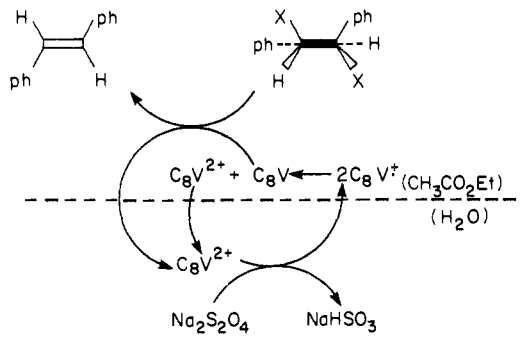

Figure 1. Reduction of 1,2-dibromostilbene in a two-phase system mediated by C_8V^{2+} (**1**).

debromination processes discussed previously is the two-electron reductant C_8V, rather than $C_8V^{+\cdot}$.

Yet, the reduction potential of dithionite and glucose in the chemical systems is only adequate for generating the one-electron reduction product $C_8V^{+\cdot}$. Therefore, the active debromination two-electron reducing agent C_8V might be formed in a disproportionation reaction (eq 3). The reduction potentials of $C_8V^{+\cdot}$

$$2C_8V^{+\cdot} \rightleftharpoons C_8V + C_8V^{2+} \quad (3)$$

and C_8V, as well as previous studies, indicate that the comproportionation constant lies overwhelmingly toward the radical cation, $C_8V^{+\cdot}$.[11] However, such a conclusion is valid only for a homogeneous phase. The success of accomplishing the debromination reaction in the two-phase system is attributed to an induced shift in the disproportionation equilibration toward the products, C_8V^{2+} and C_8V, due to reextraction of C_8V^{2+} into the aqueous phase.

The entire scheme leading to the cyclic debromination of the dibromides is displayed in Figure 1. The formation of $C_8V^{+\cdot}$ in the aqueous phase is followed by its extraction into the organic solution. Disproportionation of $C_8V^{+\cdot}$ in the organic phase is accomplished by the reextraction of C_8V^{2+} into the aqueous phase. Consequently, the two-electron reductant, C_8V, capable of reducing the dibromides is formed. Debromination recycles the mediating electron acceptor.

The photosensitized formation of 4,4′-dipyridinium radical cations by visible light is well-known.[7,8] In these systems organometallic compounds such as ruthenium tris(bipyridine), $Ru(bpy)_3^{2+}$, or zinc porphyrins are used as sensitizers, and triethanolamine, ethylenediaminetetracarboxylic acid, EDTA, or cysteine are introduced as electron donors. Thus, in the previous systems the reducing agent solubilized in the aqueous phase could be substituted by a sensitizer and electron donor. Introduction of the sensitizer $Ru(bpy)_3^{2+}$ and the electron donor $(NH_4)_3$-EDTA into the aqueous phase yields upon illumination ($\lambda > 400$ nm) the 4,4′-bipyridinium radical $C_8V^{+\cdot}$. This radical is extracted into the organic phase and mediates the previously described debromination reaction. The sensitizer $Ru(bpy)_2^{2+}$ and the mediating electron acceptor C_8V^{2+} are present in the two-phase system in catalytic amounts. The cyclic photoreaction mediated by C_8V^{2+} corresponds to the photosynthesis of stilbene *via* oxidation of $(NH_4)_3$-EDTA by dibromostilbene.

Futhermore, our previous discussion implies that the similar debromination process should be unfavorable in a homogeneous phase due to the low availability of the active reductant C_8V. Indeed, illumination of an acetonitrile solution that includes $Ru(bpy)_3^{2+}$ as sensitizer, C_8V^{2+} as electron acceptor, triethanolamine as electron donor, and dibromostilbene does not lead to stilbene (**4**), despite the effective formation of $C_8V^{+\cdot}$.

