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Lipase-catalyzed asymmetric acylation in the chemoenzymatic synthesis of furan-based alcohols

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ABSTRACT

Eight racemic 1-(furan-2-yl)ethanols were prepared from the corresponding carbonyl compounds for enantioselective acylation studies, and seven of them were used in preparative-scale kinetic resolutions with *Candida antarctica* lipase B (Novozym 435) and vinyl acetate in dried diisopropyl ether. Mechanism-based competition between the (*R*)-acetate (enzymatic acylation product), vinyl acetate (added acylating reagent), and acetic acid (enzymatic hydrolysis product) toward CAL-B, together with the residual water of the lipase were shown to be potential reasons for side reactions, which affected the course of the kinetic resolution of 1-[5-(2-chlorophenyl) and (4-bromophenyl)furan-2-yl]ethanols. Clear effects were not observed with the other alcoholic substrates. Alcoholysis of the enantiomerically enriched (*R*)-acetates with methanol and CAL-B in diisopropyl ether was shown to be a potential method for the deprotection of the (*R*)-acetates and the formation of (*R*)-alcohols.

1. Introduction

The reactivity of the aromatic furan ring in Diels–Alder cycloadditions, in reduction and oxidation reactions and in various ring opening reactions has made furans versatile building blocks for the synthesis of biologically and pharmacologically active compounds.[1] For instance, 5-aryl-2-vinyl furans have been transformed into furyl carbinols, which have been further applied to the synthesis of papulacandins;[2] glycolipid-antifungal agents which as 1,3-β-D-glucan synthase inhibitors affect the cell wall construction of fungal cells; and for the synthesis of 3-deoxy-D-manno-2-octulosonic acid,[3] vital in the growth and proliferation of Gram negative bacteria.[4] When a furyl-based structure is part of a chiral molecule, the preparation of a single enantiomer is often required, especially in the synthesis of pharmaceuticals and natural products.

The enzymatic kinetic resolution of a racemate with a suitable hydrolytic enzyme is one of the most commonly used biocatalytic methods for the preparation of enantiomers. Lipases (E.C. 3.1.1.3.) are especially attractive hydrolytic enzymes for the enantioselective acylation of racemic alcohols and for the enantioselective deacylation of the corresponding esters in organic solvents.[5] This is due to the high stability and enantioselectivity as well as good commercial availability of lipases in free and variously immobilized forms. Secondary alcohol functionalities have been subjected to lipase-catalyzed acylations, affording enantiomerically enriched resolution counterparts (one enantiomer as an unreacted alcohol and the other as an acylated product). The lipase-catalyzed acylation of benzofuranylethanols, 3-hydroxy-3-(furanyl)-propanoates as well as phenylfuran-based cyanohydrins and 3-hydroxypropanenitriles, are good examples related to the present work.[6] In these reactions, lipases such as *Candida antarctica* lipase B (CAL-B) as a Novozym 435 preparation (CAL-B adsorbed on a divinylbenzene-crosslinked hydrophobic methacrylate polymer), generally favor reaction of the (*R*)-enantiomer for alcohols with the large group at the asymmetric center having CIP priority over the medium size group, as is the case with (*R*)-1-phenylethanol.[7]

In the present chemoenzymatic work, racemic furan-based alcohols *rac*-**2a–h** were first prepared from the corresponding carbonyl compounds and then subjected to lipase-catalyzed asymmetric acylations in organic solvents, leading to the enantiomerically enriched resolution products (*R*)-**3a–h** and (*S*)-**2a–h** (Scheme 1). 1-(Furan-2-yl)ethanol *rac*-**2a** and 1-(5-bromofuran-2-yl)ethanol *rac*-**2b** were studied since they lack the phenyl substitution at the furan ring that is common to the other alcohols. Alcohols *rac*-**2b** (optimal behavior in kinetic resolutions) and *rac*-**2d** (anomalous behavior in kinetic resolutions) were used as model substrates. Specific rotations for (*R*)- and (*S*)-**2a** are known, and this together with the generally observed enantiopreferences of the lipases were used to confirm the absolute configurations of the resolution products

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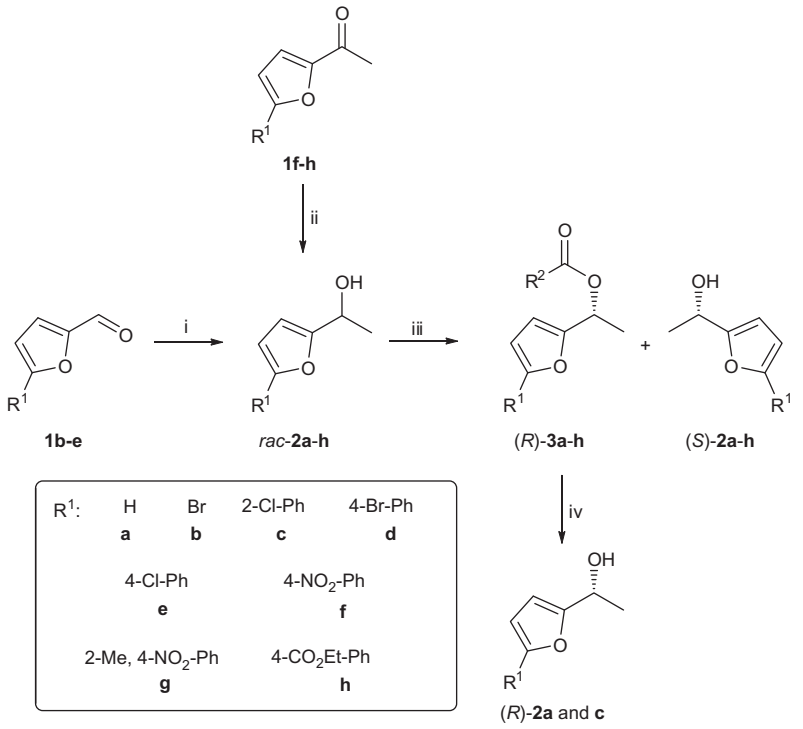


Scheme 1. Chemoenzymatic preparation of 1-furylethanols: (i) Grignard reaction with MeMgBr in Et_2O; (ii) reduction with $NaBH_4$ in ethanol; (iii) lipase-catalyzed kinetic resolution with $R^2CO_2R^3$; (iv) lipase-catalyzed alcoholysis with MeOH.

shown in Scheme 1.[7,8] We have also considered side-reactions which become possible when lipase-catalyzed acylations are performed in organic solvents. This kind of consideration is general especially when taking into account that the acyl donor and the acylated product might both be activated esters and as such might be subjected to hydrolysis by the so-called residual water (the water in the seemingly dry catalyst) of the lipase catalyst.

2. Results and discussion

2.1. Chemical synthesis

While *rac*-**2a** was a commercial product, alcohols *rac*-**2b–h** were prepared from the corresponding carbonyl compounds, usually with good chemical yields, using traditional methods [the Grignard reaction with MeMgBr (*rac*-**2b–e**) and reduction with $NaBH_4$ (*rac*-**2f**–**h**), Scheme 1]. Details of these syntheses can be found in the Section 4.

2.2. Enzymatic kinetic resolution

For the kinetic resolution, lipase screening with common commercial lipase preparations (10 mg mL^{-1}) was first performed using the acylation of *rac*-**2d** (0.05 M) with vinyl butanoate (0.1 M) as a model reaction in diisopropyl ether (DIPE, directly from a bottle, water content 800 ppm). Screening at room temperature (23 °C) gave CAL-B (Novozym 435) with $E = 27$ as the most enantioselective catalyst, while other lipases from *Burkholderia cepacia, Candida antarctica A, Pseudomonas fluorescens,* and *Thermomyces lanuginosus* gave E values ranging between 3 and 23. CAL-B was the most attractive of the enzymes; this was not unexpected since the lipase is known to catalyze the acylation of various 1-phenylethanols with excellent enantioselectivity.[9] The high enantioselectivity of CAL-B with such alcohol substrates was previously explained by the presence of the so-called stereospecificity pocket capable of accommodating groups smaller than propyl at the active site.[10] The screening results showed signs which were interpreted as hydrolytic side reactions, and accordingly the solvents were carefully dried before use in further studies. Alcohol *rac*-**2d** (in addition to other alcohols studied later) was not chemically acylated in the absence of the lipase under the otherwise identical reaction conditions.

