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Probing the Hydrophobic Pocket of the Active Site in the Particulate Methane Monooxygenase (pMMO) from *Methylococcus capsulatus* (Bath) by Variable Stereoselective Alkane Hydroxylation and Olefin Epoxidation

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pMMO from M. capsulatus *(Bath) oxidizes straight-chain C1–C5 alkanes and alkenes to form their corresponding 2-alcohols and epoxides. According to experiments performed with cryptically chiral ethane and* D,L*-[2-$^2H_1$,3-$^2H_1$]butane, the reactions proceed through the concerted O-atom insertion mechanism. However, when propene and but-1-ene are used as epoxidation substrates, the enantiomeric excesses (*ees*) of the enzymatic products are only 18 and 37%, respectively. This relatively poor stereoselectivity in the enzymatic epoxidation presumably reflects low stereochemical differentiation between the* re *and* si *faces in the hydrophobic pocket of the active site. Further insights into the reaction mechanism are now provided by studies on* trans*-but-2-ene, which reveal only the* D,L*-2,3-dimethyloxirane products, and on* cis*-but-2-ene, which yield only the* meso *product. These observations indicate that the enzymatic epoxidation indeed proceeds through electrophilic* syn *addition. To achieve better facial selectivity, we have also used 3,3,3-trifluoroprop-1-ene as the substrate. The products obtained are 90% (2S)-oxirane. When 1,1,1-trifluoropropane is the substrate, the hydroxylation at the 2-carbon exhibits an inverse chiral selectivity relative to that seen with normal butane, if we consider the size of the CF_3 group in the fluorinated propane to be comparable to one of the ethyl groups in butane. These experiments are beginning to delineate the factors that influence the orientations of various substrates in the hydrophobic cavity of the active site in the enzyme.*

Introduction

Particulate methane monooxygenase (pMMO) is the first enzyme in the C_1 metabolic pathway in methanotrophic bacteria when these microorganisms adapt to habitats enriched in copper ions (>5–10 μM).[1–3] This multi-copper protein converts methane into methanol, providing the feedstock utilized in the C_1 pathway.[3,4] As in most other C–H activation enzymes, however, methane is not the only substrate metabolized by pMMO. A number of small straight-chain aliphatic alkanes and alkenes (C_2–C_5) are also metabolized[5–7] and oxidized to their corresponding 2-alcohols and oxiranes.

As well as pMMO, certain strains of methanotrophic bacteria (type II and type X) also produce soluble methane monooxygenase (sMMO) under copper-deficient conditions as an alternative enzyme to carry out methane oxidation.[3,8] The active site of the sMMO consists of a diiron core.[9–12] Studies on cryptically chiral ethanes indicated that the oxidation of substrates mediated by the diiron core might involve both a concerted and a radical process. A significant amount of inversion of the configuration of the chiral sp^3 center was observed in these experiments.[13,14] On the other hand, experiments involving cyclopropane-based radical clock substrates suggested that the reaction was largely concerted.[13,15,16] Here, reaction mostly occurred at the primary methyl group of the corresponding methylcyclopropane,[15,16] although small amounts of rearranged products were also observed. It is possible that the reaction pathway was branched in the radical clock experiments as well.[14,15,17]

The size of the hydrophobic pocket in pMMO would not allow for the cyclopropane radical clock substrates to be used as mechanistic probes; these three-membered ring compounds are simply too bulky to enter into the active site of the enzyme. Workers in the field have therefore had to rely on the use of isotopically labeled chiral alkanes (including cryptically chiral ethanes) to ascertain the extent of the racemization of the sp^3 center(s) during the hydroxylation. Total retention of the configuration of the oxidized chiral center would suggest the conclusion that the mechanism of hydroxylation is concerted. Indeed, full retention of configuration was observed in the hydroxylation products when cryptically chiral[1-$^2H_1$,1-$^3H_1$]-ethane[18] and dideuterated D,L-[2-$^2H_1$,3-$^2H_1$]butane[7] were incu-

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bated with cells in which the membrane-bound pMMO was expressed.

Experiments on alkene epoxidation by pMMO, however, were ambiguous in terms of whether the O-atom transfer occurred either from the *re* or from the *si* faces of the alkenes, or both. In the cases of propene and but-1-ene, for example, the epoxidation products exhibited very low enantiomeric excesses of 18 and 32%, respectively, in favor of the *S* configuration.[5] These results left unsettled the question of whether the epoxidation occurred by concerted *syn* addition across the double bond, or through O-atom or hydroxyl radical addition to the 2-carbon, followed by subsequent ring-closure to form the oxirane. In the concerted mechanism, the enantiomeric excess in the propene product would reflect the different probability of insertion of the two ends of the olefin into the hydrophobic pocket, while in the case of but-1-ene it would measure the relative degrees of presentation of the *re* and *si* faces of the olefin to the attacking O atom. In the stepwise mechanism, the stereochemical outcome of the epimerization would also depend on the rotational barrier of the partially oxidized C–C bond prior to ring-closure.

To address these mechanistic issues further, we have now employed *cis*-but-2-ene and *trans*-but-2-ene as mechanistic probes of pMMO. Coon and coworkers have previously applied the same strategy to cytochrome P-450.[19] In addition, we introduce two novel substrates—1,1,1-trifluoropropane and 3,3,3-trifluoroprop-1-ene—to probe the size and shape of the hydrophobic cavity at the active site of the pMMO. In these substrates, the terminal saturated carbons of propane and propene have been replaced by bulkier CF_3 groups. We show that the stereochemical fates of these fluorinated substrates provide new insights into how the regioselectivity and the induction of the chiral selectivity is controlled by the interaction of the individual substrates with the hydrophobic pocket.

