from **4** + **5** (*Z*-series), this selective desilylation is accomplished upon exposure of the system to the aldol reaction conditions ($TiCl_4$–CH_2Cl_2, −85 °C, 30 min). For the product derived from **4** + **6** (*E*-series), a subsequent reaction of the siloxy transfer product with aqueous AcOH–THF achieves the same result. The resultant alcohols are acetylated (Ac_2O; Py; DMAP) to afford acetates **7** and **8** in the indicated yields.

The pathways from compound **7** and **8** to $PGF_{2\alpha}$ were very direct indeed. *Reaction of compound 7 with $Pd(MeCN)_2Cl_2$ led to allylic transposition of the acetate with the formation of the $E_{13,14}$ double bond and installation of the required 15S stereochemistry (see compound 9) in 72% yield.*[16-19] At this stage[20] reduction of the C_{11} ketone with sodium borohydride is stereospecific in the desired sense. Acetylation provided compound **11** in 74% yield (53% overall yield from **7**). Cleavage of the TBS group and lactonization was accomplished through the action of TBAF. Reaction of **12** with DIBAL resulted in formation of the lactol with deacylation to give compound **13** in 72% overall yield from **11**. Reaction of **13** with phosphorane **14** under the usual conditions gave, in 53% yield,[21] $PGF_{2\alpha}$ (**1**) whose infrared and NMR spectra as well as optical rotation and chromatographic properties were identical with those of an authentic sample.[22]

The same type of allylic transposition occurred even more rapidly[23] with the *E* isomer **8**. The rearrangement is unidirectional,[24] and the C_{13}–C_{14} double bond emerges cleanly trans. The stereochemistry at carbon 15 is of course *R*. Again, reduction of the C_{11} ketone with sodium borohydride is stereospecific affording compound **16** which was protected as its tetrahydropyranyl ether **17** (69% overall yield from **8**). Desilylation as above is accompanied by lactonization, and compound **18** is obtained in 84% yield. This substance is clearly a very valuable intermediate for preparing prostaglandins of the 15*R* series. We have used it to cross over to the natural series by inverting the stereochemistry at carbon 15. This was accomplished as follows. Deacylation of the **18** epiacetate afforded (98%) the 15*R* alcohol **19**, which was inverted in a standard Mitsunobu reaction[25] to the 15*S* benzoate **20** in 73% yield. Treatment of this compound with diisobutyl aluminum hydride resulted in reduction of the lactone and debenzoylation, affording compound **21**. Reaction of this compound with Wittig reagent, **14**, followed by cleavage of the THP protecting group (aqueous acetic acid), again afforded $PGF_{2\alpha}$ (**1**), this time in 46% yield from **20**.[21]

These routes offer major advantages in terms of conciseness, availability of all the building blocks, and simplicity of the reactions. Not the least advantage is the ready access to the required (*S*)-enone **2**. The diacetate **22**, available in multigram scale from cyclopentadiene,[26] is converted through the action of acetylcholinesterase[27] in 89% yield and, essentially total optical purity, to the monoacetate **23**. Protection of the alcohol as its TBS derivative through the action TBSCl and imidazole in DMF affords **24** which on simple hydrolysis (sodium methoxide) leads to **25**. The latter is oxidized with manganese dioxide to the optically pure (*S*)-enantiomer **2**. The overall conversion of **22** to **2** is achieved in 70% yield. *This chemistry provides an eminently practical route for the total synthesis of prostaglandins and congeners thereof.*[28] Experiments directed toward taking advantage of this new capability will be described in due course.

Acknowledgment. This research was supported by PHS Grant HL25848. An MEC/Fulbright Fellowship to M.P.C. is gratefully acknowledged. We thank Professor K. C. Nicolaou of the University of Pennsylvania for providing valuable correlation samples for our synthetic intermediates on the way to $PGF_{2\alpha}$. NMR spectra were obtained through the auspices of the Northeast Regional NSF/NMR facility of Yale University, which was supported by NSF Chemistry Division Grant CHE 7916210.

Supplementary Material Available: Spectral data (^1H NMR, IR, and MS) for all compounds described herein (5 pages). Ordering information is given on any current masthead page.

(15) A major complication arises if the TBS group is cleaved at this stage. With the C_{11} ketone still in place, β-elimination occurs to give the enone.

