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Expanding the Scope of Alcohol Dehydrogenases towards Bulkier Substrates: Stereo- and Enantiopreference for α,α-Dihalogenated Ketones

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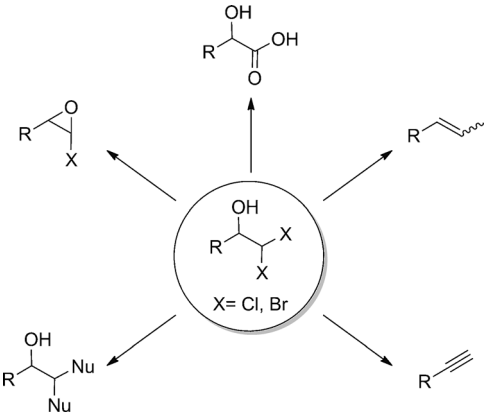
Alcohol dehydrogenases (ADHs) were identified as suitable enzymes for the reduction of the corresponding α,α-dihalogenated ketones, obtaining optically pure β,β-dichloro- or β,β-dibromohydrins with excellent conversions and enantiomeric excess. Among the different biocatalysts tested, ADHs from *Rhodococcus ruber* (ADH-A), *Ralstonia* sp. (RasADH), *Lactobacillus brevis* (LBADH), and PR2ADH proved to be the most efficient ones in terms of activity and stereoselectivity. In a further study, two racemic α-substituted ketones, namely α-bromo-α-chloro- and α-chloro-α-fluoroacetophenone were investigated to obtain one of the four possible diastereoisomers through a dynamic kinetic process. In the case of the brominated derivative, only the (1*R*)-enantiomer was obtained by using ADH-A, although with moderate diastereomeric excess (>99% *ee*, 63% *de*), whereas the fluorinated ketone exhibited a lower stereoselectivity (up to 45% *de*).

Introduction

β,β-Dihalogenated alcohols, also called *gem*-dihalo alcohols or β,β-dihalohydrins,[1] are a family of interesting compounds because of their versatility in organic synthesis,[2] and because of their role as precursors of biologically active derivatives such as antineoplastic drugs like mitotane.[3] Thus, owing to the highly activated nature of these compounds, they can be used as synthetic intermediates of interesting molecules such as alkenes,[4] terminal alkynes,[5] epoxides,[6] and α-methoxy alkyl acetic acid derivatives.[7] Additionally, owing to their reactivity in aqueous medium, they have been described as chemical analogues of α-hydroxy aldehydes, opening the scope towards other types of substrates, for example, α-hydroxy acids (Scheme 1).[8]



Scheme 1. Synthetic applicability of the *gem*-dihalo alcohol core.

The preparation of the racemic derivatives can be achieved by means of different synthetic approaches such as the Hunsdiecker reaction,[9] the decarboxylative heterodifunctionalisation of α,β-unsaturated carboxylic acids[10] or the reduction of the corresponding ketone precursors.[11] Unfortunately, these methods usually afford a mixture of products because of the formation, among others, of dehydrated, hydrolysed or over-reduced compounds. It is even more difficult to find in the literature an appropriate methodology to stereoselectively achieve these chiral precursors. Unfortunately, the selectivities obtained in these processes by reduction of the ketones using chiral oxaborolidines[12] or borane complexes were moderate (<83%),[13] and, alternatively, dichlorocarbene C–H insertion re-

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actions[14] led to incomplete conversions (< 90%) starting from an expensive enantiopure alcohol precursor.

Interestingly, the use of biocatalytic methods under mild reaction conditions has allowed the selective synthesis of difluorohydrins,[15] However, for the chlorinated or brominated counterparts, and although the formation of by-products was minimised, the enantioselectivities or yields obtained in these processes were still not high enough. For instance, (*R*)-2,2-dibromo-1-(4′-benzyloxy-3′-hydroxymethylphenyl)ethanol was obtained in 82% yield and 92% *ee* by using *Rhodotorula rubra* whole cells in the presence of a surfactant.[16] The lipase-catalysed resolution of 2,2-dichloro-1-phenylethanol (**3a**) was achieved with Amano *Pseudomonas cepacia* lipase (PSL), but 44% conversion of the final product was reached after 142 h.[17] On the other hand, the bioreduction of the α,α-dihalo ketone precursor **2a** has been tested with whole cells from *Geotrichum candidum* APG4[18] and baker's yeast,[19] but stereoselectivities remained modest (< 55% *ee*). Based on the high selectivities displayed by alcohol dehydrogenases (ADHs),[20] and as α-monohalogenated ketones are excellent substrates for these enzymes,[19,21] the bioreduction of a series of bulkier α,α-dihalogenated acetophenones is presented herein. Several partially purified/overexpressed ADHs were tested to gain access to the enantiopure β,β-dihalohydrins. Moreover, the reduction of two racemic derivatives was also tried to study the formation of two contiguous stereocentres in a dynamic kinetic resolution (DKR) process catalysed by an ADH through racemisation in basic conditions.

