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Selective Oxidations of Organoboron Compounds Catalyzed by Baeyer–Villiger Monooxygenases

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Abstract: The applicability of Baeyer–Villiger monooxygenases (BVMOs) in organoboron chemistry has been explored through testing chemo- and enantioselective oxidations of a variety of boron-containing aromatic and vinylic compounds. Several BVMOs, namely: phenylacetone monooxygenase (PAMO), M446G PAMO mutant, 4-hydroxyacetophenone monooxygenase (HAPMO) and cyclohexanone monooxygenase (CHMO) were used in this study. The degree of chemoselectivity depends on the type of BVMO employed, in which the biocatalysts prefer boron-carbon oxidation over Baeyer–Villiger oxidation or epoxidation. Interestingly, it was discovered that PAMO can be used to perform kinetic resolution of boron-containing compounds with good enantioselectivities. These findings extend the known biocatalytic repertoire of BVMOs by showing a new family of compounds that can be oxidized by these enzymes.

Keywords: Baeyer–Villiger reaction; Baeyer–Villiger monooxygenases; boron; boron compounds; oxidation

Introduction

The chemo-, regio-, and stereoselective introduction of oxygen into organic molecules represents a key chemical transformation with broad applicability in organic synthesis.[1] These features have significantly increased the interest in exploring oxidative reactions catalyzed by enzymes. One of the most versatile class of enzymes that catalyzes a variety of oxidations are the Baeyer–Villiger monooxygenases (BVMOs): flavin-containing and NAD(P)H-dependent enzymes that catalyze the incorporation of one oxygen atom into organic substrates.[2] BVMOs are known for performing the oxidation of aldehydes and ketones to their corresponding esters, the oxygenation of heteroatoms (sulfur, nitrogen, phosphorus, boron, selenium) and even epoxidation reactions.[3] In many of these reactions, the transformations of substrates occur with high enantio- and/or regioselectivity, while using environment friendly conditions.[4]

A literature survey revealed to us that only very few boron compounds have been evaluated with monooxygenases aiming at carbon-boron bond oxidation to afford the corresponding alcohols.[3b,5] This is in contrast with the fact that boron-containing compounds are versatile intermediates in synthetic organic chemistry. A variety of chemical approaches has been reported to synthesize organoboron compounds.[6] In order to increase the synthetic potential of oxidative biocatalysts for the preparation of boron-containing compounds, in this work we explored four BVMOs, namely: phenylacetone monooxygenase (PAMO) from *Thermobifida fusca,*[7] its M446G PAMO mutant,[8] 4-hydroxyacetophenone monooxygenase (HAPMO) from *Pseudomonas fluorescens* ACB[9] and cyclohexanone monooxygenase (CHMO) from *Acinetobacter* sp.[10] Achiral aromatic and vinylic boron compounds as well as racemic ones have been evaluated as target substrates in BVMO-catalyzed oxidation reaction.

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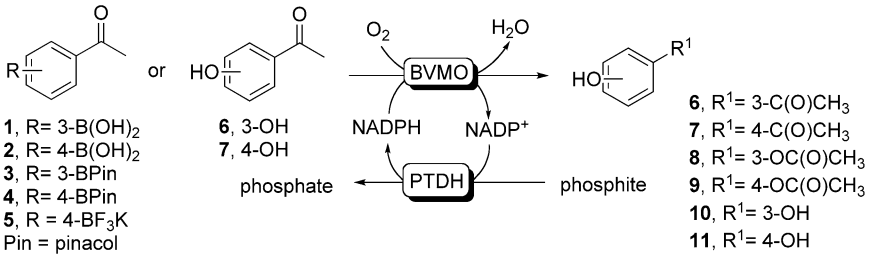
Results and Discussion

Initially, in order to evaluate the chemoselectivity between the C–B bond oxidation and the Baeyer–Villiger (BV) reaction, five boron-containing acetophenones (**1** and **2**: *meta*- and *para*-substituted boronic acids; **3** and **4**: *meta*- and *para*-substituted boronic esters; **5**: *para*-substituted potassium trifluoroborate) were selected as substrates. Reactions with 3- and 4-hydroxyacetophenones (**6** and **7**) were also performed as reference (Table 1). Purified BVMOs were used as biocatalysts in the oxidation reactions. Besides, a secondary enzymatic system containing phosphite dehydrogenase was employed for NADPH regeneration.

