SUPPORTING INFORMATION

Monoamine oxidase (MAO-N) whole cells biocatalyzed aromatization of 1,2,5,6-tetrahydropyridines into pyridines

Anita Toscani,[‡] Caterina Risi,[‡] Gary W. Black,[†] Nicola L. Brown,[†] Ali Shaaban,[‡] Nicholas J. Turner,[±] and Daniele Castagnolo[‡,*]

[‡]*School of Cancer and Pharmaceutical Sciences, King's College London, 150 Stamford Street, SE1 9NH, London, United Kingdom.* [†]*Department of Applied Sciences, Northumbria University, Ellison Place, NE1 8ST, Newcastle upon Tyne, United Kingdom.* [±]*School of Chemistry, Manchester Institute of Biotechnology, University of Manchester, 131 Princess Street, M1 7DN, Manchester, United Kingdom.*

*Corresponding Author. Email: *daniele.castagnolo@kcl.ac.uk*

Experimental section

^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR) spectra were recorded using a Bruker Ascend 400 spectrometer at 298 K operating at the frequencies indicated in the experimental section. Chemical shifts (δ) are reported in ppm, referenced to tetramethylsilane. Coupling constants (J) are reported in Hertz. Splitting patterns are abbreviated as follows: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), sextet (sxt), broad (br) or some combination of them. InsightMR® experiments were performed at 310 K. InsightMR® experiments were performed at 310 K using the Bruker InsightMR system. Biocatalytic reactions were performed by shaking the mixtures contained in 15 mL Falcon tubes using a Grant Bio™ PSU-10i Orbital Platform Shaker. HPLC analysis was carried out using a Perkin-Elmer 1100 HPLC system coupled with UV/Vis set to 254 nm. The columns used were C18 (0.5 μm, 4.6 mm X 250 mm), and Chiralpak IG® (5 μm, 4.6 mm X 250 mm), supplied by Daicel. Water and acetonitrile were used as isocratic mobile phase system for the reverse phase HPLC, whereas heptane-ethanol (9:1 or 8:2) where used for the direct phase HPLC experiments. A JouanB4 centrifuge with exchangeable buckets was used to centrifuge and isolate the biocatalytic products. The supernatant derived from the biocatalytic reactions was filtered through a 0.2 μm PTFE filters before being analysed *via* HPLC. Flash column chromatography was carried out using Sigma Aldrich silica gel particle size, 40-63 μm particle size 60 Å. All reactions were conducted under a nitrogen atmosphere in oven-dried glassware unless otherwise stated. All solvents and commercially available reagents were purchased from Sigma Aldrich and used as without further purifications. Gas chromatography analysis was performed with a chiral Supelco™ Beta-DEX 325 Capillary Column (30 m x 0.25 mm x 0.25 μm) using dichloromethane solutions of the compounds (concentration of 1 mg/mL ca.).

Compounds **5a, 5f** and **15c** were purchased and used without any further purification.

**General procedure for the synthesis of tertiary alcohols 8b-e**
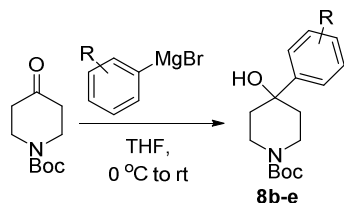
Commercially available aryl magnesium bromide regents (0.6 mmol, THF or Et$_2$O solutions) were added dropwise to a suspension of *N*-Boc piperidone (0.5 mmol) in dry THF. The addition was performed at 0 °C. The reaction was let to warm up and was then heated at reflux for 1-3 hours. The reaction was then quenched with 1M HCl solution and the THF was evaporated under vacuum. The crude mixture was extracted with EtOAc (3×15mL), and the organic layer was washed with brine, dried over Na$_2$SO$_4$, filtered and dried in vacuo. Purification by flash chromatography (petroleum ether/EtOAc 10:1) afforded the desired compounds **8b-e** as yellow oils (45-67% yield).

**tert-Butyl-4-hydroxy-4-(p-tolyl)piperidine-1-carboxylates (8b)**[1]

8b
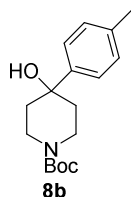
^{1}H NMR (400 MHz, CDCl$_3$) δ 7.37 (d, J = 8.1 Hz, 8 H), 7.18 (d, J = 8.1 Hz, 8 H), 4.02 (d, J = 12.8 Hz, 2 H), 3.25 (t, J = 12.8 Hz, 2 H), 2.35 (s, 3 H), 1.99 (dt, J = 4.7, 13.1 Hz, 2 H), 1.73 (d, J = 12.8 Hz, 2 H), 1.68 (s, br, 1 H), 1.49 (s, 9 H) ppm. **^{13}C NMR** (100 MHz, CDCl$_3$) δ 172.2, 137.6, 137.1, 135.7, 129.2, 124.8, 117.8, 53.5, 43.3, 41.8, 25.5, 21.1 ppm.

**tert-Butyl-4-hydroxy-4-(p-methoxyphenol)piperidine-1-carboxylate (8c)**[2]

8c
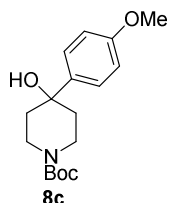
^{1}H NMR (400 MHz, CDCl$_3$) δ 7.40 (d, J = 8.9 Hz, 2 H), 6.89 (d, J = 8.9 Hz, 2 H), 4.01 - 3.99 (m, 2 H), 3.81 (s, 3 H), 3.24 (t, J = 12 Hz, 2 H), 1.96 (t, J = 11 Hz, 2 H), 1.73 (d, J = 12 Hz, 2 H), 1.64 (s, br, 1 H), 1.47 (s, 9 H). **^{13}C NMR** (100 MHz, CDCl$_3$) δ 167.80, 163.61, 156.17, 153.31, 130.88, 128.80, 83.09, 79.31, 68.14, 40.46, 28.30, 26.51 ppm.

**tert-butyl 4-hydroxy-4-(p-fluoro)piperidine-1-carboxylate (8d)**[2]

8d
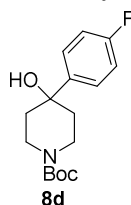
^{1}H NMR (400 MHz, CDCl$_3$) δ 7.44 - 7.40 (m, 2 H), 7.00 (t, J = 8.7 Hz, 2 H), 3.96 (d, J = 10 Hz, 2 H), 3.20 (t, J unresolved, 2 H), 2.20 (br. s., 1 H), 1.91 (t, J = 12 Hz 2 H), 1.69 (d, J = 12.1 Hz, 2 H), 1.45 (s, 9 H) ppm. **^{13}C NMR** (100 MHz, CDCl$_3$) δ 163.1, 160.6, 154.9, 144.0, 144.0, 126.3, 126.3, 115.2, 115.0, 79.6, 71.1, 38.2, 28.5 ppm.

### *tert*-Butyl-4-hydroxy-4-(p-chloro)piperidine-1-carboxylate (8e)[3]

8e
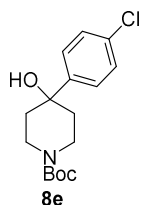
¹H NMR (400 MHz, CDCl$_3$) δ 7.31 (m, *J* = 8.6 Hz, 2 H), 7.20 (m, *J* = 8.6 Hz, 2 H), 3.87 (d, *J* = 12.7 Hz, 2 H), 3.11 (t, *J* = 12.2 Hz, 2 H), 2.66 (br. s., 1 H), 1.80 (dt, *J* = 4.6, 12.9 Hz, 2 H), 1.60 (d, *J* = 12.9 Hz, 2 H), 1.36 (s, 9 H). **¹³C NMR** (100 MHz, CDCl$_3$) δ 154.9, 146.9, 132.8, 128.4, 126.2, 79.7, 71.0, 37.9, 28.5 ppm.

### General procedure for the synthesis of 4-(*p*-R)-1,2,3,6-tetrahydropyridines (5b-e)

8b-e   **5b-e**
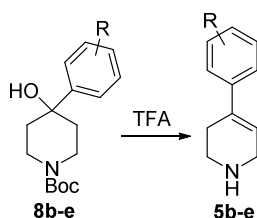
TFA (2 mL) was added dropwise to a solution of **8b-e** (0.5 mmol) in DCM (4 mL) at 0 $^{\mathrm{o}}$C under nitrogen. The resulting mixture was slowly allowed to warm to room temperature and stirred for 30 min. The reaction was then neutralised with saturated NaHCO$_3$ solution and was kept stirring for 30 minutes. The mixture was then extracted with DCM (3×10 mL), washed with saturated brine solution and dried with Na$_2$SO$_4$, filtered, evaporated in vacuo, and therefore purified by flash chromatography (EtOAc/MeOH 8:2) affording the desired compounds **5b-e** in variable yields (10-40%).

4-(*p*-tolyl)-1,2,3,6-tetrahydropyridine (5b)[4]

5b
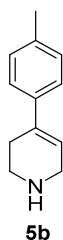
¹H NMR (400 MHz, CDCl$_3$) δ 7.27 (d, *J* = 8.3 Hz, 2 H), 7.16 (d, *J* = 8.3 Hz, 2 H), 6.02 (br. s., 1 H), 3.71 (d, *J* = 2.5 Hz, 3 H), 3.33 - 3.24 (m, 2 H), 2.69 - 2.62 (m, 2 H), 2.35 (s, 3 H) ppm. **¹³C NMR** (100 MHz, CDCl$_3$) δ 137.6, 137.1, 135.7, 129.2, 124.8, 117.8, 53.5, 43.3, 41.8, 25.5, 21.1 ppm. **LRMS** m/z (ES+) m/z: 174.2 [M+H]$^{+}$.

4-(*p*-methoxyphenyl)-1,2,3,6-tetrahydropyridine (5c)[5]

5c
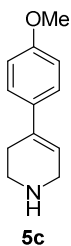
¹H NMR (400 MHz, CDCl₃) δ 7.30 (m, *J = 8.8* Hz, 2 H), 6.85 (m, *J = 8.8* Hz, 2 H), 6.01 (br. s., 1 H), 3.79 (s, 3 H), 3.51 (d, *J = 2.6 Hz*, 2 H), 3.10 (t, *J = 5.7 Hz*, 2 H), 2.89 (br. s., 1 H), 2.49 - 2.39 (m, 2 H) ppm. **¹³C NMR** (100 MHz, CDCl₃) δ 158.8, 134.7, 133.8, 125.9, 121.2, 113.7, 55.3, 45.2, 43.2, 27.5 ppm. **LRMS** m/z (ES+) m/z: 190.2 [M+H]⁺.

4-(*p*-fluorophenyl)-1,2,3,6-tetrahydropyridine (5d)[4]

5d
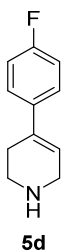
¹H NMR (400 MHz, CDCl₃) δ 9.76 (br. s., 1 H), 7.33 - 7.30 (m, 2 H), 7.04 (t, *J = 8.7* Hz, 2 H), 5.93 (br. s., 1 H), 3.82 (d, *J unresolved*, 2 H), 3.41 (t, *J = 5.9 Hz*, 2 H), 2.76 (t, *J unresolved*, 2 H) ppm. **¹³C NMR** (100 MHz, CDCl₃) δ 163.9, 161.4, 135.0, 126.8, 126.7, 115.6, 115.4, 41.9, 41.8, 40.8, 40.7, 23.9, 23.9 ppm. **¹⁹F NMR** (376.5 MHz, CDCl₃) δ -113.5 ppm. **LRMS** m/z (ES+) m/z: 178.2 [M+H]⁺.
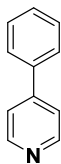
4-(*p*-chlorophenyl)-1,2,3,6-tetrahydropyridine (5e)[6]

5e
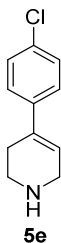
¹H NMR (400 MHz, CDCl₃) δ 7.35 - 7.27 (m, 4H) 5.98 (br. s, 1H) 3.84 (br. s, 2H) 3.43 (t, *J=5.5* Hz, 2H) 2.77 (br. s, 2H) ppm. **¹³C NMR** (100 MHz, CDCl₃) δ 138.5, 134.9, 133.6, 128.8, 128.7, 126.4, 119.7, 43.4, 41.8, 29.8 ppm. **LRMS** m/z (ES+) m/z: 194.0-196.0 [M+H]⁺.
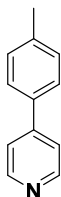
The ^{1}H NMR of pyridines **6**, **16**, **17** and **4** was compared with that of available samples or with literature data.
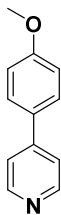
4-phenylpyridine (6a)[7]

^{1}H NMR (400 MHz, CDCl$_3$) δ 8.68 (d, *J = 5.9 Hz*, 2 H), 7.67 (d, *J = 7.8* Hz, 2 H), 7.61 (d, *J = 6.0 Hz*, 2 H), 7.51 (d, *J = 7.8 Hz*, 2H) ppm. **LRMS** m/z (ES+) m/z: 156.2 [M+H]$^+$.
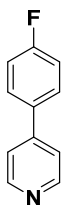
4-(4-tolyl)pyridine (6b)[8]

^{1}H NMR (400 MHz, CDCl$_3$) δ 8.57 (d, *J = 6.0 Hz*, 2 H), 7.48 (d, *J = 8.2* Hz, 2 H), 7.43 (d, *J = 6.0 Hz*, 2 H), 7.23 (d, *J = 8.2 Hz*, 2H), 2.35 (s, 3H) ppm. **LRMS** m/z (ES+) m/z: 170.2 [M+H]$^+$.
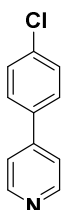
4-(4-methoxyphenyl)pyridine (6c)[8]

^{1}H NMR (400 MHz, CDCl$_3$) δ 8.62 (d, *J = 6.0 Hz*, 2 H), 7.61 (d, *J = 8.8* Hz, 2 H), 7.48 (d, *J = 6.0 Hz*, 2 H), 7.02 (d, *J = 8.8 Hz*, 2H), 3.87 (s, 3H) ppm. **LRMS** m/z (ES+) m/z: 186.2 [M+H]$^+$.
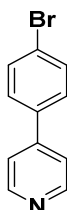
4-(4-fluorophenyl)pyridine (6d)[7]

¹H NMR (400 MHz, CDCl₃) δ 8.66 (d, *J = 6.2 Hz*, 2 H), 7.64 - 7.60 (m, 2 H), 7.47 (d, *J = 8.6 Hz*, 2 H), 7.18 (t, *J = 8.6 Hz*, 2 H) ppm. **¹⁹F NMR** (376.5 MHz, CDCl₃) δ -112.5 ppm. **LRMS** m/z (ES+) m/z: 174.2 [M+H]⁺.
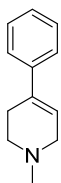
4-(4-chlorophenyl)pyridine (6e)[9]

¹H NMR (400 MHz, CDCl₃) δ 8.67 (d, *J = 6 Hz*, 2 H), 7.58 (d, *J = 8.6* Hz, 2 H), 7.48- 7.47 (m, 4H) ppm. **LRMS** m/z (ES+) m/z: 190.0-192.0 [M+H]⁺.

