

# Chemoenzymatic One-Pot Cascade for the Construction of Asymmetric C–C and C–P Bonds via Formal C–H Activation

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The integration of organocatalysis and enzyme catalysis in one-pot cascade processes allows for the efficient construction of complex molecular architectures with high levels of stereo-control. However, challenges related to reaction compatibility between both processes are often a limitation for the development of efficient synthetic routes. In this study, we describe the combination of an enzymatic aerobic oxidation followed by the squaramide-mediated asymmetric formation of C–P and C–C

bonds to access important building blocks such as chiral  $\alpha$ -hydroxyphosphonates and  $\beta$ -nitro alcohols in good yields and enantiomeric ratios. This sequential process is conducted in a one-pot fashion within a biphasic system and represents a pioneering example of a chemoenzymatic cascade involving aerobic biooxidation and an organocatalytic step operating under hydrogen-bond activation mode and under mild reaction conditions.

## Introduction

The design of synthetic routes based on the sequential combination of several steps in the same reaction vessel provides many practical, economic and environmental advantages.<sup>[1–5]</sup> These cascade processes are especially useful when highly reactive or unstable species are reaction intermediates. These compounds often present a high tendency to degradation which ultimately results in poor yields. Aldehydes, which are important feedstocks in synthetic chemistry that can be obtained from bioderived sources,<sup>[6]</sup> are a clear example of such species as they tend to degrade to the corresponding carboxylic acid over time. At the same time, they are versatile starting materials or intermediates in many useful stereoselective transformations to access important scaffolds present in natural products, active pharmaceutical ingredients (APIs) and agrochemicals.<sup>[7]</sup> Particularly, 2-nitro-1-arylethanol and  $\alpha$ -hydroxyphosphonates can be made from the corresponding benzaldehyde derivatives via asymmetric Henry<sup>[8–11]</sup> and Pudovik<sup>[12–16]</sup> reactions, respectively. Most of these reactions are

promoted by small-molecule catalysts in which hydrogen bonding and other non-covalent interactions are key in substrate activation and the stereochemical outcome.<sup>[8–16]</sup> As a consequence, small traces of acid can easily disrupt the catalyst's performance.<sup>[17]</sup> For this reason, the use of surrogate systems provides useful and practical benefits.<sup>[18]</sup> Different systems have been recently developed for the *in situ* generation of aldehydes for further asymmetric transformations to create new asymmetric C–C bonds (Scheme 1). For instance, Liu and coworkers chose the laccase-2,2,6,6-tetramethylpiperidine 1-oxyl radical (TEMPO) system to oxidise benzyl alcohols derivatives and cyclohexanol to generate the corresponding carbonyl compounds which subsequently reacted via proline-mediated aldol reaction to access chiral  $\beta$ -hydroxy ketones (Scheme 1a).<sup>[19]</sup> Also recently, Chatterjee *et al.* employed an alcohol dehydrogenase (ADH) to catalyse the nicotinamide-dependent reversible oxidation of primary alcohols (Scheme 1b).<sup>[20]</sup> In 2018, our research group developed a one-pot sequential cascade combining the stoichiometric oxidation of primary alcohols using MnO<sub>2</sub> with an asymmetric aldol and Henry reactions using different catalysts to access chiral alcohols (Scheme 1c).<sup>[9]</sup> More recently, the same strategy was applied to create C–P asymmetric bonds using a chiral squaramide as the catalyst.<sup>[13]</sup> Despite the simplicity of these reaction setups, achieving high yields required an excess of MnO<sub>2</sub>, elevated temperatures, and cryogenic conditions in the asymmetric step. Therefore, we envisioned that the replacement of the initial step with a biocatalytic approach could significantly reduce the environmental impact of the entire process as well as their safety. Moreover, with the combination of two different non-metallic catalytic processes, a fully metal-free strategy to access chiral  $\alpha$ -hydroxy phosphonates and 1,2-nitro alcohols from the corresponding primary alcohols could be achieved (Scheme 1d).

