A Mechanistic Comparison between Cytochrome P_{450}- and Chloroperoxidase-Catalyzed *N*-Dealkylation of *N*,*N*-Dialkyl Anilines

Mehul N. Bhakta[a] and Kandatege Wimalasena*[a]

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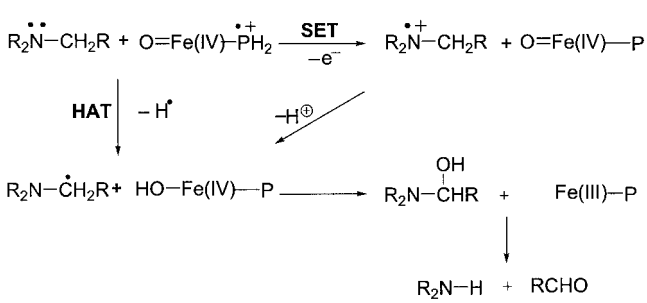
Most peroxidases use histidine as an axial ligand for heme, while chloroperoxidase (CPO) uses a thiolate, which is similar to the ligand employed by cytochrome P_{450} (P_{450}). Several studies have also shown that, unlike other peroxidases, CPO is capable of carrying out monooxygenation reactions in a similar manner to P_{450} in addition to typical peroxidase-like reactions. These observations have been attributed to the similarities of the active-site architecture of the two enzymes. Both enzymes have been shown to efficiently catalyze the oxidative *N*-dealkylation of amines. The similar magnitudes of the kinetic isotope effects determined for P_{450}- and CPO-catalyzed *N*-dealkylation of *N*,*N*-dimethylaniline have been used to propose that these reactions proceed through similar mechanisms. In this study, we have examined the mechanism of CPO-catalyzed *N*-dealkylation using a series of radical probes, 4-chloro-*N*-cyclopropyl-*N*-alkylanilines **1**–**3**, which we have recently used in the mechanistic studies of P_{450}, and compared the results with those of P_{450}-catalyzed reactions. The results show that P_{450}- and CPO-catalyzed reactions proceed through distinctly different mechanisms. As previously reported, while P_{450}-catalyzed reactions appear to proceed through a C_α-hydrogen abstraction mechanism, CPO-catalyzed reactions proceed through a single electron/proton transfer (SET/H^+) mechanism, similar to reactions catalyzed by Horseradish peroxidase (HRP). Thus, CPO may not be a good mechanistic model for P_{450}-catalyzed *N*-dealkylations.(© Wiley-VCH Verlag GmbH & Co. KGaA, 69451 Weinheim, Germany, 2005)

Introduction

Chloroperoxidase (CPO) from *Caldariomyces fumango* is an interesting heme peroxidase which uses cysteine as an axial ligand,[1–3] while most other peroxidases use histidine for this purpose. Thus, the active site of CPO is very similar to those of the ubiquitous cytochrome P_{450} (P_{450}) enzymes. Similarly, and in contrast to other peroxidases, CPO is known to carry out both typical peroxidase-type oxidations and more rare oxygen-transfer reactions including hydroxylation of alkanes,[4] sulfoxidations of sulfides, *N*-dealkylation and *N*-oxygenation of alkylamines,[5–6] and epoxidations of olefins[7–8] in a similar manner to P_{450}. The oxygen atom present in the products of these reactions has been shown to originate exclusively from the oxidant, as in the case of typical monooxygenation reactions carried out by P_{450} enzymes.[3–8] One of the major differences between CPO- and P_{450}-enzyme catalysis is the source of oxygen in their catalytic cycles. While CPO employs hydrogen peroxide as the source of oxygen, P_{450} enzymes employ molecular oxygen and an ancillary reduction system that provides reducing equivalents from NAD(P)H. These similarities, along with the convenient isolation and spectroscopic characterization of a compound I type terminal active oxygen species in the CPO catalytic cycle, make chloroperoxidase a good model for the active oxygen species of P_{450} reactions.[9–11]

P_{450}-catalyzed *N*-dealkylations of *N*,*N*-dialkylamines have been proposed to proceed through either an electron/proton transfer (SET/H^+) or a hydrogen-atom-abstraction (HAT) mechanism, as shown in Scheme 1. Evidence for the SET/H^+ mechanism has been derived from studies with chemically and mechanistically well-characterized peroxidase model systems [e.g. horseradish peroxidase. (HRP)],[12] kinetic isotope effects,[12] redox-potential correlations,[13] and irreversible enzyme inactivation by various radical probes.[14–15] However, recent mechanistic studies



Scheme 1.

