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Supporting information

Development of an efficient biocatalytic system based on bacterial laccase for the oxidation of selected 1,4-dihydropyridines

Stefan Simić¹, Sanja Jeremic², Lidija Djokic², Nataša Božić³, Zoran Vujčić¹, Nikola Lončar^{4,5}, Ramsankar Senthamarai kannan⁶, Ramesh Babu^{6,7}, Igor M. Opsenica^{1,*}, Jasmina Nikodinović-Runić^{1,2*}

¹University of Belgrade, Faculty of Chemistry, Studentski trg 16, P.O. Box 51, Belgrade, 11158, Serbia

²Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Vojvode Stepe 444a, Belgrade, 11000, Serbia

³Department of Chemistry, Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Studentski trg 12-16, 11000 Belgrade, Serbia

⁴Groningen Enzyme and Cofactor Collection (GECCO Biotech), Groningen, The Netherlands

⁵Molecular Enzymology Group, University of Groningen, Groningen, The Netherlands

⁶AMBER Centre, Trinity College Dublin, College Green, Dublin 2, Ireland

⁷BEACON SFI Bioeconomy Research Centre, O'Brien Science Centre, University College Dublin, Ireland

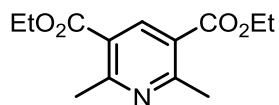
*Corresponding authors:

Tel. +381 11 3336684; E-mail: igorop@chem.bg.ac.rs

Tel. +381 11 3976034; E-mail: jasmina.nikodinovic@imgge.bg.ac.rs

Synthesis and characterization of Py1- Py6

Diethyl 2,6-dimethylpyridine-3,5-dicarboxylate (**Py1**) [1]



Following the general procedure for the laccase-catalyzed oxidation of 1,4-dihydropyridines, diethyl 2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**DHPy1**) (final concentration 26 mM) was oxidized using *TvLacc* (final concentration 1.16 U/ml; activity unit determined against catechol) during 4 h at 50 °C. Purification by dry-flash chromatography (SiO₂; hexane/ethyl acetate 9:1 v/v) yielded **Py1** as a white powder (28.4 mg, 94%).

Following the same procedure, diethyl 2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**DHPy1**) (final concentration 26 mM) was oxidized using *BliLacc* (final concentration 0.87 U/ml; activity unit determined against catechol) during 4 h at 50 °C. Purification by dry-flash chromatography (SiO₂; hexane/ethyl acetate 9:1 v/v) yielded **Py1** likewise (28.3 mg, 94%).

Following the general procedure for the oxidation of 1,4-dihydropyridines catalyzed BNC-supported laccase, diethyl 2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**DHPy1**) (final concentration 26 mM) was oxidized using *TvLacc* (5 U) immobilized on BNC (10.7 mg) during 20 h at 50 °C. Purification by dry-flash chromatography (SiO₂; hexane/ethyl acetate 9:1 v/v) yielded **Py1** (26.3 mg, 87%).

Following the same procedure, diethyl 2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**DHPy1**) (final concentration 26 mM) was oxidized using *BliLacc* (4 U) immobilized on BNC (10.7 mg) during 20 h at 50 °C. Purification by dry-flash chromatography (SiO₂; hexane/ethyl acetate 9:1 v/v) yielded **Py1** (20.8 mg, 69%).

Following the general procedure for the oxidation of 1,4-dihydropyridines catalyzed by the *E. coli* whole-cell suspension, diethyl 2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**DHPy1**) (final concentration 11 mM) was oxidized using *E. coli* (*BliLacc*) (0.2 ml, OD₆₀₀ = 150) during 7 h at 50 °C. Purification by dry-flash chromatography (SiO₂; hexane, hexane/ethyl acetate 9:1 v/v) yielded **Py1** (14.6 mg, 97%).

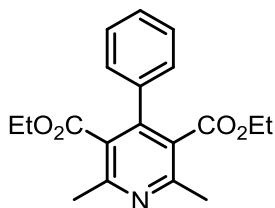
Following the same procedure, diethyl 2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**DHPy1**) (final concentration 11 mM) was oxidized using *E. coli* TOP10 (0.2 ml, OD₆₀₀ = 150) during 24 h at 50 °C. Purification by dry-flash chromatography (SiO₂; hexane, hexane/ethyl acetate 9:1 v/v) yielded **Py1** (14.3 mg, 95%).

Following the general procedure for the oxidation of 1,4-dihydropyridines catalyzed by the BNC-supported *E. coli*, diethyl 2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**DHPy1**) (final concentration 11 mM) was oxidized using *E. coli* (*BliLacc*) (0.24 ml, OD₆₀₀ = 150) immobilized on BNC (9.7 mg) during 24 h at 50 °C. Purification by dry-flash chromatography (SiO₂; hexane, hexane/ethyl acetate 9:1 v/v) yielded **Py1** (13.2 mg, 87%).

