5′-$O(\gamma S_p)$-[$\beta\gamma$-^{17}O,γ-^{17}O,^{18}O](3-thiotriphosphate)(chiral ATPγS)[8] with chicken liver pyruvate carboxylase (EC 6.4.1.1).[9] This enzyme catalyzes the overall reaction

$$\underset{\text{pyruvate}}{CH_3COCO_2^-} + HCO_3^- + ATP \rightleftharpoons \underset{\text{oxaloacetate}}{{}^-O_2CCH_2COCO_2^-} + ADP + P_i$$

via the intermediacy of N_1-carboxybiotin. The chiral (S_p)-ATPγS was incubated[10] with pyruvate, bicarbonate, Co(II), and enzyme,[11] and the absolute configuration of the product inorganic [^{16}O,^{17}O,^{18}O]thiophosphate[12] was determined to be R_p by the ^{31}P NMR method independently developed by Webb and Trentham[13] and by Tsai[14] (Figure 2). That is, of the stereochemically informative resonances (the second and third peak of each set) of the adenosine 5′-O-(2-thiotriphosphate) (ATPβS) that derives from the inorganic thiophosphate product, the second peak is larger than the third. Pyruvate carboxylase from chicken liver therefore catalyzes the conversion of ATP to ADP and P_i with overall *stereochemical inversion of the configuration at phosphorus.* Control experiments demonstrated that no ATPγS was broken down when incubated under identical conditions with enzyme that had been inactivated with avidin,[15] a potent biotin-binding protein.

Of the three mechanisms outlined above, both the stepwise (**1**) and the concerted (**2**) are predicted to go with overall inversion at phosphorus, while the composite (**3**) is predicted to go with retention. The stepwise mechanism involves the direct transfer of the phospho group of ATP to bicarbonate (with inversion[16]) followed by the expulsion of inorganic phosphate. The concerted mechanism also begins with a direct transfer of the phospho group, this time to the ureido oxygen of biotin (with inversion), followed by a six-electron $\pi_2S + \pi_2S + \sigma_2S$ electrocyclic rearrangement (with retention[7]). The composite mechanism, although chemically and enzymatically precedented, must be incorrect because it involves two direct transfers (each with inversion) and would thus result in overall retention at phosphorus.

In an effort to distinguish between mechanisms **1** and **2**, we may look to three critical results in the literature. First, when carbamoyl phosphate, a putative carboxyphosphate analogue, is allowed to react with ADP and either sheep liver pyruvate carboxylase[17] or *E. coli* acetyl-CoA carboxylase,[18] ATP is produced (however, see Kluger et al.[3]). Second, phosphonacetic acid, a stable carboxy phosphate analogue, is a noncompetitive inhibitor of ATP with the sheep liver enzyme.[17] Third, in the absence of bicarbonate, there is no positional isotope exchange of ATP[19] with chicken liver pyruvate carboxylase[20] and apparently no ADP–ATP isotope exchange with the sheep liver enzyme.[17] These results are most simply accommodated by the stepwise mechanism **1** (but do not unequivocally rule out mechanism **2**). Furthermore, it is becoming apparent that the few enzymes that might in principle catalyze concerted electrocyclic mechanisms, e.g., anthranilate synthase[21] or phosphoenolpyruvate carboxylase,[7] evidently follow stepwise pathways.

Finally, in mechanism **1**, how does the enzyme "activate" the nonnucleophilic ureido N_1 nitrogen atom of biotin so that it may attack carboxy phosphate? If by using acid–base chemistry the enzyme could tautomerize the ureido group into the enolic form (a mechanism for which has been proposed[22]), the N_1 nitrogen would become approximately 10^{10} times more nucleophilic.[6] Interestingly, Mildvan, Lane, and co-workers have recently shown that the N_1 nitrogen of biotin is unusually reactive compared with that of desthiobiotin or *O*-heterobiotin which may be due to a transannular interaction between the ureido carbonyl group and the biotin sulfur atom.[23] It thus appears that the ureido N_1 nitrogen of biotin, by whatever means, is nucleophilic enough to attack carboxy phosphate in these enzymatic reactions.

Acknowledgment. This work was supported by grants from the National Institute of Health (to J.R.K.) and the National Science Foundation (to the Department of Chemistry, toward the purchase of the Bruker WM-300 spectrometer).

