Supplementary Material (ESI) for Chemical Communications

A Template-Based Mnemonic for Monoamine Oxidase (MAO-N) Catalyzed Reactions and its Application Towards the Chemo-Enzymatic Deracemisation of the Alkaloid (±)-Crispine A

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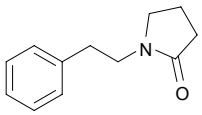
SUPPORTING INFORMATION

General methods

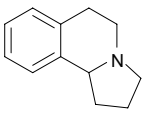
Commercially available reagents were used as received without purification. Analytical thin layer chromatography was performed with Keiselgel 60 F_{254}, in a variety of solvents on plastic-backed plates. The plates were visualised by UV light (254 nm) and potassium permanganate. Flash column chromatography was conducted with Merck silica gel 60H (40-60 μm, 230-400 mesh) under bellows pressure. Nominal mass spectra were recorded on an Agilent 1100 series spectrometer using electrospray (ES). ^{1}H and ^{13}C NMR spectra were recorded on either a Bruker Avance 300 (300 MHz) or Bruker DPX 400 (400 MHz) spectrometer as stated. All chemical shifts (δ) are quoted in parts per million (ppm) relative to a calibration reference of the 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. The following abbreviations were used to define the multiplicities: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br., broad. The coupling constants (*J*) are measured in hertz (Hz). Petroleum ether refers to the fraction of petroleum ether that boils between 40 and 60 °C. Normal phase high performance liquid chromatography (HPLC) was performed either on an Agilent 1100 or 1200 chromatograph with a Chiracel-ODH column with dimentions 25 × 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 *iso*-hexane/*iso*-propanol (90:10) for **3** or *iso*-hexane/*iso*-propanol (98:2) for **5**. Wavelengths of 210 nm and 220 nm were 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.

Preparation of 1-phenethylpyrrolidin-2-one (11)[1]



2-Phenylethylamine (16.5 mL, 131 mmol) and γ-butyrolactone (10 mL, 131 mmol) were heated together at 120 °C for 3 h. Upon allowing to cool the reaction mixture solidified to give 26.8 g of a crude yellow solid (**8**). The crude material (**8**) (20.4 g) was dissolved in dichloromethane (200 mL) and cooled to 0 °C. Thionyl chloride (36 mL, 494 mmol) was slowly added and the reaction allowed to warm to room temperature after 1 h. After a further 30 min at room temperature the reaction mixture was reduced *in vacuo*. Passing the crude product through a plug of silica gel (50% ethyl acetate in petroleum ether) gave 13.6 g of an off-white solid (**9**). The semi-pure solid (**9**) (2.51 g) was dissolved in ethanol (50 mL) and potassium *tert*-butoxide (1.87 g) was added and the reaction stirred at reflux for 2 h. The reaction was allowed to cool and the residual oil partitioned between dichloromethane (20 mL) and water (20 mL). The layers were separated and the aqueous layer extracted with dichloromethane (20 mL). The combined organic layers were then reduced *in vacuo* and purified by flash column chromatography (diethyl ether, SiO_2) to yield the title compound (**11**) (1.15 g, 55%) as a colourless oil. δ_H (300 MHz, $CDCl_3$) 1.95 (2H, m, $CH_2CH_2CH_2$), 2.36 (2H, t, *J* 8.1, CH_2CO), 2.84 (2H, t, *J* 7.4, CH_2Ar), 3.25 (2H, t, *J* 7.1, $ArCH_2CH_2N$), 3.53 (2H, t, *J* 7.4, $CO(CH_2)_2CH_2N$), 7.21-7.33 (5H, m, Ar-*H*); MS ES (+ve) found *m/z* 190.0 (MH^+, 100%).

