A short, chemoenzymatic route to chiral β-aryl-γ-amino acids using reductases from anaerobic bacteria†‡

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A short chemoenzymatic synthesis of β-aryl-γ-aminobutyric acids has been developed, based on a highly enantioselective biocatalytic reduction of β-aryl-β-cyano-α,β-unsaturated carboxylic acids.

Chiral γ-amino acids and their derivatives have been the subject of intense investigation because they can be used to treat a range of central nervous system disorders.[1] Moreover, these compounds are important building blocks in organic synthesis, they occur in biologically active natural products[2] and they are known to form γ-peptidomimetics.[3] This has led to an increased demand for provision of these compounds in an enantiopure form.[1] Various synthetic approaches towards the asymmetric synthesis of chiral γ-amino acids have been proposed,[1] but they tend to involve a large number of reaction steps or lack environmental acceptability.

Here, we report a new asymmetric methodology for the preparation of γ-amino butyric acid (GABA) derivatives (Scheme 1). The key advance is the use of an enzymatic step for asymmetric synthesis of (*S*)-β-aryl-β-cyano-propanoates (**3**), as an efficient and environmentally friendly alternative to classical catalysis. The usefulness of this approach is demonstrated by a short synthesis of the (*S*)-enantiomer of baclofen (**7**, >99% *ee*, 43% overall yield from **2** for 4 steps), which is a selective $GABA_B$ receptor agonist.[1]

Existing enzymatic methods for the production of γ-amino acids are based on kinetic resolution of GABA or their precursors by hydrolases, which by definition leads to a maximum product yield of 50%.[4] In contrast, biocatalytic asymmetric reduction of C=C bonds of activated alkenes allows synthesis of the respective products with up to two new stereogenic centres in a highly stereoselective fashion in quantitative yields.[5] Various reductases have been successfully employed for the asymmetric bioreduction of α,β-unsaturated carboxylic acids, aldehydes, ketones, maleimides, nitriles and nitroalkenes.[5–8] Unlike classical catalysts, 'ene' reductases exhibit high chemoselectivity for the reduction of C=C bonds *versus* other unsaturated bonds (*e.g.* C=O, C≡N).[5,6] Thus, we envisaged that the reductive enzymes could be employed for the synthesis of chiral precursors of γ-amino acids.

Based on the retrosynthetic analysis of β-substituted GABA derivatives, we decided to use β-aryl-β-cyano-α,β-unsaturated carboxylic acid salts (**2**) as substrates for their synthesis, as depicted in Scheme 1. Thus, β-aryl-β-cyano-propanoic acid potassium salts **2a–d** were used to screen for enzymatic activity. These substrates were synthesised conveniently in a condensation reaction of the respective arylacetonitriles (**1a–d**) with glyoxalic acid, catalysed by potassium carbonate in 32–75% yield (Scheme 1).

Initially, we tested two typical reductases from the Old Yellow Enzyme (OYE) family, namely pentaerythritol tetranitrate reductase from *Enterobacter cloacae*[8] and thermophilic old yellow enzyme from *Thermoanaerobacter pseudethanolicus*,[9] for activity towards cinnamic acid derivatives and their esters. Although the purified OYEs have proved to be useful biocatalysts for the reduction of a wide spectrum of activated α,β-unsaturated alkenes,[5–7] the enzymes we tested did not exhibit activity towards cinnamic acid derivatives and their esters (data not shown).[8,9]

A number of anaerobic bacteria are known to possess cinnamic acid reductase activity, including *Clostridium sporogenes* (DSM 795),[10] *Ruminococcus productus* (DSM 3507)[11] and *Acetobacterium woodii* (DSM 1030),[12] although the enzymes catalysing the reaction have not been purified and characterised. Therefore, we prepared crude extracts of these organisms[13] and tested them for NADH-dependent asymmetric reduction of 3-aryl-3-cyano-propenoic acid derivatives **2** (Table 1).[14]

All three crude extracts catalysed formation of the respective (*S*)-β-aryl-β-cyanopropanoic acids **3** in quantitative yields and very good enantioselectivities (Table 1). The absolute configuration of the product (*S*)-**3c** was assigned by comparing the sign of the optical rotation of its methyl ester **5c** with literature data.[1b] The configurations of other derivatives **5** were assigned as (*S*) based on circular dichroism spectra, which showed the same negative Cotton effect around 256 nm for all the cyanopropanoates **5a–d** (for details see Supplementary Information‡).

