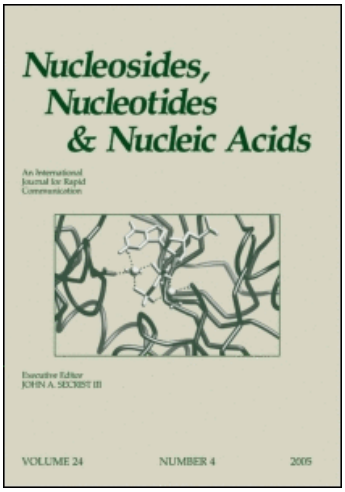

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Synthesis and Antiviral Activity of 5'-O-Sulfamoyluridine Derivatives

José Fiandor[a]; María Teresa García-López[a]; Federico G. De las Heras[a]; Paloma P. Méndez-Castrillón[a]; Carmen Gil-Fernández[b]; Sara Pérez[b]; Pilar Vilas[b]; Concepción Pérez[b]; Angel García Gancedo[b]
[a] Instituto de Química Médica, Madrid, Spain [b] Centro de Investigaciones Biológicas, Madrid, Spain

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SYNTHESIS AND ANTIVIRAL ACTIVITY OF 5'-O-SULFAMOYLURIDINE DERIVATIVES.

José Fiandor[1], María Teresa García-López[1], Federico G. De las Heras[1]*, Paloma P. Méndez-Castrillón[1], Carmen Gil-Fernández[2], Sara Pérez[2], Pilar Vilas[2], Concepción Pérez[2] and Angel García Gancedo[2]

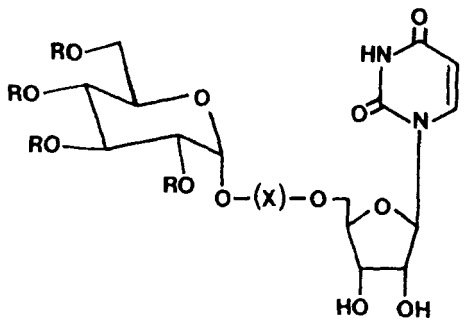
[1]Instituto de Química Médica, Juan de la Cierva 3, 28006-Madrid, Spain.
[2]Centro de Investigaciones Biológicas, Velázquez 144, 28006-Madrid, Spain

SUMMARY. A series of 5'-O-[[[[(alkyl)oxy]carbonyl] amino] sulfonyl] uridines have been synthesized by reaction of cyclohexanol, palmityl alcohol, 1,2-di-O-benzoylpropanetriol and 2,3,4,6-tetra-O-benzoyl-L-glucopyranose with chlorosulfonyl isocyanate and 2',3'-O-isopropylidene-uridine. Another series of 5'-O-(N-ethyl and N-isopropylsulfamoyl) uridines have been prepared by reaction of 2',3'-O-isopropylidene and 2',3'-di-O-acetyluridine with N-ethylsulfamoyl and N-isopropylsulfamoyl chlorides. All compounds were tested against HSV-2, VV, SV and ASFV viruses. 2',3'-Di-O-acetyl-5'-O-(N-ethyl and N-isopropylsulfamoyl) uridine showed significant activities against HSV-2. 5'-O-[[[[(2,3,4,6-Tetra-O-benzoyl-β-L-glucopyranosyl)oxy]carbonyl]amino] sulfonyl] -2',3'-O-isopropylideneuridine was very active against ASFV.

INTRODUCTION

In previous papers in this series,[1,2] we reported the synthesis and antiviral activity of 5'-O-[[[[(α -D-hexopyranosyl)oxy]carbonyl] amino]sulfonyl]uridine derivatives, such as **1**.[1] These analogues of uridine 5'-diphosphate glucose (UPD-Glc, **2**), in which the diphosphate group has been replaced by an isosteric O-CO-NH-SO_2-O group, were designed to interfere with glycosylation processes. Preliminary studies on the mode of action of **1** demonstrated that it shows selectivity in its antiviral activity and inhibits the glycosylation of viral proteins to a greater extent than glycosylation of cellular proteins. However, their mode of action is more complex since it also inhibits DNA synthesis.[3]

Studies on the structure-activity relationships in this series[1,2] revealed that a) the structure of the perbenzoylated hexopyranosyl residue does not significantly affect either the antiviral activity or the protein glycosylation inhibition, b) this residue should play an important role in the transport of the molecule and c) all compounds showing antiviral activity have a nucleotide-like 5'-O-sulfamoyluridine moiety.

