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Synthesis and Evaluation of Antiviral Activity of 2′-Deoxyuridines with 5-Methylene-2-thiohydantoin Substituents in the 5-Position

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Summary. 1-(2-Deoxy-3,5-bis-*O*-(4-methylbenzoyl)-*D-erythro*-pentofuranosyl)-5-formyluracil (**4**) was synthesized from 5-formyluracil and an appropriate methyl glycoside and condensed with 2-thiohydantoin (**5a**) and its corresponding 3-phenyl derivative **5b** to give 5-[1-(2-deoxy-3,5-bis-*O*-(4-methylbenzoyl)-*D-erythro*-pentofuranosyl)uracil-5-ylmethylene]-2-thiohydantoins **7a** and **7b**, respectively, in 65–70% yield. They were deprotected with sodium methoxide in methanol to give both anomers of the free nucleosides. In a different route 5-formyluracil (**1**) was condensed with **5b** and subsequently with an appropriate methyl glycoside to give **7b**.

Keywords. 2′-Deoxyuridines; 2-Thiohydantoins; Nucleoside synthesis; Antiviral activity.

Synthese und Testung der antiviralen Aktivität von 2′-Deoxyuridinen mit 5-Methylen-2-thiohydantoin-Substituenden in der 5-Position

Zusammenfassung. 1-(2-Deoxy-3,5-bis-*O*-(4-methylbenzoyl)-*D-erythro*-pentofuranosyl)-5-formyluracil (**4**) wurde aus 5-Formylurazil und dem entsprechenden Methylglycosid synthetisiert und mit 2-Thiohydantoin (**5a**) und seinem 3-Phenylderivat **5b** kondensiert, wobei die 5-[1-(2-Deoxy-3,5-bis-*O*-(4-methylbenzoyl)-*D-erythro*-pentofuranosyl)uracil-5-ylmethylene]-2-thiohydantoine **7a** bzw. **7b** in 65–70% Ausbeute erhalten wurden. Nach Entfernung der Schutzgruppen mit Natriummethoxid in Methanol erhielt man beide Anomeren der freien Nucleoside. Auf einem anderen Weg wurde 5-Formyluracil (**1**) mit **5b** und anschließend mit einem entsprechenden Methylglycosid zu **7b** kondensiert.

Introduction

In a recent publication Herdewijn and coworkers investigated the antiviral activity of 2′-deoxyuridines with a five-membered heterocyclic substituent in the 5-position [1]. The most important finding was that 5-(5-bromothien-2-yl)-2′-deoxyuridine

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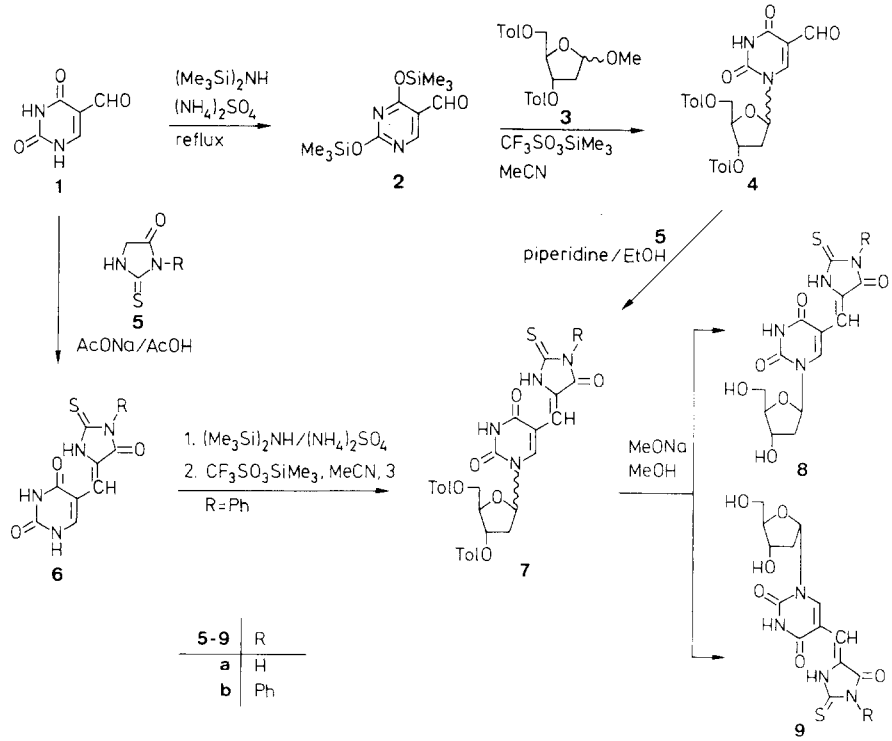
and 5-(5-chlorothien-2-yl)-2′-deoxyuridine were equipotent to (*E*)-5-(2-bromovinyl)-2′-deoxyuridine in the inhibition of herpes simplex type 1 replication. For the time being we are investigating different aspects of hydantoins in nucleoside chemistry and we were therefore prompted to investigate the antiviral activity of 2′-deoxyuridines with 5-methylene-2-thiohydantoins as the heterocyclic substituent in the 5-position. With an attachment of the pyrimidine ring to the methylene group of such a hydantoin moiety it would also be possible to fulfil some of the requirements discovered in a structure-activity relationship study of some 30 5-substituted 2′-deoxyanalogues [2]: the substituent should be unsaturated, in conjugation with the pyrimidine base, and include an electronegative hydrophobic function.

