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PALLADIUM-CATALYZED SYNTHESIS OF (E)-5-(2-ACYLVINYL)-2'-DEOXYURIDINES AND THEIR ANTIVIRAL AND CYTOTOXIC ACTIVITIES[1]

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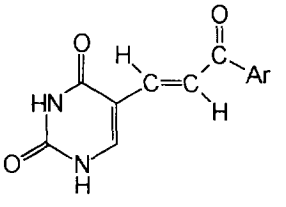
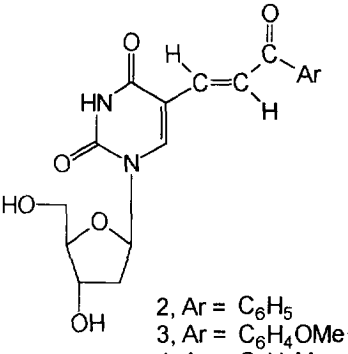
Abstract. 5-Iodo-2'-deoxyuridine was converted to (E)-5-(2-acylvinyl)-2'-deoxyuridines by palladium-catalyzed reactions in excellent yields. Compound **2** [(E)-5-(2-benzoylvinyl)-2'-deoxyuridine] had a weak activity against tumor cell lines. In contrast to BVDU, compound **2** and its (E)-5-[2-(p-methoxybenzoyl)vinyl] counterpart (compound **3**) were inactive against HSV-1 and VZV.

Introduction

5-Substituted uracils and their nucleosides have been of importance in cancer[2] and viral chemotherapy[3-5]. Of them, (E)-5-(2-bromovinyl)-2'-deoxyuridine (BVDU)[6] has been a potent agent against herpes simplex virus type 1 (HSV-1). Recently, we synthesized a number of (E)-5-(2-acylvinyl)uracils **1** (Scheme 1) which we found to have cytotoxic activities against CCRF-CEM human lymphoblastoid cells, HT-29 colon carcinoma cells and L1210 mouse leukemia cells in culture.[7] These compounds were also found to inhibit the thymidylate synthase enzyme.



1a, Ar = C_6H_5 ; 1b, Ar = C_6H_4OMe-p
1c, Ar = C_6H_4Me-p ; 1d, Ar = C_6H_4Me-m



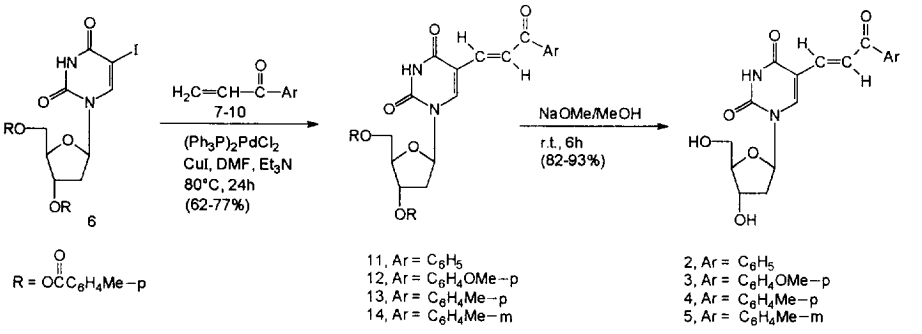
2, Ar = C_6H_5
3, Ar = C_6H_4OMe-p
4, Ar = C_6H_4Me-p
5, Ar = C_6H_4Me-m

Scheme 1

In our quest to develop better anticancer and antiviral drugs, we became interested in the nucleosides **2-5** which we believed would be more active than the corresponding bases. In this letter, we report on the chemical synthesis of compounds **2-5** and their biological properties.

Chemistry

The 2'-deoxyribonucleosides of (E)-5-(2-acylvinyl)uracil **1** were synthesized according to Scheme 2.



Scheme 2

We have recently reported the synthesis of 5-(acylethynyl)-2'-deoxyuridines[8]. We found that palladium-catalyzed reaction between protected 5-iodo-2'-deoxyuridine and α,ß-conjugated acetylenic ketones did not lead to the desired protected 5-(acylethynyl)-2'-deoxyuridines in good yields. Since it was observed that the presence of Cu(I) led to slightly better yields and cleaner products, (E)-5-(2-acylvinyl)-3',5'-di-O-p-toluoyl-2'-deoxyuridines (**11-14**) were obtained in good yields (62-77%) when a mixture of 5-iodo-3',5'-di-O-p-toluoyl-2'-deoxyuridine[9] **6** and freshly prepared vinyl ketones[7] (**7-10**) was heated in DMF at 80°C for 24 hours in the presence of 10-14 mol% of $(Ph_3P)_2Pd(II)Cl_2$ and 19-31 mol% of Cu(I) iodide with 4-4.5 equivalents of Et_3N as base. Since the starting material **6** and the products **11-14** had close R_f values, it was desirable to use excess (2.2-2.3 equivalents with respect to **6**) of the vinyl ketones to drive the reaction to completion. The products were purified by chromatography on a column of silica gel and were well characterized by their IR, UV and ^{1}H NMR spectra[10].

