

5-Alkynyl-2'-deoxyuridines: Chromatography-free synthesis and cytotoxicity evaluation against human breast cancer cells

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Abstract—Starting with 5-iodo-2'-deoxyuridine, a series of 5-alkynyl-2'-deoxyuridines (with *n*-propyl, cyclopropyl, 1-hydroxy-cyclohexyl, *p*-tolyl, *p*-*tert*-butylphenyl, *p*-pentylphenyl, and trimethylsilyl alkyne substituents) have been synthesized via the palladium-catalyzed (Sonogashira) coupling reaction followed by a simplified isolation protocol (76–94% yield). The cytotoxic activity of modified nucleosides against MCF-7 and MDA-MB-231 human breast cancer cells has been determined in vitro. 5-Ethynyl-2'-deoxyuridine, the only nucleoside in the series containing a terminal acetylene, is the most potent inhibitor with IC₅₀ (μM) 0.4 ± 0.3 for MCF-7 and 4.4 ± 0.4 for MDA-MB-231.

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1. Introduction

Modified nucleosides have acquired an important role as therapeutics.^{1–3} Cytotoxic nucleoside analogues were among the first chemotherapeutic agents to be introduced for the medical treatment of cancer.³ This family of compounds has grown to include a variety of purine and pyrimidine nucleoside derivatives with activity in both solid tumors and hematological malignancies. These agents behave as antimetabolites, compete with physiologic nucleosides, and consequently, interact with a large number of intracellular targets to induce cytotoxicity.⁴

Alkynyl modifications of uridines have been explored for diverse biochemical studies. A common approach is based upon the attachment of the active units via the ethynyl arm at position C-5 of the uracil base. Altered in such a way uridine nucleosides facilitate blue-to-red energy transfer (absorption–fluorescence emission), as well as when incorporated into oligonucleotides.⁵ 1-Ethynylpyrene derivatives help to organize the

helical-stacked arrangements along the major groove of duplex DNA.⁶ Alkynyl-modified dU:dA base pairs may stabilize duplexes and reinforce helices,⁷ or serve as tools for charge transfer excited-state dynamics DNA duplex studies,⁸ to name a few.

Medicinal-related activity of various 5-alkynyl pyrimidine analogues has been investigated for some time,^{9,10} including their oligonucleotides.¹¹ Recently potent inhibitory properties of mycobacteria were reported.^{12a} Although broad antiviral studies have been conducted, anticancer investigations of 5-alkynyl uridine analogues have been limited.^{1,12b}

Various synthetic routes to 5-alkynyl uridines have been reported,^{13,14} including a reaction of 5-trifluoromethanesulfonyloxy pyrimidine nucleosides with alkynes.¹⁵ The classical, effective, and most frequently used synthesis of 5-alkynyl uridines involves palladium-catalyzed (Sonogashira)¹⁶ coupling of unprotected 5-iodo-2'-deoxyuridine (**1**).^{14,17–19} This reaction is carried out under precisely controlled conditions, since an elevated temperature and the presence of metal/amine lead to a cyclization reaction yielding 2,3-dihydrofuro-[2,3-*d*]pyrimidin-2-one nucleoside (furopyrimidine).^{13c,20} Following the reported effect of additives,²¹ we elaborated a room temperature coupling procedure that includes

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triphenylphosphine and tetrabutylammonium iodide.^{22,23} Currently, for a somewhat larger scale, due to material economy and purification effectiveness a procedure with no additives was applied.

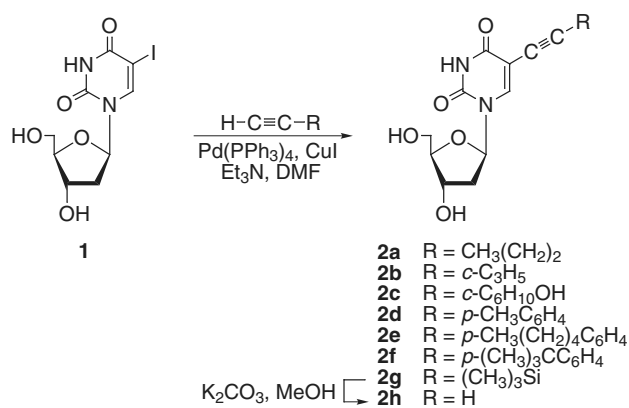
2. Results and discussion

2.1. Synthesis and characterization

The series of known and new 5-alkynyl-2'-deoxyuridines (**2**) was synthesized via Sonogashira coupling, as visualized in Scheme 1 (4.0 equiv of acetylene, 0.1 equiv of Pd(PPh₃)₄ and CuI, 2.0 equiv of Et₃N, DMF, 55 °C, 17 h). To provide a starting position for more detailed structure–activity relationship studies, diverse alkyne substituents R were selected, which include a linear alkyl chain (**2a**, R = *n*-propyl),^{13c} cycloalkyl (**2b**, R = cyclo-

propyl),²³ tertiary cycloalkanol (**2c**, R = 1-hydroxy-cyclohexyl), aromatic ring substituted with linear (**2d,e**, R = *p*-tolyl,²² *p*-pentylphenyl) and branched alkyl groups (**2f**, R = *p*-*tert*-butylphenyl),²³ and silicon (**2g**, R = trimethylsilyl).¹⁵ The nucleoside containing a terminal alkyne (**2h**, R = H)²⁴ was prepared by desilylation of **2g** with K₂CO₃/MeOH^{13c,25} or TBAF/THF.¹⁵

Practical preparative comments are shared below. TLC observation of the reaction under a UV 254 nm lamp (to determine completion) can be misleading due to the low fluorescence of the 5-iodo-2'-deoxyuridine **1**. Careful monitoring by ¹H NMR (H-6 signal) helped to establish reaction progress and revealed that conversion of **1** to its alkynyl derivatives **2** proceeded essentially in a quantitative manner. 5-Alkynyl uridines with unprotected hydroxyl groups are reportedly difficult to purify,²⁶ thus the yields are moderate.²⁷ Separation from ammonium salt is usually accomplished by protection/chromatography/deprotection,^{10a–d,26} quenching with ion-exchange resin,^{27,28} or with careful column chromatography. Thus, a convenient isolation protocol has significant practical value.²⁹ The alkynyl nucleosides **2a–g** were separated with good analytical purity from spent and unused reagents through a workup procedure that includes two subsequent crystallizations. After thorough removal of DMF, a post-reaction mixture was treated with methanol, to precipitate phosphine/palladium spent reagents.³⁰ Subsequently, after drying under vacuum, addition of chloroform precipitated the nucleoside whereas the ammonium salt (Et₃NHI) remained in solution.³¹ This protocol provided material without any detectable triethylammonium- or triphenylphosphine-derived impurities when analyzed by ¹H NMR. This workup procedure allows



Scheme 1. Synthesis of 5-alkynyl-2'-deoxyuridines **2a–h**.

