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Biorational design of herbicides: Synthesis of inhibitors of the PFP enzyme

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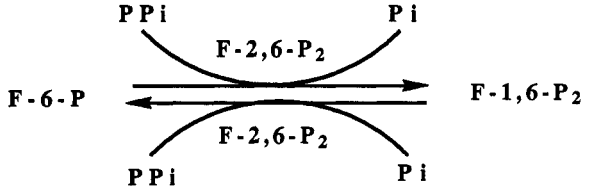
Abstract-Transition state and reaction coordinate analog inhibitors of the PFP enzyme were synthesized for the biorational design of herbicides. Some of the promising ones were scaled up and tested on whole plants.

Pyrophosphate fructose 6-phosphate 1-phosphotransferase (PFP), an enzyme that was discovered in bacteria(1), has been accorded a central role in plant carbohydrate metabolism. The enzyme catalyzes the conversion of inorganic pyrophosphate (PPi) and fructose 6-phosphate (Fru-6-P) to Fructose-1,6-bisphosphate (Fru-1,6-P_2) inorganic phosphate (Pi). The reaction is fully reversible and near equilibrium.



The enzyme is thought to serve different functions depending on the metabolic status of the cell or the environmental conditions of the system. When sucrose is actively consumed PFP can function to maintain sufficient PPi concentration(2). Under different metabolic conditions, for example when starch is mobilized, PFP may work in the opposite direction to consume PPi(3). It can also take over the role of adenylates in glycolysis under anaerobic conditions(4,5). PFP is activated by the regulatory metabolite Fructose 2,6-bisphosphate (Fru-2,6-P_2) (6).

The varied functions fulfilled by the enzyme suggest that in many different tissues and under a variety of circumstances, uninterrupted functioning of PFP is crucial for maintaining the metabolic balance of the cell. Disruption of its activity will likely lead to severe metabolic imbalance and ultimately death of the cell. Inhibition of this enzyme is therefore, a viable strategy in the biorational design of herbicides.

Purification of enzyme: Polyethylene glycol (PEG 8000) fractionation and medium pressure (FPLC) ion exchange chromatography yielded a 240 fold purified enzyme. The sample was free of enzyme activities which could interfere with the kinetic analysis of the PFP reaction.

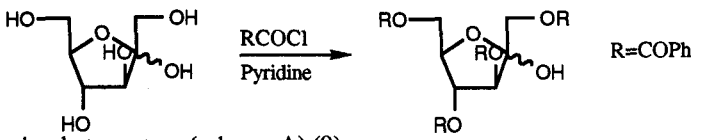
Reaction mechanism: Our results indicated that maize leaf PFP follows a ternary complex mechanism similar to the other PFPs studied(6). When the rate/[s] ([s]-concentration of the variable substrate) was plotted against [s] at different concentrations of the constant substrate, the series of lines intersected to the left of the y axis, rather than on the y axis itself. The second pattern would indicate a ping-pong or substituted enzyme mechanism(7). The reaction is likely to proceed through a PPi-Fru-6-P complex.

PFP Assay: The formation of Fru-1,6-BP (forward reaction) or Fru-6-P (reverse reaction) is coupled to the oxidation or reduction of pyridine nucleotides. The reaction is monitored at 340 nm. Inhibition data are calculated using a control assay with solvent only.

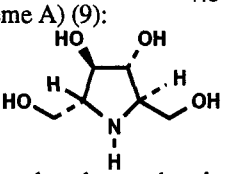
PFP Test: i) Compounds are dissolved in 80% acetone. ii) Final inhibitor concentration in the reaction mixture is adjusted to 50 ppm. iii) The effect is tested in both the forward and the reverse direction. 4) Active compounds are retested to determine the I, 50 value.

Synthesis of Transition State Analogs: Putative transition state and reaction coordinate analog inhibitors designed to mimic this structure were synthesized. Several analogs that impact "electron availability at the reactive site" were studied. The following strategies were generally pursued.