(E)-5-(2-acylvinyl)-3',5'-di-O-p-toluoyl-2'-deoxyuridines **11-14** on treatment with sodium methoxide in methanol at room temperature for 6 hours yielded the desired (E)-5-(2-acylvinyl)-2'-deoxyuridines **2-5** in excellent yields (82-93%). (E)-5-(2-Acylvinyl)-2'-deoxyuridines were characterized by their IR, UV and ^{1}H NMR spectra[11]. In ^{1}H NMR, the characteristic triplet at around δ 6.16-6.30 (J=6 Hz) was due to the C-1' H and confirmed the ß-configuration of the nucleosides. The E-configuration of the nucleosides was indicated by the coupling constant (J=16 Hz) for the vinylic protons.

Biological Results

(E)-5-(2-Acylvinyl)-2'-deoxyuridines **2** and **3** were tested for their biological activities in various cell lines and viral systems (Tables 1-5)[12-14]. As can be seen from Table 1, compound **2** only was weakly toxic to the tumor cell lines tested. From comparison of the activities of **2** and **3**, it can be seen that the methoxy substitution reduced the cytotoxic activity. Also, no activity was seen against HIV-1 and HIV-2 at subtoxic concentrations.

The antiviral activities of the compounds were determined against HSV-1, HSV-2, vaccinia virus (VV), vesicular stomatitis virus (VSV) and HSV-1 TK^- strains in E_6SM cell cultures (Table 2). The compounds did not exhibit an appreciable antiviral selectivity, i.e. they did not inhibit virus-induced cytopathicity at a concentration that

was sufficiently (i.e. ≥ 10-fold) lower than the cytotoxic concentration. Likewise, compounds **2** and **3** did not show activity against other viruses: e.g., VSV, Coxsackie virus, polio virus in HeLa cells (Table 3) and parainfluenza virus, reovirus, Sindbis virus, Coxsackie virus and Semliki forest virus in Vero cell cultures (Table 4).

Table 1. Cytostatic and anti-HIV-1 and -HIV-2 activities of (E)-5-(2-acylvinyl)-2'-deoxyuridines *in vitro*

Compound	IC_{50} (μg/ml)[a]				EC_{50} (μg/ml)[b]	
	L1210	FM3A	Molt 4/C8	CEM	HIV-1	HIV-2
2	30 ± 4.6	32 ± 4.4	23 ± 1.0	24 ± 0.7	> 20	> 20
3	> 100	> 100	> 100	> 100	> 40	> 40

[a]Concentration of compound that reduced the number of viable cells by 50%.
[b]50% Effective concentration or concentration required to protect CEM cells against the cytopathogenicity of HIV by 50%.
Abbreviations: murine leukemia cells (L1210), murine mammary carcinoma cells (FM3A) and human T-lymphocyte cells (Molt 4/C8, CEM).

Table 2. Antiviral activities of (E)-5-(2-acylvinyl)-2'-deoxyuridines in E_6 SM cell cultures

Compound	Minimum cytotoxic concentration[a] (μg/ml) against E_6SM cells	Minimum inhibitory concentration[b] (μg/ml)					
		HSV-1 (KOS)	HSV-2 (G)	VV	VSV	HSV-1 TK⁻ B2006	HSV-1 TK⁻ VMW 1837
2	40	> 10	> 10	> 10	> 10	> 10	> 10
3	40	> 10	> 10	> 10	> 10	> 10	> 10
BVDU	> 400	0.02	100	10	> 400	> 400	> 400

[a]Required to cause a microscopically detectable alteration of normal cell morphology.
[b]Required to reduce virus-induced cytopathogenicity by 50%.
Abbreviations: herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2), vaccinia virus (VV), vesicular stomatitis virus (VSV).

Table 3. Antiviral activities of (E)-5-(2-acylvinyl)-2'-deoxyuridines in HeLa cell cultures

Compound	Minimum cytotoxic concentration[a] (μg/ml)	Minimum inhibitory concentration[b] (μg/ml)		
		VSV	Coxsackie virus type B4	Poliovirus type 1
2	20	70	70	> 100
3	≥ 200	> 100	> 100	> 100
BVDU	> 400	> 400	> 400	> 400

[a,b]See footnotes to Table 2.

