5-Alkynyl Analogs of Arabinouridine and 2′-Deoxyuridine: Cytostatic Activity against Herpes Simplex Virus and Varicella-Zoster Thymidine Kinase Gene-Transfected Cells

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A group of arabinouridines (TMSEAU, EAU, IEAU-TA) and 2′-deoxyuridines (TMSEDU, EDU, IEDU) having a variety of substituents at the uracil C-5 position (trimethylsilylethynyl, TMSE; ethynyl, E; or iodoethynyl, IE), and the sugar C-2′ position (2′-arabino OH in arabinouridine, AU; or 2′-deoxyribo H in 2′-deoxyuridine, DU) were prepared to acquire antiviral structure−activity relationships. A broad-spectrum viral panel screen showed that these 5-alkynylarabino/deoxy-uridines exhibit moderate anti-HSV-1 activity, with no difference in potency between arabinouridines and 2′-deoxyuridines. The 2′-deoxyuridines TMSEDU, EDU, and IEDU, unlike the arabinouridines, exhibited potent antiviral activity against cytomegalovirus, but they were also highly cytostatic. The abilities of the 5-alkynylarabino/deoxy-uridines to inhibit nontransfected (wild-type or thymidine kinase-deficient, *tk*$^-$) and viral gene transfected (HSV-1, HSV-2, or VZV thymidine kinase-positive, *tk*$^+$) FM3A and OST (osteosarcoma) cells were determined. This group of 5-alkynylarabino/deoxy-uridines showed an enhanced ability to inhibit cells transfected with a viral thymidine kinase gene (HSV-1*tk*$^+$, HSV-2*tk*$^+$, VZV*tk*$^+$) relative to wild-type or thymidine kinase-deficient (*tk*$^-$) cells.

Introduction

The design of arabinouridines and 2′-deoxyuridines possessing novel two-carbon substituents at the C-5 position, which are potent and selective antivirals, represent an important area of antiviral drug development. The discovery of (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU,[a] Brivudin), (*E*)-5-(2-bromovinyl)arabinouridine (BVAU), and 5-(prop-1-ynyl)-arabinouridine (PAU) significantly boosted interest and contributed to the concept of a specific antiviral therapy. First generation antiherpetics, such as 5-iodo- and 5-trifluoromethyl-2′-deoxyuridine, exhibited little if any selectivity in their antiviral action. In contrast, BVDU, BVAU, and PAU are highly specific inhibitors of herpes simplex virus (HSV-1) and varicella-zoster virus (VZV) replication. This selectivity is attributed to specific phosphorylation by virus-encoded thymidine kinase (TK) in virus-infected, but not in uninfected, host cells.[1−4] Within the large group of 5-substituted pyrimidine nucleosides that have been investigated, (*E*)-5-[2-halo (iodo, IVDU; bromo, BVDU; chloro, CVDU]-vinyl[1,2] and 5-(2-chloroethyl)-2′-deoxyuridine (CEDU)[5] are among the most potent and selective in their action against HSV-1. In this regard, CEDU showed efficacy against systemic HSV-1 infection and HSV-1 encephalitis in mice at a 5−15-fold lower concentration than BVDU.[6] 5-Ethyl-2′-deoxyuridine is less potent and approximately equiactive against HSV-1 and HSV-2.[1,2]

The rational variation of substituents on the uracil base moiety of pyrimidine nucleosides can confer new properties (oral bioavailability, metabolic stability, pharmacokinetics)[7] that the natural bases lack and, when incorporated into nucleic acids by enzymatic processes, can further alter the structure and/or function of these biopolymers. For example, 2′-deoxyuridine derivatives, which possess a C-5 substituent two-carbon atoms in length, usually exhibit antiviral, but not anticancer, activity. Structure−activity relationship (SAR) correlations for a group of 5-olefinic 2′-deoxyuridines indicated that optimum inhibition of HSV-1 in vitro occurred when the 5-substituent was unsaturated and conjugated with the uracil ring, was not longer than four carbon atoms, had the (*E*)-stereochemistry, and included a hydrophobic electronegative functionality such as (*E*)-CH═CH−I).[8] 5-Alkynyl pyrimidine nucleosides subsequently received considerable attention to treat VZV infections.

Although 5-ethynyl-2′-deoxyuridine (5-C≡CH) was highly active against HSV-1, CMV, and VZV in vitro, it was also cytotoxic. On the other hand, 5-(−C≡C−CH_3) derivatives of arabinouridine and 2′-deoxyuridine exhibited highly selective anti-VZV activity and they were 2−3 orders of magnitude less cytotoxic. In this regard, changing the C-5 substituent from −C≡CH to −C≡C−CH_3 greatly increased VZV selectivity and decreased cytotoxicity, and these effects could be attributed to the inability of the −C≡C−CH_3 analogs to act as efficient substrates for cellular TK or to decreased affinity of the monophosphate for thymidylate synthase (TS).[9] The methyl (2.0 Å) and iodine (2.15 Å) substituents are often considered to be isosteres in view of their similarities in size (Van der Waals' radius). For example, 5-iodo-2′-deoxyuridine is an antimetabolite of the natural DNA substrate 5-methyl-2′-deoxyuridine (2′-deoxythymidine). A structural comparison of the linear 5-(C≡C−I) substituent in 5-(2-iodoethynyl)-2′-deoxyuridine (IEDU) with the (*E*)-5-(CH═CH−I) substituent in (*E*)-5-(2-iodovinyl)-2′-deoxyuridine (IVDU) is illustrated in

