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RESEARCH ARTICLE

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Exploring the enzyme-catalyzed synthesis of isotope labeled cyclopropanes

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Cyclopropanes are commonly employed structural moieties in drug design since their incorporation is often associated with increased target affinity, improved metabolic stability, and increased rigidity to access bioactive conformations. Robust chemical cyclopropanation procedures have been developed which proceed with high yield and broad substrate scope, and have been applied to labeled substrates. Recently, engineered enzymes have been shown to perform cyclopropanations with remarkable diastereoselectivity and enantioselectivity, but this biocatalytic approach has not been applied to labeled substrates to date. In this study, the use of enzyme catalysis for the synthesis of labeled cyclopropanes was investigated. Two readily available enzymes, a modified CYP450 enzyme and a modified *Aeropyrum pernix protoglobin,* were investigated for the cyclopropanation of a variety of substituted styrenes. For this biocatalytic transformation, the enzymes required the use of ethyl diazoacetate. Due to the highly energetic nature of this molecule, alternatives were investigated. The final optimized cyclopropanation was successfully demonstrated using *n*-hexyl diazoacetate, resulting in moderate to high enantiomeric excess. The optimized procedure was used to generate labeled cyclopropanes from ^{13}C-glycine, forming all four labeled stereoisomers of phosphodiesterase type-IV inhibitor, MK0952. These reactions provide a convenient and effective biocatalytic route to stereoselective ^{13}C-labeled cyclopropanes and serve as a proof-of-concept for generating stereoselective labeled cyclopropanes.

KEYWORDS

biocatalysis, cyclopropanation, diazoacetates, enzyme catalysis, isotopic labeling, stereoselectivity

1 | INTRODUCTION

Cyclopropanes are often metabolically stable motifs in drugs and drug candidates and make an intelligent location for labeling with stable isotopes or radionuclides in drug development studies. Cyclopropanes are featured in many natural compounds, including pheromones and terpenes,[1] and the introduction of this subunit into pharmacologically active synthetic compounds dates back to the 1960s.[2,3] Talele *et al*[2] have reviewed the frequent appearance of cyclopropyl rings in drug molecules and stated that the cyclopropane unit can be used as a less lipophilic surrogate for hydrophobic ring systems and can be used to increase affinity and rigidity, and to improve metabolic stability. This ring also offers controlled stereochemistry, with alternate stereoisomers often having very different biological activities.[2,4] Many synthetic methods have been developed for the synthesis of cyclopropanes, including the Simmons-Smith reaction and the transition-metal-catalyzed decomposition of diazo compounds.[1] Despite the synthetic utility of these methods, they can be difficult to use in the synthesis of isotopically labeled compounds due to the lack of desirable labeled starting materials.

Biocatalysis is an important tool in organic synthesis, often exhibiting exquisite chemo-, regio-, and stereoselective control,[5–7] combined with low environmental impact and improved safety parameters. Arnold and co-workers[4,8] engineered cytochrome P450 enzymes (CYP450) to form cyclopropanes with high stereo- and enantioselectivities from olefins. Similarly, Hartwig and co-workers[9] described a modified CYP450 containing iridium rather than iron in the heme. This modified enzyme is suitable for a broad range of alkenes forming cyclopropanes with high stereoselectivity. In addition to CYP450-enzymes, another iron-based heme-dependent enzyme, myoglobin, has also been engineered by Fasan and co-workers[10] to transform alkenes into cyclopropanes. The reaction exhibits broad substrate scope and has been applied to the synthesis of Tasimelteon, a TRPV1 inhibitor, and ticagrelor (Figure 1) on a gram scale.[11,12] In contrast to many synthetic procedures,[13] enzymatic cyclopropanations are performed in aqueous media. By replacing organic solvent with water and stoichiometric metal for catalytic iron, the environmental impact of the reaction should be improved. In addition, and perhaps more importantly, the reactions afford cyclopropanes in a high diastereoselectivity and

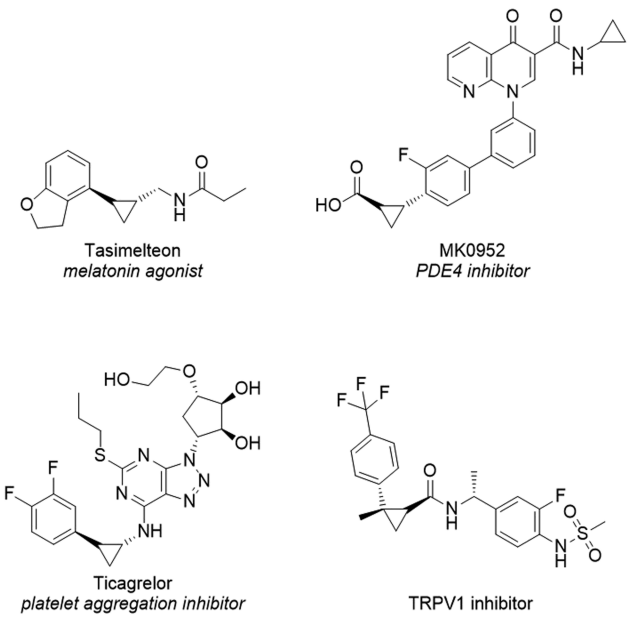
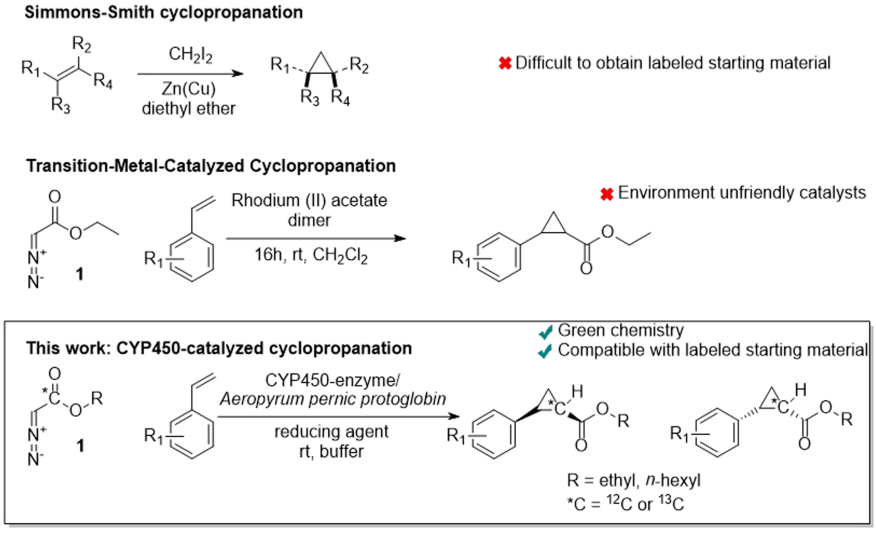


FIGURE 1 Selection of pharmaceutically important cyclopropanes



SCHEME 1 Cyclopropanation methodologies

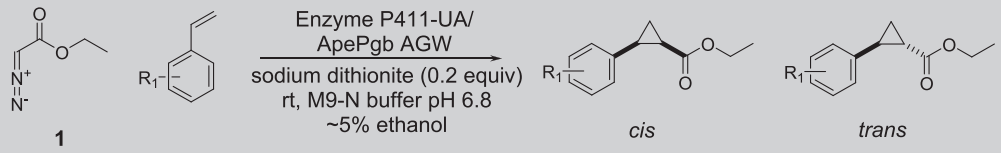
enantioselectivity, limiting the formation of any by-products. However, the enzyme-mediated cyclopropanations reported to date use ethyl diazoacetate (EDA (**1**)) as the methylene source, which exhibits high impact sensitivity (1730 J/g).[14,15]

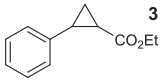
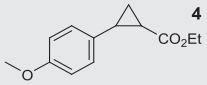
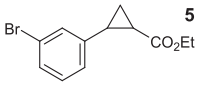
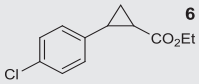
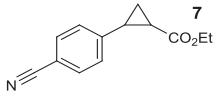
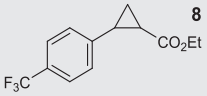
Inspired by the works of Arnold, Fasan, and Hartwig, we explored the synthesis of various substituted cyclopropanes with readily available enzymes (Scheme **1**) using EDA (**1**) as co-substrate as a first objective. For this feasibility study, enzymes previously engineered for 1-octene were chosen. As described before, enzymes engineered for a target molecule provide the ability to produce cyclopropanes with both high diastereoselectivity (*cis* or *trans*) and enantioselectivity. Our second objective was to develop a diazoacetate substrate with a higher carbon content to mitigate the impact sensitivity. With this alternative diazoacetate and optimized conditions, ^{13}C-labeled MK0952 was successfully synthesized.

