Chemistry of Natural Compounds, Vol. 42, No. 2, 2006

SYNTHESIS OF CONJUGATES OF 2′,3′-DIDEOXYNUCLEOSIDE-5′-MONOPHOSPHATES WITH α-AMINO ACIDS

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UDC 547.92+577.113.3+577.152.271

*Previously undescribed conjugates of 2′,3′-dideoxyuridine-5′-monophosphate-(L)-methoxytryptophylphosphoramidate (**5**) and 2′,3′-dideoxycytidine-5′-monophosphate-(L)-methoxytryptophylphosphoramidate (**7**) were isolated by a chemical enzymatic method in order to study their pharmacological properties and to prepare new medicinal preparations based on them.*

Key words: nucleotides, nucleosides, 2′,3′-dideoxycytidine, 2′,3′-dideoxyuridine, conjugates, L-tryptophan, cytidinedeaminase, nucleosidephosphotransferase, *Escherichia coli*, *Erwinia herbicola*.

Until now, most synthesized 2′,3′-dideoxyanalogs of natural nucleosides (2′,3′-dideoxynucleosides) have exhibited distinct antiviral activity toward human immunodeficiency virus (HIV) [1]. Certain representives of this group are preparations such as 3′-azidothymidine (AZT), 2′,3′-dideoxycytidine (**1**, ddC), 2′,3′-dideoxyinosine (ddI), and (-)-3′-thio-2′,3′-dideoxycytidine (3TC) have been used clinically to treat HIV-infected patients. The exception is 2′,3′-dideoxyuridine (**2**, ddU), which has practically no antiviral activity. On the other hand, it is known that the 5′-triphosphate derivative of this nucleoside, 2′,3′-dideoxyuridine-5′-triphosphate (ddU-5′-TP) is a potent (and specific!) inhibitor of HIV reverse transcriptase [2]. This contradiction is explained by the very low rate of the first step in metabolic activation of a nucleoside in the cell, conversion of it into the 5′-monophosphate derivative, 2′,3′-dideoxyuridine-5′-monophosphate (**3**, ddU-5′-MP). The low permeability through the cell membrane of ddU-5′-MP prevents its use in medical procedures. Furthermore, ddU-5′-MP, like other nucleoside-5′-monophosphates, is easily dephosphorylated in the intercellular medium and on the surface of cell membranes by phosphomonoesterase.

Conjugates of nucleoside-5′-monophosphates with certain natural compounds can be used to circumvent partially the deficiencies of these compounds mentioned above. The resulting "pronucleotides" possess increased lipophilicity and, as a result, permeate more easily through cell membranes. Metabolism of the conjugate within the cell liberates active nucleoside-5′-monophosphate. This avoids the monophosphorylation of the nucleoside. Furthermore, such compounds are rather stable because they are not substrates for phosphohydrolases and can be viewed as a unique storage form of the preparation. Data on the activity of certain conjugates of AZT toward viral types resistant to the starting preparation are also known [3].

The use of nucleotides bonded through a phosphoramide bond to natural aromatic α-amino acids as pronucleotides was proposed earlier [4]. Such conjugates exhibited several advantages, namely stability and good solubility in water owing to the residual negative charge. Such a conjugate of AZT with L-tryptophan methyl ester turned out to be eight times more active than starting AZT and less toxic.

We proposed and demonstrated experimentally a rational scheme for chemical enzymatic transformation of ddC (**1**) prepared in several steps from cytidine [5] into the two prodrugs 2′,3′-dideoxyuridine-5′-monophosphate-(L)-methoxytryptophylphosphoramidate (**5**) [ddU-5′-MP-(L)-TrpOMe] and 2′,3′-dideoxycytidine-5′-monophosphate-(L)-methoxytryptophylphosphoramidate (**7**) [ddC-5′-MP-(L)-TrpOMe], which are conjugates of ddU-5′-MP (**3**) and ddC-5′-MP (**6**) with L-tryptophan methyl ester (**4**).

