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]

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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

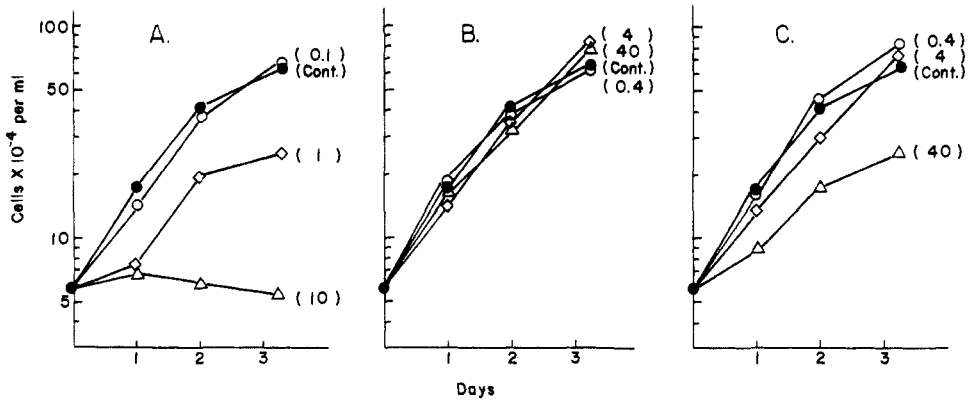


Figure 1. The effect of 5-trifluoromethyl-2',5'-dideoxyuridine (A), 5-trifluoromethyl-5'-azido-2',5'-dideoxyuridine (B), and 5-trifluoromethyl-5'-amino-2',5'-dideoxyuridine (C) on the replication of Vero (African green monkey kidney) cells in culture. The solid circles represent the cells grown in the absence of test compound. The number within the parentheses represents the μM concentration of the appropriate fluorinated nucleoside analogue.

not found (Table I, Figure 1). Thus F_3dThd produced about a 2 log decrease in virus titer at a 50-μM concentration; however, as little as 1 μM F_3dThd produced a marked cytotoxic effect on the uninfected host Vero cells. Of interest is the greater than 100-fold inhibition by F_3dThd of the neoplastic murine Sarcoma 180 cells relative to the Vero cells.

The 5'-amino analogue of F_3dThd is about fourfold less potent than the parant compound F_3dThd as an antiviral agent but is about 40-fold less toxic to the host Vero cells. Thus the therapeutic index of F_3dThd has been improved by a factor of 10 by replacement of the 5'-hydroxyl with an amino moiety. However, relative to its antineoplastic activity against Sarcoma 180 cells, the parent compound F_3dThd is more than 100-fold superior to the 5'-amino analogue.

The 5'-azido analogue of F_3dThd is considerably more toxic to Sarcoma 180 cells than to Vero cells and as an antiviral agent, against the replication of herpes simplex virus type 1, is markedly less inhibitory than either F_3dThd or the 5'-amino analogue of F_3dThd.

The antiviral activity of the various fluorinated nucleoside analogues was determined. Vero cells were grown to confluency in 25-cm^2 Falcon flasks using Dulbecco's medium supplemented with 10% fetal calf serum. The cells were then infected with herpes simplex virus, type 1 (CL-101, obtained from Dr. Wilma Summers who originally received the virus from Dr. Saul Kit), at a MOI of 10. After a 1-h absorption period at 37 °C, the viral inoculum was removed and the flask washed once with phosphate buffered saline. Medium, either test compound free or containing the concentrations of test compound indicated in Table I, was then added. The infected cultures were incubated at 37 °C for 40 h and then frozen until virus titrations were performed. Virus was released by freezing and thawing the media-cell suspension one time. The cell lysates were diluted directly and the virus yield assayed by plaque formation on Vero cells. The number of plaque forming units (pfu) of virus in the drug-treated cultures relative to that found in the drug free condition is presented in Table I.

