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Synthesis of 5′-O-Oligopeptide Derivatives of Uridine as Inhibitors of *UDP*-glucuronosyltransferase

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Summary. In order to design potential inhibitors of *UDP*-glucuronosyltransferase, the synthesis of some 5′-O-oligopeptide derivatives of uridine is presented. 5′-O-(N-*tert*.Butyloxycarbonyl-O-benzyl-*L*-seryl-*L*-valyl)-2′,3′-O-isopropylideneuridine (**1**) was synthesized by the *DCC*/HO*Bt* method from N-*tert*.butyloxycarbonyl-O-benzyl-*L*-serine and 5′-O-*L*-valyl-2′,3′-O-isopropylideneuridine in 95% yield. In a similar way, 5′-O-(N-*tert*.butyloxycarbonyl-*L*-valyl-O-benzyl-*L*-seryl)-2′,3′-O-isopropylideneuridine (**2**) was obtained from N-*tert*.butyloxycarbonyl-*L*-valine and 5′-O-(O-benzyl-*L*-seryl)-2′,3′-O-isopropylideneuridine in 93% yield. Treatment of **1** and **2** with HCl/EtOAc at room temperature for 30 min led to removal of both *Boc* and 2′,3′-O-isopropylidene groups. 5′-O-(O-Benzyl-*L*-seryl-*L*-valyl)-uridine (**3**) and 5′-O-(*L*-valyl-O-benzyl-*L*-seryl)-uridine (**4**) were obtained in 94% and 91% yields, respectively.

Keywords. Uridine, 5′-O-oligopeptide derivatives; *UDP*-glucuronosyltransferase, inhibitors.

Synthese von 5′-O-Oligopeptidderivaten des Uridins als Inhibitoren der *UDP*-Glukuronosyltransferase

Zusammenfassung. Die Synthese von 5′-O-Oligopeptidderivaten des Uridins als Inhibitoren der *UDP*-Glucuronosyltransferase wird beschrieben. 5′-O-(N-*tert*.Butyloxycarbonyl-O-benzyl-*L*-seryl-*L*-valyl)-2′-,3′-O-isopropylidenuridin (**1**) wurde nach der *DCC*/HO*Bt*-Methode aus N-*tert*.Butyloxycarbonyl-O-benzyl-*L*-serin und 5′-O-*L*-Valyl-2′-,3′-O-isopropylidenuridin in 95%iger Ausbeute hergestellt. Auf ähnliche Weise erhielt man aus N-*tert*.Butyloxycarbonyl-*L*-valin und 5′-O-(O-Benzyl-*L*-seryl)-2′,3′-O-isopropylidenuridin in 93%iger Ausbeute 5′-O-(N-*tert*.Butyloxycarbonyl-*L*-valyl-O-benzyl-*L*-seryl)-2′,3′-O-isopropylidenuridin (**2**). Beide Schutzgruppen – *Boc* und 2′,3′-O-Isopropyliden – wurden mit HCl/EtOAc bei Zimmertemperatur (30 min) abgespalten. 5′-O-(O-Benzyl-*L*-seryl-*L*-valyl)-uridin (**3**) and 5′-O-(*L*-Valyl-O-benzyl-*L*-seryl)-uridin (**4**) entstanden in Ausbeuten von 94 bzw. 91%.

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Introduction

UDP-glucuronosyltransferase (*UGT*, EC 2.4.1.17) is a large family of closely related membrane-bound isoenzymes involved in the biotransformation and detoxification of a large variety of xenobiotics and endogenous substances [1–4]. These enzymes are responsible for the transfer of glucuronic acid from *UDP*-glucuronic acid (*UDPGA*) to the respective aglycones containing hydroxyl, amino, carboxyl, or sulfhydryl groups, forming water soluble β-(*D*)-glucuronides. Various drugs are extensively converted to inactive glucuronides in this way and subsequently excreted from the organism [5]. Thus, the inhibition of *UGT* could increase the plasma level and therapeutic efficiency of a number of drugs. Specific inhibitors could also be valuable tools for studying the active sites of *UGT* isoforms.

Several classes of *UGT* inhibitors have been developed [6–9]. According to the current concepts, the *UDP* part or the uridine moiety is thought to provide most of the free binding energy of the ligand-enzyme complex [10]. Thus, the synthesis of new inhibitors was directed to structures with full analogy to either the *UDP* part [8] or the uridine moiety [11]. Linkage of lipophilic aryl or arylalkyl residues to *UDP* led to powerful selective *UGT* inhibitors, considered as possible transition state analogs [8, 9].

