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**Synthesis of pharmaceutically relevant 17-α-amino steroids using an ω-transaminase**

The synthesis of 17-α-amino steroids has been achieved in one step from the corresponding ketones in excellent yields, using a biocatalytic approach. In addition the ω-transaminase used catalysed the reaction with exceptional stereocontrol.

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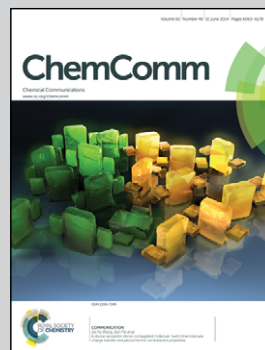
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# Synthesis of pharmaceutically relevant 17-α-amino steroids using an ω-transaminase†

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**An efficient and sustainable biocatalytic route for the synthesis of important 17-α-amino steroids has been developed using an ω-transaminase variant from *Arthrobacter* sp. Optimisation of the reaction conditions facilitated the synthesis of these valuable synthons on a preparative scale, affording excellent isolated yields and stereocontrol.**

Steroids are a large and diverse class of secondary metabolites, essential for the control of a variety of biological processes. Based on this key role in metabolism, steroids and their derivatives often exhibit biological activity and therefore have enormous potential as pharmaceuticals.[1] Indeed, approximately 300 steroidal drugs have been placed on the market since 1950 with cortisone as one prominent example.[1,2] Moreover, among the 200 top-selling drugs in 2010 13% were steroids and derivatives thereof.[3] 17-Amino steroids (1) (Fig. 1) have proven to be particularly interesting non-natural steroids that are used as intermediates in the synthesis of biologically active steroidal derivatives. For example the 17β-arylsulfonamide derivative 2 has recently been highlighted as a potent inhibitor of steroid sulfatase, a target in the treatment of breast cancer.[4–7] Moreover, a number of 17β-aminoestrogens were identified to have a prolonged anticoagulant effect in rodents.[8] In both examples the amine functionality, and derivatives thereof, attached to the C-17 position of the steroid is presumed to be crucial for the biological activity.

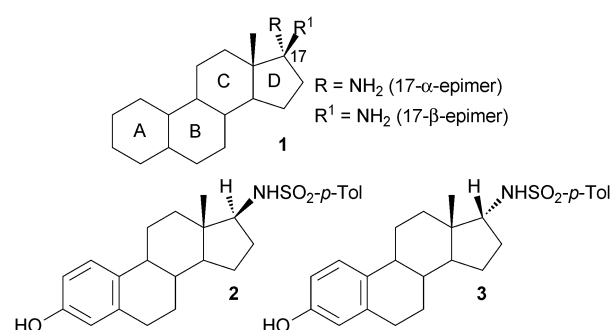In general, the 17β-amino steroid motif is accessible by a classical two-step method, *via* reductive amination and



Fig. 1 Steroid core structure 1 with amino group at C-17, and 17β- and 17α-sulfonamide derivatives 2 and 3, respectively.

deprotection, and consequently studies have focussed on derivatives of the β-epimer.[6,7] Although the synthesis of the 17α-amino steroid is less efficient, requiring a three-step synthetic route with a low yielding reduction reaction, the α-epimer derivative 3 (Fig. 1) was also found to be a potent sulfatase inhibitor.[6,8] As a consequence of the poor accessibility of the α-epimer the potential of further derivatives has to the best of our knowledge not been investigated to date. Therefore, the development of an efficient method for the synthesis of the α-epimer would be highly desirable. Here we present a novel route to access 17-α-amino steroids by applying the use of an ω-transaminase (ω-TAm). ω-TAms are continuing to attract significant attention for use in asymmetric synthesis for the generation of both (*S*)- and (*R*)-chiral amines.[9–19] Moreover, biocatalytic strategies employing ω-TAms have been used for the synthesis of pharmaceutically relevant compounds,[20–27] even leading to the development of an industrial process.[28]

In initial studies our aim was to use two previously reported ω-TAms, *Vibrio fluvialis* (Vf-TAm),[10] and *Chromobacterium violaceum* DSM30191 (CV-TAm),[16] and a particularly interesting ω-TAm variant described, from *Arthrobacter* sp. (ArRMut11).[28] CV-TAm has previously been reported for the transamination of a wide range of ketones including more sterically challenging substrates such as 1,3-dihydroxy-1-phenylpropan-2-one,[16,25]

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while Vf-TAm has been used with in general smaller substrates.[11,14] The ω-TAm ArRMut11 variant was evolved by Savile and co-workers over 11 rounds of mutation, to catalyse the amination of sterically demanding 1,3-ketoamides to generate the (R)-aminoamide functionality present in sitagliptin.[28] An additional feature of this variant is its tolerance towards high co-solvent concentrations and 2-propylamine, facilitating the use of this low cost amine donor to shift the equilibrium towards the product.[11,17,28–31] The variant ArRMut11 has also been successfully used for the transamination of tetralone and chromone bicyclic compounds.[29,30] For the generation of 17α-amino steroids TAms are required with good co-solvent tolerance, due to the limited aqueous solubilities of the substrates, and ability to accept the large tetracyclic steroidal ring system. Here we describe the use of the three ω-TAms and optimisation of the reaction conditions using ArRMut11 as a novel route to 17-α-amino steroids.