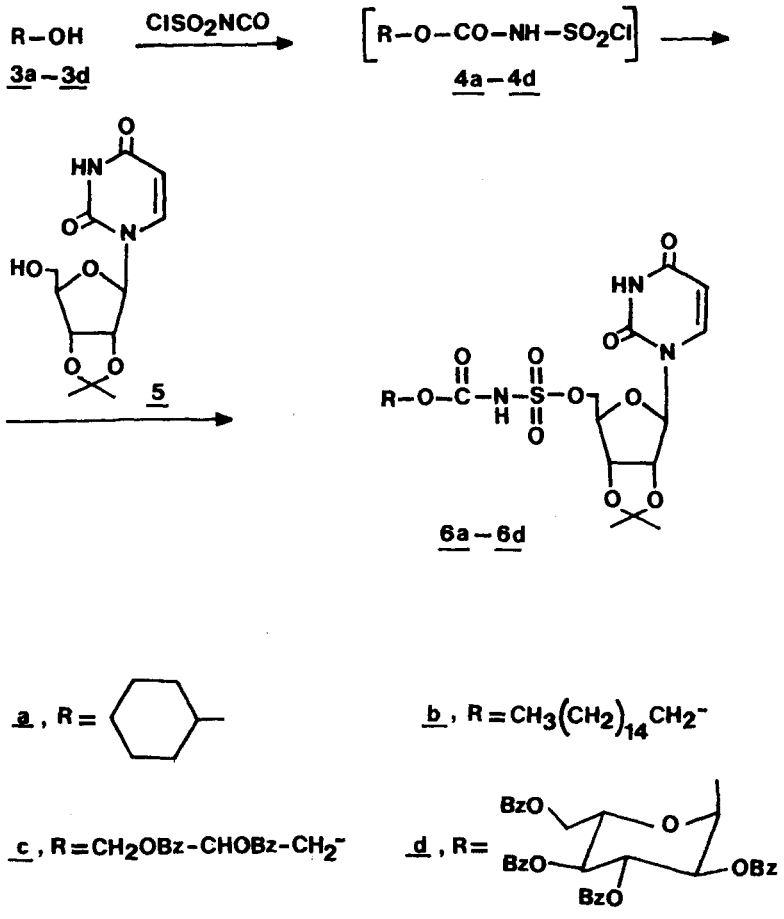


1 , R = C_6H_5CO
X = CO−NH−SO_2

2 , R = H
X = PO_2H−O−PO_2H

In relation to point c), it is interesting to note that many 5'-phosphates of nucleoside analogues are inhibitors of DNA synthesis and that some nucleotides such as CMP and the 5'-monophosphate of bromovinyldeoxyuridine inhibit protein glycosylation.[4,5] In addition, Nucleocidin,[6] Ascamycin,[7] and Dealanylascamycin[7] are naturally occuring nucleosides having a nucleotide-like structure of 5'-O-sulfamoyl nucleosides. Therefore, the nucleotide-like moiety of **1** and/or of its metabolites could be responsible for the observed inhibitions of DNA synthesis and protein glycosylation.

Based on the above hypothesis we decided to study the respective roles of the hexopyranosyl and 5'-O-sulfamoyl residues in the activity of **1** and related compounds. With this aim, we have now synthesized and tested as antivirals a series of 5'-O-(substituted sulfamoyl)uridines which do not include in their structure a perbenzoyl-D-hexose residue, but have different lipophilic substituents to facilitate the transport of the molecule into the cell. Some of these compounds (**6a-6d**) keep the diphosphate-like 5'-O-(oxycarbonylaminosulfonyl)bridge of **1**, while others, which possess a 5'-O-alkylaminosulfonyl group (**7-13**), can be considered as 5'-monophosphate analogues.



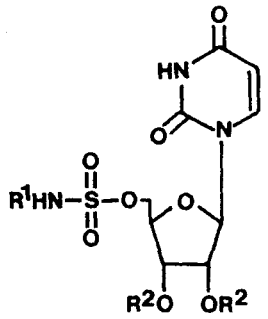
Scheme 1

SYNTHESIS

5'-O-[[[[(Alkyl) oxy]carbonyl]amino]sulfonyl]uridine derivatives **6a-6d** were prepared by reaction of the corresponding alcohol, namely cyclohexanol (**3a**) palmityl alcohol (**3b**), 1,2-di-O-benzoylpropanetriol (**3c**), and 2,3,4,6-tetra-O-benzoyl-L-glucopyranose (**3d**) with chlorosulfonyl isocyanate followed by *in situ* reaction of the unstable intermediate [[[(alkyl)oxy]carbonyl]amino]sulfonyl chloride **4a-4d** with 2',3'-O-isopropylideneuridine (**5**) (Scheme 1). The intermediates **4a-4d** were formed by reaction of the isocyanate group, more reactive toward nucleophiles than the chlorosulfonyl group of chlorosulfonyl isocyanate, with the hydroxyl group of alcohols **3a-3d**.

The attachment of the [[(alkyloxy)carbonyl]amino]sulfonyl residue to the 5'-O-position of uridine was demonstrated by the presence in the ^1H NMR spectra of broad singlets, assigned to the uridine 3-NH proton, which disappeared by treatment with D_2O, at 9.85 ppm in $CDCl_3$ for **6a**, and at $\delta \simeq$ 11.35 ppm in $(CD_3)_2SO$ for **6b-6d**, as compared to similar singlets at δ 9.85 and 11.43 ppm for the same proton of 2',3'-O-isopropylideneuridine in the same solvents, respectively. Additional evidence of this attachment was the downfield chemical shift of the H-4' ($\Delta\delta \simeq 0.2$ ppm) and H-5' ($\Delta\delta \simeq 0.5$ ppm) ribose moiety protons as compared to those of **5**. The anomeric configuration of the glucopyranosyl moiety of **6d**, was determined from the coupling constant value $J_{1'',2''} = 3.6$ Hz, which indicates the equatorial arrangement of H-1" and, thus, a β configuration for an hexopyranose of the L-series.

