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Deracemisation of Benzylisoquinoline Alkaloids Employing Monoamine Oxidase Variants

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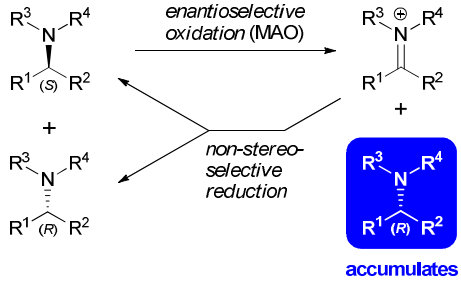
Chemo-enzymatic deracemisation was applied to obtain the (*S*)-enantiomer of 1-benzylisoquinolines from the racemate in high isolated yield (up to 85%) and excellent optical purity (*ee* >97%). The one-pot deracemisation protocol encompassed enantioselective oxidation by a monoamine oxidase (MAO-N) and concomitant reduction of the resulting iminium species by ammonia-borane. The challenge was the oxidation at the sterically demanding chiral centre. Recently developed variants of MAO-N, featuring an enlarged active-site pocket, turned out to be suitable biocatalysts for these substrates. In contrast to previous MAO-N variants, which preferentially converted the (*S*)-enantiomer, the MAO-N variant D11 used in the present study was found to oxidise all tested benzylisoquinoline substrates with (*R*)-enantiopreference. The structural determinants of enantioselectivity were investigated by means of protein–ligand docking simulations. The applicability of the deracemisation system was demonstrated on preparative scale (150 mg) for three benzylisoquinoline alkaloids (natural as well as non-natural), including the hypotensive and antispasmodic agent (*S*)-reticuline.

Introduction

1-Benzylisoquinoline alkaloids are produced by various higher plant species, for instance members of the *Papaveraceae* and *Berberidaceae* families.[1] Simple 1-benzyl-1,2,3,4-tetrahydro-isoquinolines constitute intermediates in the biosynthesis of – among others – the benzophenanthridine, protopine, and morphinan alkaloids, and as such they usually do not accumulate to substantial amounts in the plant tissue.[2] As a consequence, the availability of benzylisoquinolines from natural sources is limited, yet pharmacological studies have found diverse and potent biological effects for these substances, ranging from sedative, antispasmodic and hypotensive properties to anti-cancer and anti-HIV activity.[3] Prompted by these interesting features, many different strategies for the asymmetric synthesis of these natural products have been developed.[4] The majority of the established syntheses rely on chiral auxiliaries in the asymmetric key step,[5] and out of the few catalytic procedures that have been reported, only asymmetric hydrogenation[6] and transfer hydrogenation[7] have proven to be of general use.[8]

In Nature, benzylisoquinolines are produced in optically pure form by highly stereoselective enzymes.[2] Using these biocatalysts in synthetic organic chemistry can open up novel routes to enantiomerically pure alkaloids, often with favourable yields and enantioselectivities.[9] For instance, the broad applicability of norcoclaurine synthase (NCS) for the preparation of (*S*)-benzylisoquinolines by stereoselective Pictet–Spengler cyclisation has recently been demonstrated,[10] and berberine bridge enzyme has been used to access (*R*)-benzylisoquinolines *via* oxidative kinetic resolution of the racemate.[11] Herein, we describe the deracemisation of 1-benzylisoquinoline derivatives using engineered variants of monoamine oxidase from *Aspergillus niger* as a novel biocatalytic entry to benzylisoquinolines that offers high yields and excellent enantioselectivity.

The simultaneous one-pot combination of enantioselective oxidases with chemical reducing agents has been shown to allow the deracemisation of amino acids, keto acids, alcohols, and amines *via* a "cyclic" oxidation/reduction cascade (Scheme 1).[12]



Scheme 1 Deracemisation of amines *via* a "cyclic" oxidation/reduction cascade.

Monoamine oxidase from *Aspergillus niger* (MAO-N) has been engineered for the deracemisation of selected amines, and variants optimised for the conversion of α-chiral

primary,[13] secondary,[14] and tertiary[15] amines have been reported. The fifth-generation variant MAO-N D5 has proven suitable for preparation of the naturally occurring tetrahydroisoquinoline alkaloid (*R*)-crispine A (Fig. 1).[16]

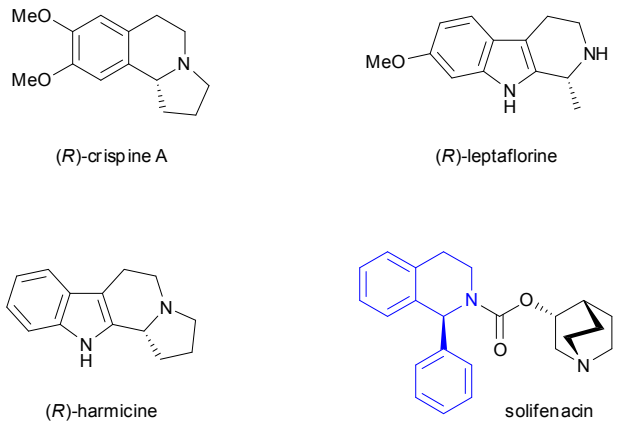


Fig. 1 Examples of alkaloids that have been obtained in optically pure form using MAO-catalysed deracemisation. The 1-phenyl-tetrahydroisoquinoline moiety of solifenacin is highlighted in blue.

Efforts to extend the scope of MAO-N to accommodate more bulky substrates have recently led to the development of variants (named D9 and D11) with a more spacious active-site pocket.[17] These biocatalysts show increased activity on crispine A,[18] and have also been used in the chemo-enzymatic asymmetric synthesis of indole alkaloids (*e.g.*, leptaflorine and harmicine) and the acetylcholine receptor antagonist solifenacin (Fig. 1).[19] Notably, the deracemisation of 1-phenyl-1,2,3,4-tetrahydroisoquinoline employing MAO-N D11 (carried out *en route* to solifenacin) afforded the (*S*)-enantiomer in 98% *ee*, which represented the first example of (*R*)-enantiopreference in amine oxidations by MAO-N.[20] This stereochemical switch prompted us to investigate the activity and enantioselectivity of MAO-N variants for benzylisoquinolines, as a structurally related class of alkaloids. Herein, we show that MAO-N D11 generally oxidises benzylisoquinolines with preference for the (*R*)-enantiomer, and that the substitution pattern on the isoquinoline ring determines the extent of enantioselectivity. Furthermore, we report on the optimisation of biocatalyst preparation, which was required for achieving satisfactory activity, and on the application of MAO-N variant D11 to the preparative-scale deracemisation of three benzylisoquinoline alkaloids.

