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Design, Synthesis and Biological Evaluation of Novel C5-Modified Pyrimidine Acyclic Nucleosides

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ABSTRACT

We report the synthesis of novel C5-arylalkynyl acyclic pyrimidine analogues with α, β-unsaturated carbonyl structures, at the acyclic portion, using a double Wittig reaction. The newly synthesized compounds were tested for their cytostatic activity against a broad panel of cancer cells. The antiviral assays showed that (E)-ethyl 4-(((E)-4-ethoxy-1-((2-fluorophenyl)ethynyl-uracil)-4-oxobout-2-en-1-yl)oxy)-5-hydropent-2-enoate (13a) had a noticeable activity against TK$^-$ VZV strain (EC_{50} 20 μM, MCC 100 μM).

Keywords: C5-pyrimidine acyclic nucleosides, Cytotoxic antiviral activity

Abbreviations: ^{13}C NMR: Carbon-13 Nuclear Magnetic Resonance Spectroscopy; ^1H NMR: Proton Magnetic Resonance Spectroscopy; AcOH: Acetic Acid; CD_3OD: Deuterated Methanol; $CDCl_3$: Deuterated Chloroform; CH_2Cl_2: Dichloromethane; CH_3CN: Acetonitrile; CuI: Copper(I) Iodide; DMF: Dimethyl Formamide; DMSO-d6: Deuterated Dimethyl Sulfoxide; Et_3N: Triethylamine; EtOAc: Ethyl Acetate; HMDS: Hexamethyldisilazane; MeOH: Methanol; MW: Microwave; Na_2SO_4: Sodium Sulfate; $NaHCO_3$: Sodium Bicarbonate; $Pd(PPh_3)_4$: Tetrakis(triphenylphosphine)palladium(0); TLC: Thin Layer Chromatography; TMS: Tetramethylsilane; TMSOTf: Trimethylsilyl Trifluoromethanesulfonate; UV-Vis: Ultraviolet-Visible Spectroscopy

INTRODUCTION

The synthesis of acyclic nucleosides such as Acyclovir (ACV) [1] and the discovery of its important antiviral properties have opened a new era in antiviral therapy, the study of acyclic nucleosides with excellent biological properties against a broad band of RNA viruses. Acyclovir is highly active against Herpes Simplex Virus (HSV), where it prevents virus replication by interacting with the DNA polymerase after metabolizing to its active triphosphate structure by disrupting DNA synthesis of the virus.

Among the numerous modified acyclic nucleosides with interesting biological properties [2-4], C5 modified acyclic nucleosides of uracil have been investigated as anticancer and antiviral agents [5-8]. Specifically Carmofur is used to treat colorectal cancer [9] and inhibits acidic ceramidase (ASAH1) [10] which plays an important role in the occurrence of metastatic breast cancer [11], while 1-[4-hydroxy-3-(hydroxymethyl)-1-butyl]-5-(1-azido-2-chloroethyl)uracil proved to be effective *in vitro* against Duck hepatitis B virus (EC_{50} 0.31-1.55 μM) and Cytomegalovirus (EC_{50} 3.1 μM) [12]. Furthermore, C5-arylalkynyl nucleosides of uracil showed antiviral activity, against Coxsackie virus B4, respiratory syncytial virus and yellow fever virus [13-16] and cytotoxic activity against murine leukemia, human T-lymphocyte, cervix carcinoma and hepatocellular carcinoma cells [17].

Considering the biological importance of acyclic nucleosides

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and the significant antiviral activity demonstrated by C5 modified uracil 3′-deoxy and 3′-deoxy-3′-C-methyl ones [16], we have decided to synthesize novel acyclic uracil nucleosides with α, β-unsaturated carbonyl structures incorporated into the acyclic fragment. The α, β-unsaturated carbonyl structures have been shown to be vital for the biological activity exhibited by both plant products and synthetic molecules [18-23], whereas introduction of a double bond into the acyclic portion of modified nucleosides appears to increase their activity [24,25]. Thus, we performed the synthesis and biological evaluation of C5-arylalkynyl uracil acyclic nucleosides bearing α, β-unsaturated carbonyl structures at the acyclic portion of the novel synthesized analogues.

EXPERIMENTAL SECTION

General methods

Melting points were recorded in a Mel-Temp apparatus and are uncorrected. Thin Layer Chromatography (TLC) was performed on Merck pre-coated 60F254 plates. Reactions were monitored by TLC on silica gel, with detection by UV light (254 nm) or by charring with sulfuric acid. Flash column chromatography was performed using silica gel (240-400 mesh, Merck). ^{1}H and ^{13}C NMR spectra were obtained at ambient temperature using a Bruker 300 spectrometer at 300 and 75.5 MHz, respectively using chloroform-d ($CDCl_3$), dimethylsulfoxide-d6 (DMSO-d_6) or methanol-d4 (CD_3OD) with internal tetramethylsilane (TMS). Chemical shifts (δ) are given in ppm measured downfield from TMS and spin-spin coupling constants in Hz. Mass spectra were obtained on a ThermoQuestFinnigan AQA Mass Spectrometer (electrospray ionization). All microwave irradiation experiments were carried out in a dedicated CEM-Explorer and CEM Discover monomode microwave apparatus, operating at a frequency of 2.45 GHz, with continuous irradiation power, from 0 to 300 W with utilization of the standard absorbance level of 300 W maximum powers. The reactions were carried out in 10 mL glass tubes, sealed with a Teflon septum and placed in the microwave cavity. Initially, microwave irradiation of requisite Watts was used, and the temperature was ramped from room temperature to the desired temperature. Once this was reached the reaction mixture was held at this temperature for the required time. The reaction mixture was continuously stirred during the reaction. The temperature was measured with an IR sensor on the outer surface of the process vial. After the irradiation period, gas jet cooling rapidly cooled the reaction vessel to ambient temperature. Dichloromethane was distilled from phosphorous pentoxide and stored over 4 Å molecular sieves. Acetonitrile and toluene were distilled from calcium hydride and stored over 3 Å molecular sieves. Diethylether (Et_2O) was freshly distilled under nitrogen from sodium/benzophenone before use. Pyridine was stored over potassium hydroxide pellets, N,N-Dimethylformamide (DMF) was stored over 3 Å molecular sieves. All reactions sensitive to oxygen or moisture were carried out under an Argon atmosphere.

Anti-proliferative assays

Compounds 4-9, 12-14, were evaluated for their cytostatic activity against the human cells: pancreatic adenocarcinoma (Capan-1), chronic myeloid leukemia (Hap-1), colorectal carcinoma (HCT-116), lung carcinoma (NCI-H460), acute lymphoblastic leukemia (DND-41), acute myeloid leukemia (HL-60), chronic myeloid leukemia (K-562) and non-Hodgkin lymphoma (Z-138). All assays were performed in 96-well microtiter plates. To each well (5-7.5) × 10^4 tumor cells were added,along withand varying concentrations of the test compounds ranging from 250, 50, 10, 2, 0.4 to 0.08 μM. The tumor cells were then allowed to proliferate at 37°C in a humidified CO_2-controlled atmosphere. To obtain optimal growth curves, 2 days of the: pancreatic adenocarcinoma (Capan-1), chronic myeloid leukemia (Hap-1), colorectal carcinoma (HCT-116), lung carcinoma (NCI-H460), acute lymphoblastic leukemia (DND-41), acute myeloid leukemia (HL-60) and 3 days for the chronic myeloid leukemia (K-562) and non-Hodgkin lymphoma (Z-138), were required. At the end of the incubation period, the cells were counted in a Coulter counter. The IC_{50} (50% inhibitory concentration) was defined as the concentration of the compound that inhibited cell proliferation by 50%.