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[a] Abbreviations: BVAU, (*E*)-5-(2-bromovinyl)arabinouridine; BVDU, (*E*)-5-(2-bromovinyl)-2′-deoxyuridine; CEDU, 5-(2-chloroethyl)-2′-deoxyuridine; CMV, cytomegalovirus; CVDU, (*E*)-5-(2-chlorovinyl)-2′-deoxyuridine; EAU, 5-ethynylarabinouridine; EDU, 5-ethynyl-2′deoxyuridine; HEL, human embryonic lung; HSV-1, herpes simplex virus type-1; HSV-2, herpes simplex virus type-2; IEAU-TA, 5-(2-iodoethynyl)arabinouridine-2′,3′,5′-tri-*O*-acetate; IEDU, 5-(2-iodoethynyl-2′-deoxyuridine; IVDU, (*E*)-5-(2-iodovinyl)-2′-deoxyuridine; PAU, 5-(prop-1-ynyl)arabinouridine; TK, thymidine kinase; TMSEAU, 5-(2-trimethylsilylethynyl)arabinouridine; TMSEAU-TA, 5-(2-trimethylsilylethynyl)arabinouridine-2′,3′,5′-tri-*O*-acetate; TMSEDU, 5-(2-trimethylsilylethynyl)-2′-deoxyuridine; TS, thymidylate synthase; VZV, varicella-zoster virus.

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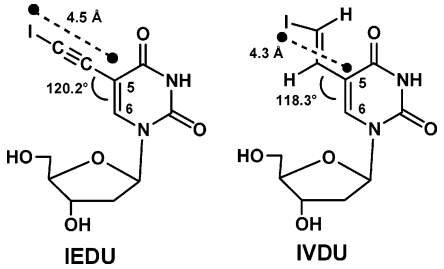
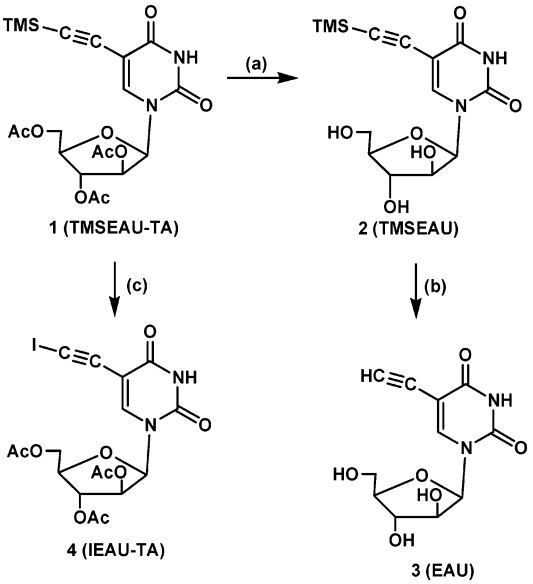


Figure 1. Calculated bond angles and distances in IEDU and IVDU.

Scheme 1[a]



[a] Reagents and conditions: (a) 0.05 N NaOMe, MeOH, 25 °C, 1h; (b) K_2CO_3, MeOH, benzene, 25 °C, 6 h; (c) $AgNO_3$, *N*-iodosuccinimide, DMF, 25 °C, 3 h (protected from light).

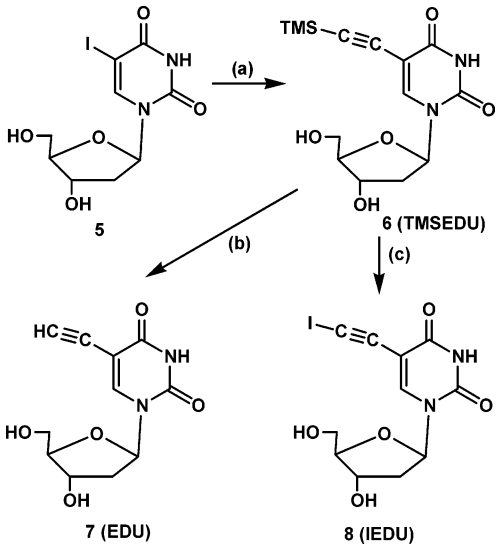
Figure 1. The calculated interspatial distances between the iodine atom and the C-5 carbon center (4.5 Å in IEDU and 4.3 Å in IVDU) and the C−C5−C6 bond angles (120.2° in IEDU and 118.2° in IVDU) are relatively similar. It was, therefore, anticipated in 1996, when this investigation was carried out, that arabinouridines and 2′-deoxyuridines possessing a linear 5-(C≡C−I) substituent may act as antiviral agents due to their selective phosphorylation by virus-infected cells,[10] as radiopharmaceutical agents for imaging,[11,12] or chemotherapeutic agents for treating[13] herpes simplex virus type-1 thymidine kinase positive (HSV-1 TK^+) gene-transfected tumors (gene therapy of cancer).[14−16] We now report the synthesis, antiviral activities, and cellular toxicities for a group of structurally related 5-alkynyl analogs of arabinouridine and 2′-deoxyuridine.

Chemistry

Treatment of TMSEAU-TA (**1**), prepared according to the method of Robins et al.,[17] with 0.05 N NaOMe selectively cleaved the acetate groups to afford the deprotected compound TMSEAU (**2**) in 81% yield (see Scheme 1). Subsequent reaction of TMSEAU with K_2CO_3 in MeOH removed the TMS moiety to furnish EAU (**3**, 70%). A similar synthesis of EAU, using the *para*-toluoyl ester analog of TMSEAU-TA, has been described.[9] Reaction of TMSEAU-TA with *N*-iodosuccinimide in the presence of $AgNO_3$ catalyst in DMF, according to a method originally developed by Isobe et al.[18] for the elaboration of R−C≡C−TMS → R−C≡C−I, furnished IEAU-TA (**4**, 78%).

