RADICAL CLOCKS: MOLECULAR STOPWATCHES FOR TIMING RADICAL REACTIONS

Reported by Nora Wang April 27, 2006

INTRODUCTION

Understanding the kinetics of radical processes has become increasingly important in the last two decades. During this time, the applications of radical reactions in synthetic organic chemistry have grown, as has the recognition that radical intermediates are common in enzymatic pathways. Standard methods employed to measure the rates of radical reactions include electron spin resonance (ESR), laser flash photolysis, and pulse radiolysis. Another tool for investigating these reactions is the radical clock.

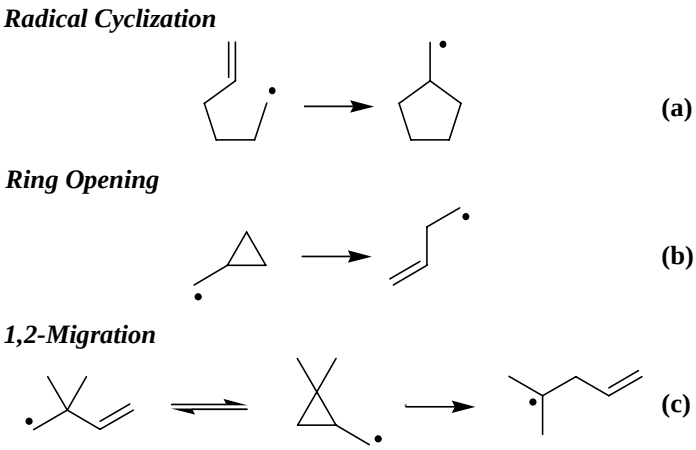


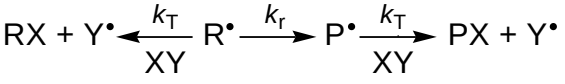
Figure 1. Examples of radical clocks.

Radical clocks are compounds that undergo a unimolecular radical reaction at a known rate. The use of these compounds in competition experiments with bimolecular radical reactions allows the measurement of unknown rates, thus functioning as a molecular "clock". Classic examples of radical clock reactions include cyclizations, ring openings, and 1,2-migrations (Figure 1). Recent examples utilize the time-dependent loss of chirality upon homolysis of a bond to a stereocenter and β-fragmentation of peroxyl radicals.[1,2] This review will discuss the use of radical clocks in the determination of reaction rate constants, examples of radical clock reactions, and reactions to which this methodology has been applied.

RATE DETERMINATION USING A RADICAL CLOCK

Rate Expression Derivation

The application of a radical clock allows the rate constant of a bimolecular reaction to be calculated from reactant concentrations, product ratios, and the known rate constant for the radical clock reaction. The kinetic experiment is summarized in Scheme 1. The bimolecular reaction of radical trap *XY* can proceed with both the radical clock reactant (*R*) and radical clock product (*P*). The ratio of products *RX* to *PX* can be used to



Scheme 1. A radical clock experiment. Radical *R* undergoes reaction to form radical *P* with known rate constant k_r. *XY*, a component of the bimolecular reaction, competitively traps radicals *R* and *P* with unknown rate constant k_T.

calculate the rate constant of the bimolecular reaction. The bimolecular trapping reaction is usually irreversible and second order, and the radical clock reaction is first order and also irreversible. Thus, when one component of the bimolecular reaction is present in large excess and the bimolecular trapping reaction is under kinetic control, a steady state approximation (Equation 1a) and the fact that the ratio of products is equal to the ratio of the rates at which they are formed (Equation 1b) are used to derive the rate expression for the reaction (Equation 1c).[3,4]

$$\frac{d[P]}{dt} = k_r[R] - k_T[XY][P] \approx 0 \qquad \textbf{(a)}$$

$$\frac{[RX]}{[PX]} = \frac{d[RX]/dt}{d[PX]/dt} = \frac{k_T[XY][R]}{k_T[XY][P]} = \frac{[R]}{[P]} \qquad \textbf{(b)}$$

$$k_T = k_r \frac{[RX]}{[XY][PX]} \qquad \textbf{(c)}$$

Equation 1a-1c. Derivation of the equation for the bimolecular reaction rate constant k_T.

