A Substituted Hypersensitive Radical Probe for Enzyme-Catalyzed Hydroxylations: Synthesis of Racemic and Enantiomerically Enriched Forms and Application in a Cytochrome P450-Catalyzed Oxidation

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The syntheses of racemic and enantiomerically enriched *trans*-1-methyl-2-(4-(trifluoromethyl)phenyl)cyclopropane (**3**) and the possible oxidation products from enzyme-catalyzed hydroxylation of **3** at the methyl group are reported. The important intermediate in the production of **3** was the Weinreb amide of the 2-arylcyclopropanecarboxylic acid which could be prepared in diastereomerically pure form and which also served as an intermediate for production of the cyclic oxidation products of **3**. Hydroxylation of **3** by the cytochrome P450 isozyme CYP2B1 gave cyclic and ring-opened products. The product ratios support an insertion mechanism for the enzyme-catalyzed hydroxylation reaction in which minor amounts of rearranged products are produced by radical fragmentation within the transition structure of the insertion and by a competing reaction involving a cationic species. Formation of cationic rearrangement products by a heterolytic fragmentation reaction of a first-formed protonated alcohol product is suggested on the basis of the apparent amounts of cationic products formed in the hydroxylation of **3**. This pathway for cation production appears to require that the activated enzyme complex (equivalent to enzyme−substrate−H_2O_2) oxidizes substrate before water dissociates to give an iron−oxo species.

Enzyme-catalyzed hydroxylations of unactivated C−H positions at ambient temperatures represent one of the most energetically difficult classes of reactions effected by Nature. Such oxidations are commonly catalyzed by cytochrome P450 enzymes which are broadly distributed in animals, plants and bacteria.[2,3] The active sites of cytochrome P450 enzymes contain a heme with thiolate from cysteine as the fifth ligand to iron. It is likely that the substrate specificity and regio- and stereoselectivity of cytochrome P450 enzymes results from the protein portions of the enzymes and that a common mechanism exists for numerous hydrocarbon hydroxylation reactions. Considerable information concerning the timing of events in cytochrome P450 hydroxylations is known, but the mechanism of the hydroxylation step remains unresolved.

For the past two decades, the consensus mechanism for cytochrome P450 hydroxylation involved pathway a in Figure 1. Hydrogen atom abstraction from substrate by a high valent iron−oxo intermediate produces an alkyl radical intermediate (hydrogen abstraction step), and subsequent homolytic displacement of OH from iron by the radical gives alcohol product (oxygen rebound step).[4−7] This mechanism, which displaced an oxygen insertion process, was deduced mainly from the results of mechanistic probe studies that implicated a radical intermediate by virtue of the formation of products expected from radical rearrangements and is permissively supported by the observation of kinetic isotope effects in hydroxylation reactions. However, attempts to "time" the oxygen rebound step with several fast, calibrated radical clocks gave incongruous results.[8−12] This suggested that an unidentified process might be occurring in competition with or as a part of the hydroxylation sequence. Because a radical and a cationic intermediate would suffer the same skeletal reorganization in each probe for which rearranged products were found, the possibility of a side-reaction involving a cation could not be excluded. We

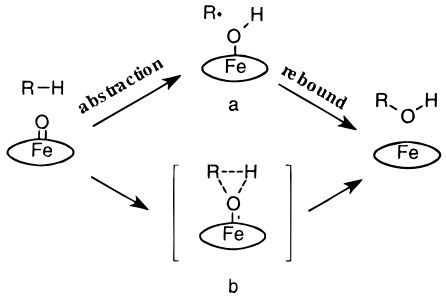

Figure 1. The hydrogen abstraction−oxygen rebound mechanism (a) and insertion mechanism (b) for cytochrome P450 catalyzed hydroxylation.

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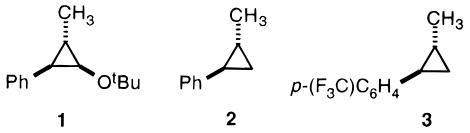
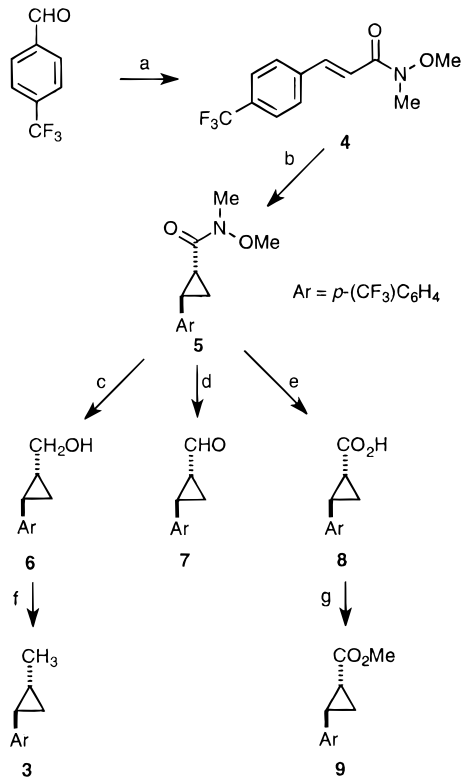
developed an ultrafast radical clock design that could be used to differentiate between radical and cationic species,[13] and cytochrome P450 oxidation of one such probe substrate, probe **1**, demonstrated that both "radical" and "cationic" rearrangements occurred in the hydroxylation process.[14] The qualitative observation of cation-derived products explained the origin of the incongruous "radical lifetimes" found from other radical probe/clocks. The quantitative results indicated that the "radical lifetime" in the cytochrome P450 hydroxylation was only 70 femtoseconds (fs), too short a lifetime for a true intermediate.[15] In other words, the "radical" could only exist in the transition state ensemble for a nonsynchronous, concerted hydroxylation (pathway b in Figure 1). Further, the very short lifetime of the radical requires that the carbon and oxygen atoms are situated nearly within bonding distance of one another in the transition state,[14] a conclusion that is supported by the regioselectivity observed in enzyme-catalyzed hydroxylations of methylcubane.[16]

The generality of the result found in the hydroxylation of one probe by one cytochrome P450 isozyme must be tested, but mechanistic studies of cytochrome P450 catalyzed hydroxylations are complicated for several reasons. Many of these enzymes are highly selective in regard to substrates and will not accept large mechanistic probes. When the probe is accepted as a substrate, oxidation often occurs at multiple sites. Further complicating the situation, the less exclusive hepatic cytochrome P450 enzymes from mammals are membrane-bound, and the structures of these enzymes are not yet known.



Phenyl groups in the cyclopropane probes provide the driving force for the very fast radical ring opening reactions ($k > 10^{11}$ s^{-1} at ambient temperature) that are necessary to time events in transition states, but the phenyl groups in probes such as **2** are oxidized to undesired phenols by cytochrome P450 enzymes[10,11] and by methane monooxygenase systems.[17] We envisioned substitution of the *para* position of the aromatic rings with nonoxidizable groups as a means to avoid phenol production and possibly to permit substituent effect studies. We report here the syntheses of diastereomerically pure *trans*-1-methyl-2-(4-(trifluoromethyl)phenyl)-cyclopropane (**3**) and the possible products from enzyme-catalyzed oxidation of probe **3** at the methyl position, resolution of **3** to high enantiomeric enrichment, and cytochrome P450 catalyzed hydroxylations of racemic **3** and its enantiomers.

