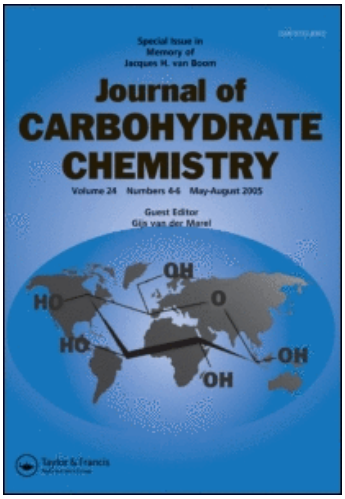

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Synthesis and Enzymic Hydrolysis of Acylated Adenosine Derivatives

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Various derivatives of adenosine were prepared by acylation of adenosine (6-amino-9-(β-D-ribofuranosyl)purine (**1**) with different molar equivalents of acetic anhydride and/or pivaloyl chloride in pyridine. Compounds 6-acetylamino-9-[(2,3,5-tri-*O*-acetyl)-β-D-ribofuranosyl]purine (**3**), 6-amino-9-[(2,3,5-tri-*O*-acetyl)-β-D-ribofuranosyl]purine (**4**), and 6-pivaloylamino-9-[(2,3,5-tri-*O*-pivaloyl)-β-D-ribofuranosyl]purine (**5**) were subsequently submitted to hydrolysis catalyzed by a number of hydrolytic enzymes. Regioselective enzymic deacetylation at the primary hydroxyl group of **3** and **4** with butyrylcholinesterase (BChE) produced 6-acetylamino-9-[(2,3-di-*O*-acetyl)-β-D-ribofuranosyl]purine (**9**) and 6-amino-9-[(2,3-di-*O*-acetyl-β-D-ribofuranosyl]purine (**10**), respectively. All structures were established by ^{1}H and ^{13}C NMR spectroscopies.

Keywords Acylated adenosine, Hydrolases, Enzymic hydrolysis

INTRODUCTION

High selectivity under mild conditions is characteristic for reactions catalyzed by enzymes. Thus, enzymes were found to be suitable biocatalysts in the development of new synthetic methods in organic synthesis.[1,2] They are especially interesting in transformations of multifunctional compounds such as nucleosides since time-consuming protection and deprotection steps, possible side reactions, tedious separation processes, low yields, and other associated problems can often be avoided. Furthermore, the regioselectivity and/or chemoselectivity observed in reactions catalyzed by enzymes add to their rising attractiveness in synthetic organic chemistry.

Nucleosides exhibit a broad spectrum of biologic activities including antiviral, anticancer, antibacterial, and antiparasitic activities.[3] These generally

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result from their ability to inhibit specific enzymes.[3] The rising focus on nucleoside chemistry has also been provoked by the discovery that some nucleoside analogs are potential anti-HIV agents[4–6] and that antisense and antigene oligonucleotides are potential and selective inhibitors of gene expression.[7–9] Although several chemical methods were used for the regioselective modifications[10,11] of nucleoside's sugar moiety, enzymic methods offer significant advantages.[1,2,12–14]

Partially deprotected 5′-adenosines are well-known common intermediates in oligonucleotide synthesis.[15,16] Their conjugates with carbohydrates were also studied. Thus, 6-acetylamino-9-[(2,3-di-*O*-acetyl)-β-D-ribofuranosyl]-purine has been used in the synthesis of 5′-*O*-glycosylnucleosides.[17] Many natural antibiotics possessing significant antitumor and antiviral activities have the structure of a nucleoside connected to oligosaccharides.[17] 6-Amino-9-[(2,3-di-*O*-acetyl)-β-D-ribofuranosyl]purine is a starting material in the synthesis of adenosine α-*P*-boranophosphoglucose, which can be useful in investigations of the stereochemistry and mechanism of action of enzymes such as glycogen and starch synthetases, glycosidyl, and nucleosidyl transferases.[18] It has also been shown that the free 5′-hydroxyl group of partially acetylated adenosines plays a crucial role in the catalytic action at the adenosine deaminase (ADA) active site.[19]

In continuation of our interest in enzymic transformations of carbohydrates,[20,21] in this work we turn to acylated adenosine derivatives in an attempt to perform regioselective enzymically catalyzed deprotection of the 5′-OH.

