Fluorinated Pyrimidine Nucleosides. 4.[1] Synthesis and Antitumor Testing of a Series of 2',5'-Dideoxy- and 2',3',5'-Trideoxynucleosides of 5-Fluorouracil

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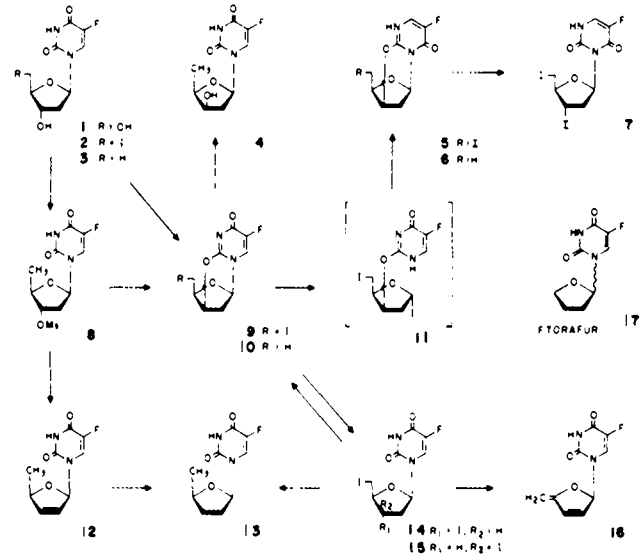
Dideoxy- and trideoxynucleosides of 5-fluorouracil have been synthesized for antitumor evaluation. 2',5'-Dideoxy-5-fluorouridine (3) was prepared from 2'-deoxy-5-fluorouridine (1) by iodination using methyltriphenoxyphosphonium iodide, followed by catalytic reduction. 1-(2',5'-Dideoxy-β-D-*threo*-pentofuranosyl)-5-fluorouracil (4) was prepared from 3 by mesylation, followed by alkaline hydrolysis. 2',3',5'-Trideoxy-5-fluorouridine (13), a methyl homologue of Ftorafur (17), was synthesized by two routes: Treatment of the 3'-mesylate 8 with potassium *tert*-butoxide yielded the 2',3'-unsaturated derivative 12, which on hydrogenation yielded 13. Alternatively, treatment of 1 with a large excess of methyltriphenoxyphosphonium iodide produced several products, including two 3'-epimeric diiodo compounds (14 and 15), each of which could be hydrogenated to 13. The major product from this iodination reaction was characterized 3-(2,3'-anhydro-2',5'-dideoxy-5'-iodo-β-D-*threo*-pentofuranosyl)-5-fluorouracil (5), presumably produced by rearrangement of the corresponding 1-isomer 9. The dideoxy compounds 3 and 4, as well as the trideoxy compound 13, were tested against sarcoma 180 in mice in comparison with 5-fluorouracil, FUDR (1), and Ftorafur (17).

The antitumor agent 5-fluorouracil was first synthesized by Duschinsky, Pleven, and Heidelberger in 1957.[2] Since that time, many attempts have been made to prepare derivatives of 5-fluorouracil in the search for compounds with improved therapeutic efficacy.[3] Interest in this area has recently been stimulated by the synthesis of Ftorafur [1-(tetrahydrofuran-2-yl)-5-fluorouracil] and its clinical use as an antitumor agent,[4] particularly in Japan. Our efforts have been directed toward the evaluation of nucleosides derived from 5-fluorouracil, and a recent paper in this series[1] described the synthesis and antitumor testing of a number of 5'-deoxy derivatives. One of these compounds, 5'-deoxy-5-fluorouridine was found to possess significant therapeutic advantages over the clinically used compounds 5-fluorouracil, Ftorafur, and 2'-deoxy-5-fluorouridine.[5] In view of the activity of this compound, it was of interest to us to examine other fluorinated pyrimidine nucleosides possessing a 5'-deoxy substituent. This paper describes the synthesis of some 2',5'-dideoxy- and 2',3',5'-trideoxynucleosides of 5-fluorouracil and their antitumor evaluation.

Scheme I



Discussion

(A) Synthesis of 2',5'-Dideoxy-5-fluorouridine. 2'-Deoxy-5-fluorouridine (1, Scheme I)[6] was employed as the starting material for this synthesis. Iodination of 1 with 1.2 equiv of methyltriphenoxyphosphonium iodide[7] yielded the 5'-iodo compound 2 as the major product; the latter could be obtained in a yield of 59% by an extraction procedure followed by crystallization. Reduction of the 5'-iodo compound 2 using hydrogen and palladium in the presence of triethylamine yielded the 2',5'-dideoxy compound 3. Hřebabecký and Beránek[8] have also reported the synthesis of this compound by reduction of a 2',5'-dichloro intermediate with tributyltin hydride. A minor product was isolated in low yield from the aqueous extracts of the iodination reaction, the elemental analysis of which indicated the presence of only one atom of iodine per molecule. Since the UV spectrum of this compound revealed the presence of an anhydronucleoside chromophore rather than a normal 5-fluoropyrimidine nucleoside, it was therefore assigned as the 5'-iodo-2,3'-anhydronucleoside 9, presumably formed from 2 by attack of the pyrimidine carbonyl function on a 3'-methyldiphenoxyphosphonium intermediate. Verheyden and Moffatt, in a study of the iodination of thymidine using methyltriphenoxy-

(1) For part 3 in this series, see Cook, A. F.; Holman, M. J.; Kramer, M. J.; Trown, P. W. *J. Med. Chem.* **1979**, *22*, 1330.
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phosphonium iodide in pyridine, isolated the closely related compound 5′-iodo-2,3′-anhydrothymidine.[7]

