VOLUME 122, NUMBER 40
OCTOBER 11, 2000
© Copyright 2000 by the
American Chemical Society

JOURNAL
OF THE
AMERICAN
CHEMICAL
SOCIETY

Biosynthesis of Isoprenoids. A Rapid Method for the Preparation of Isotope-Labeled 4-Diphosphocytidyl-2C-methyl-D-erythritol

Felix Rohdich,[†] Christoph A. Schuhr,[†] Stefan Hecht,[†] Stefan Herz,[†] Juraithip Wungsintaweekul,[‡] Wolfgang Eisenreich,[†] Meinhart H. Zenk,[‡] and Adelbert Bacher[†*]

Contribution from the Lehrstuhl für Organische Chemie und Biochemie, Technische Universität München, Lichtenbergstrasse 4, D-85747 Garching, Federal Republic of Germany, and Biozentrum-Pharmazie, Universität Halle, Weinbergweg 22, D-06099 Halle/Saale, Federal Republic of Germany

Received April 20, 2000

Abstract: 4-Diphosphocytidyl-2*C*-methyl-D-erythritol serves as an intermediate in the nonmevalonate pathway of isoprenoid biosynthesis. The compound has been prepared in millimole quantity by a sequence of one-pot reactions using ^{13}C-labeled pyruvate and dihydroxyacetone phosphate or ^{13}C-labeled glucose as starting materials and recombinant enzymes of the nonmevalonate isoprenoid pathway as catalysts. The method has been used for the preparation of various 4-diphosphocytidyl-2*C*-methyl-D-erythritol isotopomers in high yield.

Introduction

Terpenes are one of the largest groups of natural products, and include numerous medically important compounds such as cholesterol, vitamin A, carotenoids, and paclitaxel (Taxol).[1] In animals, fungi, and archaea, the universal terpene precursors, isopentenyl pyrophosphate and dimethylallyl pyrophosphate, are biosynthesized via the mevalonate pathway, which has been studied in considerable detail.[2−5]

More recently, the research groups of Rohmer and Arigoni independently observed ^{13}C-labeling patterns in terpenoids of certain bacteria such as *Rhodopseudomonas palustris* and *Escherichia coli* which could not be explained via the mevalonate pathway (refs 6−8; for reviews, see also refs 9−11). The data suggested that pyruvate and a triose serve as substrates of an alternative pathway. Arigoni and co-workers also showed that exogenous 1-deoxy-D-xylulose is utilized efficiently for terpenoid biosynthesis via this alternative pathway,[7] and that plants use both isoprenoid pathways for the biosynthesis of different terpenoids.[12]

Several enzymes of the nonmevalonate isoprenoid pathway have recently been obtained from microorganisms and plants. 1-Deoxy-D-xylulose 5-phosphate synthase, specified by the *dxs* gene, catalyzes the formation of 1-deoxy-D-xylulose 5-phosphate

* Address correspondence to this author. Phone: +49-89-289-13360. Fax: +49-89-289-13363. E-mail: adelbert.bacher@ch.tum.de.
[†] Technische Universität München.
[‡] Universität Halle.

