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Evaluation of the new protocol to enzymatic dynamic kinetic resolution of 3-hydroxy-3-(aryl)propanoic acids

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The application of tandem metal-enzyme dynamic kinetic resolution (DKR) is a powerful tool for the manufacture of high-value chemical commodities. The new protocol of kinetic resolution based on irreversible enzymatic esterification of carboxylic acids with orthoesters was introduced to obtain optically active β-hydroxy ester. This procedure was combined with metal catalyzed racemization of the target substrate providing both (*R*) and (*S*) enantiomers of ethyl 3-hydroxy-3-(4-nitrophenyl)propanoate with high yield 89% at 40 °C. A substantial influence of enzyme type, organic co-solvent, and metal catalyst on conversion and enantioselectivity of the enzymatic dynamic kinetic resolution was noted.

Introduction

The synthesis of enantiomerically pure chemical commodities is of great importance for both academia and pharmaceutical industry.[1] Large amount of natural products are chiral, non-racemic compounds which biological activity is highly dependent on their absolute configuration of stereogenic centers. Nowadays, the most convenient way to obtain enantiomerically pure compounds is still based on kinetic resolution (KR). The major disadvantage of these methods is the great loss of yield due to the nature of the kinetic resolution process, as no more than 50% yield could be theoretically achieved while maintaining high enantiomeric purity. If the racemization of the slowly reacting enantiomer can occur concurrently with the kinetic resolution, then theoretically 100% of racemic mixture can be converted into one enantiomer, what is known as dynamic kinetic resolution (DKR).[2] Over the past decade, the procedures employing enzymes have been intensively explored for the efficient DKR.[3] The racemization of a substrate can occur through various pathways, such as: thermal racemization,[4] base-catalyzed racemization,[5] acid-catalyzed racemization via Schiff bases,[6] enzyme-catalyzed racemization,[7] racemization via redox and radical reactions.[8] The classical and commonly applied tandem metal-enzyme DKR is based on sec-alcohol racemization and enzymatic acylation of preferred enantiomer.[9] It is well known, that the several complexes of rhodium, iridium and ruthenium, among others, are capable to catalyze rapid racemization of various alcohol substrates, but only few have proved compatible with an enzymatic reaction.[10] In the case of alcohols the racemization predominantly occurs through hydrogen transfer process.[11] Several problems must be addressed, e.g. interference of transition metals with enzyme structure, selection of an appropriate solvent and temperature to achieve efficient enzymatic DKR.[12]

The DKR procedure can be used also for functionalized alcohols, which are versatile building blocks in organic synthesis.[13] Enantiomerically enriched active β-hydroxy esters and the derivatives thereafter are synthetically important chiral synthons, of which the chiral β-hydroxy-β-arylpropionates are precursors of Fluoxetine,[14] β-lactam antibiotics,[15] Tuckolidean HMG CoA reductase-inhibitor.[16] Chiral β-hydroxy esters are also used as a starting materials in the preparation of enantiomerically pure β-blockers.[17] In view of these medicinal importance, synthetic studies of these compounds have attracted considerable interest. Several chemical procedures have been explored to provide the routes to them. Two common approaches include the aldol reactions[18] as well as catalytic asymmetric hydrogenation.[19]

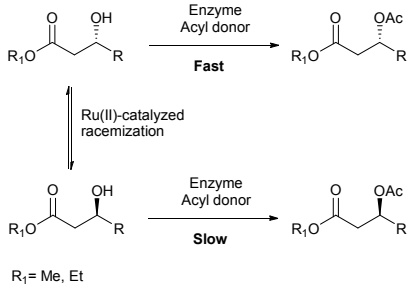

Figure 1 DKR of racemic β-hydroxy esters.