The Sonogashira[19] coupling reaction of 5-iodo-2′-deoxyuridine (**5**) with Me_3Si−C≡CH in the presence of $(PPh_3)_2PdCl_2$ and CuI in Et_3N and MeCN afforded TMSEDU (**6**, 79%), as illustrated in Scheme 2. Although we and others have protected the sugar hydroxyl groups as acetates,[17,20] *p*-chlorobenzoates,[21] or *p*-toluoates[9] when a Sonogashira coupling reaction is employed, the reaction works equally well with the unprotected 5-iodo-2′-deoxyuridine (**5**). The TMS group in TMSEDU was readily removed by treatment with 0.05 N NaOMe to yield the previously reported[9] EDU (**7**, 87%). The $AgNO_3$-catalyzed iodination of TMSEDU with *N*-iodosuccinimide in DMF yielded IEDU (**5**, 40%). This two-step reaction that was developed in 1996 in our research program is shorter than a recently reported[20] synthesis of IEDU that involved the iodination of 3′,5′-di-*O*-acetyl-5-ethynyl-2′-deoxyuriidne using iodonium di-*syn*-collidine perchlorate.

Scheme 2[a]



[a] Reagents and conditions: (a) Me_3Si−C≡CH, MeCN, Et_3N, $(PPh_3)_2PdCl_2$, CuI, 50 °C, 3 h; (b) MeOH, 0.05 N NaOMe, 25 °C, 2 h; (c) $AgNO_3$, *N*-iodosuccinimide, DMF, 25 °C, 3 h (protected from light).

Biological Results and Discussion

A group of arabinouridines (TMSEAU, EAU, IEAU-TA) and 2′-deoxyuridines (TMSEDU, EDU, IEDU) having a variety of substituents at the uracil C-5 position (trimethylsilylethynyl, TMSE; ethynyl, E; or iodoethynyl, IE) and the sugar C-2′ position (2′-arabino OH in arabinouridine, AU; or 2′-deoxyribo H in 2′-deoxyuridine, DU) were prepared (see structures in Schemes 1 and 2) to acquire an antiviral structure−activity relationship (SAR). This type of information is useful for the design of chemotherapeutic agents to treat viral diseases and radiopharmaceutical agents to monitor viral gene expression that is beneficial in the gene therapy of cancer.[11−16] Accordingly, biological evaluations were performed using an extensive battery of antiviral assays (see Tables 1−5) and specific cell lines, both wild-type and those that were transfected with a viral thymidine kinase gene (HSV-1tk^+, HSV-2tk^+, or VZVtk^+). The 2′,3′,5′-tri-*O*-acetyl (TA) derivative (IEAU-TA) of 5-(2-iodoethynyl)-arabinouridine (IEAU) was originally prepared with the view that IEAU-TA would then serve as a prodrug that undergoes in vivo cleavage by plasma esterases to release the parent compound.

The antiviral activities exhibited by the group of 5-alkynylarabino(deoxy)uridines was determined against a broad spectrum of viruses (see data in Table 1). This group of compounds, except for IEAU-TA, exhibited moderate anti-HSV-1 inhibitory activity (EC_{50} = 0.7 to 2 μg/mL range) relative to the reference drug BVDU. Antiviral activity against HSV-2 was maintained,

Table 1. Cytotoxicity and Antiviral Activity of 5-Ethynyl Analogs of Arabino/deoxy-uridine in E_6SM Cell Cultures

		minimum inhibitory concentration:[b] EC_{50} (μg/mL)				
cmpd	MCC[a] (μg/mL)	HSV-1 (KOS)	HSV-2 (G)	vaccinia virus	HSV-1 TK$^-$ B2006	HSV-1 TK$^-$ VMW1837
TMSEAU	400	0.7	2	7	150	150
EAU	400	0.7	7	7	70	>200
IEAU-TA	40	20	7	>10	>10	>10
TMSEDU	>400	2	7	0.2	4	7
EDU	>400	0.7	2	0.1	0.7	4
IEDU	≥100	2	7	0.2	2	7
brivudin[c]	≥400	0.02	150	0.2	40	40
ribavirin[d]	400	70	150	20	20	70
ganciclovir[e]	>100	0.002	0.007	>100	0.7	0.02
acyclovir[f]	>400	0.02	0.07	>400	40	7

[a] Minimum compound concentration required to cause a microscopically detectable alteration of normal cell morphology. [b] Minimum inhibitory compound concentration required to reduce virus-induced cytopathogenicity by 50%. [c] (*E*)-5-(2-Bromovinyl)-2′-deoxyuridine. [d] 1-(β-D-ribofuranosyl)-1,2,4-thiazole-3-carboxamide. [e] 9-[(1,3-Dihydroxy-2-propoxy)methyl]guanine. [f] 9-[2-Hydroxyethoxy)methyl]guanine.

Table 2. Antiviral Activity of 5-Ethynyl Analogs of Arabino/deoxy-uridine against Cytomegalovirus in Human Embryonic Lung Cells

	antiviral activity[a] EC_{50} (μM)		cytoxic/cytostatic activity	
	AD-169	davis	cell morphology[b] (MCC)	cell growth[c] (CC_{50})
TMSEAU	>50	>50	200	127
EAU	>50	>50	200	>200
IEAU-TA	>20	>20	50	20
TMSEDU	1.2	0.75	200	0.18
EDU	1.2	0.85	>200	2.5
IEDU	2.5	0.58	200	0.76
cidofovir[d]	0.66	0.66		121
ganciclovir	6.3	2.75	>200	

[a] Inhibitory concentration required to reduce virus plaque formation by 50%. Virus input was 100 plaque forming units (PFU). [b] Minimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology. [c] Cytotoxic compound concentration required to reduce cell growth by 50%. [d] 1-[(*S*)-3-Hydroxy-2-(phosphonomethoxy)propyl]cytosine.

