REVIEW

Preparation of Isotope Labeled/Unlabeled Key Intermediates in 2-Methyl-D-erythritol 4-Phosphate Terpenoid Biosynthetic Pathway

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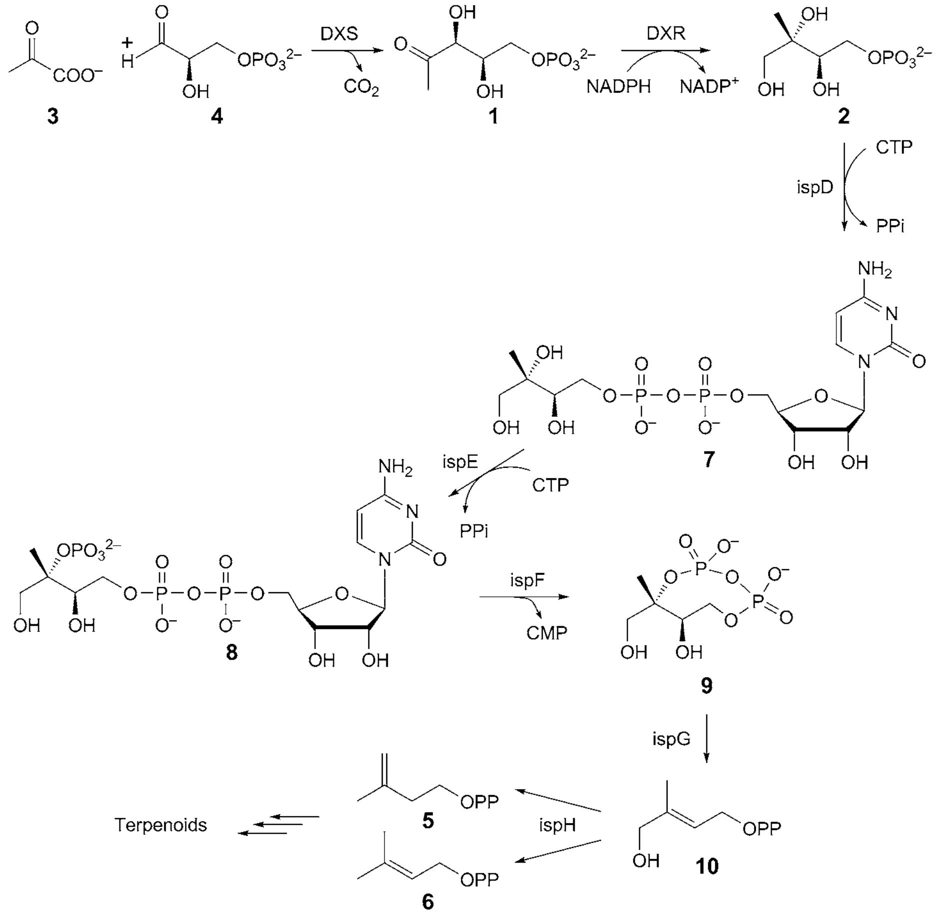
Naturally occurring terpenes constitute one of the largest groups of natural products with complicated and variable structures, and a great number of important biological activities. The 2-methyl-D-erythritol 4-phosphate (MEP) pathway is a newly found and established biosynthetic route for terpenoids, and all the enzymes involved in this pathway can be used as targets for the screening of antibiotics. Progress in chemical and enzymatic preparation of the key intermediates in this pathway is reviewed with the emphasis on the synthesis of 1-deoxy-D-xylulose 5-phosphate and 2-methyl-D-erythritol 4-phosphate with isotope labels.

Introduction. – Naturally occurring terpenoids, containing more than 45,000 members, constitute one of the largest groups of secondary metabolites with complicated and different structures, and a great variety of important biological activities. Although the biological functions of the vast majority of the known terpenes still remain largely unknown, the scrutinized and clarified functions demonstrate the extreme significance of terpenoids. These include regulation of cell-wall and glycoprotein biosynthesis (dolichol diphosphates), transportation of electron in redox chemistry (plastoquinones and ubiquinones), photosynthetic light harvesting (carotenoids), contribution to lipid membrane structure (cholesterol in eukaryotes, archaebacterial lipids), modification of proteins involved in signal transduction (prenylated proteins), intercellular signaling and developmental control (estrogens), interspecies defence (microbe–microbe, plant–microbe, plant–insect), and as antibiotics and phytoalexins (trichochecin, capsidiol), to name a few. Furthermore, a large number of other terpenoids display potent, medicinally useful activities; for example, the widely used cardiovascular agents of the ginkgolide type, antitumor diterpene taxoids, and the antimalarial compound artemisinin. Thus, a lot of research on the biosynthesis of terpenoids has been performed, and two pathways have been established, *i.e.*, the classic mevalonate (MVA) pathway and the newly established 2-methyl-D-erythritol 4-phosphate (MEP) pathway.

Recent research on the terpenoid biosynthesis has revealed how widespread the recently discovered non-mevalonate pathway is. In fact, it indicates that the new pathway may be followed in nature much more frequently than the classic one. This pathway is largely operative in bacteria, and it is also significant in higher plants. In addition, the coexistence of both pathways in two different cell compartments of higher plant cells was demonstrated, with the MVA route in the cytosol and the MEP route confined to the plastids. Further experiments confirmed that, in higher plants, the MVA pathway affords sesquiterpenes, sterols, and triterpenes, whereas the alternative pathway leads to a wide variety of hemi-, mono-, di-, and tetraterpenes.

In the MEP terpenoid biosynthetic pathway (for recent reviews, see [1]), the key intermediate 1-deoxy-D-xylulose 5-phosphate (DXP; **1**; *Scheme 1*), which is converted to MEP (**2**; *Scheme 1*), the first accepted intermediate in this route, by a two-step process catalyzed by DXP reductoisomerase (DXR) in the presence of NADPH, is biosynthesized from pyruvate (**3**; *Scheme 1*) and from D-glyceraldehyde 3-phosphate (D-GAP; **4**; *Scheme 1*) by the catalytic action of DXP synthase (DXS), a thiamine diphosphate-dependant enzyme. Subsequently, MEP (**2**) is transformed by five consecutive enzyme reactions to isopentenyl diphosphate (IPP; **5**) and dimethylallyl

Scheme 1. *MEP Pathway for Biosynthesis of Terpenoids*

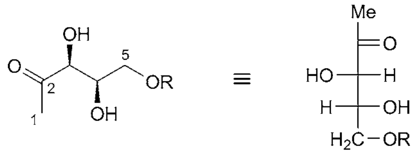


diphosphate (DMAPP; **6**), the two universal building blocks for all natural terpenes. In this reaction sequence, **2** is converted to 4-diphosphocytidyl-2-methyl-D-erythritol (CDPME; **7**) by 4-diphosphocytidyl-2-methyl-D-erythritol synthase (IspD) in the presence of CTP. Then, **7** is phosphorylated in an ATP-dependent reaction mediated by CDPME kinase (IspE) to afford 4-diphosphocytidyl-2-methyl-D-erythritol 2-phosphate (CDPMEP; **8**), which is cyclized to form 2-methyl-D-erythritol 2,4-cyclodiphosphate (cMEPP; **9**) by catalytic action of cMEPP synthase (IspF). Subsequently, **9** is transformed to the acyclic compound (*E*)-4-hydroxy-3-methylbut-2-enyl diphosphate (HDMAPP; **10**) with loss of two OH groups induced by the protein HDMAPP synthase (IspG). Finally, building blocks **5** and **6** are formed spontaneously from **10** in a reaction catalyzed by IPP/DMAPP synthase (IspH).

Although the alternative pathway for the biosynthesis of terpenoids has been well-established so far, the catalytic mechanism of some of the involved enzymes has not yet been fully elucidated. Thus, there is a high demand for all intermediates, especially isotope-labeled ones, to achieve further progress in understanding. Furthermore, research has shown that the non-mevalonate pathway serves as the unique source of terpenoids in numerous pathogenic eubacteria and in apicoplast-type protozoa, most notably *Plasmodium*, but it is absent in human beings who use the classic MVA pathway as one of the sources of terpenoids. All enzymes of the MEP route, most importantly the key proteins DXS, DXR, and IspH, therefore, represent attractive targets for the development of new biocides, which are of major interest in the present situation of bacterial resistance towards antibiotics being used clinically. As a consequence, there is also a demand for all of these compounds in large quantities.

Over the last three decades, general chemical and enzymatic methods have been established to prepare these intermediates by several research groups, due to the underlying biological importance. Here, we would like to review the published procedures for the preparation of the intermediates of the MEP pathway.

2. Synthesis of DX/DXP. – The synthesis of DXP (**1**; *Fig. 1*) was carried out earlier, because it was already established as a precursor in the biosynthesis of vitamins B_1 and B_6 in some bacteria [2][3], prior to the detection of its biological role in the MEP pathway. The dephosphorylated product of **1**, namely 1-deoxy-D-xylulose (DX; **11**), is not a direct intermediate of the pathway, but it can be phosphorylated by D-xylulokinase in plants and microbes to form **1**, and then effectively incorporated into final terpenoids by organisms [4], so it is regarded as a potential intermediate in the route.



1 R = PO_3H_2
11 R = H

Fig. 1. *Structures of DXP* (**1**) *and 1-Deoxy-*D*-xylulose* (**11**)

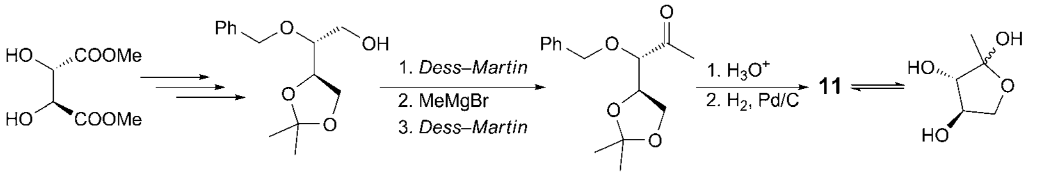
Given to the biological significance of **1** and **11**, some chemical and enzymatic methods for preparation of these two compounds, labeled with stable (^{2}H, ^{13}C) or radioactive isotopes (^{3}H, ^{14}C), have been established by several groups. Generally, chemical routes are more suitable for the preparation of unlabeled or stable isotope-labeled compounds, whereas enzymatic ways are better for the synthesis of radio-isotope-labeled precursors.

2.1. *Chemical Preparation of* **1** *and* **11**. Around ten chemical methods have been established to furnish these two compounds by adopting two different synthetic strategies. One starts with chiral materials, for instance, D-tartrate and its derivatives that possess the same stereogenic centers as the target molecules, the other starts from achiral compounds, and the stereogenic centers are introduced at a later stage.

2.1.1. *Strategy with Easily Available Chiral Compounds as Starting Materials.* Making use of a chiral pool to prepare a target molecule is a common strategy in organic synthesis. D-Tartrate is a very useful chiral raw material possessing the same absolute configurations at C(2) and C(3) as C(3) and C(4) in DX(P). Therefore, this compound and various derivatives thereof were invariably employed to prepare **1** and **11**. Similarly, D-threitol, D-mannitol, and some carbohydrates have the same structural moiety as in the target molecules, and thus they were also quite frequently chosen as starting compounds to synthesize the two targets. Actually, a great majority of the published methods employed this strategy, and the common point was a *C*-methylation step, because the starting materials, *e.g.*, D-tartrate or D-threitol, have one C-atom less than DX(P). This strategy also allowed for a ready preparation of isotope labeled **1** and **11**.

Begley and co-workers [5] reported an eight-step, stereocontrolled synthesis of **11** from dimethyl-D-tartrate in an overall yield of *ca.* 20% (*Scheme 2*). Mono-*O*-benzylation, reduction of the ester, and protection of the 1,2-diol unit with a isopropylidene group afforded alcohol **12**. *Dess–Martin* oxidation of the alcohol, followed by *Grignard* addition and reoxidation, gave ketone **13**. Subsequent acid hydrolysis and hydrogenolysis of **13** produced pentulose **11** as a mixture of ketone **11**, and hemiacetals **11a** and **11b** in a ratio of 1:1:1.

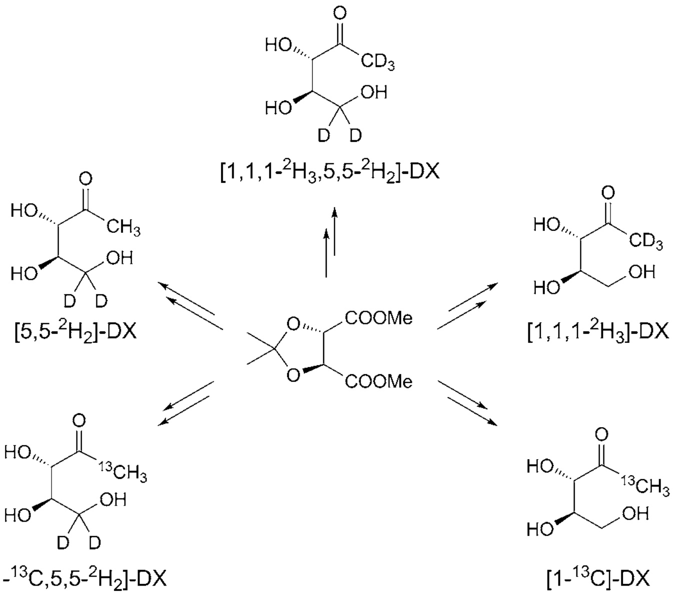
Scheme 2



12 **13** **11a/11b**

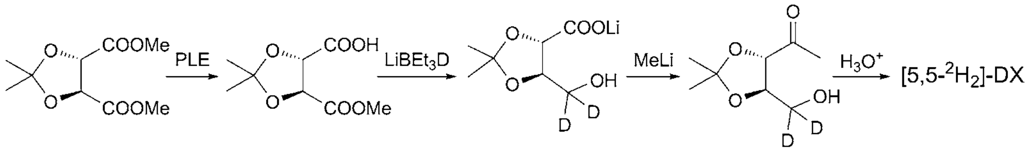
With isopropylidene-protected dimethyl D-tartrate as starting material, *Boland* and co-workers [6][7] prepared isotope labeled **11** in 31% overall yield. This protocol allows for a flexible adaptation to other labeling patterns and isotopes by exploiting different combinations of labeled and unlabeled alkylating and reducing reagents (*Scheme 3*).