(1) Saccettini, J. C.; Poulter, C. D. *Science* **1997**, *277*, 1788−1789.
(2) Qureshi, N.; Porter, J. W. *Biosynthesis of Isoprenoid Compounds*; Porter, J. W., Spurgeon, S. L., Eds.; John Wiley: New York, 1981; Vol. 1, pp 47−94.
(3) Bloch, K. *Steroids* **1992**, *57*, 378−382.
(4) Bach, T. J. *Lipids* **1995**, *30*, 191−202.
(5) Bochar, D. A.; Friesen, J. A.; Stauffacher, C. V.; Rodwell, V. W. *Comprehensive Natural Product Chemistry*; Barton, D., Nakanishi, K., Eds.; Pergamon: Oxford, 1999; Vol. 2, pp 15−44.
(6) Flesch, G.; Rohmer, M. *Eur. J. Biochem.* **1988**, *175*, 405−411.
(7) Broers, S. T. J. Ph.D Thesis Nr. 10978, ETH Zürich, Switzerland, 1994.
(8) Rohmer, M.; Knani, M.; Simonin, P.; Sutter, B.; Sahm, H. *Biochem. J.* **1993**, *295*, 517−524.
(9) Eisenreich, W.; Schwarz, M.; Cartayrade, A.; Arigoni, D.; Zenk, M.; Bacher, A. *Chem. Biol.* **1998**, *5*, R221−R233.
(10) Rohmer, M. *Nat. Prod. Rep.* **1999**, *16,* 565−574.
(11) Rohmer, M. *Comprehensive Natural Products Chemistry*; Barton, D., Nakanishi, K., Eds.; Pergamon: Oxford, 1999; Vol. 2, pp 45−68.
(12) Schwarz, M. K.; Arigoni, D. *Comprehensive Natural Products Chemistry*; Barton, D., Nakanishi, K., Eds.; Pergamon: Oxford, 1999; Vol. 2, pp 367−400.

10.1021/ja001385o CCC: $19.00 © 2000 American Chemical Society
Published on Web 09/23/2000

(**7**) from pyruvate (**6**) and D-glyceraldehyde 3-phosphate (**5**).[13-16] The enzyme product is converted into 2*C*-methyl-D-erythritol 4-phosphate (**8**) by a reductoisomerase specified by the *dxr* gene of *Escherichia coli*.[17] The branched polyol **8** is converted into 4-diphosphocytidyl-2*C*-methyl-D-erythritol (**9**) by the catalytic action of the 4-diphosphocytidyl-2*C*-methyl-D-erythritol synthase specified by the *ispD* (*ygbP*) gene of *E. coli*.[18]

Isotope-labeled precursors were crucial in the relatively short history of the investigation of the alternative terpenoid pathway which involved the reassessment of numerous erroneous claims on the universality of the mevalonate pathway. The possibility to prepare **9** in isotope-labeled form in a very high overall yield should benefit future studies in the area. More specifically, ^{13}C-labeled **9** will be helpful for in vivo and in vitro studies identifying further downstream metabolites as well as the mechanisms of the novel nonmevalonate pathway enzymes.

Results

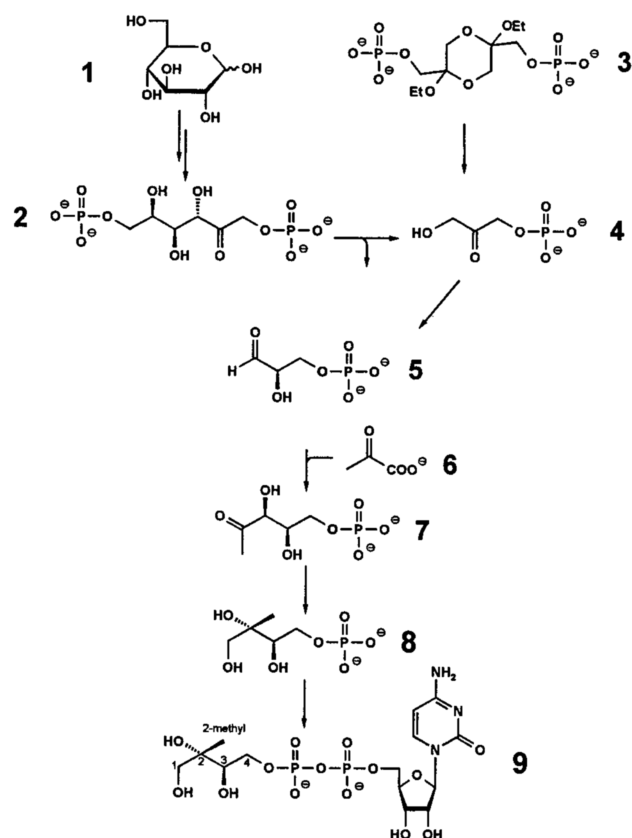
This paper describes the use of recombinant enzymes of the nonmevalonate isoprenoid pathway for the efficient preparation of ^{13}C- and ^{14}C-labeled 4-diphosphocytidyl-2*C*-methyl-D-erythritol (**9**) in millimole quantity. The synthesis can be performed as a one-pot reaction using D-glyceraldehyde 3-phosphate (**5**) and pyruvate (**6**) as starting materials (Scheme 1). Using the methods described below, a wide variety of isotopomers of **9** could also be prepared from commercially available starting materials such as glucose (**1**), respectively, pyruvate (**6**) labeled with ^{13}C or ^{14}C (Table 1).