Scheme 1[a]



[a] (a) $Ph_3P{=}CHC(O)N(OCH_3)CH_3$, CH_2Cl_2 (95%); (b) $(CH_3)_3SO^+I^-$, NaH, DMSO and then **4** (86%); (c) $LiAlH_4$, THF, 0 °C (62%); (d) *i*-Bu_2AlH, CH_2Cl_2, −78 °C (79%); (e) *t*-BuOK, Et_2O, H_2O (90%); (f) (i) MsCl, Et_3N, THF, −30 °C, (ii) $LiEt_3BH$, THF, −78 °C (79%); (g) CH_2N_2, CH_2Cl_2 (94%).

Results

Synthesis of Probe 3. Compound **3** was reported previously from a carbenoid addition to the appropriate substituted styrene.[18] This preparation afforded a mixture of diastereomers and was impractical for our use; in addition to a diastereomer separation, alternative routes to the possible oxidation products of the probe would be required. Therefore, a synthesis of **3** was designed which would yield the trans diastereomer and some of its possible oxidation products (Scheme 1).

The commercially available 4-(trifluoromethyl)benzaldehyde was first converted to the Weinreb amide **4** by Wittig olefination with *N*-methoxy-*N*-methyl-2-(triphenylphosphoranylidene)acetamide.[19] Compound **4** was prepared so that the cyclopropanation protocol developed by Rodriques[20] could be employed. Accordingly, **4** was treated with the ylide derived from trimethylsulfoxonium iodide and sodium hydride to afford **5**.

Amide **5** proved to be quite versatile in that it could be converted into three of the possible oxidation products of probe **3**. Carbinol **6** was prepared by treatment of **5** with lithium aluminum hydride. Aldehyde **7** was readily prepared by treatment of **5** with diisobutylaluminum hydride (DIBALH) at low temperature; this compound was found to be quite unstable and was prepared immediately prior to use. Saponification of **5** by Gassman's "anhydrous hydroxide" procedure[21] afforded acid **8**. To

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(15) In their excellent review of heme-containing oxygenases, Sono et al. (ref 7) state that the lifetimes we report contain calculation errors. We note that lifetimes are defined as $\tau = 1/k$. Sono et al. apparently confused half-lives ($t_{1/2} = \ln 2/k$) with lifetimes.

(16) Choi, S. Y.; Eaton, P. E.; Hollenberg, P. F.; Liu, K. E.; Lippard, S. J.; Newcomb, M.; Putt, D. A.; Upadhyaya, S. P.; Xiong, Y. *J. Am. Chem. Soc.* **1996**, *118*, 6547−6555.

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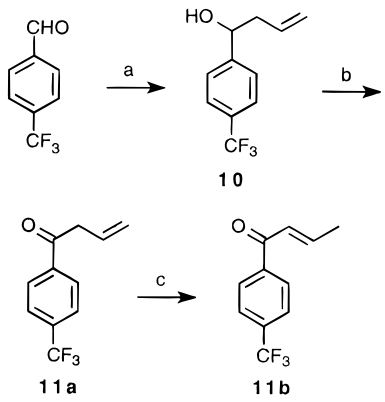
Scheme 2[a]

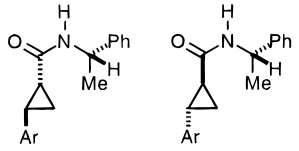


[a] (a) CH_2=$CHCH_2MgBr$, THF, 0 °C (86%); (b) PCC, CH_2Cl_2 (84%); (c) Et_3N, THF, reflux (32%).

provide a convenient GC method for assay for **8**, the acid was converted to its methyl ester **9** by treatment with diazomethane. Probe **3** was prepared from alcohol **6** by conversion to the methanesulfonate at low temperature and reduction of the mesylate with lithium triethylborohydride.[22]

The initial rearranged oxidation product from probe **3** is alcohol **10** (Scheme 2). Further oxidation of **10** would give ketone **11a**, but this β,γ-unsaturated ketone was expected to isomerize in the buffer mixture of the enzyme reactions to give the conjugated isomer **11b**. Alcohol **10** was prepared by addition of allylmagnesium bromide to 4-(trifluoromethyl)benzaldehyde.[23] Ketone **11** was prepared as a 9:1 mixture of **11a** and **11b** by pyridinium chlorochromate (PCC) oxidation of **10**. That this was a mixture of the two alkene isomers was demonstrated by treatment of the 9:1 mixture with triethylamine in refluxing tetrahydrofuran to give a 1:19 mixture of **11a** and **11b**. These compounds were also found to be unstable and were prepared immediately before use.

Highly enantiomerically enriched samples of **3** were prepared from the resolved acids **8** by the method previously used for preparation of enantiomerically enriched samples of **2**.[11] Thus, acid **8** was converted to its ethyl ester and partially hydrolyzed with a lipase. Separation of the acid from the ester and subsequent saponification of the ester provided the enantiomers of **8** with 84% and 90% ee. The enantiomerically enriched acids were converted to their dehydroabeitylamine salts which were recrystallized to effect further enrichment.[24] Samples of the recovered acids were converted to amides **12** by reaction with (*S*)-1-phenylethylamine and 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride,[25] and the diastereomeric mixtures were analyzed by ^{1}H NMR spectroscopy. Samples of the mixtures of amides in $CDCl_3$ did not display clearly resolved signals for the diastereomers, but samples in C_6D_6 did. The signals for the aryl protons adjacent to the CF_3 groups in the two diastereomers (δ 6.5 and 6.6) had baseline resolution at 500 MHz and were used to determine the diastereomeric purity of **12** prepared from each enantiomerically enriched acid **8**. From integration of these signals from the amides prepared from the enantiomerically enriched samples, the sample from (−)-**8** had a 99:1 diastereomer ratio, and the sample from (+)-**8** had a 97.5:2.5 diastereomer ratio.



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Care was taken to avoid a diastereomeric separation in preparation of the samples of amide **12**, but the possibility exists that the amide-forming reaction led to a kinetic resolution. However, such a resolution apparently did not occur on the basis of the observation that the optical rotations of the samples of resolved acids **8** were consistent with the % ee values assigned from the NMR spectra of the diastereomeric amides **12**. Specifically, the sample of (+)-**8** had $[\alpha]^{25}_D = +260.2°$, and (−)-**8** had $[\alpha]^{25}_D = -267.9°$ at comparable concentrations in $CHCl_3$. These two rotations give absolute rotations of +274° and −273°, respectively, when corrected for the diastereomeric ratios obtained from the NMR analyses of amides **12**.

The resolved acids **8** were converted to probes **3** by lithium aluminum hydride reduction to alcohols **6** followed by the mesylate formation−reduction protocol used for preparation of the racemic sample. In these sequences, intermediates were not purified, and we assume that the samples of probe **3** had the same % ee values as the original samples of acids **8**.

The various possible oxidation products from probe **3** were readily separated from one another by GC and had characteristic mass spectral fragmentation patterns that permitted conclusive identifications. The availability of the authentic samples also allowed us to run a number of control reactions to determine stability of the products to the enzyme reaction conditions and analytical conditions.