Results

The epoxidation of *cis*- and/or *trans*-but-2-ene by pMMO

We treated *cis*- and *trans*-but-2-ene (**1** and **2**, Tokyo Chemical Industrial Co., Ltd.; Scheme 1) for 2.0 h with the pMMO-enriched *Methylococcus capsulatus* (Bath) cells grown at high copper concentrations (30–40 μM).[7,20] Each of the two alkenes gave rise to a single product according to GC (Agilent 6890) on a 100 m HP-1 capillary column (100 m × 0.25 mm × 0.5 μm film thickness, isothermal 150 °C). The retention times of the two different signals were at $t_R = 34.2$ min (Figure 1 D) and

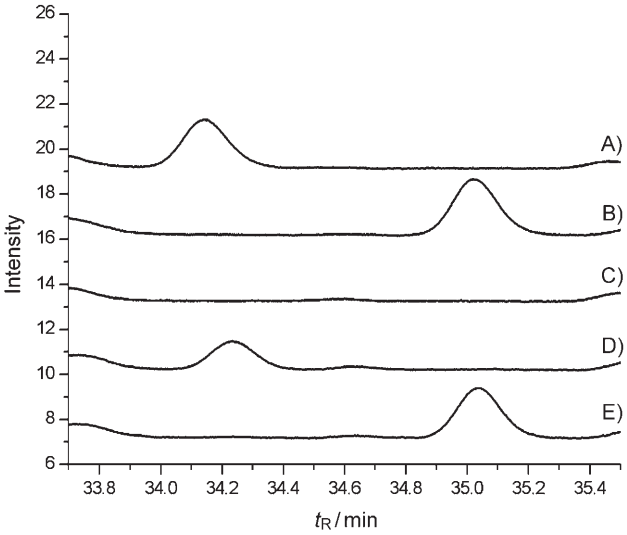
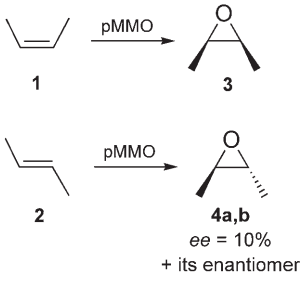


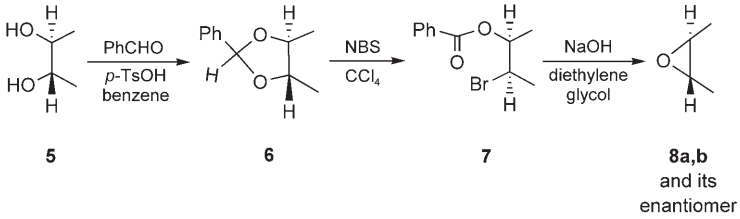
Figure 1. GC chromatograms A and B were obtained from authentic standards of (±)-*trans*-2,3-dimethyloxirane and *cis*-2,3-dimethyloxirane, respectively. Chromatograms D and E were obtained from the epoxidation products produced from pMMO with *trans*-but-2-ene and *cis*-but-2-ene as substrates. Chromatogram C was the control obtained when the bacterially mediated oxidation reaction was carried out in the absence of substrates.

35.0 min (Figure 1 E), respectively, and the products were identified as *trans*-2,3-dimethyloxirane **4a**/**4b** (D and L enantiomers, Figure 1 A) and *cis*-2,3-dimethyloxirane **3** (*meso* isomer, Figure 1 B) by comparison with authentic standards (Aldrich). The lack of diastereomeric products formed by the pMMO oxidation revealed that the olefin electrophilic epoxidation must proceed by a *syn* addition reaction mechanism.

To characterize the optical activity of the D,L-*trans*-2,3-dimethyloxirane formed from *trans*-but-2-ene in the pMMO-enriched cells further, we used published methods[21–23] (Scheme 2) to prepare (2*S*,3*S*)-2,3-dimethyloxirane for use as an authentic standard. Commercially available optically active *threo*-(2*S*,3*S*)-butane-2,3-diol (**5**, Aldrich) was used as the starting material and was treated with benzaldehyde and *p*-toluenesulfonic acid in a benzene solution under anhydrous conditions to form the corresponding (2*S*,3*S*)-4,5-dimethyl-2-phenyl-1,3-dioxolane (**6**). This was further treated with *N*-bromosuccinimide (NBS) in carbon tetrachloride to provide the optically



Scheme 1. pMMO-mediated epoxidation of *cis*- and *trans*-but-2-ene.



Scheme 2. The synthesis of authentic standards of (2*S*,3*S*)-2,3-dimethyloxirane (90%).

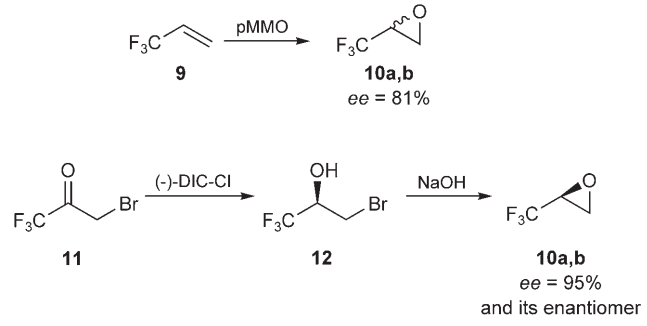
active intermediate, *erythro*-(2*S*,3*R*)-3-bromo-2-butyl benzoate (**7**) in 69% yield. On removal of the benzoyl group under basic conditions, the resulting anionic C2 alkoxy group back-attacked at the C3 carbon to form the desired authentic standard—*trans*-(2*S*,3*S*)-dimethyloxirane (**8b**)—in 90% *ee*.

The enantiomeric isomers of D- and L-dimethyloxirane could be conveniently resolved by gas chromatography on an Agilent 6890 *Plus* GC instrument fitted with a CHIRALDEX B-TA column manufactured by Astec Inc. (30 m × 0.25 mm × 0.125 μm film thickness, isothermal 30 °C). The retention times of the authentic standards of (2*R*,3*R*)- and (2*S*,3*S*)-*trans*-2,3-dimethyl oxirane isomers **8a** and **8b** were seen at $t_R = 14.2$ min and 14.5 min, respectively. The *trans*-but-2-ene epoxidation products derived from the pMMO oxidation were similarly analyzed by GC and also appeared at t_R 14.2 min and 14.5 min. The area integrations of the highlighted peaks intensity were 45 ± 1% and 55 ± 1%, respectively. On the basis of these results, we estimated an *ee* ratio of 10 ± 2% in favor of (2*S*,3*S*)-dimethyloxirane formation in the pMMO-catalyzed product, from the equation $ee = (SS-RR)/(SS+RR)$.

pMMO-mediated epoxidation of 3,3,3-trifluoroprop-1-ene and hydroxylation of 1,1,1-trifluoropropane

Because of the unique behavior of pMMO toward hydrophobic substrates, 1,1,1-trifluoropropane (**13**) and 3,3,3-trifluoroprop-1-ene (**9**) turned out to be excellent substrates for the enzyme. The fluorinated propane and propene were readily converted into the corresponding 2-alcohol and oxirane derivatives.