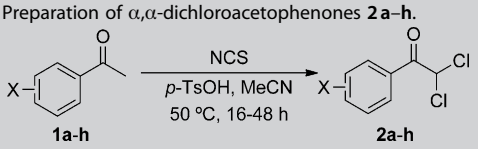
Results and Discussion

Preparation of α,α-dihaloacetophenones and the corresponding alcohols

The synthesis of α,α-dichloroacetophenones **2a–h** was performed in good to very high yields starting from commercially available acetophenones **1a–h**, bearing different substitution pattern in the aromatic ring, by reaction with a 2-fold molar excess of *N*-chlorosuccinimide (NCS) in the presence of *para*-toluenesulfonic acid (*p*-TsOH) using acetonitrile as a solvent at 50 °C (Table 1).

A lower reactivity for the *ortho*-substituted derivatives **2b–c** was observed, thus longer reaction times were required in these cases (entries 2 and 3), probably owing to steric hindrance. In addition, to achieve the synthesis of 2,2-dichloro-1-(3,4-dichlorophenyl)ethanone (**2i**), the corresponding α-chloroacetophenone derivative **1i** was used as starting material utilising 1.1 equivalents of NCS, obtaining **2i** in 83% yield. Starting from **1a** with a 3-fold molar excess of *N*-bromosuccinimide (NBS), 2,2-dibromoacetophenone **2j** was achieved in 81% isolated yield. Racemic dihalohydrins **3a–j** were obtained in good to very high yields (70–96%) by reduction of the corresponding ketones with $NaBH_4$ in MeOH at room temperature (see the Supporting Information).

Table 1. Preparation of α,α-dichloroacetophenones **2a–h**.



1a-h **2a-h**

Entry	X	*t* [h]	**2a–h** [%][a]
1	H	16	77 (**a**)
2	2-Me	48	82 (**b**)
3	2-Cl	48	77 (**c**)
4	3-OMe	16	75 (**d**)
5	3-NO_2	16	80 (**e**)
6	3-Cl	16	78 (**f**)
7	4-NO_2	16	78 (**g**)
8	4-Cl	16	90 (**h**)

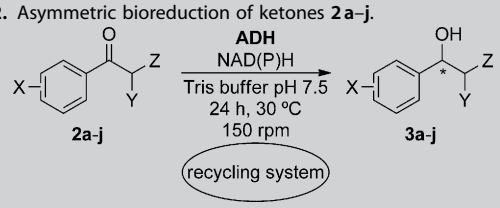
[a] Isolated yields of prochiral ketones **2a–h** after flash chromatography. In brackets appears the identification of the corresponding α,α-dihalogenated acetophenone, **2a–h** obtained from **1a–h**. For more details see the Experimental Section.

Bioreduction of prochiral α,α-dihaloketones 2a–j

Once synthesised, the asymmetric bioreduction of α,α-dihaloketones **2a–j** was studied by using commercially available and overexpressed ADHs. Owing to our previous experience with similar α-halogenated substrates,[15a,21c,f] six enzymes were used in this study, including also those accepting bulky–bulky ketones as substrates: ADH-A from *Rhodococcus ruber*,[22] RasADH from *Ralstonia* sp.[23] and SyADH from *Sphingobium yanoikuyae*,[24] which are Prelog enzymes;[25] and on the other hand LBADH from *Lactobacillus brevis*,[26] LKADH from *Lactobacillus kefir*,[27] and PR2ADH that are anti-Prelog ADHs (see the Experimental Section for more details). All these biocatalysts accept aromatic ketones bearing a small substituent at alpha position such as methyl or chloromethyl. Besides, RasADH and SyADH can also reduce bulkier substrates.[23,24] Except for RasADH and LKADH, for which glucose and glucose dehydrogenase (GDH) were used to recycle a catalytic amount of the nicotinamide cofactor, 2-propanol was employed as the hydrogen donor (5% v/v). This is owing to the fact that RasADH and LKADH work better under these conditions, as previously described.[23c,28] For clarity, the best results for the synthesis of both **3a–j** enantiomers are collected in Table 2, and in the Supporting Information more detailed information about the screening process can be found.

