SUPLEMENTARY INFORMATION

Combined chemoenzymatic strategy for sustainable continuous synthesis of the natural product hordenine

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Content:

a. General information

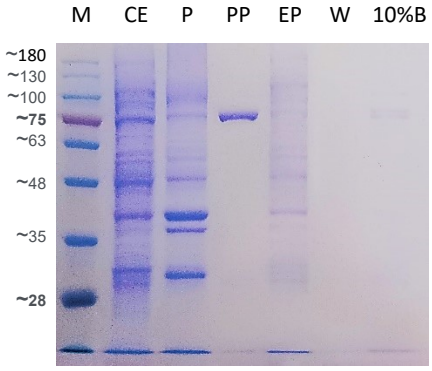
UHPLC method

HPLC Analysis were performed using a Dionex UltiMate 3000 UHPLC with RS Diode Array detector. Column: XBridge BEH C18 Column (130Å, 3.5 µm, 2.1 mm X 150 mm). Injection volume of 2 µL, at 45 °C .

For the analysis of the reductive amination production, the flow rate in the UHPLC was 0.8 mL/min. The Elution phase is reported here with A: Water 0.1% TFA, B: Acetonitrile 0.1% TFA. Method: 0-1 min 5% B, 1-5 min 95% B, 5-5.1 min 100% B, 5.1-6.6 min 100% B, 6.6-7 5% B, 7-9.5 min 5% B. λ = 280 nm. Conversions were calculated from a calibration curve following the formation of product.

For the analysis of the tyramine production and for the activity test of LbTDC, the flow rate in the UHPLC was 1 mL/min. The Elution phase is reported here with A: Water 0.1% TFA, B: Methanol. Method: 0-1 min 1% B, 1-7 min 40% B, 7-9 45% B, 9-10 min 1% B. λ = 280 nm. Conversions were calculated from a calibration curve following the formation of product.

b. Gel from the purification of *Lb*TDC



M: marker
CE: Crude extract - soluble fraction
P: sonicated pellet
PP: purified protein
EP: elution phase
W: Buffer A wash
10%B: 10% buffer B wash

Loading buffer: 25 mM Tris, 300 mM NaCl, 20 mM imidazole, 0.2 mM PLP, pH 7.4
Elution buffer: 25 mM Tris, 300 mM NaCl, 280 mM imidazole, 0.2 mM PLP, pH 7.4

Figure S1. SDS-PAGE for the purification of LbTDC.

c. Activity assay of the *Lb*TDC immobilized enzyme

The activity of immobilized biocatalyst was determined by weighing an appropriate amount of imm-LbTDC (10- 40 mg) into a 2 mL reaction tube with cap, followed by the addition of 2 mL reaction mixture (sodium acetate buffer (0.2 M), L-tyrosine disodium salt hydrate (2.5 mM), PLP (0.2 mM), pH 5). The imm-*Lb*TDC reaction mixture was shaken at 37 °C, 150 rpm and the conversion to the product was analyzed *via* HPLC at 280 nm every five minutes as single readings. The imm-*Lb*TDC specific activity (U/g) is defined as µmol of tyramine formed per minute for grams of immobilized support.

d. Improvement of the batch biocatalyst productivity

The batch biotransformations with the free version of the biocatalyst were carried out with an *Lb*TDC concentration of 0.006 mg/mL, in 1 mL with 2.5 mM L-tyrosine disodium salt hydrate. The immobilized *Lb*TDC biotransformations had an enzyme concentration of 0.075 mg/mL, in 2 mL with 5 mM L-tyrosine disodium salt hydrate. The resin was washed and resused in consecutive batch biotransformation to investigate the immobilised *Lb*TDC productivity.

e. Lactobacillus tyrosine decarboxylase immobilization

Resin

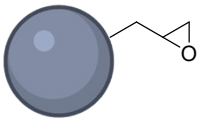
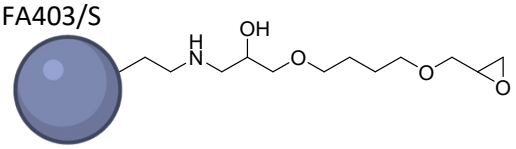
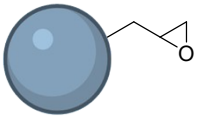
Epoxydic resin	Characteristics
EP4003/S 	Particle size: 100-300 µm Ø Pores: 40-60 nm
HFA403/S 	
EP400/SS 	Particle size: 50-150 µm Ø Pores: 40-50 nm Hydrophilic