4-(4-bromophenyl)pyridine (6f)[10]

¹H NMR (400 MHz, CDCl₃) δ 8.67 (d, *J = 6.1 Hz*, 2 H), 7.62 (d, *J = 8.5* Hz, 2 H), 7.53 (d, *J = 8.5* Hz, 2 H), 7.45 (d, *J = 6.1Hz*, 2 H) ppm. **LRMS** m/z (ES+) m/z: 233.9-235.9 [M+H]⁺.

Synthesis of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (15a)


114 μL of formaldehyde solution (1.52 mmol, 37% aq. solution) was added to a THF (5 mL) solution of 4-phenyl-1,2,3,6-tetrahydropyridine (0.15 g, 0.76 mmol) and was left stirring at room temperature for 30 minutes. Sodium cyanoborohydride (283 mg, 1.52 mmol) was then added and the mixture was allowed to stir overnight at room temperature. The solution was

then quenched with sodium hydroxide solution (1 N) and was stirred for further 30 minutes. The solution was then extracted with EtOAc (3 x 7 mL), dried over sodium sulfate and concentrated *in vacuo*. The crude was purified with flash chromatography (hexane : EtOAc = 3 : 7) to obtain a white powder. Yield: 112 mg, 85%.

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.40 − 7.38 (m, 2 H), 5.99 (dd, *J = 2.3 Hz*, 1 H), 3.84 (dd, *J^1 = 2.8 Hz, J^2 = 14.8 Hz,* 1 H) , 3.49 − 3.43 (dd, *J^1 = 1 Hz, J^2 = 13.4 Hz* 1 H), 3.31 − 3.20 (m, 2 H), 2.74 (s, 3 H) ppm . **^{13}C NMR** (100 MHz, CDCl$_3$) δ 138.4, 134.3, 128.7, 128.3, 124.9, 116.4, 57.8, 55.1, 46.1, 23.0 ppm.

1-Methyl-5-phenyl-1,2,3,6-tetrahydropyridine (15b)[11]

15b
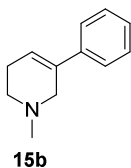
Compound **15b** was synthesised according to the procedure reported by Gessner *et al.* The product was obtained as a pale yellow oil (yield: 95%).

^{1}H NMR (400MHz ,CDCl$_3$) δ 7.39 - 7.27 (m, 5 H), 6.22 - 6.15 (m, 1 H), 3.97 (dd, *J* = 2.6, 17.0 Hz, 1 H), 3.57 (d, *J = 16.9* Hz, 1 H), 3.06 (d, *J = 5 .9* Hz, 1 H), 2.65 (s, 3 H), 2.49 - 2.41 (m, 2 H) ppm. **^{13}C NMR** (101MHz, CDCl$_3$) δ 138.6, 132.4, 128.8, 128.1, 125.2, 120.5, 60.0, 55.2, 47.0, 22.1 ppm.

Synthesis of 1,2,5,6-tetrahydropyridines 14a and 14b
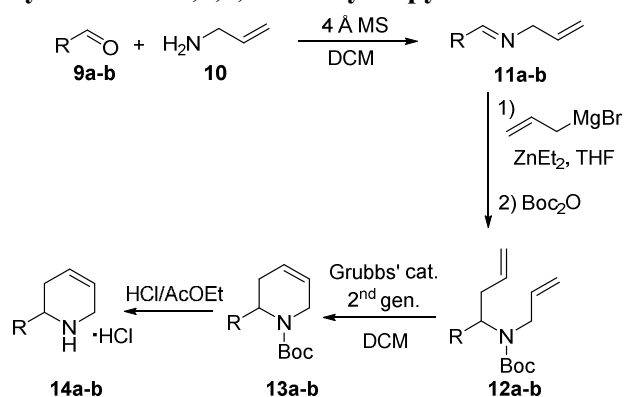
The synthesis of *N*-benzylideneprop-2-en-1-amine (**11a**) was prepared according to literature procedures.[12]

N-Allyl-1-phenylbut-3-en-1-amine[13]

To the solution of imine **11a** (6.24 mmol) in THF dry (0.1 M) was added $ZnEt_2$ (1 M in hexane, 15.6 mmol) dropwise at – 78 °C. This solution was stirred for 2 hours at – 78 °C. After 2 hours was added allyl magnesium bromide (1 M in Et_2O, 15.6 mmol) at -78 °C. The reaction was warmed to room temperature and stirred for 16 h. The reaction was then quenched with H_2O and basified with $NaHCO_3$ until pH 10. The organic layer was extracted with EtOAc (3 x 20 mL), washed with brine, dried with $MgSO_4$ and the solvent was removed under vacuum to yield the crude amine, which was used without further purification (43%yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 – 7.24 (m, 5H), 5.91 – 5.83 (m, 1 H), 5.77 – 5.67 (m, 1 H), 5.13 – 5.03 (m, 4H), 3.73 – 3.69 (m, 1H), 3.15 – 3.10 (m, 1H), 3.05 – 2.99 (m, 1H), 2.44 – 2.40 (m, 2H).

tert-Butyl allyl(1-phenylbut-3-en-1-yl)carbamate (12a)[14]

To a 8 mL of a DCM/$NaHCO_3$ mixture (1:1 v/v) of N-allyl-1-phenylbut-3-en-1-amine **12a** (2.13 mmol), Boc_2O (2.55 mmol) was added as a solid. The reaction was stirred for 16 hours at room temperature. The mixture was extracted and the aqueous layer was washed with DCM (2 x 5 ml). The organic layer dried over $MgSO_4$, filtered, evaporated *in vacuo*, and therefore purified by flash chromatography (hexane/EtOAc 8:2) affording the desired compound **13a** (99% yield). The Boc-protected carbamate **12a** was then prepared according to the procedure reported below.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.33 – 7.24 (m, 5H), 5.86 – 5.76 (m, 1 H), 5.60 (br, s, 1H), 5.16 – 5.11 (m, 1H), 5.08 – 5.04 (m, 1H), 4.95 – 4.92 (m, 2H), 3.51 (br, s, 1H), 2.76 – 2.68 (m, 2H), 1.46 (s, 9H).

tert-Butyl 6-phenyl-5,6-dihydropyridine-1(2H)-carboxylate (13a)

Carbamate **12a** (1.04 mmol) was dissolved in degassed DCM (25 ml) and was added the 2nd generation Grubbs' catalyst 2nd gen. (5mol%). The reaction was stirred for 5 hours at room temperature. The solvent of the reaction was removed under vacuum and the crude was purified by flash chromatography (hexanes/Et_2O 9:1) affording the desired compound **13a** (68%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.32 – 7.22 (m, 5H), 5.91 – 5.86 (m, 1H), 5.66 – 5.62 (m, 1 H), 5.54 (br. s, 1H), 4.25 – 4.20 (m, 1 H), 3.38 – 3.30 (m, 1H), 2.74 – 2.58 (m, 1H), 2.60 – 2.53 (m, 1H), 1.49 (s, 9 H) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$) δ 155.3, 141.3, 128.4, 127.1, 126.9, 124.7, 123.4, 80.00, 77.4, 40.5, 28.7, 27.9 ppm.

2-Phenyl-1,2,3,6-tetrahydropyridine hydrochloride (14a)[15]

tert-butyl 6-phenyl-5,6-dihydropyridine-1(2*H*)-carboxylate was dissolved in HCl/EtOAc solution and was stirred for 96 hours at room temperature. The solvent was removed under reduced pressure to yield the compound **14a**, which was used without further purification.

^{1}H NMR (CDCl$_3$, 400 MHz) δ 10.03 (br. s, 1H), 9.87 (br. s, 1H), 7.62-7.60 (m, 2H), 7.39 – 7.34 (m, 3H), 6.00 – 5.96 (m, 1H), 5.66 – 5.64 (m, 1H), 4.19 – 4.14 (m, 1H), 3.59 – 3.54 (m, 1H), 3.43 -3.39 (m, 1H), 2.87 – 2.80 (m, 1H), 2.54 – 2.48 (m, 1H) ppm. **^{13}C NMR** (100 MHz, CDCl$_3$) δ 135.6, 129.4, 129.2, 128.3, 125.9, 120.2, 56.5, 42.5, 29.3 ppm.

N-Hexylideneprop-2-en-1-amine (11b)

In a round bottom flask equipped with 4 Å molecular sieves, hexanal (2 mmol) and allylamine (8 mmol) were dissolved in dry DCM (16 ml). The mixture was stirred for 8 hours at room temperature. The solvent was removed under pressure to yield the compound **11b**, which was used without further purification.

^{1}H NMR (CDCl$_3$, 400 MHz) δ 7.66 (t, *J = 4.7 Hz*, 1H), 6.01 - 5.92 (m, 1H), 5.16 - 5.07 (m, 2H), 2.28 – 2.23 (m, 2H), 1.57 – 1.52 (m, 2H), 1.33 - 1.30 (m, 6H), 0.91 – 0.87 (m, 3H) ppm. **^{13}C NMR** (100 MHz, CDCl$_3$) δ 166.5, 136.3, 115.8, 63.6, 31.63, 25.8, 25.5, 14.1.

tert-Butyl allyl(non-1-en-4-yl)carbamate (12b)

The crude amine **11b** was protected according to the procedure described for carbamate **12a** and was purified by flash chromatography (hexanes/Et$_2$O 9:1) affording the desired compound **12b** (0.177 mmol, 20%). **^{1}H NMR** (CDCl$_3$, 400 MHz) δ 5.82 – 5.74 (m, 2H), 5.14 – 4.98 (m, 4H), 3.80 – 3.72 (m, 2H), 3.60 (br. s., 1H), 2.26 – 2.12 (m, 2H), 1.59 - 1.50 (m, 2H), 1.45 (s, 9H), 1.27 – 1.26 (m, 6H), 0.87 – 0.85 (m, 3H) ppm. **^{13}C NMR** (100 MHz, CDCl$_3$) δ 156.1, 146.9, 116.6, 115.6, 79.4, 55.6, 45.8, 38.3, 33.2, 31.8, 29.8, 27.6, 22.7, 14.2 ppm.

tert-Butyl 6-pentyl-5,6-dihydropyridine-1(2*H*)-carboxylate (13b)[14a]

Carbamate **13b** was prepared according to the procedure described for **13a**. The crude mixture was purified by flash chromatography (hexanes/Et$_2$O 95:5) affording the desired compound **13b** (0.335 mmol, 41%). **^{1}H NMR** (CDCl$_3$, 400 MHz) δ 5.69 – 5.68 (m, 1H), 5.62 (m, 1 H), 4.22 (br. s., 2H), 3.46 – 3.41 (m, 1 H), 2.43 – 2.38 (m, 1H), 1.91 – 1.87 (m, 1H), 1.58 (m, 2H), 1.46 (s, 9H), 1.30 – 1.25 (m, 6H), 0.88 (t, *J* = 6.7 Hz, 3H) ppm. **^{13}C NMR** (100 MHz, CDCl$_3$) δ 154.12, 122.4, 122.0, 78.3, 47.5, 46.6, 39.1, 38.3, 30.7, 30.5, 27.5, 25.1, 21.7, 13.0 ppm.

2-pentyl-1,2,3,6-tetrahydropyridine hydrochloride (14b)

The THP **14b** was prepared according to the procedure reported for the THP **14a**.

^{1}H NMR (CDCl$_3$, 400 MHz) δ 9.77 (br, 1H), 9.60 (br, 1H), 5.91 (d, J = 9.5 Hz, 1H), 5.68 (d, J = 9.6 Hz, 1H), 3.73 – 3.61 (m, 2H), 3.20 – 3.16 (m, 1H), 2.39 – 2.37 (m, 2H), 2.10 – 2.03 (m, 1H), 1.82 – 1.73 (m, 1H), 1.45 – 1.24 (m, 6H), 0.88 (t, J = 6 Hz, 3H). **^{13}C NMR** (CDCl$_3$, 100 MHz) δ 125.7, 119.8, 53.2, 42.0, 32.7, 31.5, 27.7, 25.1, 22.6, 14.1 ppm.

Biocatalytic aromatization of THP into pyridines 6a-e, 16a-b, 17 and 4
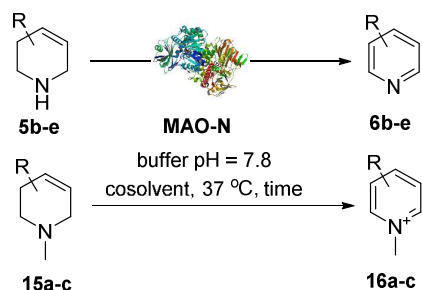
In a Falcon tube (15 mL), freeze dried whole cells of *E.coli* expressing recombinant monoamine oxidase MAO-N (variants D5, D9, D11) (140 mg obtained from bulk production) were suspended in 800 µL of potassium phosphate buffer (1 M pH = 7.8). Then, the appropriate 1,2,5,6-tetrahydropyridine (0.1 mmol) dissolved in isooctane or DMSO (co-solvent:buffer = 1:66) was added leading to a solution with a final concentration of 0.02 g/mL. The reaction mixture was incubated at 37 ºC and shaken at 160 rpm for 24h. The reaction mixture was added with EtOAc (5 mL) and then centrifuged at 4000 x g for 10 minutes. The organic layer was then separated and dried over anhydrous MgSO$_4$. After the solvent was evaporated, the crude product was analysed through ^{1}H-NMR, HPLC and/or GC and the conversion values were determined by integration.
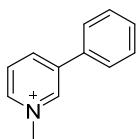
2-phenylpyridine (17a)[16]
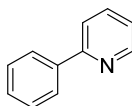
^{1}H NMR (400 MHz, CDCl$_3$) δ 8.71 (d, J = 4.4 Hz, 1H), 7.99 (d, J = 7.0 Hz, 2H), 7.78 – 7.72 (m, 2H), 7.48 (t, J = 7.3 Hz, 2H), 7.42 (t, J = 7.3 Hz, 1H), 7.24 (ddd, J_1 = 2.1, J_2 =4.8, J_3 = 6.7 Hz, 1H) ppm.

