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SYNTHESIS OF SOME 5′-AMINO-2′,5′-DIDEOXY-5-IODOURIDINE DERIVATIVES AND THEIR ANTIVIRAL PROPERTIES AGAINST HERPES SIMPLEX VIRUS

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In an attempt to improve the antiviral efficacy of 5′-amino-2′,5′-dideoxy-5-iodouridine (AIdU) the *N*-acetyl and *N*,3′-*O*-diacetyl derivatives were prepared. *N*-Acetylation of AIdU increased its ability to inhibit the phosphorylation of thymidine by the deoxypyrimidine kinase of herpes simplex virus type 1 (HSV1) while diacetylation had the converse effect. The affinity of the corresponding compounds containing uracil or thymine for virus deoxypyrimidine kinase was also determined. A range of *N*-acyl-, *N*-sulphonyl- and *N*,3′-*O*-diacyl- derivatives of AIdU were synthesised; enhanced inhibition of deoxypyrimidine kinase by a number of these compounds was observed. The previous observation that 5′-azido-2′,5′-dideoxy-5-iodouridine has antiherpetic activity in vivo led us to investigate its 3′-*O*-acetyl derivative as well as the corresponding compound containing uracil. None of the derivatives described showed antiviral activity in cell culture against HSV1; acylation failed to enhance the potency of AIdU against HSV1 in vivo.

AIdU (5′-amino-2′,5′-dideoxy-5-iodouridine) acylation herpes simplex virus

INTRODUCTION

Ideally, any antiherpetic agent should be selectively effective against the replicating virus without toxicity to the host. The herpes simplex virus type 1 (HSV1) induced deoxypyrimidine nucleoside kinase appears to be a prime target for such compounds (see Ref. 5 for review) and the selective antiviral properties of AIdU [4] have been attributed to the fact that it is *N*-phosphorylated only by the virus enzyme [3]. Similarly the antiviral activity with low cytotoxicity of 9-(2-hydroxyethoxymethyl)-guanine [18] and *E*-5-(2-bromovinyl)-2′-deoxyuridine [6] reflects their phosphorylation by the herpes virus-induced kinase without phosphorylation in normal uninfected cells [7,10].

Whilst AIdU has been shown to be effective in the treatment of experimental herpes keratitis in the rabbit when applied topically at high concentrations [1, 16], it has proved ineffective in the systemic treatment of HSV1 infections [19]. The compounds described here were synthesised in an attempt to further define the substrate requirements of the

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HSV1 deoxypyrimidine nucleoside kinase and hopefully to identify a more active compound. Acylation of known antiviral nucleosides to increase potency has been reported [2, 11, 12, 14, 17, 22–24]; such derivatisation of AIdU was investigated in an attempt to enhance activity by affording better tissue distribution of the drug or greater resistance to metabolic degradation. Whether the 5′-amides described are cleaved in vivo to regenerate AIdU remains to be established.

EXPERIMENTAL SECTION

Available nucleosides were purchased from Sigma. ^{1}H-NMR spectra were recorded using a Varian EM360 spectrometer (60 MHz). Chemical shifts in δ units are ppm downfield from internal tetramethylsilane. Mass spectra were obtained with a Finnigan 4000 instrument with an on-line Finnigan 6100 series data system (chemical ionization under NH_3 gas). All compounds gave the expected M + 1 amu peaks and M + 18 amu peaks due to the addition of $NH_4^{\oplus}$. Corresponding peaks for the expected base residues were also always observed as well as peaks attributable to the expected substituted sugar fragments (data not shown). UV spectra were recorded using a Unicam SP1800. Melting points were measured using an Electrothermal apparatus and are uncorrected. Thin-layer chromatography (TLC) was performed on precoated silica (Merck 5549) or cellulose (Merck 5574/0025) sheets eluting with mixtures of chloroform/ethanol or propan-1-ol/conc. NH_4OH/H_2O (6 : 1 : 3, v/v), respectively. (Preparative layer chromatography was performed on Merck 5717 plates.) Compounds *1, 2, 3, 10, 11* and *12* were prepared by methods cited in the literature [13, 25]. A sample of 3′,5′-di-*O*-acetyl-2′-deoxyuridine was prepared by acetylation of 2′-deoxyuridine as described below for AIdU (see Table 2). Characterisation (data not shown) was as in Table 1.

