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Synthesis of Some 5′-O-Amino Acid Derivatives of Uridine as Potential Inhibitors of *UDP*-Glucuronosyltransferase

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Summary. In an attempt to develop potential inhibitors of *UDP*-glucuronosyltransferase, some 5′-O-amino acid derivatives of uridine were synthesized. N-protected *L*-amino acids were coupled at the 5′-O-position of 2′,3′-O-isopropylideneuridine by esterification employing the method of symmetrical anhydrides in presence of 4-dimethylaminopyridine. 5′-O-(N-benzyloxycarbonyl-O-*tert*.butyl-*L*-threonyl)-2′,3′-O-isopropylideneuridine (**1**), 5′-O-(N-*tert*.butyloxycarbonyl-O-benzyl-*L*-seryl)-2′,3′-O-isopropylideneuridine (**2**), 5′-O-(N-*tert*.butyloxycarbonyl-*L*-leucyl)-2′,3′-O-isopropylideneuridine (**3**), and 5′-O-(N-*tert*.butyloxycarbonyl-*L*-valyl)-2′,3′-O-isopropylideneuridine (**4**) were obtained in good yield after column chromatography on silica gel. The treatment of **2** with *TFA*/CH_2Cl_2 (6:1) at room temperature for 30 min led to a selective removal of the *Boc* group without deblocking of the 2′,3′-O-isopropylidene group of uridine. Treatment of **2** with *TFA*/H_2O (5:1) at room temperature for 1 h, however, released both *Boc* and 2′,3′-O-isopropylidene groups. The *Z* group of **1** was deprotected by catalytic hydrogenolysis over 10% Pd/C/ammonium formate.

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

Synthese von 5′-O-Aminosäurederivaten des Uridins als potentielle Inhibitoren der *UDP*-Glukuronosyl-Transferase

Zusammenfassung. In einem Versuch, potentielle Inhibitoren der *UDP*-Glukuronosyl-Transferase zu entwickeln, wurden einige 5′-O-Aminosäurederivate des Uridins synthetisiert. N-Geschützte *L*-Aminosäuren wurden durch Veresterung mit der 5′-O-Position des 2′,3′-O-Isopropylidenuridins gekuppelt (Methode der symmetrischen Anhydride in der Gegenwart von 4-Dimethylaminopyridin). Solcherweise wurden 5′-O-(N-Benzyloxycarbonyl-O-*tert*.butyl-*L*-threonyl)-2′,3′-O-isopropylidenuridin (**1**), 5′-O-(N-*tert*.Butyloxycarbonyl-O-benzyl-*L*-seryl)-2′,3′-O-isopropylidenuridin (**2**), 5′-O-(N-*tert*.Butyloxycarbonyl-*L*-leucyl)-2′,3′-O-isopropylidenuridin (**3**) und 5′-O-(N-*tert*.Butyloxycarbonyl-*L*-valyl)-2′,3′-O-isopropylidenuridine (**4**) nach Säulenchromatographie (Kieselgel) in guter Ausbeute hergestellt. Die Behandlung von **2** mit *TFA*/CH_2Cl_2 (6:1) bei Zimmertemperatur (30 min) führte zu einer selektiven Abspaltung der *Boc*-Gruppe ohne Deblockierung der 2′,3′-O-Isopropylidengruppe des Uridins. Eine Behandlung von **2** mit *TFA*/H_2O (5:1) bei Zimmertemperatur für 1 Stunde führte hingegen zur Abspaltung sowohl der *Boc* als auch der 2′,3′-O-Isopropylidengruppe. Die *Z*-Gruppe von **1** wurde durch katalytische Hydrogenolyse auf 10% Pd/C/Ammoniumformiat abgespalten.