The cytotoxicity of the various test compounds on the uninfected host Vero cells was determined (Figure 1). Vero cells in Dulbecco's medium (2.5 ml) supplemented with 10% fetal calf serum were added to eight 25-cm^2 Falcon flasks at a concentration equivalent to 0.1 confluency. After incubation at 37° in 5% CO_2–95% air for 1 day, the test compound 1, 3, or 4, dissolved in 2.5 ml of the above growth medium, was added and two flasks were harvested immediately by decanting the medium, washing once with 5 ml of phosphate buffered saline, and then incubating at 37° for 15 min with 5-ml solution of trypsin (0.125%) and EDTA (0.02%). The cells dislodged from the flask by this latter procedure are generally in clumps and were dispersed by repeated forceful pipetting the suspension against the surface of the flask. To 1 ml of the well-dispersed cell suspension, 0.2 ml of trypan blue solution was added and the number of cells was counted using a haemocytometer. Each day for the next 3 days, two of the remaining flasks were harvested in the manner just described for determination of cell number.

The effect of the various fluorinated nucleoside analogues on neoplastic cells was determined by the following procedure and the results are shown in Figure 2. Murine Sarcoma 180 cells were maintained as suspension cultures in screw top tubes containing 5 ml of Fischer's medium supplemented with 10% horse serum at 37° in an atmosphere of 5% CO_2–95% air. Under these conditions the generation time for Sarcoma 180 cells is approximately 16 h. The various compounds were added at the indicated concentrations to Sarcoma 180 cells (~2 × 10^4 cells per ml). The increase in cell number, as determined in a Coulter counter, of the drug-free culture (control) as well as that of the cultures supplemented with the fluorinated nucleoside thymidine analogues was determined after 1, 2, and 3 days.

Chemistry. Tosylation of 1 with *p*-toluenesulfonyl chloride in dry pyridine at 4°[13] gave **2** which was converted to the 5'-azido derivative **3** by reacting with lithium azide in *N*,*N*-dimethylformamide at 85–90° for 2 h.[14] Hydrogenation[15] of compound **3** in ethanol–water (1:1, v/v) at room temperature and 35 psi of hydrogen pressure in the presence of 10% palladium on charcoal afforded 5-trifluoromethyl-5'-amino-2',5'-dideoxyuridine (**4**) in 86% yield. The reaction sequence is shown in Scheme I.

Experimental Section

Melting points were taken on a Thomas-Hoover Unimelt apparatus and are not corrected. A Perkin-Elmer 257 instrument was used to determine the ir spectra. The uv spectra were recorded on a Beckman-25 spectrophotometer. The TLC was performed on Eastman 6060 precoated silica gel plates with fluorescent indicator, using chloroform–ethanol (4:1, v/v) as the eluting solvent. The NMR spectra were recorded on a Bruker 270HX spectrometer. The elemental analyses were carried out by Galbraith Laboratories, Inc., Knoxville, Tenn.

5-Trifluoromethyl-5'-*O*-(*p*-tolylsulfonyl)-2'-deoxyuridine (2). To a solution of 5'-trifluoromethyl-2'-deoxyuridine (1, 1.00 g, 3.38 mmol) in 20 ml of dry pyridine at 0° was added *p*-