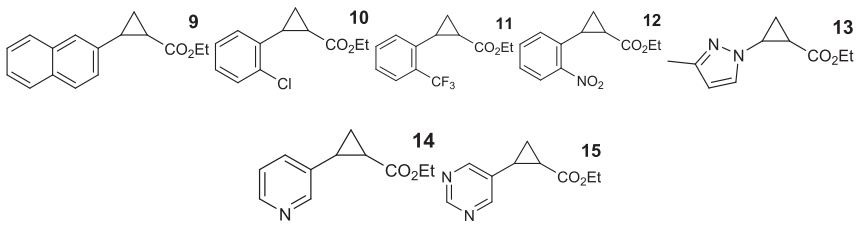
2 | RESULTS AND DISCUSSION

We initiated our optimization study with the enzyme P411-UA and enzyme ApePgb AGW, provided by

TABLE 1 Scope of cyclopropanation using EDA (**1**)



Entry[a]	Product	Enzyme P411-UA/ApePgb AGW	Conversion %	d.r. (*cis*: *trans*)	e.e. (%) cis	e.e. (%) trans
1	 **3**	*P411-UA*	>99	1:1	61	-
		ApePgb AGW	>99	7:3	82	71
2	 **4**	*P411-UA*	67	4:1	92	43
		ApePgb AGW	54	1:1	87	75
3	 **5**	*P411-UA*	49	7:3	62	0
		ApePgb AGW	92	9:1	93	93
4	 **6**	*P411-UA*	85	9:1	94	42
		ApePgb AGW	60	1:1	83	74
5	 **7**	*P411-UA*	51	7:3	92	13
		ApePgb AGW	45	3:7	90	86
6	 **8**	*P411-UA*	81	7:3	94	9
		ApePgb AGW	>99	1:1	85	66
Limitations						



[a]*Conditions: Enzyme (~150 mg), M9-N (pH 6.8, 28.5 mL), sodium dithionite (0.2 equiv), EDA (**1**) (1.06 mmol), styrene (0.53 mmol), 6% EtOH. Enzyme P411-UA showed a selectivity for one cis-enantiomer and enzyme ApePgb AGW showed a selectivity for the other cis-enantiomer.*

Professor Frances Arnold, using EDA (**1**) and styrene (**2**).[4] Various parameters were investigated, including concentration, EDA (**1**) equivalents, and reaction time. The summarized results of this optimization study can be found in the experimental section (Table E1, in the Experimental). By adjusting the parameters, a shift of the diastereomers to either *cis* or *trans* could be achieved. When using **2** equiv EDA (**1**), 15 mM styrene (**2**), and 2.8 mg/mL enzyme, a conversion of 100% is obtained with a d.r. of 7:3 (*cis*:*trans*) after shaking 22 h (Table **E1**, entry 9). Given that any optimization requires a target molecule and that this work represents a feasibility study rather than a study tailored to a specific target molecule, the conditions found for styrene (**2**) were applied to investigate the substrate scope.

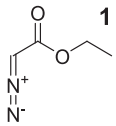
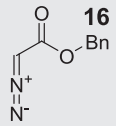
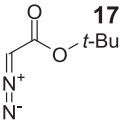
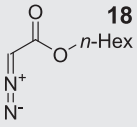
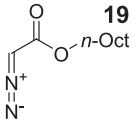
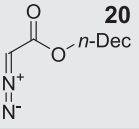
2.1 | Screening of substituted styrenes with EDA (1) using enzyme P411-UA and ApePgb AGW

The substrate scope was probed using various substituted styrenes with enzymes P411-UA and AgePgb AGW as shown in Table 1. As expected, generally more *cis*-cyclopropanes (d.r. > 50%) were obtained from the enzyme P411-UA than AgePgb AGW-catalyzed cyclopropanation.

The *cis*-cyclopropanes obtained from enzyme P411-UA also displayed a higher e.e. in comparison to the *trans*-cyclopropanes. One electron-rich and several electron-deficient styrenes provided modest to excellent conversions. In general, a higher conversion resulted in higher selectivity towards *cis*. The e.e. of the *cis*-cyclopropanes was moderate to excellent, while that of the *trans*-cyclopropanes was poor. The results using enzyme ApePgb AGW are more difficult to categorize, but the e.e. were good to excellent for both *cis*- and *trans*-cyclopropanes. In addition, *ortho* substitution and heterocycles were not tolerated for either enzyme.

A robust substrate scope was demonstrated by this study, although these readily available enzymes were evolved using 1-octene as a substrate. It is likely that by using a styrene as a substrate and further engineering of the enzyme, higher specificity for the desired product could be achieved. Nevertheless, the e.e. and d.r. obtained are useful and may be further manipulated

TABLE 2 DSC analysis of diazoacetates

Entry[a]	Diazoacetate	T onset °C	J/g (neat)
1	 **1**	83	1730
2	 **16**	116	340
3	 **17**	134	1830
4	 **18**	128	1700
5	 **19**	130	1125
6	 **20**	126	1150

[a]All diazoacetates were dissolved/stored in toluene.

by reaction optimization if a specific substrate is targeted. Moreover, a high e.e. can be obtained without having a high d.r. (Table 1, entry 3, enzyme ApePgb AGW), suggesting that the diastereoselectivity and enantioselectivity of the enzymes are not correlated.

Overall, moderate conversions were obtained. This can be explained by the limited conversion for most cyclopropanations, the small scale of the reactions, and the dimerization of EDA (**1**) forming maleate and fumarate. Additionally, the isolation of phenylcyclopropane **3** was challenging due to volatility. Unfortunately, only a small mass peak was observed for 2-vinylnaphthalene **9**, along with all *ortho*-substituted styrenes (**10–12**), suggesting that these reactions need further optimization or may represent a limitation of this enzyme. Finally, based on gas chromatography-mass spectrometry (GC-MS) analysis, heterocyclic ring systems did not provide any product formation (**13–15**).

2.2 | Finding an alternative for EDA (1)

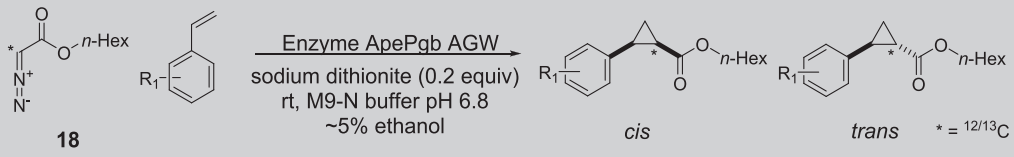
EDA (**1**) plays a central role in many cyclopropanation reactions; however, the industrial use of EDA (**1**) requires special precautions due to safety concerns associated with its instability and high reactivity.[16] To reduce safety concerns, diazoacetates with longer alkyl chains were synthesized and investigated. It was expected that extending the alkyl side chain would reduce the inherent energetics of the compound while having a minimal effect upon the enzymatic conversion. The differential scanning calorimetry (DSC) results show that while onset temperature was raised significantly by lengthening the alkyl side chain, the J/g was not significantly affected (Table 2).

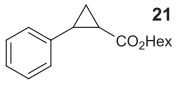
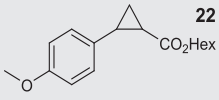
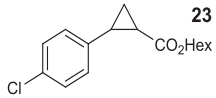
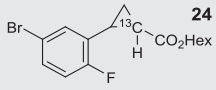
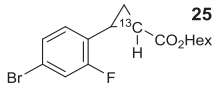
Initially, benzyl diazoacetate (**16**) was investigated as the enzymatic substrate; however, no product formation in the enzyme-catalyzed cyclopropanation was observed. We hypothesized that the benzyl chain may not fit in the pocket in the enzyme where the reaction takes place; therefore, longer alkyl chain were used instead. Product formation was observed when using *n*-hexyldiazoacetate (**18**). The following scope was performed using **18** as neither **19** nor **20** significantly increased the onset temperature, although they reduced the energetic content of the molecule.