but at a lower potency range (EC_{50} = 2−7 μg/mL). No distinct differences in activities against HSV-1 or HSV-2 between the arabinouridine (TMSEAU, EAU) and 2′-deoxyuridine (TMSEDU, EDU, IEDU) subclasses of compounds were observed. On the other hand, there was a distinct difference in antiviral activity against thymidine kinase-deficient HSV-1 *tk*$^-$ (B2006 and VMW1837 strains) cells. In this regard, the arabinouridines (TMSEAU, EAU), like BVDU, were inactive as expected, but the 2′-deoxyuridines (TMSEDU, EDU, IEDU) retained similar antiviral activity (IC_{50} = 0.7−4 μg/mL, B2006 strain) or slightly reduced potency (IC_{50} = 4−7 μg/mL, VMW1837 strain) against HSV-1 *tk*$^-$ strains. The 2′-deoxyuridines (TMSEDU, EDU, IEDU), which showed high potencies against vaccinia virus (IC_{50} = 0.1−0.2 μg/mL range) similar to the reference drug BVDU (IC_{50} = 0.2 μg/mL), were markedly more potent than the arabinouridines (TMSEAU, EAU, IC_{50} = 7 μg/mL). None of the drugs showed appreciable cytotoxicity to the monolayer E_6SM cell cultures.

Additional antiviral studies using VZV-infected human embryonic lung (HEL) cells [TK$^+$ VZV (OKA), TK$^+$ VZV (YS), TK-deficient VZV/TK$^-$ (07/1) and TK$^-$-deficient VZV/TK$^-$ (YS/R)] were carried out to determine the inhibitory test compound concentration required to reduce viral plaque formation by 50% (EC_{50}, μM) for a viral input of 20 plaque-forming units (PFU) against these VZV strains (see data in Table 3). In these studies, the arabinouridines, TMSEAU and EAU, and the 2′-deoxyuridines, TMSEDU, EDU and IEDU, all showed pronounced antiviral activity against the TK$^+$ OKA strain (IC_{50} = 0.48 to 1.0 μM) and TK$^+$ YS strain (IC_{50} = 0.29 to 1.0 μM range). In sharp contrast to the arabinouridines, TMSEAU and EAU, the 2′-deoxyuridines TMSDU, EDU, and IEDU retained appreciable antiviral activity against the TK$^-$ VZV strains 07/1 (EC_{50} = 0.69 to 0.89 μM range) and YS/R (EC_{50} = 0.75 to 0.90 μM range).

Antiviral activity against cytomegalovirus (CMV) [strains AD-169 and Davis] was determined using a cytopathicity (CPE) reduction assay (Table 2). In these anti-CMV assays, the arabino compounds TMSEAU, EAU (EC_{50}s > 50 μM), and IEAU-TA (EC_{50} > 20 μM) were all inactive. In contrast, the 2′-deoxyribo compounds TMSEDU, EDU, and IEDU exhibited potent anti-CMV activities against AD-169 (IC_{50} = 1.2 to 2.5 μM range) and Davis (IC_{50} = 0.58 to 0.85 μM range) strains similar to the reference drug cidofovir (IC_{50} = 0.66 μM). The 2′-deoxyribo compounds were virtually not cytotoxic (as their arabino counterparts) (MCC ≥ 50 μM) but markedly more cytostatic (CC_{50} = 0.18 to 2.5 μM range) than the arabino compounds (CC_{50} = 20 to >200 μM range). These anti-CMV and cell cytotoxicity (CC_{50}) data are consistent with previous studies showing that (i) EDU is more potent than EAU against CMV,[9] (ii) EDU is more cytostatic than EAU,[9] (iii) IEDU is a more potent anti-CMV agent than ribavirin,[20] and (iv) IEDU is very cytostatic to PBM and CEM cells.[20] The high cytostatic activity of the 5-alkynyl-2′-deoxyuridines may likely be due to inhibition of TS and subsequent inhibition of cellular DNA synthesis.

Other antiviral assays (data not shown) were performed to determine the ability of these 5-alkynylarabino/deoxyuridines to reduce virus-induced cytopathicity in HeLa cell cultures. All compounds were inactive at the highest test compound concentration employed against vesicular stomatitis virus (>40 to >400 μg/mL range; ribavirin IC_{50} = 20 μg/mL), Coxsackie virus B4 (>40 to >400 μg/mL range; ribavirin IC_{50} = 20 μg/mL), and respiratory syncytial virus (>40 to >400 μg/mL range; ribavirin IC_{50} = 2 μg/mL). Other antiviral assays (data not shown) carried out using Vero cell cultures also indicated that this group of arabino/deoxyuridines failed to reduce virus-induced cytopathicity by 50% at the highest test compound concentration employed against parainfluenza-3 virus (20 to >400 μg/mL range), reovirus-1 (>40 to >400 μg/mL range, ribavirin IC_{50} = 0.7 μg/mL), Sindbis virus (>40 to >400 μg/mL), and Punta Toro virus (20 to >400 μg/mL range, ribavirin IC_{50} = 20 μg/mL).

The abilities of the 5-alkynylarabino/deoxyuridines to inhibit nontransfected (wild-type and thymidine kinase deficient, *tk*$^-$) and viral gene transfected (HSV-1, HSV-2 or VZV thymidine kinase positive, *tk*$^+$) FM3A (Table 4), and OST (osteosarcoma; Table 5) cells have been investigated. The arabinouridines TMSEAU and EAU, as expected, did not inhibit (IC_{50} ≥

Table 3. Activity of 5-Ethynyl Analogs of Arabino/deoxy-uridine against Varicella-Zoster Virus in Human Embryonic Lung Cells

	antiviral activity[a] EC_{50} (μM)				cytotoxicity/cytostatic activity (μM)	
	TK^+ VZV		TK^- VZV			
cmpd	OKA strain	YS strain	07/1 strain	YS/R strain	cell morphology[b] (MCC)	cell growth[c] (CC_{50})
TMSEAU	0.48	0.33	>50/>50	>50/>50	200	127
EAU	1.0	0.29	>50/>50	>50/>50	200	>200
IEAU-TA	8.5	3.1	>20	>20	50	20
TMSEDU	0.26	0.15	0.69	0.90	>200	0.18
EDU	0.16	0.11	0.81	0.75	>200	2.5
IEDU	0.18	0.11	0.89	0.78	200	0.76
brivudin[d]	0.003	0.005	92	115	>100	>100
sorividine[d,e]	0.0001	0.0001	>57	>57	>50	>50

[a] Inhibitory compound concentration required to reduce virus plaque formation by 50%. Virus input was 20 plaque forming units (PFU). Data from two separate experiments are listed. [b] Minimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology. [c] Cytotoxic concentration required to reduce cell growth by 50%. [d] Data taken from the literature.[22] [e] (*E*)-5-(2-Bromovinyl)uridine.