Enzyme-Catalyzed Oxidations. Much of the mechanistic work with cytochrome P450 enzymes has involved mammalian hepatic enzymes induced by drug treatment. One of the rat liver enzymes induced by phenobarbital treatment (CYP2B1)[26] is known to be a relatively nonselective oxidation catalyst and has been employed widely in mechanistic studies, and that isozyme was employed in this work.

The hepatic cytochrome P450 enzymes require a reductase enzyme which delivers reducing equivalents from NADPH to the substrate−enzyme complex and to the subsequently formed substrate−enzyme−superoxide complex. The reductase enzyme is unstable and undergoes proteolysis to an inactive form even when stored at −80 °C. Fortunately, the reductase enzyme has been expressed in *E. coli*, thereby facilitating the production of large amounts of enzyme with a significant decrease in time and cost involved.[27] Most of the oxidations con-

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Table 1. Results of CYP2B1 Hydroxylation of Probe 3

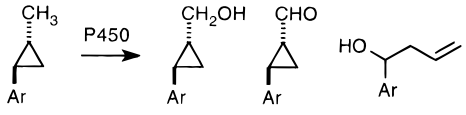
substrate	concentration	C/A[a]	% conversion[b]	turnover[c]
(±)-**3**	high[d]	4.6	0.9	154
(+)-**3**		6.9	0.9	145
(−)-**3**		4.3	0.8	144
(±)-**3**	low[e]	4.0	13	280
(+)-**3**		5.3	14	280
(−)-**3**		2.6	19	330

[a] Average ratio of cyclic products (**6** + **7**) to acyclic product **10**. See the Supporting Information for a complete table of results. [b] Average % conversion of substrate. [c] Average catalyst turnover. [d] Averages from three sets of experiments containing 7.5−14.5 μmol of substrate. [e] Averages from three sets of experiments containing 1.05−1.30 μmol of substrate.

ducted in this work employed expressed reductase. In independent studies, we found that CYP2B1 hydroxylations of probe **2** conducted with expressed reductase gave results comparable to those obtained with rat liver reductase.

Racemic probe **3** and both enantiomers of **3** were oxidized by CYP2B1 in reactions conducted using a reconstituted system consisting of the cytochrome P450 and reductase enzymes, NADPH, and dilauroyl phosphatidylcholine (DLPC). Complete results are given in the Supporting Information; Table 1 contains a summary where each listed value is the average from three independent experiments. The experiments can be divided into two groups depending on the substrate concentrations used. In Table 1 we have given the average ratios of cyclic to acyclic products found in the experiments using high and low substrate concentrations. One notes that the differences in product ratios for the two enantiomers of **3** were reproducible.

The only products observed in the enzyme catalyzed oxidations of **3** were the cyclic alcohol **6**, the aldehyde **7**, and the ring-opened alcohol **10**. Acid **8** and ketone **11** were not detected at a limit of about 1% relative to the amounts of products observed. The enzyme turnover values were a function of the concentrations of substrate **3** present, but turnover numbers in oxidations of the two enantiomers of **3** were similar when the enantiomers were present in comparable amounts. Importantly, we did not observe products at GC retention times expected for phenols from **3**.



The total amount of oxidized substrate was 0.6−2.5% in the high substrate concentration experiments and 11−23% in the low concentration experiments. Interestingly, the enzyme turnover values were larger when substrate **3** was present in low concentrations. This effect could arise from an inhibitory property of the substrate for the enzyme, but it might have a more mundane origin. The isozyme is membrane-bound in nature, and the probe at higher concentrations might have disrupted the micelles in the reconstituted system. In any event, the amount of enantiomerically enriched substrate oxidized in the low substrate concentration experiments far exceeded the amount of minor enantiomer contamination in the enriched samples thus ensuring that we were observing products from hydroxylation of each enantiomer.

A series of control reactions was performed to determine the stability of the products to the reaction conditions (Table 2). As noted above, aldehyde **7** and ketone **11** were unstable when stored at room temperature. The observed oxidation products (**6**, **7**, and **10**) and putative products **8** and **11**, in the approximate amounts isolated from the oxidations of **3**, were subjected to fully competent enzyme oxidation conditions. In the reaction of alcohol **6**, aldehyde **7** and acid **8** were obtained, and acid **8** also was obtained from oxidation of aldehyde **7**.

Table 2. Results of Product Stability Control Reactions

set[a]	substrate (nmol)	% yields of products[b] **6**	**7**	**8**	**10**	**11**
1	**6** (57)	25	20	nd[c]		
	7 (67)		48	nd[c]		
	10 (184)				39	
2	**6** (28)	39	32	15		
	7 (20)		29	24		
	8 (35)			64		
	11 (72)					36

[a] The "sets" are a group of experiments performed simultaneously with the same batch of cytochrome P450 and reductase enzymes. [b] Absolute % yields of products. [c] nd = not determined.

Table 3. Results of Product Recovery Studies

experiment[a]	substrate	nmol[b]	% recovery[c]
A	**6**	64	85
	7	28	68
	10	15	85
B	**6**	64	100
	7	28	85
	10	15	96
C	**6**	64	103
	7	28	77
	10	15	82

[a] A mixture of **6**, **7**, and **10** was studied in each experiment. The experiments were conducted in a manner similar to the enzyme oxidations described in the Experimental Section with the following changes. In A, no enzymes were present. In B, NADPH was omitted. In C, a fully competent oxidation system containing 12.2 μmol of *trans*-2-(*tert*-butoxycarbonyl)-1-methylcyclopropane was employed. [b] Amount of substrate introduced. [c] Absolute % yields of recovered products as determined by GC analysis against a standard.

Another set of control reactions was conducted to determine the extent of product recovery expected from the reactions of **3** (Table 3). Thus, mixtures of products **6**, **7**, and **10** were treated with the fully competent enzyme system and a test substrate, with the enzyme system lacking NADPH, and with the reaction mixture lacking the enzymes. Product recoveries of **6** and **10** were high in each of these reactions. Unstable aldehyde **7** was recovered in good to high yield.

The formation of aldehyde **7** in the cytochrome P450 oxidation of probe **3** is noteworthy. Oxidation of alcohol **6** to aldehyde **7** in the control reactions demonstrated that **6** was a substrate, but we and others had not previously observed aldehyde formation when methylcyclopropane probes were oxidized by CYP2B1 in low conversion experiments.[8,10−12,14] Nevertheless, Zaks and Dodds reported that probe **2** was oxidized to the cyclic aldehyde and cyclic acid in addition to the cyclic alcohol by chloroperoxidase from the fungus *Caldaromyces fumago*, a heme-containing enzyme that is closely related to cytochrome P450 enzymes.[28] Apparently, in comparison to hydrocarbon probe **3**, alcohol **6** is selectively bound and/or oxidized by CYP2B1. It is possible that the second

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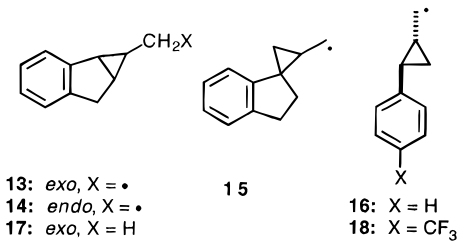
oxidation of alcohol **6** occurred in competition with release of this product from the enzyme.

