

# Regioselective Epoxidations by Cytochrome P450 3A4 Using a Theobromine Chemical Auxiliary to Predictably Produce N-Protected $\beta$ - or $\gamma$ - Amino Epoxides

Vanja Polic, Kin Jack Cheong, Fabien Hammerer and Karine Auclair\*

Department of Chemistry, McGill University, 801 Sherbrooke Street West, Montreal, QC, Canada H3A 0B8.  
Fax: (+1)-514-398-3797; E-mail: k.auclair@mcgill.ca

**Abstract.** N-Protected  $\beta$ - and  $\gamma$ -amino epoxides are useful chiral synthons. We report here that the enzyme cytochrome P450 3A4 can catalyze the formation of such compounds in a regio- and stereo-selective manner, even in the presence of multiple double bonds or aromatic substituents. To this end, the theobromine chemical auxiliary is used not only to control the selectivity of the enzyme, but also as a masked amine and to facilitate product recovery. Theobromine predictably directed epoxidation at the double bond of the fourth carbon from the theobromine group. Unlike with most catalysts, the selectivity did not depend on electronic or steric factors but rather on the position of the olefin relative to the theobromine group.

**Keywords:** Biocatalysis; Chemical Auxiliaries; Cytochrome P450 3A4; Epoxidation; Theobromine.

## Introduction

Cytochrome P450 enzymes (P450s or CYPs) are a superfamily of heme monooxygenases that are known to catalyze diverse reactions, including hydroxylations, epoxidations, sulfoxidations, aryl-aryl couplings, Baeyer-Villiger oxidations, and more.<sup>[1-7]</sup> Recently, even some non-natural enzymatic reactions such as cyclopropanations<sup>[8,9]</sup> and C-H aminations<sup>[10-15]</sup> were made available using engineered P450. Many of these reactions are otherwise difficult to achieve by traditional chemical synthesis.

Drug metabolizing P450s are particularly attractive for synthetic applications because of their high substrate promiscuity. This is, however, contrasted with our poor ability to predict their products.<sup>[16]</sup> To circumvent this challenge, we have previously reported that chemical auxiliaries can be used to afford predictable regio- and stereo-selective hydroxylations by human P450 3A4<sup>[17]</sup> and P450 2E1.<sup>[18]</sup> Thus, following attachment of the chemical auxiliary to the substrate, the auxiliary is expected to bind the enzyme in a way that orients the substrate near the reactive iron species of the P450 such that oxidation occurs consistently at the same distance from the auxiliary. For example, when theobromine is used as the chemical auxiliary with P450 3A4, it was shown that it directs hydroxylation at the fourth atom away from the auxiliary with *pro-R* facial selectivity.<sup>[17]</sup> In addition, the theobromine auxiliary can also serve as a bait for the selective and quick recovery of the product from complex biocatalytic mixtures.<sup>[19]</sup> This is substantial since product recovery typically accounts for ~80% of the production cost in fermentation.

Epoxides are valuable building blocks in chemical synthesis, with the potential to introduce two adjacent chiral centers. These three-membered heterocycles are often employed as versatile intermediates in organic synthesis.<sup>[20]</sup> Available methods for producing chiral epoxides are largely based on transition-metal catalysis and/or the requirement of pre-existing directing group in the substrate.<sup>[21-23]</sup> Vinyl epoxides in particular are useful building blocks that combine the conjugated reactivity of two functional groups. One strategy to access this class of intermediates involves direct monoepoxidation of the corresponding diene; however, these reactions often suffer from lack of regioselectivity or low selectivity between monoepoxidation and bisepoxidation, or may result in the formation of the undesired regioisomer. Typically, the regioselectivity of diene epoxidation is controlled by electronic factors, as with peracid oxidants,<sup>[24]</sup> or by a directing group, as with Sharpless epoxidation.<sup>[21,25]</sup> However, it is not uncommon to obtain mixtures of epoxides along with polymeric products.

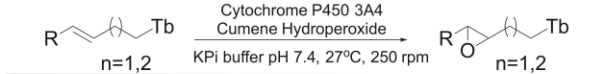
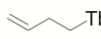
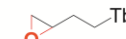
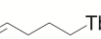
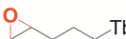
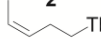
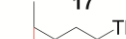
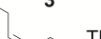









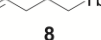
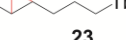
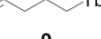
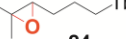
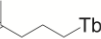
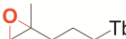


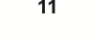



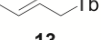
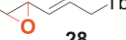
Also of interest are N-protected  $\beta$ - and  $\gamma$ -amino epoxides. Since  $\beta$ - and  $\gamma$ -amino 1,2-dioxygen motifs (and related groups) are common in natural products and in biologically active molecules,<sup>[26–31]</sup> new methods providing easy access to diverse N-protected  $\beta$ - and  $\gamma$ -amino epoxides are desirable.

