Pergamon

Tetrahedron 58 (2002) 5767–5771

TETRAHEDRON

Deamination of 5′-substituted-2′,3′-isopropylidene adenosine derivatives catalyzed by adenosine deaminase (ADA, EC 3.5.4.4) and complementary enzymatic biotransformations catalyzed by adenylate deaminase (AMPDA, EC 3.5.4.6): a viable route for the preparation of 5′-substituted inosine derivatives

Pierangela Ciuffreda, Angela Loseto and Enzo Santaniello*

Dipartimento di Scienze Precliniche, Università degli Studi di Milano, LITA Vialba Via G. B. Grassi, 74-20157 Milano, Italy

Received 27 March 2002; revised 16 May 2002; accepted 6 June 2002

Abstract—Adenosine deaminase (ADA) catalyzes the deamination of 2′,3′-isopropylidene adenosine and the corresponding 5′-amino derivative in a 3% dimethylsulfoxide aqueous solution. Whereas ADA is unable to convert other 5′-substituted derivatives (acetate, acetamido, azide), the enzyme adenylate deaminase (AMPDA) accepts all the above compounds as substrates for their biotransformation to the corresponding 5′-substituted inosine derivatives.

1. Introduction

Adenosine deaminase [ADA, EC 3.5.4.4] is an enzyme that catalyzes the rapid and irreversible deamination of adenosine **1a** to inosine **2a** and may be considered as a valuable biocatalyst for the biotransformation of a wide range of structurally modified purine nucleosides.[1] The recently reported three-dimensional structures of the enzyme[2,3] have added valuable information on the structural prerequisites that the purine and ribose moieties have to possess in order to be accepted as substrates by the enzyme. A considerable body of evidence has been gained to establish that the 5′-hydroxy group is necessarily required for the activity of ADA, whereas some flexibility is possible at the 2′,3′-positions.[4,5]

In previous papers,[6,7] we have shown that some steric hindrance at the positions 2′ and 3′ is well tolerated by the enzyme, since adenosine 2′,3′-diacetates **1b** and 2′,3′-isopropylidene adenosine **1c** may be converted by means of ADA to the corresponding inosine derivatives **2b**,**c**. We decided to extend the above observations preparing a few 5′-analogs of 2′,3′-isopropylidene adenosine **3a**, namely compounds **3b**–**e**, and submit them to the ADA-catalyzed deamination (Figs. 1 and 2).

2. Results and discussions

2.1. Synthesis of 5′-derivatives of 2′,3′-isopropylidene adenosine as possible substrates for ADA-catalyzed deamination

As expected by the crucial role played by the 5′-hydroxy group, the acetate **3b** should not be enzymatically

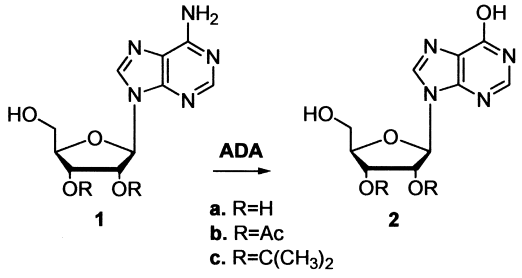


Figure 1. ADA-catalyzed deamination of adenosine derivatives **1a**–**c**.

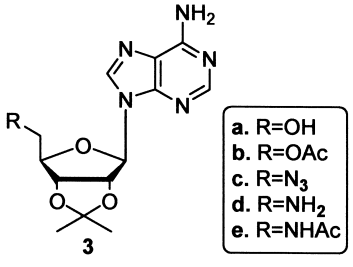
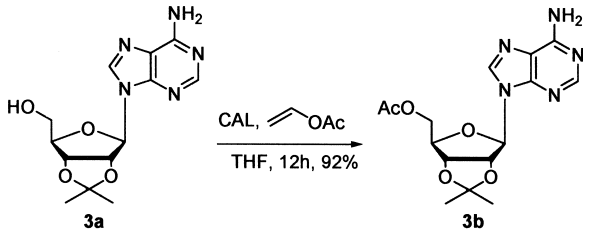


Figure 2. Structure of compounds **3a**–**e**.

Keywords: biotransformations; adenosin deaminase; deamination.

* Corresponding author. Tel.: +39-2-38210472; fax: +39-2-38210295; e-mail: enzo.santaniello@unimi.it

0040–4020/02/$ - see front matter
PII: S0040-4020(02)00575-6



Scheme 1. Lipase-catalyzed acetylation of 2′,3′-isopropylidene adenosine **3a**.

