EPSPS INHIBITOR DESIGN & SYNTHESIS

DESIGN AND SYNTHESIS OF POTENTIAL INHIBITORS OF *ENOL*PYRUVYL SHIKIMATE 3-PHOSPHATE SYNTHASE (EPSPS)

By

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Abstract

The emergence of antibiotic resistance to current treatments of bacterial infection represents a major challenge that needs to be addressed with the development of new generations of inhibitors. The enzyme 5-*enol*pyruvylshikimate 3-phosphate synthase (EPSPS) catalyses the sixth step in the shikimate biosynthetic pathway, which is essential for the synthesis of aromatic compounds such as the aromatic amino acids phenylalanine, tryptophan and tyrosine. It occurs in plants, bacteria and some parasites. Since the pathway is absent in mammals but essential for the pathogenicity of a number of organisms, EPSPS is considered a prospective target for new inhibitor design. A number of EPSPS inhibitors have been reported in the literature. What we are lacking is an understanding of the features that are important for binding EPSPS. We have synthesized compounds to probe the active site of the enzyme based on the knowledge of an enzyme-catalyzed intermediate with a high cationic character. This will include assembling bipartite/tripartite inhibitors to discover what interactions or structural motifs are important for binding. Once the features important for binding to EPSPS are understood, the possibility of elaborating them to create potent inhibitors of EPSPS will be investigated. In addition, the synthesis of two shikimate analogs [5-^{18}O]shikimic acid and 4-deoxyshikimic acid were completed for further experiments to probe the enzyme mechanism in detail, and

for transition state structure by transition state analysis. Transition state analysis using kinetic isotopic effects (KIE) will elucidate the transition state structure of the enzyme-catalyzed EPSP reaction, and provide a detailed starting point for designing EPSPS inhibitors

Acknowledgements

I am most grateful to my supervisors, Dr. Paul Berti and Dr. Fred Capretta for their support and guidance throughout this project. Thank you Dr. Berti for giving me the opportunity to work in your laboratory. My gratitude also goes to Dr. Capretta for his enthusiasm and concern for my development as a synthetic chemist.

I thank all my fellow lab members. It certainly has been interesting working with the Capretta group, especially Emelia and Silvia for their support, encouragement and the research time we have had together. I would like to thank the group for making our laboratory a great working environment. And you, Dave, you have been really wonderful to us in the lab. Aside from the Capretta group, I would like to thank all members of Berti's lab for the friendship we have enjoyed together especially to Paul Chindemi for answering all my questions. I must thank all friends in the McNulty and Harrison groups for the discussions and your generosity with chemical reagents.

I am grateful also to the Chemistry department for providing me with all I needed to make this project a success.

Finally, I would like to collectively thank all my friends and loved ones for the emotional support and friendship. I am indebted to you all.

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List of abbreviations

AIBN	2,2-azobisisobutyronitrile
aq	aqueous
AroA	*enol*pyruvyl shikimate 3-phosphate synthase
Asp	aspartate
Boc	*tert*-butyloxycarbonyl
Bu_3SnH	tributyltin hydride
t-Bu	*tert*-butyl
CI	chemical ionization
DIBALH	diisobutylaluminum hydride
DMAP	4-dimethylaminopyridine
DMF	*N,N*-dimethylformamide
DMSO	dimethyl sulfoxide
EPSP	*enol*pyruvyl shikimate 3-phosphate
equiv	equivalent
Fmoc	9-fluorenyl-methyloxy carbonyl
FPEP	(Z)-3-fluoro-PEP
h	hour
HPLC	high performance liquid chromatography
Hz	Hertz
KIE	kinetic isotope effect
MeI	methyl iodide
mp	melting point
MsCl	methanesulfonyl chloride (mesyl chloride)
MurA	UDP-*N*-acetylglucosamine *enol*pyruvyl transferase
NMR	nuclear magnetic resonance

NHMDS	sodium bis(trimethylsilyl)amide
PEP	phospho*enol*pyruvate
P_i	inorganic phosphate
Py	pyridine
S3P	shikimate 3-phosphate
THI	tetrahedral intermediate
TS	transition state
TB	tuberculosis
TBS	*tert*-butyldimethylsilyl
Tf	trifluoromethanesulfonyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilyl
UNAG	UDP-*N*-acetylglucosamine

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1.0 Introduction

1.1 Antibiotic resistance

Since the discovery of antibiotics there has been a steady emergence of bacterial resistance to these agents. For example, tuberculosis (TB) kills two million people per year in the developing world.[1] Antibiotics were first used to treat TB in the mid-1940s.[2-3] Resistance to these agents appeared in the late 1950s and has continued to increase.[2] Researchers thought that multi drug-resistant (MDR) TB was the worst form of the disease,[3] but in 2005 a new strain emerged in South Africa, an extremely drug-resistant (XDR) TB. XDR TB withstands all of the front-line drugs in the tuberculosis treatment arsenal.[4] MDR TB is defined as being resistant to at least two varieties of front-line antibiotics, while XDR strains are resistant to all of them. Doctors now turn to a number of secondary antibiotics that can be used to treat XDR and MDR TB. Most of those drugs are more expensive than the front-line varieties and often have greater side effects. Drug resistance is therefore a difficult problem in treating critically ill patients who are less able to fight off infections without the help of antibiotics. Unfortunately the development of new agents or methods to counteract this resistance has not kept up with the rate of evolution of resistance mechanisms. The need for the development of new antibacterial agents has, therefore, become more urgent if we are to overcome antibiotic resistance and be able to fight off new infectious diseases.

This thesis describes the synthesis of compounds that will be used to study the binding strategies of *enol*pyruvylshikimate 3-phosphate synthase (EPSPS), which is part of the shikimate biosynthetic pathway that is found in all bacteria, including *Mycobacterium tuberculosis*. The ultimate goal is to design effective inhibitors of EPSPS and thereby create better drugs for the treatment of these diseases.

1.2 Enzymes as good targets for novel drug development

Enzymes are critical for biological reactions. Inhibiting a key enzyme is a common means to create drugs that are targeted at a particular pathogen. Since blocking an enzyme's activity may kill a pathogen, coupled with the knowledge that when an enzyme binds to a substrate or inhibitor, it undergoes conformational changes that close its active site, we can design compounds that will bind to enzyme active sites as mimics of the transition state structure of the enzyme-catalyzed reaction. This will trap the enzyme in the closed conformational state and inhibit the enzyme's activity. These transition state mimics may be potent inhibitors of the enzyme.

1.3 Enzyme of interest

EPSPS and MurA are enzymes that have generated considerable interest as targets for inhibitor design due to their potential as targets for new antimicrobial agents.[5] They form a small enzyme family of carboxyvinyl transferases which catalyses the chemically unusual reaction of carboxyvinyl transfer from PEP to the 5-hydroxyl group of shikimate 3-phosphate (S3P) and the 3-hydroxyl group of UDP *N*-

acetylglucosamine (UNAG) respectively (FIGURE 1). This project will focus on EPSPS. EPSPS catalyzes the synthesis of *enol*pyruvyl shikimate 3-phosphate (EPSP) from phospho*enol*pyruvate (PEP) and shikimate 3-phosphate (S3P). It is part of the shikimate pathway,[6] which produces all the key aromatic compounds involved in primary metabolism in bacteria, plants and apicomplexa, including the three aromatic amino acids. Animals, in contrast, have to derive their aromatic compounds from their diet. For this reason there has been interest, extending back to more than 25 years, in the shikimate pathway enzymes as potential targets for herbicides and anti-microbial compounds.[7-9]

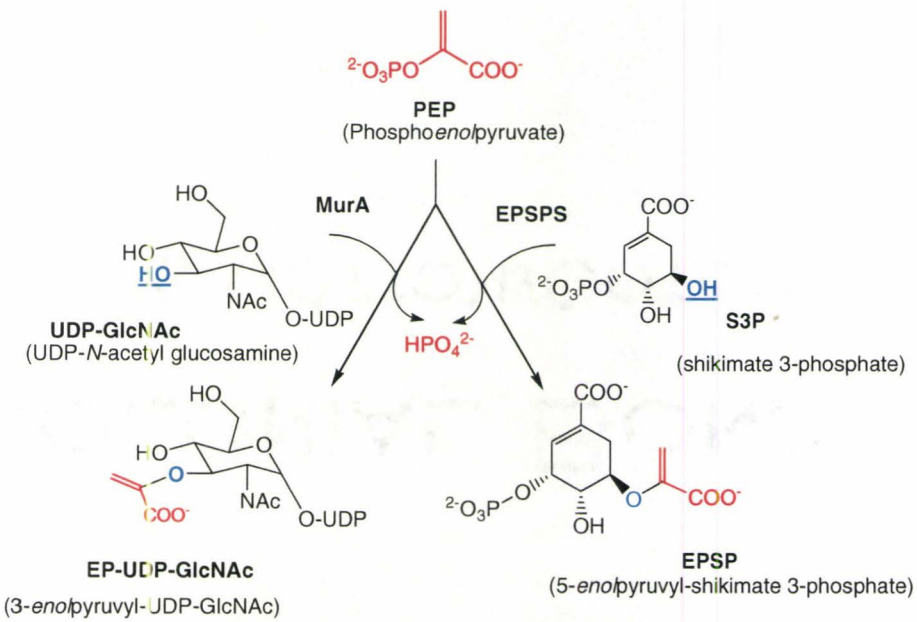


FIGURE 1. Carboxyvinyl transfer by EPSPS and MurA.

EPSPS catalyzes a two-step mechanism where the hydroxyl group of S3P adds across the double bond of PEP to form a tetrahedral intermediate (THI). Elimination of phosphate from the THI completes the reaction.[10-12]

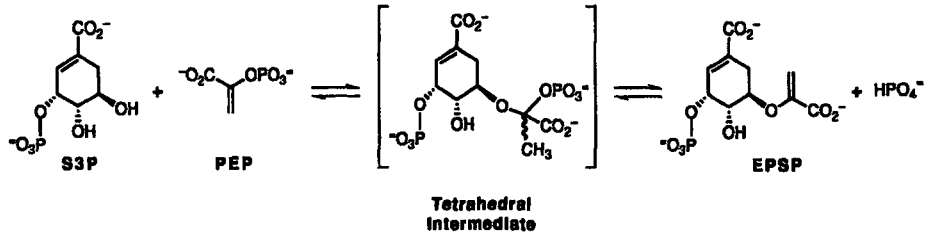


FIGURE 2. EPSPS-catalyzed reaction.[11]

1.4 Mechanism of enzyme action

The overall carboxyvinyl transfer follows an addition-elimination mechanism (FIGURE 3). The addition step involves protonation of C3 of phosphoenolpyruvate (PEP) to form a methyl group, with nucleophilic attack at C2 by the 5-OH group of shikimate 3-phosphate (S3P). The elimination step involves the elimination of phosphate and a proton from the THI.13 The addition and elimination steps of the reaction occur with opposite stereochemistry, either *anti/syn* or *syn/anti*. It remains to be determined conclusively which step is *anti* and which is *syn*. However, the absolute stereochemistry of the enzyme MurA is known.[14] Studies on the absolute stereochemistry of EPSPS showed that, both enzymes share a common stereochemical course. The stereochemistry showed that EPSPS catalyses the 2-*si* face addition of hydrogen to C3 of PEP.[14] Thus, the addition across the double bond of PEP is *anti* and

hence the elimination from the tetrahedral intermediate is *syn* elimination (FIGURE 4).

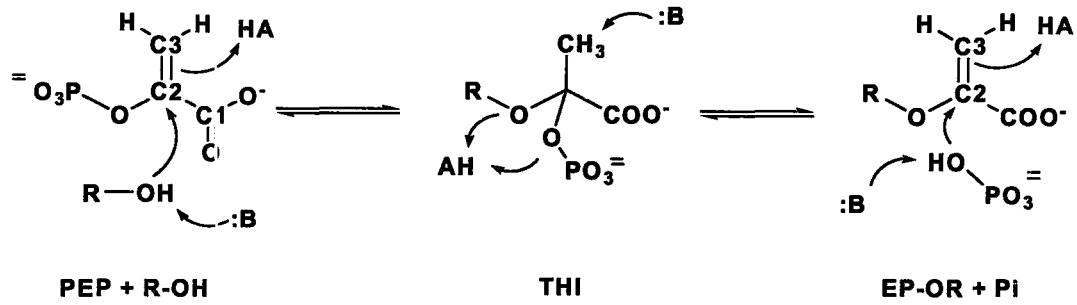


FIGURE 3. Mechanism of enzyme action.[14]

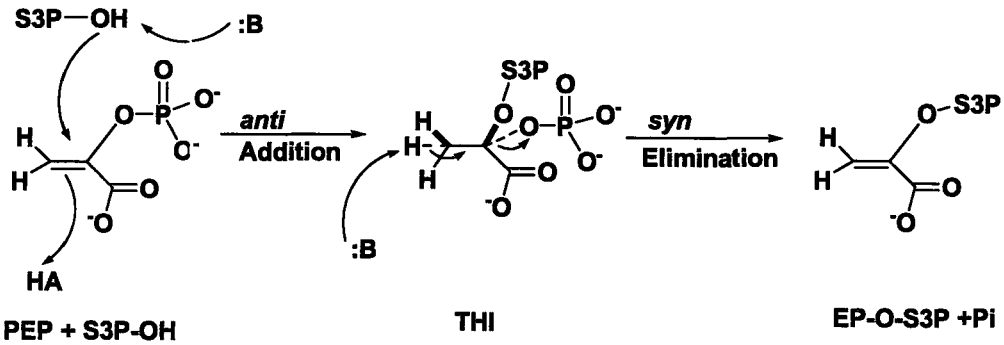


FIGURE 4. Stereochemistry of EPSPS reaction.[15]

1.5 Crystal structures of EPSPS

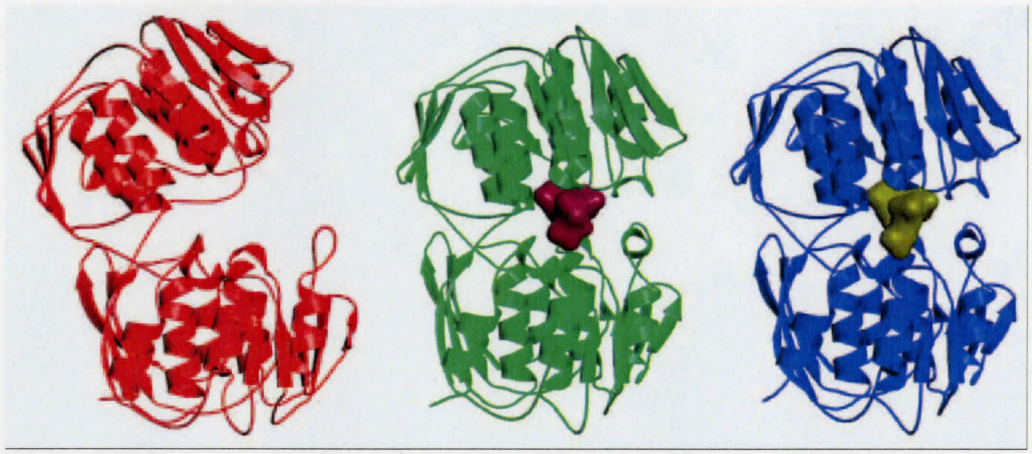


FIGURE 5. Ribbon diagram of *S. pneumoniae* EPSPS in unliganded form (red), S3PGLP complex form (green) and fluoro-THI complex form (blue).[15]

The crystal structures of *Streptococcus pneumoniae* EPSPS have been successfully solved in the unliganded state, and two differently liganded states: the S3P and glyphosate (GLP) ternary complex; and the fluoro-tetrahedral intermediate (F-THI) formed from S3P and (Z)-3-fluoro-PEP (FPEP) by Park and co workers.[15] The crystal structures of *Streptococcus pneumoniae* EPSPS was found to be similar to that of *Escherichia coli* EPSP synthase.[15] The structural study showed that the enzyme exists in an open unliganded state and that the second substrate PEP and/ inhibitor glyphosate bind to the closed state of the enzyme adjacent to the target 5-hydroxyl group. This indicates that the binding of substrate follows an induced fit mechanism. These structural studies of enzyme with substrates and inhibitors give valuable information on the enzyme ligand binding domain movement and closure, which is essential in inhibitor design.[15] This is because crystal structures of an enzyme bound

to substrates and inhibitors provide a detailed portrait of the catalytic strategies used by the enzyme.

1.6 EPSPS inhibitors

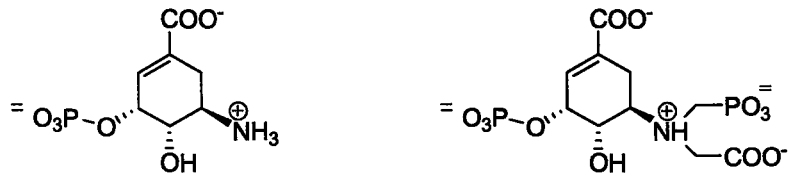
A number of EPSPS inhibitors have been reported in the literature. Glyphosate (*N*-phosphonomethylglycine)[12] is the only commercially available inhibitor of EPSPS. Glyphosate inhibits EPSPS by binding non-covalently. It binds competitively with PEP ($K_i = 0.2$ μM), and is noncompetitive with respect to S3P. Glyphosate forms a stable ternary complex with the enzyme and S3P (AroA•S3P•glyphosate). This ternary complex is responsible for its herbicidal activity.[12] Tight binding of glyphosate to the EPSPS•S3P binary complex has been rationalized based on its resemblance to the putative protonated PEP oxacarbenium ion (FIGURE 6) forming during catalysis.[12] There has been an extensive number of mimics of these simple structures, but all failed to improve upon binding affinity significantly.[12]

Glyphosate **Protonated PEP**

FIGURE 6. Structural comparisons of glyphosate and protonated PEP.[12]

Other inhibitors have been reported in the literature (FIGURE 7). The compound 5-amino-S3P is a mimic of S3P with the 5-OH moiety of S3P replaced by an amino functionality. [12] The S3P glyphosate hybrid was synthesized very recently in an attempt to incorporate and enhance the tight binding properties of

EPSPS•S3P•glyphosate.[12] These dead end inhibitors which are structural mimics of EPSPS•S3P•glyphosate ternary complex in a single molecule have been useful in solving the kinetics for the EPSPS mechanism.[12] They have also been utilized to probe the steady state kinetics mechanism for EPSPS reverse reaction.[12]



5-amino-S3P **S3P glyphosate hybrids**

K_i = 52 μM K_i = 7.4 μM

FIGURE 7. Structure of 5-amino-S3P and an S3P glyphosate hybrid [12]

Both the (*R*)- and (*S*)-diastereomers of compound **1** (FIGURE 8) have been synthesized as phosphonate analogues of the tetrahedral intermediate. They were kinetically characterized for the reverse reaction of EPSPS, and found to be competitive inhibitors with K_i's of 15 nM and 1130nM against EPSP and K_i's of 16 nM and 750 nM against S3P respectively.[16] Compound **2** (FIGURE 8) has also been synthesized and has a K_i of 1.3 μM.[17] These compounds possess several characteristics: they are difficult to synthesize; they have multiple anionic functionalities and multiple chiral centers, and none have been developed commercially.

