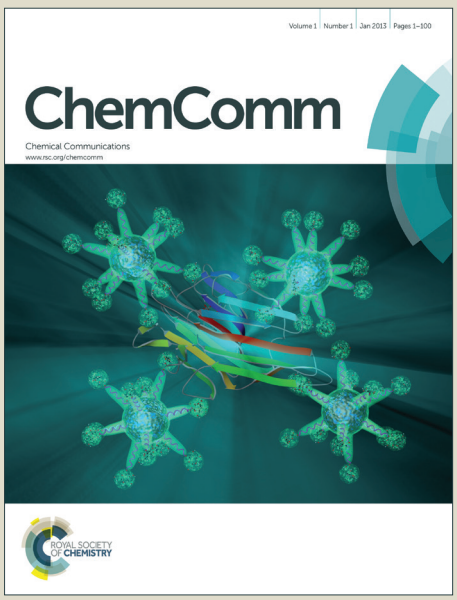
ChemComm

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/chemcomm

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

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

Nina Richter,[a,b] Robert C. Simon,[c] Wolfgang Kroutil,[b,c] John M. Ward,[d] and Helen C. Hailes*[a]

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX

DOI: 10.1039/b000000x

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 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**) (Figure 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-aminoestrogenes 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 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** (Figure 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]

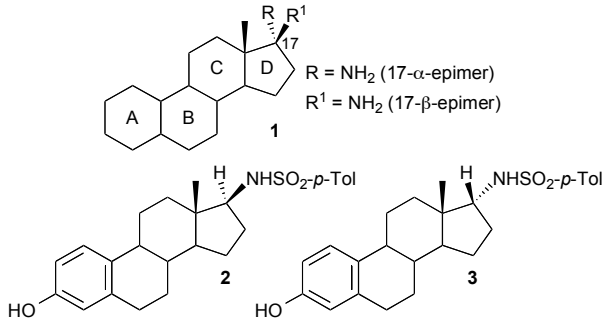
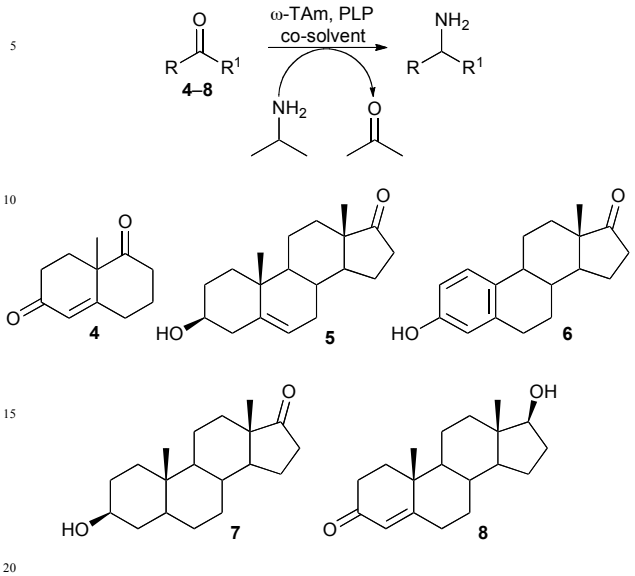


Figure 1. Steroid core structure **1** with amino group at C-17, and 17β- and 17α-sulfonamide derivatives **2** and **3**, respectively.

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] 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 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.

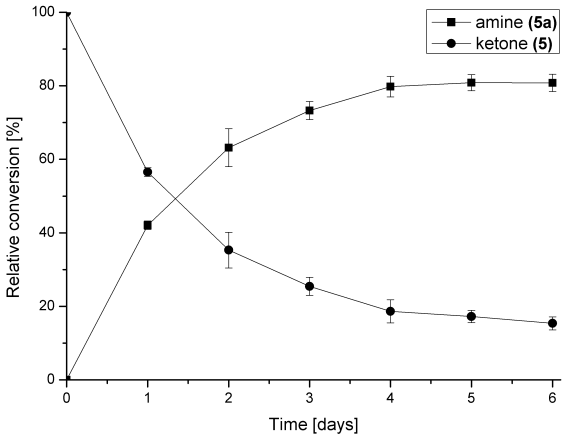


Scheme 1. TAm reaction using ArMut11 and substrates **4**–**8**.