Satisfyingly, from the twenty possible enantiopure alcohols, fifteen were obtained in enantiomerically pure form, finding ADH-A, LBADH and PR2ADH as the most versatile biocatalysts for the stereoselective reduction of α,α-dihaloacetophenones, which was achieved in eighteen cases with at least 90% conversion. Initially, α,α-dichloroacetophenone (**2a**) was studied yielding selectively either enantiomer of alcohol **3a** in conversions over 95% by using a Prelog enzyme (ADH-A, entry 1) or anti-Prelog reductases (PR2ADH and LBADH, entries 2 and 3). Then, the influence of electron-donating (Me or OMe) or electron-withdrawing substituents (Cl or NO_2), at different positions in the aromatic ring was studied for α,α-dichloroacetophenone

Table 2. Asymmetric bioreduction of ketones **2a–j**.



Entry	X	Y	Z	ADH	Conv. [%][a]	*ee* [%][b]
1 (**a**)	H	Cl	Cl	A	96	>99 (*R*)
2 (**a**)	H	Cl	Cl	PR2	99	>99 (*S*)
3 (**a**)	H	Cl	Cl	LB	99	>99 (*S*)
4 (**b**)	2-Me	Cl	Cl	*E. coli*/Ras	99	>99 (*R*)
5 (**b**)	2-Me	Cl	Cl	PR2	43	>99 (*S*)
6 (**c**)	2-Cl	Cl	Cl	*E. coli*/Ras	99	>99 (*R*)
7 (**c**)	2-Cl	Cl	Cl	LB	12	91 (*S*)
8 (**d**)	3-OMe	Cl	Cl	*E. coli*/A	99	>99 (*R*)
9 (**d**)	3-OMe	Cl	Cl	PR2	99	>99 (*S*)
10 (**d**)	3-OMe	Cl	Cl	LB	99	>99 (*S*)
11 (**e**)	3-NO_2	Cl	Cl	A	99	>99 (*R*)
12 (**e**)	3-NO_2	Cl	Cl	LB	96	>99 (*S*)
13 (**f**)	3-Cl	Cl	Cl	A	99	96 (*R*)
14 (**f**)	3-Cl	Cl	Cl	LB[c]	99	>99 (*S*)
15 (**g**)	4-NO_2	Cl	Cl	A	97	>99 (*R*)
16 (**g**)	4-NO_2	Cl	Cl	PR2	99	>99 (*S*)
17 (**h**)	4-Cl	Cl	Cl	A	98	>99 (*R*)
18 (**h**)	4-Cl	Cl	Cl	LB	91	>99 (*S*)
19 (**i**)	3,4-Cl_2	Cl	Cl	*E. coli*/A[c]	99	99 (*R*)
20 (**i**)	3,4-Cl_2	Cl	Cl	LB[c,d]	90	98 (*S*)
21 (**j**)	H	Br	Br	*E. coli*/Ras	99	95 (*R*)
22 (**j**)	H	Br	Br	LB[c,e]	99	99 (*S*)

[a] Conversion values calculated by GC. [b] Enantiomeric excess of alcohols calculated by using chiral GC or HPLC indicating their absolute configuration in brackets [note the switch in the Cahn–Ingold–Prelog (CIP) priority]. [c] DMSO (2% v/v) was added. [d] 48 h and 4.5 U of enzyme employed. [e] 4.5 U of enzyme employed.

derivatives **2b–i**. Clear trends were observed as follows: 1) ADHs led to good levels of activity and stereoselectivity to those substrates with the presence of substituents in the *meta* or *para* position (entries 8–20), whereas for *ortho*-substituted acetophenones **2b,c** (entries 4–7), only the Prelog enzyme RasADH (entries 4 and 6) allowed the isolation of the corresponding (*R*)-alcohols in quantitative yield and enantiopure form. This is especially relevant because the bioreduction of *ortho*-substituted acetophenones remains usually hampered. In a recent contribution, RasADH exhibited good activity for similar ketones;[23b] 2) bulky–bulky ADH from *Ralstonia* sp. was identified as the best enzyme for highly hindered substrates;[23] 3) ADH-A demonstrated also high versatility acting as a very selective enzyme,[22] only leading to low conversion in the case of the 2-methyl derivative **2b** (see also the Supporting Information). Thus, a correct choice between RasADH or ADH-A allowed the synthesis of the (*R*)-alcohols with excellent stereoselectivities and conversions; (iv) the poorest results were generally attained with LKADH, which seemed to be not suitable for dihalohydrins synthesis.