Enzymes offer a green alternative to transition-metal catalysts. Based on the success of theobromine as a chemical auxiliary to allow prediction of the regioselectivity of hydroxylation by P450 3A4,<sup>[17]</sup> this system was further studied for its synthetic utility in epoxidation of isolated and conjugated dienes. We report herein that the use of P450 3A4 in combination with the theobromine auxiliary provides a new route to some difficult to access N-protected  $\beta$ - and  $\gamma$ -amino epoxides and vinyl epoxides.

## Results and Discussion

### Epoxidation of isolated olefins

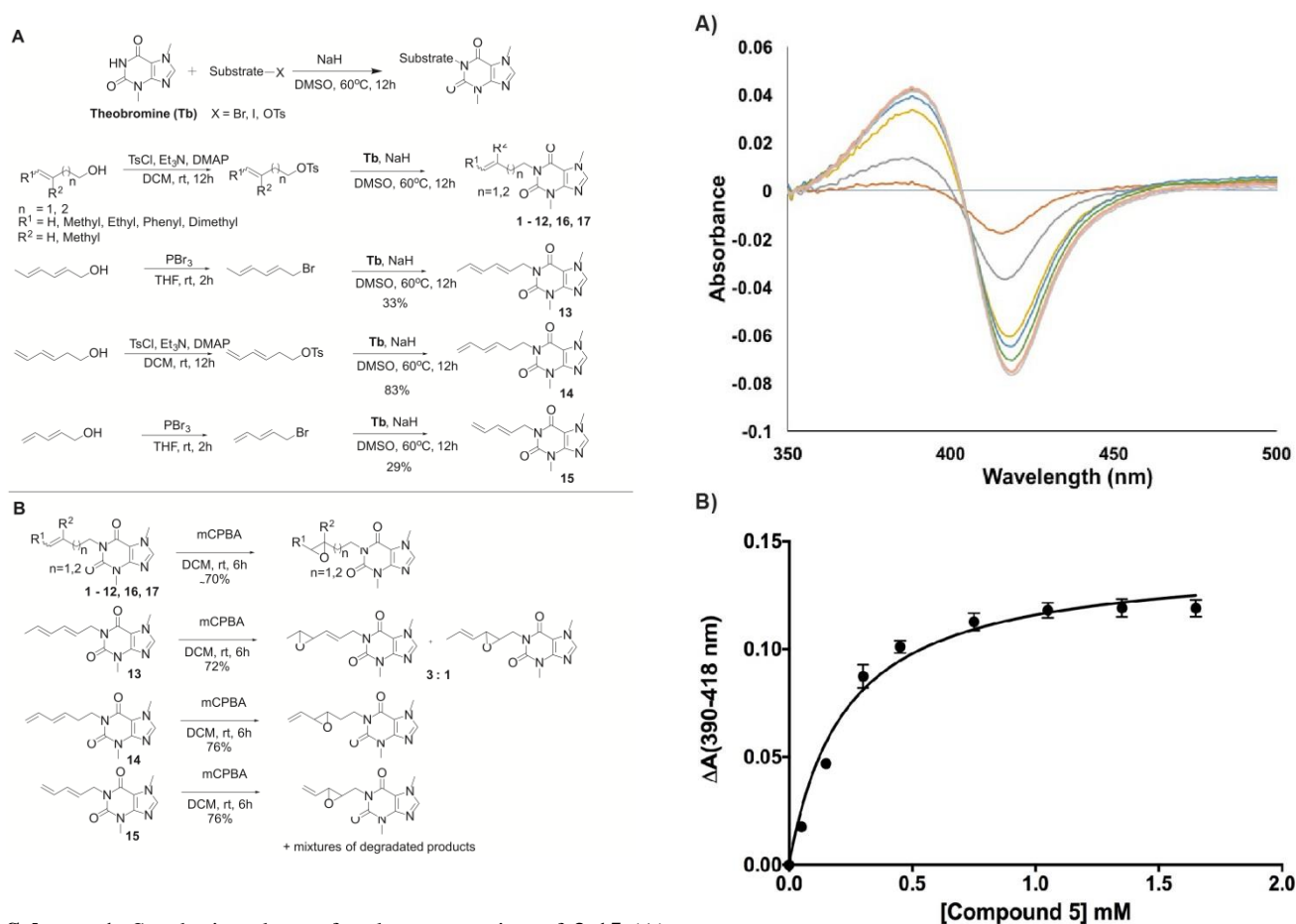
While compound **1** is oxidized to the racemic epoxide **16**, compound **2** yields the enantiopure (>99%) product **17** with the expected *R*-configuration at C4 (Table 1; structure and orientation of the theobromine group, Tb, is shown in Scheme 1A).<sup>[17]</sup> Intrigued by these results, we wondered if olefin substitution could affect the regio- and stereoselectivity of P450 3A4. Thus, a variety of theobromine derivatives with isolated olefins or conjugated dienes in the side chain were surveyed for selective epoxidation (see **3–15**, Table 1). Theobromine derivatives were prepared with side chain double bonds located on either side of the 4th carbon from theobromine, with either *E* or *Z* configuration, with or without branching, additional double bonds or aromatic substituents. The compounds were conveniently prepared by nucleophilic attack of the theobromine N-anion on the desired tosylate or bromide precursor (Scheme 1A). Encouragingly, spectral binding studies of selected compounds with P450 3A4 indicate that the theobromine derivatives display typical Type 1 ligand binding characteristics with spectral binding constant ( $K_s$ ) values ranging from 0.2 to 1.5 mM (Figure 1 and S7). Type 1 spectra are characterized by a peak around 390 nm and trough around 418 nm, resulting from a heme iron spin shift from low to high spin upon ligand binding. Type 1 binding spectra confirm that the theobromine-containing compounds do indeed bind the P450 3A4 active site, without directly coordinating to the heme iron. Theobromine alone, however, did not show evidence of active site binding nor of coordination with the heme iron