RESULTS AND DISCUSSION

Preparation of Acylated Adenosine Derivatives

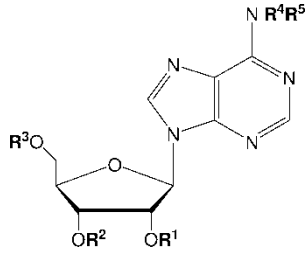
Conventional acetylation of adenosine **1** with 10 molar equivalents of Ac_2O in pyridine for 24 h followed by column chromatography on silica gel produced the pentaacetate **2** (29%) and the tetraacetate **3** (67%) as the major product (Table 1).

Treatment of **1** with six molar equivalents of the same acylating agent for 24 h resulted in the formation of the triacetate **4** (70%) as the sole reaction product.

Pivaloylation of **1** was achieved by using 10 equivalents of pivaloyl chloride in pyridine for 48 h at 50°C. It was noticed, by TLC monitoring, that during the first 24 h several products were formed. After 48 h only tetrapivalate **5** was isolated in 70% yield.

Gradual addition of seven molar equivalents of pivaloyl chloride to a solution of **4** in pyridine for 48 h produced 6-pivaloylamino-9-[(2,3,5-tri-*O*-acetyl)-β-D-ribofuranosyl]purine **6** (66%). Some unreacted **4** (33%) was recovered from the reaction mixture as well.

Table 1: Acylated adenosine derivaties.



	R^1	R^2	R^3	R^4	R^5
1	H	H	H	H	H
2	Ac	Ac	Ac	Ac	Ac
3	Ac	Ac	Ac	Ac	H
4	Ac	Ac	Ac	H	H
5	Piv	Piv	Piv	Piv	H
6	Ac	Ac	Ac	Piv	H
7[a]	H	Ac	Ac	Ac	H
8[a]	Ac	H	Ac	Ac	H
9[a]	Ac	Ac	H	Ac	H
10[a]	Ac	Ac	H	H	H

[a]Compounds prepared by enzymic hydrolysis.

Enzymic Deacylations

Tetraacetate **3**, triacetate **4**, and tetrapivalate **5** were submitted to hydrolysis catalyzed by various commercially available enzymes. To improve solubility of acylated derivatives **3** and **5**, organic solvent (Me_2SO or DMF) was added to the phosphate-buffered (pH 7) incubation mixtures (Table 2). Only in hydrolysis of **3** catalyzed by BChE the addition of organic solvents was not needed. Parallel controls containing the substrate in reaction medium but without the enzyme were also prepared to exclude any nonenzymic processes.

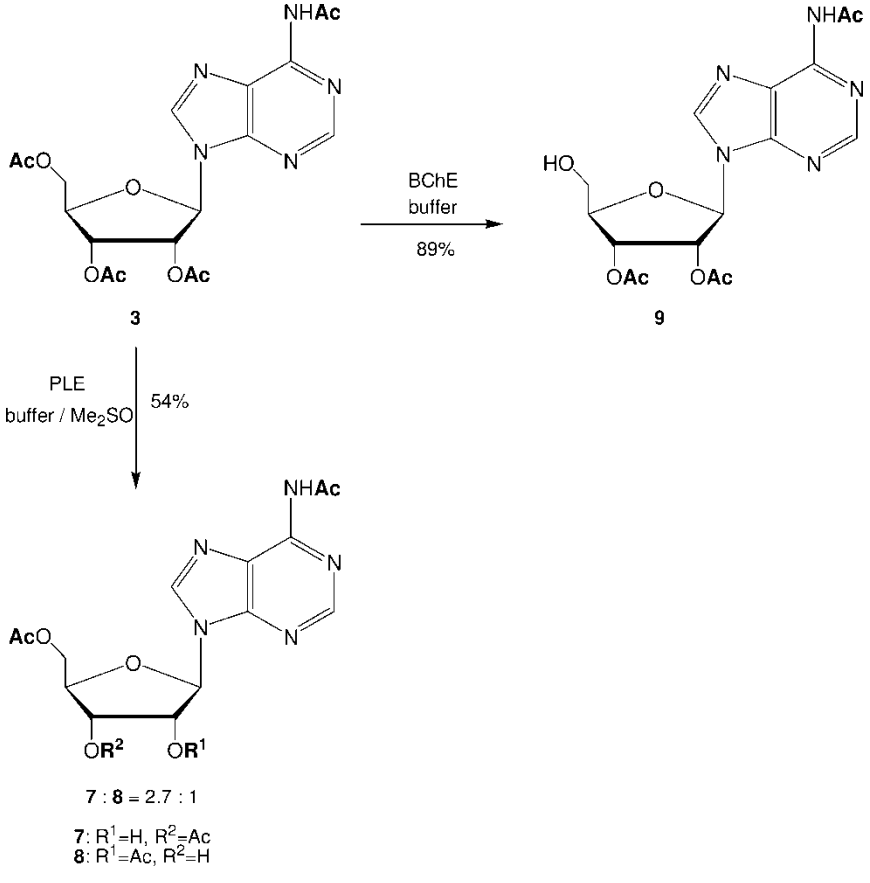
Table 2: Enzyme-catalyzed hydrolysis of tetraacetate **3**.

