Supporting Information

Access to lactone building blocks via horse liver alcohol dehydrogenase-catalyzed oxidative lactonization

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CONTENTS

1 Experimental Methods

1.1 HLADH-catalyzed oxidation of 1,4-butanediol coupled with LMS at 50 mM- and 250 mM-substrate concentration

50 mM-substrate concentration: 1,4-Butanediol stock (1,4-BD, 500 mM), NAD^+ stock (25 mM), acetosyringone stock (2 mM) and HLADH stock (1 gL^{-1}) were freshly prepared in 50 mM Tris-HCl buffer at pH 8. *Mt*laccase was used as delivered (0.2 mM solution). The mixture of buffer (940 µL), 1,4-BD stock (200 µL), acetosyringone stock (200 µL), and NAD^+ stock (40 µL) was incubated at 30 °C for 3 min. Biotransformations were initiated with the *Mt*laccase (20 µL) and HLADH solution (600 µL), and the reaction mixtures (2 mL) were orbitaly shaken at 600 rpm in 10 mL glass vessels at 30 °C. The starting concentrations were: 50 mM 1,4-BD, 0.2 mM acetosyringone, 0.5 mM NAD^+, 2 µM *Mt*laccase and 0.3 gL^{-1} HLADH.

250 mM-substrate concentration: 1,4-BD stock (1 M), NAD^+ stock (25 mM), acetosyringone stock (2 mM) and HLADH stock (1 gL^{-1}) were freshly prepared in 50 mM Tris-HCl buffer at pH 8. The mixture of buffer (640 µL), 1,4-BD stock (500 µL), acetosyringone stock (200 µL), and NAD^+ stock (40 µL) was incubated at 30 °C for 3 min. Biotransformations were initiated with the *Mt*laccase (20 µL) and HLADH solution (600 µL), and the reaction mixtures (2 mL) were orbitaly shaken at 600 rpm in 50 mL Falcon tubes at 30 °C. The starting concentrations were: 250 mM 1,4-BD, 0.2 mM acetosyringone, 0.5 mM NAD^+, 2 µM *Mt*laccase and 0.3 gL^{-1} HLADH.

Aliquots (50 µL) were removed at intervals and mixed with 200 µL of ethyl acetate (EtOAc). The mixture was vortexed for 20 sec, followed by centrifugation (16,100 × g, 0.5 min). The clear organic phase was removed and dried over anhydrous $MgSO_4$. Dodecane (5 mM) was used as an external standard in EtOAc and lactone formations were assessed using GC (see 2. Analytics).

1.2 Kinetic analysis of HLADH-catalyzed oxidation of diols

Stock solutions of diol (up to 4.2 M), NAD^+ (25 mM) and HLADH (1.2 gL^{-1}) were freshly prepared in 50 mM Tris-HCl buffer at pH 7. Reaction mixtures containing reaction buffer (V µL), diol stock (970–V µL) and NAD^+ stock (20 µL) were incubated at 30 °C for 3 min. Reactions were started by the addition of the HLADH stock (10 µL). Formation of NADH was followed by UV/Vis spectroscopy and quantified using an absorption coefficient of 6.22

mM^{-1} cm^{-1} (340 nm). The starting concentrations of NAD^+ and HLADH were 0.5 mM and 0.012 gL^{-1}, respectively. The simulation was performed using MATLAB® 2010 based on Michaelis-Menten double substrate kinetics. In case of substrate surplus inhibition the following equation was used:

$$\frac{V_{\max} \times c(\text{diol}) \times c(\text{NAD}^+)}{\left((K_{\text{M}}\text{diol} + c(\text{diol}) \times \left(1 + \frac{c(\text{diol})}{K_i\text{diol}}\right)\right) \times (K_{\text{M}}\text{NAD}^+ + c(\text{NAD}^+))}$$

