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Prodrugs of 5-Ethyl-2′-deoxyuridine, II[1)]

Syntheses and Antiviral Activities of 5′- and 3′-Ester Derivatives[+++)]

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With the aim of obtaining derivatives of the well established antiherpes compound 5-ethyl-2′-deoxyuridine **(1)** (Aedurid®, EtUdR, EDU), which are more lipophilic and therapeutically superior, 5′- and 3′-ester derivatives of **1** were synthesized. Tested in primary rabbit kidney cell cultures against various strains of herpes simplex type 1 (HSV-1) and type 2 (HSV-2), all EtUdR esters, with the exception of compounds **8** and **13**, proved almost as active as EtUdR itself, suggesting that they were readily hydrolized.

[+++)] Dedicated to Apotheker *Ernst Mauz*, managing director of Robugen GmbH, on the occasion of his 85th birthday

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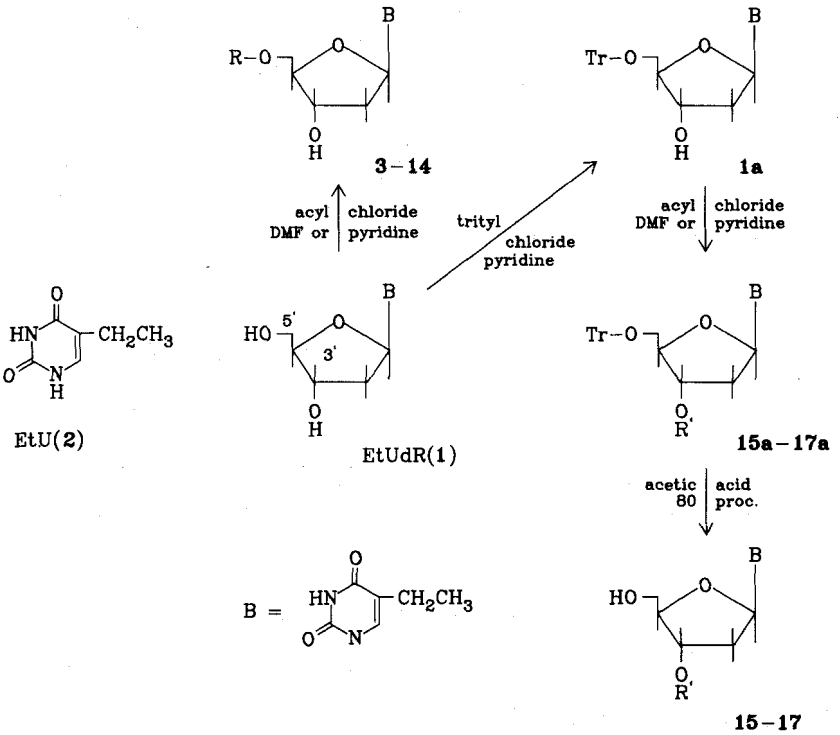
Prodrugs von 5-Ethyl-2'-desoxyuridin, 2. Mitt.:[1] Synthesen und antivirale Aktivitäten von 5'- und 3'-Ester-Derivaten

Mit dem Ziel, lipophilere und therapeutisch überlegene Derivate des Virustatikums 5-Ethyl-2'-desoxyuridin **(1)** (Aedurid®, EtUdR, EDU) zu erhalten, wurde eine Reihe von 5'- und 3'-Ester-Derivaten von **1** synthetisiert. Bei Testung in primären Kaninchennieren-Zellkulturen gegen verschiedene Stämme von Herpes Simplex Typ 1 (HSV-1) und Typ 2 (HSV-2) erwiesen sich alle EtUdR-Ester, mit Ausnahme der Substanzen **8** und **13**, als fast so aktiv wie EtUdR. Dies legt die Vermutung nahe, daß sie leicht hydrolysiert werden.

Optimization of drug delivery and, consequently, drug efficacy implies an efficient and selective delivery and transport of the drug to its site of action[2]. Especially in the treatment of herpes simplex encephalitis, it is necessary to administer compounds which are able to pass the blood brain barrier. 5-Ethyl-2'-deoxyuridine**(1) has marked activity against HSV-1 and HSV-2 and vaccinia virus in cell cultures as well as in man[3-7]. With a view to a systemic treatment of herpes virus infections, the metabolic fate of **1** has been well investigated[8-12]. Upon intravenous administration of **1** to rats infected with pseudorabies virus, a non-virostatic metabolite of **1**, namely 5-ethyluracil (EtU) **(2)**, is formed rather rapidly, as monitored by high performance liquid chromatography (HPLC)[8,10]. On the other hand, compound **1** is highly stable in aqueous solution[9]. In order to prevent the rapid degradation of EtUdR *in vivo* which may occur via enzymatic cleavage of the N-glycoside bond, a series of 5'- and 3'-esters has been prepared (Table 1)[13-15].



