Synthesis of α-Alkyl-β-Hydroxy Amides through Biocatalytic Dynamic Kinetic Resolution Employing Alcohol Dehydrogenases

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Abstract. Chiral (α-substituted) β-hydroxy amides are interesting derivatives as they are useful building blocks of many biologically active compounds. Herein, the biocatalytic stereocontrolled synthesis of various acyclic *syn*-α-alkyl-β-hydroxy amides through a dynamic kinetic resolution is shown. Hence, a series of overexpressed alcohol dehydrogenases (ADHs) in *Escherichia coli* was used to reduce the corresponding racemic β-keto amides. Among them, ADH-A from *Rhodococcus ruber* and commercial evo-1.1.200 afforded the best activities and selectivities, giving access to the opposite enantiomers with high diastereomeric excess and excellent enantiomeric excess. Some of these compounds were obtained at preparative scale.

Keywords: Alcohol dehydrogenases; Biocatalysis; Chiral synthesis; Dynamic kinetic resolutions; β-Hydroxy amides

Chiral β-hydroxy amides are highly interesting derivatives as they are versatile and useful building blocks of different biologically active compounds. Among them, oxazolines,[1] oxazoles,[2] pyrrolidines,[3] azetidines,[4] and 1,3-amino alcohols such as fluoxetine,[5] can be mentioned. They are also present in the core structure of anticancer families like bengamides,[6] and can be utilised as ligands to induce chirality in organic transformations.[7]

Different synthetic approaches have been designed in order to synthesise these derivatives (Figure 1A). Hence, the aldol condensation between a (chiral) amide and an aldehyde[4,8] or an acylsilane[9] has been demonstrated as a valuable methodology to obtain these compounds with high enantiomeric excess (*ee*). Likewise, the hydrogenation of β-keto amide precursors employing ruthenium[10] or iridium[11] complexes, the hydroboration of unsaturated amides mediated by rhodium[12] or copper[13] complexes, the regioselective ring-opening of α,β-epoxy amides with sodium bis(2-methoxyethoxy)aluminium hydride (Red-Al),[5] the palladium-catalysed β-acyloxylation of amides,[14] and the ruthenium-mediated reduction of β-keto nitriles[15] have been shown as attractive strategies.

However, the synthesis of α-substituted β-hydroxy amides in diastereo- and enantioselective manner is more challenging due to the formation of four possible diastereoisomers. Again, various synthetic protocols have been developed to successfully get access to these compounds (Figure 1A). Aldol additions have provided good results although at the expense of using very low reaction temperatures.[16] The opening of chiral epoxides with different nucleophiles has also been demonstrated as another powerful tool. However, enantiopure synthons must be previously synthesised.[6,17] A very simple and direct method is the stereoselective reduction of the racemic α-substituted β-keto amides under dynamic conditions.[18] Since these substrates can easily racemise due to the high acidity of the α-hydrogen, a dynamic kinetic resolution (DKR)[19,20] can be achieved, thus providing in the ideal case one out of four diastereoisomer products. Various chemical agents such boranes[21] and hydrides[22] have afforded the corresponding racemic β-hydroxy amides with high diastereomeric excess (*de*), while the Ru-[3,23] and Ir-mediated[24] hydrogenation of α-substituted β-keto lactams gave the alcohols with excellent *de* and *ee*. There is just one report demonstrating the asymmetric hydrogen transfer of acyclic substrates with a ruthenium complex, obtaining the final compounds with very high *de* and *ee* values after recrystallisation of the reaction crude.[25]
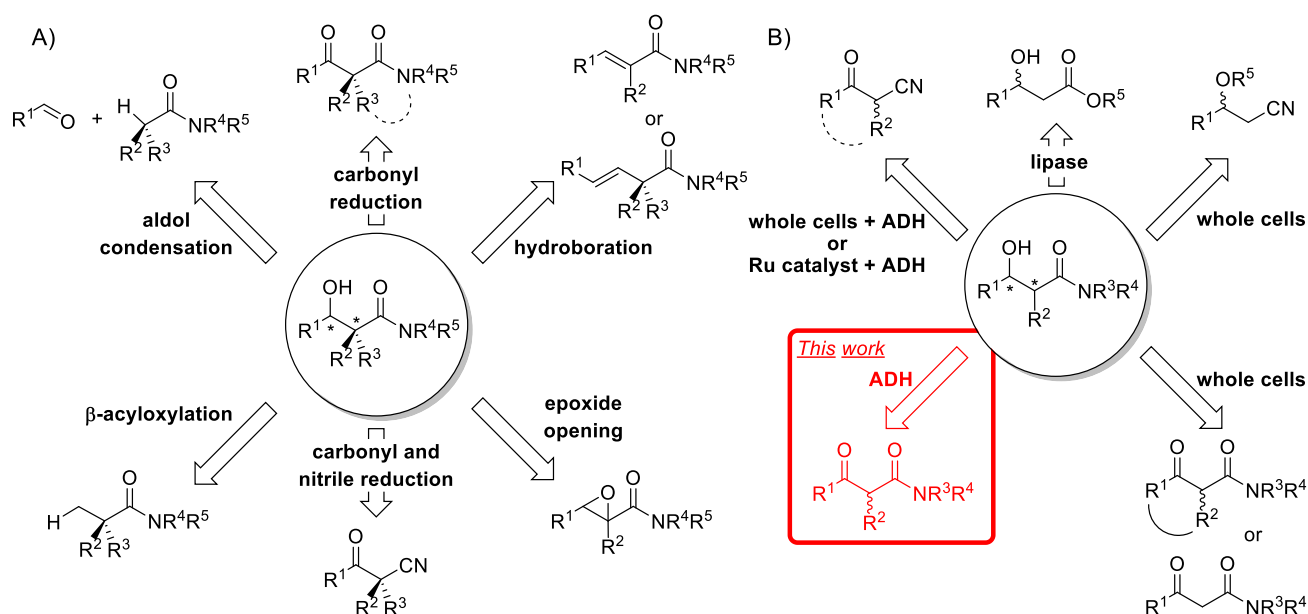
Figure 1. A) Chemical approaches; and B) (chemo)enzymatic methodologies to synthesise chiral (α-substituted) β-hydroxy amides.

