Design and Synthesis of 3′- and 5′-*O*-(3-Benzenesulfonylfuroxan-4-yl)-2′-deoxyuridines: Biological Evaluation as Hybrid Nitric Oxide Donor−Nucleoside Anticancer Agents

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Received October 24, 2003

A group of 3′-*O*- and 5′-*O*-(3-benzenesulfonylfuroxan-4-yl)-2′-deoxyuridines possessing a variety of substituents (H, Me, I, F, CF_3) at the C-5 position of the nucleoside moiety were synthesized for evaluation as hybrid anticancer agents that have the ability to simultaneously release cytotoxic nitric oxide (·NO). Incubation of these nitric oxide donor−nucleoside conjugates in the presence of 18 mM L-cysteine released a high percentage of ·NO (21−48% at 1 h; 37−86% at 16 h). The release of ·NO in the absence of the thiol cofactor was negligible. These hybrid ·NO donor−nucleosides exhibited high cellular toxicity ($CC_{50} = 10^{-6}-10^{-8}$ M range) against a battery of tumor cell lines (143B-LTK, 143B, EMT-6, KBALB-STK, and KBALB) and normal human fibroblasts (Hs578Bst). No differences in cytotoxicity between nontransfected (143B, KBALB) and the corresponding transfected (143B-LTK, KBALB-STK) cancer cell lines possessing the herpes simplex virus type 1 (HSV-1) thymidine kinase gene (TK^+) were observed, indicating that expression of the viral TK enzyme did not provide a gene therapeutic effect.

Introduction

Nitric oxide (·NO)is a mediator of many physiological and pathological processes. Its in vivo synthesis is catalyzed by neuronal, inducible, and endothelial isoforms of nitric oxide synthase (NOS) using L-arginine as substrate.[1] The biological actions of ·NO are paradoxical because it serves important regulatory functions in addition to its potentially useful cytotoxic effects.[2−4] ·NO also possesses vasoactive[5,6] and neurotransmitter[7,8] properties. The production of ·NO by macrophages, Kupfer cells, natural killer T-cells, and endothelial cells can induce cytotoxicity against a variety of tumors.[9,10] It is well documented that the 1,2,5-oxadiazole *N*-oxide ring (1,2,5-oxadiazole 2-oxide, furoxan ring) and derivatives thereof release ·NO in the presence of thiol cofactors.[11] Mechanisms for the release of ·NO from the 1,2,5-oxadiazole *N*-oxide ring system have been postulated,[12] but the structure of the resulting product(s) is still unclear. A variety of 1,2,5-oxadiazole *N*-oxides have been evaluated as vasodilators to treat cardiovascular disease.[13] Recent studies have shown that certain 1,2,5-oxadiazole *N*-oxides possessing a C-3 moiety with a good leaving group that can be displaced by biological nucleophiles exhibit in vitro cytotoxic activity.[14,15] It was therefore of interest to attach the 3-benzenesulfonylfuroxan-4-yl ·NO donor moiety to the 3′-*O*- and 5′-*O*-positions of 2′-deoxyuridine to determine whether this new type of hybrid ·NO donor−nucleoside dual prodrug combination would result in a synergestic, or additive, cytotoxic effect. We now report the synthesis, ·NO release data, and in vitro cytotoxicity for a group of 3′-*O*- (**4a**−**e**) and 5′-*O*-(3-benzenesulfonylfuroxan-4-yl)-2′-deoxyuridines (**7a**−**d**) possessing a variety of 2′-deoxyuridine C-5 substituents (H, Me, I, F, CF_3).

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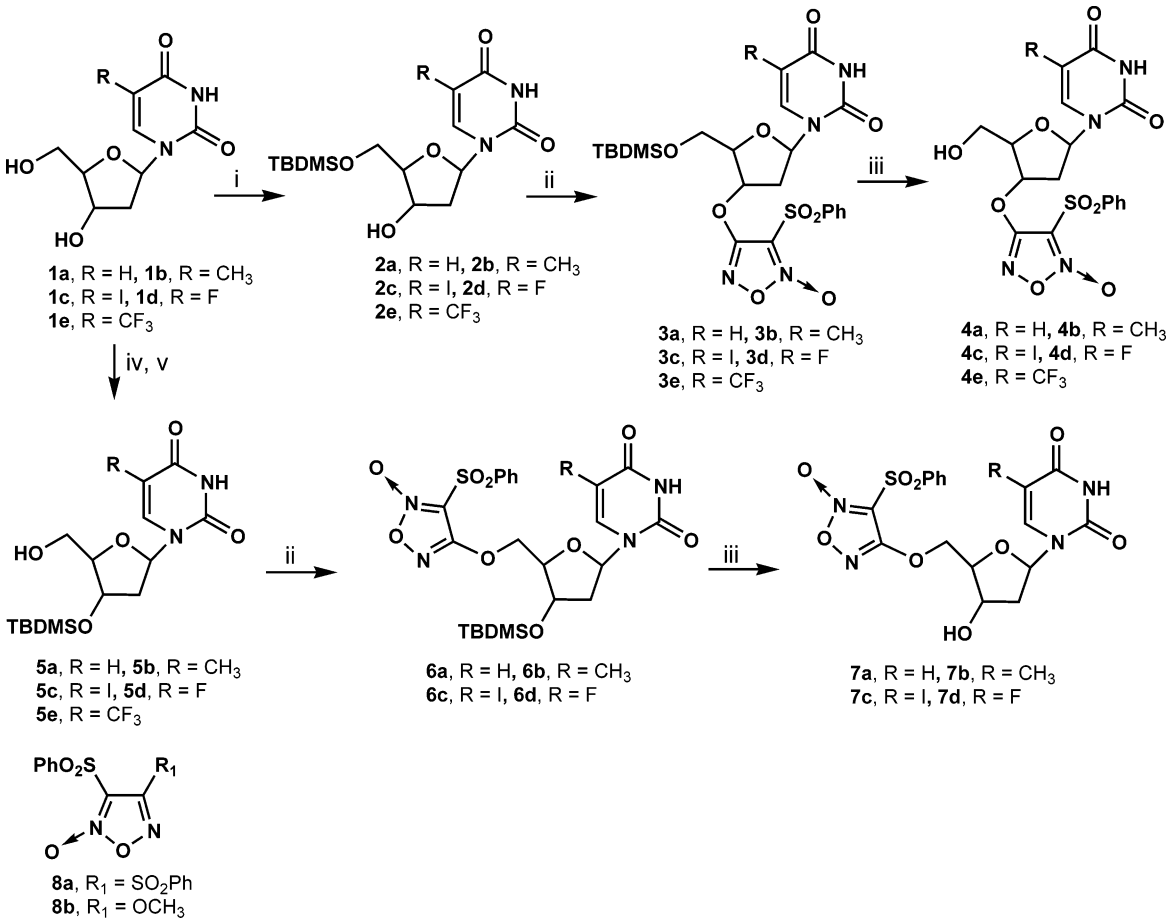
Chemistry

Selective monoprotection of the 5-substituted 2′-deoxyuridine derivatives (**1a**−**e**, R = H, Me, I, F, CF_3) by reaction with 1 equiv of *tert*-butyldimethylsilyl chloride (TBDMSCl) and 4-(dimethylamino)pyridine (DMAP) in DMF afforded the respective 5′-*O*-*tert*-butyldimethylsilyl derivatives (**2a**−**e**) in 82−93% yields (see Scheme 1).[16,17] Coupling of **2a**−**e** with 3,4-bis-(benzenesulfonyl)furoxan (**8a**)[18] in the presence of 50% aqueous NaOH in THF[11] furnished the corresponding 3′-*O*-(3-benzenesulfonylfuroxan-4-yl) derivative (**3a**−**e**) in 79−92% yield. Subsequent removal of the 5′-*O*-TBDMS protecting group by treatment with 1 N HCl in THF gave the respective target 3′-*O*-(3-benzenesulfonylfuroxan-4-yl)-5-substituted-2′-deoxyuridines (**4a**−**e**) in 88−94% chemical yields.

It was anticipated that application of a similar methodology for the synthesis of the regioisomeric 5′-*O*-(3-benzenesulfonylfuroxan-4-yl)-2′-deoxyuridines (**7a**−**d**) would require selective protection of the 3′-hydroxyl group. In this regard, after various trials using different protection−deprotection strategies, it was found that the best results were obtained by the protection of both the 3′- and 5′-hydroxyl groups of the parent nucleosides (**1a**−**e**) as the TBDMS ethers. Selective cleavage of the 5′-*O*-TBDMSO ether protecting group under mild acidic conditions (80% acetic acid/THF, 4:1, v/v) with a prolonged reaction time (up to 3 days) afforded the respective monoprotected 3′-*O*-TBDMSO ether (**5a**−**e**) in 54−70% yield. The 3′-*O*-TBDMSO ethers (**5a**−**d**; R = H, Me, I, F) were subsequently converted to the respective 5′-*O*-(3-benzenesulfonylfuroxan-4-yl) derivatives (**7a**−**d**) as illustrated in Scheme 1. In contrast, a similar attempt to convert the 5-trifluoromethyl compound (**5e**) to the corresponding 5′-*O*-(3-benzensulfonylfuroxan-4-yl) derivative (**7e**) was unsuccessful because of product decomposition under these reaction conditions.

