Activity of Adenosine Deaminase (ADA) and Adenylate Deaminase (AMPDA) Towards 6-Chloropurine Nucleosides Modified in the Ribose Moiety

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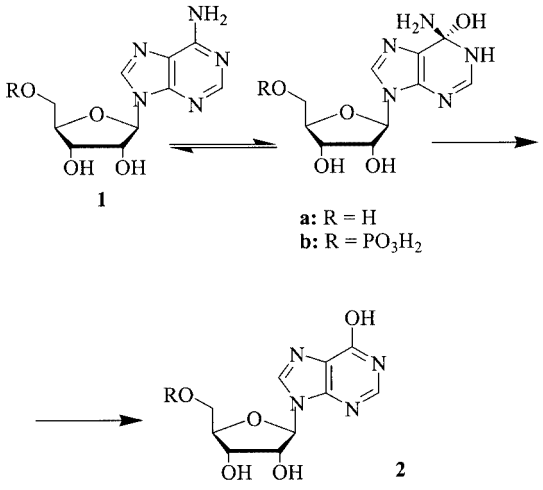
The enzymes adenosine deaminase (ADA) and adenylate deaminase (AMPDA) are able to catalyze the hydrolytic dechlorination of 6-chloropurine riboside and the corresponding 2′,3′-*O*-isopropylidene derivative, but show no activity towards the 3,4-*O*-isopropylidene-1-methylriboside of 6-chloropurine and adenine.

Introduction

Adenosine deaminase (ADA, EC 3.5.4.4) catalyzes the hydrolytic deamination of adenosine (**1a**) to inosine (**2a**). According to several studies,[1] this reaction should proceed *via* a tetrahedral intermediate. A similar mechanism probably accounts for the adenylate deaminase (5′-adenylic acid deaminase, AMPDA, EC 3.5.4.6)-catalyzed transformation of adenosine 5′-monophosphate (AMP) (**1b**) into inosine 5′-monophosphate (**2b**) (Scheme 1).[2]



Scheme 1. ADA- and AMPDA-catalyzed deamination of adenosine (**1a**) and 5′-adenylic acid (**1b**)

ADA is able to catalyze the deamination of structurally modified purine nucleosides[3] provided that a hydroxy group is present at the 5′-position.[4] The other deaminating enzyme, AMPDA, has been studied less, but recent results indicate that it can deaminate a wider range of substrates, including 5′-substituted adenosines like **3a**−**e**, that do not undergo catalyzed deamination by ADA.[5]

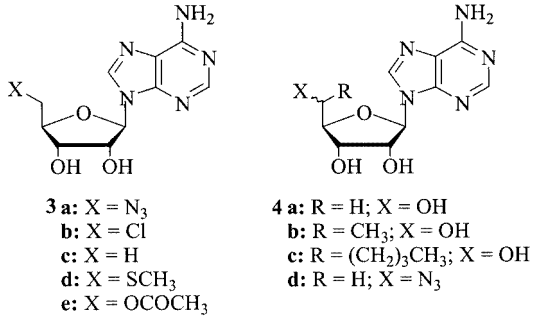


Figure 1. Structure of adenosine analogues **3a**−**e** and **4a-d**

We have previously observed that steric hindrance at the 2′- and 3′-positions of purine nucleosides is well tolerated by both enzymes, that the enzymes can also smoothly transform 2′,3′-*O*-(isopropylidene)adenosine derivatives of **4a**,**b** and behave differently if bulky alkyl groups R (compound **4c**) or moieties X other than OH are present at the 5′-position (for example, compound **4d**).[6−8]

In order to gain more information on the biocatalytic capability of ADA and AMPDA, we decided to study the conversion of analogs of 6-chloropurine riboside (**5**), a nucleoside that is widely used as an intermediate in the synthesis of adenosine derivatives.[9] It is well established that ADA can catalytically hydrolyze 6-chloropurine riboside (**5**) itself,[10] whereas the activity of AMPDA towards **5** has only recently been reported.[11] We have now decided to investigate the activity of ADA and AMPDA towards the

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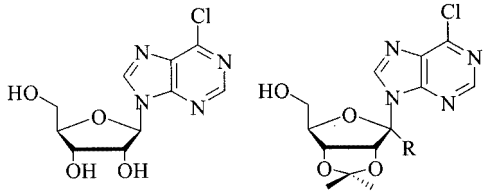
2′,3′-*O*-isopropylidene derivative **6a** and also the 1′-methyl analogue **6b** (Figure 2).