Radical Clock Calibration

The rates of radical clock reactions must be calibrated before use in kinetic studies. Newcomb and Ingold have calibrated radical clocks of various reaction rates over an extensive range of temperatures.[5,6] Calibration methods (described further below) involve the trapping of both radical species of the radical clock reaction. This requires the use of a radical trap whose rates of reaction have been measured for various radical types (primary, secondary, benzylic, etc). Thus, compilations of kinetic data exist for the reactions of commonly used radical traps with radicals of different structural characteristics.[7,8] Based on an assumption that radicals of similar structure will react with similar rates, k_T for the specific radical clock/radical trap reaction can be approximated from previously compiled data, and Equation 1c can be used to calculate k_r for the radical clock in question.[3] A plot of log(k_r) as a function of inverse temperature is often constructed from data obtained from radical clock calibrations. The data are fit to the Arrhenius equation, which allows the activation energy (E_a) and log(A) values for the radical clock reaction to be determined from the slope and *y*-intercept of the linear fit. These values allow the calculation of the rate constant for the radical clock reaction for the temperature at which it will be used.

The tin hydride trapping method, can be used to calibrate radical clocks with rates between 10^4 and 10^8 s^{-1} at 25°C.[3] This calibration method involves the trapping of the radical species of the clock reaction with Bu_3SnH, followed by analysis of the product distribution by GC or HPLC. Ingold and coworkers have used the tin hydride method to measure Bu_3SnH-trapping rates for a variety of radicals generated by laser flash photolysis.[7] Thus, rate constants for Bu_3SnH trapping of a variety of structurally diverse radicals are known and can be used to estimate the rate constant (k_T) for trapping of the radical

components of a radical clock reaction.

Newcomb and coworkers typically employ the PTOC-thiol method of radical clock calibration which utilizes Barton's pyridine-2-thione-*N*-oxycarbonyl (PTOC) esters as light-initiated sources of alkyl radicals (Figure 2). Benzenethiol and benzeneselenol, both efficient radical traps, are commonly employed. The use of efficient traps with the PTOC-thiol method allows the measurement of rates of radical clocks from 10^5 to 10^{11} s^{-1} at room temperature.[3]

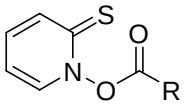


Figure 2. PTOC ester.

Nitroxyl radical couplings are used for calibration of radical clocks with rates approaching the diffusion controlled limit. Unlike the aforementioned trapping reagents, nitroxyl radicals are generated by homolytic dissociation in solution. The radical clock reactant can be generated via photolysis of a variety of radical precursors including diacyl peroxides, peroxy esters, and symmetric ketones. Nitroxyl radicals trap the radical clock species to form *N*-alkoxyamine coupling products that are usually analyzed by HPLC or HPLC/MS.[3]

RADICAL CLOCKS: CLASSIC EXAMPLES

Radical Cyclization

The cyclization of the 5-hexenyl radical (Figure 1a) and its substituted analogues are commonly used as radical clocks. The open chain radical undergoes 5-exo-cyclization to the cyclopentylcarbinyl radical. The 6-endo product is not observed, as this cyclization is a kinetically controlled process, and the transition state for the formation of the 5-exo product is lower in energy.[9] Calibrated in 1974 by Ingold and coworkers, the 5-hexenyl radical clock was one of the first to be used.[10] Since then, the clock has been recalibrated and applied in a large number of mechanistic studies including that of the formation and oxidation of Grignard reagents and the Wittig rearrangement.[9,11]