3,3,3-Trifluoroprop-1-ene: In order to characterize the enantiomeric excess determined for the pMMO-derived oxiranes **10a** and **10b**, we employed commercially available 3,3,3-trifluoroprop-1-ene as the substrate and used (−)-*B*-chlorodiisopinocamphenylborane ((−)-DIP-Cl; Aldrich), developed by H. C. Brown et al.,[24] to reduce 1-bromo-3,3,3-trifluoropropan-2-one (**11**; Scheme 3) stereoselectively to (*R*)-(−)-1-bromo-3,3,3-trifluoropropan-2-ol (**12**) as the major chiral species. After back-attack from the oxygen anionic species generated by the addition of sodium hydroxide, we successfully obtained (*S*)-(trifluoromethyl)oxirane (**10**) as our authentic chiral standard (95% *ee*).



Scheme 3. The conversion of 3,3,3-trifluoroprop-1-ene to (trifluoromethyl)oxirane (81% *ee*) by pMMO, and the synthesis of authentic standards of (*S*)-(trifluoromethyl)oxirane (95% *ee*).

The subsequent chiral resolution was carried out by gas chromatography (Agilent 6890 *Plus* GC fitted with a CHIRALDEX G-TA column manufactured by Astec Inc, 30 m × 0.25 mm × 0.125 μm film thickness, isothermal 28 °C; Figure 2). The retention times (t_R) of the racemic authentic standards (*R*)- and (*S*)-(trifluoromethyl)oxirane were 2.68 and 2.75 min, respectively. For the corresponding conversion mediated by pMMO with 3,3,3-trifluoroprop-1-ene as the substrate, the *ee* was determined to be 81% in favor of the formation of (*S*)-(trifluoromethyl)oxirane (Scheme 3).

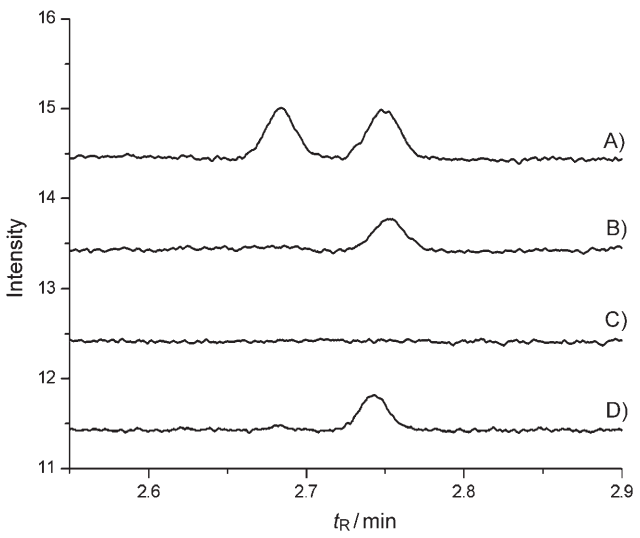
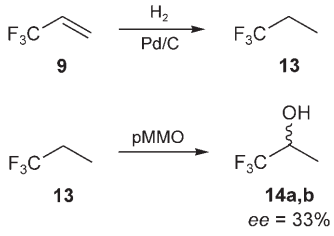


Figure 2. The epoxidation products from *M. capsulatus* (Bath) cells (grown in 30–40 μM $CuSO_4$) C) in the absence and D) in the presence of 3,3,3-trifluoroprop-1-ene. The GC chromatograms A and B are derived from the authentic standards of (±)- and (*S*)-(trifluoromethyl)oxirane synthesized by the method of H. C. Brown et al.,[24] respectively.

1,1,1-Trifluoropropane: To prepare 1,1,1-trifluoropropane (**13**; Scheme 4), we carried out a hydrogenation reaction in the presence of Pd/C catalyst to convert 3,3,3-trifluoroprop-1-ene (**9**, Aldrich) into the corresponding trifluorinated alkanes.[6]



Scheme 4. The synthesis of 1,1,1-trifluoropropane and the conversion of 1,1,1-trifluoropropane into (*S*)-1,1,1-trifluoropropan-2-ol (33% *ee*).

The absolute stereochemical identification of the product 1,1,1-trifluoropropan-2-ol obtained from pMMO oxidation was compared with the authentic standards in the form of the corresponding alcohol derivatives of mandelic acids. The 1,1,1-tri-

fluoropropan-2-ol standard **14a**/**14b** was synthesized by reduction of 1,1,1-trifluoropropan-2-one with $LiAlH_4$. The alcohols obtained were further derivatized with (*R*)-2-acetoxy-2-phenylethanoate in the presence of DCC and catalytic amount of DMAP. We separated the resultant diastereomers on an Agilent 1100 series HPLC instrument through a Spherical Silica (10 μm particle size, 10×250 mm column size) normal phase column (elution buffer: EA/hexanes 10:90). The two racemic fluorinated alcohols were resolved through the formation of the diastereomeric derivatives, which appeared separately at $t_R = 8.2$ and 9.1 min and could be fractionally collected for subsequent NMR studies. The results allowed us to verify that the methyl ^{1}H NMR absorption of the trifluoroisopropyl moiety of the corresponding mandelic acid derivative at $t_R = 8.2$ min was shifted upfield by 0.17 ppm relative to that of the derivative collected at $t_R = 9.1$ min. The same result was also obtained in the case of the butan-2-ol mandelic acid derivatives. Here the methyl group of the (*S*,*R*)-butan-2-ol derivative was shifted upfield by 0.17 ppm relative to that of the (*R*,*R*) derivative (data not shown).[7] From these data, we identified the absolute configuration of the diastereomer at $t_R = 8.2$ min as (*S*,*R*)-1′,1′,1′-trifluoroisopropyl-2-acetoxy-2-phenylacetic acid and the diastereomer at $t_R = 9.1$ min as (*R*,*R*)-1′,1′,1′-trifluoroisopropyl-2-acetoxy-2-phenylacetic acid. Chiral *O*-acetylmandelic acid has been extensively used as a chiral anisotropy reagent for the determination of the absolute configurations of chiral alcohols.[25] In the *S* configuration, the methyl moiety (the criterion is that the other ligand has priority over the methyl group for assigning chirality) will be shifted further upfield because of the shielding effects originating from the proximal phenyl ring.