2.3 | Screening of substituted styrenes with *n*-hexyldiazoacetates

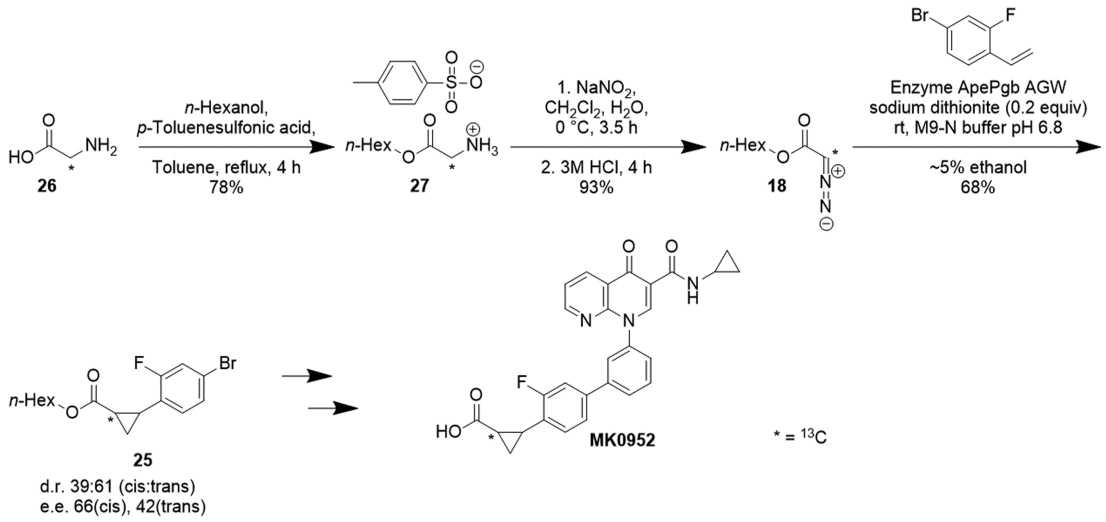
A small panel of substrates was investigated using *n*-hexyl diazoacetate (**18**) (Table 3). The conversions and

TABLE 3 Scope of cyclopropanation using *n*-hexyl diazoacetate (**18**)



Entry	Product	Conversion %	d.r. (*cis*: *trans*)	e.e. (%) cis	e.e. (%) trans
1[a]	 **21**	>99	1:1	ND	ND
2[a]	 **22**	>99	2:3	ND	ND
3[a]	 **23**	>99	3:7	81	2
4[b]	 **24**	84	2:3	ND	34
5[b]	 **25**	92(61)	2:3	66	42

[a]Conditions: Enzyme (5.6 mg), M9-N (pH 6.8, 4.3 mL), sodium dithionite (0.2 equiv), *n*-hexyl diazoacetate (**18**) (2 equiv), styrene (0.1 mmol).
[b]Conditions: Enzyme (150 mg), M9-N (pH 6.8, 28.5 mL), sodium dithionite (0.2 equiv), EDA (**1**) (2 equiv), styrene (0.53 mmol), and 6% EtOH. Isolated yield is shown in parenthesis.



SCHEME 2 Synthesis of labeled MK0952 using labeled glycine

selectivities obtained from these reactions are comparable with the results found in Table 1.

The synthesis of isomers **24** and **25** was performed using labeled *n*-hexyl diazoacetate (^{13}C-**18**). This diazoacetate is synthesized in two steps, starting from commercially available labeled glycine **26** with a yield of 73% over two steps. C-13 labeling was used here as a model for C-14 labeling, avoiding unnecessary radiochemical handling and the generation of difficult-to-dispose radiochemical waste. The two labeled isomers, **24** and **25**, were synthesized to compare the effect of halogens at two different positions, changing from *meta* to *ortho*. Both compounds gave similar results in terms of conversion and d.r., and **25** was isolated with good yield. *p*-Bromo cyclopropane **25** is a precursor for the selective phosphodiesterase type-IV inhibitor, MK0952, and is only three steps away from the final drug.[17] MK0952 was previously synthesized using an enantioselective Simmons-Smith cyclopropanation followed by a Suzuki coupling. Using labeled glycine **26** as the starting material, MK0952 was successfully synthesized with an overall yield of 27% by the enzymatic approach (Scheme 2). The metabolic profile reported by Gallant *et al*[17] indicates that the targeted cyclopropane ring is a metabolically stable position and labeling there would be desirable.

3 | CONCLUSION

In summary, the enzyme-catalyzed cyclopropanation with readily available enzymes was implemented successfully with various styrenes. This transformation was applied to a variety of substituted styrenes and afforded moderate to high levels of diastereoselectivity and enantioselectivity. The reaction could be tuned towards the desired diastereomer by adjusting the concentration of the reaction, the equivalents of EDA (**1**), and/or the amount of enzyme. Since the enzymes were designed for the transformation of 1-octene, further engineering of the enzymes towards substituted styrenes could result in considerably improved d.r. and e.e.

Moreover, a selection of diazoacetates was investigated, and *n*-hexyl diazoacetate (**18**) displayed a higher onset time by DSC, making this a safer alternative to EDA (**1**). Furthermore, *n*-hexyl diazoacetate (**18**) performed well in the biocatalytic system. The biocatalytic strategy was successfully applied to the asymmetric synthesis of labeled cyclopropanation using modified enzymes and ^{13}C-labeled diazoacetate to synthesize ^{13}C-MK0952 with good yield and moderate stereoselectivity.

4 | EXPERIMENTAL

4.1 | General information

All reagents were purchased from commercial suppliers and used without further purification unless mentioned otherwise. Anhydrous solvents were purchased from Sigma-Aldrich and stored under a nitrogen atmosphere. Racemates of the cyclopropane products were synthesized using a literature procedure.[4] For literature known compounds, the analytical data were in agreement with those reported previously. The corresponding literature reference is given for each molecule. Yields are based on styrene (as the limiting reagent) and refer to purified, isolated, homogeneous product and spectroscopically pure material (analyzed by NMR), unless stated otherwise.

Enzymes were obtained from the group of Frances Arnold from the California Institute of Technology as cell-free extracts. Enzyme P411-UA: P411 BM3-CIS L437F T438Q L75Y L181I. Enzyme ApePgb AGW: *A. pernix* protoglobin W59A Y60G F145W.[4]

The M9-N buffer was prepared by dissolving Na_2HPO_4 (3.386 g, 23.85 mmol), KH_2PO_4 (1.497 g, 11.00 mmol), NaCl (251.3 mg, 4.3 mmol), $MgSO_4$ (120.5 mg, 1.00 mmol), and $CaCl_2$ (5.5 mg, 0.05 mmol) in water (400 mL). The pH was adjusted to 6.8 by the addition of phosphoric acid and water was added up to 500 mL.

For each substrate, a 500- or 1000-mM stock solution in ethanol was prepared and used for the enzymatic cyclopropanation. Ethyl diazoacetate in ethanol was prepared by co-evaporation of the commercially available EDA in CH_2Cl_2 with ethanol (2x), without concentration to dryness. The concentration of **1** in EtOH was determined by NMR analysis.

All diazoacetates were stored in CH_2Cl_2; however, for the DSC analysis, the solvent was switched to toluene, due to solvent interference during the analysis. DSC analyses were measured on a DSC3 + by Mettler Toledo using 10–20 mg of diazoacetates in toluene (10–15 wt%) using either of the following gradients:

1. Method A: 5 K/min, 30–300°C
2. Method B: 3 K/min, 30–350°C

All reactions were carried out under a nitrogen atmosphere and were magnetically stirred or shaken. Electric heating plates and DrySyn were used for elevated temperatures, and a stated temperature corresponds to the external DrySyn temperature. Concentration was performed on a rotary evaporator with a heating bath at 40°C, unless stated otherwise.

Crude reaction mixtures were assayed by GC-MS and/or liquid chromatography-MS (LC-MS) or SFC for diastereomeric ratio and enantiomeric ratio, respectively. Diastereomeric ratio (d.r.) (%) is based on the area under the curve (AUC) for the *cis*-product and *trans*-product in GCMS analysis, and e.e. (%) is based on the AUC of the two enantiomers in chiral HPLC or chiral supercritical fluid chromatography (SFC) analysis.[18] Enantiomer 1 is the main enantiomer obtained by the enzyme P411-UA-catalyzed cyclopropanation. The four different enantiomers that could be obtained from the enzyme-catalyzed reaction are displayed in Scheme 3.

GC-MS (EI) analysis was performed on an Agilent 7890A GC system and Agilent 5975C inert MSD system equipped with an Agilent 19091S-433L (30 m × 250 μ × 0.25 μm) capillary column using a gradient: (SHALLOW) 40–150°C with a rate of 15°C/min followed by 150–300°C with a rate of 60°C/min and electron impact ionization at 70 eV or (STANDARD) gradient 40–250°C with a rate of 50°C/min, followed by 250–300°C with a rate of 13°C/min, and electron impact ionization at 70 eV.

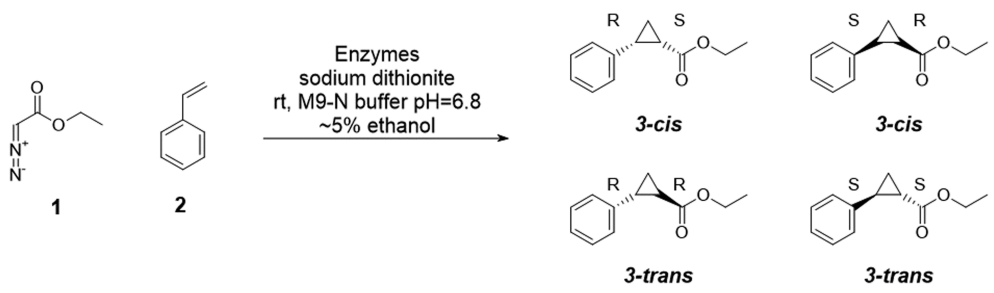
Chiral SFC: Waters Acquity UPC2 - equipped with convergence manager, PDA detector, sample manager, binary solvent manager, and 30S column managers.

Chiral HPLC: Waters Acquity ARC HPLC - equipped with PDA detector, sample Manager FTN-R, Quaternary solvent manager-R, and 30S column manager.