Enzyme/mg	Organic solvent/μL	Reaction time/h	Product (Yield)
PPL/1.7	Me_2SO/200	4	Unselective hydrolysis
PLE/1.1	Me_2SO/200	2.5	**7** + **8** (54%; **7** : **8** = 2.7 : 1)
BChE/2.6	—	2	**9** (89%)
Subtilisin/3.5	DMF/500	48	—
α-Chymotrypsin/1	DMF/200	48	—

It was shown that BChE catalyzes the selective hydrolysis of the 5′-*O*Ac in the tetraacetate **3** yielding 89% of 6-acetylamino-9-[(2,3-di-*O*-acetyl)-β-D-ribofuranosyl]purine **9**. The reaction was carried out in phosphate buffer without the addition of organic solvents (Sch. 1).

Hydrolysis of tetraacetate **3** catalyzed by porcine liver esterase (PLE) (Sch. 1) produced the regioisomeric mixture of 6-acetylamino-9-[(3,5-di-*O*-acetyl)-β-D-ribofuranosyl]purine **7** and 6-acetylamino-9-[(2,5-di-*O*-acetyl)-β-D-ribofuranosyl]purine **8** in a ratio of 2.7 : 1 as determined by ^{1}H NMR. Total yield of 54% was obtained in 2.5 h. It is possible, however, that in this reaction selective hydrolysis of the 2′-*O*Ac occurs in the first step liberating the 2′-OH group, followed by the 3′ → 2′ acetyl migration. This type of migrations are known and were previously reported.[19,22]

Hydrolysis of **3** with porcine pancreas lipase (PPL) as a catalyst in 4 h resulted in a mixture of several partially acetylated products. Some unreacted starting material was detected as well. No reaction occurred when



Scheme 1

α-chymotrypsin or subtilisin were used as possible catalysts under the same reaction conditions.

Regioselective hydrolyses of 2′,3′,5′-tri-*O*-acetylribonucleosides have already been described using subtilisin and lipase from *Candida antarctica* (CAL) as catalysts,[14,19] and the corresponding 2′,3′-di-*O*-acetylribonucleosides were obtained in relatively good yields in slow reactions (16–24 h). We found that BChE catalyzes the selective hydrolysis of the 5′-*O*Ac in the triacetate **4** as well. In 4.5 h 39% of diacetate **10** was obtained (Table 3). Prolongation of reaction times resulted in loss of selectivity. This is often the case when using enzymes as catalysts in transformations of compounds that are not their natural substrates. While stereospecific reactions occur with enzymes' natural substrates, only stereoselectivity can be expected in transformations of nonnatural substrates, and enzymes with time start recognizing, in smaller degrees, other similar structures leading to the decrease of selectivity.

Triacetate **4** underwent unselective deacetylation in reactions catalyzed by PPL and PLE and no reaction was observed using α-chymotrypsin.

Tetrapivalate **5** was also subjected to hydrolyses under similar reaction conditions and with the same enzymes as **3** and **4**, but no reactions were observed. The most probable cause for this unreactivity is the very low solubility of **5** even in incubation mixtures in which up to 30% (v/v) of organic solvent was added.

EXPERIMENTAL

General Methods for Synthesis and Characterization of Adenosine Derivatives

All solvents were reagent grade and distilled before use. Column chromatography was performed on silica gel (Merck) and TLC on Merck silica gel (60 F 254) plates (0.25 mm) with solvent A, EtOAc-C_6H_6-EtOH (10 : 2 : 1.5); solvent B, EtOAc-MeOH (5 : 1); solvent C, EtOAc-C_6H_6 (10 : 1); and solvent D, EtOAc-C_6H_6 (5 : 1). Visualisation was effected by use of UV light and by charring with H_2SO_4. Melting points were determined with a Büchi B-40 apparatus

Table 3: Enzyme-catalyzed hydrolysis of triacetate **4**.

