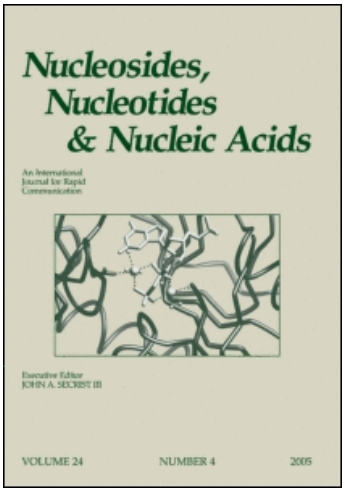


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Transglycosylation of β-D-RIbofuranosylindazoles

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

To cite this Article Boryski, Jerzy(1995) 'Transglycosylation of β-D-RIbofuranosylindazoles', Nucleosides, Nucleotides and Nucleic Acids, 14: 1, 77 — 89
To link to this Article: DOI: 10.1080/15257779508014654
URL: http://dx.doi.org/10.1080/15257779508014654

NUCLEOSIDES & NUCLEOTIDES, 14(1&2), 77-89 (1995)

TRANSGLYCOSYLATION OF β-D-RIBOFURANOSYLINDAZOLES

Jerzy Boryski

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

ABSTRACT: Irreversible, acid-catalyzed 2 → 1 transglycosylation reactions of fully acetylated β-D-ribofuranosylindazoles were studied applying HPLC analysis. Results so obtained support the intermolecular mechanism of transglycosylation *via* a 1,2-diglycosylindazole intermediate and are compared to those of the purine series.

INTRODUCTION

It has been well established that the chemical synthesis of nucleosides proceeds in two steps.[1] In the first step, reaction of the protected heterocyclic base and a sugar cation, generated from an appropriate sugar derivative, leads to a kinetic product of glycosylation. In the second step, this product undergoes rearrangement to form a thermodynamically more stable isomer. The second step of nucleoside synthesis is named "transglycosylation" and represents an intermolecular reaction catalyzed by Lewis acids.[1] Some transglycosylation reactions take place at elevated temperatures even in the absence of catalysts.[2-4]

According to the previously proposed mechanism,[1,5] the purine nucleoside formation proceeds *via* initial 3-glycosylation followed by rearrangement of the sugar moiety to N9. In my opinion, however, 3-glycosylpurine as the kinetic product of glycosylation has been well documented only in the case of adenine and its derivatives.[5] More recent studies support the hypothesis that in the case of guanine, hypoxanthine and other 6-oxopurines only N7 and N9 nitrogen atoms participate in the glycosylation and glycosyl exchange reactions and that reversible transglycosylation proceeds *via* 7,9-diglycosyl-purine intermediates.[6,7]

These observations lead to the conclusion that at least two different reaction pathways of glycosylation should be taken into consideration in the purine series: glycosylation in the sequence 3 → 9 for adenine and its derivatives, and 7 ⇄ 9 for 6-oxopurines. In both cases, however, transglycosylation reactions represent a glycosyl migration process of the type 1,3

i.e. nitrogen atoms to which a glycosyl substituent may be attached are at the distance of two chemical bonds (N3 - N9 or N7 - N9).

Consequently, it would be of general significance to also investigate transglycosylation of the type 1,2. To do this some structural analogues of purine nucleosides possessing two neighboring nitrogen atoms in a five-membered ring would be required. From this point of view indazole nucleosides seem to be excellent model compounds.

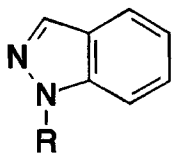
β-D-Ribofuranosylindazoles (**1a, 2a**) and their 2',3',5'-tri-O-acetyl derivatives (**1b, 2b**) have already been obtained. Synthesis and chemistry of the indazole nucleosides has been discussed in details by Preobrazhenskaya et al.[8] The fusion reaction of indazole (**1e**) and 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose in the presence of Lewis acids gives predominantly 1-substituted isomer (**1b**).[9-13] On the other hand, the silyl method and mercury procedure lead to 2-glycosylated products (compound **2b** in the ribo series).[14-17] Stereoselective synthesis of indazole nucleosides in the 2'-deoxyribo series produces exclusively β-D-anomers of both 1- and 2-substituted regioisomers.[18]

Moreover, it has already been shown that the 2-riboside of indazole (**2b**) is a kinetic product, when the corresponding 1-riboside (**1b**) is a thermodynamic product in their synthesis by the fusion method.[9,12,13] The influence of temperature and the amount of p-toluenesulfonic acid on distribution of regioisomers (**1b, 2b**) and their respective α-anomers (**1c, 2c**) has been also studied in the reaction mixture during the fusion of indazole and tetraacetylribose.[12,19]

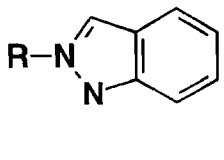
However, any direct comparison of the transglycosylation processes in the purine and indazole series cannot be made because glycosyl migration reactions have never been investigated in the case of the isolated ribofuranosides **1b** and **2b**, nor has any mechanism for their transglycosylation been proposed.

