nature chemical biology

Chemo-enzymatic fluorination of unactivated organic compounds

Andrea Rentmeister[1], Frances H Arnold[1] & Rudi Fasan[1,2]

Fluorination has gained an increasingly important role in drug discovery and development. Here we describe a versatile strategy that combines cytochrome P450–catalyzed oxygenation with deoxofluorination to achieve mono- and polyfluorination of nonreactive sites in a variety of organic scaffolds. This procedure was applied for the rapid identification of fluorinated drug derivatives with enhanced membrane permeability.

Fluorination has become an increasingly important tool for fine-tuning the pharmacokinetic and pharmacological properties of drugs and lead compounds, thus leading to a growing number of fluorine-containing pharmaceuticals on the market[1]. Benefits of hydrogen-to-fluorine substitutions arise principally from their effects on membrane permeability, metabolic stability and/or receptor-binding properties of bioactive molecules[1–3]. In many cases, fluorination of much less active precursors has yielded potent drugs with enhanced bioavailability, reduced toxicity or improved affinity for the target receptor[3]. A number of methods have been developed for synthesis of fluorinated compounds[4,5], including asymmetric fluorination strategies[6,7] and chemo-enzymatic approaches[8,9]. Despite this progress, selective incorporation of fluorine at non-activated or weakly reactive sites of a target scaffold remains difficult and may require several synthetic steps.

Here we describe a facile two-step procedure for the selective fluorination of one or more non-activated sites in an organic molecule. This approach couples the exceptional ability of cytochrome P450 monooxygenases to selectively insert oxygen into nonreactive C-H bonds with a deoxofluorination reaction in which the newly generated hydroxyl group is substituted by fluorine by means of a nucleophilic fluorinating reagent (**Fig. 1**). To test the validity of this approach, we targeted various classes of small molecules, including marketed pharmaceuticals (**Supplementary Fig. 1** online). For the enzymatic step, we used variants of the bacterial long-chain fatty acid hydroxylase $P450_{BM3}$ from *Bacillus megaterium*. Catalytic self-sufficiency, high monooxygenase activity and high expression level in *Escherichia coli* render $P450_{BM3}$ an attractive catalyst for *in vitro* and *in vivo* applications[10]. For this work, we assembled a panel of 96 P450s derived from a catalytically promiscuous $P450_{BM3}$ variant identified in the early stages of the directed evolution of a proficient alkane monooxygenase[11]. These variants were found to exhibit good activity and various degrees of selectivity on alkanes and non-alkane substrates[11].

The first group of test molecules (**1**, **2** and **3**; **Fig. 2a**,**b**) contain a cyclopentenone moiety found in several natural products (for example, jasmonoids, cyclopentanoid antibiotics and prostaglandins). The synthesis and functionalization of these scaffolds is not trivial[12]. The activities of the enzymes toward these substrates were probed in multiwell format using gas chromatography and GC-MS (**Fig. 2a**). Depending on the substrate, approximately 30 to 50% of the 96 enzyme variants displayed useful activity (>800 turnovers), while 30 to 50% of this active subset showed moderate to excellent regioselectivity (50–100%). The most active and selective variants were applied in preparative scale reactions (100–300 mg) using ~0.05 mol% catalyst. Compared with 96-well plate reactions, three to four times higher turnover numbers could be obtained using purified enzyme and longer reaction times (24–48 h). After flash chromatography purification, the hydroxylated products were subjected to deoxofluorination using the common nucleophilic fluorinating agent diethylaminosulfur trifluoride (DAST, **4**). The identities and purities of the fluorinated products were established by GC-MS, HRMS and ^{1}H, ^{13}C and ^{19}F NMR (**Supplementary Methods** online). Using this strategy, we were able to target two to three different sites on each substrate with good to excellent regioselectivity (55–100%), thereby affording the fluorinated derivatives **5**, **6**, **7**, **8**, **9**, **10** and **11** with yields of up to 80% over the two steps.

Next we tested this fluorination strategy on a methylester pro-drug of the anti-inflammatory drug ibuprofen (**12**; **Fig. 2a**,**c**). Although preparation of α-fluoro derivatives of this compound is straightforward[13], incorporating fluorine atoms in the poorly reactive isobutyl group is not. The general procedure described above enabled us to identify two chemo-enzymatic routes to achieve this goal in a selective (position 1, 75%; position 2, 100%) and efficient manner (yields over two steps for **15** and **16** were 62% and 84%, respectively) and at preparative scales (150–200 mg).

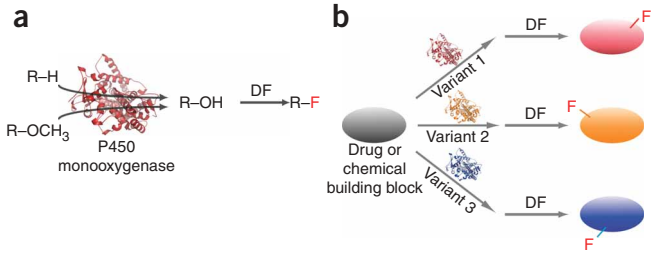


Figure 1 Cytochrome P450-based approach for selective fluorination of organic molecules. (**a**) Hydroxyl groups are introduced (via direct oxygen insertion) or exposed (via hydroxylation-demethylation) in a target scaffold using a P450 monooxygenase and substituted for fluorine using a nucleophilic fluorinating agent. DF, deoxofluorination. (**b**) Different fluorinated derivatives of a molecule of interest are obtained using P450 variants with different regioselectivities.

[1]Department of Chemistry and Chemical Engineering, California Institute of Technology 210-41, 1200 East California Boulevard, Pasadena, California 91125, USA. [2]Present address: Department of Chemistry, University of Rochester, Hutchison Hall, RC Box 270216, Rochester, New York 14627, USA. Correspondence should be addressed to R.F. (fasan@chem.rochester.edu).

Received 11 August; accepted 27 October; published online 16 November 2008; doi:10.1038/nchembio.128

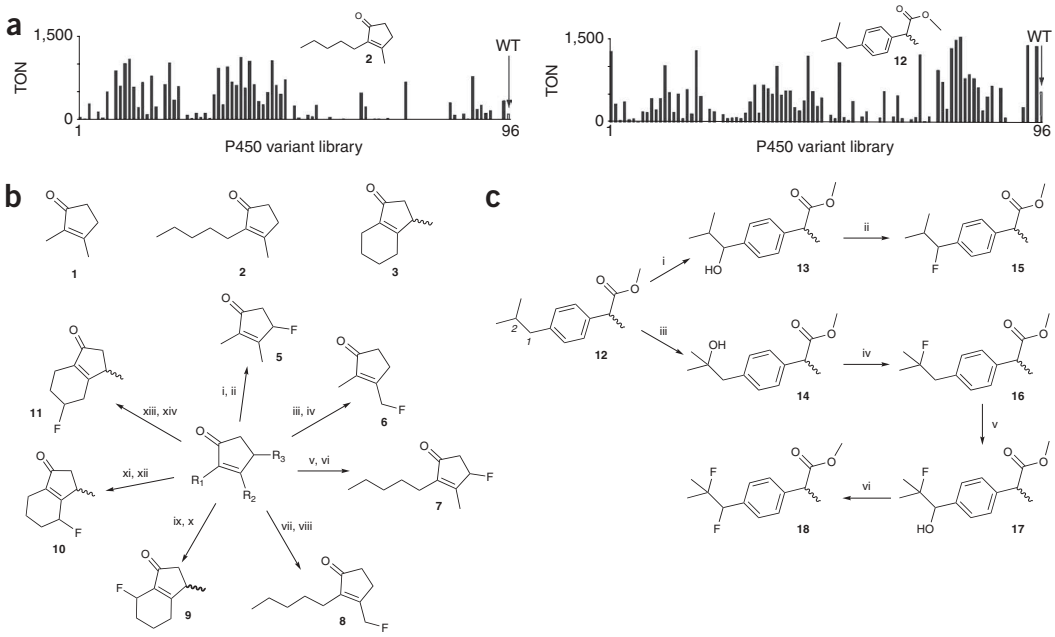


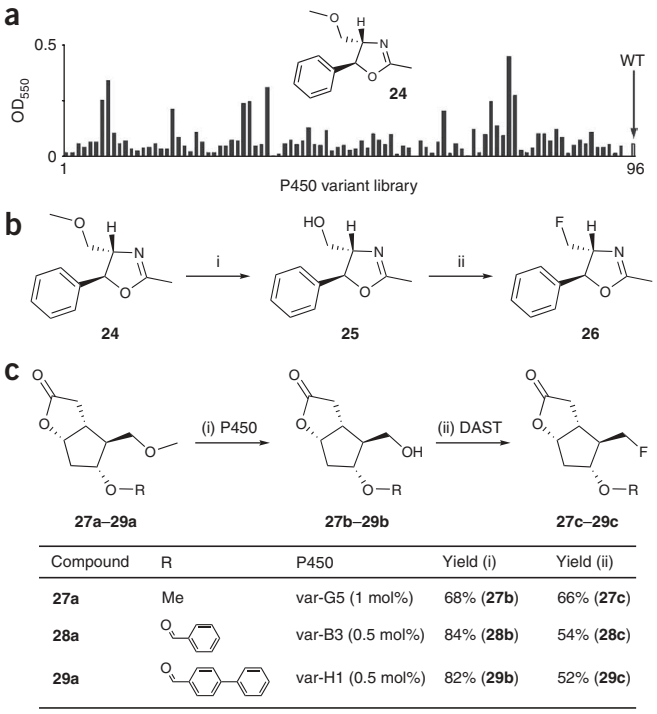
Figure 2 Chemo-enzymatic fluorination of organic molecules. (**a**) Screening of P450 library in 96-well format. Reactions were carried out in the presence of the substrate, P450 enzyme from cell lysate and a glucose-6-phosphate dehydrogenase–based NADPH regeneration system. TON, turnover number. Standard error is within 15%. WT, wild-type $P450_{BM3}$. (**b**) Selective fluorination of cyclopentenone derivatives. Reagents and conditions: (i) **1**, 0.04 mol% var-H3, 88%; (ii) DAST (1.2 equiv), CH_2Cl_2, –78 °C, 12 h, 90% (20% *ee*); (iii) **1**, 0.04 mol% var-G6, 45%; (iv) DAST (1.2 equiv), CH_2Cl_2, –78 °C, 12 h, 85%; (v) **2**, 0.05 mol% var-H3, 85%; (vi) DAST (1.3 equiv), CH_2Cl_2, –78 °C, 12 h, 92% (78% *ee*); (vii) **2**, 0.05 mol% var-G4, 42%; (viii) DAST (1.5 equiv), CH_2Cl_2, –78 °C, 12 h, 89%; (ix) **3**, 0.05 mol% var-D10, 69%; (x) DAST (1.2 equiv), CH_2Cl_2, –78 °C, 3 h, 88% (dr 1:8.5; major, 5% *ee*; minor, 71% *ee*); (xi) **3**, 0.05 mol% var-G4, 62%; (xii) DAST (1.2 equiv), CH_2Cl_2, –78 °C, 5 h, 92% (dr 4:96; major, 0% *ee*; minor, 57% *ee*); (xiii) **3**, 0.07 mol% var-G5, 32%; (xiv) DAST (1.2 equiv), CH_2Cl_2, –78 °C, 5 h, 90% (dr not measurable). (**c**) Selective mono- and difluorination of pro-drug ibuprofen methylester. Reagents and conditions: (i) 0.1 mol% var-B4, 72%; (ii) DAST (1.4 equiv), CH_2Cl_2, –78 °C, 12 h, 86% (dr 1:3.2; major, 19% *ee*; minor, 44% *ee*); (iii) 0.05 mol% var-G4, 88%; (iv) DAST (1.4 equiv), CH_2Cl_2, –78 °C, 12 h, 95%; (v) 0.06 mol% var-B2, 93%; (vi) DAST (1.2 equiv), CH_2Cl_2, –78 °C, 12 h, 98% (dr 1:3.7; major, 9% *ee*; minor, 9% *ee*). All experimental procedures are described in detail in **Supplementary Methods**. The sequences of the P450 variants are described in **Supplementary Table 2** online. Yields refer to the isolated products. Enantiomeric excess values were determined by chiral gas chromatography analysis.

We then investigated whether two sequential chemo-enzymatic transformations could be used to fluorinate multiple sites of the same molecule. P450 variant B4 (var-B4)—which was used to convert **12** to **13**—was found to retain comparable activity on **16**, providing a possible route to the desired **17** intermediate. Re-screening of **12**-active variants on **16**, however, led to the identification of a more suitable candidate, var-B2, with higher activity than var-B4 toward **16** and excellent (100%) 2-regioselectivity. Using var-B2, the synthesis of fluorohydroxy derivative **17** was afforded in higher yields (93% versus 72% for conversion of **12** to **13**) and required less catalyst (0.06 mol% versus 0.1 mol% for conversion of **12** to **13**). **17** was then converted quantitatively to the desired difluoroderivative **18**.

The value of the present approach as synthetic tool for asymmetric fluorination was also examined. In the absence of anchimeric group participation, the deoxofluorination reaction generally preserves the enantiopurity of the enzymatic products through inversion of configuration[14]. Chiral gas chromatography analysis showed appreciable stereoselectivity during preparation of **7** (78% enantiomeric excess (*ee*), **9** (diasteromeric ratio (dr) 1:8.5), **10** (dr 4:96), **15** (dr 1:3.2) and **18** (dr 1:3.7) (see **Supplementary Figs. 2–6** online for gas chromatography traces). We then extended our previous investigations on 2-phenylacetic acid esters[15], carrying out the asymmetric synthesis of the corresponding 2-fluoro-2-arylacetic acid derivatives at 100-mg scale (**19a**, **20a**, **21a**, **22a** and **23a**; **Supplementary Table 1** online). In this case, up to 89% *ee* in up to 60% yield (two steps) was achieved.

