PREPARATION OF THYMIDINE-4′-*C*-CARBOXYLIC ACID AND ITS DERIVATIVES

Hubert HREBABECKY[1] and Antonin HOLY

Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, 166 10 Prague 6, Czech Republic; e-mail: [1] hubert@uochb.cas.cz

Received April 4, 1997
Accepted May 7, 1997

3′,5′-Di-*O*-benzoyl-4′-*C*-hydroxymethylthymidine (**3**) was prepared in four steps from 3′-*O*-(*tert*-butyldimethylsilyl)-4′-*C*-hydroxymethylthymidine (**1**). Oxidation of **3** with pyridinium dichromate afforded 3′,5′-di-*O*-benzoylthymidine-4′-*C*-carboxylic acid (**4**) which on debenzoylation gave free thymidine-4′-*C*-carboxylic acid, (3*R*,2*S*,5*R*)-3-hydroxy-2-hydroxymethyl-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)tetrahydrofuran-2-carboxylic acid, (**5**). Esterification of acid **5** with diazomethane afforded the methyl ester **6**. Its isopropyl ester **7** was obtained by transesterification of the methyl ester **6**. Reaction of ester **6** with ammonia and hydrazine led to the respective amide **8** and hydrazide **9**. Upon reaction with 1,1′-carbonyldiimidazole, the protected acid **4** was converted into the corresponding imidazolide **11**, which, without isolation, was treated with glycinamide, dimethylamine and aminoethanol to give aminocarbonylmethylamide **12a**, *N*,*N*-dimethylamide **13a** and hydroxyethylamide **14a**, respectively. The free amides **12b**, **13b** and **14b** were obtained by methanolysis of corresponding benzoates with methanolic sodium methoxide. Neither of the prepared compounds exhibited significant activity against HIV.

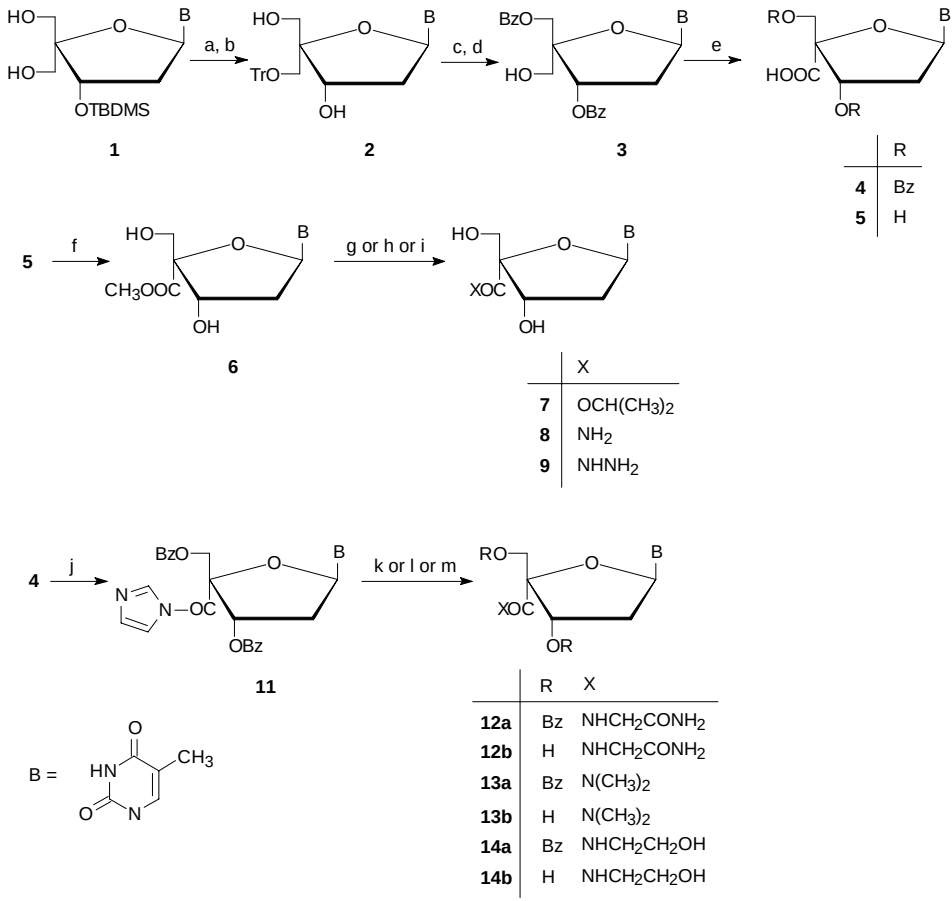
Key words: (3*R*,2*S*,5*R*)-3-Hydroxy-2-hydroxymethyl-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)-tetrahydrofuran-2-carboxylic acid; Thymidine-4′-*C*-carboxylic acid.