2-pentylpyridine (17b)[17]
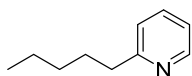
^{1}H NMR (400 MHz, CDCl$_3$) δ 8.52 (ddd, J = 0.9, 1.9, 4.9 Hz, 1H), 7.58 (td, J = 1.9, 7.6 Hz, 1H), 7.14 (d, J = 7.6 Hz, 1H), 7.09 (ddd, J_1 = 1.1, J_2 = 4.9, J_3 = 7.5 Hz, 1H), 2.78 (t, J = 7.8 Hz, 2H), 1.76 – 1.69 (m, 2H), 1.36 – 1.32 (m, 4H), 0.89 (t, J = 6.9, 3H) ppm.

1-methyl-3-phenylpyridin-1-ium (16b)[11]

^{1}H NMR (400 MHz, CDCl$_3$) δ 9.40 (s, 1H), 9.19 (d, J = 5.9 Hz, 1H), 8.57 (d, J = 8.2 Hz, 1H), 8.11 (dd, $J1$ = 6.0, 8.1 Hz, 1H), 7.88 - 7.81 (m, 2H), 7.60 - 7.49 (m, 3H), 4.78 (s, 3H) ppm.

Biocatalytic aromatization of THP 5a in the presence of catalase
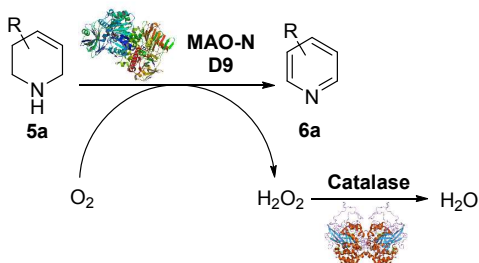
In a Falcon tube (15 mL), freeze dried whole cells of *E.coli* expressing recombinant monoamine oxidase MAO-N (variant D9) (50 mg, obtained from bulk production) and catalase from bovine liver were suspended in 3.3 mL of sodium phosphate buffer (1 M pH = 7.8). Then, the 4-phenyl-1,2,3,6-tetrahydropyridine **5a** (0.026 mmol) dissolved in isooctane (50 µL) was added. The reaction mixture (cosolvent/buffer = 1:66) was incubated at 37 ºC and shaken at 160 rpm for 24 h. The reaction mixture was then added with EtOAc (5 mL) and then centrifuged at 4000 x g for 10 minutes. The organic layer was then separated and dried over anhydrous MgSO$_4$. After the solvent was evaporated, the crude product **6a** was analysed via ^{1}H-NMR and the conversion was determined by integration (conv. = 72 %).

Large-scale biocatalytic aromatization of 5a into pyridine 6a

In a round bottom flask (500 ml), the 4-Ph-1,2,3,6-tetrahydropyridine **5a** (0.5 g, 2.6 mmol) was dissolved in a mixture of sodium phosphate buffer (270 mL, 1M, pH = 7.8) / isooctane (4.1 mL) (cosolvent/buffer = 1:66). To this solution, freeze dried whole cells of *E.Coli* expressing recombinant monoamine oxidase MAO-N (variant D9) (3.57 g obtained from bulk

production) were added. The reaction mixture was stirred at 37 °C for 24 hours. The reaction mixture was then added with EtOAc (5 mL) and then centrifuged at 4000 x g for 10 minutes. The organic layer was then separated and dried over anhydrous $MgSO_4$. After the solvent was evaporated, the crude product **6a** was analysed via ^{1}H-NMR and the conversion was determined by integration (conv. = 99 %). Crude **6a** was purified by flash chromatography (hexane/EtOAc 9:1) affording pure pyridine **6a** in 75% yield.

Preparation of Biocatalysts

MAO-N (monoamine oxidase from *Aspergillus niger*) was transformed into *E. coli* according to previously reported procedures.[18]

MAO-N D5 was produced in *E. coli* BL21(DE3)

MAO-N D9 was produced in *E. coli* BL21(DE3)

MAO-N D11 was produced in *E. coli* C43(DE3)

In all cases an overnight 10 mL starter culture of each clone was grown in LB broth + Ap (100 µg/ml) at 37 °C, 200 rpm. The starter culture was then inoculated into 1 L of Auto Induction Media Super Broth Base including trace elements (Formedium Ltd, UK) + Ap (100 µg/ml), in a 2L baffled flask, and grown at 30 °C, 180 rpm for 2 days. Cells were then harvested by centrifugation at 4000 x g for 10 min at 4 °C. The supernatant was discarded and the cell pellet was resuspended in 10 mL of 18.2 MΩ/cm H_2O. The resuspended cells were then frozen and freeze-dried. Typically 4 g of lyophilized *E. coli* cells were obtained from a 600 mL culture.

InsightMR experiment

The *in situ* real-time monitoring of the reaction was performed using a Bruker Ascend 400 MHz equipped with InsightMR set-up (hood, peristaltic pump, Julabo F25 refrigerated/heating circulator for temperature control and a tube flow) as shown in Figure S1. The experiment was carried out on 50 mg of **5d** in a mixture of PBS buffer/isooctane (60:1) according to the procedure reported in the experimental section. The temperature of both NMR probe and tube flow was set at 310 K. In situ ^{19}F-experiments were performed consequently at different time intervals using standard ^{19}F with proton decoupling sequence.
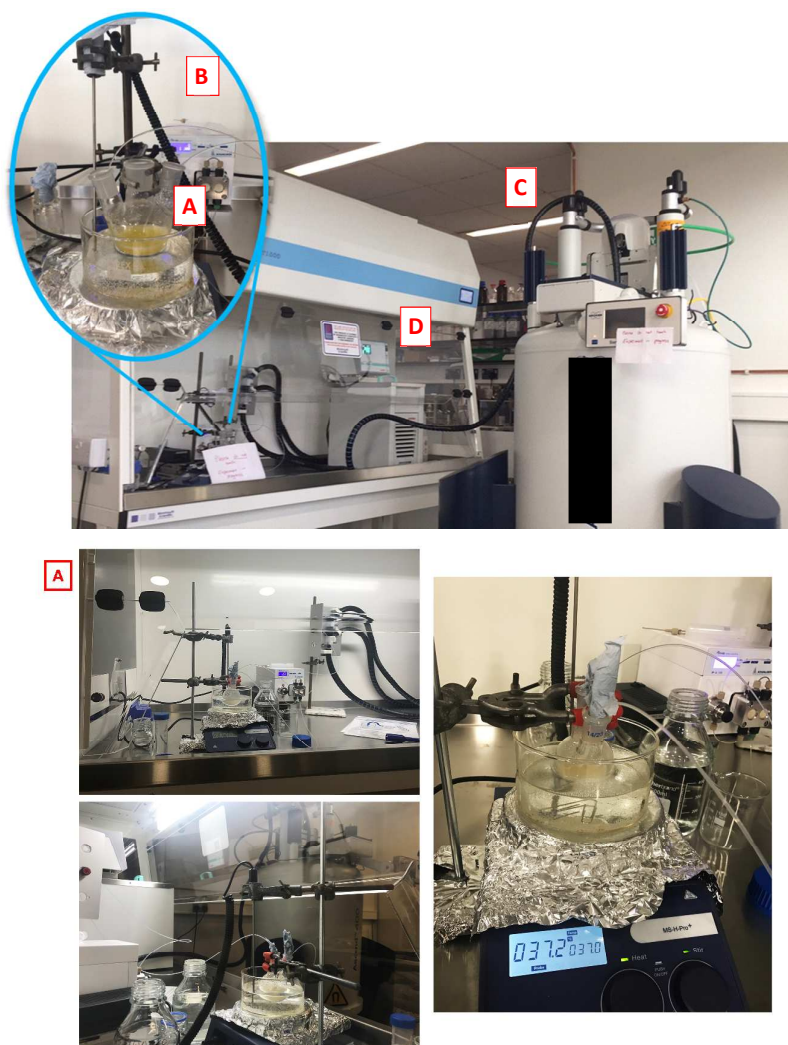
Figure S1. InsightMR hood equipped with reaction vessel (A, pictures below) connected to the peristaltic pump (B) via two tubes (inlet and outlet, as to form a closed flow circuit). The peristaltic pump let the reaction mixture flow through the inner sleeve of the flow tube (C), which is thermally regulated by the Julabo F25 heating circulator (D) that circulates a mixture of water and glycerol in the outer sleeve of the flow tube.

In situ ^{1}H NMR of THP 5a

The *in situ* ^{1}H NMR of the biocatalyzed conversion of **5a** into pyridine **6a** was recorded. The NMR was recorder in buffer/isooctane mixture, at 37 °C, using MAO-N D9 whole cells as biocatalyst. At t_0 the peak at 6.38 ppm belonging to the C3 alkene proton (green, figure B) is clearly evident and it disappears as the reaction proceeds. The other sharp peaks at >7.5ppm appear as soon as the catalyst is added to the mixture and, to our understanding, most likely belong to other amino acids, nucleic acids or molecules of the whole cell biocatalyst, not to the pyridine **6a**. In support to this statement, we report the stacked spectra of the 4-phenylpyridine **6a** and the reaction mixture (containing the MAO whole cell catalyst, substrate **5a** and isooctane) in PBS buffer (Figure S2 A) as a comparison. As aforementioned, the peaks at 8.5, 7.7 and 7.5 ppm of **6a** appear as broad peaks in the reaction mixture (areas highlighted in light blue, Figure S2 A-B), whilst the sharp doublets at 8.7, 8.2 – 8.1, 7.8 – 7.4 ppm appear as soon as the catalyst is added the solution containing **5a**. The broadness of the pyridine peaks is allegedly due to the scarce solubility of the whole-cell catalyst in solution which ultimately accumulate in the continuous-flow InsightMR tube. For these difficulties, the idea of monitoring the kinetic *via* proton NMR was abandoned and the monitoring of the ^{19}F nuclei was pursued instead. However, quite interestingly, further signals at 6.98, 7.80, and 8.32 ppm (areas highlighted in pink, Figure S2 B) could still be visible. As these signals appear upon the addition of the catalyst and subsequently disappear after 4 h, we hypothesized that they could indicate the formation of intermediates **7**.

Figure S2. A) ^{1}H NMR peaks of pyridine **6a** in buffer solution and in *in situ* ^{1}H NMR experiment. B) Peaks of **5a**, **6a** and **7a-b** in *in situ* ^{1}H NMR experiment.
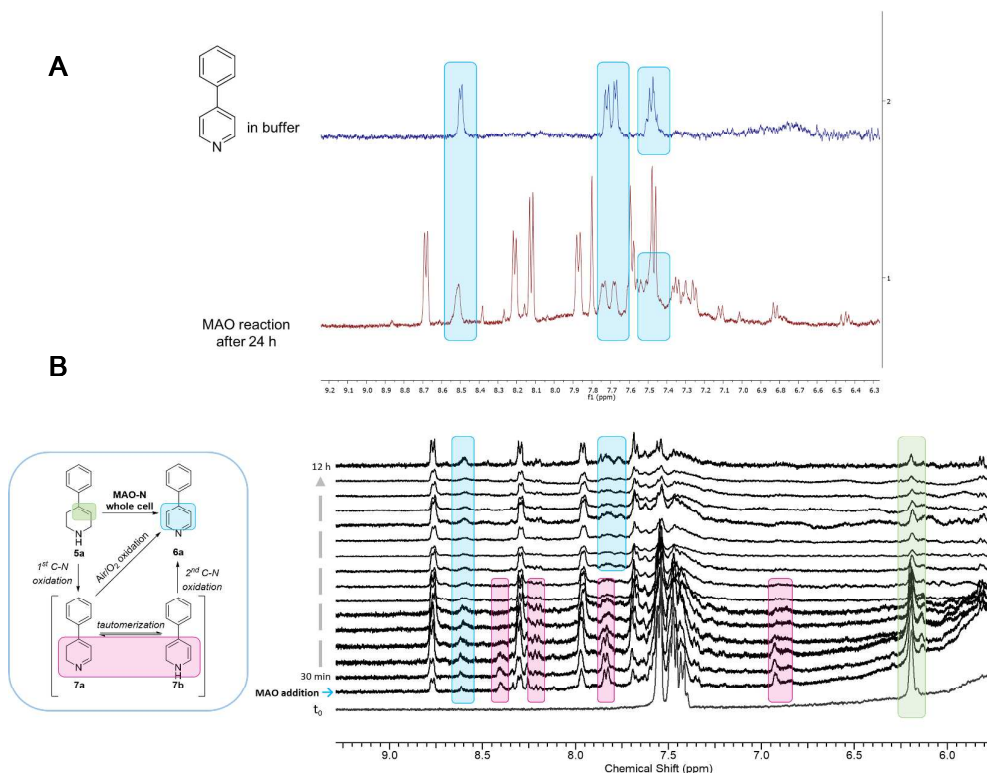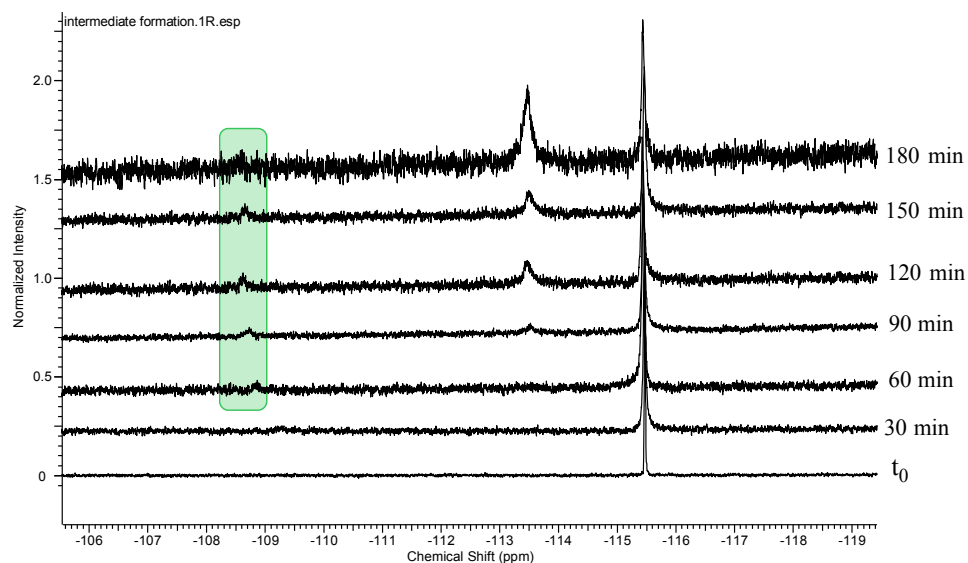
In-situ ^{19}F NMR of THP 5d