Results and discussion

Activity and enantioselectivity screenings

In a recent study aimed at producing berbine alkaloids *via* a cascade deracemisation system, we have identified four benzyl-isoquinolines that are accepted as substrates by MAO-N D11.[21] To gain more insight into the scope of this transformation, we screened MAO-N variants D5, D9 and D11 against a panel of 15 racemic 1-benzyl-1,2,3,4-tetrahydroisoquinolines using a previously reported[13b] colorimetric assay on hydrogen peroxide formation. The substrates (Fig. 2) can be classified into three groups according to their substitution pattern: Compounds **1a** and **1b** lack any substituents on the isoquinoline ring, and are thus the sterically least demanding substrates. Compounds **2a–f** share 6,7-dimethoxy substitution on the isoquinoline ring as the common structural feature, but carry different functionalities on the benzyl moiety. Compounds **3a–g** vary in the substitution on both the isoquinoline and the benzyl ring system, although they all feature a 3'-hydroxyl group.

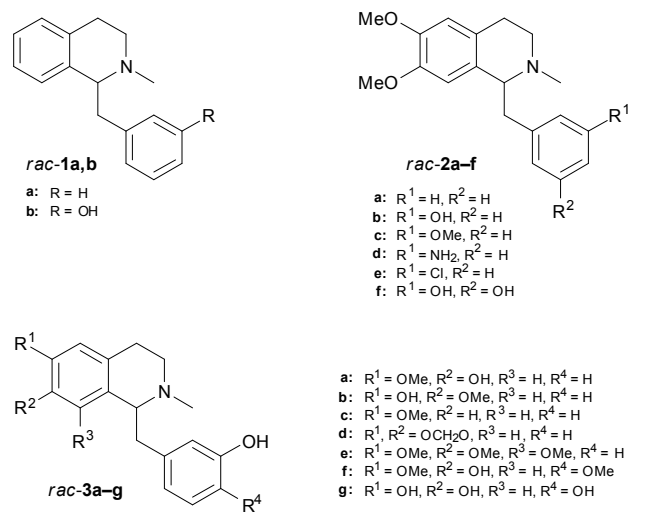


Fig. 2 Substrates used in the activity screening of MAO-N variants.

In the colorimetric assay,[13b] MAO-N D5 proved inactive on all substrates except for the positive controls *rac*-crispine A and *rac*-1-phenylethylamine (Fig. S1, ESI), while variant D9 converted five of the tested compounds, although at very limited rates (Fig. S2, ESI).[22] Variant D11 showed fair activity (1–14 U/mg at 1 mM substrate concentration) on eight benzylisoquinolines, and the rate of oxidation determined for the smallest substrate, **1a**, even exceeded that for crispine A (Fig. 3).

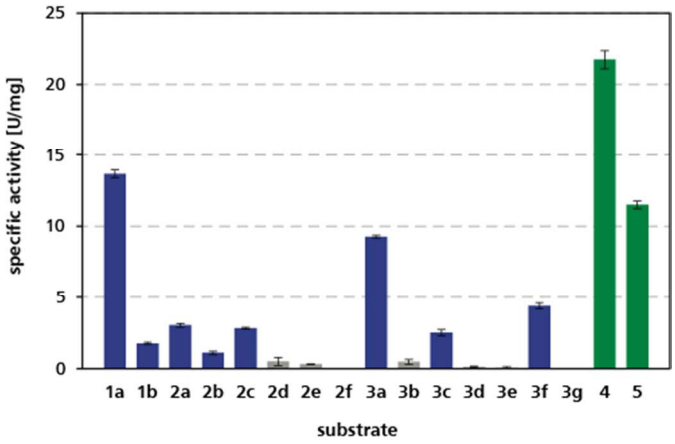


Fig. 3 Results of substrate screening with MAO-N variant D11. Activity (criterion: mean > 5 standard deviations) was found for substrates **1a**, **1b**, **2a–c**, **3a**, **3c**, and **3f** (blue) as well as the positive controls *rac*-1-phenylethylamine (**4**) and *rac*-crispine A (**5**, both green). Error ranges represent standard deviations of triplicate experiments.

Encouraged by these results, we proceeded to investigate the enantioselectivity of MAO-N D11 in the oxidation of those benzylisoquinolines that showed good activity. To this end, bacterial cells expressing the enzyme were incubated with the substrates for 24 h in the absence of a reducing agent, and the conversion as well as the *ee* of the remaining substrate were

determined by GC and/or HPLC analysis. As shown in Table 1, the (*S*)-enantiomer was in excess in all samples, indicating that the MAO-N variant preferentially oxidised the (*R*)-enantiomer of the substrates. Interestingly, this enantiopreference was excellent for six out of the eight compounds tested (E >200), while the other two – **1a** and **1b** – were converted with strikingly low selectivity (E = 4.9 and 6.5, respectively).

Table 1 Results of oxidation reactions employing *E. coli* cells expressing MAO-N variant D11

Substrate	Conversion [%]	ee^a [%]	E^b
1a	39[c]	36 (*S*)	5
1b	26[d]	24 (*S*)	7
2a	9[d]	10 (*S*)	>200
2b	2[d]	2 (*S*)	*n.d.*[e]
2c	6[d]	7 (*S*)	*n.d.*[e]
3a	29[d]	40 (*S*)	>200
3c	15[d]	18 (*S*)	>200
3f	26[d]	35 (*S*)	>200

a Enantiomeric excess of residual substrate determined by HPLC analysis on a chiral stationary phase. *b* Determined from conversion (c) and substrate *ee* (ee_S) according to the equation: $E = \ln[(1-c)\cdot(1-ee_S)]/\ln[(1-c)\cdot(1+ee_S)]$.[23] *c* Determined by GC analysis. *d* Determined by HPLC analysis using an internal standard. *e* Not determined due to low conversion.

