

# Old Yellow Enzyme-mediated reduction of $\beta$ -cyano- $\alpha,\beta$ -unsaturated esters for the synthesis of chiral building blocks: stereochemical analysis of the reaction†

Cite this: *Catal. Sci. Technol.*, 2013, **3**, 1136

Elisabetta Brenna,<sup>\*a</sup> Francesco G. Gatti,<sup>a</sup> Alessia Manfredi,<sup>a</sup> Daniela Monti<sup>b</sup> and Fabio Parmeggiani<sup>a</sup>

Baker's yeast and Old Yellow Enzyme-mediated reduction of the carbon–carbon double bonds of  $\beta$ -cyano- $\alpha,\beta$ -unsaturated esters was investigated, in order to broaden the applicability of this kind of reaction in the field of preparative organic chemistry. The synthetic significance of the enantioselective reduction of these difunctionalised substrates was shown by considering the conversion of saturated chiral cyanoesters into  $\gamma^2$ -amino acid derivatives for foldamer chemistry applications. The stereochemical outcome of the biotransformations was carefully analysed by means of deuterium labeling experiments. The results of this analysis were employed to rationalise the effects of substrate-control on the stereoselectivity of a certain class of ene-reductase-mediated reduction reactions. A simple model was developed to describe the structural prerequisites for the optimal arrangement of the substrates within the binding site of OYE1-3 enzymes.

Received 21st November 2012,  
Accepted 4th January 2013

DOI: 10.1039/c3cy20804d

[www.rsc.org/catalysis](http://www.rsc.org/catalysis)

## Introduction

Bioconversions, including biocatalytic processes with isolated enzymes and biotransformations with whole cell systems, are now becoming key components in the toolbox of the process chemist for the synthesis of chiral compounds,<sup>1</sup> with a place alongside chemocatalysis and chromatographic separation.<sup>2</sup> The importance given to the cost and profit margin in today's competitive market, and the growing societal concern for the development of clean, or at least less polluting, chemical processes have increased the need for novel, high-yield, short process routes, characterised by high selectivity, low working temperatures and pressures, and waste reduction. Biocatalysis is best suited to satisfy these requirements.

Among known enzyme mediated procedures, the stereoselective reduction of carbon–carbon double bonds promoted by ene-reductases (ERs), belonging to the Old Yellow Enzyme

(OYE) family,<sup>3</sup> is still an emerging chemistry.<sup>4</sup> If the starting olefin is suitably substituted, the reaction occurs with the creation of at least one stereogenic centre under high stereochemical control, thus making this kind of reduction of great value for the synthesis of enantiopure chiral compounds: the first large scale application of ERs for the preparation of chiral building blocks has been recently reported in the literature.<sup>5</sup>

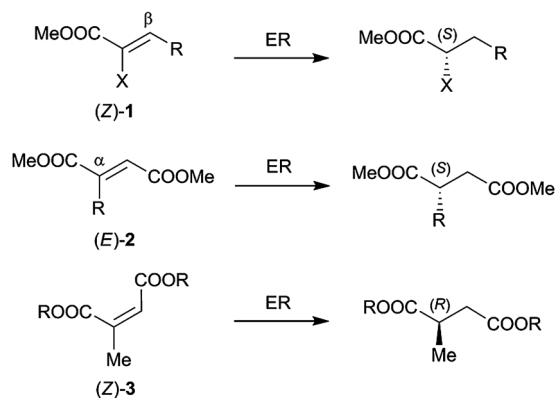
It has been established that the double bond can be effectively reduced by ERs, if it is made susceptible to the nucleophilic attack of a hydride, delivered by the reduced flavin cofactor, in the presence of an electron-withdrawing substituent, which is also able to establish hydrogen bonds with certain amino acid residues within the active site of the enzyme.<sup>6</sup>

$\alpha,\beta$ -Unsaturated aldehydes and ketones together with nitroalkenes are typical substrates for ERs, whereas the bioreduction of  $\alpha,\beta$ -unsaturated carboxylic acids and derivatives is quite a challenging task, because the presence of a single carboxylic moiety generally does not promote ER-mediated reduction: only a few exceptions are reported in the literature, consisting of  $\alpha$ -substituted  $\alpha$ -methylenic esters.<sup>7</sup> The structural prerequisites, which are needed for the double bond reduction of an  $\alpha,\beta$ -unsaturated acid or ester, are still to be clearly defined, in order to establish the synthetic usefulness of this procedure and to include it into the ready-to-use reactions available to the synthetic chemists in charge of manufacturing process development.

<sup>a</sup> Politecnico di Milano, Dipartimento di Chimica, Materiali, Ingegneria Chimica, Via Mancinelli 7, I-20131 Milano, Italy. E-mail: [elisabetta.brenna@polimi.it](mailto:elisabetta.brenna@polimi.it); Fax: +39 02 23993180; Tel: +39 02 23993077

<sup>b</sup> Istituto di Chimica del Riconoscimento Molecolare-CNR, Via M. Bianco 9, I-20131 Milano, Italy

† Electronic supplementary information (ESI) available: Full product characterisation, NMR data of deuterated compounds, analytical procedures for the determination of conversion and enantiomeric excess values. See DOI: 10.1039/c3cy20804d



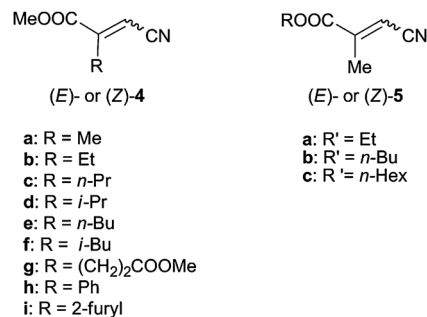
**Scheme 1** Unsaturated esters already subjected to ER-mediated reduction.

Utaka *et al.*<sup>8</sup> first observed that the presence of a chlorine atom on the same carbon atom bearing the carboxylic moiety promoted C=C reduction by baker's yeast (BY) fermentation. The same effect was not observed when the chlorine atom was either absent (*e.g.* in (*E*)-2-heptenoic acid and in its methyl ester) or on the  $\beta$  carbon atom. The successful biocatalysed reduction of methyl citraconate and mesaconate<sup>9</sup> further supported the hypothesis that the presence of a second electron-withdrawing group was necessary to make the double bond of  $\alpha,\beta$ -unsaturated esters electrophilic enough to undergo hydride attack in ene-reductase biotransformations.

We have recently investigated the OYE-mediated reduction of  $\alpha$ -haloalkenoates (*Z*)-1 (Scheme 1),<sup>10</sup> of 2-substituted methyl fumarates and maleates (*E*)- and (*Z*)-2, and of alkyl mesaconates and citraconates (*E*)- and (*Z*)-3,<sup>11</sup> in order to evaluate the effects due to the stereochemistry of the starting alkene and the nature of substituents. High values of enantioselectivity and conversions were obtained with (*Z*)-1, (*E*)-2, and (*Z*)-3, affording, respectively (Scheme 1), the (*S*)-, (*S*)-, and (*R*)-enantiomers of the corresponding reduced products. Compounds (*Z*)-2 and (*E*)-3 were found to be completely unreactive.

For substrates (*Z*)-1 a great variety of alkyl and aryl substituents could be accommodated at the carbon atom in position  $\beta$ ;<sup>10</sup> either a short chain alkyl group or a phenyl ring could be present at the  $\alpha$  carbon atom on methyl esters (*E*)-2;<sup>11</sup> the use of alkyl chains other than methyl groups at the ester moieties in derivatives 3 made (*Z*)-stereoisomers the most favourable substrates for the reduction, with a modification of the most reactive binding mode with respect to compounds (*E*)-2 and, consequently, a change in the stereochemical outcome of the reaction.<sup>11</sup>

With the aim of expanding the applications of this kind of reduction in the field of preparative organic chemistry, we now report the effect produced by a nitrile group in the  $\beta$  position with respect to the ester functionality on the reduction of the double bond of methyl 2-cyanomethylenealkanoates (*E*)- and (*Z*)-4 and alkyl 3-cyanomethacrylates (*E*)- and (*Z*)-5 (Fig. 1). Up to now, only a few examples of enzymatic carbon-carbon double bond reduction reactions of unsaturated nitriles have been reported in the literature.<sup>12</sup>



**Fig. 1**  $\beta$ -Cyano- $\alpha,\beta$ -unsaturated esters subjected to ER-mediated reduction.

The nitrile moiety is particularly versatile, and it can be easily converted into other functional groups either by classical or biocatalysed organic reactions. Thus, the enantiomerically enriched 3-cyanoalkanoates recovered from ER-mediated bio-reduction of substrates 4 and 5 represent useful chiral synthons for organic synthesis: their conversion into  $\gamma^2$ -amino acids for foldamer chemistry applications is herein considered. The results of a detailed mechanistic analysis, carried out by means of deuteration experiments to explain the stereochemical outcomes obtained with these cyanoesters, are also shown and employed to establish general criteria for the identification of optimal substrates for this kind of biocatalysts.

