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The Design and Synthesis of Inhibitors of Adenosine 5'-Monophosphate Deaminase

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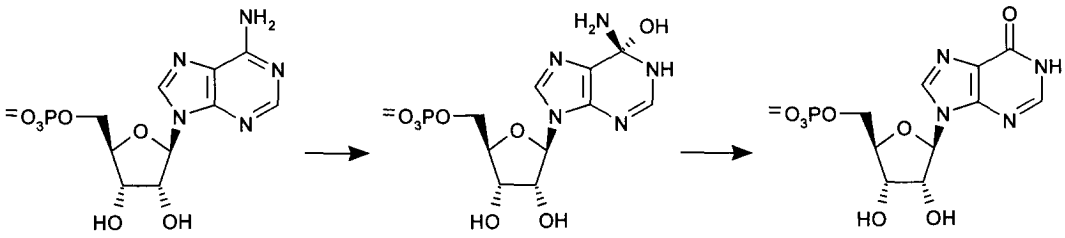
Abstract: Carbocylic coformycin (**4**) is a potent herbicide whose primary mode of action involves inhibition of adenosine 5'-monophosphate deaminase (AMPDA) following phosphorylation of the 5'-hydroxyl group *in vivo*. The search for more stable and accessible structures led to the synthesis of carbocyclic nebularine (**8**) and deaminoformycin (**10**). The latter compound is a good herbicide and its corresponding 5'-monophosphate **14** is a strong inhibitor of plant AMPDA (IC_{50} 100 nM).

Keywords: Enzyme inhibitors; Herbicides; Nucleosides; Nucleotides

Adenosine 5'-monophosphate (AMP) deaminase (AMPDA, EC 3.5.4.6) catalyses the hydrolytic deamination of AMP (**1**) to inosine 5'-monophosphate (**3**) *via* the tetrahedral intermediate **2** (Scheme 1).[1] The enzyme plays a key role in maintaining the relative concentrations of the adenylate nucleotides AMP, ADP and ATP *in vivo*.

Scheme 1



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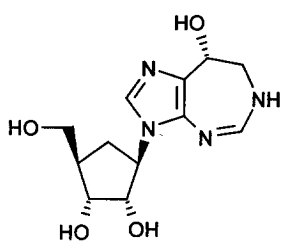
This relationship, which is quantitatively expressed as the adenylate energy charge, is of great importance in the regulation of almost every aspect of metabolism.[2] Perturbation of the intracellular ATP pool in plants through inhibition of AMPDA has recently been shown to result in a strong herbicidal effect.[3] This communication describes the results of some of our efforts to exploit this new herbicidal mode of action through the design and synthesis of novel inhibitors of AMPDA.[4]

Carbocyclic coformycin (**4**) is a naturally occurring nucleoside which exhibits strong herbicidal activity.[5] It has recently been shown that the primary herbicidal mode of action of compound **4** is due to inhibition of AMPDA following phosphorylation of the 5'-hydroxyl group.[3] Our initial attempts to find new inhibitors of AMPDA concentrated on the synthesis of analogues of carbocyclic coformycin (**4**).[6] However, this molecule is synthetically difficult to work with (due to the instability of the diazepine ring) and even close analogues were significantly less active. For these reasons we began to search for more stable and accessible structures and our attention was drawn to nebularine 5'-monophosphate (**5**) which is reported to be a good inhibitor of AMPDA (K_i 6.5μM, rabbit muscle enzyme).[7] It is believed that compound **5** binds to AMPDA as the covalent hydrate **6** which is a mimic of the tetrahedral reaction intermediate **2** (or the transition states leading to or from it).[1] Nebularine itself is not herbicidal, possibly due to a rapid enzymatic cleavage of the labile glycosyl bond.[8] Thus, in an effort to improve *in vivo* stability we identified the carbocyclic analogue **8** and the C-nucleoside **10** as potential new herbicides (Scheme 2). Compound **8** is closely related to aristeromycin (**7**) and compound **10** to formycin A (**9**), both of which were known to be enzymatically phosphorylated to give 5'-monophosphates which were efficiently deaminated by AMPDA.[9,10] These observations provided experimental support for our proposal that compounds **8** and **10** would be phosphorylated *in vivo* and would be able to undergo covalent hydration at the AMPDA active site to give the required inhibitory species **12** and **13**, respectively.