Unlabeled dihydroxyacetone phosphate (**4**) was obtained from dimeric dihydroxyacetone phosphate ethyl hemiacetal (**3**)[19] and was further converted into unlabeled **5** by the catalytic action of triose phosphate isomerase. Isotope-labeled samples of **5** were obtained from isotopomers of **1** using enzymes of the glycolytic pathway which are commercially available. Crude **5** from these respective starting materials could be converted enzymatically into **9** without prior isolation.

The enzyme-assisted synthetic reactions can be monitored conveniently in real time by ^{13}C NMR when ^{13}C-labeled starting materials are used, since the label enhances both the sensitivity and the selectivity of NMR diagnosis. Figure 1 shows the relevant sections of ^{13}C NMR spectra from an experiment for the preparation of [1,2,2-methyl,3,4-$^{13}C_5$]-**9** from [U-$^{13}C_6$]-**1** and [2,3-$^{13}C_2$]-**6**. Spectrum A was obtained at the start of the enzyme reaction, and spectrum B was recorded prior to the addition of 1-deoxy-D-xylulose 5-phosphate reductoisomerase. In Figure 1B, signals reflecting the ^{13}C-labeled starting materials ([U-$^{13}C_6$]-glucose and [2,3-$^{13}C_2$]pyruvate) have virtually disappeared, and the spectrum is dominated by the signals of [U-$^{13}C_5$]1-deoxy-D-xylulose 5-phosphate (**7**). The resulting [U-$^{13}C_5$]-**7** can be further converted into [1,2,2-methyl,3,4-$^{13}C_5$]-**9,** without prior isolation, by the sequential addition of 1-deoxy-D-xylulose 5-phosphate reductoisomerase and 4-diphosphocytidyl-2*C*-methyl-D-erythritol synthase, together with their respective cosubstrates.