Introduction

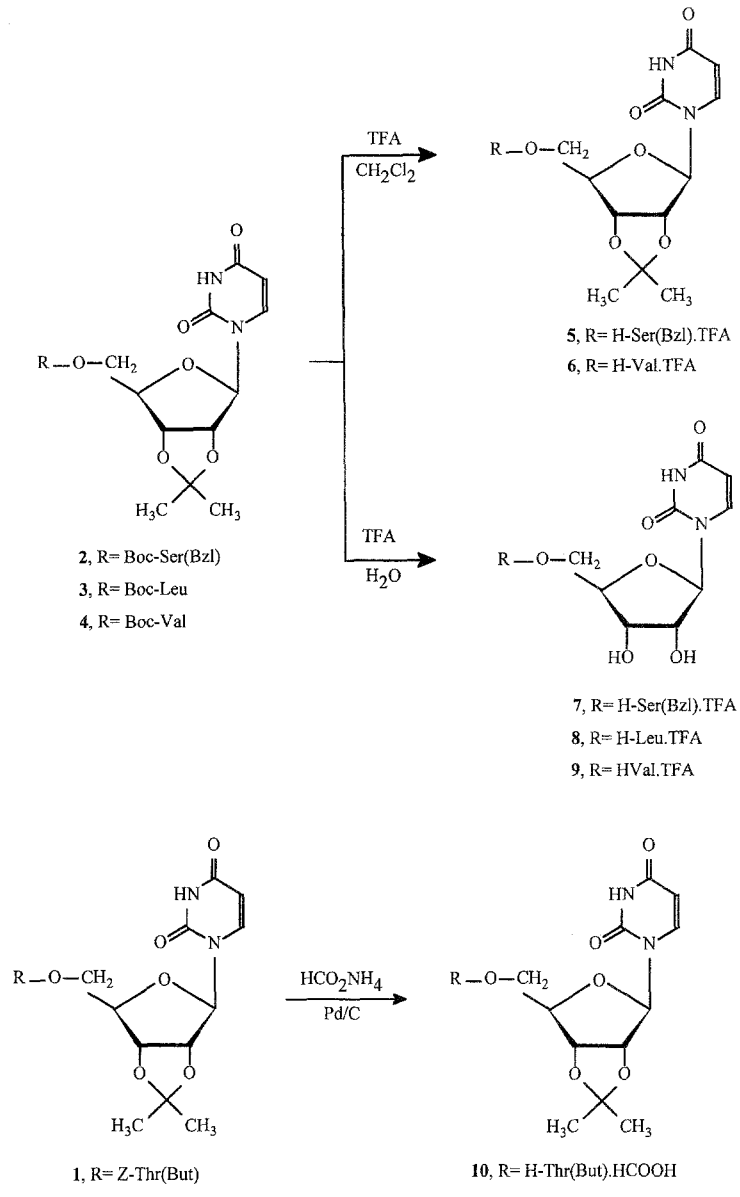
UDP-glucuronosyltransferase (*UGT*, EC 2.4.1.17) is a family of membrane-bound isoenzymes which play a key role in the biotransformation of a wide number of exogenous and endogenous compounds [1]. Glucuronidation is a major pathway for drug metabolism [2]. Various drugs, including the anti-HIV agent *AZT* (3′-azido-3′-deoxythymidine), are extensively converted to their inactive glucuronides and excreted from the organism [3–5]. It can be proposed that inhibition of the enzyme may improve the therapeutic efficacy of the drugs. The development of selective inhibitors of *UGT* represents also a useful approach in studying the active sites of different *UGT* isoenzymes.

Recently, several classes of inhibitors of *UGT* have been developed, including alkanoic and arylalkanoic acids and related derivatives [5–8]. Linkage of lipophilic aryl or arylalkyl residues to *UDP* resulted in powerful selective *UGT* inhibitors, considered as possible transition state analogs [7,8]. They contain both a hydrophilic uridine moiety and a lipophilic acceptor substrate moiety bound by a 5-atom diphosphate bridge. Thus, these compounds were designed to take advantage of both the high affinity of *UGT* for *UDPGA* and the specific structural requirements for an acceptor substrate (aglycone). It was suggested that both phosphate groups might be required for correct binding [7]. However, structurally related compounds in which this linker has been replaced by a diphosphate-like five-atom spacer (–OCONHSO$_2$O–) have been reported to be strong inhibitors of diverse *UGT* isoforms, and some of them behave as possible transition state analogs [10–13]. This finding offered the possibility-by varying the 5-atom spacer-to design and to synthesize novel active-site directed inhibitors based on the presumed transition state for the glucuronidation reaction.

Here we report the synthesis of some 5′-O-amino acid derivatives of uridine in an attempt to develop such *UGT* inhibitors. In order to determine the inhibitory potency of these compounds, they were tested on the glucuronidation of 4-nitrophenol (4-*NP*) and phenolphthalein (*PPh*) by rat liver microsomes. Some of them showed a significant inhibitory effect on *UGT* [14].

Results and Discussion

N-Protected *L*-amino acids were coupled at the 5′-O-position of 2′,3′-O-isopropylideneuridine by esterification employing the method of symmetrical anhydrides in the presence of 4-dimethylaminopyridine [15]. Symmetrical anhydrides of protected amino acids were obtained using dicyclohexylcarbodiimide (*DCC*, 0.5 equiv.) at 0°C for 30 min [16]. The esterification was carried out in dimethylformamide at room temperature for 48 hours. 4-Dimethyl-aminopyridine (0.1 equiv) was used as a catalyst. Thus, 5′-O-(N-benzyloxycarbonyl-O-*tert*.butyl-*L*-threonyl)-2′,3′-O-isopropylideneuridine (**1**) was obtained in 88% yield after column chromatography on silica gel. 5′-O-(N-*tert*.butyloxycarbonyl-O-benzyl-*L*-seryl)-2′,3′-O-isopropylideneuridine (**2**) was prepared in the same way as described for **1** in 96% yield after purification. In a similar way, 5′-O-(N-*tert*.butyloxycarbonyl-*L*-leucyl)-2′,3′-O-isopropylideneuridine (**3**) and 5′-O-(N-*tert*.butyloxycarbonyl-*L*-valyl)-2′,3′-O-isopropylideneuridine (**4**) were obtained in 92% and 97% yield, respectively.