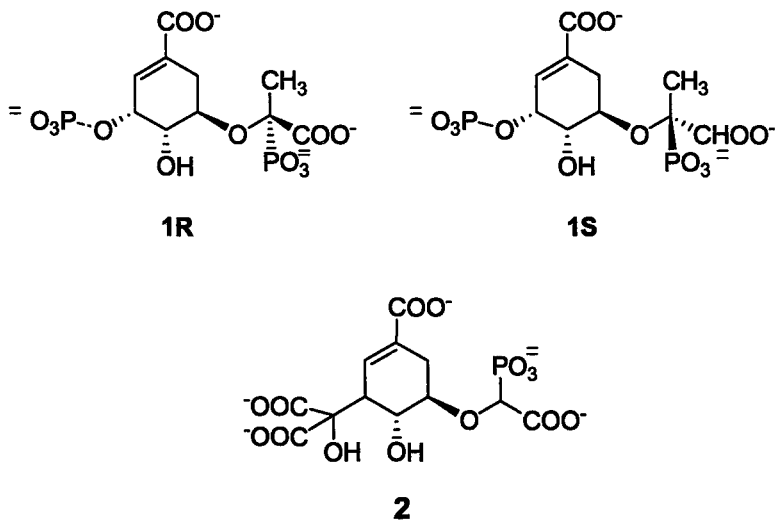


FIGURE 8. EPSPS inhibitors.[16, 17]

There are no known antibiotics that target EPSPS. The herbicide glyphosate inhibits bacterial EPSPS but does not kill bacteria. Designing an inhibitor that mimics the transition state structure of the enzyme-catalyzed reaction is an alternative approach to creating tight binding inhibitors of EPSPS. In doing this, the challenge will be to understand the major features that are important for binding, beyond "more THI-like". The TS analogue should incorporate the binding determinant of both substrates and take advantage of any TS stabilization that the enzyme provides. Using the transition state structure found by transition state analysis, which is being solved by Grace Lou in the Berti lab, it will be possible to design TS mimics that are potent inhibitors.

1.7 EPSP ketal

EPSP ketal was found and characterized previously as a side product formed by EPSPS.[18] It was first proposed to have been formed from non-enzymatic breakdown

of THI. However, work done in our lab showed that instead of being formed non-enzymatically, EPSP ketal was synthesized in the enzyme's active site in the presence of excess EPSPS, but too slowly to be a catalytic intermediate.[18] The relation between THI and ketal and the proposed EPSP cation is indicated in (FIGURE 9).

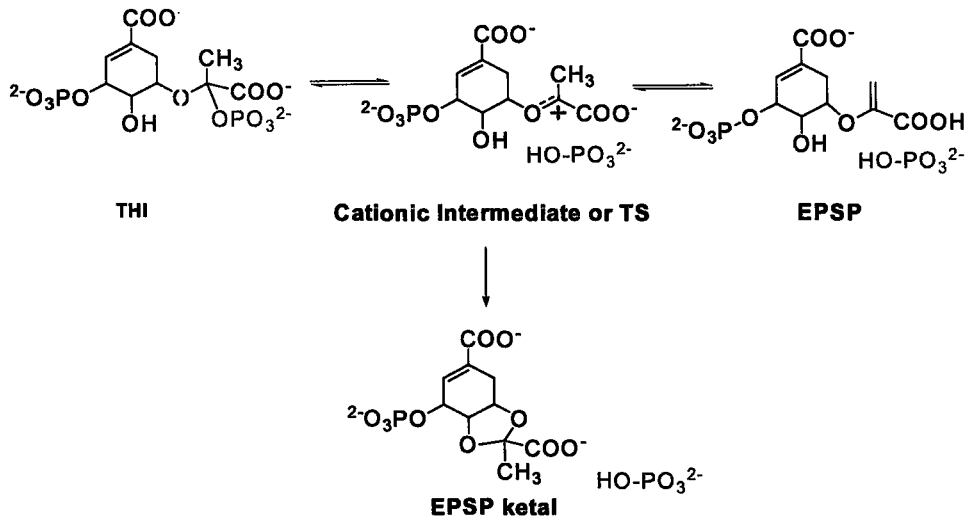


FIGURE 9. The relation between THI, proposed EPSP cation and EPSP ketal [18]

1.8 *Enol*pyruvyl activation with EPSPS

Demonstration of EPSP ketal formation by EPSPS from my lab group, as shown above, demonstrated that EPSPS activates the *enol*pyruvyl group by forming either a discrete EPSP cationic intermediate, or a transition state with high cationic character (FIGURE 9). Knowing that the enzyme stabilizes a transition state with high cationic character provides a template for our inhibitor design with cationic mimics.

1.9 Objectives

The results obtained in the demonstration of *enol*pyruvyl activation with EPSPS, which implies a cationic intermediate or cationic TS, provides a basis for our inhibitor

design of cationic mimicsThe goals of this project were to synthesize compounds to be tested as inhibitors of EPSPS. This involved assembling bipartite/tripartite inhibitors, or "inhibitors-in-pieces" (FIGURE 10). It involved synthesizing a number of S3P analogs that will be used to probe the active site of the enzyme, and then we will assemble novel inhibitors. The initial goal therefore, was to probe what interactions or structural motifs improve binding. Using bipartite or tripartite inhibitors means that there will be a large entropy cost to binding two molecules instead of one. Once we understand binding better, we will try to design unimolecular inhibitors that incorporate all the features that we have found to be important.

1.9.1 Bipartite/Tripartite Inhibitors

We propose to assemble and use bipartite inhibitors in understanding the features that are important for binding to EPSPS because it is easier to synthesize the individual parts than unimolecular inhibitors (FIGURE 11). In addition, the individual parts of the inhibitors can orient themselves optimally in the active site, reducing problems caused by non-optimal linkages. There are some examples of inhibitors designed in pieces in the literature, which are quite effective compared to their unimolecular counterparts. These have been demonstrated with ricin and uracil DNA glycosylase (UDG) (FIGURE 11), where bipartite inhibitors were more effective than single molecules [19, 20]

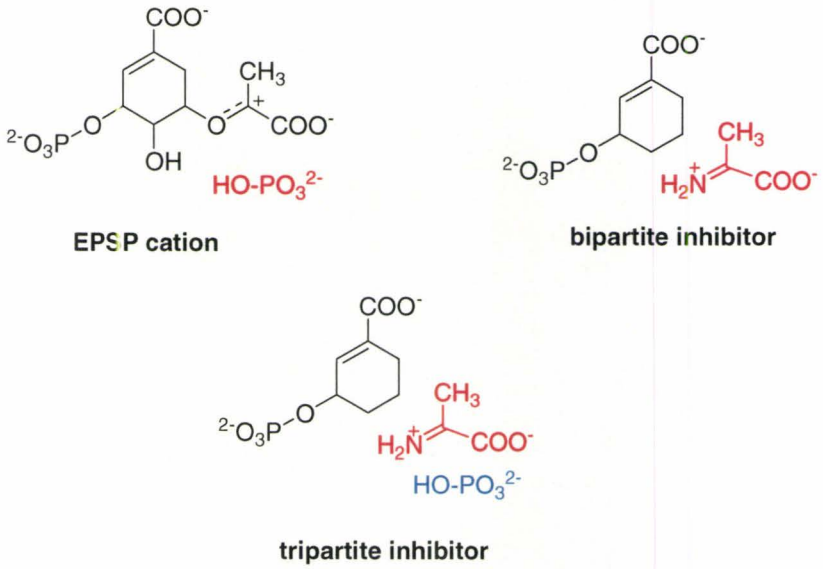


FIGURE 10. Our model of bipartite, and tripartite inhibitors compared with EPSP cation.

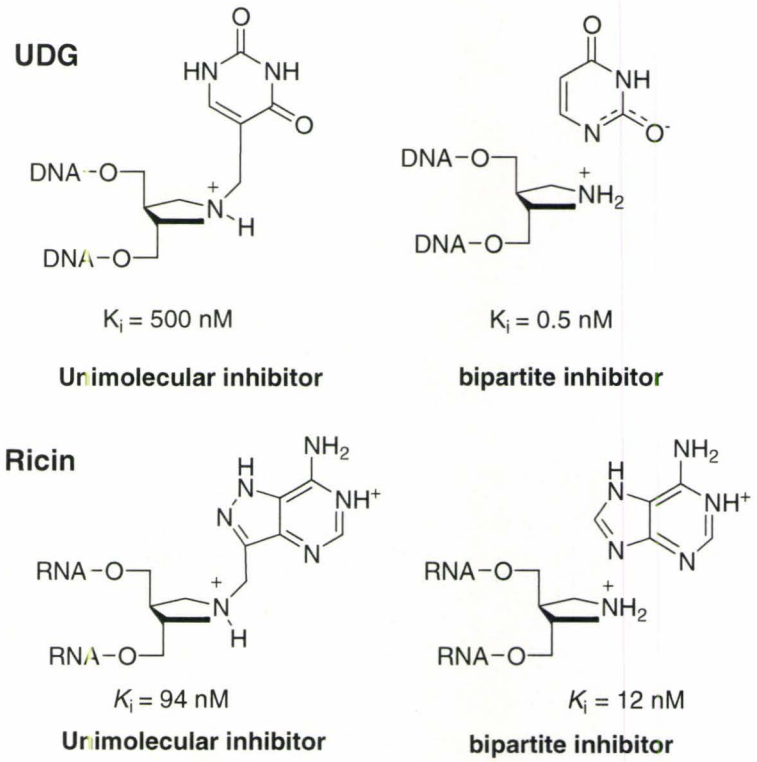


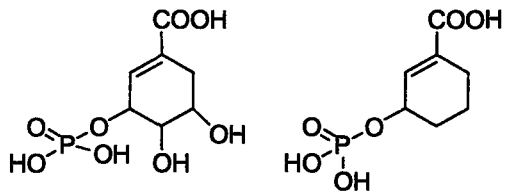
FIGURE 11. Effectiveness of some bipartite inhibitors[19]

1.9.2 Project outline

The first target for this project was the synthesis of 4,5-dideoxyshikimate 3-phosphate (ddS3P). This was followed by the synthesis of some potential cationic mimics to create bipartite inhibitors in understanding the features important for binding EPSPS. I also synthesized two analogs of shikimate, [5-^{18}O]shikimic acid and 4-deoxyshikimic acid for mechanistic studies.

1.9.3 4,5-dideoxyshikimate 3-phosphate (ddS3P)

The first analog of S3P to be synthesized and tested is 4,5-dideoxyshikimate 3-phosphate (ddS3P). It lacks the 4-OH and 5-OH groups of S3P.



S3P **ddS3P**

Studies show that 4,5-dideoxy-S3P competitively inhibits EPSPS with S3P (K_i =10^{-2} M) and causes the enzyme to exchange solvent ^{3}H at C3 of PEP.[20] This implies that ddS3P "activates" EPSPS by providing the crucial carboxylate and phosphate groups required as in the substrate S3P. No phosphate exchange is observed between PEP and Pi in the presence of ddS3P and there is also no scrambling of the bridging oxygen of PEP. These results shows that the C5-OH group of the shikimate 3-phosphate is not required to form the complex involved in the exchange of the methylene hydrogen of PEP with solvent protons.[21] With this knowledge, ddS3P was

proposed to serve as the basic core structure of the bipartite inhibitors by mimicking the cyclohexene moiety of S3P. The question that remains to be answered is: What groups of atoms or charges, when positioned in the active site in the same location as O5' will be effective inhibitors? These effects will be explored by some potential cationic mimics that will serve as the second pieces of our bipartite inhibitors. In addition, the Berti lab has shown that inorganic phosphate (Pi) could facilitate the reverse reaction by 10^5 times,[21] because the enzyme could use the binding energy of phosphate to promote its reactivity. It may be possible to design a TS mimic, which lacks the phosphate group, and exploit the high intracellular concentration of P_i. This would reduce the challenge of transporting a highly charged inhibitor with a P_i functional group across the cell membrane. As a result, these inhibitors will be analyzed in the presence and absence of Pi, which may lead to tripartite inhibitors.

1.9.4 Potential EPSPS inhibitors

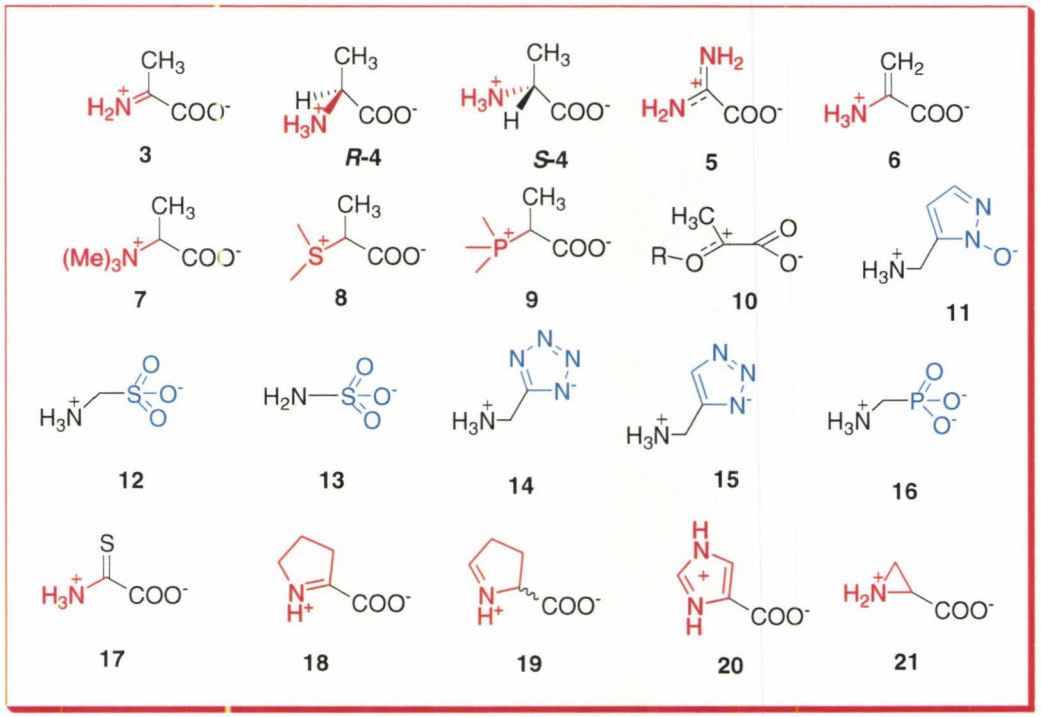


CHART 1. Potential cationic mimics

CHART 1 illustrates the potential cationic mimics that we wished to synthesize to combine with ddS3P in forming the bipartite/tripartite inhibitors. These mimics were proposed in order to answer some questions related to the features important for binding EPSPS. Most are known compounds or derivatives of known compounds. Using the tautomeric cationic mimics **3** and **6** will help in determining the importance of a planar geometry for inhibition of EPSPS, since the EPSP cation and EPSP are both planar at C2. The tetrahedral counterparts, i.e., ***R*-4** and ***S*-4** will also be tested. Even though they will introduce a chiral center into the inhibitor, they will have the advantage of being stable. Compounds **7**, **8** and **9**, will demonstrate if quaternary

amine, methyl sulfonium and phosphonium ions will be effective cationic inhibitors. The tolerance of larger groups in the active site will also be explored with three and five member rings. The hydroxyazole, compound **11** and the sulfonates may serve as effective bioisosteres of the carboxylic acid. The acidity of the tetrazole group also corresponds closely to that of the carboxylic acid (pk_a~5).[30] As such, the replacement of the C-terminal of the intermediate cationic mimic with tetrazole analogs may improve the effectiveness of the inhibitors. The cationic mimics in (CHART 2) are commercially available, while those in (CHART 3) are to be synthesized.

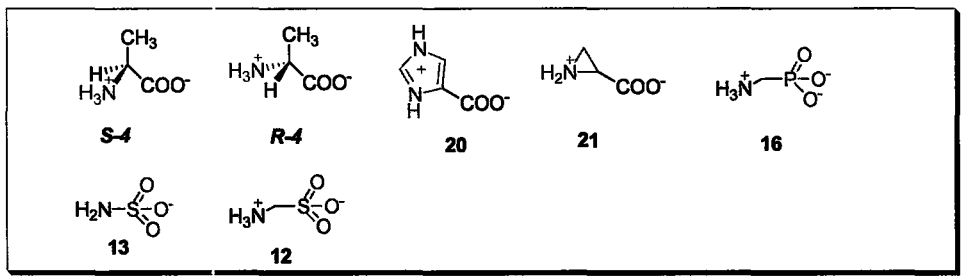


CHART 2. Commercially available cationic mimics

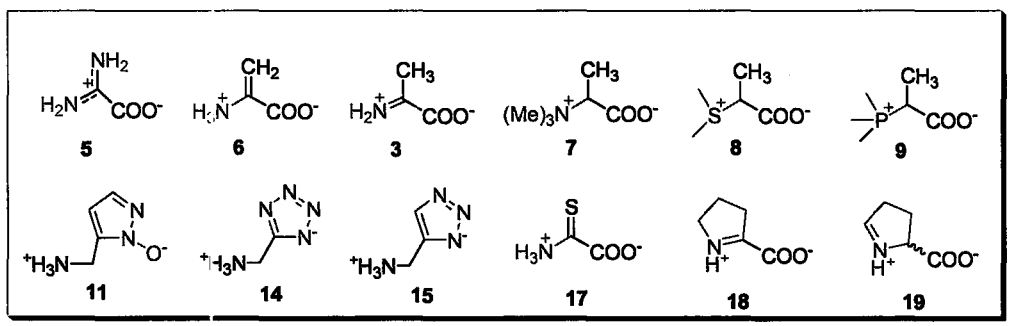
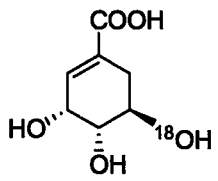
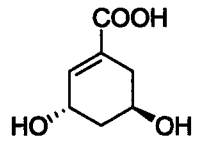


CHART 3. Cationic mimics to be synthesized

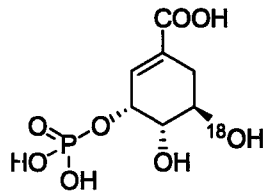
1.9.5 shikimate analogs:



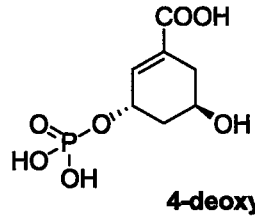
[5-^{18}O]shikimic acid



4-deoxyshikimic acid



[5-^{18}O]S3P



4-deoxyS3P

FIGURE 12. Shikimate analogs for mechanistic studies[22, 23]

As stated earlier, our research is focused on designing inhibitors of EPSPS based on the TS structure of the enzyme-catalyzed reaction. Demonstrating *enol*pyruvyl activation with EPSPS does not provide us with the atomic level detail of TS analysis.

Using techniques such as TS analysis, which involves measuring KIEs at multiple positions in the substrate and analyzing them to solve the TS structure is an effective means to clearly define the TS structure of the enzyme-catalyzed reaction. Grace Lou in the Berti lab is performing the TS analysis. [5-^{18}O]shikimic acid was therefore synthesized, from which [5-^{18}O]EPSP was synthesized. The other analog, 4-deoxyshikimic acid was also synthesized, which was then phosphorylated to give 4-deoxyS3P, and this will be used to test a hypothesis by Schonbrunn and co-workers that the elimination step of the enzyme catalyzed reaction involves an intramolecular proton transfer from C-3 of the tetrahedral intermediate to the oxyanion (4-O$^-$) formed

from the abstraction a proton by an aspartate carboxylate group from the 4-OH of S3P (FIGURE 13).[23]

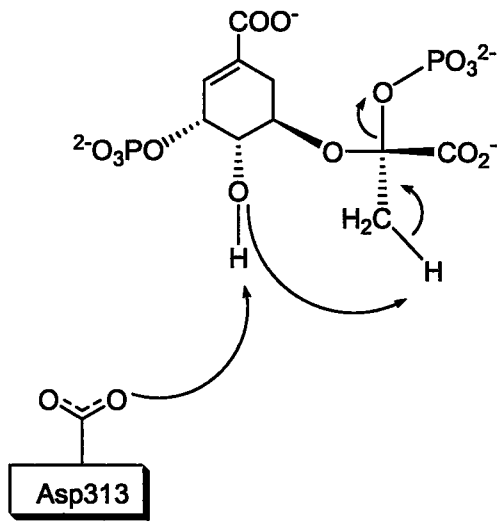
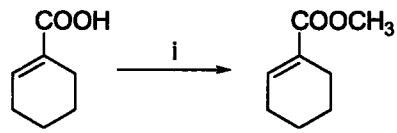


FIGURE 13 proposed intramolecular proton transfer for the elimination step of the enzyme catalyzed reaction.[23]

2.0 Results and Discussion (Shikimate analogs):

2.1 Synthesis of ddS3P (31):

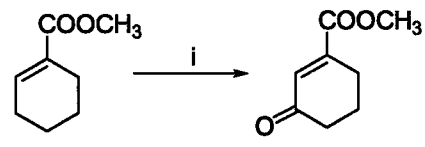
The synthesis of ddS3P, **31**, which will serve as the basic core of our bipartite inhibitors has been reported by Theoclitou and co-workers,[24] but without a complete experimental procedure. With a number of changes in reagents, the syntheses were achieved as described in (SCHEMES 1-7). The starting material for the synthesis was cyclohex-1-ene carboxylic acid **22**. The esterification of **22** to form the methyl cyclohex-1-ene carboxylate **23** is shown in (SCHEME 1).



