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Gram-Scale Synthesis of Chiral Cyclopropane-Containing Drugs and Drug Precursors with Engineered Myoglobin Catalysts Featuring Complementary Stereoselectivity

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Abstract: *Engineered hemoproteins have recently emerged as promising systems for promoting asymmetric cyclopropanations, but variants featuring predictable, complementary stereoselectivity in these reactions have remained elusive. In this study, a rationally driven strategy was implemented and applied to engineer myoglobin variants capable of providing access to 1-carboxy-2-aryl-cyclopropanes with high trans-(1R,2R) selectivity and catalytic activity. The stereoselectivity of these cyclopropanation biocatalysts complements that of trans-(1S,2S)-selective variants developed here and previously. In combination with whole-cell biotransformations, these stereocomplementary biocatalysts enabled the multigram synthesis of the chiral cyclopropane core of four drugs (Tranylcypromine, Tasimelteon, Ticagrelor, and a TRPV1 inhibitor) in high yield and with excellent diastereo- and enantioselectivity (98–99.9% de; 96–99.9% ee). These biocatalytic strategies outperform currently available methods to produce these drugs.*

Catalytic methods for the cyclopropanation of olefins cover a prominent role in organic and medicinal chemistry owing to the recurrence of cyclopropane motifs among biologically active natural products and pharmaceuticals.[1] Significant progress has been made in the development of synthetic methods for asymmetric cyclopropanation, in particular through the transition-metal-catalyzed insertion of carbenoid species into carbon–carbon double bonds.[2] More recently, the Arnold group and our own laboratory have shown that engineered cytochrome P450s[3] and myoglobins (Mb),[4] respectively, constitute promising catalysts for mediating the cyclopropanation of styrenes in the presence of α-diazoacetate reagents, thus providing a biocatalytic alternative for this valuable transformation. Variants of the bacterial cytochrome $P450_{BM3}$ were found to favor *cis* selectivity in the cyclopropanation of styrene in the presence of ethyl α-diazoacetate (EDA; $P450_{BM3}$-CIS-T438S: 86% *de* (*cis*), 97% *ee* (1*S*,2*R*)).[3a] By utilizing a different P450 enzyme, opposite enantioselectivity was achieved by Brustad and co-workers for the *cis* cyclopropanation of this substrate, albeit with more moderate diastereoselectivity ($P450_{Biol}$-T238A: 42% *de* (*cis*), 95% *ee* (1*R*,2*S*)).[5] Unfortunately, varying *cis*/*trans* ratios and degrees of stereoselectivity were observed with these enzymes in the presence of other styrene derivatives.[3a,5] We previously reported the development of an engineered myoglobin variant, Mb(H64V,V68A), that is capable of catalyzing the cyclopropanation of styrene with EDA with excellent *trans* diastereoselectivity and (1*S*,2*S*) enantioselectivity (99.9% *de*, 99.9% *ee*).[4] Promisingly, the high *trans*-(1*S*,2*S*) selectivity of this Mb variant extended to the cyclopropanation of a variety of styrene derivatives (97–99.9% *de*, 96–99.9% *ee*).[4]

The ability to access both enantiomeric forms of a target cyclopropane pharmacophore is critical in the context of the synthesis of bioactive molecules, as such stereoisomers often exhibit remarkably divergent pharmacological and/or toxicity profiles.[1] However, developing stereo- or enantiocomplementary variants of an enzyme is far from being a trivial task,[6] as mirror-image forms of these biomolecules are not readily available.[7] Reflecting this notion, cyclopropanation biocatalysts that can reliably offer complementary stereoselectivity have thus far remained unavailable.[3,5,8] Herein, we report the development and characterization of a panel of engineered Mb catalysts that provide access to *trans*-(1*R*,2*R*)-configured 1-carboxy-2-aryl-cyclopropanes with high selectivity and catalytic activity across a broad range of olefin substrates. We further demonstrate that these Mb-catalyzed reactions can be carried out and scaled up using whole-cell systems. Using these stereocomplementary biocatalysts in combination with whole-cell transformations, the asymmetric synthesis of the cyclopropane core of four different drugs, featuring both *trans*-(1*S*,2*S*) and *trans*-(1*R*,2*R*) configurations, was accomplished at the multigram scale.