P450-catalyzed hydroxylation of methoxy groups leads to exposure of a free hydroxyl group through decomposition of the hemi-acetal produced and release of formaldehyde. We reasoned that our chemo-enzymatic strategy could be extended to substitute a methoxy group for fluorine, a challenging transformation for traditional chemical methods. This approach was first tested on the 5-phenyloxazoline derivative **24** (**Fig. 3a**,**b**). The demethylation activities of the P450 variants could be easily assessed using a colorimetric screen (**Fig. 3a**). The most active variants from the screen were further analyzed with respect to the regioselectivity of oxidation using GC-MS. The highly selective P450 variant var-H1 (95%) was thus applied in combination with deoxofluorination to afford the desired fluorine-containing compound (**26**).

The same approach was tested on a set of derivatives of the synthetically important building block Corey lactone (**Fig. 3c**). The use of various Corey lactones (**27a**, **28a** and **29a**) enabled us to investigate the tolerance of the enzymatic transformation to structural variations within the target scaffold. Based on the colorimetric screen, 30 variants displayed activity on at least one Corey lactone (12 on **27a**, 17 on **28a**, 5 on **29a**). Twelve variants were found to accept both **27a** and **28a**, five were found to accept **28a** and **29a** and five were found to accept **27a** and **29a**. Notably, four variants (~10% of the Corey lactone–active variants) could be used to activate the substrate for subsequent fluorination, regardless of the size of the variable substituent. Using the most active and selective enzymes toward each of the Corey lactones, the desired fluorine-containing compounds **27c**, **28c** and **29c** were synthesized and isolated.



Compound	R	P450	Yield (i)	Yield (ii)
27a	Me	var-G5 (1 mol%)	68% (**27b**)	66% (**27c**)
28a		var-B3 (0.5 mol%)	84% (**28b**)	54% (**28c**)
29a		var-H1 (0.5 mol%)	82% (**29b**)	52% (**29c**)

Figure 3 Chemo-enzymatic methoxy-to-fluorine transformation. (**a**) Screening of P450 demethylation activities using a Purpald-based assay for detection of formaldehyde formation in 96-well plate format. Standard error is within 15%. WT, wild-type $P450_{BM3}$; OD_{550}, optical density at 550 nm. (**b**) Methoxy-to-fluorine transformation in a 5-phenyloxazoline derivative. Reagents and conditions: (i) 0.1 mol% var-H1, KPi pH 8.0, room temperature, 48 h, 92%; (ii) DAST (1.0 equiv), CH_2Cl_2, 0 °C, 12 h, 40%. (**c**) Methoxy-to-fluorine transformation in Corey lactone derivatives. All experimental procedures are described in detail in **Supplementary Methods**. The sequences of the P450 variants are described in **Supplementary Table 2**. Yields refer to the isolated products.

Ibuprofen has recently shown promising activity against amyloidogenic diseases[16]. Anti-amyloidogenic drugs with high brain permeability are intensively sought[16]. We tested **15**, **16** and **18** in a membrane permeability assay that mimics the composition of the blood brain barrier (BBB). While **12** has only modest BBB-crossing potential, monofluorinated **15** and **16** and difluorinated **18** exhibit, respectively, very good and excellent membrane permeability properties (effective permeability value $> 10 \times 10^{-6}$ cm s^{-1}; **Supplementary Fig. 7** online), which demonstrates how this procedure could be applied to rapidly screen various hydrogen-to-fluorine substitutions in a target molecule for improvement in chemophysical or biological properties.

This chemo-enzymatic approach has proven useful for fluorinating 13 of the 16 molecules tested (see **Supplementary Fig. 1**). The molecular weight of these compounds ranges between 110 and 450 Da. About 75% of commercial drugs fall within this window[17]. Much larger molecules may have restricted access to the enzyme's active site, representing suboptimal targets for this chemo-enzymatic approach. As the $P450_{BM3}$ active site is largely hydrophobic, highly polar compounds may also be poor substrates. Difficulties were mostly associated with solubilization of the substrate in aqueous media (**30**) or with the occurrence of side reactions—in particular elimination—during the deoxofluorination transformation (hydroxylated **31** and **32**), which prevented isolation of the enzymatic and fluorinated products, respectively, in satisfactory yields. These issues could be addressed, however, by using $P450_{BM3}$ variants with increased activity in the presence of organic co-solvent[18] and applying milder nucleophilic fluorination methods.

Overall, the described methodology extends the range of available tools for selective fluorination. Importantly, it provides a concise solution to selective incorporation of fluorine atoms at relatively unreactive sites in a variety of organic molecules and at a preparative scale. The regio- and stereoselectivity of this chemical transformation can be altered by engineering the protein catalyst. This strategy is versatile, as the same chemo-enzymatic route can be applied to various structurally related scaffolds, and more chemo-enzymatic transformations can be combined for selective fluorination of multiple sites on the same molecule. A common strategy for improving drugs' *in vivo* half-lives involves blocking the sites in the molecule that are susceptible to attack by human P450s with fluorine substituents[2,3]. It is worth noting that fluorination of position 2 in **15** and **18** would prevent one of the major P450-dependent routes of ibuprofen metabolism in humans[19]. An added advantage of this approach is thus to enable fluorination of metabolically vulnerable sites in the target molecule. We expect this procedure to find utility in the rapid identification of drug or lead compound derivatives with improved chemophysical or biological properties and in the preparation of fluorinated synthons for chemical synthesis or fragment-based drug discovery programs.

Note: Supplementary information and chemical compound information is available on the Nature Chemical Biology website.

ACKNOWLEDGMENTS

We are grateful to M. Shahgholi for assistance with the LC-MS and HRMS analyses. This work was supported by US National Institutes of Health grant GM068664 and US Department of Agriculture grant 2006-35505-16660 to F.H.A. and by the Jacobs Institute (Caltech). A.R. acknowledges the Deutsche Forschungsgemeinschaft for financial support.

AUTHOR CONTRIBUTIONS

R.F. and F.H.A. conceived the project; R.F. and A.R. performed the experiments; all authors discussed the results; R.F. wrote the paper with help and edits from F.H.A. and A.R.

COMPETING INTERESTS STATEMENT

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturechemicalbiology/.

Published online at http://www.nature.com/naturechemicalbiology/
Reprints and permissions information is available online at http://npg.nature.com/reprintsandpermissions/

1. Muller, K., Faeh, C. & Diederich, F. *Science* **317**, 1881–1886 (2007).
2. Park, B.K., Kitteringham, N.R. & O'Neill, P.M. *Annu. Rev. Pharmacol. Toxicol.* **41**, 443–470 (2001).
3. Bohm, H.J. *et al. ChemBioChem* **5**, 637–643 (2004).
4. Kirsch, P. *Modern Fluoroorganic Chemistry* (Wiley-VCH, Weinheim, Germany, 2004).
5. Shimizu, M. & Hiyama, T. *Angew. Chem. Int. Ed.* **44**, 214–231 (2005).
6. Ma, J.A. & Cahard, D. *Chem. Rev.* **104**, 6119–6146 (2004).
7. Bobbio, C. & Gouverneur, V. *Org. Biomol. Chem.* **4**, 2065–2075 (2006).
8. Günter, H. *J. Fluor. Chem.* **125**, 875–894 (2004).
9. Iacazio, G. & Réglier, M. *Tetrahedron Asymmetry* **16**, 3633–3639 (2005).
10. Warman, A.J. *et al. Biochem. Soc. Trans.* **33**, 747–753 (2005).
11. Fasan, R., Meharenna, Y.T., Snow, C.D., Poulos, T.L. & Arnold, F.H. *J. Mol. Biol.* **383**, 1069–1080 (2008).
12. Mikolajczyk, M., Mikina, M. & Zurawinski, R. *Pure Appl. Chem.* **71**, 473–480 (1999).
13. Fujisawa, H., Fujiwara, T., Takeuchi, Y. & Omata, K. *Chem. Pharm. Bull. (Tokyo)* **53**, 524–528 (2005).
14. Singh, R.P. & Shreeve, J.M. *Synthesis* **17**, 2561–2578 (2002).
15. Landwehr, M. *et al. J. Am. Chem. Soc.* **128**, 6058–6059 (2006).
16. Leuchtenberger, S., Beher, D. & Weggen, S. *Curr. Pharm. Des.* **12**, 4337–4355 (2006).
17. Feher, M. & Schmidt, J.M. *J. Chem. Inf. Comput. Sci.* **43**, 218–227 (2003).
18. Wong, T.S., Arnold, F.H. & Schwaneberg, U. *Biotechnol. Bioeng.* **85**, 351–358 (2004).
19. Hamman, M.A., Thompson, G.A. & Hall, S.D. *Biochem. Pharmacol.* **54**, 33–41 (1997).

CORRESPONDING AUTHOR: Fasan
MS NUMBER: NCHEMB-BC080803093A
MS TYPE: Brief Communication

Supplementary Information Titles

Journal: Nature Chemical Biology

Article Title:	Chemo-enzymatic fluorination of unactivated organic compounds
Corresponding Author:	Rudi Fasan

Supplementary Item & Number	Title or Caption
Supplementary Figure 1	Test molecules (ordered according to their molecular weight).
Supplementary Figure 2	Chiral GC traces of purified compound **5** (a) and **7** (b).
Supplementary Figure 3	Chiral GC traces of purified compound **9** (a) and **10** (b).
Supplementary Figure 4	Chiral GC traces of purified compound **15** (a) and **18** (b).
Supplementary Figure 5	Chiral GC traces of purified compound **19c** (a) and **20c** (b).
Supplementary Figure 6	Chiral GC traces of purified compound **21c** (a), **22c** (b) and **23c** (c).
Supplementary Figure 7	Membrane permeability properties of **12** and its fluorinated derivatives.
Supplementary Table 1	Stereoselective fluorination of substituted 2-aryl acetic acid esters.
Supplementary Table 2	Sequences of the $P450_{BM3}$ variants.
Supplementary Methods (or Discussion or Data)	Materials, Methods and Synthetic Procedures

Supplementary Information for

Chemo-enzymatic fluorination of unactivated organic compounds

Andrea Rentmeister[1], Frances H. Arnold[1] & Rudi Fasan[1,2]

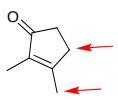
[1] *Department of Chemistry and Chemical Engineering, California Institute of Technology, 1200 E. California Blvd., Pasadena, California 91125, USA.*

[2] *Current address: Department of Chemistry, University of Rochester, Hutchison Hall, RC Box 270216, Rochester, New York 14627, USA.*

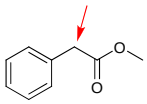
Correspondence should be addressed to R.F. (fasan@chem.rochester.edu).

Nature Chemical Biology: doi: 10.1038/nchembio.128

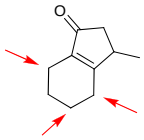
Supplementary Figure 1. Test molecules (ordered according to their molecular weight). Arrows indicate the site(s) targeted by chemo-enzymatic fluorination.



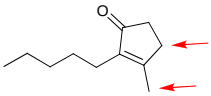
2,3-dimethylcyclopent-2-enone
(**1**)
Natural product
Mol. Wt.: 110.1



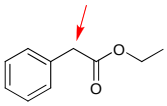
Methyl 2-phenylacetate
(**19a**)
Building block
Mol. Wt.: 150.0



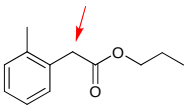
3-methyl-hexahydro-inden-1-one
(**3**)
Natural product
Mol. Wt.: 150.1



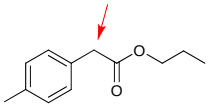
Dihydrojasmone
(**2**)
Natural product
Mol. Wt.: 166.3



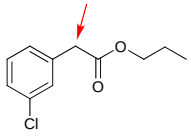
Ethyl 2-phenylacetate
(**20a**)
Building block
Mol. Wt.: 164.2



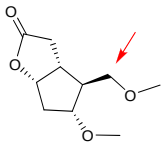
Propyl 2-(*o*-methylphenyl)acetate
(**22a**)
Building block
Mol. Wt.: 192.2



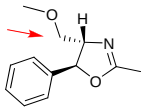
Propyl 2-(*p*-methylphenyl)acetate
(**23a**)
Building block
Mol. Wt.: 192.2



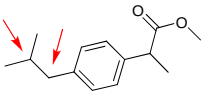
Propyl 2-(*m*-chlorophenyl)acetate
(**21a**)
Building block
Mol. Wt.: 212.7



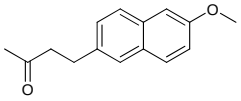
Methoxymethyl methyl Corey lactone
(**27a**)
Synthetic building block
Mol. Wt.: 200.2



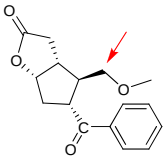
4-(methoxymethyl)-2-methyl-
5-phenyl-4,5-dihydrooxazole (**24**)
Synthetic building block
Mol. Wt.: 205.2



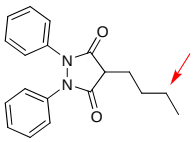
Ibuprofen methyl ester
(**12**)
Antiinflammatory
Mol. Wt.: 220.1



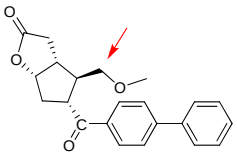
Nabumetone
(**30**)
Antiinflammatory drug
Mol. Wt.: 226.3



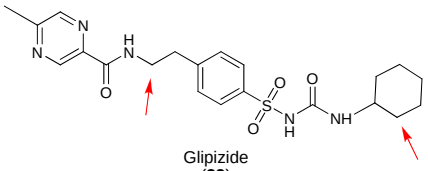
Methoxymethyl Benzoyl Corey lactone
(**28a**)
Synthetic building block
Mol. Wt.: 274.3