The enzymatic methods are available as well. They comprise the asymmetric reduction of ketones[20] and the lipase-catalyzed kinetic resolution of racemic β-hydroxy esters.[21] All of these chemical and enzymatic methods have several

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Electronic Supplementary Information (ESI) available: Experimental procedures, ^{1}H NMR, ^{13}C NMR spectra and chiral HPLC tracers. See DOI: 10.1039/x0xx00000x

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disadvantages. In particular, the enzymatic kinetic resolution has a serious limitation that the theoretical maximum yield is 50% for the wanted enantiomer. New methods providing enantiomerically pure compounds based on tandem metal-enzyme dynamic kinetic resolution are still of high interest due to the concepts of asymmetric synthesis. Bäckvall and co-workers have extensively studied DKR of α- and β-hydroxy esters (Figure 1). Authors applied transesterification in cyclohexane using immobilized PS-C as the biocatalyst and *p*-chlorophenyl acetate as the acyl donor.[22] Under these conditions appropriate products were obtained in moderate to good yields and enantioselectivity. Nevertheless, this DKR reaction requires high temperature, long time (up to seven days) and anhydrous conditions. There still remains some limitations in DKR of β-hydroxy esters: firstly, the Shvo's diruthenium complexes[23] are not easily available because of the expense; secondly, the racemic temperature is mostly higher than the optimum reaction temperature for enzyme, which may decrease the enzyme activity last but not least, the boiling point of the acyl donor *p*-chlorophenyl acetate is high what hinders product purification. The former method relies on the enzymatic acylation of β-hydroxy moiety, an obvious drawback of it is necessity to perform an additional not trivial step to recover hydroxy group towards further functionalization. Thus, it seems significant to find a method to overcome these limitations.

Results and Discussion

Herein, we report our results on the novel approach to the synthesis of optically active β-hydroxy esters applying corresponding β-hydroxy acids which are readily available from corresponding aldehydes in straightforward synthesis.[18]

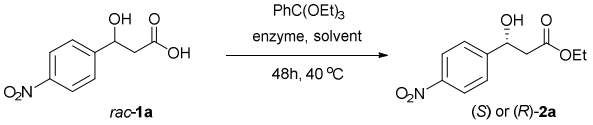
We first investigated the enzymatic kinetic resolution of selected racemic β-hydroxy acids. Recently we have shown that orthoesters can be successfully used for enzymatic esterification of carboxylic acids.[24] It is well known that orthoesters trap the water through hydrolysis and therefore prevent the reverse reaction, and at the same time provide the nucleophile for the esterification.[25] To the best of our knowledge presented approach has been never used for kinetic resolution of racemic β-hydroxy acids

For the initial studies under EKR lipase from *Pseudomonas fluorescence* (Amano AK), triethyl orthobenzoate and toluene as a solvent were employed. All reactions were conducted at 40 °C for 48 hours in 0.1 mmol scale and obtained esters **2** were isolated before subjected to further HPLC analysis (Table 1). Racemic 3-hydroxy-3-(4-nitrophenyl)propanoic acid (**1a**) which ester **2a** is a progenitor for the anti-malarial drugs[26] and hypoxia-selective cytotoxic agent (HSAs)[27] has been used as a model substrate for kinetic resolution.

As shown in Table 1, Amano AK enzyme seems to have a potential for the kinetic resolution of racemic β-hydroxy acid **1a** leading to enantiomerically enriched ester (*S*)-**2a** with 51% *ee* and 29% isolated yield (Table 1, entry 1). The choice of the proper organic solvent can be critical, because selectivity and activity of used enzyme may change significantly.[28]

In the reactions catalyzed by Amano AK polarity of the solvent did not influence the yield of the reaction, but strongly affected enantioselectivity *E* (Table 1, entries 1-4) leading in *tert*-butyl methyl ether (TBME) to the optically pure product (*S*)-**2a** with high enantioselectivity >200 (Table 1, entry 4). Additionally, different ethoxy group donors were tested; (diethoxymethyl)benzene, 1-(triethoxymethoxy)ethane and 1,1,1-triethoxyethane using TBME as a solvent (Table 1, entries 5-7). In all cases the ester (*S*)-**2a** was obtained substantially lower enantioselectivity, exceptionally reaction with (diethoxymethyl)benzene lead to product (*S*)-**2a** with slightly higher isolated yield 41% (Table 1, entry 5).

