Graphical Abstract

Baeyer-Villiger monooxygenase-catalyzed desymmetrizations of cyclobutanones. Application to the synthesis of valuable spirolactones

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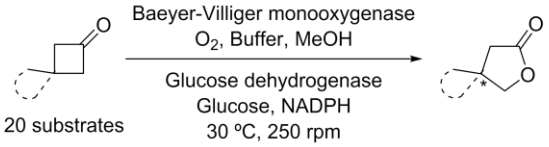

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Baeyer-Villiger monooxygenase-catalyzed desymmetrizations of cyclobutanones. Application to the synthesis of valuable spirolactones

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ABSTRACT

A series of γ-butyrolactone derivatives, including some spiranic ones, was obtained through desymmetrization of the corresponding prochiral 3-substituted cyclobutanones *via* Baeyer-Villiger monooxygenase (BVMO)-catalyzed oxidation. After reaction optimization using several commercial enzymes, both antipodes of various lactones were synthesized in most cases with >90% conversion and >80% enantiomeric excess under mild reaction conditions. In some cases alcohol formation was also observed (up to 40% conversion) as an undesired side reaction due to the presence of alcohol dehydrogenases in these preparations. Selected transformations were achieved on a 100 mg scale showing the possibilities of these oxidative biocatalysts as a new source of highly interesting compounds.

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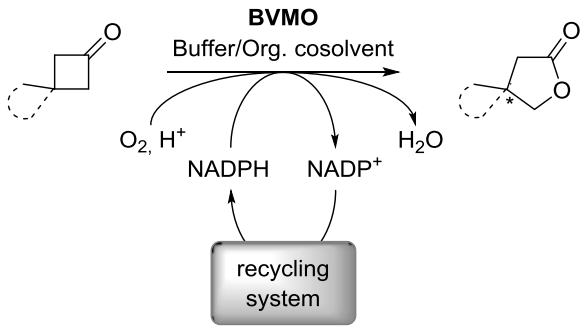
1. Introduction

Enantioenriched γ-butyrolactones are highly valuable compounds displaying a broad biological profile with applications as flavor and aroma constituents.[1] In addition, they also possess multiple uses as building blocks for polymers and natural product synthesis. As examples, γ-valerolactone is recognized as a potential intermediate for the production of fuels,[2] and 4-substituted analogues as precursors of analgesics,[3] GABA receptor inhibitors,[4] and lignans,[5] a family of compounds with broad range of activities including antiviral and antineoplastic.

The selective synthesis of spiro compounds is also highly appealing due to their interesting conformational features and structural implications on biological systems.[6] Hence, the spiro functionality is known to be present in a variety of phytochemicals such alkaloids or terpenoids.[7] In particular, spirolactones are important motifs in medicinal and natural product chemistry.[8] The intrinsic rigidity imparted by the spiro center, together with the latent lactone functionality, has been postulated as the source of these beneficial activities.

Among the different strategies to synthesize enantiopure γ-lactone derivatives, biocatalytic approaches have rapidly gained ground due to the excellent selectivities demonstrated by enzymes under mild reaction conditions.[9] Among them, hydrolase-catalyzed (dynamic) kinetic resolutions starting from the corresponding racemic lactones or hydroxy ester precursors,[10] and alcohol dehydrogenase-mediated stereoselective oxidations of racemic diols or reductions of keto esters can be cited.[11] Alternatively, the use of Baeyer-Villiger monooxygenases (BVMOs) to achieve the BV oxidation of racemic or prochiral cyclic ketones has also been studied to afford the enantiopure products.[12] This class of oxidoreductases is a promising family of biocatalysts able to perform the oxidation of carbonylic compounds as well as certain heteroatoms with excellent regio- and/or enantioselectivities, by simply using oxygen as final electron acceptor while releasing water as the only byproduct.[13] Thus, although both kinetic and dynamic kinetic resolutions of racemic ketones could also be performed, desymmetrization reactions of prochiral substrates represent one of the most appealing applications as they allow a theoretical yield of 100% of enantiopure products. More specifically, the desymmetrization of 3-substituted cyclobutanones *via* BVMO-catalyzed oxidation has been successfully performed to gain access to the corresponding enantioenriched γ-butyrolactones.[14]

As part of our ongoing interest related to the application of biological catalysts to obtain valuable compounds, herein we show the application of several commercially available BVMOs in the desymmetrization of prochiral cyclobutanones (Scheme 1), including also interesting spiro derivatives which, to the best of our knowledge, have not been previously studied under similar biocatalytic oxidative conditions. The optimization of the reaction conditions and the scale-up of the biotransformation have been considered as well.



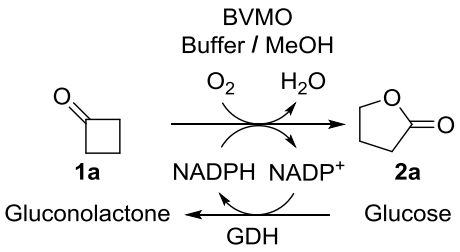
Scheme 1. General approach to enantioenriched 4-substituted γ-butyrolactones based on a BVMO-catalyzed desymmetrization process.

2. Results and discussion

Six different Baeyer-Villiger monooxygenases acquired from Codexis were studied towards the oxidation of cyclohexanone, a typical substrate for BVMOs. An initial enzyme activity screening was performed using conditions previously optimized in our research group,[15] which imply a 10 mM substrate concentration, use of co-solvent (DMSO, 1% v/v), NADPH (0.2 mM), glucose (20 mM), glucose dehydrogenase (GDH, 10 U) and phosphate buffer 100 mM pH 9. The reactions were initially carried out at 30 ºC and 250 rpm for 24 h in a 0.5 mL scale. In order to improve the obtained results, some reaction parameters were modified. Among them, addition of other prosthetic groups (FAD or FMN) and variations in the nicotinamide (NADH instead of NADPH), cofactor regeneration systems (glucose-6-phosphate/glucose-6-phosphate dehydrogenase or sodium formate/formate dehydrogenase instead of glucose/glucose dehydrogenase), buffer pH (7, 8, 9 or 10) or temperature (20, 30 or 40 ºC) can be named, but none of them improved the first reaction conditions. Remarkably, we noticed the formation of the corresponding alcohol at higher temperatures, due to the concomitant presence of alcohol dehydrogenase(s) in these enzymatic preparations. Interestingly, when different co-solvents were explored, better results were observed especially in the presence of methanol. For this reason, together with its lower boiling point compared to that of dimethylsulfoxide, which allows an easier elimination from the reaction media, methanol was the co-solvent selected from now on.

Once the reaction parameters had been optimized, our attention was focused on cyclobutanone (**1a**) for being the simplest substrate of the family. It turned to be highly reactive, affording conversions up to 98% into the corresponding lactone **2a** as can be seen in Table 1. Additional experiments were performed in order to contemplate the possibility of carrying out the biotransformations under stirring conditions. Hence, the biooxidation of cyclobutanone was performed on a 30 mg scale in the presence of BVMO-P1-D-08 using magnetic stirring, observing also a complete conversion at either 250 or 900 rpm (see conditions in Supporting Information).