				
Substrate <sup>a</sup>	Product <sup>b</sup>	Selectivity <sup>c</sup>	e.r. <sup>d</sup>	Yield <sup>e</sup>
 <b>1</b>	 <b>16</b>	>95%*	50:50	41%
 <b>2</b>	 <b>17</b>	>95%*	99:1	76%
 <b>3</b>	 <b>18</b>	74%*	80:20	91%
 <b>4</b>	 <b>19</b>	37%*	80:20	94%
 <b>5</b>	 <b>20</b>	83%*	70:30	94%
 <b>6</b>	 <b>21</b>	91%*	55:45	92%
 <b>7</b>	 <b>22</b>	66%*	55:45	88%
 <b>8</b>	 <b>23</b>	81%*	55:45	91%
 <b>9</b>	 <b>24</b>	74%*	55:45	93%
 <b>10</b>	 <b>25</b>	99%*	50:50	93%
 <b>11</b>	 <b>26</b>	99%	55:45	94%
 <b>12</b>	 <b>27</b>	99%	50:50	90%
 <b>13</b>	 <b>28</b>	99%	70:30	80%
 <b>14</b>	 <b>29</b>	53%	80:20	85%
 <b>15</b>	 <b>30</b>	99%	95:5	59%

(Figure S8).

**Table 1.** Results of Cys-depleted mutant P450 3A4-catalyzed reactions.

<sup>a</sup>Tb is used to abbreviate the theobromine auxiliary. The site of attachment is shown in Scheme 1A. <sup>b</sup>Determined by comparison with authentic standards. Refer to Supporting Information for reaction conditions. All reactions were performed at least in triplicate. <sup>c</sup>Regioselectivity is shown as percentage of epoxidation at the C3-C4 or C4-C5 double bond with the major product shown. <sup>\*</sup>denotes chemoselectivity of the enzymatic reaction where applicable. <sup>d</sup>Enantiomeric ratio of the major product. <sup>e</sup>Percent conversion of substrate to oxidized product.



**Scheme 1.** Synthetic scheme for the preparation of **3-15** (A) and their respective racemic epoxides using mCPBA (B). The alcohols were prepared as described elsewhere (see main text).

