Synthesis of 5-Substituted 2'-Deoxyuridines

Arieh Kampf, Conrad J. Pillar, Warren J. Woodford, and Mathias P. Mertes*

Department of Medicinal Chemistry, School of Pharmacy, University of Kansas, Lawrence, Kansas 66045.
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A series of thymidylate synthetase inhibitors was synthesized, some of which were potential irreversible inhibitors. 5-Formyl-2'-deoxyuridine (**9**) and its dithiolane derivative **11** were prepared by condensation of the bis(trimethylsilyl) derivative of 5-formyluracil dimethyl acetal and the protected chloro sugar followed by saponification of the protective groups. 5-Acetyl-2'-deoxyuridine (**15**) was prepared in the same way from 5-acetyluracil. Treatment of the diester of 5-allyl-2'-deoxyuridine (**17** or **22**) with *m*-chloroperbenzoic acid gave the corresponding epoxide. Dimethylamine removed the ester groups and opened the epoxide to give the amino alcohol **24**. The diester of 5-chloromethyl-2'-deoxyuridine (**27**) treated with methanol or sodium azide gave 5-methoxymethyl- (**29**) and 5-azidomethyl- (**31**) 2'-deoxyuridines. Compound **27** also was converted to 5-iodoacetamidomethyl-2'-deoxyuridine by treatment with ammonia, chloroacetyl chloride, base saponification, and finally sodium iodide.

Antimetabolite theory and applications have provided the clinician with many useful drugs for the treatment of bacterial infections and for the control of some cancers. However, in the treatment of cancer, severe toxicity is associated with all of these cytotoxic agents principally because the normal and the cancerous cell has similar metabolism. The 50-year search for metabolic pathways unique to the cancer cell has uncovered several potentially useful targets, a few of which have been exploited (asparaginase), but the overall progress in this direction has been disappointing.

Fifteen years ago, Baker proposed an approach to selectivity that remains promising.[1] This theory is based on the fact that many enzymes that catalyze the same transformation have different primary and, subsequently, tertiary structures. These isozymes are often found in different tissues. For example, lactic acid dehydrogenase from heart and skeletal muscle catalyzes the same biological reaction but has different structures. Assuming that isozymes exist for cancerous and normal cells, Baker maintained that isozyme structural differences offer the potential for selective inhibition of cancer cells.

A target enzyme for such an approach is thymidylate synthetase.[2] This enzyme catalyzes the reductive methylation of 2'-deoxyuridine 5'-phosphate (**1**) to thymidine 5'-phosphate (**2**, Scheme I). Inhibition of this enzyme has been utilized in the treatment of cancer; 5-fluorouracil is one such agent. Further, Krauss and co-workers[3] have described thymidylate synthetase isozymes in bacterial and phage-infected bacterial cells.

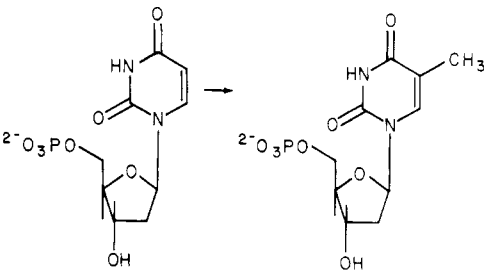
The syntheses of several agents are described in this report that probe the enzyme active site structural requirements for binding to thymidylate synthetase.

The first compounds in this series are 5-formyl-2'-deoxyuridine (**9**) and 5-acetyl-2'-deoxyuridine (**15**). The formyl derivative **9**, synthesized several years ago,[4] has been found to be a potent inhibitor of thymidylate synthetase.[5,6] The dithiolane derivative **11** has been prepared in the search for an alternate route to the 5'-phosphate required for testing since problems have been encountered in the chemical synthesis of the 5'-phosphate of **9**. Because of the high potency of the formyl **9**, the acetyl **15** was prepared.

The second series of compounds, the 5-allyl and the potential alkylating agent, 5-(2,3-oxypropyl)-2'-deoxyuridine (**21**), was prepared to examine the effect of a three-atom chain on binding and alkylation (**21**) of the enzyme.

Based on the activity of 5-benzyloxymethyl-2'-deoxyuridine (**26**)[6] the next series of target compounds, 5-methoxymethyl- and 5-azidomethyl-2'-deoxyuridines (**29** and **31**), was prepared. The latter compound was particularly attractive as an enzyme inhibitor since it contained a reactive function, the azide, that potentially could be displaced by an enzyme nucleophile to give irreversible inhibition.

Scheme I



1 **2**

The final compound, 5-iodoacetamidomethyl-2'-deoxyuridine (**35**), was prepared since the enzyme was found to be inhibited even with relatively large groups in the 5 position (the nucleotide of **26**) and to probe potential nucleophiles on thymidylate synthetase from difference sources used as isozyme models.

5-Formyl-2'-deoxyuridine (**9**) has been synthesized previously from the corresponding 5-benzyloxymethyl derivative by a series of reactions that require several chromatographic purifications.[7] Hydrogenolysis of 5-benzyloxymethyl-2'-deoxyuridine gave a mixture of the desired 5-hydroxymethyl-2'-deoxyuridine and thymidine which had to be column purified. Manganese dioxide oxidation of the hydroxymethyl derivative gave the product **9**. In this report we sought a more direct synthesis of **9** (Scheme II).

Purification of the bis(trimethylsilyl) derivative of 5-formyluracil by distillation was unsuccessful. Alternatively, the dimethyl acetal **4**, prepared by standard procedures, when treated with hexamethyldisilazane, gave a distillable product in 74% yield. Using the recently reported stannic chloride catalyzed sugar condensation of Niedbolla and Vorbruggen[8] we condensed the bis(trimethylsilyl) derivative of **4** with 3,5-di-*O*-*p*-toluoyl-2-deoxy-D-ribofuranosyl chloride (**5**). After workup, which hydrolyzed the acetal, the β anomer **6** was fractionally crystallized from the α-β mixture using acetone in yields up to 45%. Pure α anomer **7** was obtained in 15% yield.