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(9) Purified by a modification of the procedure in: Scrutton, M. C.; Fung, C.-H. *Arch. Biochem. Biophys.* **1972**, *150*, 636.
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(11) The reaction mixture (30 mL, incubated at 30 °C for 6 h) contained chiral ATPγS (20 μmol), pyruvate carboxylase (200 units[9]), $CoSO_4$, (0.5 mM), sodium pyruvate (40 mM), potassium bicarbonate (40 mM), and reduced glutathione (0.66 mM) in Hepes buffer (266 mM, pH 7.8). Acetyl-CoA (0.5 μmol) was added every 30 min.[9]
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Crystal and Band Electronic Structures of an Organic Salt with the First Three-Dimensional Radical-Cation Donor Network, (BEDT-TTF)$Ag_4(CN)_5$

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Most organic conducting salts of π-conjugated donor molecules known so far have a structure consisting of sheetlike networks of electron-donor molecules.[1] Each of these donor sheets, separated by a layer of counteranions, is typically composed of stacks of donor molecules. Thus intermolecular interactions take place within as well as between adjacent donor stacks. Despite such two-dimensional (2D) networks most conducting salts of donor molecules are one-dimensional (1D) metals because interactions between adjacent stacks are typically much weaker than those within each stack.[1,2] This situation can be significantly altered with bis(ethylenedithio)tetrathiafulvalene, $C_{10}S_8H_8$, (BEDT-TTF or simply ET), as shown by Kobayashi et al.[3] for $(ET)_2ClO_4$·

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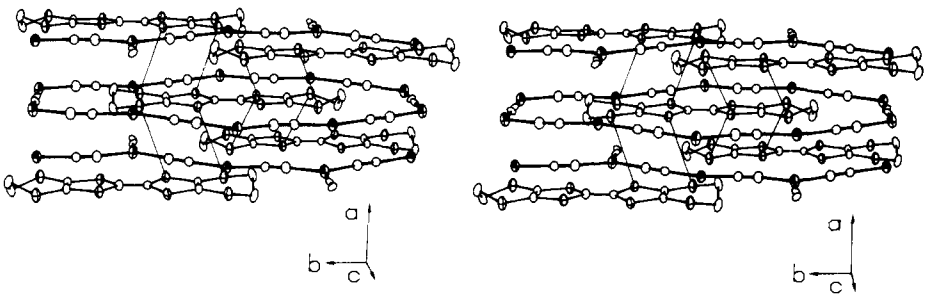

Figure 1. Stereoview of the crystal structure of $(ET)Ag_4(CN)_5$. Partially shaded ellipses, Ag; crossed, S; open, C and N. Thin lines denote the nearest intermolecular S···S contacts (3.90 Å) between ET molecules. Heavy lines indicate the "necklace" formed by the polymeric anion.

$(C_2H_3Cl_3)_{1/2}$, where ET molecules exhibit more side-by-side than face-to-face interactions within a 2D network. Subsequent synthesis and characterization of new conducting β-$(ET)_2X$ salts with linear anions, $X^- = I_3^-$,[4] IBr_2^-,[5] and AuI_2^-,[6] showed that these salts are not only 2D metals but also ambient pressure superconductors with transition temperatures (T_c's) of 1.5, 2.8, and 5 K, respectively. Our recent attempt to prepare ET salts of the $Ag(CN)_2^-$ anion led not only to an expected 2:1 salt α'-$(ET)_2Ag(CN)_2$,[7] which band electronic structure calculations reveal to be a 1D metal like β-$(ET)_2ICl_2$,[8] but also to a totally unexpected salt $(ET)Ag_4(CN)_5$, which contains a *three-dimensional* (3D) network of ET^+ cations. In this paper, we describe the crystal[9] and band electronic structures of $(ET)Ag_4(CN)_5$, the first example of a 3D donor-network organic conductor.

As shown in Figure 1, every ET^+ cation of the 3D donor network in $(ET)Ag_4(CN)_5$ is surrounded by four ET^+ cations in a quasi-tetrahedral arrangement such that every nearest-neighbor ET^+ pair interacts via two equivalent S···S contacts of 3.902 (4) Å, exceeding the van der Waals radii sum of sulfur (~ 3.6 Å).

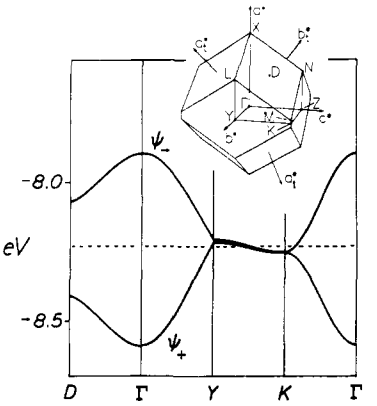

Figure 2. Dispersion of the two highest occupied bands ψ_+ and ψ_- of the 3D network of ET^+ cations, where the dashed line refers to the Fermi level. The inset shows the first Brillouin zone for the triclinic primitive unit cell with two ET^+ cations and with the primitive vectors $\vec{a}_t$, $\vec{b}_t$, and $\vec{c}_t$. In units of the corresponding reciprocal vectors $\vec{a}^*_t$, $\vec{b}^*_t$, and $\vec{c}^*_t$, the special wavevector points of the first Brillouin zone are defined as follows: $\Gamma = (0, 0, 0)$, $X = (0, 0.4790, 0.4790)$, $Y = (0.5, 0, 0.5)$, $Z = (0.5, 0.5, 0)$, $K = (1, 0.5, 0.5)$, $L = (0.5, 0.2481, 0.2481)$, $M = (1, 0.5210, 0.5210)$, $N = (0.5, 0.7519, 0.7519)$, and $D = (0.5, 0.5, 0.5)$. $\vec{a}^*$, $\vec{b}^*$, and $\vec{c}^*$ are the reciprocal vectors corresponding to the orthorhombic primitive unit cell vectors.