Table 1. Activity of different BVMOs towards cyclobutanone (**1a**).[a]



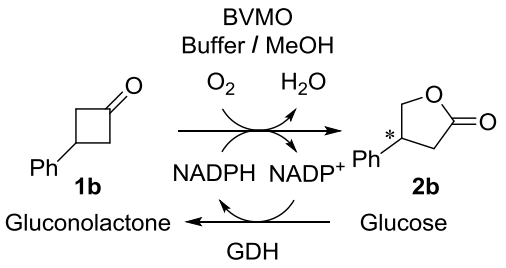
Entry	Enzyme	*c* (%)[b]
1	BVMO-P1-C06	88
2	BVMO-P1-D08	98
3	BVMO-P3-A10	19
4	BVMO-P3-A12	47
5	BVMO-P3-C07	52
6	CDX-003	2

[a] Reaction conditions (0.5 mL): Cyclobutanone (10 mM) in phosphate buffer 100 mM pH 9 with MeOH (1% v/v), NADPH (0.2 mM), glucose (20 mM), GDH (10 U) and BVMO (2 mg) for 24 h at 30 ºC and 250 rpm. [b] Determined by GC.

These promising results inspired us to employ these enzymes on useful compounds, as could be the synthesis of a broad family of enantioenriched (spiro)lactones starting from the corresponding cyclic ketones. To start with, cyclobutanone bearing a phenyl group at position 3 (**1b**)[16] was selected as model prochiral substrate. Under previously optimized conditions, the screening of the six BVMOs was thereby performed (Table 2). To our delight, a total conversion into the corresponding lactone (**2b**) was achieved in all cases, also reaching excellent enantiomeric excess for BVMO-P1-D08 and CDX-003 to obtain the (*R*)-enantiomer (entries 3 and 7). Interestingly, BVMO-P3-C07 showed an opposite selectivity although at lower extent (83% *ee*, entry 6). A more detailed study demonstrated that quantitative conversions were obtained after just 4 h of reaction, indicating the stability of the final product in the reaction media.

For comparison, other previous reports related to BVMO-catalyzed oxidations on substrate **1b** using purified or overexpressed enzymes, have been added in Table 2 (entries 8-19). As can be seen, earlier articles have employed lower substrate concentrations affording lactone **2b** with moderate to high yields and enantiomeric excess. As can be seen, cyclohexanone monooxygenases (CHMOs) coming from different sources[14e,f,g,h,j] allowed synthesizing the (*R*)-enantiomer, while other biocatalysts such as steroid monooxygenase (STMO, entry 10),[14j] cyclopentanone monooxygenase (CPMO, entry 14),[14g] and 4-hydroxyacetophenone monooxygenase (HAPMO, entry 19),[14d] afforded preferentially (*S*)-**2b**.

Table 2. Oxidation of 3-phenylcyclobutanone (**1b**) using commercial BVMOs.[a]



Entry	Enzyme	**1b** concentration (mM)	**2b** (%)[b]	ee_P (%)[c]
1	----[d]	10	0	----
2	BVMO-P1-C06	10	>99	11 (*R*)
3	BVMO-P1-D08	10	>99	98 (*R*)
4	BVMO-P3-A10	10	>99	7 (*S*)
5	BVMO-P3-A12	10	>99	53 (*S*)
6	BVMO-P3-C07	10	>99	83 (*S*)
7	CDX-003	10	>99	97 (*R*)
8	CHMO[e]	5	46	59 (*R*)
9	CAMO[f]	5	71	91 (*R*)
10	STMO[g]	5	72	76 (*S*)
11	CHMO[h]	n.f.	>90	83 (*R*)
12	CHMO[i]	3	45	93 (*R*)
13	CHMO[j]	3	73	98 (*R*)
14	CPMO[k]	3	66	37 (*S*)
15	CHMO[l]	n.f.	>90	62 (*R*)
16	CHMO[m]	n.f.	>90	96 (*R*)
17	CHMO[n]	3	54	87 (*R*)
18	CHMO[o]	3	63	50 (*R*)
19	HAPMO[p]	3	12	92 (*S*)

[a] Reaction conditions (0.5 mL): **1b** in phosphate buffer 100 mM pH 9 with MeOH (1% v/v), NADPH (0.2 mM), glucose (20 mM), GDH (10 U) and BVMO (2 mg) for 24 h at 30 ºC and 250 rpm. [b] Determined by GC. [c] Determined by HPLC. [d] Blank reaction with no enzyme. [e] Cyclohexanone monooxygenase from *A. calcoaceticus*; reference 14j. [f] Cycloalkanone monooxygenase from *C. radicicola*; reference 14j. [g] Steroid monooxygenase from *R. rhodochrous*; reference 14j. [h] Cyclohexanone monooxygenase from *Xanthobacter* sp.; reference 14h. [i] Cyclohexanone monooxygenase from *Brachymonas*; reference 14g. [j] Cyclohexanone monooxygenase from *Brevibacterium*, type 1; reference 14g. [k] Cyclopentanone monooxygenase from *Comamonas*; reference 14g. [l] Cyclohexanone monooxygenase from *Acinetobacter* sp.; reference 14f. [m] Cyclohexanone monooxygenase from *Acinetobacter* sp., mutant; reference 14f. [n] Cyclohexanone monooxygenase from *Arthrobacter* sp.; reference 14e. [o] Cyclohexanone monooxygenase from *Rhodococcus*; reference 14e. [p] 4-Hydroxyacetophenone monooxygenase from *P. fluorescens*; reference 14d. n.f.: Not found.

Prochiral 3-substituted cyclobutanones turned out to be quite reactive, most likely related to the liberation of ring-strain upon rearrangement and ring expansion. So, at this point, in order to enhance the process efficiency and synthetic applicability, we attempted to increase the substrate concentration up to 50 mM (Figure 1). However, in spite of maintaining the enantioselectivity, we came across to a dramatic decrease in the enzyme activity as well as the formation of the corresponding alcohol in appreciable amount (1-10%). Remarkably, a 73% of lactone **2b** was reached when the reaction with CDX-003 at 20 mM was allowed continuing for 48 h.

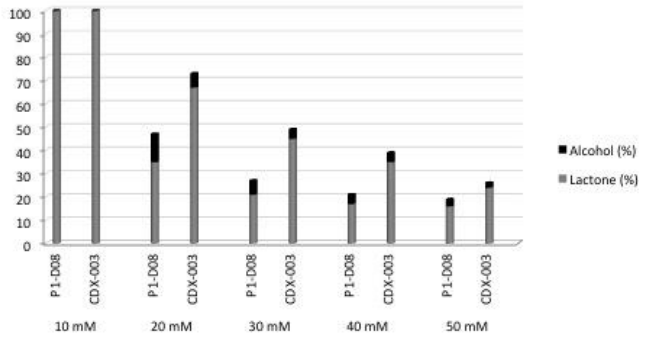


Figure 1. Evolution of lactone **2b** formation using different substrate concentration.