The present study is a part of our program aimed at the synthesis of 2′-deoxy-4′-*C*-substituted nucleoside analogues and study of relationship between structure and antiviral activity[1]. The biological activity of these derivatives depends crucially on the character of substitution in the positions 4′-C and 3′, the antiviral activity being associated with an electron-withdrawing substituent in position 4′-C and a free hydroxy group in position 3′ (see refs[2–5]). Particularly the 4′-*C*-azido derivative[2,3] and the 4′-*C*-cyano derivative[4] exhibit high anti-HIV activity.

The aim of the present communication was to synthesize thymidine-4′-*C*-carboxylic acid ((3*R*,2*S*,5*R*)-3-hydroxy-2-hydroxymethyl-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)tetrahydrofuran-2-carboxylic acid, **5**) and its derivatives and to study whether the presence of the electron-withdrawing carboxyl group in the position 4′-C also affects the antiviral activity.

As the starting compound for the synthesis of thymidine-4′-*C*-carboxylic acid and its derivatives (Scheme 1), we made use of the already described[4] 3′-*O*-silyl derivative **1**.

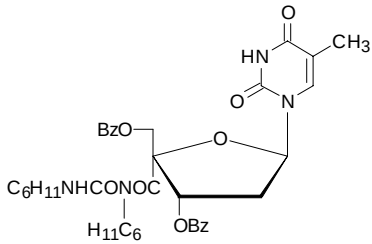
Its tritylation with trityl chloride in pyridine at room temperature and subsequent desilylation with tetrabutylammonium fluoride afforded the trityl derivative **2** in 65% overall yield. Also in this case we observed the already described[1d,4,5] higher reactivity of the 4′-*C*-hydroxymethyl group in comparison with the 5′-hydroxy group. The trityl derivative **2** was benzoylated with benzoyl chloride in pyridine and the obtained dibenzoyl trityl derivative was detritylated to give the dibenzoyl derivative **3**.



a) TrCl, pyridine; b) Bu_4NF, THF; c) benzoyl chloride, pyridine; d) 80% acetic acid, 60°C; e) pyridinium dichromate, DMF; f) CH_2N_2; g) sodium 2-propoxide, propan-2-ol; h) NH_3, MeOH; i) hydrazine; j) 1,1'-carbonyldiimidazole; k) glycinamide; l) dimethylamine; m) ethanolamine

SCHEME 1

The hydroxymethyl to carboxyl oxidation can be performed using several procedures. Oxidation with permanganate[6a] or platinum-catalyzed oxidation with air[6b,6c] usually give low yields. Moreover, these methods cannot be used for oxidation of the hydroxymethyl derivative **3** because they require an aqueous medium in which compound **3** is insoluble. Oxidation with ruthenium tetroxide, generated from ruthenium(III) chloride with periodate, can be used only for purine nucleosides[6d]. Also a modified method[6e], employing potassium peroxydisulfate in strongly alkaline medium for generation of the ruthenium tetroxide, could not be used because the (hydroxymethyl)thymidine **3** is protected by the alkali-labile benzoyl groups. Finally, oxidation with pyridinium dichromate[6f] proved to be the method of choice which afforded the acid **4** in a high yield. Acid **5** was obtained by debenzoylation of the acid **4** with methanolic sodium methoxide. Methyl ester **6** was prepared by esterification of acid **5** with diazomethane. The ethereal solution of diazomethane was added dropwise to a cool solution of the acid to avoid methylation of the base and the reaction was monitored by TLC. Transesterification of the methyl ester **6** with sodium 2-propoxide in propan-2-ol afforded the isopropyl ester **7**. The ester **6** was converted into amide **8** and hydrazide **9** by reaction with methanolic ammonia and hydrazine, respectively.



10

For the preparation of other derivatives we tried a method[7] used in the peptide chemistry. This consisted in activation of the carboxyl with 1,3-dicyclohexylcarbodiimide by forming a reactive *O*-isourea derivative. Although the reaction was carried out at –40 °C, the yield of the subsequent reaction was low and we isolated mainly the unreactive *N*-acylurea derivative **10**. We found a better way *via* imidazolide **11** which was prepared *in situ* by reaction of the protected acid **4** with 1,1′-carbonyldiimidazole in dimethylformamide, and without isolation treated with glycinamide, dimethylamine or ethanolamine. The thus obtained protected derivatives **12a**, **13a** and **14a** were methanolyzed with methanolic sodium methoxide to give the free derivatives **12b**, **13b** and **14b**.