The known 1,2-di-O-benzoylpropanetriol **3c** was prepared from glycerine in 45% total yield by a route two steps shorter than the reported procedure.[8,9] Thus, reaction of glycerine with 1 equivalent of trityl chloride gave 1-O-tritylpropanetriol, which by treatment with an excess of benzoyl chloride in pyridine afforded 1,2-di-O-benzoyl-3-O-tritylpropanetriol. Hydrolysis of the latter with acetic acid/water gave the desired **3c**. The unknown 2,3,4,6-tetra-O-benzoyl-L-glucopyranose **3d** was prepared by regioselective 1-O-deacylation of pentabenzoyl-L-glucopyranose with ammonia in tetrahydrofuran/methanol.[10]



7, $R^1 = CH_3CH_2$, $(R^2, R^2) = C(CH_3)_2$

8, $R^1 = CH_3CH_2$, $R^2 = H$

9, $R^1 = CH_3CH_2$, $R^2 = CH_3CO$

10, $R^1 = (CH_3)_2CH$, $(R^2, R^2) = C(CH_3)_2$

11, $R^1 = (CH_3)_2CH$, $R^2 = H$

12, $R^1 = (CH_3)_2CH$, $R^2 = CH_3CO$

13, $R^1 = (CH_3)_2CH$, $R^2 = C_6H_5CO$

5'-O-(Ethylsulfamoyl)uridines **7** and **9** were synthesized in quantitative yields by reaction of 2',3'-O-isopropylideneuridine (**5**) and 2',3'-O-acetyluridine (**15**) with N-ethylsulfamoyl chloride. Similarly,

5'-O-(isopropylsulfamoyl)uridine **10** was prepared by reaction of **5** with N-isopropylsulfamoyl chloride. The attachment of the sulfamoyl moiety to the uridine 5'-OH group was demonstrated, as before, by the appearance in the ^{1}H NMR spectra of the 3-NH broad singlet at 9.90 ppm in $CDCl_3$ and the downfield chemical shift of H-4' ($\Delta\delta \simeq 0.2$) and H-5' ($\Delta\delta \simeq 0.5$ ppm) of **7**, **9** and **10** as compared to the same protons of the corresponding starting products **5** and **15**. Other 5'-O-(N-alkylsulfamoyl) uridines were prepared as follows. Acidic hydrolysis of the isopropylidene group of **7** and **10** with trifluoroacetic acid/water gave the deprotected N-ethylsulfamoyl and N-isopropylsulfamoyl derivatives **8** and **11** respectively. Acylation of **11** with acetic anhydride or benzoyl chloride afforded in quantitative yields, the 2',3'-di-O-acetyl and 2',3'-di-O-benzoyluridines **12** and **13**.

ANTIVIRAL RESULTS AND DISCUSSION

The present 5'-O-(alkylsulfamoyl)uridines were tested as antivirals against Herpes simplex virus type 2 (HSV-2), Vaccinia virus (VV), Sindbis virus (SV) and African swine fever virus (ASFV) in Vero cells (Table 1). Three well known antivirals, namely, Ribavirin,[11] Acyclovir (ACV),[12-14] and 5-iodo-2'-deoxyuridine (IDU),[12-14] as well as the parent compound **1**, have also been tested under the same experimental conditions. The antiviral effect was determined by measuring the minimal inhibitory concentration required to reduce the virus yield by 50% (MIC_{50}). The toxicity of these compounds is indicated by the concentration of compound that produces a 50% inhibition of protein synthesis in uninfected Vero cells after 48 h incubation (IP_{50}). The Therapeutic Index (TI) is defined as IP_{50}/MIC_{50}.

Table 1 shows that the antiviral activities against HSV-2 and VV are similar to those of the parent compound **1**, exception being **6a**. However, only the nucleotide-like 5'-O-(N-alkylsulfamoyl)uridines **9**, **10**, and **12** showed significant TI values against HSV-2, higher than that of **1**, but lower than that of Acyclovir. Antiviral activity data for compounds **8** and **11** are not included, due to their low solubility under the standard conditions used. None of the compounds tested showed a significant activity against SV. Compound **6d** showed a high activity against ASFV with a TI value of 440, much higher than that of Ribavirin.

TABLE 1. In Vitro Toxicity and Antiviral Activity against HSV-2, VV, SV and ASFV of 5'-O-Sulfamoyluridine Derivatives.

Compound	IP_{50}[a]	MIC_{50}[b], μg/mL (TI)[c]			
	μg/mL	HSV-2	VV	SV	ASFV
6a	619	1000	150(4.1)	181(3.4)	ND[d]
6b	72	25	27	75	25
6c	369	54(6.8)	54(6.8)	95(3.9)	50(7.4)
6d	44	89	52	28	0.1(440)
9	500	25(20)	ND	ND	300
10	304	25(12.1)	26(11.7)	162	100(3.0)
12	352	25(14.0)	101(3.5)	138	100(3.5)
13	300	58(5.2)	84(3.6)	ND	60(5.0)
1	237	26(9.1)	25(9.5)	55(4.3)	18(13.2)
Ribavirin	200	ND	ND	ND	25(8.0)
Acyclovir	300	0.1(3000)	ND	ND	ND
Iododeoxy-uridine	200	ND	25(8.0)	ND	ND

a) IP_{50} is the concentrated of compound that causes a 50% inhibition of protein synthesis.
b) MIC_{50} is the concentration of compound required to reduce the virus yield by 50%.
c) TI = IP_{50}/MIC_{50}. Only values of TI > 3 are included.
d) ND = Not done.