Next *rac*-**2d** (0.05 M) was subjected to acylation conditions with vinyl butanoate (0.1 M) in dried DIPE at different CAL-B contents (from 2 to 20 mg mL^{-1}; Fig. 1). The acylation first proceeded rapidly at rates almost independent of the enzyme content until the sudden cessation in the formation of (*R*)-**3d** took place at 45–50% conversion. The acylation then continued slowly with time, although without the enantiomeric purification of the less reactive (*S*)-**2d**, as was expected. Evidently two separate processes are in-

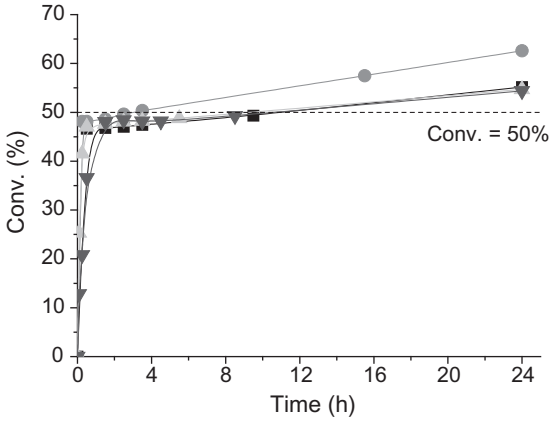


Figure 1. Formation of (*R*)-**3d** from *rac*-**2d** (0.05 M) with vinyl butanoate (0.1 M) in dry DIPE with CAL-B [2 mg mL^{-1} (▼), 5 mg mL^{-1} (▲), 10 mg mL^{-1} (■) and 20 mg mL^{-1} (●)].

volved. First, the slope of the slow stage clearly increased when increasing the lipase content. This stage, as observed for the acylation of all of the alcohol substrates, was later confirmed as the slow CAL-B-catalyzed acylation of the less reactive (*S*)-alcohols by subjecting (*S*)-**2a** (98% *ee*) to the CAL-B-catalyzed acylation conditions in dried DIPE. Second, the fast stage of the reaction of *rac*-**2d** finished too early. Moreover, the enantiomer ratio *E* (measure of enantioselectivity) for the acylation varied with conversion rather than being constant, going through a maximum value at around 45–50% conversion. This result meant that the Sih and Chen equation (see Section 4), commonly used to calculate *E* (the measure of enzymatic enantioselectivity), was not valid for the acylation of *rac*-**2d** (*E* is replaced as a suggestive '*E*' value at the given conversion in Table 1).[11] The very slow acylation of (*S*)-**2d**, although unlikely to be able to disturb the formation of (*R*)-**3d** at the fast stage of the reaction, cannot be completely ruled out as an explanation for the observed variation of *E* with conversion. Furthermore, it cannot alone explain the fact that the less reactive (*S*)-**2d** was not purified with conversion or that both $ee^{(S)\text{-}\mathbf{2d}}$ and $ee^{(R)\text{-}\mathbf{3d}}$ started to decrease after the fast stage of the acylation was over. Thus, a side-reaction connected to the amount of the residual water was suggested, leading to the need to keep the amount of the enzyme preparation low. Finally, 5 mg mL^{-1} of CAL-B (see the curve ▲) was chosen for further studies.

Table 1
Acylation of *rac*-**2d** (0.05 M) with an acyl donor (2 equiv) and CAL-B (5 mg mL^{-1}) in dried organic solvents

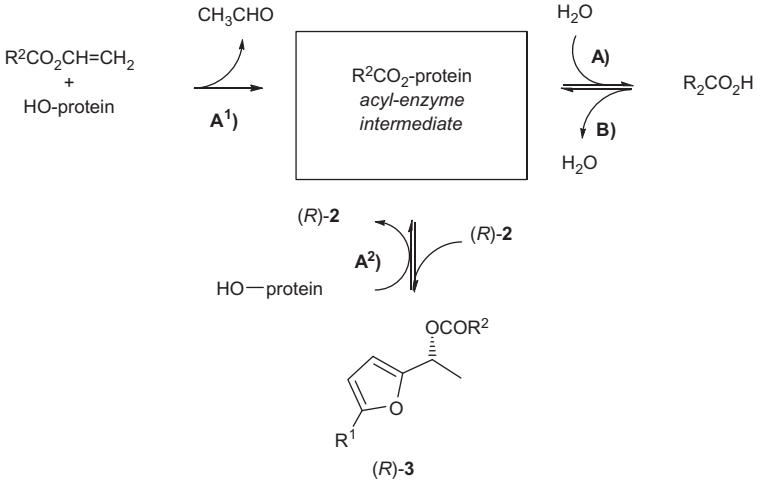
Entry	Solvent	Acyl donor	Time (h)	Conv.[a] (%)	$ee^{(R)\text{-}\mathbf{3d}}$ (%) [*E*']^{b}
1	DIPE	Vinyl butanoate	2.5	49	90 [50]
2	Toluene	Vinyl butanoate	3.5	48	86 [35]
3	TBME	Vinyl butanoate	3.5	49	85 [35]
4	Hexane	Vinyl butanoate	0.5	48[c]	87 [25]
5	DIPE	TFEB[c]	3.5	47	85 [26]
6	Ethyl butanoate		3.5	40	80 [15]
7	DIPE	Vinyl acetate	1.5	50	93 [90]
8	Toluene	Vinyl acetate	1.5	44	93 [60]
9	TBME	Vinyl acetate	1.5	47	92 [60]
10	Hexane	Vinyl acetate	1.5	51	92 [80]

[a] Calculated from the *ee*-values.
[b] $Ee^{(R)\text{-}\mathbf{3d}}$ at the given conversion; *E* value is inaccurate and marked as *E*'.
[c] 2,2,2-Trifluoroethyl butanoate.

When the acylation of *rac*-**2d** (0.05 M) carried out with vinyl butanoate (Table 1, entries 1–4), or with vinyl acetate (entries 7–10), was studied in four dried solvents (water contents 20–40 ppm), enantioselectivities according to the $ee^{(R)\text{-}\mathbf{3d}}$ values at the given conversions were high while the reaction with 2,2,2-trifluoroethyl butanoate in DIPE (TFEB, entry 5) and with ethyl butanoate as an acyl donor and a solvent (entry 6) gave low selectivities. On the basis of both the $ee^{(R)\text{-}\mathbf{3d}}$ and '*E*' values, vinyl acetate gave a slightly more enantioselective acylation than vinyl butanoate, with DIPE being the best solvent (entry 7). Thus, investigations with vinyl acetate in DIPE were continued, since vinyl acetate is also economically the most favorable choice. Vinyl esters were also preferred to make the acylation irreversible as $CH_2{=}CHOH$ liberated in step A[1] of Scheme 2 immediately tautomerizes to acetaldehyde.

For substrate scope studies, alcohols *rac*-**2a–h** were subjected to acylation with vinyl acetate in the presence of CAL-B (1 or 5 mg mL^{-1}) in dried DIPE (Table 2). Since the solubility of the alcohols varied from case to case, 0.1 M *rac*-**2a–c** and 0.05 M *rac*-**2d–h** were used. Furanyl alcohols *rac*-**2a** and **b** (entries 1 and 2) reacted rapidly and with excellent enantioselectivity (*E* >200). The acylation of phenylfuran-based alcohols mostly proceeded with good (*E* 90 to <200) to excellent (*E* >200) enantioselectivity, reaching 50% conversion without difficulty. However, the acylation of *rac*-**2c** and **d** (entries 3 and 4), although it proceeded with high $ee^{(R)\text{-}\mathbf{3}}$ values and with good enantioselectivity, tended to stop too early, with *E* tending to vary with conversion as already mentioned with *rac*-**2d**. It is worth noting that $ee^{(S)\text{-}\mathbf{2}}$ and $ee^{(R)\text{-}\mathbf{3}}$ values both stayed unchanged for at least 24 h when CAL-B was filtered off from the resolved mixture. Accordingly, the anomalous behaviors with *rac*-**2c** and **d** must be connected to the lipase catalysis.

Finally, the preparative-scale kinetic resolution of *rac*-**2a–d** and **f–h** (0.05 M or 0.1 M) with vinyl acetate (2 equiv) in the presence of CAL-B (1 or 5 mg mL^{-1}) was performed successfully in dried DIPE. The reactions proceeded as expected, and enantiomerically enriched unreacted (*S*)-**2a–d** and **f–h** together with the corresponding acetate products (*R*)-**3a–d** and **f–h** were separated mostly with good chemical yields by column chromatography (Table 3). However, in accordance with the previous kinetic resolution of *rac*-**2a**,[8] a decrease in the $ee^{(R)\text{-}\mathbf{3}}$ values was evident during the purification although it was possible to minimize the drops by adding triethylamine (0.5–1%) into the dichloromethane eluent (Table 3, compare columns 4 and 5). This decrease was exceptionally large (from 91% to 75%) with (*R*)-**3d** (entry 10). When silica (100 mg mL^{-1}) and (*R*)-**3d** (0.05 M, 56% *ee*) were mixed in dichloromethane, 0% *ee* was detected after one hour indicating that silica gel or dichloromethane was the reason for this decrease in *ee*. Since other solvents (e.g., ethyl acetate/hexane or petroleum ether/



Scheme 2. Competitive mechanistic pathways for the CAL-B-catalyzed acylation of *rac*-**2**.

