RESEARCH ARTICLE

Chemoenzymatic Cascades for the Enantioselective Synthesis of β-Hydroxysulfides Bearing a Stereocentre at the C−O or C−S Bond by Ketoreductases

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Abstract: Chiral β-hydroxysulfides are an important class of organic compounds which find broad application in organic and pharmaceutical chemistry. Herein we describe the development of novel biocatalytic and chemoenzymatic methods for the enantioselective synthesis of β-hydroxysulfides by exploiting ketoreductase (KRED) enzymes. Four KREDs were discovered from a pool of 384 enzymes identified and isolated through a metagenomic approach. KRED311 and KRED349 catalysed the synthesis of β-hydroxysulfides bearing a stereocentre at the C−O bond with opposite absolute configurations and excellent ee values by novel chemoenzymatic and biocatalytic-chemical-biocatalytic (bio-chem-bio) cascades starting from commercially available thiophenols/thiols and α-haloketones/alcohols. KRED253 and KRED384 catalysed the enantioselective synthesis of β-hydroxysulfides bearing a stereocentre at the C−S bond with opposite enantioselectivities by dynamic kinetic resolution (DKR) of racemic α-thioaldehydes.

Introduction

The number of chemical reactions catalysed by enzymes has increased exponentially in the last two decades. Biocatalysis represents a sustainable and cost-effective approach in the synthesis of drugs and chemicals as it offers advantages over other classical catalytic methods, such as the possibility to carry out reactions at mild temperatures, in turn improving the energy efficiency of chemical processes, as well as in aqueous media, allowing a reduced use of petroleum-based solvents.[1] Enzymes can also be used in chemoenzymatic cascades, in which chemo- and enzymatic reactions can be combined in one-pot sequential transformations.[2] Chemoenzymatic cascades avoid the isolation of reaction intermediates and negate the need for functional group protection/deprotection, thus resulting in safer, more efficient and sustainable chemical processes. Therefore, it is highly appealing to develop chemoenzymatic cascades for the assembly of fine chemicals.

Chiral β-hydroxysulfides, also known as 1,2-thioalcohols, are an important class of organic compounds widely found in natural products,[3] foods and fruits as flavours and aromas,[4] and pharmaceutical ingredients such as the antimycobacterial drug thiolactomycin **1**,[5] the calcium channel blocker diltiazem **2**[6] and the leukotriene analogue **3** (Figure 1a).[7]

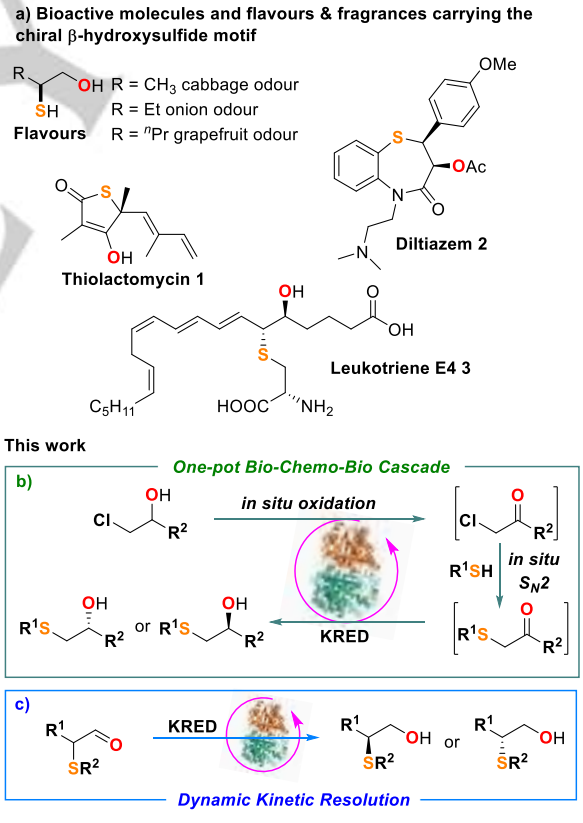


Figure 1. Examples of chiral β-hydroxysulfides and aim of this work.

In addition, chiral β-hydroxysulfides are also useful building blocks in medicinal[8] and organic chemistry.[9] Therefore, synthetic approaches towards their preparation have captured wide attention.[10] Classic routes to chiral β-hydroxysulfides

include the asymmetric ring opening of epoxides[11] or cyclic carbonates[12] with thiophenols/mercaptanes catalysed by metal complexes based on chiral BINOL,[13] salen,[14] or bipyridine ligands.[15] However, these methods show some drawbacks such as the requirement of metals and complex chiral ligands, the usage of not easily accessible starting materials and, in some cases, unsatisfactory regio- and enantioselectivities.

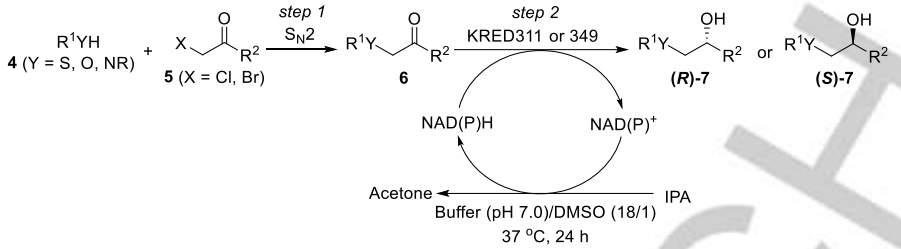
Although biocatalysis offers a green and more sustainable platform to access structurally diverse molecules,[16] only few enzymatic approaches for the synthesis of chiral β-hydroxysulfides have been reported to date. Such methods, which are limited to 1,2-thioalcohols bearing a stereocentre at the C−O bond, rely on the reduction of prochiral ketone precursors using Baker's yeast[17] or the CALB-catalysed kinetic resolution of racemic alcohols.[18] However, only (*S*)-enantiomers were achievable with Baker's yeast and less than 50% conversions were obtained using CALB. In addition, poor or limited selectivity and yields were observed in some cases with these two methods. Besides, it must be noted that no biocatalytic methods for the synthesis β-hydroxysulfides bearing a stereocentre at the C−S bond have been described to date. In fact, the construction of stereodefined centres at C−S bonds poses a major challenge in current organic chemistry and pre-existing methods generally show drawbacks like the requirement of metal catalysts, complex chiral ligands and harsh reaction conditions.[9f-g, 19]

