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Galactosylation by Use of β-Galactosidase: Enzymatic Syntheses of Disaccharide Nucleosides

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Abstract: The synthesis of various galactose containing disaccharide nucleosides has been achieved by utilizing the transgalactosylation potential of ß-galactosidase from *Aspergillus oryzae*. Thus, using *p*-nitrophenyl-ß-D-galactoside **1** as galactosyl donor, 2-deoxyuridine **2a**, uridine **2b**, thymidine **2c** and adenosine **2d** have proven to be useful acceptors for the enzyme catalyzed disaccharide nucleoside formation. The regiochemistry of the products **4a** - **4d** formed after acetylation has been assigned unambiguously by using modern NMR-techniques.

INTRODUCTION

The development of methods for stereoselective formation of glycosidic linkages remains a challenging task in organic synthesis.[1] Alternatively to chemical methods the use of enzymes has led to impressive results in addressing this problem. In particular, glycosyltransferases have been applied successfully for the stereoselective syntheses of various oligosaccharides.[2] However, the high costs and often limited accessibility of many glycosyltransferases are major drawbacks for a broad application of these biocatalysts.

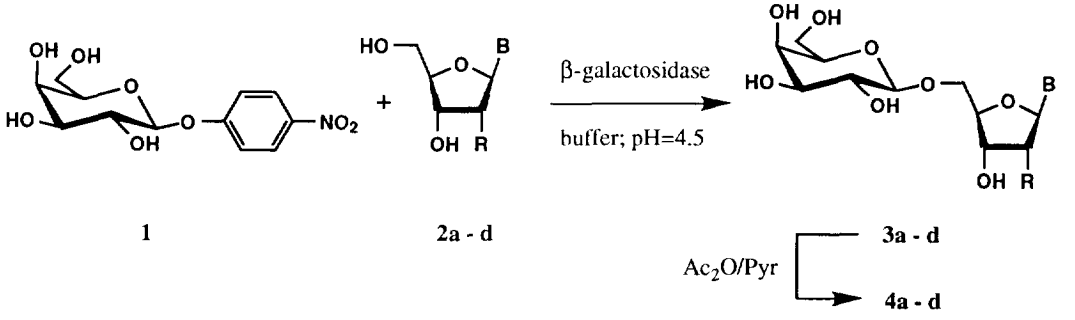
Another enzymatic approach is based on the transglycosylation potential of glycosidases. These hydrolytic enzymes are *in vivo* generally responsible for the cleavage of glycosidic linkages. However, in a kinetically controlled reaction glycosidases are able to transfer a suitable glycosyl donor to a glycosyl acceptor thus stereoselectively forming a glycosidic linkage. Although the yields obtained in reactions catalyzed by glycosidases are usually moderate to low there are some features which make the application of this class of enzymes valuable for synthesis: (i) multistep protection and deprotection sequences for the glycosyl donor and acceptor can be avoided (ii) the regiochemistry of the glycosyltransfer can be controlled within certain limits[3-5] and (iii) many glycosidases are commercially available and reasonable cheap for synthetic use. Among the class of glycosidases ß-galactosidase is one of the best investigated enzymes. This biocatalyst tolerates a variety of glycosyl acceptors including besides other monosaccharides[3-9] oximes,[10] diols,[11] allyl and propargyl alcohol,[12] steroids[13] and alcaloids.[14] Furthermore it has been successfully utilized for the synthesis of glycopeptides.[15,16]

RESULTS AND DISCUSSIONS

In our syntheses of disaccharide nucleosides we utilized ß-galactosidase from *Aspergillus oryzae* (E.C.3.2.1.23). Disaccharide nucleosides are a class of compounds with potential biological activities.[17,18] We were especially interested in the regiochemical outcome of the galactosylation reaction since it is well known that the regioselectivity of ß-galactosidase catalyzed reactions is highly dependent of the atom linked to the anomeric

carbon and the nature of the aglycon of the acceptor saccharide itself.[3-9] To the best of our knowledge nucleosides like uridine, thymidine and adenosine have not been investigated as acceptor substrates in glycosidation procedures catalyzed by ß-galactosidase.

