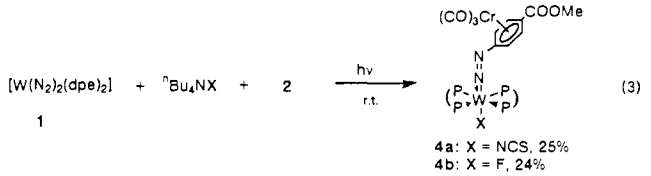
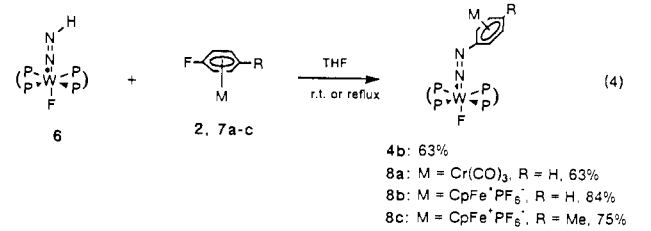
Complex **4** was also obtained by irradiation (tungsten-filament lamp) of a THF solution of **1** and **2** in the presence of $^{n}Bu_4NX$ (X = SCN or F) (eq 3). No more than a trace amount of **4** was



obtained in the absence of $^{n}Bu_4NX$ or in the dark. Although the formation of $[^{n}Bu_4N][WF(N_2)(dpe)_2]$ (**3b**) could not be confirmed, **3a** can be prepared by similar irradiation of **1** and $^{n}Bu_4NSCN$ in THF.[6] Therefore in situ generated anionic dinitrogen complexes **3a** and **3b** are considered to be the intermediates which react with **2**. Since reaction 1 does not need irradiation, light probably liberates one of the dinitrogen ligands in **1**.

Alkylation of coordinated dinitrogen in complexes **1** and **3a** with alkyl halides has been considered to proceed by the attack of alkyl radical species generated from the halides on the dinitrogen ligands.[4,6,11] The facts which support the radical mechanism include the formation of organodiazenido or organohydrazido complexes having the *N*-tetrahydrofuranyl[11a] or *N*-benzyl[4] group in the reactions using THF or toluene as solvent, respectively. Such products are rationalized by considering the incorporation of THF• and benzyl radicals generated by radical transfer between the solvent molecule and the initially formed alkyl radical. However, in reactions 1 and 3, no product incorporating the THF• radical was isolated. Furthermore, in a reaction of **3a** with **2** in THF/toluene (2:1), we observed neither a decrease in the yield of **4a** (62%) nor formation of *N*-benzyl complexes due to the participation of benzyl radicals. These results strongly suggest that the observed arylation of the dinitrogen ligand proceeds not by a radical mechanism but by direct nucleophilic substitution at the coordinated haloarene. This is also in accord with the generally accepted reactivity of haloarene complexes.[5]

Finally, it should be pointed out that arylation of a diazenido or hydrazido complex derived from a dinitrogen complex can provide an indirect method of arylating dinitrogen. In spite of the well-documented nucleophilicity of diazenido and hydrazido complexes,[2a] such reactions have been investigated to a very limited extent; the only examples are reactions between $[WX(NNH_2)(dpe)_2]^+$ (X = Br, F, or CF_3COO) and 2,4-$(NO_2)_2C_6H_3F$.[12] We have now employed haloarene complexes **2** and **7a-c** in indirect dinitrogen arylation, which resulted in smooth reaction with a diazenido complex[13] $[WF(NNH)(dpe)_2]$ (**6**) at room temperature (**2**, **7b**, **7c**) or under THF reflux (**7a**) to give the corresponding μ-aryldiazenido complexes **4b** and **8a-c** in good yields (eq 4). Spectroscopic and X-ray diffraction study[14]



of these products revealed essentially the same type of μ-aryldiazenido structure as that found for **4a**.

As a conclusion, haloarene complexes have proved to be effective reagents for the direct and indirect arylation of coordinated dinitrogen. Novel reactivities of the unique μ-aryldiazenido structure are now under investigation.

