The mevalonate and isoprene biosynthesis pathways result in the synthesis of a wide variety of compounds, including sterols, dolichols, ubiquinones, heme a, farnesyl pyrophosphate, geranylgeranyl pyrophosphate, and many terpenes and other natural products. The structures of the enzymes catalyzing the biosynthesis of such metabolites are of considerable general interest, not least from the perspective of drug design. For example, inhibitors of the enzyme hydroxymethylglutaryl CoA reductase, statins, are important as cholesterol lowering drugs, while farnesyl pyrophosphate synthase inhibitors are important in treating osteoporosis, Paget’s disease, and hypercalcemia due to malignancy. The enzyme isopentenyl pyrophosphate-dimethylallyl pyrophosphate isomerase (EC 5.3.3.2) catalyses the isomerization of isopentenyl pyrophosphate (IPP) to form dimethylallyl pyrophosphate (DMAPP), which then condenses with further IPP molecules to form FPP, which is used in, for example, protein prenylation and in cholesterol and ergosterol biosynthesis. There is, therefore, some interest in determining the structure of this enzyme, because it is a potential drug target.

In previous work, we and others reported the crystallographic structure of an IPP/DMAPP isomerase from Escherichia coli, a relatively small enzyme which appears to represent the minimal core structure required to catalyze IPP isomerization. The enzyme required one Mg$^{2+}$ or Mn$^{2+}$ to fully fold, and we proposed a tentative structure-based model for isomerization. It is, however, of course of interest to try to obtain additional structural information, on enzyme–inhibitor complexes, to more rigorously test any such mechanistic interpretations, as well as to facilitate future drug design. There are several IPP/DMAPP isomerase inhibitors known in the literature, and one of these, the epoxide of isopentenyl pyrophosphate (1), is also known to be a potent stimulator of γδ T cells. In addition, the bromohydrin of isopentenyl pyrophosphate (2) is also a potent γδ T cell activator and is being developed for use in treating multiple myeloma, renal carcinoma, and non-Hodgkin’s B cell lymphoma. Because both compounds 1 and 2 are known to stimulate γδ T cells and because the oxirane is a potent isomerase inhibitor, we investigated the possibility that the bromohydrin (2, BH) compound might also be an inhibitor. This was found to be the case, with the bromohydrin pyrophosphate having a $K_i$ of ca. 1.4 μM (Supporting Information). Using this inhibitor, we were able to obtain crystals of an isomerase–inhibitor complex and solve its structure, using basically the methods we reported previously for the apo and Mg$^{2+}$ or Mn$^{2+}$ complexes.

We show in Figure 1A the crystal structure of the isomerase–
C3 fragments are the same in both enantiomers. The bond to E116, and the structures of the pyrophosphate and C1 forms of the inhibitor bind to the enzyme, because introduction of the arginine and lysine groups present in this region. The inactivity of the E116Q and E87Q mutants can also be readily explained because E116 is used to protonate IPP, while the conserved E87 is essential for Mg2+ binding. This isomerase-inhibitor structure is thus of considerable interest because it provides the first detailed structural insights into the mechanism of action of IPP/DMAPP isomerase, which may be of use in the development of new drugs which inhibit the mevalonate/isoprene pathway.

**Acknowledgment.** We thank J. M. Sanders, C. R. Lea, F. Yin, and R. M. Coates for helpful comments. L.D. is a Research Associate of the Belgian Fonds National de la Recherche Scientifique (FNRS). This work was supported by grants from the French Community of Belgium “Action de Recherche Concertée”, from the FNRS (ISN and FRFC grants), from the E. Defay Fund (ULB), and by the UPSHS (NIH grant EB00271-24, to E.O.).

**Supporting Information Available:** $K_i$ (determination) (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

**References**

13. The bromohydrin of IPP was made by the addition of bromine water to IPP (Echelon Bioscience Inc., P.O. Box 5837, Salt Lake City, UT 84158–0537) at pH 7, ref 11. Excess bromine was removed using a stream of N2, and the product was characterized by 500 MHz 1H NMR spectroscopy.
14. Isomerase crystals were grown by equilibration of a protein solution (6 mg/mL) against PEG2000 (16%), ammonium sulphate (100 mM), and MnCl2 (10 mM) buffer to pH 5.5 with Tris/maleate. The crystals were then soaked in a solution of 2 for 2 h. A single crystal was then flash frozen, and diffraction data was collected using a Mar345 imaging plate system, from Marresearch, equipped with Osic optic and running on an FR591 rotating anode generator. Diffraction data (1.93 Å) were processed with the MarFLM suite: space group P21212121 (two molecules in the asymmetric unit) with cell parameters $a = 60.3$, $b = 78.6$, and $c = 92.5$ Å. The final model was refined to an $R$ value of 20.14% (25 353 reflections) and a $R_{	ext{ref}}$ of 25.74% using Sheldx197 (Sheldrick, G. University of Göttingen, Germany). Both enantiomers of 2 were included in the refinement. PDB submission: RCSB017448, PDB code 1N2U.

![Figure 2. Mechanistic proposals for enzyme inhibition.](image-url)