Compounds **2** and **3** were also tested for their antiviral activities against cytomegalovirus (CMV) and varicella-zoster virus (VZV) in human embryonic lung (HEL) cells (Table 5). No inhibitory effects on CMV or VZV were noted with either compound at subtoxic concentrations.

Table 4. Antiviral activities of (E)-5-(2-acylvinyl)-2'-deoxyuridines in Vero cell cultures

Compound	Minimum cytotoxic concentration[a] (μg/ml)	Minimum inhibitory concentration[b] (μg/ml)				
		Parainfluenza virus type 3	Reovirus type 1	Sindbis virus	Coxsackie virus type B4	Semliki forest virus
2	400	> 200	> 200	> 200	> 200	> 200
3	100	> 40	> 40	> 40	> 40	> 40
BVDU	> 400	> 400	> 400	> 400	> 400	> 400

[a,b]See footnotes to Table 2.

Table 5. Antiviral activities of (E)-5-(2-acylvinyl)-2'-deoxyuridines in human embryonic lung (HEL) cells

Compound	Cytotoxicity CC_{50}[a] (μg/ml)	Minimum cytotoxic concentration[b] (μg/ml)	Antiviral activity, IC_{50} (μg/ml)[c]					
			CMV	CMV	TK^+ VZV		TK^- VZV	
			AD-169 strain	Davis strain	OKA strain	YS strain	07/1 strain	YS/R strain
2	140	50	> 20	> 20	> 20	> 20	> 20	> 20
3	> 200	20	> 5	> 5	> 20	> 20	> 20	> 20
BVDU	150	> 50	-	-	0.0008	0.002	5	> 50
(*S*)-HPMPC	> 200	> 50	0.06	0.075	0.065	0.09	0.055	0.045

[a]Cytotoxic concentration required to reduce cell growth (after 3 days) by 50%.
[b]See footnote a under Table 2 (cytotoxicity scored after 7 days).
[c]Inhibitory concentration required to reduce virus plaque formation by 50%. Virus input was 100 and 20 plaque forming units (PFU) for CMV and VZV, respectively.
Abbreviations: cytomegalovirus (CMV) and varicella-zoster virus (VZV).

Conclusions

The (E)-5-(2-acylvinyl)-2'-deoxyuridine **2** was found to be weakly active against some tumor cell lines. In contrast with BVDU, neither compound **2** nor **3** showed activity against TK^+ HSV-1 and VZV strains. It appears that replacement of the bromine atom in BVDU by an acyl group completely annihilates the antiviral effects of BVDU.