The synthesized derivatives were tested for antiviral activity. Neither of them exhibited significant activity *in vitro*[8]. Although it is known that electronegative substituents in position 4′-C increase the antiviral activity, the electronegativity alone obviously is not sufficient to elicit the activity.

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. ^{1}H NMR spectra (δ, ppm; *J*, Hz) were measured on a Varian UNITY 200 instrument in hexadeuteriodimethyl sulfoxide with tetramethylsilane as internal standard. Column chromatography was performed on silica gel 30–60 μm (Service Laboratories of this Institute) and thin-layer chromatography (TLC) on Silufol UV 254 foils (Kavalier, Votice). Solvents were evaporated at 30–60 °C (bath)/2 kPa and compounds were dried at 13 Pa over phosphorus pentoxide.

1-(2-Deoxy-4-*C*-hydroxymethyl-5-triphenylmethyl-α-L-*threo*-pentofuranosyl)thymine (**2**)

Trityl chloride (3.35 g, 12 mmol) was added to a solution of silyl derivative **1** (ref.[4]) (4.64 g, 12 mmol) in pyridine (80 ml). The mixture was set aside at room temperature overnight, the solvent evaporated and the residue partitioned between water (100 ml) and ethyl acetate (300 ml). The organic layer was separated, washed with water (3 × 100 ml), dried over magnesium sulfate and taken down. The residue was dissolved in tetrahydrofuran (70 ml) and 1 M solution of tetrabutylammonium fluoride in tetrahydrofuran was added. The solution was heated at 40 °C for 4 h, concentrated to about one quarter of the original volume, diluted with ethyl acetate, washed with water (3 × 50 ml) and dried over magnesium sulfate. The solvent was evaporated and the residue chromatographed on a column of silica gel (250 g) in ethyl acetate to yield 4.01 g (65%) of trityl derivative **2** as a solid foam. For $C_{30}H_{29}N_2O_6$ (513.5) calculated: 70.16% C, 5.69% H, 5.46% N; found: 69.87% C, 5.98% H, 5.19% N. ^{1}H NMR spectrum: 1.80 s, 3 H (CH_3); 1.99–2.11 m and 2.18–2.32 m, 2 H (2 × H-2′); 3.01 d, 1 H, *J*(5a′,5b′) = 10.1 (H-5a′); 3.26 d, 1 H (H-5b′); 3.55 dd, 1 H, *J*(a,b) = 11.1, *J*(CHaH,OH) = 4.9 (CHaH-O); 3.73 dd, 1 H (CHbH-O); 4.31–4.38 m, 1 H (H-3′); 5.07–5.13 m, 2 H (3-OH, CH_2OH); 6.22 d, 1 H, *J*(1′,2a′) = 6.4, *J*(1′,2b′) = 7.3 (H-1′); 7.20–7.43 m, 15 H (H-arom.); 7.77 d, 1 H, *J* = 1.2 (H-6); 11.29 s, 1 H (H-3).

1-(3-*O*-Benzoyl-4-*C*-benzoyloxymethyl-2-deoxy-α-L-*threo*-pentofuranosyl)thymine (**3**)

A solution of benzoyl chloride (1.4 ml) in pyridine (14 ml) was added dropwise during 4 h to a stirred solution of trityl derivative **2** (2.57 g, 5 mmol) in pyridine (25 ml). Methanol (2 ml) was added and after 15 min the reaction mixture was concentrated. The residue was partitioned between ethyl acetate (100 ml) and water (30 ml), the organic phase was washed three times with water, dried over magnesium sulfate and the solvent was evaporated. The residue was dissolved in 80% aqueous acetic acid (40 ml) and boiled for 15 min. After cooling, the separated trityl alcohol was removed by filtration, washed with 80% aqueous acetic acid and the combined filtrates were taken down. Column chromatography on silica gel (150 g) in ethyl acetate–toluene (2 : 1) gave 1.72 g (72%) of dibenzoyl derivative **3** as a solid foam. For $C_{25}H_{24}N_2O_8$ (480.5) calculated: 62.49% C, 5.04% H, 5.83% N; found: 62.20% C, 5.16% H, 5.62% N. ^{1}H NMR spectrum: 1.80 s, 1 H (CH_3); 1.99–2.11 m, 2 H (2 × H-2′); 3.01 d, 1 H, *J*(a,b) = 10.1 (CHaH-O); 3.26 d, 1H (CHbH-O); 3.55 dd, 1 H, *J*(5a′,5b′) = 11.1, *J*(5′,OH) = 4.9 (H-5a′); 3.77 dd, 1 H (H-5b′); 4.31–4.38 m, 1 H (H-3′); 5.07–5.13 m, 2 H (3′-OH, 5′-OH); 6.22 dd, 1 H, *J*(1′,2a′) = 6.4, *J*(1′,2b′) = 7.3 (H-1′); 7.20–7.43 m, 15 H (H-arom.); 7.77 d, 1 H, *J* = 1.2 (H-6); 11.29 s, 1 H (H-3).