Scheme 3



An example of this approach is the synthesis of [5,5-$^2H_2$]-DX (*Scheme 4*) [7]. Compound **14** was selectively hydrolyzed to its monomethyl ester **15** by pig liver esterase (PLE) under controlled conditions. Reduction of **15** with Li^{2}HBEt$_3$ gave the lithio salt of the isopropylidene-protected dideutero-threonate **16**. Treatment of **16** with excess MeLi, followed by a non-protic workup with CO_2, provided the protected dideuterated xylulose **17**. Removal of the protecting group by acid hydrolysis furnished [5,5-$^2H_2$]-DX in an overall yield of *ca.* 37%. It should be mentioned that the methylation of **16** with excess MeLi also produced the dimethylated by-product, but this difficulty was overcome by rapidly bubbling dry CO_2 into the mixture to consume excess MeLi at the final stage of this conversion.

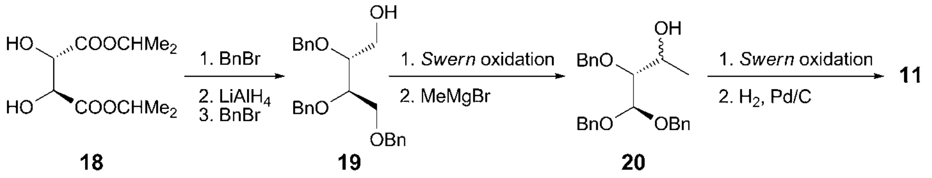
Scheme 4



14 **15** **16** **17**

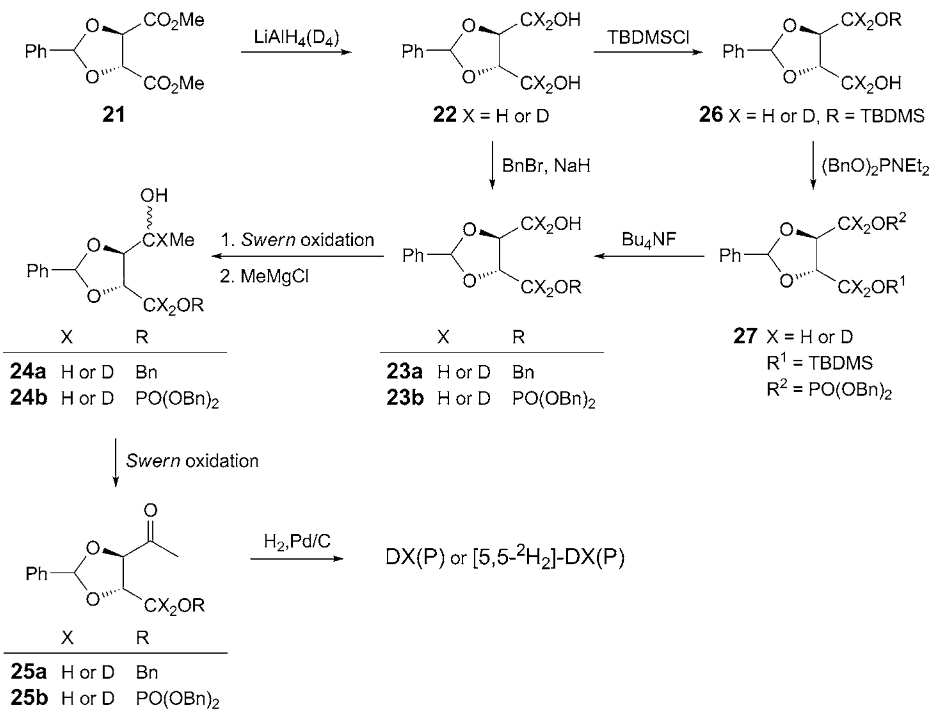
In the above discussed approaches, acetone was selected to protect the 1,2-dihydroxy group, and the main problem of this strategy was that the isopropylidene protecting group had to be removed under strictly controlled conditions, otherwise the by-products, mainly hemiacetal **11** (*Scheme 2*) and its dimer, turned out to be the major reaction products [8]. To avoid multiple deprotection steps and side reactions, *Giner* [9] developed a route with benzyl ethers as the sole protecting groups. Complete benzylation, $LiAlH_4$ reduction, and then monobenzylation of diisopropyl D-tartrate **18** gave 2,3,4-tribenzyl-D-threitol **19**; *Swern* oxidation and methylation provided fully benzylated alcohol **20**. *Swern* oxidation of **20**, followed by hydrogenation, yielded the target **11** (*Scheme 5*). A similar route was followed by *Begley* and co-workers [10] to prepare **1** in an overall yield of 5%.

Scheme 5



An improved short way for large-scale synthesis of enantiomerically pure **11**/**1** from commercially available benzylidene dimethyl-D-tartrate (=dimethyl (4*R*,5*R*)-2-phenyl-1,3-dioxolane-4,5-dicarboxylate; **21**; for ^{2}H-labeled targets) or benzylidene-D-threitol (=[(4*S*,5*S*)-2-phenyl-1,3-dioxolane-4,5-diyl]dimethanol; **22**), in high overall yields (*ca.* 60%) and the possibility of introducing ^{2}H-labeling at C(5), was described by *Rohmer* and co-workers (*Scheme 6*) [11]. $LiAlH_4(D_4)$ reduction of **21** produced protected D-threitol **22**, and benzyl protection of the OH group of **22** gave **23a**. *Swern*

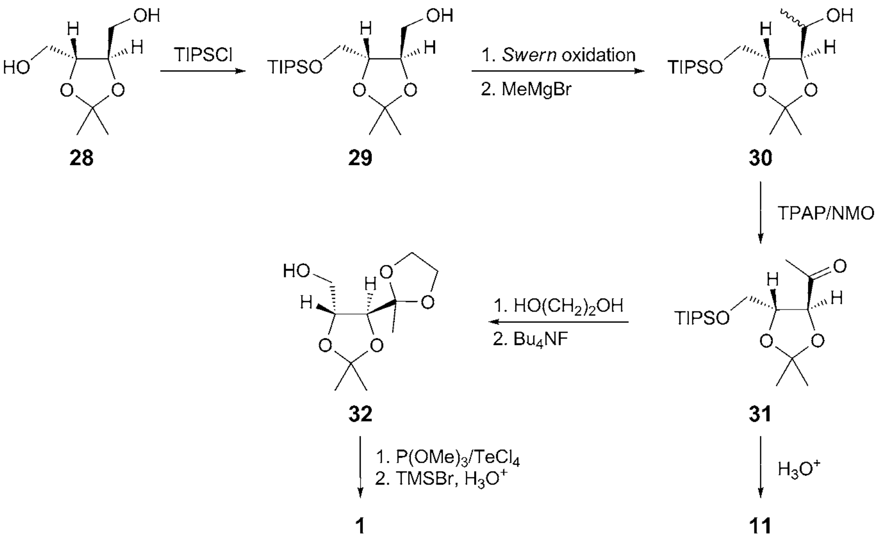
Scheme 6



oxidation of the **23a** and *Grignard* methylation of the resulting aldehyde led to the secondary alcohol **24a** as a mixture of two diastereoisomers. *Swern* oxidation of **24a** under mild conditions with tetrapropylammonium perruthenate (TPAP)/*N*-methylmorpholine *N*-oxide (NMO) furnished protected 1-deoxy-D-xylulose **25a**. Hydrogenolysis of the benzylidene and benzyl (Bn) groups quantitatively afforded DX (**11**). Preparation of DXP (**1**) was achieved in a similar way, the difference being that compound **22** was monoprotected with (*tert*-butyl)dimethylsilyl (TBDMS) rather than with a Bn group to produce **26**. The other OH group of **26** was subsequently protected with dibenzyl *N*,*N*-diethylphosphoramidite in the presence of *meta*-chloroperbenzoic acid (*m*CPBA) to afford **27**. This step not only screened the free OH of **26**, but introduced a potential phosphate group as well. Then, removal of TBDMS of **27** with Bu_4NF resulted in compound **23b**, which was consequently converted to **1** by the same reaction sequence as for **11**.

Besides the above depicted approaches from D-tartrate and its derivatives, there are some other approaches with D-threitol [8][12][13] and D-mannitol [14] as chiral sources to synthesize **1** and **11**, among which the procedure of *Poulter* was chosen as a representative (*Scheme 7*). The 3,4-acetonide of D-threitol, **28**, was monoprotected with triisopropylsilyl chloride (TIPSCl) to give the primary alcohol **29**, which was then converted to secondary alcohol **30** with one more C-atom by *Swern* oxidation and MeMgBr methylation. Further oxidation of **30** with TPAP/NMO afforded the protected deoxyxylulose **31**, both protecting groups of which could be removed under acidic conditions to give deoxyxylulose **11** (overall yield was *ca.* 70%). Meanwhile, intermediate **31** could be converted to the sugar phosphate **1** in four additional steps. Glycol protection of the ketone group, followed by Bu_4NF treatment of **31**, provided the protected primary alcohol **32**; then, phosphorylation of **32** with $P(OMe)_3/TeCl_4$ and subsequent acidic deprotection gave the target molecule **1** in an overall yield of *ca.*

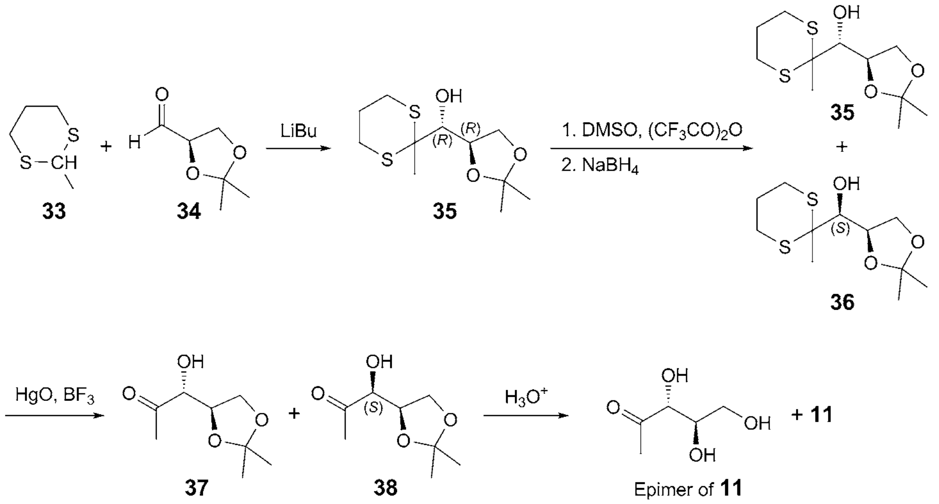
Scheme 7



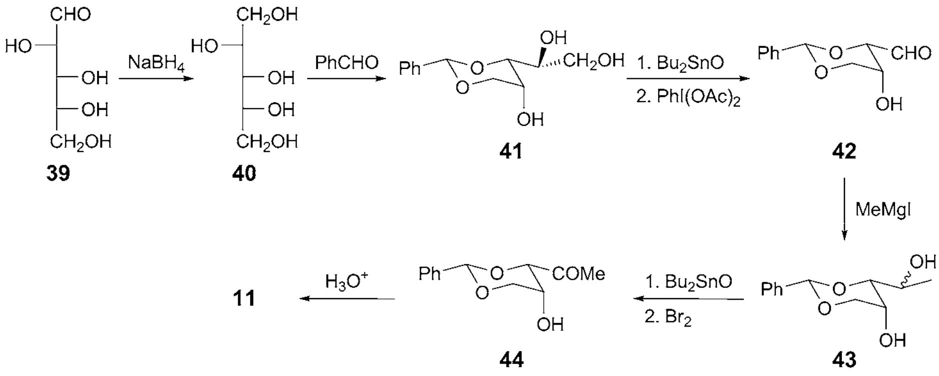
58%. The advantage of this route is that it is sufficiently versatile to permit one to incorporate isotopes of H or C in **11** and, in addition, phosphate in **1** for biosynthetic experiments.