Thin layer chromatography was carried out using E. Merck silica glass plates (60F-254) with UV light (254 nm) and/or potassium permanganate as the visualization agent.

Crude reaction mixtures were purified by either flash chromatography prepacked Biotage SNAP columns (10 g or 25 g) using a Biotage automated flash systems with UV detection, Buchi Sepacore or preparative reversed-phase high-performance liquid chromatography (HPLC) purifications using a Waters 2545 Quaternary Gradient Module equipped with a Waters 2489 UV/Vis detector with an Xbridge Prep OBM C18 10.0 μm, 30 × 250 mm column.

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AVANCE III system running at a proton frequency of 500.1 MHz with a cryogenic probe or on a Bruker Avance Nanobay system at 400.13 MHz and processed with the NMR software MestreNova (Mestrelab Research SL). ^{1}H chemical shifts are referenced relative to the residual solvent peak at 7.26 ppm, and ^{13}C chemical shifts are referenced to 77.16 ppm for $CDCl_3$. Signals are listed in ppm, and



SCHEME 3 General scheme of the reaction to display the four possible enantiomers

multiplicity was identified as s = singlet, br = broad, d = doublet, dt = doublet of triplets, t = triplet, tt = triplet of triplets, q = quartet, quin = quintet, h = hextet, and m = multiplet; coupling constants in Hz; and integration. ^{13}C NMR data are reported as chemical shifts. Purity was calculated with the NMR processing software, MestreNova. Purity assays were also performed on the aforementioned LC-MS and GC-MS systems.

4.2 | Optimization study using EDA (1) and styrene (2)

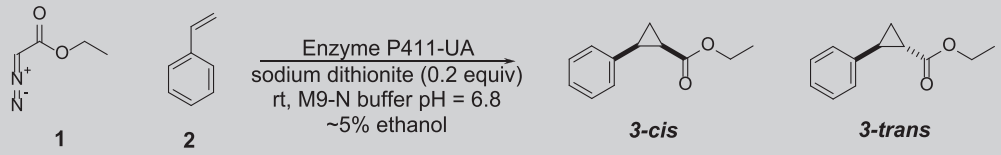


TABLE E1 Overview of the results of enzyme P411-UA-catalyzed cyclopropanation of styrene (**2**)

Entry[a]	1 (equiv)	2 (mM)	Enzyme (mg/mL)	Time (h)	Conversion %	d.r. (cis:trans)
1	1	10	1.3	23	36	3:7
2	2	10	1.3	72	100	1:1
3	4	10	1.3	72	100	3:2
4	8	10	1.3	72	100	4:1
5	2	15	1.9	44	100	1:1
6	2	20	2.6	44	100	3:7
7	2	30	3.9	44	86	2:3
8	2	40	5.2	44	93	3:7
9	2	15	2.8	22	100	7:3
10	2	15	5.1	22	100	7:3

[a]Standard conditions: Enzyme P411-UA, M9-N buffer (pH 6.8), sodium dithionite (0.2 equiv), EDA (**1**), styrene (**2**) (0.08 mmol, 1 equiv), 5% EtOH.

4.3 | General procedure A: Synthesis of alkyl 2-aminoacetates

To a solution of glycine (1 equiv) in toluene (0.27 M) were added *n*-alcohol (4 equiv) and *p*-toluenesulfonic acid (1 equiv). The resulting reaction mixture was heated to reflux (140 °C) with azeotropic removal of water using a Dean Stark apparatus for 4–24 h. Upon completion, the reaction mixture was cooled to room temperature, and Et_2O was added to induce precipitation. The crystals were filtered, washed with Et_2O, and dried under vacuum. Alkyl 2-aminoacetates were obtained as a colorless solid.

4.3.1 | 2-(hexyloxy)-2-oxoethan-1-aminium 4-methylbenzenesulfonate

2-(hexyloxy)-2-oxoethan-1-aminium 4-methylbenzenesulfonate was synthesized according to general procedure A from 3.00 g of glycine. The crude product was purified by recrystallization to provide the desired product as a colorless solid (10.68 g, 81%).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.02 (s, 3H), 7.68–7.73 (m, 2H), 7.08–7.13 (m, 2H), 3.98 (t, *J* = 6.9 Hz, 2H), 3.66 (q, *J* = 5.8 Hz, 2H), 2.33 (s, 3H), 1.44–1.52 (m, 2H), 1.17–1.30 (m, 6H), 0.86 (t, *J* = 7.0 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$): δ = 167.6, 141.2, 140.6, 129.1, 126.1, 66.5, 40.5, 31.5, 28.3, 25.5, 22.6, 21.5, 14.1.

4.3.2 | 2-(hexyloxy)-2-oxoethan-1-aminium-1-^{13}C 4-methylbenzenesulfonate (**27**)

2-(hexyloxy)-2-oxoethan-1-aminium-1-^{13}C 4-methylbenzenesulfonate (**27**) was synthesized according to general procedure A from 1.01 g of 2-^{13}C-glycine. The crude product was purified by recrystallization to provide the desired product as a colorless solid (3.79 g, 86%).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.00 (d, J = 6.3 Hz, 3H), 7.72–7.66 (m, 2H), 7.07 (d, J = 7.9 Hz, 2H), 3.96 (t, J = 6.9 Hz, 2H), 3.78 (q, J = 5.8 Hz, 1H), 3.49 (q, J = 5.7 Hz, 1H), 2.31 (s, 3H), 1.46 (t, J = 7.2 Hz, 2H), 1.29–1.13 (m, 6H), 0.84 (t, J = 7.0 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$): δ = 167.58 (d, J = 62.3 Hz) 141.4, 140.6, 129.0, 126.2, 66.5, 40.5, 31.5, 28.3, 25.5, 22.6, 21.4, 14.1.

4.3.3 | 2-(octyloxy)-2-oxoethan-1-aminium 4-methylbenzenesulfonate

2-(octyloxy)-2-oxoethan-1-aminium 4-methylbenzenesulfonate was synthesized according to general procedure A from 1.50 g of glycine. The crude product was purified by recrystallization to provide the desired product as a colorless solid (2.72 g, 38%).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.03 (t, J = 5.8 Hz, 3H), 7.69–7.75 (m, 2H), 7.11 (d, J = 8.0 Hz, 2H), 3.99 (t, J = 6.9 Hz, 2H), 3.66 (q, J = 5.8 Hz, 2H), 2.33 (s, 3H), 1.49 (p, J = 6.8 Hz, 2H), 1.17–1.34 (m, 10H), 0.88 (t, J = 7.0 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$): δ = 167.6, 141.4, 140.6, 129.1, 126.2, 66.5, 40.5, 31.9, 29.4, 29.3, 28.4, 25.8, 22.8, 21.5, 14.2.

4.3.4 | 2-(decyloxy)-2-oxoethan-1-aminium 4-methylbenzenesulfonate

2-(decyloxy)-2-oxoethan-1-aminium 4-methylbenzenesulfonate was synthesized according to general procedure A from 1.50 g of glycine. The crude product was purified by recrystallization to provide the desired product as a colorless solid (2.57 g, 33%).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.02 (t, J = 5.8 Hz, 3H), 7.72 (d, J = 8.1 Hz, 2H), 7.10 (d, J = 7.9 Hz, 2H), 3.99 (t, J = 6.9 Hz, 2H), 3.66 (q, J = 5.8 Hz, 2H), 2.33 (s, 3H), 1.49 (p, J = 6.7 Hz, 2H), 1.34–1.18 (m, 14H), 0.88 (t, J = 6.9 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$): δ = 167.6, 141.4, 140.5, 129.0, 126.2, 66.5, 40.5, 32.0, 29.7, 29.7, 29.5, 29.4, 28.4, 25.9, 22.8, 21.5, 14.9.

4.4 | General procedure B: Alkyl 2-diazoacetates

A biphasic mixture of alkyl 2-aminoacetate (1 equiv) in CH_2Cl_2 and water (1:1, 0.33 M) at 0 °C was stirred as sodium nitrite (1.5 equiv, 4.55 M in H_2O) was added dropwise over a period of 5 min, and the reaction mixture was warmed to room temperature. Upon completion, sat. aq. $NaHCO_3$ was added, and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (3x), and the combined organic layers were dried over $MgSO_4$, filtered, and partly concentrated in vacuo. The crude yellow oil was purified by flash column chromatography on silica gel (10–15% Et_2O in n-pentane) to give the alkyl 2-diazoacetate as a yellow solution. The product was stored as a solution in CH_2Cl_2.

4.4.1 | n-Hexyl 2-diazoacetate (**18**)

n-Hexyl 2-diazoacetate (**18**) was synthesized according to general procedure B from 2.65 g of 2-(hexyloxy)-2-oxoethan-1-aminium 4-methylbenzenesulfonate to give the desired product as a yellow solution in CH_2Cl_2 (0.556 g, 41%). The product was stored as solution in CH_2Cl_2.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.73 (s, 1H), 4.15 (t, J = 6.8 Hz, 2H), 1.63 (dq, J = 8.0, 6.6 Hz, 2H), 1.39–1.25 (m, 6H), 0.92–0.86 (m, 3H).