Phenylbutazone
(**31**)
Antiinflammatory drug
Mol. Wt.: 308.3



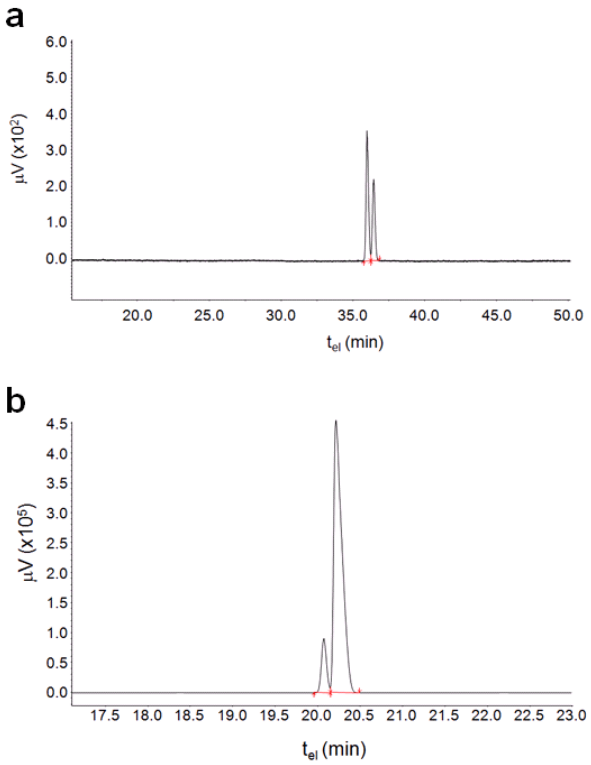
Methoxymethyl *p*-phenylbenzoyl Corey lactone
(**29a**)
Synthetic building block
Mol. Wt.: 350.4



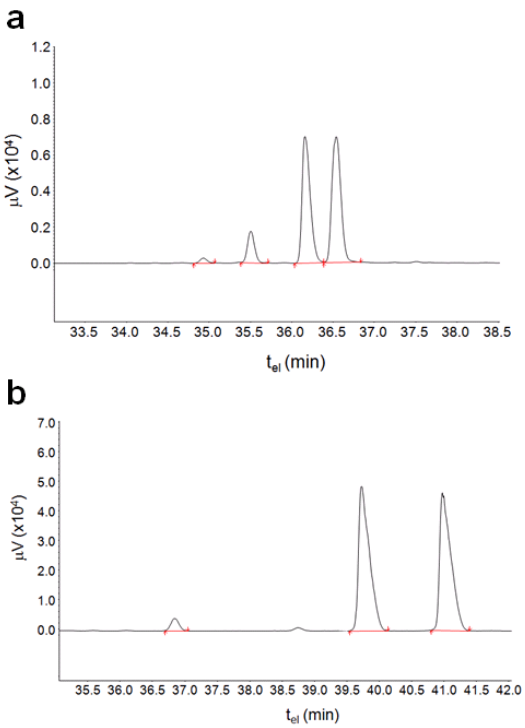
Glipizide
(**32**)
Antidiabetic drug
Mol. Wt.: 445.5

Nature Chemical Biology: doi: 10.1038/nchembio.128

Supplementary Figure 2. Chiral GC traces of purified compound **5** (a) and **7** (b).

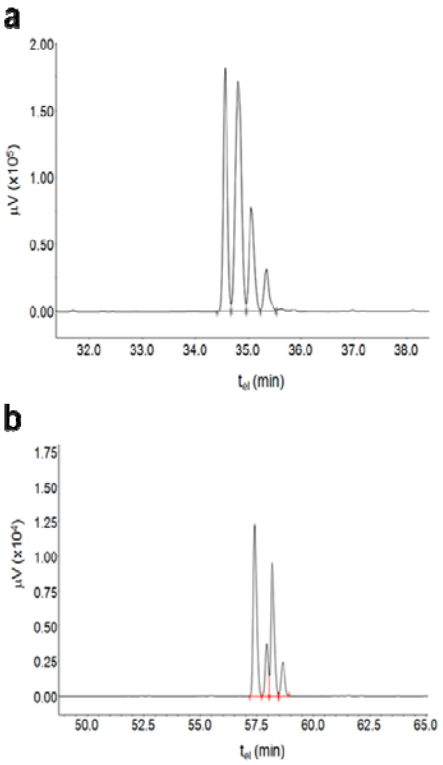


Supplementary Figure 3. Chiral GC traces of purified compound **9** (a) and **10** (b).

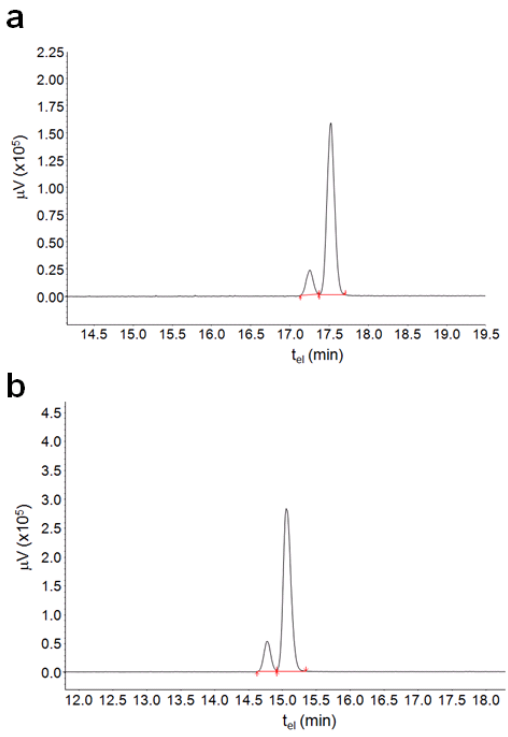


Nature Chemical Biology: doi: 10.1038/nchembio.128

Supplementary Figure 4. Chiral GC traces of purified compound **15** (a) and **18** (b).

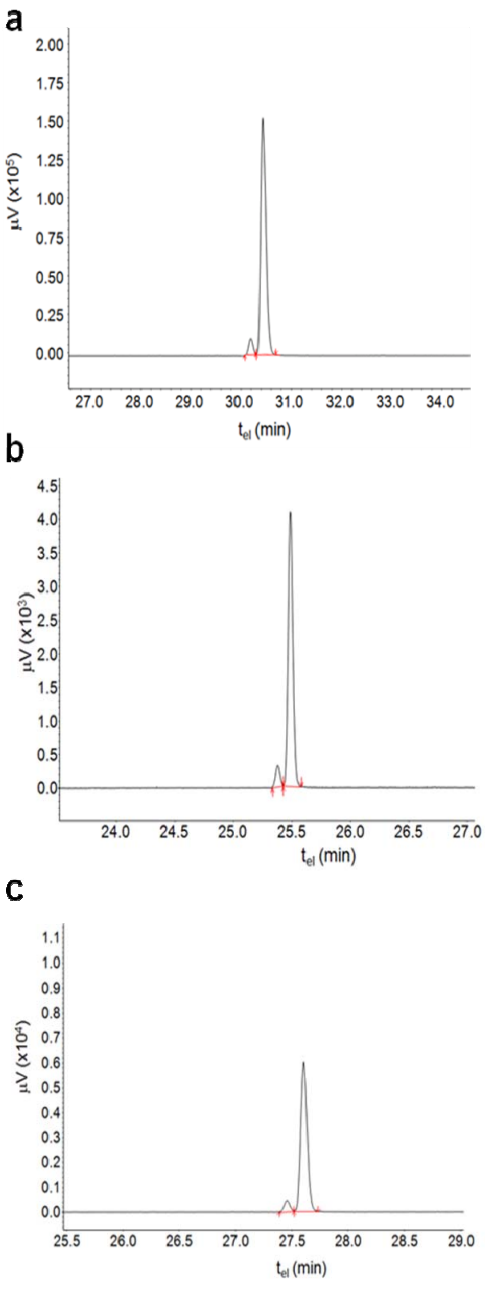


Supplementary Figure 5. Chiral GC traces of purified compound **19c** (a) and **20c** (b).



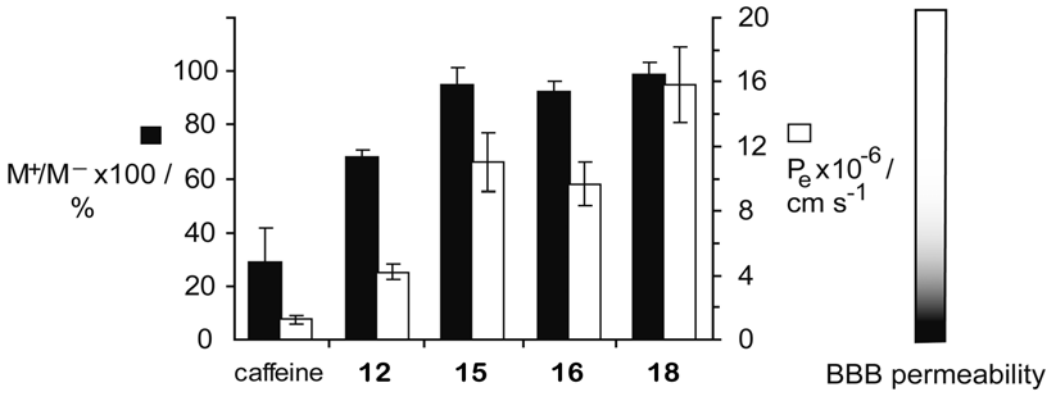
Nature Chemical Biology: doi: 10.1038/nchembio.128

Supplementary Figure 6. Chiral GC traces of purified compound **21c** (a), **22c** (b), and **23c** (c).



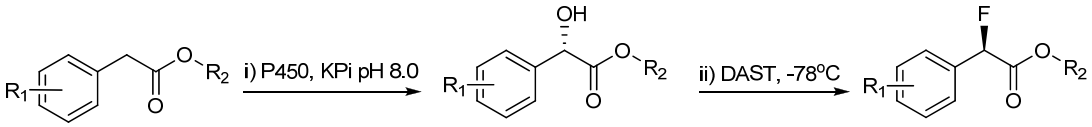
Nature Chemical Biology: doi: 10.1038/nchembio.128

Supplementary Figure 7. Membrane permeability properties of **12** and its fluorinated derivatives. Effective permeability values (P_e) are calculated by monitoring accumulation of the organic molecule in a cross-membrane compartment. The correlation between P_e value and BBB-permeability in the absence of active transport has been previously established using a wide set of commercial drugs[2] and is illustrated by the graded bar (black = not BBB-permeable; white = BBB-permeable). Caffeine served as control for assay validation (literature: 1.30 x 10^{-6} cm s^{-1}; experimental: 1.24 x 10^{-6} cm s^{-1}). M^+/M^- refers to the fraction of compound that crossed the BBB-mimic membrane as compared to system with no membrane after 16 hour incubation at room temperature. Values are means of three replicates.



Nature Chemical Biology: doi: 10.1038/nchembio.128

Supplementary Table 1. Stereoselective fluorination of substituted 2-aryl acetic acid esters.



19a - 23a **19b - 23b** **19c - 23c**

Compound	R_1	R_2	P450[a]	mol%	Yield (i)[b]	Yield (ii)[b]	*ee*[c]
19a	H	Me	var-A3	0.1	45% (**19b**)	75% (**19c**)	74%
20a	H	Et	var-B4	0.1	66% (**20b**)	78% (**20c**)	72%
21a	*m*-Cl	Pr	var-C12	0.05	75% (**21b**)	82% (**21c**)	89%
22a	*o*-Me	Pr	var-C12	0.05	65% (**22b**)	83% (**22c**)	85%
23a	*p*-Me	Pr	var-C12	0.05	72% (**23b**)	88% (**23c**)	87%

[a] The sequences of the P450 variants are reported in Supplementary Table 2. [b] Yields refer to the isolated product. [c] Enantiomeric excess determined by chiral GC analysis.

Nature Chemical Biology: doi: 10.1038/nchembio.128

Supplementary Table 2. Sequences of the $P450_{BM3}$ variants.

Enzyme	Amino acid mutations compared to wildtype $P450_{BM3}$
var-B2[a]	V78A, H138Y, T175I, V178I, A184V, H236Q, E252G, R255S, A290V, A295T, L353V
var-B3[b]	R47C, V78A, K94I, P142S,T175I, A184V, F205C, S226R, H236Q, E252G, R255S, A290V, L353V
var-B4	F87A
var-C12[c]	R47C, V78A, F87A, K94I, P142S,T175I, A184V, F205C, S226R, H236Q, E252G, R255S, A290V, L353V
var-D10[c]	R47C, V78A, A82L, K94I, P142S,T175I, A184V, F205C, S226R, H236Q, E252G, R255S, A290V, A328V, L353V
var-G4[c]	V78A, A82G, F87V, P142S,T175I, A184V, F205C, S226R, H236Q, E252G, R255S, A290V, A328V, L353V
var-G5[c]	V78A, F87I, P142S,T175I, A184V, F205C, S226R, H236Q, E252G, R255S, A290V, L353V
var-G6[c]	V78A, F81P, A82L, F87A, P142S, T175I, A180T, A184V, A197V, F205C, S226R, H236Q, E252G, R255S, A290V, L353V
var-H1[d]	R47C, L52I, V78F, A82S, K94I, P142S,T175I, A184V, F205C, S226R, H236Q, E252G, R255S, A290V, A328L, K349N, L353V, I366V, E464G, I710T
var-H3[a]	V78A, T175I, A184V, F205C, S226R, H236Q, E252G, R255S, A290V, L353V

[a] var-B2 and var-H3 correspond, respectively, to variants 139-3 and J described in Fasan *et al.*[4].

[b] var-B3 corresponds to variant 9-10A described in Meinhold *et al.*[5].

[c] These variants are derivatives of variant var-B3.

[d] var-H1 is a derivative of variant ETS8 described in Fasan *et al.*[4].