(B) 1-(2′,5′-Dideoxy-β-D-*threo*-pentofuranosyl)-5-fluorouracil (4). The 2,3′-anhydro-5′-iodonucleoside **9**, which was isolated as a byproduct in the iodination of **1**, could be used as an intermediate in the synthesis of small amounts of the D-*threo* compound **4**. Hydrogenation of **9** using hydrogen and palladium on carbon gave the 5′-deoxy-2,3′-anhydro compound **10** as a crystalline solid. The NMR spectrum of **10** revealed the presence of a three-proton doublet at δ 1.23 which is characteristic for the CH_3CH functionality, thus confirming the assignment of **9** as a 5′-iodonucleoside. Trial experiments on the hydrolysis of **10** using aqueous sodium hydroxide indicated that the D-*threo* isomer **4** could readily be produced by hydrolysis of the anhydro linkage. Since the viability of this route to **4** therefore depended upon the availability of the anhydro compound **9**, attempts were made to improve the synthesis of this compound from **1** by variation of the reaction conditions. The reaction of **1** with a large excess (5 equiv) of methyltriphenoxyphosphonium iodide produced a more complex mixture of products, and only a trace of the required anhydro compound **9** was produced. The major product was a highly crystalline material which, based on elemental analysis (C, H, F, I, N), appeared to be an isomer of the 5′-iodo-2,3′-anhydronucleoside **9**. A comparison of the NMR spectra of this material with that of **9** revealed many similarities, the major difference being in the signal for the anomeric proton in the new compound which was located at δ 6.57, whereas the signal for the $H_{1'}$ atom in **9** was observed upfield at δ 5.93. The UV spectra of these two compounds showed striking differences even when run in the same solvent. The UV spectrum for **9** in methanol revealed a maximum at 254 nm, which is consistent with that observed for other anhydronucleosides of 5-fluorouracil.[9] The new compound, on the other hand, possessed a maximum at higher wavelength (283 nm), which suggested that the anomeric linkage had been modified. This data led to the new compound being assigned as the 5′-iodo-2,3′-anhydro-3-β-D-nucleoside **5**. The location of the iodo substituent was confirmed by hydrogenation of **5** to the deoxynucleoside **6**. The NMR spectrum of **6** revealed the presence of a three-proton doublet at δ 1.25; thus, the iodo substituent must have previously been located at the 5′ position. Final conformation for the structure **5** was obtained when a suitable crystal was obtained for X-ray studies; the anomeric linkage was confirmed as being β linked via N_3, with the iodo substituent being located at the 5′ position; details of the crystallographic data are provided under Experimental Section.

Since the formation of the N_3-linked anhydronucleoside (**5**) posed an interesting mechanistic question, the synthesis of this compound was studied in more detail. Examination of the starting material **1** revealed no trace of contamination by the N_3 isomer. An isolated sample of the N_1 anhydronucleoside **9** was treated with an excess of methyltriphenoxyphosphonium iodide for 21 h at room temperature, and after column chromatography **5** was isolated in low yield. Thus, **9** is a possible intermediate in the production of **5** from **1**. One mechanistic possibility for the rearrangement of **9** to **5** is via attack of iodide ion at $C_{1'}$ with cleavage of the anomeric bond and formation of an unstable glycosyl iodide such as **11**; this intermediate would be capable of recyclization via N_3 without cleavage of the anhydro linkage to give the rearranged anhydronucleoside **5**. Smith, Robins, and Tolman[10] described the rearrangement of a 2,2′-anhydroorotidine derivative into its corresponding N_3 isomer in the presence of hydrogen bromide in trifluoroacetic acid. These authors suggested that rearrangement occurs via protonation of the pyrimidine, followed by attack of bromide ion, to give a glycosyl bromide which recyclizes to give the less hindered N_3 isomer. Polazzi, Leland, and Kotick[11] also described a related rearrangement of 2,2′-anhydronucleosides to the corresponding N_3 isomers in the presence of hydrogen fluoride. The reports of these acid-catalyzed rearrangements suggested that the rearrangement of **9** to **5** might be induced by the presence of hydrogen iodide which would be formed in situ by decomposition of the iodinating agent due to traces of water in the reaction mixture. Treatment of **9** with hydrogen iodide, however, gave no significant amounts of **5** but instead produced the diiodo compound **14**.

Table I. Effect of Fluorinated Pyrimidines against Sarcoma 180J

compd	dose, mg/kg ip × 8	% reduction in tumor growth	no. of survivors/ no. tested
5-FU	50	toxic	
	40	87	17/24
	25	38	22/22
FUDR	200	93	11/16
(1)	100	93	18/24
	50	85	16/16
	25	61	16/16
	12.5	26	8/8
Ftorafur	200	81	12/16
(17)	100	46	15/16
3	200	0	15/16
4	200	6	8/8
6	200	28	8/8
13	200	81	12/16
	100	35	7/8

Since the 2,3′-anhydro compound **9** could not be produced in relatively large amounts via the iodination of **1**, this route was not covenient for the preparation of **4**. An alternate route was therefore employed in which the dideoxy compound **3** was treated with methanesulfonyl chloride to give the 3′-mesylate **8**. Treatment of **8** with an excess of sodium hydroxide under reflux for a short period of time readily produced the D-*threo* compound **4**, via the intermediacy of the anhydro compound **10**. The latter could actually be isolated by treatment of **8** with a limited amount of sodium hydroxide.

(C) 2′,3′,5′-Trideoxy-5-fluorouridine (13). Two routes were employed for the synthesis of this compound. The first route involved elimination of methanesulfonic acid from the mesylate **8** using potassium *tert*-butoxide to give the unsaturated compound **12**, followed by catalytic reduction to **13**. Khwaja and Heidelberger[12] used this type of procedure to prepare 2′,3′-dideoxy-5-fluorouridine from 2′-deoxy-3′-mesyl-5′-trityl-5-fluorouridine.

