hydrous suspension of 1a (112.6 mg, 0.5 mmol) in dimethylformamide (3 mL). After being stirred with exclusion of moisture for 45 min, the resulting solution was treated with 90% chloroacetaldehyde in ethyl ether (50 μL, ca. 0.6 mmol). Stirring was continued at room temperature for 45 min, then at 45 °C for 1 h. The reaction mixture was made basic by addition of concentrated aqueous ammonia (2 mL), and after 1 h the obtained solution was evaporated to dryness. The residue after evaporation was dissolved in 50% aqueous ethanol and adsorbed on a portion of silica gel (ca. 3 g, 70–230 mesh) by repeated coevaporation with ethanol. The dried gel was applied onto a silica gel short column (2.8 × 9 cm) and the product was eluted with chloroform–methanol (6:1). Fractions 34–47 (at 6 mL) containing the TLC-homogeneous 3a were evaporated to give 22.4 mg (18%) of a white solid. The ultraviolet and ^{1}H NMR spectra were identical with those described in method B.

Method B. Acetal derivative 4a (400 mg, 1.17 mmol) was gently refluxed in 40% acetic acid (25 mL) with a slow distillation of solvent in order to remove ethanol formed in the reaction. During the reaction time ca. 10 mL of a distillate (bp 92–94 °C) were collected. The rest of solvent was then evaporated under diminished pressure and a resulting white residue was coevaporated twice with 2-propanol, which gave the chromatographically pure 3a as a white powder (yield 286 mg, 98%). An analytical sample was crystallized from water: mp 248.5 °C dec; ^{1}H NMR δ 3.50 (s, 4, 4'H and 5'H), 4.67 (t, 1, OH), 5.50 (s, 2, 1'H), 7.45 and 7.63 (2 d, J = 2.6 Hz, 2, HC=CH), 8.04 (s, 1, 8H), 12.48 (br s, 1, NH). Anal. ($C_{10}H_{11}N_5O_3$) C, H, N.

3,9-Dihydro-3-[(1,3-dihydroxy-2-propoxy)methyl]-9-oxo-5*H*-imidazo[1,2-*a*]purine (3b). Acetal derivative 4b (100 mg, 0.269 mmol) was hydrolyzed in 1.2 N hydrochloric acid (6 mL) at room temperature for 18 h. At this time TLC in solvent C showed that substrate 4b had completely disappeared, and the reaction mixture was made alkaline by addition of concentrated aqueous ammonia (5 mL). An excess of ammonia was gently evaporated after 1 h, and a resulting colorless solution was adsorbed on a portion of silica gel (ca. 3 g, 70–230 mesh) by evaporation. The dried gel was applied onto a silica gel short column (2.5 × 7 cm) and the product was eluted with chloroform–methanol (6:1). Fractions 35–45 (at 6 mL) contained chromatographically pure 3b and were evaporated to a crystalline solid (yield 58.4 mg, 78%). An analytical sample was recrystallized from 90% aqueous ethanol: mp 250–251 °C dec; ^{1}H NMR δ 3.41 (m, 4, 3'H and 5'H), 3.64 (p, 1, 4'H), 4.60 (t, 2, OH), 5.59 (s, 2, 1'H), 7.42 and 7.61 (2 d, J = 2.6 Hz, 2, HC=CH), 8.03 (s, 1, 8H), 12.46 (br s, 1, NH). Anal. ($C_{11}H_{13}N_5O_4$) C, H, N.

Antiviral Activity and Cytotoxicity Evaluation. The methods for measuring virus-induced cytopathogenicity,[11] viral plaque formation,[12] host cell DNA synthesis,[11] and cell growth,[12] as well as the sources of the different virus strains,[11,13,14] have been described previously.

Acknowledgment. This work was supported by the Polish Academy of Sciences (Project CPBP 01.13.1.3), the Belgian Fonds voor Geneeskundig Wetenschappelijk Onderzoek (Project no. 3.0040.83), and the Belgian Geconcerteerde Onderzoeksacties (Project no. 85/90-79). We are indebted to M. Popenda for the NMR spectra, Dr. R. Snoeck for help with the anti-VZV and anti-CMV assays, Anita Van Lierde, Frieda De Meyer, Ria Van Berwaer, and Anita Camps for excellent technical assistance with the antiviral activity and cytotoxicity evaluations, and Christiane Callebaut for her fine editorial help.

Registry No. 1a, 59277-89-3; 1b, 82410-32-0; 2a, 114199-19-8; 2b, 134287-58-4; 2c, 59327-60-5; 3a, 134287-59-5; 3b, 134287-60-8; 3c, 62462-38-8; 4a, 134287-61-9; 4b, 134287-62-0; 4c, 132131-58-9.

(11) De Clercq, E.; Descamps, J.; Verhelst, G.; Walker, R. T.; Jones, A. S.; Torrence, P. F.; Shugar, D. *J. Infect. Dis.* **1980**, *141*, 563.
(12) Snoeck, R.; Sakuma, T.; De Clercq, E.; Rosenberg, I.; Holy, A. *Antimicrob. Agents Chemother.* **1988**, *32*, 1839.
(13) De Clercq, E.; Holy, A.; Rosenberg, I.; Sakuma, T.; Balzarini, J.; Maudgal, P. C. *Nature* **1986**, *323*, 464.
(14) De Clercq, E.; Bères, J.; Bentrude, W. G. *Mol. Pharmacol.* **1987**, *32*, 286.

5-(5-Bromothien-2-yl)-2'-deoxyuridine and 5-(5-Chlorothien-2-yl)-2'-deoxyuridine Are Equipotent to (*E*)-5-(2-Bromovinyl)-2'-deoxyuridine in the Inhibition of Herpes Simplex Virus Type I Replication

P. Wigerinck, C. Pannecouque, R. Snoeck, P. Claes, E. De Clercq, and P. Herdewijn*

Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium.
Received December 28, 1990

2'-Deoxyuridines with a five-membered heterocyclic substituent in the 5-position were synthesized by palladium-catalyzed coupling reactions of 5-iodo-2'-deoxyuridine with the activated heteroaromatics. Further modification of the compound with the 5-thien-2-yl substituent gave 5-(5-bromothien-2-yl)-2'-deoxyuridine and 5-(5-chlorothienyl-2-yl)-2'-deoxyuridine. Both compounds show potent and selective activity against herpes simplex virus type 1 and varicella-zoster virus.

Introduction

Several 5-substituted pyrimidine nucleosides show potent anti herpes virus activity.[1] Among them, 5-iodo-2'-deoxyuridine (IdUrd) and 5-(trifluoromethyl)-2'-deoxyuridine have been in clinical use for years. The most active congeners among the 5-substituted 2'-deoxyuridine derivatives are (*E*)-5-(2-halogenovinyl)-2'-deoxyuridines.[2] These compounds are particularly active against herpes simplex virus type 1 (HSV-1) and varicella-zoster virus. Their antiviral selectivity is primarily due to a preferential phosphorylation by the virus-encoded thymidine kinase and a greater inhibitory effect of the 5'-triphosphates on the viral DNA polymerase than the cellular DNA polymerases.[1]

From a structure–activity relationship study of some 30 5-substituted 2'-deoxyuridine analogues, the following features for the ideal 5-substitutent were proposed:[3] the substituent should be unsaturated, in conjugation with the pyrimidine base, not more than four carbon atoms long, it should possess *E* stereochemistry, and include an electronegative hydrophobic function.

(1) De Clercq, E. *Approaches to Antiviral Agents*; Harnden, M. R., Ed.; McMillan: New York, 1985; pp 57–99.
(2) De Clercq, E. *Pure Appl. Chem.* **1983**, *55*, 623–636.
(3) Goodchild, J.; Porter, R.; Raper, R.; Sim, I.; Upton, R.; Viney, J.; Wadsworth, H. *J. Med. Chem.* **1983**, *26*, 1252–1257.

0022-2623/91/1834-2383$02.50/0

Scheme I

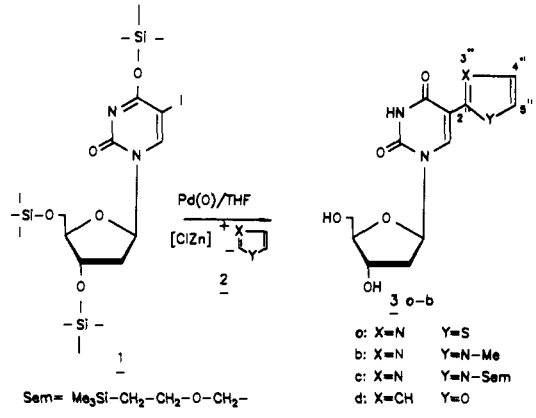


Table I. Yields of Coupling Reactions between the Zinc Chloride Salt of the Heterocycles and Silylated IdUrd[a]

X	Y	% yield	X	Y	% yield
N	S	48	N	N-Sem	<10
N	N-Me	64	CH	O	<10

[a] See Scheme I for structures.