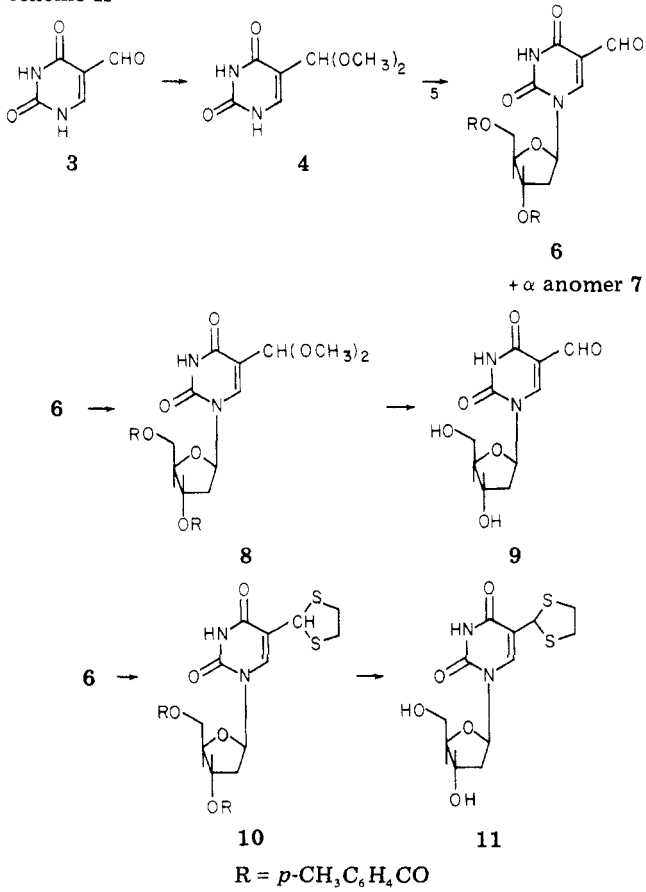
Previous attempts in these laboratories to phosphorylate 5-formyl-2'-deoxyuridine (**9**) to the 5'-phosphate using the selective phosphorylation procedure of Yoshikawa et al.[9] have failed. Although phosphorylated product is obtained, the formyl group is absent as evidenced by a shift in the ultraviolet from 279 nm for the nucleoside **9** and its 5'-phosphate[6] to 263 nm characteristic of a 5-CH_2OR sub-

Table I. Ultraviolet Absorption Characteristics of 5-Substituted 2′-Deoxyuridine[a]

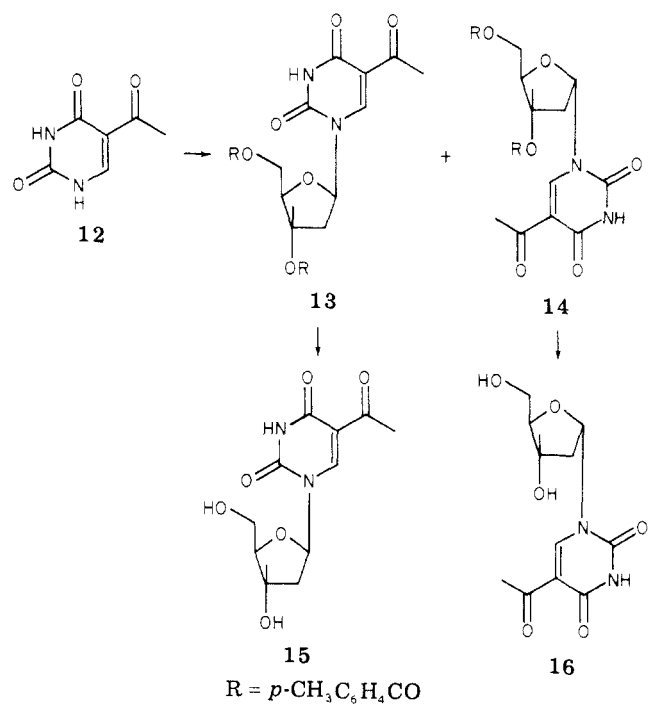
Compd	5-Substituent	pH[a]	λ_{max}	ϵ	λ_{max}	ϵ
11		1	271	16 000	239	8000
		7	270	16 000	239	7600
		13	270	13 000		
15	$COCH_3$ (β anomer)	1	282	12 400	250	2600
			229	9 500	207	3800
		7	282	13 000	250	3000
			230	10 000	207	3900
		13	274	9 800	245	4500
16	$COCH_3$ (α anomer)	1	282	11 600	247	2000
			228	7 000		
		7	282	11 800	247	1000
			227	6 000		
		13	282	8 400	258	3600
29	CH_2OCH_3[b]	7	261	11 000	230	2500
			208	10 000		
31	CH_2N_3	1	262	13 000	231	4200
		7	262	13 000	231	4000
		13	261	10 000	242	7800
34	$CH_2NHCOCH_2Cl$	1	264	8 000		
		7	263	7 200		
		13	263	6 100		

[a] Hydrochloric acid (1 M) was used for the low pH and 1 M sodium hydroxide for the high pH. [b] Reported by G. L. Bubbar and V. S. Gupta, *Can. J. Chem.*, **48**, 3147 (1970), to have a λ_{max} in 0.1 M HCl of 264 (ϵ 10 700).

Scheme II



Scheme III



stituent instead of the 5-CH=O. We have not investigated this reaction further. Instead, an alternate route to the 5′-phosphate of **9** was elected. Conversion of **6** to the dimethyl acetal **8** in quantitative yield used a cation exchange resin as the catalyst in dry methanol. Base-catalyzed hydrolysis of the protective ester groups of **8** gave the nucleoside **9**; however, the dimethyl acetal function was unstable on workup and a mixture of the formyl nucleoside **9** and its dimethyl acetal was found in a 20:80 ratio according NMR analysis and integration of the aldehyde proton compared to the C-6 proton of the ring. Attempted resolution on silica or by recrystallization resulted in hydrolysis of the acetal function to give the formyl nucleoside **9**.

Another approach to a derivative of the formyl **9** that would be more stable under phosphorylation conditions is through the dithiolane analogue. Treatment of the protected formyl nucleoside **6** with ethanedithiol in a BF_3-catalyzed reaction gave an 84% yield of **10**. Base-catalyzed transesterification converted **10** to the nucleoside **11**. The ultraviolet maxima of **11** at 270 nm is midway between the maxima for a 5-substituted CH_3 or CH_2OR derivative (260–263 nm) and that of the formyl **9** (279 nm) (Table I).

The second compound in this series of thymidylate synthetase inhibitors is 5-acetyl-2′-deoxyuridine (**15**,

Scheme III). 5-Acetyluracil[10,11] (12) was converted to its bis(trimethylsilyl) derivative which was used without purification in the condensation reaction with the protected chloro sugar 5. The mixture of the α and β anomers (14, 13) treated with acetone–Skelly B gave a solid containing both anomers and a filtrate that was essentially pure β anomer 13. Crystallization of the filtrate residue from chloroform–Skelly B gave a 20% overall yield of 1-(3,5-di-*O*-*p*-toluoyl-2-deoxy-β-D-ribofuranosyl)-5-acetyluracil (13). The corresponding α anomer 14 was isolated in low yield by chromatography on silica gel. Conversion of both the β anomer 13 and the α anomer 14 to the respective nucleosides 15 and 16 was done by base-catalyzed transesterification in methanol. The anomeric character of 15 and 16 and other compounds in this study was confirmed by the proton NMR of the anomeric C_1 sugar proton; a triplet at approximately 6.2 ppm is characteristic of the β anomer and a quartet for the α anomer.[12] The ultraviolet maxima (Table I) of both anomers are similar with the exception of the 250-nm minima for the β anomer 15 and the 247-nm minima observed for the α anomer 16. In addition, the shift to 274 nm in the base spectrum of the β anomer was not observed in the α anomer.

