

Supporting Information

Synthesis and Laccase-Mediated Oxidation of New Condensed 1,4-Dihydropyridine Derivatives

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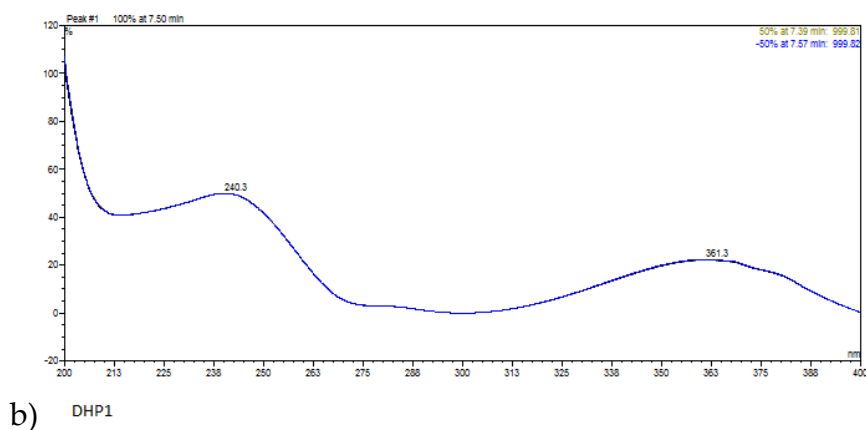
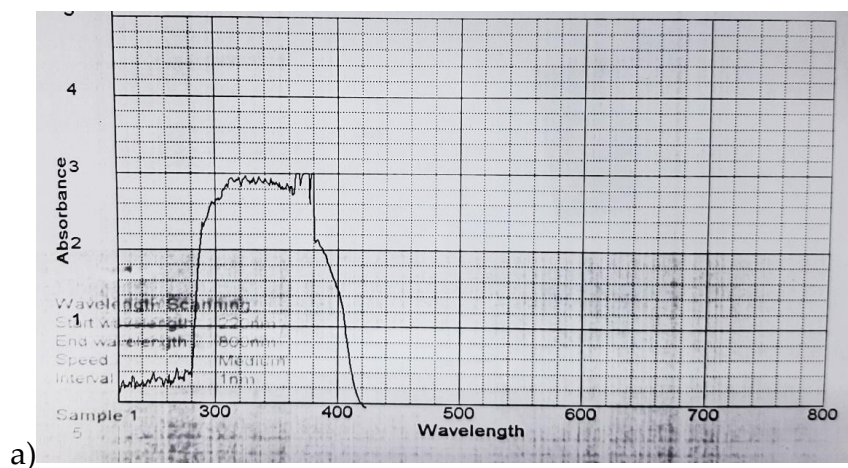
⁵ Faculty of Chemistry, University of Belgrade, Studentski trg 16, 11158 Belgrade, Serbia; vbeskoski@chem.bg.ac.rs

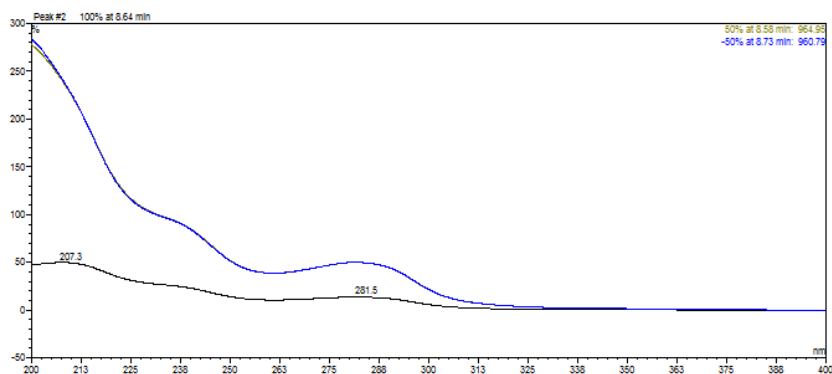
⁶ Laboratory of Analytical Chemistry, Department of Chemistry, National and Kapodistrian University of Athens, Panepistimioupolis Zografou, 15771 Athens, Greece; ntho@chem.uoa.gr

* Correspondence: jasmina.nikodinovic@gmail.com or jasmina.nikodinovic@imgge.bg.ac.rs; Tel.: +381-11-397-60-34

† Equal contribution.

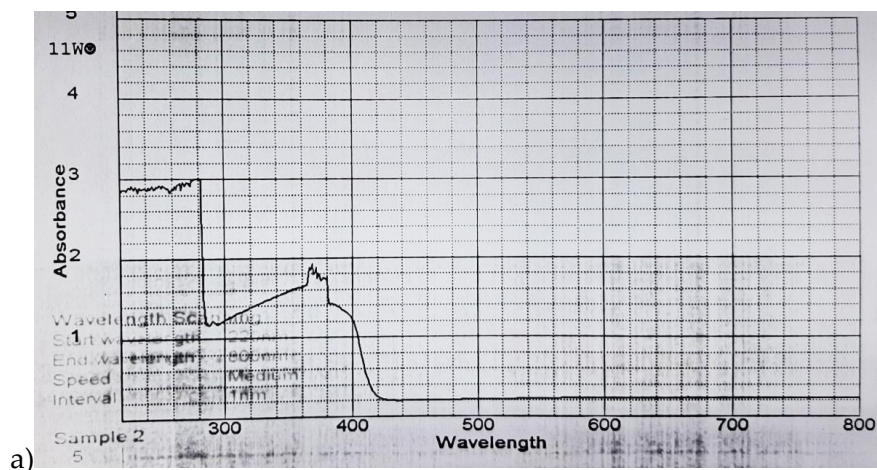
UV-Vis spectra of DHP substrates and products



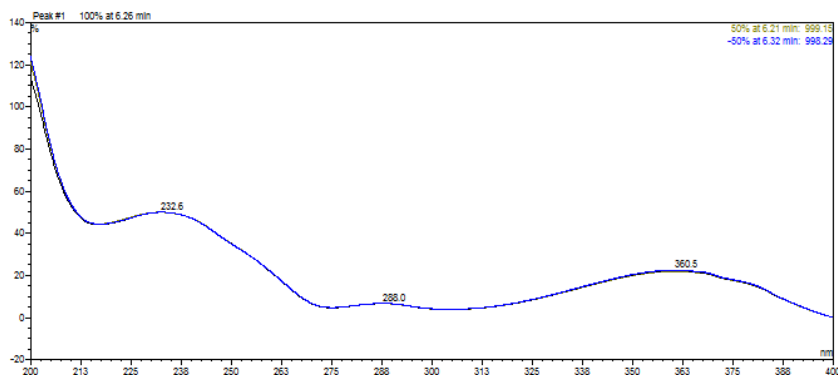


c) DHP1 product

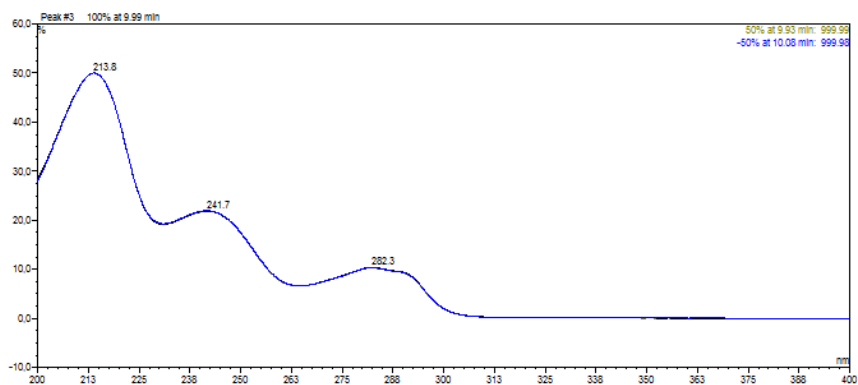
Figure S1. DHP1 **a)** wave scan 220 - 800 nm, starting compound from 290 - 410 nm (concentrated sample dissolved in MeOH, recorded at spectrophotometer); **b)** wave scan 200 - 400 nm, starting compound from 313 - 400 nm (sample conc. 1 mg/mL, dissolved in MeOH, recorded at HPLC); **c)** wave scan 200 - 400 nm, reaction product -peak of product (blue line) (sample conc. 1 mg/mL, dissolved in MeOH, recorded at HPLC) (black line is from another wavelength channel).



a)

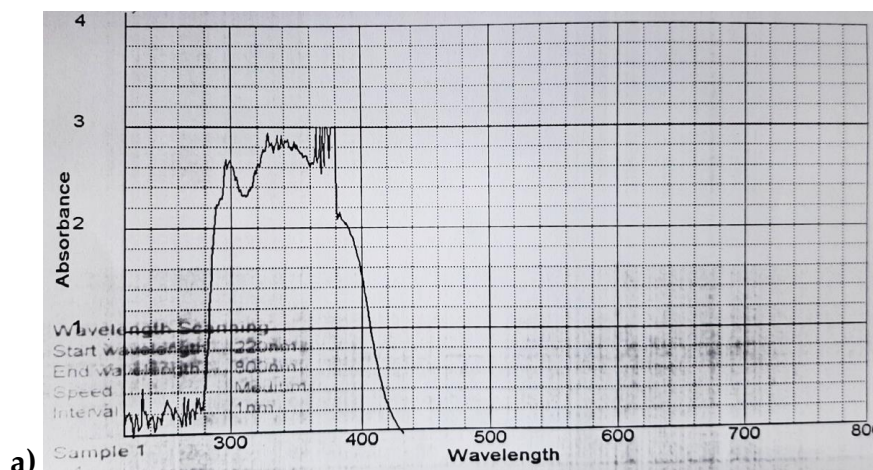


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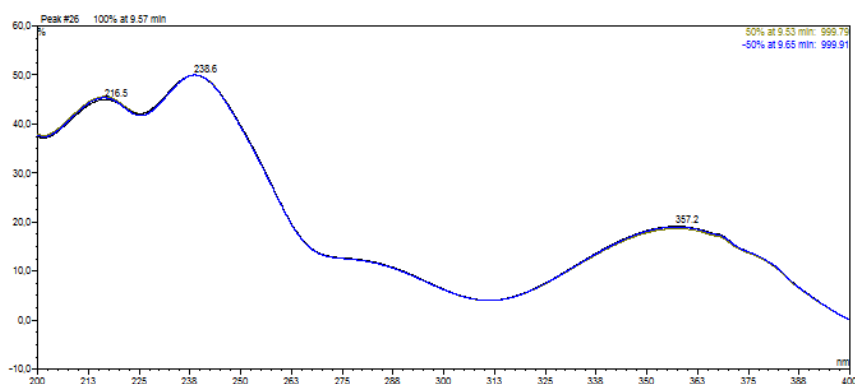


c) DHP2 product

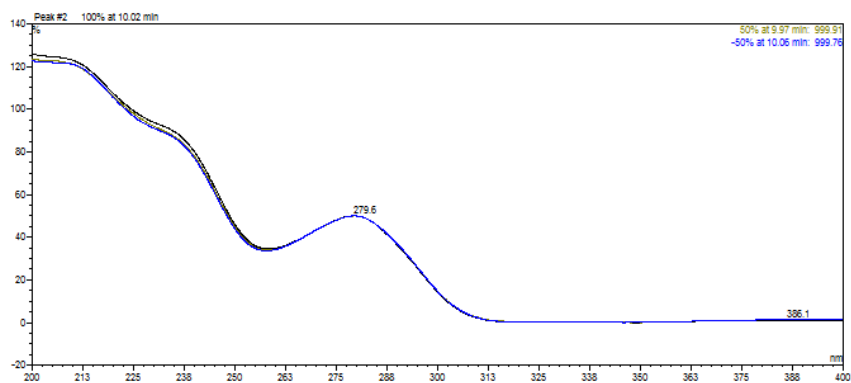
Figure S2. DHP2 **a)** wave scan 220 - 800 nm, starting compound from 290 - 410 nm (concentrated sample dissolved in MeOH, recorded at spectrophotometer); **b)** wave scan 200 - 400 nm, starting compound from 313 - 400 nm (sample conc. 1 mg/mL, dissolved in MeOH, recorded at HPLC); **c)** wave scan 200 - 400 nm, reaction product (sample conc. 1 mg/mL, dissolved in MeOH, recorded at HPLC).



a)

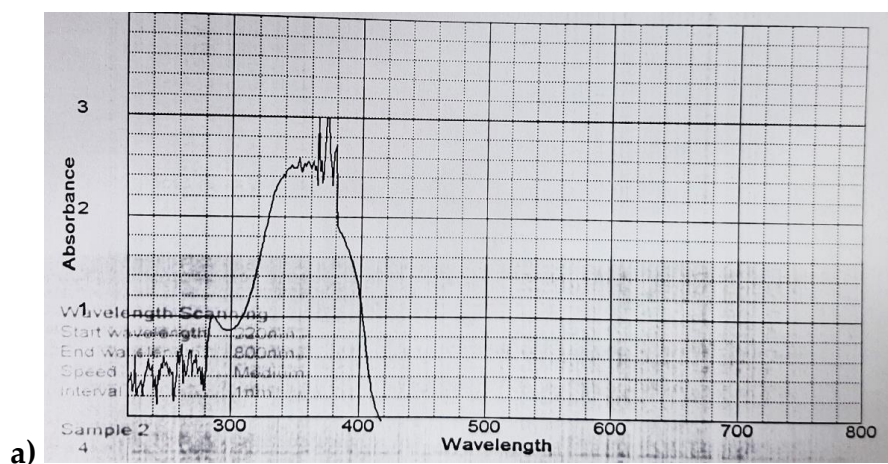


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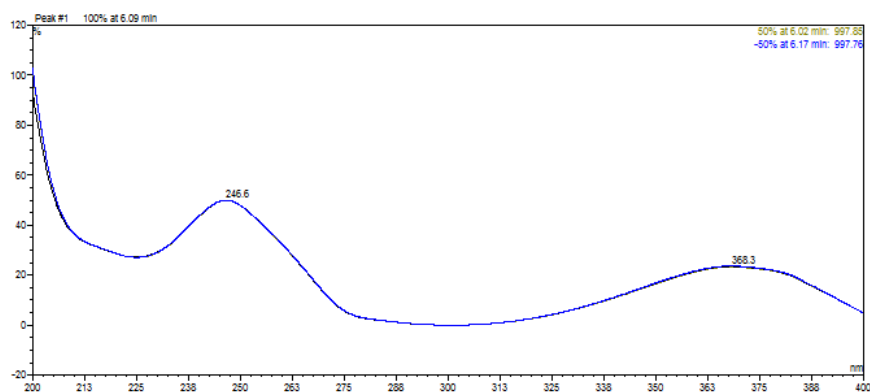


c) DHP3 product

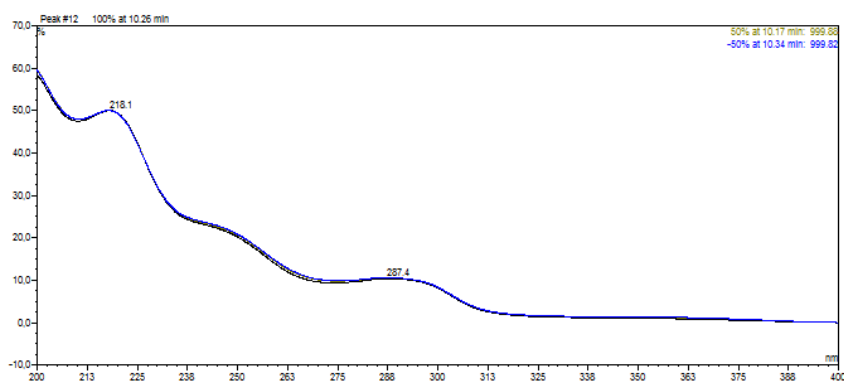
Figure S3. DHP3 **a)** wave scan 220 - 800 nm, starting compound from 290 - 410 nm (concentrated sample dissolved in MeOH, recorded at spectrophotometer); **b)** wave scan 200 - 400 nm, starting compound from 313 - 400 nm (sample conc. 1 mg/mL, dissolved in MeOH, recorded at HPLC); **c)** wave scan 200 - 400 nm, reaction product (sample conc. 1 mg/mL, dissolved in MeOH, recorded at HPLC).



a)

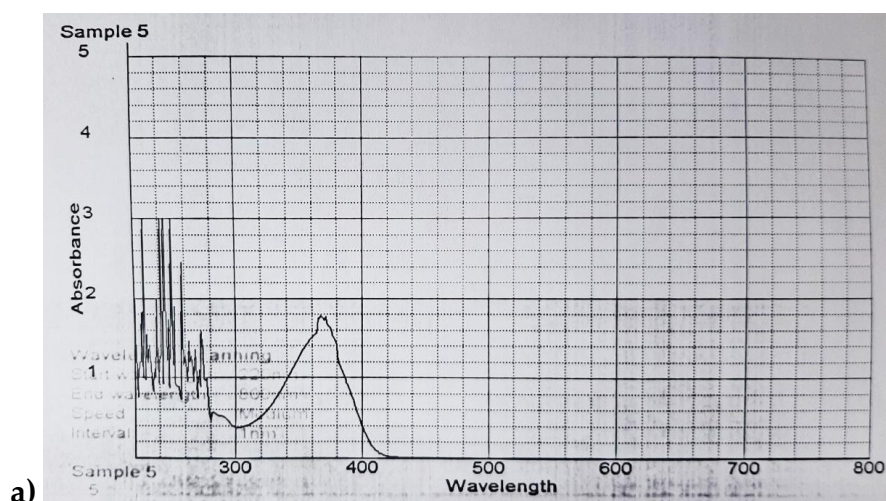


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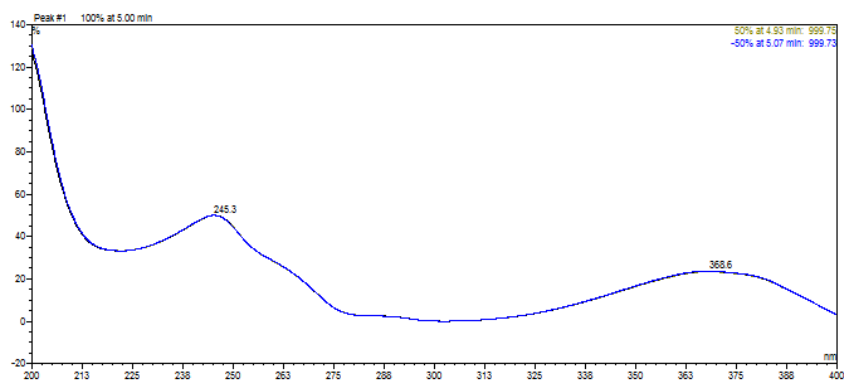


c) DHP 4 product

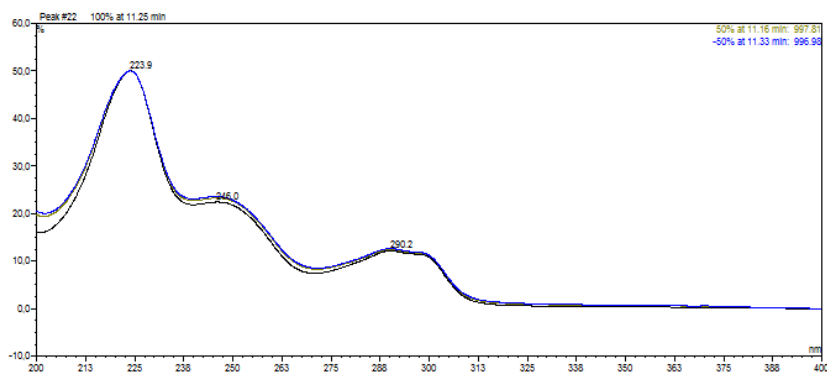
Figure S4. DHP4 **a)** wave scan 220 - 800 nm, starting compound from 290 - 410 nm (concentrated sample dissolved in MeOH, recorded at spectrophotometer); **b)** wave scan 200 - 400 nm, starting compound from 313 - 400 nm (sample conc. 1 mg/mL, dissolved in MeOH, recorded at HPLC); **c)** wave scan 200 - 400 nm, reaction product (sample conc. 1 mg/mL, dissolved in MeOH, recorded at HPLC).