10.1021/jm030544m CCC: $27.50
Published on Web 02/24/2004

Scheme 1[a]



[a] Reagents and conditions: (i) 1 equiv of TBDMSCl, 3 equiv of imidazole, 0.1 equiv of DMAP in DMF, 25 °C, 1 h; (ii) 1.5 equiv of **8a** in THF, 0 °C, aqueous 50% NaOH solution, 1 h; (iii) 1 N HCl, THF, 25 °C, 3 h; (iv) 2.1 equiv of TBDMSCl, 5 equiv of imidazole, 0.3 equiv of DMAP in DMF, 25 °C, 1 h; (v) AcOH/THF (4:1, v/v), 60 °C, 3 days.

Results and Discussion

The unstable nitric oxide (·NO) radical is readily oxidized to the nitrosonium cation (NO^+), which is moderately stable in aqueous solutions but highly reactive with nucleophiles or other nitrogen oxides. Under physiological conditions, ·NO is readily oxidized to nitrite and nitrate or it is trapped by thiols as an *S*-nitroso adduct. Under aerobic conditions, these reactive nitrogen oxides can be trapped by various amines, in particular by aromatic amines to form diazonium salts or by aromatic 1,2-diamines to form benzotriazoles. The Griess reagent[19,20] provides a simple and well-characterized colorimetric assay for nitrites, and nitrates that have been reduced to nitrites, with a detection limit of about 100 nM. Nitrites react with sulfanilic acid in acidic solution to form an intermediate diazonium salt that couples to *N*-(1-naphthyl)ethylenediamine to yield a purple azo derivative that can be monitored by absorbance at 548 nm.

The percentages of ·NO released upon incubation of the 3′-*O*- (**4a**−**e**) and 5′-*O*-(3-benzenesulfonylfuroxan-4-yl)-2′-deoxyuridines (**7a**−**d**) in the absence and presence of 18 mM L-cysteine (thiol cofactor)[21−25] are listed in Table 1. In this regard, incubation in aqueous phosphate buffer solution generally released negligible amounts of ·NO in the absence of L-cysteine (0−9.9% at 1−16 h). In contrast, in the presence of 18 mM L-cysteine as thiol cofactor, the percentage of ·NO released was high (21−48% at 1 h and 37−86% at 16 h) relative to the reference compound isosorbide dini-

Table 1. In Vitro Percent Nitric Oxide Release for 3′-*O*-(3-Benzenesulfonylfuroxan-4-yl)-5-substituted-2′-deoxyuridines (**4a-e**), 5′-*O*-(3-Benzenesulfonylfuroxan-4-yl)-5-substituted-2′-deoxyuridines (**7a**−**d**), 3,4-Bis(benzenesulfonyl)furoxan (**8a**), and 3-Benzenesulfonyl-4-methoxyfuroxan (**8b**)

	% NO release[a] (PBS)		% NO release[b] (PBS + 18 mM L-cysteine)	
compd	1 h	16 h	1 h	16 h
4a	0.9 ± 0.5	0.2 ± 0.1	45.6 ± 1.2	85.8 ± 0.6
4b	1.4 ± 0.1	0.4 ± 0.2	39.5 ± 0.2	77.3 ± 1.5
4c	1.2 ± 0.4	0.4 ± 0.3	39.8 ± 0.4	74.8 ± 0.7
4d	0.4 ± 0.1	0.2 ± 0.2	41.5 ± 1.9	73.9 ± 0.5
4e	0.3 ± 0.2	0.4 ± 0.1	48.3 ± 1.2	78.5 ± 1.2
7a	1.5 ± 0.4	0.2 ± 0.1	46.0 ± 0.6	77.9 ± 0.5
7b	2.2 ± 0.2	2.2 ± 0.1	20.9 ± 0.8	36.8 ± 1.4
7c	9.8 ± 1.2	9.9 ± 0.1	28.3 ± 0.5	71.9 ± 0.4
7d	0.8 ± 0.2	0.7 ± 0.1	41.6 ± 1.1	68.7 ± 1.9
8a	0.2 ± 0.1	0.4 ± 0.1	22.7 ± 0.4	38.8 ± 1.6
8b	1.1 ± 0.2	1.0 ± 0.4	12.3 ± 0.7	44.8 ± 0.5
ISBN[c]	3.5 ± 0.2	24.0 ± 0.2	1.9 ± 0.1	2.6 ± 0.6

[a] The percent nitric oxide released from the test compound was determined as the percent of nitrite (NO_2^-) produced in phosphate-buffered saline (PBS) as quantitated using the Griess reagent (±SD, $n = 3$). [b] The percent nitric oxide released from the test compound was determined as the percent of nitrite (NO_2^-) produced in the present of L-cysteine (18 mM) as quantitated using the Griess reagent. [c] ISDN = isosorbide dinitrate (ISDN possesses two ONO_2 groups that may release ·NO, whereas compounds **4** and **7** possess only one ONO_2 group that may release ·NO).

Table 2. In Vitro Cell Cytotoxicity for 3′-*O*-(3-Benzenesulfonylfuroxan-4-yl)-5-substituted-2′-deoxyuridines (**4a**−**e**), 5′-*O*-(3-Benzenesulfonylfuroxan-4-yl)-5-substituted-2′-deoxyuridines (**7a**−**d**), 3,4-Bis(benzenesulfonyl)furoxan (**8a**), and 3-Benzenesulfonyl-4-methoxyfuroxan (**8b**)

	cellular toxicity (CC_{50}, M) toward various cell lines[a]					
compd	KBALB[b]	KBALB−STK[c]	143B[d]	143B-LTK[c]	EMT-6[e]	Hs578Bst[f]
4a	7.9×10^{-7}	1.7×10^{-6}	9.6×10^{-7}	2.9×10^{-6}	8.0×10^{-8}	4.8×10^{-6}
4b	5.6×10^{-7}	1.0×10^{-6}	7.2×10^{-7}	8.8×10^{-7}	2.4×10^{-6}	2.0×10^{-6}
4c	3.8×10^{-7}	0.4×10^{-6}	5.6×10^{-7}	1.3×10^{-6}	8.0×10^{-8}	0.8×10^{-6}
4d	2.0×10^{-6}	8.4×10^{-7}	2.4×10^{-7}	8.4×10^{-7}	2.0×10^{-6}	4.4×10^{-6}
4e	3.2×10^{-6}	2.0×10^{-6}	5.2×10^{-7}	4.8×10^{-7}	8.8×10^{-7}	4.4×10^{-6}
7a	4.6×10^{-6}	5.4×10^{-6}	3.3×10^{-6}	6.3×10^{-6}	7.5×10^{-7}	4.8×10^{-6}
7b	2.8×10^{-6}	4.8×10^{-6}	3.6×10^{-6}	5.6×10^{-6}	2.0×10^{-6}	6.8×10^{-6}
7c	9.2×10^{-7}	9.2×10^{-7}	5.4×10^{-7}	7.1×10^{-7}	1.0×10^{-6}	8.0×10^{-7}
7d	4.8×10^{-8}	6.0×10^{-8}	3.2×10^{-6}	2.0×10^{-6}	6.0×10^{-8}	7.6×10^{-8}
8a	5.2×10^{-7}	6.0×10^{-7}	4.0×10^{-8}	2.8×10^{-8}	1.0×10^{-7}	1.0×10^{-7}
8b	4.8×10^{-7}	4.4×10^{-7}	7.0×10^{-8}	3.2×10^{-7}	5.6×10^{-7}	6.8×10^{-7}
IUDR[g]	9.7×10^{-5}	1.0×10^{-5}	7.0×10^{-3}	7.4×10^{-3}	3.8×10^{-4}	
FUDR[h]	6.0×10^{-11}	8.8×10^{-11}	9.0×10^{-5}	1.0×10^{-4}	9.0×10^{-12}	
thymidine	9.5×10^{-5}	1.0×10^{-4}			1.3×10^{-4}	
ISDN[i]	9.7×10^{-4}	6.5×10^{-4}	6.0×10^{-4}	7.0×10^{-4}		

[a] The molar concentration of the test compound that killed 50% of the cells (or 50% cell survival) upon incubation for 3−5 days at 37 °C in a humidified atmosphere of 95% air and 5% CO_2 (mean value, $n = 6$) was determined using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay. [b] Transformed fibroblast sarcoma cell line. [c] These cells were transfected by, and expressed, the herpes simplex virus type 1 thymidine kinase (HSV-1 TK) gene. [d] Human osteosarcoma cell line. [e] Mouse mammary carcinoma cell line. [f] Human fibroblast cell lines. [g] IUDR = 5-iodo-2′-deoxyuridine. [h] FUDR = 5-fluoro-2′-deoxyuridine. [i] Isosorbide dinitrate.