To discard the possibility of enzyme inhibition by the substrate, its addition was studied in two portions every 24 h, but results were even worse (61%). With the same aim, we also performed the biotransformations at 10 mM for 24 h employing a biphasic system using 10% v/v of heptane, observing that the enzymes were slightly deactivated, reaching high conversions (81-97%) and maintaining their enantioselectivity. As pH could change in the medium due to higher concentrations of glucose, and therefore formation of gluconic acid, we increased the strength of the buffer up to 200 mM, but a decrease in the enzyme activity was noticed (19% of **2b**). Finally, we considered the possibility of enzyme deactivation after a certain period of time. This problem should be avoided by adding fresh biocatalyst after 24 h, but a complete conversion could not be achieved either with an initial substrate concentration of 20 mM (84%) or the BVMO addition in two parts (74%).

In this sense, it was also studied the possibility of enzyme recycling by means of reusing the aqueous phase after extraction with heptane, which does not inhibit the biocatalyst at 10 mM substrate concentration (data not shown), but the conversion in the second cycle remained below 50%. With these results in hand, the strategy was extended to a broad panel of 3-substituted cyclobutanones (Figure 2).

Some of them were commercially available (**1f**,**g**,**i-k**), while other were synthesized through conventional chemical protocols (**1c-e**,**h**,**l-o**, see Supporting Information). Table 3 summarizes the best results found (see the Supporting Information for complete data).

Initially, the influence of different substituents in the phenyl ring at position 3 was analyzed (**1c-h**). Both *R*- and *S*-selective monooxygenases seemed to act better when they were placed at the *para* position, leading to almost complete conversions and high enantiomeric excess into **2e-h** (from 86 to 97%, depending on both substrate and biocatalyst). The *meta*-substituted compound **1d** offered worse results in terms of reactivity and the *orto*-bromo substrate **1c** led to lower conversion and stereoselectivity values in comparison with the 3-(4'-bromophenyl)cyclobutanone (**1e**). For instance, BVMO-P1-D08 and CDX-003 were able to achieve usually very high conversions and *ee* into the corresponding (*R*)-lactones, while BVMO-P3-C07 afforded the best results for *para*-substituted derivatives in order to gain access to the *S*-antipodes. We then compare our results with previous reports using purified or overexpressed BVMOs, finding that ketone **1f** was formerly studied. Hence, results obtained with P3-C07 and CDX-003 were comparable in terms of activity and selectivity with biotransformations using various CHMOs.[14g,h]

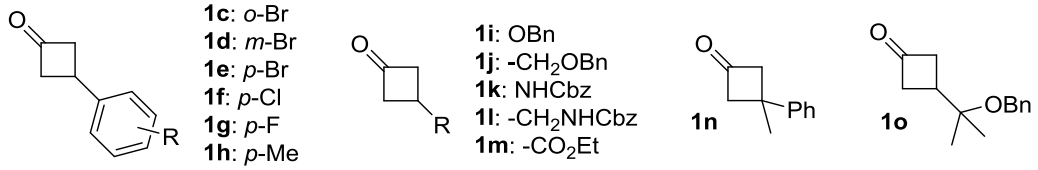


Figure 2. 3-Substituted cyclobutanones subjected to oxidation with commercial BVMOs.

Table 3. Best results found for BVMO-catalyzed oxidations of prochiral 3-substituted cyclobutanones under optimum conditions.[a]

Substrate	(*S*)-Lactone				(*R*)-Lactone			
	Ketone concentration (mM)	BVMO	Lactone (%)[b]	ee_P (%)[c]	Ketone concentration (mM)	BVMO	Lactone (%)[b]	ee_P (%)[c]
1c	10	P3-C07	51	87	10	P1-D08	95 (4)	89
1d	10	P1-C06	79	47	10	CDX-003	43	98
1e	10	P3-C07	95 (5)	97	10	P1-D08	99	93
1f	10	P3-C07	95 (2)	94	10	CDX-003	93	96
	n.f.	CHMO[d]	>90	81	2.2	CHMO[e]	47	87
	2.2	CHMO[f]	63	95				
1g	10	P3-C07	94 (4)	86	10	CDX-003	89 (11)	97
1h	10	P3-C07	99	86	10	CDX-003	98	87
1i[g]	10	P1-D08	>99	82	10	P3-C07	>99	77
1j	10	P1-C06	>99	54	10	P1-D08	>99	79
	2.4	CHMO[e]	26	55	2.4	CPMO[h]	53	63
	n.f.	CHMO[i]	>90	53	n.f.	CHMO[j]	>90	83
	n.f.	MO1[k]	98	74	3	HAPMO[l]	35	29
					n.f.	MO2[m]	95	90
1k[g]	10	P1-D08	29 (41)[n]	78	10	P1-C06	46 (43)[n]	32
1l	10	P3-A10	>99[n]	96				
1n	10	P3-C07	98	94	10	P1-D08	>99	93
1o[g]					10	P3-A10	>99	97

[a] Reaction conditions (0.5 mL): **1c-l,n,o** in phosphate buffer 100 mM pH 9 with MeOH (1% v/v), NADPH (0.2 mM), glucose (20 mM), GDH (10 U) and BVMO (2 mg) for 24 h at 30 ºC and 250 rpm. [b] Determined by GC analysis (alcohol formation in brackets). [c] Determined by chiral HPLC analysis. [d] Cyclohexanone monooxygenase from *Xanthobacter* sp.; reference 14h. [e] Cyclohexanone monooxygenase from *Brevibacterium*, type 1; reference 14g. [f] Cyclohexanone monooxygenase from *Rhodococcus*; reference 14g. [g] Change in Cahn-Ingold-Prelog (CIP) priority. [h] Cyclopentanone monooxygenase from *Comamonas*; reference 14g. [i] Cyclohexanone monooxygenase from *Acinetobacter* sp.; reference 14f. [j] Cyclohexanone monooxygenase from *Acinetobacter* sp., mutant; reference 14f. [k] Monooxygenase from *P. putida*, type 1; reference 14a. [l] 4-Hydroxyacetophenone monooxygenase from *P. fluorescens*; reference 14d. [m] Monooxygenase from *P. putida*, type 2; reference 14a. [n] Determined by ^{1}H-RMN. n.f.: Not found.