After the identification of the absolute configurations of the two components of the racemic 1,1,1-trifluoropropan-2-ol standard mixture by derivatization, we were now able to measure the retention times of the corresponding authentic diastereomeric isomers by Agilent 6890 GC and HP-5 60 m column chromatography (60 m×0.25 mm×0.25 μm film thickness, isothermal 110 °C) column chromatography (Agilent 6890 GC and HP-5, 60 m×0.25 mm×0.25 μm film thickness, isothermal 110 °C). The retention times for the corresponding (*S*,*R*)- and (*R*,*R*)-mandelic acid derivatives are 41.2 min and 40.4 min, respectively. When the products obtained from oxidation of 1,1,1-trifluoropropane (**13**) by pMMO were analyzed by GC, the *ee* ratio of the 1,1,1-trifluoropropan-2-ol products **14a** and **14b** was 33± 1% in favor of the *S* configuration.

Finally, the absolute configurations of the products obtained on oxidation of 1,1,1-trifluoropropane by pMMO were verified by the synthesis of (*S*)-1,1,1-trifluoropropan-2-ol from the trifluorinated (*S*)-oxirane **10** (Scheme 2) by reduction with $LiAlH_4$.[25] When the corresponding (*S*,*R*)-mandelic acid derivative (98% abundance) was prepared, the appearance of the GC signal at a retention time of $t_R = 41.2$ min provided further confirmation of the stereochemical assignment of the 1,1,1-trifluoropropan-2-ol obtained from pMMO oxidation. The co-injection data are supplied as Supporting Information.

Discussion

The low chiral selectivity of olefinic epoxidation (*ee* = 10 ± 2%; Table 1) determined for *trans*-but-2-ene is readily accounted for in terms of poor discrimination between the *si* face and the *re* face during the "oxene" attack.[1] There was no evidence of the formation of *meso* products. When *cis*-but-2-ene was used as the substrate, we obtained only the *meso*-2,3-dimethyloxirane. These outcomes are totally to be expected in light of our earlier experiments on propene and but-1-ene. Taken together, these results allow us to conclude beyond reasonable doubt that the epoxidation reaction within pMMO proceeds by the *syn* addition reaction pathway, as would be expected if the hydroxylation of alkanes occurs by the concerted "oxene" addition across the C–H bond.[5,7,18]

The use of fluorinated substrates as mechanistic probes and inhibitors has proven to be a powerful method for the study of enzymatic transformations.[26] In pharmaceutical applications, the C–F bond is usually considered an effective bioisosteric group of either the C–H or C–OH bond. The resemblance between fluorine and oxygen in terms of electronegativity and van der Waals radii (4.0 and 1.47 Å vs. 3.5 and 1.57 Å) makes fluorine a possible hydroxyl group mimic.[27] Over the years, there have been many examples of enzyme probes and inhibitors in which a hydrogen has been substituted with a fluorine atom.[27,28] In fact, in pharmaceutical and membrane chemistry, perfluorinated substrates or inhibitors are often chosen to increase lipophilicity and oxygen solubility.[28,29]

In this work, we have replaced one of the methyl groups of propane and the single methyl group of propene with the trifluoromethyl group. These two substrates were readily taken up by pMMO and oxidized. Presumably, the low polarity of the perfluorohydrocarbon results in weak intermolecular London dispersive interactions between the fluorinated substrates and the enzyme.[28] From molecular modeling, the trifluoromethyl group should be comparable in size to the isopropyl group, so 1,1,1-trifluoropropane should behave like the branched hydrocarbon 2-methylbutane in terms of its ability to enter the hydrophobic pocket of pMMO. In the alkane series, only *n*-butane and *n*-pentane are accommodated within the active site, aside from methane, ethane, and propane, so it is quite astonishing that the 1,1,1-trifluoropropane is oxidized to 1,1,1-trifluoropropan-2-ol. However, with a 33 ± 1% *ee* value in favor of the formation of (*S*)-1,1,1-trifluoropropan-2-ol, the chiral selectivity for the 1,1,1-trifluoropropane is the opposite of that previously reported for *n*-butane. Even more interestingly, not only is 3,3,3-trifluoroprop-1-ene oxidized by pMMO, but the chiral selectivity is surprising high, at 81 ± 2% *ee* (Table 1).

The implication is that the hydrophobic cavity at the active site within pMMO can still accommodate a CF_3 group despite the congestion encountered. Although the C–F bond is presumably acting in these substrates as a bioisosteric group of a C–H bond, there must be a energetic preference in favor of one orientation of the substrate in the hydrophobic pocket. The obvious scenario is that the trifluoromethyl substrate is inserted into the hydrophobic pocket with the trifluoromethyl

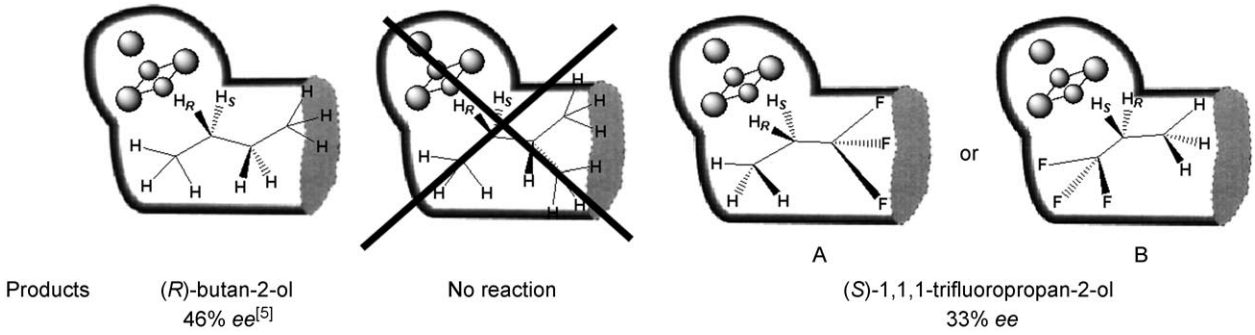


Figure 3. Spatial orientations of butane, 2-methylbutane, and 1,1,1-trifluoropropane in the putative hydrophobic pocket of pMMO. The copper atoms of the active site in pMMO are designated by ● and the oxygen atoms are designated by ●.