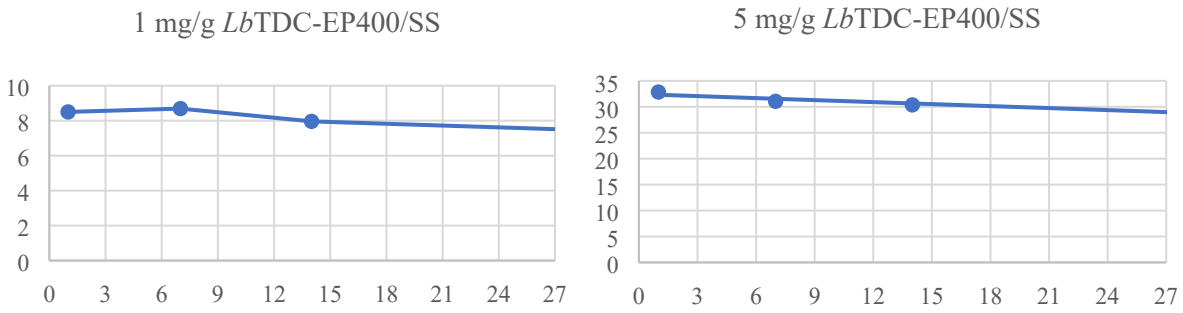
Table S1. Methacrylic supports with epoxy handles tested in the immobilization study.

Protein Loadings

The immobilization results in terms of retained activity and stability (after a week at 4 °C), at different concentration of enzyme (1, 5, 10 mg/g_{matrix}) are reported in Table 2. Data are collected in duplicates and the results are expressed as average of the obtained the data.

Epoxydic Resin	Protein loading	Activity (U/g)	Recovered activity (%)	Stability (%)
EP403/S	1 mg/g	2.2	5	5
HFA403/S	1 mg/g	4.8	11	10
EP400/SS	1 mg/g	8.8	20	20
EP400/SS	5 mg/g	32.9	15	14
EP400/SS	10 mg/g	52.3	12	12

Table S2. *Lb*TDC immobilized on different methacrylate epoxydic resins



Graph S1. Stability over 28 days of *Lb*TDC 1 mg/g and *Lb*TDC 5 mg/g immobilized on EP400/SS

f. Flow test with immobilized enzyme *Lb*TDC

Protein Loading (mg/g)	Residence time (min)	M.c. (%)
1	10	>99
1	5	87
5	5	>99
5	2.5	>99

Table S3. Flow biotransformations with the immobilized biocatalyst in a 1.3 mL PBR

g. Batch test with immobilized enzyme and surfactant for process intensification

For the intensification of the process, we performed batch test where the solubility of the starting material was increased by a co-solubilizer. We investigated three different co-solubilizers: Triton-X100, Tween 20 and PEG 400. The optimal choice was Tween 20. The data from the batch biotransformations performed in the presence of Tween 20 are reported in the Table 3. The stability of the biocatalyst against Tween 20 has been assessed by measuring the activity of the used resin, and the data related to these experiments are reported in the Table 4.

L-tyrosine disodium salt (mM)	Tween 20 (% v/v)	Control M.c. (%)	M.c. (%)
10	5	73	95
30	10	39	84
40	15	34	75

Table S4. Batch biotransformations with the immobilized biocatalyst scaling-up the concertation of L-tyrosine disodium salt hydrate with the co-solubilizer Tween 20. The controls have been set up without adding the surfactant in the biotransformations. The biocatalyst concentration was 0.05 mg/mL and the analysis of the sample was after 16 h.