Then, our interest turned into the study of the behavior of these biocatalysts when a heteroatom such as oxygen or nitrogen, was introduced at the substituent of the prochiral cyclobutanone. On the one hand, oxygenated derivatives **2i** and **2j** could be obtained with BVMO-P3-C07, P1-C06 or P1-D08 with complete conversions but with moderate enantiomeric excess [from 54% for (*S*)-**2j** to 82% for (*S*)-**2i**]. In this sense, previous reports concerning BVMO-catalyzed oxidations on substrate **1j** have demonstrated that this substrate is usually converted with moderate selectivities.[14a,d,f,g] On the other hand, nitrogenated derivatives **1k** and **1l** showed several complications. Both ketones and lactones revealed solubility problems and also displayed very low GC signals. Hence, it was necessary to work at a higher scale in order to study the transformations through ^{1}H-NMR technique. When testing substrate **1k**, apart from a low conversion degree and enantioselectivity, a huge amount of the corresponding alcohol was detected, in some cases even higher than the lactone of interest. However, when compound **1l** was treated under the same conditions, complete conversions were achieved with all tested enzymes except CDX-003. Remarkably, (*S*)-lactone **2l** was formed in quantitative conversion and 96% *ee* using BVMO-P3-A10.

Additionally, and having in mind its possible synthetic applications, we tested the transformation over ethyl ester **1m**, but only traces of lactone **2m** could be detected due to hydrolysis of the substrate. We also synthesized the benzyl ester derivative seeking a higher stability towards hydrolysis, but similar results were found.

Next, we wondered if the set of Baeyer-Villiger monooxygenases could transform compounds bearing quaternary centers. With this aim, after synthesizing ketones **1n** and **1o** (see Supporting Information), and subjecting them to enzymatic oxidation, we came across with unexpected results. On the one hand substrate **1n**, with the 3-position quaternized, could be transformed into both enantiomers of the corresponding lactone either with BVMO-P3-C07 [(*S*)-**2n**, 92% conversion and 94% *ee*] or BVMO-P1-D08 [(*R*)-**2n**, >99% conversion and 93% *ee*]. On

the other hand substrate **1o**, with the quaternary center in an adjacent position, in spite of being able to react in a more enantioselective way (97% *ee*) towards the formation of the lactone (*R*)-**2o** assisted by BVMO-P3-A10, none of the biocatalysts in the set catalyzed the formation of the other enantiomer (*S*)-**2o**.

Once evaluated the efficiency of the enzyme set with this type of substrates, we decided to explore another family with an increased degree of complexity, the spiro compounds, with potential applications in medicinal chemistry (Figure 3).[17]

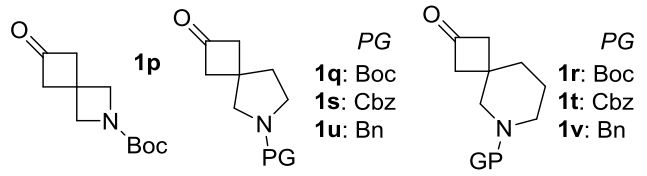


Figure 3. Family of spiro compounds subjected to oxidation with commercial BVMOs.

To begin with these unprecedented oxidations, we purchased the *N*-Boc protected compounds **1p**-**r**. To study if these spiro derivatives could be appropriate for our BVMOs, achiral compound **1p** was chosen as model substrate. Thus, employing the same conditions previously optimized, we found that only BVMO-P3-C07 could afford 90% of the lactone **2p** with a minimum amount of alcohol formation (5%), while the other monooxygenases remained below 80% with an alcohol formation up to 37% (see complete data in Supporting Information). In order to avoid the generation of such quantity of the reduction product, we attempted the use of an organic solvent (10% v/v) such as heptane, driving the reaction in a biphasic system, but the results were quite similar. The presence of heptane neither deterred or improved the activity of the enzyme, nor prevented the alcohol formation (data not shown). Then, we carried out the study with prochiral *N*-Boc spiro compounds **1q** and **1r**. Surprisingly, several biocatalysts led to more than 85% lactone formation, while only traces of the corresponding alcohol were detected (see complete data in Supporting Information). However, owing to the lack of a chromophore group, the enantiomeric excess of the final products could not be measured. For this reason, we decided to introduce other common protecting groups for amines, such as benzyloxycarbonyl (Cbz, **1s** and **1t**) and benzyl (Bn, **1u** and **1v**), which are more suitable for UV detection (see Supporting Information for detailed conditions).

When **1s** and **1t** were subjected to BVMO-catalyzed transformation in the same conditions, we realized that the effects of the enzymes were completely different on each substrate, regarding both conversion and selectivity (Table 4). With respect to **1s**, though in some cases conversions reached were acceptable, the enantiomeric excess of the lactones remained low-to-high. It can only be outlined the result with BVMO-P3-C07, which led to the lactone in 64% conversion and 86% *ee* with a minimum formation of the alcohol product. Regarding **1t** it is worth to remark that employing BVMO-P3-A10, an excellent conversion into lactone **2t** with high *ee* (87%) and only 2% of alcohol, was achieved.

Once benzylated ketones **1u** and **1v** had been synthesized, we realized the complexity of its chemical Baeyer-Villiger oxidation, as the product formed under the presence of the peracid was the *N*-oxide derivative (see Supporting Information). However, this reason confers additional value to our method employing biocatalysis. Thus, when ketone **1u** was subjected to enzymatic Baeyer-Villiger oxidation in the same conditions (Table 4), no product (neither starting material nor lactone) was detected by GC with most biocatalysts, probably due to the formation of the *N*-oxide, which remained in the aqueous phase during the extraction protocol. Luckily, BVMO-P3-C07 allowed the access to lactone **2u** with a conversion degree up to 79%, although the process proceeded with only moderate enantioselectivity (59% *ee*). Surprisingly, in spite of the *N*-oxidative competing reaction, results obtained with substrate **1v** were better, detecting the lactone formation in all cases (see complete data in the Supporting Information). Remarkably, BVMO-P3-C07 made possible the synthesis of lactone **2v** after 24 h with 82% conversion and 86% *ee*. Furthermore, BVMO-P1-D08 acted with total stereopreference, even though the side reaction still remained important.

Once the general studies came to an end, and aiming at a practical application, we thought about the possibility of scaling-up the process together with a reduction in the enzyme loading. In this sense, we handled 15-times higher scale reactions, keeping proportions in a linear manner (Figure 4). Initially, under identical conditions, we verified that the results could be reproduced in Erlenmeyer flasks thermostated at 30 ºC and 250 rpm. Even when the enzyme loading was reduced by a factor of two (2 mg/mL), complete conversions were achieved. However, when only 1 mg of enzyme per mL of reaction media was employed, it was not enough to transform all starting material after 24 h but the selectivity remained unaltered.

Table 4. Best results found for BVMO-catalyzed oxidation of spiro compounds under optimum conditions.[a]

Substrate	(+)-Lactone			(–)-Lactone		
	BVMO	Lactone (%)[b]	ee_P (%)[c]	BVMO	Lactone (%)[b]	ee_P (%)[c]
1s	P3-C07	64 (3)	86	P1-D08	66 (15)	68
1t	P3-C07	36	76	P3-A10	98 (2)	87
1u	P3-C07	79	59		n.f.	
1v	P3-C07	82	86	P1-D08	46[d]	>99

[a] Reaction conditions (0.5 mL): **1s-v** (10 mM) in phosphate buffer 100 mM pH 9 with MeOH (1% v/v), NADPH (0.2 mM), glucose (20 mM), GDH (10 U) and BVMO (2 mg) for 24 h at 30 ºC and 250 rpm. [b] Determined by GC analysis (alcohol formation in brackets). [c] Determined by chiral HPLC analysis. [d] Low peak intensity, loss of product in the aqueous phase. n.f.: Not found.