Table 1. Preparation and cytotoxic activity of 5-alkynyl-2'-deoxyuridines **2a–h**

Compound (ref.)	R	Yield (%)	IC ₅₀ (μM)	
			MCF-7	MDA-MB-231
2a ^{13c}	CH ₃ CH ₂ CH ₂ –	87(53) ^a	>50	>50
2b		80	>50	>50
2c		94	>50	>50
2d		88	0.9 ± 0.2	>50
2e		76	37.0 ± 6.8	29.2 ± 5.0
2f		94(72) ^a	9.5 ± 1.4	>50
2g ¹⁵	(CH ₃) ₃ Si–	92(28) ^a	1.6 ± 1.3	4.2 ± 1.5
2h ²⁴	H–	— ^b	0.4 ± 0.3	4.4 ± 0.4
Cisplatin ^c	—	—	2.0 ± 0.3	4.0 ± 1.5
5-Fluorouracil ^c	—	—	4.8 ± 0.6	9.6 ± 0.3

^a Yield includes second crop obtained with the aid of column chromatography; yield from first crystallization in parentheses.

^b Prepared from **2g** by two independent desilylation methods.

^c Reference.

for the synthesis to be easily carried out on a somewhat larger scale (0.4–5 g). This procedure is dependent on the thorough removal of DMF, as this will effect a clean precipitation of the product. The yields are summarized in Table 1.

The alkynyl nucleosides **2b–f** were characterized by ^1H and ^{13}C NMR, IR, MS, and UV–vis spectroscopy. The characteristic NMR ($\text{DMSO}-d_6$) signals for **2b–f** include the ^1H H-6 signal (8.36–8.11 ppm), and ^{13}C signals of C-5 (99.6–98.3) and $\text{C}\equiv\text{C}$ (97.5–68.6 ppm). ^{13}C NMR C-1' and C-4' signals were distinguished using INEPT-based HETCOR correlation spectra. Comparison of ^1H and ^{13}C NMR signals for **2a–h** is provided in the Supplementary data. The mass spectra for **2b–f** exhibited intense molecular ions. Alkynyl uridines **2a,c–h** gave correct elemental analyses, mostly as hydrates. The representative ^1H and ^{13}C NMR spectra, given in the Supplementary data, illustrate the organic homogeneity of the compounds isolated without column chromatography.

2.2. Cytotoxicity

After completion of synthesis and characterization, the nucleosides were investigated for their antitumor activity in vitro using two human breast cancer cultures MCF-7 and MDA-MB-231 that are of highly different phenotypes: hormone-sensitive and hormone-insensitive, respectively. To reduce the presence of trace impurities such as metals, all samples were additionally passed through a silica gel pad before submission for activity evaluations.³² The experiments were carried out according to a modified established microtiter assay.^{33,34} Cisplatin and 5-fluorouracil were used as references.

Testing using the method described above indicated high activity of 5-ethynyl-2'-deoxyuridine **2h** ($\text{R}=\text{H}$). Observed IC_{50} were $0.4\text{ }\mu\text{M}$ (± 0.3) for MCF-7 and $4.4\text{ }\mu\text{M}$ (± 0.4) for MDA-MB-231, exceeding or matching the IC_{50} for cisplatin or 5-fluorouracil. These results, which stand out, can be attributed to the presence of the terminal alkynyl functional group, and were consistent for two samples of **2h** prepared independently from **2g** with the use of different deprotection reagents. However, internal aromatic-substituted alkyne *p*-tolylethynyl-2'-deoxyuridine **2d** also showed high potency with MCF-7 (IC_{50} $0.9\text{ }\mu\text{M}$ (± 0.2)), but no detectable potency with MDA-MB-231. Replacement of the aromatic ring system with a trimethylsilyl group also afforded a highly active compound **2g** which matches the activity of **2h** (IC_{50} $1.6\text{ }\mu\text{M}$ (± 1.3)/ $4.2\text{ }\mu\text{M}$ (± 1.5) for MCF-7/MDA-MB-231). Longer (more lipophilic) or more bulky aliphatic substituents in the aromatic ring led to significantly lower activity such as for compounds **2e** or **2f**. Usually less lipophilic nucleosides tend to be more active, whereas more lipophilic derivatives exhibit enhanced antiviral properties. It can be concluded in general that the compounds containing internal alkyne structures were of lower activity than **2h** and MCF-7 cells were more sensitive to the presence of the nucleosides than MDA-MB-231 cells. Within the group of internal alkynes those containing an aromatic ring or a trimethylsilyl group

attached to the alkyne (**2d–g**) gave better results than those with aliphatic substituents (**2a–c**), which were devoid of activity up to the highest concentration used. The results are summarized in Table 1.

The precise mechanism of action of these compounds is not documented. However, it could be anticipated that the agents acting as DNA polymerase inhibitors might affect cellular DNA synthesis. Enzymes that are involved in DNA replication and repair have been shown to serve as targets for anticancer nucleosides such as the deoxycytidine analogue gemcitabine. Other possible modes of action involve an interaction with deoxynucleoside kinases, which are required for activation of deoxynucleosides as antiviral or anticancer drugs, or a behavior as antimetabolites and a subsequent incorporation into the DNA. Furthermore, the inhibition of thymidylate synthase, which catalyzes the reductive methylation of deoxyuridine monophosphate into deoxythymidine monophosphate, must be taken into account based on the fact that this is one of the major biochemical effects of the cytostatic agent 5-fluorouracil.^{3,35–38} The fact that the target compounds displayed higher activity in the MCF-7 cell line than in the MDA-MB-231 cell line indicates the presence of a specific molecular target or carrier system for these agents in the MCF-7 cell line.