Nature Chemical Biology: doi: 10.1038/nchembio.128

Materials, Methods and Synthetic Procedures

Reagents and Analytical Methods. Chemical reagents, substrates and solvents were purchased from Sigma-Aldrich, Acros Organics, Fluka, and Kaironkem. Silica gel chromatography purifications were carried out using AMD Silica Gel 60 230-400 mesh. Gas chromatography (GC) analyses were carried out using a Shimadzu GC-17A gas chromatograph, a FID detector, and an Agilent HP5 column (30 m x 0.32 mm x 0.1 μm film). Chiral GC analyses were carried out using a Shimadzu GC-17A gas chromatograph, a FID detector, and an Agilent Cyclosilb column (30 m x 0.52 mm x 0.25 μm film). GC-MS analyses were carried out on a Hewlett-Packard 5970B MSD with 5890 GC and a DB-5 capillary column. HPLC analyses were carried out using a Waters 2690 instrument equipped with a 996 Photodiode Array Detector and a Phenomenex Gemini 5u C_{18} column. HPLC-MS analyses were carried out using an Agilent 1100 Series LC/MSD device and Kromasil 100, 5 um C_{18} column. ^{1}H, ^{13}C, and ^{19}F NMR spectra were recorded on a Varian Mercury 300 spectrometer (300 MHz, 75 MHz, and 282 MHz, respectively), and are internally referenced to residual solvent peak. Data for ^{1}H NMR are reported in the conventional form: chemical shift (δ ppm), multiplicity (s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, br=broad), coupling constant (Hz), integration). Data for ^{13}C and ^{19}F NMR are reported in the terms of chemical shift (δ ppm) and multiplicity. High-resolution mass spectra were obtained with a JEOL JMS-600H High Resolution Mass Spectrometer at the California Institute of Technology Mass Spectral Facility.

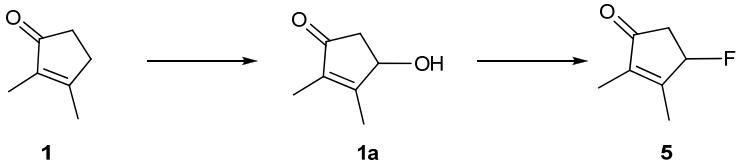
Enzyme Library Screening. Expression of $P450_{BM3}$ variants in 96-well plates and preparation of cell lysates were performed as described.[1] Screening of enzyme activity on cyclopentenone derivatives (**1**, **2**, and **3**) and ibuprofen derivatives (**12** and **16**) was

carried out in 96-well microtiter plates mixing 700 μL KPi (100 mM, pH 8.0), 200 μL cell lysate, 10 μL 200 mM substrate in EtOH, and 100 μL of a cofactor regeneration solution containing 4 mM NADPH, 100 mM glucose-6-phosphate, and 10 U/mL glucose-6-phosphate dehydrogenase. After incubation for 16 hours at room temperature in orbital shaker, reactions were extracted with chloroform and analyzed by gas chromatography on a Shimadzu GC-17A GC using an Agilent HP5 column (30 m x 0.32 mm x 0.1 μm film), 1 μL injection, FID detector, and the following separation method: 300 °C inlet, 300 °C detector, 70 °C oven, 10 °C/min gradient to 210 °C, 50 °C/min gradient to 260 °C, and 260 °C for 2 min. Screening of enzyme activity on dihydro-4-methoxymethyl-2-methyl-5-phenyl-2-oxazoline (**24**) was carried out on microtiter plates by mixing 100 μL KPi (100 mM, pH 8.0), 50 μL cell lysate, 2 μL 200 mM **24** in DMSO, and 50 μL KPi 4 mM NADPH. Reaction mixtures were incubated for 30 minutes at room temperature and then added with 50 μL 150 mM Purpald in 2 M NaOH. Absorbance at 550 nm was recorded after 30 minutes. The most active variants identified in the colorimetric screen were re-tested for selectivity towards demethylation. 1-mL scale reactions were carried out using 1 mM **24**, 0.5 μM P450, 400 μM NADPH, 10 mM glucose-6-phosphate and 1 U/mL glucose-6-phosphate dehydrogenase. After stirring for 16 hours at room temperature, the reaction mixtures were extracted with chloroform and analyzed by GC-MS on a Hewlett-Packard 5970B MSD with 5890 GC using a DB-5 capillary column, 1 μL injection, 250 °C inlet, and the following separation program: 80 °C oven, 10 °C/min gradient to 250 °C, 250 °C for 3 min. Enzyme activity on **27a**, **28a**, and **29a** was screened by mixing 50 μL cell lysate, 100 μL 100 mM KPi buffer (pH 8.0) containing 1 mM substrate (1% DMSO), and 50 μL 4 mM NADPH in microtiter plates. After 30 min, 50 μL 150 mM Purpald (Sigma)

in 2 M NaOH were added to each well. Absorbance at 550 nm was measured after 60 minutes. The most active variants were re-tested at 1-mL scale using 1 mM substrate, 0.5 μM purified P450, and the cofactor regeneration system described above. After overnight incubation at 4 °C, reactions were analyzed by HPLC-MS using an Agilent 1100 Series LC/MSD device and Kromasil 100, 5 μm-C_{18} column.

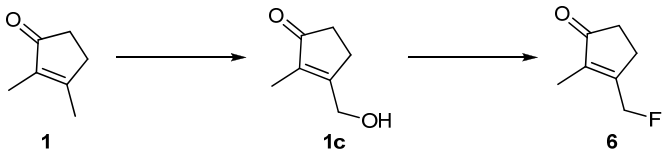
Membrane permeability assay. The membrane permeability assays were carried out adapting a described procedure[2] and using 96-well MultiScreen-IP plates from Millipore. Filter plates were rinsed with 70% EtOH and double-distilled water prior to use. BBB-mimic membranes were prepared by coating filter wells with 5 μL porcine brain lipids (PBL, Avanti Polar Lipids) in dodecane (20 mg/mL). Control wells (PBL-free) were primed in the same way but not coated with PBL. Working solutions contained 0.5 mg/mL fluorinated compound in PBS pH 7.4 (5% EtOH). Assembled plates contained 150 μL working solution in the PBL-coated (or PBL-free) well, 300 μL PBS (5% EtOH) in the acceptor well, and were incubated at room temperature and 100% humidity. The change in organic compound concentration in the donor and acceptor wells was monitored over time by HPLC. Membrane permeability values (P_e) were calculated as described.[3] All measurements were performed in triplicates. The assay was validated by comparing our experimental P_e value for caffeine with that reported by Wohnsland *et al.*[3].

Synthetic procedures:



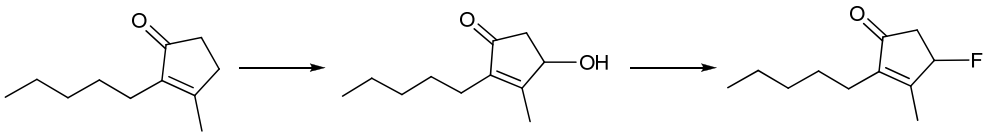
Conversion of 1 to 5. 130 mg (1.18 mmol) **1** were dissolved in 1.5 mL ethanol and added to 200 mL potassium phosphate buffer pH 8.0**.** The solution was added with var-H3 (final conc.: 2.5 μM) and a cofactor regeneration system (final conc.: 500 μM NADPH, 30 mM glucose-6-phosphate, 1 unit/mL glucose-6-phosphate dehydrogenase) and stirred at room temperature. After 48 hours, the reaction mixture was extracted with dichloromethane (4 x 50 mL). The combined organic phases were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by flash chromatography (gradient 0 to 20% ethyl acetate in hexane) to afford **1a** (130 mg, 88%, colorless oil). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.71 (m, 3H, CH_3), δ 2.08 (m, 3H, CH_3), δ 2.27 (dd, J= 18.3, 1.8 Hz, 1H), δ 2.77 (dd, J= 18.3, 6.3 Hz, 1H), δ 4.73 (m, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 8.18, δ 13.91, δ 44.44, δ 71.85, δ 138.37, δ 168.50, δ 205.96. HRMS (EI+): exact mass calculated for $C_7H_{10}O_2$ requires m/z 126.0681, found 126.0687. Under argon, 50 mg (0.22 mmol) **1a** and a catalytic amount (3 drops) of ethanol were dissolved in 3 mL dry dichloromethane. The solution was cooled to -78 °C and added with 38 μL DAST (0.26 mmol, 1.2 eq) in two aliquots. The reaction was stirred in dry ice for 12 hours. The reaction mixture was added with 5 mL saturated sodium bicarbonate and extracted with dichloromethane (3 x 15 mL). The organic phase was then dried over anhydrous magnesium sulfate and evaporated under reduced pressure. The residue was purified by flash chromatography (gradient 10% to 50% dichloromethane in pentane) to afford **5** (25 mg, 90% yield, colorless oil). ^{1}H-NMR

(300 MHz, CD_2Cl_2): δ 1.68 (dm, J= 4.2 Hz, 3H, CH_3), δ 2.04 (m, 3H, CH_3), δ 2.41 (tm, J= 20.7 1H), δ 2.63-2.76 (m, 1H), δ 5.48 (dm, J= 30.6 Hz, 1H). ^{13}C-NMR (75 MHz, CD_2Cl_2): δ 7.83, δ 13.40, δ 41.21 (d, J= 19.9 Hz), δ 91.59 (d, J= 173.7 Hz), δ 128.03. ^{19}F-NMR (282 MHz, CD_2Cl_2): δ -179.4 (dd, J= 51.6, 24.2 Hz). HRMS (EI+): exact mass calculated for C_7H_9FO requires m/z 128.0637, found 128.0642. Chiral GC analyses were carried out using a Shimadzu GC-17A gas chromatograph, FID detector, Agilent Cyclosilb column (30 m x 0.52 mm x 0.25 μm film) and the following separation program: 300 °C inlet, 300 °C detector, 60 °C oven, 0.5 °C/min gradient to 200 °C, 50 °C/min gradient to 250 °C, and 250 °C for 2 min. An enantiomeric excess of 22% was estimated based on peak areas (see **Supplementary Figure 2**).



Conversion of 1 to 6. 130 mg (1.18 mmol) **1** were dissolved in 1.5 mL ethanol and added to 200 mL potassium phosphate buffer pH 8.0**.** The solution was added with var-G6 (final conc.: 2.5 μM) and a cofactor regeneration system (final conc.: 500 μM NADPH, 30 mM glucose-6-phosphate, 1 unit/mL glucose-6-phosphate dehydrogenase) and stirred at room temperature. After 48 hours, the reaction mixture was extracted with dichloromethane (4 x 50 mL). The combined organic phases were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by flash chromatography (gradient 0 to 20% ethyl acetate in hexane) to afford **1c** (66 mg, 45%, colorless oil). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.71 (m, 3H, CH_3), δ 2.4 (dm, J= 4.5 Hz, 2H), δ 2.65 (m, 2H), δ 4.58 (s, 2H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 8.23, δ 27.18, δ 34.10, δ 61.05, δ 136.15, δ 170.05, δ 210.61. HRMS (EI+): exact mass calculated for

$C_7H_{10}O_2$ requires m/z 126.0681, found 126.0685. Under argon, 50 mg (0.22 mmol) **1c** and a catalytic amount (3 drops) of ethanol were dissolved in 3 mL dry dichloromethane. The solution was cooled to -78 °C and added with 38 µL DAST (0.26 mmol, 1.2 eq) in two aliquots. The reaction was stirred in dry ice for 12 hours. The reaction mixture was added with 5 mL saturated sodium bicarbonate and extracted with dichloromethane (3 x 15 mL). The organic phase was then dried over anhydrous magnesium sulfate and evaporated under reduced pressure. The residue was purified by flash chromatography (gradient 10% to 50% dichloromethane in pentane) to afford **6** (24 mg, 85% yield, colorless oil). ^{1}H-NMR (300 MHz, CD_2Cl_2): δ 1.67 (m, 3H, CH_3), δ 2.36 (m, 2H), δ 2.56 (m, 2H), δ 5.23 (d, J= 34.2 Hz, 2H). ^{13}C-NMR (75 MHz, CD_2Cl_2): δ 7.95, δ 26.41 (d, J= 4.3 Hz), δ 80.83 (d, J= 164.0 Hz). ^{19}F-NMR (282 MHz, CD_2Cl_2): δ -226.9 (t, J= 42.3 Hz). HRMS (EI+): exact mass calculated for C_7H_9FO requires m/z 128.0637, found 128.0643.

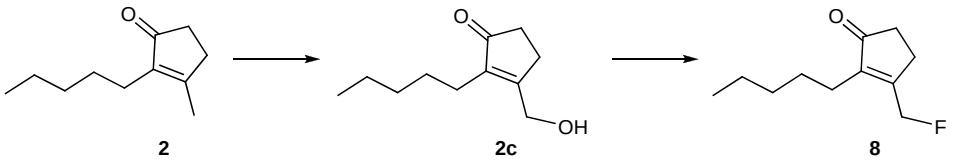


2 **2a** **7**

Conversion of 2 to 7. 270 mg (1.62 mmol) **2** were dissolved in 2.5 mL ethanol and added to 250 mL potassium phosphate buffer pH 8.0**.** The solution was added with var-H3 (final conc.: 3 µM) and a cofactor regeneration system (final conc.: 500 µM NADPH, 30 mM glucose-6-phosphate, 1 unit/mL glucose-6-phosphate dehydrogenase) and stirred at room temperature. After 48 hours, the reaction mixture was extracted with dichloromethane (4 x 50 mL). The combined organic phases were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by flash chromatography (gradient 0 to 30% ethyl acetate in hexane) to afford **2a** (250 mg, 85%,