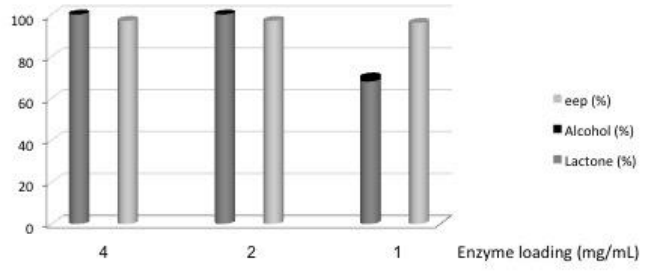


Figure 4. Optimization of the enzyme loading in 15x-scale biotransformations of **1b** with BVMO-CDX-003.

At this point, several selected Baeyer-Villiger oxidation reactions were scaled up to 100 mg employing half as much enzyme as in the screening protocols (Table 5 and Table S22), demonstrating the applicability of the process as almost complete conversions were reached for most of the studied substrates, and the stereoselectivity of the processes remained very similar. Furthermore, the enantioenriched pure lactones could be easily recovered after a simple extraction protocol when all starting material was consumed. In other cases, purification through column chromatography was necessary.

Table 5. Scale-up in the lactonization reaction of selected ketones.[a]

Substrate	BVMO	Lactone (%)[a]	Alcohol (%)[b]	ee_P (%)[c]
1b	P3-C07	81	19	82 (*S*)
	CDX-003	97 (90)	0	93 (*R*)
1n	P3-C07	87	0	94 (*S*)
	P1-D08	>99 (89)	0	92 (*R*)
1s	P3-C07	93 (77)	0	87 (+)
1t	P3-A10	99 (93)	0	86 (–)

[a] Reaction conditions: **1b**,**n**,**s**,**t** (100 mg, 10 mM) in phosphate buffer 100 mM pH 9 with MeOH (1% v/v), NADPH (0.2 mM), glucose (20 mM), GDH (1250 U) and BVMO (2 mg/mL) for 24 h at 30 ºC and 250 rpm. [b] Determined by GC analysis (isolated yields in brackets). [c] Determined by chiral HPLC analysis.

3. Conclusions

In summary, a series of commercially available BVMOs have been identified as efficient catalysts for the synthesis of enantioenriched lactones through desymmetrization of prochiral 3-substituted cyclobutanones. Thus, both enantiomers of several γ-butyrolactones could be obtained in many cases with high or excellent conversions and selectivities. These derivatives are highly important as they have found many applications in different fields as pharmaceuticals and fuel or polymer precursors, among others. While enzymatic (dynamic) kinetic resolutions of racemic lactones or hydroxy ester derivatives have offered interesting results, the desymmetrization of a prochiral precursor is an appealing process as it can give access to quantitative yield of an enantiopure product in the best case.

Herein, starting from various ketones and after optimization of the enzymatic reaction conditions, it has been demonstrated that several enzymatic preparations could be employed to efficiently synthesize chiral γ-butyrolactones substituted at the 4-position, comprising aromatic rings, (hetero)alkyl chains, and also spiranic compounds. In fact, to the best of our knowledge, this is the first report related to a biocatalytic process to get access to such heterocyclic derivatives. To obtain the (*S*)-enantiomers, BVMO-P3-C07 and BVMO-P1-C06 usually offered the best results, while for getting the (*R*)-antipodes, BVMO-P1-D08 and CDX-003 showed the best performances. These transformations show similar, or even better, performances that previous reports with these cyclobutanones in terms of activity and selectivity,[14] the possibility to carry out the biotransformations being practical at a higher substrate concentration.

It is noteworthy that when the ketone substrate was not well accepted by the BVMO, the corresponding alcohol was detected at some extent, probably due to the undesired presence of alcohol dehydrogenases in these preparations. In some cases biphasic systems were tested to improve conversions, and although enzyme inactivation was not observed at high extent, results could not be improved. Finally, efforts were focused on the scale-up of some of the biocatalytic oxidations, showing that reactions on a 100 mg scale could be easily carried out reducing the quantity of enzyme employed at smaller scale to obtain similar conversion and *ee* values. This study demonstrates a new sustainable alternative to produce interesting lactones which are not easily accessible by other conventional chemical methods.

4. Experimental part

4.1. General procedures

Chemicals and cyclobutanones **1a**,**f**,**g**,**i-k**,**p-r** were purchased from different commercial sources and were of the highest purity available. Baeyer-Villiger monooxygenases and glucose dehydrogenase (GDH-105) were acquired from Codexis. Diethyl ether and tetrahydrofuran used in chemical transformations were previously dried over sodium under inert atmosphere using benzophenone as indicator. Phosphoryl chloride ($POCl_3$) was distilled under reduced pressure before its utilization. The rest of reagents and solvents were employed without additional purification.

Chemical reactions were monitored by analytical TLC, performed on silica gel 60 F_{254} plates, and visualized by UV or revealed in a solution of potassium permanganate (1% $KMnO_4$, 5% K_2CO_3 and 5% NaOH in water) or *p*-anisaldehyde (2.5% *p*-anisaldehyde, 1% H_2SO_4 and 1% AcOH in ethanol). Flash chromatography was performed using silica gel 60 (230-400 mesh). IR spectra were recorded on an infrared Fourier transform spectrophotometer on NaCl pellets. NMR spectra were recorded at 300.13 (^{1}H), and 75.5 (^{13}C) MHz. The chemical shifts (δ) are reported in parts per million (ppm) relative to the solvent residual signals and the coupling constants (*J*) in Hertz (Hz). ESI-TOF mode was used to record high-resolution mass spectra (HRMS). Gas chromatography (GC) analyses were performed on a standard gas chromatograph equipped with a FID. HPLC analyses were performed using a standard HPLC chromatograph with UV detection. Optical rotations were measured using a standard polarimeter with a sodium lamp (D) and are reported in units of 10^{-1} deg cm^2 g^{-1}. Melting points were taken on samples in open capillary tubes and are uncorrected.