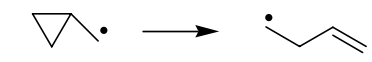
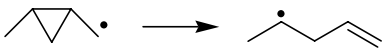
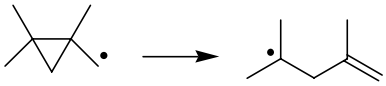
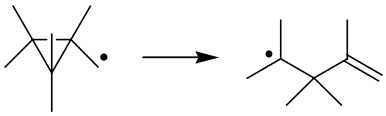
Ingold and coworkers determined that the 5-hexenyl radical cyclization proceeds at 25°C with a rate constant of 2×10^5 s^{-1}.[12] Substituted variants of the 5-hexenyl radical clock cyclize with even faster rates. For example, Newcomb and coworkers showed that 6,6-diphenyl-5-hexenyl and 6-cyano-5-hexenyl radical cyclizations proceeded at 25°C with rate constants of 5.4×10^7 s^{-1} and 1.6×10^8 s^{-1}, respectively.[13,14] Thus, the rate of the 5-hexenyl radical clock is in the middle of the range of rates spanned by the radical clocks and can be increased by introducing radical-stabilizing substituents at the 6-position.

Ring Opening

Possibly the most studied radical clock reaction is the ring opening of the cyclopropylcarbinyl

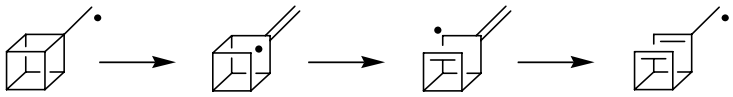
radical (Figure 1b). This reaction, driven by the relief of ring strain, proceeds with a rate constant of 8.6×10^7 s^{-1} at 25°C.[5] Using the nitroxyl radical trapping with 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO), Ingold and coworkers calibrated a variety of substituted cyclopropylcarbinyl radical clocks, some of which are presented in Table 1. The increased rates of ring opening observed upon substitution of the cyclopropyl ring were attributed to relief of steric strain introduced by eclipsing ring substituents and the stabilization of the acyclic radical product. Newcomb and coworkers found that aryl substitution on the cyclopropyl ring drastically increased the rate of ring opening, as rate constants for these clock reactions were on the order of 10^{11} s^{-1}.[15]

Table 1. Rate Constants for Ring Opening of Substituted Cyclopropylcarbinyl Radicals at 25°C.[8]

Rearrangement	k_r[a] (10^7 s^{-1})
	8.6
	55
	150
	360

a. Rate constant values converted from values reported at 37°C using the Arrhenius equation.

The fragmentation of the cubylcarbinyl radical and its substituted variants have been calibrated for use as radical clocks.[16] This highly strained radical actually undergoes two sequential ring openings (Scheme 2). Newcomb and coworkers calibrated the cubylcarbinyl radical clock using the PTOC-thiol method and a benzeneselenol radical trap. They found that at 25°C, the two successive ring fragmentations proceeded with a rate constant of 2.7×10^{10} s^{-1}. An interesting point to note is that this clock belongs to a class referred to as "round-trip radical probes."[17] After both fragmentations, the radical ends up on the same carbon from which it began. This characteristic may be useful in the study of enzymatic radical intermediates, as the static nature of the radical during the clock reaction allows continuous interaction with intermediates without tumbling within the active site.[17]

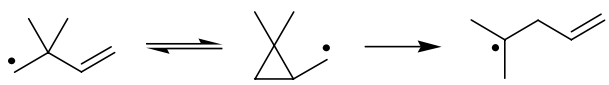


Scheme 2. Cubylcarbinyl radical is a "round-trip radical probe." Radical ring opening to give the tricyclooctadienyl product.