a)

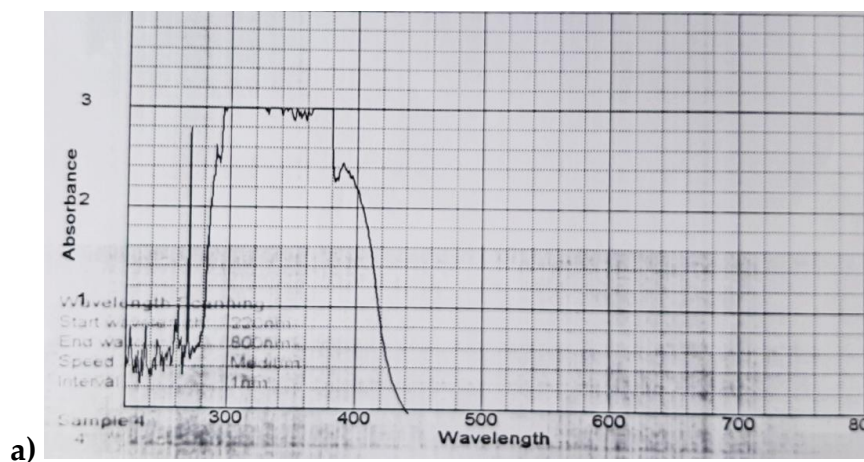


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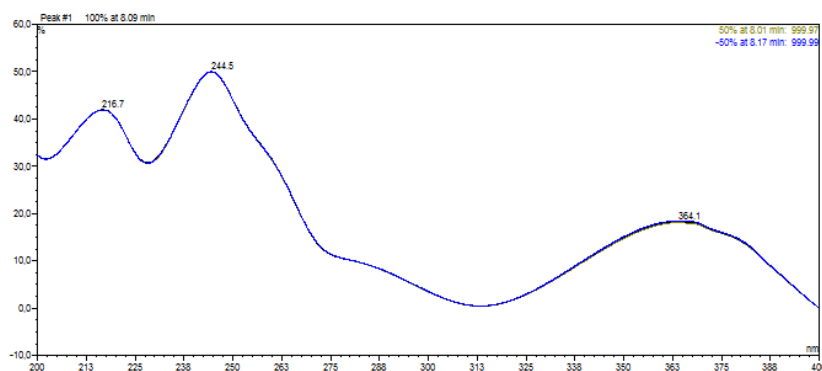


c) DHP 5 product

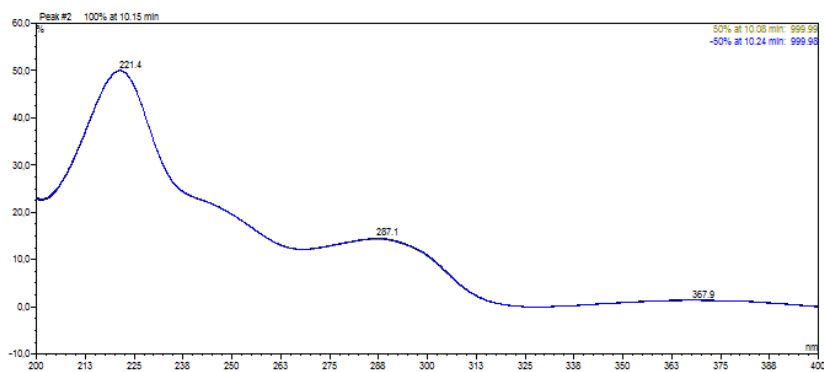
Figure S5. DHP5 a) wave scan 220 - 800 nm, starting compound from 290 - 410 nm (concentrated sample dissolved in MeOH, recorded at spectrophotometer); b) wave scan 200 - 400 nm, starting compound from 313 - 400 nm (sample conc. 1 mg/mL, dissolved in MeOH, recorded at HPLC); c) wave scan 200 - 400 nm, reaction product (sample conc. 1 mg/mL, dissolved in MeOH, recorded at HPLC).



a)



b) DHP6



c) DHP6 product

Figure S6. DHP6 **a)** wave scan 220 - 800 nm, starting compound from 290 - 410 nm (concentrated sample dissolved in MeOH, recorded at spectrophotometer); **b)** wave scan 200 - 400 nm, starting compound from 313 - 400 nm (sample conc. 1 mg/mL, dissolved in MeOH, recorded at HPLC); **c)** wave scan 200 - 400 nm, reaction product (sample conc. 1 mg/mL, dissolved in MeOH, recorded at HPLC).

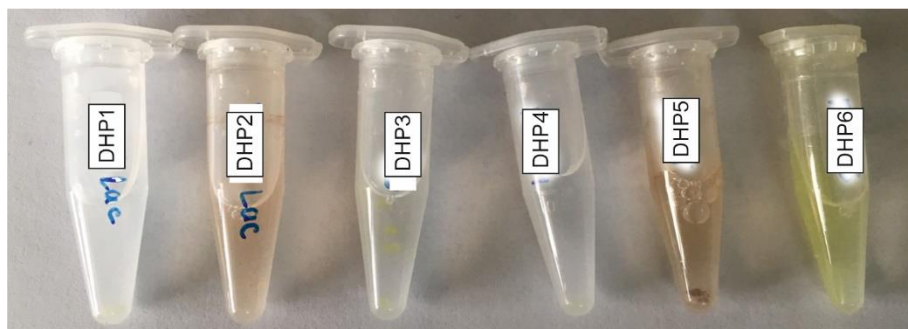
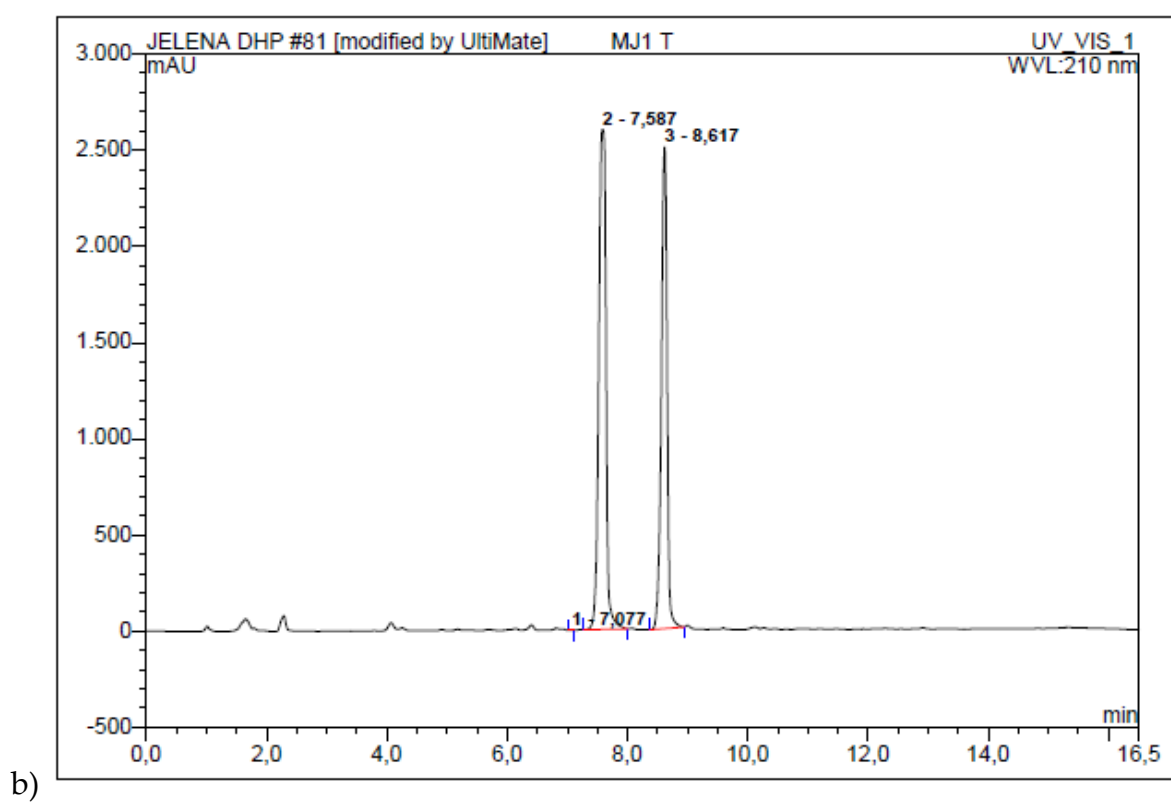
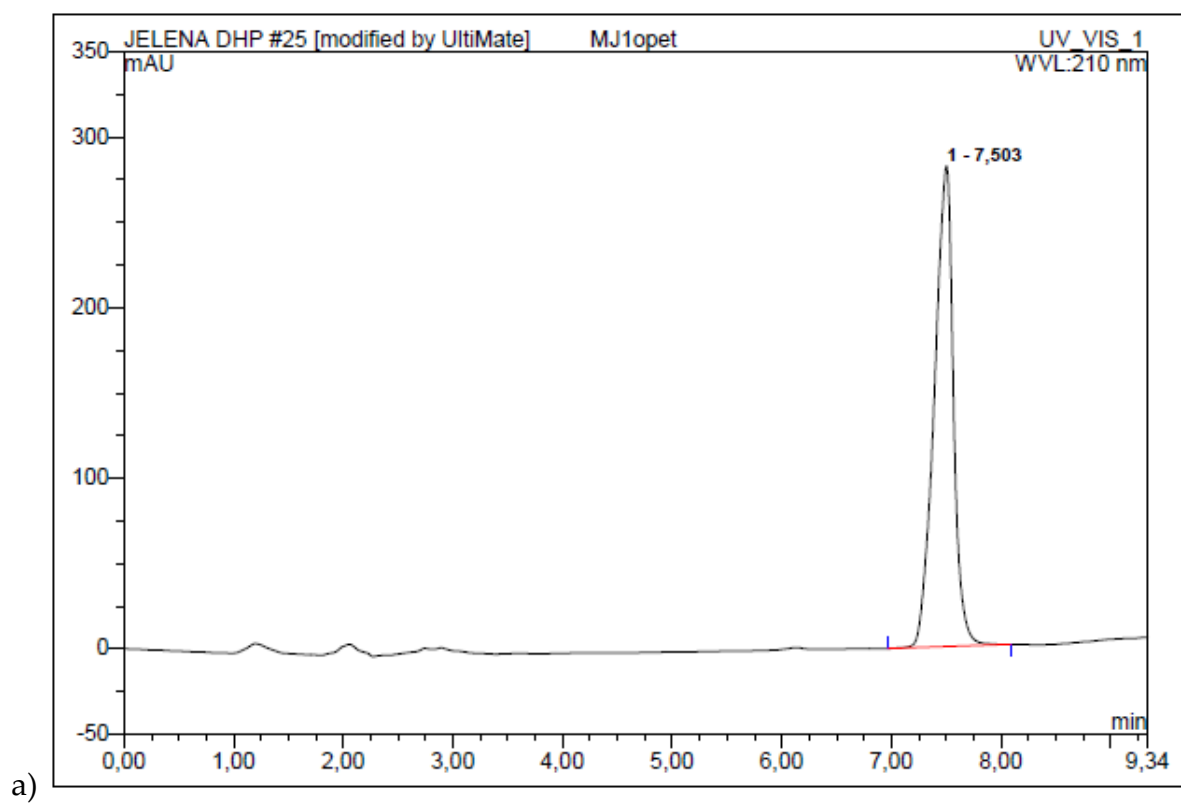
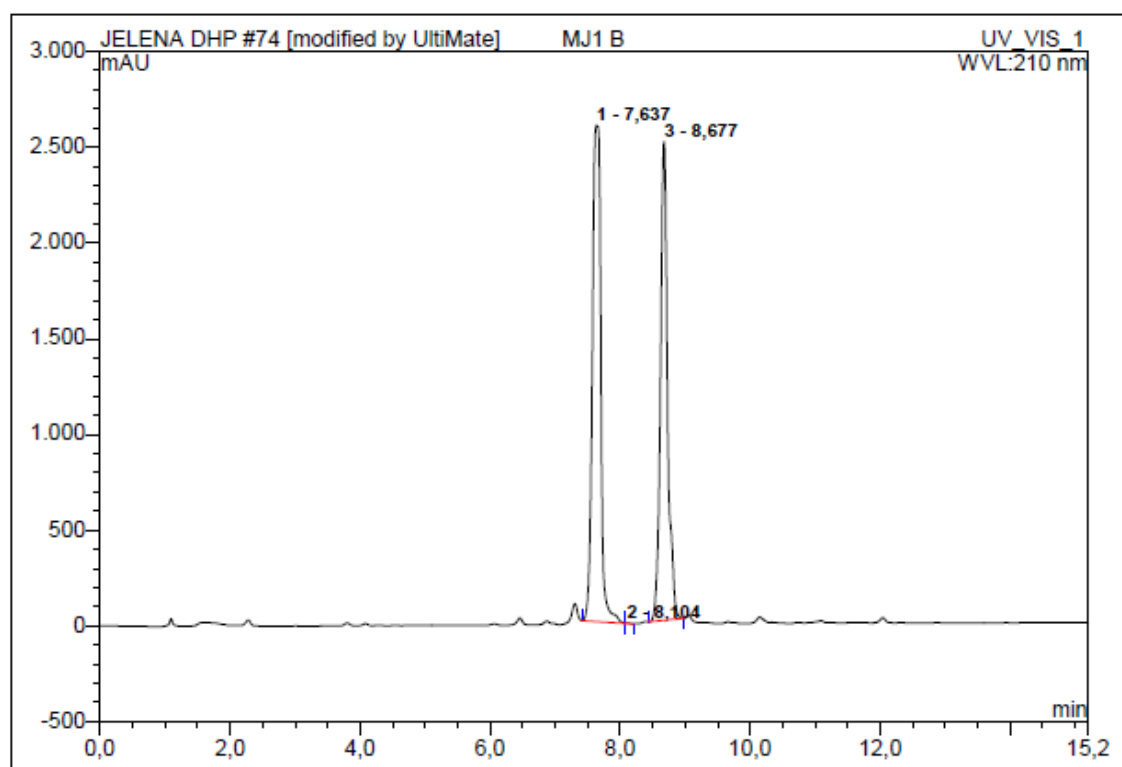


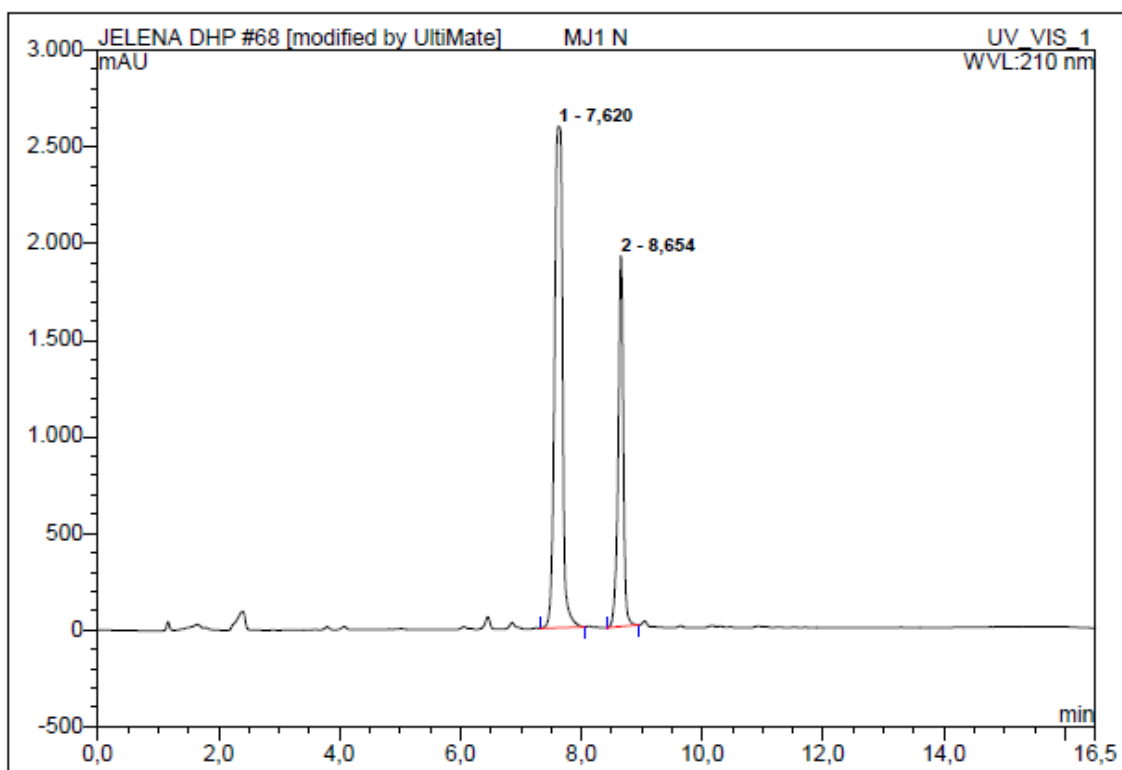
Figure S7. Colors of reactions after 18 h incubation with *Tv*Lacc without ABTS mediator using six different DHP substrates.



c)



d)