Tween 20 (% v/v)	Stability (%)
10	99
30	103
40	106

Table S5. Stability of the immobilized biocatalyst after one biotransformation with Tween 20. The activity test on the used immobilized support was performed as reported above.

h. Optimization of hordenine production

Tyramine (mM)	Formaldehyde (eq.)	STAB (eq.)	MeCN (% v/v)	t (min)	M.c. (%)
5	69	12	50	60	94
5	30	7	50	60	88
15	30	7	50	60	96

Table S6. Optimization of the equivalents ratio in batch for hordenine production *via* reductive alkylation of tyramine with STAB

Tyramine (mM)	Formaldehyde (eq.)	pic-BH_3 (eq.)	pH	t (h)	M.c. (%)
5	30	24	4.5	1	45
5	60	24	4.5	24	99
5	60	48	4.5	1	78
5	30	24	9	60	96

Table S7. Optimization of the equivalents ratio in batch for hordenine production *via* reductive alkylation of tyramine with pic-BH_3

Tyramine (mM)	Formaldehyde (eq.)	STAB (eq.)	MeCN (% v/v)	t (min)	M. c. (%)
15	25	7	50	25	>99
15	12.5	5	50	25	95
15	10	5	50	25	88
15	12.5	5	75	4.62	>99
20	12.5	5	75	4.62	>99
30	12.5	3	75	4.62	>99
40	12.5	3	75	4.62	82

Table S8. Optimization of the equivalents ratio in flow and intensification of the process with STAB.

Formaldehyde (Eq.)	STAB (Eq.)	MeCn (% v/v)	Pressure control	Temperature control	RT (min)	M.c. (%)
12.5	12	75	yes	yes	9.23	93
12.5	12	75	no	yes	9.23	83
12.5	12	75	yes	no	9.23	92
12.5	12	75	no	no	9.23	93
12.5	12	75	yes	yes	4.62	94
12.5	12	75	no	yes	4.62	87
12.5	12	75	yes	no	6.92	93
12.5	12	75	no	no	4.62	96

S i. tudying of temperature and pressure conditions for the flow coil

Table S9. Hordenine production starting from 5 mM tyramine (1eq), trialing different residence time under temperature (37°C) and pressure (8 bars) control.

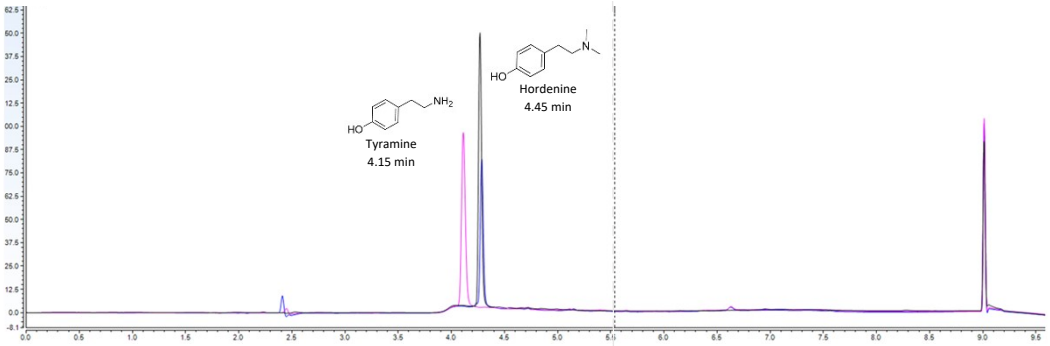
	Hordenine	Formaldehyde	Pic-BH_3	Celite	CH_3COONa	PLP	Na_2CO_3	CO_2
MW (g/mol)	165.23	30.03	211.94		82.03	247.14	60.052	44.01
Total mass (g)	0.206	0.005	0.683	0.3	4.1	0.012	1.226	0.055
Total waste (g)	g(Hordenine) + g(Formaldehyde) + g(Pic-BH_3) + g(Celite) + g(CH_3COONa) + g(PLP) + g(Na_2CO_3) + g(CO_2) + g(Immobilized catalyst) = 7.58							
E factor	36							