group located at the open edge. With the substrate embedded in this manner, steric hindrance could bias the orientation of the trifluoromethyl hydrocarbon such that the C2-H_S is more favorably directed towards the tricopper cluster at the active site for hydroxylation of the secondary carbon (Figure 3A). However, given the snugness of the fit near the open end of the pocket, when 1,1,1-trifluoropropane is inserted into the cavity with the fluorocarbon anchored more or less near the opening of the channel, the substrate might not penetrate deep enough into the cavity for O-atom transfer from the dioxygen-activated tricopper cluster to the secondary carbon of the fluorocarbon. Hydroxylation of the terminal methyl group would seem to be more likely under these circumstances, but we have obtained no evidence for the formation of 3,3,3-trifluoropropan-1-ol, the primary alcohol. The other scenario is that the 1,1,1-trifluoropropane or 3,3,3-trifluoroprop-1-ene is accommodated primarily with the bulkier CF_3 inserted into the depth of the cavity near the tricopper cluster, and that the long axis of the fluorocarbon is preferentially oriented for O-atom transfer with *si* facial addition rather than the *re* facial addition (Figure 3B). This scenario of 1,1,1-trifluoropropane being directed into the hydrophobic pocket of the active site would account for the observed 1,1,1-trifluoropropan-2-ol. In this connection, Dawson and co-workers[30] had previously reported unusual reactivity of cytochrome P450cam when 5,5-difluorocamphor was employed as the substrate. The regioselectivity of hydroxylation was found to be dramatically changed from 5-*exo* to C-9, which should be relatively higher in energy relative to activation of the primary methyl group. The amino acid residues Phe 87, Tyr96, and Phe98 within the substrate pocket of cytochrome P450cam seemed to exhibit significant control over the regioselective C–H activation of the camphor molecule. There is thus precedent for interaction of the trifluoromethyl group with aromatic side chains to redirect the orientation of a substrate within the hydrophobic pocket of the active site of an enzyme. Indeed, in our molecular modeling studies on the recent crystal structure of pMMO we have also noted that the active site of the enzyme consists of a hydrophobic pocket lined by four aromatic residues: Trp48, Phe50, Trp51, and Trp54 of the PmoA subunit (vide infra). Additionally, the size and shape of this hydrophobic cavity seem to control both the binding of hydrocarbon substrates and the regioselectivity of the oxidation. We thus surmise that the interactions between the trifluoromethyl group and the aromatic side chains forming the active site could steer the binding of the fluoro-hydrocarbon substrate so that the bulkier CF_3 becomes inserted into the depth of the cavity near the tricopper cluster.

In any case, regardless of the mode of insertion for 1,1,1-trifluoropropane or 3,3,3-trifluoroprop-1-ene, the inverse chiral selectivity observed for the hydroxylation of 1,1,1-trifluoropropane relative to *n*-butane, and the surprisingly high chiral selectivity observed for the epoxidation of 3,3,3-trifluoroprop-1-ene, are striking indeed. It is evident that substitution of the CF_3 group for a terminal methyl group in an aliphatic alkane or alkene can dramatically change the positioning of the substrate in the active site for hydroxylation and epoxidation. In this manner, interactions of the hydrophobic pocket with the substrate can dramatically change the nature of the induced stereoselectivity in the O-atom transfer reaction.

Finally, in order to provide a better structural framework for the discussion of the experimental observations reported in this study, we have made an effort to locate the hydrophobic pocket that might accommodate the various straight-chain hydrocarbons oxidized by the enzyme, including 1,1,1-trifluoropropane and 3,3,3-trifluoroprop-1-ene, within the protein structure of pMMO. To this end, we subjected the recently published crystal structure to Global Protein Surface Survey (GPSS) analysis on the GPSS web site (http://gpss.mcsg.anl.gov). The GPSS PyMOL plugin was applied to the pMMO protein monomer (PDB ID: WS_1YEW1), which was constructed from the PDB model of 1YEW. The calculations yielded 122 CASTp surfaces, but the most probable site was determined to be the hydrophobic pocket previously identified near Site D in the structure adjacent to the tricopper cluster[31] (Figure 4A). In support of this outcome of the GPSS analysis, the same binding site was also predicted by Dockligand (LigandFit) on Discovery Studio 1.7 manufactured by Accelrys Software, Inc. This putative hydrophobic pocket is sufficiently long to bind only C_1–C_5 hydrocarbons, and it is wide enough to accommodate only straight-chain alkanes or alkenes. The "channel", which appears to be more or less closed at one end, is lined by the aromatic residues Trp48, Phe50, Trp51, and Trp54 of PmoA, the 28 kDa subunit (Figure 4B). Interestingly, with the hydrocarbon substrate fully inserted into the pocket, the tricopper cluster is di-

