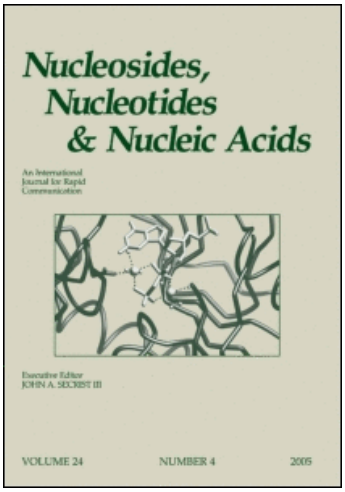

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A Novel Approach to Synthesis of 2'-Deoxy-β-D-Ribonucleosedes *Via* Transglycosylation of 6-Oxopurine Ribonucleosides

Jerzy Boryski[a]
[a] Institute of Bioorganic Chemistry, Polish Academy of Sciences ul., Poznań, Poland

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A NOVEL APPROACH TO SYNTHESIS OF 2'-DEOXY-β-D-RIBONUCLEOSIDES *VIA* TRANSGLYCOSYLATION OF 6-OXOPURINE RIBONUCLEOSIDES*

Jerzy Boryski

Institute of Bioorganic Chemistry, Polish Academy of Sciences
ul. Noskowskiego 12/14, PL-61704 Poznań, Poland

ABSTRACT: A novel method of synthesis of 2'-deoxy-β-D-ribonucleosides *via* trans-glycosylation of 6-oxopurine ribonucleosides is exemplified for conversion of inosine into 6-metylpurine 2'-deoxyriboside (**5**). The method offers high regio- and stereoselectivity as well as a good overall yield, and in these respects is superior to the fusion or anionic glycosylation procedures.

INTRODUCTION

The synthesis of β-D-ribonucleosides from a protected heterocyclic base and an appropriate sugar derivative proceeds stereoselectively in line with the Baker's rule of 1,2-*trans*-substitution.[1,2] Stereoselectivity in the ribo series can be explained by the formation of 1,2-cyclic acyloxonium sugar cation.[3] Therefore, due to the presence of 2-acyloxy group, the ribosylation of heterocyclic bases gives exclusively (or in prevailing amounts) the β-anomers of products. In the absence of 2-acyloxy group the formation of acyloxonium ions cannot take place, and 2-deoxysugars may form planar carboxonium ions. That is why the 2-deoxyribosylation of the S_N1 type leads to equimolar amounts of the α- and β-anomers. Their separation by crystallization or chromatography is usually difficult and time-consuming, and further reduces the already low yield of the synthesis.

The procedure of anionic glycosylation[4] offers much better results in the synthesis of 2'-deoxy-β-D-nucleosides. In this method, a protected α-chlorosugar reacts with the

* This paper is dedicated to Prof. Wolfgang Pfleiderer for the occasion of his 70th birthday.

anionic form of a heterocyclic base. The reaction is in line with the S_N2 mechanism, and gives the β-anomers of products. The anionic glycosylation, however, has some practical limitations, *i.e.* unstable and expensive α-chlorosugars, lower regioselectivity than in the glycosylation of the S_N1 type, and sometimes a partial lack of stereoselectivity.

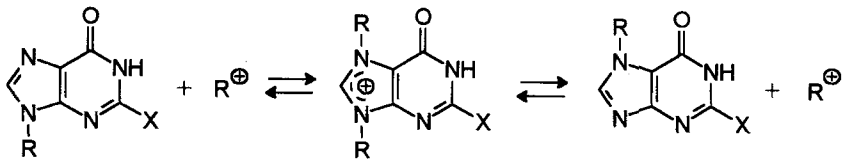
Synthetic 2'-deoxynucleosides are applied in chemistry, molecular biology and medicine, and there is still an increasing demand for new analogs of this type. For that reason, I would like to present a novel approach to the synthesis of that class of compounds. The approach combines: i) high stereoselectivity of glycosylation in the ribo series; ii) facility of 2'-deoxygenation due to the use of Markiewicz's protecting system[5] at the substrate level; iii) high yield of glycosylation, as anticipated for transglycosylation reactions of 6-oxopurine nucleosides.[6]