(16) For a most interesting precedent for this type of stereochemical adjustment in the [2,3] series, see: Miller, J. G.; Kurz, W.; Untch, K. G.; Stork, G. *J. Am. Chem. Soc.* **1974**, *96*, 6774.

(17) For the first application of the Pd(II)-mediated allylic acetate transposition to a modified prostaglandin intermediate, see: Grieco, P. A.; Takigawa, T.; Bongers, S. L.; Tanaka, H. *J. Am. Chem. Soc.* **1980**, *102*, 7588.

(18) Pd(II)-catalyzed allylic acetate transposition was first described by: Meyer, K. DOS 2513198 (1975); *Chem. Abstr.* **1976**, *84*, 89629s.

(19) For a full review of Pd(II)-catalyzed [3,3] sigmatropic rearrangements, see: Overman, L. E. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 579.

(20) Attempts to carry out the reduction of the C_{11} ketone before the allylic transposition results, at best, in modest stereoselectivity possibly due to competing directivities from the 13-oxygen function.

(21) Corey, E. J.; Weinshenker, N. M.; Schaaf, T. K.; Huber, W. *J. Am. Chem. Soc.* **1969**, *91*, 5675.

(22) The synthetic material had an optical rotation $[\alpha]_D$ +23.0° (*c* 1.01, THF) which is essentially the same as authentic $PGF_{2\alpha}$ ($[\alpha]_D$ +23.5°, *c* 1.0, THF).

(23) Not surprisingly the rate of transposition of the *Z* isomer is slower than that of the *E* isomer. For compound **7** conditions involved catalytic Pd(II) in THF at room temperature for 4 h. For compound **8**, the equivalent transformation was complete after 2 h.

(24) Compound **9** and **15** failed to show indications of undergoing back rearrangement.

(25) A solution of **19** in THF was treated with triphenylphosphine (2 equiv), benzoic acid (2 equiv), and diethylazodicarboxylate (2 equiv) at room temperature. After 5 min the reaction was quenched with a solution of saturated $NaHCO_3$. See: (a) Mitsunobu, O.; Yamada, M. *Bull. Chem. Soc. Jpn.* **1967**, *40*, 2380. (b) Mitsunobu, O. *Synthesis* **1981**, 1.

(26) Deardorff, D. R.; Myles, D. C.; MacFerrin, K. D. *Tetrahedron Lett.* **1985**, *26*, 5615.

(27) Deardorff, D. R.; Matthews, A. J.; McMeekin, D. S.; Craney, C. L. *Tetrahedron Lett.* **1986**, *27*, 1255.

(28) This assessment is not meant by way of a comparison with the efficiency of previous excellent efforts (ref 2–6). A detailed analysis of the implications of our work for commercial production relative to the existing methods has not been undertaken. The attraction of our route stems from the easy availability of all of its components and the ease of their assembly. In that vein we note that, as of this writing, the (*S*)-enone **2** is more readily obtained than is either the (*R*)-enone or, indeed, the racemate.

Hemoglobin Quaternary Structure Change Monitored Directly by Transient UV Resonance Raman Spectroscopy

Chang Su, Young D. Park,† Gang-Yu Liu, and Thomas G. Spiro*

Department of Chemistry, Princeton University Princeton, New Jersey 08544-1009

Received November 10, 1988

We report pulse-probe transient ultraviolet resonance Raman (UVRR) spectra of photolyzed carbonmonoxy hemoglobin (HbCO) which provide direct evidence that the R → T quaternary structure change occurs in ~20 μs. This process coincides with the transition from fast to slow recombining Hb[1] and with the final optical transient of photolyzed HbCO molecules.[2] The UVRR spectra are interpreted as responding to H bonding changes of aromatic groups at the $\alpha_1\beta_2$ interface of the Hb tetramer. The transient signals also indicate the formation of a structural intermediate associated with the R → T transition.

Figure 1 shows a fragment of the UVRR spectra of oxy- and deoxyHb excited at 229 nm with an H_2-Raman-shifted pulsed Nd:YAG laser.[3] The spectra contain bands which are associated with ring modes of tyrosine (Tyr), $\nu_{8a/8b}$ = 1617/1601 cm^{-1}, and tryptophan (Trp), W_3 = 1555 cm^{-1}.[4,5] These spectra have sufficient signal/noise to expose the slight differences between oxy- and deoxyHb. The difference spectrum reveals a downshift (1.5 cm^{-1}) of the Trp band and upshifts (2 cm^{-1}) in the Tyr bands.