1.3 HLADH-catalyzed oxidation of diols coupled with LMS at 50 mM-substrate concentration

Diol stocks (500 mM each), NAD^+ stock (25 mM), acetosyringone stock (2 mM) and HLADH stock (1 gL^{-1}) were freshly prepared in 50 mM Tris-HCl buffer at pH 8. The mixture of buffer (940 µL), diol stock (200 µL), acetosyringone stock (200 µL), and NAD^+ stock (40 µL) was incubated at 30 °C for 3 min. Biotransformations were initiated with the *Mt*laccase (20 µL, 0.2 mM stock) and HLADH solution (600 µL), and the reaction mixtures (2 mL) were *orbitaly* shaken at 600 rpm in 10 mL glass vessels at 30 °C. The starting concentrations were: 50 mM diol, 0.2 mM acetosyringone, 0.5 mM NAD^+, 2 µM *Mt*laccase and 0.3 gL^{-1} HLADH.

Aliquots (50 µL) were removed at intervals and mixed with 200 µL of ethyl acetate (EtOAc). The mixture was vortexed for 20 sec, followed by centrifugation (16,100 × g, 0.5 min). The clear organic phase was removed and dried over anhydrous $MgSO_4$. Dodecane (5 mM) was used as an external standard in EtOAc and lactone formations were assessed using GC (see 2. Analytics).

1.4 HLADH-catalyzed oxidation of 1,4-butanediol to γ-butyrolactone coupled with LMS in 2LPS

1,4-BD stock (1 M), NAD^+ stock (10 mM), acetosyringone stock (2 mM), ABTS stock (10 mM), and HLADH stock (0.6 gL^{-1}) were freshly prepared in 50 mM Tris-HCl buffer at pH 8. The *Mt*laccase (0.2 mM) was diluted 10-fold to 0.02 mM using aforementioned buffer. The mixture of 1,4-BD stock (50 µL), acetosyringone stock (20 µL) or ABTS stock (20 µL), and NAD^+ stock (10 µL) was incubated at 30 °C for 3 min. Biotransformations were initiated by the addition of *Mt*laccase (20 µL) and HLADH solution (100 µL) in a total of 0.2 mL of aqueous medium. After addition of the enzymes, 1.8 mL of diisopropyl ether (DIPE) containing 5 mM of dodecane was immediately added to the aqueous reaction medium. The reaction mixtures (2 mL) were *orbitaly* shaken at 600 rpm in 50 mL Falcon tubes at 30 °C.

Aqueous medium (0.2 mL) contained: 250 mM 1,4-BD, 0.2 mM acetosyringone or 1 mM ABTS, 0.5 mM NAD^+, 2 μM *Mt*laccase and 0.3 gL^{-1} HLADH. The organic phase (1.8 mL) contained: DIPE (with 5 mM dodecane). Aliquots (25 μL) were removed from the clearly separated organic phase at intervals and mixed with 225 μL of DIPE (containing 5 mM dodecane). The mixture was vortexed for 20 sec and dried over anhydrous $MgSO_4$.

1.5 HLADH-catalyzed oxidation of diols to their corresponding lactones coupled with LMS in 2LPS

Diol stock (1 M each), NAD^+ stock (10 mM), ABTS stock (10 mM), and HLADH stock (0.4 gL^{-1}) were freshly prepared in 50 mM Tris-HCl buffer at pH 8.0. The *Mt*laccase (0.2 mM) was diluted 10-fold to 0.02 mM. The mixture of diol stock (50 μL), ABTS stock (20 μL), and NAD^+ stock (10 μL) was incubated at 30 °C for 3 min. Biotransformations were initiated by the addition of *Mt*laccase (20 μL) and HLADH solution (100 μL) in a total of 0.2 mL of aqueous medium. After addition of the enzymes, 1.8 mL of DIPE (containing 5 mM of dodecane) was immediately added to the aqueous reaction medium. The reaction mixtures (2 mL) were orbitaly shaken at 600 rpm in 50 mL Falcon tubes at 30 °C. Aqueous medium (0.2 mL) contained: 250 mM diol, 1 mM ABTS, 0.5 mM NAD^+, 2 μM *Mt*laccase and 0.2 gL^{-1} HLADH. The organic phase (1.8 mL) contained: DIPE (with 5 mM dodecane). Aliquots (25 μL) were removed from clearly separated organic phase at intervals and mixed with 225 μL of DIPE (containing 5 mM dodecane). The mixture was vortexed for 20 sec and dried over anhydrous $MgSO_4$.