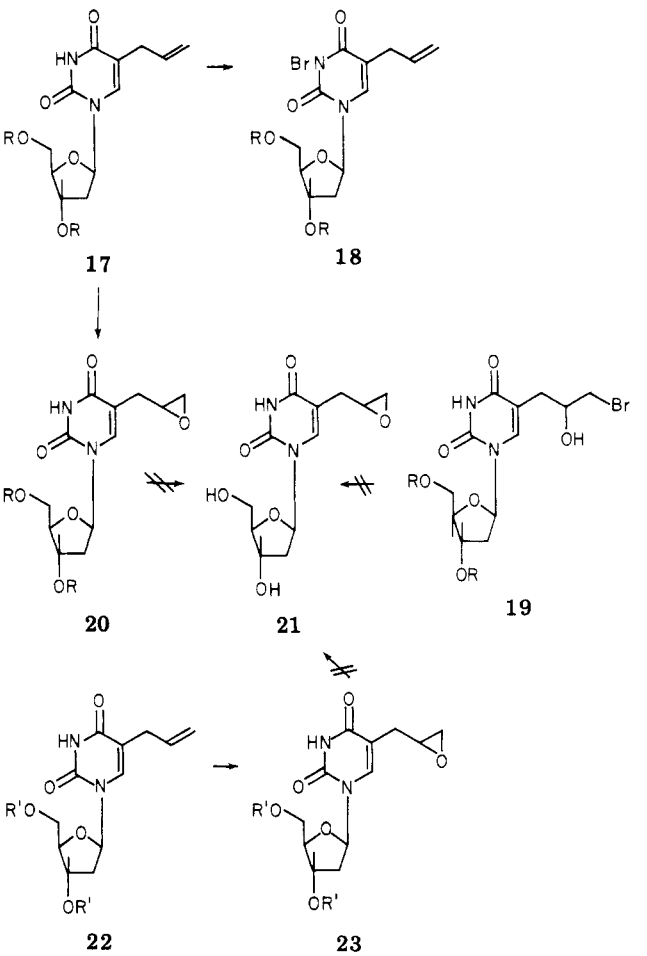
The third target compound in this series is the potentially irreversible alkylating agent 5-(2,3-epoxypropyl)-2'-deoxyuridine 5'-phosphate. Clearly, the most direct approach to this would be by epoxidation of the corresponding allyl nucleoside followed by phosphorylation. To this end 5-allyluracil,[13,14] after conversion to the bis(trimethylsilyl) derivative, was condensed with the protected chloro sugar 5 to give a mixture of anomers which was difficult to separate (Scheme IV). A series of dry column resolutions on silica was necessary to obtain an enriched (β) anomeric mixture from which the crystalline β anomer 17 could be obtained pure from ethyl acetate in 23% overall yield.

One approach to the desired epoxide 21 from the respective olefin is treatment with *N*-bromosuccinimide in an aqueous solution followed by base-catalyzed conversion of the bromohydrin to the epoxide. Treatment of the ditoluoyl-protected 5-allyl nucleoside 17 with *N*-bromosuccinimide in a water–tetrahydrofuran solution gave two products. The protected N^3-bromo nucleoside 18, formed in 68% yield, was identified by its spectral characteristics. There was no change in the ultraviolet which precludes addition to the 5,6 double bond of the pyrimidine ring. Burckhalter and co-workers[15] have found this type of reaction, addition to the 5,6 double bond of pyrimidines, occurs using *N*-halosuccinimides in the presence of alcohol. The proton NMR of 18 was identical with that of 17 with the exception of the ring N–H peak which was absent; the elemental analysis agrees with the structure (18) proposed.

The second product isolated by preparative TLC was the bromohydrin 19 in 21% yield. The characteristic allyl proton resonance was absent and two aliphatic multiplets were found in the 2.6–3.8-ppm region. Attempts to convert this compound in a one-step reaction to the epoxy nucleoside 21 were unsuccessful. Treatment of 19 with base hydrolyzed the ester groups but did not give a single product; rather, a complex mixture was observed on the analysis.

A more promising route to the desired epoxide was undertaken. Two different protected 5-allyl nucleosides 17 and 22[13,14] (Scheme IV) could be converted directly to the respective epoxides 20 and 23 by treatment with *m*-chloroperbenzoic acid.

Scheme IV

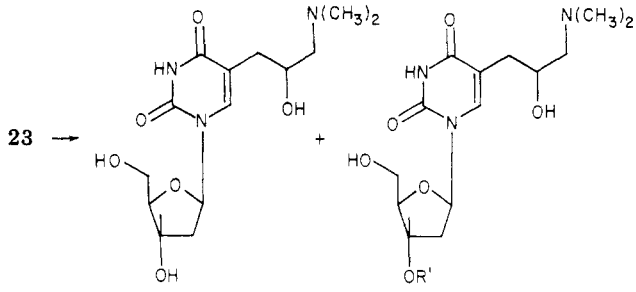


R = *p*-$CH_3C_6H_4CO$; R' = *p*-ClC_6H_4CO

Deprotection of the respective epoxides 20 or 23 to give the desired epoxy nucleoside 21 again gave a complex mixture that could not be resolved to give the expected product. Several reactions could possibly occur, two of which could account for our difficulty. The epoxide may not be stable under conditions used to remove the ester groups (catalytic amounts of sodium methoxide or potassium carbonate in methanol). Secondly, the 5'-hydroxyl of the free nucleoside 21 could add to the epoxide. Santi and Brewer[16] reported evidence for the intramolecular reaction of a 5'-hydroxyl with the C_6 in a uridine derivative. In our studies an intramolecular attack of the epoxide by the 5'-hydroxyl is possible from the observation that all attempts (recrystallization and silica chromatography) to purify the initial major product of the reaction led to breakdown of that material to give several spots on TLC. For example, a rapid resolution on a silica column gave a reasonably pure product that, on attempted crystallization from ethanol, reverted to the original complex mixture. This route of decomposition was suggested from the observation that the 5'-phosphate of 21 is stable.[6]

Treatment of the protected nucleoside 23 with dimethylamine gave two compounds resulting from attack at the epoxide and ester saponification (Scheme V). The nucleoside 24 (picrate salt) was obtained in 61% yield after reaction in dimethylamine for 40 h at 100°. The structure of the product is illustrated as the 3-dimethylamino-2-hydroxypropyl derivative; however, we cannot exclude the alternative ring-opening path to give the 2-dimethylamino-3-hydroxypropyl product. Similar treatment of 23 for 4 h gave a product from reaction at the epoxide and