Antiviral activity assays

The antiviral tests were based on inhibition of the virus-induced cytopathicity in Human Embryonic Lung (HEL) (Varicella-Zoster Virus (VZV)) cell cultures. Confluent cell cultures in microtiter 96-well plates were inoculated with 100 cell culture inhibitory dose-50 ($CCID_{50}$) of virus (1 $CCID_{50}$ being the virus dose to infect 50% of the cell cultures). After a 1 h virus adsorption period, the residual virus was removed, and the cell cultures were incubated in the presence of varying concentrations (200, 40, 8, … μM) of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures, which were not treated with the test compounds.

Synthesis of C5 alkynyl 1-(1-((1,3-dihydropropan-2-yl)oxy)-2-hydroxyethyl)-5-iodouracil (7, 9)1-(5′-O-Trityl-ribofuranozyl)-5-iodouracil (2)

A solution of trityl chloride (2.8 g, 9.90 mmol) in anhydrous dichloromethane (19 mL) was added drop wise to a solution of 1 (3.0 g, 8.26 mmol) in anhydrous pyridine (35 mL) at 0°C. Following the addition, the mixture was allowed to warm slowly to room temperature and set aside for 12 h. Methanol (7 mL) and ethyl acetate (50 mL) were added and the mixture was washed successively with saturated aqueous $NaHCO_3$ (200 mL) and H_2O (100 mL). The organic phase was dried over $MgSO_4$ and evaporated under reduced pressure. The resulting residue was crystallized from chloroform to give 6 (2.3 g; 82%). m.p. 162-163°C; $[\alpha]D^{22}$=-

8 (c 0.10, $CHCl_3$); Rf=0.27 (EtOAc/Hexane, 7:3); λ_{max} 383 nm (ε 21460); ^{1}H-NMR (300 MHz, CD_3OD) δ 8.14 (s, 1H, H-6), 7.49-7.24 (m, 15H, Tr), 5.90 (d, 1H, $J_{1',2'}$=4.8 Hz, H-1′), 5.35-4.31 (m, 2H, H-2′, H-4′), 4.10 (t, 1H, J=2.7, J=1.2 Hz, H-3′), 3.41 (dd, 1H, $J_{4'-5a'}$=3.2 Hz, $J_{5a'-5b}$=10.8 Hz, H-5b′), 4.55 (dd, 1H, $J_{4'-5b}$=2.1 Hz, H-5a′); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 161.80, 150.83, 144.19, 143.65, 129.71, 128.34, 126.28, 97.67, 94.83, 86.93, 73.22, 70.11, 68.42, 63.92; Anal. Calcd. for $C_9H_{11}IN_2O_6$: C, 59.41; H, 4.11; N, 4.57% found: C, 59.61; H, 4.41; N, 4.97%; Mass (M+H)+: 612.08.

1-(2-Hydroxy-1-(1-hydroxy-3-(trityloxy)propan-2-yl)oxy)ethyl)-5-iodouracil (3)

Compound 2 (1.1 g, 1.8 mmol) was added to a stirred solution of $NaIO_4$ (425 mg, 1.98 mmol) in H_2O (19 mL) and MeOH (19 mL) leading to immediate precipitation of $NaIO_3$. After 1 h at room temperature, any residual periodate was destroyed with a drop of ethylene glycol. The reaction mixture was stirred for 1 h at room temperature with $NaBH_4$ (500 mg, 18.5 mmol), neutralized with aqueous $NaHCO_3$ and then extracted with ethyl acetate (EtOAc) (4 × 300 mL). The organic layer was washed with $NaHSO_4$, dried over anhydrous Na_2SO_4, evaporated to dryness and purified by column chromatography with EtOAc/Hexane (7:3) to give compound 3 (650 mg, 86%) as a syrup. $[\alpha]_D^{22}$+13 (c 0.10, MeOH); Rf=0.35 (EtOAc/Hexane, 7:3); λ_{max} 305 nm (ε21400); ^{1}HNMR (300 MHz, $CDCl_3$) δ 9.97 (brs, 1H, NH), 7.84 (s, 1H, H-6), 7.39-7.25 (m, 15H,Tr), 5.99 (t, 1H, $J_{1',2a'}$=5.2 Hz, $J_{1',2b}$=5.0 Hz, H-1′), 3.87-3.72 (m, 7H, H-2′, H-3′, H-4′, OH, OH), 3.26 (dd, 1H, $J_{4'-5a'}$=3.7 Hz, $J_{5a'-5b}$=10.8 Hz, H-5a′), 3.16 (dd, 1H, $J_{4'-5b}$=6.0 Hz, H-5b′); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 161.80, 150.83, 144.19, 143.65, 129.71, 128.34, 126.28, 96.25, 94.83, 83.63, 68.42, 63.92, 61.81, 61.13; Anal. Calcd for $C_{28}H_{27}IN_2O_6$: C, 54.73; H, 4.43; N, 4.56. Found: C, 54.33; H, 4.83; N, 4.96; ESI-MS (m/z): 614.09 ($M+H^+$).

1-(1-((1,3-Dihydroxypropan-2-yl)oxy)-2-hydroxyethyl)-5-iodouracil (4)

To a solution containing 3 (1 g, 1.62 mmol) is added 1: 1 mixture of formic acid (HCOOH)/diethyl ether (Et_2O) (29 ml) and reflux for 30 min. The mixture is then neutralized with solid sodium bicarbonate ($NaHCO_3$) and extracted sequentially with sodium chloride (NaCl) and water (H_2O), dried over magnesium sulphate ($MgSO_4$), evaporated to dryness and purified by column chromatography with CH_2Cl_2/MeOH (9:1) to give compound 4 (554 mg, 92%) as a syrup. $[\alpha]_D^{22}$+9 (c 0.14, MeOH); Rf=0.25 (CH_2Cl_2/MeOH 9:1); λ_{max} 286 nm (ε18592); ^{1}HNMR (300 MHz, CD_3OD) δ 8.12 (s, 1H, H-6), 7.55-7.11 (m, 4H, Bz), 8.99 (t, 1H, J=4.9 Hz, J=4.9 Hz N-CH-C), 3.80-3.51 (m, 7H, 3x -CH_2OH, C-CH-C); ^{13}C NMR (75.5 MHz, CD_3OD) δ 161.30, 150.83, 144.19, 96.25, 94.83, 80.63, 68.22, 60.92, 58.32; Anal. Calcd for $C_9H_{13}IN_2O_6$: C, 29.05; H, 3.52; N, 7.53. Found: C, 29.45; H, 3.92; N, 7.93; ESI-MS (m/z): 372.98 ($M+H^+$).