Scheme 1

The treatment of 5′-O-(N-butyloxycarbonyl-O-benzyl-*L*-seryl)-2′,3′-O-isopropylideneuridine (**2**) with *TFA*/CH_2Cl_2 (6:1) at room temperature for 30 min led to the selective removal of the *tert.*butyloxycarbonyl (*Boc*) group without deblocking of 2′,3′-O-isopropylidene group of uridine (Scheme 1). 5′-O-(O-benzyl-*L*-seryl)-2′,3′-O-isopropylideneuridine·trifluoroacetate (**5**) was obtained in 81% yield. About 10% of 5′-O-(O-benzyl-*L*-seryl)-uridine trifluoroacetate (**7**) were obtained after column chromatography on silica gel. In a similar way, 5′-O-(*L*-valyl)-2′,3′-

O-isopropylideneuridine·trifluoroacetate (**6**) was obtained from 5′-O-(N-*tert*.butyloxycarbonyl-*L*-valyl)-2′,3′-O-isopropylideneuridine in 97% yield. In the case of 5′-O-(N-*tert*.butyloxycarbonyl-*L*-leucyl)-2′,3′-O-isopropylideneuridine (**3**), we suppose that hydrolysis of the acyl bond between the amino acid and the uridine moiety occurs. Treatment of 5′-O-(N-*tert*.butyloxycarbonyl-O-benzyl-*L*-seryl)-2′,3′-O-isopropylideneuridine with *TFA*/H_2O (5:1) at room temperature for 1 hour led to a removal of both *Boc* and 2′,3′-O-isopropylidene groups, and 5′-O-(O-benzyl-*L*-seryl)-uridine·trifluoroacetate (**7**) was obtained in a quantitative yield. At the same conditions, 5′-O-*L*-leucyl-uridine·trifluoroacetate (**8**) and 5′-O-*L*-valyl-uridine·trifluoroacetate (**9**) were obtained from the corresponding compounds.

The benzyloxycarbonyl (*Z*) group of 5′-O-(N-benzyloxycarbonyl-O-*tert*.butyl-*L*-threonyl)-2′,3′-O-isopropylideneuridine (**1**) was deprotected by catalytic hydrogenolysis over 10% Pd/C/ammonium formate [17]. The crude product 5′-O-(O-*tert*.butyl-*L*-threonyl)-2′,3′-O-isopropylideneuridine·formate (**10**) was purified by column chromatography on silica gel with CH_3Cl/MeOH (6:1) as eluent to give pure **10** in 98% yield. The new derivatives were TLC pure and were characterized by MS, ^{1}H NMR, and elemental analysis.

In order to determine the inhibitory potency of these compounds, they were tested on the glucuronidation of 4-*NP* and *PPh* catalyzed by different forms of *UGT* present in rat liver microsomes. Compound **2**, the serine derivative of isopropylideneuridine, was found to be the most potent inhibitor of both 4-*NP* and *PPh* glucuronidation with ID_{50} values of 0.45 m*M* and 0.22 m*M*, respectively [14]. We hypothesize that compound **2**, which includes a benzylresidue linked to isopropylideneuridine by a 5-atom spacer (-OCH_2CH(NH-)COO-), resembles the structure of possible transition state analogs reported recently [12, 13]. Replacement of the benzyl by a *tert*.butyl moiety (**1**) and/or shortening of the linker (**3**, **4**) led to a significant reduction of the inhibitory effect towards 4-*NP* or *PPh* glucuronidation.

It would be of interest to investigate also the inhibitory effects of the compounds obtained by removal of the N-protecting groups from the amino acid residues by trifluoroacetic acid (**5**–**10**). However, we found that at the concentrations tested (1 m*M*, 0.5 m*M* and 0.25 m*M*) the trifluoroacetate ions exerted a significant inhibition of *UGT* activities, thus hindering the proper registration of the inhibitory effect. This finding should be always taken into account when testing any compounds as *TFA* salts in enzyme systems.

Experimental

The amino acid derivatives were purchased from Bachem Biochemica GmbH (Heidelberg). 2′,3′-O-Isopropylideneuridine was obtained from Sigma. All other chemicals were of analytical grade. Melting points were measured with a Kofler hot-stage apparatus. TLC analysis was performed on aluminium sheets Silica gel 60 F_{254} (Merck) using the eluent 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 [18], 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 a Bruker DRX 250 MHz instrument. Elemental

analyses were performed by a Perkin-Elmer M 240 apparatus. Mass spectra were recorded with a Jeol JMS D 100 spectrometer.

