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The Action of Adenosine Deaminase (E.C. 3.5.4.4.) on Adenosine and Deoxyadenosine Acetates: The Crucial Role of the 5′-Hydroxy Group for the Enzyme Activity

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Abstract—From adenosine **1**, 2′-deoxyadenosine **3** and 3′-deoxyadenosine **5** all the acetates were prepared by lipase-catalyzed reactions. Only the acetates with free 5′-hydroxy group were deaminated by adenosine deaminase (ADA), confirming the crucial role of 5′-OH for the enzyme activity. © 2000 Elsevier Science Ltd. All rights reserved.

Introduction

Adenosine deaminase (adenosine aminohydrolase, E.C. 3.5.4.4, ADA) catalyzes the hydrolytic deamination of adenosine **1** to inosine **2** (Fig. 1) and the broad activity of the enzyme on nucleosides modified either on the purine base or in the ribose has long been known.[1]

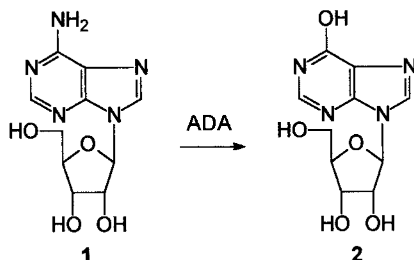

Figure 1. ADA catalysis of the hydrolytic deamination of adenosine **1** to inosine **2**.

ADA is a commercially available enzyme that can be used for preparative purposes to transform derivatives and analogs of adenosine **1** into a variety of compounds that often show interesting pharmacological properties.[2] We have already applied the ADA-catalyzed deamination of 2′-deoxyadenosine **3** to 2′-deoxyinosine **4** on a preparative scale[3] and report here that the same enzymatic reaction can be conveniently used[4] to prepare from 3′-deoxyadenosine **5**[5] the less available 3′-deoxyinosine **6** (1 h, 95% yield).[6,7] For the reported transformation of inosine **2** or 2′-deoxyinosine **4** into the anti AIDS drug 2′,3′-dideoxyinosine (DDI, **7**),[3] we had investigated the lipase-catalyzed selective acylation of the above nucleosides (Fig. 2).

However, due to the polar solvents used (pyridine or DMSO), these enzymatic reactions were slow (20–96 h) and the recovery of the products often troublesome. We decided to study the action of ADA on the acetates of adenosine **1**, 2′-deoxyadenosine **3** and 3′-deoxyadenosine **5** since this enzymatic approach could constitute a viable method for the preparation of selectively acetylated inosines and deoxyinosines.

Results and Discussion

Several methods for the enzymatic selective acylation of

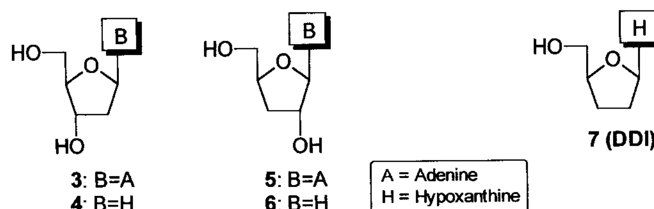

Figure 2. Compounds **3**–**7**.

Keywords: deamination; enzymes and enzyme reactions; nucleosides.
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Table 1. Enzymatic preparation of acetates of adenosine (**1**), 2′-deoxyadenosine (**3**) and 3′-deoxyadenosine (**5**). (These reactions did not take place in absence of enzyme. The acetylation is performed in THF at 60°C and the hydrolysis in acetone/buffer or water-saturated $CHCl_3$ (see Experimental))

Entry	Substrate	Lipase	Time (h)	Yield[a] (%)	Product
1	**1**	CAL	2.0	89	**8a**
2	**1**	PSL	48.0	87	**8c**[b]
3	**3**	CAL	0.5	92	**9a**
4	**3**	PSL	48.0	90	**9b**
5	**5**	CAL	0.5	85	**10a**
6	**8e**	CAL	24.0	84	**8b**
7	**10c**	CAL	24.0	88	**10b**

[a] Pure isolated product.
[b] We always observe also the formation of 2′-acetate **8d**.

the reaction at 50°C, since at this temperature the substrates are soluble in water and the ADA enzyme still shows a good activity. We observed that only the acetates with free 5′-OH groups (e.g.: **8b**, **8c**, **9b**, **10b**) quantitatively react to afford the corresponding inosine acetates **11a–d** (Fig.4) as shown in Table 2.

