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Synthesis of Enantiopure Vicinal Halohydrins Using a Sequence of Haloperoxidase and Lipase

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Abstract: Vicinal halohydrins are key building blocks to produce bioactive molecules and drugs, especially if they can be obtained in enantiomerically pure form. In this study, we present a bi-enzymatic sequence that allows to obtain *vic*-halohydrins through a photochemoenzymatic olefin hydroxy halogenation followed by a lipase catalysed kinetic resolution. The absolute configuration of the resulting products was determined using Mosher's method

Keywords: Cascade reaction; photochemoenzymatic cascade; enzymatic kinetic resolution; vanadium chloroperoxidase; *Candida antarctica* lipase B

Vicinal halohydrins (*vic*-halohydrins) are structural motifs with two spatially close functional groups that are easy to manipulate. They are useful building blocks in the synthesis of a plethora of bioactive compounds. For example, *vic*-halohydrins are reported as intermediate in the production of β-adrenoreceptor agonists *R*-(−)-denopamine, (−)-isoproterenol[1] and mirabegron,[2] cholesterol absorption inhibitors such as ezetimibe,[3] or antifungal agents miconazole, econazole, and sertaconazole.[4] For their stereoselective synthesis biocatalytic methods are increasingly considered.[3] So far, enzymes employed to obtain *vic*-halohydrins include ketoreductases (KREDs),[5–7] halohydrin dehalogenases (HHDHs),[8–10] cytochrome P450 monooxygenases,[11] and haloalkane dehalogenases.[12] In all cases, pre-installed halogens are required.

An alternative synthesis strategy starts with the functionalisation of C=C double bonds. For example, a sequence of epoxidation and nucleophilic ring opening is conceivable (Scheme 1a).[13–15] Given that at least one of the steps is stereoselective, enantioenriched *vic*-halohydrins are accessible. Also the direct hydroxyhalogenation of C=C double bonds is an established approach (Scheme 1b).[16–19] Enzymatic routes, particularly using choroperoxidase (CPO)-catalysed hydroxyhalogenations, have attracted some interest especially because of the lower waste production as compared to established *N*-halo compounds, such as *N*-halosuccinimide (NXS) (Scheme 1b).[20–27] While in the latter case stoichiometric amounts of *N*-succinimide are formed, the sole by-product of the enzymatic method is water.

Heme-[28–31] and V[32]-containing haloperoxidases (HPOs) have been reported to catalyse H_2O_2-driven hypohalite formation followed by spontaneous, non-enzymatic hydroxyhalogenation of a broad range of alkenes. Currently, two major limitations hamper the preparative application of HPO-catalysed *vic*-halohydrin formation. First, especially the heme-dependent HPOs suffer from a pronounced instability towards the stoichiometric oxygen-donor (H_2O_2), which can be overcome by suitable *in situ* H_2O_2 generation methods.[33] Furthermore, due to the chemoenzymatic

nature of the overall reaction, racemic products are formed limiting the applicability of this method for the synthesis of biologically active products.

We therefore set out to address especially the latter issue by devising a multistep cascade comprising the chemoenzymatic formation of *vic*-halohydrins followed by an **e**nzymatic **k**inetic **r**esolution (EKR) to access both enantiomers of the intermediate racemic *vic*-halohydrin. For this, we decided to use the well-known lipase B from *Candida antarctica* (CalB) in its commercially available, immobilised form (Novo435).[34] As haloperoxidase, we chose the V-containing chloroperoxidase from *Curvularia inaequalis* (*Ci*VCPO)[35–36] and for *in situ* peroxide generation we used the previously reported photochemical hydroperoxidation of 2-methyl tetrahydrofuran (2-MeTHF).[37] This method, though producing stoichiometric waste of 2-hydroxy-2-MeTHF (and its degradation products) offers the possibility of controlling the H_2O_2 generation rate and therefore will be attractive for H_2O_2-labile enzymes. Overall, a chemoenzymatic reaction scheme to obtain optically pure *vic*-halohydrins or their esters was envisaged (Scheme 1c).

