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Stereoselective anti-Prelog reduction of ketones by whole cells of *Comamonas testosteroni* in a 'substrate-coupled' approach

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ABSTRACT

Lyophilized cells of the open accessible bacterium *Comamonas testosteroni* DSM 1455 proved to be an excellent catalyst for the asymmetric reduction of different α-azido, α-bromo, and α-nitro ketones at elevated substrate concentrations (16 g/L) in a 'substrate-coupled' approach using 20% (v/v) of 2-propanol as hydrogen donor. Excellent anti-Prelog stereoselectivity was obtained, which is less common found in nature.

1. Introduction

The β-aryl ethanolamine structural motif (Fig. 1) occurs frequently in nature and has been exploited by pharmaceutical companies in the development of compounds for combating high blood pressure, hypertension, arrhythmia, asthma and angina. For example, KUR-1246 (**1**) is a highly active and selective β_2-adrenoceptor agonist arresting preterm labor in pregnant women without serious cardiovascular side effects induced by β_1-adrenoceptor stimulation [1]. Denopamine (**2**) is a relatively new β_1-receptor agonist which is effective in the treatment of congestive heart failure. It can be administered orally and has reduced toxicity which is an important advantage over many other drugs possessing positive inotropic activity [2]. The high biological activity of β-aryl ethanolamine and their widespread application as important intermediates not only in pharmaceuticals but also for chiral catalysts [3] makes them prime candidates in developing enantioselective synthetic routes for their synthesis. Other examples of β-amino alcohols of biological interest are 2-benzylmorpholine (**3**) [4] and fenfluramine (**4**) [5], both used as appetite suppressants. Enantiopure β-aza functionalised alcohols such as chiral nitro alcohols or 1-azido-2-alcohols are therefore interesting building blocks in organic synthesis since they can easily be converted into the corresponding chiral β-hydroxy amines via reduction of the nitro or azido group. Being able to control and predict the stereochemistry by using a specific catalyst is of high importance, especially for pharmaceutical and agricultural applications [6].

Furthermore, the enantioselective syntheses of chiral (ω-2)-azido-(ω-1)-alcohols possessing two vicinal chiral centers lead to 'α-bichiral' synthons. For instance, vicinal azido alcohols are direct precursors of aziridines [7] and amino alcohols, which are structural elements of numerous natural products and antifungal agents [8].

Asymmetric reduction of ketones to the corresponding enantiopure alcohols has been performed employing chiral metal complexes [9] or biocatalytic methods [10]. Alcohol dehydrogenases (ADHs) are found in many microbial strains catalyzing the stereoselective transfer of a hydride from the cofactor, NAD(P)H, to the *Si*- or *Re*-face of the carbonyl group which results in the formation of the corresponding (*S*)-or (*R*)-alcohol [11]. However, most of these ADHs follow the "Prelog's rule" [12], thus (*S*)-alcohols are