The virucide activities of all the compounds described have been tested. Exception being **6b** and **6d**, none of them showed virucide activity against HSV-2, VV, ASFV and SV. The virucide activities of **6b** and **6d** (Table 2) only affected the virions produced by infected cells. These compounds did not affect the inoculum since they were added to the culture after virus adsorption.

Comparison of the antiviral activities of the 5'-O-sulfamoyl substituted uridines of this paper with those of the parent compound **1**

TABLE 2. Virucidal Activity of 5'-O-Sulfamoyluridine Derivatives **6b** and **6d** by Direct Contact with the Virus.

	6b		6d	
Virus	C	M	C	M
HSV-2	2.0×10^5	$< 10^2$	--	--
VV	2.3×10^6	$< 10^2$	--	--
SV	6.8×10^7	$< 10^4$	--	--
ASFV	2.0×10^5	$< 10^2$	2.5×10^5	0

C = Control virus + diluent
M = Virus + compound

and related 5'-O-sulfamoyluridine derivatives,[1,2,15] and taking into account the lack of antiviral effect of structurally related 5'-O-carbonyl and 5'-O-thiocarbonyluridine derivatives[16] it can be suggested that the 5'-O-sulfamoyl substituted uridines appear to constitute a new type of antiviral compounds. The nature of the residue attached to the 5'-O-sulfamoyl group seems to be not of critical importance, but its lipophilicity may affect the transport of the molecule. These residues may also be responsible for the different toxicities.

EXPERIMENTAL

CHEMICAL METHODS

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. ^1H NMR spectra were recorded with a Varian XL-300 (300 MHz) and a Varian EM-390 (90 MHz) spectrometers using Me_4Si as the internal standard. Analytical TLC was performed on aluminium sheets coated with a 0.2 mm layer silic gel 60 F_{254} purchased from Merck and preparative TLC on glass plates coated with a 2 mm layer of silica gel

PF_{254} (Merck). Silica gel PF_{254} (Merck) was also used for column chromatography. Detection of compounds on TLC was by UV light (254 nm) and with 30% H_2SO_4 in EtOH spray followed by heating. Evaporations were carried out under reduced pressure with the bath temperature below 30 C.

1,2-Di-O-benzoylpropanetriol **(3c)**. To a solution of glycerine (2g, 21.7 mmol) in dry pyridine (20 mL), trityl chloride (6.04 g, 21.7 mmol) was added and the mixture was stirred at room temperature for 48 h. The solvent was evaporated under reduced pressure and the residue, dissolved in chloroform (30 mL), was washed with water (3 x 20 mL) and dried over anhydrous sodium sulfate. The chloroform phase was filtered and concentrated. The residue was purified by column chromatography using ethyl acetate/hexane (1:1) as the eluent, to afford 1-O-tritylpropanetriol (4.36 g, 60%): mp 108 C (from toluene) (lit[17] mp 109-110 C); ^1H NMR ($CDCl_3$, 300 MHz) δ 3.15 (d, 2H, J= 5.5 Hz, CH_2O Tr), 3,53 (d, 2H, J= 7 Hz, CH_2OH), 3.78 (m, 1H, CHOH).

To a solution of 1-O-tritylpropanetriol (1.8 g, 5.4 mmol) in methylene chloride (50 mL), pyridine (1 mL, 12.5 mmol) and benzoyl chloride (1.7 g, 12.1 mmol) were added. The mixture was stirred at room temperature for 24 h and the solvent was evaporated under reduced pressure. The residue was dissolved in chloroform (30 mL), washed with water (3 x 20 mL) and dried over anhydrous sodium sulfate. The organic solution was filtered and evaporated to dryness to yield 1,2-di-O-benzoyl-3-O-tritylpropanetriol (2.9 g, 99%) as a syrup: ^1H NMR ($CDCl_3$, 90 MHz) δ 3.46 (m, 2H, CH_2OTr), 4.69 (d, 2H, J = 6 Hz, CH_2OBz), 5.63 (m, 1H, CHOBz).

Anal. Calcd. for $C_{36}H_{30}O_5$: C, 79.70; H, 5.53. Found: C, 79.67; H, 5.46.

A solution of 1,2-di-O-benzoyl-3-O-tritylpropanetriol (2.5 g, 4.6 mmol) in acetic acid (12 mL) and water (3 mL) was heated to 80 C for 30 min. The reaction mixture was cooled to room temperature, filtered and evaporated under reduced pressure. The residue was coevaporated three times with ethanol (3 x 20 mL) and purified by column chromatrography with ethyl acetate/hexane (1:3) to give **3c** (1.0 g, 72%) as a syrup which slowly crystallized; mp 55-56 C (lit[8] mp 57.5-58 C); ^1H NMR ($CDCl_3$, 90 MHz) δ 2.50 (bs, 1H, OH), 3.95 (d, 2H, J = 5 Hz, CH_2OH), 4.64 (d, 1H, J = 5 Hz, CH_2OBz), 5.48 (m, 1H, CH_2OBz).