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 (Figure S1a), 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.

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



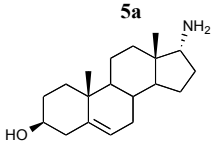
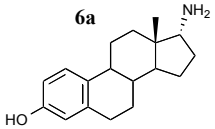
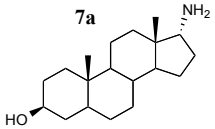
The concentration of the amine donor 2-propylamine was then investigated using 20-, 50- and 100-fold molar excesses to shift the equilibrium of the ω-TA reaction towards the aminated steroid. The conversion of **5** to 17-amino-3β-hydroxyandrost-5-ene was improved from 40% after 6 days using a 20-fold excess of 2-propylamine to 81% after 5 days using a 100-fold excess (Figure S1b). 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 Figure 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 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). 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 analysis. Interestingly, on a preparative scale all biotransformations showed enhanced conversions compared to the previous results despite a shorter reaction time (Table 1 ccf Table S1): 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% yield. 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]

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

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

*Determined by GC analysis and ^{1}H NMR spectroscopy: no β-epimer detected, ratios reflect detection limit (GC).

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.

This work has been supported by a postdoctoral fellowship of the German academic exchange service (DAAD) to N. R.; part of the work was supported by the Austrian BMWFJ, BMVIT, SFG, Standortagentur Tirol and ZIT through the Austrian FFG-COMET- Funding Program.

Notes and references

[a] *Department of Chemistry, University College London, 20 Gordon Street, London WC1H 0AJ London, UK. Tel: +44 (0)20 7679 7463. E-mail: h.c.hailes@ucl.ac.uk.*
[b] *ACIB GmbH, c/o Department of Chemistry, University of Graz, Heinrichstrasse 28, 8010 Graz, Austria.*
[c] *Department of Chemistry, University of Graz, Heinrichstrasse 28, 8010 Graz, Austria.*
[d] *Department of Biochemical Engineering, University College, London, Torrington Place, London, WC1E 7JE, UK.*

† Electronic Supplementary Information (ESI) available: [Procedures and characterisation data for **5a**, **6a**, **7a**. Supplementary Figure S1, Table S1 and NMR data for **5a**, **6a**, **7a**]. See DOI: 10.1039/b000000x/.