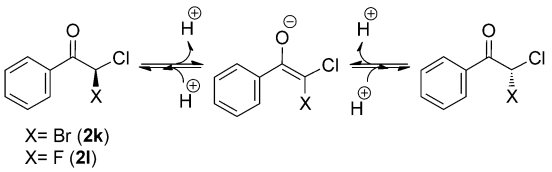
Finally, the bioreduction of a bulkier ketone possessing two bromine atoms at α-position instead of chlorines, α,α-dibromoacetophenone (**2j**), was also analysed, finding complete stereoselectivities for anti-Prelog enzymes PR2ADH and LBADH (entry 22 and see also the Supporting Information), whereas RasADH was found as the best Prelog enzyme (entry 21), ADH-A leading to low conversions and high enantiomeric excess, and SyADH produced (*R*)-**3j** with almost complete conversion but moderate *ee* (see the Supporting Information).

Preparation of racemic α,α-dihaloacetophenones

Owing to the high stereoselectivities obtained for ketones **2a–j**, and to broaden the applicability of the already tested alcohol dehydrogenases, the bioreduction of two halogenated racemic ketones was envisaged, thus 2-bromo-2-chloro-1-phenylethanone (**2k**) and 2-chloro-2-fluoro-1-phenylethanone (**2l**) were prepared by following standard procedures. For **2k**, α-chloroacetophenone was reacted with NBS in the presence of *p*-TsOH in MeCN at 50 °C for 16 h, yielding the ketone in 85% isolated yield. On the other hand, fluoro ketone **2l** was obtained by following the procedure described by Yamazaki et al., starting from ethyl chlorofluoroacetate (56% yield).[29]

Bioreduction of racemic α,α-dihaloketones 2k–l

Owing to the acidity of the α-proton, it was expected that racemisation of the substrates would occur in situ (Scheme 2), making a DKR process feasible obtaining, in the ideal case, one



Scheme 2. Interconversion of both **2k** or **2l** enantiomers through an enolate intermediate.

of the four possible diastereoisomer products.[28,30] These enantioenriched alcohols would be of high interest because in a further step they could be selectively modified to obtain more complex and valuable structures as a result of the different reactivity of both halide atoms.

Therefore, we firstly performed a screening with substrate **2k** (Table 3). In a first set of experiments, it was observed that ADH-A, RasADH[23a] and LBADH[26] were the best biocatalysts in terms of activity and selectivity (entries 1–3). Although still far away from a perfect diastereoselectivity, it is remarkable that these enzymes could distinguish between these two halide atoms, because previous results with structurally similar ketones did not show high induction levels.[23b] As ADH-A was the enzyme displaying better diastereoselectivity favouring the formation of the *syn* diastereomer (59%, entry 1), we tried to optimise the process by changing several reaction parameters such as pH or temperature, thus differentially modifying the rate of the enzymatic and the racemisation reactions leading to improved diastereomeric excess (*de*) values. In this regard, low temperatures had a negative influence in the activity of

Table 3. Bioreduction of racemic ketone **2k** ($t = 24$ h).

Entry	ADH	T [°C]	pH	**3k** [%][a]	*ee* [%][b]	*de* [%][b]
1	*E. coli*/ADH-A	30	7.5	99	>99	59 (1*R*,2*R*)
2	*E. coli*/RasADH	30	7.5	95	>99	1 (1*R*,2*RS*)
3	LBADH	30	7.5	65	>99	26 (1*S*,2*R*)
4	*E. coli*/ADH-A	4	7.5	18	>99	58 (1*R*,2*R*)
5	*E. coli*/ADH-A	40	7.5	86	>99	62 (1*R*,2*R*)
6	*E. coli*/ADH-A	30	8.5	98	>99	63 (1*R*,2*R*)
7	LBADH	30	8.5	70	>99	33 (1*S*,2*R*)

[a] Conversion values measured by GC. [b] Enantiomeric and diastereomeric excess measured by chiral HPLC (note the switch in the CIP priority).

the biocatalyst (entry 4), but a higher temperature or pH did not influence the *de* observed (entries 5–7). Higher pH values afforded the decomposition of both substrate and product forming, among other, benzoic acid.

With the aim of gaining a deeper insight in this DKR with ADH-A, we followed the reaction time course to analyse the rate of the racemisation step together with the *de*. In the case of an inefficient racemisation rate, we would detect a decrease of the diastereomeric excess of the alcohol product within the reaction progress (Figure 1). As can be seen, even at low conversions, the *de* values remained almost unaltered during the whole process, showing that the racemisation rate was fast enough for the DKR process.