Discussion

The oxygen rebound mechanism for cytochrome P450 catalyzed hydroxylation of an unactivated C−H position is shown in path a in Figure 1. In this mechanism, a radical is formed as a discrete, albeit possibly short-lived, intermediate. From the results with probe **1**, we concluded that a discrete radical intermediate could not be formed.[14] The lifetime[15] of the "radical" was only about 70 fs, which is too short a lifetime for an intermediate, and the "radical" could only be a component of the transition state in a concerted insertion process (Figure 1, path b). On the basis of the observation that cation-derived products were formed in the hydroxylation of probe **1**, we reasoned that previous studies with radical probes that gave rearranged products gave misleading results concerning the radical lifetime. Specifically, we suggested that in cases where the skeletal reorganization occurring for radical and cationic rearrangements is the same, that the rearranged products result from both species. Two possible routes to cationic species are discussed later.

Our mechanistic conclusions are open to criticism. One important point that must be considered is the possibility that the enzyme can dramatically alter the rate constants for ring opening of the radical. In fact, we specifically raised this point in an early report of the application of hypersensitive radical probe **2** in hydroxylations catalyzed by a methane monooxygenase system.[17] If the enzyme affects the radical ring opening rate constants, presumably by steric compression that forces the aromatic ring into poor alignment with the breaking C−C bond of the cyclopropyl ring, then the quantitative aspects of the studies are vitiated. However, a preponderance of evidence suggests that any influence of the enzyme on the rate constants for ring opening of cyclopropylcarbinyl radicals can only be minor. Among the more important observations in this regard are the facts (1) that enforced nonoptimal alignments of the aromatic ring with the breaking C−C bond in radicals **13**−**15** result in only minor differences in the rate constants for the fragmentation reactions in comparison to the parent radical **16**,[29] (2) that the constrained probe **17**, in which the aryl ring cannot be twisted appreciably by the enzyme, is hydroxylated by cytochrome P450 with an apparent radical lifetime of 80 fs,[12] and (3) that both enantiomers of probe **2**, which should interact differently with the chiral enzyme, give similar product ratios in cytochrome P450 hydroxylation studies.[11]



13: *exo*, X = •
14: *endo*, X = •
17: *exo*, X = H

15

16: X = H
18: X = CF_3

The mechanistic probe we are testing in this work, compound **3**, provides supporting evidence for the insertion mechanism of cytochrome P450 catalyzed hydroxylation even though it suffers from the same limitation as most of the other probes that have been employed in such studies in that both radical and cationic cyclopropylcarbinyl species will fragment in the same manner. In independent studies that will be reported in detail later, we have measured rate constants for ring opening of the radical **18** using benzeneselenol trapping as a competing reaction,[30] the same method as employed in calibrations of ring opening rate constants for other 2-arylcyclopropylcarbinyl radicals.[29,31] The rate constant for ring opening of **18** at 37 °C is 9×10^{11} s^{-1}, about three times greater than that for ring opening of parent radical **16**. The average ratio of cyclic to ring-opened products obtained with probe **3** was about 4.5, which requires that the minimum limit for the rate constant for radical capture in the hydroxylation is 4×10^{12} s^{-1} at 37 °C. Thus, the lifetime of the radical must be 250 fs or less at this temperature. The minimum rate constant for capture is only slightly smaller than the rate constant for decomposition of a transition state calculated from conventional transition state theory (6×10^{12} s^{-1}), and the free energy of activation for radical capture, a defining requirement for an intermediate, must be less than 0.3 kcal/mol. We emphasize that the 250 fs lifetime is the limiting case wherein one assumes that no cationic rearrangement occurred.

The discussion in the above paragraph is founded on the premise that the radical rearrangement rate constants are not strongly influenced by the enzyme. The studies of hydroxylations of the two enantiomers of probe **3** provide additional evidence that this assumption is reasonable. As was the case with hydroxylation of the enantiomers of probe **2**,[11] the product ratios for the enantiomers of **3** were only slightly (but reproducibly, see below) different. On the basis of the observation that the constrained radicals **13**−**15** fragment with nearly the same rate constants, one must conclude that truly dramatic enzymic constraint would be required to reduce the rate constants for ring opening of the radicals by more than 2 orders of magnitude necessary to bring the results into alignment with a radical lifetime in the enzyme-catalyzed hydroxylation of 70000 fs (the value one calculates from the results of hydroxylation of bicyclo[2.1.0]pentane[8]). The likelihood that the chiral enzyme could have such a dramatic effect on the rate constants for fragmentation of both of the enantiomeric radicals **18** seems remote as an independent fact. Taken together with the other evidence that the enzyme does not influence the cyclopropylcarbinyl radical ring-opening rate constants strongly, we believe the premise that such an effect occurs is unreasonable.

If the mechanistic model for cytochrome P450 hydroxylation we have proposed, an insertion process with a competing pathway to cationic species, is reasonable, then the question of the origin of the cationic species remains unresolved. The following possible routes to cation formation have been considered (Figure 2): (1) an oxidation event competes with collapse of the "radical" in the transition state of the hydroxylation and (2) the first formed product is not an alcohol but rather a protonated alcohol formed by insertion of "OH^+" into the C−H bond instead of "O", and proton transfer from the

(29) Martin-Esker, A. A.; Johnson, C. C.; Horner, J. H.; Newcomb, M. *J. Am. Chem. Soc.* **1994**, *116*, 9174−9181.

(30) Choi, S.-Y.; Toy, P.; Newcomb, M. Unpublished results.

(31) Newcomb, M.; Johnson, C. C.; Manek, M. B.; Varick, T. R. *J. Am. Chem. Soc.* **1992**, *114*, 10915−10921.

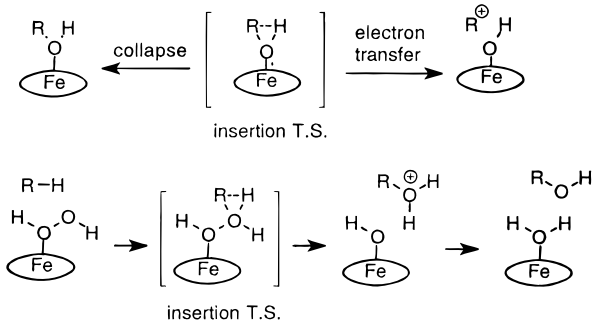
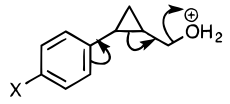


Figure 2. Two possible routes for cation formation in cytochrome P450 hydroxylation. In the upper scheme, electron-transfer competes with collapse in the transition state, and the alkyl cation formed by oxidation can rearrange. In the lower scheme, hydroxylation occurs before release of water, and the first-formed oxidation product is a protonated alcohol that can rearrange in competition with proton transfer.

protonated alcohol competes with loss of water in a solvolytic-type reaction. Taken with previous observations, the results of the present study appear to be more consistent with the latter pathway as discussed below, and this ultimately leads to a conclusion that the active oxidant in the cytochrome P450 hydroxylation might be an iron−hydrogen peroxide complex (as shown in Figure 2) rather than the commonly assumed iron−oxo intermediate. We caution that such a conclusion is speculative and that our results are only suggestive in this regard, but we believe the point warrants presentation on the basis of the current interest in the mechanism of cytochrome P450 hydroxylation.