However, the construction of such system would have to face significant challenges such as finding an enzymatic process simple enough to avoid any possible interactions with the catalyst as well as being able to operate at high substrate loadings. Concerning the organocatalytic step, the ability to

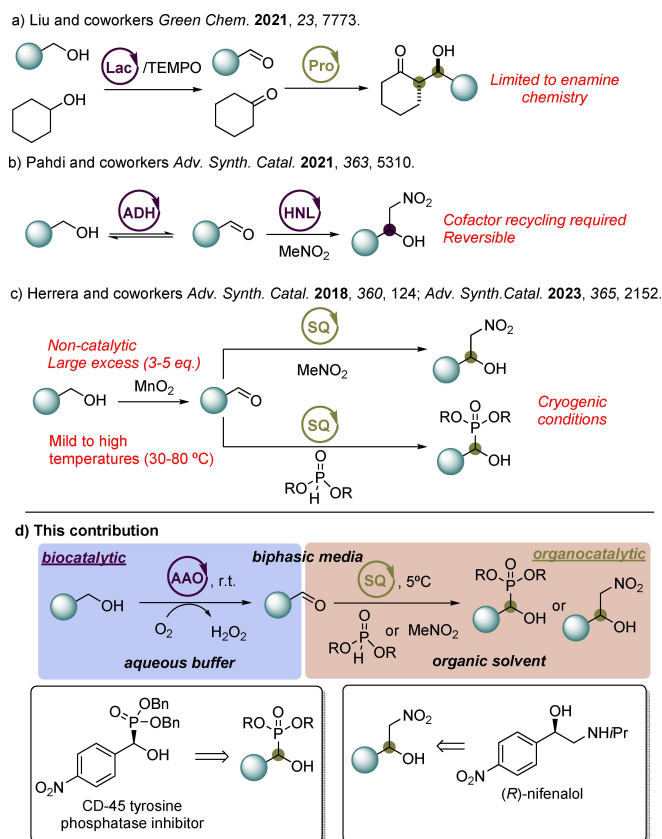
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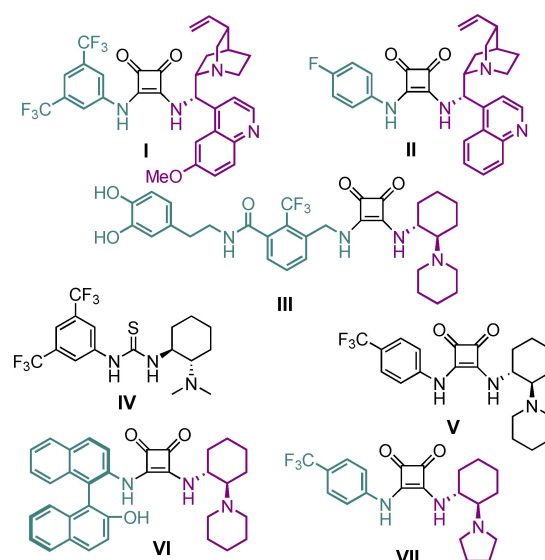


**Scheme 1.** One-pot cascade processes involving alcohol oxidation followed by asymmetric transformations facilitated by organocatalysts or enzymes. Lac: laccase; Pro: proline-derived catalyst; ADH: alcohol dehydrogenase; HNL: Hydroxy nitrile lyase; SQ: squaramide; Cin: Cinchona-based catalyst; AAO: Aryl alcohol oxidase.

work in the presence of water would be key. Organocatalysts operating through enamine activation have demonstrated high tolerance to aqueous media,<sup>[22–26]</sup> and in our recent report combining iminium catalysis and biocatalysis, the organocatalytic step was carried out in organic solvent.<sup>[27]</sup> Non-covalent activation modes, such as hydrogen-bond interactions, represent an important obstacle in the construction of synthetic pathways employing one-pot strategies that integrate diverse types of catalysis and the use of aqueous media.

