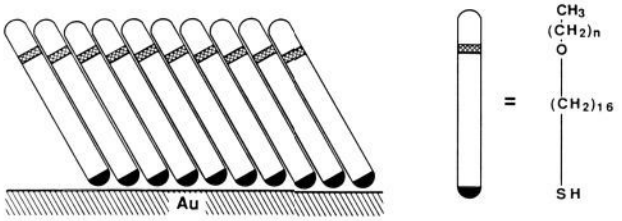

Figure 1. A schematic illustration of a monolayer of $HS(CH_2)_{16}O(CH_2)_nCH_3$ on gold.

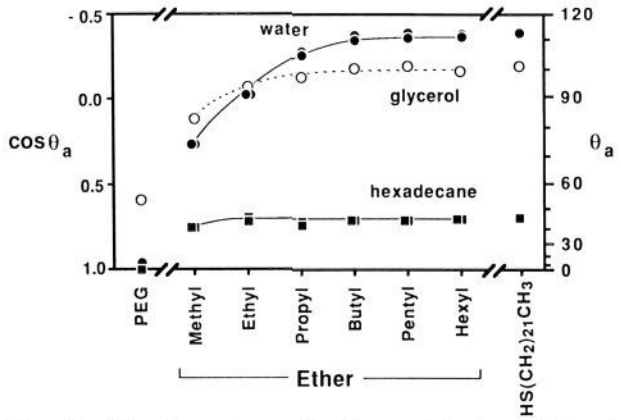

Figure 2. Advancing contact angles of water (●), glycerol (○), and hexadecane (■) on monolayers of $HS(CH_2)_{16}O(CH_2)_nCH_3$ on gold, as a function of the length of the terminal alkyl chain. PEG (poly(ethylene glycol)) is a model for a surface in which the ether linkage is exposed to the contacting liquid. A monolayer of docosanethiol ($HS(CH_2)_{21}CH_3$) on gold models the case in which the oxygen of the ether has no influence on the contact angle. Errors in measurement are within the size of the data points. The value of the contact angle of water on PEG is approximate since PEG rapidly dissolves in the water drop.

peak in XPS with increasing chain length of the alkyl group supported our proposed structural model.

We measured the advancing contact angle, θ_a, of water, glycerol, and hexadecane on the monolayers as the ether group was progressively screened from the contacting liquid by alkyl chains of increasing length.[9] Figure 2 relates cos θ_a to the length of the terminal alkyl chain. A smooth poly(ethylene glycol) (PEG) surface[10] provided a reference for a surface in which ether linkages are exposed to the contacting liquids.[11]

We note two features of Figure 2. First, for sufficiently long terminal alkyl chains, the contact angles approach those observed on monolayers of simple *n*-alkanethiols adsorbed on gold. Thus, the influence on wettability of the ether oxygen disappears entirely. Second, the length of the alkyl chain for which the ether group no longer influences the contact angle varies with the nature of the contacting liquid. Hexadecane, which interacts only by dispersion interactions, is largely screened from the influence of the ether oxygen by a single methyl group and completely screened (to within experimental precision) by an ethyl group. Water, which interacts primarily by hydrogen bonding, senses the ether group at greater depths; limiting contact angles are only reached for the butyl ether. Clearly, water cannot form hydrogen bonds through 4 Å of hydrocarbon. It is more likely that the water molecules are able to penetrate through the terminal alkyl chains, possibly by disordering the outermost part of the monolayer. Water–hydrocarbon contacts are, however, energetically unfavorable and beyond a certain depth the energy of a hydrogen bond to an ether no longer compensates for the concomitant hydrophobic interactions. Glycerol not only forms strong hydrogen bonds but also has considerable dispersive character.[12] The contact angles of glycerol reach a plateau at the propyl ether: at this point the glycerol molecules are beyond the range of significant dispersive interactions with the ether functionality but are perhaps too sterically hindered to penetrate through the terminal alkyl chain to form hydrogen bonds to the oxygen atom of the ether.[13]

In conclusion, the sensitivity of the contact angle of hexadecane to the ether group in this monolayer system extends only ~2 Å, whereas water senses the ether group down to ~5 Å beneath the surface. The greater sensing depth of water may reflect its penetration through short alkyl chains at the surface of the monolayer.