Treatment of **8** with *tert*-butoxide did produce elimination in preference to anhydronucleoside formation to give the unsaturated compound **12**, the NMR spectrum of which revealed the presence of two vinylic hydrogens as signals at δ 5.77 and 6.37. Attempts to purify **12** by crystallization led to formation of a new compound which was chromatographically almost identical but which could

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not be obtained in pure form. Examination of the NMR spectrum of the mixture revealed two doublets (δ 7.38, 7.33) due to CHCF hydrogens and overlapping doublets (δ 1.29, 1.23) due to CH_3CH hydrogens, in addition to the other protons which were observed as broadened signals. Thus, it appears that **12** underwent anomerization during attempts at recrystallization, to give a mixture of α and β isomers. Such instability was not noted by Khwaja and Heidelberger during the isolation of the corresponding 5′-hydroxy analogue of **12**.[12] Hydrogenation of crude **12** gave the trideoxy compound **13**, although the yield was poor due to the formation of several unknown byproducts.

An alternate method for the synthesis of **13** was also investigated. As discussed above, reaction of **1** with an excess of iodinating agent produced the 3-β-D-anhydronucleoside **5** as the major product. Three other compounds were isolated in low yields from this reaction. These were isomeric with each other and each contained two atoms of iodine per molecule. One compound which was chromatographically almost identical with **5** and which was separated from it with difficulty was assigned the structure **14**, since the UV spectrum indicated the presence of an N_1-linked fluorouracil chromophore. The configuration of the 3′-iodo substituent was established by treatment of **14** with sodium hydroxide to produce the 2,3′-anhydronucleoside **9**. This conversion implies that the 3′-iodo substituent has the down (*S*) configuration, since the *R* isomer would not be expected to undergo cyclization for steric reasons. The reverse reaction, i.e., the opening of the anhydro bond of **9** by iodide ion to give the diiodo compound **14**, was also carried out. The use of a dilute solution of aqueous hydrogen iodide in ethyl acetate at room temperature for 1.5 h was sufficient to cleave the anhydro bond of **9** without hydrolysis of the anomeric linkage, and the diiodo compound **14** was obtained after column chromatography in 59% yield. The formation of **14** is consistent with the stereochemistry generally observed for cleavage of anhydronucleosides in which the incoming nucleophile attacks from below the plane of the sugar ring; the anhydronucleoside **9** is presumably an intermediate in the formation of **14** from **1**.

The second diiodo compound isolated from the reaction of **1** with excess iodinating agent was designated as **15**, i.e., the 3′ epimer of **14**. Unlike **14**, **15** was not converted into the anhydronucleoside **9** by treatment with sodium hydroxide under the same conditions; only starting material was recovered, together with minor decomposition products. In this case, the stereochemistry at $C_{3'}$ is unfavorable for anhydronucleoside formation. The formation of **15** as a byproduct during the iodination reaction can be rationalized by reaction of **2** with excess reagent to give a 3′-*O*-phosphonium intermediate, which then undergoes attack by iodide ion with inversion at $C_{3'}$. Differentiation between **14** and **15** could also be achieved by a comparison of their NMR spectra. Verheyden and Moffatt[13] have observed that for a series of 3′-substituted 2′-deoxynucleosides the $C_{2'}$ protons of the erythro isomers produce very similar chemical shifts which frequently overlap. In contrast, the $C_{2'}$ protons for the threo isomers have markedly different chemical shifts, differing by 0.5–1 ppm. Thus, for **14** (erythro isomer) the 2′-protons (δ 2.6–2.8) are unresolved and almost identical, whereas for **15** (threo isomer) the signals are well separated at δ 2.62 and 3.26. The signals for the $C_{1'}$ hydrogens were also quite different; for **14**, $C_{1'}$ H was observed as a triplet, whereas **15** revealed a quartet with both signals showing additional minor coupling with the $C_{3'}$ hydrogen. A third diiodo compound from this iodination was characterized as the 3-β-D isomer **7**.

Both **14** and **15** could be hydrogenated to the same trideoxynucleoside **13**, thus providing an alternate synthesis for the target compound **13**.

Treatment of **14** with an excess of sodium hydroxide in methanol did not produce a significant amount of the anhydronucleoside **9**; instead, elimination of hydrogen iodide took place to give the unsaturated compound **16**. The $C_{5'}$ hydrogens in the NMR spectrum of **16** were observed as well-separated multiplets at δ 4.26 and 4.4 with a small (1 Hz) geminal coupling, and the $C_{2'}$ and $C_{3'}$ hydrogens were detected downfield at δ 6.76 and 6.38. The UV spectrum of **16** revealed an intense chromophore (ε 14 790), due to the additional contribution of the diene functionality. Unfortunately, only small amounts of **16** were obtained so that in vivo testing was not possible.

Antitumor Testing. The dideoxy compounds **3** and **4**, as well as the trideoxy compound **13**, were evaluated against sarcoma 180J in mice, and 5-fluorouracil (5-FU), 2′-deoxy-5-fluorouridine (FUDR, **1**), and Ftorafur (**17**) were employed as standards (Table I). This tumor model has previously been shown to be sensitive to fluorinated pyrimidines.[5] 5-FU, an extensively used antineoplastic substance, was active and slightly toxic at 40 mg/kg but inactive at 25 mg/kg. At 50 mg/kg, 5-FU was lethal for mice. FUDR (**1**), also used clinically, is usually administered by intraarterial infusion. FUDR was active at several dose levels (200, 100, 50, and 25 mg/kg) and inactive at 12.5 mg/kg, although at the higher levels (200 and 100 mg/kg) some toxicity (mouse death) was observed. Ftorafur (**17**) is commonly used in Japan, and clinical trials in the USA have been reported.[4,14] Ftorafur was active at 200 mg/kg with some toxicity and inactive at 100 mg/kg. In contrast, both of the dideoxy compounds **3** and **4** were inactive at 200 mg/kg.

