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Exploiting the Biocatalytic Toolbox for the Asymmetric Synthesis of the Heart-Rate Reducing Agent Ivabradine

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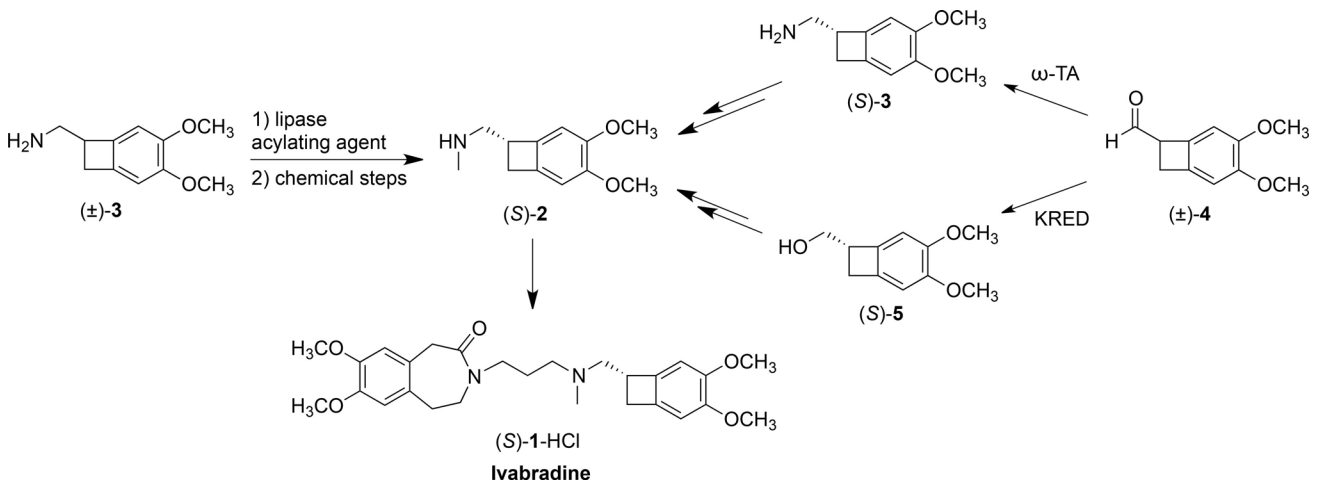
Abstract: Several chemoenzymatic routes have been evaluated for the production of the heart-rate reducing agent ivabradine. Lipases and ω-transaminases have been identified as useful biocatalysts for the preparation of key enantiopure precursors. The lipase-catalysed kinetic resolution by alkoxycarbonylation of a racemic primary amine and subsequent chemical reduction of the resulting carbamate provided an *N*-methylated (*S*)-amine, one step away from ivabradine. Alternatively, the dynamic kinetic resolution by asymmetric bioamination of an aldehyde precursor enabled, in a four-step sequence, the preparative scale synthesis of enantiopure ivabradine in 50% overall yield.

Keywords: amines; asymmetric synthesis; biotransformations; lipases; transaminases

Introduction

The conventional medical treatment of myocardial ischemia is essentially based on β-blockers, calcium channel blockers and nitrates.[1] However, some drawbacks to be circumvented concern: (i) the efficacy, which still needs to be improved as reflected by patients with angina refractory to the best present therapeutic associations, (ii) the side effects due, in particular, to the vascular effects of the drugs or other non-cardiac effects of β-blockers, (iii) safety, since the hemodynamic impact of the drugs can have severe consequences, and (iv) the insufficient prevention of mortality and major coronary events. Accordingly, and with the prospect of a higher impact of ischemic heart disease in an increasingly aging population, the discovery of new anti-ischemic drugs would be highly advisable. On the other hand, heart-rate (HR) reduction has proven to be particularly important in patients with chronic heart failure (CHF) who exhibit high values of HR in spite of the use of β-blockers. This constitutes a further reason to seek new therapeutic strategies aimed at HR modulation.

The cardiac pacemaker "funny current" (I_f) was described in cardiac pacemaker cells of the mammalian sino-atrial node (SAN) and its role in cardiac pacemaking has been investigated.[2] Thus, it has been demonstrated that the direct inhibition of the I_f current appears to be an ideal target to produce a selective reduction not only of resting HR but also of tachycardia. In this context, Servier developed a screening program aimed at identifying compounds able to inhibit the I_f current. Thus, from a series of benzocycloalkane derivatives, a benzocyclobutene emerged as the most effective, both enantiomers being equipotent in reducing the HR. After analysing other parameters, the (*S*)-enantiomer was the selected as a compound for clinical development and eventually became ivabradine (**1**, Procoralan®).[3] According to the patent literature, most synthetic approaches towards **1** use the secondary amine **2** as building block (Scheme 1).[4] Thus, once prepared as a racemate, the target (*S*)-**2** can be obtained *via* resolution with chiral acids or by preparative chiral HPLC. The former process involves several crystallisation cycles to achieve the enantiomerically pure amine, and the later is not practical for industrial-scale production.