22 **23**

SCHEME 1. Reagent and conditions: i. CH_2N_2, Et_2O, 2 h. 85%.[25]

This was achieved by first preparing the diazomethane, which was formed by reacting *N*-methyl-*N*-nitrosotoluenesulfonamide with KOH and distilling out of the reaction mixture as an azeotrope with ether.[25] Diazomethane methylation is a good way of making methyl esters from carboxylic acids on a small scale since the only by-product is nitrogen gas, and the yields are good.[33] Allylic oxidation using *tert*–butyl hydroperoxide (TBHP) and dirhodium(II)caprolactamate $Rh_2(cap)_4$ catalyst in dichloromethane formed the 3-keto compound **24** (SCHEME 2).



23 **24**

SCHEME 2. Reagent and conditions: i. 0.1 mol% $Rh_2(cap)_4$, ^tBuOOH, K_2CO_3, CH_2Cl_2, 23 °C, 50%.[26]

Doyle and co-workers[26] reported the above reaction as a high yielding process. However we were only able to obtain 45-50% of the compound. This yield, though not encouraging, was better than the 35% reported by Theoclitou's group[24] using chromium trioxide in acetic acid-water. The reactivity of the rhodium catalyst is due to its ability to undergo single electron oxidation in the presence of TBHP to form Rh_2^{5+} species as shown in the proposed mechanism for the reaction (FIGURE 14).

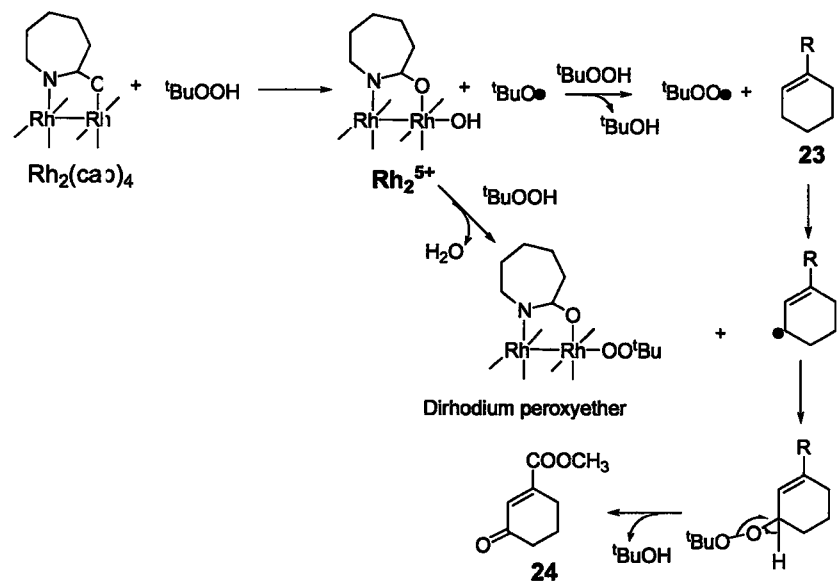
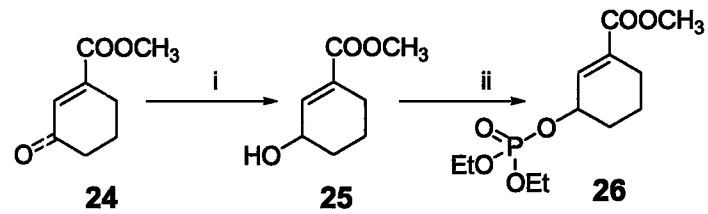


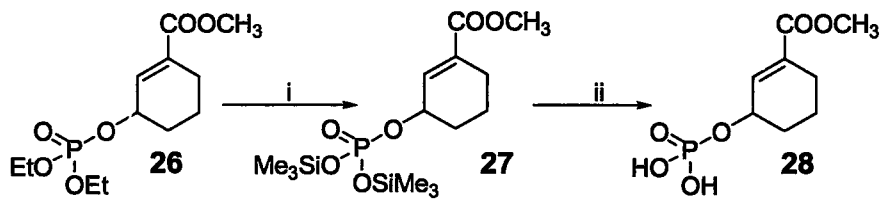
FIGURE 14. Mechanism for allylic oxidation catalyzed by $Rh_2(cap)_4$.[26]

The ketone **24** was reduced to the allylic alcohol **25** using sodium borohydride in methanol. Reacting **25** with diethyl chlorophosphate in the presence of 1-methylimidazole in anhydrous ether formed the phosphotriester **26**. The yields of the two reactions were 85% and 90% respectively.

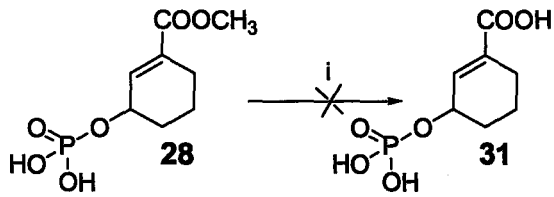


SCHEME 3. Reagents and conditions: i. $NaBH_4$, MeOH, rt, 46 h, 85% ii. $(EtO)_2POCl$, 1-methylimidazole, Et_2O, rt, 2 h, 90%.[24, 27]

Having achieved the synthesis of **26**, the fully deprotected carboxylic acid phosphate (4,5-dideoxyshikimate 3-phosphate) **31** was then obtained by a series of hydrolysis reactions (SCHEMES 6 & 7). The first attempt was the synthesis of compound **31** from **26** through (SCHEMES 4 & 5). The diethyl phosphate **26** was converted to the phosphonic acid **28** by treatment with trimethylsilyl bromide to yield the silylated product **27**, which was hydrolyzed to the acid with water. Alkaline hydrolysis of the phosphate monoester **28** did not yield compound **31** as judged by ^{31}P and ^{1}H NMR.

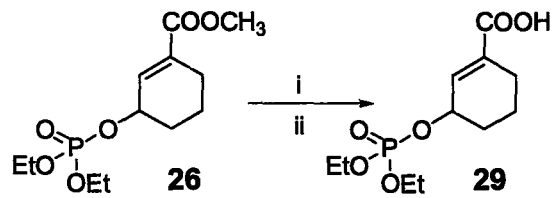


SCHEME 4. Reagents and conditions: i. TMSiBr, CH_2Cl_2, 20 h, 35 °C. ii. H_2O rt, overnight, 90%.[28]

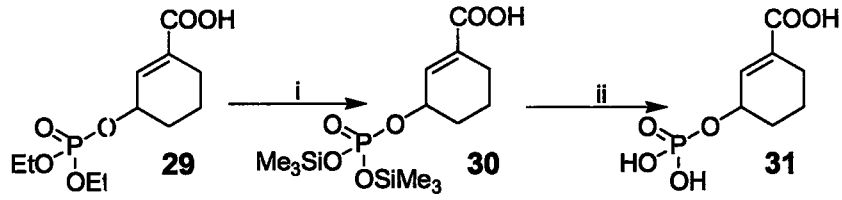


SCHEME 5. Reagents and conditions: i. NaOH, H_2O, rt, 2 h

This result was unexpected, since it is suggestive of hydrolysis by water/ hydroxide attack at the phosphorus rather than the acyl group or as well as the hydrolysis of the methyl ester, but phosphate monoesters are stable to base. And the product of such a transformation (3-hydroxycyclohex-1-enecarboxylic acid) was not observed. An unidentified product was however observed. We changed our synthetic route[24] by hydrolyzing the carboxylic ester first with K_2CO_3 before treating the diethyl esters with trimethylsilyl bromide and hydrolyzing finally with H_2O to yield compound **31** (SCHEME 6, 7).[28]



SCHEME 6. Reagents and conditions: i. K_2CO_3, H_2O, 50 °C, 72 h. ii. 1M HCl, 90%.[24]

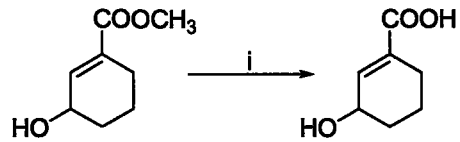


SCHEME 7. Reagents and conditions: i. TMSiBr, CH_2Cl_2, 20 h, ii. H_2O overnight, 60%.[23]

The final product was purified by ion exchange chromatography using diethylaminoethyl (DEAE) cellulose, as the anion exchanger. A (20 mL) column volume with ammonium formate buffer (pH 8) and a gradient of 30 to 970 mM ($NH_4^+HCO_2^-$), the flow rate was set at 1ml/min with a dual wavelength absorbance detection at 240 and 254 nm. The result gave two peaks with a retention time of 20 and 35 minutes. The desired product was identified by ^1H and ^{31}P NMR as the compound that eluted at 35 minutes. Yield of the pure compound was (49 mg, 60 %). Overall yield of the synthesis was 15%. It is worth mentioning that the compound is racemic and it has been proposed that one of the two enantiomers may be responsible for its activity.[22]

2.1.2 4,5-Dideoxyshikimic acid (32):

Synthesis of compound **31** (ddS3P), was achieved in a more convenient manner by enzymatic phosphorylation of 4,5-dideoxyshikimic acid **32** with shikimate kinase and adenosine triphosphate (ATP) by Jen Baker in the Berti lab. 4,5-dideoxyshikimic acid **32** was however synthesized from compound **25** (SCHEME 8).

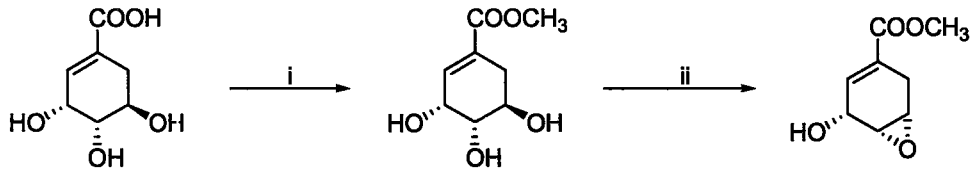


25 32

SCHEME 8. Reagent and conditions: i a. NaOH, H_2O, rt, overnight. b. 1M HCl.

2.2 Synthesis of [5-^{18}O]shikimic acid (37):

The next target of our synthesis was [5-^{18}O]shikimic acid **37**, that was used to synthesize [5-^{18}O]EPSP for KIE measurements. This was achieved following a procedure, which was used by Hilvert and co-workers22 in their synthesis of the doubly labelled ethyl [5-^{18}O, 7- ^{13}C]shikimate ester. Shikimic acid **33**, having a *cis*- and *trans*-diol was an excellent starting material for reasons that will be discussed shortly. The epoxide **35**, served as an important target for the synthesis of **37** because the regiospecific acid catalyzed cleavage of **35** provides a straightforward high-yield route for the preparation of **37**.[29] Procedure for the synthesis of **35** was reported by Berchtold and co-workers[29] (SCHEME 9). This was achieved by the esterification of shikimic acid **33** to form the methyl ester **34** by reacting **33** with thionyl chloride ($SOCl_2$) in MeOH. Reacting **34** with triphenylphosphine and diethylazodicarboxylate in THF (Mitsunobu reaction which takes place with inversion of configuration) yielded 50% of the epoxide with 30% recovery of the starting material.



33 **34** **35**

SCHEME 9. Reagents and conditions: i. $SOCl_2$, MeOH, rt, overnight, 90%. ii. DEAD, PPh_3, THF, 2 h, 50%.[29-31]

Formation of epoxide **35** from **34** by the Mitsunobu reaction has been proposed to occur by selective activation of the C-5 hydroxyl group.[29] A review of relevant literature revealed that 1,2-diols are converted into epoxides or phosphoranes, depending on whether the hydroxyl groups adopt an antiperiplanar (cyclic trans-diols and acyclic-diols) or an eclipsed conformation (cis-diol) respectively.[32] In cyclic systems, it has been observed that cis-1,2 diols afforded triphenyl phosphorane under the reaction conditions (FIGURE 15), whereas epoxides are formed from the reaction of trans diols[31]

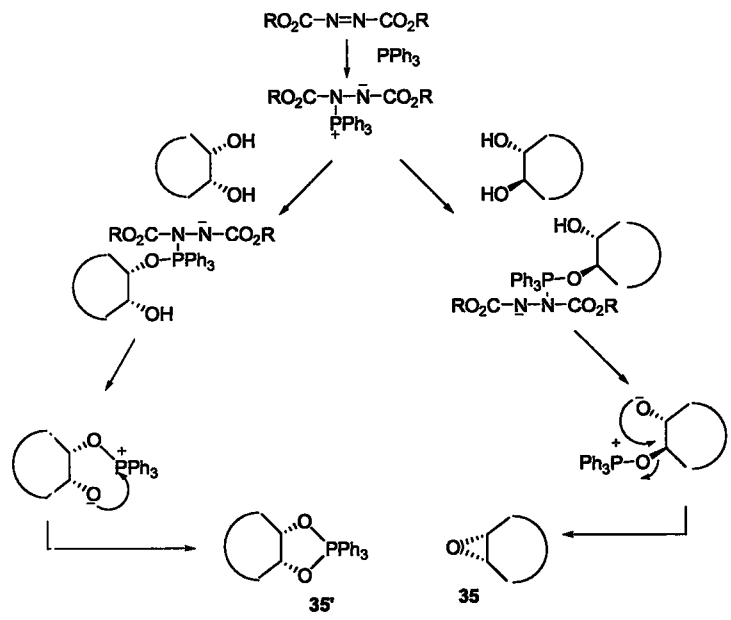
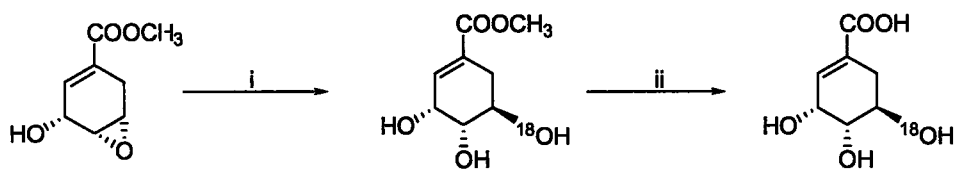


FIGURE15. Formation of triphenylphosphorane 35' and epoxide 35 by the Mitsunobu reaction.

In the presence of $^{18}OH_2$, trifluoromethanesulfonic acid (TFOH), and acetonitrile, regiospecific ring opening of the epoxide was performed to give [5-^{18}O]shikimate ester **36** (SCHEME 10) and (FIGURE 16). In addition we also observed some *in situ* generation of [5-^{18}O]shikimic acid as a side product.



35 **36** **37**

SCHEME 10. Reagents and conditions: i. $H_2{}^{18}O$, TfOH, CH_3CN, rt, 48 h, 70%. ii. NaOH, H_2O, overnight, 1.0 M HCl.[22]

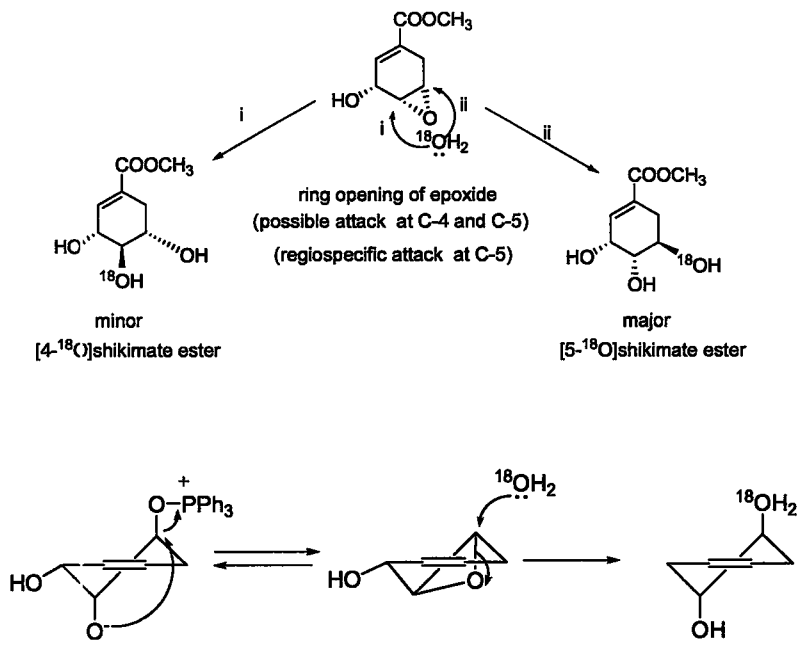


FIGURE 16. Illustrates the overwhelming preference for the formation of the [5-^{18}O]shikimate ester.[29, 33]

Since ring closure is only possible when starting material is diaxially substituted; equally, ring opening is only possible if the product is diaxial.[33] This explains the overwhelming preference for the formation of the [5-^{18}O]shikimate ester **37**. The methyl ester of compound **36** was successfully hydrolyzed to the acid **37**. Mass spectral analysis of **37** was difficult due the presence of high percentage of salt in the sample. However the ratio of product labelled (^{18}O) to (^{16}O) was observed in the mass spectral analysis of the final product after it has been enzymatically converted to [5-^{18}O]EPSP as 55.4% (FIGURE 17).

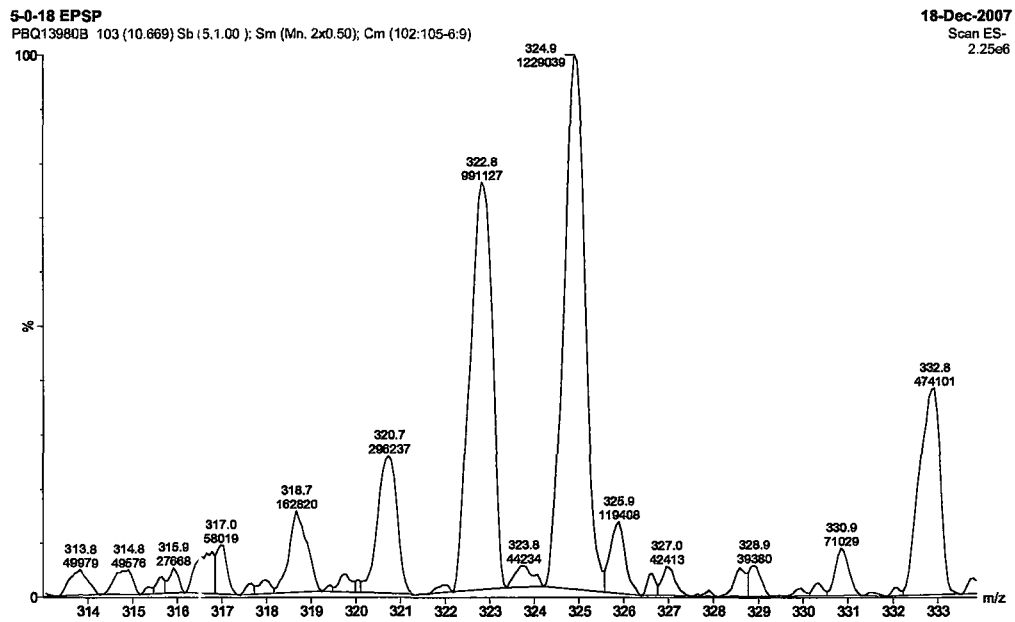


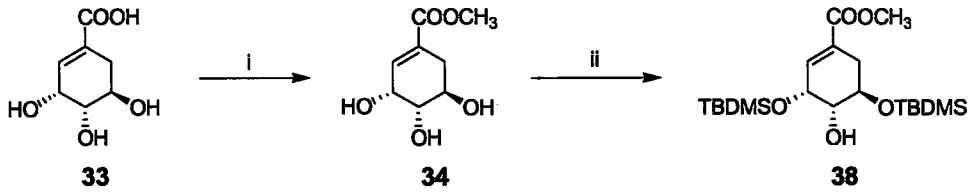
FIGURE 17. Mass spectral analysis of [5-^{18}O]EPSP

The molecular weight of EPSP is 323 Da (^{16}O), thus we expected a molecular weight of 325 Da for the labelled compound. The ratio is calculated by the peak areas of (^{18}O) /{(^{18}O) + (^{16}O)}, which equalled 55.4% [5-^{18}O]EPSP. The [5-^{18}O]EPSP was synthesized by Grace Lou.