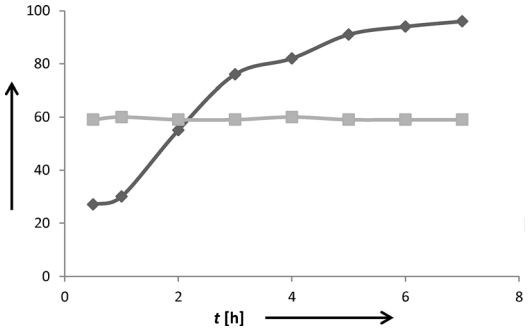


Figure 1. Conversion (◆) and *de* values (■) in the *E. coli*/ADH-A-catalysed bioreduction of ketone **2k** into **3k** at 30 °C and pH 7.5. In all cases, *ee* values were higher than 99%.

Owing to the fact that strong basic conditions decomposed both **2k** and **3k**, several bases in equimolar amounts were added into the reaction medium to study their effect in the DKR process as previously described by us.[30a] Thus, DBU (final pH ≈ 9.0), piperidine (final pH ≈ 9.0), pyridine (final pH ≈ 7.5) and triethylamine (final pH ≈ 8.8) were employed, but no remarkable improvement in the *de* was detected (see the Supporting Information for more details).

Finally, the effect of a non-miscible organic solvent such as *n*-hexane, or a miscible ionic liquid, Ammoeng 102, which already proved to be compatible with ADH-A,[31] was also measured (Figure 2). The use of an external additive could influence both ADH selectivity and reaction rates favouring, in the best scenario, the DKR outcome.[30a] From the results attained, it can be summarised that both biphasic and monophasic media did not have an influence in the enantio- and diastereoselectivity of the process, suggesting that a better biocatalyst to achieve this goal might be constructed by active-site architecture modification rather than medium engineering.

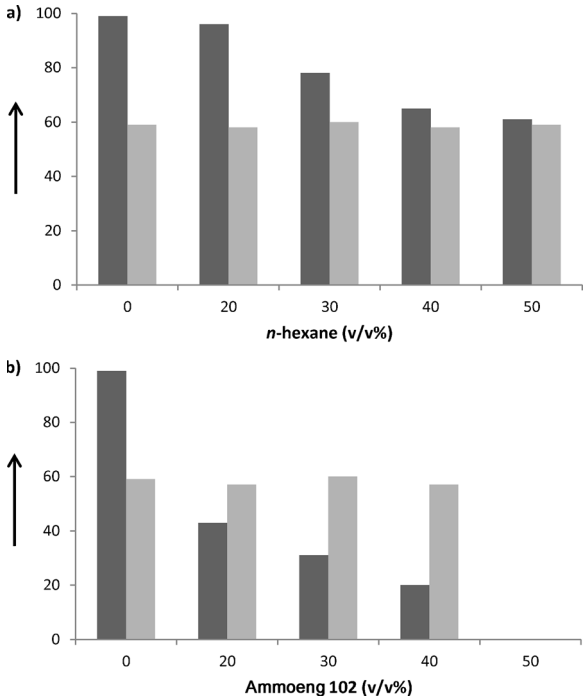


Figure 2. Conversion (■) and *de* values (■) after 24 h in the *E. coli*/ADH-A-catalysed bioreduction of ketone **2k** into **3k** at 30 °C and pH 7.5 with different proportions of: a) *n*-hexane; and b) Ammoeng 102, as cosolvents. In all cases, the *ee* values were higher than 97%.

Crystallisation of acylated alcohol 4

Different attempts were made to obtain suitable crystals for X-ray diffraction analysis to confirm the relative and absolute configuration of the stereogenic centres of **3k**. The most successful approach was achieved, converting the optically active alcohol obtained from the ADH-A-catalysed bioreduction of **2k** into ester **4**, after reaction with 4-nitrobenzoyl chloride in the presence of triethylamine in dry dichloromethane, and subsequent crystallisation using a mixture of diethyl ether and *n*-hexane. As can be seen in Figure 3, both stereogenic centres presented the (*R*)-configuration, and data for the C–OH bond are in agreement with the known stereopreference exhibited by ADH-A as a Prelog enzyme, which determined the absolute configuration at position 2 bearing both halogens.[32]

From the data shown in Table 3, it is remarkable that ADH-A produced the (1*R*,2*R*)-**3k** diastereoisomer with moderate diastereomeric excess (63% *de*), which stands for a *syn* configuration, whereas LBADH slightly preferred the formation of the (1*S*,2*R*)-**3k** diastereoisomer (33% *de*), which accounts for an *anti* configuration. On the other hand, it is noteworthy that the reduction of racemic ketone **2k** with sodium borohydride

