AN ABSTRACT OF THE DISSERTATION OF

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Title: Syntheses and Evaluation of Putative Enzyme Inhibitors of Isoprenoid Biosynthesis

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Philip Proteau

The discovery of the methylerythritol phosphate pathway (the MEP pathway) as an alternate pathway for isoprenoid biosynthesis in some organisms including most bacteria, malarial parasites and plants, but not in animals, has stimulated extensive studies in this area. Research has revealed the potential of finding novel antibacterials, antimalarial drugs, and herbicides from enzyme inhibitors of this pathway. The natural products fosmidomycin and FR900098 appear to be very promising antibacterial and antimalarial compounds. Both compounds have inhibition activities against the second enzyme in the MEP pathway, deoxyxylulose-5-phosphate reductoisomerase (DXR), which mediates the conversion of deoxyxylulose-5-phosphate (DXP) into methylerythritol-4-phosphate (MEP).

This thesis presents one aspect of the MEP pathway studies. Six different analogs of DXP were designed based on the structural features of DXP to understand the requirements of the DXR-substrate binding. Compounds with the trivial names 1-Me-DXP (containing an ethyl ketone moiety), DX-phosphonate (DXP having a phosphonate group rather than a phosphate group), 4-epi-DXP (possessing the opposite stereochemistry at the $C_4$ position compared to DXP), 4-deoxy-DXP (lacking the hydroxyl group at the $C_4$ position), 3-deoxy-DXP (lacking the hydroxyl group at the $C_3$ position), and DXP carboxamide (having a primary amide group rather than the methyl ketone) were synthesized and tested as alternate substrates and enzyme inhibitors against DXR. The compound DX-phosphonate was the only

alternate substrate among the synthesized compounds. The remaining analogs of DXP acted as weak competitive inhibitors against DXR. Kinetic studies of these compounds provided an overall picture of how the substrate DXP binds to DXR. Further studies of the compound 1-Me-DXP, using the published X-ray crystal structures of DXR and DXR mutagenesis demonstrated more detail of the DXR active site. The results present useful information for designing better enzyme inhibitors.

The mechanism for the rearrangement of DXP to MEP by DXR was also studied. Two possible mechanisms for this rearrangement have been proposed, the α-ketol rearrangement and the retroaldol/aldol rearrangement. Several approaches including the use of the potential alternate substrates, 4-deoxy-DXP and 3-deoxy-DXP were tried. Unfortunately none of the results obtained can definitively rule out either of the mechanisms. Further studies are needed to completely understand this mechanism and establish additional strategies for inhibition of DXR.

Syntheses of an intermediate from the DXR reaction, methylerythrose-4-phosphate, were also attempted in order to better understand the chemistry mediated by DXR. Even though the target compound was not successfully obtained, several synthetic approaches to this compound were useful for the syntheses of the different DXP analogs mentioned above.

# Syntheses and Evaluation of Putative Enzyme Inhibitors of Isoprenoid Biosynthesis

by

Chanokporn Phaosiri

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Dean of Graduate School

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Chanokporn Phaosiri, Author

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## CONTRIBUTION OF AUTHORS

Dr. Xihou Yin conducted initial experiments for DXR expression and purification, including DXP synthesis in Chapter Two.

Roberta Fernandes performed modeling experiments for DXR and DXR mutagenesis in Chapter Three.

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| | |
|---|---|
| Ac | Acetyl |
| aq | Aqueous |
| BnBr | Benzylbromide |
| br | Broad |
| calcd | Calculated |
| *c* | Concentration |
| CI | Chemical Ionization |
| COSY | Correlated Spectroscopy |
| d | doublet |
| DMSO | Dimethylsulfoxide |
| EtOH | Ethanol |
| FAB | Fast Atom Bombardment |
| h | Hours |
| HSQC | Heteronuclear Single Quantum Correlation |
| HMPA | Hexamethyl phosphoramide |
| HPLC | High Performance Liquid Chromatography |
| HRMS | High Resolution Mass Spectrometry |
| IR | Infrared Spectroscopy |
| *J* | Coupling Constant |
| lit | Liturature |
| LRMS | Low Resolution Mass Spectrometry |
| m | Multiplet |
| Me | Methyl |
| MeOH | Methanol |
| NMO | 4-Methylmorpholine-*N*-oxide |
| NMR | Nuclear Magnetic Resonance |
| Ph | Phenyl |
| q | Quartet |

| | |
|---|---|
| s | Singlet |
| t | Triplet |
| TBAF | Tetrabutylammonium fluoride |
| TBDMS | *tert*-Butyldimethyl silyl |
| TBDPS | *tert*-Butyldiphenyl silyl |
| TEA | Triethylamine |
| THF | Tetrahydrofuran |
| TIPS | Triisopropyl silyl |
| TLC | Thin Layer Chromatography |
| TPAP | Tetrapropylammonium perruthenate |
| $t_R$ | Retention Time |
| v | volume |

LIST OF SCHEMES

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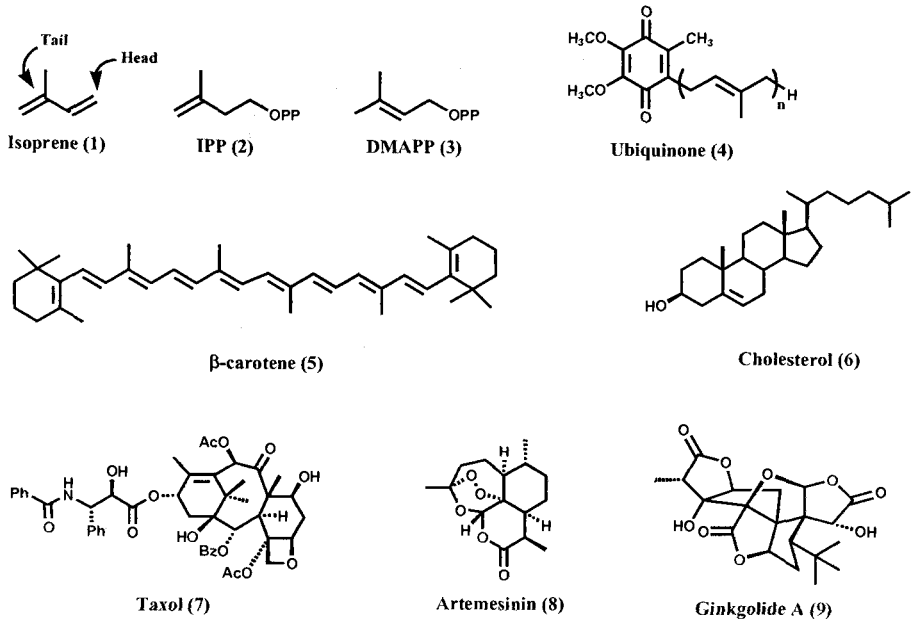
# Syntheses and Evaluation of Putative Enzyme Inhibitors of Isoprenoid Biosynthesis

## CHAPTER ONE

### ISOPRENOID NATURAL PRODUCTS AND BIOSYNTHESES

### 1) Introduction

Isoprenoids, also known as terpenoids or terpenes, compose a large group of natural products. More than 35,000 isoprenoid compounds have been characterized and new isoprenoids are reported each year.[1] All isoprenoids share a common feature in that they can be conceptually assembled from the same five-carbon isoprene unit (**1**). These building blocks are typically linked together in a head-to-tail manner. Note that the branched end of the isoprene unit is regarded as the "tail" and the unbranched end as the "head".




Isoprene (1) IPP (2) DMAPP (3) Ubiquinone (4)

β-carotene (5) Cholesterol (6)

Taxol (7) Artemesinin (8) *Ginkgolide* A (9)

**Figure 1.1. Examples of Isoprenoid Natural Products.**

Although the head-to-tail junction is the most common method of linking isoprene units together, head-to-head junctions can also occur in many isoprenoid

structures. The biological equivalents of the isoprene unit are isopentenyl diphosphate (**2**, IPP), and dimethylallyl diphosphate (**3**, DMAPP). In other words, all isoprenoids are assembled biosynthetically from only two precursors: IPP and DMAPP.

Isoprenoids have important and essential function in all living organisms. These compounds are considered as primary metabolites. Examples of isoprenoid primary metabolites include: ubiquinones (**4**), which conduct electron transport and redox chemistry, carotenoids (**5**), which provide photooxidative protection and photosynthetic light harvesting in plants, and cholesterol (**6**), which contributes to lipid membrane structure in eukaryotes.[2]

Even though some isoprenoids are known to play very crucial roles in many stages of life, the functions of most isoprenoids are still unknown. These secondary isoprenoid metabolites are mainly found in plants, fungi, marine organisms, and some bacteria. Isoprenoid natural products also appear in mosses, liverworts, algae, and lichens, although some are of insect or microbial origin.[3] A number of specific secondary metabolite terpenoids serve a role in communication and defense, for instance, as attractants for pollinators and seed dispersers (geraniol), competitive phytoalexins (capsidiol), antibiotics (trichothecin), herbivore repellents (camphor), and plant growth hormones (gibberellins).[4]

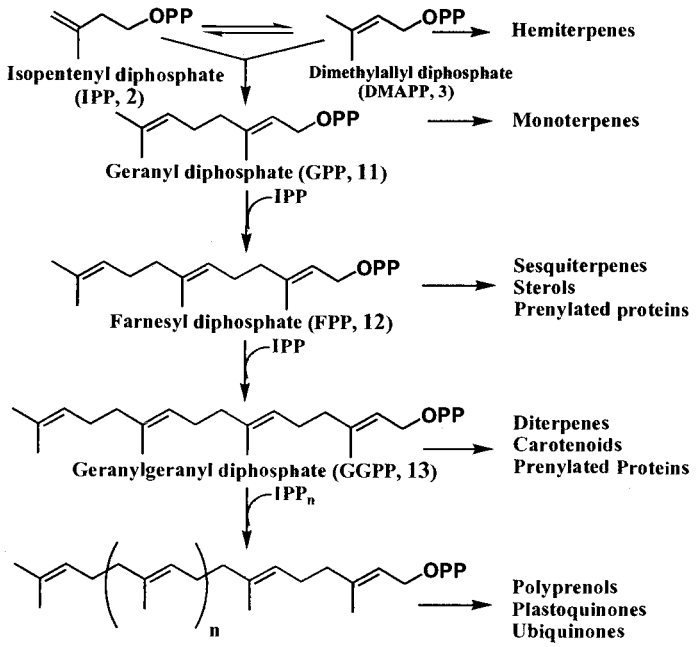
Although their ecological functions may not be known, isoprenoids often have useful properties that can be exploited. Paclitaxel or Taxol® (**7**) and Artemesinin (**8**) are examples of isoprenoid secondary metabolites which show potent and medically useful activities as an antitumor agent and antimalarial compound respectively.[5] Additionally, there is a study showed that the tree *Ginkgo biloba* extract which contains terpene lactone such as ginkgolide A (**9**) can promote short-term retention of spatial memory in rats.[6] Even though there is still no clinical prove for using *Ginkgo biloba* extract as a memory enhancer, the use of this herbal remedy has become increasingly popular during recent years.

The number of pharmacologically interesting isoprenoids is increasing with the rising number of new isoprenoids isolated each year. In the past five years, more

than fifty isoprenoids from plants have been identified as potential anti-inflammatory compounds in both *in vivo* animal models and *in vitro* cultures of cells.[7] Positive new isoprenoid leads for anti-HIV drug development were also discovered. These isoprenoids were associated with several modes of action such as anti-virus-cell fusion, reverse transcriptase inhibition, and protease inhibition.[8] Undoubtedly more isoprenoids with novel pharmacological actions will be discovered in the future.

**2) Classification of Isoprenoids**

Isoprenoids can be classified into different groups based on the number of carbon atoms in the isoprenoid portion of their structures. Hemiterpenoids, monoterpenoids, sesquiterpenoids, diterpenoids, sesterterpenoids, triterpenoids and polyterpenoids contain five, ten, fifteen, twenty, twenty-five, thirty and more than 30 carbon atoms, respectively (Scheme 1). Slight variations in the numbers of carbon atoms in isoprenoids can occur through modification of the core structures such as demethylation or methylation. For examples, there is a loss of three carbons during the transformation of the triterpenoid lanosterol to cholesterol.




**Scheme 1.1. Biosynthesis of Higher Isoprenoids.**

Hemiterpenoids such as isoprene (**1**), and methylbutenol (**10**) can be synthesized directly from DMAPP (**3**). Both of these hemiterpenoids are volatile and are released from trees.[9]

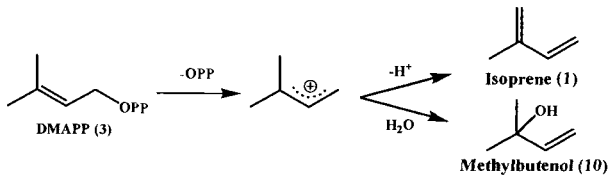



**Figure 1.2. Example of Hemiterpenoid Formation.**

The higher order isoprenoid building blocks, including geranyl diphosphate (GPP, $C_{10}$, **11**), farnesyl diphosphate (FPP, $C_{15}$, **12**), and geranylgeranyl diphosphate (GGPP, $C_{20}$, **13**), can be generated by the action of different prenyltransferses. These enzymes have been studied intensively at the mechanistic, biochemical, protein structural, and genetic level.[10] The reactions catalyzed by prenyltransferases are very unique and interesting from a mechanistic viewpoint. The reaction starts with ionization of the allylic diphosphate (DMAPP, **3**) to form an allylic cation, which is attacked by the double bond from a molecule of IPP (**2**). A new carbon-carbon bond is formed and stereospecific removal of a proton provides the product, geranyl diphosphate (GPP, **11**). By repeating this type of condensation between IPP and the allylic diphosphate product, GPP (**11**), FPP (**12**) can be synthesized. By adding one more unit of IPP, GGPP (**13**), can be generated. Addition of further IPP units results in the polyprenyl diphosphates.

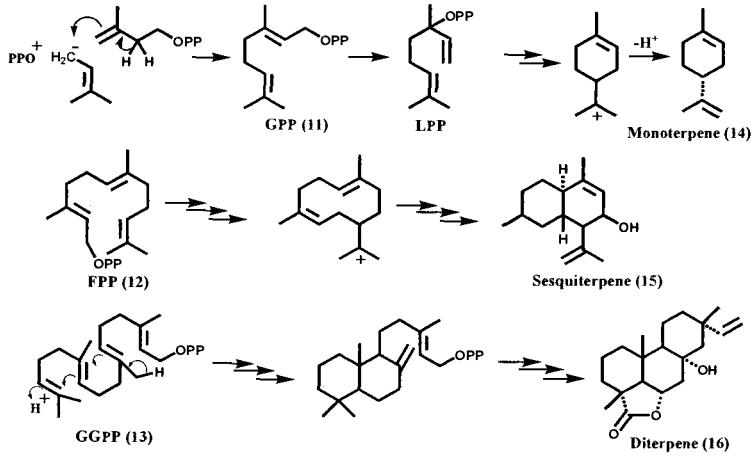



**Figure 1.3. Examples of Cyclic Monoterpenes, Sesquiterpenes and Diterpenes.**

Cyclic monoterpenoids can be generated from GPP (**11**) through cyclization of the tertiary allylic GPP isomer, linalyl diphosphate (LPP), and internal electrophilic addition to the remaining double bonds. Hydride shifts and other rearrangements before termination of the reaction sequence by proton loss or capture of the carbocation by a nucleophile provide a range of monoterpene structures.[11] A relatively simple monoterpene is (+)-limonene (**14**) which has the smell of oranges.[12]

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Farnesyl diphosphate (FPP, **12**) is the precursor to a wide variety of sesquiterpenoids from a reaction catalyzed by sesquiterpene synthases.[13] Most sesquiterpene cyclization reactions occur through variations of a common mechanism involving ionization of farnesyl diphosphate and electrophilic attack of the resultant allylic cation, subsequent cationic rearrangements and quenching of the positive charge by deprotonation or capture of a nucleophile such as water.[14] Xenitorin A (**15**) is an example of a sesquiterpenoid isolated from the formosan soft coral *Xenia puertogalerae*.[15] It showed cytotoxicity against a human lung adenocarcinoma cell line.

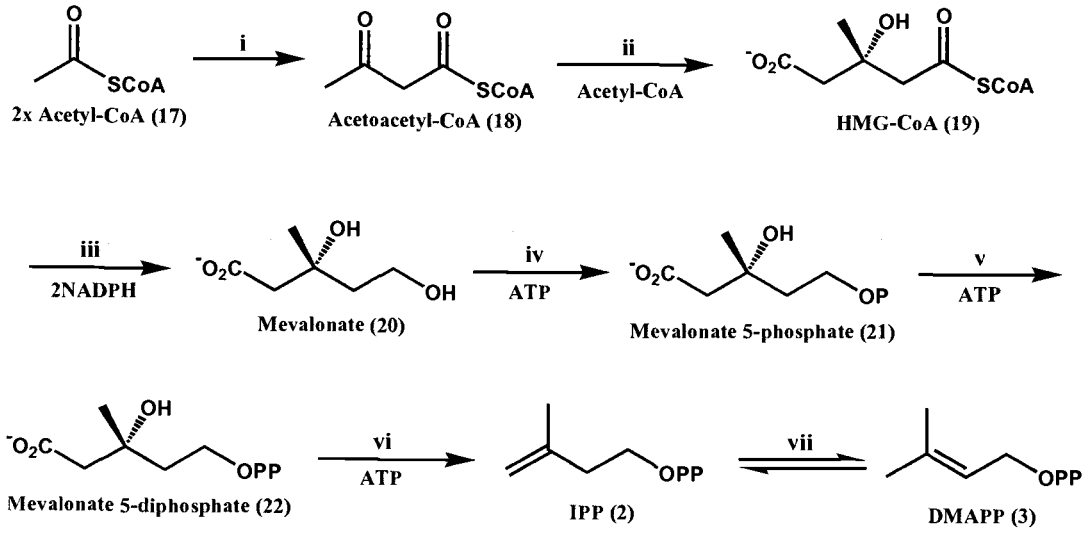
Diterpenoids can be obtained from the cyclization of the normal progenitor of diterpenoids, GGPP (**13**) by a similar manner to sesquiterpenoids. However, an additional mechanism to generate cyclic diterpenoid structures is a proton-initiated cyclization of GGPP (**13**) to generate the diterpenoid pimarane derivative (**16**) as shown in Figure 3. The pimarane derivative (**16**) is isolated from the leaves of *Tetraclinis articulate*.[16] It inhibited various human leukocyte functions mainly the degranulation process and the superoxide production.

### 3) Biosyntheses of Isoprenoid Precursors

Studies of isoprenoids at the structural, enzymatic and biosynthetic level have been done extensively.[2,10,13] The biosynthesis of the isoprenoid universal precursor, IPP (isopentenyl diphosphate) will be discussed in more detail in this chapter. There are two different pathways for biosynthesis of IPP, the mevalonate pathway and the methylerythritol phosphate pathway.

### 3.1) The Mevalonate (MVA) Pathway

In the MVA pathway, a Claisen-type condensation of two acetyl-CoA molecules by acetoacetyl-CoA thiolase yields acetoacetyl-CoA (**18**). Another Claisen-type condensation of acetyl-CoA and acetoacetyl-CoA provides hydroxymethylglutaryl-CoA (HMG-CoA, **19**).

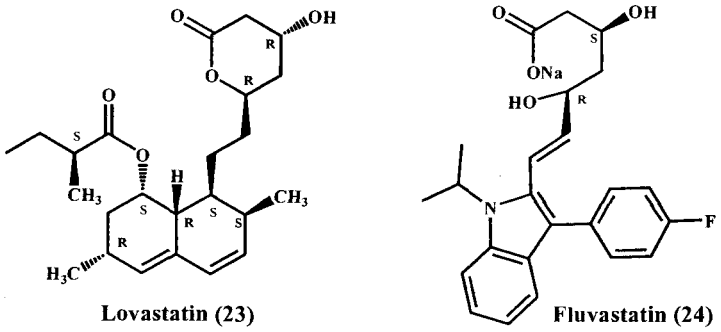



**Scheme 1.2. The mevalonate pathway.**[17]

*Enzymes*: i, acetoacetyl-CoA thiolase; ii, HMG-CoA synthase; iii, HMG-CoA reductase; iv, mevalonate kinase; v, phosphomevalonate kinase; vi, mevalonate 5-diphosphate decarboxylase; vii, IPP isomerase.

A NADPH-dependent HMG-CoA reductase (EC 1.1.1.34) catalyzes the rate-limiting step of the MVA pathway, the reduction of HMG-CoA to mevalonate (**18**). Finally, mevalonate undergoes two successive phosphorylations followed by an ATP-assisted decarboxylation to generate the universal precursor of isoprenoids, IPP.[9] Isomerization of IPP can further provide DMAPP as another precursor of isoprenoids.

The enzyme HMG-CoA reductase is a major regulatory enzyme of the MVA pathway in various organisms and has served as an important target for the control of cholesterol biosynthesis in humans.[18] Inhibitors of HMG-CoA reductase, such as the fungal metabolite, lovastatin (**23**, Mevacor®), and the synthetic compound, fluvastatin (**24**), are used efficiently in the clinical treatment and prevention of coronary artery disease.[19,20] These compounds block synthesis of cholesterol and transcription of the LDL (low-density lipoprotein) receptor genes.[21]




Lovastatin (23) Fluvastatin (24)

**Figure 1.4. Structures of HMG-CoA Reductase Inhibitors.**

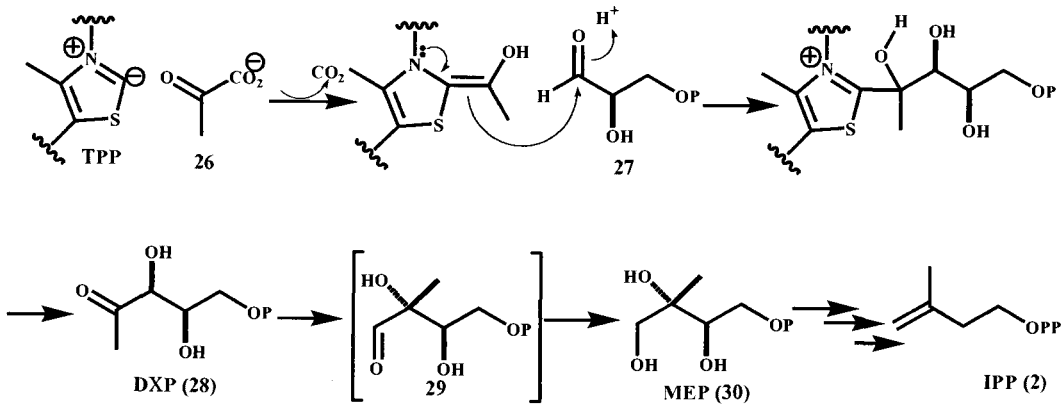
Although the MVA pathway was strongly believed to be the only pathway for isoprenoid biosynthesis for many years, there were early studies in the 1950's that showed contradictions with the universal role of mevalonate as the isoprenoid precursor. For example, labeled mevalonate and acetate were usually not, or were only very poorly, incorporated into carotenoids, monoterpenes and diterpenes in a variety of plant systems.[22,23] Moreover, mevinolin, a potent inhibitor of HMG-CoA reductase could inhibit sterol biosynthesis in plants, but had no affect on carotenoid formation.[24] Further investigations later revealed an entirely different pathway from the MVA pathway for isoprenoid biosynthesis.

### 3.2) The Methylerythritol Phosphate (MEP) Pathway

In 1993 a novel pathway for IPP biosynthesis was reported independently from the laboratories of Rohmer[25] and Arigoni[26]. Because this pathway does not involve mevalonate, it was called the non-mevalonate pathway. The early primary precursor experiments from Rohmer's laboratory used $^{13}$C-acetate and $^{13}$C-glucose as carbon sources. Isoprenoids including bacteriohopanetetrol glycoside, bacteriohopanetetrol, diplopterol and ubiquinone were isolated from cultures of *Zymononas mobilis*, *Methylobacterium fujisawaense*, *Alicyclobacillus acidoterrestris* and *Escherichia coli*.[27] The labeling patterns observed for the isoprenoids after incubation with each labeled precursor were not consistent with the classical mevalonate pathway. Independent studies by Arigoni and his research group examined the incorporation of various $^{13}$C-labelled glucose samples into the isoprenoid sidechain of ubiquinone in the bacterium *Escherichia coli* and into ginkgolides in seedlings of the tree *Ginkgo biloba*.[26] The detailed analysis of ginkgolides generated from various specifically labeled $^{13}$C isotopomers of glucose indicated that the formation of the isoprenoid precursors could not be explained by the mevalonate pathway.

From all data gathered from labeling experiments by both Rohmer's and Arigoni's groups, it was concluded that the diversion of $^{13}$C to isoprenoids has occurred via intermediates in the triose phosphate pool. Further incubation experiments from Rohmer's group showed that $^{13}$C-labelled pyruvate was incorporated into the ubiquinone of *Escherichia coli* mutants lacking enzymes of the triose phosphate metabolism.[28] Arigoni's group suggested a condensation of glyceraldehyde-3-phosphate (**26**) and "activated acetaldehyde" generated from pyruvate (**27**) by a thiamine-diphosphate dependent decarboxylation. More $^{13}$C-labeling experiments with bacteria were conducted, and both compounds were shown as the only precursors of the $C_5$-skeleton of isoprenoid units. Further investigation by Arigoni's group confirmed the incorporation of isotope-labelled deoxyxylulose into terpenoids in cell cultures of *Catharanthus roseus*.[29]

Subsequent work by Sahm *et al.*[30] and Rohmer *et al.*[31] identified 1-deoxy-D-xylulose 5-phosphate (DXP, **28)** as the product generated from the condensation of pyruvate (**26**) and glyceraldehyde-3-phosphate (**27**) by the enzyme deoxy-D-xylulose 5-phosphate synthase (DXS, EC 2.2.1.7). This intermediate is not only the precursor to isoprenoids but also to thiamine (vitamin $B_1$) and pyridoxol phosphate (vitamin $B_6$). The *dxs* gene encoding DXS was identified owing to its homology with transketolases, other thiamine diphosphate dependent enzymes.[32,33] This enzyme requires a divalent cation such as $Mg^{2+}$ or $Mn^{2+}$ and thiamine diphosphate for activity. Kinetic studies of this enzyme from different organisms revealed that this enzyme catalyzes the rate-limiting step of the non-mevalonate pathway.[34,35] A $^{14}CO_2$ trapping experiment by Eubanks *et al.* demonstrated that the binding of both substrates is required for the formation of a catalytically competent enzyme-substrate complex.[36] These studies also showed that DXS binds pyruvate before glyceraldehyde-3-phosphate.

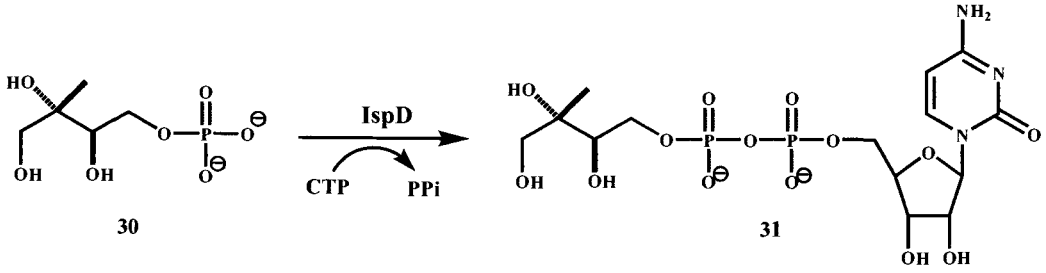



**Scheme 1.3. Mevalonate-independent glyceraldehyde-3-phosphate/pyruvate pathway for isoprenoid biosynthesis.**

In the second step of the non-mevalonate pathway, deoxyxylulose phosphate is converted into 2-*C*-methyl-D-erythritol 4-phosphate (MEP, **30**) by the NADPH-dependent enzyme, deoxyxylulose-5-phosphate reductoisomerase (DXR, EC 1.1.1.267).[37,38,39] It is encoded by the *dxr* gene. Because MEP is the first

committed intermediate in the non-mevalonate pathway, a recommendation was made to call this pathway the MEP pathway at the 4th European Symposium on Plant Isoprenoids (Barcelona, 1999).[40] The enzyme DXR requires divalent metal ions such as $Mn^{2+}$, $Co^{2+}$ or $Mg^{2+}$ as a cofactor. The compound DXP (**28**) was presumed to rearrange to form a 2-*C*-methyl-D-erythrose 4-phosphate intermediate (**29**) which was reduced to generate MEP (**30**). Mechanisms for the rearrangement of DXP to MEP by the enzyme DXR will be discussed in detail later.

Further elucidation of the MEP pathway was pursued on by several different research groups in Europe and Japan. The compound 4-diphosphocytidyl-2-*C*-methyl-D-erythritol (CDP-ME, **31**) was identified as the next intermediate after characterization of the enzyme, 4-diphosphocytidyl-2-*C*-methyl-D-erythritol synthase (EC 2.7.7.60) which is encoded by the *ispD* gene.[41,42,43] This enzyme requires a divalent cation, preferably $Mg^{2+}$, and CTP. Incubation studies showed that CDP-ME was incorporated efficiently into carotenoids of red pepper by isolated chromoplasts.[43]




**Scheme 1.4. The conversion of MEP to CDP-ME.**

The X-ray crystal structures of the enzyme, CDP-ME synthase, in the apo form and in a complex with CTP-$Mg^{2+}$ or CDP-ME-$Mg^{2+}$ were reported by Richard *et al.*[44] These structures revealed the active site features responsible for the regiochemical control of the MEP cytidyltransferase reaction. The amino acid residue, Arg-20, interacts with the α- and γ-phosphate of CTP, Lys-27 plays an essential role in catalysis, and Lys-213 can enhance the nucleophilicity of the phosphate functional group of MEP and assist nucleophilic attack on CTP as shown

in Figure 1.5. The CDP functional group on CDP-ME might be important for enzyme binding in a subsequent step in the MEP pathway.

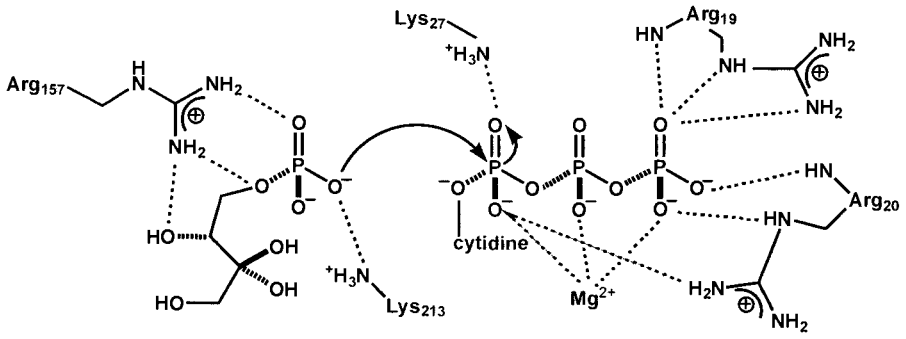
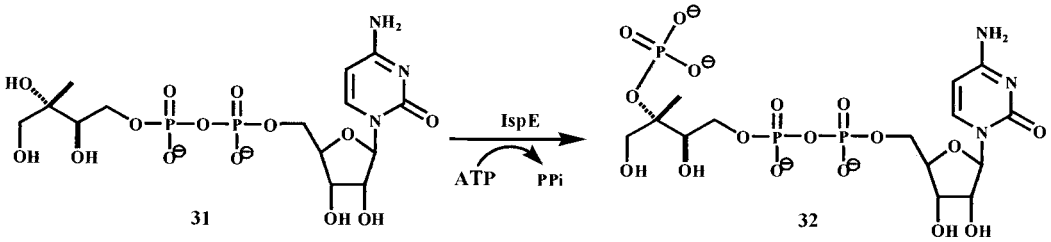


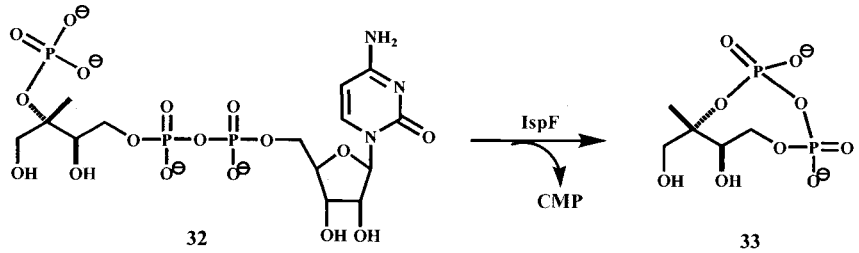

**Figure 1.5. The putative role of side chains in the mechanism of CDP-ME synthase.**

In order to further understand the MEP pathway, a search for orthologous genes with the same distribution pattern as *dxs*, *dxr*, and *ispD* genes was conducted.[43] The *ispE* and *ispF* genes were found as potential members of the MEP pathway. The *ispE* gene of *E.coli* was overexpressed in *E.coli* where it directed the synthesis of a 30 kDa peptide. The purified recombinant enzyme from *E.coli* was shown to catalyze the ATP-dependent phosphorylation of CDP-ME (**31**) at the C-2 hydroxy group yielding 4-diphosphocytidyl-2-*C*-methyl-D-erythritol 2-phosphate (CDP-MEP, **32**).[45,46] The enzyme encoded by the *ispE* gene was identified as 4-diphosphocytidyl-2-*C*-methyl-D-erythritol kinase (EC 2.7.1.148). A $^{14}C$-labelled specimen of CDP-MEP was converted into carotenoids isolated from *Capsicum annuum* very efficiently.[47]




**Scheme I.5. The conversion of CDP-ME to CDP-MEP.**

The conversion of CDP-MEP (**32**) into 2-*C*-methyl-D-erythritol 2,4-cyclodiphosphate (MEcPP, **33**) by the enzyme, MEcPP synthase (EC 4.6.1.12) encoded by the *ispF* gene, was identified as a next step in the MEP pathway.[48,49] The enzyme required $Mg^{2+}$ or $Mn^{2+}$ but no other cofactors. The MEcPP (**33**) was also shown to be transformed into carotenoids by isolated chromoplasts of *Capsicum annuum* and *Narcissus pseudonarcissus*.[50,51] This compound was previously known as it accumulates under oxidative stress caused by benzyl viologen in certain bacteria such as *Corynebacterium ammoniagenes*.[52]




**Scheme 1.6. The conversion of CDP-MEP to MEcPP.**

A three dimensional structure of the MEcPP synthase from *E.coli* was reported independently from three research groups.[53,54,55] The protein has a homotrimeric quaternary structure built around a $\beta$ prism, carrying three active sites, each of which is formed in a cleft between pairs of subunits. A tetrahedrally arranged transition metal binding site, potentially occupied by $Mn^{2+}$, sits at the base of the active site cleft. A phosphate oxygen of MEcPP synthase and the side chains of $Asp^{8}$, $His^{10}$, and $His^{42}$ occupy the metal ion sphere.

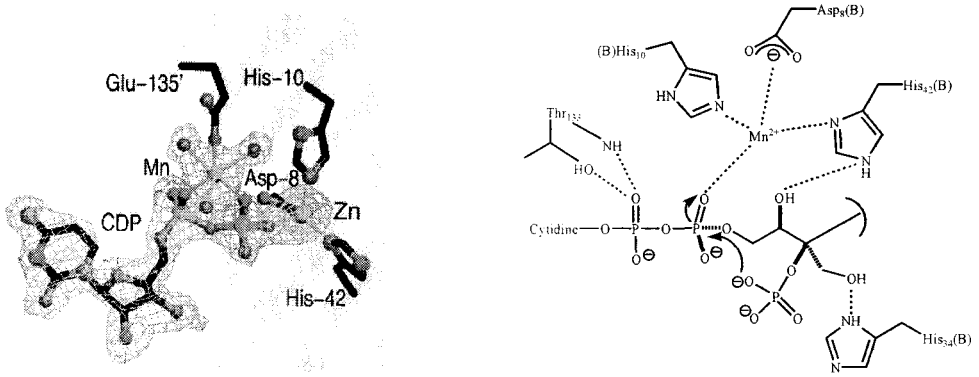
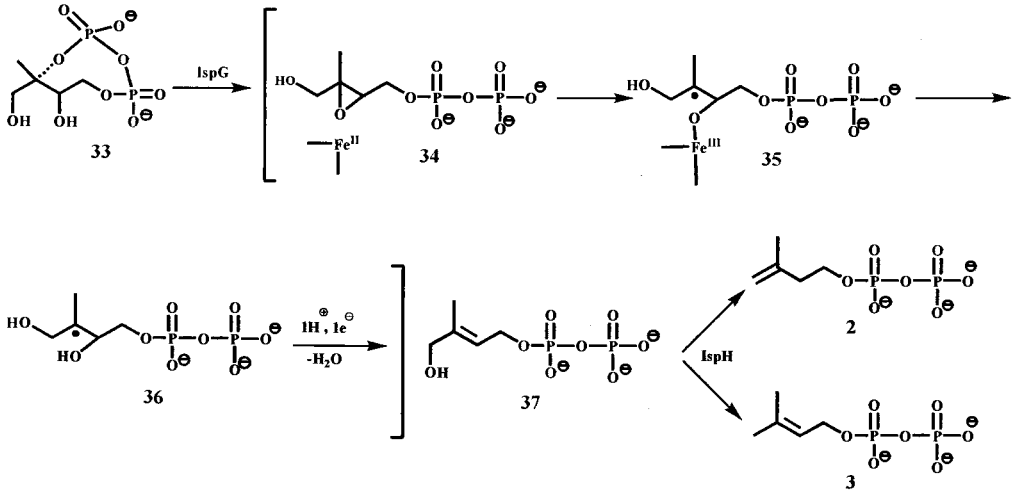



**Figure 1.6. The substrate binding site of MEcPP synthase.**

The last intermediate in the MEP pathway before IPP was proposed as (*E*)-4-hydroxy-3-methylbut-2-enyl diphosphate (HMB-PP, **37**).[56,57] The intermediate, HMB-PP (**37**) was generated from MEcPP (**33**) in a cell free system from an *E.coli* strain overexpressing the *ispG* (*gcpE* ) gene.[58] A sequence analysis of IspG was conducted and it appeared that these sequences either matched a 4Fe-4S ferredoxin signature or an aconitase signature or both. The latter enzyme is characterized by an active [4Fe-4S] cluster that mediates electron transfer. In addition, the IspG is characterized by three conserved cysteines. These features are compatible with the presence of an iron-sulfur cluster in IspG. Therefore, it was proposed that IspG contains a Fe-S cluster and may use FAD and/or NADPH as cofactor(s).[59]



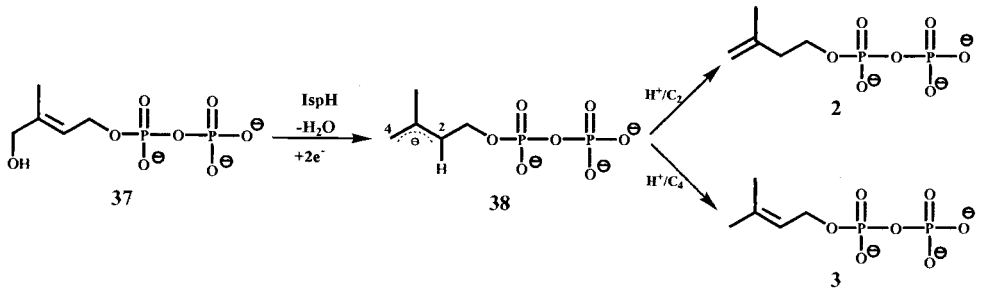

**Scheme 1.7. Late reaction steps leading to IPP (2) and DMAPP (3).**

Further experiments by Seemann *et al.* showed that this enzyme efficiently converts MEcPP into HMB-PP in the presence of flavodoxin, flavodoxin reductase, and NADPH.[60] From earlier works on isoprenoid biosynthesis of *E.coli*, it could be assumed that all of the carbon bonded hydrogen atoms of MEcPP (**33**) are preserved in HMB-PP (**37**).[61,62,63] Therefore, there are limitations on a reasonable mechanism of this reaction. However, Rohdich *et al.* were able to propose the mechanism of IspG as shown in Scheme 7.[64] An epoxide intermediate can be derived from HMB-PP by intramolecular displacement of the diphosphate at C2 by the C3 hydroxyl.

The geometry of this intermediate is dictated by the configuration of the two chiral centers in the precursor. Therefore, the exclusive formation of the (*E*) isomer of HMB-PP can occur.

As another possible mechanism, the reaction may be initiated by ring opening of cyclic diphosphate yielding a relatively stable tertiary carbocation instead of the tertiary radical **35**.[65] In the next step, the tertiary carbocation can be reduced by two successive one-electron transfer steps followed by the concerted elimination of the hydroxyl group to generate the intermediate **37**.

The last step in the MEP pathway is associated with the *ispH* gene. A study by Rohdich *et al.* showed that the crude extract from *E.coli* overexpressing the *ispH* gene catalyzed the transformation of HMB-PP (**37**) into a 5:1 mixture of IPP and DMAPP.[64,66] Recombinant IspH (LytB) protein from the thermophilic eubacterium *Aquifex aeolicus* produced in *E. coli* was purified to apparent homogeneity by Altincicek *et al.*[67] The purified IspH protein also catalyzed the reduction of HMB-PP (**37**) in a defined *in vitro* system. This enzyme possesses a dioxygen-sensitive [4Fe-4S] cluster. The reaction of IspH (EC 1.17.1.2) required NADH, FAD and a divalent cation such as $Co^{2+}$. It was proposed that the mechanism of this reaction should be a single electron transfer process based on a $[4Fe\text{-}4S]^{2+}$ cluster of IspH protein which is similar to that of IspG.[64]




**Scheme 1.8. A proposed reaction mechanism leading to IPP (2) and DMAPP (3).**[68]

However, a recent study from Wolff *et al.* suggested that the last step occurs via two successive one-electron transfers from the reduced $[4Fe\text{-}4S]^{1+}$ form, yielding

the allylic anionic intermediate (**38**).[68] In the next step, this allylic intermediate could be protonated either at $C_2$ yielding IPP (**2**) or at $C_4$ yielding DMAPP (**3**). Based on the enzymatic studies, it has been demonstrated that IspH is responsible for formations of both IPP and DMAPP in the MEP pathway.[69]

**4) Distributions of the MVA and MEP Pathways**

Since the MEP pathway was discovered, genes of this pathway including *dxs*, *dxr*, *ispD*, *ispE*, and *ispF* have been identified in many organisms: *Arabidopsis thaliana*, *Bacillus subtilis*, *Chlamydia trachomatis*, *Escherichia coli*, *Helicobacter pylori*, *Mentha piperita*, *Mycobacterium tuberculosis*, *Neisseria gonorrhoeae*, *Plasmodium falciparum*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium* and many others. There has been tremendous interest in identification and characterization of the enzymes in the MEP pathway from various organisms as shown below.

**Table 1.1. Recombinant enzymes of the MEP pathway.**[70,71,72,73]

| **Enzyme**/Organisms | $K_m$ (μM) | $V_{max}$ (μM/min.mg) |
|---|---|---|
| **DXS** | | |
| *Capsicum annuum* | 500[a] / 750[b] | 500 |
| *Escherichia coli* | 96[a] / 250[b] | 300 |
| *Streptomyces sp.strain CL190* | 65[a] / 120[b] | 370 |
| **DXR** | | |
| *Zymomonas mobilis* | 300[c] / 5.0[d] | 19.5 |
| *Escherichia coli* | 175[c] / 1.0[d] | 30-148 |
| *Streptomyces coelicolor* | 190[c] / 190[d] | 25.8 |
| *Synechocystis sp* PCC6803 | 195[c] / 3.3[d] | 20 |
| **CDP-ME Synthase** | | |
| *Arabidopsis thaliana* | 500[e] / 114[f] | 67 |
| *Escherichia coli* | 131[e] / 3.0[f] | 23 |
| **CDP-ME Kinase** | | |
| *Escherichia coli* | | 34 |
| *Lycopersicon esculentum* | | 33 |
| **MEcPP Synthase** | | |
| *Plasmodium falciparum* | | 4.3 |

Substrates are indicated by: [a]D-glyceraldehyde 3-phosphate, [b]Pyruvate, [c]DXP, [d]NADPH, [e]MEP, [f]CTP.

Many gram-negative and gram-positive eubacteria use the MEP pathway rather than the MVA pathway for isoprenoid biosynthesis. However, some bacteria such as *Borrelia burgdorferi*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Streptococcus sp.* use the MVA pathway.[71] *Streptomyces* sp. strain CL190 and *Streptomyces aeriouvifer* have been shown to use both pathways.[74,75] While red algae use both pathways, green algae such as *Scenedesmus obliquus* appear to use only the MEP Pathway.[17] Only the MVA pathway could be found in archaebacteria such as *Archaeoglobus fulgidus*, *Methanococcus jannaschii*, and *Pyrococcus furiosus*.[71] Moreover, there was only the MVA pathway present in *Saccharomyces cerevisiae*, and in higher eukaryotes such as animals including humans.[72]

In higher plants, such as *Ginkgo biloba* and *Mentha piperita*, there are both the MVA and MEP pathways conducting isoprenoid biosynthesis.[76,77] The MVA pathway is present in the cytoplasm for the biosynthesis of sterols, sesquiterpenes, and triterpenoids. But the MEP pathway is responsible for the biosynthesis of carotenoids, phytol, plastoquinone, mono-, diterpenes, and some sesquiterpenes in plastids. There is evidence that crosstalk between the two pathways in different organelles can also happen.[78] The evolutionary history of the enzymes involved in both routes and the phylogenetic distribution of their genes across genomes suggested that the MVA pathway was germane to archaebacteria and the MEP was germane to eubacteria. Therefore, eukaryotes have inherited their genes for IPP biosynthesis from prokaryotes.[79]

The discovery of the MEP pathway has stimulated much research in this area. Most of the enzymes of this pathway have been considered as targets for novel antibacterial, and antimalarial agents or even herbicides.[80] Different procedures have been used to accomplish this research including high-throughput screening technology and genetic approaches.[81,82] Moreover, genetic engineering to improve production of isoprenoids was also conducted.[83] All of these experiments will benefit mankind in the future. More studies and more detailed knowledge of the mechanisms of this pathway will help us reach the goal of finding novel drugs in a shorter period of time. This dissertation provides an example of another aspect of

the MEP pathway. One of the enzymes of the MEP pathway, deoxyxylulose phosphate reductoisomerase (DXR), is the main focus of this dissertation.

In Chapter Two, the preparation and characterization of various analogs of the DXR substrate, deoxyxylulose phosphate (DXP) will be discussed in order to understand the requirements of the DXR-substrate binding. Chapter Three will describe a study that incorporates data from a three dimensional structure of DXR and a kinetic study of a DXP analog to explain the binding specificity of DXP to DXR. Mechanistic studies for the rearrangement of DXP to methylerythritol 4-phosphate (MEP) will be discussed in Chapter Four. Chapter Five will show different procedures that were tried to prove that methylerythrose 4-phosphate is the true intermediate in the MEP pathway.

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# CHAPTER TWO

## SYNTHESES AND EVALUATION OF DXP ANALOGS

### 1) Introduction

Drug resistance is one of the major problems in the clinical treatment of infectious diseases such as malaria. About 40% of the world's population is currently at risk for contracting this disease.[1] The World Health Organization estimates that 300 to 500 million clinical cases of malaria occur each year, resulting in up to 2.7 million deaths. Most of these cases occur in Africa, Asia, Central and South America. The widespread occurrence of malaria in humans is mainly caused by the malarial parasite *Plasmodium falciparum*, which has *Anopheles* mosquitoes as carriers. Malaria was cured effectively for decades with chloroquine, but drug resistance to chloroquine is contributing to the resurgence of malaria.[2] There are many antimalarial drugs in current use, such as dihydrofolate reductase inhibitors (pyrimethamine or biguanides). However, they belong to a limited collection of chemical structures that act on a small number of partially characterized biochemical targets and resistance has emerged to many of these compounds.[3] Additionally, the mechanism of the resistance is not fully understood[4] because of the malarial parasite's complex life cycle. Moreover, the development of malaria vaccines will not be complete in the near future. Therefore, finding new targets for antimalarial drug development appears to be a good solution to this problem, and may help reduce the spread of multidrug resistant parasites. These new targets should allow for effective chemotherapy with minimal any harmful side effects for humans.

The discovery of the MEP pathway has provided an opportunity for scientists to target the pathway for finding new antimalarial drugs or possibly antibacterial drugs which are also in need due to the worsening problem of antibiotic-resistant bacteria. Enzyme inhibitors of the MEP pathway will meet the aforementioned criteria because this pathway can be found only in the malarial parasite and some bacteria, but not in humans. Examples of MEP pathway inhibitor are FR900098 (**4**) and fosmidomycin (**5**, FR-31564) which were originally isolated

as natural antibiotics from *Streptomyces rubellomurinus* sp. nov. and *Streptomyces lavendulae* respectively.[5,6] The action of FR900098 (**4**) involved interference with bacterial cell wall synthesis.[5]

Fosmidomycin (**5**) was examined in a phase II clinical trial by Fujisawa Pharmaceutical Company for the treatment of urinary tract infections in the early eighties.[7] At that time its mode of action was known only to be inhibition of bacterial isoprenoid synthesis.[8] Although there is no information in the literature explaining why this compound was not pursued through further clinical trials, it may have partially been due to the lack of broad spectrum antibacterial activity. After the MEP pathway was discovered, it was observed that only a distinct group of bacteria such as *E. coli* and *Bacillus subtilis*, known to use the MEP pathway for isoprenoid biosynthesis, were sensitive to fosmidomycin.[9] In addition to antibacterial action, fosmidomycin was shown to cause chlorosis in plants. This loss of green pigments could be explained by the impaired synthesis of the isoprenoid side chain of the chlorophyll molecules.[10] In addition, the similarity in the structures of fosmidomycin and the methylerythrose phosphate intermediate (**3**) in the MEP pathway led to an assumption that deoxyxylulose phosphate reductoisomerase (DXR) might be a specific target for fosmidomycin.[9]

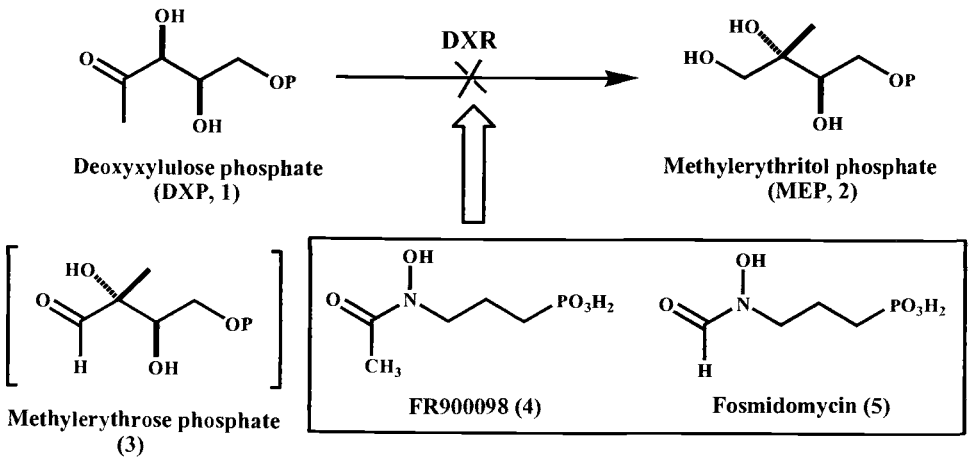



**Figure 2.1. Inhibitors of deoxyxylulose phosphate reductoisomerase (DXR).**

Further experiments showed that fosmidomycin could inhibit DXR from *E. coli* as an uncompetitive inhibitor against NADPH and a slow tight-binding competitive inhibitor against DXP. The $K_i$ values of fosmidomycin against DXP were 215 nM and 21 nM when determined from initial ($v_0$) and final ($v_s$) velocities respectively.[11] The existence of two distinguishable $K_i$ values was believed to reflect an initial binding step, followed by isomerization of the enzyme to a state which binds the inhibitor more tightly. In other words, fosmidomycin is a slow, tight- binding inhibitor of DXR. A recent X-ray crystal structure of DXR complexed with fosmidomycin revealed that fosmidomycin probably binds to DXR in the same manner as the substrate DXP.[12]

Fosmidomycin was considered for the treatment of malaria after the discovery of the MEP pathway in malarial parasites. Even though only a limited number of isoprenoids such as ubiquinones and dolichols, have been found in malaria parasites,[13,14] it has been demonstrated that they are essential for normal parasite metabolism. New light has been shed on isoprenoid metabolism in malarial parasites with the characterization of a self-replicating and plastid-like organelle called the apicoplast.[15] Organisms in the phylum *Apicomplexa*, including the malarial parasites, *Plasmodium* spp, and an important pathogen associated with AIDS, *Toxoplasma gondii*, have acquired the apicoplast, presumably through a secondary endosymbiotic event.[16] There are several metabolic pathways associated with the apicoplast including the MEP pathway. Therefore, the apicoplast pathways were considered as potential drug targets.[17] In fact, genes encoding all known enzymes of the MEP pathway could be identified in the completely sequenced human malarial parasite, *Plasmodium falciparum,* genome.[18,19] On the other hand, none of the genes involved in the MVA pathway could be detected.