## Results and Discussion

Based on prior experience, we initially investigated the two-phase asymmetric hydrophosphonylation reaction of aldehydes employing chiral squaramides as catalysts.<sup>[28–36]</sup> Squaramides are cyclobutene derivatives composed of two carbonyl moieties that act as hydrogen-bond acceptors alongside two amide-NH groups which serve as hydrogen-bond donors. Using *p*-Cl-benzaldehyde (**2a**) and dibenzyl phosphite (**i**) as the model reaction, a series of chiral squaramides as well as Takemoto's bifunctional chiral thiourea derivative (**I–V**, Figure 1) were investigated (Table 1). Initial experiments were conducted at 25 °C using CH<sub>2</sub>Cl<sub>2</sub> as the solvent, 2 eq. of dibenzyl phosphite (**i**)



**Figure 1.** Organocatalysts (I–VII) evaluated in this contribution (figures in black correspond to commercially available catalysts).

as the nucleophile in a biphasic system with NaPi pH 6 buffer serving as the aqueous layer. Under these conditions, squaramides **I**, **II** and **V** provided promising results, with the corresponding (*R*)-4-Cl-phenyl  $\alpha$ -hydroxy dibenzyl phosphonate (*R*)-**3ai** isolated in good to excellent yields (59–82%) and high enantiomeric ratios (up to 85:15). Notably, squaramides **I** and **II** exhibited reduced performance at lower temperatures, with a mere 23% yield for **I** and no observable reaction for **II**. In contrast, catalyst **V** displayed robust performance, yielding **3ai** in a 78% isolated yield and an 88:12 enantiomeric ratio.

In an effort to optimise the reaction conditions, a solvent screening was conducted (entries 8–12), revealing CHCl<sub>3</sub> and CH<sub>2</sub>Cl<sub>2</sub> (DCM) as optimal solvents for achieving high enantiomeric ratios in the two-phase asymmetric hydrophosphonylation reaction using squaramide **V** as the catalyst. Subsequently, an attempt was made under cryogenic conditions using DCM and CHCl<sub>3</sub> as solvents after water removal (entries 13 and 14), resulting in the isolation of (*R*)-**3ai** with an excellent enantiomeric ratio (up to 92:8 e.r.). However, the observed low conversion, high energy demands and the necessity for water removal prompted us to discard these reaction conditions for the construction of the one-pot sequential process. Finally, lower catalyst loadings led to a drop in reaction conversion, with no significant effect observed in the stereoselectivity of the process. Next, we tested different phosphites (**ii–v**) bearing aromatic and other alkyl substituents. A similar yield was obtained for diphenyl phosphite (**ii**) (75%, entry 15), whereas no reaction was detected when dialkyl phosphites **iii–v** were used (entries 16–18).

With the best results in hand for the asymmetric formation of C–P bond catalysed by squaramide **V** in the presence of water (Table 1, entry 9), our next focus was to explore various enzymatic strategies for the mild oxidation of primary alcohols to their corresponding carbonyls. Traditionally, alcohol dehydrogenases (ADHs) and laccases in combination with radical

**Table 1.** Organocatalysed asymmetric addition of aryl and alkyl phosphites (i–v) to *p*-chlorobenzaldehyde (2a) in biphasic systems using different organocatalysts I–V (cat).

Entry	Cat	Phosphite	T (°C)	Solvent	Yield (%)	e.r. (R:S)
1	I	i	25	DCM	59	76:24
2	II	i	25	DCM	82	78:22
3	III	i	25	DCM	75	55:45
4	IV	i	25	DCM	84	61:39
5	V	i	25	DCM	80	85:15
6	I	i	5	DCM	23	79:21
7	II	i	5	DCM	n.r.	n.a.
8	V	i	5	DCM	78	88:12
9	V	i	5	CHCl <sub>3</sub>	91	88:12
10	V	i	5	EtOAc	86	75:25
11	V	i	5	MTBE	95	74:26
12	V	i	5	Toluene	94	76:24
13	V	i	-25	DCM	60	91:9
14	V	i	-25	CHCl <sub>3</sub>	38	92:8
15	V	ii	5	DCM	75	80:20
16	V	iii	5	DCM	n.r.	n.a.
17	V	iv	5	DCM	n.r.	n.a.
18	V	v	5	DCM	n.r.	n.a.