Scheme 1. Chemoenzymatic roadmap towards ivabradine (**1**).

The excellent properties displayed by enzymes in terms of selectivity and reactivity under mild reaction conditions make biocatalysis an attractive alternative for the production of enantiopure building blocks and pharmaceuticals.[5] Thus, given the structural features of **2** and the availability of synthetic precursors, we envisaged three enzymatic approaches for the asymmetric key step: (a) the classical kinetic resolution (KR) through lipase-catalysed acylative processes of the primary amine (±)-**3**; (b) the asymmetric bioamination of the aldehyde (±)-**4** using ω-transaminases; (c) the asymmetric bioreduction of (±)-**4** mediated by ketoreductases (Scheme 1). Then, the selective follow-up chemistry in the isolated optically active compounds would enable a facile entry to the target (*S*)-**2** for its further transformation into ivabradine. Herein, we wish to report our findings on the development of chemoenzymatic routes towards this drug.

Results and Discussion

Lipase-Catalysed Acylation of (±)-3

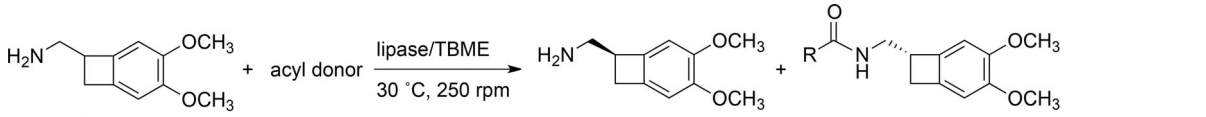
From the hydrolytic enzymes, lipases are the most popular biocatalysts and also the most useful in asymmetric synthesis.[6,7] Particularly, *Candida antarctica* lipases B and A (CAL-B and CAL-A) have been extensively applied to the resolution of primary and secondary amines, mainly those bearing the amino group in the stereocentre.[8] On the contrary, processes involving amines whose amino group is remote from the stereocentre, such as **3**, are rarely reported.[9] Thus, the lipase-catalysed resolution of (±)-**3** was firstly considered, by searching for a biocatalyst able to produce the target (*S*)-**3** in high optical purity. A representative set of commercially available, immobilised lipases was selected and screened for activity (2 mass equivalent enzyme, 30 °C, 250 rpm) in the aminolysis process under the simplest reaction conditions, namely employing ethyl acetate as acyl donor and solvent. Although the acylation took place in all cases, the enzymes showed significant differences in both activity and enantioselection (see Table S1 in the Supporting Information). Thus, half of the lipases led to conversions lower than 15% after 4 h while the other half were highly active and exceeded 50% conversion. Regarding selectivity, only five enzymes displayed modest enantioselection ($E = 6$–15), namely PPL, PSC-II, AK, PS IM and PS SD. It is worth noting the lack of enantiorecognition of CAL-B which historically is the most widely applied enzyme for the resolution of amines. On the other hand, all the biocatalysts showed stereopreference for the (*S*)-enantiomer of **3**, agreeing with the (*S*)-selectivity reported for lipases in the acylation of amines of the type R–CH_2–NH_2 and homochiral primary alcohols (R–CH_2–OH).[10] Likewise, the low enantioselectivity displayed towards **3** agreed with those measured with most of the amine and alcohol analogues.[9,11] Indeed, substrates bearing a remote stereogenic centre and without steric hindrance around the amine group could access easily the active site of the enzyme, accounting for a fast acylation but causing a detrimental effect on the enantiorecognition.[12]

Further attempts were aimed at optimising the enantioselectivity of the five best catalysts identified above, by testing the effect of different solvents and ethyl acetate as acyl donor (20 equiv.). These results revealed an important influence of the solvent on the biotransformation, leading again to modest enantioselectivity at best (see Table S2 in the Supporting Information). Likewise, conversion rates were, in general, lower than those in neat ethyl acetate due to the lower concentration of acyl donor. The best enzyme-solvent combinations were PS IM in THF and 1,4-dioxane, AK in 1,4-dioxane, and PSC-II in THF, with conversions close to 50%, and E up to 18.