Enzyme/mg	Reaction time/h	Product (Yield)
PPL/1.9	4	Unselective hydrolysis
PLE/1.2	3.5	Unselective hydrolysis
BChE/14.5	4.5	**10** (39%)
α-Chymotrypsin/1	48	—

and are uncorrected. Optical rotations were measured at ~20°C using the Optical Activity AA-10 Automatic Polarimeter. ^{1}H and ^{13}C NMR spectra (300 MHz or 600 MHz, Me_2SO or $CDCl_3$, internal Me_4Si) were recorded with a Bruker AV300 spectrometer.

6,6-Diacetylamino-9-[(2,3,5-tri-*O*-acetyl)-β-D-ribofuranosyl]purine (2) and 6-acetylamino-9-[(2,3,5-tri-*O*-acetyl)-β-D-ribofuranosyl]purine (3). To a solution of **1** (100 mg, 0.37 mmol) in dry pyridine (1 mL) acetic anhydride (354 μL, 3.74 mmol) was added. The mixture was stirred at ambient temperature for 24 h and the reaction stopped by addition of 96% EtOH. Water was added and the mixture of solvents evaporated under reduced pressure. The remaining traces of water were removed by codistillation with toluene. Column chromatography (solvent A) of the residue gave, firstly, 6,6-diacetylamino-9-[(2,3,5-tri-*O*-acetyl)-*β*-D-ribofuranosyl]purine **2** (51 mg, 29%) as a white syrup;[23] $[\alpha]_D$ −18.5° (c 0.87, $CHCl_3$); $R_f \sim 0.46$ (solvent A). ^{1}H NMR (300 MHz, $CDCl_3$): 2.12 (s, 3 H, *O*Ac) 2.14 (s, 3 H, *O*Ac), 2.17 (s, 3 H, *O*Ac), 2.38 (s, 6 H, *N*Ac$_2$), 4.37–4.51 (m, 3 H, H-4′, H-5′a, H-5′b), 5.69 (app t, 1 H, $J = 5.22$ Hz, $J = 4.94$ Hz, H-3′), 5.98 (app t, 1 H, $J = 5.50$ Hz, $J = 5.22$ Hz, H-2′), 6.27 (d, 1 H, $J = 4.95$ Hz, H-1′), 8.30 (s, 1 H, H-8), 8.99 (s, 1 H, H-2); ^{13}C NMR (600 MHz, $CDCl_3$): 20.27, 20.38, 20.61 (***C***H$_3$CO, 2′-*O*Ac, 3′-*O*Ac, 5′-*O*Ac), 26.20 (2 ***C***H$_3$CO, *N*Ac$_2$), 62.86 (***C***-5′), 70.43 (***C***-3′), 72.98 (***C***-2′), 80.54 (***C***-4′), 86.70 (***C***-1′), 130.77 (***C***-5), 141.19 (***C***-8), 151.07 (***C***-4), 152.84 (***C***-2), 153.15 (***C***-6), 169.20, 169.39, 170.10 (***C***═O, 2′-*O*Ac, 3′-*O*Ac, 5′-*O*Ac), 171.67 (2 ***C***═O, *N*Ac$_2$).

Eluted next was 6-acetylamino-9-[(2,3,5-tri-*O*-acetyl)-*β*-D-ribofuranosyl]-purine **3** as a white syrup[24] (109 mg, 67%); $[\alpha]_D$ −17.3° (c 1.33, $CHCl_3$); $R_f \sim 0.26$ (solvent A). ^{1}H NMR (300 MHz, $CDCl_3$): 2.09 (s, 3 H, *O*Ac), 2.12 (s, 3 H, *O*Ac), 2.17 (s, 3 H, *O*Ac), 2.64 (s, 3 H, *N*Ac), 4.35–4.48 (m, 3 H, H-4′, H-5′a, H-5′b), 5.68 (app t, 1 H, $J = 5.22$ Hz, $J = 4.95$ Hz, H-3′), 5.98 (app t, 1 H, $J = 5.50$ Hz, $J = 5.49$ Hz, H-2′), 6.24 (d, 1 H, $J = 5.22$ Hz, H-1′), 8.26 (s, 1 H, H-8), 8.71 (s, 1 H, H-2), 9.28 (br s, 1 H, *N*H); ^{13}C NMR (300 MHz, $CDCl_3$): 20.23, 20.36, 20.57 (***C***H$_3$CO, 2′-*O*Ac, 3′-*O*Ac, 5′-*O*Ac), 25.60 (***C***H$_3$CO, *N*Ac), 62.86 (***C***-5′), 70.41 (***C***-3′), 72.87 (***C***-2′), 80.18 (***C***-4′), 86.25 (***C***-1′), 122.05 (***C***-5), 141.49 (***C***-8), 149.38 (***C***-4), 150.91 (***C***-6), 152.43 (***C***-2), 169.24, 169.46, 170.23, 170.92 (***C***═O, 2′-*O*Ac, 3′-*O*Ac, 5′-*O*Ac, *N*Ac).

