

Cytochrome P450 Catalyzed Oxidative Hydroxylation of Achiral Organic Compounds with Simultaneous Creation of Two Chirality Centers in a Single C–H Activation Step**

Gheorghe-Doru Roiban, Rubén Agudo, and Manfred T. Reetz*

Dedicated to the MPI für Kohlenforschung on the occasion of its centenary

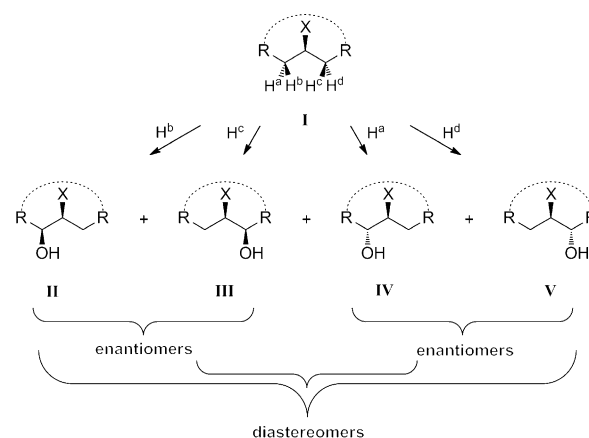
Abstract: Regio- and stereoselective oxidative hydroxylation of achiral or chiral organic compounds mediated by synthetic reagents, catalysts, or enzymes generally leads to the formation of one new chiral center that appears in the respective enantiomeric or diastereomeric alcohols. By contrast, when subjecting appropriate achiral compounds to this type of C–H activation, the simultaneous creation of two chiral centers with a defined relative and absolute configuration may result, provided that control of the regio-, diastereo-, and enantioselectivity is ensured. The present study demonstrates that such control is possible by using wild type or mutant forms of the monooxygenase cytochrome P450 BM3 as catalysts in the oxidative hydroxylation of methylcyclohexane and seven other monosubstituted cyclohexane derivatives.

Oxidative C–H activation of structurally simple and complex organic compounds that leads to the regio- and stereoselective introduction of hydroxy groups at predetermined non-activated sites constitutes a difficult yet rewarding goal in synthetic organic chemistry.^[1] Numerous synthetic reagents and catalysts have been developed for achieving this kind of selective functionalization but there are still problems regarding control of regio-, diastereo- and enantioselectivity.^[1] The use of cytochrome P450 enzymes constitutes an alternative approach.^[2] The mechanism of these heme dependent monooxygenases involves radical abstraction at a C–H site with formation of the respective alkyl radical followed by rapid C–O bond formation. When selectivity is poor, protein engineering based on directed evolution^[3,4]

provides a means to generate improved cytochrome P450 mutants.^[5] In the case of most achiral substrates, oxidative hydroxylation leads to enantiomers according to the process $R^1CH_2R^2 \rightarrow R^1CH(OH)R^2$, whereas chiral compounds provide a pair of diastereomers in the otherwise similar process $R^*CH_2R \rightarrow R^*CH(OH)R$. The first examples of the use of directed evolution to produce cytochrome P450 variants that induce high regioselectivity while also controlling stereoselectivity were recently reported with functionalized substrates such as steroids,^[6,7] 1-cyclohexene carboxylic acid ester,^[8] or *N*-benzyl pyrrolidine,^[5c,9] compounds that may undergo binding interactions at the respective hetero-atoms. Such regio- and enantioselectivity has not been achieved in reactions of alkanes that lack functional groups.

Oxidative hydroxylation produces a different stereochemical outcome when appropriate achiral substrates are used, in which case a single C–H-activating process induces the concomitant creation of more than one center of chirality. Consider, for example, the reaction of achiral compounds of the type **I**, in which oxidation at the four stereotopic H atoms of the two methylene units flanking the X-bearing C atom leads to four different stereoisomers: **II**, **III**, **IV**, and **V**, each of which has two new chirality centers (Scheme 1). Depending upon the nature of the R groups, other regioisomeric alcohols can also be formed, a fact that contributes to the overall challenge.

We chose methylcyclohexane (**1a**), which is devoid of any functional groups, as the model substrate and cytochrome



Scheme 1. The stereochemical consequences of the oxidative hydroxylation of prochiral compounds of type **I**.