Following the same procedure, diethyl 2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**DHPy1**) (final concentration 11 mM) was oxidized using *E. coli* TOP10 (0.18 ml, OD₆₀₀ = 150) immobilized on BNC (6.9 mg) during 24 h at 50 °C. Purification by dry-flash chromatography (SiO₂; hexane, hexane/ethyl acetate 9:1 v/v) yielded **Py1** (11.4 mg, 75%).

The product was obtained as a white solid. FT-IR (ATR): 2980m, 2932m, 2870w, 1722s, 1591m, 1551m, 1440m, 1369m, 1290m, 1258m, 1224m, 1110m, 1046m; ^1H NMR (500 MHz, CDCl_3): δ = 1.42 (t, J = 7.1 Hz, 6H), 2.85 (s, 6H), 4.40 (q, J = 7.1 Hz, 4H), 8.67 (s, 1H) ppm; ^{13}C NMR (125 MHz, CDCl_3): δ = 14.3, 24.9, 61.4, 123.1, 140.9, 162.2, 166.0 ppm.

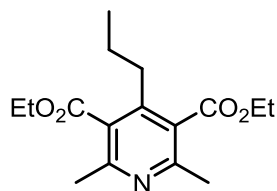
Diethyl 2,6-dimethyl-4-phenylpyridine-3,5-dicarboxylate (Py2) [1]



Following the general procedure for the laccase-catalyzed oxidation of 1,4-dihydropyridines, diethyl 2,6-dimethyl-4-phenyl-1,4-dihydropyridine-3,5-dicarboxylate (**DHPy2**) (final concentration 26 mM) was oxidized using *BliLacc* (final concentration 0.87 U/ml) during 4 h at 50 °C. Purification by dry-flash chromatography (SiO_2 ; hexane/ethyl acetate 9:1 v/v) yielded **Py2** (4.2 mg, 11%).

The product was obtained as a white solid. FT-IR (ATR): 3058w, 2981m, 2959m, 2927m, 2854w, 1727s, 1559m, 1495w, 1447w, 1410w, 1376w, 1292m, 1235s, 1211m, 1105m, 1043m; ^1H NMR (500 MHz, CDCl_3): δ = 0.90 (t, J = 7.1 Hz, 6H), 2.60 (s, 6H), 4.00 (q, J = 7.1 Hz, 4H), 7.23-7.26 (m, 2H), 7.34-7.37 (m, 3H) ppm; ^{13}C NMR (125 MHz, CDCl_3): δ = 13.5, 22.9, 61.3, 126.9, 128.0, 128.1, 128.3, 136.5, 155.4, 167.8 ppm.

Diethyl 2,6-dimethyl-4-propylpyridine-3,5-dicarboxylate (**Py3**) [2]



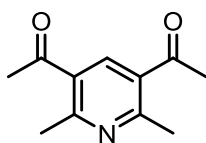
Following the general procedure for the laccase-catalyzed oxidation of 1,4-dihydropyridines, diethyl 2,6-dimethyl-4-propyl-1,4-dihydropyridine-3,5-dicarboxylate (**DHPy3**) (final concentration 26 mM) was oxidized using *BliLacc* (final concentration 0.87 U/ml) during 4 h at 50 °C. Purification by dry-flash chromatography (SiO₂; hexane/ethyl acetate 9:1 v/v) yielded **Py3** (5.4 mg, 15%).

Following the general procedure for the oxidation of 1,4-dihydropyridines catalyzed by the *E. coli* whole-cell suspension, 2,6-dimethyl-4-propyl-1,4-dihydropyridine-3,5-dicarboxylate (**DHPy3**) (final concentration 11 mM) was oxidized using *E. coli* (*BliLacc*) (0.2 ml, OD₆₀₀ = 150) during 24 h at 50 °C. The conversion of the starting material was determined to be 11% by NMR spectroscopy.

Following the general procedure for the oxidation of 1,4-dihydropyridines catalyzed by the *E. coli* whole-cell suspension, 2,6-dimethyl-4-propyl-1,4-dihydropyridine-3,5-dicarboxylate (**DHPy3**) (final concentration 11 mM) was oxidized using *E. coli* TOP10 (0.2 ml, OD₆₀₀ = 150) during 24 h at 50 °C. The conversion of the starting material was determined to be 7% by NMR spectroscopy.