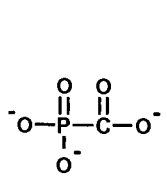
An early study by Jomaa *et al.* showed that the DXR inhibitors, fosmidomycin and FR900098 could be used to treat mice infected with the rodent malarial parasite *Plasmodium vinckei.*[20] The same studies also demonstrated that FR900098 can inhibit DXR from *Plasmodium falciparum* with an $IC_{50}$ value lower than fosmidomycin. More investigations on the antimalarial activity of

fosmidomycin have been conducted at Jomaa Pharmaka GmbH, a biotech company in Germany. A recent clinical study showed that fosmidomycin is effective and well tolerated in the treatment of adult patients from Gabon and Thailand with acute uncomplicated *Plasmodium falciparum* malaria, but there was an unacceptably high rate of recurrence of symptoms.[21] A drug combination of fosmidomycin and an antibacterial drug, clindamycin, may be a solution for this problem.[22]

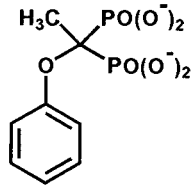
**Table 2.1. Sensitivity of different *P. falciparum* strains to fosmidomycin, FR900098, chloroquine, and pyrimethamine.**[20]

| *Plasmodium falciparum* strain | $IC_{50}$ (nM) | | | |
|---|---|---|---|---|
| | Fosmidomycin | FR-900098 | Chloroquine | Pyrimethamine |
| HB3 | 350 ± 170 | 170 ± 100 | 20 ± 5 | 60 ± 42 |
| Dd2 | 370 ± 45 | 170 ± 45 | 37 ± 7 | 4 ± 2 |
| A2 | 290 ± 130 | 90 ± 20 | 200 ± 30 | 2500 ± 1000 |

Fosmidomycin does have drug delivery problems that are common to other phosphonate containing drugs such as the antiviral agent, foscarnet (**6**, Foscavir®)[23] and the inhibitor of inositol monophosphatase, L-690,330 (**7**).[24]



Foscarnet (6)



L-690,330 (7)

**Figure 2.2. Examples of phosphonate drugs.**

There are two basic problems for the delivery of these drugs. Firstly, their highly ionic nature makes it more difficult to cross through mucosal or cellular membranes.[25] Secondly, because of the increased polarity, these agents often exhibit a low volume of distribution and therefore tend to be subject to efficient renal clearance as well as possible biliary excretion.[26] In order to overcome these problems, the polar phosphonates can be neutralized via chemical derivatization (prodrugs).[27] By increasing the lipophilicity of the drug molecule, it will improve access to cells and tissue. Diaryl ester and acyloxyalkyl ester prodrugs of FR900098 were synthesized and showed higher activity in mice infected with the rodent malaria parasite, *P. vinckei*.[28, 29] Both ester derivatives were believed to be hydrolyzed to form the active drug by non-specific plasma esterases which can be found in most mammals. Different analogs of fosmidomycin were also synthesized [30,31] and some showed very high activity when compared to the leading compound.[32]

Although fosmidomycin and its analogs are potent inhibitors of DXR, analogs of the DXR substrate, DXP, should also be considered in order to understand more about the mechanism of DXR inhibition. These compounds will allow us to develop a clearer picture of how DXR binds DXP, complementing the three dimensional structures of DXR, and help to design putative DXR inhibitors. This chapter provides detail about the design and syntheses of DXP analogs including kinetic studies.

## 2) Experimental Design

At the initiation of this project, there were no data on X-ray crystal structures of DXR available. Therefore, various features of DXP were considered as potential sites for designing analogs of DXP.

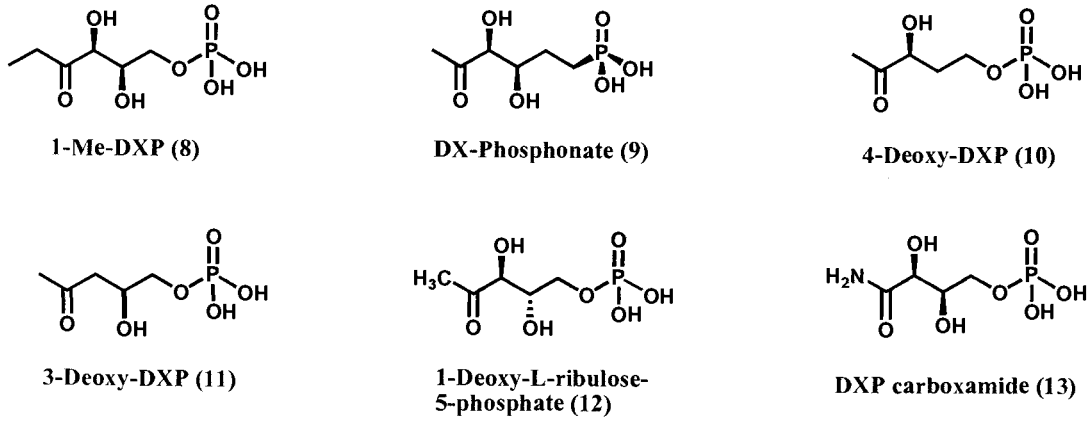


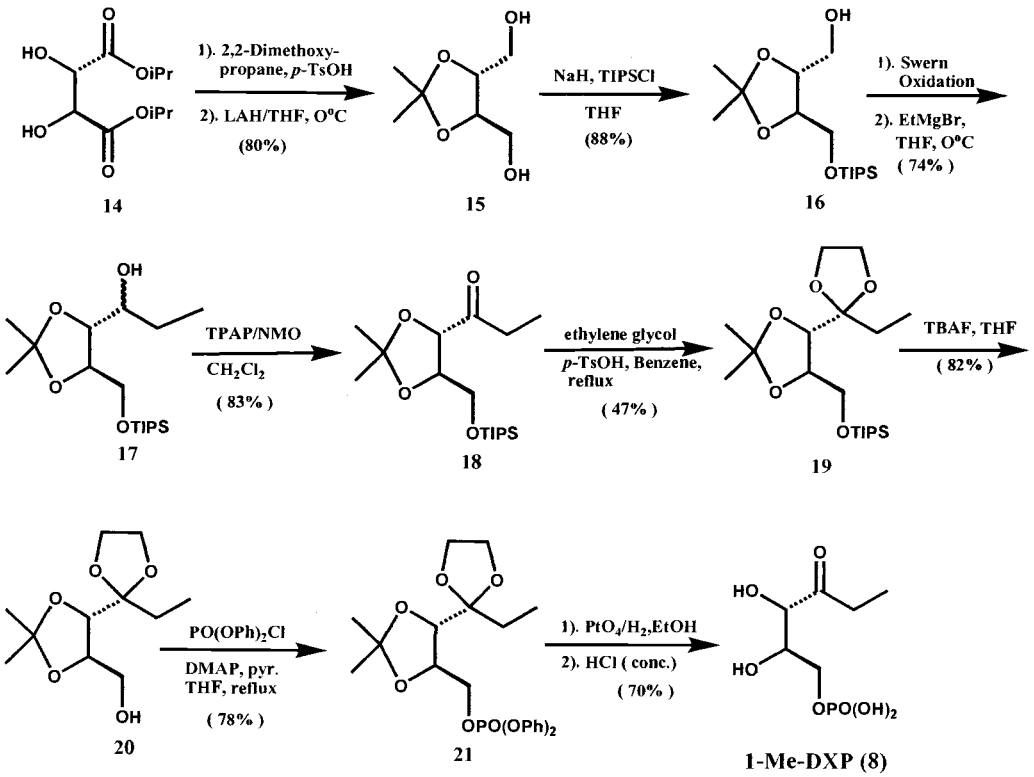

**Figure II.3. Putative inhibitors and alternate substrates of DXR.**

The compound, 1-methyl-DXP (1-Me-DXP, **8**) was designed to provide extra steric bulk at the methyl ketone moiety of DXP. It should provide details about the steric hindrance at the methyl ketone binding pocket of DXR. Another compound, DX-phosphonate (**9**) which has a phosphonate functional group instead of a phosphate functional group was proposed to act as an alternate substrate of DXR, but should provide information on the differences of phosphate versus phosphonate binding. It may also lead to inhibition further downstream in the MEP pathway. Another DXP analog, 4-deoxy DXP (**10**) which lacks the hydroxyl functional group at the C4 position was also designed. It should provide useful information about a binding effect of the C4-hydroxyl group to DXR. On the other hand, a DXP analog without a hydroxyl functional group at C3 position, 3-deoxy DXP (**11**), will reveal the importance of the C3-hydroxyl group for DXR binding. Moreover, these two compounds may provide information about the mechanism of the reaction mediated by DXR. The 4-epi-DXP compound, 1-deoxy-L-ribulose 5-

phosphate (**12**), has the opposite stereochemistry at the C-4 position from that of DXP. This compound will give information about how crucial the stereochemistry of the hydroxyl functional group is for binding to DXR. The methyl ketone moiety will be replaced with an amide in DXP carboxamide (**13**). Even though this compound has the same steric requirements as DXP, it has poorer electrophilicity at the carbonyl moiety compared to the substrate DXP. Therefore, this compound should not be a substrate for DXR and may act as a DXR inhibitor.

## 3) Experimental Syntheses

### 3.1) Synthesis of 1-methyl-DXP

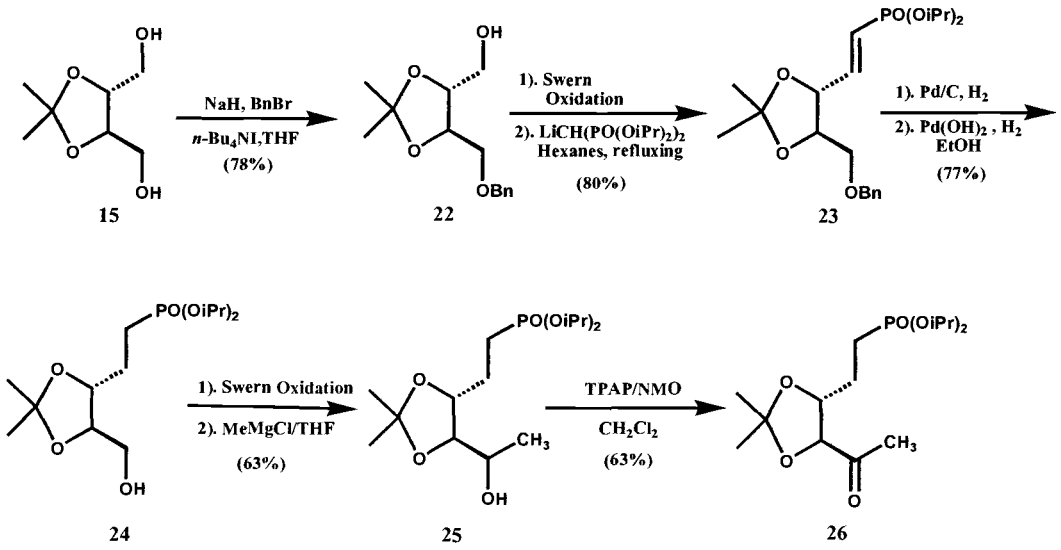



**Scheme 2.1. Synthesis of 1-methyl-DXP.**

The DXP substrate analog, 1-Me-DXP (**8**), was synthesized by modifying the synthesis of DXP from Blagg *et al.*[33] Instead of using the commercially available (-)2,3-O-isopropylidene-D-threitol (**15**) as mentioned in the literature procedure, this compound was obtained in two steps (acetonide formation and reduction)[34,35] from commercially available diisopropyl-D-tartate (**14**). The primary alcohol (**16**) was obtained from monosilylation [36] of **15** with 88% overall yield. Swern oxidation[37,38] of **16** followed by addition of the ethyl Grignard reagent to the aldehyde gave a diastereomeric mixture secondary alcohols (**17**). A direct Grignard addition to the aldehyde without a prior purification was shown to provide a better yield for this two-step process. The ketone (**18**) was produced by oxidation of the alcohols (**17**) with TPAP/NMO.[39] Protection of the ketone (**18**) as a 1,3-dioxolane[40] was conducted to provide **19** in 47% overall yield. A better yield of this reaction could not be obtained after several trials. Cleavage of the acetonide might happen under these conditions, preventing a high yield of the desired product. In the subsequent step, removal of the silyl protecting group with tetrabutylammonium fluoride[41] was conducted to obtain the alcohol (**20**). In the next step, the alcohol (**20**) was phosphorylated with diphenyl chlorophosphate. This phosphorylation procedure was shown to provide a better yield than the trimethylphosphite and 2,6-lutidine conditions indicated in the DXP synthesis.[33] Finally, 1-Me-DXP (**8**) was obtained by removal of the phenyl protecting group by catalytic hydrogenation with Pt(IV)oxide and acidic hydrolysis with aqueous HCl to cleave the acetonide and dioxolane.[42] Purification of 1-Me-DXP (**8**) was accomplished by cellulose chromatography[43, 44] with 15-40% (v/v) 0.1% TFA and THF. The sodium salt of **8** was generated by adding solid $NaHCO_3$ to pH 7 before the sample was lyophilized to obtain a white solid in a 9% overall yield in 11 steps from diisopropyl-D-tartate.

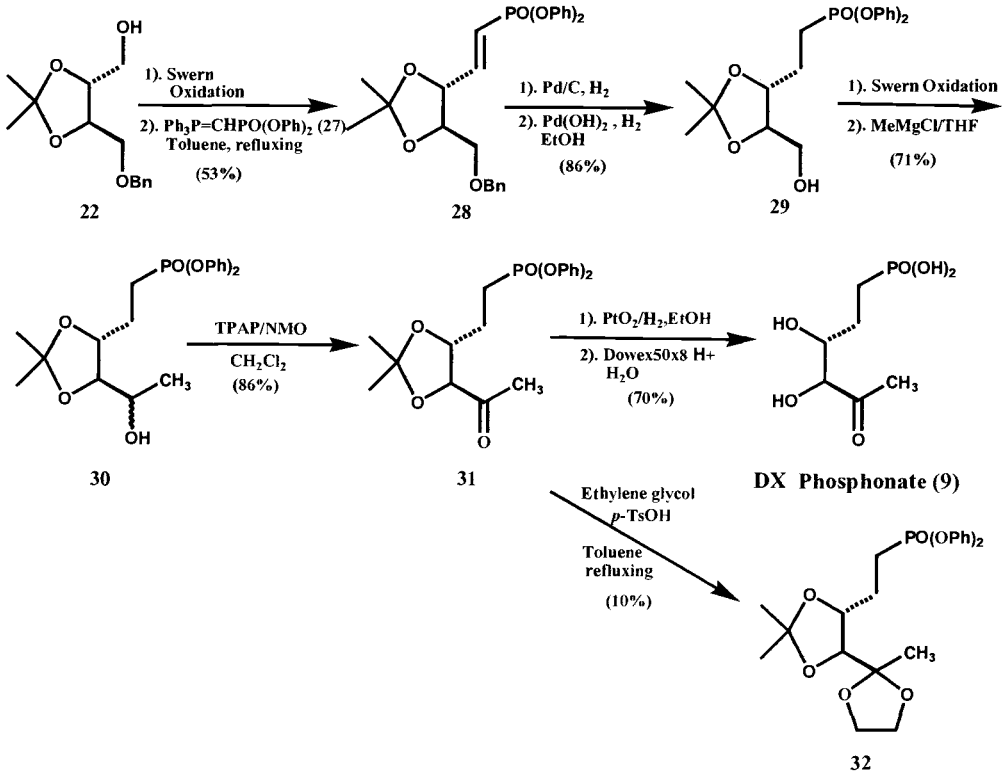
### 3.2) Synthesis of DX-Phosphonate

The synthesis of DX-phosphonate (**9**) started with monoselective benzylation of the diol **15** to get the monoprotected alcohol **22**.[45] It appeared that the dibenzylated diol by-product was observed in only a minor amount. Swern oxidation of **22** and Wittig reaction of the resulting aldehyde with tetraisopropylmethylene bisphosphonate ylide[46] gave the vinyldiisopropyl phosphonate (**23**). The primary alcohol (**24**) was obtained by cleaving the benzyl protecting group via catalytic hydrogenation with palladium on carbon while hydrogenation of the alkene required Pearlman's catalyst.[47] Palladium on carbon itself was not sufficient to successfully remove the benzyl group and hydrogenate the alkene in one step. The alcohol (**24**) was oxidized and the resulting aldehyde was reacted with the methyl Grignard reagent to give a secondary alcohol **25** as only one diastereomer. Oxidation of **25** with TPAP/NMO provided the keto diisopropyl phosphonate **26**. Different reagents such as TMSBr, TMSCl/NaI and TMSCl/$Ac_2O$ were tried for the deprotection of the diisopropyl groups on the phosphonate ,[48,49,50] but it appeared that only one isopropyl group could be cleaved. The negatively charged phosphonate arising from mono dealkylation of the diisopropyl ester might make it more difficult to undergo complete di-dealkylation.[50]




**Scheme 2.2. Synthesis of DX-Phosphonate (Route 1).**

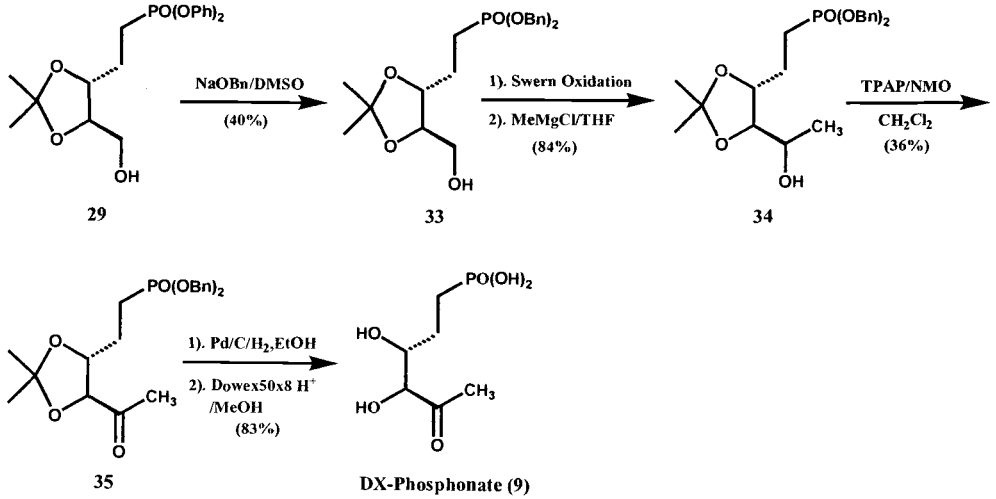
Because cleavage of the diisopropyl groups was unsuccessful, a different protecting group for the phosphonate was tried. The vinyl diphenylphosphonate **28** was generated by a Wittig reaction between diphenyl triphenylphosphoranylidene methylphosphonate (**27**)[51] and the aldehyde resulting from the Swern oxidation of alcohol **22**. The remaining reactions were performed as described for the synthesis of the diisopropyl phosphonate (**26**) synthesis. The expected DX-phosphonate (**9**) was obtained after catalytic hydrogenation and acid hydrolysis of the phenyl phosphonate **31**. Unfortunately, about 50% of the phosphonate product was found to be other related phosphonate impurities. Several purification methods including cellulose chromatography, Sephadex G-10 chromatography, cation exchange columns and HPLC with a $C_{18}$ column were tried, but the phosphonate impurities still could not be removed. Based on NMR data, the major phosphonate impurity appeared to be an alcohol phosphonate which may come from reduction of the methyl ketone using Pt(IV) oxide as the hydrogenation catalyst.




**Scheme 2.3. Synthesis of DX-Phosphonate (Route 2).**

To overcome this problem, the ketone **31** was protected with a 1,3-dioxolane functional group (**32**) before catalytic hydrogenation and the acid hydrolysis steps to generate DX-phosphonate (**9**). Even though the DX-phosphonate (**9**) that was obtained from catalytic hydrogenation and acid hydrolysis of the dioxolanephenyl-phosphonate **32** was relatively pure and there was no phosphate impurity, compound **32** could only be generated in a very low yield from the ketone **31**, likely because of competing hydrolysis of the acetonide under the ketone protection conditions.

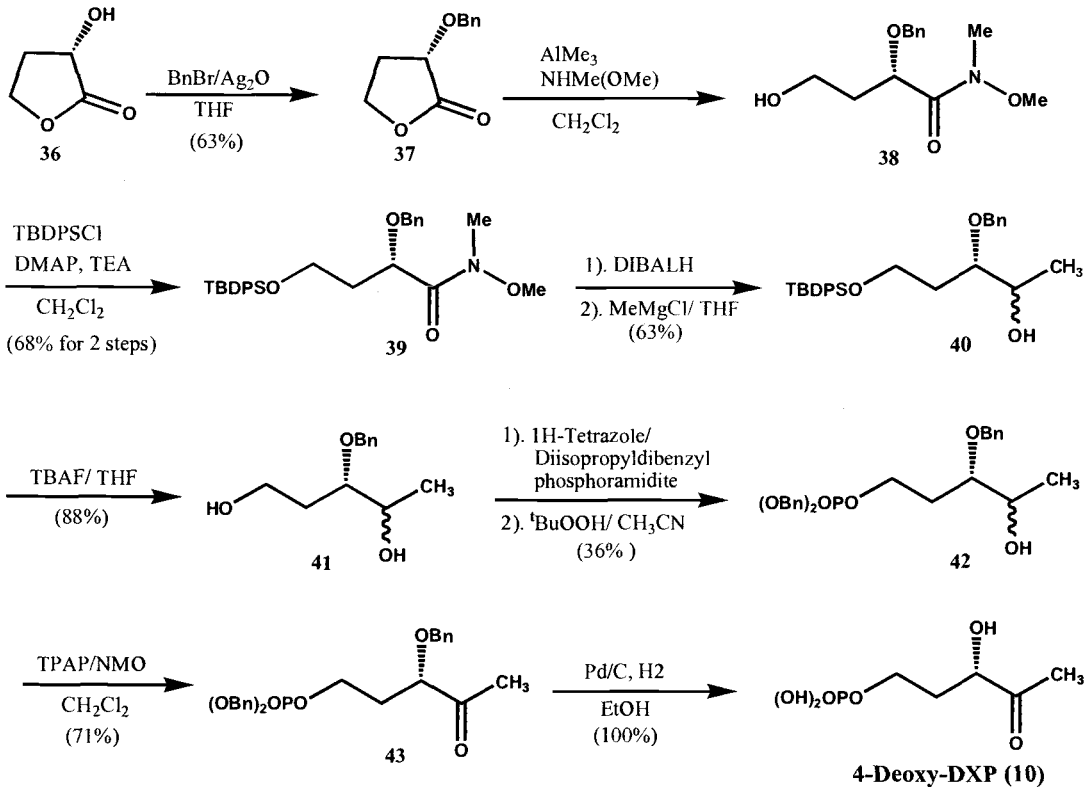
Because Pt(IV)oxide may reduce the ketone, a milder reducing agent such as palladium on carbon should be tried. The protecting groups of the phosphonate also needed to be easily removed with the Pd/C hydrogenation catalyst. Therefore, a dibenzyl phosphonate group should be used in the synthetic scheme rather than the diphenyl phosphonate. A synthesis of dibenzyl triphenylphosphoranylidene methylphosphonate ($Ph_3$=CHPO(OBn)$_2$) was attempted by reacting dibenzylchloro-phosphate[52] with methylene triphenylphosphonate,[53] but the desired product could not be obtained. A transesterification of the diphenyl ester **29** to the dibenzyl ester **33** was successfully done by using sodium benzyloxide in DMSO.[54] After processing the dibenzyl ester **33** in a manner similar to earlier schemes, DX-phosphonate (**9**) could be obtained in 3% overall yield (13 steps from diisopropyl-D-tartate) after catalytic hydrogenation and acid hydrolysis of dibenzyl phosphonate **35**. The DXP analog, DX-phosphonate (**9**) was used without further purification.




**Scheme 2.4. Synthesis of DX-Phosphonate (Route 3).**

### 3.3) Synthesis of 4-deoxy DXP

The compound 4-deoxy DXP was synthesized from the commercially available (*S*)-2-hydroxy-γ-butyrolactone (**36**). Benzylation of **37** was first tried with sodium hydride and benzyl bromide,[55] but TLC analysis of the reaction mixture showed a very complicated pattern with more than one reaction product. When benzyl bromide and silver (I) oxide[56] were used, the lactone **37** was successfully generated from **36** in moderate yield.



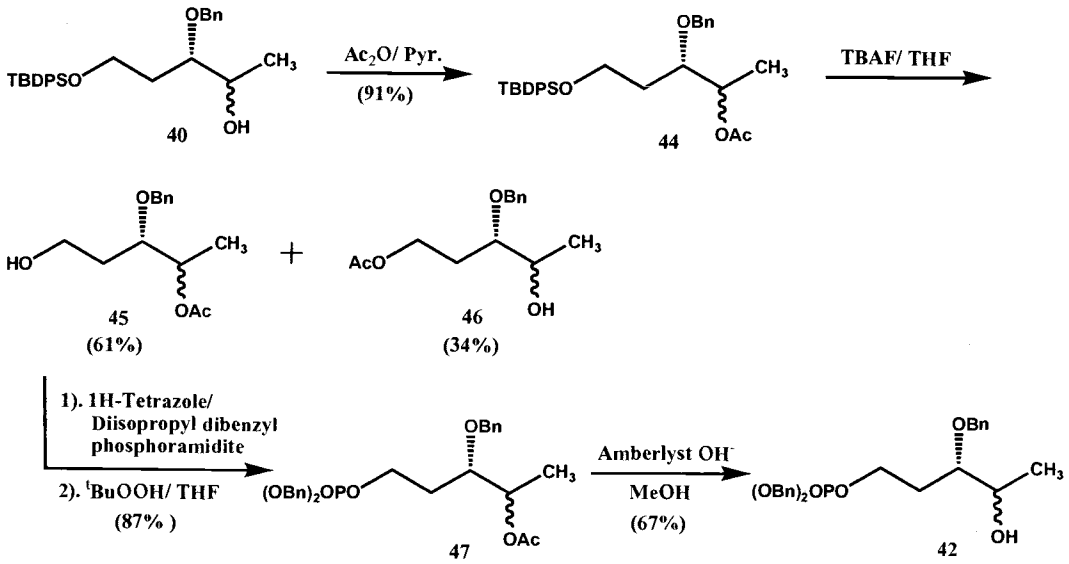

**Scheme 2.5. Synthesis of 4-deoxy DXP.**

In a subsequent step, lactone **37** was reacted with *N,O*-dimethylhydroxyl-amine in the presence of trimethylaluminum to generate the Weinreb amide with a free alcohol on the other end of the chain (**38**).[57] Without any further purification of **38**, the resulting alcohol was protected with a *tert*-butyldiphenylsilyl group to gain the Weinreb amide **39**.[58] This direct protection of the alcohol without prior

purification increased the percent yield for these two-step reactions because **38** was unstable to the slightly acidic nature of the silica used for chromatography. The detection of this compound on normal phase TLC had to be done with a basic solvent system (0.1% TEA in ethyl acetate/hexanes). Reduction of **39** was accomplished with DIBALH[59] and the resulting aldehyde was reacted with the methyl Grignard reagent to yield the secondary alcohol **40** as a mixture of diastereomers. In the next step, the *tert*-butyldiphenylsilyl group of **40** was cleaved with TBAF to generate the diol (**41**), with one primary alcohol and one secondary alcohol. The procedure from Graham *et al.* was use to selectively phosphorylate the primary alcohol of **41** with 5-methylthio-1H-tetrazole and diisopropyl phosphoramidite followed by *in situ* oxidation of the resulting phosphate triester with *tert*-butylhydroperoxide.[60] The yield of the phosphorylation and oxidation steps was disappointingly only 36%. As mentioned in the original procedure, solubility plays a very important role in this phosphorylation reaction. Acetonitrile was used as a solvent in this reaction, but the solubility of 5-methylthio-1H-tetrazole in acetonitrile was quite low. While 5-methylthio-1H-tetrazole has more solubility in THF, this solvent system provided no selective phosphorylation of the primary alcohol over the secondary alcohol.[61] Acetone was also tried as a solvent, but there was no difference in percent yield when compared to acetonitrile. However, even with the poor yield, the dibenzylphosphate **42** could be obtained in sufficient amounts to be carried on to the next step. The ketone **43** was generated by TPAP oxidation of **42**. In a final step, 4-deoxy DXP (**10**) was obtained by a catalytic hydrogenation of **43** in quantitative yield.

To improve the yield of the phosphorylation step in the 4-deoxy DXP (**10**) synthetic route, the secondary alcohol **40** was protected with an acetyl group before cleavage of the *tert*-butyldiphenylsilyl group to generate the primary alcohol **45**. However, there was a problem with transesterification of **45** to yield the secondary alcohol **46** under the slightly basic conditions of the TBAF cleavage reaction. The ratio of the expected alcohol **45** to the by-product **46** was about 2:1 and **45** and **46** could be separated on a silica column with ethyl actetate/hexanes. The benzyl

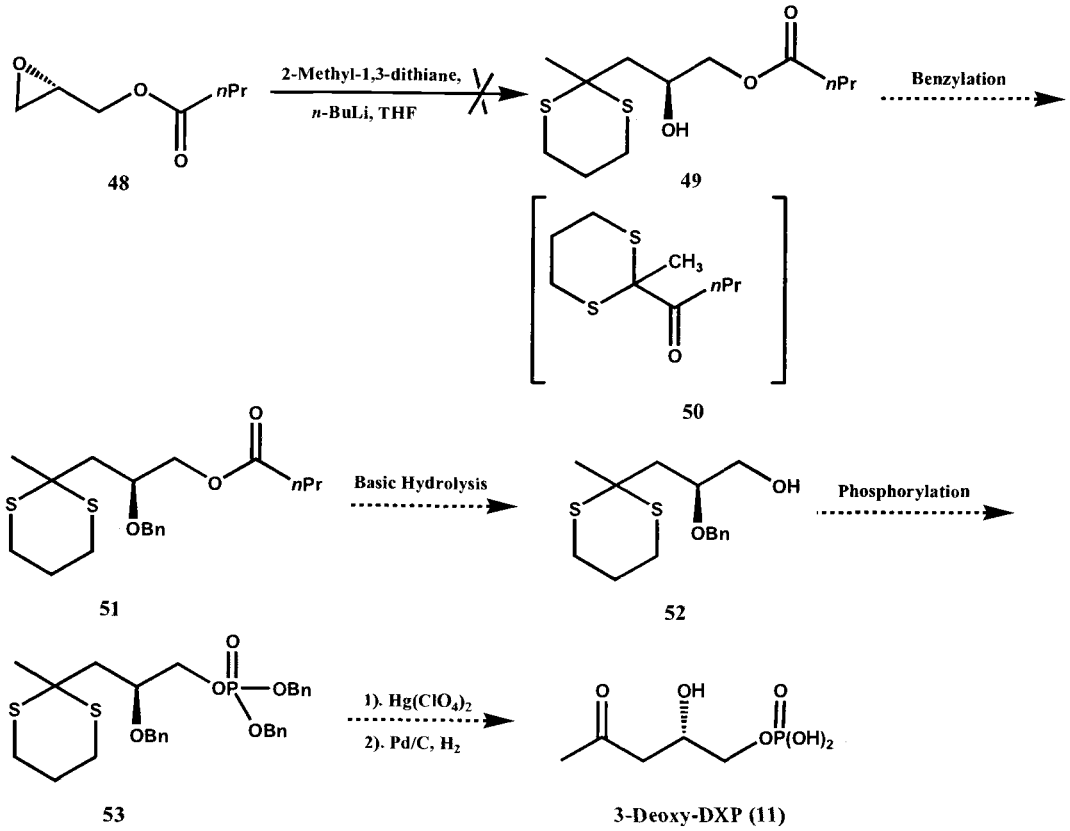
protecting group for the secondary alcohol **40** was also tried, but the desired product could not be obtained. Moreover, the *tert*-butyldiphenylsilyl group is quite stable under acidic condition, but is vulnerable under basic condition.[62] Therefore, there are limited choices for the deprotection of the *tert*-butyldiphenyl silyl group without causing the transesterification of the acetyl group. Despite the low yield, the next step was pursued to compare the percent yield for the phosphorylation step. Phosphoryaltion of **45** was performed with 1H-tetrazole and diisopropyl phosphoramidite in THF followed with *tert*-butylhydroperoxide odixation.[63] This phosphorylation step gave a better yield than the previous phosphorylation reaction. In the next step, the dibenzylphosphate **42** was generated by a basic hydrolysis of acetyl group with an anion exchange resin ($OH^-$ form). Even though the phosphorylation step was performed with a better yield, the overall yield from the secondary alcohol **40** to the dibenzylphosphate **42** was still in the same range as in the previous procedure. Moreover, this new scheme required additional work up for the added reaction steps. Therefore, the synthesis of 4-deoxy DXP (**10,** in 10 steps with 7% overall yield) was based on Scheme 2.5.




**Scheme 2.6. An alternative synthesis of 4-deoxy DXP.**

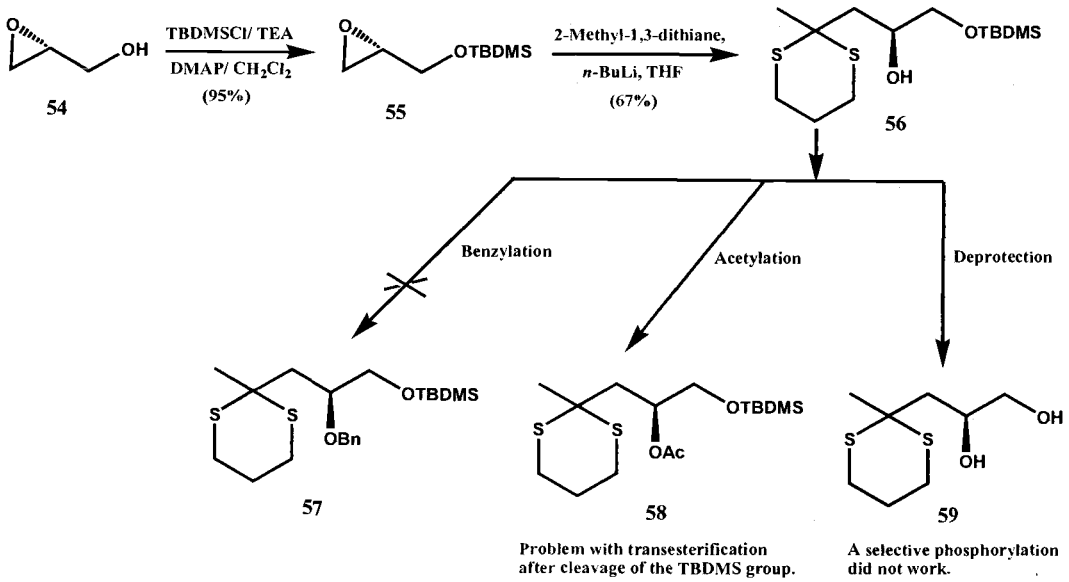
### 3.4) Synthesis of 3-deoxy DXP

The first attempt to synthesize 3-deoxy DXP started with the ring opening of the commercially available compound (*S*)-glycidyl butyrate (**48**) with the 2-methyl-1,3-dithiane anion. The dithiane compound (**53**) can be cleaved to form the ketone functional group as needed in the final product (**11**).[64,65] The butyrate group was expected to be maintained as a protecting group of the primary alcohol until the phosphorylation step. However, the dithiane anion preferentially attacked the ester carbonyl rather than the epoxide. The dithiane **50** was characterized as a by-product from this reaction. Therefore, the ester group was not a good protecting group for the primary alcohol in this synthetic scheme.



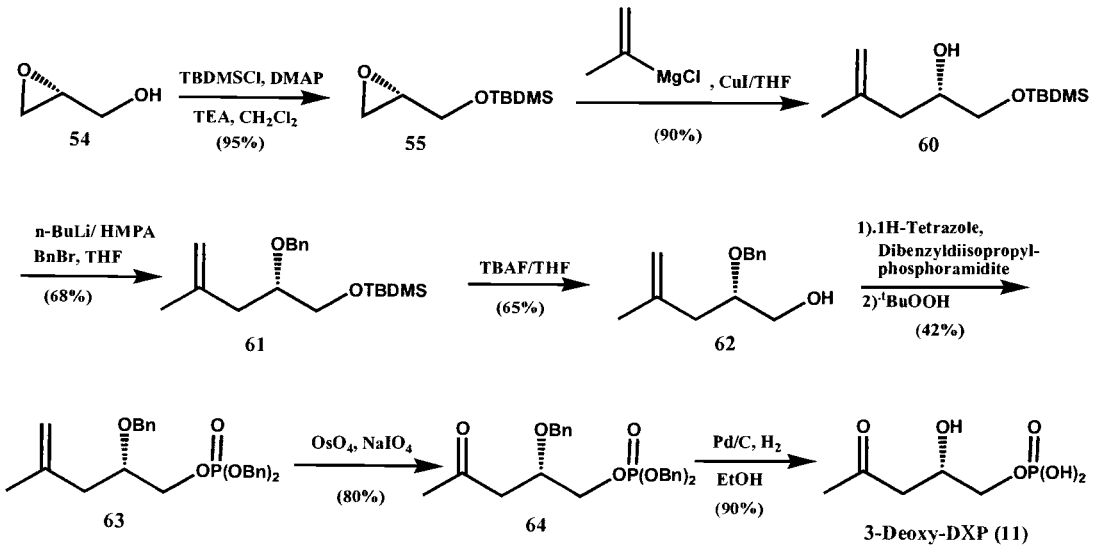

**Scheme 2.7. The first attempt for synthesis of 3-deoxy DXP.**

In order to find a better protecting group for the primary alcohol, protection of the commercially available compound (*R*)-glycidol (**54**) was conducted with *tert*-butyldimethylsilyl chloride to yield the oxirane (**55**). Ring opening of the oxirane was again tried with the dithiane derivative. However, there was a problem with this approach regarding a difficulty in protecting the secondary alcohol with the benzyl functional group. Steric hindrance of the dithiane may be a significant problem. Even though an acetyl group could be used to protect the secondary alcohol, there was a problem with transesterification as encountered in the synthesis of 4-deoxy-DXP after cleavage of the silyl group. Selective phosphorylation of the primary alcohol was also attempted, but the expected product could not be obtained. It appeared that the dithiane causes major difficulties for this synthetic scheme, so another approach was tried with a different protecting group for the ketone.




**Scheme 2.8. The second attempt for synthesis of 3-deoxy DXP.**

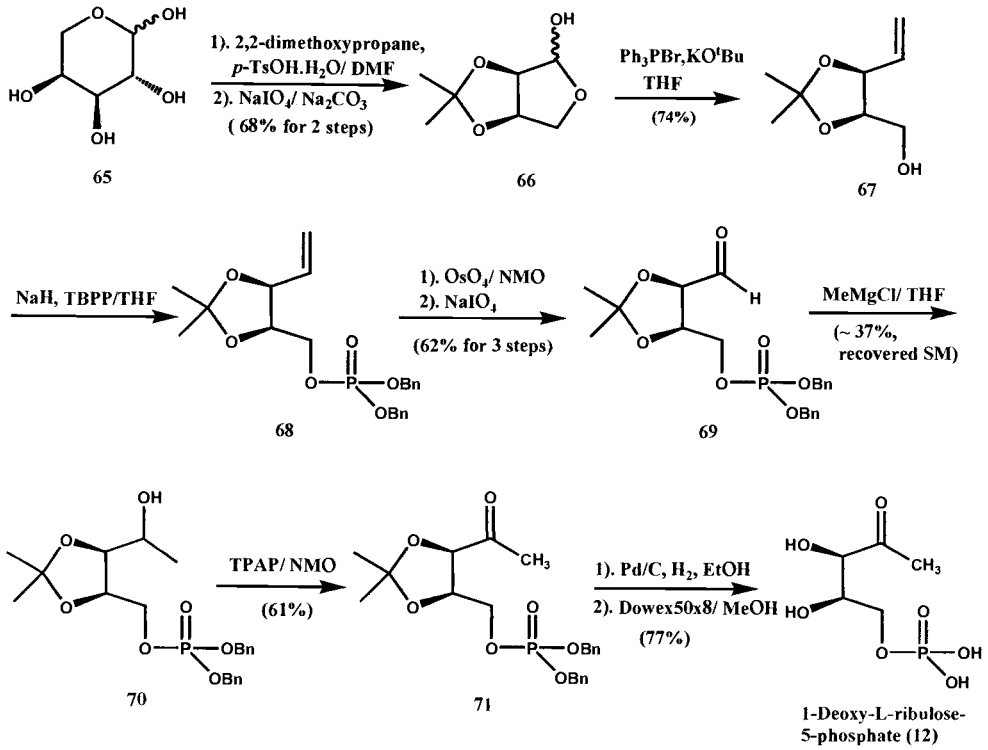
The third approach for the synthesis of 3-deoxy DXP is outlined in Scheme 2.9. The protection of (*R*)-glycidol (**54**) yielded the oxirane (**55**) which was reacted with the isoproprenyl Grignard reagent with a catalytic amount of copper (I) iodide to form the secondary alcohol **60**.[66] Benzylation of **60** was successfully conducted under *n*-BuLi and HMPA conditions[67] after the previously mentioned benzylation procedure failed. In the subsequent step, the primary alcohol **62** was obtained from cleavage of the *tert*-butyldimethylsilyl group with TBAF. Phosphorylation of the primary alcohol utilized phosphoramidite chemistry[63] to obtain **63** in 42% overalyield. Oxidative cleavage of the alkene **63** was performed with osmium tetroxide and sodium metaperiodate in one pot to yield the ketone **64** in a very good yield.[68,69] Finally, 3-deoxy DXP (**11**) was obtained by catalytic hydrogenation of the ketone **64**. In conclusion, 3-deoxy DXP (**11**) was obtained from commercially available (*R*)-glycidol in 8 steps with 11% overall yield.




**Scheme 2.9. The synthesis of 3-deoxy DXP.**

### 3.5) Synthesis of 1-deoxy-L-ribulose 5-phosphate

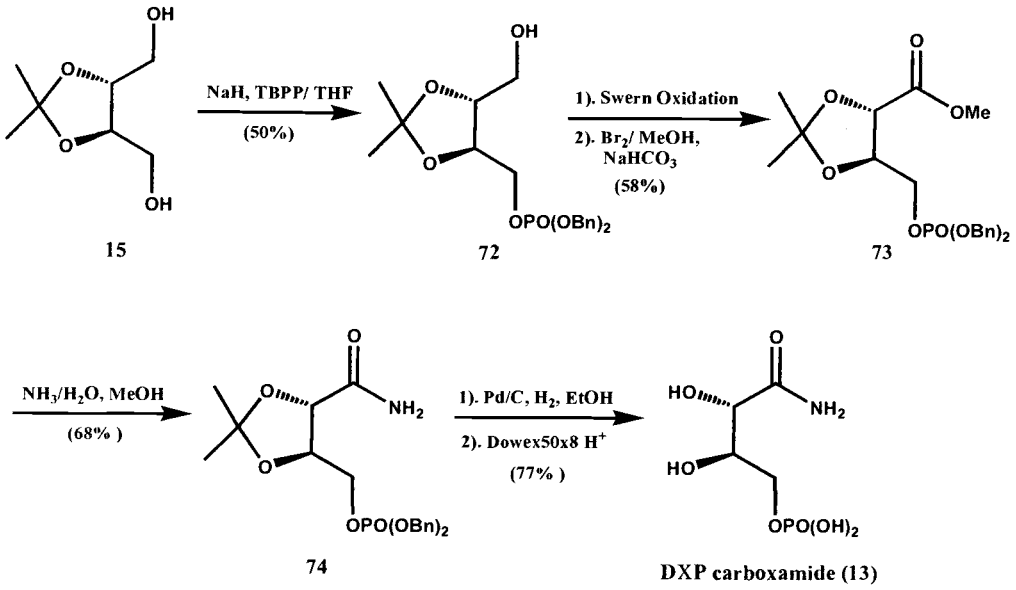
The compound, 1-deoxy-L-ribulose 5-phosphate, 4-epi-DXP (**12**) was synthesized from L-arabinose (**65**). Selective isopropylidene formation and oxidative cleavage of **65** generated a diastereomeric mixture of **66**.[70] The Wittig reaction of **66** with methyltriphenylphosphonium bromide/potassium *tert*-butoxide yielded the alkene **67**.[71] Phosphorylation of **67** was accomplished using tetrabenzyl pyrophosphate (TBPP).[72] Dihydroxylation of the resulting dibenzyl phosphate compound with osmium tetroxide followed by oxidative cleavage with periodate provided the aldehyde **69** in 62% overall yield for 3 steps. A single isomer of secondary alcohol **70** was obtained from the methyl Grignard addition to the aldehyde **69**. Oxidation of **70** using TPAP and NMO provided ketone **40** in 61% yield. Finally, 4-epi-DXP (**12**) (5% overall yield, 10 steps from L-arabinose) was obtained by catalytic hydrogenation and acid hydrolysis of **71**. The phosphate product was used without further purification.




**Scheme 2.10. Synthesis of 1-Deoxy-L-ribulose 5-phosphate.**

### 3.6) Synthesis of DXP carboxamide

Scheme 11 shows a procedure for the DXP carboxamide (**13**) synthesis. The primary alcohol **72** was obtained from monophosphorylation of the diol **15** with tetrabenzyl pyrophosphate. Even though the desired alcohol **72** could be obtained in only 50% yield, there was no by product that could be detected in an appreciable amount. Swern oxidation of **72** and oxidation of the resulting aldehyde with bromine in methanol directly afforded the ester **73**.[73] A reaction between the ester **73** and ammonia in water gave amide **74** in 68% yield.[73] Finally, catalytic hydrogenation and acid hydrolysis[74] of **74** were performed to yield the DXP carboxamide (**13**) (12% overall yield, 8 steps from diisopropyl-D-tartate) which was used without further purification.




**Scheme 2.11. Synthesis of DXP carboxamide.**

**4) Results**

The DXP analogs were tested as alternate substrates for DXR. All five phosphate analogs of DXP showed no sign of being an alternate substrate for DXR when tested at concentrations up to 30 mM. However, DX-phosphonate did show activity as an alternate substrate. In initial studies, it was turned over by DXR at concentrations as low as 33 μM. This compound has a $K_m$ = 690 μM which is three and a half-fold higher than the $K_{m(DXP)}$.

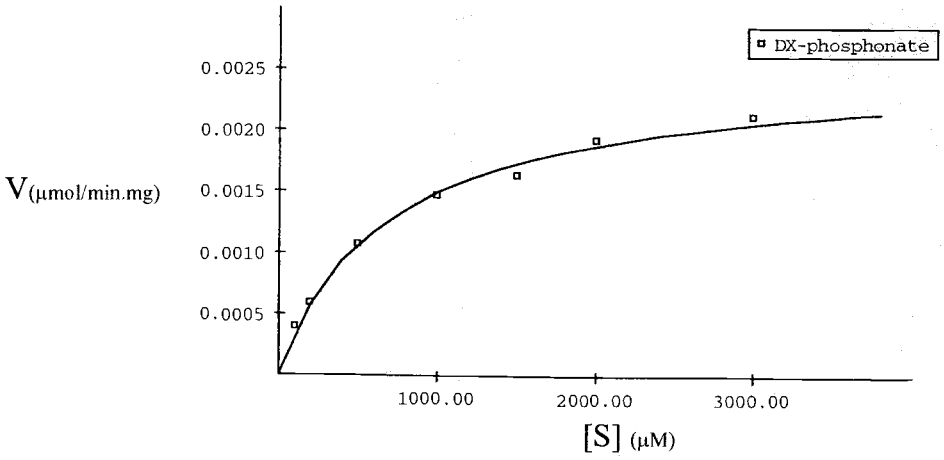



**Figure 2.4. A non-linear regression plot of DX-phosphonate as the substrate for DXR.**

When testing all five phosphate analogs of DXP as inhibitors, they appeared to be weak competitive inhibitors for DXR. The $K_i$ value of 1-Me-DXP is 630 μM, which is about three-fold higher than the $K_{m(DXP)}$. The compound 4-epi-DXP, or 1-deoxy-D-ribulose 5-phosphate, has a $K_i$ = 180 μM which is in the same range as the $K_{m(DXP)}$. The 4-deoxy-DXP, however, has a $K_i$ = 30 μM which is about seven-fold lower than the $K_{m(DXP)}$. The $K_i$ value of 3-deoxy-DXP is 150 μM which is about one and a half-fold lower than the $K_{m(DXP)}$. The DXP carboxamide was also found to be a competitive inhibitor of DXR with $K_i$ = 90 μM.