5′-O-(N-Benzyloxycarbonyl-O-tert.butyl-L-threonyl)-2′,3′-O-isopropylideneuridine (**1**; $C_{28}H_{37}N_3O_{10}$)

N-Benzyloxycarbonyl-O-*tert*.butyl-*L*-threonine·dicyclohexylamonium salt (3.44 g, 7.0 mmol) was suspended in EtOAc (100 ml) and washed with 10% citric acid until acid reaction of the water layer. The EtOAc solution was washed with water, dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The residue was dissolved in *DMF* (5ml), stirred, and cooled in an ice water bath while *DCC* (0.72 g, 3.5 mmol) was added. Stirring was continued for 30 min at 0°C. Then, 2′,3′-O-isopropylideneuridine (0.85 g, 3.0 mmol) and 4-dimethylaminopyridine (0.037 g, 0.3 mmol) were added to the reaction mixture, and stirring was continued for 48 h at room temperature. The N,N′-dicyclohexyl urea was removed by filtration. EtOAc was added to the filtrate, and the organic phase was washed with a 5% $NaHCO_3$ solution and finally with 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 *PE*/EtOAc (1:1) to give pure **1** (1.53 g, 88%) as an amorphous solid.

M.p.: 54–59°C; $[\alpha]_D^{20} = +16.0$ ($c = 1.0$, CH_3OH); R_f(A) = 0.73, R_f(B)= 0.80; ^{1}H NMR ($(CD_3)_2SO$): δ(ppm)= 11.42 (s, 1H, NH-3), 7.71 (d, 1H, H-6, $J_{6,5} = 8.1$ Hz), 7.30 – 7.37 (m, 5H, H-arom.), 6.96 (d, 1H, NH, $J_{NH,\alpha} = 8.8$ Hz), 5.81 (d, 1H, H-1′, $J_{1',2'} = 1.8$ Hz), 5.62 (d, 1H, H-5, $J_{5,6} = 8.1$ Hz), 5.06 (s, 2H, CH_2-benzyl), 5.02 (dd, 1H, H-5′, $J_{5',5'} = 6.3$ Hz, $J_{5',4'} = 2.0$ Hz), 4.81 (dd, 1H, H-5′, $J_{5',5'} = 6.3$ Hz, $J_{5',4'} = 3.7$ Hz), 4.01 – 4.35 (m, 5H, H-α, H-β, H-2′, H-3′, H-4′), 1.49 (s, 3H, isopropylidene), 1.29 (s, 3H, isopropylidene), 1.03 (s, 12H, $4CH_3$); MS: $m/z = 576(M^+)$, 475, 363, 282, 228, 164, 137, 113 (B+2H), 91 ($C_6H_5CH_2^+$, 100%), 79, 57 ($C_4H_9^+$, 98%), 41; calcd.: C 58.42, H 6.48, N 7.30; found: C 59.03, H 6.54, N 7.33.

5′-O-(N-tert.Butyloxycarbonyl-O-benzyl-L-seryl)-2′,3′-O-isopropylideneuridine (**2**; $C_{27}H_{35}N_3O_{10}$)

N-*tert*.Butyloxycarbonyl-O-benzyl-*L*-serine (2.07 g, 7.0 mmol) and *DCC* (0.72 g, 3.5 mmol) were stirred in *DMF* for 30 min at 0°C. Then, 2′,3′-O-isopropylideneuridine (0.85 g, 3.0 mmol) and 4-dimethylaminopyridine (0.037 g, 0.3 mmol) were added, and stirring was continued for 48 h at room temperature. The N,N′-dicyclohexyl urea was removed by filtration. EtOAc was added to the filtrate, and the organic phase was washed with a 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 *PE*/EtOAc (1:1) to give pure **2** (1.61 g, 96%) as an amorphous solid.