1,2,3,4,6-Penta-O-benzoyl-L-glucopyranose. A mixture of L-glucose (2 g, 11.1 mmol), dry pyridine (30 mL) and benzoyl chloride (11 g, 78.3 mmol) was stirred at room temperature for 24h and the solvent evaporated under reduced pressure. The residue was dissolved in chloroform, washed with dilute HCl and water, dried over anhydrous sodium sulfate, filtered and evaporated to dryness to give 6.22 g (80%) of a (3:1) mixture of the two anomers of the title compound: mp 177-179 C; ^{1}H NMR ($CDCl_3$, 300 MHz)δ 5.70 [dd, 1H, $J_{1,2}$ = 3.5, $J_{2,3}$= 10.4 Hz, H-2(β)], 5.88 [m, 2H, H-2 (α), H-4(β) , 6.31[d, 1H, $J_{1,2}$ = 8.0 Hz, H-1 (α)], 6.88[d, 1H, H-1 (β)].

Anal. Calcd. for $C_{41}H_{32}O_{11}$: C, 70.29; H, 4.57. Found: C, 70.58; H, 4.61.

2,3,4,6-Tetra-O-benzoyl-L-glucopyranose **(3d)**. Through a cold (ice bath) solution of pentabenzoyl-L-glucopyranose (2 g, 2.86 mmol) in tetrahydrofuran (35 mL) and methanol (15 mL), ammonia gas was bubbled for 10 min. The mixture was kept at 0 C for 3h and the solvents were evaporated under reduced pressure. The residue was purified by preparative TLC with ethyl acetate/hexane (1:3) to give **3d** (1.2 g, 70%): mp 115 C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ5.09 [d, 1H, $J_{1,2}$ = 8 Hz, H-1 (α)], 5.32 [dd, 1H, $J_{1,2}$ = 3.5, $J_{2,3}$ = 10 Hz, H-2 (β)]5.40[dd, 1H, $J_{2,3}$ = 9.7 Hz, H-2 (α)], 5.78 [d, 1H, $J_{1,2}$ = 3.5 Hz, H-1 (β)].

Anal. Calcd. for $C_{34}H_{28}O_{10}$: C, 68.46; H, 4.70. Found: C, 68.59; H, 4.99.

General Procedure for the Synthesis of 5'-O-[[[[(Alkyl) oxy]carbonyl]amino]sulfonyl]uridine derivatives. To a cold solution (~-15°C) of the alcohol **3a-3d** (2 mmol) in dry methylene chloride (20 mL), chlorosulfonyl isocyanate (0.15 mL, 2 mmol) was added in the absence of humidity. The mixture was stirred at ~ -15 C until the alcohol disappeared (~ 20 min) and then, a suspension of 2',3'-O-isopropylideneuridine (0.56 g, 2 mmol) in methylene chloride (50 mL) containing dry pyridine (0.16 mL, 2 mmol) was added. The resulting mixture was stirred for 4h, while the reaction reached room temperature, and the solvents were evaporated. The residue was purified as specified in each case.

5'-O-[[[[(Cyclohexyl)oxy]carbonyl] amino]sulfonyl] -2',3'-O-isopropylideneuridine **(6a)**. From **3a**. The residue was filtered through silica gel (20 g) using chloroform/methanol (20:1). The eluted solution was concentrated and purified by preparative TLC with ethyl acetate/

methanol (8:1)to give **6a** (0.362 g, 37%): mp 104-106 C (from methanol/ethyl ether); ^{1}H NMR ($CDCl_3$, 90 MHz) δ 1.06-1.93 (m, 10 H, cyclohexyl), 1.35, 1.55 (2s, 6H, isopropylidene), 4.27-4.60 (m, 4H, H-1", H-4', H-5'),5.65 (d, 1H, H-1', $J_{1',2'}$ = 2 Hz), 5.80 (d, 1H, H-5, $J_{5,6}$ = 8.5 Hz), 7.38 (d, 1H, H-6), 9.85 (bs, 1H, 3-NH). UV λ_{max} (EtOH) 254nm (ε 14.000).

Anal. Calcd. for $C_{19}H_{27}N_3O_{10}S$: C, 46.63; H, 5.52; N, 8.59; S, 6.54. Found: C, 46.79; H, 5.51; N, 8.63; S, 6.45.

5'-O-[[[[(Palmityl)oxy]carbonyl]amino]sulfonyl]-2',3'-O-isopropylideneuridine **(6b)**. From **3b**. The residue was purified by flash column chromatography using chloroform/acetone (1:1) as the eluent to give **6b** (0.59 g, 47%) as a white foam: ^{1}H NMR [$(CD_3)_2SO$, 300 MHz] δ 0.87 [t, 3H, CH_3(palmityl)], 1.27 [bs, 26H, $(CH_2)_{13}$], 1.30, 1.49 (2s, 6H, isopropylidene), 3.72[t, 2H, J= 7Hz, CH_2-O (palmityl)], 4.01 (m, 2H, H-5'), 4.23 (m, 1H, H-4'), 5.57 (d, 1H, $J_{5,6}$ = 8Hz, H-5), 5.86 (d, 1H, $J_{1',2'}$ = 2.8 Hz, H-1'), 7.82 (d, 1H, H-6), 11.34 (bs, 1H, 3-NH). UV λ_{max} (EtOH) 255 nm(ε 9400).