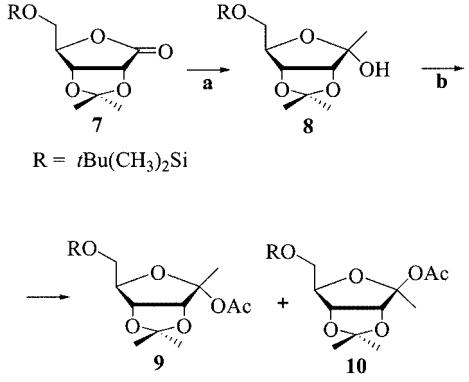


5 **6a:** R = H **b:** R = CH_3

Figure 2. Structure of 6-chloropurine (**5**) and 6-chloropurine derivatives **6a**,**b**

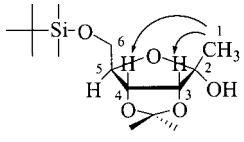
Results and Discussion

Synthesis of Substrates 6a,b: The acetonide **6a** was prepared in a straightforward manner from the commercially available nucleoside **5** (Fluka), whereas the synthesis of the 1′-methyl analogue **6b** required an ad hoc synthesis that started from the ribonolactone derivative **7**, which was prepared as described previously.[12] The key 1-*C*-methyl intermediate **8** was prepared (91% yield) by the reaction of **7** with methyllithium at −70 °C[13] and this procedure afforded exclusively the α anomer (Scheme 2). A few papers have described the above methylation reaction,[13−15] but no clear statement about the stereochemical outcome has been provided. Based on the expected β attack of the methyl anion on the 1-carbonyl group of compound **7**, the α anomer should be obtained. In this work we have been able to unequivocally assign the configuration at the 2-position of the 1-methyl compound, 6-*O*-(*tert*-butyldimethylsilyl)-3,4-*O*-isopropylidene-1-deoxy-D-psicofuranose (**8**), from the results of ^{1}H NMR studies and NOE experiments (Figure 3).



R = *t*Bu(CH_3)$_2$Si

Scheme 2. Reagents and conditions: (a) CH_3Li/Et_2O/−70 °C, 91%; (b) Ac_2O/DMAP/Py/room temp., 86%



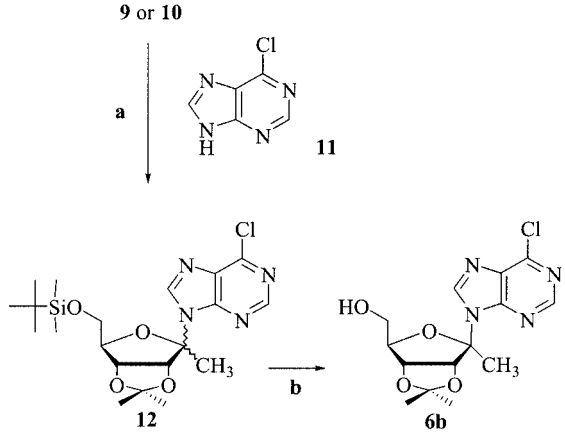
8

Figure 3. Spatial correlations determined from NOE experiments performed on **8**

Accordingly, saturation of 1-CH_3 resulted in NOEs of the H−C(3) and H−C(4) signals whereas H−C(5) was not affected. This clearly indicates that the 1-methyl group is in the β-position.