In conclusion, we have demonstrated that the amphyphylic 4,4′-bipyridinium salt C_8V^{2+} (**1**) serves as a phase-transfer electron carrier. Photoreduction of C_8V^{2+} in the aqueous layer coupled to reactions in the organic phase might be a general approach in photosynthetic applications. The induced shift in the disproportionation equilibrium of the one-electron reduction product in the two-phase system forms a two-electron reductant having a very low reduction potential, capable of reducing a variety of 1,2-dibromides. In nature, multielectron reducing mediators are very common. Thus, similar hydrophobic–hydrophilic interactions might lead to the natural intermediates via disproportionation reactions.

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Registry No. 1, 66620-94-8; **1**·Br_2, 36437-30-6; **2**, 13440-24-9; **3**, 24533-06-0; **4**, 103-30-0; **5**, 1694-19-5; **6**, 87922-24-5; **7**, 87922-25-6; **8**, 538-49-8; **9**, 5097-93-8; $C_8V^{+\cdot}$, 87922-26-7; C_8V, 87922-27-8; $Ru(bpy)_3^{2+}$, 15158-62-0; $Na_2S_2O_4$, 7775-14-6; $(NH_4)_3$-EDTA, 15934-01-7; glucose, 50-99-7.

(11) (a) Hunig, S.; Berneth, H. *Top. Curr. Chem.* **1980**, *92*, 1-44. (b) Hunig, S. *Pure Appl. Chem.* **1967**, *15*, 109-122. (c) Bard, A. J.; Ledwith, A.; Shine, H. J. *Adv. Phys. Org. Chem* **1976**, *13*, 155-278.

Kinetics of Long-Distance Ruthenium-to-Copper Electron Transfer in [Pentaammineruthenium histidine-83]azurin†

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Recent investigations have established that relatively rapid electron transfer can take place between metal centers separated by long distances (>10 Å) in proteins.[1-5] Of the systems examined to date, $a_5Ru(His\text{-}33)^{3+/2+}$–cytochrome *c* ($Fe^{3+/2+}$) ($a = NH_3$) is special in the sense that it involves electron transfer between metal centers in their electronic ground states.[1-3] Clearly, more studies of this sort are needed, because the dependences of the rate constant on separation distance and on the nature of the medium are critical factors that are yet to be elucidated. The purpose of this communication, therefore, is to report a fixed-site, long-distance electron-transfer experiment involving *Pseudomonas aeruginosa* azurin (Az), a blue copper protein whose structure and properties have been studied extensively.[6] For this experiment we have labeled His-83 of Az with a_5Ru^{3+} (Figure 1).

Samples of $a_5Ru(His\text{-}83)^{3+}$–$Az(Cu^{2+})$ were prepared by procedures developed previously for a_5Ru^{3+} protein modification.[1,2,7,8]

† Dedicated to the memory of Eraldo Natonini.

(1) (a) Winkler, J. R.; Nocera, D. G.; Yocom, K. M.; Bordignon, E.; Gray, H. B. *J. Am. Chem. Soc.* **1982**, *104*, 5798-5800; (b) *Chem. Scr.* **1983**, *21*, 29-33.

(2) Nocera, D. G.; Winkler, J. R.; Yocom, K. M.; Bordignon, E.; Gray, H. B., manuscript in preparation.

(3) Isied, S. S.; Worosila, G.; Atherton, S. J. *J. Am. Chem. Soc.* **1982**, *104*, 7659-7661.

(4) McGourty, J. L.; Blough, N. V.; Hoffman, B. M. *J. Am. Chem. Soc.* **1983**, *105*, 4470-4472.

(5) McLendon, G.; Simolo, K.; Taylor, L.; Cupo, P.; Miller, J.; Muhlks, W. *Abstr. Pap.-Am. Chem. Soc.* **1983**, 186th, INOR 19.