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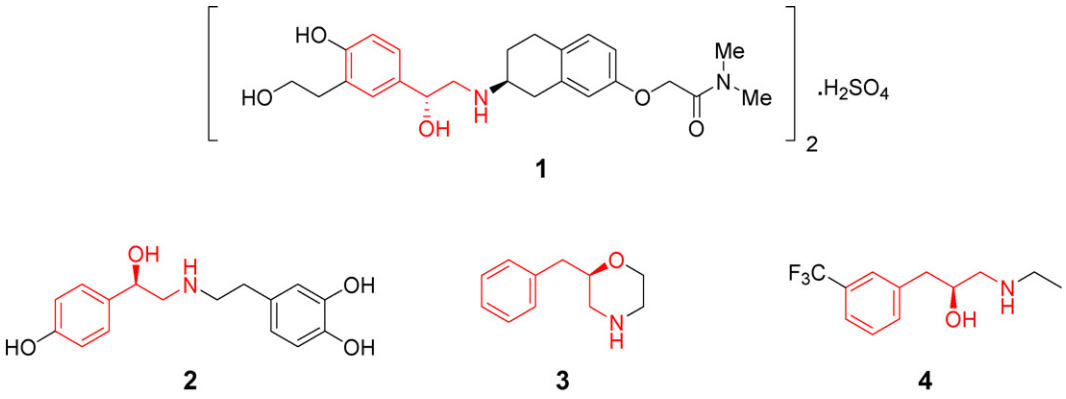
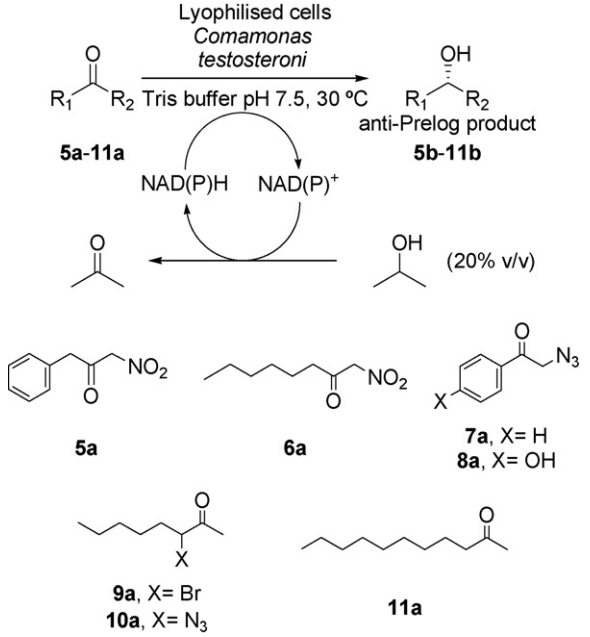


Fig. 1. Chiral β-hydroxy amines as building blocks in bioactive compounds: KUR-1246 (**1**), denopamine (**2**), 2-benzylmorpholine (**3**), and fenfluramine (**4**).

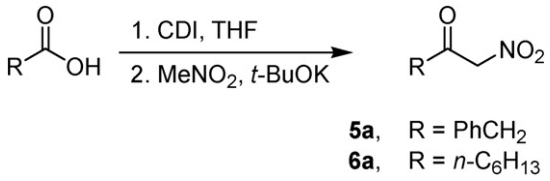
usually obtained assuming that the smaller substituent of the ketone has the lower CIP priority. Additionally, only in few cases a simultaneous recycling of the cofactor and the reduction of the desired ketone by a single enzyme in the so-called 'substrate-coupled' approach is possible [13]. Consequently, only a very limited number of ADHs is available which show anti-Prelog activity [14] and work according to the 'substrate-coupled' approach. For instance, ADHs from *Lactobacillus kefir* (LK-ADH) and *Leifsonia brevis* (LB-ADH) [15], *Leifsonia* sp. (LSADH) [16], *Pseudomonas fluorescens* (PFADH) [17], or *Pseudomonas* sp. [18] showed the desired properties. In continuation of our previous work on Prelog-enzymes [19], we searched for new organisms containing stable ADHs with anti-Prelog preference accepting 2-propanol for cofactor recycling.

2. Results and discussion

Substrates of interest (Scheme 1) were synthesized by different methodologies. Thus, α-nitro ketones **5a** and **6a** were obtained by coupling the corresponding carboxylic acids with nitromethane using 1,1′-carbonyldiimidazole (CDI) as coupling agent (Scheme 2) [20]. Nitro alcohols **5b** and **6b** were synthesized via Henry reaction coupling the aldehydes and nitromethane in the presence of Et_3N [21].



Scheme 1. 'Substrate-coupled' approach for the stereoselective reduction of ketones using *Comamonas testosteroni* DSM 1455 as catalyst.



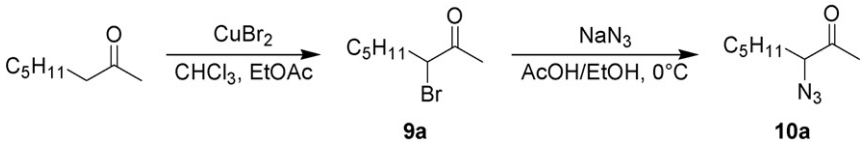
Scheme 2. Synthesis of α-nitro ketones **5a** and **6a**.