When PAMO was used as biocatalyst, the boron oxidation was observed for all substrates affording the corresponding phenols (**8**–**11**). However, the BV oxidation was achieved only for the 4-substituted substrates (Table 1, compounds **2**, **4**, **5** and **7**). All the oxidations led to only one final product with excellent conversions: 3-hydroxyacetophenone **6** for the 3-substituted compounds and 4-hydroxyphenyl acetate **9** for the 4-substituted ones. The M446G PAMO mutant presented a similar behavior for all substrates in which the boron oxidation was observed. However, for the 4-substituted compounds (**2**, **4**, **5** and **7**), the M446G PAMO mutant showed to be less effective for BV oxidation when compared with the wild-type enzyme, resulting in a final mixture of the ketones and the esters as indicated in Table 1 (entries 2, 4, 5 and 7). On the other hand, in all HAPMO-catalyzed reactions, both oxidations occurred (boron oxidation and BV oxidation). The esters **8** or **9** (3- or 4-hydroxyphenyl acetate, respectively) obtained from the BV oxidation undergo hydrolysis to give the corresponding catechols (resorcinol **10** or hydroquinone **11**, respectively). Finally, CHMO showed a high chemoselectivity in favor of boron oxidation, but low activity, affording only the 3- or 4-hydroxyacetophenones (**6** or **7**) with poor conversions. Besides the hydroxylated compounds that are obtained from a boron-carbon oxidation using BVMO, $B(OH)_3$ or HOBPin[3b,6] can be produced as by-products. In another set of reactions, we tested all BVMOs with 3- and 4-hydroxyacetophenones **6** and **7** as substrates with the aim of evaluating the BV reaction. The PAMO- and M446G PAMO-catalyzed oxidation of 3-hydroxyacetophenone **6** gave no BV reaction product, but it was observed for 4-hydroxyacetophenone **7**. These results are identical to those obtained with boron-containing acetophenones **1**–**5**. HAPMO was discovered to be an excellent biocatalyst for BV oxidation of 3- and 4-substituted acetophenones (hydroxy- or boron-substituted compounds **1**–**7**), which resulted in resorcinol **10** and hydroquinone **11** as products, respectively. Finally, when CHMO was employed as biocatalyst, no BV reaction was observed for 3- and 4-substituted acetophenones as substrates (compounds **1**–**7**).

However, CHMO was found to be highly chemoselective for C–B bond oxidation of boron-containing

Table 1. Oxidation of boron-containing acetophenones **1**–**5** and 3- and 4-hydroxyacetophenones catalyzed by BVMOs.[a]



Entry	Substrate	Products[b] (Yield) with BVMO			
		PAMO	M446G PAMO	HAPMO	CHMO
1	**1**	**6** (>99%)[c]	**6** (>99%)[c]	**10** (>99%)[c]	**6** (>99%)[c]
2	**2**	**9** (>99%)[c]	**7** and **9** (>99%, 1:4)[d]	**11** (>99%)[c]	**7** (>99%)[c]
3	**3**	**6** (>99%)[c]	**6** (>99%)[c]	**10** (>99%)[c]	–[e]
4	**4**	**9** (>99%)[c]	**7** and **9** (>99%, 5:4)[c]	**11** (74%)[d]	**7** (43%)[d]
5	**5**	**9** (>99%)[c]	**7** and **9** (>99%, 1:2)[d]	**11** (>99%)[c]	**7** (>99%)[c]
6	**6**	–[e]	–[e]	**10** (>99%)[c]	–[e]
7	**7**	**9** (>99%)[c]	**7**, **9** and **11** (>99%, 5:2:2)[c]	**11** (>99%)[c]	–[e]