The overall yield of [1,2,2-methyl,3,4-$^{13}C_5$]-**9** was 60% based

Scheme 1



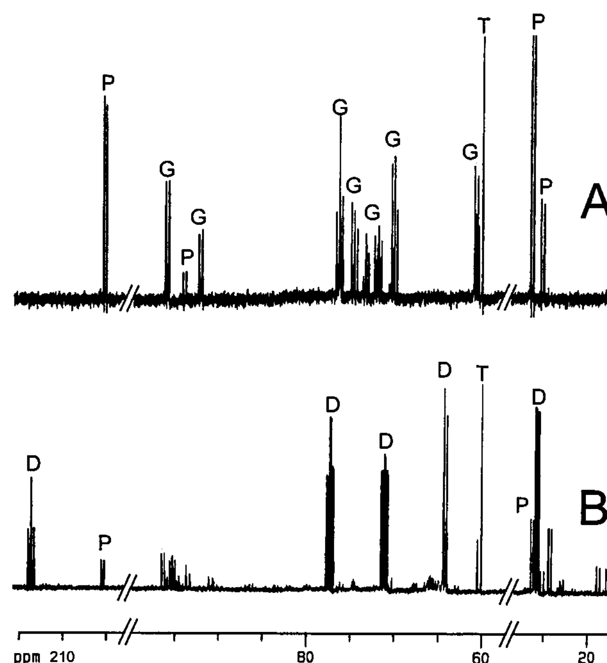


Figure 1. Parts of ^{13}C NMR spectra of a mixture containing [U-$^{13}C_6$]-**1** and [2,3-$^{13}C_2$]-**6** prior to the addition of enzymes (A) and after incubation with hexokinase, glucose 6-phosphate isomerase, fructose 6-phosphate kinase, aldolase, triose phosphate isomerase, and 1-deoxy-D-xylulose 5-phosphate synthase (B). Signals arising from glucose (**1**), pyruvate (**6**), and 1-deoxy-D-xylulose 5-phosphate (**7**) are indicated by G, P, and D, respectively. T = Tris hydrochloride.

(13) Sprenger, G. A.; Schörken, U.; Wiegert, T.; Grolle, S.; deGraaf, A. A.; Taylor, S. V.; Begley, T. P.; Bringer-Meyer, S.; Sahm, H. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 12857−12862.

(14) Lois, L. M.; Campos, N.; Putra, S. R.; Danielsen, K.; Rohmer, M.; Boronat, A. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 2105−2110.

(15) Lange, B. M.; Wildung, M. R.; McCaskill, D.; Croteau, R. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 2100−2104.

(16) Kuzuyama, T.; Takagi, M.; Takahashi, S.; Seto, H. *J. Bacteriol.* **2000**, *182*, 891−897.

(17) Takahashi, S.; Kuzuyama, T.; Watanabe, H.; Seto, H. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 9879−9884.

(18) Rohdich, F.; Wungsintaweekul, J.; Fellermeier, M.; Sagner, S.; Herz, S.; Kis, K.; Eisenreich, W.; Bacher, A.; Zenk, M. H. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 11758−11763.

(19) Mizuno, M.; Masuda, S.; Takemaru, K.; Hosono, S.; Sato, T.; Takeuchi, M.; Kobayashi, Y. *Microbiology* **1996**, *142*, 3103−3111.

Table 1. Preparation of ^{13}C-Labeled 4-Diphosphocytidyl-2*C*-methyl-D-erythritol (**9**) from Pyruvate (**6**), Glucose (**1**), and Glyceraldehyde 3-Phosphate (**5**)

C_2 precursor	C_3 precursor	product
[2,3-$^{13}C_2$]-**6**	[U-$^{13}C_6$]-**1**	[1,2-methyl,3,4-$^{13}C_5$]-**9**
[2,3-$^{13}C_2$]-**6**	5	[2,2-methyl-$^{13}C_2$]-**9**
6	[1,2,3-$^{13}C_1$]-**1**[a])	[1,3,4-$^{13}C_1$]-**9**

[a] Equimolar mixture of [1-$^{13}C_1$]-, [2-$^{13}C_1$]-, and [3-$^{13}C_1$]-**1.**

Table 2. ^{13}C NMR Data of ^{13}C-Labeled **9** Using D_2O as Solvent

		[2,2-methyl-$^{13}C_2$]-**9**		[1,3,4-$^{13}C_1$]-**9**		[1,2,2-methyl,3,4-$^{13}C_5$]-**9**		
position	δ ^{13}C ppm	J_{CH} Hz	J_{CC} Hz	J_{CH} Hz	J_{CP} Hz	J_{CH} Hz	J_{CC} Hz	J_{CP} Hz
1	66.25			141		142	40	
2	73.75		40				n.d.[a]	
2-methyl	18.13	127	40			127	38	
3	73.28			n.d.	7	135	n.d.	n.d.
4	66.86			n.d.	6	135	39	5

[a] n.d., not determined due to signal overlapping.

