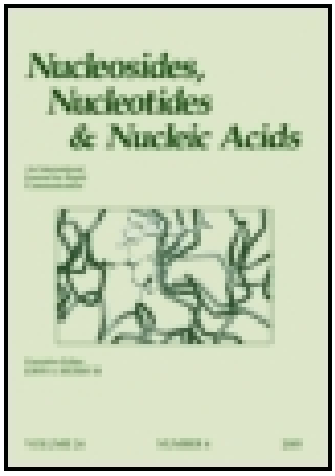

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Chemical-Enzymatic Synthesis of 3′-Amino-2′, 3′-dideoxy-β-D-ribofuranosides of Natural Heterocyclic Bases and Their 5′-Monophosphates

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CHEMICAL-ENZYMATIC SYNTHESIS OF 3'-AMINO-2',3'-DIDEOXY-β-D-RIBOFURANOSIDES OF NATURAL HETEROCYCLIC BASES AND THEIR 5'-MONOPHOSPHATES

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Abstract. Treatment of O^2,3'-anhydro-5'-O-trityl derivatives of thymidine (**1**) and 2'-deoxyuridine (**2**) with lithium azide in dimethylformamide at 150 °C resulted in the formation of the corresponding isomeric 3'-azido-2',3'-dideoxy-5'-O-trityl-β-D-ribofuranosyl N^1- (the major products) and N^3-nucleosides (**3/4** and **5/6**). 3'-Amino-2',3'-dideoxy-β-D-ribofuranosides of thymidine [Thd(3'NH_2)], uridine [dUrd(3'NH_2)], and cytidine [dCyd(3'NH_2)] were synthesized from the corresponding 3'-azido derivatives. The Thd(3'NH_2) and dUrd(3'NH_2) were used as donors of carbohydrate moiety in the reaction of enzymatic transglycosylation of adenine and guanine to afford dAdo(3'NH_2) and dGuo(3'NH_2). The substrate activity of dN(3'NH_2) *vs.* nucleoside phosphotransferase of the whole cells of *Erwinia herbicola* was studied.

INTRODUCTION

The synthesis of 3'-amino-2',3'-dideoxynucleosides [dN(3'NH_2)] from 3'-azido derivatives is generally straightforward, since there are highly effective and simple methods for conversion of an azido

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This paper is dedicated to the late Professor R.K. Robins.

group into an amino function in order to obtain the desired compounds (reviewed in [1]; for recent publications, see [2-5]). In turn, for the synthesis of 3'-azido-2',3'-dideoxynucleosides of natural pyrimidine and purine bases several methods and their modifications have been suggested. One of them consists in the use of 3'-azido-3'-deoxy-thymidine or its derivatives as donors of a carbohydrate moiety in the reaction of chemical transglycosylation of adenine and guanine derivatives [6,7]. It should be noted that the methods for the synthesis of 3'-azido-3'-deoxythymidine from thymidine *via* O^2,3'-anhydronucleoside route are rather efficient (*e.g.* [8], and Refs therein). On the contrary, transglycosylation of purine bases results in the formation of complex reaction mixtures from which the desired β-nucleosides were isolated in a yield of 20-30%. The other approach is concerned with the use of an universal sugar precursor, methyl 3-azido-2,3-dideoxy-5-O-p-toluoyl-D-ribofuranoside [9] and related compound [10] for the synthesis of both pyrimidine and purine nucleosides [10,11]. A drawback of this approach is, in the first place, a rather laborious synthesis of glycosylating agents. Besides, the glycosylation of pyrimidine bases gave the mixtures of β- and α-anomers in a ratio of ≈2:1 (in 50-60% combined yield) and moreover the attempts to separate the anomeric mixture of cytosine nucleoside into individual compounds have failed [11]. Quite unexpectedly, the formation of N^9-β-purine nucleosides as the sole products of an analogous reaction of glycosylation of purine bases have been observed, albeit the desired compounds were isolated in a low yield [11].