## Results and discussion

### Biocatalysed reduction of cyanoesters 4 and 5

Compounds (*E*)- and (*Z*)-4a-i and (*E*)- and (*Z*)-5a-c were prepared by Wittig condensation of the suitable ketoester with 2-(triphenylphosphoranylidene)acetonitrile. The (*E*)- and (*Z*)-stereoisomers were separated by column chromatography, and subjected separately to bioreduction, employing either fermenting cells of BY or isolated OYE1-3 (with *in situ* regeneration of the NAD(P)H cofactor with a glucose dehydrogenase and glucose as a sacrificial cosubstrate). The results are collected in Table 1.

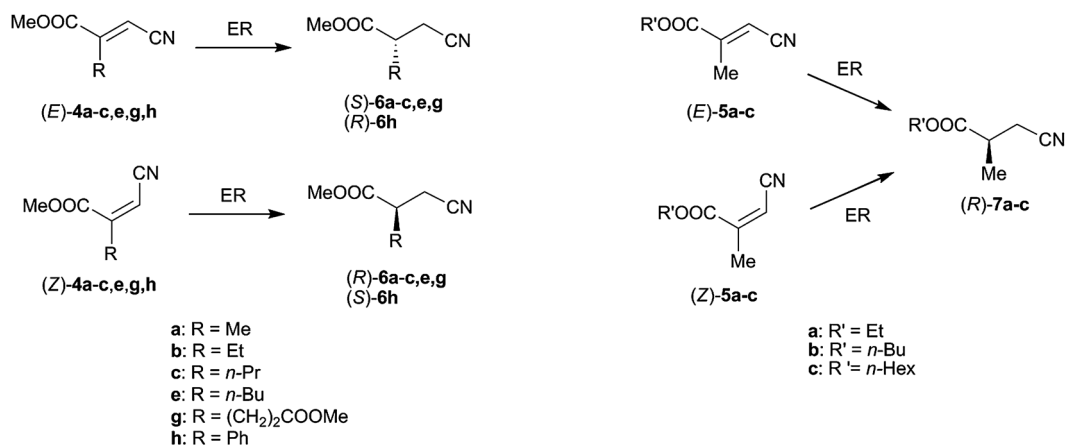
The analysis of these data highlighted that the bioreduction of methyl (*E*)-cyanomethylenealkanoates 4 occurred with higher enantioselectivity and more satisfactory yields than those of the corresponding (*Z*)-stereoisomers, except for the least hindered substrates (*E*)- and (*Z*)-4a. No reaction occurred with substrates (*E*)- and (*Z*)-4d and f, bearing a branched chain at the carbon atom in position 2.

The (*E*)-stereoisomers of compounds 4a-c, e, g, and h (Scheme 2) were converted into derivatives (*S*)-6 (Scheme 2), whereas the corresponding (*Z*)-stereoisomers gave reduced compounds of opposite configuration, with the exception of compound (*Z*)-4b, which afforded very poorly enriched (*S*)-reduction products. It should be noted that for derivative 6h (Scheme 2) a switch of Cahn-Ingold-Prelog priority of the groups linked to the stereogenic carbon atom occurs: thus, the (*R*)-configuration of derivative 6h corresponds to a spatial distribution of substituents which is identical to that of the (*S*)-enantiomers of the other reduced compounds of type 6. Furthermore, with compound (*E*)-4h the outcome of the

**Table 1** Results of BY and OYE1-3-mediated reduction of substrates (*E*)- and (*Z*)-**4a-i** and (*E*)- and (*Z*)-**5a-c**

R	R'	Substrate	BY		OYE 1		OYE 2		OYE 3	
			<i>c</i> <sup>a</sup> (%)	ee <sup>b</sup> (%)	<i>c</i> <sup>c</sup> (%)	ee <sup>b</sup> (%)	<i>c</i> <sup>c</sup> (%)	ee <sup>b</sup> (%)	<i>c</i> <sup>c</sup> (%)	ee <sup>b</sup> (%)
Me	Me	( <i>E</i> )- <b>4a</b>	100	38 ( <i>S</i> )	100	34 ( <i>S</i> )	50	40 ( <i>S</i> )	100	97 ( <i>S</i> )
		( <i>Z</i> )- <b>4a</b>	100	67 ( <i>R</i> )	100	88 ( <i>R</i> )	53	98 ( <i>R</i> )	77	52 ( <i>R</i> )
Et	Me	( <i>E</i> )- <b>4b</b>	100	99 ( <i>S</i> )	100	99 ( <i>S</i> )	100	99 ( <i>S</i> )	100	99 ( <i>S</i> )
		( <i>Z</i> )- <b>4b</b>	83	10 ( <i>S</i> )	100	68 ( <i>S</i> )	45	38 ( <i>S</i> )	55	rac
<i>n</i> -Pr	Me	( <i>E</i> )- <b>4c</b>	45	99 ( <i>S</i> )	100	99 ( <i>S</i> )	44	99 ( <i>S</i> )	87	99 ( <i>S</i> )
		( <i>Z</i> )- <b>4c</b>	43	58 ( <i>R</i> )	100	rac	45	47 ( <i>R</i> )	46	76 ( <i>R</i> )
<i>i</i> -Pr	Me	( <i>E</i> )- <b>4d</b>	0		4		0		1	
		( <i>Z</i> )- <b>4d</b>	1		2		1		2	
<i>n</i> -Bu	Me	( <i>E</i> )- <b>4e</b>	48	94 ( <i>S</i> )	100	99 ( <i>S</i> )	32	99 ( <i>S</i> )	75	99 ( <i>S</i> )
		( <i>Z</i> )- <b>4e</b>	58	91 ( <i>R</i> )	100	72 ( <i>R</i> )	50	90 ( <i>R</i> )	53	83 ( <i>R</i> )
<i>i</i> -Bu	Me	( <i>E</i> )- <b>4f</b>	0		1		0		0	
		( <i>Z</i> )- <b>4f</b>	0		3		1		2	
(CH <sub>2</sub> ) <sub>2</sub> COOMe	Me	( <i>E</i> )- <b>4g</b>	68	78 ( <i>S</i> )	99	94 ( <i>S</i> )	40	94 ( <i>S</i> )	100	94 ( <i>S</i> )
		( <i>Z</i> )- <b>4g</b>	22	35 ( <i>S</i> )	100	32 ( <i>R</i> )	36	58 ( <i>R</i> )	94	46 ( <i>R</i> )
Ph	Me	( <i>E</i> )- <b>4h</b>	26	28 ( <i>R</i> )	100	99 ( <i>R</i> )	58	99 ( <i>R</i> )	97	99 ( <i>R</i> )
		( <i>Z</i> )- <b>4h</b>	6	34 ( <i>S</i> )	85	16 ( <i>R</i> )	24	22 ( <i>R</i> )	51	7 ( <i>S</i> )
2-furyl	Me	( <i>E</i> )- + ( <i>Z</i> )- <b>4i</b>	85		100		88		100	
Me	Et	( <i>E</i> )- <b>5a</b>	10	95 ( <i>R</i> )	100	70 ( <i>R</i> )	45	96 ( <i>R</i> )	21	90 ( <i>R</i> )
		( <i>Z</i> )- <b>5a</b>	83	99 ( <i>R</i> )	100	99 ( <i>R</i> )	100	99 ( <i>R</i> )	96	99 ( <i>R</i> )
Me	<i>n</i> -Bu	( <i>E</i> )- <b>5b</b>	2		93	98 ( <i>R</i> )	26	84 ( <i>R</i> )	12	71 ( <i>R</i> )
		( <i>Z</i> )- <b>5b</b>	41	96 ( <i>R</i> )	100	98 ( <i>R</i> )	100	99 ( <i>R</i> )	94	99 ( <i>R</i> )
Me	<i>n</i> -Hex	( <i>E</i> )- <b>5c</b>	0		46	64 ( <i>R</i> )	10	42 ( <i>R</i> )	12	74 ( <i>R</i> )
		( <i>Z</i> )- <b>5c</b>	37	98 ( <i>R</i> )	100	97 ( <i>R</i> )	60	99 ( <i>R</i> )	97	99 ( <i>R</i> )

<sup>a</sup> *c* = Conversion percentage; calculated by GC analysis of the crude mixture after 72 h reaction time. <sup>b</sup> Calculated by GC analysis on a chiral stationary phase. <sup>c</sup> *c* = Conversion percentage, calculated by GC analysis of the crude mixture after 24 h reaction time; isolated yields are reported in ESI.

**Scheme 2** Products obtained by reduction of β-cyano-α,β-unsaturated esters **4** and **5**.

reduction was sensibly different when performed by means of isolated enzymes or in the presence of BY. OYE1-3 reduced substrate (*E*)-**4h** completely and with high enantioselectivity, while the product obtained by BY fermentation showed a low enantiomeric excess value, as a consequence of double bond isomerisation occurring in the fermenting medium, monitored by GC/MS analysis of the reaction. Substrate (*Z*)-**4h** was converted into poorly enriched or nearly racemic reduction products, either by means of OYE1-3 biotransformation or with the use of BY. As for substrate **4i**, it was impossible to separate

the two diastereoisomers by column chromatography: the bioreductions proceeded nearly quantitatively to afford racemic reduction products.

