Biosynthesis of Terpenes. Preparation of (*E*)-1-Hydroxy-2-methyl-but-2-enyl 4-Diphosphate, an Intermediate of the Deoxyxylulose Phosphate Pathway†

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(*E*)-1-Hydroxy-2-methyl-but-2-enyl 4-diphosphate (*E*-**6**) was synthesized in six reaction steps from hydroxyacetone (**9**) and (ethoxycarbonylmethenyl)-triphenylphosphorane (**11**) with an overall yield of 38%. The compound was shown to be identical with the product of IspG protein, which serves as an intermediate in the nonmevalonate terpene biosynthetic pathway.

Introduction

More than 30 000 representatives make terpenoids one of the largest groups of natural products that are all assembled from the universal precursors, isopentenyl diphosphate (IPP) (**7**) and dimethylallyl diphosphate (DMAPP) (**8**).[1]

The formation of IPP and DMAPP via mevalonate has been studied in considerable detail over a period of five decades.[2−5] More recently, a second pathway operating in plants[6] and certain bacteria[7,8] has been uncovered by independent studies in the research groups of Rohmer and Arigoni (for review, see also refs 9−12).

The nonmevalonate pathway (Scheme 1) starts with a thiamine diphosphate dependent condensation of pyruvate (**1**) and D-glyceraldehyde 3-phosphate (**2**) affording 1-deoxy-D-xylulose 5-phosphate (**3**).[13,14] This product is then converted into the branched polyol derivative, 2*C*-methyl-D-erythritol 4-phosphate (**4**), by isomerization and reduction of the resulting branched chain aldose; both reaction steps are catalyzed by the NADPH-dependent 2*C*-methyl-D-erythritol 4-phosphate synthase (IspC).[15] The polyol phosphate **4** is converted into the cyclic diphosphate **5** by the sequential action of three enzymes specified by the *ispDEF* genes.[16−21]

Gene targeting analysis using *Escherichia coli* and *Synechocystis* sp. had previously indicated an essential role in the new paythway for the genes *ispG* (formerly *gcpE*)[22,23] and *ispH* (formerly *lytB*).[24−26] In recent work with *E. coli* cells engineered for the overexpression of the *xylB* gene (specifying 1-deoxy-D-xylulose kinase), as well as of the *ispDEFG* genes, we observed the formation of a new intermediate, (*E*)-1-hydroxy-2-methyl-but-2-enyl 4-diphosphate (*E*-**6**),[27] upon feeding of ^{13}C-labeled 1-deoxy-D-xylulose. The additional expression of the *ispH* gene led to the in vivo formation of both IPP and DMAPP.[28]

† Dedicated to Professor Helmut Simon on the occasion of his 75th birthday.

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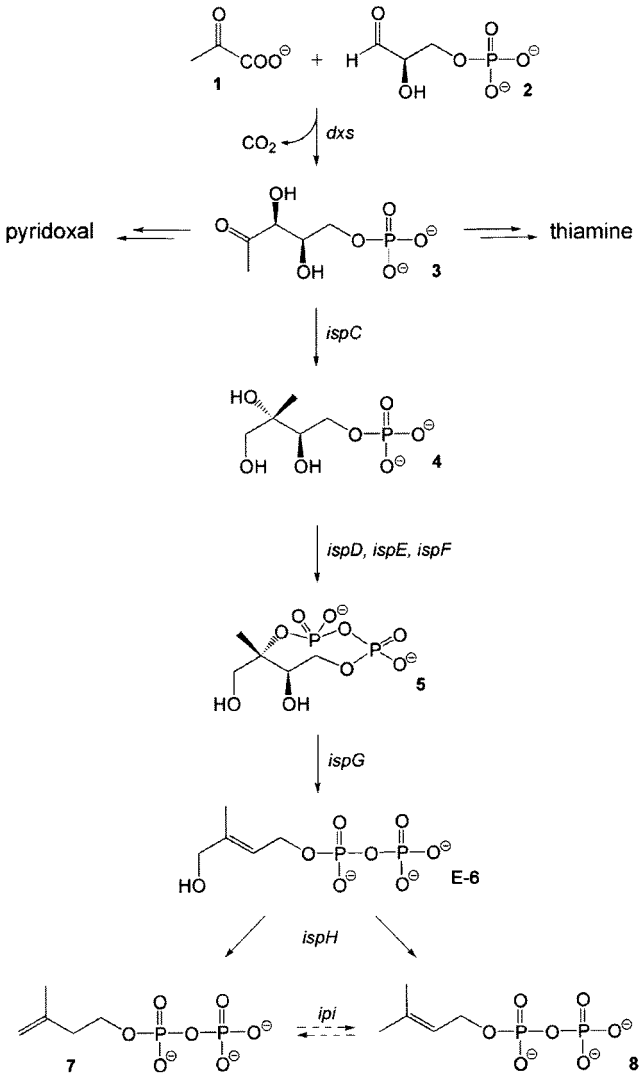
Scheme 1. Biosynthesis of IPP (7) and DMAPP (8) via 1-Deoxy-D-xylulose 5-Phosphate (3)[a]