Table 1 Kinetic resolution of racemic 3-hydroxy-3-(4-nitrophenyl)propanoic acid (**1a**) with Amano AK in various organic solvents, the partition coefficient (Log*P*) and dielectric constant (ε_r)[a]



rac-**1a** (*S*) or (*R*)-**2a**

Entry	Solvent	Log*P* (ε_r)	yield[e] (%)	ee[f] (%)	*E*[g]
1	toluene	2.30 (2.38)	29	51 (*S*)	5
2	cyclohexane	2.70 (2.20)	31	82 (*S*)	14
3	isopentylether	4.25 (2.80)	37	96 (*S*)	87
4	TBME	1.40 (2.60)	37	99 (*S*)	>200
5[b]			41	38 (S)	3
6[c]			15	90 (S)	22
7[d]			17	78 (S)	9

[a]Reaction conditions: 0.1mmolof **1a**, ethoxy group donor 2 equiv., solvent (2.0 mL), Amano AK (5 mg). [b](diethoxymethyl)benzene. [c]1-(triethoxymethoxy)ethane. [d]1,1,1-triethoxyethane. [e]Isolated yield. [f]Determined by the chiral HPLC (Chiralpak IA). [g]Enantioselectivity, E=ln[1-c(1+ee_p)]/ln[1-c(1-ee_p)][29]

Separation of two enantiomers from racemic mixture is of great importance, particularly in the pharmaceutical industry, because of the different pharmacological activities and pharmacokinetic characteristics of each enantiomer.[30] Addressing this problem we were interested in finding enzyme which can provide opposite (*R*)-enantiomer of the studied ester **2a**. Screening of biocatalysts was performed using over 20 commercially available enzymes. Only four out of these tested catalysts were active in kinetic resolution of β-hydroxy acid **1a**; immobilized lipase from *Candida antarctica* (Novozym 435), immobilized lipase from *Pseudomonas cepacia* (Amano PS-C I), lipase from wheat germ and domestic made goose liver acetone powder (GLAP) (Table 2).

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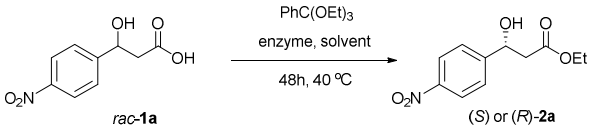
In all cases the strong influence of solvent lipophilicity (Log*P*) as well as polarity (ε_r) on the reaction course has been observed.

Table 2 Kinetic resolution of racemic 3-hydroxy-3-(4-nitrophenyl)propanoic acid (**1a**) in different organic solvents with various enzymes[a]

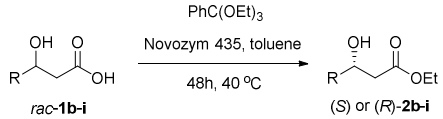


Entry	Enzyme	Solvent	yield[c] (%)	ee[d] (%)	*E*[e]
1	Novozym 435	TBME	42	19 (*R*)	-
2		isopentylether	43	51 (*R*)	4
3		cyclohexane	39	50 (*R*)	4
4		toluene	41	95 (*R*)	78
5	Amano PS-C I	TBME	31	76 (*S*)	10
6		isopentylether	32	31 (*S*)	2
7		cyclohexane	28	*rac*	-
8		toluene	29	*rac*	-
9	Wheat germ lipase	TBME	45	*rac*	-
10		isopentylether	31	9 (*R*)	1
11		cyclohexane	19	21 (*R*)	2
12		toluene	22	*rac*	-
13	GLAP[b]	TBME	39	*rac*	-
14		isopentylether	47	*rac*	-
15		cyclohexane	35	33 (*R*)	2
16		toluene	37	94 (*R*)	56