We also treated the lactone **7** with a Grignard reagent (methylmagnesium iodide, −70 °C) and the reaction proceeded with similar stereochemical control, but with a lower yield (65%).

Following the experimental protocol recently described for 1-*C*-methylribose protected with other groups at the 3-, 4-, and 6-positions,[14] the protected nucleoside **12** could be prepared by a coupling reaction of the corresponding 2-acetate of compound **8** and 6-chloropurine (**11**) in the presence of ethylaluminium dichloride (Scheme 3).

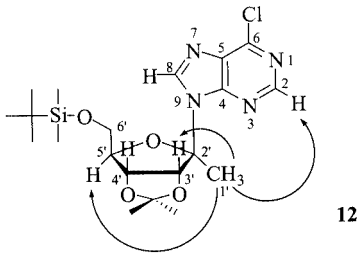


Scheme 3. Reagents and conditions: (a) $EtAlCl_2/CH_3CN$/room temp., 85%; (b) TBAF/CH_3CN/room temp., 80%

We were unable to prepare the α-acetate **9** selectively from the diastereomerically pure compound **8** by carrying out the reaction with acetic anhydride at −70 °C as described previously[13] and the traditional acetylation of anomerically pure **8** afforded a mixture of α-acetate **9** and β-acetate **10** in a ratio of 1:2.[16] Acetates **9** and **10** were separated by flash chromatography and subsequent ^{1}H NMR analysis allowed the anomeric assignment of a single compound. Ethylaluminium dichloride-catalyzed coupling of 6-chloropurine (**11**) with the isolated acetates **9** and **10** gave the same anomeric ratio of 6-chloronucleoside **12**. The results evidently indicate that there is no steric control during aluminium-mediated nucleoside formation. In any event, separation of the α and β nucleosides of **12** allowed a careful ^{1}H NMR study and NOE experiments were carried out in order to confirm the structure of the required nucleoside **12** (Figure 4).

That **12** adopts the β conformation was evident from the observation that irradiation of the 1′-CH_3 group led to H−C(3′) and H−C(5′) enhancements. Moreover, the observation of an NOE effect at H−C(2) and of a smaller one at H−C(8) led us to conclude that a "distorted" *anti* conformation characterizes the β purine **12** in chloroform.

The preparation of the nucleoside **6b** was completed after desilylation of compound **12** with tetrabutylammonium flu-



12

Figure 4. Spatial correlations determined from NOE experiments performed on **12**

oride (TBAF) and the required substrate **6b** was obtained from the protected ribonolactone **7** in 32% yield.

AMPDA- and ADA-Catalyzed Dechlorination of Modified 6-Chloropurine Nucleosides (6a,b): The enzymatic dechlorination of compounds **5**, **6a** and **6b** was carried out in a 3% DMSO aqueous solution, conditions that do not influence the deaminating activity of ADA and AMPA towards 2′,3′-*O*-isopropylideneadenosine.[6] Under these conditions we carried out the enzymatic reactions using the rate of the reaction of 6-chloropurine nucleoside **5** as a reference. The results obtained with nucleoside **6a** indicate that the introduction of the 2′,3′-*O*-isopropylidene group only slowed the ADA- and AMPDA-catalyzed dechlorination to the corresponding inosine derivative. The presence of a methyl group at the 1′-position in **6b** caused a loss of activity of both ADA and AMPDA (see Table 1) and we can attribute this effect to changes in the spatial arrangement of compound **6b** caused by the introduction of the 1′-methyl group; the classic *anti* conformation of nucleosides was changed into a "distorted" *anti* arrangement in the purine.

Table 1. ADA- and AMPDA-catalyzed transformation of adenosines **5**, **6a** and **6b**

Substrate	ADA Reaction time (hours)[a]	AMPDA Reaction time (hours)[a]
5	2	4
6a	11	14
6b	no reaction	no reaction

[a] Time for complete reaction.