2.3 Synthesis of 4-deoxyshikimic acid (51):

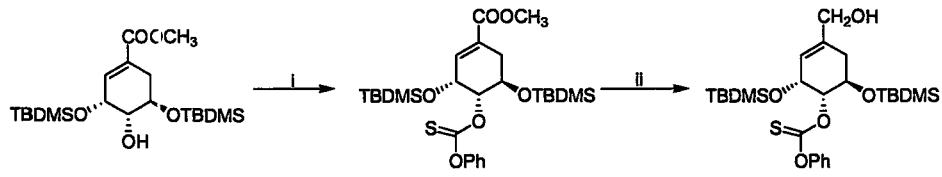
The third shikimate analog, 4-deoxyshikimic acid **51**, was also used for the synthesis of 4-deoxyS3P to test a hypothesis by Schonbrunn et al.[23] Abell and his group has synthesized this compound according to the route shown in (SCHEMES 11 & 12).[34] In our hands, Abell's synthesis from shikimic acid allowed for the preparation of compounds **38** to **40**, as described in (SCHEMES 11 & 13). Methyl shikimate **34** was selectively diprotected on the C-3 and C-5 hydroxyls using *tert*-butyldimethylsiyl chloride (TBSCl) leaving the C-4 hydroxy group in **38** free to react

with phenylchlorothionoformate to form the thionocarbonate **39.** Reduction of the ester **39** to the alcohol **40** was achieved using DIBAL-H at –78 °C.[34]



33 **34** **38**

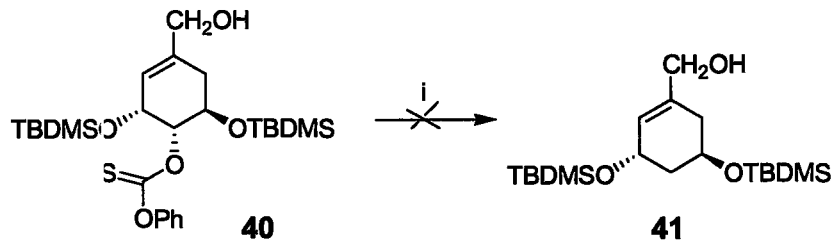
SCHEME 11. Reagents and conditions: i. $SOCl_2$, MeOH, rt, overnight, 90%. ii. TBSCl, DMAP, Bu_3NI, Et_3N, DMF, 3 h, 62%.[34]



38 **39** **40**

SCHEME 12. Reagents and conditions: i. ClC=SOPh, DMAP, rt, 24 h, 50%. ii. DIBALH, $PhCH_3$, 3 h, 95%.[34]

However, dehydroxylation of **40** to form the 4-deoxy compound **41** by thioacylation radical-induced reductive cleavage with tin hydride was not achieved. A mixture of unidentified products was observed.



40 **41**

SCHEME 13. Reagents and conditions: i. AIBN, Bu_3SnH, $PhCH_3$, 2 h.[34]

A general survey of the literature showed that the deoxygenation of alcohols by means of radical scission of corresponding thiocarbonyl derivatives (phenylthiocarbonyl ester, xanthate ester, and thiocarbonyl imidazolide, (SCHEME 13), is known as the Barton-McCombie deoxygenation.[35] The reaction was found to be especially good for hindered secondary alcohols (e.g. compound **38**). It involves conversion of the alcohol to a thiocarbonyl ester followed by radical-induced reductive cleavage of the ester by tributyltin hydride and 2,2'-azobisisobutyronitrile (AIBN).[35]

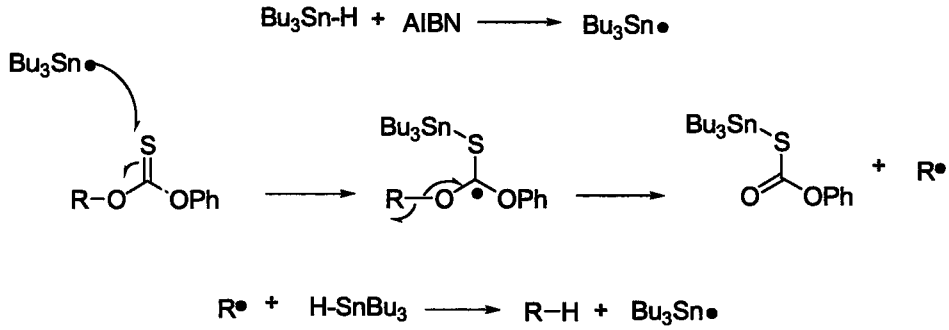
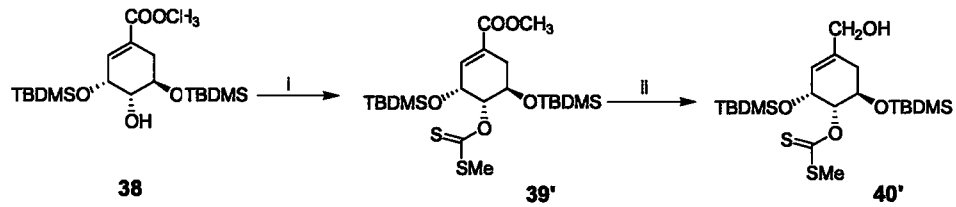


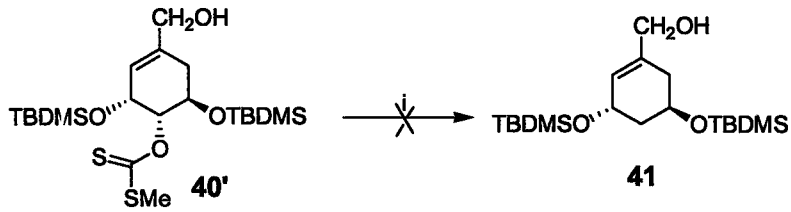
FIGURE 18. Mechanism of the Barton-McCombie reaction.

Since the reduction of the phenylthiocarbonyl ester **40** failed to give the desired result, the next attempt was the introduction of a new thiocarbonyl reagent to form the xanthate ester analog of **40**, compound **40'** as an alternative Barton-McCombie procedure to remove the 4-OH group in **38.** Unfortunately the reduction of **40'** also did not produce the desired result **41.**

2.3.1 Formation and reductive cleavage of xanthate ester (40'):



SCHEME 14. Reagents and conditions: i. MeI, CS_2, NaHMDS, THF, -78 °C, 2 h, 90%. ii. DIBAL-H, $PhCH_3$, 90 °C, 3 h, 95%.[34, 36]



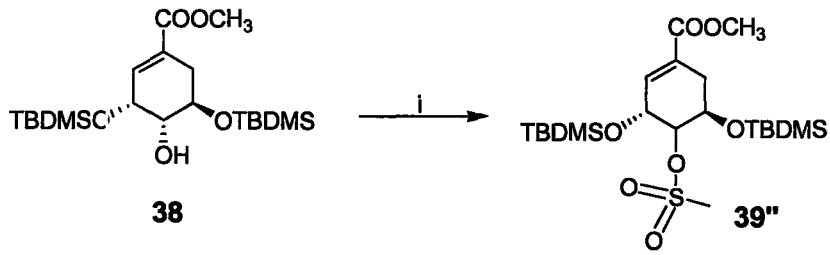
SCHEME 15. Reagents and conditions: i. AIBN, Bu_3SnH, $PhCH_3$, 90 °C, 2 h.[34]

After several unsuccessful optimizations of the reaction conditions of the Barton-McCombie procedure to obtain the desired product, other alternative approaches to the synthesis of the 4-deoxy compound **41** were followed. An explanation for the failure to obtain the desired result with the Barton-McCombie procedure with our substrate may be due to intramolecular cyclization of radical intermediates on the olefin,[37] even though we did not identify the products formed from the reaction.

2.3.2 Mesylation and subsequent reduction:

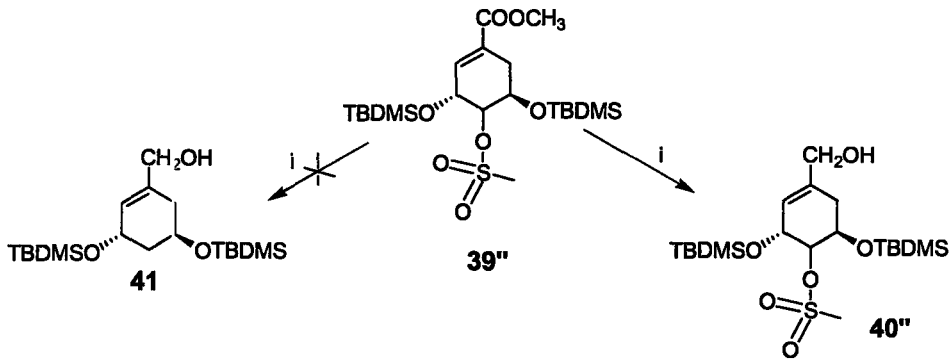
An alternative method of removing the hydroxyl group at C-4 of **38** involves transformation of the free hydroxy group in compound **38** into a good leaving group

(by mesylation) and subsequent displacement with hydride. The mesylation of compound **38** was successful but the S_N2 displacement of the mesylate **39"** by the hydride ion to form compound **41** (SCHEME 16) was not observed. A possible conformational problem in **39"** may prevent this S_N2 process. As expected, the only observed product was the reduction of the ester in compound **39"** to the alcohol **40"** (SCHEME 17).



38 **39"**

SCHEME 16. Reagents and conditions: i. MsCl, py, rt, 12 h, 90%.



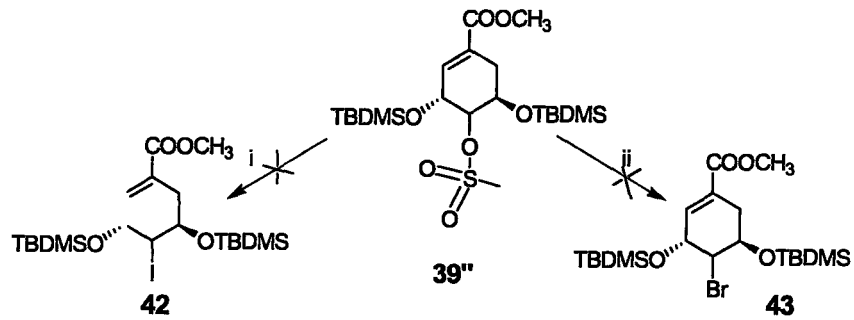
41 **39"** **40"**

SCHEME 17. Reagents and conditions: i. $LiAlH_4$, THF, 24 h, 85%[39]

2.3.3 Nucleophilic halide substitution:

Nucleophilic halide substitution of the mesylate **39"** to form compounds **42** and **43** were also unsuccessful (SCHEME 18). This result is consistent with the possibility that the conformation of compound **39"** does not allow the nucleophiles (I^- and Br^-) to

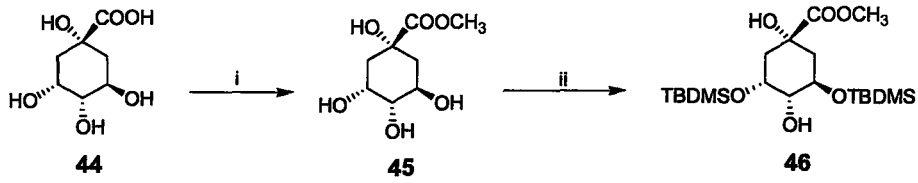
reach the σ* orbital of the carbon carrying the leaving group. The large TBS groups on compound **39''** can lock the conformation of the ring in an equatorial conformer that will be unfavourable for an S_N2 transformation.



SCHEME 18. Reagents and conditions: i. NaI, acetone, 30 °C, 72 h. ii. NaBr, DMF, 100 °C, 48 h.[40]

2.3.4 Synthesis from (-)-quinic acid:

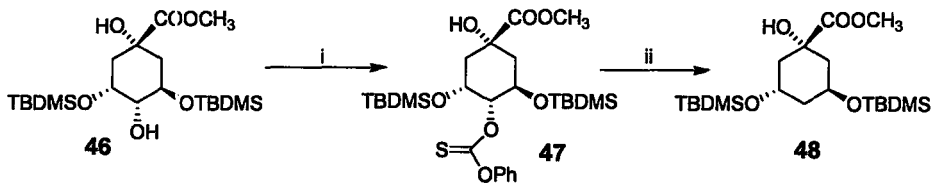
Another alternative way to the target 4-deoxy shikimate was the use of (-)-quinic acid **44** (SCHEMES 19-26). This method was used by Gotor and co-workers[41] in the synthesis of methyl 4-deoxyshikimate **50**, a key precursor in the synthesis of 1α, 25-dihyroxy-19- norprevitamin D_3, an analogue of the hormone 1α, 25-dihydroxy vitamin D_3.[42] Although this procedure also leads to the application of the Barton McCombie reaction, the substrate **48** has no double bond that may undergo intramolecular cyclization with the radical intermediates formed in the reaction. The esterification of (-)-quinic acid **44** with HCl in methanol provided the methyl quinate **45** in a quantitative yield that was diprotected at the C-3 and C-5 hydroxyls to form compound **46.**



44 45 46

SCHEME 19. Reagents and conditions: i. HCl, MeOH, 40 °C, overnight, 100%. ii. TBSCl, Et_3N, DMF, 16 h, 87%.[41]

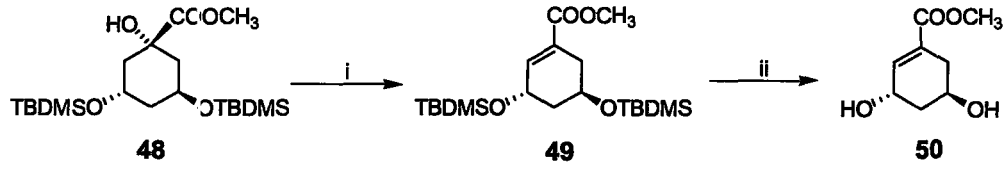
The secondary alcohol in **46** was selectively reacted to form the phenoxythionocarbonate **47** with the corresponding chloride in DMAP. This was in contrast to the thioimidazole derivative used by Gotor and his group. The phenyl chlorothionoformate was added slowly in order to avoid reaction at the tertiary hydroxyl group.[34]



46 47 48

SCHEME 20. Reagents and conditions: i. ClC=SOPh, DMAP, rt, 24 h, 40%. ii. AIBN, Bu_3SnH, $PhCH_3$, 3h, 68%.[34]

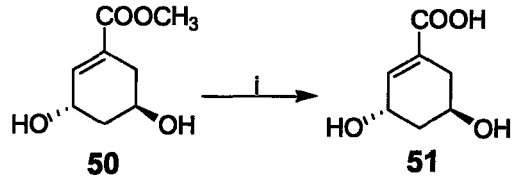
Although the introduction of the phenoxythionocarbonate was successful at the C-4 hydroxyl, the yield (40%) was very poor and a large amount of starting material was recovered. Tin radical cleavages of the thionocarbonate **47** followed by dehydration of the tertiary alcohol in compound **48** with $POCl_3$ in pyridine led to the 4-deoxy compound **49.**



48 49 50

SCHEME 21. Reagents and conditions: i. $POCl_3$, py, rt, 10 h, 83%. ii. 1% HCl, EtOH, 98%.[34, 41]

Desilylation and hydrolysis afforded the target compound **51**.



50 51

SCHEME 22. Reagents and conditions: i a. NaOH, H_2O, overnight. b. 1M HCl.

Compound 51 was then converted enzymatically to 4-deoxyS3P using shikimate kinase and ATP by Paul Chindemi.

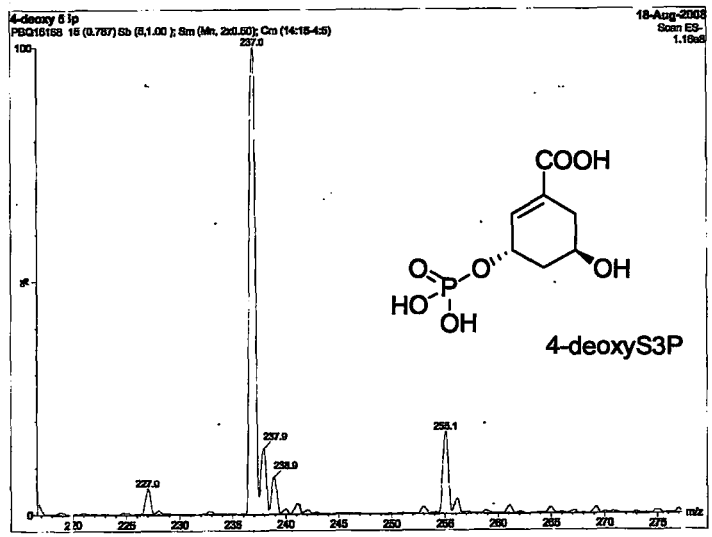


FIGURE 19. Mass spectrometry of 4-deoxyS3P. The molecular weight of 4-deoxyS3P is 238 Da, *m/z* (ESMS -ve) 237 (M-H).

3.0 Synthesis of cationic mimics (5), (14), (15), and (18)

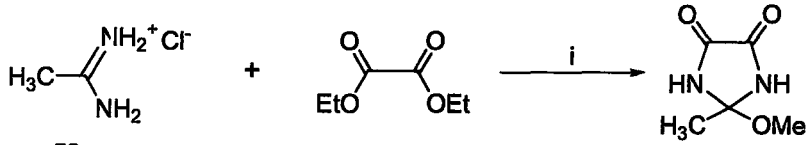
3.1 Synthesis of amidinoformic acid (5):

Amidinoformic acid[42] was the first cationic mimic to be synthesized in designing our bipartite inhibitors. The compound was synthesized from 1,1-diamino-2,2-dinitroethene (DADNE) **57** by Hervé et al., (FIGURE 20).

57 **5**

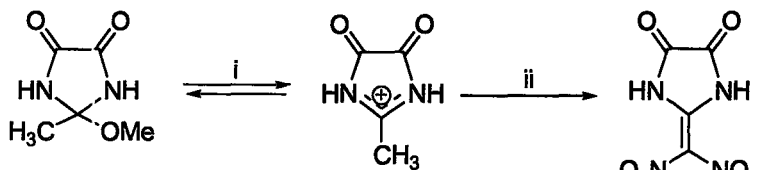
FIGURE 20. Synthesis of amidinoformic acid.

DADNE (also known as FOX-7) is not commercially available and was therefore synthesized as described by Latypov et al. (SCHEMES 23-26).[43] Starting from acetamidine hydrochloride **52** and diethyl oxalate **53**, compound **56** was successfully synthesized from nitration of compound **55** in 80–100% sulfuric acid at room temperature.



52 **53** **54**

SCHEME 23. Reagents and conditions: i. Na, CH_3OH, 3 h, 85%.[43]



54 **55** **56**

SCHEME 24. Reagents and conditions: i. H_2SO_4, ii. HNO_3, 30 min., rt, 61%.[43]

In the nitration of 2-methylimidazolidine-4,5-dione **55**, it was shown that the nitration of the methyl group is activated by the enolization through the imino group of compound **55** in sulfuric acid.[43] This tautomerization (imine-enamine) is necessary for the formation of the gem-dinitro group as illustrated in the suggested mechanism (FIGURE 21).