1.6 HLADH-catalyzed oxidation of 1,4-butanediol coupled with LMS in aqueous medium

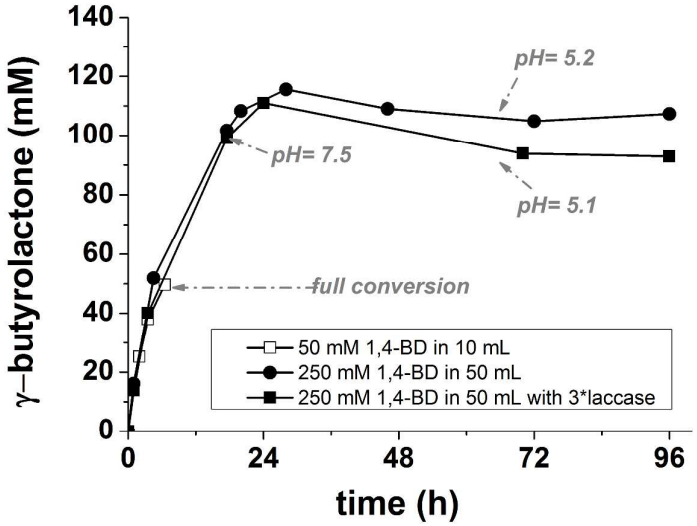


Figure S1. HLADH-catalyzed oxidation of 1,4-BD. Differing parameters: □ = 50 mM 1,4-BD; ●,■ = 250 mM 1,4-BD; □,● = 2 µM *Mt*laccase; ■ = 6 µM *Mt*laccase. Constant parameters: Tris-HCl (50 mM, pH 8), 0.2 mM acetosyringone, 0.5 mM NAD^+, 0.3 gL^{-1} HLADH, at 30 °C and 600 rpm.

1.7 Kinetic analysis of HLADH-catalyzed oxidation of diols

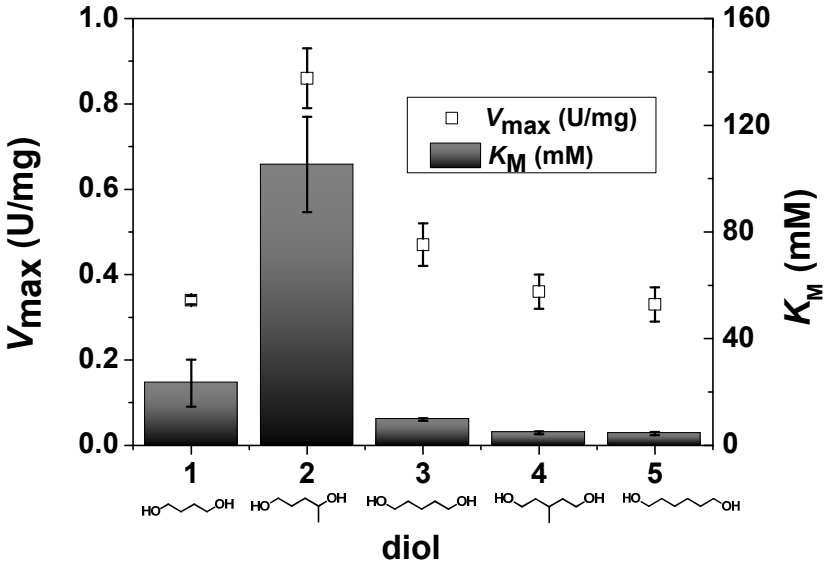


Figure S2. Kinetic analysis of oxidation of diols catalyzed by HLADH. Reaction conditions: c(diol) = 0–4200 mM, c(NAD$^+$) = 0.5 mM, c(HLADH) = 0.012 gL^{-1}, buffer: Tris-HCl (50 mM, pH 7), T = 30°C.

Table S1. Kinetic analysis of oxidation of diols catalyzed by HLADH. Diols: **1a** = 1,4-butanediol, **2a** = 1,4-pentanediol, **3a** = 1,5-pentanediol, **4a** = 3-methyl-1,5-pentanediol, **5a** = 1,6-hexanediol.