Conclusions

Our results confirm the versatility of the two deaminating enzymes ADA and AMPDA, which are also able to catalyze the dechlorination of 6-chloropurine nucleosides in the presence of a 2′,3′-*O*-isopropylidene protecting group that is well tolerated by both enzymes. Our results from experiments with the nucleoside **6b** confirm the recent observation[17] that 1′-methyladenosine is not a suitable substrate for ADA catalysis. Our ^{1}H NMR analysis of nucleoside **12** and NOE experiments confirm that the purine ring adopts a "distorted" *anti* position and this conformational difference with respect to natural nucleosides may explain the lack of activity of two otherwise versatile biocatalysts, ADA and AMPDA. Further studies are in progress to investigate the significance of modifications to the structure of adenosine on the activity of enzymes involved in purine metabolism.

Experimental Section

General Remarks: Melting points were recorded with a Stuart Scientific SMP3 instrument and are uncorrected. Optical rotations were measured in $CHCl_3$ with a Perkin−Elmer 241 polarimeter (sodium lamp, λ = 589 nm). ^{1}H NMR spectra were recorded at 303 K with a Bruker AM-500 spectrometer equipped with an Aspect 3000 computer, a process control, and an array processor. The ^{1}H NMR chemical shifts are reported in parts per million, using as reference the signal for residual solvent protons (δ = 7.24 for $CDCl_3$ and δ = 3.30 for CD_3OD); coupling constants (J) are given in Hertz. NMR signals were assigned using ^{1}H homodecoupling and COSY experiments. For the NOE experiments, samples were degassed by bubbling nitrogen through the solution and fitting sample holder with a PTFE septum cap.

The progress of all reactions and column chromatographic separations were monitored by TLC and HPLC. TLC was performed on silica gel 60 F_{254} precoated plates with a fluorescent indicator (Merck). Flash chromatography[18] was performed using silica gel 60 (230−400 mesh, Merck).

HPLC analyses were carried out with a Jasco HPLC instrument with an Uvidec 100 II UV detector operating at 260 nm using an Alltech Hypersil BDS C18 column (4.6 mm × 250 mm). Mass spectra were recorded on a Finnigan LCQ-Deca (Termoquest) in ESI positive-ion mode, KV 5.00, 225 °C, 15 V.

All reagents were obtained from commercial sources and used without further purification. Solvents were purified and dried in the usual way; in silica gel chromatography, petroleum ether (40−60 °C bp) was used as the eluent. Enzymes were obtained as follows: adenosine deaminase from calf intestinal mucosa (Sigma, type II, 2.2 units per mg protein), 5′-adenylic acid deaminase from *Aspergillus* species (Sigma, 0.107 units per mg protein). 6-Chloro-9-(2′,3′-*O*-isopropylidene-β-ribofuranosyl)-9*H*-purine (**6a**) and 5-*O*-(*tert*-butyldimethylsilyl)-2,3-*O*-isopropylidene-D-ribonolactone (**7**) were prepared according to literature procedures.[19,12]