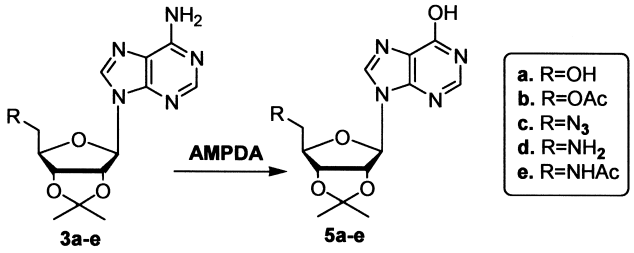
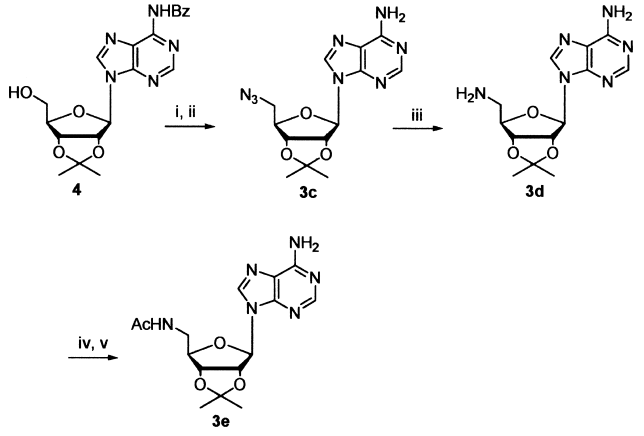


Figure 3. AMPDA-catalyzed deamination of adenosine derivatives **3a**–**c**.

transformed. We have prepared the substrate **3b** by a lipase-catalyzed acetylation in an organic solvent, since the direct chemical acetylation of compound **3a** was complicated by the formation of mono- and bis-acetamido derivatives at the N^6-amino group. For the enzymatic preparation of **3b** from **3a** the lipase from *Candida antarctica* (CAL) was preferred, due to the selectivity of this enzyme towards the 5′-position of adenosine or inosine derivatives.[8] The reaction was carried out in THF at room temperature under irreversible transesterification conditions using vinyl acetate as acyl transfer agent. The acetate **3b** was quantitatively formed from **3a** in 12 h, with no trace of N^6-acetylation (Scheme 1).

Due to low solubility of the substrate, the ADA-catalyzed deamination could be carried out either at 60°C or in a 3% DMSO aqueous solution. We have previously shown that at the above temperature the enzymatic activity is still compatible with a quantitative transformation of a substrate[6] and it has been reported that the presence of polar solvents does not inhibit the ADA activity on adenosine **1a**.[9] Preliminary experiments were carried out on a DMSO solution of isopropylidene adenosine **3a** in aqueous phosphate buffer (pH 7.4) so that a final 3% concentration of DMSO was reached. The substrate **3a** was quantitatively deaminated in 15 min to the inosine derivative **5a**, this result confirming that the above mixed DMSO-aqueous solution did not deactivate the enzyme. Therefore we decided to use this medium for the ADA-catalyzed deamination of the acetate **3b** and substrates **3c**–**e**. In these conditions, the acetate **3b** was not deaminated to any extent and this result again confirmed the essential role of the 5′-hydroxy group for the catalytic



Scheme 2. Synthesis of compounds **3c**–**e**, substrates of the deaminating enzymes ADA and AMPDA: (i) TsCl/py, 0°C, 2 h, 93%; (ii) NaN_3/DMF, 80°C, 1 h, 80%; (iii) H_2/Pt(C), rt, 5 h, 90%; (iv) Ac_2O/py, 0°C, 2 h; NH_3/MeOH, rt, 4 h, 80%.