The fact that all nucleosides with 5′-protected hydroxy groups, namely the acetates **8a**, **8e**, **9a**, **9c**, **10a**, and **10c**, failed to react with ADA confirms that in the catalytic action at the ADA active site the 5′-hydroxy group plays a crucial role. This is presumably due to the hydrogen bonding with Hys 17 and Asp 19 that is a structural prerequisite to stabilize the transition state during the catalytic process (Fig. 5).[12]

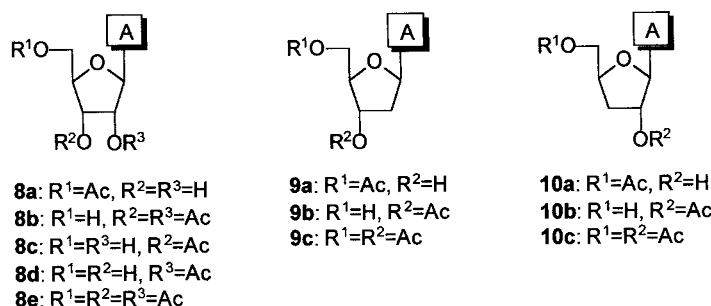

Figure 3. Compounds **8–10**.

nucleosides are currently available[8] and we have prepared all the selectively acetylated adenosine and deoxyadenosines by the lipase-catalyzed reactions previously described.[3] The enzyme *Candida antarctica* lipase (CAL) catalyzes the irreversible acetylation with vinyl acetate[9] of the parent nucleoside (**1**, **3** or **5**) at the 5′-hydroxy group, thus allowing the preparation of the monoacetates **8a**, **9a** and **10a** (Entries 1, 3 and 5, Table 1). When the enzyme used is *Pseudomonas sp.* lipase (PSL) the 3′-hydroxy group of adenosine **1** can be selectively acylated, but the well established acyl migration to the vicinal 2′-OH[10] leads to a 77:23 mixture of monoacetates **8c** and **8d** (Entry 2, Table 1). As a result of the PSL specificity for the 3′-OH, 2′-deoxyadenosine **3** selectively afforded the monoacetate **9b** (Entry 4, Table 1), whereas no product was observed from 3′-deoxyadenosine **5** after 48 h.[11] The remaining acetates bearing the non-acetylated 5′-hydroxy group, namely compounds **8b** and **10b** were prepared by selective hydrolysis of fully acetylated compounds **8e** and **10c** using CAL in acetone/buffer or water-saturated $CHCl_3$ (Entry 6 and 7, Table 1). Finally, the monoacetate **9b**, available by PSL-catalyzed acetylation of **3**, as described above, could also be prepared by CAL-catalyzed hydrolysis of the diacetate **9c** (Fig. 3).

We next studied the ADA-catalyzed hydrolytic deamination of the nucleoside acetates prepared as above and carried out

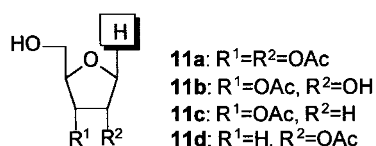

Figure 4. Inosine acetates **11a–d**.

Table 2. ADA-catalyzed deamination of adenosine, 2′-deoxyadenosine and 3′-deoxyadenosine acetates

Substrate	Time (h)	Yield[a] (%)	Product
8b	6.0	90	**11a**
8c[b]	1.0	91	**11b**[b]
9b	1.0	94	**11c**
10b	1.0	94	**11d**

[a] Pure isolated product.
[b] Substrate and product are in 77:23 mixture with the 2′-acetate.