The data in Table 1 were recorded after 3 days. While propanoate **3d** was obtained in >99% *ee* with all the crude extracts, the optical purities of the products **3a–c** were slightly lower and decreased with time.[15] The loss of enantiopurity of **3a–c** should be attributed to racemisation of these products under the reaction conditions, due to the acidity of the proton at the newly formed stereogenic centre, adjacent to the electron withdrawing group.[8] In contrast, the electron donating character of the methoxy group of propanoate **3d** might have resulted in diminished acidity of the proton at C3, hence its high enantiopurity. It has to be noted that the racemisation could not account for the low optical purity of

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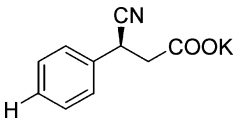
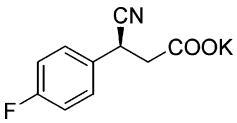
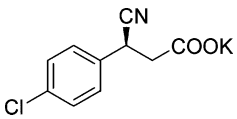
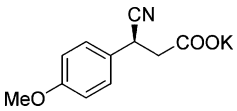
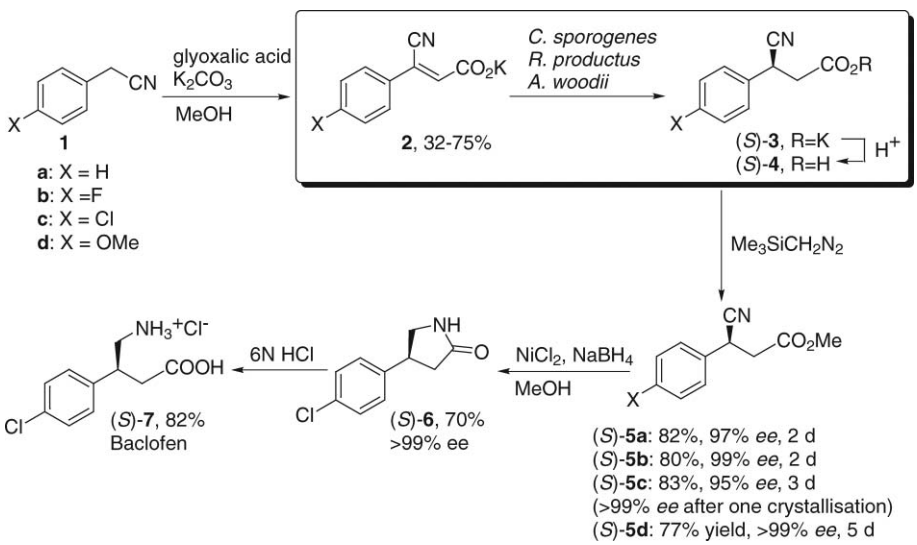
‡ Electronic supplementary information (ESI) available: Experimental details, anaerobic methodology, cell growth, circular dichroism and full characterisation data and copies of the ^1H and ^{13}C NMR spectra for the prepared compounds. See DOI: 10.1039/b919526b

Table 1 Enantioselective reduction of 3-cyano-3-aryl-propenoic acid salts (**2a–d**) by crude extracts from *C. sporogenes*, *A. woodii* and *R. productus*[14]

Substrate	Product		*C. sporogenes*[13]		*A. woodii*[13]		*R. productus*[13]	
			Conf. (*ee*)	Conv. (%)	Conf. (*ee*)	Conv. (%)	Conf. (*ee*)	Conv. (%)
2a	**3a**		*S* (93%)	>99	*S* (92%)	>99	*S* (94%)	>99
2b	**3b**		*S* (98%)	>99	*S* (57%)	>99	*S* (93%)	>99
2c	**3c**		*S* (96%)	95	*S* (89%)	95	*S* (91%)	94
2d	**3d**		*S* (≥99%)	94	*S* (≥99%)	95	*S* (≥99%)	93



Scheme 1 Chemoenzymatic asymmetric synthesis of β-aryl-γ-amino acids.