Acknowledgment. This work was supported in part by the ONR and by DARPA, employed surface instrumentation provided by DARPA/URI, and was housed in the Harvard University Materials Research Laboratory. We thank Dr. R. Nuzzo (AT&T Bell Laboratories) for obtaining the reflection IR spectra and for valuable discussions.

(8) The C–H stretching modes of the methyl ether are complex; we have not yet analyzed them thoroughly. Full spectroscopic data will be published separately.

(9) For experimental details, see: Bain, C. D.; Troughton, E. B.; Tao, Y.-T.; Evall, J.; Whitesides, G. M.; Nuzzo, R. G. *J. Am. Chem. Soc.*, in press.

(10) PEG (MW 7500 av, Polysciences) was cooled from the melt against a polished silicon wafer that had been cooled with a monolayer of $CF_3(CF_2)_7(CH_2)_2SiCl_3$ (Petrarch) to prevent adhesion to the surface. PEG dissolves rapidly in water: the contact angle of water shown in Figure 2 is an estimate of the angle immediately after application (~1 s) of the drop to the surface.

(11) An alternative reference surface—a monolayer of $HS(CH_2)_{11}OH$ on gold—is wet by all three liquids ($\theta_a < 10°$).

(12) Fowkes, F. M. *Ind. Eng. Chem.* **1964**, *56(12)*, 40–52.

(13) Relatively long-range polar interactions could also play a role in determining the contact angle. Experiments with monolayers of thiols on silver, in which the alkyl chains are less canted and hence have less freedom to become disordered in the presence of water, should help to distinguish between these two mechanisms.

Concerning the Catalytic Site of Porphobilinogen Deaminase

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Porphobilinogen (PBG) deaminase (EC4.3.1.8) catalyzes the tetramerization of PBG (**1**) to preuro'gen (hydroxymethylbilane, HMB, **2**)[1,2] which is cyclized with rearrangement to the unsymmetrical uro'gen III (**3**) by uro'gen III synthase (EC4.2.1.75) (Scheme I). In the absence of the latter enzyme, preuro'gen (**2**) cyclizes to uro'gen I (**4**),[3] which has recently been shown to be a substrate for the methylases of the vitamin B_{12} pathway.[4,5] Previous work with deaminase (from *Rhodopseudomonas spheroides*) has established that a covalent bond is formed between substrate and enzyme.[2,6,7] Application of ^3H NMR spectroscopy

(1) Burton, G.; Fagerness, P. E.; Hosazawa, S.; Jordan, P. M.; Scott, A. I. *J. Chem. Soc., Chem. Commun.* **1979**, 202. Scott, A. I.; Burton, G.; Jordan, P. M.; Matsumoto, H.; Fagerness, P. E.; Pryde, L. M. *J. Chem. Soc., Chem. Commun.* **1980**, 384.

(2) Evans; J. N. S.; Burton, G.; Fagerness, P. E.; Mackenzie, N. E.; Scott, A. I. *Biochemistry* **1986**, *25*, 897.

(3) Review: Leeper, F. J. *Nat. Prod. Rep.* **1985**, *2*, 19.

(4) Müller, G.; Schmiedl, J.; Schneider, E.; Sedlmeier, R.; Wörner, G.; Scott, A. I.; Williams, H. J.; Santander, P. J.; Stolowich, N. J.; Fagerness, P. E.; Mackenzie, N. E.; Kriemler, H.-P. *J. Am. Chem. Soc.* **1986**, *108*, 7875.

(5) Müller, G.; Schmiedl, J.; Savidis, L.; Wirth, G.; Scott, A. I.; Santander, P. J.; Williams H. J.; Stolowich, N. J.; Kriemler, H.-P. *J. Am. Chem. Soc.* **1987**, *109*, 6902.

(6) Berry, A.; Jordan, P. M.; Seehra, J. S. *FEBS Lett.* **1981**, *129*, 220.

(7) Battersby, A. R.; Fookes, C. J. R.; Matcham, G. W. J.; Macdonald, E.; Hollenstein, R, *J. Chem. Soc., Perkin Trans. 1* **1983**, 3031. Battersby, A. R.; Fookes, C. J. R.; Hart, G.; Matcham, G. W. J.; Pandey, P. S. *J. Chem. Soc., Perkin Trans. 1* **1983**, 3041.

