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Synthesis of (*R*)-[2-^{2}H]Isopentenyl Diphosphate and Determination of Its Enantiopurity by ^{2}H NMR Spectroscopy in a Lyotropic Medium

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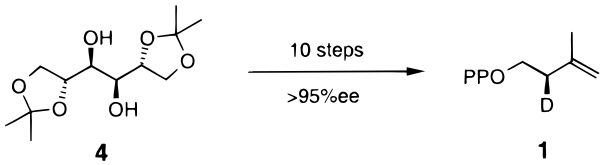
ABSTRACT



4 **1**

The synthesis of (*R*)-[2-^{2}H]isopentenyl diphosphate from D-mannitol 1,2:5,6-bis-acetonide in 10 steps is reported. Stereospecific incorporation of the label is achieved by a BF_3-catalyzed $NaCNBD_3$ reduction of the enantiomerically pure (*S*)-isopropylidene oxirane intermediate. The enantiomeric excess of the penultimate precursor [2-^{2}H]isopentenyl tosylate (>95% ee) was determined by ^{2}H NMR spectroscopy in a poly-γ-benzyl-L-glutamate/CH_2Cl_2 liquid crystal at −50 °C.

Isopentenyl diphosphate (IPP)[1] is the universal precursor of more than 23 000 isoprenoid metabolites identified to date.[2] Prominent members of this extensive family of compounds include cholesterol,[3] taxol,[4] steroid hormones,[5] and the cell membrane-stabilizing family of dolichols.[6] In eukaryotic organisms (animals, higher plants, yeast, etc.) IPP is biosynthesized by the well-characterized mevalonate route,[7] whereas most bacteria and green algae synthesize IPP from 1-deoxy-D-xylulose.[8] The latter pathway was first reported by Rohmer and co-workers[8] in 1993 and is less well characterized. Interestingly, isoprenoids synthesized in plant plastids have been shown to originate from 1-deoxy-D-xylulose[9] while those synthesized in the cytosol are from mevalonate.

The biogenesis of IPP as well as mechanistic details of the biosynthetic pathways have been elucidated primarily on the basis of in vivo and in vitro labeling experiments.[7−9] Such an approach requires precursor metabolites that are specifically labeled with either stable (^{2}H, ^{13}C) or radioactive (^{3}H, ^{14}C) isotopes. Our recent mechanistic studies of the reactions

(1) Abbreviations used: IPP, isopentenyl diphosphate; PBLG, poly-γ-benzyl-L-glutamate;.

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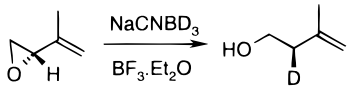
catalyzed by two enzymes involved in the isoprenoid biosynthetic pathway—isopentenyl diphosphate:dimethylallyl diphosphate isomerase[10] and farnesyl diphosphate synthase[11]—required (*R*)-[2-^{2}H]isopentenyl diphosphate (**1**). This compound was first synthesized by Popjak et al.[12] from C4-labeled mevalonate utilizing partially purified preparations of the enzymes involved in the synthesis of IPP in vivo. The purification of labeled IPP from a complex mixture of products and current commercial unavailability of the required enzymes make this approach impractical. Suga et al.[13] later synthesized **1** via Sharpless epoxidation of dimethylallyl alcohol in 15% yield and 85% ee after five steps. We now report the synthesis of **1** in >95% ee and the determination of its enantiopurity by ^{2}H NMR spectroscopy in a liquid crystal.

The key reaction in the stereospecific incorporation of deuterium at C2 of IPP is the boron trifluoride-catalyzed reduction of enantiomerically pure epoxide **2** by sodium cyanoborodeuteride[14] (Scheme 1). Studies with various model

Scheme 1



2 **3**

oxiranes showed that this reaction proceeds with high regioselectivity, affording almost exclusively the least substituted alcohol. It was also observed that hydride delivery to the oxirane ring occurs with inversion of configuration.[14]