3′,5′-Di-*O*-benzoylthymidine-4′-*C*-carboxylic Acid (**4**)

Dibenzoyl derivative **3** (2.40 g, 5 mmol) was dissolved in a solution of pyridinium dichromate (17 g, 45 mmol) in dimethylformamide (18 ml). After standing at room temperature overnight, the solution was diluted with water (20 ml) and the product was extracted with ethyl acetate (3 × 80 ml). The

combined extracts were washed with 5% hydrochloric acid and water, dried over magnesium sulfate, and the solvent was evaporated. Yield 2.02 g (82%) of chromatographically pure acid **4** as a solid foam. For $C_{25}H_{20}N_2O_9$ (492.4) calculated: 60.97% C, 4.09% H, 5.69% N; found: 60.59% C, 4.31% H, 5.42% N. ^{1}H NMR spectrum: 1.67 s, 3 H (CH_3); 2.55–2.87 m, 2 H (2 × H-2′); 4.79 d, 1 H, J(5a′,5b′) = 11.8 (H-5a′); 4.85 d, 1 H (H-5b′); 6.00 dd, 1 H, J(3′,2a′) = 4.9, J(3′,2b′) = 7.6 (H-3′); 6.55 t, 1 H, J(1′,2a′) = J(1′,2b′) = 6.9 (H-1′); 7.47–7.73 m and 7.94–7.99 m, 6 H (H-6, H-arom.); 11.41 s, 1 H (H-3); 13.68 bs, 1 H (COOH).

Thymidine-4′-*C*-carboxylic Acid (**5**)

A solution of dibenzoate **4** (1.97 g, 4 mmol) in 0.3 M methanolic sodium methoxide (20 ml) was set aside at room temperature overnight and filtered through a column of Dowex 50 (H^+ form, 10 ml). The column was washed with methanol and the combined filtrates were taken down. Crystallization from a small amount of methanol afforded 995 mg (87%) of free acid **5**, m.p. 218–221 °C. For $C_{11}H_{14}N_2O_7$ (286.2) calculated: 46.15% C, 4.93% C, 9.79% N; found: 45.97% C, 4.91% H, 9.79% N. ^{1}H NMR spectrum: 1.77 s, 3 H (CH_3); 2.17 dd, 2 H (2 × H-2′); 3.72 d, 1 H, J(5a′,5b′) = 11.9 (H-5a′); 3.79 d, 1 H (H-5b′); 4.42 t, 1 H, J(3′,2a′) = J(3′,2b′) = 5.5 (H-3′); 6.35 t, 1 H, J(1′,2a′) = J(1′,2b′) = 6.7 (H-1′); 7.70 d, 1 H, J = 1.3 (H-6); 11.30 s, 1 H (H-3).

Methyl Thymidine-4′-*C*-carboxylate (**6**)

An ethereal solution of diazomethane (1 mol/l, 3 ml) was added dropwise to a stirred and cooled (ice bath) solution of acid **5** (859 mg, 3 mmol) in methanol (12 ml). After evaporation of the solvent, the residue was chromatographed on a column of silica gel (80 g) in ethyl acetate–acetone–ethanol–water (19 : 3 : 2 : 1) to give 790 mg (88%) of ester 6 as a solid foam. For $C_{12}H_{16}N_2O_7$ (300.3) calculated: 48.00% C, 5.37% H, 9.33% N; found: 47.71% C, 5.51% H, 9.05% N. ^{1}H NMR spectrum: 1.78 d, 3 H, J(CH_3,6) = 0.6 (CH_3); 2.07–2.29 m, 2 H (2 × H-2′); 3.65 s, 3 H (CH_3O); 3.78 bd, 2 H (2 × H-5′); 4.42 m, 1 H, J(3′,2a′) = J(3′,2b′) = 4.5, J(3′,OH) = 5.1 (H-3′); 5.28 t, 1 H, J(5′,OH) = 5.5 (5′-OH); 5.70 d, 1 H (3′-OH); 6.37 t, 1 H, J(1′,2a′) = J(1′,2b′) = 6.7 (H-1′); 7.66 d, 1 H (H-6); 11.31 s, 1 H (H-3).

