A Chemo-Enzymatic Route to Enantiomerically Pure Cyclic Tertiary Amines

Colin J. Dunsmore, Reuben Carr, Toni Fleming, and Nicholas J. Turner*,†

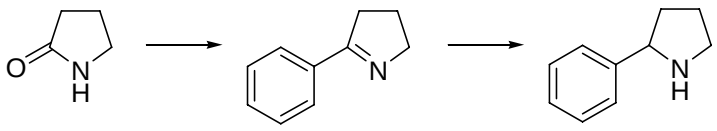
School of Chemistry, University of Edinburgh, King's Buildings, West Mains Road, Edinburgh, EH9 3JJ, UK

SUPPORTING INFORMATION

General methods

^{1}H and ^{13}C spectra were recorded on a Brüker AC250 instrument. Chemical shifts (δ) are reported in parts per million (ppm) and coupling constants (*J*) in Hz. Residual protic solvent, C*H*Cl_3 (δ_H 7.26, s) was used as the internal standard in ^{1}H NMR spectra, and ^{13}C NMR shifts were referenced using *C*DCl_3 (δ_C 77.0, t) with broad band decoupling. Electrospray (ES) nominal mass spectra were recorded using a Micromass Platform II mass spectrometer (CV=35). Microwave reactions were carried out in a CEM Discover Microwave Synthesiser. The reaction conditions were monitored using a pressure probe which was inserted through the septum of the reaction tube and black body irradiation measured to monitor the temperature of the sample. Normal phase high performance liquid chromatography (HPLC) was performed on a Waters 600 controller/pump utilising a Waters 486 tunable absorbance detector and equipped with a Chiracel-ODH column with dimensions 25 x 0.46 cm. Samples were injected via a 20 µl loop with a flow rate of 1 ml/min and eluted with an isocratic system of hexane/isopropanol (99:1). A wavelength of 254 nm was used for sample detection. Capillary electrophoresis (CE) was performed on an Agilent CE system G1601A with a capillary diameter of 50 µm and length 64.5 cm (effective length 56 cm) using a 30 kV potential and column temperature of 20 °C. Samples were injected at a pressure of 50 mbar/sec and eluted with 0.1 M potassium phosphate buffer (pH 3) containing 75 mM HP-β-cyclodextrin and 75 mM 18-crown-6. A wavelength of 195 nm was used for sample detection. Optical rotations were performed on an AA1000 polarimeter from Optical Activity Ltd. (measurements made at the sodium D-line). Concentrations are given in g/100 ml. Flash chromatography was carried out using silica gel 60H (Merck 9385, 0.04-0.063 mm, 230-400 mesh). Anhydrous solvents were purchased from Aldrich or Baker and used as received. Other solvents and reagents were standard laboratory grade and used without further purification. Commercially available substrates were purchased and used as received.

Preparation of 2-phenylpyrrolidine (2) via 2-step process from pyrrolidin-2-one



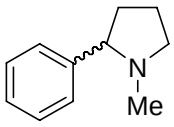
Pyrrolidin-2-one (10.0 g, 117 mmol) was dissolved in ether (150 ml) and triethylamine (17.3 ml, 123 mmol) added. The mixture was cooled to 0 °C and chlorotrimethylsilane (15.6 ml, 123 mmol) slowly added. The mixture was then refluxed for 30 min, cooled, and filtered. Phenylmagnesium bromide (39.2 ml, 117 mmol) was added to the filtrate under argon and the resulting mixture heated under reflux for 3 hours. After cooling to room temperature, the reaction was quenched with 1M HCl (60 ml) and the layers separated. The aqueous phase was basified to pH 10 with 2M NaOH solution and extracted with EtOAc (3 x 50 ml). The total organic phase was washed with brine (30 ml), dried (Na_2SO_4), and concentrated *in vacuo* to give an orange liquid (8.0 g). Purification by flash chromatography on silica gel using hexane/EtOAc (1:1) as eluent afforded 2-phenylpyrroline (5.1 g, 30 %): δ_H (250 MHz, $CDCl_3$) 2.02 (2H, m, C*H*$_2$), 2.93 (2H, m, C*H*$_2$), 4.06 (2H, m, C*H*$_2$), 7.40 (3H, m, Ar-*H*), 7.83 (2H, m, Ar-*H*); MS ES (+ve) found *m/z* 146.0 (MH^+, 100 %).