[a] Wichita State University, Department of Chemistry
1845 Fairmount St., Wichita, Kansas 67260, USA
Fax: +1-316-978-3431
E-mail: kandatege.wimalasena@wichita.edu

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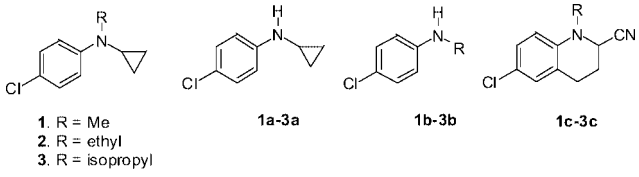
with sensitive radical probes[16] and kinetic isotope effect studies[17] have indicated that a HAT mechanism could be operative in P_{450}-catalyzed *N*-dealkylation reactions. In contrast to P_{450}-catalyzed *N*-dealkylations, relatively few mechanistic studies have been carried out with CPO-catalyzed *N*-dealkylations, and thus the detailed mechanism has not been completely elucidated. However, a mechanism similar to the SET mechanism proposed for P_{450} was thought to be operative in CPO-catalyzed *N*-dealkylations, primarily on the basis of the similar magnitudes of the kinetic isotope effects.[18–19]

We have recently shown that P_{450}- and HRP-catalyzed *N*-dealkylations of the radical probes **1**–**3** proceed through distinct HAT and SET pathways, producing cyclopropyl ring-unopened and -opened products, respectively (Scheme 2).[16] In the present study, we have investigated the product profiles of the CPO-catalyzed *N*-dealkylations of **1**–**3** in order to test the above proposal that the mechanisms of the *N*-dealkylations catalyzed by P_{450} and CPO are mechanistically similar. The results show that the CPO-catalyzed *N*-dealkylations of **1**–**3** give product profiles that are diagnostic of a SET mechanism parallel to that of HRP. These are clearly distinct from those of the P_{450}-NADPH-mediated reactions. These results suggest that the mechanism of the CPO-catalyzed *N*-dealkylation closely resembles that of HRP rather than P_{450}. Thus, the active-site constraints, rather than the electronics of the axial thiolate ligand, play an important role in CPO-catalyzed reactions, suggesting that CPO is not a good model for P_{450}-catalyzed *N*-dealkylations.



Scheme 2.

Results and Discussion

Incubation of *N*-alkyl-*N*-cyclopropyl-*p*-chloroaniline derivatives **1**–**3** with P_{450}-2B1 gave two sets of products which were identified as the *N*-cyclopropyl-*p*-chloroanilines **1a**–**3a**, and the *N*-alkyl-*p*-chloroanilines **1b**–**3b**, where the partition ratios were dependent on the nature of the *N*-substituent (Scheme 2 and Table 1). The intramolecular isotope effects (k_H/k_D) for the P_{450}-2B1-catalyzed cleavage of the *N*-ethyl and *N*-cyclopropyl groups of **2**, which were determined from the partition ratios of deuterated derivatives, were 2.6 ± 0.1 and 2.9 ± 0.1, respectively. The k_H/k_D for the cleavage of the *N*-isopropyl and *N*-cyclopropyl groups of **3** were 2.9 ± 0.1 and 3.0 ± 0.1, respectively. Furthermore, no detectable cyclopropyl ring-opened or ring-opened radical cyclized products **1c-3c**, were observed in the reaction mixture when the P_{450}-2B1 reactions were carried out in the presence or absence of CN^- in the incubation medium.[16] In contrast to the product profiles of the P_{450} reactions, the CPO-catalyzed oxidations of **1**–**3** exclusively produced the *N*-cyclopropyl-cleaved products **1b**–**3b**, with no detectable *N*-methyl-, -ethyl-, or -isopropyl-cleaved products. In addition, in the presence of CN^- in the incubation medium, the cyclopropyl ring-opened, radical-cyclized CN^- adducts[20] were detected as the major products in the CPO reaction mixtures, similar to the products of parallel HRP reactions (Table 1 and Scheme 3). These results demonstrate that while the HRP- and CPO-catalyzed oxidations of **1**–**3** exclusively proceed through a cyclopropyl ring-opening followed by a radical cyclization pathway, P_{450} reactions proceed through a pathway that does not involve the opening of the cyclopropyl ring, but involves the isotope-sensitive removal of C_α-H from both *N*-alkyl and *N*-cyclopropyl substituents (Scheme 3). On the basis of previous studies,[16] we conclude that the observed differences in the product distributions between the P_{450} and CPO reactions, with respect to the substrates above, must be due to the fundamental differences in the chemistry of these two systems. Thus, while P_{450}-catalyzed monooxygenations of these substrates exclusively proceed through a HAT mechanism, CPO-catalyzed oxidations exclusively proceed through an initial SET from the benzylic nitrogen followed by fast opening of the cyclopropyl ring. These results further suggest that the rate of the cyclopropyl ring-opening is greater than that of the C_α-deprotonation of the *N*-methyl, -ethyl, -isopropyl or -cyclopropyl groups of the nitrogen radical cation intermediate of these substrates under both CPO and HRP turnover conditions (Scheme 3). These findings are consistent with the fast ring-opening rates predicted for the nitrogen radical cation intermediates of *N*-cyclopropylamines (Scheme 3).[21–22]

