Chiral Imines

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# Enantioselective Biocatalytic Oxidative Desymmetrization of Substituted Pyrrolidines**

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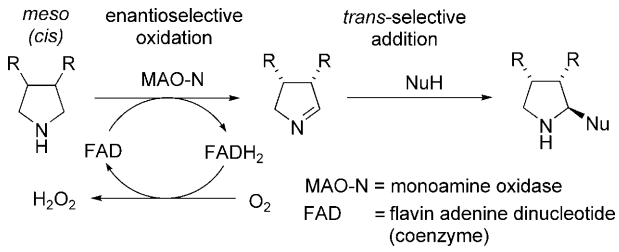
Direct activation of $sp^3$ C–H bonds α to a nitrogen atom represents an attractive strategy for functionalization of amines, especially those found in 5- and 6-membered ring heterocycles.[1] In particular, C–H activation by oxidation, followed by nucleophilic addition, generates products of an overall oxidative Strecker or Mannich process. Recent reports have described the use of various metal-based oxidants to achieve this transformation although there are few examples of catalytic and/or enantioselective processes.[2] The enantioselective oxidation of N-protected pyrrolidines has been reported using manganese–salen catalysts and iodosobenzene as the stoichiometric oxidant.[3] Herein we report the enantioselective enzyme-catalyzed desymmetrization of a range of unprotected pyrrolidines to the corresponding $\Delta^1$-pyrrolines (Scheme 1), which serve as useful building blocks for the synthesis of L-proline analogues of high enantiomeric purity.




***Scheme 1.*** Oxidative desymmetrization of pyrrolidines.

We have previously reported the use of monoamine oxidases (MAO-N from *Aspergillus niger*) for the deracemization of primary, secondary, and tertiary amines.[4] These biocatalysts, which use molecular oxygen as oxidant, have been subjected to several rounds of directed evolution such that they now possess broad substrate specificity and high enantioselectivity.[4a,b] However, to date we have not examined their ability to catalyse the desymmetrization of symmetrical amines. The MAO-N D5 variant was found to exhibit good activity towards a range of substituted pyrrolidines **1**–**10** as determined by a colorimetric assay (Figure 1). In general, increasing the substrate bulk and lipophilicity seemed to correlate with higher rates of oxidation.

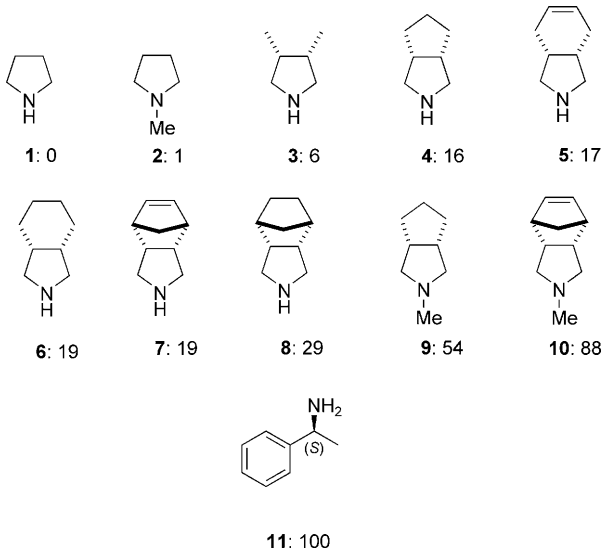



***Figure 1.*** Relative rates of oxidation with MAO-N D5/air for amines **1**–**11**. Rates were determined by absorbance measurements in a horseradish peroxidase coupled colorimetric assay with cell-free extracts. Assays were performed at 10 mM substrate concentration, 30 °C and pH 8.0. Rates are relative to (*S*)-α-methylbenzylamine (**11**; rate = 100).

To determine the enantioselectivity of the MAO-N catalysed oxidations, the required racemic imine standards were prepared by elimination of HCl from the corresponding *N*-chloroamines in methanolic KOH.[5] Interestingly, after distillation of the concentrated methanolic solution of imine **12**, spontaneous crystallization occurred in the distillate. The racemic crystals consisted of homochiral trimers as shown by X-ray crystallography.[6]

Pyrrolidine **4** was selected for initial studies, and biotransformations were carried out with *E. coli* cells expressing MAO-N in aqueous phosphate buffer (100 mM) and monitored by GC-FID. Reactions at 20 mM substrate concentra-

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tion ran to high conversion (>98%, based on **4**) within 7 h (1 mmol scale). The enantiomeric excess (*ee*) of the imine **12**, isolated by simple extraction (77% yield), was determined as 94% after derivatization to the trifluoroacetamide of the corresponding α-amino nitriles (see below for assignment of the absolute configuration).[7] Recrystallization of the trimer of **12** from EtOAc improved the *ee* to at least 98%. Optimization of this process yielded 51% of trimeric imine **12** with an *ee* of 98% (5 mmol scale). Similarly imines **13** and **14** were obtained from pyrrolidines **8** and **7**, respectively, in greater than 99% *ee* (Figure 2).

