Enantioselective oxidation of *O*-methyl-*N*-hydroxylamines using monoamine oxidase N as catalyst

Tom S. C. Eve,[a] Andrew Wells[b] and Nicholas J. Turner*[c]

Received (in Cambridge, UK) 1st December 2006, Accepted 17th January 2007
First published as an Advance Article on the web 5th February 2007
DOI: 10.1039/b617537f

Enantioselective oxidation of racemic *O*-methyl-*N*-hydroxycyclohexylethylamine, using a variant of monoamine oxidase N (MAO-N) from *Aspergillus niger*, yields unreacted (*R*)-enantiomer (e.e. = 99%) together with the oxime exclusively in the (*E*)-configuration.

Enantiomerically pure *N*-hydroxylamines are widely used as precursors for a range of nitrogen-containing compounds including amino acids, carbohydrates, nucleoside analogues and pyrrolidines.[1] Methods for their preparation include asymmetric reduction of oximes,[2] direct oxidation of chiral amines[3] and asymmetric addition to nitrones.[4] A potentially attractive biocatalytic approach would be *via* kinetic resolution using hydrolytic enzymes although suprisingly there are very few reports of this type of transformation.[5] Previously we have reported a general method for the synthesis of enantiomerically pure 1°, 2° and 3° amines using the enzyme monoamine oxidase N (MAO-N) from *Aspergillus niger* as the catalyst.[6] In view of the demonstrated broad specificity of this enzyme, which has been subjected to directed evolution, we decided to examine its possible application for the enantioselective oxidation of racemic *N*-hydroxylamines (Fig. 1).

We initially investigated the oxidation of *N*-hydroxy-α-methylbenzylamine **1** using *Escherichia coli* cells expressing the MAO-N D5 variant which has previously been shown to possess high activity towards a range of structurally different amines including α-methylbenzylamine **2**.[6b] However, not only was there no conversion, but substrate **1** was found to act as a competitive inhibitor of the oxidation of amine **2**, implying at least that the former was able to bind at the active-site of the enzyme. We therefore prepared the corresponding *O*-methyl ethers **3**–**7**[7] which gratifyingly proved to be substrates, rather than inhibitors, of the MAO-N D-5 variant (Table 1). Hydroxylamines **3**, **4** and **5** reacted at approximately equal rates whereas **6** and **7** were found to be oxidised more slowly. Determination of kinetic constants for (±)-**3** using purified enzyme revealed that k_{cat} = 10.2 min^{-1}; K_M = 5.7 mM, compared to (±)-**2** where k_{cat} = 43.4 min^{-1}; K_M = 3.5 mM. Despite the lower overall activity of MAO-N D5 towards **3** compared to amine **2**, we decided to examine the preparative scale kinetic resolution of hydroxylamine **3**.

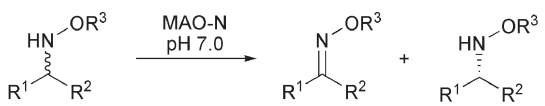
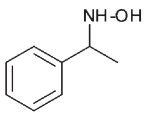
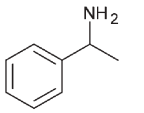
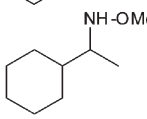
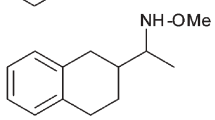
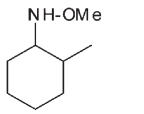
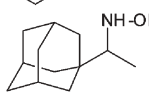
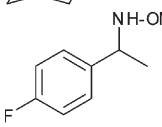


Fig. 1 Kinetic resolution of *N*-hydroxylamines using MAO-N.

Kinetic resolution of hydroxylamine **3** was carried out on a 1.2 g scale using *E. coli* cells expressing the MAO-N D-5 variant (Fig. 2).[8] After 18 h, the cells were removed by filtration, followed by extraction of the products and purification by silica chromatography to yield unreacted hydroxylamine **3** (44%; e.e. = 99% by GC) and oxime **8** (46%). The oxime **8** was found to be exclusively (*E*)-configuration by ^{1}H NMR (NOESY). The absolution configuration of recovered hydroxylamine **3** was established as (*R*)- by reduction to the amine (H_2, Pd/C, 24 h) followed by treatment with Tf_2O to give the *N*-triflamide **9** and comparison with an authentic sample by chiral GC (e.e. = 99%).