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References and Notes

1. Part 21 of our series on Studies on Uracil Derivatives and Analogues; for Part 20 see: Kundu, N.G.; Das, P. *J. Chem. Soc. Chem. Commun.* **1995**, 99-100.
2. Heidelberger, C. *Pyrimidine and Pyrimidine Antimetabolites in Cancer Medicines*; Holland, J.F., Frei, E., Eds.; Lea and Febiger: Philadelphia, **1984**, pp. 801-824.
3. Heidelberger, C.; King, D.H. *Antiviral Agents in Pharmacology and Therapeutics*; Shugar, D., Ed.; Pergamon Press: Oxford, England, 1979, vol. 6, p. 427.
4. De Clercq, E. *Pure and Appl. Chem.* **1983**, *55*, 623.
5. Hirsch, M.S.; Kaplan, J.C. *Scientific American* **1987**, *256* (4), 66.
6. De Clercq, E.; Descamps, J.; De Somer, P.; Barr, P.J.; Jones, A.S.; Walker, R.T. *Proc. Natl. Acad. Sci. USA* **1979**, *76*, 2947-2951.
7. Kundu, N.G.; Dasgupta, S.K.; Chaudhuri, L.N.; Mahanty, J.S.; Spears, C.P.; Shahinian, A.H. *Eur. J. Med. Chem.* **1993**, *28*, 473.
8. Kundu, N.G.; Dasgupta, S.K. *J. Chem. Soc. Perkin Trans.* 1 **1993**, 2657.
9. Robins, M.J.; Barr, P.J.; Giziewicz, J. *Can. J. Chem.* **1982**, *60*, 554.
10. **Experimental synthesis of (E)-5-(2-acylvinyl)-3',5'-di-O-p-toluoyl-2'-deoxyuridines. A typical procedure. (E)-5-(2-Benzoylvinyl)-3',5'-di-O-p-toluoyl-2'-deoxyuridine (11).** A mixture of 5-iodo-3',5'-di-O-p-toluoyl-2'-deoxyuridine (**6**, 80 mg, 0.1355 mmol), $(Ph_3P)_2Pd(II)Cl_2$ (10 mg, 0.014 mmol), Cu(I)iodide (5 mg, 0.026 mmol) in DMF (7 ml) was stirred under nitrogen atmosphere for 15 min. 3-Oxo-3-phenylprop-1-ene[7] (40 mg, 0.30 mmol) was then added to the mixture followed by Et_3N (60 mg, 0.59 mmol). The mixture was further stirred at room temperature for 15 min and then at 80°C for 24 hr. After removal of solvents under pressure, a light brown gum was obtained which was purified by chromatography on a column of silica gel (60-120 mesh) with 25% ethyl acetate in chloroform as eluent to obtain compound **11** (50 mg, 0.084 mmol, 62%) as a yellowish white solid; crystallized from benzene, m.p. 219-220°C; ν_{max} 1730, 1710, 1690, 1660, 1610, 1600 cm^{-1}; λ_{max} 325.8 nm (log ε = 4.28), 241.4 mm (log ε = 4.48); ^1H NMR ($CDCl_3$, 100 MHz) δ 2.12-3.00 (m, 2H, C-2'H), 2.32 (s, 3H, Ar-CH_3), 2.44 (s, 3H, Ar-CH_3), 4.52-5.00 (m, 3H, C-4'H and C-5'H), 5.52-5.78 (m, 1H, C-3'H), 6.28-6.52 (dd, 1H, C-1'H), 7.08-8.40 (m, 16H, Ar H, -CH=CH- and C-6H) and 8.58 (bs, 1H, N-3 H); Anal. Calcd. for $C_{34}H_{30}N_2O_8$; C, 68.67; H, 5.08; N, 4.71. Found: C: 68.73; H, 5.25; N, 4.41. **(E)-5-[2-(p-methoxybenzoyl)vinyl]-3',5'-di-O-p-toluoyl-2'-deoxyuridine (12).** Synthesized as described above in 62% yield; crystallized from benzene, a white solid, m.p. 210°C. **(E)-5-[2-(methylbenzoyl)vinyl]-3',5'-di-O-toluoyl-2'-deoxyuridine (13).** A white solid (78%), m.p. 225°C. **(E)-5-[2-(m-Methylbenzoyl)vinyl]-3',5'-di-O-p-toluoyl-2'-deoxyuridine (14).** A white solid (62%), mp. 220°C.
11. **Experimental. Deprotection of compounds 11-14. A typical procedure. (E)-5-(2-Benzoylvinyl)-2'-deoxyuridine (2).** (E)-5-(2-Benzoylvinyl)-3',5'-di-O-p-toluoyl-2'-deoxyuridine (**11**, 90 mg, 0.15 mmol) was added to sodium methoxide solution [7.7 ml, containing sodium (7.7 mg, 0.33 mmol)] under nitrogen atmosphere. The mixture was stirred at room temperature for 6 h and then neutralized by addition of Dowex 50-X8 (H^+) resin. The mixture was filtered and the resin was washed with methanol (3 x 10 ml). The combined filtrate on removal of solvent under reduced pressure, afforded a white solid which, after trituration with ether to remove the ester formed, yielded the title compound **2** (50 mg, 0.14 mmol, 93%), crystallized from ethanol, m.p. 189-190°C; ν_{max} 1715, 1690, 1605, 1590 cm^{-1}; λ_{max} 330.8 nm (log ε = 4.30), 276.6 nm (log ε = 3.93); ^1H NMR ([$^2H_6$]DMSO; 100 MHz); δ 2.00-2.52 (m, 2H, C-2'H), 3.80 (bs, 2H, C-5'H), 3.96 (m, 1H, C-4'H), 4.42 (bs, 1H, C-3'H), 5.16 (m, 2H, 2 OH), 6.30 (t, 1H, J=6 Hz, C-1'H), 7.40-7.68 (m, 4H, Ar $H_{m,p}$ and C=CH-CO), 7.80-8.10 (m, 2H, Ar H_o), 8.22 (d, 1H, J=16 Hz, Ura-CH=C), 8.68 (s, 1H, C-6 H) and 11.60 (bs, 1H, N-3 H). Anal. Calcd. for $C_{18}H_{18}N_2O_6$: C, 60.33; H, 5.06; N, 7.82. Found: C, 60.44; H, 5.48 and N, 7.77. **(E)-5-[2-(p-Methoxybenzoyl)vinyl]-2'-deoxyuridine (3).** This was obtained as a light yellow solid (89%) from compound **12** by