Synthesis of **11** and its phosphate **1** from carbohydrate is also a promising option. Indeed, the first preparation of **11** was achieved by this strategy (*Schemes 8* and *9*) [2]. Addition of the anion of acetaldehyde trimethylene dithioacetal **33** in the presence of BuLi to 2,3-isopropylidene-D-glyceraldehyde **34** gave practically pure D-*erythro*-isomer **35** in protected form. Oxidation of **35** to a ketone, followed by $NaBH_4$ reduction, provided a 1 : 1 mixture of **35** and D-*threo*-isomer **36**. Treatment of the mixture with BF_3 and HgO generated ketone **37** and its epimer **38** that could be resolved by column chromatography. Final acidic hydrolysis of **38** gave **11**. This method is also suitable for introduction of different isotopes at different positions of **11** when labeled starting

Scheme 8



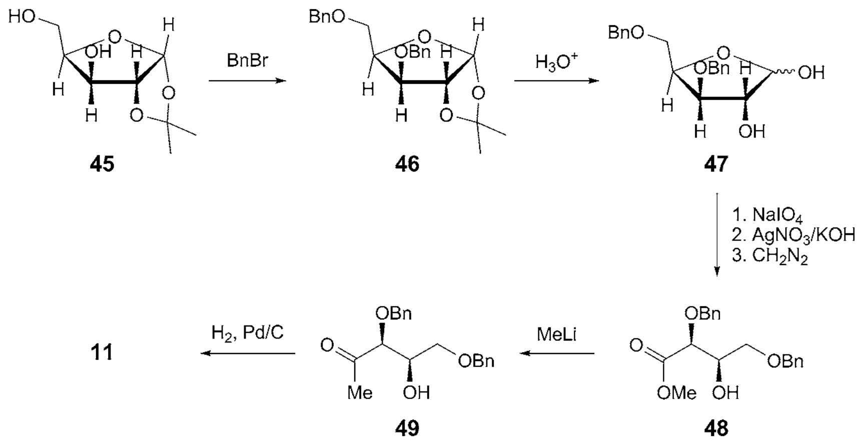
Scheme 9



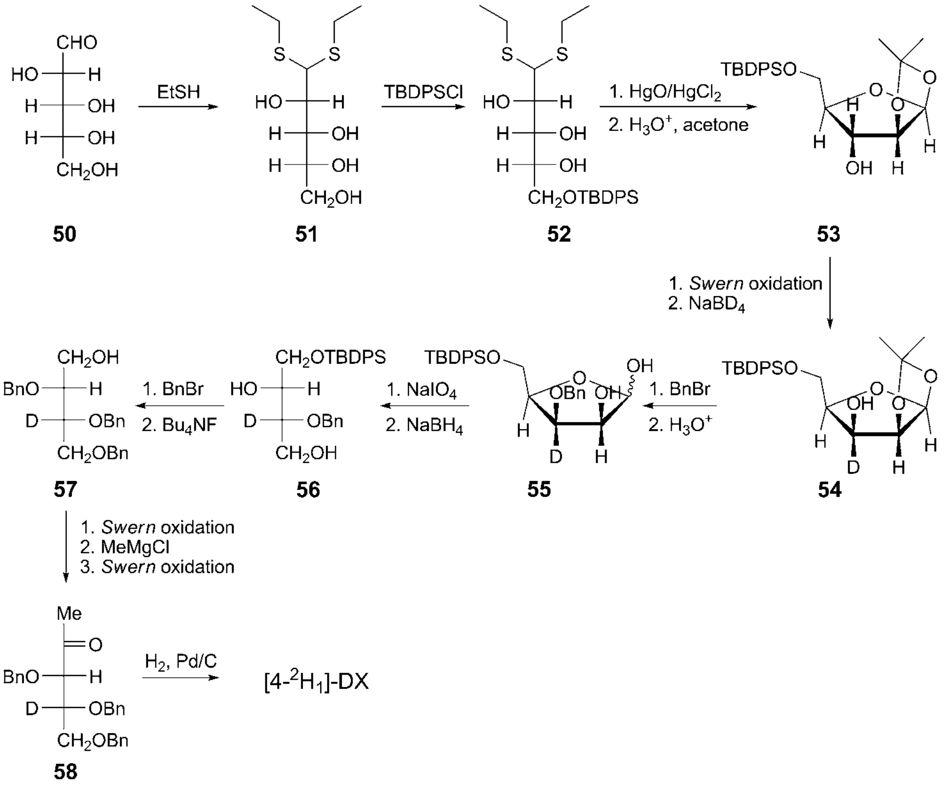
materials or $NaBD_4$ are used. In the same study, also D-arabinose (**39**) was used to prepare **11**. $NaBH_4$ Reduction of the raw material generated arabitol (**40**), which was then protected with benzylidene group to form **41**. The protected D-threose **42** was subsequently prepared by oxidation of the stannylidene derivative of **41** with (diacetoxy)iodobenzene. Methylation of the aldehyde group of **42** gave **43**, which was converted to protected target **44** by reacting first with Bu_2SnO then Br_2 in the presence of 4-Å molecular sieves. After final acidic hydrolysis, compound **11** was obtained. This route allows labeling with ^2H at both ends of the target molecule.

Starting from differently protected pentoses, *Rohmer* and co-workers [15] synthesized [1,1,1-$^2H_3$]-**11** and [4-^2H]-**11**. Benzylation of the two OH groups of the commercial 1,2-*O*-isopropylidene-α-D-xylofuranose (**45**), which has the required configurations at the two stereogenic centers of **11** (*Scheme 10*), gave compound **46**, acidic hydrolysis of which afforded 3,5-*O*-dibenzyl-D-xylofuranose (**47**). The free sugar was successively oxidized with $NaIO_4$ and $AgNO_3$/KOH to give 2,4-*O*-dibenzyl-D-*threo*-trihydroxybutanoic acid, which was subsequently esterified with CH_2N_2 to provide **48**. Addition of MeLi to **48** gave compound **49**, and, after catalytic hydrogenation, **11** was effectively obtained with an overall yield of 48%. The initial step of the synthesis of [4-^2H]-**11** was the conversion of arabinose **50** to its thioacetal **51** (*Scheme 11*). Treatment of the thioacetal with (*tert*-butyl)diphenylsilyl chloride (TBDPSCl) resulted in a highly selective protection of the primary OH group to afford silyl ether **52**. Deprotection of the thioacetal moiety in **52** by a Hg^{II} derivative, followed by protection with acetone, led to the arabinofuranose derivative **53**. After *Swern* oxidation and then highly stereoselective reduction with $NaBH_4(D_4)$, the configuration at C(3) in **53** was inverted from (*S*) to (*R*), and the xylofuranoside **54** was formed. The high stereoselectivity of this transformation is due to the presence of the 1,2-*O*-isopropylidene group on the β-face of the furanoside, directing the reduction of the oxo group by hydride (or deuteride) from the less-hindered α-face to afford **54** with the required configuration. Subsequent benzylation of the secondary OH group and removal of acetonide protection gave **55**; $NaIO_4$ oxidation and immediate $NaBH_4$

Scheme 10



Scheme 11

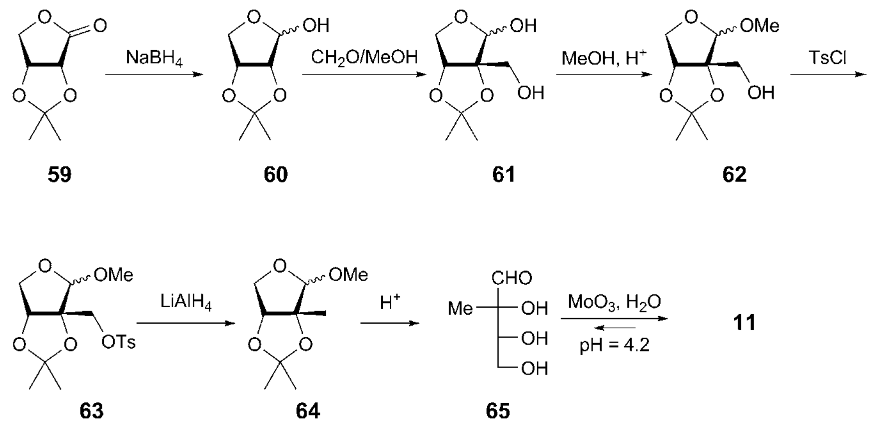


reduction yielded **56**. Benzylation of diol **56** and removal of the TBDPS groups provided 1,2,3-*O*-tribenzyl-D-threitol **57**. One-pot *Swern* oxidation, nucleophilic addition of MeMgCl, and again *Swern* oxidation afforded 3,4,5-*O*-tribenzyl-1-deoxy-D-xylulose **58**. Quantitative deprotection of **58** over 10% Pd/C gave **11** in 16% overall yield. This synthetic route allowed ^{2}H-labeling at C(1) and/or C(4).

In the route developed by *Serianni* and co-workers [16], **11** was prepared in seven steps in a 21% overall yield from commercial 2,3-*O*-isopropylidene-D-erythrono-1,4-lactone **59** (*Scheme 12*). $NaBH_4$ Reduction of **59** in H_2O gave sugar **60**, which was successively alkylated with CH_2O to **61**, and then methyl-glycosidated to afford β-furanoside **62**. Tosylation of **62** ($\rightarrow$**63**), followed by $LiAlH_4$ reduction, gave **64**, the deprotection of which yielded 2-methyl-D-erythrose (**65**). The key transformation of **65** to the desired target **11** was mediated by MoO_3 in medium acidic solution.

2.1.2. *Strategy with Achiral Compounds as Starting Materials.* This strategy involved the synthesis of the C_5 chain from achiral precursors, and the stereogenic centers were introduced by *Sharpless* asymmetric dihydroxylation/epoxidation (*Sharpless* AD/AE) of achiral α,β-unsaturated aldehyde or ketone derivatives using a chiral OsO_4 complex. Often, the α,β-unsaturated C_5 compounds were prepared by *Wittig* reaction or by

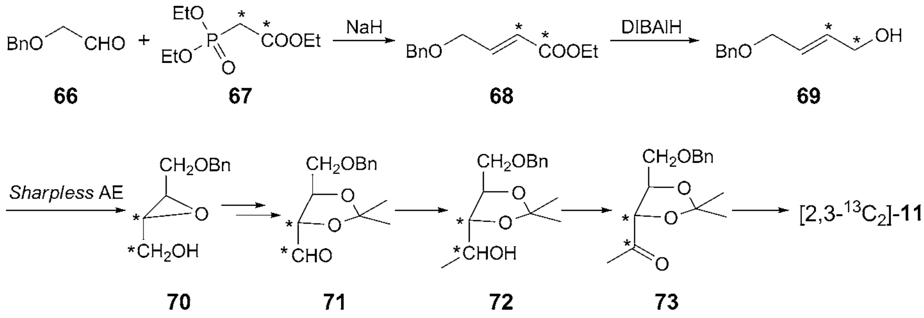
Scheme 12



reduction of substituted alkynes. The target molecules with different labels could be synthesized from labeled starting materials or by employing labeled reductants.

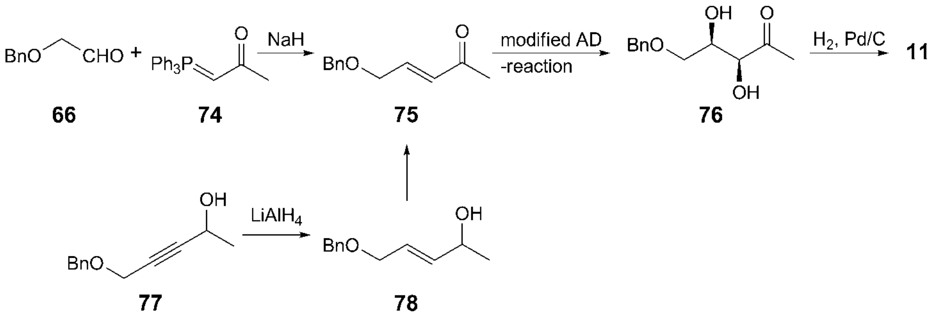
Besides the preparation of **11** from D-threitol, *Spenser* and co-workers [8] also accomplished the synthesis of [2,3-$^{13}C_2$]-DX using this strategy with a 16% overall yield (*Scheme 13*). Condensation of *O*-benzylglycolaldehyde **66** with triethyl phosphono[1,2-$^{13}C_2$]acetate (**67**) gave **68** ((*E*)-isomer exclusively). Diisobutylaluminium hydride (DIBALH) reduction of **68** afforded **69**, which was converted to chiral compound **70** by using *Sharpless*'s AE procedure. After several more steps, **70** was transformed to the labeled key intermediate of this approach, *i.e.*, protected [1,2-$^{13}C_2$]-D-threose **71**. *Grignard* reaction of **71** with MeMgCl, then pyridinium dichromate (PDC) oxidation of the product **72** to give **73**, followed by acidic hydrolysis, provided ^{13}C-labeled **11**.

Scheme 13



The protocol developed by *Giner et al.* also involved this strategy (*Scheme 14*) [9][17]. In the first route, raw material **66** with **74** in the presence of NaH produced enone **75** with a 12 : 1 (*E*)/(*Z*) ratio. Then, under modified *Sharpless* AD conditions, the enone was converted to benzylated **11**, *i.e.*, compound **76**, in high stereoselectivity and yield. Final debenzylation by catalytic hydrogenation afforded target **11**. Only three

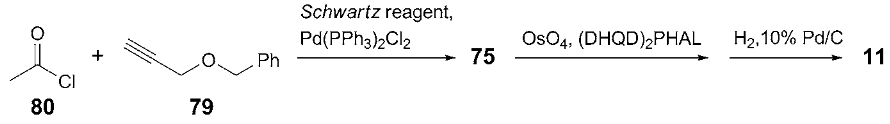
Scheme 14



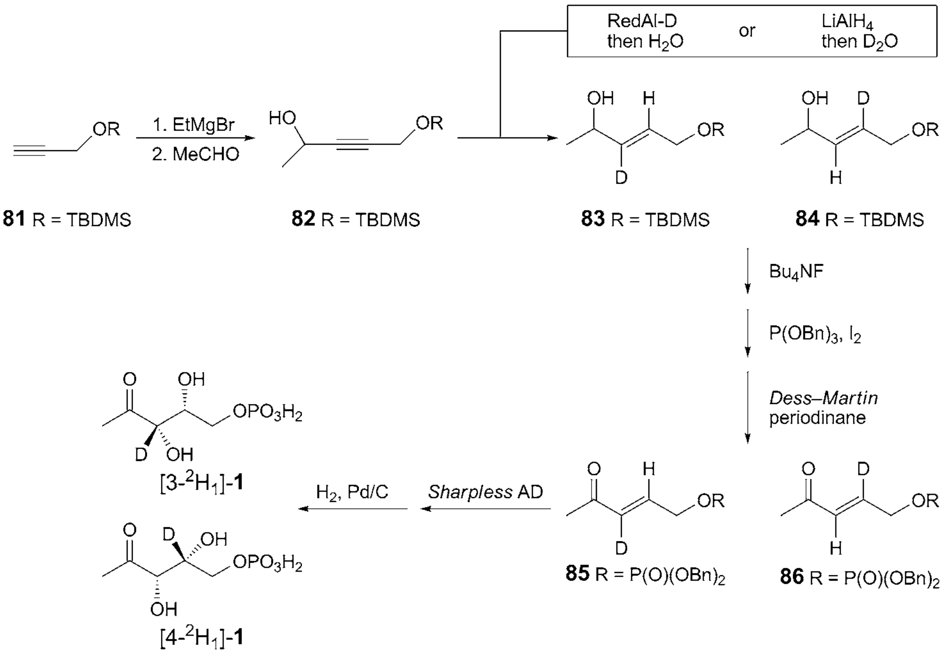
steps were involved in this synthesis, which represents the shortest one of all published procedures. Another advantage of this process is that [3-$^2H_1$]-**11** or [4-$^2H_1$]-**11** can also be obtained from deuterated **66** or **74**. A second route started from propargyl alcohol **77**, $LiAlH_4$ reduction of which gave the allylic alcohol **78** in (*E*)-configuration. Full deuteration at C(3) was achieved by using $LiAlD_4$, while C(4) was cleanly deuterated by quenching the reduction with D_2O. *Swern* oxidation of **78** generated **75**, which could be transformed to the target **11** in two more steps.