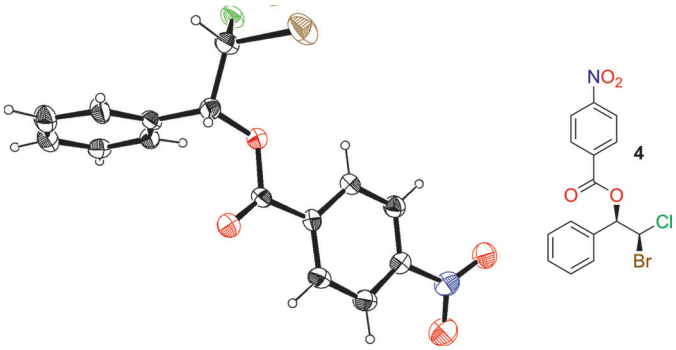


Figure 3. X-Ray structure of ester (*R*,*R*)-**4** synthesised through ADH-A-catalysed bioreduction of racemic ketone **2k**.

led to the formation of the racemic mixtures at a proportion 3:1 (50% *de*) favouring the *anti* diastereoisomer. The opposite diastereopreference displayed by ADH-A compared to $NaBH_4$ or LBADH-catalysed reductions is a very interesting feature, and will be the object of further studies.

Next, the DKR of 2-chloro-2-fluoro-1-phenylethanone (**2l**) was also tried under the best conditions found for ketone **2k**, but as shown in Table 4, although excellent conversions and enantioselectivities were observed, low *de* values were achieved. *Sphingobium yanoikuyae* ADH overexpressed in *E. coli* afforded the highest value of *de* (45%), although with low *ee* (59%, entry 4), whereas the other biocatalysts studied gave access to the enantiopure alcohol **3l** but with vanished diastereoselectivity (entries 1–3). The effect exerted by the fluorine in the diastereomeric excess values was remarkable, probably as a result of a better recognition of the bulky Br atom in substrate **2k** as that of fluorine in ketone **2l**.

Table 4. Bioreduction of racemic ketone **2l** at pH 7.5 and 30 °C ($t = 24$ h).

Entry	ADH	**3l** [%][a]	*ee* [%][b]	*de* [%][b]
1	*E. coli*/ADH-A	99	98	5 (1*R*,2*RS*)
2	*E. coli*/RasADH	99	97	< 1 (1*R*,2*RS*)
3	LBADH	99	> 99	1 (1*S*,2*RS*)
4	*E. coli*/SyADH	98	59	45 (1*R*,2*RS*)

[a] Conversion values measured by GC. [b] Enantiomeric and diastereomeric excess measured by chiral HPLC (note the switch in the CIP priority).

Conclusions

The successful preparation of α,α-dihaloacetophenones as well as an ADH selection guideline for their stereoselective reduction was provided. Enantioenriched dihalohydrins are precursors in the chemical synthesis of a wide number of valuable compounds, but their selective synthesis by traditional chemical methods is hampered by low asymmetric induction or formation of by-products. Thus, different ADHs under mild reaction conditions in aqueous medium were considered as suitable catalysts yielding both enantiomers depending on the choice of the enzyme. A series of dihalohydrins bearing different substitutions in the phenyl ring were obtained with enantiomeric excess values over 95% for both (*R*)- and (*S*)-enantiomers by the correct selection of the ADH for the bioreduction process. In addition, eighteen out of twenty of these alcohol enantiomers were achieved with over 90% conversion, finding lower conversions for the *ortho*-substituted substrates.

Moreover, the asymmetric bioreduction of two racemic ketones, namely α-bromo-α-chloroacetophenone and α-chloro-α-fluoroacetophenone, was also studied. The use of an organic base or cosolvent, and the modification of the temperature or the pH did not have a significant effect on the stereoselectivity of the DKR process. For the first substrate, the corresponding enantiopure alcohol was obtained in excellent conversions, albeit with moderate diastereomeric excess. By using X-ray diffraction, the absolute configuration of the major diastereoisomer obtained could be assigned. For the second racemic ketone, the ADHs could not differentiate between both halogen atoms. Overall, the bioreduction of α,α-dihaloacetophenones was studied by using different ADHs, giving access to valuable enantiopure β,β-dihalohydrins selecting the proper biocatalyst. This study enables the future application of these enzyme-catalysed processes in the syntheses of more complex chiral compounds.

Experimental Section

Overexpressed ADHs from *Rhodococcus ruber* (*E. coli*/ADH-A), from *Ralstonia* species (*E. coli*/RasADH) and *Sphingobium yanoikuyae* (*E. coli*/SyADH) were used as lyophilised cells.[23c,24,33] Glucose dehydrogenase (GDH 002, 30 U mg^{-1}), ADH-A (20 U mg^{-1}), PR2ADH (0.13 U mg^{-1}), and LBADH from *Lactobacillus brevis* (3.7 U μL^{-1}) were purchased from Codexis. LKADH from *Lactobacillus kefir* (0.42 U mg^{-1}) was obtained from Fluka. For the bioreduction processes, Tris–H_2SO_4 buffer was employed in all cases with α-brominated ketones to avoid undesired S_N2 reactions.