α-Azido ketones **7a** and **8a** were obtained starting from the corresponding non-functionalized ketones [19b]. Reduction with sodium borohydride resulted into the racemic alcohols **7b** and **8b**. Finally, racemic 3-bromo-2-octanone (**9a**) was synthesized from 2-octanone employing the method of King and Ostrum using $CuBr_2$ (Scheme 3) [22]. In this reaction $CuBr_2$ is reduced to CuBr which coordinates to the enolate ion. Under the reaction conditions employed, the thermodynamic product (addition in position three) is favoured over the kinetic one (addition in position one). Racemic 3-azido-2-octanone (**10a**) was obtained in good yield treating **9a** with sodium azide in a mixture of acetic acid and ethanol at low temperature [23]. Reduction using $NaBH_4$ in methanol yielded the corresponding racemic alcohols **9b** and **10b**.

Lyophilized microorganisms (41 strains from commercial sources) were tested for the reduction of 2-undecanone (**11a**) as a model substrate. This screening was performed employing 20% (v/v) of 2-propanol to enable cofactor recycling and to improve the solubility of the substrate (Scheme 1). To our delight, *Comamonas testosteroni* DSM 1455 was active and showed anti-Prelog alcohol dehydrogenase activity: 57% of conversion to (*R*)-**11b** was achieved with a very promising enantioselectivity (98% e.e.) after 24 h. This encouraged us to investigate this strain further.

In a subsequent step, various ketones **5a**–**10a** were reduced under the same 'substrate-coupled' approach conditions as described above (Table 1).

To our delight, excellent enantioselectivities were obtained for the reduction of α-nitro ketones **5a** and **6a**. However, we observed that a retro-Henry reaction took place affording the corresponding aldehydes as previously observed by other groups [24]. We noticed that the ratio of retro-Henry reaction depended on the substrate: for instance, reduction of **5a** led to 75% of alcohol **5b** with excellent stereoselectivity and 25% of the corresponding retro-Henry aldehyde was obtained. In the case of the reduction of the aliphatic substrate **6a**, the formation of the desired product **6b** was favoured, only traces of the corresponding aldehyde could be observed. Again, enantiopure (*S*)-alcohol could be obtained. Note that the (*S*)-alcohols represents the anti-Prelog product due to a switch of the CIP priority.



9a **10a**

Scheme 3. Synthesis of racemic ketones **9a** and **10a**.

Table 1
Reduction of ketones **5a**–**10a** (16 g/L) employing lyophilized cells of *C. testosteroni* DSM 1455 as catalyst with 2-propanol (20%, v/v) as hydrogen donor at 30 °C

Substrate	*t* (h)	GC-yield (%)	e.e. (%)	d.e. (%)
5a	24	75[a,b]	>97 (*S*)[a,c]	n.a.
6a	48	47[d]	>99 (*S*)[d,c]	n.a.
7a	48	43[d]	>99 (*S*)[d,c]	n.a.
8a	48	4[a]	52 (*S*)[a,c]	n.a.
9a	10	>99[d]	>99 (2*R*, 3*R*)[d] >99 (2*R*, 3*S*)[d]	12[d,e]
10a	48	>99[d]	>99 (2*R*, 3*R*)[d] >99 (2*R*, 3*S*)[d]	<1[b]
11a	24	57[d]	98[d]	n.a.

[a] Determined by HPLC employing a chiral column.
[b] 25% of aldehyde were found as side product.
[c] Switch in CIP priority.
[d] Determined by GC employing a chiral column.
[e] (2*R*, 3*R*) in excess. n.a. not applicable.

Since lyophilized cells of *C. testosteroni* DSM 1455 seemed to be highly active and selective with α-aza-functionalized ketones, this strain was used subsequently with α-azido ketones **7a** and **8a** under identical reaction conditions, showing excellent stereoselectivity in the reduction of α-aryl azidoketone **7a**, furnishing azido alcohol (*S*)-**7b** with e.e. >99%. In the case of *p*-hydroxy derivative **8a** both activity and selectivity were diminished, showing a significant influence of the phenolic hydroxy group on the bioreduction.