In the enzyme catalyzed reactions we describe here we utilized p-nitrophenyl-ß-D-galactopyranoside **1** as galactosyl donor (Scheme 1). We performed the galactosylation procedure in aqueous buffer solution at a pH of 4.5. To circumvent the problem arising from a possible galactosyltransfer to the donor substrate itself we were adding the donor galactoside **1** in small portions over a period of 30 minutes to the solution of acceptor nucleosides **2a** - **2d** thus keeping the concentrations of the glycosyl donor at any time during the reaction as low as possible.[9,16] The disaccharide nucleosides **3a** - **3d** formed in the reaction were isolated after denaturation of ß-galactosidase by heating to 90° C, removal of water and subsequent acetylation followed by purification of the peracetylated derivatives **4a** - **4c** via column chromatography over silica gel. In the case of disaccharide nucleoside **4d** this isolation protocol was not successful since derivative **4d** was not separable from peracetylated adenosine present in the crude product mixture. Therefore the material obtained was treated with sodium methoxide in dry methanol and the deprotected product **3d** could be easily purified by chromatography over Biogel® P2.



1 **2a - d**

	a	b	c	d
B	**Uracil**	**Uracil**	**Thymine**	**Adenine**
R	**H**	**OH**	**H**	**OH**

Scheme 1

The disaccharide nucleosides synthesized are summarized in Table 1. The yields obtained for the products **3a** - **3d** after deacetylation were 3 - 7% showing a dependence on the nucleobases present in the acceptor nucleosides. A further remarkable result is that by using 2´-deoxyuridine **2a** or uridine **2b** as acceptor substrate only the formation of a ß(1-5´) glycosidic linkage could be observed. Small changes in the nucleobase - as in the case of thymidine **2c** - again led predominantly to the formation of the ß(1-5´) linked disaccharide nucleoside **3c**. Additionally a rather significant byproduct with a ß(1-3´) linkage was obtained in a ratio of 2 : 1. The same observation was made for adenosine **2d**. The ratio of ß(1-5´) **3d** to ß(1-3´) linked disaccharide nucleoside

formed was 3.3 : 1. This formation of significant amounts of a ß(1-3´) linked product also reflects a sensible steric interaction of the enzyme with the acceptor aglycon. The low yields obtained are most likely due to the polar character of the nucleobases since it is known that the best acceptor monosaccharides for ß-galactosidase catalyzed reactions usually contain big, non-polar aglycons.[3-5]

Table 1. Disaccharide nucleosides synthesized by the use of ß-galactosidase

entry	acceptor nucleoside	product disaccharide nucleoside	reaction time (min)	isolated yield (%)
1	**2a**	Gal ß(1-5´) 2´-deoxyuridine (**3a**)	30	7
2	**2b**	Gal ß(1-5´) uridine (**3b**)	30	6
3	**2c**	Gal ß(1-5´) thymidine (**3c**) and Gal ß(1-3´) thymidine ß(1-5´) : ß(1-3´) = 2 : 1	30	5
4	**2d**	Gal ß(1-5´) adenosine (**3d**) and Gal ß(1-3´) adenosine ß(1-5´) : ß(1-3´) = 3.3 : 1	30	3

The regiochemistry of the reaction products was assigned unambiguously by performing extensive NMR studies.[19] In particular, phase-sensitive DQF-COSY and HMQC experiments - performed with a preliminary BIRD puls and GARP-^{13}C decoupling - allowed exact assignment of protons and carbons. By measuring long range ^{1}H - ^{13}C connectivities (using coupling constants of 4 - 5 Hz) either from the anomeric carbon to the corresponding protons of the nucleoside unit or from the anomeric proton to the corresponding connected carbon respectively utilizing HMBC spectra led to an exact evaluation of the glycosidic linkage. This assignment could be achieved even in those cases where a separation of the regioisomers by chromatography was not possible.