[a]Reaction conditions: 0.1mmolof **1a**, triethyl orthobenzoate 2 equiv., solvent(2.0 mL), enzyme (native 5 mg or immobilized 10 mg). [b]Domestic made goose liver acetone powder. [c]Isolated yield. [d] Determined by the chiral HPLC (Chiralpak IA). [e] Enantioselectivity, E=ln[1-c(1+ee_p)]/ln[1-c(1-ee_p)].[29]

As shown in Table 2, Novozym 435 catalyzed esterification of acid **1a**, in contrast to Amano AK in TBME solvent corresponding ester **2a** was obtained as a racemate (Table 2, entry 1). Also reactions performed in isopentyl ether or cyclohexane showed very low enantioselectivity below 5 (Table 2, entries 2 and 3). The highest enantioselectivity 78 has been observed applying toluene as a solvent what lead to desired product **2a** with 41% yield and high 95% enantiomeric excess (Table 2, entry 4). Finally, the catalyst used in the reaction with acid **1a** was filtered off and applied again (five times) without loss of activity. Lipase from *Pseudomonas cepacia* (Amano PS-C I) was more effective in polar solvents leading to ester (*S*)-**2a** with low enantioselectivity (Table 2, entries 5 and 6). In the reactions catalyzed by lipase from wheat germ the yield increases with the polarity of the solvent and was the highest in solvent with lowest partition coefficient. On the other hand enantioselectivity of the reaction catalyzed by this enzyme in different solvents remained very low (Table 2, entries 9-12). GLAP were most effective in nonpolar solvents considering enantioselectivity, the yield of the reaction remained similar in almost all cases leading to ester **2a** with 94% enantiomeric excess what corresponds to enantioselectivity value 56 (Table 2, entries 17-20). Novozym 435, lipase from wheat germ and GLAP provided product **2a** with (*R*)-configuration. Next, the series of experiments with different β-hydroxy acids **1b**-**i** were performed (Scheme 1). Applying previously used enzymes in TMBE and toluene, it turned out that only Novozyme 435 in toluene at 40 °C provides corresponding enantiomerically enriched esters **2b**-**i** with low enantioselectivities regardless of the groups attached to the phenyl ring. In case of three other enzymes products **2b**-**i** has not been observed on TLC after 72 hours. Obtained results are shown in Table 3.

Table 3 Kinetic resolution of *rac*-3-hydroxy-3-arylpropanoic acids**1b**-**i**[a]



Entry	R	Product	yield[b] (%)	*ee*[c] (%)	*E*[d]
1	4-CNC_6H_4	**2b**	29	58 (*R*)	5
2	2,4-$(NO_2)_2C_6H_3$	**2c**	18	83	13
3	4-F-2-$NO_2C_6H_3$	**2d**	42	52	3
4	4-CF_3-2-$NO_2C_6H_3$	**2e**	31	62	7
5	4-$MeOC_6H_4$	**2f**	39	60 (*R*)	6
6	2-BrC_6H_4	**2g**	<1	-	-
7	2-FC_6H_4	**2h**	42	68 (*R*)	4
8	Ph	**2i**	45	63 (*R*)	4

[a]Reaction conditions: 0.1mmolof **1**, triethyl orthobenzoate 2 equiv., toluene (2.0 mL), Novozym 435 (10 mg). [b]Isolated yield. [c]Determined by the chiral HPLC (CHIRALPAK IA, OD-H, OB). [d]Enantioselectivity, E=ln[1-c(1+ee_p)]/ln[1-c(1-ee_p)].[29]

Obtained results applying Novozym 435 as biocatalyst indicate that there are specific substrate requirements which cannot be easily determined. The results indicated that the most suitable substrates for the enzymatic reactions are these with strong