Previously, we found that mutations at the five amino acid positions defining the distal pocket in Mb (i.e., Leu29, Phe43, His64, Val68, and Ile107; see the Supporting Information, Figure S1) significantly affected the activity and selectivity of this hemoprotein in carbene[4,9] and nitrene[10] transfer reactions. In particular, mutation of the distal histidine residue (H64V) was shown to have a general activity-enhancing effect in these reactions, possibly owing to an increased accessibility of the heme pocket to the reactants. This mutation also slightly increases the *trans*-(1*S*,2*S*) selectivity of Mb for styrene cyclopropanation with EDA (93% *de* (*trans*), 10% *ee* (1*S*,2*S*) compared to 86% *de* (*trans*), 6% *ee* (1*S*,2*S*) for wild-type Mb). This effect could be combined with that of a stronger *trans*-(1*S*,2*S*) selectivity inducing mutation, i.e., V68A (96% *de* (*trans*), 68% *ee* (1*S*,2*S*)), to yield the aforementioned highly *trans*-(1*S*,2*S*)-selective Mb(H64V,V68A) catalyst (Figure 1, gray path).[4] These

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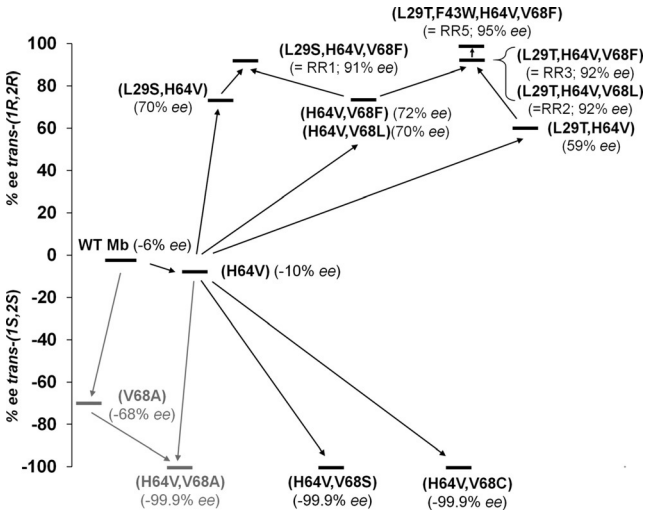
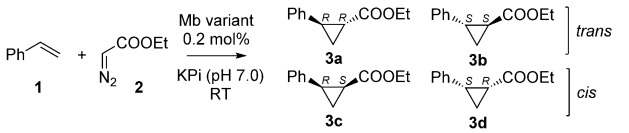


Figure 1. Structure–reactivity-guided design of Mb cyclopropanation biocatalysts with complementary stereoselectivity. The path in gray is described in Ref. [4].

Table 1: Activity and selectivity of representative myoglobin variants for styrene cyclopropanation with EDA.[a]



Catalyst	Conv. [%][b]	TON	de_{trans} [%]	ee_{trans} [%][c]	ee_{cis} [%][c]
Mb	36	180	86	−6	0
Mb(H64V)	47	235	93	−10	8
Mb(L29S,H64V)	60	300	93	70	18
Mb(L29T,H64V)	93	460	95	59	39
Mb(H64V,V68F)	57	285	>99.9	72	–
Mb(H64V,V68L)	53	265	>99.9	70	–
Mb(H64V,V68S)	82	410	>99.9	−99	–
Mb(L29T,H64V,V68L)	61	305	>99.9	92	–
Mb(L29T,H64V,V68F)	58	290	>99.9	92	–
Mb(L29T,F43W,H64V,V68F)	53	265	99	95	–

[a] Reaction conditions: 20 μM Mb variant, 10 mM styrene, 20 mM EDA, 10 mM dithionite, 16 h. See also Tables S2 and S3. [b] Based on GC analysis, relative to the olefin. [c] *trans* = (1*R*,2*R*), *cis* = (1*S*,2*R*). Values determined by gas chromatography or supercritical fluid chromatography on a chiral stationary phase.

results supported our hypothesis that additive effects can be leveraged to fine-tune the stereoselectivity of Mb in cyclopropanation reactions. At the same time, none of the active-site Mb variants (>10) examined in these earlier studies showed any preference for formation of the *trans*-(1*R*,2*R*) product, indicating that achieving this type of stereoselectivity would require exploration of a wider active-site sequence space. On the basis of these considerations, we sought to implement a systematic active-site mutagenesis approach combined with structure–reactivity-guided design to achieve our goal of developing *trans*-(1*R*,2*R*)-selective Mb catalysts.