Following our interest in the development of biocatalytic methods for the synthesis of pharmaceutical ingredients and building blocks,[20] herein we describe new chemoenzymatic approaches to obtain enantiomerically pure β-hydroxysulfides exploiting ketoreductase (KRED) biocatalysts. KREDs are enzymes increasingly used in academia and industry.[21] However, all KREDs require NAD(P)H/NAD(P)$^+$ cofactors to work, which increases the costs of their application. Therefore, external sacrificial substrates, typically isopropanol (IPA) or glucose dehydrogenase (GDH)/glucose, are commonly used as cofactor recycling reagents, making such biotransformations less atom-economic and environmentally benign. In this work, we speculated the possibility of exploiting α-haloalcohols with a dual role of internal cofactor recycling reagents as well as synthetic precursors of β-hydroxysulfides. We demonstrated that the α-haloalcohols can be oxidized *in situ* by KREDs to form α-haloketones, which can undergo S_N2 nucleophilic substitution with thiophenols/thiols to provide α-thioketones. α-Thioketones can finally be enantioselectively reduced by the same KREDs, thus affording an atom- and step-economic biocatalytic-chemical-biocatalytic (bio-chem-bio) cascade for the synthesis of chiral β-hydroxysulfides bearing a stereocentre at the C−O bond (Figure 1b). Alternatively, β-hydroxysulfides can also be generated directly *via* a chemoenzymatic cascade starting from commercially available α-haloketones and thiophenols/thiols. On the other hand, it should be noted that the application of KREDs in the asymmetric construction of other carbon-heteroatom bonds instead of C−O bond has never been explored yet. Notably, in this work, we also develop a new strategy for the enantioselective synthesis of β-hydroxysulfides bearing a stereocentre at the C−S bond through KRED biocatalysed dynamic kinetic resolution (DKR) of racemic α-thioaldehydes (Figure 1c). To the best of our knowledge, this is the first example of asymmetric C−S stereocentre construction by KREDs.

Results and Discussion

β-Hydroxysulfides bearing a stereocentre at the C−O bond. The synthesis of β-hydroxysulfides bearing a stereocentre at the C−O bond was investigated at first. A series of α-thioketones **6a-s** was prepared through the S_N2 nucleophilic substitution between the appropriate thiophenols/thiols and α-chloro/bromoketones (see Supporting Information) and used as substrates in KRED biocatalysed reduction reactions. Two enzymes, KRED311 and KRED349, were identified and selected for this biotransformation as the most effective biocatalysts *via* screening of 384 KREDs from Prozomix library. The 384 KREDs were developed through a metagenomic approach, and the best biocatalysts were selected through colorimetric and spectrophotometric assays.[22] Employing IPA as the cofactor recycling reagent, α-thioketone **6a** was initially treated with KRED311 or KRED349 in the presence of NADH or NADPH cofactor, respectively, in a buffer/DMSO (18/1) solution for 24 h (Table 1, *entry 1*). Pleasingly, the desired product **7a** was obtained with full conversions, high yields and excellent enantioselectivities with both enzymes. Remarkably, KRED311 and KRED349 showed opposite enantioselectivities. KRED311 afforded the *R* enantiomer (*R*)-**7a** with >99% ee, whereas KRED349 favoured the *S* enantiomer (*S*)-**7a** with 95% ee. The absolute configurations of (*R*)-**7a** and (*S*)-**7a** were assigned by comparison of their optical rotations with the data reported in literature.[18] Next, the biocatalytic reduction of a variety of α-thioketones **6b-n** bearing diverse substituents at R^1 was investigated (Table 1, *entries 2-14*). Impressively, all the substrates **6b-n** were reduced by KRED311 or KRED349 with high efficiency (>54-99% conversions) and enantioselectivity (>64-99% ee values) irrespective of R^1 group, providing the corresponding β-hydroxysulfides **7b-n** with opposite enantioselectivities in high yields. The reaction of substrate **6o** bearing a CF_3 group at R^2 worked well with KRED311 to give (*R*)-**7o** in >99% ee, while, surprisingly, the same reaction with KRED349 also produced (*R*)-**7o** rather than (*S*)-**7o** as the major isomer, albeit with only 11% ee (Table 1, *entry 15*). Notably, the reactions of α-thioketones **6p-s** bearing different groups at R^2 proved that KRED311 was more sensitive to the steric hindrance at R^2 position than KRED349 (Table 1, *entries 16-19*). For example, KRED311 and KRED349 both worked well on ethyl ketone **6p** to deliver (*R*)-**7p** and (*S*)-**7p** in full conversions with >99% and 96% ee values, respectively (Table 1, *entry 16*). On the other hand, the reaction of isopropyl ketone **6q** with KRED311 resulted in a moderate conversion (46%) but an excellent *R* selectivity (99% ee). By contrast, the same reaction with KRED349 still led to a high conversion (96%) and *S* selectivity (84% ee) (Table 1, *entry 17*). In the case of *tert*-butyl ketone **6r**, no conversion was observed with KRED311 at all, while low conversion (18%) but excellent *S* selectivity (95% ee) was observed with KRED349 (Table 1, *entry 18*). Similarly, no conversion of the phenyl ketone **6s** was observed with KRED311 while a good conversion (60%) and *S* selectivity (51% ee) were obtained with KRED349 (Table 1, *entry 19*). In addition to α-thioketones, the biocatalysed reduction of α-oxyketones **6t** and **6u** was also investigated. Substrates **6t** and **6u** were converted into the corresponding β-hydroxy ethers **7t** and **7u**, respectively, in a highly enantioselective manner, affording the *R* or *S* enantiomers in >99% ee values in all cases (Table 1, *entries 20 and 21*).