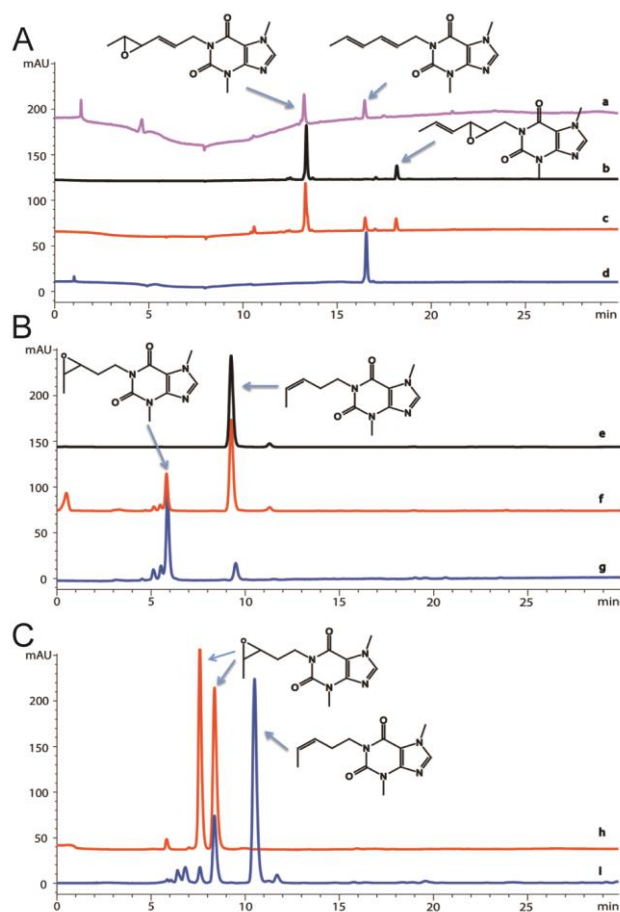
Considering that human P450 3A4 is a membrane-bound enzyme and is not very soluble in vitro, it is expected to be more efficient and more stable in a cellular environment, as in fermentation. The biotransformation of **1-15**, however, was performed with purified enzyme (as opposed to whole-cells) in order to fully demonstrate the involvement of cytochrome P450 3A4. For expression and purification from *Escherichia coli*, the enzyme is typically truncated to remove the membrane-anchoring tail and a 4×His-tag is added to facilitate purification. This variant is used here and referred to as the wild type. We

have also used a Cys-depleted P450 3A4 mutant, previously generated in our lab<sup>[32]</sup>, from which we have replaced 5 native cysteine residues to the following: C58T/C64A/C98S/C239S/C468G. This mutant shows 2 fold improved activity with the substrates 7-benzyloxy-4-trifluomethyl-coumarin and testosterone, compared to the wild type enzyme<sup>[32]</sup>, which may be attributed to conformational changes as observed for other P450 3A4 cysteine mutants.<sup>[33]</sup> Compounds **1-15** were subjected to enzymatic transformation by both the wild type and Cys-depleted P450 3A4 enzymes. For simplicity, the reactions were performed in the presence of the surrogate cofactor

**Figure 1.** Spectral binding characteristics of **5** with P450 3A4. A) Difference binding spectra of P450 3A4, with **5** displaying a peak at ~ 390 nm and a trough at ~420 nm, which are characteristic of a type 1 ligand. B) Spectral binding curve of P450 3A4 titrated with **5** to yield a spectral binding constant ( $K_S$ ) of  $0.22 \pm 0.02$  mM (fitting in GraphPad).

cumene hydroperoxide (CHP). The use of CHP was reported to replace the natural cofactor and redox partner, NADPH and cytochrome P450 reductase (CPR), and to afford higher yields.<sup>[34]</sup> No oxidation was observed in the absence of the enzyme, demonstrating that CHP is not oxidizing the double bonds. As expected, control experiments with the more expensive NADPH and CPR generated the same products, only in lower yields compared to the CHP-supported reaction (Figure 2B). In order to further demonstrate the importance of the auxiliary, substrate precursors without the theobromine moiety were exposed to the enzyme under the same conditions as above. None of them were turned over by the enzyme in the absence of the theobromine group. No oxidized product were detected (Figures S4-S6), confirming the necessity of the auxiliary for substrate recognition by P450 3A4, in agreement with our earlier study.<sup>[17]</sup> After optimization, substrate conversions of ca. 60% for the wild type enzyme and ca. 90% for the Cys-depleted P450 3A4 mutant were consistently obtained (Table 1 and Figure S3). Enzyme regio- and stereoselectivities were unaffected by the mutation. Most compounds were oxidized to give one major product with a mass increase of +16 corresponding to epoxidation, as monitored by HPLC-MS (Figure 2), with allylic hydroxylation likely being the minor product. Racemic synthetic epoxide standards were also synthesized by reaction of **3-15** with mCPBA (Scheme 1B) for HPLC comparison.