Acknowledgment. We thank Professor Yasuhiro Yamamoto and Dr. Tomoaki Tanase of Toho University for the X-ray diffraction analysis of **4a**.

Supplementary Material Available: Experimental details for the preparation of **4a** and **4b** and tables of positional parameters, anisotropic thermal coefficients, and bond lengths and angles for **4a** (13 pages); table of observed and calculated structure factors for **4a** (52 pages). Ordering information is given on any current masthead page.

(10) (a) Miyagi, H.; Ishii, Y.; Aoshima, T.; Mizobe, Y.; Hidai, M. *Chem. Lett.* **1991**, 611. (b) Head, R. A.; Hitchcock, P. B. *J. Chem. Soc., Dalton Trans.* **1980**, 1150. (c) Hidai, M.; Mizobe, Y.; Sato, M.; Kodama, T.; Uchida, Y. *J. Am. Chem. Soc.* **1978**, *100*, 5740.

(11) (a) Chatt, J.; Head, R. A.; Leigh, G. J.; Pickett, C. J. *J. Chem. Soc., Dalton Trans.* **1978**, 1638. (b) Bossard, G. E.; Busby, D. C.; Chang, M.; George, T. A. *J. Am. Chem. Soc.* **1980**, *102*, 1001.

(12) Colquhoun, H. M. *J. Chem. Res., Synop.* **1979**, 325; *J. Chem. Res., Miniprint* **1979**, 3677.

(13) Chatt, J.; Pearman, A. J.; Richards, L. *J. Chem. Soc., Dalton Trans.* **1976**, 1520.

(14) The molecular structures of **4b** and **8c** were determined by X-ray diffraction analyses. The details will be reported elsewhere.

A Stable Solid That Generates Hydroxyl Radical upon Dissolution in Aqueous Solutions: Reaction with Proteins and Nucleic Acid

P. A. King, V. E. Anderson,* J. O. Edwards, G. Gustafson, R. C. Plumb,* and J. W. Suggs

Department of Chemistry
Brown University
Providence, Rhode Island 02912

Received January 13, 1992
Revised Manuscript Received April 20, 1992

Radiolysis, photolysis of peroxide species, and Fenton chemistry have all been used to generate hydroxyl radical (HO•) in aqueous solution. Access to radiolysis is limited by availability of a radioactive source (^{60}Co or ^{137}Cs) and, except in N_2O-saturated solution, generates a mixture of reactive species.[1] Photochemical HO• generation methods involving hydrogen peroxide or alkyl hydroperoxides require irradiation at short wavelengths (254 nm) where the compounds targeted for reaction with HO• often absorb.[2] Phthalimide hydroperoxides which generate HO• from photoirradiation at longer wavelengths suffer from low photoefficiencies and require long irradiation times.[3] The iron-EDTA systems,[4] while sufficient for many purposes, become problematic in studies where addition of iron or hydrogen peroxide cannot be tolerated. The footprinting of DNA cleavage reagents such as bleomycin, which themselves utilize iron to effect cleavage, exemplifies this interference. The possible generation of oxidative species other than free HO•[5] and quantitation of the amount of HO• produced further complicate the use of these reagents. In this communication we describe the preparation and application of a solid which obviates the need for either sample irradiation or transition metal ion introduction while quickly generating an easily quantifiable amount of HO•. We demonstrate the convenient use of this solid to nonspecifically oxidize and cleave both DNA and protein.

A solid solution of potassium peroxonitrite (ONOOK) in potassium nitrate (KNO_3) can be generated by photolysis. Reagent grade KNO_3, a white crystalline solid, which has been crushed and sieved to between 250 and 420 μm is irradiated in a rotating 3 × 28 cm quartz tube mounted parallel to three UV lamps. The solid KNO_3 is irradiated at 254 nm for 2 h while $N_2(g)$ flows through the tube to maintain the temperature at about 42 °C.