Results and Discussion

5-Formyluracil (**1**) was prepared in 2 steps from uracil in 30% overall yield according to reported procedures [3, 4]. Methyl 2-deoxy-3,5-di-*O*-(4-methylbenzoyl)-*D*-*erythro*-pentofuranoside (**3**) was prepared in 2 steps from 2-deoxy-*D*-ribose in 70% overall yield by treatment with hydrogen chloride in methanol followed by toluoylation [5, 6]. The 5-formyl nucleoside **4** has previously been prepared [7] in 29% yield ($\alpha/\beta = 1:1$) by condensation of 2-deoxy-3,5-di-*O*-(4-methylbenzoyl)-α-*D*-*erythro*-pentofuranosyl chloride with 5-formyluracil in chloroform. In this investigation the nucleoside **4** was synthesized in 63% yield ($\alpha/\beta = 1:2$) by condensing the sugar **3** with the silylated 5-formyluracil **2** in the presence of the Lewis acid trimethylsilyl trifluoromethanesulfonate (*TMS* triflate) [8].



5-Formyluracil (**1**) was condensed with 2-thiohydantoins **5** by refluxing in a solution of sodium acetate and acetic acid to give **6a, b** in 74–78% yields. Compound **6b** was silylated by refluxing in 1,1,1,3,3,3-hexamethyldisilazane (*HMDS*) using ammonium sulphate as the catalyst [9] and the silylated compound was used without further purification for the coupling with the sugar **3**. This coupling was performed in anhydrous acetonitrile in the presence of *TMS* triflate to afford an anomeric mixture of the corresponding 2′-deoxy nucleoside **7b** after purification by column chromatography on silica gel. The glycosylation virtually took place on pyrimidine and not on the hydantoin ring which was proven by synthesizing the same nucleoside by an alternative route as follows. Compound **4** was condensed with 2-thiohydantoins **5a** and **5b** using piperidine as a catalyst to give the corresponding nucleosides **7a** and **7b**, respectively. Treatment of **7a, b** with sodium methoxide in methanol afforded deprotection of the 3′-OH and 5′-OH groups to give the β nucleosides **8a, b** in 11–25% yield and the corresponding α anomers **9a, b** in 9–18% yield after separation by reversed phase chromatography.

The assignment of the anomeric configuration was made by ^{1}H-NMR spectra: the anomeric protons of the α-anomers were observed further downfield than those of the corresponding β-anomers. Furthermore, the H-4′ proton of the α-anomers appears downfield from that observed for the β-anomers, and the H-5′ protons of the α-anomers appear upfield from those observed for the β-anomers [10, 11] (see Experimental Part).

The compounds **6a, b**, **7a, b**, **8a, b** and **9b** did not show any significant activity at 100 μM against Herpes Simplex Virus, type 1 (HSV-1), strain McIntyre, when tested in a continuous cell line from rabbit cornea (SIRC) which was maintained in Eagle's MEM containing 1% fetal calf serum (FCS) and the test compound. The same compounds were also devoid af any activity against HIV-1 (strain HTLV-IIIB) in MT-4 cells. MT-4 cells were incubated with virus, washed and added in a proportion of 1:10 to uninfected MT-4 cells which had been preincubated in test compound (100 μM) containing culture medium (RPM 1640 containing 10% FCS) for 2 h. The MT-4 cells were maintained in culture medium likewise containing the test compound (100 μM). Expression of HIV in culture medium was quantitated by HIV antigen detection ELISA. Only the nucleobase **6b** and the α anomer **9b** showed cytotoxicity against MT-4 cells at 100 μM. At 10 μM these compounds did not show any significant activity against HIV-1.