Table 1 lists all products obtained from the enzymatic transformations. With isolated olefins, the results indicate that the enzyme stereoselectivity is not influenced by the location of the double bond. Olefin substitution and geometry (*E* vs *Z*), however, seem to affect the stereoselectivity of the reaction. Interestingly, the *Z*-olefins (compounds **3**, **4** and **5**) yielded products with very good enantiomeric ratio (e.r.). In general, olefin derivatives with smaller substituents and *E* geometry, such as methyl and ethyl groups (see **6**, **7**, and **8**), were transformed to the corresponding epoxide (**21**, **22**, and **23** respectively) with poor enantioselectivity. 5,5-Substituted double bonds (as in **9** and **10**) or olefins conjugated to a phenyl substituent (see **11** and **12**) were also transformed with low stereoselectivity. Remarkably however, no aromatic hydroxylation products were observed in the case of **11** and **12** (see Figure S1). In most cases the chemoselectivity was good (>80%), however it was sometimes diminished with the presence of a



methylene at the fifth carbon from the theobromine moiety. In combination, these results suggest that the optimal site of oxidation may be between C4 and C5.

### Monoepoxidation of dienes

To elucidate the scope of theobromine-mediated epoxidation of dienes by P450 3A4, compounds **13**, **14** and **15** were separately subjected to enzymatic transformation. Both dienes **13** and **15** contain a double bond between C2 and C3 (from theobromine), whereas the double bonds in **14** are shifted by one carbon further from the chemical auxiliary. While the double bonds of compound **13** are all internal, compounds **14** and **15** both have an internal and a terminal olefin, the latter being more electron poor than the former. When treated with mCPBA, a commonly used reagent for epoxidation, compound **13** produced a mixture of regioisomers in a 3:1 molar ratio that could not be separated by column chromatography (Scheme 1B). On the other hand, diene **13** was efficiently transformed by both P450 3A4 variants, in the presence of either CHP or CPR/NADPH, into one single regioisomer, product **28** (Table 1, Figure 2A). Oxidation occurred at the double bond between C4 and C5, as expected due to the directing effect of theobromine that is based on distance from the auxiliary rather than electronics.<sup>[17]</sup> Exposure of diene **15** to the enzymatic reaction also yielded a single regioisomer with excellent enantioselectivity, compound **30**, where the oxygen

**Figure 2.** HPLC traces for representative P450 3A4 and chemical transformations, monitored at 273 nm. Panel A (non-chiral HPLC traces): a) P450 3A4 transformation of **13** using CHP and stopped before completion; b) product of the chemical epoxidation of **13** with mCPBA; c) co-injection of the products from the chemical and enzymatic reactions of **13**; d) unmodified compound **13**. Panel B (non-chiral HPLC traces): e) unmodified compound **5**; f) enzymatic transformation of **5** by P450 3A4 using CPR/NADPH; g) enzymatic transformation of **5** by cytochrome P450 3A4 using CHP. Panel C (chiral HPLC traces): h) product of the chemical epoxidation of **5** with mCPBA; i) P450 3A4 transformation of **5** using CHP and stopped before completion.

atom was introduced between C4 and C5. This is in sharp contrast to the reaction with mCPBA where again a mixture of products was observed. Enzymatic reaction with diene **14** however, yielded a mixture of regioisomers. Compound **29** was the main product with oxidation at the C3-C4 bond, and the reaction proceeded with very good enantioselectivity. The minor product is likely due to oxidation at the C5-C6 bond. This result is again consistent with the optimal site of oxidation being between C4 and C5.

### Product isolation using molecularly imprinted polymers

Since cytochrome P450 3A4 is a membrane-bound enzyme and is more efficient and more stable in a cellular environment, biocatalytic reactions should be done with whole-cells as in fermentation processes. We have previously demonstrated that molecularly imprinted polymers (MIPs) can be used for the selective recovery of theobromine-containing compounds from complex biocatalytic mixtures with great efficacy.<sup>[19]</sup> We thus verified if the theobromine epoxide derivatives are compatible with MIPs (see below for detailed conditions), and found that they are indeed stable under the solid phase extraction procedure used with MIPs. We achieved ca. 95% recovery of epoxide **20** with <2% epoxide opening observed under the acidic conditions.