Table 1. KRED biocatalysed enantioselective reduction of α-thio/oxy/aminoketones into β-hydroxysulfides/ethers/amines.[a]



Entry	Ketone	R^1	Y	X	R^2	KRED	Alcohol	Conv. [%][b]	Yield [%][c]	ee [%][b]
1	**6a**	Ph	S	Cl	Me	311	**7a**	99	91	>99 (*R*)
						349		99	92	95 (*S*)
2	**6b**	2-F-Ph	S	Cl	Me	311	**7b**	>99	95	>99 (*R*)
						349		>99	94	97 (*S*)
3	**6c**	2-Cl-Ph	S	Cl	Me	311	**7c**	99	92	>99 (*R*)
						349		99	90	96 (*S*)
4	**6d**	4-Cl-Ph	S	Cl	Me	311	**7d**	99	94	>99 (*R*)
						349		99	91	95 (*S*)
5	**6e**	2-Br-Ph	S	Cl	Me	311	**7e**	97	89	>99 (*R*)
						349		99	87	94 (*S*)
6	**6f**	4-Br-Ph	S	Cl	Me	311	**7f**	99	96	>99 (*R*)
						349		99	95	96 (*S*)
7	**6g**	4-Me-Ph	S	Cl	Me	311	**7g**	99	90	>99 (*R*)
						349		99	91	96 (*S*)
8	**6h**	3,5-*di*-Me-Ph	S	Cl	Me	311	**7h**	99	92	>99 (*R*)
						349		99	90	98 (*S*)
9	**6i**	Bn	S	Cl	Me	311	**7i**	99	86	>99 (*R*)
						349		>99	88	90 (*S*)
10	**6j**	4-Me-Bn	S	Cl	Me	311	**7j**	57	41	>99 (*R*)
						349		85	70	90 (*S*)
11	**6k**	4-MeO-Bn	S	Cl	Me	311	**7k**	54	42	>99 (*R*)
						349		97	85	92 (*S*)
12	**6l**	(furan-2-yl)CH_2	S	Cl	Me	311	**7l**	95	84	>99 (*R*)
						349		94	85	91 (*S*)
13	**6m**	EtO_2CCH_2	S	Cl	Me	311	**7m**	91	79	>99 (*R*)
						349		91	76	>99 (*S*)
14	**6n**	*n*-pent	S	Cl	Me	311	**7n**	99	80	>99 (*R*)
						349		99	81	64 (*S*)
15	**6o**	Ph	S	Br	CF_3	311	**7o**	>99	85	>99 (*R*)
						349		>99	81	11 (*R*)
16	**6p**	Ph	S	Br	Et	311	**7p**	98	90	>99 (*R*)
						349		99	93	96 (*S*)
17	**6q**	Ph	S	Br	*i*-Pr	311	**7q**	46	31	99 (*R*)
						349		96	85	84 (*S*)
18	**6r**	Ph	S	Br	*t*-Bu	311	**7r**	<1	ND[d]	ND[d]
						349		18	ND[d]	95 (*S*)
19	**6s**	Ph	S	Br	Ph	311	**7s**	<1	ND[d]	ND[d]
						349		60	44	51 (*S*)
20	**6t**	2-Br-Ph	O	Cl	Me	311	**7t**	>99	95	>99 (*R*)
						349		>99	96	>99 (*S*)
21	**6u**	2-I-Ph	O	Cl	Me	311	**7u**	93	82	>99 (*R*)
						349		99	86	>99 (*S*)
22	**6v**	Ph	NH	Cl	Me	311	**7v**	99	81	>99 (*R*)
						349		>99	83	97 (*S*)
23	**6w**	Bn	NMe	Cl	Me	311	**7w**	99	80	>99 (*R*)
						349		14	ND[d]	73 (*R*)

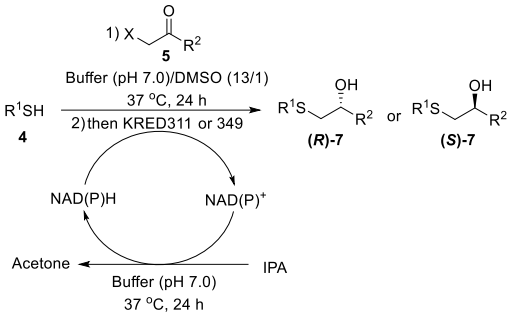
[a] Reaction conditions: **6** (0.03 mmol), 100 µL KRED311 (c = 12.296 mg/mL) or KRED349 (c = 20.782 mg/mL), NADH or NADPH (0.001 mmol), IPA (0.65 mmol), 900 µL NaPBS buffer (pH 7.0, 200 mM), DMSO (50 µL), 37 °C, 24 h. [b] Determined by HPLC equipped with ChiralPak® IG. [c] Isolated yield. [d] ND = Not determined.

Likewise, α-aminoketone **6v** was also well tolerated, leading to the desired β-hydroxy amines (*R*)-**7v** and (*S*)-**7v** in high yields with >99% and 97% ee values, respectively (Table 1, *entry 22*). Interestingly, in the case of α-aminoketone **6w**, both KRED311 and KRED349 gave (*R*)-**7w**, even though only a low conversion (14%) and moderate ee (73%) were observed with KRED349 (Table 1, *entry 23*). It is noteworthy that enantiopure β-hydroxy ethers/amines are important building blocks and structural motifs

for pharmaceutical agents,[23] which further suggests the potential application of this methodology.

Having established the protocol for the enantioselective reduction of α-thioketones into β-hydroxysulfides, and with the aim to make the synthetic method greener, we then explored the possibility of developing a two-step one-pot chemoenzymatic cascade, which combines the S_N2 nucleophilic substitution together with the KRED biocatalysed reduction to allow the synthesis of enantiopure β-hydroxysulfides directly from commercially available thiophenols/thiols and α-chloro/bromoketones (Table 2).

Table 2. Two-step one-pot chemoenzymatic cascade for the enantioselective synthesis of β-hydroxysulfides.[a]



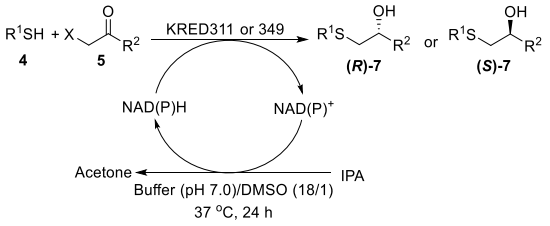
Entry	R¹	X	R²	KRED	Alcohol	Conv. [%][b]	ee [%][b]
1	Ph	Cl	Me	311	**7a**	99 [88[c]]	>99 (*R*) [>99 (*R*)[d]]
				349		>99 [84[c]]	94 (*S*) [87(*S*)[d]]
2	4-Cl-Ph	Cl	Me	311	**7d**	99	>99 (*R*)
				349		99	90 (*S*)
3	2-Br-Ph	Cl	Me	311	**7e**	86	99 (*R*)
				349		99	92 (*S*)
4	4-Me-Ph	Cl	Me	311	**7g**	98	99 (*R*)
				349		99	92 (*S*)
5	3,5-*di*-Me-Ph	Cl	Me	311	**7h**	98	98 (*R*)
				349		99	94 (*S*)
6	Ph	Br	Et	311	**7p**	98	99 (*R*)
				349		99	90 (*S*)
7	Bn	Cl	Me	311	**7i**	71	>99 (*R*)
				349		82	87 (*S*)