A different stereochemical outcome (Table 1) was observed for the biocatalysed reduction of 3-cyanomethacrylates (*E*)- and (*Z*)-**5**, which were characterised by the presence of a methyl group on carbon atom 2, and by different alkyl chains in the ester moiety. The reduction of compounds (*Z*)-**5a-c** occurred with higher yields and better enantioselectivity values than that of the (*E*)-stereoisomers, and both diastereoisomers were

converted into the (*R*)-enantiomers of the corresponding alkyl cyanopropanoates **7a–c** (Scheme 2).

Similar stereochemical outcomes have already been obtained for the bioreduction of diesters **2** and **3** (Scheme 1),<sup>11</sup> structurally related to cyanoesters **4** and **5** by substitution of the nitrile with an ester moiety: (*E*)-**2** and (*Z*)-**3** were found to afford the (*S*)- and (*R*)-reduced products, respectively, just as (*E*)-**4** and (*Z*)-**5**.

The configuration of the double bond of starting unsaturated compounds **4** and **5** was determined by analysis of the <sup>1</sup>H and <sup>13</sup>C NMR spectra of the two stereoisomers. The absolute configuration of compounds **6a–c**, **g**, **h** and **7a–c** was established by chemical correlation to the corresponding dimethyl esters: the enantiomerically enriched cyanoesters were heated in methanol solution in the presence of sulphuric acid, to afford the corresponding diesters of known absolute configuration. Compound **6e** was transformed into the corresponding diacid of known absolute configuration by reaction in 37% refluxing HCl solution.

### Synthetic exploitation of the reduction products

To add practical synthetic value to our screening study, we manipulated the nitrile functionality of saturated cyano methyl ester (*S*)-**6b**, in order to obtain the  $\gamma^2$ -amino ester (*S*)-**8** (Scheme 3).

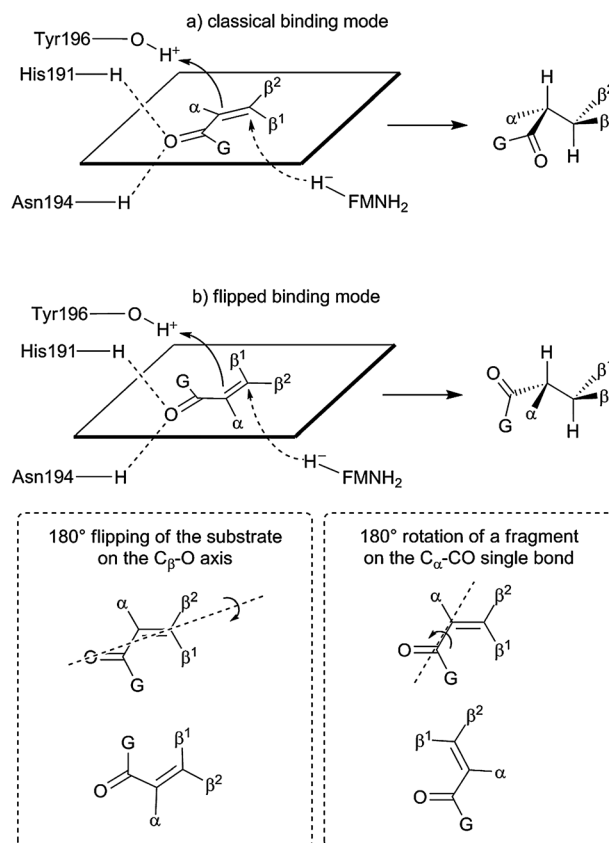
$\gamma^2$ -Amino acids are an important class of non-natural amino acids: being analogues of the neurotransmitter  $\gamma$ -aminobutyric acid, they are of potential biomedical utility for the treatment of neurological disorders.<sup>13</sup> They are also useful building blocks for  $\gamma$ -peptides<sup>14</sup> and heterogeneous backbone foldamers.<sup>15</sup> Peptides with a homogeneous backbone, *e.g.*  $\gamma$ -peptides, or  $\alpha,\gamma$ - or  $\beta,\gamma$ -hybrid peptides, are relevant synthetic targets, and their availability greatly depends on the possibility of accessing suitable  $\gamma$ -amino acids. The synthesis of optically enriched  $\gamma^2$ -amino acids is rather challenging: the known methods<sup>16</sup> consist, for example, in resolution techniques based on the use of (*R*)- and (*S*)-pantolactone,<sup>17a</sup> or (*R*)- and (*S*)-pantolactam,<sup>17b–d</sup> or in procedures involving Evans oxazolidinone.<sup>18</sup> Only a few examples of stereoselective synthesis have been reported so far, such as an enantioselective organocatalytic Michael addition of aldehydes to nitroethylene,<sup>19</sup> or an enantioselective addition of methyl cyanoacetate to allylic acetates.<sup>20</sup> Biocatalysed methods<sup>21</sup>

for  $\gamma$ -amino acids reported up to now do not involve  $\gamma^2$  derivatives.

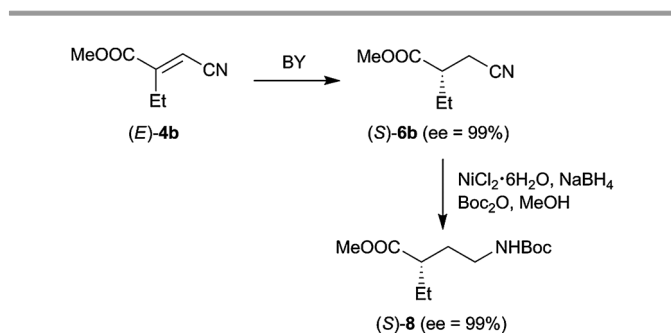
Our synthetic sequence (Scheme 3), based on the stereoselective reduction of the double bond followed by chemical reduction of the nitrile functionality, widens the choice of synthetic routes available for these valuable chiral synthons. Compound (*S*)-**6b** was obtained by BY fermentation of (*E*)-**4b** in 72% isolated yield after column chromatography. Subsequently, the chemoselective reduction of the nitrile group by treatment with a catalytic amount of NiCl<sub>2</sub>·6H<sub>2</sub>O and an excess of NaBH<sub>4</sub> in the presence of di-*tert*-butyl dicarbonate promoted the conversion of (*S*)-**6b** into the corresponding Boc protected  $\gamma^2$ -amino ester (*S*)-**8** (70% after column chromatography).

### Stereochemical analysis of the ER-catalysed reductions

A careful stereochemical analysis was necessary, in order to explain the observed enantioselectivities of the bioreduction of (*E*)- and (*Z*)-unsaturated cyanoesters **4** and **5**. The generally accepted mechanism<sup>6c</sup> of OYE-mediated reactions (Fig. 2) consists first in the transfer of a hydride ion from the flavin prosthetic group to the sp<sup>2</sup> carbon atom in the  $\beta$  position with respect to the activating substituent, which is involved in the formation of hydrogen bonds with His191 and/or Asn194 in the active site of the enzyme. A tyrosine residue then delivers a



**Fig. 2** Mechanism and possible substrate reactive binding modes for OYE-mediated reduction of C=C double bonds. G is the substituent linked to the C=O moiety of the withdrawing group;  $\alpha$ ,  $\beta^1$  and  $\beta^2$  are substituents of the double bond (numbering according to PDB structure code 1OYB).



**Scheme 3** Synthesis of  $\gamma^2$ -amino ester (*S*)-**8** from cyano methyl ester (*S*)-**6b**.

proton, which can be derived by exchange with the solvent, to the  $\alpha$  carbon atom, usually<sup>22</sup> on the opposite stereoheterotopic face of the alkene, thus affording *anti* hydrogen addition.

In the case of an electron withdrawing group containing a carbon–oxygen double bond, the “classical” binding mode is considered to be that reported in Fig. 2a,<sup>6c,23a</sup> which has been inferred from the structure of oxidised OYE1, bearing *p*-hydroxybenzaldehyde<sup>6c</sup> in the active site. Nonetheless, examples of substrates binding in more than one active conformation have been described in the literature.<sup>4d,23</sup> In particular, an alternative “flipped” binding mode (Fig. 2b)<sup>4d,23a</sup> has been described: it can be obtained by a 180° rotation of the substrate about the axis passing through the oxygen atom of the carbonyl group and the carbon atom of the double bond in the  $\beta$  position to the activating moiety. In this way, the oxygen atom, involved in hydrogen bonds with the amino acids of the binding pocket, and the  $\beta$  carbon atom, which has to be at a suitable distance and orientation from the flavin cofactor to allow H<sup>+</sup> transfer, maintain their position.