A common approach to increase the selectivity of a lipase-catalysed acylation consists of changing the acyl donor. Accordingly, three activated esters such as methyl methoxyacetate, ethyl chloroacetate and α-methylbenzyl acetate were tested. The employment of the first two is widespread, leading to fast KRs with high enantioselectivity.[13] Regarding the last one, the influence of such a bulky leaving group on the enantioselectivity has proven beneficial.[14] TBME was the solvent chosen for this set of experiments, in combination with the previous enzymes (Table 1). Unfortunately, none of these esters led to satisfactory results, substrate and product being isolated with poor *ee* after 96 h (entries 1–12). Carbonates are another kind of acyl donor, with a long history in enzymatic reactions, among them aminolysis.[15] Thus, the study was extended to some commercially available carbonates such as diallyl carbonate, dibenzyl carbonate and diethyl carbonate (entries 13–24), with TBME as solvent. Pleasingly, diallyl carbonate provided good results in the PSC-II and PS IM-catalysed acylations (E up to 40) and the remaining (R)-amine was isolated with very high *ee* and at conversions close to 55% (entries 13 and 16). Similarly, dibenzyl carbonate also led to notable results with PSC-II and PS IM, although with slightly lower enantioselectivity ($E = 26$–27, entries 17 and 20). However, reactions performed with diethyl carbonate were less selective (entries 21 and 24).

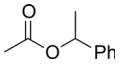
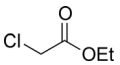
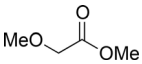
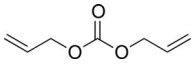
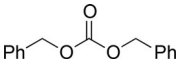
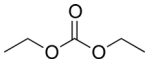
Taken together, these results identified carbonates as the best acyl donors for a chemoenzymatic ivabradine process. Importantly, the use of diethyl carbonate

Table 1. Lipase-catalysed acylation of (±)-**3** employing activated esters and carbonates.[a]



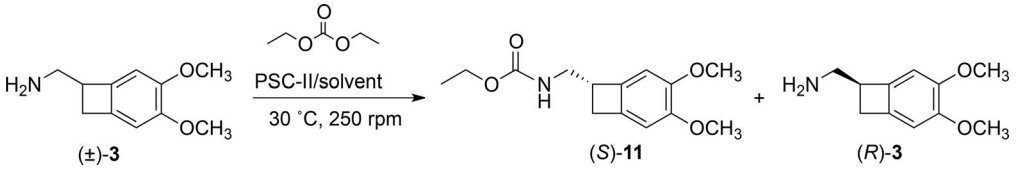
(±)-**3** (*R*)-**3**

(*S*)-**6** R= CH_3; (*S*)-**7** R= CH_2Cl;
(*S*)-**8** R= CH_2OCH_3; (*S*)-**9** R= $OCH_2CH{=}CH_2$
(*S*)-**10** R= OCH_2Ph; (*S*)-**11** R= OCH_2CH_3

Entry	Lipase	Acyl donor	Product	t [h]	c [%][b]	ee_s [%][c] of (R)-**3**	ee_p [%][c] of (S)-**6-11**	E[d]
1	PSC-II		**6**	96	62	61	37	4
2	AK		**6**	96	16	10	55	4
3	PS SD		**6**	96	21	21	78	10
4	PS IM		**6**	96	59	65	45	5
5	PSC-II		**7**	96	2	<1	4	1
6	AK		**7**	96	4	<1	<1	1
7	PS SD		**7**	96	2	<1	4	1
8	PS IM		**7**	96	2	<1	4	1
9	PSC-II		**8**	96	48	49	86	5
10	AK		**8**	96	30	23	53	4
11	PS SD		**8**	96	35	29	55	4
12	PS IM		**8**	96	53	60	53	6
13	PSC-II		**9**	24	59	>99	70	40
14	AK		**9**	24	14	12	74	7
15	PS SD		**9**	24	4	4	83	11
16	PS IM		**9**	24	52	92	84	36
17	PSC-II		**10**	24	57	97	73	26
18	AK		**10**	24	5	4	78	8
19	PS SD		**10**	24	<1	—	—	—
20	PS IM		**10**	24	33	44	89	27
21	PSC-II		**11**	24	16	18	90	22
22	AK		**11**	24	3	2	66	5
23	PS SD		**11**	24	<1	—	—	—
24	PS IM		**11**	24	12	11	85	13

[a] *Reaction conditions:* (±)-**3** (5 mg), acyl donor (10 equiv), lipase (10 mg), TBME (500 μL), shaking at 30 °C and 250 rpm.
[b] Deduced from the *ee* of the substrate (ee_s) and the product (ee_p): $c = ee_s/(ee_s + ee_p)$.
[c] The *ee* was determined by HPLC.
[d] Determined from *ee* of the substrate (ee_s) and the product (ee_p) as in ref.[16]