2-(2-Acetoxy-1-(5-iodouracil)ethoxy)propan-1,3-diyldiacetylo (5)

To a solution of 4 (84 mg, 0.23 mmol) added dry pyridine (2 ml) and acetic anhydride (1 ml). The reaction was carried out at room temperature for 1 h, then was quenched with MeOH at 0°C and was concentrated in vacuum. The residue was diluted with ethyl acetate (EtOAc), washed with saturated $NaHSO_4$, $NaHCO_3$ and H_2O. The organic extract was dried over anhydrous Na_2SO_4, filtered and evaporated to dryness to give compound 5 (80 mg, 95%) as a white crystal. M.P. 215-217°C; $[\alpha]_D^{22}$+12 (c 0.25, $CHCl_3$); Rf=0.22 (EtOAc/Hexane, 2:8); λ_{max} 286 nm (ε 16459); ^{1}HNMR (300 MHz, $CDCl_3$) δ 8.47 (brs, 1H, NH), 7.85 (s, 1H, H-6), 6.13 (t, 1H, J=5.1 Hz, J=5.3 Hz N-CH-C), 4.46-3.95 (m, 7H, 3x-CH_2OAc, C-CH-C), 2.13, 2.09, 2.07 (3s, 9H, 3x-OAc);^{13}CNMR (75.5 MHz, $CDCl_3$) δ 170.48, 170.44. 169.98, 159.46, 150.24, 144.14, 81.34, 75.76, 68.85, 63.36, 63.05, 62.50, 21.06, 20.67, 20.49; Anal. Calcd for $C_{15}H_{19}IN_2O_9$: C, 36.16; H, 3.84, N 5.62. Found: C, 36.56; H, 4.04, N 5.92; ESI-MS (m/z): 416.99 ($M+H^+$).

General procedure for the preparation of the C5-arylalkynyl uracil acyclic nucleosides-6,8

Mixtures of the appropriate alkynes (0.72 mmol), Pd (PPh_3)4 (28 mg, 0.02 mmol), CuI (5.3 mg, 0.02 mmol), triethylamine (116 μl, 0.34 mmol) and 2-(2-Acetoxy-1-(5-iodouracil)ethoxy)propan-1,3-diyldiacetylo (5) (100 mg, 0.24 mmol) in 1.0 mL of anhydrous DMF, were irradiated in a microwave apparatus (200 W maximum power) for 5 min at 50°C. The reaction mixture was concentrated under reduced pressure and the crude residue was purified by flash chromatography on silica gel. The purified material was dried in vacuo to afford the corresponding derivatives 6, 8 in 78-81% yields.

2-(2-Acetoxy-1-(5-((2-fluorophenyl)ethynyl)-uracil)ethoxy)propan-1,3-diyl diacetyl (6a)

75 mg, 78% as white foam; $[\alpha]_D^{22}$=+12 (c 0.15, $CHCl_3$); Rf=0.24 (EtOAc/Hexane 2:8); λ_{max} 286 nm (ε17254); ^{1}HNMR (300 MHz, $CDCl_3$) δ 8.70 (brs, 1H, NH), 7.80 (s, 1H, H-6), 7.52 (t, 1H, J=7.5 Hz, J=7.1 Hz, Bz), 7.35 (dd, 1H, J=7.2 Hz, J=13.8 Hz, Bz), 7.14-7.07 (m, 2H, Bz), 6.20 (t, 1H, J=5.3 Hz, J=5.6 Hz N-CH-C), 4.46-3.95 (m, 7H, 3x-CH_2OAc, C-CH-C), 2.13, 2.09, 2.08 (3s, 9H, 3xOAc); ^{13}CNMR (75.5 MHz, $CDCl_3$) δ 170.60, 170.48. 170.00, 160.49, 149.61, 142.08, 133.56, 130.74, 130.62, 124.09, 124.03, 115.70, 115.37, 100.69, 81.30, 75.55, 63.39, 63.03, 62.44, 21.06, 20.67, 20.50; Anal. Calcd. for $C_{23}H_{23}FN_2O_9$: C, 56.33; H, 4.73; N, 5.71%; Found: C, 56.73; H, 4.33; N, 5.91%; ESI-MS (m/z): Mass (M+H)+: 491.14.

2-(2-Acetoxy-1-(5-((2-chlorophenyl)ethynyl)-uracil)ethoxy)propan-1,3-diyldiacetyl (6b)

80 mg, 79% as white foam; $[\alpha]_D^{22}$=+15 (c 0.19, $CHCl_3$); Rf=0.26 (EtOAc/Hexane 2:8); λ_{max} 286 nm (ε 17845);

^{1}HNMR (300 MHz, $CDCl_3$) δ 8.56 (brs, 1H, NH), 7.80 (s, 1H, H-6), 7.55 (t, 1H, J=7.5 Hz, J=7.1 Hz, Bz), 7.41 (dd, 1H, J=7.2 Hz, J=13.8 Hz, Bz), 7.30-7.22 (m, 2H, Bz), 6.19 (t, 1H, J=5.2 Hz, J=5.5 Hz N-CH-C), 4.47-3.93 (m, 7H, 3x-CH_2OAc, C-CH-C), 2.14, 2.09 (2s, 9H, 3xOAc); ^{13}CNMR (75.5 MHz, $CDCl_3$) δ 170.53, 170.48. 170.00, 160.33, 149.55, 142.09, 135.76, 133.45, 129.90, 129.33, 126.54, 122.19, 100.73, 91.03, 84.66, 81.36, 75.70, 63.38, 63.05, 62.47, 20.78, 20.67, 20.51; Anal. Calcd. for $C_{23}H_{23}ClN_2O_9$: C, 54.50; H, 4.57; N, 5.53%; Found: C, 54.10; H, 4.27; N, 5.93%; ESI-MS (m/z): Mass (M+H)+: 507.11.

2-(2-Acetoxy-1-(5-((1,4-dimethylphenyl)ethynyl)-uracily)ethoxy)propan-1,3-diyldiacetyl (6c)

80 mg, 79% as white solid; M.P. 251-253°C; $[\alpha]_D^{22}$=+12 (c 0.22, $CHCl_3$); Rf=0.30 (EtOAc/Hexane 2:8); λ_{max} 286 nm (ε 14523); ^{1}HNMR (300 MHz, $CDCl_3$) δ 8.28 (brs, 1H, NH), 7.71 (s, 1H, H-6), 7.22 (t, 1H, J=4.5 Hz, J=5.9 Hz, Bz), 7.11 (d, 1H, J=4.5 Hz, Bz), 7.06 (d, 1H, J=7.2 Hz, Bz), 6.19 (t, 1H, J=5.6 Hz, J=5.6 Hz N-CH-C), 4.47-3.96 (m, 7H, 3x-CH_2OAc, C-CH-C), 2.44, 2.29 (2s, 6H, 2x CH3)2.14, 2.08, 2.07 (3s, 9H, 3xOAc); ^{13}CNMR (75.5 MHz, $CDCl_3$) δ 170.53, 170.48. 170.00, 160.33, 149.55, 142.09, 135.76, 133.45, 129.90, 129.33, 126.54, 122.19, 100.73, 91.03, 84.66, 81.36, 75.70, 63.38, 63.05, 62.47, 20.74, 20.70, 20.62, 20.54, 20.21; Anal. Calcd. for $C_{25}H_{28}N_2O_9$: C, 59.59; H, 5.64; N, 5.50%; Found: C, 59.59; H, 5.24; N, 5.20%; ESI-MS (m/z): Mass (M+H)+: 501.18.