Table 1 Rates of oxidation of a series of *O*-methyl-*N*-hydroxylamines **3**–**7** using the MAO-N D-5 variant

Substrate	Structure	activity/%[a]
1		0
2		100
3		17
4		16
5		13
6		4
7		3

[a] activities are relative to amine **2** (100%) and were determined by Δ_{abs} min^{-1} at 510 nm and 30 °C: substrate (10 mM), *E. coli* cells expressing MAO-N D5, aminoantipurine, horse radish peroxidase and TBHBA.

[a] *School of Chemistry, University of Edinburgh, King's Buildings, West Mains Road, Edinburgh, UK EH9 3JJ*
[b] *AstraZeneca R&D Charnwood, Bakewell Road, Loughborough, Leicestershire, UK LE11 5H*
[c] *School of Chemistry, University of Manchester, Manchester Interdisciplinary Biocentre, 131 Princess Street, Manchester, UK M1 7DN. E-mail: nicholas.turner@manchester.ac.uk; Fax: +44 161 275 1311; Tel: +44 161 306 5173*

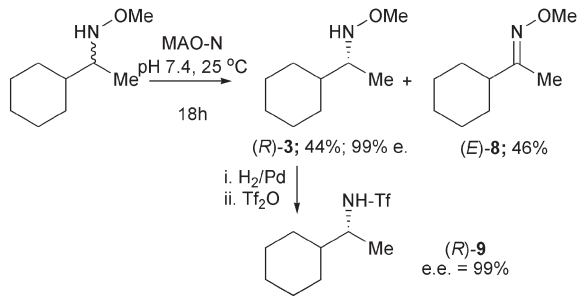


Fig. 2 Kinetic resolution of (±)-**3** using *E. coli* MAO-N D5 variant.

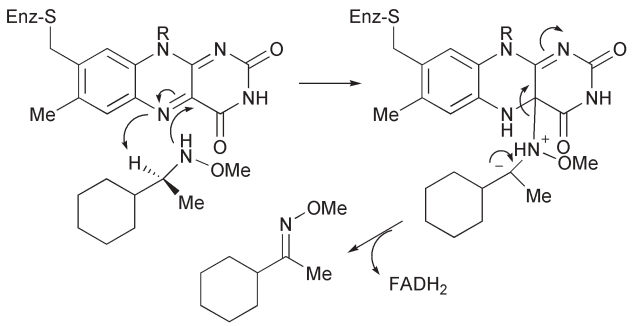


Fig. 3 Polar nucleophilic mechanism for catalysis by MAO-B/MAO-N.

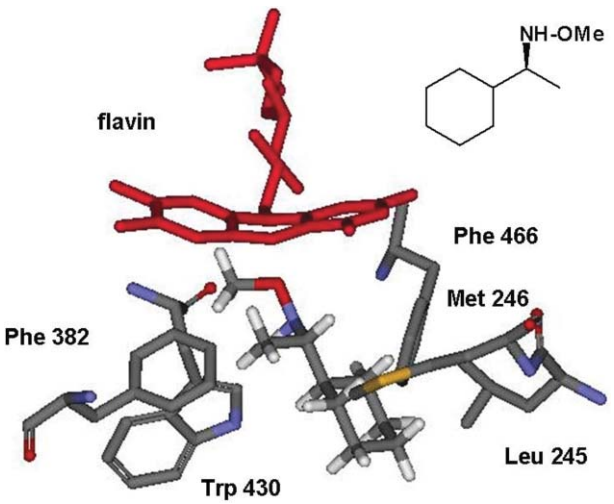


Fig. 4 Model of MAO-N with (*S*)-**3** docked at active-site.

The observed (*S*)-selectivity is in agreement with previous results obtained with the enantioselective oxidation of amines.[6]

The catalytic mechanism of the related enzyme monoamine oxidase B (MAO-B) from humans is believed to proceed *via* a polar nucleophilic mechanism as shown in Fig. 3 in which the amino group of the substrate undergoes nucleophilic attack on the C(4a) position of the flavin. The N5 atom then acts as a base to abstract the hydrogen from the substrate.[9]

Recently the structure of MAO-B has been reported[10] together with the inhibitor *para*-nitrobenzylamine bound at the active-site.[11] In order to gain insight into the high stereoselectivity obtained in the oxidation of **3** by MAO-N, we constructed an homology model of MAO-N based upon the available structure of MAO-B and used the co-ordinates of the inhibitor to position (*S*)-3 into the active-site of MAO-N (Fig. 4). Trp430 and Phe466 constitute the 'aromatic cage' of MAO-N, binding either side of the substrate, and are likely to be important in determining substrate specificity. Docking the substrate in the conformation that leads to the observed (*E*)-oxime **8** results in the *O*-Me group occupying a position just below the flavin ring and has the two hydrogen atoms that are abstracted in an *anti*-arrangement. Leu245 and Met246 appear to interact with the cyclohexyl ring and hence were targeted for further mutagenesis experiments in order to improve activity.

