

BIORATIONAL DESIGN OF HERBICIDES: SYNTHESIS OF INHIBITORS OF THE PFP ENZYME[†]

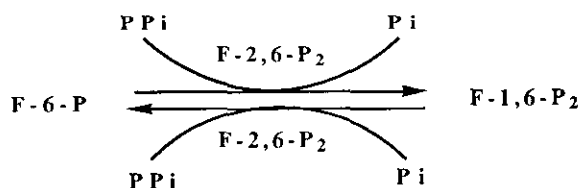
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Abstract-Transition state and reaction coordinate analog inhibitors of the PFP enzyme were synthesized for the biorational design of herbicides. Some of the promising ones were scaled up and tested on whole plants. Open chain, aza and phosphonated analogs of fructose showed significant PFP inhibitory activity.

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-P₂) and 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

[†] This paper is dedicated to Professor Rolf Huisgen.

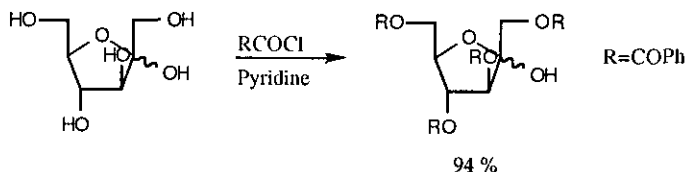
conditions.^{4,5} PFP is activated by the regulatory metabolite fructose 2,6-bisphosphate (Fru-2,6-P₂).⁶

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.

Substrate and inhibitor kinetic studies were carried out to determine the reaction mechanism. Our results⁶ 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 P_i-Fru-6-P complex.

An extensive program of synthesizing putative transition state and reaction coordinate analog inhibitors designed to mimic this structure was undertaken. Several analogs that impact "electron availability at the reactive site" were studied. The following strategies were generally pursued; many of the analogs were synthesized by appropriate modifications of literature procedures.

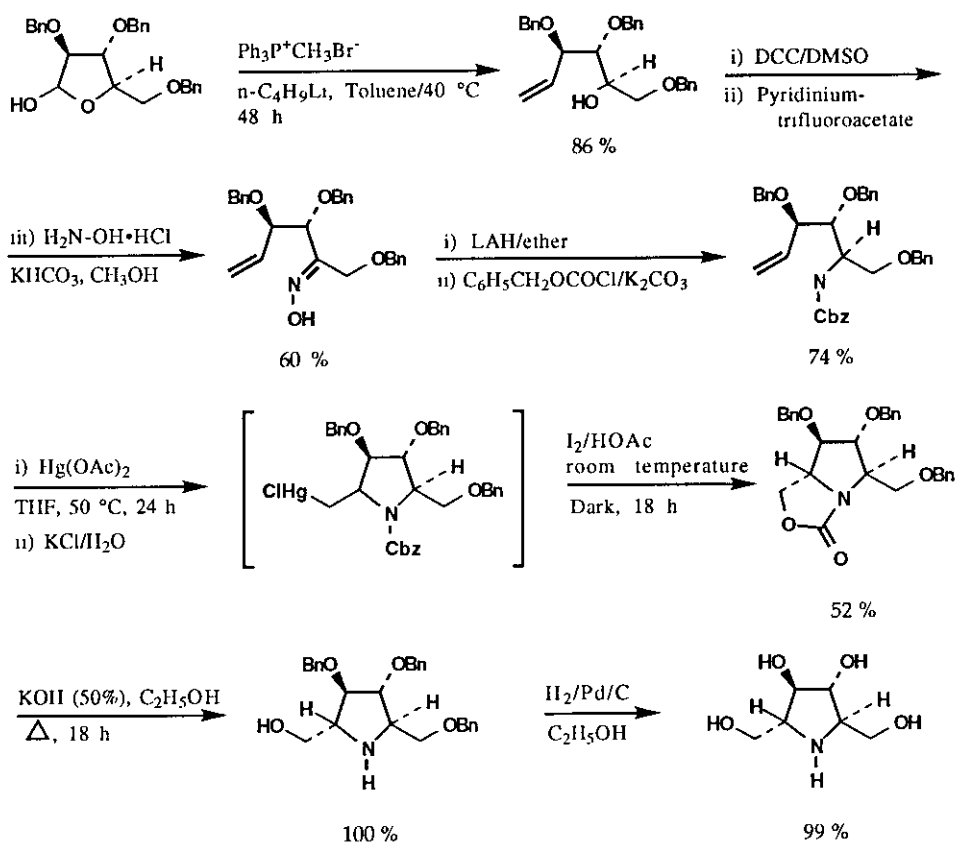
- (i) Incorporation of steric hindrance: persubstituted derivatives of fructose with a good leaving group at the reactive site.⁸



The perbenzoates and perbenzylates revealed PFP inhibitory activity but no whole plant activity. Derivatives with tosylate, mesylate, SMe and CH₂I functionalities revealed no inhibitory activity whatsoever.