The transglycosylation method, in which the starting nucleoside serves as a sugar donor for glycosylation of another heterocyclic base, has been often used for synthesis of new nucleosides.[7-14] For example, heating of fully acetylated cytidine with an excess of N^2-acetylguanine in the presence of mercuric bromide gave tetraacetylguanosine and its 7-regioisomer in a moderate yield (21% and 20%, respectively).[7] However, the trans-glycosylation method does not seem to be an optimal synthetic route to 6-oxopurine nucleosides. Their unique and facile 7 $\rightleftarrows$ 9 isomerization[15-17] must be considered as an evidence that nucleosides of 6-oxopurines are thermodynamically less stable than nucleosides of other heterocyclic bases. Consequently, equilibrium of the exchange reaction is shifted towards other nucleosides (*e.g.* adenosine, cytidine) rather than towards 6-oxopurine ones. Therefore, in the transglycosylation approach, the 6-oxopurine nucleosides should not be considered as products, but as excellent substrates.

The idea of an efficient nucleoside synthesis from 6-oxopurine nucleosides is presented in SCHEME 1. Under appropriate conditions, *i.e.* in the presence of acidic catalysts or at high temperature (>200°C) without catalysts,[15-17] the starting nucleosides (general structure **1**) undergo a reversible 7 $\rightleftarrows$ 9 isomerization (Reaction I), which leads to the corresponding 7-isomers (**2**). The mechanism of that intermolecular chain process has been discussed recently.[6]

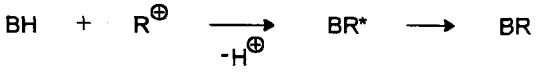
In the present approach, Reaction I serves as a source of the sugar cations (R^+). The 9- and 7-isomers (**1** and **2**, respectively) remain in a dynamic equilibrium, and their

I. 7 ⇄ 9 isomerisation of 6-oxopurine nucleoside:

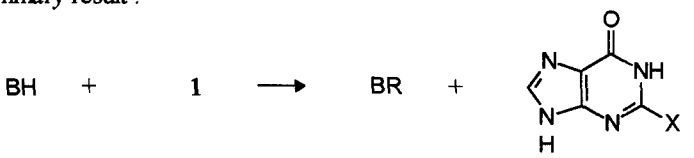


1 3 2

II. formation of a new nucleoside :



III. summary result :



X = H, NHAc 4

SCHEME 1

interconversion proceeds *via* a 7,9-diglycosylpurine intermediate (**3**).[6, 18, 19] A constant decomposition of **3** provides sugar cations, which are used for glycosylation of another heterocyclic base (Reaction II). In fact, glycosylation takes place in two steps: i) the formation of a kinetic glycosylation product (BR*); ii) its isomerization to a thermodynamic one (BR). Sites of glycosylation in BR* and BR, as well as facility of their interconversion, depend on the structure of the heterocyclic base (BH).[3] Nevertheless, in most cases (except that of 6-oxopurines), Reaction II represents an irreversible process, whereas Reaction I is fully reversible. As may be anticipated, an equilibrium of the exchange (Reaction III) should be entirely shifted towards the formation of a new nucleoside (BR) and liberation of 6-oxopurine (**4**). Therefore, Reaction III should be of a quantitative yield even at the equimolar amounts of substrates. It is worthy to note that