^{13}C NMR (126 MHz, $CDCl_3$): δ = 167.0, 65.2, 46.2, 31.5, 28.9, 25.6, 22.6, 14.1.

4.4.2 | n-Hexyl 2-diazoacetate-2-^{13}C (^{13}C-18)

n-Hexyl 2-diazoacetate-2-^{13}C (^{13}C-**18**) was synthesized according to general procedure B from 668.0 mg of 2-(hexyloxy)-2-oxoethan-1-aminium-1-^{13}C 4-methylbenzenesulfonate to give the desired product as a yellow solution in CH_2Cl_2 (320.0 mg, 93%). The product was stored as a solution in CH_2Cl_2.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.70 (d, J = 202.8 Hz, 1H), 4.12 (t, J = 6.7 Hz, 2H), 1.66–1.57 (m, 2H), 1.36–1.24 (m, 6H), 0.86 (t, J = 6.7 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$): δ = 166.8 (d, J = 76.2 Hz), 65.2, 46.3, 31.6, 28.9, 25.6, 22.7, 14.1.

4.4.3 | n-Octyl 2-diazoacetate (**19**)

n-Octyl 2-diazoacetate (**19**) was synthesized according to general procedure B from 719.0 mg of 2-(octyloxy)-2-oxoethan-1-aminium 4-methylbenzenesulfonate to give the desired product as yellow solution in CH_2Cl_2 (81.5 mg, 21%). The product was stored as a solution in CH_2Cl_2.

^{1}H NMR (500 MHz, $CDCl_3$) δ 4.70 (s, 1H), 4.12 (t, J = 6.7 Hz, 2H), 1.67–1.55 (m, 2H), 1.35–1.19 (m, 10H), 0.89–0.82 (m, 3H).

^{13}C NMR (126 MHz, $CDCl_3$): δ = 167.1, 65.2, 46.3, 31.9, 29.3, 29.3, 28.9, 26.0, 22.8, 14.2.

4.4.4 | *n*-Decyl 2-diazoacetate (**21**)

n-Decyl 2-diazoacetate (**21**) was synthesized according to general procedure B from 775.0 mg of 2-(decyloxy)-2-oxoethan-1-aminium 4-methylbenzenesulfonate to give the desired product as yellow solution in CH_2Cl_2 (60.0 mg, 13%). The product was stored as a solution in CH_2Cl_2.

^{1}H NMR (500 MHz, $CDCl_3$) δ 4.70 (s, 1H), 4.12 (t, J = 6.8 Hz, 2H), 1.65–1.57 (m, 2H), 1.35–1.19 (m, 14H), 0.85 (t, J = 6.9 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$): δ = 167.0, 65.2, 46.3, 32.0, 29.7, 29.7, 29.4, 29.4, 28.9, 26.0, 22.8, 14.3.

4.5 | General procedure C: Enzymatic cyclopropanation

Enzyme P411-Ua/ApePgb AGW (150 mg) was added to a reaction tube which was sealed and flushed with N_2 for at least 30 min. Under N_2 flow were added M9-N buffer (28.5 mL), sodium dithionite (4.5 mL, 0.11 mmol, 500 mM stock solution, 0.2 equiv), styrene (1.05 mL, 0.53 mmol, 500 mM stock solution, 1 equiv), and EDA (**1**) (1.05 mmol, 1306 mM stock solution, 2.00 equiv) with 6% EtOH as co-solvent. The N_2 flow was removed, and the reaction tube was shaken at room temperature for 24 h. The reaction was acidified by the addition of HCl (3 M, 0.7 mL), after which the pH was adjusted to 3. EtOAc (10 mL) was added to the reaction mixture, which was filtered through a pad of Celite to remove the enzyme and to break the emulsion. The Celite pad was rinsed with additional water (2 × 10 mL) and EtOAc (2 × 50 mL). The layers were separated, and the aqueous layer wasextracted with EtOAc (2 × 100 mL). The combined organic layers were dried over sodium sulfate and filtered. The organic solvents were partly removed in vacuo.

4.5.1 | Ethyl 2-phenylcyclopropane-1-carboxylate (**3**)[19]

Ethyl 2-phenylcyclopropane-1-carboxylate (**3**) was synthesized according to general procedure C from 54.7 mg of styrene. The crude product was purified by flash column chromatography on silica gel (2–20% Et_2O in *n*-pentane) to provide the scalemic mixture as a colorless oil.

Chiral HPLC was used for the e.e. determination: Chiralcel OJ column (150 × 4.6 mm, 3 μm) with 10–40% EtOH in heptane as eluent over 10 min with flow of 0.8 mL/min, wavelength 230/235 nm.

Enzyme P411-UA: 9.0 mg, 9%, d.r. (*cis*: *trans*) = 53:47, e.e. (*cis*) = 61%, e.e. (*trans*) = ND.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.24–7.29 (m, 6H), 7.17–7.23 (m, 2H), 7.07–7.12 (m, 2H), 4.17 (q, J = 7.1 Hz, 2H), 3.87 (q, J = 7.2 Hz, 2H), 2.55–2.63 (m, 1H), 2.48–2.55 (m, 1H), 2.08 (ddd, J = 9.3, 7.8, 5.7 Hz, 1H), 1.90 (ddd, J = 8.4, 5.3, 4.2 Hz, 1H), 1.71 (ddd, J = 7.5, 5.6, 5.1 Hz, 1H), 1.51–1.64 (m, 1H), 1.22–1.38 (m, 7H), 0.97 (t, J = 7.1 Hz, 3H).

Enzyme ApePgb AGW: 5.3 mg, 5%, d.r. (*cis*:*trans*) = 66:34, e.e. (*cis*) = 82%, e.e. (*trans*) = 71%.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.26–7.31 (m, 3H), 7.16–7.26 (m, 3H), 7.07–7.12 (m, 1H), 4.17 (q, J = 7.1 Hz, 1H), 3.87 (q, J = 7.1 Hz, 2H), 2.48–2.64 (m, 2H), 2.08 (ddd, J = 9.3, 7.8, 5.6 Hz, 1H), 1.90 (ddd, J = 8.4, 5.3, 4.2 Hz, 1H), 1.71 (ddd, J = 7.5, 5.6, 5.0 Hz, 1H), 1.60 (ddd, J = 9.2, 5.3, 4.5 Hz, 1H), 1.24–1.36 (m, 5H), 0.97 (t, J = 7.1 Hz, 3H).

4.5.2 | Ethyl 2-(4-methoxyphenyl) cyclopropane-1-carboxylate (**4**)[19]

Ethyl 2-(4-methoxyphenyl)cyclopropane-1-carboxylate (**4**) was synthesized according to general procedure C from 70.4 mg of 1-methoxy-4-vinylbenzene. The crude product was purified by flash column chromatography on silica gel (2–20% EtOAc in *n*-heptane) to provide the scalemic mixture as a pale-yellow solid.

Chiral SFC was used for the e.e. determination: Lux C4 column (150 × 4.6 mm, 3 μm) with 1% IPA in CO_2 (120 bar) as mobile phase, wavelength 230 nm.

Enzyme P411-UA: 54.5 mg, 47%, d.r. (*cis*:*trans*) = 81:19, e.e. (*cis*) = 92%, e.e. (*trans*) = 43%.

Enzyme ApePgB AGW: 28.9 mg, 25%, d.r. (*cis*:*trans*) = 54:46, e.e. (*cis*) = 87%, e.e. (*trans*) = 75%.

4.5.3 | Ethyl 2-(3-bromophenyl) cyclopropane-1-carboxylate (**5**)[20]

Ethyl 2-(3-bromophenyl)cyclopropane-1-carboxylate (**5**) was synthesized according to general procedure C from 96.0 mg of 1-bromo-3-vinylbenzene. The crude product was purified by flash column chromatography on silica gel (2–20% EtOAc in *n*-heptane) to provide the scalemic mixture as a colorless oil.

Chiral SFC was used for the e.e. determination: Lux C4 column (150 × 4.6 mm, 3 μm) with 1% IPA in CO_2 (120 bar) as mobile phase, wavelength 220 nm.

Enzyme P411-UA: 31.2 mg, 22%, d.r. (*cis*:*trans*) = 66:34, e.e. (*cis*) = 62%, e.e. (*trans*) = 0%.

Enzyme ApePgB AGW: 53.7 mg, 38%, d.r. (*cis*:*trans*) = 87:13, e.e. (*cis*) = 93%, e.e. (*trans*) = 93%.

4.5.4 | Ethyl 2-(4-chlorophenyl) cyclopropane-1-carboxylate (**6**)[19]

Ethyl 2-(4-chlorophenyl)cyclopropane-1-carboxylate (**6**) was synthesized according to general procedure C from 72.8 mg of 1-chloro-4-vinylbenzene. The crude product was purified by flash column chromatography on silica gel (2–20% EtOAc in *n*-heptane) to provide the scalemic mixture as a colorless oil.

