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Stereoselective deamination of (5′*RS*)-5′-methyl-2′,3′-isopropylidene adenosine catalyzed by adenosine deaminase: preparation of diastereomerically pure 5′-methyl adenosine and inosine derivatives

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Abstract—Adenosine deaminase from calf intestine catalyzes the stereoselective deamination of (5′*S*)-5′-methyl-2′,3′-isopropylidene adenosine to the corresponding inosine derivative, so that by enzymatic deamination of (5′*RS*)-5′-methyl-2′,3′-isopropylidene adenosine, the diastereomerically pure (5′*S*)-inosine and the unreacted (5′*R*)-adenosine derivatives can be efficiently prepared.
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Adenosine deaminase (adenosine aminohydrolase, E.C. 3.5.4.4, ADA) is an enzyme that catalyzes the rapid and irreversible deamination of adenosine **1a** to inosine **2a** (Fig. 1) and may exert its action on a wide range of structurally modified purine nucleosides, as such it may be considered a valuable biocatalyst in this special area of research.[1]

The recently reported three-dimensional pictures of the enzyme[2,3] have added valuable information on the structural requisites of the purine and ribose moieties of nucleosides that are 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′- and 3′-positions.[4,5] In a previous paper, we have shown that adenosine 2′,3′-diacetates **1b** may be converted by ADA to the corresponding inosine derivatives **2b**, thus confirming that some steric hindrance in this part of the molecule is well tolerated by the enzyme (Fig. 1).[6]

We have now confirmed this structural feature of the enzyme action, since ADA is also able to readily and quantitatively deaminate 2′,3′-isopropylidene adenosine **3**[7] to the corresponding inosine derivative **4**[8] in 15 min at room temperature (Fig. 2).[9]

In order to study the influence of the 5′-position on the stereoselectivity of ADA, we considered the formation of a new stereogenic center at that position by the introduction of a methyl group. The required substrate

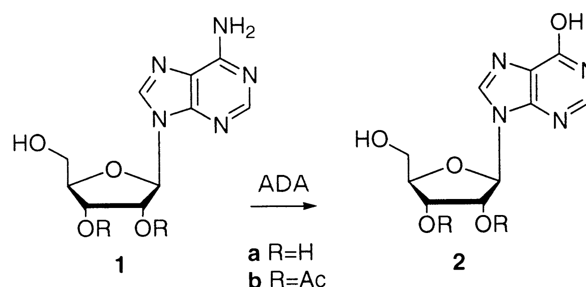

Figure 1. ADA-catalyzed deamination of adenosine **1a** and adenosine 2′,3′-diacetates **1b**.

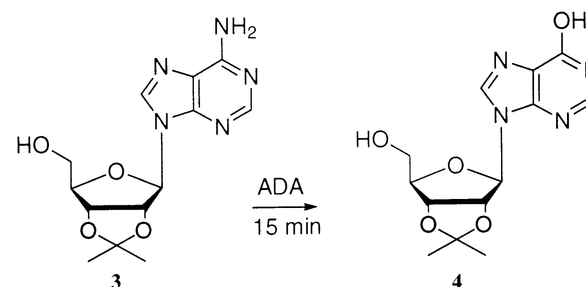

Figure 2. ADA-catalyzed deamination of 2′,3′-isopropylidene adenosine **3**.

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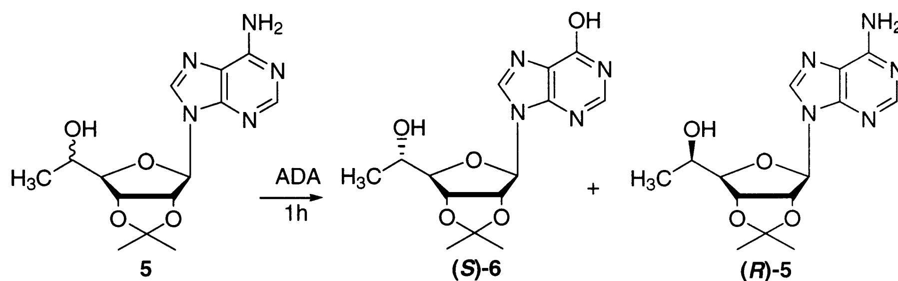

Figure 3. Diastereoselectivity of the ADA-catalyzed deamination of 5′-methyl-2′,3′-isopropylidene adenosine **5**.

