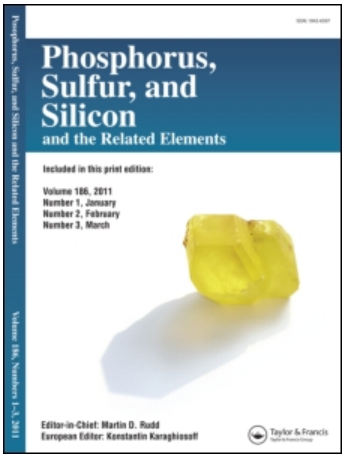

Phosphorus, Sulfur, and Silicon and the Related Elements

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EPSP Synthase: The Design and Synthesis of Bisubstrate Inhibitors Incorporating Novel 3-Phosphate Mimics

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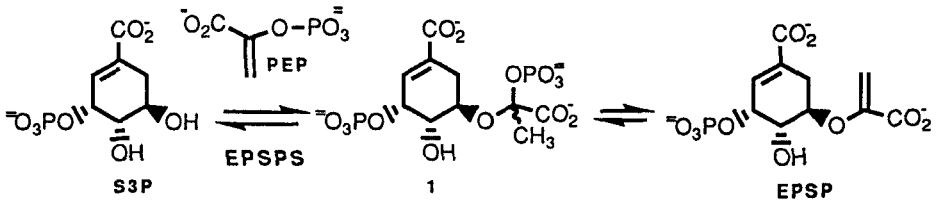
EPSP SYNTHASE: THE DESIGN AND SYNTHESIS OF BISUBSTRATE INHIBITORS INCORPORATING NOVEL 3-PHOSPHATE MIMICS

James A. Sikorski,§ Michael J. Miller,† Diane S. Braccolino,† Darryl G. Cleary,† Susan. D. Corey,† Jose' L. Font,† Kenneth J. Gruys,† C. Y. Han,† Ko-Chung Lin,† Paul D. Pansegrau,† Joel E. Ream,† Dora Schnur,† Ajit Shah,† and Mark C. Walker.† §Structural and Medicinal Chemistry Section, Corporate Research Group and †New Products Division, Agricultural Group, Units of Monsanto Company, 700 Chesterfield Parkway North, St. Louis, MO. 63198 USA

Abstract Novel aromatic bisubstrate inhibitors of the enzyme EPSP (5-enolpyruvoylshikimate-3-phosphate) synthase (EC 2.5.1.19) have been designed and synthesized as structural analogs of the single, catalytic intermediate **1** utilized by the enzyme. These aromatic inhibitors incorporate novel α-hydroxyphosphonates, malonate ethers and α-hydroxymalonates as replacements for the hydrolytically labile 3-phosphate group. These 3-phosphate mimics were much preferred to the corresponding methylene and vinylic phosphonates, malonates and phosphonomethyl ethers.

INTRODUCTION

The enzyme EPSP synthase (EPSPS) is the biological target in plants for the successful broad-spectrum herbicide, glyphosate.[1,2] As such, EPSPS has been the target of an ongoing multidisciplinary herbicide discovery effort in our laboratory.[3] EPSPS catalyzes an unusual transfer reaction of the carboxyvinyl moiety from phosphoenolpyruvate (PEP) regiospecifically to the 5-OH of shikimate-3-phosphate (S3P) to form EPSP and inorganic phosphate (P_i).[3,4] The EPSPS chemical mechanism proceeds through one,[5] kinetically competent,[6] tightly bound[7] (K_d = 50 pM), tetrahedral intermediate, **1**, which forms *via* protonation of PEP at C-3 during catalysis. Close structural analogs of **1** are potent EPSPS inhibitors.[8-10] A predictive 3-D template model for **1** was developed from NMR conformational analysis of bound



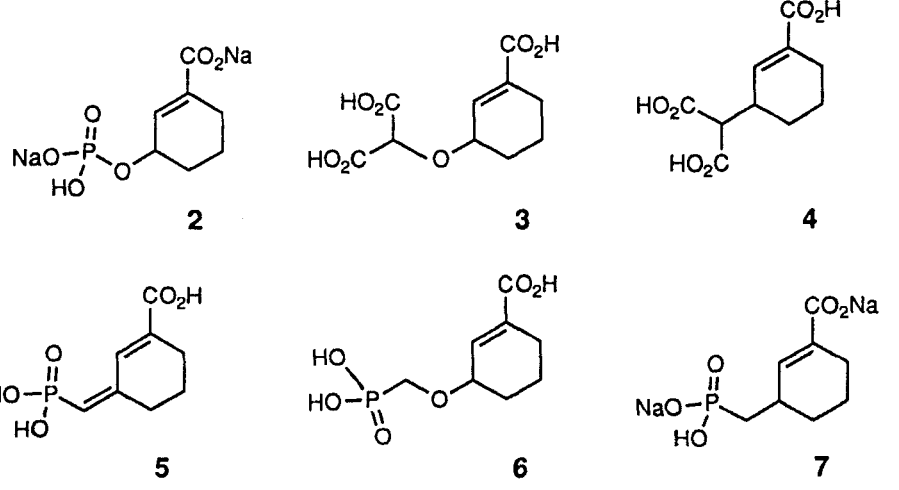
S3P[11] and substrate recognition experiments. Novel aromatic bisubstrate inhibitors of EPSPS were then designed and synthesized from this template.