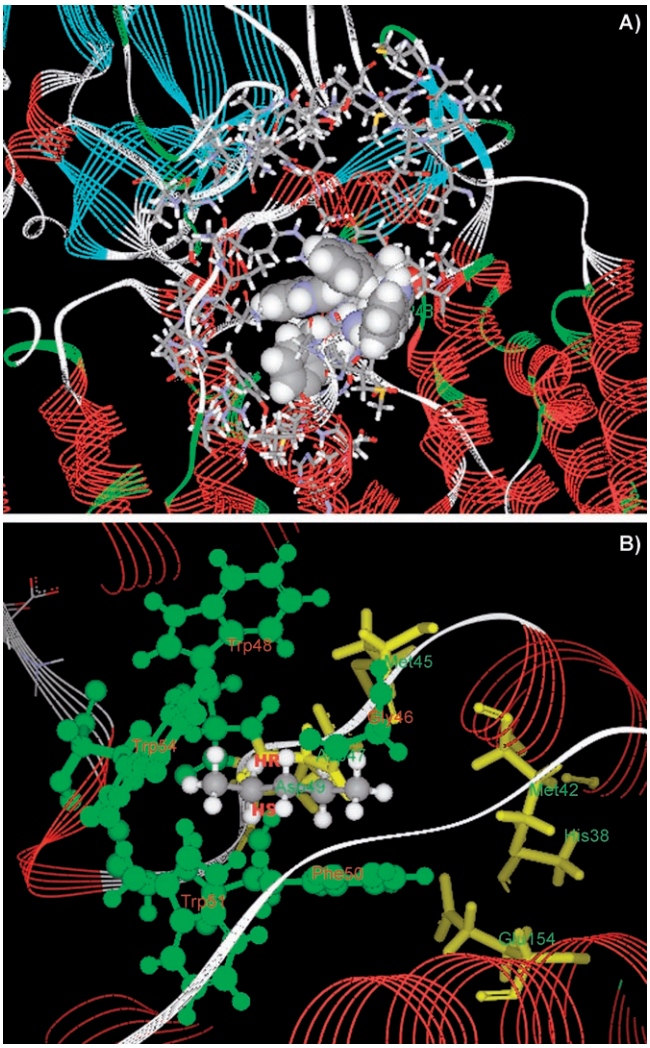


Figure 4. A) The hydrocarbon binding pocket inferred from Global Protein Surface Survey analysis of the published crystal structure of the pMMO protein monomer WS_1YEW1, of 1YEW from the RCSB Protein Data Bank on the GPSS website (http://gpss.mcsg.anl.gov). The putative hydrophobic pocket is represented by a CPK model of the aromatic side chains of the residues lining the cavity. B) The hydrophobic cavity is lined by the aromatic residues Trp48, Phe50, Trp51, and Trp54 of PmoA, with Gly46 located at the open entrance of the pocket. The dimensions of the cavity are just large enough to accommodate a C_1–C_5 straight-chain hydrocarbon. A CPK model of the pentane molecule in the hydrophobic cavity as predicted by Dockligand (LigandFit) is shown. The putative tricopper cluster is located in the D site of the crystal structure[32] just behind the pocket in close proximity. The orientation of the activated tricopper cluster controls the direct insertion of the harnessed "oxene" towards either the C2–H_R or –H_S atom of the depicted pentane.

rected at the secondary carbon of the substrate at the bottom, perfectly poised for O-atom transfer to this secondary carbon when the tricopper cluster is activated by dioxygen. Located at the open substrate entrance to the pocket is Gly46. It appears that this open end of the pocket is not flexible enough to accommodate a trifluoromethyl group or the bulky end of 2-methylbutane. In any case, our proposed mode of binding of 1,1,1-trifluoropropane or 3,3,3-trifluoroprop-1-ene with the bulkier CF_3 inserted deep in the cavity near the tricopper cluster and away from the opening end of the hydrophobic pocket is consistent with the observed stereoselectivity of the O-atom transfer chemistry. The different ability of the hydrophobic pocket to accommodate *n*-butane, 2-methylbutane, and 1,1,1-trifluoropropane is illustrated schematically in Figure 3.

Experimental Section

Whole-cell conversions of aliphatic alkanes and alkenes by pMMO:[7] The growth of pMMO-enriched *Methylococus capsulatus* (Bath) followed published procedures.[32] *M. capsulatus* (Bath) cell solution (0.5 mL, grown in the presence of 30–40 μM $CuSO_4$) was added to a Pipes buffer (25 mM, 4.0 mL) containing $CuSO_4$ (100 μM) and sodium formate (3.0 mM, 0.5 mL), and the mixture was incubated at 42 °C under a gas mixture consisting of air and compounds **1**, **2**, **9**, or **13** at a composition of 50:50 volume ratio. After 2.0–6.0 h incubation, the cell mixture was withdrawn (0.70 mL aliquots) and transferred into 1.5 mL Eppendorf tubes. Methylene chloride (0.70 mL) was then added to each Eppendorf tube, and the mixture was spun down with a microcentrifuge at 8000 rpm for 5 min. The organic layer was dried over anhydrous $MgSO_4$ and filtered through a pad of silica gel. These filtrates were subsequently used for ascertainment of stereochemistry by comparison with authentic standards.

Determination of the optical activities of epoxidation products of *trans*-but-2-ene

*Preparation of (4S,5S)-4,5-dimethyl-2-phenyl-1,3-dioxolane (**6**)*:[23] Commercial redistilled benzaldehyde (500 mg, 4.7 mmol, 1.2 equiv, Aldrich) was added to a benzene solution (10 mL) containing *trans*-(2*S*,3*S*)-butane-2,3-diol (353 mg, 3.9 mmol, 1.0 equiv, Aldrich) together with a catalytic amount of *p*-toluenesulfonic acid (ACROS, Inc.) dissolved in benzene (10 mL). The system was then fitted with a Dean–Stark water separator apparatus and heated at reflux overnight. The reaction was quenched by the addition of extra Na_2CO_3 (5 mg), and the mixture was then fractionated at reduced pressure to provide (2*S*,3*S*)-4,5-dimethyl-2-phenyl-1,3-dioxolane (642 mg, 3.6 mmol, 92% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.31 (d, J = 5.8 Hz, 2H), 1.37 (d, J = 5.8 Hz, 2H), 3.80 (m, 2H), 5.93 (s, 1H), 7.35 (m, 3H) 7.47 ppm (d, J = 7.8 Hz, 2H); ^{13}C NMR ($CDCl_3$): δ = 16.9, 17.2, 78.6, 80.4, 103, 127, 128, 129, 139 ppm.

*Preparation of erythro-(2S,3R)-3-bromo-2-butyl benzoate (**7**)*:[21] The levorotatory dioxolane (510 mg, 2.9 mmol, 1.0 equiv) in carbon tetrachloride (1.0 mL) was added dropwise to a solution of *N*-bromosuccinimide (NBS, 500 mg, 2.9 mmol, 1.0 equiv, Fluka, Inc.) in carbon tetrachloride (5 mL), cooled in an ice bath. The mixture was stirred in the dark for 48 h at room temperature, cooled in an ice bath, and filtered. Distillation of the residue yielded the corresponding product (516 mg, 2.0 mmol, 69% yield). bp 80–90 °C (0.15 mmHg); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.43 (d, J = 6.3 Hz, 2H), 1.71 (d, J = 6.9 Hz, 2H), 4.32 (m, 1H), 5.16 (m, 1H), 7.42 (t, J = 7.8 Hz, 2H), 7.54 (t, J = 7.4 Hz, 1H) , 8.05 ppm (d, J = 8.2 Hz, 2H); ^{13}C NMR ($CDCl_3$): δ = 16.4, 21.6, 51.6, 73.4, 128, 129, 130, 133, 166 ppm.