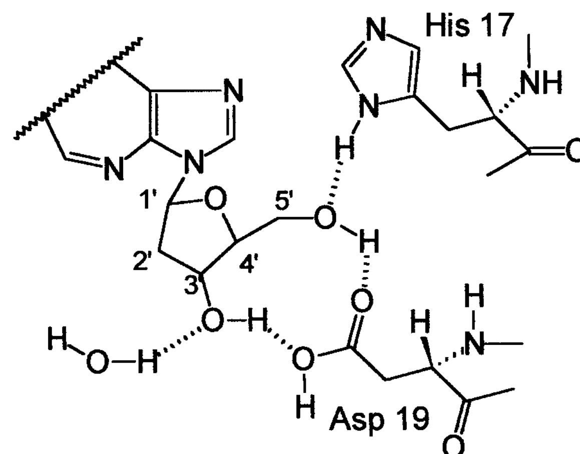

Figure 5. Schematic representation of deoxyribose interactions within the active site of the enzyme ADA (from the crystal structure of ADA-bound 2-deoxycoformicin[12]).

Additionally, the hydrogen bonding between the 3′-OH and Asp-19 appears to be non-essential for the catalysis, since the acetate **9b** is enzymatically deaminated to **11c**.

The 5′-acetate could act as an apolar group that does not allow the formation of the hydrogen bonding network with Hys 17 and Asp 19 that is necessary for the enzyme activity. On the other hand, one cannot exclude that steric hindrance of the acetyl group may disfavour the complete fitting of the substrate within the active site. A more accurate study of the chemical features of the 5′-position is needed in order to define the nature of the observed loss of reactivity of the 5′-acetates.[13]

Conclusions

We have shown that ADA can be used as a biocatalyst for the hydrolytic deamination of adenosine and deoxyadenosines acetates. Additionally, from these results we have observed that the acetylation of the 5′-hydroxy group leads to loss of enzyme reactivity and, consequently, that this position has a crucial role to play in the catalytic action of the enzyme. This observation is complementary to the existing pictures of the active site, generated by X-ray studies of the complex formed by ADA with the inhibitor 2′-deoxycoformicin.[12]

Experimental

Melting points were obtained using a Stuart Scientific SMP3 instrument and are uncorrected. IR spectra were recorded on a Nicolet 510 Fourier transform spectrophotometer. ^{1}H NMR spectra were recorded on Bruker AM-500 spectrometer operating at 500.13 MHz. The ^{1}H NMR chemical shifts are reported in parts per million, using as reference the signal for residual solvent protons (7.24 for $CDCl_3$, 3.30 for CD_3OD and 2.49 for DMSO-d_6) and coupling constants (*J*) are given in Hertz. Assignments of the NMR signals were done using ^{1}H-homodecoupling and COSY experiments. Optical rotations were measured on a Perkin–Elmer 241 polarimeter (sodium D line at 25°C) for solutions in MeOH. Optical rotations of the known compounds **10b** and **11a** have not been previously reported. For the remaining acetates the $[\alpha]_D$ values have been reported in various solvents: **8a**,[14] **8b**,[15] **9a** and **9b**.[16]

Thin-layer chromatography (TLC) was performed using Merck silica gel 60 F_{254} precoated plates with a fluorescent indicator. Flash chromatography[17] was performed using Merck silica gel 60 (230–400 mesh) using appropriate mixtures of CH_2Cl_2 and MeOH as eluents.

All reagents were obtained from commercial sources and used without further purification. THF was distilled under nitrogen from sodium benzophenone. The enzymes used were obtained as follows: lipase from *Pseudomonas sp.* (Amano Pharmaceutical Co.), an immobilised lipase from *Candida antarctica* (Novozym 435®, Novo Nordisk), adenosine deaminase (Sigma, type II, 2.2 units/mg protein). The starting nucleosides **1** and **3** were purchased from Aldrich. Compound **5** was prepared according to literature procedure[5] and the acetates **8e**, **9c**, **10c** were obtained by the usual acetylation procedure with acetic anhydride in pyridine.

