

Synthesis of Inhibitors of Imidazole Glycerol Phosphate Dehydratase

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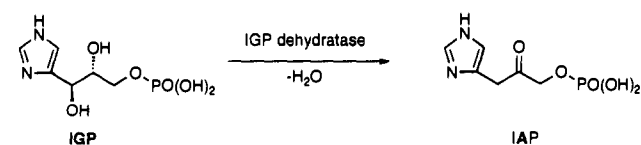
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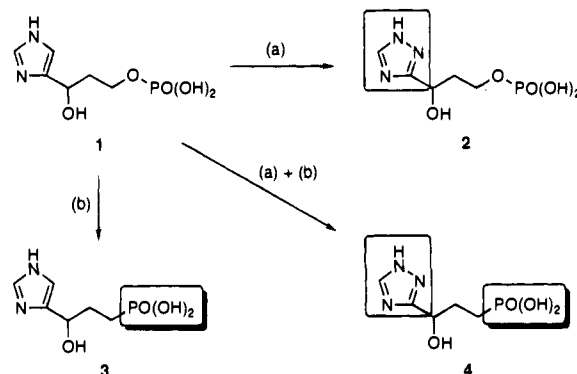
With recognition that chemical disruption of the biosynthesis of amino acids can lead to plant death, the enzymes from these pathways have become the target for rational design of inhibitors as potential herbicides.¹ Contrary to an increasing interest in the enzymes of biosynthesis of essential amino acids such as aromatic amino acids² and branched-chain amino acids,³ enzymes of histidine biosynthesis have drawn less attention until recently.⁴ Imidazole glycerol phosphate (IGP) dehydratase (EC 4.2.1.19) is an enzyme involved in the histidine biosynthesis pathway. It catalyzes the conversion of D-erythro-(2*R*,3*S*)-imidazole glycerol phosphate (IGP) to imidazole acetyl phosphate (IAP) (Scheme 1).^{4,5} This is a unique dehydration reaction characterized by the unusual mechanistic feature that the substrate requires no imine or carbonyl group α to the departing hydrogen.^{6,7} Since IGP dehydratase plays a key role in the biosynthesis of an essential amino acid, it is of interest to examine its inhibitors as potential herbicides.^{8,9} We now disclose the design and synthesis of inhibitors based on a plant IGP dehydratase isolated from wheat germ. During the course of our studies, Cox and his co-workers have reported a closely related work using recombinant yeast IGP dehydratase expressed in *Escherichia coli*.¹⁰

Although its high stereoselectivity⁷ and substrate specificity¹¹ have been reported, the mechanism of the IGP dehydratase

Scheme 1



Scheme 2



reaction is unknown. Due to the lack of detailed mechanistic studies, our inhibitor design was based on substrate analogues (Scheme 2). The simplest IGP analogue examined first was 2-deoxy-IGP (1), which showed a very weak inhibition ($\text{IC}_{50} = 2 \text{ mM}$) for the wheat IGP dehydratase ($K_m = 360 \mu\text{M}$).^{12,13} Next, we investigated the systematic replacement of the functional groups of 1 [(a) imidazole and (b) phosphate group] with their possible isosteric groups. Thus, replacement of the imidazole moiety of 1 with 1,2,4-triazole led to a remarkable (>60 times) enhancement of inhibitory activity (2; $\text{IC}_{50} = 32 \mu\text{M}$).¹⁴ On the other hand, since the phosphate moiety of 1 would be of scant utility for herbicides due to hydrolyzing phosphatases often encountered in plant tissue, it was replaced with the more stable phosphonate group.¹⁵ This structural change gave rise to a 5-fold increase of its inhibitory potency (3; $\text{IC}_{50} = 400 \mu\text{M}$). To our surprise, combination of both changes resulted in a remarkable synergetic effect in inhibitory enhancement (>15 000 times compared with 1) to give 4, a competitive inhibitor with high binding affinity ($\text{IC}_{50} = 130 \text{ nM}$; $K_i = 40 \pm 6.5 \text{ nM}$, $K_m/K_i = 9.0 \times 10^3$).¹⁶

In order to examine the effects of the C(2)-hydroxyl group, the *anti*- and *syn*-diols (5 and 6, Figure 1) were stereoselectively

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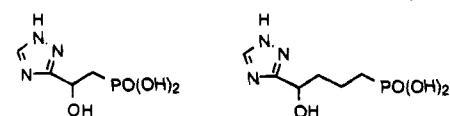
(12) IGP dehydratase was purified from wheat germ according to the methods described in ref 5b. The enzyme activity was measured by the method reported by Ames and Mitchell: Ames, B. N.; Mitchell, H. K. *J. Biol. Chem.* **1955**, *212*, 687. Thus, the assay system (for apparent K_i determination) consisted of 100 mM 2-mercaptoethanol, 1 mM MnCl_2 , 0.06–1 mM IGP, 2–5 milliunits of the enzyme in 0.25 mL of 50 mM bistris–propane–HCl buffer (pH 6.7), and an inhibitor. After incubation at 30 °C for 20 min, the formed IAP was dephosphorylated by alkaline phosphatase and quantified by measuring the absorption at 370 nm (the enol form of imidazoleacetyl) on addition of 5 N NaOH phosphate.