Recently,<sup>24</sup> a modification of the arrangement of the substrate in the enzyme active site induced by rotation of the alkenyl fragment on the C $\alpha$ –CO single bond has been hypothesised. This conformational change produces only a different orientation of the substrate in the so called “flipped” binding mode: the stereoheterotopic faces exposed to H<sup>+</sup> and H<sup>−</sup> addition are indeed the same. What is really relevant is that the switching between the two possible binding modes, either by flipping or by rotation of a fragment, causes an exchange of the stereoheterotopic faces of the alkene on which the addition of either H<sup>+</sup> and H<sup>−</sup> occurs, with formation of reduced products of opposite absolute configuration.<sup>†</sup>

It is clear that the stereochemical outcome of the reaction is controlled by the arrangement of the stereoheterotopic faces of the olefin towards hydride and proton delivery, and by the stereospecificity of the hydrogen addition step.<sup>22</sup> When two electron withdrawing groups are present on the double bond, the accommodation of the substrate within the catalytic site does not only depend on the steric and electronic effects of substituents, but also on which group acts effectively as the activating one.<sup>25</sup> Thus, for the cyanoesters of this study it was necessary to establish whether the ester or the cyano functionality was the real activating group making bioreduction feasible.

**Identification of the activating electron withdrawing group of cyanoesters 4 and 5.** The use of stoichiometric NADH and of deuterium oxide as a solvent promotes the incorporation of a hydrogen atom at C $\beta$  and of a deuterium atom at C $\alpha$  with respect to the activating group, *i.e.* the one which is in the active site of the enzyme. Derivatives (*E*)- and (*Z*)-**4a**, **4b**, **4e** and **5b** were subjected to OYE1-mediated biotransformations in D<sub>2</sub>O solution in the presence of a stoichiometric quantity of NADH.

<sup>†</sup> In the work<sup>11</sup> devoted to the investigation of unsaturated diesters 2 and 3, we have established by means of deuterium labelling that the different stereochemical outcome of their bioreduction was due to the fact that the proton was delivered, respectively, on the C $\alpha$ -*re* and C $\alpha$ -*si* face in substrates (*E*)-2 and (*Z*)-3.

Cyanoesters **4a** represented the reference compounds of this series, with the minimum steric hindrance (a methyl group) either on the double bond or at the ester moiety. The choice of other substrates was motivated by the observation that the reactions of the two diastereoisomers of compounds **4b** and **5b** afforded the same enantiomer of the corresponding reduced products, **6b** and **7b**, respectively, even though with different enantioselectivity values: (*S*)-**6b** was obtained either from (*E*)- or (*Z*)-**4b**; (*R*)-**7b** was the reduced product, starting either from (*E*)- or (*Z*)-**5b**. Compound **4e** was representative of the other substrates of type **4**, which gave opposite enantiomers upon reduction of the two starting alkene diastereoisomers.

The analysis of the <sup>1</sup>H NMR spectra of the saturated products obtained starting from (*E*)- and (*Z*)-**4a**, (*E*)- and (*Z*)-**4b**, (*E*)- and (*Z*)-**5b**, and (*E*)-**4e** highlighted that the ester moiety was indeed the activating group for these substrates. In each spectrum the intensity of the multiplet due to the hydrogen atom alpha to the ester group was very low, thus proving the delivery of a D<sup>+</sup> to this position, and, accordingly, the signal of the CH<sub>2</sub>CN fragment was reduced to an AB system.

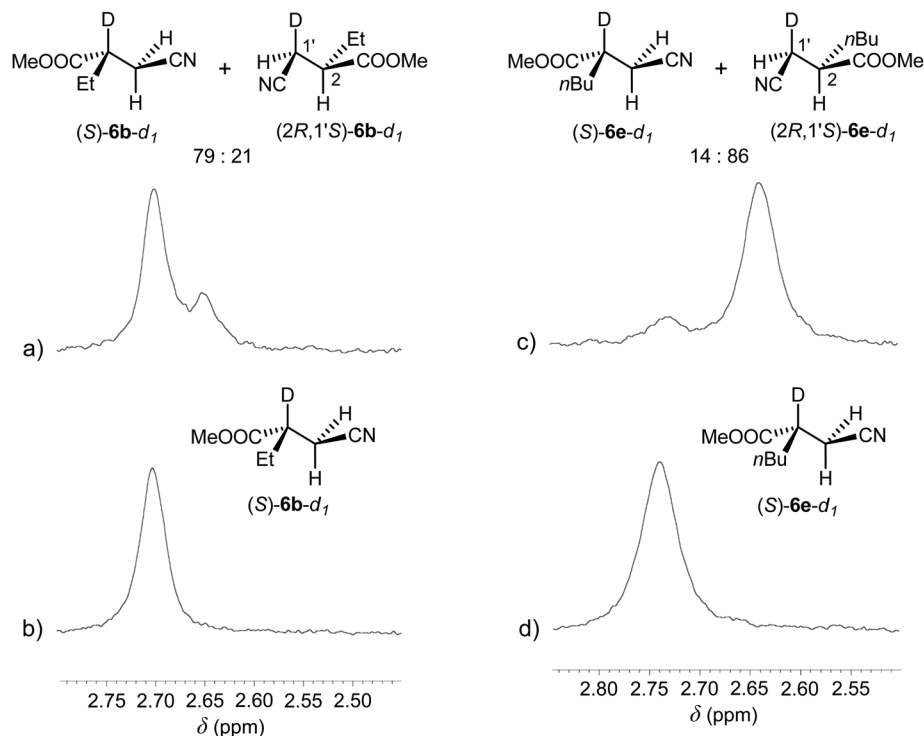
Only in the reduction of (*Z*)-**4e**, and at a decidedly minor extent in that of (*Z*)-**4b**, a reactive binding mode with the nitrile group acting as the activating functionality was deduced from the analysis of the <sup>2</sup>H NMR spectra of monodeuterated compounds.

In the case of (*Z*)-**4b**, (*S*)-**6b**-*d*<sub>1</sub> (ee = 58%) was obtained as the reduction product (*c* = 67%): a modest contribution of a binding mode characterised by the activation of the nitrile group was apparent from the corresponding <sup>2</sup>H NMR spectrum (Fig. 3a): besides the main broad singlet of CDCOOMe at 2.70 ppm, identical to that of the deuterium spectrum of (*S*)-**6b**-*d*<sub>1</sub> obtained from (*E*)-**4b** (Fig. 3b), a less intense signal due to CHDCN at 2.65 ppm was detected. The low enantioselectivity observed in the reduction of (*Z*)-**4b** was thus tentatively attributed to the concomitant realization of this alternative binding mode, affording (2*R*,1'*S*)-**6b**-*d*<sub>1</sub>. The configuration at C<sub>2</sub> in this compound was derived as a consequence of this assumption; that of C<sub>1'</sub> was determined by an *anti* reduction mechanism, and it was confirmed by further deuteration experiments (see the next section).

In the case of (*Z*)-**4e** the binding mode showing the nitrile group as the activating one was found to be the most reactive: in the <sup>1</sup>H NMR spectrum§ of the corresponding reduced product the signal of the hydrogen atom adjacent to the ester moiety resulted to be a quartet, as a consequence of the addition of a D<sup>+</sup> to the carbon atom in the  $\alpha$  position to the nitrile group. Derivative (2*R*,1'*S*)-**6e**-*d*<sub>1</sub> (ee = 72%) was recovered: the absolute configuration of C<sub>1'</sub> was determined by an *anti* reduction mechanism, which was later confirmed (see the next section). The <sup>2</sup>H NMR spectrum of this product (Fig. 3c) showed a main singlet at 2.64 ppm for CHDCN, but also a less intense signal at 2.73 ppm for CDCOOMe, identical to that of (*S*)-**6e**-*d*<sub>1</sub> (Fig. 3d). Thus, the decrease in enantioselectivity for the

§ The <sup>1</sup>H NMR spectra of the monodeuterated compounds obtained from (*Z*)-**4b** and (*Z*)-**4e** are reported as examples in ESI† as Fig. S1 and S2.





**Fig. 3** (a)  $^2\text{H}$  NMR spectra of the 79/21 mixture (determined by GC analysis on a chiral stationary phase) of  $(S)\text{-}6\text{b-d}_1$  and  $(2R,1'S)\text{-}6\text{b-d}_1$  obtained from  $(Z)\text{-}4\text{b}$ ; (b)  $^2\text{H}$  NMR spectra of  $(S)\text{-}6\text{b-d}_1$  obtained from  $(E)\text{-}4\text{b}$ ; (c)  $^2\text{H}$  NMR spectra of the 14/86 mixture (determined by GC analysis on a chiral stationary phase) of  $(S)\text{-}6\text{e-d}_1$  and  $(2R,1'S)\text{-}6\text{e-d}_1$  obtained from  $(Z)\text{-}4\text{e}$ ; (d)  $^2\text{H}$  NMR spectra of  $(S)\text{-}6\text{e-d}_1$  obtained from  $(E)\text{-}4\text{e}$ .

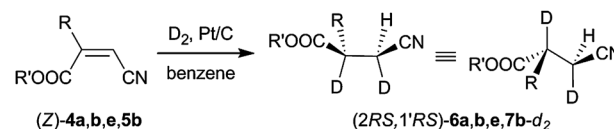
reduction of  $(Z)\text{-}4\text{e}$  was assumed to be produced by an alternative active binding conformation, affording  $(S)\text{-}6\text{e-d}_1$ .