Table 2
Acylation of *rac*-**2a–i** (0.05 M) with vinyl acetate (2 equiv) and CAL-B (5 mg mL^{-1}) in dried DIPE

Entry	Substrate	Time (h)	Conv. (%)	$ee^{(S)\text{-}\mathbf{2}}$ (%)	$ee^{(R)\text{-}\mathbf{3}}$ (%)	*E*
1	*rac*-**2a**[a]	2	50	98	99	>200
2	*rac*-**2b**[a]	2	50	98	99	>200
3	*rac*-**2c**[a]	2	39	60	96	—
4	*rac*-**2d**	1.5	49	91	93	—
5	*rac*-**2e**	1.5	48	78	83	25 ± 5
6	*rac*-**2f**	1.5	44	76	97	177 ± 21
7	*rac*-**2g**	1.5	50	95	95	150 ± 10
8	*rac*-**2h**	1.5	50	94	94	90 ± 13

[a] *rac*-**2** (0.1 M), vinyl acetate 0.2 M and CAL-B (1 mg mL^{-1}).

Table 3
Isolated yields after preparative-scale kinetic resolution of *rac*-**2** with vinyl acetate and CAL-B in DIPE

Entry	Product	Yield (%)	ee^{b} (%)	ee^{c} (%)	$[\alpha]_D^{25}$ (*c* 1, $CHCl_3$)
1	(*S*)-**2a**	42[a]	99	98	−20.8
2	(*R*)-**3a**	43[a]	98	92	+164
3	(*R*)-**2a**	82	99	99	+20.7
4	(*S*)-**2b**	45[a]	96	95	−20.1
5	(*R*)-**3b**	45[a]	95	94	+159
6	(*S*)-**2c**	48[a]	99	98	−1.6
7	(*R*)-**3c**	49[a]	98	90	+146
8	(*R*)-**2c**	76	99	99	+1.6
9	(*S*)-**2d**	21[a]	92	91	+5
10	(*R*)-**3d**	55[a]	91	75	+54
11	(*S*)-**2f**	28[a]	94	89	+2.3
12	(*R*)-**3f**	27[a]	96	90	+225
13	(*S*)-**2g**	46[a]	98	95	−2.3
14	(*R*)-**3g**	46[a]	94	88	+198
15	(*S*)-**2h**	48[a]	93	93	+5.8
16	(*R*)-**3h**	43[a]	94	86	+178.8

[a] Yield calculated from the racemic mixture.
[b] *Ee* in the resolution mixture.
[c] *Ee* after purification by column chromatography.

acetone) for the elution gave poor separations of (*S*)-**2** from (*R*)-**3**, dichloromethane was mostly used with rapid chromatography. Alcohol *rac*-**2e** was not used in the preparative scale studies owing to the poor enantioselectivity (*E* = 25; Table 2).

Alcoholysis studies in the presence of CAL-B (5 mg mL^{-1}) revealed that the reaction of *rac*-**3a** (0.1 M, *E* >>200) and **c** (0.1 M, *E* = 96) with methanol (0.5 M) proceeded with high enantioselectivity in dried DIPE. This method was used for the deprotection of the purified ester products (*R*)-**3a** (92% *ee*) and **c** (90% *ee*) to give (*R*)-**2a** and **c** with 99% *ee* at 83 and 76% isolated yields, respectively. The routine process, not repeated with the other (*R*)-esters, was expected to be usable in general.

2.3. Mechanism-based side-reactions

As discussed above, conversion tended to cease too early and *E* varied with conversion for the CAL-B-catalyzed acylation of *rac*-**2c** and **d**. With the other substrates, this behavior cannot be excluded although it was not detectable within experimental accuracy. This led us to consider reaction mechanism-based possibilities for side-reactions and the involvement of the residual water (or water otherwise in the system) for the acylation of *rac*-**2** in DIPE (Scheme 2). In the first step of the two-step reaction mechanism of serine hydrolases,[5] an acyl donor (here a vinyl ester $R^2CO_2CH{=}CH_2$) acylates the serine hydroxyl (Ser105 in CAL-B) at the active site in the formation of an ester intermediate (R^2CO_2-protein) called an acyl-enzyme intermediate and vinyl alcohol, the latter tautomerizing resulting in the formation of acetaldehyde. Accordingly, the step with vinyl esters is irreversible. In the second mechanistic step, any nucleophile [here preferably (*R*)-**2** and water] present at the active site may compete to react with the intermediate in the formation of the corresponding enantiomerically enriched (*R*)-**3** and R^2CO_2H. At high enough concentrations, both new products are potential acyl donors for the serine hydroxyl, that is, enzymatic hydrolysis of the vinyl ester through route A^1/A; enzymatic hydrolysis of (*R*)-**3** through route A^2/A; and enzymatic esterification of the carboxylic acid through route B can take place in addition to the desired acylation of (*R*)-**2**. Hydrolytic routes consume, while route B forms, water. The residual water plays a crucial role in initiating the hydrolytic process, while esterification serves as a continuous source of new water. We propose that for reactions in relatively hydrophobic organic solvents, the water produced by enzymatic esterification does not leave the active site. Instead, it effectively competes with (*R*)-**2** from the intermediate. This proposal is in accordance with the previous explanation for the high interesterification (also mechanistically an alcoholysis reaction) reactivities detected compared to the corresponding reactivities for alcoholysis in organic solvents.[5,12]

Lipase-catalyzed hydrolysis of activated acyl donors, such as the hydrolysis of vinyl esters and enantiomerically enriched acylated products by the residual water, is well recognized for reactions in organic solvents (Scheme 2).[13] It is clear that when the CAL-B-catalyzed hydrolysis of enantiomerically enriched (*R*)-**3** takes place through route A^2/A in the resolution mixture, both $ee^{(S)\text{-}\mathbf{2}}$ and $ee^{(R)\text{-}\mathbf{3}}$ decrease. The extent of the ester hydrolysis and esterification cannot be separately determined during the enzymatic acylation of *rac*-**2** whereas the total amount of the carboxylic acid in underivatized samples can be easily obtained by GC. When the CAL-B-catalyzed acylation of *rac*-**2** proceeded in DIPE, acetic (filled signs) or butanoic acid (open signs) was present in the reaction mixture depending on the acyl donor used. The amount of acetic acid was higher than that of the corresponding butanoic acid under otherwise identical conditions (Fig. 2). Acid concentrations increased with conversion and time, a burst occurring when the more reactive enantiomer (*R*)-**2** was nearly acylated at close to 50% conversion. The presence of the acid can affect the enantiomeric outcome of the lipase-catalyzed kinetic resolution, at least through esterification (route B) producing enantiomerically enriched (*R*)-**3** with its own enantioselectivity and by changing the pH of the microenvironment and accordingly the active conformation of the enzyme.

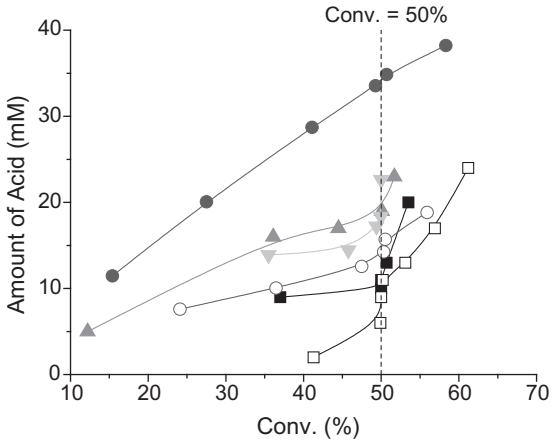


Figure 2. Acid formation in the acylation of *rac*-**2a-c** (0.1 M, *rac*-**2a** (■), *rac*-**2b** (●), *rac*-**2c**, (▲)) and *rac*-**2d** (0.05 M, ▼) with 2 equiv. of vinyl acetate (filled) or vinyl butanoate (open) in dried DIPE with CAL-B (1 mg mL^{-1} for *rac*-**2a-c**, 5 mg mL^{-1} for *rac*-**2d**).

Table 4
Esterification of *rac*-**2b** (0.1 M) and *rac*-**2d** (0.05 M) with acetic and butanoic acid in dried DIPE in the presence of CAL-B (1 mg mL^{-1})

Entry	Alcohol	RCO_2H	v_o (μmol min^{-1} mg^{-1})	Yield [(*R*)-**3**][a] (%)	$ee^{(R)\text{-}\mathbf{3b}}$ (%)
1	*rac*-**2b**	AcOH (0.05 M)	13.6 ± 0.7	10	98
2	*rac*-**2b**	AcOH (0.02 M)	6.9 ± 0.1	7	97
3	*rac*-**2b**	AcOH (0.01 M)	5.0 ± 0.4	4	95
4	*rac*-**2b**	AcOH (0.005 M)	2.5 ± 0.3	3	94
5	*rac*-**2d**	AcOH (0.05 M)	—	26	79
6	*rac*-**2b**	$PrCO_2H$ (0.05 M)	13.5 ± 0.3	11	98
7	*rac*-**2b**	$PrCO_2H$ (0.02 M)	4.4 ± 0.5	6	95
8	*rac*-**2b**	$PrCO_2H$ (0.01 M)	1.7 ± 0.3	3	98
9	*rac*-**2b**	$PrCO_2H$ (0.005 M)	0.7 ± 0.1	1	99
10	*rac*-**2d**[c]	$PrCO_2H$ (0.05 M)	—	22	85

[a] Yield of enantiomerically enriched (*R*)-**3**; reaction time 2 h.
[b] *ee* after the reaction of 2 h.
[c] CAL-B content 10 mg mL^{-1}; reaction time 1.5 h.