Recently, we have developed novel uridinyl analogs modified at the 5′-O-position by protected and unprotected amino acids and tested them as inhibitors of diverse rat liver *UGT*s [12]. Some of them, (5′-O-(N-*tert*.butyloxycarbonyl-O-benzyl-*L*-seryl)-2′,3′-O-isopropylideneuridine and 5′-O-(N-*tert*.butyloxycarbonyl-*L*-valyl)-2′,3′-O-isopropylideneuridine), were found to be very potent inhibitors of both 4-*NP* and *PPh* glucuronidation [13].

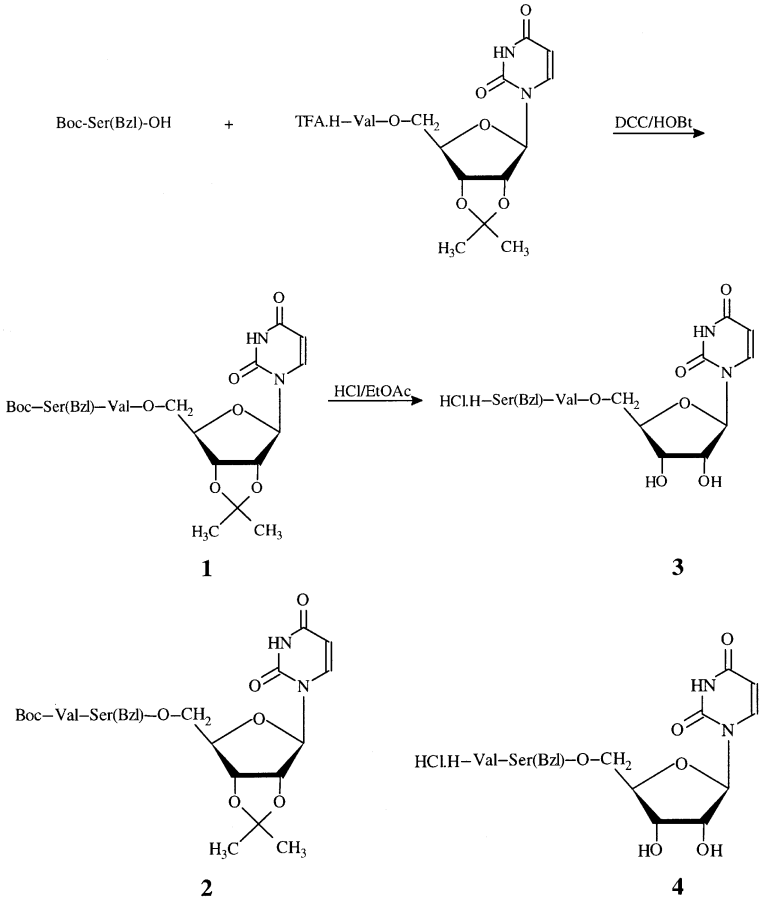
In continuation of our studies on the design of such *UGT* inhibitors and the investigation of their structure-activity relationships, we report the synthesis of some 5′-O-oligopeptide derivatives of uridine. The inhibitory potency of these compounds on the glucuronidation of 4-nitrophenol (4-*NP*) and phenolphthalein (*PPh*) by rat liver microsomes was also tested.

Results and Discussion

5′-O-(N-*tert*.Butyloxycarbonyl-O-benzyl-*L*-seryl-*L*-valyl)-2′,3′-O-isopropylideneuridine (**1**) was synthesized by the *DCC*/HO*Bt* method [14] from N-*tert*.butyloxycarbonyl-O-benzyl-*L*-serine and 5′-O-*L*-valyl-2′,3′-O-isopropylideneuridine [12] in 95% yield after gel chromatography (Scheme 1). In a similar way, 5′-O-(N-*tert*.butyloxycarbonyl-*L*-valyl-O-benzyl-*L*-seryl)-2′,3′-O-isopropylideneuridine (**2**) was obtained from N-(*tert*.butyloxycarbonyl-*L*-valine and 5′-O-(O-benzyl-*L*-seryl)-2′,3′-O-isopropylideneuridine [12] in 93% yield.

Treatment of **1** and **2** with HCl/EtOAc at room temperature for 30 min led to removal of both *Boc* and 2′,3′-O-isopropylidene groups. 5′-O-(O-Benzyl-*L*-seryl-*L*-valyl)-uridine (**3**) and 5′-O-(*L*-valyl-O-benzyl-*L*-seryl)-uridine (**4**) were obtained in 94% and 91% yield, respectively. The new derivatives were TLC pure and were characterized by MS, ^{1}H NMR, and elemental analysis.