(6) (a) "Copper Proteins"; Spiro, T. G., Ed.; Wiley-Interscience: New York, 1981. (b) Adman, E. T.; Jensen, L. H. *Isr. J. Chem.* **1981**, *21*, 8-12. (c) Freeman, H. C. In "Coordination Chemistry-21"; Laurent, J. P., Ed.; Pergamon: Oxford, 1981; pp 29-51. (d) English, A. M.; Lum, V. R.; DeLaive, P. J.; Gray, H. B. *J. Am. Chem. Soc.* **1982**, *104*, 870-871. (e) With the assistance of S. L. Mayo, several views of the azurin structure[6b] were examined by H.B.G. on a computer graphics system at the Pennsylvania State University.

(7) Yocom, K. M.; Shelton, J. B.; Shelton, J. R.; Schroeder, W. A.; Worosila, G.; Isied, S. A.; Bordignon, E.; Gray, H. B. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 7052-7055.

(8) Margalit, R.; Pecht, I.; Gray, H. B. *J. Am. Chem. Soc.* **1983**, *105*, 301-302 and references therein.

0002-7863/83/1505-7765$01.50/0

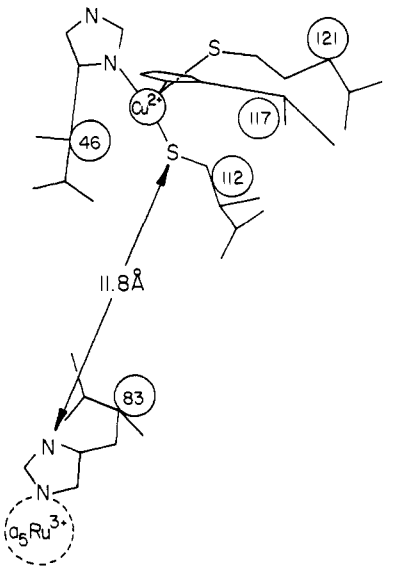

Figure 1. View of selected parts of the molecular skeleton of azurin with a_5Ru^{3+} bonded to the imidazole of His-83. The copper ligands are His-46, Cys-112, His-117, and Met-121. The closest distance between the $a_5Ru(His\text{-}83)^{3+}$ group and the blue copper center is 11.8 Å (N1 of the imidazole of His-83 to S of Cys-112).[6e]

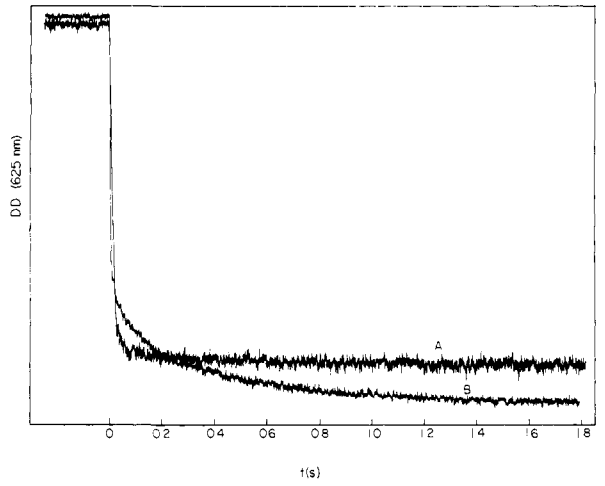

Figure 2. Changes in 625-nm absorbance upon flash photolysis of (A) $Az(Cu^{2+})$ and (B) $a_5Ru(His\text{-}83)^{3+}$–$Az(Cu^{2+})$. Conditions: 1×10^{-5} M protein, 7×10^{-5} M $[Ru(bpy)_3]Cl_2$, 5×10^{-3} M Na_2EDTA, 0.100 M phosphate buffer (pH 7.0), 23 °C. The initial OD decrease is due to reduction of Cu^{2+} by $Ru(bpy)_3^{2+*}$.