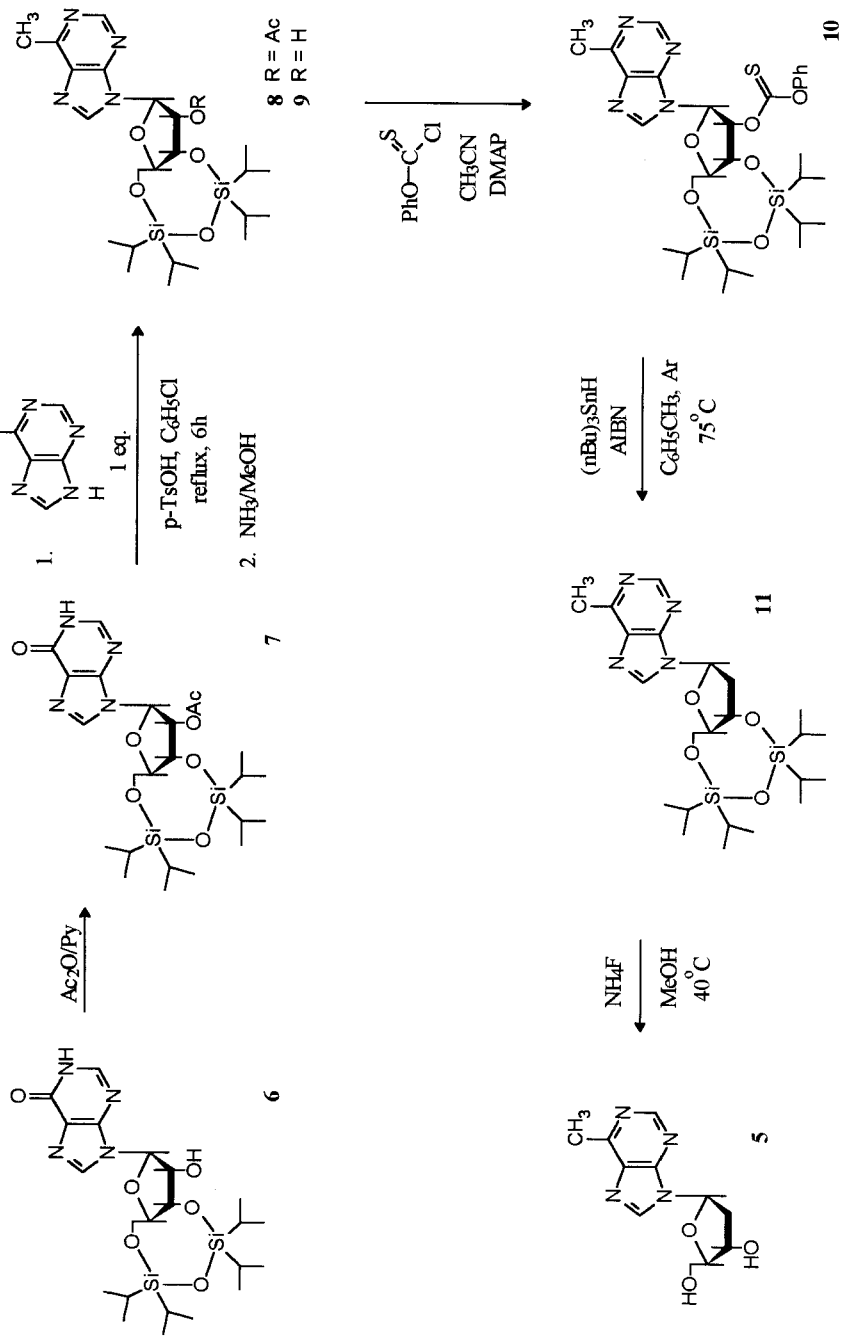
not only the 9-isomers of 6-oxopurine nucleosides (**1**), but also their 7-isomers (**2**) or their mixtures can be used as the starting material.

RESULTS AND DISCUSSION

In the present work the usefulness of the transglycosylation approach to the stereoselective synthesis of 2'-deoxy-β-D-nucleosides has been tested for transformation of inosine into 6-methylpurine 2'-deoxyriboside (MeP-dR, 6MPDR; **5**).[20] Recently, the latter nucleoside has found a promising application in the so-called 'suicide gene' therapy of colonic carcinoma. This compound is *in vitro* converted by *E. coli* purine nucleoside phosphorylase (PNP) into 6-methylpurine, an analog of adenine of high bystander toxicity.[21]