Figure S3. *In situ* ^{19}F NMR of THP **5a**

When the *in situ* ^{19}F NMR NMR of **5d** was recorded a weak signal at around -108.75 ppm appeared after 30 min (signal highlighted in green, Figure S3). We hypothesised that these signals could be due to the intermediate **7a-b** (Scheme 1 in the manuscript).
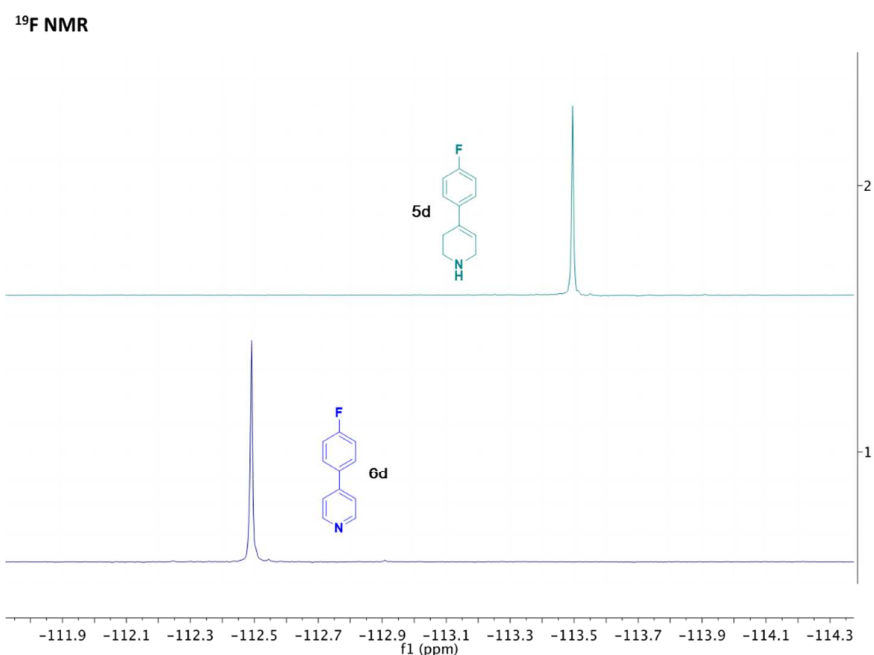

Figure S4. ^{19}F NMR stacked spectra of **5d** and **6d** in CDCl₃. The spectrum of **6d** is the crude product extracted from the MAO-N biocatalyzed aromatization of **5d** during the *in situ* InsightMR experiment.
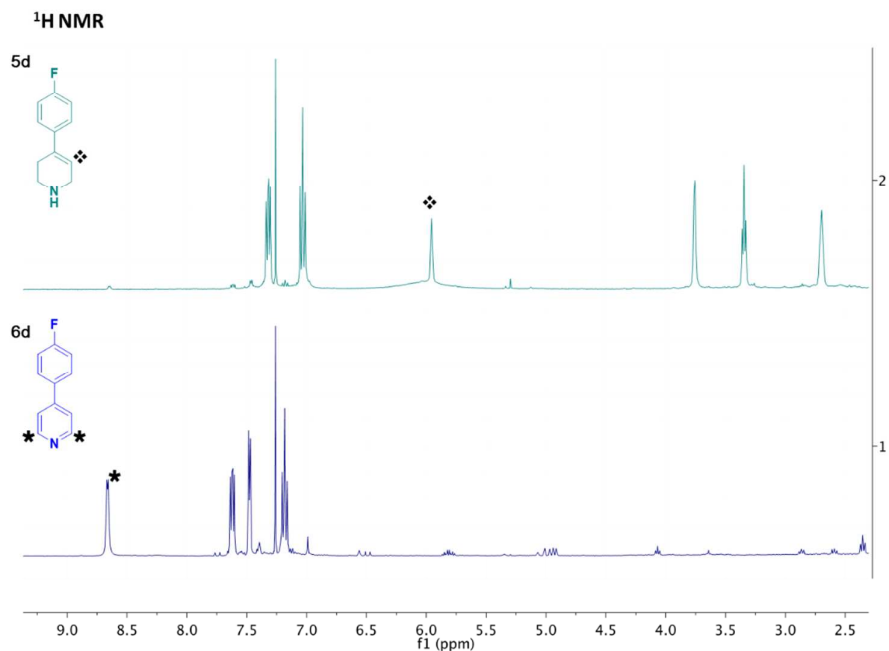
Figure S5. ^{1}H NMR stacked spectra of **5d** and **6d** in CDCl₃. The spectrum of **6d** is the crude product extracted from the MAO-N biocatalyzed aromatization of **5d** during the *in situ* InsightMR experiment.

HPLC analysis of pyridinium compound 16a

MAO-catalyzed conversion of 1-methyl-4-phenyl-THP **15a** into MPP⁺ **16a**
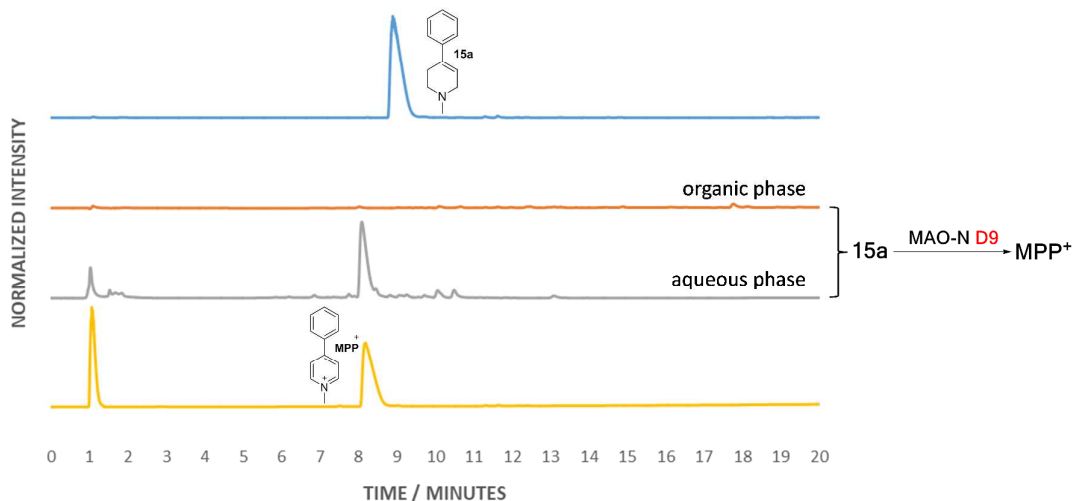
Figure S6. HPLC spectra of the biocatalyzed conversion of **15a** into **16a**. A C18 reverse phase column was used. Method: water : CH₃CN gradient 90:10 to 50:50, 0.2 mL/min.
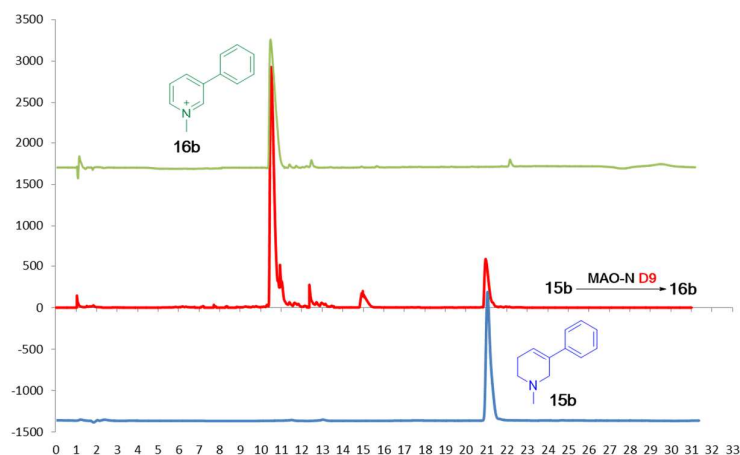
Figure S7. HPLC chromatograms showing the aqueous phase obtained from the MAO-catalyzed aromatization of **15b** (red). The starting material **15b** (blue) and the aromatized compound **16b** (green) are also reported as a reference. HPLC analysis performed using C_{18} column, method: water/acetonitrile gradient (90/10 → 80/20, 0.2 mL/min).

Examples of ^{1}H NMR spectra of the crude mixture from MAO-N biocatalyzed reactions
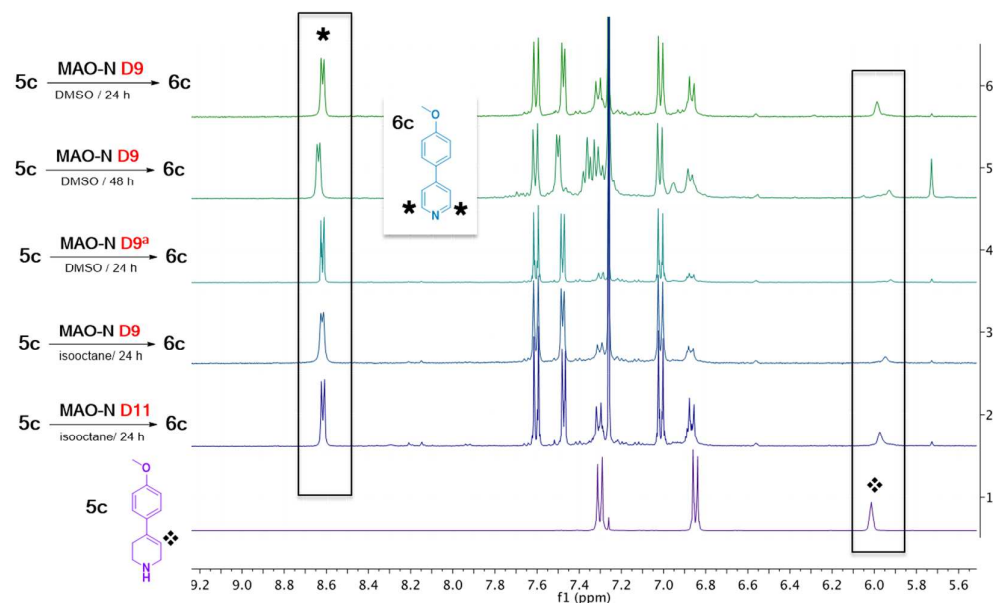
Figure S8. Stacked NMRs of the conversion of 4-methoxyphenylTHP **5c** into 4-methoxyphenylpyridine **6c**
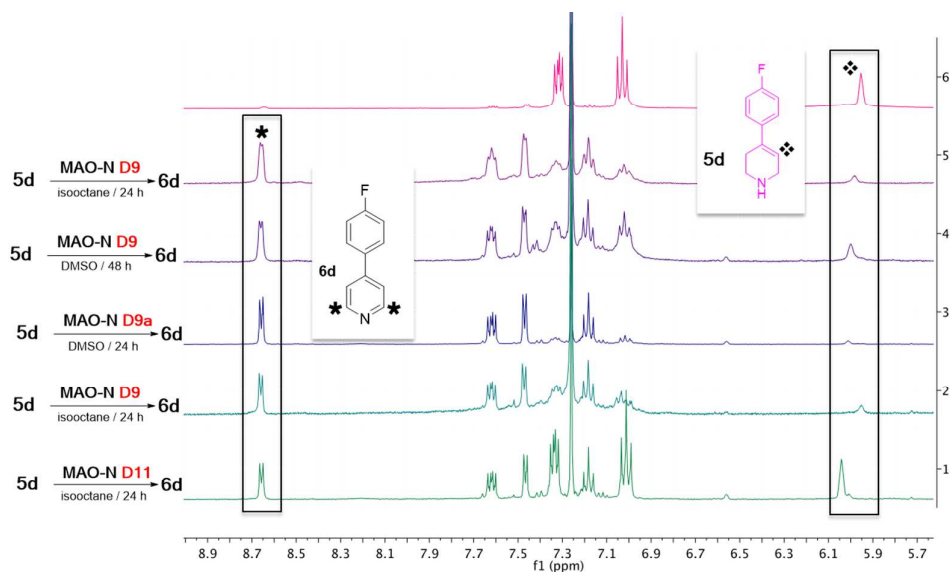
Figure S9. Stacked NMRs of the conversion of 4-F-phenylTHP **5d** into 4-F-phenylpyridine **6d**.

References

1. Chen, G.; Xia, H.; Cai, Y.; Ma, D.; Yuan, J.; Yuan, C. Synthesis and SAR Study of Diphenylbutylpiperidines as Cell Autophagy Inducers. *Bioorg. Med. Chem. Lett.* **2011**, 21, 234-239.

2. Berini, C.; Navarro, O. Ni-Catalysed, Domino Synthesis of Tertiary Alcohols from Secondary Alcohols. *Chem. Commun.* **2012**, *48*, 1538-1540.

3. Greb, A.; Poh, J.-S.; Greed, S.; Battilocchio, C.; Pasau, P.; Blakemore, D. C.; Ley, S. V. A Versatile Route to Unstable Diazo Compounds via Oxadiazolines and their Use in Aryl-Alkyl Cross-Coupling Reactions. *Angew. Chem., Int. Ed.* **2017**, *56*, 16602-16605.

4. Liegeois, J.-F.; Lespagnard, M.; Meneses Salas, E.; Mangin, F.; Scuvee-Moreau, J.; Dilly, S. Enhancing a CH−π Interaction to Increase the Affinity for 5-HT$_{1A}$ Receptors *ACS Med. Chem. Lett.* **2014**, *5*, 358-362.