### Conclusion

Chemical auxiliaries have proven popular in asymmetric synthesis; in particular, Evans oxazolidone chiral auxiliaries have shown significant utility in controlling the diastereoselectivity of various reactions such as alkylation,<sup>[35]</sup> acylation<sup>[36]</sup> and aldol condensations.<sup>[37]</sup> The auxiliaries are normally removed after the reaction; however, they are often expensive. In contrast, directing groups such as the alcohol group required in Sharpless epoxidation are typically part of the substrate and cannot be removed easily. The

results presented here show that the use of P450 3A4 with the chemical auxiliary theobromine is complementary to existing chemical methods. Not only does P450 3A4 show outstanding substrate promiscuity, theobromine is also achiral and inexpensive. Furthermore, the theobromine chemical auxiliary may also serve as a masked amine, which can be revealed under reducing conditions in high yield (80% as previously shown).<sup>[17]</sup> This is non-negligible since existing amino groups are most often protected in the presence of epoxides.

For P450 3A4 transformations, it has been shown that when the 4th carbon from theobromine is a methylene or a methine, hydroxylation is favored at the *pro-R* C-H bond, even when more reactive groups (e.g. double bonds or other tertiary carbons) are present elsewhere in the molecule.<sup>[17]</sup> We report here that when the 4th carbon from theobromine is part of a double bond, i.e. between C3 and C4, or C4 and C5, an epoxide is consistently produced in high yield. In the case of molecules with a single olefin, better stereoselectivity is observed for *Z*-double bonds or terminal double bonds between C4 and C5.

The approach reported here is especially useful with dienes and proceeds with predictable regioselectivity, in a way that is complementary to the current chemical toolbox. In contrast to chemical catalysts, the site of epoxidation by P450 3A4 is independent of electronic factors, and depends solely on the distance from the chemical auxiliary, with the double bond that includes the 4th carbon from theobromine being oxidized. Interestingly this method can differentiate electronically similar double bonds and even favor epoxidation at the electron-poorest double bond.

Besides the fact that theobromine can serve to control the regioselectivity of P450 3A4 and as a protected amine, another advantage is that it can facilitate product recovery with the use of MIPs, as shown here with epoxides and previously for hydroxylated products<sup>[19]</sup>. This is especially useful in whole-cell biotransformations where the majority of the cost goes to product recovery from the complex mixture. Although the work presented here was done with purified enzyme, large scale uses of P450 enzymes are likely to be done *via* fermentation to maximize enzyme stability. MIPs also replace the need for organic solvents during product recovery and/or purification. Thus, combining the use of MIP with whole-cell transformations should improve the sustainability of the overall process. Finally, this work promises to open up a new generation of chemical auxiliaries for diene epoxidations with complementary regioselectivities depending on the enzyme and the auxiliary used.

## Experimental Section

### Expression and Purification of Wild-Type and Mutant P450 3A4

The expression plasmid for truncated P450 3A4 (E.C. 1.14.13.-) (pSE3A4His) was generously provided by Prof. J. R. Halpert (University of Connecticut). The Cys-depleted mutant was prepared as previously described by our lab and had the following additional mutations: C58T, C64A, C98S, C239S, C468G.<sup>[32]</sup> Expression and purification were performed as previously described.<sup>[34]</sup>

### Expression and Purification of Cytochrome P450 Reductase (CPR)

The expression plasmid for cytochrome P450 reductase (pOR263) was generously provided by Prof. Charles B. Kasper (University of Wisconsin-Madison). CPR was expressed and purified as described elsewhere.<sup>[34]</sup> Protein concentration was measured by the Bradford method (Pierce 23236). The concentration of holo-CPR was estimated from its flavin content using an extinction coefficient of 21.4 mM<sup>-1</sup> cm<sup>-1</sup> at 456 nm.<sup>[38]</sup>

### Optimized Enzymatic Transformation Conditions

P450 3A4 (1.2 nmol, 12 μM final concentration) and substrate (20 nmol, 200 μM final concentration) were mixed in potassium phosphate buffer (KPi, 0.1 M, pH 7.4) and preincubated at 27°C and 250 rpm for 5 min (reaction tubes were placed on their side in an orbital shaker/incubator). The enzymatic reaction was initiated with the addition of cumene hydroperoxide (CHP, 60 nmol, 600 μM final concentration). Additional CHP (60 nmol) was added after 15 min and the reaction mixture was incubated for an additional 1.5 h. Alternatively, a mixture of cytochrome P450 reductase (16 μM) and NADPH (1 mM) can be used instead of CHP. Controls were run in the absence of each of CHP, substrate or enzyme. The reaction was terminated by extraction of the substrate and product in dichloromethane (4 × 0.5 mL). The extracts were combined and the solvent was evaporated. Alternatively, substrate and product can be extracted directly using MIP as described below. The residue was redissolved in acetonitrile (200 μL), filtered and analyzed by HPLC-MS.