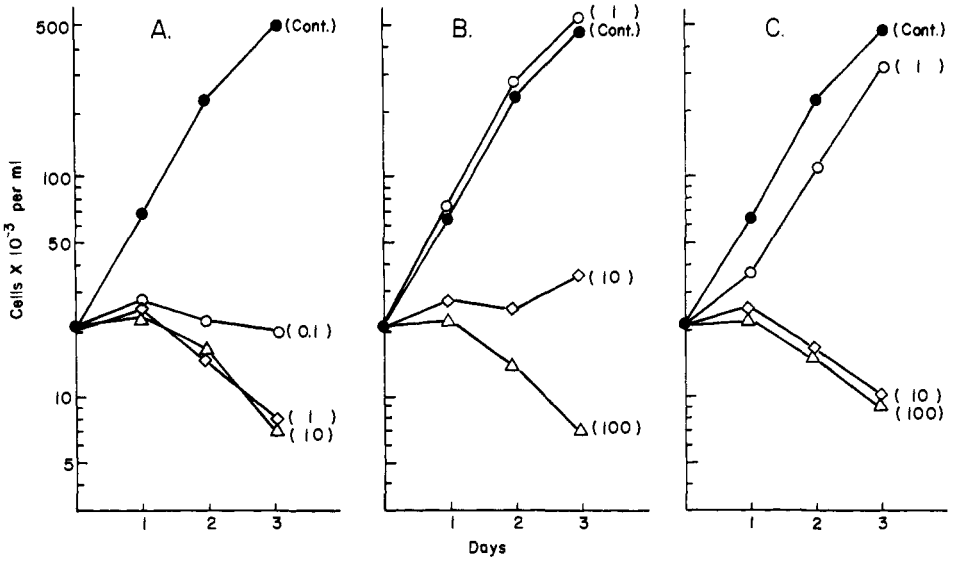
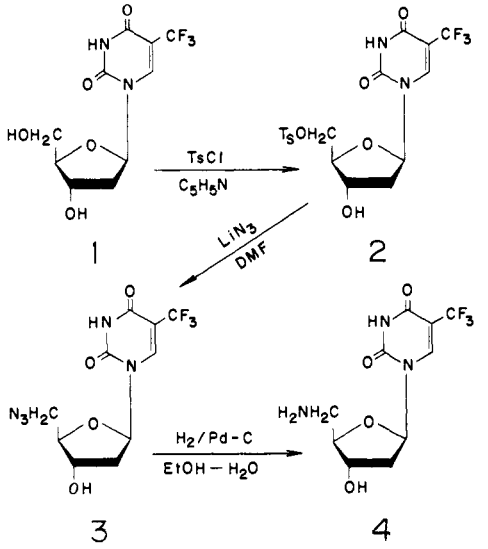


Figure 2. The effect of 5-trifluoromethyl-2′,5′-dideoxyuridine (A), 5-trifluoromethyl-5′-azido-2′,5′-dideoxyuridine (B), and 5-trifluoromethyl-5′-amino-2′,5′-dideoxyuridine (C) on the replication of murine Sarcoma 180 cells in culture. The solid circles represent the cells grown in the absence of test compound. The number within the parentheses represents the μM concentration of the appropriate fluorinated nucleoside analogue.

Scheme I



toluenesulfonyl chloride (0.95 g, 4.99 mmol). The reaction mixture was stirred at 0° for 1 h and then placed in a refrigerator (4°) for another 23 h. The solvent was removed at room temperature under reduced pressure. The gummy residue was triturated with ice-cooled water. The product crystallized out as a white solid which was collected immediately by filtration and washed with small amounts of ice-cooled ethanol and with a large quantity of ether. The product was dried over P_2O_5 at room temperature in vacuo and weighed 0.86 g (57%), mp 178–181°. Upon recrystallization from ethyl acetate–ethanol, the analytically pure sample was obtained: mp 180–181°. Anal. ($C_{17}H_{17}F_3N_2O_7S$) C, H, F, N, S.

5-Trifluoromethyl-5′-azido-2′,5′-dideoxyuridine (3). To a solution of **2** (0.86 g, 1.91 mmol) in 25 ml of dry DMF was added lithium azide (0.28 g, 5.73 mmol). The reaction mixture was heated to 85–90° in an oil bath for 2 h. The solvent was removed under diminished pressure (0.1 mmHg) at 40°. The residue was coevaporated several times with ethanol and triturated with 30 ml of ice-cooled water. The product crystallized out as fine needles which was collected by filtration, washed with ice-cooled water and ether, and recrystallized from ethanol–ether to give 0.36 g (59%) of analytically pure product: mp 199–201° dec; ir λ_{max} 4.76 μ (azide); TLC R_f 0.8; uv λ_{max}^{EtOH} 261 nm (ε 9370); uv λ_{min}^{EtOH} 230 nm; NMR (Me_2SO-d_6) δ 2.35 (m, 2, H-2′), 3.67 (m, 2, H-5′), 3.90 (m, 1, H-4′), 4.17 (m, 1, H-3′), 5.23 (br s, 1, C-3′ OH), 6.08 (t, 1, J = 5.86 Hz, H-1′), 8.25 (s, 1, H-6). Anal. ($C_{10}H_{10}F_3N_5O_4$) C, H, F, N.