[a] Reaction conditions: **4** (0.03 mmol), **5** (0.036 mmol), 650 µL NaPBS buffer (pH 7.0, 200 mM), DMSO (50 µL), 37 °C, 24 h; then 100 µL KRED311 (c = 12.296 mg/mL) or KRED349 (c = 20.782 mg/mL), NADH or NADPH (0.001 mmol), IPA (0.65 mmol), 250 µL NaPBS buffer (pH 7.0, 200 mM), 37 °C, 24 h. [b] Determined by HPLC equipped with ChiralPak® IG. [c] Isolated yield at 0.9 mmol scale of **4**. [d] The ee value at 0.9 mmol scale of **4**..

As all the reported S_N2 nucleophilic substitution methodologies between thiophenols/thiols and α-chloro/bromoketones require a base to work,[24] the main challenge of such cascade was to tune the reaction conditions in a way that the S_N2 nucleophilic substitution could be compatible with the following enzymatic reduction in buffer solution at mild temperature. Amazingly, we found that the reaction of thiophenol and α-chloroacetone in a buffer (pH 7.0)/DMSO (13/1) solution at 37 °C for 24 h led to the full conversion of thiophenol into the corresponding α-thioketone **6a**, which was subsequently reduced by the addition of KRED311 or KRED349 in one-pot to give the alcohols (*R*)-**7a** or (*S*)-**7a** with >99 and 94% ee values, respectively (Table 2, *entry 1*). We speculated that the highly electrophilic property of α-chloroacetone induced by the combination of the σ∗(C−Cl) and π∗(C=O) orbitals, together with the neutralization of HCl produced in the reaction by the buffer media, accounts for the full conversion of the nucleophilic substitution under mild reaction conditions. Remarkably, the synthesis of (*R*)-**7a** and (*S*)-**7a** via the chemoenzymatic cascade was also scaled up at 0.9 mmol of PhSH, providing the desired products in high isolated yields and ee values. Only a slight loss in the enantioselectivity was observed at preparative scale with KRED349. The scope of the two-step one-pot chemoenzymatic cascade was then investigated, leading to the enantioselective synthesis of β-hydroxysulfides **7d**, **7e**, **7g**, **7h, 7p** and **7i** (Table 2, *entries 2-7*). KRED311 and KRED349 led to *R* and *S* enantiomers, respectively, with excellent conversions (>71%) and ee values (>87%) in all cases. Noteworthy, no obvious loss in enantioselectivities was observed as compared with the direct reduction of α-thioketones, indicating the efficacy of the two-step one-pot cascade.

Furthermore, we aimed to investigate if a more convenient one-pot chemoenzymatic cascade for the enantioselective synthesis of β-hydroxysulfides was possible, by adding together in a single step thiophenols/thiols, α-chloro/bromoketones and KREDs (Table 3). The main challenge of such cascade was represented by the possibility that the nucleophilic thiophenol/thiol reagents and the strong electrophilic α-chloro/bromoketones might react and inactivate the KRED enzymes by covalent bonding.

Table 3. One-pot chemoenzymatic cascade for the enantioselective synthesis of β-hydroxysulfides.[a]



Entry	R¹	X	R²	KRED	Alcohol	Conv. [%][b]	ee [%][b]
1	Ph	Cl	Me	311	**7a**	99 [91[c]]	>99 (*R*) [>99 (*R*)[d]]
				349		>99 [87[c]]	94 (*S*) [88 (*S*)[d]]
2	2-F-Ph	Cl	Me	311	**7b**	99	>99 (*R*)
				349		>99	95 (*S*)
3	2-Cl-Ph	Cl	Me	311	**7c**	99	99 (*R*)
				349		99	95 (*S*)
4	4-Cl-Ph	Cl	Me	311	**7d**	99	99 (*R*)
				349		99	95 (*S*)
5	2-Br-Ph	Cl	Me	311	**7e**	98	99 (*R*)
				349		99	93 (*S*)

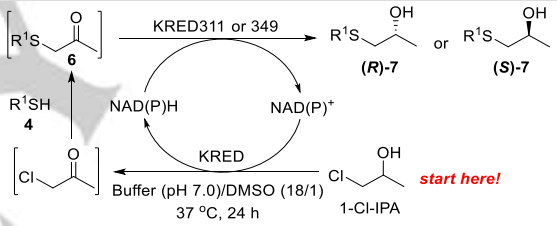
6	4-Br-Ph	Cl	Me	311 349	**7f**	65 82	>99 (*R*) 95 (*S*)
7	4-Me-Ph	Cl	Me	311 349	**7g**	99 99	>99 (*R*) 96 (*S*)
8	3,5-*di*-Me-Ph	Cl	Me	311 349	**7h**	88 99	99 (*R*) 96 (*S*)
9	Ph	Br	Et	311 349	**7p**	99 99	>99 (*R*) 95 (*S*)
10	Bn	Cl	Me	311 349	**7i**	66 24	>99 (*R*) 83 (*S*)

[a] Reaction conditions: **4** (0.03 mmol), **5** (0.036 mmol), 100 µL KRED311 (c = 12.296 mg/mL) or KRED349 (c = 20.782 mg/mL), NADH or NADPH (0.001 mmol), IPA (0.65 mmol), 900 µL NaPBS buffer (pH 7.0, 200 mM), DMSO (50 µL), 37 ºC, 24 h. [b] Determined by HPLC equipped with ChiralPak® IG. [c] Isolated yield at 0.9 mmol scale of **4**. [d] The ee value at 0.9 mmol scale of **4**.

Surprisingly, both KRED311 and KRED349 were compatible with thiophenols/thiols and α-chloro/bromoketones in a buffer/DMSO (18/1) solution. As a result, a range of α-thioketone intermediates formed through the nucleophilic substitutions of various thiophenols/thiols with α-chloro/bromoketones were reduced *in situ* by KRED311 or KRED349, producing β-hydroxysulfides (*R*)-**7a-i** or (*S*)-**7a-i**, respectively, in good to excellent conversions and ee values (Table 3, *entries 1-10*). Again, on the whole, no loss in enantioselectivities was observed. Moreover, the scale up of this one-pot chemoenzymatic cascade exemplified by (*R*)-**7a** and (*S*)-**7a** turned out to be successful, producing the target products in high isolated yields and enantioselectivities (Table 3, *entry 1*).

