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Design and Synthesis of Inhibitors of Adenosine and AMP Deaminases

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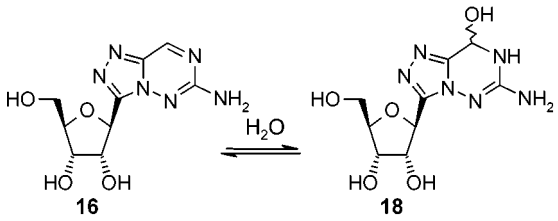
ABSTRACT

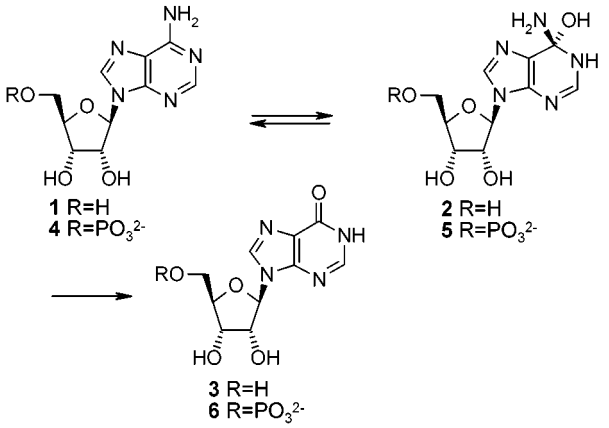


Nucleosides and nucleotides which are able to undergo covalent hydration in the aglycone ring system are potential inhibitors of the enzymes adenosine deaminase (ADA) and AMP deaminase, respectively. Calculations of the enthalpy of covalent hydration and of enzyme binding energy have been used to design new inhibitors of ADA. The ribosyl triazolotriazine 16, which was synthesized as a result of these calculations, exists predominantly as the covalent hydrate 18 in water and is a potent inhibitor of mammalian ADA (IC_{50} 50 nM).

Adenosine deaminase (ADA) (EC 3.5.4.4) catalyses the hydrolytic deamination of adenosine (**1**) to inosine (**3**) probably via the tetrahedral high energy intermediate **2** (Scheme 1). The related enzyme, AMP deaminase (AMPDA) (EC 3.5.4.6), converts adenosine 5′-monophosphate (AMP) (**4**) into inosine 5′-monophosphate (**6**) via the phosphorylated intermediate **5** (Scheme 1). In man, a deficiency of ADA is the cause of a form of severe combined immunodeficiency disease,[1] whereas AMPDA deficiency appears to be much less serious and in some cases may even be beneficial.[2] In contrast, plants do not appear to contain ADA and inhibition of AMPDA results in a strong herbicidal effect.[3]

A comparison of the amino acid sequences of ADA and AMPDA indicates that the aglycone binding pocket of both enzymes is highly conserved.[4] In agreement with this observation, nucleoside-based inhibitors of ADA also tend to be inhibitors of AMPDA following 5′-monophosphorylation to give the corresponding nucleotides.[5] For example,

Scheme 1



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(3) Dancer, J. E.; Hughes, R. G.; Lindell, S. D. *Plant Physiol*. **1997**, *114*, 119.

(4) Merkler, D. J.; Kline, P. C.; Weiss, P.; Schramm, V. L. *Biochemistry* **1993**, *32*, 12993. Merkler, D. J.; Schramm, V. L. *Biochemistry* **1993**, *32*, 5792.

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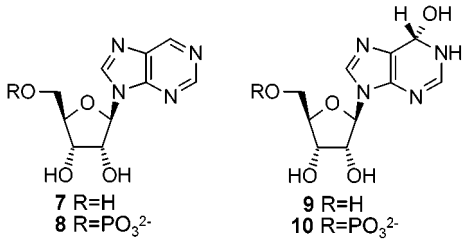
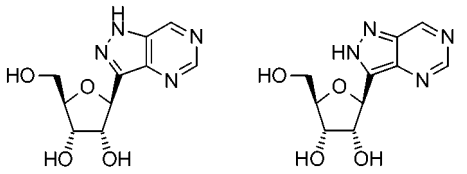
the nucleoside nebularine (**7**) is an inhibitor of ADA (K_i 4 μM,[5] 16 μM[6]) while the corresponding nucleotide **8** is a selective AMPDA inhibitor (K_i 6.5 μM[5]). The crystal structure of nebularine (**7**) bound to ADA shows that it binds as the covalent hydrate **9**,[7] and it is highly likely that **8** also binds to AMPDA as a covalent hydrate **10**.[4] The inhibitors **9** and **10** are mimics of the high energy tetrahedral intermediates **2** and **5** which are unable to undergo the normal forward reaction. However, covalent hydrates such as **9** and **10** require considerable stabilization by the enzyme in order to exist in anything more than trace amounts. Consequently,