The advantage of the syntheses presented is that starting with readily available nucleosides a variety of disaccharide nucleosides are accessible in a straightforward galactosylation procedure. Although the yields obtained in the enzyme catalyzed reactions are low, the facts that extensive protecting group strategies can be circumvent and the catalyst is reasonable cheap are very advantageous. Since a chemical synthesis towards the target molecules would require a multistep reaction sequence the enzymatic approach opens an economical alternative. In addition, the different products obtained by enzymatic transgalactosylation may lead to further insight into enzyme - substrate interactions.

EXPERIMENTAL

General: Chemicals were purchased from Aldrich and were reagent grade. ß-Galactosidase from *Aspergillus oryzae* (EC 3.2.1.23; Grade XI) was purchased from Sigma. Analytical thin layer chromatography was performed on Merck plates (silica gel F_{254}, 0.25 mm thick). Compounds were visualized by spraying with a solution of 3% $Ce(SO_4)_2$ in 2N H_2SO_4 followed by heating to 200 °C. Flash chromatography was performed using Merck silica gel 60 (0.04-0.063 mm thick).

NMR spectra were recorded on a BRUKER AM 400 spectrometer. In the spectroscopical data given we used superscripts to denote atoms or groups in disaccharide nucleosides and to refer to the individual sugar residues and aglycons respectively. They are serially indicated beginning with the non-reducing residue. For

example, in (β-D-Galactopyranosyl)-(1-5')-O-2'-deoxy-β-D-ribofuranosyluracil the Gal-residue has no superscript, the 2-deoxy-ribofuranosyl-residue is indicated with (') and the uracil aglycon with (").

Optical rotations were measured on a Perkin Elmer polarimeter 141. Abbreviations used are as follows: hexane (PE), ethyl acetate (EA), dichloromethane (MC), methanol (MeOH).

General procedure for the enzymatic galactosylation (method A)

To a solution of 1 mmol of the acceptor substrate and 0.3 mmol *p*-nitrophenyl-ß-D-galactopyranoside (p-NO_2Ph-β-Gal) **1** in 45 mL of phosphate buffer (pH = 4.5; 10 mM $MgSO_4$) 8 mg (40 U) of ß-galactosidase from *Aspergillus oryzae* were added. A solution of 1 mmol of **1** in 15 mL of phosphate buffer was continuously added to the reaction mixture over a period of 30 minutes. The progress of the reaction was followed by TLC (30% NH_3 : isopropanol : water = 3 : 7 : 2). After all of the galactopyranoside **1** has been consumed, the reaction was quenched by heating to 90 °C for 5 min. The denaturated enzyme was filtered off and the solvent was removed by coevaporation with toluene. The remaining crude material was acetylated by adding 10 mL of pyridine and 4 mL of acetic anhydride. After stirring for 10 h at room temperature, pyridine and acetic anhydride were removed in vacuum and the crude material was purified by flash chromatography (specific eluent given in each entry).

General procedure for deacetylation (method B)

The acetylated material was dissolved in 10 mL of methanol and 0.2 mL of a solution of sodium methoxide in dry methanol (0.1M) was added. After completion of the reaction (judged by TLC), the pH was adjusted to 8 by the addition of solid CO_2. The solvent was removed in vacuum and the crude product was dried in vacuum and afterwards purified by chromatography over Biogel® P2.