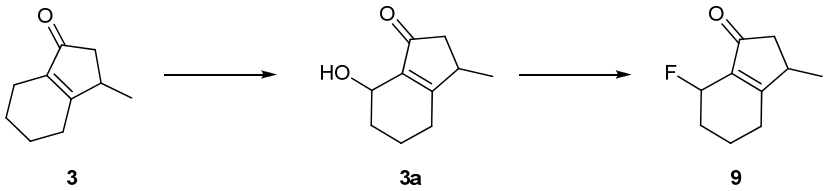
pale yellow oil). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.87 (t, J= 6.9 Hz, 3H, CH_3), δ 1.25-1.39 (m, 6H), δ 2.08 (s, 3H, CH_3), δ 2.17 (t, J= 8.1 Hz, 2H), δ 2.27 (dd, J= 18.3, 2.1 Hz, 1H), δ 2.77 (dd, J= 18.3, 6.3 Hz, 1H), δ 4.72 (dm, J= 5.1 Hz, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 13.83, δ 14.22, δ 22.68, δ 23.16, δ 28.06, δ 28.06, δ 31.99, δ 44.60, δ 71.87, δ 142.73, δ 168.32, δ 205.59. HRMS (EI+): exact mass calculated for $C_{11}H_{18}O_2$ requires m/z 182.1307, found 182.1311. Under argon, 200 mg (1.09 mmol) **2a** and a catalytic amount (4 drops) of ethanol were dissolved in 5 mL dry dichloromethane. The solution was cooled to -78 °C and added with 200 μL DAST (1.4 mmol, 1.3 eq) in four aliquots. The reaction was stirred in dry ice for 12 hours. The reaction mixture was added with 7 mL saturated sodium bicarbonate and extracted with dichloromethane (3 x 20 mL). The organic phase was then dried over anhydrous magnesium sulfate and evaporated under reduced pressure. The residue was purified by flash chromatography (gradient 0 to 30% ethyl acetate in hexane) to afford **7** (193 mg, 92% yield, pale yellow oil). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.88 (t, J= 6.6 Hz, 3H, CH_3), δ 1.25-1.40 (m, 6H), δ 2.10 (d, J= 2.1 Hz, 2H, CH_3), δ 2.20 (t, J= 7.1 Hz, 2H), δ 2.51 (tm, J= 22.5 Hz, 1H), δ 2.70-2.82 (m, 1H), δ 5.47 (dd, J= 53.7, 6.0, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 13.75, δ 14.21, δ 22.66, δ 23.13, δ 27.90, δ 29.94, δ 31.96, δ 41.46 (d, J= 19.6 Hz), δ 91.42 (d, J= 174.9 Hz), δ 167.62. ^{19}F-NMR (282 MHz, $CDCl_3$): δ -179.1 (ddd, J= 51.8, 21.4, 9.3 Hz). HRMS (EI+): exact mass calculated for $C_{11}H_{17}FO$ requires m/z 184.1263, found 184.1255. Chiral GC analyses were carried out using a Shimadzu GC-17A gas chromatograph, FID detector, Agilent Cyclosilb column (30 m x 0.52 mm x 0.25 μm film) and the following separation program: 300 °C inlet, 300 °C detector, 70 °C oven, 1 °C/min gradient to 200 °C, 50 °C/min gradient to 250 °C, and 250 °C for 2 min. An

enantiomeric excess of 78% was estimated based on peak areas (see **Supplementary Figure 2**).



Conversion of 2 to 8. 100 mg (0.60 mmol) **2** were dissolved in 1 mL ethanol and added to 150 mL potassium phosphate buffer pH 8.0**.** The solution was added with var-G4 (final conc.: 2 μM) and a cofactor regeneration system (final conc.: 500 μM NADPH, 30 mM glucose-6-phosphate, 1 unit/mL glucose-6-phosphate dehydrogenase) and stirred at room temperature. After 48 hours, the reaction mixture was extracted with dichloromethane (3 x 50 mL). The combined organic phases were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by flash chromatography (gradient 0 to 30% ethyl acetate in hexane) to afford **2c** (46 mg, 42%, pale yellow oil). GC-MS (EI^+): m/z 182. Under argon, 46 mg (0.25 mmol) **2c** and a catalytic amount (2 drops) of ethanol were dissolved in 2.5 mL dry dichloromethane. The solution was cooled to -78 °C and added with 54 μL DAST (0.38 mmol, 1.5 eq) in two aliquots. The reaction was stirred in dry ice for 12 hours. The reaction mixture was added with 5 mL saturated sodium bicarbonate and extracted with dichloromethane (3 x 15 mL). The organic phase was then dried over anhydrous magnesium sulfate and evaporated under reduced pressure. The residue was purified by flash chromatography (gradient 0 to 30% ethyl acetate in hexane) to afford **8** (62 mg, 89% yield, pale yellow oil). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.87 (t, J= 6.5 Hz, 3H, CH_3), δ 1.23-1.38 (m, 6H), δ 2.17 (t, J= 7.5 Hz, 2H), δ 2.41 (m, 1H), δ 2.60 (m, 1H), δ 5.26 (d, J= 47.1, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 14.32, δ 22.86, δ 23.33, δ 28.46, δ 31.80, δ 31.93, δ 34.87, δ

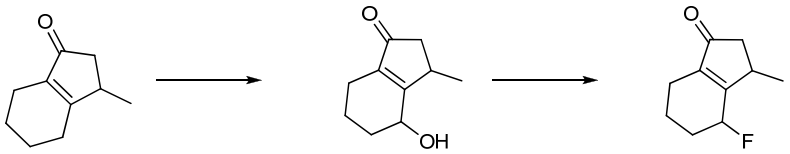
80.37 (d, J= 164.8 Hz). ^{19}F-NMR (282 MHz, $CDCl_3$): δ -227.9 (t, J= 48.2 Hz). HRMS (EI+): exact mass calculated for $C_{11}H_{17}FO$ requires m/z 184.1263, found 184.1263.



3 3a 9

Conversion of 3 to 9. 230 mg (1.53 mmol) **3** were dissolved in 2 mL ethanol and added to 300 mL potassium phosphate buffer pH 8.0**.** The solution was added with var-D10 (final conc.: 2.5 μM) and a cofactor regeneration system (final conc.: 500 μM NADPH, 30 mM glucose-6-phosphate, 1 unit/mL glucose-6-phosphate dehydrogenase) and stirred at room temperature. After 48 hours, the reaction mixture was extracted with dichloromethane (4 x 80 mL). The combined organic phases were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by flash chromatography (gradient 0 to 20% ethyl acetate in hexane) to afford **3a** (175 mg, 69%, colorless oil). GC-MS (EI^+): m/z 166. Under argon, 100 mg (0.60 mmol) **3a** and a catalytic amount (3 drops) of ethanol were dissolved in 5 mL dry dichloromethane. The solution was cooled to -78 °C and added with 103 μL DAST (0.72 mmol, 1.2 eq) in two aliquots. The reaction was stirred in dry ice for 3 hours. The reaction mixture was added with 5 mL saturated sodium bicarbonate and extracted with dichloromethane (3 x 20 mL). The organic phase was then dried over anhydrous magnesium sulfate and evaporated under reduced pressure. The residue was purified by flash chromatography (10% ethyl acetate : 90% hexane) to afford **9** (89 mg, 88% yield, colorless oil). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.84 (d, J= 6.6 Hz), δ 1.45-1.75 (m, 2H), δ 1.80-1.86 (m, 2H), δ 2.03 (m, 1H), δ 2.06 (dd, J= 18.4, 1.8 Hz, 1H), δ 2.13-2.21 (m, 1H), δ 2.65 (dd, J=18.4,6.9 Hz, 1H), δ 2.80 (m, 1H), δ 5.28 (dbr, J= 50.1 Hz, 1H). ^{13}C-NMR (75 MHz,

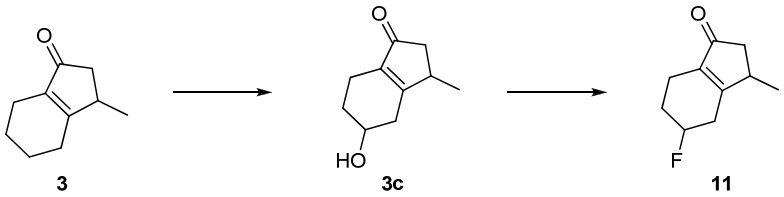
$CDCl_3$): δ 14.47, δ 18.78, δ 25.89, δ 28.67 (d, J=21.9 Hz), δ 36.29, δ 43.89, δ 79.93 (d, J= 164.2 Hz), δ 135.71 (d, J= 17.9 Hz), δ 183.76 (d, J= 5.6 Hz), δ 206.26. ^{19}F-NMR (282 MHz, $CDCl_3$): δ -171.4 (m). HRMS (EI+): exact mass calculated for $C_{10}H_{13}FO$ requires m/z 168.0950, found 168.0908. Chiral GC analyses were carried out using a Shimadzu GC-17A gas chromatograph, FID detector, Agilent Cyclosilb column (30 m x 0.52 mm x 0.25 μm film) and the following separation program: 300 °C inlet, 300 °C detector, 70 °C oven, 1 °C/min gradient to 200 °C, 50 °C/min gradient to 250 °C, and 250 °C for 2 min. A diasteromeric ratio (dr) of 1:8.5, an enantiomeric excess of 5% for the major product, and an enantiomeric excess of 71% for the minor product were estimated based on peak areas (see **Supplementary Figure 3**).



3 **3b** **10**

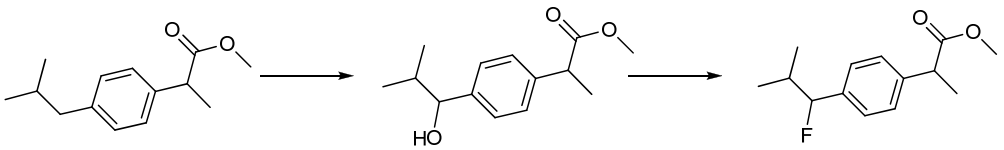
Conversion of 3 to 10. 200 mg (1.33 mmol) **3** were dissolved in 1.5 mL ethanol and added to 200 mL potassium phosphate buffer pH 8.0**.** The solution was added with var-G4 (final conc.: 3.5 μM) and a cofactor regeneration system (final conc.: 500 μM NADPH, 30 mM glucose-6-phosphate, 1 unit/mL glucose-6-phosphate dehydrogenase) and stirred at room temperature. After 36 hours, the reaction mixture was extracted with dichloromethane (4 x 80 mL). The combined organic phases were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by flash chromatography (30% ethyl acetate in hexane) to afford **3b** (137 mg, 62%, colorless oil). GC-MS (EI^+): m/z 166. Under argon, 50 mg (0.30 mmol) **3b** and a catalytic amount (3 drops) of ethanol were dissolved in 3 mL dry dichloromethane. The solution was cooled to -78 °C and added with 51 μL DAST (0.36 mmol, 1.2 eq) in two aliquots. The

reaction was stirred in dry ice for 5 hours. The reaction mixture was added with 5 mL saturated sodium bicarbonate and extracted with dichloromethane (3 x 15 mL). The organic phase was then dried over anhydrous magnesium sulfate and evaporated under reduced pressure. The residue was purified by flash chromatography (10% ethyl acetate : 90% hexane) to afford **10** (46 mg, 92% yield, colorless oil). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.27 (d, J= 6.9 Hz, 3H), δ 1.71-1.79 (m, 3H), δ 2.02 (dbr, J= 18.6, 1H), δ 2.03 (br, 1H), δ 2.10-2.13 (m, 1H), δ 2.23-2.35 (m, 1H), δ 2.67 (dd, J=18.6,6.3 Hz, 1H), δ 2.92 (br, 1H), δ 5.20 (dbr, J= 46.2 Hz, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 17.55, δ 19.96, δ 20.18 (d, J= 2.8 Hz), δ 29.80 (d, J=21.3 Hz), δ 36.12, δ 44.32, δ 86.48 (d, J= 166.5 Hz), δ 142.79, δ 168.15 (d, J= 13.9 Hz), δ 208.86. ^{19}F-NMR (282 MHz, $CDCl_3$): δ -174.8 (m). HRMS (EI+): exact mass calculated for $C_{10}H_{13}FO$ requires m/z 168.0950, found 168.0913. Chiral GC analyses were carried out using a Shimadzu GC-17A gas chromatograph, FID detector, Agilent Cyclosilb column (30 m x 0.52 mm x 0.25 μm film) and the following separation program: 300 °C inlet, 300 °C detector, 70 °C oven, 1 °C/min gradient to 200 °C, 50 °C/min gradient to 250 °C, and 250 °C for 2 min. A diasteromeric ratio (dr) of 4:96 and an enantiomeric excess of 57% for minor product were estimated based on peak areas (see **Supplementary Figure 3**).



Conversion of 3 to 11. 150 mg (1.0 mmol) **3** were dissolved in 1.5 mL ethanol and added to 200 mL potassium phosphate buffer pH 8.0**.** The solution was added with var-G5 (final conc.: 3.5 μM) and a cofactor regeneration system (final conc.: 500 μM NADPH, 30 mM glucose-6-phosphate, 1 unit/mL glucose-6-phosphate dehydrogenase)

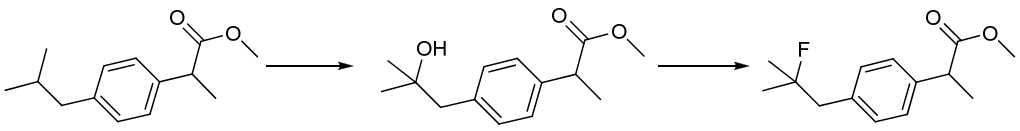
and stirred at room temperature. After 36 hours, the reaction mixture was extracted with dichloromethane (4 x 80 mL). The combined organic phases were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by flash chromatography (30% ethyl acetate in hexane) to afford **3c** (53 mg, 32%, colorless oil). GC-MS (EI^+): m/z 166. Under argon, 53 mg (0.32 mmol) **3c** and a catalytic amount (3 drops) of ethanol were dissolved in 3 mL dry dichloromethane. The solution was cooled to -78 °C and added with 51 μL DAST (0.36 mmol, 1.2 eq) in two aliquots. The reaction was stirred in dry ice for 5 hours. The reaction mixture was added with 5 mL saturated sodium bicarbonate and extracted with dichloromethane (3 x 15 mL). The organic phase was then dried over anhydrous magnesium sulfate and evaporated under reduced pressure. The residue was purified by flash chromatography (10% ethyl acetate : 90% hexane) to afford **11** (48 mg, 90% yield, colorless oil). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.18 (d, J= 7.2 Hz, 3H), δ 1.45-1.64 (m, 1H), δ 1.79-1.86 (m, 2H), δ 2.00 (dd, J= 18.6, 3.9 Hz, 1H), δ 2.11-2.24 (m, 1H), δ 2.33-2.37 (m, 1H), δ 2.67 (dd, J= 18.6, 6.3 Hz, 1H), δ 2.86 (m, 1H), δ 5.29 (dm, J= 50.1 Hz, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 17.36, δ 18.62, δ 26.58, δ 28.74 (d, J= 20.7 Hz), δ 37.08, δ 43.67, δ 79.82 (d, J= 164.2 Hz), δ 135.65, δ 208.57. ^{19}F-NMR (282 MHz, $CDCl_3$): δ -170.5 (m). HRMS (EI+): exact mass calculated for $C_{10}H_{13}FO$ requires m/z 168.0950, found 168.0923. Chiral GC analyses were carried out using a Shimadzu GC-17A gas chromatograph, FID detector, Agilent Cyclosilb column (30 m x 0.52 mm x 0.25 μm film). Complete separation of all four diasteromers was not possible.