The novel synthesis of **5** is shown in SCHEME 2. Acetylation of 3',5'-O-(tetra-isopropyldisiloxane-1,3-diyl)inosine[5,22] with acetic anhydride in pyridine gave the 2'-acetyl derivative (**7**; 100%). This product may serve as a versatile substrate of a general use in the transglycosylation approach. In the present synthesis, transglycosylation of **7** with 1.0 eq. of 6-methylpurine in chlorobenzene containing 0.1 eq. of *p*-toluenesulfonic acid yielded quantitatively the protected riboside of 6-MePu (**8;** 91% after chromatographic purification) and hypoxanthine (**4**; X=H). Product **8** was then deacetylated in methanolic ammonia to afford the 2'-OH derivative (**9**), which on further reaction with phenoxythiocarbonyl chloride in acetonitrile containing 4-dimethylaminopyridine (DMAP) gave product **10** (88%). Reductive cleavage of 2'-O-phenoxythiocarbonyl group of **10** with tri-*n*-butyltin hydride in the presence of α,α'-aza-*bis*-isobutyronitrile (AIBN) according to Barton and McCombie[23-25] afforded the 2'-deoxy compound (**11**; 98%). The final deprotection, removal of the cyclic silyloxy group, was accomplished by treatment of **11** with ammonium fluoride in methanol[26] to give the 2'-deoxynucleoside **5** in 98%.

All steps of the described synthesis proceeded nearly quantitatively; the overall yield from 6-MePu was as high as 77%. Moreover, due to the presence of 2'-acetyloxy group in **7**, the transglycosylation step was fully stereoselective, and the formation of α-anomers was not observed at the further stages of the synthesis. High anomeric purity of the obtained compound was confirmed by proton magnetic resonance spectra (TABLE 1) and high performance liquid chromatography (HPLC).



SCHEME 2

TABLE 1. 300 MHz ^{1}H NMR Chemical shifts (ref. TMS, δ, ppm).[a]

Compd	N^{1}H	8-H	2-H	1'H	2'H	3'H	4'H	5'H	2'OR[e]	3'OH	4'OH	6-Me	iPr
6[b]	12.50[d] bs, 1	8.16 s, 1	7,99 s, 1	5.82 d, 1	4.44 td, 1	4.57 dd, 1	3,91-4.11 m, 3		5,71[d] d, 1	-	-	-	1.02 m, 28
7[b]	12.52[d] bs, 1	8.22 s,1	7.96 s, 1	6.09 d, 1	5.79 dd, 1	5.00 dd, 1	3.92-4.06 m, 3		2.11 s, 3	-	-	-	1.02 m, 28
8[c]	-	8.80 s, 1	8.20 s, 1	6.08 d, 1	5.82 d, 1	5.09 dd, 1	4.01-4.20 m, 3		2.18 s, 3	-	-	2.87 s, 3	1.08 m, 28
9[c]	-	8.79 s, 1	8.18 s, 1	6.03 d, 1	4.63 t, 1	5.13 dd, 1	4.01-4.16 m, 3		5.60[d] d, 1	-	-	2.87 s, 3	1.09 m, 28
10[c]	-	8.79 s, 1	8.19 s, 1	6.21 d, 1	6.40 dd, 1	5.38 dd, 1	4.04-4.21 m, 3		7.11-7.46 3 m, 2:1:2	-	-	2.87 s, 3	1.10 m, 28
11[c]	-	8.82 s, 1	8.27 s, 1	6.35 dd, 1	2.71 m, 2	5.00 q, 1	4.05 m, 2	3.91 m, 1	-	-	-	2.88 s, 3	1.07 m, 28
5[b]	-	8.78 s, 1	8.73 s, 1	6,47 t, 1	2.79 2.34 m, 1 m, 1	4.45 m, 1	3.89 dd, 1	3.62 3.53 m, 1 m,1	-	5.39[d] d, 1	5.04[d] t, 1	2.72 s, 3	-

[a] Figures following the observed multiplicities are numbers of protons as estimated by integration. [b] In $(CD_3)_2SO$. [c] In $CDCl_3$.

[d] Exchangeable with D_2O[b] or CD_3OD[c]. [e] R=H (**6**, **9**); CH_3CO (**7**, **8**); C_6H_5OCS (**10**).