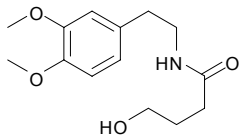
Preparation of (±)-1,2,3,5,6,10b-hexahydropyrrolo[2,1-*a*]isoquinoline (5)[1]



Phosphorous pentoxide (2.5 g) was heated at 207 °C in tetralin (10 mL) for 20 min. 1-Phenethylpyrrolidin-2-one (**11**) (1.0 g, 5.3 mmol) was dissolved in tetralin (2.5 mL) and added to the above suspension. After a further 20 min, a further portion of phosphorous pentoxide (4.0 g) and tetralin (8 mL) were added and the heating was continued. After 1 h, the cooled reaction mixture was treated with large amounts of ice and then basified with cold

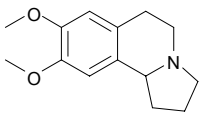
concentrated potassium hydroxide. The alkaline mixture was extracted with diethyl ether (20 mL) and the organic layer washed wih brine (10 mL), dried ($MgSO_4$) and reduced *in vacuo*. The residual oil was dissolved in ethanol (20 mL) and cooled to 0 °C, after which sodium borohydride (450 mg) and acetic acid (0.1 mL) were added and the reaction was allowed to warm to room temperature overnight. Water (10 mL) was added and the ethanol removed *in vacuo*. The product was extracted with dichloromethane (2 × 10 mL) and the combined organic layers were washed with brine (10 mL), dried ($MgSO_4$) and reduced *in vacuo*. The product was purified by flash column chromatography (60% ethyl acetate in petroleum ether + 1% triethylamine, SiO_2) to yield the title compound (**5**) (415 mg, 45%) as an orange oil. δ_H (300 MHz, $CDCl_3$) 1.67-1.81 (1H, m), 1.83-1.99 (2H, m), 2.31-2.41 (1H, m), 2.52 (1H, q, *J* 8.6), 2.64 (1H, ddd, *J* 15.3, 10.5, 5.0), 2.83 (1H, dt, *J* 16.5, 3.6), 3.06-3.14 (2H, m), 3.16-3.24 (1H, m), 3.41 (1H, t, *J* 8.1), 7.06-7.16 (4H, m); δ_C (75 MHz, $CDCl_3$) 22.1, 28.6, 30.1, 48.5, 53.4 (CH_2), 63.4, 125.5, 125.7, 125.9, 128.4 (CH), 134.2, 138.9 (C); MS ES (+ve) found *m/z* 174.3 (MH^+, 100%).

Preparation of *N*-(3,4-dimethoxyphenethyl)-4-hydroxybutanamide (10)[2]



2-(3,4-Dimethoxyphenyl)ethylamine[3] (126 mg, 0.7 mmol) and γ-butyrolactone (59 μL, 0.77 mmol) in toluene (7 mL) were heated at 110 °C for 20 h. Upon allowing to cool the solvent was reduced *in vacuo* and the crude product purified by flash column chromatography (10% MeOH in dichloromethane, SiO_2) to yield the title compound (**10**) (135 mg, 72%) as a yellow oil. δ_H (300 MHz, $CDCl_3$) 1.75-1.83 (2H, m), 2.26 (2H, t, *J* 7.2), 2.71 (2H, t, *J* 7.2), 3.43 (2H, q, *J* 6.7), 3.59 (2H, t, *J* 5.6), 3.81 (3H, s), 3.82 (3H, s), 6.16 (1H, br.s), 6.68-6.78 (3H, m); δ_C (75 MHz, $CDCl_3$) 28.0, 33.7, 35.0, 40.7(CH_2), 55.7, 55.8 (CH_3), 61.9 (CH_2), 111.2, 111.8, 120.5 (CH), 131.2, 147.5, 148.8, 173.5 (C).