12 13 15

Conversion of 12 to 15. 1.4 mL DMSO containing 150 mg (0.68 mmol) **12** were dissolved in 150 mL 100 mM KPi pH 8.0. The solution was added with var-B4 (final conc.: 4.5 μM) and a cofactor regeneration system (final conc.: 1 mM NADPH, 30 mM glucose-6-phosphate, 1 unit/mL glucose-6-phosphate dehydrogenase) and stirred at room temperature. After 48 hours, the reaction mixture was extracted with dichloromethane (4 x 50 mL). The combined organic phases were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by flash chromatography (gradient from 5 to 40% ethyl acetate in hexane) to afford **13** (114 mg, 72%, colorless oil). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.78 (d, J= 6.9 Hz, 3H, CH_3), δ 0.98 (d, J= 6.9 Hz, 3H, CH_3), δ 1.48 (d, J= 6.9 Hz, 3H, CH_3), δ 1.85-2.00 (m, 1H, CH), δ 3.65 (s, 3H, OCH_3), δ 3.72 (q, J= 6.9 Hz, 1H, CH), δ 4.33 (d, J= 6.9 Hz, 1H, CH), δ 7.25 (m, 4H, CH). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 18.43, δ 18.81, δ 19.26, δ 35.42, δ 45.32, δ 52.27, δ 79.99, δ 127.09, δ 127.52, δ 139.86, δ 142.77, δ 175.27. HRMS (EI^+): exact mass calculated for $C_{14}H_{20}O_3$ requires m/z 236.1412, found 236.1415. Under argon, 40 mg (0.17 mmol) **13** and a catalytic amount of ethanol were dissolved in 4 mL dry dichloromethane. The solution was cooled to -78 °C. After 5 minutes, 29 μL DAST (0.24 mmol, 1.4 eq) were added to the mixture. The reaction was stirred in dry ice overnight. The reaction was then added with 5 mL saturated sodium bicarbonate and extracted with dichloromethane (3 x 15 mL). The organic phase was then dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by flash chromatography (5% ethyl acetate : 95% hexane) to afford **15** (35 mg, 86% yield,

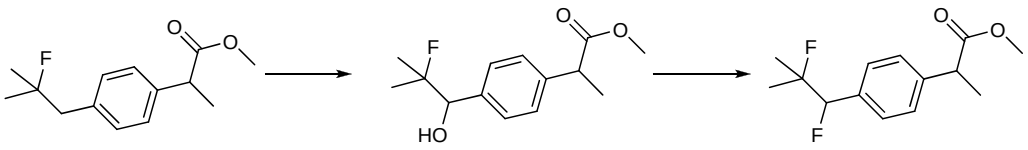
colorless oil). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.84 (d, J= 6.3 Hz, 3H, CH_3), δ 1.01 (d, J= 5.7 Hz, 3H, CH_3), δ 1.48 (d, J= 6.9 Hz, 3H, CH_3), δ 2.05-2.18 (m, 1H, CH), δ 3.66 (s, 3H, OCH_3), δ 3.72 (q, J= 7.5 Hz, 1H, CH), 5.07 (dd, J= 40.0, J= 6.9 Hz, 1H, CHF), δ 7.25 (m, 4H, CH). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 17.78 (d, J= 5.1 Hz), δ 18.58 (d, J= 6.0 Hz), δ 18.81, δ 34.48 (d, J: 85.7 Hz), δ 45.37, δ 52.31, δ 99.3 (d, J= 174 Hz), δ 126.65, δ 126.75, δ 127.56, δ 138.37, δ 140.6, δ 175.16. ^{19}F-NMR (282 MHz, $CDCl_3$): δ -179.8 (m). HRMS (EI+): exact mass calculated for $C_{14}H_{19}FO_2$ requires m/z 238.1369, found 238.1367. Chiral GC analyses were carried out using a Shimadzu GC-17A gas chromatograph, FID detector, Agilent Cyclosilb column (30 m x 0.52 mm x 0.25 μm film) and the following separation program: 300 °C inlet, 300 °C detector, 70 °C oven, 1 °C/min gradient to 200 °C, 50 °C/min gradient to 250 °C, and 250 °C for 2 min. A diasteromeric ratio (dr) of 1:3.2, an enantiomeric excess of 19% for the major product, and an enantiomeric excess of 44% for the minor product were estimated based on peak areas (see **Supplementary Figure 4**).



12 **14** **16**

Conversion of 12 to 16. 2.5 mL DMSO containing 220 mg (1 mmol) **12** were dissolved in 250 mL 100 mM KPi pH 8.0. The solution was added with var-G4 (final conc.: 2 μM) and a cofactor regeneration system (final conc.: 500 μM NADPH, 30 mM glucose-6-phosphate, 1 unit/mL glucose-6-phosphate dehydrogenase) and stirred at room temperature. After 48 hours, the reaction mixture was extracted with dichloromethane (4 x 80 mL). The combined organic phases were dried over anhydrous Na_2SO_4 and

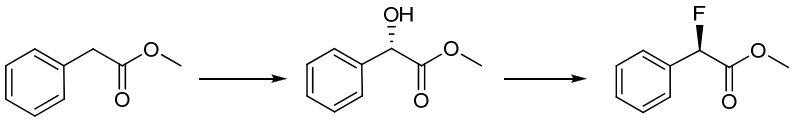
evaporated under reduced pressure. The residue was purified by flash chromatography (gradient from 5 to 40% ethyl acetate in hexane) to afford **14** (208 mg, 88%, colorless oil). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.21 (s, 6H, CH_3), δ 1.48 (d, J= 7.2 Hz, 3H, CH_3), δ 2.73 (s, 2H, CH), δ 3.65 (s, 3H, OCH_3), δ 3.70 (q, J= 7.2 Hz, 1H, CH), δ 7.15 (d, J= 8.1, 2H, CH), δ 7.23 (d, J= 8.1, 2H, CH). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 18.84, δ 29.41 (2C), δ 45.26, δ 49.51, δ 52.29, δ 71.00, δ 127.53 (2C), δ 130.98 (2C), δ 136.82, δ 138.92, δ 175.33. HRMS (EI^+): exact mass calculated for $C_{14}H_{20}O_3$ requires m/z 236.1412, found 236.1413. Under argon, 120 mg (0.51 mmol) **14** and a catalytic amount of ethanol (3 drops) were dissolved in 6 mL dry dichloromethane. The solution was cooled to -78 °C. After 5 minutes, 87 µL DAST (0.61 mmol, 1.4 eq) were added to the mixture in three aliquots. The reaction was stirred in dry ice overnight. The reaction was then added with 8 mL saturated sodium bicarbonate and extracted with dichloromethane (3 x 20 mL). The organic phase was then dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by flash chromatography (5% ethyl acetate: 95% hexane) to afford **16** (114 mg, 95% yield, colorless oil). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.32 (d, J= 23.6 Hz, 6H, CH_3), δ 1.48 (d, J= 6.9 Hz, 3H, CH_3), δ 2.87 (d, J: 20.4 Hz, 2H), δ 3.65 (s, 3H, OCH3), δ 3.70 (q, J= 7.1 Hz, 1H), δ 7.15 (d, J= 8.1 Hz, 2H, CH), δ 7.21 (d, J= 8.1 Hz, 2H, CH). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 18.80, δ 26.83 (d, J: 24.2 Hz), δ 45.24, δ 47.37 (d, J: 22.8 Hz), δ 52.25, δ 98.37 (d, J= 264 Hz), δ 129.12 (d, J: 258.8 Hz), δ 127.39, δ 130.85, δ 136.07, δ 138.92, δ 175.31. ^{19}F-NMR (282 MHz, $CDCl_3$): δ -137.7 (m). HRMS (EI^+): exact mass calculated for $C_{14}H_{19}FO_2$ requires m/z 238.1369, found 238.1370.



16 **17** **18**

Conversion of 16 to 18. 2 mL DMSO containing 80 mg (0.33 mmol) **16** were dissolved in 200 mL 100 mM KPi pH 8.0. The solution was added with var-B2 (final conc.: 1 μM) and a cofactor regeneration system (final conc.: 500 μM NADPH, 30 mM glucose-6-phosphate, 1 unit/mL glucose-6-phosphate dehydrogenase) and stirred at room temperature. After 48 hours, the reaction mixture was extracted with dichloromethane (4 x 80 mL). The combined organic phases were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by flash chromatography (5% ethyl acetate: 95% hexane) to afford **17** (78 mg, 93%, colorless oil). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.31 (d, J= 22.5 Hz, 3H, CH_3), δ 1.32 (d, J= 22.5 Hz, 3H, CH_3), δ 1.48 (d, J= 7.2 Hz, 3H, CH_3), δ 3.65 (s, 3H, OCH_3), δ 3.72 (q, J= 7.2 Hz, 1H, CH), δ 4.69 (d, J= 11.7 Hz, 1H, CH), δ 7.26 (d, J= 8.1 Hz, 2H, CH), δ 7.34 (d, J= 8.1 Hz, 2H, CH). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 18.79, δ 21.55 (d, J= 23.1 Hz), δ 24.08 (d, J= 23.7 Hz), δ 45.31, δ 52.28, δ 79.29 (d, J= 23.6 Hz), δ 98.14 (d, J= 168 Hz), δ 127.42, δ 127.91, δ 138.28 (d, J= 5.7 Hz), δ 140.55, δ 175.12. ^{19}F-NMR (282 MHz, $CDCl_3$): δ -146.8 (m). HRMS (EI^+): exact mass calculated for $C_{14}H_{20}FO_3$ requires m/z 255.1396, found 255.1394. Under argon, 30 mg (0.12 mmol) **17** and a catalytic amount of ethanol were dissolved in 3 mL dry dichloromethane. The solution was cooled to -78 °C. After 5 minutes, 20 μL DAST (0.14 mmol, 1.2 eq) were added to the mixture. The reaction was stirred in dry ice overnight. The reaction was then added with 5 mL saturated sodium bicarbonate and extracted with dichloromethane (3 x 15 mL). The organic phase was then dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue

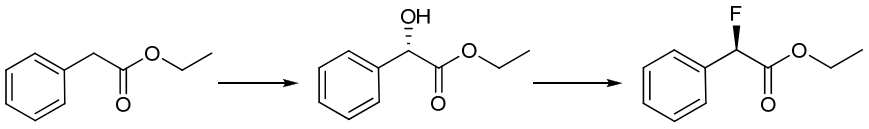
was purified by flash chromatography (5% ethyl acetate: 95% hexane) to afford **18** (30 mg, 98% yield, colorless oil). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.32 (m, 6H, CH_3), δ 1.51 (d, J= 7.2 Hz, 3H, CH_3), δ 3.66 (s, 3H, OCH_3), δ 3.74 (q, J= 7.2 Hz, 1H, CH), δ 5.29 (dd, J= 45.0, 13.8 Hz, 1H, CHF), δ 7.31 (s, 4H, CH). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 18.80, δ 23.01 (m), δ 45.38, δ 52.34, δ 96.80 (d, J= 179 Hz), δ 96.52 (d, J= 179 Hz), δ 127.50, δ 127.59, δ 134.8 (d, J= 22.5), δ 141.28, δ 175.03. ^{19}F-NMR (282 MHz, $CDCl_3$): δ -150.7 (m), δ -187.8 (dd, J= 45.6, 9.0 Hz). HRMS (EI^+): exact mass calculated for $C_{14}H_{19}F_2O_2$ requires m/z 257.1353, found 257.1356. Chiral GC analyses were carried out using a Shimadzu GC-17A gas chromatograph, FID detector, Agilent Cyclosilb column (30 m x 0.52 mm x 0.25 μm film) and the following separation program: 300 °C inlet, 300 °C detector, 70 °C oven, 0.5 °C/min gradient to 200 °C, 50 °C/min gradient to 250 °C, and 250 °C for 2 min. A diasteromeric ratio (dr) of 1:3.7, an enantiomeric excess of 9% for the major product, and an enantiomeric excess of 9% for the minor product were estimated based on peak areas (see **Supplementary Figure 4**).



19a **19b** **19c**

Conversion of 19a to 19c. 90 mg (0.6 mmol) **19a** were dissolved in 500 μL ethanol and added to 240 mL 100 mM KPi buffer pH 8.0**.** Var-A3 was added to the mixture at a final concentration of 2 μM. The mixture was added with a cofactor regeneration solution containing 0.5 mM NADPH, 30 mM glucose-6-phosphate and 1 U/mL glucose-6-phosphate dehydrogenase (final concentrations). The reaction mixtures were stirred at room temperature and extracted with chloroform (3 x 100 mL) after 4 hours. The organic phase was then dried over anhydrous $MgSO_4$ and evaporated under reduced

pressure. Purification of the resulting oil by silica gel chromatography (5% ethyl acetate : 95% hexane) afforded **19b** (45 mg). 45 mg (0.27 mmol) of **19b** and a catalytic amount (3 drops) of ethanol were dissolved in 2 mL dry dichloromethane under argon. The solution was cooled to -78 °C and added with 43 µL DAST (0.30 mmol, 1.2 eq). The reaction was stirred in dry ice for 12 hours. The reaction mixture was then added with 5 mL saturated sodium bicarbonate and extracted with dichloromethane (3 x 15 mL). The organic phases were then dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. Purification of the resulting oil by silica gel chromatography (5% ethyl acetate : 95% hexane) afforded **19c** (34 mg, 75% yield, pale yellow oil) in 74% *ee*, as determined by chiral GC analysis. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 3.75 (s, 3H), δ 5.77 (d, J= 48 Hz, 1H, -CHF), δ 7.37-7.46 (m, 5H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 52.8, δ 89.5 (d, J= 184.5 Hz), δ 126.8, δ 126.9, δ 129.0, δ 129.9, δ 134.4 (d, J= 34.5 Hz), δ 169.0. ^{19}F-NMR (282 MHz, $CDCl_3$): δ -180.29 (d, J= 48.7 Hz). HRMS (EI+): exact mass calculated for $C_9H_9FO_2$ requires m/z 168.0587, found 168.0594. Chiral GC analyses were carried out using a Shimadzu GC-17A gas chromatograph, FID detector, Agilent Cyclosilb column (30 m x 0.52 mm x 0.25 µm film) and the following separation program: 300 °C inlet, 300 °C detector, 100 °C oven, 2 °C/min gradient to 200 °C, 50 °C/min gradient to 250 °C, and 250 °C for 2 min. An enantiomeric excess of 74% was estimated based on peak areas (see **Supplementary Figure 5**).

