AMP Deaminase as a Novel Practical Catalyst in the Synthesis of 6-Oxopurine Ribosides and Their Analogs

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Adenylic acid deaminase from *Aspergillus niger* (AMP deaminase; AMPDA; EC 3.5.4.6) has been introduced as a novel practical catalyst in the synthesis of 6-oxopurine riboside and their analogs. This enzyme has a very broad substrate specificity and has been used on a preparative scale for deamination of several derivatives of adenosine including phosphorylated, cyclic, carbocyclic as well as acyclic analogs. In addition, AMPDA catalyzes dechlorination and demethoxylation of the purine ribosides. Overall substrate specificity of AMPDA is much broader than that of adenosine deaminase which can also be used for the synthesis of 6-oxopurine ribosides. Although the stereoselectivity of AMPDA is modest, this enzyme has successfully been used in the synthesis of a novel antiviral agent, carbovir phosphonate (**14**), after the carbocyclic component was resolved *via* lipase-catalyzed hydrolysis or acylation.

Nucleoside analogs possess antitumor,[1] antiinflammatory[2] and antiviral[3] activities and have long been of great interest to chemotherapy. Many of these nucleosides have a great therapeutic potential as drugs in the treatment of several viral infections including AIDS.[4] Among new potent broad-spectrum antiviral agents the analogs of adenosine, inosine and guanosine figure prominently.[5] The synthesis of these compounds can present a serious challenge.

The synthesis of guanosine and inosine analogs can be achieved by the hydrolysis of the corresponding 6-amino or 6-chloro compounds under basic conditions. The scope of this approach, however, is limited by the inherent instability of many pharmaceutically important nucleosides, especially dideoxynucleosides such as 2′,3′-dideoxyguanosine (ddG), 2′,3′-dideoxyinosine (ddI) or 2′,3′-dideoxyadenosine (ddA).[6] This problem can in principle be solved by incorporating enzyme-catalyzed steps in the synthetic routes.[7,8]

One enzyme, which has recently gained popularity as an efficient catalyst in the synthesis of 6-oxopurine ribosides from the corresponding purine ribosides, is adenosine deaminase (ADA; EC 3.5.4.4) from calf intestinal mucosa. ADA has been successfully used to convert dideoxyribosyl and 3′-azidoribosyl 6-substituted purine nucleosides to the potential anti-HIV compounds 3′-azidoguanosine and 2′,3′-dideoxyguanosine.[9] Also, ADA has recently been used to synthesize 2′-deoxy-2′,2′-difluoronucleosides in the preferred β-form,[10] 2′,3′-dideoxyguanosine (ddG)[6] and several carbocyclic guanosine analogs,[11–13] including the potent anti-HIV agent carbovir.[14]

Despite these successes the use of ADA in the synthesis of antiviral compounds is rather limited. Since ADA is involved in adenosine metabolism many of the new ribosyl and carbocyclic nucleosides are specifically designed to prevent the deactivation of these compounds by ADA-catalyzed deamination.[15] Therefore, obtaining their inosine and guanosine analogs from 6-substituted purines using ADA can be difficult. For instance, although ADA can tolerate some modifications in the purine moiety[15] and at 2′ and 3′ positions of the sugar moiety,[13,16] replacement or substitution of the 5′-hydroxyl group[16] as well as substantial changes in the cyclopentyl ring of carbocyclic analogs[17] leads to a significant loss of

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Table 1. Enzyme-Catalyzed Preparative Synthesis of 6-Oxopurine Ribosides and Their Analogs

substrate[a]	initial rate[b]	reaction time[c] (min)	V_{AMPDA}/V_{ADA}[d]	yield[e] (%)
1	100			
2	85	40	1.1	100
3	4	120	63	100
4	8	180	0.5	100
5	60	90	250	79
6	30	180	270	87
7	50	40	80	100
8	0.7	5 h	>1000	93
9	12	15 h	>1000	78
10	0.02	114 h	>1000	92
11	0.02	60 h	>1000	80
12	0.02	48 h	>1000	85