Preparation of (±)-crispine A (3)[2]



N-(3,4-Dimethoxyphenethyl)-4-hydroxybutanamide (**10**) (781 mg, 2.93 mmol) was suspended in toluene (29 mL) and phosphorous(III) oxychloride (2.81 mL, 30.7 mmol) was added. The reaction mixture was heated at 110 °C for 4 h. The reaction was allowed to cool and the solvent removed *in vacuo*. The resulting brown oil was dissolved in EtOH (11 mL) and acetic acid (1 mL) added. Sodium borohydride (225 mg, 6.0 mmol) was added and the reaction was stirred at room temperature for 16 h. The reaction was carefully quenched with water (5 mL) and the EtOH was removed *in vacuo*. Further water (5 mL) was added and the product was extracted with DCM (3 × 5 mL). The combined organic layers were washed with 2M NaOH (3 × 5 mL) and the combined aqueous layers extracted with DCM (5 mL). The organic layers were combined and washed with brine (5 mL), dried ($MgSO_4$) and reduced *in vacuo*. The product was purified by flash column chromatography (5% MeOH in DCM, SiO_2) to yield the title compound (**3**) (457 mg, 67%) as a pale yellow solid. δ_H (300 MHz, $CDCl_3$) 1.67-1.76 (1H, m), 1.83-1.95 (2H, m), 2.29-2.37 (1H, m), 2.64 (1H, q, *J* 8.5), 2.66-2.71 (1H, m), 2.73 (1H, br. dt, *J* 16.1, 3.8), 2.93-3.00 (1H, m), 3.02-3.07 (1H, m), 3.12-3.17 (1H. m), 3.51 (1H, br. t, *J* 6.0), 3.82 (6H, s), 6.54 (1H, s), 6.58 (1H, s); δ_C (75 MHz, $CDCl_3$) 22.1, 27.6, 30.5, 48.1, 53.0 (CH_2), 55.8, 55.9 (CH_3), 62.6, 108.7, 111.1 (CH), 125.8, 130.2, 147.2, 147.3 (C); MS ES (+ve) found *m/z* 234.1 (MH^+, 100%).

For HPLC analysis:

Procedure for the deracemisation of (3)

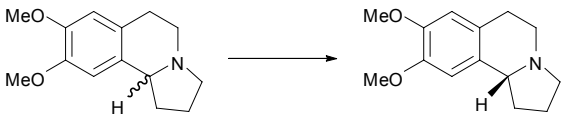
Whole wet cells (~440 mg) expressing the MAO-N-5 variant were thawed and suspended in 0.1 M potassium phosphate buffer pH 7.6 (2.46 mL). To this suspension was added the tertiary amine substrate (6.0 mg, 0.026 mmol) followed by ammonia borane (3.5 mg, 0.11 mmol). The mixture was left shaking in an incubator (30 °C) and samples (0.5 mL) taken periodically for analysis. Samples were spun at maximum speed (13,000 rpm) on a microcentrifuge, and the supernatant was decanted. The aqueous layer was filtered through a 0.2 μm inline syringe filter and analysed directly by chiral HPLC, at t = 40 h: t_R 18.6 minutes, 97% ee (OD-H column, 90% *iso*-hexane in *iso*-propanol, 1.0 mL/minute, 210 nm).

Procedure for the deracemisation of (5)

Whole wet cells (~630 mg) expressing the MAO-N-5 variant were thawed and suspended in 0.1 M potassium phosphate buffer pH 7.6 (5 mL). To this suspension was added *iso*-hexane (5 mL), the tertiary amine substrate (17 mg, 0.10 mmol) followed by ammonia borane (9.5 mg, 0.31 mmol). The mixture was left shaking in an incubator (30 °C) and samples

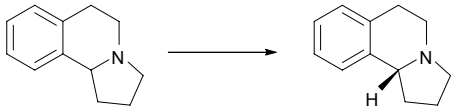
(2.0 mL) taken periodically for analysis. Samples were spun at maximum speed (13,000 rpm) on a microcentrifuge, and the supernatant was decanted. The aqueous layer was extracted with ethyl acetate and the combined organic layers reduced *in vacuo*, the residue was dissolved in *iso*-propanol and analysed by chiral HPLC, at t = 6 h: t_R 8.13 minutes, 97% ee (OD-H column, 98% *iso*-hexane in *iso*-propanol, 1.0 mL/minute, 220 nm).

