relationships of the two highest occupied bands $\psi_+$ and $\psi_-$ along some wavevector directions of the first Brillouin zone (see the inset of Figure 2). The $\psi_+$ and $\psi_-$ bands are largely represented by bonding and antibonding combinations of the HOMO's of two $ET^+$ cations in each unit cell. It is noted from Figure 2 that the $\psi_+$ and $\psi_-$ band overlap slightly. Since there are two electrons per unit cell of $(ET^+)_2$ to fill these bands, the top portion of the $\psi_+$ band is empty and the bottom portion of the $\psi_-$ band is filled. Consequently, $(ET)Ag_4(CN)_5$ is expected to be a semimetal.[13] Our calculations further reveal that the top portion of $\psi_+$ is empty for the wavevectors in the vicinity of a line from the midpoint of $\overline{KY}$ to Y, from Y to L, and from L to the midpoint of $\overline{LM}$. Likewise, the bottom portion of $\psi_-$ is filled for the wavevectors in the vicinity of a line from the midpoint of $\overline{YK}$ to K, from K to Z, from Z to N, and from N to the midpoint of $\overline{NM}$. That is, the semimetallic nature of the $\psi_+$ and $\psi_-$ bands is present in *all* directions of the $(ET)Ag_4(CN)_5$ crystal, which is therefore expected to be a 3D metal. Nevertheless, a number of nesting wavevectors exist, which could lead to charge density wave instability.

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**Supplementary Material Available:** Tables of final atom positions and anisotropic thermal parameters (Table X1) and of observed and calculated structure factors (Table X2) (11 pages). Ordering information is given on any current masthead page.

(12) The lattice parameters of the triclinic primitive cell are: $a_t = 13.80$ Å, $b_t = 11.82$ Å, $c_t = 11.75$ Å, $\alpha_t = 71.69°$, $\beta_t = 54.78°$, and $\gamma_t = 53.93°$. The primitive basis vectors are defined as $\vec{a}_t = {}^1/_2(\vec{b} + \vec{c})$, $\vec{b}_t = {}^1/_2(\vec{c} + \vec{a})$, and $\vec{c}_t = {}^1/_2(\vec{a} + \vec{b})$.

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## Isopentenyl Diphosphate:Dimethylallyl Diphosphate Isomerase. Irreversible Inhibition of the Enzyme by Active-Site-Directed Covalent Attachment

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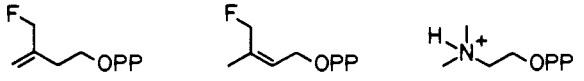
Isopentenyl diphosphate:dimethylallyl diphosphate isomerase (EC 5.3.3.2) catalyzes the conversion of the homoallylic substrate


IPP DMAPP

to its allylic isomer by an antarafacial [1.3] transposition of hydrogen.[1] This reaction provides electrophilic DMAPP[2] for subsequent prenyl transfer reactions and is a mandatory step in the biosynthesis of all isoprenoids. During the isomerization the *pro-R* proton at C2 of IPP is lost to water[3] and a proton from water is added to the *si* face of the double bond.[4] The functional groups that catalyze the antarafacial protonation–deprotonation are unknown and conclusive evidence for the mechanism of the isomerization is lacking.[1,5] We decided to investigate the mechanism for isomerization with fluorinated analogues and by inhibition with a reactive intermediate analogue patterned after our experiments conducted with farnesyl diphosphate synthetase[6-8] and squalene synthetase.[9] To our surprise these analogues were potent irreversible inhibitors of IPP:DMAPP isomerase. In this paper we report the first examples of active-site-directed inactivation of the enzyme by covalent modification.

Upon incubation with the allylic fluoro analogues FIPP and FDMAPP and with ammonium analogue NIPP,[10] IPP:DMAPP isomerase[12] was irreversibly inactivated in a pseudo-first-order time-dependent manner. Kinetic constants for the inactivation




FIPP FDMAPP NIPP

**Table I.** Kinetic Constants for Irreversible Inhibition of Isomerase[a]

| inhibitor | $k_{inact}$, $min^{-1}$ | $K_I$, μM |
|---|---|---|
| FIPP | 0.22 ± 0.07 | 0.09 ± 0.04 |
| FDMAPP | 0.4 ± 0.04 | 0.6 ± 0.09 |
| NIPP | 1.2 ± 0.3 | 15.2 ± 4.7 |

[a] Measured at 37 °C in 10 mM HEPES, pH 7.0, 1 mM DTT, 2 mM $MgCl_2$, 0.01% BSA; SA 0.7–1.2 μmol $min^{-1}$ $mg^{-1}$.

