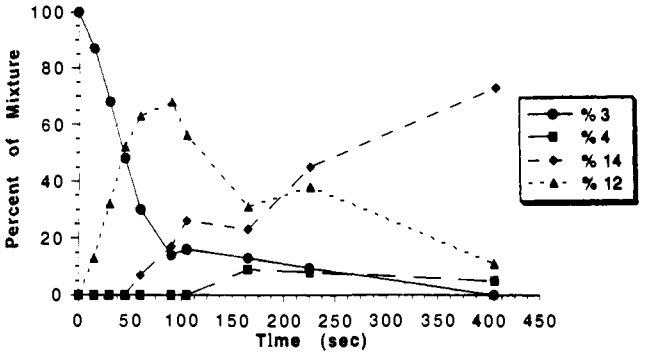
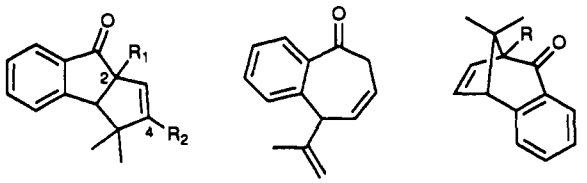



Figure 1. $^1$H NMR monitoring of the photolysis of *trans*-homotropone **3**.

isomerized via a well-precedented [1,5] sigmatropic shift,[13a] resulting in quantitative conversion to **13**.[4] The latter process also proved to be first-order, with $E_a$ = 29.9 kcal/mol[13b] and $\Delta S^{\ddagger}$ = −3.3 eu. The exceptionally low activation energy[14] for rearrangement of **3** reflects the additional strain imparted by the trans ring fusion.[15]



**12** $R_1 = R_2 = H$
**16** $R_1 = H, R_2 = D$
**18** $R_1 = D, R_2 = H$

**13**

**14** R = H
**17** R = D

Irradiation of **4** in benzene (0.4 M, 0.5 h, Pyrex)[16] led to ketone **14**[4] as the only isolable product (28% yield, 57% based on recovered **4**). In contrast, the photolysis of **3**, monitored by $^1$H NMR at 15-s intervals as illustrated in Figure 1, cleanly generated a mixture of **4**, **14**, and **12**. The data revealed fast initial formation of *cis*-homotropone **4** and suggested that **14** then derived from **4**, and **12** in turn from **14**. Support for this scheme followed from deuterium-labeling studies, wherein irradiation of **15** (82% D) afforded **16** labeled only in the C(4) vinyl position (81% D). The product presumably derived from Norrish type I cleavage of **17** and radical recombination; an excited-state vinylcyclopropane-type rearrangement[17] of **15** would have furnished **18**, containing the deuterium label at C(2).[18]

In summary, we have prepared the first *trans*-homotropone and characterized its thermal and photochemical reactivity. An experimental evaluation of Möbius antihomoaromaticity in **3** and the corresponding oxonium ions will be described in due course.

**Acknowledgment**. Support for this work was provided by the National Institutes of Health (National Cancer Institute) through Grant CA22807. We thank Drs. G. Furst and P. Carroll and Mr. J. Dykins, Directors of the University of Pennsylvania Spectroscopic Facilities, for assistance in obtaining high-field NMR spectra, X-ray crystallographic analyses, and high-resolution mass spectra.

**Supplementary Material Available:** Complete spectral data for **3**–**14** and tables of experimental details, positional parameters, and thermal parameters for **10** (11 pages). Ordering information is given on any current masthead page.

(13) (a) Ohloff, G. *Tetrahedron Lett.* **1965**, 3795. (b) This value is comparable to activation energies previously reported for similar [1,5] sigmatropic shifts.[14a]

(14) Activation energies of other vinylcyclopropane rearrangements vary from 26.3 to 54.6 kcal/mol. Reviews: (a) Mil'vitskaya, E. M.; Tarakanova, A. V.; Plate, A. F. *Russ. Chem. Rev.* **1976**, *45*, 469. (b) Hudlicky, T.; Kutchan, T. M.; Naqvi, S. M. *Org. React.* **1985**, *33*, 247.