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

Scheme A

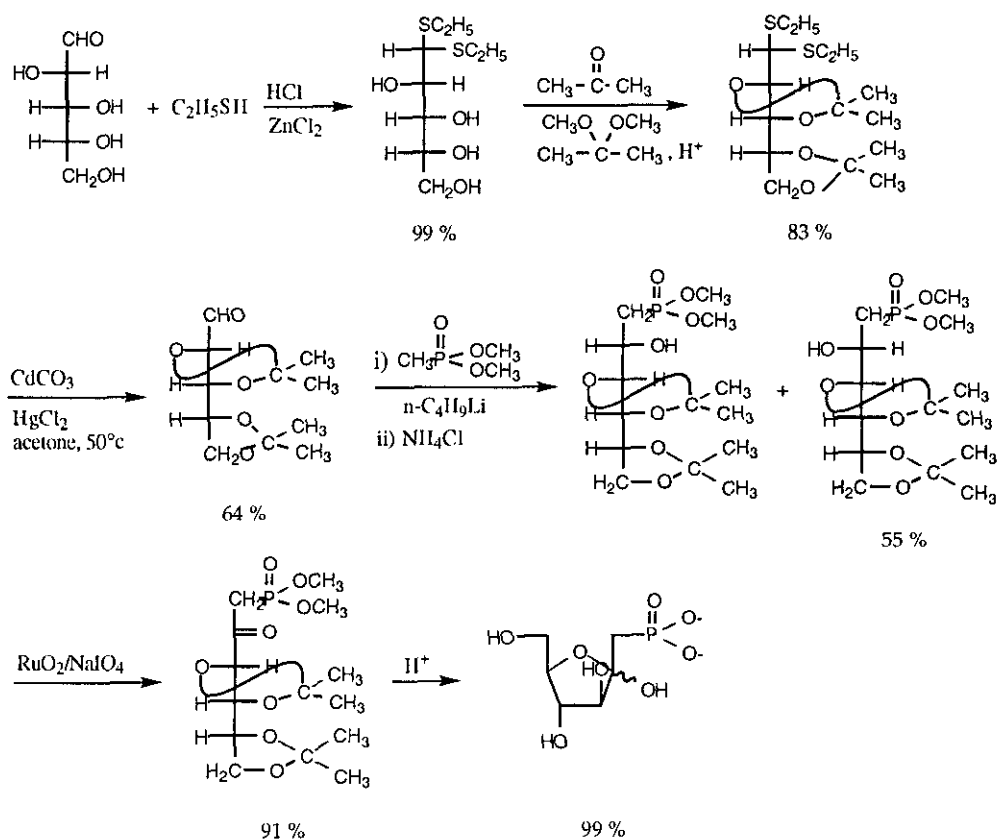


Our interest in the synthesis of this derivative was enhanced by reports of its potent glycosidase and invertase inhibition. It is also thought to function as an insect anti-feedant.¹⁰

The synthesis shown in scheme A was versatile, amenable to scale up and can be adapted to the synthesis of polyhydroxylated pyrrolidines and piperidines.¹⁰

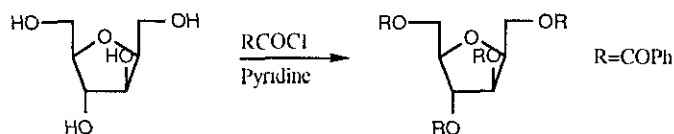
(iii) Introduction of a non-hydrolyzable carbon-phosphorous bond at the reactive site (Scheme B):¹¹

Scheme B



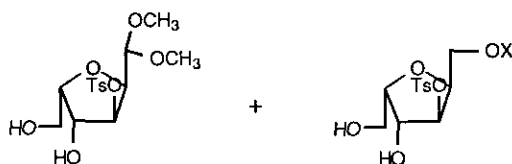
Scheme B shows the synthesis of the phosphonated analog. The aldehyde intermediate is unstable and tends to degrade rapidly. Reaction with the phosphonate anion was a capricious process; the yields were low and variable. Elimination to yield a terminal alkene was a frequent result. A mixture of the gluco and mano derivatives was obtained. These were not separated but immediately oxidized with RuO_2 . Further work is in progress in our laboratories to optimize this process and will be reported in a future communication.