(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-(1-5')-O-3'-O-acetyl-2'-deoxy-β-D-ribofuranosyluracil (4a)

228.2 mg (1.0 mmol) of 2-deoxyuridine **2a** were treated with p-NO_2Ph-β-Gal **1** and β-galactosidase in 45 mL of buffer according to **method A**. After purification by flash chromatography (PE / EA = 2 / 3) 41 mg (7 %) of **4a** were obtained as colorless oil. $[\alpha]_D^{20}$ = -0.8 (c = 0.65, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): δ 1.93; 1.98; 2.01; 2.04; 2.14 (5 s, 15H, acetyl-CH_3); 2.13 (dd, 1H, J = 8.8, 14.4 Hz, H-2a'); 2.36 (dd, 1H, J = 5.8, 14.4 Hz, H-2b'); 3.71 (dd, 1H, J = 10.1, 1.9 Hz, H-5a'); 3.87 (dt, 1H, J = 1.3, 6.7 Hz, H-5); 4.05 (dd, 1H, J = 6.7, 11.2 Hz, H-6a'); 4.12 (m, 1H, H-4'); 4.13 (dd, 1H, J = 6.7, 11.2 Hz, H-6b'); 4.15 (dd, 1H, J = 10.1, 1.9 Hz, H-5b'); 4.44 (d, 1H, J = 7.7 Hz, H-1); 4.99 (dd, 1H, J = 3.4, 10.5 Hz, H-3); 5.07 (dd, 1H, J = 7.7, 10.5 Hz, H-2); 5.11 (m, 1H, H-3'); 5.36 (dd, 1H, J = 3.4, 1.3 Hz, H-4); 5.72 (dd, 1H, J = 2.4, 8.2 Hz, H-5"); 6.32 (dd, 1H, J = 5.8, 8.8 Hz, H-1'); 7.84 (d, 1H, J = 8.2 Hz, H-6"); 8.31 (s, 1H, broad, NH). ^{13}C-NMR ($CDCl_3$): δ 170.70; 170.31; 170.07; 169.99; 169.72; 162.76 (C-4"); 150.27 (C-2"); 140.43 (C-6"); 102-44 (C-5"); 100.73 (C-1); 85.38 (C-1'); 83.82 (C-4'); 75.27 (C-3'); 70.95 (C-5); 70.27 (C-3); 69.17 (C-5'); 68.76 (C-2); 66.87 (C-4); 61.07 (C-6); 37.54 (C-2'); 20.96; 20.74; 20.71; 20.64; 20.53. Calcd. for $C_{25}H_{32}N_2O_{15}$ (600.53): C, 50.00; H, 5.37; N, 4.66. Found: C, 50.11; H, 5.42; N, 4.59.

(β-D-Galactopyranosyl)-(1-5')-O-2'-deoxy-β-D-ribofuranosyluracil (3a)

A solution of 41 mg (0.068 mmol) of **4a** in 45 mL of methanol was treated according to **method B**. After purification 25 mg (96 %) of **3a** were obtained as colorless oil. $[\alpha]_D^{20}$ = +14.7 (c = 0.3, H_2O). ^{1}H-NMR (D_2O): δ 2.51 (m, 2H, H-2'); 3.66 (dd, 1H, J = 3.5, 10.0 Hz, H-2); 3.79 (dd, 1H, J = 3.4, 10.0 Hz, H-3); 3.82 (m, 1H, J = 4.1, 7.7, 0.8 Hz, H-5); 3.91 (m, 2H, H-6); 3.95 (m, 1H, H-5a'); 4.06 (dd, 1H, J = 3.4, 0.8 Hz, H-4); 4.32 (m, 1H, H-5b'); 4.33 (m, 1H, H-4'); 4.58 (d, 1H, J = 7.7 Hz, H-1); 4.66 (dt, 1H, J = 3.7,

5.9 Hz, H-3'); 6.00 (d, 1H, J = 7.9 Hz, H-5''); 6.45 (t, 1H, J = 6.8 Hz, H-1'); 7.98 (d, 1H, J = 7.9 Hz, H-6'). ^{13}C-NMR (D_2O): δ 163.45 (C-4''); 157-81 (C-2'); 143.44 (C-6''); 105.18 (C-1); 104.66 (C-5''); 87.97 (C-1'); 87.34 (C-4'); 77.34 (C-5); 74.88 (C-3); 73.16 (C-3'); 72.96 (C-2); 71.47 (C-5'); 70.78 (C-4); 63.2 (C-6); 40.98 (C-2'). Calcd. for $C_{15}H_{22}N_2O_{10}$ (390.35): C, 46.16; H, 5.68; N, 7.18. Found: C, 46.25; H, 5.56; N, 7.09.