Table 2. PSC-II-catalysed alkoxycarbonylation of (±)-**3** with diethyl carbonate; Optimization of parameters.[a]



Entry	Solvent	[Substrate]	t [h]	c [%][b]	ee_s [%][c] of (*R*)-**3**	ee_p [%][c] of (*S*)-**11**	E[d]
1	TBME	0.05 M	24	28	34	87	20
2	THF	0.05 M	24	60	78	53	7
3	dioxane	0.05 M	24	10	10	86	15
4	toluene	0.05 M	24	10	9	77	8
5	TAA	0.05 M	24	23	24	81	12
6	2-Me-THF	0.05 M	24	38	58	92	45
7	CPME	0.05 M	24	30	40	92	34
8	CH_3CN	0.05 M	24	8	7	85	14
9	2-Me-THF	0.1 M	24	18	20	90	24
10	2-Me-THF	0.15 M	24	18	21	91	25
11	2-Me-THF	0.26 M	24	22	26	91	28
12	2-Me-THF	0.1 M	96	51	93	89	58
13	2-Me-THF	0.15 M	96	53	97	87	60
14	2-Me-THF	0.26 M	96	57	>99	76	69

[a] *Reaction conditions:* (±)-**3** (5 mg), acyl donor (10 equiv), PSC-II (5 mg), 30 °C and 250 rpm.
[b] Deduced from the *ee* of the substrate (ee_s) and the product (ee_p): $c = ee_s/(ee_s + ee_p)$.
[c] The *ee* was determined by HPLC.
[d] Determined from *ee* of the substrate (ee_s) and the product (ee_p) as in ref.[12]

led to (*S*)-**11**, a carbamate already implemented on the industrial scale.[4] Consequently, the optimisation study was narrowed to this acylating agent and PSC-II and PS IM lipases. In a first approach, a panel of solvents was screened to identify the optimal reaction medium for the aminolysis of (±)-**3** with diethyl carbonate (Table 2). PSC-II was particularly active in THF but led to poor selectivity. On the other hand, 2-methyltetrahydrofuran (2-Me-THF), provided not only good conversion ($c = 38\%$) but also a enantioselectivity increase ($E = 45$, entry 6).[17] Similarly, cyclopentyl methyl ether (CPME) was also a good option although both conversion and enantioselectivity were slightly lower (entry 7).[18] Once having identified PSC-II and 2-Me-THF as the most appropriate lipase and solvent, respectively, the substrate concentration effect on the biotransformation was evaluated since the previous attempts were conducted at high dilution (0.05 M). Hence, the acylation of (±)-**3** was tested to 0.1 M, 0.15 M and 0.26 M under the found optimal conditions (entries 9–14) and monitoring at 24 h and 96 h revealed a negligible impact on the KR outcome although the biotransformations occurred. Interestingly, enantioselectivity gradually increased along with conversion until a maximum value close to 50% conversion (entries 12–14). Thus, the experiment performed at 0.26 M proceeded with an optimised E value of 69, the remaining (*R*)-amine being isolated with >99% *ee* to 57% conversion (entry 14). The finding of a progressive increase of the enantioselectivity with the conversion degree was remarkable since the E value is inherent to each biotransformation and remains constant along the process. Likewise, this phenomenon was also observed in parallel studies performed with different combinations of lipases and acyl donors.

Taking into account the high load of lipase employed (100% w/w of enzyme/substrate) and to make the process more attractive, the recyclability of the biocatalyst was investigated (Table 3). Thus, the immobilised PSC-II was collected by filtration, washed with TBME and submitted to successive biotransformations. Gratifyingly, the lipase could be recycled for five cycles without loss of activity. After the initial

Table 3. Recyclability of PSC-II in the acylation of (±)-**3**.[a]

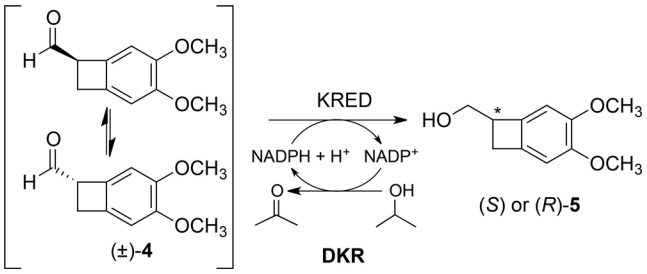
Entry	Cycle	c [%]	ee_s [%] of (*R*)-**3**	ee_p [%] of (*S*)-**11**	E
1	0	22	26	91	27
2	1	16	17	88	18
3	2	15	16	89	19
4	3	13	13	87	16
5	4	14	14	86	15
6	5	14	13	85	14

