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The Utility of 2,5-Dideoxy-2,5-imino-D-mannitol as a PFP Enzyme Inhibitor

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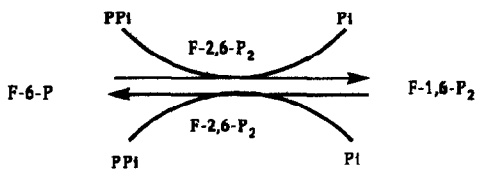
Abstract: 2,5-Dideoxy-2,5-imino-D-mannitol was synthesized from an arabinofuranose derivative. Mercuric acetate cyclization of an ene-carbamate was the key step. The compound proved to be an inhibitor of the pyrophosphate-fructose-6-phosphate-1-phosphotransferase (PFP) enzyme and has potential utility in the biorational design of herbicides.

Fructose 2,6-bis-phosphate is a regulatory metabolite that was first isolated as a liver phosphofructokinase activation factor.[1] It has been accorded a central role in the hormonal regulation of glycolysis and gluconeogenesis in animal cells.[2,3,4] In plants, fructose 2,6-bis-phosphate activates inorganic pyrophosphate-D-fructose-6-phosphate-1-phosphotransferase (PFP),[4,5] an enzyme that catalyzes a reversible reaction at an initial point in the synthesis of sucrose in young tissue and its utilization in meristems. Inhibitors of the enzyme were expected to be useful in the biorational design of herbicides.[6]

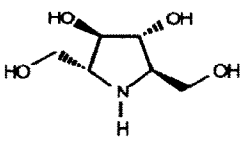
PFP Reaction



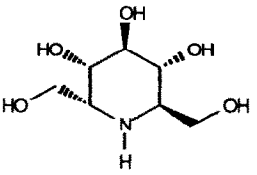
The enzyme was isolated from maize and rigorously purified by fractionation over polyethylene glycol.[6] Substrate interaction studies indicated that the PFP reaction follows a ternary complex comprising fructose-6-phosphate, inorganic pyrophosphate and the enzyme rather than a ping-pong (or substituted enzyme) mechanism. Further details of the mechanism were elucidated by determining and analyzing product inhibition patterns. These tests were carried out by assaying the enzyme both in the forward (fructose-1,6-bisphosphate formation) and in the reverse (fructose-6-phosphate formation) direction, respectively. The results[6] are consistent with an ordered reaction pattern for both substrate addition and product release. Based on the ternary complex mechanism one can propose that 1-pyrophospho, 6-phospho fructose may be formed as a central complex along the reaction coordinate. Based on the transition state theory, this putative intermediate would be expected to have high binding affinity to the enzyme. Analogs of this intermediate would be expected to inhibit the PFP reaction. Numerous transition state analogs that were

design to mimic this structure were synthesized[6] and shown to be inhibitors of the enzyme in the forward and reverse directions. Data on this and related testing on whole corps will be presented elsewhere.

During the course of these studies, we screened some polyhydroxylated piperidines and pyrrolidines for PFP inhibitory activity. One such compound, DMDP I, proved to be particularly interesting. This compound had originally been isolated form *Omphalea diandra L.*, *Derris elliptica* and *Lonchocarpus SPP* and was reported to possess activity as an invertase and glycosidase inhibitor and as an insect anti-feedant.[7] Syntheses of DMDP have been reported[8]; however, scale up considerations prompted us to look for a synthesis more amenable to multi-gram synthesis.