trate (1.9% and 2.6% release at 1 and 16 h) that possesses two ONO_2 ·NO donor moieties. These ·NO release data are consistent with reports that buffers containing reduced thiols such as L-cysteine or glutathione, which serve as a source of thiols equivalent to the mercapto groups present in plasma, are required for the release of ·NO from a furoxan moiety.[6,22]

Compounds **4a**−**e** and **7a**−**d** (**a**, R = H; **b**, R = Me; **c**, R = I; **d**, R = F; **e**, R = CF_3) and the reference compounds IUDR, FUDR, and thymidine were evaluated for their tumor cell cytotoxicity against a battery of cancer cell lines (KBALB, KBALB-STK, 143B, 143B-LTK, EMT-6) using the MTT cytotoxicity assay[26] (Table 2). This group of furoxanyl deoxynucleosides generally exhibited more potent cytotoxicities ($CC_{50} = 10^{-6}$−10^{-8} M range) than the reference compounds IUDR ($CC_{50} = 10^{-3}$−10^{-5} M range), FUDR ($CC_{50} = 10^{-4}$−10^{-12} M range), and thymidine ($CC_{50} = 10^{-4}$−10^{-5} M range). The only exceptions to this general observation was FUDR, which exhibited a greater cytotoxicity against KBALB, KBALB-STK, and EMT-6 cells than the furoxanyl compounds **4** and **7**. It is anticipated that the potent cytotoxicities exhibited by the 5-substituted compounds **4a** (R = H) and **4b** (R = Me) are predominantly, or exclusively, due to the furoxanyl moiety because the 2′-deoxyuridine (**4a**) and thymidine (**4b**) nucleoside moieties are expected to make little, or no, contribution to the overall cytotoxic effect. The observation that compounds **4a**−**e** and **7a**−**d** exhibited similar cytotoxicity against nontransfected (KBALB, 143B) and the corresponding transfected (KBALB-STK, 143B-LTK) cancer cell lines possessing the herpes simplex virus type 1 (HSV-1) thymidine kinase gene (TK^+) indicates that expression of the viral TK enzyme did not provide a gene therapeutic effect.

The high amount of ·NO release, and potent cytotoxicities exhibited, by these furoxanyl deoxynucleosides prompted us to further investigate the role of the furoxan moiety with respect to the cytotoxic effect. It was found that 3,4-bis(benzenesulfonyl)furoxan (**8a**) released ·NO readily in the presence of 18 mM L-cysteine (23% in 1 h; 39% in 16 h) and that **8a** was highly cytotoxic ($CC_{50} = 10^{-7}$−10^{-8} M range). Similarly, 3-benzenesulfonyl-4-methoxyfuroxan (**8b**), which is considered to be a very close structural mimic of the furoxany-4-yl moiety present in compounds **4a**−**e** and **7a**−**d**, was also highly cytotoxic ($CC_{50} = 10^{-7}$−10^{-8} M range). A plausible explanation for these results is that the high cytotoxicity exhibited by the hybrid nucleoside−·NO donor compounds **4** and **7** is due predominantly to the furoxan moiety, with a minimal synergistic or additive contribution from the nucleoside component. It is expected that the furoxans **8a** and **8b** exert their cytotoxic effect by an alternative mechanism such as DNA alkylation, since **8a**,**b** cannot serve as nucleotide substrates for DNA polymerase catalyzed incorporation into DNA. In this regard, covalent alkylation by the N^7- or O^6-atom of a guanine base in DNA could result in the covalent attachment of DNA to a furoxan-4-yl ring carbon by nucleophilic displacement of the 3-benzenesulfonyl moiety present in compounds **4a**−**e**, **7a**−**d**, and **8a**,**b**. To obtain further evidence to support this postulate, attempts were made to isolate the byproduct(s) produced after ·NO release. Accordingly, when compound **4a** was incubated with 18 mM L-cysteine in phosphate buffer for 16 h, thin-layer chromatography (TLC) showed complete disappearance of **4a** and the appearance of a more polar compound with a different TLC R_f value than the parent nucleoside 2′-deoxyuridine. Several attempts to isolate this incubation product were unsuccessful because a progressive decomposition occurred during both the isolation and attempts to purify the product. Irrespective of the mechanism(s) by which ·NO is released from the furoxanyl ring system, the carbon component of the furoxan ring system would be retained at the C-3′ (**4**) or C-5′ (**7**) position of the nucleoside. The product(s) produced after elimination of ·NO likely exhibits negligible cytotoxic activity because 3-benzenesulfonyl-4-methoxyfuroxan (**8b**) exhibits cytotoxic activity comparable to that of the nucleoside analogues **4** and **7**. The 3′-*O*- (**4a**−**d**) and 5′-*O*-(3-benzenesulfonylfuroxan-4-yl) (**7a**−**d**) compounds are

also cytotoxic (CC_{50} = 10^{-6}–10^{-8} M range) against human fibroblast cells (Hs578Bst cell line). This observation indicates that any drug design study incorporating a 3-benzenesulfonylfuroxan moiety should examine their toxicity against normal cells.

Conclusions

A group of 3′- (**4**) and 5′-*O*-(3-benzenesulfonylfuroxan-4-yl)-2′-deoxyuridines (**7**) were designed for evaluation as hybrid nucleoside–•NO donor conjugates. Biological evaluation showed that these compounds are effective •NO donor agents in the presence of 18 mM L-cysteine and exhibit cytotoxic activity against a battery of cancer cell lines (KBALB, KBALB-STK, 143B, 143B-LTK, EMT-6) as well as a contraindicated cytotoxic effect against normal nonmalignant human fibroblast cells. The observation that the non-nucleoside •NO donor (**8b**) exhibits cytotoxicity similar to that of the 3′-*O*- and 5′-*O*-furoxan-4-yl deoxynucleosides (**4** and **7**) suggests that cytoxicity is due predominantly to the effect of the 3-benzenesulfonylfuroxan-4-yl moiety that may alkylate DNA.

Experimental Section

General. Melting points were determined with a Thomas-Hoover capillary apparatus and are uncorrected. Nuclear magnetic resonance (^{1}H NMR, ^{13}C NMR) spectra were recorded on a Bruker AM-300 spectrometer. Proton chemical shifts (δ) are given relative to internal TMS (δ 0), and the assignment of exchangeable protons (N*H*, O*H*) was confirmed by addition of D_2O. ^{13}C NMR spectra were acquired using the *J* modulated spin–echo technique where methyl and methine carbon resonances appear as positive peaks and methylene and quaternary carbon resonances appear as negative peaks. Carbon chemical shifts (δ) are given relative to $CDCl_3$ (δ 77). Elemental analyses were performed by the MicroAnalysis Service Laboratory, Department of Chemistry, University of Alberta, and the results were within ±0.4% of theoretical values for all elements listed. Silica gel 60A (Silicycle Co., 230–400 mesh) was used for all flash column chromatography separations. 3,4-Bis(benzenesulfonyl)furoxan (**8a**)[18] and 3-benzenesulfonyl-4-methoxyfuroxan (**8b**)[11] were prepared according to literature procedures. All other reagents were purchased from Aldrich Chemical (Milwaukee, WI).

General Method for the Preparation of 5′-*O*-(*tert*-Butyldimethylsilyl)-5-substituted-2′-deoxyuridines (2a–e). To a stirred solution of a compound selected from the group **1a**–**e** (1 mmol), imidazole (206 mg, 3 mmol), and DMAP (12.2 mg, 0.1 mmol) in DMF (5 mL) was added TBDMSCl (158 mg, 1.05 mmol) at 0 °C, and the mixture was allowed to warm to room temperature and stirred for 1 h. The reaction mixture was quenched using a saturated aqueous solution of ammonium chloride (10 mL) and extracted with ethyl acetate (3 × 5 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated in vacuo to give an oil that was purified by flash chromatography using EtOAc/hexane (3:1, v/v) as eluant to give the respective title product listed below.

5′-*O*-(*tert*-Butyldimethylsilyl)-2′-deoxyuridine (2a): white solid; yield, 90%; mp 127–129 °C; ^{1}H NMR ($CDCl_3$) δ 9.70 (br s, 1H, N*H*), 7.94 (d, *J* = 8.2 Hz, 1H, H-6), 6.37 (t, *J* = 6.4 Hz, 1H, H-1′), 5.7 (d, *J* = 8.2 Hz, 1H, H-5), 4.46–4.50 (m, 1H, H-3′), 4.03–4.10 (m, 1H, H-4′), 3.89 (dd, *J* = 11.9, 1.8 Hz, 1H, H-5′), 3.84 (dd, *J* = 11.9, 1.8 Hz, 1H, H-5′), 2.38–2.48 and 2.06–2.16 (two m, 1H each, H-2′), 0.90 (s, 9H, *t-Bu*), 0.09 [s, 6H, Si(CH_3)$_2$]; ^{13}C NMR ($CDCl_3$) δ 163.57 (C-4 *C*=O), 150.45 (C-2 *C*=O), 140.26 (C-6), 102.23 (C-5), 87.43 (C-4′), 85.37 (C-1′), 71.94 (C-3′), 63.30 (C-5′), 41.50 (C-2′), 25.88 [C(CH_3)$_3$], 18.34 [*C*(CH_3)$_3$], −5.50 [d, $J_{Si,CH3}$ = 6.6 Hz, Si(CH_3)$_2$]. Anal. ($C_{15}H_{26}N_2O_5Si$) C, H, N.