To evaluate the potential of the selected ω-TAms in the asymmetric amination of steroid precursors, the truncated analogue 4 and steroid 5 (10 mM) were initially screened against the three ω-TAms using either (R) or (S)-α-methylbenzylamine as the amine donor, depending on the selectivity of the ω-TAm used, and acetophenone production was monitored by HPLC analysis.

Only for ArRMut11 were conversions > 5% detected, so this ω-TAm was used in further experiments with 4–8 (10 mM) using isopropylamine as a low cost donor (Scheme 1) and 20% of either 1,2-dimethoxyethane (DME) (4 and 5) or dimethylsulfoxide (DMSO) (6–8) as co-solvent. LC-MS analysis indicated that 5–7 were good substrates for the ω-TAm. Although substrates 4 and 8 seemed to be accepted by the enzyme and m/z peaks corresponding to the product were detected by LC-MS, it was not possible to isolate a single product. This was not surprising due to the two ketone moieties in 4 complicating product analysis, and α,β-unsaturated enones in both 4 and 8 which can form conjugated enamines in the presence of amines. Compounds 4 and 8 were therefore not explored further.
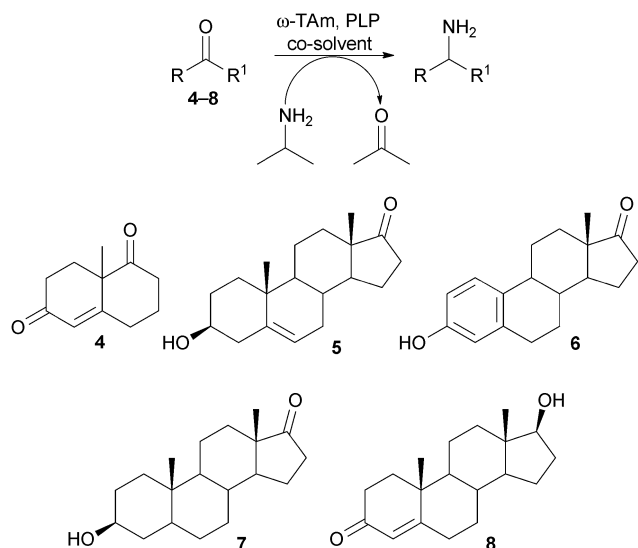
The reaction conditions were then optimised in order to maximise reaction rates and conversion yields using trans-dehydroandrosterone (5) as a model substrate (1 mL, 10 mM). Parameters including the employment of co-solvents, to increase the substrate solubility, as well as the amount of amine donor 2-propylamine, to maximise the conversion rate were explored. DMSO is frequently used as a co-solvent in biocatalysis, but the substrates were not fully soluble in mixtures of DMSO–water. DME and dimethylformamide (DMF) have recently been applied successfully as co-solvents in ω-TAm reactions so their potential here was investigated.[32] Both DME and DMF enhanced the solubility of the substrates in mixed aqueous systems giving rise to clear rather than cloudy solutions, whereas substrates exhibited the best solubilities in water–DMF mixtures. Comparative studies using 25% of DME or DMF as co-solvents led to similar conversions (~60%) after a reaction time of 6 days (Fig. S1a, ESI†), however since higher reaction rates were observed with DMF it was selected as a co-solvent for all further experiments. The impact of different DMF concentrations (25–50%) on reaction rate and conversion were investigated as well. While conversions after 6 days were again rather similar (64–68%) the fastest reaction rate and highest conversion (68%) were reached using 35% of DMF as an optimal co-solvent concentration.

The concentration of the amine donor 2-propylamine was then investigated using 20-, 50- and 100-fold molar excesses to shift the equilibrium of the ω-TAm reaction towards the aminated steroid. The conversion of 5 to 17-amino-3β-hydroxyandrost-5-ene was improved from 40% after 6 days using a 20-fold excess of 2-propylamine to 81% after 5 days using a 100-fold excess (Fig. S1b, ESI†). The reaction profile for the biotransformation using the optimised conditions (35% DMF and 100-fold molar excess of 2-propylamine) over a period of 6 days is shown in Fig. 2. The data indicated that the reaction conditions were well tolerated by ω-TAm ArRMut11.