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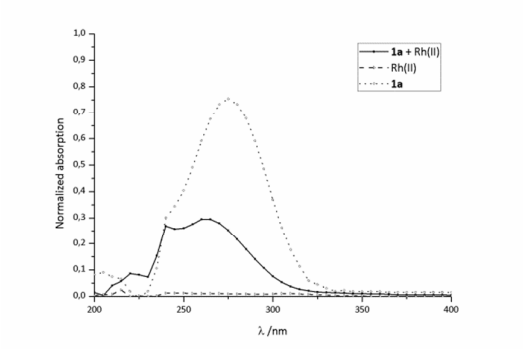
electron withdrawing group, which offer the best compromise between conversion and selectivity (Table 3, entry 7). In case of derivative **1c** with two strong electron withdrawing nitro groups corresponding ester **2c** was isolated with substantially lower yield and 83% enantiomeric excess (Table 1, entry 7). Moreover, the size of substituent attached to the *ortho* position of the phenyl ring cannot exceed the size of the active pocket in the enzyme (Table 1, entries 3 and 4).

To the best of our knowledge, DKR of β-hydroxy acids **1** has not been reported but corresponding β-hydroxy esters **2** is well recognized and is based on enzymatic acylation of hydroxyl group.[22]

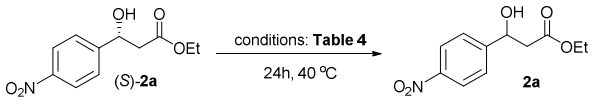
In the next step we investigated the racemization of (*S*)-3-hydroxy-3-(4-nitrophenyl)propanoic acid ((*S*)-**1a**). Optically active acid (*S*)-**1a** ($[\alpha]_D^{20}$= −30.2) obtained via hydrolysis of ester (*S*)-**2a** has been placed in TMBE at 40 °C together with rhodium(II) acetate ($Rh_2(OOCCH_3)_4$). This catalysts has been used with success by Williams *et al*. in enzymatic DKR of 1-phenylethanol.[12a] Applying rhodium(II) acetate together with KOH, *o*-phenanthroline and acetophenone caused partial racemization of (*S*)-**1a**($[\alpha]_D^{20}$= −12.7) within 24 hours in TMBE at 40 °C. Detailed studies shown that full racemization of (*S*)-**1a** within 4 hours in TMBE at 40 °C can be achieved by applying nothing but rhodium(II) acetate. Since the earliest preparations of $Rh_2(O_2CR)_4L_2$ complexes, it was recognized that rhodium(II) acetate readily exchange the bridging acetate groups in the presence of other carboxylic acids what may explain high efficiency of Rh(II) catalyst in DKR of β-hydroxy acid.[33] The exchanges of the bridging acetate groups with acid **1a** can be followed by Uv-Vis spectroscopy. Hypsochromic shift together with decreasing in infelicity can be observed in case of formed rhodium complex with acid **1a** (Fig. 1). Presented absorption spectra were recorded after 2 hours of agitation at ambient temperature in $CDCl_3$ using the same concentration of acid **1a**.

Figure 1 Absorption spectra of free and rhodium complex with acid **1a** recorded in $CDCl_3$.



Some more aspects of racemization process has been studied extensively. In order to determine the influence of rhodium catalyst, enzyme and triethyl orthobenzoate on the racemization of enantiomerically pure ester (*S*)-**2a** additional experiments were performed (Table 4). Ester (*S*)-**2a** does not racemize in the presence of Amano AK lipase (Table 4, entry 1). Under conditions proposed by Williams *et al*.[12a] significant racemization of (*S*)-**2a**proceeded(Table 4, entry 2).Also, in the presence of triethyl orthobenzoate and ruthenium catalyst exclusively, slight decrease in enantiomeric excess was observed (Table 4, entries3 and 6).