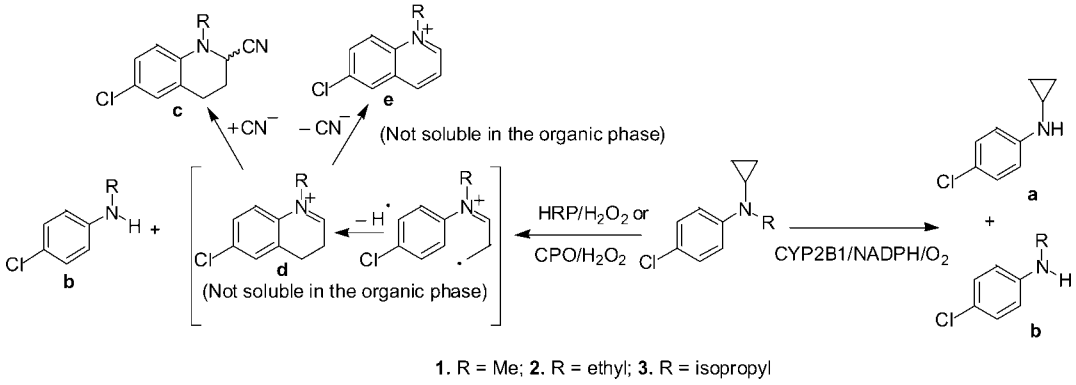
The proposed SET/H^+ mechanism of hemo-protein-catalyzed *N*-dealkylations assumes that a $[P–FeO]^{2+}$ species, rather than a protein-derived base, is responsible for the C_α-deprotonation of the nitrogen radical cation intermediate.[19,23] Since the above results strongly suggest that CPO-catalyzed *N*-dealkylations of **1**–**3** proceed through a SET mechanism, the $[P–FeO]^{2+}$ species of CPO could also be capable of abstracting a proton from the SET intermediate, the nitrogen radical cation, under normal circumstances. However, the observed product profiles show that the rate of C_α-deprotonation of the nitrogen radical cation intermediate of **1**–**3** is much slower than that of the cyclopropyl ring-opening in CPO-catalyzed reactions. Therefore, if the $[P–FeO]^{2+}$ species is in fact the putative base in CPO, which is responsible for the deprotonation of C_α-H, then it must be a relatively weak base.[17,24] On the other hand, the preferential opening of the cyclopropyl ring in the CPO-catalyzed oxidations of **1**–**3** could also be due to the inaccessiblity of the C_α-H to the $[P–FeO]^{2+}$ species of the CPO active site because of steric constraints. Therefore, further studies are certainly necessary to distinguish between these possibilities.

Taken together, the above results show that CPO- and P_{450}-catalyzed *N*-dealkylations of **1**–**3** are mechanistically distinct. In contrast to previous proposals, while P_{450}-cata-

Table 1. Product distribution of P_{450}-, HRP-, and CPO-catalyzed *N*-dealkylations of **1**–**3**.