6-*O*-(*tert*-Butyldimethylsilyl)-3,4-*O*-isopropylidene-1-deoxy-D-psicofuranose (8): A solution of 1.6 M methyllithium in diethyl ether (10.8 mL) was added dropwise to a stirred solution of 5-*O*-(*tert*-butyldimethylsilyl)-2′,3′-*O*-isopropylidene-D-ribonolactone (**7**) (3 g, 9.91 mmol) in anhydrous diethyl ether (90 mL) at −70 °C under argon. The mixture reacted for 1 h and then warmed to 0 °C and treated with 10% aqueous NH_4Cl (90 mL). Then the mixture was extracted with diethyl ether (3 × 90 mL), the combined organic layers were washed with ice-cold water (2 × 70 mL) and dried with anhydrous Na_2SO_4. Removal of solvent under reduced pressure and purification of the residue by flash chromatography (petroleum ether/ethyl acetate, 9:1) afforded **8** (2.86 g, 91%) as a colorless syrup; R_f = 0.29 (petroleum ether/ethyl acetate, 9:1). $[\alpha]_D^{22}$ = −12.2 (c = 2, $CHCl_3$) {ref.[14] $[\alpha]_D^{22}$ = −10 (c = 1.5, $CHCl_3$)}. ^{1}H NMR ($CDCl_3$): δ = 0.10 (s, 3 H, SiMe), 0.11 (s, 3 H, SiMe), 0.89 (s, 9 H, $SiCMe_3$), 1.31 (s, 3 H, 1-Me), 1.47 (s, 3 H, CMe), 1.48 (s, 3 H, CMe), 3.72 (dd, $J_{6a,5}$ = 2.1 Hz, $J_{6a,6b}$ = 11.2 Hz, 1 H, 6a-H), 3.76 (dd, $J_{6b,5}$ = 2.1 Hz, $J_{6b,6a}$ = 11.2 Hz, 1 H, 6b-H), 4.23 (ddd, $J_{5,4}$ = 1.4 Hz, $J_{5,6a}$ = 2.1 Hz, $J_{5,6b}$ = 2.1 Hz, 1 H, 5-H), 4.40 (d, $J_{3,4}$ = 6.3 Hz, 1 H, 3-H), 4.47 (dd, $J_{4,5}$ =

1.4 Hz, $J_{4,3}$ = 6.3 Hz, 1 H, 4-H), 5.12 (br. s, 1 H, OH) ppm. MS (ESI): m/z = 341 $[M + Na]^+$.

2-*O*-Acetyl-6-*O*-(*tert*-butyldimethylsilyl)-3,4-*O*-isopropylidene-1-deoxy-D-psicofuranose (9) and (10): A mixture of compound **8** (2.80 g, 8.79 mmol), 4-(dimethylamino)pyridine (0.040 g, 0.33 mmol) and acetic anhydride (0.63 mL, 7.30 mmol) in pyridine (22 mL) was stirred at room temperature for 5 h. Ice-cold water (50 mL) was then added, the aqueous phase was extracted with $CHCl_3$ (3 × 30 mL) and the combined organic layers were washed with a cold saturated $NaHCO_3$ solution (3 × 30 mL), dried with anhydrous Na_2SO_4 and evaporated under reduced pressure. Flash chromatography on silica gel (petroleum ether/ethyl acetate, 9:1) afforded the β anomer **10** (1.82 g, 58%) and the α anomer **9** (0.88 g, 28%) as white foams. **Compound 9:** R_f = 0.29 (petroleum ether/ethyl acetate, 9:1). $[\alpha]_D^{22}$ = −12.8 (c = 2, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ = 0.06 (s, 3 H, SiMe), 0.07 (s, 3 H, SiMe), 0.86 (s, 9 H, $SiCMe_3$), 1.34 (s, 3 H, CMe), 1.55 (s, 3 H, CMe), 2.00 (s, 3 H, 1-Me), 2.20 (s, 3 H, COMe), 3.76 (dd, $J_{6a,5}$ = 4.2 Hz, $J_{6a,6b}$ = 11.2 Hz, 1 H, 6a-H), 3.81 (dd, $J_{6b,5}$ = 3.5 Hz, $J_{6b,6a}$ = 11.2 Hz, 1 H, 6b-H), 4.51 (d, $J_{3,4}$ = 7.7 Hz, 1 H, 3-H), 4.64 (dd, $J_{4,5}$ = 7.7 Hz, $J_{4,3}$ = 7.7 Hz, 1 H, 4-H), 4.80 (ddd, $J_{5,6b}$ = 3.5 Hz, $J_{5,6a}$ = 4.2 Hz, $J_{5,4}$ = 7.7 Hz, 1 H, 5-H). MS (ESI): m/z = 383 $[M + Na]^+$. **Compound 10:** R_f = 0.51 (petroleum ether/ethyl acetate, 9:1). $[\alpha]_D^{22}$ = −34.7 (c = 1, $CHCl_3$) {ref.,[20] = $[\alpha]_D^{22}$ = −25.5 ($CHCl_3$)}. ^{1}H NMR ($CDCl_3$): δ = 0.04 (s, 3 H, SiMe), 0.05 (s, 3 H, SiMe), 0.87 (s, 9 H, $SiCMe_3$), 1.31 (s, 3 H, CMe), 1.46 (s, 3 H, CMe), 1.72 (s, 3 H, 1-Me), 1.97 (s, 3 H, COMe), 3.54 (dd, $J_{6a,5}$ = 9.1 Hz, $J_{6a,6b}$ = 10.5 Hz, 1 H, 6a-H), 3.68 (dd, $J_{6b,5}$ = 5.6 Hz, $J_{6b,6a}$ = 10.5 Hz, 1 H, 6b-H), 4.19 (ddd, $J_{5,4}$ = 1.4 Hz, $J_{5,6b}$ = 5.6 Hz, $J_{5,6a}$ = 9.1 Hz, 1 H, 5-H), 4.72 (dd, $J_{4,5}$ = 1.4 Hz, $J_{4,3}$ = 6.3 Hz, 1 H, 4-H), 4.75 (d, $J_{3,4}$ = 6.3 Hz, 1 H, 3-H) ppm. MS (ESI): m/z = 383 $[M + Na]^+$.