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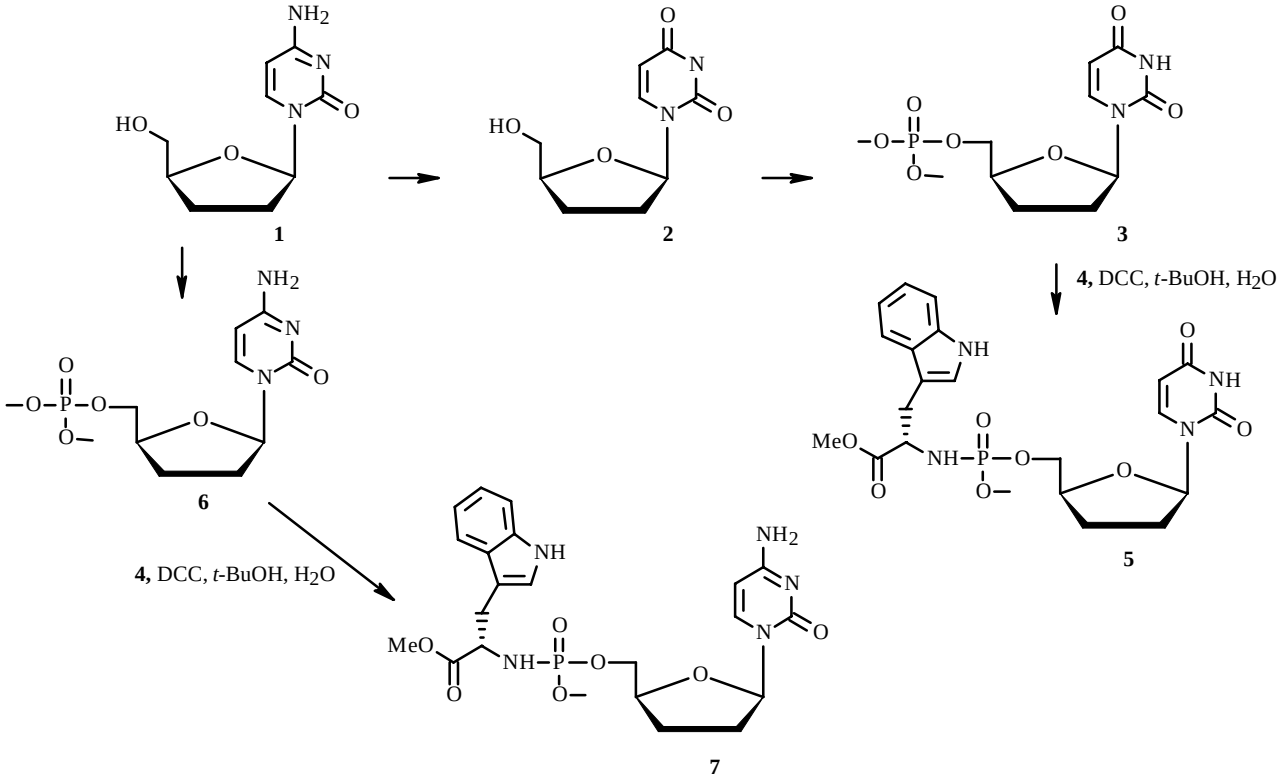
 0009-3130/06/4202-0208

The preparative scheme includes deamination of **1** by *Escherichia coli* BM-11 cells [6], which exhibit cytidinedeaminase (KF 3.5.4.5) activity, 5′-monophosphorylation of **1** and the product of its deamination ddU (**2**) by nucleosidephosphotransferase (KF 2.7.1.77) within *Erwinia herbicola* 47/3 cells [7], and, finally, chemical synthesis of the target products **5** and **7** by dicyclohexylcarbodiimide (DCC) mixed with ddU-5′-MP (**3**) or ddC-5′-MP (**6**) and **4** in aqueous *t*-butanol according to the modified procedure proposed in the literature [8].



The structures of the prepared compounds were confirmed by spectral data. Thus, PMR spectra of conjugates **5** and **7** contained signals for the carbohydrate fragment and the pyrimidine base, for the indole group at 6.9-7.8 ppm, and a signal for the *O*-methyl. The UV spectra of the synthesized compounds exhibited absorption bands near 220 nm, characteristic of tryptophan.

A study of the enzymatic deamination of **7** by bacterial cytidinedeaminase under the conditions described in the literature [6] showed that this conjugate is stable to cytidinedeaminase of *E. coli* because even traces of its deamination were not found after 24 h whereas starting **1** under these same conditions was transformed into **2** almost quantitatively after 2 h.

Thus, we prepared for the first time new conjugates of ddU and ddC with L-tryptophan methyl ester (**5** and **7**) and developed a microbiological method to prepare ddU-5′-MP (**3**) and ddC-5′-MP (**6**) starting from **1**. The synthesized **5** and **7** were sent for biotesting of their antiviral activity.