5-Trifluoromethyl-5′-amino-2′,5′-dideoxyuridine (4). The 5′-azido derivative **3** (0.20 g, 0.62 mmol) was dissolved in 30 ml of EtOH–H_2O (1:1, v/v) and hydrogenated at 35 psi of hydrogen pressure for 3 h in the presence of 0.20 g of 10% palladium on charcoal. The catalyst was removed by filtration through a Celite pad and the filtrate was concentrated to one-tenth of its original volume. Ether was added to the solution and the product crystallized out as a white powder which was collected by filtration, washed with ether, and dried to give 0.16 g (86%) of **4**. This material was recrystallized from ethanol–ether to afford the analytically pure sample which effervesced above 170° and completely decomposed at 200°: uv $\lambda_{max}^{0.1\ N\ HCl}$ 260 nm (ε 6690); uv $\lambda_{min}^{0.1\ N\ HCl}$ 230 nm; uv $\lambda_{max}^{0.1\ N\ NaOH}$ 260 nm (ε 5090); uv $\lambda_{min}^{0.1\ N\ NaOH}$ 245 nm; uv $\lambda_{max}^{H_2O\ (pH\ 6.8)}$ 260 nm (ε 6480); uv $\lambda_{min}^{H_2O\ (pH\ 6.8)}$ 230 nm; NMR (Me_2SO-d_6) δ 2.20 (m, 2, H-2′), 3.39 (m, 2, H-5′), 3.82 (m, 1, H-4′), 4.14 (m, 1, H-3′), 6.08 (m, 4, H-1′, C-3′ OH, C-5′ NH_2), 8.55 (s, 1, H-6); TLC R_f 0.07. Anal. ($C_{10}H_{12}F_3N_3O_4$) C, H, F, N.

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Alkyl Streptozotocin Analogues with Improved Biological Activities

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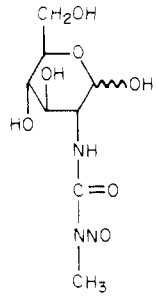
Alkyl 16α- and -β-glycosides of a series of N^3-alkyl homologues of streptozotocin were synthesized from glucosamine hydrochloride. These compounds, when tested against ascites Sarcoma 180, Ehrlich ascites carcinoma, or leukemia L1210, exhibited potent antitumor activities, and antibacterial and diabetogenic activities were eliminated. Furthermore, the acute toxicities of these compounds were lower than that of streptozotocin. The methyl, ethyl, *n*-propyl, and *n*-butyl glycosides of streptozotocin, whether α- or β-anomers, all showed higher antitumor activities than streptozotocin itself. The most active compound was found to be the methyl β-streptozotocin.

Streptozotocin is a broad-spectrum antibiotic[1] and has been shown by degradation[2,3] and synthesis[3-5] to have structure 1. It exhibits marked antileukemic activity[6] but suffers from its observed damaging effects on the β cells of the islets of Langerhans and its diabetogenic activity.[7,8]