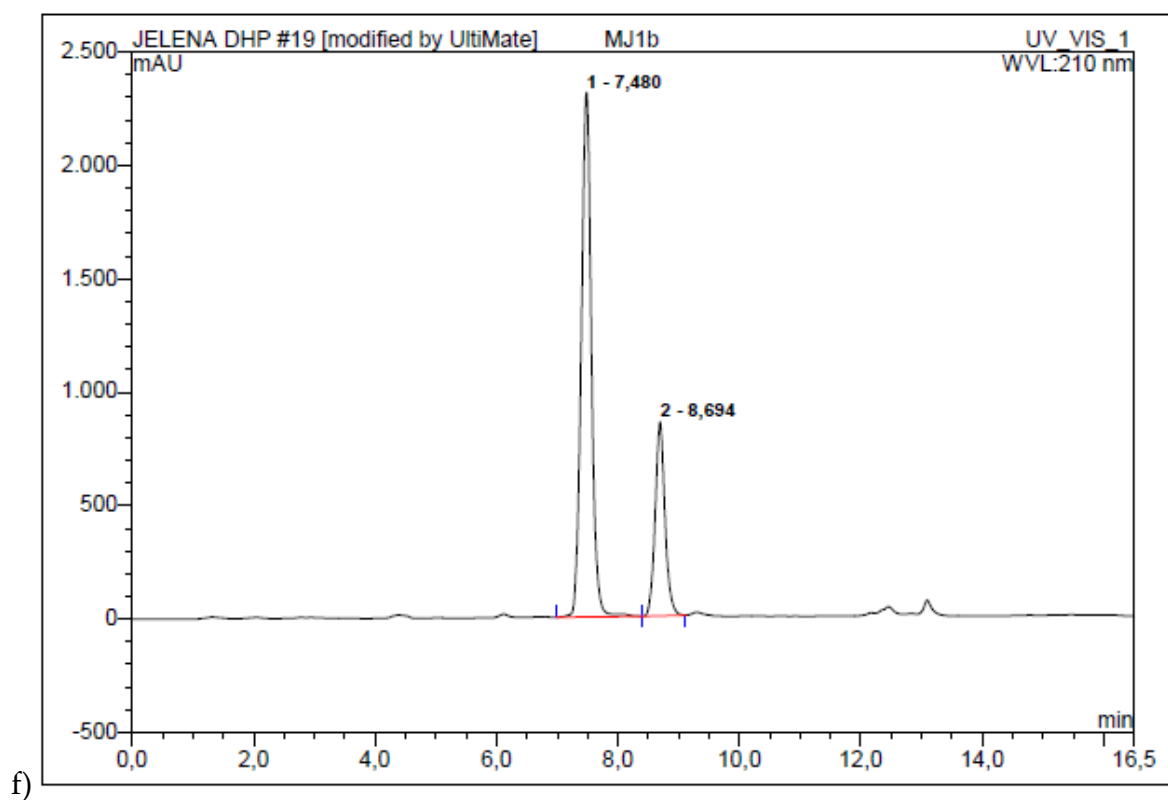
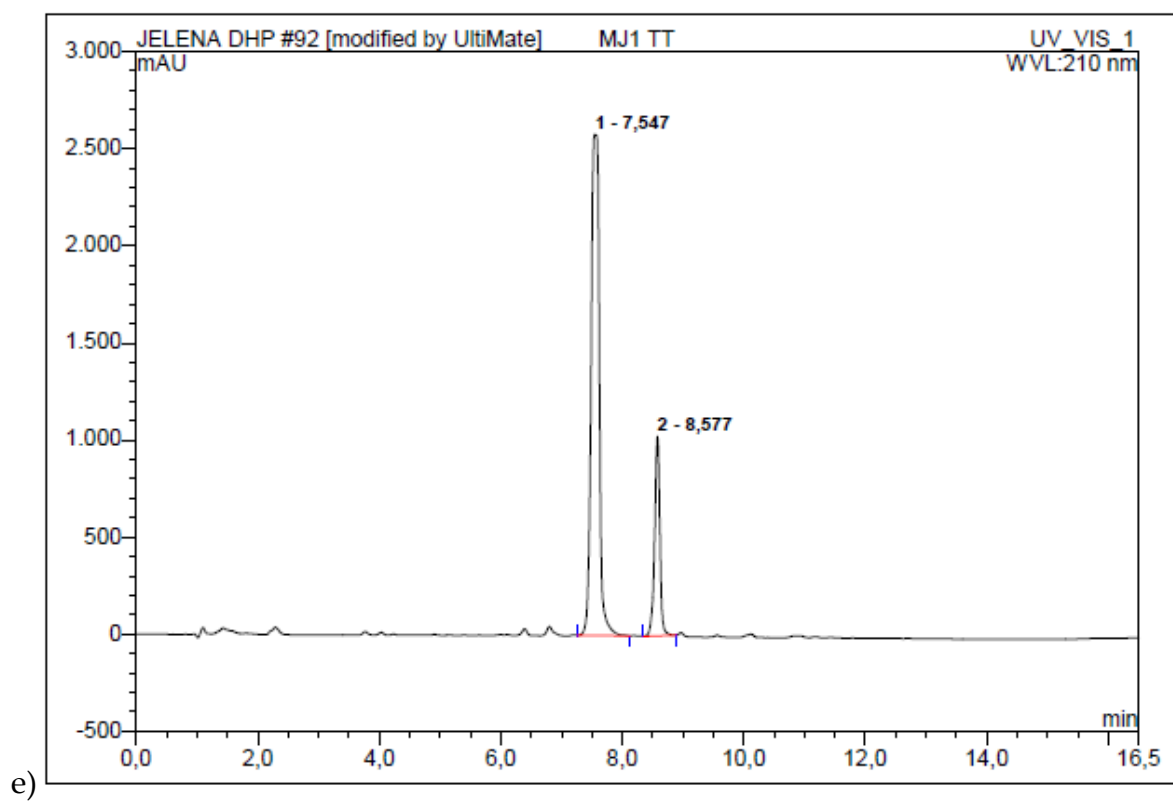
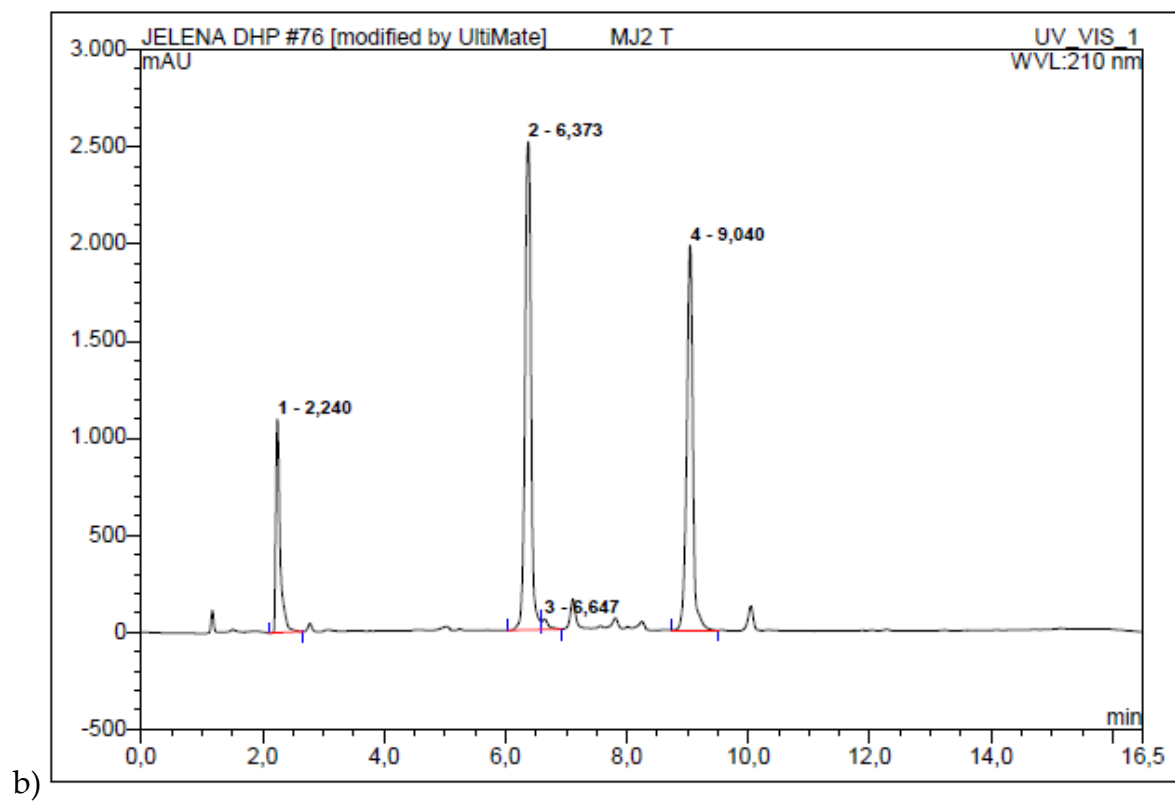
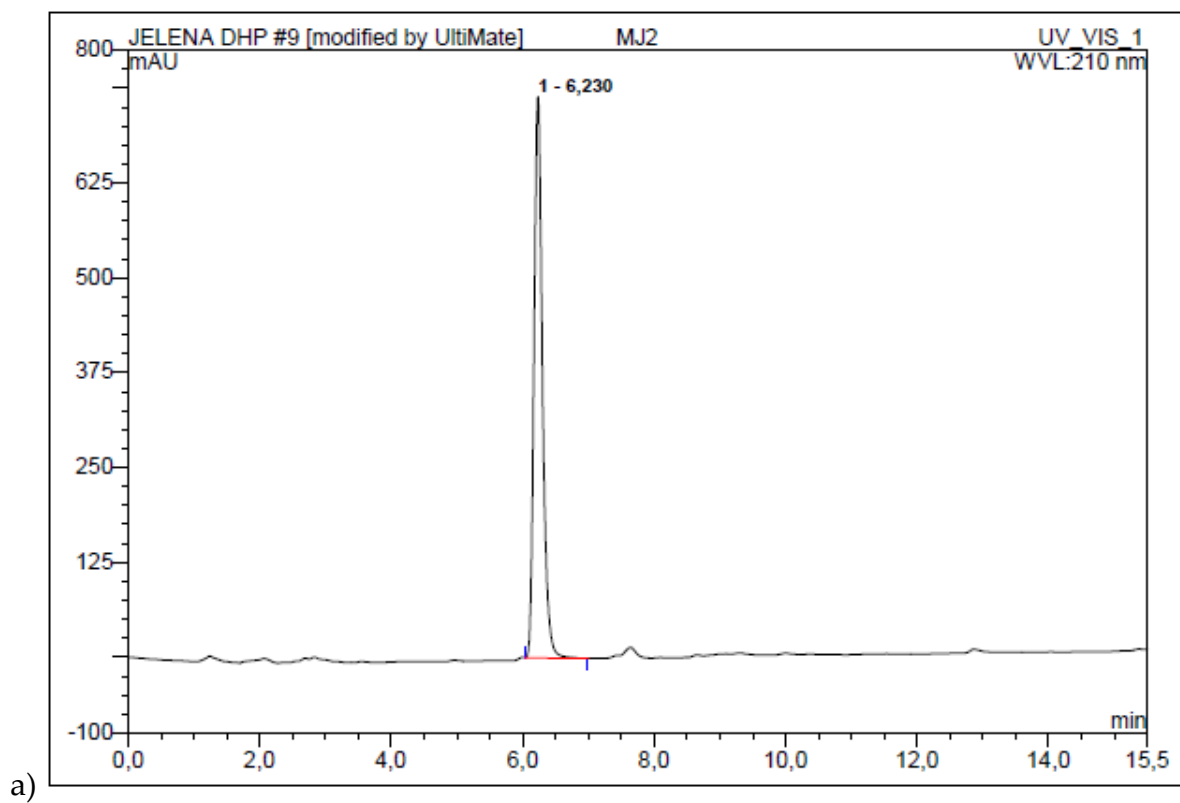
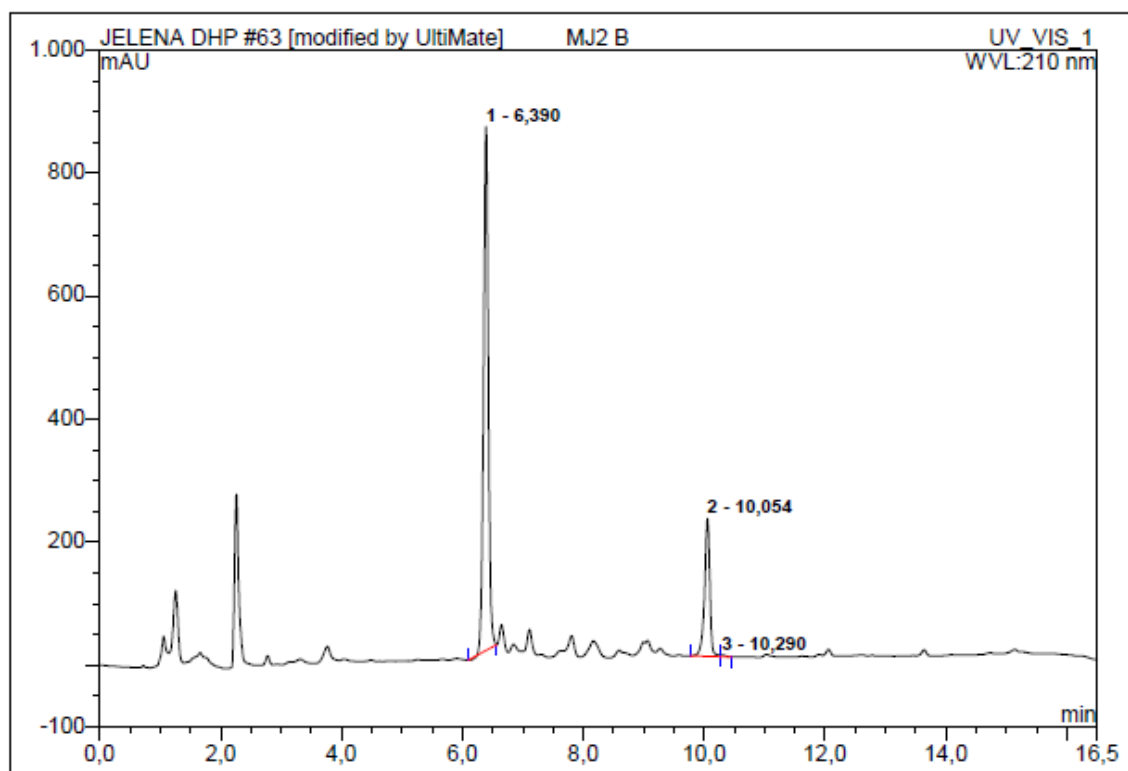


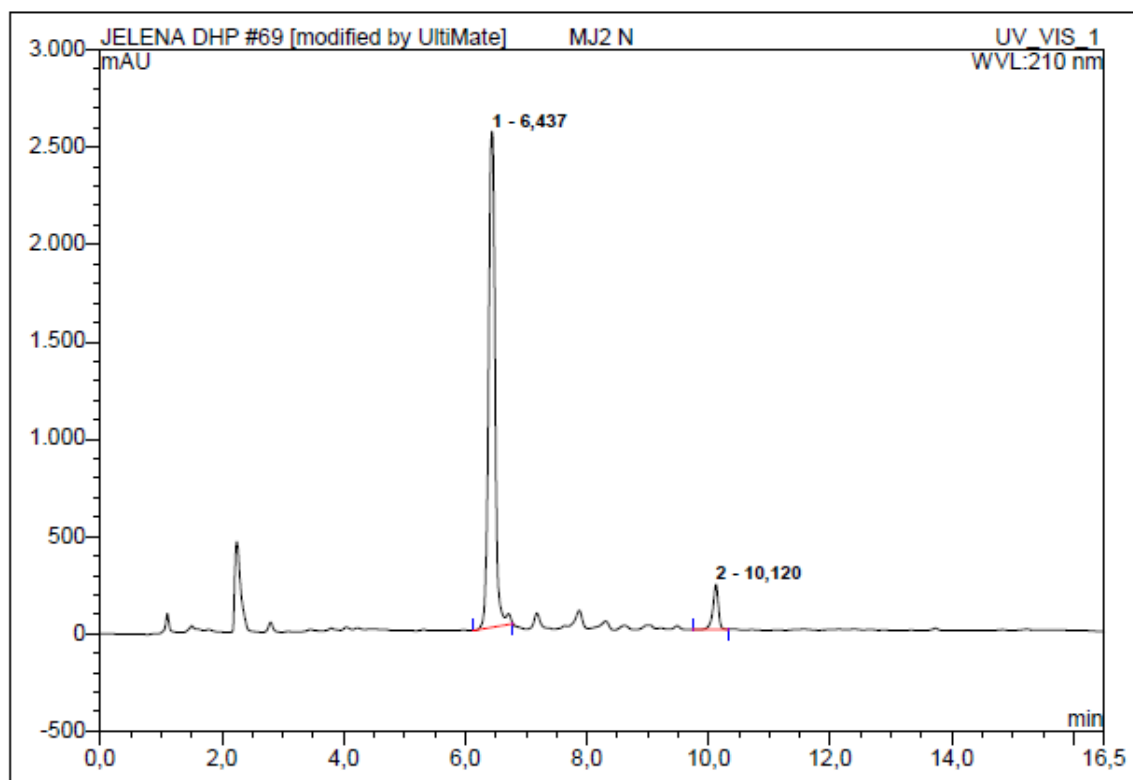
Figure S8. HPLC chromatograms of DHP1 before (a) and after oxidation with different enzymes b) *TvLacc*, c) *BacillusLacc*, d) *Novozym51003*, e) *TtLMCO1* and f) *TvLacc* without ABTS



c)



d)