For isolated material:
Deracemisation of (±)-crispine A (3)

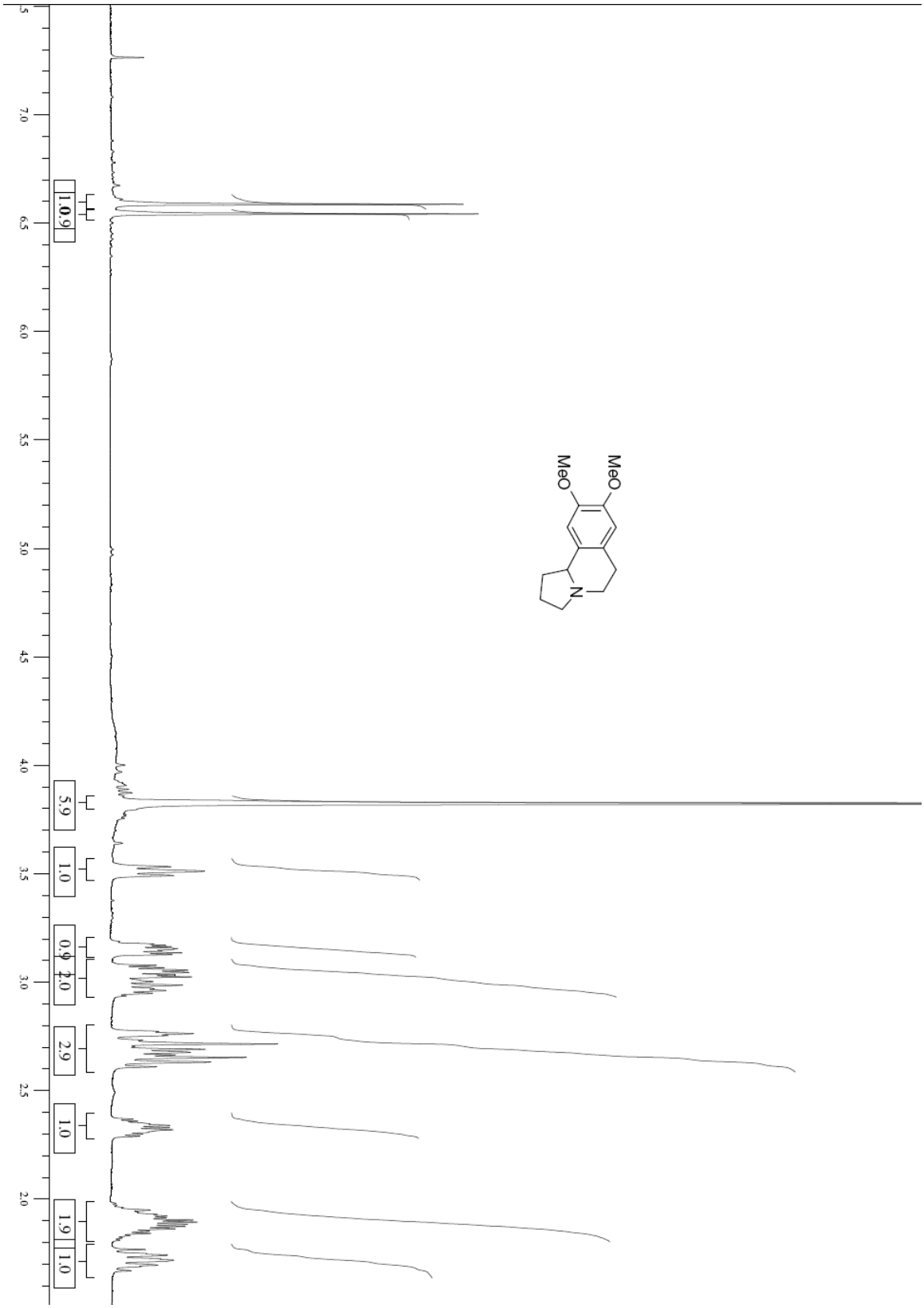


Whole wet cells (~680 mg) expressing the MAO-N-5 variant were thawed and suspended in 0.1 M potassium phosphate buffer pH 7.6 (3.85 mL). To this suspension was added the tertiary amine substrate (9.4 mg, 0.04 mmol) followed by ammonia borane (5.5 mg, 0.18 mmol). The mixture was left shaking in an incubator (30 °C) for 144 h. The reaction mixture was spun at maximum speed (13,000 rpm) on a microcentrifuge, and the supernatant was decanted and extracted with dichloromethane. The dichloromethane phase was dried ($MgSO_4$) and concentrated *in vacuo* to yield the title compound as a colourless oil (4 mg, 43%): $[\alpha]_D$ +88.4 (c1.0, $CHCl_3$, 97% ee) [lit.[2] +91.0 (MeOH)]; 1H NMR was identical to that described above for (±)-crispine A (**3**).

Deracemisation of (±)-1,2,3,5,6,10b-hexahydropyrrolo[2,1-*a*]isoquinoline (5)