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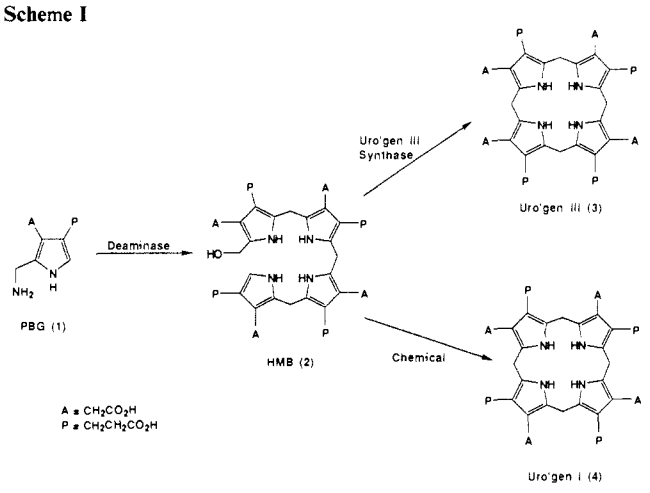
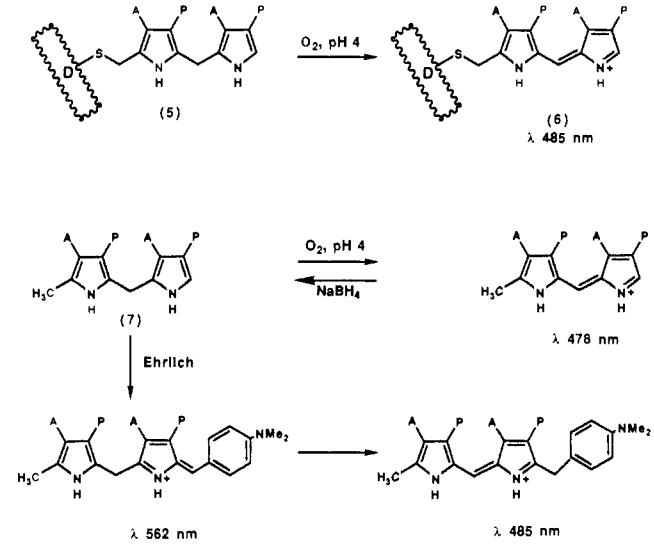
Scheme I



Scheme II



to the mono PBG adduct (ES_1) revealed that, in contrast to a claim[7,8] (since withdrawn[9]) that the ϵ-NH_2 of a lysine residue is covalently attached to substrate, the observed ^3H chemical shift indicated bond formation with a cysteine thiol group at the active site.

In this communication we present evidence that a novel cofactor, derived from 5-amino levulinic acid (ALA) during the biosynthesis of deaminase, is covalently attached to one of the four cysteine residues of the enzyme in the form of a dipyrromethane which, in turn, becomes the site of attachment of the succeeding 4 mol of substrate during the catalytic cycle.

First it was shown that at pH < 4 PBG deaminase (**5**) rapidly developed a chromophore (λ_{max} 485 nm) diagnostic of a pyrromethene (as **6**), whilst reaction with Ehrlich's reagent generated a chromophore typical of a dipyrromethane (λ_{max} 560 nm) changing to 490 nm after 5–10 min (Scheme II). The latter chromophoric interchange was identical with that of the Ehrlich reaction of the synthetic model pyrromethane (**7**) and can be ascribed to the isomerization shown (Scheme II) for the model system **7**. Proof that the catalytic site involves covalent attachment of a dipyrromethane (as **5**) came from the following NMR experiments, now made possible by the cloning of the *Escherichia coli* hem C gene[10,11] for deaminase. Incubation of *E. coli* SASX41B (hemA$^-$ requiring ALA for growth) previously