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. UV spectra of solutions were taken on a Specord M-400 instrument; PMR spectra, on a Bruker AC-200 NMR spectrometer at working frequency 200 M Hz with TMS internal standard. Biomass of *Erw. herbicola* 47/3 bacteria cells, which exhibit nucleosidephosphotransferase activity (capability to transfer phosphate from *p*-nitrophenylphosphate to nucleoside 5′-hydroxyl), were prepared as before [7]. Cells of *E. coli* BM-11, which possess high cytidinedeaminase activity, were grown as before [6]. The sorbent for preparative column chromatography was silica gel 60H (70-230 mesh, Merck). The course of reactions was monitored by TLC using Silufol-UV_{254} plates (Serva).

2′,3′-Dideoxyuridine (ddU) (2). A reaction mixture (11.0 mL) containing **1** (70.4 mg, 0.33 mol), Tris-HCl-buffer (30 mM, pH 7.25), and *E. coli* BM-11 cells (0.2% dry mass) was incubated and stirred on a magnetic stirrer for 3.5 h at 50°C. The course of the deamination was monitored using TLC of aliquots of the reaction mixture and $CHCl_3$:CH_3OH (4:1). Based on UV spectroscopy, the transformation of ddC into ddU was quantitative. After the reaction was complete, the mixture was evaporated, placed on a silica-gel column, and eluted by $CHCl_3$:CH_3OH (5:1, 200 mL). Fractions containing product were evaporated to afford **2** (66.1 mg, 94%), mp 113-115°C (acetone:diethylether), lit. [9] mp 116-117°C (acetone:diethylether). TLC, R_f 0.75 ($CHCl_3$:CH_3OH, 4:1). UV spectrum (dist. H_2O, λ, nm, ε): 262 max (9600), 232 min (3500).

PMR spectrum (CD_3OD, δ, ppm, J/Hz): 8.08 (1H, d, $J_{5,6}$ = 8, H-6), 6.04 (1H, m, H-1′), 5.70 (1H, d, $J_{5,6}$ = 8, H-5), 4.16 (1H, m, H-4′), 3.88 (1H, dd, $J_{4',5'}$ = 4, $J_{5',5''}$ = 11, H-5′), 3.68 (1H, dd, $J_{4',5''}$ = 4, $J_{5',5''}$ = 11, H-5″), 2.40 (1H, m, H-2′), 2.16-1.9 (3H, m, H-2″, H-3′, H-3″). The reaction mixture was used in the next step without work up or product isolation.

2′,3′-Dideoxyuridine-5′-monophosphate (3). A reaction mixture (11.0 mL) containing **2** (70.7 mg, 0.33 mM), *p*-nitrophenylphosphate (0.15 M), sodium-acetate buffer (0.2 M, pH 4.2), and *Erw. herbicola* 47/3 cells (0.5% dry mass) was incubated and stirred on a magnetic stirrer for 19 h at 35°C. The course of the reaction was monitored using TLC and $CHCl_3$:C_2H_5OH (4:1) and then *i*-propanol:conc. NH_4OH:H_2O (7:1:2). Based on UV data, the transformation of **2** into its 5′-monophosphate **3** was 49%. After the reaction was finished, cells were removed from the mixture by centrifugation (10,000 g, 5 min). The supernatant was placed on a column (10 mL) with Dowex 2×10 resin in the Cl^- form. Washing the column with NaCl solution (0.1 M) and HCl gradient (60 mL, 0→40 mM) produced **3**, which was then desalted over a column (2 mL) of activated carbon and eluted with ethanol (30%) containing ammonia (1 M). The effluent was evaporated. The resulting monophosphate **3** was converted to the H^+-form by a standard procedure (over Dowex 50W × 2 resin, 200-400 mesh in the H^+-form) to produce **3** (30 mg, 31%). TLC, R_f 0.18 (isopropanol:NH_4OH:H_2O, 7:1:2). UV spectrum (H_2O, λ, nm, ε): 262 max (9700), 230 min (3800).