[a] See Experimental Section.
[b] All products were obtained commercially or synthetically, they were then injected on GC and GC-MS for comparison purposes.
[c] This compound was detected as sole product by GC and GC-MS analysis, after extraction with organic solvent.
[d] Conversion was determined by GC and GC-MS analysis. The ratio between the products formed is also shown in brackets, when necessary.
[e] No reaction was observed.

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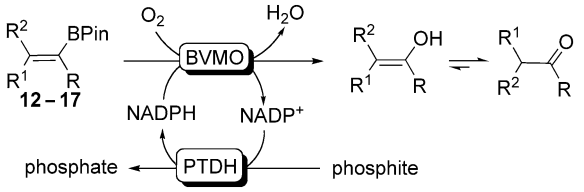
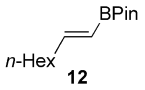
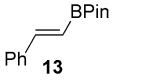
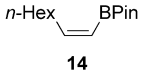
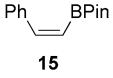
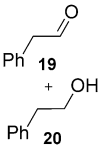
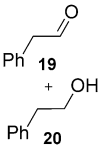
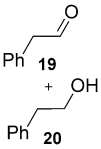
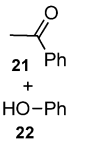
Table 2. Oxidation of vinyl boron compounds **12**–**17** catalyzed by BVMOs.[a]



Entry	Substrate	Products (Yield) with BVMO							
		PAMO		HAPMO		M446G PAMO		CHMO	
1	 **12**	–[b]		–[b]		–[b]		(–)[b]	
2	 **13**	–[b]		–[b]		–[b]		(–)[b]	
3	 **14**	–[b]		–[b]		–[b]		(–)[b]	
4	 **15**	 **18**	(>99%)	 **19** + **20**	(>99%, 3:2)[c]	 **18**	(>99%)[c]	 **19** + **20**	(>99%,1:4)[c]
5	 **16**	 **18**	(>99%)[c]	 **19** + **20**	(>99%, 7:3)[c]	 **18**	(>99%)[c]	 **18**	(>99%)[c]
6	 **17**	 **21**	(>99%)[c]	 **21** + HO–Ph **22**	(68%, 1:2)[c]	 **21**	(>99%)[c]	 **21**	(>99%)[c]

[a] See Experimental Section.
[b] No reaction was observed.
[c] Conversion was determined by GC and GC-MS analysis. The ratio between the products formed is also shown in brackets, when necessary.

acetophenones (substrates **1**, **2**, **4** and **5**) affording the corresponding hydroxyacetophenones in up to complete conversions after 24 h (Table 1, entries 1, 2, 4, and 5). These findings point out that BVMOs can catalyze the BV oxidation of 3- or 4-hydroxyacetophenones as well as boron-containing acetophenones. In order to identify the intermediates described above, the oxidation of the compounds **2** and **4** by BVMOs over time was monitored using GC and GC-MS analysis (1, 4, 8 and 24 h, see Supporting Information). For both compounds, the boron oxidation is the first reaction to give the corresponding 3- or 4-hydroxyacetophenones and boron by-products [$B(OH)_3$ or HOBPin].[3b,6]

With the aim of gaining a better view on the catalytic performance of BVMOs in the boron atom oxidation, we decided to perform steady-state kinetic studies for the M446G PAMO mutant with compounds **1** and **3** (see Supporting Information). This biocatalyst was chosen due to its high activity and exclusive chemoselectivity in favor of boron oxidation. The results revealed that the turnover rates (k_{cat} = 2.6 s^{-1} and 1.1 s^{-1} for boronic acid **1** and boronic ester **3**, respectively) were similar to those observed for the best substrates reported for the M446G PAMO mutant. The boronic acid **1** and boronic ester **3** are also well recognized by the enzyme (K_M = 1.4 and 0.4 mM for **1** and **3**, respectively).