Each ET^+ cation is located at the intersection of three twofold rotation axes so that only one-quarter of an ET^+ is crystallographically unique. The 3D network of ET^+ cations is interwoven with honeycomblike polymeric networks of silver cations and cyanide anions parallel to each plane of ET^+ cations (i.e., *bc* plane). Each ET^+ cation is surrounded by a silver cyanide "necklace" consisting of 10 Ag^+ cations and 10 CN^- anions. There exist two kinds of Ag^+ cations in the structure: one silver is bonded to the carbon atoms of two CN^- anions in linear coordination, while the other has trigonal-planar geometry. Two coordination sites of the latter are occupied by nitrogen atoms of two CN^- anions, and the third one by either end of a disordered CN^- anion.[10] Each Ag^+ cation has two long Ag···S contacts (3.18 and 3.34 Å, for the two Ag^+ sites, respectively) with sulfur atoms of the ethylenedithio bridges of ET^+ cations.

It is important to examine whether or not $(ET)Ag_4(CN)_5$, with its 3D network of ET^+ cations, is a 3D metal as well. To date, crystals of insufficient size for electrical property measurements have been obtained, so that we have probed this question by performing tight-binding band electronic structure calculations.[4g,11] The $(ET)Ag_4(CN)_5$ crystal structure has eight ET^+ cations per face-centered unit cell. Our calculations, which are summarized in Figure 2, were based upon the primitive triclinic unit cell containing two ET^+ cations.[12] Figure 2 shows the dispersion

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(9) X-ray data from two crystals (grown by electrocrystallization[5a] using BEDT-TTF and $(Bu_4N)Ag(CN)_2$ in THF) were collected on an automated Picker and on a Nicolet P3/F diffractometer. The cell data are (298 K): space group *Fddd*, $Z = 8$, $a = 13.24$ (1) Å, $b = 19.48$ (2) Å, $c = 19.62$ (2) Å, $V = 5060$ (8) Å^3, $\rho_o = 2.6$ (1) g/cm^3, $\rho_c = 2.48$ g/cm^3. 5215 reflections (θ–2θ scan, Mo Kα radiation, graphite monochromator, $\lambda = 0.7107$ Å) were collected in the range $4° < 2\theta < 50°$ to yield two sets of 1128 unique, allowed reflections each from the two diffractometers ($R_{ave} = 0.041$, $wR_{ave} = 0.021$) which were corrected for absorption ($\mu = 37$ cm^{-1}, $T_{min} = 0.44$, $T_{max} = 0.71$). Full-matrix least-squares refinement (two scale factors, all non-hydrogen atoms anisotropic, hydrogen included, but fixed) yielded $R = 0.075$, $wR = 0.043$, GOF = 2.542 for 1690 (of the total 2256 allowed reflections from the two sets) observed ($F > 2\sigma$) reflections. Some systematically absent reflections carry very weak intensity, suggesting short-range order of the disordered CN^- anion.

(10) We have refined the crystal structure by assuming 50% C and 50% N for each of the atomic positions of the disordered CN^- anion.

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relationships of the two highest occupied bands ψ_+ and ψ_- along some wavevector directions of the first Brillouin zone (see the inset of Figure 2). The ψ_+ and ψ_- 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 ψ_+ and ψ_- band overlap slightly. Since there are two electrons per unit cell of $(ET^+)_2$ to fill these bands, the top portion of the ψ_+ band is empty and the bottom portion of the ψ_- 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 ψ_+ 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 ψ_- 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 ψ_+ and ψ_- 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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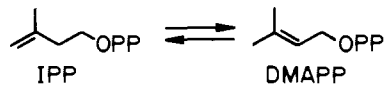
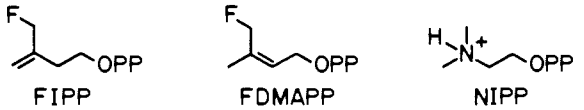
Isopentenyl diphosphate:dimethylallyl diphosphate isomerase (EC 5.3.3.2) catalyzes the conversion of the homoallylic substrate



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



FIPP FDMAPP NIPP

isomerase[12] was irreversibly inactivated in a pseudo-first-order time-dependent manner. Kinetic constants for the inactivation

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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(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.

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