*Preparation of trans-(2S,3S)-2,3-dimethyloxirane (**8**)*:[23] (2*S*,3*R*)-2-(3-Bromobutyl) benzoate (257 mg, 1.0 mmol, 1.0 equiv) was added under ambient conditions to a solution containing sodium hydroxide (91 mg, 2.3 mmol) in diethylene glycol (1.0 mL) in a 10 mL round-bottomed flask. The mixture was heated at reflux for 3.0 h and then cooled in ice bath, and methylene chloride (2.0 mL) was added. The reaction mixture was then distilled in a 10 mL round-

bottomed flask fitted with a −70 °C condenser to afford the product solution, consisting of *trans*-(2*S*,3*S*)-dimethyloxirane in methylene chloride. The identification and characterization of the synthetic stereoisomer was ascertained by co-injection with authentic standards of enantiomeric *trans*-dimethyloxirane (Aldrich). The t_R values of the *trans*-(2*R*,3*R*)- and *trans*-(2*S*,3*S*)-dimethyloxirane as determined by gas chromatography on a CHIRALDEX B-TA column (30 °C, isothermal) are 14.20 min and 14.48 min, respectively.

Preparation of the authentic standards of (*S*)-(−)-(trifluoromethyl)oxirane and (±)-(trifluoromethyl)oxirane

*Synthesis of (S)-(−)-2-(trifluoromethyl)oxirane (**10**)*:[24] The preparation of chiral (*R*)-(−)-1-bromo-3,3,3-trifluoropropan-2-ol followed the procedure developed by H. C. Brown et al.[24] (−)-DIP-Cl (3.60 g, 11 mmol, 1.2 equiv, Aldrich) was placed in a 50 mL round-bottomed flask containing anhydrous ether (15 mL) under nitrogen gas at room temperature. The mixture was stirred until the solids were completely dissolved. The reaction temperature was then lowered to −20 °C, and 1-bromo-3,3,3-trifluoropropan-2-one (1.80 g, 9.4 mmol, 1.0 equiv, Aldrich) was added to the reaction mixture. The solution was stirred for another 72 h to allow the reaction to continue. After the reaction was complete, the mixture was exposed to the air, and a white precipitation formed. The mixture was then filtered, and the precipitate was washed three times with diethyl ether. The filtrates were dried over anhydrous $MgSO_4$ and concentrated to afford a light yellow and viscous oil containing the corresponding alcohols. The mixture was then added dropwise at 40 °C to preheated NaOH (50% *w/w*, 15 mL) in a two-necked 25 mL round-bottomed flask fitted with a stirrer and short-path distillation apparatus. While the addition was proceeding, the reaction temperature was maintained below 65 °C. The formation of a yellow-orange solid precipitate indicated that the epoxide products had been formed. We obtained (*S*)-(trifluoromethyl)oxirane as a colorless liquid (330 mg, 2.9 mmol, 95% *ee*) in 31% yield (not optimized) by distillation at 32–42 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): $\delta = 2.90$ (td, $J = 3.75$ Hz, 1.23 Hz, 1H), 2.96 (dd, $J = 4.88$ Hz, 2.09 Hz, 1H), 3.40 ppm (m, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz): $\delta = 43.2$ (t, $J = 2.69$ Hz), 48.2 (q, $J = 41.3$ Hz), 123 ppm (q, $J = 273$ Hz); EI-MS *m/z* (relative intensity): 112 $[M]^+$ (100), 93 (17), 82 (31), 69 (42), 64 (27), 51 (26), 43 (17), 29 (31).

*Synthesis of racemic 2-(trifluoromethyl)oxirane (**10**)*: 1-Bromo-3,3,3-trifluoropropan-2-one (500 mg, 2.6 mmol, 1.0 equiv) was added to dried methanol (5.0 mL). A reaction mixture containing calcium chloride (290 mg, 2.7 mmol, 1.0 equiv) and sodium borohydride (200 mg, 5.4 mmol, 2.1 equiv) in tetrahydrofuran (5.0 mL) was then added to the methanol solution mixture. The reaction mixture was incubated in an ice-bath for 3 h, and saturated ammonium chloride solution (5.0 mL) and ethyl acetate (10 mL) were then added. After 30 min, concentrated hydrochloric acid (3–5 mL) was slowly added. The reaction mixture was extracted twice with ethyl acetate, and the organic layer was washed with saturated sodium bicarbonate solution (1 ×), water (1 ×), and brine (1 ×). The washed organic layer was dried in an oven with magnesium sulfate, and was concentrated to yield a crude mixture of 1-bromo-3,3,3-trifluoropropan-2-ols. Treatment of the corresponding alcohols with sodium hydroxide led to the desired colorless liquid, (±)-(trifluoromethyl)-oxirane (133 mg, 1.2 mmol, 46% yield, not optimized). ^{1}H NMR ($CDCl_3$, 500 MHz): $\delta = 2.90$ (tt, $J = 3.78$ Hz, 1.18 Hz, 1H), 2.96 (dd, $J = 5.02$ Hz, 2.19 Hz, 1H), 3.40 ppm (m, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz): $\delta = 43.2$ (s), 48.2 (q, $J = 41.0$ Hz), 122 ppm (q, $J = 273$ Hz); EI-MS: *m/z* (relative intensity): 112 $[M]^+$ (100), 93 (16), 82 (30), 69 (41), 64 (26), 51 (25), 43 (16), 29 (31).

*The synthesis of 1,1,1-trifluoropropane (**13**)*:[6] The hydrogenation of 3,3,3-trifluoroprop-1-ene followed the method developed earlier by D.-H. Huang et al.[6] A pad of palladium on activated carbon (Pd/C) was placed in a glass tube with two open ends stopped by septa. Two balloons were connected to the two ends of the Pd/C glass tube. One of the balloons was filled with hydrogen gas (1 atm), and the other with 3,3,3-trifluoroprop-1-ene gas (Aldrich). The contents of the two balloons were pushed from one side to the other through the glass tube three to five times. The completion of hydrogenation reaction could be monitored by the heat released from the Pd/C filled glass tube. While the sizes of the two balloons had reduced to half of their initial volumes, it indicated that the reaction was finished: ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 1.09$ (t, $J = 7.56$ Hz, 3H), 2.07 ppm (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 6.15$ (s), 27.4 (q, $J = 29.0$ Hz), 128 ppm (q, $J = 275$ Hz); EI-MS: *m/z* (relative intensity): 98 $[M]^+$ (0.51), 79 (100), 78 (42), 77 (80), 69 (53), 59 (37), 51 (43), 29 (75).