The initial product, $a_5Ru(His\text{-}83)^{2+}$–$Az(Cu^{+})$, was formed by mixing a 50-fold excess of $[a_5RuH_2O](PF_6)_2$ with $Az(Cu^{2+})$ for 3 h in aqueous solution at pH 7. Peptide-mapping experiments have established that the a_5Ru^{3+} group is bonded to His-83, and a variety of spectroscopic measurements (UV–visible, resonance Raman, CD, EPR) have shown that the blue copper site is virtually unperturbed by the $a_5Ru(His\text{-}83)^{3+}$ label.[9] Reduction potentials are as follows:[10] $a_5Ru(His\text{-}83)^{3+}$–$Az(Cu^{2+/+})$, 0.320 (2) V; $a_5Ru(His\text{-}83)^{3+/2+}$–$Az(Cu^{+})$, 0.040 (10) V.

Production of $a_5Ru(His\text{-}83)^{2+}$–$Az(Cu^{2+})$ was achieved by flash photolysis[11] of $a_5Ru(His\text{-}83)^{3+}$–$Az(Cu^{2+})$/$Ru(bpy)_3^{2+}$/EDTA solutions ($Ru(bpy)_3^{2+*}$ reacts rapidly with $a_5Ru(His\text{-}83)^{3+}$ to give $a_5Ru(His\text{-}83)^{2+}$). The bleaching at 625 nm that continues long

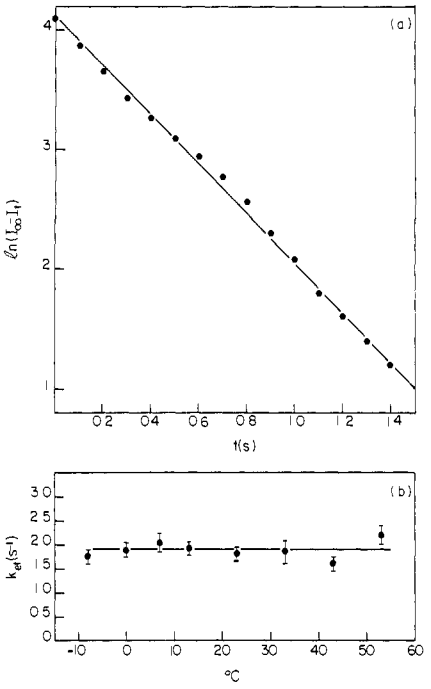

Figure 3. (a) First-order kinetic plot for the reduction of Cu^{2+} in flash-generated $a_5Ru(His\text{-}83)^{2+}$–$Az(Cu^{2+})$ at 23 °C; $t = 0$ taken at 0.2 s after the flash. (b) Rate constant (k_{et}) vs. temperature.

after the flash for the modified protein, but not for native azurin, is attributable to intramolecular electron transfer from $a_5Ru(His\text{-}83)^{2+}$ to the blue copper (Figure 2). The Cu^{2+} reduction closely follows first-order kinetics, and the rate constant was found not to vary over 3- (by dilution) or 5-fold (by repeated flashing) changes in the protein concentration. The $a_5Ru(His\text{-}83)^{2+}$–$Az(Cu^{2+}) \rightarrow a_5Ru(His\text{-}83)^{3+}$–$Az(Cu^{+})$ rate constant is 1.9 (4) s^{-1} over the entire temperature range investigated (Figure 3).

Again we have found that electron transfer can occur at a reasonably rapid rate between metal centers separated by a relatively long (and fixed) distance in a protein. Strikingly, but in accord with results found previously for ruthenium-modified horse heart cytochrome *c*,[1,2] electron transfer from $a_5Ru(His\text{-}83)^{2+}$ to the copper center in azurin is independent of temperature within our experimental error of 20%. These results allow us to place an upper limit of 1 kcal mol^{-1} on the activation enthalpy for the intramolecular $Ru^{2+} \rightarrow Cu^{2+}$ electron transfer. Thus the reorganizational enthalpy associated with electron transfer to blue copper cannot be very large ($\leq$7 kcal mol^{-1}),[9] a conclusion that had been anticipated from spectroscopic and structural studies.[12]