[a] Ad, *N*-adeninyl. AMPDA from Sigma was employed in all cases. The hydrolysis of **5**, **8**, and **11** was also carried out with AMPDA from Amano (Deamyzyme 5000). [b] Initial rates of the enzyme-catalyzed hydrolysis of the substrates were measured by HPLC (see Experimental Section for details). Conditions: 25 °C, 0.1 M phosphate buffer pH 6.5, substrate concentrations 5 mM for **9** and **10** and 10 mM for other substrates. AMPDA concentrations were 0.16 units/mL for **1**, **2**, **7**, and **9**, 0.38 units/mL for **3** and **4**, 0.032 units/mL for **5** and **6**, 0.48 units/mL for **8**, 1.9 units/mL for **10**, 0.38 units/mL for **11**, and 0.96 units/mL for **12**. AMPDA was added to initiate the reaction, and aliquots (10 μL) was taken at 5 min intervals, diluted 200-fold, and assayed by HPLC. [c] Time for complete reaction. The starting amounts of substrates were 35 mg for **12**, 50 mg for **3**, **8**, and **11**, and 100 mg for other substrates. Enzyme concentrations were as in footnote *b*. [d] The ratio of initial rates of AMPDA (V_{AMPDA})- and ADA (V_{ADA})-catalyzed hydrolysis was determined by HPLC in 0.1 M phosphate buffer pH 6.5 at 25 °C for the substrate concentrations denoted in footnote *b*. In the cases where V_{AMPDA}/V_{ADA} is more than 1000, no reaction with ADA was detected after several days of incubation with high ADA concentration (more than 10 units of ADA per mg of a substrate). [e] In all cases the enzyme-catalyzed reactions proceeded quantitatively, without formation of byproducts (HPLC). The isolated yields less than 100% reflect mostly mechanical losses during the purification procedure which was not optimized.

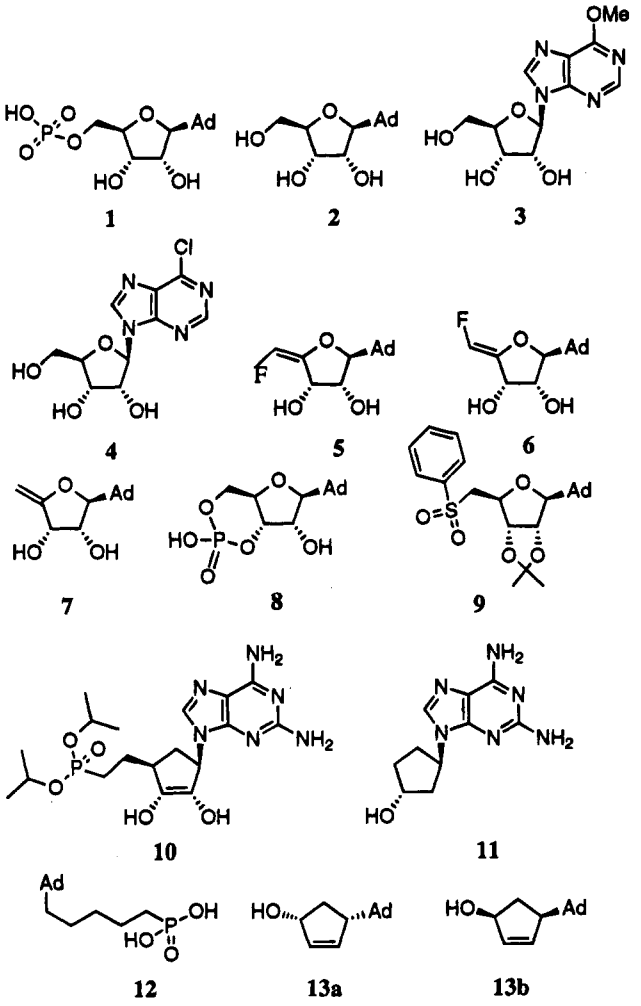
enzymatic activity. It has been reported that microbial ADA has somewhat broader substrate specificity[18] hydrolyzing some 5′-thioadenosine analogs but not adenosine sulfonium compounds.[19]

Here we introduce a different enzyme, AMP deaminase from *Aspergillus* sp.(AMPDA; EC 3.5.4.6), as a novel and practical catalyst in the synthesis of a variety of inosine and guanosine analogs. We will show that this enzyme has a very broad substrate specificity and can be used on a preparative scale for quantitative deamination, dechlorination or demethoxylation of a variety of purine ribosides including carbocyclic and acyclic compounds. This enzyme is available commercially, is inexpensive and can be used for large-scale synthesis of 6-oxopurine ribosides and their carbocyclic and acyclic analogs.