(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-(1-5')-O-2',3'-di-O-acetyl-β-D-ribofuranosyluracil (4b)

A solution of 244 mg (1 mmol) uridine **2b** in 45 mL of buffer was reacted with p-NO_2Ph-β-Gal and β-galactosidase according to **method A**. After purification by flash chromatography (PE / EA = 1 / 1) 38 mg (6 %) of **4b** were obtained as colorless oil. $[\alpha]_D^{20}$ = -20.0 (c = 1.65, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): δ 1.97; 2.02; 2.06; 2.08; 2.09; 2.17 (6 s, 18H, acetyl-CH_3); 3.66 (dd, 1H, J = 2.4, 11.2 Hz, H-5a'); 3.91 (dt, 1H, J = 1.1, 6.6 Hz, H-5); 4.08 (dd, 1H, J = 6.6, 11.3 Hz, H-6a'); 4.15 (dd, 1H, J = 6.6, 11.3 Hz, H-6b'); 4.20 (m, 1H, H-4'); 4.21 (dd, 1H, J = 2.2, 11.3 Hz, H-5b'); 4.53 (d, 1H, J = 7.8 Hz, H-1); 5.03 (dd, 1H, J = 10.5, 3.4 Hz, H-3); 5.04 (dd, 1H, J = 6.9, 5.8 Hz, H-2'); 5.13 (dd, 1H, J = 7.8, 10.5 Hz, H-2); 5.31 (dd, 1H, J = 5.8, 3.7 Hz, H-3'); 5.38 (dd, 1H, J = 1.2, 3.4 Hz, H-4); 5.80 (dd, 1H, J = 8.2, 2.2 Hz, H-5''); 6.27 (d, 1H, J = 6.9 Hz, H-1'); 7.75 (d, 1H, J = 8.2 Hz, H-6''); 9.37 (s, broad, 1H, NH). ^{13}C-NMR ($CDCl_3$): δ 170.26; 170.09; 170.02; 169.94; 169.90; 169.30; 162.99 (C-4''); 150.66 (C-2''); 140.03 (C-6''); 103.25 (C-5''); 100.53 (C-1); 85.53 (C-1'); 81.81 (C-4'); 73.22 (C-2'); 71.06 (C-3'); 70.90 (C-5); 70.32 (C-3); 68.56 (C-2); 67.84 (C-5'); 66.86 (C-4); 61.03 (C-6); 20.68 (2C); 20.60 (2C); 20.49; 20.34. Calcd. for $C_{27}H_{34}N_2O_{17}$ (658.57): C, 49.24; H, 5.20; N, 4.25. Found: C, 49.10; H, 5.02; N, 4.33.

(β-D-Galactopyranosyl)-(1-5')-O-β-D-ribofuranosyluracil (3b)

A solution of 38 mg (0.057 mmol) of **4b** in 10 mL of methanol was treated according to **method B**. After purification 22.3 mg (95 %) of **3b** were obtained as colorless oil. $[\alpha]_D^{20}$ = +2.5 (c = 0.75, H_2O). ^{1}H-NMR (D_2O): δ 3.70 (dd, 1H, J = 7.8, 9.9 Hz, H-2); 3.79 (dd, 1H, J = 3.3, 9.9 Hz, H-3); 3.81 (m, 1H, H-5); 3.94 (m, 2H, H-6); 4.01 (m, 1H, H-5'); 4.07 (dd, 1H, J = 3.3, 0.8 Hz, H-4); 4.38 (m, 1H, H-5b'); 4.4 (m, 1H, H-4'); 4.46 (t, 1H, J = 5.0 Hz, H-3'); 4.51 (t, 1H, J = 4.9 Hz, H-2'); 4.60 (d, 1H, J = 7.7 Hz, H-1); 6.02 (d, 1H, J = 7.9 Hz, H-5''); 6.08 (d, 1H, J = 4.5 Hz, H-1'); 8.02 (d, 1H, J = 7.9 Hz, H-6''). ^{13}C-NMR (D_2O): δ 172.18 (C-4''); 156.57 (C-2''); 143.67 (C-6''); 105.12 (C-1); 104.59 (C-5''); 91.58 (C-1'); 85.00 (C-4'); 77.35 (C-5); 75.86 (C-2'); 74.91 (C-3); 73.03 (C-2); 71.88 (C-3'); 70.85 (C-5'); 70.76 (C-4); 63.24 (C-6). Calcd. for $C_{15}H_{22}N_2O_{11}$ (406.35): C, 44.34; H, 5.46; N, 6.89. Found: C, 44.21; H, 5.37; N, 6.71.