M.p.: 50–55°C; $[\alpha]_D^{20} = +4.0$ ($c = 1.0$, CH_3OH); R_f (A) = 0.75, R_f (B) = 0.79; ^{1}H NMR ($(CD_3)_2SO$): δ (ppm) = 11.42 (s, 1H, NH-3), 7.65 (d, 1H, H-6, $J_{6,5} = 8.0$ Hz), 7.25 – 7.37 (m, 5H, H-arom.), 7.23 (d, 1H, $J_{NH,\alpha} = 7.6$ Hz), 5.81 (d, 1H, H-1′, $J_{1',2'} = 2.1$ Hz), 5.63 (d, 1H, H-5, $J_{5,6} = 8.0$ Hz), 4.96 (dd, 1H, H-5′, $J_{5',5'} = 6.3$ Hz, $J_{5',4'} = 1.9$ Hz), 4.76 (dd, 1H, H-5′, $J_{5',5'} = 6.3$ Hz, $J_{5',4'} = 3.6$ Hz), 4.47 (s, 2H, CH_2-benzyl), 4.24–4.40 (m, 4H, H-α, H-2′, H-3′, H-4′), 3.65 (d, 2H, 2H-β, $J_{\alpha,\beta} = 2.0$ Hz), 1.48 (s, 3H, isopropylidene), 1.38 (s, 9H, $3CH_3$), 1.27 (s, 3H, isopropylidene); MS: $m/z = 460$, 446, 430, 355, 340, 269, 173, 137, 113 (B+2H), 108, 91 ($C_6H_5CH_2^+$, 100%), 79, 68, 57 ($C_4H_9^+$), 43; calcd.: C 57.70, H 6.28, N 7.48; found: C 57.74, H 6.22, N 7.18.

5′-O-(N-tert. Butyloxycarbonyl-L-leucyl)-2′,3′-O-isopropylideneuridine (**3**; $C_{23}H_{35}N_3O_9$)

The title compound was prepared from N-*tert*.butyloxycarbonyl-*L*-leucine (0.50 g, 2.0 mmol), *DCC* (0.21 g, 1.0 mmol), 2′,3′-O-isopropylideneuridine (0.28 g, 1.0 mmol), and 4-dimethylaminopyridine (0.028 g, 0.1 mmol) in accordance with the procedure described for **2** in 92% (0.46 g) yield.

M.p.: 70–75°C; $[\alpha]_D^{20} = -6.1$ ($c = 1.0$, CH_3OH); R_f(A) = 0.77, R_f(B) = 0.80; ^{1}H NMR ($(CD_3)_2SO$): δ (ppm) = 11.4 (s, 1H, NH-3), 7.65 (d, 1H, H-6, $J_{6,5} = 8.0$ Hz), 7.24 (d, 1H, NH, $J_{NH,\alpha} = 7.7$ Hz), 5.79 (d, 1H, H-1′, $J_{1',2'} = 2.1$ Hz), 5.62 (d, 1H, H-5, $J_{5,6} = 8.0$ Hz), 5.0 (dd, 1H, H-5′, $J_{5',5''} = 6.3$ Hz, $J_{5',4'} = 1.8$ Hz), 4.75 (dd, 1H, H-5″, $J_{5''5'} = 6.3$ Hz, $J_{5'',4'} = 3.1$ Hz), 4.29–4.16 (m, 3H, H-2′, H-3′, H-4′), 4.03–3.86 (m, 1H, H-α), 1.47 (s, 3H, CH_3-isopropylidene), 1.35 (m, 18H, $5CH_3$, H-γ, 2H-β), 1.27 (s, 3H, CH_3-isopropylidene), 0.84 (d, 3H, CH_3-δ, $J_{\gamma,\delta} = 6.5$ Hz), 0.81 (d, 3H, CH_3-δ, $J_{\gamma,\delta} = 6.4$ Hz); MS: m/z = 408, 398, 382, 330, 312, 267, 173, 137, 130, 113 (B+2H), 86 (100%), 69, 57 ($C_4H_9^+$, 95%), 43 ($C_3H_7^+$); calcd.: C 55.52, H 7.09, N 8.44; found: C 54.72, H 7.46, N 8.49.

5′-O-(N-tert.Butyloxycarbonyl-L-valyl)-2′,3′-O-isopropylideneuridine (**4**; $C_{22}H_{33}N_3O_9$)

The title compound was prepared from N-*tert*.butyloxycarbonyl-*L*-valine (1.52 g, 7.0 mmol), *DCC* (0.72 g, 3.5 mmol), 2′,3′-O-isopropylideneuridine (0.85 g, 3.0 mmol), and 4-dimethylaminopyridine (0.037 g, 0.3 mmol) in accordance with the procedure described for **2** in 97% (1.41 g) yield.