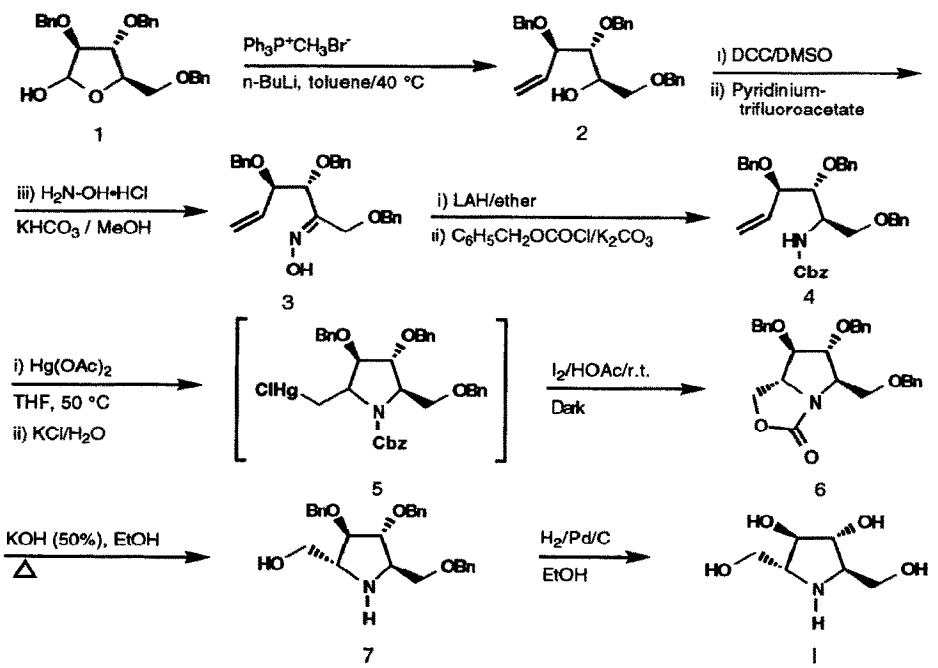
I



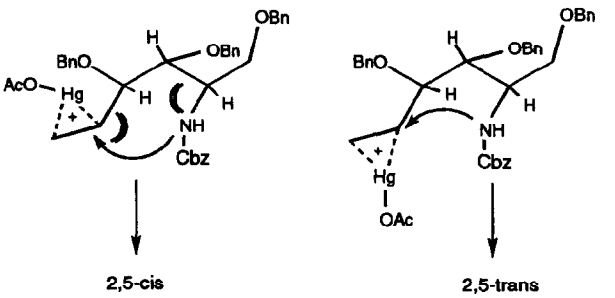
II

We report herein a synthesis of DMDP and the hitherto unexplored potential of the product as a biorationally designed herbicide. The general methodology had earlier been applied to the synthesis of α'-homonojirimycin II.[9]

Scheme 1



Arabinofuranose derivative **1** was converted to the ene-alcohol **2** in 86% yield by a modification of Pougny's procedure.[10] Oxidation of **2** by the DMSO based procedure of Moffatt or Swern[11] gave an unstable ene-ketone which was immediately converted to oxime **3** in 60% yield. Reduction of the oxime was influenced by choice of solvents and reducing agents. An optimal ratio (7:1) of the desired D-amine (Cram product) to the undesired L-amine was obtained with $LiAlH_4$ in anhydrous ether. The mixture of amines was converted to the carbamates and separated by hplc. Carbamate **4** underwent intramolecular cyclization upon treatment with mercuric acetate in THF to yield **5**. Interestingly, the 2,5-trans stereochemisty, identical to that observed in the ring closure of piperidine **II**[9] is probably due to the differential steric requirements in the possible transition states.



Steric interaction between the axially oriented substituents at C-2 and C-5 disfavors the formation of the transition state leading to the 2,5-cis product. The alternate path, devoid of such interactions, provides the observed product. The chloromercurial obtained after ligand exchange with KCl was converted to **I** by reductive oxygenation ($NaBH_4$-DMF-O_2)[12] and subsequent hydrogenation. This transformation proceeded in only 37% yield. However, treatment of the mercuric complexes originating from **4** and its L-isomer with iodine and acetic acid provided bicyclic carbamates from which **6** was obtained in 52% yield by fractional crystallization. The structure of **6** was confirmed by x-ray crystallography. Base hydrolysis yielded **7** which was catalytically hydrogenated to **I** in quantitative yield.

The product was tested for PFP activity by dissolving them in 80% acetone (aqueous), adjusting the inhibitor concentration to 50 ppm and testing the effect in the forward and reverse directions. The progress of the reaction was monitored at 340 nm. Inhibition data were calculated using a control assay containing solvent only. DMDP **I** inhibited the PFP enzyme to the extent of 48% in the forward direction and 52 % in the reverse direction.

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13. All isolated compounds were chromatographically pure and gave satisfactory spectroscopic and microanalytical data, e.g., (I) is a amorphous, colorless solid. M.p. 116-118 °C; ^{1}H nmr (D_2O) δ 3.80 (2H, dt, H-3,4), 3.55-3.40 (4H, 2dd, H-1, 1',6, 6'), 3.0 (2H, m, H-2,5); ^{13}C nmr (D_2O) δ 61.9, 62.2, 78.1; ms (CI-CH_4) 164 ($M+H^+$); $[\alpha]_D$ +56.4 (c, 7.0, H_2O); literature reported value, $[\alpha]_D$ +53.8 (c, 0.3, H_2O). Analytical; calculated for $C_6H_{13}NO_4$: C,44.17; H, 8.03; N, 8.58. Found C, 44.11; H,8.03; N, 8.50.

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