following procedure for **2**; m.p. 209-210°C; ν_{max} 1705, 1670, 1610, 1580 cm^{-1}; λ_{max} 333.6 (log ε = 4.46), 226.6 nm (log ε = 4.02); ^1H NMR ([$^2H_6$]DMSO, 100 MHz) δ 2.22 (apparent triplet, 2H, C-2'H), 3.68 (bs, 2H, C-5'H), 3.88 (s, 4H, $ArOCH_3$ and C-4'H), 4.30 (bs, 1H, C-3'H), 5.28 (m, 2H, 2 OH), 6.18 (t, 1H, J=6 Hz, C-1'H), 7.12 (d, 2H, J=8 Hz, Ar H_m), 7.48 (d, 1H, J=16 Hz, C=CH-CO), 7.88-8.26 (m, 3H, Ar H_o and Ura-CH=C), 8.60 (s, 1H, C-6 H) and 11.64 (bs, 1H, N-3 H); Anal. Calcd. for $C_{19}H_{20}N_2O_7$: C, 58.75; H, 5.19; N, 7.21; Found: C, 58.49; H, 5.35 and N, 7.23. **(E)-5-[2-(p-Methylbenzoyl)vinyl]-2'-deoxyuridine (4)**. This was obtained as a white solid (91%) from **13**, crystallized from ethanol, mp. 200-202°C; ν_{max} 1700, 1690, 1610, 1580 cm^{-1}; λ_{max} 330 nm (log ε = 4.215), 283 nm (log ε = 3.96); ^1H NMR ([=$^2H_6$]DMSO, 100 MHz) δ 2.22 (app.t, 2H, C-2'H), 2.40 (s, 3H, $ArCH_3$), 3.66 (bs, 2H, C-5'H), 3.84 (m, 1H, C-4'H), 4.30 (bs, 1H, C-3'H), 5.28 (m, 2H, 2.0H), 6.16 (t, 1H, J=6 Hz, C-1'H), 7.28-7.64 (m, 3H, Ar H_m and C=CH-CO), 7.90 (d, 2H, J=8 Hz, Ar H_o), 8.08 (d, 1H, J=16 Hz, Ura-CH=C), 8.60 (s, 1H, C-6 H) and 11.72 (bs, 1H, N-3 H); Anal. Calcd. for $C_{19}H_{20}N_2O_6$: C, 61.28; H, 5.41; N, 7.52; Found: C, 61.64; H, 5.76; N, 7.35. **(E)-5-[2-(m-Methylbenzoyl)vinyl]-2'-deoxyuridine (5)**. The title compound was obtained as a white solid (82%) from **14**, crystallized from ethanol, m.p. 203-204°C; ν_{max} 1710, 1690, 1645, 1605 cm^{-1}; λ_{max} 331 nm (log ε = 4.44); ^1H NMR ([$^2H_6$]DMSO, 100 MHz), δ 2.20 (apparent triplet, 2H, C-2'H), 2.40 (s, 3H, $ArCH_3$), 3.64 (m, 2H, C-5'H), 3.80 (m, 1H, C-4'H), 4.28 (m, 1H, C-3'H), 5.24 (m, 2H, 2 OH), 6.16 (t, 1H, J=6 Hz, C-1'H), 7.32-7.88 (m, 5H, Ar H and C=CH-CO), 8.08 (d, 1H, J=16 Hz, Ura-CH=C), 8.60 (s, 1H, C-6 H) and 11.76 (bs, 1H, N-3 H); Anal. Calcd. for $C_{19}H_{20}N_2O_6$; C, 61.28; H, 5.41; N, 7.52; Found: C, 61.17; H, 5.38, N, 7.47.

12. De Clercq, E.; Balzarini, J.; Torrence, P.F.; Mertes, M.P.; Schmidt, C.L.; Shugar, D.; Barr, P.J.; Jones, A.S.; Verhelst, G.; Walker, R.T. *Mol. Pharmacol.* **1981**, *19*, 321.

13. De Clercq, E.; Descamps, J.; Verhelst, G.; Walker, R.T.; Jones, A.S.; Torrence, P.F.; Shugar, D. *J. Infect. Dis.* **1980**, *141*, 563.

14. Balzarini, J.; Naesens, L.; Slachmuylders, J.; Niphuis, H.; Rosenberg, I.; Holy, A.; Schellekens, H.; De Clercq, E. *AIDS* **1991**, *5*, 51.

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