5′-*O*-(*tert*-Butyldimethylsilyl)-2′-deoxythymidine (2b): white solid; yield, 84%; mp 198–200 °C; ^{1}H NMR ($CDCl_3$) δ 9.70 (br s, 1H, N*H*), 7.54 (s, 1H, H-6), 6.43 (dd, *J* = 8.1, 5.5 Hz, 1H, H-1′), 4.43–4.45 (m, 1H, H-3′), 4.01 (d, *J* = 2.2 Hz, 1H, H-4′), 3.84 (dd, *J* = 11.4, 2.2 Hz, 1H, H-5′), 3.79 (dd, *J* = 11.4, 2.2 Hz, 1H, H-5′), 2.34–2.44 and 2.06–2.16 (two m, 1H each, H-2′), 1.85 (s, 3H, *CH*$_3$), 0.86 (s, 9H, *t-Bu*), 0.11 (s, 3H, Si*CH*$_3$), 0.10 (s, 3H, Si*CH*$_3$); ^{13}C NMR ($CDCl_3$) δ 163.93 (C-4 C=O), 150.59 (C-2 C=O), 135.44 (C-6), 110.90 (C-5), 87.36 (C-4′), 85.06 (C-1′), 72.48 (C-3′), 63.62 (C-5′), 41.12 (C-2′), 25.94 [C(CH_3)$_3$], 18.36 [*C*(CH_3)$_3$], 12.53 (C-5 *C*H$_3$), −5.38 [d, $J_{Si,CH3}$ = 6.6 Hz, Si(*C*H$_3$)$_2$]. Anal. ($C_{16}H_{28}N_2O_5Si$) C, H, N.

5′-*O*-(*tert*-Butyldimethylsilyl)-5-iodo-2′-deoxyuridine (2c): white solid; yield, 89%; mp 218–219 °C; ^{1}H NMR ($CDCl_3$) δ 10.66 (s, 1H, N*H*), 8.02 (s, 1H, H-5), 6.22 (dd, *J* = 8.1, 5.9 Hz, 1H, H-1′), 4.45 (br d, *J* = 3.3 Hz, 1H, O*H*), 4.20–4.30 (m, 1H, H-3′), 3.95–3.99 (m, 1H, H-4′), 3.79 (dd, *J* = 11.4, 2.2 Hz, 1H, H-5′), 3.70 (dd, *J* = 11.4, 2.2 Hz, 1H, H-5′), 2.24–2.34 and 1.88–2.00 (two m, 1H each, H-2′), 0.84 (s, 9H, *t-Bu*), 0.06 (s, 3H, Si*CH*$_3$), 0.04 (s, 3H, Si*CH*$_3$); ^{13}C NMR ($CDCl_3$) δ 160.28 (C-4 C=O), 149.89 (C-2 C=O), 144.03 (C-6), 87.68 (C-4′), 85.56 (C-1′), 71.70 (C-3′), 68.33 (C-5), 63.37 (C-5′), 41.47 (C-2′), 25.96 [C(*C*H$_3$)$_3$], 18.26 [*C*(CH_3)$_3$], −5.35 [d, $J_{Si,CH3}$ = 10.9 Hz, Si-(*C*H$_3$)$_2$]. Anal. ($C_{15}H_{25}IN_2O_5Si$) C, H, N.

5′-*O*-(*tert*-Butyldimethylsilyl)-5-fluoro-2′-deoxyuridine (2d): white solid; yield, 82%; mp 201–202 °C; ^{1}H NMR (CD_3OD) δ 8.10 (d, *J* = 6.2 Hz, 1H, H-6), 7.8 (br s, 1H, N*H*), 6.37 (dd, *J* = 7.2, 5.9 Hz, 1H, H-1′), 4.43–4.58 (m, 1H, H-3′), 4.08–4.12 (m, 1H, H-4′), 3.93 (dd, *J* = 11.5, 1.7 Hz, 1H, H-5′), 3.82 (dd, *J* = 11.5, 1.7 Hz, 1H, H-5′), 2.40–2.50 and 2.00–2.16 (two m, 1H each, H-2′), 0.90 (s, 9H, *t-Bu*), 0.11 (s, 3H, Si*CH*$_3$), 0.10 (s, 3H, Si*CH*$_3$); ^{13}C NMR (CD_3OD) δ 157.47 (d, J_{CCF} = 26.4 Hz, C-4 *C*=O), 149.34 (C-2 *C*=O), 140.53 (d, J_{CF} = 236.2 Hz, C-5), 124.43 (d, J_{CCF} = 39.5 Hz, C-6), 87.87 (C-4′), 85.73 (C-1′), 72.03 (C-3′), 63.48 (C-5′), 41.32 (C-2′), 25.86 [C(*C*H$_3$)$_3$], 18.35 [*C*(CH_3)$_3$], −5.60 [Si(*C*H$_3$)$_2$]. Anal. ($C_{15}H_{25}FN_2O_5Si$) C, H, N.

5′-*O*-(*tert*-Butyldimethylsilyl)-5-trifluoromethyl-2′-deoxyuridine (2e): white solid; yield, 93%; mp 105–107 °C; ^{1}H NMR (CD_3OD) δ 8.07 (s, 1H, H-6), 6.14 (dd, *J* = 7.3, 5.9 Hz, 1H, H-1′), 4.31–4.37 (m, 1H, H-3′), 4.03–4.09 (m, 1H, H-4′), 3.90 (dd, *J* = 11.7, 2.2 Hz, 1H, H-5′), 3.84 (dd, *J* = 11.7, 2.2 Hz, 1H, H-5′), 2.43 (ddd, *J* = 13.5, 5.9, 2.2 Hz, 1H, H-2′), 2.13 (ddd, *J* = 13.5, 7.3, 2.2 Hz, 1H, H-2′), 0.89 (s, 9H, *t-Bu*), 0.09 [s, 6H, Si(*CH*$_3$)$_2$]; ^{13}C NMR (CD_3OD) δ 160.94 (C-4 *C*=O), 151.03 (C-2 *C*=O), 142.55 (q, J_{CCCF} = 3.3 Hz, C-6), 123.78 (q, J_{CF} = 268.1 Hz, *C*F$_3$), 105.09 (q, J_{CCF} = 33.0 Hz, C-5), 89.82 (C-4′), 88.31 (C-1′), 72.84 (C-3′), 64.60 (C-5′), 42.62 (C-2′), 26.44 [C(*C*H$_3$)$_3$], 19.28 [*C*(CH_3)$_3$], −5.42 (d, $J_{Si,CH3}$ = 5.5 Hz, Si(*C*H$_3$)$_2$]. Anal. ($C_{16}H_{25}F_3N_2O_5Si$) C, H, N.

General Method for the Preparation of 3′-*O*-(3-Benzenesulfonylfuroxan-4-yl)-5′-*O*-(*tert*-butyldimethylsilyl)-5-substituted-2′-deoxyuridines (3a–e). To a stirred solution of the compound selected from the group **2a**–**e** (1 mmol) and **8a** (550 mg, 1.5 mmol) in THF (5 mL) at 0 °C was added a 50% aqueous sodium hydroxide solution (3 mmol). The mixture was stirred for 1 h at 25 °C, diluted with water (5 mL), and extracted with ethyl acetate (3 × 5 mL). The combined organic extracts were dried (Na_2SO_4), and the solvent was removed in vacuo to give an oil that was purified by flash chromatography using EtOAc/hexane (1:1, v/v) as eluant to give the respective title product listed below.

3′-*O*-(3-Benzenesulfonylfuroxan-4-yl)-5′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyuridine (3a): white solid; yield, 82%; mp 188–190 °C; ^{1}H NMR ($CDCl_3$) δ 8.90 (br s, 1H, N*H*), 8.08 (d, *J* = 7.7 Hz, 2H, *o*-Ph), 7.88 (d, *J* = 8.1 Hz, 1H, H-6), 7.80 (t, *J* = 7.7 Hz, 1H, *p*-Ph), 7.68 (t, *J* = 7.7 Hz, 2H, *m*-Ph), 6.42 (dd, *J* = 8.8, 5.5 Hz, 1H, H-1′), 5.74 (d, *J* = 8.1 Hz, 1H, H-5), 5.35–5.41 (m, 1H, H-3′), 4.40–4.43 (m, 1H, H-4′), 3.90–3.98 (m, 2H, H-5′), 2.75 (dd, *J* = 14.6, 5.5 Hz, 1H, H-2′), 2.28 (ddd, *J* = 14.6, 8.8, 6.2 Hz, 1H, H-2′), 0.92 (s, 9H, *t-Bu*), 0.13 [s, 6H, Si(*CH*$_3$)$_2$]. Anal. ($C_{23}H_{30}N_4O_9SSi$) C, H, N, S.