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(16) Pyrex test tubes (20 mL) or NMR tubes (Wilmad no. 507) were charged with reactant solutions and irradiated with a 450-W Hanovia mercury vapor lamp (part no. 679A0360) suspended in a water-cooled Pyrex immersion well.

(17) Paquette has described an isomerization, analogous to the conversion of **15** to **16**, which proceeds via central cyclopropane bond cleavage; cf., Paquette, L. A.; Meehan, G. V.; Henzel, R. P.; Eizember, R. F. *J. Org. Chem.* **1973**, *38*, 3250 and references cited therein.

(18) The estimated detection threshold for **18** is ≤5%.

## Blue to Type 2 Binding. Copper(II) and Cobalt(II) Derivatives of a Cys112Asp Mutant of *Pseudomonas aeruginosa* Azurin

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*Received June 29, 1992*

Of the five invariant residues that surround the copper in azurins,[1] the ligand cysteine at position 112 (Cys112) is believed to be especially important in the bonding interactions responsible for the unusual blue copper absorption and electron paramagnetic resonance (EPR) spectra.[2] It is striking that mutagenesis studies of Met121,[3] His46,[3c] and His117[4] have shown that these ligands are not required for a blue copper center, thereby reinforcing the feeling that Cys112 is absolutely essential.[5] To address this issue directly, we have replaced Cys112 with Asp by site-directed mutagenesis.

Cys112 of *Pseudomonas aeruginosa* azurin was substituted with an aspartate using a synthetic azurin gene.[3c] The mutant (Cys112Asp) azurin was expressed in *Escherichia coli* using a T7 RNA polymerase expression system[6] in which azurin is secreted into the periplasm. $Cu^{II}$- and $Co^{II}$-Cys112Asp azurins were made by adding the appropriate metal ion to 1 mM to the periplasmic fraction containing crude apoazurin. In contrast to previously published protocols,[3c,7] protein purifications were performed at room temperature under basic conditions by FPLC. Concentrated crude protein solution was passed through Q-Sepharose fast flow resin with 50 mM Tris-Cl buffer (pH 7.8) containing 50 mM

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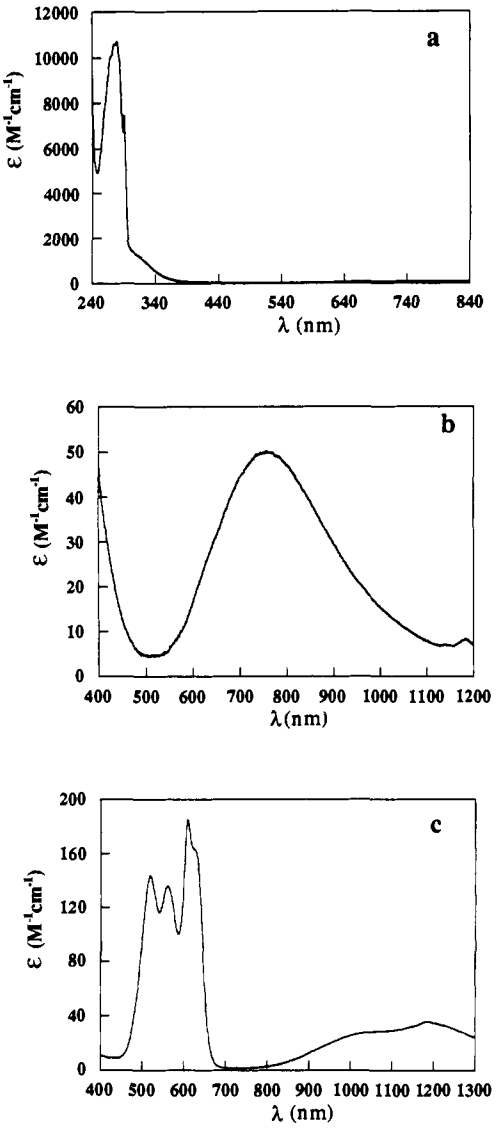


**Figure 1.** Electronic absorption spectra of $Cu^{II}$- and $Co^{II}$-Cys112Asp derivatives of *P. aeruginosa* azurin: (a) $Cu^{II}$-Cys112Asp azurin in 10 mM DEA-Cl buffer (pH 9.0); (b) $Cu^{II}$-Cys112Asp azurin in $D_2O$ containing 25 mM NaCl; (c) $Co^{II}$-Cys112Asp azurin in $D_2O$ containing 25 mM NaCl. Spectra were measured at room temperature.