**Assessment of the stereochemistry of hydrogen addition and considerations on the reactive binding modes of the substrates.** After the identification of the activating group, the stereospecificity of hydrogen addition has to be assessed, in order to understand the diverse stereochemical outcome of these bioreductions.

Thus, OYE1-mediated reduction of derivatives  $(E)\text{-}$  and  $(Z)\text{-}4\text{a}$ ,  $4\text{b}$ ,  $4\text{e}$  and  $5\text{b}$  was also performed in the presence of  $(R)\text{-NADPD}$  (regenerated *in situ* by alcohol dehydrogenase and *i*-PrOH- $d_8$ ) in  $\text{D}_2\text{O}$  solvent,<sup>26</sup> in order to incorporate two deuterium atoms. The  $^2\text{H}$  NMR spectra of the doubly deuterated reduced compounds were compared with those of the derivatives obtained upon reduction of the same starting substrates with deuterium gas in the presence of Pt/C in benzene solution.

Metal-catalysed *syn* addition of deuterium to  $(Z)\text{-}4\text{a}$ ,  $4\text{b}$ ,  $4\text{e}$  and  $5\text{b}$  afforded the  $(2RS,1'RS)$  stereoisomer of the corresponding reduced compounds  $6\text{a}$ ,  $6\text{b}$ ,  $6\text{e}$ , and  $7\text{b}$  (Scheme 4): with this relative configuration, the deuterium atom introduced in the  $\alpha$  position to the nitrile was the one characterised by the lowest chemical shift ( $\delta_{\text{D}} = 2.54, 2.55, 2.55, 2.53$  ppm, respectively, for  $6\text{a}$ ,  $6\text{b}$ ,  $6\text{e}$ , and  $7\text{b}$ ).

In Fig. 4a and d the  $^2\text{H}$  NMR spectra of  $(2RS,1'RS)\text{-}6\text{e-d}_2$  and  $(2RS,1'RS)\text{-}7\text{b-d}_2$  are reported. The relative configuration of the stereogenic centres created upon insertion of two deuterium atoms by OYE1 biotransformation of substrates  $(E)\text{-}$  and  $(Z)\text{-}4\text{a}$ ,  $4\text{b}$ ,  $4\text{e}$  and  $5\text{b}$  was obtained by analysis of their  $^2\text{H}$  NMR spectra, using as a reference the chemical shifts of the stereoisomer



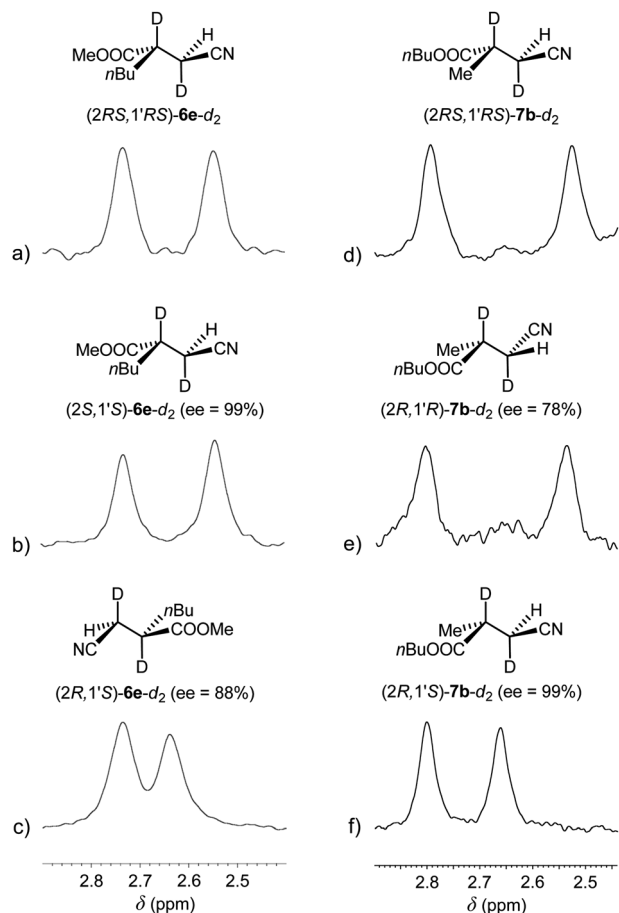
**Scheme 4** *syn* chemical double deuteration of  $(Z)$ -substrates.

produced by deuterium *syn* addition to the  $(Z)$ -double bond. The  $^2\text{H}$  NMR spectra of the doubly deuterated compounds recovered from the enzymatic reduction of both diastereoisomers of substrates  $4\text{e}$  and  $5\text{b}$  are reported in Fig. 4b, c and e, f, respectively, and compared with those of the chemically reduced products obtained from  $(Z)\text{-}4\text{e}$  and  $(Z)\text{-}5\text{b}$  (Fig. 4a and d).

$^2\text{H}$  NMR spectra showed that biocatalysed *anti* addition to  $(E)$ -alkenes gave the same stereoisomers recovered from chemical *syn* deuteration of  $(Z)$ -substrates. Furthermore, it was found that the deuterium atom introduced in the  $\alpha$  position to the nitrile by *anti* addition to  $(Z)$ -compounds was characterised by a higher chemical shift ( $\delta_{\text{D}} = 2.67, 2.64, 2.64, 2.66$  ppm, respectively, for  $6\text{a}$ ,  $6\text{b}$ ,  $6\text{e}$ , and  $7\text{b}$ ) than that inserted by *syn* deuteration. The latter information also confirmed the configuration of  $\text{C}_{1'}$  in compounds  $(2R,1'S)\text{-}6\text{b-d}_1$  and  $(2R,1'S)\text{-}6\text{e-d}_1$  depicted in Fig. 3.

A stereospecific *anti* addition mechanism was confirmed for all the four pairs of diastereoisomers.

The information on the absolute configuration of  $\text{C}_2$  and that on the activating group were combined, in order to establish the preferred reactive binding mode for the



**Fig. 4** (a) and (d)  $^2\text{H}$  NMR spectra of  $(2R,1'S)\text{-6e-d}_2$  and  $(2R,1'RS)\text{-7b-d}_2$  obtained from metal catalyzed deuterium *syn* addition to  $(Z)\text{-4e}$  and **5b**; (b) and (e)  $^2\text{H}$  NMR spectra of  $(2S,1'S)\text{-6e-d}_2$  and  $(2R,1'R)\text{-7b-d}_2$  obtained from enzymatic reduction of  $(E)\text{-4e}$  and **5b**; (c) and (f)  $^2\text{H}$  NMR spectra of  $(2R,1'S)\text{-6e-d}_2$  and  $(2R,1'S)\text{-7b-d}_2$  obtained from enzymatic reduction of  $(Z)\text{-4e}$  and **5b**.

investigated substrates. These conclusions are collected in Fig. 5: exclusive arrangements affording bioreductions with enantioselectivity values higher than 99% are highlighted with shaded boxes, and the predominant activating group is shown in bold on the left side.

The opposite enantioselectivity observed for the reduction of the two stereoisomers  $(E)\text{-}$  and  $(Z)\text{-4a}$  (Fig. 5a) could be attributed to a change in the preferred reactive binding mode of the two substrates, with delivery of  $\text{D}^+$  on the  $\text{C}_\alpha\text{-re}$  and  $\text{C}_\alpha\text{-si}$  face, respectively.

For both the stereoisomers  $(E)\text{-}$  and  $(Z)\text{-4b}$  (Fig. 5b) the stereochemical course could be justified by the same arrangement of the stereoheterotopic face of the alkene in the active site, with addition of  $\text{D}^+$  to the  $\text{C}_\alpha\text{-re}$  face. The low enantioselectivity of the bioreduction of  $(Z)\text{-4b}$  was attributed to the competition of a binding mode activated by the nitrile moiety, affording the enantiomer  $(2R,1'S)\text{-6b-d}_2$ , with addition of a  $\text{D}^+$  to the *si* face of the olefinic carbon atom linked to the CN group.

In the reduction of compound  $(E)\text{-4e}$  (Fig. 5c), addition of  $\text{D}^+$  on the  $\text{C}_\alpha\text{-re}$  face afforded  $(2S,1'S)\text{-6e-d}_2$  (ee = 99%).

The formation of  $(2R,1'S)\text{-6e-d}_2$  (ee = 88%) from  $(Z)\text{-4e}$  was explained by the fact that the nitrile group became the activating one, and the substrate was arranged in the active site in such a way that  $\text{D}^+$  was delivered on the *si* face of the olefinic carbon atom linked to the CN group. The non-exclusive formation of  $(2R,1'S)\text{-6e-d}_2$  was explained by the possibility of a binding mode being activated by the ester group with addition of  $\text{D}^+$  on the *re* face of the adjacent carbon atom, giving  $(2S,1'R)\text{-6e-d}_2$ .