Synthesis of compounds 8a, 8c, 8d, 9a, 9b, 10a. General procedure. Adenosine **1**, 2′-deoxyadenosine **3** or 3′-deoxyadenosine **5** (2.0 mmol), vinyl acetate (4.5 mmol) and 1.0 g of CAL (or PSL) were suspended in 20 ml of THF (in the case of **3**, 0.5 g of molecular sieve activated powder was added to remove hydration water from starting nucleoside) under nitrogen atmosphere. The mixture was allowed to react at 60°C and the progress of the reaction monitored by TLC ($CHCl_3$/MeOH, 4:1 for compounds **1** and **3**, $CHCl_3$/MeOH, 9:1 for **5**). For time, see Table 1. The enzyme was filtered off and washed with MeOH, the solvents were removed under vacuum and the products, after flash chromatography (CH_2Cl_2/MeOH 95:5), crystallized from the suitable solvents as white solids.

5′-*O*-Acetyladenosine (8a). Yield: 0.550 g (89%); Mp 132–133°C (from MeOH) (lit.[14] 131–132°C); $[\alpha]_D^{25}=-51.8$ (*c* 1, MeOH); ^{1}H NMR (CD_3OD) δ 8.24 (1H, s, H-2), 8.20 (1H, s, H-8), 6.02 (1H, d, *J*=4.8 Hz, H-1′), 4.74 (1H, dd, *J*=4.8, 5.2 Hz, H-2′), 4.40 (1H, dd, *J*=3.5, 12.0 Hz, H-5′a), 4.38 (1H, dd, *J*=5.2, 5.2 Hz, H-3′), 4.34 (1H, dd, *J*=4.9, 12.0 Hz, H-5′b), 4.25 (1H, ddd, *J*=3.5, 4.9, 5.2 Hz, H-4′), 2.05 (3H, s, OCOCH_3).

3′-*O*-Acetyladenosine (8c) and 2′-*O*-acetyladenosine (8d). Yield: 0.538 g (87%); The regioisomeric mixture shows: ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.29 (1H, s, H-2), 8.18 (1H, s, H-8), 5.96 (1H, d, *J*=7.0 Hz, H-1′), 5.40 (1H, dd, *J*=2.0, 5.6 Hz, H-3′), 4.97 (1H, dd, *J*=5.6, 7.0 Hz, H-2′), 4.26 (1H, ddd, *J*=2.0, 2.0, 2.0 Hz, H-4′), 3.87 (1H, dd, *J*=2.0, 12.6 Hz, H-5′a), 3.77 (1H, dd, *J*=2.0, 12.6 Hz, H-5′b), 2.16 (3H, s, OCOCH_3) for the 3′-acetate **8c** (77%) and δ 8.32 (1H, s, H-2), 8.18 (1H, s, H-8), 6.19 (1H, d, *J*=5.6 Hz, H-1′), 5.66 (1H, dd, *J*=5.6, 5.6 Hz, H-2′), 4.63 (1H, dd, *J*=3.5, 5.6 Hz, H-3′), 4.17 (1H, ddd, *J*=3.5, 3.5, 3.5 Hz, H-4′), 3.90 (1H, dd, *J*=3.5, 12.6 Hz, H-5′a), 3.75 (1H, dd, *J*=3.5, 12.6 Hz, H-5′b), 2.07 (3H, s, OCOCH_3) for the 2′-isomer **8d** (23%).