General procedure for acetylation of 5′-amino-2′,5′-dideoxy-pyrimidine nucleosides (4, 5, 6)

Aminonucleoside (*1, 2* or *3*, 2 mM) dried overnight in vacuo at 110°C was stirred with acetic anhydride (5 ml, approx. 50 mM) in dry pyridine (15 ml) at room temperature. Reaction was complete within 3 h by TLC and volatile materials were removed in vacuo. After PLC where necessary (see Discussion) purified material was dissolved in CH_2Cl_2 and precipitated from a large volume of stirred diethyl ether/hexane (3 : 2, v/v). The resulting material (Table 1) was collected by centrifugation and dried in vacuo. Attempted recrystallisation of the analytically pure material (*4, 5* or *6*) was unsuccessful from a variety of solvents.

General synthesis of N-acetyl-5′-amino-2′,5′-dideoxypyrimidine nucleosides (7,8,9)

Diacetyl derivatives (*4, 5* or *6*, approx. 5 mM) were stirred at 0°C for 15 min in ethanol (50 ml) and 2 N NaOH (50 ml). After neutralisation with Dowex 50 X2 (pyridinium

TABLE 1.

CHARACTERISATION OF NOVEL COMPOUNDS.

Compound	Elemental analysis[a] (% yield)[b]	MPt	UV (H_2O:EtOH) λ_{max} (ε)	λ_{min} (ε)	NMR (δ) H-6	5′-NH	H-1′	H-5	H-3′	[H-4′]	[H-2′]	H-5′	5′N-Acyl	3′O-Acyl	CH_3-5	Solvent
4	$C_{13}H_{16}N_3O_8I$ (92%)	-	288 (6,080)	249 (1,810)	8.10 (s,1H)	8.05 (t,1H)	6.06 (t,1H)	- -	5.13 (m,1H)	3.95 (m,1H)	3.40 (m,2H)	2.30 (m,2H)	2.02 (s,3H)	1.82 (s,3H)	-	Me_2SO-d_6
5	$C_{13}H_{17}N_3O_6 \cdot \frac{1}{2}H_2O$ (76%)	-	262 (9,400)	231 (2,240)	7.50 (d,1H,J=4Hz)	6.77 (t,1H)	6.17 (t,1H,J 53.5Hz)	5.81 (d,1H,J=4Hz)	5.19 (m,1H)	4.13 (m,1H)	3.58 (m,2H)	2.42 (t,2H)	2.10 (s,2H)	2.02 (s,3H)	-	$CDCl_3$
6	$C_{14}H_{19}N_3O_6 \cdot H_2O$ (62%)	-	268 (8,770)	236 (2,340)	7.55 (s,1H)	-	6.30 (t,1H)	-	5.29 (m,1H)	4.25 (m,1H)	3.58 (m,2H)	2.47 (m,2H)	2.16 (s,3H)	2.06 (s,3H)	1.97 (s,3H)	D_2O
7	$C_{11}H_{14}N_3O_5I$ (76%)	205° (dec)	289 (7,240)	249 (2,020)	8.01 (s,1H)	8.05 (t,1H)	6.10 (t,1H)	-	4.19 (m,1H)	3.75 (m,1H)	3.34 (m,2H)	2.20 (m,2H)	1.90 (s,3H)	-	-	Me_2SO-d_6
8	$C_{11}H_{15}N_3O_5 \cdot H_2O$ (98%)	149°	263 (9,200)	232 (2,200)	7.69 (d,1H,J=8Hz)	8.00 (t,1H)	6.11 (t,1H)	5.61 (d,1H,J=8Hz)	4.12 (m,1H)	3.72 (m,1H)	3.30 (m,2H)	2.10 (t,2H)	1.82 (s,3H)	-	-	Me_2SO-d_6
9	$C_{12}H_{17}N_3O_5$ (84%)	228%	269 (8,310)	236 (2,020)	7.56 (s,1H)	-	6.30 (t,1H)	-	4.48 (q,1H)	4.12 (m,1H)	3.57 (m,2H)	2.46 (t,2H)	2.10 (s,3H)	-	1.98 (s,3H)	D_2O
13	$C_{11}H_{12}N_5O_5I$ (72%: IR 2,110cm^{-1})	156-8° (dec)	284 (7,690)	248 (2,230)	8.10 (s,1H)	-	6.28 (t,1H,J=2Hz)	-	5.20 (m,1H)	4.15 (m,1H)	3.75 (m,2H)	2.40 (m,2H)	-	2.10 (s,3H)		$CDCl_3$
14	$C_{11}H_{13}N_5O_5$ (63%: IR 2,105cm^{-1})	118-9°	262 (8,970)	232 (2,360)	7.60 (d,1H,J8Hz)	-	6.30 (t,1H)	5.80 (d,1H,J=8Hz)	5.18 (m,1H)	4.10 (m,1H)	3.70 (d,2H)	2.35 (m,2H)	-	2.10 (s,3H)		$CDCl_3$
15	$C_{17}H_{25}N_3O_6I$ (35%)	-	289 (6,680)	251 (1,990)	7.95 (s,1H)	6.49 (t,1H)	6.16 (t,1H)	-	5.19 (m,1H)	4.14 (m,1H)	3.60 (m,2H)	2.70-2.20 (m,4H,H-5′,CHCO)		1.18 (d,12H,CH_3)		$CDCl_3$
16	$C_{19}H_{28}N_3O_6I$ (55%)	-	289 (6,620)	250 (2,140)	7.97 (s,1H)	6.78 (t,1H)	6.12 (t,1H)	-	5.20 (m,1H)	4.12 (m,1H)	3.63 (m,2H)	2.42 (m,2H)	2.18 (d,4H,CH_2)	1.30 (m,2H,CH)	0.91 (d,12H,J=6Hz,CH_3)	$CDCl_3$