5. Davidson, R.; Hsu, Y.-T.; Batchelor, T.; Yufit, D.; Beeby, A. The Use Of Organolithium Reagents For The Synthesis Of 4-Aryl-2-Phenylpyridines And Their Corresponding Iridium(III) Complexes. *Dalton Trans.* **2016**, *45*, 11496-11507.

6. Yue, L.; Du, J.; Ye, F.; Chen, Z.; Li, L.; Lian, F.; Zhang, B.; Zhang, Y.; Jiang, H.; Chen, K.; Li, Y.; Zhou, B.; Zhang, N.; Yang, Y.; Luo, C. Identification Of Novel Small-Molecule Inhibitors Targeting Menin-MLL Interaction, Repurposing The Antidiarrheal Loperamide. *Org. Biomol. Chem.* **2016**, *14*, 8503-8519.

7. Panda, S.; Coffin, A.; Nguyen, Q. N.; Tantillo, D. J.; Ready, J. M. Synthesis and Utility of Dihydropyridine Boronic Esters. *Angew. Chem. Int. Ed*. **2016**, *55*, 2205 - 2206.

8. Abe, T.; Mino, T.; Watanabe, K.; Sakamoto, M. Suzuki–Miyaura Coupling of Aryl Chlorides with Arylboronic Acids Using the Morpholine–NiCl$_2$ Catalyst System. *Eur. J. Org. Chem.* **2014**, *6*, 6983 - 6991.
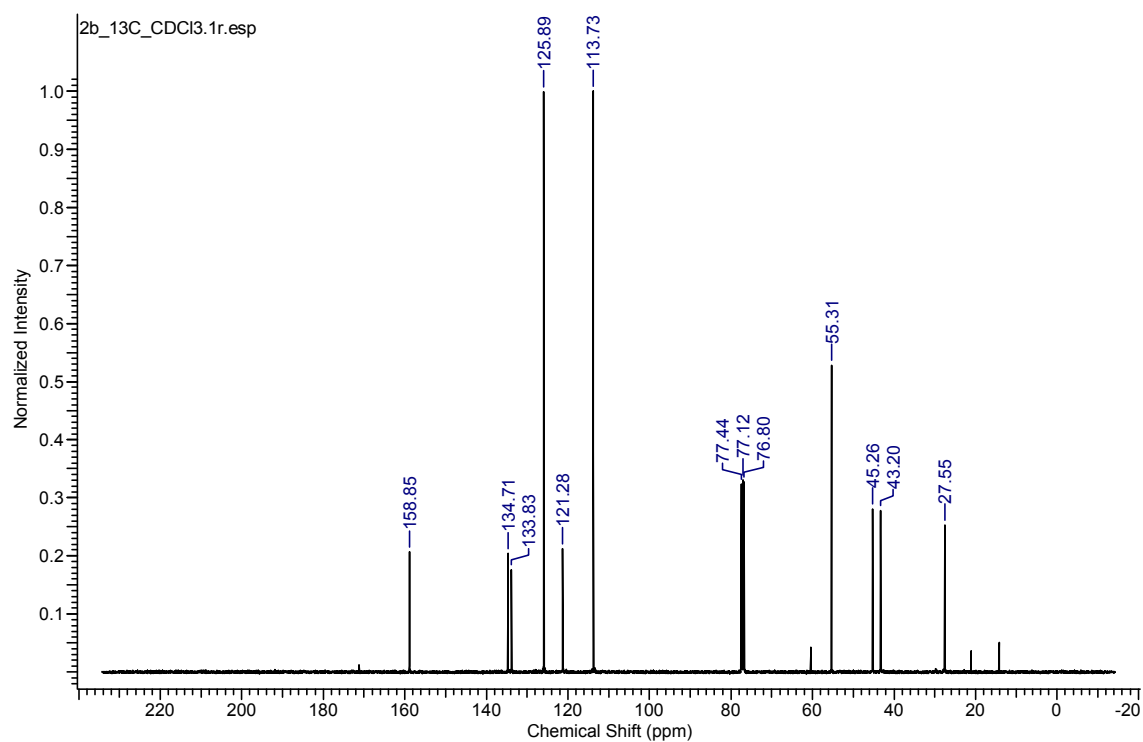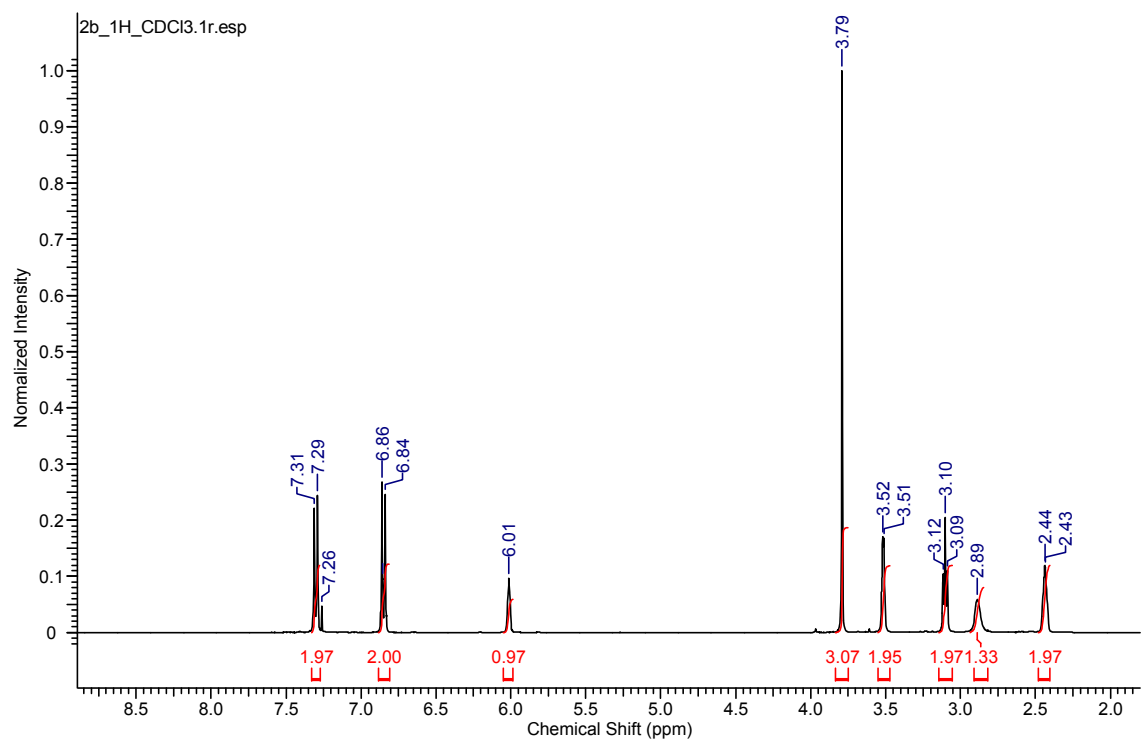
9. Ammirati, M.; Bagley, S. W.; Bhattacharya, S. K.; Buckbinder, L.; Carlo, A. A.; Conrad, R.; Cortes, C.; Dow, R. L.; Dowling, M. S.; El-Kattan, A.; Ford, K.; Guimarães, C. R.; Hepworth, D.; Jiao, W.; LaPerle, J.; Liu, S.; Londregan, A.; Loria, P. M.; Mathiowetz, A. M.; Munchhof, M.; Orr, S. T.; Petersen, D. N.; Price, D. A.; Skoura, A.; Smith, A. C.; Wang, J. Discovery of an in Vivo Tool to Establish Proof-of-Concept for MAP4K4-Based Antidiabetic Treatment. *ACS Med Chem. Lett.* **2015**, *6*, 1128 - 1133.

10. Wang, Y.; Frattarelli, D. L.; Facchetti, A.; Cariati, E.; Tordin, E.; Ugo, R.; Zuccaccia, C.; Macchioni, A.; Wegener, S. L.; Stern, C. L.; Ratner, M. A.; Marks T. J. Twisted π-electron system electrooptic chromophores. Structural and electronic consequences of relaxing twist-inducing nonbonded repulsions *J. Phys. Chem. C* **2008**, *112*, 8005 - 8015.

11. Gessner, W.; Brossi, A. Reduction of Phenyl-Substituted Pyridinium Methoiodides with Sodium Borohydride. Formation of Amine-Borane Complexes in Water. *Synth. Commun.* **1985**, *15*, 911-916

12. Tait, B. M.; Butterworth, S.; Clayden, J. 2,2- and 2,6-Diarylpiperidines by Aryl Migration within Lithiated Urea Derivatives of Tetrahydropyridines. *Org. Lett.* **2015**, *17*, 1236 – 1239.

13. Katritzky, A. R.; Nair, S. K.; Silina, A. Easy Access to *N,N*-Bis(but-3-enyl)-, *N*-Allyl-*N*-(but-3-enyl)-, and *N*-(But-3-ynyl)-*N*-(but-3-enyl)-amines. *J. Org. Chem.* **2002**, *67*, 7530-7532.

14. a) Wang, G.; Mao, Y.; Liu, L. Diastereoselectively Complementary C–H Functionalization Enables Access to Structurally and Stereochemically Diverse 2,6-Substituted Piperidines. *Org. Lett.* **2016**, *18*, 6476 – 6579; b) Coombs, T. C.; Lushigton, G. H.; Douglas, J.; Aubé, J. 1,3☐Allylic Strain as a Strategic Diversification Element for Constructing Libraries of Substituted 2☐Arylpiperidines. *Angew. Chem., Int. Ed*. **2011**, *50*, 2734-2737.

15. Wang, Z. J.; Jackson, W. R.; Robinson, A. J. A Simple and Practical Preparation of an Efficient Water Soluble Olefin Metathesis Catalyst. *Green Chem*. **2015**, *17*, 3407-3114.

16. a) Zhang, E.; Tang, J.; Li, S.; Wu, P.; Moses, J. E. Sharpless, K. B. Chemoselective Synthesis of Polysubstituted Pyridines from Heteroaryl Fluorosulfates. *Chem. Eur. J.* **2016**, *22*, 5692 – 5697; b) Peng, H.; Chen, Y-Q.; Mao, S-L.; Pi, Y-X.; Chen, Y.; Lian, Z-Y.; Tong, M.; Liu, S-H.; Yu, G-A. A General Catalyst for Suzuki–Miyaura and Sonogashira Reactions of Aryl And Heteroaryl Chlorides in Water. *Org. Biomol. Chem*., **2014**, *12*, 6944–6952.

17. Vilas, M.; Rocha, M. A. A.; Fernandes, A. M.; Tojo, E.; Santos, L. M. N. B. F. Novel 2-Alkyl-1-Ethylpyridinium Ionic Liquids: Synthesis, Dissociation Energies and Volatility. *Phys. Chem. Chem. Phys.* **2015**, *17*, 2560-2572.

18. a) Ghislieri, D.; Green, A. P.; Pontini, M.; Willies, S. C.; Rowles, I.; Frank, A.; Grogan, G.; Turner, N. J. Engineering an Enantioselective Amine Oxidase for the Synthesis of Pharmaceutical Building Blocks and Alkaloid Natural Products. *J. Am. Chem. Soc.* **2013**, *135*, 10863. b) Ghislieri, D.; Houghton, D.; Green, A. P.; Willies, S. C.; Turner, N. J. Monoamine Oxidase (MAO-N) Catalyzed Deracemization of Tetrahydro-β-carbolines: Substrate Dependent Switch in Enantioselectivity. *ACS Catal.* **2013**, *3*, 2869–2872.