In recent years, considerable success has been achieved in the synthesis of modified nucleosides using a combination of chemical and enzyme-catalyzed procedures (for reviews, see Refs [12,13]; some recent publications are also noted [14-18]). With this in mind, the present study was undertaken, first of all, to develop an enzymatic synthesis of dAdo(3'NH_2) and dGuo(3'NH_2) using Thd(3'NH_2) or dUrd(3'NH_2) as donors of the carbohydrate moiety in the reaction of enzymatic transglycosylation of adenine and guanine. The required Thd(3'NH_2) and dUrd(3'NH_2) were prepared from thymidine and 2'-deoxyuridine, respectively. The scheme of the synthesis was modified at the stage of O^2,3'-anhydroring closure

and a detailed study on transformation of O^2,3'-anhydronucleosides under the action of lithium azide was performed. A preliminary account of this work has been published [19]. Finally, we have studied the substrate activity of 3'-amino-2',3'-dideoxynucleosides in relation to the nucleoside phosphotransferase of the whole cells of *Erwinia herbicola*.

EXPERIMENTAL

General. The UV spectra were recorded on a Specord UV-VIS spectrometer (Carl Zeiss, Germany). ^1H NMR spectra were recorded with a Bruker WM 360 spectrometer (Bruker, Germany) with tetramethylsilane as an internal standard (s = singlet; d = doublet; t = triplet; m = multiplet; br.s = broad signal). Chemical shifts (δ) are reported in ppm, J values are given in Hz. The CD spectra were recorded on a J-20 spectropolarimeter (JASCO, Japan) in H_2O (pH 7.0). Thin layer chromatography (TLC) was performed on a silica gel [A] Silufol UV_{254} (Kavalier, Czechoslovakia) and [B] Kieselgel 60 F_{254} (Merck, Germany). As solvent systems were used (v/v): chloroform/ethanol, 18:1 (1); chloroform/ethanol, 10:1 (2); chloroform/ethanol, 5:1 (3); isopropanol/25% aq. ammonia/water, 7:1:2 (4); chloroform/ethanol, 40:1 (5). Silica gel L, 40/100 μ (Czechoslovakia) was used for column chromatography. Melting points were determined with a Boethius (Germany) apparatus and are uncorrected. Lithium azide was prepared according to [20]. The reactions were performed at room temperature, unless stated otherwise.

Glutaraldehyde-treated cells of *Escherichia coli* BM-11 were obtained as described [21]. The strain *E. coli* BMT-38 was selected according to [22] and the biomass was prepared as described for *E. coli* BM-11 cells. The biomass of *Erw. herbicola* 47/3 cells was prepared as described [23,24] and the synthesis of dNMP(3'NH_2) was monitored as described previously [25]. In brief, the reaction mixture for 5'-monophosphorylation of nucleosides (1 mL) contained 15 mM nucleoside, 90 mM p-nitrophenylphosphate, 0.2 M sodium acetate buffer (pH 4.5), and wet paste of intact cells (10 mg, calculated as abs. dry weight). The mixture was incubated at 40 ^0C with gentle stirring. Aliquots of the reaction medium were analyzed by the TLC on Silufol UV_{254} plates (isopropanol/25% aq. ammonia/water, 7:2:1,

Table. Synthesis of 5'-Monophosphates of 3'-Amino-2',3'-dideoxy-β-D-ribofuranosides of Thymine, Cytosine, Adenine, and Guanine by the whole Cells of *Erw. herbicola*[a)]

Initial compound	Phosphotransferase activity of *Erw. herbicola* cells[b)]		Max. degree of conversion to 5'-monophosphate (%)		Time of obtaining max. conversion (h)	
	nmol/min·mg[c)]	rel. to the corresp. natl 2'-deoxynucl.				
Thd($3'NH_2$) **13**	22.8	153	73		2.0	
Thd	14.9			82		3.0
dCyd($3'NH_2$) **15**	26.0	218	76		1.5	
dCyd	11.9			78		3.0
dAdo($3'NH_2$) **16**	23.3	224	75		2.5	
dAdo	10.4			65		3.5
dGuo($3'NH_2$) **17**	20.0	74	62		2.5	
dGuo	27.0			73		1.0

a) The values presented are mean values of 4-5 experiments.
b) The initial rate values of the reactions were analyzed; p-nitrophenylphosphate was employed as a phosphate group donor.
c) The activity was measured as the rate of 5'-monophosphate formation and expressed as nmole of product formed per min per mg of dry cells [25].

v/v). The starting nucleoside and its 5'-monophosphate were extracted with 50 mM potassium phosphate buffer (pH 7.0) and quantified spectrophotometrically using a molar extinction coefficient for long-wavelength band. The data are presented in Table. Enzymatic dephosphorylation of 5'-mono-phosphates under the action of alkaline phosphatase (Boehringer, Germany) [24] gave quantitatively the corresponding aminodeoxy nucleosides (TLC).