5-Fluoropyrimidine compounds are generally considered to exert their antitumor effects primarily by conversion to 2′-deoxy-5-fluorouridine 5′-phosphate (FdUMP), which in turn has been shown to inhibit the enzyme thymidylate synthetase.[15] Other metabolic pathways, such as incorporation into the RNA of the tumor cell, have also been considered to be of significance in the cytostatic effects of these substances. Compounds **3** and **4** structurally closely resemble FUDR, although they lack the possiblity for activation by direct phosphorylation since the 5′-hydroxyl group has been removed. Conversion of **3** or **4** into 5-FU might be achieved via degradation by the enzyme uridine phosphorylase. Although the substrate specificity requirements of this enzyme are currently being investigated, preliminary evidence indicates that **3** and **4** are not degraded to 5-FU by extracts of sarcoma 180 containing this enzyme.[16]

The trideoxynucleoside **13** bears a closer structural resemblance to Ftorafur (**17**) and it can actually be considered as a methyl homologue of the latter. Activity of **13** against S180J was detected at the 200 mg/kg level, but it was inactive at 100 mg/kg. Thus, the activity of **13** was similar to, but not superior to, that of Ftorafur. In view of the activity of **13** against sarcoma 180J, it was also tested against leukemia L1210 ascites and colon carcinoma 38 in

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mice. This compound was inactive against both tumors.

The 2,3′-anhydro-5′-deoxy compound **6** was also tested against sarcoma 180J and was inactive at 200 mg/kg.

Experimental Section

General. Melting points were determined using a Thomas-Hoover apparatus and are uncorrected. Proton magnetic resonance spectra were obtained using either a Varian XL-100 or HA-100 spectrometer and IR spectra with a Perkin-Elmer 621 or a Beckman IR-9 instrument. UV spectra were obtained using a Cary Model 14 recording spectrometer. Hydrogenations were carried out at atmospheric pressure and room temperature using a Vibromix vibrator for agitation of the reaction mixture.

2′,5′-Dideoxy-5′-iodo-5-fluorouridine (2). A solution of 1 (20 g) in DMF (250 mL, dried over 4 Å molecular sieve) was treated with methyltriphenoxyphosphonium iodide (45.6 g) for 1 h at room temperature. Methanol (75 mL) was added and after 75 min the solution was concentrated to an oil and partitioned between ethyl acetate (750 mL) and aqueous sodium thiosulfate (750 mL, 5%). The organic layer was washed with water (3 × 750 mL) and the aqueous phase was washed with ethyl acetate (3 × 750 mL). The organic layers were combined and evaporated to an oil, which was treated with chloroform (400 mL) and water (400 mL) and chilled overnight. The crystals were filtered and washed with chloroform and dried in vacuo to give **2** (14.5 g). The organic phase was washed with water (2 × 500 mL), and the aqueous layers were combined and concentrated to incipient crystallization (~40 mL) and stored at 5 °C to yield additional **2** (2.7 g): total yield 17.2 g (59%). An analytically pure sample was obtained by recrystallization from ethyl acetate/hexane: mp 155–156 °C; NMR (Me_2SO-d_6) δ 11.78 (d, 1, NH), 7.90 (d, 1, J = 6 Hz, CHCF), 6.16 (m, 1, $C_{1'}$ H), 5.39 (d, 1, J = 4 Hz, OH), 4.13, 3.80 (m, 2, 2 × CH), 2.18 (m, 2, CH_2); UV (H_2O) λ_{max} 267 nm (ε 9200). Anal. ($C_9H_{10}FIN_2O_4$) C, H, I, N.

The mother liquor from **2** was concentrated to 25 mL and stored at 5 °C to yield **9**, 251 mg (1%). Recrystallization from methanol/ethyl acetate gave analytically pure material: mp 198–199 °C dec; NMR (Me_2SO-d_6) δ 8.10 (d, 1, J = 5 Hz, CHCF), 5.93 (d, 1, J = 3 Hz, $C_{1'}$ H), 5.35 (d, 1 J = 1 Hz, $C_{3'}$ H), 4.52 (m, 1, $C_{4'}$ H), 3.2 (m, 2, CH_2I), 2.65 (m, 2, CH_2); UV (H_2O) λ_{max} 254 nm (ε 8920), 231 (6600). Anal. ($C_9H_8FIN_2O_3$) C, H, I, N.

2′,5′-Dideoxy-5-fluorouridine (3). A solution of **2** (5 g) in methanol (100 mL) and triethylamine (2 mL) was treated with hydrogen in the presence of palladium on carbon (2.5 g, 5%) for 50 min. The catalyst was removed by filtration and washed with methanol, and the combined filtrate and washings were evaporated to dryness and stirred with ethyl acetate (100 mL). After 0.5 h the solid was removed by filtration and the filtrate was evaporated to half volume and filtered to remove additional solids. The filtrate was evaporated to dryness and recrystallized from ethanol to give **3**, 3.55 g (79%). A second crop was obtained from the mother liquor (339 mg): mp 171–173 °C; NMR (Me_2SO-d_6) δ 11.7 (br s, 1, NH), 7.82 (d, 1, J = 7 Hz, CHCF), 6.06 (m, 1, $C_{1'}$ H), 5.2 (br s, 1, OH), 3.7–4.0 (m, 2, $C_{3'}$ H, $C_{4'}$ H), 2.1 (m, 2, CH_2), 1.27 (d, 3, J = 6 Hz, CH_3CH); UV (CH_3OH) λ_{max} 269 nm (ε 8650). Anal. ($C_9H_{11}FN_2O_4$) C, H, F, N.