[a] *Reaction conditions:* (±)-**3** (50 mg), diethyl carbonate (10 equiv.), PSC-II (Amano) (50 mg), 2-Me-THF (1.0 mL), 24 h at 30 °C and 250 rpm.

biotransformation, the conversion rate remained almost constant (14–16%) along the five cycles, with a minor drop in the enantioselectivity.[19]

KRED-Catalysed Bioreduction of (±)-4

Despite being operationally simple and working well on a laboratory scale, the previous lipase-based approach suffers from two important drawbacks: (a) the recalcitrant 50% maximum yield inherent to KRs; (b) since the lipases exhibited moderate enantioselectivity and the target enantiomer of **3** was the acylated product [(*S*)-**3**], the biotransformation had to be quenched at 40% conversion to reach high *ee*, hampering even more the overall yield. Consequently, we turned our attention to another enzymatic approach, namely the bioreduction of the racemic α-chiral aldehyde (±)-**4** employing ketoreductases (KREDs). Since **4** is a racemate, a stereoselective reduction would lead to a 50% maximum yield *via* KR. However, the main feature of this precursor is the lability of its chiral centre which is in both a benzylic position and an α-position to a carbaldehyde unit. Thus, it is expected to spontaneously racemise in the buffer, triggering a dynamic kinetic resolution (DKR) with a 100% theoretical yield (Scheme 2). Actually, several optically active 2-arylpropanols have been obtained through a DKR of 2-arylpropanals catalysed by alcohol dehydrogenases.[20]

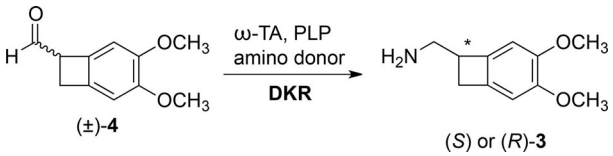


Scheme 2. Asymmetric bioreduction of (±)-**4** involving a dynamic kinetic resolution.

The bioreduction of (±)-**4** was tested using ketoreductases from the Codex® KRED Screening Kit with 2-propanol for cofactor recycling and as co-solvent. The initial screening was performed at pH 7.0 (Table S3 in the Supporting Information), enough to promote the desired racemisation. Thus, all the KREDs were very active and reached complete conversion towards the alcohol **5** after 24 h. However, the stereoselectivity was very disappointing with racemic **5** being isolated in all cases. Given the absence of enantiorecognition by this set of enzymes, we discarded further optimisation by medium engineering.

Transaminase-Catalysed Amination of (±)-4

Finally, the last enzymatic approach consisted in the bioamination of the aldehyde (±)-**4** employing ω-transaminases (ω-TA$_s$).[21] As explained above, the lability of **4** would enable a DKR process and led directly to the primary amine **3** in 100% yield (Scheme 3). In this case, the reported examples of bioamination of α-aldehydes *via* DKR processes are very scarce.[22]



Scheme 3. Asymmetric bioamination of (±)-**4** involving a dynamic kinetic resolution.

In a first set of experiments, the screening kit of ω-TAs from Codexis (Codex® ATA Screening Kit, v2) was tested at 30 °C in 100 mM phosphate buffer, at pH 7.5, supplemented with pyridoxal phosphate (PLP, 1 mM) and isopropylamine (1 M). Table 4 shows selected results of this screening (entries 1–6). Thus, all the ω-TAs were very active and (±)-**4** was totally consumed after 24 h. Regarding enantioselectivity, results depended on the biocatalyst but the DKR was successfully performed leading to both antipodes of the product, namely (*S*)-**3** and (*R*)-**3** with *ee* up to 67 and

Table 4. Asymmetric bioamination of (±)-**4**.[a]

Entry	ω-TA	pH	*c* (%)[b]	*ee* **3** (%)[b]
1	ATA-412	7.5	>99	58 (*R*)
2	ATA-P2A01	7.5	>99	76 (*R*)
3	ATA-117	7.5	>99	78 (*R*)
4	ATA-P2A07	7.5	>99	76 (*R*)
5	ATA-P1A06	7.5	>99	67 (*S*)
6	ATA-P1G06	7.5	>99	56 (*S*)
7	WTA716	10	86	77 (*S*)
8	WTA740	8	89	86 (*S*)
9	E120	8	100	68 (*S*)

[a] *Reaction conditions for entries 1–6:* (±)-**4** (25 mM) in 0.1 M KPi, pH 7.5 (500 μL, 1 mM PLP, 1 M *i*-PrNH$_2$), ω-TA (2 mg), DMSO (2.5%), 24 h at 30 °C and 250 rpm; *for entries 7 and 8:* (±)-**3** (10 mM) in 0.1 M KPi, pH 8.0 or 10.0 (500 μL, 1 mM PLP), L-alanine (5 equiv.), ω-TA (2 mg), DMSO (2.5%), 24 h at 30 °C and 250 rpm; *for entry 9:* (±)-**3** (25 mM) in 0.1 M KPi, pH 8.1 (500 μL, 0.5 mM PLP, 100 mM alanine), ω-TA (2 mg), DMSO (2.5%), 24 h at 30 °C and 250 rpm.