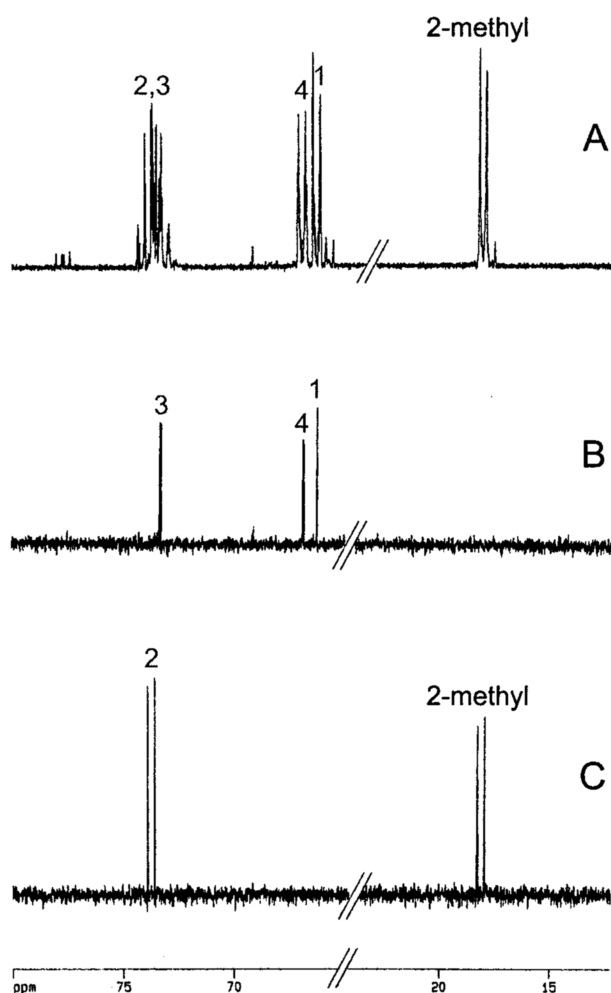


Figure 2. ^{13}C NMR signals of [1,2,2-methyl,3,4-$^{13}C_5$]-**9** (A), [1,3,4-$^{13}C_1$]-**9** (B), and [2,2-methyl-$^{13}C_2$]-**9** (C). $^{13}C^{13}C$ and $^{13}C^{31}P$ coupling constants are given in Table 2.

on ^{13}C-labeled **1**. [2,2-Methyl-$^{13}C_2$]-**9** and [1,3,4-$^{13}C_1$]-**9** were prepared from [2,3-$^{13}C_2$]-**6** and [1,2,3-$^{13}C_1$]-**1**, respectively, in similar yields. The purity of the compounds was assessed by NMR (Table 2, Figure 2) and mass spectrometry.

No specific adaptations are required for the preparation of different isotopomers since a wide variety of ^{13}C-labeled pyruvate and glucose isotopomers are commercially available. The enzymes used in this study are either commercially available (hexokinase, glucose 6-phosphate isomerase, fructose 6-phosphate kinase, aldolase, and glucose dehydrogenase) or can be prepared by recombinant DNA techniques.[13,14,16,17]

One limitation for the scale-up of the procedure is the necessity to purify the final product by chromatographic procedures. The purification of 1 mmol of **9** using semipreparative HPLC columns (2 × 25 cm) requires about 15 h. The efficient purification of larger batches would be possible using preparative scale HPLC columns.

The method can be scaled down to very small volumes to prepare radiolabeled samples of high specific activity. Thus, radioactive labels can be introduced into **9** virtually without dilution. As an example, 7.4 μmol of [2-^{14}C]-**9** have been prepared from [2-^{14}C]pyruvate at a specific activity of 15.8 mCi $mmol^{-1}$ with a yield of 47%.

Synthetic procedures for the preparation of intermediates of the nonmevalonate pathway were reported in the literature for 1-deoxy-D-xylulose 5-phosphate (**7**)[21,22] and 2*C*-methyl-d-erythritol 4-phosphate (**8**).[23,24] The chemical synthesis of **8** involves 14 steps[23] and can be used for the preparation of the [1-^{3}H]-isotopomer with high specific activity, but the introduction of isotopes at other positions would not be achieved easily. In contrast, a wide variety of isotopomers carrying stable or radioactive labels in the methylerythritol moiety of **9** can be obtained with the method reported in this paper.