j. Simple Environmental Factor calculation

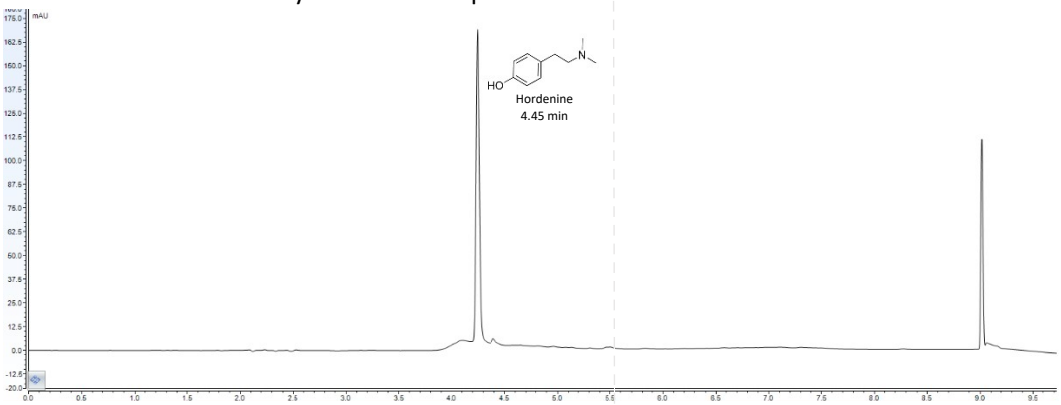
Table S10. Calculation of the process E-factor with picoline borane application. Calculations are made to consider the processing of 250 mL of starting materials.

For the calculation of the simple E-factor, we added up the total masses (g) of each reaction component. To this value we added 1.2 g, corresponding to the support where the biocatalyst was immobilized. Finally, we divided the resulting value for the produced hordenine mass.

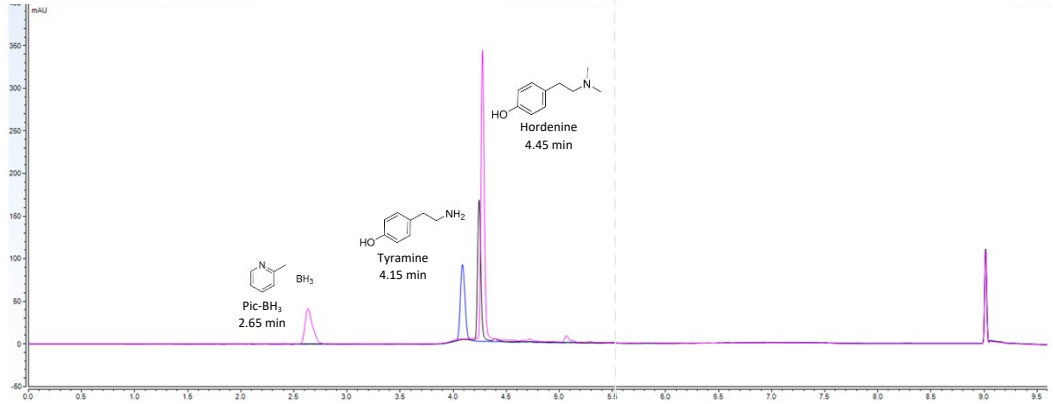
HPLC chromatograms



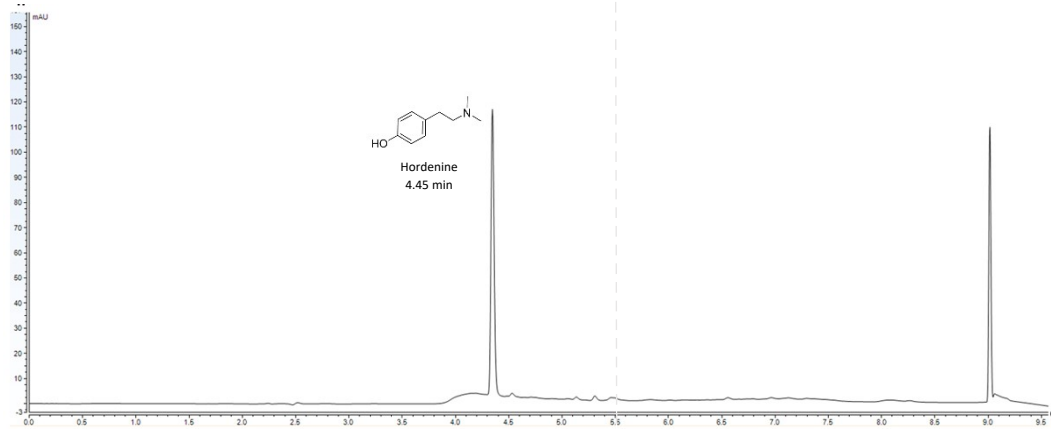
Spectrum 1: Full conversion to hordenine in continuous flow in the system with STAB application. Hordenine STD in black. Tyramine STD in pink.