Isolated yields for (R)-3a after column chromatography. Enantiomeric ratios (e.r.) determined by HPLC on a chiral stationary phase. n.r.: no reaction observed. n.a.: not applicable.

mediators like 2,2,6,6-tetramethylpiperidine 1-oxyl radical (TEMPO), have been the preferred options for developing (chemo) enzymatic cascades involving alcohol oxidation.<sup>[37–46]</sup> Since the use of ADHs introduces additional complexity due to the need for cofactor regeneration as well as the use of a sacrificial substrate, we initially opted for the well-established and robust laccase-TEMPO system employing the commercially available laccase from *Trametes versicolor*. While this combination effectively facilitated alcohol oxidation, control experiments showed no reaction when this oxidation system was employed, preventing the establishment of a one-pot sequential cascade (Table S2).

As a result, we decided to explore the use of alcohol oxidases (AO) instead. AOs are flavin-dependent enzymes that catalyse the non-reversible oxidation of alcohols into their corresponding carbonyl compounds with atmospheric O<sub>2</sub> as the final electron acceptor and H<sub>2</sub>O<sub>2</sub> as the by-product.<sup>[47,48]</sup> Notably, these enzymes have recently proven effective in multi-step enzyme cascades, successfully overcoming the limitations associated with ADHs.<sup>[49–51]</sup> Inspired by these achievements, and intrigued to explore whether oxidases could likewise be useful

to improve our process, we selected the FX9 variant of the aryl alcohol oxidase from *Pleurotus eryngii* (PeAAO FX9) which shows a remarkable activity towards the oxidation of benzylic and allylic alcohols.<sup>[52]</sup> Given that this enzyme had not previously been challenged at high substrate concentrations or exposed to organic solvents, an initial assessment of the oxidative process for preparative-scale biotransformations was carried out (Table S1), finding optimal performance at 180 mM substrate concentration using 15.2 μM FX9, 10% DCM and in the presence of catalase to eliminate H<sub>2</sub>O<sub>2</sub>, resulting in remarkable turnover numbers exceeding 12,000. Control experiments demonstrated full compatibility between FX9 and squaramide V.

With the identification of the appropriate biocatalyst for aerobic oxidation as well as the operational window in which both catalytic systems could work, the one-pot sequential process starting from 1a was constructed. Firstly, the aerobic biooxidation step was carried out for 24 h at 30 °C until full consumption of the starting material. Subsequently, reactions were cooled down to 5 °C, and a solution containing catalyst V (20 mol%) in DCM and dibenzyl phosphite (i, 2 eq.) were added