In these respects the transglycosylation approach, at least for synthesis of **5**, is superior to the previously reported methods of synthesis of 2'-deoxy-β-D-nucleosides. Namely, the classical fusion reaction of 6-methylpurine and 1,3,5-tri-O-acetyl-2-deoxy-D-ribofuranose resulted in a mixture of 9α and 9β anomers, and the desired β-anomer was isolated in 13% yield.[27] More recently, coupling of the sodium salt of 6-MePU with 1-chloro-3,5-ditoluoyl-2-deoxy-α-D-ribofuranose gave a mixture of 9β, 9α, and 7β compounds in a ratio of *ca* 8:2:1, respectively; the overall yield for conversion of 6-MePu into **5** was 56%.[28] A similar yield (55%) was reported for synthesis of the deoxyribonucleoside **5** by enzymatic transglycosylation of deoxyuridine.[29,30] It is important to note that the 2'-OH product (**9**), still possessing 3',5'-cyclosilyloxy protection, may be easily modified at C2' to get new nucleoside analogs. The described 2'-deoxygenation is just an example of possible modifications.

EXPERIMENTAL

Melting points were determined on a Laboratory Devices Mel-Temp II macromelting points apparatus and are uncorrected. UV spectra were measured in methanol on a Beckman DU-65 spectrophotometer. ^{1}H NMR were recorded on a Varian Unity 300 FT NMR spectrometer with tetramethylsilane as an internal standard. Elemental analyses were performed on a Perkin-Elmer 240 Elemental Analyzer. TLC was conducted on Merck silica gel F_{254} 60 plates using the following solvent systems (measured by volume): A, chloroform - methanol (95:5); B, dichloromethane - ethanol (98:2); C, toluene - ethanol (4:1). For preparative short-column chromatography Merck TLC gel H 60 was used.

Analytical high performance liquid chromatography (HPLC) was performed using the following components from Waters Division of Millipore: Nova Pak C_{18} column (8 x 100 mm Radial-Pak Cartridge), 600E Multisolvent Delivery System with U6K Universal Liquid Chromatography Injector, 486 Tunable Absorbance Detector and 746 Data Module. 3',5'-O-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)inosine (**6**) was prepared according to Ref. 22.

2'-O-Acetyl-3',5'-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)inosine (7). The 3',5'-protected inosine (**6**; 3.2 g, 6.265 mmol) was dissolved in anhydrous pyridine (100 mL) and the solution was concentrated under reduced pressure to a volume of *ca* 50 mL.

Acetic anhydride (1.77 mL; 1.92 g, 18.8 mmol) was then added, and after 8 h of stirring at room temperature the reaction was quenched by addition of absolute methanol (20 mL). After 20 min the solvents were evaporated, the resulting oil was coevaporated with toluene - chloroform (2:1, 2 x 50 mL) and with absolute methanol (2 x 30 mL). Yield 3.47 g (100%) of a white solid foam. This preparation may be applied in the nucleoside synthesis without further purification. An analytical sample was crystallized from ethanol, mp 225° (dec). R_F 0.42(A); 0.15(B); 0.51(C). Anal. Calcd for $C_{24}H_{40}N_4O_7Si_2$ (522.78): %C, 52.15; %H, 7.29; %N, 10.14. Found: %C, 52.28; %H, 7.41; %N, 9.86. λ_{max} 247 nm.

6-Methyl-9-[2-O-acetyl-3,5-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl]purine (8). The fully protected derivative of inosine **7** (0.830 g, 1.5 mmol), 6-methylpurine (0.201 g, 1.5 mmol), and *p*-toluenesulfonic acid monohydrate (0.029 g, 0.15 mmol) were refluxed in chlorobenzene (25 mL; dried over molecular sieve 4A) for 6 h. The solvent was then removed by evaporation, and a resulting residue was treated with dichloromethane - ethanol (98:2). The precipitated hypoxanthine was filtered off and washed with dichloromethane. The filtrate and washings were combined and concentrated to an oil, which was chromatographed on a silica gel short column (5 x 6 cm) in a gradient of dichloromethane - ethanol (from 98:2 to 96:4). Fractions containing **8** were pooled and evaporated to a solid foam. Yield 0.752 g (91%). R_F 0.67(A); 0.38(B); 0.72(C). λ_{max} 244 nm (sh, ε 6800), 261 (7400).