(13) Satisfactory elemental analyses or high-resolution mass spectral data were obtained for all new compounds.

(14) Among several N-containing heteroaromatic isosteres synthesized, 1,2,4-triazole was found to show the most enhanced binding to the enzyme.

(15) (a) Engel, R. *Chem. Rev.* **1977**, *77*, 349. (b) Blackburn, G. M. *Chem. Ind. (London)* **1981**, *7*, 134. (c) Myrvold, S.; Reimer, M.; Pompliano, D. L.; Frost, J. W. *J. Am. Chem. Soc.* **1989**, *111*, 1861. See also ref 2b.

(16) *Nor*- and homophosphonate derivatives (i and ii) were also synthesized and found to show much weaker inhibitory activities than 4



$\text{IC}_{50} = 380 \mu\text{M}$

45% inhibition at 0.1 mM

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(1) Kishore, G. M.; Shah, D. M. *Annu. Rev. Biochem.* **1988**, *57*, 627.

(2) For more recent studies, see: (a) Bartlett, P. A.; Nakagawa, Y.; Johnson, C. R.; Reich, S. H.; Luis, A. J. *Org. Chem.* **1988**, *53*, 3195. (b) Bender, S. L.; Widlanski, T.; Knowles, J. R. *Biochemistry* **1989**, *28*, 7560. (c) Montchamp, J.-L.; Pehler, L. T.; Frost, J. W. *J. Am. Chem. Soc.* **1992**, *114*, 4453. (d) Marzabadi, M. R.; Fond, J. L.; Gruys, K. J.; Pansegrau, P. D.; Sikorski, J. A. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 1435.

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(6) Walsh, C. *Enzymatic Reaction Mechanisms*; W. H. Freeman: San Francisco, CA, 1979.

(7) Moore, J. A.; Parker, A. R.; Davisson, V. J.; Schwab, J. M. *J. Am. Chem. Soc.* **1993**, *115*, 3338.

(8) Aminotriazole, a commercial herbicide (Amitrole), has been the only IGP dehydratase inhibitor ($\text{IC}_{50} = 20 \mu\text{M}$)¹ known so far. However, its herbicidal activity is not solely due to the IGP dehydratase inhibition: Heim, D. R.; Larrinua, I. M. *Plant Physiol.* **1989**, *91*, 1226.

(9) Patents related to this work: (a) Cox, J. M. EP 0078613, **1983**. (b) Mori, I.; Iwasaki, G.; Scheidegger, A.; Koizumi, S.; Hayakawa, K.; Mano, J. WO9219629, **1992**. (c) Hayakawa, K.; Mori, I.; Iwasaki, G.; Matsunaga, S. EP 528760, **1993**. (d) Cox, J. M.; Bellini, P.; Barrett, R.; Ellis, R. M.; Hawkes, T. R. WO9315610, **1993**. (e) Mori, I.; Matsunaga, S.; Kimura, Y. GB 2271113, **1994**.

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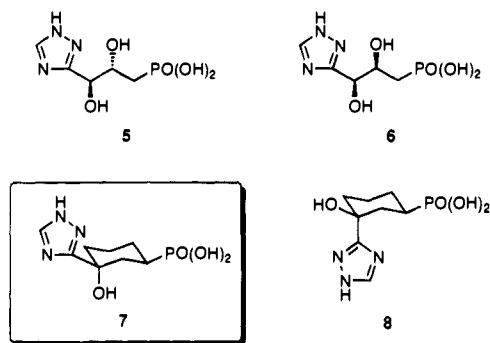
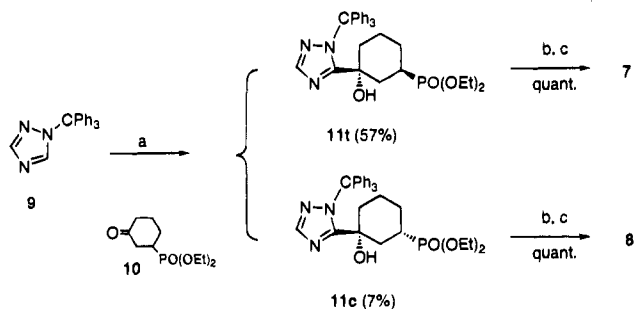


Figure 1.