The question of the preferred pathway for electron transfer over long distance (>10 Å) in a protein is still very much an open one.[13-17] In the two experiments involving ground-state electron transfer, ($a_5Ru(His\text{-}83)^{2+} \rightarrow (Cys\text{-}112)Cu^{2+}$ $d(83\text{-}112) = 11.8$ Å, $k = 1.9$ s^{-1}, $\Delta E° = 0.28$ V; $a_5Ru(His\text{-}33)^{2+} \rightarrow (His\text{-}18)Fe^{3+}$ $d(33\text{-}18) = 12.1$ Å, $k = 25$ s^{-1}, $\Delta E° = 0.18$ V^2), it is of interest that the lower rate constant is found in the system having the higher driving force. The difference could be attributable to protein–medium effects in a simple through-space mechanism; however, it is worth noting that the closest peptide-chain excursion in modified azurin (His-83 to the nearest copper ligand, Cys-112) involves twice as many peptide bonds as that in modified cytochrome *c* (His-33 to the nearest iron ligand, His-18), thereby raising the intriguing possibility of a through-bond pathway (at

(9) Margalit, R.; Kostič, N. M.; Che, C.-M.; Chiang, H.-J.; Pecht, I.; Shelton, J. B.; Shelton, J. R.; Schroeder, W. A.; Gray, H. B., manuscript in preparation.

(10) $E°$ vs. NHE, 25 °C, pH 7 (ref 9); $E°$ for native azurin is 0.308 (2) V (Taniguchi, V. T.; Sailasuta-Scott, N.; Anson, F. C.; Gray, H. B. *Pure Appl. Chem.* **1980**, *52*, 2275–2281).

(11) Experimental procedures followed ref 1 (xenon flash, ~5 μs; optical path length, 15 cm).

(12) Gray, H. B.; Malmström, B. G. *Comments Inorg. Chem.* **1983**, *2*, 203–209.

(13) DeVault, D. *Q. Rev. Biophys.* **1980**, *13*, 387–564 and references therein.

(14) Sutin, N. *Acc. Chem. Res.* **1982**, *15*, 275–282.

(15) Larsson, S. *J. Chem. Soc., Faraday Trans. 2* **1982**, 1375–1388 and references therein.

(16) Freed, K. F. *Chem. Phys. Lett.* **1983**, *97*, 489–493.

(17) Hush, N., manuscript in preparation.

least in the latter case). Thus our results to date highlight the great need for kinetic experiments on fixed-site systems (at constant $\Delta E°$) in which systematic variations in through-space and through-bond distances can be investigated thoroughly.

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Stereochemistry of Oxidative Addition of an Optically Active Allyl Acetate to a Palladium(0) Complex

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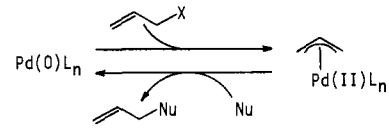
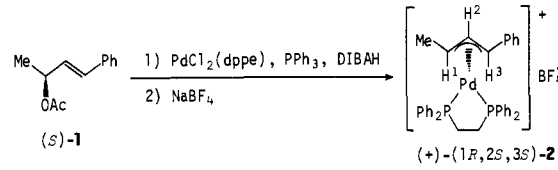
There has been considerable synthetic and mechanistic interest in palladium-catalyzed allylation of nucleophiles with allylic compounds represented by allyl acetates.[1] The catalytic cycle of the allylation is generally accepted to involve a π-allylpalladium(II) complex as a key intermediate, which is formed by oxidative addition of an allyl acetate to palladium(0) and undergoes nucleophilic attack to yield allylation product and to regenerate palladium(0)[1] (Scheme I). The nucleophilic attack has been reported to proceed with either inversion[2-6] or retention[7-9] of configuration depending on the nature of nucleophiles, and the stereochemistry of the oxidative addition has been deduced[3,10] to be inversion by stereochemical results obtained for the catalytic allylation[3,10-19] and stoichiometric reaction of π-allylpalladium complexes with nucleophiles.[2-9] However, there has been no direct evidence to support the stereochemistry of the oxidative addition. Here we report the isolation of an optically active π-allylpalladium