Using the Quikchange (Stratagene) kit, randomised libraries at positions 245 and 246 were generated and the colonies expressing enzyme variants screened for activity against **3** using a colorimetric solid-phase assay previously described.[6d] 6 colonies from the 245 library showing improved activity were picked and sequenced and all found to have Ile → Met mutation. Comparison of this variant with the parent D5 showed that it possessed *ca.* 20% greater activity, the main effect being on K_M which was lowered from 5.7 to 4.2 mM. The corresponding saturation library at 246 failed to yield any variants with enhanced activity.

In summary, *O*-methyl-*N*-hydroxyamines have for the first time been shown to be substrates for the enzyme monoamine oxidase enabling preparation of these compounds in enantiomerically pure form *via* kinetic resolution.

We are grateful to EPSRC, AstraZeneca and the Wellcome Trust for funding.

Notes and references

1 D. A. Efremov, *Comprehensive Organic Functional Group Transformations II*, 2005, **2**, 301.
2 M. P. Krzeminski and M. Zaidlewicz, *Tetrahedron: Asymmetry*, 2003, **14**, 1463.
3 H. Tokuyama, T. Kuboyama and T. Fukuyama, *Org. Synth.*, 2003, **80**, 207.
4 R. Fassler, D. E. Frantz, J. Oetiker and E. M. Carreira, *Angew. Chem., Int. Ed.*, 2002, **41**, 3054.
5 M. A. P. J. Hacking, F. Van Rantwijk and R. A. Sheldon, *J. Mol. Cat. B: Enzym.*, 2001, **11**, 315.
6 (*a*) C. J. Dunsmore, R. Carr, T. Fleming and N. J. Turner, *J. Am. Chem. Soc.*, 2006, **128**, 2224; (*b*) R. Carr, M. Alexeeva, M. J. Dawson, V. Gotor-Fernández, C. E. Humphrey and N. J. Turner, *ChemBioChem*, 2005, **6**, 637; (*c*) R. Carr, M. Alexeeva, A. Enright, T. S. C. Eve, M. J. Dawson and N. J. Turner, *Angew. Chem., Int. Ed.*, 2003, **42**, 4807; (*d*) M. Alexeeva, A. Enright, M. J. Dawson, M. Mahmoudian and N. J. Turner, *Angew. Chem., Int. Ed.*, 2002, **41**, 3177.
7 Substrates **3**–**7** were prepared by reduction ($NaCNBH_3$/MeOH) of the corresponding *O*-methyl oximes.
8 To a solution of (±)-*O*-methyl-*N*-hydroxycyclohexylethylamine **3** (1.21 g) in potassium phosphate buffer (pH 7.4, 0.1 M, 20 mL) was added a pellet of *E. coli* BL21 cells expressing MAO-N D5 (2 g wet weight). The reaction mixture was incubated at 25 °C for 18 h with shaking (200 rpm) after which the cells were removed by centrifugation (3000 rpm for 10 min) and the supernatant decanted. The cell pellet was resuspended in phosphate buffer (pH 7.4, 0.1 M, 10 mL) and then centrifuged (3000 rpm for 5 min). The combined aqueous supernatant fractions were extracted with EtOAc (3 × 25 mL) and the combined organic layers dried over anhydrous magnesium sulphate. The solvent was removed *in vacuo* to yield a residue which was purified on silica chromotagraphy (*n*-hexane 70 : 30 EtOAc to EtOAc 30 : 70 *n*-hexane) to yield (*E*)-1-cyclohexylethanone-*O*-methyl-oxime **8** [R_f 0.59 (*n*-hexane 50 : 50 EtOAc), 0.55 g, 46% yield] and (*R*)-*O*-methyl-*N*-hydroxycyclohexylethylamine **3** [R_f 0.64 (*n*-hexane 50 : 50 EtOAc), 0.53 g, 44% yield].
9 J. R. Miller and D. E. Edmondson, *Biochemistry*, 1999, **38**, 13670.
10 C. Binda, P. Newton-Vinson, F. Hubalek, D. E. Edmonson and A. Mattevi, *Nat. Struct. Biol.*, 2002, **9**, 22.
11 M. Li, C. Binda, A. Mattevi and D. E. Edmondson, *Biochemistry*, 2006, **45**, 4475.