Spectrum 2: Hordenine peak from purification in the system with STAB application.



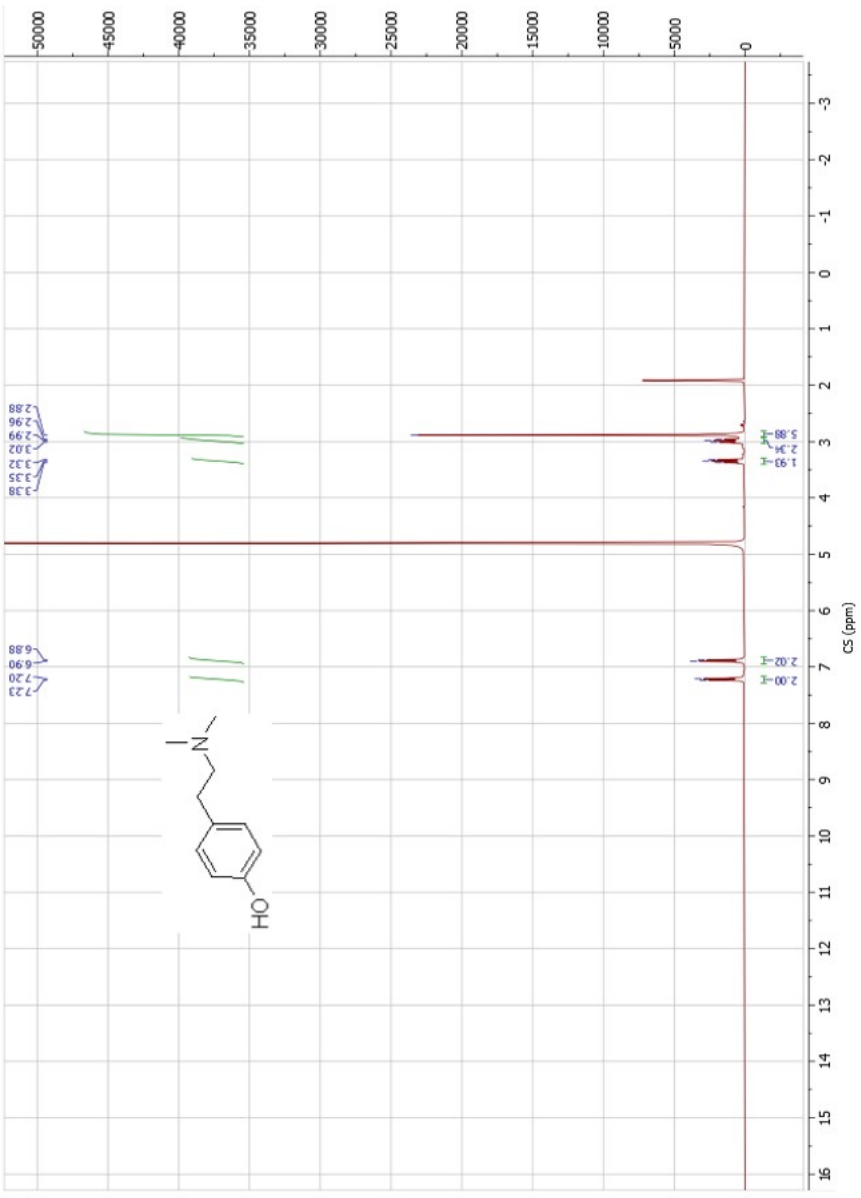
Spectrum 3: full conversion to hordenine in continuous flow in the system with pic-BH_3 application. Hordenine STD in black. Tyramine STD in blue.