(1) Bielski, B. H.; Gebicki, J. M. *Free Radicals Biol.* **1977**, *3*, 18-21.
(2) Ogata, Y.; Tomizawa, K.; Furuta, K. *The Chemistry of Peroxides*; Patai, S., Ed.; Wiley: New York, 1983.
(3) Saito, I.; Takayama, M.; Matsuura, T.; Matsugo, S.; Dawanishi, S. *J. Am. Chem. Soc.* **1990**, *112*, 883-884.
(4) (a) Hertzberg, R. P.; Dervan, P. B. *J. Am. Chem. Soc.* **1982**, *104*, 313-315. (b) Tullius, T. D.; Dombroski, B. A. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 5469-5473.
(5) (a) Rush, J. D.; Koppenol, W. H. *J. Biol. Chem.* **1986**, *261*, 6730-6733. (b) Imlay, J. A.; Chin, S. M.; Linn, S. *Science* **1988**, *240*, 640-642.

0002-7863/92/1514-5430$03.00/0

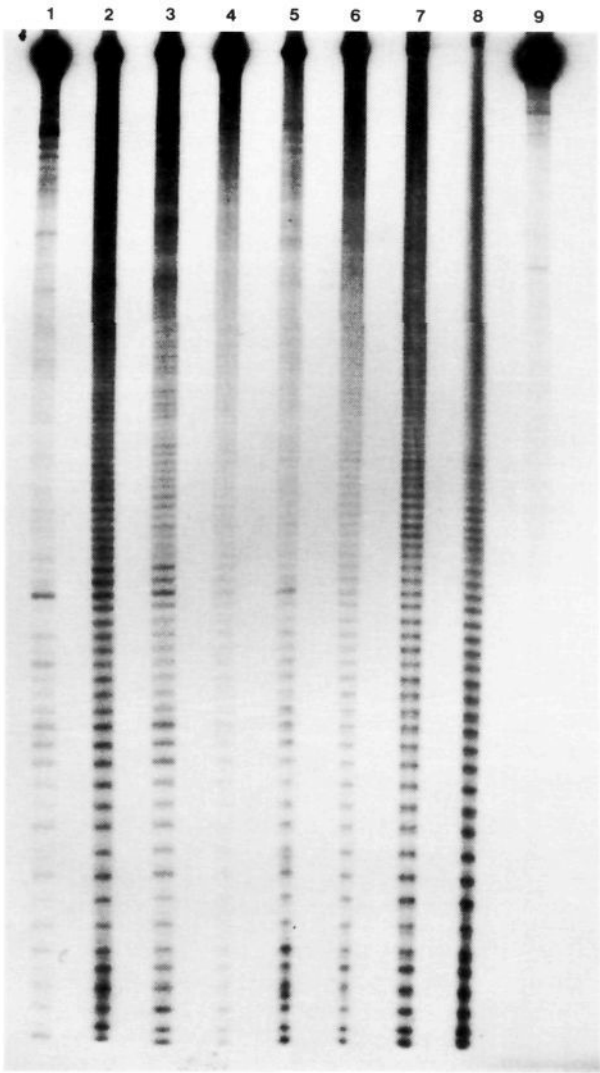


Figure 1. DNA cleavage. The 227-base-pair DNA restriction fragments labeled at the 3′-end with ^{32}P (4.3×10^4 cpm/lane) were incubated with the following. Lane 1: 10 mM Tris, pH 7.0. Lane 2: 10 mM Tris, pH 7.0; 10 μM Fe(II), 20 μM EDTA, 1 mM ascorbate, 0.06% H_2O_2. Lane 3: 10 mM Tris, pH 7.0; 3.3 μM Fe(II), 6.7 μM EDTA, 0.33 mM ascorbate, 0.03% H_2O_2. Lane 4: 10 mM Tris, pH 7.0; 10 mg of ONOOK/KNO_3 (110 nmol of HO•). Lane 5: 10 mM Tris, pH 7.0; 50 mg of ONOOK/KNO_3 (550 nmol of HO•). Lane 6: 10 mM Tris, pH 7.0; 100 mg of ONOOK/KNO_3 (1100 nmol of HO•). Lane 7: 10 mM NaH_2PO_4, pH 7.0; 10 mg of ONOOK/KNO_3 (110 nmol of HO•). Lane 8: 10 mM NaH_2PO_4, pH 7.0; 50 mg of ONOOK/KNO_3 (550 nmol of HO•). Lane 9: end-labeled restriction fragment only. Except for lane 9, an addition of 50 μL of 50 mM β-mercaptoethanol was made after a 2-min reaction time to stop the cleavage reaction. Such an addition was also made to lane 1 despite no addition of cleavage reagent.