3b (57% *ee*) formed using extracts of *A. woodii*, and this suggests that this enzyme preparation exhibits poor stereoselectivity for this substrate.

Preparative scale biotransformations (0.7-1.0 mmol) were performed using the *C. sporogenes* crude extract, because it gave the best enantioselectivities (Table 1). We have previously reported that crude extracts from *C. sporogenes* exhibit a broad pH optimum for 'ene' reductase activity, giving similar yields and conversions at pH 7.0–8.5.[16] Therefore, all the reactions were performed at pH 7.0 to minimise the racemisation of the product under aqueous reaction conditions. The reactions were continued until the substrates **2** had been fully converted (see Supplementary Information), and the resulting products **3** were converted into their respective acids, methylated as before and purified to give products **5** in good overall yields (77–83%) and high enantioselectivities (95–≥99% *ee*, Scheme 1). Single recrystallisation of (*S*)-**5c** (95% *ee*) resulted in an enantiopure compound (≥99% *ee*, 90% yield) which was used in further synthesis reactions.

In order to demonstrate the synthetic utility of this approach, we sought to apply it to the synthesis of a chiral pharmaceutical compound. For this purpose, a highly enantioselective synthesis of β-(4′-chlorophenyl)-γ-GABA (**7**) serves as an example (Scheme 1). The reduction of the nitrile group to the respective amine using nickel borohydride, with subsequent ring formation, produced the γ-lactam (*S*)-**6** in 70% yield with no loss of the optical purity, as described previously.[1b] This was converted in one step to (*S*)-baclofen (**7**) in high yield (82%), according to an established procedure.[1] Hence, the applicability of this chemoenzymatic approach for

the efficient synthesis of an illustrative pharmaceutically relevant compound has been demonstrated. Our methodology should be applicable in the synthesis of a range of analogous enantiopure lactams and γ-amino acids.

Among the anaerobic microorganisms tested for cinnamate reductase activity in this study, none catalysed the formation of the (*R*)-enantiomer. The opposite stereochemical course of the reaction could be obtained by identifying an enzyme/microorganism that catalyses the formation of the opposite enantiomer[5–7] or, as recently demonstrated, stereocomplementary variants of the enzyme can be obtained by a protein engineering strategy.[17] Screening for the organisms and enzymes possessing opposite stereoselectivity, as well as studies on the extended substrate specificity, are ongoing in our laboratory.

Conclusions

We have developed a short, high-yielding preparative method for the synthesis of chiral β-aryl-β-cyano-propanoic acid derivatives with high optical purity. This is also the first example of chemoselective and highly enantioselective reduction of β-aryl-β-cyano-propanoic acid derivatives (up to >99% *ee*) using organisms possessing 'ene' reductase activity. Our approach can be applied to the synthesis of β-aryl substituted γ-aminobutyric acid derivatives, like baclofen **7**, in good overall yields and high enantiopurity. The synthetic strategy is convenient and much shorter than many other synthetic methods for the production of GABA derivatives.[1]

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13 Total protein concentration of crude extracts was determined to be: for *C. sporogenes*: 36 mg mL^{-1}; for *Acetobacterium woodii*: 10.7 mg mL^{-1} and for *Ruminococcus productus*: 5.8 mg mL^{-1}.

14 **Typical experimental procedure for analytical assays:** salt **2** (0.09 mmol), NADH (0.10 mmol, 76 mg), crude extracts of *C. sporogenes*, *R. productus* and *A. woodii* (1 mL), phosphate buffer (20 mL, 50 mM, pH 7.0) were incubated under H_2 atmosphere at 30 °C for 3 days. The reactions were monitored by GC (conversion) and HPLC (*ee*, Chiralcel OD), after acidification and derivatization of the product and unreacted substrate to their respective methyl esters **5**, using a (trimethylsilyl)diazomethane solution (2 M in hexanes). For 0.7–1 mmol scale experiments, all the reaction components were used proportionally.

15 After prolonged incubation (up to 6 days) the products **3a–c** were seen to undergo racemisation at a rate of approximately 1–2% *ee* loss per day at pH 7.0 (see Supplementary Information, Table S1).

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