Table 4. Inhibitory Effects of 5-Ethynyl Analogs of Arabino/deoxy-uridine on the Proliferation of Murine Mammary Carcinoma (FM3A) Cell Lines Transfected with the HSV-1 or HSV-2 Thymidine Kinase Gene[a]

cmpd	FM3A/O	FM3A*tk*$^-$	FM3A*tk*$^-$/HSV-1*tk*$^+$	FM3A*tk*$^-$/HSV-2*tk*$^+$
TMSEAU	≥250	≥250	3.8 ± 0.81	0.29 ± 0.07
EAU	≥250	≥250	3.9 ± 1.64	0.30 ± 0.06
IEAU-TA	18 ± 0.06	20 ± 3.4	5.4 ± 0.02	0.96 ± 0.32
TMSEDU	0.23 ± 0.04	≥250	0.16 ± 0.02	0.48 ± 0.14
EDU	0.18 ± 0.02	≥250	0.13 ± 0.02	0.39 ± 0.16
IEDU	0.30 ± 0.08	87 ± 37	0.16 ± 0.02	0.35 ± 0.17
brivudin[b]	13 ± 0.1	0.19 ± 0.15	0.004 ± 0.001	0.002 ± 0.001

[a] IC_{50} (μM), 50% inhibitory compound concentration required to inhibit FM3A cell proliferation by 50%; mean value (±s.d., $n \geq 3$). [b] Data taken from the literature.[23]

Table 5. Cytostatic Activity of 5-Ethynyl Analogs of Arabino/deoxy-uridine Against the Osteosarcoma Cell Lines OST*tk*$^-$, OST*tk*$^-$/VZV*tk*$^+$-a and OST*tk*$^-$/VZV*tk*$^+$-b Cells[a]

cmpd	OST*tk*$^-$	OST*tk*$^-$/VZV*tk*$^+$-a	OST*tk*$^-$/VZV*tk*$^+$-b
TMSEAU	≥176	0.75 ± 0.12	1.9 ± 0.33
EAU	≥250	0.71 ± 0.13	2.2 ± 0.93
IEAU-TA	5.40 ± 1.00	3.6 ± 0.22	3.9 ± 0.13
TMSEDU	23 ± 6.02	0.041 ± 0.044	0.20 ± 0.13
EDU	23 ± 5.62	0.083 ± 0.016	0.16 ± 0.04
IEDU	34 ± 22.86	0.063 ± 0.016	0.12 ± 0.02
brivudin	≥250	0.60 ± 0.33	0.35 ± 0.15
sorivudine	≥250	0.15 ± 0.09	0.20 ± 0.09

[a] IC_{50} (μM), compound concentration required to inhibit cell proliferation by 50%; mean value (±s.d., $n \geq 3$). OST*tk*$^-$/VZV*tk*$^+$-a and OST*tk*$^-$/VZV*tk*$^+$-b are two different, independently obtained, VZV*tk* gene-transfected OST*tk*$^+$ cell lines.

250 μM) wild-type FM3A/O or FM3A*tk*$^-$ (cells deficient in cytosol *tk*) cells, but they did inhibit proliferation of FM3A*tk*$^-$/HSV-1*tk*$^+$ (IC_{50} = 3.8 to 4.0 μM range) and FM3A*tk*$^-$/HSV-2*tk*$^+$ (0.29 to 0.30 μM range) cells (Table 4). The 2′-deoxyuridines TMSEDU, EDU, and IEDU did not appreciably inhibit FM3A*tk*$^-$ cell proliferation (IC_{50} = 87 to ≥250 μM range), but they markedly did inhibit FM3A*tk*$^-$/HSV-1*tk*$^+$ (IC_{50} = 0.13 to 0.16 μM range) and FM3A*tk*$^-$/HSV-2*tk*$^+$ (0.35 to 0.48 μM range) cell proliferation. However, in contrast with the arabinouridines, the 2′-deoxyribosides TMSDU, EDU, and IEDU were also highly cytostatic to wild-type FM3A/O cells (CC_{50} = 0.18 to 0.30 μM range). A similar antiproliferative activity was also observed for HEL cells (see Tables 2 and 3; CC_{50} = 0.18 to 2.5 μM). Similar cytostatic activity profiles were observed using osteosarcoma cells transfected with the VZV*tk* gene (see Table 5). For example, the arabinouridines TMSEAU and EAU were inactive against OST*tk*$^-$ cells, but they were potent inhibitors of cell proliferation of two VZV*tk* gene-transfected cell lines (OST*tk*$^-$/VZV*tk*$^+$-a, IC_{50} = 0.71 to 0.75 μM range and OST*tk*$^-$/VZV*tk*$^+$-b, IC_{50} = 1.9 to 2.2 μM range). The 2′-deoxyuridines TMSEDU, EDU, and IEDU were weak inhibitors of thymidine kinase-deficient cells (OST*tk*$^-$, IC_{50} = 22 to 34 μM range), as expected, but they are extremely potent inhibitors of the *tk* gene-transfected cell lines OST*tk*$^-$/VZV*tk*$^+$-a (IC_{50} = 0.041 to 0.083 μM range) and OST*tk*$^-$/VZV*tk*$^+$-b (IC_{50} = 0.12 to 0.20 μM range). These latter data are consistent with an earlier study that showed that EDU is a more potent inhibitor than EAU of VZV-infected cells.[9] The observation that IEDU-TA is more cytostatic to E_6SM, HEL, and FM3A/O cells than TMSEAU and EAU could be due to its greater lipophilic character that may enhance its ability to cross the cell membrane by passive diffusion. The fact that IEAU-TA exhibits similar inhibitory effects against wild-type FM3A, transfected FM3A/HSV-1*tk*$^+$ or FM3A/HSV-2*tk*$^+$ cells (Table 4), and OST*tk*$^-$ and OST*tk*$^-$/VZV*tk*$^+$ cells (Table 5) is in agreement with the belief that the high cellular toxicity may be due to its high lipophilic character and lack of significant further intracellular or extracellular metabolization by HSV-1, HSV-2, or VZV TK.