There is some evidence that cytochrome P450 enzymes with iron bound to an alkoxy or hydroxyl group can oxidize a carbon radical to a cation. For example, prostacyclin (PGI_2) and thromboxane (TxA_2) synthases are cytochrome P450 enzymes that might produce their respective products from prostaglandin PGH_2 by routes that include oxidations of carbon radical intermediates by iron−alkoxy moieties.[32] In a similar manner, an iron−hydroxy species might oxidize a DNA radical to a cation in one of the competing reactions resulting in DNA strand scission by the anticancer agent bleomycin.[33] To accommodate such an oxidation with the results of many cyclopropane probe studies, however, one would need to rationalize that oxidation of a cyclopropylcarbinyl radical with an aryl group at C2 is considerably more favorable than oxidation of a simple cyclopropylcarbinyl radical, which seems to be unlikely. This rationalization is necessary because simple cyclopropane probes such as methylcyclopropane are hydroxylated at the methyl position without formation of ring-opened products.[8,9] An important point casting further doubt on the radical oxidation route to a cation is the report that cytochrome P450 hydroxylation of isopropylcyclopropane to the tertiary alcohol occurs without formation of rearranged products;[9] the putative tertiary cation from this species is much more stable than the primary cation that would have to be produced from methylcyclopropane probes.

In the alternative route to rearranged products, insertion of "OH^+" into the C−H bond, the first formed products of the hydroxylation process would be a protonated alcohol and hydroxide ligated to iron. Rapid proton transfer to hydroxide would give the alcohol product and water. The attraction of this pathway is that one could postulate that aryl-substituted cyclopropane probes would have an anchimeric assistance route for ionic fragmentation not available to the simple cyclopropane probes (i.e. **19**). In addition, there is computational support for a low energy pathway for insertion of OH^+ into C−H bonds from simple models for the heme−iron−H_2O_2 complex such as the hydroperoxonium ion $(H_2O-OH)^+$;[34] one awaits advanced computations of reactions of an Fe−H_2O_2 complex. The problem with this pathway is that it apparently requires that the insertion occurs before loss of water from the enzyme−H_2O_2 complex as shown in Figure 2. Although the precise timing of the oxidation step relative to loss of water in a cytochrome P450 hydroxylation has not been established, it is commonly thought that the actual oxidizing entity is an iron−oxo species similar to compound I observed in decompositions of H_2O_2 by peroxidase enzymes; that is, loss of water precedes oxidation.



19

If one again assumes that the insertion model of Figure 1, path b is correct and further assumes that the radical lifetime in the transition state for P450 insertion is essentially constant for hydroxylation of any probe, then the amount of rearranged products obtained in probe studies can be divided into the amount resulting from the radical process and that from the ionic process. On the basis of the rate constants for ring opening of the cyclopropylcarbinyl radicals, three times as much rearranged product should have been found from hydroxylation of probe **3** as was found in the oxidations of probe **2**, but, in fact, more acyclic product was obtained in the oxidations of probe **2**. This qualitative observation indicates that the ionic route to rearranged products was more important in the case of probe **2**.

One can attempt to quantitate the results by assuming that the radical capture rate constant in the hydroxylation insertion transition state is 1.5×10^{13} s^{-1}, the value obtained in study of probe **1**. In that case, the ratio of cyclic to acyclic products resulting solely from the radical route would be 97:3 for probe **2** and 92:8 for probe **3**. Hydroxylation of probe **2** gave ratios of cyclic to acyclic products that averaged about 75:25,[11] and the ratio found in this work for probe **3** averaged about 82:18. Thus, the amount of *ionic* rearrangement that apparently occurred with probe **2** was about 2.5 times as great as that with probe **3**.

Both of the possible pathways we envision for production of cationic products (Figure 2) could exhibit a response to the trifluoromethyl substituent on the aromatic ring. This substituent has a stabilizing effect on a radical (witness the 3-fold acceleration in ring opening of radical **18** relative to radical **16**), and it is strongly destabilizing for a cation. Nevertheless, a remote effect on the oxidation potential of the radical center on the methylene group in **18** (as opposed to the benzylic radical center in the rearranged product) might be expected to

(32) Ullrich, V.; Brugger, R. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1911−1919.

(33) Stubbe, J.; Kozarich, J. W. *Chem. Rev. (Washington, D.C.)* **1987**, *87*, 1107−1136.

(34) Bach, R. D.; Mintcheva, I.; Estevez, C. M.; Schlegel, H. B. *J. Am. Chem. Soc.* **1995**, *117*, 10121−10122.

be quite small, and we have already noted that the radical oxidation pathway is inconsistent with the observation that hydroxylation of the secondary and tertiary positions of simple alkylcyclopropanes proceeds with no rearrangement.[9,10] Alternatively, the rate of the aryl-assisted solvolysis of protonated alcohol (i.e. **19**) should be reduced by the CF_3 group. On balance, the results appear to be more in accord with a solvolysis pathway than a radical oxidation pathway. This leads us to the speculation that water dissociation from the enzyme−H_2O_2 complex might not precede the oxidation event.

Finally, we comment on the slight differences in product ratios observed for the two enantiomers of **3**. Similar small but reproducible differences in product ratios were also found in hydroxylation of probe **2**.[11] We argued above that drastic effects of the enzyme on the unimolecular radical rearrangement rate constants are not expected; essentially, unimolecular collapse of the transition state ensemble competes with unimolecular radical rearrangement, and neither process should be influenced significantly by the enzyme. However, there is little reason to believe that minor kinetic effects for cation production will not be found in the diastereomeric ensembles of the two enantiomers with the chiral enzyme because one of the competing reactions in each possible route to the cation is "bimolecular" in nature. If radical oxidation occurs, then the electron transfer must compete with the collapse of the transition structure that results in alcohol product. If assisted solvolysis of a protonated alcohol is the origin of cationic products, then this solvolysis step must compete with proton transfer from protonated alcohol to hydroxide bound to iron. The kinetics of both electron transfer and proton transfer events will be affected by the distances between the reactive centers. Thus, we propose that differences in the assembly of the diastereomeric enzyme−substrate complexes result in small differences in the distances between reactive centers that ultimately affect the rate of a competition reaction and thus the amount of cation-derived products.

Conclusions

The hypersensitive radical probe **3** containing the *p*-(trifluoromethyl)phenyl group functions well in studies of hydroxylations by the cytochrome P450 isozyme CYP2B1. Good yields of products from oxidation at the methyl position were obtained from both enantiomers of **3**, and aromatic ring oxidation apparently did not occur to an appreciable extent. The similar product ratios obtained from the two enantiomers of **3** provides more evidence that the enzyme does not have a significant effect on the rate constants for radical rearrangements of aryl-substituted cyclopropylcarbinyl radicals. The small amount of rearranged product formed is consistent with an insertion pathway for the enzyme-catalyzed hydroxylation with a competing process that involves production of a cationic species. From comparison of the amount of apparent cation-derived product produced in hydroxylation of **3** to that produced in hydroxylation of the analogous probe not containing the trifluoromethyl substituent (**2**), a solvolytic-type pathway with anchimeric assistance from the aromatic group is suggested for production of the cation-derived rearranged products. Such a pathway suggests that the first formed species in the hydroxylation reaction is a protonated alcohol formed by "OH^+" insertion into a C−H bond, and this leads to speculation that the enzyme−H_2O_2 complex might be the active oxidizing moiety rather than the "iron−oxo" intermediate formed by loss of water from the complex.