ribosides containing aglycones which per se form more stable hydrates are likely to be stronger inhibitors.[6] In this Letter we report the use of calculated covalent hydrate stability, in combination with modeling into the ADA crystal structure, to design new *C*-nucleoside-based inhibitors of ADA. *C*-Nucleosides were chosen because of the improved biological efficacy associated with their stability to nucleosidases and phosphorylases. It was hoped that the compounds would undergo in vivo 5′-phosphorylation to give herbicidal *C*-nucleotidal AMPDA inhibitors.[3,8]

The starting point for our present work was the *C*-nucleoside, deaminoformycin (**11**), which we have previously shown to be a good ADA inhibitor (IC_{50} 5 μM)[8] and which under our test conditions bound 18 times more strongly than nebularine (**7**; IC_{50} 90 μM).[9] In an attempt to try and understand this result we calculated the enthalpy of covalent hydration for nebularine (**7**) and the *N*(1)H and *N*(2)H tautomers of deaminoformycin **11** and **11a**.[10] In addition, the three covalent hydrates were modeled into the active site of ADA and their binding energies, relative to the known ligand **9**, were calculated.[11] Hydration of the less stable *N*(2)H tautomer **11a** was found to be 3.8 kcal mol^{-1} more favorable than hydration of the *N*(1)H tautomer **11** and 5.4 kcal mol^{-1} more favorable than hydration of nebularine (**7**) (Table 1).

Table 1. Calculated and Experimental Parameters Relating to Covalent Aglycone Hydration and ADA Inhibition

compd no.	calcd ΔH(hydration),[10] kcal mol^{-1}	% covalent hydration in D_2O	calcd BE[11] of hydrate to ADA,[a] kcal mol^{-1}	measd IC_{50}(ADA),[9] μM
7[b]	7.1	0	0	90
11	5.5	0	4.5	5
11a	1.7		1.6	
12	−1.6		−1.9	
13	2.0	0	29.9	40
14	−4.3		6.7	
15[c]	−1.7	45	72.0	180
16[d]	−2.1	90	6.2	0.05

[a] Relative to compound **9**. [b] Covalent hydrate is compound **9**. [c] Covalent hydrate is compound **17**. [d] Covalent hydrate is compound **18**.

The covalent hydrate of tautomer **11a** was also predicted to bind more tightly to ADA than the hydrate of tautomer **11** by 2.9 kcal mol^{-1}. These data suggest that the *N*(2)H tautomer **11a** is responsible for the observed enhancement in ADA inhibition even though deaminoformycin normally exists almost exclusively as the *N*(1)H tautomer **11**.[12] If this hypothesis is correct, then nucleosides containing nontautomeric aglycone heterocycles with electronic properties similar to those of tautomer **11a** should be more potent inhibitors of ADA than deaminoformycin (**11**). To test this proposal we calculated the enthalpy of hydration, and the relative binding energy of the equivalent covalent hydrates to ADA, for a number of heterocycles including the imidazotriazine **12** and the triazolotriazine **14**. The calculations predicted that both of these compounds should react exothermically with water to give stable covalent hydrates (Table 1).[10] The calculated binding energy for the covalent hydrate derived from **12** was slightly more negative than that for ligand **9** while that for the hydrate derived from **14** was 6.7 kcal mol^{-1} more positive (Table 1).[11] However, the large difference in the ΔH(hydration) values means that overall the binding of the covalent hydrates derived from both **12**