4.2. General method for the preparation of lactones 2b-t

To an ice-cooled solution of the corresponding cyclobutanone **1b-t** (0.25 mmol) in dichloromethane (1.0 mL), *m*-chloroperbenzoic acid (MCPBA, 88 mg, 0.50 mmol) was added, and the resulting mixture stirred for 20 h at room temperature. Then, more dichloromethane was added (5 mL) and the solution washed repeatedly with a saturated aqueous solution of sodium bicarbonate (6×5 mL). The resulting organic phase was dried over anhydrous sodium sulfate, filtered and the solvent evaporated under reduced pressure. Purification by column chromatography on silica gel (10-30% EtOAc/hexane) afforded

lactones **2b-t** in 38-99% yield (83% for **2b**; 72% for **2c**; 99% for **2d**; 85% for **2e**; 86% for **2f**; 88% for **2g**; 44% for **2h**; 96% for **2i**; 77% for **2m**; see below for **2j-l**,**n-t**).[18]

4.2.1. 4-((Benzyloxy)methyl)dihydrofuran-2(3*H*)-one (2j). 58% yield; yellow oil; IR (NaCl) ν 3061, 2913, 2859, 1777, 1495, 1479, 1454, 1421, 1366, 1266, 1174, 1101, 1023, 736 cm^{-1}; ^{1}H-NMR (300.13 MHz, $CDCl_3$) δ 2.37 (dd, ^3J = 6.3 Hz, ^2J = 17.7 Hz, 1H, C*H*H), 2.61 (dd, ^3J = 8.9 Hz, ^2J = 17.7 Hz, 1H, CH*H*), 2.77-2.91 (m, 1H, CH), 3.45-3.52 (m, 2H, CHCH_2O), 4.18 (dd, ^3J = 5.5 Hz, ^2J = 9.2 Hz, 1H, OCH*H*), 4.40 (dd, ^3J = 7.5 Hz, ^2J = 9.2 Hz, 1H, OCH*H*), 4.53 (s, 2H, OCH_2Ph), 7.27-7.39 (m, 5H, Ph); ^{13}C-NMR (75.5 MHz, $CDCl_3$): δ 31.3 (CH_2), 35.6 (CH), 70.5, 70.9 (OCH_2, CHCH_2O), 73.5 (OCH_2Ph), 127.8 (2 CH Ar), 128.0 (CH Ar), 128.7 (2 CH Ar), 137.8 (C_{ipso}), 176.9 (COO); HRMS (ESI^+, *m*/*z*): calcd for $C_{12}H_{15}O_3$ [$(M+H)^+$]: 207.1016, found: 207.1007.

4.2.2. Benzyl (5-oxotetrahydrofuran-3-yl)carbamate (2k). 43% yield; white solid; mp: 104-105 ºC; IR (NaCl) ν 3055, 2987, 1786, 1723, 1514, 1422, 1170, 1066, 1016 cm^{-1}; ^{1}H-NMR (300.13 MHz, $CDCl_3$) δ 2.43 (dd, ^3J = 2.9 Hz, ^2J = 17.8 Hz, 1H, C*H*H), 2.74 (dd, ^3J = 7.8 Hz, ^2J = 17.8 Hz, 1H, CH*H*), 4.16-4.19 (m, 1H, OC*H*H), 4.38-4.49 (m, 2H, OCH*H* + CH), 5.07 (s, 2H, OCH_2Ph), 5.84 (br d, ^3J = 3.6 Hz, 1H, NH), 7.28-7.37 (m, 5H, Ph); ^{13}C-NMR (75.5 MHz, $CDCl_3$): δ 34.8 (CH_2), 48.0 (CH), 67.2 (OCH_2Ph), 73.7 (OCH_2), 128.2 (2 CH Ar), 128.4 (CH Ar), 128.6 (2 CH Ar), 136.0 (C_{ipso}), 155.9 (NCOO), 175.6 (COO); HRMS (ESI^+, *m*/*z*): calcd for $C_{12}H_{14}NO_4$ [$(M+H)^+$]: 236.0917, found: 236.0897.

4.2.3. Benzyl ((5-oxotetrahydrofuran-3-yl)methyl)carbamate (2l). 38% yield; colorless oil; IR (NaCl) ν 3338, 3063, 3033, 2929, 1773, 1701, 1534, 1454, 1417, 1376, 1254, 1174, 1019, 738 cm^{-1}; ^{1}H-NMR (300.13 MHz, $CDCl_3$) δ 2.25-2.33 (m, 1H, C*H*H), 2.55-2.64 (m, 1H, CH*H*), 2.76-2.82 (m, 1H, CH), 3.24-3.28 (m, 2H, CH_2NH), 4.05-4.15 (m, 1H, OC*H*H), 4.33-4.39 (m, 1H, OCH*H*), 5.09 (s, 2H, OCH_2Ph), 5.21 (br s, 1H, NH), 7.29-7.42 (m, 5H, Ph); ^{13}C-NMR (75.5 MHz, $CDCl_3$): δ 32.1 (CH_2), 36.0 (CH), 43.0 (CH_2NH), 67.2 (OCH_2Ph), 71.0 (OCH_2), 128.3 (2 CH Ar), 128.4 (CH Ar), 128.7 (2 CH Ar), 136.2 (C_{ipso}), 156.8 (NCOO), 176.6 (COO); HRMS (ESI^+, *m*/*z*): calcd for $C_{13}H_{15}NNaO_4$ [$(M+Na)^+$]: 272.0893, found: 272.0889.

4.2.4. 4-Methyl-4-phenyldihydrofuran-2(3H)-one (2n): 93% yield; White solid; Mp: 49-51ºC; IR (nujol) 3024, 2969, 2930, 2904, 1773, 1601, 1497, 1305, 1173,1094, 1020, 767 cm^{-1}; ^{1}H-NMR (300.13 MHz, $CDCl_3$) δ 1.54 (s, 3H, CH_3), 2.68 (dd, ^2J = 16.8, ^4J = 0.4, 1H, C*H*H), 2.93 (dd, ^2J = 16.8, ^4J = 0.6, 1H, CH*H*), 4.39-4.47 (m, 2H, OCH_2), 7.18-7.23 (m, 2H, Ph), 7.27-7.33 (m, 1H, Ph), 7.36-7.42 (m, 2H, Ph); ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ 28.1 (CH_3), 42.1 (CH_2), 44.2 (C), 78.5 (OCH_2), 125.2 (2CH), 127.3 (CH), 129.1 (2CH), 144.4 (C), 176.2 (CO); HRMS (ESI^+, m/z): calcd for $C_{11}H_{12}NaO_2$ [$(M+Na)^+$]: 199.0730, found: 199.0781.

4.2.5. 4-(2-(Benzyloxy)propan-2-yl)dihydrofuran-2(3*H*)-one (2o): 75% yield; pale yellow oil; IR (NaCl) ν 3055, 2986, 1775, 1422, 1180, 1026, 706 cm^{-1}; ^{1}H-NMR (300.13 MHz, $CDCl_3$) δ 1.26, 1.27 (2 s, 6H, 2 CH_3), 2.44-2.54 (m, 1H, C*H*H), 2.65-2.77 (m, 3H, CH*H* + CH), 4.32-4.43 (m, 2H, OCH_2), 4.46 (s, 2H, OCH_2Ph), 7.25-7.37 (m, 5H, Ph); ^{13}C-NMR (75.5 MHz, $CDCl_3$): δ 23.0, 23.3 (2 CH_3), 29.7 (CH_2), 46.1 (CH), 63.9 (OCH_2Ph), 69.2 (OCH_2), 74.2 (*C*Me_2), 127.1 (2 CH Ar), 127.5 (CH Ar), 128.5 (2 CH Ar), 139.1 (C_{ipso}), 177.5 (COO); HRMS (ESI^+, *m*/*z*): calcd for $C_{14}H_{19}O_3$ [$(M+H)^+$]: 235.1329, found: 235.1329.