Anal. Calcd. for $C_{29}H_{49}N_3O_{10}S$: C, 55.15; H, 7.77;N, 6.66; S, 5.07. Found: C, 55.50; H, 8.00; N, 6.39; S, 5.15.

5'-O-[[[[(2",3"-Dibenzoyloxypropyl)oxy]carbonyl]amino]sulfonyl]-2',3'-O-isopropylideneuridine **(6c)**. From **3c**. The residue was purified by preparative TLC with ethyl acetate/methanol (14:1) to give **6c** (1.1 g, 80%) as a white foam: ^{1}H NMR[$(CD_3)_2SO$, 300 MHz] δ 1.27, 1.46 (2s, 6H, isopropylidene), 4.02 (m, 2H, H-1"), 4.20 (m, 3H, H-3", H-4'), 4.52 (dd, 1H, $J_{5'a,\ 5'b}$ = 12.0 $J_{4',5'a}$ = 6.8 Hz, H-5'a), 4.63 (dd, 1H, $J_{4',5'b}$ = 3.3 Hz, H-5'b), 5.53 (m, 1H, H-2"), 5.59 (d, 1H, $J_{5,6}$ = 8.1 Hz, H-5), 5.85 (d, 1H, $J_{1',2'}$= 2.7 Hz, H-1'), 11.37 (bs, 1H, 3-NH). UV λ_{max} (EtOH) 227 nm (ε 17750), 256 (6700).

Anal. Calcd. for $C_{30}H_{31}N_3O_{14}S$: C, 52.25; H, 4.50; N, 6.10; S, 4.64. Found: C, 52.72; H, 4.58; N, 5.85: S, 4.58.

5'-O-[[[[(2",3",4",6"-Tetra-O-benzoyl-β-L-glucopyranosyl)oxycarbonyl]amino]sulfonyl]-2',3'-O-isopropylideneuridine **(6d)**. From **3d**. The residue was purified by flash column chromatography with chloroform/acetone (2:1) to give **6d** (0.59 g, 30%): mp 128°C (from ethyl acetate/hexane); ^{1}H NMR [$(CD_3)_2SO$, 300 MHz] δ 1.26, 1.50 (2s, 6H, isopropylidene), 4.04 (m, 2H, H-5'), 4.20 (m, 1H, H-4'), 5.51 (dd, 1H, $J_{1",2"}$ =

3.6, $J_{2",3"}$ = 9.9 Hz, H-2"), 5.58 (d, 1H, $J_{5,6}$ = 8.3 Hz, H-5), 5.83 (d, 1H, $J_{1',2'}$ = 2.8 Hz, H-1'), 6.32 (d, 1H, H-1"), 11.35 (bs, 1H, 3-NH). UV λ_{max} (EtOH) 229 nm (ε 64.460), 261 (12.800).

Anal. Calcd. for $C_{47}H_{43}N_3O_{19}S$: C, 57.26: H, 4.37; N, 4.26; S, 3.25. Found: C, 57.56; H, 4.00; N, 4.01; S, 3.17.

5'-O-[(N-Ethyl)sulfamoyl]-2',3'-O-isopropylideneuridine (7). To a stirred suspension of 5 (2g, 7 mmol) in methylene chloride (50 mL), pyridine (0.7 mL, 8.9 mmol) and ethylsulfamoyl chloride[18] (1.0, 7 mmol) were added at room temperature. After the instantaneous reaction, the solvents were evaporated under reduced pressure. The residue, dissolved in chloroform, was washed with dilute HCl and with water up to pH 7, dried over anhydrous sodium sulfate, filtered and evaporated to dryness to afford 7 (2.71 g, 98%) as a syrup: ^{1}H NMR ($CDCl_3$, 90 MHz) δ 1.16 (t, 3H, $CH_2\underline{CH_3}$) 1.33, 1.54 (2s, 6H, isopropylidene), 3.18 (dq, 2H, $\underline{CH_2}CH_3$), 5.50 (t, 1H, J = 6 Hz, $\underline{NH}$-CH_2CH_3), 5.66 (d, 1H, $J_{1',2'}$ = 2 Hz, H-1'), 5.77 (d, 1H, $J_{5,6}$ = 8.5 Hz, H-5), 7.33 (d, 1H, H-6), 9.86 (bs, 1H, 3-NH). UV λ_{max} (EtOH) 254 nm (ε 9800).

Anal. Calcd. for $C_{14}H_{21}N_3O_8S$: C, 42.97; H, 5.37; N, 10.74, S, 8.18. Found: C, 43.09; H, 5.50; N, 10.86; S, 8.21.