(iv) Synthesis of derivatives of glucitol to avoid free acetaldehyde-hemiacetal equilibrium:¹²

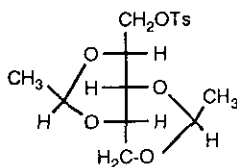


These derivatives were simpler to synthesize from the available D-glucitol.¹² The dibenzoate was found to possess significant PFP inhibitory activity.

(v) Preparation of derivatives with a "trans" side chain to avoid anchimeric assistance of the -OH group:¹³



(vi) Testing of open chain analogs:¹⁴

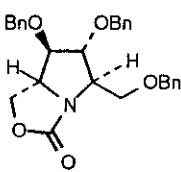
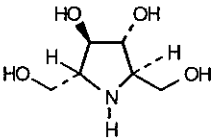
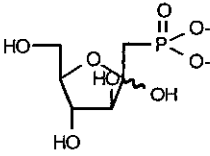
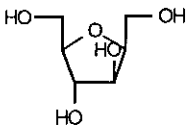
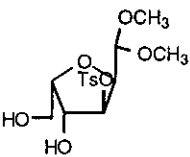
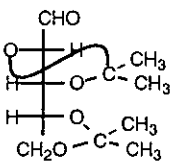
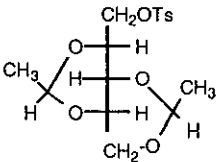


Of the numerous open chain analogs tested, the ones involving a free aldehyde at position 1 were most inhibitory of PFP. Osazone derivatives such as L-xylosazone also revealed ability to inhibit PFP.

Preliminary results of enzyme inhibition studies are shown in Table I.

Table I

<u>Compound</u>	<u>ppm</u>	<u>% Inhibition forward reaction</u>	<u>% Inhibition reverse reaction</u>
 $R = \text{COPh}$	50	0	75
	50	21	33
 N-Cbz	50	18	33

	50	0	70
	50	48	52
	50	62	67
	50	25	25
	50	8	52
	50	32	68
	50	18	84



So far, some of the active compounds have been tested on whole plants and have not revealed activity.

EXPERIMENTAL

Plant material:

Maize (*Zea mays*) seedlings were grown in a greenhouse.

Purification of PFP:

Stems of 7-10 days old seedlings (20 g fresh weight) were rinsed with distilled water and homogenized in a blender in 40 ml Buffer A (20 mM Tricine KOH pH 8.0, 10 % glycerol, 0.1 % 2-mercaptoethanol, 1 % polyvinylpyrrolidone).

The homogenate was filtered through 4 layers of cheesecloth. The filtrate was adjusted to 6 % polyethylene glycol (PEG) concentration using solid PEG 8000 (Sigma). The solution was stirred for 30 min and centrifuged in a Sorval RC5-C centrifuge (SS-34 rotor) at 30,000xg for 15 min. The pellet was discarded and the supernatant was adjusted to a PEG concentration of 18 %. The solution was treated as for the previous PEG fractionation step but the pellet was resuspended in 5 ml Buffer A. This solution was applied to a 1 x 12 cm phosphocellulose (P11, Whatman) column that had been equilibrated with Buffer A. The column was washed with 50 ml of Buffer A and then eluted with 150 ml, 0 - 10 mmol linear sodium pyrophosphate gradient in Buffer A. Fractions containing PFP activity were pooled and used in the inhibition studies.

PFP Test:

- i) Compounds were dissolved in 80% acetone. ii) Final inhibitor concentration in the reaction mixture was adjusted to 50 ppm. iii) The effect was tested in both the forward and the reverse direction.

PFP Assay:

Forward reaction: The reaction mixture contained, in 1 ml final volume, 50 mmol Tris HCl pH 7.8, 5 mmol MgCl₂, 1 mmol ethylenediamine tetraacetic acid (EDTA), 0.2 mmol PPI, 1 mmol Fru 6-P, 1 μ mol Fru-2,6-BP, coupling enzyme mixture (1 U aldolase, 10 U triose phosphate isomerase and 1 U glycerophosphate dehydrogenase), 0.1 mmol NADH, 0.1 mg enzyme fraction and inhibitor as indicated in the text. Fru-6-P was added to start the reaction.