Isopropyl Thymidine-4′-*C*-carboxylate (**7**)

A suspension of methyl ester **6** (150 mg, 0.5 mmol) in 0.1 M solution of sodium 2-propoxide in propan-2-ol (5 ml) was stirred at room temperature for 24 h and then neutralized with Dowex 50 (H^+ form). Water was added until the product dissolved. The ion-exchange resin was filtered off and washed with water, the combined filtrates were concentrated and the residue was chromatographed on a column of silica gel (15 g) in ethyl acetate–acetone–ethanol–water (40 : 6 : 2 : 1). Crystallization from propan-2-ol–ether afforded 125 mg (76%) of isopropyl ester **7**, m.p. 198–201.5 °C. For $C_{14}H_{20}N_2O_7$ (328.3) calculated: 51.21% C, 6.14% H, 8.53% N; found: 50.97% C, 6.30% H, 8.53% N. ^{1}H NMR spectrum: 1.20 d, 6 H, J(CH_3,CH) = 6.0 ((CH_3)$_2$C); 1.77 s, 3 H (CH_3); 2.08–2.28 m, 2 H (2 × H-2′); 3.68–3.85 m, 2 H (2 × H-5′); 4.39–4.47 m, 1 H (H-3′); 4.91 m, 1 H ((CH_3)$_2$CH); 5.23 t, 1 H, J(OH,5′) = 5.5 (5′-OH); 5.26 d, 1 H, J(OH,3′) = 4.9 (3′-OH); 6.33 t, 1 H, J(1′,2a′) = J(1′,2b′) = 6.7 (H-1′); 7.68 d, 1 H, J = 1.2 (H-6); 11.31 s, 1 H (H-3).

Thymidine-4′-*C*-carboxamide (**8**)

A solution of ester **6** (150 mg, 0.5 mmol) in 12% methanolic ammonia (2 ml) was set aside at room temperature for 6 days. After evaporation, the residue was crystallized from water to give 130 mg

(91%) of amide **8**, m.p. 187–189 °C. For $C_{11}H_{15}N_3O_6$ (285.3) calculated: 46.31% C, 5.30% H, 14.73% N; found: 46.03% C, 5.48% H, 14.76% N. ^{1}H NMR spectrum: 1.77 d, 3 H, J = 0.7 (CH_3); 2.02–2.17 m, 2 H (2 × H-2′); 3.60–3.84 m, 2 H (2 × H-5′); 4.26–4.32 m, 1 H (H-3′); 5.27 t, 1 H, J(OH,5′) = 4.3 (5-OH); 5.34 d, 1 H, J(OH,3′) = 4.6 (3′-OH); 6.38 dd, 1 H, J(1′,2a′) = 5.5, J(1′,2b′) = 8.85 (H-1′); 7.17 s and 7.36 s, 2 H (NH_2); 7.89 d, 1 H, J = 1.2 (H-6); 11.18 s, 1 H (H-3).

Thymidine-4′-*C*-carbohydrazide (**9**)

Hydrazine sulfate (195 mg, 1.5 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (0.3 ml) were added to a solution of ester **6** (150 mg, 0.5 mmol) in methanol (3 ml). The mixture was stirred at 55 °C for 4 days. The crystalline product was collected on a filter and crystallized from aqueous methanol. Yield 113 mg (75%) of hydrazide **9**, m.p. 221–224 °C. For $C_{11}H_{16}N_4O_6$ (300.3) calculated: 44.00% C, 5.37% H, 18.66% N; found: 43.76% C, 5.44% H, 18.94% N. ^{1}H NMR spectrum: 1.77 d, 3 H, J = 0.8 (CH_3); 2.01–2.24 m, 2 H (2 × H-2′); 3.63 d, 1 H J(5a′,5b′) = 11.6 (H-5a′); 3.82 d, 1 H (H-5b′); 4.29–4.33 m, 1 H (H-3′); 6.33 dd, 1 H, J(1′,2a′) = 5.5, J(1′,2b′) = 8.8 (H-1′); 7.82 d, 1 H, J = 1.2 (H-6); 9.08 s, 1 H (NH).

N-[(Aminocarbonyl)methyl]-3′,5′-di-*O*-benzoylthymidine-4′-*C*-carboxamide (**12a**)