3-PHOSPHATE MIMICS

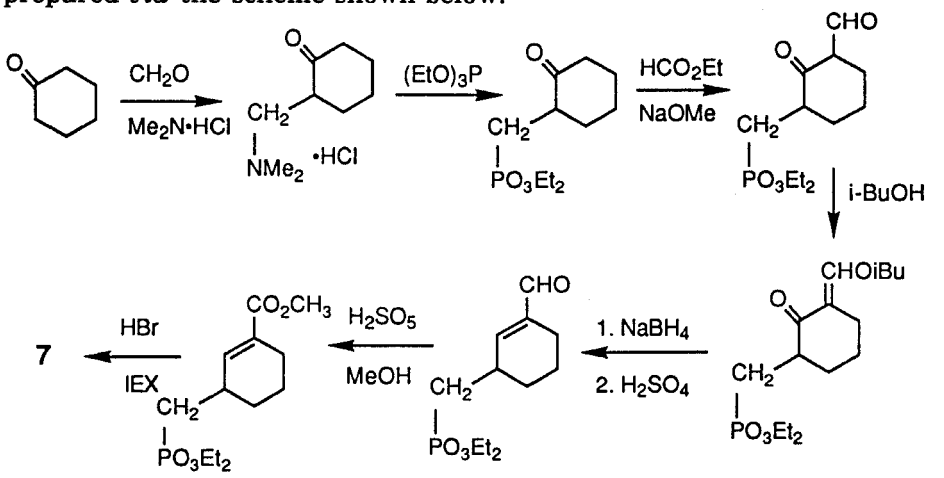
A series of substrate analog recognition experiments demonstrated that the ionic groups present in S3P are more important for binding than the OH groups. In particular, the 3-phosphate moiety in S3P contributes significantly (> 8 kcal/mol) to its binding with enzyme and its catalytic turnover to **1**.[12] Thus, loss of this hydrolytically labile allylic 3-phosphate group greatly diminishes the potency of any potential bisubstrate inhibitor. A variety of α-hetero phosphonates have been described previously in the literature as potential phosphate replacements.[13] Molecular modeling experiments suggested that isosteric phosphonates might be reasonable solutions to this problem.

4,5-Dideoxy-S3P Derivatives

In order to identify functionalities with increased stability to replace the 3-phosphate group, a series of 4,5-dideoxy-shikimate derivatives (**3-7**) were prepared and evaluated as EPSPS inhibitors versus 4,5-dideoxy-S3P (**2**).

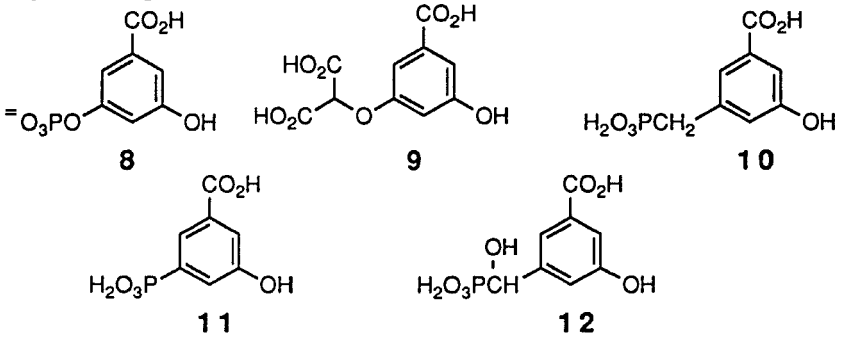


Only the malonate ether (**3**) exhibited EPSPS inhibition comparable to **2**, suggesting that the oxygen linkage between the carbon and phosphorus atoms is very important for enzyme recognition. The synthesis of most of these targets will be reported separately.[14] Reference compound **7** was prepared *via* the scheme shown below:



4-Deoxy-Aromatic S3P Derivatives

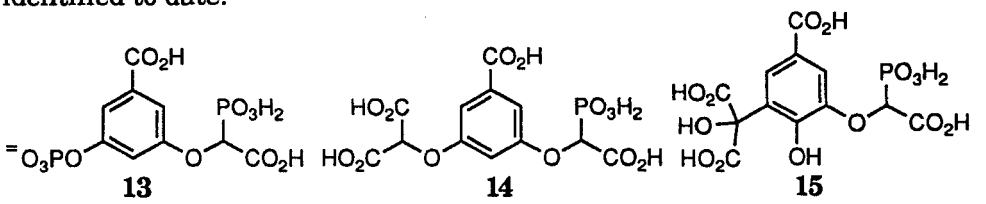
Similar results were obtained using a series of planar 4-deoxy aromatic derivatives (**8-12**) containing various replacements for the 3-phosphate group. Again, the malonate ether (**9**) and an α-hydroxyphosphonate (**12**) were the only effective phosphate mimics providing significant interaction with enzyme comparable to **2** and **8**.



AROMATIC TETRAHEDRAL INTERMEDIATE MIMICS

Given these results a series of 4-deoxy aromatic analogs (**13-15**) of **1** were prepared which incorporate either malonate ethers or α-hydroxy-malonates and were found to be potent EPSPS inhibitors[14] with K_i's in the mid to high

nanomolar range. These compounds represent some of the most potent aromatic inhibitors of the aromatic amino acid biosynthetic pathway identified to date.



CONCLUSIONS

Thus, α-hydroxyphosphonates, malonate ethers and α-hydroxymalonates can function as suitable 3-phosphate replacements for the EPSP synthase system. These functionalities should have applicability for other phosphate dependent enzymes and the design of their relevant potent enzyme inhibitors.

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