The O^2,3'-anhydronucleosides **1** and **2** were synthesized in high yields from 5'-O-trityl-thymidine and -2'-deoxyuridine, respectively, by treatment with morpholinosulfur trifluoride (MSTF) in dry dioxane as described previously [26-28].

Interaction of O^2,3'-anhydro-5'-O-tritylthymidine (1) with lithium azide. A mixture of **1** (2.2 g, 4.7 mmol) and lithium azide (1.15 g, 23.5 mmol) in 10 mL of anhydrous freshly distilled DMF was heated at 150 ^{0}C for 3 h and evaporated *in vacuo*. The residue was treated with chloroform (100 mL), insoluble material was filtered off, washed with chloroform (100 mL) and combined filtrates were evaporated. The residue was purified by column chromatography on silica gel (80 mL) using chloroform as eluent to yield the mixture of isomers **3** and **4** (2.0 g; 83%) as a syrup. This mixture was dissolved in 80% acetic acid (25 mL), kept at 55 ^{0}C for 4 h, cooled to 4 ^{0}C and kept for 12 h. Trityl alcohol precipitated was filtered off, filtrate was evaporated and the residue was chromatographed on a silica gel column (110 mL), eluting with chloroform (150 mL) and then with a linear ethanol gradient (0-10%, v/v; 2 x 330 mL) in chloroform. The fractions, containing the individual products, were collected and evaporated to yield 0.10 g (8%) of 3-(3-azido-2,3-dideoxy-β-D-ribofuranosyl)thymine (**8**) as a syrup that could not be obtained in crystalline form {R_f 0.17 (A, double elution in system 5); UV, λ_{max} nm ($\varepsilon \cdot 10^{-3}$): (pH 7.0), 209 (7.0), 272 (6.6); (pH 12.0), 299 (7.2); CD, λ nm ($[\Theta] \cdot 10^{-3}$): 220 (-1.1), 248 (+3.3), 275 (-1.95); λ nm, [Θ] = 0: 228, 263; ^{1}H NMR (DMSO-d_6): 10.90 (br.s, 1H, NH), 7.28 (br.s, 1H, H-6), 6.54 (dd, 1H, $J_{1',2'}$ = 4.8, $J_{1',2''}$ = 9.0, H-1'), 4.79 (t, 1H, OH), 4.41 (dt, 1H, $J_{2',3'}$ = 9.0, $J_{2'',3'}$ = 6.6, $J_{3',4'}$ = 6.0, H-3'), 3.73 (dt, 1H, $J_{4',5'}$ = $J_{4',5''}$ = 6.0, H-4'), 3.55 (m, 2H, H-5', H-5''), 2.79 (m, 1H, $J_{2',2''}$ = 13.2, H-2'), 2.17 (m, 1H, H-2'')} and 0.85 g (81%) of 3'-azido-3'-deoxythymidine (**7**), m.p. 121-122 ^{0}C (after crystallization from ethanol)[reported [29], m.p. 120-122 ^{0}C (from water)]; R_f 0.14 (A, double elution in system 5); UV, λ_{max} nm ($\varepsilon \cdot 10^{-3}$): (pH 7.0), 209 (9.5), 268 (9.7); (pH 12.0), 209, 268; CD, λ nm ($[\Theta] \cdot 10^{-3}$): 274 (+6.1); λ nm, [Θ] = 0: 250; ^{1}H NMR (DMSO-d_6): 9.90 (br.s, 1H, NH), 7.67 (d, 1H, $J_{6,Me}$ = 1.1, H-6), 6.09 (t, 1H, $J_{1',2'}$ = $J_{1',2''}$ = 6.3, H-1'), 5.23 (t, 1H, OH), 4.40 (dt, 1H, $J_{2',3'}$ = 6.6, $J_{2'',3'}$ = $J_{3',4'}$ = 5.1, H-3'), 3.83 (dt, 1H, $J_{4',5'}$ = $J_{4',5''}$ = 4.2, H-4'), 3.62 (m, 2H, H-5', H-5''), 2.37 (m, 1H, $J_{2',2''}$ = 13.8, H-2'), 2.28 (m, 1H, H-2'').

