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Biocatalysis

Enzymatic Desymmetrising Redox Reactions for the Asymmetric Synthesis of Biaryl Atropisomers

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Abstract: Atropisomeric biaryls carrying *ortho*-hydroxymethyl and formyl groups were made enantioselectively by desymmetrisation of dialdehyde or diol substrates. The oxidation of the symmetrical diol substrates was achieved using a variant of galactose oxidase (GOase), and the reduction of the dialdehydes using a panel of ketoreductases. Either *M* or *P* enantiomers of the products could be formed, with absolute configurations assigned by time-dependent DFT calculations of circular dichroism spectra. The differing selectivities observed with different biaryl structures offer an insight into the detailed structure of the active site of the GOase enzyme.

Atropisomeric biaryls are a family of axially chiral compounds with a long history of providing outstandingly effective chiral ligands for asymmetric synthesis.[1] They are also important components of the structures of several biologically active natural products, including vancomycin, knipholone, the korupensamines and the michellamines.[2] Typically, enantiomerically enriched atropisomeric ligands are obtained by kinetic resolution,[1] and their asymmetric synthesis presents significant challenges.[3] Strategies which have been developed recently include the use of dynamic kinetic resolution[4] and dynamic thermodynamic resolution,[5] resolutions based on sulfoxide chemistry,[5,6] and atropselective transition-metal-catalysed coupling.[7] Enzymatic desymmetrisation is an appealing method for asymmetric synthesis,[8,9] but it is noteworthy that only three examples of enzymatic desymmetrisation applied to atropisomers have been reported,[9–11] and only two of these involve the most common class of atropisomers, the biaryls.[9]

We now report that simple symmetrical biaryls may be desymmetrised by a rapid enantioselective oxidation catalysed by a variant of galactose oxidase (GOase M_{3-5}) developed in our group previously,[12] or alternatively (and complementarily) by enantioselective reduction by using a panel of ketoreductases (KREDs). This pair of enzymatic methods gives access to products that are atropisomeric biaryl aldehydes bearing chemically versatile substituents amenable to further transformation.

Six biaryl substrates **1**–**6** were studied, with the aim of probing the tolerance of the enzymes to the structural features of substituted biaryl skeletons typically found in target ligands. The substrates were synthesised by the method outlined in Scheme 1 and reported in detail in the Supporting Information. Generally, a Suzuki coupling between halobenzenes **7** and the hindered boronic acid **8**[13] gave the biaryls **1d**–**6d**, which were oxidised to the dialdehydes **1c**–**6c** through their tetrabrominated derivatives **1e**–**6e**. Reduction gave the diols **1a**–**6a**.

Two complementary enzymatic methods for the desymmetrisation of prochiral biaryls were employed in this study. Firstly, galactose oxidase (GOase), an enzyme that has been applied in a broad range of areas including biosensors,[14] cancer detection,[15] chemical synthesis[16] and recently glycoprotein labelling,[17] was employed in the oxidation of the achiral diol **1a**. After 3 h of incubation with GOase M_{3-5} at 30 °C, monoaldehyde (*M*)-**1b** was obtained in 50% yield and 99% enantiomeric excess (*ee*; Scheme 2). Secondly, a panel of ketoreductases (KREDs), which are known to be highly active and selective for reduction of a range of carbonyl compounds,[18] were screened for the reduction of the achiral dialdehyde **1c**. After incubation with KRED119 for 24 h at 30 °C, the monoaldehyde (*P*)-**1b** was formed in 67% yield and 89% *ee* (Scheme 2).

Absolute configuration was assigned to the enantiomerically enriched products (*M*)- and (*P*)-**1b** by comparison of their circular dichroism (CD) spectra with that calculated for (*P*)-**1b**. The experimental CD spectrum of the sample of **1b** (89% *ee*) produced by reduction of dialdehyde **1c** by KRED 119 aligns closely with the CD spectrum of the minimum-energy conformation of (*P*)-**1b** calculated by time-dependent (TD)-DFT (Figure 1 and the Supporting Information). As a result,