[a] Dashed arrows under *ipi* are meant to indicate that an isomerization step is not essential.[25]

In agreement with these findings, accumulation of a compound identified spectroscopically as *E*-**6** was independently observed in an *ispH*-deficient mutant of *E. coli*.[29] The possible cooperation of additional but yet unidentified proteins in channeling the electron flow required by the reductive nature of the last two steps of Scheme 1 remains to be investigated.

In our original work on the role of the IspG protein, identification of the reaction product was greatly helped by the availability of an authentic specimen of *E*-**6** of synthetic origin. This paper describes the synthesis of *E*-**6** and its spectroscopic comparison with the biosynthetic material. The synthetic route outlined in this paper could stimulate future work on the synthesis of isotope-labeled *E*-**6** as a substrate for biosynthetic studies.

Results and Discussion

Treatment of hydroxyacetone (**9**) with an excess of 3,4-dihydro-2*H*-pyran under catalysis by pyridinium toluene 4-sulfonate[30] afforded the known tetrahydropyranyl (THP) derivative **10** (Scheme 2). Wittig reaction of **10** with (ethoxycarbonylmethylene)triphenylphosphorane (**11**)[31] in toluene under reflux conditions gave a 6:1 mixture of (*E*)- and (*Z*)-ethyl-2-methyl-1-tetrahydropyranyloxy-but-2-enoate (*E*/*Z*-**12**), from which the pure *E*-isomer was separated by preparative HPLC (for stereochemical assignment of the two isomers cf. below).

Reduction of *E*-**12** by DIBALH[32] in CH_2Cl_2 afforded (*E*)-2-methyl-1-tetrahydropyranyloxy-but-2-ene-4-ol (*E*-**13**). To avoid the formation of side products, it was important to maintain the temperature at −78 °C. During workup, it was essential to remove aluminum salts by extraction with copious amounts of methanol; otherwise, the oily product could not be purified by chromatography on silica gel.

The allyl alcohol *E*-**13** was transformed into the chloride *E*-**14** with DMAP and *p*-TsCl in CH_2Cl_2. The chloride *E*-**14** is unstable at room temperature.

The diphosphate group was introduced by reaction of *E*-**14** with 1.2 equiv of tris(tetra-*n*-butylammonium) hydrogen diphosphate in MeCN,[33] and the resulting *E*-**15** could be purified by two chromatographic steps using columns of DOWEX 50 WX8 and cellulose. Alternatively, the mixture was subjected to deprotection without prior purification.

The THP group of *E*-**15** was removed by acid hydrolysis (pH 1.3) at ambient temperature. Prolonged incubation of the reaction mixture resulted in progressive hydrolysis of the diphosphate motif of *E*-**6**. (*E*)-1-Hydroxy-2-methyl-but-2-enyl 4-diphosphate (*E*-**6**) was purified by cation exchange chromatography on DOWEX 50 WX8 followed by chromatography on cellulose. The overall yield for the last two steps was 66%.

A 6:1 mixture of (*E*)- and (*Z*)-1-hydroxy-2-methyl-but-2-enyl 4-diphosphate (*E*/*Z*-**6**) was prepared by the same route from the original unresolved mixture of *E*/*Z*-**12** and used for the spectroscopic characterization of both isomers. NMR data are summarized in Table 1. E/Z assignments are based on NOESY experiments. Specifically, the geometry of the double bond was established by strong NOE interactions between H-3/H-1 and H-4/H-2-methyl for *E*-**6** and H-3/H-2-methyl and H-4/H-1 for *Z*-**6**, respectively.

For comparison with the synthetic specimen of *E*-**6**, a sample of the biosynthetic material was produced following the published procedure[27] by incubating [U-$^{13}C_5$]1-deoxy-D-xylulose with an *E. coli* strain engineered for expression of the *xylB* and *ispCDEFG* genes. An extract prepared by ultrasonic treatment of the cells was used without purification for NMR analysis of the biosynthetic product. The relevant ^{13}C NMR signals are shown in Figure 1A; in agreement with published data,[27] they form complex multiplets due to $^{13}C-^{13}C$ coupling.