action of the enzyme. We then prepared 5′-deoxy-5′-amino adenosine **3d** in order to verify if an amino group at the position 5′ could provide the hydrogen bonding network that seems necessary to a nucleoside structure for an ADA-catalyzed deamination. It was also expected that the corresponding 5′-acetamide **3e** should not be a substrate of the enzyme. For the preparation of the required 5′-amino and 5′-acetamido compounds **3d** and **3e**, we started with the N^6-benzoylated compound **4**, that was converted into the corresponding tosylate (Scheme 2). Displacement by sodium azide of the above intermediate in dimethyl formamide at 80°C afforded the debenzoylated azide **3c**. Among several methods described for the reduction of a nucleoside azide,[10,11] we were able to obtain a quantitative yield of the required amino compound **3d** by catalytic hydrogenation (10% Pd–C)[12] of the azide **3c**. A selective enzymatic acetylation of the 5′-amino group of **3d** was attempted, but no reaction occurred in the presence of CAL. Therefore, the 5′-acetamido derivative **3e** was prepared by chemical acetylation of **3d**, that afforded a mixture of 5′- and N^6-benzoylated products. Subsequent treatment of this mixture with ammonia solution in methanol afforded the required acetamido derivative **3e**.

The 5′-amino nucleoside **3d** was deaminated rather slowly, if compared to the corresponding adenosine **3a** (6 h versus 15 min), thus confirming the essential role played by the hydrogen bond network around the 5′-position. In fact, we have later observed that the 5′-azido and the 5′- acetamido derivatives **3c** and **3e** were not substrates for the ADA-catalyzed deamination.

AMPDA-catalyzed deamination of 5′-derivatives of 2′,3′-isopropylidene adenosine (compounds **3a**–**e**).

Compared with ADA, the properties and the possible use of adenylate deaminase (5′-adenylic acid deaminase, AMPDA, EC 3.5.4.6) as biocatalyst are much less explored. This enzyme catalyzes the deamination of adenylic acid (adenosine 5′-phosphate, AMP) to inosine 5′-phosphate (IMP), a reaction that is of considerable commercial value, due to the use of IMP as a flavoring in the food industry (Fig. 3).

Information available from the literature seems to indicate that, compared to ADA, AMPDA is able to accept a wider range of substrates, but is less stereoselective.[13] AMPDA from *Aspergillus* sp. is commercially available and we decided to use this enzyme on compounds **3a**–**e**, previously used as substrates for ADA-catalyzed deamination. For this enzyme we again used a 3% DMSO aqueous solution as the

Table 1. ADA- and AMPDA-catalyzed deamination of adenosine derivatives **3a–e**

Substrate	AMPDA reaction time (min)[a]	ADA reaction time (min)[a]	V_o(AMPDA)/V_o(ADA)[b]	Product (yield, %)[c]
3a	45	15	0.2	**5a** (98)
3b	20	No reaction	>1000	**5b** (92)
3c	30	No reaction	>1000	**5c** (94)
3d	180	360	9.3	**5d** (98)
3e	10	No reaction	>1000	**5e** (95)

[a] Time for complete reaction.
[b] Initial rates of the enzyme-catalyzed reactions were measured by HPLC (see Section 4 for details). V_o(AMPDA)/V_o(ADA)>1000 means that at high ADA concentration no reaction was detected after several days of incubation.
[c] In all case enzyme-catalyzed reactions proceeded quantitatively without formation of by-products (HPLC); yields refer to isolated products.

medium, once it was established that isopropylidene adenosine **3a** was readily deaminated by AMPDA in 45 min (Table 1).

It was gratifying to us to find that all compounds were substrates for the catalytic action of AMPDA and that this enzymatic deamination allows a viable preparation of the inosine derivatives **5b–e** from the 5′-substituted adenosine derivatives **3b–e**.

3. Conclusion

We have shown that the two deaminating enzymes, ADA and AMPDA, show a difference in their substrate selectivity. Both enzymes can tolerate substitution at the 2′,3′-positions, but, as a salient difference, the presence of a hydroxy group at the 5′-position is necessary for ADA-catalyzed reactions. This can be substituted by another polar group such as amino, although the reaction is much slower. AMPDA seems a more versatile biocatalyst in the nucleoside field, since it is able to convert all the compounds **3a–e** to the corresponding inosine derivative **5a–e** in a time suitable for preparative purposes. The results obtained may find explanation through knowledge of the three-dimensional structure of the enzymes. However, this information is not available for AMPDA. Further research is needed in order to define the structural features that a nucleoside derivative must possess in order to become a substrate for AMPDA. We have shown that AMPDA is a more versatile biocatalyst than ADA for the preparation of a great variety of 6-oxopurine ribosides.