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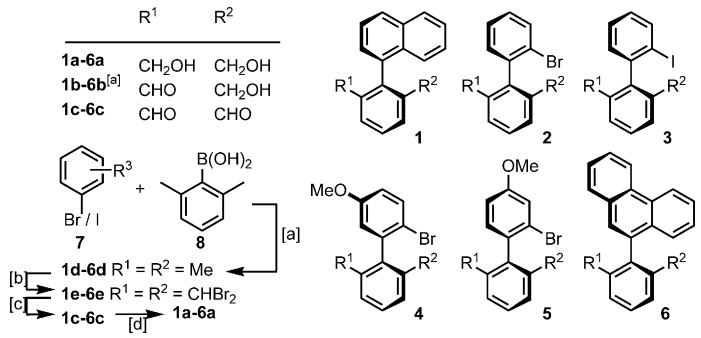
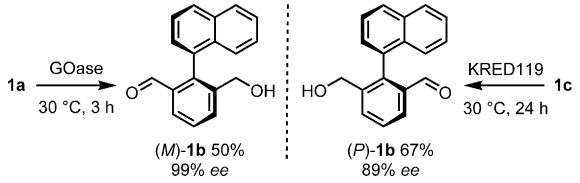


Scheme 1. Structures and preparation of the biaryl substrates. a) *M*(=R_a) enantiomer illustrated. Reagents: [a] [$Pd_2(dba)_3$] (dba = dibenzylideneacetone; 0.05 equiv), 2-dicyclohexylphosphino-2′,6′-dimethoxybiphenyl (SPhos; 0.2 equiv), K_3PO_4 (3 equiv), toluene, reflux 16 h, or [$PdCl_2$(Amphos)] (0.01 equiv), K_2CO_3 (3 equiv), reflux 5 h, toluene/water (10:1) (83–96%). [b] *N*-Bromosuccinimide (NBS), azobisisobutyronitrile (AIBN) or dibenzoyl peroxide, dichloroethane (DCE) or CCl_4, reflux 16 h (45–98%). [c] $AgNO_3$ (4.5–16 equiv), THF/H_2O, reflux 24 h, or EtOH/H_2O, 50 °C (42–93%), 5 h. [d] $NaBH_4$ (2 equiv), MeOH or THF/MeOH (3:1), RT, 4 h (24–99%).



Scheme 2. Enzymatic desymmetrisation for the enantioselective synthesis of **1b**. Yields and *ee* were determined by HPLC.

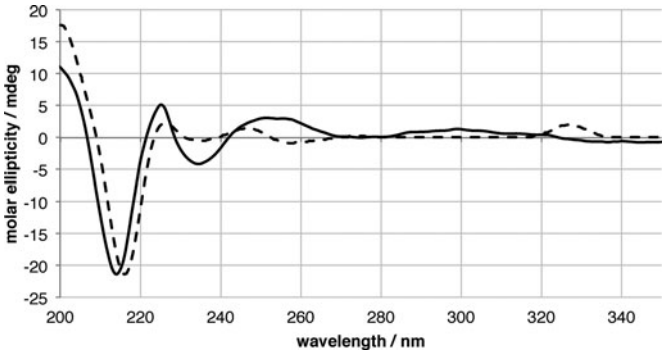


Figure 1. Experimental (solid line) and calculated (dotted line) CD spectra of *P*-**1b**.

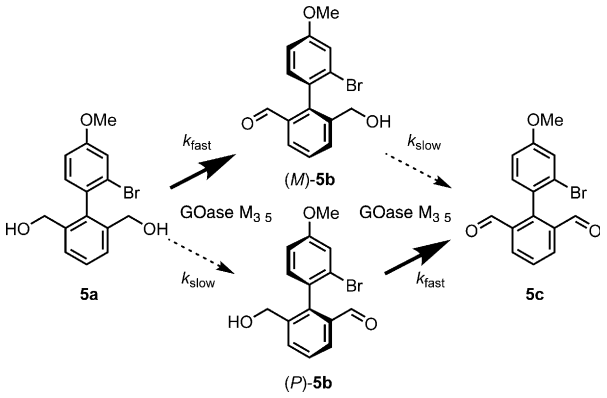
KRED 119 can be confirmed to be *P* selective in reduction of dialdehyde **1c**, and GOase M_{3-5} is *M* selective in oxidation of diol **1a**. The slower eluting peak of the two enantiomers in the HPLC trace of (±)-**1b** (Chiralcel ADH column; isohexane/isopropyl alcohol (IPA) 88:12) may thus be assigned to *P* stereochemistry.