Treatment of 1 with an Excess of Methyltriphenoxyphosphonium Iodide. A solution of 1 (5 g, 20.3 mmol) in DMF (100 mL) was treated with methyltriphenoxyphosphonium iodide (46 g, 0.1 mol) for 6 h at room temperature. Methanol (100 mL) was added and the solution was stored at 5 °C overnight and evaporated to an oil. The oil was partitioned between methylene chloride (400 mL) and aqueous sodium thiosulfate (400 mL), and the organic layer was washed with water (300 mL) and applied to the top of a silica column (700 g) packed in methylene chloride/ethyl acetate (10:1). The column was eluted with the same solvent (4.5 L), followed by methylene chloride/ethyl acetate (5:1), and fractions of 15 mL were collected.

Fractions 66–95, 96–195, and 196–365 were each combined and evaporated to dryness. Each residue was triturated with methylene chloride (20 mL) to provide crystalline material. The batches of crystals were collected, washed with methylene chloride, and combined to give almost pure **5**, 1.55 g (23%). Recrystallization from ethyl acetate/hexane gave analytically pure material: mp 219–220 °C dec; NMR (Me_2SO-d_6) δ 7.83 (d, 1, J = 3 Hz, CHCF), 6.57 (m, 1, $C_{1'}$ H), 5.35 (m, 1, $C_{3'}$ H), 4.49 (m, 1, $C_{4'}$ H), 3.3 (m, 2, $C_{5'}$ H), 2.57 (m, 2, $C_{2'}$ H); UV (dioxane) λ_{max} 281 nm (ε 6400). Anal. ($C_9H_8FIN_2O_3$) C, H, F, I, N.

Crystals of **5** are monoclinic, space group $P2_1$, with a = 9.352 (2) Å, b = 10.406 (3) Å, c = 5.327 (1) Å, β = 95.02 (1)°, and d_{calcd} = 2.173 g cm^{-3} for z = 2. X-ray crystallographic intensity data were measured on a Hilger–Watts diffractomer (Ni-filtered Cu Kα radiation, θ–2θ scans). The approximate size of the crystal used for data collection was 0.04 × 0.28 × 0.45 mm; the data were corrected for absorption. There were 1023 accessible reflections with θ <70°, of which 1003 were considered to be observed. The structure was solved by the heavy atom method and was refined by full matrix least squares. The final discrepancy indices were R = 0.055 and WR = 0.067 for the 1003 observed reflections.

After fractions 66–95 had been triturated with methylene chloride to remove crystalline material as described above, the filtrate was evaporated to dryness and dissolved in ethyl acetate (5 mL) containing DMF (0.1 mL) and applied to the top of a silica column (65 g) packed in and eluted with methylene chloride/ethyl acetate (5:1). Fractions of 12 mL were collected, and tubes 24–39 were combined and evaporated to dryness to give an amorphous solid (0.2 g). Recrystallization gave **7**, 137 mg (2%). A second crop was obtained from the mother liquor: yield 50 mg (1%); mp 102–103 °C; NMR (Me_2SO-d_6) δ 11.09 (br, d, 1, J = 7 Hz, NH), 7.82 (dd, 1, J = 6 and 6 Hz, C_6 H), 6.50 (dd, 1, J = 4 and 4 Hz, $C_{1'}$ H), 4.41 (m, 1, $C_{3'}$ H), 4.12 (m, 1, $C_{4'}$ H), 3.43 (m, 2, CH_2I), 3.05 (m, 1, CH_2), 2.63 (m, 1, CH_2); UV (MeOH) λ_{max} 269 nm (ε 7210); UV (0.01 N KOH) λ_{max} 303 nm (ε 7820). Anal. ($C_9H_9FI_2N_2O_3$) C, H, F, I, N.

After methylene chloride trituration of fractions 96–195, the filtrate was evaporated to dryness to give a solid (2.2 g). This material was divided into four portions, each was dissolved in methylene chloride/ethyl acetate (10 mL, 4:1), applied to the top of a silica column (125 g), and eluted with methylene chloride/ethyl acetate (5:1), and fractions (12 mL) were collected. Typically, tubes 47–53 contained pure **7**, tubes 54–60 contained a mixture of 14 and **7**, and tubes 61–80 contained pure 14. The fractions containing pure **7** were combined and evaporated to give pure material, 0.15 g (2%). The fractions containing pure 14 were combined, evaporated to dryness, and recrystallized from ethyl acetate/hexane to give 14, 1.64 g (17%): mp 117–129 °C indef dec; NMR (Me_2SO-d_6) δ 11.85 (br d, 1, NH), 7.96 (d, 2, J = 7 Hz, CHCF), 6.17 (m, 1, $C_{1'}$ H), 4.0–4.4 (m, 2, $C_{3'}$ H), 2.6–2.8 (m, 2, $C_{2'}$ H); UV (MeOH) λ_{max} 268 nm (ε 10 100). Anal. ($C_9H_9FI_2N_2O_3$) C, H, N, I.

After removal of crystalline material from fractions 196–365 by trituration, the filtrate was evaporated to dryness, and the residue was taken up in ethyl acetate (20 mL). This solution was filtered and applied to the top of a silica column (250 g) which was packed in and eluted with methylene chloride/ethyl acetate (5:1); fractions of 15 mL were collected. Tubes 81–125 were combined and evaporated to dryness to give 14, 0.15 g (1.6%). Fractions 141–190 were combined and evaporated to dryness to give **15** (0.7 g) as an amorphous solid. Repeated recrystallization from aqueous ethanol and treatment with charcoal yielded pure **15**, 293 mg (3%): mp 72–76 °C; NMR (Me_2SO-d_6) δ 11.61 (br d, 1, J = 5 Hz, NH), 7.94 (d, 1, J = 7 Hz, CHCF), 6.02 (m, 1, $C_{1'}$ H), 3.2–3.8 (m, 4, CH_2I, 2 × CH), 2.62 (m, 1, $C_{2'}$ H); UV (methanol) λ_{max} 267–268 nm (ε 9720). Anal. ($C_9H_9FI_2N_2O_3$) C, H, F, I, N.