Interaction of O^2,3'-anhydro-5'-O-trityl-2'-deoxyuridine (2) with lithium azide. The mixture of blocked nucleosides **5** and **6** (0.45 g) was obtained as described above starting from 0.42 g

(0.93 mmol) of **2** after treatment with chloroform. This mixture was separated into the individual isomers by chromatography on a column of silica gel (60 mL) using chloroform as eluent to yield 0.3 g (65%) of **5**, m.p. 176-178 °C (from ethanol){reported [30], m.p. 175-176 °C (from ethanol); R_f 0.28 (A, 5)} and 60 mg (13%) of **6** as a syrup, R_f 0.25 (A, 5). Both compounds were detritylated as described above to yield, respectively, 0.13 g (85%) of N^1-isomer **9**, m.p. 166-167 °C (from acetone){reported [31], m.p. 164-166 °C (from acetone); {R_f 0.30 (A, double elution in system 1); UV, λ_{max} nm ($\varepsilon \cdot 10^{-3}$): (pH 7.0), 208 (9.1), 262 (10.1); (pH 12.0), 208, 262 ; CD, λ nm ($[\Theta] \cdot 10^{-3}$): 240 (-1.74), 267 (+10.2); λ nm, $[\Theta]$ = 0: 248; ^{1}H NMR (D_2O): 7.79 (d, 1H, $J_{5,6}$ = 8.0, H-6), 6.15 (t, 1H, $J_{1',2'} = J_{1',2''}$ = 6.3, H-1'), 5.83 (d, 1H, H-5), 4.31 (dt, 1H, $J_{2',3'} = J_{2'',3'} = J_{3',4'}$ = 6.0, H-3'), 3.99 (m, 1H, $J_{4',5'}$ = 3.6, $J_{4',5''}$ = 4.8, H-4'), 3.82 (dd, 1H, $J_{5',5''}$ = 12.0, H-5'), 3.78 (dd, 1H, H-5"), 2.50 (m, 2H, H-2', H-2")} and 25 mg (82%) of N^3-isomer **10** as a chromatographically homogeneous syrup that failed to crystallize, {R_f 0.33 (A, double elution in system 1); UV, λ_{max} nm ($\varepsilon \cdot 10^{-3}$): (pH 7.0), 207 (7.5), 264 (6.6); (pH 12.0), 208, 294 (9.1); CD, λ nm ($[\Theta] \cdot 10^{-3}$): 215 (-1.5), 245 (+4.1), 274 (-1.85); λ nm, $[\Theta]$ = 0: 222, 263; ^{1}H NMR (D_2O): 7.41 (d, 1H, $J_{5,6}$ = 7.8, H-6), 6.60 (dd, 1H, $J_{1',2'}$ = 4.8, $J_{1',2''}$ = 9.0, H-1'), 5.74 (d, 1H, H-5), 4.41 (dt, 1H, $J_{2',3'}$ = 9.0, $J_{2'',3'}$ = 6.6, $J_{3',4'}$ = 6.0, H-3'), 3.88 (m, 1H, $J_{4',5'}$ = 3.6, $J_{4',5''}$ = 6.0, H-4'), 3.80 (dd, 1H, $J_{5',5''}$ = 12.6, H-5'), 3.73 (dd, 1H, H-5"), 2.87 (m, 1H, $J_{2',2''}$ = 13.2, H-2',) 2.33 (m, 1H, H-2")}.

3'-Amino-3'-deoxythymidine (13), 3'-amino-2',3'-dideoxyuridine (14), and -cytidine (15). Compound **11** was obtained as described [32] starting from **5**. Standard detritylation of **11** (80% acetic acid; 55 °C; 5 h) and subsequent chromatography on a Dowex 1 x 8 (OH^--form) ion-exchange resin eluting with water gave **12** in 58% overall yield.

Azides **7**, **9**, and **12** were reduced by treatment with Ph_3P/pyridine/aq. NH_3 to give the corresponding aminodeoxy nucleosides **13** (78%), **14** (67%), and **15** (68%). The physical properties of all compounds were in agreement with these recorded in the literature [28, 30, 31, 33].