Experimental Part

1-[2-Deoxy-3,5-bis-O-(4-methylbenzoyl)-D-erythro-pentofuranosyl]-5-formyluracil
[**4**; $C_{26}H_{24}N_2O_8 \cdot 0.25\,H_2O$]

A mixture of 5-formyluracil **1** (1.4 g, 10 mmol), anhydrous $(NH_4)_2SO_4$ (125 mg, 0.05 mmol) and 1,1,1,3,3,3-hexamethyldisilazane (75 ml) was refluxed overnight. The solvent was removed under reduced pressure and the resulting oily residue of compound **2** was then dissolved in anhydrous MeCN (30 ml), cooled to $-50\,^\circ$C. A solution of the sugar **3** (2.6 g, 6.7 mmol) in anhydrous MeCN (20 ml) was added and trimethylsilyl trifluoromethanesulfonate (1.5 ml, 7.8 mmol) in anhydrous MeCN (10 ml) was added dropwise to the mixture. The mixture was stirred at $-50\,^\circ$C for 2 h and then overnight at $-10\,^\circ$C. The temperature was allowed to raise to room temperature and stirring was continued for 4 h. The mixture was diluted with CH_2Cl_2 (300 ml), washed with cold saturated aqueous $NaHCO_3$ (300 ml), cold water (2 × 150 ml) and dried over Na_2SO_4. The solvent was removed in vacuo and the residue

chromatographed on silica gel with a gradient from 0–2% MeOH in $CHCl_3$ to obtain the anomeric mixture **4** as a white foam ($\alpha/\beta = 1:2$); yield 2.1 g (63%). MS, *m/z* 492 (M^+, 0.35).

5-(Uracil-5-ylmethylene)-2-thiohydantoins **6.** *General Procedure*

A mixture of 5-formyluracil **1** (0.50 g, 3.6 mmol), anhydrous sodium acetat (1 g, 12.2 mmol) and the appropriate 2-thiohydantoin **5a, b** (3.5 mmol) in glacial acetic acid (20 ml) was refluxed for 4 h until the starting material was consumed (TLC). The reaction mixture was poured into cold H_2O. The yellow solid obtained was filtered off and recrystallized from acetic acid to give the products **6a, b.**

5-(Uracil-5-ylmethylene)-2-thiohydantoin [**6a**; $C_8H_6N_4O_3S$ (*HRMS*)]

Yield 0.65 g (78%). M.p. >320 °C. ^{1}H-NMR (*DMSO-d*$_6$) δ 6.30 (1H, s, =CH), 8.16 (1 H, s, H-6), 11.74 (4H, s, 4 NH). ^{13}C-NMR (*DMSO-d*$_6$) δ 105.52 (C-5), 106.61 (=CH), 125.58 (C-5 hydantoin), 146.83 (C-2), 150.28 (C-6), 163.81 (C-4), 165.19 (C-4 hydantoin), 175.89 (C=S). MS, *m/z* 238 (M^+, 78).

5-(Uracil-5-ylmethylene)-3-phenyl-2-thiohydantoin [**6b**; $C_{14}H_{10}N_4O_3S$]

Yield 0.81 g (74%). M.p. >320 °C. ^{1} H-NMR (*DMSO-d*$_6$) δ 6.46 (1H, s, =CH), 7.34–7.52 (5H, m, Phenyl), 8.32 (1H, s, H-6), 9.0–10.5 (2H, broad s, 2 NH). ^{13}C-NMR (*DMSO-d*$_6$) δ 106.72 (C-5), 106.80 (=CH), 125.37 (C-5 hydantoin), 128.35, 128.60 133.17, 133.64, (Phenyl), 148.30 (C-2), 150.94 (C-6), 163.68 (C-4), 163.89 (C-4 hydantoin), 175.25 (C=S); MS, *m/z* 314 (M^+, 100).

5-[1-(2-Deoxy-3,5-bis-O-(4-methylbenzoyl)-D-erythro-pentofuranosyl)uracil-5-ylmethylene]-2-thiohydantoins **7**

Method A: To a mixture of the appropriate 2-thiohydantoin **5a, b** (2 mmol), piperidine (2 mmol) and absolute ethanol (30 ml) was added **4** (1.0 g, 2 mmol). The reaction mixture was stirred overnight at r.t. The solvent was removed in vacuo and the residue chromatographed on a silica gel column with a gradient from 0–2% MeOH in $CHCl_3$ to obtain the anomeric mixture of compounds **7a, b** in 65–70% yield as yellow foams.