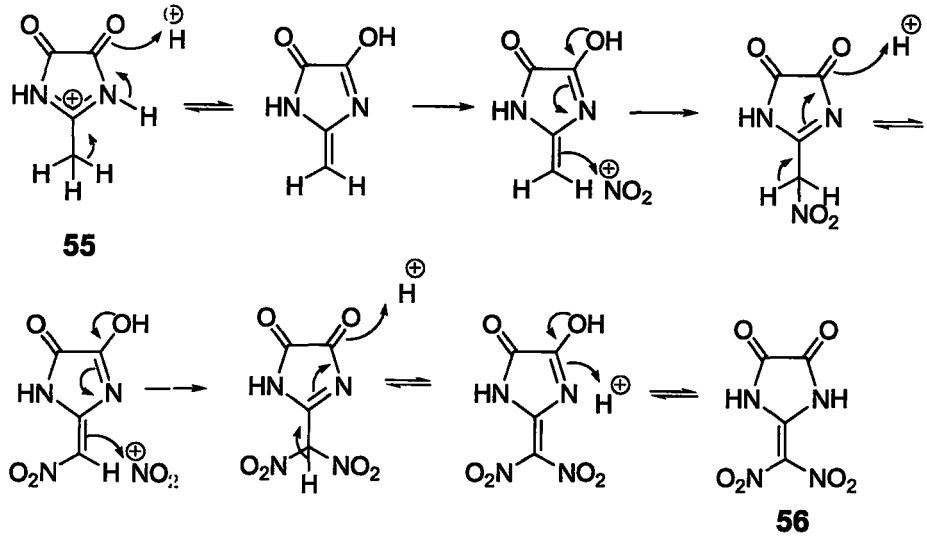
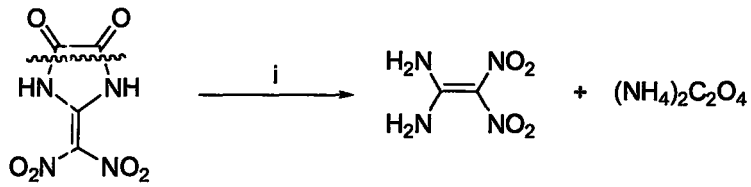


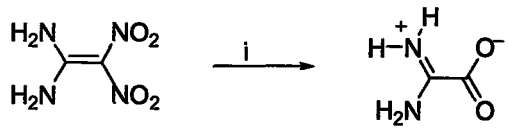
FIGURE 21 Mechanism for the formation of the gem-dinitro group in compound 56[43]

Addition of compound **56** to water and neutralizing with aqueous ammonia at pH 8-9 resulted in a fast dissolution and formation of the target compound **57**, from which amidinoformic acid was synthesized by oxidation in a mixture of hydrogen peroxide (30% H_2O_2) and concentrated sulfuric acid (SCHEME 26).



56 57

SCHEME 25. Reagents and conditions: i. NH_3 (aq), rt, 5 min., 75%.[43]



57 5

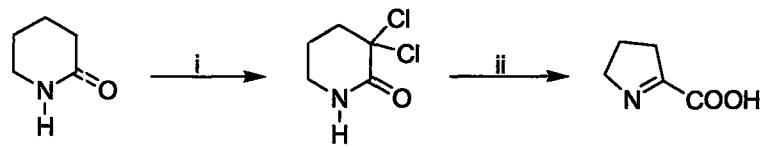
SCHEME 26. Reagents and conditions: i. 30% H_2O_2, H_2SO_4, rt, 18 h, 85%.[44]

The resulting product was a white solid which has a very low solubility in all solvents tested including water and DMSO, making full analytical characterization of amidinoformic acid difficult. The low solubility may be rationalized by the formation of salt bridges between positive and negative charges on the molecule. We were able to obtain the ^1H NMR spectrum in DMSO. The infrared (IR) spectrum and melting point were also obtained. The insolubility of this compound in most solvents implies that it is unlikely to inhibit EPSPS at concentrations achievable in aqueous solution.

3.2 Synthesis of Pyrroline-2-carboxylic acid (18):

Pyrroline-2-carboxylic acid, **18**, is one of the compounds that will serve as our five membered ring cationic mimics whose effect we wish to test in the enzyme catalyzed reaction. Wright et al.[46] have shown that it could be synthesized by the hydrolysis 3,3-dichloro-2-piperidone **59** with hot barium hydroxide solution. The

synthesis of **59** was achieved by treating commercially available 2-piperidone **58** with phosphoryl chloride, phosphorus pentachloride and thionyl chloride following a procedure by Rickenbacher and co-workers[45] in their synthesis of a similar compound.



58 **59** **18**

SCHEME 27. Reagents and conditions: i. $POCl_3$, PCl_5, $SOCl_2$, 0-40 °C, 8 h, 50%. ii a. $Ba(OH)_{2\,(aq)}$, overnight.[45, 46] b. 1M H_2SO_4.

The mechanism of this reaction is still being debated, and it is believed to occur through an imino chloride or equivalent intermediate.[46] Despite the simple procedure in the synthesis of **59**, the reaction sequence leads to a very messy TLC at the end of the reaction and hence the product was very tedious to purify. Treatment of compound **59** with hot 10% aqueous barium hydroxide solution and subsequent acidification with 1.0 M H_2SO_4 yielded the acid **18** after filtration of the insoluble $BaSO_4$ formed. The product, a red-orange liquid, was also difficult to purify. Purification using 1% acetic acid, 9% methanol in DCM removed some impurities. Due to the fact that literature data on this specific compound is sparse, full characterization (^1H, ^{13}C NMR and high resolution mass spectrometry) was undertaken to confirm the synthetic product. The isomeric structure of compound **18,** pyrroline-5-carboxylic acid **19** is synthesized by a different procedure that will be done later.

3.3 Synthesis of 3H-1,2,3-triazole-4-methylamine (15):

The triazole **15** as well as the tetrazole **14** may serve as effective bioisosteres of the carboxylic acid in designing our bipartite inhibitors. 1,2,3-triazoles are formed from the cycloadditions between azide and acetylenes.[47] Two synthetic routes were applied for the synthesis of this compound. Both routes relied on the same starting material but with different synthetic approaches. In the first route, the target was compound **62** as a direct starting material on which 1,3–dipolar cycloaddition with NaN_3 should give the desired product **15**.

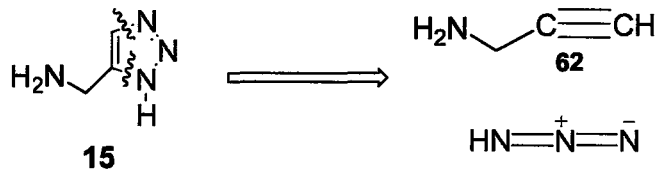
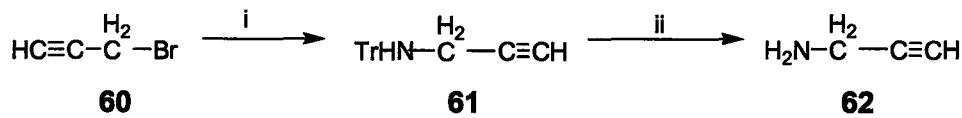


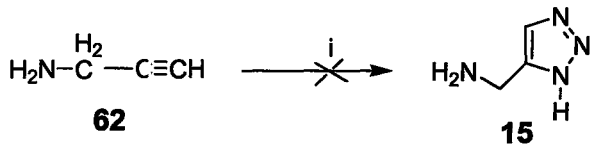
FIGURE 22. Retrosynthetic analysis of compound 15

As a starting material, tritylamine was alkylated with propargyl bromide **60** according to the procedure of Dimitriou and co workers[48] to form the tritylated amino acetylene **61**, which was detritylated to give the propargyl amine **62**.



SCHEME 28. Reagents and conditions: i. $TrNH_2$, CH_3CN, rt, 72 h, 50%. i. CF_3COOH, CH_2Cl_2, rt, 10 min. 70%.[48]

Reacting **62** with sodium azide catalyzed by NH_4Cl in DMF resulted in a white unidentified solid product, but not the desired product **15**.



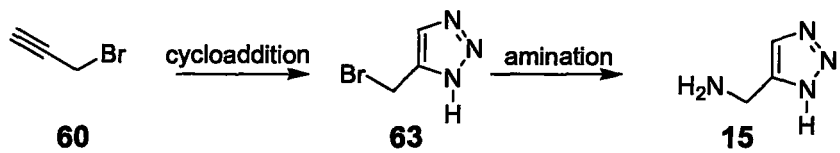
62 15

SCHEME 29. Reagents and conditions: i. NaN_3, NH_4Cl, DMF, reflux, 100 °C, 24 h. 70%.[49]

Using compound **62** as a starting material for this reaction was based on my own retrosynthetic analysis of the target compound **15** (FIGURE 22) since literature concerning this specific reaction is sparse. However, if reaction conditions are optimized, this reaction should work effectively by a direct azide cycloaddition to the C-C triple bond.

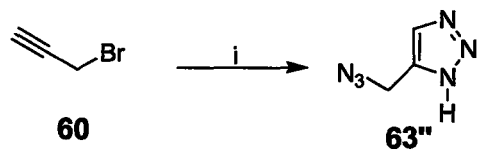
3.3.1 Synthesis from propargyl bromide:

In the second route the 1,3–dipolar cycloaddition with NaN_3 was done directly onto the propargyl bromide **60** relying on the nucleophilicity of ammonia to reach the final target **15**.



60 63 15

Reacting propargyl bromide with sodium azide (NaN_3), NH_4Cl, and DMF at reflux, led to the formation of an oily product after preparative column chromatography. Spectral analysis of the product including HRMS confirmed the formula $C_3H_5N_6$ and absence of Br, which conforms to compound **63"**, instead of compound **63**.



SCHEME 30. Reagents and conditions: i. NaN_3, NH_4Cl, DMF, reflux, 100 °C, 24 h, 77%.

This result can be rationalized by the fact that the nucleophilic azide ion (N_3^-) can easily displace the bromide in an S_N2 reaction, but this would lead to the formation of propargyl azide **60'** (FIGURE 23), if it was the only reason.

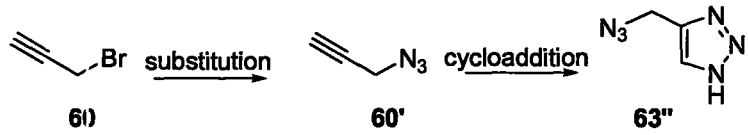
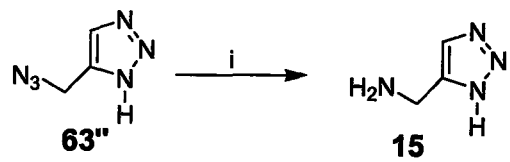


FIGURE 23. Conversion of propargyl bromide to triazoles 63"

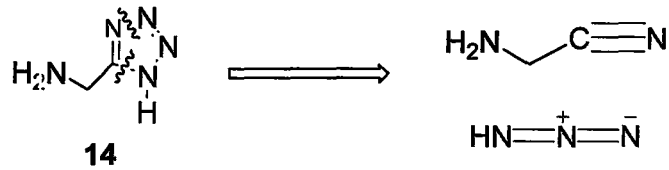
It is also obvious that a direct displacement reaction coupled with azide cycloaddition will lead to the formation of compound **63".** However the formation of **63"** is in agreement with the proposed mechanism for the conversion of propargyl azide to 1,2,3-triazoles, by Klaus Banert.[47] Catalytic hydrogenation of compound **63"** furnished compound **15** as a white solid.



SCHEME 31. Reagents and conditions: i. H_2 / Pd, rt overnight, 80%.[50]

3.4. Synthesis of 1H-tetrazole-5-methylamine (14):

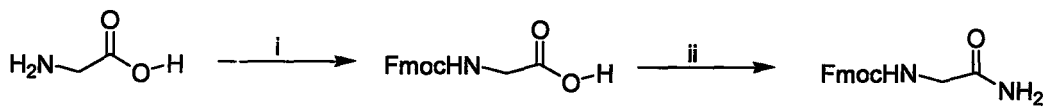
1H-tetrazole-5-methylamine **14** was synthesized by azide cycloaddition onto a nitrile, similar to the triazole **15**. The synthesis of compound **14** was reported by Chennacrishnareddy and co workers[51] using the amino acid glycine as a starting material.



14

FIGURE 24. Retrosynthetic analysis of compound 14.

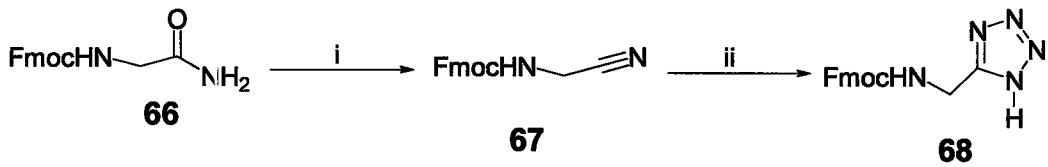
The synthesis began with protection of the amino group of glycine **64** with Fmoc-Osu, which gave a quantitative yield. Fmoc was a good choice for the protection process since it can be easily removed under mild basic conditions.[51] Amidation of **65** to the amide **66** was achieved with NH_4HCO_3, applying Vladimir's procedure[53] by activating the carboxylate group of the starting material with Boc anhydride.



64 **65** **66**

SCHEME 32. Reagents and conditions: i. Fmoc-Osu, $NaHCO_3$, dioxane, 0-rt, overnight, 100%[52] ii. NH_4HCO_3, Boc_2O, Py, DMF, rt, 5 h.[53]

Purification was quite tedious and as a consequence, the compound was used unpurified to the next step. Dehydration of the amide to the nitrile **67** was achieved using trifluoroacetic anhydride. This reagent was found to be the best, since DCC mediated dehydration required the use of pyridine as solvent, which does not support the chemistry of the Fmoc group.[51]



66 **67** **68**

SCHEME 33. Reagents and conditions: i. $(CF_3CO)_2O$, Py, dioxane, 3 h.[54] ii. NaN_3, H_2O/2-propanol, 80 °C, 16 h.[51]

The nitrile was treated with sodium azide and catalytic amount of zinc bromide in water/2-propanol mixture at reflux to form the 1,2,3,4-tetrazole, compound **68**. Although the ^1H and ^{13}C spectral analysis of compound **68** seemed to show the desired compound, the mass spectra did not show the expected m/z of the compound. It will be necessary to analyze compound **68** carefully in order to continue the synthesis to the target compound **14**, which is one step away from **68** (i.e. the deprotection of the Fmoc group of **68** with diethyl amine in dichloromethane).

4.0 Conclusion

In summary, we have synthesized dideoxyshikimic acid and three cationic mimics, which will be tested in the enzyme-catalyzed reaction to aid in understanding the features that are needed for tight binding to EPSPS. In addition, we have successfully synthesized [5-^{18}O]EPSP and 4-deoxyS3P, which were needed for further studies to understand the enzyme catalysis in detail. Future efforts should be aimed at testing these mimics together with those that are commercially available. This will help to ascertain the importance of cationic mimics in the enzyme active site and will as well help define clearly the features that are important for binding EPSPS. Doing this will successfully take us a step towards designing potent inhibitors of EPSPS.

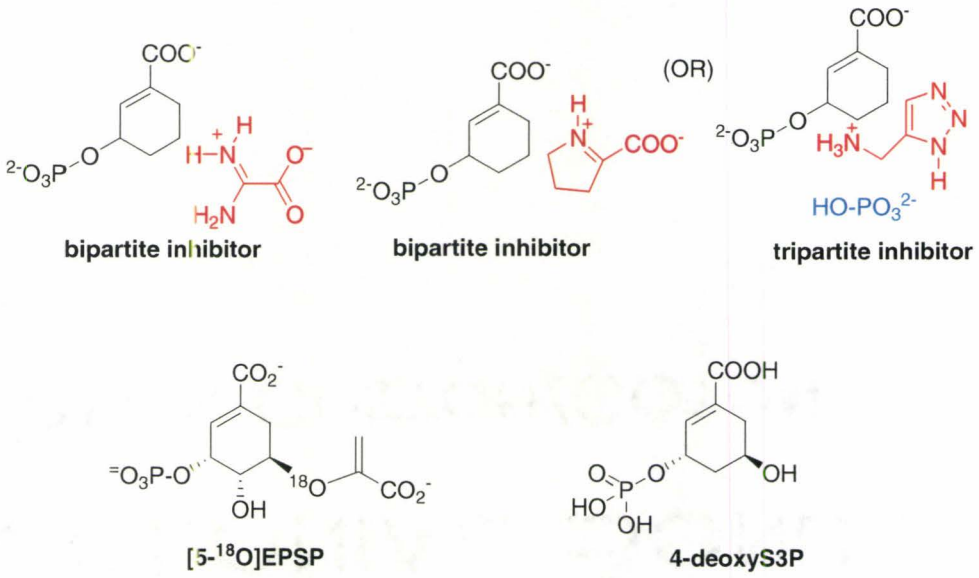


FIGURE 25. Summary of synthesis

5.0 Future work:

All inhibitors will be tested in the presence and absence of inorganic phosphate (Pi), with rates measured for the reverse reaction by other members of the lab group. Coupled reaction with pyruvate kinase/ lactate dehydrogenase to oxidize NADH will give the analytical signal (ΔA_{340}) and the thermodynamic driving force to run the reaction in the reverse direction. Future work will be needed to complete the synthesis of the remaining cationic mimics in (CHART 4) and their effects in the enzyme active site will also be tested. This will provide the answers to our questions and hence the necessary information we need to design inhibitors of EPSPS.

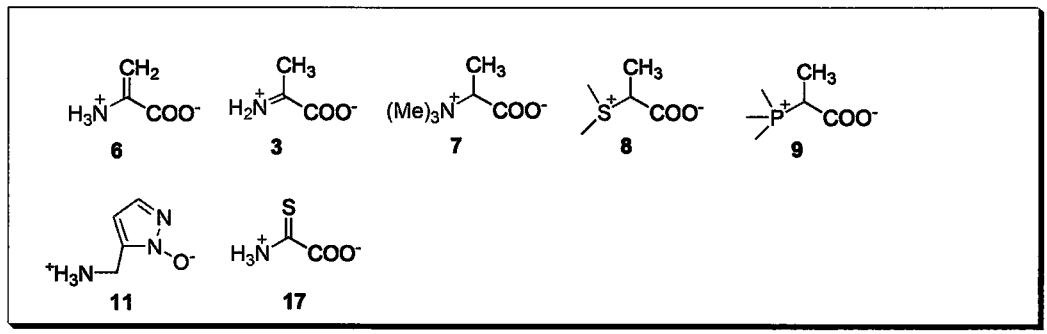


Chart 4. Cationic mimics left to be synthesized.

A long-term goal after exploring the features important for binding to EPSPS with these inhibitors, if successful, is to investigate the possibility of elaborating them to create potent inhibitors of EPSPS.

6.0 Experimental methods:

6.1 General:

Unless stated otherwise, all reactions were performed in flame-dried glassware under an argon atmosphere, and magnetically stirred using freshly distilled solvents. All reagents were commercially obtained from Sigma-Aldrich, Canada, and used as received. Organic preparative column chromatography purification was carried out using silica gel (60A, 40-60 mesh). Thin-layer chromatography was performed using aluminum-backed plates coated with silica gel 60 (Machery-Nagel Alugram®, Sil G/UV_{254}, 0.20 mm) and visualized using a combination of UV fluorescence and p-anisaldehyde staining. The solvents used for NMR analysis: $CDCl_3$, D_2O, and DMSO-d6 were supplied by Cambridge Isotopes and Isotec. ^1H NMR (200.23 MHz) ^{13}C NMR (50.35 MHz) and ^{31}P NMR (80.01 MHz) spectra were obtained on a Bruker DRX – 200 NMR spectrometer. Chemical shifts (δ) are reported in ppm relative to internal TMS (1H and 13C, δ 0.00 ppm). Coupling constants (*J*) are expressed in Hz. Mass spectra (CIMS) were obtained on a Micromass Quattro Ultima spectrometer fitted with a direct injection probe (DIP) with ionization energy set at 70 eV and HRMS (CI) were performed with a Micromass Q-Tof Ultima spectrometer.

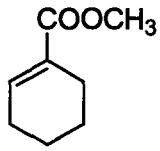
ES-HRMS were obtained using a Kratos Concept 1S double focusing mass spectrophotometer interfaced to a Kratos DART acquisition system optically linked to a SUN SPARC workstation.