2-phenylpyrroline (1.00 g, 6.89 mmol) was dissolved in MeOH/H_2O (4:1, 25 ml) and sodium borohydride (0.29 g, 7.71 mmol) added. The mixture was then stirred at room temperature overnight. The mixture was acidified to pH 1-3 with 2M HCl and left for 30 min. 2M NaOH solution was then added until the pH was 13-14 and the product extracted with DCM (3 x 25 ml). The total organic phase was dried (Na_2SO_4) and concentrated *in vacuo* to give a colorless liquid which was purified by Kugelrohr distillation to yield 2-phenylpyrrolidine (**2**) (0.98 g, 97 %): δ_H (250 MHz, $CDCl_3$) 1.78 (3H, m, C*H*$_2$ + C*H*^{A}H^B), 2.09 (1H, s, N*H*), 2.19 (1H, m, CH^A*H*^{B}), 3.01 (1H, ddd, *J* 10.2, 8.1, 6.7, NHC*H*^{A}H^B), 3.21 (1H, ddd, *J* 10.2, 7.6, 5.4, NHCH^A*H*^{B}), 4.12 (1H, dd. *J* 7.6, 7.6, NHC*H*), 7.29 (5H, m, Ar-*H*); MS ES (+ve) found *m/z* 148.0 (MH^+, 100 %).

General procedure for the methylation of secondary amines

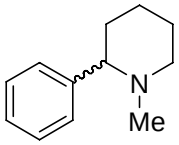
The secondary amine (0.50 g) was suspended in water (4 ml) in a microwave test tube and formic acid (1.1 eq) and formaldehyde (1.1 eq, 37 % solution in H_2O) added. The tube was sealed with a septum and heated using microwave radiation at 150 °C (120 W) for 5 min. Once cool, the mixture was basified to pH 14 with 2M NaOH solution and extracted with DCM (3 x 10 ml). The total organic phase was dried (Na_2SO_4) and concentrated *in vacuo* to give the crude tertiary amine product, which was purified by Kugelrohr distillation.

***N*-Methyl-2-phenylpyrrolidine (3)**[1]



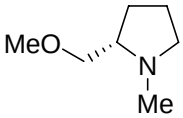
Colorless liquid, (0.32 g, 58 %): δ_H (250 MHz, $CDCl_3$) 1.98 (4H, m, 2 x C*H*$_2$), 2.18 (3H, s, NC*H*$_3$), 2.29 (1H, m, NC*H*^{A}H^B), 3.04 (1H, m, NC*H*), 3.26 (1H, m, NCH^A*H*^{B}), 7.29 (5H, m, Ar-*H*); MS ES (+ve) found *m/z* 162.0 (MH^+, 100 %).

***N*-Methyl-2-phenylpiperidine (5)**[1]



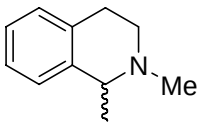
Colorless liquid, (0.22 g, 41 %): δ_H (250 MHz, $CDCl_3$) 1.35 (1H, m, C*H*^{A}H^B), 1.69 (5H, m, 2 x CH_2 + CH^A*H*^{B}), 1.99 (3H, s, NCH_3), 2.11 (1H, dd, *J* 10.7, 3.1, NC*H*^{A}H^B), 2.75 (1H, m, NC*H*), 3.03 (1H, m, NCH^A*H*^{B}), 7.26 (5H, m, Ar-*H*); MS ES (+ve) found *m/z* 175.9 (MH^+, 100 %).