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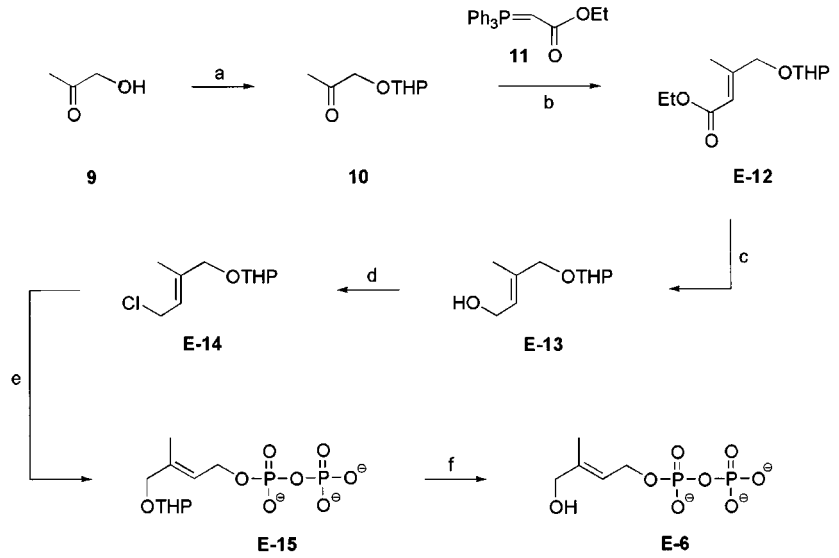
Scheme 2[a]



[a] Reagents and conditions: (a) DHP, PPTS, 25 °C (2.5 h); (b) toluene, reflux (23 h); (c) (1) DIBALH, CH_2Cl_2, −78 °C (2.5 h), (2) 1 M NaOH/H_2O; (d) *p*-TsCl, DMAP, CH_2Cl_2, 25 °C (3 h); (e) tris(tetra *n*-butylammonium) hydrogendiphosphate, MeCN, 25 °C (3 h); (f), HCl/H_2O pH 1.3, 25 °C (20 min).

Table 1. NMR Data of (*E*)-1-Hydroxy-2-methyl-but-2-enyl 4-Diphosphate (*E*-6) and (*Z*)-1-Hydroxy-2-methyl-but-2-enyl 4-Diphosphate (*Z*-6)[e] in D_2O at pH 7

position	chemical shifts [ppm]			coupling constants [Hz]			^{13}C isotope shifts[c] [ppb]	NMR correlation pattern	
	^{1}H[a]	^{13}C[a]	^{31}P[b]	J_{HH}	J_{PC}	J_{PP}		NOESY (rel NOE)[d]	HMBC
(*E*)-1-Hydroxy-2-methyl-but-2-enyl 4-Diphosphate (*E*-**6**)									
1	3.88	68.6					−8	2-methyl (53), 3 (100)	2, 2-methyl, 3
2		141.6					−67		
2-methyl	1.57	15.1					−15	4 (79), 1 (53)	1, 2, 3
3	5.52	122.7		6.89, 1.2	8.0		−100	1 (100)	
4	4.39	64.3		7.2	5.1		−30	2-methyl (79)	2, 3
P_β			−3.49 (−4.48)[e]			22.3 (20.8)[e]			
P_α			−7.17 (−7.06)[e]			22.0 (20.8)[e]			
(*Z*)-1-Hydroxy-2-methyl-but-2-enyl 4-Diphosphate (*Z*-**6**)[e]									
1	4.03	62.0						2-methyl (34), 4 (100)	2, 2-methyl, 3
2		141.8							
2-methyl	1.70	22.7						1 (34), 3 (88)	1, 2, 3
3	5.49	125.6		6.8	7.7			2-methyl (88)	
4	4.41	63.8		7.3	5.1			1 (100)	2, 3
P_β			−4.48			20.8			
P_α			−7.06			20.8			

[a] Referenced to external trimethylsilylpropane sulfonate. [b] Referenced to external 85% orthophosphoric acid. [c] Determined with [U-$^{13}C_5$]1-hydroxy-2-methyl-but-2-enyl 4-diphosphate. [d] Intensities of cross-signals are given as relative units. [e] Determined from *E*/*Z*-**6**.