Whole wet cells (~600 mg) expressing the MAO-N-5 variant were thawed and suspended in 0.1 M potassium phosphate buffer pH 7.6 (14.45 mL). To this suspension was added *iso*-hexane (14.45 mL), the tertiary amine substrate (50 mg, 0.29 mmol) followed by ammonia borane (26 mg, 0.84 mmol). The mixture was left shaking in an incubator (30 °C) for 22 h. The reaction mixture was spun at maximum speed (13,000 rpm) on a microcentrifuge, and the supernatant was decanted and the product extracted with ethyl acetate. The ethyl acetate phase was concentrated *in vacuo* and the product was purified by flash column chromatography (60% ethyl acetate in petroleum ether, SiO_2) to yield the title compound (+)-(**3**) (17 mg, 34%) as a colourless oil: $[\alpha]_D$ + 97.0 (c1.0, MeOH, 97% ee) [lit.[4] +97.6 (c0.6, MeOH)]; 1H NMR was identical to that described above for (±)-1,2,3,5,6,10b-hexahydropyrrolo[2,1-*a*]isoquinoline (**5**).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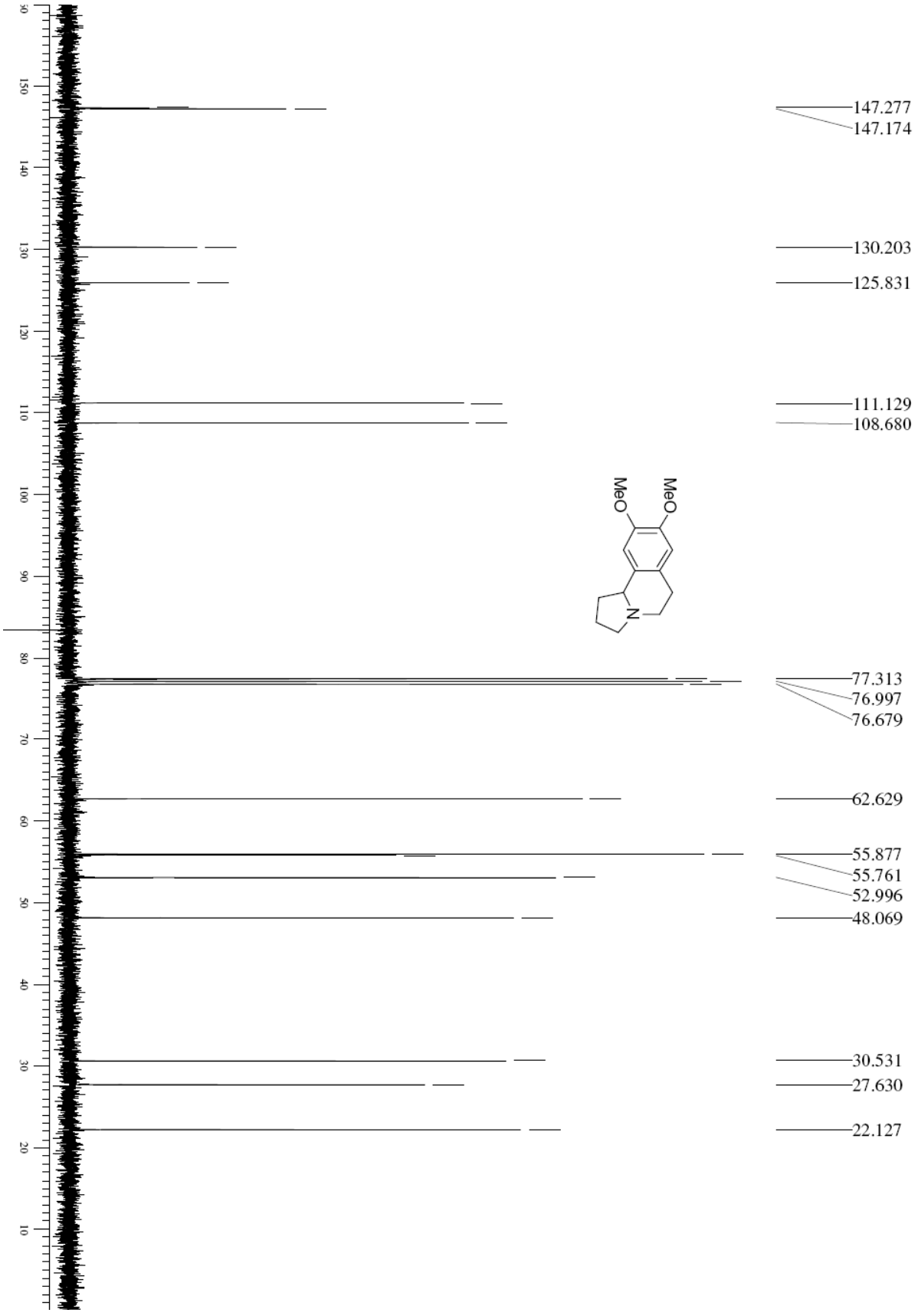
2.0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MeO
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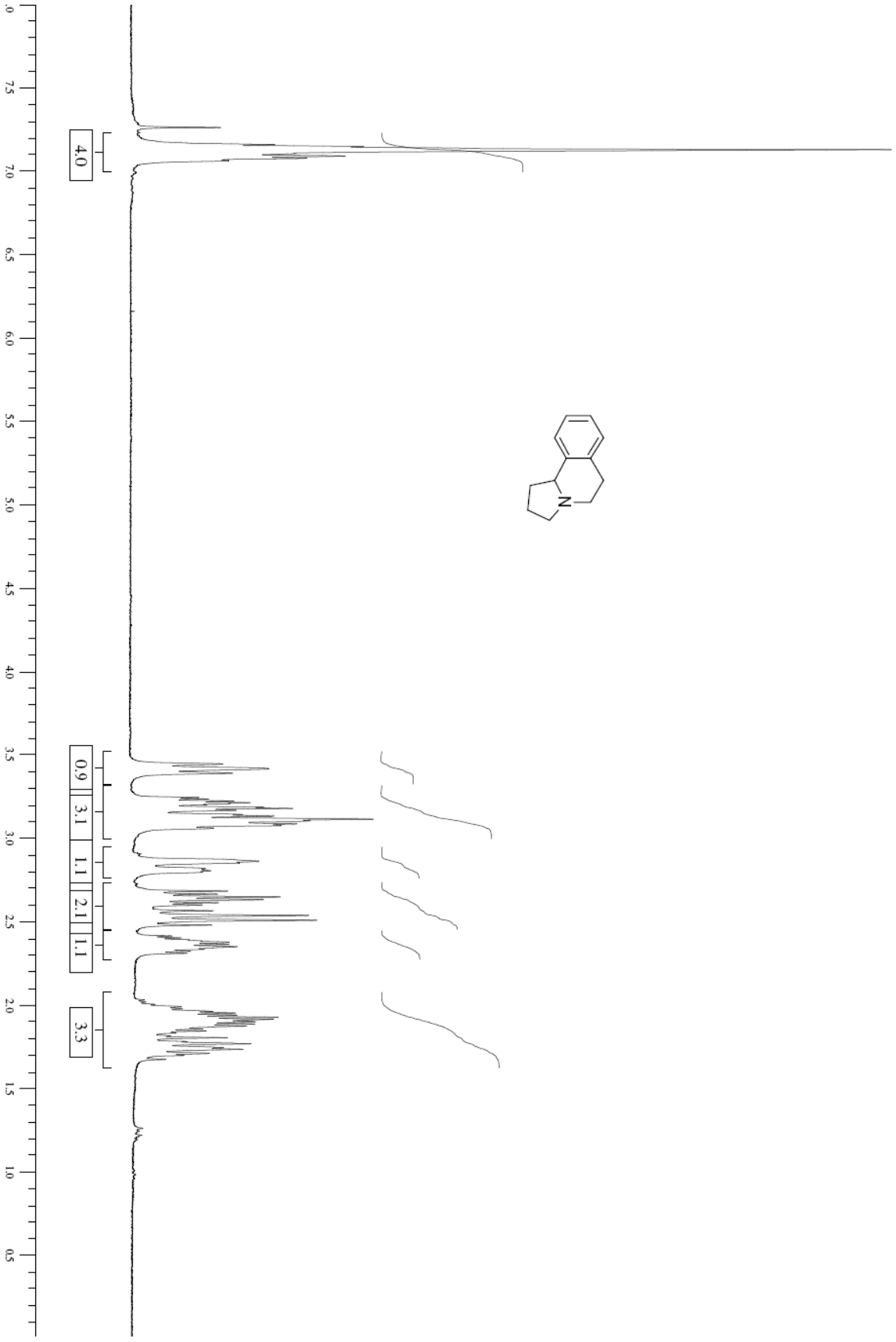