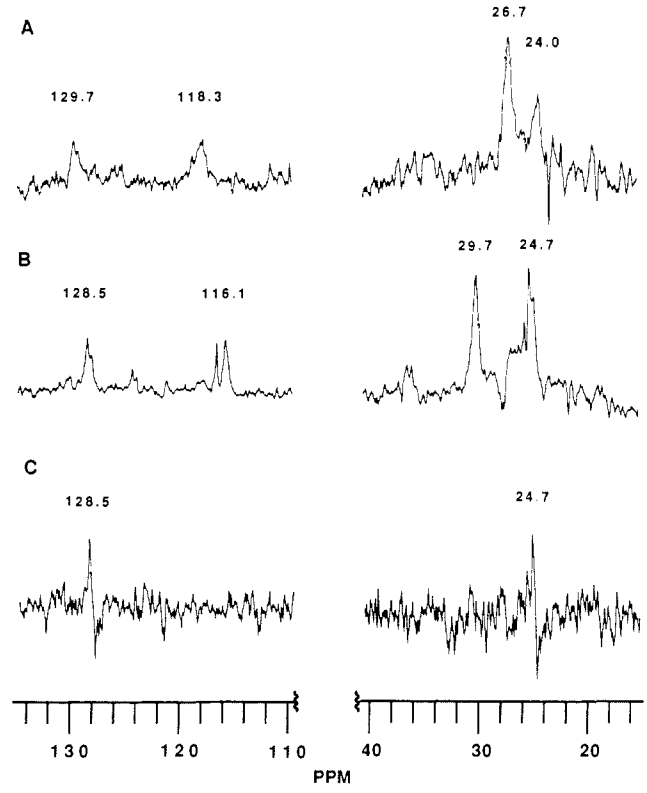


Figure 1. Proton decoupled 125 MHz ^{13}C NMR spectra of *E. coli* PBG deaminase, 20 °C: (A) difference spectrum between deaminase samples grown in the presence and absence of (5-^{13}C)ALA (100 mg/L; 90 atom ^{13}C %), pH 8, (B) difference spectrum as is (A), recorded at pH 12, (C) ^{13}C INADEQUATE spectrum of ^{13}C-enriched deaminase, pH 12 (see text for details).

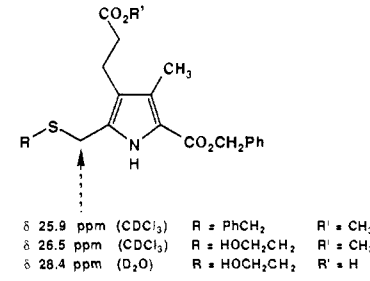
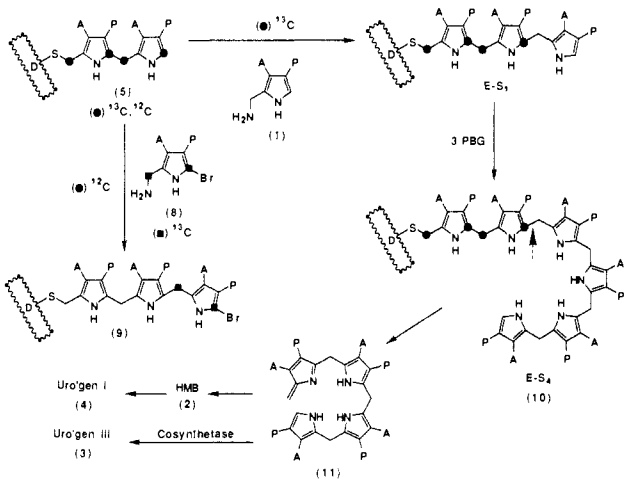


Figure 2. ^{13}C chemical shift range for R-S-CH_2-pyrrole in synthetic model pyrroles.

transformed with the plasmid pBG101[12] in the presence of 5-^{13}C-ALA afforded highly enriched (>80% ^{13}C) enzyme for NMR studies. At pH 8, the enriched carbons of the dipyrromethane (py-CH_2-py) are clearly recognized at 24.0 ppm (py-CH_2-py), 26.7 ppm (py-CH_2-X), 118.3 ppm (α-free pyrrole), and 129.7 ppm (α-substituted pyrrole) (Figure 1A). Comparison with synthetic models reveals that a shift of 26.7 ppm is in the range expected for an α-thiomethylpyrrole (py-CH_2-SR) (see Figure 2). The spectrum of ^{13}C-enriched deaminase was then recorded at pH 12. As can be seen from Figure 1B, the pyrrolic carbons now appear as sharp signals at 24.7 (doublet; 1C), 29.7 (singlet; 1C), 116.1 (singlet; 1C), and 128.5 ppm (broad doublet; 1C). Final confirmation of the dipyrromethane (rather than oligopyrromethane)[9] constitution **5** came from the ^{13}C INADEQUATE spectrum (Figure 1C) which reveals the expected coupling only between py-CH_2-py (δ 24.7) and the adjacent substituted pyrrole carbon (δ 128.5 ppm). The signals were unchanged after resto-