[b] Measured by HPLC.

78%, respectively. Similarly, other kits from Libragen and Daicel were tested. From the first one, two ω-TAs (WTA716 and WTA740) which use a 5-fold excess of L-alanine as amino donor were identified, displaying good enantioselectivity towards the target (*S*)-**3** with *ee* up to 86% and high conversion (entries 7 and 8). Regarding Daicel's kit, only one ω-TA (E120) turned out to be active and led to (*S*)-**3** with 68% *ee* (entry 9).

Once having selected the previous nine ω-TAs, the efforts were focused on optimising the reaction parameters. Particularly, the goal was the enantioselectivity enhancement, although other important aspects for process scale up such as substrate loading or substrate:enzyme ratio were also considered.[23]

The first parameter studied was the amount of enzyme. Initial screenings of ω-TAs were run with a 1:1 or 1:2 substrate:enzyme weight ratio. Thereafter, the gradually decreasing ratios were tested, such as 2:1, 4:1 and 10:1. Thus, ω-TAs from Codexis and Daicel at 2:1 and 4:1 ratios remained active and led to complete conversion and negligible changes in enantioselectivity after 24 h. On the contrary, the activity of the ω-TAs from Libragen drastically dropped even with a 2:1 ratio. Regarding the desirable 10:1 ratio, many side products were formed concomitantly to the product amine **3**. Next, the substrate concentration range was investigated, increasing from 25 mM to 100 mM. Thus, at 50 mM and 100 mM, some of the Codexis' enzymes worked well while ω-TAs from Libragen and Daicel rendered lower conversions and enantioselectivities.

Although (±)-**4** spontaneously racemises at pH 7.5, the pH profile was also optimised for the DKR. Thus, the bioamination was tested at pH 8.5 and 10 (LibraGen's enzymes were ruled out since they were previously applied in these optimal pH values). Moreover, these reactions were conducted in a 4:1 substrate:ω-TA ratio, which provided excellent results above. At pH 8.5, the ω-TAs reached complete conversion and the *ee* of **3** decreased in all cases with the exception of ATA-412, which notably showed a rise from 68% to 80% *ee*. Likewise, a basic medium such as pH 10 inactivated ATA-P2A07 and ATA-P1G06 while the rest preserved their activity. More interestingly, ATA-412 confirmed the previous improvement to higher pH with an enhanced value of 88% *ee*. Even more surprisingly, Daicel 's E120 reached an excellent 94% *ee* with complete conversion, although at a 1:1 substrate:enzyme ratio.

In order to test the influence of a co-solvent on the bioamination, DMSO was selected and added at 10% and 20% v/v (Table 5). In general, for the enzymes investigated it was observed that an increase of co-solvent led to improved *ee* values. This effect was especially remarkable in the case of WTA740 for which the optical purity of **3** increased from 86% *ee* at 2.5% v/v to >99% *ee* at 10 and 20% v/v with very high conversion (entries 10–12). In a similar fashion, the *ee* of **3** produced by E120 rose from 68% to >95% with quantitative conversion (entries 13–15).

Table 5. Influence of DMSO on the bioamination of (±)-**4**.[a]

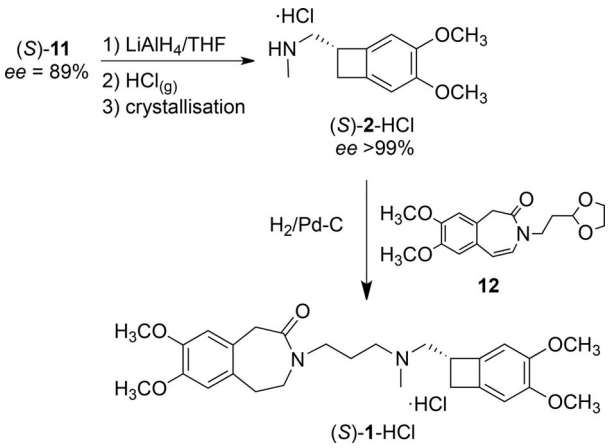
Entry	ω-TA	DMSO [%]	*c* [%][b]	*ee* of **3** [%][b]
1	ATA-412	2.5	>99	58 (*R*)
2	ATA-412	10	>99	76 (*R*)
3	ATA-412	20	>99	78 (*R*)
4	ATA-P1A06	2.5	>99	67 (*R*)
5	ATA-P1A06	10	>99	85 (*S*)
6	ATA-P1A06	20	>99	88 (*S*)
7	ATA-P1G06	2.5	>99	56 (*S*)
8	ATA-P1G06	10	>99	72 (*S*)
9	ATA-P1G06	20	>99	77 (*S*)
10	WTA740	2.5	89	86 (*S*)
11	WTA740	10	86	>99 (*S*)
12	WTA740	20	86	>99 (*S*)
13	E120	2.5	>99	68 (*S*)
14	E120	10	>99	>95 (*S*)
15	E120	20	>99	>95 (*S*)