Results and Discussion

The properties of AMPDA from *Aspergillus* sp. and other microorganisms are virtually unknown.[20,21] Yet AMPDA from *Aspergillus* sp. is an important commercially available enzyme[22] which is used in the food industry for the large-scale production of flavors.[20,23] We decided to investigate the synthetic utility of this enzyme in the preparation of novel antiviral agents.[24]

In a typical experiment 100 mg of a substrate (Table 1) were dissolved in 30 mL of 0.1 M phosphate buffer pH 6.5 and then 50–100 mg of crude enzyme preparation (0.096 unit/mg solid) was added. The solution was incubated at room temperature for the period of time indicated in Table 1. When the reaction was complete (HPLC), the solution was lyophilized and the residue was purified by flash-chromatography on silica with MeOH/CH_2Cl_2 (15:85) as an eluent (**5**, **6**, **7**, **9**, **10**, **11**, **13**) or semi-preparative HPLC (Hamilton PRP-1, 305 × 7 mm; H_2O/CH_3CN (4:1); 2 mL/min; **2**, **3**, **4**, **8**, **12**). All AMPDA-catalyzed reactions were run under mild conditions (pH 6.5 and 25 °C). The reactions are clean and proceed without formation of by-products. In all cases the complete conversion of the starting substrates to the corresponding 6-oxopurine derivatives took place.



The results presented in Table 1 demonstrate a very broad substrate specificity for AMPDA.[25] In addition to its natural reaction (deamination of AMP; Scheme 1) AMPDA catalyzes the hydrolysis of a wide variety of synthetic substrates (Table 1). It easily deaminates adenosine **2**, its analogs modified at 5′-position **5**, **6** and

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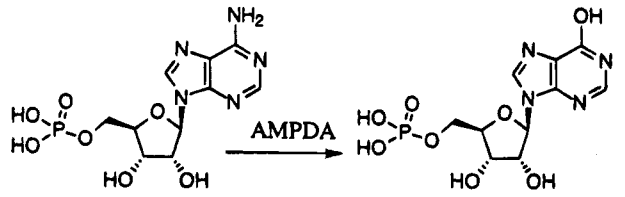
(22) AMPDA is available from Sigma ($133 per 10 000 units) and from Amano as Deamizyme 5000 ($180 per kg). This work was mostly done with Sigma enzyme, although several syntheses were repeated with AMPDA supplied by Amano (see Table 1). In our hands both enzymes had similar activities per mg solid despite the fact that Amano uses different activity units.
(23) See description of AMPDA supplied by Amano.

Scheme 1

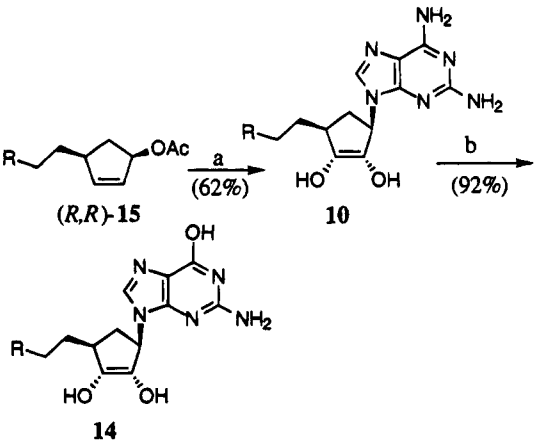


7, compounds modified at both 5′, 2′ and 3′ carbon atoms **9**, 5′,3′-cyclic analog **8**, as well as carbocyclic **10**, **11** and most surprisingly acyclic **12** analogs. Although the activity of AMPDA toward carbocyclic and acyclic compounds is much lower, complete preparative hydrolysis can be achieved in reasonable time. In addition, AMPDA efficiently dechlorinates and demethoxylates adenosine analogs **3**, **4** modified at C6 position. AMPDA also exhibits a modest *E*/*Z* selectivity with *Z*-isomer **5** being twice more reactive than its E-counterpart **6**.