2′,3′-Dideoxyuridine-5′-monophosphate-(L)-methoxytryptophylphosphoramidate (5). A solution of L-tryptophan methyl ester (**4**, 25 mg, 0.115 mmol) and **3** (16 mg, 0.05 mmol) in a mixture of *t*-butanol and water (4:1, 1 mL) was treated with DCC (30 mg, 0.135 mmol). The reaction mixture was heated at 65-70°C for 7 h with stirring on a magnetic stirrer. The course of the reaction was monitored by TLC. After the reaction was finished, the mixture was cooled, evaporated, and chromatographed over silica gel with elution by $CHCl_3$:CH_3OH:H_2O (40:10:2, 100 mL) and then $CHCl_3$:CH_3OH:H_2O (30:15:3, 250 mL) to produce **5** (20 mg, 71%, oil), R_f 0.39 ($CHCl_3$:CH_3OH:H_2O, 15:5:1). UV spectrum (H_2O, λ, nm, ε): 220 max (31,000), 266 max (9700), 205 min (22,000), 243 min (5300).

PMR spectrum (CD_3OD, δ, ppm, J/Hz): 7.92 (1H, d, $J_{5,6}$ = 8, H-6), 7.52 (1H, d, J = 8, H-4, indole), 7.30 (1H, d, J = 8, H-7, indole), 7.10 (1H, s, H-2, indole), 7.06 (1H, t, J = 8, H-6, indole), 6.98 (1H, t, J = 8, H-5, indole), 5.96 (1H, m, H-1′), 5.72 (1H, d, $J_{5,6}$ = 8, H-5), 4.12 (1H, m, $\underline{CH}CO_2CH_3$), 3.98 (1H, m, H-4′), 3.82 (1H, m, H-5′), 3.70 (1H, m, H-5″), 3.60 (3H, s, OCH_3), 3.16 (2H, d, J = 7, $\underline{CH_2}$-indole), 2.26 (1H, m, H-2′), 2.04-1.74 (3H, m, H-2″, H-3′, H-3″).

2′,3′-Dideoxycytidine-5′-monophosphate (6). A reaction mixture (20 mL) containing ddC (**1**, 84 mg, 0.4 mmol), *p*-nitrophenylphosphate (0.15 M), sodium-acetate buffer (0.2 M, pH 4.2), and *Erw. herbicola* 47/3 cells (0.5% dry mass) was incubated for 12 h at 35°C. After the reaction was finished (TLC), the target product **6** was isolated as described above for the synthesis of **3** to produce **6** (32 mg, 28%) Yield 43% according to UV spectroscopy, R_f 0.27 (isopropanol:NH_4OH:H_2O, 7:1:2). UV spectrum (H_2O, λ, nm, ε): 278 max (10,400), 243 min (2500).

2′,3′-Dideoxycytidine-5′-monophosphate-(L)-methoxytryptophylphosphoramidate (7). A solution of **4** (50 mg, 0.23 mmol) and **6** (50 mg, 0.15 mmol) in a mixture of *t*-butanol and water (4 mL, 4:1) was treated with DCC (30 mg, 0.135 mmol). The reaction mixture was heated at 65-70°C for 3 h with stirring on a magnetic stirrer and treated with another portion of DCC (30 mg, 0.135 mmol). The course of the reaction was monitored by TLC. After the reaction was finished, the reaction mixture was worked up analogously to **5** to produce **7** (62 mg, 70%, oil), R_f 0.23 ($CHCl_3$:CH_3OH:H_2O, 10:6:1), R_f 0.75 [$(CH_3)_2CHOH$:NH_4OH:H_2O, 7:1:2]. UV spectrum (H_2O, λ, nm, ε): 220 max (30,600), 248 min (5400), 272 max (10,000).

PMR spectrum (CD_3OD, δ, ppm, J/Hz): 8.06 (1H, d, $J_{5,6}$ = 8, H-6), 7.52 (1H, d, J = 8, H-4, indole), 7.30 (1H, d, J = 8, H-7, indole), 7.10 (1H, s, H-2, indole), 7.06 (1H, t, J = 8, H-6, indole), 6.96 (1H, t, J = 8, H-5, indole), 5.98 (1H, m, H-1′), 5.94 (1H, d, $J_{5,6}$ = 8, H-5), 4.12 (1H, m, $\underline{CH}CO_2CH_3$), 4.02 (1H, m, H-4′), 3.92 (1H, m, H-5′), 3.74 (1H, m, H-5″), 3.56 (3H, s, OCH_3), 3.16 (2H, d, J = 7, $\underline{CH_2}$-indole), 2.30 (1H, m, H-2′), 2.04-1.76 (3H, m, H-2″, H-3′, H-3″).

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