5′-*O*-Acetyl-2′-deoxyadenosine (9a). Yield: 0.540 g (92%); Mp 142–143°C (from EtOH) (lit.[18] 140–141°C); $[\alpha]_D^{25}=-6.8$ (*c* 1, MeOH); ^{1}H NMR (CD_3OD) δ 8.24 (1H, s, H-2), 8.19 (1H, s, H-8), 6.42 (1H, dd, *J*=6.7, 6.7 Hz, H-1′), 4.58 (1H, ddd, *J*=4.7, 4.7, 6.7 Hz, H-3′), 4.31 (1H, dd, *J*=4.0, 11.4 Hz, H-5′a), 4.27 (1H, dd, *J*=4.7, 11.4 Hz, H-5′b), 4.14 (1H, ddd, *J*=4.0, 4.7, 4.7 Hz, H-4′), 2.86 (1H, ddd, *J*=6.7, 6.7, 14.0 Hz, H-2′a), 2.49 (1H, ddd, *J*=4.7, 6.7, 14.0 Hz, H-2′b), 1.99 (3H, s, OCOCH_3).

3′-*O*-Acetyl-2′-deoxyadenosine (9b). Yield: 0.527 g (90%); Mp 214–216°C (from EtOH) (lit.[18] 211–212.5°C); $[\alpha]_D^{25}=-28.6$ (*c* 1, MeOH); ^{1}H NMR (CD_3OD) δ 8.30 (1H, s, H-2), 8.18 (1H, s, H-8), 6.42 (1H, dd, *J*=5.8, 9.0 Hz, H-1′), 5.46 (1H, ddd, *J*=2.0, 2.0, 6.2 Hz, H-3′), 4.21 (1H, ddd, *J*=2.0, 2.8, 3.2 Hz, H-4′), 3.86 (1H, dd, *J*=2.8, 12.4 Hz, H-5′a), 3.81 (1H, dd, *J*=3.2, 12.4 Hz, H-5′b), 2.95 (1H, ddd, *J*=6.2, 9.0, 14.0 Hz, H-2′a), 2.54 (1H, ddd, *J*=2.0, 5.8, 14.0 Hz, H-2′b), 2.11 (3H, s, OCOCH_3).

5′-*O*-Acetyl-3′-deoxyadenosine (10a). Yield: 0.498 g (85%); Mp 165°C (from $CHCl_3$) (lit.[19] 166–168°C); $[\alpha]_D^{25}=-23.7$ (*c* 1, MeOH) (lit.[19] −21.2, *c* 0.5 in MeOH); ^{1}H NMR (CD_3OD) δ 8.25 (1H, s, H-2), 8.20 (1H, s, H-8), 5.99 (1H, d, *J*=2.0 Hz, H-1′), 4.79 (1H, ddd, *J*=2.0, 2.0, 5.6 Hz, H-2′), 4.68 (1H, dddd, *J*=2.7, 3.4, 6.0, 9.8 Hz, H-4′), 4.35 (1H, dd, *J*=3.4, 10.0 Hz, H-5′a), 4.32 (1H, dd, *J*=2.7, 10.0 Hz, H-5′b), 2.29 (1H, ddd, *J*=5.6, 9.8, 14.0 Hz, H-3′a), 2.09 (1H, ddd, *J*=2.0, 6.0, 14.0 Hz, H-3′b), 2.03 (3H, s, $OCOCH_3$).

Synthesis of compounds 8b and 10b: General procedure. A typical hydrolysis was performed dissolving compounds **8e** or **10c** (1.2 mmol) in acetone (2.0 ml) and adding to this solution 1.0 g of CAL dispersed in 0.1 M phosphate buffer pH=7.0 (10 ml). The mixture was stirred at room temperature, monitoring the reactions by TLC ($CHCl_3$/MeOH, 4:1). Products were extracted with CH_2Cl_2 and, after removal of the solvent, purified by flash chromatography (CH_2Cl_2/MeOH, 95:5) to afford white solids. For time see Table 1. Alternatively, to compounds **8e** or **10c** (1.2 mmol) in water-saturated $CHCl_3$ (10 ml) 1.0 g of CAL was added. The mixture was stirred at room temperature (48 h for both **8e** and **10c**) affording, after usual work-up, compounds **8b** and **10b** in yields comparable to the acetone/buffer hydrolysis.