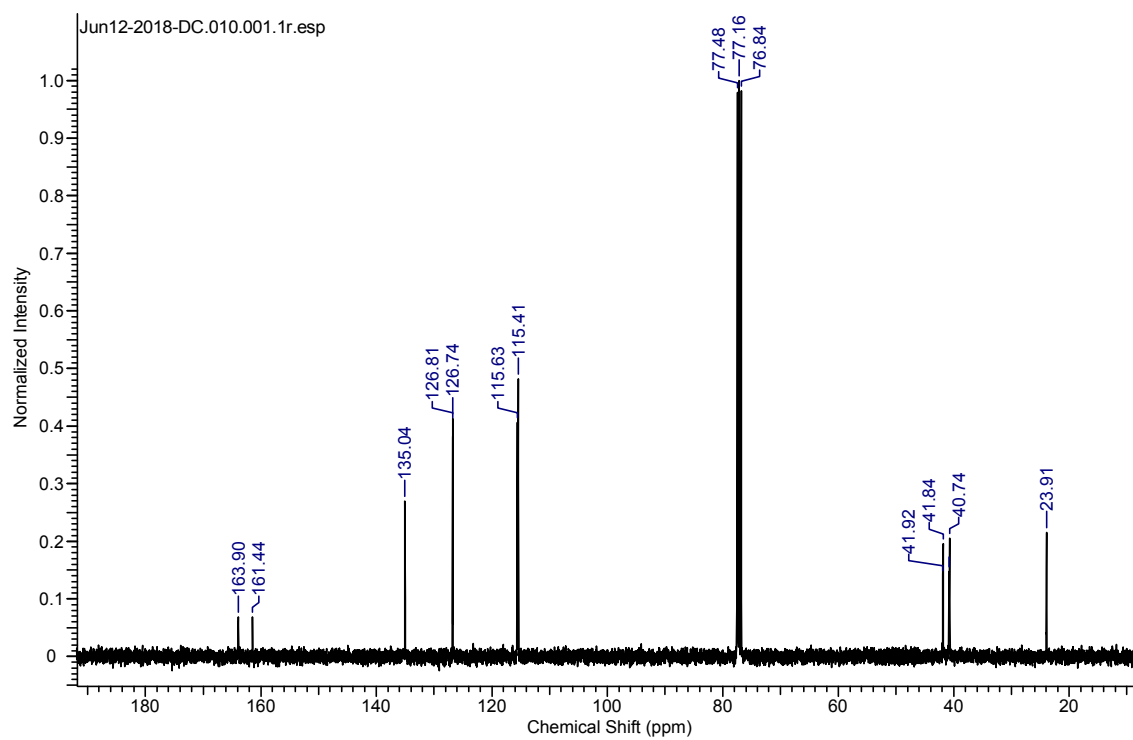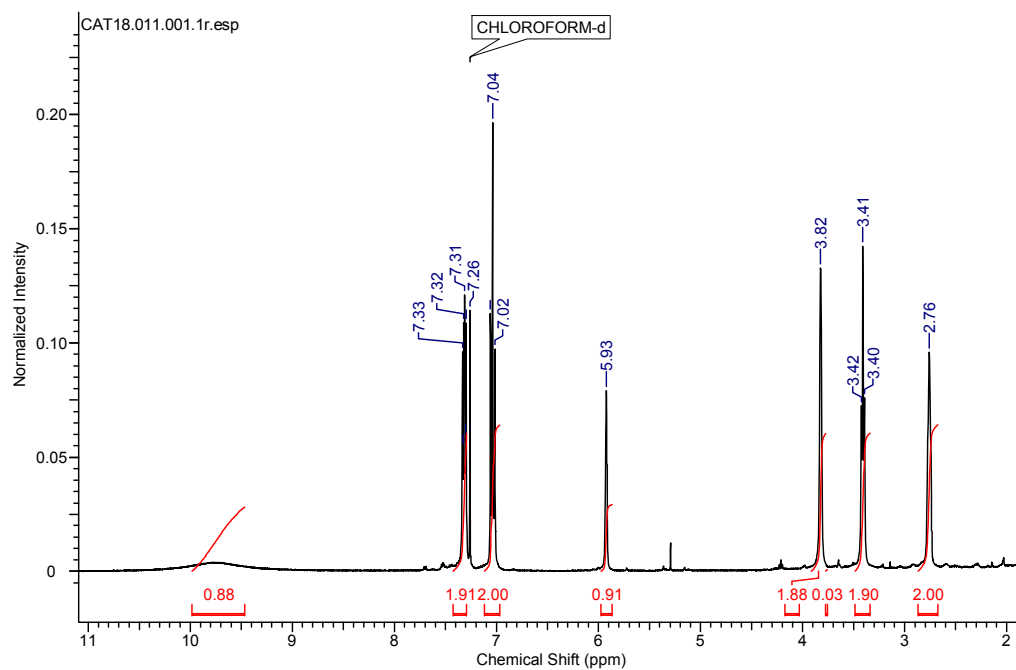# Copies of Spectra of synthesized key substrates for biocatalytic reactions and new compounds
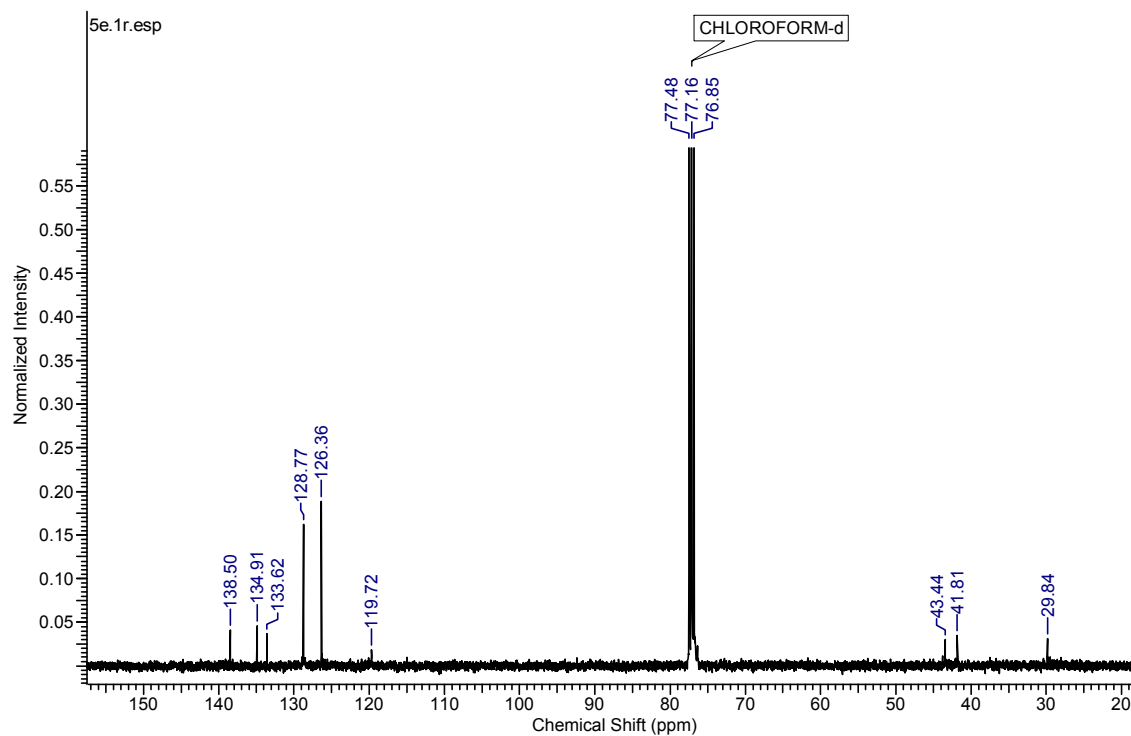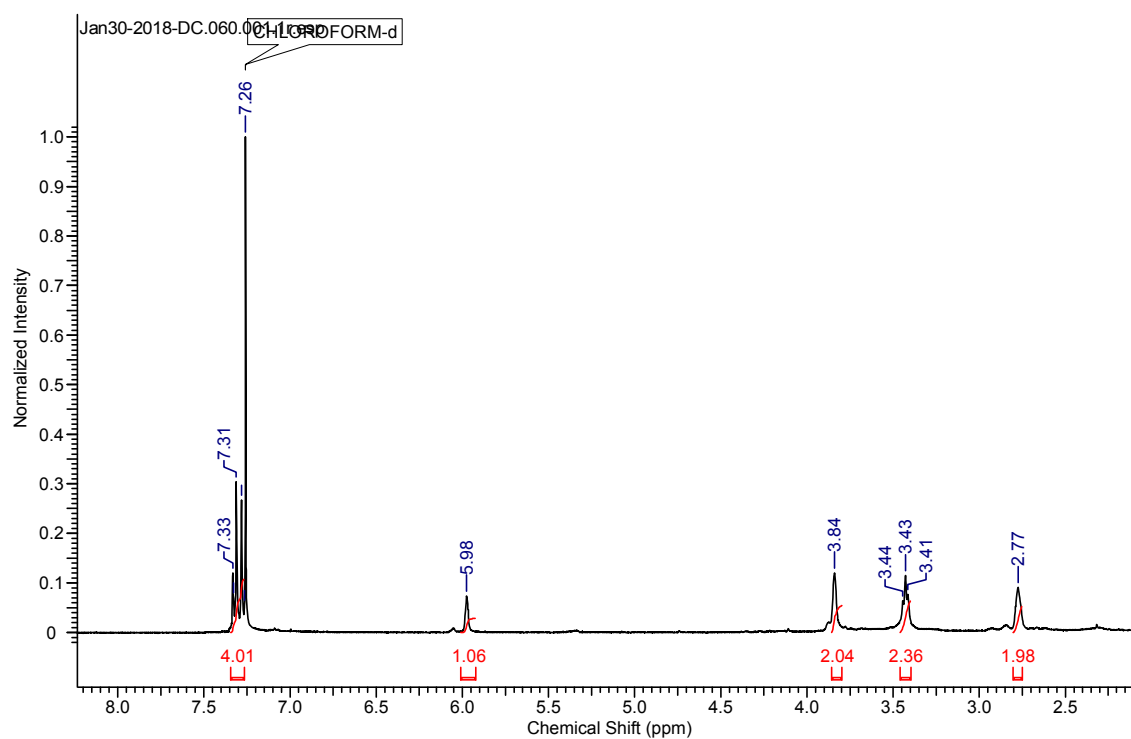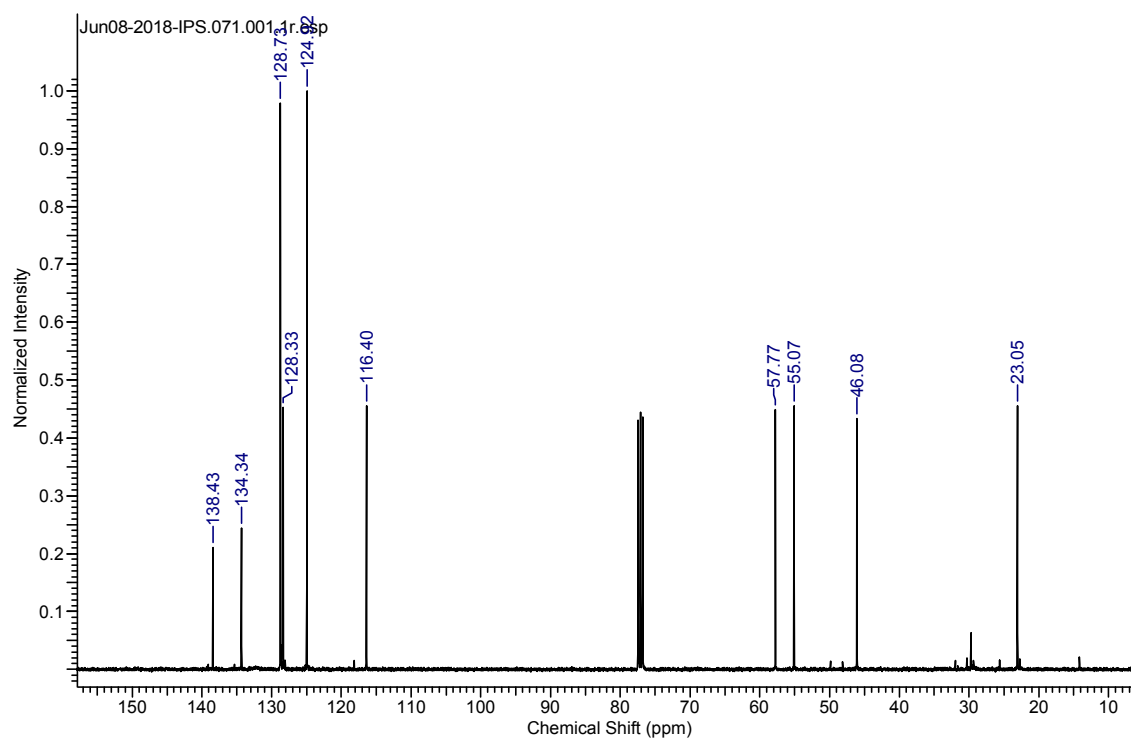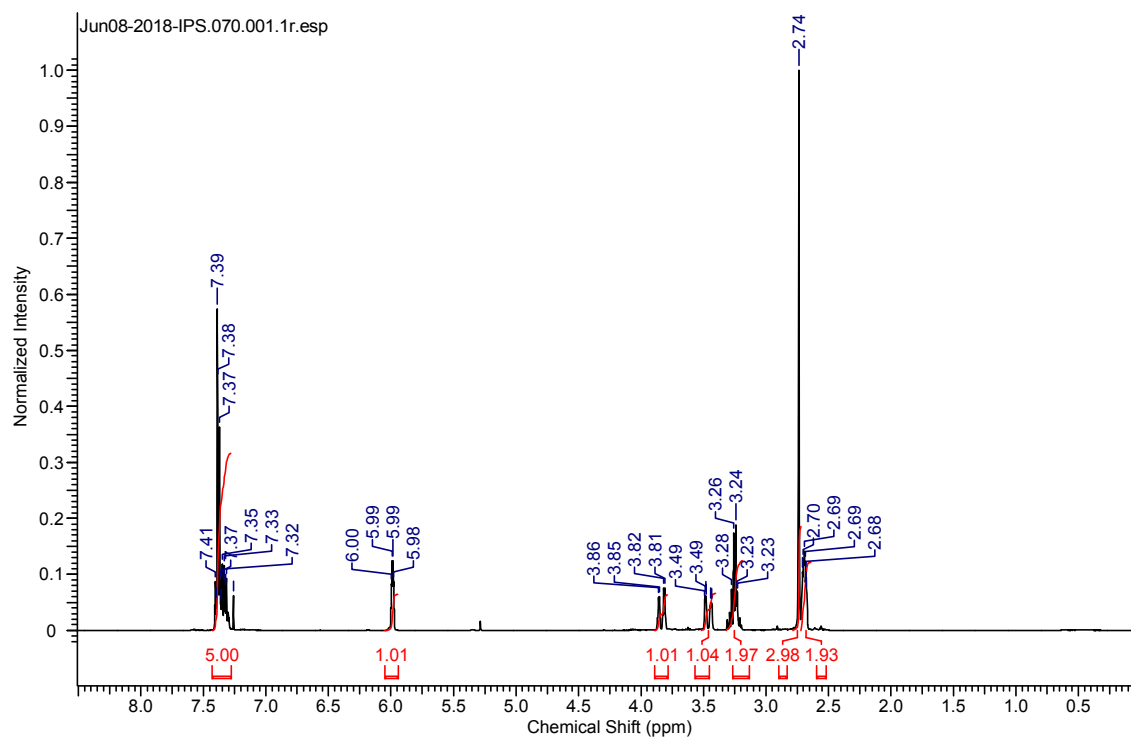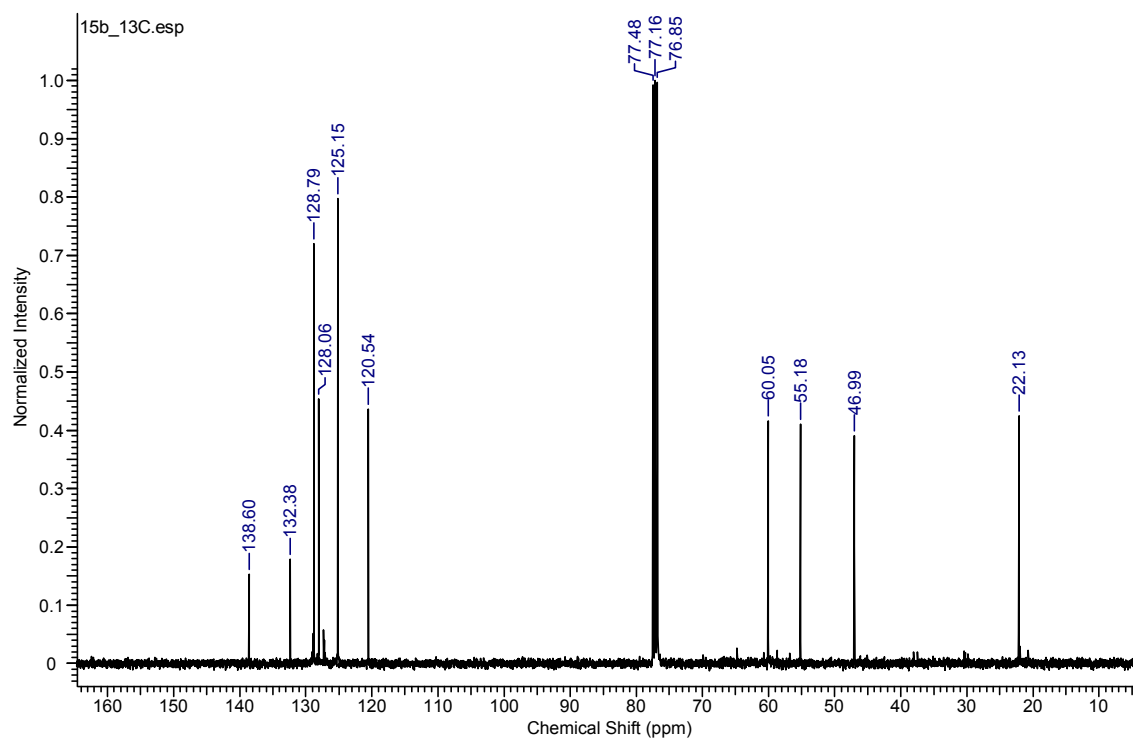
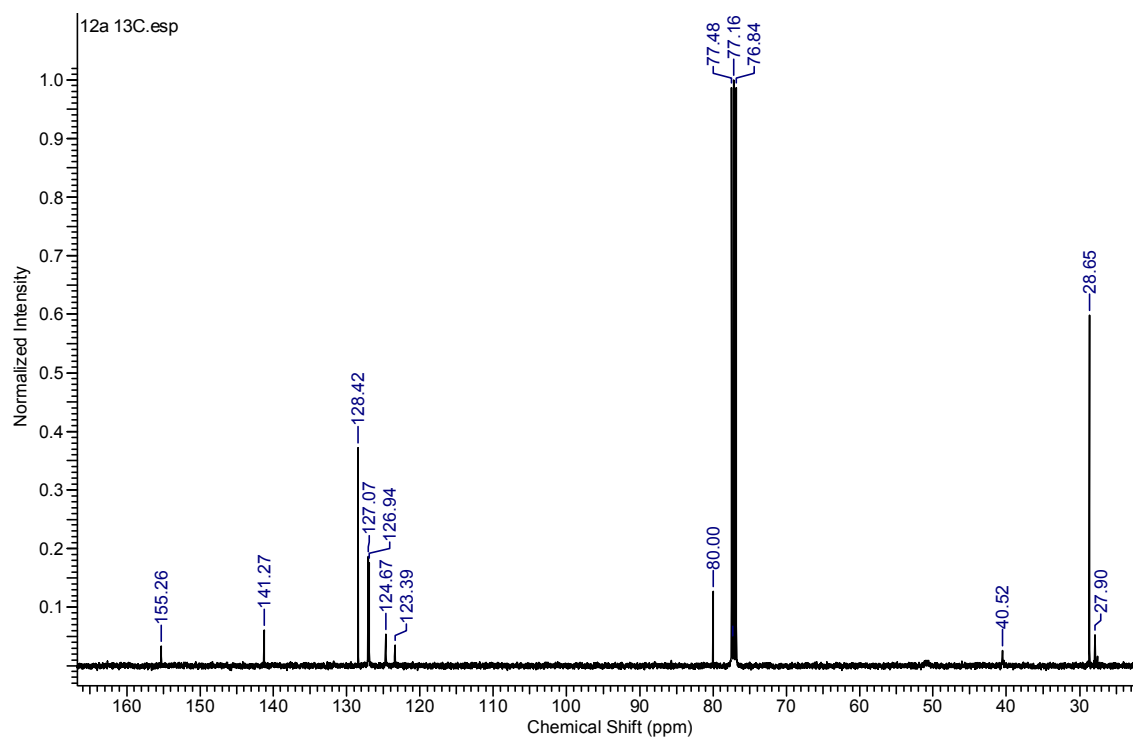## Tetrahydropyridine 5b