6.2 Solvent preparation:

All solvents were obtained from commercial suppliers and were used without further purification except as indicated below. THF, toluene, and dioxane were distilled from sodium metal with benzophenone indicator; dichloromethane and chloroform were distilled over calcium hydride.

6.3 Reactions:

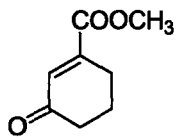
Methyl cyclohex-1-ene carboxylate 23:



Cyclohex-1-ene carboxylic acid (2.085g, 14.90 mmol)[25] was dissolved in ether (1mL) and an ethereal solution of CH_2N_2 (75 mL) was added in small portions, until gas evolution ceased and the solution was pale yellow in colour. The solvent was evaporated under reduced pressure to afford (1.97 g, 85 %) as an oily product.

^{1}H NMR ($CDCl_3$) δ 7.09 (m, 1H), 3.83 (s, 3H), 2.27-2.29 (m, 4H), 1.66-1.80 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 176.2, 146.6, 138.3, 58.2, 30.5, 30.9, 28.8, 28.2. CI (HRMS) calculated for $C_8H_{13}O_2$, $[M]^+$, 140.0874, found 140.0874.

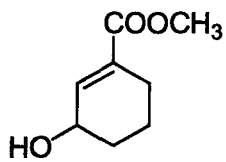
Methyl 3-oxocyclohex-1-ene carboxylate 24:



Methyl cyclohex-1-ene carboxylate **23** (380 mg, 2.71 mmol),[26] K_2CO_3 (187 mg, 1.36 mmol) and $Rh_2(cap)_4$ (0.85 mg, 0.0027 mmol) were dissolved in CH_2Cl_2 (10 mL). To the mixture was added TBHP (2.27 mL, 13.60 mmol) in one portion via syringe and the colour of the solution immediately turned from light to deep purple. After 3 hours, the solution was filtered through a short plug of silica gel to remove the catalyst. Preparative column chromatography using 30% ethyl acetate in hexane as the eluent afforded an analytically pure compound (216 mg, 50%) as an oily product.

^{1}H NMR ($CDCl_3$) δ 6.74 (s, 1H), 3.79 (s, 3H), 2.52 (t, J = 6 Hz, 2H), 2.16 (t, J = 8.6 Hz, 2H), 1.65-1.95 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 190.2, 172.5, 158.3, 139.9, 59.6, 44.5, 31.6, 28.9. CI (HRMS) calculated for $C_8H_{10}O_3$, $[M]^+$, 154.0630, found 154.0630.

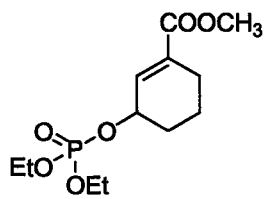
Methyl 3-hydroxycyclohex-1-ene carboxylate 25:



$NaBH_4$ (96 mg, 2.59 mmol)[24] was dissolved in methanol (1 mL), in a 50 mL flask equipped with a stir bar. The ketone **24** (100 mg, 0.65 mmol), also dissolved in methanol (1 mL), was added to the flask slowly. The reactants were mixed slowly to

prevent over heating on an ice bath. After 46 h of stirring and monitoring by TLC, the mixture was quenched with 1M HCl to destroy excess $NaBH_4$. The aqueous solution was extracted with DCM. The methylene chloride extract was dried with saturated sodium chloride solution and anhydrous sodium sulphate. The solvent was evaporated under reduced pressure. Preparative column chromatography (SiO_2) using 10% ethyl acetate in DCM afforded an analytically pure compound (89 mg, 88%). ^1H NMR ($CDCl_3$) δ 6.86 (d, J = 2.0 Hz, 1H), 4.34 (m, 1H), 3.72 (s, 3 H), 2.23 (s, 1H), 1.77 (m, 6 H). ^{13}C NMR ($CDCl_3$) δ167.9, 139.9, 131.1 66.1, 52.0, 31.3, 26.4, 19.2. CI (HRMS) calculated for $C_8H_{13}O_3$, $[M]^+$, 156.0874, found 156. 0929.

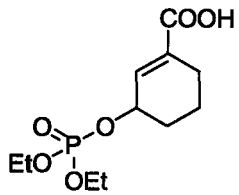
3-(methoxylcarbonyl)cyclohex-2-enyl diethyl phosphate 26:



Methyl 3-hydroxycyclohex-1-ene carboxylate **25** (150 mg 0.96 mmol)[24] was dissolved in ether (1 mL) at 0-5 °C. Simultaneously from two syringes, diethyl phosphochloride (0.4 mL, 2.89 mmol) and methylimidazole (0.3 ml, 1.92 mmol) were added slowly while stirring. The mixture was stirred for 3h. After the reaction was completed, the mixture was washed with 10% NaOH solution and water (20 mL) and dried with anhydrous sodium sulphate. The solvent (DCM) was evaporated under reduced pressure. Preparative column chromatography using 10% ethyl acetate in

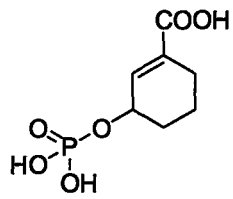
dichloromethane afforded an analytically pure compound. (223 mg, 80%). ^{1}H NMR ($CDCl_3$) δ 6.86 (m, 1H), 4.98 (m, 1H), 4.14 (m, 4H), 3.75 (s, 3H), 2.25 (m, 6H), 1.77 (t, J = 6.0 Hz, 6H). ^{13}C NMR ($CDCl_3$) δ 174.0, 142.6, 141.1, 78.6, 70.6, 58.7, 35.9, 30.8, 25.4, 22.9. ^{31}P NMR ($CDCl_3$) δ 2.64. CI (HRMS) calculated for $C_{12}H_{22}O_6P$, $[M]^+$, 292.1118, found 292.1118.

1-cyclohexene-1-carboxylic acid, 3-phosphodiester 29:



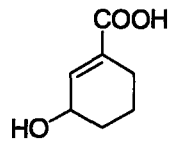
The phosphotriester **26** (200 mg, 0.68 mmol)[24] was dissolved in water, and a solution of K_2CO_3 (284 mg, 2.05 mmol,) in water (1 mL) was added. The solution was stirred for 72 h at 50°C. After the reaction was complete, the mixture was acidified with 1.0 M HCl, to afford the crude product (173 mg, 90%). ^{1}H NMR ($CDCl_3$) δ 7.85 (s, 1H), 6.97 (s, 1H), 4.38 (s, 1H), 4.07 (m, 4H), 2.24 (s, 2H), 1.88 (m, 4H), 1.34 (t, J = 6.8 Hz, 6H). ^{13}C NMR ($CDCl_3$) δ 171.5, 140.6, 131.5, 65.6, 62.2, 31.1, 24.1, 19.3, 15.6. ^{31}P NMR ($CDCl_3$) δ 4.54.

4,5-dideoxyshikimate 3-phosphate 31:



Diethyl phosphate **29** (100 mg, 0.35 mmol)[27] was treated with trimethylsilyl bromide (0.4 mL, 3.50 mmol). The mixture was heated in dichloromethane (4 mL) at 40°C under argon for 20h. The solvent was evaporated and water (2 mL) was added. The mixture was stirred overnight at room temperature. The water was evaporated under reduced pressure. Purification by ion exchange chromatography afforded the product (49 mg, 60%). ^{1}H NMR (D_2O) δ 6.45 (s, 1H), 4.41 (br s, 1H), 2.23 (br s, 2H), 2-1.5 (br m, 4H), ^{13}C NMR (D_2O) δ 171.3, 140.1, 132.3, 65.3, 29.6, 23.5, 18.4. ^{31}P NMR (D_2O) δ 5.28. *m/z* (ESMS) 222.6.

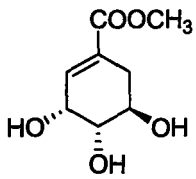
3-hydroxycyclohex-1-enecarboxylic acid 32:



Methyl 3-hydroxycyclohex-1-ene carboxylate, **25**, (180 mg, 0.12 mmol)[24,26] in 5% solution of NaOH in H_2O (1 mL) was stirred overnight. The mixture was acidified with 1M HCl. The solvent was evaporated under reduced pressure to afford 4,5-dideoxyshikimic acid **32**. ^{1}H NMR (D_2O) δ 6.83 (br s, 1H), 4.38 (br s, 1H), 2.17-1.68

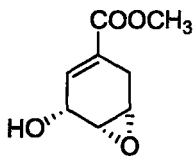
(br m, 6H), ^{13}C NMR (D_2O) δ 170.9, 140.7, 132.1, 65.5, 29.8, 23.6, 18.7. EI (HRMS) calculated for $C_7H_{10}O_3$, [M^+], 142.0589 found 142.0589.

(3R,4S,5R)-3,4,5-Trihydroxy-1-cyclohexene-1-carboxylate methyl ester 34:



Shikimic acid **33** (500 mg, 2.75 mmol)[31], was dissolved in methanol, and $SOCl_2$ (0.375 mL, 5.25 mmol) was added dropwise during 1 h at 0 °C. The reaction mixture was warmed to room temperature and stirred overnight. Removal of the solvent and recrystallization from ethyl acetate afforded methyl shikimate (520 mg, 96 %) as a white solid. ^1H NMR (D_2O) δ 6.78 (m, 1H), 4.41 (m, 1H), 3.98 (td, 1H), 3.73 (s, 3H), 3.70 (m, 1H), 2.72 (dd, J = 15.4, 5.6 Hz, 1H), 2.19 (dd, J = 17.8, 4.4 Hz, 1H). ^{13}C NMR (D_2O) δ 168.8, 137.0, 129.4, 70.9, 66.4, 65.6, 52.4, 30.0. CI (HRMS) calculated for $C_8H_{12}O_5$, [M]$^+$, 188.0687, found 188.0687.

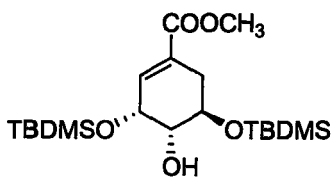
Methyl *cis*-3-Hydroxy-4,5-oxycyclohex-1-enecarboxylate 35:



Triphenylphosphine[22,29] (170 mg, 0.64 mmol) and methyl shikimate (110 mg, 0.59 mmol) were dissolved in THF, and the mixture was cooled to 0 °C under argon. Dimethyl azodicarboxylate (94 mg, 0.64 mmol) was added dropwise with stirring. The

mixture was kept at 0 °C for 30 min and then at room temperature for 1 h. The solvent was removed under reduced pressure. The residue was distilled (Kugelrohr), and distillate coming over up to 165 °C was collected. The distillate was dissolved in a minimum amount of diethyl ether, cooled, filtered, and the filtrate was concentrated. Preparative column chromatography using diethyl ether afforded compound **35**, (52 mg, 50%) as an oily product that crystallized on being allowed to stand. ^{1}H NMR ($CDCl_3$) δ 6.71 (br s, 1 H), 4.56 (m d, J = 10.7 Hz, 1 H), 3.76 (s, 3 H), 3.55 (br s, 2 H), 3.01 (md, J = 19.9 Hz, 1 H), 2.60 (d, J = 10.6 Hz, 1 H), 2.48 (md, J = 19.9 Hz, 1 H). ^{13}C NMR ($CDCl_3$) δ 167.2, 136.0, 130.2, 65.7, 65.7, 54.7, 52.3, 24.4. CI (HRMS) calculated for $C_8H_{10}O_4$, $[M]^+$, 170.0579, found 170.0579.

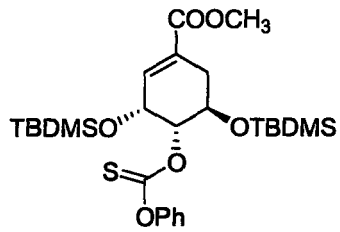
Methyl(3*S*,4*S*,5*S*)-3,5-bis(*tert*-butyldimethylsilyloxy)-4-hydroxycyclohex-1-enecarboxylate 38:



Tert-butyldimethylsilyl chloride[34] (895 mg, 5.90 mmol) was added under argon at 0 °C to a stirred solution of methyl shikimate **34** (520 g, 2.77 mmol), DMAP (95 mg, 0.77 mmol) and Bu_4NI (102 mg, 0.27 mmol) in dry DMF (4.5 mL) and dry triethylamine (0.8 mL, 6.08 mmol). The solution was stirred at this temperature for 1 h and then 3 h at room temperature. The resultant suspension was eluted with ethyl

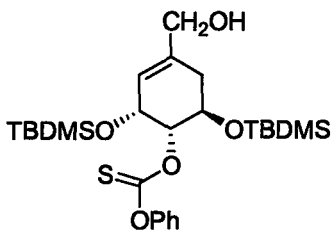
acetate and filtered over Celite. The solution was washed with 1 M HCl and brine, dried (Na_2SO_4), filtered and evaporated. The yellow residue was purified by flash chromatography, eluting with 20% diethyl ether in hexane to yield the disilyl ether (800 mg, 69%) as a white solid. The spectral data was identical to that reported.[34] CI (HRMS) calculated for $C_{20}H_{41}O_5Si_2$, $[M]^+$, 417.2464, found 417.2493.

Methyl(3*S*,4*S*,5*S*)-3,5-bis(*tert*-butyldimethylsilyloxy)-4-phenoxythiocarbonyloxycyclohex-1-enecarboxylate 39:



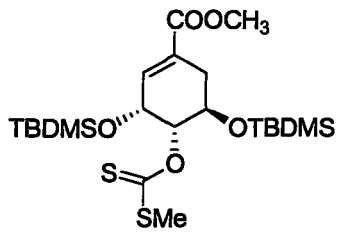
Phenyl chlorothionoformate (0.16 mL, 1.16 mmol) was added slowly during 1 h, to a stirred solution of the alcohol **38** (400 mg, 0.96 mmol) and DMAP (177 mg, 1.45 mmol) in dry acetonitrile (9.6 mL) under argon. The yellow solution was stirred at room temperature for 24 h. The solvent was evaporated, the crude redissolved in diethyl ether and washed with HCl, and brine, dried (Na_2SO_4), filtered and evaporated. The residue was purified by flash chromatography eluting with 10% diethyl ether in hexane to yield the thionocarbonate **39** (250 mg, 47%). The spectral data was identical to that reported.[34] CI (HRMS) calculated for $C_{27}H_{44}O_6SSi_2$, $[M]^+$, 552.2390, found 552.2390.

(3*S*,4*S*,5*S*)-3,5-Bis(*tert*-butyldimethylsilyloxy)-1-hydroxymethyl-4-phenoxythiocarbonyloxycyclohex-1-ene 40:



1.0 M diisobutylaluminum hydride in toluene (0.3 mL, 0.32 mmol)[34] was added to a stirred solution of the ester **39** (80 mg, 0.14 mmol) in dry toluene (1.6 mL) under argon at -78 °C. The mixture was stirred for 15 min and then quenched with water. The organic layer was extracted with diethyl ether. The combined organic layers were dried (Na_2SO_4), filtered and evaporated under reduced pressure. The residue was purified by flash chromatography, eluting with 25% diethyl ether in hexane to yield alcohol **40** (70 mg, 92%). The spectral data were identical to those reported.[34] CI (HRMS) calculated for $C_{26}H_{43}O_5Si_2S$, $[M]^+$, 523.2350, found 523.2370.

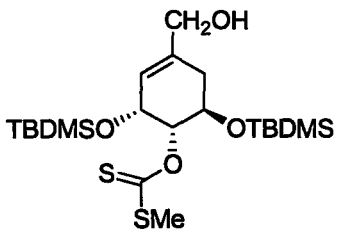
Methyl(3*S*,4*S*,5*S*)-3,5-bis(*tert*-butyldimethylsilyloxy)-4-methoxythiocarbonyloxycyclohex-1-enecarboxylate 39':



Carbon disulfide[36] (0.3 mL, 9.0 mmol) was added to a solution **38** (150 mg, 0.36 mmol) in dry THF (2 mL) then sodium bis(trimethylsilyl)amide (NHMDS) (1M

in THF, 0.3 mL, 0.36 mmol) was added under argon at –78 °C. The reaction mixture was stirred gradually warming up to –40 °C for 1 h, then cooled to –78 °C and MeI (0.3 mL, 0.36 mmol) was added at –78 °C and the solution was stirred at –78 °C for 1 h. The mixture was poured into saturated aqueous ammonium chloride and extracted with ethyl ether. The organic layer was washed with brine, dried (Na_2SO_4), filtered and evaporated. The residue was purified by flash chromatography eluting with 20% diethyl ether in hexane to yield xanthate ester, **39'** (170 mg, 93%). ^{1}H NMR ($CDCl_3$) δ 6.76 (d, J = 1.6 Hz, 1 H), 5.70 (dd, J = 8.9, 3.8 Hz, 1 H), 4.77 (t, 1 H), 4.39 (m, 1 H), 3.74 (s, 3 H), 2.75 (dd, J = 15.5, 5.3 Hz, 1 H), 2.52 (s, 3 H), 2.30 (dd, J = 18.4, 4.8 Hz, 1 H), 0.92 (s, 9 H), 0.88 (s, 9 H), 0.16, (s, 3 H), 0.15, (s, 3 H), 0.14 (s, 3 H), 0.12 (s, 3 H). ^{13}C NMR ($CDCl_3$) δ 215.0, 167.1, 134.1, 129.7, 82.0, 65.5, 65.2, 52.2, 32.0, 25.9, 19.2, 18.1, -4.6, -4.7. CI (HRMS) calculated for $C_{22}H_{42}O_5Si_2S_2$, $[M]^+$, 506.2011, found 506.2011.

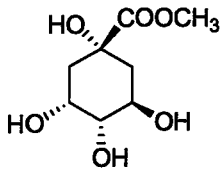
(3*S*,4*S*,5*S*)-3,5-Bis(*tert*-butyldimethylsilyloxy)-1-hydroxymethyl-4-methoxythiocarbonyloxycyclohex-1-ene 40':



1.0 M diisobutylaluminum hydride in toluene[34] (0.6 mL, 0.59 mmol) was added to a stirred solution of the ester **39'** (150 mg, 0.30 mmol) in dry toluene (2 mL) under

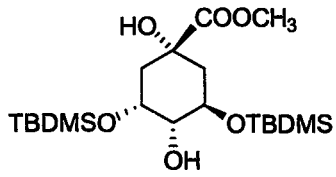
argon at –78 °C to – 40 °C. The mixture was stirred for 2 h and then quenched with water. The organic layer was extracted with diethyl ether. The combined organic layers were dried (Na_2SO_4), filtered and evaporated under reduced pressure. The residue was purified by flash chromatography, eluting with 25% diethyl ether in hexane, to yield alcohol **40'** (70 mg, 92%). ^{1}H NMR ($CDCl_3$) δ 5.68 (m, 2 H), 4.62 (t 1 H), 4.43 (q, J = 14.8, 7.2, 6.0 Hz, 1 H), 4.05 (d, J = 5.8 Hz, 2 H), 2.61 (s, 3 H), 2.52 (dd, J = 17.2, 6.2 Hz, 1 H), 2.12 (dd, J = 17.2, 8.8 Hz, 1 H), 0.88 (s, 9 H), 0.87 (s, 9 H), 0.11 (s, 6 H), 0.06 (s, 6 H). ^{13}C NMR ($CDCl_3$) δ 215.2, 122.2, 84.4, 65.7, 66.1, 65.5, 34.9, 26.0, 25.9, 19.4, 18.2, -4.4, -4.5, -4.6 -4.8. CI (HRMS) calculated for $C_{21}H_{42}O_4Si_2S_2$, $[M]^+$, 478.2070, found 478.2070.