M.p.: 65–70°C; $[\alpha]_D^{20} = +11.4$ ($c = 1.0$, CH_3OH); R_f(A) = 0.73, R_f(B) = 0.79; ^{1}H NMR ($(CD_3)_2SO$): δ (ppm) = 11.41 (s, 1H, NM-3), 7.75 (d, 1H, H-6, $J_{6,5} = 8.1$ Hz), 7.17 (d, 1H, NH, $J_{NH,\alpha} = 7.8$ Hz), 5.81 (d, 1H, H-1′, $J_{1',2'} = 1.9$ Hz), 5.64 (d, 1H, H-5, $J_{5,6} = 8.1$ Hz), 5.04 (dd, 1H, H-5′, $J_{5',5''} = 6.3$ Hz, $J_{5',4'} = 1.9$ Hz) 4.78 (dd, 1H, H-5″, $J_{5'',5'} = 6.3$ Hz, $J_{5'',4'} = 3.1$ Hz), 4.32–4.21 (m, 3H, H-2′, H-3′, H-4′), 3.83 (t, 1H, H-α, $J_{\alpha,\beta} = 6.7$ Hz, $J_{\alpha,NH} = 7.8$ Hz), 1.99 (m, 1H, H-β), 1.48 (s, 3H, CH_3-isopropylidene), 1.37 (s, 9H, $3CH_3$), 1.29 (s, 3H, CH_3-isopropylidene), 0.86–0.84 (m, 6H, $2CH_3$-γ); MS: m/z = 428, 410, 394, 384, 369, 352, 316, 267, 228, 191, 173, 137, 116, 113 (B + 2H), 97, 85, 72, 57 ($C_4H_9^+$, 100%), 43 ($C_3H_7^+$, 85%); calcd.: C 54.65, H 6.88, N 8.69; found: C 54.82, H 6.99, N 8.76.

5′-O-(O-Benzyl-L-seryl)-2′,3′-O-isopropylideneuridine·trifluoroacetate (**5**; $C_{24}H_{28}N_3O_{10}F_3$)

5′-O-(N-*tert*.Butyloxycarbonyl-O-benzyl-*L*-seryl)-2′,3′-O-isopropylidene-uridine (1.12 g, 2.0 mmol) was dissolved in 6 ml *TFA*/CH_2Cl_2 (6:1), and the solution was stirred at room temperature for 30 min. The solution was evaporated *in vacuo* (bath temperature below 30°C), and the residue was treated with diethylether. A small spot of 5′-O-(O-benzyl-*L*-seryl)-uridine (**7**) was observed at TLC. The solid product was chromatographed on silica gel with $CHCl_3$/MeOH (6:1) as eluent to afford **5** (0.93 g, 81%) as a chromatographically homogeneous white solid.

R_f(A)= 0.59; $[\alpha]_D^{20} = +11.39$ ($c = 1.0$, CH_3OH); ^{1}H NMR (D_2O): δ (ppm) = 7.51 (d, 1H, H-6, $J_{6,5} = 8.08$ Hz), 7.39–7.31 (m, 5H, H-arom.), 5.71 (d, 1H, H-5, $J_{5,6} = 8.08$ Hz), 5.69 (d, 1H, H-1′, $J_{1',2'} = 2.0$ Hz), 4.94 (dd, 1H, H-2′, $J_{2',1'} = 2.0$ Hz, $J_{2',3'} = 6.5$ Hz), 4.43 (m, 5H, H-α, H-β, CH_2-benzyl, H-4′) 4.40 (dd, 1H, H-3′, $J_{3',2'} = 6.5$ Hz, $J_{3',4'} = 3.1$ Hz), 4.04 (dd, 1H H-5′(A), $J_{5'(A),5'(B)}$, = 11.19 Hz, $J_{5'(A),4'} = 3.46$ Hz), 3.93 (dd, 1H, H-5′(B), $J_{5'(B),5'(A)} = 11.19$ Hz, $J_{5'(B),4'} = 2.94$ Hz), 1.58 (s, 3H, isopropylidene), 1.37 (s, 3H, isopropylidene); MS: m/z = 381, 365, 338, 322, 304, 294, 269, 253, 173, 137, 113 (B + 2H), 91 ($C_6H_5CH_2^+$), 79, 69 (100%) 43.

5′-O-(L-Valyl)-2′,3′-O-isopropylideneuridine·trifluoroacetate (**6**; $C_{19}H_{26}N_3O_9F_3$)

5′-O-(N-*tert*.Butyloxycarbonyl-*L*-valyl)-2′,3′-O-isopropylideneuridine (0.97 g, 2.0 mmol) was treated using the procedure described for **5** to give compound **6** as a white, chromatographically homogeneous foam (0.81 g, 82%).

R_f(A) = 0.55; $[\alpha]_D^{20} = -9.41$ ($c = 1.0$, CH_3OH); ^{1}H NMR ($CDCl_3$): δ (ppm) = 7.26 (d, 1H, H-6, $J_{6,5} = 8.04$ Hz), 5.73 (d, 1H, H-5, $J_{5,6} = 8.04$ Hz), 5.56 (d, 1H, H-1′, $J_{1',2'} = 1.67$ Hz), 5.07 (dd, 1H, H-2′, $J_{2',1'} = 1.67$ Hz, $J_{2',3'} = 6.39$ Hz), 4.87 (dd, 1H, H-3′, $J_{3',2'} = 6.39$ Hz, $J_{3',4'} = 3.67$ Hz), 4.44–

4.28 (m, 3H, H-5′, H-4′), 3.99 (bs, 3H, NH, NH_2), 3.40 (d, 1H, H-α, $J_{\alpha,\beta} = 4.82$ Hz), 2.07 (m, 1H, H-β), 1.56 (s, 3H, isopropylidene), 1.36 (s, 3H, isopropylidene), 0.98 (d, 3H, H-γ, $J_{\gamma,\beta} = 6.85$ Hz), 0.91 (d, 3H, H-γ, $J_{\gamma,\beta} = 6.83$ Hz); MS: $m/z = 385$ (M+), 369, 341, 298, 281, 241, 224, 215, 137, 97, 73, 43 (100%).