Such irradiation produces a yellow solid solution containing 30 μmol of ONOOK/g of solid.

Generation of HO• is accomplished by direct addition of the solid to a solution buffered at pH 7.0. This results in protonation of a significant fraction of the peroxonitrite, as peroxonitrous acid has a pK_a of 6.8.[6] The peroxonitrous acid formed undergoes homolytic fission to form HO• and NO_2•.[7,8]

$$ONOO^- + H^+ \rightarrow ONOOH \rightarrow NO_2^{\bullet} + HO^{\bullet}$$

The peroxonitrite has a half-life of 1.9 s at pH 7.4.[9] Recombination of HO• and NO_2• to form nitric acid in the solvent cage reduces the amount of free HO• that may be trapped in solution by two-thirds.[8]

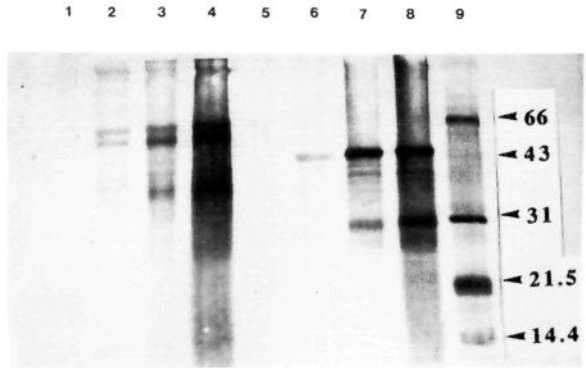


Figure 2. Radiolysis of BSA and enolase. Samples were exposed for either 15 or 30 min to a ^{137}Cs source at a dose rate of 1150 rad/min in O_2-saturated 10 mM NaH_2PO_4, pH 7.0. This corresponds to generation of 50 or 100 nmol of HO•, respectively. BSA was irradiated at a concentration of 0.30 mg/mL and enolase at a concentration of 0.23 mg/mL. After biotin hydrazide (BHZ) derivatization, 0.5 μg of protein/lane was loaded, separated, and then Western blotted. The strepavidin–AP/BCIP/NBT visualized blots are shown. Lane 1: unirradiated BSA control (biotinylation omitted). Lane 2: unirradiated, BHZ-derivatized BSA control. Lane 3: 50 nmol of HO•/0.15 mg of BSA. Lane 4: 100 nmol of HO•/0.15 mg of BSA. Lane 5: unirradiated enolase control (biotinylation omitted). Lane 6: unirradiated, BHZ-derivatized enolase control. Lane 7: 50 nmol of HO•/0.10 mg of enolase. Lane 8: 100 nmol of HO•/0.10 mg of enolase. Lane 9: biotinylated molecular weight standards.

The ability of this reagent to cleave duplex DNA has been tested and its reactivity compared with an untethered iron(II)–EDTA reagent also believed to produce HO•.[10] A singly 3′-^{32}P end-labeled 227-bp *Hin*dIII–*Pvu*II restriction fragment from pUC18 was subjected to increasing amounts of the solid reagent added directly to the air-saturated DNA solution (in either 10 mM Tris-HCl (pH 7.0) or 10 mM sodium phosphate (pH 7.0)). After desalting, the samples were electrophoresed on 10% polyacrylamide sequencing gels containing 50% urea. The gel was then dried onto Whatman 3M filter paper and autoradiographed at −78 °C using an intensifying screen. The resulting autoradiogram (Figure 1) indicates that the peroxonitrite reagent produces nonspecific DNA strand cleavage directly analogous to that induced by the iron(II)–EDTA reagent. The relative amounts of cleavage in lanes 4 and 7 indicate that Tris is effectively inhibiting cleavage by scavenging HO• as would be expected in the presence of any primary alcohol containing molecule.[11]