3. Conclusion

In conclusion, an effective purification protocol for 5-alkynyl-2'-deoxyuridines **2** that avoids the use of column chromatography, ion exchange chromatography, or protection/deprotection steps was established. The series of modified nucleosides **2** was tested for antiproliferation properties against MCF-7 and MDA-MB-231 human mammary carcinoma cell lines. A terminal alkyne, 5-ethynyl-2'-deoxyuridine **2h**, showed the highest potency exceeding cisplatin and 5-fluorouracil. Further testing of **2h** toward other cancer cell lines would be of interest.

We think that the structure–activity relationship studies that will involve incorporation of CH_2 spacers between the uracil base and alkyne, to follow an established active fragment of $\text{CH}_2\text{C}\equiv\text{CH}$ (as the $\text{Co}_2(\text{CO})_6$ complex),³⁹ may further improve the activity.

The reported nucleosides are also converted into their organometallic analogues, and the synthesis and cytotoxic activity of dicobalt hexacarbonyl derivatives will be reported on in due course. Moreover, more extensive structure–activity relationship studies concerning different substituents in the aromatic ring as well as more detailed investigations on the molecular drug targets are envisaged.

4. Experimental

4.1. General

Commercial chemicals were treated as follows: DMF distilled from CaH_2 and degassed (freeze and thaw)

three times prior to use; Et₃N distilled from P₂O₅. 5-Iodo-2'-deoxyuridine (Berry&Associates), 1-pentyne, cyclopropylacetylene, 1-ethynyl-1-cyclohexanol, 4-ethynyl-toluene, 4-(*tert*-butyl)phenylacetylene, and (trimethylsilyl)acetylene (GFS), Pd(PPh₃)₄ (Pressure Chemical), 1-ethynyl-4-pentylbenzene and CuI 99.999% (Aldrich), silica gel (J.T. Baker, 60–200 mesh), and TLC plates Analtech GF, cat. no. 2521 or Merck 60, cat. No. 5715, used as received. Other materials not listed were used as received.

IR and UV–vis spectra were recorded on a Bio-Rad FTS-175C and Cary 50 spectrometers. UV–vis absorbances are in nm (ϵ , M^{−1}cm^{−1}). NMR spectra were obtained on a Bruker Avance DPX-200 spectrometer (¹H of 200 MHz and ¹³C of 50 MHz). Chemical shifts (δ) values are in ppm and coupling constants (*J*) values are in Hz. Mass spectra were recorded on a Finnigan MAT 95 or Micromass ZQ instruments; *m/z* for most intense peak of isotope envelope. Microanalyses were conducted by Atlantic Microlab.

4.2. 5-Alkynyl-2'-deoxyuridines 2a–f; general procedure (1 mmol scale)

A Schlenk flask was charged with 5-iodo-2'-deoxyuridine (1.18 mmol), Pd(PPh₃)₄ (0.118 mmol), CuI (0.118 mmol), DMF (5 mL), Et₃N (2.4 mmol), and acetylene (4.7 mmol). The yellow mixture was stirred at 55 °C for 17 h (oil bath; ¹H NMR showed complete conversion of the substrate). Solvent was removed (by oil pump vacuum) and the residue was extracted (ultrasonicated) with MeOH (6 mL). The solid was filtered off and washed with MeOH (3 × 3 mL). The solvent was removed from combined filtrates by rotary evaporation. The residue (yellow oil) was kept under oil pump vacuum for 2 h, dissolved (ultrasonicated) in CHCl₃ (5 mL),³¹ and kept at −6 °C (freezer) for 12 h. The precipitate was filtered off and washed with cold CHCl₃ (3 × 3 mL). The product was dried by oil pump vacuum for 6 h to give 2.

4.2.1. 5-Pent-1-yn-1-yl-2'-deoxyuridine (2a).^{13c} From 5-iodo-2'-deoxyuridine (1.00 g, 2.82 mmol), Pd(PPh₃)₄ (0.326 g, 0.282 mmol), CuI (0.0539 g, 0.282 mmol), DMF (5 mL), Et₃N (0.82 mL, 5.65 mmol), and 1-pentyne (1.40 mL, 14.1 mmol). The yellow mixture was stirred at 40 °C for 38 h. Crystallization from CHCl₃ gave **2a** as a white powder (0.440 g, 1.50 mmol, 53%). A second crop, obtained after concentration and silica gel column chromatography (15 × 2.5 cm; CHCl₃ → CHCl₃/MeOH 95:5), increased the yield to a total of 0.723 g (2.46 mmol, 87%).

NMR (DMSO-*d*₆): ¹H data matched those reported earlier.^{13c} ¹³C 161.8 (d, *J* = 9.4, C-4), 149.5 (d, *J* = 8.1, C-2), 142.7 (d, *J* = 182.1, C-6), 99.1 (s, C-5), 93.1 (m, dU-C≡C), 87.5 (d, *J* = 144.9, C-4'), 84.6 (d, *J* = 163.4, C-1'), 73.0 (d, *J* = 4.4, dU-C≡C), 70.2 (d, *J* = 148.4, C-3'), 61.0 (t, *J* = 140.3, C-5'), 40.0 (t, *J* = 134.0, C-2'), 21.7 (t, *J* = 130.0, C-2''), 20.8 (t, *J* = 130.8, C-1''), 13.4 (q, *J* = 124.5, C-3'').