2-(2-Acetoxy-1-(5-((6-methoxynapthlene)ethynyl-uracil)ethoxy)propan-1,3-diyldiacetyl (8)

83 mg, 81% as white solid; M.P. 286-289°C; $[\alpha]_D^{22}$=-2 (c 0.16, $CHCl_3$); Rf=0.30 (EtOAc/Hexane 2:8); λ_{max} 286 nm (ε 16895); ^{1}HNMR (300 MHz, $CDCl_3$) δ 8.43 (brs, 1H, NH), 7.96 (s, 1H, H-6), 7.77 (s, 1H, napthalene), 7.70 (t, 2H, J=7.7 Hz, J=8.2 Hz, napthalene), 7.49 (d, 1H, J=8.7 Hz, napthalene), 7.16 (d, 1H, J=8.7 Hz, napthalene), 7.11 (s, 1H, napthalene), 6.20 (t, 1H, J=5.4 Hz, J=5.6 Hz N-CH-C), 4.48-3.97 (m, 7H, 3x-CH_2OAc, C-CH-C), 3.93 (s, 3H, OCH_3), 2.14, 2.11, 2.09 (3s, 9H, 3xOAc); ^{13}CNMR (75.5 MHz, $CDCl_3$) δ 170.53, 170.48. 170.00, 160.33, 158.92, 149.55, 142.09, 135.76, 133.45, 129.90, 129.33, 126.54, 122.19, 119.89, 117.50, 105.96, 100.73, 91.03, 84.66, 81.36, 75.70, 63.38, 63.05, 62.47, 55.41, 20.74, 20.70, 20.62; Anal. Calcd. for $C_{28}H_{28}N_2O_{10}$: C, 60.87; H, 5.11; N, 5.07%; Found: C, 60.47; H, 5.31; N, 5.27%; ESI-MS (m/z): Mass (M+H)+: 553.17.

General procedure for the preparation of the unprotected C5-arylalkynyl uracil acyclic nucleosides-7,9

The protected nucleosides 6, 8 (0.12 mmol), were treated with methanolic ammonia (saturated at 0°C, 6.7 mL). The solution was stirred overnight at room temperature and then evaporated under reduced pressure. The residue obtained was purified by flash column chromatography to afford the unprotected derivatives 7, 9 in 65-85% yields, as white solids.

1-(1-((1,3-Dihydroxypropan-2-yl)oxy)-2-hydroxyethyl)-5-[(2-fluorophenyl) ethynyl] uracil (7a)

80 mg, 85% as white solid; M.P. 189-192°C; $[\alpha]_D^{22}$=+8 (c 0.24, MeOH); Rf=0.19 (CH_2Cl_2/MeOH 9:1); λ_{max} 286 nm (ε 13564); ^{1}HNMR (300 MHz, CD_3OD) δ 8.12 (s, 1H, H-6), 7.55-7.11 (m, 4H, Bz), 8.99 (t, 1H, J=4.9 Hz, J=4.9 Hz N-CH-C), 3.80-3.51 (m, 7H, 3x-CH_2OH, C-CH-C); ^{13}CNMR (75.5 MHz, CD_3OD) δ 162.79, 150.64, 144.70, 133.25, 130.25, 130.12, 123.99, 123.93, 115.24, 114.90, 98.71, 85.62, 84.36, 81.11, 62.21, 61.13, 60.69; Anal. Calcd. for $C_{17}H_{17}FN_2O_6$: C, 56.04; H, 4.70; N, 7.69%; Found: C, 56.44; H, 4.30; N, 8.09%; ESI-MS (m/z): Mass (M+H)+: 365.11.

1-(1-((1,3-Dihydroxypropan-2-yl)oxy)-2-hydroxyethyl)-5-[(2-chlorophenyl) ethynyl] uracil (7b)

84 mg, 85% as white solid; M.P. 195-197°C; $[\alpha]_D^{22}$=+9 (c 0.23, MeOH); Rf=0.22 (CH_2Cl_2/MeOH 9:1); λ_{max} 286 nm (ε 15732); ^{1}HNMR (300 MHz, CD_3OD) δ 8.12 (s, 1H, H-6), 7.59-7.27 (m, 4H, Bz), 5.99 (t, 1H, $J_{1'-2a'}$=4.9 Hz, $J_{1'-2b}$=4.9 Hz N-CH-C), 3.79-3.53 (m, 7H, 3x-CH_2OH, C-CH-C); ^{13}CNMR (75.5 MHz, CD3OD) δ 164.16, 152.08, 146.15, 136.60, 134.53, 130.85, 127.90, 124.21, 100.19, 90.49, 87.23, 85.88, 82.58, 63.70, 62.81, 62.20; Anal. Calcd. for $C_{17}H_{17}ClN_2O_6$: C, 53.62; H, 4.50; N, 7.36%; Found: C, 54.02; H, 4.30; N, 7.76%; ESI-MS (m/z): Mass (M+H)+: 381.08.

1-(1-(((1,3-Dihydroxypropan-2-yl)oxy)-2-hydroxyethyl)-5-[(1,4-dimethylphenyl) ethynyl] uracil (7c)

88 mg, 85% as white solid; M.P. 220-221°C; $[\alpha]_D^{22}$=+12 (c 0.18, MeOH); Rf=0.28 (CH_2Cl_2/MeOH 9:1); λ_{max} 282 nm (ε 18563); ^{1}HNMR (300 MHz, CD_3OD) δ 8.07 (s, 1H, H-6), 7.25 (s, 1H, ArH), 7.10 (d, 1H, J=7.6, Bz), 7.04 (d, 1H, Bz), 5.99 (t, 1H, J=4.9 Hz, J=4.9 Hz N-CH-C), 3.80-3.52 (m, 7H, 3x-CH_2OH, C-CH-C), 2.40, 2.26 (2s, 6H, 2xCH_3); ^{13}CNMR (75.5 MHz, CD_3OD) δ 164.16, 152.08, 146.15, 136.60, 134.53, 130.85, 127.90, 124.21, 100.19, 90.49, 87.23, 85.88, 82.58, 63.70, 62.81, 62.20, 19.37, 18.94.; Anal. Calcd. for $C_{19}H_{22}N_2O_6$: C, 60.95; H, 5.92; N, 7.48%; Found: C, 60.55; H, 5.62; N, 7.08%; ESI-MS (m/z): Mass (M+H)+: 375.15.

1-(1-((1,3-Dihydroxypropan-2-yl)oxy)-2-hydroxyethyl)-5-[(6-methoxynapthalene) ethynyl] uracil (9)

88 mg, 65% as white solid; M.P. 162-163°C; $[\alpha]_D^{22}$=+15 (c 0.17, MeOH); Rf=0.15 (CH_2Cl_2/MeOH 9:1); λ_{max} 286 nm (ε 19654); ^{1}HNMR (300 MHz, CD_3OD) δ 8.12 (s, 1H, H-6), 7.94-7.12 (m, 6H, naphthalene), 5.99 (t, 1H, J=5.15 Hz, J=5.13 Hz N-CH-C), 3.90 (s, 3H, OCH_3), 3.82-3.53 (m, 7H, 3x-CH_2OH, C-CH-C); ^{13}CNMR (75.5 MHz, CD_3OD) δ 164.57, 160.07, 152.13, 145.48, 135.85, 132.14, 130. 38, 129.95, 129.69, 128.09, 120.60, 119.17, 106.90, 100.77, 94.59, 85.79, 82.55, 81.41, 63.69, 62.82, 62.16, 55.89; Anal. Calcd. for $C_{22}H_{22}N_2O_7$: C, 61.97; H, 5.20; N, 6.57%; Found:

C, 62.27; H, 5.60; N, 6.97%; ESI-MS (m/z): Mass (M+H)+: 427.14.

