution (7 mL) and filtered from precipitates through a Celite pad. The filtrate was diluted with CH_2Cl_2 (100 mL) and saturated $NaHCO_3$ solution. The organic layer was separated, washed with water, dried (Na_2SO_4), and evaporated to dryness to a syrup which was chromatographed on a column of silica gel G60 (300 g) using $CHCl_3$–MeOH (5:1) as the eluent. Compound 13 was obtained as a foam (582 mg, 49%): ^1H NMR ($CDCl_3$) δ 2.05 (6 H, s, 2 Ac), 2.16 (3 H, s, Ac), 3.77 (3 H, s, ester Me), 3.94 (1 H, m, H-4'), 4.11 (1 H, d, H-5'', $J_{4'',5''}$ = 8.5 Hz), 4.74 (1 H, d, H-1'', $J_{1'',2''}$ = 7.4 Hz), 5.0–5.4 (4 H, m, H-3', 2'', 3'', 4''), 5.68 (1 H, q, H-2', $J_{1',2'}$ = 6.0, $J_{2',3'}$ = 3.2 Hz), 6.67 (1 H, dd, H-1', $J_{1',2'}$ = 6.0, $J_{1',F}$ = 2.0 Hz), 7.3–9.2 (10–12 H, m, aromatic). Upon saponification of this compound, 5'-*O*-(sodium β-D-glucopyranosyluronate)-5-fluorocytidine (21), which was identical with an authentic sample (^1H NMR and IR) prepared by saponification of the penta-*O*-acetate 19 (vide infra), was obtained.

Benzyl 2,3-*O*-Isopropylidene-β-D-ribofuranoside (14). A mixture of benzyl β-D-ribofuranoside[29] (8; 24 g, 0.1 mol), 2,2-dimethoxypropane (12 mL), and *p*-toluenesulfonic acid (1 g) in acetone (480 mL) was stirred for 2 h and then neutralized with solid $NaHCO_3$ (5 g). Inorganic salts were removed by filtration and the filtrate was evaporated to dryness. The residue was crystallized from EtOH–Et_2O to give 25.1 g (90%) of 14, mp 104–105 °C (lit.[30] mp 104–105 °C).

Benzyl 5-*O*-(Methyl 2',3',4'-tri-*O*-acetyl-β-D-glucopyranosyluronate)-2,3-*O*-isopropylidene-β-D-ribofuranoside (15). A suspension of 9 (8.2 g, 0.034 mol) and $Hg(CN)_2$ (20 g, 0.079 mol) in a mixture of C_6H_6 (180 mL) and $MeNO_2$ (180 mL) was dried azeotropically by distillation of approximately 80 mL of solvent. A solution of the halogen sugar 2 (21 g, 0.05 mol) in dry C_6H_6 (150 mL) was added dropwise to the vigorously stirred and refluxing suspension while distilling the solvent so that the volume of solvent in the reaction vessel remained constant. After the addition of 2, approximately 80 mL of solvent was removed by distillation. The reaction mixture was allowed to cool to room temperature and filtered from inorganic salts, and the filtrate was evaporated in vacuo to near dryness. The residue was shaken in a mixture of C_6H_6 (150 mL) and 30% KI solution (100 mL). The organic layer was separated and washed again with 30% KI (100 mL) and water (100 mL × 3) and then dried (Na_2SO_4) and

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(30) Rauch, E. B.; Lipkin, D. *J. Org. Chem.* **1962**, *27*, 403.

evaporated in vacuo to dryness. The solid residue was crystallized from EtOH to give 11.5 g (66%) of 15: mp 180.5–181 °C; ^{1}H NMR ($CDCl_3$) δ 1.30 (3 H, s, isopropylidene Me), 1.46 (3 H s, isopropylidene Me), 2.02 (6 H, s, 2 Ac), 2.07 (3 H, s, Ac), 3.73 (3 H, s, ester Me), 5.16 (2 H, s, CH_2Ph), 7.33 (5 H, CH_2Ph).

The mother liquor of crystallization was evaporated in vacuo to dryness, and the residue was chromatographed over a column of silica gel G (C_6H_6–EtOH, 40:1). An additional crop of 15, mp 180–181 °C (4.5 g), was obtained to give a total yield of 92%.

Benzyl 5-*O*-(Methyl 2′,3′,4′-tri-*O*-acetyl-β-D-glucopyranosyluronate)-β-D-ribofuranoside (16). A suspension of 15 (10 g, 0.017 mol) in 80% aqueous AcOH (100 mL) was heated to reflux with stirring for 20 min (clear solution was obtained within 5 min). After evaporation of solvent in vacuo, traces of AcOH were removed by several azeotropic distillations with toluene. TLC ($CHCl_3$–MeOH, 9:1) showed the presence of 14 and 15 in addition to the desired product 16, which was isolated after chromatography on a silica gel column (G60; 300 g; $CHCl_3$–EtOH, 19:1) to give 6.3 g (68%): mp 127–128.5 °C; ^{1}H NMR ($CDCl_3$) δ 2.02 (6 H, s, 2 Ac), 2.05 (3 H, s, Ac), 3.71 (3 H, s, ester Me), 5.01 (2 H, s, CH_2Ph), 7.32 (5 H, s, CH_2Ph).