1,2-Migration

During the course of the calibration of the 2,2-dimethyl-3-butenyl radical clock, Newcomb and coworkers observed an intermediate that served as definitive evidence of the accepted mechanism of 1,2-migration today.[18] The 2,2-dimethyl-3-butenyl radical was calibrated using the PTOC-thiol method with a benzenethiol radical trap for temperatures below 37°C and the tin hydride method for

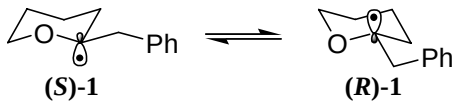
temperatures at and above 37°C. They concluded that the rate constant for the 1,2-migration of the 2,2-dimethyl-3-butenyl radical was 4.8×10^{10} s^{-1} at 25°C. In the course of this experiment, they were also able to trap a cyclopropylcarbinyl radical intermediate, an observation consistent with the proposed cyclization-fragmentation mechanism through which 1,2-migration occurs (Scheme 3).



Scheme 3. Mechanism of 1,2-migration in 2,2-dimethyl-3-butenyl radical.

CONFORMATIONAL RADICAL CLOCKS

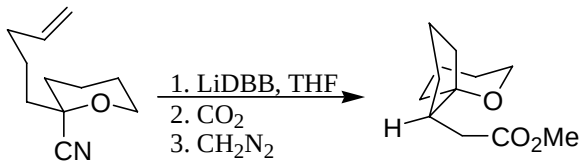
A recent advance in the development of new radical clocks is the exploitation of the higher-than-normal barrier to inversion of tetrahydropyran-2-yl radical (***S*)-1** formed by reduction of enantiopure radical precursor. Rychnovsky and coworkers developed this radical clock based on the relatively high inversion barriers of these chiral radicals (Scheme 4).[1] Upon calibration of the conformational radical clock using the PTOC-thiol method and a *tert*-butylthiol radical trap, they calculated the racemization rate constant as 3.9×10^6 s^{-1} at -78°C.



(*S*)-1 **(*R*)-1**

Scheme 4. Conformational radical clock.

Rychnovsky and coworkers applied this radical clock, along with 5-hexenyl cyclization kinetic data, to investigate the mechanism of cyclization of terminal olefin **2** to form ester **3** upon subsequent reaction (Scheme 5).[19] The conformational radical clock was used to determine if the cyclization occurred through a pentenyllithium or pentenyl radical intermediate. The rate of radical-mediated cyclization was estimated from the known rates of hexenyl radical cyclizations. The calculated radical lifetime was too short for a hexenyl cyclization mechanism; thus, Rychnovsky and coworkers concluded that cyclization proceeded through a pentenyllithium intermediate.

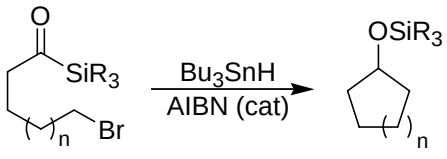


2 **3**

Scheme 5. Conformation radical clock methodology was used to determine the mechanism of cyclization of the terminal olefin 2 to form spirocyclic ester 3. Radical species were generated by reductive decyanation by LiDBB (lithium di-*tert*-butylbiphenylide).

RADICAL CYCLIZATION OF ACYLSILANES

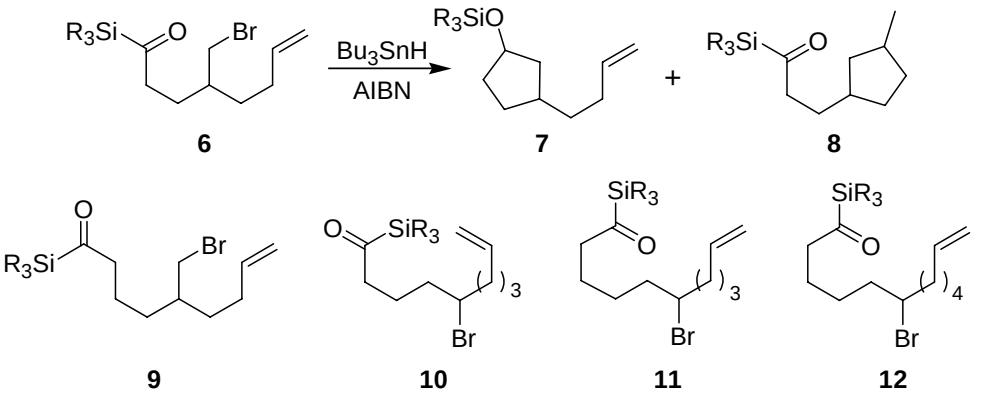
Bromo-substituted acylsilanes undergo radical cyclization in the presence of suitable initiators followed by radical-Brook rearrangement to yield cyclic silyl ethers (Scheme 6). To study the effects of silyl substitution, radical substitution, and ring size on the