3′-*O*-(3-Benzenesulfonylfuroxan-4-yl)-5′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxythymidine (3b): white solid; yield, 86%; mp 146–148 °C; ^{1}H NMR ($CDCl_3$) δ 9.37 (br s, 1H, N*H*), 8.08 (d, *J* = 7.3, 2H, *o*-Ph), 7.80 (t, *J* = 7.3 Hz, 1H, *p*-Ph), 7.68

(t, J = 7.3, 2H, *m*-Ph), 7.51 (s, 1H, H-6), 6.38 (dd, J = 9.2, 5.2 Hz, 1H, H-1′), 5.34−5.40 (m, 1H, H-3′), 4.34−4.39 (m, 1H, H-4′), 3.90−4.00 (m, 2H, H-5′), 2.67 (dd, J = 14.0, 5.2 Hz, 1H, H-2′), 2.24 (ddd, J = 14.0, 9.2, 5.5 Hz, 1H, H-2′), 1.92 (s, 3H, C-5 CH_3), 0.92 (s, 9H, *t-Bu*), 0.134 (s, 3H, SiCH_3), 0.130 (s, 3H, SiCH_3). Anal. ($C_{24}H_{32}N_4O_9SSi$) C, H, N, S.

3′-*O*-(3-Benzenesulfonylfuroxan-4-yl)-5′-*O*-(*tert*-butyldimethylsilyl)-5-iodo-2′-deoxyuridine (3c): viscous pale-yellow oil; yield, 79%; ^{1}H NMR ($CDCl_3$) δ 8.02 (d, J = 7.6 Hz, 2H, *o*-Ph), 7.91 (s, 1H, H-6), 7.74 (t, J = 7.6 Hz, 1H, *p*-Ph), 7.62 (t, J = 7.6 Hz, 2H, *m*-Ph), 6.34 (t, J = 4.9 Hz, 1H, H-1′), 5.28−5.38 (m, 1H, H-3′), 4.32−4.40 (m, 1H, H-4′), 3.88−3.98 (m, 2H, H-5′), 2.65−2.80 (m, 1H, H-2′), 2.00−2.20 (m, 1H, H-2′), 0.92 (s, 9H, *t-Bu*), 0.14 (s, 3H, SiCH_3), 0.13 (s, 3H, SiCH_3). Anal. ($C_{23}H_{29}IN_4O_9SSi$) C, H, N, S.

3′-*O*-(3-Benzenesulfonylfuroxan-4-yl)-5′-*O*-(*tert*-butyldimethylsilyl)-5-fluoro-2′-deoxyuridine (3d): white solid; yield, 87%; mp 194−195 °C; ^{1}H NMR ($CDCl_3$) δ 11.98 (d, J = 4.8 Hz, 1H, N*H*), 7.73−8.05 (m, 6H, C_6H_5, H-6), 6.18 (t, J = 5.9 Hz, 1H, H-1′), 5.30−5.37 (m, 1H, H-3′), 4.35−4.42 (m, 1H, H-4′), 3.90 (dd, J = 11.7, 2.9 Hz, 1H, H-5′), 3.81 (dd, J = 11.7, 2.9 Hz, 1H, H-5′), 2.64 (dd, J = 14.3, 5.9 Hz, 1H, H-2′), 2.32 (ddd, J = 14.3, 5.9, 5.5 Hz, 1H, H-2′), 0.86 (s, 9H, *t-Bu*), 0.08 [s, 6H, Si(CH_3)$_2$]. Anal. ($C_{23}H_{29}FN_4O_9SSi$) C, H, N, S.

3′-*O*-(3-Benzenesulfonylfuroxan-4-yl)-5′-*O*-(*tert*-butyldimethylsilyl)-5-trifluoromethyl-2′-deoxyuridine (3e): white solid; yield, 92%; mp 225−226 °C; ^{1}H NMR ($CDCl_3$) δ 8.25 (s, 1H, H-6), 8.04 (d, J = 7.9 Hz, 2H, *o*-Ph), 7.82 (t, J = 7.9 Hz, 1H, *p*-Ph), 7.68 (t, J = 7.9 Hz, 2H, *m*-Ph), 6.20 (dd, J = 8.4, 5.9 Hz, 1H, H-1′), 5.40−5.46 (m, 1H, H-3′), 4.46−4.52 (m, 1H, H-4′), 3.92−3.99 (m, 2H, H-5′), 2.82 (dd, J = 14.3, 5.9 Hz, 1H, H-2′), 2.32 (ddd, J = 14.3, 8.4, 5.5 Hz, 1H, H-2′), 0.88 (s, 9H, *t-Bu*), 0.10 (s, 3H, SiCH_3), 0.09 (s, 3H, SiCH_3). Anal. ($C_{24}H_{29}F_3N_4O_9SSi$) C, H, N, S.

General Method for the Preparation of 3′-*O*-(3-Benzenesulfonylfuroxan-4-yl)-5-substituted-2′-deoxyuridines (4a−e). To a stirred solution of a compound selected from the group **3a**−**e** (1 mmol) in THF (10 mL) was added 1 N HCl solution (3 mL), and the mixture was stirred at room temperature for 3 h. The mixture was extracted with ethyl acetate (3 × 5 mL), the combined organic extracts were dried (Na_2SO_4), and the solvent was removed in vacuo to afford an oil that was purified by flash chromatography using EtOAc/hexane (3:1, v/v) as eluant to give the respective title product listed below.

3′-*O*-(3-Benzenesulfonylfuroxan-4-yl)-2′-deoxyuridine (4a): white solid; yield, 94%; mp 206−207 °C; ^{1}H NMR (DMSO-d_6) δ 11.40 (s, 1H, N*H*), 8.00 (d, J = 7.7 Hz, 2H, *o*-Ph), 7.90 (t, J = 7.7 Hz, 1H, *p*-Ph), 7.88 (d, J = 8.0 Hz, 1H, H-6), 7.75 (t, J = 7.7 Hz, 2H, *m*-Ph), 6.21 (dd, J = 8.8, 5.9 Hz, 1H, H-1′), 5.69 (d, J = 8.0 Hz, 1H, H-5), 5.38−5.43 (m, 1H, H-3′), 5.32 (t, J = 5.1 Hz, 1H, O*H*), 4.22−4.31 (m, 1H, H-4′), 3.36−3.51 (m, 2H, H-5′), 2.56 (dd, J = 14.6, 5.9 Hz, 1H, H-2′), 2.37 (ddd, J = 14.6, 8.8, 5.1 Hz, 1H, H-2′); ^{13}C NMR (DMSO-d_6) δ 163.03 (C-4 C═O), 157.80 (furoxan C-4), 150.42 (C-2 C═O), 140.19 (C-6), 136.97 (phenyl C-1), 136.21 (phenyl C-4), 130.00 and 128.47 (phenyl C-2, C-3), 110.81 (furoxan C-3), 102.26 (C-5), 84.05 and 82.81 (C-4′, C3′, C-1′), 61.20 (C-5′), 36.54 (C-2′). Anal. ($C_{17}H_{16}N_4O_9S$) C, H, N, S.

3′-*O*-(3-Benzenesulfonylfuroxan-4-yl)-2′-deoxythymidine (4b): white solid; yield, 91%; mp 138−139 °C; ^{1}H NMR (DMSO-d_6) δ 8.88 (br s, 1H, N*H*), 8.07 (d, J = 7.9 Hz, 2H, *o*-Ph), 7.80 (t, J = 7.6 Hz, 1H, *p*-Ph), 7.67 (t, J = 7.6 Hz, 2H, *m*-Ph), 7.46 (s, 1H, H-6), 6.21 (dd, J = 8.5, 5.8 Hz, 1H, H-1′), 5.52−5.58 (m, 1H, H-3′), 4.37−4.42 (m, 1H, H-4′), 3.90−4.05 (m, 2H, H-5′), 2.9 (br s, 1H, O*H*), 2.73 (ddd, J = 14.6, 8.5, 5.8 Hz, 1H, H-2′), 2.62 (dd, J = 14.6, 5.8 Hz, 1H, H-2′), 1.94 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 164.15 (C-4 C═O), 158.10 (furoxan C-4), 150.79 (C-2 C═O), 137.22 (phenyl C-1), 136.65 and 136.21 (C-6, phenyl C-4), 130.40 and 128.76 (phenyl C-2, C-3), 111.08 and 110.43 (C-5, furoxan C-3), 84.22, 84.15, and 83.10 (C-4′, C-3′, C-1′), 61.47 (C-5′), 36.63 (C-2′), 12.60 (CH_3). Anal. ($C_{18}H_{18}N_4O_9S$) C, H, N, S.