(*E*)-5-(2-Bromovinyl)-2′-deoxyuridine (BrVdUrd) is a compound that meets these requirements. In vitro, BrVdUrd is a potent inhibitor of HSV-1 [minimum inhibitory concentration (MIC) = 0.02 μg/mL] and VZV (MIC = 0.003 μg/mL), but a rather weak inhibitor of HSV-2 (MIC = 1 μg/mL).[4]

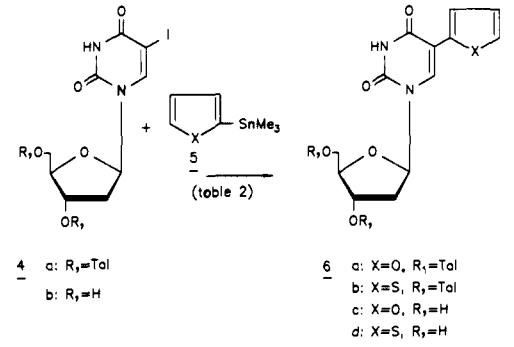
In a previous study,[5] we have reported the synthesis and antiviral activity of 5-isoxazole-, 5-thiazole-, and 5-pyrrole-substituted 2′-deoxyuridines. From this series of compounds 5-(3-bromoisoxazol-5-yl)-2′-deoxyuridine emerged as a selective anti-HSV-1 and anti-VZV agent. These results prompted us to broaden this structure–activity relationship study to other heterocyclic substituents.

Here we report on the synthesis and antiviral activity of 5-thien-2-yl- and of 5-furan-2-yl-substituted 2′-deoxyuridines, which were synthesized through Pd(0)-catalyzed C–C bond-formation reaction.

Chemistry

Several 5-aryl-substituted 2′-deoxyuridines were synthesized previously by Mertes et al.[6] with either a Pd(0) coupling reaction between silylated IdUrd and the $[ZnCl]^+$ salt of the aromatic or a Pd(0)-catalyzed reaction between 5-(chloromercuri)-2′-deoxyuridine and a halogenated aromatic. Direct introduction of a heteroaryl substituent in the 5-position of 2′-deoxyuridine was described previously by Pichat et al.[7] Their procedure was based on the coupling of $[ZnCl]^+$ salts of the heteroaromatic with silylated IdUrd and a Pd(II) catalyst. However, the yields of both 5-thien-2-yl-2′-deoxyuridine and 5-thien-3-yl-2′-deoxyuridine were very poor.[7] No biological or spectroscopic data were provided for these compounds. Variable results were obtained when we tried to adapt the previously described condensation reaction[6] to the synthesis of 2′-deoxyuridine analogues with a five-membered heterocyclic substituent in the 5-position. Therefore, silylated IdUrd and palladium(0) tetrakis(triphenylphosphine) were added at once to the freshly prepared $[ZnCl]^+$ salt of the heterocycle in THF (Scheme I). As originally suggested by Bell et al.,[8] the reaction was carried out in the presence of an excess of $ZnCl_2$ in order to dissociate intermolecular complexes between Zn^{2+} and the heterocycle. Using this strategy (Table I), we obtained fairly good yields of 5-thiazol-2-yl and 5-(*N*-methylimidazol-2-yl)-2′-deoxyuridine (48% and 64%, respectively). The method, however, failed with isoxazole and 2-(trimethylsilyl)thiazole as heterocycles and only poor yields were obtained with furan and *N*-[[2-(trimethylsilyl)ethoxy]methyl]imidazole. Apart from the fact that the method is not general applicable, it also suffers from the disadvantage that strictly anhydrous conditinos have to be used and that the elimination of excess of $ZnCl_2$ during the workup procedure can be problematic.

Scheme II



Scheme III

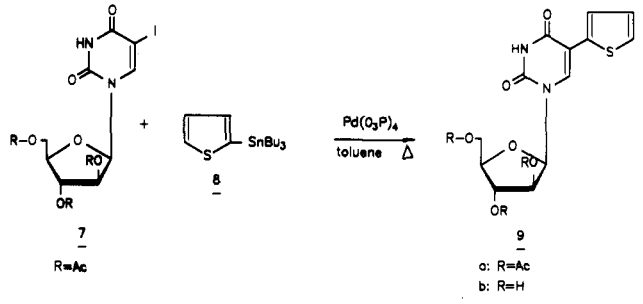


Table II. Coupling Reaction between Stannylated Heterocycles and IdUrd[c]

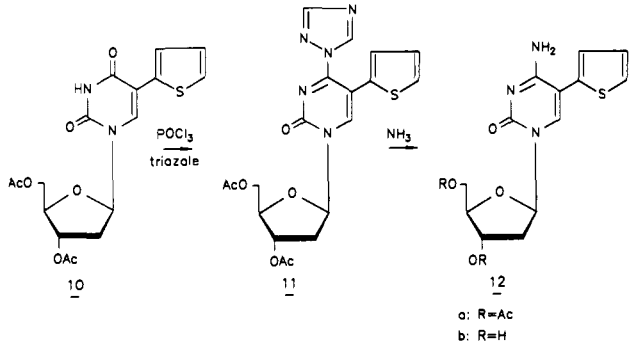
R_1	X	solvent	catalyst[a]	% yield
tol[b]	O	toluene	A	95
tol	O	THF	A	0
tol	O	THF	B	73
tol	S	THF	B	87
H	O	dioxane	B	89
H	S	dioxane	B	77

[a] Catalyst A, $Pd(Ph_3P)_4$; B, $PdCl_2(Ph_3P)_2$. [b] tol = $CH_3C_6H_4CO$. [c] See Scheme II for structures.

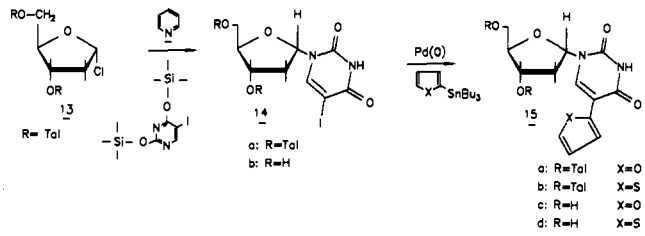
Therefore, we attempted the coupling reaction between 5-iodo-2′-deoxyuridine and the trialkylstannyl derivatives of the heterocycles[9] (Scheme II). The trimethylstannyl derivatives are much more stable than the corresponding $[ZnCl]^+$ salts, and they can be easily prepared. 2-(Trimethylstannyl)thiophene and 2-(trimethylstannyl)furan react in good yields with both protected and unprotected IdUrd. When the reaction was carried out in a coordinating solvent (THF, dioxane) a Pd(II) catalyst is needed, and partial reduction to dUrd was noted. When a noncoordinating solvent (toluene) was used, a Pd(0) catalyst

(4) De Clercq, E.; Walker, R. T. *Pharmacol. Ther.* **1984**, *26*, 1-44.
(5) Wigerinck, P.; Snoeck, R.; Claes, P.; De Clercq, E.; Herdewijn, P. *J. Med. Chem.*, in press.
(6) Al-Razzak, L. A.; Schwepler, D.; Decedue, C. J.; Balzarini, J.; De Clercq, E.; Mertes, M. P. *J. Med. Chem.* **1987**, *30*, 409-419.
(7) Vincent, P.; Beaucourt, J.; Pichat, L. *Tetrahedron Lett.* **1984**, *25*, 201-202.
(8) Bell, A.; Roberts, D.; Ruddoch, K. *Tetrahedron Lett.* **1988**, *29*, 5013-5016.
(9) Bailey, T. R. *Tetrahedron Lett.* **1986**, *37*, 4407-4410.

Scheme IV



Scheme V



is needed, and no side reaction was observed (Table II). A more straightforward synthesis could start from unprotected IdUrd making 5-thien-2-yl-1-(β-D-2-deoxyribofuranos-1-yl)uracil in one step. This conversion can be done in 77% isolated yield by reaction of IdUrd with 2-(trimethylstannyl)thiophene in the presence of $PdCl_2$/Ph_3P/Et_3N in dioxane.