When the esterification of *rac*-**2b** was studied with the carboxylic acids and CAL-B (1 mg mL^{-1}) in dried DIPE, esterification took place even at 0.005 M acid concentrations (Table 4, entries 4 and 9). Reactivity (initial rate v_o) and ester yields of (*R*)-**3b** after 2 h, increased when the acid concentration increased (entries 1–4 and 5–9). Esterifications typically attained their equilibrium positions in the 2 h samples. The high $ee^{(R)\text{-}\mathbf{3b}}$ values (94-99%) indicated excellent enantioselectivity for the esterification**.** Thus, visible effects on the outcome of the highly enantioselective (*E* >200) acylation of *rac*-**2b** with vinyl esters were not detected (Table 2, entry 2). For the acylation of *rac*-**2d** (0.05 M) with the acids (0.05 M), the esterification yields of (*R*)-**3d** were doubled when compared to those of (*R*)-**3b** at the same time (for instance 26%, entry 5 vs 10%, entry 1); the values of $ee^{(R)\text{-}\mathbf{3d}}$ = 79% and 85% indicate moderate enantioselectivity for the esterifications (entries 5 and 10). These results suggest that depending on the structure of *rac*-**2**, ester hydrolysis/esterification side-reactions for lipase-catalyzed acylations in organic solvents may well stop the CAL-B catalyzed acylation too early and affect the enantiomeric outcome of the acylation with conversion.

Lipase promiscuity, also based on the catalytic properties of the active site, is well known to lead to C–C bond forming side-reactions for reactions in organic solvents and is thus noteworthy.[14] However, the formation of new compounds other than the ester products (*R*)-**3** were not observed after chromatography for the CAL-B catalyzed acylation of *rac*-**2**.

3. Conclusions

The CAL-B-catalyzed kinetic resolution of racemic 1-(furan-2-yl)ethanols has been studied in dried diisopropyl ether. Seven enantiomerically enriched counterparts, (*S*)-**2** as the unreacted alcohol enantiomers and (*R*)-**3** as the produced acetate enantiomers were prepared.

Acylations of *rac*-**2** with vinyl esters in DIPE revealed two noteworthy aspects. First, the progression curves showed a rapid stage when mainly the (*R*)-alcohols reacted, which was followed by a slow stage when the less reactive (*S*)-enantiomers reacted. Second, progress for the kinetic resolution of 1-[5-(2-chlorophenyl)furan-2-yl]ethanol *rac*-**2c** and 1-[5-(4-bromophenyl)furan-2-yl]ethanol *rac*-**2d** ceased at 45–50% conversion, while *E* varied with conversion and $ee^{(S)\text{-}\mathbf{2c/d}}$ was not further purified. This behavior was anomalous to the above. With the other alcohols studied, the reactions did not undergo this behavior. We concluded that mechanism-based side-reactions, initiated by the residual water of CAL-B and leading to complicated enzymatic ester hydrolysis/esterification equilibria (Scheme 2) might be a potential reason for the anomalous behavior. We have shown that acetic (or butanoic) acid as an enzymatic hydrolysis product, was present in the reaction mixture and that CAL-B-catalyzed the enantioselective esterification between the acid and *rac*-**2** and proceeded even at very low acid concentrations (0.005 M) with possible effects on kinetic resolution.

4. Experimental part

4.1. Materials and methods

Candida antarctica lipase B (CAL-B) as a Novozym 435 preparation was from Novo Nordisk. Methanol and other solvents used were of the highest analytical grade from J.T. Baker, Lab-Scan Ltd, Aldrich and Mallinckrodt. Vinyl butanoate and 1-(furan-2-yl)ethanol *rac*-**2a** were purchased from Fluka, vinyl acetate and the aldehydes from Aldrich. MeMgBr (3 M in diethyl ether) and aldehydes **1b–e** were products of Aldrich. For analytical purposes and for alcoholysis experiments, the esters (acetates **3**, propanoates **4,** and butanoates **5**) of *rac*-**2a–h** were prepared from the corresponding acid anhydride and alcohol using traditional methods.

The progress of the enzymatic acylation of *rac*-**2** was followed by taking samples (50 or 100 μL) at intervals and analyzing them with an Agilent 6850 GC/FID equipped with a Chrompack CP-Chirasil-DEX CB column (25 m × 0.25 mm) (Table 5). The unreacted (*S*)-**2** in the samples (50 or 100 μL) was derivatized as ester **3–5** with the corresponding anhydride (5–10 μL) in the presence of 4-dimethylaminopyridine (DMAP, 1% in pyridine) prior to injection to achieve good baseline separation. Underivatized samples were separately injected in order to determine the acetic and butanoic acid contents in the samples against an internal standard.

The ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance 500 Spectrometer at 25 °C. Tetramethylsilane (TMS) was used as an internal standard in chloroform ($CDCl_3$). Mass spectra (MS) were measured on a VG ZabSpec mass spectrometer (EI+). DIPE was dried by refluxing with CaH_2 followed by distillation before use or by molecular sieves (4 Å) to attain the water content of 20-40 ppm. Water contents were measured using a Metrohm 831 KF Coulometer. The determination of '*E*' was based on the equation $E = \ln[(1-c)(1-ee_S)]/\ln[(1-c)(1+ee_S)]$ (ee_S is the enantiomeric excess of the unreacted substrate at conversion *c*) and *E* values on the use of linear regression (*E* as the slope of the line $\ln[(1-c)(1-ee_S)]$ versus $\ln[(1-c)(1+ee_S)]$ and).[11] Analytical thin layer chromatography (TLC) was carried out on Merck Kieselgel $60F_{254}$ sheets. Spots were visualized by treatment with 5% ethanolic phosphomolybdic acid solution and heating. Chromatographic separations were performed using column chromatography on Kieselgel 60 (0.063–0.200 μm). Optical rotations were determined with a Perkin Elmer 341 Polarimeter (*c* 1, $CHCl_3$,

Table 5
GC methods and retention times for compound analysis.

R^1	R^2 in 3-**4a–h** /acid	t_R (*S*/*R*) [min]	t_R (acid) [min]	t_R (Std) [min]
a	**3**[a]	5.06/5.90		
	4[a]	7.28/7.66		
	5[a]	11.25/11.65		
	AcOH[b]		3.04	10.22 (dodecane)
	Butanoic acid[a]		5.93	8.47 (acetophenone)
b	**3**[c]	10.17/12.40		
	4[c]	15.20/16.64		
	5[c]	24.44/26.20		
	AcOH[d]		2.90	6.40 (decane)
	Butanoic acid[c]		4.07	6.91 (dodecane)
c	**3**[e]	28.85/30.28		
	4[e]	39.96/41.13		
	5[e]	60.96/62.30		
	AcOH[f]		7.47	19.54 (dodecane)
	Butanoic acid[g]		4.31	6.06 (acetophenone)
d	3[h]	72.44/77.46		
	4[h]	103.18/108.03		
	AcOH[i]		2.04	12.66 (dihexyl ether)
e	**3**[j]	35.58/37.77		
	4[j]	51.12/52.37		
	5[j]	70.81/72.48		
f	**3**[k]	112.02/117.79		
	5[k]	155.47/159.52		
g	**3**[l]	119.06/126.39		
	4[l]	167.44/171.15		
h	**3**[m]	119.46/125.42		
	5[m]	167.69/171.88		

