A template-based mnemonic for monoamine oxidase (MAO-N) catalyzed reactions and its application to the chemo-enzymatic deracemisation of the alkaloid (±)-crispine A†

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A template-based mnemonic has been developed for the enzyme monoamine oxidase from *Aspergillus niger* and has been used to successfully identify the alkaloid (±)-crispine A as a target for chemo-enzymatic deracemisation yielding the biologically active (*R*)-enantiomer in 97% e.e.

We have recently reported a general method for the chemo-enzymatic deracemisation of primary,[1] secondary[2] and tertiary[3] amines in high yield and enantiomeric excess. The deracemisation process involves a two-step, one-pot reaction and employs an enantioselective amine oxidase in combination with a non-selective chemical reducing agent (Fig. 1). In the reaction shown, the enzyme enantioselectively oxidizes (*S*)-**1** to the corresponding imine or iminium ion **2**, which is then reduced *in situ* by ammonia–borane back to the racemic amine (*R*/*S*)-**1**. Starting from racemic amine substrate, repeated catalytic cycles result in eventual accumulation of (*R*)-**1** in high yield and enantiomeric excess.

An essential feature of any biocatalytic process is the ability to transform a structurally diverse range of substrates. By subjecting the monoamine oxidase from *Aspergillus niger* (MAO-N) to several rounds of directed evolution,[4] a number of variant enzymes have been identified that possess enhanced catalytic activity and considerably broader substrate specificity compared to the wild-type enzyme. For example, the previously reported MAO-N-5 variant, which contains five important mutations (Ile246Met/Asn336Ser/Met348Lys/Thr384Asn/Asp385Ser), shows high activity and enantioselectivity towards a broad range of primary,[1] secondary[2] and tertiary amines.[3] In order to encourage future applications of MAO-N as a biocatalyst for the preparation of enantiomerically pure chiral amines, we now propose a simple template-based mnemonic that should prove useful in substrate design and selection.

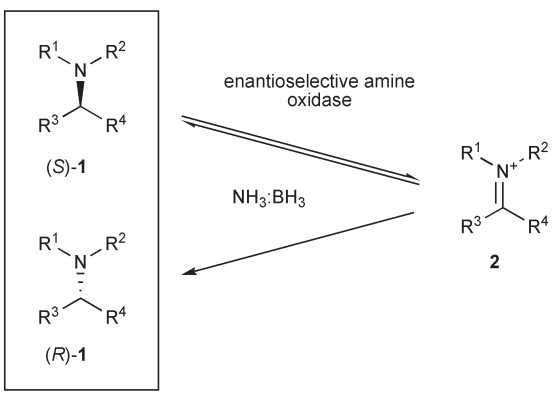

Fig. 1 Enzymatic deracemisation of racemic amines.

The template shown in Fig. 2 was generated in the following manner: (i) all substrates known to be active with MAO-N were successively superimposed onto each other in order to build up a hypothetical structure which represents an amalgam of all known reactive amines; (ii) in each case the substrates were positioned such that the N–C–H fragment (highlighted in bold) of each substrate was closely aligned. This alignment is a necessary consequence of the fact that the substrate needs to adopt a precise geometric position in order to interact with the active-site flavin prior to oxidation. All variants of MAO-N are highly (*S*)-selective so in each case only the (*S*)-enantiomer is included. The dashed bonds indicate that an aromatic ring is not an absolute requirement at this position (*e.g.* the aromatic ring can be replaced by cyclohexyl or *tert*-butyl). X indicates that a heteroatom can be tolerated at this position. Finally, the arrows indicate areas of the active-site where there is further scope for mutation (*e.g.* by targeting residues Leu245, Phe382 and Phe466) to accommodate bulkier substrates; the larger the arrow (green > black > blue > red), the greater the opportunity for accommodating amine substrates that exert a greater level of steric demand at these positions.

In order to assess the usefulness of the model in terms of predicting reactive substrates, we identified the alkaloid crispine A (**3**) as a potentially suitable target compound for preparation by deracemisation. Crispine A was first isolated from extracts of the

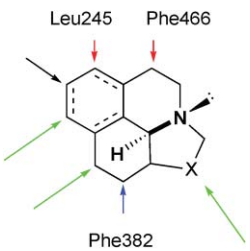

Fig. 2 A template-based mnemonic for the design of substrates for monoamine oxidase N (MAO-N).

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plant *Carduus crispus* (welted thistle) along with crispine B (**4**) and three other bicyclic isoquinoline alkaloids (Fig. 3).[5]

Crispine A (**3**) has been shown to possess certain biological activity, *i.e.* it shows cytotoxic activity against HeLa human cancer cell lines,[5] whilst compound **5** and its 5-phenyl derivatives exhibit antidepressant-like activity.[6] The deoxygenated compound **5**, which constitutes the parent framework of crispines A and B, is a known degradation product of the alkaloid norsecurinine[7] and has been previously synthesized, including an elegant route to both enantiomers from L-malic or L-tartaric acid.[8] A number of syntheses of racemic crispine A (**3**) have been reported, which utilize different protocols for the construction of the pyrrolidine ring.[9–11] Recently, the first enantioselective syntheses of this alkaloid were described, whereby an asymmetric transfer hydrogenation was employed in the key step,[12] or an asymmetric allylboration of cyclic imines was utilised.[13]

Both crispine A (**3**) and the less functionalised analogue **5** were found to map well onto the template shown in Fig. 2 and hence were predicted to be suitable candidates for chemo-enzymatic deracemisation by MAO-N. In the case of crispine A (**3**), the two methoxy groups occupy the space depicted by one of the green arrows and the black arrow and hence it was felt that this substrate would be a good probe of the steric limits imposed on the substrate by the available space at the enzyme active-site.