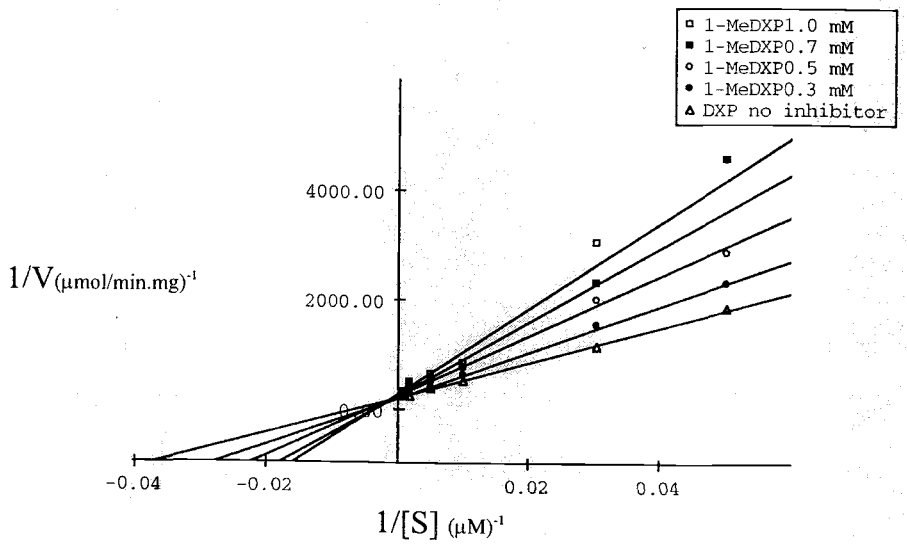



**Figure 2.5. Double reciprocal plots for inhibition of DXR by 1-Me-DXP.**

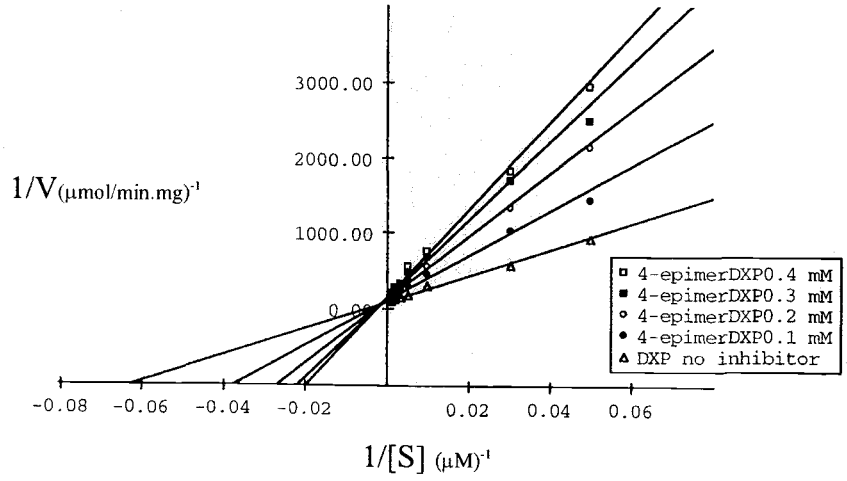



**Figure 2.6. Double reciprocal plots for inhibition of DXR by 1-deoxy-D-ribulose 5-phosphate.**

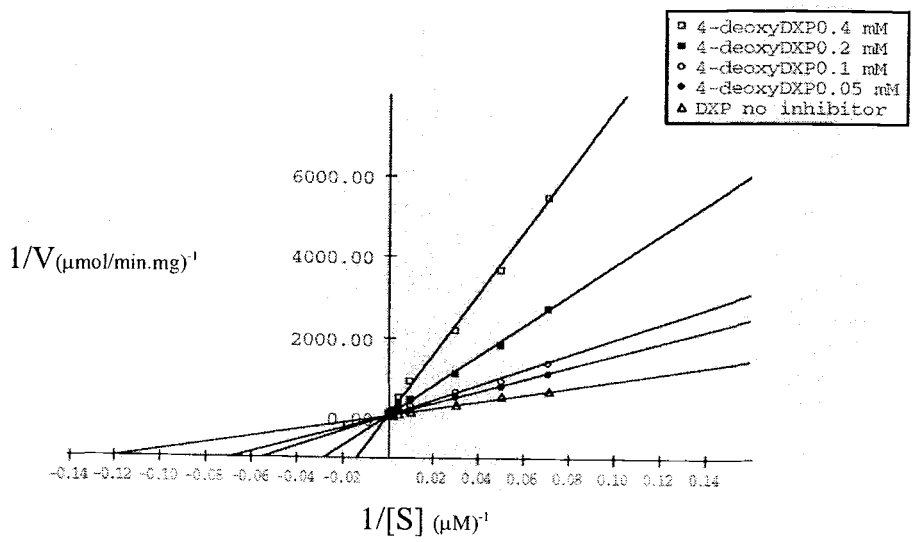



**Figure 2.7. Double reciprocal plots for inhibition of DXR by 4-deoxy-DXP.**

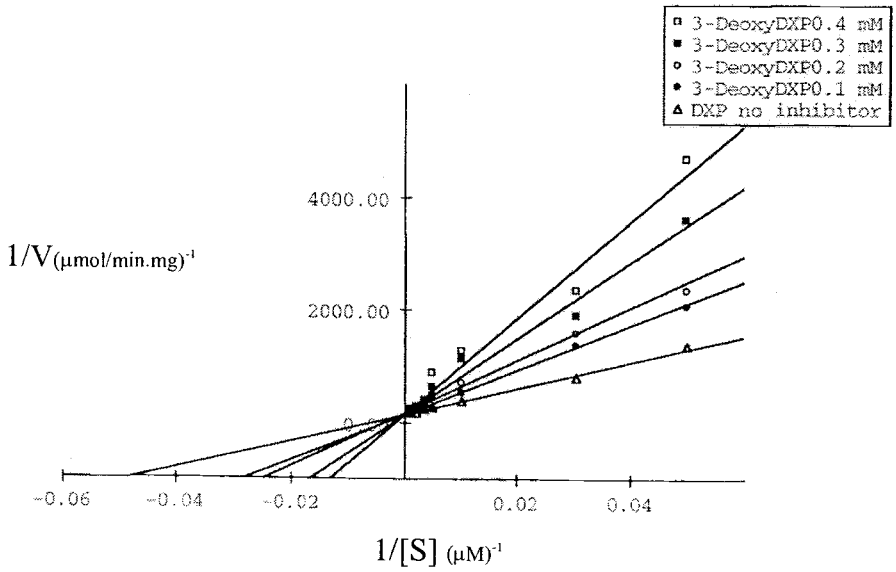



**Figure 2.8. Double reciprocal plots for inhibition of DXR by 3-deoxy-DXP.**

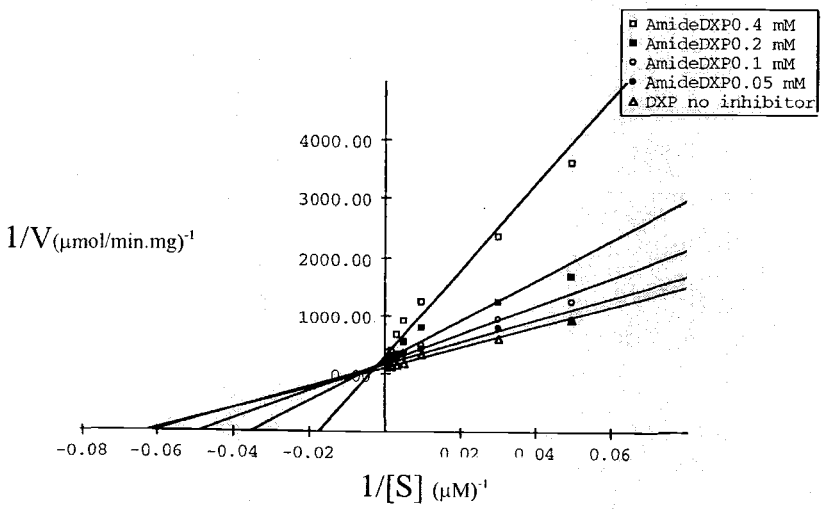
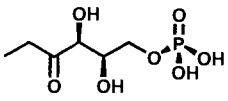
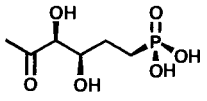


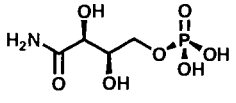

**Figure 2.9. Double reciprocal plots for inhibition of DXR by DXP carboxamide.**



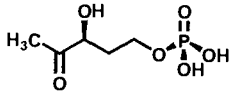
**1-Methyl-DXP (1)**
**( $K_i$ = 630 μM)**



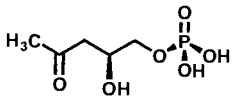
**Deoxyxylulose phosphonate (2)**
**( $K_m$ = 690 μM)**



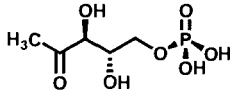
**DXP carboxamide (3)**
**( $K_i$ = 90 μM)**



**4-Deoxy-DXP (4)**
**( $K_i$ = 32 μM)**



**3-Deoxy-DXP (5)**
**( $K_i$ = 150 μM)**



**1-Deoxy-L-ribulose-5-phosphate (6)**
**( $K_i$ = 180 μM)**

**$K_{m(DXP)}$ = 190 ± 20 μM**

**Figure 2.10. The alternate substrate and the inhibitors against DXR.**

**5) Discussion**

The DXP-analog, 1-Me-DXP, which has an ethyl ketone group rather than the methyl ketone moiety of DXP, could not act as an alternate substrate. From the inhibition test, the results indicate that 1-Me-DXP acts as only a weak competitive inhibitor against DXR with a $K_i$ about three and a half-fold higher than the $K_{m(DXP)}$. This result demonstrates that there are steric limitations in the vicinity of the methyl ketone moiety of DXP toward DXR binding. The methyl ketone group of DXP appears to be the optimal size of this moiety for binding to DXR and for designing a better inhibitor.

Another DXP analog, 1-deoxy-D-ribulose 5-phosphate, did not get turned over by DXR when it was tested as an alternate substrate. This result indicates that the stereochemistry of the hydroxyl group at the $C_4$ position of DXP is a major factor for DXR-substrate specificity. The binding affinity of 1-deoxy-D-ribulose 5-phosphate is similar to that of DXP based on the $K_i$ value which is in the same range with the $K_{m(DXP)}$. Using the positioning of the hydrophobic chain of fosmidomycin in the X-ray structure as a guide,[12] a hydroxyl group at the carbon equivalent to $C_4$ does not appear to have strong interaction with any amino acid sidechains. On the other hand, the same data suggested a significant role of the hydroxyl group at the $C_3$ position of DXP as a ligand for the divalent cation cofactor. However, the hydroxyl at the $C_4$ position is quite important for the rearrangement which will be discussed in more detail in Chapter Four.

The compound 4-deoxyDXP, which is the analog of DXP lacking the hydroxyl at the $C_4$ position, was not an alternate substrate for DXR. However, it was a competitive inhibitor against DXR with the lowest $K_i$ value of the tested analogs which is about seven-fold lower than $K_m$ of DXP. The absence of the $C_4$ hydroxyl clearly does not have a negative effect on binding to DXR. This result also confirms the significance of the hydroxyl at the $C_4$ position of DXP not for the binding, but for successful turnover. The hydroxyl at the $C_3$ position of DXP also is not crucial for binding to DXR according to the $K_i$ value of 3-deoxy-DXP which is about one and a half-fold lower than the $K_m$ of DXP. The 3-deoxy-DXP also acted

as a competitive inhibitor. Like all previously mentioned compounds, 3-deoxyDXP was not an alternate substrate for DXR.

When the DXP carboxamide was tested as an alternate substrate, no turnover was observed. In addition to the difference in electrophilicity at the carbonyl moiety, there is a difference in properties between a methyl group in DXP and the amide-$NH_2$ group in the DXP carboxamide that should be considered. The methyl group is more hydrophobic when compared to the amide nitrogen group, while the amide-$NH_2$ could participate in hydrogen bonding interactions. The different interactions between these two groups toward the amino acid residues at the binding pocket of DXR may play an important role in this case. The DXP carboxamide was a competitive inhibitor against DXR with the $K_i$ value about two-fold lower than the $K_m$ of DXP. It can be concluded that the DXP carboxamide with the amide-$NH_2$ group does not have any major binding interactions at the active site that differ from those of DXP. The DXP carboxamide would be expected to bind better than 1-Me-DXP based on steric factors alone.

Unlike the above mentioned phosphate compounds, the phosphonate analog of DXP was the only compound tested that could act as an alternate substrate. The $K_m$ of DX-phosphonate is about three and a half-fold higher than the $K_{m(DXP)}$. This result actually is not far from expected because the structural change is remote from the site of rearrangement in the molecule. The result suggests a similarity in the enzyme binding pocket for the phosphate functional group of DXP, and a phosphonate functional group of DX-phosphonate as would be expected. From the X-ray crystal structure of DXR complexed to fosmidomycin,[12] the phosphonate moiety of fosmidomycin is anchored by numerous hydrogen bonds to Ser186, Ser222, Asn227, and Lys228. The phosphonate group of DX-phosphonate should also be coordinated by the same amino acid residues in the same binding pocket.

The only difference between these two functional groups is the linker that connects the phosphorus atom to the rest of a deoxyxylulose chain. Rather than having a hydrophilic oxygen atom as a linker, DX-phosphonate has a hydrophobic methylene for connecting the phosphorus atom to another carbon atom. There

should be amino acid residue(s) that coordinates the hydrophilic oxygen and assists the binding of DXP to DXR. This hydrophilic interaction will disappear when the oxygen is replaced with the methylene group. Unfortunately, the data from the X-ray crystal structure of DXR[12] did not clearly show any amino acid residues that can be a candidate for this interaction.

Moreover, there may be different charges for the phosphate and phosphonate groups present under physiological pH. The phosphate analog, DXP should bind to DXR as dianion while the DX-phosphonate might bind as monoanion.[75] These differences clearly contribute to the difference in binding affinity of both compounds. From the $K_m$ value of DX-phosphonate which is about three and a half-fold higher than the $K_{m(DXP)}$, it can be concluded that the binding affinity of the phosphonate group is slightly lower than that of the phosphate functional group. Independent studies from another member in this laboratory showed that the $K_i$ value of a phosphate analog of fosmidomycin is about three-fold lower than the $K_i$ of fosmidomycin itself.[32] This perhaps suggests a trend that the affinity of a phosphate containing molecule is about three to three and a half fold greater than an otherwise identical phosphonate containing compound. Interestingly, during the preparation of this dissertation, there was a report from Meyer *et al.* indicating that DX-phosphonate was an alternate substrate for DXR from *E. coli*.[76] The same study also showed the $K_i$ value of DX-phosphonate (120 μM) is four fold higher than the $K_{m(DXP)}$ (30 μM) when testing against DXR from *E. coli*.

Although DX-phosphonate is not an inhibitor of DXR, DX-phosphonate may still act as an inhibitor of isoprenoid biosynthesis. The expected DXR turnover product from DX-phosphonate is methylerythritol phosphonate (**75**) which was also isolated and characterized by the same research group.[77] This product will probably be carried on further downstream by enzymes from the MEP pathway, possibly forming the phosphonate analogs of DMAPP and IPP. These derivatives should not be turned over by farnesyl diphosphate synthase, because this enzyme requires the allylic phosphate, DMAPP, which can be ionized. The phosphonate analog of DMAPP is not capable of forming the necessary allylic cation.

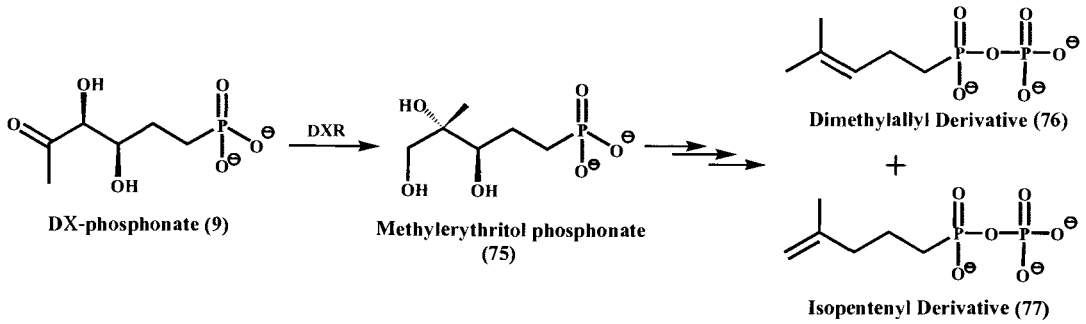



**Figure 2.11. The reactions of DX-phosphonate along the MEP pathway.**

In order to test this hypothesis, DX-phosphonate along with the rest of DXP analogs were tested for antibiotic activity against two different bacteria including *E. coli*, and *P. aeruginosa*. By using the antibiotic disk diffusion test and chloramphenicol as a standard, the discs on *E. coli* and *P.aeruginosa* agar plates showed an inhibition zone with chloramphenicol, while no sign of growth inhibition was detected with all DXP analogs when up to 500 μg of each compound were used. These analogs, therefore, show no antibiotic activity. Meyer *et al.* also indicated that DX-phosphonate does not prevent the growth of *E. coli* even at the highest amount tested (100 μg).[76] It may be that the uptake process for these highly charged phosphate and phosphonate compounds might be a major problem for delivery these compounds into the bacterial cells. Derivatization of these compounds as prodrugs could help increase the rate of uptake, potentially allowing for better assessment of these compounds as antibiotics.

## 6) Experimental Section

### 6.1) DXP Analog Concentrations

In order to obtain accurate concentrations of phosphate compounds, a phosphate analysis was needed. Because phosphate compounds can easily bind with water molecules which are often very difficult to remove and because phosphates can exist in several ionized states, concentrations obtained from the crude weight might be higher than the real concentrations. The concentrations of phosphate compounds from phosphate analysis should be closer to the actual concentrations.[78]

After all compounds were obtained, a stock solution of each compound was prepared in $ddH_2O$. Solid $NaHCO_3$ was added to the solution to adjust the pH to 7.0. In the next step, 1.0 mM solution of the phosphate compounds, including 1-Me-DXP, 1-deoxy-D-ribulose 5-phosphate, and DXP carboxamide, were prepared based on the dry weight. The phosphate analysis of these compounds was conducted according to the procedure from Martin *et al.*[78] Briefly, perchloric acid was used to cleave the phosphate group and a complex of the phosphate anion with molybdate was formed. The absorbance of this complex was measured at 310 nm. A standard curve was obtained from a monobasic potassium phosphate stock solution. Concentrations of 1-Me-DXP and 1-deoxy-D-ribulose 5-phosphate calculated from the phosphate analysis were very close to those based on the weighed amount, with values being 5-10% lower. However, the DXP carboxamide appeared to have a very low concentration based on the phosphate analysis. A time course experiment was performed to check the possibility that the phosphate group on DXP carboxamide might not be completely cleaved. There was no different result obtained from varied reaction times of perchloric acid with DXP carboxamide. The standard phosphate analysis does not work for all phosphate compounds. It might be that the amide functional group interferes with the complexation of phosphate with molybdate or that the phosphate group is not cleaved under these conditions. Therefore, the concentration of amide-DXP was based on the crude weight. The concentration of DX-phosphonate was measured by a $^1$H-NMR technique by comparing to an internal standard, *p*-toluenesulfonic acid

monohydrate. This value is also very close to that obtained from the direct weight of the solid sample.

### 6.2) DXR Preparation and Purification

The enzyme DXR was obtained by Dr. Xihou Yin according established procedures.[79] A pBAD expression plasmid containing the *Synechocystis* sp. PCC6803 *dxr* was constructed previously.[80] The plasmid pBAD-Bgl-Sdxr was transformed into *E.coli* Top 10 for overproduction of H-DXR (Histidine-tagged DXR). Single colonies isolated from fresh transformation plates were grown in Luria-Bertani medium supplemented with ampicillin. An aliquot of the culture was used to inoculate 500 mL of the same medium. Induction of expression was initiated by adding L-arabinose. The bacterial cells were harvested by centrifugation 5 h after induction. The cells were washed with extraction buffer containing Tris buffer, $MgCl_2$, dithiothreitol (DTT), and phenylmethanesulfonyl fluoride (PMSF) and stored at -80 °C. When the H-DXR was needed, the frozen cells were thawed on ice and resuspended in Tris buffer. The resulting suspension was sonicated and centrifuged to remove cellular debris. The supernatant was used as the cell-free extract.

The cell-free extract for the H-DXR enzyme was mixed with a slurry of Talon® resin (Invitrogen) following the manufacturer's protocol (Batch/Gravity-flow Column Purification) using a Tris buffer system as the extraction/washing buffer (Buffer B) instead of the phosphate buffer. The elution was conducted with a stepwise gradient from 20-250 mM imidazole in buffer B. The eluate was collected as 1-mL fractions. The purity of each fraction was verified by SDS-PAGE. The high purity fractions were combined and dialyzed with Tris buffer (50 mM Tris pH 7.8 and 300 mM NaCl) at 4 °C. The enzyme solution was stored at 4 °C and used within four weeks. The concentration of DXR was determined by Bradford analysis using bovine serum albumin as a standard.[81]

**6.3) DXR Assay**

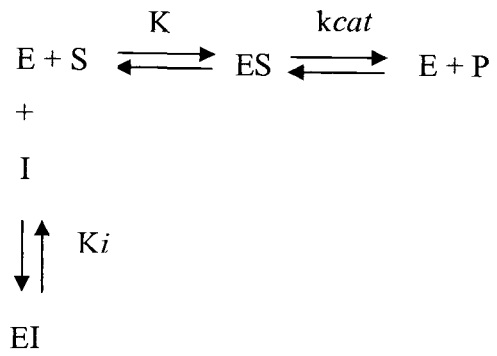
The activity of DXR was monitored by measuring the decrease in absorbance at 340 nm as NADPH was oxidized. These assays were based on Seto's protocol.[82] The standard assays were performed at 37 °C with a total volume of either 1 mL or 500 μL, containing 50 mM Tris buffer, pH 7.8, 1 mM $Mn^{2+}$, 0.2 mM NADPH, the substrate (DXP), and the enzyme DXR containing 1 mg/mL BSA. The reactions were initiated by the addition of DXR. Serial dilutions of DXR were made into 50 mM Tris buffer pH 7.5 containing 1 mg/mL BSA. To determine the Michaelis-Menten constants ($K_m$), assays were performed in triplicate at different concentrations of DXP including 1000, 500, 300, 200, 100, 33, and 20 μM. Rates of the assays were measured in the linear range and less than 10% of substrate was consumed which were typically the first four minutes of the reactions. The Enzyme Kinetics Program from Trinity software was used to calculate $K_m$. The $K_{m(DXP)}$ value obtained from *Synechocystis* sp. PCC6803 DXR in this experiment was 190 ± 20 μM. This value is similar to those previously reported for the *Synechocystis* sp. PCC6803 ($K_{m(DXP)}$ = 180±15 μM),[80] as well as to those of DXR's from other sources: *Z. mobilis* ($K_{m(DXP)}$ = 300 μM),[83] *S. coelicolor* ($K_{m(DXP)}$ = 190 μM),[84] and *E.coli* ($K_{m(DXP)}$ = 175 μM).[11] The DXR substrate, DXP, was enzymatically synthesized as previously described.[85] The phosphate analysis was used to determine the concentration of the DXP stock solution.

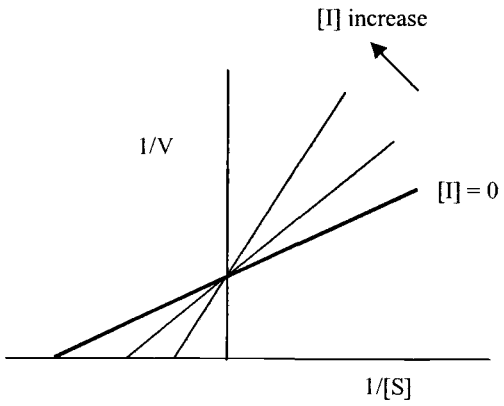
### 6.4) Alternate Substrate and Inhibition of DXR Studies

All compounds were tested as possible substrate for DXR by using the assay mentioned above by using concentration of all compounds up to 30 mM. For inhibition studies of the DXP analogs, all compounds were tested with the UV assay mentioned above with minor modifications. Rates of reaction were measured for seven different concentrations of the substrate (DXP) at four different concentrations of the DXP analogs. The Enzyme Kinetics Program from Trinity software was used to calculate the $K_i$ of each compound based on a non-linear regression method. Double reciprocal plots (Lineweaver Burk plots) of each compound were prepared to determine the nature of the inhibition: competitive, uncompetitive, linear mixed type or noncompetitive.[86] If any of the compounds displays characteristics of more than one type of inhibitor, it will be categorized as a mixed-type inhibitor.

A) Competitive Inhibitors

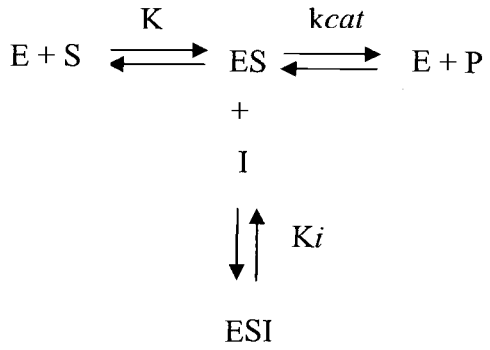
If an inhibitor I binds reversibly to the active site of the enzyme and prevents substrate S binding and vice versa, I and S compete with the same active site and I is said to be a competitive inhibitor. Competitive inhibition affects $K_m$ only and not $V_{max}$, since infinitely high concentrations of S displace I from the enzyme.

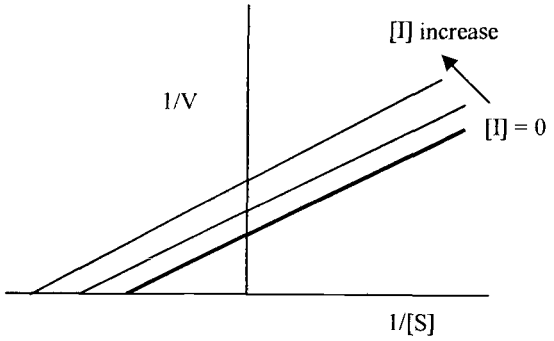


B) Uncompetitive Inhibitors

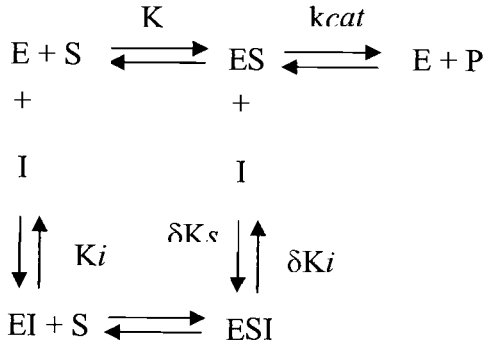
Uncompetitive inhibition occurs when I binds to ES (an enzyme-substrate intermediate) at a site other than the active site. This results in the decrease in both $K_m$ and $V_{max}$.

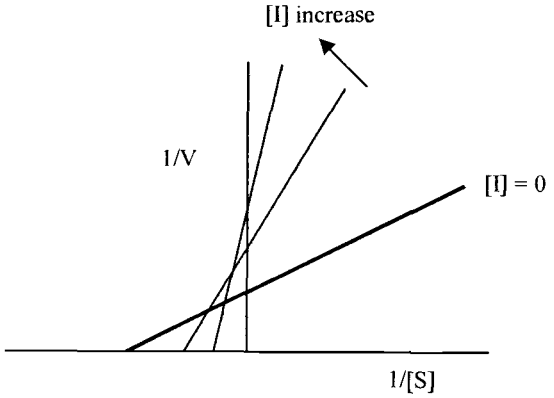



C) Linear Mixed Type Inhibitors

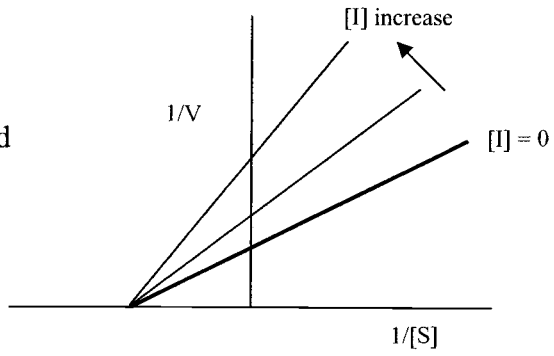
In this type of inhibition, I can interact with both the free enzyme and the enzyme-substrate intermediate at a site other than the active site. This results in an apparent decrease in $V_{max}$ and an apparent increase in $K_m$.



D). Noncompetitive Inhibitors

Noncompetitive inhibition is a special case of linear mixed inhibition. An apparent decrease in $V_{max}$ is observed while $K_m$ remains unaffected.




(All description were cited from Ref. 86)

**6.5) Antibiotic Diffusion Tests**

In addition to the biochemical characterization of these compounds, each DXP analog was also tested to see if inhibition might translate into antibacterial action.

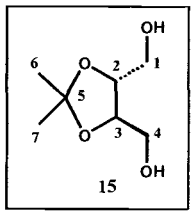
Antibiotic diffusion tests of all DXP analogs were determined by the agar diffusion method with *E. coli*, and *P.aeruginosa*. The Luria-Bertani agar plates containing the bacteria were prepared. Chloramphenicol (10 μg) was used as a positive control. For each DXP analog, amounts of 50 μg, and 500 μg were used. The diffusion zones were determined after incubation at 37 °C for 20 hours.

**6.6) General Methods and Materials.**

Reagents were purchased from Sigma-Aldrich. Solvents were purchased from Fisher Scientific or VWR. All solvents and volatile reagents were distilled prior to use or kept with 4 Å molecular sieves under an argon atmosphere. Tetrahydrofuran (THF) and toluene were distilled from sodium/benzophenone; $CH_2Cl_2$ and acetonitrile were distilled from $CaH_2$. All non-aqueous reactions were run in dry solvents under an argon atmosphere. "Dried and concentrated" refers to removal of residual amounts of water with anhydrous $Na_2SO_4$ followed by evaporation of solvent on a rotary evaporator. Flash chromatography[44] was conducted on ICN silica 60 Å, 230-400 mesh with the solvent system indicated. Cellulose flash chromatography was performed on Whatman CF-11 fibrous cellulose and cellulose TLC was visualized with *p*-anisaldehyde.[87] Normal phase thin layer chromatography plates were developed with phosphomolybdic acid/ cerium sulfate/ sulfuric acid solution. $^1H$, $^{13}C$, and $^{31}P$ NMR spectra were recorded in the indicated solvents on either a Bruker AM400 or AC300 spectrometer, where noted. When $CDCl_3$ was used as a solvent, the 77.00 ppm (center line) signal was the reference for $^{13}C$ NMR, and residual $CHCl_3$ ($^1H$ NMR, 7.26 ppm) was used as an internal standard. When $D_2O$ was used as a solvent, residual HOD ($^1H$ NMR, 4.80 ppm), and internal MeOH ($^{13}C$ NMR, 50.00 ppm) were used as standards. An external phosphoric acid (0 ppm) in $CHCl_3$ or $D_2O$ was used as a standard for $^{31}P$ NMR. Optical rotations were determined at 25 °C (*c* in g per 100 mL solvent) with Perkin Elmer Model 141 Polarimeter. High- resolution mass spectra (HRMS) were recorded using chemical ionization (CI) or FAB with a Kratos MS TC spectrometer. IR spectra were recorded on a Nicolet 5DXB FT-IR spectrophotometer. Registry numbers of known compounds were obtained from the Scifinder Scholar program.

**(*4S*, *5R*)-4,5-Dihydroxyhexan-3-one-6-phosphate (1-Methyl-DXP) Syntheses:**

**(-)-2,3-*O*-Isopropylidene-D-threitol (15); Registry No. 73346-74-4.**

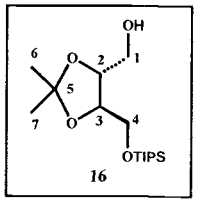


15

To a solution of 2,2-dimethoxypropane (26 mL, 220 mmol, 10 equivalents) in 20 mL of THF at room temperature was added diisopropyl-D-tartrate (4.5 mL, 22 mmol, 1 equivalent), and *p*-toluenesulfonic acid monohydrate (20 mg, 0.11 mmol, 0.05 equivalent). The reaction mixure was heated to reflux. After 12 h, the reaction mixture was cooled and 3 mL of TEA was added prior to concentration to yield 5.80 g of a yellow oil. This crude reaction mixture of 2,3-*O*-isopropylidene-diisopropyl-D-tartate (5.80 g, 21 mmol) was dissolved in 20 mL of THF and added into a slurry of lithium aluminium hydride (0.80 g, 21 mmol, 1 equivalent) in 100 mL of THF at 0 °C over 15 min period. The slurry was slowly warmed to room temperature and stirring was continued for 2 h. The reaction mixture was cooled to 0 °C, quenched by slow sequential addition of THF (30 mL), water (1 mL), 15% sodium hydroxide (1 mL), and water (3 mL). The reaction mixture was stirred for 30 min before filtration over Celite and the Celite layer was washed with 100 mL of diethyl ether. The filtrate was dried and concentrated. Purification by flash chromatography (6:1 (v/v) ethyl acetate/hexanes) gave 2.75 g (80% for 2 steps) of a yellow oil.

$[\alpha]^{25}_{D}$ -3.0° (*c* 2.0, $CHCl_3$), lit = -4.5°( *c* 5.0, $CHCl_3$)[88]; IR (thin film) 3405 (br), 2989, 2935, 2873, 1644, 1456, 1378, 1219, 1185, 1102, 1049 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 3.94 (m, $2H_{2,3}$), 3.68 (dd, 2H, $J$ = 2.8, 1.5 Hz, $H_{1a,1b}$), 3.74 (dd, 2H, $J$ = 2.8, 1.5 Hz, $H_{4a,4b}$), 1.39 (s, $3H_6$, $3H_7$); $^{13}C$ NMR (75 MHz, $CDCl_3$) 109.22 ($C_5$),78.21 ($C_2$,$C_3$), 62.06 ($C_1$,$C_4$), 26.91 ($C_6$,$C_7$); HRMS(CI) calcd. for $C_7H_{15}O_4$ 163.0970 found 163.0970.

**(*2R*, *3R*)-4-[(Triisopropylsilyl)oxy]-2,3-(isopropylidenedioxy)butanol (16); Registry No. 221154-50-3.**

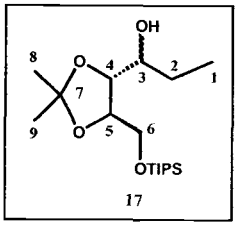


16

To a solution of NaH (60% in mineral oil, 0.38 g, 9.7 mmol, 1.05 equivalents) in 100 mL of THF at 0 °C was added a solution of (-)-2,3-*O*-isopropylidene-D-threitol **15** (1.50 g, 9.26 mmol) in 20 mL of THF over a 15 min period. The slurry was warmed to room temperature and stirred for 45 min before triisopropylsilyl chloride (1.80 mL, 9.26 mmol, 1.0 equivalent) was added over a 15 min period. After being stirred for 4 h, 10 mL of water was added slowly to the reaction mixture, which was extracted with diethylether (3×50 mL). The combined organic layers were dried and concentrated. Purification by flash chromatography (1:5 (v/v) ethyl acetate/hexanes) gave 2.60 g (88%) of a yellow oil.

$[\alpha]^{25}_{D}$ -9.2° (*c* 2.4, $CH_2Cl_2$), lit = -9.2° (*c* 17.0, $CH_2Cl_2$)[33]; IR (thin film) 3468 (br), 2946, 2863, 1461, 1378, 1248, 1224, 1073, 1004 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 3.95 (m, $4H_{2,3,4a,4b}$), 3.74 (dd, $J$ = 9.6, 7.2 Hz, $2H_{1a,1b}$), 2.40 (br, 1H, hydroxyl proton), 1.42 (s, $3H_6$), 1.40 (s, $3H_7$), 1.03-1.11 (m, 21H, isopropyl group); $^{13}C$ NMR (75 MHz, $CDCl_3$) 109.08($C_5$), 80.49($C_3$), 78.16($C_2$), 64.19($C_4$), 62.83($C_1$), 27.02($C_6$), 26.90($C_7$), 17.89(6×$CH_3$ of isopropyl group), 11.83 (3×CH of isopropyl group); HRMS(CI) calcd. for $C_{16}H_{35}SiO_4$ 319.2305 found 319.2295.

**(*4S*, *5R*)-6-[(Triisopropylsilyl)oxy]-4,5- (isopropylidenedioxy)hexan-3-ol (17).**



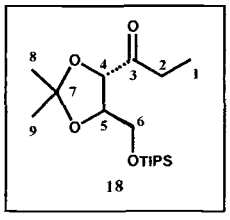
17

A solution of DMSO (0.64 mL, 9.08 mmol, 2.6 equivalents) in 20 mL of $CH_2Cl_2$ was added to a solution of oxalyl chloride (2M in $CH_2Cl_2$, 2.30 mL, 4.54 mmol, 1.3 equivalents) at -78 °C. After the solution was stirred for 5 min, a solution of 1.10 g (3.49 mmol) of the alcohol **16** in 30 mL of $CH_2Cl_2$ was added via cannula. The reaction mixture was stirred for 15 min at -78 °C, and 2.40 mL of TEA (17.5 mmol, 5.0 equivalents) was added. The reaction mixture was warmed to room temperature and stirred for 1 h, before 30 mL of water was added. The organic layer was removed, dried and concentrated to give 1.0 g of a yellow oil. The aldehyde

1.0 g (3.50 mmol) was dissolved in 100 mL of THF, cooled to -78 °C, and 10.5 mL of ethylmagnesium bromide (1.0 M in THF, 10.5 mmol, 3.0 equivalents) was added at -78 °C. The reaction mixture was stirred at -78 °C for 4 h, warmed to room temperature, and stirred for an additional 12 h. The reaction mixture was quenched with 10 mL of saturated $NH_4Cl$. The aqueous layer was extracted with diethylether (3×20 mL), and the combined organic layers were dried and concentrated. The residue was purified by flash chromatography (1:5 (v/v) ethyl acetate/hexanes) to give 0.90 g of an alcohol (74% for 2 steps) as a mixture of diastereomers.

IR (thin film) 3458 (br), 2936, 2863, 1460, 1373, 1243, 1214, 1136, 1079 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 3.94 (m, $2H_{4,5}$), 3.78 (m, $2H_{6a,6b}$), 3.60 (ddd, $J$ = 11.2, 7.5, 3.5 Hz, $1H_3$), 1.69 (m, $1H_{2a}$), 1.51(m, $1H_{2b}$), 1.39 (s, $3H_8$), 1.38 (s, $3H_9$), 1.01-1.11 (m, 24H, isopropyl group and $H_1$ ); $^{13}C$ NMR (75 MHz, $CDCl_3$) 108.57 ($C_7$), 82.24 ($C_5$), 79.33 ($C_4$),72.97($C_3$), 64.46 ($C_6$), 26.96 ($C_8$), 26.78 ($C_9$), 26.17 ($C_2$), 17.86 (6×$CH_3$ of isopropyl group), 11.81 (3×CH of isopropyl group), 9.46 ($C_1$); HRMS(CI) calcd. for $C_{18}H_{39}SiO_4$ 347.2618 found 347.2608.

**(*4S, 5R*)-6-[(Triisopropylsilyl)oxy]-4,5-(isopropylidenedioxy)hexan-3-one (18).**



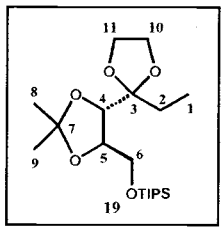
18

To a solution of the alcohols **17** (0.9 g, 2.6 mmol) in 30 mL of $CH_2Cl_2$ were added NMO (0.43g, 3.6 mmol, 1.5 equivalents), 3 g of finely crushed and activated 4 Å molecular sieves, and TPAP (46 mg, 0.13 mmol, 0.05 equivalents). The reaction mixture was stirred for 2 h, filtered over Florisil and the Florisil was washed with 50 mL of $CH_2Cl_2$. The filtrate was dried and concentrated. Purification by flash chromatography (1:9 (v/v) ethyl acetate/hexanes) provided 0.75 g (84%) of a colorless oil.

$[\alpha]^{25}_D$ -12° (*c* 3.9, $CH_2Cl_2$); IR (thin film) 2935, 2868, 2357, 1717, 1461, 1379, 1248, 1224, 1147, 1093 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 4.41 (d, $J$ = 7.5 Hz, $1H_4$), 4.06 (ddd, $J$ = 11.2, 7.5, 3.7 Hz, $1H_5$), 3.95 (dd, $J$ = 11.0, 3.6 Hz, $1H_{6a}$), 3.85 (dd, $J$ = 11.0, 3.8 Hz, $1H_{6b}$), 2.68 (dd, 2H, $J$ = 10.5, 7.3, 3.3 Hz, $H_{2a,2b}$), 1.50 (2s, $6H_{8,9}$), 1.05 (m, 24H, isopropyl group and $H_1$); $^{13}C$ NMR (75 MHz, $CDCl_3$) 210.85

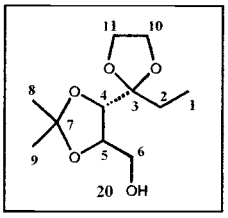
($C_3$), 111.20 ($C_7$), 81.69 ($C_5$), 79.67 ($C_4$), 63.84 ($C_6$), 32.72 ($C_2$), 27.48 ($C_8$), 27.05 ($C_9$), 18.50 (6×$CH_3$ of isopropyl group), 12.52 (3×CH of isopropyl group), 7.57 ($C_1$); HRMS(CI) calcd. for $C_{18}H_{35}SiO_4$ 343.2304 found 343.2305.

**(*4S,5R*)-6-[(Triisopropylsilyl)oxy]-4,5 (isopropylidenedioxy)hexan-3-(1,3-dioxolane) (19).**




To a solution of the ketone **18** (0.75 g, 2.20 mmol) in 60 mL of toluene was added 0.82 g of ethylene glycol (13 mmol, 6 equivalents) and 20 mg of *p*-toluenesulfonic acid monohydrate (0.11 mmol, 0.05 equivalents). The reaction mixture was heated to reflux and water was removed azeotropically in a Dean-Starke trap. After 5 h, the reaction was cooled and 0.5 mL of TEA was added before concentration. Purification by flash chromatography (1:9 (v/v) ethyl acetate/hexanes) gave 0.4 g (47%) of a colorless oil. $[\alpha]^{25}_D$ +14° (*c* 2.0, $CH_2Cl_2$); IR (thin film) 2946, 2858, 1735, 1464, 1373, 1249, 1221, 1150, 1078 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 4.17 (d, $J$ = 7.7 Hz, $1H_4$), 4.05 (m, $5H_{10a,10b,11a,11b,5}$), 3.94 (dd, $J$ = 11.0, 2.8 Hz, $1H_{6a}$), 3.77 (dd, $J$ = 11.0, 3.8 Hz, $1H_{6b}$), 1.67 (m, $H_{2a}$), 1.82 (m, $H_{2b}$), 1.43 (s, $3H_8$), 1.41 (s, $3H_9$), 1.08 (m, 24H, isopropyl group and $H_1$); $^{13}C$ NMR (75 MHz, $CDCl_3$) 110.45 ($C_3$), 109.43 ($C_7$), 78.73 ($C_{4,5}$), 66.23 ($C_{11}$), 65.89 ($C_{10}$), 63.91($C_6$), 27.28 ($C_8$), 26.92 ($C_2$), 26.91 ($C_9$), 17.96 (6×$CH_3$ of isopropyl group), 11.93 (3×CH of isopropyl group), 6.86 ($C_1$); HRMS(CI) calcd. for $C_{20}H_{41}SiO_5$ 389.2723 found 389.2712.

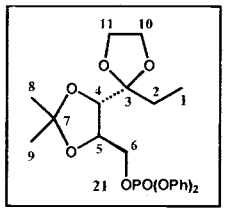
**(*4S,5R*)-(Isopropylidenedioxy)-3-(1,3-dioxolane)-6-hexanol (20).**




To a solution of the dioxolane **19** (400 mg, 1.0 mmol) in 30 mL of THF was added TBAF (1.0 M in THF, 1.55 mL, 1.55 mmol, 1.5 equivalents). The reaction mixture was stirred at room temperature for 14 h and then concentrated to a thick orange oil. Purification by flash chromatography (1:1 (v/v) ethyl acetate/hexanes) gave 0.19 g (82%) of a colorless oil.

$[\alpha]^{25}_D$ +11° (*c* 0.5, $CH_2Cl_2$); IR (thin film) 3440 (br), 2974, 2888, 1638, 1466, 1384, 1247, 1165, 1075 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 4.05 (m, $5H_{10a,10b,11a,11b,5}$), 3.93 (d, *J* = 8.2 Hz, $1H_4$), 3.80 (dd, *J* = 11.7, 4.0 Hz, $1H_{6a}$), 3.67 (dd, *J* = 11.7, 4.5 Hz, $1H_{6b}$), 1.80 (m, $H_{2a}$), 1.70 (m, $H_{2b}$), 1.43 (s, $3H_8$), 1.41 (s, $3H_8$), 0.93 (t, *J* = 14.8, 7.4 Hz, $3H_1$); $^{13}C$ NMR (75 MHz, $CDCl_3$) 110.08 ($C_3$), 109.53 ($C_7$), 79.42 ($C_{4,5}$), 66.24 ($C_{11}$), 65.69 ($C_{10}$), 63.27 ($C_6$), 27.25 ($C_8$), 26.91 ($C_9$), 26.67($C_2$) 6.91 ($C_1$); HRMS(CI) calcd. for $C_{11}H_{21}O_5$ 233.1389 found 233.1396.

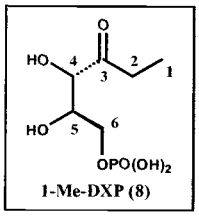
**Diphenyl (*4S,5R*)-4,5-(Isopropylidenedioxy)-3-(1,3-dioxolane)-6-oxohexyl phosphate (21).**




To a solution of the alcohol **20** (100 mg, 0.43 mmol) in 15 mL of THF was added pyridine (0.14 μL, 0.65 mmol, 1.5 equivalents), diphenylchlorophosphate (0.14 μL, 0.65 mmol, 1.5 equivalents), and 80 mg of DMAP. The reaction mixture was heated to reflux. After 10 h the reaction mixture was cooled, 10 mL of water was added, and the reaction mixture was extracted with diethylether (3×50 mL). The combined organic layers were dried and concentrated. Purification by flash chromatography (1:1 (v/v) ethyl acetate/hexanes) gave 160 mg (78%) of a colorless oil.

$[\alpha]^{25}_D$ +20° (*c* 1.5, $CH_2Cl_2$); IR (thin film) 3070, 2978, 2886, 1737, 1588, 1492, 1379, 1297, 1165, 1043 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.25 (m, 10H, Ph), 4.50 (ddd, *J* = 8.6, 6.3, 2.1 Hz, $1H_{6a}$), 4.25 (m, $2H_{6b,5}$), 3.96 (s, $4H_{10a,10b,11a,11b}$), 3.94 (d, *J* = 8.0 Hz, $1H_3$), 1.80 (m, $H_{2a}$), 1.71 (m, $H_{2b}$), 1.43 (s, $3H_8$), 1.38 (s, $3H_9$), 0.91 (t, *J* = 14.8, 11.4 Hz, $3H_1$); $^{13}C$ NMR (75 MHz, $CDCl_3$) 130.16, 125.78, 120.57, 120.51 (Ph), 110.05 ($C_3$), 109.52 ($C_7$), 79.06 ($C_4$), 76.01 (d, *J* = 7.5 Hz, $C_5$), 69.21(d, *J* = 6.8 Hz, $C_6$), 66.69 ($C_{10}$), 66.50 ($C_{11}$), 27.28 ($C_8$), 27.19 ($C_9$), 27.10($C_2$) 7.32 ($C_1$); $^{31}P$ NMR (121 MHz, $CDCl_3$) δ −10.60; HRMS(CI) calcd. for $C_{23}H_{30}O_8P$ 465.1678 found 465.1675.

**(*4S*, *5R*)-4,5-dihydroxyhexan-3-one-6-phosphate (1-Methyl-DXP, 8).**



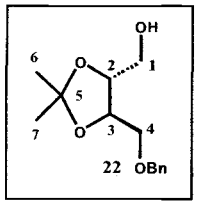
1-Me-DXP (8)

To a solution of the diphenylphosphate **21** (0.150 g, 0.32 mmol) in 10 mL of EtOH was added 20 mg of Pt(IV) oxide. The reaction flask was stirred for 5 h at room temperature under a hydrogen atmosphere (1 atm). The reaction mixture was filtered over Celite and washed with 50 mL of EtOH. After concentration, 3.0 mL of water and 200 μL concentrated HCl were added. The resulting solution was stirred for an additional 5 h, before adjustment to pH 7 with solid $NaHCO_3$. The mixture was frozen and lyophilized to provide a white solid. The residue was purified by cellulose chromatography (15-40% (v/v) 0.1% trifluoroacetic acid in THF) to give 45 mg (90%) of a white solid as sodium salt.

$[\alpha]^{25}_D$ -1.7° (*c* 3.0, $H_2O$); lit.$[\alpha]^{25}_D$ -1.2° (*c* 4.2, $H_2O$) for DXP;[33] $^1H$ NMR (300 MHz, $D_2O$) δ 4.53 (d, $J$ = 1.8 Hz, $1H_4$), 4.33 (dt, $J$ = 6.5, 1.8 Hz, $1H_5$), 3.88 (dt, $J$ = 13.8, 7.1 Hz, $2H_{6a,6b}$), 2.71 (m, $2H_{2a,2b}$), 1.07 (t, $J$ = 14.4, 7.2 Hz, $3H_1$); $^{13}C$ NMR (75 MHz, $D_2O$/MeOH, 1:100) 216.98 ($C_2$), 76.78 ($C_4$), 70.96 (d, $J$ = 6.5 Hz, $C_5$), 65.56 (d, $J$ = 3.8 Hz, $C_6$), 32.31 ($C_2$), 7.13 ($C_1$); $^{31}P$ NMR (121 MHz, $D_2O$) δ 1.05; HRMS(-FAB) calcd. for $C_6H_{13}O_7P$ 228.0399 found 228.0392.

**DX-Phosphonate Synthesis:**

**(2*R*, 3*R*)-2,3-(Isopropylidenedioxy)-4-benzyloxybutanol (22); Registry No. 78469-77-9.**

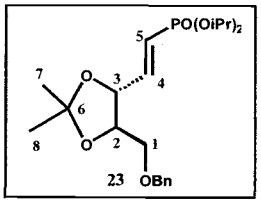



To a suspension of NaH (60% in mineral oil, 2.6 g, 6.4 mmol, 1.0 equivalents) in 150 mL of THF at 0 °C was added a solution of (-)-2,3-*O*-isopropylidene-D-threitol **15** (10.4 g, 6.4 mmol) in 20 mL of THF over a 15 min period. The slurry was warmed to room temperature and stirred for 10 min before BnBr (9.2 mL, 77 mmol, 1.2 equivalents) was added over a 15 min period. After the BnBr was added for 5 min, 2 mg of *n*-$Bu_4NI$ was added. After being stirred for 6 h, 20 mL of water was added slowly to the reaction mixture which was extracted with diethylether (3×50 mL). The combined organic layers

were dried and concentrated. Purification by flash chromatography (1:1 (v/v) ethyl acetate/hexanes) gave 11.9 g (78%) of a light yellow oil.

$[\alpha]^{25}_{D}$ +5.0° (*c* 5.0, $CH_2Cl_2$), lit = +8.7° (*c* 1.2, $CHCl_3$)[73]; IR (thin film) 3443, 3027, 2989, 2883, 1499, 1451, 1373, 1252, 1209, 1161 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.33 (m, 5H, Ph), 4.58 (s, 2H, benzylic protons), 4.05 (ddd, *J* = 5.7, 5.0, 2.7 Hz, $1H_3$), 3.95 (ddd, *J* = 8.6, 4.2, 3.9 Hz, $1H_2$), 3.77 (dd, *J* = 11.7, 4.3 Hz, $1H_{5a}$), 3.69 (dd, *J* = 9.7, 5.0 Hz, $1H_{1a}$), 3.68 (dd, *J* = 11.7, 4.3 Hz, $1H_{5b}$), 3.56 (dd, *J* = 9.8, 5.7 Hz, $1H_{1b}$), 2.18 (s, 1H, hydroxyl proton), 1.42 (s, $3H_6$), 1.41 (s, $3H_7$); $^{13}C$ NMR (75 MHz, $CDCl_3$) 137.54, 128.45, 127.84, 127.74 (Ph), 109.33 ($C_5$), 79.66 ($C_3$), 76.65 ($C_2$), 73.69 (benzylic carbon), 70.34 ($C_4$), 62.41($C_1$), 26.83 ($C_6$), 26.93 ($C_7$); HRMS(CI) calcd. for $C_{14}H_{20}O_4$ 252.1362 found 252.1359.

**(2*R*, 3*R*)-4-Ene-2,3-(isopropylidenedioxy)-1-benzyloxypent-5-diisopropyl-phosphonate (23).**



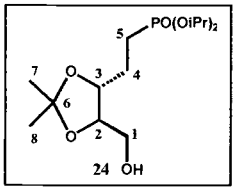

A solution of DMSO (2.1 mL, 30 mmol, 2.6 equivalents) in 20 mL of $CH_2Cl_2$ was added to a solution of oxalyl chloride (2M in $CH_2Cl_2$, 7.5 mL, 15 mmol, 1.3 equivalents) at -78 °C. After the solution was stirred for 5 min, a solution of the alcohol **22** (2.7g, 11 mmol, 1 equivalent) in 30 mL of $CH_2Cl_2$ was added via cannula. The reaction mixture was stirred for 15 min at -78 °C, and 8.0 mL of TEA (60 mmol, 5.0 equivalents) was added. The reaction mixture was warmed to room temperature and stirred for 1 h before 30 mL of water was added. The organic layer was removed, dried and concentrated to give 3.0 g of the corresponding aldehyde as a yellow oil which was used without further purification.

To a solution of tetraisopropylmethane phosphonate (3.8 mL, 11.8 mmol, 1.2 equivalents) in 30 mL of hexanes was added *n*-BuLi (1.6M in hexanes, 11.8 mmol, 1.2 equivalents) at 0 °C. After being stirred at room temperature for 2 h, the reaction mixture was cooled to 0 °C and a solution of the crude aldehyde from the previous Swern oxidation (2.3 g, 9.83 mmol) in 20 mL of hexanes was added. The reaction mixture was heated to reflux for 14 h. After being cooled to room temperature, the

reaction mixture was filtered over Celite and washed with 30 mL of hexanes. The filtrate was washed with 3×10 mL of water, 2×10 mL of saturated sodium chloride, dried, and concentrated. Purification by flash chromatography (2:1 (v/v) ethyl acetate/hexanes) gave 3.22 g (80%) of a light yellow oil.

$[\alpha]^{25}_{D}$ +10° (*c* 1.1, $CH_2Cl_2$); IR (thin film) 2980, 2936, 1717, 1446, 1384, 1214, 1176, 1103, 992 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.32 (m, 5H, Ph), 6.73 (dd, $J$ = 17.0, 4.6 Hz, $1H_5$), 5.99 (dd, $J$ = 17.1, 1.5 Hz, $1H_4$), 4.65 (m, 2H, CH of isopropyl group and $H_3$), 4.59 (s, 2H, benzylic protons), 4.42 (m, 1H, CH of isopropyl group), 3.95 (m, $1H_2$), 3.62 (d, $J$ = 4.8 Hz, $2H_{1a,1b}$), 1.45 (s, $3H_7$), 1.41 (s, $3H_8$), 1.27 (m, 12H, isopropyl group); $^{13}C$ NMR (75 MHz, $CDCl_3$) 147.37 (d, $J$ = 5.9 Hz, $C_4$), 137.71, 128.43, 127.73 (Ph), 119.93 (d, $J$ = 188.5 Hz, $C_5$), 110.19 ($C_6$), 79.51 ($C_2$), 78.29 (d, $J$ = 22.5 Hz, 2×CH of isopropyl group), 73.67 (benzylic carbon), 70.47 ($C_3$), 69.39 ($C_1$), 26.99 ($C_7$), 26.65 ($C_8$), 24.01 (4×$CH_3$ of isopropyl group); $^{31}P$ NMR (121 MHz, $CDCl_3$) δ 16.50; HRMS(CI) calcd. for $C_{21}H_{34}O_6P$ 413.2093 found 413.2087.

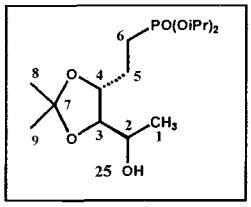
**(2*R*, 3*R*)-2,3-(Isopropylidenedioxy)-1-hydroxypent-5-diisopropyl-phosphonate (24).**




To a solution of **23** (1.4 g, 3.4 mmol) in 20 mL of EtOH was added 340 mg of 10% Pd on carbon. The reaction mixture was stirred for 24 h at room temperature under a hydrogen atmosphere (1 atm). The mixture was filtered over Celite which was washed with 50 mL of EtOH. In the next step, 350 mg of $Pd(OH)_2$ on carbon was added to the crude reaction mixture and the reaction mixture was stirred for 24 h at room temperature under a hydrogen atmosphere (1 atm). The reaction mixture was filtered over Celite and the Celite was washed with 50 mL of EtOH. The crude reaction mixture was concentrated to give 890 mg (77% for 2 steps) of a colorless oil which was used without further purification.