2′,3′-*O*-Diacetyladenosine (8b). Yield: 0.564 g (84%); Mp 179–180°C (from acetone) (lit.[20] 180–181°C); $[\alpha]_D^{25}=-49.5$ (*c* 1, MeOH); ^{1}H NMR ($CDCl_3$) δ 8.22 (1H, s, H-2), 7.85 (1H, s, H-8), 6.00 (1H, d, *J*=7.5 Hz, H-1′), 5.97 (1H, dd, *J*=5.0, 7.5 Hz, H-2′), 5.65 (1H, dd, *J*=1.5, 5.0 Hz, H-3′), 4.32 (1H, ddd, *J*=1.5, 1.5, 1.5 Hz, H-4′), 3.93 (1H, dd, *J*=1.5, 12.6 Hz, H-5′a), 3.81 (1H, dd, *J*=1.5, 12.6 Hz, H-5′b), 2.12 (3H, s, $OCOCH_3$), 1.97 (3H, s, $OCOCH_3$).

2′-*O*-Acetyl-3′-deoxyadenosine (10b). Yield: 0.515 g (88%); Mp 230–231°C (from MeOH) (lit.[21] 231–232°C); $[\alpha]_D^{25}=-16.4$ (*c* 1, MeOH); ^{1}H NMR (CD_3OD) δ 8.35 (1H, s, H-2), 8.18 (1H, s, H-8), 6.12 (1H, d, *J*=2.7 Hz, H-1′), 5.61 (1H, ddd, *J*=2.7, 2.7, 6.0 Hz, H-2′), 4.48 (1H, dddd, *J*=2.7, 3.4, 6.0, 9.4 Hz, H-4′), 3.90 (1H, dd, *J*=2.7, 12.0 Hz, H-5′a), 3.67 (1H, dd, *J*=3.4, 12.0 Hz, H-5′b), 2.65 (1H, ddd, *J*=6.0, 9.0, 14.0 Hz, H-3′a), 2.20 (1H, ddd, *J*=2.7, 6.0, 14.0 Hz, H-3′b), 2.09 (3H, s, $OCOCH_3$).

Enzymatic deaminating hydrolysis of adenosine derivatives 5, 8b, 9b, 10b to inosine derivatives 6, 11a, 11c–d. Compounds **5**, **8b**, **9b** and **10b** (0.1 g) in water (10 ml) were treated with ADA (10% by weight, 10 mg) and reacted at 50°C for the time indicated in Table 2. The reactions were monitored by TLC ($CHCl_3$/MeOH, 4:1). The solution was lyophilized and the residue crystallized as white solids (compounds **6** and **11a**). The acetates **11c** and **11d** resisted several attempts at crystallization.

3′-Deoxyinosine (6). Yield: 0.095 g (95%); Mp 203°C (from MeOH) (lit.[6] 197–199°C). R*f*=0.20 ($CHCl_3$/MeOH 4:1); $[\alpha]_D^{25}=-42.3$ (*c* 1, H_2O); ^{1}H NMR (DMSO-d_6) δ 8.31 (1H, s, H-2), 8.04 (1H, s, H-8), 5.85 (1H, d, *J*=2.0 Hz, H-1′), 5.66 (1H, d, *J*=3.3 Hz, 2′-O*H*), 4.99 (1H, dd, *J*=4.7, 4.7 Hz, 5′-O*H*), 4.49 (1H, dddd, *J*=2.0, 2.0, 3.3, 5.4 Hz, H-2′), 4.35 (1H, dddd, *J*=4.7, 4,7, 6.0, 9.4 Hz, H-4′), 3.68 (1H, ddd, *J*=4.7, 4.7, 12.0 Hz, H-5′a), 3.52 (1H, ddd, *J*=4.7, 4.7, 12.0 Hz, H-5′b), 2.20 (1H, ddd, *J*=5.4, 9.4, 12.7 Hz, H-3′a), 1.89 (1H, ddd, *J*=2.0, 6.0, 12.7 Hz, H-3′b).