As the alkene products of interest were all rather hydrophobic, we decided to evaluate a two-liquid-phase-system (2LPS) comprising 2-MeTHF as substrate reservoir and product sink as well as O_2 activating reagent. The labile tertiary C_2-H bond of 2-MeTHF readily reacts with the photochemically (*meso*-TPP-catalysed) generated singlet oxygen ($^1O_2$) forming the corresponding 2-hydroperoxide as stoichiometric oxidant. As the majority of reagents can be expected to reside in the organic layer, we also applied catalytic amounts of tetrabutylammonium bromide ($N(Bu)_4Br$, TBAB) as phase transfer catalyst (Scheme 2).[37–38]

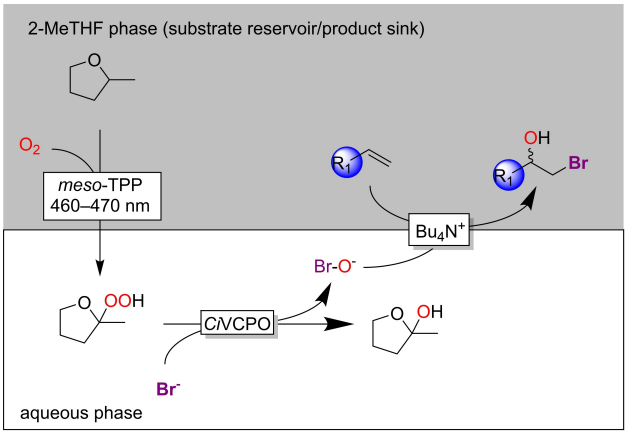
In a first set of experiments, we investigated a photochemoenzymatic procedure for the synthesis of bromohydrins using 4-vinylanisole **1 a** as model substrate (Figure 1). Using 25 mM_{org} of **1 a** (0.012 mmol) in the presence of 1 eq. of TBAB (serving as phase transfer catalyst and Br-source), 50 μM_{org} *meso*-TPP and 15 nM_{aq} *Ci*VCPO in a biphasic system (2-Me-THF and citrate buffer, 1:1 v/v) and irradiating the mixture with blue LED light for 72 h resulted in near-full (98%) conversion of **1 a** into the desired **2 a** (Figure 1). **2 a** was not formed using thermally inactivated *Ci*VCPO, in the absence of illumination or photocatalyst or upon replacing 2-MeTHF by ethyl acetate (SI#1) In the absence of the phase transfer catalysis (albeit in the presence of 25 mM_{aq} KBr), a significantly reduced yield in **2 a** was observed within the timeframe of this experiment (Figure 1). Reducing the TBAB concentration by a factor of 10 did not negatively influence the product concentration (Figure 1).

As shown in Figure 1 full conversion could be achieved translating into turnover numbers (TN = $mol_{2a} \times mol_{Cat}^{-1}$) of 10, 500 and > 1600000 for TBAB, *meso*-TPP, and *Ci*VCPO, respectively. In case of *Ci*VCPO this corresponds to an average turnover frequency over 72 h (TF=TN × t^{-1}) of > 6 s^{-1}. This value is in line with previous findings.[32]

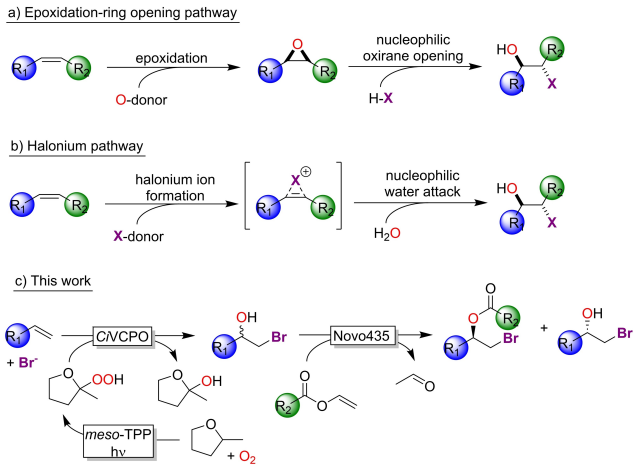
Already without further optimisation, a semi-preparative (0.1 mmol) reaction was successfully carried out (data not shown).

Using these reaction conditions, we further expanded the product scope of the proposed photochemo-enzymatic reaction (Figure 2).

A panel of styrene compounds (**1 a–g**) was converted into the respective bromohydrins (**2 a–g**) in acceptable to high NMR-yields. The procedure was tested also on the aliphatic terminal alkene 1-octene **1 i**,



Scheme 2. Envisioned two-liquid phase system for the chemoenzymatic hydroxyhalogenation of alkenes.