RESULTS AND DISCUSSION

Synthesis of the model compounds for these transglycosylation studies (**1b** and **2b**) was carried out as presented in SCHEME 1. As mentioned above, reaction of indazole (**1e**) and 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose in the presence of p-toluenesulfonic acid gave mainly **1b** under thermodynamically controlled conditions.[12,19] In the present studies, however, the same reaction performed in refluxing chlorobenzene afforded the desired 2-ribosylated isomer **2b** as the major product. This product was then separated from the small amount (8%) of **1b** using silica gel chromatography, but compound **2b** was still contaminated by unreacted tetraacetylribose, which might influence the isomerization studies. Therefore, the

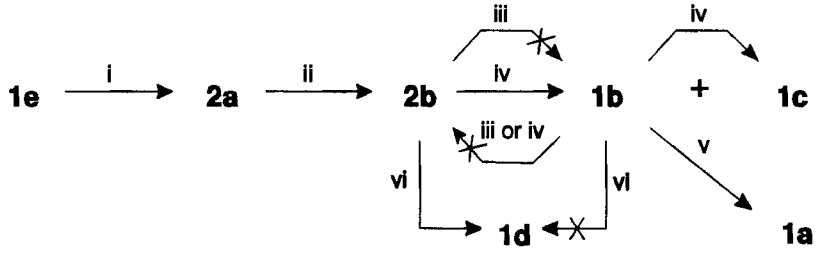


1a - e



2a - d

a: R = β-D-ribofuranosyl
b: R = 2',3',5'-tri-O-acetyl-β-D-ribofuranosyl
c: R = 2',3',5'-tri-O-acetyl-α-D-ribofuranosyl
d: R = (2-acetoxyethoxy)methyl
e: R = H



SCHEME 1. *Reagents and conditions*: i, 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose, p-TsOH, chlorobenzene, reflux; then NH_3/MeOH; ii, Ac_2O, pyridine, room temp.; iii, 200-260°C; iv, p-TsOH, 160°C; v, NH_3/MeOH; vi, $AcOCH_2CH_2OCH_2OAc$, p-TsOH, chlorobenzene, 130°C.

acetylated ribonucleoside **2b** was deprotected to 2-(β-D-ribofuranosyl)indazole (**2a**) in saturated methanolic ammonia. The product was purified by chromatography and crystallized from toluene to give a good yield of a very pure material (71% from **1e**). Nucleoside **2a** was then acetylated with acetic anhydride in pyridine which yielded quantitatively pure **2b**.

The reaction mixtures after the transglycosylation experiments were analyzed by reverse phase high performance liquid chromatography (see EXPERIMENTAL).

Surprisingly, 2-(2',3',5'-tri-O-acetyl-β-D-ribofuranosyl)indazole (**2b**) did not undergo thermal rearrangement to its 1-substituted isomer (**1b**) in the absence of catalysts even at 260°C, while the corresponding thermal 7 ⇄ 9 isomerization of peracetylated guanosine or inosine progressed easily from as little as 200°C.[3] However, glycosyl migration 2 → 1 took place readily in the presence of catalytical amounts of p-toluenesulfonic acid at 160°C (FIG. 1). In this case transglycosylation was irreversible: the fully acetylated 2-riboindazol (**2b**) could be transformed to 1-riboindazole, but not *vice versa*.

In contrast to the series of purine ribonucleosides,[1-7] 2 → 1 transglycosylation of riboindazoles proceeded with a high degree of anomerization. Thus, heating **2b** in the presence of an 8% mol of p-toluenesulfonic acid gave 6% of the 2-substituted α-anomer (**2c**) after 1 min, and 13% of the 1-α-product (**1c**) after 15 min of reaction (FIG. 1). A similar degree of anomerization had been observed previously during the synthesis of indazole nucleosides by the fusion method.[9-12,19]

It seems to be possible that glycosyl exchange reactions of indazole ribonucleosides are stereocontrolled, i.e. every single step of glycosylation and/or transglycosylation proceeds with retention of β-configuration of the sugar according to the "trans rule",[1] and the observed anomerization is a postglycosylation process catalyzed by p-toluenesulfonic acid. In order to prove this hypothesis the 1-β-isomer (**1b**) was heated to 160°C in the presence of p-toluenesulfonic acid for 10 min, which resulted in the formation of the 1-α-isomer (**1c**; 6% of yield based on HPLC) and free indazole (**1e**; 3.5%). This experiment indicates that riboindazoles undergo facile acid-catalyzed anomerization and underlines the irreversibility of 2 → 1 transglycosylation. The anomerization of riboindazoles is reversible. Heating of the 1-α-isomer (**1c**) in the presence of p-toluenesulfonic acid also resulted in a mixture of the α and β anomers (**1c** and **1b**, respectively).