3'-Amino-2',3'-dideoxyadenosine (16). To a suspension of **13** (477 mg, 1.98 mmol), adenine (0.8 g, 5.92 mmol) and glutaraldehyde-treated cells of *E. coli* BM-11 (3.0 g, dry wt.) in 150 mL 50 mM Na-acetate buffer (pH 6.75), 50 mL of 20 mM phosphate buffer (pH 6.75) were added and the mixture was incubated at 50 °C for 24 h. Cells were removed by centrifugation (5,000 x g, 5 min), the supernatant was diluted two-fold with water and loaded onto a column (60 mL) with Dowex AG 50W x 8 (H^+-form) ion-exchange resin. The column was eluted with a linear ammonium hydroxide gradient (0.0-0.5 M, 2 x 600 mL). The fractions, containing amine **16**, were collected, evaporated and crystallized from ethanol to give 228 mg (46%; referred to the starting compound **13**) of **16**, m.p. 184-186 °C (reported [6], m.p. 188-189 °C (from ethanol)); R_f 0.70 (B, 4); UV, λ_{max} nm ($\varepsilon \cdot 10^{-3}$): (pH 7.0), 259 (15.0); (pH 1.0), 257 (14.7); ^{1}H NMR (DMSO-d_6): 8.37 (s, 1H, H-2), 8.15 (s, 1H, H-8), 7.34 (br.s, 2H, NH_2-6), 5.99 (t, 1H, $J_{1',2'} = J_{1',2''} = 6.5$, H-1'), 5.36 (br.s, 1H, OH), 4.13 (m, 1H, $J_{3',4'} = 4.5$, H-4') 4.07 (m, 1H, $J_{2',3'} = J_{2'',3'} = 7.0$, H-3'), 3.66 (m, 4H, H-5', H-5", NH_2-3'), 2.90 (m, 1H, $J_{2',2''} = 14.0$, H-2"), 2.38 (m, 1H, H-2')

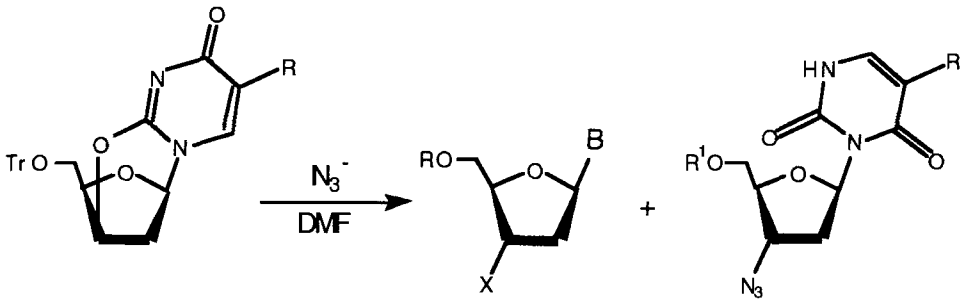
The fractions, containing the starting compound **13**, were collected, evaporated, dissolved in water and loaded onto a column (50 mL) with Dowex 1 x 8 (HCO_3^--form) ion-exchange resin. Elution with water (600 mL), followed by crystallization from ethanol gave 110 mg (0.457 mmol) of **13**.

3'-Amino-2',3'-dideoxyguanosine (17). To a suspension of **13** (250 mg, 1.04 mmol), guanosine (320 mg, 1.13 mmol) and *E.coli* BMT-38 cells (2 g, dry wt.) in 25 mL 50 mM Na-acetate buffer (pH 6.75), 5 mL of 30 mM phosphate buffer (pH 6.75) were added and the mixture was incubated at 50 °C for 28 h. Cells were removed by centrifugation (5,000 x g, 10 min), ethanol (20 mL) and silica gel (15 mL) were added to the supernatant, evaporated and silica gel with the products was placed onto the top of a column with silica gel (70 mL) which was preequilibrated in chloroform-methanol (24:1, v/v). Elution with a linear methanol gradient (4-20%, v/v; 2 x 500 mL) in chloroform, and then with a linear gradient of chloroform/methanol (4:1, v/v) in methanol/25% aq. ammonia hydroxide (9:1, v/v; 2 x 300