A. A solution of 1,3-dicyclohexylcarbodiimide (113 mg, 0.55 mmol) in dimethylformamide (0.5 ml) was added dropwise at –40 °C to a stirred solution of acid **4** (246 mg, 0.5 mmol) in dimethylformamide (1.5 ml). The mixture was stirred at –15 °C for 30 min and then a solution of glycinamide hydrochloride (61 mg, 0.55 mmol) in dimethylformamide (0.5 ml) was added, followed by *N*,*N*-diisopropylethylamine (0.1 ml, 0.6 mmol). The mixture was allowed to warm to room temperature and set aside for 24 h. The solvent was evaporated and the residue dissolved in ethyl acetate (30 ml). The solution was washed with water (3 × 15 ml), dried and taken down and the residue was chromatographed on a column of silica gel (25 g) in ethyl acetate–toluene (2 : 1) to give 245 mg (70%) of the dicyclohexylurea derivative **10** as a solid foam. For $C_{38}H_{44}N_4O_9$ (700.8) calculated: 65.13% C, 6.33% H, 8.00% N; found: 65.32% C, 6.50% H, 7.80% N. ^{1}H NMR spectrum: 0.95–1.83 m, 20 H (10 × CH_2 in cyclohexyl); 1.40 s, 3 H (CH_3); 2.39–2.65 m, 2 H (2 × H-2′); 3.76–3.87 m, 2 H (2 × CH in cyclohexyl); 4.83 d, 1 H, J(5a′,5b′) = 11.9 (H-5a′); 4.88 d, 1 H (H-5b′); 6.05 d, 1 H, J(3′,2b′) = 4.0 (H-3′); 6.46 dd, 1 H, J(1′,2a′) = 5.4, J(1′,2b′) = 9.5 (H-1′); 7.34 s, 1 H (NH); 7.45–8.14 m, 11 H (H-6, H-arom.); 11.42 s, 1 H (H-3).

Elution with ethyl acetate–acetone–ethanol–water (40 : 6 : 3 : 1) afforded 60 mg (22%) of glycinamide derivative **12a** as solid foam. For $C_{27}H_{26}N_4O_9$ (550.5) calculated: 58.90% C, 4.76% H, 10.18% N; found: 58.67% C, 4.91% H, 9.89% N. ^{1}H NMR spectrum: 1.66 s, 3 H (CH_3); 2.47–2.83 m, 2 H (2 × H-2′); 3.37–3.88 m, 2 H (NCH_2); 4.70 d, 1 H, J(5a′,5b′) = 11.6 (H-5a′); 4.98 d, 1 H (H-5b′); 5.89 d, 1 H, J(3′,2b′) = 4.3 (H-3′); 6.55 dd, 1 H, J(1′,2a′) = 5.5, J(1′,2b′) = 9.2 (H-1′); 7.12 s, 1 H and 7.33 s, 1 H (NH_2); 7.48–8.03 m, 11 H (H-6, H-arom.); 8.54 t, 1 H, J(NH,CH_2) = 5.6 (NH); 11.43 s, 1 H (H-3).

B. 1,1′-Carbonyldiimidazole (90 mg, 0.55 mmol) was added to a solution of acid **4** (246 mg, 0.5 mmol) in dimethylformamide (2 ml). After standing for 1 h at room temperature, glycinamide hydrochloride (61 mg, 0.55 mol) and diisopropylethylamine (42 μl, 0.3 mmol) were added. The solution was allowed to stand for 3 h, the solvent was evaporated and the residue was partitioned between water (10 ml) and ethyl acetate (20 ml). The organic layer was separated, washed with water, 2% hydrochloric acid, water, 5% sodium hydrogen carbonate solution, dried and the solvent was evaporated. Yield 220 mg (80%) of chromatographically pure derivative **12a** as solid foam.

N-[(Aminocarbonyl)methyl]thymidine-4′-*C*-carboxamide (**12b**)

A solution of benzoyl derivative **12a** (220 mg, 0.4 mmol) in 0.1 M methanolic sodium methoxide (3 ml) was allowed to stand at room temperature overnight. The mixture was neutralized with Dowex 50 (H^+ form), the ion-exchange resin was filtered off, washed with methanol and the combined filtrates were concentrated. The residue was mixed with ether and the amorphous solid was filtered and washed with ether. Yield 120 mg (88%) of derivative **12b**. For $C_{13}H_{18}N_4O_7$ (342.3) calculated: 45.61% C, 5.30% H, 16.37% N; found: 45.39% C, 5.44% H, 16.09% N. ^{1}H NMR spectrum: 1.78 s, 3 H (CH_3); 2.06–2.28 m, 2 H (2 × H-2′); 3.31–3.88 m, 4 H (NCH_2, 2 × H-5′); 4.32–4.37 m, 1 H (H-3′); 5.36 t, 1 H, *J*(OH,5′) = 5.5 (5′-OH); 5.86 d, 1 H, *J*(OH,3′) = 4.1 (3′-OH); 6.47 dd, *J*(1′,2a′) = 5.7, *J*(1′,2b′) = 8.9 (H-1′); 7.12 s, 1 H and 7.19 s, 1 H (NH_2); 7.85 s, 1 H (H-6); 8.37 t, 1 H, *J*(NH,CH_2) = 5.6 (NH); 10.82 bs, 1 H (H-3).