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(6) Shewach, D. S.; Krawczyk, S. H.; Acevedo, O. L.; Townsend, L. B. *Biochem. Pharmacol.* **1992**, *44*, 1697.
(7) Wang, Z.; Quiocho, F. A. *Biochemistry* **1998**, *37*, 8314. Wilson, D. K.; Rudolph, F. N.; Quiocho, F. A. *Science* **1991**, *252*, 1278.
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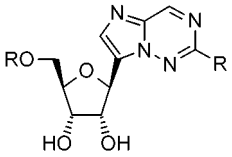
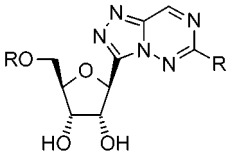
(9) Experimental details describing our ADA assay procedure can be found in ref 3. The enzyme source was calf intestinal mucosa (Sigma). Adenosine exhibited a K_m value of 35 μM and its assay concentration was 60 μM.
(10) **Enthalpy of Hydration Calculations:** Calculations were performed using the AM1 Hamiltonian in VAMP (Clark, T.; Alex, A.; Beck, B.; Chandrasekhar, J.; Gedeck, P.; Horn, A.; Hutter, M.; Rauhut, G.; Sauer, W.; Steinke, T. VAMP-version 7.0, Erlangen, 1998, distributed by Oxford Molecular Limited) on a model system in which a methyl group replaced the sugar ring. An aqueous environment was simulated using the SCRF method (Rauhut, G.; Clark, T.; Steinke, T. *J. Am. Chem. Soc.* **1993**, *115*, 9174). The enthalpies of hydration were calculated from the individual heats of formation of the parent aglycones and the covalent hydrates (ΔH(hydration) = ΔH_f(hydrate) − (ΔH_f(aglycone) + ΔH_f(water)). This format is superior to the $\Delta\Delta H$ format used in our earlier work ($\Delta\Delta H = \Delta H_f$(hydrate) − ΔH_f(aglycone): ref 8 and Le, V.-D. Ph.D. Thesis, University of London, 1997, p 26) as it makes it easier to see whether the hydration reactions are likely to be exo- or endothermic. No entropic or explicit hydrogen bonding factors are taken into account. The absolute values of the enthalpy of hydration are consequently only approximate, but are adequate for comparisons of similar structures (Table 1). For related calculations carried out by other workers, see: Erion, M. D.; Reddy, M. R. *J. Am. Chem. Soc.* **1998**, *120*, 3295.

and **14** were predicted to be 10.6 and 4.7 kcal mol^{-1}, respectively, more favorable than that for the hydrate **9**.

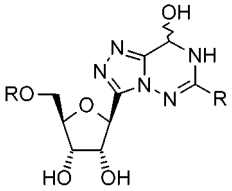
Unfortunately, the target molecules **12** and **14** proved difficult to synthesize, though we were able to achieve the related 6-substituted analogues **13**[13] and **15**.[14] The calculated enthalpy of hydration for compound **13** was similar to that for tautomer **11a**, while that for compound **15** was more negative, and indeed exothermic (Table 1).[10] In close agreement with these calculations, the triazolotriazine **15** exists to the extent of 45% as the covalent hydrate **17** in aqueous solution.[14] No covalent hydration of nebularine (**7**),



12 R=H
13 R=SMe



14 R=H
15 R=NMe_2
16 R=NH_2



17 R=NMe_2
18 R=NH_2

deaminoformycin (**11**), or the imidazotriazine **13** could be detected under similar conditions (D_2O, pH 7). Molecular modeling calculations indicated that the presence of the 6-substituents in compounds **13** and **15** would probably have a large negative effect on the binding affinity to ADA, to the extent that the 6-dimethylamino compound **15** would not be expected to bind at all (Table 1).[11] In the event, however, both **13** and **15** are ADA inhibitors (IC_{50} 40 and 180 μM, respectively), presumably because the 6-substitutent is able to displace one of the two water molecules bound within this region of the enzyme.[7,11] Nonetheless, it was clear that the 6-SMe group in **13** and the 6-NMe_2 group in **15** were too large for optimal binding to ADA and that smaller substituents were required. Because of its more negative enthalpy of hydration, we decided to concentrate our synthetic efforts on the triazolotriazine system. Since we were unable to synthesize the 6-unsubstituted compound **14**, we selected the 6-amino compound **16** as a new target. Calculations indicated that **16** should form the stable covalent hydrate **18**, which was predicted to bind by 3.0 kcal mol^{-1} more strongly to ADA than ligand **9** (Table 1).[10,11] A schematic representation of **18** bound at the ADA active site