Protein–ligand docking simulations

To elucidate the dramatic differences in enantiopreference of MAO-N towards the investigated benzylisoquinolines, protein–ligand docking simulations were carried out using the X-ray crystal structure of MAO-N D11 (PDB: 3ZDN)[19a] as receptor molecule and substrates **1a** and **3a** as ligands (for details, see Experimental section). Consistent with the experimental results, both enantiomers of **1a** were docked in conformations that allow productive interaction with the FAD prosthetic group (*i.e.*, the lone pair on N2 of the isoquinoline ring pointing towards C4a of the flavin cofactor, and the hydrogen atom at C1 positioned close to N5 of FAD; see Fig. 4, A). (*S*)-**1a** adopts a binding mode in which the benzyl substituent points into the active site, while in the docked conformation of the (*R*)-enantiomer the benzyl ring points towards amino acids Thr246 and Tyr365, located near the active-site entrance channel. These results are in good agreement with recently reported docking studies of various C1-substituted tetrahydro-β-carbolines to MAO-N D11.[19b]

In contrast, only the (*R*)-enantiomer of **3a** was successfully docked into the enzyme structure, with the substituents on the isoquinoline ring tightly accommodated in a large pocket created by the Trp430Gly mutation (Fig. 4, B, blue structure). When docking of (*S*)-**3a** is forced in a position analogous to (*S*)-**1a**, the 6-methoxy and 7-hydroxy substituents produce steric clashes with the side chains of Thr246 and Phe382, respectively, which seems to prevent (*S*)-**3a** from binding in a productive conformation. Consequently, the isoquinoline substitution pattern governs the enantioselectivity in MAO-N catalysed benzylisoquinoline oxidation.

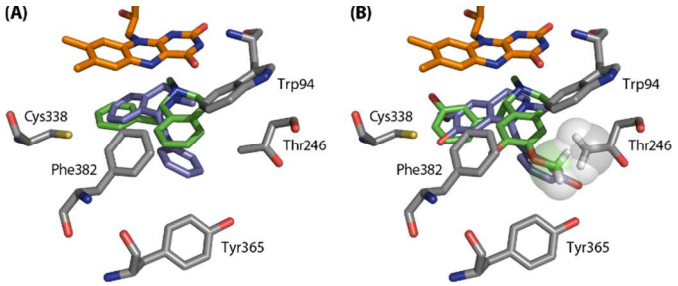


Fig. 4 Docked structures of **(A)** (*R*)-**1a** (blue) and (*S*)-**1a** (green), and **(B)** (*R*)-**3a** (blue) and (*S*)-**3a** (green, forced docking) in the active site of MAO-N D11. Active-site residues are shown in grey and the FAD cofactor is shown in orange. The steric clash between the methoxy group of (*S*)-**3a** and the side chain of Thr246 is illustrated using space-filling models.

Improvement of biocatalyst preparation

After investigating the substrate scope and enantioselectivity of the MAO-N-catalysed oxidation of benzylisoquinolines, we sought to optimise the preparative aspect of this transformation. Although six of the eight substrates were oxidised with high enantioselectivity, the conversions after 24 h of reaction time (≤ 29%, Table 1) suggested that higher biocatalyst loadings would be required. The low expression level of MAO-N (below 0.5 mg of protein per g of wet cell weight) was identified as the main source of the limited catalytic activity, and hence we decided to test alternative expression hosts and cultivation conditions in order to optimise protein production and thus the specific activity of the cells. The key to success in this regard was the use of *E. coli* C43 (DE3) as expression host[24] in combination with cultivation under auto-inducing conditions (for details on the optimisation study, see ESI).[25] These modifications resulted in a more than 3-fold improvement in the specific activity of the whole cell biocatalyst compared to the previously reported expression protocol, along with a ~5-fold increase in cell yield (Tables S4 and S5, ESI). Furthermore, it was found that lyophilised cells could be used instead of wet cells with no loss of specific activity (corrected for the weight loss caused by lyophilisation), and hence we chose to use lyophilised cells prepared by the auto-induction protocol for all further experiments.

Time-course experiments

Time-course studies of deracemisation reactions were carried out for all substrates to evaluate the stability of the biocatalyst under the process conditions (37 °C, 10% v/v DMSO, 40 mM $NH_3{\cdot}BH_3$; for details see Experimental section) and also to confirm the enantioselectivity values determined in the oxidation experiments. An initial set of experiments using 30 mg/mL cell mass revealed a good long-term performance of the system (as indicated by the linear increase in substrate *ee* over a period of 90 h; Fig. 5, green squares), but complete deracemisation was only achieved for two substrates (**3a** and **3f**). The study was therefore repeated employing 100 mg/mL of lyophilised cells. Under these conditions, borane was quickly depleted in the samples where oxidation took place with low enantioselectivity, and had to be supplemented as the reaction proceeded. Using the higher amount of MAO-N expressing cells, four substrates (**2a**, **3a**, **3c**, **3f**) could be deracemised to enantiomeric purity (*ee* > 97% by HPLC) within 2–9 days. In two cases (**1a**, **1b**) the *ee* levelled out at a

lower value (55% and 68%, respectively), and two substances (**2b**, **2c**) were still turned over too slowly to reach a plateau within 9 days of reaction time (Fig. 5). Addition of fresh biocatalyst after 7 days (where applicable) assured that the enantiomeric excess did not level out because of enzyme inactivation. Enantioselectivity values *E* of 3 and 5 were determined from the final *ee* reached in the deracemisation reactions of substrates **1a** and **1b**, respectively.[26] These values are comparable with those calculated from the substrate *ee* and conversion observed in the oxidation reactions (Table 1).