4.2.2. 5-(Cyclopropylethynyl)-2'-deoxyuridine (2b). From 5-iodo-2'-deoxyuridine (0.418 g, 1.18 mmol), Pd(PPh₃)₄ (0.1364 g, 0.1180 mmol), CuI (0.0225 g, 0.118 mmol), DMF (5 mL), Et₃N (0.34 mL, 2.4 mmol), and cyclopropylacetylene (0.40 mL, 4.7 mmol). The yellow mixture was stirred at 55 °C for 17 h. White powder (0.277 g, 0.948 mmol, 80%). Calcd for C₁₄H₁₆N₂O₅·0.75H₂O: C, 54.99; H, 5.77. Found: C, 54.64; H, 5.31. IR (ν , cm^{−1}, KBr) 3414 br s, 2235 w, 1709 vs, 1689 vs, 1630 m. UV–vis (CH₃OH, 4.7 × 10^{−5} M) 226 sh (18000), 296 (15000). MS (ES⁺, KCl, MeOH) 623 ((2M+K)⁺, 61%), 607 ((2M+Na)⁺, 26%), 585 ((2M+H)⁺, 8%), 331 ((M+K)⁺, 100%), 315 ((M+Na)⁺, 19%), 293 ((M+H)⁺, 15%); no other peaks above 200 of >4%.

NMR (DMSO-*d*₆): ¹H 11.55 (s, 1H, N-3), 8.11 (s, 1H, H-6), 6.10 (t, *J* = 5.6, 1H, H-1'), 5.23 (d, *J* = 4.2, 1H, OH-3'), 5.10 (t, *J* = 4.9, 1H, OH-5'), 4.29–4.16 (m, 1H, H-3'), 3.83–3.74 (m, 1H, H-4'), 3.68–3.48 (m, 2H, H-5'), 2.18–2.03 (m, 2H, H-2'), 1.55–1.40 (m, 1H, H-1''), 0.92–0.59 (2m, 2 × 2H, H-2'' and H-3''); ¹³C 162.5 (d, *J* = 9.2, C-4), 150.1 (d, *J* = 6.2, C-2), 143.6 (d, *J* = 183.7, C-6), 99.6 (d, *J* = 1.9, C-5), 96.9 (d, *J* = 2.6, dU-C≡C), 88.2 (d, *J* = 146.1, C-4'), 85.2 (d, *J* = 163.3, C-1'), 70.8 (d, *J* = 148.8, C-3'), 68.6 (d, *J* = 4.3, dU-C≡C), 61.6 (t, *J* = 140.3, C-5'), 40.1 (t, *J* = 132.9, C-2'), 9.0 (t, *J* = 164.3, C-2'' and C-3''), 0.5 (d, *J* = 168.7, C-1'').

4.2.3. 5-[(1-Hydroxycyclohexyl)ethynyl]-2'-deoxyuridine (2c). From 5-iodo-2'-deoxyuridine (0.418 g, 1.18 mmol), Pd(PPh₃)₄ (0.1364 g, 0.1180 mmol), CuI (0.0225 g, 0.118 mmol), DMF (5 mL), Et₃N (0.34 mL, 2.4 mmol), and 1-ethynyl-1-cyclohexanol (0.59 mL, 4.7 mmol). The yellow mixture was stirred at 50 °C for 7 h. White powder (0.390 g, 1.11 mmol, 94%). Calcd for C₁₇H₂₂N₂O₆·1/3H₂O: C, 57.29; H, 6.41. Found: C, 57.64; H, 6.29. IR (ν , cm^{−1}, KBr) 3356 br s, 2221 vw, 1710 vs, 1674 s. UV–vis (MeOH, 3.4 × 10^{−5} M) 232 (7400), 291 (9400). MS (ES⁺, MeOH) 739 ((2M+K)⁺, 15%), 723 ((2M+Na)⁺, 100%), 389 ((M+K)⁺, 23%), 373 ((M+Na)⁺, 97%); no other peaks above 100 of >10%.

NMR (DMSO-*d*₆): ¹H 11.55 (s, 1H, N-3), 8.14 (s, 1H, H-6), 6.10 (t, *J* = 6.5, 1H, H-1'), 5.32 (s, 1H, HO-C₆H₁₀), 5.22 (d, *J* = 4.2, 1H, OH-3'), 5.07 (t, *J* = 4.7, 1H, OH-5'), 4.30–4.16 (m, 1H, H-3'), 3.83–3.74 (m, 1H, H-4'), 3.64–3.52 (m, 2H, H-5'), 2.20–2.05 (m, 2H, H-2'), 1.87–1.68 (m, 2H, *c*-C₆H₁₀), 1.68–1.33 (m, 7H, *c*-C₆H₁₀), 1.30–1.05 (m, 1H, *c*-C₆H₁₀); ¹³C 161.6 (d, *J* = 9.4, C-4), 149.5 (d, *J* = 8.0, C-2), 143.0 (d, *J* = 183.0, C-6), 98.6 (d, *J* = 4.8, C-5), 97.5 (s, dU-C≡C), 87.6 (d, *J* = 145.0, C-4'), 84.7 (d, *J* = 168.3, C-1'), 75.3 (d, *J* = 4.5, dU-C≡C), 70.1 (d, *J* = 148.4, C-3'), 67.1 (s, C-1''), 60.9 (t, *J* = 140.6, C-5'), 40.2 (C-2'),⁴⁰ 39.7 (C-2''),⁴⁰ 24.9 (t, *J* = 120.2, C-4''), 22.7 (t, *J* = 124.6, C-3'').

4.2.4. 5-*p*-Tolylethynyl-2'-deoxyuridine (2d). From 5-iodo-2'-deoxyuridine (3.000 g, 8.472 mmol), Pd(PPh₃)₄ (0.9790 g, 0.8472 mmol), CuI (0.1618 g, 0.8472 mmol), DMF (15 mL), Et₃N (2.45 mL, 16.9 mmol), and

4-ethynyltoluene (3.65 mL, 28.8 mmol). The yellow mixture was stirred at 40 °C for 20 h. White powder (2.56 g, 7.48 mmol, 88%). Calcd for $C_{18}H_{18}N_2O_5$: C, 63.15; H, 5.30. Found: C, 62.73; H, 5.58. IR (ν , cm^{-1} , KBr) 3433 br s, 3190 m br, 2220 vw, 1722 vs, 1669 vs, 1652 vs, 1618 m, 817 m. UV–vis (CH_3OH , 5.5×10^{-5} M) 252 (9500), 266 (11000), 280 (9900), 311 (12000). MS (FAB, gly, positive) 435 ((M+gly+1)⁺, 10%), 343 ((M+1)⁺, 42%), 227 ((M–C \equiv CC $_6$ H $_4$ CH $_3$)⁺, 100%); no other peaks above 200 of >6%; (negative) 433 ((M+gly–1)[–], 13%), 341 ((M–1)[–], 65%), 227 ((M–C \equiv CC $_6$ H $_4$ CH $_3$ –2)[–], 100%); no other peaks above 200 of >9%.