Figure 1C displays the ^{13}C NMR signals of synthetic (*E*)-1-hydroxy-2-methyl-but-2-enyl 4-diphosphate (*E*-**6**), and Figure 1B shows the signals of the sample of Figure 1A after addition of the synthetic compound. The singlets representing the synthetic compound are slightly downfield-shifted from the center of the multiplets representing the uniformly ^{13}C-labeled biosynthetic compound as a consequence of heavy isotope chemical shift effects in the latter (cf. Table 1). It should also be noted that the line widths of the ^{13}C-labeled biosynthetic material exceed those of the unlabeled synthetic material as a consequence of long-range $^{13}C-^{13}C$ couplings, which are too small to be fully resolved but can be gleaned from the slight line broadening. It could also be shown that the ^{1}H NMR signals of synthetic *E*-**6** coincide with those of the ^{13}C-decoupled ^{1}H NMR spectrum of the biosynthetic product (Figure 2). These findings provide conclusive proof for the correctness of the structure assigned to the new intermediate of the nonmevalonate terpene pathway. Not surprisingly, the synthetic species has also displayed the full biological activity described by other authors[29] for the novel compound, i.e., the stimulation of the proliferation of $\gamma\delta$ T cells (data not shown).

Experimental Section

General. Commercially available reagents were used without further purification. Chromatography was performed on silica gel 60 (230−400 mesh), DOWEX 50 WX8 (200−400 mesh), and cellulose. TLC plates were stained with a 1:2:100 v/v mixture of anisaldehyde/H_2SO_4/HOAc. NMR spectra were recorded at room temperature.

Acetonyl Tetrahydropyranyl Ether (10). A mixture of 339 mg (1.35 mmol) of pyridinium toluene-4-sulfonate, 9.35 mL (10.0 g, 0.135 mol) of hydroxyacetone, and 24.7 mL (22.7 g, 0.270 mol) of 3,4-dihydro-2*H*-pyran was stirred at room temperature for 2.5 h. Residual 3,4-dihydro-2*H*-pyran was removed by evaporation under reduced pressure. The crude mixture was purified by flash chromatography on a column of silicagel (6 × 20 cm; 4:1 hexane/acetone) to yield 18.7 g (0.118 mol, 88%) of **10** as a colorless liquid: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.62 (t, J = 3.6 Hz, 1H), 4.22 (d, J = 17.3 Hz, 1H),

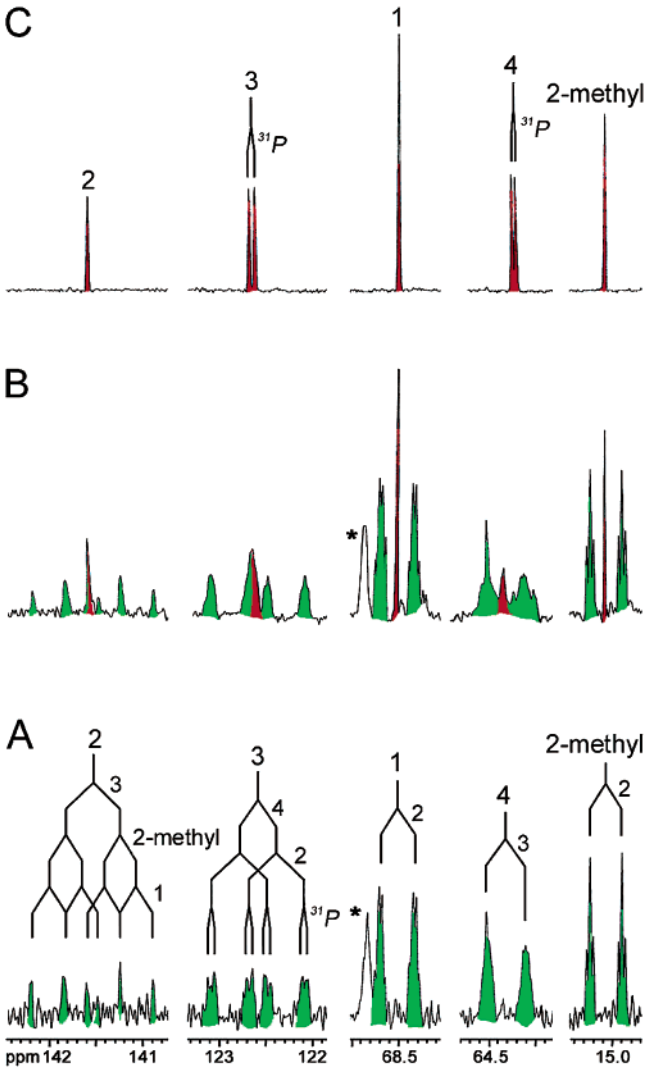


Figure 1. ^{13}C NMR signals of 1-hydroxy-2-methyl-but-2-enyl 4-diphosphate: A, obtained with a crude cell extract of an *E. coli* strain engineered for expression of the *xylB* and *ispCDEFG* genes and proffered with [U-$^{13}C_5$]1-deoxy-D-xylulose; B, obtained with the sample in A after addition of synthetic (*E*)-1-hydroxy-2-methyl-but-2-enyl 4-diphosphate (*E*-**6**); C, synthetic (*E*)-1-hydroxy-2-methyl-but-2-enyl 4-diphosphate (*E*-**6**). An * indicates signals of residual [U-$^{13}C_5$]2*C*-methyl-D-erythritol 2,4-cyclodiphosphate (**5**) present in the crude *E. coli* extract. Couplings with coupling constants below 5 Hz are not indicated. The concentration of biosynthetic [U-$^{13}C_5$]-*E*-**6** was 0.1 mM. The acquisition time was approximately 1 h for each of the ^{13}C NMR experiments.