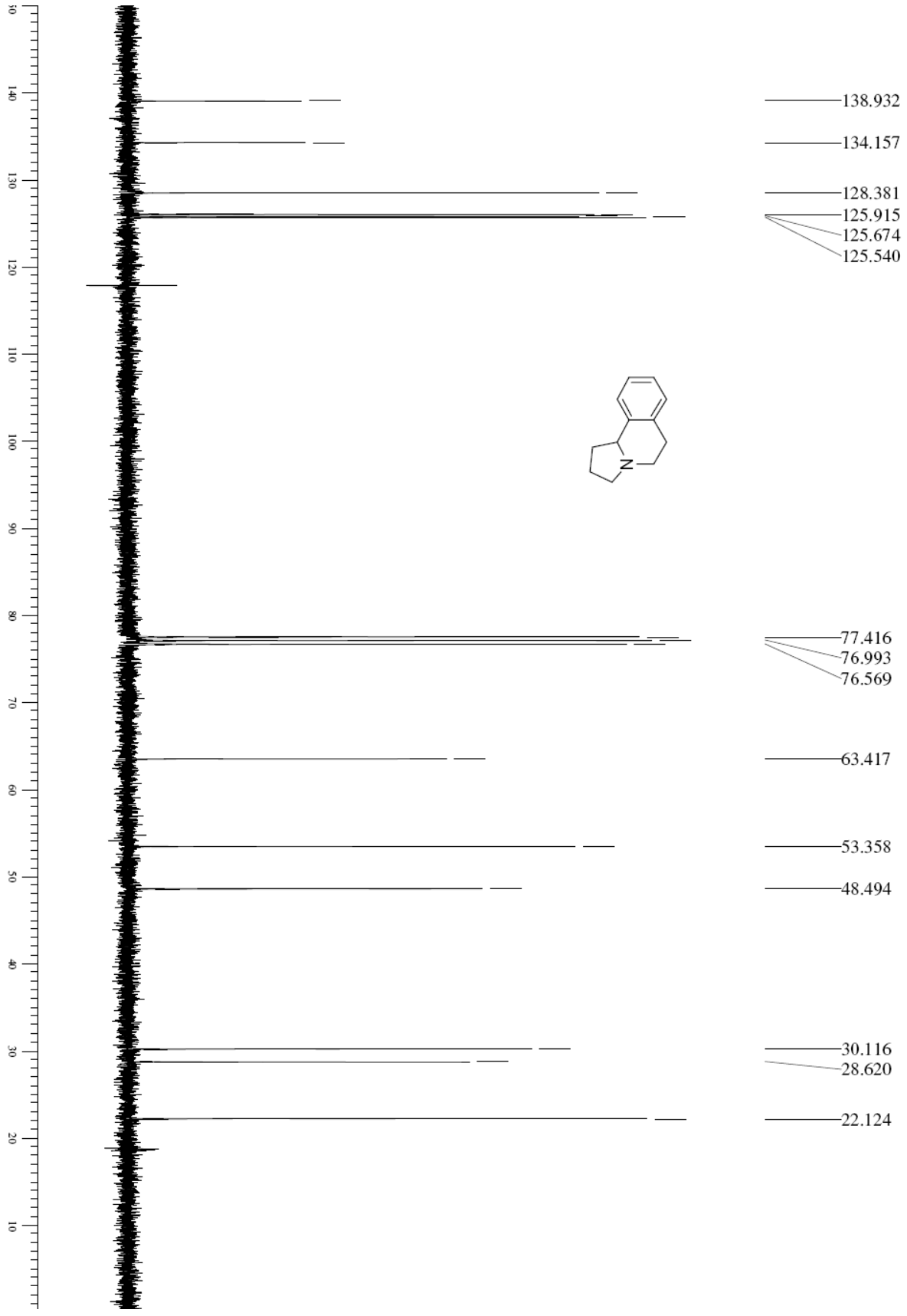
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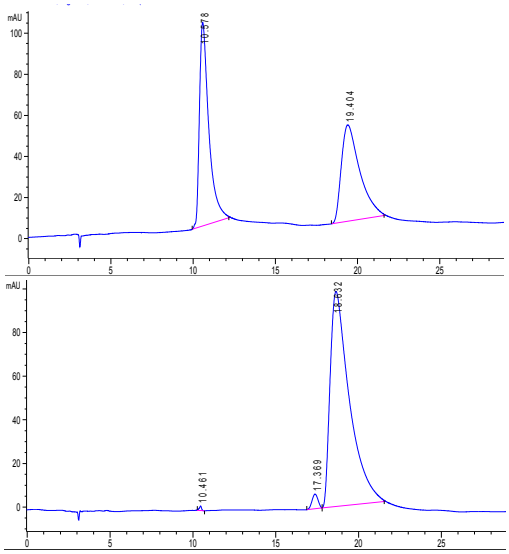
HPLC traces for crispine A (**3**)



Racemic crispine A

(*R*)-(+)-crispine A (**3**), e.e. = 97%

References

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3 This compound is commercially available (Aldrich), alternatively, see: www.syntheticpages.org/pages/262 for its synthesis from 3,4-dimethoxyphenylacetonitrile.
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