NMR (DMSO- d_6):⁴¹ 1H 11.68 (s, 1H, N-3), 8.33 (s, 1H, H-6), 7.35 (d, J = 8.1, 2H, *o*-C $_6$ H $_4$ C \equiv C), 7.21 (d, J = 8.1, 2H, *m*-C $_6$ H $_4$ C \equiv C), 6.13 (t, J = 6.5, 1H, H-1'), 5.27 (d, J = 3.8, 1H, OH-3'), 5.17 (t, J = 4.3, 1H, OH-5'), 4.26 (q, J = 4.2, 1H, H-3'), 3.81 (q, J = 3.6, 1H, H-4'), 3.71–3.52 (m, 2H, H-5'), 2.33 (s, 3H, CH $_3$), 2.16 (t, J = 5.6, 2H, H-2'); ^{13}C 162.1 (d, J = 9.3, C-4), 149.9 (d, J = 7.5, C-2), 143.6 (dd, J = 181.2, 2.2, C-6), 138.3 (m, *p*-C $_6$ H $_4$ C \equiv C), 131.1 (dd, J = 162.3, 6.2, *o*-C $_6$ H $_4$ C \equiv C), 129.4 (dq, J = 159.6, 5.4, *m*-C $_6$ H $_4$ C \equiv C), 119.5 (t, J = 8.2, *i*-C $_6$ H $_4$ C \equiv C), 98.3 (s, C-5), 91.8 (t, J = 5.4, dU–C \equiv C), 87.6 (d, J = 147.9, C-4'), 84.8 (d, J = 170.8, C-1'), 82.1 (d, J = 5.5, dU–C \equiv C), 69.9 (d, J = 148.0, C-3'), 60.9 (t, J = 140.4, C-5'), 40.2 (t, J = 133.5, C-2'), 21.1 (qt, J = 126.2, 4.3, CH $_3$).

4.2.5. 5-[(*p*-Pentylphenyl)ethynyl]-2'-deoxyuridine (2e). From 5-iodo-2'-deoxyuridine (5.01 g, 14.1 mmol), Pd(PPh $_3$) $_4$ (1.63 g, 1.41 mmol), CuI (0.269 g, 1.41 mmol), DMF (30 mL), Et $_3$ N (4.00 mL, 28.0 mmol), and 1-ethynyl-4-pentylbenzene (6.32 mL, 32.5 mmol). The yellow mixture was stirred at 40 °C for 35 h. White powder (4.29 g, 10.8 mmol, 76%). Calcd for $C_{22}H_{26}N_2O_5$: C, 66.32; H, 6.58. Found: C, 66.20; H, 6.57. IR (ν , cm^{-1} , KBr) 3418 br s, 2224 vw, 1699 vs, 1678 vs. UV–vis (CH_3OH , 2.8×10^{-5} M) 253 (18000), 266 (21000), 281 (18000), 311 (24000). MS (ES⁺, MeOH) 835 ((2M+K)⁺, 15%), 819 ((2M+Na)⁺, 100%), 797 ((2M+H)⁺, 11%), 453 (unassigned, 51%), 437 ((M+K)⁺, 32%), 421 ((M+Na)⁺, 80%), 399 ((M+H)⁺, 8%); no other peaks above 300 of >7%.

NMR (DMSO- d_6):⁴¹ 1H 11.65 (s, 1H, N-3), 8.36 (s, 1H, H-6), 7.32 (d, J = 8.1, 2H, *o*-C $_6$ H $_4$ C \equiv C), 7.14 (d, J = 8.1, 2H, *m*-C $_6$ H $_4$ C \equiv C), 6.13 (t, J = 6.3, 1H, H-1'), 5.24 (d, J = 4.2, 1H, OH-3'), 5.14 (t, J = 4.5, 1H, OH-5'), 4.26 (p, J = 3.5, 1H, H-3'), 3.86–3.75 (m, 1H, H-4'), 3.71–3.51 (m, 2H, H-5'), 2.50 (t, J = 7.2, 2H, H-1''), 2.14 (t, J = 5.1, 2H, H-2'), 1.48 (p, J = 6.7, 2H, H-2''), 1.30–1.10 (m, 4H, H-3'', H-4''), 0.77 (t, J = 6.3, 3H, H-5''); ^{13}C 161.4 (d, J = 9.3, C-4), 149.3 (d, J = 8.0, C-2), 143.4 (d, J = 184.3, C-6), 143.1 (s, *p*-C $_6$ H $_4$ C \equiv C), 131.0 (dd, J = 162.3, 6.1, *o*-C $_6$ H $_4$ C \equiv C), 128.4 (dd, J = 159.2, 5.4, *m*-C $_6$ H $_4$ C \equiv C), 119.5 (t, J = 8.2, *i*-C $_6$ H $_4$ C \equiv C), 98.4 (s, C-5), 91.9 (t, J = 4.7, dU–C \equiv C), 87.5 (d, J = 147.6, C-4'), 84.7 (d, J = 169.5, C-1'), 81.6 (d, J = 5.5, dU–C \equiv C), 70.0 (d, J = 148.5, C-3'), 60.8 (t, J = 140.3, C-5'), 40.2 (t, J = 133.7, C-2'), 34.9 (t, J = 113.2, C-1''), 30.7 (t, J = 120.3, C-3''), 30.3

(t, J = 120.1, C-2''), 21.8 (t, J = 124.3, C-4''), 13.7 (q, J = 123.2, C-5'').