4.2.6. *tert*-butyl 7-oxo-6-oxa-2-azaspiro[3.4]octane-2-carboxylate (2p): 57% yield; white solid; mp: 154-155 ºC; IR (NaCl) ν 2977, 2880, 1786, 1692, 1470, 1406, 1366, 1323, 1286, 1250, 1165, 1150, 1097, 1023, 1016 cm^{-1}; ^{1}H-NMR (300.13 MHz, $CDCl_3$) δ 1.44 (s, 9H, ^{t}Bu), 2.76 (s, 2H, H-8), AB system (δ_A= 3.94, δ_B= 3.98, $^2J_{AB}$= 8.9 Hz, 4H, H-1 and H-3), 4.40 (s, 2H, H-5); ^{13}C-NMR (75.5 MHz, $CDCl_3$): δ 28.4 (3C, C-11), 37.3 (C-4), 39.3 (C-8), 59.0 (C-1 and C-3), 77.4 (C-5), 80.5 (C-10), 156.0 (NCOO), 174.8 (COO); HRMS (ESI^+, *m*/*z*): calcd for $C_{11}H_{17}NNaO_4$ [$(M+H)^+$]: 250.1050, found: 250.1097.

4.2.7. *tert*-Butyl 3-oxo-2-oxa-7-azaspiro[4.4]nonane-7-carboxylate (2q): 71% yield; white solid; mp: 65-67 ºC; IR (KBr) ν 1782, 1691, 1483, 1463, 1402, 1362, 1307, 1261, 1233, 1172, 1152, 1120, 1088, 1026, 1018, 854, 775 cm^{-1}; ^{1}H-NMR (300.13 MHz, $CDCl_3$) δ 1.45 (s, 9H, ^{t}Bu), 1.93-1.98 (m, 2H, H-9), 2.46-2.61 (m, 2H, H-4), 3.37-3.46 (m, 4H, H-6 and H-8), AB system (δ_A= 4.16, δ_B= 4.20, $^2J_{AB}$= 9.2 Hz, 2H, H-1); ^{13}C-NMR (75.5 MHz, $CDCl_3$): δ 28.5 (3C, C-12), 34.5, 35.6 (C-9), 38.7 (C-4), 44.5, 44.8 (C-8), 45.6, 46.5 (C-5), 54.2, 55.0 (C-6), 75.9 (C-1), 80.1 (C-11), 154.4 (NCOO), 175.4 (COO) (presence of rotamers); HRMS (ESI^+, *m*/*z*): calcd for $C_{12}H_{19}NNaO_4$ [$(M+Na)^+$]: 264.1206, found: 264.1201.

4.2.8. *tert*-Butyl 3-oxo-2-oxa-7-azaspiro[4.5]decane-7-carboxylate (2r): 63% yield; colorless oil; IR (NaCl) ν 3059, 3026, 2958, 2921, 1785, 1602, 1496, 1445, 1381, 1080, 764 cm^{-1}; ^{1}H-NMR (300.13 MHz, $CDCl_3$) δ 1.44 (s, 9H, ^{t}Bu), 1.60-1.65 (m, 2H, H-9), 1.67-1.69 (m, 2H, H-10), AB system (δ_A= 2.29, δ_B= 2.43, $^2J_{AB}$= 17.6 Hz, 2H, H-4), 3.34-3.40 (m, 4H, H-6 and H-8), 3.96 (d, ^2J = 9.3 Hz, 1H, H-1), 4.11 (d, ^2J = 9.3 Hz, 1H, H-1); ^{13}C-NMR (75.5 MHz, $CDCl_3$): δ 22.5 (C-9), 28.5 (3C, C-13), 33.8 (C-10), 38.6 (C-4), 40.4 (C-5), 43.5 (C-8), 51.2 (C-8), 75.4 (C-1), 80.4 (C-12), 154.7 (NCOO), 175.9 (COO); HRMS (ESI^+, *m*/*z*): calcd for $C_{13}H_{21}NNaO_4$ [$(M+Na)^+$]: 278.1363, found: 278.1360.

4.2.9. Benzyl 3-oxo-2-oxa-7-azaspiro[4.4]nonane-7-carboxylate (2s): 58% yield; colorless oil; $[\alpha]_D^{20}$= +11.6 (*c* 1, $CHCl_3$); IR (NaCl) ν 3054, 2984, 2889, 1782, 1701, 1420, 1361, 1267, 1170, 1109, 1029, 736 cm^{-1}; ^{1}H-NMR (300.13 MHz, $CDCl_3$) δ 1.97-2.04 (m, 2H, H-9), 2.41-2.63 (m, 2H, H-4), 3.41-3.58 (m, 4H, H-6 and H-8), 4.11-4.23 (m, 2H, H-1), 5.13 (s, 2H, H-11), 7.32-7.38 (m, 5H, Ph); ^{13}C-NMR (75.5 MHz, $CDCl_3$): δ 34.5, 35.6 (C-9), 38.6 (C-4), 44.7, 45.1 (C-8), 45.7, 46.6 (C-5), 54.7 (C-6), 67.3 (C-11), 75.8 (C-1), 128.2, 128.3, 128.7 (5C, Ph), 136.5 (C_{ipso}), 154.8 (NCOO), 175.2 (COO) (presence of rotamers); HRMS (ESI^+, *m*/*z*): calcd for $C_{15}H_{17}NNaO_4$ [$(M+Na)^+$]: 298.1050, found: 298.1079.

4.2.10. Benzyl 3-oxo-2-oxa-7-azaspiro[4.5]decane-7-carboxylate (2t): 51% yield; pale yellow oil; $[\alpha]_D^{20}$= −32.0 (*c* 1, $CHCl_3$); IR (NaCl) ν 2936, 2860, 1777, 1698, 1470, 1433, 1365, 1349, 1255, 1238, 1217, 1176, 1101, 1028, 763, 737 cm^{-1}; ^{1}H-NMR (300.13 MHz, $CDCl_3$) δ 1.53-1.61 (m, 2H, H-9), 1.67-1.71 (m, 2H, H-10), 2.27-2.45 (m, 2H, H-4), 3.35-3.55 (m, 4H, H-6 and H-8), 4.08-4.15 (m, 2H, H-1), 5.13 (s, 2H, H-12), 7.28-7.40 (m, 5H, Ph); ^{13}C-NMR (75.5 MHz, $CDCl_3$): δ 22.4 (C-9), 33.7 (C-10), 38.6 (C-4), 40.2 (C-5), 44.0 (C-8), 51.2 (C-6), 67.6 (C-12), 75.2 (C-1), 128.1 (2 CH Ar), 128.3 (CH Ar), 128.7 (2 CH Ar), 136.5 (C_{ipso}), 155.4 (NCOO), 175.7 (COO); HRMS (ESI^+, *m*/*z*): calcd for $C_{16}H_{20}NO_4$ [$(M+H)^+$]: 290.1387, found: 290.1394.

