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SYNTHESIS OF INHIBITORS OF IMIDAZOLE GLYCEROL PHOSPHATE DEHYDRATASE[1]

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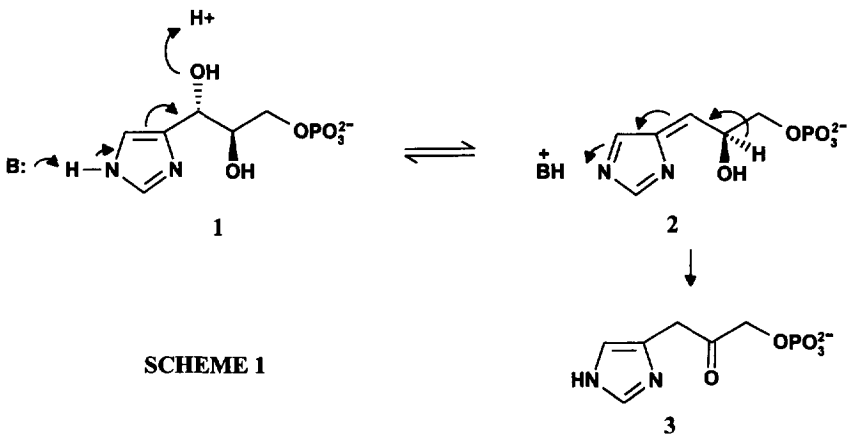
Abstract: Novel inhibitors of the newly discovered herbicide target enzyme imidazole glycerol phosphate dehydratase are described. The most potent inhibitor, compound **6** (IC_{50} < 1μM), arose by linking a 1,2,4-triazole and phosphonic acid *via* a five atom chain. The use of malonate as a phosphate equivalent also gave rise to inhibitors, the best being compound **12** (IC_{50} ~ 6μM).

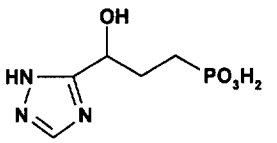
Inhibition of essential amino acid biosynthesis is now a well established mode of herbicidal action. Commercial herbicides have been developed which inhibit enzymes involved in the biosynthesis of aromatic amino acids and branched-chain amino acids.[2] Such a mode of action is attractive from a toxicological viewpoint since the enzymes involved in the biosynthesis of essential amino acids are not present in mammals. Consequently, agrochemical companies have expended some considerable effort in devising inhibitors of established and novel target enzymes. Most recently, several companies including ourselves,[3] Ciba-Geigy[4] and Zeneca[5] have become interested in inhibitors of imidazole glycerol phosphate dehydratase (IGPD) (EC 4.2.1.19) as potential herbicides, and possibly also as fungicides. IGPD is one of the enzymes in the biosynthetic pathway leading to histidine and converts imidazole glycerol phosphate (**1**) to imidazole acetol phosphate (**3**) probably *via* the diazafulvene intermediate **2** (Scheme 1).[6]



1 2 3

SCHEME 1

Currently, the most potent inhibitors of IGPD contain either a C- or N-linked triazole joined *via* a three carbon chain to a phosphonic acid, as exemplified by structures **4** (IC_{50} = 0.13μM; K_i = 40nM)[4] and **5** (K_i = 0.6nM).[5] It has been proposed[3,5] that these are transition state type inhibitors in which the triazole ring is mimicking the diazafulvene moiety in the reaction intermediate **2**. Less potent inhibitors are obtained when the chain linking the triazole and phosphonate is lengthened to four atoms, as found in the natural substrate.[3,4,5] It has been suggested[3,5] that the shortened chain in inhibitors **4** and **5** mimics a folded conformation of intermediate **2** in which the phosphate acts as an internal base abstracting the C-2 hydrogen to give the product.



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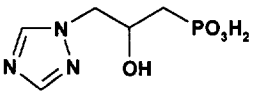

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In this communication we describe the results of recent studies into the design and synthesis of new inhibitors of IGPD. These have led to the discovery that use of a five atom linking chain (i.e. one atom longer than in the natural substrate **1**) can result in potent inhibitors of IGPD. In addition we have found that replacement of the phosphonate group with a malonate moiety can also give rise to inhibitors.

During our early work on IGPD inhibitors we had concentrated on compounds in which the heterocycle and phosphonate were joined *via* a three or four atom chain.[3] In our subsequent search for more diverse structures we have discovered that the phosphonate **6**, which possesses a five atom linker, is a good inhibitor of the yeast enzyme (IC_{50} < 1μM).[7] Molecular modelling studies show that this longer structure may prefer to adopt a folded conformation in which the phosphonate and triazole groups are separated by almost the same distance