5'-O-[(N-Ethyl)sulfamoyl]uridine (8). A mixture of 7 (0.738 g, 1.88 mmol), trifluoroacetic acid (5 mL) and water (1 mL) was stirred at room temperature for 30 min. The solvents were evaporated under reduced pressure and coevaporated 3 times with ethanol (3 x 10 mL). The residue was purified by column chromatography using ethyl acetate as the eluent to yield 8 (0.53 g, 80%) as a white foam: ^{1}H NMR [$(CD_3)_2SO$, 90 MHz] δ 1.26 (t, 3H, $CH_2\underline{CH_3}$), 3.26 (dq, 2H, $\underline{CH_2}CH_3$), 5.78 (d, 1H, $J_{5,6}$ = 8.5 Hz, H-5), 6.01 (d, 1H, $J_{1',2'}$ = 2.7 Hz, H-1'), 6.86 (t, 1H, J = 6.2 Hz, $\underline{NH}$-CH_2CH_3), 7.79 (d, 1H, H-6), 10.1 (bs, 1H, 3-NH). UV λ_{max} (EtOH) 258 nm (ε 8050).

Anal. Calcd. for $C_{11}H_{17}N_3O_8S$: C, 37,60; H, 4.84; N, 11.96; S, 9.11. Found: C, 37.73; H, 4.90; N, 11.81; S, 9.28.

2',3'-Di-O-acetyl-5'-O-[(N-ethyl)sulfamoyl]uridine (9). A solution of 2',3'-di-O-acetyluridine (1 g, 3.0 mmol) in methylene chloride (40 mL) reacted with ethylsulfamoyl chloride[18] (0.43 g, 3.0 mmol) and was worked up as indicated above for 7 to give 9 (1.25 g, 94%) as a white foam: ^{1}H NMR ($CDCl_3$, 90 MHz) δ 1.30 (t, 3H, $CH_2\underline{CH_3}$), 2.13, 2.17 (2s, 6H,

OAc), 3.31 (dq, 2H, $C\underline{H}_2CH_3$), 5.50 (t, 1H, $N\underline{H}$-CH_2CH_3), 5.92 (d, 1H, $J_{5,6}$ = 8.4 Hz, H-5), 6.10 (d, 1H, $J_{1',2'}$ = 5 Hz, H-1'), 7.56 (d, 1H, H-6), 9.53 (bs, 1H, 3-NH). UV λ_{max} (EtOH) 256 nm (ϵ 8500).

Anal. Calcd. for $C_{15}H_{21}N_3O_{10}S$: C, 41.38; H, 4.83; N, 9.65; S, 7.36. Found: C, 41.62; H, 4.98; N, 9.39; S, 7.43.

5'-O-[(N-Isopropyl)sulfamoyl]-2',3'-O-isopropylideneuridine **(10)**. A suspension of **5** (2g, 7 mmol) in methylene chloride (50 mL) reacted with isopropylsulfamoyl chloride[18] (1.1 g, 7 mmol) and pyridine (0.7 mL, 8.9 mmol) and was worked up as indicated before for **7**, to afford **10** (2.8 g, 100%) as a syrup: ^{1}H NMR ($CDCl_3$, 90 MHz) δ 1.18[d, 6H, $CH(C\underline{H}_3)_2$], 1.32, 1.53 (2s, 6H, isopropylidene), 3.55 m, 1H, $C\underline{H}CH_3)_2$, 5.66 (d, 1H, $J_{1',2'}$ = 1.5 Hz, H-1'), 5.70 (bs, 1H, $N\underline{H}CH$), 5.75 (d, 1H, $J_{5,6}$ = 8.5 Hz, H-5), 7.35 (d, 1H, H-6), 9.90 (bs, 1H, 3-NH). UV λ_{max} (EtOH) 254 nm (ϵ 11350).

Anal. Calcd. for $C_{15}H_{23}N_3O_8S$: C, 44.44; H, 5.70; N, 10.37; S, 7.70. Found: C, 44.61; H, 5.85; N, 10.41; S, 8.02.

5'-O-[(N-Isopropyl)sulfamoyl]uridine **(11)**. A mixture of **10** (1g, 2.47 mmol), trifluoroacetic acid (5 mL) and water (1 mL) reacted and was worked up as indicated for **8** to give **11** (0.873 g, 97%) as a white foam: ^{1}H NMR[$(CD_3)_2SO$, 90 MHz] δ 1.13[d, 6H, $CH(CH_3)_2$], 3.37[m, 1H, $C\underline{H}(CH_3)_2$], 5.60 (d, 1H, $J_{5,6}$ = 7.5 Hz, H-5), 5.77 (d, 1H, $J_{1',2'}$ = 4 Hz, H-1'), 7.60 (d, 1H, H-6), 5.24, 5.40, 7.75 (3bs, 3H, 2'-OH, 3'-OH, $N\underline{H}$-CH), 11.33 (bs, 1H, 3-NH). UV λ_{max} (EtOH) 259 nm (ϵ 7900).

Anal. Calcd. for $C_{12}H_{19}N_3O_8S$: C, 39.45; H, 5.21; N, 11.51; S, 8.77. Found: C, 39.49; H, 5.51; N, 11.28; S, 8.53.

