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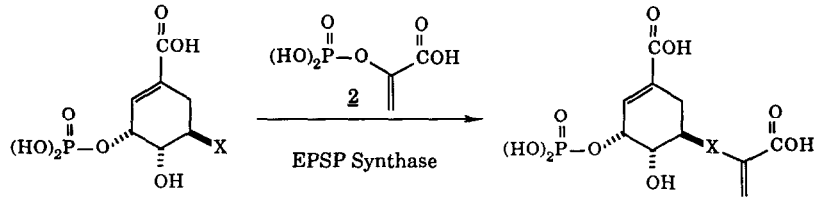
SYNTHESIS AND EVALUATION OF TWO NEW INHIBITORS OF EPSP SYNTHASE

Paul D. Pansegrau*, Karen S. Anderson[1a]*, Theodore Widlanski[1b]§, Joel E. Ream*, R. Douglas Sammons*, James A. Sikorski* and Jeremy R. Knowles§.

*Technology Division, Monsanto Agricultural Company, A Unit of Monsanto Company, 800 N. Lindbergh Boulevard, St. Louis, Mo. 63167, and §Department of Chemistry, Harvard University, 12 Oxford Street, Cambridge, Massachusetts, 02138.

Abstract: The enzyme EPSP synthase, EPSPS, (EC 2.5.1.19) catalyzes an unusual transfer reaction of the enolpyruvoyl moiety from phosphoenol pyruvate (2, PEP) regiospecifically to the 5-OH of shikimate 3-phosphate (1, S3P) to form 5-enol-pyruvoylshikimate 3-phosphate (3, EPSP). Two new inhibitors, (4, and 5) were prepared to probe the S3P binding site.

EPSPS occupies a prominent position in the shikimate pathway by which plants and bacteria synthesize the aromatic amino acids.[2] This pathway is present in all plants and micro-organisms, but is completely absent in mammals, birds, fish and insects. EPSPS is effectively inhibited by glyphosate,[3] the active ingredient in the herbicide, Roundup®. The unusual chemistry of EPSPS catalysis has prompted a series of mechanistic investigations which have recently been concluded with a clear definition of a single kinetically competent tetrahedral intermediate.[4] The design of new mechanism-based EPSPS inhibitors could in principle provide another herbicide of commercial significance, and low environmental impact. Structural mimics of this intermediate are indeed potent EPSPS inhibitors.[5]



1, X = OH
4, X = H
5, X = NH_2

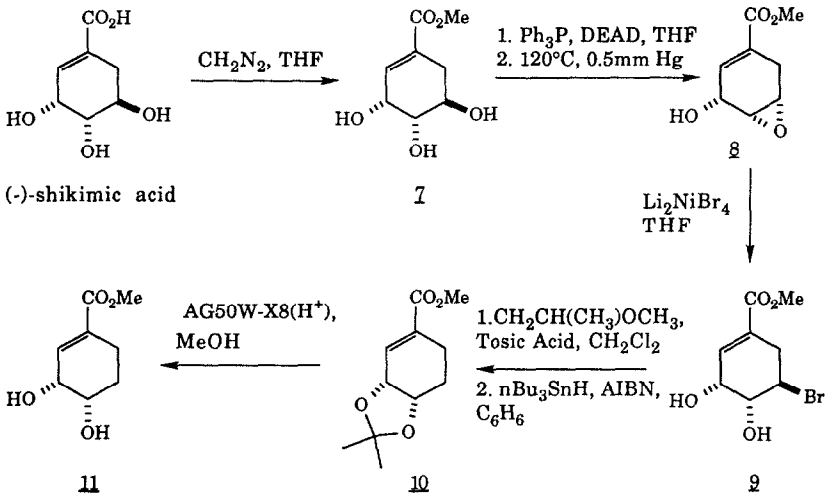
3, X = O
6, X = NH

Additional analogs of S3P were sought to provide insight to the functional requirements for S3P binding and to probe new approaches for mechanism-based inhibitors. The only other known probe of the S3P binding site is 4,5-deoxy-S3P.[6] The mono-deoxy analog, 4, was sought to determine what, if any, hydrogen bonding interactions were contributing to S3P recognition. Derivative 4 should also be a better probe for examining the glyphosate and PEP binding sites.