Experimental Section

Materials. ATP, CTP, NADPH, and TPP were purchased from Sigma (Deisenhofen, Germany). [1-$^{13}C_1$]-, [2-$^{13}C_1$]-, [3-$^{13}C_1$]-, and [U-$^{13}C_6$]glucose were obtained from Omicron, South Bend, IN. [2,3-$^{13}C_2$]Pyruvate was purchased from Isotec, Miamisburg, OH. [2-^{14}C]-Pyruvate was obtained from NEN, Boston, MA.

Proteins. Hexokinase from yeast (E.C.2.7.1.1), glucose 6-phosphate isomerase from yeast (1.1.1.49), fructose 6-phosphate kinase from rabbit muscle (E.C.2.7.1.11), aldolase from rabbit muscle (E.C.5.3.1.1), glucose dehydrogenase from *Bacillus megaterium* (E.C.1.1.1.47), triose phosphate isomerase from rabbit muscle (E.C.5.3.1.1), and inorganic pyrophosphatase from *E. coli* (E.C.3.6.1.1.) were purchased from Sigma (Deisenhofen, Germany). Recombinant 4-diphosphocytidyl-2*C*-methyl-D-erythritol synthase from *E. coli* was prepared by a published procedures.[18] The preparation of recombinant 1-deoxy-D-xylulose 5-phosphate synthase from *B. subtilis* and recombinant 1-deoxy-D-xylulose 5-phosphate reductoisomerase from *E. coli* will be published elsewere.

General Procedures. NMR spectra were obtained using a Bruker AVANCE DRX 500 spectrometer (Karlsruhe, Germany). Chemical shifts were referenced to external trimethylsilylpropane sulfonate or 85% ortho-phosphoric acid, respectively. UV/VIS spectra were determined with an Ultrospec 2000 spectrometer (Amersham Pharmacia Biotech, Heidelberg, Germany). ESI mass spectra were recorded on a LCQ Finnigan mass spectrometer (San Jose, CA) in the negative ion detection mode. Optical rotation was measured on a Perkin-Elmer 241 MC polarimeter (Weiterstadt, Germany).

Crude [U-$^{13}C_5$]1-Deoxy-D-xylulose 5-Phosphate. A reaction mixture containing 120 mM Tris hydrochloride, pH 8.0, 6 mM $MgCl_2$, 31 mM ATP, 1.6 mM thiamine pyrophosphate, 15 mM [U-$^{13}C_6$]glucose, 30 mM [2,3-$^{13}C_2$]sodium pyruvate, 174 U of hexokinase, 106 U of glucose 6-phosphate isomerase, 48 U of fructose 6-phosphate kinase, 26 U of aldolase, 284 U of triose phosphate isomerase, and 2.8 mg

(20) Effenberger, F.; Straub, A. *Tetrahedron Lett.* **1987**, *28*, 1641.
(21) Thiel, R.; Adam, K.-P. *Tetrahedron Lett.* **1999**, *40*, 5307−5308.
(22) Proteau, P. J.; Woo, Y.-H.; Williamson, R. T.; Phaosiri, C. *Org. Lett.* **1999**, *1*, 921−923.
(23) Kis, K.; Wungsintaweekul, J.; Eisenreich, W.; Zenk, M. H.; Bacher, A. *J. Org. Chem.* **2000**, *65*, 587−592.
(24) Koppisch, A. T.; Blagg, B. S. J.; Poulter, C. D. *Org. Lett.* **2000**, *2*, 215−217.

(6.2 U) of 1-deoxy-D-xylulose 5-phosphate synthase in a total volume of 122 mL was incubated at 37 °C. The pH was maintained at 8.0 by the addition of 1 M NaOH. The formation of 1-deoxy-D-xylulose 5-phosphate was monitored by ^{13}C NMR and was found to be virtually complete after 22 h.