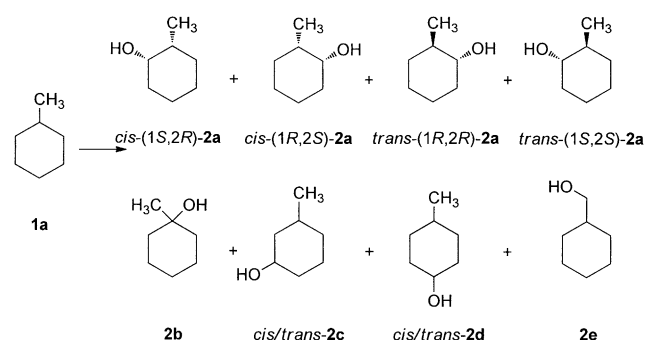
[*] Dr. G.-D. Roiban,^[‡] Dr. R. Agudo,^[‡] Prof. Dr. M. T. Reetz
Max-Planck-Institut für Kohlenforschung
Kaiser-Wilhelm-Platz 1, 45470 Mülheim an der Ruhr (Germany)
and
Department of Chemistry, Philipps-Universität Marburg
Hans-Meerwein-Strasse, 35032 Marburg (Germany)
E-mail: reetz@mpi-muelheim.mpg.de

[†] These authors contributed equally to this work.

[**] Financial support by the Max-Planck-Society and the Arthur C. Cope Foundation is gratefully acknowledged. We thank Dr. Pamela Torres-Salas for assistance in TON and TTN measurements, Stephanie Dehn and Corinna Heidgen for numerous GC analyses, Julian Kuttner (group of Prof. G. Hilt) for providing several starting cyclohexane derivatives, and Jonas Schwaben (group of Prof. U. Koert) for support with optical rotation measurements.

Supporting information for this article is available on the WWW under <http://dx.doi.org/10.1002/anie.201310892>.

P450 BM3 (CYP102A1) from *Bacillus megaterium* as the enzyme.^[2,10,11] P450 BM3 is a self-sufficient fusion protein composed of a cytochrome P450 monooxygenase and an NADPH diflavin reductase for cofactor regeneration. Hydroxylation at the two methylene units flanking the CH₃-bearing C atom provides four possible products, *cis*-(1*S*,2*R*)-**2a**, *cis*-(1*R*,2*S*)-**2a**, *trans*-(1*R*,2*R*)-**2a** and *trans*-(1*S*,2*S*)-**2a**, with regioisomers **2b–e** resulting from oxidative attack at other C atoms also being possible (Scheme 2).



Scheme 2. Possible products of the P450 BM3 catalyzed oxidative hydroxylation of methylcyclohexane (**1a**).

Wild type (wt) P450 BM3 was found to be notably regio- (78 %), diastereo- (≥ 97 %), and enantioselective (e.r. = 91:9) in favor of *cis*-(1*S*,2*R*)-**2a** (Table 1, entry 1). This is a remarkable result not possible when utilizing synthetic catalysts or oxidants.^[12] In the hope of boosting enantioselectivity in the regioselective formation of *cis*-(1*S*,2*R*)-**2a**, directed evolution by using the combinatorial active-site saturation test (CAST) was applied.^[4] Accordingly, four CAST sites were chosen for saturation mutagenesis (Figure 1): A (V78/T88), B (V78/L181) and C (T268/A328), which were randomized by using a highly reduced amino acid alphabet composed of six building blocks (Phe, Tyr, Trp, Lys, Arg, His) and the corresponding wt amino acids, and site D (F87) for which NNK codon degeneracy was chosen to encode all 20 canonical amino acids. Upon screening a total of 760 transformants, variant F87A (identified from library D), which is the “standard” mutant generated in previous protein engineering studies involving other substrates,^[2,10] led to an undesired shift in regioselectivity (71 %) in favor of the tertiary alcohol **2b** (Table 1, entry 2). This reaction involves the weakest C–H