2',3'-O-Di-O-acetyl-5'-O-[(N-isopropyl)sulfamoyl]uridine **(12)**. To a solution of **11** (1 g, 2.74 mmol) in pyridine (10 mL), acetic anhydride (0.6 g, 5.9 mmol) was slowly added. The mixture was stirred at room temperature for 24 h and the solvents evaporated under reduced pressure. The residue was dissolved in chloroform, washed with dilute HCl and with water up to neutral pH, dried over anhydrous sodium sulfate, filtered and evaporated to dryness to afford **12** (1.20 g, 98%) as a syrup: ^{1}H NMR ($CDCl_3$, 90 MHz) δ1.20 [d, 6H, $CH(C\underline{H}_3)_2$], 2.05, 2.10 (2s, 6H, OAc), 3.63 [m, 1H, $C\underline{H}(CH_3)_2$], 5.50 [bs, 1H, $N\underline{H}CH(CH_3)_2$], 5.75 (d, 1H, $J_{5,6}$ = 8 Hz, H-5), 5.98 (d, 1H, $J_{1',2'}$ = 4.5 Hz, H-1'), 7.44 (d, 1H, H-6), 9.70 (bs, 1H, 3-NH). UV λ_{max} (EtOH) 256 nm (ϵ 8900).

Anal. Calcd. for $C_{16}H_{23}N_3O_{10}S$: C, 42.76; H, 5.12; N, 9.35; S, 7.13. Found: C, 42.98; H, 5.37; N, 8.97; S, 7.19.

2',3'-Di-O-benzoyl-5'-O- [(N-isopropyl)sulfamoyl] uridine **(13)**. A mixture of **11** (1 g, 2.74 mmol), pyridine (10 mL), and benzoyl chloride (0.9 g, 6.4 mmol) was reacted and was worked up as indicated before for **12**. The residue was purified by preparative TLC using ethyl acetate/ hexane (1:1) as the eluent to give **13** (1.35 g, 85%) as a white foam: ^1H NMR[$(CD_3)_2SO$, 90 MHz]δ 1.18 [d, 6H, $CH(C\underline{H}_3)_2$], 3.45 [m, 1H, $C\underline{H}(CH_3)_2$], 5.72 (d, 1H, $J_{5,6}$ = 8 Hz, H-5), 6.25 (d, 1H, $J_{1',2'}$ = 4.5 Hz, H-1'), 11.48 (bs, 1H, 3-NH). UV λ_{max} (EtOH) 229 nm (ϵ 27300), 254 (13100).

Anal. Calcd. for $C_{26}H_{27}N_3O_{10}S$: C, 54.45; H, 4.71; N, 7.33; S, 5.58. Found: C, 54.32; H, 4.43; N, 7.32; S, 5.22.

BIOLOGICAL METHODS

Cells and cell culture. Vero cells were grown in Dulbecco's modified Eagle medium with glutamine, supplemented with 10% calf serum, and either 0.85% sodium bicarbonate for flask cultures or 3.7% for cultures in 24- and 96- well plates (Costar, Cambridge, Ma. USA), incubated in a 5% CO_2 atmosphere, 95% humidity and 37 C. The maintenance medium was supplemented with 2% calf serum. Vero cells, media and sera were supplied by Flow Labs. Scotland, UK.

Viruses. Virus stocks were prepared in Vero cell culture, their titres were determined by plaque assay, and they were stored in aliquots at -70 C until used.

Cellular toxicity assay. Confluent Vero cell monolayers growing in 96-well plates were treated with various concentrations of the test compound. After 48 h. of incubation at 37 C the medium was removed and methionine-free medium supplemented with 10 µCi of [^{35}S] methionine was added to the cells for 1 h. The medium was then removed, the culture washed with PBS, treated with 5% TCA during 5 min., washed three times with 95% ethanol, air dried, and dissolved with 0.1 N NaOH plus 1% SDS. Samples of 50 µl with scintillation fluid were counted for radioactivity in an intertechnique scintillation spectrometer SL-32. The cytotoxicity is expressed as the inhibitory concentration required to inhibit [^{35}S] methionine incorporation by 50% (IP_{50}).

In vitro antiviral assay. Confluent Vero cell monolayers in 24-well plates were virus infected with a MOI (multiplicity of infection) of 0.5. After 90 min of adsorption (except SV 60 min) the virus was removed and the cells were further incubated in maintenance medium containing various concentrations of the test compound (0.01, 0.1, 1, 25, 50, 100, 150, 200, 400, 600, 800, 1000, 2000). Virus infected cultures without compound and uninfected cells treated with the different compounds were included as controls. When virus controls showed 100% cytopathic effect (48 h post infection for the viruses except for ASFV at 72 h) cells plus medium were removed and sonicated. Cell debris was discarded by centrifugation, virus yields of the supernatants were determined by plaque formation in Vero cell mono-layers growing in 24-well plates. The concentration of compound resulting in 50% inhibition of virus replication was calculated (MIC_{50}).

Virucidal assay by direct contact virus + drug. The virucidal effect on virus particles in the presence of drugs was tested by incubation of the virus suspension in Hanks' media with the drug solution (MIC_{50} dose) for 4 h at 37°C. The control sample was kept in the absence of drug substituted by diluent. After incubation period with periodical shaking of the mixtures, aliquots were removed, diluted in order to obtain a drug dilution in which the drug does not show inhibitory effect against the viruses, and titrations were performed in Vero cells by plaque assay. After the virus adsorption period the rest of unadsorbed virus and drug was carefully washed off.

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