$[\alpha]^{25}_D$ +13° (*c* 0.2, $CH_2Cl_2$); IR (thin film) 3376 (br), 2980, 2931, 2878, 1456, 1376, 1224, 1176, 1103, 1055 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 4.60 (m, 2H, 2×CH of isopropyl group), 3.88 (m, $1H_3$), 3.70 (m, $2H_{2,1a}$), 3.62 (m, $1H_{1ab}$), 1.33 (s, $6H_{7,8}$), 1.25 (m, 12H, isopropyl group); $^{13}C$ NMR (75 MHz, $CDCl_3$) 109.13 ($C_6$), 81.33 ($C_2$), 77.71 (d, $J$ = 16.1 Hz, 2×CH of isopropyl group), 70.52 (d, $J$ = 6.5 Hz, $C_3$), 62.54 ($C_1$), 27.64 ($C_7$), 27.41 ($C_8$), 26.71 (d, $J$ = 4.6 Hz, $C_4$), 24.39 (4×$CH_3$ of isopropyl group), 23.59 (d, $J$ = 142.9 Hz, $C_5$ ); $^{31}P$ NMR (121 MHz, $CDCl_3$) δ 30.71; HRMS(CI) calcd. for $C_{14}H_{30}O_6P$ 325.1780 found 325.1787.

**(3*S*, 4*R*)-3,4-(Isopropylidenedioxy)-2-hydroxyhexan-6-diisopropyl-phosphonate (25).**

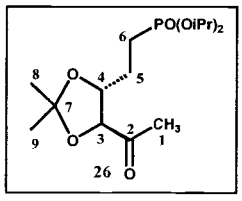



A solution of DMSO (0.19 mL, 2.65 mmol, 2.6 equivalents) in 20 mL of $CH_2Cl_2$ was added to a solution of oxalyl chloride (2M in $CH_2Cl_2$, 0.66 mL, 1.32 mmol, 1.3 equivalents) at -78 °C. After the solution was stirred for 5 min, a solution of 340 mg (1.01 mmol) of the alcohol **24** in 10 mL of $CH_2Cl_2$ was added via cannula. The reaction mixture was stirred for 15 min at -78 °C and 1.3 mL of TEA (5.07 mmol, 5.0 equivalents) was added. The reaction mixture was warmed to room temperature and stirred for 1 h, cooled to -78 °C and 1.70 mL of methylmagnesium chloride/THF (3.0 M, 5.07 mmol, 5.0 equivalents) was added. The reaction mixture was stirred at -78 °C for 4 h, warmed to room temperature, and stirred for an additional 12 h. The reaction was quenched with 10 mL of saturated $NH_4Cl$. The aqueous layer was extracted with diethylether (3×20 mL), and the combined organic layers were dried and concentrated. The residue was purified by flash chromatography on a Sep-Pack® (vac 6 cc, $C_{18}$- 500 mg) with 1:3 (v/v) water in methanol as an eluent to give 224 mg (63% for 2 steps) as a yellow oil.

IR (thin film) 3386 (br), 2979, 2931, 2878, 1456, 1379, 1229, 1171, 1103, 1069 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 4.68 (m, 2H, 2×CH of isopropyl group), 4.04 (ddd, $J$ = 10.9, 7.6, 3.3 Hz, $1H_4$), 3.86 (m, $1H_2$), 3.54 (dd, $J$ = 7.5, 5.6 Hz, $1H_3$), 2.65 (s, 1H, hydroxyl proton), 1.94 (m, $4H_{5a,5b,6a,6b}$), 1.38 (s, $3H_8$), 1.37 (s, $3H_9$), 1.30

(d, $J$ = 6.1 Hz, 12H, isopropyl group), 1.22 (d, $J$ = 6.4 Hz, $3H_1$); $^{13}C$ NMR (75 MHz, $CDCl_3$) 108.59 ($C_7$), 84.57 ($C_2$), 77.75 (d, $J$ = 14.1 Hz, 2×CH of isopropyl group), 70.15 ($C_4$), 67.81 ($C_2$), 27.74 ($C_5$), 27.29 ($C_8$), 27.09 ($C_9$), 23.28 (d, $J$ = 142.4 Hz, $C_6$), 24.05 (2×$CH_3$ of isopropyl group), 23.99 (2×$CH_3$ of isopropyl group), 19.16 ($C_1$); $^{31}P$ NMR (121 MHz, $CDCl_3$) δ 30.93; HRMS(CI) calcd. for $C_{15}H_{32}O_6P$ 339.1937 found 339.1935.

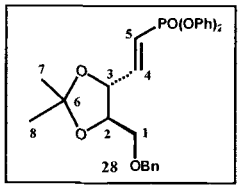
**(3*S*, 4*R*)-3,4-(Isopropylidenedioxy)-2-one-hexan-6-diisopropylphosphonate (26).**




To a solution of the alcohol **25** (194 mg, 0.56 mmol) in 15 mL of $CH_2Cl_2$ were added NMO (90 mg, 0.78 mmol, 1.5 equivalents), 150 mg of finely crushed and activated 4 Å molecular sieves, and TPAP (15 mg, 0.03 mmol, 0.05 equivalents). The reaction mixture was stirred for 5 h, filtered over Florisil and the Florisil layer was washed with 30 mL of $CH_2Cl_2$. The filtrate was dried and concentrated. Purification by flash chromatography (3:1 (v/v) ethyl acetate/hexanes) yielded 123 mg (63%) of a colorless oil.

$[\alpha]^{25}_D$ +2.4° (*c* 6.1, $CH_2Cl_2$); IR (thin film) 2979, 2936, 1795, 1727, 1451, 1379, 1229, 1176, 1108 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 4.66 (m, 2H, 2×CH of isopropyl group), 3.98 (ddd, $J$ = 11.5, 7.7, 3.8 Hz, $1H_4$), 3.95 (d, $J$ = 7.8 Hz, $1H_3$), 2.27 (s, $3H_1$), 1.90 (m, $4H_{5a,5b,6a,6b}$), 1.43 (s, $3H_8$), 1.41 (s, $3H_9$), 1.30 (d, $J$ = 6.2 Hz, 12H, 4×$CH_3$ of isopropyl group); $^{13}C$ NMR (75 MHz, $CDCl_3$) 208.15 ($C_2$), 110.35 ($C_7$), 84.86 ($C_3$), 77.31 (d, $J$ = 18.0 Hz, 2×CH of isopropyl group), 69.91 (d, $J$ = 3 Hz, $C_4$), 26.97($C_1$), 26.88 (d, $J$ = 4.2 Hz, $C_5$), 26.20 ($C_8$), 26.11 ($C_9$), 23.92 (2×$CH_3$ of isopropyl group), 23.88 (2×$CH_3$ of isopropyl group), 22.90 (d, $J$ = 143.1 Hz, $C_6$); $^{31}P$ NMR (121 MHz, $CDCl_3$) δ 29.78; HRMS(CI) calcd. for $C_{15}H_{30}O_6P$ 337.1780 found 337.1774.

**(2*R*, 3*R*)-4-Ene-2,3-(isopropylidenedioxy)-1-benzyloxypent-5-diphenylphosphonate (28).**



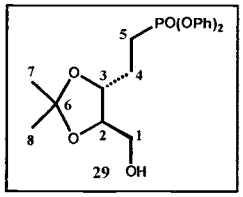

A solution of DMSO (2.0 mL, 28 mmol, 2.6 equivalents) in 100 mL of $CH_2Cl_2$ was added to a solution of oxalyl chloride (2M in $CH_2Cl_2$, 6.7 mL, 14 mmol, 1.3 equivalents) at -78 °C. After the solution was stirred for 5 min, a solution of 2.5 g (10 mmol) of the alcohol **26** in 10 mL of $CH_2Cl_2$ was added via cannula. The reaction mixture was stirred for 15 min at -78 °C, prior to the addition of TEA (7.4 mL, 53 mmol, 5.0 equivalents). The reaction mixture was warmed to room temperature and stirred for 1 h before 20 mL of water was added. The organic layer was removed, dried and concentrated to a yellow oil. The crude reaction mixture was dissolved in 20 mL toluene, diphenyl triphenylphosphoranylidene methylphosphonate ($Ph_3P$=CHP(O)($OPh_2$)) (5.4 g, 10 mmol, 1.0 equivalent) was added, and the reaction mixture was heated to reflux for 3 h. The crude reaction mixture was concentrated and purified by flash chromatography (1:3 (v/v) ethyl acetate/hexanes) to give 2.8 g (53% for 2 steps) of a colorless oil.

$[\alpha]^{25}_D$ +8.6° (*c* 3.0, $CH_2Cl_2$); IR (thin film) 3061, 2989, 2931, 2863, 1630, 1591, 1489, 1456, 1378, 1267, 1185 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.25 ( m, 15H, Ph), 6.97 (ddd, $J$ = 21.2, 17.1, 4.2 Hz, $1H_5$), 6.20 (ddd, $J$ = 17.1, 1.7, 0.5 Hz, $1H_4$), 4.57 (ABq, $J$ = 10.0, 3.0 Hz, 2H, benzylic proton), 4.45 (m, $1H_3$), 3.85 (m, $1H_2$), 3.60 (d, $J$ = 4.3 Hz, $2H_{1a,1b}$), 1.35 (s, $3H_7$), 1.45 (s, $3H_8$); $^{13}C$ NMR (75 MHz, $CDCl_3$) 151.65 (d, $J$ = 6.3 Hz, $C_4$), 150.15 (d, $J$ = 7.5 Hz), 129.73, 128.45, 127.85, 125.15, 120.55 (Ph), 116.35 (d, $J$ = 191.6 Hz, $C_5$), 110.35 ($C_6$), 79.16 ($C_1$), 78.31 (d, $J$ = 22.9 Hz, $C_2$), 73.58 (benzylic carbon), 69.15 ($C_1$), 26.88 ($C_7$), 26.43 ($C_8$); $^{31}P$ NMR (121 MHz, $CDCl_3$) δ 8.52; HRMS(CI) calcd. for $C_{27}H_{30}O_6P$ 481.17800 found 481.17883.

**Diphenyl triphenylphosphoranylidene methylphosphonate ($Ph_3P$=CHP(O)$(OPh)_2$) (27); Registry No. 22400-41-5.**

Methyltriphenylphosphonium bromide (30 g, 8.4 mmol, 2.2 equivalents) was added to a suspension of KO$^t$Bu (9.4 g, 8.4 mmol, 2.2 equivalents) in 150 mL of THF at 0 °C. The reaction mixture was stirred for 15 min, and 7.9 mL of diphenylchlorophosphate (38 mmol, 1.0 equivalent) was added. The reaction mixture was warmed to room temperature and stirred for 12 h, then filtered over Celite which was washed with 100 mL diethylether. The filtrate was washed with 3N HCl (100 mL) and neutralized with 3N NaOH (100 mL) to give yellow crystals. The crystals were collected by filtration and dried at room temperature to give 13.7 g (79%).

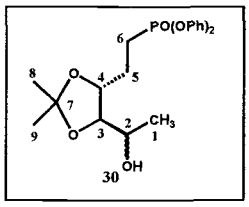
**(2*R*, 3*R*)-2,3-(Isopropylidenedioxy)-1-hydroxypent-5-diphenylphosphonate (29).**




To a solution of **28** (2.8 g, 5.83 mmol) in 50 mL of EtOH was added 500 mg of 10% Pd on carbon. The reaction mixture was stirred for 4 h at room temperature under a hydrogen atmosphere (1 atm). The reaction mixture was filtered over Celite and washed with 50 mL of EtOH. Then 300 mg of $Pd(OH)_2$ on carbon was added to the crude reaction mixture and the reaction mixture was stirred for 12 h at room temperature under a hydrogen atmosphere (1 atm). The reaction mixture was filtered over Celite and washed with 50 mL of EtOH. The crude reaction mixture was concentrated and purified by flash chromatography (2:1 (v/v) ethyl acetate/hexanes) to give 2.0 g of the alcohol (86% for 2 steps) as a colorless oil. $[\alpha]^{25}_D$ +12° (*c* 7.8, $CH_2Cl_2$); IR (thin film) 3419 (br), 3066, 2984, 2931, 2877, 1597, 1485, 1373, 1214, 1194 cm$^{-1}$; $^1$H NMR (300 MHz, $CDCl_3$) δ 7.25 (m, 10H, Ph), 3.98 (td, $J$ = 8.0, 3.5 Hz, $1H_2$), 3.76 (m, $2H_{1a,1b}$), 3.62 (dd, $J$ = 13.0, 5.4 Hz, $1H_3$), 2.15 (m, $4H_{4a,4b,5a,5b}$), 1.40 (s, $6H_{7,8}$); $^{13}$C NMR (75 MHz, $CDCl_3$) 151.54 (d, $J$ = 6.4 Hz), 129.72, 125.25, 120.52, 120.40 (Ph), 109.03 ($C_6$), 80.83 ($C_2$), 76.81 (d, $J$ = 8.30 Hz, $C_3$), 61.74 ($C_1$), 27.23 ($C_7$), 26.94 ($C_8$), 25.95 (d, $J$ = 4.6 Hz, $C_4$), 22.48 (d, $J$ = 141.9

Hz, $C_5$ ); $^{31}P$ NMR (121 MHz, $CDCl_3$) δ 25.81; HRMS(CI) calcd. for $C_{20}H_{26}O_6P$ 393.1473 found 393.1467.

**(3*S*, 4*R*)-3,4-(Isopropylidenedioxy)-2-hydroxyhexan-6-diphenylphosphonate (30).**

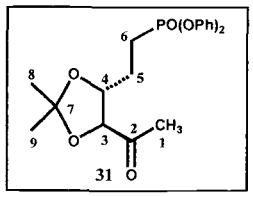



A solution of DMSO (1.1 mL, 15 mmol, 2.6 equivalents) in 60 mL of $CH_2Cl_2$ was added to a solution of oxalyl chloride (2M in $CH_2Cl_2$, 3.80 mL, 7.6 mmol, 1.3 equivalents) at -78 °C. After the solution was stirred for 5 min, a solution of the alcohol **29** (2.0 g, 5.9 mmol) in 20 mL of $CH_2Cl_2$ was added via cannula. The reaction mixture was stirred for 15 min at -78 °C, and 4.3 mL of TEA (29.0 mmol, 5.0 equivalents) was added. The reaction mixture was warmed to room temperature and stirred for 1 h, cooled to -78 °C and 10 mL of methylmagnesium chloride/THF

(3.0 M, 29.0 mmol, 5.0 equivalents) was added. The reaction mixture was stirred at -78 °C for 4 h, warmed to room temperature, and stirred overnight for an additional 12 h. The reaction mixture was quenched with 15 mL of saturated $NH_4Cl$. The aqueous layer was extracted with diethylether (3×50 mL), and the combined organic layers were dried and concentrated. The residue was purified by flash chromatography (2:1 (v/v) ethyl acetate/hexanes) to give 1.7 g of the alcohol (71% for 2 steps) as a mixture of diastereomers.

IR (thin film) 3419 (br), 3066, 2984, 2931, 2873, 1741, 1596, 1489, 1373, 1243, 1190, 1161, 1064 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.27 (m, 10H, Ph), 4.10 (m, $1H_2$), 3.83 (m, $1H_4$), 3.53 (dd, $J$ = 7.5, 5.7 Hz, $1H_3$), 2.55 (s, 1H, hydroxyl proton), 2.25 (m, $4H_{5a,5b,6a,6b}$), 1.37 (s, $3H_8$), 1.36 (s, $3H_9$), 1.19 (d, $J$ = 6.4 Hz, $3H_1$); $^{13}C$ NMR (75 MHz, $CDCl_3$) 150.12 (d, $J$ = 6.4 Hz), 129.73, 125.14, 120.51, 120.46 (Ph), 108.74 ($C_7$), 83.95 ($C_3$), 77.42 (d, $J$ = 16.43 Hz, $C_4$), 67.71 ($C_2$), 27.37 ($C_8$), 27.24 ($C_9$), 27.02 ($C_5$), 23.45 (d, $J$ = 141.75 Hz, $C_6$ ), 19.32 ($C_1$); $^{31}P$ NMR (121 MHz, $CDCl_3$) δ 26.30; HRMS(CI) calcd. for $C_{21}H_{28}O_6P$ 407.1624 found 407.1622.

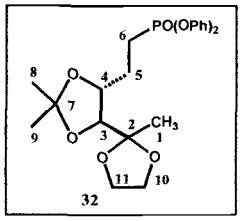
**(3*S*, 4*R*)-3,4-(Isopropylidenedioxy)-2-one-hexan-6-diphenylphosphonate (31).**




To a solution of the mixture of alcohol **30** (1.7 g, 4.2 mmol) in 30 mL of $CH_2Cl_2$ were added NMO (0.75 g, 6.3 mmol, 1.5 equivalents), 1.0 g of finely crushed and activated 4 Å molecular sieves, and TPAP (75 mg, 0.2 mmol, 0.05 equivalents). The reaction mixture was stirred for 2 h, filtered over Florisil and the Florisil layer was washed with 50 mL of $CH_2Cl_2$. The filtrate was dried and concentrated. Purification by flash chromatography (1:1 (v/v) ethyl acetate/hexanes) provide 1.47 g of the ketone **31** (86%) as a colorless oil.

$[\alpha]^{25}_D$ -6° (*c* 0.5, $CH_2Cl_2$); IR (thin film) 3066, 2979, 2935, 1794, 1722, 1591, 1489, 1378, 1185, 1073 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.20 (m, 10H, Ph), 4.05 (dd, $J$ = 7.7, 3.2 Hz, $1H_4$), 3.92 (d, $J$ = 7.7 Hz, $1H_3$), 2.25 (s, $3H_1$), 2.15 (m, $4H_{5a,5b,6a,6b}$),1.45 ( s, $3H_8$), 1.35 ( s, $3H_9$); $^{13}C$ NMR (75 MHz, $CDCl_3$) 208.31($C_2$), 150.12 (d, $J$ = 8.25 Hz), 129.74, 125.15, 120.48 (Ph), 110.59 ($C_7$), 84.82 ($C_3$), 77.25 (d, $J$ = 18.23 Hz, $C_4$), 27.02 ($C_8$), 26.74 (d, $J$ = 18.0 Hz, $C_5$), 26.46 ($C_9$), 26.15 ($C_1$), 22.55 (d, $J$ = 143.57 Hz, $C_6$ ); $^{31}P$ NMR (121 MHz, $CDCl_3$) δ 25.35; HRMS(CI) calcd. for $C_{21}H_{26}O_6P$ 405.1459 found 405.1467.

**(3*S*, 4*R*)-3,4-(Isopropylidenedioxy)-2-(1,3-dioxolane)hexan-6-diphenylphosphonate (32).**

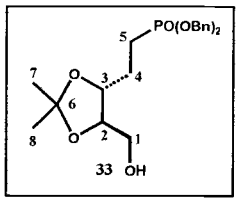



To a solution of the ketone **31** (430 mg, 1.1 mmol) in 20 mL of toluene were added 660 mg of ethylene glycol (11 mmol, 10 equivalents) and 10 mg of *p*-toluenesulfonic acid monohydrate. The reaction mixture was heated to reflux using a Dean-Starke trap to remove water. After 5 h, the reaction mixture was cooled and 0.5 mL of TEA was added prior to concentration. Purification by flash chromatography (1:1 (v/v) ethyl acetate/hexanes) gave 63 mg (13%) of a colorless oil.

$[\alpha]^{25}_D$ +16° (*c* 0.4, $CH_2Cl_2$); IR (thin film) 2984, 2931, 2892, 1596, 1489, 1378, 1262, 1219, 1190, 1078 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.25 (m, 10H, Ph), 4.05

(m, $1H_4$), 3.95 (s, $4H_{10a,10b,11a,11b}$), 3.65 (d, $J$ = 7.7 Hz, $1H_3$), 2.15 (m, $4H_{5a,5b,6a,6b}$), 1.45 (s, $3H_8$), 1.40 (s, $3H_9$), 1.35 (s, $3H_1$); $^{13}C$ NMR (75 MHz, $CDCl_3$) 150.26 (d, $J$ = 8.7 Hz), 129.72, 120.48 (Ph), 109.58 ($C_7$), 108.45 ($C_2$), 83.53 ($C_3$), 77.05 (d, $J$ = 17.4 Hz, $C_4$), 65.36 ($C_{10}$), 65.00 ($CH_{11}$), 27.44 ($C_8$), 27.35 (d, $J$ = 4.7 Hz, $C_5$), 26.68 ($C_9$), 22.50 (d, $J$ = 142.46 Hz, $C_6$ ), 20.42 ($C_1$); $^{31}P$ NMR (121 MHz, $CDCl_3$) δ 26.05; HRMS(CI) calcd. for $C_{23}H_{29}O_7P$ 448.1651 found 448.1653.

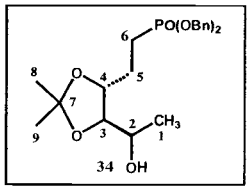
**(2*R*, 3*R*)-2,3-(Isopropylidenedioxy)-1-hydroxypent-5-dibenzylphosphonate (33).**




To a solution of the alcohol **29** (1.47 g, 3.75 mmol, 1 equivalent) in 10 mL of DMSO was added 13 mL of sodium benzyloxide (1.0 M in BnOH, 13 mmol, 3.5 equivalents). The reaction mixture was stirred for 10 min at room temperature before 10 mL of water was added. The aqueous layer was extracted with ethyl acetate (3×50 mL), and the combined organic layers were dried and concentrated. The residue was purified by flash chromatography (100 % ethyl acetate) to give 680 mg (43%) of a colorless oil.

$[\alpha]^{25}_D$ -11° (*c* 0.2, $CH_2Cl_2$); IR (thin film) 3399 (br), 2986, 2932, 2883, 1456, 1373, 1230, 1048, 994 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.34 (m, 10H, Ph), 5.04 (m, 4H, benzylic protons), 3.85 (dd, $J$ = 7.8, 4.1 Hz, $1H_2$), 3.66 (m, $2H_{1a,1b}$), 3.54 (m, $1H_3$), 2.04 (s, 1H, hydroxyl proton), 1.80 (m, $4H_{4a,4b,5a,5b}$), 1.36 (s, $6H_{7,8}$); $^{13}C$ NMR (75MHz, $CDCl_3$) 136.22, 128.51, 128.36, 127.87 (Ph), 108.78 ($C_6$), 80.76 ($C_2$), 76.73 (d, $J$ = 16.4 Hz, $C_3$), 67.16 (d, $J$ = 4.1 Hz, benzylic carbons), 61.84 ($C_1$), 27.18 ($C_7$), 26.91 ($C_8$), 25.75 ($C_4$), 22.30 (d, $J$ = 141.5 Hz, $C_5$); $^{31}P$ NMR (121 MHz, $CDCl_3$) δ 33.47; HRMS(CI) calcd. for $C_{22}H_{30}O_6P$ 421.1780 found 421.1787.

**(3*S*, 4*R*)-3,4-(Isopropylidenedioxy)-2-hydroxyhexan-6-dibenzylphosphonate (34).**

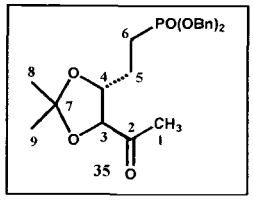



A solution of DMSO (180 μL, 2.5 mmol, 3.0 equivalents) in 10 mL of $CH_2Cl_2$ was added to a solution of oxalyl chloride (2M in $CH_2Cl_2$, 630 μL, 1.3 mmol, 1.5

equivalents) at -78 °C. After the solution was stirred for 5 min, a solution of the alcohol **33** (350 mg (0.8 mmol) in 20 mL of $CH_2Cl_2$ was added via cannula. The reaction mixture was stirred for 15 min at -78 °C, and 580 μL of TEA (4.2 mmol, 5.0 equivalents) was added. The mixture was warmed to room temperature and stirred for 1 h, cooled to -78 °C and 830 μL of methylmagnesium chloride/THF (3.0 M, 2.5 mmol, 5.0 equivalents) was added. The reaction mixture was stirred at -78 °C for 4 h, warmed to room temperature, and stirred for an additional 12 h. The reaction mixture was quenched with 5 mL of saturated $NH_4Cl$. The aqueous layer was extracted with diethylether (3×20 mL), and the combined organic layers were dried and concentrated. The residue was purified by flash chromatography (100% ethyl acetate) to give 145 mg (40%) of a colorless oil.

IR (thin film) 3375 (br), 3065, 3028, 2981, 2933, 2891, 1729, 1499, 1457, 1378, 1236, 1220, 1052 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.34 (m, 10H, Ph), 5.02 (m, 4H, benzylic protons), 4.10 (m, $1H_2$), 3.85 (m, $1H_4$), 3.47 (dd, $J$ = 7.6, 5.4 Hz, $1H_3$), 1.90 (m, $4H_{5a,5b,6a,6b}$), 1.35 (s, $3H_8$), 1.34 (s, $3H_9$), 1.16 (d, $J$ = 6.5 Hz, $3H_1$); $^{13}C$ NMR (75 MHz, $CDCl_3$) 136.32, 128.55, 128.37, 127.87 (Ph), 108.61 ($C_7$), 83.93 ($C_3$), 77.30 (d, $J$ = 15.15 Hz, $C_4$), 67.59 ($C_2$), 67.16 (benzylic carbons), 27.25 ($C_8$), 27.02 ($C_9$), 26.85 ($C_5$), 22.41 (d, $J$ = 141.0 Hz, $C_6$ ), 19.07 ($C_1$); $^{31}P$ NMR (121 MHz, $CDCl_3$) δ 34.01; HRMS(CI) calcd. for $C_{23}H_{32}O_6P$ 435.1937 found 435.1952.

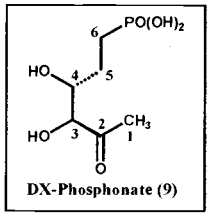
**(3*S*, 4*R*)-3,4-(Isopropylidenedioxy)-2-one-hexan-6-dibenzylphosphonate (35).**




To a solution of the alcohol **34** (145 mg, 0.3 mmol) in 10 mL of $CH_2Cl_2$ were added NMO (50 mg, 0.5 mmol, 1.5 equivalents), 200 mg of finely crushed and activated 4 Å molecular sieves, and TPAP (10 mg, 0.02 mmol, 0.05 equivalents). The reaction mixture was stirred for 2 h, filtered over Florisil and the Florisil was washed with 20 mL of $CH_2Cl_2$. The filtrate was dried and concentrated. Purification by flash chromatography (3:1 (v/v) ethyl acetate/hexanes) provided 52 mg of the ketone (36%) as a colorless oil.

$[\alpha]^{25}_D$ -10° (*c* 0.2, $CH_2Cl_2$); IR (thin film) 2984, 2936, 2883, 1717, 1495, 1456, 1379, 1243, 1209, 1079 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.34 (m, 10H, Ph), 5.03 (m, 4H, benzylic protons), 3.95 (m, $1H_4$), 3.85 (d, $J$ = 7.7 Hz, $1H_3$), 2.22 (s, $3H_1$), 1.90 (m, $4H_{5a,5b,6a,6b}$), 1.39 (s, $3H_8$), 1.36 (s, $3H_9$); $^{13}C$ NMR (75 MHz, $CDCl_3$) 208.19($C_2$), 136.28, 128.57, 128.37, 127.93 (Ph), 110.46 ($C_7$), 84.86 ($C_3$), 77.24 (d, $J$ = 17.7 Hz, $C_4$), 67.15 (benzylic carbons), 27.03 ($C_8$), 26.65 ($C_5$), 26.34 ($C_9$), 26.13 ($C_1$), 22.12 (d, $J$ = 141.9 Hz, $C_6$); $^{31}P$ NMR (121 MHz, $CDCl_3$) δ 33.45; HRMS(CI) calcd. for $C_{23}H_{30}O_6P$ 433.1780 found 433.1791.

**(3*S*, 4*R*)-3,4-(Dihydroxy)-2-one-hexan-6-phosphonate (DX-Phosphonate, 9)**



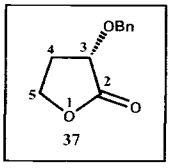
DX-Phosphonate (9)

To a solution of the ketone **35** (20 mg) in 5 mL of EtOH was added 10 mg of 10% Pd/C. The reaction mixture was stirred for 2 h at room temperature under a hydrogen atmosphere (1 atm). The reaction mixture was filtered over Celite and washed with 20 mL of EtOH. After concentration, 5.0 mL of MeOH and 100 mg of Dowex50x8($H^+$) were added. The reaction mixture was stirred for an additional 20 h, and was filtered over Celite and washed with 10 mL of MeOH to give 10 mg (77%) of a colorless oil. The reaction product was dissolved in 1 mL of water and the pH was adjusted to 7 with solid $NaHCO_3$. The mixture was frozen and lyophilized to give a white solid of DX-phosphonate sodium salt.

$[\alpha]^{25}_D$ +48° (*c* 2.5, $H_2O$); $^1H$ NMR (300 MHz, $D_2O$) δ 4.31 (d, $J$ = 1.3 Hz, $1H_3$), 4.15 (t, $J$ = 10.3, 5.3 Hz, $1H_4$), 2.24 (s, $3H_1$), 1.85 (m, $4H_{5a,5b,6a,6b}$); $^{13}C$ NMR (75 MHz, $D_2O$/MeOH, 100:1) 214.09 ($C_2$), 80.16 ($C_3$), 72.42 (d, $J$ = 18.47 Hz, $C_4$), 27.39 ($C_5$), 26.87 ($C_1$), 24.13 (d, $J$ = 135.52 Hz, $C_6$); $^{31}P$ NMR (121 MHz, $D_2O$) δ 31.37; HRMS(-FAB) calcd. for $C_6H_{14}O_6P$ 213.0528 found 213.0533.

**4-Deoxy-DXP Synthesis.**

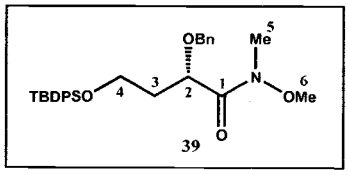
**(*S*)-α-Benzyloxy –γ-butyrolactone (37); Registry No. 78469-77-9.**




To a solution of (*S*)-α-hydroxy -γ-butyrolactone **36** (1.4 g, 13.5 mmol) in 20 mL of THF was added silver(I) oxide (8.0 g, 34 mmol, 2.5 equivalents) and BnBr (2.4 mL, 20 mmol, 1.5 equivalents). The reaction mixture was stirred for 12 h and filtered over Celite which was then washed with 100 mL of diethylether. After concentration, the crude reaction was purified by flash chromatography (1:1 (v/v) ethyl acetate/hexanes) to give 2.5 g (63%) of a colorless oil.

$[\alpha]^{25}_{D}$ -49° (*c* 3.4, $CH_2Cl_2$), lit = -83.4° (*c* 0.8, $CHCl_3$)[90]; IR (thin film) 3032, 2911, 2868, 1794, 1455, 1344, 1219, 1177, 1135, 1024 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.45 (m, 5H, Ph), 4.96 (d, $J$ = 11.8 Hz, 1H, benzylic proton), 4.75 (d, $J$ = 11.8 Hz, 1H, benzylic proton), 4.45 (m, $1H_3$), 4.15 (m, $2H_{5a,5b}$), 2.45 (m, $1H_{4a}$), 2.25 ( m, $1H_{4b}$); $^{13}C$ NMR (75 MHz, $CDCl_3$) 175.02 ($C_2$), 136.87, 128.53, 128.14 (Ph), 72.34 ($C_3$), 72.09 (benzylic carbon), 65.47 ($C_5$), 29.84 ($C_4$); HRMS(CI) calcd. for $C_{11}H_{13}O_3$ 193.0865 found 193.0861.

**(2*S*)-2-Benzyloxy-4-(*tert*-buthyldiphenylsiloxy)-*N*-methoxy-*N*-methylbutanamide (39).**

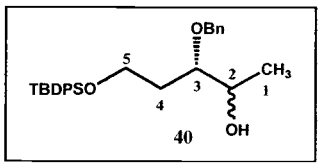



To a solution of *N*,*O*-dimethylhydroxylamine (0.58 g, 5.90 mmol, 2.0 equivalents) in 10 mL of $CH_2Cl_2$ was added trimethylaluminum (2.0 M in toluene, 3.0 mL, 5.90 mmol, 2.0 equivalents) at 0 °C. After the reaction mixture was stirred for 5 min, a solution of the butyrolactone **37** (0.53 g, 2.95 mmol) in 10 mL of $CH_2Cl_2$ was added via cannula. The reaction mixture was stirred for 2 h at room temperature, and 3.0 mL of water was added at 0 °C. The reaction mixture was warmed to room temperature and stirred for 1 h. The organic layer was removed, dried and concentrated to give a yellow oil. The crude reaction mixture was dissolved in 10 mL of $CH_2Cl_2$ and TEA (2.5 mL, 18.0 mmol, 6.0 equivalents), *tert*-butyldiphenylsilylcholoride (2.0 mL, 7.40 mmol, 2.5 equivalents), and DMAP (20 mg) were added and the reaction mixture

was stirred for 24 h. The reaction mixture was quenched with 3 mL of water and extracted with $CH_2Cl_2$ (3×20 mL), and the combined organic layers were dried and concentrated. The residue was purified by flash chromatography (1:2 (v/v) ethyl acetate/hexanes) to give 1.16 g (80% for 2 steps) of a colorless oil.

$[\alpha]^{25}_D$ -35° (*c* 2.1, $CH_2Cl_2$), IR (thin film) 3431, 3068, 2928, 2854, 1675, 1466, 1431, 1391, 1112, 1003 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.50 (m, 15H, Ph), 4.75 (m, $1H_2$), 4.72 (d, $J$ = 11.7 Hz, 1H, benzylic proton), 4.44 (d, $J$ = 11.7 Hz, 1H, benzylic proton), 4.02 (td, $J$ = 4.2, 9.9 Hz, $1H_{4a}$), 3.83 (m, $1H_{4b}$), 3.59 (s, $3H_6$), 3.23 (s, $3H_5$), 2.00 (m, $2H_{3a,3b}$), 1.05 (s, 9H, 3×$CH_3$ of TBDPS group); $^{13}C$ NMR (75 MHz, $CDCl_3$) 137.72, 136.59, 135.33, 129.54, 128.23, 127.78, 127.49 (Ph), 128.16 ($C_6$), 127.82 ($C_5$), 71.46 (benzylic carbon), 61.16 ($C_2$), 59.55 ($C_4$), 35.24 ($C_3$), 26.68 (3×$CH_3$ of TBDPS group), 19.08 (quaternary carbon of TBDPS group); HRMS(CI) calcd. for $C_{29}H_{38}O_4NSi$ 492.2570 found 492.2579.

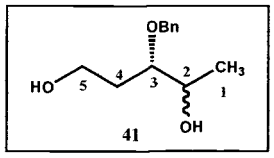
**(3*S*)-2-Hydroxy-3-benzyloxy-5-(*tert*-buthyldiphenylsiloxy)pentane (40).**




To a solution of the Weinreb amide **39** (1.16 g, 2.37 mmol) in 15 mL of THF was added diisobutyl aluminum hydride (1.0 M in THF, 4.00 mL, 4.03 mmol, 1.7 equivalents) at -78 °C. The reaction mixture was stirred at -78 °C for 2 h before 5 mL of MeOH was added. In a next step, the reaction mixture was stirred at room temperature for 30 min before filtration over Celite and washing with 100 mL of hot MeOH. The filtrate was concentrated to obtain a sticky colorless oil. The crude reaction mixture was dissolved in 15 mL of THF and methylmagnesium chloride (3.0 M in THF, 2.5 mL, 7.41 mmol, 3.2 equivalents) was added at -78 °C. The reaction mixture was stirred at -78 °C for 1 h and at room temperature for an additional 1 h. In the next step, the reaction mixture was quenched with 7 mL of saturated $NH_4Cl$. The aqueous layer was extracted with diethylether (3×20 mL), and the combined organic layers were dried and concentrated. The residue was purified by flash chromatography (1:3 (v/v) ethyl acetate/hexanes) to give 740 mg of the alcohol (70% for 2 steps) as a mixture of diastereomers.

$[\alpha]^{25}_{D}$ -6° (*c* 0.5, $CH_2Cl_2$); IR (thin film) 3446, 3076, 2943, 2854, 1461, 1426, 1386, 1107 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.55 (m, 15H, Ph), 4.65 (d, *J* = 11.3 Hz, 1H, benzylic proton), 4.52 (d, *J* = 11.7 Hz, 1H, benzylic proton), 3.95 (m, $1H_3$), 3.85 (m, $2H_{5a,5b}$), 3.55 (dq, *J* = 11.6, 10.0, 6.0, 4.0 Hz, $1H_2$), 2.55 (2d, *J* = 4.3 Hz, hydroxyl proton), 1.85 (m, $2H_{4a,4b}$), 1.20 (d, *J* = 6.4 Hz, $3H_1$), 1.05 (s, 9H, 3×$CH_3$ of TBDPS group); $^{13}C$ NMR (75 MHz, $CDCl_3$) 138.48, 138.35, 135.53, 133.48, 129.73, 128.43, 128.37, 127.82, 127.68 (Ph), 81.12, 80.28 ($C_3$), 72.79, 72.03 (benzylic carbon), 69.33, 68.05 ($C_2$), 60.35, 60.19 ($C_5$), 33.47, 31.76 ($C_4$), 26.84 (3×$CH_3$ of TBDPS group), 19.12 (quaternary carbon of TBDPS group), 19.01, 18.09 ($C_1$); HRMS(CI) calcd. for $C_{28}H_{36}O_3Si$ 448.2434 found 448.2445.

**(3*S*)-2-Hydroxy-3-benzyloxy-5-pentanol (41).**

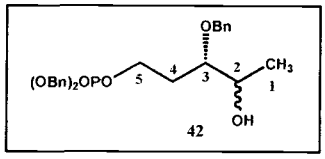


41

To a solution of the mixture of alcohols **40** (0.29 g, 0.65 mmol) in 10 mL of THF was added TBAF (1.0 M in THF, 1.0 mL, 1.5 equivalents). The reaction mixture was stirred for 14 h at room temperature and concentrated. The residue was purified by flash chromatography (3:1 (v/v) ethyl acetate/hexanes) to give 120 mg (88%) of a colorless oil as a mixture of diastereomers.

$[\alpha]^{25}_{D}$ -30° (*c* 0.1, $CH_2Cl_2$); IR (thin film) 3352 (br), 2962, 2888, 1650, 1594, 1497, 1451, 1367, 1057 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.35 (m, 10H, Ph), 4.60 (4d, *J* = 11.7 Hz, 4H, benzylic proton), 4.05 (m,$1H_3$), 3.80 (m, $5H_{3,2,2',5b,5b'}$), 3.50 (dq, *J* = 15.5, 11.4, 7.7, 4.0, $2H_{5a,5a'}$), 1.82 (m, $4H_{4a,4a',4b,4b'}$), 1.20 (2d, *J* = 6.4, 6H, 2×$3H_1$); $^{13}C$ NMR (75 MHz, $CDCl_3$) 133.66, 128.58, 127.98 (Ph), 81.61, 81.16 ($C_3$), 72.90, 71.79 ($C_2$), 69.28, 67.71 (benzylic carbon), 59.57, 59.29 ($C_5$), 32.91, 30.78 ($C_4$),19.26, 18.07 ($C_1$); HRMS(CI) calcd. for $C_{12}H_{18}O_3$ 210.1256 found 210.1251.

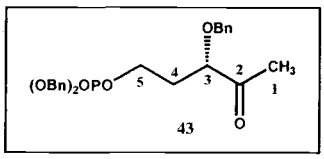
**(3*S*)-2-Hydroxy-3-benzyloxypentan-5-dibenzylphosphate (42).**




To a solution of the diol **41** (100 mg, 0.51 mmol) in 10 mL of $CH_3CN$ was added 5-methylthio-1H-tetrazole (70 mg, 0.61 mmol, 1.2 equivalents) in one portion at room temperature. The reaction mixture was stirred at room temperature for 15 min before cooling to 0 °C and a solution of diisopropyldibenzyl phosphoramidite (0.25 mL, 0.75 mmol, 1.5 equivalents) in 15 mL of $CH_3CN$ was added via cannula within 20 min. The reaction mixture was stirred at room temperature for 12 h and $^t$BuOOH (1 mL) was added. After being stirred for an additional 30 min, the reaction mixture was concentrated and purified by flash chromatography (3:1 (v/v) ethyl acetate/hexanes) to give 100 mg (36%) of a colorless oil as a diastereomeric mixture.

$[\alpha]^{25}_D$ -6° (*c* 0.4, $CH_2Cl_2$); IR (thin film) 3417 (br), 3050, 3027, 2972, 2893, 1460, 1376, 1265, 1015 $cm^{-1}$; $^1$H NMR (300 MHz, $CDCl_3$) δ 7.30 (m, 15H, Ph), 5.05 (ABq, $J$ = 8.3, 3.2 Hz, 4H, benzylic protons), 4.50 (4d, $J$ = 11.3 Hz, 2H, benzylic protons), 4.15 (m, $2H_{5a,5b}$), 3.85 (m, $1H_3$), 3.35(m, $1H_2$), 1.85 (m, $2H_{4a,4b}$), 1.10 (dd, $J$ = 6.3, 6.5 Hz, $3H_1$); $^{13}$C NMR (75 MHz, $CDCl_3$) 138.05 (d, $J$ = 8.2 Hz), 135.81, 128.54, 128.45, 127.91, 127.82, 127.75 (Ph), 79.93, 78.99 ($C_3$), 73.29, 72.19 (benzylic carbon), 69.22 (benzylic carbon), 69.08, 67.42($C_2$), 64.82, 64.47 (d, $J$ = 5.7 Hz, $C_5$), 30.37, 29.35 (d, $J$ = 6.8 Hz, $C_4$), 19.01, 17.75 ($C_1$); $^{31}$P NMR (121 MHz, $CDCl_3$) δ **0**.32, **0**.28; HRMS(CI) calcd. for $C_{26}H_{32}O_6P$ 471.1937 found 471.1918.

**(3*S*)-3-Benzyloxypentan-2-one-5-dibenzylphosphate (43).**

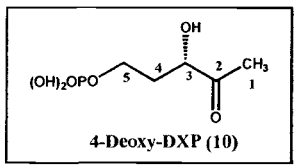



To a solution of the mixture of alcohols **42** (90 mg, 0.19 mmol) in 10 mL of $CH_2Cl_2$ were added NMO (40 mg, 0.29 mmol, 1.5 equivalents), 100 mg of finely crushed and activated 4 Å molecular sieves, and TPAP (5 mg, 9.9 μmol, 0.05 equivalents). The reaction mixture was stirred for 2 h, filtered over Florisil and washing with 30 mL of $CH_2Cl_2$. The filtrate was dried and concentrated.

Purification by flash chromatography (3:1 (v/v) ethyl acetate/hexanes) to give 65 mg (71%) of a colorless oil.

$[\alpha]^{25}_D$ -2° (*c* 0.7, $CH_2Cl_2$); IR (thin film) 3060, 3027, 2953, 2897, 1720, 1455, 1362, 1228 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.35 (m, 15H, Ph), 5.02 (dd, *J* = 2.0, 8.2 Hz, 4H, benzylic protons), 4.54 (d, *J* = 11.4 Hz, 1H, benzylic proton), 4.42 (d, *J* = 11.4 Hz, 1H, benzylic proton), 4.13 (m, $2H_{5a,5b}$), 3.90 (dd, *J* = 4.4, 8.5 Hz, $1H_3$), 2.15 (s, $3H_1$), 1.95 ( m, $2H_{4a,4b}$); $^{13}C$ NMR (75 MHz, $CDCl_3$) 210.04 ($C_2$), 137.16, 135.83, 128.64, 128.02, 127.93 (Ph), 80.93 ($C_3$), 72.72 (benzylic carbon), 69.30 (d, *J* = 5.4 Hz, benzylic carbon), 63.65 (d, *J* = 5.7 Hz, $C_5$), 32.48 (d, *J* = 7.3 Hz, $C_4$), 25.61 ($C_1$); $^{31}P$ NMR (121 MHz, $CDCl_3$) δ 0.12 ; HRMS(CI) calcd. for $C_{26}H_{30}O_6P$ 469.1780 found 469.1747.

**(3*S*)-3-Hydroxypentan-2-one-5-phosphate (4-Deoxy DXP, 10).**



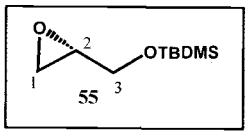
4-Deoxy-DXP (10)

To a solution of the ketone **43** (55 mg) in 5 mL of EtOH was added 10 mg of 10% Pd/C. The reaction mixture was stirred for 12 h at room temperature under a hydrogen atmosphere (1 atm). The reaction mixture was filtered over Celite and washed with 25 mL of EtOH. The crude product solution was concentrated to give 25 mg (99%) of a colorless oil. The reaction product was dissolved in 1 mL of water and the pH was adjusted to 7 with solid $NaHCO_3$. The mixture was frozen and lyophilized to give a white solid of 4-deoxy DXP sodium salt.

$[\alpha]^{25}_D$ +3.2° (*c* 1.3, $H_2O$); $^1H$ NMR (300 MHz, $D_2O$) δ 4.47 (dd, *J* = 3.7, 8.0 Hz, $1H_3$), 4.05 (dd, *J* = 6.0, 12.2 Hz, $2H_{5a,5b}$), 2.28 (s, $3H_1$), 2.10 (m, $2H_{4a,4b}$); $^{13}C$ NMR (75 MHz, $D_2O$/ MeOH, 100:1) 215.95 ($C_2$), 74.79 ($C_3$), 62.67 ($C_5$), 34.32 (d, *J* = 7.5 Hz, $C_4$), 26.50 ($C_1$); $^{31}P$ NMR (121 MHz, $D_2O$) δ 0.12 ; HRMS(-FAB) calcd. for $C_5H_{10}O_6P$ 197.0215 found 197.0215.

**3-Deoxy-DXP Synthesis.**

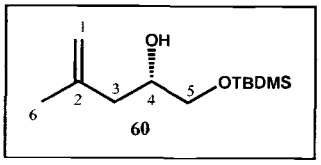
**(*S*)-(*tert*-Butyldimethylsiloxymethyl)oxirane (55); Registry No. 124150-87-4.**




To a solution of (*R*)-glycidol **54** (1.7g, 22.0 mmol) and TEA (3.1 mL, 22.0 mmol, 1 equivalent) in dry $CH_2Cl_2$ (40 mL) was added *tert*-butyldimethylsilylchloride (3.3 g in 10 mL of $CH_2Cl_2$, 22.0 mmol, 1 equivalent) and DMAP (75 mg) at room temperature. After being stirred at room temperature for 12 h, 10 mL of water was added to the reaction mixture which was extracted with 3×20 mL of $CH_2Cl_2$. The combined extracts were dried, filtered, concentrated and purified by flash chromatography (1:9 (v/v) ethyl acetate/hexanes) to give 4.0 g (95%) of a colorless oil.

$[\alpha]^{25}_D$ +6° (*c* 0.5, $CH_2Cl_2$), lit = +6.11° (*c* 2.75, $CH_2Cl_2$)[91]; IR (thin film) 2965, 2931, 2892, 2858, 1465, 1257, 1096 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 3.87 (dd, *J* = 11.9, 3.5 Hz, $1H_{3a}$), 3.65 (dd, *J* = 11.9, 4.8 Hz, $1H_{3b}$), 3.08 (m,$1H_2$), 2.75 (dd, *J* = 5.2, 4.2 Hz, $1H_{1a}$), 2.63 (dd, *J* = 5.2, 2.7 Hz, $1H_{1b}$), 0.85 (s, 9H, 3×$CH_3$ of TBDMS group), 0.05 (s, 6H, 2×$CH_3$ of TBDMS group); $^{13}C$ NMR (75 MHz, $CDCl_3$) 63.72 ($C_3$), 52.41 ($C_2$), 44.46 ($C_1$), 25.85 (3×$CH_3$ of TBDMS group), 18.26 (quaternary carbon of TBDMS group), -5.32 (2×$CH_3$ of TBDMS group); LRMS(CI) calcd. for $C_9H_{21}O_2Si$ 189.1 found 189.1

**(4*S*)-1-Ene-2-methyl-4-hydroxy-5-(*tert*-butyldimethylsiloxy)-pentane (60). Registry No. 226703-84-0.**

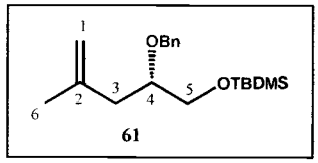



To a solution of the oxirane **55** (1.3 g, 6.9 mmol) in 30 mL of THF was added CuI at -50 °C (0.53 g, 2.8 mmol, 0.4 equivalents) and isoproprenylmagnesium chloride (0.5 M in THF, 28 mL, 14.0 mmol, 2.0 equivalents). The mixture was stirred at -50 °C for 1 h and quenched with 5 mL of saturated $NH_4Cl$. The reaction mixture was extracted with diethylether (3×20 mL). The combined organic layers were dried and concentrated. The residue was purified by flash chromatography (1:9 (v/v) ethyl acetate/hexanes) to give 1.43 g (90%) of a colorless oil.

$[\alpha]^{25}_D$ +3° (*c* 0.5, $CH_2Cl_2$); IR (thin film) 3405 (br), 3066, 2923, 2855, 1711, 1643, 1463, 1382, 1259, 1122, 1079 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 4.80 (d, $J$ = 16.5 Hz, $2H_{1a,1b}$), 3.82 (m, $1H_4$), 3.61 (dd, $J$ = 9.9, 3.8 Hz, $1H_{5a}$), 3.46 (dd, $J$ = 9.9, 6.8 Hz, $1H_{5b}$), 2.35 (s, 1H, hydroxyl proton), 2.16 (d, $J$ = 6.7 Hz, $2H_{3a,3b}$), 1.75 (s, $3H_6$), 0.85 (s, 9H, 3×$CH_3$ of TBDMS group), 0.05 (s, 6H, 2×$CH_3$ of TBDMS group); $^{13}C$ NMR (75 MHz, $CDCl_3$) 142.35 ($C_2$), 112.88 ($C_1$), 69.59 ($C_4$), 66.82 ($C_5$), 41.55 ($C_3$), 25.87 (3×$CH_3$ of TBDMS group), 22.55 ($C_6$), 18.29 (quaternary carbon of TBDMS group), -5.35 (2×$CH_3$ of TBDMS group); LRMS(CI) calcd. for $C_{12}H_{27}O_2Si$ 231.1 found 231.1

**(4*S*)-1-Ene-2-methyl-4-benzyloxy-5-(*tert*-butyldimethylsiloxy)-pentane (61).**

To a solution of the alcohol **60** (0.2 g, 0.8 mmol) in 0.5 mL of THF at -78 °C was added *n*-butyllithium (1.6 M, 0.82 mL, 1.3 mmol, 1.5 equivalents). After being stirred at -78 °C for 10 min, HMPA (0.23 mL, 1.3 mmol, 1.5 equivalents) and BnBr (0.15 mL, 1.3 mmol, 1.5 equivalents) were added. The reaction mixture was allowed to warm slowly to room temperature and stirred for 20 h. In the next step, 20 mL of diethylether was added and the reaction mixture was washed with 3×5 mL of water. The organic layer was dried and concentrated. The residue was purified by flash chromatography (3% ethyl acetate/hexanes) to give 0.19 g (68%) of a colorless oil.

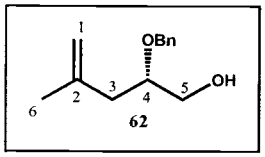


61

$[\alpha]^{25}_D$ -7° (*c* 0.6, $CH_2Cl_2$); IR (thin film) 2923, 2855, 1647, 1463, 1357, 1259, 1096 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.32 (m, 5H, Ph), 4.80 (d, $J$ = 7.8 Hz, $2H_{1a,1b}$), 4.64 (dd, $J$ = 27.2, 11.8 Hz, 2H, benzylic protons), 3.63 (m, $3H_{4,5a,5b}$), 2.26 (m, $2H_{3a,3b}$), 1.74 (s, $3H_6$), 0.91 (s, 9H, 3×$CH_3$ of TBDMS group ), 0.06 (s, 6H, 2×$CH_3$ of TBDMS group); $^{13}C$ NMR (75 MHz, $CDCl_3$) 142.75 ($C_2$), 138.97, 128.23, 127.70, 127.38 (Ph), 112.65 ($C_1$), 78.46 ($C_4$), 72.14 (benzylic carbon), 65.56 ($C_5$), 40.19 ($C_3$), 25.91 (3×$CH_3$ of TBDMS group), 22.89 ($C_6$), 18.29 (quaternary carbon

of TBDMS group), -5.33 (2×$CH_3$ of TBDMS group); HRMS(CI) calcd. for $C_{19}H_{32}O_2Si$ 320.2172 found 320.2165.

**(4*S*)-1-Ene-2-methyl-4-benzyloxy-5-pentanol (62).**

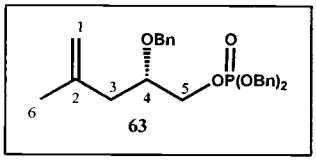


62

To a solution of **61** (0.18 g, 0.56 mmol) in 10 mL of THF was added TBAF (1.0 M in THF, 0.68 mL, 0.67 mmol, 1.2 equivalents). The reaction mixture was stirred for 13 h at room temperature, and concentrated. The residue was purified by flash chromatography (1:2 (v/v) ethyl acetate/hexanes) to give 75 mg (65%) of a colorless oil.