Compound	Elemental analysis[a] (% yield)[b]	MPt	UV (H_2O/EtOH) λ max (ε)	λ min (ε)	NMR (δ) H-6	5′-NH	H-1′	H-5	H-3′	H-4′	H-2′	H-5′	5′N-Acyl	Solvent
17	$C_{23}H_{20}N_3O_6I$ (94%)	209° (dec)	283* (7,414)	274* (7,272)	8.40 (s,1H)	8.56 (t,1H)	6.20 (t,1H)	-	5.60 (m,1H)	4.37 (m,1H)	3.80 (m,2H)	2.56 (m,2H)	7.9 and 7.5 (2m,10H,C_6H_5)	$CDCl_3$
18	$C_{12}H_{16}N_3O_5I$ (76%)	205° (dec)	283* (7,580)	245* (2,050)	8.02 (s,1H)	7.97 (t,1H)	6.08 (t,1H)	-	4.15 (m,1H)	3.75 (m,1H)	3.34 (m,2H)	2.15 (m,4H,H-5′,CH_2)	1.02 (t,3H,J8Hz,CH_3)	Me_2SO-d_6
19	$C_{13}H_{18}N_3O_5I$ (71%)	199° (dec)	284* (6,920)	244* (940)	8.02 (s,1H)	7.90 (t,1H)	6.10 (t,1H)	-	4.18 (m,1H)	3.77 (m,1H)	3.34 (m,2H)	2.40-2.00 (m,3H,H-5′,CHCO)	0.99 (d,6H,J7Hz,CH_3)	Me_2SO-d_6
20	$C_{14}H_{20}N_3O_5I$ (25%)	203° (dec)	290* (7,080)	250* (2,140)	8.05 (s,1H)	7.97 (t,1H)	6.08 (t,1H)	-	4.14 (m,1H)	3.72 (m,1H)	3.35 (m,2H)	2.10 (m,4H,H-5′,CH_2CO)	1.40 (m,4H,CH_2) 0.90 (m,3H,CH_3)	Me_2SO-d_6
21	$C_{14}H_{20}N_3O_5I$ (45%)	199° (dec)	285 (7,510)	246 (1,700)	8.02 (s,1H)	7.94 (t,1H)	6.07 (t,1H)		5.25 (m,1H)	4.14 (m,1H)	3.73 (m,2H)	2.05 (m,4H,H-5′,CH_2)	0.85 (d,6H,J6Hz,CH_3)	Me_2SO-d_6
22	$C_{16}H_{16}N_3O_5I$ (97%)	195-6° (dec)	283* (7,310)	257* (3,890)	8.10 (s,1H)	8.64 (t,1H)	6.09 (t,1H)	-	4.28 (m,1H)	3.90 (m,1H)	3.56 (m,2H)	2.18 (m,2H)	7.9 and 7.5 (2m,5H,C_6H_5)	Me_2SO-d_6
23	$C_{12}H_{16}N_3O_6I$ (27%)	209-10° (dec)	289* (6,900)	250* (2,000)	7.96 (s,1H)	7.18 (t,1H)	6.00 (t,1H)	-	5.20 (m,1H)	3.80 (m,1H)	3.16 (m,2H)	2.10 (m,2H)	4.00 (q,2H,J6.5Hz,CH_2O) 1.15 (t,3H,J6.5Hz,CH_3)	Me_2SO-d_6
24	$C_{12}H_{16}N_3O_5SI$ (34%)	205-6° (dec)	290* (7,300)	251* (2,160)	7.94 (s,1H)	8.16 (t,1H)	6.00 (t,1H)	-	5.18 (m,1H)	3.80 (m,1H)	3.28 (m,2H)	2.08 (m,2H)	2.78 (q,2H,J7Hz,CH_2S) 1.15 (t,3H,J7Hz,CH_3)	Me_2SO-d_6
25	$C_{10}H_{14}N_3O_6SI$ (12%)	191° (dec)	289* (7,070)	250* (1,960)	7.80 (s,1H)	-	5.87 (t,1H)	-	4.16 (m,1H)	3.60 (m,1H)	3.04 (m,2H)	2.34 (m,2H)	2.70 (s,3H,CH_3SO_2)	$Me_2SO-d_6/CDCl_3$
26	$C_{16}H_{18}N_3O_6SI$ (12%)	214° (dec)	290* (6,130)	253* (2,310)	7.69 (s,1H)	-	5.78 (t,1H)	-	3.98 (m,1H)	3.52 (m,1H)	2.80 (m,2H)	2.34 (m,2H)	7.22 (q,4H,$C_6H_4SO_2$) 2.13 (s,3H,CH_3)	$Me_2SO-d_6/CDCl_3$