N,*N*-Dimethyl-3′,5′-di-*O*-benzoylthymidine-4′-*C*-carboxamide (**13a**)

1,1′-Carbonyldiimidazole (90 mg, 0.55 mmol) was added to a solution of acid **4** (246 mg, 0.5 mmol) in dimethylformamide (2 ml). After standing at room temperature for 1 h, the solution was slowly saturated with dimethylamine and the reaction was monitored by TLC. When the reaction was complete, the solvent was evaporated and the residue was dissolved in ethyl acetate (20 ml). The solution was washed with water (3 × 10 ml), dried over magnesium sulfate and the solvent was evaporated. Column chromatography on silica gel (20 g) in ethyl acetate–toluene (3 : 1) afforded 182 mg (70%) of compound **13a** as a solid foam. For $C_{27}H_{27}N_3O_8$ (521.5) calculated: 62.18% C, 5.22% H, 8.06% N; found: 61.91% C, 5.44% H, 7.85% N. ^{1}H NMR spectrum: 1.65 s, 3 H (CH_3); 2.50 dd, 1 H, *J*(2a′,1′) = 5.0, *J*(2a′,2b′) = 13.7 (H-2a′); 2.75–2.90 m, 1 H (H-2b′); 2.85 s, 3 H (NCH_3); 3.31 s, 3 H (NCH_3); 4.81 d, 1 H, *J*(5a′,5b′) = 11.3 (H-5a′); 4.90 d, 1 H (H-5b′); 5.92 d, 1 H, *J*(3′,2b′) = 4.6 (H-3′); 6.51 dd, 1 H, *J*(1′,2a′) = 5.0, *J*(1′,2b′) = 9.9 (H-1′); 7.49–7.72 m and 7.86–7.96 m, 7 H and 4 H (H-arom., H-6); 11.43 s, 1 H (H-3).

N,*N*-Dimethylthymidine-4′-*C*-carboxamide (**13b**)

Dibenzoyl derivative **13a** (156 mg, 0.3 mmol) was methanolyzed with 0.1 M methanolic sodium methoxide as described for compound **12b**; yield 76 mg (81%) of dimethylamide **13b**, m.p. 215–218 °C. For $C_{13}H_{19}N_3O_6$ (313.3) calculated: 49.83% C, 6.11% H, 13.41% N; found: 49.61% C, 6.29% H, 13.20% N. ^{1}H NMR spectrum: 1.78 s, 1 H (CH_3); 2.01–2.35 m, 2 H (2 × H-2′); 2.83 bs, 3 H (NCH_3); 3.11 bs, 3 H (NCH_3); 3.64–3.86 m, 2 H (2 × H-5′); 4.37 t, 1 H, *J*(3′,2b′) = 3.7, *J*(3′,OH) = 4.0 (H-3′); 5.32 t, 1 H, *J*(OH,5′) = 5.5 (5′-OH); 5.44 d, 1 H, *J*(OH,3′) = 4.0 (3′-OH); 6.37 dd, 1 H, *J*(1′,2a′) = 5.5, *J*(1′,2b′) = 9.5 (H-1′); 7.83 s, 1 H (H-6); 11.33 s, 1 H (H-3).

N-(2-Hydroxymethyl)-3′,5′-di-*O*-benzoylthymidine-4′-*C*-carboxamide (**14a**)

1,1′-Carbonyldiimidazole (90 mg, 0.55 mmol) was added to a solution of acid **4** (246 mg, 0.5 mmol) in dimethylformamide (2 ml). After standing at room temperature for 1 h, the solution was slowly added to a stirred solution of 2-aminoethanol (61 mg, 1 mmol) in dimethylformamide (1 ml). The reaction mixture was set aside at room temperature for 3 h and the solvent was evaporated. The residue was dissolved in ethyl acetate and the solution was washed with 5% hydrochloric acid, water, 5% solution of sodium hydrogen carbonate, dried and the solvent was evaporated. Column chromatography on silica gel in ethyl acetate gave 190 mg (71%) of hydroxyethylamide **14a** as a solid foam. For $C_{27}H_{27}N_3O_9$ (537.5) calculated: 60.33% C, 5.06% H, 7.82% N; found: 60.05% C, 5.11% H, 7.56% N. ^{1}H NMR spectrum: 1.68 s, 3 H (CH_3); 2.51 dd, 1 H, *J*(2a′,1′) = 5.3, *J*(2a′,2b′) = 14.7 (H-2a′); 2.76 m, 1 H (H-2b′); 3.03–3.50 m, 4 H (CH_2CH_2); 4.70 d, 1 H (H-5a′); 4.73 t, 1 H,

J(OH,CH_2) = 5.5 (CH_2OH); 4.98 d, 1 H, J(5a′,5b′) = 11.6 (H-5b′); 5.87 d, 1 H, J(3′,2b′) = 4.3 (H-3′); 6.54 dd, 1 H, J(1′,2b′) = 9.3 (H-1′); 7.48–7.97 m, 11 H (H-6, H-arom.); 8.40 t, 1 H, J(NH,CH_2) = 5.3 (NHCH_2); 11.42 s, 1 H (H-3).