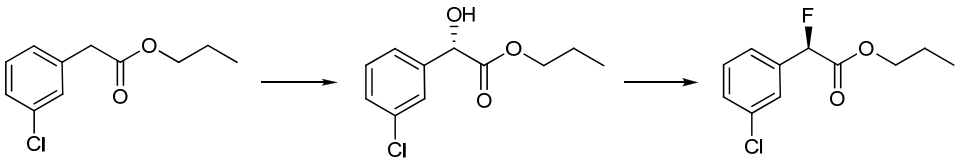


20a **20b** **20c**

Conversion of 20a to 20c. 100 mg (0.61 mmol) **20a** were dissolved in 500 µL ethanol and added to 250 mL 100 mM KPi buffer pH 8.0**.** var-B4 was added to the mixture at a

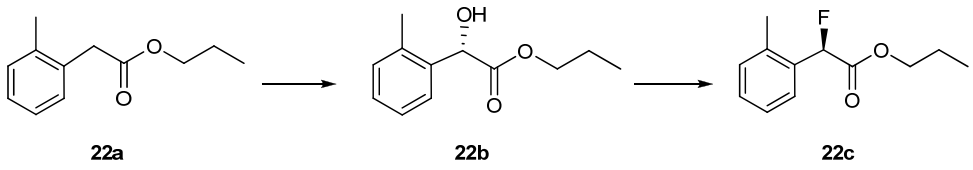
final concentration of 2 μM. The mixture was added with a cofactor regeneration solution containing 0.5 mM NADPH, 30 mM glucose-6-phosphate and 1 U/mL glucose-6-phosphate dehydrogenase (final concentrations). The reaction mixtures were stirred at room temperature and extracted with chloroform (3 x 100 mL) after 3 hours. The organic phases were collected, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. Purification of the resulting oil by silica gel chromatography (5% ethyl acetate : 95% hexane) afforded **20b** (72 mg, 66%). 72 mg (0.4 mmol) of **20b** and a catalytic amount (3 drops) of ethanol were dissolved in 2 mL dry dichloromethane under argon. The solution was cooled to -78 °C and added with 61 μL DAST (0.43 mmol, 1.2 eq). The reaction was stirred in dry ice for 12 hours. The reaction mixture was then added with 5 mL saturated sodium bicarbonate and extracted with dichloromethane (3 x 15 mL). The organic phases were collected, dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. Purification of the resulting oil by silica gel chromatography (5% ethyl acetate : 95% hexane) afforded **20c** (57 mg, 78% yield, pale yellow oil) in 72% *ee*, as determined by chiral GC analysis. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.24 (t, J= 7.2 Hz, 3H, -CH_3), δ 4.16-4.27 (m, 2H, -OCH_2), δ 5.75 (d, J= 48 Hz, 1H, -CHF), δ 7.37-7.46 (m, 5H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 14.2, δ 62.0, δ 81.2, δ 89.6 (d, J= 184.5 Hz), δ 126.8, δ 126.9, δ 128.9, δ 129.8, δ 134.4 (d, J= 34.5 Hz), δ 169.0. ^{19}F-NMR (282 MHz, $CDCl_3$): δ -180.27 (d, J= 48.7 Hz). HRMS (EI+): exact mass calculated for $C_{10}H_{11}FO_2$ requires m/z 182.0743, found 182.0750. Chiral GC analyses were carried out using a Shimadzu GC-17A gas chromatograph, FID detector, Agilent Cyclosilb column (30 m x 0.52 mm x 0.25 μm film) and the following separation program: 300 °C inlet, 300 °C detector, 100 °C oven, 2 °C/min gradient to

200 °C, 50 °C/min gradient to 250 °C, and 250 °C for 2 min. An enantiomeric excess of 72% was estimated based on peak areas (see **Supplementary Figure 5**).



Conversion of 21a to 21c. 95 mg (0.45 mmol) **21a** were dissolved in 500 μL ethanol and added to 250 mL 100mM KPi buffer pH 8.0**.** Var-C12 was added to the mixture at a final concentration of 1 μM. The mixture was added with a cofactor regeneration solution containing 0.5 mM NADPH, 30 mM glucose-6-phosphate and 1 U/mL glucose-6-phosphate dehydrogenase (final concentrations). The reaction mixtures were stirred at room temperature and extracted with chloroform (3 x 100 mL) after 4 hours. The organic phases were collected, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. Purification of the resulting oil by silica gel chromatography (5% ethyl acetate : 95% hexane) afforded **21b** (71 mg, 75%). 70 mg (0.34 mmol) of **21b** and a catalytic amount (3 drops) of ethanol were dissolved in 2 mL dry dichloromethane under argon. The solution was cooled to -78 °C and added with 64 μL DAST (0.45 mmol, 1.5 eq). The reaction was stirred in dry ice for 12 hours. The reaction mixture was then added with 5 mL saturated sodium bicarbonate and extracted with dichloromethane (3 x 15 mL). The organic phases were collected, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. Purification of the resulting oil by flash chromatography (5% ethyl acetate : 95% hexane) afforded **21c** (63 mg, 82% yield, colorless oil) in 89% *ee*, as determined by chiral GC analysis. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.85 (t, J= 7 Hz, 3H, -CH_3), δ 1.56-1.68 (m, 2H, CH_2), δ 4.12 (t, J= 6 Hz, 2H,

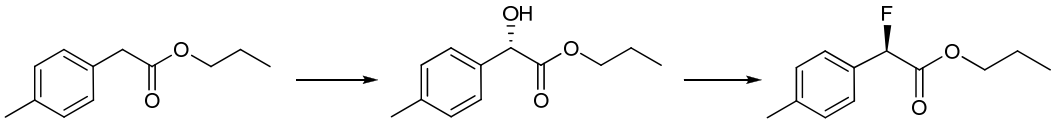
-OCH_2), δ 5.72 (d, J= 48 Hz, 1H, -CHF), δ 7.32 (br, 3H), δ 7.44 (br, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 10.3, δ 21.9, δ 67.7, δ 88.7 (d, J= 186.5 Hz), δ 124.8, δ 126.9, δ 129.9, δ 130.3, δ 134.9. ^{19}F-NMR (282 MHz, $CDCl_3$): δ -182.8 (d, J= 48.7 Hz). HRMS (EI+): exact mass calculated for $C_{11}H_{12}ClFO_2$ requires m/z 230.0510, found 230.0502. Chiral GC analyses were carried out using a Shimadzu GC-17A gas chromatograph, FID detector, Agilent Cyclosilb column (30 m x 0.52 mm x 0.25 μm film) and the following separation program: 300 °C inlet, 300 °C detector, 100 °C oven, 2 °C/min gradient to 200 °C, 50 °C/min gradient to 250 °C, and 250 °C for 2 min. An enantiomeric excess of 89% was estimated based on peak areas (see **Supplementary Figure 6**).



22a **22b** **22c**

Conversion of 22a to 22c. 100 mg (0.52 mmol) of **22a** were dissolved in 500 μL ethanol and added to 250 mL 100 mM KPi buffer pH 8.0**.** Var-C12 was added to the mixture at a final concentration of 1 μM. The mixture was added with a cofactor regeneration solution containing 0.5 mM NADPH, 30 mM glucose-6-phosphate and 1 U/mL glucose-6-phosphate dehydrogenase (final concentrations). The reaction mixtures were stirred at room temperature and extracted with chloroform (3 x 100 mL) after 4 hours. The organic phases were collected, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. Purification of the resulting oil by silica gel chromatography (5% ethyl acetate : 95% hexane) afforded **22b** (78 mg, 65%). 78 mg (0.37 mmol) of **22b** and a catalytic amount (3 drops) of ethanol were dissolved in 4 mL dry dichloromethane

under argon. The solution was cooled to -78 °C and added with 63 µL DAST (0.44 mmol, 1.5 eq). The reaction was stirred in dry ice for 12 hours. The reaction mixture was then added with 5 mL saturated sodium bicarbonate and extracted with dichloromethane (3 x 15 mL). The organic phases were collected, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. Purification of the resulting oil by flash chromatography (5% ethyl acetate : 95% hexane) afforded **22c** (68 mg, 88%, colorless oil) in 85% *ee*, as determined by chiral GC analysis. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.83 (t, J= 7.5 Hz, 3H, -CH_3), δ 1.52-1.68 (m, 2H, CH_2), δ 2.43 (s, 3H, -CH_3), δ 4.12 (m, 2H, -OCH_2), δ 5.96 (d, J= 48 Hz, 1H, -CHF), δ 7.16-7.30 (m, 4H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 10.3, δ 19.3, δ 22.0, δ 29.9, δ 67.4, δ 87.4 (d, J= 183 Hz), δ 126.5, δ 127.5, δ 129.8, δ 131.0. ^{19}F-NMR (282 MHz, $CDCl_3$): δ -180.1 (d, J= 48.7 Hz). HRMS (EI+): exact mass calculated for $C_{12}H_{15}FO_2$ requires m/z 210.1056, found 210.1070. Chiral GC analyses were carried out using a Shimadzu GC-17A gas chromatograph, FID detector, Agilent Cyclosilb column (30 m x 0.52 mm x 0.25 µm film) and the following separation program: 300 °C inlet, 300 °C detector, 100 °C oven, 2 °C/min gradient to 200 °C, 50 °C/min gradient to 250 °C, and 250 °C for 2 min. An enantiomeric excess of 85% was estimated based on peak areas (see **Supplementary Figure 6**).

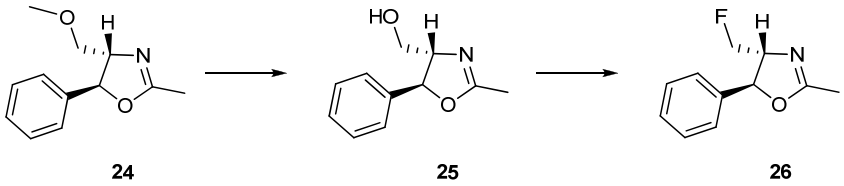


23a **23b** **23c**

Conversion of 23a to 23c. 100 mg (0.52 mmol) of **23a** were dissolved in 500 µL ethanol and added to 250 mL 100mM KPi buffer pH 8.0**.** Var-C12 was added to the

mixture at a final concentration of 1 μM. The mixture was added with a cofactor regeneration solution containing 0.5 mM NADPH, 30 mM glucose-6-phosphate and 1 U/mL glucose-6-phosphate dehydrogenase (final concentrations). The reaction mixtures were stirred at room temperature and extracted with chloroform (3 x 100 mL) after 4 hours. The organic phases were collected, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. Purification of the resulting oil by silica gel chromatography (5% ethyl acetate : 95% hexane) afforded **23b** (70 mg, 65%). 70 mg (0.34 mmol) of **23b** and a catalytic amount (3 drops) of ethanol were dissolved in 4 mL dry dichloromethane under argon. The solution was cooled to -78 °C and added with 58 μL DAST (0.4 mmol, 1.5 eq). The reaction was stirred in dry ice for 12 hours. The reaction mixture was then added with 5 mL saturated sodium bicarbonate and extracted with dichloromethane (3 x 15 mL). The organic phases were collected, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. Purification of the resulting oil by flash chromatography (5% ethyl acetate : 95% hexane) afforded **23c** (59 mg, 83%, colorless oil) in 87% ee, as determined by chiral GC analysis. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.84-0.91 (m, 3H, -CH_3), δ 1.57-1.68 (m, 2H, CH_2), δ 2.37 (s, 3H, -CH_3), δ 4.08-4.16 (m, 2H, -OCH_2), δ 5.75 (d, J= 48 Hz, 1H, -CHF), δ 7.18-7.27 (m, 2H), δ 7.27-7.44 (m, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 10.4, δ 19.4, δ 22.1, δ 29.9, δ 67.5, δ 87.2 (d, J= 185 Hz), δ 126.5, δ 131.1. ^{19}F-NMR (282 MHz, $CDCl_3$): δ -178.5 (d, J= 48.7 Hz). HRMS (EI+): exact mass calculated for $C_{12}H_{15}FO_2$ requires m/z 210.1056, found 210.1062. Chiral GC analyses were carried out using a Shimadzu GC-17A gas chromatograph, FID detector, Agilent Cyclosilb column (30 m x 0.52 mm x 0.25 μm film) and the following separation program: 300 °C inlet, 300 °C detector, 100 °C oven, 2 °C/min gradient to 200 °C, 50 °C/min gradient to 250 °C, and 250 °C for 2 min. An

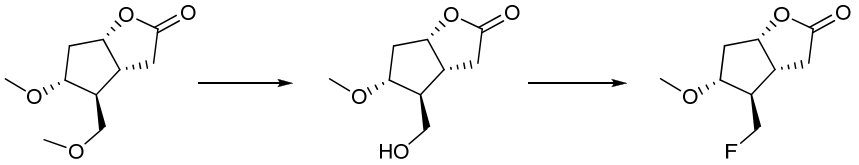
enantiomeric excess of 87% was estimated based on peak areas (see **Supplementary Figure 6**).