(8) Leeper, F. J. *Nat. Prod. Rep.* **1985**, *2*, 561.
(9) Hart, G. J.; Miller, A. D.; Leeper, F. J.; Battersby, A. R. *J. Chem. Soc., Chem. Commun.* **1987**, 1762.
(10) Raich, N.; Romeo, P. H.; Dubart, A.; Beaupain, D.; Cohen-Solal, M.; Goossens, M. *Nucleic Acid Res.* **1986**, *14*, 5955.
(11) Thomas, S. D.; Jordan, P. M. *Nucleic Acid Res.* **1986**, *14*, 6215.
(12) Expression of pBG101 from a suitable *E. coli* host results in a 200-fold overproduction of deaminase. For a full account of the molecular cloning and expression of deaminase, see: Scott, A. I.; Baldwin, T. O.; Treat, M.; Roessner, C. A.; Grant, S. K.; Stolowich, N. J.; Williams, H. J. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, in press.

Scheme III



ration of the denatured enzyme solution to pH 8. Accordingly, the environment of the thioether in the native enzyme at pH 8 (δ py-CH_2-SR 26.7 ppm) relative to pH 12 (δ 29.7) must involve shielding by an adjacent residue, an effect which is removed by denaturation at pH 12.

Finally, the monocovalent inhibitor complex[13] (**9**, Scheme III) prepared by incubation of deaminase with [2,11-$^{13}C_2$]-2-bromo PBG (**8**) was analyzed by CMR. The difference spectrum at pH 12 (not shown) displays signals at 24.6 ppm [py-CH_2-py (Br)] and 97.5 ppm (α-Br pyrrole-C) consistent with loss of ammonia from **8** to give structure **9**. The site of covalent attachment of substrate (and inhibitor) is therefore the free α-pyrrole carbon at the terminus of the dipyrromethane in the native enzyme. The above evidence, together with our previous ^{3}H NMR results, leads to the structural and mechanistic proposal shown in Scheme III. We suggest that PBG is incorporated into the apoenzyme *before* folding and that the first (kinetic) encounter of PBG deaminase with substrate involves attachment of PBG (with loss of NH_3) to the α-free pyrrole position of the dipyrromethane to form the ES_1 complex (Scheme III). The process is repeated until the "tetra PBG" (ES_4) adduct **10** is formed. At this juncture site-specific cleavage of the *hexapyrrole* chain (at →) releases the azafulvene bilane (**11**) which *either* becomes the substrate of uro'gen III synthase, or, in the absence of the latter enzyme, is stereospecifically hydrated[3,8] to HMB (**2**) at pH 12, or is cyclized chemically to uro'gen I (**4**) at pH $\leq$ 8.

The previously reported ^{3}H NMR spectra[2] of the ES_1 complex are now reinterpreted to accommodate the unexpected finding of a C-C bond between substrate and enzyme, in terms of a broad ^{3}H signal at δ 3.28[2] which could be due to initial (and transitory) attachment of the substrate at the second cysteine residue[10,11] conserved in human and *E. coli* deaminase. Recent independent and complementary work from two other laboratories[9,14] has reached similar conclusions regarding the catalytic site but does not address the question of the covalent linkage to the enzyme or the exact chain length of the oligopyrrolic cofactor. The present study defines both the *number* of PBG units (two) attached to the native enzyme at pH 8 and their head-to-tail relationship (AP-AP) as well as revealing the identity of the nucleophilic group (Cys-SH) which anchors the dipyrromethane (and hence the growing oligopyrrolic chain) to the enzyme. Confirmation of these proposals by X-ray crystallography is in progress.

Acknowledgment. We thank the National Institutes of Health for generous support of this work (Grants GM32596 and DK32034) and the Robert A. Welch Foundation for a Fellowship (to M.D.G.). We also thank Dr. G. Müller (Stuttgart) for a reference specimen of 2-bromo PBG[15] and Dr. B. Bachman (*E. coli* Genetic Stock Center, Yale University) for *E. coli* strain SASX41B.

(13) A 1:1 adduct between enzyme and inhibitor was confirmed by nondenaturing polyacrylamide gel electrophoresis described in a forthcoming paper.[12]

(14) Warren, M. J.; Jordan, P. M. *FEBS Lett.* **1987**, *225*, 87.

(15) The use of 2-bromo PBG as covalent inhibitor of deaminase was first suggested to us by Dr. G. Müller whom we thank for details regarding its preparation.