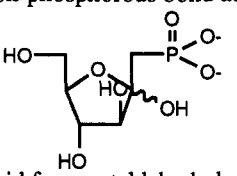
- Incorporation of steric hindrance: persubstituted derivatives of fructose with a good leaving group at the reactive site (8).



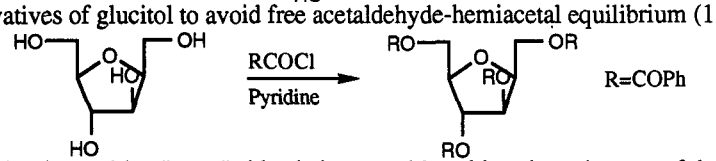
- Substitution of the ring hetero atom (scheme A) (9):



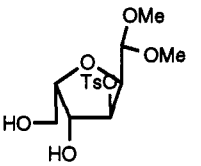
- Introduction of a non-hydrolyzable carbon-phosphorous bond at the reactive site (scheme B) (10):



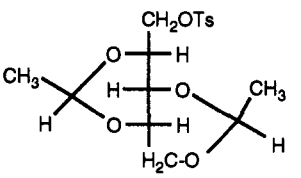
- Synthesis of derivatives of glucitol to avoid free acetaldehyde-hemiacetal equilibrium (11):



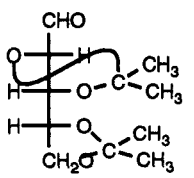
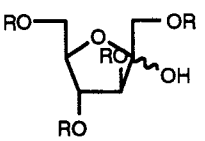
- Preparation of derivatives with a "trans" side chain to avoid anchimeric assistance of the -OH group (12):

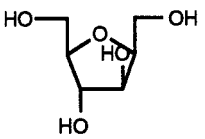
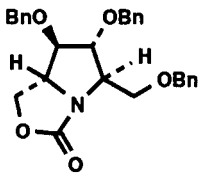
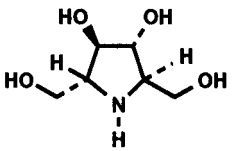


- Testing of open chain analogs (13):



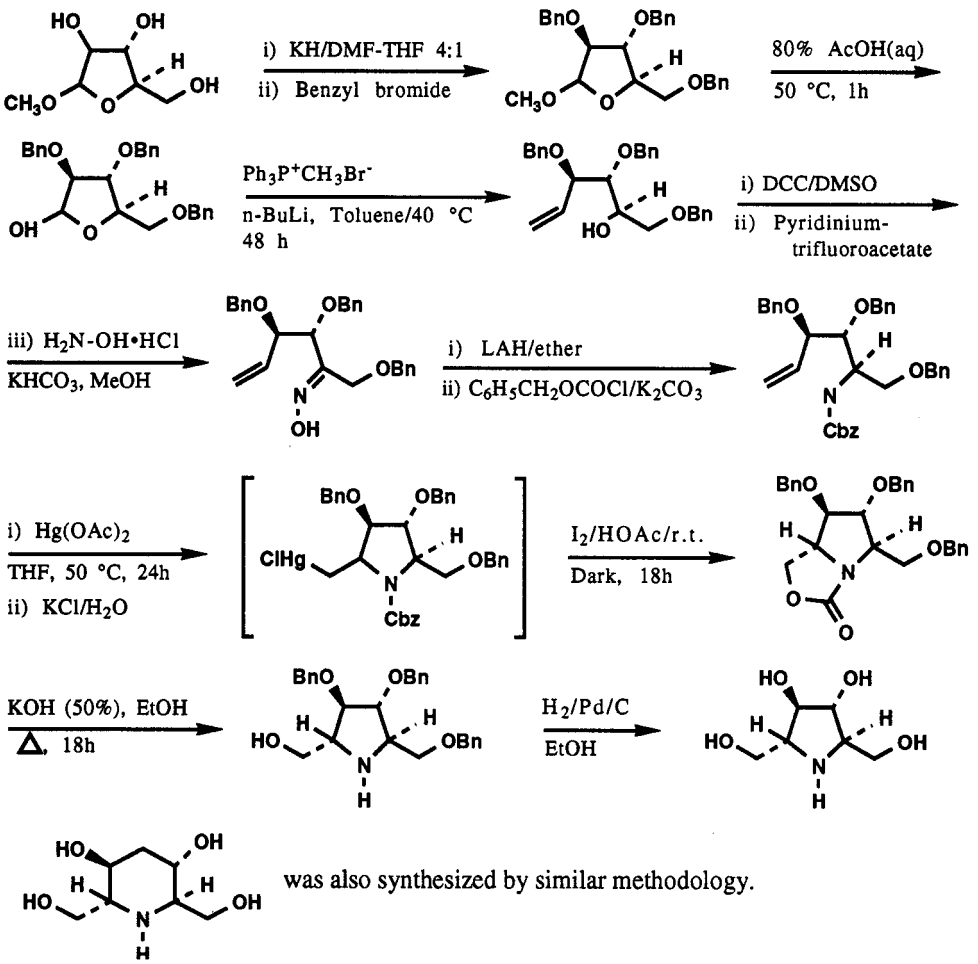
Results of enzyme inhibition studies:

Compound	ppm	% Inhibition forward reaction	% Inhibition reverse reaction
	50	32	68
 R=COPh	50	0	75

	50	25	25
	50	0	70
	50	93	86

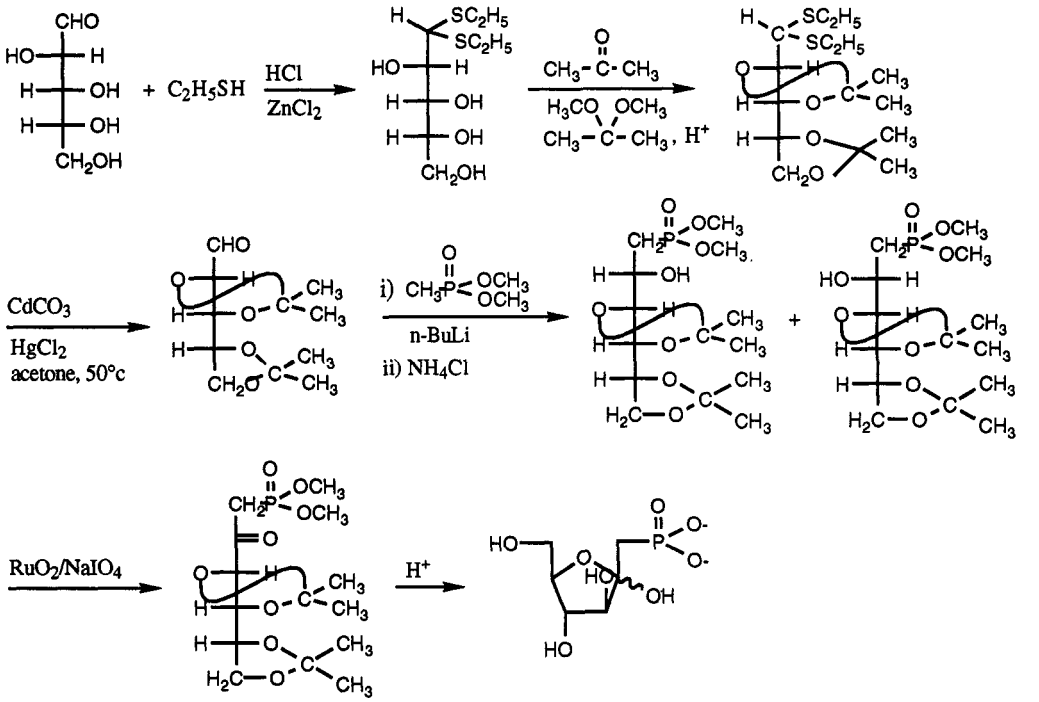
The active compounds are being tested on whole plants.

Scheme A



 was also synthesized by similar methodology.

Scheme B



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