6-Amino-9-[(2,3,5-tri-*O*-acetyl)-β-D-ribofuranosyl]purine (4). To a solution of **1** (110 mg, 0.41 mmol) in dry pyridine (1 mL) acetic anhydride (235 μL, 2.47 mmol) was added. The mixture was stirred at ambient temperature for 24 h and the reaction stopped by addition of 96% EtOH. After usual workup of the reaction mixture, as described in preparation of **2** and **3**, column chromatography (solvent B) of the residue gave white crystalline[24,25] 6-amino-9-[(2,3,5-tri-*O*-acetyl)-*β*-D-ribofuranosyl]purine **4**; (137 mg, 85%); mp 174–175°C (from abs. EtOH), lit.[24] 174°C; $[\alpha]_D$ −20.9° (c 1.00, $CHCl_3$),

lit.[24] -27.9° ($CHCl_3$); $R_f \sim 0.44$ (solvent B). ^1H NMR (600 MHz, $CDCl_3$): 2.09 (s, 3 H, *O*Ac), 2.13 (s, 3 H, *O*Ac), 2.15 (s, 3 H, *O*Ac), 4.36–4.40 (m, 1 H, H-4′), 4.44–4.48 (m, 2 H, H-5′a, H-5′b), 5.68 (dd, 1 H, $J = 5.45$ Hz, $J = 4.54$ Hz, H-3′), 5.74 (br s, 2 H, NH_2), 5.94 (app t, 1 H, $J = 5.43$ Hz, $J = 5.36$ Hz, H-2′), 6.19 (d, 1 H, $J = 5.30$ Hz, H-1′), 7.97 (s, 1 H, H-8), 8.37 (s, 1 H, H-2); ^{13}C NMR (600 MHz, $CDCl_3$): 20.37, 20.51, 20.74 (***C***H_3CO, 2′-*O*Ac, 3′-*O*Ac, 5′-*O*Ac), 63.05 (***C***-5′), 70.63 (***C***-3′), 73.28 (***C***-2′), 80.44 (***C***-4′), 86.41 (***C***-1′), 120.08 (***C***-5), 139.42 (***C***-8), 149.64 (***C***-4), 151.38 (***C***-2), 154.60 (***C***-6), 169.32, 169.52, 170.25 (***C***=O, 2′-*O*Ac, 3′-*O*Ac, 5′-*O*Ac).

6-Pivaloylamino-9-[(2,3,5-tri-*O*-pivaloyl)-β-D-ribofuranosyl]purine (5). To a solution of **1** (200 mg, 0.75 mmol) in dry pyridine (2 mL) pivaloyl chloride (920 μL, 7.5 mmol) was added. The mixture was stirred at 50°C under reflux for 48 h and the reaction stopped by addition of 96% EtOH. After evaporation, as described in preparation of **2** and **3**, column chromatography (solvent C) of the residue gave as a main product 6-pivaloylamino-9-[(2,3,5-tri-*O*-pivaloyl)-β-D-ribofuranosyl]purine **5**; yellow oil (317 mg, 70%); $[\alpha]_D$ -3° (c 1.00, $CHCl_3$); $R_f \sim 0.55$ (solvent C). ^1H NMR (300 MHz, $CDCl_3$): 1.13 (s, 9 H, *O*Piv), 1.23 (s, 9 H, *O*Piv), 1.27 (s, 9 H, *O*Piv), 1.40 (s, 9 H, *N*Piv), 4.35–4.46 (m, 3 H, H-4′, H-5′a, H-5′b), 5.58 (dd, 1 H, $J = 4.95$ Hz, $J = 2.75$ Hz, H-3′), 5.83 (app t, 1 H, $J = 6.04$ Hz, $J = 5.77$ Hz, H-2′), 6.23 (d, 1 H, $J = 6.04$ Hz, H-1′), 8.15 (s, 1 H, H-8), 8.51 (br s, 1 H, *N*H), 8.76 (s, 1 H, H-2); ^{13}C NMR (300 MHz, $CDCl_3$): 26.86, 27.00, 27.09, 27.20 ((***C***$H_3)_3CCO$, 2′-*O*Piv, 3′-*O*Piv, 5′-*O*Piv, *N*Piv), 38.67 ($(CH_3)_3$***C***CO, Piv), 38.73 (2 $(CH_3)_3$***C***CO, Piv), 40.43 ($(CH_3)_3$***C***CO, Piv), 63.12 (***C***-5′), 70.60 (***C***-3′), 73.25 (***C***-2′), 81.18 (***C***-4′), 85.92 (***C***-1′), 122.71 (***C***-5), 140.83 (***C***-8), 149.43 (***C***-4), 151.48 (***C***-6), 152.18 (***C***-2), 175.68, 176.80, 177.01, 177.89 (***C***=O, 2′-*O*Piv, 3′-*O*Piv, 5′-*O*Piv, *N*Piv).