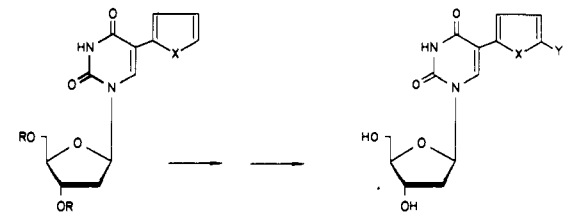
Since trimethylstannylated heterocycles are noxious, we prefer to carry out the reaction with the tributylstannyl derivatives. The yields were comparable. 5-Thien-2-yl-1-β-D-arabinofuranos-1-yluracil was obtained in the same way starting from 5-iodo-1-(2,3,5-triacetyl-β-D-arabinofuranos-1-yl)uracil[10] (Scheme III). The protective groups were removed with ammonia in methanol.

During the preparation of this paper, Crisp and Macolino reported on the palladium-catalyzed coupling of protected 5-iodo-2′-deoxyuridine with functionalized and nonfunctionalized arylboronic acids and aryltrimethylstannanes.[11] However, coupling reactions with heteroaryltrimethylstannanes were not described.

5-Thien-2-yl-2′-deoxycytidine was synthesized from 5-thien-2-yl-2′-deoxyuridine (**6d**) by a classical procedure[12] (Scheme IV). Both hydroxyl groups were protected by acetyl esters (**10**); the uracil base was converted to the cytosine base with $POCl_3$/triazole and aqueous ammonia; the deprotection was carried out with ammonia in methanol.

As a recent report[13] mentioned that α-nucleosides may also show antiviral activity, we also synthesized the α-analogues of 5-thien-2-yl-2′-deoxyuridine and 5-furan-2-yl-2′-deoxyuridine. A few years ago, Aoyama[14] reported that the reactions of silylated uracil bases and a crystalline derivative of α-D-2-deoxyribofuranosyl chloride gave predominantly the α-product when they were carried out in the presence of *p*-nitrophenol (0.32 equiv) and pyridine

Scheme VI



start. mater.	reagent (1)	16 X	16 Y	yield after deprotection
X=O, R=Tol	Br_2	a: O	Br	32%
X=S, R=Ac	Br_2	b: S	Br	83%
X=O, R=Tol	N.C.S.	c: O	Cl	85%
X=S, R=Ac	N.C.S.	d: S	Cl	28%

(0.28 equiv). The role of *p*-nitrophenol in this reaction is not very clear. When we tried the reaction without *p*-nitrophenol, using 5-iodouracil as the pyrimidine base, the yields were lower (50–70%) (Scheme V). The use of a higher amount of 5-iodouracil (2.5 equiv) gave higher overall yields but lower stereoselectivity.

The α-anomer of 5-IdUrd was isolated by chromatography on silica gel using a 9/1 mixture of CH_2Cl_2 and CH_3CN. Reaction of **14** with the 2-(tributylstannyl)-thiophene in the usual way and deprotection of the hydroxyl functions gave 5-thien-2-yl-1-(α-D-2-deoxyribofuranos-1-yl)uracil. The composition of the anomers was determined by HPLC analysis. Although it is not an absolute rule, a distinction between the α- and the β-isomer could be made by NMR spectroscopy. The H-1′ signal of the β-anomers appeared as triplets (J = ±6 Hz); the H-1′ signal of the α-anomers all appeared as double doublets (J = ±8 Hz and J = ±2 Hz). Final proof was provided by comparing the obtained compound with the independently synthesized β-anomer starting from IdUrd.

Halogenation of the furan-2-yl (**6a**) substituent with Br_2 (1 equiv) in CCl_4 and with *N*-chlorosuccinimide (2 equiv) in pyridine gave mainly the 5-bromofuran-2-yl and 5-chlorofuran-2-yl derivatives (Scheme VI). Chlorination of the thien-2-yl substituent (**10**), with 1 equiv of *N*-chlorosuccinimide, however, gave a mixture of the mono- and the dichlorinated derivatives. The mixture was purified by column chromatography and the acetyl groups were removed with ammonia in methanol giving 5-(5-chlorothien-2-yl)-1-(β-D-2-deoxyribofuranos-1-yl)uracil. In contrast, bromination with 1 equiv of Br_2 gave only the monobrominated product in high yield. The ^1H NMR spectrum of 5-(5-chlorofuran-2-yl)-1-(2-deoxy-β-D-ribofuranos-1-yl)uracil showed two doublets at δ 6.47 (H-4″) and 6.86 (H-3″) (J = 3.1 Hz). The magnitude of the coupling constant excludes the formation of a 4-chlorofuran-2-yl substituent.

Antiviral Activity

Substitution at the C-5 position of 2′-deoxyuridine with a thiazol-2-yl group, as in **3a**, led to some activity against HSV-1, whereas a *N*-methylimidazol-2-yl substitution gave a totally inactive product (**3b**) (Table III).

Introduction of a furan-2-yl or thien-2-yl group at the C-5 position afforded compounds (**6c** and **6d**, respectively) with marked activity against HSV-1. To a lesser extent, these compounds were also inhibitory to VZV, and **6c** was also active against vaccinia virus (VV). However, neither **6c** nor **6d** proved active against HSV-2 or thymidine kinase deficient (TK^-) mutants of HSV-1 or VZV.

A thiophene ring at the C-5 position of 1-β-D-arabinofuranos-1-yluracil (**9b**) and 1-(β-D-2-deoxyribofuranos-1-yl)cytosine (**12b**) led to compounds that were as active as **6c** against TK^+ strains of HSV-1 and VZV. Again, these

(10) Verheyden, J.; Wagner, D.; Moffatt, J. *J. Org. Chem.* **1971**, *36*, 250–254.
(11) Crisp, G.; Macolino, V. *Synth. Commun.* **1990**, *20* (3), 413–422.
(12) Van Aerschot, A.; Everaert, D.; Balzarini, J.; Augustijns, K.; Liu, J.; Janssen, G.; Peeters, O.; Blaton, M.; De Ranter, C.; De Clercq, E.; Herdewijn, P. *J. Med. Chem.* **1990**, *33*, 1833–1839.
(13) Johansson, K.; Malmberg, H.; Noreen, R.; Sahlberg, S.; Sohn, D.; Gronowitz, S. *Chem. Abstr.* **1990**, *112*, 235778e.
(14) Aoyama, H. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 2073–2077.

Table III. Antiviral Activity and Cytotoxicity in Vitro

virus (strain)	cell	minimum inhibitory concentration, μg/mL[a]													
		BrVdUrd	3a	3b	6c	6d	9b	12b	14b	15c	15d	16a	16b	16c	16d
HSV-1 (KOS)	E_6SM	0.02	45	>200	0.2	0.45	0.2	1.3	300	55	>200	0.15	0.03	0.2	0.02
HSV-1 (F)	E_6SM	0.02	10	>200	0.2	0.2	0.2	0.4	300	40	ND[b]	0.1	0.07	0.2	0.04
HSV-1 (McIntyre)	E_6SM	0.02	20	>200	0.2	0.1	0.07	0.07	300	70	ND	0.2	0.02	0.4	0.004
HSV-2 (G)	E_6SM	20	>200	>200	200	250	20	45	300	300	>300	150	150	150	>200
HSV-2 (196)	E_6SM	>400	>200	>200	>400	150	100	20	300	150	150	150	>200	150	>200
HSV-2 (Lyons)	E_6SM	10	>200	>200	>400	100	20	20	300	150	100	300	150	150	>200
TK⁻ HSV-1 (B2006)	E_6SM	20	>200	>200	150	300	>400	>100	300	>200	>200	100	150	150	>200
TK⁻ HSV-1 (VMW 1837)	E_6SM	4	>200	>200	300	150	>400	>100	150	70	150	300	150	150	>200
VZV (Oka)	HEL	0.001	>100	>400	4	18	5.5	3	15	210	75	3	0.03	2.5	0.02
VZV (YS)	HEL	0.001	>100	>400	7	41	6.5	4	10	240	>100	3	0.03	3	0.02
TK⁻ VZV (YS-R)	HEL	>40	>100	>400	220	>400	>400	30.0	170	240	80	>100	>40	>100	65
TK⁻ VZV (07-1)	HEL	>40	>100	>400	155	180	370	8	90	260	85	>100	>40	>100	35
CMV (AD169)	HEL	250	>100	>400	>400	250	250	60	400	>100	>100	>100	>100	>100	>40
CMV (Davis)	HEL	250	>100	>400	>400	250	250	40.0	ND	>100	ND	>100	>100	>100	ND[b]
VSV	E_6SM	400	>200	>200	300	>400	>400	>200	300	>400	>200	>200	>200	>200	>200
VV	E_6SM	0.07	>200	>200	7	>400	>400	20	>400	300	>200	70	70	45	>200
morphologic alteration	E_6SM	>400	>200	>200	>400	>400	>400	>200	>400	>400	>400	>400	>400	>400	>400
cell growth	HEL	200	>50	>200	100	>200	200	45	>200	110	180	125	155	105	>50

[a] Concentration required to reduced virus-induced cytopathogenicity (HSV, VZV, VSV, VV) or plaque formation (CMV) or cell growth by 50%; for morphologic alteration, it corresponded to the minimum concentration required to cause a microscopically detectable alteration of normal cell morphology. [b] Not determined.

compounds were inactive against TK⁻ mutants of HSV-1 and VZV.