Methyl-1, 3, 4, 5-tetrahydroxycyclohexanecarboxylate 45:



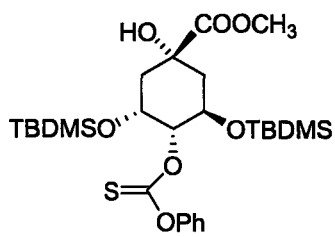
Concentrated HCl (3 drops)[41] was added to a suspension of (-)- quinic acid (1 g, 5.2 mmol) in methanol (30 mL). The resulting solution was stirred overnight at 40 °C. The solvent was evaporated under reduced pressure to afford compound **45**. The spectral data were identical to those reported.[41]

Methyl(3*S*,4*S*,5*S*)-3,5-di[(tert-Butyldimethylsiyl)oxy]-1,4-dihydroxycyclohexanecarboxylate 46:



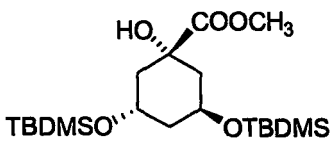
Tert-butyldimethylsilyl chloride[41] (1.85 g, 12.3 mmol) was added to a stirred solution of methyl (-)-quinate **45** (1.00 g, 4.9 mmol) and triethylamine (0.7 mL, 4.9 mmol) in dry DMF (10 mL) at 0 °C under argon. After 1 h, the solution was allowed to warm to room temperature and stirred overnight. The solvent was evaporated. Water was added and the resulting suspension was extracted with Et_2O. The organic layer was dried over Na_2SO_4, filtered and concentrated. Preparative column chromatography using 20% EtOAc in hexane afforded compound **46** after vacuum drying (1.6 mg, 87%) as a white solid. The spectral data were identical to those reported.[33]

Methyl (3*S*,4*S*,5*S*)-3,5-di[(tert-Butyldimethylsiyl)oxy]-1-hydroxy-4-phenoxythiocarbonyloxycyclohexanecarboxylate 47:



Phenyl chlorothionoformate[34] (0.34 mL, 2.53 mmol) was added slowly during 1 h to a stirred solution of compound **46** (1 g, 2.3 mmol) and DMAP (422 mg, 3.45 mmol) in dry acetonitrile (10 mL) under argon. The resulting solution was stirred at room temperature for 24 h. 1.0 M HCl (10 mL) was added and the crude mixture was extracted with diethyl ether, The organic layer was dried with Na_2SO_4, filtered and evaporated. The residue was purified by flash chromatography eluting with 20% EtOAc in hexane to yield thionocarbonate **47** (500 mg, 40%) with 40% recovery of starting material. ^1H ($CDCl_3$) δ 7.40 (t, J = 7.0 Hz, 2 H), 7.28 (m, 1 H), 7.02 (d, J = 7.4 Hz, 2 H), 5.16 (d, J = 9.4 Hz, 1 H), 4.65 (s, 1 H), 4.55 (ddd, J = 5.0, 4.2, 5.4 Hz, 1 H), 3.75 (s, 3 H), 2.27 (ddd, J = 12.8, 10.8, 11.8, Hz, 1 H), 2.11 (d, J = 15.4 Hz, 1 H,), 1.99 (dd, J = 13.0, 12.0, 1 H), 0.99 (s, 9 H), 0.94 (s, 9 H), 0.2, 0.15, 0.12 (m, 6 H), 0.10 (s, 6 H). ^{13}C ($CDCl_3$) δ 195.5, 173.9, 153.8, 130.0, 127.1, 122.4, 88.1, 69.9, 65.5, 53.2, 44.3, 38.2, 26.1, 18.4, 18.3, -3.1, -4.2, -4.4 -4.7. CI (HRMS) calculated for $C_{26}H_{43}O_7SSi_2$, $[M\text{-}Me]^+$ 555.2447, found 555.2447.

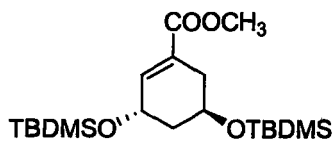
Methyl(3*S*,5*S*)-3,5-di[(*tert*-butyldimethylsilyl)oxy]-1-hydroxycyclohexanecarboxylate 48:



A solution of thiocarbonate **47** (200 mg, 0.35 mmol)[34] in dry toluene (8 mL) was added dropwise during 1 h to a refluxing solution of Bu_3SnH (0.7 mL, 2.5 mmol) and

AIBN (58 mg, 0.35 mmol) in dry toluene (15mL) under argon. The solution was refluxed for 3 h. The solvent was evaporated and the crude was purified by flash chromatography eluting with dichloromethane to yield the 4-deoxy compound **48** (100 mg, 68%) as an oily product. ^{1}H ($CDCl_3$) δ 4.78 (br s, 1 H), 4.40 (m, 1 H), 4.32 (m, 1 H), 3.76 (s, 3 H), 2.25-1.95 (m, 4 H), 1.71 (dd, J = 12.8, 10.9 Hz, 1H), 1.50 (ddd, J = 13.3, 10.9, 2.3 Hz, 1 H) 0.90 (s, 9 H), 0.88 (s, 9 H), 0.11 (s, 3 H), 0.10 (s, 3 H), 0.07 (s, 3H), 0.05 (s, 3 H). ^{13}C ($CDCl_3$) δ 174.1, 76.2, 69.8, 63.5, 52.7, 44.8, 42.3, 38.5, 26.0, 25.8, 18.3, 18.2, -4.1, -4.5, -5.3 -5.4. CI (HRMS) calculated for $C_{20}H_{43}O_5Si_2$, $[M]^+$ 419.2659, found 419.2649.

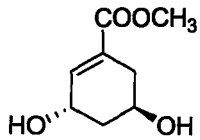
(3*S*,5*S*)-3,5-di[(*tert*-butyldimethylsilyl)oxy]methylcyclohex-1-ene 49:



Phosphoryl chloride[41] (0.2 mL, 0.25 mmol) was added slowly to a solution of compound **48** (100 mg, 0.24 mmol) in pyridine (1.6 mL) at 0 °C. The resulting solution was stirred at room temperature overnight. NH_4Cl (aq) was added at 0 °C to quench the reaction, and the extracted with Et_2O. The organic layer was dried over Na_2SO_4. The solvent was evaporated and the crude mixture was purified by flash chromatography eluting with dichloromethane to yield compound **49** (80 mg, 83%). ^{1}H NMR ($CDCl_3$) δ 6.79 (m, 1H), 4.54 (m, 1H), 4.19 (m, 1H), 3.74 (s, 3H), 2.54 (dd, J = 17.8, 4.5 Hz, 1H), 2.15 (dd, J = 17.8, 5.6 Hz, 1H), 2.03 (ddd, J = 12.9, 7.9, 4.9 Hz,

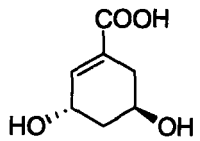
1H), 1.69 (ddd, J = 12.9, 6.4, 2.8 Hz, 1H), 0.90 (s, 9 H), 0.87 (s, 9 H), 0.09 (s, 3 H), 0.07 (s, 3 H), 0.06 (s, 3H), 0.05 (s, H). ^{13}C ($CDCl_3$) δ 167.8, 140.3, 128.9, 65.6, 65.2, 53.6, 39.9, 33.9, 26.0, 18.2, -4.6. CI (HRMS) calculated for $C_{20}H_{40}O_4Si_2$, $[M]^+$, 400.2465, found 400.2456.

(3*S*,5*S*)-3,5-Dihydroxymethylcyclohex-1-enecarboxylate 50:



Compound **49** (80 mg, 0.2 mmol)[34] was stirred for 4 h in a 1% solution of HCl in ethanol (1.6 mL). The solvent was evaporated under reduced pressure and the residue was purified by flash chromatography eluting with 10% MeOH in DCM to yield compound **50** (33 mg, 98 %). ^1H NMR (MeOD) δ 6.84 (s, 1H), 4.50 (m, 1H), 4.16 (m, 1H), 3.93 (s, 3H), 2.77 (dd, J = 12.0 Hz, 1H), 2.19 (dd, J = 12.4, 5.4 Hz, 1H), 2.03 (m, 1H), 1.8 (m, 1H). ^{13}C NMR (MeOD) δ 169.3, 138.7, 129.1, 63.8, 63.7, 52.3, 36.5, 31.6. CI (HRMS) calculated for $C_8H_{12}O_4$, $[M]^+$, 172.0736, found 172.0739.

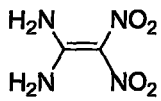
(3*S*,5*S*)-3,5-Dihydroxycyclohex-1-enecarboxylic acid (4-deoxyshikimic acid) 51:



Compound **50** (30 mg, 0.17 mmol) in a 5% solution of NaOH in H_2O (1 mL) was stirred overnight. The mixture was acidified with 1.0 M HCl. The solvent was

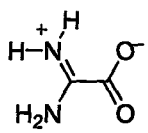
evaporated under reduced pressure to afford 4-deoxyshikimic acid **51**. ^{1}H NMR ($CDCl_3$) δ 6.62 (br s, 1H), 4.56 (m, 1H), 2.61 (dm, J = 15.3 Hz, 1H), 3.71 (m, 1H), 3.63 (s, 3H), 2.57 (dd, J = 5.2 Hz, 1H), 2.15 (dd, J = 11.2, 6.3 Hz, 1H), 1.95 (m, 1H), 1.63 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 176.3, 134.5, 134.0, 64.2, 64.1, 37.8, 33.6. *m/z* (ESMS -ve) 157 (M-H).

1,1 -Diamino-2,2-dinitroethylene 57:



2-(Dinitromethylene)-4,5-imidazoledione[43] (400 mg, 1.98 mmol) was suspended in water (1.6 mL) and 25 % aqueous ammonia (0.64 mL) was added to obtain pH 8-9. The white solid dissolved immediately and in a few seconds bright yellow crystals precipitated. The precipitate was washed with water and dried at 50 °C to give (180 mg, 60 %) of l,l-diamino-2,2-dinitroethylene. The spectral data was identical to that reported.[39]

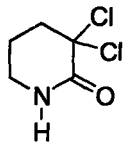
Amidinoformic acid 5:



DADNE **57** (50 mg, 0.3 mmol)44 was rapidly added to a stirred solution containing 30% hydrogen peroxide (0.5 g) and concentrated sulfuric acid (4.4 g) at room temperature. The mixture was kept at room temperature for 18 h and poured into

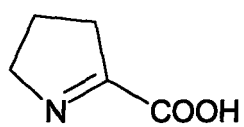
iced water (8 mL). The precipitate was collected by filtration. It was washed twice with cold water and dried over P_2O_5. A white solid was obtained (30 mg, 85%). Mp 273 °C. ^{1}H NMR (DMSO) δ 9.08 (2s 1H, 1H). IR (KBr) 3318, 3023, 1714 (C=N), 1639 (C=O), 1488, 1356, 1130, 1105, 1052 882, 851 cm-1.

3,3-dichloro-2-piperidone 59:



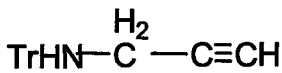
Piperidone **58** (400 mg, 4.08 mmol),[45] was added to phosphoryl chloride (0.8 ml, 8.16 mmol) at 0 °C –10 °C during 30 min. A clear solution was obtained. Phosphorus pentachloride (425 mg, 2.02 mmol) was added also at 0 –10 °C to obtain a suspension. The suspension was stirred for 2 - 3 h at 2 - 5 °C. Sulfonyl chloride (1.1 mL, 16 mmol) was added at 0 °C - 5 °C and stirred another 2 h at 0 - 5 °C. The solution was slowly warmed up to 25 °C. The resulting product was distilled at 40 °C to produce an oily product. Purification with 10% MeOH in DCM afforded compound **59**. ^{1}H NMR (D_2O) δ 3.01-3.35 (m, 4H), 2.50 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 165.9, 82.2, 43.6, 42.4, 19.4. CI (HRMS) calculated for $C_5H_7NOCl_2$, $[M]^+$, 166.9911 found 166.9911.

Pyrroline-2-carboxylic acid 18:



Hot 10% Barium hydroxide[46] solution (1 mL) was added to 3,3-dichloro-2-piperidone (30 mg, 0.18mmol) and the solution was stirred overnight at 40 °C. At the end of the reaction as monitored by TLC, the mixture was acidified with 1.0 M H_2SO_4. The precipitated salt ($BaSO_4$) was removed by filtration; the solvent was evaporated under reduced pressure. The residue was purified on a *combi* flash chromatography eluting with 1% acetic acid, 9% methanol in DCM to yield compound **18** as a red-orange liquid (0.1 mL). ^{1}H NMR (D_2O) δ 0.61-0.96 (m, 2H), 1.92-2.02 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 176.0, 170.5, 61.5, 38.4, 24.0. CI (HRMS) calculated for $C_5H_8NO_2$, $[M]^+$, 114.0525, found 114.0555.

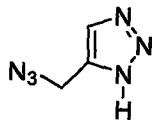
N-tritylprop-2-yn-1-amine 61:



Propargyl bromide48 (1.0g, 8 mmol) was added to a solution of tritylamine47 (414 mg, 1.6 mmol) in CH_3CN (5 mL) and the resulting solution was left at rt for 3 days. The precipitated salt was removed by filtration. The solvent was evaporated under reduced pressure. The residue was purified by column chromatography 50% ether in hexane, to afford compound **61** (300 mg, 70%) as a colourless solid.

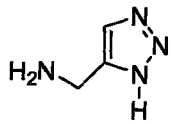
^{1}H NMR ($CDCl_3$) δ 7.75 (d, J = 7.0 Hz, 5 H), 7.36 (m, 10 H), 2.94 (s, 2 H), 2.24 (s, 1H), 2.07 (br s, 1H). ^{13}C NMR ($CDCl_3$) δ 145.2, 128.6, 128.1, 126.6, 82.0, 71.0, 70.9, 33.6.

4-(azidomethyl)-1H-1,2,3-triazole 63'':



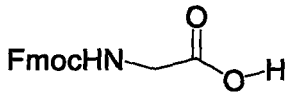
Propargyl bromide (100 mg, 0.84mmol), NaN_3 (273mg, 0.42 mmol) and NH_4Cl, (227mg, 0.42mmol) were dissolved in DMF, and the resulting solution was reflux at 100 °C for 24 h. Solvent was evaporated under reduced pressure and the residue was purified by column chromatography, 50% ether in hexane afforded the desired compound **63** (105 mg, 77%) as an oily product. ^1H NMR ($CDCl_3$) δ 7.69 (s, 1H), 4.55 (s, 2H). ^{13}C ($CDCl_3$) δ 142.4, 130. 3, 45.3. ES-HRMS calculated for $C_3H_5N_6$, $[M]^+$, 125.0577, found 125.0577.

3H-1,2,3-triazole-4-methylamine 15:



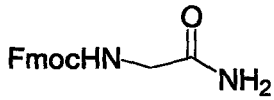
Compound **63''** (40 mg, 0.32 mmol),[50] and 10% palladium-charcoal (10 mg) in methanol (3.5 mL) was stirred overnight under H_2 gas. The catalyst was removed by filtration and washed with methanol. The solvent was evaporated under reduced pressure. The residue was washed with DCM to yield amino triazole **15** (26 mg, 80%) as white solid. ^1H NMR (D_2O) δ 7.62 (s, 1H), 4.06 (s, 2H). ^{13}C NMR ($CDCl_3$) δ 134.1, 127.5, 33.8. CI (HRMS) calculated for $C_3H_7N_4$, 99.0659, $[M]^+$, found 99.0671.

Fmoc-glycine 65:



Glycine[52] (100 mg, 1.33mmol) was dissolved in water and sodium bicarbonate (224 mg, 2.66mmol) was added with stirring. The resulting solution is cooled to 5 °C and Fmoc-OSu (674 mg, 2.00 mmol) was added slowly as a solution in para-dioxane. The resulting mixture was stirred at 0°C for 1 h and allowed to warm to room temperature overnight. Water was then added and the aqueous layer was extracted 2 times with EtOAc. The organic layer was back extracted twice with saturated sodium bicarbonate solution. The combined aqueous layers was acidified to a pH of 1 with 10% HCl, then extracted 3 times with EtOAc. The combined organic layers was dried (Na_2SO_4) and concentrated. The white residue formed was then washed with diethyl ether to afford compound **65** (302 mg, 90%). ^{1}H NMR (DMSO-d6) δ 8.0 (br s, 1H), 7.91 (m, 2 H), 7.73 (m, 1 H), 7.17 (m, 2 H), 4.30 (m, 3 H), 3.66 (dd, J = 6.4 Hz, 2 H). ^{13}C NMR (DMSO-d6) δ 171.6, 156.5, 143.8, 140.7, 127.6, 127.1, 65.7, 46.6, 42.2. *m/z* (ESMS -ve) 296 (M-H).

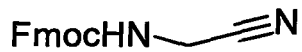
Fmoc-glycinamide 66:



Ammonium hydrogen carbonate[53] (40 mg, 0.51 mmol) was added to a stirred solution of *N*-protected amino acid (100mg, 0.34 mmol), pyridine (0.02 mL), and

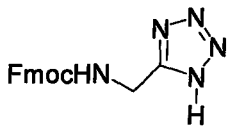
Boc_2O (118.5 mg, 0.50 mmol) in DMF (1 mL) and the mixture was stirred for 4-16 h. Ethyl acetate was added and after washings with water and 5% H_2SO_4 the solution was dried, (Na_2SO_4) the solvent was evaporated and the product was triturated with ether. ^1H NMR (DMSO-d6) δ 7.94 (br s, 1H), 7.90 (m, 2 H), 7.66 (m, 1 H), 7.28 (m, 2 H), 6.20 (br s, 1H), 4.26 (m, 2H), 3.72 (m, 1H), 3.33 (m, 1H). ^{13}C NMR (DMSO-d6) δ 163.16, 157.1, 146.1, 144.7, 141.5, 129.7, 128.4, 128.2, 128.0, 127.6, 126.1, 122.3, 120.9. 120.7, 64.6, 47.5. CI (HRMS) calculated for $C_{17}H_{16}N_2O_3$, $[M]^+$, 296.1088, found 296.1161.

Fmoc-amino nitrile 67:



Trifluoroacetic anhydride[54] (0.012 mL, 0.1mmol) was added dropwise to a stirred ice cooled solution or suspension of the amide (100 mg, 0.34 mmol) in anhydrous dioxane (2 mL) and anhydrous pyridine (0.06 mL, 0.1 mmol) at 0 °C. The pyridinium salt was removed by filtration and the filtrate was diluted with chloroform washed with water and saturated brine, dried with Na_2SO_4 and evaporated under vacuum. ^1H NMR (DMSO-d6) δ 8.0 (br s, 1H), 7.96 (d, J = 10.9 Hz, 2 H), 7.78 (d, J = 6.6 Hz, 2 H), 7.29 (m, 4 H), 4,35 (d, J = 7.4 Hz, 2 H), 3.87 (m, 1H), 3.72 (d, 5.3 Hz, 2 H,). ^{13}C NMR (DMSO-d6) δ 157.8, 143.6, 141.5, 128.1, 127.3, 125.1, 120.3, 114.1, 67.3, 47.2, 31.6. CI (HRMS) calculated for $C_{17}H_{15}N_2O_2$, $[M]^+$, 279.1088, found 279.1088.