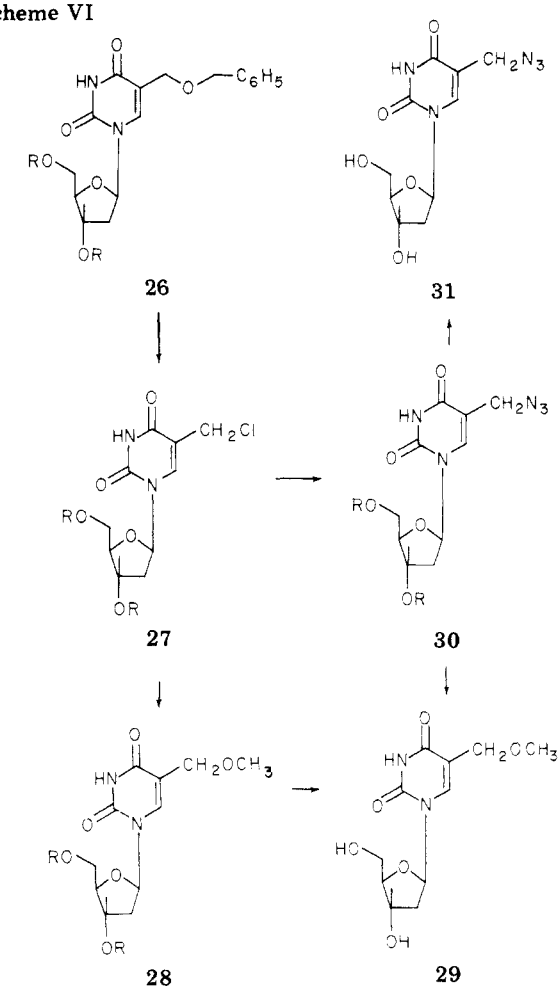
Scheme V



24 25

R′ = p-ClC$_6$H$_4$CO

Scheme VI



26 31 27 30 28 29

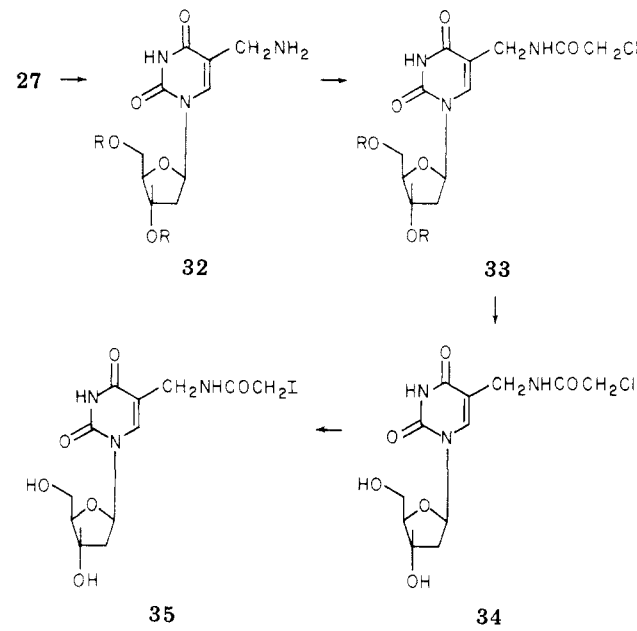
R = p-CH$_3$C$_6$H$_4$CO

only partial saponification; the elemental analysis corresponds to the monoester **25**. The 3′-ester is proposed because the NMR of **25** clearly showed the C_3 sugar proton in the same downfield position (5.22 ppm) as is noted in the diester **22**.

Assuming, from these results, that the epoxy nucleoside **21** was unstable because of reaction of either solvent or the 5′-sugar hydroxyl with the epoxide function, the final desired product, 5-(2,3-epoxypropyl)-2′-deoxyuridine 5′-phosphate, was prepared by treatment of the 5-allyl nucleotide with *m*-chloroperbenzoic acid. Apparently the presence of a 5′-phosphate instead of the hydroxyl prevents the decomposition noted in the nucleoside **21**.[6]

A convenient starting point for the preparation of the substituted 5-methyl nucleosides is the 5-benzyloxymethyl derivative **26**.[17] Conversion to the chloromethyl derivative (Scheme VI) by treatment of **26** in dioxane with dry hydrogen chloride gave **27**.[17,18] Refluxing **27** in dry methanol gave an 81% yield of the protected 5-methoxy derivative **28**; potassium carbonate catalyzed transesterification of **28** gave 5-methoxymethyl-2′-deoxyuridine (**29**) in good yield.[19]

Scheme VII



32 33 35 34

R = p-CH$_3$C$_6$H$_4$CO

The protected azidomethyl derivative **30** was prepared by the method of Brossmer and Rohm[17] from the chloro compound **27** by treatment with sodium azide. Conversion of **30** to the nucleoside by potassium carbonate–methanol treatment for 1 h, neutralization, and recrystallization from butanone gave **31** in 55% yield. Treatment of **30** for longer periods gave the methoxymethyl compound **29** via methanol displacement of the azide.

The final compound sought in this series was 5-iodoacetamidomethyl-2′-deoxyuridine (**35**), a potentially irreversible inhibitor of thymidylate synthetase. The synthesis of the first intermediate **32** was accomplished by treatment of the chloromethyl derivative **27** with liquid ammonia (Scheme VII). Separation on silica gave **32** in 62% yield. The amine **32** (1 equiv) and 2 equiv of trimethylamine in chloroform added to chloroacetyl chloride gave a 79% yield of the protected 5-chloroacetamidomethyl derivative **33**, which was stable on recrystallization from ethanol. Compound **33** was remarkably stable in potassium carbonate in methanol used to remove the ester groups; a quantitative yield of 5-chloroacetamidomethyl-2′-deoxyuridine (**34**) was obtained.

The conversion of an organic chloro compound to the corresponding iodo compound is frequently accomplished by treatment with sodium iodide in acetone. This method was used to convert the chloroacetamide **34** to the corresponding iodo compound **35**; a quantitative yield of sodium chloride was found. The same conversion was effected using dimethylformamide. Elemental analysis of **35** obtained from several reactions was acceptable for nitrogen and hydrogen; however, carbon was 1% high. We believe that the product obtained by recrystallization from acetone is contaminated by a small amount of the starting material **34**. Successive recrystallizations from acetone lowered the melting point and gradually increased the percentage of carbon in the product, presumably by en-

richment of starting material.