Synthesis of (E)-ethyl 4-(((E)-4-ethoxy-1-(5-iodo-uracil)-4-oxobout-2-en-1-yl)oxy)-5-(trityloxy)pent-2-enoate (11)

A solution of sodium periodate (0.23 g, 1.09 mmol) in water (5 mL) was slowly added to a cooled stirred solution of the protected nucleoside 2 (0.612 g, 1 mmol) in methanol (10 mL). The reaction mixture was stirred at room temperature for 1 h and then filtered to remove the salts. The solution was diluted with ethyl acetate (15 mL), washed with a saturated solution of NaCl (2 × 15 mL) and dried over $MgSO_4$. The solvent was removed by evaporation under reduced pressure to yield a white powder of the corresponding dialdehyde derivative. The crude dialdehyde was dissolved in freshly distilled tetrahydrofuran (5 mL) and (ethoxycarbonylmethylene) triphenylphosphorane (0.871 g, 2.5 mmol) added and the mixture was heated at 40°C for 1 h under nitrogen. The reaction mixture washed twice with a saturated solution of ammonium chloride (2 × 30 mL), dried over $MgSO_4$, evaporated to a yellow syrup and purified by column chromatography (ethyl acetate/hexane, 2:8) to afford the protected nucleoside 11 (0.435 g, 53%) as a foam. $[\alpha]_D^{22}$ +22 (c 0.25, $CHCl_3$); Rf=0.52 (EtOAc/Hexane, 2:8); λ_{max} 286 nm (ε 24538); ^{1}HNMR (300 MHz, $CDCl_3$) δ 8.62 (s, 1H, NH), 7.61 (s, 1H, H-6), 7.41-7.25 (m, 15H, trityl), 6.72 (dd, 1H, J=3.5 Hz, J=15.6 Hz, H-3), 6.69 (dd, 1H, J=6.7 Hz, J=15.8 Hz, H-2), 6.35 (dd, 1H, J=6.7 Hz, J=15.8 Hz, H-2′), 6.29 (t, 1H, J=3.4 Hz, H-1), 6.11 (dd, 1H, J=1.2 Hz, J=15.8 Hz, H-3′), 4.28 (dd, 2H, J=6.1 Hz, J=13.3 Hz, CH_2), 4.21 (dd, 2H, J=7.1 Hz, J=14.2 Hz, CH_2), 4.03-3.99 (m, 1H, H-4), 3.38 (dd, 1H, J=7.4 Hz, J=11.0 Hz, -H-5), 3.23 (dd, 1H, J=3.4 Hz, H-5′), 1.35 (t, 3H, J=7.2 Hz, CH_3), 1.29 (t, 3H, J=7.2 Hz, CH_3); ^{13}CNMR (75.5 MHz, $CDCl_3$) δ 165.32, 164.96, 159.38, 150.07, 144.07, 143.36, 141.21, 139.76, 128.64, 128.47, 128.05, 127.96, 127.90, 127.30, 127.23, 127.14, 126.10, 125.27, 87.38, 79.79, 77.24, 70.25, 65.56, 61.26, 60.90, 14.15; Anal. Calcd for $C_{36}H_{35}IN_2O_8$: C, 57.61; H, 4.70, N 3.73. Found: C, 58.01; H, 5.10, N 4.13; ESI-MS (m/z): 751.14 ($M+H^+$).

Synthesis of (E)-ethyl 4-(((E)-4-ethoxy-1-(5-iodo-uracil)-4-oxobout-2-en-1-yl)oxy)-5-hydroxypent-2-enoate (12)

Compound 12 was synthesized from 11 by a similar procedure to that described for the preparation of 4. The crude product was purified by flash column chromatography (EtOAc/Hexane, 3:7) to give analogue 12 (88 mg, 95%) as a white solid. M.P. 182-184°C; $[a]_D^{22}$=23 (c 0.31, $CHCl_3$); Rf=0.35 (EtOAc/ Hexane, 3:7); λ_{max} 286 nm (ε 24167)); ^{1}HNMR (300 MHz, $CDCl_3$) δ 8.80 (s, 1H, NH), 7.71 (s, 1H, H-6), 6.79 (dd, 1H, J=6.7 Hz, J=15.3 Hz, H-2), 6.75 (dd, 1H, J=3.9 Hz, J=15.8 Hz, H-3), 6.38 (t, 1H, J=3.3 Hz,H-1), 6.35 (dd, 1H, J=1.2 Hz, J=15.8 Hz, H-3′), 6.11 (dd, 1H, J=1.2 Hz, J=15.8 Hz, H-2′), 4.32 (s, 1H, OH), 4.28-4.18 (m, 4H, 2xCH_2, H-4), 3.74 (dd, 1H, J=2.9 Hz, J=11.9 Hz, -H-5), 3.67 (dd, 1H, J=7.5 Hz, H-5′), 1.34 (t, 3H, J=7.2 Hz, CH_3), 1.31 (t, 3H, J=7.2 Hz, CH_3); ^{13}CNMR (75.5 MHz, $CDCl_3$) δ 165.48, 165.01, 159.96, 150.61, 144.68, 144.04, 139.63, 126.23, 125.15, 80.59, 79.07, 69.76, 64.25, 61.29, 60.98, 14.14; Anal. Calcd.for $C_{17}H_{21}IN_2O_8$: C, 40.17; H, 4.16, N 5.51. Found: C, 40.57; H, 4.36, N 5.81; ESI-MS (m/z): 509.03 ($M+H^+$).

General procedure for the preparation of the C5-arylalkynyl (E)-ethyl 4-(((E)-4-ethoxy-1-(5-iodo-uracil)-4-oxobout-2-en-1-yl)oxy)-5-hydroxypent-2-enoate (13, 14)

Mixtures of the appropriate alkynes (0.72 mmol), Pd (PPh^3)^4 (28 mg, 0.02 mmol), CuI (5.3 mg, 0.02 mmol), triethylamine (116 μl, 0.34 mmol) and (E)-ethyl 4-(((E)-4-ethoxy-1-(5-iodo-uracil)-4-oxobout-2-en-1-yl)oxy)-5-hydroxypent-2-enoate (12) (100 mg, 0.20 mmol) in 1.0 mL of anhydrous DMF, were irradiated in a microwave apparatus (200 W maximum power) for 5 min at 50°C. The reaction mixture was concentrated under reduced pressure and the crude residue was purified by flash chromatography on silica gel. The purified material was dried in vacuo to afford the corresponding derivatives 13, 14 in 69-82% yields.