From an enzymatic point of view (Figure 1B), the kinetic resolution of racemic β-hydroxy esters through lipase-catalysed aminolysis,[26] or β-O-protected nitriles with *Rhodococcus erythropolis* whole cells[27] and the reduction of β-keto amides using yeasts or fungi,[28] have provided the chiral alcohols but with low yields and/or selectivities. Very recently, the combination of *Rhodococcus rhodochrous* whole cells[29] or a ruthenium catalyst[30] with alcohol dehydrogenases (ADHs)[31] delivered different cyclic β-hydroxy amides starting from the corresponding racemic β-keto nitriles through a DKR process with excellent *de* and *ee*.

While the bioreduction under dynamic conditions of α-substituted β-keto esters using whole cells or isolated ADHs has been recurrently studied,[20,32] this has not been the case for the amide analogues. Herein (Figure 1B, red), a set of α-alkyl-β-keto amides has been successfully reduced with different lyophilised preparations containing overexpressed ADHs in *E. coli* affording the corresponding β-hydroxy amides in many cases with outstanding enantio- and diastereoselectivities.

For this purpose, the chemical synthesis of a wide panel of α-substituted β-keto amides was developed (Scheme 1). They differed in the amide protecting group and the substitution pattern at α-position. We used as starting materials commercially available β-keto esters **1a** and **1b** following two independent synthetic methodologies in order to obtain the corresponding β-keto amides **2a-d**. Better results were found for the synthesis of N-benzylated keto amides **2a** and **2b** (89% yield) through the formation of 1,3,2-dioxaborinane intermediates,[33] while to get access to compounds **2c** (91% yield) and **2d** (90% yield) the lipase-mediated approach was preferred,[34] finding less than 20% yield when benzylamine was