(*S*)-*N*-Methyl-2-methoxymethylpyrrolidine (8)[2]



Colorless liquid, (0.06 g, 11 %): δ_H (250 MHz, $CDCl_3$) 1.74 (4H, m, 2 x CH_2), 2.18 (1H, m, NC*H*^{A}H^B), 2.34 (1H, m, NCH^A*H*^{B}), 2.38 (3H, s, NCH_3), 3.04 (1H, m, NC*H*), 3.31 (1H, dd, *J* 9.4, 5.5, MeOC*H*^{A}H^B), 3.34 (3H, s, CH_3O), 3.40 (1H, dd, *J* 9.4, 5.1, MeOCH^A*H*^{B}); δ_C (63 MHz, DEPT, $CDCl_3$) 22.7 (*C*H_2), 28.5 (*C*H_2), 41.4 (N*C*H_3), 57.7 (N*C*H_2), 59.1 (*C*H_3O), 64.8 (N*C*H), 75.7 (MeO*C*H_2); MS ES (+ve) found *m/z* 129.9 (MH^+, 100 %), 98.1 (MH^+-OMe, 8).

***N*-Methyl-2-methyl-1,2,3,4-tetrahydroisoquinoline (10)**[3]



Colorless liquid, (0.93 g, 58 %) [based on 1.47 g of starting material]: δ_H (250 MHz, $CDCl_3$) 1.40 (3H, d, *J* 6.5, CHCH_3), 2.49 (3H, s, NCH_3), 2.64 (1H, m, NCH$_2$C*H*^{A}H^B), 2.86 (2H, m, NC*H*^{A}H^{B}CH^A*H*^{B}), 3.05 (1H, m, NCH^A*H*^{B}CH$_2$), 3.62 (1H, q, *J* 6.5, C*H*CH$_3$), 7.12 (4H, m, Ar-*H*); MS ES (+ve) found *m/z* 161.9 (MH^+, 100 %).

Expression and purification of variant amine oxidase (MAO-N-5)

BL21 star was transformed with the variant MAO-N-5 amine oxidase gene and plated onto LB (70μg/ml ampicillin) petri dishes. A single colony was added to 6 x 300ml LB media containing ampicillin and grown at 30ºC for 24h. The cells were spun and the cell pellet stored at -20ºC. Lysis of the cells was performed in 25mM Tris/HCl pH 7.8, 10mM imidazole, 1mM β-mercaptoethanol, 1mM PMSF and 300mM NaCl and the lysate centrifuged. The cell-free extract from a 1g pellet was loaded onto a 1 ml Ni-NTA column. Column wash (5 column volumes); 25mM Tris/HCl pH 7.8, 60mM imidazole, 1mM β-

mercaptoethanol, 1mM PMSF and 300mM NaCl. Protein elution (the amine oxidase elutes in ~2nd-7th 1ml fractions); 25mM Tris/HCl pH7.8, 200mM imidazole, 1mM β-mercaptoethanol, 1mM PMSF and 300mM NaCl. The protein was desalted in 25mM Tris/HCl pH7.8, 1mM DTT, 1mM PMSF and 300mM NaCl using a Pharmacia PD10 column. Samples were stored frozen at -80ºC and thawed prior to use.

General procedure for determination of the relative activity of amine substrates

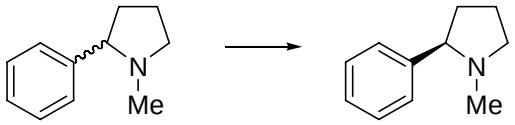
The following *assay* solution was prepared; 1M 4-aminoantipyrine (37.5µl), 2% (w/v) 2,4,6-tribromo-3-hydroxybenzoic acid in DMSO (500µl), 10mM (after dilution) of amine (54µl), 1M potassium phosphate buffer pH 7.6 (5ml) and finally water to give a final volume of 50ml.

5µl of 1mg/ml HRP (Horseradish peroxidase, Sigma P-6782) was added to 10µl of the purified MAO-N protein sample in a U-shaped 96-well microtitre plate. To this was added 185µl of the *assay* solution giving a final volume of 200µl. The colorimetric product formation was monitored by at λ = 510nm with a plate reader (Molecular Devices, VersaMax tunable microplate reader) at 30˚C (ε = 29400 M^{-1} cm^{-1})[178]. The activity of MAO-N towards the amine was calculated from the rate of change of absorbance. Relative activities were determined by dividing the rate measured for the specific amine by the rate for the reference substrate α-methylbenzylamine **1**.