	Product Distribution[a]								
Reaction	**1a**	**1b**	**1c**	**2a**	**2b**	**2c**	**3a**	**3b**	**3c**
P_{450}[b]	90(2)	10(2)	–	47(1)	53(1)	–	30(1)	70(1)	–
P_{450}/CN[c]	94(1)	6(1)	ND	48(1)	52(1)	ND	27(2)	73(2)	ND
HRP[b]	ND	>98	–	ND	>98	–	ND	>98	–
HRP/CN[c]	ND	5(1)	95(1)	ND	8(2)	92(2)	ND	32(1)	68(1)
CPO[b]	ND	>98	–	ND	>98	–	ND	>98	–
CPO/CN[c]	ND	4(1)	96(1)	ND	17(2)	83(2)	ND	35(2)	65(2)

[a] The product ratios were averages of at least three independent determinations. The standard deviations for the last significant figures are given in parentheses. **a**. 4-chloro-*N*-cyclopropylaniline; **b**. 4-chloro-*N*-alkylaniline; **c**. CN adduct. [b] The% products were calculated on the basis of the detectable products **a** and **b** (Scheme 3). [c] The% product ratios were calculated assuming 100% trapping of **d** (Scheme 3) by CN^-. ND: not detected.



Scheme 3.

lyzed reactions proceed through a C_α-hydrogen-abstraction mechanism, CPO-catalyzed reactions proceed through a SET mechanism. Therefore, the magnitudes of the kinetic isotope effects alone could clearly not be used to distinguish between these two mechanisms. The mechanistic similarity of *N*-dealkylation catalyzed by CPO to that catalyzed by HRP rather than P_{450}, suggests that the steric rather than electronic constraints of the active site may play an important role in CPO-catalyzed reactions. Therefore, although the active-site architecture of CPO, especially with respect to the axial heme ligand, is similar to that of P_{450}, it is not a good model for P_{450}-catalyzed *N*-dealkylations of *N*,*N*-dialkylanilines.[27] Detailed isotope effect studies of the P_{450}-, CPO-, and HRP-catalyzed *N*-dealkylations of *N*,*N*-dialkylaniline derivatives are in progress to determine the origin of the similarities and differences of the kinetic isotope effects between the *N*-dealkylation reactions catalyzed by these systems.

Experimental Section

Materials: Substrates and chromatographic standards were synthesized according to literature procedures as described previously[25] and purified by preparatory reverse-phase HPLC. Dilauryl-L-α-phosphatidylcholine (DLPC), NADPH, chloroperoxidase (CPO; EC 1.11.1.10), and horseradish peroxidase (HRP; EC 1.11.1.7, RZ = 3.1) were purchased from Sigma–Aldrich. Catalase was purchased from Roche Applied Sciences. Cytochrome P_{450}-2B1 and NADPH-P_{450}-reductase were purified as reported previously.[26] ^{1}H- and ^{13}C NMR spectra were recorded with a Varian/Inova-300 spectrometer or a Varian/Nicolet-400 spectrometer. All NMR spectra were recorded in $CDCl_3$ using TMS (δ =0.0 ppm) as an internal standard. Proton chemical shifts (δ) are reported in parts per million (ppm) downfield from TMS. Proton–proton coupling constants are reported in Hertz (Hz) and reflect assumed first-order behavior. Splitting patterns are designated as *s*, *singlet*; *d*, *doublet*; *t, triplet*; *q*, *quartet*; *sept*, *septet*; *m*, *multiplet*; and *br*, *broad*. Carbon chemical shifts are reported in ppm relative to $CDCl_3$ (δ = 77.30 ppm) as an internal standard. GC analyses were performed using a flame ionization detector with an Agilent 6890 series instrument (30 m × 0.320 mM carbon-packed capillary column) with a temperature program of 100 °C for 3 min, 15 °C/min gradient from 100 °C to 280 °C and 280 °C for 10 min. GC–MS analyses were performed with a Varian CP 3800 GC interfaced with a Varian-Saturn Model 2200 GC/MS/MS (30 m × 0.25 mM carbon-packed capillary column) and the product mixtures were separated using a temperature gradient similar to that above, and the spectra of desired products were recorded and analyzed using standard software.