The differences in cytostatic and cytotoxic activity of the arabinouridine versus the 2′-deoxyuridine derivatives are striking. Both series of compounds were not cytotoxic. That means that in metabolically poorly active (resting, confluent) cell cultures, the compounds are not converted to potentially toxic metabolites and the compounds are not incorporated into DNA due to lack of any significant DNA synthesis in these cells. However, in actively growing (S-phase) thymidine kinase-competent cell cultures, the arabinouridines were poorly cytostatic, whereas the 2′-deoxyuridine counterparts were highly cytostatic. These findings suggest that the 2′-deoxyuridines are good substrates for cytosolic TK-1 and efficiently converted to their active metabolites in the proliferating cells. Targets for cytostatic activity may be TS and incorporation into DNA. Indeed, it has been demonstrated earlier that EDU-5′-monophosphate is a potent inhibitor of TS, resulting in efficient inhibition of cell proliferation.[24] TMSEDU and IEDU may have a similar mechanism of antiproliferative action. Our data also revealed that the arabinouridines are likely a poor substrate for cytosolic TK-1, for one of the next phosphorylating (nucleotide kinase) enzymes, and poorly inhibit their eventual cytostatic target (i.e., TS or DNA synthesis). Instead, they are well-recognized by the viral TKs, resulting in highly selective antivirals that can also be of use as radiopharmaceuticals to monitor viral *tk* gene expression in combined gene/chemotherapy of cancer.

Conclusions

A group of 5-alkynyl analogs of arabinouridine and 2′-deoxyuridine were synthesized to evaluate their ability to inhibit viral infection and their potential application for imaging/monitoring viral gene thymidine kinase expression in the gene therapy of cancer. In vitro SARs indicate that (i) high cellular toxicity is a deterrent to the potential use of the highly potent 5-alkynyl-2′-deoxyuridines (TMSEDU, EDU) to treat CMV infection and (ii) the high potency of the 5-trimethylsilylethynyl analogs of arabinouridine (TMSEAU) against osteosarcoma or mammary carcinoma VZVtk^+ and HSV tk^+ gene-transfected cells, relative to VZVtk^- or HSV tk^- cells, suggests that these precursors may be suitable candidates for radioiodination using electrophilic radioiodine to prepare the radiopharmaceuticals [^{124}I]-IEAU and [^{124}]-IEDU to monitor viral gene expression during gene therapy cancer protocols.[25]

Experimental Section

General. All chemicals and solvents used in this study were purchased from Aldrich Chemical. 1-(2,3,5-Tri-*O*-acetyl-β-D-arabinofuranosyl)-5-[2-(trimethylsilyl)ethynyl]uracil (**1**) was prepared according to a literature procedure.[17] Reactions requiring anhydrous conditions were performed under an atmosphere of argon. All solvents were dried by standard methods[26] and distilled just prior to use or were purchased as anhydrous solvents in Sure-Sealed bottles. Tetrahydrofuran was dried over sodium and benzophenone. Dichloromethane, triethylamine, and acetonitrile were dried over calcium hydride. Melting points were determined with a Thomas−Hoover capillary apparatus. Flash column chromatography was performed on silica gel 60 (E. Merck, 230−400 mesh). All columns were dry packed and eluted using positive air pressure, as recommended by Still et al.[27] A Bruker AM-300 NMR spectrometer was used to acquire ^{1}H NMR spectra with TMS as internal standard. Coupling constant (*J*) values are estimated in hertz (Hz), and spin multiples are given as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad). Microanalyses determined for C, H, and N were within ±0.4% of theoretical values.

1-(β-D-Arabinofuranosyl)-5-[2-(trimethylsilyl)ethynyl]uracil (2, TMSEAU). In an argon purged flask, TMSEAU-TA (**1,** 0.27 g, 0.58 mmol) was dissolved in anhydrous MeOH (5 mL). A solution of 0.05 N NaOMe in MeOH (46 mL) was added, and the reaction was allowed to proceed with stirring at 25 °C for 1 h. The pH of the solution was adjusted to pH 5−6 using prewashed Amberlite IR-120 acidic resin, and the mixture was filtered through a Celite pad. Removal of the solvent from the filtrate in vacuo furnished a residue that was purified by silica gel flash column chromatography using $CHCl_3$/MeOH (4:1, v/v) as eluent to afford TMSEAU (**2**; 114 mg, 81%) as a white crystalline solid, mp 115−118 °C; ^{1}H NMR (DMSO-d_6) δ 11.65 (s, 1H, N*H*), 7.93 (s, 1H, H-6), 5.95 (d, *J* = 4.5 Hz, 1H, H-1′), 5.61 (d, *J* = 5.0 Hz, 1H, C-2′ O*H*), 5.48 (d, *J* = 4.5 Hz, 1H, C-3′ OH), 5.12 (t, *J* = 5.3 Hz, 1H, C-5′ O*H*), 4.00 (br q, *J* = 4.4 Hz, 1H, H-2′), 3.90 (br q, *J* = 3.9 Hz, 1H, H-3′), 3.73 (br q, *J* = 3.4 Hz, 1H, H-4′), 3.62 (br m, 2H, C-5′), 0.18 [s, 9H, -Si(CH_3)$_3$]; Exact mass (HRMS) calcd for $C_{14}H_{20}N_2O_6Si$, 340.1091; found, 340.1078. Anal. ($C_{14}H_{20}N_2O_6Si$) C, H, N.