Besides anomerization, the final yield of the transglycosylation product (**1b**) was additionally lessened due to the cleavage of the N-glycosylic bond (23% of indazole after 15 min. under the discussed conditions). Therefore, compound **1b** was isolated from the reaction mixture in the yield lower than expected (63%). This product was then deacetylated in methanolic ammonia to 1-(β-D-ribofuranosyl)indazole (**1a**).

Transglycosylation of **2b** performed as a molecular-sieve catalyzed reaction[8,20] was less effective. A solution of **2b** in dry chlorobenzene containing molecular sieves 4Å was heated under reflux for 2 h to give 6% of the 1-substituted isomer (**1b**), the unreacted substrate (8%), and as much as 85% of indazole (**1e**). After 8 h of reaction indazole was found to be a single product. The same reaction in toluene[20] gave only traces of **1b**. These results lead to the conclusion that the use of molecular sieves as a substitute for acid catalysts facilitates the cleavage of the N-glycosylic bond rather than transglycosylation of riboindazoles.

Another important detail about the transglycosylation mechanism came from the glycosyl exchange experiment. As shown in the guanine series,[7] the reaction of fully protected nucleosides with 2-acetoxyethyl acetoxymethyl ether made possible to monitor the regioselectivity of transglycosylation. In this case, fully acetylated 2-riboindazole (**2b**) was transformed directly to 1-[(2-acetoxyethoxy)methyl]indazole (**1d**) when treated with an excess

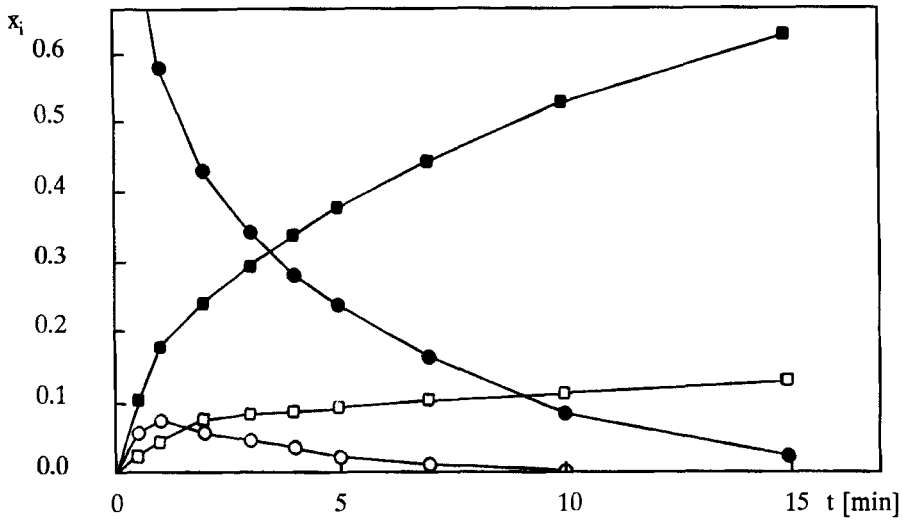


FIG. 1. Distribution of isomers during transglycosylation reaction of 2-(2',3',5'-tri-O-acetyl-β-D-ribofuranosyl)indazole (**2b**) in the presence of p-toluenesulfonic acid (8% mol) at 160°C: **1b** ■; **1c** □; **2b** ●; **2c** ○. The mole fractions are based on HPLC data.

of the ether in the presence of p-toluenesulfonic acid. Structure of **1d** was confirmed by elemental analysis, ^{1}H NMR spectrum, and mass spectrometry. In particular, the indicative for 1-alkylindazoles ultraviolet spectrum of this product proved the site of substitution. The second possible isomeric product, 2-substituted acyclonucleoside (**2d**),[21] was not detected in the reaction mixture (FIG. 2). In a counter experiment, 1-(2',3',5'-tri-O-acetyl-β-D-ribofuranosyl)indazole (**1b**) did not undergo glycosyl exchange reaction when subjected to the action of 2-acetoxyethyl acetoxymethyl ether.

All these experimental data support the mechanism of transglycosylation of riboindazoles shown in SCHEME 2. Protonation of the substrate (**2b**) at N1 (structure **3**) facilitates the cleavage of the N-glycosylic bond, which results in the formation of the sugar cation (structure **4**; either the carboxonium cation shown here or the acyloxonium cation may be taken under consideration)[1] and the liberation of free indazole (**1e**). This process initiates a chain reaction. A nucleophilic attack of the unsubstituted nitrogen atom N1 of another molecule of **2b** at C1 of the sugar cation may lead to the formation of 1,2-diribofuranosyl intermediate (**5**), which then decomposes to the more stable 1-substituted isomer (**1b**) with the liberation of the sugar cation (**4**). According to this mechanism the formation of the new glycosylic bond (N1 - C1'$_b$) takes place before the former one (N2 - C1'$_a$) is cleaved. The two