Diol	V_{max} (Umg^{-1})	K_M (mM)	K_i/K_M[a]
1a (1,4-BD)	3.4 ± 0.1	23.3 ± 8.8	57
2a (1,4-PD)	8.6 ± 0.7	105.3 ± 17.9	12
3a (1,5-PD)	4.7 ± 0.5	9.6 ± 0.5	215
4a (3-Me-1,5-PD)	3.6 ± 0.4	4.7 ± 0.5	390
5a (1,6-HD)	3.3 ± 0.4	4.4 ± 0.6	–

[a] Inhibition was observed at concentrations of: c(**1a**) > 250 mM, c(**2a**) > 900 mM, c(**3a**) > 600 mM, c(**4a**) > 250 mM, **5a**: no inhibition observed up to 1 M.

1.8 HLADH-catalyzed oxidation of 1,4-butanediol at different pH values

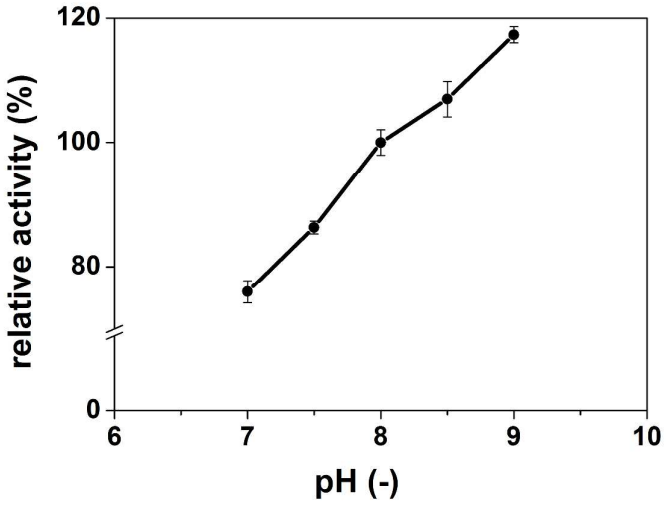


Figure S3. HLADH-catalyzed oxidation of 1,4-BD at different pHs. Reaction conditions: *c*(1,4-BD) = 250 mM, *c*(NAD^+) = 0.5 mM, *c*(HLADH) = 0.012 gL^{-1}, buffer: Tris-HCl (50 mM, pH 7–9), *T* = 30°C.

1.9 HLADH-catalyzed oxidation of 1,4-butanediol (1a) in the presence of γ-butyrolactone (1b)

Stock solutions of 1,4-BD (1 M), γ-butyrolactone (GBL, 1 M), NAD^+ (25 mM), and HLADH (1.2 gL^{-1}) were freshly prepared in 50 mM Tris-HCl buffer at pH 8.0. Reaction mixture containing reaction buffer (V µL), 1,4-BD stock (250 µL), GBL stock (720–V µL), and NAD^+ stock (20 µL) was incubated at 30 °C for 3 min. Biotransformations were initiated by the addition of the HLADH stock (10 µL). Formation of NADH was followed as described above. The starting concentrations of NAD^+, HLADH and 1,4-BD were 0.5 mM, 0.012 gL^{-1} and 250 mM, respectively, whereas GBL concentration differed between 0 and 250 mM. Measurements were triplicated whereby standard deviations were less than 4%. The following graph (Figure S4) shows oxidation activity of HLADH towards 1,4-BD in the presence of different GBL amounts.