The product was obtained as a colorless solid. FT-IR (ATR): 2965m, 2932m, 2875w, 1728s, 1568m, 1449m, 1412w, 1380w, 1283m, 1236s, 1200m, 1104m, 1040m; ^1H NMR (500 MHz, CDCl_3): δ = 0.93 (t, J = 7.3 Hz, 3H), 1.39 (t, J = 7.1 Hz, 6H), 1.58 (m, 2H), 2.51 (s, 6H), 2.55 (m, 2H), 4.41 (q, J = 7.1 Hz, 4H) ppm; ^{13}C NMR (125 MHz, CDCl_3): δ = 14.1, 14.4, 22.9, 24.2, 33.4, 61.5, 127.2, 146.3, 155.0, 168.5 ppm.

1,1'-(2,6-dimethylpyridine-3,5-diyl)diethanone (**Py4**) [1]



Following the general procedure for the laccase-catalyzed oxidation of 1,4-dihydropyridines, 1,1'-(2,6-dimethyl-1,4-dihydropyridine-3,5-diyl)diethanone (**DHPy4**) (final concentration 26 mM) was oxidized using *BliLacc* (final concentration 0.87 U/ ml) during 4 h at 50 °C.

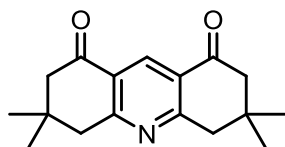
Purification by dry-flash chromatography (SiO_2 ; hexane/ethyl acetate 8:2 v/v, hexane/ethyl acetate 7:3 v/v) yielded **Py4** (21.7 mg, 94%).

Following the general procedure for the oxidation of 1,4-dihydropyridines catalyzed by the *E. coli* whole-cell suspension, 1,1'-(2,6-dimethyl-1,4-dihydropyridine-3,5-diyl)diethanone (**DHPy4**) (final concentration 11 mM) was oxidized using *E. coli* (*BliLacc*) (0.2 ml, OD_{600} = 150) during 24 h at 50 °C. The conversion of the starting material was determined to be >99% by NMR spectroscopy.

Following the general procedure for the oxidation of 1,4-dihydropyridines catalyzed by the *E. coli* whole-cell suspension, 1,1'-(2,6-dimethyl-1,4-dihydropyridine-3,5-diyl)diethanone (**DHPy4**) (final concentration 11 mM) was oxidized using *E. coli* TOP10 (0.2 ml, OD₆₀₀ = 150) during 24 h at 50 °C. The conversion of the starting material was determined to be >99% by NMR spectroscopy.

The product was obtained as a white solid. FT-IR (ATR): 2970w, 2925w, 1682s, 1593m, 1531m, 1434m, 1358m, 1266s, 1206m, 1072m, 1025m, 952m, 928m; ¹H NMR (500 MHz, CDCl₃): δ = 2.63 (s, 6H), 2.77 (s, 6H), 8.23 (s, 1H) ppm; ¹³C NMR (125 MHz, CDCl₃): δ = 24.9, 29.3, 130.1, 137.7, 160.2, 199.2 ppm.

3,3,6,6-tetramethyl-3,4,6,7-tetrahydroacridine-1,8(2H,5H)-dione (**Py5**) [3]



Following the general procedure for the laccase-catalyzed oxidation of 1,4-dihydropyridines, 3,3,6,6-tetramethyl-3,4,6,7,9,10-hexahydroacridine-1,8(2H,5H)-dione (**DHPy5**) (final concentration 26 mM) was oxidized using *BliLacc* (final concentration 0.87 U/ml) during 4 h at 50 °C. Purification by dry-flash chromatography (SiO₂; hexane/ethyl acetate 9:1 v/v, hexane/ethyl acetate 7:3 v/v) yielded **Py5** (5.1 mg, 16%).

Following the general procedure for the oxidation of 1,4-dihydropyridines catalyzed by the *E. coli* whole-cell suspension, 3,3,6,6-tetramethyl-3,4,6,7,9,10-hexahydroacridine-1,8(2H,5H)-dione (**DHPy5**) (final concentration 11 mM) was oxidized using *E. coli* (*BliLacc*) (0.2 ml,

OD600 = 150) during 24 h at 50 °C. The conversion of the starting material was determined to be 9% by NMR spectroscopy.

Following the general procedure for the oxidation of 1,4-dihydropyridines catalyzed by the *E. coli* whole-cell suspension, 3,3,6,6-tetramethyl-3,4,6,7,9,10-hexahydroacridine-1,8(2H,5H)-dione (**DHPy5**) (final concentration 11 mM) was oxidized using *E. coli* TOP10 (0.2 ml, OD600 = 150) during 24 h at 50 °C. The conversion of the starting material was determined to be 7% by NMR spectroscopy.

The product was obtained as a white solid. FT-IR (ATR): 2957s, 2928s, 2871m, 1699s, 1588s, 1563w, 1465m, 1417m, 1389w, 1371w, 1335w, 1296w, 1260w, 1234m, 1120w; ¹H NMR (500 MHz, CDCl₃): δ = 1.12 (s, 12H), 2.57 (s, 4H), 3.05 (s, 4H), 8.81 (s, 1H) ppm.