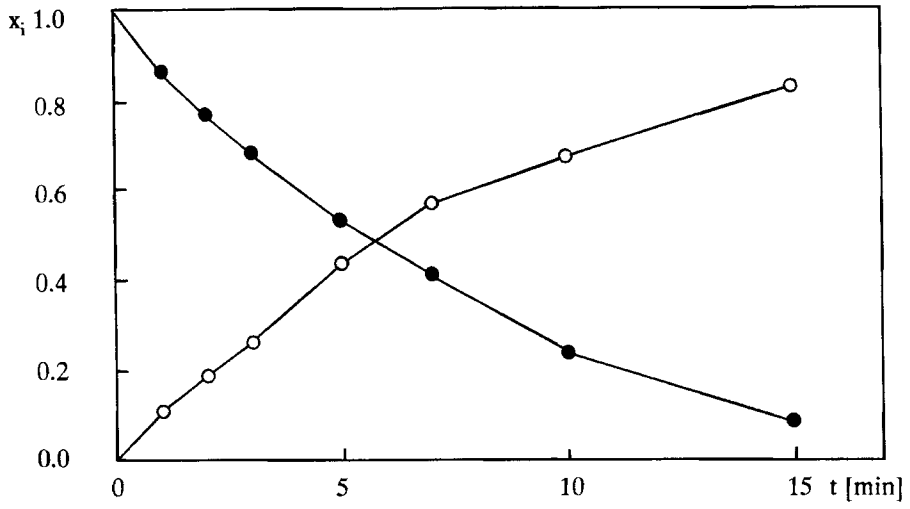
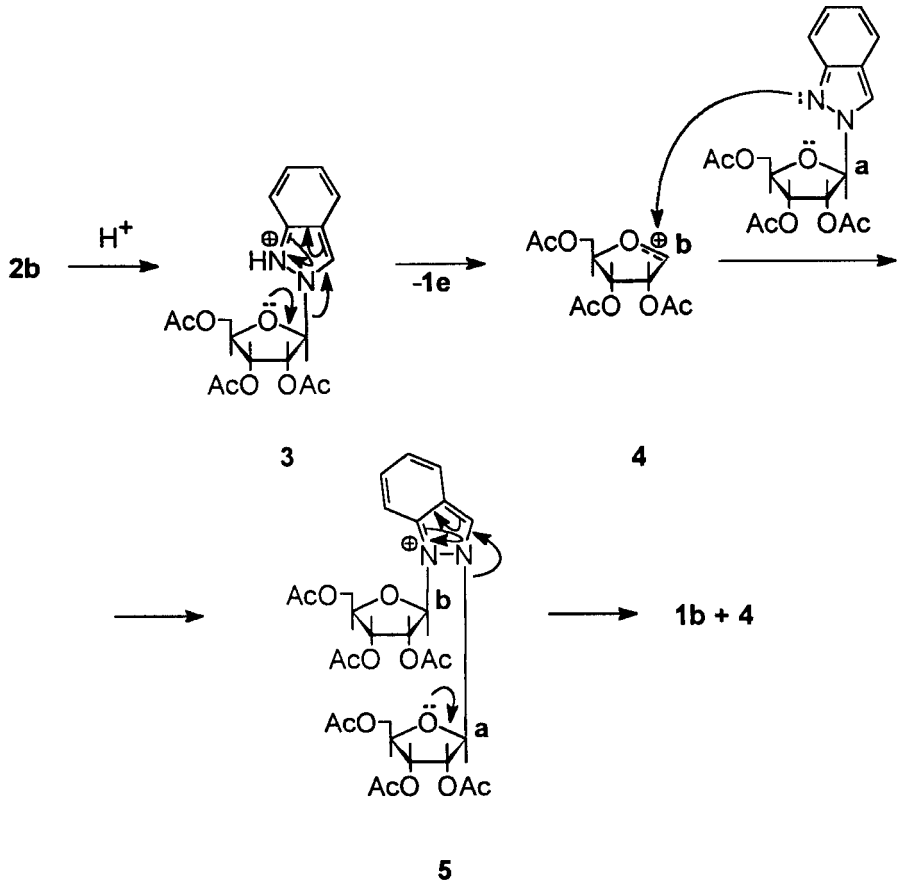


FIG. 2. Formation of 1-[(2-acetoxyethoxy)methyl]indazole (**1d**) in the reaction of **2b** with 2-acetoxyethyl acetoxymethyl ether (see Experimental): **2b** ●; **1d** ○.

latter reactions represent an elongation step of this chain process. A similar mechanism may be depicted for the reaction of **2b** with carboxonium cation generated from 2-acetoxyethyl acetoxymethyl ether.