used as nucleophile in the biotransformations for the synthesis of **2a** and **2b**. Later on, the β-keto amides were alkylated at α-position by treatment with different alkyl halides in basic medium using acetone as solvent.[32] After purification by column chromatography, α-alkylated β-keto amides **3a-h** were obtained in moderate yields (38-60%). Finally, the racemic β-hydroxy amides (**4a-d**) and α-substituted β-hydroxy amides (**5a-h**) were synthesised by addition of sodium borohydride to a solution of the corresponding keto amide in dry dichloromethane, obtaining the products after extraction in high yields (89-98%) and excellent purities.
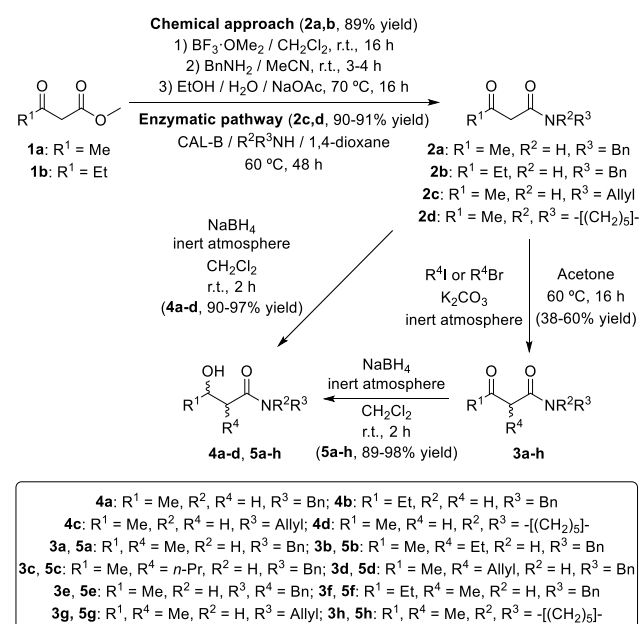
Scheme 1. Synthesis of (α-substituted) β-keto amides and racemic β-hydroxy amides.

In order to study the suitability of the DKR processes with the α-substituted substrates, different ADHs were tested first towards the bioreduction of β-keto amides **2a-d**. Thus, the effect of the amide protecting group could be considered. This way, lyophilised *E. coli* preparations containing overexpressed (*S*)-selective ADHs from *Rhodococcus ruber* (ADH-A),[35] *Thermoanaerobacter ethanolicus* (TeSADH),[36] *Thermoanaerobium* sp. (ADH-T),[37] *Sphingobium yanoikuyae* (SyADH),[38] *Ralstonia* sp. (RasADH),[39] and the (*R*)-selective ADH from *Lactobacillus brevis* (LbADH)[40] were screened. Also, commercially available evo-1.1.200[41] was studied.

The bioreductions were performed at 25 mM concentration of the substrate in a reaction mixture containing Tris·HCl 50 mM pH 7.5 and 1 mM of the nicotinamide cofactor NAD(P)H. DMSO was used as cosolvent in all cases in a 2.5% v/v ratio, and either large amounts of 2-propanol (*i*PrOH) or the glucose/GDH system were employed to regenerate the cofactor. All reactions were incubated at 30 ºC for 24 hours (Table 1). After this time, ADH-A and ADH-T revealed good results with all substrates, leading to the synthesis of the (*S*)-alcohols with high conversions and *ee* values (entries 1, 2, 4, 5, 7, 8, 10 and 11). Furthermore, the commercial evo-1.1.200 fully converted **2a-c** into the enantiopure (*R*)-alcohols **4a-c** (entries 3, 6 and 9). However, it led to the formation of enantiopure (*R*)-**4d** with a moderate conversion value (entry 12). The other ADHs afforded worse results (see SI, Table S1).

From these results, it was clear that even though these substrates were suitable for some ADHs, the amide moiety (R^2 and R^3) and the substitution pattern in R^1 had an important influence in some ADHs behaviour. For this reason, the next step was to study the influence that the substitution pattern at the α-position had in the bioreduction, and also to look if DKR transformations were possible.

The α-methylated β-keto amide **3a** was chosen as model substrate (Table 2 and Table S2 in the Supporting Information). Under the same reaction conditions previously described, it was observed that full conversion into **5a** was achieved when using ADH-A (entry 1), RasADH (entry 2) or evo-1.1.200 (entry 3). On the one hand, ADH-A and evo-1.1.200 led to very high diastereo- and enantioselectivity, obtaining in both cases the *syn* diastereoisomer as the major one. This way, (2*R*,3*S*)-**5a** was obtained with 90% *de* and 99% *ee* using ADH-A as biocatalyst and evo-1.1.200 led to the formation of (2*S*,3*R*)-**5a** with 94% *de* and >99% *ee*. On the other hand, even though RasADH showed good results in terms of conversion, it achieved the synthesis of the *anti*-diastereoisomer (2*S*,3*S*)-**5a** but with only 30% *de* and 28% *ee*.