[a] 0.1 μL inj, vol, inlet temp 300 °C, 12.21 psi, 25:1 split, 28.2 mL min^{-1} total flow, oven temp 110 °C (12.5 min), then +50 °C min^{-1} up to 165 °C (4 min).
[b] 0.1 μL inj, vol, inlet temp 300 °C, 12.21 psi, 25:1 split, 28.2 mLmin^{-1} total flow, oven temp60 °C (6 min), +40 °C min^{-1}, 100 °C (5 min), +50 °C min^{-1}, 165 °C (3 min).
[c] 1.0 μL inj, vol, inlet temp 300 °C, 11.42 psi, 70:1 split, 66.6 mL min^{-1} total flow, oven temp 114 °C (21 min).
[d] 1.0 μL inj, vol, inlet temp 300 °C, 11.42 psi, 70:1 split, 66.6 mL min^{-1} total flow, oven temp 80 °C (5 min), +20 °C min^{-1}, 120 °C (15 min).
[e] 0.1 μL inj, vol, inlet temp 300 °C, 12.21 psi, 25:1 split, 28.2 mL min^{-1} total flow, oven temp 160 °C (45 min).
[f] 0.1 μL inj, vol, inlet temp 300 °C, 12.21 psi, 25:1 split, 28.2 mLmin^{-1} total flow, oven temp (70 °C (10 min), +40 °C min^{-1}, 110 °C (21 min).
[g] 0.1 μL inj, vol, inlet temp 300 °C, 12.21 psi, 25:1 split, 28.2 mL min^{-1} total flow, oven temp 120 °C (7 min).
[h] 0.2 μL inj, vol, inlet temp 150 °C, 12.42 psi, 20:1 split, 21.7 mL min^{-1} total flow, oven temp 150 °C (115 min).
[i] 0.2 μL inj, vol, inlet temp 150 °C, 12.42 psi, 20:1 split, 21.7 mL min^{-1} total flow, oven temp 80 °C (5 min), + 20 °C min^{-1}, 120 °C (10 min).
[j] 0.5 μL inj, vol, inlet temp 300 °C, 12.21 psi, 10:1 split, 22.4 mL min^{-1} total flow, oven temp 165 °C (77 min).
[k] 0.5 μL inj. vol, inlet temp 300 °C, 13.42 psi, 8:1 split, 44.3 mL min^{-1} total flow, oven temp 165 °C (170 min).
[l] 0.5 μL inj, vol, inlet temp 300 °C, 13.42 psi, 20:1 split, 21.7 mL min^{-1} total flow, oven temp 165 °C (175 min).
[m] 0.5 μL inj, vol, inlet temp 300 °C, 12.42 psi, 20:1 split, 21.7 mL min^{-1} total flow, oven temp 165 °C (175 min).

25 °C), and $[\alpha]_D^{25}$ values are given in units of 10^{-1} deg cm^2 g^{-1}. All enzymatic reactions were performed at room temperature (23 °C).

4.2. Preparation of racemic alcohols

4.2.1. Synthesis of racemic alcohols *rac*-2b–e

Alcohols *rac*-**2b–e** were prepared by a Grignard reaction from the corresponding aldehydes (Scheme 1) as exemplified for the preparation of 1-(5-(2-chlorophenyl)furan-2-yl)ethanol *rac*-**2c**.

Aldehyde **1c** (2.5 g, 12.10 mmol) in diethyl ether (60 mL) was added to 10 mL of MeMgBr (3 M solution in diethyl ether, 30.25 mmol) in an ice-water bath and the mixture was stirred for 1 h at room temperature. The reaction mixture was cooled to 0 °C and satd NH_4Cl aq (100 mL) with 30 mL of ice was added. The organic layer was separated and the aqueous layer washed with diethyl ether (3 × 75 mL). The combined organic layers were washed with sat. $NaHCO_3$ aq (1 × 50 mL) and water (3 × 50 mL), dried over anhydrous $MgSO_4$, filtered, and evaporated. When impurities were observed by TLC, the crude product was purified by filtering the product through silica (dichloromethane) to yield 1-(5-(2-chlorophenyl)furan-2-yl)ethanol *rac*-**2c** as a light yellow semisolid: (2.47 g, 11.10 mmol, 92%). ^{1}H NMR (500 MHz, 25 °C, $CDCl_3$): δ = 1.60 (d, *J* = 6.6 Hz, 3H, –CH(OH)C*H*$_3$), 2.10 (br, 1H, O*H*), 4.95 (q, *J* = 6.6 Hz, –C*H*(OH)CH_3), 6.36 (dd, *J* = 0.6 Hz, *J* = 3.4 Hz, 1H, 3-*H*), 7.05 (d, *J* = 3.4 Hz, 1H, 4-*H*), 7.18 (ddd, *J* = 1.7 Hz, *J* = 7.5 Hz, *J* = 7.9 Hz, 1H, 4′-*H*), 7.30 (ddd, *J* = 1.3 Hz, *J* = 7.5 Hz, *J* = 7.9 Hz, 1H, 5′-*H*), 7.42 (dd, *J* = 1.3 Hz, *J* = 8.0 Hz, 1H, 3′-*H*), 7.84 (dd, *J* = 1.7 Hz, *J* = 7.9 Hz, 1H, 6′-*H*) ppm. ^{13}C NMR (126 MHz, 25 °C, $CDCl_3$): δ = 21.38 (–CH(OH)*C*H$_3$), 63.75 (–*C*H(OH)CH_3), 107.32 (3-*C*), 111.56 (4-*C*), 126.82 (5′-*C*), 127.81 (6′-*C*), 128.01 (3′-*C*), 129.07 (2′-*C*), 130.06 (1′-*C*), 130.69 (4′-*C*), 149.48 (2-*C*), 157.20 (5-*C*) ppm. MS(EI+): *m*/*z* (rel. int.) = 222 (53) [M^+], 207 (100), 179 (5), 149 (14), 139 (9), 125 (2), 115 (20); HRMS: M^+ found (M^+ calculated for $C_{12}H_{11}O_2Cl$): 222.04450 (222.04476).

4.2.1.1. 1-(5-Bromofuran-2-yl)ethanol, *rac*-2b. From aldehyde **1b** (1.0 g, 5.71 mmol) a light yellow semisolid *rac*-**2b** (1.02 g, 5.31 mmol, 93%) was obtained as presented for *rac*-**2c** above. ^{1}H NMR (500 MHz, 25 °C, $CDCl_3$): δ = 1.53 (d, *J* = 6.6 Hz, 3H, –CH(OH)C*H*$_3$), 2.04 (br, 1H, –O*H*), 4.83 (q, *J* = 6.6 Hz, 1H, –C*H*(OH)CH_3), 6.21 (dd, *J* = 0.6 Hz, 3.2 Hz, 1H, 4-*H*), 6.24 (d, *J* = 3.3 Hz, 1H, 3-*H*) ppm. ^{13}C NMR (126 MHz, 25 °C, $CDCl_3$): δ = 20.03 (–CH(OH)*C*H$_3$), 62.48 (–*C*H(OH)CH_3), 106.91 (3-*C*), 110.76 (4-*C*), 120.15 (2-*C*), 158.54 (5-*C*) ppm. MS(EI+): *m*/*z* (rel. int.) = 190 (13) [M^+], 175 (100), 160 (2), 145 (9), 95 (99), 65 (47); HRMS: M^+ found (M^+ calculated for $C_6H_7O_2Br$): 189.96240 (189.96294).

4.2.1.2. 1-[5-(4-Bromophenyl)furan-2-yl]ethanol, *rac*-2d. From aldehyde **1d** (1.0 g, 3.98 mmol) a light golden yellow semisolid *rac*-**2d** (1.00 g, 3.75 mmol, 94%) was obtained as presented for *rac*-**2c** above. ^{1}H NMR (500 MHz, 25 °C, $CDCl_3$): δ = 1.59 (d, *J* = 6.6 Hz,

3H, –CH(OH)CH_3), 2.01 (br, 1H, –O*H*), 4.93 (q, *J* = 6.6 Hz, 1H, –C*H*(OH)CH_3), 6.31 (d, *J* = 3.2 Hz, 1H, 4-*H*), 6.58 (d, *J* = 3.4 Hz, 1H, 3-*H*), 7.50 (m, 4H, 2′-*H*, 6′-*H*, 5′-*H*, 3′-*H*) ppm. ^{13}C NMR (126 MHz, 25 °C, $CDCl_3$): δ = 21.36 (–CH(OH)CH_3), 63.77 (–*C*H(OH)CH_3), 106.13 (4-*C*), 107.47 (3-*C*), 121.09 (2-*C*), 125.21 (2′-*C*, 6′-*C*), 129.65 (4′-*C*), 131.79 (3′-*C*, 5′-*C*), 152.29 (1′-*C*), 157.57 (5-*C*) ppm. MS: *m*/*z* (rel. int.) = 267 (52) [^{81}Br-M$^+$], 266 (58) [^{79}Br-M$^+$], 251 (100), 185 (7), 172 (9), 144 (18), 122 (4), 115 (23), 76 (9); HRMS: M$^+$ found (M$^+$ calculated for $C_{12}H_{11}BrO_2$): 265.99320 (265.99424).

4.2.1.3. 1-[5-(4-Chlorophenyl)furan-2-yl]ethanol, *rac*-2e. From aldehyde **1e** (1.5 g, 7.26 mmol) a light yellow semisolid *rac*-**2e** (1.45 g, 6.53 mmol, 90%) was obtained as presented for *rac*-**2c** above. ^{1}H NMR (500 MHz, 25 °C, $CDCl_3$): δ = 1.58 (d, *J* = 6.6 Hz, 3H, –CH(OH)CH_3), 2.14 (br, 1H, –O*H*), 4.92 (q, *J* = 6.6 Hz, –C*H*(OH)CH_3), 6.31 (d, *J* = 3.2 Hz, 1H, 4-*H*), 6.56 (d, *J* = 3.2 Hz, 1H, 3-*H*), 7.33 (d, *J* = 8.4 Hz, 2H, 2′-*H*, 6′-*H*), 7.57 (d, *J* = 8.4 Hz, 2H, 3′-*H*, 5″-*H*) ppm. ^{13}C NMR (126 MHz, 25 °C, $CDCl_3$): δ = 21.35 (–CH(OH)*C*H_3), 63.73 (–*C*H(OH)CH_3), 106.00 (3-*C*), 107.44 (4-*C*), 124.93 (3′-*C*, 5′-*C*), 128.85 (2′-*C*, 6′-*C*), 129.22 (1′-*C*), 132.96 (4′-*C*), 152.25 (2-*C*), 157.50 (5-*C*) ppm.