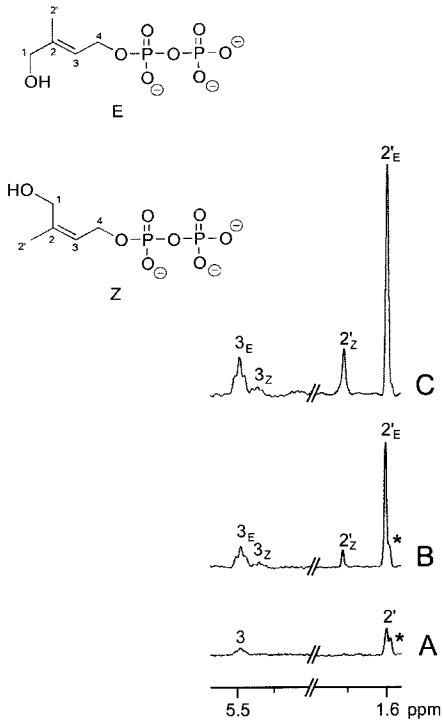


Figure 2. ^{13}C-decoupled ^{1}H NMR signals of 1-hydroxy-2-methyl-but-2-enyl 4-diphosphate: A, a crude cell extract of an *E. coli* strain engineered for expression of the *xylB* and *ispCDEFG* genes and proffered with [U-$^{13}C_5$]1-deoxy-D-xylulose; B, the sample in A after addition of a 6:1 mixture of (*E*)- and (*Z*)-1-hydroxy-2-methyl-but-2-enyl 4-diphosphate; C, the sample in B after addition of a second equivalent of the 6:1 mixture of (*E*)- and (*Z*)-1-hydroxy-2-methyl-but-2-enyl 4-diphosphate. An * indicates signals of an impurity.

4.09 (d, J = 17.3 Hz, 1H), 3.83−3.79 (m, 1H), 3.51−3.47 (m, 1H), 2.15 (s, 3H), 1.87−1.49 (m, 6H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 206.7, 98.7, 72.3, 62.3, 30.2, 26.5, 25.2, 19.2; MS (CI, isobutane) m/z 159 $[M + 1]^+$. Anal. Calcd for $C_8H_{14}O_3$: C, 60.74; H, 8.92. Found: C, 60.39; H 8.65.

Ethyl (*E*)-2-Methyl-1-tetrahydropyranyloxy-but-2-enoate (*E*-12). Acetonyl tetrahydropyranyl ether (**10**, 3.88 g, 24.5 mmol) was dissolved in 50 mL of dry toluene under a nitrogen atmosphere at room temperature. A suspension of (ethoxycarbonylmethylene)triphenylphosphorane (**11**, 12.8 g, 36.8 mmol) in 150 mL of dry toluene was added, and the mixture was heated under reflux for 23 h. The solvent was evaporated under reduced pressure to yield an orange oil. Triphenylphosphinoxide was precipitated by the addition of 100 mL of 9:1 hexane/acetone. The mixture was filtered, and the filtrate was concentrated to a volume of about 1 mL. A 9:1 mixture of hexane/acetone (100 mL) was added. The precipitate was filtered off, and the solvent was evaporated under reduced pressure to yield 9 g of an orange-colored oil that was purified by FC on a column of silica gel (6.5 × 28 cm, 9:1 hexane/acetone) to afford 7.03 g (30.8 mmol, 89%) of a mixture of (E)/(Z) stereoisomers of **12** in a ratio of 86:14. The isomers were separated by preparative reversed-phase HPLC (column, 250 × 40.0 mm; eluent, 33:22:45 methanol/2-propanol/water; flow rate, 76 mL/min). The retention time of *E*-**12** was 13 min. Fractions were combined, and the volume was reduced to about one-half by evaporation under reduced pressure. The solution was extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4, filtered, and evaporated to dryness under reduced pressure to yield 5.64 g (24.7 mmol, 71%) of isomerically pure *E*-**12**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.93 (q, J = 1.4 Hz, 1H), 4.59 (t, J = 3.4 Hz, 1H), 4.17 (dd, J = 15.6 Hz, 1.4 Hz, 1H), 4.12 (q, J = 7.1 Hz, 2H), 3.90 (dd, J = 15.5, 1.4 Hz, 1H), 3.81−3.76 (m, 1H), 3.49−3.45 (m, 1H), 2.06 (s, 3H), 1.87−1.78 (m, 2H), 1.72−1.50 (m, 4H), 1.23 (t, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 166.8, 154.7, 114.5, 98.0, 70.6, 62.0, 59.7, 30.3, 25.3, 19.1, 15.9, 14.3; MS (CI, isobutane) m/z 229 $[M + 1]^+$. Anal. Calcd for $C_{12}H_{20}O_4$: C, 63.14; H, 8.83. Found: C, 63.13; H, 8.44.