† Present address: Harrison Spectroscopy Laboratory, MIT, Cambridge, Massachusetts 02139.

(1) Sawicki, C. A.; Gibson, Q. H. *J. Biol. Chem.* **1976**, *251*, 1533.

(2) Hofrichter, J.; Sommer, J. H.; Henry, E. R.; Eaton, W. A. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 2235.

(3) Fodor, S. P. A.; Rava, R. P.; Copeland, R. a.; Spiro, T. G. *J. Raman Spectrosc.* **1986**, *17*, 471.

(4) Rava, R. P.; Spiro, T. G. *J. Phys. Chem.* **1985**, *89*, 1856.

(5) Takeuchi, H.; Harada, I. *Spectrochim. Acta* **1986**, *42A*, 1069.

0002-7863/89/1511-3457$01.50/0

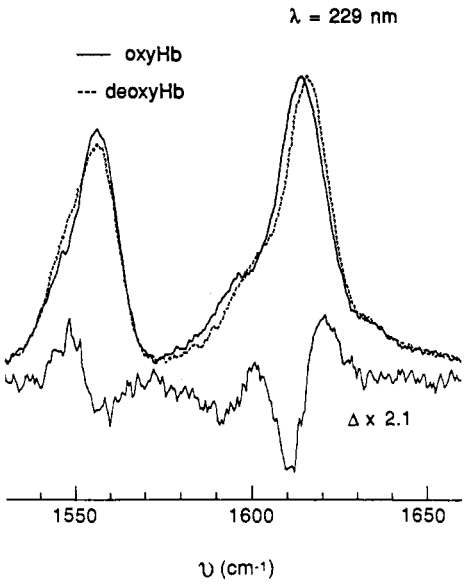


Figure 1. UVRR spectra of oxy- and deoxyHb (1 mM in heme), recirculated in a flowing free stream, obtained with 229-nm excitation from H_2-Raman-shifted pulsed Nd:YAG laser. The experimental arrangement is described in ref 3. The scattered light was dispersed with a 1.25 m single monochrometer (Spex 1269) equipped with a 2400 groove/nm grating operating in second order. The monochromator was scanned in 0.5-cm^{-1} increments with 1-s accumulation time; 180 scans were co-added to produce the spectra. The computer-subtracted deoxy-oxyHb difference spectrum is shown below, with a factor of 2.1 amplification. (The difference signals become detectable at 20 scans.) OxyHb was prepared from fresh human red blood cells using the standard method described in ref 15. DeoxyHb was prepared by flushing high purity N_2 over a stirred oxyHb sample. For the deoxyHb spectrum, the flowing cell was enclosed and purged with N_2.

Figure 2 shows transient UVRR difference spectra as a function of time after photolysis of HbCO. These spectra were generated with 229-nm laser pulses which were electronically delayed with respect to 532-nm photolysis pulses from a second Nd;YAG laser. The spectra were converted to difference spectra by subtracting the HbCO spectrum obtained with the photolyzing laser turned off. This latter spectrum was identical with that of oxyHb. The transient difference spectrum is featureless at zero delay and for several delays (not shown) in the nanosecond region. Difference bands begin to appear at ~5 μs, and at a 20-μs delay one sees a difference spectrum which closely resembles the deoxyHbCO static difference spectrum. The amplitudes are diminished in the 20-μs transient difference spectrum because by this time a significant fraction (~30%) of the Hb molecules have recombined either geminately or with CO from solution, according to the available kinetic data.[1,2] For the Tyr bands, there is a smooth growth of signal from 10 to 20 μs, but the Trp band shows an altered difference signal at 5 to 10 μs from that seen at 20 μs. Instead of a downshift an amplitude loss is seen on the high-frequency side of the W_3 band (arrow in Figure 2), indicating the formation and decay of a ~10 μs intermediate species.