4. Experimental

4.1. General

Melting points were recorded on 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). Coupling constants (*J*) are given in Hz and the NMR signals were assigned by ^{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. The progress of all reactions and column cromatography were monitored by TLC and HPLC. HPLC analyses were carried out on a Jasco HPLC instrument with an Uvidec 100 II UV detector operating at 260 nm using an Alltech Hypersil BDS C18 (4.6 mm×250 mm). The eluant was phosphate buffer at pH 6.0 containing CH_3CN in the following ratio: 80/20 for **3a** and **3b**, 70/30 for **3c**, 85/15 for **3d** and **3e** at a flow rate of 1 mL/min. Thin-layer chromatography (TLC) was performed using Merck silica gel 60 F_{254} precoated plates with a fluorescent indicator. Flash chromatography[14] was performed using Merck silica gel 60 (230–400 mesh) with appropriate mixtures of CH_2Cl_2 and MeOH as eluant. All reagents were obtained from commercial sources and used without further purification. The enzymes were obtained as follows: immobilized lipase from *C. antarctica* (Novozym 435®, Novo Nordisk), adenosine deaminase from calf intestinal mucosa (Sigma, type II, 2.2 units/mg protein), 5′-adenylic acid deaminase from *Aspergillus* sp. (Sigma, 0.107 units/mg protein). The starting nucleoside **1a** was purchased from Aldrich, compounds **1c** and **4** were prepared according to literature procedures.[15,16]

4.1.1. 5′-Acetyl 2′,3′-*O*-isopropylidene adenosine (3b). 2′,3′-*O*-Isopropylidene adenosine **1c** (154 mg, 0.5 mL), vinyl acetate (1.5 mmol) and CAL (200 mg) were suspended in THF (10 mL). The mixture was allowed to react at 60°C for 12 h, the progress of the reaction being monitored by TLC (CH_2Cl_2/MeOH, 9:1). The enzyme was filtered off, washed with MeOH, and the solvents were removed under reduced pressure. The residue was purified by flash chromatography (CH_2Cl_2/MeOH, 95:5) to give 5′-acetyl 2′,3′-*O*-isopropylidene adenosine **3b** (160 mg, 92%) as a white powder: mp 164–165°C (lit.[17] mp 167°C); $[\alpha]_D^{25}=-29.8$ (*c* 1, MeOH); ^{1}H NMR (CD_3OD) δ 8.22 (1H, s, H-2), 8.21 (1H, s, H-8), 6.19 (1H, d, *J*= 2.7 Hz, H-1′), 5.51 (1H, dd, *J*=2.7, 6.7 Hz, H-2′), 5.07 (1H, dd, *J*=3.4, 6.7 Hz, H-3′), 4.43 (1H, ddd, *J*=3.4, 4.7, 6.0 Hz, H-4′), 4.26 (1H, dd, *J*=4.7, 12.0 Hz, H-5′a), 4.22 (1H, dd, *J*=6.0, 12.0 Hz, H-5′b), 1.95 (3H, s, OCOCH_3), 1.59 (3H, s, CCH_3), 1.36 (3H, s, CCH_3).

4.1.2. 5′-Deoxy-5′-azido-2′,3′-*O*-isopropylidene adenosine (3c). N^6-Monobenzoyl 2′,3′-*O*-isopropylidene adenosine **4** (1 g, 2.4 mmol) was evaporated three times with pyridine, then dissolved in pyridine (15 mL) and the solution cooled with an external ice bath. Tosyl chloride (1.1 g, 6.0 mmol) was added and the solution stirred for 2 h at 0°C. After addition of cold water, the mixture was extracted with ethyl acetate. The resulting solution was sequentially washed with HCl, $NaHCO_3$, brine, dried over anhydrous