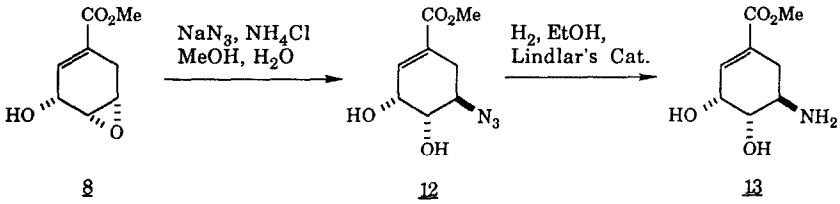
Amino-shikimates such as 5 might serve as substrates for EPSPS forming the EPSP analog 6, but 6 would be expected to readily decompose to 5 and pyruvate. Thus, 5-amino-S3P could be viewed as a new mechanism-based inhibitor which readily cycles PEP to pyruvate. No 5-amino-shikimates have been previously reported.

The synthesis of 4 and 5 began from (-)-shikimic acid. Esterification with diazomethane provided methyl shikimate (7) which was converted to the shikimate epoxide (8) according to the procedure of Berchtold.[7] At this point the syntheses of 4 and 5 diverged.

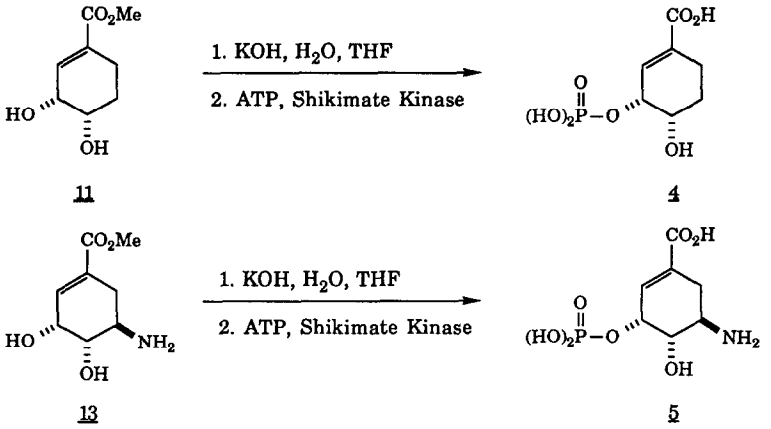
The synthesis of 5-deoxy-S3P continued by treatment of 8 with dilithiotetrabromonickelate[8], which provided the 5-bromo-shikimate (9) as the major product. Removal of the bromine was efficiently accomplished via a tri-n-butyltinhydride reduction after protection to the diols in the form of an acetonide, affording 10. Deprotection of the diol was smoothly accomplished by treatment with Dowex (AG50W-8X) in methanol. Thus, methyl 5-deoxy-shikimate (11) was obtained in 14% overall yield.



The synthesis of 5-amino-S3P began from the shikimate-epoxide 8, by opening of the epoxide with sodium azide.[9] The 5-azido-shikimate (12) was obtained as the major product. Reduction with Lindlar's catalyst[10] provided methyl 5-amino-shikimate, 13, in 21% overall yield from (-)-shikimic acid.



The completion of the synthesis of the desired targets was accomplished by saponification of the esters of 11 and 13, followed by treatment of the crude acids with shikimate kinase[11] and ATP. Purification of the crude broths by ion-exchange chromatography (DEAE Sephadex, triethylammoniumbicarbonate buffer gradient) provided the desired targets 4 and 5 as homogenous, analytically pure materials.



The K_i values for 4 and 5 were determined using standard kinetic assays and are shown below. The K_i for 4 is a factor of seven weaker than the K_d for S3P.[4b] Derivative 4 also will permit slow deuterium exchange into PEP as observed previously with the dideoxy analog.[6] A comparison of 4 with 4,5-dideoxy shows that each hydroxyl group in the shikimate ring contributes at least one hydrogen bond during recognition at the EPSPS active site.

The K_i for 5 corresponds closely to that of S3P. This new amino derivative also will permit slow deuterium exchange into PEP. However, no new products corresponding to 6 or to pyruvate are observed after prolonged incubation with PEP and EPSPS. Both 4 and 5 were shown to be competitive EPSPS inhibitors with respect to S3P.

More detailed biochemical studies of the interaction of these compounds with PEP and EPSPS are currently underway.

Determined K_i Values.

Compound	K_i
4	51μM
5	22μM

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