1-(β-D-Arabinofuranosyl)-5-ethynyluracil (3, EAU). A solution of TMSEAU (**2**, 0.27 g, 0.58 mmol) in benzene (6.0 mL) was added to a solution of K_2CO_3 (70 mg, 0.63 mmol) in MeOH (12.0 rnL) with stirring at 25 °C, and the reaction was allowed to proceed for 6 h. Removal of the solvent in vacuo afforded a residue that was purified by silica gel column flash chromatography using $CHCl_3$/MeOH (4:1, v/v) as eluent to yield EAU (**3**) as a white solid (0.14 g, 90%), mp > 230 °C (dec) [lit[9] mp > 250 °C]; ^{1}H NMR (DMSO-d_6) δ 11.70 (s, 1H, N*H*), 7.98 (s, 1H, H-6), 5.98 (d, *J* = 4.5 Hz, 1H, H-1′), 5.62 (d, *J* = 5.3 Hz, 1H, C-2′ OH), 5.48 (d, *J* = 4.5 Hz, 1H C-3′ OH), 5.14 (t, *J* = 5.1 Hz, 1H, C-5′ OH), 4.08 (s, 1H, acetylene proton), 3.98 (dd, *J* = 8.8, 4.5 Hz, 1H, H-2′), 3.90 (br q, 1H, *J* = 4.4, 3.9 Hz, H-3′), 3.73 (br q, *J* = 5.4, 4.4 Hz, 1H, H-4′), 3.59 (br m, 2H, H-5′).

1-(2,3,5-Tri-*O*-acetyl-β-D-arabinofuranosyl)-5-(2-iodoethynyl)-uracil (4, IEAU-TA). $AgNO_3$ (15 mg, 0.086 mmol) and *N*-iodosuccinimide (136 mg, 0.60 mmol) were added to a solution of TMSEAU-TA (200 mg, 0.43 mmol) in anhydrous DMF (4 mL). The reaction flask was covered with aluminum foil and the reaction was allowed to proceed at 25 °C with stirring for 3 h. The reaction flask was then immersed in an ice bath, distilled water (15 mL) was added, and the mixture was stirred for 5 min. This mixture was extracted with chloroform (4 × 15 mL), the combined chloroform extracts were washed with brine (3 × 50 mL), and the chloroform fraction was dried (Na_2S0_4). Removal of the solvent in vacuo gave IEAU-TA (**4**), which was recrystallized from MeOH to give yellow crystals (176 mg, 78%), mp 182−183 °C; ^{1}H NMR ($CDCl_3$) δ 8.56 (s, 1H, N*H*), 7.79 (s, 1H, H-6), 6.28 (d, 1H, *J* = 4.5 Hz, H-1′), 5.46 (m, 1H, H-2′), 5.17 (dd, 1H, *J* = 4.2, 2.8 Hz, H-3′), 4.44 (dd, *J* = 11.2, 4.2 Hz, 1H, H-5′), 4.41 (dd, *J* = 11.2, 5.7 Hz, 1H, H-5″), 4.22 (dd, *J* = 9.8, 4.2 Hz, 1H, H-4′), 2.19 (s, 3H, OAc), 2.17 (s, 3H, OAc), 2.15 (s, 3H, OAc). Anal. ($C_{17}H_{17}IN_2O_9$) C, H, N.

1-(2-Deoxy-β-D-ribofuranosyl)-5-[2-(trimethylsilyl)ethynyl]uracil (6, TMSEDU). 5-Iodo-2′-deoxyuridine (**5**, 1.0 g, 2.82 mmol) was dissolved in MeCN/Et_3N (66 mL of 1:1, v/v) under an argon atmosphere. Trimethylsilylacetylene (1.6 mL, 11.3 mmol), bis-(triphenylphosphine)palladium(II) chloride (42.2 mg, 0.60 mmol), and CuI (28 mg, 0.15 mmol) were added, a condenser was fitted to the flask, and the reaction flask was immersed into a preheated oil bath (50 °C). The reaction was allowed to proceed for 3.5 h, and the solvents were removed in vacuo to give a residue that was purified by silica gel flash column chromatography. Elution with $CHCl_3$/MeOH (9:1, v/v) afforded TMSEDU as a beige solid (0.73 g, 79%), mp 63−64 °C; ^{1}H NMR (MeOH-d_4) δ 8.52 (s, 1H, H-6), 6.21 (dd, *J* = 4.5, 4.5 Hz, 1H, H-1′), 4.38 (ddd, *J* = 5.5, 4.2, 3.9 Hz, 1H, H-3′), 3.92 (dd, *J* = 4.7, 3.1 Hz, 1H, H-4′), 3.81 (dd, *J* = 10.9, 3.1 Hz, 1H, H-5′), 3.72 (dd, *J* = 12.8, 3.7 Hz, 1H, H-5″), 2.28 (m, 2H, H-2′), 0.19 [s, 1H, Si(CH_3)$_3$]; Exact mass (HRMS) calcd for $C_{14}H_{20}N_2O_5Si$, 324.1141; found, 324.1134. Anal. ($C_{14}H_{20}N_2O_5Si$) C, H, N.