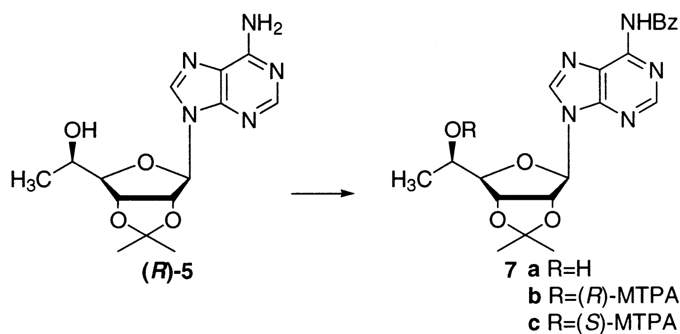

Figure 4. (*R*)- and (*S*)-MTPA esters **7b** and **7c**.

5 was prepared from compound **3** essentially by a published procedure,[10] involving oxidation to the corresponding 5′-carboxaldehyde and Grignard reaction with MeMgCl. By this sequence, compound **5** was formed as a diastereomeric mixture. According to the above report, the more abundant diastereomer should possess D-configuration. We therefore assumed that our procedure had afforded product with an *R*/*S* ratio of 3:1.[11] The obtained substrate (*RS*)-**5** was then enzymatically deaminated: the reaction was monitored by HPLC and we found that in 1 h the less abundant diastereomer was completely converted into the corresponding (5′*S*)-5′-methyl-2′,3′-isopropylidene inosine (*S*)-**6**, leaving a quantitative amount of unreacted (5′*R*)-5′-methyl-2′,3′-isopropylidene adenosine (*R*)-**5** (Fig. 3).

The two compounds could be separated by silica gel column chromatography; HPLC and ^{1}H NMR analysis of single compounds confirmed the diastereomeric purity.[12]

In order to unequivocally assign the configuration of the C(5′)-secondary alcohol, we applied the modified Mosher's method[13] to the unreacted (*R*)-**5**, which was N^6-protected as its benzamide to afford compound **7a** (Fig. 4).[14] Treatment of **7a** with (*S*)- and (*R*)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride (MTPACl) in the presence of 4-dimethylaminopyridine in acetonitrile afforded the corresponding (*R*)- and (*S*)-MTPA esters **7b** and **7c**. The $\Delta\delta$ ($\delta_S-\delta_R$) values, expressed in hertz, obtained from ^{1}H NMR spectra at 500 MHz, are shown in Fig. 5. All protons of the ribose moiety have $\Delta\delta$ <0 values and the C(5′)-methyl protons have a $\Delta\delta$ >0 value of +10.00 Hz. According to the model adopted to determine the absolute configuration

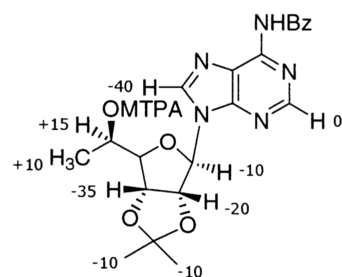

Figure 5. Values of $\Delta\delta$ (Hz) for MPTA esters **7b** and **7c**.

of secondary alcohols,[13] we conclude that the 5′-configuration of the unreacted diastereomer is *R* and this result confirmed the original configurational assignment[10] that was based on chemical correlations.

The results so far obtained show that ADA is able to catalyse the deamination of adenosine analogs containing the ribose moiety modified at positions 2′, 3′ and 5′. A hydroxyl group has to be present at C(5′) for the correct binding at the transition state and the substitution with a methyl group is compatible with the catalytic action of the enzyme, if the newly generated stereogenic center possesses the (5′*S*)-configuration. This conclusion is in agreement with previous observations on the stereoselectivity exhibited by ADA on the diastereomeric mixture of the carbocyclic nucleoside neplanocin[15] and widens the opportunities offered by the enzyme as a valuable biocatalyst in the field of natural and synthetic nucleosides. With this method, diastereomerically pure (5′*S*)-inosine and (5′*R*)-adenosine derivatives (*S*)-**6** and (*R*)-**5** may be prepared from **5**.