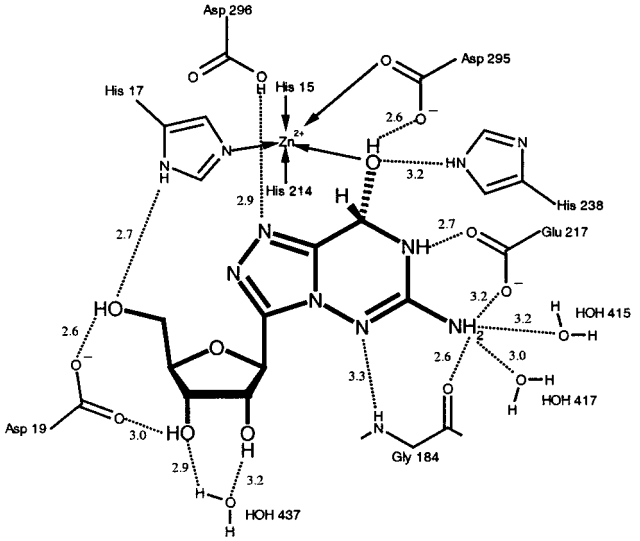


Figure 1. Proposed binding mode of the covalent hydrate **18** to ADA. H bonds are shown as dashed lines, and the distances between H bond donor and acceptor atoms are given in angstroms. Arrows indicate coordination bonds to the zinc cation. Numbering scheme of amino acids and water molecules refers to PDB 2ADA.

and showing the most important interactions is shown in Figure 1. Of particular interest is the observation that, in contrast to the 6-NMe_2 group, the 6-NH_2 group does not interact unfavorably with the two water molecules bound in

(11) **ADA Binding Energy Calculations:** Force-field calculations were performed using an augmented AMBER force field (Weiner, S. J.; Kollman, P. A.; Case, D. A.; Singh, U. C.; Ghio, C.; Alagona, G.; Profeta, S.; Weiner, P. *J. Am. Chem. Soc.* **1984**, *106*, 765). AMBER standard atomic charges were assigned to the amino acids of the enzyme. Gasteiger's method (Gasteiger, J.; Marsili, M. *Tetrahedron* **1980**, *36*, 3219) was used to compute approximate atomic charges for the ligands. The structure of the binding site of ADA[7] was taken from the Protein Data Bank (entry 2ADA; Berman H. M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat T. N.; Weissig, H.; Shindyalov I. N.; Bourne P. E. *Nucl. Acids Res.* **2000**, *28*, 235). Only those residues (including amino acids, water molecules and the zinc ion) with at least one atom within 8 Å of any atom of the complexed ligand 6-hydroxy-1,6-dihydropurine ribonucleoside (**9**) were included in the calculations. The geometries of the covalent hydrates derived from structures **11**−**16** were fully optimised by force-field methods before being docked into the binding site, which had previously been occupied by compound **9**. The optimizations of the enzyme−ligand complexes were performed in dihedral space by permitting rotation of specified bonds and free translation and rotation of specified molecules in the system. The specified molecules were the docked ligand and the water molecules HOH 402, 415, 417, 437, and 438 (using the numbering in the PDB file 2ADA). The specified bonds were all of the rotatable single bonds in the corresponding ligand as well as those of the side chains of amino acids Asp 19, Leu 58, Phe 61, Phe 65, Ser 103, Leu 106, Met 155, Ala 183, His 214, Glu 217, His 238, Asp 295, and Cys 514. The X-ray structure served as a reference geometry to compute conformational energies due to geometry changes of the enzyme upon binding of a ligand. Conformational energies of the ligands were determined relative to their fully optimized geometries. The interaction energy was calculated as the sum of the van der Waals and electrostatic interactions between a ligand and the enzyme. Water molecules were treated as part of the enzyme. The binding energies (BE) were then computed by adding together the interaction energy, the conformational energy of the enzyme, and the conformational energy of the ligand. Solvation effects and entropic contributions were not taken into account. The resulting BEs are only an approximate measure of a ligand's binding affinity and cannot be directly compared to the free energy of binding, but the difference between the BEs of any two ligands indicates which should bind most strongly. Therefore, the results are expressed as a relative binding energy which was calculated by subtracting the BE for compound **9** from the BE for each specific compound (Table 1).

(12) Chenon, M.-T.; Panzica, R. P.; Smith, J. C.; Pugmire, R. J.; Grant, D. M.; Townsend, L. B. *J. Am. Chem. Soc.* **1976**, *98*, 4736.