Na_2SO_4 and the solvent evaporated under reduced pressure. The residue (1.26 g, 93%) was sufficiently pure by TLC (CH_2Cl_2/MeOH, 95:5) to be used in the next step without purification; ^{1}H NMR ($CDCl_3$) δ 8.69 (1H, s, H-2), 8.12 (1H, s, H-8), 8.03–8.00 (4H, m, 2×benzoyl *o* and 2×tosyl *o*), 7.58 (1H, dd, *J*=7.0, 7.0 Hz, benzoyl *p*), 7.50 (2H, dd, *J*=7.0, 7.0 Hz, benzoyl *m*), 7.50 (2H, d, *J*=8.4 Hz, tosyl *m*), 6.13 (1H, d, *J*=2.8 Hz, H-1′), 5.34 (1H, dd, *J*=2.8, 6.3 Hz, H-2′), 5.02 (1H, dd, *J*=2.8, 6.3 Hz, H-3′), 4.49 (1H, ddd, *J*=2.8, 4.2, 5.6 Hz, H-4′), 4.25 (1H, dd, *J*=4.2, 10.5 Hz, H-5′a), 4.22 (1H, dd, *J*=5.6, 10.5 Hz, H-5′b), 2.36 (3H, s, tosyl CH_3), 1.58 (3H, s, CCH_3), 1.35 (3H, s, CCH_3). To a solution of tosylate (1.25 g, 2.2 mmol) in dry DMF (25 mL), NaN_3 was added (572 mg, 8.8 mmol) and the reaction heated at 80°C for 1 h. Excess NaN_3 was removed by filtration and the solution was diluted with ethyl acetate then washed with water. The organic solution was dried over anhydrous Na_2SO_4 and the solvent evaporated at reduced pressure. The residue was purified by flash chromatography (CH_2Cl_2/MeOH, 95:5) to afford 5′-deoxy-5′-azido-2′,3′-*O*-isopropylidene adenosine **3c** as an amorphous solid (585 mg, 80%): IR (ν_{max}, cm^{-1}, KBr) 3434, 2924, 2109, 1637, 1384, 1249, 1092; ^{1}H NMR ($CDCl_3$) δ 8.34 (1H, s, H-2), 7.94 (1H, s, H-8), 6.09 (1H, d, *J*=2.1 Hz, H-1′), 5.42 (1H, dd, *J*=2.1, 6.3 Hz, H-2′), 5.03 (1H, dd, *J*=3.5, 6.3 Hz, H-3′), 4.37 (1H, ddd, *J*=3.5, 5.6, 5.6 Hz, H-4′), 3.60–3.53 (2H, m, part AB of system ABX, H-5′a and H-5′b), 1.60 (3H, s, CCH_3), 1.37 (3H, s, CCH_3). Anal. calcd for $C_{13}H_{16}N_8O_3$: C, 46.98; H, 4.85; N, 33.72. Found: C, 46.74; H, 4.62; N, 33.68.

4.1.3. 5′-Deoxy-5′-amino-2′,3′-*O*-isopropylidene adenosine (3d). Compound **3c** (200 mg, 0.60 mmol) was dissolved in ethanol (10 mL) and 10% Pd/C (10 mg) was added. The solution was left under of a hydrogen atmosphere (5 h) and then the catalyst removed by filtration. The solvent was evaporated at reduced pressure and the residue purified by flash chromatography (CH_2Cl_2/MeOH, 80:20) to afford 5′-deoxy-5′-amino-2′,3′-*O*-isopropylidene adenosine **3d** (166 mg, 90%) as a white powder: mp 206–208°C (dec), (lit.[18] mp 204–205°C); $[\alpha]_D^{25}$=−35.8 (*c* 1, MeOH); ^{1}H NMR (CD_3OD) δ 8.26 (1H, s, H-2), 8.20 (1H, s, H-8), 6.13 (1H, d, *J*=3.4 Hz, H-1′), 5.46 (1H, dd, *J*=3.4, 6.0 Hz, H-2′), 5.00 (1H, dd, *J*=3.4, 6.0 Hz, H-3′), 4.22 (1H, ddd, *J*=3.4, 6.0, 6.0 Hz, H-4′), 2.92–2.84 (2H, m, part AB of system ABX, H-5′a and H-5′b), 1.58 (3H, s, CCH_3), 1.36 (3H, s, CCH_3).

4.1.4. 5′-Deoxy-5′-acetamido-2′,3′-*O*-isopropylidene adenosine (3e). To a solution of compound **3d** (100 mg, 0.33 mmol) in dry pyridine (0.5 mL), acetic anhydride (67 mg, 0.66 mmol) was added and the solution stirred for 2 h at 0°C. After usual work-up, the resulting mixture of acetates was dissolved in methanol saturated with ammonia (10 mL). After stirring for 4 h at room temperature, the solvent was evaporated to leave a yellow oil. The residue was purified by flash chromatography (CH_2Cl_2/MeOH, 95:5) to afford title compound **3e** as a white solid (100 mg, 80%): mp 169–170°C, $[\alpha]_D^{25}$=−16.9 (*c* 1, MeOH); ^{1}H NMR (CD_3OD) δ 8.25 (1H, s, H-2), 8.24 (1H, s, H-8), 6.13 (1H, d, *J*=3.3 Hz, H-1′), 5.43 (1H, dd, *J*=3.3, 6.7 Hz, H-2′), 4.97 (1H, dd, *J*=3.0, 6.7 Hz, H-3′), 4.30 (1H, ddd, *J*=3.0, 5.4, 6.0 Hz, H-4′), 3.55 (1H, dd, *J*=6.0, 14.0 Hz, H-5′a), 3.45 (1H, dd, *J*=5.4, 14.0 Hz, H-5′b), 1.95 (3H, s, NHCOCH_3), 1.58 (3H, s, CCH_3), 1.36 (3H, s, CCH_3). Anal. calcd for $C_{15}H_{20}N_6O_4$: C, 51.72; H, 5.79; N, 24.12. Found: C, 51.60; H, 5.73; N, 24.09.