The solid ONOOK solution was also used to oxidize bovine serum albumin and enolase. The results are compared with those of γ-irradiated solutions of the same proteins. Exposure of O_2-saturated protein solutions to HO• results in both main-chain scission and side-chain oxidation, giving rise to both fragmentation and carbonyl formation.[12] In order to detect both occurrences, protein samples exposed to HO• produced both radiolytically and via addition of the peroxonitrite reagent were derivatized using biotin hydrazide (BHZ) prior to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). Following electrophoresis, the gels were Western blotted, and the blocked poly(vinylidene difluoride) (PVDF) membranes were subsequently probed using a streptavidin–alkaline phosphatase (AP) conjugate and visualized using 5-bromo-4-chloro-3-indolyl phosphate (BCIP)

(6) (a) Radi, R.; Beckman, J. S.; Bush, K. M.; Freeman, B. A. *J. Biol. Chem.* **1991**, *266*, 4244–4250. (b) P. Jones reports a pK_a of 6.75 ± 0.1 (personal communication).
(7) Halfpenny, E.; Robinson, P. L. *J. Chem. Soc. A* **1952**, 928–938.
(8) Mahoney, L. R. *J. Am. Chem. Soc.* **1970**, *92*, 5262–5263.
(9) Beckman, J. S.; Beckman, T. W.; Chen, J.; Marshall, P. A.; Freeman, B. A. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 1620–1624.
(10) Tullius, T. D.; Dombroski, B. A. *Science* **1985**, *230*, 679–681.
(11) Farhataziz; Ross, A. B. *Natl. Stand. Ref. Data Ser. (U.S., Natl. Bur. Stand.)* **1977**, *NSRDS-NBS 59*, 47–48.
(12) (a) Garrison, W. M.; Jayko, M. E.; Bennet, W. *Radiat. Res.* **1962**, *16*, 483–502. (b) Wolff, S. P.; Dean, R. T. *Biochem. J.* **1986**, *234*, 399–403. (c) Davies, K. J. A.; Delsignore, M. E. *J. Biol. Chem.* **1987**, *262*, 9908–9913. (d) Garrison, W. M. *Chem. Rev.* **1987**, *87*, 381–398.

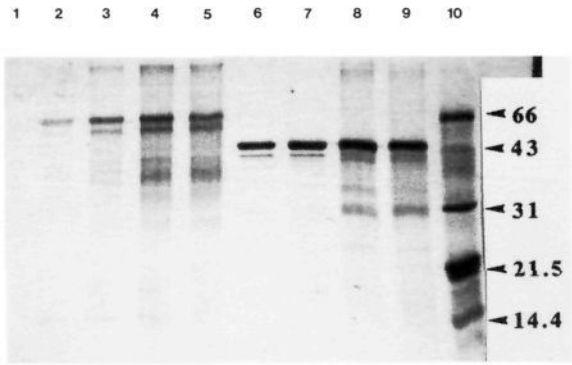


Figure 3. Peroxonitrite-generated hydroxyl radical. To samples of BSA and enolase in O_2-saturated 10 mM NaH_2PO_4, pH 7.0, was added directly either 2, 20, or 40 mg of ONOOK/KNO_3 generating 22, 220, and 440 nmol of HO•, respectively. BSA was present at a concentration of 0.30 mg/mL and enolase at a concentration of 0.23 mg/mL. After BHZ derivatization, 0.5 μg of protein/lane was loaded, separated, and then Western blotted. The strepavidin-AP/BCIP/NBT visualized blots are shown. Lane 1: untreated BSA control (biotinylation omitted, no bands apparent). Lane 2: untreated, BHZ-derivatized BSA control. Lane 3: 22 nmol of HO•/0.15 mg of BSA. Lane 4: 220 nmol of HO•/0.15 mg of BSA. Lane 5: 440 nmol of HO•/0.15 mg of BSA. Lane 6: untreated, BHZ-derivatized enolase control. Lane 7: 22 nmol of HO•/0.10 mg of enolase. Lane 8: 220 nmol of HO•/0.10 mg of enolase. Lane 9: 440 nmol of HO•/0.10 mg of enolase. Lane 10: biotinylated molecular weight standards.

and *p*-nitro blue tetrazolium chloride (NBT). Comparison of the Western blots for the two proteins using samples exposed to the two different HO•-generating methods (Figures 2 and 3) indicates that addition of the peroxonitrite reagent results in protein damage directly analogous to that produced radiolytically.