Experimental Section

All melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Ir spectra were measured with a Beckmann IR33, uv spectra with a Cary 14 recording spectrophotometer or Beckman DU, and NMR spectra with a Varian Model A-60 or T-60. Microanalyses were obtained from an F and M 185 or a Hewlett-Packard 185B. Unless specified the elemental analyses for C, H, and N are ±0.4% of theory.

5-Formyluracil Dimethyl Acetal (4). A solution of 5-formyluracil[20] (3, 12.0 g, 0.085 mol) in dry methanol (500 ml) containing *p*-toluenesulfonic acid monohydrate (400 mg) was refluxed overnight. After cooling to room temperature 6.5 g of solid 4 was collected (42% yield), mp 194–195°. Evaporation of the mother liquor to a volume of 90 ml yielded an additional 3.8 g of 4 (25%), mp 194–195°. After three recrystallizations from methanol the melting point rose to 199–200°: uv λ_{max} (ethanol) 259 nm (ε 4300); NMR (Me_2SO-d_6) δ 3.3 (s, 6, 2 methyls), 5.2 [s, 1, $-CH(OMe)_2$], 7.0 (s, 1, H-6), 10.66 ppm (br s, 2, 2NH). Anal. ($C_7H_{10}N_2O_4$) C, H, N.

1-(3,5-Di-*O*-*p*-toluoyl-2-deoxy-β-D-ribofuranosyl)-5-formyluracil (6) and the α Anomer 7. 5-Formyluracil dimethyl acetal (4, 6.0 g, 0.032 mol) was suspended in 70 ml of 1,1,1,3,3,3-hexamethyldisilazane containing 50 mg of ammonium sulfate and refluxed with stirring, excluding moisture, for 4 h. The pale yellow solution was evaporated under reduced pressure (bath temperature 80°) and the oily residue was distilled at 105–110° (0.2 mmHg). The bis(trimethylsilyl) derivative of 4 was obtained as a colorless oil, 7.9 g (74%). To a solution of the silyl derivative of 4 (7.9 g, 24 mmol) and of 3,5-di-*O*-*p*-toluoyl-2-deoxy-D-ribofuranosyl chloride[21] (5, 7.5 g, 0.019 mol) in 190 ml of dry 1,2-dichloroethane[8] at 2° was added dropwise, with stirring and exclusion of moisture, a solution of stannic chloride (0.41 ml) in 95 ml of 1,2-dichloroethane. A clear solution was obtained after a short time. The mixture was stirred for an additional 2 h at the same temperature, diluted with 100 ml of 1,2-dichloroethane, and shaken with 500 ml of saturated sodium bicarbonate solution. The layers were filtered through moist Celite; the organic layer was washed with water, dried, and evaporated to yield a mixture of 6 and 7 as a white solid (12.0 g). The anomers were separated by fractional recrystallization from hot acetone. Yields of pure β isomer 6, which separates first, ranged from 25 to 45%: mp 195–196°. The yield of the pure α isomer was only 15%: mp 188–189°. The physical properties of 6 and 7 were identical with those reported in previous papers.[4,7]

1-(3,5-Di-*O*-*p*-toluoyl-2-deoxy-β-D-ribofuranosyl)-5-formyluracil Dimethyl Acetal (8). 1-(1,3-Di-*O*-*p*-toluoyl-2-deoxy-β-D-ribofuranosyl)-5-formyluracil (6, 1.97 g, 4 mmol) and Amberlite IR-120 (H^+) (400 mg) in 130 ml of dry methanol were refluxed with stirring for 2 h. The mixture was filtered and evaporated to a volume of 25 ml, from which 1.7 g of crystalline 8 was collected: mp 143–144°. From the mother liquor an additional 0.35 g was collected: mp 139–140°; the total yield was 95%. The product was recrystallized from methanol: mp 143–145°; uv λ_{max} (methanol) 243 nm (ε 38000), λ_{min} 216 (ε 11500); NMR ($CDCl_3$) δ 3.15, 3.36 [2 s, 6, $-CH(OCH_3)_2$], 5.22 [s, 1, $-CH(OCH_3)_2$], 7.75 (s, 1, H-6), 9.4 ppm (br s, 1, NH). The spectrum of the sugar protons resembled that of thymidine 3',5'-di-*O*-*p*-toluate.[22] Anal. ($C_{28}H_{30}N_2O_9$) C, H, N.

1-(3,5-Di-*O*-*p*-toluoyl-2-deoxy-β-D-ribofuranosyl)-5-[2-(1,3-dithiolane)]uracil (10). A solution of the formyl compound 6 (984 mg, 2 mmol) and 1,2-ethanedithiol (0.7 ml) in 15 ml of glacial acetic acid containing boron trifluoride–etherate (1.4 ml) was stirred overnight at room temperature. The reaction mixture was slowly added to 225 ml of an ice-cold 5% sodium hydroxide solution; the solid precipitate was dissolved in chloroform and the resulting solution was washed with water, dried, and evaporated. The gummy residue was crystallized from acetone–cyclohexane to give 0.96 g of 10 (84%), mp 172–175°. Two crystallizations raised the melting point to 175–176°: uv λ_{max} (chloroform) 244 nm (ε 37600); NMR ($CDCl_3$) δ 3.03 (s, 4, $-SCH_2CH_2S-$), 5.38 (s, 1, H-2 of dithiolane ring), 7.98 (s, 1, H-6), 9.63 ppm (br s, 1, NH). Anal. ($C_{28}H_{28}N_2O_7S_2$) C, H, N.

5-[2-(1,3-Dithiolane)]-2'-deoxyuridine (11). A suspension of the protected dithiolane 10 (420 mg, 0.74 mmol) and potassium carbonate (300 mg) in dry methanol (40 ml) was stirred at room temperature. After 30–40 min a clear solution was obtained. After an additional 4 h, the solution was neutralized with dry Amberlite IR-120 (H^+), filtered, and evaporated. The methyl *p*-toluate was extracted by trituration of the residue with Skelly B and the product crystallized from ethanol to give 135 mg (55%) of 11, mp 185–187°. An additional 50 mg (37%) was collected from the mother liquor. After two recrystallizations the melting point rose to 194–195°: uv (H_2O) λ_{max} 270 nm (ε 16000), λ_{min} 239 (ε 8000); uv (1 M hydrochloric acid) λ_{max} 271 nm (ε 16000), λ_{min} 239 (ε 7600); uv (1 M sodium hydroxide) λ_{max} 270 nm (ε 13000), λ_{min} 262 (ε 11600). Anal. ($C_{12}H_{16}N_2O_5S_2$) H, N; C: calcd, 43.36; found, 43.81.