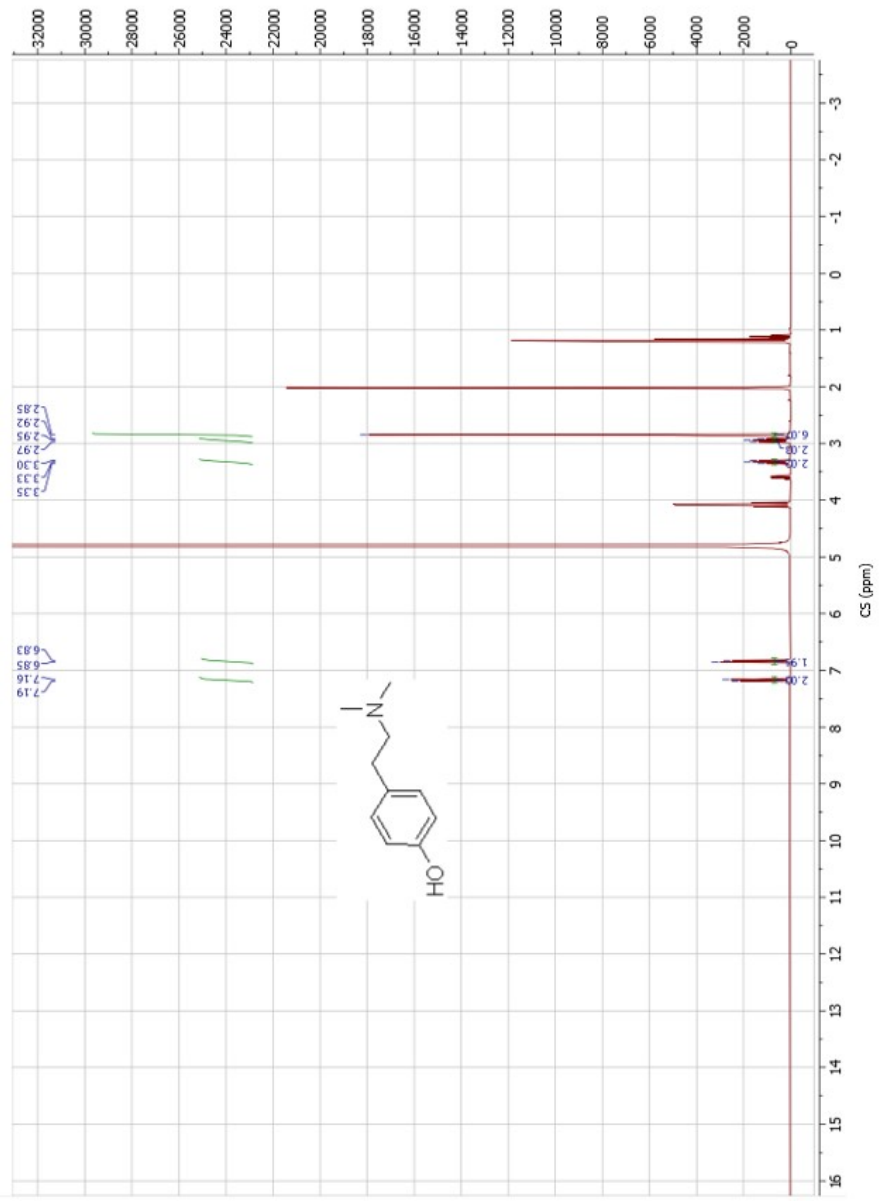
Spectrum 3: Hordenine peak from purification in the system with STAB application.