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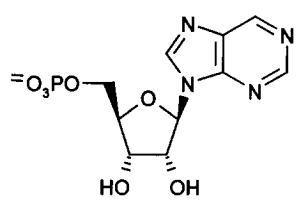

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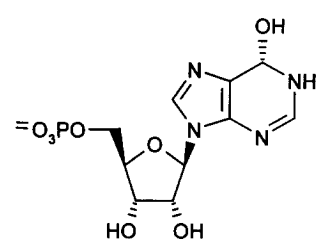
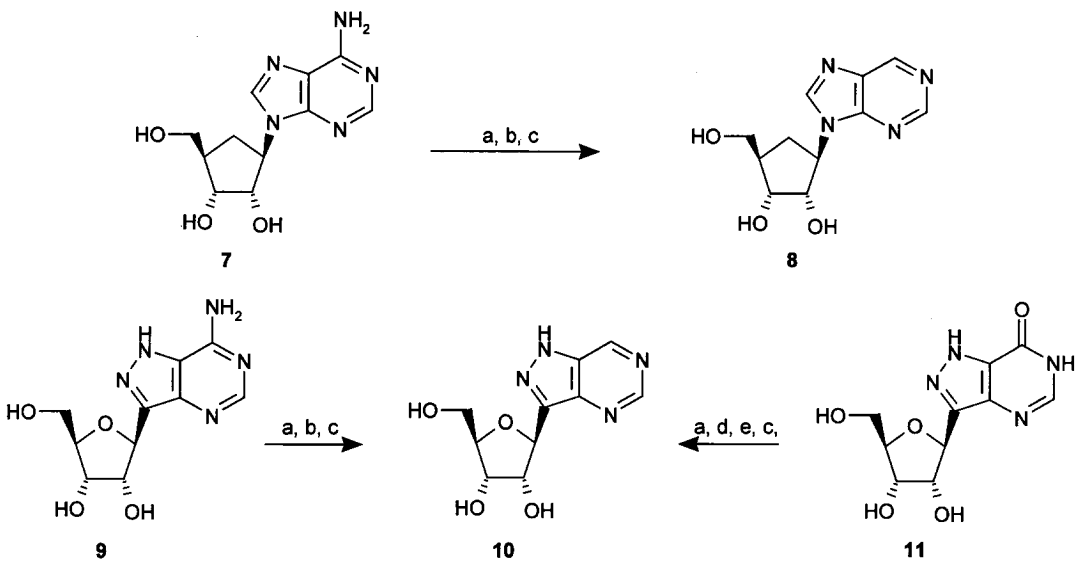


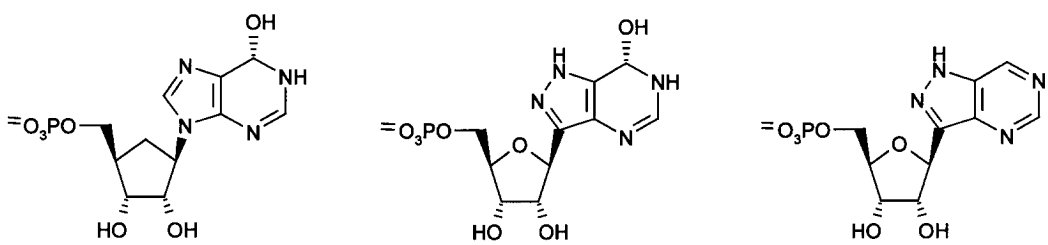
6

The synthesis of the target nucleosides **8** and **10** was achieved in three steps starting from the natural products aristeromycin (**7**) and formycin A (**9**) by adapting the procedure used by Nair and Chamberlain in their synthesis of nebularine (Scheme 2).[11,12] This route furnished carbocyclic nebularine (**8**) and deaminoformycin (**10**) in an overall yield of 61% and 42%, respectively. Compound **10** was also prepared in four steps and in 38% overall yield from formycin B (**11**) *via* a similar route to that previously described by Townsend and coworkers (Scheme 2). [11,13]

Scheme 2



Reagents: (a) 12 eq. Ac_2O, Py, 0°C, 1h then r.t., 5 h. Evaporate repeatedly from EtOH; (b) 7 eq. n-BuONO, THF, 50°C, 24 h; (c) NH_3 in EtOH (sat.), r.t., 4 days; (d) 0.2 M soln. in $POCl_3$, Δ, 0.5 h; (e) H_2 (I atm.), Pd/C, 2.5 eq MgO, EtOAc, 4 days.