1-(2-Deoxy-β-D-ribofuranosyl)-5-ethynyluracil (7, EDU). A solution of NaOMe in MeOH (33 mL of 0.05 N) was added to a solution of TMSEDU (**6**, 159 mg, 0.34 mmol) in anhydrous MeOH (4 mL) under an argon atmosphere, and the reaction was allowed to proceed with stirring at 25 °C for 2 h. The pH of the solution was adjusted to pH 5−6 using prewashed Amberlite IR-120 acidic resin, and the mixture was filtered through a Celite pad. Removal of the solvent from the filtrate in vacuo furnished a residue that was purified by silica gel column flash chromatography using $CHCl_3$/MeOH (4:1, v/v) as eluent to yield EDU (**7**) as a white crystalline solid (80 mg, 87%), mp 178−180 °C (dec) [lit[9] 175 °C]; ^{1}H NMR (DMSO-d_6) δ 11.60 (s, 1H, N*H*), 8.30 (s, 1H, H-6), 6.11 (dd, *J* = 3.6, 3.6 Hz, 1H, H-1′), 5.28 (d, *J* = 4.5 Hz, 1H, C-3′ OH), 5.16 (t, *J* = 4.5 Hz, 1H, C-5′ OH), 4.24 (m, 1H, H-3′), 4.12 (s, 1H, acetylene proton), 3.79 (m, 1H, H-4′), 3.58 (m, 2H, H-5′), 2.10 (m, 2H, H-2′).

1-(2-Deoxy-β-D-ribofuranosyl)-5-(2-iodoethynyl)uracil (8, IEDU). TMSEDU (**6**, 1.2 g. 3.70 mmol) was added, under an argon atmosphere, to the reaction flask containing anhydrous DMF (24 mL), *N*-iodosuccinimide (1.17 g, 5.18 mmol), and $AgNO_3$ (130 mg, 0.74 mmol) with stirring. The reaction flask was covered with aluminum foil, and the reaction was allowed to proceed at 25 °C for 3 h with stirring. The solvent was coevaporated using toluene (3 × 25 mL), and the crude product was purified by silica gel flash column chromatography using $CHCl_3$/MeOH (9:1, v/v) as eluent. Recrystallization of the eluted product from MeOH−H_2O afforded IEDU (**8**, 56 mg, 40%) as a pale yellow solid, mp 140 °C (dec) [lit[20] mp 135−137 °C]; ^{1}H NMR (MeOH-d_4) δ 8.32 (s, 1H, H-6), 6.21 (dd, *J* = 3.2, 3.2 Hz, 1H, H-1′), 4.38 (ddd, *J* = 6.5, 5.9, 3.5 Hz, 1H, H-3′), 3.93 (q, *J* = 3.5 Hz, 1H, H-4′), 3.81 (dd, *J* = 12.0, 3.0 Hz, 1H, H-5′), 3.72 (dd, *J* = 12.0, 3.5 Hz, 1H, H-5″), 2.25 (m, 2H, H-2′). Anal. ($C_{l1}H_{l1}IN_2O_5$) C, H, N.

Calculation of Bond Angles and Distances. The C−C5−C6 bond angles and the interspatial distances between iodine and the C5 carbon atom in IEDU and IVDU (see structures in Figure 1) were calculated following PM3 geometry optimization using the Alchemy 2000 program (Version 2.0, Tripos Inc., St. Louis, MO).

Antiviral Activity Assays. The antiviral assays were based on an inhibition of virus-induced cytopathicity in E_6SM, HeLa, Vero, or HEL cell cultures, following previously established procedures.[28−31] HSV-1 (KOS), HSV-2 (G), vaccinia virus, vesicular stomatitis virus (VSV), the thymidine kinase-deficient HSV-1 TK^- (B2005), and HSV-1 TK^-/TK^+ (VMW 1837) strains were exposed to E_6SM cell cultures, parainfluenza-3 virus, reovirus-1, Sindbis virus, Coxsackie B4 virus, and Punta Toro virus to Vero cell cultures, respiratory syncytial virus to HeLa cell cultures, and CMV (AD-169, Davis), and VZV (YS, OKA), and the thymidine kinase-deficient VZV (07/1, YS/R) strains to HEL cell cultures. Briefly, confluent cell cultures in 96-well microtiter trays were incubated with 100 $CCID_{50}$ of virus, with one $CCID_{50}$ being the virus dose required to infect 50% of the cell cultures. After a 1 h virus adsorption period, residual virus was removed, and the cell cultures were incubated in the presence of varying concentrations of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures.

Cytostatic and Cytotoxic Activity Assays. The cytostatic assays for HEL cells were performed as follows. Aliquots (100 μL) of the HEL cell suspensions were added to the wells of a 96-well microtiter plate containing 100 μL of varying concentrations of the test compounds. After a 3-day incubation period at 37 °C in a humidified CO_2-controlled incubator, the number of viable cells was determined using a Coulter counter. Cytostatic activity was expressed as the compound concentration that reduced the number of viable cells by 50% (CC_{50}). The cytotoxicity measurements were based on a microscopically visible alteration of normal cell morphology (E_6SM, HeLa, Vero, HEL) or inhibition of cell growth (HEL), as previously described.[31]

The cytostatic activity assays for the various FM3A cell lines were as follows: 6×10^4 cells suspended in growth medium were allowed to proliferate in 200-μL wells of microtiter plates in the presence of 5-fold dilutions of the test compounds at 37 °C in a humidified CO_2-controlled atmosphere. After 48 h, the number of cells was counted in a Coulter counter. The IC_{50} value was defined as the compound concentration required to inhibit cell proliferation by 50%.

To evaluate the cytostatic activity of the nucleoside analogues against the osteosarcoma cell lines, 10^4 cells/well were plated in 96-well microtiter plates (Falcon) and allowed to adhere. Cells were subsequently incubated at 37 °C in a humidified CO_2-controlled atmosphere in the presence of 5-fold dilutions (in normal cell growth medium) of the compounds. After 3 days, the cells were detached with trypsin solution (Gibo) and counted in a Coulter counter (Coulter Electronics Ltd., Harpenden Hertz, U.K.). The IC_{50} was defined as the drug concentration required to inhibit cell proliferation by 50%.

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