Syntheses

Prochiral ketones **2a–h** (general procedure): To a solution of the corresponding acetophenone **1a–h** (3.7 mmol) and *p*-TsOH (708.8 mg, 3.7 mmol) in acetonitrile (10 mL), NCS was added (1 g, 7.6 mmol). The reaction mixture was heated at 50 °C and stirred till disappearance of the starting material (16–48 h). After completion, the solvent was evaporated under reduced pressure and the crude was purified using flash chromatography (50–80% CH_2Cl_2/hexane) yielding the corresponding prochiral ketones (see Table 1).[34]

2,2-Dichloro-1-(3,4-dichlorophenyl)ethanone (**2i**): To a solution of α-chloroacetophenone **1i** (280 mg, 1.25 mmol) and *p*-TsOH (238 mg, 1.25 mmol) in acetonitrile (4 mL), NCS was added (184 mg, 1.37 mmol). The reaction mixture was heated at 50 °C and stirred till disappearance of the starting material (16 h). After completion of the reaction, the solvent was evaporated under reduced pressure and the crude was purified using flash chromatography (20% CH_2Cl_2/hexane) yielding product **2i** as a white solid (0.27 g, 83%).

2,2-Dibromo-1-phenylethanone (**2j**): To a solution of acetophenone (**1a**, 250 mg, 2.08 mmol) and *p*-TsOH (400 mg, 2.08 mmol) in acetonitrile (10 mL), NBS was added (1.11 g, 6.24 mmol). The reaction

mixture was heated at 50 °C and stirred till disappearance of the starting material (16 h). After completion of the reaction, the solvent was evaporated under reduced pressure and the crude was purified using flash chromatography (33% CH_2Cl_2/hexane) yielding product **2j** as a white solid (467 mg, 81%).

Racemic 2-bromo-2-chloro-1-phenylethanone (**2k**): To a solution of α-chloroacetophenone **1k** (500 mg, 3.2 mmol) and *p*-TsOH (615 mg, 3.2 mmol) in acetonitrile (14 mL), NBS was added (863 mg, 4.85 mmol). The reaction mixture was heated at 50 °C and stirred till disappearance of the starting material (16 h). After completion of the reaction, the solvent was evaporated under reduced pressure and the crude was purified using flash chromatography (20% CH_2Cl_2/hexane) yielding **2k** as a white solid (0.64 g, 85%).

Racemic 2-chloro-2-fluoro-1-phenylethanone (**2l**): To a solution of ethyl chlorofluoroacetate (4.35 mmol, 0.5 mL) in dry toluene (5 mL) at −78 °C under nitrogen atmosphere, 1.1 equiv. of phenyl magnesium bromide (1.6 mL of a 3 M solution in Et_2O) was added dropwise and the reaction was stirred for one hour. Following that time, the reaction mixture was warmed up to 0 °C and then left for 10 min prior to the quenching with ammonium chloride (saturated solution). The crude was extracted with Et_2O (3×10 mL), dried over anhydrous Na_2SO_4 and was slowly evaporated under reduced pressure in an ice bath to prevent the loss of the volatile product. The crude mixture was purified using flash chromatography (100% pentane to 70% pentane/CH_2Cl_2) yielding **2l** as a white crystal solid (0.42 g, 56% yield).[29]

Bioreductions

Ketones **2a**–**l** with *E. coli*/ADH-A: To a 15 mg portion of overexpressed *E. coli*/ADH-A (lyophilised cells) in an Eppendorf vial (1.5 mL), Tris–HCl or Tris–H_2SO_4 buffer (510 μL, 50 mM, pH 7.5), NADH (60 μL of a 10 mM solution, final concentration: 1 mM), 2-propanol (30 μL, 5% v/v), and the corresponding ketone (**2a**–**l**, 25 mM) were added. The reaction tubes were shaken horizontally at 30 °C for 24 h and 150 rpm. After that time, the reaction products were extracted with EtOAc (2×0.5 mL). The organic layers were separated by centrifugation (1.5 min, 13000 rpm) and dried over anhydrous Na_2SO_4. Conversion and *ee* values were determined by GC or HPLC analysis.