2′,3′-*O*-Diacetylinosine (11a). 0.090 g (90%); Mp 212–213°C (from MeOH) (lit.[22] 215°C). R*f*=0.34 ($CHCl_3$/MeOH 4:1); $[\alpha]_D^{25}=-38.6$ (*c* 1, MeOH); ^{1}H NMR (CD_3OD) δ 8.35 (1H, s, H-2), 8.09 (1H, s, H-8), 6.25 (1H, d, *J*=6.3 Hz, H-1′), 5.88 (1H, dd, *J*=6.3, 6.3 Hz, H-2′), 5.61 (1H, dd, *J*=3.5, 6.3 Hz, H-3′), 4.33 (1H, ddd, *J*=2.8, 2.8, 3.5 Hz, H-4′), 3.89 (1H, dd, *J*=2.8, 12.6 Hz, H-5′a), 3.81 (1H, dd, *J*=2.8, 12.6 Hz, H-5′b), 2.15 (3H, s, $OCOCH_3$), 2.03 (3H, s, $OCOCH_3$).

3′-*O*-Acetyl-2′-deoxyinosine (11c). 0.094 g (94%); Non crystalline white solid. Mp 116°C. R*f*=0.33 ($CHCl_3$/MeOH 9:1); $[\alpha]_D^{25}=-22.5$ (*c* 1, MeOH); ν_{max} (KBr) 3431, 2926, 1689, 1576 cm^{-1}; ^{1}H NMR (CD_3OD) δ 8.31 (1H, s, H-2), 8.06 (1H, s, H-8), 6.42 (1H, dd, *J*=6.0, 8.7 Hz, H-1′), 5.43 (1H, ddd, *J*=2.0, 2.0, 6.0 Hz, H-3′), 4.19 (1H, ddd, *J*=2.0, 3.4, 4.0 Hz, H-4′), 3.83 (1H, dd, *J*=3.4, 12.0 Hz, H-5′a), 3.79 (1H, dd, *J*=4.0, 12.0 Hz, H-5′b), 2.88 (1H, ddd, *J*=6.0, 8.7, 14.8 Hz, H-2′a), 2.59 (1H, ddd, *J*=2.0, 6.0, 14.8 Hz, H-2′b), 2.10 (3H, s, $OCOCH_3$). Anal. Calcd for $C_{12}H_{14}N_4O_5$: C, 48.98; H, 4.80; N, 19.04. Found: C, 48.87; H, 4.86; N, 19.00.

2′-*O*-Acetyl-3′-deoxyinosine (11d). 0.094 g (94%); Non crystalline white solid. Mp 143°C. R*f*=0.20 ($CHCl_3$/MeOH 9:1); $[\alpha]_D^{25}=-26.8$ (*c* 1, MeOH); ν_{max} (KBr) 3427, 2925, 1689, 1586 cm^{-1}; ^{1}H NMR (CD_3OD) δ 8.34 (1H, s, H-2), 8.03 (1H, s, H-8), 6.14 (1H, d, *J*=2.0 Hz, H-1′), 5.59 (1H, ddd, *J*=2.0, 2.0, 6.7 Hz, H-2′), 4.46 (1H, dddd, *J*=3.3, 4.0, 6.0, 10.0 Hz, H-4′), 3.88 (1H, dd, *J*=3.3, 12.1 Hz, H-5′a), 3.68 (1H, dd, *J*=4.0, 12.1 Hz, H-5′b), 2.60 (1H, ddd, *J*=6.7,10.0, 14.1 Hz, H-3′a), 2.18 (1H, ddd, *J*=2.0, 6.0, 14.1 Hz, H-3′b), 210 (3H, s, $OCOCH_3$). Anal. Calcd for $C_{12}H_{14}N_4O_5$: C, 48.98; H, 4.80; N, 19.04. Found: C, 48.76; H, 4.91; N, 19.02.

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unsuccessfully tried to prepare the classical 2′,3′-O-(methoxymethylene) derivative that was needed for further elaboration. Due to insolubility of **2** in the reaction medium, poor yields of the required compound were obtained using trimethylorthoformate as reagent and solvent, whereas addition of DMSO or DMF improved the yields but complicated the work-up.

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