mL) gave 56.7 mg (20.5%; referred to the starting compound **13**) of **17**, m.p. ≈250 °C (decomp., from water) {reported [6], m.p. 210 °C, decomp., from water; R_f 0.54 (B, 4); UV, λ_{max} nm ($\varepsilon \cdot 10^{-3}$): (pH 7.0), 253 (13.2); (pH 12.0), 265 (11.3); (pH 1.0), 255.5 (12.1); ^{1}H NMR (DMSO-d_6): 7.91 (s, 1H, H-8), 6.58 (br.s, 2H, NH_2-2), 6.06 (t, 1H, $J_{1',2'} = J_{1',2''} = 6.6$, H-1'), 5.00 (br.s, 1H, OH), 3.58 (m, 6H, H-3', H-4', H-5', H-5", NH_2-3'), 2.41 (m, 1H, $J_{2'',3'} = 6.0$, $J_{2',2''} = 13.2$, H-2"), 2.13 (m, 1H, $J_{2',3'} = 6.0$, H-2')} and 147 mg (0.61 mmol) of the starting nucleoside **13**.

RESULTS AND DISCUSSION

We have recently shown that the treatment of 5'-O-trityl derivatives of thymidine and 2'-deoxyuridine with morpholinosulfur trifluoride (MSTF) or diethylaminosulfur trifluoride (DAST) in dry dioxane at room temperature results in the formation of the corresponding O^2,3'-anhydronucleosides **1** and **2** in a yield of more than 90% [26-28](*cf.* [34]). Application of this method permits considerable simplification of the scheme of synthesis of pyrimidine 3'-azido-2',3'-dideoxynucleosides. Treatment of the compounds **1** and **2** with lithium azide in anhydrous DMF at 150 °C , surprisingly, gave the mixtures of isomeric nucleosides **3** and **4** (the ratio of **3**/**4** was 9:1 and the combined yield of 83%), and **5** and **6** (5:1; 78%), respectively. We have failed to separate the mixture of isomers **3** and **4** into individual compounds. Therefore, the mixture was detritylated and then chromatographed on silica gel to afford 3'-azido-3'-deoxythymidine (**7**) and its N^3-isomer **8**. On the contrary, the compounds **5** and **6** were obtained after chromatography on silica gel and their detritylation gave the corresponding uracil nucleosides **9** and **10**. The structure of isomeric pairs of compounds **7**/**8** and **9**/**10** was confirmed by ^{1}H NMR analysis and by UV- and CD-spectroscopy (see Experimental). By going from N^1- to N^3-isomers the H1' and H2' resonance signals are shifted downfield due to an additional deshielding effect of 4-keto group. The $\Delta\delta$ variations observed in the pentofuranose fragment in going from N^1- to N^3-isomer show close agreement for both pairs of compounds. Further, the UV and CD data for N^3-isomers **8** and **10** were found to be in good coincidence with those for the related compounds [35].



1, R = Me
2, R = H

Compd	B	R	X
3	Thy	Tr	N_3
5	Ura	Tr	N_3
7	Thy	H	N_3
9	Ura	H	N_3
11	Cyt	Tr	N_3
12	Cyt	H	N_3
13	Thy	H	NH_2
14	Ura	H	NH_2
15	Cyt	H	NH_2

Compd	R	R^1
4	Me	Tr
6	H	Tr
8	Me	H
10	H	H

Chart 1

A thorough study of the reaction of 3'-O-mesyl-5'-O-trityl-thymidine, 2'-deoxy-3'-O-mesyl-5'-O-trityl-uridine, -5-iodouridine and -5-fluorouridine with lithium or sodium azide in DMF at different temperatures has revealed the $N^1 \rightarrow N^3$ isomerization only in the case of 5-halide derivatives [31]. It has been suggested that the reaction of the latter compounds proceeds preferentially *via* the O^2,3'-anhydro intermediates. Of interest is the fact that in all studied conditions the $N^1 \rightarrow N^3$ isomerization has not been accompanied by subsequent opening of O^2,3'-anhydroring to give the compounds like **4** and **6**. The authors suggested that the O^2,3'-anhydro intermediates like **1** and **2** transform into O^2,3'-anhydro-3-(2,3-dideoxy-5-O-trityl-β-D-*threo*-pentofuranosyl)-5-halideuracil in consequence of initial protonation of the base. In our cases such an acidic catalysis is not possible. We assume, therefore, the possibility of the $N^1 \rightarrow N^3$ isomerization *via* the nucleophilic attack