4.2.6. 5-[(4-*tert*-Butylphenyl)ethynyl]-2'-deoxyuridine (2f). From 5-iodo-2'-deoxyuridine (1.00 g, 2.82 mmol), Pd(PPh $_3$) $_4$ (0.326 g, 0.282 mmol), CuI (0.0539 g, 0.282 mmol), DMF (5 mL), Et $_3$ N (0.82 mL, 5.65 mmol), and 4-(*t*-butyl)phenylacetylene (1.5 mL, 8.47 mmol). The yellow mixture was stirred at 40 °C for 25 h. Crystallization from CHCl $_3$ gave **2f** as a white powder (0.780 g, 2.03 mmol, 72%). A second crop, obtained after concentration and silica gel column chromatography (15 \times 2.5 cm; CHCl $_3$ \rightarrow CHCl $_3$ /MeOH 95:5), increased the yield to a total of 1.02 g (2.65 mmol, 94%). Calcd for $C_{21}H_{24}N_2O_5 \cdot 1.5H_2O$: C, 60.64; H, 5.82. Found: C, 60.71; H, 5.90. IR (ν , cm^{-1} , KBr) 3429 br s, 2227 vw, 1701 vs br, 835 w. UV–vis (CH_3OH , 4.9×10^{-5} M) 253 (13000), 266 (15000), 280 (13000), 309 (16000). MS (MeOH) 791 ((2M+Na)⁺, 78%), 407 ((M+Na)⁺, 100%), 385 ((M+H)⁺, 12%); no other peaks of >12%.

NMR (DMSO- d_6):⁴¹ 1H 11.69 (s, 1H, N-3), 8.35 (s, 1H, H-6), 7.41 (s, 4H, C $_6$ H $_4$), 6.13 (t, J = 6.3, 1H, H-1'), 5.26 (d, J = 3.7, 1H, OH-3'), 5.16 (t, J = 4.4, 1H, OH-5'), 4.33–4.17 (m, 1H, H-3'), 3.86–3.74 (m, 1H, H-4'), 3.72–3.51 (m, 2H, H-5'), 2.17 (t, 2H, H-2'), 1.28 (s, 9H, C(CH $_3$) $_3$); ^{13}C 161.5 (d, J = 9.3, C-4), 151.4 (d, J = 3.8, *p*-C $_6$ H $_4$ C \equiv C), 149.4 (d, J = 8.1, C-2), 143.7 (dd, J = 184.6, 2.0, C-6), 130.9 (dd, J = 162.0, 4.0, *o*-C $_6$ H $_4$ C \equiv C), 125.6 (dd, J = 158.9, 3.9, *m*-C $_6$ H $_4$ C \equiv C), 119.5 (t, J = 6.1, *i*-C $_6$ H $_4$ C \equiv C), 98.3 (s, C-5), 91.9 (s, dU–C \equiv C), 87.6 (d, J = 147.2, C-4'), 84.8 (d, J = 170.6, C-1'), 81.8 (d, J = 5.6, dU–C \equiv C), 70.0 (d, J = 149.9, C-3'), 60.8 (t, J = 140.2, C-5'), 40.2 (t, J = 133.0, C-2'), 34.6 (s, C(CH $_3$) $_3$), 30.9 (qt, J = 125.8, 4.4, C(CH $_3$) $_3$).

4.2.7. 5-[(Trimethylsilyl)ethynyl]-2'-deoxyuridine (2g).¹⁵ From 5-iodo-2'-deoxyuridine (3.96 g, 11.2 mmol), Pd(PPh $_3$) $_4$ (1.29 g, 1.12 mmol), CuI (0.213 g, 1.12 mmol), DMF (25 mL), Et $_3$ N (3.20 mL, 22.4 mmol), and (trimethylsilyl)acetylene (7.90 mL, 55.9 mmol). The yellow mixture was stirred at 55 °C for 24 h. Crystallization from CHCl $_3$ gave **2g** as a white powder (1.02 g, 3.16 mmol, 28%). A second crop, obtained after concentration and silica gel column chromatography (15 \times 2.5 cm; CHCl $_3$ \rightarrow CHCl $_3$ /MeOH 95:5), increased the yield to a total of 3.34 g (10.3 mmol, 92%).

NMR (DMSO- d_6): 1H data matched those reported earlier.¹⁵ ^{13}C 161.5 (d, J = 9.3, C-4), 149.5 (d, J = 8.0, C-2), 144.7 (d, J = 184.2, C-6), 98.3 (s, C-5), 98.0 (d, J = 5.4, dU–C \equiv C), 97.0 (s, dU–C \equiv C), 87.6 (d, J = 147.6, C-4'), 84.8 (d, J = 169.4, C-1'), 69.9 (d, J = 140.7, C-3'), 60.8 (t, J = 140.3, C-5'), 40.2 (t, J = 133.8, C-2'), –0.03 (q, J = 99.2, Si(CH $_3$) $_3$).

4.3. Cytotoxicity experiments (IC $_{50}$)

Nucleoside samples were additionally passed through a silica gel column, to exclude contamination with metals. The experiments were performed according to established procedures with some modifications.^{39a} A

100 μ L of 10000 cells/mL (MCF-7) or 5000 cells/mL (MDA-MB-231) was incubated in 96-well plates at 37 °C in 5% CO₂/95% air atmosphere for 72 h. One plate was used for the determination of the initial cell biomass and was treated in the following way: the medium was removed, cells were fixed by a 20–30 min incubation with 100 μ L glutardialdehyde solution (0.5 mL glutardialdehyde + 12.5 mL phosphate-buffered saline solution of pH 7.4), the wells were emptied, phosphate-buffered saline solution (180 μ L, pH 7.4) was added, and the plate was stored at 4 °C until further treatment. In the 'experimental' plates the medium was replaced with a medium containing the drugs in graded concentrations (six replicates). After further incubation for 72 h (MDA-MB-231) or 96 h (MCF-7) these plates were treated as described above. The cell biomass was determined by crystal violet staining according to the following procedure: the phosphate-buffered saline solution (pH 7.4) was removed, 0.02 M crystal-violet solution was added (100 μ L), and plates were incubated for 30 min at room temperature, washed three times with water, and incubated on a softly rocking rotary shaker with ethanol (70%, 180 μ L) for a further 3–4 h. Absorption was recorded in a microplate reader at 590 nm (Flashscan Analytik Jena AG). The mean absorption of the initial cell biomass plate was subtracted from the mean absorption of each experiment and control. The corrected control was set to 100%. IC₅₀ was determined as that concentration causing 50% inhibition of cell proliferation and calculated as a mean of at least two independent experiments.