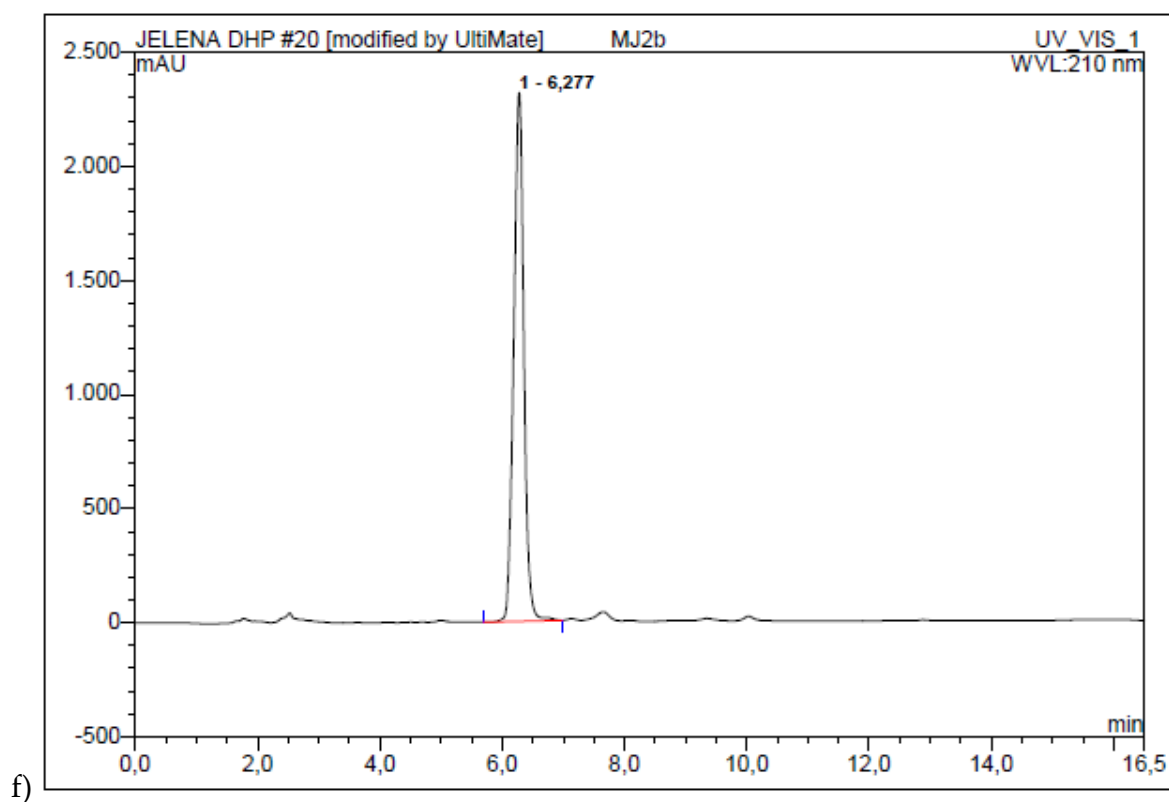
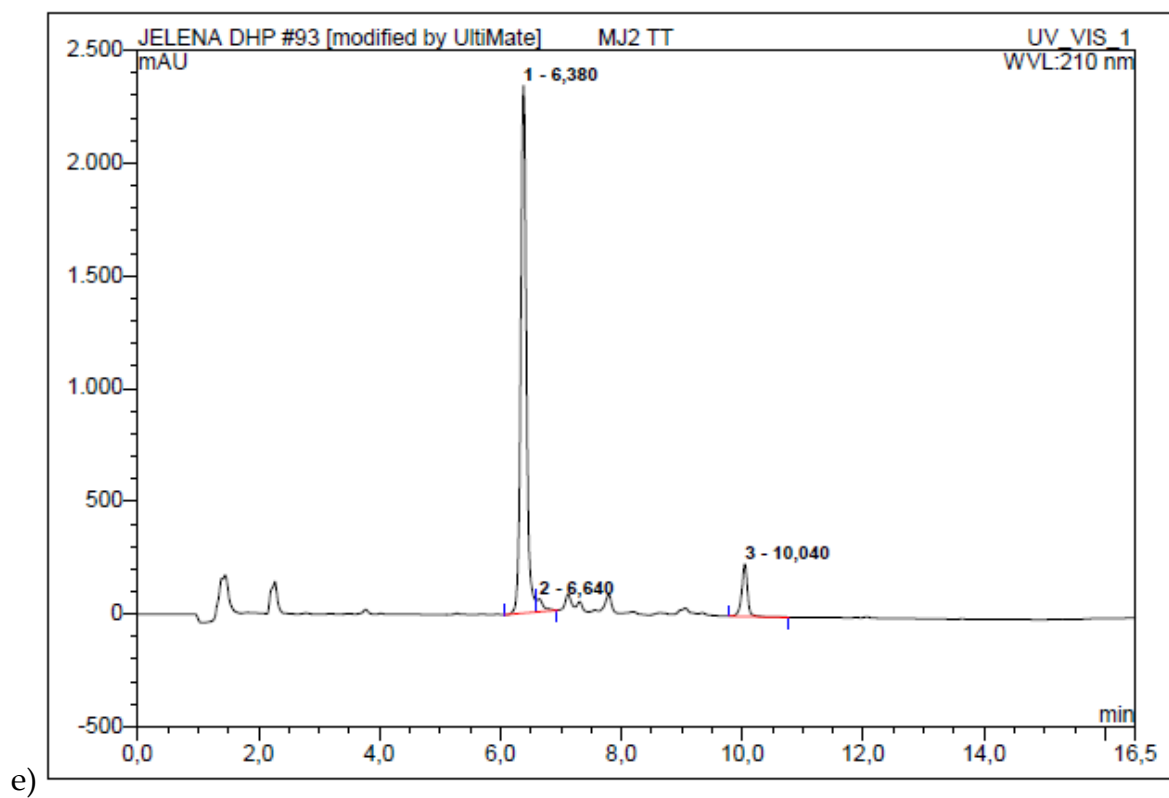
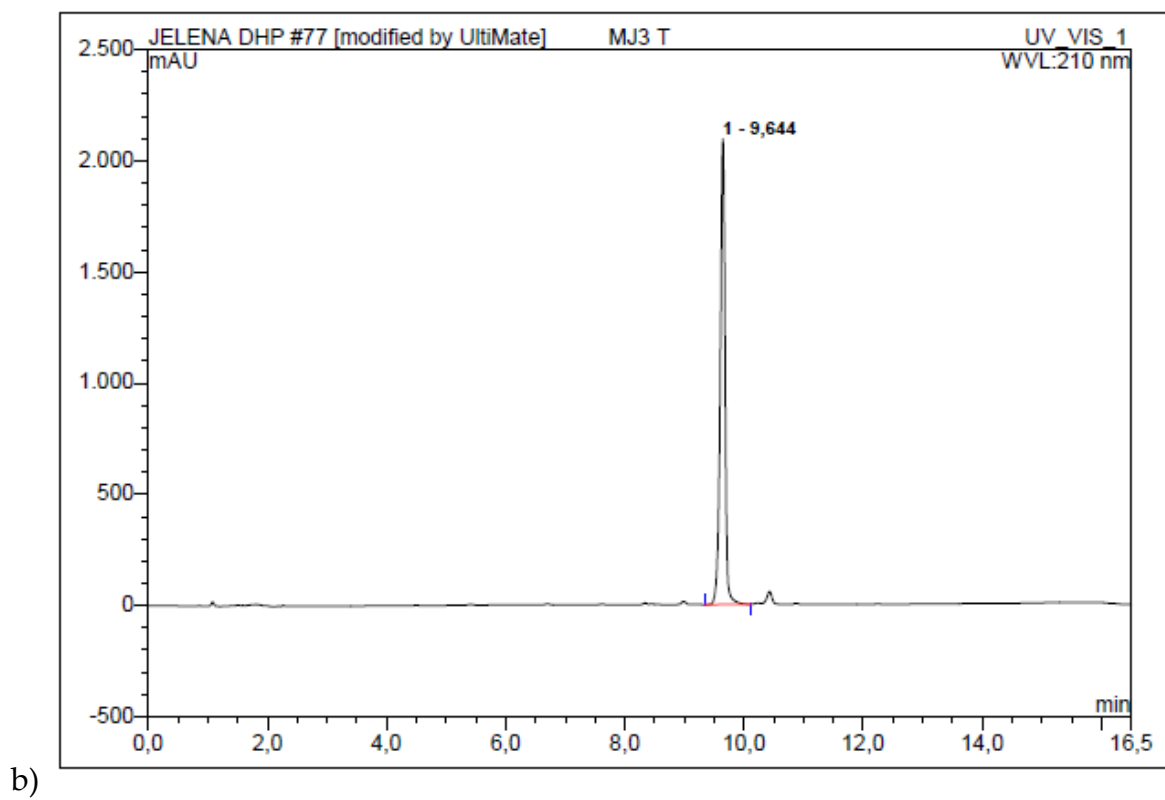
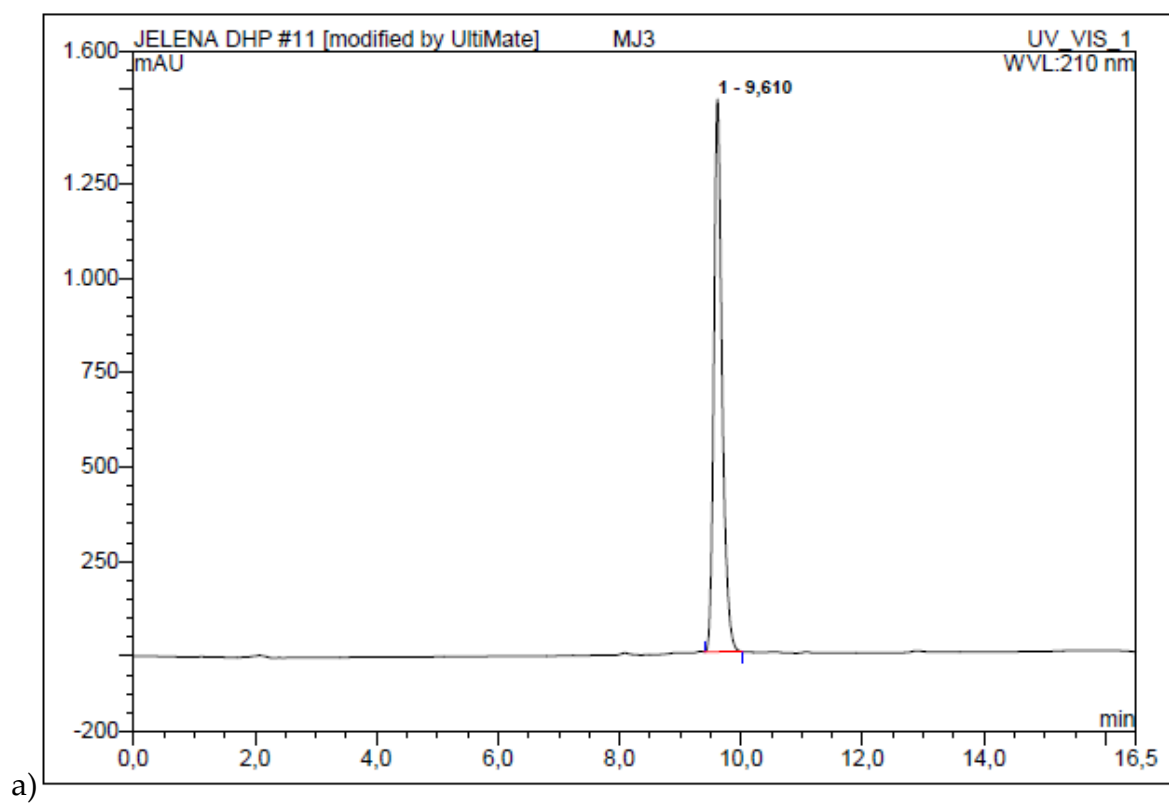
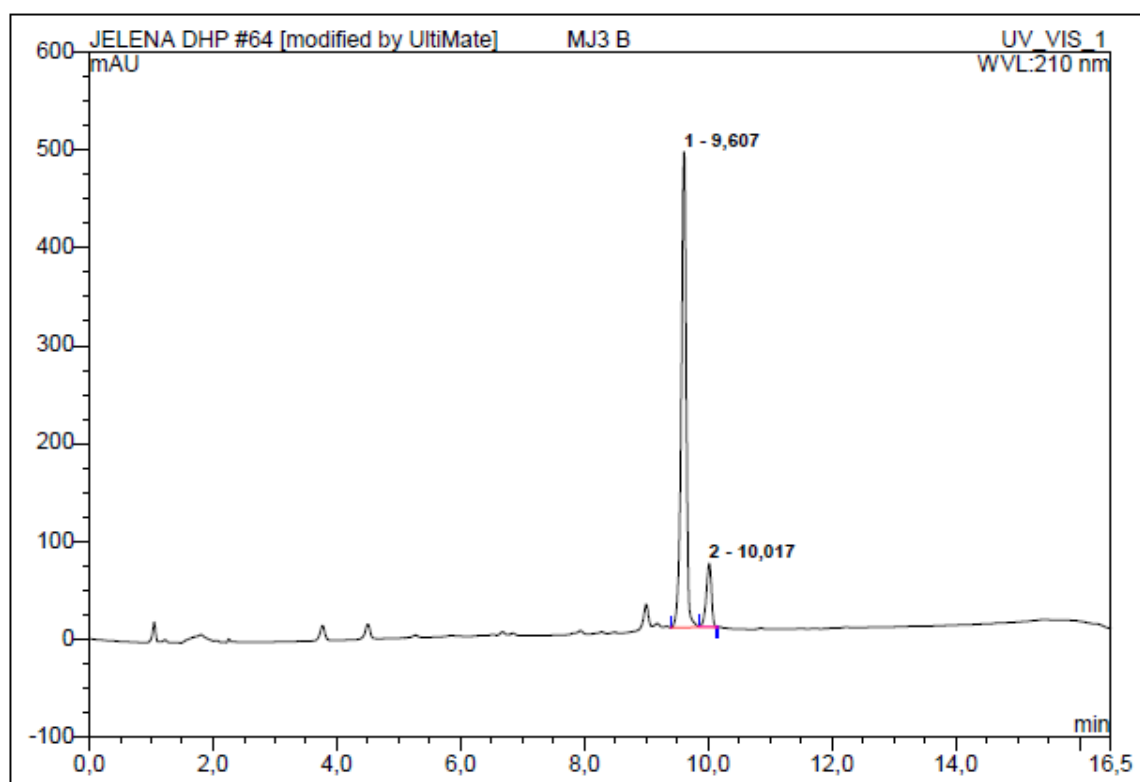


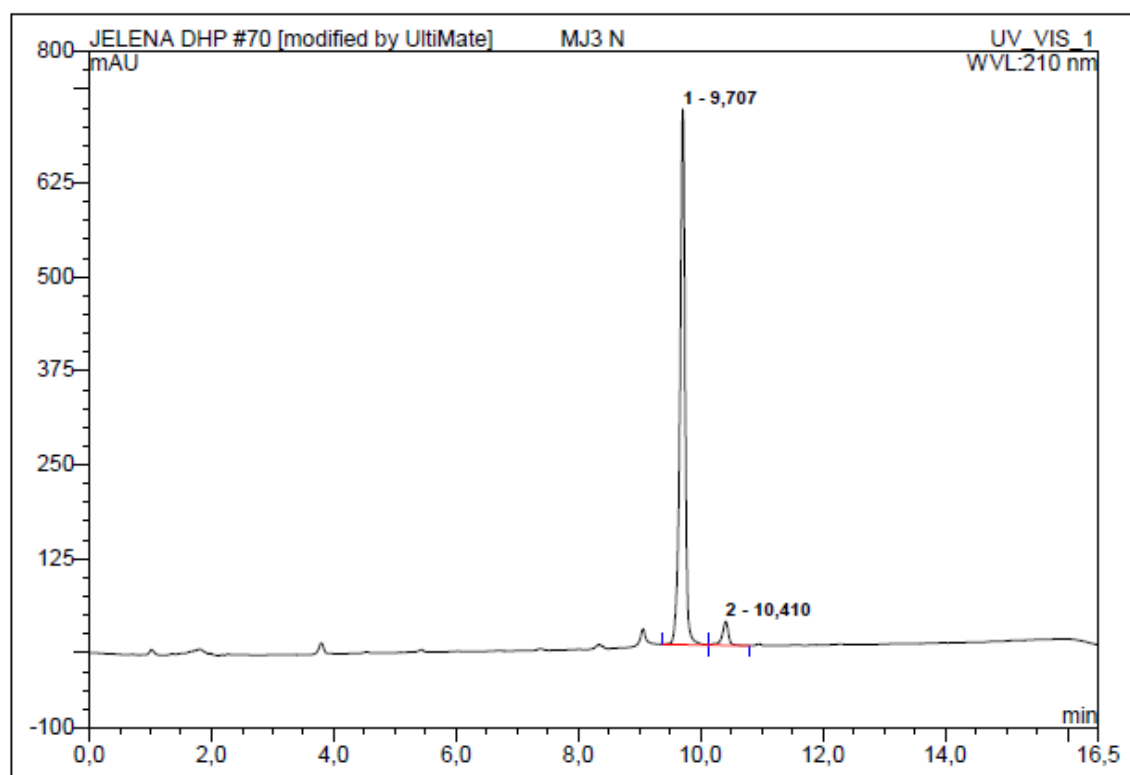
Figure S9. HPLC chromatograms of DHP2 before (a) and after oxidation with different enzymes b) *TvLacc*, c) *BacillusLacc*, d) *Novozym51003*, e) *TtLMCO1* and f) *TvLacc* without ABTS



c)



d)



