5-Arylethynyl-2′-deoxyuridines, compounds active against HSV-1

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Three new 5-arylethynyl-2′-deoxyuridines containing bulky aryls have been prepared and tested against HSV-1 in *Vero* cells. The introduction of a substituent in the phenyl group of an inactive compound, 5-phenylethynyl-2′-deoxyuridine, leads to the appearance of anti-HSV properties. The most active compounds are those containing a polycyclic aromatic hydrocarbon residue attached to the 5 position of 2′-deoxyuridine through a rigid triple bond.

Introduction

The *Herpes simplex* virus type 1 (HSV-1) belongs to a group of viruses for which vaccines are not developed yet. The known anti-HSV-1 drugs (*e.g.* acyclovir (ACV), brivudin) are inhibitors of viral DNA polymerase.[1] The search for new antiviral compounds, especially those having a different mechanism of action, is important in terms of potential treatment of diseases caused by drug-resistant strains of HSV-1.

The first 5-arylethynyl-2′-deoxyuridine, 5-phenylethynyl-2′-deoxyuridine **1**, was prepared more than two decades ago and showed no activity against HSV-1.[2] Recently, we found that 5-arylethynyl-2′-deoxyuridines with bulky aryl substituents are able to inhibit HSV-1 replication in *Vero* cell culture; for instance, nucleoside **2** displays considerable activity (ID_{50} 7.8 μg cm^{-3}) along with moderate cytotoxicity (CD_{50} 250 μg cm^{-3} for *Vero* cells). Interestingly, compound **2** retains pronounced activity against the ACV-resistant strain of HSV-1 (ID_{50} 31.2 μg cm^{-3}).[3] The rigid ethynyl connection of an aryl to uracil seems to be a crucial feature for biological activity: the introduction of a flexible spacer between aryl and triple bond usually increases the cytotoxicity and dramatically reduces the antiviral activity. For example, nucleoside **3** containing an additional CH_2OCH_2 spacer as compared to compound **2**, shows CD_{50} 62.5 μg cm^{-3} and no detectable activity against HSV-1 below this concentration[4] (*cf.* Table 1).

There are no obvious structural reasons to anticipate that 5-(perylen-3-ylethynyl)-2′-deoxyuridine **2** is phosphorylated in cells with cellular or HSV-1 thymidine kinases much faster than nucleosides **1** and **3** (it is known that the increase of the size of the 5-substituent R in 5-R-2′-deoxyuridines generally decreases their binding to HSV-1 thymidine kinase[5]). It is also doubtful that the 5′-triphosphate of **2** is a better substrate for viral DNA polymerase. Hence, the usual, so-called nucleoside way of virus inhibition,[1] is unlikely for compound **2**. Perhaps, a non-nucleoside mechanism[6] is the case here, and the HSV-1 DNA polymerase is not a target.

This makes reasonable the search for antiviral nucleosides among analogues of **2** in order to reveal the structure–activity relationship and to elucidate comprehensively their antiviral action mechanism. It was also of interest, whether the introduction of a substituent into the phenyl ring in **1** can render it active against HSV-1.

In this article we describe the synthesis of nucleosides **4–6** and evaluation of their activity against HSV-1. The nucleoside **4** differs from **1** by the presence of the adamantyl group in the phenyl ring, and the nucleoside **5** contains a trityl group. The nucleoside **6** contains a flat and bulky tricyclic anthracene residue instead of a phenyl one. The comparison of cytotoxicity and antiviral activity of compounds **4–6** will allow the discovery of empirical criteria to help to find new anti-HSV compounds among the 5-arylethynyl-2′-deoxyuridines.

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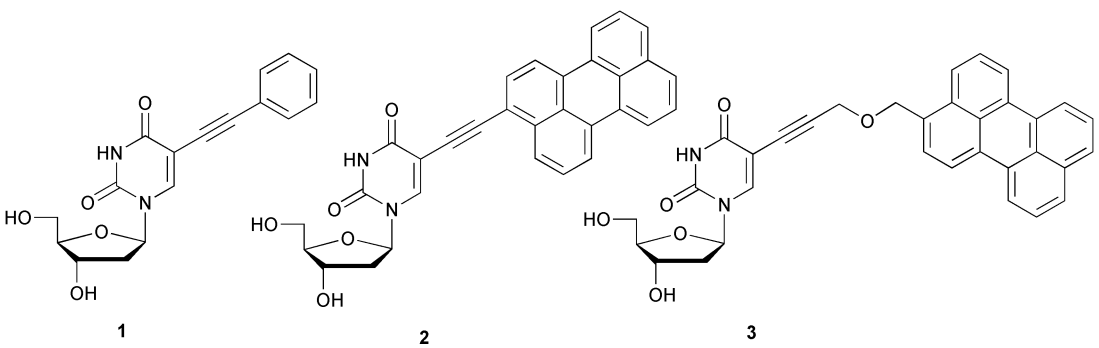
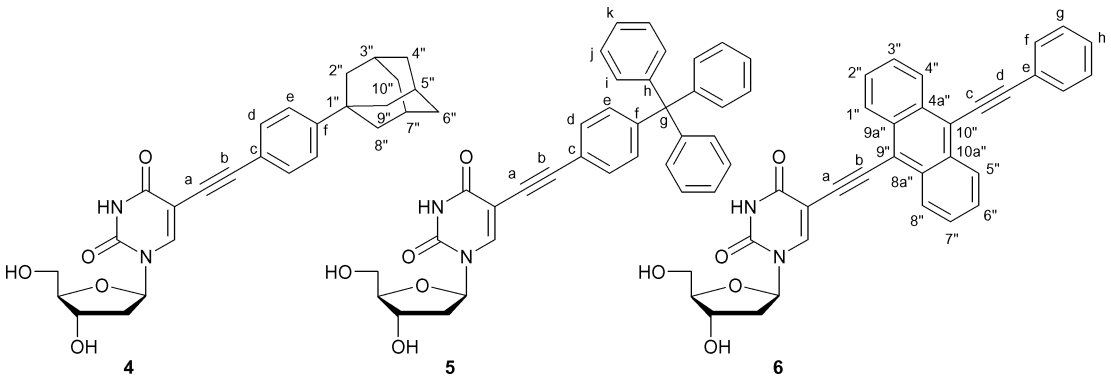




