Biocatalysis

Enzymatic Desymmetrising Redox Reactions for the Asymmetric Synthesis of Biaryl Atropisomers

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Abstract: Atropisomeric biaryls carrying ortho-hydroxy-methyl 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 koru-pensamines and the michellamines. [2] Typically, enantioselectively enriched atropisomeric ligands are obtained by kinetic resolution, [3] and their asymmetric synthesis presents significant challenges. [4] Strategies which have been developed recently include the use of dynamic kinetic resolution [5] and dynamic thermodynamic resolution, [6] resolutions based on sulfoxide chemistry, [7] and atroposelective transition-metal-catalysed coupling. [8] Enzymatic desymmetrisation is an appealing method for asymmetric synthesis, [9] but it is noteworthy that only three examples of enzymatic desymmetrisation applied to atropisomers have been reported [10] and only two of these involve the most common class of atropisomers, the biaryl. [9]

We now report that simple symmetrical biaryls may be desymmetrised by a rapid enantioselective oxidation catalysed by a variant of galactose oxidase (GOase M₃₃) developed in our group previously, [11] 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 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 biaryl were employed in this study. Firstly, galactose oxidase (GOase), an enzyme that has been applied in a broad range of areas including biosensors, [12] cancer detection, [13] chemical synthesis [14] and recently glycoprotein labelling, [15] was employed in the oxidation of the achiral diol 1a. After 3 h of incubation with GOase M₃₃ 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, [16] were screened for the reduction of the achiral dialdehyde 1c. After incubation with KRED119 for 24 h at 30 °C, the monoaaldehyde (P)-1b was formed in 67% yield and 89% ee (Scheme 2).

Absolute configuration was assigned to the enantioselectively 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 KRED119 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,
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 found 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 [%] | Config. | Dialdehyde Xc [%] |
|-------|------------------|---------------------|---------------------|---------|------------------|
| 1     | 1a 99 180 50 ± 5 | M^N 50 ± 5          |                     |         |                  |
| 2     | 2a 48 120 15 ± 5 | M^N 24 ± 5          |                     |         |                  |
| 3     | 3a 21 180 37 ± 5 | M^N 7 ± 5           |                     |         |                  |
| 4     | 4a 10 10 73 ± 5 | M^N 17 ± 5          |                     |         |                  |
| 5     | 5a 85 60 75 ± 5 | M^N 21 ± 5          |                     |         |                  |
| 5     | 5b 92 180 66 ± 5 | M^N 20 ± 5          |                     |         |                  |
| 7     | 7a 96 300 81 ± 5 | M^N 17 ± 5          |                     |         |                  |

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

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; isooctane/isopropyl alcohol (IPA) 88:12) may thus be assigned to P stereochimistry.

After these successful desymmetrisation reactions of the napthyl 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 found 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.

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
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 diadehyde 5c. As shown in Figure 1, this 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 diadehyde 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 hydroxaldehyde 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 M₃₋₅ 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 K₂PO₄ 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 M₃₋₅, 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. Enzymatic desymmetrisation using ketoreductases (KREDs).[a]

| Entry | Sub. | Enzyme[C] | ee (%) | Yield [%][d] | 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 ± 6      | 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[c]   | P          |
| 15    | 6c   | KRED 112 (R) | 62     | 27 ± 5[c]   | M          |
| 16    | 6c   | KRED 114 (S) | 86     | 39 ± 5[c]   | M          |
| 17    | 6c   | KRED 123     | 95     | 20 ± 5[c]   | 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.[16] [c] Based on composition by HPLC. [d] Configuration of the major enantiomer of the product. [e] Incubated for 48 h at 30°C.
diaryl ether substrates (for example, 7) are oxidised to the P-enantiomer by GOase M₃.⁷ 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 stereo-ecenic centres. The GOase M₃₋₅-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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