Since the biological activity of nucleoside analogs normally resides in one (natural) enantiomer,[11,12] the synthesis of these, as well as other new pharmaceuticals in optically pure form presents an important and urgent task.[8] AMPDA deaminates the natural enantiomers of the carbocyclic substrate **13a** six times faster than their unnatural counterpart **13b**.[26] Since the stereoselectivity of AMPDA in the reaction with **13** is modest, we decided to use the convergent approach[12,27] for the synthesis of 6-oxopurine ribosides and their analogs in optically pure form. We have successfully employed this approach to the synthesis of a new potent antiviral agent carbovir phosphonate **14** in optically pure form (Scheme 2). In this synthesis we combined high enantioselectivity of lipases in both water and organic solvents with broad substrate specificity of AMPDA. The carbocyclic component **15** can be obtained in any stereochemical configuration via lipase-catalyzed hydrolysis in water or by stereoselective acylation in organic solvents (Scheme 3). Both reactions proceed with excellent stereoselectivity (ee > 98%)[28] and give the resulting acetate in high yield (>45%). After coupling with the base, the resulting diamino compound **10** was subjected to AMPDA-catalyzed hydrolysis to give the natural biologically active enantiomer of carbovir phosphonate **14**.

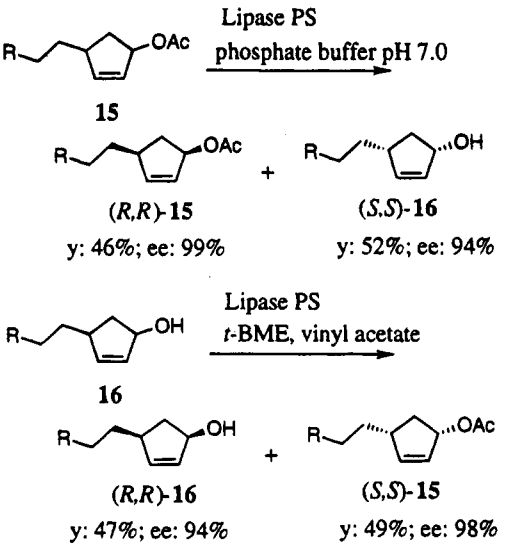
The extremely broad substrate specificity, the ability to work under mild reaction conditions and the low price make AMPDA an excellent catalyst in the synthesis of many nucleoside analogs including optically pure compounds. We believe that the introduction of this novel

Scheme 2[a]



[a] Key: (a) adenine, NaH, $Pd(PPh_3)_4$; (b) AMPDA, 0.1 M phosphate, pH 6.5, rt. R = P(O)(*i*-PrO)$_2$.

Scheme 3



efficient catalyst to the synthetic practice will facilitate the synthesis of new biologically active 6-oxopurine ribosides and their analogs.

Experimental Section

AMPDA from *Aspergillus* sp. was purchased from Sigma (0.096 units/mg solid) and from Amano (Deamyzyme 5000). Lipase PS from *Pseudomonas cepacia* was a product of Amano

(24) We have purified AMPDA from the extract of lyophilized powder of *Aspergillus* sp. 206-fold in 24% yield. The molecular weight of a subunit determined by SDS-PAGE was 77 kDa. The detailed results of purification and initial kinetics study will be published elsewhere.

(25) The comparison of AMPDA and ADA is instructive. The activity of both enzymes toward the typical ADA substrates adenosine **1** and 6-chloropurine riboside **4** is similar. The methoxy modification, however, at the 6-position in the purine ring **3** and any modifications in the sugar moiety which we have investigated, lead to a significant drop in ADA activity. It is worth mentioning that the rate of ADA-catalyzed hydrolysis of even the most reactive substrates **5**, **6**, **7** in this series is less than 2% of that for AMPDA. ADA has no activity whatsoever toward cyclic **8**, carbocyclic **10**,**11**, acyclic **12** nor the phenylsulphonyl **9** derivative.

(26) The initial rates were 16 μmol/min for **13a** and 2.6 μmol/min for **13b** per unit of enzyme activity in 0.1 M phosphate buffer, pH 6.5 and 25 °C.