(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-(1-5')-O-3'-O-acetyl-2'-deoxy-β-D-ribofuranosylthymine (4c)

256 mg (1 mmol) of thymidine **2c** in 50 mL of buffer were treated with p-NO_2Ph-β-Gal **1** and β-galactosidase according to **method A**. After purification by flash chromatography (PE / EA = 1 / 1) 33 mg (5 %) of **4c** were obtained as an inseparable mixture of the β(1-5') and β(1-3') linked regioisomers in a ration of 2 : 1. Full assignment of all resonances could be achieved for both regioisomers.

β(1-5'): ^{1}H-NMR ($CDCl_3$): δ 1.94 (d, 3H, J = 1.1 Hz, CH_3); 2.00; 2.05; 2.10; 2.11; 2.12 (5 s, 15H, acetyl-CH_3); 2.26 (m, 1H, H-2a'); 2.60 (m, 1H, J = 4.1, 6.7, 14.2 Hz, H-2b'); 3.92 (dt, 1H, J = 6.6, 0.8 Hz, H-5); 4.31 (m, 2H, H-5'); 4.15 (m, 1H, H-4'); 4.19 (m, 2H, H-6'); 4.31 (m, 1H, H-3'); 4.57 (d, 1H, J = 8.0 Hz, H-1); 5.03 (dd, 1H, J = 3.4, 10.5 Hz, H-3); 5.23 (dd, 1H, J = 8.0, 10.5 Hz, H-2); 5.40 (dd, 1H, J = 3.4, 0.8 Hz, H-4); 6.10 (t, 1H, J = 6.5 Hz, H-1'); 7.21 (d, 1H, J = 1.1 Hz, H-6''); 9.11 (s, 1H, NH). ^{13}C-NMR

($CDCl_3$): δ 170.69; 170.28; 169.94; 169.93; 169.87; 163.63 (C-4''); 149.99 (C-2''); 135.69 (C-6''); 111.03 (C-5''); 101.06 (C-1); 86.20 (C-1'); 82.15 (C-4'); 78.86 (C-3'); 70.92 (C-5); 70.60 (C-3); 68.33 (C-2); 66.78 (C-4); 63.46 (C-5'); 61.10 (C-6); 38.76 (C-2'); 20.93; 20.65; 20.59; 20.50; 20.46; 12.57 (CH_3).