The influence of these compounds on the glucuronidation of 4-*NP* and *PPh* by rat liver microsomal *UGT*s was tested. A marked suppression of *PPh*



Scheme 1

glucuronidation was registered with the protected derivatives 5′-O-(N-*tert*.butyloxycarbonyl-O-benzyl-*L*-seryl-*L*-valyl)-2′,3′-O-isopropylideneuridine (**1**) and 5′-O-(N-*tert*.butyloxycarbonyl-*L*-valyl-O-benzyl-*L*-seryl)-2′,3′-O-isopropylideneuridine (**2**; 70% and 65%, respectively). The same inhibitory potency against *PPh* and 4-*NP* convertion (77% and 75% inhibition) has been previously shown [13] with the most powerful inhibitor among the 5′-O-amino acid derivatives of uridine – (5′-O-(N-*tert*.butyloxycarbonyl-O-benzyl-*L*-seryl)-2′,3′-O-isopropylideneuridine. However, the four oligopeptide derivatives of uridine caused less decrease in 4-*NP*-*UGT* activity (21–34% inhibition).

Experimental

The amino acid derivatives were purchased from Bachem Biochemica GmbH (Heidelberg). All other chemicals were of analytical grade. Melting points were measured with a Kofler hot-stage apparatus. TLC analysis was performed on aluminum sheets (Silica gel $60\,F_{254}$, Merck) using the chromatographic systems A: BuOH:AcOH:H_2O (3:1:1) and B: $CHCl_3$:MeOH (9:1). The compounds were visualized by UV light or by spraying with the appropriate reagents (*Reindel* [15], ninhidrin). For column chromatography, Merck Kieselgel 60 (76–230 mesh ASTM) was used. Optical rotation

was determined with a Polamat A Carl-Zeiss instrument. The ^{1}H NMR spectra were obtained on Bruker DRX 250 MHz instrument. Elemental analyses were performed using a Perkin-Elmer M 240 apparatus. Mass spectra were recorded with a Jeol JMS D100 spectrometer.

5′-O-(N-tert.Butyloxycarbonyl-O-benzyl-L-seryl-L-valyl)-2′,3′-O-isopropylideneuridine (**1**; $C_{32}H_{44}N_4O_{11}$)

5′-O-*L*-valyl-2′,3′-O-isopropylideneuridine · trifluoroacetate (2.0 g, 5.0 mmol), triethylamine (0.69 ml, 5.0 mmol), N-*tert*.butyloxycarbonyl-O-benzyl-*L*-serine (1.48 g, 5.0 mmol), and 1-hydroxybenzotriazole (0.81 g, 6.0 mmol) were dissolved in 10 ml dimethylformamide. The solution was stirred and cooled in an ice water bath while dicyclohexylcarbodiimide (*DCC*) (1.24 g, 6.0 mmol) was added. Stirring was continued for 1 h at 0°C and 24 hours at room temperature. The formed N,N′-dicyclohexylurea was removed by filtration. EtOAc was added to the filtrate, and the organic phase was washed with 10% citric acid solution, 5% $NaHCO_3$ solution, and water. The EtOAc solution was dried over anhydrous Na_2SO_4 and evaporated to dryness *in vacuo*. The residue was chromatographed on silica gel with EtOAc/*PE* (1:1) to give pure **1** (3.14 g, 95%) as an amorphous solid.

