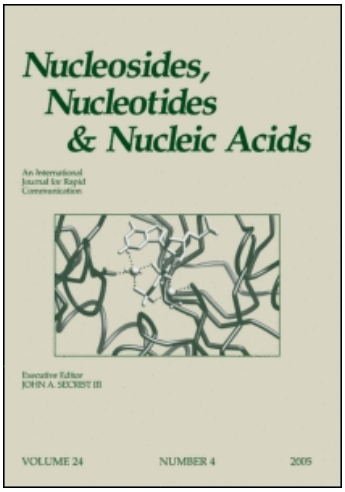

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2',3'-Isopropylidene Group, A Molecular Scaffold to Study the Activity of Adenosine and Adenylate Deaminase on Adenosine Analogues Modified in the Ribose Moiety

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2′,3′-ISOPROPYLIDENE GROUP, A MOLECULAR SCAFFOLD TO STUDY THE ACTIVITY OF ADENOSINE AND ADENYLATE DEAMINASE ON ADENOSINE ANALOGUES MODIFIED IN THE RIBOSE MOIETY

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□ *2′,3′-Isopropylidene group can be used as a molecular scaffold for the introduction of modifications at 5′ and 1′ positions of adenosine and these modified nucleosides are used to evaluate the biocatalytic activity of adenosine and adenylate deaminase.*

Keywords Nucleosides; biocatalysis; deamination; adenosine deaminase; adenylate deaminase

INTRODUCTION

Adenosine deaminase (adenosine aminohydrolase, ADA, EC 3.5.4.4) and adenylate deaminase (5′-adenylic acid deaminase, AMP deaminase, AMPDA, EC 3.5.4.6), which catalyze the hydrolytic deamination of adenosine and adenylic acid (adenosine 5′-phosphate, AMP) to inosine and its 5′-phosphate,[1] also can be considered valuable biocatalysts to transform many modified nucleosides, including 2′,3′-isopropylidene adenosine **1a**.[2] The isopropylidene group is a convenient scaffold in ribonucleoside chemistry, because it can be used to protect 2′- and 3′-hydroxy groups, thus allowing the introduction of specific modifications at 5′ and 1′ positions of the adenosine structure. By this way, we have prepared a few analogues of 2′, 3′-isopropylidene adenosine **1a** (Figure 1) and studied the influence of the afore-mentioned modifications on the activity of ADA and AMPDA.

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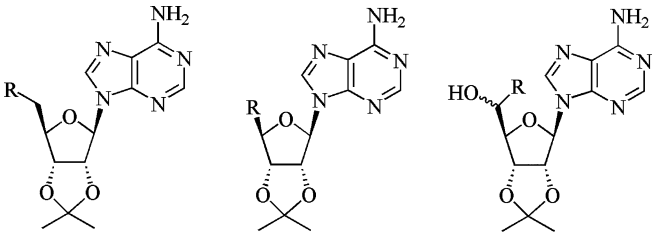


FIGURE 1 Structures of 5′-modified-2′,3′-isopropylidene adenosine analogues, **1a-e**, **2a,b**, and **3a,b**.

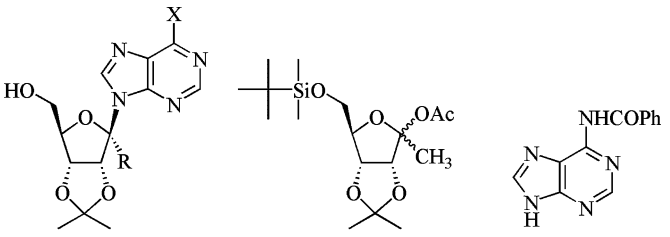
5′-Modified-2′,3′-Isopropylidene Adenosine Analogues

ADA is capable to deaminate modified nucleosides only if the 5′-hydroxy group is present in the molecule and cannot transform 2′,3′-isopropylidene adenosine analogues (compounds **1b–d**) lacking the 5′-hydroxy moiety.[3] Instead, all compounds are substrates for AMPDA which, in this respect, seems to be a biocatalyst more versatile than ADA. Interestingly, ADA and AMPDA are able to deaminate compounds **2a** and **2b** where the CH_2OH group is substituted by CH_2NH_2[3] and COOH[4] groups. For ADA a lower reaction rate has been observed, whereas AMPDA is active on both compounds with no significant difference in the reaction rate. We have also synthesized (5′ *R,S*)-5′-alkyl compounds **3a** and **3b** (Figure 1) and reported that both enzymes catalyze the selective deamination of (5′ *S*)-5′-methyl-2′, 3′-isopropylidene adenosine **3a**,[5] but only AMPDA is active on the (5′ *R,S*)-5′-butyl analogue **3b**[6]

1′-Methyl- 2′,3′-Isopropylidene Purine Ribosides

Our studies on 1′-methyl-2′,3′-*O*-isopropylidene compounds (Figure 2) began with the synthesis of 1′-methyl-6-chloro analogue **4a**. We observed that, differently from 2′,3′-isopropylidene 6-chloropurine riboside **4b**, ADA and AMPDA are not able to hydrolyze **4a**.[7] We now have prepared 2′, 3′-isopropylidene 1′-methyl-adenosine (**4c**) that has been previously synthesized by Cappellacci et al.[8] For this purpose, we started from D-ribose and prepared in five steps the 1-deoxy-psicofuranosyl derivative **5**[7] (Figure 2) that was reacted with N^6-benzoyl adenine **6** in the presence of $EtAlCl_2$.

Desilylation and debenzoylation of the intermediate protected nucleoside, afforded 2′,3′-isopropylidene 1′-methyl-adenosine (**4c**). No deaminating activity of AMPDA or ADA was observed and this result is in agreement with the reported lack of activity of ADA on 1′-methyladenosine.[8] Results so far obtained with ADA and AMPDA on substrates **4a** and **4c** may be



4 a. R=CH_3, X=Cl
b. R=H, X=Cl
c. R=CH_3, X=NH_2

5

6

FIGURE 2 Structures of compounds **4a–c**, **5**, and **6**: 1′-methyl-2′,3′-isopropylidene derivatives **4a** and **4c** are not transformed by ADA or AMPDA.

explained by a distorted *anti*-conformation of the purin ring caused by the introduction on the methyl group at 1′-position. ^{1}H-NMR studies have confirmed this conformational effect with the observation of an evident NOE effect at H-C(2) and of a smaller one at H-C(8) produced by irradiation of 1′-CH_3.

All together our data confirm the reliability of 2′,3′-isopropylidene as a convenient scaffold either to prepare adenosine analogues modified at 1′ and 5′ positions and to study the influence of these modifications on the activity of deaminating enzymes.

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