4.2.2. Synthesis of racemic alcohols *rac*-2f–h

The reaction between 1-(furan-2-yl)ethanone and the corresponding diazonium salts by a variation of the Meerwein method[15] produced ketones **1f**–**h**, which were transformed into alcohols *rac*-**2f–h** by $NaBH_4$ reduction (Scheme 1, ii), as exemplified for the synthesis of 1-[5-(-carboxyethylphenyl)furan-2-yl]ethanol, *rac*-**2h**.

Ketone **1h** (0.66 g, 2.56 mmol) was dissolved in ethanol (25 mL) after which $NaBH_4$ (0.15 g, 3.84 mmol) in 15 mL of ethanol was added slowly while stirring the mixture at room temperature. The reaction mixture was poured into a glass with ice, water, and HCl (1 M, 1 mL). The aqueous solution was extracted three times with diethyl ether. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified on a short column (dichloromethane) to afford a yellow semisolid 1-[5-(4-carboxyethylphenyl)furan-2-yl]ethanol *rac*-**2h**: (0.61 g, 2.36 mmol, 92%). ^{1}H NMR (500 MHz, 25 °C, $CDCl_3$): δ = 1.40 (t, *J* = 7.1 Hz, 3H, –$CO_2CH_2CH_3$), 1.60 (d, *J* = 6.6 Hz, 3H, –CH(OH)CH_3), 2.25 (br, 1H, –O*H*), 4.37 (q, *J* = 7.1 Hz, 2H, –$CO_2CH_2CH_3$), 4.95 (q, *J* = 6.6 Hz, 1H, –C*H*(OH)CH_3), 6.35 (d, *J* = 3.4, 1H, 3-*H*), 6.71 (d, *J* = 3.6 Hz, 1H, 4-*H*), 7.69 (d, *J* = 8.5 Hz, 2H, 3′-*H*, 5′-*H*); 8.03 (d, *J* = 8.5 Hz, 2H, 2′-*H*, 6′-*H*) ppm. ^{13}C NMR (126 MHz, 25 °C, $CDCl_3$): δ = 14.35 (–$CO_2CH_2CH_3$), 21.42 (–CH(OH)*C*H_3), 61.01 (–$CO_2CH_2CH_3$), 63.76 (–*C*H(OH)CH_3), 107.64 (3-*C*), 107.82 (4-*C*), 123.26 (2′-*C*, 6′-*C*), 128.83 (1′-*C*), 130.04 (3′-*C*, 5′-*C*), 134.57 (4′-*C*), 152.24 (5-*C*), 158.40 (2-*C*), 166.40 (–$CO_2CH_2CH_3$) ppm. MS(EI+): *m*/*z* (rel. int.) = 260 (66), [M]$^+$, 245 (100), 217 (25), 197 (7), 172 (11), 145 (7), 115 (21), 100 (5), 77 (5); HRMS: M$^+$ found (M$^+$ calculated for $C_{15}H_{16}O_4$): 260.10470 (260.10486).

4.2.2.1. 1-[5-(4-Nitrophenyl)furan-2-yl]ethanol, *rac*-2f. From ketone **1f** (1.0 g, 4.33 mmol) a brown semisolid *rac*-**2f** (0.97 g, 4.14 mmol, 96%) was obtained as presented for *rac*-**2h** above. ^{1}H NMR (500 MHz, 25 °C, $CDCl_3$): δ = 1.62 (d, *J* = 6.6 Hz, 3H, –CH(OH)CH_3), 2.12 (br, 1H, –O*H*), 4.97 (q, *J* = 6.6 Hz, 1H, –C*H*(OH)CH_3), 6.41 (d, *J* = 3.4 Hz, 1H, 3-*H*), 6.82 (d, *J* = 3.4 Hz, 1H, 4-*H*), 7.77 (d, *J* = 7.0 Hz, 2H, 2′-*H*, 6′-*H*), 8.23 (d, *J* = 8.9 Hz, 2H, 3'-*H*, 5′-*H*) ppm. ^{13}C NMR (126 MHz, 25 °C, $CDCl_3$): δ = 21.48 (–CH(OH)*C*H_3), 63.78 (–*C*H(OH)CH_3), 108.08 (3-*C*), 109.70 (4-*C*), 123.82 (3′-*C*, 5′-*C*), 124.33 (2′-*C*, 6′-*C*), 136.35 (1′-*C*), 146.35 (4′-*C*), 151.02 (5-*C*), 159.56 (2-*C*) ppm. MS(EI+): *m*/*z* (rel. int.) = 233 (45) [M$^+$], 218 (100), 172 (36), 160 (6), 150 (6), 115 (22), 83 (10), 76(7); HRMS: M$^+$ found (M$^+$ calculated for $C_{12}H_{11}NO_4$): 233.06880 (233.06881).

4.2.2.2. 1-[5-(2-Methyl-4-nitrophenyl)furan-2-yl]ethanol, *rac*-2g. From ketone **1g** (0.77 g, 3.14 mmol) a golden brown semisolid *rac*-**2g** (0.74 g, 3.00 mmol, 96%) was obtained as presented for *rac*-**2h** above. ^{1}H NMR (500 MHz, 25 °C, $CDCl_3$): δ = 1.62 (d, *J* = 6.6 Hz, 3H, –CH(OH)CH_3), 2.27 (br, 1H, –O*H*), 2.59 (s, 3H, –CH_3), 4.98 (q, *J* = 6.6 Hz, 1H, –C*H*(OH)CH_3), 6.42 (d, *J* = 3.4 Hz, 1H, 3-*H*), 6.71 (d, *J* = 3.4 Hz, 1H, 4-*H*), 7.85 (d, *J* = 8.7 Hz, 1H, 6′-*H*), 8.06 (dd, *J* = 2.3 Hz, *J* = 8.7 Hz, 1H, 5′-*H*), 8.10 (d, *J* = 2.0 Hz, 1H, 3′-*H*) ppm. ^{13}C NMR (126 MHz, 25 °C, $CDCl_3$): δ = 21.50 (–*C*H_3), 22.36 (–CH(OH)CH_3), 63.75 (–*C*H(OH)CH_3), 107.79 (3-*C*), 112.76 (4-*C*), 121.27 (5′-*C*), 126.30 (3′-*C*), 126.87 (6′-*C*), 135.18 (2′-*C*), 135.71 (1′-*C*), 145.96 (4′-*C*), 150.56 (5-*C*), 158.78 (2-*C*) ppm. MS(EI+): *m*/*z* (rel. int.) = 247 (49) [M$^+$], 232 (100), 204 (3), 186 (22), 164 (5), 141 (3), 128 (20) 115 (16), 102 (6), 77 (10); HRMS: M$^+$ found (M$^+$ calculated for $C_{13}H_{13}NO_4$): 247.08500 (247.08446).

4.3. Analytical-scale enzymatic reactions

In a typical small-scale experiment, CAL-B as the commercial Novozym 435 preparation (1–20 mg mL^{-1}) was added to a solution of one of the substrates *rac*-**2a–h** (0.05 or 0.1 M) and an acyl donor (2 equiv) in an organic solvent. The mixture was then shaken at room temperature. The progress and ee values of the products were followed by taking samples at intervals and analyzing the samples after derivatization by GC.