[a] *Reaction conditions:* identical to those described in Table 4 for each kit.
[b] Measured by HPLC.

The synthetic applicability of the approach was demonstrated on a 150-mg scale (25 mM) for the bioamination of (±)-**4** catalysed by ATA-P1A06. This biocatalyst had exhibited high selectivity and robustness to various reaction conditions. Thus, using a 4:1 substrate:ω-TA ratio and 20% v/v DMSO, the amine (*S*)-**3** was obtained in 85% isolated yield and 90% *ee*, without the need of further purification. Likewise, WTA740 was also selected by the very high selectivity exhibited, despite demanding a higher load of biocatalyst. Thus, 100 mg of (±)-**4** (10 mM) were subjected to biocatalytic transamination using a 1:2 substrate:ω-TA ratio and 20% v/v DMSO. The reaction was quenched after 20 hours and reached a conversion of 89%. After work-up, enantiopure (*S*)-**3** was isolated with 75% yield.

Chemoenzymatic Synthesis of Ivabradine

The overall synthetic sequence towards **1** is depicted in Scheme 4. In the lipase-based approach, after the enzymatic alkoxycarbonylation of (±)-**3**, the resulting mixture formed by the carbamate (*S*)-**11** and the remaining amine (*R*)-**3** was separated by flash chromatography. For example, in a typical process described in Table 2 (entry 12), (*S*)-**11** was isolated in 89% *ee*



Scheme 4. Chemoenzymatic synthesis of ivabradine hydrochloride [(*S*)-**1**-HCl].

and 44% yield. Then, reduction of (*S*)-**11** with $LiAlH_4$ yielded the secondary amine (*S*)-**2** which was isolated as hydrochloride salt in 92% yield. Further crystallisation allowed us to achieve (*S*)-**2** with >99% *ee*. Finally, ivabradine hydrochloride [(*S*)-**1**-HCl] was obtained by one-step reductive alkylation of **2** with the protected aldehyde **12** in the presence of hydrogen and Pd/C catalyst. In parallel, the dehydrobenzazepine moiety is saturated to the tetrahydrobenzazepine structure during the process. A final crystallisation provided enantiopure (*S*)-**1**-HCl with an overall yield >30% for the three-step sequence.

Regarding the transaminase-base approach, the resulting primary amine (*S*)-**3** obtained in both scaled bioaminations was easily converted into the key synthon (*S*)-**2** by successive acylation with ethyl cloroformate and further reduction with $LiAlH_4$ in quantitative yield. Following the procedure depicted in Scheme 4, enantiopure ivabradine was isolated in 50% and 45% overall yield (without the need of chromatography) for the ATA-P1A06- and WTA740-catalysed processes, respectively.

Conclusions

Two chemoenzymatic syntheses of ivabradine, an alternative drug for the treatment of stable angina pectoris in cases of intolerance or contraindications for β-blockers, were developed. In the first approach, the key asymmetric step was the KR of the amine (±)-**3** *via* lipase-catalysed alkoxycarbonylation with diethyl carbonate. Further reduction of the resulting carbamate (*S*)-**11** provided the secondary amine (*S*)-**2** which was submitted to reductive alkylation with a benzazepine moiety. As a result, enantiopure ivabradine was isolated in 30% overall yield for the three-step sequence. On the other hand, the biocatalytic amination of the aldehyde (±)-**4**, catalysed by ω-TAs, emerged as a powerful alternative approach. Thus, the spontaneous racemisation of the aldehyde enabled a DKR process which tackled the yield hurdles of the lipase-based approach. Particularly, from the resulting amine (*S*)-**3** (90% *ee*), enantiopure ivabradine was reached by means of a four-step sequence with 50% overall yield without the need of chromatographic purification.