For both substrates  $(E)\text{-}$  and  $(Z)\text{-5b}$  (Fig. 5d), the stereochemistry of the two bioreductions could be justified by delivery of  $\text{D}^+$  to the  $\text{C}_\alpha\text{-si}$  face, with formation of  $(2R,1'R)\text{-7b-d}_2$  (ee = 78%) and  $(2R,1'S)\text{-7b-d}_2$  (ee = 99%), respectively.

The non-exclusive formation of a single enantiomer in the bioreduction of  $(E)\text{-4a}$  and  $(E)\text{-5b}$  could be attributed neither to a binding mode activated by the nitrile nor to the contribution of *syn* addition which were not highlighted by  $^2\text{H}$  NMR analysis. Only a competition between the flipped and classical binding modes of the substrate could account for the formation of the minor enantiomer.

### General considerations on the stereochemical course of OYE-mediated reduction of unsaturated compounds

The data so far collected allowed us to provide some general considerations on the stereochemical course of the biocatalysed reduction of  $\alpha,\beta$ -unsaturated derivatives with a carbonyl or carboxyl moiety as the activating group and a substituent at  $\text{C}_\alpha$ , which is described to occur with high values of enantioselectivity. This analysis highlighted the structural requisites necessary for the optimal arrangement of these substrates in the binding site of OYE1-3 enzymes, which make them adopt a single preferred reactive conformation.

For unsaturated methyl esters **4**, showing an increasing steric hindrance at the carbon-carbon double bond, the most satisfactory values of conversion and enantioselectivity were obtained for the  $(E)$ -stereoisomers. The experimental information, gathered within this work on the stereochemistry of their reduction, showed that the  $\text{H}^+$  addition took place on the stereoheterotopic face of the olefin established by a flipped binding mode, obtained either by flipping of the whole substrate or by rotation of the alkene fragment (Table 2, entry 1). This binding mode is very likely to be preferred also by haloalkenoates  $(Z)\text{-1}$  (entry 2), affording  $(S)$ -enantiomers (ee = 94–99%),<sup>10</sup> unsaturated esters  $(E)\text{-2}$  (entry 3), converted into  $(S)$ -reduction products,<sup>11</sup>  $\alpha$ -methylenic methyl esters (entry 4), giving  $(R)$ -enantiomers (ee >99% with OYE1-3),<sup>7a-c</sup> and some dimethyl *N*-acylamino fumarates (entry 5), affording reduction products with  $(S)$ -configuration (ee >99% with OYE1-3).<sup>25</sup> The same flipped arrangement can be deduced for  $\alpha$ -alkylcinnamaldehydes (entry 6),<sup>27</sup> and substituted benzylacrylaldehydes (entry 7),<sup>28</sup> affording  $(S)$ - and  $(R)$ -enantiomers, respectively.

As for cyanoesters **5**, the bioreduction of  $(Z)$ -stereoisomers afforded the highest values of enantioselectivity. The stereochemical analysis, herein performed for these substrates, highlighted  $\text{H}^+$  addition on the stereoheterotopic face established by a classical binding mode (entry 8). This arrangement was

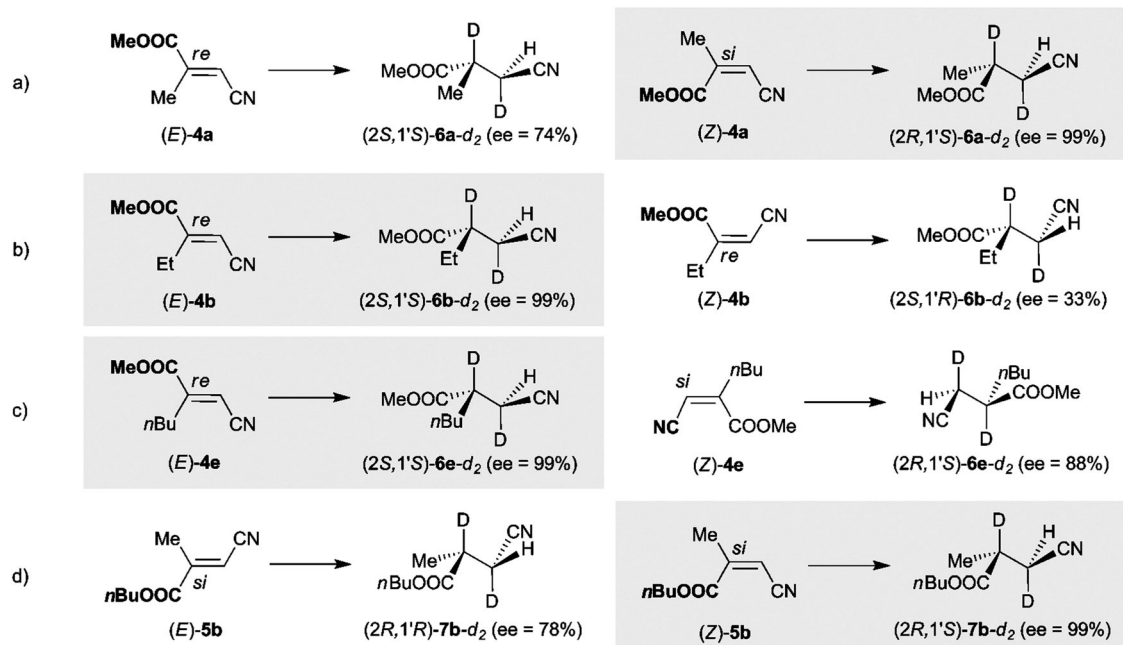


Fig. 5 Identification of the reactive binding modes of the investigated substrates.

Table 2 Reactive arrangements of optimal substrates for ene-reductases and the suggested empirical model for their rationalisation

Entry	Arrangement	G	$\beta^1$	$\beta^2$	$\alpha$	Empirical model
1	 $\beta_1$ $\beta_2$ $\alpha$ <i>flipped</i>	OMe	H	CN	R, Ar	
2		OMe	H	R, Ar	Cl, Br	
3		OMe	H	COOMe	R, Ar	
4		OMe	H	H	CH <sub>2</sub> OR	
5		OMe	H	COOMe	NHCOR	
6		H	H	Ar	R	
7		H	H	H	ArCH <sub>2</sub>	
8	 $\alpha$ $\beta_1$ $\beta_2$ $\alpha$ $\beta_1$ $\beta_2$ <i>classical</i>	OR	CN	H	Me	
9		OR	COOR	H	Me	
10		CH <sub>2</sub> CH <sub>2</sub>	CH <sub>2</sub>	H	Me	

found to be the preferred one also for unsaturated esters (*Z*)-3, transformed into (*R*)-reduction compounds (entry 9),<sup>11</sup> and for 2-methyl-2-cyclohexenone (entry 10),<sup>9,23a</sup> converted into the (*R*)-enantiomer of the reduced product.

For all these compounds, which are optimal substrates for OYE1-3 enzymes, with a carbonyl or carboxyl moiety as the activating group and a substituent at  $C_\alpha$ , the preferred reactive binding mode seems to be determined by the relative size of the groups labelled G and  $\alpha$  (Table 2): if G has a modest steric hindrance (S, small), and  $\alpha$  is bulky or more hindered (L, large), then a *flipped binding mode* is preferred; if G is large, and  $\alpha$  is small, then a *classical binding mode* is adopted.

When (*E*)- and (*Z*)-derivatives are compared, one diastereoisomer is usually found to be more suitable for bioreduction than the other one: for a flipped binding mode the favoured stereochemistry of the double bond is the one with the smallest

group at  $C_\beta$  on the same side of the activating group; for a classical binding mode the favoured stereochemistry of the double bond is the one with the smallest group at  $C_\beta$  on the same side of the substituent at  $C_\alpha$  (Table 2).

If the stereochemistry of the starting alkene is not the optimal one for the particular binding mode which is induced by the bulkiness of G and  $\alpha$ , an exclusive arrangement within the active site cannot be achieved and enantioselectivity decreases.

In the case of (*E*)- and (*Z*)-4a, and of methyl mesaconate and citraconate (diesters of type 3), G and  $\alpha$  are a methyl and a methoxy group of similar dimensions. A flipped binding mode is assumed by (*E*)-4a and by methyl mesaconate, and a classical binding mode by the corresponding (*Z*)-stereoisomers.

As for compounds (*Z*)-4 and (*E*)-5, which were found to be less favourable substrates for ER-mediated reductions than the



corresponding diastereoisomers, the spatial disposition of substituents prevented them from finding an exclusive optimal binding mode, of the types described in Table 2, and their bioreduction proceeded with modest yields and enantioselectivity. The competition between two binding modes assumed by (*Z*)-**4b** and **4e**, and by (*E*)-**5b** resulted in a less efficacious control on the efficiency and the stereochemistry of the bioreduction.

## Conclusions

The investigation of the effects of substituents on the activation of C=C double bonds towards bioreduction is still of great relevance, with the aim of broadening and better understanding the applicability of the reaction in the field of preparative synthetic chemistry. The experimental data collected in this work have shown that  $\beta$ -cyano- $\alpha,\beta$ -unsaturated esters are useful substrates for ER-mediated reactions. The cyano derivatives obtained upon reduction can be easily converted into valuable  $\gamma^2$ -amino acids by simple manipulation of the nitrile functionality, without altering the absolute configuration of the molecule. This fact highlights the significance of this bioreduction from the practical synthetic point of view.