However, reaction intermediate **5**, the structure of which explains regioselectivity observed in the glycosyl exchange experiment, has not been detected in the reaction mixture by using the HPLC technique. It may be accounted for the great instability of such a compound, or for steric reasons at least. Regioselectivity of transglycosylation can be also rationalized by a one-step mechanism of elongation, in which the cleavage of the former glycosylic bond and the formation of the new one are synchronized, like in reactions of the S_N2 type. On the other hand, the two-step mechanism of transglycosylation *via* diglycosyl intermediates is well documented in the series of purine and pyrimidine nucleosides.[1,6,7]

Another possible mechanism of transglycosylation, assuming a simple acid-catalyzed dissociation of **2b** to **1e** and sugar oxonium ion (**4**) with reassociation to form **1b** (i.e. first cleavage of the former glycosylic bond, then formation of the new one) may be excluded because reaction of **1e** and sugar cations gives directly **2b**, not **1b**.[8-12,19]. This reversible process may take place under the reactions conditions but it does not lead to the 1-substituted compound (**1b**). Moreover, the above presented glycosyl exchange experiment applying 2-acetoxyethyl acetoxymethyl ether also rules this type of mechanism out. If the acid-



SCHEME 2

catalyzed dissociation of **2b** gave substantial amounts of indazole (**1e**), it would react with an excess of the ether to form 2-[(2-acetoxyethoxy)methyl]indazole (**2d**),[21] which in fact has not been detected in the disscussed reaction (FIG. 2).

In summary, glycosylation of indazole closely resembles that of adenine. The kinetic glycosylation products, 2-substituted indazole and 3-substituted adenine, are very unstable and undergo fast and irreversible transglycosylation to form the thermodynamic products, 1-glycosylindazol and 9-glycosyladenine. Their structures correspond to the structure of the most stable tautomer of the starting heterocyclic bases. In contrast to guanine nucleosides,[2-4,7] the fully acetylated derivatives of either 1-riboindazole or adenosine[3,4] do not undergo thermal transglycosylation or react with 2-acetoxyethyl acetoxymethyl ether. This

can be rationalized by the fact, that the main driving force for 2 → 1 transglycosylation of indazole is aromatization: the quinonoid system of the 2-substituted isomer is converted to the more stable aromatic system of 1-isomer.

EXPERIMENTAL

Melting points were determined on a Laboratory Devices Mel-Temp II micromelting point apparatus in open capillaries and are uncorrected. Elemental analyses were performed on a Perkin-Elmer 240 Elemental Analyzer. Mass spectra were taken on a Varian MAT 312 spectrometer at 70 eV. ^1H magnetic resonance spectra were recorded on a Bruker WM-250 or on a Varian Unity 300 spectrometer with tetramethylsilane as an internal standard and are reported on the δ scale. UV spectra were measured in methanol on a Perkin-Elmer Lambda 5 spectophotometer. Thin-layer chromatography (TLC) was conducted on Merck silica gel F_{254} 60 plates using the following solvent systems (measured by volume): A, toluene - ethanol (95:5); B, chloroform - methanol (9:1). For preparative short-column chromatography Merck TLC gel HF_{254} 60 was used.

Analytical HPLC was performed using the following components from Waters Division of Milipore: Nova Pak C_{18} column (8 x 100 mm cartridge), 600E Multisolvent Delivery System with U6K Universal Liquid Chromatography Injector, 486 Tunable Absorbance Detector and 746 Data Module. The flow rate was set at 1 mL/min and UV absorption was measured at 290 nm. In order to differentiate 1- and 2-substituted derivatives of indazole some HPLC separations were also performed at 250 nm. The following average ϵ_{290} values [L x mol^{-1} x cm^{-1}] were taken for the calculations of molar ratios: 3900 for 1-substituted indazoles and 8300 for 2-substituted isomers.

2-(β-D-Ribofuranosyl)indazole (**2a**)

A mixture of indazole (591 mg, 5.0 mmol), 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose (2.03 g, 6.5 mmol), and p-toluenesulfonic acid monohydrate (48 mg, 0.25 mmol) was dissolved in dry toluene and evaporated in vacuo to dryness. The residue after evaporation was gently refluxed (oil bath temp. 142-144°C) in dry chlorobenzene (35 mL) for 5 h. After evaporation of the solvent under diminished pressure, the reaction mixture was chromatographed on a silica gel short column (5.5 x 10 cm) in toluene - ethanol 95:5. Evaporation of fractions # 18-20 (@ 20 mL) gave pure 1-substituted isomer (**1b**); 146 mg (8%) of a colorless syrup. The next fractions (# 26-38) were homogenous under a UV 254