Results and discussion

Synthesis of nucleosides

Phenols **7a,b** were converted into corresponding triflates[7] **8a,b**, which were reacted with dimethylpropargyl alcohol under conditions of the Sonogashira reaction.[8] The obtained protected acetylenes **9a,b** were deblocked with potassium hydroxide in the presence of 18-crown-6 as a catalyst to yield ethynylarenes **10a,b** (Scheme 1).

Acetylenes **10a,b**, as well as 10-ethynyl-9-phenylethynylanthracene **11**[9] were coupled with 3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-5-iodo-2′-deoxyuridine[4] under conditions optimized for nucleosides.[10] The Markiewicz' protection of 3′ and 5′ hydroxyls allows easy chromatographic purification of coupling products **13a–c**. The silyl group was then removed with triethylamine trihydrofluoride in THF[11] to yield the desired nucleosides **4–6** (Scheme 2).

The identity and purity of the final compounds and intermediates were confirmed by high resolution mass spectrometry (HRMS) and NMR. The signals in ^{13}C NMR spectra were assigned using HMQC and HMBC ^{1}H-^{13}C correlations.

The nucleoside **6** is highly fluorescent (Fig. 1). The feature could probably help to monitor its cellular distribution using fluorescence microscopy.

Pharmacology

The nucleosides **4–6** were tested for their cytotoxicity and anti-HSV-1 activity in *Vero* cells.[12] The results are given in Table 1.

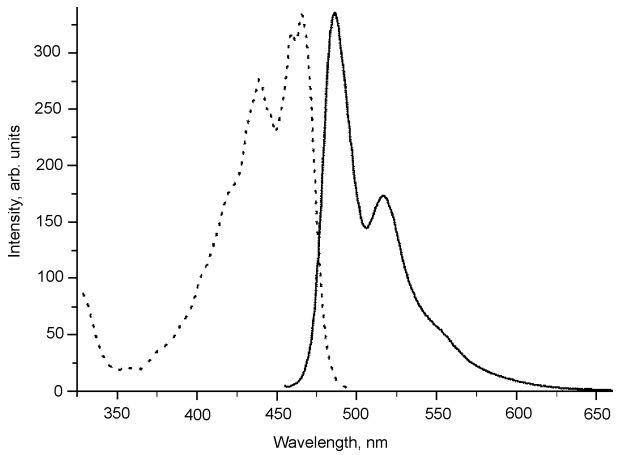
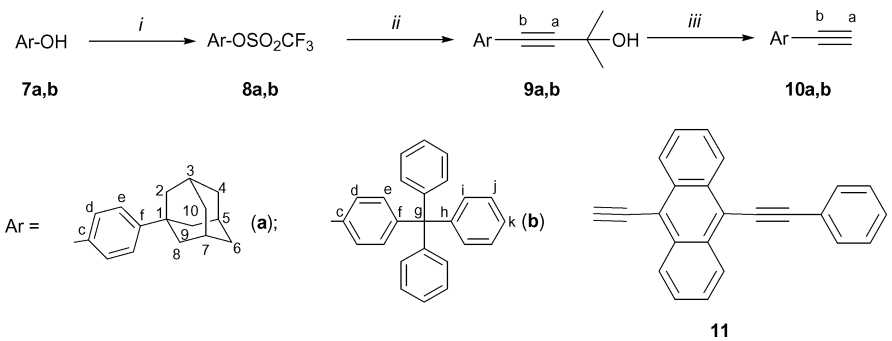
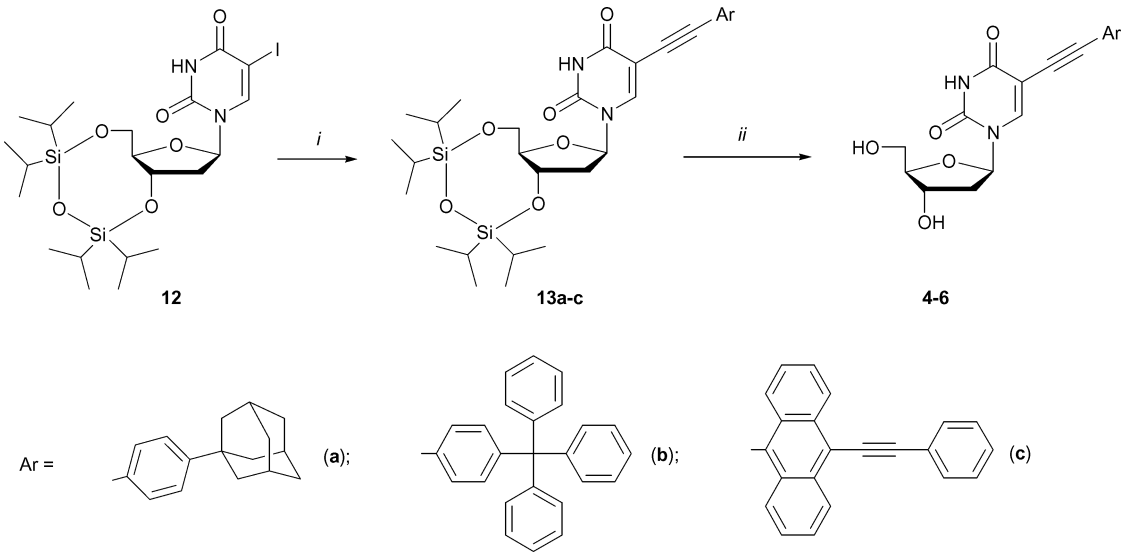


Fig. 1 Normalized emission (solid line, λ_{ex} 440 nm) and excitation (dashed line, λ_{em} 505 nm) spectra of nucleoside **6** in MeOH.