(*E*)-2-Methyl-1-tetrahydropyranyloxy-but-2-ene-4-ol (*E*-13). A solution of *E*-**12** (3.00 g, 13.1 mmol) in 30 mL of dry CH_2Cl_2 was cooled to −78 °C. A 1.0 M solution of DIBALH (31.5 mL, 31.5 mmol) in hexane was added over a period of 2 h under an atmosphere of nitrogen. The solution was stirred for 30 min at −78 °C. The reaction was terminated by the addition of 0.5 mL of 1 M NaOH. The solvent was removed by evaporation under reduced pressure. Methanol (200 mL) was added, and the mixture was passed through a column of Na_2SO_4/silica gel (3.5 × 5; 21 cm). The column was developed with 600 mL of methanol. The effluent was concentrated under reduced pressure. The resulting oil was dissolved in 5 mL of CH_2Cl_2. The solution was loaded on a column of silica gel (3.5 × 12 cm), which was developed with 450 mL of methanol.

Evaporation of the solvent gave 2.39 g (12.8 mmol, 98%) of *E*-**13** as a colorless liquid: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.66 (tq, J = 6.8, 1.3 Hz, 1H), 4.59 (t, J = 3.6 Hz, 1H), 4.17 (t, J = 5.0 Hz, 2H), 4.10 (d, J = 12.5 Hz, 1H), 3.86−3.81 (m, 1H), 3.84 (d, J = 12.5 Hz, 1H), 3.50−3.46 (m, 1H), 1.86−1.66 (m, 2H), 1.67 (s, 3H), 1.61−1.48 (m, 4H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 135.5, 125.6, 97.8, 71.9, 62.1, 59.0, 30.5, 25.4, 19.3, 14.1; MS (CI, isobutane) m/z 169 [M − H_2O + 1]$^+$.

(*E*)-4-Chloro-2-methyl-1-tetrahydropyranyloxy-but-2-en (*E*-14). To a solution of *E*-**13** (1.10 g, 5.91 mmol) in 12 mL of dry CH_2Cl_2, solutions of 1.01 g (8.27 mmol) of DMAP in 12 mL of dry CH_2Cl_2 and of 1.35 g (7.09 mmol) of *p*-TsCl in 12 mL of dry CH_2Cl_2 were added. The resulting solution was stirred at room temperature for 3 h. After evaporation of the solvent under reduced pressure, the residue was purified by FC on silica gel (3.5 × 12.5 cm, CH_2Cl_2 100%) to obtain 1.13 g (5.52 mmol, 93%) of *E*-**14** as a colorless liquid: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.72 (tq, J = 8.0, 1.5 Hz, 1H), 4.59 (t, J = 3.6 Hz, 1H), 4.18 (d, J = 12.8 Hz, 1H), 4.15 (d, J = 8.0 Hz, 2H), 3.92 (d, J = 12.8 Hz, 1H), 3.90−3.86 (m, 1H), 3.59−3.52 (m, 1H), 1.92−1.52 (m, 6H), 1.77 (s, 3H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 138.7, 121.8, 97.9, 71.3, 62.1, 40.2, 30.5, 25.4, 19.4, 13.8; MS (CI, isobutane) m/z 205 [M + 1]$^+$. Anal. Calcd for $C_{10}H_{17}ClO_2$: C, 58.68; H, 8.37; Cl, 17.32. Found: C, 58.56; H, 8.54; Cl, 16.96.