$[\alpha]^{25}_D$ +17° (*c* 0.8, $CH_2Cl_2$); IR (thin film) 3426 (br), 3061, 3027, 2927, 2870, 1646, 1451, 1346, 1212, 1095 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.32 (m, 5H, Ph), 4.80 (d, $J$ = 15.6 Hz, $2H_{1a,1b}$), 4.67 (d, $J$ = 11.5 Hz, 1H, benzylic proton), 4.54 (d, $J$ = 11.5 Hz, 1H, benzylic proton), 3.69 (m, $2H_{4,5a}$), 3.52 (dd, $J$ = 12.2, 7.4 Hz, $1H_{5b}$), 2.40 (dd, $J$ = 14.0, 5.5 Hz, $1H_{3a}$), 2.21 (dd, $J$ = 14.5, 7.8 Hz, $1H_{3b}$), 1.87 (s, 1H, hydroxyl proton), 1.76 (s, $3H_6$); $^{13}C$ NMR (75 MHz, $CDCl_3$) 141.96($C_2$), 138.29, 128.49, 127.79 (Ph), 113.27 ($C_1$), 78.01 ($C_4$), 76.69 (benzylic carbon), 64.27 ($C_5$), 39.41 ($C_3$), 22.85 ($C_6$); HRMS(CI) calcd. for $C_{13}H_{18}O_2$ 206.1307 found 206.1309.

**(4*S*)-1-Ene-2-methyl-4-benzyloxypentan-5-dibenzylphosphate (63).**



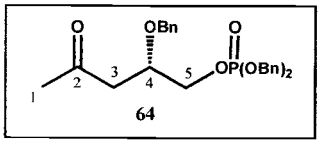
63

To a solution of the alcohol **62** (68 mg, 0.33 mmol, 1 equivalent) in 7 mL of THF was added 1H-tetrazole (28 mg, 0.39 mmol, 1.2 equivalents) in one portion at room temperature. The reaction mixture was stirred at room temperature for 15 min before cooling to 0 °C and a solution of diisopropyl dibenzylphospho-ramidite (0.13 mL, 0.39 mmol, 1.2 equivalents) was added. The reaction mixture was stirred at room temperature for 18 h and $^t$BuOOH (1 mL) was added. After being stirred for an additional 30 min, the reaction mixture was concentrated and purified by flash chromatography (1:2 (v/v) ethyl acetate/hexanes) to give 65 mg (42%) of a colorless oil.

$[\alpha]^{25}_D$ +13° (*c* 0.1, $CH_2Cl_2$); IR (thin film) 3068, 3038, 2944, 2889, 1647, 1498, 1455, 1374, 1263, 1212, 998 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.34 (m,15H, Ph), 5.04 (d, *J* = 15.6 Hz, 2H, benzylic protons), 4.78 (d, *J* = 24.0 Hz, $2H_{1a,1b}$), 4.57 (dd, *J* = 28.9, 11.5 Hz, 2H, benzylic protons), 4.09 (m, $1H_{5a}$), 4.09 (m, $1H_{5b}$), 3.73 (m, $1H_4$), 2.29 (dd, *J* = 14.2, 6.9 Hz, $1H_{3a}$), 2.20 (dd, *J* = 14.3, 6.2 Hz, $1H_{3b}$), 1.70 (s, $3H_6$); $^{13}C$ NMR (75 MHz, $CDCl_3$) 141.96($C_2$), 138.19, 135.79 (d, *J* = 6.9 Hz), 128.51, 128.45, 128.37, 128.27, 127.86, 127.70, 127.56, 127.39 (Ph), 113.46 ($C_1$), 75.95 (d, *J* = 7.4 Hz, $C_4$), 71.87 (benzylic carbon), 69.22 (d, *J* = 5.4 Hz, $C_5$), 68.72 (d, *J* = 6.2 Hz, benzylic carbon), 39.55 ($C_3$), 22.75 ($C_6$); $^{31}P$ NMR (121 MHz, $CDCl_3$) δ 0.26; HRMS(CI) calcd. for $C_{27}H_{31}O_5P$ 466.1909 found 466.1896.

**(4*S*)-2-One-4-benzyloxypentan-5-dibenzylphosphate (64).**

To a solution of the alkene **63** (65 mg, 0.14 mmol, 1 equivalent) in 5 mL of THF was added osmium tetroxide (5 mg, 0.007 mmol, 0.05 equivalents). After the reaction mixture was stirred for 15 min at room temperature, NMO (20 mg, 0.15 mmol, 1.1 equivalents) was added. After stirring for an additional 1 h, 2 mL of water and $NaIO_4$ (120 mg, 0.56 mmol, 4 equivalents) were added. The reaction mixture was stirred at room temperature for 15 min, quenched by addition of saturated $Na_2CO_3$ (3 mL) and extracted with 2×10 mL of diethylether. The combined organic layer was dried, concentrated, and purified by flash chromatography (2:1 (v/v) ethyl acetate/hexanes) to give 52 mg (80%) of a colorless oil.

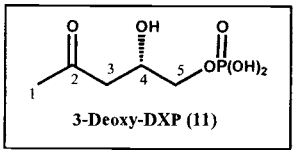


64

$[\alpha]^{25}_D$ +16° (*c* 0.1, $CH_2Cl_2$); IR (thin film) 1707, 1451, 1357, 1271, 1002 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.35 (m, 15H, Ph), 5.04 (dd, *J* = 8.4, 2.8 Hz, 2H, benzylic protons), 4.56 (d, *J* = 27.5, 11.3 Hz, 2H, benzylic protons), 4.05 (m, $3H_{4,5a,5b}$), 2.70 (dd, *J* = 17.0, 7.3 Hz, $1H_{3a}$), 2.54 (dd, *J* = 17.1, 4.5 Hz, $1H_{3b}$), 2.10 (s, $3H_1$); $^{13}C$ NMR (75 MHz, $CDCl_3$) 206.02($C_2$), 137.81, 135.71 (d, *J* = 7.0 Hz), 128.54, 128.33, 127.92, 127.84, 127.73 (Ph), 73.48 (d, *J* = 7.4 Hz, $C_4$), 72.35

(benzylic carbon), 69.32 (d, $J$ = 5.2 Hz, $C_5$), 67.81 (d, $J$ = 6.1 Hz, benzylic carbon), 45.19 ($C_3$), 30.82 ($C_1$); $^{31}P$ NMR (121 MHz, $CDCl_3$) δ 0.32 ; HRMS(CI) calcd. for $C_{26}H_{30}O_6P$ 469.1780 found 469.1777.

**(4*S*)- 2-One- 3-hydroxypentan-5-phosphate (3-Deoxy-DXP, 11).**

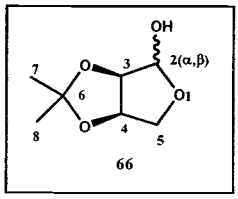


3-Deoxy-DXP (11)

To a solution of the ketone **64** (52 mg) in 4 mL of EtOH was added 20 mg of 10% Pd/C. The reaction mixture was stirred for 5 h at room temperature under a hydrogen atmosphere (1 atm). The reaction mixture was filtered over Celite and washed with 15 mL of EtOH. The filtrate was concentrated to give 20 mg (91%) of a colorless oil. The free phosphate form of 3-deoxy DXP was characterized and was dissolved in 1 mL of water and the pH was adjusted to 7 with solid $NaHCO_3$. The mixture was frozen and lyophilized to give a white solid of monosodium salt. $[\alpha]^{25}_D$ -73° (*c* 0.2, $CH_2Cl_2$); $^1H$ NMR (300 MHz, $D_2O$) δ 4.25 (m, $1H_4$), 3.85 (m, $2H_{5a,5b}$), 2.73 (m, $2H_{3a,3b}$), 2.19 (s, $3H_1$); $^{13}C$ NMR (75 MHz, $D_2O$/ MeOH, 100:1) 214.08 ($C_2$), 70.02 (d, $J$ = 4.3 Hz, $C_5$), 67.39 (d, $J$ = 7.9 Hz, $C_4$), 47.07 ($C_3$), 31.06 ($C_1$); $^{31}P$ NMR (121 MHz, $D_2O$) δ 1.32; HRMS(-FAB) calcd. for $C_5H_{10}O_6P$ 197.0215 found 197.0209.

**Deoxy-L-ribulose 5-phosphate Synthesis:**

**3,4-*O*-Isopropylidene-L-erythrose (66); Registry No. 92761-41-6.**



66

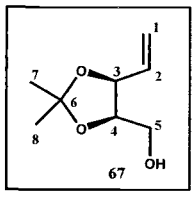
To a solution of 2,2-dimethoxypropane (4.0 mL, 30 mmol, 3 equivalents) in 20 mL of THF were added L-arabinose **65** (1.5 g, 10 mmol, 1 equivalent) and 20 mg of *p*-toluenesulfonic acid monohydrate at room temperature. After being stirred at room temperature for 2 h, the reaction mixture was quenched by adding 1 mL of TEA (10 mmol, 1 equivalent). The reaction mixture was concentrated to dryness in vacuo. To the crude reaction mixture were added hexanes (15 mL), water (30 mL), and sodium periodate (4.0 g, 25 mmol, 2 equivalents). After being stirred at room temperature for 1.5 h, the reaction was

quenched by adding solid $Na_2CO_3$ (2.5 g, 30 mmol, 3 equivalents) and the reaction mixture was stirred for an additional 1.5 h. The organic layer was removed and the aqueous layer was extracted with ethyl acetate (3×20 mL). The combined organic layers were dried and concentrated. Purification by flash chromatography (1:1 (v/v) ethyl acetate/hexanes) gave 1.10 g (68% for 2 steps) of a colorless oil with 7:1 ratio of β:α epimer of the cyclic hemiacetal.[70]

IR (thin film) 3424 (br), 2979, 2935, 2882, 1456, 13173, 1209, 1161, 1064 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) δ 5.36 (s, $1H_{2\beta}$), 4.95 (d, $J$ = 3.6 Hz, $0.15H_{2\alpha}$), 4.79 (dd, $J$ = 5.9, 3.5 Hz, $1H_{4\beta}$), 4.72(m, $0.15H_{4\alpha}$), 4.51 (d, $J$ = 6.0 Hz, $1H_{3\alpha}$), 4.46 (m, $0.15H_{3\beta}$), 3.95 (m, $2.3H_{5\alpha,5\beta}$), 3.65 (s, hydroxyl proton), 1.50 (s, $0.5H_{7\alpha}$), 1.42 (s, $3H_{7\beta}$), 1.33 (s, $0.5H_{8\alpha}$), 1.27 (s, $3H_{8\beta}$); $^{13}C$ NMR (100 MHz, $CDCl_3$) 113.33, 112.19 ($C_{6\alpha,6\beta}$), 101.57, 97.38 ($C_{2\alpha,2\beta}$), 85.05, 79.44 ($C_{3\alpha,3\beta}$), 79.82, 78.13 ($C_{5\alpha,5\beta}$), 71.69, 67.49 ($C_{7\alpha,7\beta}$), 26.05, 25.81($C_{8\alpha,8\beta}$), 24.59, 24.75 ($CH_3$); HRMS(CI) calcd. for $C_6H_9O_4$ 145.0501 found 145.0501.

**(*3R*, *4S*)-1-Ene-3,4-(isopropylidenedioxy)-5-pentanol (67).**

**Registry No. 99902-67-7**



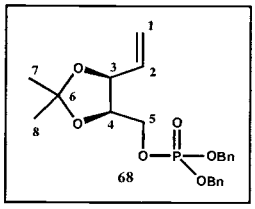
67

To a suspension of methyltriphenylphosphonium bromide (3.7 g, 10 mmol, 1.5 equivalent) in THF (40 mL) at -78 °C was added potassium *tert*-butoxide (1.2 g, 10 mmol, 1.5 equivalents). After the reaction mixture was stirred for 1 h at -20 °C, a solution of the erythrose **66** (1.10 g, 6.9 mmol, 1 equivalent) in 15 mL of THF was added via cannula. The reaction mixture was stirred for 12 h at room temperature before 5 mL of water and 50 mL of diethylether were added. The organic layer was removed, dried, and concentrated. Purification by flash chromatography (1:2 (v/v) ethyl acetate/hexanes) gave 0.8 g (74%) of a colorless oil.

$[\alpha]^{25}_D$ -47° (*c* 0.8, $CH_2Cl_2$), lit = -44° (*c* 4.89, $CHCl_3$)[92]; IR (thin film) 3448 (br), 3081, 2978, 2931, 1643, 1459, 1378, 1246, 1212, 1169 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 5.87 (2dd, $J$ = 10.3, 7.3 Hz, $1H_2$), 5.30 (m, $2H_{1a,1b}$), 4.62 (t, $J$ = 7.3, 5.9

Hz, $1H_3$), 4.24 (dd, $J$ = 12.5, 5.8 Hz, $1H_4$), 3.55 (d, $J$ = 5.8 Hz, $2H_{5a,5b}$), 2.20 (br, 1H, hydroxyl proton), 1.49 (s, $3H_7$), 1.37 (s, $3H_8$); $^{13}C$ NMR (100 MHz, $CDCl_3$) 132.94 ($C_2$), 118.87 ($C_1$), 108.81 ($C_6$), 78.22 ($C_{3,4}$), 61.97 ($C_5$), 27.72 ($C_7$), 25.17 ($C_8$); HRMS(CI) calcd. for $C_8H_{13}O_3$ 157.0865 found 157.0863.

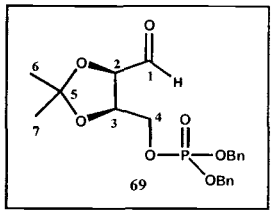
**(*3R*, *4S*)-1-Ene-3,4-(isopropylidenedioxy)pent-5-dibenzylphosphate (68).**




To a solution of the alcohol **67** (100 mg, 0.65 mmol, 1 equivalent) in THF (10 mL) was added NaH (60% in mineral oil, 30 mg, 0.67 mmol, 1.2 equivalents) in one portion at 0 °C. The slurry was stirred for 10 min before crystals of tetrabenzylpyrophosphate (290 mg, 0.54 mmol, 1.0 equivalent) were added in one portion. After being stirred for 2 h at room temperature, 10 mL of diethylether and 5 mL of water were added slowly to the reaction mixture which was further extracted with diethylether (2×5 mL). The combined organic layers were dried and concentrated. A small amount of the crude product was purified by flash chromatography (1:5 (v/v) ethyl acetate/hexanes) for a characterization and the remaining crude product was used without further purification.

$[\alpha]^{25}_D$ -4.1° (*c* 1.3, $CH_2Cl_2$); IR (thin film) 3056, 3030, 2983, 2927, 2889, 1496, 1459, 1378, 1280, 1212, 1169 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.34 (m, 10H, Ph), 5.73 (2dd, $J$ = 10.3, 7.1 Hz, $1H_2$), 5.35 (m, $1H_{1a}$), 5.22 (m, $1H_{1b}$), 5.04 (dd, $J$ = 8.2, 0.9 Hz, 4H, benzylic protons), 4.58 (t, $J$ = 13.7, 6.8 Hz, $1H_3$), 4.27 (m, $1H_4$), 3.94 (m, $2H_{5a,5b}$), 1.47 (s, $3H_7$), 1.35 (s, $3H_8$); $^{13}C$ NMR (75 MHz, $CDCl_3$) 135.82, 135.76 (d, $J$ = 6.8 Hz), 128.52, 127.92 (Ph), 132.24 ($C_2$), 119.07 ($C_1$), 109.26 ($C_6$), 77.94 ($C_3$), 76.28 (d, $J$ = 8.1 Hz, $C_4$), 69.32 (benzylic carbons), 66.52 (d, $J$ = 5.7 Hz, $C_5$), 27.72 ($C_7$), 25.27 ($C_8$); $^{31}P$ NMR (121 MHz, $CDCl_3$) δ 0.06; HRMS(CI) calcd. for $C_{22}H_{28}O_6P$ 419.1626 found 419.1625.

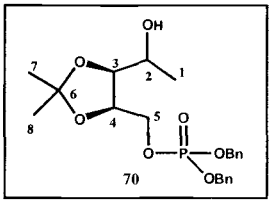
**(*2S*, *3S*)-1-al-2,3-(isopropylidenedioxy)but-4-dibenzylphosphate (69).**




To a solution of the crude dibenzylphosphate **68** (100 mg, 0.65 mmol, 1 equivalent) in THF (5 mL) was added osmium tetroxide (10 mg, 0.03 mmol, 0.05 equivalents) at 0 °C. After being stirred for 5 min, NMO (85 mg, 0.72 mmol, 1.1 equivalents) was added. The reaction mixture was stirred for 1 h at room temperature before 2 mL of saturated $NaHSO_3$ and 10 mL of diethylether were added. The organic layer was removed and 2 mL of water was added into the organic layer. In the next step, sodium periodate (560 mg, 2.6 mmol, 4 equivalents) was added at room temperature. After being stirred for 20 min, 2 mL of saturated $Na_2CO_3$ was added and the reaction mixture was extracted with diethylether (2×10 mL). The combined organic layers were dried and concentrated. Purification by flash chromatography (3:1 (v/v) ethyl acetate/hexanes) gave 169 mg (62% for 3 steps) of a colorless oil.

$[\alpha]^{25}_D$ -26° (*c* 1.0, $CH_2Cl_2$); IR (thin film) 3055, 2978, 2927, 2889, 1732, 1498, 1455, 1378, 1250, 1216, 1165 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 9.63 (d, $J$ = 2.1 Hz, $1H_1$), 7.35 (m, 10H, Ph), 5.05 (m, 4H, benzylic protons), 4.48 (m, $1H_3$), 4.40 (dd, $J$ = 7.6, 2.1 Hz, $1H_2$), 4.17 (dd, $J$ = 6.5, 4.0 Hz, $1H_{4a}$), 3.99 (dd, $J$ = 6.0, 4.6 Hz, $1H_{4b}$), 1.54 (s, $3H_6$), 1.37 (s, $3H_7$); $^{13}C$ NMR (75 MHz, $CDCl_3$) 200.39 ($C_1$), 135.63, 135.59 (d, $J$ = 6.1 Hz), 128.56, 127.93 (Ph), 111.26 ($C_5$), 80.39 ($C_2$), 76.71 (d, $J$ = 8.5 Hz, $C_3$), 69.43 (d, $J$ = 5.4 Hz, benzylic carbons), 64.49 (d, $J$ = 5.5 Hz, $C_4$), 26.86 ($C_6$), 24.93 ($C_7$); $^{31}P$ NMR (121 MHz, $CDCl_3$) δ −0.42; HRMS(CI) calcd. for $C_{21}H_{26}O_7P$ 421.1416 found 421.1428.

**(*3R*, *4S*)-2-ol-3,4-(isopropylidenedioxy)pent-5-dibenzylphosphate (70).**

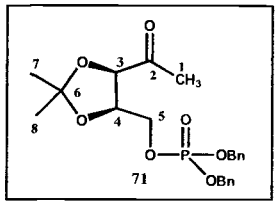



To a solution of the aldehyde **69** (159 mg, 0.38 mmol, 1 equivalent) in THF (5 mL) at -78 °C was added 0.25 mL of methylmagnesium chloride (3.0 M in THF, 0.76 mmol, 2.0 equivalents) was added. The reaction mixture was warmed to room temperature slowly. After being stirred for 1 h, the reaction was quenched

with 2 mL of saturated $NH_4Cl$. The aqueous layer was extracted with diethylether (3×5 mL), and the combined organic layers were dried and concentrated. The residue was purified by flash chromatography (3:1 (v/v) ethyl acetate/hexanes) to give 50 mg of an alcohol (37%) of a colorless oil.

$[\alpha]^{25}_D$ -1.3° (*c* 2.0, $CH_2Cl_2$); IR (thin film) 3395(br), 2979, 2926, 2889, 1456, 1374, 1243, 1219 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.34 (m, 10H, Ph), 5.05 (m, 4H, benzylic protons), 4.27 (m, $2H_{3,4}$), 4.06 (m, $1H_2$), 3.85 (m, $2H_{5a,5b}$), 1.39 (s, $3H_7$), 1.32 (s, $3H_8$), 1.26 (d, $J$ = 5.9 Hz, $3H_1$); $^{13}C$ NMR (75 MHz, $CDCl_3$) 135.70 (d, $J$ = 5.9 Hz), 128.58, 127.99 (Ph), 108.78 ($C_6$), 80.87 ($C_3$), 75.84 (d, $J$ = 9.3 Hz, $C_4$), 69.51, 69.42 (benzylic carbons), 66.26 (d, $J$ = 5.7 Hz, $C_5$), 65.53 ($C_2$), 27.89 ($C_7$), 25.41($C_8$), 20.99 ($C_1$); $^{31}P$ NMR (121 MHz, $CDCl_3$) δ 0.15; HRMS(CI) calcd. for $C_{22}H_{30}O_7P$ 437.1729 found 437.1723.

**(*3S*, *4S*)-2-one-3,4-(isopropylidenedioxy)pent-5-dibenzylphosphate (71).**

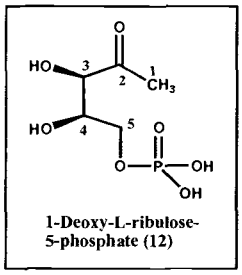



To a solution of the alcohol **70** (50 mg, 0.11 mmol) in 5 mL of $CH_2Cl_2$ were added NMO (20 mg, 0.17 mmol, 1.5 equivalents), 50 mg of finely crushed and activated 4 Å molecular sieves, and TPAP (5 mg, 6.0 μmol, 0.05 equivalents). The reaction mixture was stirred for 2 h, filtered over Florisil and the Florisil was washed with 20 mL of $CH_2Cl_2$. The filtrate was dried and concentrated. Purification by flash chromatography (2:1 (v/v) ethyl acetate/hexanes) provided 30 mg (61%) of a colorless oil.

$[\alpha]^{25}_D$ -13° (*c* 0.2, $CH_2Cl_2$); IR (thin film) 3482, 3064, 3030, 2978, 2931, 2889, 1796, 1493, 1459, 1378, 1263 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.34 (m, 10H, Ph), 5.05 (dd, $J$ = 7.8, 1.2 Hz, 4H, benzylic protons), 4.47 (s, $2H_{3,4}$), 4.12 (m, $1H_{5a}$), 3.94 (m, $1H_{5b}$), 2.23 (s, $3H_1$), 1.55 (s, $3H_7$), 1.35 (s, $3H_8$); $^{13}C$ NMR (75 MHz, $CDCl_3$) 208.27($C_2$), 135.65, 128.54, 127.92 (Ph), 110.42 ($C_6$), 80.56 ($C_3$), 76.32 (d, $J$ = 8.5 Hz, $C_4$), 69.31 (benzylic carbons), 65.27 ($C_5$), 28.03 ($C_7$), 26.74 ($C_8$),

24.67 ($C_1$); $^{31}P$ NMR (121 MHz, $CDCl_3$) δ 0.42; HRMS(CI) calcd. for $C_{22}H_{26}O_7P$ 433.1416 found 433.1414.

**1-Deoxy-L-ribulose-5-phosphate (12).**



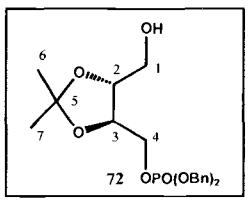
1-Deoxy-L-ribulose-5-phosphate (12)

To a solution of the dibenzylphosphate **71** (27 mg, 60 μmol) in 5 mL of EtOH was added 10 mg of 10% Pd/C. The reaction mixture was stirred for 5 h at room temperature under a hydrogen atmosphere (1 atm). The reaction mixture was filtered over Celite and washing with 20 mL of EtOH. After concentration, 3.0 mL of MeOH and 100 mg of Dowex50x8($H^+$) were added. The reaction mixture was stirred for an additional 12 h, filtered over Celite and washing with 10 mL of MeOH to give 12 mg (90%) of a light yellow oil. The free phosphate form of 1-Deoxy-L-ribulose-5-phosphate was characterized and was dissolved in 1 mL of water and the pH was adjusted to 7 with solid $NaHCO_3$. The mixture was frozen and lyophilized to give a white solid of monosodium salt.

$[\alpha]^{25}_D$ -4° (*c* 0.7, $H_2O$); $^1H$ NMR (300 MHz, $CDCl_3$) δ 4.26 (d, $J$ = 5.0 Hz, $1H_3$), 4.09 (q, $J$ = 10.9, 5.6 Hz, $1H_4$), 3.90 (t, $J$ = 11.7, 5.9 Hz, $2H_{5a,5b}$), 2.18 (s, $3H_1$); $^{13}C$ NMR (300 MHz, $CDCl_3$) 213.93 ($C_2$), 78.31 ($C_3$), 71.50 (d, $J$ = 8.8 Hz, $C_4$), 66.57 (d, $J$ = 5.3 Hz, $C_5$), 27.84 ($C_1$); $^{31}P$ NMR (121 MHz, $D_2O$) δ 0.54; HRMS(CI) calcd. for $C_5H_{10}O_7P$ 213.0164 found 213.0167.

**DXP carboxamide Synthesis:**

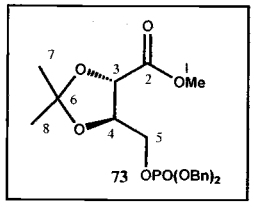
**(*2R, 3R*)-4-(Dibenzylphosphate)-2,3-(isopropylidenedioxy)butanol (72).**




To a solution of (-)-2,3-O-isopropylidene-D-threitol **15** (410 mg, 2.54 mmol) in 10 mL of THF was added NaH (60% in mineral oil, 150 mg, 3.05 mmol, 1.2 equivalents) at 0 °C in one portion. After being stirred for 5 min, a solution of tetrabenzylpyrophosphate (1.50 g, 2.78 mmol, 1.1 equivalents) in 5 mL of THF was added over a 5 min period. After being stirred for 4 h, 10 mL of water was added slowly to the reaction mixture, which was extracted with diethylether (3×50 mL).

The combined organic layers were dried and concentrated. Purification by flash chromatography (100 % ethyl acetate) gave 260 mg (50%) of a colorless oil. $[\alpha]^{25}_{D}$ +8° (*c* 0.9, $CH_2Cl_2$); IR (thin film) 3409 (br), 2984, 2935, 2887, 1456, 1378, 1252, 1214, 1165 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.35 (m, 10H, Ph), 5.04 (m, 4H, benzylic protons), 4.07 (m, $3H_{3,4a,4b}$), 3.90 (m, $1H_2$), 3.72 (dd, $J$ = 12.0, 4.0 Hz, $1H_{1a}$), 3.59 (dd, $J$ = 11.9, 4.3 Hz, $1H_{1b}$), 1.40 (s, $3H_6$), 1.37 (s, $3H_7$); $^{13}C$ NMR (75 MHz, $CDCl_3$) 135.65, 128.59, 127.98 (Ph), 109.72 ($C_5$), 77.92 ($C_2$), 75.68 (d, $J$ = 7.58 Hz, $C_3$), 69.49 (d, $J$ = 5.40 Hz, benzylic carbons), 66.88 (d, $J$ = 5.40 Hz, $C_4$), 61.95 ($C_1$), 27.01 ($C_6$), 26.84 ($C_7$); $^{31}P$ NMR (121 MHz, $CDCl_3$) δ 0.205; HRMS(CI) calcd. for $C_{21}H_{28}PO_7$ 423.1573 found 423.1579.

**(*3S, 4R*)-5-(Dibenzylphosphate)-3,4-(isopropylidenedioxy)-1-methylpentanoate (73).**

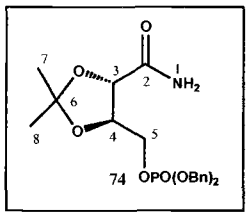



A solution of DMSO (0.18 mL, 2.46 mmol, 4.0 equivalents) in 10 mL of $CH_2Cl_2$ was added to a solution of oxalyl chloride (2M in $CH_2Cl_2$, 0.62 mL, 1.23 mmol, 2.0 equivalents) at -78 °C. After the solution was stirred for 5 min, a solution of 0.26 g (0.62 mmol) of the alcohol **72** in 25 mL of $CH_2Cl_2$ was added via cannula. The reaction mixture was stirred for 15 min at -78 °C and 0.52 mL of TEA (3.70 mmol, 6.0 equivalents) was added. The reaction mixture was warmed to room temperature and stirred for 1 h, before 10 mL of water was added. The organic layer was removed, dried and concentrated to give 0.30 g of a yellow oil. Without further purification, a 2.0 M bromine solution in 9:1 MeOH-$H_2O$ (2 mL) was added to a solution of the resulting aldehyde 0.30 g (0.70 mmol) in 9:1 MeOH-$H_2O$ (10 mL) buffered with $NaHCO_3$ (1.3 g). After being stirred at room temperature for 12 h, solid sodium thiosulfate (0.50 g) was added to quench excess bromine. The undissolved material was remove by filtration and washed with diethylether (20 mL). The filtrate was washed with water (2×5 mL) and saturated $NH_4Cl$ (2 × 5 mL) and the organic layer was dried and concentrated. The residue was purified by flash

chromatography (1:1 (v/v) ethyl acetate/hexanes) to give 160 mg (58% for 2 steps) as a mixture of a colorless oil.

$[\alpha]^{25}_D$ +11° (*c* 0.7, $CH_2Cl_2$); IR (thin film) 3032, 2989, 2950, 2897, 1755, 1499, 1456, 1378, 1277, 1209, 1112 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.34 (m, 10H, Ph), 5.05 (m, 4H, benzylic protons), 4.33 (m, $3H_{3,4,5a}$), 4.15 (m, $1H_{5b}$), 3.74 (s, $3H_1$), 1.43 (s, $3H_7$), 1.41 (s, $3H_8$); $^{13}C$ NMR (75 MHz, $CDCl_3$) 170.49 ($C_2$), 135.62, 128.50, 127.88 (Ph), 111.90 ($C_6$), 76.33 (d, $J$ = 13.6 Hz, $C_4$), 74.88 ($C_3$), 69.38 (d, $J$ = 5.27 Hz, benzylic carbons), 66.30 (d, $J$ = 5.33 Hz, $C_5$), 52.44 ($C_1$), 26.69 ($C_7$), 25.71 ($C_8$); $^{31}P$ NMR (121 MHz, $CDCl_3$) δ -0.47; HRMS(CI) calcd. for $C_{22}H_{28}PO_8$ 451.1522 found 451.1516.

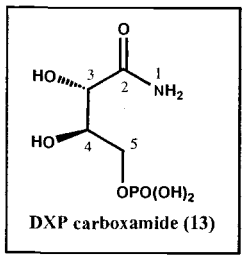
**(*3S, 4R*)-5-(Dibenzylphosphate)-3,4-(isopropylidenedioxy)-pentanamide (74).**




To a solution of the ester **73** (155 mg, 0.36 mmol) in MeOH (2 mL) was added 36 % aq $NH_3$ (2 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 3 h, prior to concentration in vacuo. The residue was purified by flash chromatography (100 % ethyl acetate) to give 98 mg (68%) of a colorless oil.

$[\alpha]^{25}_D$ -5° (*c* 0.3, $CH_2Cl_2$); IR (thin film) 3465, 3307, 2987, 1690, 1591, 1451, 1378, 1263, 1037 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.34 (m, 10H, Ph), 6.55 (s, $1H_{1a}$), 6.20 (s, $1H_{1b}$), 5.05 (m, 4H, benzylic protons), 4.40 (ddd, $J$ = 8.3, 6.4, 2.1 Hz, $1H_4$), 4.20 (m, $3H_{3,5a,5b}$), 1.43 (s, $3H_7$), 1.41 (s, $3H_8$); $^{13}C$ NMR (75 MHz, $CDCl_3$) 172.82 ($C_2$), 135.68 (d, $J$ = 7.0 Hz), 128.50, 128.45, 127.89 (Ph), 111.29 ($C_6$), 77.80 (d, $J$ = 7.7 Hz, $C_4$), 75.08 ($C_3$), 69.31 (benzylic carbons), 66.73 (d, $J$ = 5.30 Hz, $C_5$), 26.77 ($C_7$), 26.02 ($C_8$); $^{31}P$ NMR (121 MHz, $CDCl_3$) δ -0.47; HRMS(CI) calcd. for $C_{21}H_{27}O_7PN$ 436.1525 found 436.1519.

**(*3S*, *4R*)-4,5-dihydroxypentanamide-5-phosphate (DXP carboxamide, 12).**



DXP carboxamide (13)

To a solution of the dibenzylphosphate **74** (53 mg, 0.12 mmol) in 4 mL of EtOH was added 10 mg of 10 % Pd/C. The reaction mixture was stirred for 4 h at room temperature under a hydrogen atmosphere (1 atm). The reaction mixture was filtered over Celite and washed with 10 mL of EtOH. After concentration, 5.0 mL of MeOH and 250 mg of Dowex50x8($H^+$) were added. The reaction mixture was stirred for an additional 12 h, and the reaction mixture was filtered over Celite and washed with 10 mL of MeOH to give 26 mg (77%) of a light yellow oil.

$[\alpha]^{25}_D$ -26° (*c* 2.0, $H_2O$): measured as sodium salt; $^1H$ NMR (300 MHz, $D_2O$) δ 4.28 (d, $J$ = 2.1 Hz, $1H_3$), 4.19 (t, $J$ = 6.8, 2.0 Hz, $1H_4$), 4.00 (m, $2H_{5a,5b}$); $^{13}C$ NMR (75 MHz, $D_2O$/MeOH, 100:1) 178.75 ($C_3$), 71.80 ($C_3$), 71.34 (d, $J$ = 8.3 Hz, $C_4$), 67.18 (d, $J$ = 4.01 Hz, $C_5$); $^{31}P$ NMR (121 MHz, $H_2O$) δ 0.62; HRMS(-FAB) calcd. for $C_4H_9O_7PN$ 214.0117 found 214.0129.

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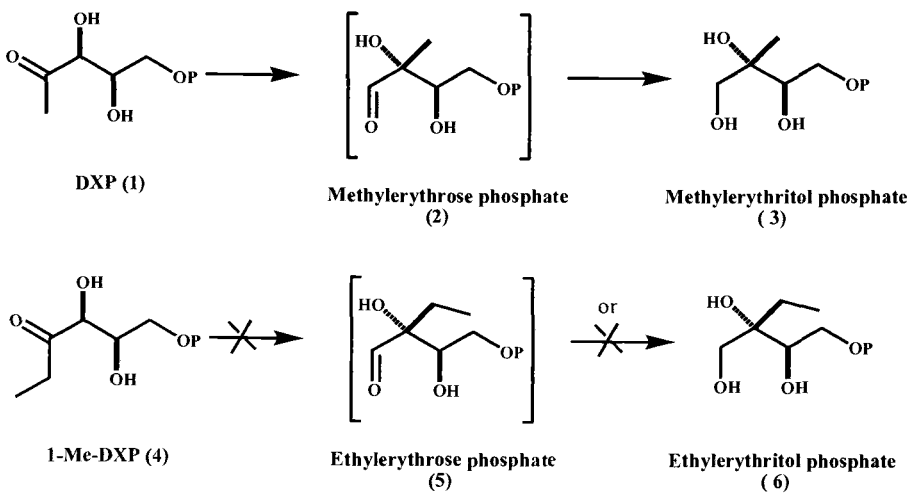
# CHAPTER THREE

## ENZYMATIC STUDIES OF 1-METHYL-DXP

### 1) Introduction

The results from the kinetic studies of the DXP analogs mentioned in Chapter 2 provide further insight into the DXR binding pocket. The results with 1-Me-DXP (**4**) provided evidence about the steric requirements for the methyl ketone binding pocket. Based on the competitive nature of the inhibition, it can be concluded that 1-Me-DXP binds at the same site as the substrate, DXP. One may assume from these results that there is a steric hindrance at the methyl ketone binding pocket of DXR which leads to an improper position of 1-Me-DXP for catalysis. However, there is another possible explanation for the lack of activity. The DXR reaction consists of two steps which are the rearrangement of 1-Me-DXP to generate the corresponding aldehyde (**5**) and the reduction to form ethylerythritol phosphate (**6**) as proposed in Scheme 1. If there is steric hindrance at the $C_2$ position of the aldehyde intermediate (**5**) rather than at the methyl ketone binding pocket of DXR, the corresponding aldehyde (**5**) may not be generated by DXR. Unfortunately the DXR assay only monitors the oxidation of NADPH in the reduction step,[1] preventing any conclusions about the isomerization step.




**Scheme 3.1. The DXR mediated reaction of DXP and proposed DXR mediated reaction of 1-Me-DXP.**

In order to further pursue the molecular basis for 1-Me-DXP inhibition, a collaborative project was conducted in our lab with Ms. Roberta Fernandes by using information from the available X-ray crystal structures of DXR. At the starting point of this project, there were two X-ray crystal structures of DXR from *E.coli* reported. The first DXR crystal structure without cofactors or substrate (apo form) was reported by Reuter *et al.*[2] This structure revealed that the enzyme is a homodimer. Each monomer consists of an amino terminal dinucleotide binding domain, a connective domain responsible for dimerization and harboring most of the active site, and a carboxyl-terminal four-helix bundle domain. The conserved acidic residues are clustered at the putative active site and might be involved in the binding of divalent cations for enzyme activity. There also is a flexible loop spanning residues 186 to 216 proposed to be involved in an induced fit during substrate binding. This flexible loop most likely folded over the active site upon substrate binding and shields the reactants from the aqueous environment. Based on the first X-ray crystal structure, the flexible loop of DXR was proposed to be a very important part for DXR activity.

The second X-ray crystal structure of DXR from *E.coli* was published by Yajima *et al.*[3] shortly after the first structure appeared. The second structure was of DXR complexed with NADPH and a sulfate ion. The sulfate ion in the crystal presumably represented where the phosphate group of DXP binds to the DXR protein. The sulfate ion was fixed through a hydrogen bond with the conserved residue His209 in the flexible loop. The hydrogen bonding between His209 and the phosphate function may act to close the hatch and fix the substrate effectively for DXR activity. Besides the hydrogen bond from His209, the flexible loop also provided hydrophobic conditions with Trp212 and Met214. These conditions were proposed to be preferable for DXR to interact with the hydrophobic backbone of a substrate DXP.[2] In conclusion, the second X-ray crystal structure of DXR also suggested that the flexible loop covering the substrate binding sites play a crucial role in the enzymatic reaction and the determination of substrate specificity.

In order to gain knowledge about the differences in amino acid sidechain interactions between DXP and 1-Me-DXP, the data from the X-ray crystal structure of *E.coli* DXR was used for further investigations. By using the data from the X-ray crystal structure of *E.coli* DXR from Yajima *et al.*[3], DXP (**1**) and the aldehyde intermediate, 2-*C*-methylerythrose-4-phosphate (**2**) were positioned at the active site of DXR relative to NADPH by using the ViewerPro® molecular visualization program.

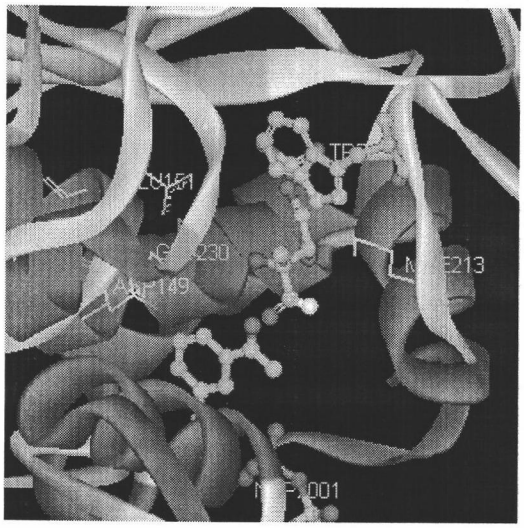


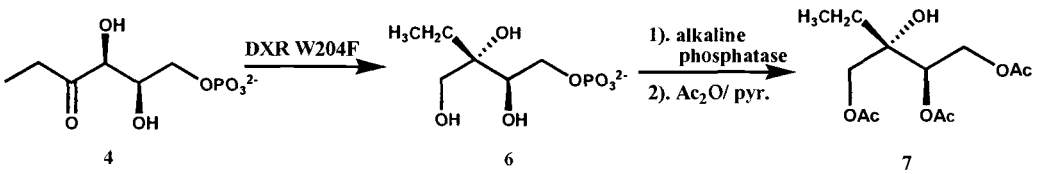
**Figure 3.1. The active site geometry of DXR complexed with 2-*C*-methyl erythrose-4-phosphate.**

When DXP was replaced with 1-Me-DXP, it did not appear that there were any major steric interactions with active site residues. However, when the 2-*C*-methylerythrose-4-phosphate intermediate was replaced with the corresponding 2-*C*-ethylerythrose-4-phosphate, a potential steric clash was identified. The indole ring of the amino acid residue Trp212 of *E.coli* DXR may be an amino acid in the flexible loop that prevents DXR from using 1-Me-DXP as the substrate. After DXP binds to DXR, the flexible loop presumably folds over to protect DXP from the environment. The indole ring of Trp212 shows close proximity to the methyl group at the C2 position of the aldehyde intermediate, methylerythrose phosphate (**2**). With an ethyl, rather than a methyl group in 1-Me-DXP (**4**) the rearrangement to form the corresponding aldehyde, ethylerythrose phosphate (**5**), could be blocked

because of the bulky Trp212. Therefore, mutation of Trp212 in *E.coli* DXR into a smaller amino acid residue such as phenylalanine or leucine may allow conversion of 1-Me-DXP (**4**) into the ethylerythrose phosphate (**5**) and then into the corresponding product ethylerythritol phosphate (**6**). If this mutant DXR can use 1-Me-DXP as the substrate, it would be strong evidence that Trp212 plays a key role in preventing the turnover of 1-Me-DXP. The Trp212 in *E.coli* DXR corresponds to Trp204 in *Synechocystis* sp. PCC6803 DXR. The DXR from the organism *Synechocystis* sp. PCC6803 has been used in our laboratory throughout the entire studies.

**2) Experimental Design**

In order to assess if the mutant DXR can convert 1-Me-DXP (**4**) to 2-*C*-ethylerythritol-4-phosphate (**6**), a product analysis will be necessary. A large scale incubation of the mutant DXR and 1-Me-DXP (**4**) should be conducted. In the next step, characterization of the incubation product would indicate whether the formation of 2-*C*-ethylerythritol-4-phosphate (**6**) occurs. However purification of the phosphate compound directly from the incubation mixture is often a very difficult procedure as mentioned in Chapter 2. In order to overcome this problem, the phosphate product from the incubation reaction can be dephosphorylated and derivatized to form the acetate derivative. The resulting acetate derivative can be readily purified by silica column chramatography. Finally, the acetate derivative can be compared to the corresponding acetate standard (**7**). Therefore, a synthesis of the ethylerythritol triacetate standard (7) is needed.

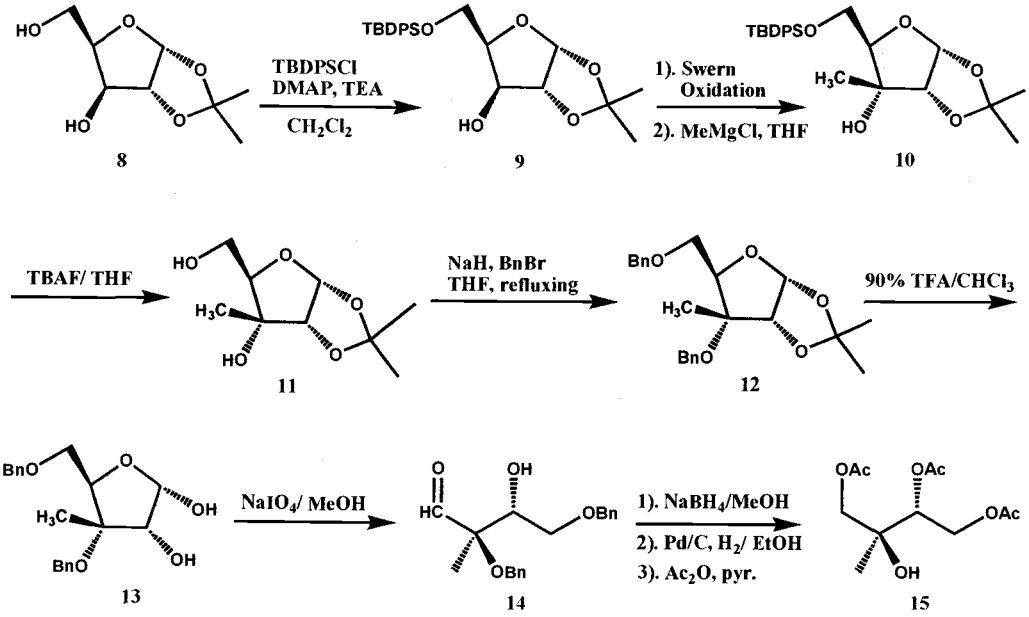



4 6 7

**Scheme 3.2. Conversion of 1-Me-DXP into ethylerythritol phosphate and ethylerythritol triacetate.**

### 3) Results

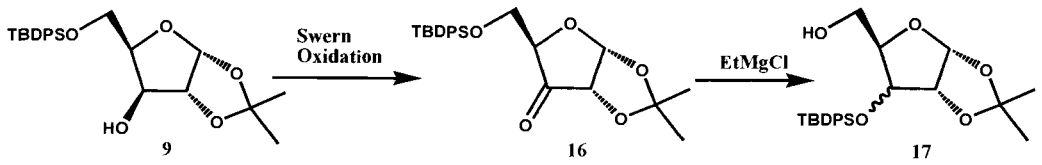
The synthesis of the ethylerythritol triacetate standard was similar to a procedure reported by Hoeffler *et al.* for methylerythritol triacetate (**15**) as shown in Scheme 3.[5] In this published procedure, the methyl group was introduced into the structure by using a methyl Grignard addition to obtain the desired configuration in **15**. To obtain the ethylerythritol triacetate, the same strategy can be followed. The ethyl group should be readily introduced by the ethyl Grignard addition to provide the ethylerythritol triacetate standard. Therefore, selective primary alcohol protection of the commercially available 1,2-*O*-isopropylidene-D-xylofuranose (**8**) was conducted with *tert*-butyldiphenylsilyl chloride.




**Scheme 3.3. The synthesis of 2-*C*-methylerythritol triacetate Hoeffler *et al.*[5]**

The TBDPS protection alcohol (**9**) was obtained in 96% yield and ready for the next reaction step. Unfortunately, the bulky TBDPS group hindered the approach of the ethyl Grignard reagent in the next step. Although most ketones react well with most Grignard reagents, there are several types of side reactions that can occur mostly with hindered ketones and bulky Grignard reagents.[6] By having a β hydrogen, the ethyl Grignard reagent can predominantly cause a reduction of the

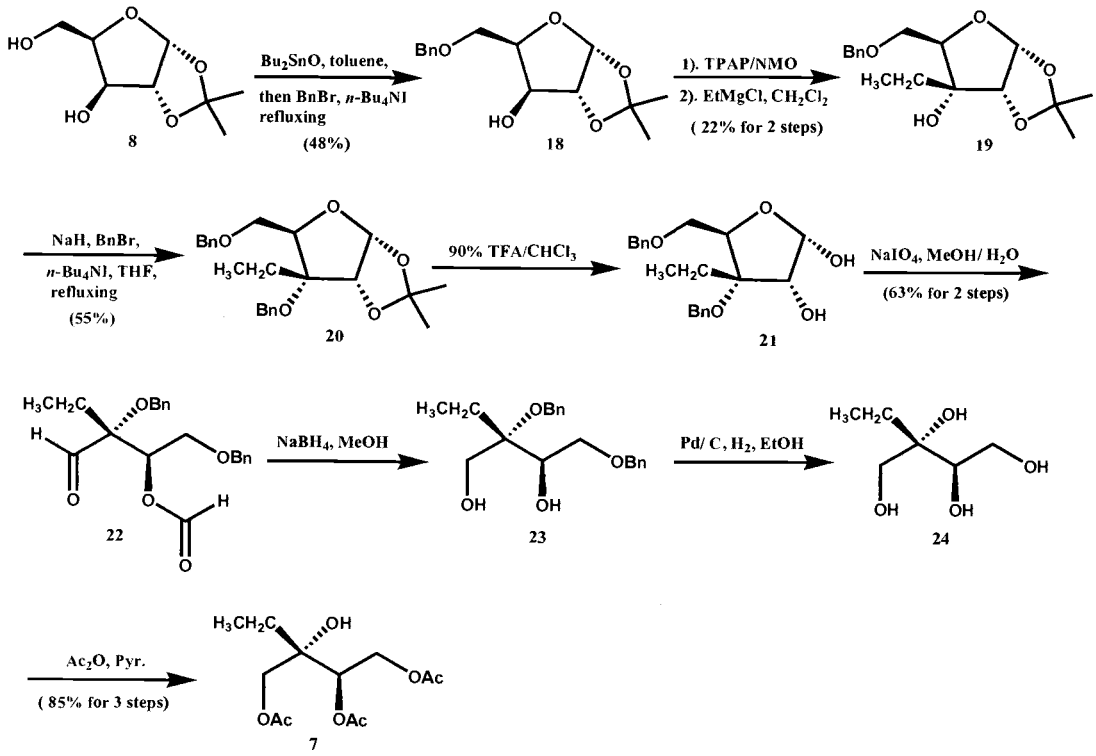
ketone. In this case, the TBDPS group was also transferred from the primary alcohol to the secondary alcohol and the undesired product **17** was obtained.




9 16 17

**Scheme 3.4. The undesired reaction from the ethyl Grignard addition.**

Because the TBDPS group prevented transfer of the ethyl group to the ketone, smaller protecting groups were tried to protect the primary alcohol of **8**. The triisopropylsilyl group and *tert*-butyldimethylsilyl group still caused the same problem. However, a benzyl group proved to be an appropriate protecting group for **8**. As shown in Scheme 5, the alcohol **18** was obtained by Stannyl ester activation and subsequent benzylation of the commercially available 1,2-*O*-isopropylidene-D-xylofuranose (**8**).[7] Surprisingly, a general procedure for benzylation (NaH/BnBr) caused the benzylation of the secondary alcohol in **8** rather than the primary alcohol. In the next step, the TPAP oxidation of **18** followed by ethyl Grignard addition to the resulting ketone gave **19** as a single diastereomer with the desired configuration.[8] Using the less hindered benzyl protecting group still did not completely avoid the reduction of the ketone, but the alcohol **19** could be obtained in a low yield and was able to be carried on to the next step. Benzylation of the tertiary alcohol **19** was performed under NaH/BnBr conditions with refluxing.[9] The acetonide protecting group was removed under acidic conditions and an anomeric mixture of hemiacetal **21** was obtained. The resulting was oxidatively cleavage with sodium metaperiodate into the aldehyde **22**. Note that the basic aqueous work up with saturated $Na_2CO_3$ of **22** did not cleave the formate ester. Reduction of **22** with sodium borohydride provided the dibenzylated ethylerythritol **23**. Ethylerythritol **24** was obtained by catalytic hydrogenation to remove the benzyl groups. Finally, ethylerythritol triacetate **7** was generated after acetylation of **24** with acetic anhydride and pyridine.




**Scheme 3.5. Synthesis of Ethylerythritol triacetate.**

The mutant DXR, W204F of *Synechocystis* sp. PCC6803 was prepared by Roberta Fernandes and the compound 1-Me-DXP (**4**) was incubated with the mutant DXR under standard assay conditions.[4] Although the reaction rate was slower than for native DXR with DXP, oxidation of NADPH was clearly observed. The enzymatic reaction mixture was dephosphorylated with alkaline phosphatase and the proteins were precipitated with ethanol. The resulting supernatant was concentrated and treated with acetic anhydride and pyridine. The ethylerythritol triacetate product was purified by flash chromatography (50% ethyl acetates/hexanes) and was compared by GC/MS and $^1$H NMR spectroscopy with an authentic standard.

## 4) Results

Both the authentic triacetate standard and the product from the enzymatic reaction were analyzed by GC/MS. The authentic ethylerythritol triacetate eluted at an $R_t$ of 26.0 min; the triacetate derivative from the enzymatic reaction eluted at an $R_t$ of 26.2 min. A co-injection of both samples showed a higher intensity of the peak eluting at an $R_t$ of 26.0 min, confirming that the enzymatic product was the expected triacetate.