Crude [3,4,5-$^{13}C_1$]1-Deoxy-D-xylulose 5-Phosphate. [3,4,5-$^{13}C_1$]-**7** was prepared as described above with an equimolar mixture of [1-$^{13}C_1$]-, [2-$^{13}C_1$]-, [3-$^{13}C_1$]glucose and unlabeled pyruvate as starting materials.

Crude [1,2-$^{13}C_2$]1-Deoxy-D-xylulose 5-Phosphate. Dimeric dihydroxyacetone phosphate ethyl hemiacetal (barium salt, 1.9 g, 3.2 mmol) was treated with 19 mL of Dowex 50 WX8 resin (H^+-form) in 44 mL of water at 65 °C for 4 h. The resin was filtered off, and the pH of the solution was adjusted to 8 by the addition of 1 M NaOH.[19] The solution was added to 70 mL of a solution containing 150 mM Tris hydrochloride, pH 8.0, 57 mM sodium [2,3-$^{13}C_2$]pyruvate, 10 mM $MgCl_2$, and 2.5 mM thiamine pyrophosphate. Triose phosphate isomerase (17 000 U) was added, and the solution was incubated for 105 min at 37 °C. 1-Deoxy-D-xylulose 5-phosphate synthase (3.3 mg, 7.4 U) was added. The reaction was found to be virtually complete after 8 h.

[2,2-methyl-$^{13}C_2$]-, [1,2,2-methyl,3,4-$^{13}C_5$]-, and [1,3,4-$^{13}C_1$]4-Diphosphocytidyl-2*C*-methyl-D-erythritol. Crude [U-$^{13}C_5$]-, [3,4,5-$^{13}C_1$]-, or [1,2-$^{13}C_2$]-**7** obtained by either of the two procedures described above can be used for the following steps without purification. Glucose (0.66 g, 3.7 mmol), 46 U of glucose dehydrogenase, 51.5 mg of $NADP^+$ (0.5 mmol), and 4 mM $MgCl_2$ were added to a solution containing approximately 3.7 mmol of **7**. The mixture was incubated for 5 min at 37 °C, and a solution (1.4 mL) containing 2.6 mg (18 U) of 1-deoxy-D-xylulose 5-phosphate reductoisomerase was added. The formation of ^{13}C-labeled **8** was monitored by ^{13}C NMR and was found to be virtually complete after 18 h.

CTP (2.0 g, 3.6 mmol) was added. The pH of the solution was brought to 8.0 with 2 N NaOH. A solution (3 mL) containing 4-diphosphocytidyl-2*C*-methyl-D-erythritol synthase (2.1 mg, 51 U) was added. The reaction mixture was incubated for 15 h and was then lyophilized.

The crude lyophilized product (8 g) was dissolved in 80 mL of 100 mM ammonium formate containing 40% methanol. Aliquots of several milliliters were placed on a HPLC column of Nucleosil SB10 (Macherey & Nagel, Germany; 2 × 25 cm) which was developed with 100 mM ammonium formate containing 40% methanol at a flow rate of 10 mL min^{-1}. The effluent was monitored photometrically (270 nm). The retention volume of **9** was about 150 mL. Fractions were combined and lyophilized. One millimole of product could be processed in about 25 runs requiring a total time of about 15 h.

The lyophilized powder (1.5 g) was dissolved in 15 mL of 10 mM ammonium formate. Aliquots of 1 mL were placed on a semipreparative HPLC column of Nucleosil 10 RP18 (Macherey & Nagel, Germany; 2 × 25 cm) which was developed with 10 mM ammonium formate at a flow rate of 6 mL. The retention volume was 60 mL. Fractions were combined and lyophilized repeatedly to remove ammonium formate. Pure **9** was obtained as a white powder with an overall yield of about 60%. Analytical data of **9** were as follows: ϵ_{268} = 7410 M^{-1} cm^{-1} (pH 7.0); $[\alpha]_{589}$, = 9.5° (*c* 0.3 g 100 mL^{-1} in H_2O). Mass spectrometry: **9** (unlabeled), *m/e* 520.4; [1,2,2-methyl,3,4-$^{13}C_5$]-**9**, *m/z* 525.6; [2,methyl-$^{13}C_2$]-**9**, *m/z* 522.6; [1,3,4-$^{13}C_1$]-**9**, *m/z* 521.4.