[a] All compounds gave C,H,N,(S) and I elemental analyses within ± 0.3% of expected values except compound 17. (± 0.6% I) which was not further examined due to its insolubility in the biological assay systems.

[b] Yields are not optimised.

TABLE 2

Inhibition of HSV1-induced thymidine kinase by various thymidine analogues

Form of nucleoside	Concentration of inhibitor (mM) which reduces phosphorylation of 20 μM [^{14}C]thymidine by 50%		
	5-Iodouracil	Thymine	Uracil
Pyrimidine base	> 2	> 2	> 5
Ribo-	0.1	0.38	> 5
2'-Deoxyribo-	0.015	0.02[a]	0.83
2',3'-Dideoxyribo-	n.d.	1.7	n.d.
3',5'-Diacetyl-2'-deoxyribo-	1.2	13.2	> 5
5'-Amino-2',5'-dideoxyribo-	0.63	1.4	> 5
5'-*N*-acetyl-5'-amino-2',5'-dideoxyribo-	0.56	2.9	> 5
5'-*N*,3'-*O*-Diacetyl-5'-amino-2',5'-dideoxyribo-	0.91	13.2	> 5
5'-Azido-2',5'-dideoxyribo-	0.28	0.6	3.7
5'-Azido-3'-*O*-acetyl-2',5'-dideoxyribo-	> 0.2	n.d.	> 2

n.d. = not done.

[a] Theoretical value; observed value from 6 experiments = 0.019 ± 0.001 mM.

form, approx. 300 ml), filtration and washing with 50% aqueous ethanol (300 ml), the filtrate and washings were evaporated in vacuo. The resulting white solid showed a single spot on TLC (silica, $CHCl_3/C_2H_5OH$, 3 : 1) with the expected UV spectrum. Recrystallisation from aqueous ethanol afforded good yields of analytically pure material (Table 1).

General procedure for acetylation of 5'-azido-2',5'-dideoxypyrimidine nucleosides (synthesis of 13 and 14)

Compound *10* or *11* (2 mM) was stirred with acetic anhydride (50 mM) in pyridine (10 ml) at room temperature for 2 h. After removal of volatile materials (and PLC in the case of *14*) the homogenous (by TLC) material was recrystallised from aqueous ethanol (30%) (Table 1).