Besides the *Wittig* reaction, *Cox* and *Evitt* [18] described a route to construct the enone **75**. Benzyl propargyl ether (**79**) was reacted first with *Schwartz*'s reagent, then the mixture was treated with AcCl (**80**) and $Pd(PPh_3)_2Cl_2$ to yield the α,β-unsaturated ketone **75**. OsO_4 Oxidation in the presence of hydroquinidine phthalizidine-1,4-diyl diether ($(DHQD)_2PHAL$), followed by catalytic hydrogenation, afforded **11** in 42% overall yield and 93% ee (*Scheme 15*). Meanwhile, the same authors prepared [3-^{2}H]-**1** and [4-^{2}H]-**1** from TBDMS-protected propargyl alcohol **81** (*Scheme 16*) [19][20]. In this reaction sequence, **81** was deprotonated with EtMgBr and then treated with MeCHO to prepare alcohol **82**, which was subsequently treated with RedAl-D (Sodium bis(2-methoxyethoxy)aluminium deuteride), followed by a workup with H_2O, to afford the deuterated olefin **83**, or with $LiAlH_4$, followed by D_2O workup, to yield its isomer **84**. Removal of TBDMS with Bu_4NF, selective phosphorylation of the resulting primary OH group, followed by *Dess–Martin* oxidation, gave the ketones **85** and **86**, which were asymmetically dihydroxylated under the *Sharpless* AD conditions. The protected DXP isotopomers thus obtained were hydrogenolyzed to afford the differently deuterated **1** ([3-^{2}H]: yield 15.5%, er 92:8; [4-^{2}H]: yield 6.5%, er 92.5:7.5). Shortly after, *Liu* and co-workers [21] reported the synthesis of these two isotopomers by applying a similar strategy. The overall yields for both compounds were low ([3-^{2}H]: 8.5%, [4-^{2}H]: 7.0%), but the stereoselectivity in the *Sharpless* AD reaction was slightly higher than in the procedure of *Cox* and *Evitt* (er > 20:1).

Scheme 15

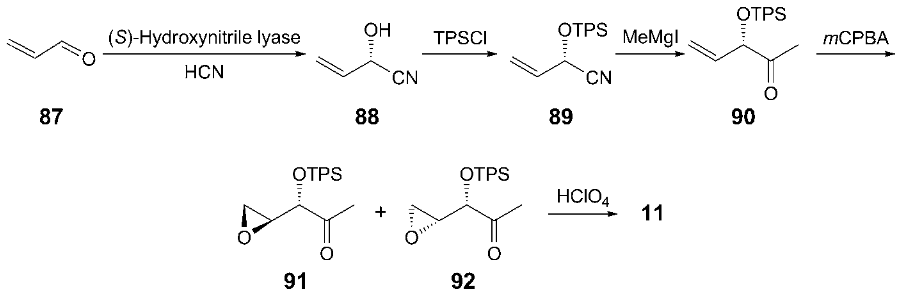


Scheme 16



In the method developed by *Fechter et al.* [22], commercial acrolein (**87**) was used as the α,β-unsaturated aldehyde to prepare **11** and, after a five-step conversion, the target molecule was obtained in a 47% overall yield and with 86% ee (*Scheme 17*). The biocatalytic transformation of **87** to cyanohydrin **88** by using the (*S*)-hydroxynitrile lyase, followed by TPS protection of the OH group to yield **89**, and *Grignard* C-elongation, gave ketone **90**, asymmetric epoxidation of which with *m*CPBA provided **91**/**92** 1:4 with the desired *threo*-epoxide **92** being the main product. Nucleophilic ring-opening of **92** afforded **11**.

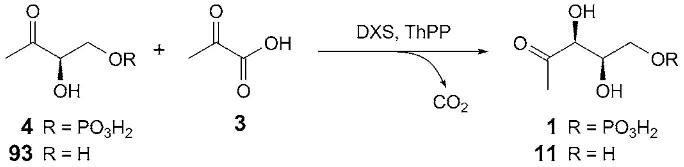
Scheme 17



2.2. *Enzymatic Preparation of* **1** *and* **11**. In comparison to chemical syntheses, the enzymatic procedures always have the combined advantages of *i*) short reaction time, *ii*) easy access to isotope-labeled products from commercially available precursors, *iii*) virtually perfect stereochemical control, and *iv*) the simplicity of the one-pot reaction conditions in aqueous solution. Therefore, enzymatic synthesis of **1**/**11**, especially multiple isotope labeled or radioactive isotope labeled **1**/**11** by using the recombinant 1-deoxy-D-xylulose 5-phosphate synthase (DXS) from D-GAP **4** and pyruvate **3** represents an attractive alternative to chemical synthesis. However, the disadvantages of enzymatic procedures, *e.g.*, *i*) the commercial unavailability of DXS, and *ii*) relative small preparation scale, are also obvious drawbacks.

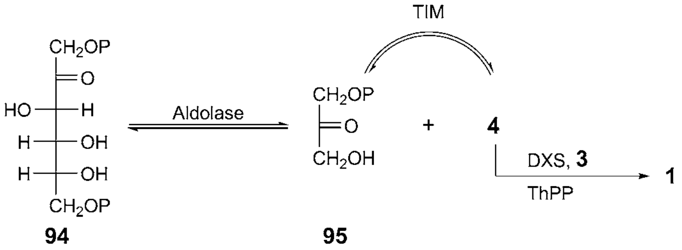
Generally, DXS catalyzes the condensation reaction of **3** with **4** with the release of the carboxylic group of **3** as CO_2 in the presence of ThPP to produce **1**. It can also mediate the reaction of D-glyceraldehyde (D-GA; **93**) with **3** to form **11** under same conditions (*Scheme 18*). Although **4** is the natural substrate of DXS, it is seldom used to prepare **1**, since commercially available preparations of **4** are either racemic, or enantiomerically impure and expensive. Therefore, the frequently employed enzymatic procedures for DXP synthesis usually involved the *in situ* generation of **4** from D-fructose 1,6-biphosphate (**94**) in the mixture.

Scheme 18



Rohmer and co-workers [23] applied the strategy outlined in *Scheme 18* directly and prepared [2-^{13}C]-**1**, [2,3-$^{13}C_2$]-**1**, and [2,4-$^{13}C_2$]-**1** from [2-^{13}C]-**3**, and unlabeled, as well as [1-^{13}C]-**4** or [2-^{13}C]-**4**. The yield was *ca.* 30% after preparative TLC purification. In the approach developed by *Begley* and co-workers [10], **94** was employed to prepare compound **1** with a yield of 47% (*Scheme 19*). In this 'one-pot' reaction sequence, **94** was first split by aldolase to give the two triose phosphates, *i.e.*, **4** and its isomer 1,3-dihydroxyacetone 3-phosphate (DHAP; **95**). This *in situ* produced compound **4** immediately reacted with **3** to afford **1** in the presence of DXS and thiamine diphosphate (ThPP). The other *in situ* produced triose phosphate **95** was continuously interconverted to **4** by triose phosphate isomerase (TIM) until the end of the reaction. According to this scheme, differently labeled **1** can be easily synthesized, if isotopically labeled starting materials are used.

Scheme 19



Kis, *Eisenreich*, and co-workers [24] subsequently established an improved 'one-pot' synthesis of **1** from D-glucose (**96**) using the upstream enzymes of glycolysis pathway (*Scheme 20*). Phosphorylation of **96** with hexokinase under the assistance of ATP produced D-glucose 6-phosphate (**97**), which was transformed to D-fructose 6-phosphate (**98**) by catalytic action of D-glucose 6-phosphate isomerase (G6PI). Further ATP phosphorylation of **98** with fructose-6-phosphate kinase (F6PK) gave **94**. In the following steps, **94** was converted to **1** by combined actions of aldolase, triose-phoshate isomerase (TIM), and DXS in the presence of ThPP and **3** (*Scheme 19*). Compound **1** could be then purified through ion-exchange chromatography with a yield of *ca.* 50%. In the same way as outlined in *Scheme 19*, differently labeled **1** can also be easily synthesized starting from labeled materials. Preparations of some ^{13}C-labeled **1** starting from D-glucose and pyruvate with distinct labels are compiled in the *Table.*

Scheme 20

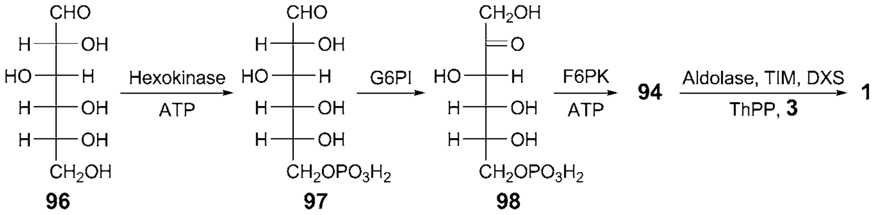


Table. *Preparation of Differently Labeled Compounds* **1**

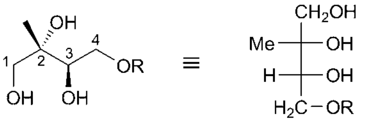
Glucose	Pyruvate	Products
Unlabeled	Unlabeled	Unlabeled **1**
Unlabeled	[2-^{13}C]	[2-^{13}C]-**1**
Unlabeled	[2,3-$^{13}C_2$]	[1,2-$^{13}C_2$]-**1**
[U-$^{13}C_6$]	Unlabeled	[3,4,5-$^{13}C_3$]-**1**
[U-$^{13}C_6$]	[2,3-$^{13}C_2$]	[U-$^{13}C_5$]-**1**
Unlabeled	[2-^{14}C]	[2-^{14}C]-**1**

We assume that it should be the most straightforward way to prepare **1** enzymatically from **3** and **4**, or its isomer DHAP (**95**), which can be synthesized chemically just prior to the enzymatic conversion. Based on this idea, we developed two methods [25][26] to prepare the target compound **1**. The first route used **4** as raw material, which was chemically prepared from Pb(OAc)$_4$ oxidation of **98** just before use. The second route started from **95** that could be obtained by the chemical procedure described by *Grosdemange–Billiard* and co-workers [27]. By both routes, **1** could be obtained in more than 80% yield and high purity (> 95%) after purification by ion-exchange chromatography.

3. Synthesis of 2-Methyl-D-erythritol (Me; 99)/2-Methyl-D-erythritol 4-Phosphate (MEP; 2).

– As discussed above, sugar phosphate **1** is not only a precursor for the biosynthesis of terpenoids, but it is a precursor for the biosynthesis of vitamins B$_1$ and B$_6$ in some bacteria as well [2][3]. Therefore, compound MEP (**2**) is regarded as the first committed intermediate of the non-mevalonate pathway, and several syntheses of

2 have been already reported. ME (**99**) is the dephosphorylated form of **2** (see *Fig. 2*). Different from compound **11** that can be phosphorylated by D-xylulokinase in plants and microbes to form **1** and then effectively incorporated into final terpenoids by organisms [4], **99** can not be converted to its phosphate **2** because no kinase that can phosphorylate **99** has been characterized in all the creatures investigated. Moreover, high concentration of compound **99** is even poisonous to hosts [28][29]. The only exception is that the wild type *E. coli* can modestly incorporate **99** into the final terpenoids, but the mechanism of this incorporation still remains unknown [28]. There are also, however, some synthetic routes developed to prepare it.



2 R = PO_3H_2
99 R = H

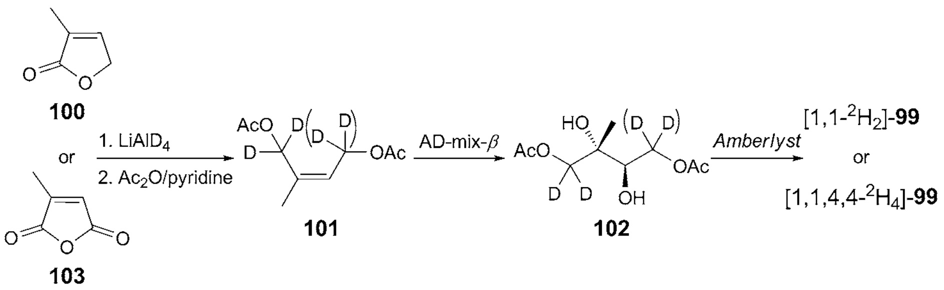
Fig. 2. *Structures of MEP* (**2**) *and ME* (**99**)

A number of chemical syntheses of **2/99** were reported up to date, which adopted quite similar strategies as used in the preparation of DXP/DX. But the structural difference between **1/11** and **2/99** (**1** and **11** have an unbranched C_5-chain, whereas **2** and **99** possess a branced C_5-skeleton), leads to different synthetic schemes and distinct ways for the introduction of isotope labels. Generally, two strategies were developed: *1*) construction of substituted 2-methylbut-2-ene derivatives from achiral compounds, then to introduction of stereogenic centers by *Sharpless* AD or *Sharpless* AE reaction; *2*) directly using chiral compounds as starting materials. A couple of enzymatic methods, which are used only for the preparation of **2**, have been published, too.