β(1-3'): ^{1}H-NMR ($CDCl_3$): δ 1.99; 2.04; 2.08; 2.14; 2.17 (5 s, 15H, acetyl-CH_3); 2.06 (d, 3H, CH_3); 2.17 (m, 1H, J = 6.1, 9.2, 14.1 Hz, H-2a'); 2.32 (m, 1H, J = 5.7, 14.1 Hz, H-2b'); 3.73 (dd, 1H, J = 1.8, 10.3 Hz, H-5a'); 3.97 (dt, 1H, J = 6.56, 0.8 Hz, H-5); 4.17 (m, 3H, H-6, H-4'); 4.28 (dd, 1H, J = 1.9, 10.4 Hz, H-5b'); 4.52 (d, 1H, J = 7.9 Hz, H-1); 5.09 (dd, 1H, J = 3.5, 10.5 Hz, H-3); 5.17 (t, 1H, J = 6.1 Hz, H-3'); 5.22 (dd, 1H, J = 7.9, 10.5 Hz, H-2); 5.45 (dd, 1H, J = 3.5, 0.8 Hz, H-4); 6.48 (dd, 1H, J = 5.7, 0.2 Hz, H-1'); 7.68 (d, 1H, J = 1.0 Hz, H-6''); 8.98 (s, 1H, NH). ^{13}C-NMR ($CDCl_3$): δ 170.31; 170.24; 170.13; 170.03; 169.36; 163.70 (C-4''); 150.57 (C-2''); 135.92 (C-6''); 111-39 (C-5''); 101.21 (C-1); 84-58 (C-1'); 83.35 (C-4'); 75.39 (C-3'); 71.06 (C-5); 70.22 (C-3); 70.09 (C-5'); 68.72 (C-2); 66.89 (C-4); 61.02 (C-6); 36.93 (C-2'); 20.78; 20.62; 20.59 (2C); 20.50; 12.47 (CH_3). Calcd. for $C_{26}H_{34}N_2O_{15}$ (614.56): C, 50.81; H, 5.58; N, 4.56. Found: C, 50.67; H, 5.43; N, 4.61.

(β-D-Galactopyranosyl)-(1-5')-O-2'-deoxy-β-D-ribofuranosylthymine (3c)

33 mg (0.049 mmol) of the mixture of regioisomers **4c** were treated according to **method B**. After purification 20 mg (97 %) of **3c** were obtained as a unseparable mixture of the β(1-5') and β(1-3') linked regioisomers in a ration of 2 : 1. Full assignment of all resonances could be achieved for both regioisomers.

β(1-5') : ^{1}H-NMR (D_2O): δ 2.04 (d, 3H, J = 1.2 Hz, CH_3); 2.52 (m, 2H, H-2'); 3.70 (dd, 1H, J = 7.8, 10.0 Hz, H-2); 3.80 (dd, 1H, J = 3.4, 10.0 Hz, H-3); 3.83 (ddd, 1H, J = 4.2, 0.9, 7.8 Hz, H-5); 3.91 (m, 2H, H-6); 3.96 (m, 1H, H-5a'); 4.07 (dd, 1H, J = 3.4, 0.9 Hz, H-4); 4.31 (m, 1H, H-4'); 4.33 (m, 1H, H-5b'); 4.59 (d, 1H, J = 7.8 Hz, H-1); 4.68 (m, 1H, H-3'); 6.44 (t, 1H, J = 6.7 Hz, H-1'); 7.76 (d, 1H, J = 1.2 Hz, H-6''). ^{13}C-NMR (D_2O): δ 169.21 (C-4''); 154.25 (C-2''); 139.54 (C-6''); 113.7 (C-5''); 105.31 (C-1); 87.46 (C-1'); 87.31 (C-4'); 77.39 (C-5); 74.93 (C-3); 73.11 (C-3'); 73.01 (C-2); 71.60 (C-5'); 70.79 (C-4); 63.21 (C-6); 40.52 (C-2'); 13.87 (CH_3).

β(1-3') : ^{1}H-NMR (D_2O): δ 2.02 (d, 3H, J = 1.2 Hz, CH_3); 2.56 (ddd, 1H, J = 2.5, 6.3, 14.4 Hz, H-2a'); 2.73 (ddd, 1H, J = 3.4, 6.3, 14.4 Hz, H-2b'); 3.68 (dd, 1H, J = 7.8, 10.2 Hz, H-2); 3.80 (dd, 1H, J = 10.2, 3.4 Hz, H-3); 3.84 (m, 1H, H-5); 3.91 (m, 2H, H-6); 5.93 (m, 2H, H-5'); 4.07 (m, 1H, H-4); 4.33 (m, 1H, H-4'); 4.62 (d, 1H, J = 7.8 Hz, H-1); 4.72 (dt, 1H, J = 3.3, 6.6 Hz, H-3'); 6.43 (t, 1H, J = 6.3 Hz, H-1'); 7.76 (d, 1H, J = 1.2 Hz, H-6''). ^{13}C-NMR (D_2O): δ 169.21 (C-4''); 154.30 (C-2''); 139.52 (C-6''); 116.62 (C-5''); 104.58 (C-1); 87.43 (C-1'); 87.10 (C-3'); 87.02 (C-4'); 77.43 (C-5); 74.73 (C-3); 72.78 (C-2); 70.72 (C-4); 63.52 (C-5'); 63.12 (C-6); 39.41 (C-2'); 13.77 (CH_3).