Synthesis of 15, 16 and 17

Compound *1* (1 g, 2.83 mmol) was stirred with anhydrous pyridine (25 ml) and the appropriate acid chloride (17 mmol, 3 equivalents) at 4°C. After 20 h the mixture was evaporated in vacuo to half the original volume and crystals of the acid pyridinium salt were removed by filtration. The filtrate was evaporated in vacuo, taken up into CH_2Cl_2, extracted twice with saturated $NaHCO_3$ solution, purified by PLC as necessary and taken up in a minimum volume of CH_2Cl_2 which was dropped into stirred hexane. The precipitate was collected by centrifugation, washed with hexane and dried in vacuo. *15* and *16*, although analytically pure, could not be recrystallised from a number of solvents. *17* was recrystallised from $CHCl_3$ (see Table 1).

Synthesis of 18, 19, 20, 21 and 22

Compound *1* (6 g, 17 mmol) was stirred with anhydrous pyridine (150 ml) and the appropriate acid chloride (34 mmol, 1 equivalent) at 4°C for 16 h. After removal of volatile materials the solid was dissolved in ethanol (90 ml) at 0°C. An equal volume of aqueous 2 N NaOH solution was added at 0°C and after 20 min the solution was neutralised with Dowex 50 X 2 (pyridinium form). The resin was removed by filtration and the filtrate evaporated to dryness in vacuo. After two co-evaporations with ethanol to remove traces of pyridine, the products were recrystallised from aqueous ethanol systems (Table 1).

Synthesis of 23 and 24

Compound *1* (5 g, 14.16 mmol) was stirred in anhydrous tetrahydrofuran (200 ml) with triethylamine (17.2 mmol, 1.2 equivalents). The acid chloride (15.5 mmol, 1.1 equivalents) in anhydrous tetrahydrofuran (50 ml) was added dropwise with stirring at room temperature. After 18 h the solution was filtered and the desired products isolated by multiple PLC. The resulting solids were recrystallised (*23* from ethanol, *24* from $CHCl_3/CH_3OH$, 10 : 1 v/v) and characterised (Table 1).

Synthesis of 25

Compound *1* (5 g, 14.16 mmol) was stirred with anhydrous pyridine (125 ml) and methanesulphonyl chloride (1.79 g, 15.63 mmol, 1.1 equivalents) at 4°C. After 24 h the product (silica TLC; $CHCl_3/C_2H_5OH$, 9 : 1, R_f 0.07) was purified on a column of silica (400 g, 6 cm diameter) and the resulting solid recrystallised from ethanol (Table 1).

Synthesis of 26

Compound *1* (4 g, 11.33 mmol) was stirred in dimethylformamide (50 ml) and pyridine (50 ml) with toluenesulphonyl chloride (2.38 g, 12.46 mmol, 1.1 equivalents) at 4°C for 4 days. The desired product (silica TLC; $CHCl_3/C_2H_5OH$, 9 : 1, R_f 0.20) was purified as above and the resulting solid recrystallised from $CHCl_3$ (Table 1).

Tissue culture assays

Antiviral activity of compounds was assessed by a micro-plaque reduction method in which virus infectivity end-point titrations were determined by inoculation of serial 0.5 $\log_{10}$ dilutions of virus on monolayers of BHK cells in flat bottomed microtitre plates (Flow Laboratories, Irvine, Scotland). Test compounds were included at the required concentration in the overlay (Eagle's minimal essential medium, Dulbecco's modification,

containing 10% donor calf serum (Flow Laboratories) and 0.5% carboxymethyl cellulose (Sigma Chemical Co.)). A compound which gave a 1 $\log_{10}$ reduction in the virus infectivity end-point compared with non-drug treated controls was considered significantly antiviral.