The four molecules containing an additional bromine or chlorine atom at the 5-position of the thiophene (**16b** and **16d**) or furan moiety (**16a** and **16c**) showed a clear increase in antiviral activity (i.e. 10-fold for HSV-1 and at least 600-fold for VZV) when compounds **16b** and **16d** were compared to the parent molecule compound **6d**. Yet, this halogenation of the thiophene or furan ring did not make the compounds **16a–d** active against either HSV-2 or TK⁻ mutants of HSV-1 or VZV.

Our data indicate that introduction of an halogen (bromine or chlorine) in the heterocycle substituted at C-5 of the pyrimidine ring of 1-(β-D-2-deoxyribofuranos-1-yl)uracil imparts strong activity against HSV-1 and VZV. The 5-bromothien-2-yl and 5-chlorothien-2-yl derivatives (**16b,d**) conferred a similar potency against HSV-1 (MIC ≈ 0.02 μg/mL) as BrVdUrd, which is one of the most potent in vitro anti-HSV-1 agents that has ever been described.[1,2]

The α-anomeric forms of the furan-2-yl (**15c**) and the thien-2-yl (**15d**) derivatives were also evaluated for antiviral activity. They exhibited little, if any, activity against any of the viruses examined. Likewise, 5-iodo-1-(α-D-2-deoxyribofuranos-1-yl)uracil failed to show any antiviral activity.

Except for 5-thien-2-yl-1-(β-D-2-deoxyribofuranos-1-yl)cytosine (**12b**), which showed slight activity against CMV (IC_{50} = 50 μg/mL), none of the test compounds proved active against CMV. None of the compounds showed activity against VSV, caused a microscopically detectable alteration of cell morphology, or inhibited cell growth at the highest concentrations tested. None of the compounds showed any activity against human immunodeficiency virus type 1 (HIV-1) or type 2 (HIV-2) (data not shown). This is in contradiction with the abstract of a recent patent by Johansson et al.,[13] who claim strong activity (0.05–10 μM) for the α-anomer of 5-thien-2-yl-2′-deoxyuridine (**16d**).

The mechanism of antiviral action of 5-(5-bromothien-2-yl)-2′-deoxyuridine and 5-(5-chlorothien-2-yl)-2′-deoxyuridine has not been assessed in the present study. According to their spectrum of antiviral activity (and in analogy with the activity spectrum of the (*E*)-5-(2-halovinyl)-2′-deoxyuridines (i.e. BrVdUrd), for which the mode of action has been established,[15–18] it can be postulated that 5-(5-bromothien-2-yl)-2′-deoxyuridine and 5-(5-chlorothien-2-yl)-2′-deoxyuridine are preferentially phosphorylated by the HSV-1- and VZV-encoded thymidine kinases and, once converted to their 5′-triphosphate form, they interact with viral DNA synthesis. Whether the 5-(5-halothien-2-yl) derivatives are eventually incorporated into (viral) DNA is an interesting question that should be addressed in further studies.

Experimental Section

Melting points were determined in capillary tubes with a Büchi-Tottoli apparatus and are uncorrected. Ultraviolet spectra were recorded with a Philips PU 8700 UV/vis spectrophotometer. The ^1H NMR and ^{13}C NMR spectra were determined with a JEOL FX 90Q spectrometer with tetramethylsilane as internal standard for the ^1H NMR spectra and DMSO-d_6 (39.6 ppm) for the ^{13}C NMR spectra (s = singlet, d = doublet, t = triplet, br s = broad signal, m = multiplet. Precoated Merck silica gel F254 plates were used for TLC, and the spots were examined with UV light and sulfuric acid–anisaldehyde spray. Column chromatography was performed on Merck silica gel (0.063–0.200 mm). Anhydrous solvents were obtained as follows: tetrahydrofuran and dioxane were refluxed overnight on lithium aluminum hydride and distilled; dichloromethane was stored for 1 week on anhydrous calcium chloride, filtered, and distilled; water was removed from *N,N*-dimethylformamide by distillation with benzene followed by distillation in vacuo; toluene was refluxed overnight on sodium and distilled. Pyridine was dried by distillation after it had been refluxed for 24 h on potassium hydroxide.

5-Thiazol-2-yl-1-(β-D-2-deoxyribofuranos-1-yl)uracil (3a). With the same procedure as described for **3b**, thiazole was coupled to the 5-position of IdUrd in 48% yield. The final purification consisted of chromatography on an XAD column (instead of benzoylation, extraction, and deprotection). This product was crystallized from 2-propanol. Mp: 217–219 °C.[6] UV (MeOH): λ_{max} = 261 nm (ε = 2850), λ_{max} = 320 nm (ε = 14100). Anal. ($C_{13}H_{13}N_3O_5S$): C, H, N.

5-(*N*-Methylimidazol-2-yl)-1-(β-D-2-deoxyribofuranos-1-yl)uracil (3b). A solution of 5-iodo-1-(β-D-2-deoxyribofuranos-1-yl)uracil (280 mg, 0.76 mmol) and hexamethyldisilazane (1.15 g, 7.1 mmol) in 5 mL of anhydrous pyridine was stirred at

(15) Allaudeen, H. S.; Kozarich, J. W.; Bertino, J. R.; De Clercq, E. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 2698–2702.
(16) Descamps, J.; De Clercq, E. *J. Biol. Chem.* **1981**, *256*, 5973–5976.
(17) Mancini, W. R.; De Clercq, E.; Prusoff, W. H. *J. Biol. Chem.* **1983**, *258*, 792–795.
(18) Balzarini, J.; Bernaerts, R.; Verbruggen, A.; De Clercq, E. *Mol. Pharmacol.* **1990**, *37*, 402–407.

room temperature overnight. The solution was evaporated in vacuo at 30 °C. The residue and tetrakis(triphenylphosphine)-palladium(0) (450 mg, 0.39 mmol) were mixed in dry tetrahydrofuran (20 mL), and the suspension was stirred vigorously at room temperature in the dark. A clear yellow solution was obtained after 20 min. This solution was transferred to another flask containing 10 mmol of the zinc chloride complex of *N*-methylimidazole. The latter was prepared by treating a solution of *N*-methylimidazole (0.74 mL, 9.5 mmol) in dry tetrahydrofuran (20 mL) at −70 °C with *n*-butyllithium (6.3 mL, 10.1 mmol of a 1.6 M solution in hexane) followed by a solution of freshly fused zinc chloride (1.33 g, 9.5 mmol) in THF (4 mL). A heavy white precipitate was formed which was warmed slowly to room temperature over 0.5 h. Then the palladium(0) complex of silylated IdUrd was added. After 5 min 2 equiv of $ZnCl_2$ (2.66 g, 20 mmol) in THF (8 mL) was added. This mixture was refluxed for 4 h. Chromatographic purification of the reaction mixture could not remove $ZnCl_2$ completely. Therefore, the product-containing fractions were evaporated and treated with an excess of benzoyl chloride (1.5 mL, 1.3 mmol) in dry pyridine (30 mL) at room temperature for 6 h. This mixture was concentrated, diluted with 100 mL of $CHCl_3$, and extracted four times with 100 mL of water to remove the excess of zinc salts. The organic layer was dried, evaporated, and taken up in 30 mL of methanol saturated with ammonia. Finally a second chromatographic purification yielded 150 mg (64%) of 5-(*N*-methylimidazol-2-yl)-1-(β-D-2-deoxyribofuranos-1-yl)uracil. The product was crystallized from MeOH/Et_2O. Mp: 209–211 °C. UV (MeOH): λ_{max} = 228 nm (ε = 10400), λ_{max} = 285 nm (ε = 8600). ^1H NMR (DMSO-d_6): δ 2.23 (m, 2 H, H-2′), 3.51 (br s, 5 H, CH_3 and H-5′), 3.84 (m, 1 H, H-4′), 4.26 (m, 1 H, H-3′), 5.02 (br s, 1 H, 5′-OH), 5.31 (br s, 1 H, 3′-OH), 6.20 (t, 1 H, H-1′), 6.89 (d, 1 H, H-5″), 7.20 (d, 1 H, H-4″), 8.08 (s, 1 H, H-6), 11.32 (s, 1 H, NH) ppm. ^{13}C NMR (DMSO-d_6): δ 33.7 (CH_3), 40.2 (C-2′), 61.3 (C-5′), 70.6 (C-3′), 85.0 (C-1′), 87.8 (C-4′), 105.8 (C-5), 123.1 (C-5″), 127.5 (C-4″), 141.9 (C-2″), 142.3 (C-6), 150.2 (C-2), 161.7 (C-4) ppm. Anal. ($C_{13}H_{16}N_4O_5 \cdot {}^1/_4H_2O$): C, H, N.