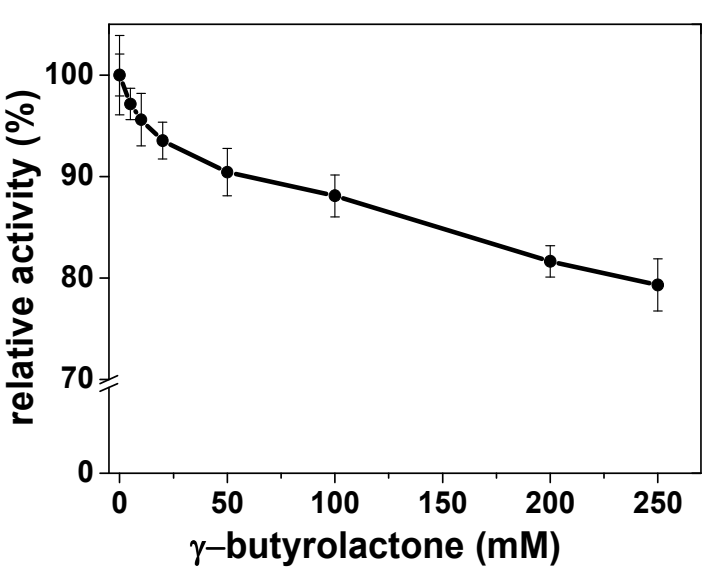


Figure S4. HLADH-catalyzed oxidation of 1,4-BD in the presence of γ-butyrolactone. Reaction conditions: *c*(1,4-BD) = 250 mM, *c*(GBL) = 0–250 mM, *c*(NAD^+) = 0.5 mM, *c*(HLADH) = 0.012 gL^{-1}, buffer: Tris-HCl (50 mM, pH 8), *T* = 30°C.

1.10 Determination of partition coefficients of solvents for 1,4-butanediol (1a) and γ-butyrolactone (1b)

Table S2. Partition coefficients of organic solvents for **1b** and **1a**.

Organic Solvent	log *P*[a]	Partition Coefficient[b]		Ratio[c]
		GBL	1,4-BD	
EtOAc	0.7	0.99	0.08	12
MTBE	1.0	0.13	0.02	7
DIPE	1.4	0.11	0.01	11

[a] Filho et al. 2003. [1]

[b] Partition coefficient is the molar ratio of each compound determined in the organic phase to the same compound in the aqueous phase. The ratio of phases was kept to $V_{organic}/V_{aqueous}$ = 9/1.

[c] The ratio of partition coefficients of GBL/1,4-BD in corresponding organic solvents.

[d] Standard deviations in partition coefficients were ≤5%.

1.11 Comparison of acetosyringone and ABTS in 2LPS

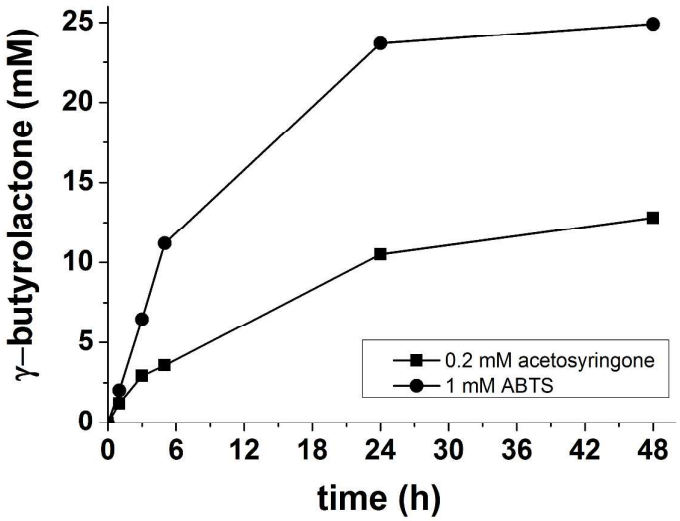


Figure S5. HLADH-catalyzed oxidation of 1,4-BD to GBL in 2LPS. Reaction conditions (aqueous, 0.2 mL): *c*(1,4-BD) = 250 mM, *c*(NAD^+) = 0.5 mM, *c*(HLADH) = 0.3 gL^{-1}, *c*(*Mt*laccase) = 2 µM, *c*(acetosyringone) = 0.2 mM (●) / *c*(ABTS) = 1 mM (■), organic phase: DIPE (containing 5 mM dodecane, 1.8 mL), $V_{organic}/V_{aqueous}$ = 9/1, buffer: Tris-HCl (50 mM, pH 8), *T* = 30 °C, 600 rpm. Concentrations are average values of duplicates.