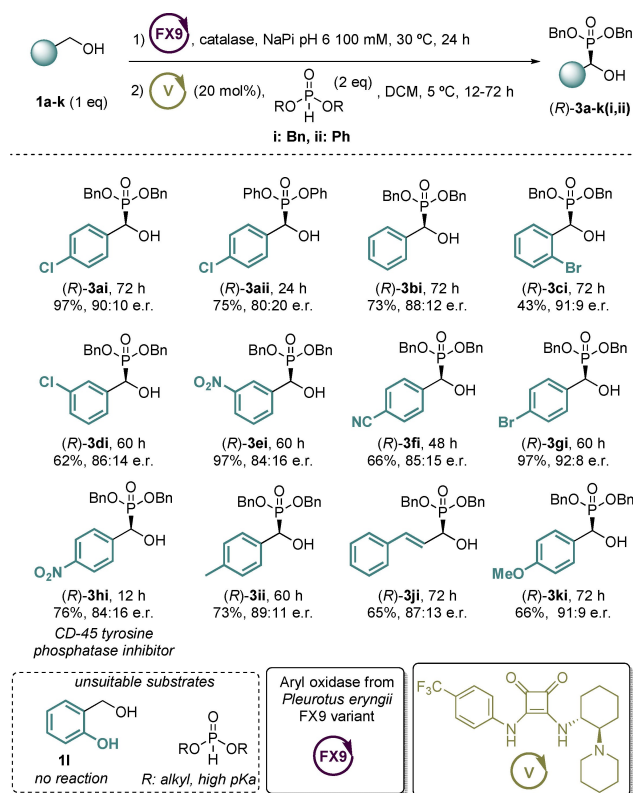
to the reaction vessel. Reaction monitoring was done by TLC until the disappearance of aldehyde intermediate **2a**. Under this set-up,  $\alpha$ -hydroxy dibenzyl phosphonate (*R*)-**3ai** was isolated in 97% yield and in 90:10 e.r. after 72 h (Scheme 2). We then examined the reaction scope. A collection of benzyl alcohols **1b–j** was selected and subjected to our novel approach to construct asymmetric C–P bonds via C–H formal activation via aerobic oxidation. Even though no significant difference was found between  $\text{CHCl}_3$  and DCM as the solvent in the initial screening starting from **1a**, higher enantiomeric ratios were obtained using DCM as the solvent across the panel. Generally, aryl alcohols containing electron-withdrawing groups were well-tolerated, with yields ranging from moderate to excellent (43–97%). However, 4-CN and 4- $\text{NO}_2$  derivatives (*R*)-**3fi,hi** could be initially isolated in yields ranging 38–45%. Previous research suggested that benzaldehydes bearing electron withdrawing groups are prone to form the corresponding hydrates, and therefore, the AAO can also catalyse the oxidation of the aldehydes to the corresponding acids.<sup>[53]</sup> This process can potentially limit both the final yield and enantiomeric ratio due to possible interactions with the catalyst. By reducing the oxidation time to 8 h, (*R*)-**3fi,hi** could be isolated in high yields (up to 76%) and in high enantiomeric ratios (up to 85:15). Reaction starting from cinnamyl alcohol (**1j**) provided the corresponding (*R*)-**3ji** derivative in 65% yield and 87:13 e.r. In

general, neutral and electron deficient aryls are well tolerated, with high yields and enantiomeric ratios observed across the panel. Concerning electron-rich aryls, the 4-OMe derivative (*R*)-**3ki** was isolated in good yield and excellent enantiomeric ratio (66%, 91:9 e.r.). Unfortunately, no product formation was observed starting from the 2-OH hydroxy derivative **1l**. Control experiments revealed that FX9 is able to oxidise **1l** but no product was observed in the asymmetric hydrophosphonylation of **2l** using **V** as the catalyst. Furthermore, reaction using dialkyl phosphites **iii–v** proved unsuccessful due to the higher pKa presented by these compounds compared to aromatic analogues.<sup>[54]</sup>

Encouraged by these results, and following on our ongoing search to develop new methods to make chiral amino alcohol derivatives,<sup>[55–57]</sup> we decided to study our oxidase-squaramide strategy to build molecular complexity with other nucleophiles. Specifically, we decided to investigate the asymmetric formation of C–C bonds through the Henry reaction to obtain chiral 2-nitro-1-arylethanol **4**, as these compounds are key building blocks in the synthesis of important active pharmaceutical ingredients such as the  $\beta$ -blocking agents (*R*)-nifenalol and (*R*)-sotalol, or the adrenergic agonist (*S*)-norphenylephrine (Scheme 1d).