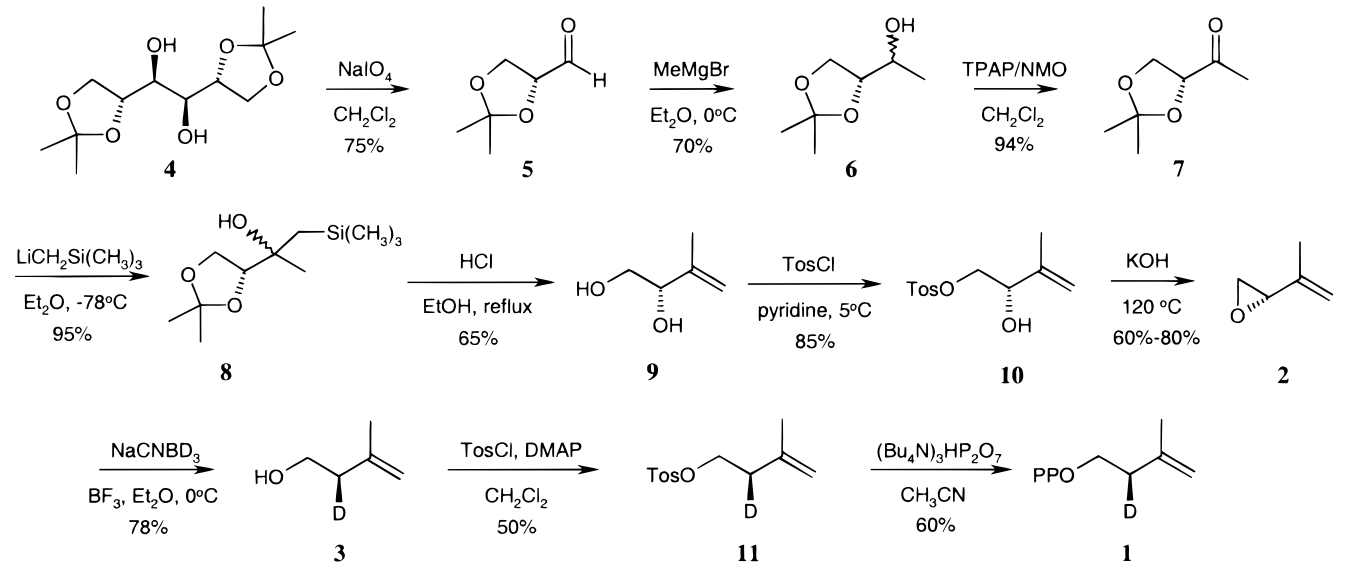
We synthesized oxirane **2** by a modification of the procedure reported by Wistuba et al.[15] (Scheme 2). Commercially available D-mannitol 1,2:5,6-bis-acetonide (**4**) was oxidatively cleaved with $NaIO_4$ to afford the acetonide-protected D-glyceraldehyde **5**. Addition of methylmagnesium bromide and subsequent oxidation of the resulting diastereomeric alcohols (**6**) afforded the ketone **7** in >99% ee on the basis of optical rotation measurements.[16]

Wistuba et al.[15] and others[17] working with similar carbonyl-containing oxiranes used Wittig reactions to introduce the olefin. We found that the high basicity of the ylide promoted epimerization and gave low yields, and instead used the nonbasic Peterson reagent. When ketone **7** was treated with (trimethylsilyl)methyllithium, β-silylacohol **8** was obtained in 95% yield. Reaction of **8** with HCl in refluxing ethanol afforded enediol **9** in >98% ee, as judged by its optical rotation[18] and analysis of the ^{1}H and ^{19}F NMR spectra of its Mosher ester derivative.[19] The enediol was tosylated in dry pyridine at 0 °C for 24 h in 80% yield. Exclusive tosylation of the primary hydroxyl group was observed under these conditions. In contrast, tosyl chloride and (dimethylamino)pyridine in methylene chloride[20] gave both primary and secondary tosylates.

The epoxide ring was formed by thoroughly mixing the semisolid tosylate **10** with excess powdered KOH previously cooled to 0 °C. The mixture was warmed to 110−120 °C, and the volatile (*S*)-2-isopropylideneoxirane **2** (bp 81 °C) was collected in a U-shaped tube placed in a dry ice−acetone bath (60−80%). The enantiomeric purity of the epoxide was not determined because of its volatility.

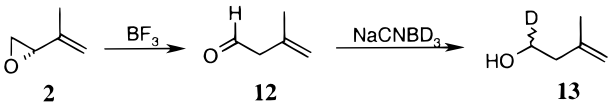
Epoxide **2** was reduced using the protocol of Hutchins et al.[14] with minor modifications. Treatment of **2** with $NaCNBD_3$ in diethyl ether at 0 °C in the presence of BF_3 afforded (*R*)-[2-^{2}H]isopentenol (**3**) in 50% yield and >95% ee (see below). A competing BF_3-catalyzed rearrangement[21] of **2** to aldehyde **12** followed by $NaCNBD_3$ reduction of **12** gave approximately 10% (see below) of racemic [1-^{2}H]isopentenol[22] **13** (Scheme 3) as a byproduct. We were not able to suppress this side reaction with an excess of the reducing agent.

Scheme 2



4 **5** **6** **7** **8** **9** **10** **2** **3** **11** **1**

Scheme 3



Alcohol **3** was tosylated and then phosphorylated by the procedure of Davisson et al.[20] to afford the (*R*)-[2-^{2}H]-isopentenyl diphosphate in overall 5% yield from **4**.