(E)-ethyl 4-(((E)-4-ethoxy-1-((2-fluorophenyl)ethynyl-uracil)-4-oxobout-2-en-1-yl)oxy)-5-hydropent-2-enoate (13a)

75 mg, 74% as white foam; $[\alpha]_D^{22}$=+21 (c 0.14, $CHCl_3$); Rf=0.36 (EtOAc/Hexane3:7); λ_{max} 286 nm (ε 19547); ^{1}HNMR (300 MHz, $CDCl_3$) δ 8.83 (s, 1H, NH), 7.66 (s, 1H, H-6), 7.51 (dd, 1H, J=8.2 Hz, J=7.4 Hz, Bz), 7.32 (dd, 1H, J=8.2 Hz, J=7.4 Hz, Bz), 7.01 (dd, 2H, J=6.7 Hz, J=15.3 Hz, Bz), 6.81 (dd, 1H, J=3.9 Hz, J=15.8 Hz, H-3), 6.77(dd, 1H, J=3.9 Hz, J=15.8 Hz, H-2), 6.46 (t, 1H, J=3.3 Hz, H-1), 6.39 (dd, 1H, J=1.2 Hz, J=15.8 Hz, H-3′), 6.13 (dd, 1H, J=1.2 Hz, J=15.8 Hz, H-2′), 4.36 (s, 1H, OH), 4.28-4.20 (m, 5H, 2xCH_2, H-4), 3.74 (dd, 1H, J=2.9 Hz, J=11.9 Hz , H-5), 3.67 (dd, 1H, J=7.5 Hz, H-5′), 1.33 (t, 3H, J=7.2 Hz, CH_3), 1.30 (t, 3H, J=7.2 Hz, CH_3); ^{13}CNMR (75.5 MHz, $CDCl_3$) δ 165.48, 165.01, 160.07, 159.96, 150.61, 144.68, 144.04, 139.63, 133.11, 126.23, 126.08, 125.15, 123.09, 115.01, 109.32, 93.25, 86.43, 80.59, 79.07, 69.76, 64.25, 61.29, 60.98, 14.14; Anal. Calcd. for $C_{25}H_{25}FN_2O_8$: C, 60.00; H, 5.03; N, 5.60%; Found: C, 60.20; H, 5.13; N, 5.90%; ESI-MS (m/z): Mass (M+H)+: 501.16.

(E)-ethyl 4-(((E)-4-ethoxy-1-((2-chlorophenyl)ethynyl-uracil)-4-oxobout-2-en-1-yl)oxy)-5-hydropent-2-enoate (13b)

75 mg, 78% as white foam; $[\alpha]_D^{22}$=+19 (c 0.15, $CHCl_3$); Rf=0.42 (EtOAc/ Hexane 3:7); λ_{max} 286 nm (ε 21547); ^{1}HNMR (300 MHz, $CDCl_3$) δ8.70 (s, 1H, NH), 7.66 (s, 1H, H-6), 7.56 (dd, 1H, J=8.2 Hz, J=7.4 Hz, Bz), 7.40 (dd, 1H, J=8.2 Hz, J=7.4 Hz, Bz), 7.21 (dd, 2H, J=6.7 Hz, J=15.3 Hz, Bz), 6.80 (dd, 1H, J=3.9 Hz, J=15.8 Hz, H-3), 6.78 (dd, 1H, J=3.9 Hz, J=15.8 Hz, H-2), 6.45 (t, 1H, J=3.3 Hz, H-1), 6.39 (dd, 1H, J=1.2 Hz, J=15.8 Hz, H-3′), 6.14 (dd, 1H, J=1.2 Hz,

J=15.8 Hz, H-2′), 4.34 (s, 1H, OH), 4.28-4.21 (m, 5H, 2xCH_2, H-4), 3.74 (dd, 1H, J=2.9 Hz, J=11.9 Hz, H-5), 3.67 (dd, 1H, J=7.5 Hz,H-5′), 1.34 (t, 3H, J=7.2 Hz, CH_3), 1.31 (t, 3H, J=7.2 Hz, CH_3); ^{13}CNMR (75.5 MHz, $CDCl_3$) δ 165.48, 165.01, 160.07, 159.96, 150.61, 144.68, 144.04, 139.63, 133.11, 126.23, 126.08, 125.15, 123.09, 115.01, 109.32, 93.25, 86.43, 80.59, 79.07, 69.76, 64.25, 61.29, 60.98, 14.14; Anal. Calcd. for $C_{25}H_{25}ClN_2O_8$: C, 58.09; H, 4.87; N, 5.42%; Found: C, 58.49; H, 4.47; N, 5.82%; ESI-MS (m/z): Mass (M+H)+: 517.13.

(E)-ethyl4-(((E)-4-ethoxy-1-((1,4-dimethylphenyl)ethynyl-uracil)-4-oxobout-2-en-1-yl)oxy)-5-hydropent-2-enoate (13c)

79 mg, 82% as white foam; $[\alpha]_D^{22}$=+24 (c 0.18, $CHCl_3$); Rf=0.42 (EtOAc/Hexane 3:7); λ_{max} 286 nm (ε 21574); ^{1}HNMR (300 MHz, $CDCl_3$) δ 8.83 (s, 1H, NH), 7.57 (s, 1H, H-6), 7.27 (s, 1H, Bz), 7.07 (d, 1H, J=7.7 Hz, Bz), 7.02 (d, 1H, J=7.7 Hz, Bz), 6.80 (dd, 1H, J=3.9 Hz, J=15.8 Hz, H-3), 6.77 (dd, 1H, J=3.9 Hz, J=15.8 Hz, H-2), 6.45 (t, 1H, J=3.3 Hz, H-1), 6.39 (dd, 1H, J=1.2 Hz, J=15.8 Hz, H-3′), 6.14 (dd, 1H, J=1.2 Hz, J=15.8 Hz, H-2′), 4.35 (s, 1H, OH), 4.26-4.20 (m, 5H, 2xCH_2, H-4), 3.73 (dd, 1H, J=2.9 Hz, J=11.9 Hz H-5), 3.67 (dd, 1H, J=7.5 Hz,H-5′), 2.42, 2.27 (2s, 6H, 2xCH_3), 1.32 (t, 3H, J=7.2 Hz, CH_3), 1.29 (t, 3H, J=7.2 Hz, CH_3); ^{13}CNMR (75.5 MHz, $CDCl_3$) δ 165.48, 165.01, 160.07, 159.96, 150.61, 144.68, 144.04, 139.63, 133.11, 126.23, 126.08, 125.15, 123.09, 115.01, 109.32, 93.25, 86.43, 80.59, 79.07, 69.76, 64.25, 61.29, 60.98, 20.75, 20.22, 14.34, 14.14; Anal. Calcd. for $C_{27}H_{30}N_2O_8$: C, 63.52; H, 5.92; N, 5.49%; Found: C, 63.92; H, 5.62; N, 5.89%; ESI-MS (m/z): Mass (M+H)+: 511.20.

(E)-ethyl4-(((E)-4-ethoxy-1-((6-methoxynaphtalene)ethynyl-uracil)-4-oxobout-2-en-1-yl)oxy)-5-hydropent-2-enoate (14)

68 mg, 69% as white solid; M.P. 192-193°C; $[\alpha]_D^{22}$=+24 (c 0.10, $CHCl_3$); Rf=0.28 (EtOAc/Hexane 3:7); λ_{max} 286 nm (ε 26413); ^{1}HNMR (300 MHz, $CDCl_3$) δ 8.83 (s, 1H, NH), 7.57 (s, 1H, H-6), 7.27 (s, 1H, Bz), 7.07 (d, 1H, J=7.7 Hz, Bz), 7.02 (d, 1H, J=7.7 Hz, Bz), 6.80 (dd, 1H, J=3.9 Hz, J=15.8 Hz, H-3), 6.77 (dd, 1H, J=3.9 Hz, J=15.8 Hz, H-2), 6.45 (t, 1H, J=3.3 Hz, H-1), 6.39 (dd, 1H, J=1.2 Hz, J=15.8 Hz, H-3′), 6.14 (dd, 1H, J=1.2 Hz, J=15.8 Hz, H-2′), 4.00 (s, 3H, OCH_3), 4.35 (s, 1H, OH), 4.26-4.20 (m, 5H, 2xCH_2, H-4), 3.73 (dd, 1H, J=2.9 Hz, J=11.9 Hz , H-5), 3.67 (dd, 1H, J=7.5 Hz, H-5′), 1.32 (t, 3H, J=7.2 Hz, CH_3), 1.29 (t, 3H, J=7.2 Hz, CH_3); ^{13}CNMR (75.5 MHz, $CDCl_3$) δ 165.48, 165.01, 160.07, 159.96, 150.61, 144.68, 144.04, 139.63, 133.11, 129.64, 129.16, 126.23, 126.08, 125.15, 123.09, 115.01, 109.32, 105. 93, 93.25, 86.43, 80.59, 79.07, 69.76, 64.25, 61.29, 60.98, 55.82, 14.28; Anal. Calcd. for $C_{30}H_{30}N_2O_9$: C, 64.05; H, 5.38; N, 4.98%; Found: C, 64.25; H, 5.58; N, 4.78%; ESI-MS (m/z): Mass (M+H)+: 563.20.