Scheme 1. Strategies for the synthesis of halohydrin derivatives. Panel a) C=C double bond epoxidation followed by nucleophilic epoxide opening; panel b) addition of halogen donor and oxygen-based nucleophile to an alkene; panel c) olefin hydroxyhalogenation and hydrolase-mediated KR.

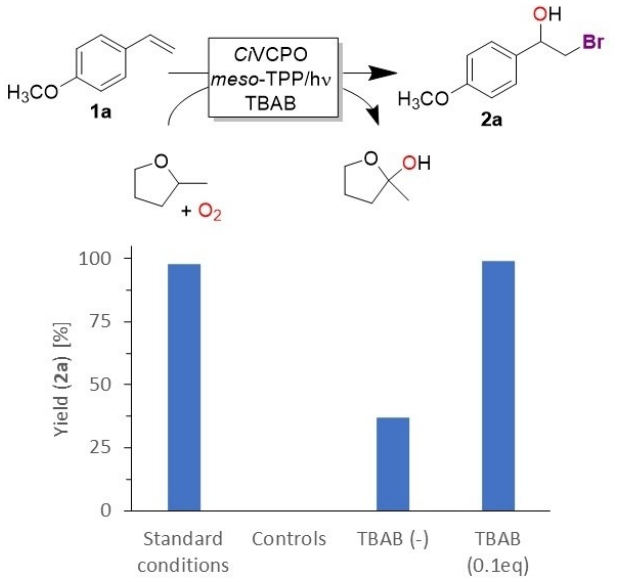


Figure 1. Reaction parameters influencing the chemoenzymatic hydroxyhalogenation of **1a**. Standard reaction conditions: 4-vinylanisole **1a** (0.012 mmol, 25 mM), TBAB (25 mM), meso-TPP (50 µM) and *Ci*VCPO (15 nM) were dissolved in organic solvent and citrate buffer (0.1 M, pH 5). The solution was gently stirred (200 rpm) under blue-LED irradiation (470 nm) for 72 h. Yields were determined by HPLC analysis (SI#4.1); Controls were performed under the same reaction conditions with the exceptions 1) using thermally inactivated *Ci*VCPO or 2) in absence of meso-TPP or 3) under dark conditions. TBAB (−): as under standard conditions but without TBAB and rather 25 mM KBr; TBAB (0.1 eq.): as under standard conditions but in the presence of 25 mM KBr and 2.5 mM TBAB. Experiments were performed in triplicates.

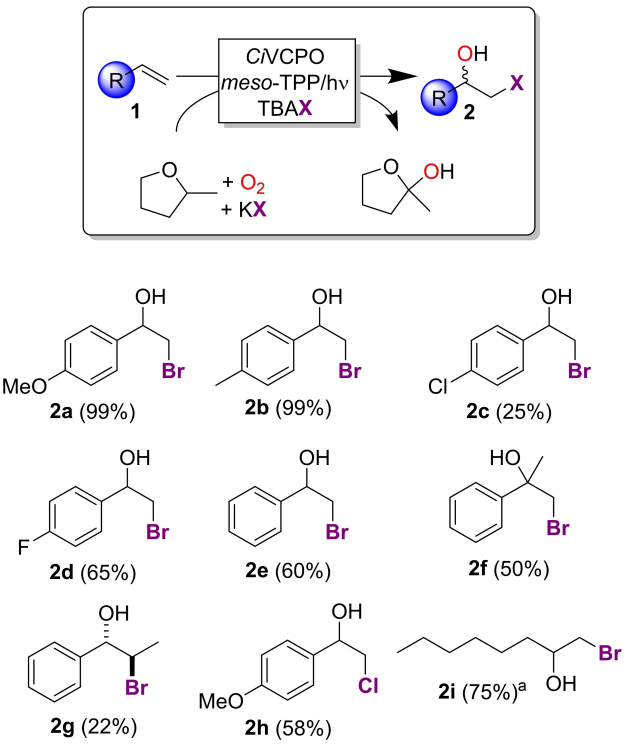


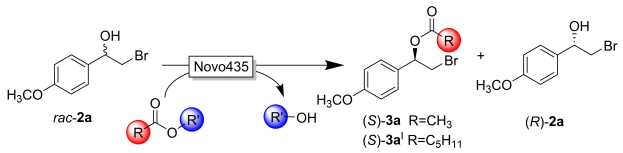
Figure 2. Product scope of the photochemo-enzymatic hydroxyhalogenation. NMR-yields were calculated on the crude reaction mixtures by ^{1}H-NMR analyses and using ethylene carbonate as internal standard (SI#4.2). [a]Reaction carried out for 24 h.