The streptavidin-AP-probed Western blots show that the majority of the newly formed BHZ-derivatized carbonyl moieties are present in the unfragmented protein, indicating that side-chain oxidation giving carbonyl substitution occurs more frequently than protein scission. This is consistent with the observation that side-chain H atom abstraction by HO• predominates over α-hydrogen abstraction in amino acids.[13]

The advantages of the peroxonitrite solid solution as a HO• source are numerous. The solid solution is remarkably stable; irradiated KNO_3 solid has been kept under ambient conditions for months with no observed decrease in yellow color or reactivity. The peroxonitrite can be quantified by dissolution in 0.1 M NaOH using $\epsilon_{302} = 1670\ cm^{-1}\ M^{-1}$.[14] This quantitation permits reproducible amounts of HO• to be generated in separate experiments.

Another advantage is the rapid disproportionation of the other radical product, NO_2•, which proceeds by the two steps

$$2NO_2^{\bullet} \rightarrow N_2O_4$$

$$N_2O_4 + H_2O \rightarrow NO_3^- + NO_2^- + 2H^+$$

which have rate constants $9 \times 10^8\ M^{-1}\ s^{-1}$ and $1 \times 10^3\ s^{-1}$, respectively.[15] By contrast, Fenton chemistry, which requires H_2O_2, generates HO_2• by the reaction of HO• with H_2O_2[16] and potentially generates hypervalent iron-oxo and nucleophilic iron-coordinated peroxo moieties. The large number of reactive species makes it difficult to determine the reaction sequences that generate the observed products. If the site-directed iron-EDTA protein cleavage systems[17] generate diffusible HO•, significant oxidative damage to neighboring amino acids should be observed in addition to cleavage. The lack of such surrounding damage would corroborate the proposal of Rana and Meares[18] that the protein fragmentation observed with a site-specific iron-EDTA conjugate is the result of a nucleophilic reaction.

A final major advantage of the single reagent system is enhanced control over the time and place of HO• generation. The time scale of exposure to HO• radical with the peroxonitrite reagent is on the order of several seconds beyond the time required for dissolution. This provides the potential for probing transient phenomena with half-lives as short as 10 s. The present results suggest the interesting possibility of generating HO• inside a cell by microinjection of the peroxonitrite-containing solid and investigating damage to the cellular components. This localization of effect would be impossible with either radiolysis or Fenton chemistry.

Acknowledgment. We thank Frank Johnson for initial investigations and Dr. John Leith for his assistance with the ^{137}Cs irradiations. The ^{137}Cs source is supported by Grant RR-01997. V.E.A. is a fellow of the Sloan Foundation.

(13) Gajewski, E.; Fuciarelli, A. F.; Dizdaroglu, M. *Int. J. Radiat. Biol.* **1988**, *54*, 445-459.
(14) Hughes, M. N.; Nicklin, H. G. *J. Chem. Soc. A* **1968**, 450-452.
(15) Forni, L. G.; Mora-Arellano, V. O.; Packer, J. E.; Willson, R. L. *J. Chem. Soc., Perkin Trans. 2* **1986**, 1-6.
(16) (a) Czapski, G. *Annu. Rev. Phys. Chem.* **1971**, *22*, 171-208. (b) Walling, C. *Acc. Chem. Res.* **1975**, *8*, 125-131.
(17) (a) Schepartz, A.; Cuenoud, B. *J. Am. Chem. Soc.* **1990**, *112*, 3247-3249. (b) Hoyer, D.; Cho, H.; Schultz, P. G. *J. Am. Chem. Soc.* **1990**, *112*, 3249-3250. (c) Rana, T. M.; Meares, C. F. *J. Am. Chem. Soc.* **1990**, *112*, 2457-2458.
(18) Rana, T. M.; Meares, C. F. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 10578-10582.