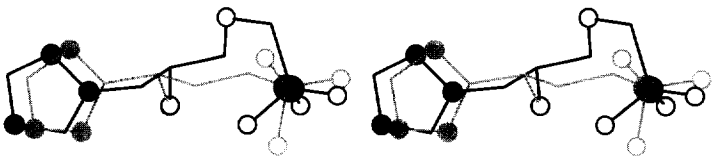
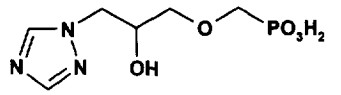
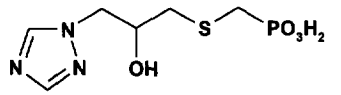
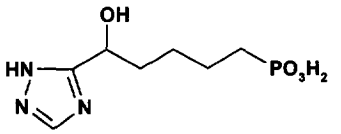
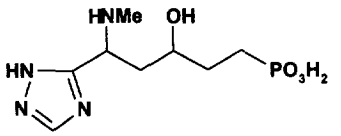
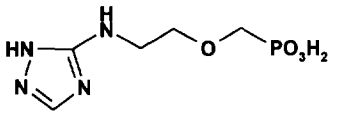


FIGURE 1: Comparison of the folded conformation of compound **6** (black) with compound **4** (grey)

as in the shorter compounds (Figure 1). Interestingly, the hydroxyl groups of **4** and **6** can also occupy very similar positions. Most importantly, compound **6** exhibits herbicidal properties very similar to those shown by **4** and **5**. Based on these results, a number of other compounds with five atom linkers between the triazole and phosphonate were made and tested for IGPD inhibition (Table 1).[7,8] The ether oxygen in the linker chain of compound **6** appears to be important for maintaining good enzyme inhibition. Thus, replacing this

TABLE 1: NOVEL IGPD INHIBITORS POSSESSING A FIVE ATOM LINKER

Compound[8]	Structure	IC_{50} (μM)[7]
6		< 1[a]
7		7
8		80
9[b]		10
10		5

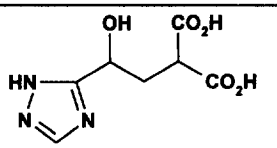
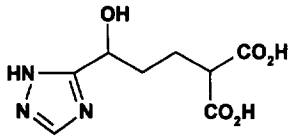
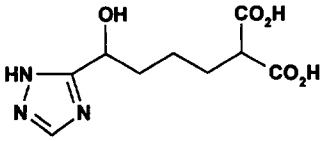
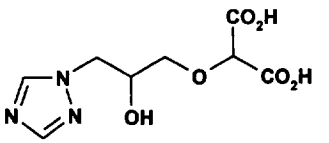
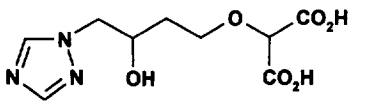
a - Compound **6** showed 80% inhibition at a concentration of 1μM[7]

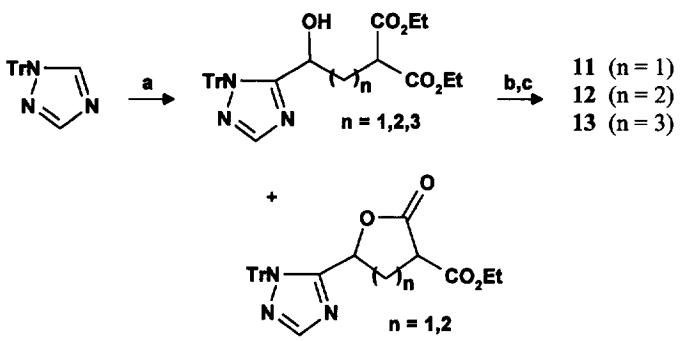
b - Compound **9** is a 3:1 mixture of *threo*:*erythro* diastereoisomers

oxygen with sulfur gave a less potent inhibitor **7** (IC_{50} ~ 7μM). Although the structural analogy is not exact, the results for compound **8** (IC_{50} ~ 80μM) suggest that replacement of the oxygen by carbon may be even more deleterious to binding. The amitrole derivative **10**, which is structurally close to our original proposals,[3] is a reasonable inhibitor of the enzyme (IC_{50} ~ 5μM), even though it does not possess a hydroxyl group on the linker.

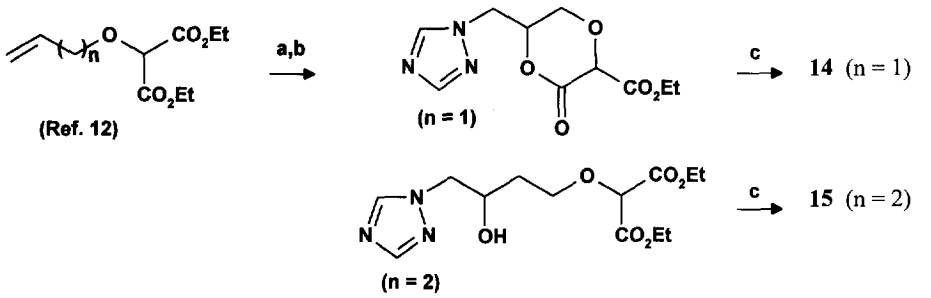
Concurrently with our studies on varying the chain length of inhibitors we have investigated the effect of replacing the phosphonate group with a different acid. Wiater *et al.* have shown that a number of dibasic acids, including oxalate, can bind at the IGPD phosphate binding site.[9] More recently, reports have appeared in which malonate has been used as a phosphate analogue in unrelated enzymes.[10] The use of a carboxylic diacid, like malonate, is attractive because carboxylate esters are more readily hydrolysed *in vivo* than phosphonate esters and hence offer more scope for the synthesis of propesticides. Consequently, we determined to try and substitute malonate for the phosphonate in our inhibitors. The required malonates **11-15** were synthesised as shown in Schemes 2 and 3 and tested for IGPD inhibition (Table 2).[7] In general the malonates were found to be less powerful inhibitors of IGPD than the phosphonates. However, in one case