5-Furan-2-yl-1-(β-D-2-deoxyribofuranos-1-yl)uracil (6c). Method A. To a solution of 5-iodo-1-(3,5-di-*O*-toluoyl-β-D-2-deoxyribofuranos-1-yl)uracil (**4a**) (150 mg, 0.26 mmol) in 30 mL of THF was added 2-(trimethylstannyl)furan (160 mg, 2 mmol) and a catalytic amount of dichlorobis(triphenylphosphine)palladium (20 mg). The mixture was refluxed for 2 h, cooled, and evaporated to give 103 mg (73%) of 5-furan-2-yl-1-(3,5-di-*O*-toluyl-β-D-2-deoxyribofuranos-1-yl)uracil as an oil.

Method B. A solution of 5-iodo-1-(3,5-di-*O*-toluoyl-β-D-2-deoxyribofuranos-1-yl)uracil (150 mg, 0.26 mmol), 2-(trimethylstannyl)furan (460 mg, 2 mmol), and tetrakis(triphenylphosphine)palladium(0) (20 mg) in toluene (30 mL) was refluxed for 7 h. The volatiles were removed by evaporation and the mixture was purified chromatographically ($CHCl_3$/MeOH, 99/1). Yield: 130 mg (95%).

The combined fraction of A and B (232 mg, 0.44 mmol) were treated for 2 h at room temperature with 20 mL of a 0.1 M sodium methoxide solution in MeOH. The solution was neutralized with acetic acid and evaporated to dryness. Chromatographic purification yielded 110 mg (85%) of pure **6c** as a foam.

Method C. To a suspension of IdUrd (270 mg, 0.76 mmol) in dioxane (20 mL) was added 2-(trimethylstannyl)furan (1 g, 3.4 mmol) and dichlorobis(triphenylphosphine)palladium (20 mg) catalyst. The mixture was heated for 1 h at 90 °C, cooled, and evaporated. Column chromatography [(1) $CHCl_3$, (2) $CHCl_3$/MeOH, 92/8] yielded 230 mg (89%) of pure **6c** as a foam.

Method D. By starting with 280 mg (0.76 mmol) and following the same method as that described for **3b**, only 23 mg (10% yield) of **6c** was obtained. The product was crystallized from acetone. Mp: 206–208 °C. UV (MeOH): λ_{max} = 249 nm (ε = 13850), λ_{max} = 316 nm (ε = 12000). ^1H NMR (DMSO-d_6): δ 2.18 (dd, 2 H, H-2′), 3.61 (t, 2 H, H-5′), 3.85 (m, 1 H, H-4′), 4.29 (m, 1 H, H-3′), 5.06 (t, 1 H, 5′-OH), 5.25 (d, 1 H, 3′-OH), 6.22 (t, 1 H, H-1′), 6.52 (dd, 1 H, H-4″), 6.96 (dd, 1 H, H-3″), 7.61 (dd, 1 H, H-5″), 8.33 (s, 1 H, H-6), 11.60 (s, 1 H, NH) ppm. ^{13}C NMR (DMSO-d_6): δ 40.2 (C-2′), 61.3 (C-5′), 70.6 (C-3′), 85.1 (C-1′), 87.8 (C-4′), 105.9 (C-5), 108.2 (C-3″), 111.8 (C-4″), 135.0 (C-6), 141.8 (C-5″), 146.6 (C-2″), 149.6 (C-2), 160.3 (C-4) ppm. Anal. ($C_{13}H_{14}N_2O_6$): C, H, N.

5-Thien-2-yl-1-(β-D-2-deoxyribofuranos-1-yl)uracil (6d). Method A. To a solution of 5-iodo-1-(3,5-di-*O*-toluyl-β-D-2-deoxyribofuranos-1-yl)uracil (300 mg, 0.52 mmol) in THF (20 mL) was added 2-(trimethylstannyl)thiophene (440 mg, 1.79 mmol) and dichlorobis(triphenylphosphine)palladium(II) (20 mg, 0.04 mmol). The mixture was refluxed overnight, cooled, and evaporated. The product was purified by column chromatography (241 mg, 87%) and then treated with a 0.1 M solution of sodium methoxide in methanol (20 mL) for 3 h at room temperature. The solution was neutralized with HOAc and evaporated. The resulting oil was purified by chromatography on a silica gel column [(1) $CHCl_3$/MeOH, 95/5, (2) $CHCl_3$/MeOH, 93/7]; 130 mg (93%) of **6d** was obtained as a foam.

Method B. A mixture of IdUrd (270 mg, 0.76 mmol), 2-(trimethylstannyl)thiophene (1 g, 4.1 mmol) and dichlorobis(triphenylphosphine)palladium(II) in dioxane (20 mL) was heated at 95 °C for 4 h. The solution was cooled and evaporated and the mixture was purified chromatographically [(1) $CHCl_3$/MeOH, 95/5, (2) $CHCl_3$/MeOH, 93/7]; 106 mg (45%) of **6d** was obtained as a foam.

Method C. 5-Iodo-1-(β-D-2-deoxyribofuranos-1-yl)uracil (470 mg, 1.33 mmol) and 2-(trimethylstannyl)thiophene (1 g, 4.1 mmol) were added to a hot solution of palladium acetate (15 mg, 0.07 mmol), triphenylphosphine (35 mg, 0.13 mmol), and triethylamine (0.5 mL, 3.5 mmol) in 25 mL of dioxane, which had been refluxed for 10–15 min. The reaction mixture was then stirred further for 4 h at 85 °C, a red solution was formed, and a palladium mirror on the walls of the reaction flask appeared at the end of the reaction. The reaction flask was cooled and evaporated and 320 mg (77%) of **6d** was obtained after chromatographic purification ($CHCl_3$/MeOH, 93/7). The product was crystallized from acetonitrile. Mp: 206–208 °C. UV (MeOH): λ_{max} = 262 nm (ε = 10300), λ_{max} = 319 nm (ε = 10000); ^1H NMR (DMSO-d_6): δ 2.24 (t, 2 H, H-2′), 3.65 (br s, 2 H, H-5′), 3.86 (m, 1 H, H-4′), 4.39 (m, 1 H, H-3′), 5.23 (br s, 2 H, 3′-OH and 5′-OH), 6.24 (t, 1 H, H-1′), 7.09 (d, 1 H, H-4″), 7.42 (m, 2 H, H-3″ and H-5″), 8.58 (s, 1 H, H-6), 11.60 (s, 1 H, NH) ppm. ^{13}C NMR (DMSO-d_6): δ 40.5 (C-2′), 61.0 (C-5′), 70.1 (C-3′), 85.0 (C-1′), 87.7 (C-4′), 108.4 (C-5), 122.7, 125.6 and 126.5 (C-3″, C-4″, C-5″), 134.1 (C-2″), 135.7 (C-6), 149.4 (C-2), 161.4 (C-4) ppm. Anal. ($C_{13}H_{14}N_2O_5S$): C, H, N.