In contrast to the parent nucleoside **1**, compounds **4–6** show appreciable anti-HSV-1 activity. This demonstrates that the size of 5-arylethynyl group is important for the biological activity of 2′-deoxyuridine nucleosides. The introduction of a bulky substituent of either aliphatic (adamantyl in the nucleoside **4**) or aromatic (trityl in the nucleoside **5**) nature in the *para*-position of the phenyl ring of the inactive 5-phenylethynyl-2′-deoxyuridine leads to appearance of HSV-1-inhibiting properties. Adamantyl derivative **4** is much more cytotoxic and slightly more active than trityl derivative **5**. Anthracene nucleoside **6** shows the highest anti-HSV-1 activity (even better than 5-(perylen-3-ylethynyl)-2′-deoxyuridine **2**). Nucleosides **4** and **6** display the same activity



Scheme 1 Preparation of terminal acetylenes **10a,b** from 1-(4-hydroxyphenyl)adamantane (**7a**) and 4-tritylphenol (**7b**). Reaction conditions and yields: *i*) $(CF_3SO_2)_2O$–Et_3N–DCM; 99% (**8a**), 89% (**8b**); *ii*) 2-methyl-3-butyn-2-ol–$Pd(PPh_3)_4$–CuI–Et_3N–DMF; 75% (**9a**), 90% (**9b**); *iii*) KOH–18-crown-6–benzene; 77% (**10a**), 84% (**10b**).



Scheme 2 Synthesis of 5-arylethynyl-2′-deoxyuridines. Reaction conditions and yields: *i*) ArC≡CH (**10a**, **10b**, **11**)–Pd(PPh$_3$)$_4$–CuI–Et$_3$N–DMF; 70% (**13a**), 53% (**13b**), 47% (**13c**); *ii*) Et$_3$N·3HF–THF; 81% (**4**), 73% (**5**), 77% (**6**).

Table 1 Cytotoxic and antiviral properties of 5-arylethynyl-2′-deoxyuridines in *Vero* cell culture (μg cm^{-3})[a]

		HSV-1		HSV-1/ACV^R	
Compound	CD_{50}	ID_{50}	ID_{95}	ID_{50}	ID_{95}
1[b]	—	>400	—	—	—
2[b]	250	7.8	15.6	31.2	62.5
3[b]	62.5	62.5	>62.5	62.5	> 62.5
4	13.8	7.8	15.6	7.8	15.6
5	218.7	31.2	125	>220	>220
6	38.8	3.9	31.2	3.9	31.2
ACV	>400	0.45	0.90	>400	>400

[a] HSV-1—*Herpes simplex* virus type 1, strain L_2; HSV-1/ACV^R—ACV-resistant strain of HSV-1; CD_{50}—cytotoxic dose causing 50% growth inhibition of *Vero* cells; ID_{50} and ID_{95}—doses inhibiting the cytopathogenic effect of virus by 50 and 95%, respectively. [b] Data for the compounds **1** (in PRK cell culture),[2] **2**,[3] and **3**[4] were taken from the literature.

against ACV-resistant (thymidine kinase deficient) strain of HSV-1. This supports the assumption that the mechanism of antiviral action of nucleosides **4** and **6** does not involve their phosphorylation by viral thymidine kinase. Unfortunately, the nucleoside **6** has considerable cytotoxicity and therefore its selectivity index (SI) is approx. 10 (for comparison, for the nucleoside **2** SI is 32).

The data obtained show that 5-arylethynyl-2′-deoxyuridines with flat and bulky polycyclic aryls reveal pronounced activity against HSV-1 and thus are promising candidates for massive antiviral screening. The synthesis of other 5-arylethynyl-2′-deoxypyrimidine nucleosides is now in progress.

Conclusions

The 5-arylethynyl-2′-deoxyuridines with bulky aryl groups constitute a new class anti-HSV-1 agents. The nucleosides containing polycyclic aryls show the highest antiviral activity. The compounds are also able to inhibit the ACV-resistant strain of HSV-1. Further investigations are needed to find structure–activity and structure–cytotoxicity relationships and to elucidate mechanism of action and cellular targets for this class of antiviral nucleosides.

Experimental

Instrumentation

500 MHz ^{1}H and 125.7 MHz ^{13}C NMR spectra were recorded on a Bruker DRX-500 spectrometer and referenced to $CDCl_3$ (7.25 ppm for ^{1}H and 77.00 ppm for ^{13}C) and DMSO-d_6 (2.50 ppm for ^{1}H and 39.60 ppm for ^{13}C). ^{1}H-^{13}C gradient-selected HMQC and HMBC spectra were obtained by using 2048 (t_2) × 256 (t_1) complex point data sets, zero filled to 2048 (F_2) × 1024 (F_1) points. The spectral widths were 13 ppm and 200 ppm for ^{1}H and ^{13}C dimensions, respectively. HMBC spectra were measured with 50 ms delay for evolution of long-range couplings. EI-MS analyses in positive ion mode were performed using a Finnigan Polaris Q ion trap mass spectrometer (the temperature of ion source 150 °C, the energy of ionization 70 eV). EI-TOF HRMS and ESI-TOF HRMS spectra in positive ion mode were obtained using Micromass LCT reflection TOF mass spectrometer. UV spectra were recorded using an LKB Ultrospec III spectrophotometer. The fluorescence spectrum was obtained using a Varian Cary Eclipse fluorescence spectrophotometer (excitation and emission slits 5 nm, concentration 2 × 10^{-7} M). Melting points were determined using a Boetius heating table and are uncorrected. Analytical thin-layer chromatography was performed on Kieselgel 60 F_{254} precoated aluminium plates (Merck), spots were visualized under UV light (254 nm). Silica gel column chromatography was performed using Merck Kieselgel 60 0.040–0.063 mm.