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Supplementary data

Information available: Tables of comparison of ¹H, ¹³C NMR signals for compounds **2a–h**. ¹H, ¹³C, or INEPT-based HETCOR correlation NMR spectra for compounds **2a, c–f**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.01.048.

References and notes

- (a) *Nucleosides and Nucleotides as Antitumor and Antiviral Agents*; Chu, C. K., Baker, D. C., Eds.; Plenum Press: New York, 1993; (b) *Deoxynucleoside Analogs in Cancer Therapy*; Godefrideus, J. P., Ed.; Humana Press: Totowa, 2006.
- (a) Ferrero, M.; Gotor, V. *Chem. Rev.* **2000**, *100*, 4319–4347; (b) *Chemistry of Nucleosides and Nucleotides*; Townsend, L. B., Ed.; Plenum Press: New York, 1994; Vol. 3.
- (a) Galmarini, C. M.; Mackey, J. R.; Dumontet, C. *Lancet Oncol.* **2002**, *3*, 415–424; (b) Galmarini, C. M. *Electr. J. Oncol.* **2002**, *1*, 22–32.
- Hatse, S.; De Clercq, E.; Balzarini, J. *Biochem. Pharmacol.* **1999**, *58*, 539–555.
- (a) Jiao, G.-S.; Kim, T. G.; Topp, M. R.; Burgess, K. *Org. Lett.* **2004**, *6*, 1701–1704; See also: (b) Skorobogatyi, M. V.; Malakhov, A. D.; Pchelintseva, A. A.; Turban, A. A.; Bondarev, S. L.; Korshun, V. A. *ChemBioChem* **2006**, *7*, 810–816; (c) Saito, Y.; Motegi, K.; Bag, S. S.; Saito, I. *Bioorg. Med. Chem.* **2007**, *5*, 10.1016/j.bmc.2006.07.025.
- (a) Barbaric, J.; Wagenknecht, H.-A. *Org. Biomol. Chem.* **2006**, *4*, 2088–2090; (b) Znosko, B. M.; Barnes, T. W., III; Krugh, T. R.; Turner, D. H. *J. Am. Chem. Soc.* **2003**, *125*, 6090–6097.
- (a) Kottysch, T.; Ahlborn, C.; Brotzel, F.; Richert, C. *Chem. Eur. J.* **2004**, *10*, 4017–4028; (b) Baumhof, P.; Griesang, N.; Bächle, M.; Richert, C. *J. Org. Chem.* **2006**, *71*, 1060–1067.
- (a) Gaballah, S. T.; Collier, G.; Netzel, T. L. *J. Phys. Chem. B* **2005**, *109*, 12175–12181; (b) Gaballah, S. T.; Vaught, J. D.; Eaton, B. E.; Netzel, T. L. *J. Phys. Chem. B* **2005**, *109*, 5927–5934.
- (a) De Clercq, E.; Descamps, J.; Balzarini, J.; Gziewicz, J.; Barr, P. J.; Robins, M. J. *J. Med. Chem.* **1983**, *26*, 661–666; (b) Sharma, R. A.; Kavai, I.; Hughes, R. G., Jr.; Bobek, M. J. *Med. Chem.* **1984**, *27*, 410–412.
- Recent representative studies: (a) Skorobogatyi, M. V.; Ustinov, A. V.; Stepanova, I. A.; Pchelintseva, A. A.; Petrunina, A. L.; Andronova, V. L.; Galegov, G. A.; Malakhov, A. D.; Korshun, V. A. *Org. Biomol. Chem.* **2006**, *4*, 1091–1096; (b) Skorobogatyi, M. V.; Pchelintseva, A. A.; Petrunina, A. L.; Stepanova, I. A.; Andronova, V. L.; Galegov, G. A.; Malakhov, A. D.; Korshun, V. A. *Tetrahedron* **2006**, *62*, 1279–1287; (c) Andronova, V. L.; Pchelintseva, A. A.; Ustinov, A. V.; Petrunina, A. L.; Korshun, V. A.; Skorobogatyi, M. V.; Galegov, G. A. *Voprosy Virusologii* **2006**, *51*, 34–38 (in Russian); (d) Pchelintseva, A. A.; Skorobogatyi, M. V.; Petrunina, A. L.; Andronova, V. L.; Galegov, G. A.; Astakhova, I. V.; Ustinov, A. V.; Malakhov, A. D.; Korshun, V. A. *Nucleosides Nucleotides Nucleic Acids* **2005**, *24*, 923–926; (e) Amblard, F.; Aucagne, V.; Guenot, P.; Schinazi, R. F.; Agrofoglio, L. A. *Bioorg. Med. Chem.* **2005**, *13*, 1239–1248.
- Flanagan, M. W.; Wagner, R. W. In *Applied Antisense Oligonucleotide Technology*; Stein, C. A., Krieg, A. M., Eds.; Wiley-Liss: New York, 1998; pp 174–191.
- (a) Rai, D.; Johar, M.; Manning, T.; Agrawal, B.; Kunitomo, D. Y.; Kumar, R. *J. Med. Chem.* **2005**, *48*, 7012–7017; (b) 5-Alkynyl uridine and 2-deoxyuridine conjugated with estradiol were tested against MCF-7 and MDA-MB-231 cells in vitro. None showed activity. Ali, H.; Ahmed, N.; Tessier, G.; van Lier, J. E. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 317–319.
- (a) Barr, P. J.; Jones, A. S.; Serafinowski, P.; Walker, R. T. *J. Chem. Soc., Perkin Trans. 1* **1978**, 1263–1267; (b) Robins, M. J.; Barr, P. J. *Tetrahedron Lett.* **1981**, *22*, 421–424; (c) Robins, M. J.; Barr, P. J. *J. Org. Chem.* **1983**, *48*, 1854–1862; (d) Vincent, P.; Beaucourt, J.-P.; Pichat, L. *Tetrahedron Lett.* **1981**, *22*, 945–947; (e) Robins, M. J.; Vinayak, R. S.; Wood, S. G. *Tetrahedron Lett.* **1990**, *31*, 3731–3734.
- Reviews: (a) Agrofoglio, L. A. *Curr. Org. Chem.* **2006**, *10*, 333–362; (b) Agrofoglio, L. A.; Gillaizeau, I.; Saito, Y. *Chem. Rev.* **2003**, *103*, 1875–1916; (c) Korshun, V. A.; Manasova, E. V.; Berlin, Yu. A. *Bioorg. Khim.* **1997**, *23*, 324–387 (in Russian).
- Crisp, G. T.; Flynn, B. L. *J. Org. Chem.* **1993**, *58*, 6614–6619.
- Sonogashira, K. In *Metal Catalyzed Cross-Coupling Reactions*; Diederich, F., Stang, P. J., Eds.; Wiley-VCH: Weinheim, 1998; pp 203–230.