**) Available as Aedurid®-Gel 0,3 % and Aedurid® forte Gel 1,2 %

Chemistry

Synthesis of the 5′-esters starts with 5-ethyl-2′-deoxyuridine **(1)** using an acid chloride, dissolved in either pyridine or DMF, to give the corresponding 5′-esters **3–14**. Extraction with dichloromethane yields the crystalline 5′-esters (Scheme 1). One recrystallization is sufficient to obtain the pure esters in yields of 18–86 %. It was found that furanoyl chloride, adamantoyl chloride, the di-chloro-substituted benzoyl chlorides, and pivaloyl chloride formed complexes with pyridine which were insoluble in that solvent. In these cases, use of DMF as solvent led to the 5′-esters in good yields (Table 1). Compound **1** and the acyl chloride were used in a molecular ratio of 1:1 as described recently[1]. In the case of the 3′-esters **15–17**, synthesis started with the intermediate 5′-*O*-trityl-5-ethyl-2′-deoxyuridine **(1a)**[17,18] (Scheme 1) to give the crude compounds **15a–17a**, which were used for the next step without further purification. Subsequent detritylation was done as described previously by *Šmrt* et al[19]. The crude 3′-esters were purified by column chromatography in yields of 21–54 %. The purity of all compounds was checked on silica gel plates $60F_{254}$.

Table 1: *5′- and 3′-Ester Derivatives of 5-Ethyl-2′-deoxyuridine*

Compd.-Nr.	Name m.p. °C yield (%)[a]	Formula (MW) λ_{max}, log ϵ	log P[b] sol. H_2O[c] (mg/ml)	Analyses Calculated: Found: C	H	N	Cl
3	5′-*O*-Butyryl-(1) 171–172,5 32,2	$C_{15}H_{22}N_2O_6$ (326,4) 268, 3.97	0.89 0,80	55,2 54,9	6,79 6,65	8,6 8,5	
4	5′-*O*-Isovaleryl-(1) 165 85,9	$C_{15}H_{22}N_2O_6$ (326,4) 268, 3.95	0.74 1,20	55,2 55,0	6,79 6,72	8,6 8,4	
5	5′-*O*-Valeryl-(1) 161–163 49,3	$C_{16}H_{24}N_2O_6$ (340,4) 267, 3.95	1.65 0,37	56,5 56,2	7,10 7,00	8,2 8,0	
6	5′-*O*-Isovaleryl-(1) 161–163 50,0	$C_{16}H_{24}N_2O_6$ (340,4) 267, 3.95	1.72 0,56	56,5 56,1	7,10 7,05	8,2 8,1	
7	5′-*O*-Pivaloyl-(1) 185–187 65,0	$C_{16}H_{24}N_2O_6$ (340,4) 267, 3.99	1.27 0,79	56,5 56,4	7,10 6,95	8,2 8,0	
8	5′-*O*-Lauryl-(1) 159–161,5 45,6	$C_{23}H_{38}N_2O_6$ (438,6) 267, 4.04[d]	2.51 0,01	62,9 62,6	8,73 8,69	6,4 6,2	