Table 4 Racemization of ethyl (*S*)-3-hydroxy-3-(4-nitrophenyl)propanoate ((*S*)-**2a**)[a]



Entry	Conditions	yield[b] (%)	ee[c] (%)
1	Amano AK	>99	99 (*S*)
2	PhCOMe, *o*-phenanthroline	97	63(*S*)
3	Rh(II)	>99	94 (*S*)
4	Amano AK, Rh(II)	>99	99 (*S*)
5	$PhC(OEt)_3$, Amano AK	>99	99 (*S*)
6	$PhC(OEt)_3$, Rh(II)	>99	96 (*S*)
7	$PhC(OEt)_3$, Amano AK, Rh(II)	>99	99 (*S*)

[a]Reaction conditions: 0.1mmolof (*S*)-**2a**, ethoxy group donor 2 equiv., TMBE (2.0 mL), Amano AK (5 mg), metal catalyst (10 mol%). [b]Isolated yield. [c]Determined by the chiral HPLC (Chiralpak IA).

Surprisingly, application of lipase from Amano AK suppresses the racemization of product (*S*)-**2a** (Table 4, entries 4, 5 and 7). Presence of triethyl orthobenzoate does not promote the racemization course of ester (*S*)-**2a** which after all was isolated with any loss in enantiomer excess (Table 5, entry 4 and 5). In all cases substrate **2a** was recovered quantitatively.

The next step our studies was to combine the racemization and the enzymatic transformation and run the reaction in one pot. Further experiments were assigned with Amano AK lipase and rhodium(II) acetate in four different organic solvents at 40 °C for 72 hours (Table 4).In all cases product (*S*)-**2a** was obtained with higher yield (more than 67%) comparing to EKR. The type of the solvent strongly influences the yield and enantiomeric excess (Table 5, entries 1-3). Enantiomerically pure ester (*S*)-**2a** with 89% isolated yield was provided from the reaction performed in TBME which was also the best solvent for kinetic resolution applying lipase from Amano AK (Table 5, entry 4). Lipase Amano AK was easily recovered by filtration and rhodium catalyst by recrystallization from methanol[34] with 90% yield and reused up to 3 times, without loss of activity. In the course of the studied reaction acylation of the free hydroxyl group of ester **2a** by a second hydroxy ester molecule did not take place what was verified by mass spectroscopy.

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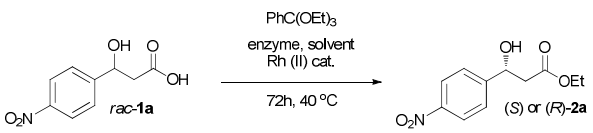
Table 5 Dynamic kinetic resolution of racemic3-hydroxy-3-(4-nitrophenyl)propanoic acid (**1a**) catalyzed by Amano AK and Novozym 435[a]



Entry	Enzyme	Solvent	yield[b](%)	*ee*[c] (%)
1	Amano AK	toluene	78	48 (*S*)
2		cyclohexane	73	84 (*S*)
3		isopentylether	67	56 (*S*)
4		TBME	89	99 (*S*)
5	Novozyme 435	toluene	78	97 (*R*)
6		cyclohexane	87	48 (*R*)
7		isopentylether	91	50 (*R*)
8		TBME	69	9 (*R*)

Reaction conditions: 0.1 mmol of **1a**, triethyl orthobenzoate 2 equiv., solvent (2.0 mL), enzyme (Amano AK 5 mg or Novozym 435 10 mg), metal catalyst (10 mol%). [b]Isolated yield. [c]Determined by the chiral HPLC (Chiralpak IA).

Further experiments were assigned with immobilized lipase from *Candida antarctica* (Novozyme 435).