4.2. Enzymatic deamination of adenosine derivatives 3a–e to inosine derivatives 5a–e

Compounds **3a**–**e** (20 mg) in phosphate buffer (50 mM, 10 mL, pH 7.4 for ADA and pH 6.5 for AMPDA) containing 3% DMSO were treated with ADA (2 mg) or AMPDA (20 mg) for the time indicated in Table 1. The progress of reactions was monitored by HPLC (**3a** and **3b**: phosphate buffer pH 6.0/CH_3CN, 80:20; **3c**: phosphate buffer pH 6.0/CH_3CN, 70:30; **3d** and **3e**: phosphate buffer pH 6.0/CH_3CN, 85:15). The solution was lyophilized and the residue crystallized from methanol–water as white solids (compounds **5b**,**e**). Compounds **5c** and **5d** resisted to several attempts of crystallization. 2′,3′-isopropylidene inosine (**5a**) showed physical characteristics in agreement with published data.[7]

4.2.1. 5′-Acetyl 2′,3′-*O*-isopropylidene inosine (5b). White powder, mp 229–230°C, $[\alpha]_D^{25}$=−22.6 (*c* 1, MeOH); ^{1}H NMR (CD_3OD) δ 8.18 (1H, s, H-2), 8.06 (1H, s, H-8), 6.21 (1H, d, *J*=2.7 Hz, H-1′), 5.42 (1H, dd, *J*=2.7, 6.7 Hz, H-2′), 5.04 (1H, dd, *J*=3.4, 6.7 Hz, H-3′), 4.45 (1H, ddd, *J*=3.4, 4.7, 6.0 Hz, H-4′), 4.27 (1H, dd, *J*=4.7, 12.0 Hz, H-5′a), 4.24 (1H, dd, *J*=6.0, 12.0 Hz, H-5′b), 1.97 (3H, s, OCOCH_3), 1.59 (3H, s, CCH_3), 1.38 (3H, s, CCH_3). Anal. calcd for $C_{15}H_{18}N_4O_6$: C, 51.43; H, 5.18; N, 15.99. Found: C, 51.34; H, 5.06; N, 14.83.

4.2.2. 5′-Deoxy-5′-azido-2′,3′-*O*-isopropylidene inosine (5c). Amorphous solid, ^{1}H NMR (CD_3OD) δ 8.36 (1H, s, H-2), 8.12 (1H, s, H-8), 6.23 (1H, d, *J*=2.7 Hz, H-1′), 5.43 (1H, dd, *J*=2.7, 6.3 Hz, H-2′), 5.02 (1H, dd, *J*=3.4, 6.3 Hz, H-3′), 4.37 (1H, ddd, *J*=3.4, 5.6, 5.6 Hz, H-4′), 3.61–3.54 (2H, m, part AB of system ABX, H-5′a and H-5′b), 1.59 (3H, s, CCH_3), 1.37 (3H, s, CCH_3). Anal. calcd for $C_{13}H_{15}N_7O_4$: C, 46.85; H, 4.54; N, 29.42. Found: C, 46.75; H, 4.38; N, 29.26.