Scheme I



complex from a mixture of an optically active allyl acetate and a palladium(0) complex,[20] which unambiguously demonstrates, for the first time, that the oxidative addition forming π-allylpalladium(II) proceeds with inversion of configuration.[21]

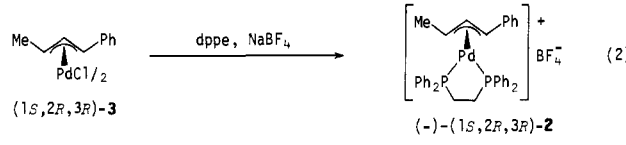
An excess of (*S*)-(*E*)-3-acetoxy-1-phenyl-1-butene (**1**) (58% ee)[22] was added to an ethereal solution containing a palladium(0) complex, presumably Pd(dppe)(PPh_3) (dppe = 1,2-bis(diphenylphosphino)ethane),[23] generated in situ by treatment of a mixture of $PdCl_2$(dppe) and 1 equiv of triphenylphosphine with 2 equiv of diisobutylaluminum hydride (DIBAH). The mixture was stirred at room temperature for 12 h, and sodium tetrafluoroborate was added (eq 1). Aqueous workup (extraction with



(*S*)-**1** (+)-(1*R*,2*S*,3*S*)-**2** (1)

chloroform) followed by preparative TLC on silica gel (hexane/EtOAc (1/4), R_f 0.1–0.2) gave 44% yield[25] of cationic π-allylpalladium complex **2** with optical rotation of $[\alpha]^{20}_D$ +57° (*c* 0.8, chloroform). **2**: Anal. Calcd for $C_{36}H_{35}P_2BF_4Pd$: C, 59.82; H, 4.88; P, 8.57. Found: C, 59.88; H, 4.82; P, 8.55. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.66 (dt, 3 H, Me), 2.28–2.72 (m, 4 H, PCH_2CH_2P), 4.42 (ddq, 1 H, H^1), 5.11 (dd, 1 H, H^3), 6.16 (t, 1 H, H^2), 6.76–7.73 (m, 25 H, Ph); $J(H^1-H^2) = J(H^2-H^3) = 12.8$, $J(H^1-Me) = 6.3$, $J(H^1-P) = 9.5$, $J(H^3-P) = 10.8$, $J(Me-P) = 9.4$ Hz. The NMR shows that both methyl and phenyl substituents in the π-allyl system are located in the syn positions with respect to the central hydrogen, and both the diphenylphosphino groups in the dppe coordinate to the palladium to form a chelate.

The absolute stereochemistry and enantiomeric purity of **2** obtained above were conveniently determined by comparison of its optical rotation with that of an authentic sample prepared through a different pathway (eq 2). Thus, (1*S*,2*R*,3*R*)-di-μ-



(1*S*,2*R*,3*R*)-**3** (-)-(1*S*,2*R*,3*R*)-**2** (2)

chlorobis(1-methyl-3-phenyl-π-allyl)dipalladium (**3**)[26] (86% ee, $[\alpha]^{20}_D$ −604° (*c* 1.0, chloroform)) was treated with dppe and sodium tetrafluoroborate in chloroform[2] to give quantitatively the palladium complex **2** with $[\alpha]^{20}_D$ −105° (*c* 1.1, chloroform), which must have the same configuration and enantiomeric purity as those of the starting **3**. It follows that the π-allylpalladium **2** obtained

(1) For reviews: (a) Trost, B. M. *Acc. Chem. Res.* **1980**, *13*, 385. (b) Tsuji, J. "Organic Synthesis with Palladium Compounds"; Springer-Verlag: New York, 1980. (c) Trost, B. M.; Verhoeven, T. R. In "Comprehensive Organometallic Chemistry"; Wilkinson, G., Stone, F. G. A., Abel, E. W., Ed.; Pergamon: New York, 1982; Vol. 8, p 799.