5-*O*-(Methyl 2′,3′,4′-tri-*O*-acetyl-β-D-glucopyranosyluronate)-D-ribofuranose (17). A solution of 16 (12.0 g, 0.022 mol) in 80% aqueous AcOH (200 mL) was hydrogenated in a Parr apparatus over 10% Pd/C (1.0 g) with an initial pressure of 2.8 kg/cm^2 for 5 days. The reaction mixture was filtered through a Celite bed and the filtrate was concentrated to dryness in vacuo. The solid residue was crystallized from EtOH–C_6H_6 to give 7.7 g (77%) of greenish crystals suitable for use in the subsequent reaction.

5-*O*-(Methyl 2′,3′,4′-tri-*O*-acetyl-β-D-glucopyranosyluronate)-1,2,3-tri-*O*-acetyl-D-ribofuranose (18). A solution of 17 (7.00 g, 0.015 mol) in Ac_2O (50 mL) and pyridine (50 mL) was stirred at room temperature overnight. The reaction mixture was concentrated to dryness in vacuo. The syrupy residue was crystallized from MeOH to give 6.07 g of the β anomer as colorless crystals, mp 145–146 °C.

The concentrated mother liquor was applied to a silica gel column. Elution with C_6H_6–EtOAc (4:1) afforded the β anomer (1.45 g), followed by the syrupy α anomer (1.02 g): total yield 8.54 g (96%); ^{1}H NMR for the β anomer ($CDCl_3$) δ 2.03 (6 H, s, 2 Ac), 2.07 (3 H, s, Ac), 2.09 (3 H, s, Ac), 2.12 (6 H, s, 2 Ac), 3.76 (3 H, s, ester Me), 4.66 (1 H, d, H-1′, $J_{1',2'}$ = 6.6 Hz), 6.11 (1 H, s, H-1); ^{1}H NMR for the α anomer ($CDCl_3$) δ 2.02 (6 H, s, 2 Ac), 2.07 (3 H, s, Ac), 2.08 (3 H, s, Ac), 2.11 (6 H, s, 2 Ac), 3.76 (3 H, s, ester Me), 4.61 (1 H, d, H-5′, $J_{4',5'}$ = 7.3 Hz), 6.41 (1 H, d, H-1, $J_{1,2}$ = 4.3 Hz).

2′,3′-Di-*O*-acetyl-5′-*O*-(methyl 2″,3″,4″-tri-*O*-acetyl-β-D-glucopuranosyluronate)-5-fluorocytidine (19). $SnCl_4$ (0.9 mL, 7.7 mmol) was added to a mixture of the β-anomer 18 (3.1 g, 5.23 mmol) and tris(trimethylsilyl)-5-fluorocytosine (prepared from 970 mg, 7.64 mmol of 5-fluorocytosine) in dry 1,2-dichloroethane (40 mL). The reaction mixture was heated at reflux for 35 min. The cooled solution was diluted with CH_2Cl_2 (50 mL and poured into aqueous $NaHCO_3$ solution (100 mL). The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (100 mL). The combined organic layers were filtered through a Celite pad, dried (Na_2SO_4), and evaporated to dryness in vacuo. The residue was purified by chromatography on a silica gel column (G60, 20 × 5 cm) with 8% EtOH in $CHCl_3$ as the eluent. The main UV-absorbing fractions were evaporated to give a foam (3.38 g, 98%), which was homogeneous by TLC: ^{1}H NMR ($CDCl_3$) δ 2.04 (6 H, s, 2 Ac), 2.09 (9 H, s, 3 Ac), 3.76 (3 H, s, ester Me), 4.68 (1 H, d, H-1″, $J_{1'',2''}$ = 7.6 Hz), 6.32 (1 H, dd, H-1′, $J_{1',2'}$ = 6.1, $J_{1',F}$ = 2.1 Hz), 7.87 (1 H, d, H-6, $J_{6,F}$ = 6.4 Hz).

5′-*O*-(Sodium β-D-glucopyranosyluronate)-5-fluorocytidine (21). A solution of 19 (1.0 g, 1.5 mmol) in EtOH (10 mL) was treated with 0.1 N NaOH (30 mL) at room temperature. After 2 h, the mixture was neutralized with IRC-50 (H^+), and the resin was removed by filtration and washed well with water. The combined filtrate and washings were evaporated to dryness in vacuo below 35 °C. The residue was dissolved in water and applied to a column of Amberlite IRC-50 (Na^+ form, 10 × 4 cm), which was eluted with water. The UV-absorbing fractions were collected and evaporated in vacuo to dryness, and the residue was crystallized from MeOH to give 21 (662 mg, 96%): mp 228 °C (eff); ^{1}H NMR (Me_2SO-d_6) δ 3.67 (1 H, d, H-5″, $J_{4'',5''}$ = 9.8 Hz), 4.25 (1 H, d, H-1″, $J_{1'',2''}$ = 7.9 Hz), 5.69 (1 H, narrow m, H-1′), 8.13 (1 H, d, H-6, $J_{6,F}$ = 7.3 Hz).