Initially, the catalytic performance of **V** was evaluated. In our previous report, cryogenic conditions were applied. However, while effective, these conditions are not viable for achieving a fully one-pot sequential cascade, as they present challenges at the laboratory scale as well as reproducibility issues in two-phase systems resulting from water removal. Based on prior experience, neat  $\text{MeNO}_2$  was selected as the solvent and 5 °C as the temperature with the addition of  $\text{MeNO}_2$  over *p*-Cl-benzaldehyde **2a** as the model reaction. Initial catalyst screening (Table S3) revealed poor catalytic performance of **V** towards this transformation, the (*R*)-**4a** obtained in 49% isolated and 66:34 e.r. The cyclopentylamine derivative **VII** was prepared although no improvement was observed. Squaramide **I** was able to catalyse the addition very efficiently although with poor stereoselectivity (96% yield, 53:47 e.r.). Interestingly, (*S*)-**4a** was obtained using **II** in 34:66 e.r. and in 72% yield. Unfortunately, the rest of the catalysts provided unsatisfactory results.

We then turned our attention towards NOBIN-based squaramide **VI** featuring a “push-pull  $\pi + \pi$ ” ( $\text{PP}\pi\pi$ ) system which triggers a mode of activation based on a  $\pi$ - $\delta +$  atom ( $\pi$ -H) and a  $\pi$ - $\delta$ -atom ( $\pi$ -O) interactions with the electrophile, enabling a more efficient and selective attack of  $\text{MeNO}_2$  over the aldehyde.<sup>[56,57]</sup> Using 10 mol % of **VI** in neat  $\text{MeNO}_2$ , chiral 1,2-nitro alcohol (*S*)-**4a** was obtained in 80% isolated yield with a promising 80:20 e.r. When the reaction was performed in the presence of water, a slight drop in stereoselectivity was observed (80:20 to 71:29 e.r.). We next examined the catalyst loading since we hypothesised that catalyst self-assembly could alter its catalytic performance and therefore, lower concentrations could have a positive effect in both efficiency and selectivity. To our delight, lower catalyst loadings led to higher enantiomeric ratios, obtaining (*S*)-**4a** in 87:13 e.r. and 77% isolated yield using 1 mol % catalyst, 200 eq. of  $\text{MeNO}_2$  in a two-phase system for 96 h at 5 °C. With the optimised conditions in



**Scheme 2.** Two-step one-pot cascade to access chiral  $\alpha$ -hydroxyphosphonates in a two-phase system combining biooxidation catalysed by a variant of the alcohol oxidase from *Pleurotus eryngii* (FX9, 15.2  $\mu\text{M}$ ) and squaramide **V** (20 mol %) catalysed asymmetric addition of phosphites. Yields are for the isolated product after column chromatography. Enantiomeric ratios (e.r.) were determined by HPLC on a chiral stationary phase.



hand, the one-pot sequential process starting from **1a** was constructed using 15.2  $\mu$ M FX9 as oxidation catalyst (Scheme 3).

Initial attempts to perform this reaction were unsuccessful. We observed the formation of an emulsion whose presence led us to speculate on its potential interference with the organo-catalyst performance. We wondered whether commercial catalase (bovine liver, C9322), which comes in a semi purified formulation, could be responsible for such emulsions. To investigate further, we decided to assess a purer formulation also sourced from bovine liver (C40). Gratifyingly, through this approach, we could isolate (S)-**4a** in 85% overall yield and in 88:12 e.r.

The process scope was evaluated starting from our panel of primary alcohols **1b–i**. Initial experiments with benzyl alcohol derivatives bearing electron withdrawing groups **1f,h** resulted in poor results due to aldehyde overoxidation. For instance, (S)-**4h** was initially isolated in 30% yield and 71:29 e.r. By reducing the oxidation time, an isolated yield of 50% was obtained with (S)-**4h** in 88:12 e.r. In a similar manner, (S)-**4f** could be obtained in excellent enantiomeric ratio (88:12) although the shorter oxidation time resulted in a 42% isolated yield due to incomplete oxidation of **1f**. The 4-Br derivative (S)-**4g** was isolated in 75% yield and 88:12 e.r. In general, good results were obtained with compounds bearing electron deficient aryls