4.4. Preparative scale kinetic resolutions

4.4.1. (*R*)-3a, (*S*)-2a and (*R*)-2a

CAL-B (1 mg/mL) was added to the solution of *rac*-**2a** (1.0 g, 8.93 mmol) and vinyl acetate (1.64 mL, 18.0 mmol) in dried DIPE (89 mL). The reaction was stopped after 5 h at 50% conversion by filtering off the enzyme. The product mixture was evaporated and purified by filtering through silica (dichloromethane), affording light yellow oils (*S*)-**2a** [0.42 g, 3.75 mmol, 42%, 98% *ee*, $[\alpha]_D^{25} = -20.8$ (*c* 1, $CHCl_3$); literature data[8] $[\alpha]_D^{25} = -24.4$ (neat), 98–99% *ee*] and (*R*)-**3a** [0.58 g, 3.78 mmol, 43%, 92% *ee*, $[\alpha]_D^{25} = +164$ (*c* 1, $CHCl_3$)]. Compound (*R*)-**3a** was further purified by adding CAL-B (5 mg mL^{-1}) to a solution of (*R*)-**3a** (0.50 g, 3.25 mmol, 92% *ee*) and methanol (0.66 mL, 16 mmol) in dried DIPE (32.5 mL). The reaction was stopped after 6 h at 95% conversion by filtering off the enzyme. The filtrate was poured through silica (dichloromethane) to afford (*R*)-**2a** as a light yellow oil [0.30 g, 2.66 mmol, 82%, 99% *ee*, $[\alpha]_D^{25} = +20.7$ (*c* 1, $CHCl_3$); literature data[8] $[\alpha]_D^{25} = +24.3$ (neat), 96% *ee*]. For (*R*)-**3a**: ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.58 (d, *J* = 6.7 Hz, 3H, –CH(OCOCH_3)CH_3), 2.06 (s, 3H, –CH(OCOCH_3)CH_3), 5.96 (q, *J* = 6.7 Hz, 1H, –C*H*(OCOCH_3)CH_3), 6.32 (d, *J* = 3.3 Hz, 1H, 3-*H*), 6.34 (dd, *J* = 1.8 Hz, *J* = 3.2 Hz, 1H, 4-*H*), 7.39 (d, *J* = 1.7 Hz, 1H, 5-*H*) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ = 18.23 (–CH(OCOCH_3)CH_3), 21.22 (–CH(OCOCH_3)CH_3), 65.07 (–CH(OCOCH_3)CH_3), 107.75 (3-*C*), 110.20 (4-*C*), 142.49 (5-*C*), 153.47 (2-*C*), 170.30 (–CH(O*C*OCH_3)CH_3) ppm. MS(EI+): *m*/*z* (rel. int.) = 154 (51) [M$^+$], 127 (1), 115 (1), 110 (6), 95 (13), 43 (100); HRMS: M$^+$ found (M$^+$ calculated for $C_8H_{10}O_3$): 154.06310 (154.06299). For (*S*)-**2a**: ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.54 (d, *J* = 6.5 Hz, 3H, –CH(OH)CH_3), 2.05 (br, 1H, –O*H*), 4.88 (q, *J* = 6.5 Hz, 1H, –C*H*(OH)CH_3), 6.22 (d, *J* = 3.2 Hz, 1H, 3-*H*), 6.32 (dd, *J* = 1.3 Hz, *J* = 3.2 Hz, 4-*H*), 7.37 (d, *J* = 1.0 Hz, 1H, 5-*H*) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ = 21.27 (–CH(OH)*C*H_3), 63.63 (–*C*H(OH)CH_3), 105.11 (3-*C*), 110.13 (4-*C*), 141.92 (5-*C*), 157.61 (2-*C*) ppm. MS(EI+): *m*/*z* (rel. int.) = 112 (51) [M$^+$], 97 (100), 94 (45), 84 (11), 81 (6), 65 (24), 55 (16), 44 (92); HRMS: M$^+$ found (M$^+$ calculated for $C_6H_8O_2$): 112.05240 (112.05243). NMR and MS spectra of (*R*)-**2a** were found identical to (*S*)-**2a**.

4.4.2. (*R*)-3b and (*S*)-2b

As above, the kinetic resolution of *rac*-**2b** (0.50 g, 2.62 mmol) with CAL-B (1 mg mL^{-1}) afforded a light brown semisolid (*S*)-**2b** [0.23 g, 1.19 mmol, 45%, 95% *ee*, $[\alpha]_D^{25} = -20.1$ (*c* 1, $CHCl_3$)] and a yellow-brown oily acetate (*R*)-**3b** [0.27 g, 1.17 mmol, 45%, 94% *ee*, $[\alpha]_D^{25} = +159$ (*c* 1, $CHCl_3$)] after purification by column chromatography (dichloromethane). For (*R*)-**3b**: ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.56 (d, *J* = 6.7 Hz, 3H, –CH(OCOCH$_3$)C*H*$_3$), 2.06 (s, 3H, –CH(O-COC*H*$_3$)CH$_3$), 5.88 (q, *J* = 6.7 Hz, 1H, –C*H*(OCOCH$_3$)CH$_3$), 6.26 (d, *J* = 3.2 Hz, 1H, 4-*H*), 6.30 (d, *J* = 3.1 Hz, 3-*H*) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ = 18.03 (–CH(OCOCH$_3$)*C*H$_3$), 21.21 (–CH(O-*C*OCH$_3$)CH$_3$), 64.76 (–*C*H(OCOCH$_3$)CH$_3$), 110.64 (3-*C*), 111.94 (4-*C*), 121.97 (5-*C*), 155.35 (2-*C*), 170.16 (–CH(O*C*OCH$_3$)CH$_3$) ppm. MS(EI+): *m*/*z* (rel. int.) = 234 (18) [M^{81}Br$^+$], 232 (18) [M^{79}Br$^+$], 192 (10), 190 (10), 175 (41), 173 (34), 145 (6), 119 (2), 111 (4), 93 (62), 82 (3), 65 (28); HRMS M$^+$ found (M$^+$ calculated for $C_8H_9O_3Br$) 231.97360 (231.97351). Spectroscopic data for (*S*)-**2b** were identical to the data of *rac*-**2b**.

4.4.3. (*R*)-3c, (*S*)-2c and (*R*)-2c

As above, the kinetic resolution of *rac*-**2c** (1.0 g, 4.49 mmol) with CAL-B (1 mg mL^{-1}) yielded an amber semisolid (*S*)-**2c** [0.48 g, 2.17 mmol, 48%, 98% *ee*, $[\alpha]_D^{25} = -1.6$ (*c* 1, $CHCl_3$)] and a yellow semisolid (*R*)-**3c** [0.58 g, 2.19 mmol, 49%, 90% *ee*, $[\alpha]_D^{25} = +146$ (*c* 1, $CHCl_3$)] after purification by column chromatography (dichloromethane). The enzymatic methanolysis of the enantiomerically enriched (*R*)-**3c** (0.50 g, 1.89 mmol) under the conditions (stopped at 24 h) used for (*R*)-**3a** above produced an amber semisolid (*R*)-**2c** [0.32 g, 1.43 mmol, 76%, 99% *ee*, $[\alpha]_D^{25} = +1.6$ (*c* 1, $CHCl_3$)] after column chromatography (dichloromethane). For (*R*)-**3c**: ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.64 (d, *J* = 6.7 Hz, 3H, –CH(OCOCH$_3$)C*H*$_3$), 2.08 (s, 3H, –CH(OCOC*H*$_3$)CH$_3$), 6.02 (q, *J* = 6.7 Hz, 1H, –CH(O-COCH$_3$)CH$_3$), 6.45 (d, *J* = 3.4 Hz, 1H, 3-*H*), 7.07 (d, *J* = 3.4 Hz, 1H, 4-*H*), 7.20 (ddd, *J* = 1.6 Hz, *J* = 7.6 Hz, *J* = 7.7 Hz, 1H, 4′-*H*), 7.31 (ddd, *J* = 1.2 Hz, *J* = 6.6 Hz, *J* = 7.5 Hz, 1H, 5′-*H*), 7.43 (dd, *J* = 1.1 Hz, *J* = 8.0 Hz, 1H, 3′-*H*), 7.85 (dd, *J* = 1.6 Hz, *J* = 7.9 Hz, 1H, 6′-*H*) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ = 18.28 (–CH(O-COCH$_3$)*C*H$_3$), 21.28 (–CH(OCO*C*H$_3$)CH$_3$), 65.11 (–*C*H(OCOCH$_3$)CH$_3$), 109.84 (3-*C*), 111.52 (4-*C*), 126.86 (5′-*C*), 127.97 (6′-*C*), 128.18 (3″-*C*), 128.97 (2′-*C*), 130.18 (1″-*C*), 130.71 (4′-*C*), 149.96 (2-*C*), 152.99 (5-*C*), 170.29 (–CH(O*C*OCH$_3$)CH$_3$) ppm. MS(EI+): *m*/*z* (rel. int.) = 264 (32) [M$^+$], 220 (33), 205 (100), 194 (9), 176 (4), 169 (9), 149 (31); HRMS: M$^+$ found (M$^+$ calculated for $C_{14}H_{13}O_3Cl$): 264.05520 (264.05532). Spectroscopic data for (*R*)- and (*S*)-**2c** were identical to the data for *rac*-**2c**.