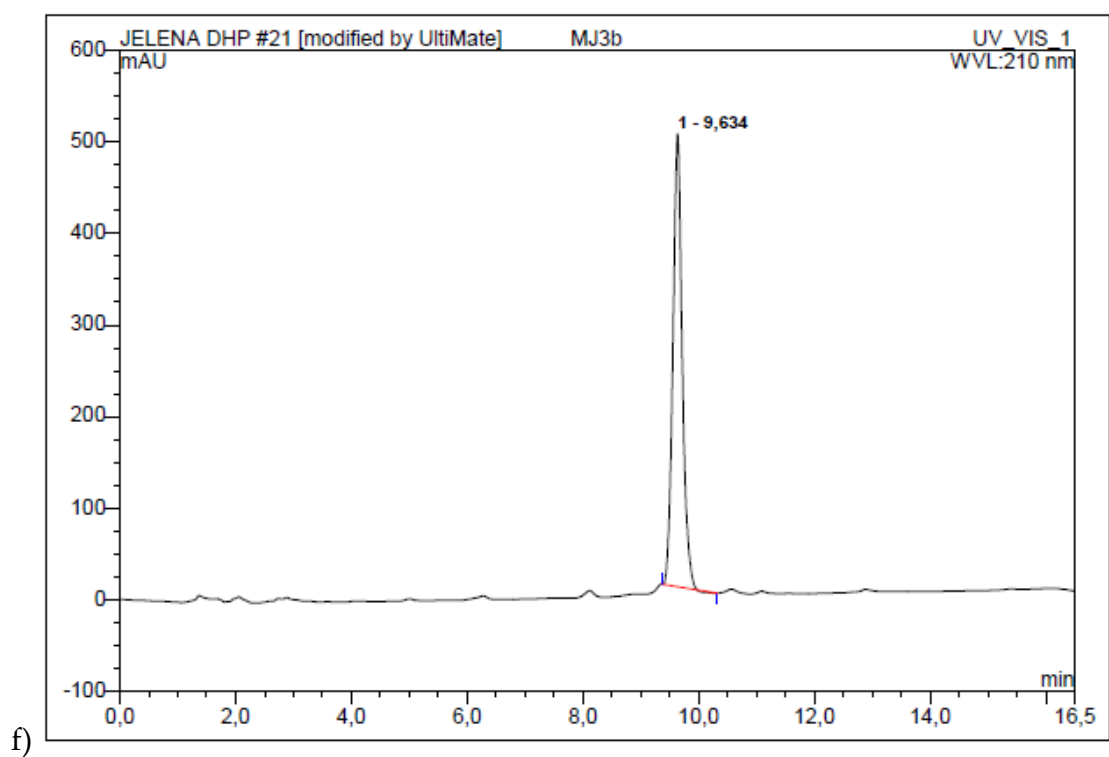
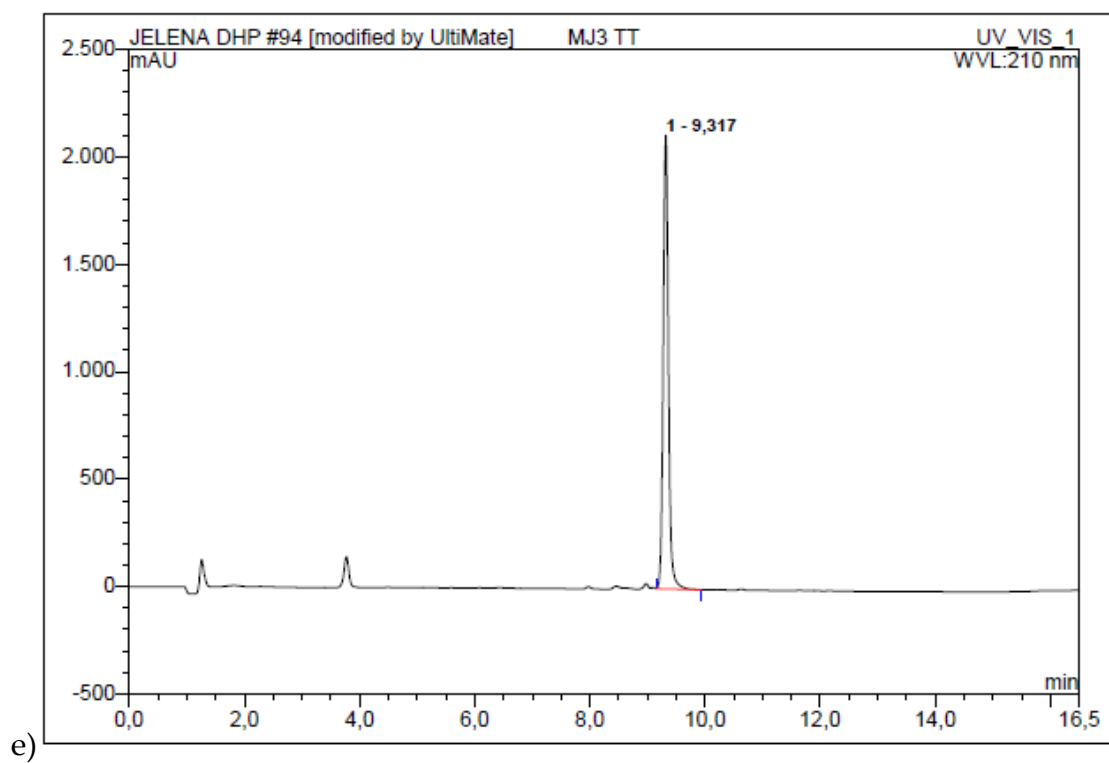
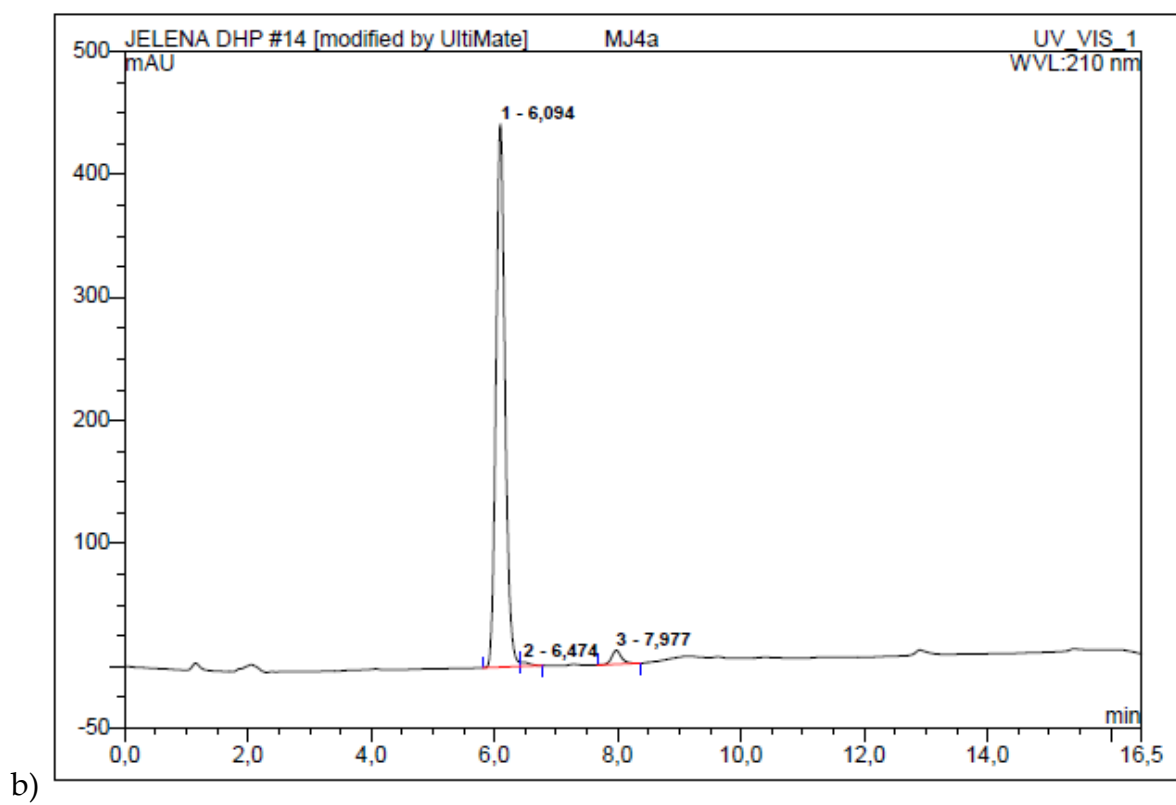
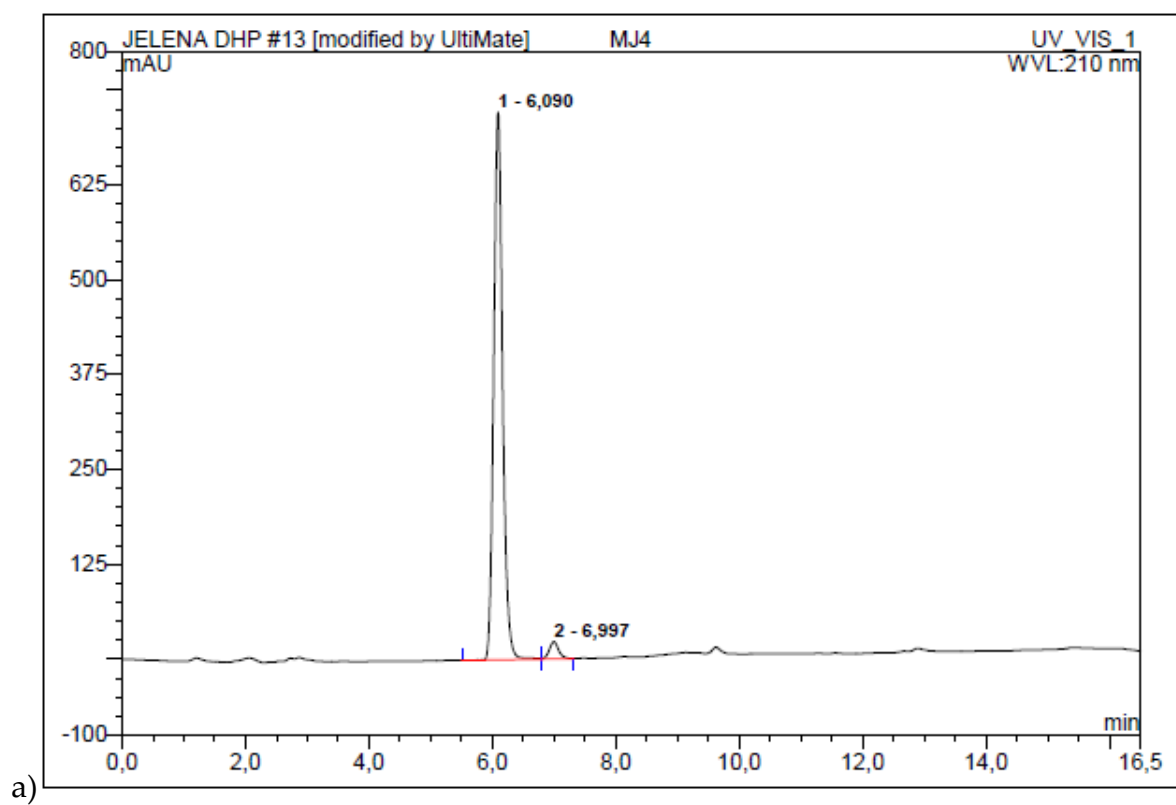
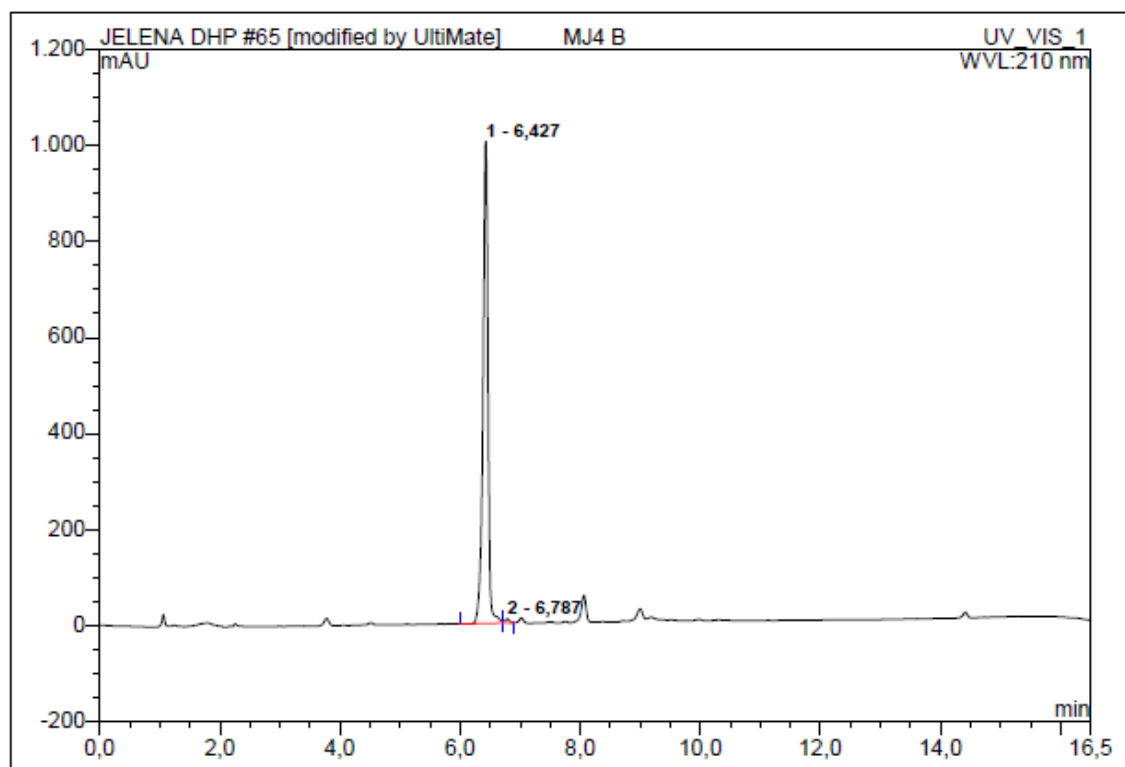


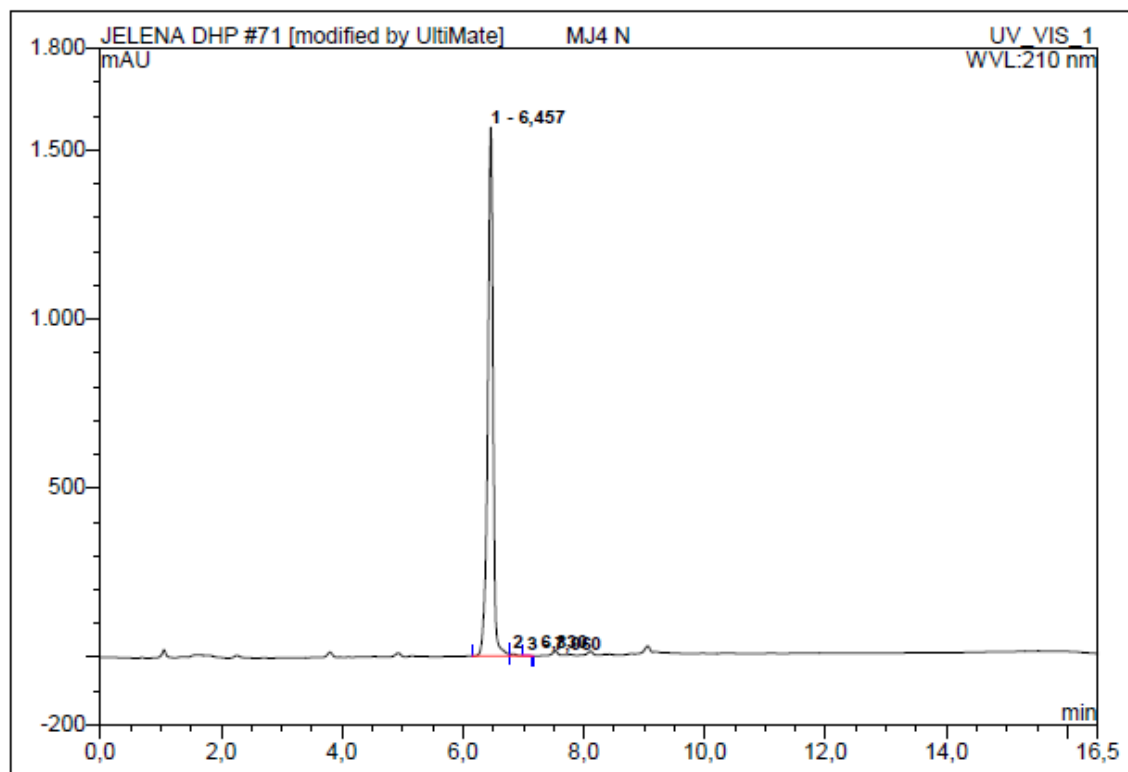
Figure S10. HPLC chromatograms of DHP3 before (a) and after oxidation with different enzymes b) *TvLacc*, c) *BacillusLacc*, d) *Novozym51003*, e) *TtLMCO1* and f) *TvLacc* without ABTS



c)



d)