2,3′-Anhydro-3-(2′,5′-dideoxy-β-D-*threo*-pentofuranosyl)-5-fluorouracil (6). A solution of **5** (4.75 g) in ethyl acetate (1.6 L) and triethylamine (4.3 mL) was treated with hydrogen in the presence of palladium on carbon (2.2 g, 5%) for 1.5 h. The catalyst was removed by filtration through Celite and washed with ethyl acetate, and the combined filtrate and washings were evaporated to dryness. The solid was crystallized from ethanol to give **6** (2.18 g). A second crop (300 mg) was obtained from the mother liquor: total yield 2.48 g (83%); mp 235–238 °C dec; NMR (Me_2SO-d_6) δ 7.69 (d, 1, J = 3 Hz, CHCF), 6.41 (d, 1, J = 3 Hz, $C_{1'}$ H), 5.08 (d, 1, J = 2 Hz, $C_{3'}$ H), 4.40 (m, 1, $C_{4'}$ H), 2.51 (m, 2, $C_{2'}$ H), 1.25 (d, 3, J = 7 Hz, CH_3CH); UV (MeOH) λ_{max} 217 nm (ε 6250), 279 (6150). Anal. ($C_9H_9FN_2O_3$) C, H, F, N.

Treatment of 9 with Methyltriphenoxyphosphonium Iodide. A solution of 9 (645 mg) in DMF (12 mL) was treated with methyltriphenoxyphosphonium iodide (4.25 g) for 21 h at room temperature. Methanol (55 mL) was added, and after 1.5 h the solution was evaporated to an oil and partitioned between ethyl acetate (100 mL) and aqueous sodium thiosulfate (100 mL). The organic layer was evaporated to dryness, and the residue was dissolved in methylene chloride (10 mL), filtered, and applied to a silica column (130 g). The column was eluted with methylene chloride/ethyl acetate (3:1), and fractions of 10 mL were collected. Tubes 81–110 were evaporated to dryness and recrystallized from ethyl acetate/hexane to give 5, 59 mg (9%); mp 215–216 °C.

Cyclization of 14. A suspension of 14 (539 mg) in 0.024 N NaOH (50 mL) was heated under reflux for 1 h. The solution was stored at 5 °C to give 9, 225 mg (57%), as large white needles, mp 191–195 °C dec.

Treatment of 9 with Hydrogen Iodide. A suspension of 9 (104 mg, 0.3 mmol) in ethyl acetate (0.5 L) was treated with aqueous hydriodic acid (0.3 mL, 47%, 1.65 mmol) for 1.5 h at room temperature. The product was washed with aqueous sodium bicarbonate (0.5 L, 5%), followed by aqueous sodium thiosulfate (0.5 L) and water (0.5 L). The organic layer was evaporated to dryness, dissolved in methylene chloride (10 mL), and applied to a silica column (60 g). The column was eluted with methylene chloride/ethyl acetate (3:1) and fractions of 12 mL were collected. Tubes 21–30 were combined and evaporated to dryness to give 14, 85 mg (59%).

2′,5′-Dideoxy-3′-*O*-(methanesulfonyl)-5-fluorouridine (8). A solution of 3 (2.35 g) in dry pyridine (10 mL) was treated at 0 °C with methanesulfonyl chloride (1.2 mL) and stored for 18 h at 5 °C. Ethanol (10 mL) was added, and after 40 min at 0 °C the solution was evaporated to dryness and treated with water (200 mL). After storage for 2 h, the crystals were collected and dried in vacuo to give 8, 2.84 g (90%): mp 148–149 °C dec; NMR (Me_2SO-d_6) δ 11.85 (br, s, 1, NH), 7.95 (d, 1, J = 7 Hz, CHCF), 6.08 (m, 1, $C_{1'}$ H), 5.05 (m, 1, $C_{3'}$ H), 4.10 (m, 1, $C_{4'}$ H), 3.26 (s, 3, CH_3SO_2), 1.34 (d, 3, J = 7 Hz, CH_3CH); UV (MeOH) λ_{max} 267 nm (ϵ 8630). Anal. ($C_{10}H_{13}FN_2O_6S$) C, H, F, N.

2,3′-Anhydro-1-(2′,5′-dideoxy-β-D-*threo*-pentofuranosyl)-5-fluorouracil (10). (A) From 9. A solution of 9 (402 mg) in methanol (70 mL) containing triethylamine (0.4 mL) was treated with hydrogen in the presence of palladium on carbon (200 mg, 5%) for 1 h. The catalyst was removed by filtration through Celite, and the filtrate was evaporated to dryness and recrystallized from ethanol to give 10, 142 mg (56%): mp 217–218 °C; NMR (Me_2SO-d_6) δ 8.09 (d, 1, J = 5 Hz, CHCF), 5.79 (d, 1, J = 4 Hz, $C_{1'}$ H), 5.20 (m, 1, $C_{3'}$ H), 4.38 (m, 1, $C_{4'}$ H), 2.5 (m, 2, CH_2), 1.23 (d, 3, J = 6 Hz, CH_3CH); UV (MeOH) λ_{max} 232 nm (ϵ 7930), 251 (7910). Anal. ($C_9H_9FN_2O_3$) C, H, F, N.

(B) From 8. A suspension of 8 (0.6 g) in water (60 mL) containing 1 N sodium hydroxide (2.04 mL) was heated under reflux for 70 min and then evaporated to dryness. The residue was dissolved in DMF/MeOH (1:1, 15 mL) and applied to a silica column (100 g) which had been packed in ethyl acetate/methanol (10:1). The column was eluted with the same solvent (600 mL), followed by ethyl acetate/methanol (5:1, 600 mL), and fractions 26–111 were pooled and evaporated to dryness. The residue was dissolved in methanol (100 mL), filtered through Celite, evaporated to dryness, and crystallized from ethanol to give 10, 246 mg (60%); mp 217–218 °C.