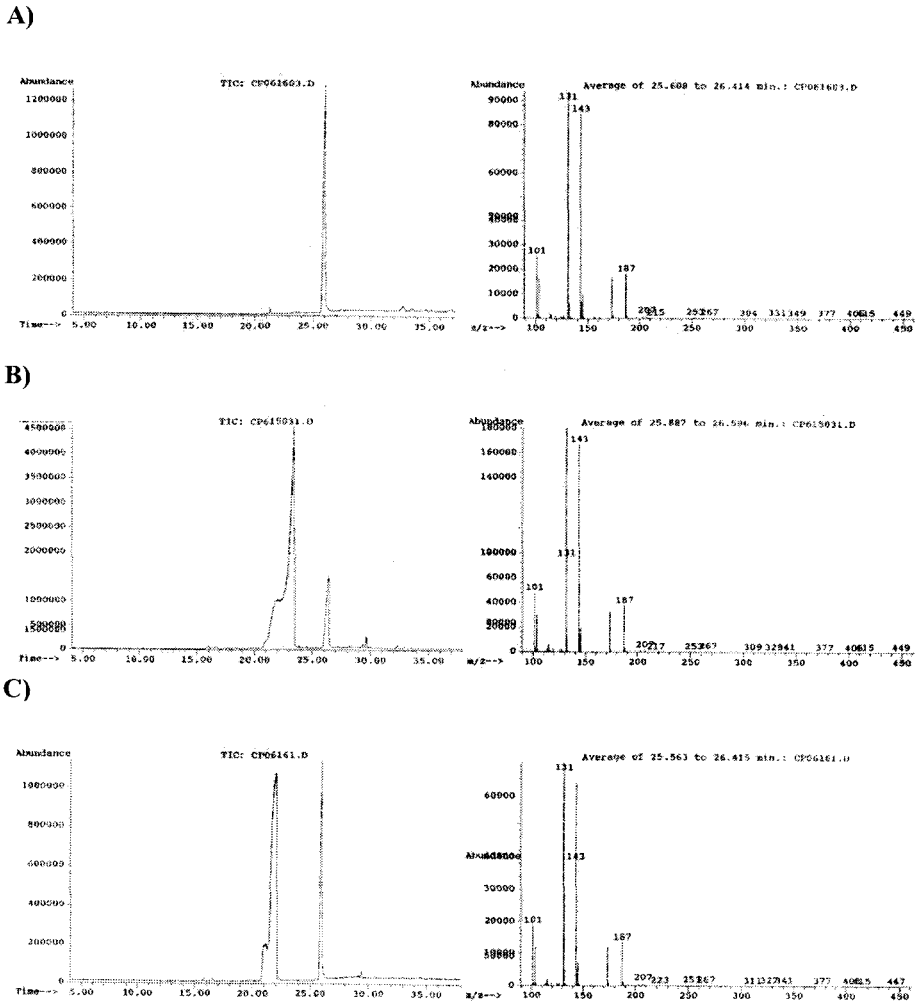



**Figure 3.2:** GC/MS analysis of A) Synthetic ethylerythritol triacetate. B) The putative ethylerythritol triacetate derivative from the enzymatic reaction. C) Co-injection of synthetic and enzymatically prepared triacetates. (The large peak at 21-23 min is an impurity from the acetylation procedure).

For the MS analysis of both samples, the patterns of mass cleavage were clearly identical, even though the molecular peak of the ethylerythritol triacetate ($C_{12}H_{20}O_7$ : MW 276) could not be detected. Mass fragmentation of the peak eluting at an $R_t$ 26.0 min showed ions m/z at 131 and 143 as dominant peaks including smaller peaks at 101 and 187. Partial fragmentation of ethylerythritol triacetate can be predicted as shown in Figure 3.3. Some ions may be obtained by McLafferty rearrangement and bond cleavage next to C=O. However, others ions require additional carbon-carbon cleavage to generate the peaks that appeared in the mass cleavage spectrum. Data from the GC/MS analysis fully support the formation of the ethylerythritol phosphate from the enzymatic reaction of 1-Me-DXP with the mutant DXR, W204F.

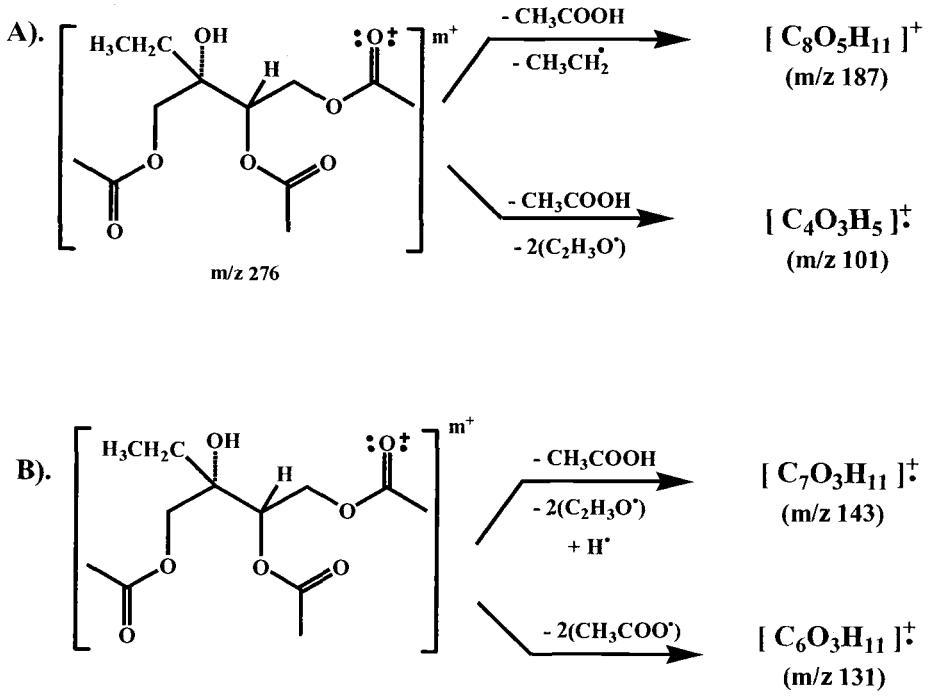



**Figure 3.3. The predicted mass fragmentation patterns of ethylerythritol triacetate.**

After the small scale incubation of 1-Me-DXP and the mutant DXR was conducted and the GC/MS analysis confirmed the formation of ethylerythritol triacetate, a larger scale incubation was performed in order to obtain enough product for NMR analysis. After purification by silica chromatography, the triacetate derivative from the incubation reaction showed an identical $^{1}H$ NMR spectrum with the standard ethylerythritol triacetate (Fig. 3.4).

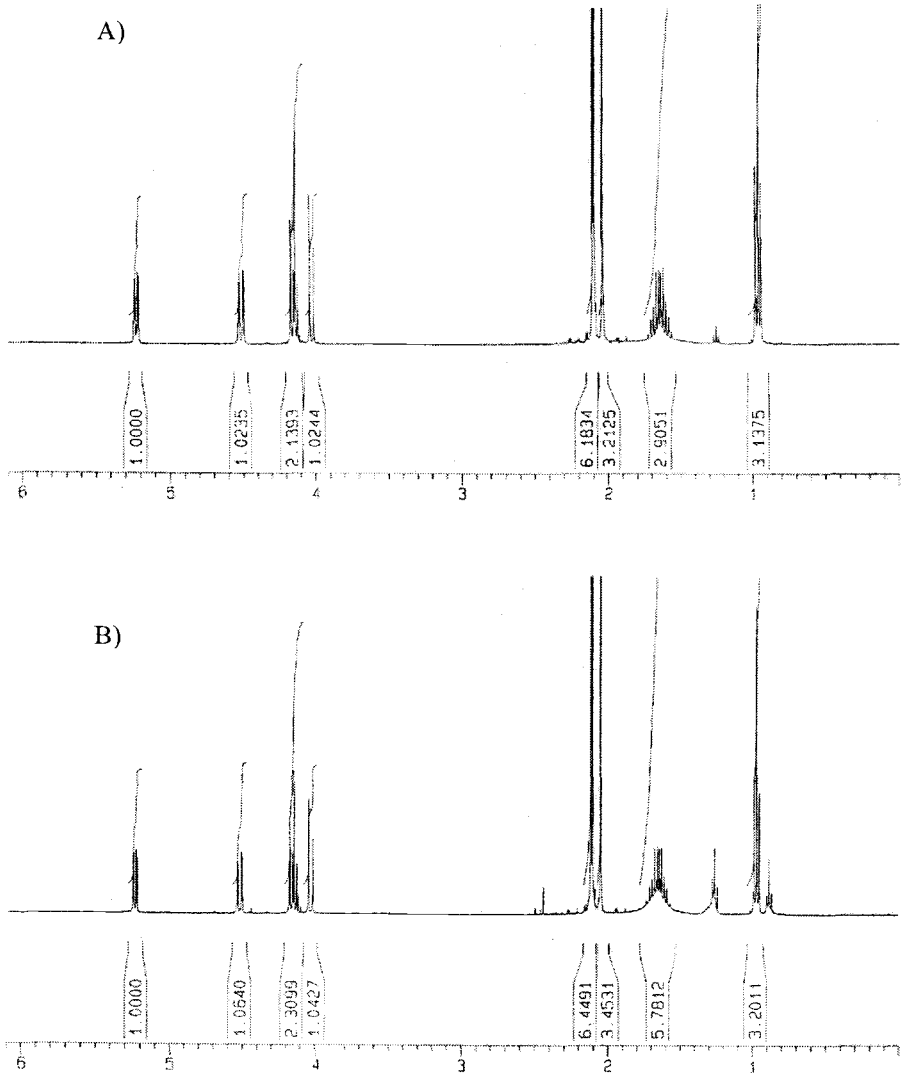
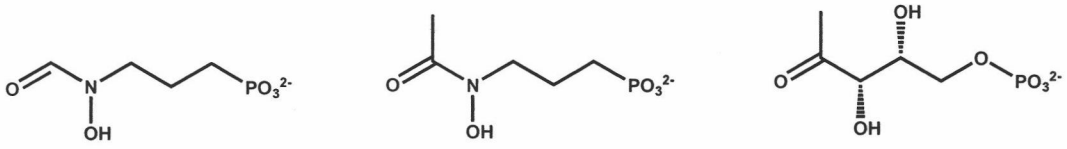



**Figure 3.4:** $^{1}H$ NMR spectrum of ethylerythritol triacetates A) Synthetic Standard B) The product from the enzymatic reaction.

**4) Discussion**

The amino acid residue Trp212 from *E.coli* DXR or Trp204 from *Synechocystis* sp. PCC6803 is highly conserved in DXRs from various organisms and appears to be an amino acid residue that plays an important role for substrate specificity. The native DXR fails to use 1-Me-DXP, an analog of DXP with an ethyl ketone moiety, as a substrate. When the Trp212 was mutated into a phenylalanine residue, it was able to convert 1-Me-DXP into the corresponding product. Moreover, Trp212 does not appear to block 1-Me-DXP binding, but interferes with the rearrangement of 1-Me-DXP into the corresponding intermediate, ethylerythrose phosphate. This result is consistent with data from the latest X-ray crystal structure of *E.coli* DXR complexed with the inhibitor fosmidomycin (**25**) and $Mn^{2+}$ from Steinbacher *et al.*[10] The data suggested that the amino acid residue Trp212 is a residue that has strong interaction with the methyl group on DXP. This assumption also explained the increased affinity of FR-900098 (**26**) compared to fosmidomycin (**25**).




**Fosmidomycin (25)** **FR-900098 (26)** **1-Deoxyxylulose 5-phosphate (1)**

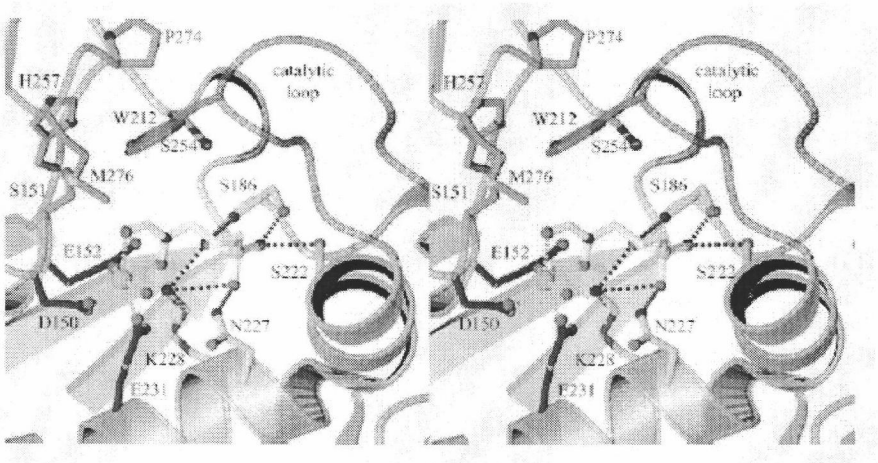
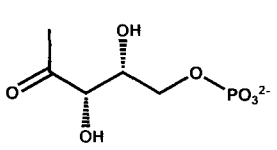


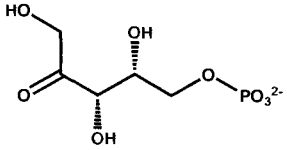

**Figure 3.5. Stereoview of the active site geometry of DXR complexed with fosmidomycin by Steinbacher *et al.*[10]**

As previously mentioned, the replacement of the formyl-hydrogen of fosmidomycin by a methyl group yielded the inhibitor FR-900098 which has a lower $IC_{50}$ value or an increased affinity for DXR.[11,12] The methyl group of FR-900098 was predicted to have a closer distance to the side chain of Trp212. Therefore, the methyl would have more interaction with Trp212 when compared to the hydrogen. The same study also proposed that the methyl group in FR900098 corresponds to the methyl group in the substrate 1-deoxyxylulose 5-phosphate (DXP, **1**). The crystal structure data, however, need to be considered carefully because the structure does not have NADPH bound to it. By not having NADPH in the crystal structure, fosmidomycin might not be optically bound at the active site of DXR. Therefore, these important details will change the overall picture of the DXR active site. It is known that NADPH binds first to DXR,[13] so the structure of fosmidomycin bound alone may not exactly reflect the binding mode of DXP.

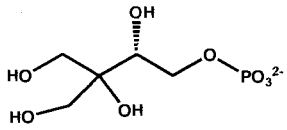
In conclusion, the Trp212 residue plays a key role in controlling the specificity of DXR toward DXP but not 1-Me-DXP. Furthermore, the Trp212 residue may also control the specificity of DXR toward DXP rather than the structurally related natural compound, xylulose-5-phosphate (X-5-P, **27**). This phosphorylated sugar is known to be an intermediate in the pentose phosphate pathway which provides ribose-5-phosphate for nucleotide and nucleic acid biosynthesis. Because the extra methyl group of 1-Me-DXP and the $C_1$ hydroxyl of X-5-P (**27**) are similar in size, the Trp212 residue may also block the turnover of X-5-P **(27)** by DXR.




**1-Deoxyxylulose 5-phosphate (1)**




**Xylulose 5-phosphate (27)**




**2-Hydroxymethylerythritol-4-phosphate (28)**

However, a mutation of DXR at Trp212 may occur in the some organisms. In this case, xylulose-5-phosphate (X-5-P, **27**) may be turned over by DXR to form the corresponding product, 2-*C*-hydroxy-methylerythritol phosphate (**28**). Recently,

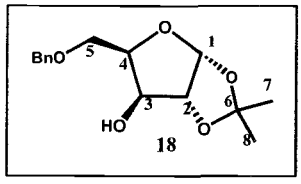
2-*C*-hydroxylmethylerythritol itself was isolated from *Torillis japonica D.C.* fruit.[14] This natural product may result from turnover of xylulose-5-phosphate (X-5-P, **27**) by a mutant DXR. Although no sequence data is available from this plant, it would be interesting to find out whether or not *Torillis japonica D.C.* has a DXR that lacks Trp at the position corresponding to Trp212.

**5) Experimental Section**

**General Methods:** Materials and general methods are listed in Chapter Two (see page 59).

**Ethylerythritol Triacetate Synthesis:**

**1,2-*O*-Isopropylidene-5-benzyloxy-α-D-xylofuranose (18) (Registry No. 155345-99-6).**

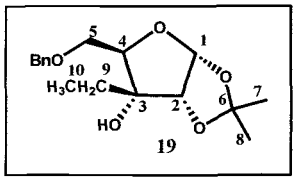



To a solution of 1,2-*O*-isopropylidene-α-D-xylofuranose **8** (500 mg, 2.63 mmol, 1.0 equivalent) in 30 mL of toluene was added $Bu_2SnO$ (690 mg, 2.76 mmol, 1.05 equivalents). The reaction mixture was heated to reflux with azeotropic removal of water. After being refluxed for 12 h, the reaction flask was cooled to room temperature, and BnBr (470 μL, 3.95 mmol, 1.5 equivalents) and 10 mg of *n*-$Bu_4NI$ (catalytic amount) were added slowly to the mixture. The reaction mixture was heated to reflux for an additional 12 h, cooled to room temperature and quenched with 10 mL of water. The mixture was extracted with ethyl acetate (3×10 mL). The combined organic layers were washed with saturated $NaHCO_3$ (2×10 mL) and saturated NaCl (2×10 mL). The organic layer was dried and concentrated. Purification by flash chromatography (1:1 (v/v) ethyl acetate/hexanes) gave 350 mg (48%) of a colorless oil.

$[\alpha]^{25}_D$ -15° (*c* 0.1, $CH_2Cl_2$); IR (thin film) 3447 (br), 2979, 2926, 2888, 1451, 1379, 1248, 1209, 1165, 1078 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.33 (m, 5H, Ph), 5.97 (d, 1H, $J$ = 4.0 Hz, $H_1$), 4.65 (d, 1H, $J$ = 11.9 Hz, benzylic proton), 4.57 (d, 1H, $J$ = 11.9 Hz, benzylic proton), 4.51 (d, $J$ = 3.7 Hz, $1H_3$), 4.28 (d, $J$ = 2.6 Hz, $1H_4$), 4.24

(dd, 1H, $J$ = 6.5, 3.8 Hz, $H_2$), 3.94 (t, 2H, $J$ = 3.0 Hz, $H_{5a,5b}$), 3.60 (s, 1H, hydroxyl proton), 1.48 (s, $3H_7$), 1.32 (s, $3H_8$); $^{13}C$ NMR (75 MHz, $CDCl_3$) 137.03, 128.57, 128.07, 127.88 (Ph), 111.57 ($C_6$), 104.85 ($C_1$), 85.35 ($C_2$), 77.99 ($C_4$), 76.68 ($C_3$), 74.16 ($C_5$), 68.24 (benzylic carbon), 26.74 ($C_7$), 26.15 ($C_8$); HRMS(CI) calcd. for $C_{15}H_{20}O_5$ 280.1311 found 280.1310.

**1,2-*O*-Isopropylidene-3-*C*-ethyl-5-benzyloxy-α-D-ribofuranose (19).**

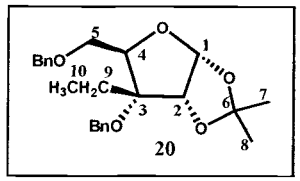



To a solution of the alcohol **18** (350 mg, 1.25 mmol, 1 equivalent) in 10 mL of $CH_2Cl_2$ were added NMO (175 mg, 1.50 mmol, 1.2 equivalents), 300 mg of finely crushed and activated 4 Å molecular sieves, and TPAP (40 mg, 0.06 mmol, 0.10 equivalents). The reaction mixture was stirred for 2 h, filtered over Florisil and washed with 30 mL of $CH_2Cl_2$. The filtrate was dried and concentrated to give the crude ketone product as a yellow. The crude product was dissolved in 20 mL of THF and 820 μL of ethylmagnesium chloride solution (2.0 M in THF, 1.63 mmol, 1.3 equivalents) was added at -78 °C. The reaction mixture was stirred at -78 °C for 12 h, warmed to room temperature, and stirred for an additional 12 h. The reaction mixture was quenched with 5 mL of saturated $NH_4Cl$. The aqueous layer was washed with diethylether (3×10 mL), and the combined organic layers were dried and concentrated. The residue was purified by flash chromatography (1:1 (v/v) ethyl acetate/hexanes) to give 85 mg (22% for 2 steps) of a colorless oil.

$[\alpha]^{25}_D$ -11° (*c* 0.05, $CH_2Cl_2$); IR (thin film) 3453 (br), 2984, 2831, 2868, 1775, 1726, 1451, 1373, 1257, 1214, 1461, 1083 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.33 (m, 5H, Ph), 5.78 (d, 1H, $J$ = 4.0 Hz, $H_1$), 4.63 (d, 1H, $J$ = 12.1 Hz, benzylic proton), 4.52 (d, 1H, $J$ = 12.1 Hz, benzylic proton), 4.28 (d, 1H, $J$ = 3.9 Hz, $H_2$), 4.03 (dd, 1H, $J$ = 7.6, 3.2 Hz, $H_4$), 3.68 (dd, 1H, $J$ = 10.5, 3.2 Hz, $H_{5a}$), 3.56 (dd, 1H, $J$ = 10.5, 7.6 Hz, $H_{5b}$), 1.58 (s, $3H_7$), 1.50 (m, $2H_9$), 1.36 (s, $3H_8$), 0.97 (t, 3H, $J$ = 7.4 Hz, $H_{10}$); $^{13}C$ NMR (75 MHz, $CDCl_3$) 137.86, 128.30, 127.74, 127.59 (Ph), 112.26 ($C_6$), 103.82 ($C_1$), 81.84 ($C_2$), 80.07 ($C_4$), 78.89 ($C_3$), 73.45 ($C_5$), 68.04 (benzylic carbon),

26.49 ($C_{7,8}$), 23.34 ($C_9$), 7.19 ($C_{10}$); HRMS(CI) calcd. for $C_{17}H_{24}O_5$ 308.1624 found 308.1622.

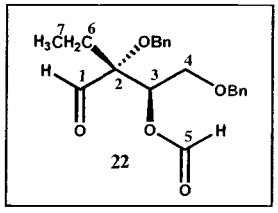
**3-*O*-Benzyl-1,2-*O*-isopropylidene-3-*C*-ethyl-5-benzyloxy-α-D-ribofuranose (20).**




To a solution of the alcohol **19** (85 mg, 0.28 mmol, 1.0 equivalent) in 12 mL of THF was added NaH (60% in mineral oil; 15 mg, 0.36 mmol, 1.3 equivalents) at 0 °C in one portion. The slurry was warmed to room temperature and stirred for 10 min before BnBr (50 μL, 1.3 mmol, 1.3 equivalents) was added. After 10 min, 10 mg of *n*-$Bu_4NI$ (catalytic amount) was added. The mixture was heated to reflux for 12 h. The reaction mixture was quenched by adding 5 mL of water and extracted with diethyl ether (3×10 mL). The combined organic layers were dried and concentrated. Purification by flash chromatography (1:4 (v/v) ethyl acetate/hexanes) gave 60 mg (55%) of a colorless oil.

$[\alpha]^{25}_D$ +21 ° (*c* 0.07, $CH_2Cl_2$); IR (thin film) 3027, 2921, 2883, 1494, 1451, 1373, 1306, 1248, 1209, 1165, 1003 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.33 (m, 10H, Ph), 5.79 (d, 1H, $J$ = 3.8 Hz, $H_1$), 4.61 (m, 4H, benzylic protons), 4.46 (dd, 1H, $J$ = 7.8, 2.8 Hz, $H_4$), 4.43 (d, 1H, $J$ = 2.7 Hz, $H_2$), 3.76 (dd, 1H, $J$ = 10.6, 2.9 Hz, $H_{5a}$), 3.65 (dd, 1H, $J$ = 10.5, 7.9 Hz, $H_{5b}$), 1.80 (m, $1H_{9a}$), 1.64 (s, $3H_7$), 1.60 (m, $1H_{9b}$), 1.38 (s, $3H_8$), 1.01 (t, 3H, $J$ = 7.4 Hz, $H_{10}$); $^{13}C$ NMR (75 MHz, $CDCl_3$) 138.74, 138.06, 128.23, 128.13, 127.65, 127.43, 127.31 (Ph), 112.48 ($C_6$), 103.96 ($C_1$), 83.84 ($C_3$), 82.58 ($C_2$), 80.58 ($C_4$), 73.31 ($C_5$), 68.77 (benzylic carbon), 66.52 (benzylic carbon), 26.85 ($C_7$), 26.59 ($C_8$), 23.13 ($C_9$), 7.85 ($C_{10}$); HRMS(CI) calcd. for $C_{24}H_{30}O_5$ 398.2093 found 398.2088.

**2-*O*-Benzyl-2-*C*-ethyl-3-*O*-formyl-4-benzyloxy-D-erythrose (22).**

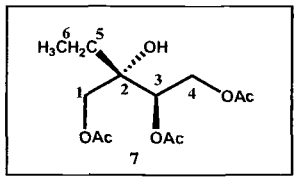



To a solution of **20** (60 mg, 0.15 mmol) in 3 mL of $CHCl_3$ was added 90% of TFA in water (1 mL) at 0 °C. After being stirred at 0 °C for 30 min, the reaction mixture was diluted with 5 mL of $CHCl_3$ and extracted with 2×10 mL

of $CHCl_3$. The combined extracts were dried and concentrated. The crude product was dissolved in 3 mL of MeOH and 1 mL of water before sodium periodate (50 mg, 0.23 mmol, 1.5 equivalents) was added at 0 °C. After being stirred at 0 °C for 30 min, the reaction mixture was diluted with 5 mL of $CHCl_3$ and neutralized with saturated $Na_2CO_3$. The reaction mixture was extracted with 2×10 mL of $CHCl_3$. The combined organic layers were dried and concentrated. Purification by flash chromatography (1:4 (v/v) ethyl acetate/hexanes) gave 34 mg (63% for 2 steps) of a colorless oil.

$[\alpha]^{25}_D$ -11° (*c* 0.1, $CH_2Cl_2$); IR (thin film) 2979, 2931, 2873, 1726, 1499, 1451, 1368, 1161cm$^{-1}$; $^1$H NMR (300 MHz, $CDCl_3$) δ 9.66 (s, $1H_1$), 8.18 (s, $1H_5$), 7.36 (m, 10H, Ph), 5.58 (t, 1H, $J$ = 6.4 Hz, $H_3$), 4.70 (d, 1H, $J$ = 11.2 Hz, benzylic proton), 4.59 (d, 1H, $J$ = 11.1 Hz, benzylic proton), 3.65 (m, $2H_{4a,4b}$), 1.95 (m, $2H_6$), 0.93 (t, 3H, $J$ = 7.4 Hz, $H_7$); $^{13}$C NMR (75 MHz, $CDCl_3$) 202.96 ($C_1$), 159.91 ($C_5$), 137.74, 137.25, 128.43, 128.40, 127.78, 127.72, 127.66, 127.26 (Ph), 84.67 ($C_2$), 74.41 ($C_3$), 73.11 ($C_4$), 67.44 (benzylic carbon), 65.46 (benzylic carbon), 22.58 ($C_6$), 6.94 ($C_7$); LRMS(CI) calcd. for $C_{21}H_{25}O_5$ 356.2 found 356.2.

**2-*C*-Ethylerythritol triacetate (7).**



7

To a solution of the aldehyde **22** (34 mg, 0.1 mmol, 1 equivalent) in 4 mL of MeOH was added $NaBH_4$ (45 mg, 0.12 mmol, 1.2 equivalents) at 0 °C in one portion. After being stirred for 1 h, the reaction was quenched by adding HCl (1.0 M, 0.5 mL) and diluted with 5 mL of $CHCl_3$. The reaction mixture was extracted with 2×5 mL of $CHCl_3$. The combined organic layers were dried and concentrated. Without further purification, the crude reaction product was dissolved in 3 mL of EtOH and 5 mg of 10% Pd/C was added. The reaction mixture was stirred at room temperature under a hydrogen atmosphere (1 atm) for 12 h. In the next step, the reaction mixture was filtered over Celite and washed with 10 mL of EtOH. The crude product was acetylated with $Ac_2O$/Pyr (1:1 mL) at room

temperature for 10 h before concentration. Purification by flash chromatography (1:1 (v/v) ethyl acetate/hexanes) gave 22 mg (85% for 3 steps) of a colorless oil. $[\alpha]^{25}_{D}$ +19° (*c* 2.0, $CH_2Cl_2$); IR (thin film) 3488 (br), 2979, 2940, 2892, 1735, 1437, 1374, 1224, 1055 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 5.22 (dd, *J* = 8.2, 2.8 Hz, $1H_3$), 4.50 (dd, *J* = 12.0, 2.7 Hz, $1H_{4a}$), 4.41 (m, $2H_{1a,4b}$), 4.01 (d, *J* = 11.8 Hz, $1H_{1a}$), 2.44 (s, 1H, hydroxyl proton), 2.09 (s, 3H, acetyl $CH_3$), 2.08 (s, 3H, acetyl $CH_3$), 2.03 (s, 3H, acetyl $CH_3$), 1.63 (m, $2H_5$), 0.95 (t, 3H, *J* = 7.5 Hz, $H_6$); $^{13}C$ NMR (75 MHz, $CDCl_3$) 170.97, 170.76, 170.17 (3×CO of acetyl groups), 74.04 ($C_2$), 72.95 ($C_3$), 65.76 ($C_1$), 62.72 ($C_2$), 26.72 ($C_5$), 20.87, 20.75 (3×$CH_3$ of acetyl groups), 7.06 ($C_6$); HRMS(CI) calcd. for $C_{12}H_{20}O_7$ 277.1287 found 277.1293.

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# CHAPTER FOUR

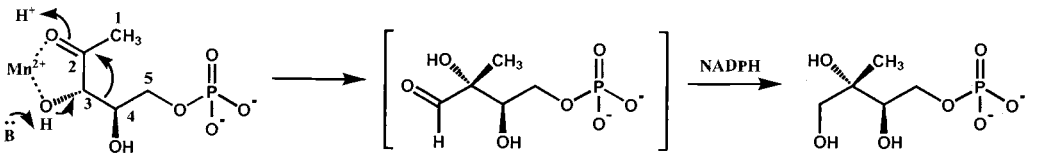
## MECHANISMS FOR THE REARRANGEMENT OF DXP TO MEP

### 1) Introduction

The enzyme DXR which mediates the rearrangement of DXP to MEP was classified as a class B dehydrogenase according to independent studies by Proteau *et al.*[1] and Arigoni *et al.*[2, 3] They had shown that, with the recombinant enzymes from *Synechocystis sp.* PCC6803 and *E. coli* , respectively, the C1 *pro-S* hydrogen in MEP derives from C3 of DXP which indicates that hydride attack occurs on the *re* face of the intermediate aldehyde and the 4*S*-hydride from NADPH is delivered to the intermediate aldehyde. Despite this knowledge about the stereochemistry of the reaction, the reaction mechanism itself has not clearly been defined.

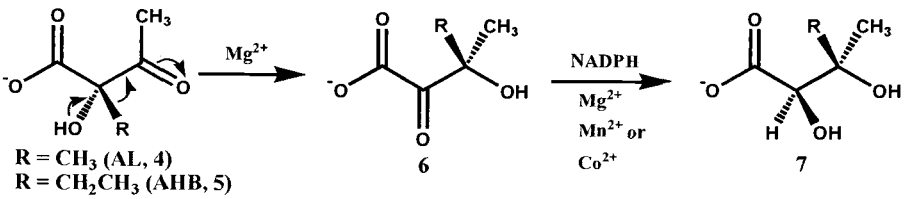
The evidence for a possible mechanism for the rearrangement of DXP to MEP was obtained from several earlier incorporation studies of isotope labeled compounds. A study by Arigoni *et al.* indicated that [1-$^{13}$C] and [2,3,4,5-$^{13}C_4$] -1-deoxy-D-xylulose can be incorporated into β-carotene, lutein, phytol, and sitosterol in a cell culture of *Catharanthus roseus*.[4] They showed that [2,3,4,5-$^{13}C_4$] -1-deoxy-D-xylulose is transformed into [1,2,3,4-$^{13}C_4$]IPP and [1,2,3,4-$^{13}C_4$]DMAPP. This exclusive formation of quadruple-labeled isotopomers from [2,3,4,5-$^{13}C_4$] -1-deoxy-D-xylulose proved that DXP should be transformed into the isoprenoid precursors, IPP and DMAPP, by a strictly intramolecular fragmentation and reassociation of the fragments.

Therefore, it was initially proposed by Rohmer *et al.* that MEP might be synthesized from DXP by an intramolecular rearrangement.[5] After that Kuzuyama *et al.* proposed the reaction mechanism to be as the ketol acid reductoisomerase (EC 1.1.1.86)[6] which is an α-ketol rearrangement. This mechanism involves the deprotonation of the hydroxyl group at C3 of DXP (**1**) followed by the migration of the C4-C5 phosphate-bearing subunit to the activated C2 position affording methylerythrose phosphate (**2**). In the next step, the resulting aldehyde is reduced by NADPH to form MEP (**3**).




**Scheme 4.1. An α-ketol rearrangement of DXP to MEP by DXR.**

The ketol acid reductoisomerase (KARI) also known as acetohydroxy acid isomeroreductase, is involved in the biosynthetic pathway of the amino acids isoleucine, valine, and leucine.[7] Like DXR, this enzyme has been found in bacteria and plants, but not in animals or humans, although it does also exist in fungi. The natural substrates of KARI are 2-acetolactate (AL), and 2-aceto-2-hydroxybutyrate (AHB). The reduction step also involves transfer of the *pro-S* hydrogen atom from NADPH.[8]




**Scheme 4.2. The reaction mediated by ketol acid reductoisomerase.**

While the KARI reaction happens in two stages, the formation of the expected intermediate (**6**) has never been shown directly. A study by Arfin *et al.* detected none of this intermediate and also showed that there was no equilibration of the intermediate with that added to the reaction mixture.[8] However, they did show that the synthetic intermediate was catalytically competent. These results suggested that the intermediate is tightly bound to the enzyme or that the reduction takes place during the alkyl transfer so that the intermediate is never really formed.[8] Site directed mutagenesis[9] and crystal structure determination[10] revealed two distinct metal ion sites in the enzyme. The reaction intermediate analogues 2-dimethylphosphinoyl-2-hydroxyacetate (Hoe 704)[11] and *N*-hydroxy-*N*-isopropyl-

oxamate (IpOHA)[12] can act as nearly irreversible inhibitors of KARI. These two compounds have attracted attention as potential herbicides and fungicides.

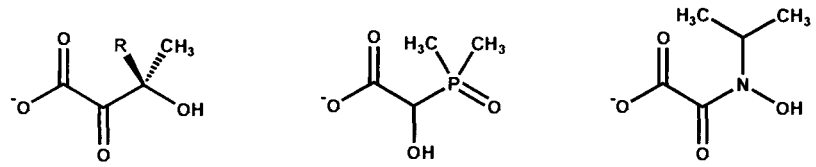



**Figure 4.1. Inhibitors of ketol acid reductoisomerase.**

The X-ray crystal structure of KARI complexed with its reaction product, $Mn^{2+}$ and (phospho)-ADP-ribose has been determined.[13] The active site of the enzyme sits at the interface between two domains of the dimer. The active site is very tightly packed, and the divalent cations interact closely with the product molecule.

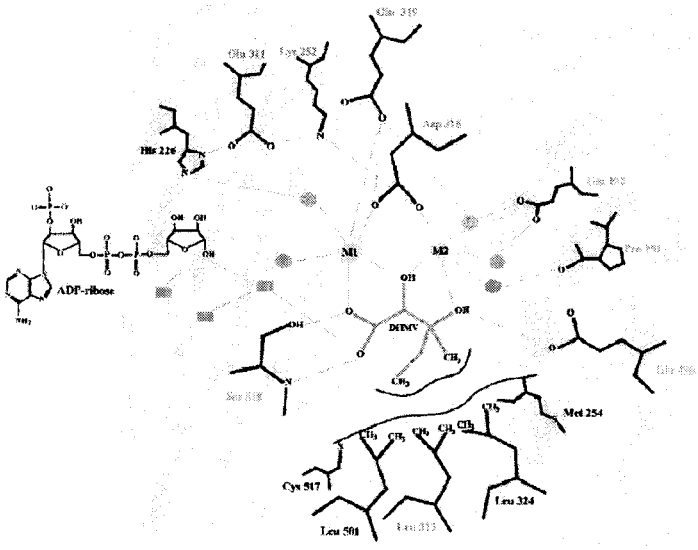



**Figure 4.2. Active site of KARI complexed with 2,3-dihydroxy-3-methylvalerate.**[13]

There are five different regions of KARI involving in ligand binding. Region I corresponds to a α-helix with a partial positive charge interacting with the negative charge of the diphosphate moiety of NADPH. In region II, His226 and Lys252 interact with cations Mn1 and Mn2 respectively. For region III, Glu319

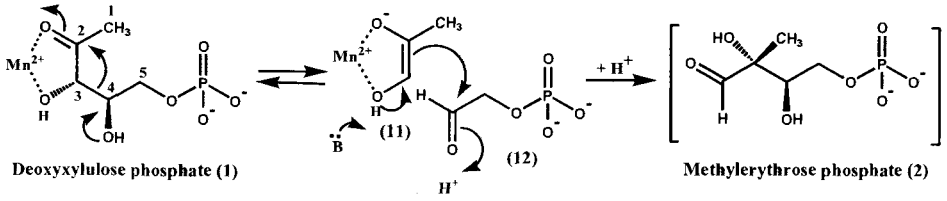
interacts with one cation (Mn1), whereas Asp315 bridges both cations. The amino acid residues, Glu492 and Glu496 from region IV interact with only one cation (Mn2). Furthermore, Glu496 interacts with the part of the substrate that is involved in the alkyl transfer. Finally, Ser518 from region V stabilizes the carboxylate moiety of the substrate via two hydrogen bonds. Kinetic studies showed that KARI obeys an ordered mechanism in which NADPH binds first and then $Mg^{2+}$ binds, followed by substrate binding.[14]

Even though DXR is proposed to catalyze a reaction that parallels that of KARI, there is little sequence similarity between these two enzymes except for the NADPH binding motif.[15] The pairwise sequence alignments of DXR and KARI from *E.coli* showed only 15% identity.

```
DXR             ----------MKQLTILGSTGSIGCSTLDVVRHNPEHFRVVALVAGKN----VTRMVEQC 46
KARI            MANYFNTLNLRQQLAQLGKCRFMGRDEFADGASYLQGKKVVIVGCGAQGLNQGLNMRDSG 60
                          :**: **.   :* . :       :  :** : .* :      .* :.

DXR             LEFS---PRYAVMDDEASAKLLKTMLQQQGSRTEVLSGQQAACDMAALEDVDQVMAAIV- 102
KARI            LDISYALRKEAIAEKRASWRKATENGFKVGTYEELIPQADLVINLTPDKQHSDVVRTVQP 120
                *::*    : *: :..** :  .    : *:  *::.  : . :::. :: .:*: ::

DXR             -----GAAGLLPTLAAIRAGKTILLANKESLVTCGRLFMDAVKQSK-----AQLLPVDSE 152
KARI            LMKDGAALGYSHGFNIVEVGEQIRKDITVVMVAPKCPGTEVREEYKRGFGVPTLIAVHPE 180
                     .* *    :  :..*: *    .  :*:      :. :: *     . *:.*..*

DXR             HNAIFQSLPQPIQHNLGYADLEQNGVVSILLTGSGGPFRETPLRDLATMTPDQACRHPNW 212
KARI            NDPKGEGMAIAKAWAAATGGHRAGVLESSFVAEVKSDLMGEQTILCGMLQAGSLLCFDKL 240
                ::.  :.:. .     . .. . . : * :::   . :        . : ...   . :

DXR             SMGRKISVDSATMMNKGLEYIEARWLFNASASQMEVLIHPQSVIHSMVRYQDGSVLAQLG 272
KARI            VEEGTDPAYAEKLIQFGWETITEALKQGGITLMMDRLSNPAKLRAYALSEQLKEIMAPLF 300
                    . .. : .::: * * *      .. :  *: * :* .:    :  *  .::* *

DXR             EPDMRTPIAHTMA------WPN--------RVNSGVKPLDFCKLSALTFAAPDYDRYPCL 318
KARI            QKHMDDIISGEFSSGMMADWANDDKKLLTWREETGKTAFETAPQYEGKIGEQEYFDKGVL 360
                : .*   *:  ::      *.*        * ::* ..:: .     .:.  :*     *

DXR             KLAMEAFEQGQAATTALN----------------AANEITVAAFLAQQIRFTDIAALN 360
KARI            MIAMVKAGVELAFETMVDSGIIEESAYYESLHELPLIANTIARKRLYEMNVVISDTAEYG 420
                 :**       *  * ::                   ** *:   :   :: ::* *  .

DXR             ---------------LSVLEKMDMREPQ---CVDD--VLSVDASAREVARKEVMRLAS-- 398
KARI            NYLFSYACVPLLKPFMAELQPGDLGKAIPEGAVDNGQLRDVNEAIRSHAIEQVGKKLRGY 480
                              :: *:  *: :.    .**:  : .*: : *. * ::* :

DXR             ----------
KARI            MTDMKRIAVAG 491
```

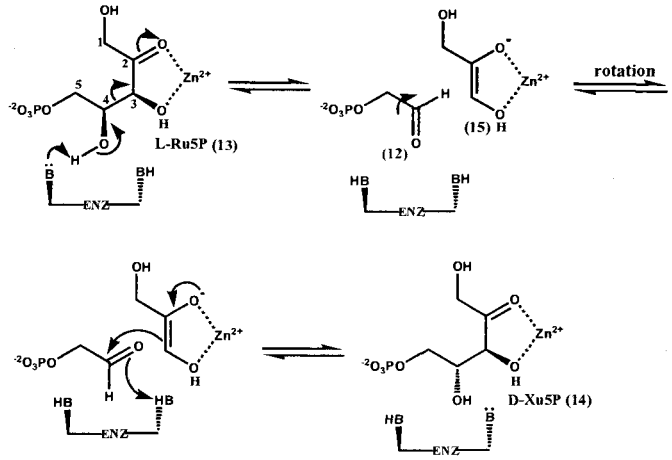
**Figure 4.3. The pairwise sequence alignments of DXR and KARI from *E.coli* based on the ClustalW (1.82) program.**

Although KARI and DXR catalyze similar overall reactions, another mechanism for the action of DXR, a retroaldol/aldol rearrangement, is possible. This mechanism involves the deprotonation of the C4 hydroxy group of DXP, followed by the cleavage of the carbon-carbon bond between the carbon atoms C3 and C4 to give the enediolate of hydroxyacetone or acetol (**11**) and glycolaldehyde phosphate (**12**). An aldol reaction of these two resulting intermediates, using the opposite end of the enediol (**11**) generates a carbon-carbon bond between the carbon atoms derived from C2 and C4 of DXP forming methylerythrose phosphate (**2**).




**Scheme 4.3. The reaction mediated by DXR with retroaldol/aldol rearrangement.**

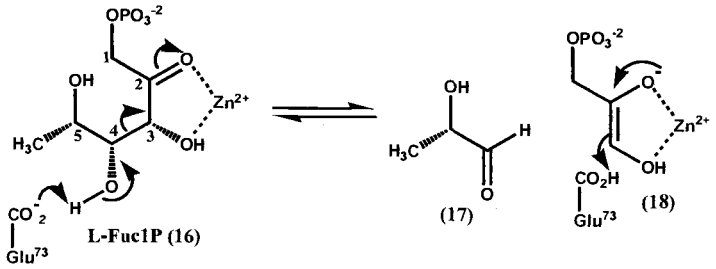
This type of mechanism has been proposed for the reaction catalyzed by the bacterial enzyme L-ribulose 5-phosphate-4-epimerase (AraD, EC 5.1.3.4). This enzyme catalyzes the interconversion of L-ribulose 5-phosphate (L-Ru5P, **13**) and D-xylulose-5-phosphate (D-Xu5P, **14**), a pentose phosphate pathway intermediate, by changing the stereochemistry at the C4 position.[16]




**Scheme 4.4. The reaction mediated by AraD with a retroaldol/aldol rearrangement.**

In the reaction mediated by the epimerase AraD, carbon-carbon bond cleavage occurs between C3 and C4 to generate the enolate of dihydroxyacetone (**15**) and glycolaldehyde phosphate (**12**). The metal ion, $Zn^{2+}$, serves as a Lewis acid catalyst and stabilizes the enolate intermediate. A reorientation can occur that exposes the opposite face of the aldehyde to the same face of the enolate (perhaps only a simple bond rotation), and an aldol addition would reform the C-C bond to generate D-xylulose-5-phosphate (D-Xu5P, **14**).

Indirect evidence suggesting the retrolaldol/adol mechanism of the epimerase AraD, came from an article in 1993 that reported that the epimerase AraD shares 26% sequence identity with L-fuculose 1-phosphate aldolase (FucA, EC 4.1.2.17).[17,18] This enzyme catalyzes the reversible aldol condensation between L-lactaldehyde (**17**) and dihydroxyacetone phosphate (**18**) to yield L-fuculose 1-phosphate (L-Fuc1P, **16**). The reaction proceeds via deprotonation of the hydroxyl group at C4 with C-C bond cleavage to produce L-lactaldehyde (**17**) and the metal bound enolate. Protonation of the enolate gives the second product, dihydroxyacetone phosphate (**18**).




**Scheme 4.5. The retroaldol reaction mediated by FucA.**

Both enzymes, the epimerase (AraD) and the aldolase (FucA), use divalent cations in the formation and stabilization of enolates during catalysis. They are also able to deprotonate the C4-hydroxyl group of a substrate bearing both ketone and phosphate groups. The sequence identity between the aldolase and the epimerase also suggests that they have evolved from a common ancestor and have retained the ability to form and cleave carbon-carbon bonds via metal-promoted aldol chemistry. To probe the relationship between these two enzymes, the X-ray crystal structures of

both enzymes were compared.[19,20] Like the aldolase, the epimerase is a homotetramer composed of four single domain subunits. A comparison of the epimerase secondary structure with that of aldolase showed a remarkable degree of similarity.[20] This agreement indicates that two enzymes belong to a superfamily of aldolases/epimerases and have evolved from a common ancestor. Furthermore, mutagenesis studies showed that these two enzymes employ the same phosphate binding pocket.[21]

From the results mentioned above, it can be concluded that the evolutionary link between the epimerase and the aldolase is evident from sequence comparisons, mechanistic studies, and structural studies. By using the same strategy for the comparison between AraD and FucA, the enzyme DXR should be compared in the same way with KARI and AraD in order to discern a reasonable mechanism for the DXR-mediated reaction. The pairwise sequence alignment of DXR and AraD (epimerase) from *E. coli* was obtained and a comparison between the three different enzymes was made as shown in Table 4.1 based on information obtained so far.

```
DXR          MKQLTILGSTGSIGCSTLDVVRHNPEHFRVVALVAGKNVTRMVEQCLEFSPRYAVMDDEA 60
Epimerase    ----MLEDLKRQVLEANLALPKHN-----LVTLTWGN---------------VSAVDRER 36
                  : . . .:  :.* : :**     :*:*. *:                :.:* *

DXR          SAKLLKTMLQQQGSRTEVLSGQQAACDMAALEDVDQVMAAIVGAAGLLPTLAAIRAGKTI 120
Epimerase    GVFVIK---------------PSGVDYSIMTADDMVVVS-------------IETGEVV 67
             .. ::*                  :. * : :   * *:.:             *.:*:.:

DXR          LLANKESLVTCGRLFMDAVKQSKAQLLPVDSEHNAIFQSLPQPIQHNLGYADLEQNGVVS 180
Epimerase    EGAKKPSSDTPTHRLLYQAFPSIGGIVHTHSRHATIWAQAGQSIPATG------------ 115
               *:* *  *  : ::  .  * . :: ..*.* :*: .  *.*  .

DXR          ILLTGSGGPFRETPLRDLATMTPDQACRHPNWSMGRKISVDSATMMNKGLEYIEARWLFN 240
Epimerase    ----TTHADYFYGTIPCTRKMTDAEINGEYEWETG---NVIVETFEKQGID--------- 159
                  : . :   .:    .** :   . :*. *   .*   *: ::*::

DXR          ASASQMEVLIHPQSVIHSMVRYQDGSVLAQLGEPDMRTPIAHTMAWPNRVNSGVKPLDFC 300
Epimerase    -AAQMPGVLVHSHG---------------------------PFAWGKNAEDAVH----- 185
              :*.   **:*.:.                          .:** :..:..*:

DXR          KLSALTFAAPDYDRYPCLKLAMEAFEQGQAATTALNAANEITVAAFLAQQIRFTDIAALN 360
Epimerase    --NAIVLEEVAYMGIFCRQLAPQLPDMQQTLLDKHYLR-KHGAKAYYGQ---------- 231
               .*:.:    *    * :** :  :  *:         :  . *: .*

DXR          LSVLEKMDMREPQCVDDVLSVDASAREVARKEVMRLAS 398
Epimerase    ------------------------------------
```

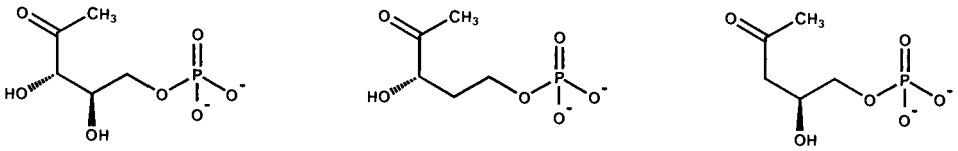
**Figure 4.4. The pairwise sequence alignment of DXR and AraD (epimerase) from *E. coli* based on the ClustalW (1.82) program.**

**Table 4.1. A comparison of *E.coli* DXR, KARI and the epimerase, AraD.**

| Enzyme from *E.coli* | DXR | KARI | Epimerase, AraD |
|---|---|---|---|
| The quaternary structure[a] | Homodimer, 3 regions and a flexible loop | Homodimer, 5 regions | Homotetramer, 4 regions |
| Identity to DXR[b] | - | 15% | 12% |
| Metal ion binding pocket[a] | Asp150, Glu152, and Glu231 | Glu319, Asp315, Glu492, and Glu496 | Glu73, His92, His94, and His155 |
| Phosphate binding pocket[c] | Ser186, Ser222, Asn227, and Lys228 | No phosphate group in KARI substrate | Asn28, Ser44, Gly45, Ser774, and Ser75 |

[a] Ref. 4,19, 22.
[b] Based on the ClustalW (1.82) program.
[c] Ref. 19, 23.

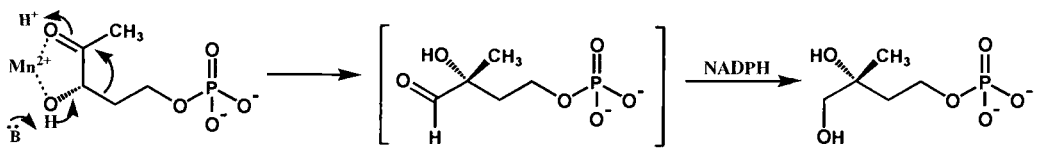
Data from Table 4.1 show some similarities between these three enzymes, for example, they require metal ions for the activity. However, there are also some differences that need to be considered, for example, DXR and KARI use $Mn^{2+}$,but the epimerase, AraD needs $Zn^{2+}$ as a cofactor. Moreover, there is no common metal binding motif between DXR, KARI and the epimerase, AraD. Another approach to provide support for one of the mechanisms would be to use analogs of DXP: 4-deoxy DXP (**19**) which is a DXP analog without a hydroxyl functional group at C4, and 3-deoxy DXP (**20**) which is a DXP analog without a hydroxyl functional group at C3 position that were synthesized in Chapter Two for discerning the mechanism. These two compounds might be processed differently based on the two mechanisms.



Deoxyxylulose phosphate (1) 4-Deoxy DXP (19) 3-Deoxy DXP (20)

**Figure 4.5. The deoxy analogs of DXP.**

These compounds had the potential to be either alternate substrates or inhibitors of DXR. The results of the assay could provide detail of how the mechanism of the rearrangement occurs. If 4-deoxy DXP (**19**) was turned over by DXR, this result would rule out the retroaldol/aldol rearrangement mechanism. In Scheme 4.6, the possible DXR mediated reaction of 4-deoxy DXP (**19**) by the α-ketol rearrangement mechanism is presented. The reaction starts with the deprotonation of the hydroxyl at C3 of 4-deoxy DXP followed by the C3-C4 bond breaking. The migration of the phosphate containing subunit can occur to generate the corresponding aldehyde intermediate **21**. The reduction of **21** by NADPH provides the phosphate compound **22**. The phosphate **22** will not be generated by the retroaldol/aldol rearrangement because 4-deoxy DXP lacks of the hydroxyl group at C4 position which is the requirement for the retroaldol rearrangement.

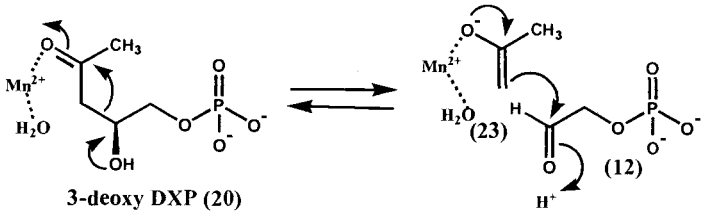



4-Deoxy DXP (19) (21) (22)

**Scheme 4.6. A putative α-ketol rearrangement of 4-deoxy DXP by DXR.**

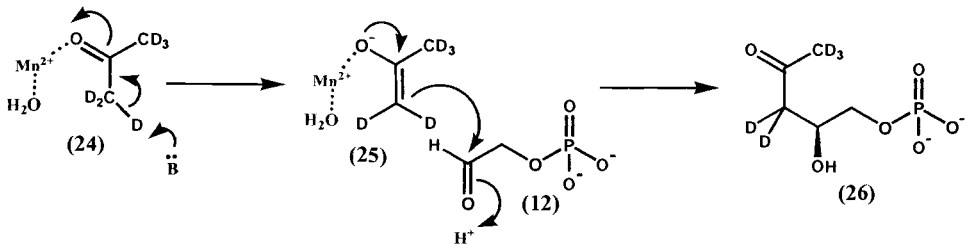
In contrast, 3-deoxy DXP (**20**) will not get turned over by DXR via the α-ketol rearrangement mechanism because there was no hydroxyl functional group at C3 position which needs to be deprotonated. Even though 3-deoxy DXP (**20**) contains the hydroxyl group at C4 position which can be deprotonated by DXR according to the retroaldol rearrangement mechanism, the aldol rearrangement will

only regenerate the starting compound, 3-deoxy DXP (**20**). There will be no novel product from this aldol rearrangement because an intermediate aldehyde cannot be formed, so no reduction is possible. However, it is still possible to probe whether or not these reactions are occurring.