Experimental Section

General Methods. Commercially available reagents were purchased form either the Sigma or Aldrich Chemical Co. unless otherwise noted and were used as received. All moisture sensitive reactions were carried out in flame-dried glassware under a nitrogen atmosphere. Tetrahydrofuran (THF) and diethyl ether were distilled under a nitrogen atmosphere over sodium and benzophenone ketyl. Methylene chloride was distilled under a nitrogen atmosphere over phosphorus pentoxide. Dimethyl sulfoxide (DMSO) was distilled in vacuo from calcium hydride.

NMR spectra were acquired on either a Varian Gemini 300 or a General Electric QE-300 spectrometer. Gas chromatography analyses were performed using flame ionization detection on a Varian 3400 chromatograph (15 m × 0.54 mm bonded phase Carbowax column, Alltech). Gas chromatography/mass spectral (GC/MS) analyses were performed using a Hewlett-Packard Model 5890 GC interfaced to a Hewlett-Packard Model 5971 mass selective detector (30 m × 0.25 mm capillary bonded phase Carbowax column, Alltech). High-resolution mass spectral analyses were performed by the Central Instrumentation Facility at Wayne State University (Detroit, MI). Melting points were determined using a Unimelt capillary melting point apparatus (Thomas-Hoover) and are uncorrected. Radial chromatography was performed on a Chromatotron Model 7294T (Harrison Research Corp.) using plates coated with TLC grade silica gel (Merck) with gypsum binder and fluorescent indicator.

Enzymatic oxidation reactions were performed in reconstituted systems using the purified isozyme CYP2B1[35] and expressed reductase[27] at 37 °C in 50 mM potassium phosphate buffer, pH 7.4, containing 1 mM desferrioxamine and 0.075 M KCl, with a total reaction volume of 2 mL. A mixture of 0.6 nmol of CYP2B1 and 1.2 nmol of reductase in buffer was allowed to stand on ice for 5 min. A suspension of DLPC (0.96 nmol) in buffer was sonicated. The enzyme and micelle mixtures were combined, and this mixture was allowed to stand on ice for 5 min. The mixture was diluted with buffer, and the substrate was added either neat (high substrate concentration) or as a solution in ca. 10 μL of methanol (low substrate concentration). The reaction mixture was incubated for 2 min at 37 °C, and the reaction was initiated by the addition of NADPH (final concentration of 1.2 mM). The reaction mixture was shaken gently at 37 °C for 30 min and then extracted with methylene chloride (3 × 2 mL). The combined organic phases were dried over anhydrous $MgSO_4$ and filtered through a plug of glass wool. An internal standard was added, and the solution was concentrated under a slow stream of nitrogen at room temperature to a final volume of approximately 0.2 mL. Quantitation of the product ratios was performed using gas chromatography with flame ionization detection, and product identities were determined by comparison with authentic samples using selected ion monitoring (SIM) GC/MS analysis.

Control reactions in Table 2 were performed by dissolving the substrates in methanol and adding aliquots of these solutions to two reaction mixtures prepared as above, one reaction mixture containing enzymes and one without the enzymes. The reactions were conducted and extracted as above. The amounts of recovered material were determined by comparison of the ratios of the GC areas of the compounds of interest to those of standards in the two reactions.

***N*-Methoxy-*N*-methyl-*trans*-4-(trifluoromethyl)cinnamamide (4).** A solution of 4-(trifluoromethyl)benzaldehyde (10.0 g, 57.4 mmol) and *N*-methoxy-*N*-methyl-2-(triphenylphosphoranylidene)acetamide (23.1 g. 63.6 mmol) in CH_2Cl_2 (150 mL) was stirred at room temperature for 16 h. The solvent was

(35) Saito, T.; Strobel, H. W. *J. Biol. Chem.* **1981**, *256*, 984−988.

then removed in vacuo, and the crude product was chromatographed on silica gel (40% ethyl acetate in hexanes) to afford **4** (14.1 g, 54.3 mmol, 95%) as a white solid. Mp 50−52 °C. ^{1}H NMR ($CDCl_3$): δ 3.32 (3H, s), 3.78 (3H, s), 7.10 (1H, d, J = 15.9 Hz), 7.65 (4H, dd, J_1 = 12.0 Hz, J_2 = 9.0 Hz), 7.74 (1H, d, J = 15.9 Hz). ^{13}C NMR ($CDCl_3$): δ 32.5, 61.9, 123.8 (q, J_{C-F} = 276.9 Hz), 125.6 (2C, q, J_{C-F} = 3.7 Hz), 128.1 (2C), 131.3 (q, J_{C-F} = 33.1 Hz), 138.6, 141.5, 166.2. HRMS: calcd for $C_{12}H_{12}F_3NO_2$, 259.0812; found, 259.0820.

***N*-Methoxy-*N*-methyl-*trans*-2-(4-(trifluoromethyl)-phenyl)cyclopropanecarboxamide (5).** A solution of trimethylsulfoxonium iodide (24.1 g, 110 mmol) in DMSO (100 mL) was cooled with a room-temperature water bath, and NaH (2.6 g, 108 mmol) was added portion wise over 15 min. After the addition was complete, the suspension was allowed to stir for 1 h, during which time it became homogeneous. A solution of **4** (14.1 g, 54.3 mmol) in DMSO (50 mL) was added via cannula, and the reaction mixture was stirred for 6 h. The reaction mixture was quenched by pouring it into saturated aqueous NH_4Cl soln (500 mL). The mixture was extracted with CH_2Cl_2 (3 × 250 mL). The combined organic layers were washed with brine (200 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude material was chromatographed on silica gel (40% ethyl acetate in hexanes) to afford **5** (12.8 g, 46.8 mmol, 86%) as a clear, colorless oil. ^{1}H NMR ($CDCl_3$): δ 1.30 (1H, ddd, J_1 = 8.4 Hz, J_2 = 6.3 Hz, J_3 = 4.5 Hz), 1.65 (1H, ddd, J_1 = 9.0 Hz, J_2 = 5.4 Hz, J_3 = 4.2 Hz), 2.38−2.46 (1H, m), 2.50 (1H, ddd, J_1 = 9.3 Hz, J_2 = 6.6 Hz, J_3 = 4.2 Hz), 3.19 (3H, s), 3.65 (3H, s), 7.18 (2H, d, J = 9.0 Hz), 7.48 (2H, d, J = 9.0 Hz). ^{13}C NMR ($CDCl_3$): δ 16.5, 21.8, 25.2, 32.4, 61.5, 124.1 (q, J_{C-F} = 270.3 Hz), 125.2 (2C, q, J_{C-F} = 3.3 Hz), 126.3 (2C), 128.3 (q, J_{C-F} = 32.4 Hz), 145.0, 172.2. HRMS: calcd for $C_{13}H_{14}F_3NO_2$, 273.0977; found, 273.0974.