Reverse reaction: The reaction mixture contained, in 1 ml final volume, 50 mmol Tris HCl pH 7.8, 5 mmol MgCl₂, 1 mmol ethylenediamine tetraacetic acid (EDTA), 0.05 mmol Fru-1,6-BP, 10 mmol sodium phosphate (Pi), 1 μ mol Fru-2,6-BP and the coupling enzyme mixture (0.1 U phosphoglucose isomerase, 0.2 U glucose-6-phosphate dehydrogenase), 0.5 mmol NAD, 0.1 mg enzyme fraction and inhibitor as indicated in the text. The reaction was started by adding of Pi.

For both the forward and the reverse direction, the progress of the reaction was monitored at 340 nm. Inhibition data were calculated using a control assay containing solvent only.

REFERENCES

1. R. E. Reeves, *Trends Biochem Sci.*, 1976, 1, 53.
2. J. Dnacer, W. D. Hatzfeld, and M. Stitt, *Planta*, 1990, 180, 205.
3. M. Hajirezaei and M. Stitt, *Plant Sci.*, 1991, 77, 177.
4. J. Dnacer, W. D. Hatzfeld, and M. Stitt, *FEBS Lett.*, 1989, 254, 215.
5. E. Mertens, *FEBS Lett.*, 1991, 285, 1.
6. i) M. Stitt, *Annu Rev Plant Physiol Plant Mol Biol.*, 1990, 41, 153. ii) M. S. Chorghade and C. T. Cseke, manuscript in preparation for *Plant Physiology*.
7. Cornish-Bowden, A. *Fundamentals of enzyme kinetics*, Butterworth, 1979.
8. i) J. W. Van Cleve, *Methods in Carbohydrate Chemistry II*, 62, 237. ii) R. S. Tipson, *ibid.*, 252. iii) P. Brigl and H. Gruner, *Ber.*, 1932, 65, 641.

9. i) M. Chmielewski and R. L. Whistler, *J. Org. Chem.*, 1975, **40**, 639. ii) C. S. Wilcox and J. J. Gaudino, *J. Am. Chem. Soc.*, 1986, **108**, 3102. iii) P. A. Benkovic, S. J. Benkovic, B. E. Maryanoff, S. J. Pilgis, A. B. Reitz, and G. F. Tutwiler, *J. Am. Chem. Soc.*, 1984, **106**, 7851. iv) P. S. Liu, *J. Org. Chem.*, 1987, **52**, 4717.
10. i) L. E. Fellows, G. W. J. Fleet, G. C. Kite, P. S. Liu, A. M. Scofield, and N. G. Smith, *Tett. Lett.*, 1988, **29**, 6483. ii) M. S. Chorghade, C. T. Cseke, and P. S. Liu, manuscript submitted to *Tetrahedron Letters*.
11. i) W. Bartsch and H. Paulsen, *Ber.*, 1975, **108**, 1229. ii) H. Kuhne, and H. Paulsen, *Ber.*, 1974, **107**, 2635. iii) P. H. Griswold and J. English, *J. Am. Chem. Soc.*, 1948, **70**, 1390. iv) idem, *ibid.*, 1945, **67**, 2039. v) H. Brandner, G. Rembarz, and H. Zinner, *Ber.*, 1956, **89**, 800. vi) S. W. Waisbrot, D. I. Weisblat, M. L. Wolfrom, and W. H. Zophy, *J. Am. Chem. Soc.*, 1941, **63**, 201.
12. i) T. A. W. Koerner, R. J. Voll, and E. S. Younathan, *Carb. Res.*, 1977, **59**, 403. ii) H. W. Diehl and H. G. Fletcher, *J. Am. Chem. Soc.*, 1952, **74**, 3175. iii) H. G. Fletcher, *Methods in Carbohydrate Chem.* **II**, 696. iv) H. Appel, *J. Chem. Soc.*, 1935, 425.
13. i) S. Emoto, H. Kuzuhara, M. Matsui, T. Ogawa, and H. Ohru, *Agr. Biol. Chem.*, 1972, **36**, 1449. ii) *ibid.*, 1972, **36**, 1655. iii) M. M. Joulie, Z. Lysenko, and P. C. Wang, *Heterocycles*, 1978, **9**, 753.
14. W. Greve and H. Paulsen, *Chem. Ber.*, 1973, **106**, 2112.

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