The requisite racemic substrates (±)-**3** and (±)-**5** were prepared as shown in Scheme 1 based upon known procedures.[14–17] Each substrate was then tested in turn with the MAO-N-5 variant amine oxidase using previously reported assay procedures.[4] In agreement with the prediction by the model, both crispine A (**3**) and the deoxygenated analogue **5** proved to be good substrates for the variant amine oxidase MAO-N-5. Initial deracemisation reactions were carried out at 10 mM substrate concentration with 3–4 equivalents of ammonia–borane as the reducing agent and washed whole cells (*Escherichia coli*) expressing the MAO-N-5 variant amine oxidase. The progress of the reactions was monitored by chiral HPLC as shown in Fig. 4. The deracemisation of (±)-**5** took only 6 hours to reach completion (e.e. = 97%) whereas (±)-crispine A (**3**) required 40 hours to reach the same enantiomeric purity (e.e. = 97%). The longer reaction time for deracemisation of (±)-**3** is presumably a consequence of the increased steric demand of the substrate and might be improved by further directed evolution of MAO-N in order to select for variants with enhanced activity towards (±)-**3**.

The specific optical rotation obtained for crispine A (**3**) ($[\alpha]_D$ = +88.4) compares favourably with the value reported for the natural material ($[\alpha]_D$ = +91.0)[12] assigning the product from the deracemisation reaction as the (*R*)-enantiomer. This result is in accordance with previous observations that MAO-N is highly

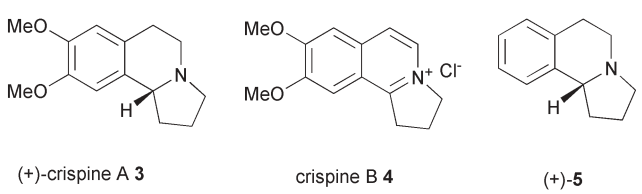
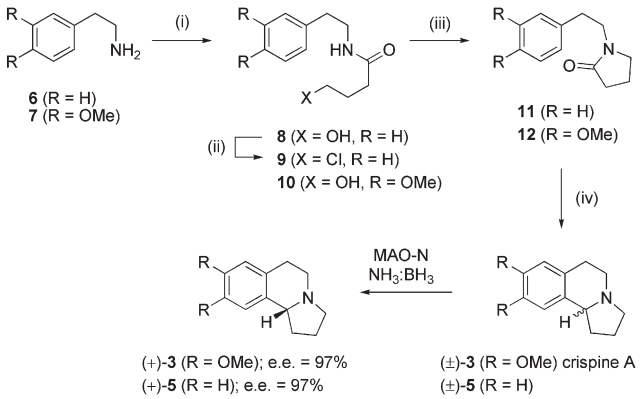


Fig. 3 The pyrrolo[2,1-*a*]isoquinoline alkaloids (**3** and **4**) from *C. crispus* and the parent framework (+)-**5**.



Scheme 1 Synthesis of (±)-crispine A (**3**) and the parent framework (±)-**5**. *Reagents and conditions*: For R = H: (i) γ-butyrolactone, neat, 120 °C, then (ii) $SOCl_2$, DCM, r.t. (87% over 2 steps); (iii) t-BuOK, EtOH, 78 °C (67%); (iv) P_2O_5, tetraline, 207 °C then $NaBH_4$, AcOH, EtOH, 0 °C (45% over 2 steps). For R = OMe: (i) γ-butyrolactone, PhMe, 110 °C (72%); (iii) and (iv) $POCl_3$, PhMe, 110 °C then $NaBH_4$, AcOH, EtOH, 0 °C (67% over 4 steps).

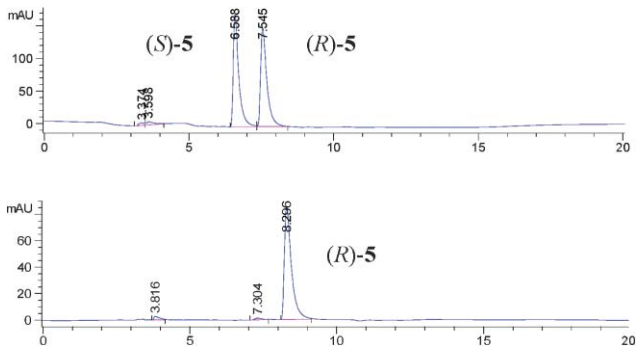


Fig. 4 Deracemisation of racemic **5** to (*R*)-(+)-**5** using the MAO-N-5 variant, as monitored by chiral HPLC.

selective for oxidation of the (*S*)-enantiomer of the substrate as shown in Fig. 1.[1–3] Similarly the specific optical rotation of **5** ($[\alpha]_D$ = +97.0) matches very well with the value previously reported ($[\alpha]_D$ = +97.6).[8]

In summary we have developed a simple template-based mnemonic for the enzyme monoamine oxidase (MAO-N) which we hope will serve as a useful guide to identify racemic amine substrates that can be subjected to chemo-enzymatic deracemisation. The template is simply based upon the steric demand of the substrate and at this stage it is not designed to take into account other properties of the substrate which could affect reactivity. However, as demonstrated above with the example of crispine A (**3**), this mnemonic can aid in the application of deracemisation as a tool not only to prepare enantiomerically pure chiral amine building blocks, but also biologically active target molecules in their own right.

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