The reduction of *rac*-3-bromo-2-octanone **9a** by *C. testosteroni* afforded a very good conversion to enantiopure *syn*-(2*R*, 3*R*)- and *anti*-(2*R*, 3*S*)-3-bromo-2-octanol **9b** with low diastereoselectivity excess, indicating that the stereo-recognition of the chiral center in α-position to the ketone is low. The biocatalytic reduction of *rac*-3-azido-2-octanone **10a** occurred with excellent activity as well as enantioselectivity and with low diastereoselectivity similarly as previously described [23].

3. Conclusions

The number of alcohol dehydrogenases which work according to the 'substrate-coupled' approach and which show rare anti-Prelog stereoselectivity is rather limited. We reported that lyophilized cells of the bacterial strain *C. testosteroni* DSM 1455 showed excellent anti-Prelog stereopreference. Several ketones of biological interest were reduced to the corresponding alcohols at elevated substrate concentration (16 g/L) with excellent stereoselectivity in a hydrogen transfer process using 2-propanol (20%, v/v) as hydrogen donor.

4. Experimental

4.1. General

All reagents were used as received either from Sigma–Aldrich–Fluka (Vienna, Austria) or Lancaster (Frankfurt am Main, Germany). Compounds **5a**–**8a** and **5b**–**6b** were synthesized as previously described [19b,20,21]. Compounds **9a** and **10a** were synthesized in a two-step fashion using methods already reported [22,23]. Racemic alcohols **7b**, **8b**, **9b**, and **10b** were obtained by conventional reduction of the corresponding ketones using $NaBH_4$. For anhydrous reactions, flasks were dried and flushed with dry argon just before use. Standard syringe techniques were applied to transfer dry solvents and reagents in an inert atmosphere of dry argon. Anhydrous THF was distilled from potassium. Petroleum ether (bp. 60–90 °C) and EtOAc used for chromatography were distilled prior to use.

Lyophilized cells of *C. testosteroni* DSM 1455 were obtained by cultivating the organism in 250 mL of a complex medium (10 g/L yeast extract (Oxoid L21), 10 g/L bacteriological peptone (Oxoid L37), 10 g/L glucose (Fluka 49150), 2 g/L NaCl (Roth 9265.1), 0.15 g/L $MgSO_4 \cdot 7H_2O$ (Fluka 63140), 1.3 g/L NaH_2PO_4 (Fluka 71496), 4.4 g/L K_2HPO_4 (Merck 5101), and distilled water) in 1-L baffled shake flasks at 30 °C at 120 rpm. After three days, the cells were harvested by centrifugation (8000 rpm, 20 min), washed with phosphate buffer (50 mM, pH 7.5), shock frozen in liquid nitrogen, lyophilized and stored at +4 °C.

4.2. General method for the biocatalytic reduction of ketones employing lyophilized cells of C. testosteroni DSM 1455

Lyophilized cells of *C. testosteroni* (20 mg) were rehydrated in Tris/HCl buffer (500 μL, 50 mM, pH 7.5) for 30 min at 30 °C and 120 rpm on a rotary shaker in an Eppendorf vial (1.5 mL). Then, 2-propanol (130 μL, 20%, v/v) and the corresponding ketone (**5a**–**11a**, 8 mg, 16 g/L) were added. Reactions were shaken at 30 °C and 120 rpm for 24 h or 48 h, respectively and stopped by extraction with ethyl acetate (2× 0.5 mL). The organic layer was separated from the cells by centrifugation (2 min, 13,000 rpm) and dried (Na_2SO_4). Conversions and enantiomeric excesses of the corresponding alcohols **7b**–**10b** were determined by chiral HPLC or GC analysis as previously described [19b,23]. Absolute configuration of alcohols **9b** and **10b** were determined by comparison with a bioreduction using commercial baker's yeast [23].

HPLC analysis for **5a** and **5b**: Chiralpak OD-H, *n*-heptane/isopropanol (70/30), flow 0.5 mL/min, 25 °C. **5a**: 23.4 min; (*S*)-**5b**: 14.1 min; (*R*)-**5b**: 16.8 min.

GC analysis for **6a** and **6b**: CP-Chiralsil-DEX CB, 14.5 psi, H_2, 110 °C/hold 25 min–50 °C per min until 170 °C/hold 2 min. **6a**: 3.8 min; (*S*)-**6b**: 19.2 min; (*R*)-**6b**: 20.7 min.

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