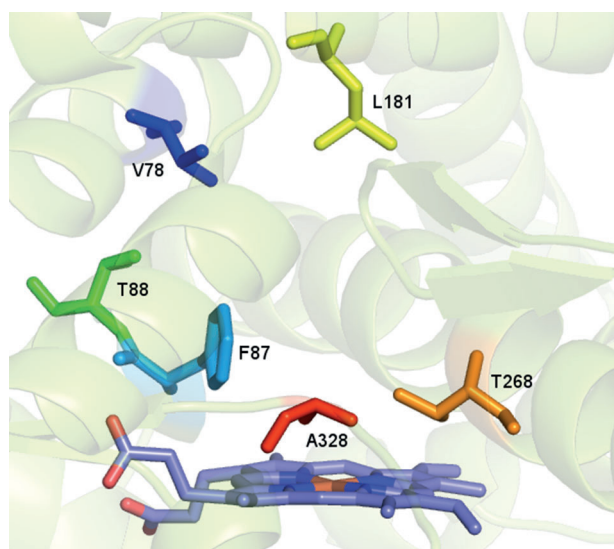


Figure 1. Selected CAST sites A (V78/T88; dark blue/green), B (V78/L181; dark blue/yellow), C (T268/A328; brown/red), and D (F87; light blue) in P450 BM3, chosen with the help of the crystal structure (PDB: 1JPZ).^[11]

bond in the molecule and is more easily achieved by synthetic reagents such as dioxiranes.^[1a,c] Several improved variants were detected (see the Supporting Information), the best of which was A328F^[2,10] from library C. It affords *cis*-(1*S*,2*R*)-**2a** with enhanced enantioselectivity (e.r. = 96:4) at the expense of a slight decrease in regioselectivity (71 %, Table 1, entry 3).

We then subjected substrates **1b–h** to oxidative hydroxylation using wt P450 BM3 and some of the mutants previously screened for **1a** (Table 2). The results point to remarkable effects, the most prominent of which is consistently high diastereoselectivity in favor of the *cis* configuration. This diastereoselectivity is often accompanied by high enantioselectivity, although at the expense of regioselectivity, as in the case of *cis*-2-halocycloalkanols (1*S*,2*R*)-**3a** and (1*S*,2*R*)-**4a**. Synthetic strategies that lead to the enantioselective production of *cis* halo alcohols in a single step from halocycloalkanes have not been considered to date; such compounds are generally synthesized by the reduction of α -halo ketones.^[13] When comparing the stereochemical results with the absolute configuration of compound **2a**, it can be seen that the sense of the enantioselectivity does not change. Mutants F87A and A328F do not result in appreciable

Table 1: Oxidative hydroxylation of methylcyclohexane (**1a**) catalyzed by wt P450 BM3 or mutants thereof.^[a]

Entry	P450 Variant					
1	wt	2a /78, d.r. = 97:3 (<i>cis</i>), (1 <i>S</i> ,2 <i>R</i>), (e.r. = 91:9)	2b /17	2c /4, d.r. = 95:5 (<i>trans</i>)	2d /1, d.r. = 69:31 (<i>cis</i>)	–
2	F87A	2a /13, d.r. = 97:3 (<i>cis</i>), (1 <i>S</i> ,2 <i>R</i>), (e.r. = 68:32)	2b /71	2c /8, d.r. > 99:1 (<i>trans</i>)	2d /8, d.r. = 68:32 (<i>cis</i>)	–
3	A328F	2a /71, d.r. = 97:3 (<i>cis</i>), (1 <i>S</i> ,2 <i>R</i>), (e.r. = 96:4)	2b /20	2c /9, d.r. = 56:44 (<i>cis</i>)	–	–

[a] Values were obtained by averaging at least three independent experiments performed as described in the Supporting Information. The numbers following the slash after the product designation represent % regioselectivity. Owing to the tendency of methylcyclohexane to evaporate under the reaction conditions, it is difficult to measure the exact % conversion, which may vary considerably when the reaction is performed in plastic or glass plates (see the Supporting Information).

Table 2: Oxidative hydroxylation of compounds **1b–h** by using wt P450 BM3 and mutants evolved for the reaction of **1a**.^[a]