Anal. Calcd. for $C_{30}H_{45}O_8N_5$: C, 59.68; H, 7.51; N, 11.60. Found: C, 60.03; H, 7.82; N, 11.21.

6-Pivaloylamino-9-[(2,3,5-tri-*O*-acetyl)-β-D-ribofuranosyl]purine (6). To a solution of **4** (30 mg, 0.076 mmol) in dry pyridine (0.5 mL) pivaloyl chloride (66 μL, 0.53 mmol) was added gradually while the mixture was stirred at ambient temperature for 48 h. The reaction was stopped by addition of 96% EtOH. After evaporation, as described in preparation of **2** and **3**, column chromatography (solvent D) of the residue gave as a main product 6-pivaloylamino-9-[(2,3,5-tri-*O*-acetyl)-β-D-ribofuranosyl]purine **6**; yellow oil (24 mg, 66%); $[\alpha]_D$ -6.5° (c 1.24, $CHCl_3$); $R_f \sim 0.55$ (solvent D). ^1H NMR (600 MHz, $CDCl_3$): 1.40 (s, 9 H, *N*Piv), 2.08 (s, 3 H, *O*Ac), 2.13 (s, 3 H, *O*Ac), 2.16 (s, 3 H, *O*Ac), 4.38–4.48 (m, 3 H, H-4′, H-5′a, H-5′b), 5.66 (dd, 1 H, $J = 5.45$ Hz, $J = 4.24$ Hz, H-3′), 5.94 (app t, 1 H, $J = 5.53$ Hz, $J = 5.53$ Hz, H-2′), 6.24 (d, 1 H, $J = 5.56$ Hz, H-1′), 8.18 (s, 1 H, H-8), 8.59 (br s, 1 H, *N*H), 8.76 (s, 1 H, H-2); ^{13}C NMR (300 MHz, $CDCl_3$): 20.24, 20.42, 20.65 (***C***H_3CO, 2′-*O*Ac,

3′-*O*Ac, 5′-*O*Ac), 27.28 ((***C***H$_3$)$_3$CCO, *N*Piv), 40.38 ((CH$_3$)$_3$***C***CO, *N*Piv), 62.93 (***C***-5′), 70.59 (***C***-3′), 73.01 (***C***-2′), 80.46 (***C***-4′), 86.22 (***C***-1′), 123.27 (***C***-5), 141.11 (***C***-8), 149.59 (***C***-4), 151.60 (***C***-6), 152.50 (***C***-2), 169.20, 169.44, 170.17 (***C***═O, 2′-*O*Ac, 3′-*O*Ac, 5′-*O*Ac), 175.69 (***C***═O, *N*Piv).

Anal. Calcd. for $C_{21}H_{27}O_8N_5$: C, 52.82; H, 5.70; N, 14.67. Found: C, 52.74; H, 6.07; N, 14.30.

Eluted next was some unreacted **4** (10 mg, 33%).