17. Cruickshank, K. A.; Stockwell, D. L. *Tetrahedron Lett.* **1988**, 29, 5221–5224.
18. Recent representative protocol development: Solid support: (a) Aucagne, V.; Berteina-Raboin, S.; Guenot, P.; Agrofoglio, L. A. *J. Comb. Chem.* **2004**, 6, 717–723; (b) Rist, M.; Amann, N.; Wagenknecht, H.-A. *Eur. J. Org. Chem.* **2003**, 2498–2504; Microwave-enhanced: (c) Petricci, E.; Radi, M.; Corelli, F.; Botta, M. *Tetrahedron Lett.* **2003**, 44, 9181–9184.
19. Recent relevant representative syntheses: (a) Flasche, W.; Cismas, C.; Herrmann, A.; Liebscher, J. *Synthesis* **2004**, 2335–2341; Uridines: (b) Ghilagaber, S.; Hunter, W. N.; Marquez, R. *Tetrahedron Lett.* **2007**, 48, 483–486; (c) González Moa, M. J.; Besada, P.; Terán, C. *Synthesis* **2006**, 3973–3978; (d) Bouamaied, I.; Stulz, E. *Synlett* **2004**, 1579–1583; Uracils: (e) Hudson, R. H. E.; Moszynski, J. M. *Synlett* **2006**, 2997–3000.
20. Recent review of antiviral activity of furopyrimidines: McGuigan, C.; Balzarini, J. *Antiviral Res.* **2006**, 71, 149–153.
21. Nakamura, K.; Okubo, H.; Yamaguchi, M. *Synlett* **1999**, 549–550.
22. Esho, N.; Davies, B.; Lee, J.; Dembinski, R. *Chem. Commun.* **2002**, 332–333.
23. Rao, M. S.; Esho, N.; Sergeant, C.; Dembinski, R. *J. Org. Chem.* **2003**, 68, 6788–6790.
24. Perman, J.; Sharma, R. A.; Bobek, M. *Tetrahedron Lett.* **1976**, 17, 2427–2430.
25. Yu, C.-S.; Oberdorfer, F. *Synlett* **2000**, 86–88.
26. Korshun, V. A.; Manasova, E. V.; Balakin, K. V.; Malakhov, A. D.; Perepelov, A. V.; Sokolova, T. A.; Berlin, Yu. A. *Nucleosides Nucleotides* **1998**, 17, 1809–1812.
27. McGuigan, C.; Barucki, H.; Blewett, S.; Carangio, A.; Erichsen, J. T.; Andrei, G.; Snoeck, R.; De Clercq, E.; Balzarini, J. *J. Med. Chem.* **2000**, 43, 4993–4997.
28. See for example: Hashimoto, H.; Nelson, M. G.; Switzer, C. *J. Am. Chem. Soc.* **1993**, 115, 7128–7134.
29. A commercial price of **2h** (\$1070/0.1 g) perhaps reflects the laborious purification.
30. ¹H NMR analysis of the precipitate revealed a small amount of the nucleoside present.
31. The use of chloroform stabilized with hexane, as opposed to the solvent with alcohol additives, increases yields. In some cases column chromatography was used to facilitate crystallization of a second crop. Precipitation of 5-alkynyluridines has been noticed (Ref. 19a).
32. Presence of metals (Pd, Cu) was determined for most active compounds using atomic absorption spectrophotometer. A Pd content was negligible (3.5×10^{-4} % (m/m) for **2g** and 6.0×10^{-5} % (m/m) for **2h**). A Cu content was not significantly higher than that of blank solvent.
33. Bednarski, P. J. *Biochem. Pharmacol.* **1992**, 43, 2609–2620.
34. Reile, H.; Birnböck, H.; Bernhardt, G.; Spruß, T.; Schönenberger, H. *Anal. Biochem.* **1990**, 187, 262–267.
35. Miura, S.; Izuta, S. *Curr. Drug Targets* **2004**, 5, 191–195.
36. Al-Madhoun, A. S.; Tjarks, W.; Eriksson, S. *Mini-Rev. Med. Chem.* **2004**, 4, 341–350.
37. Van Rompay, A. R.; Johansson, M.; Karlsson, A. *Pharmacol. Ther.* **2003**, 100, 119–139.
38. Jordheim, L. P.; Galmarini, C. M.; Dumontet, C. *Bull. Cancer* **2005**, 92, 239–248 (in French).
39. (a) Ott, I.; Schmidt, K.; Kircher, B.; Schumacher, P.; Wiglenda, T.; Gust, R. *J. Med. Chem.* **2005**, 48, 622–629; (b) Schmidt, K.; Jung, M.; Keilitz, R.; Schnurr, B.; Gust, R. *Inorg. Chim. Acta* **2000**, 306, 6–16.
40. When the C-2' signal was obscured by the solvent, the chemical shift was taken from HETCOR experiment.
41. The *ilolm/p* positions were designated with respect to the alkynyl group. Assignments with the aid of HETCOR spectra.