Entry	1	R	P450 variant						Other oxid. prod. [%]	Conv. [%]
1	1b	Cl	wt	3a /49, d.r. = 96:4 (<i>cis</i>), (1 <i>S</i> ,2 <i>R</i>), (e.r. = 96:4)	– (7) ^[b]	3c /21, d.r. = 99:1 (<i>trans</i>)	3d /18, d.r. = 99:1 (<i>cis</i>)	–	5	58
2	1c	Br	wt	4a /67, d.r. = 94:6 (<i>cis</i>), (1 <i>S</i> ,2 <i>R</i>), (e.r. = 98:2)	– (13) ^[c]	4c /11, d.r. = 99:1 (<i>trans</i>)	4d /7, d.r. = 99:1 (<i>cis</i>)	–	2	86
3	1d	CN	wt	5a /47, d.r. = 95:5 (<i>cis</i>), (1 <i>S</i> ,2 <i>S</i>), (e.r. = 96:4)	– (23) ^[d]	5c /16	5d /10	–	4	62
4	1d	CN	F87A	5a /4	– (86) ^[d]	5c /3	5d /2	–	5	10
5	1e	<i>i</i> Pr	wt	6a /77, d.r. = 97:3 (<i>cis</i>), (1 <i>S</i> ,2 <i>S</i>), (e.r. = 63:37)	6b /8	6c /7	6d /2	6e /3 ^[f]	3	12
6	1e	<i>i</i> Pr	A328F	6a /48, d.r. = 98:2 (<i>cis</i>), (1 <i>S</i> ,2 <i>S</i>), (e.r. = 96:4)	6b /3	6c /18	6d /9	6e /18 ^[f]	4	30
7	1f	<i>t</i> Bu	wt	7a /59, d.r. = 98:2 (<i>cis</i>), (1 <i>R</i> ,2 <i>R</i>), (e.r. = 95:5)	7b /8	7c /25	7d /7, d.r. = 54:46 (<i>cis</i>)	–	1	19
8	1g	COCH ₃	wt	8a /58, d.r. = 95:5 (<i>cis</i>), (1 <i>R</i> ,2 <i>S</i>), (e.r. = 77:23)	8b /34	n.a. ^[e]	n.a. ^[e]	–	8	88
9	1g	COCH ₃	A328F	8a /78, d.r. = 98:2 (<i>cis</i>), (1 <i>R</i> ,2 <i>S</i>), (e.r. = 98:2)	8b /7	n.a. ^[e]	n.a. ^[e]	–	15 ^[g]	85 (97) ^[h]
10	1g	COCH ₃	F87A	8a /2	8b /84	n.a. ^[e]	n.a. ^[e]	–	14 ^[g]	14
11	1h	CH ₂ COCH ₃	wt	9a /67, d.r. = 97:3 (<i>cis</i>), (1 <i>R</i> ,2 <i>R</i>), (e.r. = 78:22)	9b /14	n.a. ^[e]	9d /13, d.r. = 62:38 (<i>cis</i>)	–	6	86 (98) ^[h]

[a] Values were obtained by averaging at least three independent experiments performed with resting cells as described in the Supporting Information. The numbers following the slash after the product designation represent % regioselectivity. [b] The crude reaction products contain 7% cyclohexanone, which is formed by dehydrohalogenation of *cis*-**3a** and/or **3b**. [c] The crude reaction products contain 13% cyclohexanone, which is formed by dehydrohalogenation of *cis*-**4a** and/or **4b**. [d] The crude reaction products contain 23% (entry 3)/86% (entry 4) cyclohexanone, which is derived from the tertiary cyanohydrin. [e] n.a. = not assigned (concentration too low/control not available). [f] **6e** = 2-cyclohexyl-2-propanol. [g] The other oxidation products consist mainly of *cis/trans* 3- and 4-hydroxy substituted methylcyclohexyl ketone but the exact structure was not identified. [h] Conversion obtained in a scaled up experiment, thus showing that the screening process leads to lower conversion values.

improvements in regio- or enantioselectivity. Cyanocyclohexane (**1d**) is also hydroxylated with *cis* diastereoselectivity at the same enantiotopic H atom with preferential formation of (1*S*,2*S*)-**5a**, with the stereochemical nomenclature changing in this case according to the CIP convention. It is also interesting to note that 86% regioselectivity in favor of hydroxylation at the cyano-bearing C atom can be achieved by using mutant F87A, in which case the intermediate cyanohydrin liberates cyclohexanone (Table 2, entry 4). This is a convenient biocatalytic method for transforming an alkyl nitrile into the respective ketone. Reasonable activity was typically observed, as in the case of substrate **1c**, which gave a turnover frequency (TOF) of 8.5 min^{−1} and a total turnover number (TTN) of 1616 (see the Supporting Information).