General Methods for Enzymic Deacylations of Adenosine Derivatives

Lipase from porcine pancreas (PPL, 27 U/mg), butyrylcholinesterase from equine serum (BChE, 17.6 U/mg), proteinase from *Bacillus subtilis* (subtilisin, 13.1 U/mg) and α-chymotrypsin from bovine pancreas (61 U/mg) were purchased from Fluka/BioChemika. Esterase from porcine liver (PLE, 41 U/mg) was obtained from Sigma. All solvents were reagent grade and distilled before use. Column chromatography was performed on silica gel (Merck) and TLC monitoring on Merck silica gel (60 F 254) plates (0.25 mm) with solvents B, C, and solvent E, EtOAc-MeOH (5 : 2). Visualization was effected by use of UV light and by charring with H_2SO_4. All experiments were carried out in phosphate-buffered solutions (0.1 M, pH 7) at 37°C. To improve solubility of adenosine derivatives in this media organic solvents (Me_2SO or DMF) were added in some reaction mixtures (Table 2). Control reactions were performed parallel to every enzymic hydrolysis and contained all reactants except the enzyme. pH values of reaction mixtures were monitored periodically by pH-indicator paper Neutralit (pH = 5.5–9.0, Merck) and adjusted with 0.1 M NaOH. Reactions were stopped by the addition of 96% EtOH and the solvents evaporated under reduced pressure, if not stated otherwise.

*Enzymic Deacetylations of Tetraacetate **3***

Tetraacetate **3** (20 mg, 0.046 mmol) was suspended in a mixture of organic solvent and phosphate buffer (2 mL) in all experiments except the one with BChE where only aqueous buffered solution was used (Table 2). Enzymes were added next. The reactions were monitored by TLC (solvent B).

Column chromatography (solvent B) of the residue with PLE gave the regioisomeric mixture of two triacetates **7** and **8** (9.8 mg, 54%); $R_f \sim 0.41$ (solvent B) as a main product. ^{1}H NMR (600 MHz, Me_2SO): 2.04 (s, 3 H, *O*Ac), 2.13 (s, 3 H, *O*Ac), 2.26 (s, 3 H, *N*Ac), 4.24–4.40 (m, 3 H, H-4′, H-5′a, H-5′b), 5.05–5.08 (m, 1 H, H-2′), 5.34 (dd, 1 H, $J = 5.34$ Hz, $J = 3.80$ Hz, H-3′), 6.03 (d, 1 H, $J = 6.17$ Hz, H-1′), 8.68 (s, 1 H, H-8), 8.70 (s, 1 H, H-2), 10.76 (br s, 1 H, *N*H); ^{13}C NMR (600 MHz, Me_2SO): 20.42, 20.59 (***C***H$_3$CO, 3′-*O*Ac, 5′-*O*Ac), 24.16 (***C***H$_3$CO, *N*Ac), 63.15 (***C***-5′), 70.76 (***C***-2′), 72.23 (***C***-3′), 79.41 (***C***-4′), 87.57 (***C***-1′), 123.71 (***C***-5), 142.91 (***C***-8), 149.61 (***C***-4), 149.64

(***C***-6), 151.67 (***C***-2), 168.70, 169.50, 169.96 (***C***═O, 3′-*O*Ac, 5′-*O*Ac, *N*Ac) for the 6-acetylamino-9-[(3,5-di-*O*-acetyl)-β-D-ribofuranosyl]purine **7** and ^{1}H NMR (600 MHz, Me_2SO): 1.98 (s, 3 H, *O*Ac), 2.08 (s, 3 H, *O*Ac), 2.26 (s, 3 H, *N*Ac), 4.24–4.40 (m, 3 H, H-4′, H-5′a, H-5′b), 4.62–4.65 (m, 1 H, H-3′), 5.78 (app t, 1 H, $J = 5.08$ Hz, $J = 4.98$ Hz, H-2′), 6.26 (d, 1 H, $J = 4.53$ Hz, H-1′), 8.66 (s, 1 H, H-8), 8.68 (s, 1 H, H-2), 10.76 (br s, 1 H, *N*H); ^{13}C NMR (600 MHz, Me_2SO): 20.37, 20.46 (***C***H_3CO, 2′-*O*Ac, 5′-*O*Ac), 23.55 (***C***H_3CO, *N*Ac), 63.15 (***C***-5′), 68.41 (***C***-3′), 74.30 (***C***-2′), 81.73 (***C***-4′), 85.69 (***C***-1′), 123.60 (***C***-5), 142.91 (***C***-8), 149.61 (***C***-4), 149.64 (***C***-6), 151.67 (***C***-2), 168.70, 169.37, 169.92 (***C***═O, 2′-*O*Ac, 5′-*O*Ac, *N*Ac) for the 6-acetylamino-9-[(2,5-di-*O*-acetyl)-β-D-ribofuranosyl]purine **8**.