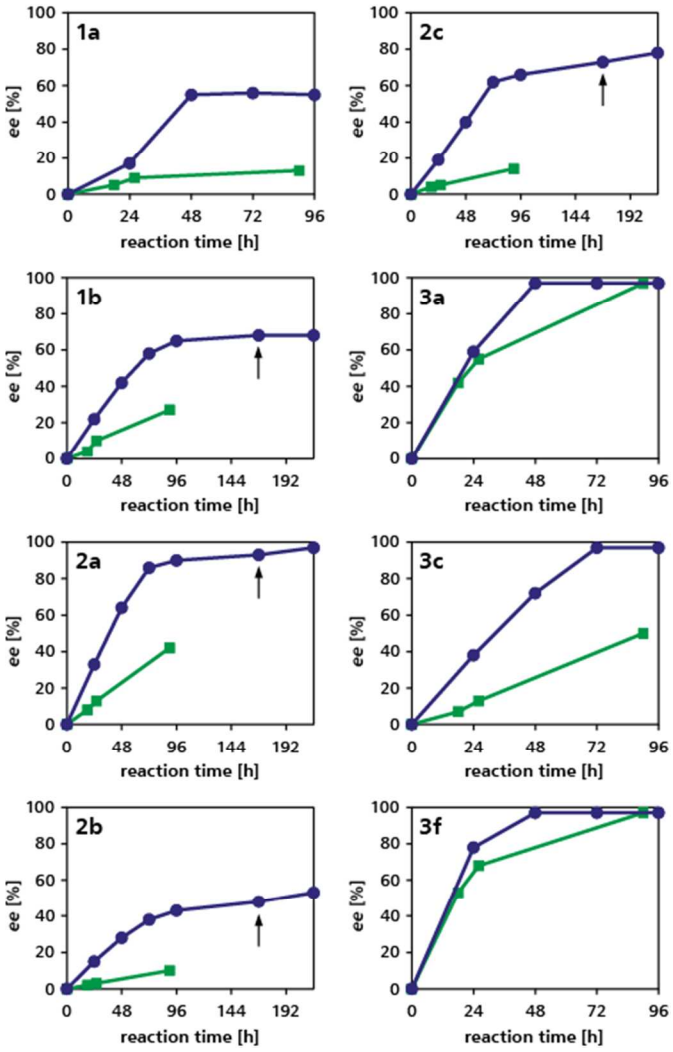
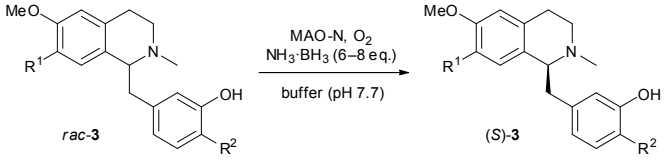


Fig. 5 Time studies of deracemisation reactions employing 30 mg/mL (green squares) or 100 mg/mL (blue circles) of lyophilised *E. coli* cells containing overexpressed MAO-N variant D11. Mind the different time scales for **1a**, **3a**, **3c** and **3f** *vs*. **1b** and **2a–c**. Arrows indicate time points where fresh biocatalyst (20 mg/mL) was added. The intermediate iminium species was not detected in any experiment.

Preparative-scale deracemisation reactions

Finally, we scaled up three deracemisation reactions to a preparative batch size, converting 0.5 mmol (142–165 mg) of the racemic benzylisoquinoline substrates. As expected based on the time-study results, the deracemisation was completed within 48 h for substrates **3a** and **3f**, while **3c** required 72 h for the reaction to reach optical purity. The deracemised alkaloids were recovered in high yields and enantiomerically pure form after column chromatography (Table 2), which demonstrates the synthetic potential of the investigated transformation. In particular, the deracemisation of reticuline **3f**, in combination with the previously reported preparation of the racemate,[11d] establishes an asymmetric total synthesis of this sedative,[3h] hypotensive,[3g] and antispasmodic[3j] natural product in 9 linear steps and with an overall yield of 13%.

Table 2 Results of preparative-scale deracemisation reactions employing ammonia-borane and lyophilised *E. coli* cells containing overexpressed MAO-N variant D11



3a: R^1 = OH, R^2 = H; **3c:** R^1 = H, R^2 = H; **3f:** R^1 = OH, R^2 = OMe

Substrate	Reaction time [h]	Yield[a] [%]	*ee*[b]
3a	48	77	>97 (*S*)
3c	72	85	>97 (*S*)
3f	48	80	>97 (*S*)

a Isolated yield after column chromatography. *b* Enantiomeric excess determined by HPLC analysis.

Conclusions

In summary, the scope of chemo-enzymatic deracemisation by combining monoamine oxidase (MAO-N) from *Aspergillus niger* and ammonia-borane has been extended to (*S*)-benzylisoquinolines, thereby establishing a novel biocatalytic synthetic route to these alkaloids. Previous studies had found that MAO-N variant D11 can oxidise amines with (*S*)- or (*R*)-enantioselectivity, depending on the substrate structure. In our present report, benzylisoquinoline alkaloids are identified as an entire class of substrates that are oxidised by MAO-N D11 with (*R*)-enantiopreference, although not all investigated compounds were converted with high selectivity. Insights into the structural determinants of enantioselectivity were gained by relating the results of extensive substrate screening to *in silico* docking simulations.

Improvement of the MAO-N expression level proved important for achieving a satisfactory specific activity of the whole-cell biocatalyst, which enabled reactions on a preparative scale. Three benzylisoquinolines were deracemised to optical purity (>97% by HPLC) and were recovered from preparative biotransformations in good yields.

Overall, our study once more demonstrates the versatility of monoamine oxidase from *Aspergillus niger* as a biocatalyst and highlights its potential for the asymmetric synthesis of alkaloids.

Experimental section

General methods and materials

^{1}H- and ^{13}C-NMR spectra were recorded using a 300 MHz instrument. Chemical shifts are given in parts per million (ppm) relative to TMS ($\delta = 0$ ppm) and coupling constants (*J*) are reported in Hertz (Hz). Melting points were determined in open capillary tubes and are uncorrected. Thin layer

chromatography was carried out on silica gel 60 F_{254} plates and compounds were visualised either by spraying with Mo reagent [100 g/L $(NH_4)_6Mo_7O_{24} \cdot 4\ H_2O$, 4 g/L $Ce(SO_4)_2 \cdot 4\ H_2O$, in 10% H_2SO_4] or by UV. Unit resolution GC–MS analyses were performed using electron impact (EI) ionisation at 70 eV and quadrupole mass selection. High resolution MS analyses were performed using electron impact (EI) ionisation at 70 eV and TOF mass selection. Optical rotation values $[\alpha]_D^{20}$ were measured at 589 nm (Na line) using a cuvette of 1 dm path length.