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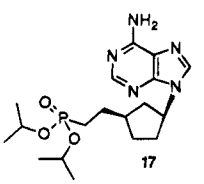
(28) The optical purity of the enantiomers of **15** was assayed by chiral GC (Cyclodex-B, Alltech; He as a carrier gas 68 mL/min, isothermal regime 145 °C. The retention times were 40 and 41.5 min for (*R*,*R*)-**15** and (*S*,*S*)-**15** respectively. To determine the optical purity of the (*R*,*R*) and (*S*,*S*) alcohol **16**, it was first converted to **15** (acetic anhydride/pyridine). The absolute configuration of enantiomers of both **15** and **16** were assigned by chemical correlation. The compound **17** was synthesized from (*R*,*R*)-**15** via **10** by reduction on Pd/C as well as from the natural product aristeromycin via 5′-silyl ether following Exall, A. M. et al *J. Chem. Soc., Perkin Trans.* **1991**, 2467–2477. Both preparations of **17** resulted in the same absolute configuration (chiral HPLC on Chiralpack AD by Daicel: MeOH:EtOH:heptane (9:5:6); 0.5 mL/min; 254 nm).



and ADA (Type II, 1.2 units/mg solid) from calf intestinal mucosa was a product of Sigma. Adenosine, 6-chloropurine riboside, 6-methoxypurine riboside, AMP and cAMP were purchased from Sigma. Compounds **5**,**6**,**7** and **9** were synthesized by D. Matthews (Marion Merrell Dow (MMD), Cincinnati) as described previously.[29] The compounds **10**, **11** and **12** were synthesized by D. Kugel (MMD, Strasbourg), D. Borcherding (MMD, Cincinnati) and S. Halazy[30] (MMD, Strasbourg) respectively.

All melting points are uncorrected. ^1H and ^{13}C chemical shifts (δ) are in parts per million (ppm) relative to Me_4Si, ^{19}F chemical shifts are in ppm relative to $SiFCl_3$ and ^{31}P chemical shifts are in ppm relative to external H_3PO_4. Elemental analysis was performed by MMD, Cincinnati. HPLC was performed on a Waters system consisting of a pump, a controller (Model 600E) and a UV-detector (Model 484) equipped with a Kromasil C-18 column (250 × 4.6 mm) for reverse phase chromatography. All analyses were run with a MeOH/H_2O mixture as eluent under isocratic conditions at a flow rate of 1 mL/min with UV-detection at 280 nm.

Inosine. Inosine was prepared from substrates **2**, **3** and **4** (See text for the experimental details). Analytical data for inosine (mp, optical rotation, ^1H NMR, MS and microanalysis) are in agreement with a commercial sample of inosine.

(4′*E*)-4′,5′-Didehydro-5′-deoxy-5′-fluoroinosine from 5: white lyophilized solid (79 mg; 79%); mp (lyophilized powder) 117 °C foam; $[\alpha]^{25}_D = -78.0$ (*c* 0.332, MeOH); ^1H NMR (CD_3OD) 8.29 (s,1H), 8.07 (s, 1H), 7.02 (d, 1H, J = 78.4 Hz), 6.22 (d, 1H, J = 7.2 Hz), 5.04–4.95 (m, 2H); ^{13}C NMR (CD_3OD) 158.84, 150.50, 149.48 (d, J = 37,8 Hz), 147.06, 140.87, 137.56 (d, J = 233.4 Hz), 125.99, 89.32, 74.43, 67.58; $^{19}FNMR$ (CD_3OD) −180.75 (dd, J = 78 and 2.4 Hz); MS (CI, methane) 269 (M + H, base), 137; UV λ_{max} = 244 (MeOH).

(4′*Z*)-4′, 5′-Didehydro-5′-deoxy-5′-fluoroinosine from 6: white lyophilized powder (87 mg; 87%); mp (lyophilized powder) 132 °C foam; $[\alpha]^{25}_D = -54.3°$ (*c* 0.499, MeOH); ^1H NMR (CD_3OD) 8.24 (s, 1H), 8.07(s, 1H), 6.56 (dd, 1H, J = 74.9 and 0.5 Hz), 6.28 (d, 1H, J = 7.0 Hz), 4.96 (dd, 1H, J = 6.9 and 5.0 Hz), 4.68 (dm, 1H, J = 5.0 Hz); ^{13}C NMR (CD_3OD) 162.00, 151.22, 149.55, 144.00 (d, J = 4 Hz), 140.74, 133.15 (d, J = 250 Hz), 126.55, 90.33, 75.02 (d, J = 3 Hz), 69.70 (d, J = 5 Hz); $^{19}FNMR$ (CD_3OD) δ −165.3 (d, J = 75 Hz); MS (CI, methane) 269 (M + H, base), 137; UV λ_{max} = 243 (MeOH).