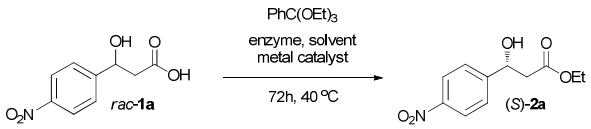
Considering strong influence of organic solvent on the reaction course observed in EKR the next set of experiments were performed in different organic solvents with Novozym 435. In the case of reactions with rhodium catalyst the most effective were cyclohexane and isopentyl ether leading to optically enriched (48% and 50% *ee*) ester (*R*)-**2a** with 87% and 91% yield, respectively (Table 5, entries 6 and 7). Only in case of using TBME the yield and enantiomeric excess of the reaction were lower and dropped to 69% and 9%, respectively (Table 6, entry 8). Nevertheless, ester (*R*)-**2a** was obtained with isolated yield 78% and very high enantiomeric excess 97%(Table 6, entry 1).

Also other commonly used in DKR metal catalysts(e.g. Shvo's Catalyst,[31] $[Pd(PPh_3)_4]$/dppf, $PdCl_2$(dppf), and $[Ru(p\text{-cymene})Cl_2]_2$/dppb)[32] were tested. In all cases, reaction performing under DKR conditions leads to enhancement in the reaction yield (Table 6).

At the same time the enantiomeric excess of the ester **2b** was much lower than in EKR procedure what was anticipated due to literature reports about DKR of the β-hydroxy esters.[12,22]

Finally, we applied this transformation concept on a preparative scale. Thus, 212 mg (1 mmol) of racemic 3-hydroxy-3-(4-nitrophenyl)propanoic acid (**1a**) was transformed into optically pure ester (*S*)-**2a** within 72 h, and with 94% yield of isolated product. Scaling up to 10 mmol of the substrate **1a** is possible and also very effective.

Table 6 Racemization of ethyl (*S*)-3-hydroxy-3-(4-nitrophenyl)propanoate ((*S*)-**2a**) with various metal catalysts[a]



Entry	Metal catalyst	yield[b] (%)	ee[c] (%)
1	$[Pd(PPh_3)_4]$/dppf	91	35 (*S*)
2	$PdCl_2$(dppf)	88	41 (*S*)
3	$Ru(p\text{-cymene})Cl_2]_2$	69	55 (*S*)
4	Shvo's catalyst	82	49 (*S*)

[a]Reaction conditions: 0.1 mmol of (*S*)-**2a**, triethyl orthobenzoate 2 equiv., TMBE (2.0 mL), Amano AK (5 mg), metal catalyst (10 mol%), t-BuOK (10 mol%). [b]Isolated yield. [c]Determined by the chiral HPLC (Chiralpak IA).

To show the applicability of this concept, other β-acids **1c** and **1i** were subjected to DKR with Novozyme 435 leading to corresponding esters (*R*)-**2c** and (*R*)-**2i** with 98% yield and enantiomeric excess values identical with those obtained via enzymatic kinetic resolution (Table 3, entries 2 and 8). Ethyl (*R*)-3-hydroxy-3-phenylpropanoate (**2i**) is a precursor of the antidepressant Fluoxetine also known by the trade names Prozac.[35]

As can be notice, appropriate combination of enzyme, metal and organic solvent is required for successful enzymatic DKR procedure. Further studies under optimization and amplification of herein proposed protocol are still underway. In spite of described limitations proposed approach diversifies already knew methods and may lead to optically pure β-hydroxy ester with very high yields.

Conclusions

In summary, we have established the new protocol to enantiomerically enriched β-hydroxy esters based on enzymatic kinetic (EKR).Afterwards this protocol has been extended to tandem metal-enzyme dynamic kinetic resolution (DKR) of β-hydroxy acids providing optically pure product. The whole process does not require anhydrous conditions, moreover dehydration of the product is not observed. The role of organic solvent and biocatalyst is crucial. Change of biocatalyst and polarity of the solvent makes possible to control (in respect to enantioselectivity) EKR and DKR. Under optimal conditions both enantiomers of ester **2a** were obtained with very high isolated yield. It was demonstrated that the high enantioselectivity of the enzyme is compatible with the rhodium catalyzed racemization of β-hydroxy acids. The protocol reported here should be a useful complement to known methods. Moreover, the easy recovery of both catalyst makes this process suitable for up scaling.