Reagents and solvents

Reagents obtained from commercial suppliers were used as received. Triethylamine was from Acros; 4-(1-adamantyl)phenol, copper(I) iodide, triethylamine trihydrofluoride, 4-tritylphenol were from Aldrich; trifluoromethanesulfonic anhydride was from Avocado; 18-crown-6, 2-methyl-3-butyn-2-ol were from

Fluka. $Pd(PPh_3)_4$[13] and 3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-5-iodo-2′-deoxyuridine[4] were prepared as described. Solvents were from Chimmed (Russia), mainly HPLC grade and used without further purification unless otherwise noted. DCM was always used freshly distilled over CaH_2. THF was distilled over powdered $LiAlH_4$ and stored over 4 Å molecular sieves under nitrogen. DMF was freshly distilled under reduced pressure.

General procedure for the preparation of aryl triflates (8a,b)

To an ice cooled solution or a suspension of the corresponding phenol (15 mmol) in dry DCM (100 cm^3), Et_3N (12.5 cm^3, 90 mmol) and trifluoromethanesulfonic anhydride (5.05 cm^3, 30 mmol) were added. The mixture was stirred for 30 min, then warmed to room temperature and filtered through a silica gel layer (5 cm). The latter was washed several times with toluene; the resulting solutions were combined with filtered reaction mixture and evaporated. The residue was chromatographed on silica gel in toluene.

1-(4-Trifluoromethanesulfonyloxyphenyl)adamantane (8a). Compound **8a** was prepared from 4-(1-adamantyl)phenol, yield 5.35 g (99%); colourless crystals. R_f 0.57 (5% EtOAc in hexane (v/v)), mp 59–60 °C (methanol). EI MS: m/z = 360 $[M]^+$, calc. for $[C_{17}H_{19}F_3O_3S]^+$ 360. EI-TOF HRMS: m/z = 360.1014 $[M]^+$, calc. for $[C_{17}H_{19}F_3O_3S]^+$ 360.1002. ^{1}H NMR ([D$_6$]DMSO): δ_H 7.54 (d, 2H, J = 8.8 Hz, Ar*H*); 7.39 (m, 2H, J = 8.8 Hz, Ar*H*); 2.06 (m, 3H, H-3,5,7); 1.86 (m, 6H, H-2,8,9); 1.73 (m, 6H, H-4,6,10). ^{13}C NMR ([D$_6$]DMSO): δ_C 151.8 (Cf); 147.1 (Cc); 127.2 (2C, Ce); 120.9 (2C, Cd); 118.3 (q, $^1J_{CF}$ = 321 Hz, *C*F$_3$); 42.4 (3C, C2,8,9); 36.0 (3C, C4,6,10); 35.9 (C1); 28.2 (3C, C3,5,7).

4-(Trifluoromethanesulfonyloxy)tetraphenylmethane (8b). Compound **8b** was prepared from 4-tritylphenol, yield 6.23 g (89%); colourless crystals. R_f 0.40 (5% EtOAc in hexane (v/v)), mp 183 °C (hexane). EI MS: m/z = 468 $[M]^+$, calc. for $[C_{26}H_{19}F_3O_3S]^+$ 468. EI-TOF HRMS: m/z = 468.1010 $[M]^+$, calc. for $[C_{26}H_{19}F_3O_3S]^+$ 468.1002. ^{1}H NMR ($CDCl_3$): δ_H 7.31 (m, 2H, Hd); 7.27 (m, 6H, Hj); 7.22 (m, 2H, He); 7.16 (m, 9H, Hi,k). ^{13}C NMR ($CDCl_3$): δ_C 147.6, 147.5 (Cc and Cf); 146.0 (3C, Ch); 132.09 (3C, Ck); 131.0 (6C, Ci); 127.7 (6C, Cj); 126.3 (2C, Ce); 120.1 (2C, Cd); 118.7 (q, $^1J_{CF}$ = 321 Hz, *C*F$_3$); 64.7 (Cg).

General procedure for the preparation of protected alkynes (9a,b)

To a solution of corresponding triflate (5.0 mmol) and 2-methyl-3-butyn-2-ol (0.59 cm^3, 6.0 mmol) in dry DMF (30 cm^3) under argon, $Pd(PPh_3)_4$ (0.58 mg, 0.5 mmol), CuI (0.19 mg, 1.0 mmol) and triethylamine (1.05 cm^3, 7.5 mmol) were successively added, and the reaction mixture was stirred for 24 h at room temperature. The mixture was then diluted with EtOAc (200 cm^3), washed with 3% aqueous EDTA-$(NH_4)_2$ (2 × 150 cm^3) and water (4 × 150 cm^3), dried over Na_2SO_4, and evaporated. The residue was chromatographed on silica gel in toluene.

