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Supporting information

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

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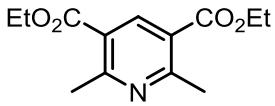
Synthesis and characterization of Py1- Py6

*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 *Tv*Lacc (final concentration 1.16 U/ml; activity unit determined against catechol) during 4 h at 50 ˚C. Purification by dry-flash chromatography (SiO_2; 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 *Bli*Lacc (final concentration 0.87 U/ml; activity unit determined against catechol) during 4 h at 50 ˚C. Purification by dry-flash chromatography (SiO_2; 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 *Tv*Lacc (5 U) immobilized on BNC (10.7 mg) during 20 h at 50 ˚C. Purification by dry-flash chromatography (SiO_2; 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 *Bli*Lacc (4 U) immobilized on BNC (10.7 mg) during 20 h at 50 ˚C. Purification by dry-flash chromatography (SiO_2; 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* (*Bli*Lacc) (0.2 ml, OD_{600} = 150) during 7 h at 50 ˚C. Purification by dry-flash chromatography (SiO_2; hexane, hexane/ethyl acetate 9:1 v/v) yielded **Py1** (14.6 mg, 97%).

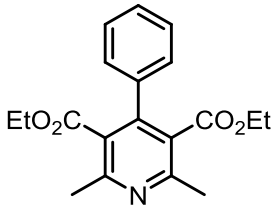
Following the same procedure, diethyl 2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**DHPy1**) (final concentration 11 mM) was oxidized using *E. coli* TOP10 (0.2 ml, OD_{600} = 150) during 24 h at 50 ˚C. Purification by dry-flash chromatography (SiO_2; hexane, hexane/ethyl acetate 9:1 v/v) yielded **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* (*Bli*Lacc) (0.24 ml, OD_{600} = 150) immobilized on BNC (9.7 mg) during 24 h at 50 ˚C. Purification by dry-flash chromatography (SiO_2; 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, OD_{600} = 150) immobilized on BNC (6.9 mg) during 24 h at 50 ˚C. Purification by dry-flash chromatography (SiO_2; 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; ^{1}H 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.

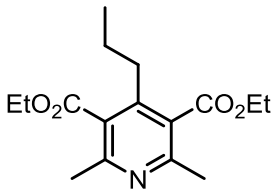
*Diethyl 2,6-dimethyl-4-phenylpyridine-3,5-dicarboxylate (**Py2**)* [1]



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 *Bli*Lacc (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; ^{1}H 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]



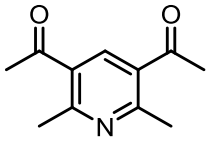
Following the general procedure for the laccase-catalyzed oxidation of 1,4-dihydropyridines, diethyl 2,6-dimethyl-4-propyl-1,4-dihydropyridine-3,5-dicarboxylate (**DHPy3**) (final concentration 26 mM) was oxidized using *Bli*Lacc (final concentration 0.87 U/ml) during 4 h at 50 ˚C. Purification by dry-flash chromatography (SiO_2; hexane/ethyl acetate 9:1 v/v) yielded **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* (*Bli*Lacc) (0.2 ml, OD_{600} = 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, OD_{600} = 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; ^{1}H 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]



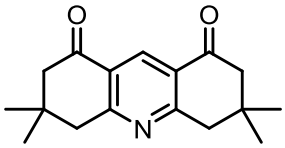
Following the general procedure for the laccase-catalyzed oxidation of 1,4-dihydropyridines, 1,1'-(2,6-dimethyl-1,4-dihydropyridine-3,5-diyl)diethanone (**DHPy4**) (final concentration 26 mM) was oxidized using *Bli*Lacc (final concentration 0.87 U/ ml) during 4 h at 50 ˚C. Purification by dry-flash 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* (*Bli*Lacc) (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, OD_{600} = 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; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.63 (s, 6H), 2.77 (s, 6H), 8.23 (s, 1H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): 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 *Bli*Lacc (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, 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* (*Bli*Lacc) (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; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.12 (s, 12H), 2.57 (s, 4H), 3.05 (s, 4H), 8.81 (s, 1H) ppm.

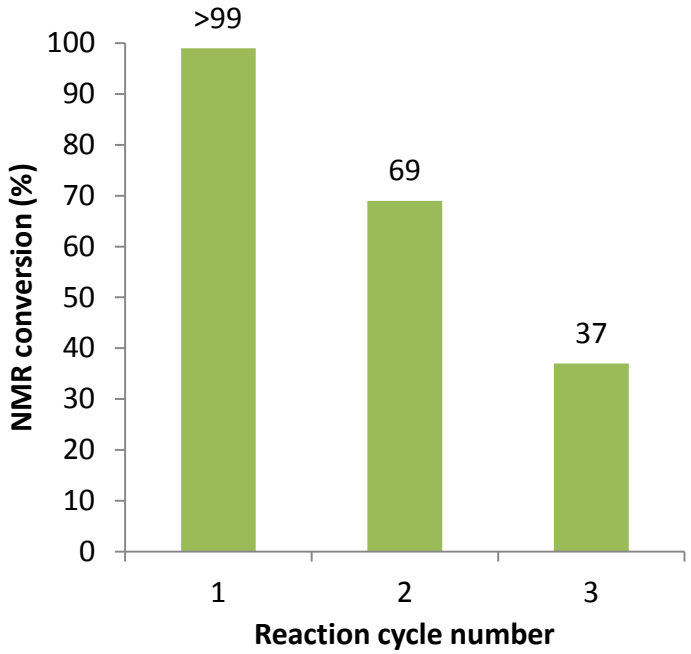


Fig. S1. Recycling of the *E. coli* (*Bli*Lacc)/BNC catalyst in the oxidation of **1a**. The reactions were run with 0.03 mmol of the substrate, according to general procedure. Substrate amount/CDW = 8.55.

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

Substrate	Catalyst	ABTS (mol%)	$CuSO_4$ (mol%)	Product yield (%)
DHPy1[d]	*Bli*Lacc	1.5	–	94[a]
DHPy1	*E. coli* (*Bli*Lacc)	1.6	1.7	97[b]
DHPy1	*E. coli* TOP10	1.6	1.7	95[b]
DHPy2[d]	*Bli*Lacc	1.5	–	11[a]
DHPy2	*E. coli* (*Bli*Lacc)	1.6	1.7	0[b]
DHPy2	*E. coli* TOP10	1.6	1.7	0[b]
DHPy3[d]	*Bli*Lacc	1.5	–	15[a]
DHPy3	*E. coli* (*Bli*Lacc)	1.6	1.7	11[b]
DHPy3	*E. coli* TOP10	1.6	1.7	7[b]
DHPy4[d]	*Bli*Lacc	1.5	–	94[a]
DHPy4	*E. coli* (*Bli*Lacc)	1.6	1.7	>99[b]
DHPy4	*E. coli* TOP10	1.6	1.7	>99[b]
DHPy5[d]	*Bli*Lacc	1.5	–	16[a]
DHPy5	*E. coli* (*Bli*Lacc)	1.6	1.7	9[b]
DHPy5	*E. coli* TOP10	1.6	1.7	7[b]
DHPy6[d]	*Bli*Lacc	1.5	–	0[c]
DHPy6	*E. coli* (*Bli*Lacc)	1.6	1.7	0[b]
DHPy6	*E. coli* TOP10	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] $CuSO_4$ was added during the production of the recombinant protein

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