(*E*)-2-Methyl-1-tetrahydropyranyloxy-but-2-enyl 4-Diphosphate Triammonium Salt (*E*-15). To a solution of *E*-**14** (475 mg, 2.32 mmol) in 2.5 mL of MeCN was slowly added a solution of 2.51 g (2.78 mmol) of tris(tetra-*n*-butylammonium) hydrogen pyrophosphate in 5.5 mL of MeCN at room temperature. After 3 h, the orange-colored solution was concentrated by evaporation under reduced pressure. The resulting orange-colored oil was dissolved in 2.5 mL of water. The solution was passed through a column of DOWEX 50 WX8 (2.5 × 11 cm, NH_4^+ form) which had been equilibrated with 100 mL of 25 mM NH_4HCO_3. The column was developed with 250 mL of 25 mM NH_4HCO_3. Fractions were combined and lyophilized. The residue was dissolved in 5 mL of a 1:1 v/v mixture of 2-propanol/100 mM NH_4HCO_3. The solution was loaded on a cellulose column (2 × 18 cm), which was developed with the same mixture of 2-propanol/100 mM NH_4HCO_3. The effluent was lyophilized affording 444 mg (1.12 mmol, 88%) of *E*-**15** as a white powder: ^{1}H NMR (D_2O, 500 MHz) δ 5.52 (tq, J = 6.8 Hz, 1H), 4.65 (s, 1H), 4.34 (t, J = 7.0 Hz, 2H), 3.98 (d, J = 12.3 Hz, 1H), 3.84 (d, J = 12.1 Hz, 1H), 3.74−3.70 (m, 1H), 3.42−3.38 (m, 1H), 1.61−1.57 (m, 2H), 1.54 (s, 3H), 1.40−1.32 (m, 4H); ^{13}C NMR (D_2O, 126 MHz) δ 136.4, 123.9 (dd, J = 8.0, 2.3 Hz), 98.5, 72.5, 63.2, 62.2 (d, J = 5.3 Hz), 29.9, 24.5, 19.0, 13.4; ^{31}P NMR (D_2O, 101 MHz) δ −5.62 (d, J = 20.9 Hz), −7.57 (d, J = 20.8 Hz).

(*E*)-1-Hydroxy-2-methyl-but-2-enyl 4-Diphosphate Triammonium Salt (*E*-6). For the following reaction step, crude *E*-**15** could be used with omission of the chromatographic purification described above. A solution of orange-colored, crude *E*-**15** (2.32 mmol) in 8 mL of MeCN was diluted by the addition of 8 mL of water. The pH was adjusted to pH 1.3 by addition of 400 μL of 37% HCl. The reaction was monitored by NMR and terminated by addition of 350 μL of 40% NaOH. The solution was passed through a column of DOWEX 50 WX8 (2.5 × 6 cm; NH_4^+ form). The column was developed with 90 mL of 25 mM NH_4HCO_3. Fractions were combined and lyophilized. The residue was dissolved in 3 mL of a 1:1 v/v mixture of 2-propanol/100 mM NH_4HCO_3. The solution was loaded on a cellulose column (2.5 × 19 cm), which was developed with the same mixture of 2-propanol/100 mM NH_4HCO_3. The effluent was lyophilized affording 516 mg (1.65 mmol, 66% based on *E*-**14**) of *E*-**6** as a white powder: ^{1}H NMR (D_2O, 500 MHz) δ 5.52 (t, J = 6.9 Hz, 1H), 4.39 (t, J = 7.2 Hz, 2H), 3.88 (s, 2H), 1.57 (s, 3H); ^{13}C NMR (D_2O, 126 MHz) δ 141.6, 122.7 (d, J = 8.0 Hz), 68.6, 64.3 (d, J = 5.1 Hz), 15.1; ^{31}P NMR (D_2O, 101 MHz) δ −3.49 (d, J = 22.3 Hz), −7.17 (d, J = 22.0 Hz).

(*E*/*Z*)-1-Hydroxy-2-methyl-but-2-enyl 4-Diphosphate Triammonium Salt (*E*/*Z*-6). An *E*/*Z* mixture of **6** was prepared by the reaction sequence described above using the *E*/*Z* mixture of **12** as a starting material. Whereas no attempt was made to isolate the *Z* compounds in pure form, the pertinent NMR data could be easily collected from the spectra of the mixtures and are summarized below.

Ethyl (*Z*)-2-Methyl-1-tetrahydropyranyloxy-but-2-enoate (*Z*-12): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.71 (q, J = 1.4 Hz, 1H), 4.60 (t, J = 3.6 Hz, 1H), 4.20 (dd, J = 15.5 Hz, 1.3 Hz, 1H), 4.11 (q, J = 7.1 Hz, 2H), 3.93 (dd, J = 15.6, 1.3 Hz, 1H), 3.84−3.79 (m, 1H), 3.52−3.48 (m, 1H), 1.97 (d, J = 1.4 Hz, 3H), 1.88−1.50 (m, 6H), 1.24 (t, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 165.9, 156.8, 116.9, 98.7, 66.5, 62.3, 59.8, 30.6, 25.3, 21.9, 19.5, 14.3; MS (CI, isobutane) m/z 229 [M + 1]$^+$; Anal. Calcd for $C_{12}H_{20}O_4$: C, 63.14; H, 8.83. Found: C, 63.13; H, 8.44.