nm lamp and contained 2-ribosylated product (**2b**), but according to ^{1}H NMR this material was contaminated by unreacted tetraacetylribose (ca 15%). Evaporation of these fractions yielded 1.44 g of oil, which was dissolved in saturated methanolic ammonia (25 mL). After 16 h at room temperature the solution was evaporated back to an oil, which was redissolved in chloroform - methanol 9:1 (20 mL) and the product was purified on a silica gel short column (4.2 x 9 cm) in this solvent system. Fractions containing **2a** were evaporated to dryness and the resulting oily residue was crystallized from hot toluene - methanol (10:1). The obtained crystalline **2a** was washed with toluene and ethyl ether, then dried in a vacuum desiccator to yield 886 mg (71%) of needles, mp 131°C (Ref.[11] 131-132°C). R_F 0.03(A), 0.14(B). Anal. Calcd for $C_{12}H_{14}N_2O_4$ (250.25): %C, 57.59; %H, 5.64; %N, 11.19. Found: %C, 57.62; %H, 5.64; %N, 11.18. λ_{max} (MeOH) 269 nm (shoulder, ϵ=8550), 276 (9700), 292 (sh, 8400). ^{1}H NMR (d_6DMSO): δ 3.56 (m, 1H, H_a-5'), 3.69 (m, 1H, H_b-5'), 3.99 (q, 1H, H-4'), 4.20 (q, 1H, H-3'), 4.38 (q, 1H, H-2'), 5.03 (t, 1H, OH-5'), 5.16 (d, 1H, OH-3'), 5.55 (d, 1H, OH-2'), 5.96 (d, J=3.7 Hz, 1H, H-1'), 7.04 (pseudotriplet, 1H, H-5), 7.25 (pt, 1H, H-6), 7.61 (dd, 1H, H-7), 7.70 (dd, 1H, H-4), 8.64 (d, 1H, H-3).

2-(2',3',5'-Tri-O-acetyl-β-D-ribofuranosyl)indazole (**2b**)

Crystalline 2-riboindazole (**2a**; 250mg, 1.0 mmol) was dissolved in dry pyridine (10 mL) and this solution was concentrated by evaporation to a volume of ca 3 mL. Acetic anhydride (0.57 mL, 6.0 mmol) was then added and after 1.5 h at room temperature the reaction was quenched by the addition of absolute methanol (5 mL). After 30 min solvents were evaporated under diminished pressure and the resulting oil was coevaporated with toluene (4 x 10 mL) to remove the rest of pyridine. Yield 377 mg (100%) of a colorless syrup. R_F 0.47(A). Anal. Calcd for $C_{18}H_{20}N_2O_7$ (376.37): %C, 57.44; %H, 5.36; %N, 7.44. Found: %C, 57.25; %H, 5.49; %N 7.50. λ_{max} 269 nm (sh, ϵ=8600), 277 (9750), 293 (sh, 8450). ^{1}H NMR ($CDCl_3$): δ 1.97, 2.05, 2.07 (3s, 9H, CH_3CO), 4.19 (q, 1H, H_a-5'), 4.43 (m, 2H, H_b-5', H-4'), 5.73 (t, 1H, H-3'), 5.81 (q, 1H, H-2'), 6.11 (d, J=2.7 Hz, 1H, H-1'), 7.01 (pt, 1H, H-5), 7.22 (pt, 1H, H-6), 7.57 (d, 1H, H-7), 7.62 (d, 1H, H-4), 8.05 (s, 1H, H-3).

Attempted thermal isomerization of **2b**

Four samples of **2b** (37.6 mg, 0.1 mmol each) were heated on an oil bath at 200, 220, 240 and 260°C for 10 min. TLC in solvent A, HPLC and ultraviolet spectra in methanol did not show any changes of the starting material after heating.

1-(2',3',5'-Tri-O-acetyl-β-D-ribofuranosyl)indazole (**1b**)

2-Substituted isomer (**2b**; 376 mg, 1.0 mmol) and p-toluenesulfonic acid monohydrate (15.2 mg, 0.08 mmol) were dissolved in dichloromethane-methanol (9:1; 8 mL) and this solution was evaporated to an oil. The oil was heated at 160°C (oil bath temperature) for 15 min. The resulting dark melt was dissolved in toluene - ethanol 98:2 (5 mL) and chromatographed on a silica gel short column (3 x 10 cm) in a toluene - ethanol gradient (from 98:2 to 95:5, respectively). Evaporation of fractions #9-14 (@ 8 mL) containing the main product gave 237 mg (63%) of **1b** in the form of a colorless oil. R_F 0.54 (A). Anal. Calcd for $C_{18}H_{20}N_2O_7$ (376.37): %C, 57.44; %H, 5.36; %N, 7.44. Found: %C, 57.22; %H, 5.45; %N, 7.28. λ_{max} 249 nm (ϵ=4500), 256 (sh, 4050), 287 (4150), 291 (4100), 297 (3400). ^{1}H NMR ($CDCl_3$): δ 2.02, 2.12, 2.14 (3s, 9H, CH_3CO), 4.13 (q, 1H, H_a-5'), 4.42 (m, 2, H_b-5', H-4'), 5.86 (t, 1H, H-3'), 6.07 (q, 1H, H-2'), 6.31 (d, J=3.3 Hz, 1H, H-1'), 7.20 (pt, 1H, H-5), 7.42 (pt, 1H, H-6), 7.52 (d, 1H, H-7), 7.73 (d, 1H, H-4), 8.07 (s, 1H, H-3).

The next fractions (#15-16) contained 1-α-isomer (**1c**); 48.8 mg (13%) of an oil. This product was identical with a sample obtained according to the procedure of Kam and Imbach[11] (TLC, HPLC, UV, ^{1}H,NMR). R_F 0.45 (A). λ_{max} 250, 256(sh), 287, 291, 298 nm. ^{1}H NMR ($CDCl_3$) δ 6.66 (d, J=5.0 Hz, H-1'), 8.05 (s, 1H, H-3).