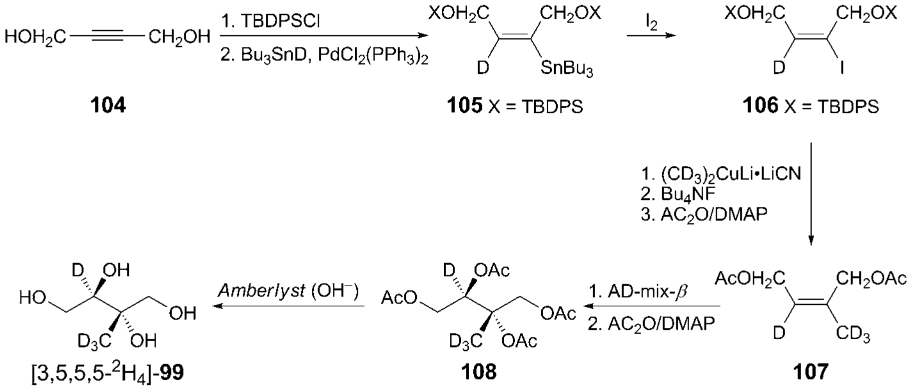
3.1. *Chemical Preparation of* **2** *and* **99**. 3.1.1. *From Easily Available Achiral Compounds.* Generally, a substituted 2-methylbut-2-ene needs to be constructed in this method before incorporation of stereogenic centers. The alkene is usually generated by a *Wittig* or modified *Wittig* reaction. It can also be obtained from reduction of proper but-2-yne derivatives or from butene derivatives that are available. The introduction of the two stereogenic centers is mostly accomplished by *Sharpless* asymmetric oxidation, except in the procedure developed by *Raghavan* and *Sreekanth* [30], in which the target **2** was acquired in *ca.* 4% overall yield after a twelve-step process.

In the method depicted by *Rohmer* and co-workers [28], 3-methylfuran-2(*5 H*)-one (**100**) was the starting material, and, after three steps, deuterated target molecule $[1,1\text{-}^2H_2]$-**2** was obtained in a high overall yield of 82% and an ee value of 80% (*Scheme 21*). $LiAlD_4$ Reduction of **100**, followed by acetylation, gave **101**. Subsequent *Sharpless* AD reaction yielded **102**, deacetylation of which afforded **99** with C(1) labeled with ^2H. Meanwhile, the authors prepared $[1,1,4,4\text{-}^2H_4]$-**99** from citraconic anhydride (**103**) employing the same process. But the 25% yield of $LiAlD_4$ reduction step rendered the preparation not practical. In another report [31], they described the synthesis of $[3,5,5,5\text{-}^2H_4]$-**99** from butyne-1,4-diol (**104**) in an eight-step procedure with a 64% overall yield and an 80% ee value (*Scheme 22*). TBDPS Protection of the OH groups of **104**, followed by a Pd^{II}-catalyzed hydrostannation of the resulting symmetric

Scheme 21



Scheme 22

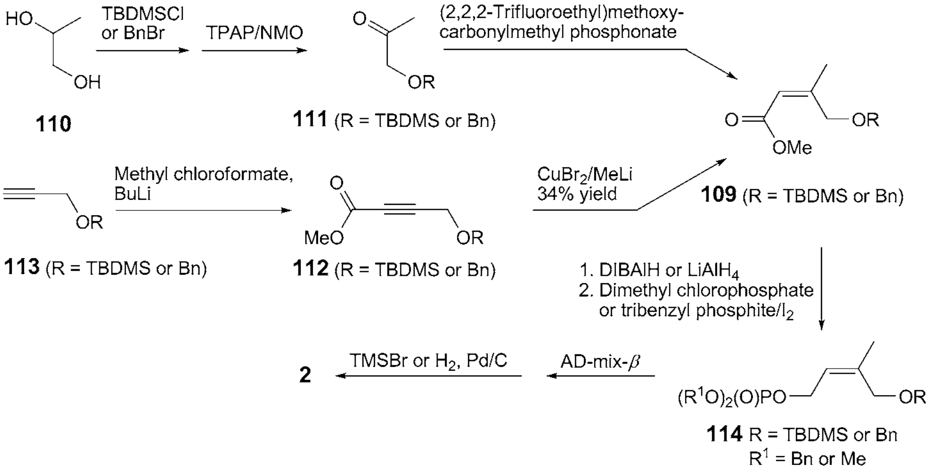


acetylenic derivative by using Bu_3SnD gave the monodeuterated compound **105** with (*E*)-configuration with up to 98% selectivity. Treatment of **105** with I_2 quantitatively led to the iodo derivative **106**. The introduction of the CD_3 group was achieved by exposing **106** to cyanocuprate coupling conditions. Bu_4NF Deprotection and acetylation gave the deuterated diacetate **107** with the required (*Z*)-configuration. Enantioselective *Sharpless* dihydroxylation of this compound, followed by acetylation, provided the ME tetraacetate **108**. Subsequent removal of the Ac groups under basic condition yielded the desired compound **99**.

The two routes dicussed above afford deuterated **99** from simple and readily available raw materials with high yields and acceptable ee values. They would be more practical methods if they can be improved for the preparation of labeled **2**, because the phosphate **2** serves more extensive purposes in the studies on the enzyme mechanisms, such as transformation of intermediates *etc.* of the MEP pathway.

Poulter and co-workers [32] [33] developed a method starting with the construction of the *α*,*β*-unsaturated ester derivatives **109**, and **2** was obtained in an overall yield of *ca.* 30% and acceptable ee value (78%) (*Scheme 23*). Two distinct ways were attempted to furnish the key **109**. In the first one, propane-1,2-diol **110** was treated with TBDMSCl or BnBr to afford the monoprotected alcohol, which was subsequently oxidized with TPAP/NMO to give the protected ketone **111**. *Still* modifications of the

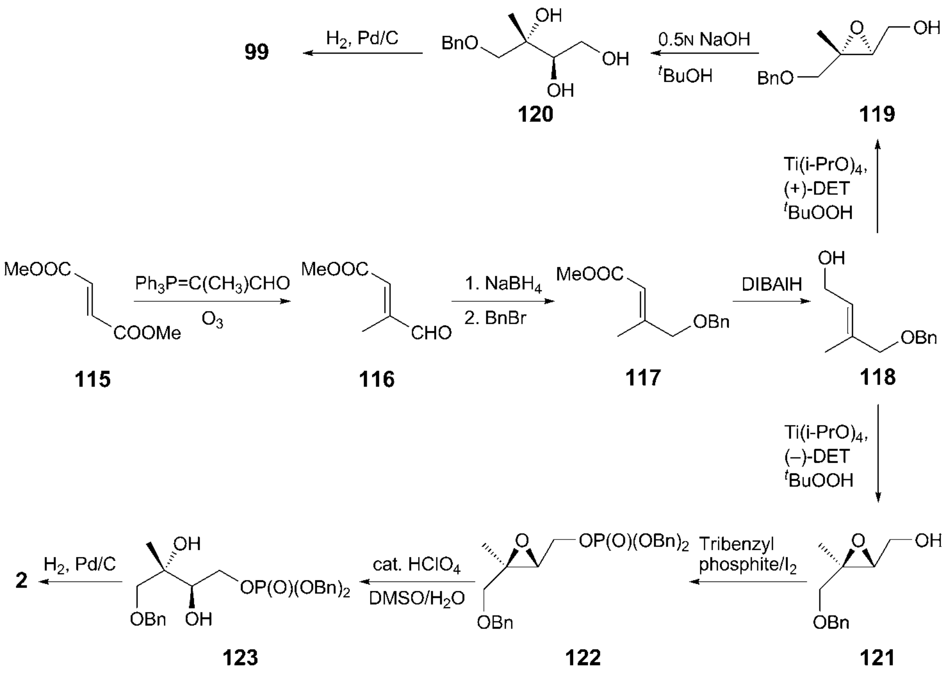
Scheme 23



Horner–Emmons reaction were appield to convert **111** to the protected olefin, which was a mixture with the (*Z*)-isomer being the main component. Flash silica-gel column chromatography gave the (*Z*)-isomer **109** in an acceptable yield. The second synthesis of **109** started from compound **112**, which was obtained from methyl ClCOOMe and the TBDMS-protected propargyl alcohol **113**. This reaction gave the (*Z*)-isomer exclusively with a relatively low yield (34%). Then, reduction of **109** with DIBAlH or $LiAlH_4$, followed by phosphorylation with dimethyl chlorophosphate or with tribenzyl phosphite in the presence of I_2, gave substituted 2-methylbut-2-enyl phosphate **114**. AD-mix-*β* Oxidation of **114**, followed by deprotection with TMSBr (removal of TBDMS or Me) or catalytic hydrogenation (removal of Bn) afforded target compound **2**.

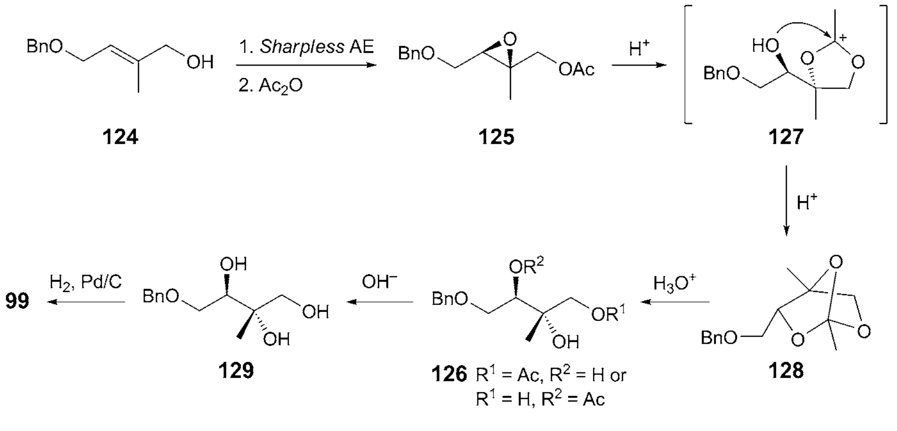
Starting from dimethyl fumarate **115**, *Fontana et al.* [34][35] completed the syntheses of **2** and **99** by applying the same strategy used before by *Poulter* and co-workers (*Scheme 24*). The difference is that the key precursor, *α*,*β*-unsaturated ester derivative **109**, in *Scheme 23* has a (*Z*)-configuration, whereas the key precursor monobenzylated methylbut-2-enol **118** in this route possesses an (*E*)-configuration. In this synthesis, ozonolysis of **115**, followed by *Wittig* reaction with the commercial triphenylphosphoranylidene compound gave the (*E*)-isomer of the formyl derivative **116** with 99% selectivity. Direct $NaBH_4$ reduction and subsequent benzylation of **116** yielded ester **117**. Reduction of **117** with DIBAlH led to desired substrate **118** required for the *Sharpless* epoxidation. A *Payne* rearrangement was used to open the epoxide **119** with very high stereoselectivity. Hydrogenolysis of the triol **120** gave **99** in 84% ee and 31% overall yield. To obtain **2**, compound **118** was converted to *threo*-epoxide **121** by *Sharpless* epoxidation with $Ti(i\text{-}PrO)_4$ and (−)-diethyl tartrate ((−)-DET), which was easily phosphorylated by using freshly prepared $(BrO)_3P$ and I_2 to result in dibenzyl phosphate **122**. Acidic ring opening of **122** in $DMSO/H_2O$ inverted the configuration at the quaternary C-atom to give the desired *erythro*-derivative **123**. Final removal of the Bn groups by catalytic hydrogenation gave **2** with an overall yield of 51% and an ee value of 72%.

Scheme 24



Giner et al. [36] constructed monobenzylated methylbutenol **124** in the same way as described above (*Scheme 25*). *Sharpless* AE reaction, followed by acetylation, led to the epoxy ester **125**, which was then converted to a 1:1 mixture of primary and secondary acetates **126** in epoxy ester/orthoester rearrangement under acidic conditions *via* intermediates **127** and **128**. Successive removal of the protecting groups furnished, *via* **129**, compound **99** with an 86% ee value.

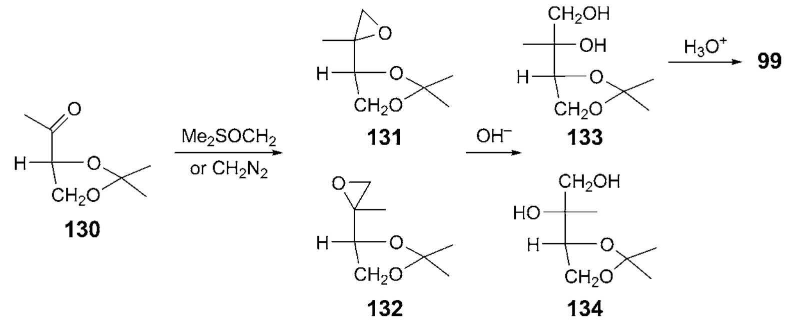
Scheme 25



3.1.2. *From Easily Available Chiral Compounds.* Same of the strategies utilized for the preparation of DX/DXP started from readily available chiral compounds, such as carbohydrates and sugar alcohols, which are choices with highest priority for the synthesis of ME/MEP (**99**/**2**).

The first synthesis of **99**, published in 1980, commenced from 1-deoxy-D-erythrulose acetonide (**130**; *Scheme 26*) [37]. Compound **130** was converted to a epoxide mixture **131**/**132** by the *Johnson–Corey–Chaykovsky* reaction or by reaction with CH_2N_2. Base hydrolysis of the epoxides provided a mixture of diols, **133**/**134**, from which the dominating **133** crystallized, leaving an oil that was fairly pure **134**. Acid hydrolysis of **133** afforded the target **99** in an overall yield of *ca.* 40%.