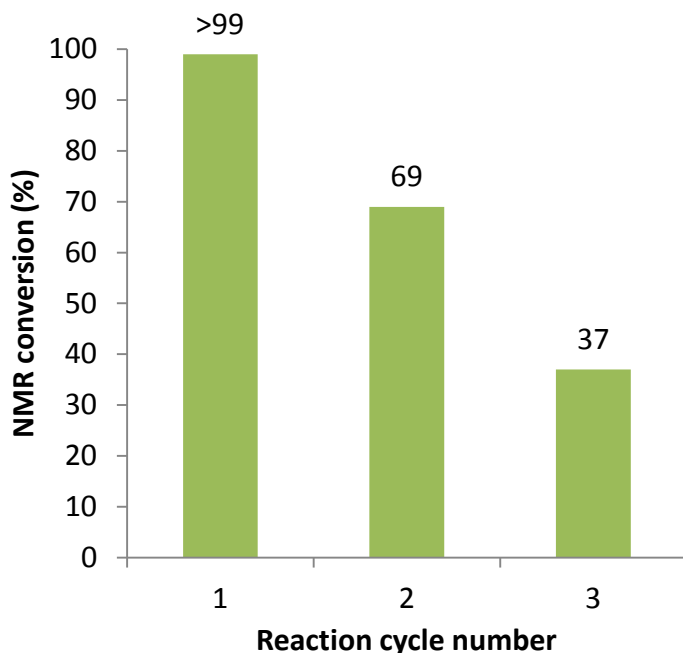


Fig. S1. Recycling of the *E. coli* (*Bli*Lacc)/BNC catalyst in the oxidation of **1a**. The reactions were run with 0.03 mmol of the substrate, according to general procedure. Substrate amount/CDW = 8.55.

Table S1. The substrate scope of the biocatalytic oxidation of 1,4-dihydropyridines.

Substrate	Catalyst	ABTS (mol%)	CuSO ₄ (mol%)	Product yield (%)
DHPy1 ^d	<i>BliLacc</i>	1.5	–	94 ^a
DHPy1	<i>E. coli (BliLacc)</i>	1.6	1.7	97 ^b
DHPy1	<i>E. coli TOP10</i>	1.6	1.7	95 ^b
DHPy2 ^d	<i>BliLacc</i>	1.5	–	11 ^a
DHPy2	<i>E. coli (BliLacc)</i>	1.6	1.7	0 ^b
DHPy2	<i>E. coli TOP10</i>	1.6	1.7	0 ^b
DHPy3 ^d	<i>BliLacc</i>	1.5	–	15 ^a
DHPy3	<i>E. coli (BliLacc)</i>	1.6	1.7	11 ^b
DHPy3	<i>E. coli TOP10</i>	1.6	1.7	7 ^b
DHPy4 ^d	<i>BliLacc</i>	1.5	–	94 ^a
DHPy4	<i>E. coli (BliLacc)</i>	1.6	1.7	>99 ^b
DHPy4	<i>E. coli TOP10</i>	1.6	1.7	>99 ^b
DHPy5 ^d	<i>BliLacc</i>	1.5	–	16 ^a
DHPy5	<i>E. coli (BliLacc)</i>	1.6	1.7	9 ^b
DHPy5	<i>E. coli TOP10</i>	1.6	1.7	7 ^b
DHPy6 ^d	<i>BliLacc</i>	1.5	–	0 ^c
DHPy6	<i>E. coli (BliLacc)</i>	1.6	1.7	0 ^b
DHPy6	<i>E. coli TOP10</i>	1.6	1.7	0 ^b

^a Isolated yield

^b Judged by conversion of starting material (based on NMR analysis)

^c Judged by conversion of starting material (based on TLC analysis)

^d CuSO₄ was added during the production of the recombinant protein

References

- [1] H.T. Abdel-Mohsen, J. Conrad, U. Beifuss, Laccase-catalyzed oxidation of Hantzsch 1,4-dihydropyridines to pyridines and a new one pot synthesis of pyridines. *Green Chem.* 14 (2012) 2686-2690.
- [2] O. De Paolis, J. Baffoe, S.M. Landge, B. Toeroek, Multicomponent Domino Cyclization-Oxidative Aromatization on a Bifunctional Pd/C/K-10 Catalyst: An Environmentally Benign Approach toward the Synthesis of Pyridines. *Synthesis* 21 (2008) 3423-3428.
- [3] V. Hegde, C.Y. Hung, P. Madhukar, R. Cunningham, T. Hopfner, R.P. Thummel, Design of receptors for urea derivatives based on the pyrido [3, 2-g] indole subunit. *J. Am. Chem. Soc.* 115 (1993) 872-878.