Forts. **Tab. 1:**

Compd.-Nr.	Name m.p. °C yield (%)[a]	Formula (MW) λ_{max}, log ϵ	log P[b] sol. H_2O[c] (mg/ml)	Analyses Calculated: Found: C	H	N	Cl
9	5'-*O*-Furanoyl-(1) 182–183 34,3	$C_{16}H_{18}N_2O_7$ (350,3) 258, 4.29	0.06 1,23	54,8 54,5	5,18 5,15	8,0 8,0	
10	5'-*O*-Adamantoyl-(1) 169–171,5 61,0	$C_{22}H_{30}N_2O_6$ (418,5) 270, 4.28	2.69 0,04	63,1 63,0	7,23 7,14	6,7 6,8	
11	5'-*O*-(2,4-Dichlorobenzoyl)-(1) 185 (decomp.) 46,6	$C_{18}H_{18}N_2O_6Cl_2$ (429,3) 245, 4.11[d]	2.41 0,01	50,4 50,0	4,23 4,20	6,5 6,3	16,5 16,2
12	5'-*O*-(3,4-Dichlorobenzoyl)-(1) 182–184 25,6	$C_{18}H_{18}N_2O_6Cl_2$ (429,3) 245, 4.22[d]	2.42 0,01	50,4 50,1	4,23 4,19	6,5 6,2	16,5 16,3
13	5'-*O*-(2,6-Dichlorobenzoyl)-(1) 240–241 18,6	$C_{18}H_{18}N_2O_6Cl_2$ (429,3) 267, 3.96[d]	2.15 0,01	50,4 50,1	4,23 4,21	6,5 6,2	16,5 16,3
14	5'-*O*-(p-Chlorophenoxyacetyl)-(1) 172–173 54,1	$C_{19}H_{21}N_2O_7Cl$ (424,8) 270, 4.15[d]	2.32 0,01	53,7 53,3	4,98 4,80	6,6 6,3	8,3 8,1
15	3'-*O*-Pivaloyl-(1) 64–66 54,7	$C_{16}H_{24}N_2O_6$ (340,4) 268, 3.68	1.81 3,27	56,5 56,3	7,10 7,00	8,2 8,1	
16	3'-*O*-Adamantoyl-(1) 134–138 21,5	$C_{22}H_{30}N_2O_6$ (418,4) 268, 4.20[d]	2.50 0,01	63,1 62,9	7,23 7,15	6,7 6,5	
17	3'-*O*-Acetyl-(1) 149–152 48,9	$C_{13}H_{18}N_2O_6$ (298,3) 265, 3.96	0.96 23,44	52,3 52,0	6,08 5,95	9,4 9,2	

[a] Yields are based on pure components, and are derived from single experiments.

[b] For methodology see exp. part, log P (EtUdR) = −0.35, solubility = 70,0 mg/ml.

[c] For methodology see[1].

[d] λmax was determined in n-octanol.

Physical Properties

As expected, the aqueous solubility of the esters (Table 1) showed a dramatic decrease as compared to that of **1**, with exception of compound **17**. The melting points increased by ca. 10 °C **(8)** to 90 °C **(13)** as compared to that of **1** (m. p. 152–153 °C). Only **15** showed a decrease of the m. p. by 90 °C. The lipophilicities measured *via* n-octanol/water distribution showed increases commensurate with increasing chain length **(3, 5** and **8)**. Comparison of the 5′-*O*-pivaloylester **7** with the 3′-*O*-pivaloylester **15** showed an increased solubility of the latter.

Biological Results

Except for the two compounds **8** and **13** all 5′- and 3′-esters of **1** inhibited the cytopathic effect of HSV-1 and HSV-2 at a concentration which was similar to, or only slightly higher than that of **1** itself (Table 2). It is conceivable, therefore, that under our *in vitro* assay conditions, the 5′- and 3′-esters of EtUdR were readily hydrolized.

Not only viral cytopathogenicity but also virus yield was reduced to a significant extent by the esters, as shown in Table 3, suggesting that their inhibitory effects on virus-induced cytopathogenicity reflected an inhibition of virus replication.

Table 2: *Antiviral Activity of 5′- and 3′-Esters of 5-Ethyl-2′-deoxyuridine in PRK Cell Cultures*

Compd.-	Minimum inhibitory concentration (MIC) (μg/ml)[a]				
	HSV-1[b]		HSV-2[c]		Vaccinia virus
1	0.33	(0.1–0.7)	0.41	(0.1–0.7)	2.7
3	0.22	(0.07–0.4)	0.27	(0.2–0.4)	2
4	0.17	(0.1–0.2)	0.2	(0.1–0.4)	2
5	1.7	(1–2)	1	(0.4–2)	40
6	3.3	(2–4)	2.7	(2–4)	20
7	1.1	(0.4–2)	0.43	(0.2–0.7)	2
8	20		27	(20–40)	70
9	0.6	(0.4–0.7)	0,37	(0.2–0.7)	20
10	2		0.43	(0.2–0.7)	20
11	0.8	(0.7–1)	0.8	(0.7–1)	2
12	2		2		2
13	⩾ 33	(20–40)	>40		> 40
14	1.6	(0.7–2)	0.6	(0.4–1)	10
15	1.2	(0.7–2)	3.6	(2–7)	7
16	0.8	(0.4–1)	1		2
17	2		2.6	(2–4)	2

[a] Required to reduce virus-induced cytopathogenicity by 50 %.