In all the aforementioned reactions, IPA was used as the external sacrificial substrate for the recycling of the cofactor NAD(P)H. The KREDs acted as bifunctional enzymes, catalysing the enantioselective reduction of α-thioketones in the presence of NAD(P)H, as well as the oxidation of IPA into acetone using the $NAD(P)^+$ formed *in situ* in the biocatalytic reductions and in turn leading to the regeneration of NAD(P)H. We wondered if the structurally similar 1-chloro-2-propanol (1-Cl-IPA) could work as the internal cofactor recycling reagent as well as synthetic precursor of β-hydroxysulfides. We assumed that KRED enzymes could catalyse the synthesis of α-chloroacetone *via* oxidation of 1-Cl-IPA using $NAD(P)^+$ as cofactor. If so, the α-chloroacetone formed *in situ* could react with thiophenols/thiols to afford α-thioketones **6**, which could be further reduced into the enantiopure β-hydroxysulfides **7** by the same KREDs in a bio-chem-bio cascade manner. Notably, the last step could eventually allow the regeneration of the $NAD(P)^+$ cofactor for a new catalytic cycle (Table 4). Moreover, another challenge of such one-pot bio-chem-bio cascade was also represented by the possible competitive racemic background reactions between 1-Cl-IPA and thiophenols/thiols. Our concept of such one-pot bio-chem-bio cascade was firstly examined by reacting PhSH and an excess of racemic 1-Cl-IPA with KRED349 in the presence of $NADP^+$ as the cofactor (Table 4, *entry 1*). Remarkably, the desired product (*S*)-**7a** was obtained in a high conversion (91%) and ee (94%). This transformation was easily scaled up without the loss of efficiency, and excellent isolated yield (90%) and ee (95%) were still observed for (*S*)-**7a**. Interestingly, the racemic background reaction between PhSH and 1-Cl-IPA, which was confirmed by control experiments (see Supporting Information), did not affect the cascade and did not cause any loss of enantioselectivity. This indicates that the reaction rate of the whole bio-chem-bio cascade is faster than that of the racemic background reaction.[25] Interestingly, when enantiomerically pure (*R*)-1-Cl-IPA or (*S*)-1-Cl-IPA were used in place of racemic 1-Cl-IPA, (*S*)-**7a** was still obtained in comparable conversions and ee values (Table 4, *entries 2 and 3*). Such data suggest that both (*R*)-1-Cl-IPA and (*S*)-1-Cl-IPA are suitable substrates for KRED349 and both of them can be converted into chloroacetone.[26] It is noteworthy that when 1 equivalent of racemic 1-Cl-IPA was used in the bio-chem-bio cascade (Table 4, *entry* 1), the desired alcohol (*S*)-**7a** was obtained with low conversion (66%) and ee (34%). The similar bio-chem-bio cascade reaction between PhSH and racemic 1-Cl-IPA with KRED311 using NAD^+ as the cofactor led to (*R*)-**7a** in only 57% conversion and 68% ee (Table 4, *entry 4*). Such result indicates that 1-Cl-IPA may be a suitable substrate for KRED349 but a relatively poor substrate for KRED311. The scope of the bio-chem-bio cascade was then investigated by treating various thiophenols/thiols and racemic 1-Cl-IPA with KRED349. In general, a wide range of thiophenols/thiols were smoothly converted into the desired β-hydroxysulfides (*S*)-**7b-i** in good to high conversions (21-92%) and ee values (78-93%), highlighting the efficiency of this atom- and step-economic one-pot bio-chem-bio cascade (Table 4, *entries 5-12*).

Table 4. One-pot bio-chem-bio cascade for the enantioselective synthesis of β-hydroxysulfides.[a]

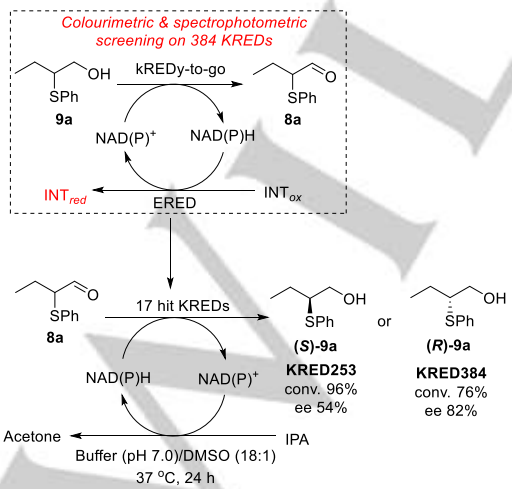


Entry	R^1	KRED	Alcohol	Conv. [%][b]	ee [%][b]
1	Ph	349	**7a**	91 66[c] [90[d]]	94 (*S*) 34 (*S*)[c] [95 (*S*)[e]]
2[f]	Ph	349	**7a**	93	91 (*S*)
3[g]	Ph	349	**7a**	89	95 (*S*)
4	Ph	311	**7a**	57	68 (*R*)
5	2-F-Ph	349	**7b**	92	93 (*S*)
6	2-Cl-Ph	349	**7c**	84	81 (*S*)
7	4-Cl-Ph	349	**7d**	62	87 (*S*)
8	2-Br-Ph	349	**7e**	90	82 (*S*)
9	4-Br-Ph	349	**7f**	55	80 (*S*)
10	4-Me-Ph	349	**7g**	72	93 (*S*)
11	3,5-*di*-Me-Ph	349	**7h**	77	93 (*S*)
12	Bn	349	**7i**	21	78 (*S*)

[a] Reaction conditions: **4** (0.03 mmol), racemic 1-Cl-IPA (tech. 75%, remainder mainly 2-chloro-1-propanol, 100 µL) (0.88 mmol), 200 µL KRED311 (c = 12.296 mg/mL) or KRED349 (c = 20.782 mg/mL), NAD^+ or $NADP^+$ (0.002 mmol), 900

µL NaPBS buffer (pH 7.0, 200 mM), DMSO (50 µL), 37 °C, 24 h. [b] Determined by HPLC equipped with ChiralPak® IG. [c] Data obtained with 0.03 mmol racemic 1-Cl-IPA. [d] Isolated yield at 0.9 mmol scale. [e] The ee value at 0.9 mmol scale. [f] (*R*)-1-Cl-IPA was used instead of racemic 1-Cl-IPA. [g] (*S*)-1-Cl-IPA was used instead of racemic 1-Cl-IPA.