Method B: A mixture of **6b** (1 g, 3.2 mmol), anhydrous $(NH_4)_2SO_4$ (50 mg, 0.02 mmol) and 1,1,1,3,3,3-hexamethyldisilazane (30 ml) was refluxed overnight. The solvent was removed under reduced pressure and the resulting oily residue was dissolved in anhydrous MeCN (15 ml) and cooled to −50 °C. The sugar **3** (1.0 g, 2.6 mmol) in anhydrous MeCN (10 ml) was added. Trimethylsilyl trifluoromethanesulfonate (0.77 ml, 4 mmol) in anhydrous MeCN (5 ml) was added dropwise to the mixture. The mixture was stirred at −50 °C for 2 h and overnight at −10 °C. The temperature was allowed to raise to r.t. and stirring was continued overnight. The mixture was diluted with CH_2Cl_2 (200 ml), washed with cold sat. aqueous $NaHCO_3$ (200 ml), cold H_2O (2 × 100 ml) and dried over Na_2SO_4. The solvent was removed in vacuo and the residue chromatographed on a silica gel column with a gradient from 0–2% MeOH in $CHCl_3$ to give the anomeric mixture of compound **7b** as a yellow foarm ($\alpha/\beta = 1:4$); yield 0.8 g (46%).

5-[1-(2-Deoxy-β-D-erythro-pentofuranosyl)uracil-5-ylmethylene]-2-thiohydantoin [**8a**; $C_{13}H_{14}N_4O_6 \cdot H_2O$] *and 5-[1-(2-Deoxy-α-D-erythro-pentofuranosyl)uracil-5-ylmethylene]-2-thiohydantoin* [**9a**; $C_{13}H_{14}N_4O_6S \cdot 1.5\,H_2O$]

A suspension of the protected nucleoside **7a** (0.5 g, 0.85 mmol), absolute MeOH (30 ml) and NaOMe (1.7 mmol) was stirred overnight. The reaction mixture was concentrated to dryness in vacuo. H_2O (15 ml) was added to the residue and extracted several times with CH_2Cl_2 to remove the ester formed

during the deprotection reaction. The resulting solution was treated with ion exchange resin (Dowex 50 W × 2, H^+-form), previously washed with MeOH and stirred for 5 min. The solution was filtered from the resin, the filtrate evaporated in vacuo, and the residue choromatographed on a silica gel column with a gradient from 5–10% MeOH in $CHCl_3$ to give a yellow solid. The solid was further separated on HPLC with 25% EtOH in water on a reversed phase column (RP-18, 15 µm, 200 A) to give **8a** and **9a**.

8a: Yield 75 mg (25%). M.p. > 300 °C dec. ^{1}H-NMR (*DMSO-d_6*) δ 2.20 (2H, m, H-2'), 3.61 (1H, dd, J = 3.1, 12.6 Hz, H-5'), 3.73 (1H, dd, J = 3.3, 12.1 Hz, H-5'), 3.85 (1H, m, H-4'), 4.28 (1H, d, J = 3.2 Hz, H-3'), 4.80 (1H, s, 5'-OH), 5.28 (1H, s, 3'-OH), 6.12 (1H, t, J = 6.4 Hz, H-1'), 6.19 (1H, s, =CH), 8.59 (1H, s, H-6), 10.60–11.03 (3H, broad s, 3 NH). ^{13}C-NMR (*DMSO-d_6*) δ 38.77 (C-2') 60.89 (C-5'), 69.99 (C-3'), 85.51 (C-1'), 87.93 (C-4'), 104.48 (C-5), 108.22 (=CH), 128.80 (C-5 hydantoin), 144.39 (C-2), 149.10 (C-6), 162.95 (C-4), 166.01 (C-4 hydantoin), 176.38 (C=S); FAB MS (*DMSO* + 1% CH_3COOH in 3-nitrobenzylalcohol), *m/z* 355 ($M + H^+$).