Experimental Section

General Remarks

Lipase B from *Candida antarctica* (CAL-B) was a kind gift from Novo Nordisk. Lipase A from *Candida antarctica* (CAL-A) was purchased from c-Lecta. Immobilised lipase from *Pseudomonas cepacia* (PSC-II) re-classified as *Burkholderia cepacia* lipase and lipase PS SD were purchased from Amano Pharmaceutical Co. Lipases from *Pseudomonas fluorescens* (AK), *Rhizomucor miehei* (Lipozyme RM IM), porcine pancreas (PPL), *Aspergillus niger* (ANL) and *Candida rugosa* (CRL) were purchased from Sigma–Aldrich. Kits of ω-TAs were purchased from Codexis, Libragen and Daicel, respectively. The kit of KREDs was purchased from Codexis. For the enzymatic reactions, commercially available solvents and acyl donors were used. Thin-layer chromatography was performed on precoated TLC plates of Merck silica gel $60F_{254}$, using potassium permanganate as developing reagent. For column chromatography, Merck silica gel 60 (particle size, 40–63 μm) was used. ^{1}H NMR and proton-decoupled ^{13}C NMR spectra were obtained using 300 MHz and 400 MHz spectrometers using the δ scale (ppm) for chemical shifts; calibration was made on the $CDCl_3$ (^{13}C; 76.95 ppm) or the residual $CHCl_3$ (^{1}H; 7.26 ppm) signals. HPLC analyses were performed using a normal phase Chiralpak IC column (Daicel).

Enzymatic Acylation of (±)-3 with Activated Esters and Carbonates

To a mixture of (±)-**3** (100 mg) and lipase (200 mg) under a nitrogen atmosphere, were added TBME (10 mL) and the corresponding acyl donor. The resulting mixture was shaken at 30 °C and 250 rpm for the time shown in Table 1. The enzyme was filtered off through a 2 cm pad of celite, washed with TBME and the solvent evaporated under reduced pressure. The crude material was purified by flash chromatography (hexane:AcOEt and AcOEt:MeOH mixtures) to obtain the enantioenriched (*S*)-amide or carbamate and the amine (*R*)-**3**. In order to prevent oxidation, the amine was isolated under a nitrogen atmosphere.

Bioreduction of (±)-4 (Analytical Scale)

In a 2.0-mL Eppendorf tube, KRED (2.0 mg) and (±)-**4** (20 mM) were added in phosphate buffer 125 mM, pH 7.0 (900 μL, 1.25 mM $MgSO_4$, 1 mM $NADP^+$) and *i*-PrOH (190 μL). The reaction mixture was shaken at 30 °C and 250 rpm for 24 h. To determine the conversion, 10 μL of the mixture were diluted with 90 μL of Mili Q water and analysed by achiral reverse phase with previous centrifuging

and filtering of the sample Then the mixture was extracted with ethyl acetate (2×500 μL), the organic layers separated by centrifugation (90 sec, 13000 rpm), combined and dried over Na_2SO_4. Then, the *ee* was measured on a chiral normal phase.

Bioamination of (±)-4 (Analytical Scale)

Representative example with ω-TAs from the Codexis' kit: In a 2-mL Eppendorf tube, ω-TA (2.0 mg) and (±)-**4** (2.4 mg) were added in phosphate buffer 100 mM, pH 7.5 (500 μL, 1 mM PLP, 1 M *i*-PrNH$_2$), and DMSO (12.5 μL). The reaction mixture was shaken at 30 °C and 250 rpm for 24 h. To determine the conversion, 10 μL of the mixture were diluted with 90 μL of Mili Q water and analysed on an achiral reverse phase column with previous centrifuging and filtering of the sample. Then, the reaction was quenched by addition of 50 μL of 1 N aqueous NaOH and extracted twice with AcOEt (2×400 μL). The combined organic phases were evaporated to dryness in a vacuum concentrator and diluted with 1 mL of ethanol. Then, the *ee* was measured on a chiral normal phase.

Bioamination of (±)-4 (Preparative Scale)

In a 50-mL Falcon tube, (±)-**4** (150 mg) was dissolved in DMSO (750 μL) and phosphate buffer 100 mM, pH 7.5 (30 mL, 1 mM PLP, 1 M *i*-PrNH$_2$) and ATA-P1A06 (38 mg) were added. The reaction mixture was shaken at 30 °C and 250 rpm and aliquots were taken periodically to measure the conversion. After 8 h, the reaction was stopped by addition of AcOEt (25 mL) and extracted with 3 N aqueous HCl (2× 25 mL). The combined aqueous phases were basified to pH 12 with 10 N aqueous NaOH at 0 °C and extracted with AcOEt (2×25 mL). The combined organic phases were dried over Na_2SO_4 and evaporated to dryness in a vacuum concentrator to yield pure (*S*)-**3**; yield: 85%; 90% *ee*.

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