In the case of isopropylcyclohexane (**1e**), wt P450 BM3 is 77% regioselective and 97% diastereoselective favoring *cis*-(**6a**) but the enantioselectivity is poor (e.r. = 63:37). When using mutant A328F, a pronounced increase in enantioselectivity resulted (e.r. = 96:4), albeit at the expense of some regioselectivity (Table 2, entries 5 and 6). *tert*-Butylcyclohexane (**1f**) is also hydroxylated with *cis* selectivity, but in this case the other stereotopic H atom is hydroxylated with formation of the opposite absolute configuration (1*R*,2*R*, e.r. = 95:5; Table 2, entry 7). Upon changing from wt P450 BM3 to mutant A328F in the reaction of cyclohexyl methyl ketone (**1g**), regioselectivity in favor of alcohol **8a** is boosted from 58% to 78%, with diastereoselectivity in favor of the *cis* isomer amounting to 98% with high enantioselectivity (e.r. = 98:2) in favor of (1*R*,2*S*)-**8a**.

Catalytic activity is acceptable (TOF = 11.9 and 12.1 min^{−1} for wt and mutant, respectively; TTN = 1535 and 1707 for wt and mutant, respectively). This result means that the sense of the enantioselectivity is the same as in the reaction of **2a**. The reaction was scaled up to 1.85 mmol of substrate **1g** (97% conversion; 65% yield of isolated **8a** following chromatography). By contrast, mutant F87A delivers primarily the tertiary alcohol **8b** in a fairly slow reaction. Wt P450 BM3 catalyzes the hydroxylation of ketone **1h** with 67% regioselectivity, 97% *cis* diastereoselectivity, and reversed enantioselectivity in favor of (1*R*,2*R*)-**9a** (e.r. = 78:22; Table 2, entry 11) with good activity (TOF = 27 min^{−1}; TTN = 1616). Similarly, a scaled up reaction of **1h** (1.85 mmol) led to 98% conversion and provided **9a** with a yield of 60% after column chromatography. On scaling up the reactions of substrates **1a–e**, the conversion values generally proved to be higher than those observed under screening conditions. However, this does not apply to compounds **1a**, **1e**, and **1f**, in which cases such characteristics as volatility and solubility influence the yields of the hydroxylated products following column chromatography. Nevertheless, sufficient amounts of the respective *cis* alcohols could be prepared, thus confirming, even in these cases, the synthetic utility of the catalytic system. Further directed evolution and appropriate process engineering constitute ways to optimize the reaction of each substrate.

In summary, we have shown that the desymmetrization of prochiral monosubstituted cyclohexane derivatives by means of oxidative hydroxylation catalyzed by wt P450 BM3 or mutants thereof can be highly regio-, diastereo- and enantioselective, thus leading to the creation of two centers of chirality in a single C–H-activation event. The regioselective formation of the vicinal *cis* diastereomers appears to be the rule, with enantioselectivity also being high in many cases. This constitutes the first strategy for accessing highly enantio-enriched vicinal disubstituted *cis*-cyclohexanols in one step, a method that fulfills the requirements of a “green” process.^[14] Some of the products arising from the hydroxylation of substrates **1a–h**, which have previously been prepared by multistep routes, are of potential interest as building blocks in the synthesis of certain biologically active compounds as therapeutic drugs.^[15] Although the C–H bond strength in all of the methylene units can be expected to be similar, those flanking the R-bearing C atom seem to be the preferred sites for oxidative hydroxylation despite the fact that the reaction might be expected to be disfavored at these sites owing to steric effects.

The present work also suggests that in the quest to transform simple achiral compounds into value-added chiral products of higher structural complexity, it may be useful to screen further P450 BM3 mutant libraries as well as other cytochrome P450 enzymes. By using appropriate substrates, it should also be possible to use the same type of cytochrome P450 catalyzed oxidative hydroxylation to bring about the simultaneous creation of three or more new chirality centers in a single C–H-activation step.