4′,5′-Didehydro-5′-deoxyinosine from 7: white lyophilized powder (78 mg; 78%); ^1H NMR (CD_3OD) 8.25 (s, 1H), 8.05 (s, 1H), 6.12 (d, 1H, J = 5.4 Hz), 4.75(t, 1H, J = 5.3 Hz), 4.66 (d, 1H, J = 5.1Hz), 4.31 (m, 1H), 4.21 (m, 1H); MS (CI, methane) 433 (M + H), 297, 239, 101(base); UV λ_{max} = 244 (MeOH); mp (lyophilized powder) 249 °C dec; $[\alpha]_D = -54.7$ (*c* 0.428, MeOH). Anal. ($C_{19}H_{20}N_4O_6S{\cdot}1/4H_2O$) Calcd: C, 52.23; H, 4.62; N, 12.96. Found: C, 52.22; H, 4.73; N, 12.63.

Inosine 3′,5′-cyclic monophosphate from 8: ^1H NMR (DMSO-d_6) 8.22 (s, 1H), 8.10 (s, 1H), 5.92 (s, 1H), 4.64 (ddd, 1H, J = 9.3, 5.0 and 2.5 Hz), 4.46 (d, 1H, J = 5.0 Hz), 4.19–3.92 (m, 3H); ^{31}P NMR (DMSO-d_6) −3.50 (dm, J = 19.6 Hz); ^{13}C NMR (DMSO-d_6) 156.93, 147.68, 146.45, 138.95, 124.65, 91.86, 76.96, (d, J = 4.7 Hz), 72.53 (d, J = 3.5 Hz), 72.15 (d, J = 7.8 Hz), 65.32 (d, J = 6.5 Hz).

5′-Deoxy-2′,3′-*O*-(1-methylethylidene)-5′-(phenylsulfonyl)inosine from 9: white lyophilized powder (78 mg; 78%); mp (lyphilized powder) 249 °C decomp; $[\alpha]_D = +21.0°$ (*c* 1.02; DMSO); ^1H NMR (DMSO-d_6) 8.10 (s, 1H), 8.07 (s, 1H), 7.69 (d, 2H, J = 7.8 Hz), 7.54 (tm, 1H, 7.2 Hz), 7.40 (t, 2H, J = 7.3 Hz), 6.07 (d, 1H, J = 2.3 Hz), 5.33 (dd, 1H, J = 6.1 and 2.2 Hz), 5.01 (dd, 1H, J = 6.1 and 2.4 Hz), 4.49 (td, 1H, J = 6.6 and 2.3 Hz), 3.90 (d, 2H, J = 6.7 Hz), 1.50 (s, 3H), 1.29 (s, 3H); ^{13}C NMR (DMSO-d_6) 156.33, 146.99, 145.85, 139.36, 139.21, 133.48, 128.69, 127.49, 124.81, 113.27, 89.94, 83.58, 83.23, 81.42, 57.45, 26.73, 25.01; MS (CI, methane) 433 (M + H), 297, 239, 101(base); UV λ_{max} = 244 (MeOH). Anal. ($C_{19}H_{20}N_4O_6S{\cdot}1/4H_2O$) Calcd: C, 52.23; H, 4.62; N, 12.96. Found: C, 52.22; H, 4.73; N, 12.63.