affording product **2i** with 75% NMR-yield. Furthermore, the photochemoenzymatic cascade worked also with chloride salts (KCl) and phase transfer catalysts (TBACl), leading to the formation of chlorohydrin **2h**. In all cases the regio chemistry of the process exclusively followed Markovnikov's rule and reaction of (*E*)-alkenes such as *trans*-β-methylstyrene (**1g**) yielded the anti-bromohydrin product. Electron-donating groups –OCH_3 and –CH_3 activated the C=C double bound (compounds **2a**, **b** and **h**, Figure 2) whereas electron-withdrawing groups Cl- and F- deactivated the olefins and resulted in lower yields (compounds **2c** and **d**, Figure 2). The presence of a –CH_3 substituent on the α- and even more on the β-carbon of the C=C double bound likewise reduced the bromohydrin formation (compounds **2f** and **g**, Figure 2, respectively).

Expectedly, the bromohydrins **2a–i** were obtained in racemic form. Therefore, we advanced to identifying a suitable hydrolase for the EKR of *rac*-**2a** as model compound (Table 1). Starting with hexanoic acid as acylating agent, we screened different lipases, including pancreatin lipase, lipase from *Aspergillus niger*, Amano lipase PS from *Burkholderia cepacia*, Amano lipase M from *Mucor javanicus*, lipase from *Candida rugosa* and *Candida antarctica* lipase B (Novo435). Initial EKR experiments were set at a substrate/enzyme ratio of 0.1 mmol (25 mM in MeTHF) per 20 mg of immobilisate. Under these conditions, only Novo435 was able to catalyse the enantioselective esterification of bromohydrin *rac*-**2a**, providing the hexanoate ester (*S*)-**3a**[I] in 20% conversion and 99% enantiomeric excess (ee), corresponding to an enantioselectivity (*E*) of 217 (Table 1, entry 1). The use of hexanoic acid for acetylation was only effective at elevated temperatures, possibly to the facilitated evaporation of the water by-product. At ambient temperature, no conversion was observed even upon prolonged reaction times. Therefore, we evaluated vinyl acetate as alternative acyl donor[39] (Table 1, entries 2–3).

Finally, we adjusted our initial reaction setup changing the solvent from 2-MeTHF (logP 1.36) to diisopropyl ether (DIPE; logP 1.82).[40–42] In particular, the desired 50% conversion of starting material was obtained performing the reaction at r.t. for 8 days with a substrate/immobilisate ratio of 1:200, a substrate concentration of 50 mM in DIPE, and 2 eq. of vinyl acetate (Table 1, entry 4). Additional attempts to

Table 1. Novo435-catalysed EKR of racemic bromohydrin *rac*-**2 a**.[a]

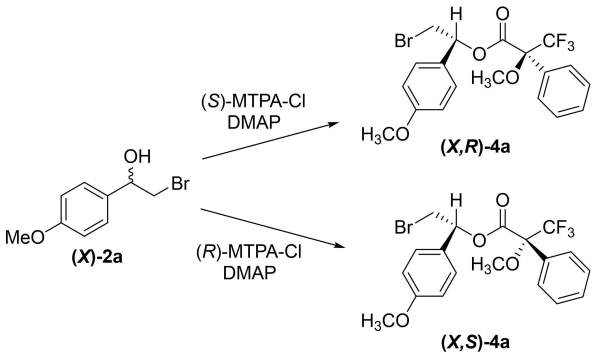


Entry	Solvent	T [°C]	t [h]	Conv. [%][b]	*ee* [%][c] (*X*)-**3 a**/(*X*)-**3 a'**	(*X*)-**2 a**	E[d]
R=C5H11, R'=H							
1	2-MeTHF	60	144	20	99	10	> 200
R=CH3, R'=C2H4							
2[e]	2-MeTHF	rt	48	30	99	12	> 200
3[f]	2-MeTHF	rt	48	26	99	12	> 200
4[e]	DIPE	rt	192	50	99	98	> 200
5[e]	DIPE	35	96	54	97	99	> 200