General procedure for small-scale deracemization or stereoinversion of tertiary amines

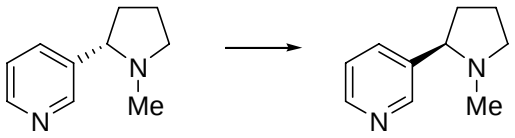
Whole cells (1.0 g) expressing the MAO-N-5 variant were thawed on ice and then suspended in 0.1 M potassium phosphate buffer pH 7 (10 ml). To this suspension was added the tertiary amine substrate (10 mM) followed by ammonia borane (100 mM). The mixture was left shaking in an incubator (37 °C, 200 rpm) and samples (200 µl) taken periodically for analysis. Samples were placed on a hot-plate set to 100 °C for 5 min to denature the enzyme, and then spun at maximum speed (13,000 rpm) on a microcentrifuge. The supernatant was decanted and analysed directly by CE, or basified to pH 13 with 2M NaOH solution and extracted into TBME for analysis by chiral HPLC.

Deracemization of *N*-methyl-2-phenylpyrrolidine (3)



Reaction monitored by CE (see General methods section for conditions): t_R 33.0 min, 99 % ee after 24 hours [t_R (*R*)-**3** = 33.0 min, t_R (*S*)-**3** = 34.0 min].

Stereoinversion of *S*-nicotine (4)

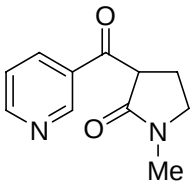


Reaction monitored by chiral HPLC (see General methods section for conditions): t_R 11.9 min, 99 % ee after 24 hours [t_R (*S*)-**4** = 10.1 min, t_R (*R*)-**4** = 11.9 min].

Procedure for preparative deracemization of *N*-methyl-2-phenylpyrrolidine (3)

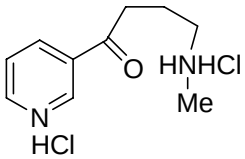
Whole cells (10.0 g) expressing the MAO-N-5 variant were thawed on ice and then suspended in 0.1 M potassium phosphate buffer pH 7 (100 ml). To this suspension was added *N*-methyl-2-phenylpyrrolidine **3** (0.40 g, 25 mM) followed by ammonia borane (0.39 g, 125 mM). The mixture was left shaking in an incubator (37 °C, 200 rpm) and samples (200 µl) taken periodically for analysis by CE. When the reaction had reached completion, the mixture was acidified to pH 1-3 with 2M HCl and centrifuged at 4000 rpm for 10 min. The supernatant was decanted and centrifuged at 4000 rpm for a further 10 min. The supernatant was decanted, basified to pH 13-14 with 2M NaOH solution, and continuously extracted with TBME overnight. The TBME phase was concentrated *in vacuo* to afford the product as a colorless liquid (0.30 g, 75 %): $[\alpha]^{RT}_D$ = +162° (*c* 1.0, $CHCl_3$, 99 % ee) [lit.[4] $[\alpha]^{RT}_D$ = +156.5° (neat)]; ^{1}H NMR identical to that described above for *N*-methyl-2-phenylpyrrolidine **3**.

Preparation of 3-(3'-pyridyl)-1-methylpyrroldin-2-one[5]