5′-O-(O-Benzyl-L-seryl)-uridine·trifluoroacetate (**7**; $C_{21}H_{24}N_3O_{10}F_3$)

5′-O-(N-*tert.*Butyloxycarbonyl-O-benzyl-*L*-seryl)-2′,3′-O-isopropylideneuridine (0.56 g, 1.0 mmol) was dissolved in 6 ml *TFA*/H_2O (6:1), and the solution was stirred at room temperature for 1 h. The solution was evaporated *in vacuo* (bath temperature below 30°C), and the residue was coevaporated three times with absolute MeOH and treated with diethyl ether. The solid product was chromatographed on silica gel with $CHCl_3$/MeOH (6:1) as eluent to give **7** (0.51 g, 94%) as a white hygroscopic solid.

R_f(A) = 0.44, R_f(B) = 0.44; $[\alpha]_D^{20} = +8.0$($c = 1.0$, CH_3OH); ^{1}H NMR (D_2O): δ (ppm) = 7.53 (d, 1H, H-6, $J_{6,5} = 8.1$ Hz), 7.38–7.31 (m, 5H, H-arom.), 5.73 (d, 1H, H-5, $J_{5,6} = 8.1$ Hz), 5.63 (d, 1H, H-1′, $J_{1',2'} = 2.74$ Hz), 4.67–4.30 (m, 4H, CH_2-benzyl, H-3′, H-α), 4.28 (m, 1H, 1H, H-4′), 4.13 (dd, 1H, H-2′, $J_{2',1'} = 2.74$ Hz, $J_{2',3'} = 5.39$ Hz), 4.02 (dd, 1H, H-5′(A), $J_{5'(A),5'(B)} = 11.05$ Hz, $J_{5'(A),4'} = 3.28$ Hz), 3.92 (dd, 1H, H-5′(B), $J_{5'(B),5'(A)} = 11.05$ Hz, $J_{5'(B),4'} = 2.44$ Hz), 3.95 (m, 2H, CH_2-β); MS: $m/z = 248$, 229, 216, 204, 191, 175, 140, 113 (B + 2H), 108, 91 ($C_6H_5CH_2^+$), 79, 69, 44 (100%).

5′-O-L-Leucyl-uridine·trifluoroacetate (**8**; $C_{17}H_{24}N_3O_9F_3$)

5′-O-(N-*tert.*Butyloxycarbonyl-*L*-leucyl)-2′,3′-O-isopropylideneuridine (0.50 g, 1.0 mmol) was treated in accordance with the procedure described for **7** to give **8** as a white, chromatographically homogeneous solid (0.45 g, 96%).

R_f(A) = 0.57; $[\alpha]_D^{20} = +30.72$ ($c = 1.0$, CH_3OH); ^{1}H NMR ($(CD_3)_2SO$): δ (ppm) = 11.38 (d, 1H, $J_{NH,5} = 1.8$ Hz), 8.42 (bs, 2H, NH_2), 7.63 (d, 1H, H-6, $J_{6,5} = 8.1$ Hz), 5.75 (d, 1H, H-1′, $J_{1',2'} = 5.0$ Hz), 5.64 (dd, 1H, H-5, $J_{5,6} = 8.1$ Hz, $J_{5,NH} = 1.8$ Hz), 4.42 (dd, 1H, H-5′(A), $J_{5'(A),5'(B)} = 12.0$ Hz, $J_{5'(A),4'} = 3.2$ Hz), 4.33 (dd, 1H, H-5′(B), $J_{5'(B),5'(A)} = 12.0$ Hz, $J_{5'(B),4'} = 6.0$ Hz), 4.14 (t, 1H, H-2′, $J_{2',1'} = 5.0$ Hz, $J_{2',3'} = 5.0$ Hz), 4.02–3.96 (m, 3H, H-3′, H-4′, H-α), 3.84 (bs, 2H, OH), 1.70 (m, 1H, H-γ, $J_{\gamma,\delta} = 5.8$ Hz), 1.63 (m, 2H, H-β), 0.88 (d, 6H, H-δ, $J_{\delta,\gamma} = 5.8$ Hz); MS: $m/z = 358$ (M+), 342, 324, 300, 265, 245, 227, 195, 182 (100%), 170, 140, 113 (B+2H), 97, 85, 69, 57 ($C_4H_9^+$), 45.