Unless otherwise noted, reagents and organic solvents were obtained from commercial suppliers in reagent grade quality and used without further purification. Toluene, methanol and acetonitrile used for anhydrous reactions were dried over appropriate molecular sieves (4Å for toluene, 3Å for MeOH and MeCN) for at least 48 hours. THF used for anhydrous reactions was distilled from potassium/benzophenone directly before use. For anhydrous reactions, flasks were oven-dried and flushed with dry argon just before use. Standard syringe techniques were applied to transfer dry solvents and reagents in an inert atmosphere of dry argon.

Substrates **1b**, **2b**, and **3a–f** were synthesised as previously described.[11a, 11c, d] Laudanosoline **3g** is commercially available. Synthetic procedures and full characterisation data for all other substrates are provided in the ESI.

Catalase (Lot 81H7146, from bovine liver), lysozyme from chicken egg white (cat. L6876, min. 40,000 U/mg protein), and horseradish peroxidase (cat. P6782, type VI-A, 250–330 U/mg solid) were purchased from *Sigma-Aldrich*. Pyrogallol red (cat. 207540050) was obtained from *Acros*. The MAO-N variants D5, D9, and D11 were heterologously expressed in *E. coli* as described in the ESI.

The analytical methods used for determination of conversion and enantiomeric excess are described in the ESI.

Activity screening of MAO-N variants

Stock solutions of the test substrates (20 mM; final concentration in the reaction mixture 1 mM) were prepared in DMSO. For each colorimetric reaction, the substrate stock solution (5 µL) was mixed with potassium phosphate buffer (20 µL; 100 mM, pH 7.7), pyrogallol red solution (50 µL; 0.3 mM in buffer), horseradish peroxidase solution (5 µL; 1 mg/mL in buffer) and purified MAO-N solution (20 µL; 0.4–1.1 mg/mL) in a 96-well microtitre plate. In addition, reactions with *rac*-1-phenylethylamine (1 mM) and *rac*-crispine A (1 mM) as well as negative controls (lacking substrate and MAO, respectively) were set up. All reactions, including the blanks, were performed in triplicate. Reactions were followed by measuring the absorbance at 550 nm every minute for a period of 4 h using a *Molecular Devices* SpectraMax M2 plate reader. For the faster transformations (full conversion in <30 min), reactions with diluted enzyme solution were also performed, so as to obtain at least 20 data points in the linear range of absorbance decrease. Slopes were determined by applying a linear fit to the linear range of the absorbance curve using the built-in function of the plate reader's *Molecular Devices* Softmax Pro v5.0 software. Slopes were corrected for spontaneous decolourisation of pyrogallol red (rate obtained from the blank samples) and the specific MAO activity was calculated using the formula given below.

$$A = \frac{\Delta OD \cdot Y}{\varepsilon \cdot L \cdot c_P}$$

Biotransformations

Oxidation of benzylisoquinolines catalysed by MAO-N D11. Frozen cells of *E. coli* BL21 (DE3) [pET16b MAO-N (D11)] (50 mg) were thawed and resuspended in potassium phosphate buffer (450 µL; 100 mM, pH 7.7) in Eppendorf vials (2 mL). Substrate (5 µmol, final concentration 10 mM) was dissolved in DMSO (50 µL; containing 4 mg/mL 2,3-dimethoxy-9-hydroxyberbine as internal standard) and added to the cell suspension. The reaction mixture was shaken at 37 °C and 150 rpm for 24 h. Samples using **1a**, **2a** and **2c** as substrate were basified by addition of 2 M aq. NaOH solution (100 µL). Afterwards, all samples were extracted with EtOAc (2 × 500 µL) and dried over Na_2SO_4. The solvent was evaporated under a stream of air, the sample was re-dissolved in HPLC-grade methanol (700 µL) and conversion as well as substrate *ee* were determined by GC-FID and/or HPLC analysis.

Deracemisation of benzylisoquinolines by MAO-N D11 and $NH_3 \cdot BH_3$ (time-course experiments). *Using 30 mg/mL lyophilized cells:* In Falcon tubes (15 mL), lyophilized cells of *E. coli* C43 (DE3) [pET16b MAO-N (D11)] from auto-induction cultures (75 mg) were resuspended in potassium phosphate buffer (2.25 mL; 100 mM, pH 7.7). Borane-ammonia complex (3.1 mg, final concentration 40 mM) and a solution of substrate (25 µmol, final concentration 10 mM) in DMSO (250 µL) were added to the cell suspension. The reaction mixture was shaken at 37 °C and 150 rpm for 90 h. Samples (500 µL) were taken after 18, 26 and 90 h and samples with **1a**, **2a** and **2c** as substrate were basified by addition of 2 M aq. NaOH solution (50 µL). Afterwards, all samples were extracted with EtOAc (2 × 500 µL) and dried over Na_2SO_4. The solvent was evaporated under a stream of air, the sample was re-dissolved in HPLC-grade methanol (700 µL) and the substrate *ee* was determined by HPLC analysis.

Using 100 mg/mL lyophilized cells: In Falcon tubes (15 mL), lyophilized cells of *E. coli* C43(DE3) [pET16b MAO-N (D11)] from auto-induction cultures (250 mg) were resuspended in potassium phosphate buffer (2.25 mL; 100 mM, pH 7.7). Borane-ammonia complex (3.1 mg, final concentration 40 mM) and a solution of substrate (25 µmol, final concentration 10 mM) in DMSO (250 µL) were added to the cell suspension. The reaction mixture was shaken at 37 °C and 150 rpm for 216 h. Additional borane-ammonia complex (1.5 mg) was added after 24, 48, 96, and 168 h. Fresh cell lyophilisate (50 mg) was added after 200 h. Samples (500 µL) were taken after 24, 48, 72, 96, 168 and 216 h and samples with **1a**, **2a** and **2c** as substrate were basified by addition of 2 M aq. NaOH solution (50 µL). Afterwards, all samples were extracted with EtOAc (2 × 500 µL) and dried over Na_2SO_4. The solvent was evaporated under a stream of air, the sample was re-dissolved in HPLC-grade methanol (700 µL) and the substrate *ee* was determined by HPLC analysis.