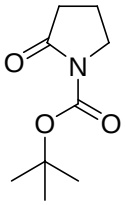
N-Methylpyrrolidin-2-one (5.00 g, 0.05 mol) in anhydrous THF (5 ml) was added dropwise to a suspension of sodium hydride (60 % in oil, 6.05 g, 0.15 mol) in anhydrous THF (40 ml) and the resulting mixture stirred for 15 min at room temperature. A solution of ethyl nicotinate (13.80 ml, 0.10 mol) in anhydrous THF (5 ml) was then added dropwise and the mixture heated under reflux for 24 hours. After allowing to cool, the mixture was poured into ice-cold 4M HCl (50 ml). Solid NaOH was used to carefully adjust the pH of the solution to 4, and the aqueous was then extracted with DCM (6 x 15 ml). The total organic phase was concentrated *in vacuo* and the residue taken-up in DCM (50 ml), dried ($MgSO_4$), and concentrated *in vacuo* to give a brown oil (10.0 g). Purification by flash chromatography on silica gel using EtOAc/MeOH (9:1) as eluent gave a yellow oil (5.30 g, 51 %): δ_H (250 MHz, $CDCl_3$) 2.19 (1H, m, C*H*^{A}H^B), 2.63 (1H, m, CH^A*H*^{B}), 2.79 (3H, s, NC*H*$_3$), 3.44 (2H, m, C*H*$_2$), 4.39 (1H, dd, *J* 9.2, 5.3, COC*H*CO), 7.34 (1H, ddd, *J* 8.0, 4.8, 0.5, Ar-*H*), 8.36 (1H, dt, *J* 8.0, 1.9, Ar-*H*), 8.72 (1H, dd, *J* 4.8, 1.6, Ar-*H*), 9.24 (1H, d, *J* 1.9, Ar-*H*); MS ES (+ve) found *m/z* 226.9 (MNa^+, 100 %), 204.9 (MH^+, 72).

Preparation of 4-(methylamino)-1-(3'-pyridyl)-1-butanone dihydrochloride (11)[5]



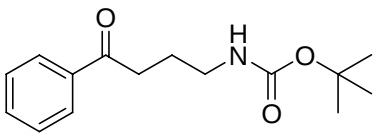
3-(3'-pyridyl)-1-methylpyrroldin-2-one (5.30 g, 25.95 mmol) was dissolved in 5M HCl and heated under reflux for 72 hours. The solution was allowed to cool and concentrated *in vacuo* and the residue taken-up in water (100 ml). The mixture was filtered and the filtrate concentrated *in vacuo* to give a brown residue that was purified by recrystallization from EtOH/H_2O to afford a pale brown solid (3.15 g, 48 %): δ_H (250 MHz, D_2O) 1.94 (2H, m, C*H*$_2$), 2.54 (3H, s, NC*H*$_3$), 2.95 (2H, t, *J* 7.8, C*H*$_2$), 3.17 (2H, t, *J* 6.8, C*H*$_2$), 8.04 (1H, dd, *J* 8.2, 6.0, Ar-*H*), 8.79 (1H, br-d, *J* 6.0, Ar-*H*), 8.90 (1H, dt, *J* 8.2, 1.7), 9.13 (1H, d, *J* 1.7, Ar-*H*); MS ES (+ve) found *m/z* 178.8 (MH^+-2HCl, 53 %), 160.8 (89), 147.8 (100).

Preparation of *t*-Boc protected pyrrolidin-2-one (15)[6]



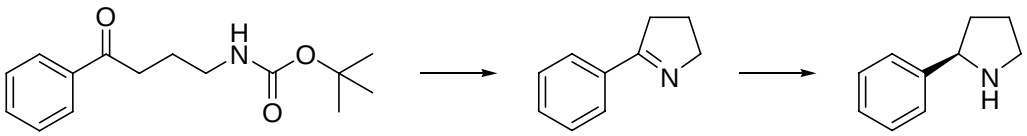
To a stirred solution of pyrrolidin-2-one (1.80 ml, 23.53 mmol) in anhydrous THF (50 ml) at -78 °C was added *n*-butyllithium (1.6 M, 14.69 ml, 23.53 mmol) dropwise, maintaining the temperature below -70 °C. The mixture was allowed to stir for 1 hour before a solution of di-*tert*-butyldicarbonate (5.13 g, 23.53 mmol) in anhydrous THF (10 ml) was slowly added over 30 min. The reaction was stirred for a further 2 hours and then allowed to warm to room temperature. Saturated NH_4Cl solution (25 ml) was added together with water (12 ml) and the organic layer separated. The aqueous phase was extracted with diethyl ether (3 x 25 ml) and the total organic phase washed with brine (50 ml), dried ($MgSO_4$), and concentrated *in vacuo* to give a yellow oil (4.44 g). Purification by flash chromatography on silica gel using hexane/EtOAc (1:4) as eluent afforded a pale yellow oil (2.69 g, 62 %): δ_H (250 MHz, $CDCl_3$) 1.49 (9H, s, C(C*H*$_3$)$_3$), 1.97 (2H, m, C*H*$_2$), 2.48 (2H, m, C*H*$_2$), 3.72 (2H, m, C*H*$_2$); MS ES (+ve) found *m/z* 186.2 (MH^+, 18 %), 208.1 (MNa^+, 100).