N-(2-Hydroxyethyl)thymidine-4′-*C*-carboxamide (**14b**)

Benzoyl derivative **14a** (161 mg, 0.3 mmol) was methanolyzed in the same manner as described for the derivative **12b**; yield 84 mg (85%) of amorphous hydroxyethylamide **14b**. For $C_{13}H_{19}N_3O_7$ (329.3) calculated: 47.41% C, 5.82% H, 12.76% N; found: 47.68% C, 6.01% H, 12.51% N. ^{1}H NMR spectrum: 1.78 s, 3 H (CH_3); 2.02–2.27 m, 2 H (2 × H-2′); 3.01–3.43 m, 4 H (CH_2CH_2); 3.59–3.68 m, 1 H (H-5a′); 3.79–3.87 m, 1 H (H-5b′); 4.29 m, 1 H (H-3′); 4.60 t, 1 H, J(OH,CH_2) = 5.6 (CH_2OH); 5.30 t, 1 H, J(OH,5′) = 5.0 (5′-OH); 5.37 d, 1 H, J(OH,3′) = 4.6 (3′-OH); 6.40 dd, 1 H, J(1′,2a′) = 5.5, J(1′,2b′) = 8.9 (H-1′); 7.78 t, J(NH,CH_2) = 5.6 (NH); 7.87 s, 1 H (H-6); 11.32 s, 1 H (H-3).

The authors are indebted to Professor E. De Clercq and to Dr J. Balzarini (Rega Institut, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium) for antiviral activity assays. The excellent technical assistance of Mrs A. Sterecova is gratefully acknowledged. The authors' thanks are also due to Mrs M. Snopkova for the NMR measurements and to the staff of the Analytical Laboratory (Dr V. Pechanec, Head) of this Institute for elemental analyses. This study was supported by the Grant Agency of the Czech Republic (Grant No. 203/94/0099).

REFERENCES

1. a) Hrebabecky H., Holy A.: Collect. Czech. Chem. Commun. *58*, 409 (1993); b) Hrebabecky H., Holy A.: Collect. Czech. Chem. Commun. *58*, 1668 (1993); c) Hrebabecky H., Holy A.: Collect. Czech. Chem. Commun. *59*, 1654 (1994); d) Hrebabecky H., Budesinsky M., Masojidkova M., Havlas Z., Holy A.: Collect. Czech. Chem. Commun. *62*, 957 (1997); e) Hrebabecky H., Balzarini J., Holy A.: Collect. Czech. Chem. Commun. *62*, 1114 (1997).
2. Maag H., Prisbe E. J., Verheyden J. P. H.: Eur. Pat. Appl. 457,326 (1991); Chem. Abstr. *116*, 129507 (1992).
3. Maag H., Chu N., Crawford-Ruth D., Eugui E., McRoberts M. J., Mirkovich A., Pettibone M., Prisbe E. J., Rydzewski R. M., Verheyden J. P. H.: Antiviral Res., Suppl. *1*, 43 (1991).
4. O-Yang C., Wu H. Y., Frazer-Smith E. B., Walker K. A. M.: Tetrahedron Lett. *33*, 37 (1992).
5. O-Yang C., Kurz W., Eugui E. M., McRoberts M. J., Verheyden J. P. H., Kurz L. J., Walker K. A. M.: Tetrahedron Lett. *33*, 41 (1992).
6. a) Harper P. J., Hampton A.: J. Org. Chem. *35*, 1688 (1970); b) Zemlicka J., Horwitz J. P. in: *Nucleic Acid Chemistry* (L. B. Townsend and R. S. Tipson, Eds), Part 1, p. 303. Wiley, New York, Chichester, Brisbane, Toronto 1978; c) Moss G. P., Reese C. B., Schofield K., Shapiro R., Todd L.: J. Chem. Soc. *1963*, 1149; d) Singh A. K., Varma R. S.: Tetrahedron Lett. *33*, 2307 (1992); e) Varma R. S., Hogan M. E.: Tetrahedron Lett. *33*, 7719 (1992); f) Corey E. J., Schmidt G.: Tetrahedron Lett. *1979*, 399.
7. a) Sheehan J. C., Hess G. P.: J. Am. Chem. Soc. *77*, 1067 (1955); b) Goodman M., Kenner G. W.: Adv. Protein Chem. *12*, 488 (1957); c) Albertson N. F.: Org. Reac. *12*, 205 (1962).
8. Balzarini J.: Unpublished results.