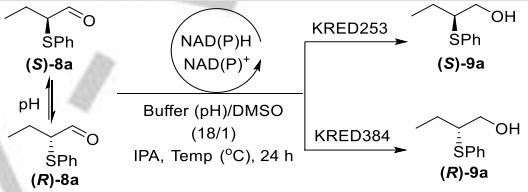
β-Hydroxysulfides bearing a stereocentre at the C−S bond. The biocatalytic synthesis of β-hydroxysulfides **9** bearing a stereocentre at the C−S bond *via* KRED-catalysed kinetic resolution of racemic α-thioaldehydes **8** was then explored (Scheme 1). Compound **8a** was chosen as the model substrate for the screening of the most suitable enzymes among the 384 KRED biocatalysts from the Prozomix metagenomic library. A kREDy-to-go colourimetric assay followed by spectrophotometric analysis led to the identification of 17 hit KREDs as potential biocatalysts for the reduction of **8a** into β-hydroxysulfide **9a** (Figures S1 and S2, Supporting Information). Interestingly, neither KRED311 or KRED349 arose from this screening. Despite the similar structures of compounds **6a** and **8a**, it is plausible that KRED311 and KRED349 are not able to recognise **8a** as a substrate, due to electronic or steric factors. A further small-scale biocatalytic screening of 17 hit KREDs on aldehyde **8a** disclosed that KRED253 and KRED384 were the most promising biocatalysts for the kinetic resolution of aldehyde **8a** (Table S3, Supporting Information). Interestingly, KRED253 afforded β-hydroxysulfide (*S*)-**9a** with 96% conversion and 54% ee, while KRED384 provided the opposite enantiomer (*R*)-**9a** with 76% conversion and 82% ee.[27] The high conversions suggested that both enzymes may be able to catalyse the dynamic kinetic resolution (DKR) rather than enzymatic kinetic resolution (EKR) of **8a**. It is plausible that each enzyme was able to preferentially reduce one of the two enantiomers of **8a** (*S* or *R*) into the corresponding alcohol **9a** (*S* or *R*), leaving the other enantiomer unreacted. Then, the unreacted enantiomer racemised under the reaction conditions, due to a combination of factors such as the acidic hydrogen at the α position of the aldehyde group and the d-orbitals in the sulfur atom which may facilitate the *in situ* racemization, leading in turn to a DKR process. Previous literature[28] showed that α-thioaldehydes are configurationally unstable in non-acid conditions, which supports such mechanism of reaction.



Scheme 1. Identification of KRED253 and KRED384 for the dynamic kinetic resolution of **8a**.

Following these promising results and hypotheses, the KRED biocatalysed reduction of α-thioaldehyde **8a** was further optimised (Table 5). In particular, the variation of the pH and temperature of the biotransformation was investigated. We reasoned that an increased pH of the reaction media might accelerate the rate of racemization at the stereocentre of **8a**, thus improving the selectivity of KREDs toward each enantiomer and in turn the final ee of the products. Expectedly, the increase of the pH from 7.0 to 9.0 led to an improvement of ee for the biocatalytic reactions with both KRED253 and KRED384 (Table 5, *entries 1-5* and *8-11*). The activity of KRED253 was not significantly affected by the change of the pH (Table 5, *entries 2-5*) and the optimal reaction conditions (95% conversion and 66% ee for *S* enantiomer) were found at 30 °C and pH 8.5 (Table 5, *entry 7*). Despite the excellent conversion observed, further attempts to improve the ee failed. By contrast, KRED384 proved to be more sensitive to the variation of the pH and temperature (Table 5, *entries 8-13*). The best conditions for KRED384 (Table 5, *entry 12*) were finally found at 25 °C and pH 9.0 (45% conversion and 92% ee for *R* enantiomer).[29]

Table 5. Optimization of the DKR of aldehyde **8a**.[a]

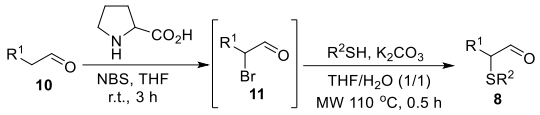


Entry	KRED	Temp. [°C]	pH	Enantiomer 9a	Conv. [%][b]	ee [%][b]
1		37	7.0	(*S*)	96	54
2		37	7.5	(*S*)	95	61
3		37	8.0	(*S*)	95	64
4	253	37	8.5	(S)	94	66
5		37	9.0	(*S*)	95	63
6		25	8.5	(*S*)	91	68
7		**30**	**8.5**	**(*S*)**	**95**[c]	**66**[c]
8		37	7.0	(*R*)	76	82
9		37	7.5	(*R*)	ND	85
10		37	8.5	(*R*)	ND	86
11	384	37	9.0	(*R*)	56	88
12		**25**	**9.0**	**(*R*)**	**45**[c]	**92**[c]
13		30	9.0	(*R*)	42	93

[a] Reaction conditions: **8a** (0.011 mmol), 2.0 mg KRED253 or KRED384 (cell-free extract), NAD(P)H (0.5 nmol, 1:1 mixture of NADH/NADPH), IPA (0.52 mmol), 910 µL NaPBS buffer (pH indicated, 100 mM), DMSO (50 µL), temp. (°C), 24 h; [b] Determined by HPLC equipped with ChiralPak® IG. [c] The use of a 1:1 mixture of NADH/NADPH or the single NADH as cofactor provided identical results.

Once the best reaction conditions were identified, the substrate scope of the KRED biocatalysed DKR of α-thioaldehydes **8** was explored. A variety of α-thioaldehydes **8** were synthesised

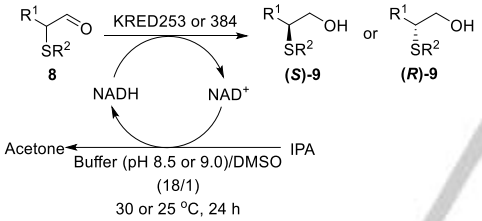
through an organocatalytic/microwave sequence as shown in Scheme 2. Aldehydes **10** were treated with NBS and DL-proline in THF to afford the bromo-derivatives **11**. Compounds **11** proved to be unstable and were immediately treated with appropriate thiophenols in THF/H_2O (1:1) under microwave irradiation, affording the desired α-thioaldehydes **8** with good overall yields.