Enzymatic Incubation and Reactions

P_{450}-2B1 Reactions: Enzymatic reactions were carried out in a total volume of 250 μL. The enzyme reaction mixtures containing (in the order of addition) cytochrome P_{450} (2 μM), cytochrome P_{450}-reductase (4 μM), and DLPC (360 μM) were pre-incubated for 45 min on ice. After the pre-incubation, catalase (50 μL, 1 mg/mL), potassium phosphate buffer (50 μL, 0.5 M, pH 7.4), H_2O (28 μL), and substrate (4 μL, 0.5 M) were added and incubated at 30 °C for 5 min. The reactions were initiated by adding NADPH to a final concentration of 1.2 mM and incubated for 30 min at 30 °C. The reactions were terminated by adding saturated K_2CO_3 (150 μL), and the products were extracted with ethyl acetate (500 μL) with *N*,*N*-diisopropylaniline as the internal standard, dried with a

stream of air, and dissolved in methanol. The products of this procedure were analyzed by GC and GC–MS.

Horseradish Peroxidase (HRP) Reactions: Enzymatic oxidations with HRP were carried out in potassium phosphate buffer (0.4 M, pH 5.5) containing HRP (52 nM) and substrate (4 mM) in a total volume of 250 μL. The reactions were initiated by adding hydrogen peroxide to a final concentration of 4 mM. The reaction mixtures were incubated for 10 min at room temperature and were quenched. The products were extracted and analyzed as described above.

Chloroperoxidase (CPO) Reactions: Chloroperoxidase reactions were carried out in a total volume of 250 μL of potassium phosphate buffer (0.1 M, pH, 6.0) containing CPO (20 units) and substrate (4 mM). The reactions were initiated by adding hydrogen peroxide to a final concentration of 4 mM, and the reaction mixtures were incubated at room temperature for 30 min. Products were extracted, identified, and quantified by GC–FID and GC–MS.

Determination of Intramolecular Kinetic Isotope Effects (k_H/k_D)

The intramolecular kinetic isotope effects (k_H/k_D) for the cleavages of *N*-alkyl and *N*-cyclopropyl groups were determined from the partition ratios of the corresponding proto- and deutero-substrates. The substrates (**1**–**3**) used in this study primarily gave two partition products, an *N*-alkyl-cleaved product, 4-chloro-*N*-cyclopropylaniline, and an *N*-cyclopropyl-cleaved product, 4-chloro-*N*-alkylaniline. Substitution of deuterium at the C_α-position of the substrate alters the product partition ratios because of the kinetic deuterium isotope effect. The intramolecular k_H/k_D values for the two pathways were obtained from the corresponding partition ratios. For example, if specifically C_α-H and C_α-D cyclopropyl derivatives were used:

$$k_{C-H}/k_{R-H} = PR_{C-H/R-H}; \quad k_{C-D}/k_{R-H} = PR_{C-D/R-H}$$

$$k_H/k_D = k_{C-H}/k_{C-D} = PR_{C-H/R-H}/PR_{C-D/R-H}$$

where $PR_{C-H/R-H}$ and $PR_{C-D/R-H}$ are the partition product ratios for the proteo- and deutero-derivatives, and k_{C-H}, k_{R-H}, and k_{C-D} are the rates of the cyclopropyl C_α-H, cyclopropyl C_α-D, and alkyl C_α-H abstraction steps, respectively.

Syntheses of 4-Chloro-*N*-isopropyl-*N*-cyclopropylanilines (General Procedures): The starting material, 4-chloro-*N*-isopropylaniline, was prepared by refluxing 4-chloroaniline with 2-bromopropane (1.2 equiv.) for about 1 h. The *N*-isopropyl C_α-deuterated derivative was obtained by the same procedure, except that 2-bromopropane was replaced with 2-deutero-2-bromopropane. 4-Chloro-*N*-ethylaniline was obtained by treating the corresponding acetanilide with borane–methyl sulfide complex (2.0 equiv.) in THF at 0 °C followed by refluxing for 2 h. Deuterium was incorporated at the C_α-position of the ethyl group by treating the desired acetanilide with $LiAlD_4$ (>98% deuterium content) in THF (25 mL) at 0 °C, followed by refluxing overnight. The corresponding 4-chloro-*N*-alkylformanilides of these *N*-alkyl derivatives were obtained by treating with formic acid (1.5 equiv.). The corresponding C_α-deuteroformanilides were obtained using the same procedure, except that formic acid was replaced with C_α-D formic acid. The *N*-alkyl-*N*-formanilide derivatives were converted into the corresponding *N*-cyclopropyl derivatives by using the method of Chaplinski and de Mejeire.[25]