1.12 Determination of partition coefficients of DIPE for diol and lactone couples

Solutions of diols and their corresponding lactones were prepared in DIPE (5 mM dodecane) at concentrations of 10–100 mM and were analyzed with GC. The same concentrations were separately prepared in 50 mM Tris-HCl buffer at 8.0 having the volume of V µL and extracted with 9V µL of DIPE (containing 5 mM dodecane). The partition coefficients are the molar ratio of each compound (diol or lactone) determined in the organic phase to the same compound in the aqueous phase (Table S3). The ratio of phases was kept to $V_{organic}/V_{aqueous}$ = 9/1. Standard deviations in partition coefficients were ≤5%.

Table S3. Partition coefficients of DIPE for diol and lactone couples.

Lactone	Diol	Partition Coefficient		Ratio[a]
		Lactone	Diol	
1b	**1a**	0.11	0.01	11
2b	**2a**	0.14	0.01	14
3b	**3a**	0.09	0.01	9
4b	**4a**	–	0.03	–
5b	**5a**	0.34	0.04	9

[a] The ratio of partition coefficients of lactone/diol in DIPE.

1.13 Synthesis of racemic 3-methyl-δ-valerolactone (4b)

The reference racemic **4b** was synthesized according to the procedure of Phillips and Graham (2008)[2]. A mixture of **4a** (24.7 mmol, 2.9 g) and MnO_2 (20 equiv., 494 mmol, 43 g) in $CHCl_3$ (120 mL) was stirred under reflux conditions for 48 hours. After 48 hours the reaction mixture was aliquoted (30 mL each) into 50 mL Falcon tubes, centrifuged (10,000 rpm, 5 min), and the precipitate was washed with $CHCl_3$ (3 ×10 mL each, 120 mL total). The solvent was removed under reduced pressure to give a colorless oily compound (1.99 g). Column chromatography (silica gel 60; petroleum ether:EtOAc, 6:1) gave 0.707 g (6.2 mmol, 25% isolated yield) of rac-**4b**. ^{1}H-NMR: (400 MHz, $CDCl_3$) δ 1.06 (d, J = 6.3 Hz, 3H), 1.56–1.48 (m, 1H), 1.94–1.86 (m, 1H), 2.14–2.04 (m, 2H), 2.71–2.63 (m, 1H), 4.29–4.22 (m, 1H), 4.44–4.39 (m, 1H). However, ^{1}H-NMR analysis revealed also the presence of a by-product (28%), which was identified as the corresponding hemiacetal intermediate (4-methyltetrahydro-2*H*-pyran-2-ol). Separation of the intermediate from rac-**4b** could not be achieved by column chromatography. The baseline separation of the peaks of enantiomers was established with GC analysis (Table S5, entry 4). The following chromatograms (Figure S6) were obtained for the synthesized rac-**4b**.

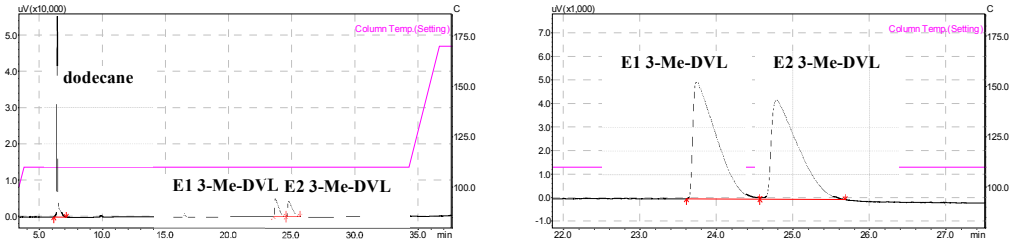


Figure S6. Left: The GC chromatogram of the synthesized rac-**4b** (E1 t_R = 23.8 min, E2 t_R = 24.9 min) using dodecane (t_R = 6.4 min) as the internal standard. Right: baseline-separated enantiomers.