We have also explored the BVMO-catalyzed oxidation of vinyl boron compounds **12**–**17** (Table 2). These reactions were performed in order to evaluate chemoselectivity of BVMO between boron oxidation and the possible epoxidation reaction.[3] It was observed that no epoxidation was achieved for any of the substrates. Moreover, no reaction at all was observed for the aliphatic boron compounds **12**, **13** and **14** used as substrates (Table 2, entries 1, 2 and 3). Nevertheless, all the aromatic compounds were transformed by the

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BVMOs, revealing the exclusive boron oxidation preference (Table 2, entries 4, 5 and 6). When PAMO and its M446G mutant were employed as biocatalysts in the oxidations of substrates **15** and **16**, benzyl alcohol **18** (entries 4 and 5) was obtained as sole product with complete conversion. For these enzymatic oxidations, phenylacetaldehyde was initially produced by a BVMO-catalyzed boron oxidation and then the BV oxidation and subsequent hydrolysis led to the final product, benzyl alcohol **18**. On the other hand, the HAPMO oxidation of compounds **15** and **16** as well as the CHMO oxidation of **15** led to a mixture of 2-phenylacetaldehyde (**19**) (a boron oxidation product), and surprisingly, to 2-phenylethanol (**20**), a reduction product of phenylacetaldehyde. In order to evaluate the role of CHMO and HAPMO in the above-mentioned reduction process, we carried out another set of enzymatic reactions, but excluding the respective BVMOs from the incubation mixtures. The results pointed out that such reductions did not occur. This suggests that the employed BVMO preparations presumably still contained trace amounts of a reductase from *Escherichia coli*, which can be the responsible biocatalyst for the reduction of phenylacetaldehyde to 2-phenylethanol. However, we cannot rule out a role of BVMOs in this reduction. While the enzymatic oxidation of *trans*-substituted vinyl boronic ester **15** catalyzed by HAPMO led to phenylacetaldehyde (**19**) as major product, the oxidation catalyzed by CHMO produced mainly 2-phenylethanol (**20**). The PAMO- and CHMO-catalyzed oxidations of *cis*-substituted vinyl boronic ester **16** allowed us to obtain exclusively benzyl alcohol (**18**).

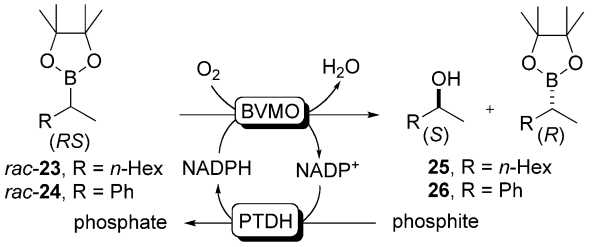
The reaction of *gem*-substituted vinyl boronic ester **17** with PAMO, M446G PAMO and CHMO led only to the respective boron oxidation product (acetophenone **21**). However, when the oxidation reaction was catalyzed by HAPMO some amount of phenol **22**, produced by hydrolysis of phenyl acetate (a BV oxidation product from acetophenone) was achieved. In order to identify the intermediates described above, additional reactions were carried out and monitored in time (after 1 and 4 h, see Supporting Information).

Based on the intriguing results above, which revealed that BVMOs can be effective in boron oxidations, we decided to evaluate the enzymatic kinetic resolution of chiral boron-compounds (**23** and **26**) catalyzed by PAMO (Table 3).