^{1}H, ^{13}C, and ^{31}P NMR data agree with previously published results.[18] More specifically, the presence of a cytidyl moiety was gleaned from the ^{1}H NMR data. The ^{31}P NMR spectrum displayed two doublets at −7.2 and −7.8 ppm with $^{31}P^{31}P$ couplings of 20 Hz reflecting the diphospho moiety. The ^{13}C NMR data and the coupling patterns of [2,2-methyl-$^{13}C_2$]-, [1,2,2-methyl,3,4-$^{13}C_5$]-, and [1,3,4-$^{13}C_1$]-**9** are summarized in Table 2.

[2-^{14}C]4-Diphosphocytidyl-2*C*-methyl-D-erythriol. A reaction mixture containing 150 mM Tris hydrochloride, 5 mM dithiothreitol, 21.7 mM dihydroxyacetone phosphate, 17 mM NADPH, 1.6 mM thiamine pyrophosphate, 18.8 mM CTP, 15.8 mM [2-^{14}C]pyruvate (15.8 mCi $mmol^{-1}$), 406 U of triose phosphate isomerase, 1.3 U of inorganic pyrophosphatase, 170 μg (0.4 U) of 1-deoxy-D-xylulose 5-phosphate synthase from *B. subtilis*, 70 μg (0.5 U) of 1-deoxy-D-xylulose 5-phosphate reductoisomerase from *E. coli*, and 100 μg (2.4 U) of 4-diphosphocytidyl-2*C*-methyl-D-erythritol synthase from *E. coli* in a total volume of 1 mL was incubated at 37 °C. At intervals of 1 h, aliquots (50 μL) of the reaction mixture were retrieved and applied to a Multospher 120 RP 18-5 column (4.6 × 250 mm, CS-Chromatographie Service GmbH, Langerwehe, Germany) which had been equilibrated with 10 mM tetra-*n*-butylammonium hydrogen sulfate, pH 6.0, at a flow rate of 0.75 mL min^{-1}. The column was developed with 15 mL of 10 mM tetra-*n*-butylammonium hydrogen sulfate, pH 6.0, followed by a linear gradient of 0−42% (v/v) methanol in 45 mL of 10 mM tetra-*n*-butylammonium hydrogen sulfate, pH 6.0. The effluent was monitored using a radiodetector (Beta-RAM, Biostep GmbH, Jahnsdorf, Germany). The retention volume of [2-^{14}C]-**9** was 29 mL. After 8 h the reaction was stopped by passing the solution through a Nanosep 10 K membrane (Pall Gelman, Rondorf, Germany). Crude [2-^{14}C]-**9** was purified by semipreparative HPLC using a column of Nucleosil SB5 (20 × 250 mm, CS-Chromatographie Service GmbH, Langerwehe, Germany). The column was equilibrated and developed with 100 mM ammonium formate as eluent at a flowrate of 5 mL min^{-1}. The effluent was monitored using a radiodetector. The retention volume of [2-^{14}C]-**9** was 600−650 mL. Fractions were combined and lyophilized. [2-^{14}C]-**9** (15.8 mCi $mmol^{-1}$) (7.4 μmol) was obtained from [2-^{14}C]pyruvate with a yield of 47%.

Acknowledgment. This work was supported by the Deutsche Forschungsgemeinschaft, the Fonds der Chemischen Industrie, and the Hans-Fischer-Gesellschaft. We thank Angelika Werner and Fritz Wendling for help with the preparation of the manuscript.

JA001385O