Scheme 2. Organocatalytic-microwave assisted synthesis of racemic aldehydes **8**.

Substrates **8b-p** were then treated with KRED253 or KRED384 under optimal conditions using IPA as the cofactor recycling reagent. Results are reported in Table 6. Overall, with the exception of **8b**, all the butyraldehydes **8c-h** were converted by KRED253 into β-hydroxysulfides (*S*)-**9c-h** with good to excellent conversions (70-98%) and good to high ee values (63-94%) (Table 6, *entries 3-8*). It is noteworthy that β-hydroxysulfides (*S*)-**9c-f** bearing *p*-substituted phenyl groups were obtained with excellent ee values (83-94%).

Table 6. Substrate scope of the KRED biocatalysed DKR of α-thioaldehydes.[a]



Entry	Alcohol	R^1	R^2	KRED	Conv. [%][b]	ee [%][b,c]
1	**9a**	Et	Ph	253	95	66 (*S*)
				384	45	92 (*R*)
2	**9b**	Et	3,5-*di*-Me-Ph	253	86	27 (*R*)
				384	67	>99 (*R*)
3	**9c**	Et	4-Br-Ph	253	90	86 (*S*)
				384	55	95 (*R*)
4	**9d**	Et	4-Me-Ph	253	70 [53[d]]	94 (*S*) [94 (*S*)[e]]
				384	87 [73[d]]	84 (*R*) [85 (*R*)[e]]
5	**9e**	Et	4-MeO-Ph	253	98	83 (*S*)
				384	62	90 (*R*)
6	**9f**	Et	4-Cl-Ph	253	94	83 (*S*)
				384	52	93 (*R*)
7	**9g**	Et	2-F-Ph	253	94	63 (*S*)
				384	24	75 (*R*)
8	**9h**	Et	2-Br-Ph	253	90	68 (*S*)
				384	14	24 (*R*)
9	**9i**	Me	Ph	253	94	85 (*S*)
				384	63	85 (*R*)
10	**9j**	Me	2-Br-Ph	253	94	93 (*S*)
				384	7	9 (*S*)
11	**9k**	Me	2-Cl-Ph	253	96	91 (*S*)
				384	11	11 (*R*)
12	**9l**	Me	4-Me-Ph	253	71	99 (*S*)
				384	58	99 (*R*)
13	**9m**	Me	3,5-*di*-Me-Ph	253	91	22 (*S*)
				384	ND[f]	ND[f]
14	**9n**	*n*-Pr	Ph	253	65	37 (*R*)
				384	54	83 (*R*)
15	**9o**	*i*-Pr	Ph	253	52	1
				384	39	90 (*R*)
16	**9p**	Bn	Et	253	68	5 (*R*)
				384	67	29 (*R*)

[a] Reaction conditions: for KRED253: **8** (0.011 mmol), 2.0 mg KRED253 (cell-free extract), NADH (0.5 nmol), IPA (0.52 mmol), 910 µL NaPBS buffer (pH 8.5, 100 mM), DMSO (50 µL), 30 °C, 24 h; for KRED384: **8** (0.011 mmol), 2.0 mg KRED384 (cell-free extract), NADH (0.5 nmol), IPA (0.52 mmol), 910 µL NaPBS buffer (pH 9.0, 100 mM), DMSO (50 µL), 25 °C, 24 h. [b] Determined by HPLC equipped with ChiralPak® IG. [c] Absolute configurations assessed by comparing α_D values of **9i, 9l** and **9o** with those reported in literature and through circular dichroism (CD) experiments.[27] [d] Isolated yield at 0.44 mmol scale of **8d**. [e] The ee value at 0.44 mmol scale of **8d**. [f] Not detected by HPLC.

Interestingly, in the case of 2-((3,5-dimethylphenyl)thio)butanal **8b**, a slightly preference for the *R* enantiomer was observed with KRED253 (Table 6, *entry 2*). On the other hand, KRED384 led to desired β-hydroxysulfides (*R*)-**9b-f** with good to high conversions (52-87%) and excellent ee values (84-99%) (Table 6, *entries 2-6*). By contrast, lower conversions and ee values were observed for (*R*)-**9g** and (*R*)-**9h** with KRED384 (Table 6, *entries 7 and 8*).[30] It is worth noting that the DKR process could also be scaled up without the loss of efficiency. The DKR of **8d** at 0.44 mmol scale with KRED253 or KRED384 led to the desired (*S*)-**9d** or (*R*)-**9d**, respectively, in good yields and high ee values. The propyl alcohols (*S*)-**9i-m** were all obtained with KRED253 in excellent conversions (71-96%) and ee values (85-99%), with the exception of 2-((3,5-dimethylphenyl)thio)propan-1-ol **9m** (Table 6, *entries 9-13*). The low ee values observed for **9b** and **9m** with KRED253 may be ascribable to steric factors. On the other hand, KRED384 proved to be less effective in the synthesis of propyl alcohols, with the exception of β-hydroxysulfides (*R*)-**9i** and (*R*)-**9l**, which were obtained with excellent ee values (85% and 99%, respectively) and good conversions (63% and 58%, respectively) (Table 6, *entries 9 and 12*). Finally, products (*R*)-**9n** and (*R*)-**9o** were obtained with moderate conversions but high ee values (83% and 90%, respectively) with KRED384 (Table 6, *entries 14-15*). Interestingly, (*R*)-**9n** was also obtained as the major enantiomer with KRED253, albeit with 37% ee. Finally, compound **9p**, which carries an Et group at the sulfur atom, was obtained with good conversion but only a slight preference on the *R* enantiomer with both KRED253 and KRED384 (Table 6, *entry 16*). On the whole, opposite enantioselectivities were observed for KRED253 and KRED384 in the DKR of α-thioaldehydes **8**, with KRED253 generally favouring (*S*)-enantiomers while KRED384 preferring (*R*)-enantiomers. In an attempt to further improve the ee values of some alcohols **9**, two additional KRED enzymes, namely KRED001 and KRED170 arising from the initial KRED screening (Table S3, Supporting Information), were tested on selected α-thioaldehydes **8** (Table S4). In all cases, unsatisfactory ee values were observed, with the only exceptions of alcohol (*R*)-**9g** which was obtained with 75% ee with KRED170, and alcohol (*S*)-**9n** which was obtained with 76% ee with KRED001.