3′-*O*-(3-Benzenesulfonylfuroxan-4-yl)-5-iodo-2′-deoxyuridine (4c): white solid; yield, 89%; mp 140−141 °C; ^{1}H NMR ($CDCl_3$) δ 11.37 (s, 1H, N*H*), 8.42 (s, 1H, H-6), 7.98 (d, J = 7.3, 2H, *o*-Ph), 7.71 (t, J = 7.3 Hz, 1H, *p*-Ph), 7.62 (t, J = 7.3, 2H, *m*-Ph), 6.27 (dd, J = 8.8, 5.5 Hz, 1H, H-1′), 5.40−5.45 (m, 1H, H-3′), 4.25−4.33 (m, 1H, H-4′), 3.72−3.82 (m, 2H, H-5′), 2.54 (dd, J = 14.6, 5.5 Hz, 1H, H-2′), 2.35 (ddd, J = 14.6, 8.8, 5.5 Hz, 1H, H-2′). Anal. ($C_{17}H_{15}IN_4O_9S$) C, H, N, S.

3′-*O*-(3-Benzenesulfonylfuroxan-4-yl)]-5-fluoro-2′-deoxyuridine (4d): white solid; yield, 90%; mp 206−208 °C; ^{1}H NMR ($CDCl_3$) δ 11.92 (d, J = 4.7 Hz, 1H, N*H*), 7.73−8.24 (m, 6H, H-6, C_6H_5), 6.20 (dd, J = 8.7, 5.8 Hz, 1H, H-1′), 5.37−5.45 (m, 1H, H-3′), 4.26−4.50 (m, 1H, H-4′), 3.62−3.77 (m, 2H, H-5′), 2.30−2.66 (m, 2H, H-2′); ^{13}C NMR ($CDCl_3$) δ 157.70 (furoxan C-4), 156.83 (d, J_{CCF} = 26.4 Hz, C-4 C═O), 148.92 (C-2 C═O), 140.00 (d, J_{CF} = 230.7 Hz, C-5), 136.93 (phenyl C-1), 136.05 (phenyl C-4), 129.85 and 128.51 (phenyl C-2, C-3), 123.60 (J_{CCF} = 39.5 Hz, C-6), 110.71 (furoxan C-3), 84.29, 84.12, and 82.69 (C-4′, C-3′, C-1′), 61.10 (C-5′), 36.53 (C-2′). Anal. ($C_{17}H_{15}FN_4O_9S$) C, H, N, S.

3′-*O*-(3-Benzenesulfonylfuroxan-4-yl)-5-trifluoromethyl-2′-deoxyuridine (4e): white solid; yield, 88%; mp 115−116 °C; ^{1}H NMR (CD_3OD) δ 8.76 (s, 1H, H-6), 8.07 (d, J = 7.6 Hz, 2H, *o*-Ph), 7.83 (t, J = 7.6 Hz, 1H, *p*-Ph), 7.70 (t, J = 7.6 Hz, 2H, *m*-Ph), 6.34 (dd, J = 8.4, 5.9 Hz, 1H, H-1′), 5.48−5.54 (m, 1H, H-3′), 4.40−4.45 (m, 1H, H-4′), 3.83−3.88 (m, 2H, H-5′), 2.71 (dd, J = 14.3, 5.9 Hz, 1H, H-2′), 2.47 (ddd, J = 14.3, 8.4, 5.5 Hz, 1H, H-2′). Anal. ($C_{18}H_{15}F_3N_4O_9S$) C, H, N, S.

General Method for the Preparation of 3′-*O*-(*tert*-Butyldimethylsilyl)-5-substituted-2′-deoxyuridines (5a−e). To a stirred solution of the compound selected from the group **1a**−**e** (1 mmol), imidazole (344 mg, 5 mmol), and DMAP (37 mg, 0.3 mmol) in DMF (10 mL) was added TBDMSCl (330 mg, 2.2 mmol) at 0 °C, and the mixture was allowed to warm to 25 °C and stirred for 1 h. The reaction was quenched using a saturated aqueous solution of ammonium chloride, and the mixture was extracted with ethyl acetate (3 × 5 mL). The combined organic extracts were dried (Na_2SO_4), and the solvent was removed in vacuo to give a viscous oil. The crude oil was dissolved in THF (15 mL), 80% acetic acid in THF (4:1, v/v) was added, and the mixture was stirred at 60 °C for 3 days. The mixture was extracted with ethyl acetate (3 × 5 mL), the combined organic extracts were dried (Na_2SO_4), and the solvent was removed in vacuo to afford an oil that was purified by flash chromatography with EtOAc/hexane (3:1, v/v) as eluant to give the respective title product listed below.

3′-*O*-(*tert*-Butyldimethylsilyl)-2′-deoxyuridine (5a): white solid; yield, 62%; mp 205−207 °C; ^{1}H NMR ($CDCl_3$) δ 9.20 (br s, 1H, N*H*), 7.57 (d, J = 8.1 Hz, 1H, H-6), 6.18 (t, J = 6.6 Hz, 1H, H-1′), 5.73 (d, J = 8.1 Hz, 1H, H-5), 4.49 (dd, J = 8.8, 4.8 Hz, 1H, H-3′), 3.94−3.91 (m, 2H, H-4′, H-5′), 3.73−3.77 (m, 1H, H-5′), 2.70 (br s, 1H, O*H*), 2.29 (t, J = 6.6 Hz, 2H, H-2′), 0.89 (s, 9H, *t-Bu*), 0.13 [s, 6H, Si(CH_3)$_2$]; ^{13}C NMR ($CDCl_3$) δ 163.29 (C-4 C═O), 150.19 (C-2 C═O), 140.98 (C-6), 102.40 (C-5), 87.59 (C-4′), 86.63 (C-1′), 71.40 (C-3′), 61.81 (C-5′), 40.93 (C-2′), 25.73 [C(CH_3)$_3$], 17.99 [C(CH_3)$_3$], −4.72 [d, $J_{Si,CH3}$ = 12.1 Hz, Si(CH_3)$_2$]. Anal. ($C_{15}H_{26}N_2O_5Si$) C, H, N.

3′-*O*-(*tert*-Butyldimethylsilyl)-2′-deoxythymidine (5b): white solid; yield, 67%; mp 93−94 °C; ^{1}H NMR ($CDCl_3$) δ 8.60 (br s, 1H, N*H*), 7.36 (s, 1H, H-6), 6.13 (t, J = 6.9 Hz, 1H, H-1′), 4.5 (dt, J = 6.9, 3.7 Hz, 1H, H-3′), 3.90−3.94 (m, 2H, H-4′, H-5′), 3.73−3.79 (m, 1H, H-5′), 2.60 (br s, 1H, O*H*), 2.37 (dd, J = 13.5, 6.9 Hz, 1H, H-2′), 2.25 (ddd, J = 13.5, 6.9, 3.7 Hz, 1H, H-2′), 1.93 (s, 3H, C-5 CH_3), 0.90 (s, 9H, *t-Bu*), 0.09 [s, 6H, Si(CH_3)$_2$]; ^{13}C NMR ($CDCl_3$) δ 163.57 (C-4 C═O), 150.11 (C-2 C═O), 136.73 (C-6), 110.73 (C-5), 87.36 (C-4′), 86.59 (C-1′), 71.36 (C-3′), 61.75 (C-5′), 40.32 (C-2′), 25.54 [C(CH_3)$_3$], 17.78 [C(CH_3)$_3$], 12.31 (C-5 CH_3), −4.92 [d, $J_{Si,CH3}$ = 12.1 Hz, Si(CH_3)$_2$]. Anal. ($C_{16}H_{28}N_2O_5Si$) C, H, N.

3′-*O*-(*tert*-Butyldimethylsilyl)-5-iodo-2′-deoxyuridine (5c): white solid; yield, 55%; mp 187−188 °C; ^{1}H NMR ($CDCl_3$) δ 11.66 (br s, 1H, N*H*), 8.34 (s, 1H, H-6), 6.07 (t, J = 6.2 Hz, 1H, H-1′), 5.18 (br s, 1H, O*H*), 4.40 (ddd, J = 6.2, 3.7, 2.6 Hz, 1H, H-3′), 3.77 (dd, J = 6.6, 3.7 Hz, 1H, H-4′), 3.50−3.64 (m,

2H, H-5′), 2.22 (dd, J = 13.2, 6.2 Hz, 1H, H-2′), 2.08 (ddd, J = 13.2, 6.2, 2.6 Hz, 1H, H-2′), 0.86 (s, 9H, *t-Bu*), 0.07 [s, 6H, Si(CH_3)$_2$]; ^{13}C NMR ($CDCl_3$) δ 160.25 (C-4 C=O), 149.93 (C-2 C=O), 144.73 (C-6), 87.50 (C-4′), 84.51 (C-1′), 71.57 (C-3′), 69.40 (C-5), 60.40 (C-5′), 25.62 [C(CH_3)$_3$], 17.63 [C(CH_3)$_3$], −4.87 (d, $J_{Si,CH3}$ = 5.5 Hz, Si(CH_3)$_2$]. Anal. ($C_{15}H_{25}IN_2O_5Si$) C, H, N.