9a: Yield 55 mg (18%). M.p. > 300 °C dec. ^{1}H-NMR (*DMSO-d_6*) δ 2.05 (1H, m, H-2'), 2.53 (1H, m, H-2'), 3.46 (2H, m, H-5') 4.22 (1H, m, H-3'), 4.34 (1H, m, H-4'), 6.01 (1H, m, H-1'), 6.15 (1H, s, =CH), 8.66 (1H, s, H-6). ^{13}C-NMR (*DMSO-d_6*) δ 40.31 (C-2'), 61.41 (C-5'), 70.16 (C-3'), 87.12 (C-1'), 89.19 (C-4'), 105.47 (C-5), 107.88 (=CH), 128.15 (C-5 hydantoin), 144.49 (C-2), 149.73 (C-6), 163.90 (C-4), 167.66 (C-4 hydantoin), 177.30 (C=S); FAB MS (*DMSO* + 1% CH_3COOH in 3-nitrobenzylalcohol), *m/z* 355 ($M + H^+$).

5-[1-(2-Deoxy-β-D-erythro-pentofuranosyl)uracil-5-ylmethylene]-3-phenyl-2-thiohydantoin (**8b**) *and 5-[1-(2-Deoxy-α-D-erythro-pentofuranosyl)uracil-5-ylmethylene]-3-phenyl-2-thiohydantoin]* (**9b**)

The protected nucleoside **7b** (0.5 g, 0.75 mmol) from *Method A* was treated similarly as described for the preparation of **8a** and **9a**. The mixture was chromatographed on a silica gel column with a gradient from 5–10% MeOH in $CHCl_3$ to give a yellow solid. The solid was further separated on HPLC with 25% EtOH in water on a reversed phase column (RP-18, 15 µm, 300 A) to give **8b** and **9b**.

8b: Yield 35 mg (11%). M.p. > 300°. ^{1}H-NMR (*DMSO-d_6*) δ 2.22 (2H, m, H-2'), 3.62 (1H, dd, J = 3.9; 12.0 Hz, H-5'), 3.74 (1H, dd, J = 3.1, 12.0 Hz, H-5'), 3.86 (1H, m, H-4'), 4.28 (1H, m, H-3'), 5.25 (2H, s, 3'-OH, 5'-OH), 6.14 (1H, t, J = 6.4 Hz, H-1'), 6.38 (1H, s, =CH), 7.32–7.52 (5H, m, Phenyl), 8.70 (1H, s, H-6), 11.25–12.25 (2H, broad s, 2 NH). ^{13}C-NMR (*DMSO-d_6*) δ 39.77 (C-2'), 60.91 (C-5'), 70.03 (C-3'), 85.52 (C-1'), 87.94 (C-4'), 105.50 (C-5), 108.35 (=CH), 126.60, 127.10, 128.22, 128.53, 133.22 (C-5 hydantoin, Phenyl), 145.82 (C-2), 149.03 (C-6), 162.89 (C-4), 164.50 (C-4 hydantoin), 175.00 (C=S); FAB MS (*DMSO* + 1 *M* trichloroacetic acid in glycerol), *m/z* 431 ($M + H^+$).

9b: 30 mg (9%). M.p. > 300 °C. ^{1}H-NMR (*DMSO-d_6*) δ 2.07 (1H, m, H-2'), 2.54 (1H, m, H-2') 3.47 (1H, dd, J = 4.2, 12.1 Hz, H-5'), 3.55 (1H, dd, J = 4.3, 11.9 Hz, H-5'), 4.23 (1H, td, J = 3.1 Hz, 6.1 Hz, Hz-3'), 4.38 (1H, m, H-4'), 4.83 (1H, s, 5'-OH), 5.41 (1H, s, 3'-OH), 5.99 (1H, dd, J = 3.0, 6.6 Hz, H-1'), 6.26 (1H, s, =CH), 7.24 – 7.45 (5H, m, Phenyl), 9.23 (1H, s, H-6), 10.94–12.75 (2H, broad s, 2NH). ^{13}C-NMR (*DMSO-d_6*) δ 39.10 (C-2'), 61.32 (C-5'), 69.99 (C-3'), 87.16 (C-1'), 89.59 (C-4'), 105.42 (C-5), 108.62 (=CH), 126.64, 128.02, 128.54, 128.86, 135.45 (C-5 hydantoin, Phenyl), 147.14 (C-2), 149.21 (C-6), 162.41 (C-4), 163.05 (C-4 hydantoin), 174.46 (C=S); FAB MS (*DMSO* + 1 *M* trichloroacetic acid in glycerol), *m/z* 431 ($M + H^+$).

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