In previous reports from this laboratory,[8,9] other markers of the R–T transition were found, including an augmented intensity ratio of the 830/850 cm^{-1} Tyr Fermi doublet[7] with 200-nm excitation, and disappearance of the Trp 880-cm^{-1} band with 218-cm^{-1} excitation. In repeated trials, however, we have been unable to reproduce these early results. We have determined that the 880-cm^{-1} band is present in 218-nm excited spectra of deoxy- as well as oxyHb and that the 830/850-cm^{-1} feature in 200-nm

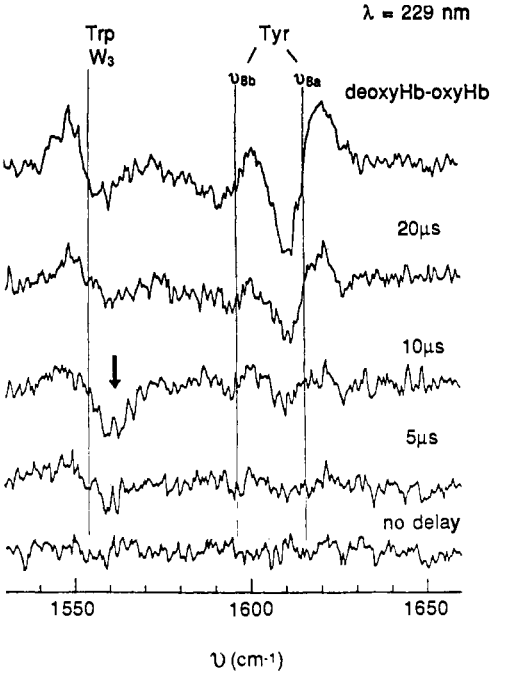


Figure 2. Excited UVRR difference spectra (229 nm) obtained by subtracting the HbCO spectrum from the spectrum of the HbCO photoproduct obtained at the indicated delays following a 532-nm photolysis pulse from a second Nd:YAG laser. Pulse and probe lasers produced 7 ns pulses at 10 Hz and were focused colinearly onto the sample stream, the 532 nm spot being substantially larger than the 229-nm spot. The delay interval was controlled electronically. HbCO was prepared by circulating CO gas over a stirred oxyHb sample. and spectra were obtained with a 10% CO atmosphere in a closed flowing cell. 100% photolysis was ensured by examining the heme RR spectrum generated by the 532-nm pulses under the same experimental conditions. The infinite time spectrum is shown at the top as the static deoxyHb–oxyHb difference spectrum. The vertical lines mark the positions of the Raman bands due to the ν_{8a} and ν_{8b} modes of tyrosine and the W_3 mode of tryptophan. The arrow points to the tryptophan difference band, which reveals an intermediate structure at 10 μs. Signal accumulation as in Figure 1.

excited spectra has essentially the same shape in both forms. Figure 3 compares 200-nm excited Tyr 830/850-cm^{-1} bands of oxy- and deoxyHb. (The doublet structure is obscured, presumably because of overlapping contributions from the six inequivalent Tyr residues in Hb.) The difference spectrum is featureless, even though the signal/noise level is appreciably higher than in our previous reports,[8,9] thanks to advances in instrumental technique and to longer accumulation times (100 scans vs 20–40 in the spectra of ref 8 and 9). We therefore conclude that the previous results were artifacts associated with insufficiently controlled conditions in early versions of the UVRR experiment. The cause of these artifacts is uncertain. One possibility is that protein precipitation, difficult to detect in its early stages in the dark Hb solutions, may have induced fluctuations in the scattering intensity which were recorded as peaks. We have since taken pains to cool the circulatory sample cell and minimize the agitation of the solution, and we check the sample frequently for integrity, changing it for a fresh sample as necessary during the successive scans required to build up the spectrum.

Kinetic processes in the evolution of the HbCO photoproduct have previously been characterized by absorption spectroscopy[2,10,11] and visible excitation RR spectroscopy of the heme group.[12]

(6) Rousseau, D. L. *J. Raman Spectrosc.* **1981**, *10*, 94.
(7) Siamwiza, M. N.; Lord, R. C.; Chen, M. C.; Takamatsu, T.; Harada, I.; Matsuura, H.; Shimanouchi, T. *Biochemistry* **1975**, *14*, 4870.
(8) Copeland, R. A.; Dasgupta, S.; Spiro, T. G. *J. Am. Chem. Soc.* **1985**, *107*, 3370.
(9) Dasgupta, S.; Copeland, R. A.; Spiro, T. G. *J. Biol. Chem.* **1986**, *261*, 10960.
(10) (a) Noe, L. J.; Eisert, W. G.; Rentzepis, P. M. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 573. (b) Greene, B. I.; Hochstrasser, R. M.; Weisman, R. B.; Eaton, W. A. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 5255.
(11) Martin, J. L.; Migus, A.; Poyart, C.; Lecarpentier, Y.; Astier, R.; Antonetti, A. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 173.
(12) (a) Dasgupta, S.; Spiro, T. G. *Biochemistry* **1986**, *25*, 5941. (b) Findsen, E. W.; Friedman, J. M.; Ondrias, M. R.; Simon, S. *Science* **1985**, *229*, 661. (d) Terner, J.; Spiro, T. G.; Nagumo, M.; Nicol, M. F.; El-Sayed, M. A. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 1313.