Compounds **23** and **24** were synthesized from the reduction of **14** and **17** catalyzed by Rh,[12] respectively. The oxidative kinetic resolution reaction was carried out by the same methodology applied for compounds **1–5** and **12–17** using pH 9.0. After 24 h no reaction was observed for 4,4,5,5-tetramethyl-2-(octan-2-yl)-1,3,2-dioxaborolane **23.**

However, the enzymatic oxidation of **24** showed excellent results in which the (*S*)-borane was oxidized to the corresponding (*S*)-alcohol. The (*R*)-borane was recovered in a process with a decent enantioselectivity ($E=23$)[13] and good conversion after 3 h. Moreover, in order to evaluate the pH influence in the kinetic resolution process, we performed the enzymatic reaction in a low pH (pH 7.5) (Table 3). The results revealed that the enantioselective behavior is superior at more neutral pH, as previously shown for this biocatalyst when performing the BV oxidation of racemic aldehydes and ketones.[14] (*S*)-**26** can be obtained with 91% *ee*, while a 49% conversion was achieved in a process with a good enantioselectivity ($E=33$).

Table 3. Enzymatic kinetic resolution of chiral boron-compounds (**23** and **24**) catalyzed by PAMO.



Entry	pH	Time [h]	Conv. [%]	*ee* (*S*)-**26**[a] [%]	*ee* (*R*)-**24**[a] [%]	E[b]
1	9	3	48	77	70	23
2	7.5	5	49	91	85	33

[a] The *ee* values were determined by HPLC analysis using a chiral stationary phase column. In the case of **24**, chemical oxidation (H_2O_2/NaOH) was performed and then the *ee* values were determined.

[b] The E values were calculated by using the formula $E=\ln[(1-ee_s)/(1+ee_s/ee_p)]/\ln[(1+ee_s)/(1+ee_s/ee_p)]$.

[c] Absolute configuration was determined by comparison with data described in ref.[11]

Conclusions

In summary, we have found that for a set of substrates with two oxidizable moieties, boron oxidation catalyzed by the studied BVMOs occurs rather than the BV oxidation or epoxidation reaction. Based on these promising synthetic properties of BVMOs concerning boron chemistry, one of the studied biocatalysts, PAMO, has also been examined in the enzymatic kinetic resolution of racemic boron compounds. This revealed that PAMO is very well suited to perform enantioselective boron oxidations, demonstrating for the first time that a BVMO can be employed in the kinetic resolution of boron compounds, affording chiral alcohols and chiral boron compounds in high enantiomeric excess. This study demonstrates that BVMOs may be very elegant tools in boron chemistry through their chemo- and stereoselective behavior.

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Experimental Section

General Procedure for Enzymatic Oxidation Reactions

To a flask (2 mL, Eppendorf®) containing a solution of the starting material (1 M in DMSO, 5 μL), Tris/Cl buffer (50 mM, pH 9.0 or 7.5, 440 μL), phosphite solution (500 mM, 20 μL), NADPH (100 mM, 10 μL), PTDH (100 μM, 5 μL) and enzyme (BVMO) (100 μM, 20 μL) were added. Reaction mixtures were shaken at 200 rpm and 30 °C (PAMO and M446G PAMO mutant) or 150 rpm and 24 °C (HAPMO and CHMO) for the times established. The reactions were then stopped, the mixture extracted with EtOAc (3×0.5 mL), dried over Na_2SO_4 and analyzed by GC, GC-MS or chiral HPLC. The absolute configurations of chiral compounds were established by comparison of HPLC chromatograms with the patterns described in previous experiments for the known configurations.[11] Control experiments in absence of enzyme were performed for all substrates tested, and no reaction was observed.

Steady-State Kinetics

Steady-state kinetic parameters for substrates **1** and **3** with M446G PAMO mutant were estimated using substrate concentrations from 0.5 mM to 5 mM, 0.1 mM of NADPH, 0.05 μM of BVMO in Tris/HCl buffer (pH 9.0) and 1% DMSO. Kinetic measurements by measuring the absorbance decrease at 340 nm were performed on a Perkin–Elmer Lambda Bio40 spectrophotometer at 25 °C. The obtained data were fitted using the SigmaPlot program, version 10.0 for Windows.

Acknowledgements

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