### MIP Extraction of Epoxides

The MIP was prepared as previously described<sup>[19]</sup> using as the template a theobromine derivative with a 6-carbon chain hydroxylated at C4, as a stable mimic of the epoxide. The template was removed as usual.<sup>[19]</sup> To test the method, a mixture containing a known amount of **20** (1 mg, 2.5 mL, 1.5 mM in H<sub>2</sub>O) was loaded onto the MIP (1.5 mL) by centrifugation (3,000 × g for 5 min at 4°C), leaving a dry solid phase. The MIP was then washed with H<sub>2</sub>O (3 × 2.5 mL) by centrifugation (3,000 × g at 4°C for 5 min each). The aqueous eluate was discarded, leaving a dry solid phase. Finally, the epoxide was eluted from the solid phase with ethanol:acetic acid (9:1, 3 × 2.5 mL). The eluate was pooled and diluted to 25 mL with H<sub>2</sub>O, before analysis by LC-UV-MS (injecting 100 µL).

## Spectral Binding Studies

All UV/Vis spectra of the enzyme-ligand complexes were obtained on a Cary 5000 UV/Vis spectrophotometer. P450 3A4 (2 µM, 22 µL from a 17.8 µM stock), KPi buffer (178 µL, 0.1 M at pH = 7.4) were added to both the reference and sample cuvettes, and a blank was taken. In separate experiments, substrates **3**, **5**, **13**, **14** and **15** were titrated into the sample cuvette from stock solutions in acetonitrile such that the final acetonitrile concentration never exceeded 3% (v/v). This acetonitrile concentration had no detectable effect on the P450 spectra. Equal volumes of acetonitrile were added to the reference cuvette and the difference spectra were acquired from 300–500 nm. The difference in absorbance at the peak and the trough was plotted against substrate concentration to obtain a binding curve. Spectral dissociation constants ( $K_s$ ) were extracted from the binding curves by fitting to the following equation:

$$|\Delta A| = (|\Delta A_{\max}| \cdot [S]) / (K_s + [S])$$

Here  $\Delta A$  is the difference in absorbance between the peak and the trough,  $\Delta A_{\max}$  is the maximum reachable value of  $\Delta A$  at saturating substrate concentrations,  $[S]$  is the substrate concentration, and  $K_s$  is the apparent spectral dissociation constant. Fitting was performed by using the GraphPad Software.

## Acknowledgements

*This work was funded by grants to K.A. from the National Science and Engineering Research Council of Canada (NSERC) and the Center in Green Chemistry and Catalysis (CGCC). K.J.C thanks the CGCC for a scholarship. V.P. was supported by scholarships from the Dr. Richard H. Tomlinson Foundation and CGCC. We would also like to thank Dr. J. R. Halpert for providing the cytochrome P450 3A4 plasmid, and Dr. C. B. Kasper for the CPR plasmid.*

## References

- [1] R. Bernhardt, *J. Biotechnol.* **2006**, *124*, 128–145.
- [2] F. P. Guengerich, in *Cytochrome P450 Struct. Mech. Biochem.* (Ed.: P.R. Ortiz de Montellano), Kluwer Academic/Plenum Publishers, New York, **2005**, pp. 377–530.
- [3] F. P. Guengerich, A. W. Munro, *J. Biol. Chem.* **2013**, *288*, 17065–17073.
- [4] F. P. Guengerich, *Chem. Res. Toxicol.* **2001**, *14*, 611–650.
- [5] E. M. Isin, F. P. Guengerich, *Biochim. Biophys. Acta* **2007**, *1770*, 314–329.
- [6] R. Fasan, *ACS Catal.* **2012**, *2*, 647–666.
- [7] H. M. Girvan, A. W. Munro, *Curr. Opin. Chem. Biol.* **2016**, *31*, 136–145.
- [8] M. Carta, R. Malpass-Evans, M. Croad, Y. Rogan, J. C. Jansen, P. Bernardo, F. Bazzarelli, N. B. McKeown, *Science*. **2013**, *339*, 303–307.
- [9] P. S. Coelho, Z. J. Wang, M. E. Ener, S. A. Baril, A. Kannan, F. H. Arnold, E. M. Brustad, *Nat. Chem. Biol.* **2013**, *9*, 485–487.
- [10] J. A. McIntosh, P. S. Coelho, C. C. Farwell, Z. J. Wang, J. C. Lewis, T. R. Brown, F. H. Arnold, *Angew. Chemie - Int. Ed.* **2013**, *52*, 9309–9312.
- [11] Z. J. Wang, N. E. Peck, H. Renata, F. H. Arnold, *Chem. Sci.* **2014**, *5*, 598–601.
- [12] T. K. Hyster, C. C. Farwell, A. R. Buller, J. A. McIntosh, F. H. Arnold, *J. Am. Chem. Soc.* **2014**, *136*, 15505–15508.
- [13] R. Singh, J. N. Kolev, P. A. Sutera, R. Fasan, *ACS Catal.* **2015**, *5*, 1685–1691.