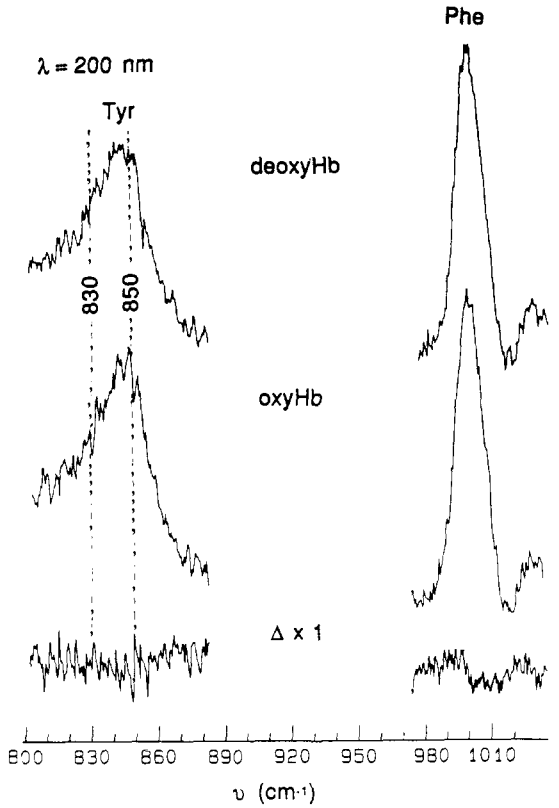


Figure 3. UVRR spectra of oxy- and deoxyHb as in Figure 1 but obtained with 200-nm excitation; a 3600 groove/mm grating was used in first order, and 100 scans were coadded. (Spectra reported in ref 8 and 9 were obtained with 20–40 scans.) The sharp 1000-cm^{-1} band is a Phe breathing mode, while the broad band at ~840 cm^{-1} contains 830/850 cm^{-1} Fermi doublet contributions from the six inequivalent Tyr residues. The difference spectrum is featureless at this signal/noise level.

Photoexcitation of HbCO molecules is followed by very rapid electronic relaxation, with a time constant of 0.35 ps.[8] Subsequent relaxaton to the final deoxyHb spectrum has been determined[2] to occur in at least three kinetically distinguishable steps, with time constants of ~100 ns, ~1 μs, and ~20 μs. This last step is coincident with the transition from fast to slow recombining forms of hemoglobin,[1] which has been assumed to involve the quaternary rearrangement of the Hb tetramer between the R and T states.[13] As shown in Figure 2, the rise time of the deoxyHbCO UVRR difference spectrum likewise coincides with this transition, and we therefore attribute the difference bands to changes in the Tyr and Trp environments which are associated with the quaternary change. Indeed, these spectra represent the first direct probe of this structural rearrangement, since the signals arise from aromatic residues rather than the heme group itself.

It is likely that the R–T difference UVRR signals are associated with aromatic residues at the critical $\alpha_1\beta_2$ subunit interface where most of the relative motion occurs in the quaternary switch.[14] In particular, the Trp β37 indole NH proton is H-bonded to the backbone carbonyl group of Asn-102 in the R state but to the carboxylate side chain of Asp α94 (a stronger interaction) in the T state.[4] The Tyr α42 OH group accepts an H-bond from the peptide NH of Asp 94 in both the R and T states, but in the T state it also forms a donor interaction with the side chain of Asp 99. We tentatively attribute the W_3 and ν_{8a}/ν_{8b} difference signals to these H-bond changes. The intermediate W_3 signal seen in the 10 μs transient spectrum suggests that the R–T transition occurs in a step-wise fashion, the intermediate step perhaps affecting the environment of Trp β37 but not Tyr α42.

These preliminary results are encouraging with respect to the development of UVRR spectroscopy as a probe of protein dynamics. By monitoring the environment of residues in various parts of the protein, one can probe for specific conformational changes along a reaction pathway, following rapid initiation of the reaction.

Acknowledgment. This work was supported by NIH Grant GM 25158.