**Table II.** Stability of Isomerase–Inhibitor Complexes[a]

| inhibitor | treatment | $^3H$ dpm retained | $^3H$ dpm released | % dpm released |
|---|---|---|---|---|
| FIPP | 7 days, 4 °C[b] | 23 000 | 558 | 2.4 |
| | 70% ethanol[c] | 4 400 | 72 | 1.6 |
| | 6 M urea[d] | 20 900 | 54 | 0.3 |
| | 0.5% SDS, 100 °C, 90 s[e] | 4 400 | 93 | 2.1 |
| | 5% BME, 0.5% SDS[e] | 23 000 | 16 700 | 73 |
| FDMAPP | 70% ethanol[c] | 8 700 | 69 | 0.8 |
| | 6 M urea[d] | 15 300 | 139 | 0.9 |
| | 0.5% SDS, 100 °C, 90 s[e] | 8 700 | 87 | 1.0 |
| | 5% BME, 0.5% SDS[e] | 18 900 | 13 200 | 69 |

[a] Filtration requires 45 min. [b] Buffer A, 10 mM HEPES, 10 mM BME, pH 7. [c] In 100 μL of buffer A diluted to 2 mL with 70% ethanol. [d] In 100 μL of buffer A diluted to 2 mL with 6 M urea. [e] In standard electrophoresis sample buffer, 30 mM imidazole, 20 mM HCl, 15% glycerol, pH 7.0.

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(2) Abbreviations used: BME, β-mercaptoethanol; BSA, bovine serum albumin; DMAPP, dimethylallyl diphosphate; DTT, dithiothreitol; FIPP, 3-(fluoromethyl)-3-buten-1-yl diphosphate; FDMAPP, (*Z*)-4-fluoro-3-methyl-2-buten-1-yl diphosphate; IPP, isopentenyl diphosphate; NIPP, 2-(dimethylamino)ethyl diphosphate; SDS, sodium dodecyl sulfate.

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(10) Syntheses of 3-(fluoromethyl)-3-buten-1-ol and (*Z*)-4-fluoro-3-methyl-2-buten-1-ol will be reported elsewhere. 2-(Dimethylamino)ethanol was purchased from Aldrich Chemical Co. Diphosphates were synthesized by the procedure of Davisson et al.[11] and were fully characterized by IR and $^1H$, $^{13}C$, and $^{31}P$ NMR spectroscopy.

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(12) Isomerase was isolated from the mycelia of *Claviceps* sp. strain SD58, by a modification[13] of the published procedure.[14] As a final step, ion-exchange chromatography of enzyme on a Waters Protein PAK 5PW column yielded isomerase, SA 1.5 μmol $min^{-1}$ $mg^{-1}$, which gave a single predominant band upon SDS gel electrophoresis.

(13) Bruenger, E.; Rilling, H. C., unpublished results.

(14) Satterwhite, D. M. *Methods Enzymol.* **1985**, *110*, 92–99.

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reactions were measured by incubation of the enzyme with the inhibitors, periodic removal of portions which were then diluted 25-fold, addition of [1-$^{14}$C]IPP,[15] and measurement of residual isomerase activity using the acid-lability assay.[14,16] Control experiments without addition of the inhibitors established that the enzyme lost less than 1% of its initial catalytic activity. The results are given in Table I.

As expected for an active-site-directed process, IPP protected isomerase from inhibition. In the presence of 1.0 $\mu$M IPP, the rates of inactivation by 0.10 $\mu$M FIPP and 0.22 $\mu$M FDMAPP decreased by 42% and 37%, respectively. With 2 $\mu$M IPP in the buffer, the rate of inactivation by 5.0 $\mu$M NIPP decreased by 75%.[17]