Translocation of Radical Sites by Intramolecular 1,5-Hydrogen Atom Transfer

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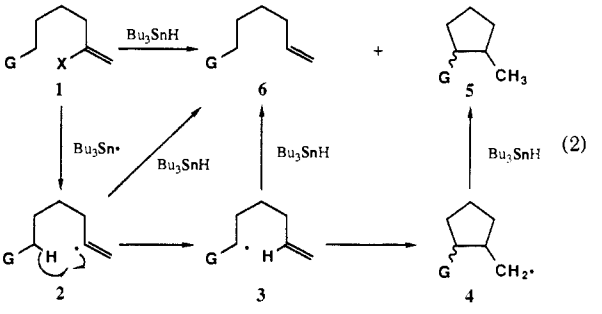
Modern free radical-based synthetic methods often apply trialkyltin hydrides to mediate chain reactions.[2] In the tin hydride method, a wide variety of carbon-heteroatom bonds serve as radical precursors (eq 1). Carbon-hydrogen bonds, by far the

$$R\text{-}X + Bu_3Sn^{\bullet} \rightarrow R^{\bullet} + Bu_3SnX \qquad (1)$$

X = halogen, thiophenyl, selenophenyl, xanthate, etc. X $\neq$ H

simplest "radical precursors", can never be directly used since the transfer of hydrogen atoms to tin radicals is significantly endothermic. We now report a collection of related reactions in which a free radical is produced indirectly from a carbon-hydrogen bond following initial generation of a radical at a remote site. The site of the radical is then translocated by intramolecular 1,5-hydrogen atom transfer[3] prior to the occurrence of a hexenyl radical cyclization.[4,5]

There are two variations on this theme. In one, the radical is first generated on the alkene that is destined to become the acceptor for the subsequent cyclization.[6] In the other, the radical is first generated in a protecting group. Equation 2 provides a



(2)

detailed sequence of propagation steps for the first variation.

(1) Recipient of a Sloan Foundation Fellowship, 1985-1987. Dreyfus Teacher-Scholar, 1985-1989. Eli Lilly Grantee, 1985-1987. Merck Faculty Development Awardee, 1986-1987. NIH Research Career Development Awardee, 1987-1992.

(2) Reviews: Curran, D. P. *Synthesis* **1988**, 417, 489. Neumann, W. P. *Synthesis* **1987**, 665. Giese, B. *Radicals in Organic Synthesis: Formation of Carbon-Carbon Bonds*; Pergamon Press: Oxford, 1986. Ramaiah, M. *Tetrahedron* **1987**, *43*, 3541.

(3) Hydrogen atom transfer is a fundamental reaction of organic free radicals which is a key step in transformations such as the Kharasch, Barton, and Hofmann-Löffler-Freytag reactions. Remote functionalization by intramolecular H-atom transfer has been extensively studied by Breslow. For a leading reference, see: Breslow, R.; Heyer, D. *J. Am. Chem. Soc.* **1982**, *104*, 2045.

(4) (a) An example of 1,5-H atom transfer prior to an addition reaction has been reported by Giese. Giese, B.; Dupuis, J.; Hasskerl, T.; Meixner, J. *Tetrahedron Lett.* **1983**, *24*, 703. (b) For a Kharasch reaction where cyclization follows H-atom transfer, see: Heiba, E. I.; Dessau, R. M. *J. Am. Chem. Soc.* **1967**, *89*, 3772.

(5) For some interesting examples where 1,5-hydrogen atom transfer has intervened in reactions conducted by the tin hydride method, see: Beckwith, A. L. J.; O'Shea, D. M.; Gerba, S.; Westwood, S. W. *J. Chem. Soc., Chem. Commun.* **1987**, 666. Choi, J.-K.; Hart, D. J. *Tetrahedron* **1985**, *41*, 3959. Bennett, S. M.; Clive, D. L. J. *J. Chem. Soc., Chem. Commun.* **1986**, 878. Chenera, B.; Chuang, C.-P.; Hart, D. J.; Hsu, L.-Y. *J. Org. Chem.* **1985**, *50*, 5409.

(6) Very recently, an example of this approach has appeared in which an allylic hydrogen is transferred. Lathbury, D. C.; Parsons, P. J.; Pinto, I. *J. Chem. Soc., Chem. Commun.* **1988**, 81.

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