NaCl. Under these conditions, azurin flows through while highly anionic contaminants adhere to the resin. The flow-through was reconcentrated and desalted with 10 mM DEA-Cl buffer (pH 9.0). The repeated concentration steps aided in removing the low molecular weight contaminants. Azurin was purified to homogeneity on a Mono Q column using a NaCl gradient. Both $Cu^{II}$- and $Co^{II}$-Cys112Asp azurins were found to be stable to metal loss and denaturation in the pH 5–9 range.[8]

Strong absorption in the 600-nm region of the electronic spectrum of $Cu^{II}$-Cys112Asp azurin is conspicuously absent (Figure 1a). The only observable charge-transfer band ($\sim$302 nm; $\epsilon \approx 1540$ $M^{-1}$ $cm^{-1}$) is attributable to an imidazole → Cu(II) transition in a tetragonal coordination site.[9,10] This band is blue-shifted considerably from a related ligand-to-metal

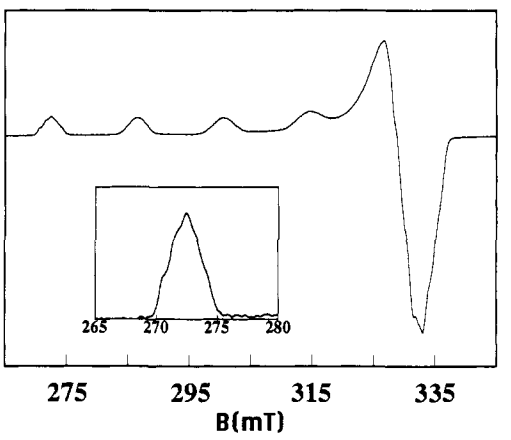


**Figure 2.** X-band (9.506 GHz) EPR spectrum of a frozen solution of $^{63}Cu^{II}$-Cys112Asp azurin in a 10 mM DEA-Cl buffer (pH 9.0)–glycerol mixture (1:1) at 85 K. The spin Hamiltonian parameters are as follows: $g_\parallel = 2.315$ (1), $A_\parallel = 152$ (1) $\times 10^{-4}$ $cm^{-1}$; $g_\perp = 2.075$ (5), $A_\perp = 10$ (2) $\times 10^{-4}$ $cm^{-1}$; $A_\parallel^N = 10$ (2) $\times 10^{-4}$ $cm^{-1}$, $A_\perp^N = 10$ (1) $\times 10^{-4}$ $cm^{-1}$.

charge-transfer absorption at 481 nm in wild-type azurin.[11] A ligand–field (*d–d*) system centered at 756 nm ($\epsilon \approx 50$ $M^{-1}$ $cm^{-1}$) in the $Cu^{II}$–Cys112Asp azurin spectrum (Figure 1b) also is substantially blue-shifted from the *d–d* bands found in the native protein.[11] The inferred tetragonal structure of Cu(II) in the Cys112Asp protein is confirmed by the EPR spectrum (Figure 2) attributable to a type 2 Cu(II) center.[12–14] The EPR spectrum of the isotopically ($^{63}Cu$) enriched $Cu^{II}$–Cys112Asp azurin exhibits superhyperfine structure in the lowest hyperfine line (Figure 2, inset), which indicates the presence of two equivalent equatorial nitrogen ligands.[13]

The spectroscopic properties of $Co^{II}$–Cys112Asp azurin suggest that the metal ion is five-coordinate in this derivative. The intensities of the *d–d* absorption bands in the 600-nm region (Figure 1c) fall closer to those of five- than of four-coordinate Co(II) complexes.[15] The pattern of band energies and intensities is similar to those of Co(II)-substituted carbonic anhydrase (acetate adduct) and carboxypeptidase A;[15] Co(II) in the latter enzyme is coordinated to two histidines, a bidentate glutamate, and a water molecule in a square pyramidal arrangement.[16]