5′-O-L-Valyl-uridine·trifluoroacetate (**9**; $C_{16}H_{22}N_3O_9F_3$)

5′-O-(N-*tert.*Butyloxycarbonyl-L-valyl)-2′,3′-O-isopropylideneuridine (0.48 g, 1.0 mmol) was treated using the procedure described for **7** to afford **9** as a white, chromatographically homogeneous foam (0.44 g, 96%).

R_f(A) = 0.33; $[\alpha]_D^{20} = +23.33$($c = 1.0$, CH_3OH); ^{1}H NMR ($(CD_3)_2SO$): δ (ppm) = 11.37 (d, 1H, $J_{NH,5} = 2.12$ Hz), 8.37 (broad s, 2H, NH_2), 7.64 (d, 1H, H-6, $J_{6,5} = 8.10$ Hz), 5.75 (d, 1H, H-1′, $J_{1',2'} = 5.06$ Hz), 5.65 (dd, 1H, H-5, $J_{5,6} = 8.10$ Hz, $J_{5,NH} = 2.12$ Hz), 4.70 (broad signal, 2H, OH), 4.41 (m, 2H, H-5′), 4.15 (t, 1H, H-2′, $J_{2',1'} = 5.06$ Hz, $J_{2',3'} = 5.14$ Hz), 4.07–3.95 (m, 3H, H-3′, H-4′; H-α), 2.50 (m, 1H, H-β, $J_{\beta,\gamma} = 7.30$ Hz), 0.97 (d, 3H, H-γ, $J_{\gamma,\beta} = 7.3$ Hz), 0.95 (d, 3H, H-γ, $J_{\gamma,\beta} = 7.25$ Hz); MS: $m/z = 343$ (M+), 328, 310, 300, 285, 282, 245, 226, 214, 208, 195 (100%), 183.

5′-O-(O-tert.Butyl-L-threonyl)-2′,3′-O-isopropylideneuridine·formate (**10**; $C_{21}H_{33}N_3O_{10}$)

5′-O-(N-Benzyloxycarbonyl-O-*tert.*butyl-*L*-threonyl)-2′,3′-O-isopropylideneuridine (0.58 g, 1.0 mmol) was dissolved in absolute MeOH (10 ml). Then, 10% Pd/C and ammonium formate

(0.25 g, 4.0 mmol) were added. The reaction mixture was stirred at room temperature for 10 min. The catalyst was removed by filtration, and the filtrate was concentrated *in vacuo*. The raw product was chromatographed on silica gel with $CHCl_3$/MeOH (6:1) as eluent to give **10** (0.48 g, 98%) as a chromatographically homogeneous foam.

R_f(A) = 0.53, R_f(B) = 0.45; $[\alpha]_D^{20} = -37.73$ ($c = 1.0$, CH_3OH); ^{1}H NMR ($CDCl_3$): δ (ppm) = 7.28 (d, 1H, H-6, $J_{6,5} = 8.02$ Hz), 5.72 (d, 1H, H-5, $J_{5,6} = 8.02$ Hz), 5.59 (d, 1H, H-1′, $J_{1',2'} = 1.79$ Hz), 5.05 (dd, 1H, H-2′, $J_{2',1'} = 1.79$ Hz, $J_{2',3'} = 6.44$ Hz), 4.88 (dd, 1H, H-3′, $J_{3',2'} = 6.44$ Hz, $J_{3',4'} = 4.22$ Hz), 4.46 (dd, 1H, H-5′(A), $J_{5'(A),5'(B)} = 11.52$ Hz, $J_{5'(A),4'} = 3.35$ Hz), 4.51 (dd, 1H, H-5′(B), $J_{5'(B),5'(A)} = 1.52$ Hz, $J_{5'(B),4'} = 5.81$ Hz), 4.00 (ddd, 1H, $J_{4',5'(A)} = 3.35$ Hz, $J_{4',5'(B)} = 5.81$ Hz, $J_{4',3'} = 4.22$ Hz), 3.52 (broad s, 2H, NH_2), 3.39 (d, 1H, H-α, $J_{\alpha,\beta} = 3.5$ Hz), 2.70 (m, 1H, H-β), 1.56 (s, 3H, isopropylidene), 1.36 (s, 3H, isopropylidene), 1.23 (d, 3H, H-γ, $J_{\gamma,\beta} = 6.26$ Hz), 1.11 (s, 9H, 3×CH_3); MS: $m/z = 442$ (M+), 426, 386, 370, 341, 312, 283, 267, 256, 243, 229, 173, 113 (B+2H), 97, 74, 57 ($C_4H_9^+$, 100%), 43.

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