(1*R-cis*)-Bis(1-methylethyl) [2-[4-(2-Amino-1,6-dihydro-6-oxo-9*H* purin-9-yl)-2-cyclopenten-1-yl]ethyl]-1-phosphonate from 10. Compound **14** was prepared from **10** by AMPDA-catalyzed hydrolysis, as described in the text. After lyophilization, the residue was purified by column chromatography on silica gel with MeOH/CH_2Cl_2 15:85 as an eluent to afford white powder (120 mg; 92%): mp 267 °C; $[\alpha]^{25}_D = -52.2$ (*c* 0.50, MeOH); R_f 0.19 (chloroform/methanol:90:10); IR (KBr) 3359, 3186, 2974, 1697, 1643, 1385, 1228, 1007, 988 cm^{-1}; ^1H NMR ($CDCl_3$) 7.68 (s, 1H), 6.62 (s, 2H, NH2), 6.12–6.06 (ddd, 1 H, J = 5.0, 3.0 and 2.0 Hz,), 5.78–5.72 (dt, 1H, J = 5, 2 and 2 Hz), 5.50–5.41 (m, 1H), 4.72–4.59 (m, 2 H), 2.92–2.81 (m, 2 H), 1.91–1.75 (m, 5 H), 1.28 (d, 12H); ^{13}C (50 MHz, $CDCl_3$) 158.81, 153.71, 151.14, 139.75, 135.68, 129.06, 116.97, 70.20 ($J_{C\text{-}P}$ = 6.71 Hz), 28.54 ($J_{C\text{-}P}$ = 5.04 Hz), 28.84 ($J_{C\text{-}P}$ = 142.75 Hz), 24.00 ($J_{C\text{-}P}$ = 3.98 Hz); $^{31}PNMR$ (81 MHz, $CDCl_3$) 29.94 Anal. Calcd for $C_{18}H_{28}N_5O_4P$: C, 52.8; H, 6.89 ; N, 17.11. Found: C, 52.74; H, 6.84; N, 16.86.

***trans*-(1*R*,3*R*)-1-(2-Amino-1,6-dihydro-6-oxo-9H-purin-9-yl)cyclopentan-3-ol from 11:** white lyophilized solid (40 mg; 80%); mp 200 °C dec; $[\alpha]^{20}_D = -7.31°$ (*c* 0.471, MeOH); ^1H NMR (DMSO-d_6) 10.54 (bs, 2H), 7.78(s, 1H), 6.43 (bs, 2H), 4.85 (p, 1H, J = 8.4 Hz), 4.71 (d, 1H, J = 3.4 Hz), 4.34 (m, 1H), 2.29–1.94 (m, 4H), 1.82 (m, 1H), 1.58 (m, 1H); ^{13}C NMR (DMSO-d_6): 156.78, 153.23, 151.00, 135.67, 116.84, 70.12, 52.89, 41.53, 33.49, 28.34; MS (CI, methane) 236 (M + 1); UV λ_{max} = 254 (MeOH).

[5-(1,6-Dihydro-6-oxo-9*H*-purin-9-yl)pentyl]phosphonic acid from 12: white lyophilized solid (39 mg; 95%); ^1H NMR (D_2O) 8.19 (s, 1H), 8.14 (s, 1H), 4.27 (t, 2H, J = 7.0 Hz), 1.97–1.85 (m, 2H), 1.63–1.46 (m, 4H), 1.43–1.31 (m, 2H); ^{31}-PNMR (D_2O) 26.62 (m); ^{13}C NMR (D_2O) 161.27, 151.46, 148.05, 144.95, 125.89, 46.92, 31.53, 30.43 (d, J = 114.6 Hz), 29.65, 25.29 (d, J = 4.5 Hz); MS (FAB, glycerol) 285.1 (M − 1), 183 (base). Anal. ($C_{10}H_{13}N_4Na_2O_4P{\cdot}3H_2O$) Calcd: C, 28.85; H, 4.57; N, 13.46. Found: C, 28.78; H, 4.47; N, 13.35.

Lipase-Catalyzed Resolution. Lipase from *Pseudomonas cepacia* (1.25 g) was added to the suspension of **15** (2 g) in 100 mL 0.1 M phosphate buffer pH 7, and the mixture was stirred at room temperature for 7 h. The reaction mixture was extracted by EtOAc (2 × 100 mL), dried over $MgSO_4$, evaporated under vacuum, and separated by flash chromatography with CH_2Cl_2/MeOH (98:2) as an eluent. This procedure gave (*RR*)-**15** (0.92 g, 46%, 99% ee) and (*SS*)-**16** (0.90 g, 52%, 94%ee).

Acylation. For the resolution *via* acylation, 95 mg (0.34 mmol) of the alcohol **16** and 95 μL (1mmol) vinyl acetate were mixed with 35 mg of the same lipase in 3.5 mL *t*-butyl methyl ether and the suspension was stirred for 4 h at room temperature. The enzyme was filtered off, the mixture was evaporated and the residue was separated by flash chromatography with CH_2Cl_2/MeOH (95:5) as an eluent. This procedure gave (*SS*)-**15** (54 mg, 49%, 98%ee) and (*RR*)-**16** (45 mg, 47%, 94% ee).