After this screening, the influence of different alkyl chains at α-position was studied. For this purpose, the bioreduction of substrates **3b-e** into the corresponding alcohols (**5b-e**) was attempted, finding remarkable results only with ADH-A and evo-1.1.200 (Tables S3 and S4 in the Supporting Information; Table 2, entries 4-11). As observed with the model substrate **3a**, both enzymes favoured the formation of the *syn*-alcohols. In this manner, (2*R*,3*S*)-enantiomers were obtained with high conversions (78->99% conv) and moderate to high diastereoselectivity (72-92% *de*) and very high enantioselectivity (99% *ee*) with ADH-A. Additionally, (2*S*,3*R*)-**5b-d** were synthesised in moderate to high conversion values (60-98% conv) and high diastereo- and enantioselectivities (90-92% *de* and 99->99% *ee*) with evo-1.1.200. However, this biocatalyst lost its activity towards the synthesis of the α-benzylated β-hydroxy amide **5e** (entry 11).

Encouraged by these results and looking for a further exploitation of the synthetic approach, we decided to undertake the ADH-catalysed DKR of substrate **3f**, bearing an ethyl group at R^1 position. Using the same reaction conditions, all enzymes were screened (Table S5 in the Supporting Information and Table 2, entries 12 and 13). Unfortunately, only evo-1.1.200 led to the preferential formation of alcohol (2*S*,3*R*)-**5f** with high conversion (98%), moderate *de* (77%) and excellent *ee* (>99%) values.

Table 1. ADH-catalysed bioreduction of β-keto amides **2a-d**.[a]

Entry	Substrate	ADH	*c* [%][b]	*ee* [%][c]
1	**2a**	ADH-A	>99	96 (*S*)
2	**2a**	ADH-T	>99	>99 (*S*)
3	**2a**	evo-1.1.200	>99	>99 (*R*)
4	**2b**	ADH-A	96	96 (*S*)
5	**2b**	ADH-T	96	>99 (*S*)
6	**2b**	evo-1.1.200	>99	>99 (*R*)
7	**2c**	ADH-A	>99	>99 (*S*)
8	**2c**	ADH-T	>99	>99 (*S*)
9	**2c**	evo-1.1.200	>99	>99 (*R*)
10	**2d**	ADH-A	>99	>99 (*S*)
11	**2d**	ADH-T	>99	>99 (*S*)
12	**2d**	evo-1.1.200	40	>99 (*R*)

[a] For reaction conditions and the complete set of data, see the Supporting Information.
[b] Conversion values were measured by GC analyses.
[c] Enantiomeric excess values were measured by HPLC analyses. Major enantiomer in parentheses.

Table 2. Dynamic kinetic resolution of α-substituted β-keto amides **3a-h** bearing different pattern substitution at α-position.[a]


Entry	Substrate	ADH	c [%][b]	de [%][c],[d]	ee anti [%][c]	ee syn [%][c]
1	**3a**	ADH-A	>99	90	n.d.	99 (2*R*,3*S*)
2	**3a**	RasADH	>99	30	28 (2*S*,3*S*)	30 (2*R*,3*S*)
3	**3a**	evo-1.1.200	>99	94	n.d.	>99 (2*S*,3*R*)
4	**3b**	ADH-A	>99	88	n.d.	99 (2*R*,3*S*)
5	**3b**	evo-1.1.200	96	92	n.d.	>99 (2*S*,3*R*)
6	**3c**	ADH-A	99	72	99 (2*S*,3*S*)	99 (2*R*,3*S*)
7	**3c**	evo-1.1.200	60	90	n.d.	99 (2*S*,3*R*)
8	**3d**	ADH-A	99	92	n.d.	99 (2*R*,3*S*)
9	**3d**	evo-1.1.200	98	92	n.d.	>99 (2*S*,3*R*)
10	**3e**	ADH-A	78	78	99 (2*S*,3*S*)	99 (2*R*,3*S*)
11	**3e**	evo-1.1.200	<1	n.d.	n.d.	n.d.
12	**3f**	ADH-A	<1	n.d.	n.d.	n.d.
13	**3f**	evo-1.1.200	98	77	5 (2*R*,3*R*)	>99 (2*S*,3*R*)
14	**3g**	ADH-A	99	82	n.d.	99 (2*R*,3*S*)
15	**3g**	evo-1.1.200	98	86	n.d.	>99 (2*S*,3*R*)
16	**3h**	ADH-A	96	12	99 (2*S*,3*S*)	99 (2*R*,3*S*)
17	**3h**	evo-1.1.200	8	n.d.	n.d.	n.d.