RESULTS AND DISCUSSION

Chemistry

Our first synthetic efforts focused on the preparation of the C5-substituted uracil 1-(1-((1,3-dihydroxyprapan-2-yl)oxy)-2-hydroxyethyl) **(Figure 1)**. Uridine 1 was treated in pyridine with an excess of trityl chloride (TrCl), in the presence of a catalytic amount of 4,4-dimethylaminopyridine (DMAP) to give the corresponding trityl derivative 2 in 82% yield [26]. The oxidative cleavage of the cis-diol in the 2′,3′-position of compound 2 followed by borohydride reduction of the resulting aldehyde (one pot), furnished the corresponding acyclic nucleoside 3. Deprotection of the 5′-O-trityl 3 by treatment with formic acid (HCOOH) in diethylether (Et_2O) gave the acyclic nucleoside of iodouracil 4 [27]. The next step of the synthesis involves the acetylation of the hydroxyl groups of the nucleoside 4 using acetic anhydride in the presence of pyridine led to acetylated derivative 5. In order to extract more detailed structure-activity relationships, diverse alkyne substituents R were selected, which included a phenyl ring substituted with halogens (6a, R=2-fluoro, 6b, R=2-chloro) or methyl groups (6c, R=2,5-dimethyl) and a polycyclic aromatic hydrocarbon substituted with a methoxyl group (8, R=6-methoxynaphthalene). In a typical experiment, the acetylated acyclic nucleoside of 5-iodouracil (5) was mixed with N,N-dimethylformamide (DMF), the appropriate alkyne, triethylamine (base), copper(I) iodide (CuI) (co-catalyst) and tetrakis(triphenylphosphine) palladium (0) ($Pd(PPh_3)_4$) (catalyst) and were irradiated at 50°C for 5 min. After removing all the volatile materials in vacuo, the solid obtained was purified by flash chromatography to provide the C5-alkynyl acyclic nucleosides 6, 8, which upon treatment with saturated methanolic ammonia afforded the unprotected derivatives 7 and 9, in good yields (65-85%).

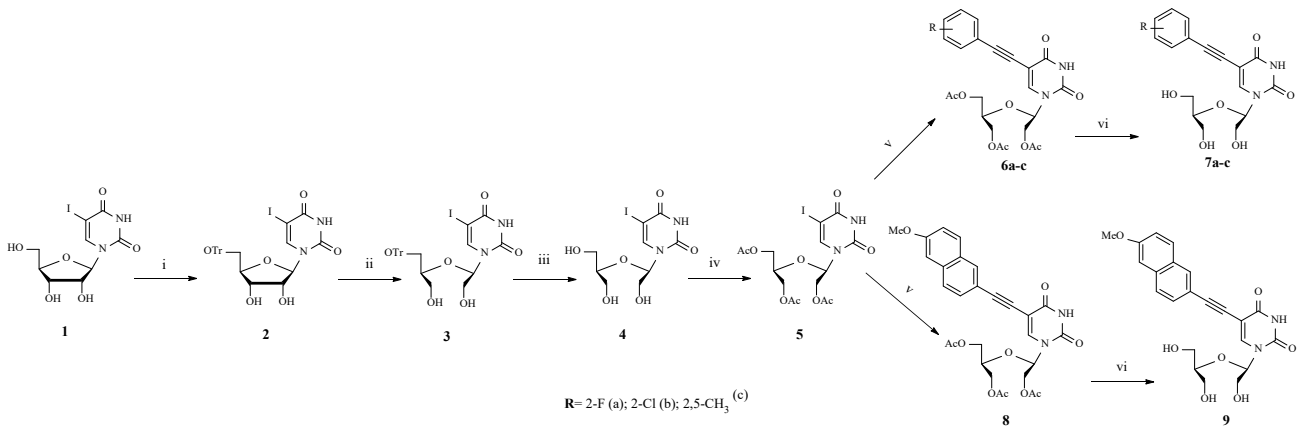

Figure 1. i) TrCl, pyridine, DMAP; ii) a) $N\alpha IO_4$, MeOH, H_2O, 1h, b) $N\alpha BH_4$, 1h; iii) HCOOH, Et_2O; iv) Ac_2O, pyridine, 1h; v) DMF, CuI, Et_3N, $Pd(PPh_3P)_4$ and the appropriate alkyne; vi) $MeOH/NH_3$.

The next objective was to synthesize C5 modified acyclic nucleosides of uracil, wherein the acyclic moiety would have been introduced into α, β-unsaturated carbonyl structures that have been shown to confer particular biological activity on the molecules carrying them. The synthesis of the C5-substituted uracil α, β-unsaturated carbonyl acyclic nucleosides is outlined in **Figure 2**. The oxidative cleavage of the cis-diol in the 2′,3′-position of compound 2 was achieved with sodium periodate in a mixture of methanol/water. The dialdehyde were unstable, hence after isolation they were directly subjected to a double Wittig olefination, using (ethoxycarbonylmethylene) triphenylphosphorane in tetrahydrofurane (THF) at 40°C for 1 h afforded compound 11in 58% yield. It was of particular note, that under such reaction conditions no diastereoisomeric by products observed, by ^{1}HNMR and COSY spectroscopy, indicating that complete chiral integrity was retained at carbon atoms C1 and C4 of 11, the iodouracil base retains the β configuration and finally the protons of the two double bonds are in trans position as we observe large coupling constants (15.8 Hz) [28-30]. Deprotection of the 5′-O-trityl 11 by treatment with formic acid (HCOOH) in diethylether (Et_2O) gave the acyclic nucleoside of iodouracil 12. Using the same one-pot Sonogashira protocol as previously discussed, the novel aryl alkynyl acyclic nucleosides 13 and 14 were obtained, in satisfying yields (69-82%).