Purification of the residue (solvent B) obtained from the incubation mixture with BChE gave 6-acetylamino-9-[(2,3-di-*O*-acetyl)-β-D-ribofuranosyl]purine **9** as a main product; white syrup[17] (16 mg, 89%); $[\alpha]_D$ −27.6° (c 0.73, acetone); $R_f \sim 0.33$ (solvent B). ^{1}H NMR (300 MHz, Me_2SO): 1.99 (s, 3 H, *O*Ac), 2.15 (s, 3 H, *O*Ac), 2.26 (s, 3 H, *N*Ac), 3.67–3.77 (m, 2 H, H-5′a, H-5′b), 4.23–4.27 (m, 1 H, H-4′), 5.55 (dd, 1 H, $J = 5.18$ Hz, $J = 2.95$ Hz, H-3′), 5.96 (app t, 1 H, $J = 6.00$ Hz, $J = 5.94$ Hz, H-2′), 6.31 (d, 1 H, $J = 6.55$ Hz, H-1′), 8.68 (s, 1 H, H-8), 8.72 (s, 1 H, H-2), 10.79 (br s, 1 H, *N*H); ^{13}C NMR (600 MHz, Me_2SO): 20.47, 20.66 (***C***H_3CO, 2′-*O*Ac, 3′-*O*Ac), 24.18 (***C***H_3CO, *N*Ac), 60.80 (***C***-5′), 70.99 (***C***-3′), 72.49 (***C***-2′), 83.62 (***C***-4′), 85.01 (***C***-1′), 123.56 (***C***-5), 142.51 (***C***-8), 149.70 (***C***-4), 151.45 (***C***-6), 152.54 (***C***-2), 169.07, 169.42, 169.64 (***C***═O, 2′-*O*Ac, 3′-*O*Ac, *N*Ac).

*Enzymic Deacetylations of Triacetate **4***

Triacetate **4** (20 mg, 0.051 mmol) was suspended in phosphate buffer (2 mL) in all experiments except the one with BChE where **4** (100 mg, 0.254 mmol) was dissolved in 5 mL of phosphate buffer. Enzymes were added next (Table 3) and the reactions were monitored by TLC (solvents B and E).

After evaporation the residue with BChE was extracted with the mixture of solvents EtOAc/MeOH 5 : 2 (3 × 10 mL) and combined organic extracts were again evaporated under reduced pressure. Column chromatography of the resulting residue (solvent B) gave white crystalline 6-amino-9-[(2,3-di-*O*-acetyl)-β-D-ribofuranosyl]purine **10** as a main product; (35 mg, 39%); mp 180.3–181.2°C (from acetone/pentane), lit.[26] 181–182°C; $[\alpha]_D$ −70.8° (c 0.72, acetone), lit.[26] −78.7° (acetone); $R_f \sim 0.32$ (solvent B), ^{1}H NMR (300 MHz, Me_2SO): 1.98 (s, 3 H, *O*Ac), 2.14 (s, 3 H, *O*Ac), 3.62–3.76 (m, 2 H, H-5′a, H-5′b), 4.23–4.26 (m, 1 H, H-4′), 5.51 (dd, 1 H, $J = 5.42$ Hz, $J = 2.43$ Hz, H-3′), 5.93 (dd, 1 H, $J = 6.96$ Hz, $J = 5.50$ Hz, H-2′), 6.21 (d, 1 H, $J = 7.05$ Hz, H-1′), 7.45 (br s, 2 H, NH_2), 8.16 (s, 1 H, H-8), 8.39 (s, 1 H, H-2); ^{13}C NMR (300 MHz, Me_2SO): 20.10, 20.43 (***C***H_3CO, 2′-*O*Ac, 3′-*O*Ac), 61.17 (***C***-5′), 71.32 (***C***-3′), 72.25 (***C***-2′), 83.82 (***C***-4′), 85.11 (***C***-1′), 119.16 (***C***-5),

139.55 (***C***-8), 148.84 (***C***-4), 152.60 (***C***-2), 156.18 (***C***-6), 169.16, 169.52, (***C***═O, 2′-*O*Ac, 3′-*O*Ac).

Starting unreacted compound **4** was recovered as well (52 mg, 52%).

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