Thymidine kinase assay

HSV1-induced thymidine kinase was prepared as described elsewhere [21]. For drug inhibition assays, reactions were performed under conditions where the reaction velocity was V_{max} and $V_{initial}$ was maintained throughout the period of incubation. Samples (50 μl) of enzyme were added to a reaction mixture (200 μl) to give a final mixture containing 0.02 M sodium phosphate buffer, pH 6.0, 5 mM $MgCl_2$, 5 mM ATP, 20 μM [2-^{14}C]thymidine (approx. 60 Ci/mol, Radiochemical Centre, Amersham, U.K.) and inhibitor at an appropriate concentration. Mixtures incubated with no inhibitor (100% control) and mixtures which were not incubated (blanks) were included in all assays. After 10 min at 37°C the reaction mixtures were boiled (2 min), centrifuged (3000 × *g*, 10 min) and samples (50 μl) spotted on to DEAE-cellulose paper discs (Whatman), dried and washed for 1 h in 3 changes of 1 mM ammonium formate. Discs were dried, placed in a toluene base scintillation fluid and counted in a Packard liquid scintillation counter. From plots of (cpm with inhibitor × 100)/ cpm without inhibitor against inhibitor concentration, a 50% inhibitory dose (ID_{50}) was determined.

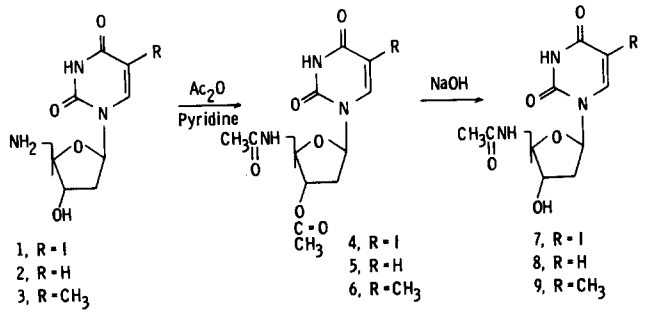
In vivo studies

Female C3H/He mice were bred in our own SPF breeding unit and were used between 6 and 10 weeks old when they weighed between 18 and 25 g. Mice were maintained at 22°C with unlimited access to water and a diet of RHM PMD cubes from Labsure Ltd., Poole, U.K. Mice were infected intraperitoneally with 10–100 × LD_{50} (HSV1). Compounds were given by the indicated route 8 h postinfection and twice daily (approx. 9 a.m. and 5 p.m.) for 5 days thereafter. The survival time (t) of mice in hours was obtained from records prepared twice daily. Records were made for 21 days from the day of infection although no further deaths occurred after 16 days postinfection in any of the studies reported here. Significant differences in the survival times of different groups of mice were tested for by calculating χ^2 values by the logrank method [15].

RESULTS AND DISCUSSION

Compounds *1*, *2*, *3*, *10*, *11* and *12* (Scheme 1) were prepared by methods described elsewhere [13, 25]. Treatment with excess acetic anhydride in anhydrous pyridine for 2–4 h at room temperature afforded the corresponding *N*,3′-*O*-diacetyl compounds (*4*, *5* and *6*) or the 5′-azido-3′-*O*-acetyl compounds (*13* and *14*). Under these conditions, *2* and *3* also gave minor amounts (about 10%) of triacetyl derivatives (*N*-3-substituted)