Scheme 3



^a (a) *n*-BuLi, THF, $-78\text{ }^{\circ}\text{C}$, then **10**; (b) Me_3SiBr , CH_2Cl_2 ; (c) propylene oxide, MeOH.

synthesized via osmium-catalyzed dihydroxylation¹⁷ of the corresponding (*Z*)- and (*E*)-allylphosphonate diethyl esters. Although *syn*-**6** ($\text{IC}_{50} = 110\text{ nM}$) was a much better inhibitor than *anti*-**5** ($\text{IC}_{50} = 1.45\text{ }\mu\text{M}$), its inhibitory activity was only slightly improved compared to that of **4**. These results show that the introduction of the second C(2) hydroxy group does not dramatically boost the binding ability of inhibitors.

Further optimization of inhibitory activity of **4** was achieved by freezing its conformational flexibility. As the conformationally restricted analog of **4**, the cyclohexane derivatives (**7** and **8**) were synthesized as shown in Scheme 3. Addition of 5-lithio-1-trityl-1,2,4-triazole,¹⁸ prepared from 1-trityl-1,2,4-triazole (**9**) and *n*-BuLi, to diethyl 3-oxocyclohexylphosphonate (**10**) gave a 9:1 mixture of **11t** and **11c**. Deprotection of these adducts afforded *trans*- and *cis*-phosphonates **7** and **8**, respectively. The ¹H NMR analysis of **7** and **8** revealed that the phosphonic acid group is positioned *equatorially* in both isomers.¹⁹ While *cis*-**8** showed a markedly weaker inhibition for IGP dehydratase ($\text{IC}_{50} = 10\text{ }\mu\text{M}$), *trans*-**7** was a 4-fold better

(17) Van Rheenen, V.; Kelly, R. C.; Cha, D. Y. *Tetrahedron Lett.* **1976**, 1973.

(18) Anderson, D. K.; Sikorski, J. A.; Reitz, D. B.; Pilla, L. T. *J. Heterocycl. Chem.* **1986**, *23*, 1257.

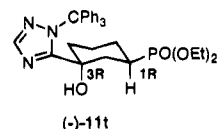


Figure 2.

inhibitor ($\text{IC}_{50} = 40\text{ nM}$, $K_i = 10 \pm 1.6\text{ nM}$) than **4**. This finding suggests that the inhibitory activity of **4** may be due to its *extended* conformer. The individual enantiomers of **7** could be obtained by HPLC separation of its precursor **11t** using a chiral column²⁰ followed by deprotection. The two enantiomers thus obtained showed remarkably different inhibition; (+)-**7** ($\text{IC}_{50} = 18\text{ nM}$) derived from (-)-**11t**, and (-)-**7** ($\text{IC}_{50} = 1800\text{ nM}$) derived from (+)-**11t**. The absolute configuration of (+)-**7** was determined by the X-ray analysis of its protected form (-)-**11t**, confirming that the potent inhibitor (+)-**7** has the (1*R*,3*R*)-configuration as shown in Figure 2.

Examination of the herbicidal activity of these inhibitors showed that **4** and **7** were slow-acting broad spectrum herbicides at postemergent application rates of 1–4 kg/ha.²¹ The triazole phosphonates developed in this study are a new class of substrate-based inhibitors of IGP dehydratase and show promising herbicidal activities. This is the first example of a rationally designed herbicide based on the inhibition of an enzyme involved in histidine biosynthesis, confirming the principle that disruption of histidine biosynthesis can lead to plant death.²²

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Supplementary Material Available: Full experimental procedures, including ¹H NMR spectral data, of compounds **4**, **5**, and **7**, ¹H NMR spectral data of compounds **1**, **2**, **6**, and **8** and crystallographic data of (-)-**11t** (8 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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(19) The assignment of the *equatorial* phosphonic acid group was based on the observation (¹H NMR) of two axial–axial proton couplings ($J = 13\text{ Hz}$ for both) for the proton α to the phosphonate group both in **7** and in **8**.

(20) A Chiralcel OD "dynamic axial compression" column obtained from Daicel was used (solvent: 2% 2-propanol in hexane). (-)-**11t**: $[\alpha]_D = -45.2^{\circ}$ (0.94 in chloroform). (+)-**11t**: $[\alpha]_D = +44.7^{\circ}$ (0.97 in chloroform). (+)-**7**: $[\alpha]_D = +10.3^{\circ}$ (0.94 in ethanol). (-)-**7**: $[\alpha]_D = -9.7^{\circ}$ (1.03 in ethanol).

(21) Biological studies using basil cell culture indicated that the cytotoxic effects of **4** and **7** are due to histidine starvation; Mori, I.; Fonné-Pfister, R.; Matsunaga, S.; Tada, S.; Kimura, Y.; Iwasaki, G.; Hatano, H.; Nakano, T.; Koizumi, S.; Hayakawa, K.; Ohta, D. *Plant Physiol.*, in press.

(22) A part of this paper was presented at the 8th International Congress of Pesticide Chemistry, Washington, DC, July 4–9, 1994.