(*trans*-2-(4-(Trifluoromethyl)phenyl)cyclopropyl)methanol (6). A solution of **5** (2.0 g, 7.3 mmol) in THF (75 mL) was cooled to 0 °C, and $LiAlH_4$ (0.5 g, 20.8 mmol) was added. The mixture was stirred for 2 h, after which time the reaction mixture was quenched by the sequential addition of water (0.5 mL), 15% NaOH (0.5 mL), and water (1.5 mL). After stirring for an additional 2 h, the suspension was filtered, and the filtrate was washed with additional THF (100 mL). The combined organic solutions were concentrated in vacuo, and the resulting residue was dissolved in dry THF (75 mL). This mixture was cooled to 0 °C, and $LiAlH_4$ (0.5 g, 20.8 mmol) was added. This mixture was stirred for 2 h, the reaction was quenched, and the crude product was isolated as above. The crude product was chromatographed on silica gel (40% ethyl acetate in hexanes) to afford **6** (1.0 g, 4.5 mmol, 62%) as a white solid. Mp 61 °C. ^{1}H NMR ($CDCl_3$): δ 1.01−1.06 (2H, m), 1.41 (1H, bs), 1.46−1.57 (1H, m), 1.87−1.93 (1H, m), 3.66 (2H, d, J = 6.6 Hz), 7.17 (2H, d, J = 8.4 Hz), 7.52 (2H, d, J = 8.4 Hz). ^{13}C NMR ($CDCl_3$): δ 14.3, 21.1, 25.9, 66.0, 124.3 (q, J_{C-F} = 269.2 Hz), 125.2 (2C, q, J_{C-F} = 3.5 Hz), 125.9 (2C), 127.8 (q, J_{C-F} = 33.1 Hz), 146.9. HRMS: calcd for $C_{11}H_{11}F_3O_2$, 216.0762; found, 216.0759. The enantiomerically enriched alcohols **6** from (+)-**8** and (−)-**8**, prepared from the acids by $LiAlH_4$ reduction as above, were obtained in 77% and 83% yields, respectively.

(*trans*-2-(4-(Trifluoromethyl)phenyl)cyclopropyl)methanal (7). A solution of **5** (1.13 g, 4.1 mmol) in CH_2Cl_2 (25 mL) was cooled to −78 °C, and a solution of DIBALH (1.5 M in toluene, 4.0 mL, 6.0 mmol) was added via syringe over 5 min. The mixture was stirred for 3 h, and then methanol (5 mL) was added. The reaction mixture was allowed to warm slowly to room temperature and was then diluted with ether (100 mL). The ethereal solution was then washed successively with 10% aqueous HCl soln (100 mL), saturated aqueous $NaHCO_3$ soln (100 mL), and brine (100 mL). The organic layer was then dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude product was chromatographed on silica gel (20% ethyl acetate in hexanes) to afford **7** (0.70 g, 3.3 mmol, 79%) as a clear, colorless oil that decomposed on standing. ^{1}H NMR ($CDCl_3$): δ 1.54 (1H, ddd, J_1 = 8.7 Hz, J_2 = 6.9 Hz, J_3 = 5.4 Hz), 1.77 (1H, dt, J_1 = 9.3 Hz, J_2 = 5.4 Hz), 2.21 (1H, ddd, J_1 = 8.7 Hz, J_2 = 5.4 Hz, J_3 = 4.5 Hz), 2.66 (1H, ddd, J_1 = 9.3 Hz, J_2 = 6.6 Hz, J_3 = 3.9 Hz), 7.21 (2H, d, J = 8.1 Hz), 7.53 (2H, d, J = 8.1 Hz), 9.36 (1H, d, J = 4.2 Hz). ^{13}C NMR ($CDCl_3$): δ 16.7, 25.9, 33.7, 124.1 (q, J_{C-F} = 270.2 Hz), 125.5 (2C, q, J_{C-F} = 4.4 Hz), 126.5 (2C), 128.9 (q, J_{C-F} = 32.0 Hz), 143.3, 199.1. MS: calcd for $C_{11}H_9F_3O$, 214; $(M)^+$ found at *m/e* = 214.

***trans*-2-(4-(Trifluoromethyl)phenyl)cyclopropanecarboxylic Acid (8).** A suspension of **5** (7.71 g, 28.2 mmol) and potassium *tert*-butoxide (17.11 g, 152 mmol) in ether (200 mL) and water (1.0 mL, 55.6 mmol) was stirred at room temperature for 16 h. The mixture was acidified by the slow addition of concentrated HCl, and the aqueous mixture was extracted with CH_2Cl_2 (3 × 200 mL). The combined organic layers were washed with brine (200 mL), dried over Na_2SO_4, filtered, and concentrated in vacuo to afford **8** (5.85 g, 25.4 mmol, 90%) as a white solid. Mp 110−111 °C. ^{1}H NMR ($CDCl_3$): δ 1.45 (1H, ddd, J_1 = 8.4 Hz, J_2 = 6.6 Hz, J_3 = 4.8 Hz), 1.73 (1H, dt, J_1 = 9.3 Hz, J_2 = 5.1 Hz), 1.96 (1H, ddd, J_1 = 9.0 Hz, J_2 = 5.4 Hz, J_3 = 4.2 Hz), 2.65 (1H, ddd, J_1 = 9.3 Hz, J_2 = 6.6 Hz, J_3 = 3.9 Hz), 7.21 (2H, d, J = 8.4 Hz), 7.55 (2H, d, J = 8.4 Hz), 12.08 (1H, bs). ^{13}C NMR ($CDCl_3$): δ 17.6, 24.2, 26.5, 124.1 (q, J_{C-F} = 270.2 Hz), 125.5 (2C, q, J_{C-F} = 3.3 Hz), 126.5 (2C), 129.0 (q, J_{C-F} = 33.1 Hz), 143.6, 179.4. HRMS: calcd for $C_{11}H_9F_3O_2$, 230.0555; found, 230.0560.

The enantiomerically enriched carboxylic acids **8** were prepared by P-30 Lipase[36] (Amano International Enzyme Co., from *Pseudomonas cepacia*, formerly classified as *Pseudomonas fluorescens*) hydrolysis of the corresponding ethyl esters by the method previously reported.[11] The reaction was approximately 50% complete after 8 days. From 9.0 g (34.9 mmol) of the ester, 2.0 g (8.7 mmol) of acid and 4.2 g (16.3 mmol) of ester were isolated following the standard workup. The ester was saponified, and the acid was isolated. Three recrystalizations of the acids as their dehydroabietylamine salts (ethyl acetate) afforded optically enriched acids **8**. The enantiomeric excesses were determined by derivatizing the acids with (*S*)-1-phenylethylamine, 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride, and 4-(dimethylamino)pyridine in CH_2Cl_2. ^{1}H NMR analysis (500 MHz, C_6D_6) of the resulting amides showed that the diastereomeric mixtures were 97.5:2.5 and 1:99. The optical rotations of the acids were $[\alpha]^{25}_D$ = +260.2° (c = 2.26, $CHCl_3$) and $[\alpha]^{25}_D$ = −267.9° (c = 2.18, $CHCl_3$).