[a] For reaction conditions and the complete set of data, see the Supporting Information.

[b] Conversion values were measured by GC analyses.

[c] Diastereomeric and enantiomeric excess values were measured by HPLC analyses. Major diastereoisomer shown in parentheses.

[d] ADH-A and evo-1.1.200 produced preferentially the *syn*-diastereoisomer, while RasADH (entry 2) led preferentially to the formation of the *anti*-diastereoisomer.

n.d. not determined.
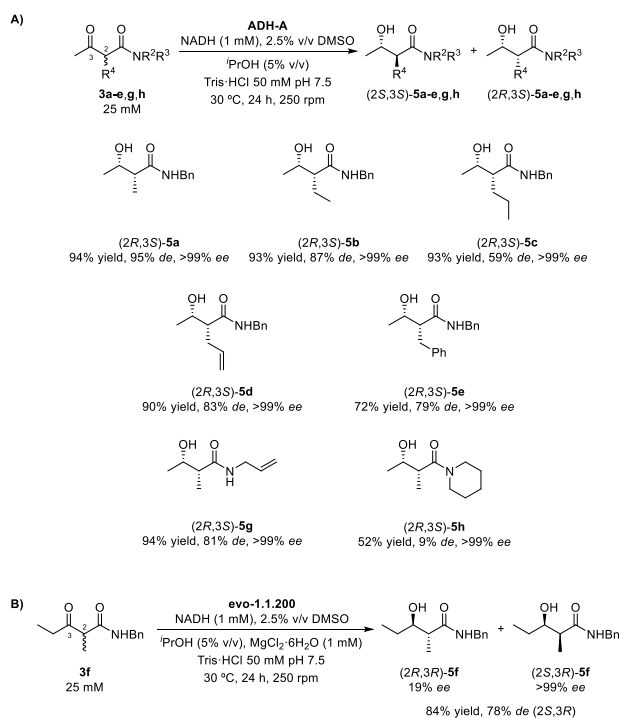
Scheme 2. Semipreparative DKR of α-substituted β-keto amides: A) **3a-e,g,h** catalysed by overexpressed ADH-A; and B) **3f** catalysed by evo-1.1.200.

At this point, the influence that different amide moieties had in the process was studied. For this reason, the bioreduction protocol was set up using α-methylated substrates **3g** and **3h** (Table S5 in the Supporting Information; Table 2, entries 14-17). On the one hand, as previously observed with compound **3a**, the keto amide **3g** was a suitable substrate for ADH-A and evo-1.1.200, being the *syn*-alcohol the major product in both cases (entries 14 and 15). Hence, (2*R*,3*S*)- and (2*S*,3*R*)-**5g** were obtained with high conversion and selectivity (99% conv., 82% *de* and 99% *ee* and 98% conv., 86% *de* and >99% *ee*, respectively). On the other hand, **3h** led to high conversion and low *de* values, while high enantioselectivity towards the formation of (2*R*,3*S*)-**5h** (96% conv, 12% *de* and 99% *ee*, entry 16) when using ADH-A as biocatalyst and a completely loss of activity when utilising evo-1.1.200. From these results it became clear that the N-protecting had a large effect in the enzyme recognition, being the benzyl and the allyl moieties the most appropriate ones to perform these DKR transformations.

The relative *syn* configuration of the final products was assigned based on the use of NMR homonuclear decoupling experiments (see the Supporting Information). This result was confirmed with the method showed by Kalaitzakis and Smonou with α-alkyl-β-hydroxy carbonyl compounds,[42] together with the measured $^{3}J_{H2H3}$ for similar derivatives[25]

4

and the known diastereopreference with the same enzymes with α-alkyl-β-keto esters.[32] The absolute configuration was determined due to the known stereospecificity of these ADHs.[32,35-41]