Tetrahydropyridine 5c

2b_1H_CDCl3.1r.esp

7.31
7.29
7.26
6.86
6.84
6.01
3.79
3.52
3.51
3.12
3.10
3.09
2.89
2.44
2.43

1.97 2.00 0.97 3.07 1.95 1.97 1.33 1.97

Chemical Shift (ppm)

2b_13C_CDCl3.1r.esp

158.85
134.71
133.83
125.89
121.28
113.73
77.44
77.12
76.80
55.31
45.26
43.20
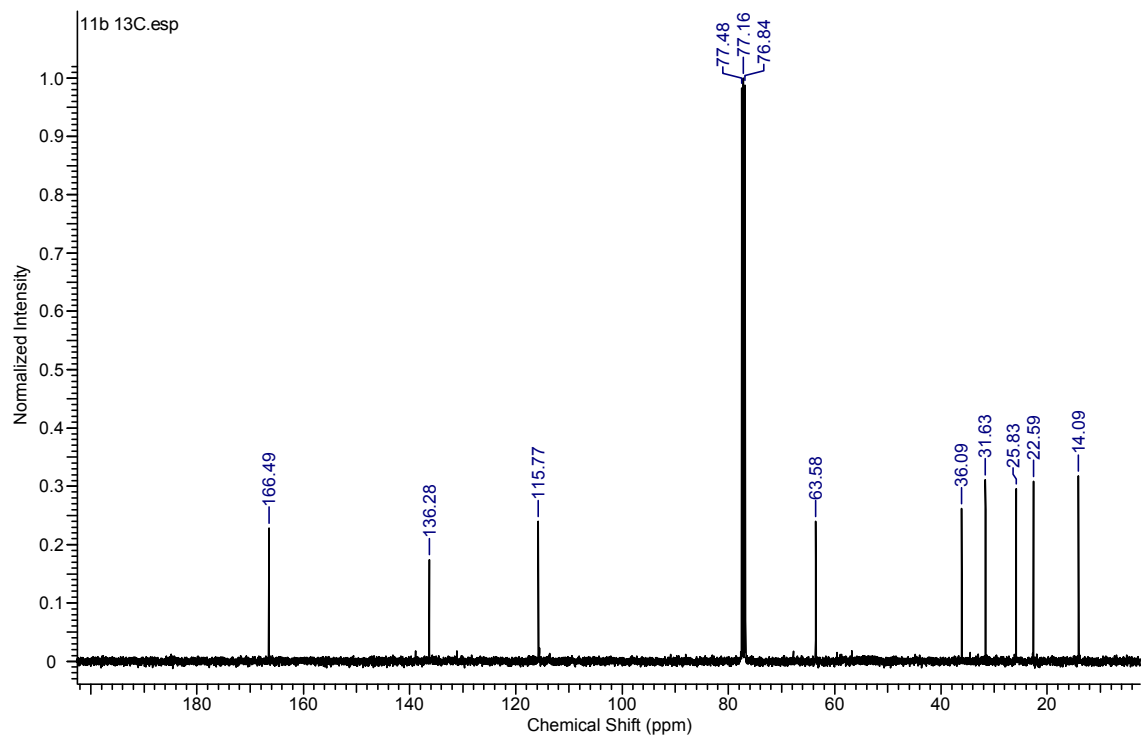27.55

Chemical Shift (ppm)
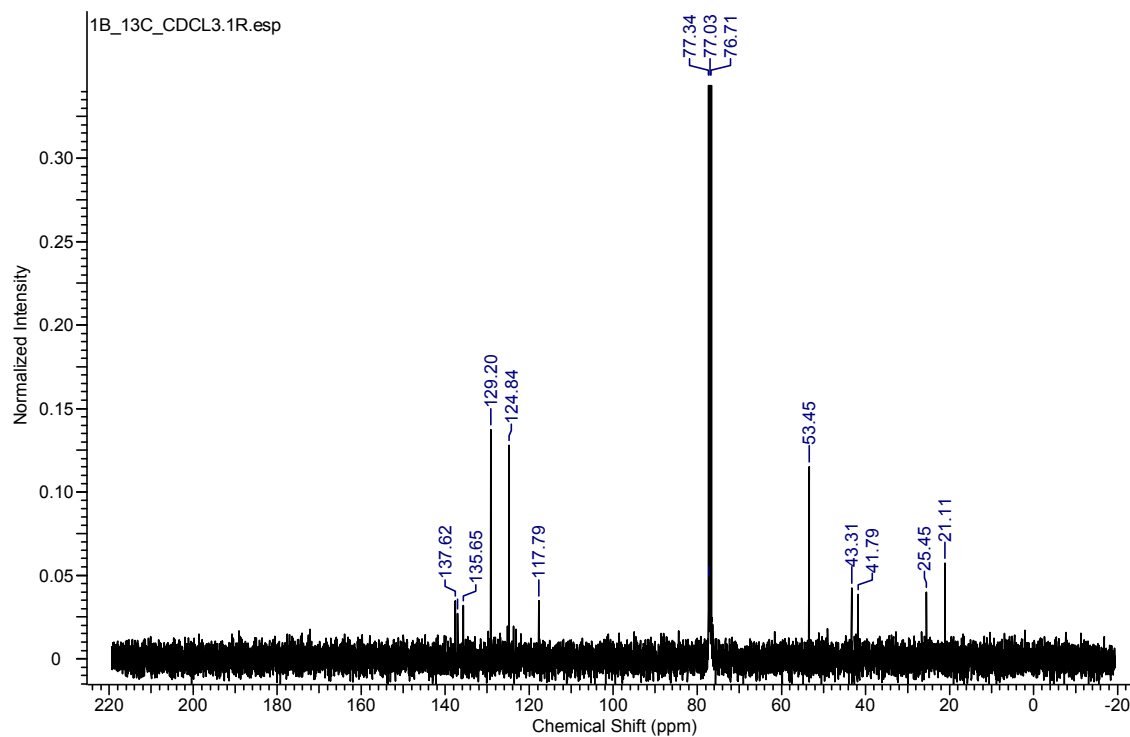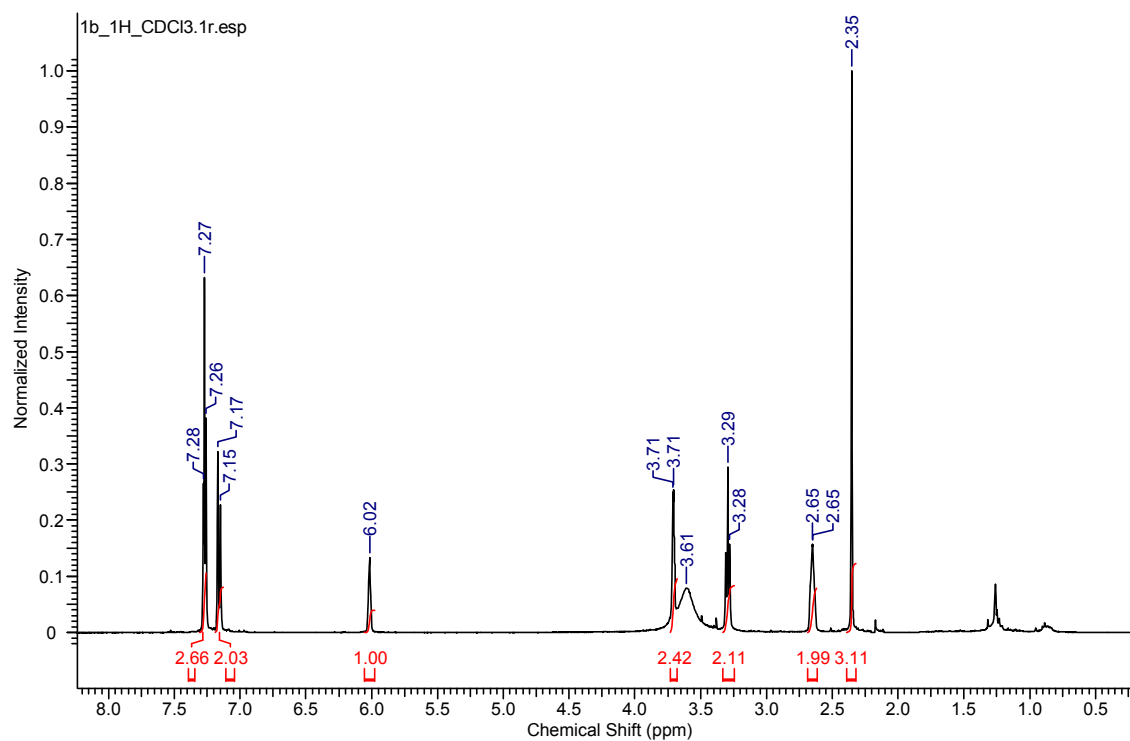
Tetrahydropyridine 5d