5-Thien-2-yl-1-β-D-arabinofuranos-1-yluracil (9b). A mixture of 590 mg (1.19 mmol) of 1-(2,3,5-triacetyl-β-D-arabinofuranos-1-yl)-5-iodouracil, 500 mg (1.34 mmol) of 2-(tributylstannyl)thiophene and 30 mg (0.026 mmol) of palladium tetrakis(triphenylphosphine) in 30 mL of toluene was refluxed overnight. After 16 h TLC evaluation ($CHCl_3$/MeOH, 97/3) showed that only 60% of the starting material had been transformed into a more liphophilic and fluorescent product. Another 500 mg of 2-(tributylstannyl)thiophene and 20 mg of palladium catalyst were added, and the mixture was refluxed for six more hours. After cooling, the black palladium precipitate was filtered off, and the filtrate was evaporated to dryness. The protective groups were removed by treatment of this oil with 20 mL of MeOH saturated with ammonia. After evaporation of the volatiles the mixture was separated by column chromatography; 130 mg (33% yield) of 5-thienyl-2-yl-1-β-D-arabinofuranos-1-yluracil was isolated. The product was taken up in acetone and precipitated by addition of heptane. Mp: 225–228 °C. UV (MeOH): λ_{max} = 262 nm (ε = 10200), λ_{max} = 317 nm (ε = 9900). ^1H NMR (DMSO-d_6): δ 3.28 (br s, 2 H, H-5′), 3.72 (br s, 2 H, H-3′ and H-4′), 4.08 (m, 1 H, H-2′), 5.29, 5.49, and 5.65 (2 × d and 1 × t, D_2O exchangeable, 2′-, 3′- and 5′-OH), 6.10 (d, 1 H, H-1′), 7.08 (m, 1 H, H-4″), 7.39 (m, 2 H, H-3″ and H-5″), 8.26 (s, 1 H, H-6), 11.65 (s, 1 H, NH) ppm. ^{13}C NMR (DMSO-d_6): δ 60.3 (C-5′), 74.7 (C-3′), 75.5 (C-2′), 84.3 (C-4′), 85.2 (C-1′), 107.2 (C-5), 122.8, 125.5, and 126.7 (C-3″, C-4″, and C-5″), 134.4 (C-2″), 137.5 (C-6), 149.5 (C-2), 161.4 (C-4) ppm. Anal. ($C_{13}H_{14}N_2O_6S$): C, H, N.

5-Thien-2-yl-1-(β-D-2-deoxyribofuranos-1-yl)cytosine (12b). 310 mg (1 mmol) of 5-thien-2-yl-1-(β-D-2-deoxyribofuranos-1-yl)uracil was taken up in pyridine (10 mL) and acetic anhydride (2 mL, 21 mmol) was added. The mixture was evaporated and coevaporated twice with toluene (10 mL); TLC evaluation ($CHCl_3$/MeOH, 90/10) revealed that all of the **6d** had been transformed in a more lipophilic product (**10**). The solution was evaporated to a foam. This foam was dried by coevaporation with pyridine (2 × 10 mL) and taken up in pyridine (50 mL). $POCl_3$

(0.27 mL, 3 mmol) and crystalline triazole (828 mg, 12 mmol) were added. The solution was stirred at room temperature for 3 h; cooled in an ice bath, and then ammonia (5 mL, 33% in H_2O) was added dropwise. After 10 min the mixture was evaporated and purified by flask chromatography [(1) $CHCl_3$/MeOH, 99/1, (2) $CHCl_3$/MeOH, 95/5)]. The protective groups were removed by treatment with methanol saturated with ammonia. After evaporation the resulting oil was taken up in methanol and crystallized. Yield: 180 mg, 60%. Mp: 194 °C dec. UV (MeOH): λ_{max} = 242 nm (ϵ = 14 400), λ_{max} = 287 nm (ϵ = 8150). ^{1}H NMR (DMSO-d_6): δ 2.10 (t, 2 H, H-2'), 3.65 (br s, 2 H, H-5'), 3.79 (m, 1 H, H-4'), 4.20 (m, 1 H, H-3'), 4.96 (t, 1 H, 5'-OH), 5.18 (d, 1 H, 3'-OH), 6.16 (t, 1 H, H-1'), 6.90 (br s, 2 H, NH_2), 7.10 (m, 2 H) and 7.55 (t, 1 H) (H-3'', H-4'', H-5''), 8.00 (s, 1 H, H-6) ppm. ^{13}C NMR (DMSO-d_6): δ 41.0 (C-2'), 61.2 (C-5'), 70.3 (C-3'), 85.8 (C-1'), 87.6 (C-4'), 101.1 (C-5), 126.6, 127.5 and 128.4 (C-3'', C-4'', and C-5''), 134.6 (C-2''), 141:1 (C-6), 154.7 (C-2), and 163.4 (C-4) ppm. Anal. ($C_{13}H_{15}O_4N_3S\cdot{}^1/_2H_2O$) C, H, N.

5-Iodo-1-(3,5-di-*O*-toluoyl-α-D-2-deoxyribofuranos-1-yl)uracil (14a). To a suspension of 5-iodouracil (1.19 g, 5.12 mmol) in 15 mL of dichloroethane was added 4 mL of hexamethyldisilazane and a catalytical amount of ammonium sulfate. The mixture was refluxed for 16 h, cooled, evaporated, and coevaporated three times with 20 mL of *m*-xylene.

To this oil was added 400 mg (1.024 mmol) of 2,5-di-*O*-toluyl-α-D-2-deoxyribofuranos-1-yl chloride and 15 mL of dry CH_2Cl_2. Finally 0.1 mmol (8 mg) of pyridine was added and the mixture was stirred at room temperature. After 1 h 30 mL of a saturated aqueous sodium bicarbonate solution and 30 mL of dichloromethane were added. The organic phase was dried on Na_2SO_4, filtrated, and evaporated. Flash chromatography ($CHCl_3$/MeOH, 95/5) yielded 700 mg (91% yield) of an α/β mixture (1/1).

The pure α-anomer was obtained by a second chromatography on silica gel using a 9/1 mixture of CH_2Cl_2 and CH_3CN. The structure of the compound was proven after deprotection. UV (MeOH): λ_{max} = 246 and 283 nm.

5-Iodo-1-(α-D-2-deoxyribofuranos-1-yl)uracil (14b). The protective groups were removed by treatment of 364 mg (0.61 mmol) of **14a** with 15 mL of 0.1 N NaOMe in MeOH for 2 h at room temperature. Chromatographic purification yielded 130 mg (60% yield) of pure 5-iodo-1-(α-D-2-deoxyribofuranos-1-yl)uracil, which was crystallized from acetone. Mp: 204–205 °C. UV (MeOH): λ_{max} = 287 nm (ϵ = 8200). ^{1}H NMR (DMSO-d_6): δ 1.99 (m, 1 H, H-2'), 2.70 (m, 1 H, H-2'), 3.52 (br s, 2H, H-5'), 4.19 (m, 2 H, H-3' and H-4'), 4.83 (br s, 1 H, 5'-OH), 5.41 (br s, 1 H, 3'-OH), 6.09 (dd, 1 H, J = 7.9 and 1.8 Hz, H-1'), 8.29 (s, 1 H, H-6), 11.70 (s, 1 H, NH) ppm. ^{13}C NMR (DMSO-d_6): δ 40.9 (C-2'), 62.0 (C-5'), 68.2 (C-5), 70.8 (C-3'), 86.8 (C-1'), 90.0 (C-4'), 146.4 (C-6), 150.6 (C-2), 161.0 (C-4) ppm. Anal. ($C_9H_{11}N_2O_5I$): C, H, N.

5-Furan-2-yl-1-(α-D-2-deoxyribofuranos-1-yl)uracil (15c). Following the same method as for **6c** but with tributyl instead of (trimethylstannyl)furan **15c** was obtained in 45% overall yield. The product was crystallized from ethyl acetate. Mp: 193–195 °C. UV (MeOH): λ_{max} = 251 nm (ϵ = 12 000), λ_{max} = 315 nm (ϵ = 11 200). ^{1}H NMR (DMSO-d_6): δ 1.99 (m, 1 H, H-2'), 2.73 (m, 1 H, H-2'), 3.45 (d, 2 H, H-5'), 4.24 (m, 2 H, H-3' and H-4'), 5.35 (br s, 2 H, 3' and 5'-OH), 6.19 (dd, 1 H, J = 5.8 and 1.8 Hz, H-1'), 6.52 (dd, 1 H, J = 3.1 and 0.9 Hz, H-4''), 6.86 (d, 1 H, J = 3.1 Hz, H-3''), 7.63 (d, 1 H, J = 0.9 Hz, H-5''), 8.34 (s, 1 H, H-6), 11.32 (s, 1 H, NH) ppm. ^{13}C NMR (DMSO-d_6): δ 40.7 (C-2'), 61.9 (C-5'), 70.8 (C-3'), 86.7 (C-1'), 90.1 (C-4'), 105.2 (C-5), 108.0 (C-3''), 111.8 (C-4''), 135.8 (C-5''), 141.6 (C-6), 146.8 (C-2''), 149.6 (C-2), 160.4 (C-4) ppm. Anal. ($C_{13}H_{14}N_2O_6$) C, H, N.