We examined whether the inhibition was through covalent modification of the enzyme or slow release of a tightly bound inhibitor. Isomerase (15-60 $\mu$g, SA 0.3-0.6 $\mu$mol min$^{-1}$ mg$^{-1}$) was incubated at 37 °C with [4-$^3H_2$]FIPP and [1-$^3$H]FDMAPP in 100 $\mu$L of buffer under conditions that resulted in >98% loss of catalytic activity. The solutions were then diluted to a final volume of 2 mL and treated as described in Table II. The samples were concentrated with a microconcentrator (Centricon-10 Amicon Corp., MW cutoff 10 000 daltons). The radioactivity of concentrate (100 $\mu$L) and filtrate (1.9 mL) was measured. The concentrate was repeatedly diluted to 2 mL and reconcentrated until the radioactivity in the filtrate dropped to background levels. The results in Table II show that radioactivity from the fluorinated analogues remains with the enzyme under the denaturing conditions of 6 M urea, 70% ethanol/water, and 0.5% SDS at 100 °C for 90 s. Radioactivity is, however, released when the isomerase–inhibitor adducts are treated with high levels of BME under denaturing conditions in buffer containing 0.5% SDS. Furthermore, we found that catalytic activity could be restored to inactivated isomerase by thiol reagents. To restore activity, excess inhibitor was removed from a sample of isomerase inactivated by FIPP and FDMAPP by microconcentration. A portion of each sample was treated for 20 min at 37 °C with buffer containing 100 mM DTT. Control samples treated in a similar manner in buffer without DTT had no detectable activity, while those treated with high levels of DTT regained 10% of their original activity.[18]

Our data clearly support inactivation of isomerase by FIPP and FDMAPP through an active-site-directed covalent modification of the enzyme. The enzyme–adduct complexes are stable to a variety of conditions that denature the protein. Presumably covalent modification involves displacement of the allylic fluorides in the inhibitors by a nucleophile(s) in the catalytic site by $S_N2$ or $S_N2'$ processes to generate catalytically inactive adducts.[19] Although we have not demonstrated covalent attachment for NIPP, the behavior of ammonium inhibitor is similar to the allylic fluorides, including partial restoration of activity upon treatment of inhibited enzyme with DTT. Alternatively, NIPP may function as a transition state or reactive intermediate analogue of a carbocation that binds extremely tightly.[17] Regardless of the nature of the inhibition, the fluoro and ammonium pyrophosphate analogues are impressive inhibitors of isomerase. Work is in progress to determine the mechanisms of the inhibition reactions including the nature of the covalent attachments.

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**Registry No.** IPP, 358-71-4; FIPP, 99282-16-3; FDMAPP, 99282-17-4; NIPP, 96555-67-8; isopentyl diphosphate isomerase, 9033-27-6.

(15) Available from Amersham.

(16) In the same buffer $K_M^{IPP}$ = 1.1 ± 0.3 $\mu$M and $K_M^{DMAPP}$ = 0.9 ± 0.2 $\mu$M.

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## Synthesis of Aromatic Schiff Base Oligomers at the Air/Water Interface

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While built-up films of organic monolayers formed at the air–water interface (Langmuir–Blodgett films) have great potential for use in electronic devices,[1] their fragility is a serious limitation.[2] Work on polymerizable monolayers has produced more structurally stable films but has been limited to aliphatic materials.[3] A few studies on small molecules, such as anthracene[4] and porphyrins,[5] avoided using long alkyl chains. We report that aromatic Schiff base oligomers formed at the air–water interface when dialkylterephthalaldimines were spread onto acidic solutions of *p*-phenylenediamine. Built-up films of poly(*p*-phenyleneterephthalaldimine) (PPTA)[6] contained only a trace of aliphatic material, the original alkyl chains having served as disposable aids to surface layer formation. PPTA itself does not dissolve in known spreading solvents and cannot be spread on the water surface.

Surface pressure studies of dialkylterephthalaldimines indicated that they have sufficiently strong interactions with the water surface to spread on it.[7] The equilibrium spreading pressure (ESP) of liquid dihexylterephthalaldimine was 17.2 mN/m at 20 °C. The excess aldimine formed lenses on the surface. This ESP is slightly less than the 20.7 mN/m of ethyl tetradecanoate.[8] The pressure–area curves of dioctadecylterephthalaldimine indicated that while it might form a true monolayer at large surface areas, upon compression it collapsed to areas too small for a monolayer. The observed zero-pressure-extrapolated (zpe) areas[9] were temperature-dependent (0.38 ± 0.02 nm$^2$/molecule [sd of measurement, 2 determinations] at 5 °C, 0.34 ± 0.01 [2] at 10 °C, 0.24 ± 0.02 [9] at 20 °C, 0.17 ± 0.02 [2] at 30 °C, 0.14 ± 0.02 [2] at 40 °C). This was an equilibrium effect, not an artifact of spreading, since a film spread on a 40 °C subphase gave a zpe area of 0.34 nm$^2$/molecule if the subphase was cooled to 10 °C

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(8) Fukuda, K.; Ishii, Y. In "Shin Jikken Kagaku Koza (Lectures on New Experimental Chemistry)"; Iguchi, Iguchi, H., Ed.; Maruzen: Tokyo, 1975; Vol. 18, p 500.

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