Conclusion

This work demonstrates the efficacy of KREDs biocatalysts in the synthesis of chiral β-hydroxysulfides through chemoenzymatic cascades and dynamic kinetic resolution reactions. Four KREDs were identified from a pool of 384 enzymes and successfully

employed for the synthesis of enantiomerically pure β-hydroxysulfides **7** and **9** bearing a stereocentre at the C−O or C−S bond, respectively. KRED311 or KRED349 catalysed the enantioselective reduction of prochiral α-thioketones into β-hydroxysulfides (*R*)-**7** or (*S*)-**7**, respectively, with excellent ee values, conversions and yields. In addition, both KRED311 and KRED349 were employed in chemoenzymatic cascades to afford the desired enantiomerically pure β-hydroxysulfides **7** with opposite enantioselectivities directly from commercially available thiophenols/thiols and α-haloketones. Furthermore, KRED311 and KRED349 has been further used in a novel bio-chem-bio cascade, in which the typical cofactor recycling agent IPA was replaced by 1-Cl-IPA. Such bio-chem-bio cascade is of particular significance in terms of atom- and step-economy as it exploits 1-Cl-IPA with a dual role of internal NAD(P)H/NAD(P)$^+$ cofactor recycling reagent and synthetic precursor of β-hydroxysulfides, avoiding the use of external sacrificial IPA, the formation of the side product acetone and a multi-step synthesis. Finally, the utility of KREDs was further demonstrated by the synthesis of β-hydroxysulfides **9** bearing a stereocentre at the C−S bond through KRED253 or KRED384-catalysed DKR of racemic α-thioaldehydes. In general, KRED253 favoured (*S*)-enantiomers while KRED384 preferred (*R*)-enantiomers. Such biotransformation represents, to the best of our knowledge, the first example of asymmetric C−S bond resolution by KREDs, and it shows how KREDs can be employed also for the asymmetric construction of stereocentres at other carbon-heteroatom bonds than C−O bond.

In summary, this work shows that the screening of rationally prepared enzyme panels, obtained through metagenomic approaches, represent an efficient and convenient protocol for the discovery of selective biocatalysts for biocatalytic and chemoenzymatic cascades, leading in this specific case to the identification of four highly active KREDs for the assembly of chiral β-hydroxysulfides bearing a stereocentre at C−O or C−S bond with excellent enantioselectivities.

Experimental Section

Experimental procedures, ^{1}H NMR, ^{13}C NMR spectra and HPLC spectra are reported in the Supporting Information.

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Keywords: biocatalysis • ketoreductase • β-hydroxysulfides • dynamic kinetic resolution • chemoenzymatic cascades

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[25] Control experiments (see Supporting Information) suggests that a futile self-cycling of **7a** is possible in principle, but negligible under the bio-chem-bio cascade conditions, since an excess of 1-Cl-IPA was used. The incomplete conversions for the desired products observed in the bio-chem-bio cascade as compared with those observed in the one-pot reactions were ascribable to the formation of the disulfide byproducts.

[26] Interestingly, the GC analysis of the reaction mixtures arising from the bio-chem-bio cascades with (*R*)-1-Cl-IPA and (*S*)-1-Cl-IPA revealed that the racemization of (*R*)-1-Cl-IPA and (*S*)-1-Cl-IPA occurred under the biotransformation conditions (see Supporting Information). On the other hand, it was proved that both (*R*)-1-Cl-IPA and (*S*)-1-Cl-IPA were configurationally stable when left in NaPBS buffer (pH 7.0, 200 mM) at 37 ^{o}C for 24 h and no racemization occurred (see Supporting Information). This suggests that KRED349 may reduce the α-chloroacetone intermediate without enantioselectivity under the biotransformation conditions leading to racemic 1-Cl-IPA.

[27] The absolute configurations of β-hydroxysulfides **9** were assigned by comparison of the a_D values of **9i, 9l** and **9o** with those reported in literatures, see: a) R. Breitschuh, D. Seebach, *Synthesis* **1992**, 1170–1178; b) K. C. Nicolaou, W. M. Dai, S.-C. Tsay, W. Wrasidlo, *Bioorg. Med. Chem. Lett*. **1992**, *2*, 1155–1160; c) G. Poli, L. Belvisi, L. Manzoni, C. Scolastico, *J. Org. Chem.* **1993**, *58*, 3165–3168; d) F. Rota, L. Benhamou, T. D. Sheppard, *Synlett* **2015**, *27*, 33–36; e) D. A. Evans, K. R. Campos, J. S. Tedrow, F. E. Michael, M. R. Gagné, *J. Am. Chem. Soc.* **2000**, *122*, 7905–7920. In addition, circular dichroism (CD) experiments on β-hydroxysulfides **9d, 9f** and **9o** were also carried out to confirm the assignment of the absolute configurations (Supporting Information, Figures S3, S4, S5 and S6).

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[29] The moderate conversion is likely due to the formation of side products arising from the decomposition of aldehyde **8a**. With the conversion 45%, we cannot exclude in this case that an EKR occurred rather than a DKR, even if no aldehyde **8a** was recovered from the reaction mixture.

[30] In most cases the aldehyde substrates **8** were fully consumed in the biocatalytic reactions with KRED384, while in some cases a number of uncharacterized side products arising from the decomposition of aldehydes in the buffer solution were formed, thus eventually leading to unsatisfactory conversions.

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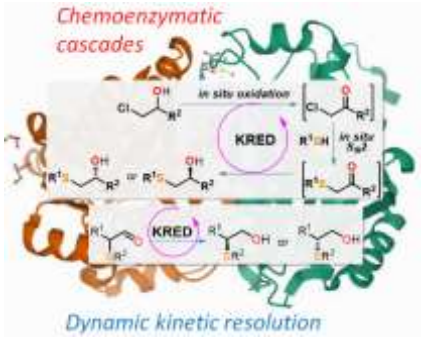

Four ketoreductases (KREDs) were identified for the enantioselective synthesis of β-hydroxysulfides. KRED311 and KRED349 catalyse the synthesis of β-hydroxysulfides bearing a stereocentre at the C−O bond via chemoenzymatic cascades from thiophenols/thiols and α-haloketones/alcohols. KRED253 and KRED384 catalyse the synthesis of β-hydroxysulfides bearing a stereocentre at the C−S bond by dynamic kinetic resolution (DKR) of racemic α-thioaldehydes.

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