Deracemisation of benzylisoquinolines by MAO-N D11 and $NH_3 \cdot BH_3$ (preparative scale). In an Erlenmeyer flask (250 mL), lyophilized cells of *E. coli* C43 (DE3) [pET16b–MAO-N(D11)] from auto-induction cultures (5.0 g) were resuspended in phosphate buffer (45 mL; 100 mM K-P_i, pH 7.7). $NH_3 \cdot BH_3$ (62 mg, 2 mmol) and a solution of substrate

(142–165 mg, 0.5 mmol) in DMSO (5 mL) were added and the mixture was shaken at 37 °C and 150 rpm. After 24 h (and in case of **3c** after 48 h), further $NH_3{\cdot}BH_3$ (31 mg, 1 mmol) was added and shaking was continued. After the appropriate time (**3a**, **3f**: 48 h; **3c**: 72 h), a sample (250 µL) was taken, extracted with EtOAc and analysed for substrate *ee*. HPLC analysis indicated complete deracemisation, and the reaction mixture was aliquoted into Falcon tubes (50 mL) and centrifuged (1,800 × *g*, 45 min, rt) to remove the cell mass. The supernatant was extracted with EtOAc (3 × 20 mL) and the cell pellets were suspended in EtOAc, centrifuged again (1,800 × *g*, 10 min, rt) and the EtOAc phase was combined with the extracts. The combined organic phases were dried over Na_2SO_4 and the solvent was evaporated under reduced pressure to give the crude benzylisoquinoline as a yellowish liquid. Column chromatography (silica; CH_2Cl_2/MeOH/NH_3(aq) = 96/3/1) afforded (*S*)-**3a**, (*S*)-**3c**, and (*S*)-**3f**, respectively.

(*S*)-1-(3-Hydroxybenzyl)-7-hydroxy-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (*S*)-3a. 115 mg (77%) as a white solid foam. mp: 88–90°C. TLC (CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1): R_f = 0.33. $[\alpha]_D^{25}$ = +37.8 ($CHCl_3$, *c* = 0.28); lit.[27] +43 (MeOH, *c* = 0.5). ^{1}H-NMR (DMSO-d_6, 300 MHz): δ = 2.30 (3H, s, NCH_3), 2.40–2.66 (3H, m, CH_2), 2.73 (1H, dd, J_1 = 14.4 Hz, J_2 = 5.1 Hz, CH_2), 2.87 (1H, dd, J_1 = 14.3 Hz, J_2 = 7.0 Hz, CH_2), 3.02–3.10 (1H, m, CH_2), 3.56 (1H, t, *J* = 6.0 Hz, CH), 3.71 (3H, s, OCH_3), 6.43 (1H, s, Ar), 6.52–6.60 (4H, m, Ar), 6.99 (1H, t, *J* = 7.9 Hz, Ar), 8.54 (1H, s, OH), 9.11 (1H, s, OH). ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 24.9, 41.3, 42.8, 47.1, 55.9, 64.2, 112.3, 113.0, 114.8, 116.8, 120.5, 124.9, 129.0, 130.4, 142.1, 144.7, 146.3, 157.3. MS (EI, 70 eV): *m*/*z* = 298 (M^+–H, <1), 192 (100), 177 (21).

(*S*)-1-(3-Hydroxybenzyl)-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (*S*)-3c. 121 mg (85%) as a yellowish oil. TLC (CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1): R_f = 0.40. $[\alpha]_D^{25}$ = +61.9 ($CHCl_3$, *c* = 1.0) lit.[11d] (*R*): –76.3 ($CHCl_3$, *c* = 0.63). ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 2.40 (3H, s, NCH_3), 2.61–2.93 (4H, m, CH_2), 3.06 (1H, dd, J_1 = 13.8 Hz, J_2 = 5.7 Hz, CH_2), 3.15–3.23 (1H, m, CH_2), 3.68 (3H, s, OCH_3), 3.79 (1H, t, *J* = 6.5 Hz, CH), 6.46–6.54 (6H, m, Ar), 6.99 (1H, dd, J_1 = 8.7 Hz, J_2 = 7.8 Hz, Ar). ^{13}C-NMR ($CDCl_3$, 75 MHz): δ = 24.8, 41.7, 45.8, 55.2, 64.5, 111.9, 113.3, 113.8, 116.4, 121.2, 128.9, 129.1, 129.3, 129.4, 134.4, 140.9, 156.7, 157.9. MS (EI, 70 eV): *m*/*z* = 282 (M^+–H, <1), 176 (100), 132 (11).

(*S*)-1-(3-Hydroxy-4-methoxybenzyl)-7-hydroxy-6-methoxy-2-methyl-1,2,3,4-tetrahydro-isoquinoline (*S*)-3f. 132 mg (80%) as a white solid foam. mp: 75–76°C. TLC (CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1): R_f = 0.31. $[\alpha]_D^{25}$ = +58.2 ($CHCl_3$, *c* = 1.0) lit.[28] +57 ($CHCl_3$, *c* = 1.2). ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 2.38 (3H, s, NCH_3), 2.48–2.55 (1H, m, CH_2), 2.64–2.74 (3H, m, CH_2), 2.95 (1H, dd, J_1 = 14.1 Hz, J_2 = 6.1 Hz, CH_2), 3.06–3.13 (1H, m, CH_2), 3.60 (1H, t, *J* = 6.2 Hz, CH), 3.77 (6H, 2 × s overlap, 2 × OCH_3), 6.30 (1H, s, Ar), 6.46 (1H, s, Ar), 6.51 (1H, dd, J_1 = 8.2 Hz, J_2 = 2.1 Hz, Ar), 6.64–6.68 (2H, d + t overlap, Ar). ^{13}C-NMR ($CDCl_3$, 75 MHz): δ = 25.0, 40.9, 42.4, 46.7, 55.8, 55.9, 64.5, 110.4, 110.6, 113.7, 115.6, 120.9, 125.2, 130.2, 133.2, 143.3, 145.0, 145.2, 145.3. MS (EI, 70 eV): *m*/*z* = 328 (M^+–H, <1), 192 (100), 177 (21).

Protein–ligand docking simulations

Docking simulations were performed using the Discovery Studio 3.1 software from *Accelrys* with the MAO-N D11 X-ray crystal structure (PDB: 3ZDN) as template.[19a] Substrates were docked into the active site using the protocol Ligand Fit with default settings and the CHARMm force field. "Forced docking" of (*S*)-**3a** was performed by positioning the substrate into the active site, followed by energy minimisation.