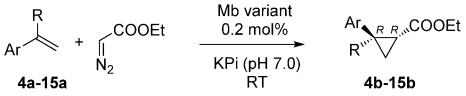
Accordingly, starting from Mb(H64V) as the parent protein, each of the remaining four active-site positions was systematically mutated to any of the other 19 amino acids by site-directed mutagenesis. This process resulted in a library of 76 Mb(H64V)-derived variants, which were tested individually for their activity (turnover number, TON) and selectivity in the model cyclopropanation reaction with styrene and EDA (Table S2). Nearly half of these Mb variants (33/76; 43%) could be expressed in *E. coli* in correctly folded form as determined by the signature Soret band for their CO-bound complex ($\lambda_{max} \approx 420$ nm). Moreover, the large majority of these proteins (75%) was catalytically competent (TON > 50) towards styrene cyclopropanation (Table S2), highlighting the robustness of the Mb active site to mutagenesis. More importantly, screening of the library led to the identification of a number of Mb variants with significantly improved *trans*-(1*R*,2*R*) selectivity (Table 1) compared to the parent protein Mb(H64V) or wild-type Mb. Most effective in favoring such selectivity was the introduction of a leucine or phenylalanine residue in place of Val68 (i.e., 86→99% *de*, (−6)→70–72% *ee* (1*R*,2*R*)). Residue 68 lies along the side of the heme group and is expected to be in close proximity to the putative heme-bound carbene intermediate implicated in the cyclopropanation reaction.[4] Interestingly, substitution of the more remote Leu29 with either serine or threonine was also beneficial towards enhancing *trans*-(1*R*,2*R*) selectivity, as indicated by the 93–95% *de* and 59–70% *ee* values obtained with Mb(L29S,H64V) and Mb(L29T,H64V) (Table 1). Mutations of positions Phe43 or Ile107 had noticeable but comparatively weaker effects on favoring *trans*-(1*R*,2*R*) selectivity (16–55% *ee*; Table S2). Moreover, a number of highly *trans*-(1*S*,2*S*)-selective variants were identified among the Val68 site-saturation sub-library, including Mb(H64V,V68S) and Mb(H64V,V68C), which produce **3b** in >99% *de* and *ee* (Tables 1 and S2).

To obtain Mb catalysts with further improved *trans*-(1*R*,2*R*) selectivity, the beneficial mutations at position 68 (V68L, V68F) were combined with those at position 29 (L29T, L29S) and 107 (I107H) to yield a set of triple-site variants (Table S3). Gratifyingly, this process resulted in the identification of two closely related variants, Mb(L29T,H64V,V68L) and Mb(L29T,H64V,V68F), with excellent *trans* diastereoselectivity (99% *de*) along with high 1*R*,2*R* stereoselectivity (92% *ee*; Table 1). Next, these Mb variants were further mutated at position Ile107 or Phe43, with the choice of the target substitutions being guided by the structure–reactivity relationship (SRR) data collected during screening of the initial library. Although none of the resulting quadruple-site variants with substitutions at position 107 showed improved *trans*-(1*R*,2*R*) selectivity, the introduction of the F43W mutation in the Mb(L29T,H64V,V68F) background led to the desired additivity effect. Indeed, the corresponding Mb(L29T,F43W,H64V,V68F) variant catalyzes the transformation of styrene and EDA into **3a** with excellent diastereo- and enantioselectivity (>99% *de*, 95% *ee*; Table 1). It should be noted that refinement of the *trans*-(1*R*,2*R*) selectivity came with no loss in catalytic efficiency as indicated by the comparable TONs achieved with the *trans*-(1*R*,2*R*)-selective Mb variants and the parent protein (265–460 vs. 235 TON). Figure 1 provides a graphical representa-