Chiral SFC was used for the e.e. determination: Lux A1 (AD) column (150 × 4.6 mm, 3 μm) with 3% IPA in CO_2 (120 bar) as mobile phase, wavelength 224/235 nm.

Enzyme P411-UA: 64.6 mg, 54%, d.r. (*cis*:*trans*) = 85:15, e.e. (*cis*) = 94%, e.e. (*trans*) = 42%.

Enzyme ApePgb AGW: 35.5 mg, 30%, d.r. (*cis*:*trans*) = 51:49, e.e. (*cis*) = 83%, e.e. (*trans*) = 74%.

4.5.5 | Ethyl 2-(4-cyanophenyl) cyclopropane-1-carboxylate (**7**)[21]

Ethyl 2-(4-cyanophenyl)cyclopropane-1-carboxylate (**7**) was synthesized according to general procedure C from 67.8 mg of 4-vinylbenzonitrile. The crude product was purified by flash column chromatography on silica gel (2–20% EtOAc in *n*-heptane) to provide the scalemic mixture as a colorless oil.

Chiral SFC was used for the e.e. determination: Lux A1 (AD) column (150 × 4.6 mm, 3 μm) with 5% EtOH in CO_2 (120 bar) as mobile phase, wavelength 220/235 nm.

Enzyme P411-UA: 33.0 mg, 29%, d.r. (*cis*:*trans*) = 71:29, e.e. (*cis*) = 92%, e.e. (*trans*) = 13%.

Enzyme ApePgb AGW: 25.4 mg, 22%, d.r. (*cis*:*trans*) = 33:67, e.e. (*cis*) = 90%, e.e. (*trans*) = 86%.

4.5.6 | Ethyl 2-(4-(trifluoromethyl)phenyl) cyclopropane-1-carboxylate (**8**)[22]

Ethyl 2-(4-(trifluoromethyl)phenyl)cyclopropane-1-carboxylate (**8**) was synthesized according to general procedure C from 90.0 mg of 1-(trifluoromethyl)-4-vinylbenzene. The crude product was purified by flash column chromatography on silica gel (2–20% EtOAc in *n*-heptane) to provide the scalemic mixture as a colorless oil.

Chiral HPLC was used for the e.e. determination: Chiralcel OJ column (150 × 4.6 mm, 3 μm) with 2% EtOH in heptane as eluent for 14.5 min with flow of 0.5 mL/min, wavelength 235 nm.

Enzyme P411-UA: 7.5 mg, 5%, d.r. (*cis*:*trans*) = 73:27, e.e. (*cis*) = 94%, e.e. (*trans*) = 9%.

Enzyme ApePgb AGW: 17.1 mg, 33%, d.r. (*cis*:*trans*) = 44:56, e.e. (*cis*) = 85%, e.e. (*trans*) = 66%.

4.6 | General procedure D: Enzymatic cyclopropanation

Enzyme ApePgb AGW (5.6 mg) was added into a round bottom flask which was sealed and flushed with N_2 for 10 min. Under N_2 flow were added M9-N buffer (4.3 mL), sodium dithionite (425 μL, 20.00 μmol, 47.1 mM stock solution, 0.2 equiv), styrene (0.100 mL, 100 μmol, 1000 mM stock solution, 1 equiv), and the diazo acetate (173 μL, 300 μmol, 1156 mM stock solution, 2 equiv) with 5% EtOH as a co-solvent. The reaction was shaken at room temperature overnight. After completion, CH_2Cl_2 (4 mL) was added, the layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were dried over sodium sulphate, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography (2–20% EtOAc in *n*-heptane) to afford the desired product.

4.6.1 | Hexyl 2-phenylcyclopropane-1-carboxylate (**21**)[23]

Hexyl 2-phenylcyclopropane-1-carboxylate (**21**) was synthesized according to general procedure D from 10.4 mg of styrene to provide the scalemic mixture as a colorless oil.

Enzyme ApePgb AGW: 1.3 mg, 5%, d.r. (*cis*:*trans*) = 51:49, e.e. (*cis*) = ND, e.e. (*trans*) = ND^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.32–7.23 (m, 5H), 7.19 (ddt, *J* = 8.4, 6.5, 2.6 Hz, 2H), 7.12–7.08 (m, 2H), 4.10 (t, *J* = 6.8 Hz, 2H), 3.88–3.73 (m, 1H), 2.57 (td, J = 9.0, 7.5 Hz, 1H), 2.51 (ddd, J = 9.3, 6.5, 4.1 Hz, 1H), 2.08 (ddd, J = 9.3, 7.8, 5.6 Hz, 1H), 1.90 (ddd, *J* = 8.4, 5.3, 4.1 Hz, 1H), 1.73–1.57 (m, 4H), 1.41–1.22 (m, 14H), 1.22–1.10 (m, 3H), 0.91–0.85 (m, 6H).

4.6.2 | Hexyl 2-(4-methoxyphenyl) cyclopropane-1-carboxylate (**22**)

Hexyl 2-(4-methoxyphenyl)cyclopropane-1-carboxylate (**22**) was synthesized according to general procedure D

from 13.4 mg of 1-methoxy-4-vinylbenzene to provide the scalemic mixture as a colorless oil.

Enzyme ApePgb AGW: 4.0 mg, 14%, d.r. (*cis*:*trans*) = 42:58, e.e. (*cis*) = ND, e.e. (*trans*) = ND

22-cis

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.17–7.13 (m, 2H), 6.80–6.74 (m, 2H), 3.86–3.76 (m, 2H), 3.75 (s, 3H), 2.49 (q, *J* = 8.5 Hz, 1H), 2.01 (ddd, *J* = 9.2, 7.8, 5.6 Hz, 1H), 1.62 (dt, *J* = 7.5, 5.3 Hz, 1H), 1.38–1.30 (m, 2H), 1.30–1.20 (m, 3H), 1.20–1.10 (m, 4H), 0.84 (t, *J* = 7.2 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$): δ = 171.4, 158.5, 130.4, 128.7, 113.5, 64.6, 55.3, 31.6, 28.7, 25.6, 25.0, 22.6, 21.9, 14.2, 11.4.

22-trans

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.03–6.99 (m, 2H), 6.82–6.77 (m, 2H), 4.07 (t, *J* = 6.8 Hz, 2H), 3.76 (s, 3H), 2.45 (ddd, *J* = 9.2, 6.5, 4.2 Hz, 1H), 1.80 (ddd, *J* = 8.4, 5.2, 4.2 Hz, 1H), 1.65–1.57 (m, 2H), 1.54–1.50 (m, 1H), 1.38–1.25 (m, 6H), 1.22 (ddd, *J* = 8.4, 6.6, 4.5 Hz, 1H), 0.90–0.84 (m, 3H).

^{13}C NMR (126 MHz, $CDCl_3$): δ = 173.8, 158.5, 132.3, 127.5, 114.1, 65.0, 55.5, 31.6, 28.8, 25.8, 25.7, 24.1, 22.7, 16.9, 14.2.

4.6.3 | Hexyl 2-(4-chlorophenyl) cyclopropane-1-carboxylate (**23**)

Hexyl 2-(4-chlorophenyl)cyclopropane-1-carboxylate (**23**) was synthesized according to general procedure D from 13.9 mg of 1-chloro-4-vinylbenzene to provide the scalemic mixture as a colorless oil.

Chiral SFC was used for the e.e. determination: Whelk-O1 column (150 × 4.6 mm, 3 μm) with 1% IPA/DEA (10:2) in CO_2 (120 bar) as mobile phase, wavelength 230 nm.

Enzyme ApePgb AGW: 2.1 mg, 7%, d.r. (*cis*:*trans*) = 30:70, e.e. (*cis*) = 81%, e.e. (*trans*) = 2%

23-cis

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.22–7.13 (m, 4H), 3.81 (qt, *J* = 10.8, 6.7 Hz, 2H), 2.49 (q, *J* = 8.5 Hz, 1H), 2.06 (ddd, *J* = 9.2, 7.9, 5.7 Hz, 1H), 1.64 (dt, *J* = 7.5, 5.4 Hz, 1H), 1.37–1.27 (m, 4H), 1.27–1.19 (m, 2H), 1.17–1.10 (m, 3H), 0.85 (t, *J* = 7.2 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$): δ = 171.0, 135.3, 132.6, 130.7, 128.2, 64.7, 31.5, 28.7, 25.6, 25.0, 22.6, 22.1, 14.2, 11.5.

23-trans

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.23–7.19 (m, 2H), 7.03–6.97 (m, 2H), 4.08 (td, *J* = 6.8, 1.2 Hz, 2H), 2.46 (ddd, *J* = 9.2, 6.5, 4.2 Hz, 1H), 1.84 (ddd, *J* = 8.4, 5.3, 4.2 Hz, 1H), 1.69–1.55 (m, 3H), 1.39–1.21 (m, 7H), 0.87 (td, *J* = 6.9, 2.3 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$): δ = 173.4, 138.8, 132.6, 128.7, 127.7, 65.2, 31.6, 28.8, 25.7, 25.6, 24.3, 22.7, 17.2, 14.1.