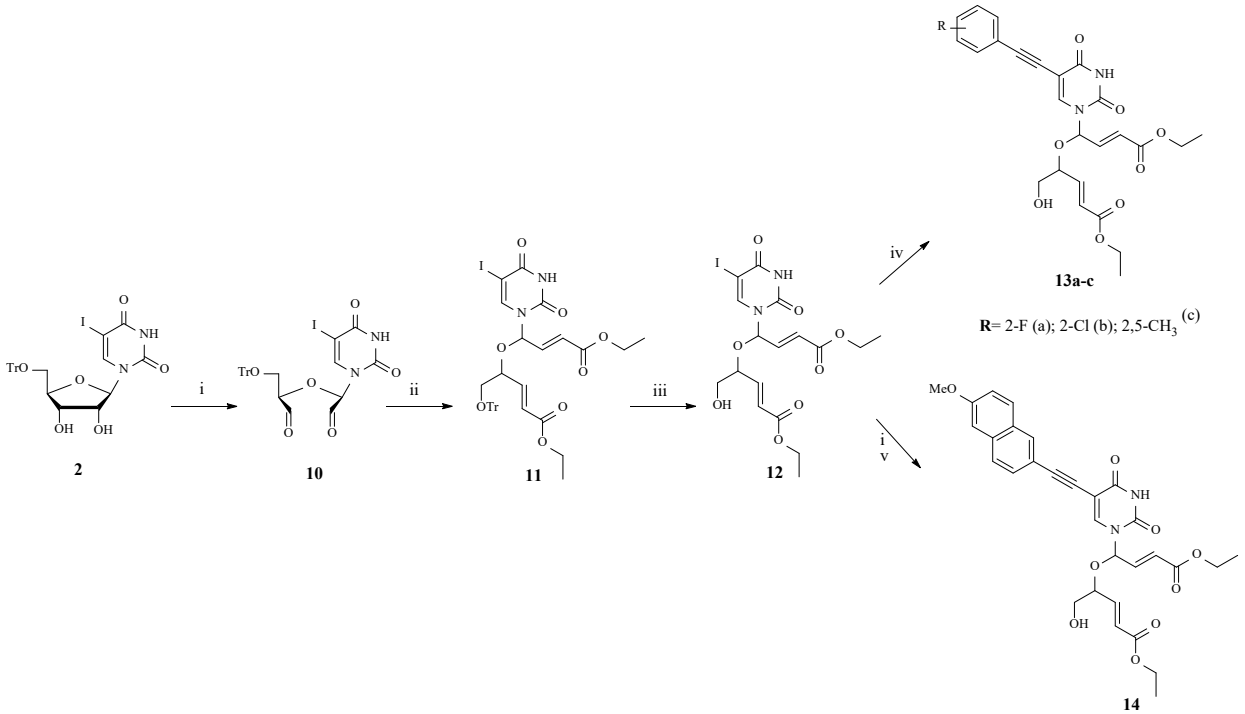

Figure 2. i) $N\alpha IO_4$, MeOH, H_2O, 1h; ii) $(C_6H_5)_3P{=}CHCO_2CH_2CH_3$, THF, 40°C, 1h; iii) HCOOH, Et_2O; iv) DMF, CuI, Et_3N, $Pd(PPh_3P)_4$ and the appropriate alkyne.

Biological evaluation

Compounds 4-9, 12-14, were evaluated for their cytostatic activity against the human cells: pancreatic adenocarcinoma (Capan-1), chronic myeloid leukemia (Hap-1), colorectal carcinoma (HCT-116), lung carcinoma (NCI-H460), acute lymphoblastic leukemia (DND-41), acute myeloid leukemia (HL-60), chronic myeloid leukemia (K-562) and non-Hodgkin lymphoma (Z-138) and their antiviral activity varicella-zoster virus (VZV) in human embryonic lung (HEL) cell cultures.The results of cytotoxic and antiviral activity are shown in **Tables 1 and 2**, respectively. Unfortunately none of the tested compound showed any significant cytostatic activity at a broad panel of cancer cell lines. The antiviral assays showed that (E)-ethyl 4-(((E)-4-ethoxy-1-((2-fluorophenyl)ethynyl-uracil)-4-oxobout-2-en-1-yl)oxy)-5-hydropent-2-enoate (13a) had a noticeable activity against TK^- VZV strain (EC_{50} 20 μM, MCC 100 μM).

Table 1. Cytostatic activity of compounds 4-9, 12-14, against a panel of tumor cell lines.

Compounds	IC_{50} (μM)	Pancreatic adenocarcinoma Capan-1	Chronic myeloid leukemia Hap-1	Colorectal carcinomaHCT-116	Lung carcinomaNCI-H460	Acute lymphoblastic leukemia DND-41	Acute myeloid leukemia HL-60	Chronic myeloid leukemia K-562	Non-Hodgkin lymphomaZ-138
		Average							
4	μM	>100	>100	>100	44,6	>100	>100	>100	>100
7a	μM	>100	>100	>100	>100	>100	>100	>100	>100
7b	μM	>100	>100	>100	47,0	>100	>100	>100	>100
7c	μM	>100	>100	56,2	46,1	>100	>100	>100	>100
9	μM	>100	>100	>100	>100	>100	>100	>100	>100
5	μM	58.0	>100	79.0	>100	96.6	>100	>100	40.0
6a	μM	>100	>100	>100	>100	>100	>100	>100	>100
6b	μM	>100	>100	>100	>100	>100	>100	>100	>100
6c	μM	64.7	>100	>100	>100	>100	>100	>100	>100
8	μM	>100	88.9	>100	>100	>100	>100	>100	>100
12	μM	37.2	23.8	55.7	57.9	22.5	45.8	62.4	21.1
13a	μM	47.4	49.1	44.5	68.5	34.1	48.6	51.6	23.4
13b	μM	>100	>100	93.3	>100	>100	>100	80.7	>100
13c	μM	44.1	45.7	35.0	>100	37.9	52.4	42.2	36.6
14	μM	>100	>100	>100	>100	>100	>100	>100	>100
Docetaxel	nM	21.9	3.0	19.3	4.9	1.7	5.4	2.1	1.1
Staurosporine	nM	49.5	46.1	66.5	60.0	28.1	52.9	39.1	9.9

**50% inhibitory concentration or compound concentration required to inhibit cell proliferation by 50%*

Table 2. Antiviral activity of compounds 4-9, 12-14 varicella-zoster virus (VZV).

Compounds	Antiviral activity EC_{50} (μM)[a]		Cytotoxicity (μM)	
	TK^+ VZV strain	TK^- VZV strain	Cell morphology	Cell growth
	OKA	07-1	(MCC)[b]	(CC50)[c]
4	>100	>100	>100	ND[d]
7a	>100	>100	>100	ND
7b	>100	>100	>100	ND
7c	>100	>100	>100	ND
9	>100	>100	>100	ND
5	>100	>100	>100	ND
6a	>100	>100	>100	ND
6b	>100	>100	>100	ND
6c	>100	>100	>100	ND
8	>100	>100	>100	ND
12	54.69	41.37	>100	ND
13a	>20	>20	100	ND
13b	>100	>100	100	ND
13c	38.07	32.82	100	ND
14	>100	>100	100	ND
Acyclovir	0.58	36.63	>440	ND
Brivudin	0.039	4.65	>300	ND

[a]*Effective concentration required to reduce virus plaque formation by 50%. Virus input was 20 plaque forming units (PFU)*
[b]*Minimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology*
[c]*Cytotoxic concentration required to reduce cell growth by 50%*
[d]*Not determined*

CONCLUSION

In the present study, we report the synthesis of novel C5-arylalkynyl pyrimidine acyclic nucleosides, by developing highly efficient synthetic routes. Nucleoside (E)-ethyl 4-(((E)-4-ethoxy-1-((2-fluorophenyl)ethynyl-uracil)-4-oxobout-2-en-1-yl)oxy)-5-hydropent-2-enoate (13a) had a noticeable activity against TK^- VZV strain (EC_{50} 20 μM, MCC 100 μM) **(Table 2)**.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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