4 **5**

Scheme 6. Radical cyclization of acylsilanes. R = Me, Ph, etc.

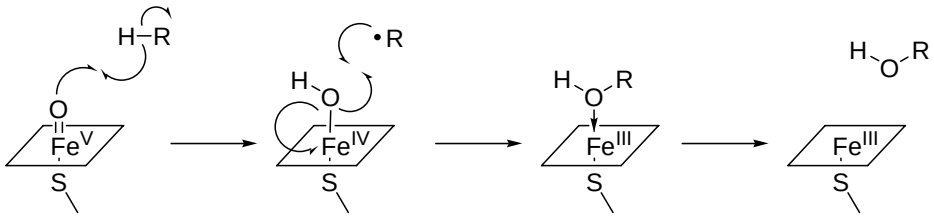
kinetics of this reaction, Tsai and coworkers synthesized compounds containing both acylsilane moieties and 5-hexenyl bromides as radical clock precursors.[20] As shown in Scheme 7, the primary radical from **6** may undergo 5-exo cyclizations with either the acylsilane to form the silyl ether **7** or the butenyl side chain to afford the cyclopentane **8**. The rate constant for the acylsilane cyclization was calculated from the rate constant for the 5-hexenyl cycliza-tion, product distri-bution, and reagent concentration. Radical clock precursors **9-12** were synthesized to obtain rate constants for 5-exo/6-exo acylsilane cyclizations in the presence of competitive terminal alkenyl cyclization onto primary and secondary radicals. In general, Tsai and coworkers concluded that 5-exo acylsilane cyclizations proceed more rapidly than do 6-exo cyclizations and that primary radicals cyclize more rapidly than do secondary radicals. In addition, while bulky substituents on the silyl group slowed cyclization, the presence of radical-stabilizing phenyl substituents accelerated the cyclization rate. While these results are not surprising, this work affords a good example of a small subset of radical clocks designed such that competing reactions exist within the same molecule.



Scheme 7. Radical clocks designed to study acylsilane cyclizations.[20]

MECHANISM OF CYTOCHROME P450 HYDROXYLATION OF C-H BONDS

The mechanism of cytochrome P450 oxidases has been actively studied for forty years; however, the means by which these enzymes catalyze the hydroxylation of C-H bonds at ambient temperature remain debatable. Groves and McGlusky first proposed the "oxygen rebound" mechanism in 1976 (Scheme 8).[21] Substrate enters the catalytic cycle when a C-H bond of the substrate is homolytically cleaved by an ion-oxo ferryl species within the active site of the enzyme. In a reaction referred to as oxygen rebound, the resulting hydroxyl group is then transferred from iron to the