- [14] J. N. Kolev, K. M. O'Dwyer, C. T. Jordan, R. Fasan, *ACS Chem. Biol.* **2014**, *9*, 164–173.
- [15] R. Singh, M. Bordeaux, R. Fasan, *ACS Catal.* **2014**, *4*, 546–552.
- [16] B. C. Jones, D. S. Middleton, K. Youdim, in *Prog. Med. Chem.*, Elsevier, **2009**, pp. 239–263.
- [17] A. T. Larsen, E. M. May, K. Auclair, *J. Am. Chem. Soc.* **2011**, *133*, 7853–7858.
- [18] A. Menard, C. Fabra, Y. Huang, K. Auclair, *Chembiochem a Eur. J. Chem. Biol.* **2012**, *13*, 2527–2536.
- [19] A. T. Larsen, T. Lai, V. Polic, K. Auclair, *Green Chem.* **2012**, *14*, 2206–2211.
- [20] D. J. Ramon, M. Yus, *Curr. Org. Chem.* **2004**, *8*, 149–183.
- [21] T. Katsuki, K. B. Sharpless, *J. Am. Chem. Soc.* **1980**, *102*, 5974–5976.
- [22] W. Zhang, J. L. Loebach, S. R. Wilson, E. N. Jacobsen, *J. Am. Chem. Soc.* **1990**, *112*, 2801–2803.
- [23] A. Murphy, G. Dubois, T. D. P. Stack, *J. Am. Chem. Soc.* **2003**, *125*, 5250–5251.
- [24] F.-D. Boyer, I. Hanna, L. Ricard, *Org. Lett.* **2004**, *6*, 1817–1820.
- [25] J. He, J. Ling, P. Chiu, *Chem. Rev.* **2014**, *114*, 8037–8128.
- [26] S. Yu, D. Qin, S. Shangary, J. Chen, G. Wang, K. Ding, D. McEachern, S. Qiu, Z. Nikolovska-Coleska, R. Miller, et al., *J. Med. Chem.* **2009**, *52*, 7970–7973.
- [27] Y.-C. Jeong, M. Anwar, Z. Bikadi, E. Hazai, M. G. Moloney, *Chem. Sci.* **2013**, *4*, 1008–1015.
- [28] K. S. A. Kumar, *Bioorganic Med. Chem.* **2013**, *21*, 3609–3613.
- [29] K. Wei, W. Li, K. Koike, Y. Pei, Y. Chen, T. Nikaido, *J. Nat. Prod.* **2004**, *67*, 1005–1009.
- [30] T. Wakamiya, T. Shiba, T. Kaneko, *Bull. Chem. Soc. Jpn.* **1972**, *45*, 3668–3672.
- [31] G. De Micheli, M. De Amini, F. Platini, D. Della Bella, *Farm. Ed. Sci.* **1986**, *41*, 913–925.
- [32] V. Polic, K. Auclair, *Bioconjug. Chem.* **2017**, *28*, 885–889.
- [33] I. F. Sevrioukova, *Biochemistry* **2017**, *56*, 3058–3067.
- [34] A. Chefson, J. Zhao, K. Auclair, *ChemBioChem* **2006**, *7*, 916–919.
- [35] D. A. Evans, M. D. Ennis, D. J. Mathre, *J. Am. Chem. Soc.* **1982**, *104*, 1737–1739.
- [36] D. A. Evans, M. D. Ennis, T. Le, N. Mandel, G. Mandel, *J. Am. Chem. Soc.* **1984**, *106*, 1154–1156.
- [37] D. A. Evans, J. S. Tedrow, J. T. Shaw, C. W. Downey, *J. Am. Chem. Soc.* **2002**, *124*, 392–393.
- [38] D. D. Oprian, M. J. Coon, *J. Biol. Chem.* **1982**, *257*, 8935–8944.