After these successful desymmetrisation reactions of the naphthyl compounds **1a** and **1c**, our attention turned to synthetically useful halo-substituted compounds, in which the halogen substituent could provide a handle for further transformations. Bromobiaryl **2a** and iodobiaryl **3a** were incubated with GOase but showed poor enantioselectivity, giving (*M*)-**2b** and (*M*)-**3b** with only 48% *ee* and 21% *ee*, respectively (Table 1, entries 2 and 3). Compounds **4a** and **5a** bearing a further methoxy substituent were also explored, aiming to probe further the tolerance of the GOase enzyme's active site to additional substituents, and their potential role in enhancing selectivity. Biaryl **4a** proved to be a poor substrate for the GOase enzyme, giving (*M*)-**4b** with only 10% *ee* (Table 1, entry 4). However, biaryl **5a**, with the methoxy group located *para* to the second aromatic ring, showed excellent enantioselectivity. Monoaldehyde (*M*)-**5b** of 85% *ee* was formed remarkably rapidly, making up 75% of the reaction composition after 1 h. The remaining material was 4% diol **5a** and 21% of the dialdehyde **5c** (Table 1, entry 5). Separation of the product mixtures by column chromatography is described in the Supporting Information. Monoaldehydes **1b**–**6b** were stable, though **4b** decomposed in chloroform.

Table 1. Desymmetrisation of biaryl substrates using GOase M_{3-5}; typical substrate concentration 10 mM.

Entry	Substrate	*ee* [%]	Reaction time [min]	Monoaldehyde **Xb** [%][a]	Config.	Dialdehyde **Xc** [%][a]
1	**1a**	99	180	50±5	*M*[b]	50±5
2	**2a**	48	120	15±5	*M*[b]	24±5
3	**3a**	21	180	37±5	*M*[b]	7±5
4	**4a**	10	10	73±5	*M*[c]	17±5
5	**5a**	85	60	75±5	*M*[c]	21±5
6	**6a**	92	180	66±5	*M*[b]	20±5
7	**1a**	96	300	81±5	*M*[b]	17±5

[a] Based on composition by HPLC. [b] Assigned by comparison of experiment and calculated CD spectra (see the Supporting Information). [c] Assumed by analogy with **1–3** and **6**.



Scheme 3. Partial kinetic resolution as the minor monoaldehyde product (*P*)-**5b** is consumed in the reaction leading to an enhancement in *ee*.

Enantioselective oxidation of the minor enantiomer of **5b** to dialdehyde **5c** contributes to the enrichment in *ee* observed during the reaction (Scheme 3). This partial kinetic resolution process was demonstrated in the oxidation of diol **5a** by monitoring the reaction at regular time intervals (Figure 2). Rapid

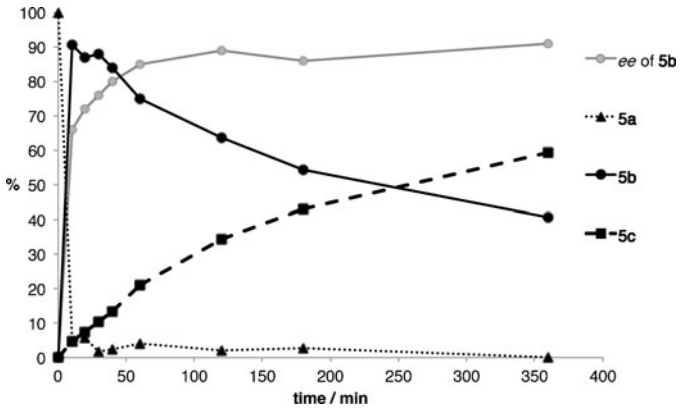


Figure 2. Evolution of the composition of the reaction mixture during the oxidation of **5a** with GOase M_{3-5}.

desymmetrising oxidation of **5a** meant that after 10 min there remained only 5% of diol **5a**, accompanied by 90% of the monoaldehyde (*M*)-**5b** with an *ee* of 66% and 5% of dialdehyde **5c**. As shown in Figure 1, this initial rapid desymmetrisation was followed by slow oxidative removal of the minor enantiomer of the product **5c**. After 6 h the *ee* of (*M*)-**5b** was dramatically enhanced to 91% at the expense of yield, which dropped from 90 to 41%, because more of the material was converted to the dialdehyde **5c** (59%).