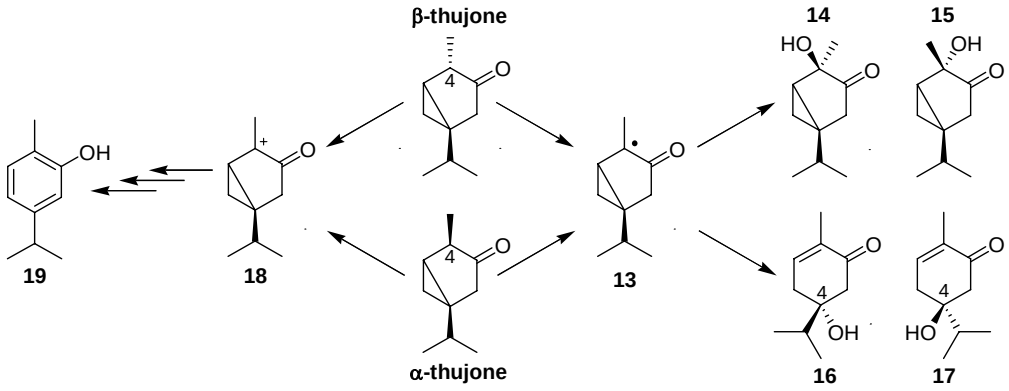


Scheme 8. Oxygen rebound mechanism for cytochrome P450-catalyzed C-H bond hydroxylation.

proximal carbon-centered radical.[22] The existence of the carbon radical intermediate resulting from H-abstraction has been supported by the observation of a large deuterium isotope effect upon oxidation, significant loss in stereochemistry at the oxidation site,[23] and the formation of rearrangement products upon oxidation of various radical probes such as bicyclo[2.1.0]pentane.[24]

As the development of faster radical clocks progressed, their use to measure the rate of oxygen rebound in P450 hydroxylation increased, albeit with confusing, inconsistent results. Rates varied greatly with the use of different clocks, and radical clocks with "ultrafast" rearrangements gave rates of oxygen rebound more consistent with that of transition state decomposition - not that expected for a radical intermediate.[25] Newcomb and coworkers attributed these inconsistencies to competitive cationic rearrangements. Thus, they designed radical clocks in such a way that reactions involving cationic intermediates could be identified by the presence of distinct cationic rearrangement products.[6]

One such radical clock based on the rearrangement of α- and β-thujone was used by Ortiz de Montellano and coworkers to test Newcomb's previous proposal of a cationic intermediate in cytochrome P450 hydroxylations (Scheme 9).[25,26] Upon homolytic cleavage of the C4 C-H bond of α- or β-thujone, the C4 radical can be trapped without rearrangement, or it can force a cyclopropane ring opening. However, should the reaction proceed through a cationic intermediate in which a cation is formed at the C4 carbon, carvacrol **19** is the expected product. Upon incubation of α- and β-thujone with cytochrome P450, Ortiz de Montellano and coworkers observed the formation of both the 4-hydroxy thujone epimers (**14** and **15**) and the ring opened 7-hydroxy dihyd-rocarvones (**16** and **17**) but not carvacrol (**19**). Based on these observations, they concluded that hydroxylation of the C4 C-H bond of thujone by cytochrome P450 proceeded through a radical intermediate, and, contrary to Newcomb's mechanistic proposal, this transformation did not involve a cationic intermediate. The trapping of this radical intermediate from α- and β-thujone to form the hydroxylated product proceeded with a rate of 0.2 $\times 10^{10}$ to 2.8 $\times 10^{10}$ s^{-1}.[25]



Scheme 9. Radical and Cationic Rearrangements of α- and β-thujone.

CONCLUSION

The radical clock field has seen significant advances in the last two decades. A large collection of classically-designed radical clocks has been calibrated and used to determine reaction rate constants ranging from 10^{-1} to 10^{12} s^{-1}.[2] In addition, emerging radical clock methodology is promising as new strategies for the development of clocks are being utilized. Radical clocks are useful tools for studying both chemical and enzymatic reactions, and their application could prove to be a unique source of mechanistic insights for such reactions in the future.