Preparation of (5-oxo-5-phenyl-butyl)-carbamic acid *tert*-butyl ester (16)[6]



To a stirred solution of *t*-Boc protected pyrrolidin-2-one **15** (2.60 g, 14.04 mmol) in anhydrous THF (55 ml) at -78 °C was added phenylmagnesium bromide (1.0 M, 16.80 ml, 16.84 mmol) dropwise over 1 hour, maintaining the temperature below -70 °C. The solution was allowed to warm to room temperature and the pH adjusted to 1-3 with 2M HCl. The layers were separated and the aqueous extracted with DCM (3 x 25 ml). The total organic phase was dried ($MgSO_4$) and concentrated *in vacuo* to give a viscous yellow oil (2.99 g). Trituration with diethyl ether and filtration afforded a white solid (0.95 g, 26 %): mp 78-80 °C [lit.[6] mp 95-96 °C]; δ_H (250 MHz, $CDCl_3$) 1.41 (9H, s, C(CH_3)$_3$), 1.93 (2H, m, CH_2), 3.01 (2H, t, *J* 7.2, CH_2), 3.21 (2H, m, CH_2), 4.70 (1H, br-s, N*H*), 7.50 (3H, m, Ar-*H*), 7.94 (2H, m, Ar-*H*); MS ES (+ve) found *m/z* 264.0 (MH^+, 100 %), 281.1 (M^++H_2O, 66).

Preparation of (*R*)-2-phenylpyrrolidine via intramolecular reductive amination



To a solution of (5-oxo-5-phenyl-butyl)-carbamic acid *tert*-butyl ester **16** (0.20 g, 0.76 mmol) in DCM (5 ml) at 0 °C was added TFA (1 ml). The mixture was stirred at room temperature and the reaction progress monitored by TLC. Once complete, the reaction was basified with 4M NaOH solution to pH 13-14, the layers separated, and the aqueous phase extracted with DCM (3 x 10 ml). The total organic phase was dried ($MgSO_4$) and concentrated *in vacuo* to afford the crude 2-phenylpyrroline. To a suspension of whole cells (1.0 g) expressing the MAO-N-5 variant in 0.1 M potassium phosphate buffer (pH 7) was added the crude 2-phenylpyrroline (14.5 mg, 10 mM) followed by ammonia borane (30.9 mg, 100 mM). The mixture was left shaking in an incubator (37 °C, 200 rpm) overnight. A sample (200 µl) was taken and placed on a hot-plate set to 100 °C for 5 min to denature the enzyme. The sample was then spun at maximum speed (13,000 rpm) on a microcentrifuge and the supernatant decanted and analysed directly by CE (see General methods section for conditions): t_R 24.8 min, 99 % ee, [t_R (*R*)-**2** = 24.8 min; t_R (*S*)-**2** = 25.1 min].

References

(1) Lewis, F. D.; Reddy, G. D.; Schneider, S.; Gahr, M. *J. Am. Chem. Soc.* **1991**, *113*, 3498.

(2) Perry, R. A.; Chen, S. C.; Menon, B. C.; Hanaya, K.; Chow, Y. L. *Can. J. Chem.* **1976**, *54*, 2385.

(3) Ebden, M. R.; Simpkins, N. S.; Fox, D. N. A. *Tetrahedron* **1998**, *54*, 12923.

(4) Malmberg, H.; Nilsson, M.; Ullenius, C. *Acta Chem. Scand. B* **1981**, *35*, 625.

(5) Pathak, T.; Thomas, N. F.; Akhtar, M.; Gani, D. *Tetrahedron* **1990**, *46*, 1733.

(6) Williams, G. D.; Pike, R. A.; Wade, C. E.; Wills, M. *Org. Lett.* **2003**, *5*, 4227.