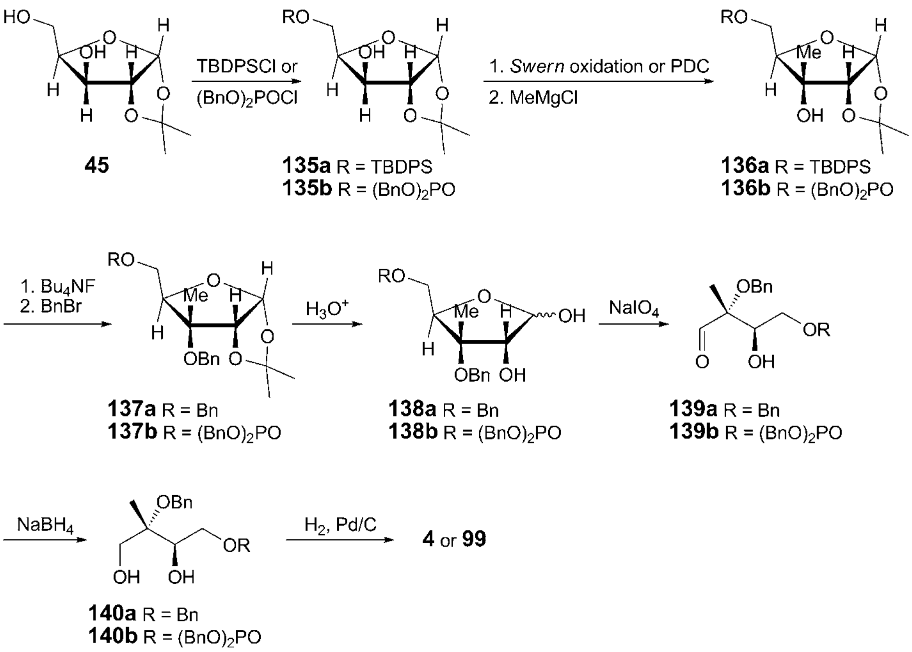
Scheme 26



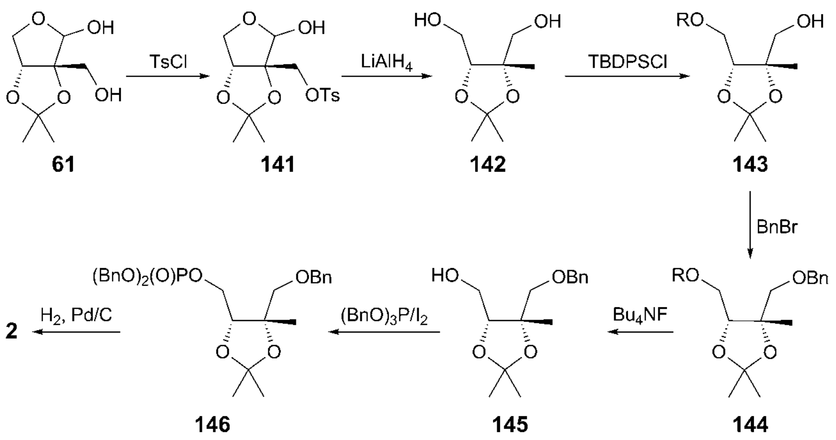
In the protocol developed by *Rohmer* and co-workers [38][39], 1,2-*O*-isopropylidene-α-D-xylofuranose (**45**) was the chiral source, and, after eight steps, the target molecules **2** and **99** were obtained in overall yields of 31 and 41%, respectively, by employing different protection strategies (*Scheme 27*). Selective protection of primary alcohol **45** with TBDPSCl or $(BnO)_2POCl$ afforded compound **135**. The $(BnO)_2PO$ protecting group introduced in **135b** is also a potential phosphate moiety in target **2**. *Swern* (→**135a**) or PDC (→**135b**) oxidation, followed by stereoselective methylation, furnished compound **136** with the desired configuration at C(3). The high stereoselectivity of the methylation step was due to the higher hindrance of the α-face of **136**, which resulted in the addition of the Me group from the less-hindered β-face. Subsequent removal of TBDPS protecting group of **136a** with Bu_4NF and then benzylation led to compound **137**. Acid hydrolysis cleaved the acetonide group to give compound **138**, which was oxidized with $NaIO_4$ to aldehyde **139**. Reduction of the aldehyde group with $NaBH_4$ yielded compound **140**, and debenzylation by standard hydrogenation afforded 2-methyl-D-erythritol (**99**) or its phosphate **2**.

Preparation of compound **2** from D-arabinose also has been reported. *Koumbis et al.* [40] synthesized lactol **61** easily from D-arabinose acetonide. Tosylation of the *C*-hydroxymethyl derivative **61** gave tosylate **141**. $LiAlH_4$ Reduction of **141** afforded methyl-D-erythritol acetonide **142**, which was then treated with TBDPSCl to provide alcohol **143**. Benzylation of the OH group of **143** gave compound **144**, and by removal of the TBDPS protecting group with Bu_4NF, **145** was obtained with its free OH group at C(4). Subsequent phosphorylation of the OH with $(BnO)_3P/I_2$ gave **146**. After catalytic

Scheme 27



Scheme 28

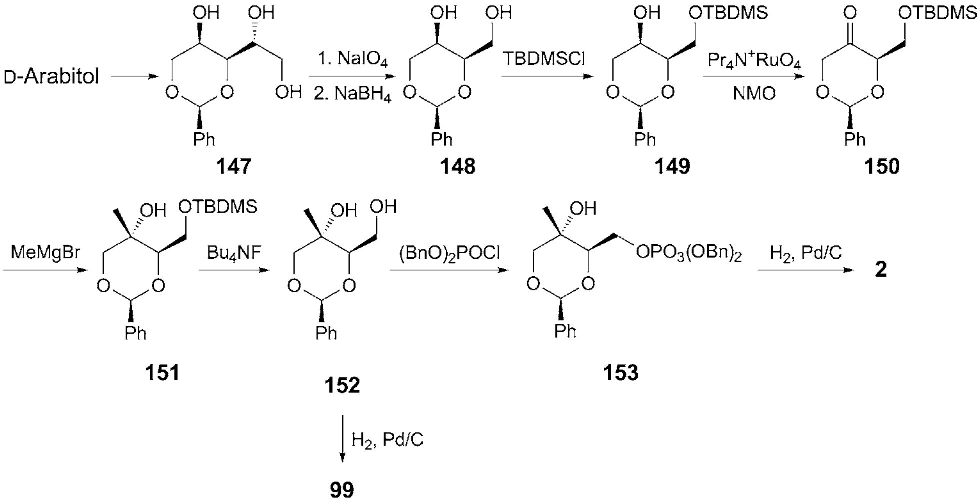


hydrogenation, **146** was converted to the target molecule **2**. The reaction sequence involved seven steps with an overall yield of 37% (*Scheme 28*).

In the method developed by *Coates* and co-workers [41][42], D-arabitol was used for the synthesis of the compounds **2** and **99**, and their derivatives (*Scheme 29*). Acetylation of D-arabitol with benzaldehyde gave the 1,3-benzylidene derivative **147**. Oxidative cleavage of the vicinal diol with $NaIO_4$ and immediate reduction of the resulting unstable aldehyde with $NaBH_4$ afforded 1,3-*O*-benzylidene-D-threitol (**148**).

Selective protection of the primary OH group with *tert*-butyldimethylsilyl chloride (TBDMSCl) produced dioxanol **149**, which was then oxidized with TPAP in the presence of NMO to give dioxanone **150**. Attempts to convert **149** to **150** by the *Swern* procedure, and with PCC and PDC oxidants either failed completely or afforded unacceptably low yields. Methylation of **150** with MeMgBr gave rise to compound **151** with high stereoselectivity (ee > 90%) due to the presence of Bn and TBDMS directing groups. Cleavage of the TBDMS group in **151** with Bu_4NF afforded 1,3-benzylidene-2-methylerythritol (**152**). Subsequent catalytic hydrogenation provided the desired tetrol **99** (seven steps, 33% overall yield). Regioselective monophosphorylation of **152** with $(BnO)_2POCl$ resulted in phosphorylated alcohol **153**. Simultaneous hydrogenolysis of the primary dibenzyl phosphate and the benzylidene ring of **153** generated **2** (eight steps, 30% overall yield).

Scheme 29



Bacher, *Zenk*, and co-workers [43] prepared **2** from D-mannitol as a chiral source. But the long route (14 steps) and the low overall yield (*ca.* 9%) rendered it impractical.

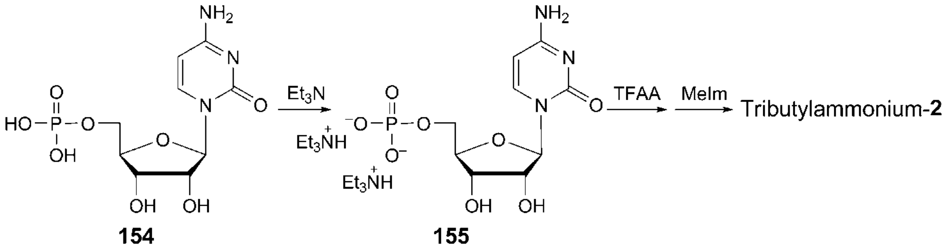
3.2. *Enzymatic Preparation of* **2**. DXP (**1**) and MEP (**2**) can also be prepared by an enzymatic method. But, because MEP synthase DXR is yet not commercialized, and its substrate **1** is expensive, it is not economically favorable to synthesize **2** by the direct enzymatic conversion. *Rohdich* and co-workers reported the enzymatic preparation of **2** from D-glucose in a quite similar manner as the enzymatic synthesis of **1** by the same authors [44]. The only difference is that DXR and coenzyme NADPH need to be supplemented to the mixture (*Scheme 20*).

4. Synthesis of CDPME (7), CDPMEP (8), and cMEPP (9).

– 4-Diphosphocytidyl-2-methyl-D-erythritol (CDPME; **7**; *Scheme 1*), 4-diphosphocytidyl-2-methyl-D-erythritol 2-phosphate (CDPMEP; **8**; *Scheme 1*), and 2-methyl-D-erythritol 2,4-cyclodiphosphate (cMEPP; **9**; *Scheme 1*) are three other important intermediates in the MEP pathway. The biological significance of these three intermediates is that, through them, the mono-phosphate moiety of MEP (**2**) is converted to pyrophosphate group which is essential for the biological activities of the downstream intermediates IPP (**5**) and DMAPP (**6**), and OH groups at C(2) and C(3) of **2** are eliminated. Compound **9** has been isolated earlier from cultures of *Desulfovibrio desulfuricans* and *Corynebacterium ammoniagenes* bacteria, and its biological role was assigned in terms of a protective function due to its accumulation in bacteria, exposed to oxidative stress [45][46]. It had been determined tentatively as a dead-end product derived from **2**, before it was established as a genuine intermediate of MEP pathway [47]. From the viewpoint of chemical structure, these three compounds can be considered as the derivatives of **2**, and thus the synthetic methods for them could be an extension of or related to the methods for the preparation of **2** or **99**.

4.1. *Synthesis of CDPME* (**7**). *Koppisch* and *Poulter* [33] developed a five-step method to synthesize MEP (**2**) from (benzyloxy)acetone **111** (*Scheme 23*) and extended it to the preparation of **7**. Cytidine monophosphate (CMP; **154**) was first converted to its triethylammonium salt **155** by titration with Et_3N. The nucleoside phosphate **155** thus obtained was activated by successive treatment with trifluoroacetic anhydride (TFAA) and 1-methyl-1*H*-imidazole (MeIu) and then coupled with the tributylammonium salt of **2** to give compound **7** in a yield of 40% (*Scheme 30*).

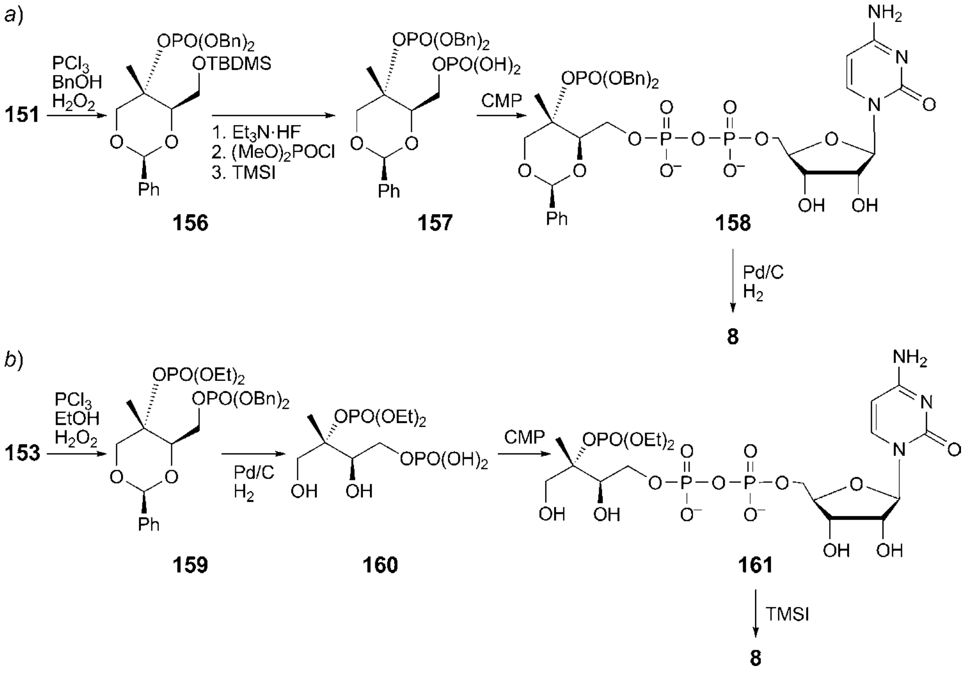
Scheme 30



Bacher and co-workers [48] reported the enzymatic preparation of **7** from D-glucose using by strategies similar to those for the syntheses of **1** (*Scheme 20*) [24] and **2** [44], which were developed by the same group. By employing this protocol, different ^{13}C-labeled compounds **7** were prepared. *Crick* and co-workers [49] set up a chemoenzymatic procedure to obtain **7**, by which MEP (**2**) was synthesized chemically almost in the same way as published by *Rohmer* and co-workers (*Scheme 27*) [38], and **7** was subsequently obtained enzymatically by coupling of **2** and cytidine triphosphate in the presence of enzyme IspD.