1-[4-(3-Hydroxy-3-methylbutyn-1-yl)phenyl]adamantane (9a). Compound **9a** was prepared from **8a**; colourless crystals (0.78 g, 75%). R_f 0.39 (toluene–EtOAc, 4 : 1 (v/v)), mp 139 °C (hexane–toluene). EI MS: m/z = 294 $[M]^+$, calc. for $[C_{21}H_{26}O]^+$ 294. EI-TOF HRMS: m/z = 294.1977 $[M]^+$, calc. for $[C_{21}H_{26}O]^+$ 294.1978. ^{1}H NMR ([D$_6$]DMSO): δ_H 7.32 (m, 4H, J = 8.7 Hz, Ar*H*); 5.40 (s, 1H, O*H*); 2.05 (m, 3H, H-3,5,7); 1.83 (d, 6H, J = 2.5 Hz, H-2,8,9); 1.73 (m, 6H, H-4,6,10); 1.45 (s, 6H, C*H*$_3$). ^{13}C NMR ([D$_6$]DMSO): δ_C 151.0 (Cf); 131.0 (2C, Ce); 125.0 (2C, Cd); 119.8 (Cc); 95.5 (Ca); 80.4 (Cb); 63.6 (*C*OH); 42.4 (3C, C2,8,9); 36.1 (3C, C4,6,10); 35.9 (C1); 31.7 (2C, *C*H$_3$); 28.3 (3C, C3,5,7).

4-(3-Hydroxy-3-methylbutyn-1-yl)tetraphenylmethane (9b). Compound **9b** was prepared from **8b**; colourless crystals (1.81 g, 90%). R_f 0.38 (toluene–EtOAc, 4 : 1 (v/v)), mp 212–213 °C (hexane). EI MS: m/z = 402 $[M]^+$, calc. for $[C_{30}H_{26}O]^+$ 403. EI-TOF HRMS: m/z = 402.1974 $[M]^+$, calc. for $[C_{30}H_{26}O]^+$ 402.1978. ^{1}H NMR ($CDCl_3$): δ_H 7.32 (m, 2H, Hd); 7.26 (m, 6H, Hj); 7.23–7.17 (m, 11H, He,i,k); 2.01 (br. s, 1H, O*H*); 1.62 (s, 6H, C*H*$_3$). ^{13}C NMR ($CDCl_3$): δ_C 147.2 (Cf); 146.4 (3C, Ch); 131.1 (9C, Ci,k); 130.8 (2C, Cd); 127.6 (6C, Cj); 126.0 (2C, Ce); 120.2 (Cc); 93.8 (Ca); 82.0 (Cb); 65.6 (*C*OH); 64.9 (Cg); 31.5 (2C, *C*H$_3$).

General procedure for the preparation of terminal alkynes (10a,b)

To a solution of corresponding protected alkyne (5.00 mmol) in benzene (50 cm^3), freshly powdered KOH (0.42 g, 7.50 mmol) and 18-crown-6 (330 mg, 1.25 mmol) were added under argon. The mixture was refluxed under argon for 10 min, cooled, filtered through silica gel (5 cm). The latter was washed several times with toluene, the resulting solutions were combined with the filtered reaction mixture and evaporated. The residue was chromatographed on silica gel in an appropriate solvent.

1-(4-Ethynylphenyl)adamantane (10a). Compound **10a** was purified in hexane; colourless crystals (0.91 g, 77%). R_f 0.42 (hexane), mp 116 °C (hexane). EI MS: m/z = 236 $[M]^+$, calc. for $[C_{18}H_{20}]^+$ 236. EI-TOF HRMS: m/z = 236.1563 $[M]^+$, calc. for $[C_{18}H_{20}]^+$ 236.1560. ^{1}H NMR ([D$_6$]DMSO): δ_H 7.40 (m, 2H, J = 8.3 Hz, Ar*H*); 7.36 (m, 2H, J = 8.3 Hz, Ar*H*); 4.07 (s, 1H, ≡C*H*); 2.05 (m, 3H, H-3,5,7); 1.84 (m, 6H, H-2,8,9); 1.72 (m, 6H, H-4,6,10). ^{13}C NMR ([D$_6$]DMSO): δ_C 151.8 (Cf); 131.5 (2C, Ce); 125.1 (2C, Cd); 118.9 (Cc); 83.6 (Ca); 80.1 (Cb); 42.3 (3C, C2,8,9); 36.1 (3C, C4,6,10); 35.9 (C1); 28.3 (3C, C3,5,7).

4-(Ethynyl)tetraphenylmethane (10b). Compound **10b** was purified in toluene; colourless crystals (1.45 g, 84%). R_f 0.48 (toluene–hexane, 1 : 1 (v/v)), mp 192–193 °C (hexane–toluene) (lit.,[14] 187–188 °C). EI MS: m/z = 344 $[M]^+$, calc. for $[C_{27}H_{20}]^+$ 344. EI-TOF HRMS: m/z = 344.1561 $[M]^+$, calc. for $[C_{27}H_{20}]^+$ 344.1560. ^{1}H NMR ($CDCl_3$): δ_H 7.39 (d, 2H, J = 8.5 Hz, Hd); 7.26 (m, 6H, Hj); 7.21 (m, 11H, He,i,k); 3.05 (s, 1H, ≡C*H*). ^{13}C NMR ($CDCl_3$): δ_C 147.8 (Cf); 146.3 (3C, Ch); 131.3 (2C, Cd); 131.1 (9C, Ci,k); 127.6 (6C, Cj); 126.1 (2C, Ce); 119.6 (Cc); 83.6 (Cb); 77.1 (Ca); 65.0 (Cg).

General procedure for the preparation of 3′,5′-*O*-silyl protected 5-arylethynyl-2′-deoxyuridines (13a–c)

To a solution of 5-iodo-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-2′-deoxyuridine **12** (1.19 g, 2.00 mmol) and the appropriate acetylene (2.5 mmol) in dry DMF (30 cm^3) under argon $Pd(PPh_3)_4$ (231 mg, 0.20 mmol), CuI (76 mg, 0.40 mmol) and triethylamine (560 μL, 4.0 mmol) were successively added, and the reaction mixture was stirred for 16 h at room temperature. The mixture was then diluted with EtOAc (200 cm^3), washed with 3% aq. EDTA-$(NH_4)_2$ (4 × 100 cm^3) and water (4 × 100 cm^3), dried over Na_2SO_4,

and evaporated to dryness. The residue was chromatographed on a silica gel column in appropriate solvent.