4-Chloro-*N*-cyclopropyl-*N*-methylaniline (1): A mixture of 4-chloro-*N*-methylformanilide (1.0 g, 5.90 mmol) and $Ti(OiPr)_4$ (1.86 g, 6.49 mmol) was dissolved in THF (20 mL) and treated with ethylmagnesium chloride (5.19 mL, 25% in THF, 14.75 mmol) and stirred overnight at room temperature. The reaction mixture was quenched by adding saturated ammonium chloride solution, and filtered. The supernatant liquid was extracted with ethyl acetate, dried with Na_2SO_4, and concentrated. The crude reaction mixture was purified using silica gel with hexane as the solvent to afford **1** (450 mg, 42%). ^1H NMR (300 MHz, $CDCl_3$): δ = 7.18 (dt, ^1J = 3.3, 9.1 Hz, 2 H, 2 CH), 6.89 (dt, ^1J = 3.3, 9.1 Hz, 2 H, 2 CH), 2.94 (s, 3 H, CH_3), 2.35 (tt, ^1J = 3.8, 6.6 Hz, 1 H, CH), 0.82 (m, 2 H, CH_2), 0.60 (m, 2 H, CH_2) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 149.6, 128.8, 122.4, 115.1, 39.3, 33.6, 9.3 ppm.

4-Chloro-*N*-cyclopropyl-*N*-ethylaniline (2): A mixture of 4-chloro-*N*-ethylformanilide (1.0 g, 5.4 mmol) and $Ti(OiPr)_4$ (1.7 g, 6.0 mmol) in THF (20 mL) was treated with ethylmagnesium chloride (4.8 mL, 25% in THF, 13.6 mmol) and stirred overnight. The desired product was obtained as described above for **1** in 45% yield. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.14 (td, ^1J = 3.0, 9.1 Hz, 2 H, 2 CH), 6.87 (dt, ^1J = 3.0, 9.1 Hz, 2 H, 2 CH), 3.42 (q, ^1J = 7.2 Hz, 2 H, CH_2), 2.37 (tt, ^1J = 3.85, 6.6 Hz, 1 H, CH), 1.06 (t, ^1J = 6.9 Hz, 3 H, CH_3), 0.79 (m, 2 H, CH_2), 0.56 (m, 2 H, CH_2) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 148.1, 128.9, 122.2, 115.7, 45.7, 31.2, 11.6, 9.2 ppm. MS: *m*/*z* = 195.

4-Chloro-*N*-cyclopropyl-*N*-[1,1-D_2]ethylaniline: A mixture of 4-chloro-*N*-[2,2-D_2]ethylformanilide (1.0 g, 5.4 mmol) and $Ti(OiPr)_4$ (1.7 g, 6.0 mmol) in THF (20 mL) was treated with ethylmagnesium chloride (4.8 mL, 25% in THF, 13.6 mmol) and stirred overnight. The desired product was obtained as described above for **1** in 35% yield. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.14 (td, ^1J = 3.5, 9.1 Hz, 2 H, 2 CH), 6.87 (dt, ^1J = 3.5, 9.1 Hz, 2 H, 2 CH), 2.37 (tt, ^1J = 3.85, 6.6 Hz, 1 H, CH), 1.05 (s, 3 H, CH_3), 0.79 (m, 2 H, CH_2), 0.56 (m, 2 H, CH_2) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 148.14, 128.94, 122.24, 115.60, 31.04, 11.31, 9.04 ppm. MS: *m*/*z* = 197, deuterium content >98%.