1-(2′,5′-Dideoxy-β-D-*threo*-pentofuranosyl)-5-fluorouracil (4). A suspension of 8 (3 g) in aqueous sodium hydroxide (1 N, 48 mL) was heated under reflux for 15 min and then cooled and neutralized with IRC 50 (H^+) resin. The resin was removed by filtration through Celite, and silica (50 g) was added to the filtrate. The mixture was evaporated to dryness and dried by evaporation of ethanol (3 × 100 mL) over the residue. This material was suspended in chloroform (100 mL) and applied to the top of a silica column (450 g) which had been packed in chloroform/methanol (12:1). The column was eluted with chloroform/methanol (12:1), and fractions 140–200 were pooled and evaporated to dryness. The residue was crystallized from ethanol and recrystallized from ethyl acetate to give 4, 1.4 g (66%): mp 187–188 °C; NMR (Me_2SO-d_6) δ 11.70 (s, 1, NH), 8.09 (d, 1, J = 7 Hz, CHCF), 6.02 (m, 1, $C_{1'}$ H), 5.35 (d, 1, J = 4 Hz, OH), 3.90 (m, 1, CH), 4.02 (m, 1, CH), 2.55 (m, 1, $C_{2'}$ H), 1.89 (dd, 1, J = 2 and 15 Hz, $C_{2'}$ H), 1.22 (d, 3, J = 6 Hz, CH_3CH); UV (H_2O) λ_{max} 205 nm (ϵ 9700), 271 (8980). Anal. ($C_9H_{11}FN_2O_4$) C, H, F, N.

1-(2′,3′,5′-Trideoxy-β-D-*glycero*-pent-2-enofuranosyl)-5-fluorouracil (12). A solution of 8 (8.2 g) in Me_2SO (200 mL, dried over molecular sieve) and potassium *tert*-butoxide (6.6 g) was stirred at room temperature for 1.5 h. Methanol (500 mL) was added and the reaction was neutralized with Amberlite IRC-50 (H^+) ion-exchange resin. The mixture was filtered through Celite and washed with methanol, and the filtrate and washings were combined and evaporated to dryness. The residue was treated with methylene chloride (100 mL) and filtered through Celite to remove solids. One-half of this solution was applied to a silica column (125 g) which was eluted with methylene chloride/ethyl acetate (6:1), and fractions of 15 mL were collected. Tubes 74–170 were combined and evaporated to give 12, 2.4 g (85%), as a white solid: mp 148–149 °C with subsequent solidification and mp 270–278 °C dec; NMR (Me_2SO-d_6) δ 11.66 (br s, 1, NH), 7.34 (d, 1, J = 7 Hz, CHCF), 6.71 (br s, 1, $C_{1'}$ H), 6.37 (m, 1, $C_{2'}$ H), 5.77 (m, 1, $C_{3'}$ H), 4.88 (m, 1, $C_{4'}$ H), 1.33 (d, 3, J = 7 Hz, CH_3CH); UV (MeOH) λ_{max} 267 nm (ϵ 8400).

2′,3′,5′-Trideoxy-5-fluorouridine (13). (A) From 14. A solution of 14 (455 mg) in methanol (90 mL) and triethylamine (0.9 mL) was treated with hydrogen in the presence of palladium on carbon (452 mg, 5%) for 1 h. The catalyst was removed by filtration through Celite and washed with methanol, and the combined filtrate and washings were evaporated to dryness. The residue was dissolved in methylene chloride (50 mL) and applied to a silica column (50 g) which was eluted with methylene chloride/acetone (10:1). Tubes 38–61 were combined and evaporated to dryness, and the residual solid was recrystallized from ethyl acetate/hexane to give 13, 133 mg (64%). An analytically pure sample was obtained by recrystallization from ethyl acetate/hexane: mp 158 °C; UV (CH_3OH) λ_{max} 207 nm (ϵ 8890), 270 (8640); NMR (Me_2SO-d_6) δ 11.73 (br s, 1, NH), 7.72 (d, 1, J = 6 Hz, CHCF), 5.58 (m, 1, $C_{1'}$ H), 4.05 (m, 1, $C_{4'}$ H), 1.45–2.45 (m, 4, $C_{2'}$ H, $C_{3'}$ H), 1.29 (d, 3, J = 6 Hz, CH_3CH). Anal. ($C_9H_{11}FN_2O_3$) C, H, F, N.

(B) From 15. A solution of 15 (328 mg) in methanol (50 mL) and triethylamine (0.6 mL) was treated with hydrogen in the presence of palladium on carbon (306 mg, 5%) for 45 min. The catalyst was removed by filtration through Celite and the filtrate was evaporated to dryness. The residue was dissolved in methylene chloride (5 mL) and applied to the top of a silica column (75 g) which was eluted with methylene chloride/ethyl acetate (3:1). Tubes 49–75 (20-mL fractions) were combined and evaporated to dryness, and recrystallization of the residue from ethyl acetate/hexane gave 13, 77 mg (51%).

(C) From 12. A solution of 12 (2 g) in dioxane (155 mL) was treated with hydrogen in the presence of palladium on carbon (2 g, 5%) for 2.5 h. The catalyst was removed by filtration through Celite and washed with dioxane, and the combined filtrate and washings were evaporated to a syrup. The residue was dissolved in methylene chloride/ethyl acetate (50 mL, 1:1) and applied to a silica column (250 g) which was eluted with methylene chloride/ethyl acetate (3:1). Tubes 72–140 were combined and evaporated to dryness to yield 1.2 g of crude material. Repeated crystallization gave 13, 0.78 g (39%); mp 156–158 °C.