(*Z*)-2-Methyl-1-tetrahydropyranyloxy-but-2-ene-4-ol (*Z*-13): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.64 (tq, J = 6.6, 1.3 Hz, 1H), 4.63 (t, J = 3.3 Hz, 1H), 4.20 (d, J = 6.8 Hz, 2H), 4.15 (d, J = 11.8 Hz, 1H), 3.87−3.82 (m, 1H), 3.83 (d, J = 11.3 Hz, 1H), 3.52−3.48 (m, 1H), 1.86−1.48 (m, 6H), 1.79 (s, 3H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 136.2, 128.6, 96.6, 65.1, 61.8, 58.1, 30.3, 25.3, 21.9, 19.0; MS (CI, isobutane) m/z 169 [M − H_2O + 1]$^+$.

(*Z*)-4-Chloro-2-methyl-1-tetrahydropyranyloxy-but-2-en (*Z*-14): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.65 (t, J = 8.1 Hz, 1H), 4.61 (t, J = 3.6 Hz, 1H), 4.18 (d, J = 12.8 Hz, 1H), 4.15 (d, J = 8.0 Hz, 2H), 3.92 (d, J = 12.8 Hz, 1H), 3.90−3.86 (m, 1H), 3.59−3.52 (m, 1H), 1.92−1.52 (m, 6H), 1.86 (s, 3H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 138.3, 124.6, 97.5, 64.7, 62.2, 40.1, 30.5, 25.4, 21.8, 19.4; MS (CI, isobutane) m/z 205 [M + 1]$^+$. Anal. Calcd for $C_{10}H_{17}ClO_2$: C, 58.68; H, 8.37; Cl, 17.32. Found: C, 58.56; H, 8.54; Cl, 16.96.

(*Z*)-2-Methyl-1-tetrahydropyranyloxy-but-2-enyl 4-Diphosphate Triammonium Salt (*Z*-15): ^{1}H NMR (D_2O, 500 MHz) δ 5.52 (t, J = 6.8, 1H), 4.65 (s, 1H), 4.31 (t, J = 7.1 Hz, 2H), 3.98 (d, J = 12.3 Hz, 1H), 3.84 (d, J = 12.1 Hz, 1H), 3.74−3.70 (m, 1H), 3.42−3.38 (m, 1H), 1.64 (s, 3H), 1.61−1.57 (m, 2H), 1.40−1.32 (m, 4H); ^{13}C NMR (D_2O, 126 MHz) δ 136.3, 125.8 (d, J = 8.6 Hz), 98.6, 72.5, 63.2, 61.8 (d, J = 5.1 Hz), 29.9, 24.5, 20.8, 19.0; ^{31}P NMR (D_2O, 101 MHz) δ −5.69 (d, J = 20.8 Hz), −7.68 (d, J = 20.8 Hz).

(*Z*)-1-Hydroxy-2-methyl-but-2-enyl 4-Diphosphate Triammonium Salt (*Z*-6): ^{1}H NMR (D_2O, 500 MHz) δ 5.49 (tm, J = 6.8 Hz, 1H), 4.41 (t, J = 7.3 Hz, 2H), 4.03 (s, 2H), 1.70 (s, 3H); ^{13}C NMR (D_2O, 126 MHz) δ 141.8, 125.6 (d, J = 7.7 Hz), 63.8 (d, J = 5.1 Hz), 62.0, 22.7; ^{31}P NMR (D_2O, 101 MHz) δ −4.48 (d, J = 20.8 Hz), −7.06 (d, J = 20.8 Hz).

[U-$^{13}C_5$]1-Hydroxy-3-methyl-but-2-enyl 4-Diphosphate. Biosynthetic [U-$^{13}C_5$]-**6** was obtained as described earlier using the *E. coli* strain XL1-pBSxispC-G[27] engineered for expression of the *xylB* and *ispCDEFG* genes. About 300 μL of cells were suspended in 440 μL of 20 mM NaF in D_2O and 210 μL of MeOD-d_3. The suspension was ultrasonically treated at 0 °C and centrifuged. The supernatant was used directly for NMR characterization.

Note Added in Proof. In a paper published after submission of this manuscript, M. Wolff et al. have reported an independent synthesis of (*E*)-1-hydroxy-2-methyl-but-2-enyl 4-diphosphate.[35]

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Supporting Information Available: NMR spectra of compounds *E*-**6**, *E*/*Z*-**6**, and *E*-**12** through *E*-**15**. This material is available free of charge via the Internet at http://pubs.acs.org.

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