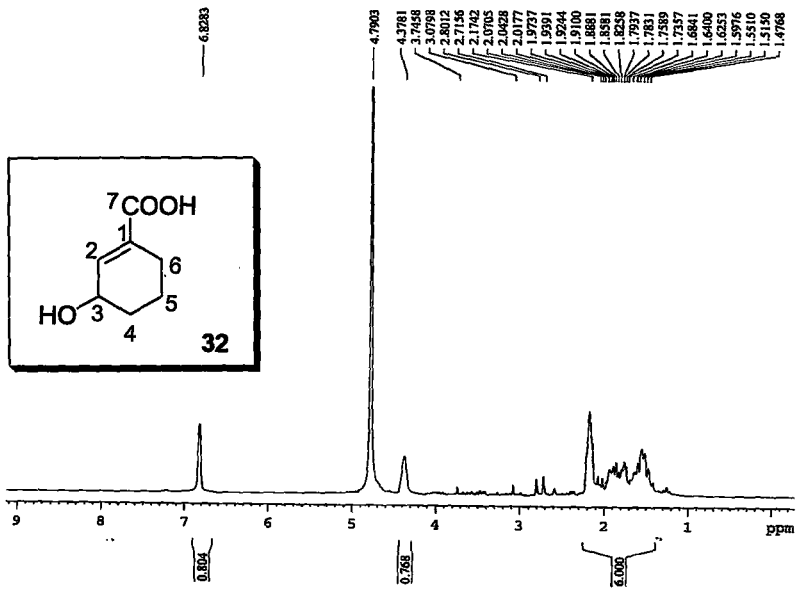
Fmoc-amino tetrazole 68:

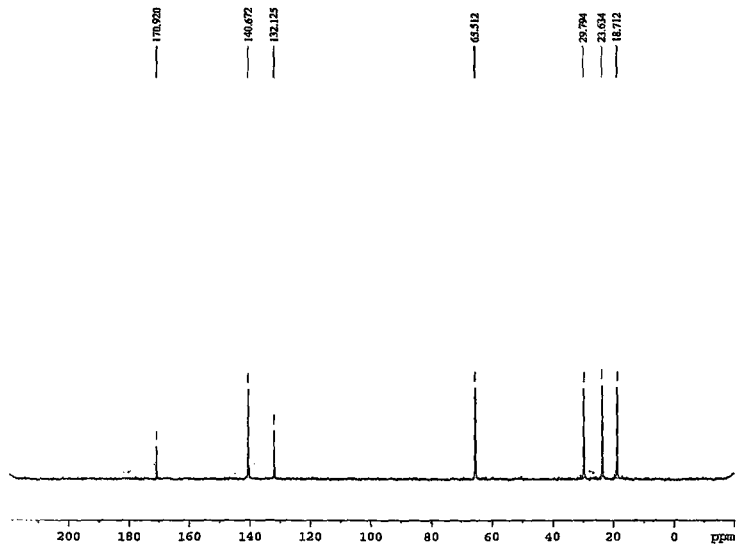


Fmoc-amino nitrile[51] (30 mg, 0.11 mmol), sodium azide (14 mg, 0.22 mmol) and zinc bromide (3 mg, 0.05 mmol) were dissolved in a mixture of 2-propanol (2 mL) and water (4 mL), and stirred at reflux for 16 h. After completion of the reaction (0.5 mL) of 3 N HCl and (1 mL) of ethyl acetate was added and stirring was continued until no solid remained. The aqueous layer was extracted twice with ethyl acetate. The combined organic layer was washed with water and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure and the residue was washed with diethyl ether to yield Fmoc-amino tetrazole **68** (25 mg, 70%). ^{1}H (DMSO-d6) δ 8.01 (br s, 1H), 7.88 (d, J = 7.4 Hz, 2 H), 7.70 (d, J = 6.8 Hz, 2 H), 7.37 (m, 4 H), 4.48 (d, J = 6.0 Hz, 2 H), 4.25 (m, 3 H), 3.32 (s, NH). ^{13}C (DMSO-d6) δ 156.7, 143.6, 140.6, 127.5, 126.9, 125. 0, 120.0, 65.7, 46.4, 34.2.

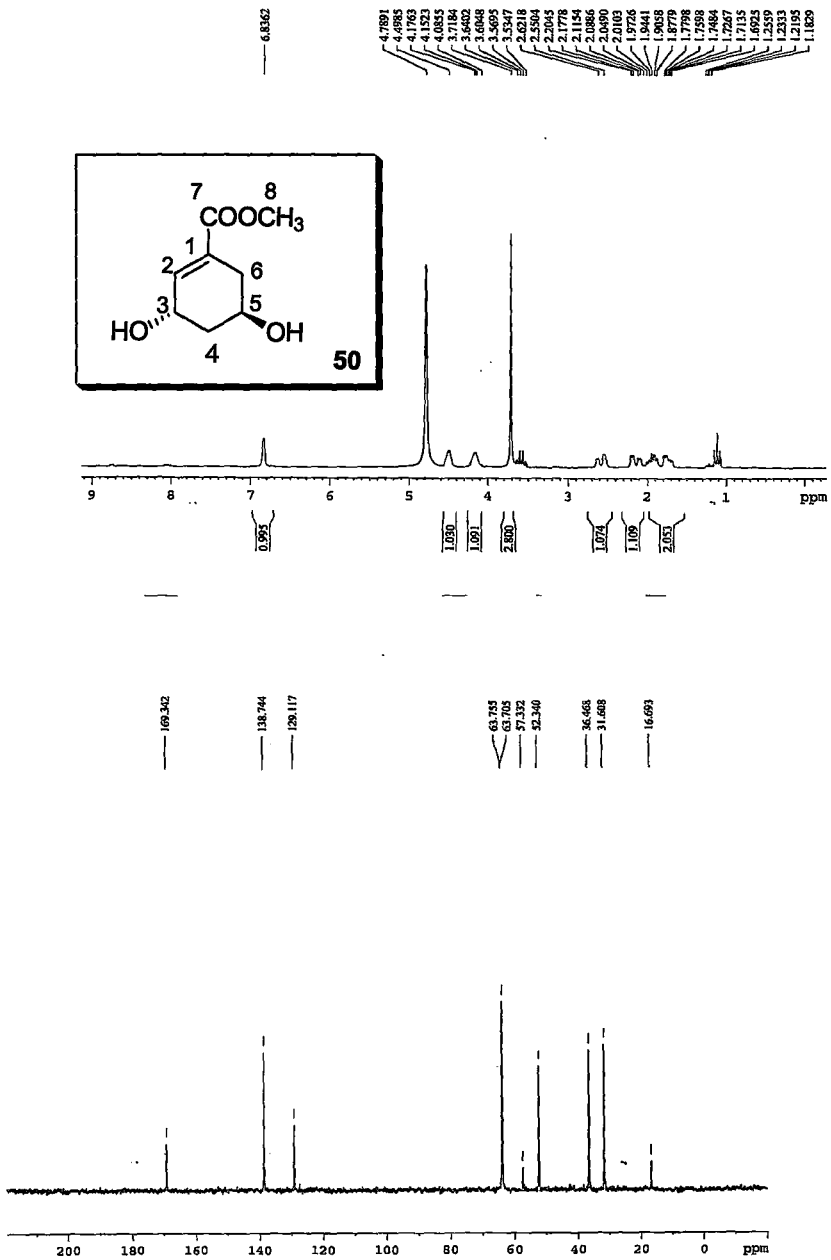
7.0 Appendix

Appendix 1. ^{1}H & ^{13}C NMR spectra of 4,5-dideoxyshikimic acid (32)

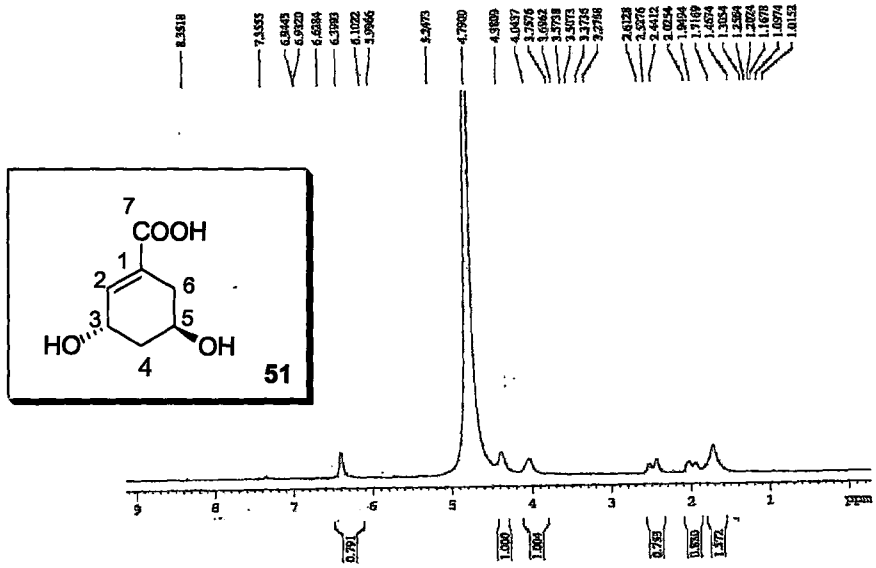


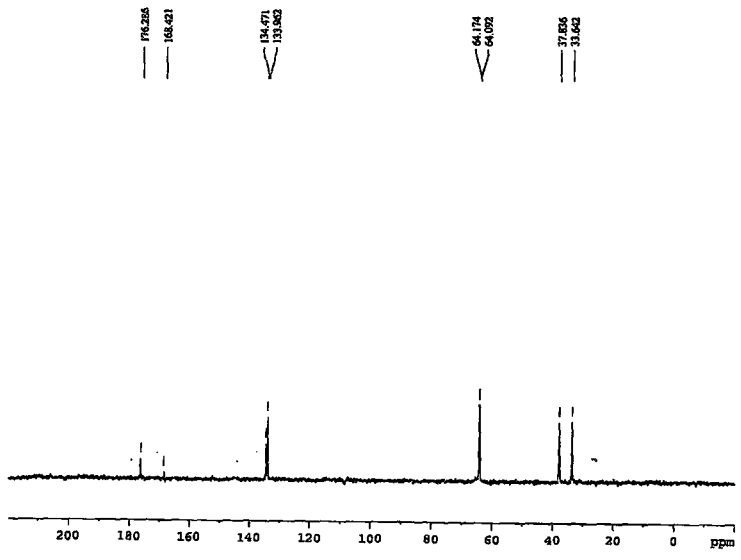


Appendix 2. ^{1}H & ^{13}C NMR spectra of Methyl 4-deoxyshikimic acid (50)

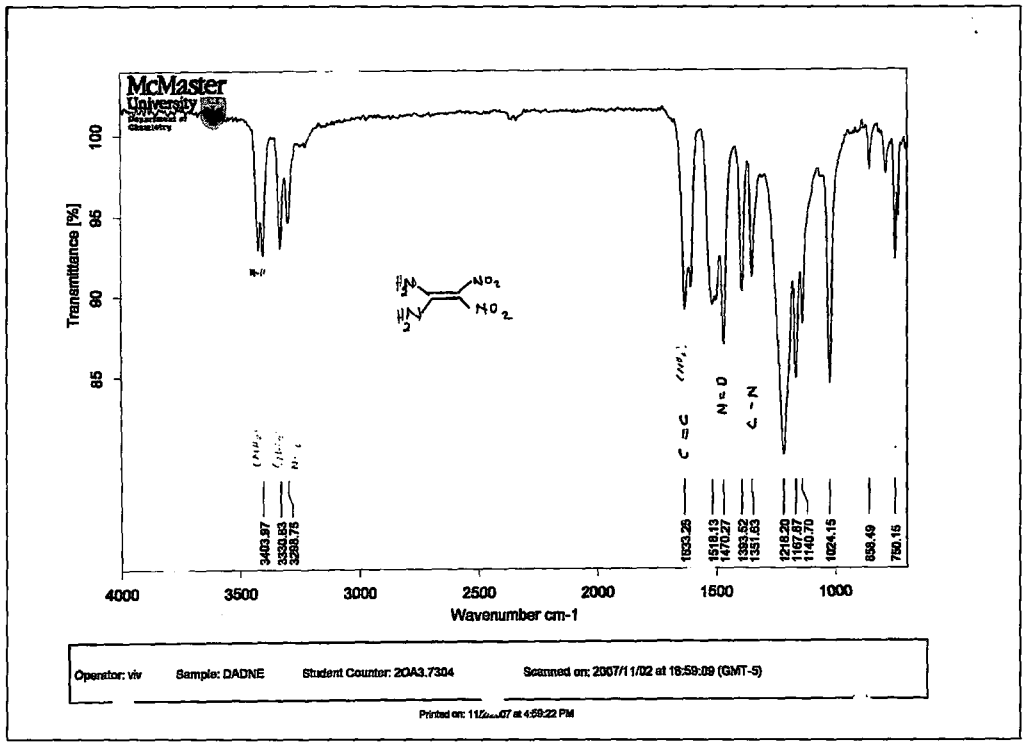


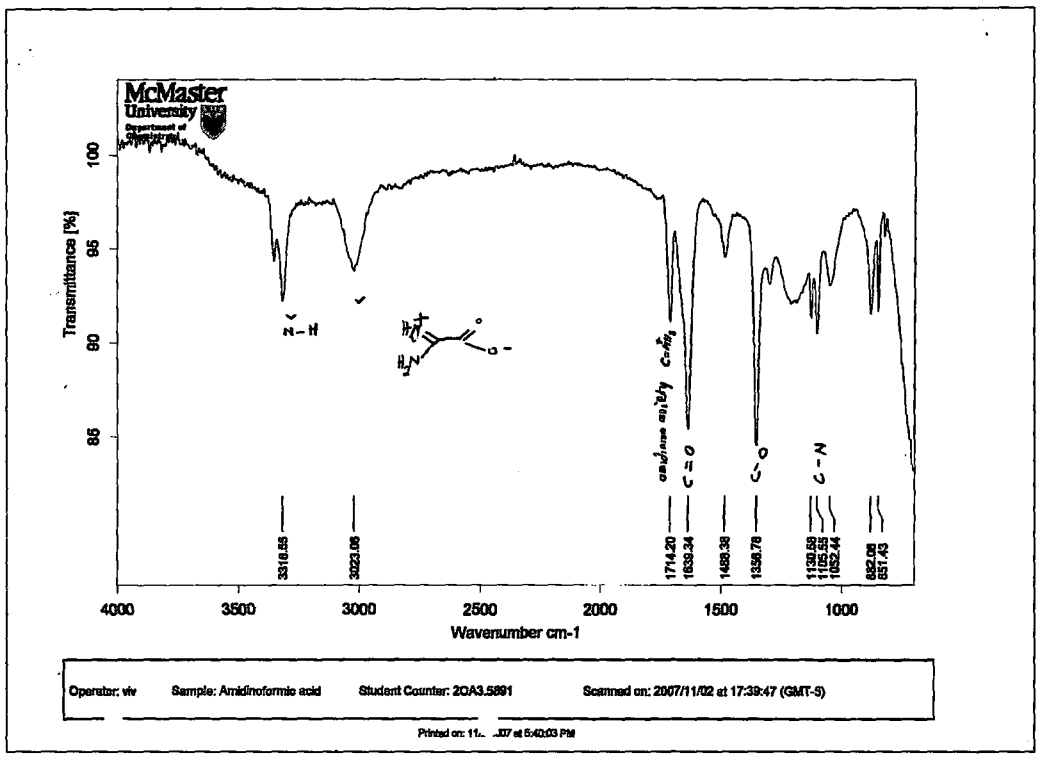
Appendix 3. ^{1}H & ^{13}C NMR spectra of 4-deoxyshikimic acid (51)



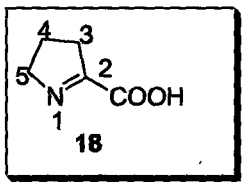


Appendix 4. Infrared spectra (IR) of DADNE and amidinoformic acid (5)

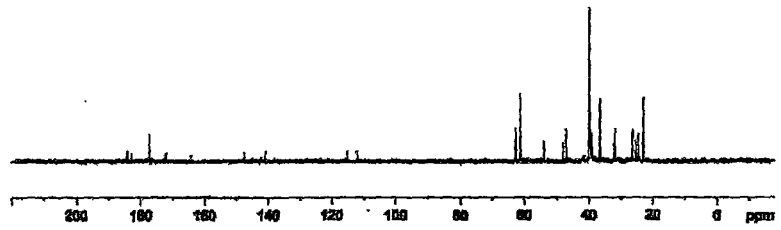




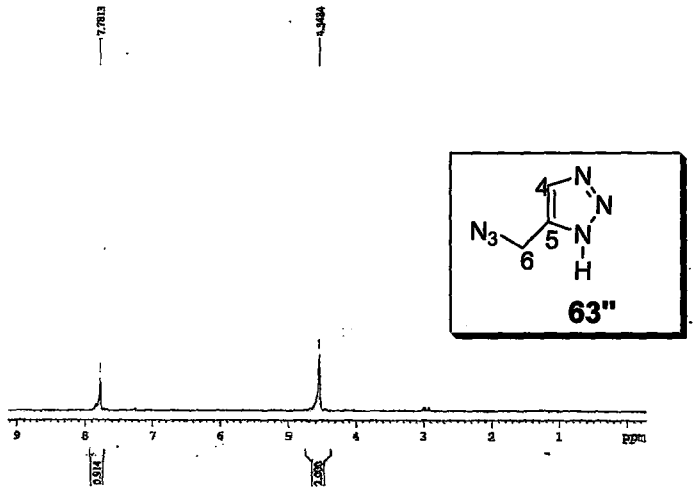
Appendix 5. ^{1}H & ^{13}C NMR spectra of Pyrroline-2-carboxylic acid (18)

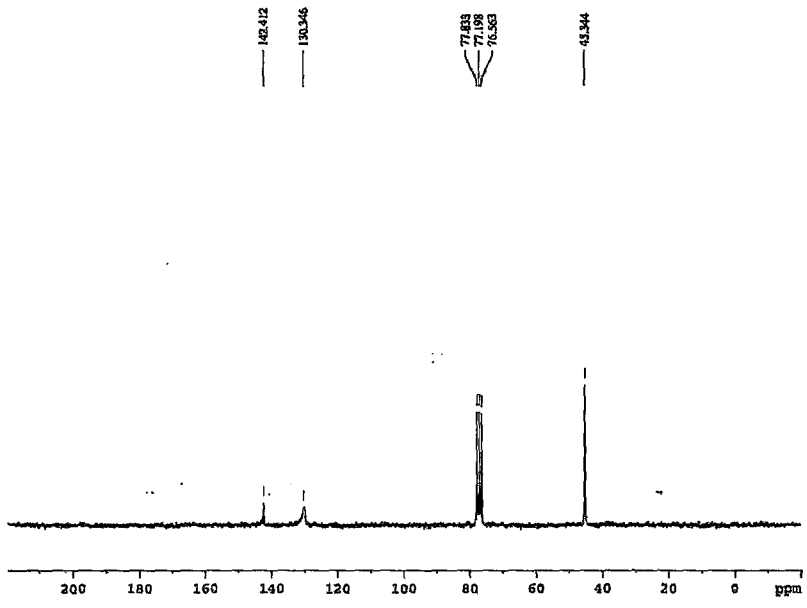


18

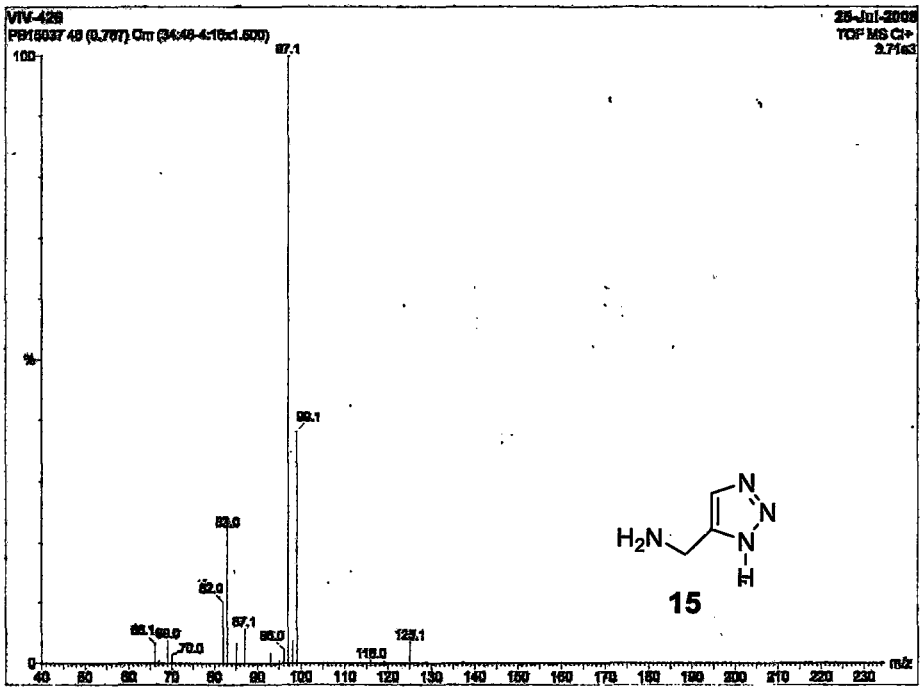


Appendix 6. ^{1}H & ^{13}C NMR spectra of 1,2,3-triazole methyl azide (63")





Appendix 7. Mass spectra of 3H-1,2,3-triazole-4-methylamine (15)



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