Received: December 16, 2013

Revised: January 22, 2014

Published online: March 3, 2014

Keywords: biocatalysis · C–H activation · cytochrome P450 · hydroxylation · stereoselectivity

- [1] Recent representative studies of oxidative hydroxylation utilizing synthetic reagents or catalysts for the preparation of simple and complex organic compounds: a) T. Newhouse, P. S. Baran, *Angew. Chem.* **2011**, *123*, 3422–3435; *Angew. Chem. Int. Ed.* **2011**, *50*, 3362–3374; b) M. C. White, *Science* **2012**, *335*, 807–809; c) S. R. Neufeldt, M. S. Sanford, *Acc. Chem. Res.* **2012**, *45*, 936–946; d) W. Adam, C.-G. Zhao, R. R. Saha-Möller, K. Jakka, *Oxidation of Organic compounds with Dioxiranes*, Wiley-VCH, New York, **2009**; e) K. Kamata, K. Yonehara, Y. Nakagawa, K. Uehara, N. Mizuno, *Nat. Chem.* **2010**, *2*, 478–483; f) R. A. Periana, *Acc. Chem. Res.* **2012**, *45*, 885–898; g) M. Sun, J. Zhang, P. Putaj, V. Caps, F. Lefebvre, J. Pelletier, J. M. Basset, *Chem. Rev.* **2014**, *114*, 981–1019; h) E. Roduner, W. Kaim, B. Sarkar, V. B. Urlacher, J. Pleiss, R. Gläser, W.-D. Einicke, G. A. Sprenger, U. Beifuß, E. Klemm, C. Liebner, H. Hieronymus, S.-F. Hsu, B. Plietker, S. Laschat, *ChemCatChem* **2013**, *5*, 82–112.
- [2] Reviews of P450 enzymes: a) P. R. Ortiz de Montellano, *Cytochrome P450: Structure, Mechanism, and Biochemistry*, 3rd ed., Springer, Berlin, **2005**; b) E. M. Isin, F. P. Guengerich, *Biochim. Biophys. Acta Gen. Subj.* **2007**, *1770*, 314–329; c) A. W. Munro, H. M. Girvan, K. J. McLean, *Nat. Prod. Rep.* **2007**, *24*, 585–609; d) P. R. Ortiz de Montellano, *Chem. Rev.* **2010**, *110*, 932–948; e) S. Shaik, S. Cohen, Y. Wang, H. Chen, D. Kumar, W. Thiel, *Chem. Rev.* **2010**, *110*, 949–1017; f) V. B. Urlacher, S. Eiben, *Trends Biotechnol.* **2006**, *24*, 324–330; g) *Handbook of Porphyry Science* (Eds.: K. M. Kadish, K. M. Smith, R. Guilard), Vol. 5, World Scientific Publishing, **2010**; h) B. Meunier, S. P. de Visser, S. Shaik, *Chem. Rev.* **2004**, *104*, 3947–3980; i) C. J. C. Whitehouse, S. G. Bell, L.-L. Wong, *Chem. Soc. Rev.* **2012**, *41*, 1218–1260; j) J. C. Lewis, P. S. Coelho, F. H. Arnold, *Chem. Soc. Rev.* **2011**, *40*, 2003–2021; k) E. O'Reilly, V. Köhler, S. L. Flitsch, N. J. Turner, *Chem. Commun.* **2011**, *47*, 2490–2501; l) R. Fasan, *ACS Catal.* **2012**, *2*, 647–666; m) Y. Khatir, F. Hannemann, M. Girhard, R. Kappl, A. Meme, M. Ringle, S. Janocha, E. Leize-Wagner, V. B. Urlacher, R. Bernhardt, *Biotechnol. Appl. Biochem.* **2013**, *60*, 18–29.