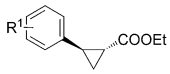
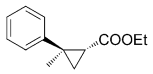
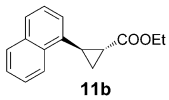
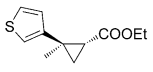
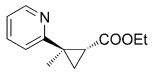
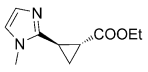
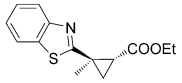
tion of the SRR-driven strategy implemented here for the development of stereocomplementary Mb cyclopropanation catalysts. As anticipated, achieving high *trans*-(1*R*,2*R*) selectivity involved a more extensive remodeling of the Mb active site (4 mutations) than required for obtaining high *trans*-(1*S*,2*S*) selectivity (2 mutations).

The substrate scope of the *trans*-(1*R*,2*R*)-selective Mb catalysts (called RR1–RR5, Table S3) was then probed against substituted styrene derivatives. As shown in Table 2, quantitative product conversion (95–99%) was achieved with different *para*-, *meta*-, *ortho*-, and *alpha*-functionalized styrenes (**4b–6b**, **8b–10b**), with more moderate conversion (45%) being observed only with 4-trifluoromethylstyrene (product **7b**). Importantly, for all of these substrates, the desired *trans*-(1*R*,2*R*)-configured cyclopropane products were obtained with good to excellent diastereoselectivity (92–99% *de*) and enantioselectivity (49–99% *ee*) with one or more of the Mb variants. Next, we extended these analyses to other aryl-substituted olefins, including pyridine, thiophene, *N*-methylimidazole, and benzothiazole derivatives. These molecules comprise N- and S-containing heterocycles, which are notoriously problematic in the context of cyclopropanation reactions with rhodium- or other metal-based complexes owing to catalyst poisoning effects. For example, the reaction of 1-methyl-2-vinylimidazole with EDA and $Rh_2(OAc)_4$ as the catalyst failed to give any product (see the Supporting Information for details). Notably, all of these olefins could be efficiently processed by the engineered Mb catalysts, resulting in product conversions of up to 84% (**11b–15b**, Table 2). Furthermore, the corresponding *trans*-(1*R*,2*R*) cyclopropane products could be obtained with good to very good selectivities (65–99% *de*, 65–98% *ee*). For all of these transformations, complementary stereoselectivity is provided by the *trans*-(1*S*,2*S*)-selective Mb(H64V,V68A) variant (Table S4).

Table 2: Substrate scope for *trans*-(1*R*,2*R*)-selective Mb variants.[a]



4a-15a 4b-15b

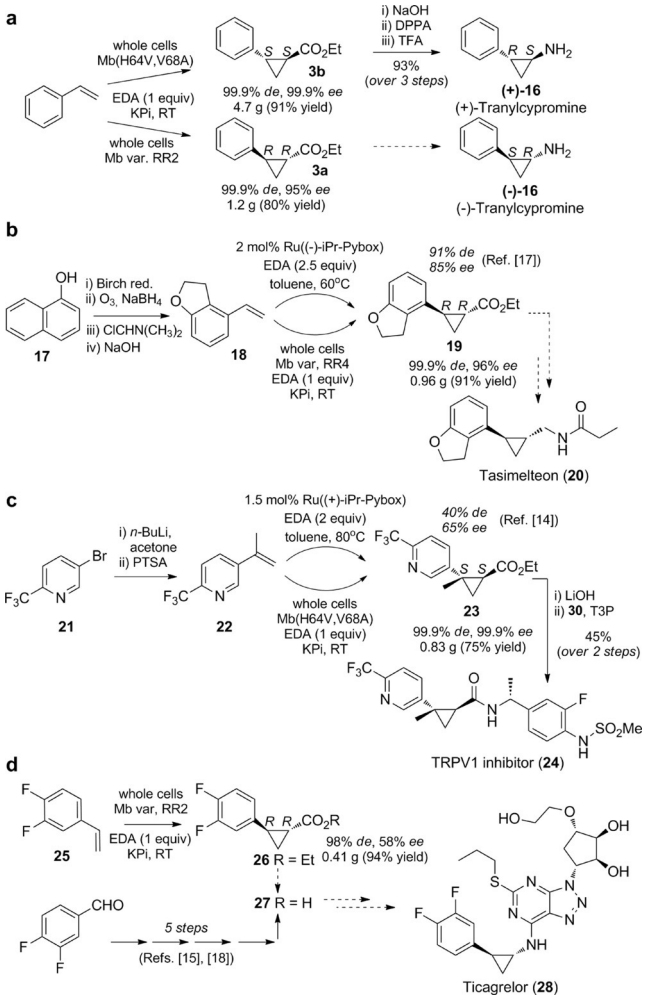
Product	Cat.[b]	Conv. [%]	TON	*de* [%]	*ee* [%]
					