with, for instance, (S)-**4e** obtained in 82% yield and 94:6 e.r. and (S)-**4d** in 71% yield and 91:9 e.r. Notably, excellent enantiomeric ratios (up to 89:11 e.r.) were also achieved the asymmetric addition starting from **1b** (R:H) and **1i** (R:4-Me) although in lower yields ranging from 28% to 32%. Efforts to access the cinnamyl alcohol derivative (S)-**4j** were not effective, with the final product obtained in 12% yield. Unfortunately, attempts to make the chiral 1,2-nitro alcohol derivatives **4k,l** using this methodology were unsuccessful. Control experiments revealed that **VI** is not able to catalyse the asymmetric Henry reaction over electron-rich benzaldehyde derivatives **2k,l**.

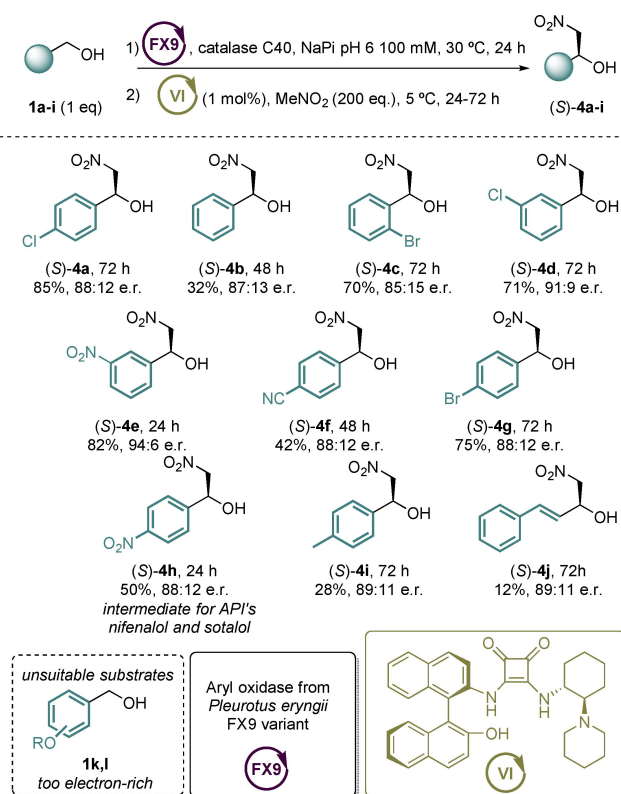
## Conclusions

In summary, in this communication we showed how alcohol oxidases can be efficiently combined with squaramide-mediated processes to make important chiral scaffolds such as 1,2-hydroxyphosphonates and 1,2-nitro alcohols in a simple manner and under mild reaction conditions. The FX9 variant of the aryl alcohol oxidase from *Pleurotus eryngii* has proved to be an excellent catalyst for the oxidation of benzylic and aryl allyl alcohols with turnovers exceeding 12,000. Remarkably, squaramides **V** and **VI** have been demonstrated to work in two-phase systems at mild temperatures with yields and stereoselectivities sometimes superior to those displayed in pure organic solvents (Schemes S2 and S3). Moreover, the synthesis of chiral 1,2-nitro alcohols was successfully carried out without the use of additional organic solvents, with MeNO<sub>2</sub> acting as both the nucleophile and reaction medium. This methodology is effective for neutral and electron-deficient aryl groups although the asymmetric MeNO<sub>2</sub> addition proved unsuitable for electron-rich aryls. Efforts to explore other catalysts for this transformation are currently underway in our laboratory.

The discovery of novel alcohol oxidases to accept a broader range of primary and secondary alcohols as well as the development of more efficient and selective squaramide-type catalysts in aqueous conditions, will allow the construction of a synthetic platform which will constitute a significant step towards the streamlined and more efficient production of fundamental chiral building blocks.