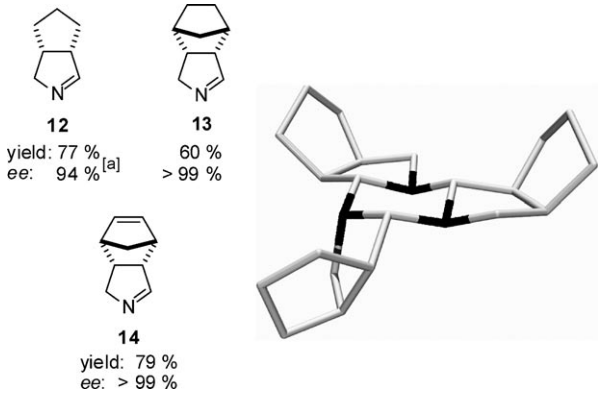



***Figure 2.*** Left: Imines isolated by extraction from biotransformation mixtures. Biotransformation conditions: substrate: 20 mM (**4**, **8**) or 10 mM (**7**), wet cells containing MAO-N D5: 100 g $L^{-1}$, 100 mM $KPO_4$ buffer, 37°C, 250 rpm, pH 8.0 at start. Reactions were carried out on a 1 mmol scale (**7**, **8**) or on a 2 mmol scale (**4**). The *ee* values were determined after derivatization with: 1) TMSCN/MeOH (1.3 equiv) and 2) TFAA (5 equiv) by chiral GC. [a] As determined in the concentrated extract, see Supporting Information. Right: Crystal structure of trimeric **12** (single enantiomer). TMSCN = cyanotrimethylsilane; TFAA = trifluoroacetic anhydride.

To demonstrate the application of these $\Delta^1$-pyrrolines of high enantiomeric purity we decided to investigate the addition of HCN, which allows access to 3,4-substituted proline analogues as exemplified by those found in hepatitis C virus protease inhibitors telaprevir (**15**; Vertex Pharmaceuticals)[8] and boceprevir (**16**; Schering Plough; Figure 3).[9] The

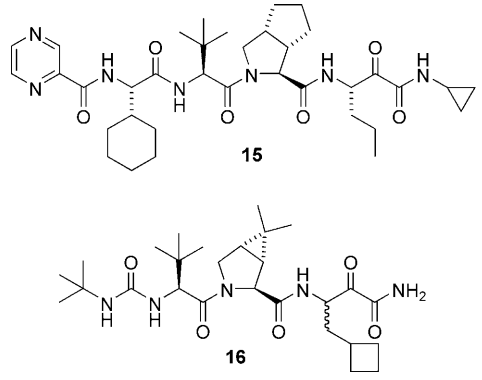
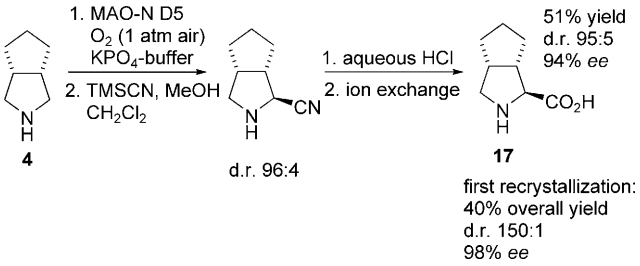



***Figure 3.*** Hepatitis C virus protease inhibitors telaprevir (**15**) and boceprevir (**16**).

diastereoselective formation of racemic bicyclic amino acids or derivatives starting from 3,4-substituted *meso*-pyrrolidines has been demonstrated previously[5a] and has also been described in a recent patent for the synthesis of the proline analogue in boceprevir (**16**).[9b]

The diastereomeric ratio (d.r.) values obtained in the addition of cyanide to the imines turned out to be strongly dependent on the reaction conditions. In the buffered aqueous medium of the biotransformation, addition of HCN occurs initially with high diastereoselectivity (≥96:4) but the kinetic product epimerizes rapidly at 37°C (d.r. at equilibrium = 66:34). On the other hand, treatment of a solution of the racemic imine **12** in $CH_2Cl_2$ with TMSCN/MeOH at room temperature gave a d.r. of 97:3.

A similar d.r. was observed when the concentrated TBME (methyl *tert*-butyl ether) extracts of the biotransformation reaction were treated with TMSCN/MeOH in $CH_2Cl_2$ (d.r. = 96:4). Hydrolysis of the amino nitrile in aqueous HCl led, after ion exchange chromatography, to the free amino acid **17** in 94% *ee* and 51% overall yield. Recrystallization of **17** from EtOH/TBME gave the amino acid **17** in a d.r. of 150:1 and an *ee* of 98% (Scheme 2).