[a] The reaction was performed at a substrate concentration of 25 mM in 2-MeTHF, using 2 eq. of the opportune acylating agent (hexanoic acid or vinyl acetate) and Novo435 (200 mg $mmol^{-1}$).
[b] Conversion determined by ^1H NMR spectroscopy.
[c] Enantiomeric excess (*ee*) determined by HPLC analysis on a Chiralpak® AD–H 5 µm (250×4.6 mm) column.
[d] E was calculated by using the formula $E = \ln[(1 - ee_S)/(1 + ee_S/ee_P)]/\ln[(1 + ee_S)/(1 + ee_S/ee_P)]$.
[e] Substrate concentration: 50 mM.
[f] Reaction carried out in absence of organic solvent, using 20 eq. of acylating agent. Reactions were performed in triplicate.

reduce the reaction time were made, such as increasing the temperature to 35 °C, and in this latter case the final product was obtained in 4 days without compromising conversion and enantiomeric excess (Table 1, entry 5).

The absolute configuration of bromohydrin **2 a** was determined using the Mosher method.[43] The first step involved the synthesis of the diastereomers **(*X,R*)-4 a** and **(*X,S*)-4 a** by coupling of (*X*)-**2 a** with (*R*)- and (*S*)-α-methoxy-α-trifluoromethylphenylacetic acid chloride (MTPA–Cl), respectively (Scheme 3 and SI#5.1).[44] The respective products were then analysed via ^1H, ^{13}C and ^{19}F NMR spectroscopy.



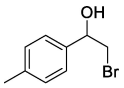
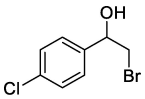
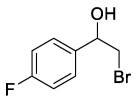
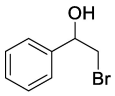
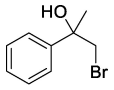
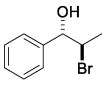
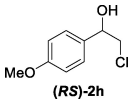
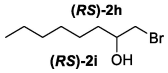
Scheme 3. Coupling reactions between bromohydrin (X)-**2 a** and enantiomerically pure Mosher acid chlorides ((*S*)- and (*R*)-MTPA–Cl).

The ^1H NMR-spectra show a strong shielding effect (+135.6 and +31.6 Hz, using the Mosher d(X,S) – d(X,R) convention) and signals are downfield for the protons of the methoxyphenyl group of **(*X,S*)-4 a** (7.48 and 6.93 ppm) compared to the ones of **(*X,R*)-4 a** (7.14 and 6.85 ppm) (SI#5.2). This behaviour is due to anisotropic effect of the aromatic ring of MTPA and indicates that this group is on the same side as the methoxyphenyl of the bromohydrin. The ^{13}C spectra were in accordance with ^1H experiments since the methylene protons of the -CH_2Br group (33.19 and 33.40) are affected by anisotropic effect of the phenyl ring of the MTPA with upfield shifting (−21 Hz) of the peak in the (XS) diastereomer (SI#5.3). Lastly, in ^{19}F NMR of **(*X,S*)**, signal of -CF_3 substituent is shifted upfield, compatible with the larger group of MTPA (phenyl) and the smaller of the bromohydrin (–CH_2Br) on the same side, assuming that methoxyphenyl exerts a higher steric hinderance compared to the bromomethylene (SI#5.4).

All these data allow us to assign *R* configuration to the chiral carbinol carbon of **(*X,S*)-4 a**. As a result, we conclude that the Novo435-catalysed KR was indeed (S)-selective producing ester **(*S*)-3 a** and leaving the hydrohalogenated compound **(*R*)-2 a** unreacted.

Finally, we extended the Novo435-catalysed KR to halohydrins *rac*-**2 b–i** produced previously (Figure 2). Pleasingly, the desired haloalcohols and haloesters were obtained in high yields and high enantiomeric purity (Table 2, entries 1–4), except for compounds *rac*-**2 f–g**, which showed poorer conversions and E

Table 2. Kinetic resolution of bromohydrins *rac*-**2 b–g** mediated by *Candida antarctica* lipase B (Novo435).[a]

Entry	Substrate	Conv. (%)[b]	*ee* (%)[c]		E[d]
			(*S*)-3	(*R*)-2	
1	 **(*RS*)-2b**	50	99	99	1060
2	 **(*RS*)-2c**	50	99	99	1060
3	 **(*RS*)-2d**	54	99	93	692
4	 **(*RS*)-2e**	50	96	97[e]	207
5	 **(*RS*)-2f**	32[f]	n.d.	7	n.d.
6	 **(*RS*)-2g**	36	72	19	7.5
7	 **(*RS*)-2h**	50[g]	99	88	584
8	 **(*RS*)-2i**	48[h]	n.d.	n.d.	n.d.