4.4.4. (*R*)-3d and (*S*)-2d

As above, the kinetic resolution of *rac*-**2d** (300 mg, 1.12 mmol) with CAL-B (5 mg mL^{-1}) yielded a light golden brown semisolid (*S*)-**2d** [62 mg, 0.232 mmol, 21%, 91% *ee*, $[\alpha]_D^{25} = +5.0$ (*c* 1, $CHCl_3$)] and a light golden brown oil (*R*)-**3d** [(192 mg, 0.621 mmol, 55%, 75% *ee*, $[\alpha]_D^{25} = +54$ (*c* 1, $CHCl_3$)] after purification by column chromatography (dichloromethane with 0.5% TEA). For (*R*)-**3d**: ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.62 (d, *J* = 6.7 Hz, 3H, –CH(OCOCH$_3$)C*H*$_3$), 2.07 (s, 3H, –CH(OCOC*H*$_3$)CH$_3$), 5.96 (q, *J* = 6.7 Hz, 1 H, –C*H*(O-COCH$_3$)CH$_3$), 6.39 (d, *J* = 3.4 Hz, 1H, 4-*H*), 6.58 (d, *J* = 3.4, 1H, 3-*H*), 7.48 (m, 4H, 2′-*H*, 3′-*H*, 5′-*H*, 6′-*H*) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ = 18.24 (–CH(OCOCH$_3$)*C*H$_3$), 21.28 (–CH(OCOC*H*$_3$)CH$_3$), 65.15 (–*C*H(OCOCH$_3$)CH$_3$), 106.10 (3-*C*), 110.06 (4-*C*), 121.31 (4′-*C*), 125.36 (2′-*C*, 6′-*C*), 129.52 (1′-*C*), 131.80 (3′-*C*, 5′-*C*), 152.74 (2-*C*), 153.33 (5-*C*), 170.28 (–CH(O*C*OCH$_3$)CH$_3$) ppm. MS(EI+): *m*/*z* (rel. int.) 310 (44) [MBr-81]$^+$, 308 (47) [MBr-79]$^+$, 266 (3), 250 (100), 170 (9), 141 (12), 115 (9), 76 (3), 43 (23); HRMS: M$^+$ found (M$^+$ calculated for $C_{14}H_{13}O_3Br$): 308.00460 (308.00481). Spectroscopic data for (*S*)-**2d** were identical to the data for *rac*-**2d**.

4.4.5. (*R*)-3f and (*S*)-2f

As above, the kinetic resolution of *rac*-**2f** (500 mg, 2.14 mmol) yielded golden brown semisolids (*S*)-**2f** [140 mg, 0.597 mmol, 28%, 89% *ee*), $[\alpha]_D^{25} = +2.3$ (*c* 1, $CHCl_3$)] and (*R*)-**3f** [161 mg, 0.585 mmol, 27%, 90% *ee*, $[\alpha]_D^{25} = +225$ (*c* 1, $CHCl_3$)] after purification by column chromatography (22:3 petroleum ether/acetone). For (*R*)-**3f**: ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.66 (d, *J* = 6.7 Hz, 3H, –CH(OCOCH$_3$)C*H*$_3$), 2.10 (s, 3H, –CH(OCOC*H*$_3$)CH$_3$), 6.02 (q, *J* = 6.7 Hz, 1H, –C*H*(OCOCH$_3$), 6.48 (d, *J* = 3.4 Hz, 1H, 3-*H*), 6.83 (d, *J* = 3.4 Hz, 1H, 4-*H*), 7.79 (d, *J* = 8.9 Hz, 2H, 2′-*H*, 6′-*H*), 8.25 (d, *J* = 8.9 Hz, 2H, 3′-*H*, 5′-*H*) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ = 18.29 (–CH(OCOCH$_3$)CH$_3$), 21.23 (–CH(OCOCH$_3$)CH$_3$), 64.98 (–*C*H(OCOCH$_3$)CH$_3$), 109.55 (3-*C*), 110.52 (4-*C*), 124.02 (3′-*C*, 5′-*C*), 124.32 (2′-*C*, 6′-*C*), 136.19 (1′-*C*), 146.53 (4′-*C*), 151.44 (5-*C*), 155.28 (2-*C*), 170.20 (–CH(OCOCH$_3$)CH$_3$) ppm. MS(EI+): *m*/*z* (rel. int.) = 275 (42), [M]$^+$, 233 (21), 216 (100), 185 (8), 170 (36), 141 (13), 115 (14); HRMS: M$^+$ found (M$^+$ calculated for $C_{14}H_{13}O_5N$) 275.07890 (275.07937). Spectroscopic data for (*S*)-**2f** were identical to the data for *rac*-**2f**.

4.4.6. (*R*)-3g and (*S*)-2g

As above, the kinetic resolution of *rac*-**2g** (500 mg, 2.02 mmol) with CAL-B (5 mg mL^{-1}) yielded a yellow semisolid (*S*)-**2g** [232 mg, 0.94 mmol, 46%, 93% *ee*, $[\alpha]_D^{25} = -2.3$ (*c* 1, $CHCl_3$)] and a yellow semisolid (*R*)-**3g** [272 mg, 0.94 mmol, 46%, 88% *ee*, $[\alpha]_D^{25} = +198$ (*c* 1, $CHCl_3$)] after purification by column chromatography (dichloromethane). For (*R*)-**3g**: ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.66 (d, *J* = 6.7 Hz, 3H, –CH(OCOCH$_3$)C*H*$_3$), 2.10 (s, 3H, –CH(O-COC*H*$_3$)CH$_3$), 2.61 (s, 3H, –C*H*$_3$), 6.03 (q, *J* = 6.7 Hz, 1H, –C*H*(O-COCH$_3$)CH$_3$), 6.50 (d, *J* = 3.3 Hz, 1H, 3-*H*), 6.73 (d, *J* = 3.3 Hz, 1H, 4-*H*), 7.87 (d, *J* = 8.6 Hz, 1H, 6′-*H*), 8.09 (d, *J* = 8.7 Hz, 1H, 5′-*H*), 8.12 (s, 1H, 3′-*H*) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ = 18.30 (–CH(OCO*C*H$_3$)CH$_3$), 21.23 (–*C*H$_3$), 22.32 (–CH(OCOCH$_3$)*C*H$_3$), 64.97 (–*C*H(OCOCH$_3$)CH$_3$), 110.16 (3-*C*), 112.50 (4-*C*), 121.31 (5′-*C*), 126.34 (3′-*C*), 127.14 (6′-*C*), 135.44 (2′-*C*), 135.55 (1′-*C*), 146.20 (4′-*C*), 151.15 (5-*C*), 154.52 (2-*C*), 170.22 (–CH(O-*C*OCH$_3$)CH$_3$) ppm. MS(EI+): *m*/*z* (rel. int.) = 289 (30), [M]$^+$, 277 (5), 247 (10), 230 (100), 214 (5), 201 (5), 184 (23), 155 (8), 141 (8), 128 (16), 115 (12), 102 (4); HRMS: M$^+$ found (M$^+$ calculated for $C_{15}H_{15}NO_5$): 289.09510 (289.09502). Spectroscopic data for (*S*)-**2g** were identical to the data for *rac*-**2g**.

4.4.7. (*R*)-3h and (*S*)-2h

As above, the kinetic resolution of *rac*-**2h** (500 mg, 1.92 mmol) with CAL-B (5 mg mL^{-1}) yielded a slightly yellow oil (*S*)-**2h** [239 mg, 0.92 mmol, 48%, 93% *ee*, $[\alpha]_D^{25} = +5.8$ (*c* 1, $CHCl_3$)] and a white semisolid (*R*)-**3h** [250 mg, 0.83 mmol, 43%, 86% *ee*, $[\alpha]_D^{25} = +178$ (*c* 1, $CHCl_3$)] after purification by column chromatography (dichloromethane with 1% TEA). For (*R*)-**3h**: ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.41 (t, *J* = 7.1 Hz, 3H, –$CO_2CH_2CH_3$), 1.65 (d, *J* = 6.7 Hz, 3H, –CH(OCOCH$_3$)C*H*$_3$), 2.09 (s, 3H, –CH(O-COC*H*$_3$)CH$_3$), 4.39 (q, *J* = 7.1 Hz, 2H, –$CO_2CH_2CH_3$), 6.01 (q, *J* = 6.7 Hz, 1H, –C*H*(OCOCH$_3$)CH$_3$), 6.44 (d, *J* = 3.4 Hz, 1H, 3-*H*), 6.73 (d, *J* = 3.4 Hz, 1H, 4-*H*), 7.71 (d, *J* = 8.5 Hz, 2H, 3′-*H*, 5′-*H*), 8.05 (d, *J* = 8.5 Hz, 2H, 2′-*H*, 6′-*H*) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ = 14.36 (–$CO_2CH_2CH_3$), 18.28 (–CH(OCOCH$_3$)*C*H$_3$), 21.28 (–CH(O-COCH$_3$)CH$_3$), 61.01 (–$CO_2CH_2CH_3$), 65.14 (–*C*H(OCOCH$_3$)CH$_3$), 107.74 (3-*C*), 110.23 (4-*C*), 123.44 (2′-*C*, 6′-*C*), 129.09 (1′-*C*), 130.05 (3′-*C*, 5′-*C*), 134.41 (4′-*C*), 152.74 (5-*C*), 154.06 (2-*C*), 166.32 (–$CO_2CH_2CH_3$), 170.28 (–CH(OCOCH$_3$)CH$_3$) ppm. MS(EI+): *m*/*z* (rel. int.) = 302 (60), [M]$^+$, 257 (13), 243 (100), 215 (20), 197 (9), 170 (13), 141 (6), 128 (3), 115 (8); HRMS: M$^+$ found (M$^+$ calculated for $C_{17}H_{18}O_5$) 302.11550 (302.11542). Spectroscopic data for (*S*)-**2h** were identical to the data for *rac*-**2h**.

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