3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-5-[4-(adamantan-1-yl)phenylethynyl]-2′-deoxyuridine (13a). Compound **13a** was purified in 10% of EtOAc in chloroform (v/v); yellow amorphous solid (0.99 g, 70%). R_f 0.29 ($CHCl_3$–EtOAc, 4 : 1 (v/v)). ESI-TOF HRMS: m/z = 705.3943 $[M + H]^+$, 727.3622 $[M + Na]^+$, calc. for $[C_{39}H_{56}N_2O_6Si_2 + H]^+$ 705.3750, $[C_{39}H_{56}N_2O_6Si_2 + Na]^+$ 727.3569. ^{1}H NMR ([D$_6$]DMSO): δ_H 11.68 (s, 1H, N*H*); 7.92 (s, 1H, H-6); 7.38 (m, 4H, Ar*H*); 5.98 (dd, 1H, $J_{1',2'\alpha}$ = 7.6 Hz, $J_{1',2'\beta}$ = 2.7 Hz, H-1′); 4.53 (m, 1H, H-3′); 4.07 (m, 1H, $^2J_{5'a,5'b}$ = 12.7 Hz, $J_{4',5'a}$ = 4.0 Hz, H-5′a); 3.94 (m, 1H, $^2J_{5'a,5'b}$ = 12.7 Hz, $J_{4',5'b}$ = 2.9 Hz, H-5′b); 3.74 (m, 1H, H-4′); 2.52 (m, 1H, H-2′α); 2.34 (m, 1H, H-2′β); 2.06 (br. s, 3H, H-3″,5″,7″); 1.86 (m, 6H, H-2″,8″,9″); 1.55 (m, 6H, H-4″,6″,10″); 1.08–0.96 (m, 28H, Pr^i). ^{13}C NMR ([D$_6$]DMSO): δ_C 161.5 (C4); 151.6 (Cf); 149.2 (C2); 143.0 (C6); 131.0 (2C, Ce); 125.1 (2C, Cd); 119.5 (Cc); 98.4 (C5); 92.2 (Cb); 84.4 (C4′); 84.2 (C1′); 81.6 (Ca); 68.9 (C3′); 60.8 (C5′); 42.4 (3C, C2″,8″,9″); 38.6 (C2′); 36.1 (3C, C4″,6″,10″); 36.0 (C1″); 28.3 (3C, C3″,5″,7″); 17.4, 17.3 (2C), 17.2, 17.1, 16.9 (2C), 16.8 (*C*H$_3$); 12.8, 12.5, 12.1, 12.0 (Si*C*).

3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-5-[4-(triphenylmethyl)phenylethynyl]-2′-deoxyuridine (13b). Compound **13b** was purified in 5% of EtOAc in chloroform (v/v); yellowish amorphous solid (0.86 g, 53%). R_f 0.38 ($CHCl_3$–EtOAc, 4 : 1 (v/v)). ESI-TOF HRMS: m/z = 813.3857 $[M + H]^+$, calc. for $[C_{48}H_{56}N_2O_6Si_2 + H]^+$ 813.3750. ^{1}H NMR ([D$_6$]DMSO): δ_H 11.68 (s, 1H, N*H*); 7.93 (s, 1H, H-6); 7.38 (d, 2H, J = 8.6 Hz, Ha); 7.31 (m, 6H, Hd); 7.22 (m, 3H, He); 7.17 (d, 2H, J = 8.6 Hz, Hb); 7.13 (d, 6H, J = 7.6 Hz, Hc); 5.97 (dd, 1H, $J_{1',2'\alpha}$ = 7.3 Hz, $J_{1',2'\beta}$ = 2.7 Hz, H-1′); 4.52 (m, 1H, H-3′); 4.05 (m, 1H, $^2J_{5'a,5'b}$ = 12.6 Hz, $J_{4',5'a}$ = 4.2 Hz, H-5′a); 3.93 (m, 2H, $^2J_{5'a,5'b}$ = 12.6 Hz, $J_{4',5'b}$ = 2.7 Hz, H-5′b); 3.74 (m, 1H, H-4′); 2.51 (m, 1H, H-2′α); 2.33 (m, 1H, H-2′β); 1.06–0.94 (m, 28H, Pr^i). ^{13}C NMR ([D$_6$]DMSO): δ_C 161.5 (C4); 149.2 (C2); 147.0 (Cf); 146.0 (3C, Ch); 143.4 (C6); 130.8, 130.6 (2C and 2C, Cd,e); 130.5 (6C, Ci); 127.9 (6C, Cj); 126.2 (3C, Ck); 120.1 (Cc); 98.1 (C5); 91.6 (Cb); 84.4 (C4′); 84.3 (C1′); 82.4 (Ca); 69.0 (C3′); 64.5 (Cg); 60.9 (C5′); 38.6 (C2′); 17.4, 17.3 (2C), 17.2, 17.1, 16.9 (2C), 16.8 (*C*H$_3$); 12.8, 12.5, 12.1, 12.0 (Si*C*).