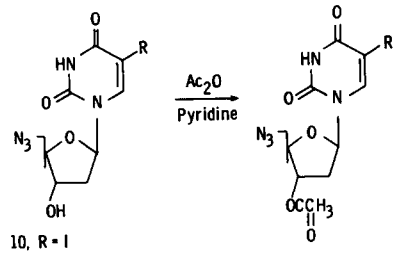
Scheme 1



1, R = I
2, R = H
3, R = CH_3

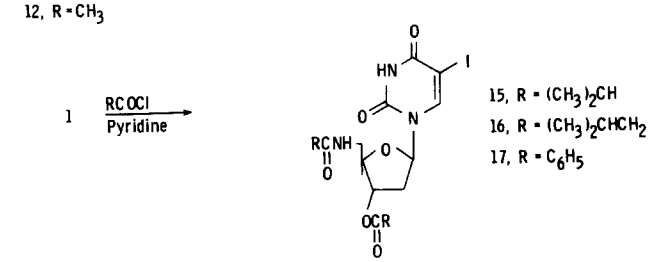
4, R = I
5, R = H
6, R = CH_3

7, R = I
8, R = H
9, R = CH_3

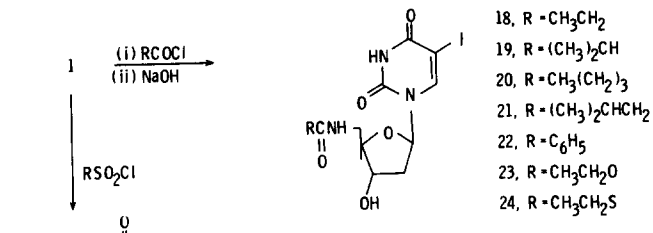


10, R = I
11, R = H
12, R = CH_3

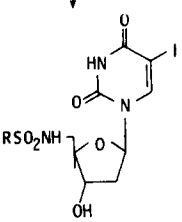
13, R = I
14, R = H



15, R = $(CH_3)_2CH$
16, R = $(CH_3)_2CHCH_2$
17, R = C_6H_5



18, R = CH_3CH_2
19, R = $(CH_3)_2CH$
20, R = $CH_3(CH_2)_3$
21, R = $(CH_3)_2CHCH_2$
22, R = C_6H_5
23, R = CH_3CH_2O
24, R = CH_3CH_2S



25, R = CH_3
26, R = $CH_3C_6H_4$

which were identified by their UV, ^{1}H-NMR and TLC properties. The triacetyl derivatives yielded the corresponding 5′-*N*-acetyl derivatives exclusively on treatment with mild base (see below). Similarly, an *N*-3-*O*-3′-substituted diacetyl compound was obtained from *11*. Compounds of this type were not detected when the base moiety was 5-iodouracil (*1* and *10*). Analytically pure samples of *4*, *5* and *6* were obtained by preparative layer chromatography (PLC). They could not be obtained crystalline from a number of solvents. Other longer chain *N*,3′-*O*-diacylated AIdU derivatives (*15* and *16*) could also not be recrystallised when pure. The dibenzoyl derivative (*17*) is the only member of this class of compounds to have been obtained crystalline. Compounds *13* and *14* were recrystallised without difficulty.

The 3′-*O*-acetyl functions of *4*, *5* and *6* were selectively removed in quantitative yield using aqueous 2 M NaOH/ethanol (1 : 1, v/v) for 15 min at 0°C. After neutralisation and evaporation *7*, *8* and *9* (Scheme 1) were readily recrystallised from aqueous ethanol. Preparation of longer chain diacylated AIdU derivatives using the relevant acid chlorides proved less straightforward. Use of 2.5 equivalents in pyridine at 4°C afforded mainly the *N*-acyl compounds with traces of the 3′-*O*-acyl derivatives but negligible diacylated material. Use of increased amounts of iso-butyryl, iso-valeryl or benzoyl chlorides under these conditions afforded reasonable yields of the required diacylated compounds (*15*, *16*, *17*) after PLC. However, use of the straight chain propionyl or valeryl chlorides under identical conditions led to loss of the 5-iodo substituent and only the corresponding *N*,3′-*O*-diacyl-2′,5′-dideoxyuridine derivatives were obtained (λ_{max} shift, 286 nm to 262 nm). The *N*-monoacylated AIdU derivatives *18*–*24* (Scheme 1) were prepared by reaction with 1 equivalent of acid chloride as above then precautionary treatment with aqueous 2 M NaOH/DMF (1 : 1, v/v) at 0°C after removal of volatile materials. Again recrystallisation after neutralisation afforded good yields of these *N*-acylated compounds. The sulphonylated derivatives *25* and *26* (Scheme 1) were prepared by reaction with one equivalent of the respective sulphonyl chlorides in pyridine at 4°C [20]. Compounds *23* and *24* were synthesised by reaction with ethyl chloroformate or ethyl chlorothiolformate in the presence of triethylamine in tetrahydrofuran [20].

Despite the apparent antiviral specificity and low toxicity of AIdU in tissue culture, it has proved to be ineffective against HSV1 infections of the mouse [19]. Here we have attempted to produce derivatives of AIdU with enhanced antiviral activity. Such enhanced activity might arise if acylated AIdU acts as a prodrug for AIdU with possibly enhanced oral absorption, duration of action or more desirable profiles of tissue distribution. In this context we note that 5′-*O*-valeryl-ara-A was considerably more potent than ara-A in an antiherpes assay in cell culture [2]. Alternatively, acylated AIdU derivatives may act as antagonists for the virus thymidine kinase, being strongly bound but unable to undergo 5′-phosphorylation.