Evaporation of fractions #18-23 yielded 27.2 mg (23%) of indazole (**1e**). The described reaction was also monitored by HPLC performed in 52% aqueous methanol and the products were identified according to their retention time (in order of elution): **1e** (retention time 6.4 min), **2c** (11.1 min), **1c** (11.9 min), **2b** (13.3 min) and **1b** (16.1 min). The results are presented in FIG. 1.

1-(β-D-Ribofuranosyl)indazole (**1a**)

Compund **1b** obtained in the above presented experiment (150 mg, 0.4 mmol) was dissolved in saturated methanolic ammonia (5 mL) and this solution was stirred at ambient temperature for 16 h. The solvent was then evaporated to dryness, and the resulting oil was crystallized from a mixture of toluene - methanol (9:1), which yielded 91 mg (91%) of needles, mp 172-173°C (Ref.[11] 170-172°C). Anal. Calcd for $C_{12}H_{14}N_2O_4$ (376.37): %C, 57.59; %H, 5.64; %N, 11.19. Found: %C, 57.58; %H, 5.71; %N, 11.38. λ_{max} 250 nm (ϵ=4500), 257 (sh, 4100), 287 (4100), 291 (4050), 298 (3400). ^{1}H NMR (d_6DMSO): δ 3.41 (dd, 1H, H_a-5'), 3.57 (dd, 1H, H_b-5'), 3.93 (q, 1H, H-4'), 4.23 (t, 1H, H-3'), 4.66 (t, 1H, H-2'), 6.11 (d, J=4.5 Hz, H-1'), 7.19 (pt, 1H, H-5), 7.42 (pt, 1, H-5), 7.79 (d, 2H, H-4, H-5), 8.18 (s, 1H, H-3).

Molecular-sieve catalyzed transglycosylation of **2b**

A sample of **2b** (37.6 mg, 0.1 mmol) was dissolved in dry chlorobenzene (5 mL) and heated under reflux in the presence of molecular sieves 4Å (ca 2 g; supplied by Union Carbide). According to HPLC analysis in 52% aqueous methanol the reaction mixture contained compounds **1b** (6%), **2b** (8%) and **1e** (85%) after 2h, and 99% of **1e** after 8h of heating. In a similar experiment performed in dry toluene, a maximal concentration of **1b** (1.3%) was observed after 4 h of reaction.

Reaction of **2b** with 2-acetoxyethyl acetoxymethyl ether. 1-[(2-Acetoxyethoxy)methyl]-indazole (**1d**)

A mixture of ribonucleoside 2b (376.4 mg, 1 mmol), 2-acetoxyethyl acetoxymethyl ether[22] (880 mg, 5 mmol) and p-toluenesulfonic acid monohydrate (9.5 mg, 0.05 mmol) in dry chlorobenzene (6 mL) was stirred at 130°C (temperature of an oil bath) for 15 min. The solution was then concentrated under diminished pressure to an oil, which was chromatographed on a silica gel short column in solvent A. Fractions containing the product (**1d**) were pooled and evaporated to a colorless syrup. This material was crystallized by cooling down to -40°C, which afforded 190 mg (81%) of crystalline **1d**, mp 39-39.5°C. Anal. Calcd for $C_{12}H_{14}N_2O_3$ (234.26): %C, 61.53; %H, 6.02; %N, 11.96. Found: %C, 61.53; %H, 6.04; %N, 11.87. λ_{max} 250 nm (ϵ=4400), 258 (4000), 286 (4100), 291 (4050), 297 (3400).
MS m/z: 234 (M^+), 175 (M-CH_3COO), 148 (BCH_2OH), 131 (BCH_2), 118 (BH), 103, 87, 77.
^{1}H NMR ($CDCl_3$): δ 1.97 (s, 3H, CH_3CO), 3.67 (m, 2H, CH_2), 4.13 (m, 2H, CH_2), 5.80 (s, 2H, CH_2N), 7.21 (pt, 1H, H-5), 7.43 (pt, 1H, H-6), 7.59 (dd, 1H, H-7), 7.75 (dd, 1H, H-4), 8.03 (s, 1H, H-3). This experiment was repeated in the scale of 0.1 mmol and the progress of reaction was monitored by HPLC. Samples of the reaction mixtures (23 nmol each) were analyzed in a methanol - water linear gradient (from 47% to 53% of aq. MeOH). Retention times: 12.2 min for **1d**, and 18.5 min for **2b** (see FIG. 2).

Attempted transglycosylation reactions of **1b**

A. Thermal isomerization

A sample of **1b** (37.6 mg, 0.1 mmol) was maintained at 260°C for 10 min. TLC, HPLC and ultraviolet spectrum did not show any changes of the starting material after heating.