3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-5-[10-(phenylethynyl)anthracen-9-ylethynyl]-2′-deoxyuridine (13c). Compound **13c** was purified using a gradient of EtOAc (4 → 8%) in $CHCl_3$ (v/v); orange amorphous solid (725 mg, 47%). R_f 0.54 (15% EtOAc in $CHCl_3$ (v/v)). ESI-TOF HRMS: m/z = 771.3218 $[M + H]^+$, calc. for $[C_{45}H_{50}N_2O_6Si_2 + H]^+$ 771.3280. ^{1}H NMR ([D$_6$]DMSO): δ_H 11.89 (s, 1H, N*H*); 8.69 (m, 4H, H-1″,4″,5″,8″); 8.25 (s, 1H, H-6); 7.88 (m, 2H, Hf); 7.79 (m, 2H), 7.74 (m, 2H) (H-2″,3″,6″,7″); 7.55 (m, 3H, Hg,h); 6.04 (dd, 1H, $J_{1',2'\alpha}$ = 7.6 Hz, $J_{1',2'\beta}$ = 2.4 Hz, H-1′); 4.62 (m, 1H, H-3′); 4.14 (m, 1H, $^2J_{5'a,5'b}$ = 12.5 Hz, $J_{4',5'a}$ = 4.6 Hz, H-5′a); 3.99 (m, 1H, $^2J_{5'a,5'b}$ = 12.5 Hz, $J_{4',5'b}$ = 2.7 Hz, H-5′b); 3.81 (m, 1H, H-4′); 2.64 (m, 1H, H-2′α); 2.41 (m, 1H, H-2′β); 1.07–0.91 (m, 28H, Pr^i). ^{13}C NMR ([D$_6$]DMSO): δ_C 161.8 (C4); 149.3 (C2); 143.8 (C6); 131.7 (2C, Cf); 131.4 (2C, C4a″,10a″); 131.0 (2C, C8a″,9a″); 129.4 (Ch); 129.0 (2C, Cg); 127.8 (2C, C3″,6″); 127.4 (2C, C2″,7″); 127.0 (2C, C1″,8″); 126.8 (2C, C4″,5″); 122.3 (Ce); 118.1 (C9″); 117.2 (C10″); 102.7 (Cd); 98.3 (C5); 96.1 (Ca); 89.6, 85.8 (Cb and Cc); 85.0 (C4′); 84.6 (C1′); 69.2 (C3′); 61.1 (C5′); 38.6 (C2′); 17.3, 17.2 (3C), 17.1, 17.0, 16.9, 16.8 (*C*H$_3$); 12.8, 12.5, 12.1, 12.0 (Si*C*).

General procedure for the preparation of 5-arylethynyl-2′-deoxyuridines (4–6)

To a solution of the corresponding 5-arylethynyl-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-2′-deoxyuridine **13a–c** (1.00 mmol) in dry THF (3 cm^3) in a Teflon flask triethylamine trihydrofluoride (0.41 cm^3, 2.50 mmol) was added in one portion and the mixture was left for 12 h at room temperature, then diluted with hexane (25 cm^3). The upper layer was removed, and the residue was washed with toluene–hexane mixture, 1 : 1 (v/v) (25 cm^3) by decantation, and the residue was dissolved in chloroform (50 cm^3). The solution was washed with water (3 × 50 cm^3), dried over Na_2SO_4, and evaporated. The residue was chromatographed on a silica gel column using gradient of methanol (3 → 6%; v/v) in chloroform.

5-[4-(Adamantan-1-yl)phenylethynyl]-2′-deoxyuridine (4). Compound **4** was prepared from **13a**; colourless crystals (375 mg, 81%). R_f 0.45 ($CHCl_3$–methanol, 4 : 1 (v/v)), mp 182–184 °C (toluene–methanol). ESI-TOF HRMS: m/z = 463.2324 $[M + H]^+$, 485.2118 $[M + Na]^+$, calc. for $[C_{27}H_{30}N_2O_5 + H]^+$ 463.2227, $[C_{27}H_{30}N_2O_5 + Na]^+$ 485.2047. UV (MeOH): λ_{min}, nm (log ε) 235 (4.05), 275 (4.17), 289 (4.15); λ_{max}, nm (log ε) 266 (4.24), 281 (4.19), 311 (4.30); shoulder, nm (log ε) 256 (4.17). ^{1}H NMR ([D$_6$]DMSO): δ_H 11.65 (br.s, 1H, N*H*); 8.34 (s, 1H, H-6); 7.40 (m, 4H, Hd,e); 6.13 (apparent t, 1H, $J_{1',2'\alpha}$ = $J_{1',2'\beta}$ = 6.4 Hz, H-1′); 5.24 (d, 1H, $J_{3',OH}$ = 4.3 Hz, 3′-O*H*); 5.13 (t, 1H, $J_{5',OH}$ = 4.9 Hz, 5′-O*H*); 4.26 (m, 1H, H-3′); 3.81 (m, 1H, H-4′); 3.68–3.55 (m, 2H, H-5′); 2.16 (m, 2H, H-2′); 2.06 (br. s, 3H, H-3″,5″,7″); 1.86 (m, 6H, H-2″,8″,9″); 1.74 (m, 6H, H-4″,6″,10″). ^{13}C NMR ([D$_6$]DMSO): δ_C 161.6 (C4); 151.6 (Cf); 149.6 (C2); 143.8 (C6); 131.1 (2C, Ce); 125.3 (2C, Cd); 119.7 (Cc); 98.5 (C5); 92.1 (Cb); 87.8 (C4′); 85.0 (C1′); 82.0 (Ca); 70.1 (C3′); 61.0 (C5′); 42.5 (3C, C2″,8″,9″); 40.2 (C2′); 36.2 (3C, C4″,6″,10″); 36.1 (C1″); 28.4 (3C, C3″,5″,7″).