4.3. Alternative method for the preparation of lactones 2u and 2v

Owing to the availability of the free electron pair in the nitrogen atoms in substrates **1u**,**v**, the treatment with MCPBA led to their *N*-oxidation. In order to circumvent this problem, a partial solution for the synthesis of **2v** was found in the use of

Oxone® ($2KHSO_5.KHSO_4.K_2SO_4$, potassium peroxomonosulfate) as oxidizing agent. Unfortunately, **2u** could not be synthesized.

Thus, Oxone® (17 mg, 0.06 mmol) was added over a solution of the ketone **1v** (10 mg, 0.04 mmol) in a mixture of acetonitrile and water (10:1, 0.1 M) and stirred at room temperature for 36 h. Then, the solvents were evaporated and the product purified by column chromatography on silica gel (2-5% $MeOH/CH_2Cl_2$), affording lactone **2v** in 33% yield.

7-Benzyl-2-oxa-7-azaspiro[4.5]decan-3-one (2v). Yellow oil; IR (NaCl) ν 3054, 2931, 1774, 1453, 1421, 1377, 1352, 1180, 1158, 1075, 1019, 739 cm^{-1}; ^{1}H-NMR (300.13 MHz, $CDCl_3$) δ 1.25-1.68 (m, 4H, H-9 and H-10), 2.17-2.53 (m, 6H, H-4, H-6 and H-8), 3.44-3.54 (m, 2H, H-11), 4.04 (d, ^2J = 9.1 Hz, 1H, H-1), 4.20-4.23 (m, 1H, H-1), 7.23-7.31 (m, 5H, Ph); ^{13}C-NMR (75.5 MHz, $CDCl_3$): δ 22.9 (C-9), 33.8 (C-10), 39.9 (C-4), 40.4 (C-5), 53.5 (C-8), 61.4 (C-6), 63.0 (C-11), 76.7 (C-1), 127.4 (CH Ar), 128.5 (2 CH Ar), 128.9 (2 CH Ar), 138.3 (C_{ipso}), 176.9 (COO); HRMS (ESI^+, *m/z*): calcd for $C_{15}H_{19}NNaO_2$ [$(M+Na)^+$]: 268.1308, found: 268.1326.

4.4. Enzymatic procedures

4.4.1. General procedure for the BVMO-catalyzed oxidation of prochiral cyclobutanones 1a-j, m-v

In a typical experiment carried out in 1.5 mL tubes (total volume of 500 µL), the substrate **1a-j, m-v** (10 mM) was dissolved in methanol (5 µL, 1% v/v) and KPi buffer (100 mM, pH 9.0, 482 µL), containing glucose (20 mM), glucose dehydrogenase (GDH-105, 10 U, from stock solution of 1.275 U/µL), NADPH (0.2 mM, from a 20 mM stock solution) and the corresponding Baeyer-Villiger monooxygenase (2 mg). The mixture was shaken at 250 rpm at 30 ºC for 24 h. The reaction was stopped by extracting with diethyl ether (2×400 µL) and centrifuged at 13000 rpm in order to separate both phases and pellet the suspended protein. The organic phases were combined, dried over anhydrous sodium sulfate and analyzed by GC in order to determine the conversion values. Then, the solvent in GC samples was evaporated with a continuous flow of nitrogen, the residue re-dissolved in a mixture of hexane:ethanol 90:10 and the new sample filtered and analyzed by HPLC, leading to the measurement of the enantiomeric excess of the lactones. Control experiments in the absence of enzyme were performed for all substrates, not observing any reaction product after similar periods of time (Tables S1-S19).

4.4.2. Alternative procedure for the BVMO-catalyzed oxidation of prochiral cyclobutanones 1k and 1l

Owing to the problems that both ketones **1k,l** and their corresponding lactones **2k,l** showed when analyzed by GC, as well as their poor solubility in diethyl ether or ethyl acetate, their enzymatic transformations were carried out in a bigger scale (×3, 2 mL tubes) but maintaining identical reaction conditions. After 24 h, products were extracted with deuterated chloroform (2×400 µL). The organic phases were dried over anhydrous sodium sulfate, filtered and conversion values were determined by ^{1}H-NMR experiments. Similarly, samples were evaporated and the residue re-dissolved in a mixture of hexane:ethanol 90:10 for HPLC analyses (see Tables S20-S21).

4.4.3. Procedure for the BVMO-catalyzed oxidation at 100 mg scale of cyclobutanontes 1b, 1n, 1s and 1t

Prochiral ketones **1b, 1n, 1s** or **1t** (100 mg, 10 mM) were dissolved in methanol (1% v/v) and KPi buffer (100 mM, pH 9.0), containing glucose (20 mM), glucose dehydrogenase (GDH-105, 1250 U), NADPH (0.2 mM) and the corresponding Baeyer-Villiger monooxygenase (2 mg/mL). The suspensions were placed in Erlenmeyer flasks and shaken at 250 rpm in an orbital shaker at 30 ºC for 24 h. 1 mL aliquots were taken for conversion and enantiomeric excess analysis (same treatment previously described). The reactions were stopped by removing the solid protein through decantation and extracting the aqueous phase with diethyl ether (3×50 µL). The organic fractions were combined, dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure affording pure lactones when total conversion were achieved. When some starting product remained, an additional purification by column chromatography in silica gel was needed (50-100% EtOAc/hexane).

4.4.4. Procedure for the enzyme-catalyzed Baeyer-Villiger oxidation of cyclobutanone (1a) using magnetic stirring

In order to check whether mechanical stirring can influence the BVMO activity, cyclobutanone (**1a**, 30 mg, 10 mM) was dissolved in methanol (428 µL, 1% v/v) and KPi buffer (42.4 mL, 100 mM, pH 9.0), containing glucose (154 mg, 20 mM). Then, NADPH (7.1 mg, 0.2 mM), glucose dehydrogenase (GDH-105, 850 U) and BVMO-P1-D08 (86 mg, 2 mg/mL) were subsequently supplemented. The mixture was placed in a 250 mL round-bottom flask and shaken at 30 ºC at either 250 or 900 rpm with a magnetic stir bar for 24 h. After this time, complete conversions were achieved as demonstrated by GC analysis.

5. Acknowledgments

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Supplementary data

General information, synthesis of cyclobutanones, characterization data, extensive enzymatic screening results, and analytical data are provided.

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