1-(3,5-Di-*O*-*p*-toluoyl-2-deoxy-β-D-ribofuranosyl)-5-acetyluracil (13) and the α Anomer 14. A mixture of 5-acetyluracil[10,11] (6.2 g, 0.04 mol) and ammonium sulfate (50 mg) in 70 ml of 1,1,1,3,3,3-hexamethyldisilazane was refluxed with stirring for 5 h. The resulting clear solution was evaporated to a thick syrup (water bath at 80°) which was used in next step without further purification. To a solution of the bis(trimethylsilyl) derivative and 3,5-di-*O*-*p*-toluoyl-2-deoxy-D-ribofuranosyl chloride[21] (10 g, 0.025 mol) in dry benzene (100 ml) was added molecular sieve (Linde 3A, 5 g) and the mixture was stirred at room temperature for 4 days. After the addition of 5 ml of ethanol the mixture was filtered and evaporated to give 12.7 g of the anomeric products 13 and 14. Partial separation could be effected by crystallization from acetone–Skelly B (1:1). An anomeric mixture separates, leaving a filtrate containing almost pure β anomer 13, from which the product was collected. This was recrystallized from $CHCl_3$–Skelly B to give 2.5 g of 13 (20%): mp 193.5–194.5°; uv λ_{max} (chloroform) 281 nm; NMR (CF_3COOH) δ 2.68 ppm (s, 3, CH_3CO). Anal. ($C_{27}H_{26}N_2O_8$) C, H, N.

The α anomer 14 was isolated in low yield by separation on a silica gel column, eluted with 2% methanol in chloroform: mp 188–189°. Anal. ($C_{27}H_{26}N_2O_8$) C, H, N.

5-Acetyl-2'-deoxyuridine (15). A suspension of compound 13 (1.0 g, 2 mmol) in 50 ml of 0.03 M sodium methoxide was stirred for 6 h at room temperature, then heated for 1 h to 50°, cooled, neutralized with Amberlite IR-120 (H^+), filtered, and evaporated. The residue was triturated with ether and the solid product filtered to give 400 mg of 15 (73%), mp 160–170°. After three recrystallizations from ethanol–Skelly B the melting point rose to 169–170°: uv (H_2O) λ_{max} 282 nm (ε 13000), λ_{min} 250 (ε 3000), λ_{max} 230 (ε 10000), λ_{min} 207 (ε 3900); uv (1 M hydrochloric acid) λ_{max} 282 nm (ε 12400), λ_{min} 250 (ε 2600), λ_{max} 229 (ε 9500), λ_{min} 207 (ε 3800); uv (1 M sodium hydroxide) λ_{max} 274 nm (ε 9800), λ_{min} 245 (ε 4500), shoulder at 223 nm. Anal. ($C_{11}H_{14}N_2O_6$) C, H, N.

1-(2'-Deoxy-α-D-ribofuranosyl)-5-acetyluracil (16). Compound 14 (1 g, 2 mmol) was allowed to react as described above for 15. A yield of 0.24 g (45%) was collected: mp 183–185°. After two recrystallizations from ethanol–Skelly B the melting point rose to 198°: uv (H_2O) λ_{max} 282 nm (ε 11800), λ_{min} 247 (ε 1000), λ_{max} 227 (ε 6000); uv (1 M hydrochloric acid) λ_{max} 282 nm (ε 11600), λ_{min} 247 (2000), λ_{max} 228 (7000); uv (1 M sodium hydroxide) λ_{max} 282 nm (ε 8400), λ_{min} 258 (ε 3600), shoulder at 231 (ε 8400). Anal. ($C_{11}H_{14}N_2O_6$) C, H, N.

1-(3,5-Di-*O*-*p*-toluoyl-2-deoxy-β-D-ribofuranosyl)-5-allyluracil (17) and the α Anomer. A mixture of 5-allyluracil[13] (4.7 g, 31 mmol) and ammonium sulfate (50 mg) in 70 ml of 1,1,1,3,3,3-hexamethyldisilazane was refluxed with stirring for 5 h. The resulting clear solution was evaporated to a thick syrup which was used in next step without further purification. To a solution of the bis(trimethylsilyl) derivative and 3,5-di-*O*-*p*-toluoyl-2-deoxy-D-ribofuranosyl chloride[21] (5, 11.0 g, 28 mmol) in dry benzene (100 ml) was added molecular sieve (5 g, Linde 4A) and the mixture was stirred at room temperature for 5 days. After the addition of 5 ml of ethanol the mixture was filtered and evaporated to yield a brown syrup (15.4 g) that was purified on a dry silica gel column. A white anomeric mixture (12.6 g, 88%) was collected from the column. Partial separation of the anomers was effected on a series of dry columns (silica gel) yielding 3.3 g (23%) of pure β anomer 17: mp 174–175° (from ethyl acetate); uv ($CHCl_3$) λ_{max} 249 nm; NMR ($CDCl_3$) δ 4.85 (d, 2, $-CH_2CH=CH_2$), 5.2–6.0 (m, 3, $-CH_2CH=CH_2$). Anal. ($C_{28}H_{28}N_2O_7$) C, H, N.

The α anomer was obtained in similar yield and had mp 146–147° (from methyl ethyl ketone–Skelly B). The spectral data were similar to compound **17**. Anal. ($C_{28}H_{28}N_2O_7$) C, H, N.

***N*-Bromo-1-(3-di-*O*-toluoyl-2-deoxy-β-D-ribofuranosyl)-5-allyluracil (18) and 1-(3,5-Di-*O*-toluoyl-2-deoxy-β-D-ribofuranosyl)-5-(3-bromo-2-hydroxypropyl)uracil (19).** To a solution of compound **17** (1.01 g, 2 mmol) in a mixture of tetrahydrofuran (20 ml) and water (10 ml), cooled in an ice bath, was added *N*-bromosuccinimide (370 mg, 2.08 mmol). After 3 h at the same temperature, the mixture was diluted with water (20 ml) and twice extracted with dichloromethane. The organic layer was washed with saturated sodium bicarbonate solution, followed by water, dried, and evaporated to yield a syrup (1.3 g). TLC (dichloromethane–methanol 30:1) indicated two major products. Crystallization from butanone–Skelly B produced compound **18** (660 mg, 57%), mp 196–197°. The NMR of this compound is identical with the spectrum of compound **17**, except for the NH band that is missing. Anal. ($C_{28}H_{27}BrN_2O_7$) C, H, N.

Compound **19** was isolated from the mother liquor of compound **18** by preparative TLC using dichloromethane–methanol (30:1) on silica. The mixture was separated into two bands, the high R_f being **18**. The bands were eluted from the silica with ethyl acetate and crystallized from butanone–Skelly B. Thus an additional crop of **18** was collected (130 mg), mp 196°, increasing the total yield to 68%. A yield of 250 mg of **19** was collected (21%), mp 172–175°. After two recrystallizations from butanone–Skelly B the melting point rose to 188°. The characteristic proton NMR allyl absorption disappeared and instead two aliphatic multiplets in the δ 2.6–3.8 region were recorded. Anal. ($C_{28}H_{29}BrN_2O_8$) C, H, N.