4.6.4 | Hexyl 2-(5-bromo-2-fluorophenyl) cyclopropane-1-carboxylate-1-^{13}C (**24**)

Hexyl 2-(5-bromo-2-fluorophenyl)cyclopropane-1-carboxylate-1-^{13}C (**24**) was synthesized according to general procedure C from 101.0 mg of 4-bromo-1-fluoro-2-vinylbenzene. The crude product was purified by preparative HPLC (50–95% MeCN in H_2O/NH_4OH 99.8/0.2 buffer over 13 min with a flow of 40 mL/min, UV = 230 nm) to provide separated diastereomers as a colorless oil.

d.r. (*cis*:*trans*) = 36:64.

Chiral SFC was used for the e.e. determination: Whelk-O1 column (150 × 4.6 mm, 5 μm) with 1% IPA/DEA (10:2) in CO_2 (120 bar) as mobile phase, wavelength 276 nm.

24-cis

9.7 mg, 6%, e.e. (*cis*) = ND

^{1}H NMR (400 MHz, CDCl3): δ = 7.34 (ddd, *J* = 6.6, 2.5, 0.9 Hz, 1H), 7.30–7.25 (m, 1H), 6.84 (dd, *J* = 9.5, 8.7 Hz, 1H), 3.86 (qt, *J* = 10.8, 6.7 Hz, 2H), 2.48–2.39 (m, 1H), 2.13 (dddd, *J* = 168.5, 9.0, 8.0, 5.7 Hz, 1H), 1.58 (dtd, *J* = 7.5, 5.4, 3.3 Hz, 1H), 1.45–1.33 (m, 3H), 1.30–1.13 (m, 6H), 0.85 (t, *J* = 7.0 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$): δ = 171.1 (d, $J_{C\text{-}C}$ = 76.8 Hz), 161.6 (d, $J_{C\text{-}F}$ = 247.0 Hz), 134.1 (dd, $J_{C\text{-}F}$ = 4.0, 1.7 Hz), 131.4 (d, $J_{C\text{-}F}$ = 8.3 Hz), 126.8, 126.62 (d, $J_{C\text{-}F}$ = 2.0 Hz), 116.7 (d, $J_{C\text{-}F}$ = 23.3 Hz), 116.1 (d, $J_{C\text{-}F}$ = 3.4 Hz), 64.9, 31.6, 28.6, 25.6, 22.6, 21.1, 19.4 (dd, $J_{C\text{-}C}$ = 8.7, 3.0 Hz), 14.2, 11.4 (d, $J_{C\text{-}C}$ = 12.6 Hz).

24-trans

20.6 mg, 12%, e.e. (*trans*) = 34%

^{1}H NMR (400 MHz, CDCl3): δ = 7.31–7.26 (m, 1H), 7.07 (dd, *J* = 6.6, 2.5 Hz, 1H), 6.91 (dd, *J* = 9.7, 8.7 Hz, 1H), 4.12 (t, *J* = 6.7 Hz, 2H), 2.61 (dddd, *J* = 9.3, 6.8, 4.3, 2.8 Hz, 1H), 1.93 (dddd, *J* = 168.9, 8.4, 5.4, 4.3 Hz, 1H), 1.57–1.68 (m, 3H), 1.40–1.28 (m, 7H), 0.95–0.85 (m, 3H).

^{13}C NMR (101 MHz, CDCl3): δ = 173.1 (d, $J_{C\text{-}C}$ = 76.5 Hz), 160.9 (d, $J_{C\text{-}F}$ = 246.8 Hz), 133.8, 130.9 (d, $J_{C\text{-}F}$ = 8.2 Hz), 130.1 (dd, $J_{C\text{-}F}$ = 4.0, 2.0 Hz), 129.7 (d, $J_{C\text{-}F}$ = 14.7 Hz), 117.3 (d, $J_{C\text{-}F}$ = 23.6 Hz), 116.7 (d, $J_{C\text{-}F}$ = 3.4 Hz), 65.3, 31.6, 28.8, 25.7, 23.0 (d, $J_{C\text{-}C}$ = 1.3 Hz), 21.1, 19.6 (dd, $J_{C\text{-}C}$ = 10.6, 4.5 Hz), 15.8 (d, $J_{C\text{-}C}$ = 11.4 Hz), 14.1.

4.6.5 | Hexyl 2-(5-bromo-2-fluorophenyl) cyclopropane-1-carboxylate-1-^{13}C (**25**)

Hexyl 2-(5-bromo-2-fluorophenyl)cyclopropane-1-carboxylate-1-^{13}C (**25**) was synthesized according to general procedure C from 101.0 mg of 4-bromo-2-fluoro-1-vinylbenzene. The crude product was purified by preparative HPLC (50–95% MeCN in H_2O/NH_4OH 99.8/0.2 buffer over 13 min with a flow of 40 mL/min, UV = 230 nm) to provide separated diastereomers as a colorless oil.

d.r. (*cis*:*trans*) = 39:61.

Chiral SFC was used for the e.e. determination: Whelk-O1 column (150 × 4.6 mm, 3 μm) with 3% IPA/DEA (10:2) in CO_2 (120 bar) as mobile phase, wavelength 240 nm.

25-cis

41.6 mg, 24%, e.e. (*cis*) = 66%

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.24–7.08 (3H, m), 6.59–5.86 (1H, m), 3.95–3.82 (2H, m), 2.48–2.40 (1H, m), 2.15 (1H, dddd), 1.66 (3H, dd), 1.46–1.32 (6H, m), 0.88–0.85 (3H, m).

^{13}C NMR (101 MHz, $CDCl_3$): δ = 171.0 (d, $J_{C\text{-}C}$ = 76.8 Hz), 162.1 (d, $J_{C\text{-}F}$ = 250.9 Hz), 133.6, 132.0 (dd, $J_{C\text{-}F}$ = 4.4, 1.7 Hz), 129.7, 126.8 (d, $J_{C\text{-}F}$ = 3.7 Hz), 123.4 (dd, $J_{C\text{-}F}$ = 14.8, 2.1 Hz), 120.6 (d, $J_{C\text{-}F}$ = 9.4 Hz), 118.4 (d, $J_{C\text{-}F}$ = 25.1 Hz), 64.7, 31.4, 28.5, 25.4, 22.5, 19.1 (dd, $J_{C\text{-}C}$ = 8.5, 3.2 Hz), 14.0, 11.1 (d, $J_{C\text{-}C}$ = 13.1 Hz).

25-trans

80.2 mg, 47%, e.e. (*trans*) = 42%

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.20 (dt, *J* = 9.7, 2.3 Hz, 2H), 6.84 (t, *J* = 8.2 Hz, 1H), 4.12 (t, *J* = 6.7 Hz, 2H), 2.59 (dddd, *J* = 9.4, 6.9, 4.3, 2.8 Hz, 1H), 2.17–1.54 (m, 4H), 1.43–1.23 (m, 7H), 0.94–0.85 (m, 3H).

^{13}C NMR (101 MHz, $CDCl_3$): δ = 173.2 (d, $J_{C\text{-}C}$ = 76.4 Hz), 161.6 (d, $J_{C\text{-}F}$ = 251.3, 1.3 Hz), 128.3 (d, $J_{C\text{-}F}$ = 4.7, 2.0 Hz), 127.5 (d, $J_{C\text{-}F}$ = 3.7 Hz), 126.6 (d, $J_{C\text{-}F}$ = 14.1, 1.3 Hz), 123.2, 120.2 (d, $J_{C\text{-}F}$ = 9.5 Hz), 119.1 (d, $J_{C\text{-}F}$ = 25.2 Hz), 65.3, 31.6, 28.7, 25.7, 22.9, 22.7, 19.7 (dd, $J_{C\text{-}C}$ = 10.7, 4.2 Hz), 15.6 (dd, $J_{C\text{-}C}$ = 11.4, 1.6 Hz), 14.1.

4.6.6 | Hexyl (1S,2S)-2-(2-fluoro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) phenyl)cyclopropane-1-carboxylate-1-^{13}C

To a microwave vial was added ***25-trans*** (56.5 mg, 0.16 mmol, 1 equiv), *bis* (pinacolato)diboron (45.8 mg, 0.18 mmol, 1.1 equiv), Pd (dppf)Cl_2 (12.01 mg, 0.02 mmol, 0.1 equiv), and KOAc (48.3 mg, 0.49 mmol, 3 equiv). The vial was capped and evacuated/backfilled with N_2 (3×) before the addition of 1,4-dioxane (3 mL). The reaction mixture was place into the microwave (120 °C, 1.5 h). Upon cooling to room temperature, the reaction mixture was diluted with water (4 mL) and filtered through a pad of Celite. The layers were separated, and the aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude brown oil was purified by flash column chromatography on silica gel (5% EtOAc in *n*-heptane) to give hexyl (1S,2S)-2-(2-fluoro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)cyclopropane-1-carboxylate-1-^{13}C (40.7 mg, 63%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.50–7.40 (m, 2H), 6.94 (t, *J* = 7.4 Hz, 1H), 4.11 (t, *J* = 6.7 Hz, 2H), 2.68 (dddd, *J* = 9.4, 6.8, 4.2, 2.8 Hz, 1H), 2.20–1.71 (m, 1H), 1.62 (ddd, *J* = 13.0, 8.9, 5.7 Hz, 3H), 1.33 (s, 19H), 0.93–0.85 (m, 3H).