R^1 = 4-CH_3 (**4b**)	RR5	>99[c]	165	96	63
R^1 = 4-MeO (**5b**)	RR5	95[c]	160	97	74
R^1 = 4-Cl (**6b**)	RR2	>99	>500	97	49
R^1 = 4-CF_3 (**7b**)	RR4	45[c]	75	97	64
R^1 = 3-Me (**8b**)	RR4	>99	>500	92	84
R^1 = 2-Me (**9b**)	RR3	>99	>500	>99	>99
 **10b**	RR3	>99[c]	165	78	78
 **11b**	RR4	45[c]	75	80	88
 **12b**	RR2	84	420	96	65
 **13b**	RR2	78	390	88	67
 **14b**	RR1	56[c]	95	99	98
 **15b**	RR4	54[c]	90	65	71

[a] Reaction conditions: 20 μM Mb variant (0.2 mol%), 10 mM styrene, 20 mM EDA, 10 mM dithionite, 16 h. [b] For the nomenclature of the RR1–RR5 variants, see Figure 1 and Table S3. [c] With 0.6 mol% Mb catalyst.

Whole-cell biotransformations constitute a convenient approach to enhance the scalability of biocatalytic processes supported by heme-dependent enzymes.[3b,11] Importantly, we established that the Mb(H64V,V68A)-catalyzed cyclopropanation of styrene with EDA to give **3b** can be readily achieved using *E. coli* cells (BL21DE3) expressing this Mb variant, resulting in high product conversion (68–100%) even under aerobic conditions (Table S5). Furthermore, the substrate loading in these whole-cell reactions could be increased up to 0.5 M styrene and 0.5 M EDA in a 1:1 ratio while maintaining high conversion (62%) and excellent selectivity (99.9% *de*, 99.9% *ee*; Table S5).

Aryl-substituted *trans* cyclopropanes are found in several marketed and investigational drugs (Scheme 1),[1] including the MAO inhibitor Tranylcypromine ((±)-**16**, formerly Parnate),[12] the melatonin receptor agonist Tasimelteon (**20**),[13] the TRPV1 antagonist **24**,[14] and the platelet aggregation inhibitor Ticagrelor (**28**, Brilinta).[15] Although Tranylcypromine has been used in clinical settings in racemic form, the two enantiomers display different biological potencies.[16] The other drugs are used in enantiomerically pure form. Each of these molecules features a cyclopropane unit that can be retrosynthetically derived from either a *trans*-(1*R*,2*R*)- ((−)-**16**, **19**, **28**) or *trans*-(1*S*,2*S*)-configured 1-carboxy-2-aryl-cyclopropane ((+)-**16**, **24**). As such, these drugs were chosen as relevant targets to assess the synthetic utility of the stereocomplementary cyclopropanation biocatalysts developed herein.

Accordingly, to obtain the dextrorotatory form of Tranylcypromine, a large-scale reaction with *E. coli* cells expressing Mb(H64,V68A) was carried out in the presence of 2.9 g styrene and 3.1 g EDA (1 equiv), resulting in the isolation of **3b** as the only product (99.9% *de*, 99.9% *ee*) in 91% yield (4.7 g; Scheme 1a). Under similar reaction conditions, but using cells expressing Mb(L29T,H64V,V68L), 1.2 g of the opposite enantiomer **3a** were obtained in 80% yield and excellent selectivity (99.9% *de*, 95% *ee*). Both intermediates can be converted into the desired (+)- and (−)-Tranylcypromine, respectively, by conversion into the corresponding acyl