When assayed for antiviral activity against HSV1 on cell cultures, none of the compounds described showed any significant antiviral activity apart from AIdU. With such a diverse group of compounds the reasons for this lack of activity may be many and complex. In particular with respect to the *N*-acyl and *N*,3′-*O*-diacyl derivatives of AIdU,

unlike the parent compound the acylated derivatives may not be taken up into the virus-infected cell or, more likely, the compounds enter the cell but, whilst 5′- or 3′-*O*-esters are cleaved from the nucleoside within the cell as occurs with derivatives of adenine arabinoside [2], *N*-acyl linkages are resistant to enzymatic cleavage and thus the active drug form is not generated. Alternatively, it might be argued that the acyl-derivatives of AIdU and the 5′-azido compounds described here might act as inhibitors of thymidine kinase. Indeed, the 5′-*N*-valeryl AIdU (*20*) described here had the highest affinity of all the compounds synthesised for the HSV1 induced kinase. In fact, an increase in chain length of the 5′-amino substituents gave a general increase in the affinity of the derivative for the enzyme. Other factors (sulphur or aryl groups in the molecule) did not enhance this binding (Table 3). Tables 2 and 3 show that although *N*-acylation yielded compounds with up to 4-fold greater affinity for the kinase than AIdU, the maximum affinity achieved was still only about 10% that of IdU itself. Also, in any series of compounds (varying the sugar component), their affinity for the enzyme followed the order 5-iodouracil>thymine>uracil. Moreover, all *N*-acylated AIdU derivatives had higher affinities for the enzyme than their *N*,3′-*O*-diacylated counterparts. However, inhibitors of thymidine kinase need not necessarily be active antivirals in cell culture systems. Indeed, the existence of virus mutants which lack the ability to induce thymidine kinase

TABLE 3

Inhibition of HSV1-induced thymidine kinase by, and antiviral activity in cell cultures of various derivatives of AIdU

Compound	Thymidine kinase (ID_{50} mM)[a]	Antiviral activity (MIC mM)[b]	Compound	Thymidine kinase (ID_{50} mM)[a]	Antiviral activity (MIC mM)[b]
1	0.63	0.1	14	> 2	n.d.
2	> 5	> 2	15	0.36	> 1
3	1.4	n.d.	16	0.23	> 0.2[c]
4	0.91	> 1	17	insoluble	insoluble
5	> 5	> 1.6	18	0.41	> 1
6	13.2	> 1.5	19	0.19	> 1
7	0.56	> 1	20	0.15	> 0.7[c]
8	> 5	n.d.	21	0.16	> 0.5[c]
9	2.9	> 1.8	22	> 0.05[c]	> 0.04[c]
10	0.28	> 0.5	23	0.29	> 0.2
11	3.7	> 2	24	> 0.5	> 0.2
12	0.6	> 1	25	0.5	> 0.2
13	> 0.2[c]	> 1	26	insoluble	insoluble

n.d. = not done.

[a] Concentration of inhibitor which reduces phosphorylation of 20 μM [^{14}C]thymidine by 50%.

[b] Concentration of inhibitor which reduces virus infectivity end-point by 1 $\log_{10}$ compared to non-drug treated control.

[c] Maximum concentration of drug tested limited by compound solubility in test medium.

[8] but which replicate as well as wild-type virus in cell cultures indicates the absence of a requirement for an active salvage pathway of thymidine metabolism in such systems. However, in vivo the expression of virus thymidine kinase seems to be required for virus growth. Thus it has been shown that mutants of HSV1 which lack the ability to code for thymidine kinase are less virulent than wild type virus in mice [9]. Furthermore, one compound described here (*10*) is an inhibitor of thymidine kinase and has antiviral activity in vivo but not in cell culture (I.S. Sim, unpublished observations). Compounds *1*, *4*, *7*, and *15–21* were also tested for anti-HSV1 activity in vivo. AIdU failed to show a therapeutic effect and the possible reasons for this have been discussed elsewhere [19]. The acylated derivatives of AIdU also failed to afford significant protection to mice infected with a lethal dose of HSV1. Clearly the chemical modifications described failed to enhance the in vivo potency of AIdU. It is uncertain as to whether this was due to a failure of the compounds to afford better absorption, tissue distribution or resistance to metabolic breakdown of the drug, or whether considerations of deacylation to yield the active drug form, discussed in relation to activity in cell culture, were also applicable in vivo.

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