In an attempt to suppress the secondary oxidation, the reaction of **1a** with GOase was repeated at a lower temperature of 20 °C. The percentage of hydroxyaldehyde **1b** was increased to 81% after 5 h with only a slight decrease in *ee* (96%) compared with the reaction carried out at 30 °C, indicating that the secondary oxidation takes place at a lower rate (Table 1, entry 7). Only 2% of the starting material **1a** remained in the system, and the remaining 17% was **1c**.

To explore the substrate scope further, we investigated the more hindered phenanthryl substituted biaryl **6** (Table 1, entry 6). GOase M_{3-5} was employed in the oxidation of the symmetrical diol, and fast formation of (*M*)-**6b** was observed. The *ee* reached 92% after 3 h, with **6b** making up 66% of the reaction composition.

For comparison with the results obtained by using GOase, a panel of KREDs were screened against the symmetrical dialdehydes **1c**, **2c**, **3c** and **6c** (Table 2), incubating at 24–48 h at 30 °C (5 mg substrate, (17 mM), DMSO (30% v/v), KRED enzyme (5 mg), glucose dehydrogenase (CDX-901, 1 mg), nicotinamide adenine dinucleotide phosphate (NADP; 1 mg), glucose (3.8 mg, 1.25 mol excess relative to substrate), in K_3PO_4 buffer (100 mM, pH 7.0)). Good results were obtained with a number of KREDs, notably KRED 114 (entry 2) and KRED 119 (entry 4) which produced (*P*)-**1b** in 84% *ee* and 89% *ee*, respectively and in 50 and 67% yields, respectively, determined by HPLC.

The two halo-substituted biaryl dialdehydes **2c** and **3c**, which showed poor results with GOase M_{3-5}, were treated with KREDs under the same conditions and in a number of cases the products **2b** and **3b** were produced in high *ee*. For example, KRED 120 gave **2b** (*ee* 88%, HPLC yield 50%: Table 2, entry 8), and KRED 121 gave **3b** (*ee* 92%, HPLC yield 49%: Table 2, entry 13). Aldehydes **4c** and **5c** were unstable, and the reductions could not be performed on these substrates but bulky dialdehyde **6c** was reduced with excellent *ee* values and moderate to good conversions. KRED 110 was noted to provide (*P*)-**6b** with outstanding selectivity (*ee* 97%, Table 2, entry 14). In several cases, complementary selectivity between GOase and the KREDs was evident, and in some cases between different KREDs. Interestingly, there was no correlation between the enzymes' sense of enantioselectivity in the reductions of prochiral ketones and in the atroposelective desymmetrisations Thus, entries **4** and **5** show typically *R*- and *S*-selective KREDs respectively both giving the *P* atropisomer, suggesting that this atroposelective reaction is probing a different part of their active site.

Table 2. Enzymatic desymmetrisation using ketoreductases (KREDs).[a]

Entry	Sub.	Enzyme[b]	*ee* [%]	Yield [%][c]	Config.[d]
1	**1c**	KRED 108 (S)	86	54±5	*P*
2	**1c**	KRED 114 (S)	88	50±5	*P*
3	**1c**	KRED 118 (S)	84	86±5	*P*
4	**1c**	KRED 119 (S)	89	67±5	*P*
5	**1c**	KRED 121 (R)	29	91±5	*P*
6	**2c**	KRED 108 (*S*)	83	59±5	*P*
7	**2c**	KRED 114 (*S*)	86	43±5	*M*
8	**2c**	KRED 120 (*S*)	88	50±5	*P*
9	**2c**	KRED 121 (*R*)	87	69±5	*P*
10	**3c**	KRED 108 (*S*)	84	27±5	*P*
11	**3c**	KRED 114 (*S*)	65	34±5	*M*
12	**3c**	KRED 120 (*S*)	78	83±5	*P*
13	**3c**	KRED 121 (*R*)	92	49±5	*P*
14	**6c**	KRED 110	97	23±5[e]	*P*
15	**6c**	KRED 112 (R)	62	27±5[e]	*M*
16	**6c**	KRED 114 (S)	86	39±5[e]	*M*
17	**6c**	KRED 123	95	20±5[e]	*M*

[a] Unless otherwise stated, the compounds were incubated for 24 h at 30 °C. A full list of results for all of the screened KREDs is given in the Supporting Information. [b] The typical reported selectivity of the KRED in the reduction of unsymmetrical ketones is shown in parentheses.[19] [c] Based on composition by HPLC. [d] Configuration of the major enantiomer of the product. [e] Incubated for 48 h at 30 °C.