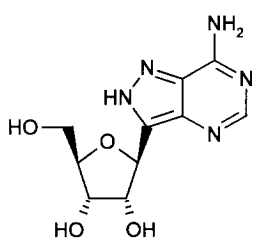
Subsequent biological testing indicated that carbocyclic nebularine (**8**) was essentially inactive whereas deaminoformycin (**10**) exhibited strong herbicidal properties. As expected neither of these nucleosides inhibited AMPDA and 5'-monophosphorylation of compound **8** also gave only a poor inhibitor. However, deaminoformycin 5'-monophosphate (**14**), obtained after phosphorylation of compound **10**, was an encouragingly potent inhibitor of AMPDA (IC_{50} 70nM, rabbit muscle enzyme; IC_{50} 100nM, pea enzyme),[14] which bound approximately two orders of magnitude more strongly than the isomeric inhibitor **5** (K_i 6.5 μM, rabbit muscle enzyme).[7] This result is consistent with inhibition of AMPDA being responsible for the

herbicidal activity of deaminoformycin (**10**) following 5'-monophosphorylation *in vivo* to give the nucleotide **14**. Additional evidence for this mode of action was provided by the results of transpiration feeding studies conducted with excised pea seedlings. In the presence of compound **10** a rapid and dramatic increase in ATP levels was observed (Table 1). A similar increase in ATP levels was previously observed in analogous feeding studies conducted with carbocyclic coformycin (**4**).[3] Mammalian erythrocytes deficient in AMPDA have also been reported to exhibit increased cellular ATP concentrations.[15] Increased AMP concentrations were not observed, presumably because any excess AMP was rapidly converted to ATP as the cell sought to stabilize its intracellular adenylate energy charge.[2]

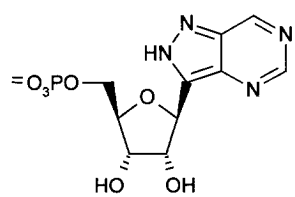
Table 1

Results of Transpiration Feeding Studies Conducted with Deaminoformycin (**10**).		
	ATP (nmol/g frozen weight)	
	4 hours	24 hours
Treated	450	693
	423	693
Control	253	213
	267	226

Method: 14-day-old pea seedlings were transpiration fed with either 100μM compound **10** in aqueous solution or water as control. At the times shown, the seedlings were rapidly frozen in liquid nitrogen and extracted in 0.4 M $HClO_4$. ATP was determined using a luciferase based assay. Each data point represents an individual seedling. For full description of method see reference 3.



9a



14a

The active sites of AMPDA and the closely related enzyme, adenosine deaminase (ADA), appear to be highly conserved.[1] Formycin A (**9**) is a substrate for ADA and is believed to bind and react in the less stable N8-H tautomeric form **9a**.[16] Based on the analogy between AMPDA and ADA, it seems reasonable to propose that deaminoformycin 5'-monophosphate (**14**) may also bind in the N8-H tautomeric form **14a** to AMPDA. Calculations of the heat of formation differences between the N8-H and N7-H tautomers of **14** and their corresponding hydrates using MOPAC[17] indicate that the N8-H tautomer **14a** forms a considerably more stable covalent hydrate than the N7-H tautomer **14** or than nebularine 5'-monophosphate (**5**).[18] This may explain why deaminoformycin 5'-monophosphate (**14**) binds to AMPDA more strongly than the isomeric compound **5** and should enable the rational design of more potent AMPDA inhibitors in the future.[19]

In conclusion, carbocyclic nebularine (**8**) and deaminoformycin (**10**) have been synthesized as potential herbicides. The latter compound exhibits good herbicidal activity which we propose is due to inhibition of AMPDA by the covalent hydrate of the corresponding 5'-monophosphate **14** (IC_{50} 100nM, pea enzyme).

Acknowledgements

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References and Notes

[1] Merkler, D.J.; Kline, P.C.; Weiss, P.; Schramm, V.L. *Biochemistry,* **1993**, *32*, 12993. Merkler, D.J.; Schramm, V.L. *ibid.,* 5792.

[2] Chapman, A.G.; Atkinson, D.E. *J. Biol. Chem.,* **1973**, *248*, 8309. Pradet, A.; Raymond, P. *Ann. Rev. Plant Physiol.,* **1983**, *34*, 199.