The stereochemical course of these biotransformations has been analysed carefully, providing a wealth of information about possible stereocontrol strategies. The reduction of cyanoesters **4** is favoured when the double bond has an (*E*)-configuration, and it affords the (*S*)-enantiomers of the corresponding saturated products. The reaction does not take place when the alkyl chain in the  $\alpha$  position contains a branched carbon atom. The increase of the steric hindrance of the alkyl residue of the ester moiety has a different effect: the (*Z*)-stereoisomer becomes the best substrate, and the same (*R*)-enantiomer is obtained starting from either stereoisomer. These results further support the idea, already expressed in recent literature,<sup>4,10,22,26</sup> that the stereochemistry of bioreductions can be controlled by substrate engineering with great efficacy.

The work also suggests a rationalization of the stereochemical outcomes of highly enantioselective bioreduction of activated unsaturated compounds having a carbonyl or a carboxyl group as the activating moiety and a substituent at  $C_{\alpha}$ . These substrates are characterized by the exclusive preference for a certain reactive arrangement within the enzyme active site, which can be explained by using an empirical model. This model takes into consideration both the bulkiness of the groups linked to the C=O moiety and to  $C_{\alpha}$ , and the stereochemistry of the double bond. Up to now, the effects due to the steric hindrance of substituents and to the configuration of the alkene have been considered as affecting separately the course of the bioreductions. This model shows that they are strictly interconnected: each binding mode shows a favoured double bond configuration related to the occupancy of the whole enzyme active site. Useful indications can be obtained, to drive the selection of optimal substrates for ER-mediated reactions, and to exploit all the effects of substrate-control on the stereochemical outcome of the reactions.

## Experimental section

### General procedure for the OYE-mediated bioreduction

The substrate (5  $\mu$ mol) dissolved in DMSO (10  $\mu$ L) was added to a KPi buffer solution (1.0 mL, 50 mM, pH 7.0) containing glucose (20  $\mu$ mol), NADP<sup>+</sup> (0.1  $\mu$ mol), GDH (4 U) and the required OYE (*ca.* 40  $\mu$ g dissolved in 100–200  $\mu$ L of H<sub>2</sub>O). The mixture was incubated for 24 h in an orbital shaker (160 rpm, 30 °C). The solution was extracted with EtOAc (2  $\times$  250  $\mu$ L), centrifuged after each extraction (15 000 g, 1.5 min), and the combined organic solutions were dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>.

### Bioreduction procedure for the preparation of monodeuterated samples

The substrate (50  $\mu$ mol) dissolved in *i*-PrOH (100  $\mu$ L) was added to a KPi buffer solution (5.0 mL, 50 mM in D<sub>2</sub>O, pH 7.0) containing glucose (20  $\mu$ mol), NADH (75  $\mu$ mol) and the required OYE (*ca.* 250  $\mu$ g, dissolved in 500–700  $\mu$ L of H<sub>2</sub>O). The mixture was incubated for 24 h in an orbital shaker (160 rpm, 30 °C). The solution was extracted with EtOAc (3  $\times$  5.0 mL), centrifuged after each extraction (3000 g, 1.5 min), and the combined organic solutions were dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>.

### Bioreduction procedure for the preparation of doubly deuterated samples

The substrate (50  $\mu$ mol) dissolved in *i*-PrOH-*d*<sub>8</sub> (100  $\mu$ L) was added to a KPi buffer solution (5.0 mL, 50 mM in D<sub>2</sub>O, pH 7.0) containing NADP<sup>+</sup> (15  $\mu$ mol), TBADH (4 U, 3 mg) and the required OYE (*ca.* 250  $\mu$ g, dissolved in 500–700  $\mu$ L of H<sub>2</sub>O). The mixture was incubated for 24 h in an orbital shaker (160 rpm, 30 °C). The solution was extracted with EtOAc (3  $\times$  5.0 mL), centrifuged after each extraction (3000 g, 1.5 min), and the combined organic solutions were dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>.

### General procedure for baker's yeast fermentation

A mixture of baker's yeast (100 g) and D-glucose (40 g) in tap water (1 L) was prepared. After 10 min stirring at 30 °C the suitable cyanoester (3.0 g) adsorbed on a hydrophobic resin (60 g, polystyrene XAD-1180N) was added in one portion. The mixture was kept under stirring for 72 h at r.t. and then filtered on a cotton plug. The collected mass was washed with tap water repeatedly, to remove most of the cells. The resin was then collected and extracted twice in sequence with acetone (200 mL) and ethyl acetate (200 mL). The organic phase was concentrated to 1/3 and then washed with brine. The residue obtained upon evaporation of the dried (Na<sub>2</sub>SO<sub>4</sub>) extract was chromatographed on a silica gel column with increasing amounts of ethyl acetate in hexane.

### Synthesis of the $\gamma^2$ -aminoacid derivative: (*S*)-methyl 4-(*t*-butoxycarbonylamino)-2-ethylbutanoate [(*S*)-**8**]

To a stirring solution of (*S*)-**6b** (ee = 99%, 0.340 g, 2.4 mmol) in MeOH (30 mL) Boc<sub>2</sub>O (1.05 g, 4.8 mmol) and a catalytic amount of NiCl<sub>2</sub>·6H<sub>2</sub>O were first added; then, at 0 °C, NaBH<sub>4</sub> (0.64 g, 16.8 mmol) was added portionwise over 30 min.

† GC methods are reported in ESI.†

The reaction mixture was stirred for 24 h. After the usual workup, column chromatography eluting with hexane and an increasing amount of ethyl acetate gave compound (*S*)-**8** (0.41 g, 70%): ee = 99%,  $[\alpha]_D^{20} + 16.5$  (c 1.96 in CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ = 3.66 (3H, s, COOCH<sub>3</sub>), 3.09 (2H, m, CH<sub>2</sub>NH), 2.31 (1H, m, CHCOOCH<sub>3</sub>), 1.80–1.45 (4H, m, 2CH<sub>2</sub>), 1.41 (9H, s, (CH<sub>3</sub>)<sub>3</sub>C), 0.86 (3H, t, *J* = 7.4 Hz, CH<sub>3</sub>); <sup>13</sup>C NMR (100.6 MHz, CDCl<sub>3</sub>) δ = 176.1, 155.8, 79.1, 51.4, 44.6, 38.8, 32.0, 28.4, 25.3, 11.5; GC-MS (EI) *t*<sub>R</sub> = 20.0 min: *m/z* (%) = 189 (*M*<sup>+</sup> – 56, 7), 172 (13), 144 (27), 57 (100).

## Acknowledgements

Prof. Claudio Fuganti and Prof. Giovanni Fronza are warmly acknowledged for fruitful discussions. Prof. Neil C. Bruce (Department of Biology, University of York) is kindly acknowledged for the gift of plasmid pT7-OYE1. Prof. Sven Panke and Christian Femmer (ETH Zürich Department of Biosystems Science and Engineering, Basel) are kindly acknowledged for the help provided in the preparation of the plasmids and the overexpressing strains.