3′-*O*-(*tert*-Butyldimethylsilyl)-5-fluoro-2′-deoxyuridine (5d): white solid; yield, 70%; mp 165−166 °C; ^{1}H NMR ($CDCl_3$) δ 9.13 (br s, 1H, N*H*), 7.97 (d, J = 6.2 Hz, 1H, H-6), 6.23 (t, J = 6.2 Hz, 1H, H-1′), 4.48 (ddd, J = 6.2, 4.0, 2.2 Hz, 1H, H-3′), 3.98−3.96 (m, 2H, H-4′, H-5′), 3.95−3.81 (m, 1H, H-5′), 2.31−2.35 (m, 2H, H-2′), 0.90 (s, 9H, *t-Bu*), 0.10 [s, 6H, Si(CH_3)$_2$]; ^{13}C NMR ($CDCl_3$) δ 156.73 (d, J_{CCF} = 26.4 Hz, C-4 C=O), 148.67 (C-2 C=O), 140.44 (d, J_{CF} = 236.2 Hz, C-5), 124.89 (d, J_{CCF} = 34.0 Hz, C-6), 87.61 (C-4′), 86.27 (C-1′), 71.39 (C-3′), 61.82 (C-5′), 41.27 (C-2′), 25.75 [C(CH_3)$_3$], 18.00 [C(CH_3)$_3$], −4.71 [d, $J_{Si,CH3}$ = 13.2 Hz, Si(CH_3)$_2$]. Anal. ($C_{15}H_{25}FN_2O_5Si$) C, H, N.

3′-*O*-(*tert*-Butyldimethylsilyl)-5-trifluoromethyl-2′-deoxyuridine (5e): white solid; yield, 54%; mp 225−226 °C; ^{1}H NMR ($CDCl_3$) δ 9.80 (br s, 1H, N*H*), 8.58 (s, 1H, H-6), 6.20 (t, J = 6.1 Hz, 1H, H-1′), 4.45−4.53 (m, 1H, H-3′), 3.93−3.98 (m, 2H, H-4′, H-5′), 3.77−3.81 (m, 1H, H-5′), 2.20−2.42 (m, 2H, H-2′), 0.87 (s, 9H, *t-Bu*), 0.07 [s, 6H, Si(CH_3)$_2$]; ^{13}C NMR ($CDCl_3$) δ 158.96 (C-4 C=O), 149.50 (C-2 C=O), 142.18 (q, J_{CCCF} = 5.5 Hz, C-6), 121.93 (q, J_{CF} = 270.3 Hz, CF_3), 104.84 (d, J_{CCF} = 33.0 Hz, C-5), 88.03 (C-4′), 86.78 (C-1′), 71.13 (C-3′), 61.29 (C-5′), 41.75 (C-2′), 25.64 [C(CH_3)$_3$], 17.90 [C(CH_3)$_3$], −4.87 [d, $J_{Si,CH3}$ = 14.2 Hz, Si(CH_3)$_2$]. Anal. ($C_{16}H_{25}F_3N_2O_5Si$) C, H, N.

General Method for the Preparation of 5′-*O*-(3-Benzenesulfonylfuroxan-4-yl)-3′-*O*-(*tert*-butyldimethylsilyl)-5-substituted-2′-deoxyuridines (6a−d). To a stirred solution of a compound selected from the group **5a−d** (1 mmol) and **8a** (550 mg, 1.5 mmol) in THF (5 mL) at 0 °C was added a 50% aqueous sodium hydroxide solution (3 mmol). The mixture was stirred for 1 h, diluted with water, and extracted with ethyl acetate (3 × 5 mL). The combined organic extracts were dried (Na_2SO_4), and the solvent was removed in vacuo to give an oil that was purified by flash chromatography using EtOAc/hexane (1:1, v/v) to give the respective title product listed below.

5′-*O*-(3-Benzenesulfonylfuroxan-4-yl)-3′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyuridine (6a): white solid; yield, 83%; mp 132−134 °C; ^{1}H NMR ($CDCl_3$) δ 9.06 (br s, 1H, N*H*), 7.80 (d, J = 8.0, 2H, *o*-Ph), 7.77 (t, J = 8.0 Hz, 1H, *p*-Ph), 7.74 (d, J = 8.1 Hz, 1H, H-6), 7.64 (t, J = 8.0 Hz, 2H, *m*-Ph), 6.46 (t, J = 6.9 Hz, 1H, H-1′), 5.84 (d, J = 8.1 Hz, 1H, H-5), 4.57−4.72 (m, 3H, H-3′, H-5′), 4.14−4.20 (m, 1H, H-4′), 2.34−2.44 (m, 2H, H-2′), 0.92 (s, 9H, *t-Bu*), 0.13 (s, 3H, SiCH_3), 0.11 (s, 3H, SiCH_3); ^{13}C NMR (DMSO-d_6) δ 162.97 (C-4 C=O), 158.59 (furoxan C-4), 150.30 (C-2 C=O), 140.13 (C-6), 137.11 (phenyl C-1), 135.89 (phenyl C-4), 129.70 and 128.56 (phenyl C-2 and C-3), 110.23 (furoxan C-3), 103.25 (C-5), 84.36 and 83.84 (C-4′, C-1′), 70.87 (C-3′), 69.18 (C-5′), 40.64 (C-2′), 25.69 [C(CH_3)$_3$], 17.96 [C(CH_3)$_3$], −4.70 [d, $J_{Si,CH3}$ = 18.6 Hz, Si(CH_3)$_2$]. Anal. ($C_{23}H_{30}N_4O_9SSi$) C, H, N, S.

5′-*O*-(3-Benzenesulfonylfuroxan-4-yl)-3′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxythymidine (6b): white solid; yield, 86%; mp 110−112 °C; ^{1}H NMR ($CDCl_3$) δ 8.80 (br s, 1H, N*H*), 8.00 (d, J = 7.7 Hz, 2H, *o*-Ph), 7.79 (t, J = 7.7 Hz, 1H, *p*-Ph), 7.63 (t, J = 7.7 Hz, 2H, *m*-Ph), 7.46 (s, 1H, H-6), 6.47 (t, J = 7.0 Hz, 1H, H-1′), 4.56−4.70 (m, 3H, H-3′, H-5′), 4.16−4.22 (m, 1H, H-4′), 2.26−2.43 (m, 2H, H-2′), 1.92 (s, 3H, C-5 CH_3), 0.92 (s, 9H, *t-Bu*), 0.14 (s, 3H, Si$C-H_3$), 0.12 (s, 3H, Si$C-H_3$). Anal. ($C_{24}H_{32}N_4O_9SSi$) C, H, N, S.

5′-*O*-(3-Benzenesulfonylfuroxan-4-yl)-3′-*O*-(*tert*-butyldimethylsilyl)-5-fluoro-2′-deoxyuridine (6d): white solid; yield, 96%; mp 225−226 °C; ^{1}H NMR (DMSO-d_6) δ 11.29 (br s, 1H, N*H*), 8.04 (d, J = 7.9 Hz, 2H, *o*-Ph), 7.81 (t, J = 7.9 Hz, 1H, *p*-Ph), 7.75 (d, J = 7.1 Hz, 1H, H-6), 7.63 (t, J = 7.9 Hz, 2H, *m*-Ph), 6.45 (t, J = 6.9 Hz, 1H, H-1′), 4.58−4.70 (m, 3H, H-3′, H-5′), 4.21−4.26 (m, 1H, H-4′), 2.30−2.45 (m, 2H, H-2′), 0.92 (s, 9H, *t-Bu*), 0.14 (s, 3H, SiCH_3), 0.12 (s, 3H, SiCH_3); ^{13}C NMR (DMSO-d_6) δ 158.55 (furoxan C-4), 156.77 (d, J_{CCF} = 26.4 Hz, C-4 C=O), 148.93 (C-2 C=O), 140.65 (J_{CF} = 237.3 Hz, C-5), 136.65 (phenyl C-1), 135.89 (phenyl C-4), 129.63 and 128.74 (phenyl C-2 and C-3), 127.20 (d, J_{CCF} = 33.0 Hz, C-6), 110.60 (furoxan C-3), 85.08 and 84.45 (C-4′, C-3′), 71.87 (C-3′), 69.86 (C-5′), 40.39 (C-2′), 25.72 [C(CH_3)$_3$], 17.96 [C(CH_3)$_3$], −4.76 [d, $J_{Si,CH3}$ = 6.7 Hz, Si(CH_3)$_2$]. Anal. ($C_{23}H_{29}FN_4O_9SSi$) C, H, N, S.

General Method for the Preparation of 5′-*O*-(3-Benzenesulfonylfuroxan-4-yl)]-5-substituted-2′-deoxyuridines (7a−d). To a stirred solution of a compound selected from the group of **6a−d** (1 mmol) in THF (10 mL) was added a 1 N HCl solution (3 mL), and the mixture was stirred at 25 °C for 3 h. The mixture was extracted with ethyl acetate (3 × 5 mL), the combined organic extracts were dried (Na_2SO_4), and the solvent was removed in vacuo to give an oil that was purified by flash chromatography using EtOAc/hexane (3:1, v/v) as eluant to give the respective title product listed below.