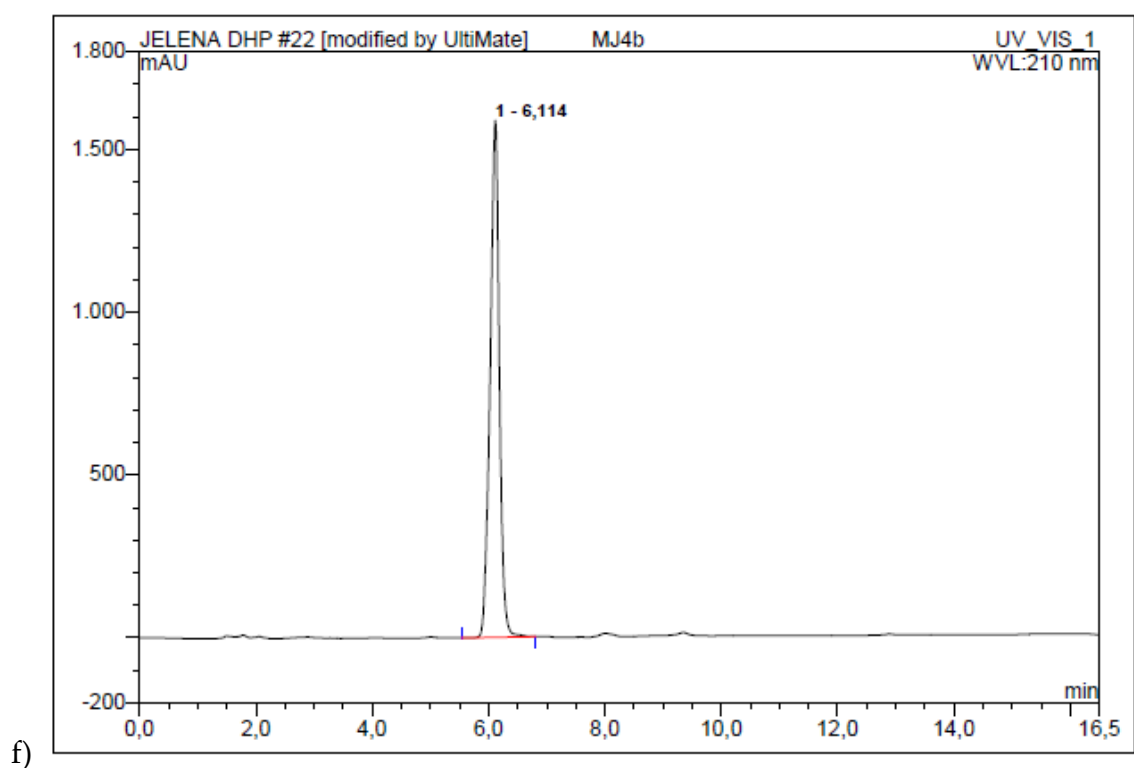
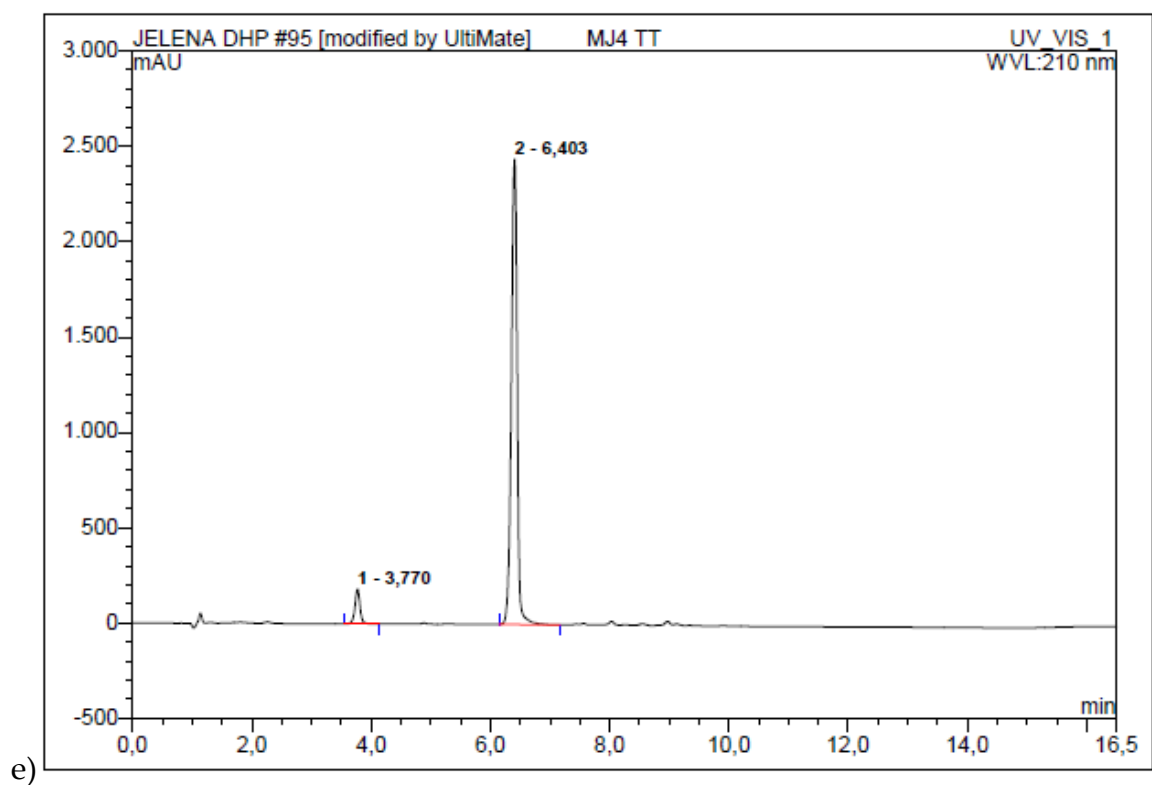
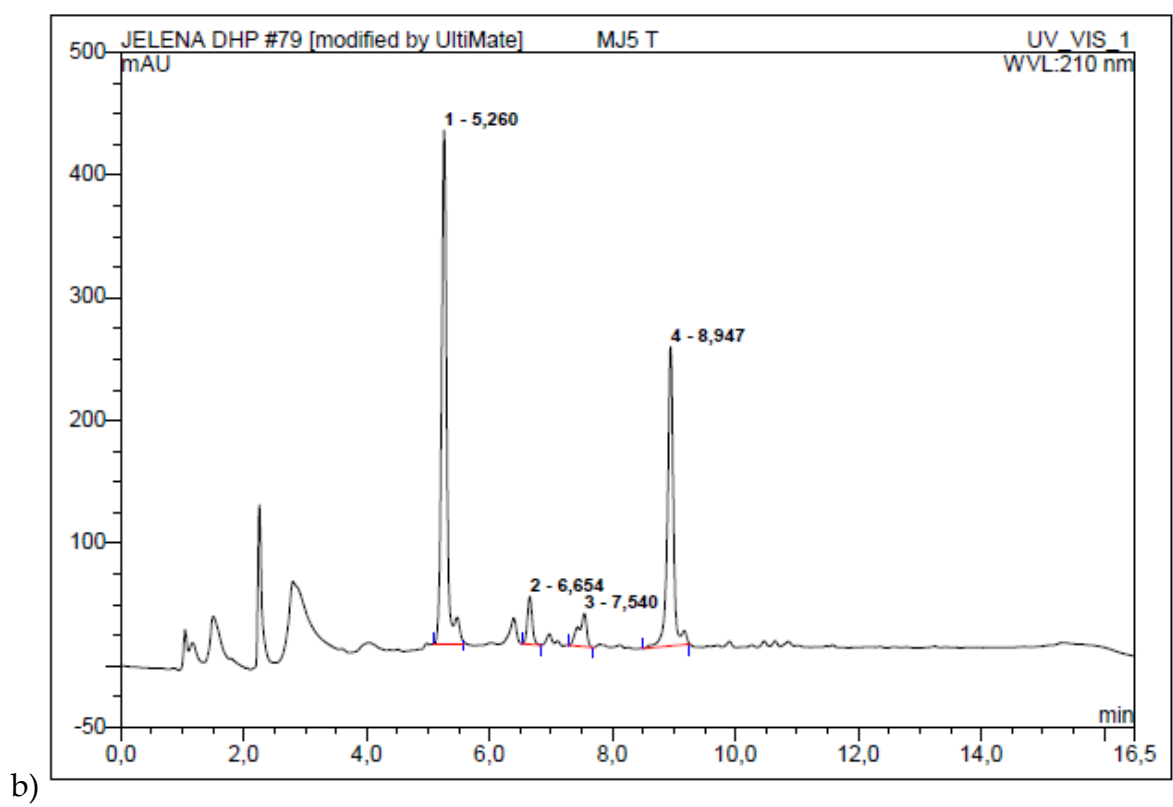
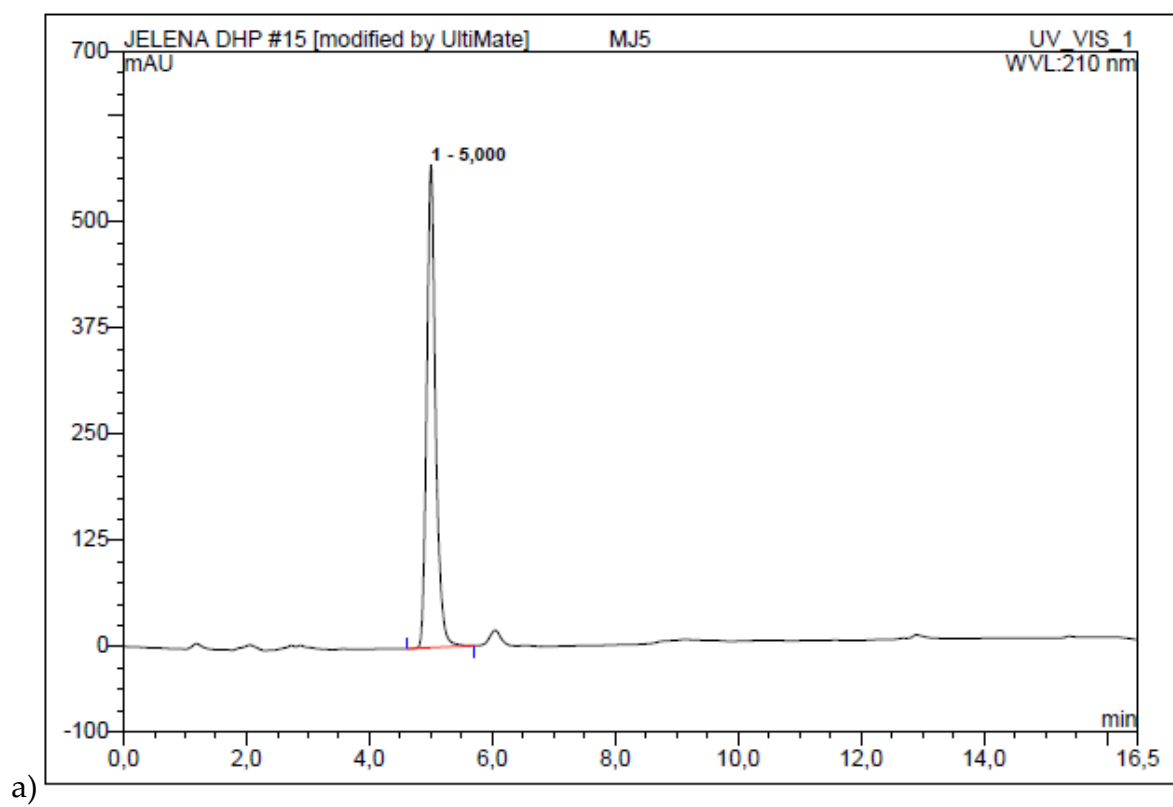
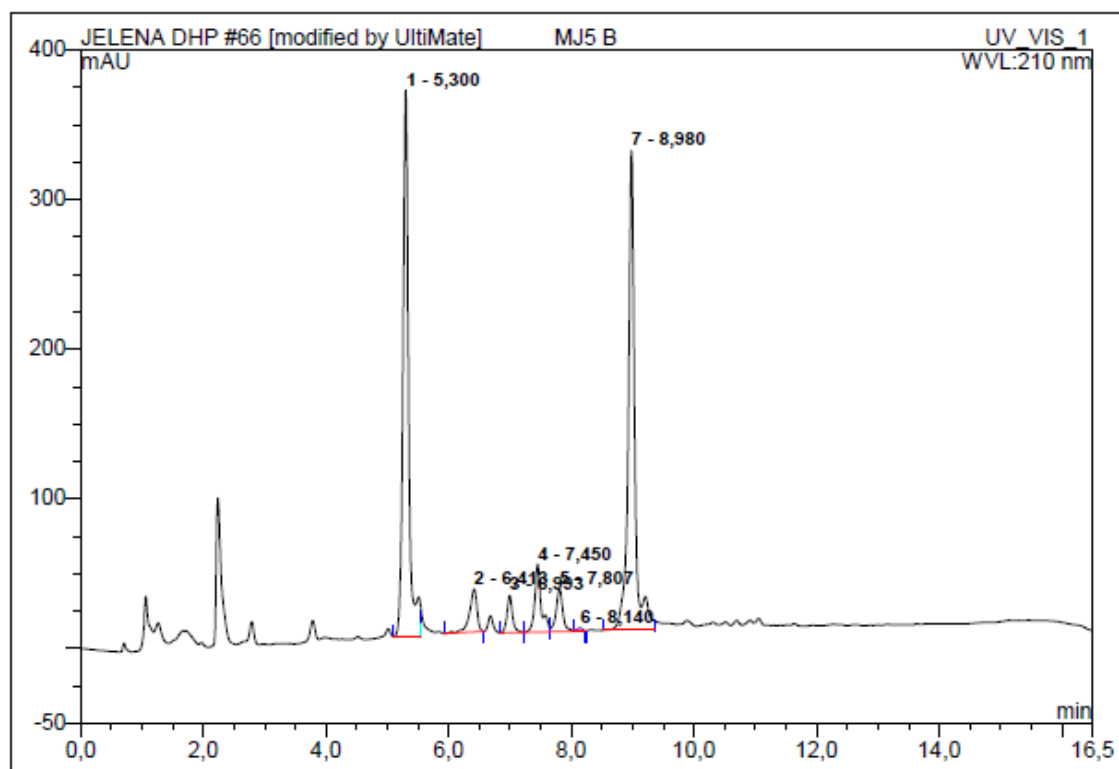


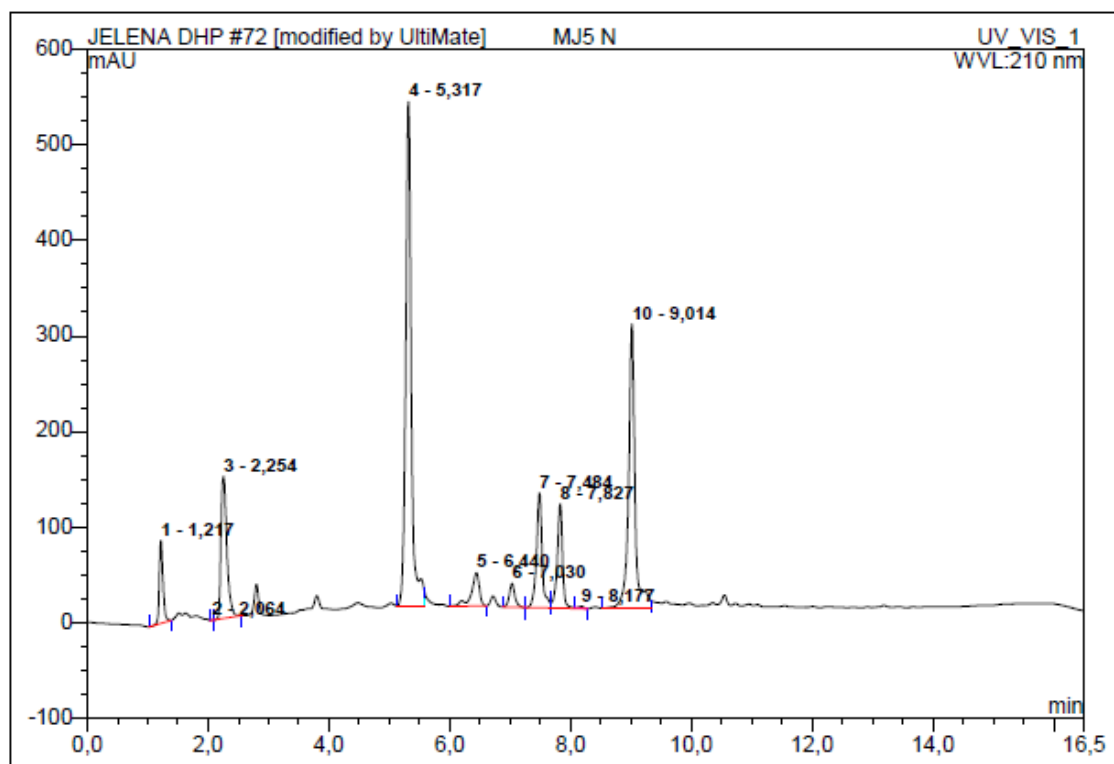
Figure S11. HPLC chromatograms of DHP4 before (a) and after oxidation with different enzymes b) *TvLacc*, c) *BacillusLacc*, d) *Novozym51003*, e) *TtLMCO1* and f) *TvLacc* without ABTS



c)



d)