## References and notes

- C. M. Clouthier and J. N. Pelletier, *Chem. Soc. Rev.*, 2012, **41**, 1585–1605; E. Brenna, C. Fuganti, F. G. Gatti and S. Serra, *Chem. Rev.*, 2011, **111**, 4036–4072.
- D. J. Pollard and J. M. Woodley, *Trends Biotechnol.*, 2006, **25**, 66–73.
- O. Warburg and W. Christian, *Biochem. Z.*, 1933, **266**, 377–411.
- (a) M. Hall and A. S. Bommarius, *Chem. Rev.*, 2011, **111**, 4088–4110; (b) M. Hall, C. K. Winkler, G. Tasnádi and K. Faber, in *Practical Methods for Biocatalysis and Biotransformations Vol. 2*, ed. J. Whittall and P. W. Sutton and J. Wiley & Sons, 2012, ch. 3.1, pp. 87–95; (c) E. Brenna, F. G. Gatti and F. Parmeggiani, in *Practical Methods for Biocatalysis and Biotransformations Vol. 2*, ed. J. Whittall and P. W. Sutton and J. Wiley & Sons, 2012, ch. 3.2, pp. 96–99; (d) H. Toogood, J. M. Gardiner and N. S. Scrutton, *ChemCatChem*, 2010, **2**, 892–914.
- D. Mangan, I. Miskelly and T. S. Moody, *Adv. Synth. Catal.*, 2012, **354**, 2185–2190.
- (a) K. M. Fox and P. A. Karplus, *Structure*, 1994, **2**, 1089–1105; (b) B. J. Brown, Z. Deng, P. A. Karplus and V. Massey, *J. Biol. Chem.*, 1998, **273**, 32753–32762; (c) R. M. Kohli and V. Massey, *J. Biol. Chem.*, 1998, **273**, 32763–32770.
- (a) C. Stueckler, C. K. Winkler, M. Bonnekessel and K. Faber, *Adv. Synth. Catal.*, 2010, **352**, 2663–2666; (b) A. Z. Walton, W. C. Conerly, Y. Pompeu, B. Sullivan and J. D. Stewart, *ACS Catal.*, 2011, **1**, 989–993; (c) Y. A. Pompeu, B. Sullivan, A. Z. Walton and J. D. Stewart, *Adv. Synth. Catal.*, 2012, **354**, 1949–1960; (d) G. Tasnádi, C. K. Winkler, D. Clay, N. Sultana, W. M. F. Fabian, M. Hall, K. Dittrich and K. Faber, *Chem.–Eur. J.*, 2012, **18**, 10362–10367.
- M. Utaka, S. Konishi, T. Ohkubo, S. Tsuboi and A. Takeda, *Tetrahedron Lett.*, 1987, **28**, 1447; M. Utaka, S. Konishi, A. Mizouka, T. Ohkubo, T. Sakai, S. Tsuboi and A. Takeda, *J. Org. Chem.*, 1989, **54**, 4989–4992.
- M. Hall, C. Stueckler, B. Hauer, R. Stuermer, T. Friedrich, M. Breuer, W. Kroutil and K. Faber, *Eur. J. Org. Chem.*, 2008, 1511–1516.
- E. Brenna, G. Fronza, C. Fuganti, D. Monti and F. Parmeggiani, *J. Mol. Catal. B: Enzym.*, 2011, **73**, 17–21; E. Brenna, F. G. Gatti, A. Manfredi, D. Monti and F. Parmeggiani, *Eur. J. Org. Chem.*, 2011, 4015–4022; E. Brenna, F. G. Gatti, A. Manfredi, D. Monti and F. Parmeggiani, *Org. Process Res. Dev.*, 2012, **16**, 262–268.
- E. Brenna, F. G. Gatti, A. Manfredi, D. Monti and F. Parmeggiani, *Adv. Synth. Catal.*, 2012, **354**, 2859–2864.
- A. J. Smallridge, A. Ten and M. A. Trehwella, *Tetrahedron Lett.*, 1998, **39**, 5121–5124; B. Kosjek, F. J. Fleitz, P. G. Dormer, J. T. Kuethe and P. D. Devine, *Tetrahedron: Asymmetry*, 2008, **19**, 1403–1406; A. Fryszkowska, K. Fisher, J. M. Gardiner and G. M. Stephens, *Org. Biomol. Chem.*, 2010, **8**, 533–535; WO 2012/02586 A1.
- J. S. Bryans and D. J. Wustrow, *Med. Res. Rev.*, 1999, **19**, 149; G. L. Sammins and E. N. Jacobsen, *J. Am. Chem. Soc.*, 2003, **125**, 4442; S. L. Poe, M. Kobašljia and T. D. McQuade, *J. Am. Chem. Soc.*, 2007, **129**, 9216; L. S. Zu, H. X. Xie, H. Li, J. Wang and W. Wang, *Adv. Synth. Catal.*, 2007, **349**, 2660.
- S. Hanessian, X. Luo, R. Schaum and S. Michnick, *J. Am. Chem. Soc.*, 1998, **120**, 8569; T. Hintermann, K. Gademann, B. Jaun and D. Seebach, *Helv. Chim. Acta*, 1998, **81**, 983; M. G. Woll, J. R. Lai, I. A. Guzei, S. J. C. Taylor, M. E. B. Smith and S. H. Gellman, *J. Am. Chem. Soc.*, 2001, **123**, 11077; D. Seebach, M. Brenner, M. Rueping and B. Jaun, *Chem.–Eur. J.*, 2002, **8**, 573.
- A. Hayen, M. A. Schmitt, F. N. Ngassa, K. A. Thomasson and S. H. Gellman, *Angew. Chem., Int. Ed.*, 2004, **43**, 505; M. Hagihara, N. J. Anthony, T. J. Stout, J. Clardy and S. L. Schreiber, *J. Am. Chem. Soc.*, 1992, **114**, 6568; P. G. Vasudev, K. Ananda, S. Chatterjee, S. Aravinda, N. Shamala and P. Balaram, *J. Am. Chem. Soc.*, 2007, **129**, 4039.
- M. Ordóñez and C. Cativiela, *Tetrahedron: Asymmetry*, 2007, **18**, 3–99.
- (a) R. K. Duke, M. Chebib, D. E. Hibbs, K. N. Mewett and G. A. R. Johnston, *Tetrahedron: Asymmetry*, 2004, **15**, 1745–1751; (b) P. Camps, D. Muñoz-Torrero and L. Sánchez, *Tetrahedron: Asymmetry*, 2004, **15**, 311–321; (c) P. Camps, S. Giménez, M. Font-Bardia and X. Solans, *Tetrahedron: Asymmetry*, 1995, **6**, 985–990; (d) P. Camps, F. Pérez and N. Soldevilla, *Tetrahedron Lett.*, 1999, **40**, 6853–6856.
- S. Azam, A. A. D'Souza and P. B. Wyatt, *J. Chem. Soc., Perkin Trans. 1*, 1996, 621–627; T. R. Bellioti, T. Capiris, I. V. Ekhatu, J. J. Kinsora, M. J. Field, T. G. Heffner, L. T. Meltzer, J. B. Schwars, C. P. Taylor, A. J. Thorpe,

- M. G. Vartanian, L. D. Wise, T. Zhi-Su, M. L. Weber and D. J. Wustrow, *J. Med. Chem.*, 2005, **48**, 2294–2307; D. A. Evans, J. R. Gage, J. L. Leighton and A. S. Kim, *J. Org. Chem.*, 1992, **57**, 1961–1963; D. A. Evans, J. R. Gage and J. L. Leighton, *J. Org. Chem.*, 1992, **57**, 1964–1966; D. A. Evans and J. R. Gage, *J. Org. Chem.*, 1992, **57**, 1958–1961; A. B. Smith III, B. A. Salvatore, K. G. Hull and J. J.-W. Duan, *Tetrahedron Lett.*, 1991, **32**, 4859–4862.
- 19 Y. Chi, L. Guo, N. K. Kopf and S. Gellman, *J. Am. Chem. Soc.*, 2008, **130**, 5608–5609.
- 20 C. J. Martin, D. J. Rawson and J. M. J. Williams, *Tetrahedron: Asymmetry*, 1998, **9**, 3723–3730.
- 21 C. Mazzini, J. Lebreton, V. Alphand and R. Furstoss, *Tetrahedron Lett.*, 1997, **38**, 1195–1196; E. Forró and F. Fülöp, *Eur. J. Org. Chem.*, 2008, 5263–5268; M. Winkler, A. C. Knall, M. R. Kulterer and N. Klempier, *J. Org. Chem.*, 2007, **72**, 7423–7426; A. Fryszkowska, K. Fisher, J. M. Gardiner and G. M. Stephens, *Org. Biomol. Chem.*, 2010, **8**, 533–535.
- 22 E. Brenna, F. G. Gatti, D. Monti, F. Parmeggiani and S. Serra, *Adv. Synth. Catal.*, 2012, **354**, 105–112.
- 23 (a) S. K. Padhi, D. J. Bougioukou and J. D. Stewart, *J. Am. Chem. Soc.*, 2009, **131**, 3271–3280; (b) A. Müller, B. Hauer and B. Rosche, *Biotechnol. Bioeng.*, 2007, **98**, 22–29; (c) H. S. Toogood, A. Fryszkowska, V. Hare, K. Fisher, A. Roujeinikova, D. Leys, J. M. Gardiner, G. M. Stephens and N. S. Scrutton, *Adv. Synth. Catal.*, 2008, **350**, 2789–2803.
- 24 G. Oberdorfer, K. Gruber, K. Faber and M. Hall, *Synlett*, 2012, 1857–1864.
- 25 C. Stueckler, C. K. Winkler, M. Hall, B. Hauer, M. Bonnekessel, K. Zangger and K. Faber, *Adv. Synth. Catal.*, 2011, **353**, 1169–1173.
- 26 D. J. Bougioukou and J. D. Stewart, *J. Am. Chem. Soc.*, 2008, **130**, 7655–7658.
- 27 C. Stueckler, N. J. Mueller, C. K. Winkler, S. M. Glueck, K. Gruber, G. Steinkellner and K. Faber, *Dalton Trans.*, 2010, **39**, 8472–8476; E. Brenna, G. Fronza, C. Fuganti and F. G. Gatti, *Eur. J. Org. Chem.*, 2010, 5077–5084; E. Brenna, F. G. Gatti, D. Monti, F. Parmeggiani and A. Sacchetti, *Chem. Commun.*, 2012, **48**, 79–81; E. Brenna, F. G. Gatti, D. Monti, F. Parmeggiani and A. Sacchetti, *ChemCatChem*, 2012, **4**, 653–659.
- 28 E. Brenna, G. Fronza, C. Fuganti, F. G. Gatti, A. Manfredi, F. Parmeggiani and P. Ronchi, *J. Mol. Catal. B: Enzym.*, 2012, **84**, 94–101.