***trans*-1-Methyl-2-(4-(trifluoromethyl)phenyl)cyclopropane (3).** A solution of **6** (0.284 g, 1.31 mmol) in THF (8 mL) was cooled to −30 °C. To this was added sequentially via syringe triethylamine (0.45 mL, 3.23 mmol) and methanesulfonyl chloride (0.11 mL, 1.42 mmol). The mixture was stirred at −30 °C for 30 min and then cooled to −78 °C. A solution of lithium triethylborohydride (1.0 M in THF, 4.0 mL, 4.0 mmol) was added via syringe. The mixture was allowed to warm slowly to room temperature and was stirred for 13 h. The reaction mixture was quenched by the addition of 30% H_2O_2 (2 mL) and 15% aqueous NaOH (2 mL). The resulting mixture was heated at reflux for 1 h and then cooled to room temperature. The organic layer was separated, and the aqueous layer was extracted with ether (3 × 25 mL). The combined organic phase was washed with brine (50 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo at 0 °C. The crude material was purified by radial chromatography (pentane) to afford **3** (0.209 g, 1.04 mmol, 79%) as a clear, colorless oil. ^{1}H NMR ($CDCl_3$): δ 0.80−0.86 (1H, m), 0.94 (1H, dt, J_1 = 8.1 Hz, J_2 = 4.8 Hz), 1.06−1.16 (1H, m), 1.20 (3H, d, J = 5.7 Hz), 1.62 (1H, dt, J_1 = 8.7 Hz, J_2 = 4.5 Hz), 7.10 (2H, d, J = 8.4 Hz), 7.48 (2H, d, J = 8.4 Hz). ^{13}C NMR ($CDCl_3$): δ 18.3, 18.9, 18.9, 24.2, 124.6 (q, J_{C-F} = 270.2 Hz), 125.1 (2C, q, J_{C-F} = 2.9 Hz), 125.6 (2C), 127.4 (q, J_{C-F} = 31.6 Hz), 148.6. HRMS: calcd for $C_{11}H_{11}F_3$, 200.0813; found, 200.0808. The enantiomerically enriched samples of compounds (+)-**3** and (−)-**3** were synthesized from the corresponding methanols (**6**) by the same method and obtained in 80% and 86% yields, respectively.

Methyl *trans*-2-(4-(Trifluoromethyl)phenyl)cyclopropanecarboxylate (9). To a solution of **5** (0.25 g, 1.1 mmol)

(36) Xie, Z. F. *Tetrahedron: Asymmetry* **1991**, *2*, 733−750.

in CH_2Cl_2 (50 mL) was added dropwise a solution of diazomethane in ether until a slight yellow color persisted. The mixture was stirred open to the atmosphere for 6 h. The solvent was removed in vacuo, and the crude material was chromatographed on silica gel (10% ethyl acetate in hexanes) to afford **7** (0.25 g, 1.0 mmol, 94%) as a clear, colorless oil. ^{1}H NMR ($CDCl_3$): δ 1.34 (1H, ddd, $J_1 = 8.4$ Hz, $J_2 = 6.3$ Hz, $J_3 = 4.5$ Hz), 1.65 (1H, ddd, $J_1 = 9.0$ Hz, $J_2 = 5.4$ Hz, $J_3 = 4.8$ Hz), 1.94 (1H, ddd, $J_1 = 8.4$ Hz, $J_2 = 5.4$ Hz, $J_3 = 4.2$ Hz), 2.56 (1H, ddd, $J_1 = 9.3$ Hz, $J_2 = 6.3$ Hz, $J_3 = 3.9$ Hz), 3.72 (3H, s), 7.17 (2H, d, $J = 8.4$ Hz), 7.51 (2H, d, $J = 8.4$ Hz). ^{13}C NMR ($CDCl_3$): δ 17.1, 24.2, 25.7, 51.9, 124.1 (q, $J_{C-F} = 270.2$ Hz), 125.4 (2C, q, $J_{C-F} = 3.3$ Hz), 126.4 (2C), 128.7 (q, $J_{C-F} = 32.0$ Hz), 144.2, 173.2. HRMS: calcd for $C_{12}H_{11}F_3O_2$, 244.0711; found, 244.0715.

1-(4-(Trifluoromethyl)phenyl)but-3-ene-1-ol (10) was prepared by the previously reported method.[23] ^{1}H NMR ($CDCl_3$): δ 2.14 (1H, d, $J = 3.3$ Hz), 2.41−2.60 (2H, m), 4.79−4.84 (1H, m), 5.15−5.22 (2H, m), 5.73−5.87 (1H, m), 7.48 (2H, d, $J = 8.4$ Hz), 7.61 (2H, d, $J = 8.4$ Hz). ^{13}C NMR ($CDCl_3$): δ 43.5, 72.66, 118.48, 124.1 (q, $J_{C-F} = 270.4$ Hz), 125.1 (2C, q, $J_{C-F} = 3.2$ Hz), 126.0 (2C), 129.5 (q, $J_{C-F} = 32.4$ Hz), 133.6, 147.8.

1-(4-(Trifluoromethyl)phenyl)but-3-ene-1-one (11a) and *trans*-1-(4-(trifluoromethyl)phenyl)but-2-en-1-one (11b). A solution of **10** (0.53 g, 2.45 mmol) and PCC (1.00 g, 4.65 mmol) in CH_2Cl_2 (25 mL) was stirred at room temperature for 8 h. The suspension was then diluted with ether (75 mL) and filtered through a pad of silica gel. The organic solution was concentrated in vacuo, and the crude material was purified by radial chromatography (15% ethyl acetate in hexanes) to afford a 9:1 mixture of **11a** and **11b** (0.44 g, 2.05 mmol, 84%). The ratio of the product mixture was determined by gas chromatography. This mixture was then dissolved in THF (25 mL) and triethylamine (0.5 mL), and the solution was heated at reflux for 14 h. The solution was concentrated in vacuo, and the crude material was purified by radial chromatography (15% ethyl acetate in hexanes) to afford a 1:19 mixture of **11a** and **11b** (0.14 g, 0.654 mmol, 32%). Compounds **11** decomposed on standing.

Characterization data for **11a**: ^{1}H NMR ($CDCl_3$): δ 3.78 (2H, dt, $J_1 = 6.6$ Hz, $J_2 = 1.2$ Hz), 5.20−5.29 (2H, m), 6.01−6.14 (1H, m), 7.74 (2H, d, $J = 8.4$ Hz), 8.07 (2H, d, $J = 8.4$ Hz). MS: calcd for $C_{11}H_9F_3O$, 214; $(M)^+$ found at $m/e = 214$.

Characterization data for **11b**: ^{1}H NMR ($CDCl_3$): δ 2.02 (3H, dd, $J_1 = 7.2$ Hz, $J_2 = 1.8$ Hz), 6.87 (1H, dq, $J_1 = 15.6$ Hz, $J_2 = 1.8$ Hz), 7.12 (1H. dq, $J_1 = 15.6$ Hz, $J_2 = 6.6$ Hz), 7.72 (2H, dd, $J_1 = 8.7$ Hz, $J_2 = 0.6$ Hz), 8.00 (2H, dd, $J_1 = 8.7$ Hz, $J_2 = 0.6$ Hz). MS: calcd for $C_{11}H_9F_3O$, 214; $(M)^+$ found at $m/e = 214$.

Supporting Information Available: Table of results from CYP2B1 hydroxylation of probe **3**, ^{1}H and ^{13}C NMR spectra of **3**−**10**, and ^{1}H NMR spectra of mixtures of **11a**/**11b** (19 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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