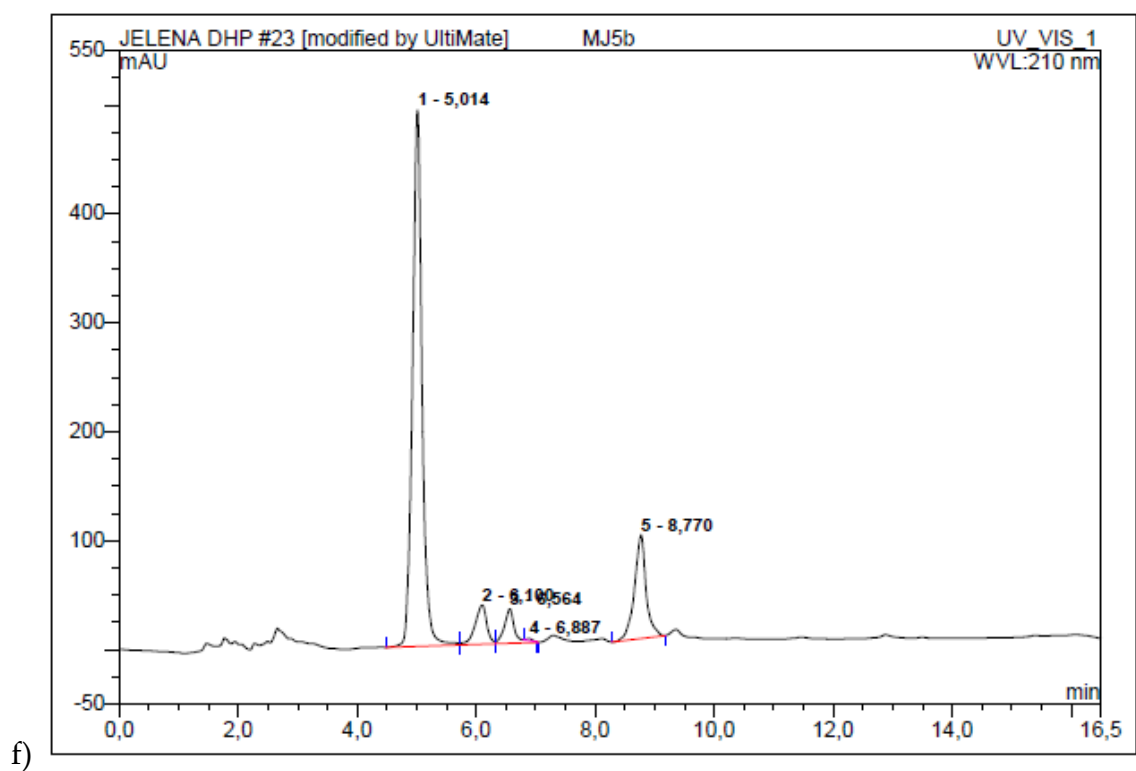
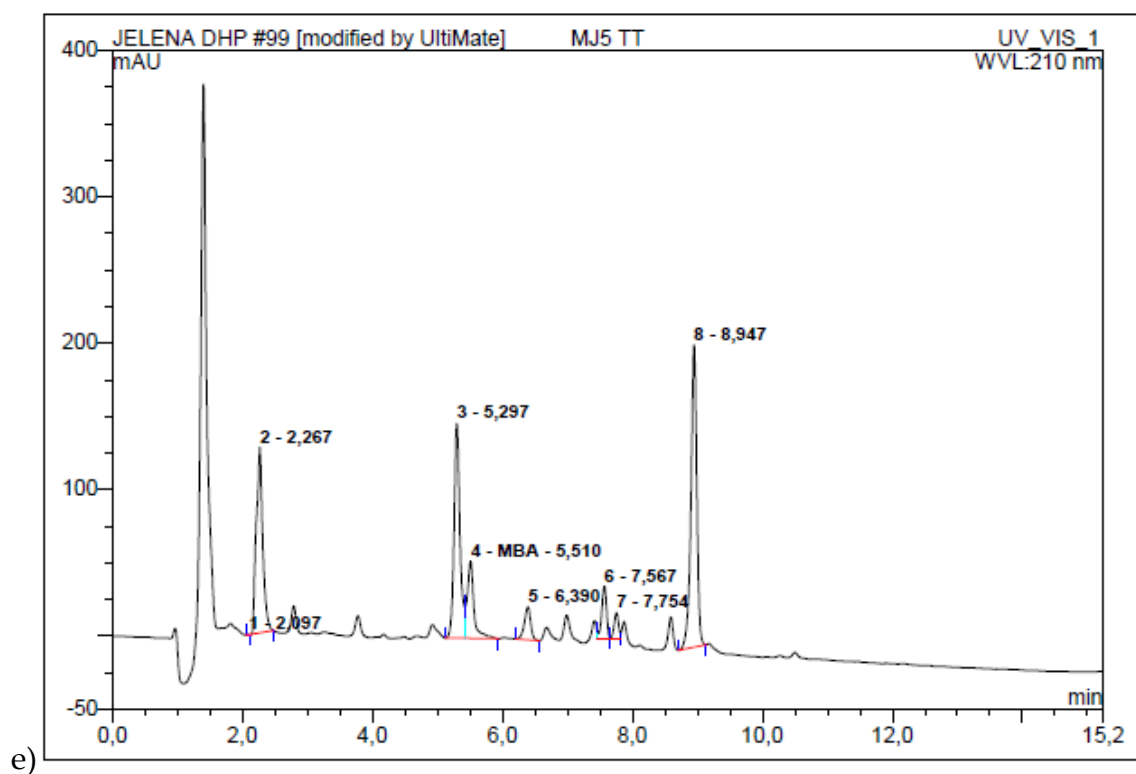
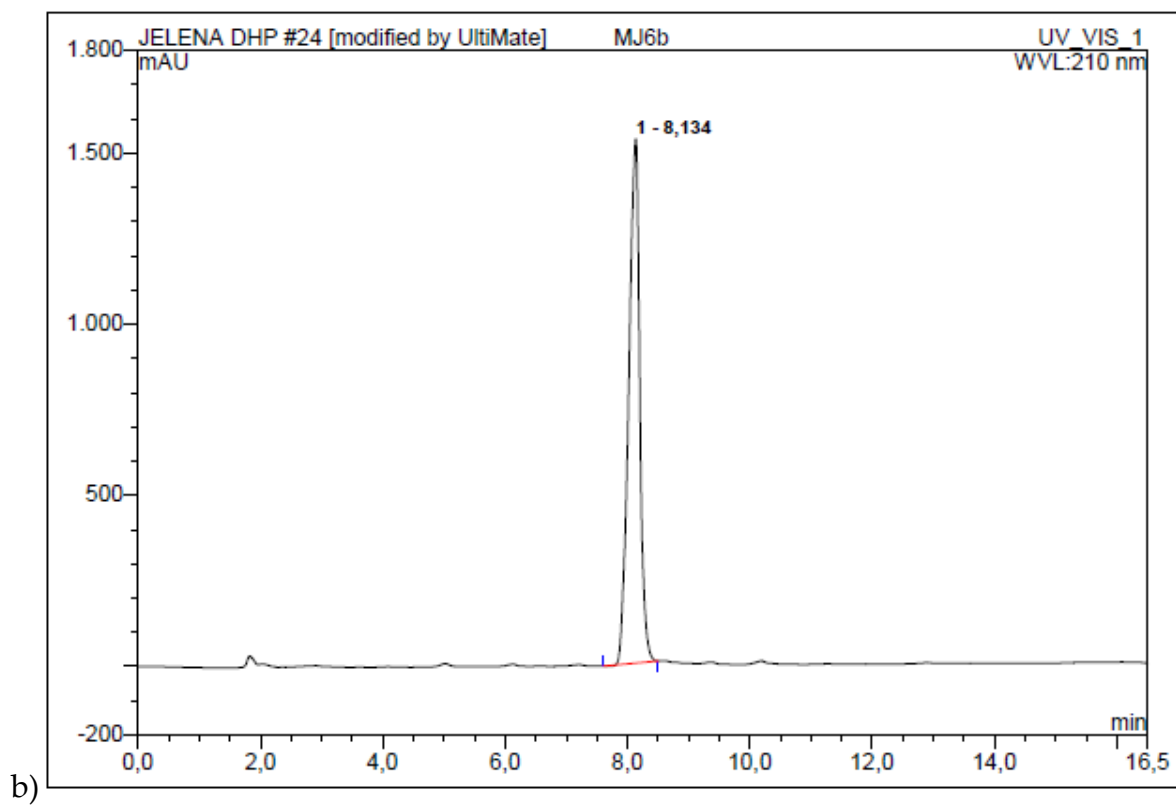
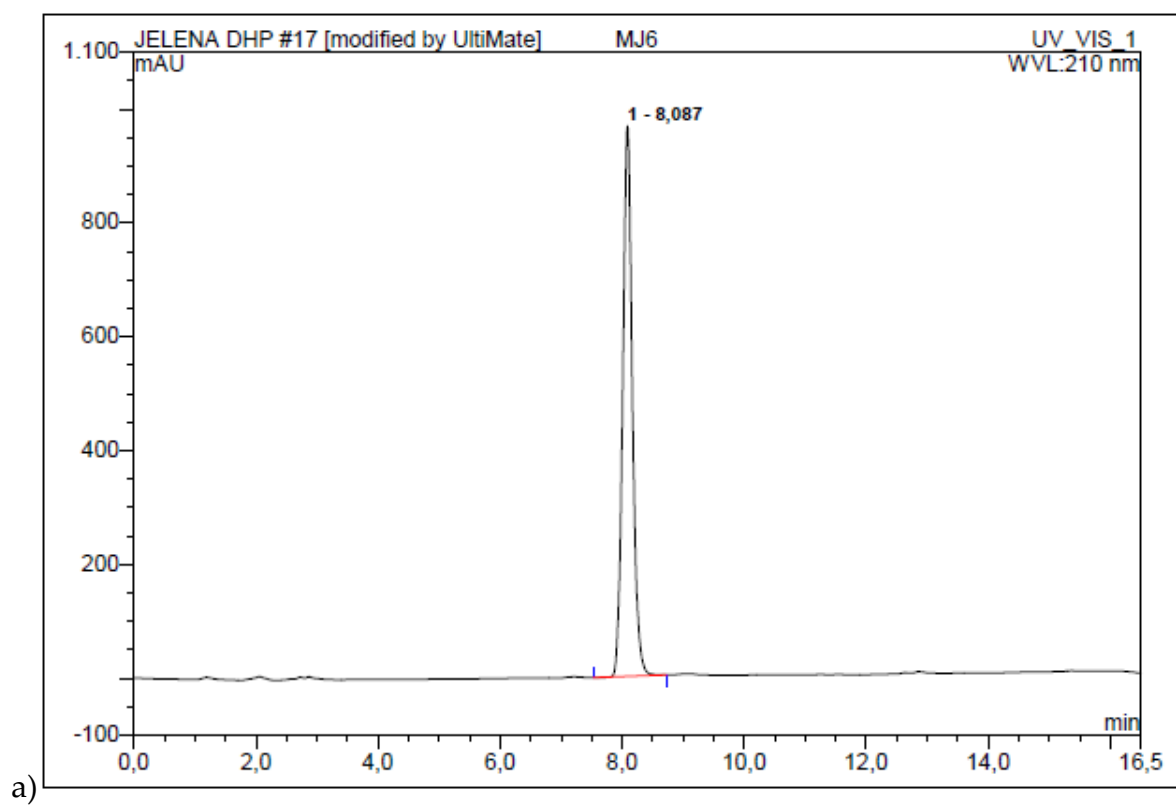
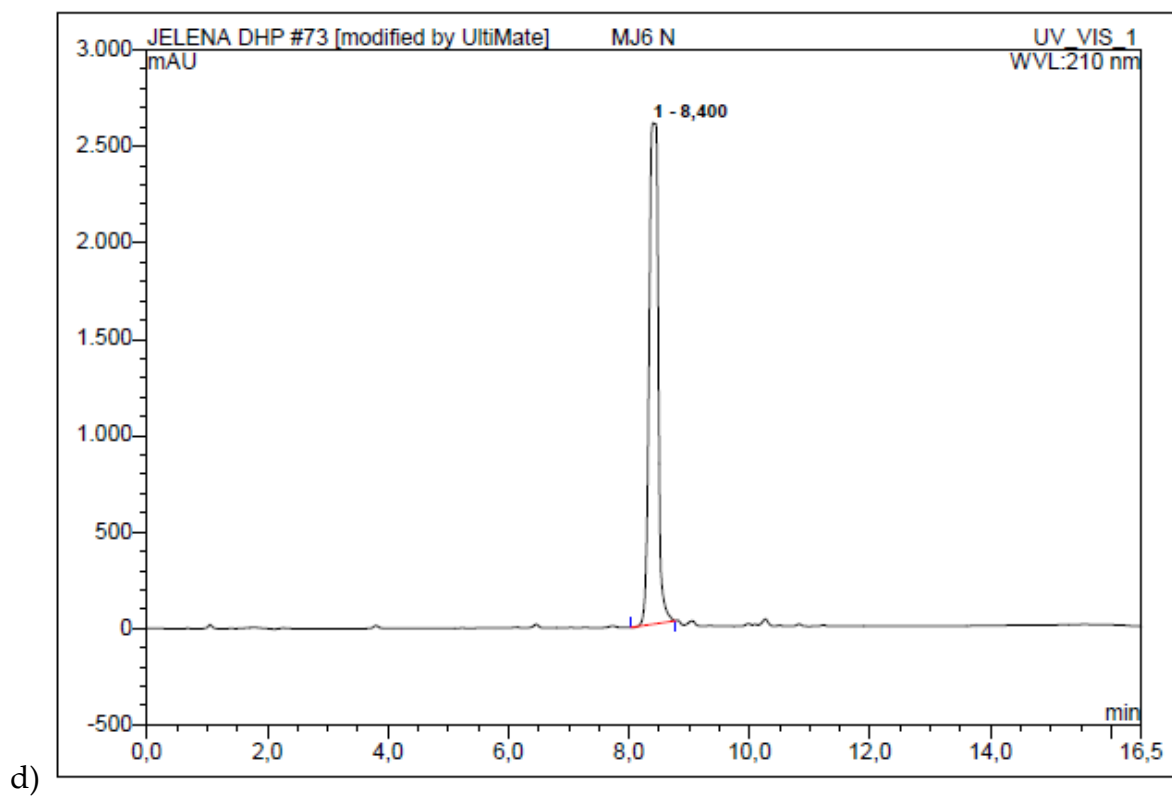
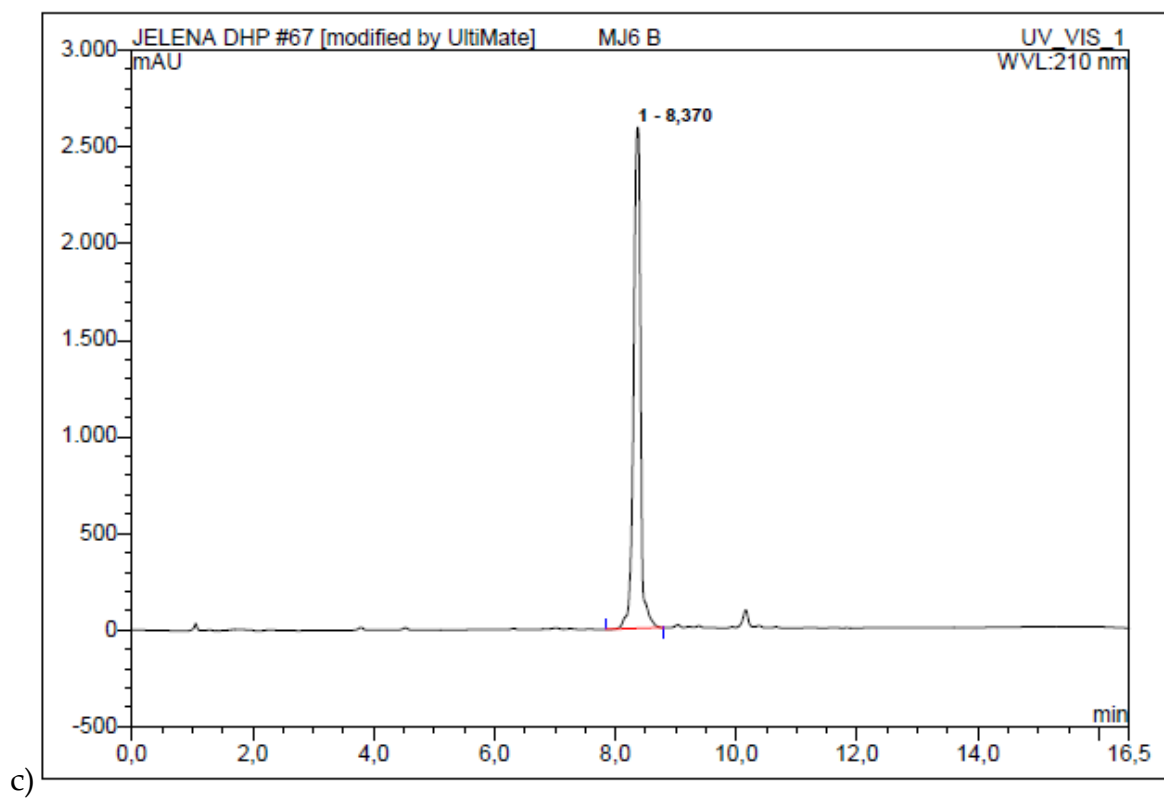


Figure S12. HPLC chromatograms of DHP5 before (a) and after oxidation with different enzymes b) *TvLacc*, c) *BacillusLacc*, d) *Novozym51003*, e) *TtLMCO1* and f) *TvLacc* without ABTS





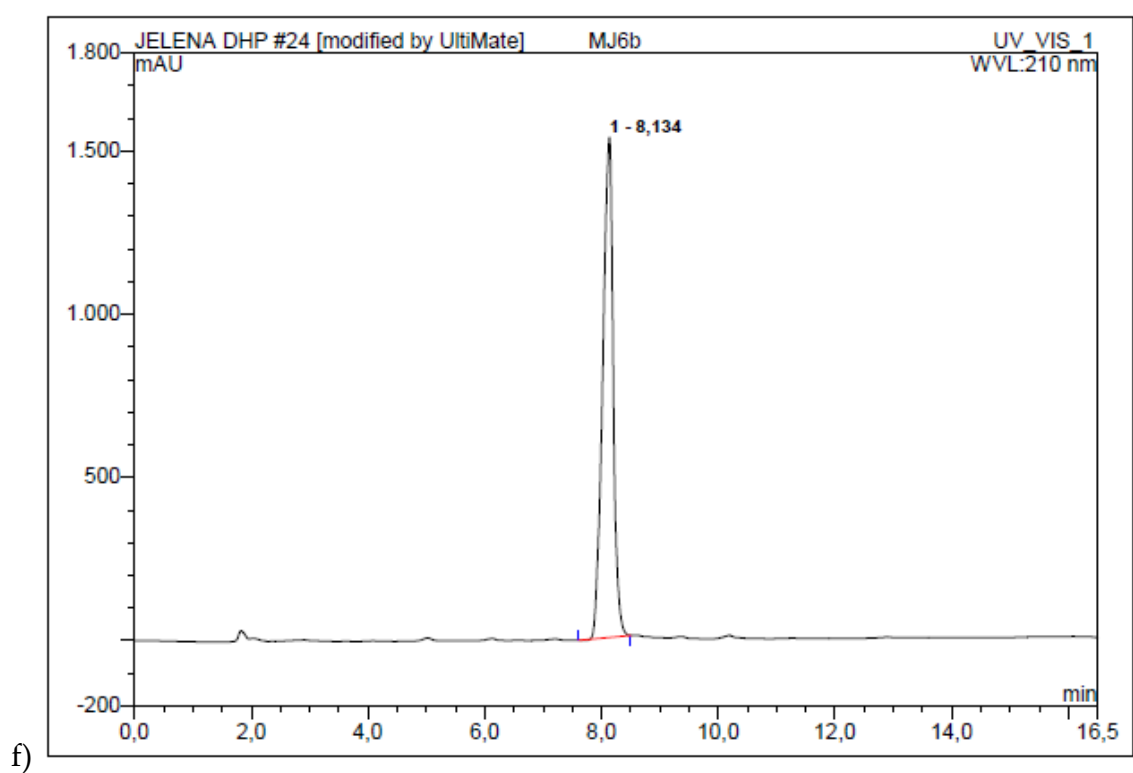
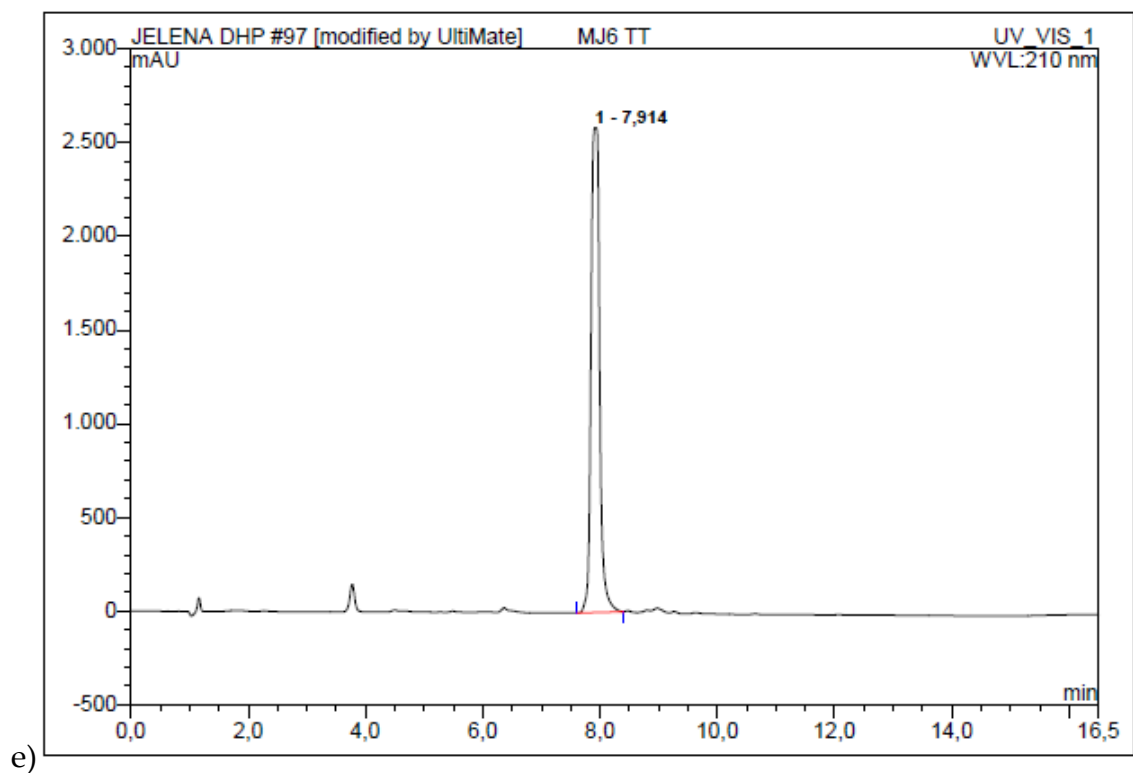
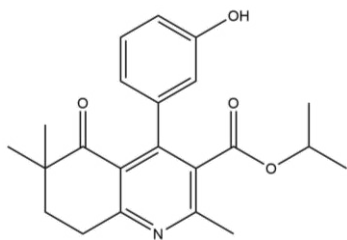


Figure S13. HPLC chromatograms of DHP6 before (a) and after oxidation with different enzymes b) *TvLacc*, c) *BacillusLacc*, d) *Novozym51003*, e) *TtLMCO1* and f) *TvLacc* without ABTS

NMR spectra



^1H NMR (400 MHz, DMSO) δ 9.45 (s, 1H), 7.10 (t, J = 7.7 Hz, 1H), 6.71 (d, J = 7.3 Hz, 1H), 6.40 (d, J = 7.9 Hz, 1H), 4.79 (dd, J = 12.3, 6.1 Hz, 1H), 3.10 (t, J = 6.2 Hz, 2H), 2.45 (s, 3H), 1.94 (t, J = 6.4 Hz, 2H), 1.06 (s, 3H), 0.94 (d, J = 6.2 Hz, 3H).