REFERENCES

(1) Buckmelter, A. J.; Kim, A. I.; Rychnovsky, S. D. *J. Am. Chem. Soc.* **2000**, *122*, 9386-9390.
(2) Roschek, B.; Tallman, K. A.; Rector, C. L.; Gillmore, J. G.; Pratt, D. A.; Punta, C.; Porter, N. A. *J. Org. Chem.* **2006**, *71*, 3527.
(3) Newcomb, M. *Tetrahedron* **1993**, *49*, 1151-1176.
(4) Lowry, T. H.; Richardson, K. S. *Mechanism and Theory in Organic Chemistry*, 3rd Ed; HarperCollins: NY, 1987.
(5) Bowry, V. W.; Lusztyk, J.; Ingold, K. U. *J. Am. Chem. Soc.* **1991**, *113*, 5687-5698.
(6) Newcomb, M.; Toy, P. H. *Acc. Chem. Res.* **2000**, *33*, 449-455.
(7) Chatgilialoglu, C.; Ingold, K. U.; Scainano, J. C. *J. Am. Chem. Soc.* **1981**, *103*, 7739-7742.
(8) Bowry, V. W.; Ingold, K. U. *J. Am. Chem. Soc.* **1992**, *114*, 4992-4996.
(9) Beckwith, A. L. J.; Ingold, K. U. Free Radical Rearrangements. In *Rearrangements in Ground and Excited States*; de Mayo, P., Ed.; Academic Press: NY, 1980; Vol. 1, pp 161-310.
(10) Schmid, P.; Griller, D.; Ingold, K. U. *Int. J. Chem. Kin.* **1979**, *11*, 333-338.
(11) Griller, D.; Ingold, K. U. *Acc. Chem. Res.* **1980**, *13*, 317-323.
(12) Chatgilialoglu, C.; Ingold, K. U.; Scaiano, J. C. *J. Am. Chem. Soc.* **1981**, *103*, 7739-7742.
(13) Ha, C.; Horner, J. H.; Newcomb, M.; Varick, T. R.; Arnold, B. R., Lusztyk, J. *J. Org. Chem.* **1993**, *58*, 1194-1198.
(14) Newcomb, M.; Varick, T. R.; Ha, C.; Manek, M. B.; Yue, X. *J. Am. Chem. Soc.* **1992**, *114*, 8158-8163.
(15) Martin-Esker, A. A.; Johnson, C. C.; Horner, J. H.; Newcomb, M. *J. Am. Chem. Soc.* **1994**, *116*, 9174-9181.
(16) Choi, S.; Eaton, P. E.; Newcomb, M.; Yip, Y. C. *J. Am. Chem. Soc.* **1992**, *114*, 6326-6329.
(17) Branchaud, B. P.; Glenn, A. G.; Stiasny, H. C. *J. Org. Chem.* **1991**, *56*, 6656-6659.
(18) Newcomb, M.; Glen, A. G.; Williams, W. G. *J. Org. Chem.* **1989**, *54*, 2675-2681.
(19) Rychnovsky, S. D.; Hata, T.; Kim, A. I.; Buckmelter, A. J. *Org. Let.* **2001**, *3*, 807-810.
(20) Jiaang, W.; Lin, H.; Tang, K.; Chang, L.; Tsai, Y. *J. Org. Chem.* **1999**, *64*, 618-628.
(21) Groves, J. T.; McClusky, G. A. *J. Am. Chem. Soc.* **1976**, *98*, 859-861.
(22) Auclair, K.; Hu, Z.; Little, D. M.; Ortiz de Montellano, P. R.; Groves, J. T. *J. Am. Chem. Soc.* **2002**, *124*, 6020-6027.
(23) Gelb, M. H.; Heimbrook, D. C.; Mälkönen, P.; Sligar, S. G. *Biochemistry* **1982**, *21*, 370-377.
(24) Ortiz de Montellano, P. R.; Stearns, R. A. *J. Am. Chem. Soc.* **1987**, *109*, 3415-3420.
(25) He, X.; Ortiz de Montellano, P. R. *J. Biol. Chem.* **2004**, *279*, 39479-39484.
(26) He, X.; Ortiz de Montellano, P. R. *J. Org. Chem.* **2004**, *69*, 5684-5689.