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Recently, there have been reports of studies on isomers and analogues of 1 with results showing varying degrees of antileukemic, diabetogenic, and antibacterial activities.[1,9-11] Notable among these, analogues of 1 obtained by reacting either methyl α- and β-glycosides[9-11] or various other sugars with the methylnitrosoureido group[10,11] were studied. These analogues exhibited activity against Ehrlich ascites carcinoma and leukemia L1210 in mice.[10,11] Furthermore, in vitro studies with the methyl glycosides of 1 revealed that the cytotoxic activities of the β-anomers were the same as that of 1, whereas the activity of the α-anomers was twice that of 1 against cultures of leukemia L1210; yet neither derivative showed any diabetogenic activity.[10] These studies were followed by reports of the synthesis of six isomers of 1 containing the methyl α-glycosidic linkage[11,12] which were also found to exhibit activity against Ehrlich ascites carcinoma in mice,[12] in addition to being 20–40% more active against leukemia L1210 than 1.[11]

Independently, we were interested in the biological effects of alkyl streptozotocin analogues, their in vivo antitumor activities, and also their toxicities. Accordingly, we synthesized 16 compounds and studied their antitumor activities, toxicities, and diabetogenic and antibacterial activities. The melting points and the specific rotations were also recorded and compared with the known values for the methyl α- and β-streptozotocins[9,10] in order to explore their relationships.

Chemistry. All of the streptozotocin analogues were synthesized by the procedure depicted in Scheme I. The starting material, glucosamine hydrochloride (**2**), was allowed to react with carbobenzyloxy chloride in an aqueous solution of sodium carbonate according to the method of Chargaff et al.[14] to yield *N*-carbobenzyloxy-D-glucosamine (**3**). Employing Fischer's[15] procedure, **3** was methylated with anhydrous methanol with a catalytic amount of hydrogen chloride to obtain methyl *N*-carbobenzyloxy-D-glucosaminide. Similarly, methanol was replaced by ethanol, 1-propanol, or 1-butanol to give the corresponding alkyl *N*-carbobenzyloxy-D-glucosaminide. In the above reaction, it has been reported[16] that if the reaction were carried out at an elevated temperature, the α-anomer predominated, whereas if carried out below room temperature, the β-anomer predominated. This phenomenon was verified with the primary alcohols used in our reaction. The products from the above reactions were purified by column chromatography to separate the anomers; and the alkyl *N*-carbobenzyloxy-α- and -β-D-glucosaminides (**4a–7a** = α-anomers; **4b–7b** = β-anomers), identified by their optical rotation values, were obtained. After catalytic decarbobenzoxylation, the products were allowed to react, according to the method of Suami et al.,[9] with various alkyl isocyanates to obtain the alkyl 2-deoxy-2-(3-alkylureido)-α- and -β-D-glucopyranosides (**8a**, **10a–16a** = α-anomers; **8b–15b** = β-anomers). These were then allowed to react with a slight excess of sodium nitrite in dilute acetic acid solution at 0–10 °C. After treatment with a cation exchanger (H^+ type) to remove sodium ions, the alkyl 2-deoxy-2-(3-alkyl-3-nitrosoureido)-α- and -β-D-glucopyranosides (**17a**, **19a–25a** = α-anomers; **17b–24b** = β-anomers) were obtained by concentration of the resultant solutions. The products could be stored in a desiccator for 20–30 months without any apparent signs of decomposition.

As shown in Tables I–III, respectively, the β-anomers of the same compound group exhibited higher melting points or decomposition temperatures than the α-anomers. That is to say, **4b–7b** had melting points 20–32 °C higher than **4a–7a** among the alkyl *N*-carbobenzyloxy-α- and -β-D-glucosaminides (Table I); **8b–15b** had melting points 9–50 °C higher than **8a** and **10a–16a** among the alkyl 2-deoxy-2-(3-alkylureido)-α- and -β-D-glucopyranosides (Table II); and compounds **17b–24b** were likewise 8–55 °C higher in their decomposition temperatures than **17a** and **19a–25a** among the alkyl 2-deoxy-2-(3-alkyl-3-nitrosoureido)-α- and -β-D-glucopyranosides (Table III).

Furthermore, as can be seen in Tables I–III, compared with each alkyl derivative in Table III, the corresponding derivative in Table II invariably exhibited 38–87 °C higher melting points or decomposition temperatures. The