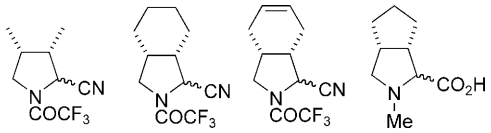



***Scheme 2.*** Stereoselective synthesis of amino acid **17**. Diastereomeric ratios were determined by $^1$H NMR spectroscopy, the *ee* of **17** by chiral HPLC.

Biotransformations of amines **3**, **5**, and **6** led to the formation of the corresponding imines in high *ee* (≥98%) with some side products, presumably due to decomposition of the $\Delta^1$-pyrrolines. The addition of 2 equivalents HCN at the start of the biotransformation allowed the isolation of the corresponding α-amino nitriles as diastereomeric mixtures, which were converted to trifluoroacetamides **18**–**20** for analysis. Similarly the *N*-methylated amino acid **21** can be obtained starting from *N*-methylated amine **9** in 98% *ee* (Figure 4). The diastereoselectivity of HCN addition determined for the preparation of standards *rac*-**18**–**20** in $CH_2Cl_2$ with TMSCN/MeOH was consistently higher (Figure 5).

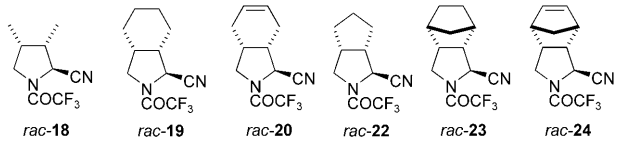
To establish the absolute configuration of the imines derived from the MAO-N reactions, a sample of D,L-**17** was treated with D-amino acid oxidase and analysed by chiral HPLC. Oxidation of the peak corresponding to D-**17** assigned the product derived from the biotransformation as L-**17** (Scheme 2). In summary, a method for the preparation of $\Delta^1$-pyrrolines with very high enantiomeric excesses by enzymatic oxidation of 3,4-substituted *meso*-pyrrolidines has been developed and applied to the synthesis of amino nitriles and nonproteinogenic amino acids.



| | 18 | 19 | 20 | 21 |
|---|---|---|---|---|
| d.r.: | 60:40 | 82:18 | 69:31 | 60:40 [a] |
| yield: | 54 % | 68 % | 70 % | 53 % |
| *ee*: | 99 % | 99 % | 98 % | 98 % |

***Figure 4.*** Biotransformation conditions: substrate: 20 mM (**5**, **6**, **9**) or 10 mM (**3**), 2 equiv KCN, wet cells containing MAO-N D5: 100 g $L^{-1}$, 100 mM $KPO_4$ buffer, 37 °C, 250 rpm, pH 8.0 at start. Reactions were carried out on a 1 mmol scale. Secondary amino nitriles were subsequently treated with TFAA for analysis purposes. The amino acid **21** was obtained by hydrolysis of the amino nitrile with aqueous HCl and subsequent ion exchange chromatography. Diastereomeric ratios were determined by GC-FID (**19**, **20**) and $^1$H NMR spectroscopy (free amino nitrile of **18** and **21**), the *ee* values by chiral GC (**18**-**20**) and chiral HPLC (**21**). Yields of isolated product based on corresponding pyrrolidines are reported. [a] d.r. of amino nitrile.



| | *rac*-18 | *rac*-19 | *rac*-20 | *rac*-22 | *rac*-23 | *rac*-24 |
|---|---|---|---|---|---|---|
| d.r.: | 86 : 14 | 96 : 4 | 90 : 10 | 97 : 3 | > 99 : 1 [a] | > 99 : 1 [a] |

***Figure 5.*** Diastereoselectivity of HCN addition for racemic samples with TMSCN/MeOH in $CH_2Cl_2$ as determined by GC-FID (**18**–**22**) in the crude products. [a] Only one diastereomer was detected by NMR spectroscopy (free amino nitrile) and GC-MS.