5-[4-(Triphenylmethyl)phenylethynyl]-2′-deoxyuridine (5). Compound **5** was prepared from **13b**; colourless crystals (417 mg, 73%). R_f 0.49 ($CHCl_3$–methanol, 4 : 1 (v/v)), mp 285–287 °C with decomp. (toluene–methanol). ESI-TOF HRMS: m/z = 571.2352 $[M + H]^+$, calc. for $[C_{36}H_{30}N_2O_5 + H]^+$ 571.2227. UV (MeOH): λ_{min}, nm (log ε) 248 (4.06), 278 (4.22), 292 (4.23); λ_{max}, nm (log ε) 270 (4.24), 286 (4.24), 313 (4.34). ^{1}H NMR ([D$_6$]DMSO): δ_H 11.66 (s, 1H, N*H*); 8.36 (s, 1H, H-6); 7.41 (d, 2H, J = 8.4 Hz, Hd); 7.31 (m, 6H, Hj); 7.22 (m, 3H, Hk); 7.17 (d, 2H, J = 8.4 Hz, He); 7.14 (m, 6H, Hi); 6.12 (apparent t, 1H, $J_{1',2'\alpha}$ = $J_{1',2'\beta}$ = 6.4 Hz, H-1′); 5.24 (m, 1H), 5.12 (m, 1H) (3′-O*H*, 5′-O*H*); 4.25 (m, 1H, H-3′); 3.81 (m, 1H, H-4′); 3.64 (m, 1H, $^2J_{5'a,5'b}$ = 11.9 Hz, $J_{4',5'a}$ = 3.3 Hz, H-5′a); 3.57 (m, 1H, $^2J_{5'a,5'b}$ = 11.9 Hz, $J_{4',5'b}$ = 3.3 Hz, H-5′b); 2.15 (m, 1H, H-2′). ^{13}C NMR ([D$_6$]DMSO): δ_C 161.5 (C4); 149.6 (C2); 147.1 (Cf); 146.1 (3C, Ch); 144.0 (C6); 130.9, 130.8 (2C and 2C, Cd,e); 130.6 (6C, Ci); 128.0 (6C, Cj); 126.9 (3C, Ck); 120.2 (Cc); 98.3 (C5); 91.6 (Cb); 87.8 (C4′); 85.0 (C1′); 82.7 (Ca); 70.1 (C3′); 64.6 (Cg); 61.0 (C5′); 40.3 (C2′).

5-[10-(Phenylethynyl)anthracen-9-ylethynyl]-2′-deoxyuridine (6). Compound **6** was prepared from **13c**; orange crystals (407 mg, 77%). R_f 0.51 ($CHCl_3$–methanol, 4 : 1 (v/v)), mp > 260 °C with decomp. (toluene–methanol). ESI-TOF HRMS: m/z = 529.1849

$[M + H]^+$, calc. for $[C_{33}H_{24}N_2O_5 + H]^+$ 529.1758. UV (MeOH): λ_{min}, nm (log ε) 242 (4.19), 287 (4.13), 298 (4.11), 354 (3.11), 450 (4.37); λ_{max}, nm (log ε) 272 (4.77), 291 (4.14), 306 (4.26), 440 (4.43), 465 (4.49); shoulder, nm (log ε) 326 (3.94). Fluorescence, (MeOH): excitation, λ_{min}, nm 449, 462, λ_{max}, nm 439, 458, 466; emission, λ_{min}, nm 507, λ_{max}, nm 486, 517. ^{1}H NMR ([D$_6$]DMSO): δ_H 11.83 (s, 1H, N*H*); 8.75 (s, 1H, H-6); 8.71 (d, 2H, $J = 8.0$ Hz, H-1″,8″); 8.67 (d, 2H, $J = 7.8$ Hz, H-4″,5″); 7.89 (m, 2H, Hf); 7.78 (m, 4H, H-2″,3″,6″,7″); 7.54 (m, 3H, Hg,h); 6.20 (m, 1H, H-1′); 5.30 (m, 2H, 3′-O*H*, 5′-O*H*); 4.35 (m, 1H, H-3′); 3.87 (m, 1H, H-4′); 3.76 (m, 1H, H-5′a); 3.68 (m, 1H, H-5′b); 2.33 (m, 1H, H-2′α); 2.25 (m, 1H, H-2′β). ^{13}C NMR ([D$_6$]DMSO): δ_C 161.8 (C4); 149.6 (C2); 144.3 (C6); 131.8 (2C, Cf); 131.5 (2C, C4a″,10a″); 131.0 (2C, C8a″,9a″); 129.5 (Ch); 129.1 (2C, Cg); 128.0 (2C, C3″,6″); 127.7 (2C, C2″,7″); 127.2 (2C, C1″,8″); 126.9 (2C, C4″,5″); 122.4 (Ce); 118.2 (C9″); 117.1 (C10″); 102.8 (Cd); 98.4 (C5); 96.6 (Ca); 89.5 (Cc or Cb); 87.9 (C4′); 86.0 (Cb or Cc); 85.4 (C1′); 69.7 (C3′); 60.8 (C5′); 40.5 (C2′).

Pharmacology

Cells. *Vero* cells culture (green monkey kidney cells) was grown in Eagle's medium (Institute of Poliomyelitis and Viral Encephalitides, Moscow, Russia) supplemented with 10% fetal calf serum ("PanEco", Moscow).

Viruses. *Herpes simplex* virus type 1, strain L_2 (HSV-1) was from the Laboratory of Virus Museum (Ivanovsky Institute of Virology, Moscow, Russia). The acyclovir-resistant strain of HSV-1 was isolated as described elsewhere.[15]

Cytotoxicity assays. *Vero* cells in 96-well microtiter plates were treated with different concentrations of the experimental drugs (1.4×10^5 cells in 185 μL of the medium per well). Cell cultures were incubated for 72 h. At the indicated time, the cells were coloured with Trypan Blue, and the cell number was determined. The 50% cytostatic concentration (CD_{50}) was defined as the compound concentration required to reduce the cell number by 50%.

Antiviral assays. *Vero* cells were inoculated with various strains of HSV-1 at an input of 0.1 PFU (plaque formation units) per cell and then incubated with a medium containing various concentrations of modified nucleosides for 48 h (95–100% virus-inducted cytopathicity in the untreated control). Antiviral activity was expressed as the compound concentration required to reduce virus-inducted cytopathicity by 50% (ID_{50}) or 95% (ID_{95}) compared to untreated control.

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