(β-D-Galactopyranosyl)-(1-5')-O-β-D-ribofuranosyladenine (3d)

A solution of 267 mg (1 mmol) adenosine **2d** in 60 mL of buffer was treated with p-NO_2Ph-β-Gal **1** and β-galactosidase according to **method A**. After purification by flash chromatography (EA / 2-propanol = 20 / 1) there was an inseparable mixture of the acetylated product **4d** with peracetyl-adenosine obtained. This mixture was deacetylated according to **method B** and afterwards purified by chromatography over Biogel® P2 to obtain 13 mg (3 %) of an inseparable mixture of the β(1-5') and β(1-3') linked regioisomers of **3d** in a ratio of 3,3 : 1. Exact assignment of all the ^{1}H and ^{13}C-signals was possible with exception of the signals of the nucleobase-part in the β(1-3')-isomer.

β(1-5') : ^{1}H-NMR (D_2O): δ 3.71 (dd, 1H, J = 7.7, 9.9 Hz, H-2); 3.79 (dd, 1H, J = 9.9, 3.3 Hz, H-3); 3.83 (m, 1H, H-5); 3.89 (m, 2H, H-6); 4.06 (dd, 1H, J = 3.3, 0.7 Hz, H-4); 4.07 (dd, 1H, J = 4.8, 11.4 Hz, H-

5a'); 4.38 (dd, 1H, J = 2.8, 11.4 Hz, H-5b'); 4.52 (m, 1H, H-4'); 4.60 (d, 1H, J = 7.7 Hz, H-1); 4.64 (t, 1H, J = 4.8 Hz, H-3'); 4.91 (m, 1H, H-2'); 6.22 (d, 1H, J = 5.3 Hz, H-1'); 8.31 (s, 1H, H-7"); 8.54 (s, 1H, H-2"). ^{13}C-NMR (D_2O): δ 157.63 (C-8"); 154.92 (C-7"); 150.95 (C-5"); 142.10 (C-2"); 120.77 (C-4"); 105.27 (C-1); 89.63 (C-1'); 85.69 (C-4'); 77.37 (C-5); 76.14 (C-2'); 74.95 (C-3); 73.01 (C-2); 72.40 (C-3'); 71.18 (C-5'); 70.83 (C-4); 63.19 (C-6).

β(1-3') : ^{1}H-NMR (D_2O): δ 3.79 (m, 1H, H-2); 3.81 (m, 1H, H-3); 3.82 (m, 1H, H-5); 3.92 (m, 2H, H-6); 4.03 (m, 2H, H-5'); 4.07 (m, 1H, H-4); 4.56 (m, 1H, H-4'); 4.70 (d, 1H J = 7.2 Hz, H-1); 4.76 (m, 1H, H-4'); 5.00 (m, 1H, H-2'); 6.22 (d, 1H, J = 5.5 Hz, H-1'). ^{13}C-NMR (D_2O): δ 104.49 (C-1); 90.58 (C-1'); 86.03 (C-4'); 79.88 (C-3'); 77.42 (C-5); 75.52 (C-2'); 74.70 (C-3); 72.70 (C-2); 70.79 (C-4); 63.44 (C-5'); 63.14 (C-6).

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19. The special NMR techniques used for assignment will be published elsewhere.

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