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Experimental

Materials and general methods

All the chemicals were obtained from commercial sources and the solvents were of analytical grade. Amano lipase PS-C I from *Pseudomonas cepacia* (immobilized on ceramic) and Amano lipase AK from *Pseudomonas fluorescens* were purchased from Sigma-Aldrich. Novozym 435 was purchased from Novo Nordisk. Goose liver was converted to the acetone powder (GLAP) by the method of Connors *et al*.[36] Column chromatography was performed on Merck silica gel 60/230-400 mesh. The enantioselectivity parameter *E* was calculated using equation: E=ln[1-c(1+*ee*$_p$)]/ln[1-c(1-*ee*$_p$)]. *E* calculations were performed for isolated yields of corresponding products 2**a**-**i**. Enzymatic reactions were performed in a vortex (Heidolph Promax 1020) equipped with incubator (Heidolph Inkubator 1000). To prove the ability of the established protocol each reaction *was* repeated at least three times.

General procedure for enzymatic kinetic resolution of racemic 3-hydroxy-3-(aryl)propanoic acids

To the solution of acid **1a**-**i** (0.1 mmol) in organic solvent (2 mL), triethyl orthobenzoate (2 equiv.) and enzyme (native 5 mg or immobilized 10 mg) were added in 5 mL screwed vial. The reaction mixture was stirred for 48 hours at 40 °C. After cooling, crude product was purified by column chromatography (ethyl acetate/hexanes). The ^{1}H NMR data were in accordance with those recorded for racemates; (*S*)-(-)-**2a**: $[\alpha]_D^{20}$= −59.5 (c 1.5, $CHCl_3$, 99% *ee*), (*R*)-(+)-**2a**: $[\alpha]_D^{20}$= +57.5 (c 1.0, $CHCl_3$, 95% *ee*); Lit (*R*)-enantiomer: $[\alpha]_D^{20}$= +23.1 (c 1.0, $CHCl_3$);[37](+)-**2b**: $[\alpha]_D^{20}$= +23.0 (c 1.00 $CHCl_3$, 58% *ee*); (+)-**2c**: $[\alpha]_D^{20}$= +25.2 (c 1.00 $CHCl_3$, 83% *ee*); (+)-**2d**: $[\alpha]_D^{20}$= +16.3 (c 1.00 $CHCl_3$, 52% *ee*); (+)-**2e**: $[\alpha]_D^{20}$= +12.8 (c 1.00 $CHCl_3$, 62% *ee*); (*R*)-(+)-**2f**: $[\alpha]_D^{20}$= +21.5 (c 1.00 $CHCl_3$, 60% *ee*), Lit (*R*)-enantiomer: $[\alpha]_D^{20}$= +28.8 (c 1.0, $CHCl_3$);[38](*R*)-(+)-**2h**: $[\alpha]_D^{20}$= +34.6 (c 1.00 $CHCl_3$, 68% *ee*), Lit (*S*)-enantiomer: $[\alpha]_D^{20}$= -49.7 (c 2.71, $CHCl_3$);[39](*R*)-(+)-**2i**: $[\alpha]_D^{20}$= +40.2 (c 1.00 $CHCl_3$, 63% *ee*), Lit (*R*)-enantiomer: $[\alpha]_D^{20}$= +48.9 (c 0.89, $CHCl_3$).[40]

General procedure for enzymatic dynamic kinetic resolution of racemic 3-hydroxy-3-(aryl)propanoic acids

To the solution of acid **1** (0.1 mmol) in organic solvent (2 mL), triethyl orthobenzoate (2 equiv.), enzyme (native 5 mg or immobilized 10 mg) and metal catalyst (10 mol%) were added in 5 mL vial. The reaction mixture was stirring for 72 hours at 40 °C. After cooling, crude product was purified by column chromatography (ethyl acetate/hexanes).

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Notes and references

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