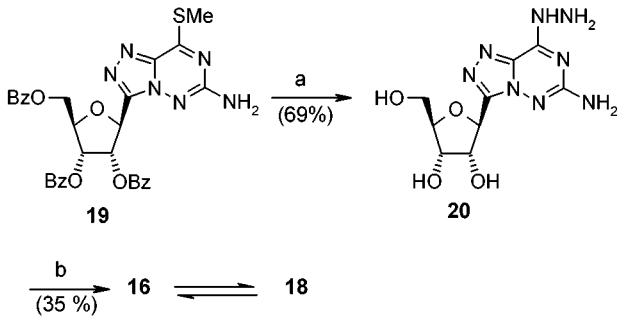
(13) Dudfield, P. J.; Le, V.-D.; Lindell, S. D.; Rees, C. W. *J. Chem. Soc., Perkin Trans. 1* **1999**, 2929.

(14) Dudfield, P. J.; Le, V.-D.; Lindell, S. D.; Rees, C. W. *J. Chem. Soc., Perkin Trans. 1* **1999**, 2937.

this area of the active site but rather is able to form hydrogen bonds with both. Other interactions are very similar to those previously seen with ligand **9**.[7]

The 6-amino target **16** was eventually prepared in just two steps from the known intermediate **19**[15] as shown in Scheme 2.[16] Treatment of **19** with excess hydrazine hydrate in refluxing ethanol effected displacement of the 8-SMe group together with removal of the benzoyl protecting groups to give the hydrazine **20** in 69% yield. Subsequent oxidation with yellow mercuric oxide in ethanol/DMF at 100 °C yielded the 8-unsubstituted target compound **16** in 35% yield.[16] This substance exists to the extent of 90% as the covalent hydrate **18** as assessed by ^{1}H NMR spectroscopy in deuterium oxide, an observation which is consistent with the calculated exothermic ΔH(hydration) value. More importantly, biochemical testing indicated that the mixture of **16** and **18** was a potent inhibitor of ADA (IC_{50} 50 nM),[9] binding 1800 and 100 times more strongly than nebularine (**7**) and deaminoformycin (**11**), respectively. Subsequent biological testing of the **16**/**18** mixture showed that it possessed postemergence herbicidal activity at rates of 320 g ha^{-1} and below, depending on the species. We believe that this activity results from in vivo phosphorylation to the corresponding 5′-monophosphate, which we would expect to be a potent inhibitor of AMPDA. The herbicidal nucleosides deaminoformycin (**11**)[8] and carbocyclic coformycin[3] have been shown to exert their biological activity via such a mechanism. However, the comparative instability of **16**/**18** has unfortunately prevented experimental confirmation of this hypothesis in the current case.

Scheme 2[a]



[a] Reagents: (a) 30 equiv of $NH_2NH_2.H_2O$, EtOH, Δ, 2 h; (b) 5 equiv of HgO, EtOH:DMF 2:5, 100 °C, 4 h.

In conclusion, calculations of the enthalpy of covalent aglycone hydration and of binding energy to the ADA crystal structure have been used to design new inhibitors of ADA. Potent inhibition is achieved by ribosides that contain easily hydrated 8-unsubstituted aglycones with small substituents at C-6. Covalent hydration is favored in aglycones with reduced aromatic character and which contain additional electron-withdrawing heteroatoms. This work culminated in the synthesis of the triazolotriazine **16**, which under aqueous conditions exists predominantly as the covalent hydrate **18** and is a potent inhibitor of mammalian ADA (IC_{50} 50nM).

Acknowledgment. We wish to thank Dr. B. Laber and Ms. T. Monk for performing the ADA enzyme assays and Dr. T. Auler for the biological testing results.

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(16) New compounds were characterized by 300 MHz ^{1}H NMR spectroscopy, electrospray mass spectrometry, and analytical HPLC. Spectral data for **16**/**18** are as follows: ^{1}H NMR (D_2O) δ 9.37 (0.1H, s, H-8 of **16**), 6.33 (0.9H, d, J = 2.5 Hz, H-8 of **18**), 5.41 (0.1H, d, J = 6.2 Hz, H-1′of **16**), 5.16 (0.9H, dd, J = 2.5 and 6.5 Hz, H-1′of **18**), 4.74 (1H, m, H-2′), 4.40 (0.1H, t, J = 6 Hz, H-3′ of **16**), 4.35 (0.9H, m, H-3′ of **18**), 4.17 (1H, m, H-4′), 3.68−3.85 (2H, m, H-5′); *m*/*z* (electrospray) 287 (M + H of **18**, 100%), 269 (M + H of **16**, 20%).