Scheme 1. Total and formal syntheses of a) (+)- and (−)-Tranylcypromine, b) Tasimelteon, c) TRPV1 inhibitor **24**, and d) Ticagrelor by myoglobin-catalyzed cyclopropanation reactions in whole cells. See the Supporting Information for details on the synthetic steps. DPPA = diphenylphosphoryl azide, PTSA = *para*-toluenesulfonic acid, T3P = propane phosphonic acid anhydride, TFA = trifluoroacetic acid.

azides followed by a stereoretentive Curtius rearrangement[16] and hydrolysis (93% yield over three steps for (+)-**16**; see the Supporting Information for details). By comparison, the same cyclopropanation reaction catalyzed by two prominent Cu(Box) catalysts was reported to proceed with high stereoselectivity (90–99% *ee*) but only moderate diastereoselectivity (46–50% *de* (*trans*)).[2d,e]

The asymmetric cyclopropanation of olefin **18** to give **19** represents a key step in the synthesis of Tasimelteon as reported by chemists at Bristol–Myers Squibb.[17] Under best performing conditions, this transformation was realized in 91% *de* and 85% *ee* using Nishiyama's Ru(*i*Pr-PyBox) catalyst in the presence of excess EDA. After affording olefin intermediate **18** according to published procedures,[17] this compound was subjected to Mb-catalyzed cyclopropanation using whole cells expressing the *trans*-(1*R*,2*R*)-selective variant Mb(L29T,H64V,V68F,I107L) in the presence of stoichiometric amounts of EDA (Scheme 1b). Notably, the desired 1*R*,2*R*-configured cyclopropanation product **19** was obtained in 99.9% *de* and 96% *ee* and in 91% isolated yield (0.96 g), thus furnishing a key intermediate en route to Tasimelteon (**20**).[17]

The stereoselective cyclopropanation of **22** into **23** was denoted by Pfizer chemists as "the most challenging step" in the preparation of the advanced drug candidate **24**,[14] owing to the presence of a pyridine functional group and formation of a trisubstituted cyclopropane.[2k] Upon screening over 120 different catalytic conditions, the best results were obtained using Ru(*i*Pr-PyBox) as the catalyst to give **23** in 40% *de* and 65% *ee*.[14] Using whole cells expressing Mb(H64V,V68A), 0.83 g of **23** could be obtained in high yield (75%) with much greater diastereo- and enantioselectivity (99.9% *de*, 99.9% *ee*; Scheme 1c). The cyclopropanation product was further processed to afford **24** (see the Supporting Information for details).

Lastly, we targeted the synthesis of (1*R*,2*R*)-ethyl 2-(3,4-difluorophenyl)cyclopropanecarboxylate **26** (Scheme 1d), which can be readily hydrolyzed to **27**, a key synthetic intermediate for the preparation of Ticagrelor.[15] Of note, this intermediate is currently accessible through synthetic routes involving no less than four to five steps.[15,18] Conveniently, the key cyclopropane building block **26** could be obtained in a single step in high yield (94%) and selectivity (98% *de*, 58% *ee*) by whole-cell cyclopropanation of commercially available 3,4-difluorostyrene with cells expressing the (1*R*,2*R*)-selective Mb(L29T,H64V,V68L) variant. Taken together, these results demonstrate the promise and scalability of stereoselective myoglobin-catalyzed cyclopropanations in the context of relevant and challenging building blocks for drug synthesis.

In summary, we have reported the successful design and application of myoglobin-based cyclopropanation biocatalysts that are capable of offering high *trans* selectivity along with complementary stereoselectivity across a broad panel of aryl-substituted olefins. Furthermore, we have demonstrated that these myoglobin-mediated transformations can be performed in the context of whole-cell systems, which further simplifies their use for synthetic applications. The biocatalytic systems developed here have enabled the stereoselective synthesis of multiple cyclopropane-containing drugs at the preparative scale, offering superior performance over currently available methods for asymmetric cyclopropanation (i.e., (+)- and (−)-**16**, **20**, and **24**) or granting a more concise route to their preparation (**28**). Along with the growing number of abiotic reactions accessible using engineered myoglobins,[4,9,10,19] these results support the promise of these metalloproteins for the asymmetric synthesis of chiral drugs and synthons at a practical scale.