1. C. H. L. Shackleton, *Lipids*, 2012, **47**, 1-12.
2. A. S. Chapelon, D. Moraleda, R. Rodriguez, C. Ollivier and M. Santelli, *Tetrahedron*, 2007, **63**, 11511-11616.
3. D. J. Mack, M. L. Weinrich, E. Vitaku and J. T. Njarðarson, 'Top 200 Brand Name Drugs by US Retail Sales in 2010'. (http://cbc.arizona.edu/njardarson/group/top-pharmaceuticals-poster).
4. J. Geisler, H. Sasano, S. A. Chen and A. Purohit, *J. Steroid Biochem. Mol. Biol.*, 2011, **125**, 39-45.
5. R. Maltais and D. Poirier, *Steroids*, 2011, **76**, 929-948.
6. Y. A. Mostafa and S. D. Taylor, *Bioorg. Med. Chem.*, 2012, **20**, 1535-1544.
7. S. D. Taylor and J. Harris, *Steroids*, 2011, **76**, 1098-1102.
8. C. Lemini, E. Cruz-Ramos, R. A. Toscano and R. Cruz-Almanza, *Steroids*, 1998, **63**, 556-564.
9. J. Ward and R. Wohlgemuth, *Curr. Org. Chem.*, 2010, **14**, 1914-1927.
10. J. S. Shin, H. Yun, J. W. Jang, I. Park and B. G. Kim, *Appl. Microbiol. Biotechnol.*, 2003, **61**, 463-471.
11. M. S. Malik, E. S. Park and J. S. Shin, *Appl. Microbiol. Biotechnol.*, 2012, **94**, 1163-1171.
12. G. Shin, S. Mathew, M. Shon, B. G. Kim and H. Yun, *Chem. Commun.*, 2013, **49**, 8629-8631.
13. M. Höhne and U. T. Bornscheuer, *ChemCatChem*, 2009, **1**, 42-51.
14. S. Mathew and H. Yun, *ACS Catal.*, 2012, **2**, 993-1001.
15. B. Wang, H. Land and P. Berglund, *Chem. Commun.*, 2013, **49**, 161-163.
16. U. Kaulmann, K. Smithies, M. E. B. Smith, H. C. Hailes and J. M. Ward, *Enzyme Microb. Technol.*, 2007, **41**, 628-637.
17. K. E. Cassimjee, C. Branneby, V. Abedi, A. Wells and P. Berglund, *Chem. Commun.*, 2010, **46**, 5569-5571.
18. M. D. Truppo, J. D. Rozzell and N. J. Turner, *Org. Process Res. Dev.*, 2010, **14**, 234-237.
19. M. D. Truppo, J. D. Rozzell and N. J. Turner, *Org. Biomol. Chem.*, 2010, **8**, 1280-1283.
20. E. S. Park, M. S. Malik, J. Y. Dong and J. S. Shin, *ChemCatChem*, 2013, DOI:10.1002/cctc.201300571.
21. I. K. Mangion, B. D. Sherry, J. J. Yin and F. J. Fleitz, *Org. Lett.*, 2012, **14**, 3458-3461.
22. M. Fuchs, D. Koszelewski, K. Tauber, W. Kroutil and K. Faber, *Chem. Commun.*, 2010, **46**, 5500-5502.
23. E. Siirola, F. G. Mutti, B. Grischek, S. F. Hoefler, W. M. F. Fabian, G. Grogan and W. Kroutil, *Adv. Synth. Catal.*, 2013, **355**, 1703-1708.
24. M. E. B. Smith, B. H. Chen, E. G. Hibbert, U. Kaulmann, K. Smithies, J. L. Galman, F. Baganz, P. A. Dalby, H. C. Hailes, G. J. Lye, J. M. Ward, J. M. Woodley and M. Micheletti, *Org. Proc. Res. Dev.*, 2010, **14**, 99-107.
25. K. Smithies, M. E. B. Smith, U. Kaulmann, J. L. Galman, J. M. Ward and H. C. Hailes, *Tetrahedron: Asymmetry*, 2009, **20**, 570-574.
26. C. Molinaro, P. G. Bulger, E. E. Lee, B. Kosjek, S. Lau, D. Gauvreau, M. E. Howard, D. J. Wallace and P. D. O'Shea, *J. Org. Chem.*, 2012, **77**, 2299-2309.
27. T. Sehl, H. C. Hailes, J. M. Ward, R. Wardenga, E. von Lieres, H. Offermann, R. Westphal, M. Pohl and D. Rother, *Angew. Chem. Int. Edit.*, 2013, **52**, 6772-6775.
28. C. K. Savile, J. M. Janey, E. C. Mundorff, J. C. Moore, S. Tam, W. R. Jarvis, J. C. Colbeck, A. Krebber, F. J. Fleitz, J. Brands, P. N. Devine, G. W. Huisman and G. J. Hughes, *Science*, 2010, **329**, 305-309.
29. F. G. Mutti, C. S. Fuchs, D. Pressnitz, J. H. Sattler and W. Kroutil, *Adv. Synth. Catal.*, 2011, **353**, 3227-3233.
30. D. Pressnitz, C. S. Fuchs, J. H. Sattler, T. Knaus, P. Macheroux, F. G. Mutti and W. Kroutil, *ACS Catal.*, 2013, **3**, 555-559.
31. E. S. Park, J. Y. Dong and J. S. Shin, *Org. Biomol. Chem.*, 2013, **11**, 6929-6933.
32. R. C. Simon, C. S. Fuchs, H. Lechner, F. Zepeck and W. Kroutil, *Eur. J. Org. Chem.*, 2013, 3397-3402.