5-Thien-2-yl-1-(α-D-2-deoxyribofuranos-1-yl)uracil (15d). Starting from 400 mg (0.66 mmol) of **14a** and following the same method as for **9a**, 203 mg (55% yield) of **16b** was obtained. Deprotection with 0.1 M sodium methoxide gave **16d** in 70% yield. The product was crystallized from acetone. Mp: 197–199 °C. UV (MeOH): λ_{max} = 262 nm (ϵ = 12 000), λ_{max} = 317 nm (ϵ = 11 700); ^{1}H NMR (DMSO-d_6): δ 2.08 (m, 1 H, H-2'), 2.70 (m, 1 H, H-2'), 3.44 (br s, 2 H, H-5'), 4.25 (m, 2 H, H-3' and H-4'), 4.84 (t, 1 H, 5'-OH), 5.47 (d, 1 H, 3'-OH), 6.24 (dd, 1 H, J = 7.9 and 1.8 Hz, H-1'), 7.05 (dd, J = 4.0 and 4.8 Hz, H-4''), 7.28-7.53 (m, 2 H, H-3' and H-5''), 8.49 (s, 1 H, H-6), 11.60 (s, 1 H, NH) ppm. ^{13}C NMR (DMSO-d_6): δ 40.5 (C-2'), 62.1 (C-5'), 71.1 (C-3'), 86.7 (C-1'), 90.2 (C-4'), 108.3 (C-5), 123.3, 125.9, and 127.0 (C-3'', C-4'', and C-5''), 134.5 (C-2''), 137.4 (C-6), 149.9 (C-2), 161.7 (C-4) ppm. Anal. ($C_{13}H_{14}N_2O_5S$): C, H, N.

5-(5-Bromofuran-2-yl)-1-(β-D-2-deoxyribofuranos-1-yl)uracil (16a). To a solution of 5-furan-2-yl-1-(3,5-di-*O*-toluoyl-β-D-2-deoxyribofuranos-1-yl)uracil (250 mg, 0.48 mmol) in 10 mL of CH_2Cl_2 was added dropwise at room temperature a dilution of Br_2 (0.49 mmol) in 5 mL of CCl_4. After 30 min the green solution was extracted with water (2 × 20 mL), dried, and evaporated. The resulting oil was treated with 20 mL of sodium methoxide 0.1 M for 2 h at room temperature. Then the solution was neutralized with HOAc and evaporated. Chromatographic purification yielded 120 mg of a white product that was crystallized from EtOAc. A first crystalline fraction contained 57 mg of pure **16a**. The filtrate contained a 6/4 mixture (NMR) of mono and dibrominated product. Mp: 109 °C. UV (MeOH): λ_{max} = 252 nm (ϵ = 12 950), λ_{max} = 319 nm (ϵ = 11 800). ^{1}H NMR (DMSO-d_6): δ 2.18 (t, 2 H, H-2'), 3.61 (br s, 2 H, H-5'), 3.84 (m, 1 H, H-4'), 4.29 (m, 1 H, H-3'), 5.05 (t, 1 H, 5'-OH), 5.25 (d, 1 H, 3'-OH), 6.19 (t, 1 H, H-1'), 6.83 and 6.60 (2 × d, 2 × 1 H, J = 3.3 Hz, H-3'' and H-4''), 8.28 (s, 1 H, H-6), 11.61 (s, 1 H, NH) ppm. ^{13}C NMR (DMSO-d_6): δ 40.2 (C-2'), 61.1 (C-5'), 70.2 (C-3'), 85.0 (C-1'), 87.7 (C-4'), 104.6 (C-5), 110.4 and 113.4 (C-3'' and C-4''), 120.1 (C-5''), 134.2 (C-6), 148.9 and 149.2 (C-2'' and C-2), 159.8 (C-4) ppm. Anal. ($C_{13}H_{13}BrN_2O_6\cdot{}^1/_2H_2O$): C, H, N.

5-(5-Bromothien-2-yl)-1-(β-D-2-deoxyribofuranos-1-yl)uracil (16b). 5-Thien-2-yl-1-(β-D-2-deoxyribofuranos-1-yl)uracil (200 mg, 0.66 mmol) was dissolved in 10 mL of pyridine; 2 mL of acetic anhydride was added. The mixture was evaporated to dryness and coevaporated twice with toluene. TLC analysis ($CHCl_3$/MeOH, 90/10) showed that the transformation to 5-thien-2-yl-1-(3,5-di-*O*-acetyl-β-D-2-deoxyribofuranos-1-yl)uracil (**10**) was complete. The oil was taken up in 25 mL of $CHCl_3$. At 0 °C a dilution of 0.66 mmol Br_2 in 5 mL of CCl_4 was added dropwise (15 min). The red of bromine disappeared immediately, and TLC evaluation revealed that all of the **10** had been transformed into a more lipophilic product. The solution was extracted with water (2 × 25 mL), dried, and evaporated to an oil. This oil was taken up in 20 mL of MeOH saturated with ammonia and set aside for 16 h. Chromatographic purification yielded 210 mg (83%) of 5-(5-bromothien-2-yl)-1-(β-D-2-deoxyribofuranos-1-yl)uracil. The product was precipitated as an amorphous solid from acetone–heptane. UV (MeOH): λ_{max} = 273 nm (ϵ = 8500), λ_{max} = 327 nm (ϵ = 12 100); ^{1}H NMR (DMSO-d_6): δ 2.23 (t, 2 H, H-2'), 3.68 (br s, 2 H, H-5'), 3.84 (m, 1 H, H-4'), 4.33 (m, 1 H, H-3'), 5.29 (br s, 2 H, D_2O exchangeable, 3' and 5'-OH), 6.20 (t, 1 H, H-1'), 7.20 (m, 2 H, H-3'' and H-4''), 8.64 (s, 1 H, H-6), 11.77 (s, 1 H, NH) ppm. ^{13}C NMR (DMSO-d_6): δ 40.5 (C-2'), 60.7 (C-5'), 69.8 (C-3'), 85.0 (C-1'), 87.7 (C-4'), 107.5 (C-5'), 111.1 (C-5''), 122.1 and 129.2 (C-3'' and C-4''), 135.7 (C-6 and C-2''), 149.1 (C-2), 161.3 (C-4) ppm. Anal. ($C_{13}H_{13}BrN_2O_5S$) C, H, N.

5-(5-Chlorofuran-2-yl)-1-(β-D-2-deoxyribofuranos-1-yl)uracil (16c). A mixture of 5-furan-2-yl-1-(3,5-di-*O*-toluoyl-β-D-2-deoxyribofuranos-1-yl)uracil (250 mg, 0.48 mmol) *N*-chlorosuccimide (NCS) (65 mg, 0.48 mmol) in 10 mL of pyridine was heated for 1 h at 90 °C. TLC evaluation (CH_2Cl_2/CH_3CN, 9/1) revealed a 50% conversion of the starting material into a more lipophilic product. Another 65 mg of NCS was added; after 2 h all of the **6b** was transformed. The solution was cooled, evaporated, and taken up in 100 mL of $CHCl_3$. The solution was extracted with a saturated sodium bicarbonate solution (2 × 100 mL). The organic layer was dried and evaporated. The protective groups were removed by treatment at room temperature for 2 h with 20 mL of 0.1 M sodium methoxide in methanol. Column chromatographic purification yielded 130 mg (85%) of a yellow foam which was crystallized from EtOAc. Mp: 175 °C. UV (MeOH): λ_{max} = 252 nm (ϵ = 12 400), λ_{max} = 318 nm (ϵ = 11 500); ^{1}H NMR (DMSO-d_6): δ 2.18 (t, 2 H, H-2'), 3.61 (br s, 2 H, H-5'), 3.84 (m, 1 H, H-4'), 4.28 (m, 1 H, H-3'), 5.04 (t, 1 H, 5'-OH), 5.25 (d, 1 H, 3'-OH), 6.18 (t, 1 H, H-2'), 6.47 and 6.86 (2 × d, 2 × 1 H, J = 3.1 Hz, H-3'' and H-4''), 8.27 (s, 1 H, H-6), 11.65 (s, 1 H, NH) ppm. ^{13}C NMR (DMSO-d_6): δ 40.6 (C-2'), 61.2 (C-5'), 70.4 (C-3'), 85.2 (C-1'), 87.8 (C-4'), 104.8 (C-5), 108.7 and 110.4 (C-3'' and C-4''), 134.0 (C-5''), 135.5 (C-6), 146.9 (C-2''), 149.4 (C-2), 160.0 (C-4) ppm. Anal. ($C_{13}H_{13}N_2O_6Cl\cdot{}^1/_2H_2O$) C, H, N.