(2) Trost, B. M.; Weber, L.; Strege, P. E.; Fullerton, T. J.; Dietsche, T. J. *J. Am. Chem. Soc.* **1978**, *100*, 3416.

(3) Trost, B. M.; Verhoeven, T. R. *J. Am. Chem. Soc.* **1978**, *100*, 3435.

(4) Collins, D. J.; Jackson, W. R.; Timms, R. N. *Aust. J. Chem.* **1977**, *30*, 2167.

(5) Akermark, B.; Jutand, A. *J. Organomet. Chem.* **1981**, *217*, C41.

(6) Akermark, B.; Bäckvall, J.-E.; Löwenborg, A.; Zetterberg, K. *J. Organomet. Chem.* **1979**, *166*, C33.

(7) Temple, J. S.; Riediker, M.; Schwartz, J. *J. Am. Chem. Soc.* **1982**, *104*, 1310.

(8) Castanet, Y.; Petit, F. *Tetrahedron Lett.* **1979**, 3221.

(9) Jones, D. N.; Knox, S. D. *J. Chem. Soc., Chem. Commun.* **1975**, 165.

(10) Trost, B. M.; Verhoeven, T. R. *J. Am. Chem. Soc.* **1980**, *102*, 4730.

(11) Fiaud, J. C.; Malleron, J. L. *Tetrahedron Lett.* **1981**, *22*, 1399.

(12) Trost, B. M.; Schmuff, N. R. *Tetrahedron Lett.* **1981**, *22*, 2999.

(13) Trost, B. M.; Klun, T. P. *J. Am. Chem. Soc.* **1979**, *101*, 6756.

(14) Trost, B. M.; Keinan, E. *Tetrahedron Lett.* **1980**, *21*, 2591.

(15) Fiaud, J.-C.; Malleron, J.-L. *J. Chem. Soc., Chem. Commun.* **1981**, 1159.

(16) Trost, B. M.; Keinan, E. *J. Am. Chem. Soc.* **1978**, *100*, 7779.

(17) Hayasi, Y.; Riediker, M.; Temple, J. S.; Schwartz, J. *Tetrahedron Lett.* **1981**, *22*, 2629.

(18) Matsushita, H.; Negishi, E. *J. Chem. Soc., Chem. Commun.* **1982**, 160.

(19) Keinan, E.; Greenspoon, N. *Tetrahedron Lett.* **1982**, *23*, 241.

(20) Isolation of π-allyl(acetato)palladium from a palladium(0) complex and allyl acetate has been reported: Yamamoto, T.; Saito, O.; Yamamoto, A. *J. Am. Chem. Soc.* **1981**, *103*, 5600.

(21) Oxidative addition of benzyl halides to $Pd(PPh_3)_4$ has been reported to proceed with inversion. Lau, K. S. Y.; Wong, P. K.; Stille, J. K. *J. Am. Chem. Soc.* **1976**, *98*, 5832.

(22) The acetate **1** ($[\alpha]^{20}_D$ − 78.5° (*c* 1.17, CCl_4)) was prepared by acetylation (Ac_2O, pyridine, DMAP) of (*S*)-(*E*)-3-hydroxy-1-phenyl-1-butene ($[\alpha]^{20}_D$ − 15.5° (*c* 4.8, $CHCl_3$)). (Terashima, S.; Tanno, N.; Koga, K. *J. Chem. Soc., Chem. Commun.* **1980**, 1026).

(23) Use of isolated $Pd(dppe)(PPh_3)_2$[24] or palladium(0) prepared in situ from $PdCl_2$(dppe) and DIBAH in the absence of PPh_3 gave unsuccessful results.

(24) Milstein, D.; Stille, J. K. *J. Am. Chem. Soc.* **1979**, *101*, 4992.

(25) The modest yield is ascribed in part to partial decomposition of **2** on the TLC.

(26) Hayashi, T.; Konishi, M.; Kumada, M. *J. Chem. Soc., Chem. Commun.* **1983**, 736.

0002-7863/83/1505-7767$01.50/0