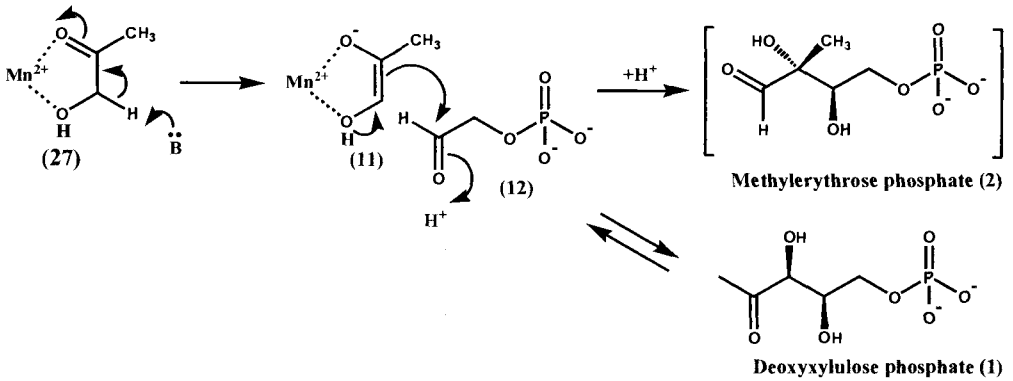

**Scheme 4.7. The possible retroaldol/aldol rearrangement of 3-deoxy DXP by DXR.**

If the retroaldol/aldol rearrangement of 3-deoxy DXP by DXR as shown in Scheme 4.7 can happen, it may be possible that the intermediate **23** may leave the active site of DXR. Intermediate **23** is the enolate of acetone, the common solvent. If **23** is released from the active size, it could exchange with acetone added to the incubation mixture. If deuterated acetone (**24**) is used in the assay, then aldol addition of **25** to glycoladehyde phosphate (**12**) could produce deuterated 3-deoxy DXP product **26** as shown in Scheme 4.8. In the analysis phase, **26** can be dephosphorylated and derivatized as an acetate and then its acetate derivative can be compared to an acetate standard of 3-deoxy DX by GC/MS. If **26** is proved to be formed from the incubation of 3-deoxy DXP and deuterated acetone (**24**) with DXR, this result would strongly support that DXR mediates the reaction via the retroaldol/aldol rearrangement mechanism.



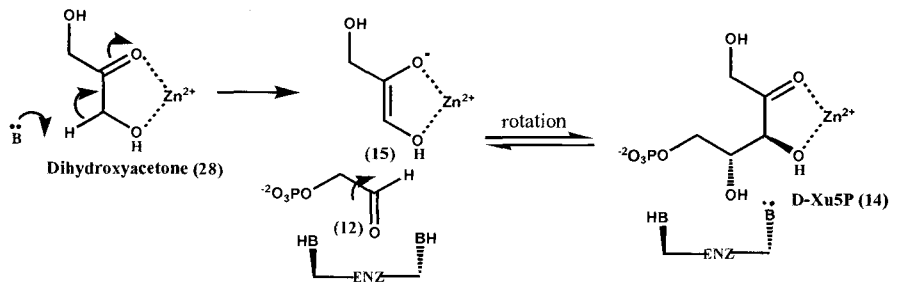

**Scheme 4.8. The possible aldol rearrangement of glycoladehyde phosphate and deuterated acetone by DXR.**

Furthermore, another experiment was designed to support the retroaldol/adol rearrangement mechanism if the α-ketol rearrangement appeared not to be the correct mechanism for DXR. The intermediate glycolaldehyde phosphate (**12**) can be synthesized[24] and reacted with the commercially available acetol (**27**) in the presence of DXR. If DXR catalyzes the reaction by the retroaldol/adol rearrangement mechanism, the enediolate (**11**), derived from acetol, can react with glycolaldehyde phosphate (**12**) to generate methylerythrose phosphate (**2**).




**Scheme 4.9. The proposed aldol rearrangement of acetol and glycoladehyde phosphate by DXR.**

This postulated experiment was based on a reported experiment with the epimerase, AraD from Luo *et al.*[19] The commercially available compound dihydroxyacetone (**28**) was used as the precursor for the enolate of dihydroxyacetone (**15**) which could react with glycolaldehyde phosphate (**12**) to generate D-xylulose-5-phosphate (D-Xu5P, **14**). Under appropriate conditions, it was shown that **14** could be prepared from the two intermediates **28** and **12**.




**Scheme 4.10. The reaction mediated by AraD with an aldol reaction.**

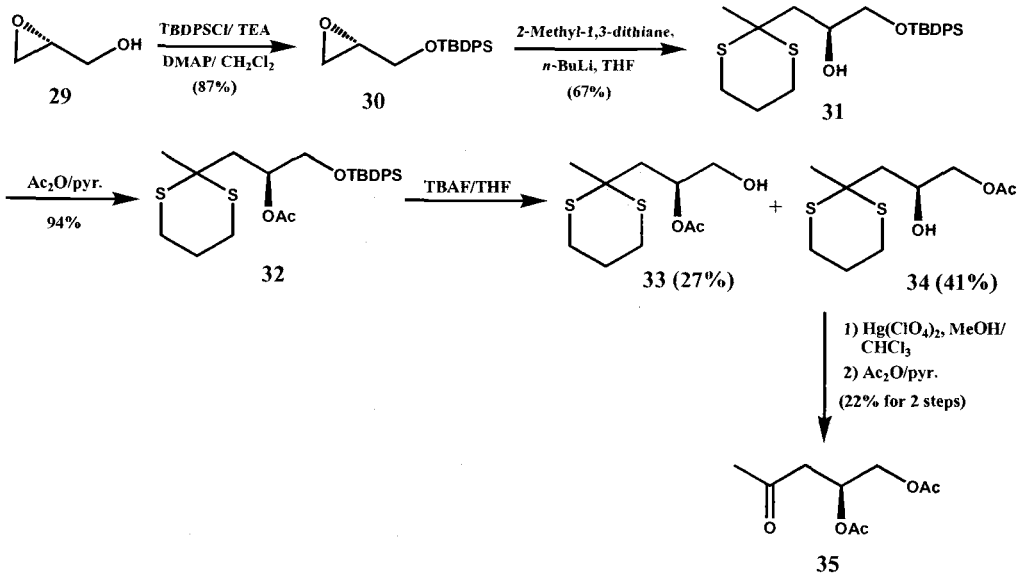
**2) Results**

The compounds, 4-deoxy DXP and 3-deoxy DXP were synthesized and tested against DXR as mentioned in Chapter 2. As was previously indicated both compounds were not alternate substrates for DXR but they acted as competitive inhibitors with the $K_i$ = 30 μM for 4-deoxy DXP and $K_i$ = 150 μM for 3-deoxy DXP.

The incubation of 3-deoxy DXP and acetone-$D_6$ was performed in parallel with an incubation of 3-deoxy DXP and unlabeled acetone for comparison. Preliminary assays of DXR with DXP indicate that 3% acetone causes a 50% decrease in the activity of DXR so 1% of acetone-$D_6$ or acetone were used in the incubation. The incubation reactions (total volume 3 mL) consisted of 50 mM Tris buffer pH 7.8, 1 mM $Mn^{2+}$, 1.2 mM NADPH (3 mg), 5 mM 3-deoxy DXP (3 mg), 1% of acetone-$D_6$ or acetone (30 μL) and DXR (30 μg). The incubations were conducted at 37 °C for 6 h before treatment with alkaline phosphatase at 37 °C for an additional 3 h. In the next step, the proteins were precipitated with ethanol and the supernatant was collected. The supernatant was concentrated and the crude residue was treated with acetic anhydride and pyridine at room temperature for 2 h. The crude reaction mixture was concentrated in vacuo and the crude products were passed though silica column using 1:2 (v/v) ethylacetate/hexanes as an eluent. The reaction products were compared to a standard diacetate of 3-deoxy DX. The synthesis of the standard diacetate of 3-deoxy DX is outlined in Scheme 4.12. This procedure was used during the attempt to synthesize 3-deoxy DXP.

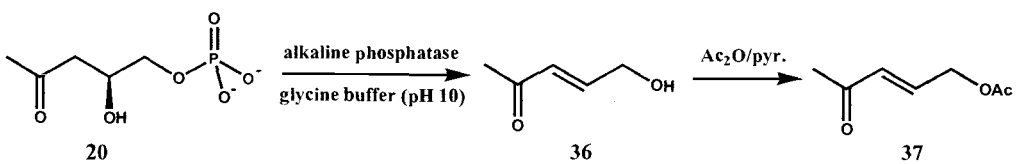
The commercially available compound (*R*)-glycidol (**29**) was protected with the *tert*-butyldiphenylsilyl group to yield the oxirane (**30**). Ring opening of the oxirane was conducted with a dithiane derivative which can be cleaved to form the ketone functional group as expected in the final product. Acetylation of **31** was accomplished in 94% yield. Deprotection of the TBDPS group in **32** provided the primary alcohol **33** and the secondary alcohol **34** in 27% and 41% yield respectively. The secondary alcohol **34** was carried on further while the primary alcohol **33** was used for the attempt to synthesize 3-deoxy DXP as mentioned in Chapter 2.

Deprotection of the dithiane functional group of **34** yielded the ketone which could be further acetylated to give the diacetate **35** with 22% yield for 2 steps.




**Scheme 4.11 The synthesis of diacetate of 3-deoxy DX.**

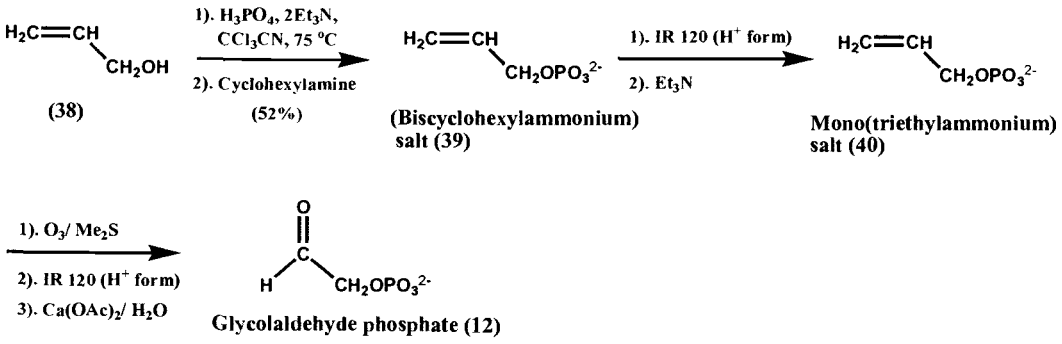
The acetate derivatives of the products from the incubations of 3-deoxy DXP with acetone and acetone-$D_6$ were compared to the diacetate standard of 3-deoxy DX. The normal phase TLC of the acetate derivatives showed only minor apparent product generated from both incubation reactions that correspond to the acetate standard. The major products from both reactions, possibly **37**, showed a different $R_f$ compared to the standard. It may be possible that there was elimination reaction occurs under basic condition of dephosporylation reaction. Moreover, the elimination product **36** might not be stable under the basic condition. Therefore the desired acetate derivative might not be generated.




**Scheme 4.12 The elimination reaction of 3-deoxy DXP.**

The acetate derivatives and the diacetate standard of 3-deoxy DX were injected into the GC/MS for the comparison despite the apparent absence of the desired product by TLC. There was no significant peak from the incubations that matched with the standard. Modified isolation procedures will be required to successfully prepare the diacetate of 3-deoxy DX.

The synthesis of glycolaldehyde phosphate (**12**) was based on a procedure from Muller *et al.*[24] As outlined in Scheme 4.9, allyl alcohol (**38**) was phosphorylated with phosphoric acid after activation as the trichloroacetimidate under triethylamine and trichloroacetonitrile conditions. The resulting allyl phosphate was transformed into a stable biscyclohexyl ammonium-salt (**39**). In the next step, the mono(triethylammonium) salt of allyl phosphate (**40**) was generated before ozonolysis to form the glycolaldehyde phosphate (**12**). For stability, the glycolaldehyde phosphate was stored in a calcium salt form. It was converted into the disodium salt for preparing a stock solution for testing against DXR due to increased solubility of the sodium salt relative to the calcium salt.[19]




**Scheme 4.13. The synthesis of glycolaldehyde phosphate.**

The glycolaldehyde phosphate (**12**) and acetol (**27**) were incubated with DXR. The assay were conducted at 37 °C with a total volume of 1 mL containing 50 mM Tris buffer pH 7.8, 1 mM $Mn^{2+}$, 0.2 mM NADPH, glycolaldehyde phosphate and acetol. Both glycolaldehyde phosphate and acetol were tested at concentrations

up to 40 mM and the absorbance at 340 nm was observed. Negative controls of both compounds were also tested to rule out any possible reactions between each compound with DXR. The decreases of the absorbance at 340 nm from the DXR assay with glycolaldehyde phosphate (**12**) and acetol (**27**) are shown in Table 2. The assay was also monitored longer than the 4 min standard assay, but the changes in absorbance for the GALP (glycolaldehyde phosphate) and acetol with DXR mixture decreased to be almost the same with the negative control. By using a higher concentration of the enzyme DXR, the change in absorbance from the assay of GALP and acetol increased a small amount, but the change was not convincing considering the high background observed with some of the negative controls.

**Table 4.2. The results from DXR assay of acetol (27) and glycolaldehyde phosphate (12, GALP).**

| Assay | Decreases in $OD_{340nm}$[a] |
|---|---|
| Negative Control/ NADPH and DXR | 0.013 |
| Positive Control/ 160 μM DXP | 0.201 |
| 40 mM of GALP w/o DXR | 0.004 |
| 40 mM of GALP w/ DXR | 0.014 |
| 40 mM of Acetol w/o DXR | 0.008 |
| 40 mM of Acetol w/ DXR | 0.018 |
| 40 mM of GALP and Acetol w/o DXR | 0.019 |
| 40 mM of GALP and Acetol w/ DXR | 0.041 |
| Negative Control/ NADPH and DXR | 0.009[b] |
| 40 mM of GALP and Acetol w/ DXR | 0.073[b] |

The decreases in the absorbance in the assays were corrected from the negative control.
[a] The assay were monitored over 4 min; 7.2 μg of N-DXR were used.
[b] 22.4 μg of N-DXR were used.

**3) Discussion**

The fact that 4-deoxy DXP is not a substrate for DXR, but rather acts as an inhibitor, does not provide any positive support for either of the mechanisms. The lack of turnover could be due to the inability to undergo the α-ketol rearrangement, but it could also reflect the importance of the 4-hydroxy group to correctly position the substrate for a successful rearrangement. Rohmer and co-workers also considered the possibility that 4-deoxy DXP might support the α-ketol rearrangement for the *E. coli* DXR, but they also found it to be a competitive inhibitor.[25]

The negative results from the incubation of 3-deoxy DXP and acetone-$d_6$ with DXR should be viewed as preliminary, and cannot be considered as ruling out the retroaldol/aldol reaction. The conditions for this assay may not have been optimal to recover and characterize the 3-deoxy DXP product. A possible solution would be to repeat the phosphatase cleavage reaction using acid phosphatase rather than alkaline phosphatase, thus preventing the proposed elimination problems with 3-deoxy DX. It also may be that the intermediate products of the retroaldol/aldol mechanism are held very tightly at the enzyme active site and cannot be released to the bulk solvent to exchange with externally supplied compounds.

Even though the reaction between acetol and glycolaldehyde phosphate showed a slight decrease in absorbance at 340 nm, the changes were quite small considering the very high concentrations of the intermediates (40 mM) and the relatively high background observed when the intermediates were incubated either separately with DXR or together in the presence of NADPH. Much more extensive investigation would be necessary to prove that this decrease in absorbance is truly due to DXR mediated condensation of these components and subsequent reduction. One route that would have to be ruled out is the non-enzymatic condensation of acetol and glycolaldehyde phosphate to produce DXP, which is then processed normally by DXR. Although it is not expected that this would be a facile non-enzymatic reaction, it would have to be tested under the incubation conditions. In addition to our findings, a report appeared in the literature that described the

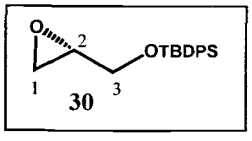
identical approach to probe the DXR mechanism.[25] The article indicated that when the enzyme was incubated in the presence of acetol and glycolaldehyde phosphate at concentrations up to 1 mM, no decrease in absorbance was observed. These concentrations were lower than in our studies, but the conclusion was essentially the same. Because it was clear that others were using this same approach to study the DXR mechanism, and because the data at this point were not convincing, a decision was made to stop this aspect of the project and proceed with other aspects of the dissertation research.

From the data for DXR obtained to date including the X-ray crystal structures and the interactions of the DXP analogs with DXR, neither of the two possible enzymatic reaction mechanisms can be ruled out. While it is tempting to favor the α-ketol rearrangement from the overall similarity of the chemical steps to ketol acid reductoisomerase, the lack of significant identity between the two enzymes does not support this conclusion. Further experiments will be necessary to resolve this question of mechanism for DXR.

**General Methods:** Materials and general methods are listed in Chapter Two (see page 59).

**Diacetate of 3-deoxy DX Synthesis:**

**(*S*)-(*tert*-Butyldiphenylsiloxymethyl)oxirane (30).**

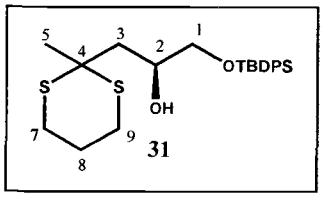


**30**

To a solution of (*R*)-glycidol **29** (450 μL, 6.8 mmol) and TEA (1.0 mL, 7.4 mmol, 1.1 equivalents) in dry $CH_2Cl_2$ (20 mL) was added *tert*-butyldiphenylsilylchloride (1.3 mL,7.4 mmol, 1.1 equivalents) and DMAP (5 mg) at room temperature. After being stirred at room temperature for 4 h, the reaction mixture was filtered over Celite and washed with 20 mL of $CH_2Cl_2$. The filtrate was washed with saturated $NH_4Cl$ (10 mL) and saturated NaCl (10 mL). The organic phase was dried, filtered, concentrated and purified by flash chromatography (1:9 (v/v) ethyl acetate/hexanes) to give 1.8 mg (87%) of a colorless oil.

$^1$H NMR (300 MHz, $CDCl_3$) δ 7.55 (m, 10H, Ph), 3.86 (dd, $J$ = 11.8, 3.3 Hz, $1H_{3a}$), 3.71 (dd, $J$ = 11.9, 4.7 Hz, $1H_{3b}$), 3.13 (m,$1H_2$), 2.75 (dd, $J$ = 5.2, 4.0 Hz, $1H_{1a}$), 2.61 (dd, $J$ = 5.2, 2.7 Hz, $1H_{1b}$), 1.05 (s, 9H, 3×$CH_3$ of TBDPS group); $^{13}$C NMR (75 MHz, $CDCl_3$) 135.62, 133.29, 129.74, 127.71 (Ph), 64.30 ($C_3$), 52.27 ($C_2$), 44.45 ($C_1$), 26.74 (3×$CH_3$ of TBDPS group), 19.23 (quaternary carbon of TBDPS group).

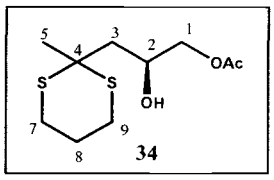
**(*S*)-1-(*tert*-Butyldiphenylsiloxymethyl)-3-(2-methyl-1,3-dithian-2-yl) propan-2-ol (31).**




To a solution of 2-methyl-1,3-dithiane (380 μL, 3.1 mmol, 1.2 equivalents) in 15 mL of THF at -78 °C was added *n*-BuLi (1.6 M, 2.8 mL, 4.4 mmol, 1.7 equivalents). The reaction mixture was stirred for 2 h at room temperature. The reaction mixture was cooled to -78 °C and a solution of **30** (810 mg, 2.6 mmol, 1 equivalent) in 15 mL of THF via cannula. After being stirred for an additional 1 h at room temperature, 10 mL of water was added slowly to the reaction mixture, which was extracted with diethyl ether (3×20 mL). The combined organic layers were dried and concentrated. Purification by flash chromatography (1:6 (v/v) ethyl acetate/hexanes) gave 760 mg (67%) of a yellow oil.

$^1$H NMR (300 MHz, $CDCl_3$) δ 7.50 (m, 10H, Ph), 4.05 (m, $1H_2$), 3.59 (d, $J$ = 6.0 Hz, $2H_1$), 2.80 (m, $4H_{7,9}$), 2.10 (m, $6H_{3,8}$), 1.65 (s, $3H_5$), 1.05 (s, 9H, 3×$CH_3$ of TBDPS group); $^{13}$C NMR (75 MHz, $CDCl_3$) 135.57, 133.19, 129.79, 127.76 (Ph), 69.49 ($C_2$), 67.98 ($C_1$), 47.80 ($C_4$), 43.91 ($C_3$), 28.42 ($C_5$), 26.85 (3×$CH_3$ of TBDPS group), 26.73 ($C_7$), 26.49 ($C_9$), 24.81 ($C_8$), 19.23 (quaternary carbon of TBDPS group).

**(S)-2-hydroxy-3-(2-methyl-1,3-dithian-2-yl)propyl acetate (34).**

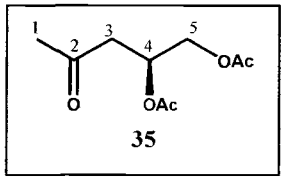



To the alcohol **31** (250 mg) was added acetic anhydride/pyridine (1:1 mL) at room temperature. The reaction mixture was stirred for 2 h at room temperature

before concentration. Purification by flash chromatography (1:9 (v/v) ethyl acetate/hexanes) gave 250 mg (94%) of the acetate derivative as a colorless oil. To a solution of the acetate (230 mg, 0.7 mmol) in 5 mL of THF was added TBAF (1.0 M in THF, 600 μL, 0.7 mmol, 1 equivalent). The reaction mixture was stirred at room temperature for 2 h and then concentrated to a thick orange oil. Purification by flash chromatography (1:1 (v/v) ethyl acetate/hexanes) gave 60 mg (41%) of **34** as a colorless oil.

$^1$H NMR (300 MHz, $C_6D_6$) δ 4.15 (m, $1H_2$), 4.00 (m, $2H_1$), 2.45 (m, $4H_{7,9}$), 2.20 (m, $2H_8$), 1.65 (s, $CH_3$ of acetyl group), 1.55 (s, $3H_5$), 1.40 (m, $2H_3$); $^{13}$C NMR (75 MHz, $C_6D_6$) 170.21 (CO of acetyl group), 68.55 ($C_2$), 67.39 ($C_1$), 47.81 ($C_4$), 44.35 ($C_3$), 28.50 ($C_5$), 26.36 ($C_7$), 26.49 ($C_9$), 24.81 ($C_8$), 20.38 ($CH_3$ of acetyl group).

**3-Deoxy-deoxyxylulose diacetate (35).**



**35**

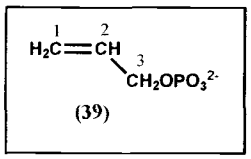
To a solution of dithiane **34** (27 mg, 0.1 mmol, 1 equivalent) in MeOH/$CHCl_3$ (1:3 mL) was added a solution of mercury perchlorate (100 mg, 0.3 mmol, 3.0 equivalents in 3 mL of MeOH) at room temperature. The reaction mixture was stirred at room temperature for 15 min before filtered over Celite. The filtrate was neutralized with $Na_2CO_3$ solution and extracted with 3×10 mL of $CHCl_3$. The combined organic layer was dried and concentrated. The crude reaction product was acetylated with acetic anhydride/pyridine (1:1 mL) at room temperature. The reaction mixture was stirred for 2 h at room temperature before concentration. Purification by flash chromatography (1:2 (v/v) ethyl acetate/hexanes) gave 5 mg (22% for 2 steps) of a colorless oil.

$^1$H NMR (300 MHz, $CDCl_3$) δ 5.42 (m, $1H_4$), 4.28 (dd, $J$ = 12.0, 3.5 Hz, $1H_{5a}$), 4.12 (dd, $J$ = 12.0, 5.3 Hz, $1H_{5b}$), 2.81 (dd, $J$ = 17.2, 7.0 Hz, $1H_{3a}$), 2.70 2.81 (dd, $J$ = 17.2, 6.0 Hz, $1H_{3b}$), 2.18 (s, $3H_1$), 2.07, 2.04 (s, 2×$CH_3$ of acetyl groups);

$^{13}$C NMR (75 MHz, $CDCl_3$) 204.38 ($C_2$), 170.58, 170.07 (2×CO of acetyl groups), 67.58 ($C_4$), 64.38 ($C_5$), 44.09 ($C_3$), 30.34 ($C_1$), 20.93, 20.72 (2×$CH_3$ of acetyl groups).

**Glycolaldehyde Phosphate Synthesis.**

**Bis(cyclohexylammonium)-allyl-phosphate (39); Registry No. 25022-72-4.**

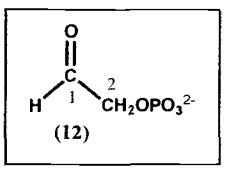


(39)

Allyl alcohol (**38**, 18.0 mL, 260 mmol, 5.2 equivalents) was added to colorless crystals of phosphoric acid (950 mg, 9.7 mmol, 0.2 equivalents), triethylamine (2.8 mL, 20 mmol, 0.4 equivalents), and trichloroacetonitrile (5.0 mL, 50 mmol, 1 equivalent) at room temperature. The reaction mixture was heated to 80 °C for 4 h. After cooling to room temperature, excess trichloroacetonitrile and allyl alcohol were distilled off under reduced pressure. The distillation was terminated when about half of the reaction mixture volume was removed. After cooling to room temperature, 50 mL of water was added to the reaction mixture and the mixture was extracted with diethyl ether (3×50 mL). Cyclohexylamine (7.5 mL, 66 mmol, 1.3 equivalents) was first added to the aqueous layer and then acetone was added dropwise. The solution sat at 4 °C overnight to recrystallize bis(cyclohexylammonium)-allylphosphate (**39**). The white crystals were filtered off, and 4.4 g (52%) of the allyl phosphate (**39**) were obtained.

$^1$H NMR (300 MHz, $D_2O$) δ 6.02 (m, $1H_2$), 5.36 (dd, $J$ = 17.2, 1.8 Hz, $1H_{1a}$), 5.19 (dd, $J$ = 10.4, 1.6 Hz, $1H_{1b}$), 4.29 (t, $J$ = 6.7, 5.3 Hz, $2H_{3a,3b}$), 3.16 (m, 2H, 2CH of cyclohexylamine), 1.16-2.02 (m, 20H, $10CH_2$ of biscyclohexylamine); $^{13}$C NMR (75 MHz, $D_2O$) 138.05 (d, $J$ = 7.2 Hz, $C_2$), 118.76 ($C_1$), 68.00 (d, $J$ = 4.4 Hz, $C_3$), 53.03 (2CH of cyclohexylamine), 33.04, 26.99, 26.50 ($10CH_2$ of cyclohexylamine); $^{31}$P NMR (121 MHz, $D_2O$) 4.30.

**Calcium glycolaldehyde phosphate (12); Registry No. 227750-56-3.**



(12)

Bis(cyclohexylammonium)-allyl phosphate (**39**, 4 g, 12 mmol) in 15 mL of water and IR 120 cation exchange resin from Fluka (30 g, activated with HCl) were stirred for 40 min. The resin was packed into a column (1.5″×10″) and the column was eluted with water (40 mL) until the pH of the eluent was 7. The aqueous eluent was extracted with diethyl ether (2×30 mL) to remove cyclohexylamine. TEA (5.3 mL) was added

to the aqueous layer to generate the mono(triethylammonium)allyl phosphate (2.3 g) which was characterized by $^{1}H$ NMR. The product was concentrated and dried under vacuum overnight. A solution of mono(triethylammonium)allyl phosphate (2.2 g) in MeOH (60 mL) was purged with ozone (flow rate 2.0-2.5 $cm^3/s$) for 3 min at -78 °C. After being stirred at -78 °C for an additional 20 min, dimethylsulfide (4 mL) was added. The reaction mixture was stirred for 17 h at room temperature and concentrated to give a light yellow precipitate. Water (60 mL) and IR-120 resin ($H^+$ form, 30 g) were added to the precipitate. The mixture was stirred for 30 min before the resin was packed into a column (1.5″×10″). The column was eluted with water (240 mL). The aqueous eluent was concentrated to about 30 mL and a solution of calcium acetate (1.5 g in 15 mL of water) was added followed by a dropwise addition of acetone (30 mL) at 4 °C over a 30 min period. The solution sat at 4 °C for 18 h and was filtered to obtain calcium glycolaldehyde phosphate as a white precipitate (1.1 g, 47%). Conversion of calcium glycolaldehyde phosphate into sodium salt was accomplished by passing calcium glycolaldehyde phosphate (1.0 g/ 10 mL $H_20$) through the column(1.5″×10″) of Dowex-50 ($Na^+$ form, 40 g). The column was washed with 100 mL of water and the eluate was frozen and lyophilized to obtain 800 mg of glycolaldehyde phosphate disodium salt.

$^{1}H$ NMR (300 MHz, $D_2O$) δ 5.16 (t, $J$ = 4.9 Hz, $1H_1$), 3.82 (dd, $J$ = 6.8, 4.8 Hz, $2H_{2a,2b}$); $^{13}C$ NMR (75 MHz, $D_2O$) 92.80 (d, $J$ = 7.7 Hz, $C_1$), 70.48 (d, $J$ = 4.6 Hz, $C_2$); $^{31}P$ NMR (121 MHz, $D_2O$) 1.16.

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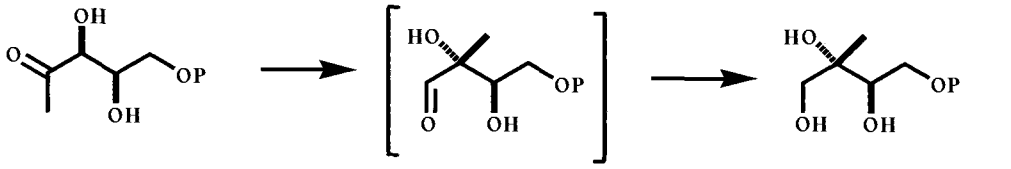
# CHAPTER FIVE

## METHYLERYTHROSE PHOSPHATE AS AN INTERMEDIATE IN THE DXR-MEDIATED REACTION

### 1) Introduction

After the MEP pathway was discovered in 1993 and 2-*C*-methyl-D-erythritol-4-phosphate (**3**) was proved to be the product from the deoxyxylulose phosphate reductoisomerase (DXR) reaction, 2-*C*-methyl-D-erythrose-4-phosphate (**2**) was no longer considered to be an intermediate in the pathway.[1,2] It was postulated to be generated only as an intermediate in the rearrangement of deoxyxylulose-5-phosphate (**1**, DXP) to MEP (**3**) by the action of the enzyme, DXR as mentioned in Chapter Four. Despite efforts to identify **2**, the methylerythrose phosphate had never been generated or isolated for a complete characterization. It appeared that this compound was not released from the enzyme active site during a catalysis.[3] In order to understand more about the mechanism of the DXR catalyzed-reaction, it is important to identify the real intermediate of this reaction step. This chapter describes procedures that were tried to synthesize 2-*C*-methyl-D-erythrose-4-phosphate (**2**), so it could be tested as the intermediate for the DXR-catalyzed reaction.




**Deoxyxylulose phosphate (DXP, 1)** — **Methylerythrose phosphate (2)** — **Methylerythritol phosphate (MEP, 3)**

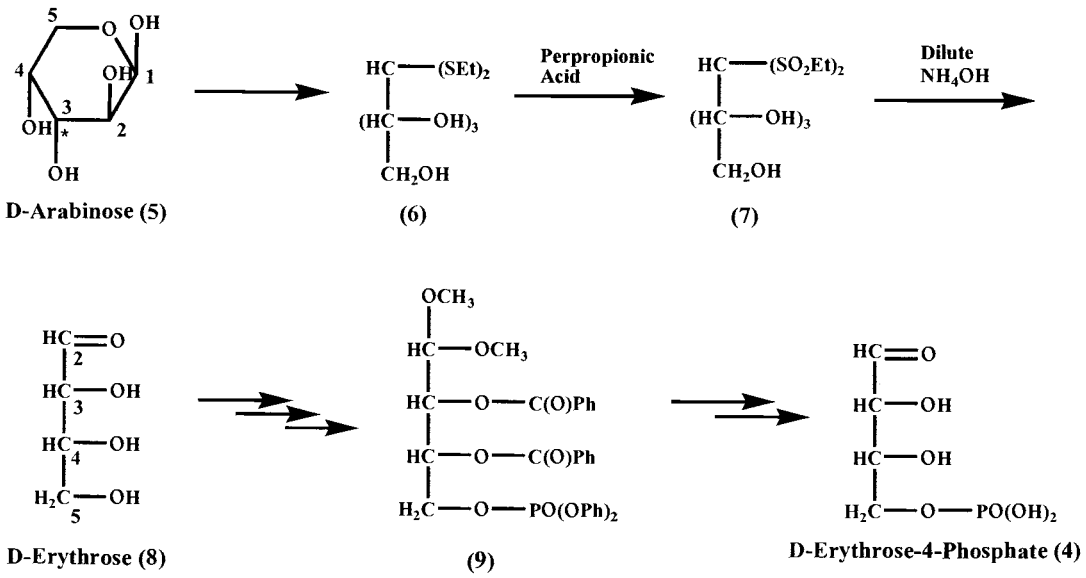
**Scheme 5.1. The reaction mediated by the enzyme DXR.**

The approach for this investigation was to synthesize the standard 2-*C*-methylerythrose-4-phosphate and incubate this compound with DXR. If this compound could be turned over by DXR and the product was identified as

methylery-thritol phosphate (**3**), this result would definitely confirm that methylerythrose phosphate (**2**) is the true intermediate in the DXR reaction.

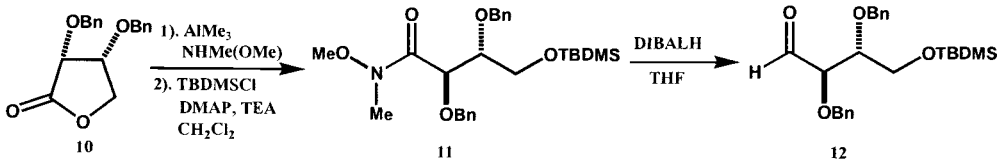
## 2) Results and Discussion

As a starting point for the synthesis of 2-*C*-methyl-D-erythrose-4-phosphate (**2**), syntheses of D-erythrose-4-phosphate (**4**) were considered because of the similarity in their structures. The compound, D-erythrose-4-phosphate (**4**) was synthesized from D-arabinose (**5**) as shown in Scheme 5.2.[4,5] This procedure, however, could not be readily modified and used for the synthesis of methylerythrose phosphate. In order to obtain 2-*C*-methyl-D-erythrose-4-phosphate (**2**) based on the synthesis of D-erythrose-4-phosphate (**4**), a methyl group would have to be introduced at the C3 position of D-arabinose (**5**) probably by oxidation of the secondary alcohol at C3 followed by methyl Grignard addition to the resulting ketone. Many protecting groups would need to be tried to get a selective protection of hydroxyl functional group at C1, C2, and C4 position. This would make the synthesis less efficient. Therefore, a different synthetic approach was proposed.



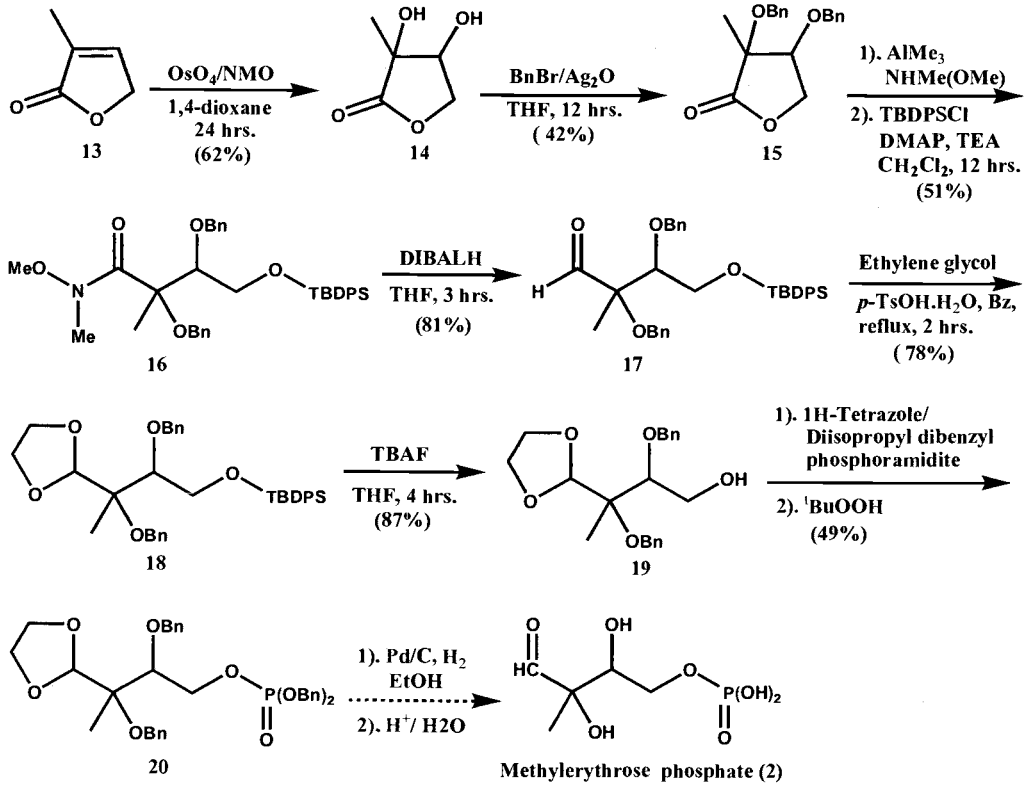

**Scheme 5.2. The synthesis of D-erythrose-4-phosphate.**[4,5]

A different synthetic approach was considered based on a formation of the aldehyde **12** from Marshall *et al.*[6] In this published procedure, the dibenzylated D-erythronolactone (**10**) was converted into the Weinreb amide **11**. In the next step, the Weinreb amide was reduced by DIBALH to provide the aldehyde **12** as shown in Scheme 5.3.




**Scheme 5.3. The model synthesis of the aldehyde from the latone.**[6]

By using the same synthetic strategy as Scheme 3, the first route that was tried for the synthesis of 2-*C*-methyl-D-erythrose-4-phosphate (**2**) used the commercially available compound, 3-methyl-2(5H)-furanone (**13**) as a starting material (Scheme 5.4). Hydroxylation of furanone **13** with osmium tetroxide yielded an enantiomeric mixture of diol **14**.[7]

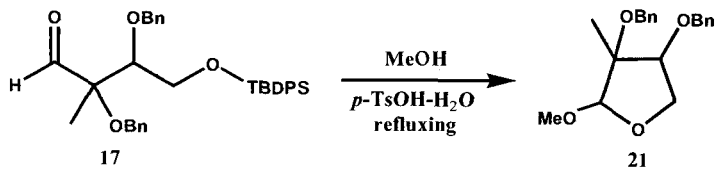



**Scheme 5.4. Potential synthetic route to methylerythrose phosphate (Route I).**

At this point, the correct stereochemistry was not considered as a main issue for the synthesis. After conditions for the entire scheme were worked out, an enantiomerically pure diol **14** might be obtained from hydroxylation of furanone **13** with Sharpless ADmixβ (Asymmetric Dihydroxylation).[8] In a subsequent step, dibenzylation of diol **14** was performed using benzyl bromide in the presence of silver(I) oxide in about 40 % yield.[9] Treatment of compound **15** with trimethylaluminum and methoxymethyl amine provided a Weinreb amide with a free alcohol at the other end of the chain. The resulting primary alcohol was protected with a *tert*-butyldiphenylsilyl (TBDPS) group without prior purification to form **16**.[6,10] Direct protection of the primary alcohol provided a better yield and a more stable compound to handle. The TBDPS group was preferred to the more common TBDMS or TMS groups because it should be quite stable under the acidic conditions which would be used later in the synthetic procedure. In a subsequent step, the aldehyde (**17**) was obtained from the reduction of the Weinreb amide (**16**) with DIBALH.[11,12] Protection of the aldehyde **17** was conducted with ethylene glycol and acidic condition to yield the 1,3-dioxolane (**18**).[13] The aldehyde **17** was protected prior to cleavage of the TBDPS group with *tert*-butylaluminium fluoride in order to prevent cyclization of the aldehyde to form a cyclic acetal. If formation of the cyclic acetal occurred, the phosphate functional group could not be introduced at the $C_4$ position of the final product (**2**) as planned. Phosphorylation of the primary alcohol **19** was conducted with diisopropylphosphoramidite and 1H-tetrazole followed by oxidation of the initially formed phosphite with *tert*-butylhydroperoxide. In theory, methylerythrose phosphate (**2**) should be obtained readily by catalytic hydrogenation of **20** and acidic hydrolysis of the 1,3-dioxolane group in the corresponding free phosphate. However, when different acidic conditions were used including pyridinium tosylate,[14] hydrochloric acid,[15] acetic acid,[13] methylerythrose phosphate (**2**) could not be obtained as expected. Under these acidic conditions for deprotection of the 1,3-dioxolane functional group, the aldehyde functional group in the product may not survive as wanted. To overcome this problem, milder conditions such as bisacetonitrile-chloro Pd(II),

($PdCl_2(CH_3(CN)_2)$,[16] were also tried to remove the aldehyde protecting group. However, the desired product still could not be obtained. When the order of the final reactions was changed by having the 1,3-dioxolane removed before catalytic hydrogenation to remove benzyl groups, a different result was observed. The 1,3-dioxolane in **20** could be cleaved with acetic acid, but the reaction was sluggish and an only small peak of the resulting aldehyde was observed along with recovered starting material **20**. It appeared that the 1,3-dioxolane might not be the appropriate protecting group of the aldehyde for the synthesis of 2-*C*-methyl-D-erythrose-4-phosphate (**2**).

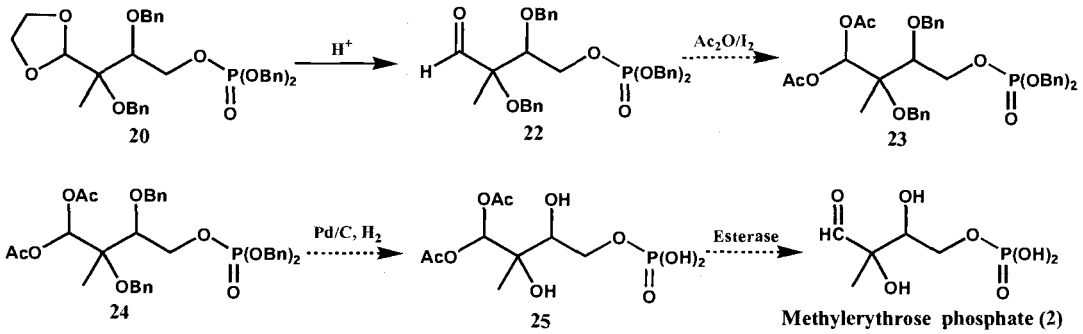
A different protecting group of the aldehyde, the dimethyl acetal,[17] was considered as an option. This group could be deprotected in a final step under mild conditions such as dichlorodicyano quinone (DDQ) or immediately hydrolyzed by the acidic phosphate group after the free phosphate was generated as mentioned in the synthesis of D-erythrose-4-phosphate.[18] Unfortunately, the *tert*-butyldiphenylsilyl protecting group could not survive under the methanol and *p*-toluenesulfonic acid conditions used to form the dimethyl acetal and an unexpected product, the methyl glycoside **21** was generated.




**Scheme 5.5. Conditions for protection of the aldehyde as a dimethyl acetal.**

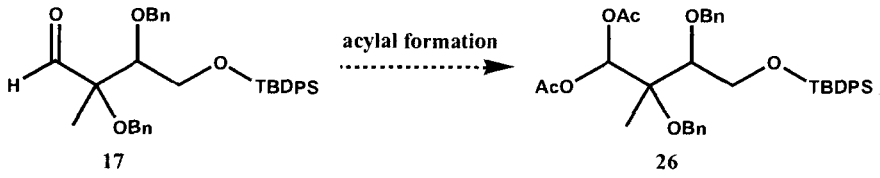
Another type of protecting group for the aldehyde, an acylal or diacetyl acetal, was proposed as a more suitable protecting group. The acylal can be cleaved under very mild aqueous conditions with a hydrolytic enzyme, an esterase,[19,20] and the expected product could be generated in a mixture that already contained DXR, removing any isolation steps for the 2-*C*-methylerythrose-4-phosphate. The formation of an acylal from the small amount of the aldehyde **22** was first tried with acetic anhydride and iodine at ambient temperature.[21] There was no acylal

generated under these conditions. Stronger conditions such as Lewis acids, phosphorus trichloride ($PCl_3$),[22] cobalt(II) chloride ($CoCl_2$),[23] ferric trichloride ($FeCl_3$),[24] and bismuth triflate ($Bi(OTf)_3$)[25] along with acetic anhydride were also tried, but no expected product was formed.



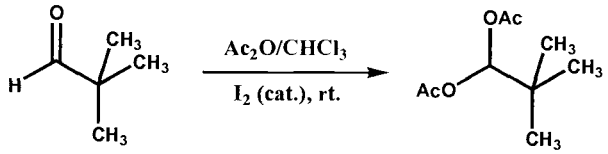

**Scheme 5.6. Proposed acylal formation and cleavage.**

The acylal formation was also tried with an aldehyde that had a structure similar to the aldehyde **22**. The aldehyde intermediate **17** from the synthetic Scheme 5.4 was chosen as the model compound. The acylal formation from **17** was tried with the aforementioned conditions. However, the corresponding acylal **26** could not be detected.




**Scheme 5.7. Proposed acylal formation from the aldehyde.**

Steric hindrance near the aldehyde was considered as a possible difficulty for acylal formation. A model reaction with pivaldehyde (**27**) which has a similar bulky group next to the aldehyde functional group, was tried under the same conditions. It turned out that the acylal of pivaldehyde (**28**) could be generated under the $Ac_2O/I_2$ conditions. Therefore, steric hindrance did not appear to be the major problem for formation of the acylal from the aldehyde. A different modification needed to be considered.

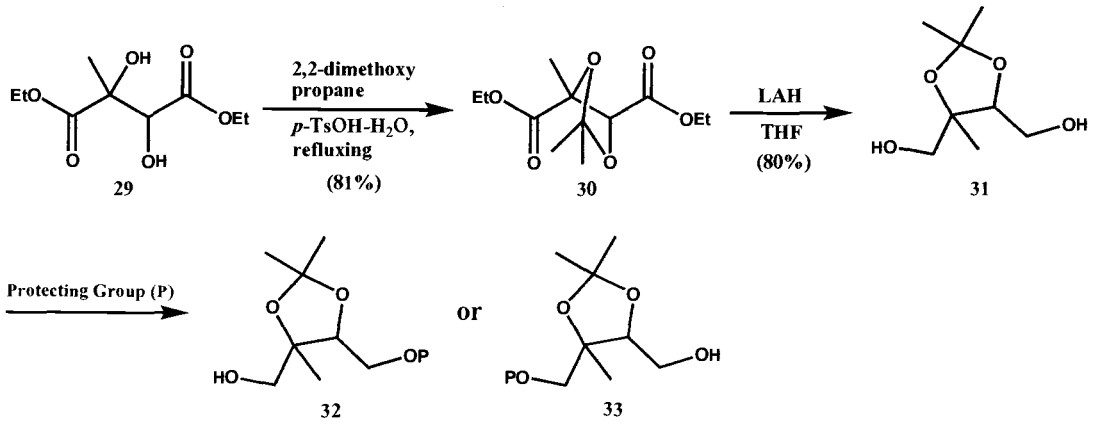



27 28

**Scheme 5.8. Acylal formation from pivaldehyde.**

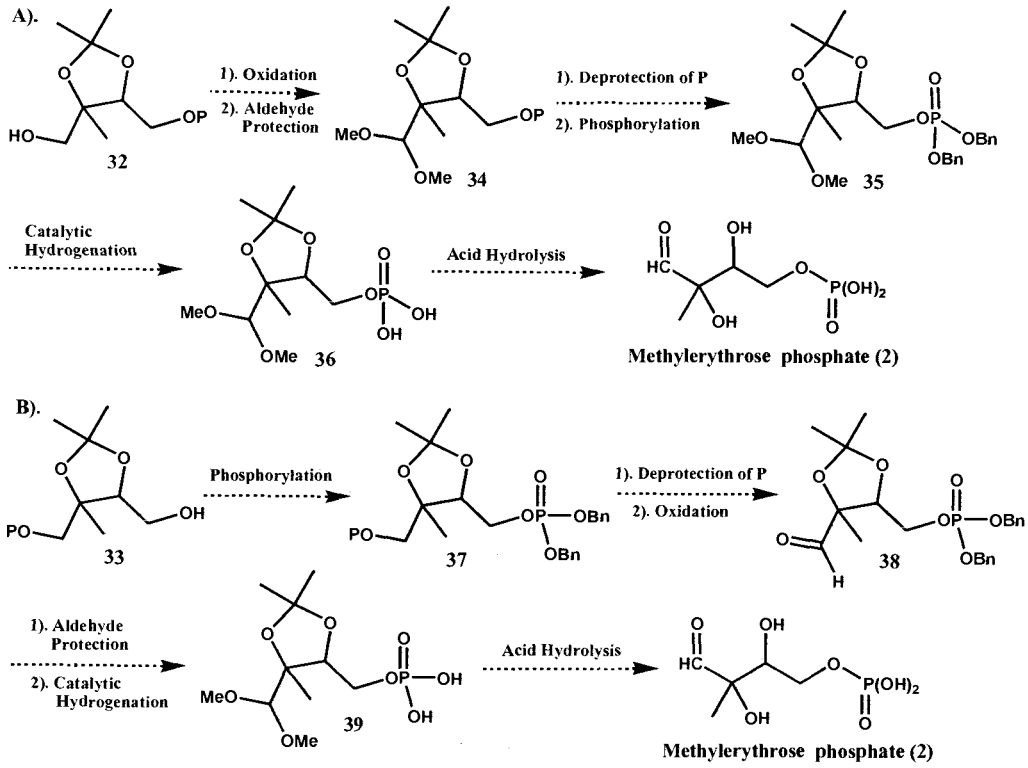
Even though further modifications of Scheme 5.4 were considered to finally obtain the 2-*C*-methyl-D-erythrose 4-phosphate, different synthetic strategies were planned due to the cost and inefficiency of Scheme 5.4. Scheme 5.4 contained many reaction steps which made the synthesis less effective and the costs of the reagents, including osmium tetroxide and silver(I) oxide were considered higher than necessary.

Another synthetic scheme for methylerythrose phosphate was proposed by using the readily obtained 2-*C*-methyl-(1,3-dioxolane) erythritol (**31**) which could be prepared in two steps from the diol **29**. The compound **29** was available in gram quantity from a different synthetic route by Dr. Younhi Woo.[26] Several protecting groups were tried in order to selectively monoprotect the diol **31**. Based on which monoprotected diol is obtained (**32** and/or **33**) the subsequent multistep reactions would be modified accordingly.



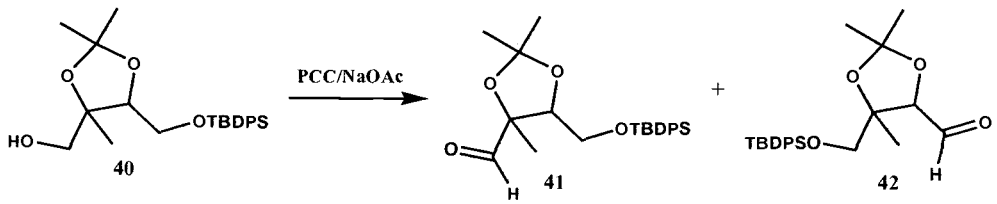

29 30 31 32 33

**Scheme 5.9. The synthesis of 2-*C*-methyl-(1,3-dioxolane) erythritol.**



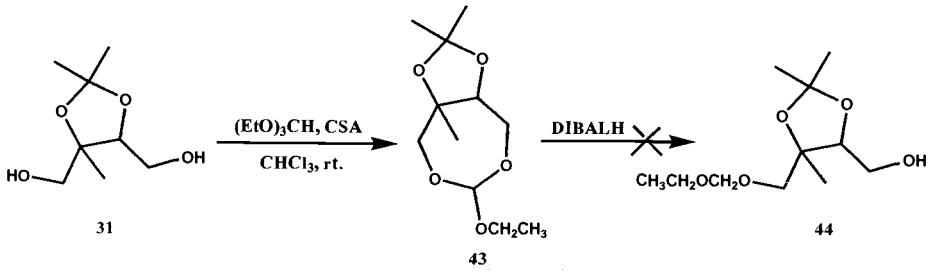

**Scheme 5.10. Proposed generic routes for methylerythrose phosphate.**

Formation of the benzyl protecting group showed no selectivity toward the diol **31**. The dibenzylated compound was obtained as the major product even when only a half equivalent of benzyl bromide was used. Silyl protecting groups such as the TBDPS- and TIPS- groups showed selectivity toward the less hindered alcohol, but a mixture of two different aldehydes **41,42** was obtained from silyl transfer process when the compound was carried on to the next step. Moreover, **41** and **42** could not be separated efficiently by silica column chromatography. When the non-basic oxidation procedure such as TPAP was used, the aldehyde **41** was not obtained.