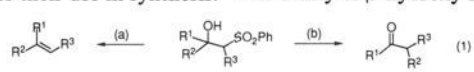
Cyclorearrangement and Cycloolefination of Keto Bis-sulfones. A Sulfone Analogue of a Pinacol Reduction-Rearrangement

Barry M. Trost,*,† J. Bryant Neilsen,† and Karst Hoogsteen‡

Department of Chemistry, Stanford University
Stanford, California 94305-5080
Merck Sharp & Dohme Research Laboratories
P.O. Box 2000, Rahway, New Jersey 07065-0900

Received February 5, 1992

The utility of organosulfones as basic building blocks initially stemmed from their ease of deprotonation to generate nucleophiles followed by reductive cleavage.[1] The recent discovery of the displacement of an arylsulfonyl group by a nucleophile mediated by a Lewis acid[2-4] or a transition metal complex[5] significantly enhances their use in synthesis. The utility of β-hydroxy sulfones



as olefination intermediates (eq 1a)[6] and their prospects for Wagner-Meerwein shifts (eq 1b)[7] suggest versatile cyclization

† Stanford University.
‡ Merck Sharp & Dohme.
(1) Simpkins, N. S. *Tetrahedron* **1990**, *46*, 6951. Roberts, D. W.; Williams, D. C. *Tetrahedron* **1987**, *43*, 1027. Kocienski, P. J. *Chem. Ind. (London)* **1981**, 548. Vogtle, F.; Rossa, L. *Angew. Chem., Int. Ed. Engl.* **1979**, *18*, 515. Magnus, P. D. *Tetrahedron* **1977**, *33*, 2019.
(2) For an overview, see: Trost, B. M. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 107.
(3) Trost, B. M.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1984**, *106*, 7260; **1986**, *108*, 1098. Trost, B. M.; Mikhail, G. K. *J. Am. Chem. Soc.* **1987**, *109*, 4124. Also see: Harmata, M.; Gamlath, G. B. *J. Org. Chem.* **1988**, *53*, 6154. Adams, J. P.; Bowler, J.; Collins, M. A.; Jones, D. N.; Swallow, S. *Tetrahedron Lett.* **1990**, *31*, 4355.
(4) For some examples wherein the sulfone is activated by the presence of heteroatom substituents, see: Simpkins, N. S. *Tetrahedron* **1991**, *47*, 323. Brown, D. S.; Charreau, P.; Ley, S. W. *Synlett* **1990**, 749. Brown, D. S.; Bruno, M.; Davenport, R. J.; Ley, S. V. *Tetrahedron* **1989**, *45*, 4293. Torisawa, Y.; Satoh, A.; Ikegami, S. *Tetrahedron Lett.* **1988**, *29*, 1729.
(5) Trost, B. M.; Merlic, C. A. *J. Am. Chem. Soc.* **1990**, *112*, 9590. Masaki, Y.; Sakuma, K.; Kaji, K. *J. Chem. Soc., Perkin Trans. 1* **1985**, 1171. Fabre, J. L.; Julia, M.; Verpeaux, J. N. *Bull. Soc. Chim. Fr.* **1985**, 772. Julia, M.; Verpeaux, J. N. *Tetrahedron* **1983**, *39*, 3289. Cuvigny, T.; Julia, M. *J. Organomet. Chem.* **1983**, *250*, C21. Trost, B. M.; Schmuff, N. R.; Miller, M. J. *J. Am. Chem. Soc.* **1980**, *102*, 5979.
(6) Kocienski, P. J. *Chem. Ind. (London)* **1981**, 548. Cf. Kocienski, P. J. *J. Chem. Soc., Perkin Trans. 1* **1978**, 829, 834. Julia, M.; Paris, J.-M. *Tetrahedron Lett.* **1973**, 4833.

0002-7863/92/1514-5432$03.00/0