Cys112 was replaced with aspartate because this residue is likely to be anionic above pH 5 and of the other 19 natural amino acids is closest in structure (though potentially bidentate) to an anionic cysteine.[17] While the absence of the intense S(Cys112) → Cu(II) absorption in $Cu^{II}$-Cys112Asp azurin was expected, we have demonstrated that Cys112 of *P. aeruginosa* azurin is not an obligatory ligand for copper binding. Both Cu(II) and Co(II) were retained throughout the rigorous purification scheme, suggesting that they are tightly associated with the protein. Since Cys112 is an internal residue,[1] its substitution is unlikely to create a strong new metal binding site that is not located at the wild-type site. Therefore, we conclude that the metals are bound to imidazole nitrogens of the native histidine ligands (His46, His117)

(8) Protein solutions were stored at 4 °C where they were stable for several months. Reversible metal loss was observed at pH <5 for $Co^{II}$-Cys112Asp azurin in acetate buffer.

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as well as a bidentate Asp112 carboxylate and possibly an axial carbonyl oxygen of Gly45 or a water molecule.[10]

It is well established that the unusual distorted trigonal pyramidal (pseudotetrahedral) coordination geometry of blue copper is forced on the metal ion by the rigidity of the polypeptide scaffold in the binding-site region (rack-induced bonding).[1c,d,18,19] Owing to the geometrical constraints imposed by the aspartate side chain, however, formation of a planar copper carboxylate group would require substantial rearrangement of the protein structure.[20] Since a large distortion of the protein structure is energetically unfavorable, it is likely that the copper is displaced significantly from the plane of the Asp112 carboxylate group in forming the Cu-$N_2O_2(O)$ structure.

**Acknowledgment.** We thank Dr. David B. Goodin of the Scripps Research Institute for assistance with the EPR measurements. This work was supported by grants from the National Institutes of Health (DK19038 to H.B.G.; GM16424 to J.H.R.; GM07616 traineeship to T.J.M.).

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(20) Computer modeling of $Cu^{II}$-Cys112Asp azurin coordination was based on the 2.7 Å resolution structure of *P. aeruginosa* azurin (ref 1a). The Cys112 side chain of wild-type azurin was replaced by an aspartate side chain using Biograf (Version 2.20) from BIODESIGN, Inc. The backbone atoms were fixed while the $C_\beta$ and $C_\gamma$ atoms of the aspartate side chain were positioned as closely as possible to the $C_\beta$ and $S_\gamma$ atoms of cysteine, and the $C_\beta$-$C_\gamma$ bond was rotated to make a pseudo square planar base containing the imidazole nitrogens of His46 and His117 and the carboxylate oxygens of Asp112. In this configuration, $C_\gamma$ of Asp112 is forced well out of the plane defined by the copper center and the carboxylate oxygens. For a discussion of metal binding to isolated carboxylate groups in proteins, see: Glusker J. P. *Adv. Protein Chem.* **1991**, *42*, 1-76.

## Formyltriisopropylsilane: The Synthesis and Chemistry of a Stable Formylsilane

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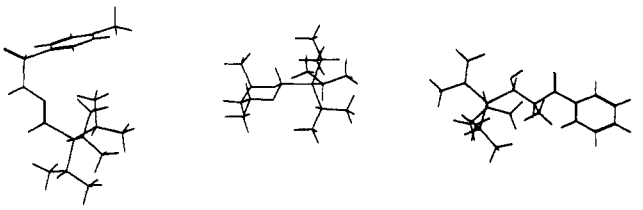
*Received July 3, 1992*

The only formylsilane isolated as a stable compound, $(Me_3Si)_3SiCHO$, reported by Tilley et al.[2] in 1988, represented an impressive synthetic achievement, being prepared from a mixed cyclopentadienyl acylzirconium precursor. Unlike $Me_3SiCHO$,[3,4] $(Me_3Si)_3SiCHO$ was found to be thermally stable although it decomposes exothermically in air.[2] We wish to report the convenient preparation of formyltriisopropylsilane (**2**) from a modified dithiane-based approach and its fascinating chemistry.