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

[4] This work represents part of an ongoing interest within AgrEvo in using mechanism-based biochemical reasoning to generate leads for the disccovery of new agrochemicals. For other recent examples see Dancer, J.E.; Ford, M.J.; Hamilton, K.; Kilkelly, M.; Lindell S.D.; O'Mahony, M.J.; Saville-Stones, E.A. *Bioorg. Med. Chem. Lett.,* **1996**, *6*, 2131. Lindell, S.D.; Earnshaw, C.G.; Wright, B.J.; Carver, D.S.; O'Mahony, M.J.; Saville-Stones, E.A. *ibid.,* 547 and references cited therein.

[5] Bush, B.D.; Fitchett, G.V.; Gates, D.A.; Langley, D. *Phytochemistry*, **1993**, *32*, 737.

[6] Saville-Stones, E.A.; Turner, R.M.; Lindell, S.D.; Jennings, N.S.; Head, J.C.; Carver, D.S. *Tetrahedron,* **1994**, *50*, 6695.

[7] Frieden, C.; Kurz, L.C.; Gilbert, H.R. *Biochemistry,* **1980**, *19*, 5303.

[8] Nuclesides are well known to be rapidly degraded *in vivo* by various nucleosidases and phosphorylases providing the *raison d'être* for much of the current interest in the synthesis of carbocyclic- and C-nucleosides. For reviews see: Crimmins, M.T. *Tetrahedron*, **1998**, *54*, 9229 and Shaban, M. A. E. *Adv. Heterocycl. Chem.*, **1998**, *70*, 163. In this context it is also of interest to note that carbocyclic coformycin (**4**) is over 100 times more potent as a herbicide than coformycin even though the 5'-monophosphate derivative of **4** is a less potent inhibitor of AMPDA than coformycin 5'-monophosphate.[3,7]

[9] Bennett, L.L,; Allan, P.W.; Rose, L.M.; Comber, R.N.; Secrist, J.A. *Molecular Pharmacology,* **1986**, *29*, 383.

[10] Dye, F.J.; Rossomando, E.F. *Bioscience Reports,* **1982**, *2*, 229. Bzowska, A.; Shugar, D. *Z. Naturforschung,* **1989**, *44c*, 581.

[11] We wish to thank Glaxo Group Research, Greenford for supplying us with a sample of aristeromycin. Formycins A and B were purchased from the Sigma Chemical Company.

[12] Nair, V.; Chamberlain, S.D. *Synthesis,* **1984,** 401.

[13] Long, R.A.; Lewis, A.F.; Robins, R. K.; Townsend, L. B. *J. Chem. Soc.(C),* **1971**, 2443. Milne, G.H.; Townsend, L.B. *J. Chem. Soc. Perkin Trans. 1,* **1972**, 2677.

[14] The phosphate **14** (disodium salt) was synthesized in a single step from **10** by adapting the method of Bennett *et al.*[9] Details describing our AMPDA enzyme assay procedure can be found in reference 3. AMP had K_m values of 50 and 400μM and its concentrations were 100 and 640 μM in the rabbit muscle and pea enzyme assays, respectively. The unphosphorylated nucleoside **10** is a good inhibitor of the enzyme adenosine deaminase (IC_{50} 5μM, calf intestinal mucosa enzyme; K_m 35 μM, [adenosine]= 60 μM).

[15] Dale, G.L.; Norenberg, S.L. *Blood,* **1989**, *74,* 2157. Paglia, D.E.; Valentine, W.N.; Nakatani, M.; Brockway, R.A. *ibid.,* 2161.

[16] Orozco, M.; Canela, E.I.; Franco, R. *Molecular Pharmacology*, **1989**, *35*, 257.

[17] MOPAC 6.0 (QCPE 455): Stewart, J.J.P. *J. Comp.-Aided Mol. Des.,* **1990**, *4*, 1.

[18] Calculations were carried out using the AM1 Hamiltonian on isolated molecules in the gas phase using a model system in which the sugar ring was replaced by a methyl group. With these approximations hydration of **14a** is ca. 5.1 kcal mol^{-1} more favourable than hydration of **14** and 6.3 kcal mol^{-1} more favourable than hydration of nebularine 5'-monophosphate (**5**).

[19] A similar explanation has been proposed to account for the enhanced binding of 8-azapurine riboside (compared with nebularine) to the enzyme adenosine deaminase: Erion, M.D.; Reddy, M.R. *J. Am. Chem. Soc.*, **1998**, *120*, 3295. Shewach, D.S.; Krawczyk, S.H.; Acevedo, O.L.; Townsend, L.B. *Biochem. Pharmacol.*, **1992**, *44*, 1697.