9-(6′-*O*-*tert*-Butyldimethylsilyl-3′,4′-*O*-isopropylidene-1′-deoxy-D-psicofuranos-2-yl)-6-chloro-9*H*-purine (12): A 1.8 M solution of ethylaluminium dichloride in toluene (1.3 mL) was added dropwise to a stirred mixture of acetate **9** (or **10**) (0.86 g, 2.38 mmol) and 6-chloroadenine **11** (0.8 g, 5.19 mmol) in anhydrous acetonitrile (25 mL) under argon. The reaction mixture was stirred overnight at room temperature and then poured into an ice-cold mixture of a saturated $NaHCO_3$ solution (65 mL) and CH_2Cl_2 (125 mL). The mixture was stirred for 10 min and the resulting solution filtered through a Celite pad. The organic solution was washed with a saturated $NaHCO_3$ solution (50 mL) and brine (2 × 50 mL), dried with anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product, purified by flash chromatography on silica gel (petroleum ether/ethyl acetate, 8:2), afforded the β (0.57 g, 53%) and α anomers (0.35 g, 32%) as colorless oils. **Compound 12 (β anomer):** R_f = 0.44 (petroleum ether/ethyl acetate, 8:2). $[\alpha]_D^{22}$ = −68.2 (c = 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ = −0.12 (s, 3 H, SiMe), −0.13 (s, 3 H, SiMe), 0.66 (s, 9 H, $SiCMe_3$), 1.41 (s, 3 H, CMe), 1.64 (s, 3 H, CMe), 1.79 (s, 3 H, 1′-Me), 3.66 (dd, $J_{6'a,5'}$ = 4.2 Hz, $J_{6'a,6'b}$ = 11.2 Hz, 1 H, 6′a-H), 3.80 (dd, $J_{6'b,5'}$ = 3.5 Hz, $J_{6'b,6'a}$ = 11.2 Hz, 1 H, 6′b-H), 4.54 (ddd, $J_{5',4'}$ = 1.4 Hz, $J_{5',6'b}$ = 3.5 Hz, $J_{5',6'a}$ = 4.2 Hz, 1 H, 5′-H), 4.75 (dd, $J_{4',5'}$ = 1.4 Hz, $J_{4',3'}$ = 5.6 Hz, 1 H, 4′-H), 5.42 (d, $J_{3',4'}$ = 5.6 Hz, 1 H, 3′-H), 8.46 (s, 1 H, 2-H), 8.77 (s, 1 H, 8-H) ppm. MS (ESI): m/z = 477 $[M + Na]^+$. $C_{20}H_{31}ClN_4O_4Si$ (454.18): calcd. C 52.79, H 6.87, N 12.31; found C 52.66, H 6.71, N 12.42. **Compound 12 (α Anomer):** R_f = 0.33 (petroleum ether/ethyl acetate, 8:2). $[\alpha]_D^{22}$ = −77.9 (c = 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ = 0.10 (s, 3 H, SiMe), 0.11 (s, 3 H, SiMe), 0.59 (s, 3 H, CMe), 0.93 (s, 9 H, $SiCMe_3$), 1.17 (s, 3 H, CMe), 2.15 (s, 3 H, 1′-Me), 3.78 (dd, $J_{6'a,5'}$ = 2.8 Hz, $J_{6'a,6'b}$ = 11.2 Hz, 1 H, 6′a-H), 3.86 (dd, $J_{6'b,5'}$ = 2.8 Hz, $J_{6'b,6'a}$ = 11.2 Hz, 1 H, 6′b-H), 4.62 (dd, $J_{5',6'b}$ = 2.8 Hz, $J_{5',6'a}$ = 2.8 Hz, 1 H, 5′-H), 4.86 (s, 2 H, 3′-H and 4′-H), 8.34 (s, 1 H, 2-H), 8.72 (s, 1 H, 8-H) ppm. MS (ESI): m/z = 477 $[M + Na]^+$. $C_{20}H_{31}ClN_4O_4Si$ (454.18): calcd. C 52.79, H 6.87, N 12.31; found C 52.70, H 6.61, N 12.46.