TABLE 2: NOVEL IGPD INHIBITORS CONTAINING A MALONATE MOIETY

Compound	Structure	IC_{50} (μM)[7]
11		>500
12		6
13		>500
14		100
15		30



SCHEME 2: (a) (i) 1.1 eq. *n*-BuLi, THF, -78°C, 1h; (ii) 1 eq. $MgBr_2$, -78°C, 15 min; (iii) 1 eq. $OHC(CH_2)_n$-$CH(CO_2Et)_2$ (Ref. 11), -78°C, 2.5h. (b) 60% aq. CF_3CO_2H, DME, r.t., 1.5h. (c) (i) 2M NaOH, r.t., 40h; (ii) 'Amberlite' IR-120, H^+ form; (iii) 2 eq. LiOH. (Tr = triphenylmethyl).



SCHEME 3: (a) 1.5 eq. MCPBA, CH_2Cl_2, r.t., 18h. (b) 1 eq. 1,2,4-Triazole, 1 eq. K_2CO_3, MEK, Δ, 24h. (c) (i) 2M NaOH, r.t., 40h; (ii) 'Amberlite' IR-120, H^+ form; (iii) 2 eq. LiOH.

(compound **12**) a reasonably good inhibitor did result (IC_{50} ~ 6μM), which encouragingly also exhibited herbicidal activity at 3kg ha^{-1}. As with the phosphonates, the best malonate inhibitors result from joining the triazole and phosphate mimic *via* a 3 or 5 atom chain (compounds **12** and **15**).

In conclusion, we have demonstrated that potent inhibitors of IGPD can be made by linking a triazole and phosphonate moiety *via* a five atom chain. In addition we have shown that the phosphonate can be replaced with an alternative diacid functionality. These discoveries further expand the range of structures known to inhibit IGPD and offer new opportunities for the synthesis of novel inhibitors.

Acknowledgements

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References and Notes

1 This work represents part of an ongoing interest within AgrEvo in using biochemical reasoning to generate leads for the discovery of new agrochemicals. For other recent examples see Baillie, A.C.; Wright, K.; Wright, B.J.; Earnshaw, C.G. *Pest. Biochem. Physiol.* **1988**, *30*, 103. Schulz, A.; Spönemann, P.; Köcher, H.; Wengenmayer, F. *FEBS Lett.*, **1988**, *238*, 375. Lindell, S.D.; Turner, R.M. *Tetrahedron Letters* **1990**, *31*, 5381. Baillie, A.C.; Cornell, C.L.; Wright, B.J.; Wright, K. *ibid* **1992**, *33*, 5133. Harde, C.; Neff, K.H.; Nordhoff, E; Gerbling, K.P.; Laber, B; Pohlenz, H.D. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 273. Laber, B.; Gerbling, K.P.; Harde, C.; Neff, K.H.; Nordhoff, E.; Pohlenz, H.D. *Biochemistry* **1994**, *33*, 3413. Laber, B; Lindell, S.D.; Pohlenz, H.D. *Arch. Microbiol.* **1994**, *161*, 400.

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3 The results of our early work were presented at the Eighth International Congress of Pesticide Chemistry, Washington, DC, 4-9 July, **1994**. See Pillmoor, J.B.; Lindell, S.D.; Briggs, G.G.; Wright, K. in *Eighth International Congress of Pesticide Chemistry : Options 2000*, Ragsdale, N.N.; Kearney, P.C.; Plimmer, J.R., Eds. ACS Symposium Series, American Chemical Society, Washington, DC, **1995**, p 292. Patent: Wright, B.J.; Lindell, S.D.; Foster, S.G.; Milling, R.J. WO 95/14385, **1995**.

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7 Experimental details describing our enzyme assay conditions can be found in our patent. See reference 3. Imidazole glycerol phosphate (**1**) had K_m = 330μM and its concentration was 1mM. The enzyme source was recombinant *Saccharomyces cerevisiae* IGPD expressed in *Escherichia coli*.

8 Experimental details describing the preparation of phosphonates **6-10** can be found in our patent. See reference 3.

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11 The required aldehydes were prepared by alkylation of diethyl malonate with the appropriate halo-alkyl acetal (EtONa, EtOH, r.t.) followed by acid hydrolysis (5% aq. HCl, THF, r.t.).

12 The required alkenyl ethers were prepared by adapting the methodology described in Chouinard, P.M.; Bartlett, P.A. *J. Org. Chem.* **1986**, *51*, 75.

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