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† Electronic Supplementary Information (ESI) available: results of MAO-N activity screenings for variants D5 and D9, detailed results and procedures for protein expression and purification, experimental procedures for substrate synthesis including full characterisation data for all new compounds, analytical methods, copies of NMR and MS spectra (and, where applicable HPLC traces of chiral separations) for new chemically synthesised compounds and products of biotransformations. See DOI: 10.1039/b000000x/

1 K. W. Bentley, *The Isoquinoline Alkaloids*, Harwood Academic Publishers, Amsterdam, 1998.

2 P. J. Facchini, *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 2001, **52**, 29–66.

3 (a) B. Sung, K. S. Ahn and B. B. Aggarwal, *Cancer Res.*, 2010, **70**, 3259–3268; (b) C.-P. Liu, W.-J. Tsai, C.-C. Shen, Y.-L. Lin, J.-F. Liao, C.-F. Chen and Y.-C. Kuo, *Eur. J. Pharmacol.*, 2006, **531**, 270–279; (c) W. Cui, K. Iwasa, H. Tokuda, A. Kashihara, Y. Mitani, T.

Hasegawa, Y. Nishiyama, M. Moriyasu, H. Nishino, M. Hanaoka, C. Mukai and K. Takeda, *Phytochemistry*, 2006, **67**, 70–79; (d) J. Liu and W. Couldwell, *Neurocrit. Care*, 2005, **2**, 124–132; (e) Y. Kashiwada, A. Aoshima, Y. Ikeshiro, Y.-P. Chen, H. Furukawa, M. Itoigawa, T. Fujioka, K. Mihashi, L. M. Cosentino, S. L. Morris-Natschke and K.-H. Lee, *Bioorg. Med. Chem.*, 2005, **13**, 443–448; (f) G.-M. Jow, Y.-C. Wu, J.-H. Guh and C.-M. Teng, *Life Sci.*, 2004, **75**, 549–557; (g) K. L. G. Dias, C. da Silva Dias, J. M. Barbosa-Filho, R. N. Almeida, N. de Azevedo Correia and I. A. Medeiros, *Planta Med.*, 2004, **70**, 328–333; (h) L. C. S. L. Morais, J. M. Barbosa-Filho and R. N. Almeida, *J. Ethnopharmacol.*, 1998, **62**, 57–61; (i) S. Chuliá, M. D. Ivorra, C. Lugnier, E. Vila, M. A. Noguera and P. D'Ocon, *Br. J. Pharmacol.*, 1994, **113**, 1377–1385; (j) M. L. Martin, M. T. Diaz, M. J. Montero, P. Prieto, L. San Roman and D. Cortes, *Planta Med.*, 1993, **59**, 63–67.

4 M. Chrzanowska and M. D. Rozwadowska, *Chem. Rev.*, 2004, **104**, 3341–3370.

5 For examples, see: (a) A. L. Zein, O. O. Dakhil, L. N. Dawe and P. E. Georghiou, *Tetrahedron Lett.*, 2010, **51**, 177–180; (b) Y.-C. Wang and P. E. Georghiou, *Org. Lett.*, 2002, **4**, 2675–2678; (c) A. Watanabe and J.-I. Kunitomo, *Heterocycles*, 1998, **48**, 1623–1630; (d) N. Yamazaki, H. Suzuki, S. Aoyagi and C. Kibayashi, *Tetrahedron Lett.*, 1996, **37**, 6161–6164; (e) A. I. Meyers, *Tetrahedron*, 1992, **48**, 2589–2612.

6 (a) A. Iimuro, K. Yamaji, S. Kandula, T. Nagano, Y. Kita and K. Mashima, *Angew. Chem. Int. Ed.*, 2013, **52**, 2046–2050; (b) Z.-S. Ye, R.-N. Guo, X.-F. Cai, M.-W. Chen, L. Shi and Y.-G. Zhou, *Angew. Chem. Int. Ed.*, 2013, **52**, 3685–3689; (c) P.-C. Yan, J.-H. Xie, G.-H. Hou, L.-X. Wang and Q.-L. Zhou, *Adv. Synth. Catal.*, 2009, **351**, 3243–3250; (d) S.-M. Lu, Y.-Q. Wang, X.-W. Han and Y.-G. Zhou, *Angew. Chem. Int. Ed.*, 2006, **45**, 2260–2263; (e) T. Morimoto, N. Suzuki and K. Achiwa, *Heterocycles*, 1996, **43**, 2557–2560; (f) M. Kitamura, Y. Hsiao, M. Ohta, M. Tsukamoto, T. Ohta, H. Takaya and R. Noyori, *J. Org. Chem.*, 1994, **59**, 297–310; (g) R. Noyori, M. Ohta, Y. Hsiao, M. Kitamura, T. Ohta and H. Takaya, *J. Am. Chem. Soc.*, 1986, **108**, 7117–7119.

7 N. Uematsu, A. Fujii, S. Hashiguchi, T. Ikariya and R. Noyori, *J. Am. Chem. Soc.*, 1996, **118**, 4916–4917.

8 For recent asymmetric total syntheses of benzylisoquinolines employing asymmetric transfer hydrogenation, see: (a) M. K. Pyo, D.-H. Lee, D.-H. Kim, J.-H. Lee, J.-C. Moon, K. C. Chang and H. S. Yun-Choi, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 4110–4114; (b) F. Werner, N. Blank and T. Opatz, *Eur. J. Org. Chem.*, 2007, 3911–3915; (c) D. Mujahidin and S. Doye, *Eur. J. Org. Chem.*, 2005, 2689–2693; (d) G. J. Meuzelaar, M. C. A. Van Vliet, L. Maat and R. A. Sheldon, *Eur. J. Org. Chem.*, 1999, 2315–2321.

9 J. H. Schrittwieser and V. Resch, *RSC Adv.*, 2013, **3**, 17602–17632.

10 (a) T. Pesnot, M. C. Gershater, J. M. Ward and H. C. Hailes, *Adv. Synth. Catal.*, 2012, **354**, 2997–3008; (b) B. M. Ruff, S. Bräse and S. E. O'Connor, *Tetrahedron Lett.*, 2012, **53**, 1071–1074; (c) A. Bonamore, I. Rovardi, F. Gasparrini, P. Baiocco, M. Barba, C. Molinaro, B. Botta, A. Boffi and A. Macone, *Green Chem.*, 2010, **12**, 1623–1627.