2′,3′-Di-*O*-acetyl-5′-*O*-(methyl 2″,3″,4″-tri-*O*-acetyl-β-D-glucopyranosyluronate)-5-fluorouridine (5). $SnCl_4$ (0.5 mL, 4.3 mmol) was added to a mixture of 18 (3.0 g, 5 mmol) and bis(trimethylsilyl)-5-fluorouracil (prepared from 1.07 g, 8.2 mmol, of 5-fluorouracil) in CH_2ClCH_2Cl (40 mL). The mixture was heated at reflux for 30 min. The reaction mixture was allowed to cool to room temperature, diluted with CH_2Cl_2 (50 mL), and poured into saturated $NaHCO_3$ solution (100 mL). The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (100 mL). The combined organic layers were filtered through a Celite pad, dried (Na_2SO_4), and evaporated to dryness. The residue was chromatographed on a silica gel column (20 × 5 cm) with 4% MeOH in $CHCl_3$ as the eluent. The main UV-absorbing fractions were evaporated to dryness to give a colorless foam (3.09 g, 92%) which was homogeneous by TLC. The ^{1}H NMR spectrum was identical with that of compound 5 prepared by an alternate route (vide supra).

5′-*O*-(Sodium β-D-glucopyranosyluronate)-5-fluorouridine (20). A solution of 5 (1.35 g, 2.04 mmol) in aqueous 50% MeOH (40 mL) was treated with 1 N NaOH (18 mL) with stirring at room temperature for 1.5 h. The mixture was neutralized with Dowex 50 (H^+). The resin was removed by filtration, and the filtrate evaporated to dryness in vacuo. The residue was dissolved in water and applied to a column of Amberlite IRC-50 (Na^+) which was eluted with water. The UV-absorbing fractions were evaporated to dryness, and the residue was suspended in a mixture of MeOH and EtOH. The suspension was stirred for 1 h, and fine crystals of 20 were collected by filtration: yield 897 mg (92%); mp 224 °C (eff); ^{1}H NMR (Me_2SO-d_6) δ 4.24 (1 H, d, H-1″, $J_{1'',2''}$ = 6.6 Hz), 5.76 (1 H, narrow m, H-1′), 7.88 (1 H, d, H-6, $J_{6,F}$ = 7.3 Hz).

Evaluation of 5′-*O*-Glucuronides of FUR and FCR as Substrates of β-Glucuronidases. The Fishman procedure[31] was modified to determine the extent of enzymatic cleavage of the glucuronides of FUR and FCR. One micromole of the synthetic glucuronide of FUR (20) or of FCR (21) was mixed either with 185 units (Fishman units; one unit = hydrolysis of 1 micromole of phenolphthalein glucuronide per hour at 37 °C) of bacterial β-glucuronidase (Sigma Type VI A from *E. coli*) in a volume of 15 μL (buffer: 0.1 M Na_2HPO_4, pH 7.0) or with 200 units of bovine β-glucuronidase (Sigma Type B-1) in a volume of 15 μL (0.2 M NaOAc, pH 5.0). The mixtures were incubated at 25 °C; 6-μL samples were removed at intervals for examination by TLC on silica gel plates using the *n*-BuOH–AcOH–H_2O (5:2:3) system and the spots were detected under UV light. R_f values of FCR and FUR were 0.66–0.70 and 0.79–0.80, respectively. The values for 20 and 21 were both 0.35–0.40. Each spot was extracted with water (3 mL), and the UV absorption spectrum of the extract was recorded on a Carey 15 spectrophotometer.

After incubation with bacterial β-glucuronidase for 20 h, about 25% of 20 was hydrolyzed to a product with similar R_f and UV absorption characteristics to FUR. Similarly, about 25% of 21 was converted into a product identical with the product from 20. Thus, most of the FCR that was liberated from 21 appeared to have also undergone enzymatic deamination by deaminases present in the bacterial enzyme preparation. After 90 h, about 50% of 20 was hydrolyzed, whereas hydrolysis of 21 proceeded to about 65% completion.

Incubation of 20 and 21 with bovine liver β-glucuronidase for 20 h resulted in about 15% hydrolysis of 21 and less than 5% hydrolysis of 20. After 90 h, about 35% of 21 was cleaved, liberating FCR, and about 20% of 20 was converted into a compound identical with the product of hydrolysis of 20 and 21 by the bacterial enzyme. No such product was detectable after incubation of 21 with bovine liver β-glucuronidase. Thus, the latter enzyme preparation apparently is not contaminated with the deaminase necessary to convert FCR to FUR.

(31) Fishman, W. H. *Methods Enzymol.* **1957**, *3*, 55.