^{13}C NMR (101 MHz, $CDCl_3$): δ = 173.4 (d, $J_{C\text{-}C}$ = 76.4 Hz), 161.5 (d, $J_{C\text{-}F}$ = 247.2 Hz), 130.5 (d, $J_{C\text{-}F}$ = 3.5 Hz), 126.3 (dd, $J_{C\text{-}F}$ = 3.4, 1.9 Hz), 121.2 (d, $J_{C\text{-}F}$ = 20.4 Hz), 84.2, 65.2, 31.6, 28.8, 25.7, 25.0, 23.2 (d, $J_{C\text{-}C}$ = 1.4 Hz), 22.9, 20.2 (dd, $J_{C\text{-}C}$ = 10.5, 4.8 Hz), 16.0 (d, $J_{C\text{-}C}$ = 11.2 Hz), 14.1.

4.6.7 | Hexyl (1R,2S)-2-(3′-(3-(cyclopropylcarbamoyl)-4-oxo-1,8-naphthyridin-1(4H)-yl)-3-fluoro-[1,1′-biphenyl]-4-yl)cyclopropane-1-carboxylate-1-^{13}C

To a microwave vial was added hexyl (1S,2S)-2-(2-fluoro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl) cyclopropane-1-carboxylate-1-13C (40.7 mg, 0.10 mmol, 1 equiv), 1-(3-bromophenyl)-N-cyclopropyl-4-oxo-1,-4-dihydro-1,8-naphthyridine-3-carboxamide (44.0 mg, 0.11 mmol, 1.1 equiv), cesium carbonate (50.8 mg, 0.16 mmol, 1.5 equiv), and Pd (PPh_3)$_4$ (12.02 mg, 10.4 μmol, 0.1 equiv). The vial was capped and evacuated/backfilled with N_2 (3×) before the addition of DMF (3 mL). The reaction mixture was place into the microwave (120 °C, 1.5 h). Upon cooling to room temperature, the reaction mixture was diluted with water (4 mL) and EtOAc (4 mL) and filtered through a pad of Celite. The layers were separated, and the aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude orange oil was purified by preparative HPLC (50–95% MeCN in H_2O/NH_4OH 99.8/0.2 buffer over 17 minutes with a flow of 40 mL/min, UV = 254 nm) to provide hexyl (1R,2S)-2-(3′-(3-(cyclopropylcarbamoyl)-4-oxo-1,8-naphthyridin-1(4H)-yl)-3-fluoro-[1,1′-biphenyl]-4-yl)cyclopropane-1-carboxylate-1-^{13}C (42.8 mg, 72%) as a colorless solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.78 (d, J = 4.1 Hz, 1H), 9.05 (s, 1H), 8.80 (dd, J = 8.0, 2.0 Hz, 1H), 8.69 (dd, J = 4.5, 2.0 Hz, 1H), 7.71 (dt, J = 7.8, 1.4 Hz, 1H), 7.67–7.56 (m, 2H), 7.49–7.38 (m, 2H), 7.34–7.24 (m, 2H), 7.03 (t, J = 7.8 Hz, 1H), 4.11 (t, J = 6.7 Hz, 2H), 2.97 (tq, J = 7.7, 4.0 Hz, 1H), 2.67 (ddt, J = 9.5, 6.8, 3.3 Hz, 1H), 1.97 (ddt, J = 168.9, 9.2, 4.9 Hz, 1H), 1.63 (ddt, J = 11.3, 9.0, 5.7 Hz, 3H), 1.41–1.25 (m, 7H), 0.93–0.80 (m, 5H), 0.69–0.61 (m, 2H).

^{13}C NMR (101 MHz, $CDCl_3$): δ = 177.3, 173.2 (d, $J_{C\text{-}C}$ = 76.4 Hz), 165.5, 162.0 (d, $J_{C\text{-}F}$ = 246.8 Hz), 153.3, 150.1, 148.5, 141.4 (d, $J_{C\text{-}F}$ = 2.0 Hz), 141.0, 139.6 (d, $J_{C\text{-}F}$ = 7.8 Hz), 136.5, 130.2, 128.0, 127.6 (dd, $J_{C\text{-}F}$ = 4.6, 1.9 Hz), 127.2 (d, $J_{C\text{-}F}$ = 14.0 Hz), 126.6, 126.0, 122.8 (d, $J_{C\text{-}F}$ = 3.3 Hz), 122.2, 121.5, 114.1 (d, $J_{C\text{-}F}$ = 23.2 Hz), 113.4, 65.2, 31.5, 28.7, 25.6, 23.1 (d, $J_{C\text{-}C}$ = 1.2 Hz), 22.9 (d, $J_{C\text{-}C}$ = 1.3 Hz), 22.6, 19.8 (dd, $J_{C\text{-}C}$ = 10.5, 4.3 Hz), 15.8 (d, $J_{C\text{-}C}$ = 11.3 Hz), 14.1, 6.7.

4.6.8 | (1R,2S)-2-(3′-(3-(cyclopropylcarbamoyl)-4-oxo-1,8-naphthyridin-1(4H)-yl)-3-fluoro-[1,1′-biphenyl]-4-yl)cyclopropane-1-carboxylic-1-^{13}C acid (MK0952)

To a solution of hexyl (1R,2S)-2-(3i′-(3-(cyclopropylcarbamoyl)-4-oxo-1,8-naphthyridin-1(4H)-yl)-3-fluoro-[1,1′-biphenyl]-4-yl)cyclopropane-1-carboxylate-1-^{13}C (18.4 mg, 32.0 μmol, 1 equiv) in THF/MeOH (1:1, 0.3 mL) was added aqueous NaOH solution (2 M, 81 μL, 160.0 μmol, 5 equiv). The reaction mixture was stirred at room temperature overnight. The solvents were removed under reduced pressure, and the residue was dissolved in water (2 mL). The solution was transferred to a phase separator and washed with CH_2Cl_2 (20 mL). The aqueous layer was acidified by the addition of aqueous HCl solution (2 M, 0.5 mL) and extracted with CH_2Cl_2 (30 mL). The organic layer was concentrated in vacuo to afford MK0952 (15.1 mg, 96%) as a yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.89 (d, J = 4.0 Hz, 1H), 9.11 (s, 1H), 8.82 (dd, J = 8.0, 2.0 Hz, 1H), 8.72 (dd, J = 4.5, 1.9 Hz, 1H), 7.73 (dt, J = 7.9, 1.4 Hz, 1H), 7.69–7.60 (m, 2H), 7.48 (dd, J = 8.0, 4.5 Hz, 1H), 7.43 (ddd, J = 7.7, 2.2, 1.1 Hz, 1H), 7.37–7.28 (m, 2H), 7.02 (t, J = 7.8 Hz, 1H), 3.00 (tq, J = 7.7, 4.0 Hz, 1H), 2.68 (ddt, J = 10.0, 6.9, 3.4 Hz, 1H), 1.95 (ddt, J = 169.6, 9.1, 4.8 Hz, 1H), 1.64 (dq, J = 8.9, 4.4 Hz, 1H), 1.41 (dddd, J = 8.4, 6.5, 4.6, 1.5 Hz, 1H), 0.86 (td, J = 7.1, 5.1 Hz, 3H), 0.72–0.64 (m, 2H).

^{13}C NMR (101 MHz, $CDCl_3$): δ = 177.6 (d, $J_{C\text{-}C}$ = 74.1 Hz), 177.4, 165.9, 162.1 (d, $J_{C\text{-}F}$ = 247.6 Hz), 153.4, 150.2, 148.9, 141.5 (d, $J_{C\text{-}F}$ = 2.0 Hz), 141.0, 139.8 (d, $J_{C\text{-}F}$ = 7.9 Hz), 136.6, 130.2, 128.1, 127.7, 127.0–126.6 (m), 122.9 (d, $J_{C\text{-}F}$ = 3.3 Hz), 122.3, 121.6, 114.2 (d, $J_{C\text{-}F}$ = 23.2 Hz), 113.2, 22.7 (d, $J_{C\text{-}C}$ = 1.4 Hz), 22.6 (d, $J_{C\text{-}C}$ = 1.5 Hz), 20.6–20.4 (m), 16.3 (d, $J_{C\text{-}C}$ = 10.8 Hz), 6.8.

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CONFLICT OF INTEREST

The authors declare no competing financial interest.

DATA AVAILABILITY STATEMENT

Data are available in the experimental section of the article.

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