4.2.3. 5′-Deoxy-5′-amino-2′,3′-*O*-isopropylidene inosine (5d). $[\alpha]_D^{25}$=−39.4 (*c* 1, MeOH); ^{1}H NMR (CD_3OD) δ 8.21 (1H, s, H-2), 8.06 (1H, s, H-8), 6.14 (1H, d, *J*=3.4 Hz, H-1′), 5.39 (1H, dd, *J*=3.4, 6.0 Hz, H-2′), 4.98 (1H, dd, *J*=3.4, 6.0 Hz, H-3′), 4.23 (1H, ddd, *J*=3.4, 6.0, 6.0 Hz, H-4′), 2.96–2.88 (2H, m, part AB of system ABX, H-5′a and H-5′b), 1.58 (3H, s, CCH_3), 1.36 (3H, s, CCH_3). Anal. calcd for $C_{13}H_{17}N_5O_4$: C, 50.81; H, 5.58; N, 22.79. Found: C, 50.63; H, 5.46; N, 22.62.

4.2.4. 5′-Deoxy-5′-acetamido-2′,3′-*O*-isopropylidene inosine (5e). Mp 178–179°C, $[\alpha]_D^{25}$=−49.6 (*c* 1, MeOH); ^{1}H NMR (CD_3OD) δ 8.19 (1H, s, H-2), 8.09 (1H, s, H-8), 6.14 (1H, d, *J*=2.7 Hz, H-1′), 5.39 (1H, dd, *J*=2.7, 6.7 Hz, H-2′), 4.96 (1H, dd, *J*=3.4, 6.7 Hz, H-3′), 4.27 (1H, ddd, *J*=3.4, 6.0, 6.7 Hz, H-4′), 3.50 (1H, dd, *J*=6.0, 14.0 Hz, H-5′a), 3.45 (1H, dd, *J*=6.7, 14.0 Hz, H-5′b), 1.93 (3H, s, NHCOCH_3), 1.57 (3H, s, CCH_3), 1.35 (3H, s, CCH_3). Anal. calcd for $C_{15}H_{19}N_5O_5$: C, 51.57; H, 5.48; N, 20.05. Found: C, 51.39; H, 5.29; N, 19.98.

Acknowledgements

This work has been financially supported by Università degli Studi di Milano (Fondi ex 60%) and the Italian National Council for Research (CNR, *Target Project in Biotechnology*).

References

1. Ferrero, M.; Gotor, V. *Chem. Rev.* **2000**, *100*, 4319–4347.
2. Marrone, T. J.; Straatsma, T. P.; Briggs, J. M.; Wilson, D. K.; Quiocho, F. A.; McCammon, J. A. *J. Med. Chem.* **1996**, *39*, 277–284.
3. Wilson, D. K.; Quiocho, F. A. *Biochemistry* **1993**, *32*, 1689–1694.
4. Bloch, A.; Robins, M. J.; McCarthy, Jr., J. R. *J. Med. Chem.* **1967**, *10*, 908–912.
5. Maury, G.; Daiboun, T.; Elalaoui, A.; Génu-Dellac, C.; Périgaud, C.; Bergogne, C.; Gosselin, G.; Imbach, J.-L. *Nucleosides Nucleotides* **1991**, *10*, 1677–1692.
6. Ciuffreda, P.; Casati, S.; Santaniello, E. *Tetrahedron* **2000**, *56*, 3239–3243.
7. Ciuffreda, P.; Loseto, A.; Santaniello, E. *Tetrahedron: Asymmetry* **2002**, *13*, 239–241.
8. Prasad, A. K.; Wengel, J. *Nucleosides Nucleotides* **1996**, *15*, 1347–1359.
9. Bolen, D. W.; Fisher, J. R. *Biochemistry* **1969**, *11*, 4239–4246.
10. Scriven, E.; Turnbull, K. *Chem. Rev.* **1988**, *88*, 297–368.
11. Samano, M. C.; Robbins, M. J. *Tetrahedron Lett.* **1991**, *32*, 6293–6296.
12. Ceulemans, G.; Vandendriessche, F.; Rozensky, J.; Herdewijn, P. *Nucleosides Nucleotides* **1995**, *14*, 117–127.
13. Margolin, A. L.; Borcherding, D. R.; Wolf-Kugel, D.; Margolin, N. A. *J. Org. Chem.* **1996**, *59*, 7214–7218.
14. Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923–2925.
15. Hampton, A. *J. Am. Chem. Soc.* **1961**, *83*, 3640–3645.
16. Chládek, S.; Smrt, J. *Collect. Czech. Chem. Commun.* **1964**, *29*, 214–232.
17. Brown, D. M.; Haynes, L. J.; Todd, A. R. *J. Chem. Soc.* **1950**, 3299–3304.
18. Reeve, A. M.; Townsend, C. A. *Tetrahedron* **1998**, *54*, 15959–15975.