2-Methylene-5-(*R*)-(5-fluorouracil-1-yl)-2,5-dihydrofuran (16). A solution of 14 (204 mg) in methanol (5 mL) was treated with 1 N NaOH (2.2 mL) for 24 h at room temperature. The reaction was neutralized with Amberlite IRC-50 (H^+), filtered through Celite, and washed with methanol. The combined filtrate and washings were evaporated and dried by evaporation of ethanol over the residue. Methylene chloride was added to the residue, and the solution was filtered through Celite to remove suspended solids. The filtrate was applied to a silica column (75 g) which was eluted with methylene chloride/ethyl acetate (3:1). Tubes 38–55 were combined and evaporated to dryness, and the residue was recrystallized from ethyl acetate/hexane to give 16, 33.2 mg (38%); mp 150–153 °C dec. A second crop of less pure material (19.7 mg, 17%) was obtained from the liquors: NMR (Me_2SO-d_6) δ 11.98 (br s, 1, NH), 7.41 (d, 1, J = 6 Hz, CHCF), 7.08 (m, 1, $C_{1'}$ H), 6.76 (dd, 1, J = 2 and 6 Hz, $C_{2'}$ H), 6.38 (m, 1, $C_{3'}$ H), 4.40 (m, 1, CH_2), 4.26 (m, 1, CH_2); UV (MeOH) λ_{max} 263 nm (ϵ 14790). Anal. ($C_9H_7FN_2O_3$) C, H, N, F.

Antitumor Testing. CD_1 mice, weighing 18–20 g, were implanted subcutaneously in the right ventrolateral area with 4×10^7 sarcoma 180J tumor cells. Test substances were dissolved or suspended in sterile deionized water, and treatment, 1.0 mL, was given intraperitoneally shortly after implantation and once daily thereafter for a total of eight treatments. Mice were sacrificed 1 day after the last treatment. An antitumor effect was defined as ≥50% reduction in tumor growth.

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Antihypertensive Ureidopiperidines

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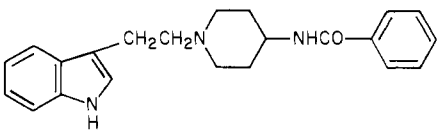
The synthesis of a series of 1-aralkyl-4-ureidopiperidines is reported. These compounds are related to the benzamidopiperidines exemplified by indoramin. Some of the ureidopiperidines are more potent antihypertensive agents than their benzamidopiperidine counterparts. Two examples, 1-(2-thenoyl)-3-[1-[2-(3-indolyl)ethyl]piperid-4-yl]urea and 1-(2-thenoyl)-3-[1-[4-(4-fluorophenyl)-4-oxobutyl]piperid-4-yl]urea (**19** and **58**), emerged as the most potent antihypertensive agents in this series.

Previous publications from these laboratories[1-3] have dealt with the origins and development of indoramin and related benzamidopiperidines. Indoramin is an antihypertensive agent incorporating competitive postsynaptic α-adrenoceptor antagonist and myocardial membrane stabilizing properties. Therapeutic advantages of this mechanism of action have been reviewed.[4,5] As an extension of this work, we now report the synthesis and pharmacological activities of a variety of ureidopiperidines, in which the benzamido group of indoramin (**67**, Table II) and related compounds has been replaced by aryl or aroylureido substituents.



indoramin (**67**)

It has been found that some of these compounds show equivalent or enhanced antihypertensive activities as compared with their benzamidopiperidine counterparts. Testing for antihypertensive activities has been carried out in DOCA/saline or renal hypertensive rats.[6,7,9] The general structure at the head of Table I indicates the range of modifications covered in this work. Compounds are listed in Table I in order of increasing length of the –A– chain which links the R_1 and piperidine moieties. An important limitation in scope is that compounds where –A– is CH_2 are excluded. This is because a profound shift in biological profile has been discovered among these latter compounds, which are virtually devoid of antihypertensive activity, suggesting potential therapeutic utility as psychotropic agents, and they will therefore be the subject of a separate publication.

Chemistry. Methods used to prepare the compounds described in this publication can be grouped into eight general types. These are illustrated in Scheme I by representative examples for each of the methods (A to H), which are the same examples as are exemplified under Experimental Section. Methods used for individual compounds are indicated by code letters in Table I. Method A involves reaction of an isocyanate (R_3NCO) or isothiocyanate (R_3NCS) with an appropriately substituted 4-aminopiperidine. This is the most generally applicable and widely used method. Method B involves hydrolysis of a 1-(4-piperidyl)-3-acylurea or thiourea to give the 3-unsubstituted urea or thiourea. The former can also be obtained directly from the corresponding aminopiperidine by reaction with potassium cyanate (method A′). Method C involves reacylation of a primary urea, as obtained by method A′ or B, with an acid chloride (R_3COCl). Method D involves alkylation of a 1-unsubstituted 4-ureidopiperidine with an aralkyl halide or tosylate, such as 3-(2-bromoethyl)indole. Method E involves reaction of an aroyl cyanamide with an appropriately substituted 4-aminopiperidine. Method F involves reduction of a carbonyl-containing A chain to give a hydroxy-substituted A chain. Method G involves reaction of an epoxide with an appropriately substituted 4-aminopiperidine. Method H involves reduction of a carbonyl group in the A chain to a methylene group. The most widely used methods are A to D. In all but three instances (compounds **1**, **33**, and **44**), compounds were isolated and tested as hydrochloride salts. Diastereoisomeric benzodioxans (**28–38**) were either obtained by fractional crystallization or by starting with a pure diastereoisomeric bromo alcohol or epoxide precursor. Stereochemical assignments were based on analysis of NMR data by methods similar to those of Howe et al.[8]

Results

An evaluation of the antihypertensive activities of compounds in Table I was carried out in conscious renal hypertensive (RHR) or DOCA/saline hypertensive rats.[6,7] Systolic blood pressure was measured by an indirect tail-cuff technique.[9] Results are presented in Table II.

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