1-(3,5-Di-*O*-*p*-toluoyl-2-deoxy-β-D-ribofuranosyl)-5-(2,3-epoxypropyl)uracil (20). A solution of **17** (1.26 g, 2.5 mmol) and *m*-chloroperbenzoic acid (0.69 g, 3 mmol) in chloroform (20 ml) was allowed to stand at room temperature for 48 h. The reaction mixture was diluted with chloroform (100 ml) and successively washed with 10% sodium bisulfite, 10% sodium thiosulfate, saturated sodium bicarbonate, and water. After evaporation the product was purified by preparative TLC (silica, dichloromethane–methanol, 30:1) and crystallized from methanol to give 450 mg of **20** (35%), mp 158°. A $CDCl_3$ solution of **20** did not have allyl adsorption; all the bands of the 5 side chain shifted to δ 1.8–3.2 as a broad multiplet. Anal. ($C_{28}H_{28}N_2O_8$) C, H, N.

1-(3,5-Di-*O*-*p*-chlorobenzoyl-2-deoxy-β-D-ribofuranosyl)-5-(2,3-epoxypropyl)uracil (23). 1-(3,5-Di-*O*-*p*-chlorobenzoyl-2-deoxy-β-D-ribofuranosyl)-5-allyluracil[14] (2.18 g, 4 mmol) in chloroform (60 ml) was oxidized with *m*-chloroperbenzoic acid (2.0 g, 10 mmol) at 4° for 5 days. The reaction mixture was worked up as described above for **20**. Crops of 1.5–1.7 g were obtained (67–76%): mp 138°. The NMR pattern was similar to **20**. Anal. ($C_{26}H_{22}Cl_2N_2O_8$) C, H, N.

1-(2-Deoxy-β-D-ribofuranosyl)-5-(3-dimethylamino-2-hydroxypropyl)uracil (24). The epoxide **23** (2.0 g, 3.6 mmol) in liquid dimethylamine (20–30 ml) was heated at 100° for 40 h in a glass-lined steel bomb. The reaction mixture was evaporated and the residue partitioned between water and chloroform. The aqueous layer was evaporated to dryness, yielding 1.13 g of **24** (96%) as a solid foam; TLC (1-butanol–ethanol–water, 40:10:12) on silica showed a single product. As the product (**24**) could not be crystallized 550 mg was treated with a saturated ethanol solution of picric acid (5 ml) and refluxed for 15 min. Ether was added to give a slight turbidity. The picrate was collected as yellow pellets (560 mg, 61%): mp 166–168°. The salt was recrystallized from absolute ethanol: mp 167–168°. Anal. ($C_{20}H_{26}N_6O_{13}$) C, H, N.

1-(3-*O*-*p*-Chlorobenzoyl-2-deoxy-β-D-ribofuranosyl)-5-(3-dimethylamino-2-hydroxypropyl)uracil (25). When the epoxide **23** was treated as described above for **24** for only 4 h and the residue obtained after dimethylamine evaporation was partitioned between ether and water, compound **25** was isolated from the ether layer: mp 176–178° (from ethanol–Skelly B). The 3′ position for the single *p*-chlorobenzoyl in the molecule was assigned as the NMR (Me_2SO-d_6) shows a single proton at δ 5.22 for the C_3 sugar proton. Anal. ($C_{21}H_{26}ClN_3O_7$) C, H, N.

1-(3,5-Di-*O*-*p*-toluoyl-2-deoxy-β-D-ribofuranosyl)-5-methoxymethyluracil (28). The chloromethyl derivative **27**[17,18] (1.0 g, 2 mmol) was refluxed in dry methanol (75 ml) for 2 h. After cooling overnight 0.8 g (81%) of the crystalline product **28** was isolated: mp 175–176° (lit.[19] 170–172°); NMR ($CDCl_3$) δ 3.22 (s, 3, –OMe), 4.05 ppm (s, 2, $-CH_2OMe$). Anal. ($C_{27}H_{28}N_2O_8$) C, H, N.

5-Methoxymethyl-2′-deoxyuridine (29). A mixture of **28** (520 mg, 1 mmol), potassium carbonate (400 mg), and absolute methanol (75 ml) was stirred overnight. The resulting clear solution was neutralized with Amberlite IR-120 (H^+), the solvent evaporated, and the residue partitioned between benzene and water. The aqueous layer was evaporated and the residue crystallized from butanone–Skelly B to give 210 mg of **29** (75%), mp 124–126°, after two crystallizations (lit.[19] mp 120–125°): uv (H_2O) λ_{max} 261 nm (ϵ 11000), λ_{min} 230 (ϵ 2500), λ_{max} 208 (ϵ 10000); NMR (D_2O) δ 3.5 (s, 3, OMe), 4.3 ppm (s, 2, CH_2OMe). Anal. ($C_{11}H_{16}N_2O_6$) C, H, N.

5-Azidomethyl-2′-deoxyuridine (31). The azidomethyl derivative **30**[17] (294 g, 5.6 mmol) and potassium carbonate (2.0 g) were stirred in absolute methanol (200 ml) for 1 h and worked up as described for compound **29**. The product was crystallized from butanone to give 870 mg of **31** (55%), mp 130–132°. After two recrystallizations from acetone–ethyl acetate, the melting point rose to 133–135°: ir 2100 cm^{-1} ($-N_3$); uv (H_2O) λ_{max} 262 nm (ϵ 13000), λ_{min} 231 (ϵ 4000); uv (1 M hydrochloric acid) λ_{max} 262 nm (ϵ 13000), λ_{min} 231 (ϵ 4200); uv (1 M sodium hydroxide) λ_{max} 261 nm (10000), λ_{min} 242 (ϵ 7800). Anal. ($C_{10}H_{13}N_5O_5$) C, H, N.

When **30** was allowed to react as described above for a longer period than 1 h a second product could be detected by TLC (butanone on silica). After a 24-h reaction time only the second product could be detected. It was identified as **29** (~70% yield).

1-(3,5-Di-*O*-*p*-toluoyl-2-deoxy-β-D-ribofuranosyl)-5-aminomethyluracil (32). A solution of **27** (2 g, 3.9 mmol) in liquid ammonia (125 ml) was stirred in a dry ice–acetone bath for 3 h and then allowed to evaporate at room temperature. TLC (ethyl acetate–methanol–concentrated ammonium hydroxide, 94:5:1) showed one major low R_f (0.2) product and two minor high R_f products. Separation was effected on a silica column using the same eluent as for TLC. Fractions containing the low R_f product were combined and evaporated to give 1.2 g of **32** (62.4%), mp 163–168°. After recrystallizing from absolute ethanol the melting point rose to 176–178°: NMR ($CDCl_3$) δ 3.36 (s, 2, $-CH_2NH_2$), 5.20 ppm (br s, 2, $-CH_2NH_2$, exchangeable with D). Anal. ($C_{26}H_{27}N_3O_7$) C, H, N.