1.14 Synthesis of (*S*)-4b catalyzed by HLADH

A stock of **4a** (0.5 M), NAD^+ stock (25 mM), acetosyringone stock (2 mM), and HLADH stock (3 gL^{-1}) were freshly prepared in 50 mM Tris-HCl buffer at pH 8. The *Mt*laccase was used as delivered (0.2 mM solution). The mixture of **4a** stock (1 mL), acetosyringone stock (1 mL), NAD^+ stock (0.2 mL) and buffer (6.7 mL) was incubated at 30 °C for 3 min. Finally, *Mt*laccase (0.1 mL) and HLADH solution (1 mL) were added. The starting concentrations were: 50 mM **4a**, 0.5 mM NAD^+, 200 µM acetosyringone, 0.3 gL^{-1} HLADH and 2 µM *Mt*laccase. The reaction mixture (10 mL) was *orbitaly* shaken at 600 rpm in 50 mL Falcon tubes at 30 °C. Aliquots (50 µL) were removed at definite time intervals and mixed with 200 µL of EtOAc (containing 5 mM dodecane). The mixture was vortexed for 20 sec and dried over anhydrous $MgSO_4$. 67% of conversion of **4a** to the enantiopure (*S*)-**4b** (*ee* >99% based on GC analysis) was achieved in 5 hours and complete consumption of the substrate was detected after 20 hours. The reaction mixture (10 mL) was saturated with NaCl and extracted with EtOAc (4 × 10 mL). After each extraction step the mixture was centrifuged (4000 rpm, 10 min). The collected clear organic phase was dried over anhydrous $MgSO_4$ and the solvent was removed under reduced pressure to give a colorless oily compound (42 mg, with impurities as specified below). Purification of the crude product was attempted by column chromatography (Silica gel 60, 70-230 mesh particle size; solvent petroleum ether: ethyl acetate 9:1) but was not successful. Due to the impurities (specified below) detected in **4b** the optical rotation could not be determined. The absolute configuration of **4b** formed by HLADH was already reported as (*S*) in literature $[\alpha]_D^{25}$ = –23.6° ($CHCl_3$)[3]; $[\alpha]_D^{27}$ = –24.8° (*c* 5.6, $CHCl_3$)[4]; $[\alpha]_D^{20}$ = –27.8° ($CHCl_3$)[5]. The following chiral-phase GC chromatogram (Fig. SI 7) was obtained for the synthesized (*S*)-**4b**.

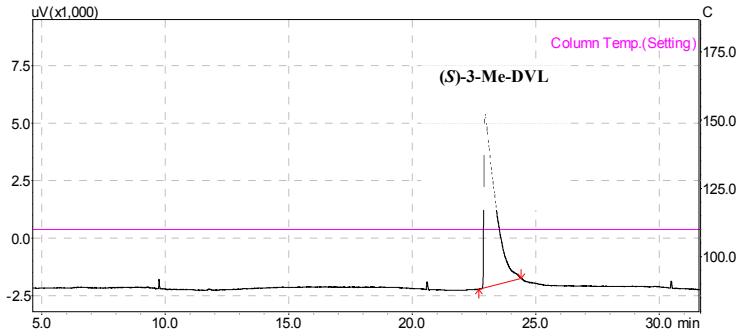


Figure S7. The GC chromatogram of the synthesized (*S*)-3-Me-DVL (t_R = 23.8 min).

(*S*)-**4b**: ^{1}H-NMR: (400 MHz, $CDCl_3$) δ 1.07 (d, J = 6.3 Hz, 3H), 1.56–1.47 (m, 1H), 1.95–1.89 (m, 1H), 2.15–2.07 (m, 2H), 2.70–2.64 (m, 1H), 4.30–4.23 (m, 1H), 4.44–4.39 (m, 1H). Isolated (*S*)-**4b** contained 0.4% of hemiacetal intermediate (4-methyltetrahydro-2*H*-pyran-2-ol), proved by achiral-phase GC analysis using CP-Wax 52 CB column. ^{1}H-NMR also revealed the corresponding acid product at 9.76 ppm however the corresponding acid could not be detected by GC–MS.

1.15 GC Chromatograms of 2b

The following graph shows the baseline separated enantiomers of **2b** (Figure S8, left); however, enantiomers of **2a** could not be separated (Figure S8, right). During the course of the reaction, the enantiomeric excess of **2b** decreases as shown in Figure S9.