Scale-up (**2a** with *E. coli*/ADH-A): In an Erlenmeyer flask (10 mL), *E. coli*/ADH-A (100 mg) was suspended in Tris–HCl buffer (3.6 mL, 50 mM, pH 7.5, 1 mM NADH) and preincubated for 30 min at 30 °C. Then, ketone **2a** (50 mg, 0.26 mmol) and 2-propanol (0.4 mL, 10% v/v) were added to the mixture. The reaction was shaken at 30 °C and 250 rpm for 48 h. After incubation, the enzymatic reaction was stopped by extraction with EtOAc (3×5 mL). The organic layers were combined and dried over Na_2SO_4. The solvent was concentrated under vacuum, furnishing the enantiopure alcohol (*R*)-**3a** (isolated yield: 65%).

Ketones **2a**–**l** with LBADH: In an Eppendorf vial (1.5 mL), LBADH (10 μL, 3 U) was added to a 450 μL volume of Tris–HCl or Tris–H_2SO_4 buffer (50 mM, pH 7.5), followed by NADPH (60 μL of a 10 mM solution, final concentration: 1 mM), $MgCl_2$ (60 μL of a 10 mM solution, final concentration: 1 mM), 2-propanol (30 μL, 5% v/v) and the corresponding ketone (**2a**–**l**, 25 mM). The reaction tubes were shaken horizontally at 30 °C for 24 h and 150 rpm. After that time, the reaction products were extracted with EtOAc (2× 0.5 mL). The organic layers were separated by centrifugation (1.5 min, 13000 rpm) and dried over anhydrous Na_2SO_4. Conversion and *ee* values were determined by GC or HPLC analysis.

Ketones **2a**–**l** with LKADH: In an Eppendorf vial (1.5 mL), LKADH (7 mg, 3 U) were added to a 510 μL volume of Tris–HCl or Tris–H_2SO_4 buffer (50 mM, pH 7.5), NADPH (60 μL of a 10 mM solution, final concentration: 1 mM), glucose (30 μmol of a 1 M solution, 50 mM), glucose dehydrogenase (10 μL, 3 U) and the corresponding ketone (**2a**–**l**, 25 mM). The reaction tubes were shaken horizontally at 30 °C for 24 h and 150 rpm. After that time, the reaction products were extracted with EtOAc (2×0.5 mL). The organic layers were separated by centrifugation (1.5 min, 13000 rpm) and dried over anhydrous Na_2SO_4. Conversion and *ee* values were determined by GC or HPLC analysis.

Ketones **2a**–**l** with PR2 ADH: In an Eppendorf vial (1.5 mL), PR2ADH (23 mg, 3 U), a 510 μL volume of Tris–HCl or Tris–H_2SO_4 buffer (50 mM, pH 7.5) were added, NADH (60 μL of a 10 mM solution, final concentration: 1 mM), 2-propanol (30 μL, 5% v/v) and the corresponding ketone (**2a**–**l**, 25 mM). The reaction tubes were shaken horizontally at 30 °C for 24 h and 150 rpm. After that time, the reaction products were extracted with EtOAc (2×0.5 mL). The organic layers were separated by centrifugation (1.5 min, 13000 rpm) and dried over anhydrous Na_2SO_4. Conversion and *ee* values were determined by GC or HPLC analysis.

Ketones **2a**–**l** with *E. coli*/RasADH: To a 15 mg portion of overexpressed *E. coli*/RasADH (lyophilised cells) in an Eppendorf vial (1.5 mL), Tris–HCl or Tris–H_2SO_4 buffer (510 μL, 50 mM, pH 7.5) were added, NADPH (60 μL of a 10 mM solution, final concentration: 1 mM), glucose (30 μmol of a 1 M solution, 50 mM), glucose dehydrogenase (10 μL, 3 U) and the corresponding ketone (**2a**–**l**, 25 mM). The reaction tubes were shaken horizontally at 30 °C for 24 h and 150 rpm. After that time, the reaction products were extracted with EtOAc (2×0.5 mL). The organic layers were separated by centrifugation (1.5 min, 13000 rpm) and dried over anhydrous Na_2SO_4. Conversion and *ee* values were determined by GC or HPLC analysis.

Ketones **2a**–**l** with *E. coli*/SyADH: To a 15 mg portion of overexpressed *E. coli*/SyADH (lyophilised cells) in an Eppendorf vial (1.5 mL), Tris-HCl or Tris-H_2SO_4 buffer (510 μL, 50 mM, pH 7.5) were added, NADPH (60 μL of a 10 mM solution, final concentration: 1 mM), 2-propanol (30 μL, 5% v/v) and the corresponding ketone (**2a**–**l**, 25 mM). The reaction tubes were shaken horizontally at 30 °C for 24 h and 150 rpm. After that time, the reaction products were extracted with EtOAc (2×0.5 mL). The organic layers were separated by centrifugation (1.5 min, 13000 rpm) and dried over anhydrous Na_2SO_4. Conversion and *ee* values were determined by GC or HPLC analysis.

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