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Angew. Chem. **2016**, *128*, 16344–16348

[1] T. T. Talele, *J. Med. Chem.* **2016**, *59*, 8712–8756.
[2] a) M. P. Doyle, D. C. Forbes, *Chem. Rev.* **1998**, *98*, 911–936; b) H. Lebel, J. F. Marcoux, C. Molinaro, A. B. Charette, *Chem. Rev.* **2003**, *103*, 977–1050; c) H. Pellissier, *Tetrahedron* **2008**, *64*, 7041–7095; d) R. E. Lowenthal, A. Abiko, S. Masamune, *Tetrahedron Lett.* **1990**, *31*, 6005–6008; for representative examples with acceptor-only diazo reagents, see: e) D. A. Evans, K. A. Woerpel, M. M. Hinman, M. M. Faul, *J. Am. Chem. Soc.* **1991**, *113*, 726–728; f) M. P. Doyle, W. R. Winchester, J. A. A. Hoorn, V. Lynch, S. H. Simonsen, R. Ghosh, *J. Am. Chem. Soc.* **1993**, *115*, 9968–9978; g) H. Nishiyama, Y. Itoh, H. Matsumoto, S. B. Park, K. Itoh, *J. Am. Chem. Soc.* **1994**, *116*, 2223–2224; h) T. Uchida, R. Irie, T. Katsuki, *Synlett* **1999**, 1163–1165; i) C. M. Che, J. S. Huang, F. W. Lee, Y. Li, T. S. Lai, H. L. Kwong, P. F. Teng, W. S. Lee, W. C. Lo, S. M. Peng, Z. Y. Zhou, *J. Am. Chem. Soc.* **2001**, *123*, 4119–4129; j) L. Y. Huang, Y. Chen, G. Y. Gao, X. P. Zhang, *J. Org. Chem.* **2003**, *68*, 8179–8184; k) S. Fantauzzi, E. Gallo, E. Rose, N. Raoul, A. Caselli, S. Issa, F. Ragaini, S. Cenini, *Organometallics* **2008**, *27*, 6143–6151; l) D. M. Carminati, D. Intrieri, A. Caselli, S. Le Gac, B. Boitrel, L. Toma, L. Legnani, E. Gallo, *Chem. Eur. J.* **2016**, *22*, 13599–13612.
[3] a) P. S. Coelho, E. M. Brustad, A. Kannan, F. H. Arnold, *Science* **2013**, *339*, 307–310; b) 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.
[4] M. Bordeaux, V. Tyagi, R. Fasan, *Angew. Chem. Int. Ed.* **2015**, *54*, 1744–1748; *Angew. Chem.* **2015**, *127*, 1764–1768.
[5] J. G. Gober, A. E. Rydeen, E. J. Gibson-O'Grady, J. B. Leuthaeuser, J. S. Fetrow, E. M. Brustad, *ChemBioChem* **2016**, *17*, 394–397.
[6] a) Q. Wu, P. Soni, M. T. Reetz, *J. Am. Chem. Soc.* **2013**, *135*, 1872–1881; b) A. Z. Walton, B. Sullivan, A. C. Patterson-Orazem, J. D. Stewart, *ACS Catal.* **2014**, *4*, 2307–2318; c) P. N. Scheller, S. Fademrecht, S. Hofelzer, J. Pleiss, F. Leipold, N. J. Turner, B. M. Nestl, B. Hauer, *ChemBioChem* **2014**, *15*, 2201–2204; d) J. Y. van der Meer, H. Poddar, B. J. Baas, Y. F. Miao, M. Rahimi, A. Kunzendorf, R. van Merkerk, P. G. Tepper, E. M. Geertsema, A. M. W. H. Thunnissen, W. J. Quax, G. J. Poelarends, *Nat. Commun.* **2016**, *7*, 10911.
[7] P. F. Mugford, U. G. Wagner, Y. Jiang, K. Faber, R. J. Kazlauskas, *Angew. Chem. Int. Ed.* **2008**, *47*, 8782–8793; *Angew. Chem.* **2008**, *120*, 8912–8923.