[a] Reactions were performed at a substrate concentration of 50 mM in DIPE, using 2 eq. of vinyl acetate and Novo435 at an enzyme (mg)/substrate (mmol) ratio: 200 mg $mmol^{-1}$. The solution was gently stirred (200 rpm) at 35 °C for 96 h.
[b] Conversion determined by ^{1}H NMR spectroscopy.
[c] Enantiomeric excess (ee) determined by HPLC analysis on a Chiralpak® AD–H 5 µm (250×4.6 mm) column (SI#6).
[d] E was calculated by using the formula $E = \ln[(1 - eeS)/(1 + eeS/eeP)]/\ln[(1 + eeS)/(1 + eeS/eeP)]$.
[e] Enantiomeric excess (ee) determined by Mosher ^{19}F NMR experiments (SI#7).
[f] Conversion calculated from purified recovered substrate.
[g] Reaction ended after 48 h.
[h] Reaction ended after 24 h. Standard *rac*-**2 b–i** and *rac*-**3 b–i** were synthesised as depicted in SI#8 and used for the determination of chromatographic methods and chiral HPLC analyses.
N.d. = not detected

values, probably due to steric hindrance exerted by the methyl substituent geminal or vicinal to the hydroxyl group (Table 2, entries 5 and 6, respectively). Interestingly, chlorohydrin **2 h** and aliphatic bromohydrin **2 i** were processed by Novo435 faster (full theoretical conversion within 48 h and 24 h, respectively, Table 2, entries 7 and 8), compared to the other substrates (96 h).

In this contribution, a novel access to enantiomerically pure *vic*-halohydrins is proposed. Combining a non-selective but very versatile photoenzymatic hydroxyhalogenation with a lipase-catalysed kinetic resolution afforded both enantiomers in >97% ee. Admittedly, the individual steps, particularly, the Novo435-catalysed KR reaction needs further improvement to attain shorter reaction times with lower catalyst loadings. We hope that this proof-of-concept study will inspire enzyme engineers to design optimised CalB variants with higher activity. It would also be very interesting to obtain hydrolases capable of converting *vic*-halohydrins obtained from internal alkenes.

Experimental Section

Commercially available reagents were used without further purification. *Ci*VCPO was prepared following a literature procedure.[37] Chromatographic separations were performed on Merck silica gel 60 (230–400 mesh). R_f values are referred to TLC carried out on 0.25 mm silica gel plates (F254) using the eluent indicated for column chromatography. All products were dried in high vacuum (10^{-3} mbar) before characterization. ^{1}H NMR, ^{13}C NMR, and ^{19}F NMR were recorded on a Bruker Avance DRX400 (400 MHz/100 MHz) spectrometer. HPLC measurements were performed using an Ultimate 3000 Rapid Resolution UHPLC system (ThermoFisher scientific) equipped with a Chiralpak® AD–H 5 µm (250×4.6 mm) column and a multiwavelength detector.

Photochemoenzymatic Procedure for the Synthesis of Bromohydrins 2 a–g

In a 4 mL glass vial, 500 µL of 2-MeTHF containing styrenic compound (25 mM) and *meso*-TPP (50 µM) were combined with 500 µL of citrate buffer pH 5.0, 0.1 M, containing *Ci*VCPO (15 nM) and TBAB (2.5 mM) and KBr (25 mM). The reaction was gently stirred at 200 rpm at room temperature for 72 hours in a jacketed beaker with commercial blue-LEDs (24 W) wrapped around. The reaction was diluted with EtOAc (1 mL). The organic layer was then separated from the aqueous one and the latter extracted EtOAc (3×1 mL). Organic fractions were combined, and the solvent evaporated under vacuum. The crude mixture has been analysed by ^{1}H NMR, using ethylene carbonate (EC) as internal standard.

Lipase-Catalysed Kinetic Resolution of Bromohydrins 2 a–g

Novo435 (20 mg) was added to a solution of *rac*-**2 a–g** (0.1 mmol; 50 mM) in anhydrous DIPE at 35 °C and, after 10 min, vinyl acetate (0.2 mmol; 0.1 M) was added. The reaction mixture was gently stirred at 200 rpm for 96 h. The reaction was stopped by filtration through a thin layer of Celite® and the filter cake was washed with EtOAc (3×2 mL). After

evaporation of the solvent, the crude product was purified by column chromatography to give (*R*)-**3 a–g** and (*S*)-**2 a–g**.

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