6-Chloro-9-(3′,4′-*O*-isopropylidene-1′-deoxy-D-psicofuranos-2-yl)-9*H*-purine (6b): A 1 M solution of tetrabutylammonium fluoride in THF (3.5 mL) was added to a stirred mixture of compound **12** (0.57 g, 1.25 mmol) in acetonitrile (35 mL). The reaction solution was left at room temperature for 3 h and then the solvent was evaporated under reduced pressure to give the crude product, which was purified by flash chromatography on silica gel (petroleum ether/ethyl acetate, 4:6) to give the product **6b** as a white crystal (0.34 g, 80%). $[\alpha]_D^{22}$ = −61.2 (c = 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ = 1.38 (s, 3 H, CMe), 1.63 (s, 3 H, CMe), 1.93 (s, 3 H, 1′-Me), 3.78 (dd, $J_{6'a,5'}$ = 2.8 Hz, $J_{6'a,6'b}$ = 11.9 Hz, 1 H, 6′a-H), 3.94 (dd, $J_{6'b,5'}$ = 2.8 Hz, $J_{6'b,6'a}$ = 11.9 Hz, 1 H, 6′b-H), 4.50 (ddd, $J_{5',4'}$ = 2.8 Hz, $J_{5',6'b}$ = 2.8 Hz, $J_{5',6'a}$ = 2.8 Hz, 1 H, 5′-H), 5.02 (dd, $J_{4',5'}$ = 2.8 Hz, $J_{4',3'}$ = 6.3 Hz, 1 H, 4′-H), 5.31 (d, $J_{3',4'}$ = 6.3 Hz, 1 H, 3′-H), 8.43 (s, 1 H, 2-H), 8.76 (s, 1 H, 8-H) ppm. MS (ESI): m/z = 363 $[M + Na]^+$. $C_{14}H_{17}ClN_4O_4$ (340.09): calcd. C 49.35, H 5.03, N 16.44; found C 49.56, H 5.18, N 16.32.

Enzymatic Deamination of Purine Nucleosides 5, 6a, and 6b: Nucleosides **5**, **6a**, and **6b** (0.02 g) in phosphate buffer (50 mM, 6 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 reaction progress was monitored by HPLC using phosphate buffer (pH 6.0)/acetonitrile mixture as eluent in the following ratios: **5**, 90:10; **6a**, 75:25; **6b**, 80:20. When the reaction was complete the solution was lyophilized to afford inosine from **5** and 2′,3′-*O*-(isopropylidene)inosine from **6a**.[21]

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