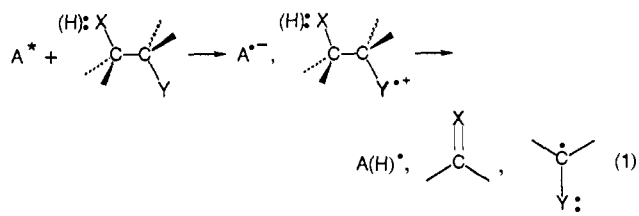
Photofragmentation via Single-Electron Transfer: Selective Labilization of C–C Bonds in Amino Alcohols with Several Bonds between Heteroatom Substituents

Xiaohong Ci and David G. Whitten*

Department of Chemistry, University of Rochester Rochester, New York 14627

Received January 6, 1989

A number of recent studies have shown that oxidative photofragmentation of α,β-diheteroatom-substituted organics can occur as a consequence of single-electron transfer as outlined in eq 1, where X = N or O and Y = N, O, or S.[1-13] The photoredox process, although involving radical ion intermediates, shows close



(1)

analogies to the "2-electron" Grob fragmentation.[14] Mechanistic studies with a variety of electron acceptors and aminoalcohols indicate that both acceptor anion–radical basicity and a drastically reduced C–C bond energy in the cation–radical contribute to the low (2.5–5 kcal/mol) activation energy[5,7] for fragmentation in the ion–radical pair. Since reaction of α,β-diheteroatom-substituted compounds is observed to proceed very cleanly via relatively low energy excitation, an interesting question is whether a correspondingly clean C–C bond fragmentation can occur in molecules in which two heteroatoms are separated by greater distances. Here we report results that indicate selective cleavage can occur for vinylogous aminoalcohols and related compounds via pathways that involve both net reduction and catalytic roles for the photoexcited acceptor.

Amines **1–3** were prepared by catalytic hydrogenation or chemical reduction of 4,4′-dimethylaminobenzoin. The reduction

(1) Davidson, R. S.; Orton, S. P. *J. Chem. Soc., Chem. Commun.* **1974**, 209 and references therein.
(2) Davidson, R. S.; Goodwin, G. D.; Pratt, J. E. *Tetrahedron* **1983**, *39*, 2373.
(3) Lee, L. Y. C.; Ci, X.; Giannotti, C.; Whitten, D. G. *J. Am. Chem. Soc.* **1986**, *108*, 175.
(4) Ci, X.; Lee, L. Y. C.; Whitten, D. G. *J. Am. Chem. Soc.* **1987**, *109*, 2536.
(5) Ci, X.; Whitten, D. G. *J. Am. Chem. Soc.* **1987**, *109*, 7215.
(6) Ci, X.; Whitten, D. G. In *Photoinduced Electron Transfer*, Part C; Fox, M. A., Chanon, M., Eds.; Elsevier: Amsterdam, 1988; p 553.
(7) Kellett, M. A.; Whitten, D. G. *J. Am. Chem. Soc.* **1989**, *111*, 2314.
(8) Haugen, C. H.; Whitten, D. G. Submitted for publication.
(9) Torii, S.; Inokuchi, T.; Takagishi, S.; Sato, E.; Tsujiyama, H. *Chem. Lett.* **1987**, 1469.
(10) Shono, T.; Hamaguchi, H.; Matsumura, Y.; Yoshida, K. *Tetrahedron Lett.* **1977**, *41*, 3625.
(11) Albini, A.; Mella, M. *Tetrahedron* **1986**, *42*, 6219.
(12) Davis, H. F.; Das, P. K.; Reichel, L. W.; Griffin, G. W. *J. Am. Chem. Soc.* **1884**, *106*, 6968.
(13) Reichel, L. W.; Griffin, G. W.; Muller, A. T.; Das, P. K.; Ege, S. N. *Can. J. Chem.* **1984**, *62*, 424.
(14) Grob, G. A. *Angew Chem., Int. Ed. Engl.* **1969**, *8*, 535 and references therein.
(13) Perutz, M. F. *Br. Med. Bull.* **1976**, *32*, 195.
(14) Baldwin, J.; Chothia, C. *J. Mol. Biol.* **1979**, *129*, 175.
(15) Antonini, E.; Brunori, M. *Hemoglobin and Myoglobin in Their Reactions with Ligands*; North-Holland Publishing Company: Amsterdam, The Netherlands, 1971; p 2.

0002-7863/89/1511-3459$01.50/0