^{13}C NMR (101 MHz, DMSO) δ 201.74, 166.81, 163.39, 157.16, 156.86, 148.45, 138.91, 129.94, 129.17, 123.73, 118.69, 115.02, 114.81, 69.19, 42.58, 34.59, 29.51, 24.09, 22.96, 21.38.

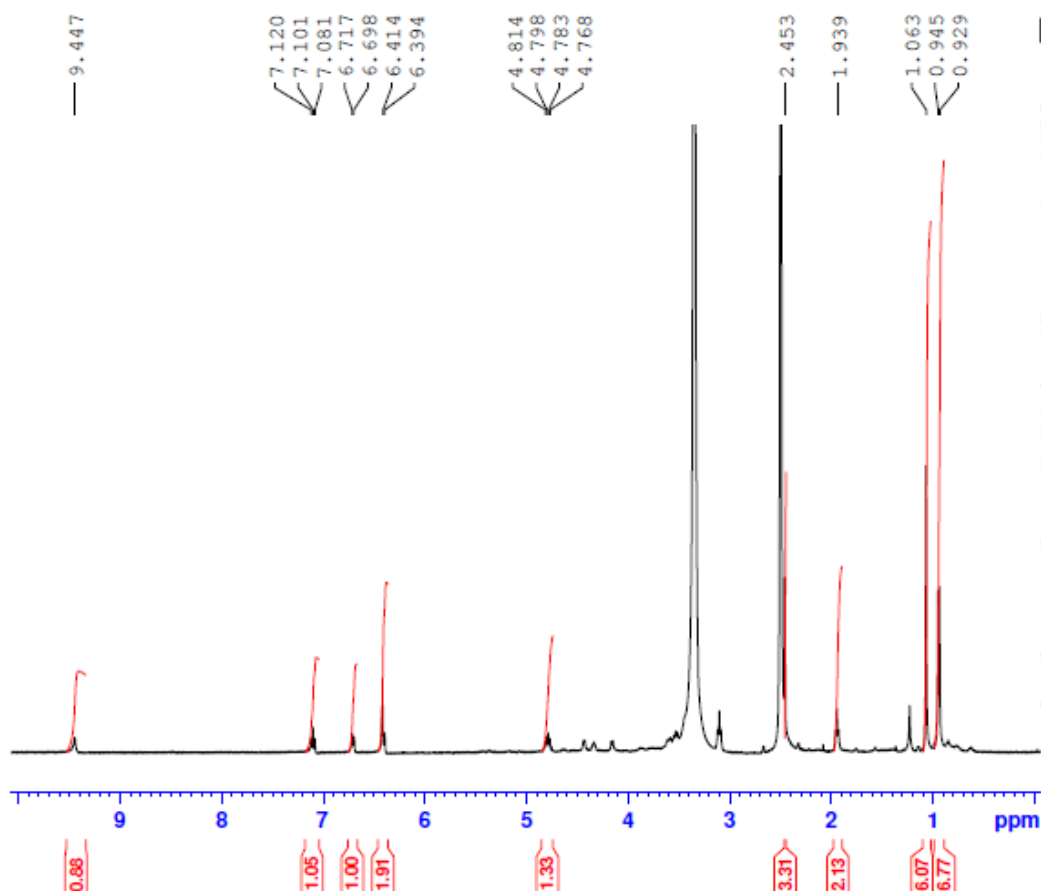


Figure S14. ^1H NMR spectra of isolated product from biocatalytic oxidation of DHP1

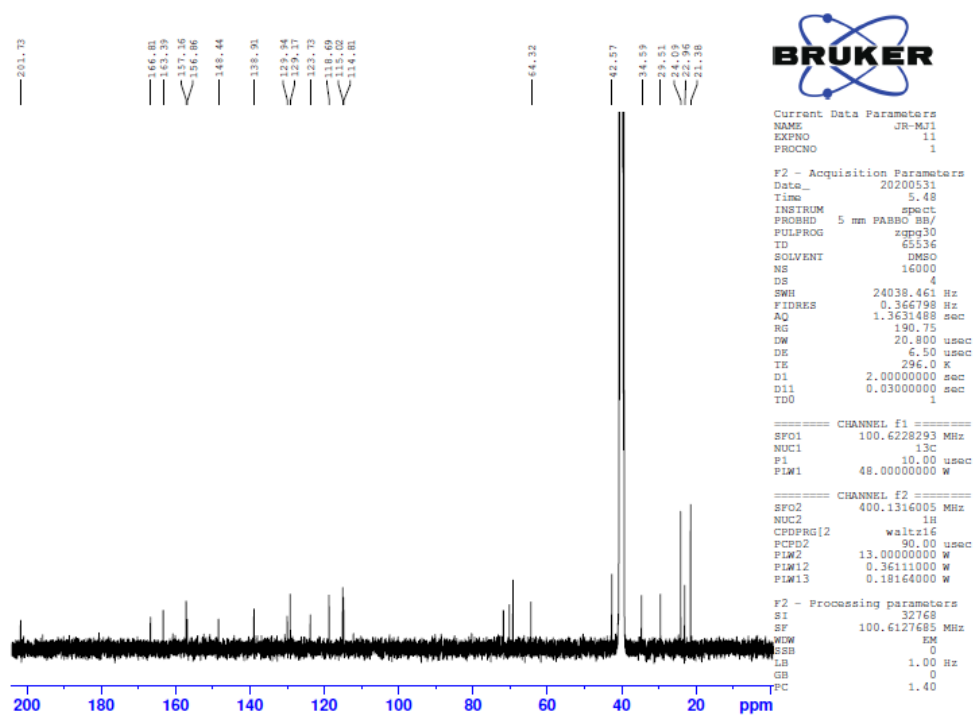


Figure S15. ^{13}C NMR spectra of isolated product from biocatalytic oxidation of DHP1

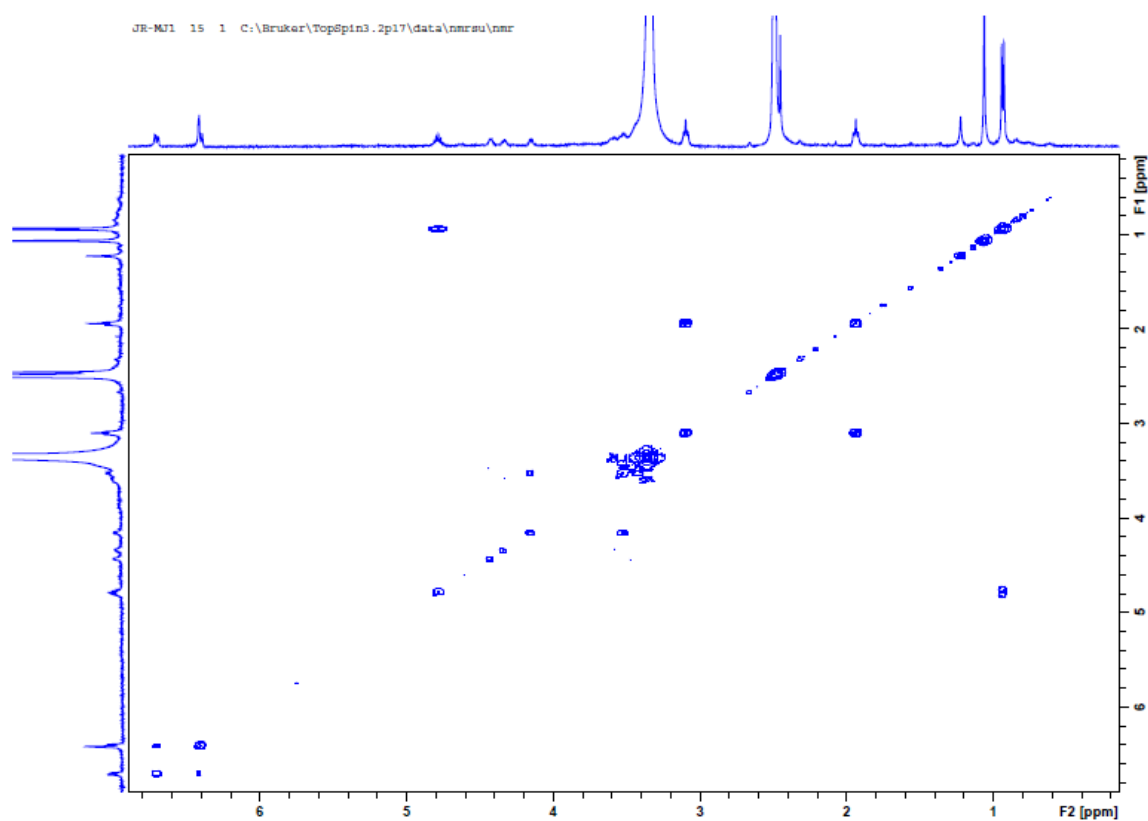


Figure S16. 2D COSY NMR spectra of isolated product from biocatalytic oxidation of DHP1

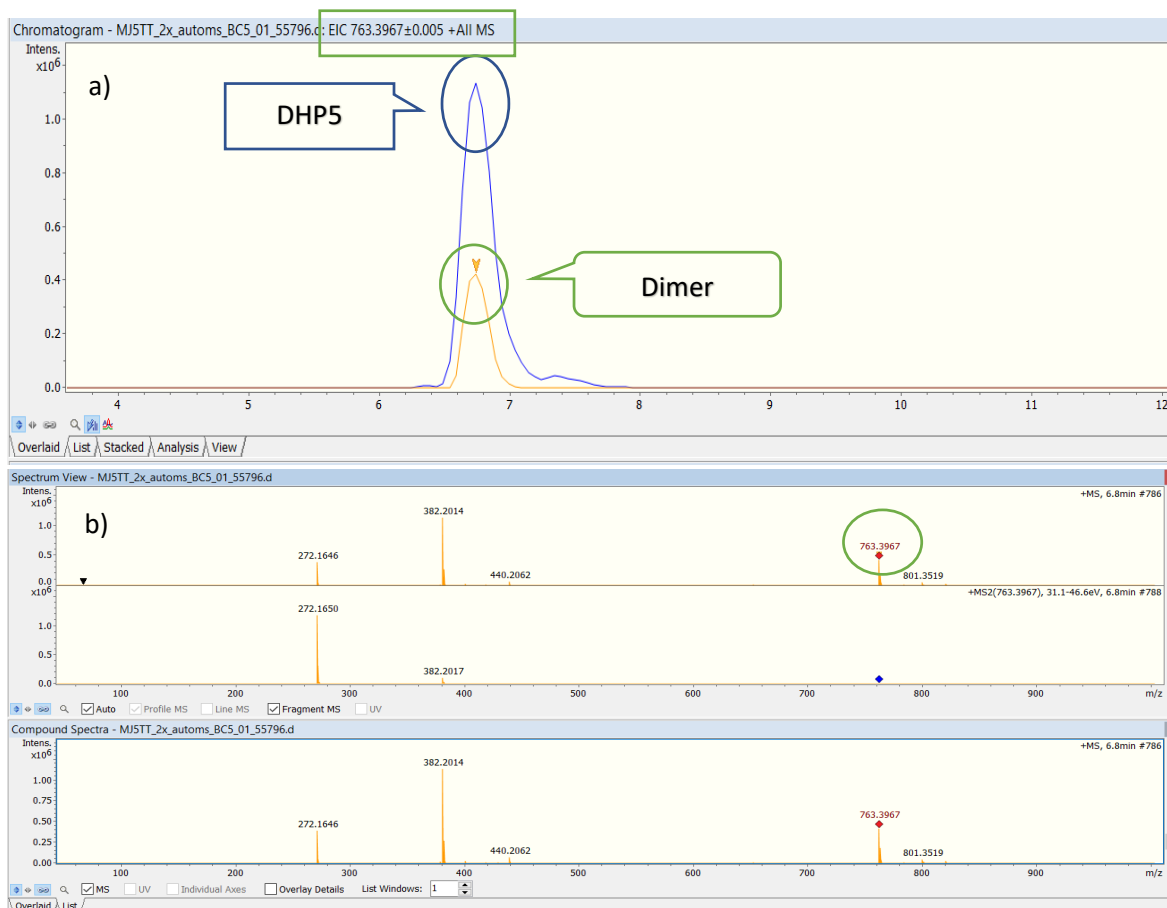


Figure S17. a) Overlaid chromatograms of DHP5 (in blue, for the m/z value 382.2013 (± 5 mDa) and its corresponding dimer (in orange, for the m/z value 763.3952 (± 5 mDa) eluted at 6.80 min in positive ionization mode. b) MS/MS spectra of the dimer of DHP5 in autoMS mode.

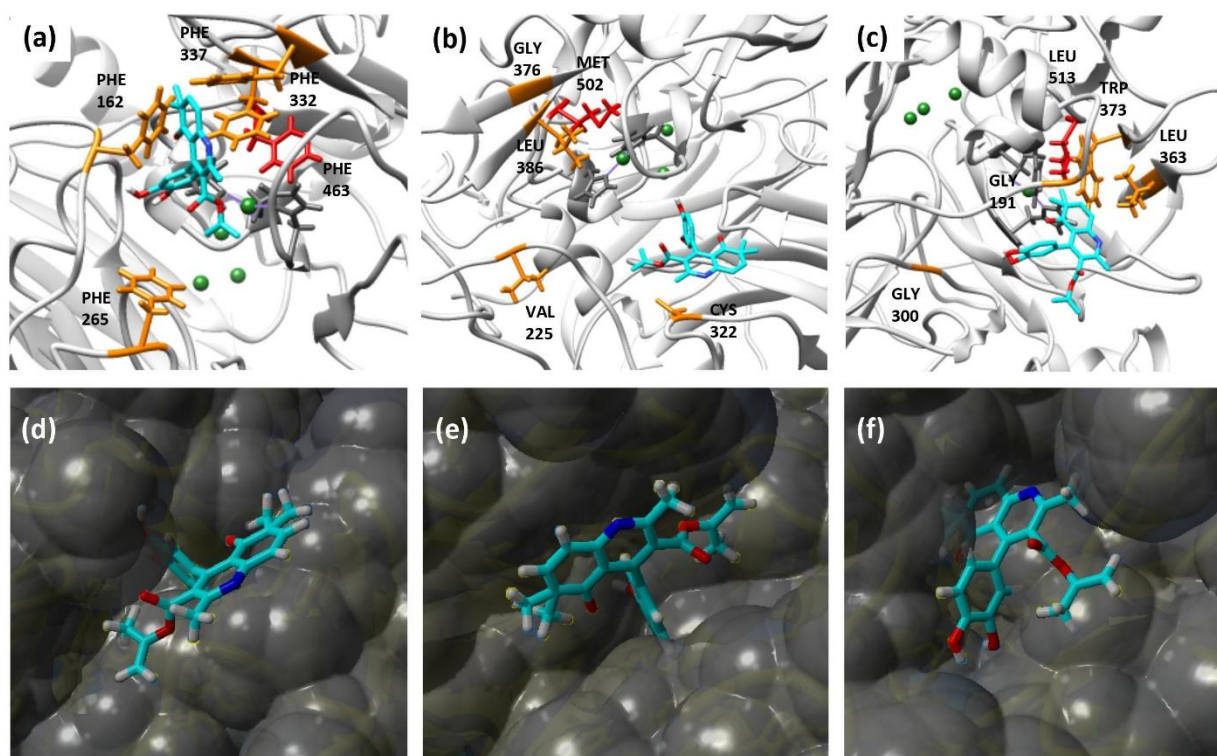


Figure S19. Models of DHP2 interacting with laccases *TvLacc* (a, d), *BacillusLacc* (b, e), and Novozym 51003 (c, f). a-c: The interaction model of DHP2 with the tested laccases. Ligand is coloured in cyan, copper atoms are coloured in green, equatorial T1 copper ligands are coloured in dark grey, an axial copper ligand is coloured in red, binding pocket residues are coloured in orange. d-f: The surface binding model of DHP2 with the tested laccases.

Table S1: Specific activities of the different laccases used in this study

Enzyme preparation	
<i>Tv</i> Lacc	0.5 U*/mg
<i>Bacillus</i> Lacc	1.2 U/mg
Novozyme51003	0.06 U/μL
<i>Tt</i> LMCO1	5 U/mg

* One unit corresponds to the amount of enzyme which converts 1 μmole of catechol per minute at pH 5.0 and 25 °C

Table S2. DHP1-DHP6 biotransformation with four laccases, without mediator ABTS, assessed by HPLC with the amount of substrate remaining after 18 h reaction expressed as %. (Chromatograms for *Tv*Lacc are provided in Figure S2-S7 as panel f))

Substrate Enzyme	DHP1	DHP2	DHP3	DHP4	DHP5	DHP6
<i>Tv</i> Lacc	73.7	88.0	98.4	98.2	71.7	98.2
<i>Bacillus</i> Lacc	68.3	78.4	100	100	80.0	100
Novozym51003	75.4	93.4	98.2	100	66.5	100
<i>Tt</i> LMCO1	90.2	94.6	100	98.2	58.2	98.0

Table S3. The results of molecular docking for DHP5 in the active site of the tested laccases.

Enzyme	Binding energy (kcal/mol)	Dissociation constant (μM)	Contacting receptor residues
<i>Tv</i> Lacc	6.898	8.78	PHE 162 B LEU 164 B PHE 332 B GLY 334 B PHE 337 B PRO 391 B GLY 392 B ALA 393 B ILE 455 B HIS 458
<i>Bacillus</i> Lacc	7.409	3.71	LEU 374 A ALA 375 A GLY 376 A THR 377 A GLN 378 A PRO 384 A LEU 386 A PRO 414 A THR 415 A ARG 416 A GLY 417 A HIS 419 A GLN 442
Novozym 51003	6.97	7.81	GLY 191 A ALA 192 A LEU 297 A LEU 363 A PHE 371 A TRP 373 A PHE 427 A THR 428 A LEU 429

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Table S4. *The results of molecular docking for DHP2 in the active site of the tested laccases.*

Enzyme	Binding energy (kcal/mol)	Dissociation constant (μ M)	Contacting receptor residues
TvLacc	6.22	27.5	PHE 162 B PRO 163 B LEU 164 B ASP 206 B PHE 332 B GLY 334 B PHE 337 B PRO 391 B GLY 392 B ALA 393 B HIS 458
<i>Bacillus</i> Lacc	6.72	11.8	ALA 227 A THR 260 A ARG 261 A THR 262 A ASN 264 A SER 293 A PRO 296 A ALA 320 A GLY 321 A CYS 322 A GLY 323 A GLY 324 A GLY 417 A THR 418 A HIS 419 A PRO 420 A ILE 494
Novozym 51003	5.62	76.07	SER 190 A GLY 191 A ALA 192 A PHE 194 A GLU 235 A LEU 296 A LEU 297 A GLY 299 A LEU 363 A PHE 371 A TRP 373 A PHE 427 A LEU 429 A HIS 508