Tetrahydropyridine 5e

Tetrahydropyridine 15a

Tetrahydropyridine 15b

Tetrahydropyridine 13a

Apr12–2018-DC.10.fid — CAT52 fr2

12a 13C.esp
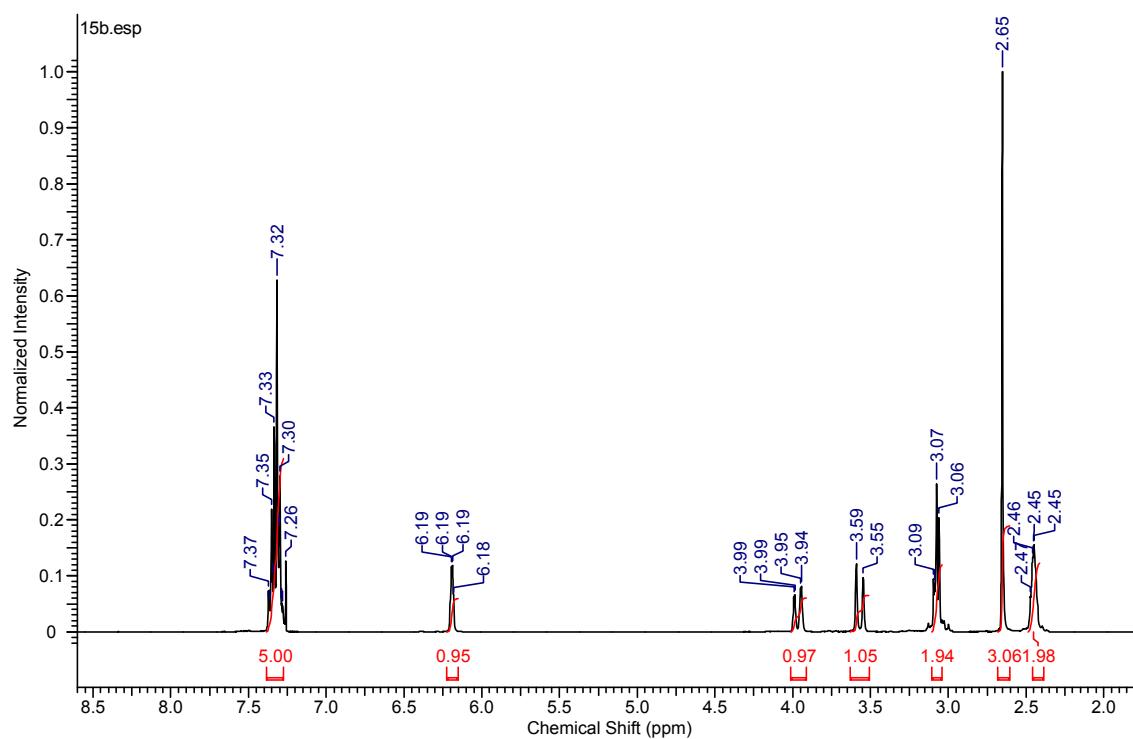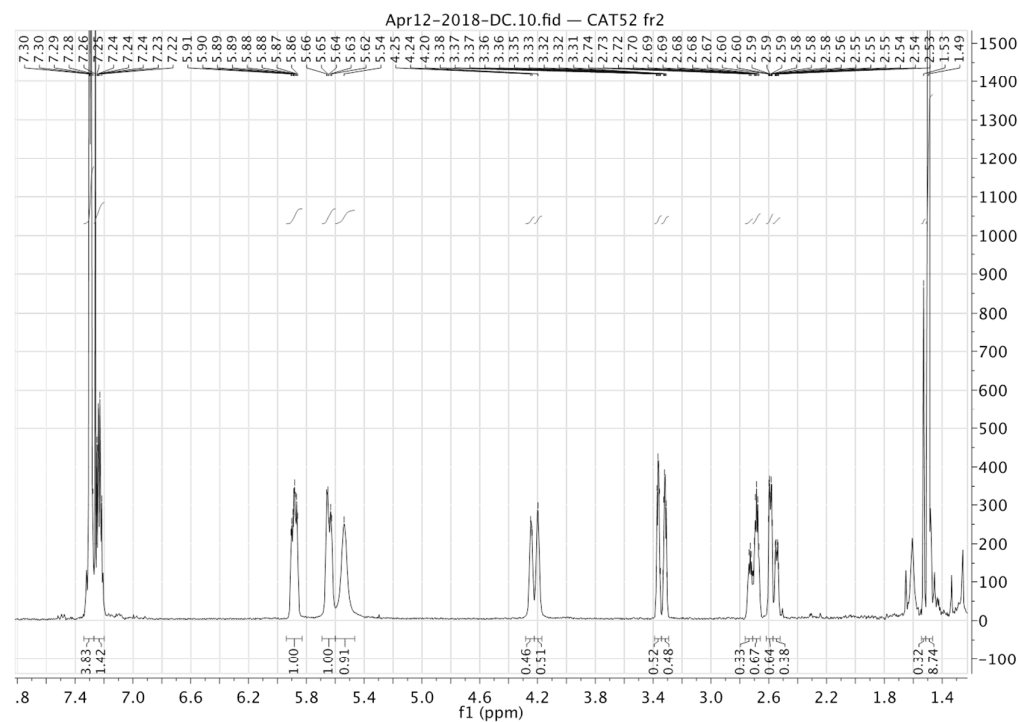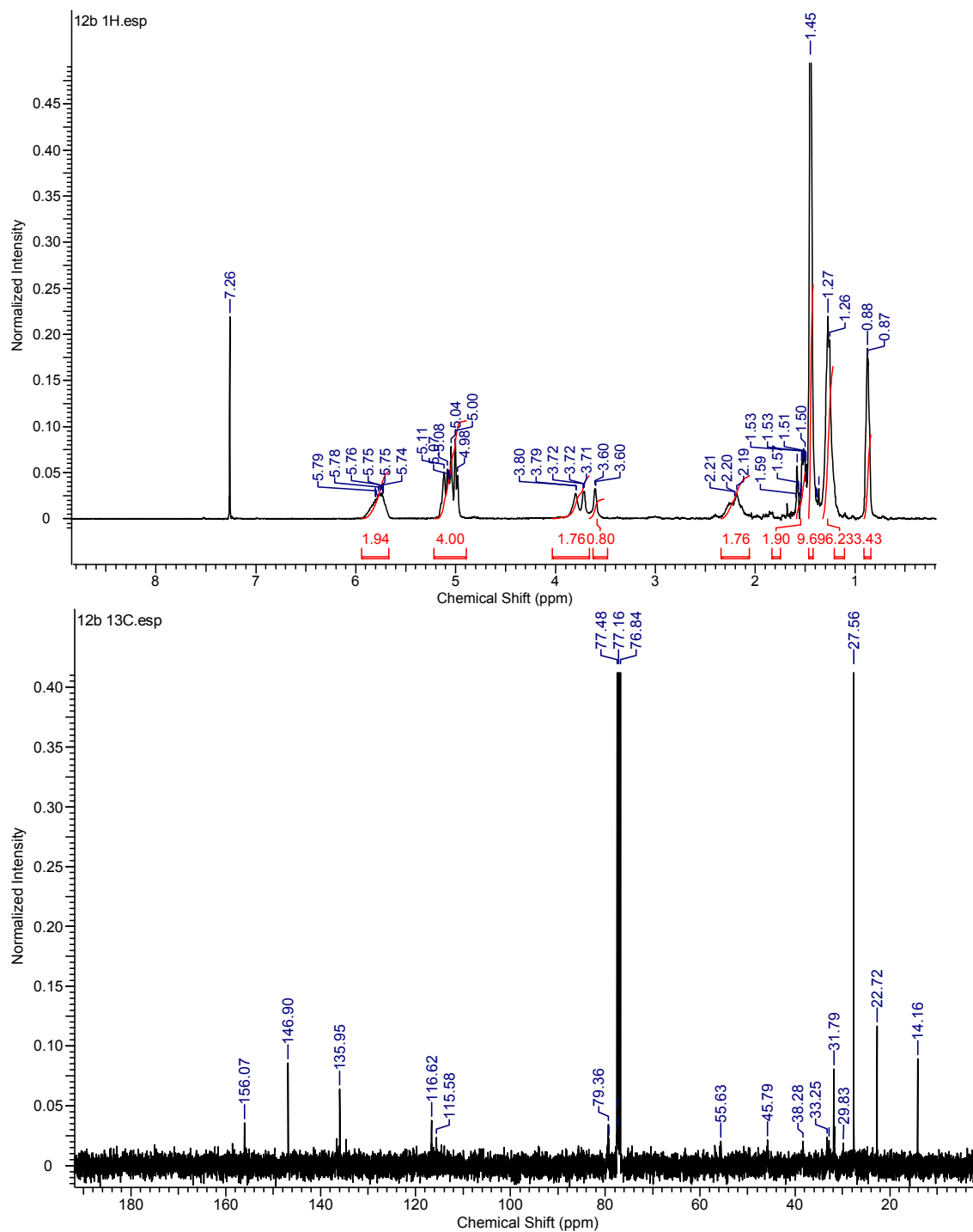
Tetrahydropyridine 14a
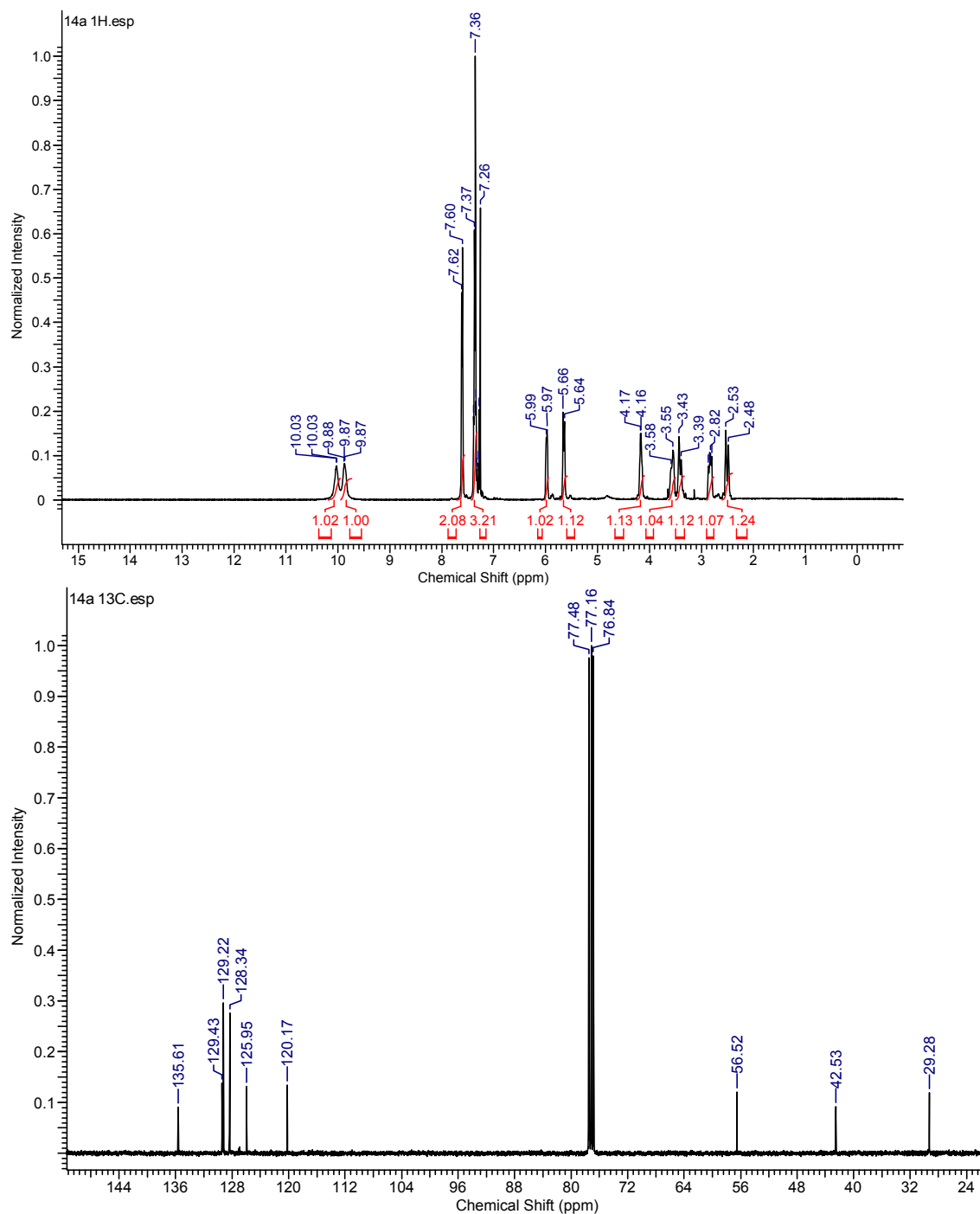
Imine 11b
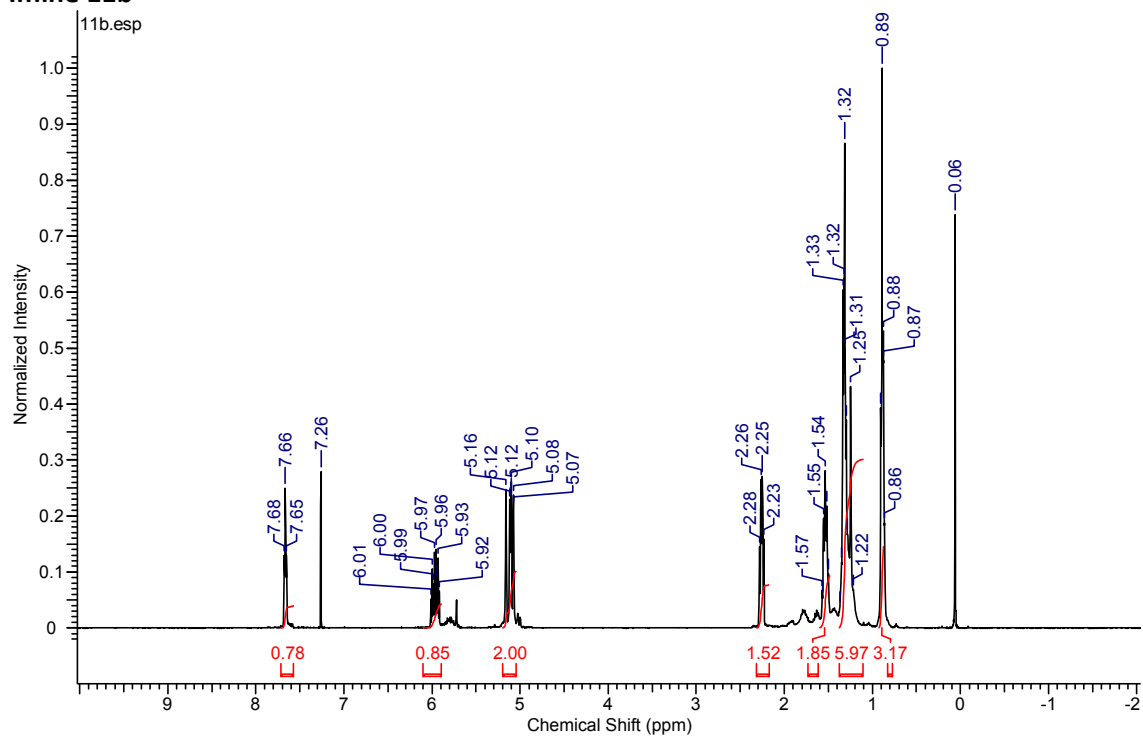
Diene 12b

Tetrahydropyridine 13b

May23-2018-DC.20.fid — CAT78 fr 36-57

13b 13C.esp

Tetrahydropyridine 14b