As a consequence these optimised conditions were used for the asymmetric amination of steroids 6 and 7 (1 mL, 10 mM). These were successfully transformed to the corresponding 17-amino steroids with excellent conversions, yielding


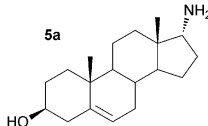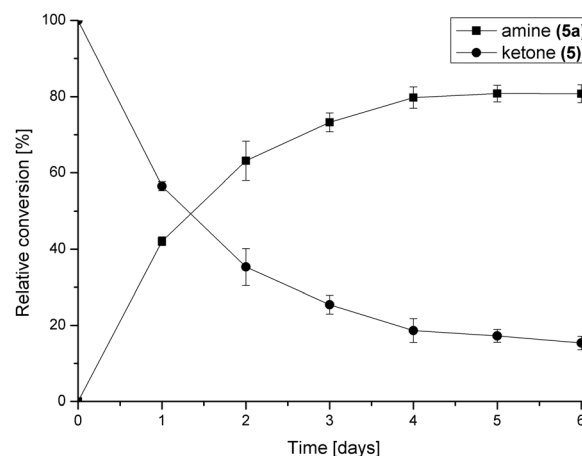
Scheme 1  TAm reaction using ArRMut11 and substrates 4–8.



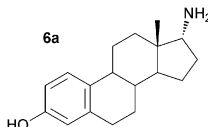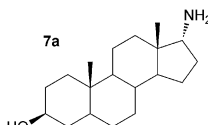Fig. 2  Reaction profile of the asymmetric amination of 5 (1 mL, 10 mM) using the optimised conditions: ω-TAm ArRMut11, 35% v/v DMF–water and 100-fold molar excess of 2-propylamine (1 M).

**Table 1** ω-TAm ArRMut11 catalysed transamination of steroids **5–7**

| Steroid | Product | Conversion (%) | Isolated yield (%) | Syn : anti ratio[a] (major isomer) |
|---|---|---|---|---|
| **5** | | 88 | 83 | 1 : 99 (17-α-R) |
| **6** | | 96 | 85 | 1 : 99 (17-α-R) |
| **7** | | 90 | 89 | 1 : 99 (17-α-R) |

[a] Determined by GC analysis and $^1$H NMR spectroscopy: no β-epimer detected, ratios reflect detection limit (GC).

17-amino-1,3,5(10)-estratrien-3-ol from **6** in 68% yield and 17-amino-5α-androstan-3β-ol from **7** in 71% yield (Table S1, ESI†). The transamination of **5–7** was then performed on preparative scale (50 mg substrate; 20 mL, 10 mM). Reactions were stopped after three days and the conversion and stereoselectivity of the reaction determined by GC and NMR analysis. Interestingly, on a preparative scale all biotransformations showed enhanced conversions compared to the previous results despite a shorter reaction time (Table 1 *cf.* Table S1, ESI†): however for the synthesis of **5a** this could be ascribed to the slightly higher amounts of enzyme used. In particular, the amination of steroid **6** was improved 1.4 fold, giving amine **6a** with a conversion of 96%. Amines **5a** and **7a** were also produced with excellent conversions of 88% and 90%, respectively. To establish the stereoselectivities reaction products were purified by flash silica chromatography, and isolated in yields of 83% (**5a**), 85% (**6a**) and 89% (**7a**).

Assignment of the absolute configuration of the products was carried out by comparing the previously reported NMR spectroscopic data to that of **6a** and using $^1$H NMR NOESY experiments (between 17-H and the methyl group at C-13).[8] These indicated that the 17-α-epimer (*anti*) was formed with full stereocontrol in all three cases. The newly formed chiral amine moiety has an (*R*)-configuration, in agreement with previous stereoselectivities reported using the (*R*)-selective ω-TAm ArRMut11. This biocatalytic approach gives the 17-α-amino steroids in one step, starting from the corresponding carbonyl compound, in excellent isolated yields of 83–89%, and highlights the tolerance of the ω-TAm ArRMut11 to such sterically challenging substrates. Compared to the classical chemical synthesis of **6a** reported not only was the number of synthetic steps reduced from three to one, but also the overall yield was improved 9-fold.[8]

In summary, a highly stereoselective, efficient and sustainable biocatalytic route, facilitating access to a variety of highly desirable 17-α-amino steroids has been developed. After optimisation of the reaction parameters the 17-α-amino steroids were synthesised in high isolated yields of 83–89% *via* a one-step procedure on a preparative scale. This novel biocatalytic methodology enables access to the

α-epimer of key intermediates in the synthesis of biologically active steroidal derivatives. Moreover, the described method represents the shortest routes towards 17-α-amino steroids published to date.

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