In order to demonstrate the applicability of the method, ADH-catalysed DKR transformations were performed at semipreparative scale. For this purpose, ADH-A was the enzyme of choice as it was the most efficient ketoreductase, providing good or excellent results in the bioreduction of substrates **3a-e,g,h**. This way, 100 mg of model β-keto amide **3a** and 20 mg of the other compounds were transformed into the corresponding enantioenriched alcohols. 2.5% v/v of DMSO was employed as co-solvent and the reaction media (50 mM Tris.HCl pH 7.5) was implemented with NADH (1 mM). 2-Propanol (5% v/v) was employed to regenerate the nicotinamide cofactor. After 24 hours, similar results to those obtained at analytical scale were found (Scheme 2). Thus, the *syn*-(2*R*,3*S*)-diastereoisomers of the alcohols were obtained as the major one in moderate to high yields (52-94%) and excellent enantioselectivities (>99% *ee*). The diastereoselectivity of ADH-A remained high with the exception of β-hydroxy amide **5c** (59% *de*) and, especially, alcohol **5h** (9% *de*). Finally, the transformation of **3f** (20 mg) with evo-1.1.200 was performed, obtaining the enantiopure (2*S*,3*R*)-**5f** diastereoisomer with 78% *de*.

Overall, herein the reduction of various acyclic α-alkyl-β-keto amides has been described, affording the corresponding *syn*-α-alkyl-β-hydroxy amides with high diastereo- and enantioselectivities through DKR processes, employing lyophilised *E. coli* cells containing overexpressed ADHs. The high acidity of the α-proton ensured a fast substrate racemisation yielding the enantioenriched products at conversions close to 100% even at almost neutral pH. Enantiocomplementary ADH-A from *Rhodococcus ruber* and commercially available evo-1.1.200 afforded the best results. An important effect of the alkyl chain at α-position and also of the amide protecting group was observed in these bioreductions. Thus, higher *de* values were obtained for short alkyl moieties and N-benzylated amides. This methodology allows to get access to a new family of compounds with selectivities comparable to the ones obtained with metal catalysts,[25] thus demonstrating the great potential of enzymes to obtain valuable derivatives under straightforward, simple, and environmentally-friendly conditions.

Experimental Section

Alcohol dehydrogenases from *Ralstonia* sp. (RasADH), *Lactobacillus brevis* (LbADH), *Sphingobium yanoikuyae* (SyADH), *Thermoanaerobacter ethanolicus* (TeSADH), *Thermoanaerobacter* sp. (ADH-T), *Rhodococcus ruber* (ADH-A) overexpressed on lyophilised *E. coli* cells were obtained as previously described in the literature.[32] evo-1.1.200 was acquired from evoxx technologies GmbH. Chemical reagents were purchased from different commercial sources and used without further purification.

*Preparative scale bioreduction of α-substituted β-keto amides **3a-e,g,h** using the alcohol dehydrogenase from* Rhodococcus ruber *(ADH-A)*

Lyophilised *E. coli*/ADH-A cells (100 mg for β-keto amide **3a** and 50 mg for β-keto amides **3b-e,g,h**), DMSO (2.5% v/v), NADH (1 mM) and *i*PrOH (5% v/v) were successively added into an Erlenmeyer flask containing β-keto amide (100 mg for **3a** and 20 mg for **3b-e,g,h**, 25 mM) in Tris·HCl buffer 50 mM pH 7.5. The reaction was shaken at 30 °C and 250 rpm for 24 h and then extracted with EtOAc (3 x 15 mL). The organic layers were separated by centrifugation (5 min, 4900 rpm), combined and finally dried over Na_2SO_4. The solvent was concentrated under vacuum, furnishing the α-substituted β-hydroxy amides **5a-e,g,h** in moderate to excellent isolated yields (52-94%).

Preparative scale bioreduction of α-substituted β-keto amide 3f using the commercial alcohol dehydrogenase evo-1.1.200.

20 mg of β-keto amide **3f** (25 mM) was added in an Erlenmeyer flask containing DMSO (2.5% v/v), $MgCl_2·6H_2O$ (1 mM), NADH (1 mM) and *i*PrOH (5% v/v) in Tris·HCl buffer 50 mM pH 7.5 (final volume: 3.6 mL). Finally, 75 mg of lyophilised evo-1.1.200 were added and the reaction was shaken at 30 ºC and 250 rpm for 24 h. After this time, the reaction was extracted with EtOAc (3 x 5 mL). The organic layers were separated by centrifugation (5 min, 4900 rpm), combined and finally dried over Na_2SO_4. The solvent was concentrated under vacuum, achieving the α-substituted β-hydroxy amide **5f** in high isolated yield (17 mg, 84%).

Acknowledgements

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Synthesis of α-Alkyl-β-Hydroxy Amides through Biocatalytic Dynamic Kinetic Resolution Employing Alcohol Dehydrogenases

Adv. Synth. Catal. **Year**, *Volume*, Page – Page

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