Determination of the Enantiomeric Purity of 11. Chiral derivatization reagents are normally not successful for determining ee's of chiral β-deuterioalcohols.[23] Indeed, a Mosher ester derivative of racemic [2-^{2}H]isopentenol[24] failed to show discrimination of diastereotopic nuclei both in ^{1}H and ^{19}F NMR spectroscopy. Recently,^{2}H NMR spectroscopy in a lyotropic medium was shown to be a sensitive technique for resolving enantiotopic deuterons in methylene groups.[25] As explained in detail elsewhere,[25] the technique exploits the fact that the deuterium quadrupolar coupling constants *do not* average to zero in an ordered environment such as a liquid crystal. Thus, the signal for each deuterium is split into a doublet with a separation given by the quadrupolar coupling constant, $\Delta\nu_Q$. Enantiotopic deuterium atoms are likely to have different $\Delta\nu_Q$ values since the latter is a geometrical parameter that depends on the angle between the electric field gradient at the site of the atom and the magnetic field vector. In the vast majority of cases, a ^{2}H NMR spectrum in a liquid crystal of a sample containing a mixture of two enantiomers shows a doublet for each antipod, centered around the observed frequency for that deuterium in an isotropic medium (e.g., CH_2Cl_2). Integration of the area under the peaks affords a straightforward measure of enantiomeric composition.

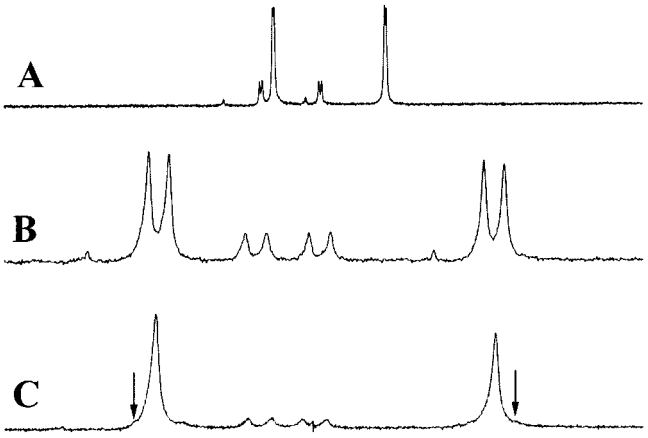


Figure 1. 46 MHz ^{2}H NMR spectra of racemic [2-^{2}H]isopentenyl tosylate at 20 °C (A), racemic [2-^{2}H]isopentenyl tosylate at −50 °C (B), and (*R*)-[2-^{2}H]isopentenyl tosylate (C) at −50 °C in a 20% PBLG/CH_2Cl_2 liquid crystal. Arrows indicate expected resonance frequencies for the *S* enantiomer, ν_S. Each peak for racemic [1-^{2}H]-isopentenyl tosylate (small set of doublets) represents ca. 6% of the signals for the *R* enantiomer.

Figure 1 shows the ^{2}H NMR spectrum of racemic **11** at 20 °C, and of racemic and (*R*)-**11** at −50 °C, in a 20% (w/v) poly-γ-benzyl-L-glutamate[26] (PBLG)/CH_2Cl_2 liquid crystal. Enantiotopic discrimination at room temperature was poor (Figure 1) as a consequence of the small difference in the quadrupolar coupling constants of the enantiomers ($\Delta\Delta\nu_Q \approx 3.5$ Hz) as compared to the corresponding line widths (ca. 3−4 Hz). As the temperature was lowered, $\Delta\Delta\nu_Q$ increased more rapidly than line width, resulting in better discrimination at lower temperatures. A series of spectra taken at different temperatures (see Supporting Information) indicated that the best resolution was obtained between −40 and −50 °C. However, even in this best case scenario, baseline resolution was not achieved.[27]

The enantiomeric composition of **11** was estimated as follows. Each peak of the small doublets corresponding to racemic [1-^{2}H]isopentenyl tosylate (spectrum C) represents ca. 6% of the intensity of the peaks for (*R*)-**11**. We reasoned that a peak of half that size (i.e., 3%) should still be visible at the expected resonance frequencies for the *S* enantiomer of **11**, ν_S (Figure 1). Hence one can set the enantiomeric composition of the sample at 97% *R*/3% *S* or better. Since the phosphorylation reaction does not promote epimerization, we conclude that for that portion of the sample with deuterium at C2, ≥97% of the label is at the *R* locus of the methylene carbon.

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(27) Attempts were made to reach baseline resolution by working in a liquid crystal of lower viscosity obtained by use of lower molecular weight PBLG (PD 562, MW 123 000) in which narrower lines are expected. Unfortunately a less viscous, less ordered medium also affords a lower intrinsic discrimination ($\Delta\Delta\nu_Q$), resulting in no net improvement.

Supporting Information Available: Complete experimental procedures for the synthesis of compounds **1**−**3** and **5**−**11** and ^{2}H NMR spectra depicting temperature dependence of the enantiomeric discrimination of [2-^{2}H]isopentenyl tosylate in a liquid crystal. This material is available free of charge via the Internet at http://pubs.acs.org.

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