24 **25** **26**

Conversion of 24 to 26. 103 mg (0.5 mmol) **24** were dissolved in 2 mL DMSO and added to 200 mL potassium phosphate buffer pH 8.0. The solution was added with var-H1 (final conc.: 2.5 μM) and a cofactor regeneration system (final conc.: 500 μM NADPH, 30 mM glucose-6-phosphate, 2 units/mL glucose-6-phosphate dehydrogenase) and stirred at room temperature. After 48 hours, the reaction mixture was extracted with dichloromethane (4 x 70 mL). The combined organic phases were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by flash chromatography (20% ethyl acetate: 80% hexane) to afford **25** (88 mg, 92%, colorless oil). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.08 (s, 3H, CH_3), δ 3.65 (dt, J=11.7, 3.6 Hz, 1H), δ 3.85-3.92 (m, 2H), δ 5.34 (d, J= 7.8 Hz, 1H), δ 7.26-7.40 (m, 5H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 14.22, δ 56.83, δ 63.18, δ 83.27, δ 125.99, δ 128.50, δ 129.12, δ 140.58, δ 166.60. HRMS (EI^+): exact mass calculated for $C_{11}H_{13}NO_2$ requires m/z 191.0946, found 191.0943. 88 mg (0.46 mmol) **25** and a catalytic amount (3 drops) of ethanol were dissolved in 3 mL dry dichloromethane under argon. The solution was placed in ice. After ten minutes, 66 μL DAST (0.46 mmol, 1 eq) were added to the mixture in aliquots of 22 μL every 30 minutes. The reaction was stirred in ice overnight. The reaction mixture was added with 5 mL saturated sodium bicarbonate and extracted with dichloromethane (3 x 15 mL). The organic phase was then dried over anhydrous magnesium sulfate and evaporated under reduced pressure. The residue was purified by

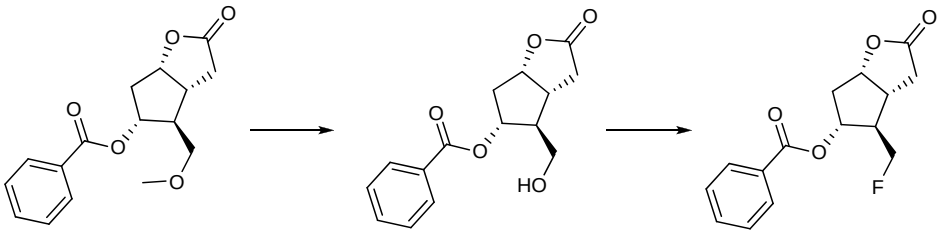
flash chromatography (gradient 10 to 30% ethyl acetate in hexane) to afford **26** (35 mg, 40% yield, colorless oil). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.12 (s, 3H, CH_3), δ 4.2 (m, 1H), δ 4.57 (dm, J= 47.4 Hz, 2H), δ 5.35 (d, J= 7.2 Hz, 1H), δ 7.25-7.40 (m, 5H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 14.39, δ 60.64, δ 82.34 (d, J= 19.6 Hz), δ 83.92 (d, J= 155.7 Hz) δ 125.74 (2C), δ 128.72, δ 129.14 (2C), δ 140.34, δ 166.50. ^{19}F-NMR (282 MHz, $CDCl_3$): δ -230.8 (dt, J=24.2, 45.1 Hz). HRMS (EI+): exact mass calculated for $C_{11}H_{12}FNO$ requires m/z 193.0903, found 193.0917.



27a **27b** **27c**

Conversion of 27a to 27c. To a solution containing potassium phosphate buffer pH 8.0 and 0.5 mM **27a** in 1% DMSO was added with var-G5 (final conc.: 5 μM), 1 mg/mL bovine serum albumin (BSA), 1000 U/mL catalase, 4.1 U/mL superoxide dismutase (SOD) and a cofactor regeneration system (final conc.: 500 μM NADPH, 30 mM glucose-6-phosphate, 2 units/mL glucose-6-phosphate dehydrogenase). The reaction was stirred for 48 hours at 4 °C then worked-up as described above (e.g. compound 16). The residue was purified by flash chromatography (33% ethyl acetate: 67% hexanes, then 50% ethyl acetate: 50% hexanes) to afford **27b** (60%, colorless oil). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.2 (m, 3H), δ 2.51 (d J=17.1, 1H), δ 2.76 (m, 2H), δ 3.30 (s, 3H), δ 3.60 (d, J=5.7, 2H), δ 3.75 (q, J= 4.8 Hz, 1H), δ 4.96 (m, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 26.14, δ 27.4, δ 30.8, δ 48.73, δ 52.09, δ 60.40, δ 73.24, δ 85.57, δ 200.18. HRMS (EI+): exact mass calculated for $C_9H_{14}O_4$ requires m/z 186.0892, found 186.0885. 14 mg (0.075 mmol) **27b** were dissolved in 3 mL of dry dichloromethane and 2.5 μL of dry ethanol were added. The mixture was cooled to -80 °C and 15 μL DAST

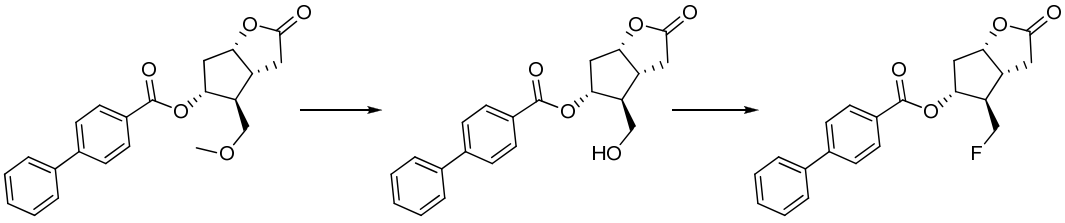
(0.11 mmol, 1.5 eq) were added. The reaction was allowed to come to room temperature slowly (16 h) and then quenched by addition of 3 mL of saturated $NaHCO_3$ solution. The mixture was extracted with dichloromethane (4 x 15 mL), the organic phases were collected, dried over anhydrous magnesium sulfate, and evaporated under reduced pressure. The residue was purified by flash chromatography (20% ethyl acetate : 80% hexanes, then 33% ethyl acetate: 67% hexanes) to afford **27c** (9.4 mg, 66% yield, colorless oil). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.21 (m, 2H), δ 2.52 (m, 1H), δ 2.81 (m, 2H), δ 3.30 (s, 3H), δ 3.78 (q, J= 7.2, 1H), δ 4.40 (ddd, J= 47.4, 24.6, 9.6 Hz, 1H, CHF), δ 4.41 (ddd, J= 47.4, 24.6, 9.6 Hz, 1H, CHF), δ 5.00 (m, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 27.05, δ 35.68, δ 36.85, δ 39.28, δ 52.98 (d, J= 18.3 Hz), δ 56.83, δ 83.18 (d, J= 139 Hz), δ 84.51, δ 179.29. ^{19}F-NMR (282 MHz, $CDCl_3$): δ -225.8 (td, J= 48.2, 27.1 Hz). HRMS (EI^+): exact mass calculated for $C_9H_{13}FO_3$ requires m/z 188.0849, found 188.0817.



28a **28b** **28c**

Conversion of 28a to 28c. To a solution containing potassium phosphate buffer pH 8.0 and 1 mM **28a** in 1% DMSO was added with var-B3 (final conc.: 5 μM) and a cofactor regeneration system (final conc.: 500 μM NADPH, 30 mM glucose-6-phosphate, 2 units/mL glucose-6-phosphate dehydrogenase). The reaction was stirred for 48 hours at room temperature, then worked-up as described above. The residue was purified by flash chromatography (25% ethyl acetate: 75% hexanes, then 33% ethyl acetate: 67% hexanes) to afford **28b** (75%, white powder). LC-MS (*m/z*): $[M+H]^+$ 279.0. ^{1}H and ^{13}C

NMR data were identical to those of the authentic standard. Two reaction vessels were prepared dissolving, under argon, 30 mg (0.11 mmol) **28b** in 3 mL dry dichloromethane in each vessel. 18.5 μL DAST (0.13 mmol, 1.2 eq) were added each vessel in aliquots of 6 μL aliquots every 30 minutes. The reaction was stirred at room temperature. After 4 hours, the reaction mixture was added with 5 mL saturated sodium bicarbonate and extracted with dichloromethane (3 x 15 mL). The organic phases were collected, dried over anhydrous magnesium sulfate, and evaporated under reduced pressure. The residue was purified by flash chromatography (20% ethyl acetate : 80% hexane) to afford **28c** (33 mg, 55% yield, white powder). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.50 (m, 4H), δ 2.96 (m, 2H), δ 4.54 (ddd, J= 47.4, 21.0, 9.6 Hz, 1H, CHF), δ 4.55 (ddd, J= 47.4, 21.0, 9.6 Hz, 1H, CHF), δ 5.12 (m, 1H), δ 5.41 (m, 1H), δ 7.44 (m, 2H), δ 7.56 (m, 1H), δ 8.01 (m, 2H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 36.20, δ 38.97, δ 40.30, δ 53.66 (d, J= 18.5 Hz), δ 73.16, δ 83.62 (d, J= 170 Hz), δ 84.92, δ 128.79, δ 129.89, δ 133.66, δ 166.22, δ 176.46. ^{19}F-NMR (282 MHz, $CDCl_3$): δ -226.7 (td, J= 48.0, 30.1 Hz). HRMS (EI^+): exact mass calculated for $C_{15}H_{16}FO_4$ requires m/z 279.1033, found 279.1041.



29a **29b** **29c**

Conversion of 29a to 29c. To a solution containing potassium phosphate buffer pH 8.0 and 0.5 mM **29a** in 1% DMSO was added var-H1 (final conc.: 5 μM), 1 mg/mL BSA, 1000 U/mL catalase, 4.1 U/mL SOD and a cofactor regeneration system (final conc.: 500 μM NADPH, 30 mM glucose-6-phosphate, 2 units/mL glucose-6-phosphate

dehydrogenase). The reaction was stirred for 48 hours at room temperature, then worked-up as described above (e.g. compound 16). The residue was purified by flash chromatography (25% ethyl acetate: 75% hexanes, then 50% ethyl acetate: 50% hexanes) to afford **29b** (60%, colorless oil). LC-MS (*m/z*): $[M+H]^+$ 355.0. ^1H and ^{13}C NMR data were identical to those of the authentic standard. Two reaction vessels were prepared dissolving, under argon, 20 mg (0.05 mmol) **29b** in 2 mL dry dichloromethane in each vessel. 10 μL DAST (0.07 mmol, 1.2 eq) were added to each vessel in aliquots of 3 μL aliquots every 30 minutes. The reaction was stirred at room temperature. After 6 hours, the reaction mixture was added with 5 mL saturated sodium bicarbonate and extracted with dichloromethane (3 x 15 mL). The organic phases were collected, dried over anhydrous magnesium sulfate, and evaporated under reduced pressure. The residue was purified by flash chromatography (20% ethyl acetate : 80% hexane) to afford **29c** (18 mg, 52% yield, white powder). ^1H-NMR (300 MHz, $CDCl_3$): δ 2.44 (m, 2H), δ 2.50-2.61 (m, 2H), δ 2.95-3.02 (m, 2H), δ 4.48 (ddd, J= 47.1, 21.3, 11.7 Hz, 1H, CHF), δ 4.64 (ddd, J= 47.1, 21.3, 11.7 Hz, 1H, CHF), δ 5.15 (m, 1H), δ 5.44 (m, 1H), δ 7.35-7.50 (m, 3H), δ 7.55-7.69 (m, 4H), δ 8.02-8.08 (m, 2H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 36.26, δ 39.00, δ 40.28 (d, J= 4.27 Hz), δ 53.76 (d, J= 18.2 Hz), 83.77 (d, J= 183.9 Hz), δ 84.81, δ 127.45 (2C), δ 127.52 (2C), δ 128.35, δ 128.45, δ 129.17 (2C), δ 130.44 (2C), δ 140.08, δ 146.35, δ 166.11, δ 176.55. ^{19}F-NMR (282 MHz, $CDCl_3$): δ -226.6 (td, J= 45.4, 30.2 Hz). HRMS (EI^+): exact mass calculated for $C_{21}H_{20}FO_4$ requires m/z 355.1346, found 355.1332.

P450 expression and purification. For the chemo-enzymatic transformations, P450 enzymes were used in purified form. Enzyme batches were prepared as follows. Two liters Terrific Broth medium were inoculated with an overnight culture (10 mL) of recombinant *E. coli* DH5α cells harboring a pCWori plasmid encoding for the P450 variant under the control of Plac promoter. At OD_{600}=1.1, cultures were added with 0.5 mM IPTG and 0.5 mM δ-aminolevulinic acid (ALA) and transferred to 25˚C. Cells were harvested after 24 hours. The cell pellet was resuspended in 25 mM TRIS (pH 8.0) and cell membranes were disrupted by sonication. Cell lysate was loaded onto a Q resin and the column was washed with 3 column volumes of 25 mM TRIS (pH 8.0), 150 mM NaCl. Bound protein was eluted with 25 mM TRIS (pH 8.0), 340 mM NaCl and concentrated using Millipore Centricon. After buffer exchange with 100 mM KPi (pH 8.0), protein samples were frozen and stored at -80 °C. Protein concentration was determined in duplicate from CO-difference spectra. Yields typically ranged between 100 and 500 mg protein per liter depending on the variant.

References

1. Fasan, R., Chen, M.M., Crook, N.C. & Arnold, F.H. *Angew. Chem. Int. Ed. Engl.* **46**, 8414-8418 (2007).
2. Di, L., Kerns, E.H., Fan, K., McConnell, O.J. & Carter, G.T. *Eur. J. Med. Chem.* **38**, 223-32 (2003).
3. Wohnsland, F. & Faller, B. *J. Med. Chem.* **44**, 923-30 (2001).
4. Fasan, R., Meharenna, Y.T., Snow, C.D., Poulos, T.L. & Arnold, F.H. *J. Mol. Biol.* **383**, 1069-1080 (2008).
5. Meinhold, P., Peters, M.W., Chen, M.M., Takahashi, K. & Arnold, F.H. *Chembiochem* **6**, 1765-8 (2005).