B. Acid-catalyzed transglycosylation

A solution of **1b** (37.6 mg, 0.1 mmol) and p-toluenesulfonic acid monohydrate (1.9 mg, 0.01 mmol) in acetonitrile (3 mL) was evaporated to dryness and the resulting oil was heated

to 160°C for 10 min. The obtained melt was redissolved in acetonitrile; according to HPLC performed in 52% aq. MeOH the reaction mixture contained indazole (**1e**, 3.5%), the 1-α-substituted product (**1c**, 6%), and the substrate (**1b**, 90%).

C. Treatment with 2-acetoxyethyl acetoxymethyl ether

A mixture of **1b** (37.6 mg, 0.1 mmol), 2-acetoxyethyl acetoxymethyl ether (35 mg, 0.2 mmol) and p-toluenesulfonic acid monohydrate (1.9 mg, 0.01 mmol) was refluxed in dry chlorobenzene (4 mL) for 45 min. TLC analysis after 2, 5, 20 and 45 min of the reaction showed a single spot of the starting material.

In a counter experiment, refluxing in chlorobenzene of the 1-α compound (**1c**) in the presence of p-toluenesulfonic acid (10% mol) for 20 min resulted in a mixture of **1b** (56%), **1e** (12%), and **1c** (30%).

Acknowledgments. This work was supported by the Komitet Badań Naukowych, Project No. 2 2671 92 03. The synthetic part of this work was performed in laboratory of Prof. Wolfgang Pfleiderer at the Universität Konstanz, Germany, and was supported by the Deutscher Akademischer Austauschdienst.

REFERENCES

1. Watanabe, K. A.; Hollenberg, D. H.; Fox, J. J. *J. Carbohydrates Nucl. Nucl.* **1974**, *1*, 1 and references cited therein.
2. Boryski, J.; Golankiewicz, B. *Nucleosides Nucleotides* **1987**, *6*, 385.
3. Boryski, J.; Golankiewicz, B. *Nucleic Acids Res.* **1987**, Symposium Ser. No 18, 45.
4. Boryski, J.; Golankiewicz, B. *Nucleosides Nucleotides* **1989**, *8*, 529.
5. Miyaki, M.; Shimidzu, B. *Chem. Pharm. Bull.* **1970**, *18*, 1446 and references cited therein.
6. Dudycz, L. W.; Wright, G. E. *Nucleosides Nucleotides* **1984**, *3*, 33.
7. Boryski, J. *Collect. Czech. Chem. Commun.* **1993**, *58*, Special Issue, 5.
8. Preobrazhenskaya, M. N.; Korbukh, I. A.; Tolkachev, V. N.; Dobrynin, J. V; Vornovitskaya, G. I, in "*Les Colloques de l'INSERM. Nucleosides, Nucleotides*" J. -L. Barascut and J. -L. Imbach (eds). INSERM, Octobre **1978**, vol. *81*, 85.
9. Korbukh, I.; Blanco, F.; Preobrazhenskaya, M. *Tetrahedron Lett.* **1973**, 4619.
10. Korbukh, I.; Blanco, F.; Preobrazhenskaya, M. *Zh. Org. Khim.* **1974**, *10*, 1091
11. Kam, B. L.; Imbach, J. L. *J. Carbohydrates Nucl. Nucl.* **1974**, *1*, 287.
12. Barascut J. L.; Imbach, J. L. in "Synthesis, Structure and Chemistry of tRNAs and their Components", M. Wiewiórowski (ed.), Poznań 1976, p. 408.

13. Kazimierczuk, Z.; Lönnberg, H; Vilpo, J.; Pfleiderer, W. *Nucleosides Nucleotides* **1989**, *8*, 599.

14. Brauninger, H.; Koine, A. *Pharmazie* **1965**, *20*, 457.

15. Alonso, G.; Garcia-Munoz, G.; Madronero, R. *J. Heterocycl. Chem.* **1970**, *7*, 1435.

16. Revankar, G. R.; Townsend, L. B. *J. Heterocycl. Chem.* **1970**, *7*, 117.

17. Revankar, G. R.; Townsend, L. B. *J. Heterocycl. Chem.* **1970**, *7*, 1329.

18. Seela, F.; Bourgeois, W. *Helv. Chim. Acta* **1991**, *74*, 315.

19. Barascut, J. L.; Kam, B. L.; Imbach, J. L. *Bull. Soc. Chim. France*, **1976**, 1983.

20. Montgomery, J. A.; Elliott, R. D. *JCS Chem. Commun.* **1972**, 1279

21. 2-[(2-Acetoxyethoxy)methyl]indazole (**2d**), obtained from indazole and 2-acetoxyethyl acetoxymethyl ether, undergoes irreversible acid-catalyzed isomerization to the 1-substituted product (**1d**): Boryski, J., Presented at *14th International Congress of Heterocyclic Chemistry*, Antwerp, Semptember 1993, Abstracts p. PO1-32.

22. Rosowsky, A.; Kim, S. H.; Wick, M. *J. Med. Chem.* **1984**, *24*, 1177.

Received March 11, 1994
Accepted September 23, 1994