- [3] Recent reviews of directed evolution:^[4] a) S. Lutz, U. T. Bornscheuer, *Protein Engineering Handbook*, Vol. 1–2, Wiley-VCH, Weinheim, **2009**; b) N. J. Turner, *Nat. Chem. Biol.* **2009**, *5*, 567–573; c) C. Jäckel, P. Kast, D. Hilvert, *Annu. Rev. Biophys. Biomol. Struct.* **2008**, *37*, 153–173; d) S. Bershtein, D. S. Tawfik, *Curr. Opin. Chem. Biol.* **2008**, *12*, 151–158; e) P. A. Romero, F. H. Arnold, *Nat. Rev. Mol. Cell Biol.* **2009**, *10*, 866–876; f) L. G. Otten, F. Hollmann, I. W. C. E. Arends, *Trends Biotechnol.* **2010**, *28*, 46–54; g) A. V. Shivange, J. Marienhagen, H. Mundhada, A. Schenk, U. Schwaneberg, *Curr. Opin. Chem. Biol.* **2009**, *13*, 19–25; h) G. A. Strohmeier, H. Pichler, O. May, M. Gruber-Khadjawi, *Chem. Rev.* **2011**, *111*, 4141–4164; i) U. T. Bornscheuer, G. Huisman, R. Kazlauskas, S. Lutz, J. C. Moore, K. Robins, *Nature* **2012**, *485*, 185–194; j) M. T. Reetz, *J. Am. Chem. Soc.* **2013**, *135*, 12480–12496.
- [4] Review of the directed evolution of stereoselective enzymes as catalysts in synthetic organic chemistry: M. T. Reetz, *Angew. Chem.* **2011**, *123*, 144–182; *Angew. Chem. Int. Ed.* **2011**, *50*, 138–174.
- [5] Recent accounts of protein engineering of P450 enzymes by using traditional site-specific mutagenesis or directed evolution:^[7] a) B. M. A. van Vugt-Lussenburg, M. C. Damsten, D. M. Maasdijk, N. P. E. Vermeulen, J. N. M. Commandeur, *Biochem. Biophys. Res. Commun.* **2006**, *346*, 810–818; b) S. T. Jung, R. Lauchli, F. H. Arnold, *Curr. Opin. Biotechnol.* **2011**, *22*, 809–817; c) W. L. Tang, Z. Li, H. Zhao, *Chem. Commun.* **2010**, *46*, 5461–5463; d) P. R. Ortiz de Montellano, *Chem. Rev.* **2010**, *110*, 932–948; e) V. B. Urlacher, M. Girhard, *Trends Biotechnol.* **2012**, *30*, 26–36; f) A. Dennig, J. Marienhagen, A. J. Ruff, L. Guddat, U. Schwaneberg, *ChemCatChem* **2012**, *4*, 771–773; g) E. Weber, A. Seifert, M. Antonovici, C. Geinitz, J. Pleiss, V. B. Urlacher, *Chem. Commun.* **2011**, *47*, 944–946; h) H. Venkataraman, S. B. A. de Beer, L. A. H. van Bergen, N. van Essen, D. P. Geerke, N. P. E. Vermeulen, J. N. M. Commandeur, *ChemBioChem* **2012**, *13*, 520–523; i) J. C. Lewis, S. M. Mantovani, Y. Fu, C. D. Snow, R. S. Komor, C. H. Wong, F. H. Arnold, *ChemBioChem* **2010**, *11*, 2502–2505; j) K. Zhang, S. E. Damaty, R. Fasan, *J. Am. Chem. Soc.* **2011**, *133*, 3242–3245; k) G.-D. Roiban, R. Agudo, M. T. Reetz, *Tetrahedron* **2013**, *69*, 5306–5311.
- [6] S. Kille, F. E. Zilly, J. P. Acevedo, M. T. Reetz, *Nat. Chem.* **2011**, *3*, 738–743.
- [7] a) S. H. Mao, X. J. Hu, B. Y. Hua, N. Wang, X. G. Liu, F. P. Lu, *Biotechnol. Lett.* **2012**, *34*, 2113–2117; b) K. T. Nguyen, C. Virus, N. Gunnewich, F. Hannemann, R. Bernhardt, *ChemBioChem* **2012**, *13*, 1161–1166; c) A. Braun, M. Geier, B. Buhler, A. Schmid, S. Mauersberger, A. Glieder, *Microb. Cell Fact.* **2012**, *11*, 106; d) M. Geier, A. Braun, P. Fladischer, P. Stepniak, F. Rudroff, C. Hametner, M. D. Mihovilovic, A. Glieder, *FEBS J.* **2013**, *280*, 3094–3108; e) P. Bracco, D. B. Janssen, A. Schallmeyer, *Microb. Cell Fact.* **2013**, *12*, 95.
- [8] R. Agudo, G.-D. Roiban, M. T. Reetz, *ChemBioChem* **2012**, *13*, 1465–1473.