M.p.: 57–62°C; $[\alpha]_D^{20} = -32.19$ ($c = 1.0$, CH_3OH); $R_f(A) = 0.89$, $R_f(B) = 0.80$; ^{1}H NMR ($CDCl_3$): δ (ppm) = 9.86 (bs, 1H, NH(U)), 7.66 (d, 1H, NH(Val), $J_{NH,\alpha} = 9.07$ Hz), 7.38–7.27 (m, 5H, H-arom), 7.19 (d, 1H, H-6, $J_{6,5} = 8.06$ Hz), 5.71 (d, 1H, H-5, $J_{5,6} = 8.06$ Hz), 5.42 (d, 1H, H-1′, $J_{1',2'} = 1.13$ Hz), 5.38 (d, 1H, NH(Ser), $J_{NH,\alpha} = 8.29$ Hz), 5.12 (dd, 1H, H-2′, $J_{1',2'} = 1.13$ Hz, $J_{2',3'} = 6.40$ Hz), 5.00 (bt, 1H, H-3′, $J_{3',2'} = 6.40$ Hz, $J_{3',4'} = 5.11$ Hz), 4.63 (dd, 1H, H-α(Val), $J_{\alpha,NH} = 9.07$ Hz, $J_{\alpha,\beta} = 4.68$ Hz), 4.56 (s, 2H, CH_2-benzyl), 4.59 (m, 1H, H-α(Ser)), 4.45 (m, 1H, H-5′(A)), 4.23 (m, 2H, H-5′(B), H-4′), 3.76 (m, 1H, H-β(A) (Ser)), 3.61 (m, 1H, H-β(B) (Ser)), 2.14 (m, 1H, H-β(Val)), 1.55 (s, 3H, isopropylidene), 1.45 (s, 9H, $3CH_3$), 1.32 (s, 3H, isopropylidene), 0.87 (d, 3H, H-γ(Val), $J_{\gamma,\beta} = 6.85$ Hz), 0.79 (d, 3H, H-γ(Val), $J_{\gamma,\beta} = 6.83$ Hz); MS: $m/z = 528$, 385, 367, 318, 277, 246, 203, 173, 127, 113 (B+2H), 91 ($C_6H_5CH_2^+$, 100%), 72, 43; $C_{32}H_{44}N_4O_{11}$ (660.7); calcd.: C 58.17, H 6.71, N 8.48; found: C 58.25, H 7.32, N 8.32.

5′-O-(N-tert.Butyloxycarbonyl-L-valyl-O-benzyl-L-seryl)-2′,3′-O-isopropylideneuridine (**2**; $C_{32}H_{44}N_4O_{11}$)

2 was prepared from N-*tert*.butyloxycarbonyl-*L*-valine dicyclohexylamonium salt (1.20 g, 3.0 mmol), 5′-O-(O-benzyl-*L*-seryl)-2′,3′-O-isopropylideneuridine · trifluoroacetate (1.80 g, 3.0 mmol), 1-hydroxybenzotriazole (0.45 g, 3.3 mmol), and *DCC* (0.68 g, 3.3 mmol) in analogy to the procedure described for **1** in 93% (1.85 g) yield.

M.p.: 56–61°C; $[\alpha]_D^{20} = -5.22$ ($c = 1.0$, CH_3OH); $R_f(A) = 0.92$, $R_f(B) = 0.72$; ^{1}H NMR ($CDCl_3$): δ (ppm) = 9.91 (bs. 1H, NH(U)), 7.63 (d, 1H, NH(Val), $J_{NH,\alpha} = 9.07$ Hz), 7.37–7.25 (m, 5H, H-arom), 7.19 (d, 1H, H-6, $J_{6,5} = 8.13$ Hz), 5.71 (d, 1H, H-5, $J_{5,6} = 8.13$ Hz), 5.42 (d, 1H, H-1′, $J_{1',2'} = 1.20\,H_2$), 5.37 (d, 1H, NH(Ser), $J_{NH,\alpha} = 8.28$ Hz), 5.10 (dd, 1H, H-2′, $J_{1',2'} = 1.20$ Hz, $J_{2',3'} = 7.44$ Hz), 5.01 (bt, 1H, H-3′, $J_{3',2'} = 7.44$ Hz, $J_{3',4'} = 5.97$ Hz), 4.63 (dd, 1H, H-α(Val), $J_{\alpha,NH} = 9.07$ Hz, $J_{\alpha,\beta} = 4.57$ Hz), 4.56 (s, 2H, CH_2-benzyl), 4.54 (m, 1H, H-α (Ser), 4.45 (m, 1H, H-5′(A)), 4.21 (m, 2H, H-5′(B), H-4′), 3.82 (m, 1H, H-β(A) (Ser)), 3.60 (m, 1H, H-β(B) (Ser)), 2.14 (m, 1H, H-β(Val)), 1.55 (s, 3H, isopropylidene), 1.46 (s, 9H, $3CH_3$), 1.32 (s, 3H, isopropylidene), 0.87 (d, 3H, H-γ(Val), $J_{\gamma,\beta} = 6.80$ Hz), 0.78 (d, 3H, H-γ(Val), $J_{\gamma,\beta} = 6.85$ Hz); MS; $m/z = 385$, 367, 277, 246, 173, 155, 127, 113 (B+2H), 99, 91 ($C_6H_5CH_2^+$, 100%), 85, 72, 55, 43; $C_{32}H_{44}N_4O_{11}$ (660.7); calcd.: C 58.17, H 6.71, N, 8.48; found: C 58.81, H 7.17, N 8.78.