[8] a) Z. J. Wang, H. Renata, N. E. Peck, C. C. Farwell, P. S. Coelho, F. H. Arnold, *Angew. Chem. Int. Ed.* **2014**, *53*, 6810–6813; *Angew. Chem.* **2014**, *126*, 6928–6931; b) H. Renata, Z. J. Wang, R. Z. Kitto, F. H. Arnold, *Catal. Sci. Technol.* **2014**, *4*, 3640–3643; c) P. Srivastava, H. Yang, K. Ellis-Guardiola, J. C. Lewis, *Nat. Commun.* **2015**, *6*, 7789.
[9] a) G. Sreenilayam, R. Fasan, *Chem. Commun.* **2015**, *51*, 1532–1534; b) V. Tyagi, R. B. Bonn, R. Fasan, *Chem. Sci.* **2015**, *6*, 2488–2494; c) V. Tyagi, R. Fasan, *Angew. Chem. Int. Ed.* **2016**, *55*, 2512–2516; *Angew. Chem.* **2016**, *128*, 2558–2562; d) S. Giovani, R. Singh, R. Fasan, *Chem. Sci.* **2016**, *7*, 234–239.
[10] M. Bordeaux, R. Singh, R. Fasan, *Bioorg. Med. Chem.* **2014**, *22*, 5697–5704.
[11] a) M. T. Lundemo, J. M. Woodley, *Appl. Microbiol. Biotechnol.* **2015**, *99*, 2465–2483; b) R. J. Sowden, S. Yasmin, N. H. Rees, S. G. Bell, L. L. Wong, *Org. Biomol. Chem.* **2005**, *3*, 57–64; c) R. Fasan, M. M. Chen, N. C. Crook, F. H. Arnold, *Angew. Chem. Int. Ed.* **2007**, *46*, 8414–8418; *Angew. Chem.* **2007**, *119*, 8566–8570; d) R. Fasan, N. C. Crook, M. W. Peters, P. Meinhold, T. Buelter, M. Landwehr, P. C. Cirino, F. H. Arnold, *Biotechnol. Bioeng.* **2011**, *108*, 500–510; e) M. Ringle, Y. Khatri, J. Zapp, F. Hannemann, R. Bernhardt, *Appl. Microbiol. Biotechnol.* **2013**, *97*, 7741–7754; f) C. A. Müller, A. Dennig, T. Welters, T. Winkler, A. J. Ruff, W. Hummel, H. Groger, U. Schwaneberg, *J. Biotechnol.* **2014**, *191*, 196–204; g) F. Tieves, I. N. Erenburg, O. Mahmoud, V. B. Urlacher, *Biotechnol. Bioeng.* **2016**, *113*, 1845–1852.
[12] A. Burger, W. L. Yost, *J. Am. Chem. Soc.* **1948**, *70*, 2198–2201.
[13] J. Catt, G. Johnson, D. Keavy, R. Mattson, M. Parker, K. Takaki, J. Yevich (BMS), U.S. Pat. 5981571, **1999**.
[14] K. J. Butcher, S. M. Denton, S. E. Field, A. T. Gillmore, G. W. Harbottle, R. M. Howard, D. A. Laity, C. J. Ngono, B. A. Pibworth, *Org. Process Res. Dev.* **2011**, *15*, 1192–1200.
[15] B. Springthorpe et al., *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6013–6018.
[16] T. N. Riley, C. G. Brier, *J. Med. Chem.* **1972**, *15*, 1187–1188.
[17] J. H. Simpson, J. Godfrey, R. Fox, A. Kotnis, D. Kacsur, J. Hamm, M. Totelben, V. Rosso, R. Mueller, E. Delaney, R. P. Deshpande, *Tetrahedron: Asymmetry* **2003**, *14*, 3569–3574.
[18] a) H. Zhang, J. Liu, L. Zhang, L. Kong, H. Yao, H. Sun, *Bioorg. Med. Chem. Lett.* **2012**, *22*, 3598–3602; b) S. Guile, D. Hardern, B. Springthorpe, P. Willis, WO/2000/034283, **2000**; c) A. Clark, E. Jones, U. Larsson, A. Minidis, WO/2001/092200, **2001**; d) K. G. Hugentobler, H. Sharif, M. Rasparini, R. S. Heath, N. J. Turner, *Org. Biomol. Chem.* **2016**, *14*, 8064–8067.
[19] H. M. Key, P. Dydio, D. S. Clark, J. F. Hartwig, *Nature* **2016**, *534*, 534–537.

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