of the azide anion at C1' followed by reclosure of the glycosidic bond with the formation of initial N^1-(O^2,3'-anhydro) nucleosides **1** and **2**, and the corresponding isomeric N^3 nucleosides and release of an azide anion (*cf.* [31]). An attack of the azide anion at C3' of both pairs of N^1- and N^3-(O^2,3'-anhydro) nucleosides resulted in formation of isomeric compounds **3/4** and **5/6**. The supposed mechanism of $N^1 \rightarrow N^3$ isomerization was substantiated by the results of an analogous reaction of O^2,3'-anhydro-2'-deoxy-5'-O-tritylcytidine with lithium azide [28]. In this case we have observed the formation of expected product, 3'-azido-2',3'-dideoxy-5'-O-tritylcytidine (**11**), as well as 3-O-(4-amino-1,3-pyrimidin-2-yl)-5-O-trityl-2-deoxy-α-D-*threo*-pentofuranosyl azide resulting from the nucleophilic attack of an azide anion at C1'. The structure of the former was proved by an alternative synthesis starting from **5** according to the procedure described by Divakar and Reese [32].

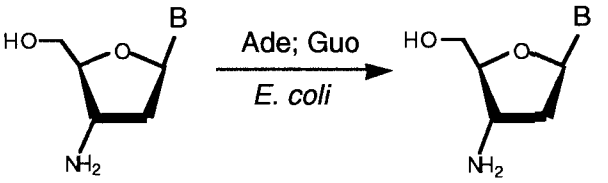
The compounds **7**, **9**, and **12** were converted into the corresponding aminodeoxy nucleosides **13**, **14**, and **15** by the treatment with triphenylphosphine/pyridine/ammonia [36].

During recent years, a large number of analogs of purine nucleosides have been synthesized by using the enzymatic transglycosylation methods. In general, two types of enzymes have been used as catalysts: *trans*-N-deoxyribosylases and nucleoside phosphorylases. The former enzymes catalyze the exchange of the deoxyribosyl moiety between 2'-deoxyribonucleosides and purine or pyrimidine bases with the intervention of a deoxyribosyl-enzyme intermediate [37,38]. Recently, this transglycosylation reaction has been extended to the preparation of purine 2',3'-dideoxynucleosides [39]. It was simultaneously found that no product formation occurs using 3'-azido- or 3'-amino-3'-deoxythymidine as donors of carbohydrate moiety and adenine as an acceptor.

In contrast to the *trans*-N-deoxyribosylases, the pentofuranosyl transfer reaction catalyzed by nucleoside phosphorylases has been shown to involve generation *in situ* of D-pentofuranose-1-α-phosphate (PFP)(detailed discussion see [40]). Two methods have successfully been employed for the preparation of purine nucleosides by using the pyrimidine nucleosides as donors of

pentofuranose moiety and purine bases as acceptors. One of these makes use of the purified enzymes, *viz.*, uridine (UPase) or thymidine (TPase) phosphorylase that catalyze the phosphorolysis of a pyrimidine nucleoside with the formation of PFP, and purine nucleoside phosphorylase (PNPase) catalyzes the synthesis of a purine nucleoside from PFP generated and a purine base [17,40-42]. The second method is based on the use of the whole bacterial cells displaying UPase and/or TPase and PNPase activities as a biocatalyst [14,15,18,21,43-52].