5′-O-(O-Benzyl-L-seryl-L-valyl)-uridine · hydrochloride (**3**; $C_{24}H_{33}N_4O_9Cl$)

5′-O-(N-*tert*.Butyloxycarbonyl-O-benzyl-*L*-seryl-*L*-valyl)-2′,3′-O-isopropylideneuridine (0.50 g, 0.76 mmol) was dissolved in 6 ml HCl/EtOAc, and the solution was stirred at room temperature

for 30 min. After evaporation *in vacuo* (bath temperature below 30°C), the residue was treated with diethyl ether. The solid product was dried *in vacuo* over P_2O_5 to yield **3** (0.40 g, 94%) as a chromatographically homogeneous hygroscopic foam.

R_f(A) = 0.49; $[\alpha]_D^{20} = +23.3$ ($c = 1.0$, CH_3OH); ^{1}H NMR (*DMSO*-d_6): δ (ppm) = 11.33 (d, 1H, $J_{NH,5} = 1.69$ Hz), 8.91 (d, 1H, NH(Val), $J_{NH,\alpha} = 7.74$ Hz), 8.35 (bs, 1H, NH_2), 8.27 (bs, 2H, 2XOH), 7.65 (d, 1H, H-6, $J_{6,5} = 8.05$ Hz), 7.33–7.26 (m, 5H, H-arom), 5.76 (d, 1H, H-1′, $J_{1',2'} = 5.20$ Hz), 5.69 (dd, 1H, H-5, $J_{5,6} = 8.05$ Hz, $J_{5,NH} = 1.69$ Hz), 4.52 (s, 2H, CH_2-benzyl), 4.50 (m, 1H, H-5′(A)), 4.32–3.93 (m, 6H, H-5′(B), H-4′, H-α (Ser), H-2′, H-3′, H-α(Val)), 3.84–3.67 (m, 2H, H-β (Ser)), 2.06 (m, 1H, H-β(Val)), 0.92 (d, 3H, H-γ(Val), $J_{\gamma,\beta} = 6.62$ Hz), 0.90 (d, 3H, H-γ(Val), $J_{\gamma,\beta} = 6.60$ Hz); MS: $m/z = 277$, 246, 204, 185, 170, 155, 127, 113 (B+2H), 99, 91 ($C_6H_5CH_2^+$, 100%), 85, 72, 55, 43, 36.

5′-O-(-L-valyl-O-benzyl-L-seryl)-uridine · hydrochloride (**4**; $C_{24}H_{33}N_4O_9Cl$)

5′-O-(N-*tert*.Butyloxycarbonyl-*L*-valyl-O-benzyl-*L*-seryl)-2′,3′-O-isopropylideneuridine (0.80 g, 1.2 mmol) was treated using the procedure described for **3** to afford **4** as a white, chromatographically homogeneous hygroscopic foam (0.61 g, 91%).

R_f(A) = 0.47; $[\alpha]_D^{20} = +36.7$ ($c = 1.0$, CH_3OH); ^{1}H NMR (*DMSO*-d_6): δ (ppm) = 11.33 (d, 1H, $J_{NH,5} = 2.10$ Hz), 8.91 (d, 1H, NH(Ser), $J_{NH,\alpha} = 7.62$ Hz), 8.35 (bs, 4H, 2OH+NH_2), 7.65 (d, 1H, H-6, $J_{6,5} = 8.13$ Hz), 7.33–7.23 (m, 5H, H-arom), 5.77 (d, 1H, H-1′, $J_{1',2'} = 4.26$ Hz), 5.68 (dd, 1H, H-5, $J_{5,6} = 8.13$ Hz, $J_{5,NH} = 2.10$ Hz), 4.52 (m, 2H, CH_2-benzyl), 4.24–4.18 (m, 5H, H-2′, H-3′, H-4′, H-α(Ser), H-2′, H-3′, H-α(Val)), 4.10–3.60 (m, 4H, H-β(Ser), CH_2-benzyl(Ser)), 2.09 (m, 1H, H-β(Val)), 0.93 (d, 3H, H-γ(Val), $J_{\gamma,\beta} = 6.8$ Hz), 0.90 (d, 3H, H-γ(Val), $J_{\gamma,\beta} = 6.9$ Hz); MS: $m/z = 277$, 246, 204, 225, 170, 127, 113 (B+2H), 91 ($C_6H_5CH_2^+$, 100%), 72, 43.

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