4.2. *Synthesis of CDPMEP* (**8**). *Crick* and co-workers [50] tried two different ways to synthesize **8** in which compounds **151** and **153** (*Scheme 29*) were prepared from D-arabitol by using the alike pathway set up by *Coates* and co-workers [41][42] for MEP (**2**). In one way (*Scheme 31*, *Path a*), the tertiary OH group of **151** was phosphorylated by PCl_3, followed by benzylation with BnOH and subsequent oxidation with a H_2O_2 solution to yield dibenzyl phosphate **156**. Removal of the TBDMS group in **156** by $Et_3N \cdot HF$ and phosphorylation of the primary OH group thus produced using $(MeO)_2POCl$ and deprotection of the Me group in the dimethyl phosphate moiety by iodotrimethylsilane (TMSI) gave **157**. Then, coupling the Bn-protected methylerythritol **157** with cytidine monophosphate (CMP), followed by hydrogenolysis of **158**, afforded the final product **8** in an overall yield of only 3%. The very low yield of this pathway was due to the 8% yield of the step from **157** to **158**, which might be largely improved by activating CMP beforehand by the method developed by *Marlow* and *Kiessling* [51]. The alternative way (*Scheme 31*, *Path b*) gave the target compound **8** in an acceptable overall yield (26%). The tertiary OH group of **153** was phosphorylated by PCl_3, followed by ethylation and subsequent oxidation with H_2O_2, to yield biphosphate **159**. Debenzylation resulted in phosphorylated 2-hydroxy-MEP **160**. Coupling **160** with CMP afforded compound **161**; after removal of the ethyl in diethyl phosphate moiety with TMSI, **8** was obtained.

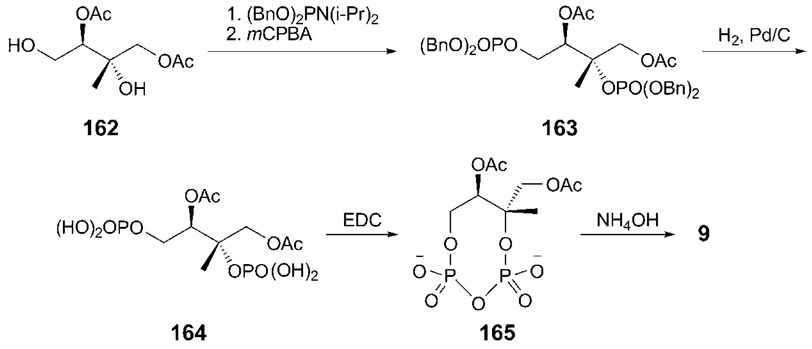
Scheme 31



The enzymatic route for preparation of compound **8** was also reported by *Illarionova et al.* [52], as an extension of the procedures developed for the synthesis of its precursors **1** (*Scheme 20*) [24], **2** [44], and **7** [48].

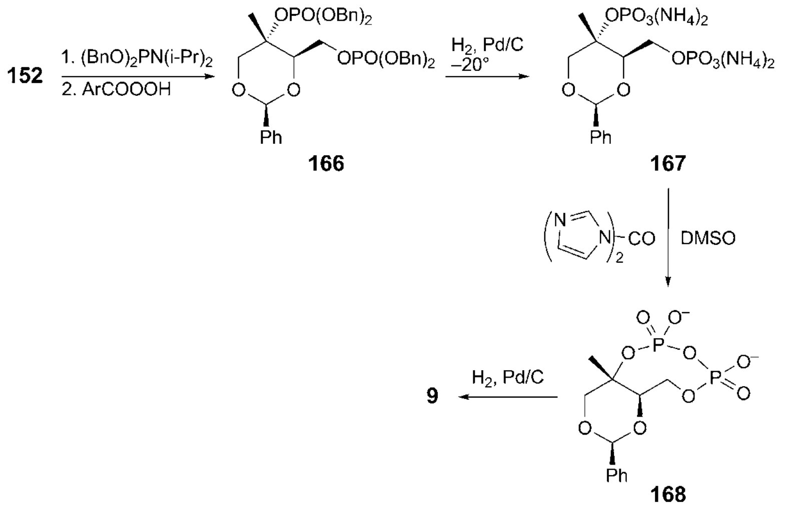
4.3. *Synthesis of cMEPP* (**9**). The first preparation of **9** was published by *Giner* and *Ferris* [53], which was based on their approach for ME (**99**) from monobenzylated methylbutenol **124** *via* biomimetic epoxy ester/ortho ester rearrangement [36]. 1,3-Diacetylated ME **162** was bisphosphorylated by the phosphoramidite method to provide Bn-protected diphosphate **163**, which, upon hydrogenolysis gave 2-methyl-D-erythritol 1,3-diacetate 2,4-diphosphate (**164**). Carbodiimide coupling of **164** with 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDC) smoothly resulted in the formation of the protected cyclic pyrophosphate **165** that could be converted to **9** by saponification of the acetates with NH_4OH. The overall yield of this route was *ca.* 42% from compound **162** (*Scheme 32*).

Scheme 32



When preparing **2**, *Coates* and co-workers [41][42] prepared compound **152** (*Scheme 29*), from which they also achieved the synthesis of **9**. Double phosphorylation of **152** by the phosphoramidite method furnished Bn-protected bisphosphate **166** (*Scheme 33*). Selective removal of the Bn groups produced bisphosphate **167** as its tetraammonium salt, which was readily transformed to cyclic diphosphate **168** in the presence of 1,1-carbonyldiimidazole in anhydrous DMSO. Further hydrogenolysis provided the target compound **9** with an overall yield of little less than 50% calculated from **150**.

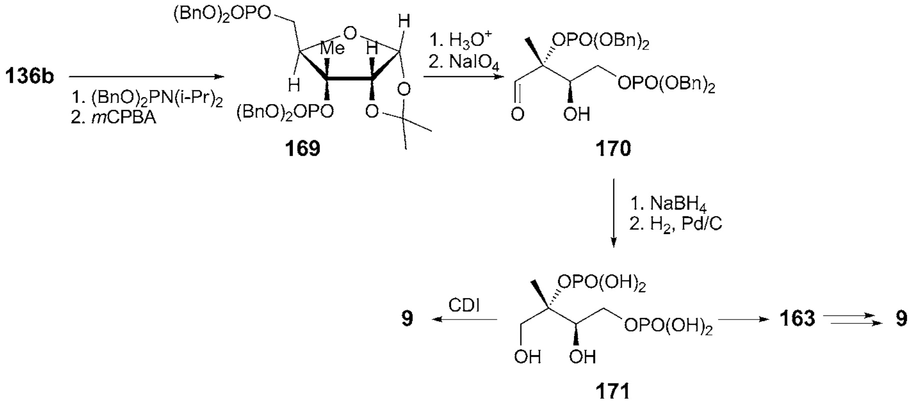
Scheme 33



The most recent chemical synthesis of **9** was reported by *Narayanasamy* and *Crick* [54]. 1,2-*O*-Isopropylidene-α-D-xylofuranose (**45**; *Scheme 27*) was taken as the chiral source, and after ten steps, **9** was obtained in an overall yield of less than 10%.

Compound **45** was first converted to **136b** by the same strategy reported by *Rohmer* and co-workers [38][39]. Phosphorylation of the tertiary OH group with dibenzyl diisopropyl phosphoramidite and then oxidation gave bisphosphate **169**, which was further transformed to formyl-bisphosphate **170** by acid hydrolysis and subsequent $NaIO_4$ oxidation. Reduction of **170** with $NaBH_4$, followed by hydrogenolysis afforded **171** with phosphorylated OH groups at C(2) and C(4) (*Scheme 34*). Subsequent cyclization using 1,1′-carbonyldiimidazole (CDI) provided **9** in only 20% yield due to the presence of the free OH groups in **171**. So, a different route that was similar to the published process [38][39] was utilized to prepare the cyclic diphosphate from **171** by protecting its free OH groups with Ac_2O leading to **163** (*Scheme 27*), which further underwent hydrogenolysis, cyclization, and deprotection to give **9** (*Scheme 34*).

Scheme 34

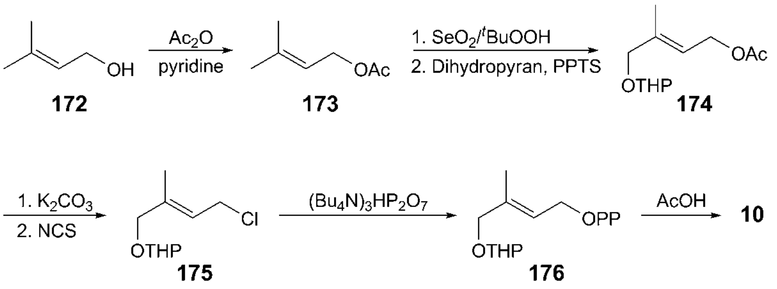


The first enzymatic preparation of **9** was established by *Rohdich* and co-workers [55]. This rapid one-pot strategy developed from the enzymatic synthesis of isotope-labeled DXP (**1**; *Scheme 20*) [24] afforded a wide variety of isotopomers of **9** in relatively high quantity. The reaction sequences involving up to ten forward reaction steps and up to 15 enzymes led to a 50–80% overall yield of purified product. *Zenk* and co-workers [56] developed a facile enzymatic preparation of **9** in highly radioactive form by using spinach (*Spinacea oleracea*) chloroplast stroma that contained all the MEP-pathway enzymes from highly radioactive **1**. Incubation of [^{14}C]-**1** with NADPH, CTP, ATP, Mg^{2+}, Mn^{2+}, as cofactors necessary for the pathway enzymes DXR, ispD, ispE, and ispF, as well as NaF, an inhibitor of phosphatases, led to the formation of the desired cyclic diphosphate **9** in a yield of more than 80% after purification. Meanwhile, this method could also be adapted to prepare ^{13}C-labeled **9**, when [^{13}C]-**1** was taken as starting material. Recently, *Oldfield* and co-workers [57] published an enzymatic synthesis of [U-^{13}C]-**9** utilizing the phenomenon that some kind of bacterium, for example, *C. ammoniagenes*, can accumulate **9** when it was exposed to oxidative stress. Growth of the bacterium on [U-^{13}C]-D-Glucose under oxidative stress condition afforded [U-^{13}C]-**9**, but the yield was not reported.

5. Synthesis of HDMAPP (10). – By using the NMR technology, *Rohdich*, *Eisenreich*, and co-workers [58] determined that the cyclic diphosphate **9** was converted to IPP/DMAPP (**5**/**6**) through (*E*)-4-hydroxy-3-methylbut-2-enyl diphosphate (HDMAPP; **10**; *Scheme 1*). Almost at same time, *Zenk* and co-workers [59] completed the synthesis of isotope-labeled **10** and verified its intermediacy in the MEP pathway by incorporating tritiated **10** into phytoene. From then on, compound **10** has drawn a lot of attention as the last MEP pathway-specific intermediate, and several chemical and enzymatic syntheses of this compound have been published.

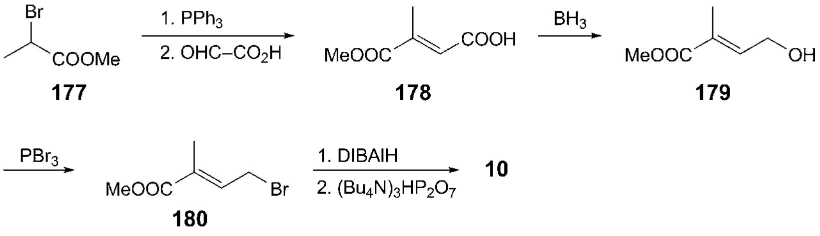
Ward and *Beale* [60] accomplished the first total synthesis of unlabeled **10** starting from 3-methylbut-2-enol (**172**) with low yield, because the pyrophosphorylation step gave only a 14% yield of purified product (*Scheme 35*). Acetylation of alcohol **172** with Ac_2O almost quantitatively afforded **173**. Regioselective allylic hydroxylation at the (*E*)-methyl group of **173** using SeO_2 and ^{t}BuOOH, followed by protection of the resulted primary OH group with dihydropyran, led to the ester **174**. Base hydrolysis of **174** and subsequent treatment of the product with *N*-chlorosuccinimide (NCS) furnished the chloro derivative **175**. Then, after reaction with tris(tetrabutylammonium) hydrogen diphosphate to yield compound **176** and removal of the THP group with aqueous acid, the target compound **10** was obtained.

Scheme 35



Shortly after, *Rohmer* and co-workers [61] described a four-step process for unlabeled **10** starting from commercial methyl 2-bromopropionate (**177**) in a 24% overall yield (*Scheme 36*). The C-atom framework of the target molecule was constructed by a *Wittig* reaction. The *Wittig* reagent derived from **177** was condensed with glyoxylic acid monohydrate to produce only acid **178** with the desired configuration. Selective BH_3/THF reduction of **178** yielded **179**, which was brominated

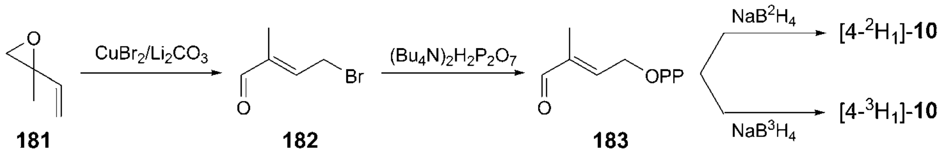
Scheme 36



with PBr_3 to afford bromido ester **180**. Reduction of **180** with DIBAlH, followed by pyrophosphorylation, resulted in the diphosphate **10**.