4-Chloro-*N*-[1-D]cyclopropyl-*N*-ethylaniline: A mixture of 4-chloro-*N*-ethyl[D]formanilide (1.0 g, 5.4 mmol) and $Ti(OiPr)_4$ (1.7 g, 6.0 mmol) in THF (20 mL) was treated with ethylmagnesium chloride (4.8 mL, 25% in THF, 13.6 mmol) and stirred overnight. The desired product was obtained as described above for **1** in 37% yield. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.14 (td, ^1J = 3.3, 9.1 Hz, 2 H, 2 CH), 6.87 (dt, ^1J = 3.3, 9.1 Hz, 2 H, 2 CH), 3.44 (q, ^1J = 6.9 Hz, 2 H, CH_2), 1.06 (t^1J = 6.9 Hz, 3 H, CH_3), 0.82 (m, 2 H, CH_2), 0.58 (m, 2 H, CH_2) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 148.1, 128.9, 122.2, 115.7, 45.6, 11.6, 9.0 ppm. MS: *m*/*z* = 196, deuterium content >98%.

4-Chloro-*N*-cyclopropyl-*N*-isopropylaniline (3): A mixture of 4-chloro-*N*-isopropylformanilide (0.98 g, 5 mmol) and $Ti(OiPr)_4$ (1.5 mL, 5.4 mmol) in THF (25 mL) was treated with ethylmagnesium chloride (4.3 mL, 25% in THF, 12.4 mmol) and stirred overnight. The desired product was obtained as described above for **1** in 40% yield. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.16 (td, ^1J = 3.3, 8.9 Hz, 2 H, CH_2), 6.93 (td, ^1J = 3.3, 8.9 Hz, 2 H, CH_2), 3.84 (septet, ^1J = 6.7 Hz, 1 H, CH), 2.25 (tt, ^1J = 3.8, 6.4 Hz, 1 H, CH), 1.24 (d, ^1J = 6.7 Hz, 6 H, 2 CH_3), 0.81 (m, 2 H, CH_2), 0.48 (m, 2 H, CH_2) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 150.2, 128.4, 123.5, 118.8, 54.8, 26.1, 21.0, 9.2 ppm. MS: *m*/*z* = 210.

4-Chloro-*N*-cyclopropyl-*N*-[1-D]isopropylaniline: A mixture of 4-chloro-*N*-[2-D]isopropylformanilide (0.57 g, 2.9 mmol) and $Ti(OiPr)_4$ (0.91 g, 3.2 mmol) in THF (25 mL) was treated with ethylmagnesium chloride (2.5 mL, 25% in THF, 7.2 mmol) and stirred overnight. The desired product was obtained as described above for **1** in 38% yield. ^1H NMR (300 MHz, $CDCl_3$): δ = 7.14 (td, ^1J = 3.0, 9.0 Hz, 2 H, CH_2), 6.92 (dt, ^1J = 3.0, 9.0 Hz, 2 H, CH_2), 2.52 (tt, ^1J = 3.85, 6.6 Hz, 1 H, CH), 1.23 (s, 6 H, 2 CH_3), 0.78 (m, 2 H, CH_2), 0.48 (m, 2 H, CH_2) ppm. ^{13}C NMR (75.4 MHz,

$CDCl_3$): δ = 150.3, 128.5, 123.4, 119.0, 54.4 (t), 26.5, 20.9, 9.3 ppm. MS: *m*/*z* = 211, deuterium content >98%.

4-Chloro-*N*-[1-D]cyclopropyl-*N*-isopropylaniline: A mixture of 4-chloro-*N*-isopropyl-*N*-[D]formanilide (0.48 g, 2.4 mmol) and $Ti(OiPr)_4$ (0.76 g, 2.6 mmol) in THF (20 mL) was treated with ethylmagnesium chloride (2.1 mL, 25% in THF, 6.1 mmol) and stirred overnight. The desired product was obtained as described above for **1** in 39% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.14 (td, ^1J = 3.5, 9.0 Hz, 2 H, CH_2), 6.92 (dt, ^1J = 3.3, 9.0 Hz, 2 H, CH_2), 3.84 (septet, ^1J = 6.6 Hz, 1 H, CH), 1.24 (d, ^1J = 6.6 Hz, 6 H, 2 CH_3), 0.77 (m, 2 H, CH_2), 0.48 (m, 2 H, CH_2) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 150.4, 128.5, 123.4, 119.1, 54.8, 26.1 (t), 21.0, 9.2 ppm. MS: *m*/*z* = 211, deuterium content >98%.

Supporting Information (see footnote on the first page of this article)**:** Selected GC traces of the products of P_{450}-, HRP-, and CPO-catalyzed *N*-dealkylation of 4-chloro-*N*-cyclopropyl-*N*-alkylanilines.

Acknowledgments

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