6-Methyl-9-[3,5-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl]-purine (9) To a solution of **8** (0.720 g, 1.307 mmol) in methanol (30 mL) was added methanolic ammonia (6 mL; saturated at 0°C). After 3.5 h at room temperature the solution was concentrated in vacuo to afford **9** as a white solid foam. Yield 0.665 g (100%). R_F 0.50(A); 0.26(B); 0.65(C). Anal. Calcd for $C_{23}H_{40}N_4O_5Si_2$ (508.77): %C, 54.30; %H, 7.92; %N, 11.01. Found: %C, 54.38; %H, 7.72; %N, 10.80. λ_{max} 243 nm (sh, ε 6700), 261 (7300).

6-Methyl-9-[2-O-phenoxythiocarbonyl-3,5-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl]purine (10). Phenoxythiocarbonyl chloride (0.261 g, 1.51

mmol) was added with the aid of syringe to a solution of **9** (0.590 g, 1.160 mmol) and 4-N,N-dimethylaminopyridine (0.425 g, 3.48 mmol) in dry acetonitrile (12 mL). After stirring the reaction mixture for 2 h, the solvent was removed by evaporation. The residue after evaporation was chromatographed on a silica gel short column (3.5 x 12 cm) in a dichloromethane - ethanol gradient (from 99:1 to 97:3). Fractions containing product **10** were pooled and evaporated to give 0.657 g (88%) of a crystallizing oil. R_F 0.78(A); 0.44(B); 0.89(C). Further fractions contained unreacted **9** (0.056 g, 9.5%). Homogenous by TLC **10** was used in the next step without further purification.

6-Methyl-9-[2-deoxy-3,5-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl]purine (11). The phenoxythiocarbonyl derivative **10** (0.411g, 0.637 mmol) was dissolved in dry toluene (50 mL) and the solution was concentrated in vacuo to a volume of *ca* 25 mL. α,α'-Aza-*bis*-isobutyronitrile (0.091 g, 0.127 mmol) was then added and the solution was purged with dry argon. Tri-*n*-butyltin hydride (0.59 mL; 0.65 g, 2.23 mmol) was injected through a septum and the reaction flask was placed in a preheated oil bath (75°C). After 2 h of heating the solvent was evaporated, and the resulting oil was chromatographed on a silica gel column (3.5 x 9 cm) in chloroform - methanol (98:2). Evaporation of fractions containing the single reaction product gave 0.308 g (98%) of **11** as a colorless oil. R_F 0.58(A); 0.30(B); 0.69(C). λ_{max} 247 nm (sh, ε 6500), 262 (7300).

6-Methyl-9-(2-deoxy-β-D-ribofuranosyl)purine (5). To a solution of **11** (0.365 g, 0.741 mmol) in absolute methanol (20 mL) was added ammonium fluoride (0.357 g, 9.63 mmol). The reaction mixture was stirred at 40°C for 16 h, and then adsorbed on an added portion of silica gel (*ca* 8 g, Merck 60, 70-230 mesh) by evaporation of methanol. The dried gel was applied onto a silica gel short column (3.5 x 9 cm) and the product was eluted with chloroform - methanol (9:1). Fractions containing the product were evaporated to afford **5** as a crystallizing colorless oil. Yield 182 mg (98%). mp 152-153°. Reversed-phase HPLC in 40% aq. methanol showed a single peak of the β-anomer at retention time 3.42 min (integration 99.68%, at 260 nm). An analytical sample of **5** was recrystallized from acetone,[27] mp 156.1-156.4°. Anal. Calcd for $C_{11}H_{14}N_4O_3$ (250.26):

%C, 52.79; %H, 5.64; %N, 22.39. Found: %C, 52.86; %H, 5.62; %N, 22.19. λ_{max} 247 nm (sh, ε 6900), 260 (7500).

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