Table 1. The hydroxylation and epoxidation products derived from reactions of a number of substrates with pMMO.

Substrates	Products	Dominant stereoisomer	*ee* [%]
cis-but-2-ene	*cis*-2,3-dimethyloxirane	n.d.[a]	n.d.[a]
trans-but-2-ene	(2*S*,3*S*)-*trans*-2,3-dimethyloxirane	*S*,*S*	10 ± 2
1,1,1-trifluoropropane	1,1,1-trifluoropropan-2-ol	*S*	33 ± 1
3,3,3-trifluoroprop-1-ene	(trifluoromethyl)oxirane	*S*	81 ± 2

[a] The products here appeared only as *meso* products.

Preparation of authentic standards of 1,1,1-trifluoropropan-2-ol (14) and trifluorinated propanol mandelic acid derivatives

*Synthesis of the authentic standards of 1,1,1-trifluoropropan-2-ol (**14**)*: 1,1,1-Trifluoropropan-2-one (2.50 g, 22 mmol, 1.0 equiv) was added dropwise under argon to a slurry of lithium aluminum hydride ($LiAlH_4$, 1.10 g, 29 mmol, 1.3 equiv) in anhydrous diethyl ether (20 mL) in a 250 mL round-bottomed flask in an ice bath. After 18 h stirring at room temperature, the reaction mixture was cooled in ice bath again. Deionized water (0.5 mL), sodium hydroxide (10%, 1.0 mL), and deionized water (5.0 mL) were then slowly added in sequence; 10 min later, the pale color of the precipitate had changed to white. The resulting suspension was filtered. The filtrate was dried over anhydrous $MgSO_4$, and was then concentrated to afford a colorless solution of the desired product (1.37 g, 12 mmol, 55% yield, not optimized.): ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 1.30$ (d, $J = 6.6$ Hz, 3H), 4.02 ppm (m, 1H); EI-MS: *m/z* (relative intensity): 113 $[M-1]^+$ (0.72), 99 (19), 79 (18), 69 (15), 51 (16), 47 (17), 45 (100), 43 (20), 29 (13).

*Synthesis of the authentic standard of (S)-1,1,1-trifluoropropan-2-ol (**14**)*: By the method of H. C. Brown et al.,[24] a solution of (*S*)-oxirane **10** (110 mg, 1.0 mmol, 1.0 equiv) (95% *ee*) in diethyl ether (5 mL) was added dropwise under argon to a stirred solution of $LiAlH_4$ (49 mg, 1.3 mmol, 1.3 equiv) in anhydrous diethyl ether (5 mL). After 1.5 h stirring at room temperature, the reaction mixture was added dropwise to wet diethyl ether (10 mL), treated with sodium hydroxide solution (0.1 N), and washed with diethyl ether. The organic layers were collected, dried over anhydrous $MgSO_4$, and concentrated to afford the alcohol **14** (77 mg, 0.68 mmol) in 68% yield.

Synthesis of the diastereomeric authentic standards of the trifluorinated propanol mandelic acid derivatives:[7] (*R*)-*O*-Acetylmandelic acid ((*R*)-2-acetoxy-2-phenylethanoic acid, 83 mg, 0.43 mmol, 1.0 equiv) and 4-(dimethylamino)pyridine (DMAP, 4.0 mg) were mixed at −40 °C in dichloromethane (2.0 mL) in a 10 mL round-bottomed flask. Over the next 5 min period, a solution of dicyclohexylcarbodiimide (DCC, 95.8 mg, 0.47 mmol, 1.1 equiv) in methylene chloride (0.50 mL) was added dropwise to the flask. A white precipitate had resulted after 10 min. A solution of 1,1,1-trifluoropropan-2-ol (50 mg, 0.43 mmol, 1.0 equiv) in dichloromethane (1.0 mL) was then added over a second 5 min period, and the mixture was stirred overnight at room temperature. The suspension was filtered through a pad of silica gel and washed with methylene chloride (5–10 mL). The filtrate was evaporated to near dryness under a dinitrogen stream, and the residue was suspended in methylene chloride (0.5 to 1.0 mL).

This mixture was further purified by HPLC column chromatography (Agilent 1100 series adapted with Spherical Silica, 10 μm, 250× 10 mm, Silicycle Co.). EA/hexanes 10:90 was used as the elution buffer, and elution of the products was monitored by the UV absorption at 270 nm. The retention times of the resolved diastereomers (*S,R*)-1′,1′,1′-trifluoroisopropyl-2-acetoxy-2-phenylacetic acid and (*R,R*)-1′,1′,1′-trifluoroisopropyl-2-acetoxy-2-phenylacetic acid appeared at 8.2 and 9.1 min, respectively.

(*S,R*)-1′,1′,1′-Trifluoroisopropyl-2-acetoxy-2-phenylacetic acid: ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 1.24$ (d, $J = 6.52$ Hz, 3H), 2.18 (s, 3H), 5.27 (m, 1H), 5.92 (s, 1H), 7.42 ppm (m, 5H); EI-MS: *m/z* (relative intensity): 290 $[M]^+$ (0.64), 248 (28), 176 (17), 149 (37), 107 (100), 105 (30), 77(25), 43 (63).

(*R,R*)-1′,1′,1′-Trifluoroisopropyl-2-acetoxy-2-phenylacetic acid: ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 1.41$ (d, $J = 6.56$ Hz, 3H), 2.19 (s, 3H), 5.25 (s, 1H), 5.90 (s, 1H), 7.35 ppm (m, 5H); EI-MS *m/z* (relative intensity): 290 $[M]^+$ (0.64), 248 (28), 176 (17), 149 (36), 107 (100), 105 (31), 77 (25), 43 (63).

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Keywords: C–H activation · chemoselectivity · enantioselectivity · fluorinated alkanes and olefins · olefin epoxidation

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