[b] Average values for three HSV-1 strains (KOS, F and Mc Intyre).

[c] Average values for three HSV-2 strains (Lyons, G and 196). The range of individual values is indicated in parentheses.

Table 3: *Antimetabolic Activity and Inhibitory Effects of 5'- and 3'-Esters of 5-Ethyl-2'-deoxyuridine on the Multiplication of HSV-1 (Strain KOS) in PRK Cell Culture*

Compd.-Nr.	Inhibitory dose-50(ID_{50}) (μg/ml)[a]		virus yield (log_{10}PFU/ml)[b]
	(^{3}H-methyl)dThd incorporation	(^{3}H-1',2')dUrd incorporation	
Control			7.3
1	46	4.6	3.8
3	52	5	3.8
4	19	4	3.9
5	40	7	4.1
6	27	27	4.2
7	9	7	3.9
8	ND	ND	ND[d]
9	16	9	4.1
10	6	3	3.9
11	35	24	4.2
12	20	10	4.1
13	ND	ND	ND[d]
14	92	9	3.8
15	87	43	4.0
16	17	13	4.1
17	41	13[c]	4.1

[a] Required to inhibit (^{3}H-methyl)dThd or (^{3}H-1', 2')dUrd incorporation by 50%. Input of the radiolabelled precursor (per 10^5 PRK cells): 10 pmoles (0.38 μCi) of (^{3}H-methyl)dThd and 6 pmoles (0.25 μCi) of (^{3}H-1', 2')dUrd. Average values for three separate determinations.

[b] The cell cultures were incubated with HSV-1 (Strain KOS) at $10^{4.5}$ PFU per petri dish (10^6 cells). The compounds were added at a conc. of 10 μg/ml (which is well above their MIC for virus-induced cytopathogenicity: see Table 2). Virus yield was determined at 24 h after virus infection by plaque formation in Vero cell cultures. Average values for two separate experiments.

[c] (^{3}H-6)dUrd incorporation instead of (^{3}H-1', 2')dUrd incorporation.

[d] Not determined since MIC for virus-induced cytopathogenicity was > 10 μg/ml (see Table 2).

ND: Not determined

The compounds were also evaluated for their cytotoxic properties as monitored by inhibition of 2'-deoxythymidine (dThd) and 2'-deoxyuridine (dUrd) incorporation into host cell DNA. The esters inhibited dThd and dUrd incorporation at a concentration which was comparable to that of **1** (Table 3). In no case was a marked (more than 10-fold) increase in ID_{50} for dThd for dUrd incorporation achieved, which implies that none of the compounds **3–17** lost a significant part of cytotoxic potential as compared to **1**.

Experimental Part

General data see[1]. Column chromatography: Silica gel 60 (70–230 mesh ASTM) (E. Merck).

General Procedure for Acylation

Compounds **3–14** were synthesized as described previously by *Keppeler* et al[1].

Compounds **15–17** were synthesized as follows: To a solution of 4,98 g (0,01 mol) 5'-*O*-trityl-5-ethyl-2'-deoxyuridine[17,18] in 10 ml dry pyridine, a mixture of the appropriate acyl chloride (0,01 mol) dissolved in 10 ml DMF was added dropwise with stirring. The reaction mixture was stirred for 48 h, and finally poured slowly into a mixture of 100 ml water and 100 ml dichloromethane. The aqueous layer was extracted with 100 ml dichloromethane, and the combined organic layers were washed with 50 ml 3 N-H_2SO_4, 50 ml water, 50 ml saturated aqueous $NaHCO_3$, and again 50 ml water. The dichloromethane layer was dried over Na_2SO_4, filtered and evaporated to dryness to yield the crude compounds **15a–17a**. To the residue 50 ml 80 proc. acetic acid were added, and the mixture was gently refluxed for 10 min. After cooling, the solution was concentrated and dissolved in 5 ml chloroform. The solution was applied to a silica gel column (100 g) and eluted with 500 ml chloroform, 500 ml chloroform ether (8:2) and finally with 500 ml chloroform/ether (7:3). Appropriate fractions were collected and evaporated under reduced pressure to yield compounds **15–17**.

Determination of Partition Coefficients

A suspension of each compound in 10 ml distilled water was shaken with 10 ml of n-octanol in a 125-ml separatory funnel for 1 h. After filtration, layers were separated, the concentration was determined by UV.

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