^{1}H-NMR for characterization and purity evaluation of the produced hordenine

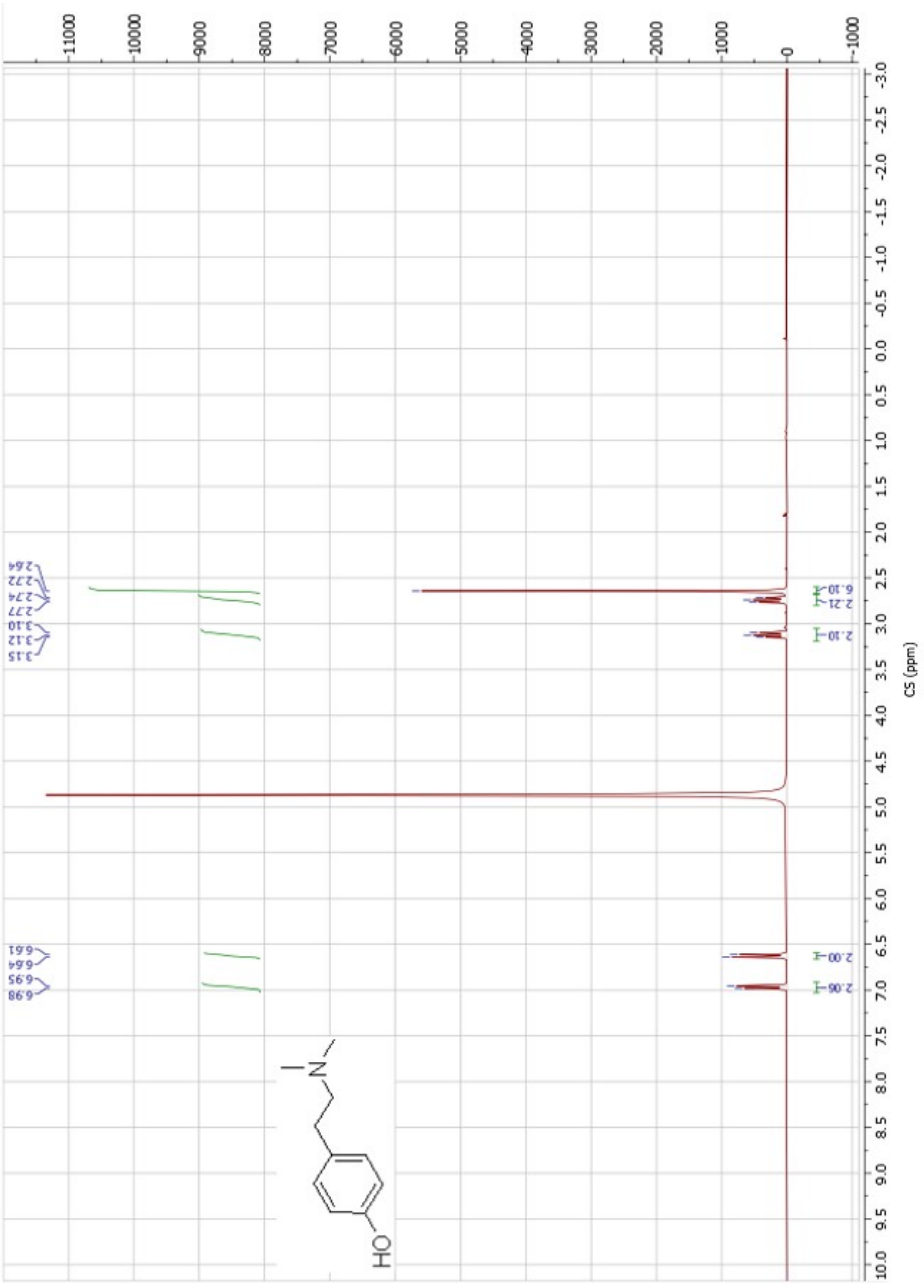
δ_H (400 MHz, D_2O) 2.88 (6 H, s, $N(CH_3)_2$), 2.99 (2H, at, *J* = 8.3 Hz, CH_2), 3.35 (2H, at, *J* = 7.3 Hz, CH_2), 6.89 (2H, d, *J* = 8.4 Hz, CH), 7.22 (2H, d, *J* = 8.6 Hz, CH).



^{1}H-NMR Spectrum 1: Hordenine from product purification in the system with STAB application



^{1}H-NMR Spectrum 2: Hordenine from product purification in the system with pic-BH_3 application



^{1}H-NMR Spectrum 3: Hordenine standard