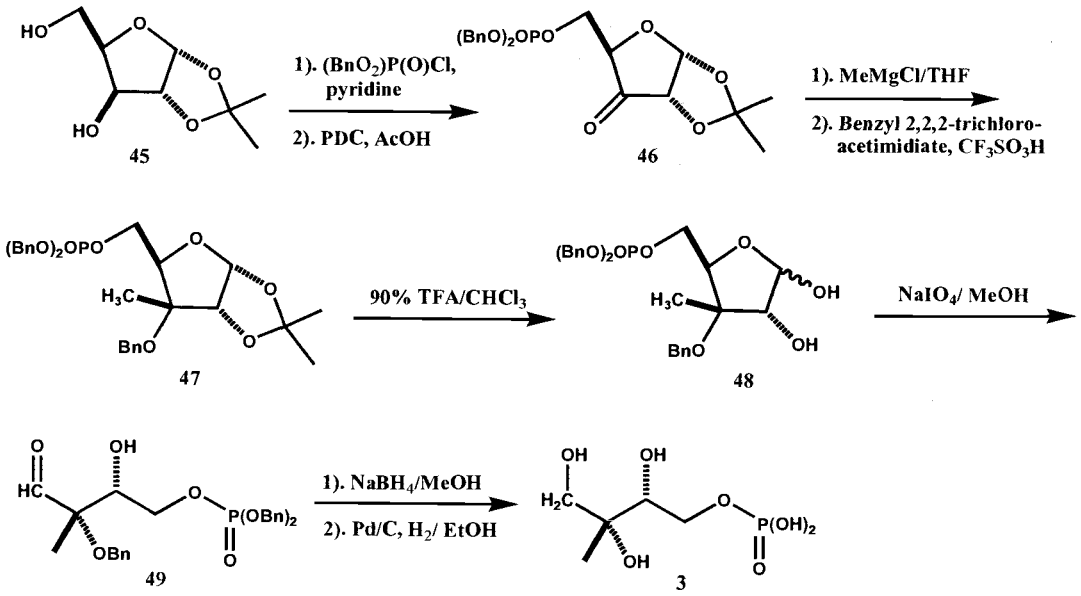

**Scheme 5.11. The undesired product from the silyl transfer process.**

Selective monoprotection of the more hindered hydroxyl group was also tried based on a procedure from Takasu *et al.*[27] This process is based on the regioselective cleavage of an orthoester with DIBALH. Unfortunately, the seven-member ring ortho ester (**43**) was very stable and could not be reduced with DIBALH as expected.



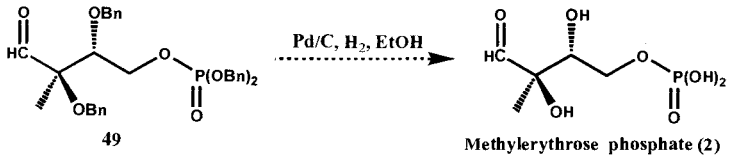

**Scheme 5.12. Another proposed selective protection of diol (31).**

During these attempts to synthesize of 2-*C*-methylerythrose phosphate, there was a report for the synthesis of 2-*C*-methylerythritol phosphate (**3**) from Hoeffler *et al.*[28] as shown in Scheme 5.13.



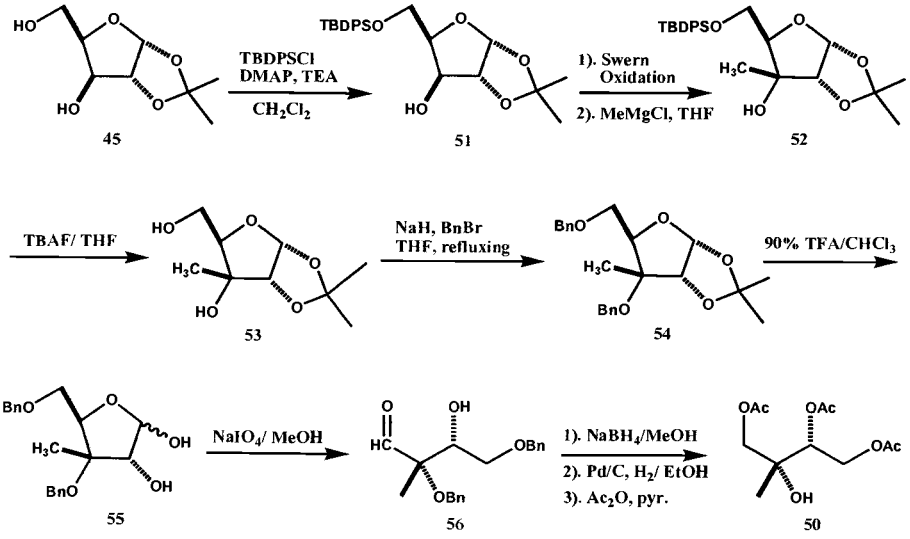

**Scheme 5.13. The synthesis of 2-*C*-methylerythritol phosphate by Hoeffler *et al.*[28]**

One of the intermediates in this synthetic scheme, the aldehyde **49** could possibly be converted into 2-C-methylerythrose phosphate by catalytic hydrogenation. The aldehyde functional group of this intermediate should be stable under these neutral conditions. Reduction of the aldehyde by catalytic hydrogenation typically only occurs under acidic condition of catalytic hydrogenation.[29]



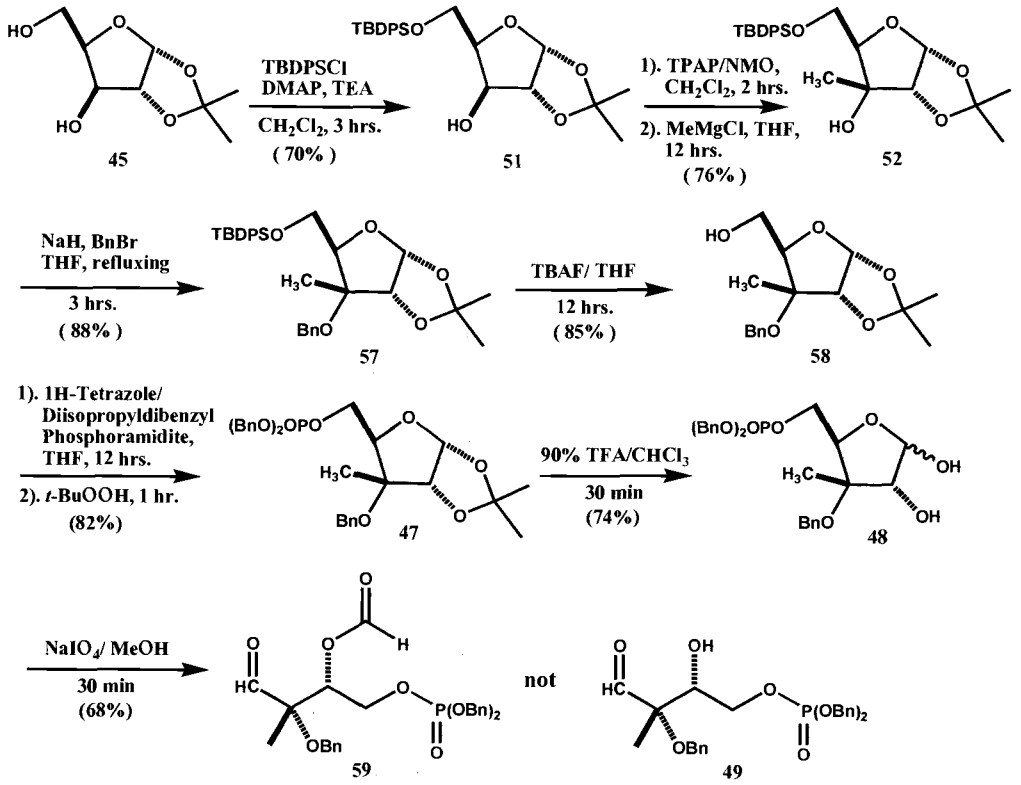

**Scheme 5.14. Proposed catalytic hydrogenation to form 2-*C*-methyl-erythrose phosphate.**

However, the synthetic Scheme 5.13 could not be repeated because of difficulty in benzylation of the tertiary alcohol with benzyl 2,2,2-trichloroacetimidate under acidic catalytic condition using trifluoromethane sulfonic acid.[30] The same research group also published a different procedure for benzylation of a tertiary alcohol for the synthesis of 2-*C*-methylerythritol triacetate **(50)**.[31] This procedure used sodium hydride/benzyl bromide in refluxing THF as the benzylation conditions for the tertiary alcohol.[32] This reaction is milder and more practical to conduct when compared to the previous procedure.




**Scheme 5.15. The synthesis of 2-*C*-methylerythritol triacetate by Rohmer *et al.*[31]**

Therefore, a modified combination of the 2-*C*-methylerythritol phosphate (**3**) and 2-*C*-methylerythritol triacetate (**50**) syntheses by Hoeffler *et al.*[29,31] was conducted to obtain the expected dibenzylphosphate aldehyde (**49**) as shown in Scheme 5.16.

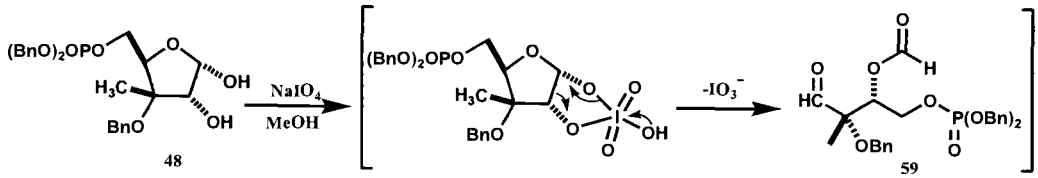



**Scheme 5.16. An attempt to synthesize methylerythrose phosphate (Route II).**

The alcohol **51** was obtained by selective protection of the commercially available 1,2-*O*-isopropylidene-D-xylofuranose (**45**) with a *tert*-butyldiphenylsilyl group. The oxidation of **51** with TPAP followed by a methyl Grignard addition to the resulting ketone gave **52** as a single diastereomer with the desired configuration.[31] Benzylation of the tertiary alcohol **52** was performed with 88% yield.[32] The primary alcohol **58** was obtained from deprotection of the *tert*-butyldiphenylsilyl group of **57**. In the next step, the primary alcohol **58** was phosphorylated to yield the protected dibenzylphosphate group. According to the procedure from Hoeffler *et al.*[28], dibenzylchlorophosphate was used as a

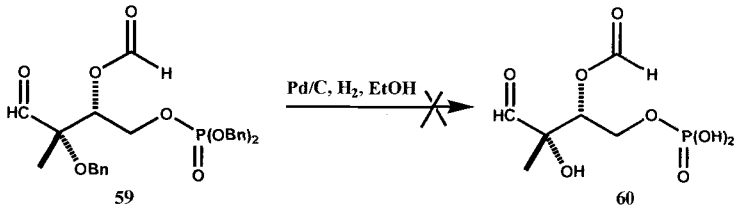
phosphorylating reagent. However, this reagent is quite unstable and needs to be prepared immediately prior to use. As an alternative, phosphoramidite chemistry was shown to work very well in this case. The acetonide protecting group in **47** was removed under acidic conditions to obtain a 3:1 anomeric mixture of hemiacetals **48** which were oxidatively and cleaved with sodium metaperiodate to presumably form the aldehyde **49**.

As mentioned in the published procedure from Hoeffler *et al.*,[28] oxidation of hemiacetal **48** with sodium metaperiodate followed by basic work up provided the aldehyde **49** and its hydrate in a 1:1 ratio. The reported chemical shift for the aldehyde hydrate proton was 7.99 ppm and the aldehyde hydrate carbon was assigned as 159.60 ppm. After we performed the same procedure with **48**, the spectrum of the product appeared to be the same as reported. However, the integration ratio of protons did not perfectly match. Rather than having 0.5:0.5 integration ratio for the aldehyde and the hydrate protons relative to the C3 proton, the ratio appeared to be 1:1. This ratio suggested that there is one more proton in the structure of the product. Moreover, the reported chemical shifts for the hydrate proton and carbon were further downfield than normal hydrate proton and carbon shifts which are about 5.5 ppm and 100 ppm respectively.[33] Because of these inconsistencies, additional NMR experiments including COSY and HSQC of the product were performed. The data seemed to fit perfectly with the formate ester **59** of the aldehyde **49**. The IR spectra also support the formate ester structure. The reaction mechanism in Scheme 17 explains the formation of the formate ester **59** from the oxidative cleavage of hemiacetal **48** with sodium metaperiodate.[34] Therefore, the reported 1:1 mixture of the aldehyde **49** and its hydrate by Hoeffler *et al.*[28] was in fact the formate ester **59**.




**Scheme 5.17. The oxidative cleavage of hemiacetal 48.**

In order to gain the aldehyde **49** as expected, basic hydrolysis of **60** was conducted with saturated $NaHCO_3$. Surprisingly, the formate functional group was quite stable under different basic and acidic conditions that were tried including $KHCO_3$ solution, $NH_4OH$ and 1 M HCl.[35] Despite the presence of the formate, a model catalytic hydrogenation reaction was tried with **59** in order to gain information about the stability of the aldehyde to the hydrogenation reaction.




59 60

**Scheme 5.18. Catalytic hydrogenation in the presence of an aldehyde.**

The catalytic hydrogenation of **59** was tried using a variety of reaction times. The $^{31}P$ NMR of the product showed only one broad peak at 0 ppm, presumably indicating only one phosphate compound was generated. However, there was no significant sign of an aldehyde, a formate or a hydrate peak from $^{1}H$ NMR. There was also a broad peak around 4-5 ppm beside the HOD peak which was very difficult to identify. The $^{13}C$ NMR spectrum of the product also did not reveal any sign of an aldehyde carbon either. Purification of the crude product was tried with cellulose column, but no desired product was obtained.

It appeared that decomposition of the aldehyde **59** occurred sometime during the catalytic hydrogenation reaction or upon workup. Unfortunately, at this stage in our synthesis of 2-*C*-methyl-D-erythrose 4-phosphate, a publication appeared by Hoeffler *et al.*[36] showing that 2-*C*-methyl-D-erythrose 4-phosphate could be synthesized and it was proved to be a true intermediate in the DXR reaction. Because of this publication, our synthetic efforts were stopped.

Surprisingly, the same catalytic hydrogenation method that was proposed in Scheme 5.14 was used to successfully synthesize 2-*C*-methyl-D-erythrose 4-phosphate. Hoeffler *et al.*[36] also indicated that this compound cannot be stored pure or in concentrated solution. Therefore, it was accordingly kept in a water solution.

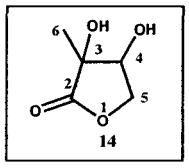
This instability might be a major reason that all previous experiments failed to obtain 2-*C*-methyl-D-erythrose 4-phosphate as the final product. Hoeffler *et al.*[36] did not mention the formate derivative **59** that was observed. It is possible that the formate group might be cleaved under the acidic condition after the free phosphate was generated.

Even though 2-*C*-methyl-D-erythrose 4-phosphate was not successfully obtained after many attempts, some of the synthetic methods were applied to the syntheses of several different compounds described in earlier Chapters. For example, the formation of the Weinreb amide in Scheme 5.4 was applied to the synthesis of 4-deoxy DXP in Chapter Two, and ethylerythritol triacetate in Chapter Three was obtained from the synthetic strategy outlined in Scheme 5.16.

### 3) Experimental Section

**General Methods:** General methods and materials are described in Chapter Two.

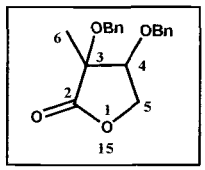
**3,4-Dihydroxy-3-methyldihydrofuran-2-one (14); Registry No. : 63700-30-1.**




To a stirred solution of 3-methyl-2(5H)-furanone **13** (1.0 g, 11.5 mmol, 1 equivalent) in 1,4-dioxane (25 mL) at room temperature were added osmiun tetroxide (150 mg, 0.58 mmol, 0.05 equivalent) and NMO (1.5 g, 12.7 mmol, 1.1 equivalents). After 24 h, saturated aqueous $NaHSO_3$ (20 mL) was added and the reaction mixture was stirred for 30 min. The solution was filtered over Celite and washed with ethyl acetate (200 mL). The organic layer was dried, filtered, concentrated and purified by flash chromatography (3:1 (v/v) ethyl acetate/hexanes) to give 940 mg (62%) of a colorless oil.

IR(thin film) 3419 (br), 2974, 2931, 1784, 1639, 1470, 1363, 1199, 1098, 1035 $cm^{-1}$; $^1H$ NMR (300 MHz, $CD_3OD$) $\delta$ 4.45 (dd, $J$ = 10.2, 4.2 Hz, $H_{5a}$), 4.15 (dd, $J$ = 10.2, 1.9 Hz, $H_{5b}$), 4.06(dd, $J$ = 4.2, 1.9 Hz, $H_4$), 1.40(s, $3H_6$); $^{13}C$ NMR (75 MHz, $CD_3OD$) 181.39 ($C_2$), 87.66 ($C_3$), 75.55 ($C_4$), 74.30 ($C_5$), 22.59 ($C_6$); HRMS(CI) calcd. for $C_5H_9O_4$ 133.0501 found 133.0504.

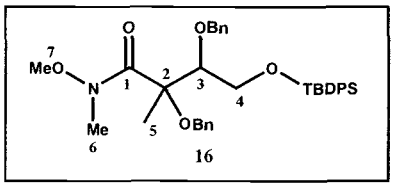
**3,4-Bisbenzyloxy-3-methyldihydrofuran-2-one (15).**




To a solution of dihydroxy furanone **14** (870 mg, 6.6 mmol, 1 equivalent) in THF (25 mL) at room temperature was added benzyl bromide (2.1 mL, 18.0 mmol, 2.7 equivalents). The reaction flask was wrapped with aluminum foil and silver(I) oxide (5.0 g, 22 mmol, 3.3 equivalents) was added in one portion. The reaction mixture was stirred for 12 h, filtered over Celite and the Celite washed with 30 mL of ethyl acetate. The filtrate was dried and concentrated. Purification by flash chromatography (1:2 (v/v) ethyl acetate/hexanes) gave 870 mg (42%) of a colorless oil.

IR(thin film) 3067, 3028, 2926, 2878, 1779, 1451, 1390, 1216, 1103, 1020 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.35 (m, 10H, Ph), 4.75 (m, 4H, benzylic protons), 4.30 (dd, $J$ = 4.8, 1.6 Hz, $2H_5$), 4.02 (t, $J$ = 5.0 Hz, $1H_4$), 1.56 (s, $3H_6$); $^{13}C$ NMR (75 MHz, $CDCl_3$) 174.57 ($C_2$), 138.11, 137.13, 128.58, 128.28, 128.17,127.63, 127.58 (Ph), 96.73 ($C_3$), 80.39 ($C_4$), 72.71 ($C_5$), 69.43, 67.88 (benzylic carbon), 20.13 ($C_6$); HRMS(CI) calcd. for $C_{19}H_{21}O_4$ 313.1440 found 313.1441.

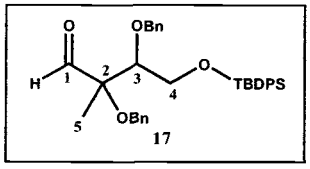
**2,3-Bisbenzyloxy-4-(*tert*-butyldiphenylsilanyloxy)-*N*-methoxy-2-*N*-dimethyl-butyramide (16).**




To a suspension of *N,O*-dimethylhydroxy-amine-HCl (390 mg, 4.0 mmol, 2.3 equivalents) in $CH_2Cl_2$ (10 mL) at 0 °C was added trimethyl aluminium (2.0 M in toluene, 2.0 mL, 2.3 equivalents). After the addition was complete, the colorless solution was stirred at room temperature for 15 min and then cooled to 0 °C, and a solution of the dibenzylated furanone **15** (540 mg, 1.7 mmol, 1 equivalent) in 15 mL of $CH_2Cl_2$ was added. After 45 min, the reaction mixture was quenched by adding 3 mL of water. The reaction mixture was filtered over Celite, which was then washed with 50 mL of ethyl acetate. The filtrate was dried and concentrated. Water in the crude reaction product was removed azeotropically by concentrating from toluene three times and the crude desired product was used

without further purification. To a solution of the crude amide in 10 mL of $CH_2Cl_2$ were added TEA (1.2 mL, 5 equivalents), TBDPSCl (1.0 mL, 2.3 equivalents), and 5 mg of DMAP. After being stirred at room temperature for 12 h, 3 mL of water was added and the product was extracted with $CH_2Cl_2$ (3×20 mL). The combined organic layers were dried and concentrated. Purification by flash chromatography (1:3 (v/v) ethyl acetate/hexanes) provided 270 mg (51% for 2 steps) of a yellow oil. IR(thin film) 3024, 2929, 2859, 1955, 1881, 1821, 1656, 1592, 1453,1366, 1210, 1111, 1059 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.30 (m, 20H, Ph), 5.12 (d, $J$ = 11.5 Hz, 1H, benzylic proton), 4.81(d, $J$ = 11.6 Hz, 1H, benzylic proton), 4.62 (d, $J$ = 11.5 Hz, 1H, benzylic proton), 4.32 (d, $J$ = 11.6 Hz, 1H, benzylic proton), 4.13 (m, $2H_{4a,3}$), 3.95 (dd, $J$ = 10.8, 7.9 Hz, $1H_{4b}$), 3.57 (s, $3H_6$), 3.22 (s, $3H_7$), 1.52 (s, $3H_5$), 1.14 (s, 9H, *t*-butyl); $^{13}C$ NMR (75MHz, $CDCl_3$) 175.30 ($C_1$), 135.62, 133.45, 129.59, 128.18, 127.67, 127.52, 127.23, 126.89 (Ph), 82.97 ($C_2$), 82.39($C_3$), 74.22($C_4$), 65.84, 65.06 (benzylic carbons), 60.37($C_7$), 35.06 ($C_6$), 26.83 (3×$CH_3$ of *t*-butyl), 19.17 (quaternary carbon of *t*-butyl), 16.49 ($C_5$); LRMS(CI) calcd. for $C_{37}H_{46}NO_5Si$ 612.3 found 612.1.

**2,3-Bisbenzyloxy-4-(*tert*-butyldiphenylsilanyloxy)-2-methylbutyraldehyde (17).**

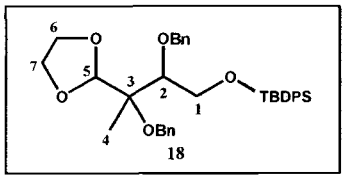



To a solution of the amide **16** (270 mg, 0.4 mmol, 1 equivalent) in 10 mL of THF was added DIBALH (1.0 M/THF, 880 μL, 0.8 mmol, 2 equivalents) at -78 °C. After being stirred at -78 °C for 3 h, 3 mL of methanol was added at 0 °C and the mixture was stirred for an additional 1 h. The reaction mixture was filtered over Celite and the Celite was washed with 30 mL of hot methanol. The filtrate was dried and concentrated. Purification by flash chromatography (1:5 (v/v) ethyl acetate/hexanes) yielded 198 mg (81%) of a colorless oil.

IR(thin film) 3067, 2929, 2863, 2699, 1954, 1891, 1818, 1731, 1591, 1461, 1432, 1388, 1098 $cm^{-1}$; $^1H$ NMR (300MHz, $CDCl_3$) δ 9.67 (s, $1H_1$), 7.50 (m, 20H, Ph), 4.78 (d, $J$ = 11.6 Hz, 1H, benzylic proton), 4.60(d, $J$ = 11.8 Hz, 2H, benzylic proton), 4.47 (d, $J$ = 11.5 Hz, 1H, benzylic proton), 4.41 (dd, $J$ = 9.0, 3.7 Hz, $H_{4a}$),

3.85 (m, $H_{4b,3}$), 1.41 (s, $3H_5$), 1.06 (s, 9H, *t*-butyl); $^{13}C$ NMR (75MHz, $CDCl_3$) 202.86 ($C_1$), 138.43, 138.10, 135.59, 132.99, 129.74, 128.31, 127.76, 127.48, 127.31 (Ph), 83.33 ($C_2$), 83.11 ($C_3$), 74.09 ($C_4$), 66.35, 63.15 (benzylic carbons), 26.69 ($3\times CH_3$ of *t*-butyl), 19.05 (quaternary carbon of *t*-butyl), 14.64 ($C_5$); HRMS(CI) calcd. for $C_{35}H_{41}O_4Si$ 553.2774 found 553.2753.

**(2,3-Bisbenzyloxy-3-[1,3]dioxolan-2-ylbutoxy)-*tert*-butyldiphenylsilane (18).**

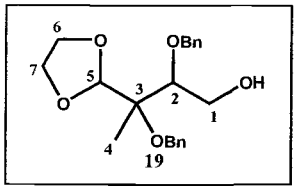


18

To a solution of the aldehyde **17** (198 mg, 0.4 mmol, 1 equivalent) in 15 mL of benzene was added ethylene glycol (200 μL, 4 mmol, 10 equivalents), and *p*-toluenesulfonic acid monohydrate (10 mg, 0.05 mmol, 0.1 equivalent). The reaction mixture was heated to reflux under an argon atmosphere for 2 h before quenching with TEA (1 mL). The reaction mixture was concentrated and purified by flash chromatography (1:5 (v/v) ethyl acetate/hexanes) to yield 166 mg (78%) of a colorless oil.

IR(thin film) 3364, 3069, 3024, 2929, 2884, 2857, 1731, 1603, 1453, 1430, 1369, 1179, 1112, 1051 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.55 (m, 20H, Ph), 5.03 (d, $J$ =11.6 Hz, 1H, bezylic proton), 5.02 (s, $1H_5$), 4.70 (d, $J$ = 11.3 Hz, 2H, benzylic protons), 4.64 (d, $J$ = 3.5 Hz, $1H_{1a}$), 4.13 (d, $J$ = 8.1 Hz, 1H, benzylic proton ), 3.90 (m, $6H_{1b,\ 2,6,7}$), 1.32 (s, $3H_4$), 1.06 (s, 9H, *t*-butyl); $^{13}C$ NMR (75MHz, $CDCl_3$) 139.96, 139.35, 135.74, 133.52, 133.39, 129.58, 128.62, 128.57, 128.09, 128.08, 127.99, 127.68, 126.88 (Ph), 105.89 ($C_5$), 83.59 ($C_2$), 79.04 ($C_3$), 74.63 ($C_1$), 66.26 (benzylic carbon), 65.29 (benzylic carbon and $C_6$), 64.99 ($C_6$), 26.79 ($3\times CH_3$ of *t*-butyl), 19.12 (quaternary carbon of *t*-butyl), 13.81 ($C_4$); HRMS(CI) calcd. for $C_{37}H_{45}O_5Si$ 597.3036 found 597.3053.

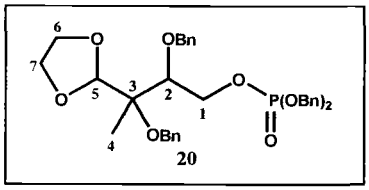
**2,3-Bisbenzyloxy-3-[1,3]dioxolan-2-ylbutan-1-ol (19).**




To a solution of the acetal **18** (166 mg, 0.3 mmol, 1 equivalent) in 10 mL of THF was added TBAF (1.0 M in THF, 600 μL, 2.0 equivalents). The reaction mixture was stirred for 4 h at room temperature and concentrated. The crude reaction mixture was purified by flash chromatography (3:1 (v/v) ethyl acetate/hexanes) to give 87 mg (87%) of a colorless oil.

IR(thin film) 3430(br), 3062, 3027, 2948, 2878, 1950, 1877, 1811, 1750, 1601, 1457, 1395, 1212, 1098, 1028 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.35 (m, **10**H, Ph), 5.05 (s, $H_5$), 4.74 (m, 4H, benzylic protons), 3.90 (m, 7H, benzylic protons, $H_{1a,1b,2,6,7}$), 2.49 (br, 1H, hydroxyl proton), 1.45 (s, $3H_4$); $^{13}C$ NMR (75MHz, $CDCl_3$) 139.15, 138.39, 128.29, 127.63, 127.57, 127.22 (Ph), 106.01 ($C_5$), 81.60 ($C_2$), 80.41 ($C_3$), 73.39 ($C_1$), 66.46, 65.30 (benzylic carbons), 64.91, 61.30 ($C_{6,7}$), 13.57 ($C_4$); HRMS(CI) calcd. for $C_{21}H_{27}O_5$ 359.1859 found 359.1856.

**Phosphoric acid dibenzyl ester 2,3-bisbenzyloxy-3-[1,3]dioxolan-2-ylbutyl ester (20).**

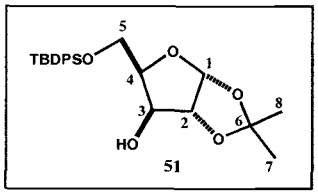



To a solution of the alcohol **19** (68 mg, 0.19 mmol, 1 equivalent) in 10 mL of THF was added 1H-tetrazole (20 mg, 0.23 mmol, 1.2 equivalents) in one portion at room temperature. The reaction mixture was stirred at room temperature for 15 min before cooling to 0 °C and a solution of diisopropyl dibenzylphospho-ramidite (100 μL, 0.29 mmol, 1.5 equivalents) was added. The reaction mixture was stirred at room temperature for 20 h and then $^t$BuOOH (1 mL) was added. After being stirred for an additional 30 min, the reaction mixture was concentrated, and purified by flash chromatography (1:1 (v/v) ethyl acetate/hexanes) to give 58 mg (49%) of a colorless oil.

IR(thin film) 3440, 3059, 3024, 2953, 2887, 1960, 1880, 1810, 1726, 1611, 1504, 1464, 1389, 1265, 1216, 1110, 1012 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.30

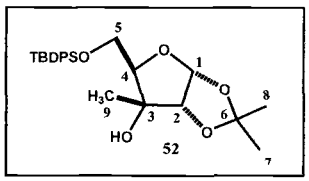
(m, 20H, Ph), 5.10 (m, 6H, benzylic protons and $H_5$), 4.70 (m, 3H, benzylic protons), 4.50 (m, $H_{1a}$), 4.25 (m, $H_{1b}$), 3.90 (m, 5H, $H_{2,6,7}$), 1.35 (s, $3H_4$);
$^{13}C$ NMR (75MHz, $CDCl_3$) 138.60, 128.50, 128.38, 128.19, 127.81, 127.60, 127.40, 127.11 (Ph), 105.93 ($C_5$), 81.34 ($C_3$), 81.30 ($C_2$), 79.14 ($C_1$), 74.51 (benzylic carbons), 69.11, 66.29 (benzylic carbons), 65.35, 65.07 ($C_{6,7}$), 13.92 ($C_4$);
$^{31}P$ NMR (121MHz, $CDCl_3$) -0.21; HRMS(CI) calcd. for $C_{35}H_{39}O_8P$ 619.2461 found 619.2415.

**1,2-*O*-Isopropylidene-5-(*tert*-Butyldiphenylsilanyloxy) -α-D-xylofuranose (51); Registry No. : 11486-14-2.**




To a solution of 1,2-*O*-isopropylidene-α-D-xylofuranose **45** (1.0 g, 5.26 mmol, 1.0 equivalent) in 30 mL of THF at 0 °C was added a solution of TEA (1.8 mL, 13.2 mmol, 2.5 equivalents), TBDPSCl (1.6 mL, 6.32 mmol, 1.2 equivalents), and DMAP (10 mg, catalytic amounts). After being stirred for 3 h at room temperature, 10 mL of water was added slowly to a reaction mixture and the reaction mixture was extracted with $CH_2Cl_2$ (3×20 mL). The combined organic layers were dried and concentrated. Purification by flash chromatography (1:4 (v/v) ethyl acetate/hexanes) gave 1.74 g (70%) of a yellow oil.
$[\alpha]^{25}_D$ -1.8° (*c* 2.5, $CH_2Cl_2$), lit = -2.3° (*c* 2.5, $CHCl_3$)[37]; IR (thin film) 3463 (br), 3076, 2936, 2883, 2859, 1466, 1427, 1379, 1253, 1219, 1166, 1113, 1079 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.55 (m, 10H, Ph), 6.00 (d, $J$ = 3.7 Hz, $H_1$), 4.54 (d, $J$ = 3.6 Hz, $H_2$), 4.36 (m, $H_3$), 4.12 (m, 3H, $H_{4,5a,5b}$), 1.47 (s, $3H_8$), 1.33 (s, $3H_7$), 1.05 (s, 9H, *t*-butyl); $^{13}C$ NMR (75 MHz, $CDCl_3$) 135.69, 135.49, 130.04, 127.89 (Ph), 111.43 ($C_6$), 104.94 ($C_1$), 85.39 ($C_2$), 78.38 ($C_4$), 76.75 ($C_3$), 62.71 ($C_5$), 26.73 ($C_8$), 26.64 (3×$CH_3$ of *t*-butyl), 26.13 ($C_7$), 19.03 (quaternary carbon of *t*-butyl); HRMS(CI) calcd. for $C_{24}H_{32}SiO_5$ 428.2019 found 428.1998.

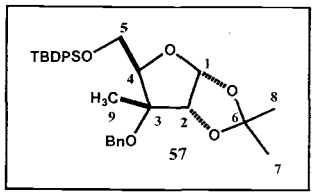
**1,2-*O*-Isopropylidene-3-*C*-methyl-5-(*tert*-Butyldiphenylsilanyloxy)-α-D-ribofuranose (52); Registry No. :286408-85-3.**




To a solution of the alcohol **51** (1.6 g, 3.36 mmol, 1 equivalent) in 30 mL of $CH_2Cl_2$ were added NMO (0.59 g, 5.04 mmol, 1.5 equivalents), 1 g of finely crushed and activated 4 Å molecular sieves and TPAP (60 mg, 0.17 mmol, 0.05 equivalents). The reaction mixture was stirred for 2 h, and filtered over Florisil. The Florisil was washed with 50 mL of $CH_2Cl_2$. The filtrate was dried and concentrated to give a yellow oil of the crude ketone product. The crude product was dissolved in 30 mL of THF and methylmagnesium chloride (3.0 M in THF, 3.4 mL, 10.0 mmol, 3.0 equivalents) was added at -78 °C. The reaction mixture was stirred at -78 °C for 4 h, warmed to room temperature, and stirred overnight for an additional 12 h. The reaction mixture was quenched with 10 mL of saturated $NH_4Cl$ and extracted with diethyl ether (3 × 30 mL). The combined organic layers were dried and concentrated. The residue was purified by flash chromatography (1:5 (v/v) ethyl acetate/hexanes) to give 1.25 g (76% for 2 steps) of a light yellow oil.

$[\alpha]^{25}_D$ +30° (*c* 1.4, $CH_2Cl_2$); IR (thin film) 3559 (br), 3061, 2941, 2859, 1775, 1466, 1427, 1374, 1209, 1118 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.55 (m, 10H, Ph), 5.76 (d, $J$ = 3.8 Hz, $H_1$), 4.08 (d, $J$ = 3.8 Hz, $H_2$), 3.89 (dd, $J$ = 6.2, 4.5 Hz, $H_4$), 3.83 (s, $H_{5a}$), 3.81 (d, $J$ = 1.4 Hz, $H_{5a}$), 2.05 (s, OH), 1.58 (s, $3H_8$), 1.36 (s, $3H_7$), 1.12 (s, $3H_9$), 1.07 (s, 9H, *t*-Bu); $^{13}C$ NMR (75 MHz, $CDCl_3$) 135.65, 133.17, 129.69, 127.66 (Ph), 112.42 ($C_6$), 103.54 ($C_1$), 84.36 ($C_2$), 81.82($C_4$), 76.95 ($C_3$), 62.31 ($C_5$), 26.79 ($C_8$), 26.62 (3×$CH_3$ of *t*-butyl), 26.55 ($C_7$), 19.16 (a quaternary carbon of *t*-butyl), 18.57 ($C_9$); HRMS(CI) calcd. for $C_{25}H_{34}SiO_5$ 442.2176 found 442.2168.

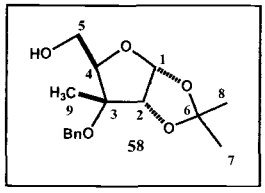
**3-*O*-Benzyl-1,2-*O*-isopropylidene-3-*C*-methyl-5-(*tert*-Butyldiphenylsilanyloxy)-α-D-ribofuranose (57).**




To a suspension of NaH (60% in mineral oil, 0.15 g, 3.38 mmol, 1.5 equivalent) in 30 mL of THF at 0 °C was added a solution of the alcohol **52** (1.25 g, 2.55 mmol, 1.0 equivalent) in 20 mL of THF over a 15 min period. The slurry was warmed to room temperature and stirred for 10 min before benzyl bromide (0.46 mL, 3.38 mmol, 1.5 equivalents) was added over a 15 min period. After 5 min, 20 mg of *n*-$Bu_4NI$ was added. The reaction mixture was heated to reflux for 3 h and was further stirred for 12 h at room temperature. The reaction mixture was quenched by adding 10 mL of water and extracted with diethyl ether (3×20 mL). The combined organic layers were dried and concentrated. Purification by flash chromatography (1:6 (v/v) ethyl acetate/hexanes) gave 1.30 g (79%) of a light yellow oil.

$[\alpha]^{25}_D$ +47° (*c* 1.0, $CH_2Cl_2$); IR (thin film) 3070, 2927, 2858, 1461, 1427, 1382, 1215, 1141, 1101, 1013 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.55 (m, 15H, Ph), 5.78 (d, $J$ = 3.8 Hz, $H_1$), 4.56 (ABq, $J$ = 11.0, 18.7 Hz, 2H, benzylic protons), 4.29 (d, $J$ = 3.7 Hz, $H_2$), 4.25 (dd, $J$ = 6.6, 3.6 Hz, $H_4$), 3.87 (dd, $J$ = 11.3, 3.7 Hz, $H_{5a}$), 3.80 (dd, $J$ = 11.4, 6.7 Hz, $H_{5b}$), 1.60 (s, $3H_8$), 1.33 (s, $3H_7$), 1.15 (s, $3H_9$), 1.05 (s, 9H, *t*-Bu); $^{13}C$ NMR (75 MHz, $CDCl_3$) 138.86, 135.69, 133.51, 129.50, 128.09, 127.56, 127.44, 127.26 (Ph), 112.69 ($C_6$), 104.17 ($C_1$), 83.20 ($C_2$), 81.98 ($C_3$), 81.76 ($C_4$), 66.38 (benzylic carbons), 62.67 ($C_5$), 26.92 ($C_8$), 26.76 ($4CH_3$, $3\times CH_3$ of *t*-butyl and $C_7$), 19.19 (quaternary carbon of *t*-butyl), 16.43 ($C_9$); LRMS(CI) calcd. for $C_{32}H_{40}SiO_5$ ($-CH_3$) 517.3 found 517.2.

**3-*O*-Benzyl-1,2-*O*-isopropylidene-3-*C*-methyl-α-D-ribofuranose (58).**

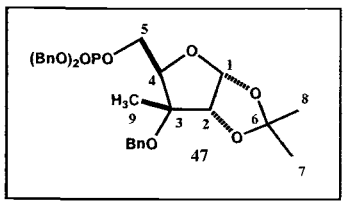



To a solution of the benzylated compound **57** (1.3 g, 2.24 mmol, 1 equivalent) in 30 mL of THF was added TBAF (1.0 M in THF, 4.40 mL, 4.48 mmol, 2.0 equivalents). The reaction mixture was stirred at room temperature for 12 h, and

concentrated to a thick orange oil. Purification by flash chromatography (1:1 (v/v) ethyl acetate/hexanes) gave 710 mg (76%) of a colorless oil.

$[\alpha]^{25}_D$ +40° (*c* 3.0, $CH_2Cl_2$); IR (thin film) 3439 (br), 2984, 2936, 1451, 1379, 1219, 1171, 1137, 1088 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.35 (m, 5H, Ph), 5.78 (d, $J$ = 3.8 Hz, $H_1$), 4.60 (ABq, $J$ = 18.6, 10.8 Hz, 2H, benzylic protons), 4.35 (d, $J$ = 3.8 Hz, $H_2$), 4.21 (dd, $J$ = 6.6, 4.3 Hz, $H_4$), 3.82 (dd, $J$ = 11.7, 4.3 Hz, $H_{5a}$), 3.76 (dd, $J$ = 11.5, 6.7 Hz, $H_{5b}$), 1.85 (s, 1H, hydroxyl proton), 1.60 (s, $3H_8$), 1.40 (s, $3H_7$), 1.23 (s, $3H_3$); $^{13}C$ NMR (75 MHz, $CDCl_3$) 138.39, 128.24, 127.58 (Ph), 112.99 ($C_6$), 104.23 ($C_1$), 83.01 ($C_2$), 82.16 ($C_3$), 80.84 ($C_4$), 66.68 (benzylic carbons), 60.99 ($C_5$), 26.78 ($C_8$), 26.62 ($C_7$), 16.36 ($C_9$); HRMS(CI) calcd. for $C_{16}H_{23}O_5$ 295.1546 found 295.1550.

**3-*O*-Benzyl-1,2-*O*-isopropylidene-3-*C*-methyl-α-D-ribofuranose-5-dibenzyl-phosphate (47); Registry No. : 270076-05-6.**

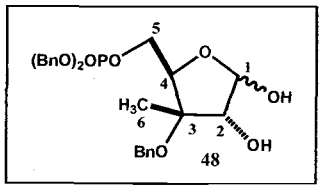



To a solution of the alcohol **58** (470 mg, 1.59 mmol, 1 equivalent) in 20 mL of THF was added 1H-tetrazole (170 mg, 2.40 mmol, 1.5 equivalents) in one portion at room temperature. The reaction mixture was stirred at room temperature for 15 min before cooling to 0 °C and a solution of diisopropyl dibenzylphosphoramidite (0.81 mL, 2.40 mmol, 1.5 equivalents) was added. The reaction mixture was stirred at room temperature for 12 h and $^t$BuOOH (1 mL) was added. After being stirred for an additional 1 h, the reaction mixture was concentrated, and the crude product was purified by flash chromatography (1:1 (v/v) ethyl acetate/hexanes) to give 730 mg (82%) of a colorless oil.

$[\alpha]^{25}_D$ +21° (*c* 2.8, $CH_2Cl_2$); IR (thin film) 3030, 2981, 2937, 2908, 1496, 1456, 1382, 1254, 1215, 1166, 1136, 1087, 1013 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.35 (m, 15H, Ph), 5.78 (d, $J$ = 3.8 Hz, $H_1$), 5.03 (2d, $J$ = 8.3 Hz, 4H, benzylic protons), 4.57 (ABq, $J$ = 21.3,10.7 Hz, 2H, benzylic protons), 4.33 (d, $J$ = 3.8 Hz, $H_2$), 4.30 (dd, $J$ = 7.8, 2.8 Hz, $H_4$), 4.23 (ddd, $J$ = 10.2, 6.4, 2.8 Hz, $H_{5a}$), 4.13 (ddd, $J$ = 11.0, 7.8, 6.5 Hz, $H_{5b}$), 1.55 (s, $3H_8$), 1.35 (s, $3H_7$), 1.25 (s, $3H_9$); $^{13}C$ NMR (75 MHz,

$CDCl_3$) 138.31, 135.81, 128.45, 128.35, 128.23, 127.97, 127.88, 127.64, 127.55 (Ph), 112.99 ($C_6$), 104.29 ($C_1$), 82.71 ($C_2$), 81.91($C_3$), 79.65 (d, $J$ = 7.9 Hz, $C_4$), 69.30 (d, $J$ = 6.9 Hz, $C_{5a}$), 69.20 (d, $J$ = 6.9 Hz, $C_{5b}$), 66.69 (benzylic carbons), 66.14 (d, $J$ = 4.9 Hz, benzylic carbons), 26.82 ($C_8$), 26.65 ($C_7$), 16.19 ($C_9$); $^{31}$P NMR (121 MHz, $CDCl_3$) 0.03; HRMS(CI) calcd. for $C_{30}H_{36}O_8P$ 555.2148 found 555.2141.

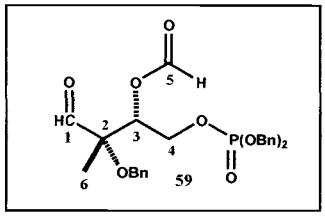
**3-*O*-Benzyl-3-*C*-methyl-D-ribofuranose 5-dibenzylphosphate (48).**




To a solution of the dibenzylphosphate compound **47** (730 mg, 1.32 mmol) in 15 mL of $CHCl_3$ was added a 90% aqueous solution of TFA (4 mL) at 0 °C. After being stirred at 0 °C for 30 min, the reaction mixture was extracted with 3×20 mL of $CHCl_3$. The combined organic layers were dried and concentrated. Purification by flash chromatography (4:1 (v/v) ethyl acetate/hexanes) gave 500 mg (74%) of the two anomers as a colorless oil.

IR (thin film) 3459 (br), 3065, 3036, 2937, 2883, 1736, 1500, 1461, 1387, 1219, 1160, 1023 cm$^{-1}$; $^1$H NMR (300 MHz, $CDCl_3$) δ 7.35 (m, 15H, Ph), 5.26 (d, $J$ = 3.1 Hz, 1/3$H_1$), 5.16 (d, $J$ = 4.5 Hz, $H_1$), 5.05 (m, 5H, benzylic protons), 4.46 (m, 2+2/3H, 1$H_4$), 4.34 (td, $J$ = 4.8, 1.2 Hz, 1/3$H_4$), 4.13 (m, 2+2/3$H_5$), 3.93 (ddd, $J$ = 11.1, 6.1, 5.0 Hz, 1+1/3$H_5$), 3.77 (d, $J$ = 4.5 Hz, 1/3$H_2$), 3.75 (d, $J$ = 3.7 Hz, 1$H_2$), 1.43 (s, 1$H_6$), 1.35 (s, 3$H_6$); $^{13}$C NMR (75 MHz, $CDCl_3$) 137.58, 137.11, 135.61, 135.54, 128.69, 128.58, 128.50, 128.37, 128.14, 128.08, 127.98, 127.85, 127.66, 127.39 (Ph), 103.02, 96.69 ($C_1$), 82.37, 82.07 ($C_2$), 81.66, 80.57($C_3$), 75.84, 79.64 (d, $J$ = 3.0 Hz, $C_4$), 69.91, 69.50 (d, $J$ = 5.8 Hz, $C_5$), 66.53 (d, $J$ = 5.6 Hz, benzylic carbon), 66.09, 65.53 (benzylic carbon), 16.21, 15.54 ($C_6$); $^{31}$P NMR (121 MHz, $CDCl_3$) 0.37, 0.05; HRMS(CI) calcd. for $C_{27}H_{32}O_8P$ (-$H_2O$) 497.1729 found 497.1710.

**2-*O*-Benzyl-2-*C*-methyl-3-*O*-formyl-D-erythrose-4-dibenzylphosphate (59).**



To a solution of hemiacetal **48** (200 mg, 0.39 mmol, 1 equivalent) in 5 mL of methanol and 3 mL of water was added sodium metaperiodate (130 mg, 0.58 mmol, 1.5 equivalents). The resulting suspension was stirred for 30 min at room temperature, neutralized with sodium hydrogen carbonate and then extracted with $CHCl_3$ (3×10 mL). The combined extracts were dried, filtered and concentrated to give 128 mg (68%) of a colorless oil.

IR (thin film) 2984, 2902, 1729, 1446, 1388, 1234, 1176, 1026 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 9.55 (s, $H_1$), 8.00 (s, $H_5$), 7.35 (m, 15H, Ph), 5.51 (ddd, $J$ = 7.6, 4.3, 3.4, 0.9 Hz, $H_3$), 5.02 (ABq, $J$ = 8.4, 2.3 Hz, 4H, benzylic protons), 4.49 (ABq, $J$ = 21.5, 11.1 Hz, 2H, benzylic protons), 4.15 (m, $H_{4a,4b}$), 1.30 (s, $3H_6$); $^{13}C$ NMR (75 MHz, $CDCl_3$) 200.82 ($C_1$), 159.55 ($C_5$), 137.64, 136.01, 135.92, 129.03, 128.95, 128.44, 127.89 (Ph), 81.89 ($C_2$), 72.30 (d, $J$ = 7.8 Hz, $C_5$), 69.55 (d, $J$ = 5.3 Hz, $C_4$), 66.53 (benzylic carbons), 64.90 (d, $J$ = 5.0 Hz, benzylic carbons), 14.45 ($CH_3$); $^{31}P$ NMR (121 MHz, $CDCl_3$) -0.1; HRMS(CI) calcd. for $C_{26}H_{28}O_7P$ (-CO) 483.1573 found 483.1571.

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# CHAPTER SIX

## CONCLUSION

The main focus in this dissertation was to study the enzyme deoxyxylulose phosphate reductoisomerase (DXR) from a chemical perpective to gain a better understanding of how this enzyme functions. The enzyme DXR from *Synechocystis* sp. PCC6803 has been used for previous studies in our laboratory and was therefore used in this current research. Different analogs of the DXR substrate, DXP were considered as tools for understanding the requirements of the DXR-substrate binding in depth. Six analogs of DXP were designed based on the individual structural features of DXP. Compounds with trivial names 1-Me-DXP (containing an ethyl ketone moiety), DX-phosphonate (DXP having a phosphonate group rather than a phosphate group), 4-epi-DXP (possessing the opposite stereochemistry at the $C_4$ position compared to DXP), 4-deoxy-DXP (lacking the hydroxyl group at the $C_4$ position), 3-deoxy-DXP (lacking the hydroxyl group at the $C_3$ position), and DXP carboxamide (having an amide group rather than the methyl ketone) were synthesized and tested as alternate substrates and enzyme inhibitors against DXR. While DX-phosphonate was the only compound that acted as the alternate substrate for DXR with the $K_m$ about three and a half-fold higher than the $K_{m(DXP)}$, the remaining compounds behaved as weak competitive inhibitors against DXR with the $K_i$ values in the micromolar range. Antibiotic tests for all DXP analogs were conducted, however, no antibiotic activity was observed with up to 500 μg of each compounds.

In summary, the analogs have provided several important pieces of information regarding the roles of various parts of the DXP structure. The 1-Me-DXP analog indicated that additional steric bulk in the region of the methyl ketone group of DXP prevents turnover. Although the 1-Me-DXP compound could bind with a several-fold lower affinity to DXR, this result suggests that future inhibitors should not contain large groups in this region of the molecule. The presence of both of the hydroxyls at C3 and C4, with the correct stereochemistry, appears to be

essential for enzymatic activity. The lack of these hydroxyl, however, does not prevent binding to DXR, as was previously known from the structure of fosmidomycin, which lacks any backbone hydroxyls. The DX-phosphonate compound shows that an appropriate replacement for the phosphate group will not interfere with catalysis, but there is a penalty in that the binding affinity is reduced when a phosphate-mimic is used. Finally, reducing the electrophilicity of the carbonyl group at C2 of DXP definitely hinders turnover, as shown by the carboxamide analog. These results, along with the activity of known fosmidomycin analogs and the DXR X-ray crystal structures will be useful for guiding the design of future DXR inhibitors.

Further details about the requirements of the DXR-substrate binding were also revealed from a modeling and mutagenesis study of DXR. One of the DXP analogs, 1-Me-DXP, with the bulkier ketone moiety was chosen for studying the DXR active site because it was of interest to understand why this compound was not a substrate. The modeling study of DXR showed that the amino acid residue Trp204 may be responsible for preventing turnover of 1-Me-DXP. The mutant DXR, W204F, with the smaller amino acid residue was able to use 1-Me-DXP as a substrate. This mutant enzyme mediated the conversion of 1-Me-DXP into the corresponding phosphate product, ethylerythritol phosphate which was characterized by derivatization to form acetate compound and then the acetate was compared to the standard compound. This result demonstrated that the highly conserved Trp204 residue plays a key role in DXR substrate specificities.

The mechanism for the conversion of deoxyxylulose phosphate (DXP) into methylerythritol phosphate (MEP) mediated by DXR was also studied. Two possible mechanisms, the α-ketol rearrangement and the retroaldol/aldol rearrangement were proposed. The DXP analogs 4-deoxy DXP and 3-deoxy DXP were originally considered as alternate substrates which might provide valuable information to rule out one of the mechanisms. Because DXR failed to use 4-deoxy DXP as a substrate, the α-ketol rearrangement was not supported as for the mechanism of DXR. However, no opposite results were obtained from experiments

designed to support either, so the α-ketol rearrangement cannot be ruled out. Further investigations are needed to provide more depth information about the mechanism. Different approaches and techniques should be considered.

The attempt to synthesize the intermediate from the DXR reaction, 2-*C*-methylerythrose-4-phosphate provided valuable approaches for the syntheses of previously mentioned compounds. The compound, 2-*C*-methylerythrose-4-phosphate appeared to be unstable under the various conditions that were tried. Even though this compound was not obtained from these studies, a report from a different research group did confirm that 2-*C*-methylerythrose-4-phosphate is the intermediate for the DXR reaction.

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