Acknowledgements

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9. Compound **3** (0.05 g) in water (20 mL) was treated with ADA (10% by weight, 5 mg) and reacted at room temperature for 15 min. The reaction was monitored by HPLC (phosphate buffer pH 6.0/CH_3CN, 8:2). The solution was lyophilized and the residue crystallized from MeOH to afford 2′,3′-isopropylidene inosine **4** (0.045 g), mp 260–262°C, $[\alpha]_D^{25}$ −68.6 (*c* 1, MeOH); ^1H NMR (CD_3OD) δ 8.30 (1H, s, H-2), 8.05 (1H, s, H-8), 6.18 (1H, d, *J*=3.3 Hz, H-1′), 5.26 (1H, dd, *J*=3.3, 6.7 Hz, H-2′), 5.01 (1H, dd, *J*=2.7, 6.7 Hz, H-3′), 4.34 (1H, ddd, *J*=2.7, 4.0, 4.7 Hz, H-4′), 3.75 (1H, dd, *J*=4.0, 12.0 Hz, H-5′a), 3.69 (1H, dd, *J*=4.7, 12.0 Hz, H-5′b), 1.59 (3H, s, CCH_3), 1.36 (3H, s, CCH_3).
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11. Major diastereomer: HPLC t_r=10.9 (phosphate buffer pH 6.0/CH_3CN, 8:2); ^1H NMR (CD_3OD) δ 8.29 (1H, s, H-2), 8.18 (1H, s, H-8), 6.11 (1H, d, *J*=3.4 Hz, H-1′), 5.21 (1H, dd, *J*=3.4, 6.0 Hz, H-2′), 5.07 (1H, dd, *J*=2.7, 6.0 Hz, H-3′), 4.12 (1H, dd, *J*=2.7, 3.4 Hz, H-4′), 3.97 (1H, dq, *J*=3.4, 6.0 Hz, H-5′), 1.61 (3H, s, CCH_3), 1.38 (3H, s, CCH_3), 1.19 (3H, d, *J*=6.0 Hz, CH_3). Minor diastereomer: HPLC t_r=10.2 (phosphate buffer pH 6.0/CH_3CN, 8:2); δ 8.36 (1H, s, H-2), 8.19 (1H, s, H-8), 6.15 (1H, d, *J*=3.4 Hz, H-1′), 5.18 (1H, dd, *J*=3.4, 6.0 Hz, H-2′), 4.98 (1H, dd, *J*=2.7, 6.0 Hz, H-3′), 4.16 (1H, dd, *J*=2.7, 3.4 Hz, H-4′), 3.93 (1H, dq, *J*=3.4, 6.0 Hz, H-5′), 1.59 (3H, s, CCH_3), 1.36 (3H, s, CCH_3), 1.21 (3H, d, *J*=6.0 Hz, CH_3).
12. Compound (*S*)-**6**: HPLC t_r=5.8 (phosphate buffer pH 6.0/CH_3CN, 8:2); decomposed without melting at 220°C, $[\alpha]_D^{25}$ −35.4 (*c* 1, MeOH); ^1H NMR (CD_3OD) δ 8.38 (1H, s, H-2), 8.06 (1H, s, H-8), 6.19 (1H, d, *J*=3.4 Hz, H-1′), 5.17 (1H, dd, *J*=3.4, 6.0 Hz, H-2′), 4.96 (1H, dd, *J*=3.4, 6.0 Hz, H-3′), 4.11 (1H, dd, *J*=3.4, 4.0 Hz, H-4′), 3.92 (1H, dq, *J*=3.4, 6.0 Hz, H-5′), 1.60 (3H, s, CCH_3), 1.37 (3H, s, CCH_3), 1.22 (3H, d, *J*=6.0 Hz, CH_3). Compound (*R*)-**5**: mp 248–250°C, $[\alpha]_D^{25}$ −25.6 (*c* 1, MeOH).
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