To gain insight into the observed enantioselective oxidations with GOase M_{3-5}, we carried out docking studies with diol **6a** (Figure 3a). A homology model of the M3-5 mutant was created by using the X-ray crystal structure of the E1 mutant as a template. [17] The substrate was docked into the active site of the protein by using the CHARMm force field.[20] The active site of the enzyme is located on the surface of the protein, and is thus accessible to the relatively bulky alcohol substrates used in this study. Inspection of the active site architecture suggested that the enantioselectivity derives from the local surface features that make intimate contact with the substrate diol. On one side of the active site is a "cliff", and on the other side a "plateau", suggesting that bulky substituents will be preferentially orientated towards the plateau (Figure 3b). Substrates **1a**–**6a** all carry an *ortho*-substituent on the upper aromatic ring, and can enter the active site only if this substituent occupies the "plateau" region, leading to formation of the *M*-enantiomer in all cases. A schematic representation of the binding of the biaryl substrate **6a** is shown in Figure 3b. Interestingly,

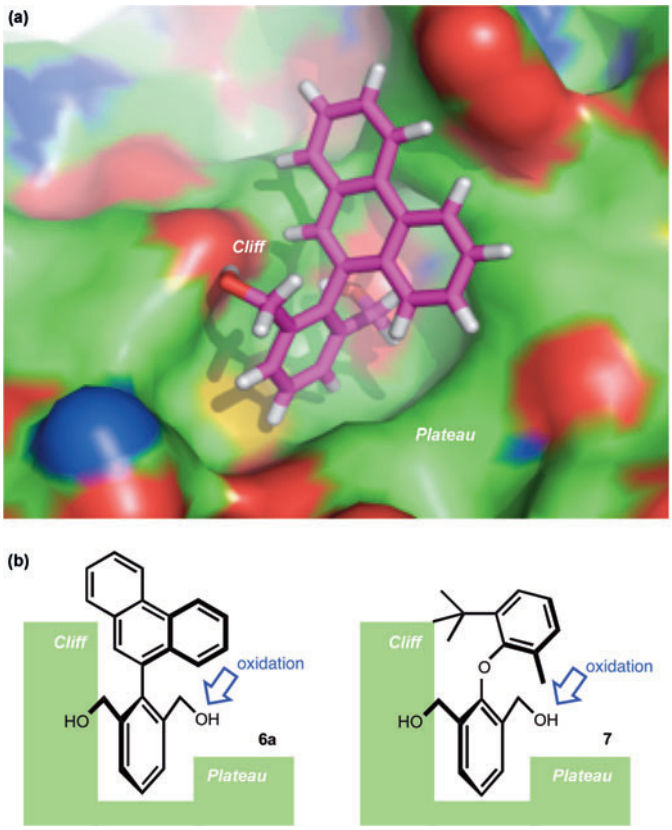


Figure 3. a) Modelling of diol **6a** into the active site of GOase M_{3-5}. b) Schematic representation for docking models of biaryl **6a** and diaryl ether **7** in the active site of GOase M_{3-5}.

diaryl ether substrates (for example, **7**) are oxidised to the *P*-enantiomer by GOase M_{3-5}.[10] We reason that the ether linkage in **7** allows the upper ring to lean over into the space above this plateau, thereby allowing the *tert*-butyl group in **7** to be placed towards the "cliff".

In summary, two complementary biocatalytic routes have been developed for the synthesis of enantioenriched biaryl atropisomers. Both GOase and KREDs have been shown to be capable of recognising axial chirality in addition to their more conventional application in the generation of OH-bearing stereogenic centres. The GOase M_{3-5}-catalysed reactions suggest that substituent effects are important in determining the enantioselectivity of the oxidation process such that with carefully designed substrates (e.g., **1a**, **5a**, **6a**) high levels of stereocontrol can be achieved. Although these substrates exhibit poor water solubility, in many cases, they are turned over rapidly by the GOase and KRED biocatalysts. Further optimisation of yields and enantioselectivity should be possible for these and other biaryl atropisomers by successive rounds of protein engineering and directed evolution of these enzymes.

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