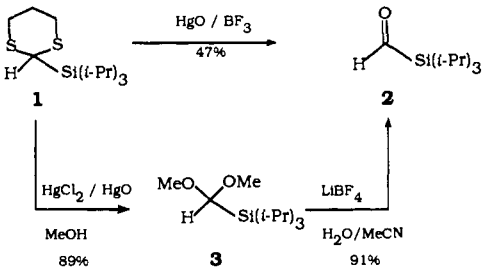
Previously, we have found that the triisopropylsilyl (TIPS) group not only significantly retards nucleophilic substitution at silicon but also greatly impedes reactions at adjacent centers.[5] This suggested that **2** should be stable, and **3** appeared to us to be its ideal precursor. By modifying the Corey-Seebach approach[6] to acylsilanes[7] to include an intermediate dithiane → acetal step,[6b] exposure of this sensitive system[8,9] to the dithiane hydrolysis conditions could be essentially avoided.



**4b** **(+)-8** **12**

**Figure 1.** MMX minimum energy structures for **4b**, **(+)-8**, and **12**.

The reaction of 2-lithio-1,3-dithiane[6] with TIPSCl (3 h, -78 → 25 °C) gives **1** cleanly (96%, >99% GC purity, bp 120 °C, 0.1 Torr, 87% from $MeOH/H_2O$, mp 45.5-47.5 °C). The solvolysis of **1** was carried out ($HgCl_2$, HgO, MeOH),[6b] which afforded **3** as a colorless liquid (bp 72 °C, 0.6 Torr) in 89% yield. The hydrolysis of **3** on a 50-mmol reaction scale was optimized employing $LiBF_4$ (0.37 M, 1.04 equiv)[10] in refluxing aqueous MeCN (9:91, 15 s), providing pure **2** as a greenish-yellow liquid in 91% yield (bp 85 °C, 3 Torr, 99% GC purity). By contrast, even the mild Vedejs-Fuchs hydrolysis conditions[11] gave **2** in significantly lower yield and purity (47%, 97% GC purity containing 3% TIPSOH), and the standard aqueous MeOH conditions[6] result in a mixture of **2** and **3**.




(1)

As anticipated,[2] the spectral properties of **2** are considerably Si-shifted (e.g., $^1H$ NMR $\delta$ 12.10 (CHO) ppm; $^{13}C$ NMR $\delta$ 249.0 (CHO) ppm; IR (neat) 2588 ($\nu_{CH}$), 1651 ($\nu_{CO}$) $cm^{-1}$; UV (THF) 375 (sh, 28), 390 (sh, 55), 406 (86), 426 (87) nm)). The electron-impact MS of **2** provides a weak $M^{\bullet+}$ (0.2%), with $TIPS^+$ (157, 63%) and its degradation products ($m/z$ 73 (67) and 59 (100)) being the major ion fragments.

Upon exposure to atmospheric oxygen, **2** spontaneously ignites! Limiting the amount of oxygen produces TIPSOH as the major Si-containing product, and in $CDCl_3$ solution, minor amounts of TIPSH(D) and TIPSCl are also observed (GCMS), implicating the intermediacy of TIPS radicals in the process. However, air-stable crystalline derivatives of **2** were easily prepared (**4a**, 2,4-DNP (75%, mp 109-110 °C); **4b**, tosylhydrazone (87%, mp 63.5-64.5 °C)) as single geometric isomers (anti)[12] (Figure 1).

(1) U.S. Department of Education Graduate Fellowship (P200A90203).

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(12) ΔMMXE (anti vs syn) = 3 kcal/mol (see Figure 1). Since the submission of this manuscript, a single-crystal X-ray structure of **4b** has been obtained (with Dr. C. L. Barnes, University of Missouri) which confirms its anti configuration and the general structural features which are depicted in Figure 1.

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