[2-[4-Hydroxy-2-cyclopenten-1-yl]ethyl-bis(1-methylethyl) ester, (1*S-cis*)-phosphonic acid (*SS*)-16: oil, $[\alpha]^{25}_D = 14.2°$ (*c* 0.934, MeOH); ^1H NMR (DMSO-d_6) 5.83 (s, 2H), 4.81 (dd, 1H, J = 7.1 and 5.0 Hz), 7.76–4.60 (m, 2H), 2.62 (m, 1H), 2.49 (dt, 1H, J = 13.5 and 7.6 Hz), 1.86–1.54 (m, 4H), 1.31 (d, 12H, J = 6.1 Hz), 1.25 (m, 1H); ^{31}P NMR ($CDCl_3$) 30.7 (bs); MS (CI, methane) 277 (M + H, base), 259.

[2-[4-(Acetyloxy)-2-cyclopenten-1-yl]ethyl]bis(1-methylethyl) ester, (1*R-cis*)-phosphonic acid (*RR*)-15: oil, $[\alpha]^{25}_D = -8.14°$ (*c* 0.884, MeOH); ^1H NMR ($CDCl_3$): 5.97 (ddd, 1H, J = 5.7, 1.9 and 1.0 Hz), 5.82 (dt, 1H, J = 5.6 and 2.0 Hz), 5.62 (m, 1H), 4.69 (m, 2H), 2.69 (m, 1H), 2.52 (dt, 1H, J = 14.0 and 7.9 Hz), 2.03 (s, 3H), 1.85–1.60 (m, 4H), 1.40 (dt, 1H, J = 13.9 and 4.4 Hz), 1.31 (d, 12H, J = 6.0 Hz); ^{31}P NMR ($CDCl_3$) 30.4 (bs); MS (CI, methane) 319 (M + 1), 259.

(1*R-cis*)-Bis(1-methylethyl) [2-[4-(2,6-diamino-9*H*-purin-9-yl)-2-cyclopenten-1-yl]ethyl]-1-phosphonate (10). 2,6-Diaminopurine (188 mg, 0.94 mmol) was added to a suspension of sodium hydride (57 mg, 1.42 mmol) in anhydrous DMF (5 mL), under nitrogen. The reaction was stirred at room temperature for 1 h. Palladium tetrakis(triphenylphosphine)

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(272 mg ; 0.23 mmol) and the acetate (*RR*)-**15** (150 mg, 0.47 mmol) were dissolved in DMF (1 mL), and triphenylphosphine (16 mg; 0.061 mmol) was added. The mixture was stirred at 45 °C for 3 h. The solvent was removed *in vacuo*, and the product was purified by column chromatography on silica gel with a gradient of methanol in chloroform to afford the product as a beige solid (120 mg, 62%): mp 189–191 °C; $[\alpha]^{25}_D = -56.3$ (*c* 0.69, MeOH); R_f 0.25 (chloroform/methanol: 90/10) IR (KBr) 3335, 3194, 1663, 1591, 1633, 1403, 1238, 1207, 1013, 982 cm^{-1}; ^1H NMR ($CDCl_3$) 7.58 (s, 1H), 6.12–6.09 (m, 1H,), 5.96 (s, 2H, NH2), 5.85–5.68 (ddd, 1H, J = 6, 2 and 1 Hz), 5.51–5.44 (m, 1H), 5.24 (s, 2H, NH2), 4.78–4.62 (m, 2H), 2.94–2.68 (m, 2 H), 1.95–1.68 (m, 4H), 1.63–1.57 (m, 1H), d 1.28 (d, 12 H); ^{13}C NMR (50 MHz; $CDCl_3$) 159.73, 155.77, 152.72, 139.53, 136.08, 129.29, 114.67, 69.98 ($J_{C\text{-}P}$ = 6.5 Hz), 59.10, 45.29 ($J_{C\text{-}P}$ = 17.6 Hz), 37.97, 28.34 ($J_{C\text{-}P}$ = 5.0 Hz), 24.99 ($J_{C\text{-}P}$ = 142.9 Hz); ^{31}P NMR (81 MHz; $CDCl_3$) 30.24; MS (CI, methane) 409 (M + H), 151 (B + 1). Anal. Calcd for $C_{18}H_{29}N_6O_3P$: C, 52.81; H, 6.89; N, 17.11. Found: C, 52.74; H, 6.84; N, 16.86.

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