11 (a) V. Resch, H. Lechner, J. H. Schrittwieser, S. Wallner, K. Gruber, P. Macheroux and W. Kroutil, *Chem. Eur. J.*, 2012, **18**, 13173–13179; (b) V. Resch, J. H. Schrittwieser, S. Wallner, P. Macheroux and W. Kroutil, *Adv. Synth. Catal.*, 2011, **353**, 2377–2383; (c) J. H. Schrittwieser, V. Resch, J. H. Sattler, W. D. Lienhart, K. Durchschein, A. Winkler, K. Gruber, P. Macheroux and W. Kroutil, *Angew. Chem. Int. Ed.*, 2011, **50**, 1068–1071; (d) J. H. Schrittwieser, V. Resch, S. Wallner, W. D. Lienhart, J. H. Sattler, J. Resch, P. Macheroux and W. Kroutil, *J. Org. Chem.*, 2011, **76**, 6703–6714.

12 For reviews, see: (a) H. Kohls, F. Steffen-Munsberg and M. Höhne, *Curr. Opin. Chem. Biol.*, 2014, **19**, 180–192; (b) W. Kroutil, E. M. Fischereder, C. S. Fuchs, H. Lechner, F. G. Mutti, D. Pressnitz, A. Rajagopalan, J. H. Sattler, R. C. Simon and E. Siirola, *Org. Process Res. Dev.*, 2013, **17**, 751–759; (c) N. J. Turner, *Chem. Rev.*, 2011, **111**, 4073–4087; (d) J. H. Schrittwieser, J. Sattler, V. Resch, F. G. Mutti and W. Kroutil, *Curr. Opin. Chem. Biol.*, 2011, **15**, 249–256; (e) N. J. Turner, *Curr. Opin. Chem. Biol.*, 2010, **14**, 115–121; (f) T. Matsuda, R. Yamanaka and K. Nakamura, *Tetrahedron: Asymmetry*, 2009, **20**, 513–557.

13 (a) M. Alexeeva, A. Enright, M. J. Dawson, M. Mahmoudian and N. J. Turner, *Angew. Chem. Int. Ed.*, 2002, **41**, 3177–3180; (b) R. Carr, M. Alexeeva, A. Enright, T. S. C. Eve, M. J. Dawson and N. J. Turner, *Angew. Chem. Int. Ed.*, 2003, **42**, 4807–4810.

14 R. Carr, M. Alexeeva, M. J. Dawson, V. Gotor-Fernández, C. E. Humphrey and N. J. Turner, *ChemBioChem*, 2005, **6**, 637–639.

15 C. J. Dunsmore, R. Carr, T. Fleming and N. J. Turner, *J. Am. Chem. Soc.*, 2006, **128**, 2224-2225.

16 K. R. Bailey, A. J. Ellis, R. Reiss, T. J. Snape and N. J. Turner, *Chem. Commun.*, 2007, 3640–3642.

17 Amino acid exchanges in the investigated MAO-N variants with respect to the wild-type: MAO-N D5: I246M, N336S, M348K, T384N, D385S; MAO-N D9: F210L, L213T, M242E, I246T, N336S, M348K, T384N, D385S, W430H; MAO-N D11: F210L, L213T, M242E, I246T, N336S, M348K, T384N, D385S, W430G.

18 I. Rowles, K. J. Malone, L. L. Etchells, S. C. Willies and N. J. Turner, *ChemCatChem*, 2012, **4**, 1259–1261.

19 (a) D. Ghislieri, A. P. Green, M. Pontini, S. C. Willies, I. Rowles, A. Frank, G. Grogan and N. J. Turner, *J. Am. Chem. Soc.*, 2013, **135**, 10863–10869; (b) D. Ghislieri, D. Houghton, A. P. Green, S. C. Willies and N. J. Turner, *ACS Catal.*, 2013, **3**, 2869–2872.

20 More recently, reversed enantioselectivity of MAO-N variants has also been observed in the oxidation of tetrahydro-β-carbolines. See ref. 19b.

21 J. H. Schrittwieser, B. Groenendaal, V. Resch, D. Ghislieri, S. Wallner, E. M. Fischereder, E. Fuchs, B. Grischek, J. H. Sattler, P. Macheroux, N. J. Turner and W. Kroutil, *Angew. Chem. Int. Ed.*, 2014, **53**, 3731–3734.

22 Interestingly, in the samples using **2f** and **3g** as substrates, the decrease in absorbance measured was slower than in the negative controls. Thus, these substances apparently have an anti-oxidative effect and interfere with the colorimetric assay.

23 C. S. Chen, Y. Fujimoto, G. Girdaukas and C. J. Sih, *J. Am. Chem. Soc.*, 1982, **104**, 7294–7299.

24 *E. coli* C43 (DE3) has been reported to be more suitable for expression of genes with rare codons: (a) H. P. Sørensen, H. U. Sperling-Petersen and K. K. Mortensen, *J. Chromatogr. B*, 2003, **786**, 207–214. In addition, the C43 (DE3) strain has been found to be advantageous when toxic proteins are to be expressed: (b) L. Dumon-Seignovert, G. Cariot and L. Vuillard, *Protein Expression Purif.*,

2004, **37**, 203–206; (c) B. Miroux and J. E. Walker, *J. Mol. Biol.*, 1996, **260**, 289–298.

25 (a) F. W. Studier, *Protein Expression Purif.*, 2005, **41**, 207–234; (b) S. E. Deacon and M. J. McPherson, *ChemBioChem*, 2011, **12**, 593–601.

26 In "cyclic deracemization" systems, the enantioselectivity E can be calculated from the final *ee* using the formula $E = (1 + ee)/(1 - ee)$. See: W. Kroutil and K. Faber, *Tetrahedron: Asymmetry*, 1998, **9**, 2901–2913.

27 J.-M. Oger, A. Fardeau, P. Richomme, H. Guinaudeau and A. Fournet, *Can. J. Chem.*, 1993, **71**, 1128–1135.

28 K. Iwasa, Y. Doi, T. Takahashi, W. Cui, Y. Nishiyama, C. Tode, M. Moriyasu, K. Takeda, H. Minami, N. Ikezawa and F. Sato, *Phytochemistry*, 2009, **70**, 198–206.