## *Experimental Section*

Representative procedure for the direct formation of α-amino nitriles from pyrrolidines: *cis*-8-azabicyclo[4.3.0]non-3-ene (**5**; 124 mg, 1.01 mmol) and KCN (130 mg, 2.00 mmol) were dissolved in $KPO_4$ buffer (5.0 mL, 1M, pH 8.0) and demineralized water, and the pH value of the resulting solution was adjusted to pH 8.0 by addition of aqueous HCl. This solution was combined with the cell pellet from *E. coli* cultures (5.0 g) expressing MAO-N D5. The mixture was homogenized by shaking, transferred to a 500 mL screw cap bottle and the total volume adjusted to 50 mL by addition of demineralized water. The mixture was agitated in a shaking incubator at 37 °C/250 rpm. Workup was performed after 2 h: the mixture was centrifuged at 3220 g and 4 °C for 60 min, and the supernatant was subsequently separated and extracted with *tert*-butyl methyl ether (3 × 50 mL). The combined organic extracts were dried with $Na_2SO_4$ and concentrated by means of a rotary evaporator. The crude α-amino nitrile was treated with TFAA (5 equiv) in dry $CH_2Cl_2$ at room temperature for 1 h. Removal of volatiles on the rotary evaporator left the crude trifluoroacetamide **20**, which was purified by column chromatography over silica (EtOAc/petrol ether (b.p. 40–60 °C) = 1:5). Yield: 70 % (based on **5**, mixture of diastereomers).

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[1] a) K. R. Campos, *Chem. Soc. Rev.* **2007**, *36*, 1069–1084; b) C.-J. Li, *Acc. Chem. Res.* **2009**, *42*, 335–344; c) I. Prochnow, R. Kubiak, O. N. Frey, R. Beckhaus, S. Doye, *ChemCatChem* **2009**, *1*, 162–172.

[2] a) R. Kubiak, I. Prochnow, S. Doye, *Angew. Chem.* **2009**, *121*, 1173–1176; *Angew. Chem. Int. Ed.* **2009**, *48*, 1153–1156; b) S.-i. Murahashi, T. Nakae, H. Terai, N. Komiya, *J. Am. Chem. Soc.* **2008**, *130*, 11005–11012; c) M. North, *Angew. Chem.* **2004**, *116*, 4218–4220; *Angew. Chem. Int. Ed.* **2004**, *43*, 4126–4128; d) I. Ibrahem, J. S. M. Samec, J. E. Bäckvall, A. Córdova, *Tetrahedron Lett.* **2005**, *46*, 3965–3968; e) H. Li, Z. He, X. Guo, W. Li, X. Zhao, Z. Li, *Org. Lett.* **2009**, *11*, 4176–4179.

[3] T. Punniyamurthy, T. Katsuki, *Tetrahedron* **1999**, *55*, 9439–9454.

[4] a) N. J. Turner, *Nat. Chem. Biol.* **2009**, *5*, 567–573; b) C. J. Dunsmore, R. Carr, T. Fleming, N. J. Turner, *J. Am. Chem. Soc.* **2006**, *128*, 2224–2225; c) for a recent review on biocatalytic routes to optically active amines see: M. Höhne, U. T. Bornscheuer, *ChemCatChem* **2009**, *1*, 42–51.

[5] a) D. A. Wood, R. F. Mason, J. A. Day, R. J. G. Searle (Shell Internationale Research Maatschappij B.V.), EP0010799, **1980**; b) for a general review of synthesis and chemical transformations of $\Delta^1$-pyrrolines see: M.-G. A. Shvekhgeimer, *Chem. Heterocycl. Compd.* **2003**, *39*, 405–448 (engl. transl. of *Khim. Geterotsikl. Soedin.*).

[6] a) A dimeric structure of **12** has been reported previously based on mass spectrometry and NMR spectroscopy results: M. Elkhatib, L. Peyrot, J. P. Scharff, H. Delalu, *Int. J. Chem. Kinet.* **1998**, *30*, 129–136. For studies of the parent compound $\Delta^1$-pyrroline see: b) H. Poisel, *Monatsh. Chem.* **1978**, *109*, 925–928; c) J. D. Baker, R. R. Heath, J. G. Millar, *J. Chem. Ecol.* **1992**, *18*, 1595–1602; d) C. Struve, C. Christophersen, *Heterocycles* **2003**, *60*, 1907–1914; e) CCDC 755434 [(*rac*-**12**)$_3$·$CH_3OH$] and 755435 [(**12**)$_3$, single enantiomer] contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

[7] M. S. Sigman, E. N. Jacobsen, *J. Am. Chem. Soc.* **1998**, *120*, 5315–5316.

[8] a) P. Revill, N. Serradell, J. Bolós, E. Rosa, *Drugs Future* **2007**, *32*, 788–798; b) G. J. Tanoury, M. Chen, J. E. Cochran (Vertex Pharmaceuticals Inc.), WO2007022459, **2007**.

[9] a) F. G. Njoroge, K. X. Chen, N.-Y. Shih, J. J. Piwinski, *Acc. Chem. Res.* **2008**, *41*, 50–59; b) G. Wu, F. X. Chen, P. Rashatasakhon, J. M. Eckert, G. S. Wong, H. C. Lee, N. C. Erickson, J. A. Vance, P. C. Nirchio, J. Weber, J.-S. D. Tsai, Z. Nanfei (Schering Corporation), WO2007075790, **2007**.

  