5′-*O*-(3-Benzenesulfonylfuroxan-4-yl)-2′-deoxyuridine (7a): white solid; yield, 80%; mp 163−164 °C; ^{1}H NMR (DMSO-d_6) δ 11.29 (s, 1H, N*H*), 7.64−8.01 (m, 6H, C_6H_5, H-6), 6.28 (t, J = 6.7 Hz, 1H, H-1′), 5.50 (d, J = 7.9 Hz, 1H, H-5), 4.56−4.65 (m, 2H, H-5′), 4.40−4.48 (m, 1H, H-3′), 4.12−4.19 (m, 1H, H-4′), 2.20−2.45 (m, 2H, H-2′); ^{13}C NMR (DMSO-d_6) δ 163.18 (C-4 C=O), 158.83 (furoxan C-4), 150.41 (C-2 C=O), 140.54 (C-6), 136.84 (phenyl C-1), 136.13 (phenyl C-4), 129.91 and 128.29 (phenyl C-2 and C-3), 110.71 (furoxan C-3), 102.13 (C-5), 84.47 and 83.55 (C-4′ and C-1′), 70.87 (C-5′), 70.24 (C-3′), 56.25 (C-2′). Anal. ($C_{17}H_{16}N_4O_9S$) C, H, N, S.

5′-*O*-(3-Benzenesulfonylfuroxan-4-yl)]-2′-deoxythymidine (7b): white solid; yield, 85%; mp 245−246 °C; ^{1}H NMR (DMSO-d_6) δ 11.35 (s, 1H, N*H*), 8.00 (d, J = 7.7 Hz, 2H, *o*-Ph), 7.88 (t, J = 7.7 Hz, 1H, *p*-Ph), 7.73 (t, J = 7.7 Hz, 2H, *m*-Ph), 7.51 (s, 1H, H-6), 6.29 (t, J = 6.6 Hz, 1H, H-1′), 5.54 (d, J = 4.4 Hz, 2H, O*H*), 4.56−4.66 (m, 2H, H-5′), 4.40−4.49 (m, 1H, H-3′), 4.10−4.19 (m, 2H, H-4′), 2.11−2.40 (m, 2H, H-2′), 1.75 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 163.94 (C-4 C=O), 159.07 (furoxan C-4), 150.66 (C-2 C=O), 136.84 (phenyl C-1), 136.30 and 136.04 (phenyl C-4, C-6), 130.17 and 128.40 (phenyl C-2, C-3), 110.87 and 110.17 (furoxan C-3, C-5), 84.05 and 83.5o (C-4′, C-1′), 71.22 (C-5′), 70.54 (C-3′), ∼38−39 (C-2′, buried under solvent peaks), 12.21 (CH_3). Anal. ($C_{18}H_{18}N_4O_9S$) C, H, N, S.

5′-*O*-(3-Benzenesulfonylfuroxan-4-yl)-5-iodo-2′-deoxyuridine (7c): white solid; yield, 89%; mp 213-215 °C; ^{1}H NMR (DMSO-d_6) δ 11.75 (s, 1H, N*H*), 8.00 (d, J = 7.7 Hz, 2H, *o*-Ph), 7.98 (s, 1H, H-6), 7.88 (t, J = 7.7 Hz, 1H, *p*-Ph), 7.72 (t, J = 7.7 Hz, 2H, *m*-Ph), 6.18 (t, J = 7.0 Hz, 1H, H-1′), 5.51 (d, J = 4.0 Hz, 2H, O*H*), 4.67 (dd, J = 10.9, 2.9 Hz, 1H, H-5′), 4.60 (dd, J = 10.9, 5.1 Hz, 1H, H-5′), 4.36−4.45 (m, 1H, H-3′), 4.15−4.22 (m, 1H, H-4′), 2.31−2.43 (m, 1H, H-2′), 2.07−2.25 (m, 2H, H-2′). Anal. ($C_{17}H_{15}FN_4O_9S$) C, H, N, S.

5′-*O*-(3-Benzenesulfonylfuroxan-4-yl)-5-fluoro-2′-deoxyuridine (7d): white solid; yield, 79%; mp 198−200 °C; ^{1}H NMR (DMSO-d_6) δ 11.88 (s, 1H, N*H*), 7.69−8.01 (m, 6H, C_6H_5, H-6), 6.25 (dd, J = 7.6, 5.9 Hz, 1H, H-1′), 4.57−4.66 (m, 2H, H-5′), 4.39−4.47 (m, 1H, H-3′), 4.13−4.20 (m, 1H, H-4′), 2.14−2.38 (m, 2H, H-2′); ^{13}C NMR (DMSO-d_6) δ 158.71 (furoxan C-4), 156.75 (d, J_{CCF} = 26.4 Hz, C-4 C=O), 148.81 (C-2 C=O), 139.90 (d, J_{CF} = 231.8 Hz, C-5), 136.71 (phenyl C-1), 135.93 (phenyl C-4), 129.71 and 128.15 (phenyl C-2, phenyl C-3), 124.48 (d, J_{CCF} = 33.0 Hz, C-6), 110.58 (furoxan C-3), 84.58 and 83.94 (C-4′, C-1′), 70.93 (C-5′), 70.13 (C-3′), ∼38−39 (C-2′, buried under solvent peaks). Anal. ($C_{17}H_{15}IN_4O_9S$) C, H, N, S.

In Vitro Nitric Oxide Release Assays. 1. Incubation with 18 mM L-Cysteine in Phosphate Buffer (pH 7.4). In vitro nitric oxide release was assayed using a modification of the previously reported procedure.[25] Briefly, a solution of the test compound (1 mL of a 0.2 mM solution in 0.1 M phosphate buffer, pH 7.4) was mixed thoroughly with a freshly prepared solution of L-cysteine (1 mL of a 3.6 mM solution in 0.1 M phosphate buffer, pH 7.4), and the mixture was incubated at

37 °C for 1 and 16 h in the absence of air. After exposure to air for 10 min at 25 °C, an aliquot of the Griess reagent (1 mL) [freshly prepared by mixing equal volumes of 1.0% sulfanilamide and 0.1% *N*-naphthylethylenediamine dihydrochloride in water] was added to an equal volume (1 mL) of each test compound's incubation solution with mixing. After 10 min had elapsed, absorbance was measured at 540 nm using a Philips PU 8740 UV−vis scanning spectrophotometer. Solutions of 0−100 μM sodium nitrite were used to prepare a nitrite absorbance versus concentration curve under the same experimental conditions. The percent nitric oxide released (quantitated as nitrite ion) was calculated (±SEM, $n = 3$) from the standard nitrite versus concentration curve.

2. Incubation with Phosphate Buffer (pH 7.4). This assay was performed as described under procedure 1 above except that a solution of the test compound (2 mL of a 2 mM solution in 0.1 M phosphate buffer pH 7.4) was used and no L-cysteine was added.

In Vitro Cell Cytotoxicity (MTT Assay). Human 143B and human 143B-LTK cells were cultured in complete MEME with 10% fetal bovine serum (FBS). Murine KBALB, KBALB-STK, and Hs578Bst human fibroblast cells were cultured in complete DMEM medium supplemented with 10% FBS, and murine EMT-6 cells were cultured in complete WAYMOUTH medium with 12.5% FBS. Exponentially growing cells were trypsinized, centrifuged, and resuspended in growth medium, and the cell number was readjusted to 8×10^3 cells/mL. Cells were seeded into 96-well plates at 8×10^2 cells/well and incubated at 37 °C in a humidified 5% CO_2 atmosphere for 24 h.

The test compound was dissolved in the medium indicated above, and 100 μL of this solution was added to the cells in 96-well plates to produce the preselected test compound concentration. Complete growth medium (100 μL) was added to control wells. The plates were incubated for 3 days at 37 °C in a humidified atmosphere consisting of 95% air and 5% CO_2. At the end of the incubation, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT, Sigma) was dissolved in phosphate-buffered saline (PBS) to produce a concentration of 5 mg/mL, filtered through a 0.45 μm membrane filter, and diluted (1:5) with prewarmed medium. An aliquot of this solution (50 μL) was added to each well, and the plates were incubated at 37 °C for 4 h. The medium was removed from the wells, dimethyl sulfoxide (150 μL) was added to each well, and the plates were placed on a shaker for 15 min to dissolve the formazan crystals. The absorbance at 540 nm (A_{540}) was measured immediately in each well using a scanning multiwell spectrophotometer (ELISA reader). A_{540} values, corrected for the absorbance in medium blanks, reflected the concentration of viable cells. The CC_{50} values reported refer to the test drug concentrations that reduced the A_{540} to 50% of the control value (mean value, $n = 6$). This assay,[26] which depends on the metabolic reduction of MTT to colored formazan, measures cytostatic and cytotoxic effects of the test drug.

Acknowledgment. We are grateful to the Alberta Cancer Board (Grant No. RI-124) for financial support of this research.

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JM030544M