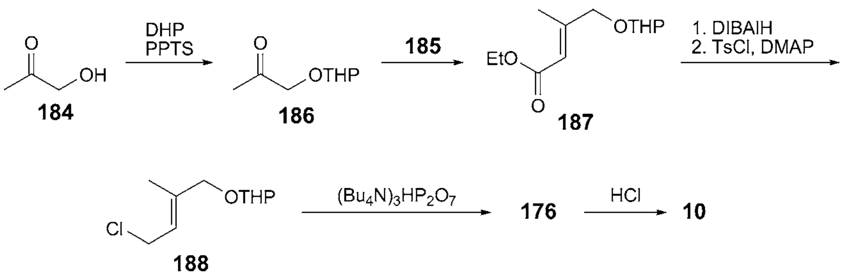
Chemical preparation of labeled **10** was first completed by *Zenk* and co-workers [59] from commercial 2-ethenyl-2-methyloxirane (**181**; *Scheme 37*). Treatment of **181** with $CuBr_2$ in the presence of Li_2CO_3 gave bromoaldehyde **182**, which was subsequently pyrophosphorylated with bis(tetrabutylammonium) dihydrogen pyrophosphate to afford formyl-pyrophosphate **183**, the key intermediate of the route. Reduction of the CHO group of **183** with NaB^2H_4 provided **10** with its C(4) being deuterated. Tritiation at the same location was achieved by reduction with NaB^3H_4. The reported overall yield of this method was only *ca.* 7%, which could be highly improved by purifying compound **183** before the reduction. Actually, the best overall yield we have ever achieved was *ca.* 30%. The advantage of this procedure is that one can introduce the isotope at the very last step of the synthesis, which can *i*) lower isotope loss during preparation; *ii*) reduce the possibility of radioactive contamination; and *iii*) ease the synthetic operation, although the overall yield is only medium.

Scheme 37



Almost at same time, *Eisenreich* and co-workers [62] reported their six-step synthesis of **10** from hydroxy-acetone **184** and (ethoxycarbonylmethenyl)(triphenyl)-phosphorane (**185**) with an overall yield of 38% (*Scheme 38*). Protection of the primary OH group of **184** with dihydropyran gave tetrahydropyranyl (THP) derivative **186**. *Wittig* reaction of **186** with **185** led to a 6:1 mixture of (*E*)- and (*Z*)-**187**, from which the pure (*E*)-isomer was separated by preparative HPLC. DIBAlH Reduction of (*E*)-**187**, followed by treatment with TsCl in the presence of 4-(dimethylamino)pyridine (DMAP), provided the chloro derivative **188**, which was subsequently converted to **10** first by pyrophosphorylation and then acid deprotection of the THP group.

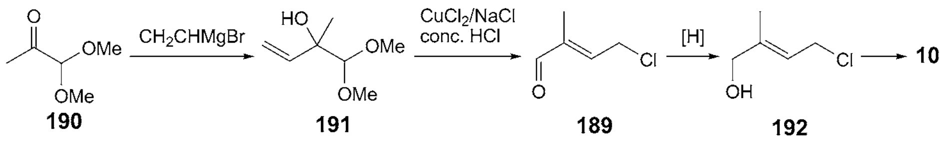
Scheme 38



Fox and *Poulter* [63] constructed chloro aldehyde **189**, an analog of compound **182** (*Scheme 37*), and, from **189**, target **10** was obtained after reduction of the aldehyde group with $NaBH_4$ (or NaB^2H_4) and pyrophosphorylation with a yield of *ca.* 65%

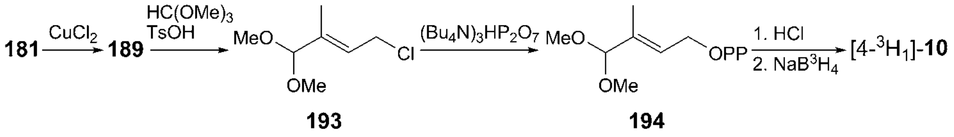
(*Scheme 39*). Compound **189** was obtained from compound **190** *via* compound **191**. The yield of the two steps from **189** to **10** was much higher than that of the steps from **182** to **10** in *Scheme 37*. So, reduction of the halo aldehyde prior to the introduction of pyrophosphate group should be a good choice for the preparation of unlabeled and ^{2}H-labeled **10**, but it is better to apply *Scheme 37* when preparing tritiated compound **10**.

Scheme 39



Taking the advantages of the processes of *Zenk* and *Poulter* together, *Rohdich* and co-workers [64] synthesized **10** from compound **181** (*Scheme 37*). Unlabeled **10** was prepared in a two-step procedure, in which **181** was first converted into **192** (*Scheme 39*), then pyrophosphorylation of the chloro alcohol afforded **10** in an overall yield of 72%. For tritiated **10**, chloro aldehyde **189** obtained from **181** without any detectable (*Z*)-isomer was protected by conversion to acetal **193**, which was then reacted with tris(tetrabutylammonium) pyrophosphate to afford acetal pyrophosphate **194**. Acid hydrolysis of **194**, followed by reduction with NaB^{3}H$_4$, gave [4-^{3}H]-**10** in an overall yield of 15% (*Scheme 40*).

Scheme 40

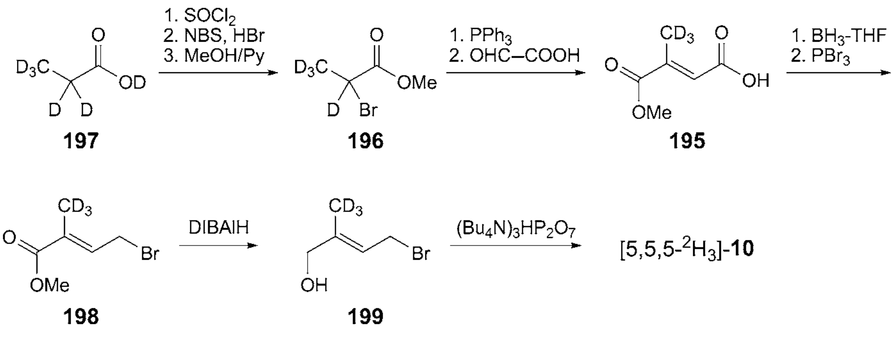


Methyl group-deuterated **10** ([5,5,5-^{2}H$_3$]-**10**) was prepared by *Oldfield* and co-workers [57] by a nine-step procedure with a low overall yield (*Scheme 41*). Compound **195** (C$_5$ skeleton with (*E*)-configration) was built up by *Wittig* reaction of bromo ester **196** that was transformed from (D$_6$)propanoic acid (**197**), by three consecutive conversions, and glyoxylic acid monohydrate. Borane reduction of **195**, followed by bromination with PBr$_3$ gave deuterated bromo ester **198**. Further reduction of the ester group with DIBAlH produced bromo alcohol **199** that was subsequently pyrophosphorylated to afford the deuterated **10**. In addition, the authors also prepared [U-^{13}C]-**10** from [U-^{13}C]-**9** by using *A. aeolicus* IspH protein as catalyst.

Zenk and co-workers [65] found that the chromoplasts from *Capsium annuum*, the enzyme activity of which was impaired by freeze-thawing, accumulate this intermediate. Based on this observation, they developed a cell-free system allowing the synthesis of **10** with labels in various positions from upstream intermediates. With the cyclic diphosphate **9** as substrate, **10** could be obtained with yields of *ca.* 50%.

6. Openings and Further Direction. – It has been more than two decades since the MEP terpenoid biosynthetic pathway was found. Although several aspects of the pathway, for example, genes, enzymes, intermediates *etc.*, have been characterized up

Scheme 41



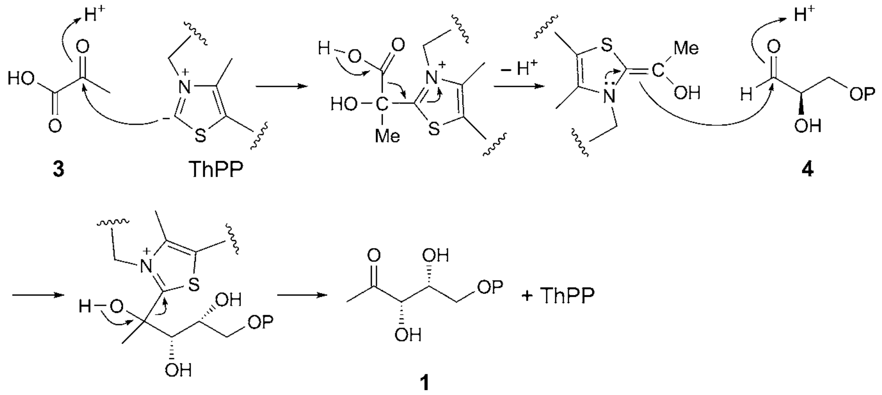
to date, there are still unclear issues that deserve further investigation. The main problems lie in two aspects:

1) The mechanical insights into the MEP pathway enzymes;

2) Screening of antimicrobial drugs using the MEP pathway as a target.

So far, several mechanical studies of the MEP pathway enzymes have been carried out, and their catalytic mechanisms have been basically elucidated. However, the details of catalysis of the enzymes still need to be complemented. DXS is one of the key enzymes of this pathway which is responsible for the formation of the key intermediate DXP (**1**) that is also a precursor for the biosynthesis of vitamins B_1 and B_6 in some bacteria [2][3]. However, little research on its catalytic function has been carried out. *Rohmer et al.* [66] proposed a biogenetic process of DXS according to the catalytic action of known ThPP-dependent enzymes (*Scheme 42*). *Eubanks* and *Poulter* [67] found out that DXS follows an ordered kinetic mechanism which is in contrast to the classical ping-pong kinetic mechanism of DXS homolog including transketolase and acetolactate synthase. Crystal structures of *E. coli* and *Deinococcus radiodurans* DXS showed that the subunit of the enzyme is formed by three domains with its active site being located between domains I and II of the same monomer of the homodimer,

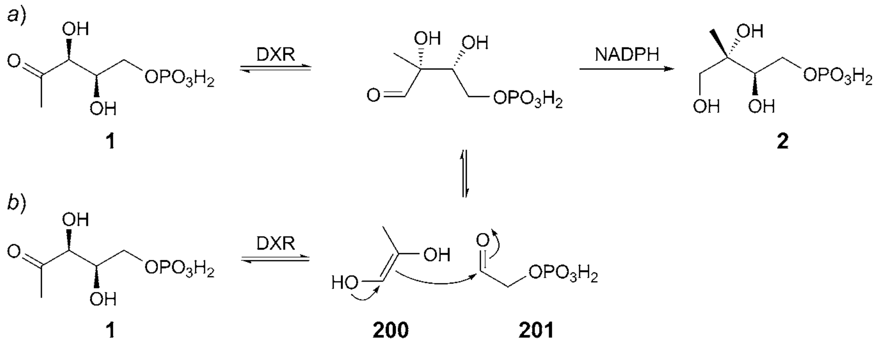
Scheme 42



whereas that of transketolase is located at the interface of the dimer [68]. A more recent study of DXS uncovered flexibility in the acceptor substrate binding pocket for nonpolar substrates and disclosed that pyruvate **3** can act as both donor and acceptor substrate [69]. No more details of this protein is available up to date, and the lack of information not only hampers further understanding of its catalytic mechanism, but also hinders the search for its inhibitors.

Although the catalytic mechanisms of DXR has been intensively investigated, and a *retro*-aldol/aldol mechanism has been accepted (*Scheme 43*, *Path b*) [19][21], some discrepancies need further interpretation. *Rohmer* and co-workers [70] incubated hydroxy-acetone **200** and glycolaldehyde phosphate **201**, the two putative fragments expected from the *retro*-aldol cleavage, with DXR and all the cofactors, and found that no MEP was formed. Furthermore, they observed that the two compounds, either alone or together at concentrations of up to 1 mM, did not inhibit the production of MEP (**2**) from DXP (**1**). Based on this observation, they concluded that these two compounds seemingly are not recognized by DXR. *Rohdich*, *Eisenreich*, and co-workers [71] found that, when a mixture of [1-$^{13}C_1$]-**2** and [3-$^{13}C_1$]-**2** was used as substrate, no fragment exchange could be detected by ^{13}C-NMR spectroscopy in the reverse reaction. In addition, they also found that exogenous **200** was not incorporated in the enzyme product. These two results seem more likely to support the α-ketol mechanism (*Scheme 43*, *Path a*), and their explanation in the framework of the *retro*-aldol/aldol mechanism remains unsolved.

Scheme 43



As for the screening of antimicrobial drugs, a number of studies have been performed, mainly taking DXR as a target, and a long-known antibiotic fosmidomycin and its acetyl congener FR900098 have been established as its inhibitors, which are also active against bacteria as well as the malaria parasite. But, unfortunately, no MEP-pathway inhibitor is presently in clinical use despite large screening programs and many attempts to set up fast enzymatic tests designed for high-throughput screening. Currently, only one report from the screening of natural-product libraries was found [72]. Therefore, seeking MEP pathway inhibitors from natural sources represents an unexplored field.

Based on the above discussion, it is quite clear that the substrates of the MEP-pathway enzymes, particularly the isotope labeled ones, are absolutely indispensable for the complete interpretation of the enzyme mechanisms and for the screening of the MEP-pathway inhibitors. Thus, establishing more practical methods for the preparation of all the intermediates of the pathway with higher efficiency is a future direction.

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