It is evident that the substrate specificity of the enzymes involved in transglycosylation process is the crucial factor. We have found that 3'-azido-3'-deoxythymidine (**7**) and 3'-azido-2',3'-dideoxyuridine (**9**) are essentially devoid of substrate activity in the phosphorolysis reactions catalyzed by UPase and/or TPase of different bacterial strains of *E.coli*. At the same time, it has been shown that TPase purified from *E.coli* catalyzes the transfer of aminodeoxypentosyl moiety of Thd(3'NH_2) to 5-fluoro-, -chloro- and -bromo-uracil, albeit with a low efficiency [53]. Further, from the substrate specificity of TPase or/and UPase and PNPase of the whole cells of *E.coli* BM-11 selected by us [50], it was reasoned that this biocatalyst may be used for the synthesis of dAdo(3'NH_2) and dGuo(3'NH_2). It was also shown that the utilization of glutaraldehyde-treated cells offers some experimental advantages over unmodified *E.coli* BM-11 cells [21,54]. Taking these data into account, we initially studied synthesis of dAdo(3'NH_2) from Thd(3'NH_2) and adenine catalyzed by glutaraldehyde-treated cells of *E.coli* BM-11 under the experimental conditions optimized earlier for the preparation of 9-(β-D-arabinofuranosyl)guanine [21]. Incubation of Thd(3'NH_2) and adenine (molar ratio was 3:1) in the presence of glutaraldehyde-treated cells in 50 mM Na-acetate buffer (pH 6.75) and 5 to 10 mM phosphate at 50 °C for 25 h, followed by subsequent withdrawal of cells by centrifugation and ion-exchange chromatography of the supernatant, gave dAdo(3'NH_2) in 60% isolated yield based on the consumed starting nucleoside. One-fifth of the initial amount of Thd(3'NH_2) was recovered from the reaction mixture.



13 , B = Thy
14, B = Ura

16 , B = Ade
17 , B = Gua

Chart 2

As demonstrated earlier, the use of guanine as an acceptor in the transglycosylation reaction was not successful due to its very low solubility in the buffer solutions employed [21,46]. Preference has been given to guanosine or 2'-deoxyguanosine which were subjected to phosphorolysis by PNPase to generate *in situ* guanine [20,45]. Unexpectedly, when adenine was replaced by guanosine in the above transglycosylation reaction, dGuo(3'NH_2) was formed in a very low yield by the utilization of both unmodified or glutaraldehyde-treated cells. We have, therefore, selected a new strain of *E.coli*, BMT-38, which turned out to be more effective for the synthesis of dGuo(3'NH_2). Thus, incubation of Thd(3'NH_2) and guanosine (molar ratio was 1:1.09) in the presence of unmodified cells of *E.coli* BMT-38 under the conditions described for the synthesis of dAdo(3'NH_2), followed by silica gel column chromatography, afforded dGuo(3'NH_2) in 50% isolated yield based on the consumed starting nucleoside, part of which (≈60%) was also recovered. One of the most unexpected findings was that an increase of guanosine amount with respect to Thd(3'NH_2) does not result in a higher yield of dGuo(3'NH_2) under the reaction conditions studied. It should also be noted that the use of dUrd(3'NH_2) as a donor of carbohydrate moiety in the synthesis of both dAdo(3'NH_2) and dGuo(3'NH_2) provides similar results.

Recently, we have reported on the enzymatic synthesis of nucleoside 5'-monophosphates by using the whole cells of *Erw. herbicola* 47/3 for conversion of natural nucleosides and their fluorodeoxy analogs into 5'-monophosphates [25]. In the present paper, this work has been further extended to the dN(3'NH_2) synthesized in order to evaluate the role of a substituent at C3' in

the nucleoside phosphotransferase-catalyzed phosphorylation. The results of 5'-monophosphate synthesis are listed in Table. Natural 2'-deoxyribonucleosides have been examined earlier [25] and are included in Table for comparative purposes. The most interesting finding is that substitution of an amino function for the hydroxyl group at C3' leads to some increase of the effectiveness of 5'-monophosphate synthesis, except for dGuo(3'NH_2)(*cf.* [25]). We have also found that 3'-deoxythymidine and 3'-deoxy-2',3'-didehydrothymidine are good substrates for nucleoside phosphotransferase of *Erw. herbicola* (Zinchenko *et al.*, unpublished observation). These results tend to confirm our previous observation [25] that the substituents at C2' and C3' play a minor role in the reaction under consideration.

In conclusion, the transglycosylation method described in this communication is shown to be rather efficient for obtaining purine 3'-amino-2',3'-dideoxy-β-D-ribofuranosides using readily available by chemical methods corresponding pyrimidine nucleosides. It was found that the reaction of **1** and **2** with lithium azide in DMF leads to the formation of the corresponding isomeric N^1 (the major products) and N^3 3'-azido-2',3'-dideoxy-5'-O-trityl-β-D-ribofuranosides. It is evident from the present study that the whole cells of *Erw. herbicola* 47/3 might be used for the synthesis of dNMP(3'NH_2). This method represents an advantageous alternative to the chemical syntheses.

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