5-(5-Chlorothien-2-yl)-1-(β-D-2-deoxyribofuranos-1-yl)-uracil (16d). 5-Thien-2-yl-1-(2-β-D-deoxyribofuranos-1-yl)uracil (385 mg) was acetylated as described for **16b**. This foam was dissolved in 10 mL of pyridine; 160 mg (1.18 mmol) of NCS was added and the mixture was heated for 4 h at 70 °C. Chromatographic purification (CH_2Cl_2/CH_3CN, 87/13) yielded 190 mg of a foam. The acetyl groups were removed by treatment with 20 mL of methanol saturated with ammonia. After evaporation and purification 120 mg (28% yield) of **13b** was obtained. The product was crystallized from acetone. Mp: 220–222 °C. UV (MeOH): λ_{max} = 274 nm (ε = 8800), λ_{max} = 327 nm (ε = 11 500). ^{1}H NMR (DMSO-d_6): δ 2.23 (t, 2 H, H-2′), 3.68 (br s, 2 H, H-5′), 3.87 (m, 1 H, H-4′), 4.31 (m, 1 H, H-3′), 5.29 (br s, 2 H, 3′-OH and 5′-OH), 6.19 (t, 1 H, H-1′), 7.03 and 7.23 [2 × (d, 1 H, J = 3.95 Hz, H-3″ and H-4″)], 8.64 (s, 1 H, H-6), 11.76 (s, 1 H, NH) ppm. ^{13}C NMR (DMSO-d_6): δ 40.5 (C-2′), 60.7 (C-5′), 69.8 (C-3′), 85.1 (C-1′), 87.7 (C-4′), 107.5 (C-5), 121.1 and 125.7 (C-3″ and C-5″), 127.4 (C-5″), 133.0 (C-2″), 135.7 (C-6), 149.1 (C-2), 161.3 (C-4) ppm. Anal. ($C_{13}H_{13}ClN_2O_5S$): C, H, N.

Antiviral Activity. The different molecules were evaluated for their antiviral in vitro activity according to well-established procedures.[17,18] The origin of the viruses [herpes simplex type 1 (HSV-1) (strains KOS, F and McIntyre), thymidine kinase deficient (TK$^-$) HSV-1 strains (B2006 and VMW 1837), herpes simplex virus type 2 (HSV-2) (strains G, 196 and Lyons), varicella-zoster virus (VZV) (strains Oka and YS), TK$^-$ VZV (strains 07-1 and YS-R), vaccinia virus (VV), vesicular stomatitis virus (VSV), and cytomegalovirus (CMV) (strains AD169 and Davis)] has been described previously.[18,19] Cytotoxicity measurements were based on either microscopically examination of detectable alteration, normal cell morphology, or inhibition of cell growth. Two different lines of human fibroblasts [HEL (human embryonic lung) and E_6SM (human embryonic skin–muscle)] were used for both the antiviral activity and cytotoxicity assays. All assays were done in 96-well microtiter plates.

Acknowledgment. This study was supported in part by the AIDS Basic Research Programme of the European Community and by grants from the Belgian Fonds voor Geneeskundig Wetenschappelijk Onderzoek (Projects no 3.0040.83 and 3.0097.97) and the Belgian Geconcerteerde Onderzoeksacties (Project no 85/90-79). Robert Snoeck is a senior research assistant from the Belgian National Fund for Scientific Research. We thank Dominique Brabants for her dedicated editorial assistance and Anita Van Lierde, Frieda De Meyer, and Anita Camps for their efficient technical assistance.

Registry No. **3a**, 133040-36-5; **3b**, 134333-64-5; **4a**, 31356-86-2; **4b**, 54-42-2; **5** (X = O), 51583-40-5; **5** (X = S), 37496-13-2; **6c**, 92233-50-6; **6d**, 89647-10-9; **7**, 84500-33-4; **8**, 54663-78-4; **8** (X = O), 118486-94-5; **9b**, 134333-65-6; **10**, 133787-21-0; **11**, 134333-66-7; **12b**, 134333-67-8; **13**, 4330-21-6; α-**14a**, 104267-95-0; β-**14a**, 31356-86-2; **14b**, 60110-67-0; α-**15a**, 127235-39-6; β-**15a**, 127235-40-9; α-**15b**, 134333-68-9; α-**15c**, 127235-62-5; α-**15d**, 127235-63-6; β-**15d** diacetyl derivative, 133787-21-0; **16a**, 134333-69-0; **16b**, 134333-70-3; **16c**, 134333-71-4; **16d**, 134333-72-5; *N*-methylimidazole, 616-47-7; 5-iodouracil, 696-07-1; 1,2,4-triazole, 288-88-0; thiazole, 288-47-1; 5-iodo-bis-*O*-(trimethylsilyl)uracil, 38953-72-9.

(19) De Clercq, E.; Descamps, J.; Verhelst, G.; Walker, R. T.; Jones, A. S.; Torrence, P. F.; Shugar, D. *J. Infect. Dis.* **1980**, *141* (5), 563–574.

Synthesis and Binding of [$^{125}I_2$]Philanthotoxin-343, [$^{125}I_2$]Philanthotoxin-343-lysine, and [$^{125}I_2$]Philanthotoxin-343-arginine to Rat Brain Membranes

R. A. Goodnow, Jr.,[†] R. Bukownik,[†] K. Nakanishi,*[,†] P. N. R. Usherwood,[§] A. T. Eldefrawi,[‡] N. A. Anis,[‡] and M. E. Eldefrawi*[,‡]

Department of Chemistry, Columbia University, New York, New York 10027, Department of Pharmacology and Experimental Therapeutics, School of Medicine, University of Maryland, Baltimore, Maryland 21201, and University of Nottingham, Nottingham NG7 2RD, U.K. *Received September 28, 1990*

$^{125}I_2$-iodinated philanthotoxin-343 (PhTX-343) (**10**), [$^{125}I_2$]PhTX-343-arginine (**11**), and [$^{125}I_2$]PhTX-343-lysine (**12**) were synthesized and evaluated as probes for glutamate receptors in rat brain synaptic membranes. It was found that these probes were not specific for the glutamate receptors but may be useful for investigating the polyamine binding site. Filtration assays with Whatman GF/B fiber glass filters were unsuitable because the iodinated PhTX-343 analogues exhibited high nonspecific binding to the filters, thus hindering detection of specific binding to membranes. When binding was measured by a centrifugal assay, [$^{125}I_2$]PhTX-343-lysine (**12**) bound with low affinity (K_D = 11.4 ± 2 μM) to a large number of sites (37.2 ± 9.1 nmol/mg of protein). The binding of [$^{125}I_2$]PhTX-343-lysine was sensitive only to the polyamines spermine and spermidine, which displaced [$^{125}I_2$]PhTX-343-lysine (**12**) with K_i values of (3.77 ± 1.4) × 10^{-5} M and (7.51 ± 0.77) × 10^{-5} M, respectively. The binding was insensitive to glutamate receptor agonists and antagonists. Binding results with [$^{125}I_2$]PhTX-343-arginine (**11**) were similar to those of [$^{125}I_2$]-PhTX-343-lysine. Considering the high number of toxin binding sites (10000-fold more than glutamate) in these membranes and the insensitivity of the binding to almost all drugs that bind to glutamate receptors, it is evident that most of the binding observed is not to glutamate receptors. On the other hand, PhTX analogues with photoaffinity labels may be useful in the isolation/purification of various glutamate and nicotinic acetylcholine receptors; they could also be useful in structural studies of receptors and their binding sites.

Introduction

Glutamate receptor pharmacology has attracted a great deal of attention in the last few years[1–4] because of the possible involvement of glutamate receptors in degenerative brain diseases,[5] mechanism of memory,[6] and ischemic damage.[7] Glutamate receptors are classified into three types according to their sensitivity to the exogenous excitatory amino acids quisqualate, kainate, and *N*-methyl-D-aspartate (NMDA). A subunit of a kainate type

[†] Columbia University.
[§] University of Nottingham.
[‡] University of Maryland.

(1) Robinson, M. B.; Coyle, J. T. *Fed. Am. Soc. Exp. Biol.* **1987**, *1*, 446.
(2) Collingridge, G. L.; Lester, R. A. L. *Pharmacol. Rev.* **1989**, *41*, 143.
(3) Lodge, D. *Trends Pharmacol. Sci.* **1987**, *8*, 243.
(4) Monaghan, D. T.; Bridges, R. J.; Cotman, C. W. *Annu. Rev. Pharmacol. Toxicol.* **1989**, *29*, 365.
(5) Greenamyre, J. T.; Penny, J. B.; Young, A. B.; D'Amato, C. J.; Hicks, S. P.; Shoulson, I. *Science* **1985**, *227*, 1496.

0022-2623/91/1834-2389$02.50/0