## Experimental Section

**General procedure for the one-pot cascade process for the synthesis of chiral  $\alpha$ -hydroxy phosphonates **3ai–ki**.** To a mixture of the corresponding alcohol **1a–k** (0.1 mmol, 1.00 equiv) in dichloromethane (55  $\mu$ L, 10% v/v) placed in an open-to-air test tube, catalase (2000 U, 0.2 mg), 100 mM phosphate buffer pH 6 (395  $\mu$ L) and the FX9 variant of the aryl alcohol oxidase (AAO) from *Pleurotus eryngii* were added and the reaction mixture was stirred at 30 °C for 24 h. After that time, the reaction mixture was cooled to 5 °C and squaramide **V** (8.87 mg, 0.02 mmol, 0.2 equiv), commercially available, dichloromethane (600  $\mu$ L) and phosphite **i** (0.2 mmol, 46  $\mu$ L, 2.00 equiv) were added. The reaction mixture was stirred at that temperature until no starting material was detected by TLC analysis. Then, the adducts **3ai–ki** were purified by flash



**Scheme 3.** One-pot sequential cascade for the synthesis of chiral 1,2-nitro alcohols from primary alcohols combining biooxidation catalysed by a variant of the alcohol oxidase from *Pleurotus eryngii* (FX9, 15.2  $\mu$ M) and the NOBIN-based squaramide **VI** (1 mol%) catalysed asymmetric Henry reaction. Yields are for the isolated product after column chromatography. Enantiomeric ratios (e.r.) were determined by HPLC on a chiral stationary phase. N.d.: not determined.

column chromatography using a mixture of *n*-hexane and ethyl acetate.

**General procedure for the one-pot cascade process for the synthesis of chiral  $\beta$ -nitro alcohols 4a–j** To a mixture of the corresponding alcohol 1a–j (0.1 mmol, 1.00 equiv) in dichloromethane (55  $\mu$ L, 10% v/v) placed in an open-to-air test tube, catalase (2000 U, 0.2 mg), 100 mM phosphate buffer pH 6 (395  $\mu$ L) and the FX9 variant of the aryl alcohol oxidase (AAO) from *Pleurotus eryngii* (2.2 U, 100  $\mu$ L) were added and the reaction mixture was stirred at 30 °C for 24 h. After that time, the reaction mixture was cooled to 5 °C and squaramide VI (0.001 mmol, 0.55 mg, 0.01 equiv), and nitromethane (1 mL) were added. The reaction mixture was stirred at that temperature until no starting material was detected by TLC analysis. Then, the adducts 4a–j were purified by flash column chromatography using a mixture of *n*-hexane and ethyl acetate.

## Supporting Information

The authors have cited additional references within the Supporting Information.<sup>[58–60]</sup>

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## Conflict of Interests

The authors declare no conflict of interest.

## Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Keywords:** asymmetric synthesis • cascade • oxidases • one-pot • squaramide

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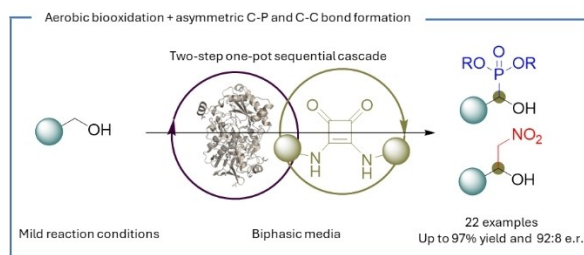
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The integration of organo- and enzyme catalysis in one-pot processes represents an efficient way to build complex molecular structures with high stereocontrol. This study demonstrates a sequential process involving enzymatic aerobic oxidation followed by squaramide-mediated asymmetric formation of C–P and C–C bonds to make important chiral synthons in

good yields and enantiomeric ratios under mild reaction conditions. This process operates within a biphasic system and represents a pioneering example of a chemoenzymatic cascade combining aerobic biooxidation and organocatalytic steps operating under non-covalent activation modes.

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**Chemoenzymatic One-Pot Cascade  
for the Construction of Asymmetric  
C–C and C–P Bonds via Formal C–H  
Activation**