1-(3,5-Di-*O*-*p*-toluoyl-2-deoxy-β-D-ribofuranosyl)-5-chloroacetamidomethyluracil (33). To an ice-cold solution of the amine **32** (1.98 g, 4 mmol) and triethylamine (1.2 ml, 8 mmol) in chloroform (20 ml) was added dropwise a solution of chloroacetyl chloride (0.7 ml, 8 mmol) in chloroform (10 ml) (5–10 min addition). The mixture was stirred at room temperature for 1 h and then washed with water and saturated sodium bicarbonate. After drying, the solvent was evaporated and the residue crystallized from ethanol to give 1.8 g of **33** (79%): mp 112–117°. After two recrystallizations the melting point was 115–118°. Anal. ($C_{28}H_{28}ClN_3O_8$) C, H, N.

5-Chloroacetamidomethyl-2′-deoxyuridine (34). A suspension of **33** (570 mg, 1 mmol) and potassium carbonate (280 mg, 2 mmol) in absolute methanol was stirred at room temperature for 80 min. The resulting clear solution was neutralized with Amberlite IR-120 (H^+) and evaporated. The residue was triturated with ether to give a quantitative yield (310 mg) of **34**, mp 183–185°. The product was recrystallized from ethanol: mp 194–195°; uv (H_2O) λ_{max} 263 nm (ϵ 7200); uv (1 M hydrochloric acid) λ_{max} 264 nm (ϵ 8000); uv (1 M sodium hydroxide) λ_{max} 263 nm (ϵ 6100). Anal. ($C_{12}H_{16}N_3ClO_6$) C, H, N.

5-Iodoacetamidomethyl-2′-deoxyuridine (35). An acetone (25 ml) solution of sodium iodide (300 mg, 2 mmol) and 5-chloroacetamidomethyl-2′-deoxyuridine (**34**, 167 mg, 0.5 mmol) was refluxed for 26 h. After cooling, filtration yielded 29 mg of sodium chloride (30 mg, theory). The filtrate was evaporated and the residue crystallized from acetone to give 81 mg (38%) of the product **35**, mp 181–182°. Two more recrystallizations from acetone gave material melting first at 179–180° and finally at 178°. This material was found, on elemental analysis, to be high in

carbon. Anal. ($C_{12}H_{16}N_3IO_6$) H, N; C: calcd, 33.90; found, 34.81.

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Synthesis and Biological Activities of 5-Trifluoromethyl-5'-azido-2',5'-dideoxyuridine and 5-Trifluoromethyl-5'-amino-2',5'-dideoxyuridine[16]

Tai-Shun Lin, Caroline Chai, and William H. Prusoff*

Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut 06510. *Received October 21, 1975*

5-Trifluoromethyl-2'-deoxyuridine (1) was tosylated with *p*-toluenesulfonyl chloride in dry pyridine at 3° to give 5-trifluoromethyl-5'-*O*-(*p*-tolylsulfonyl)-2'-deoxyuridine (2), which was converted to 5-trifluoromethyl-5'-azido-2',5'-dideoxyuridine (3) by reacting with lithium azide in *N*,*N*-dimethylformamide at 85–90° for 2 h. Compound 3 was then hydrogenated in ethanol–water (1:1, v/v) at room temperature and 35 psi of hydrogen pressure, using 10% palladium on charcoal as catalyst, to yield 5-trifluoromethyl-5'-amino-2',5'-dideoxyuridine (4). Compound 4 is about fourfold less potent than compound 1 as an antiviral agent but is about 40-fold *less* toxic to the host Vero cells. Thus the therapeutic index of compound 1 has been improved by a factor of 10 by replacement of the 5'-hydroxyl with an amino group. Compound 1, however, is more than 100-fold more inhibitory to Sarcoma 180 cells in culture relative to compound 4. Compound 3 is markedly less potent than compound 1 or 4 as either an antiviral or an antineoplastic compound.

The antiviral activity of nucleosides has been reviewed recently.[1-5] Although 5-iodo-2'-deoxyuridine (IdUrd), 5-trifluoromethyl-2'-deoxyuridine (F_3dThd), 1-β-D-arabinofuranosylcytosine (ara-C), and 9-β-D-arabinofuranosyladenine (ara-A) possess antiviral activity, they also induce moderate to severe cytotoxicity. The 5'-amino analogue of IdUrd [5-iodo-5'-amino-2',5'-dideoxyuridine (AIU)] is a novel nucleoside analogue which exhibits significant antiviral activity in the absence of detectable cytotoxicity to the host Vero cells.[6] AIU is neither cytotoxic to a variety of murine, avian, simian, and human cells[7] nor mutagenic to L-5178 cells.[8] Studies in newborn and 8-day-old mice reveal no gross or histological activity.[9] Comparative therapy with IdUrd and AIU of experimental herpetic keratitis in rabbits indicates AIU has similar efficacy but less potency.[10] Thus the replacement of the 5'-hydroxyl moiety of IdUrd by an amino group has resulted in retention of antiviral activity with concomitant loss of cytotoxic properties. The corresponding modification of F_3dThd[11,12] produced 5-trifluoromethyl-5'-amino-2',5'-dideoxyuridine and the intermediate 5-trifluoromethyl-5'-azido-2',5'-dideoxyuridine. The synthesis and antiviral and antineoplastic activity of these analogues of F_3dThd are described.

Table I. Effect of 5-Trifluoromethyl-2'-deoxyuridine and Its 5'-Azido and 5'-Amino Analogues on the Replication of Herpes Simplex Virus in Vero Cells

Compound	Concn, μM	Virus titer[a] (pfu)
None		1.0×10^7
5-Trifluoromethyl-2'-deoxyuridine (1)	50	1.5×10^5
5-Trifluoromethyl-5'-azido-2',5'-dideoxyuridine (3)	200	1.7×10^6
5-Trifluoromethyl-5'-amino-2',5'-dideoxyuridine (4)	200	1.0×10^{5b}

[a] Titers were performed in duplicate with agreement within 10%. [b] In separate experiments the titer was 0.68 ± 0.26% of the control.

Biological. Although 5-iodo-5'-amino-2',5'-dideoxyuridine exerted a potent inhibition of the replication of herpes simplex virus type 1 with no cytotoxic effect to the uninfected host Vero cells,[6] a similar finding with the fluorinated nucleoside analogues in the present study was