- [9] S. Q. Pham, G. Pompidor, J. Liu, X. D. Li, Z. Li, *Chem. Commun.* **2012**, 48, 4618–4620.
- [10] a) L. O. Narhi, A. J. Fulco, *J. Biol. Chem.* **1986**, 261, 7160–7169; b) A. W. Munro, D. J. Leys, K. J. McLean, K. R. Marshall, T. W. B. Ost, S. Daff, C. S. Miles, S. K. Chapman, D. A. Lysek, C. C. Moser, C. C. Page, P. L. Dutton, *Trends Biochem. Sci.* **2002**, 27, 250–257; c) T. Jovanovic, R. Farid, R. A. Friesner, A. E. McDermott, *J. Am. Chem. Soc.* **2005**, 127, 13548–13552; d) K. H. Clodfelter, D. J. Waxman, S. Vajda, *Biochemistry* **2006**, 45, 9393–9407; e) U. Schwaneberg, A. Sprauer, C. Schmidt-Dannert, R. D. Schmid, *J. Chromatogr. A* **1999**, 848, 149–159.
- [11] a) D. C. Haines, D. R. Tomchick, M. Machius, J. A. Peterson, *Biochemistry* **2001**, 40, 13456–13465; b) D. C. Haines, B. Chen, D. R. Tomchick, M. Bondlela, A. Hegde, M. Machius, J. A. Peterson, *Biochemistry* **2008**, 47, 3662–3670; see also c) H. Li, T. L. Poulos, *Nat. Struct. Biol.* **1997**, 4, 140–146.
- [12] a) See Ref. [1e]; b) S. I. Murahashi, N. Komiya, Y. Oda, T. Kuwabara, T. Naota, *J. Org. Chem.* **2000**, 65, 9186–9193; c) H. Fuwa, T. Kobayashi, T. Tokitoh, Y. Torii, H. Natsugari, *Tetrahedron* **1984**, 40, 4297–4312; d) J. R. Lindsay Smith, G. B. Shul'pin, *Tetrahedron Lett.* **1998**, 39, 4909–4912; e) M. V. Kirillova, A. M. Kirillov, D. Mandelli, W. A. Carvalho, A. J. L. Pombeiro, G. B. Shul'pin, *J. Catal.* **2010**, 272, 9–17; f) G. B. Shul'pin, C. C. Golfeto, G. Süß-Fink, L. S. Shul'pin, D. Mandelli, *Tetrahedron Lett.* **2005**, 46, 4563–4567.
- [13] a) M. Marigo, S. Bachmann, N. Halland, A. Branton, K. A. Jørgensen, *Angew. Chem.* **2004**, 116, 5623–5626; *Angew. Chem. Int. Ed.* **2004**, 43, 5507–5510; b) A. Ros, A. Magriz, H. Dietrich, R. Fernandez, A. Alvarez, J. M. Lassaletta, *Org. Lett.* **2006**, 8, 127–130.
- [14] a) B. M. Trost, *Angew. Chem.* **1995**, 107, 285–307; *Angew. Chem. Int. Ed. Engl.* **1995**, 34, 259–281; b) R. A. Sheldon, *Chem. Soc. Rev.* **2012**, 41, 1437–1451; c) P. A. Wender, V. A. Verma, T. J. Paxton, T. H. Pillow, *Acc. Chem. Res.* **2008**, 41, 40–49; d) T. Newhouse, P. S. Baran, R. W. Hoffmann, *Chem. Soc. Rev.* **2009**, 38, 3010–3021.
- [15] For the use of compound **2a** in the synthesis of analgesics: a) Y. Tsuzuki, T. Morie, T. Nakamura, I. Shimizu, M. Miyauchi, US2012/28937A1, **2012**; b) Dainippon Sumitomo Pharma Co., Ltd. EP2277861A1, **2011**; synthesis of androgen receptors and binding affinity substrates: c) Warner-Lambert Company LLC US2006/9427A1, **2006**; compounds **3a** and **4a** are key intermediates for the synthesis of many bioactive compounds: d) N. De Kimpe, R. Verhe, *The Chemistry of α -Haloketones, α -Halaldehydes, and α -Haloimines*, Wiley, New York, **1990**; e) “Halohydrins and derivatives”: *Fiesers' Reagents for Organic Synthesis* (Eds.: J. G. Smith, M. Fieser), Wiley, New York, **1990**; compound **5a** can be used in the synthesis of inhibitors for the viruses hepatitis B and C, and HIV-1: f) Institute of Medicinal Biotechnology, EP2253621A1, **2010**; g) P. Beaulieu, P. Bonneau, R. Coulombe, P. Forgione, J. Gillard, A. Jakalian, J. Rancourt, WO2010/37210A1, **2010**; kinase inhibitors: h) D. Kay, R. Knegtel, J.-D. Charrier, H. Twin, US2008/167289A1, **2008**; i) Amgen Inc., WO2009/11871A2, **2009**.