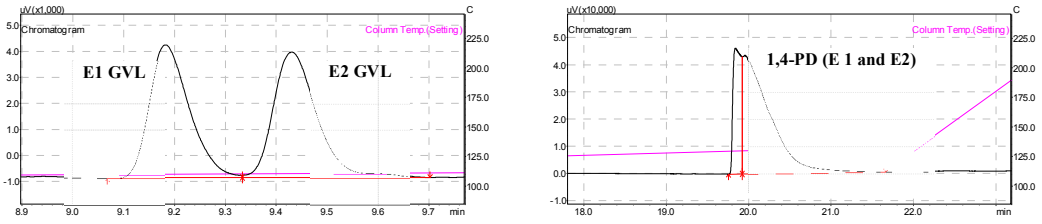


Figure S8. Left: The GC chromatogram of **2b** synthesized from 50 mM **2a** in aqueous medium (t = 24 h). Baseline separated enantiomers: E1, t_R = 9.20 min; E2, t_R = 9.44 min. Right: **2a**, no baseline separation.

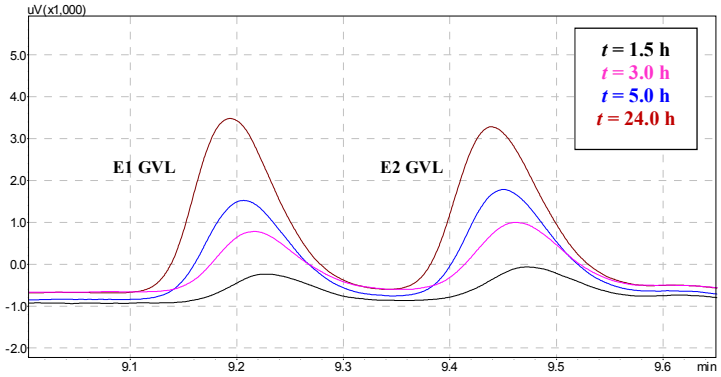


Figure S9. The GC chromatograms of **2b** synthesized from 250 mM (concentration in aqueous phase) **2a** in 2LPS. The peak areas are given in the following table (Table S4).

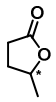
Table S4. The peak areas of enantiomers of GVL during the course of reaction.

Time (h)	E1 (Peak Area)	E2 (Peak Area)	*ee* (%)
1.5	2475.1	3480.0	17
3	6041.2	7587.8	11
5	9365.6	11047.6	8
24	23001.1	23891.9	2

2 Analytics

The reaction progress and the optical purity of the products were determined using GC.

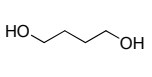
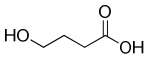
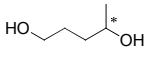
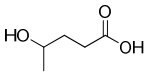
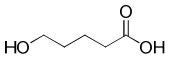
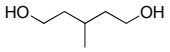
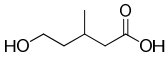
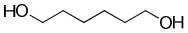
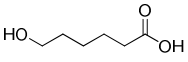
Table S5. Details for the analytics used in HLADH-catalyzed oxidation of diols to their corresponding lactones.

Entry	Product	Analysis – Column	Temperature Profile			t_R[min]
1	 **1b**	GC[a] CP-Wax 52 CB, 50 m × 0.53 mm × 2 µm	R [°C/min]	T [°C]	H [min]	GBL = 5.9
			-	60	1.0	
			50	150	2.2	
			50	160	1.5	
			50	190	2.0	1,4-BD = 8.4
			50	250	1.0	
2	 **2b**	GC[b] CP-Chiralsildex CB, 25 m × 0.32 mm × 0.25 µm	R [°C/min]	T [°C]	H [min]	
			-	100	5.0	GVL E1 = 9.2
			2	130	2.0	
			50	220	2.0	GVL E2 = 9.4
						1,4-PD = 20.8[i]
3	 **3b**	GC[a] CP-Wax 52 CB, 50 m × 0.53 mm × 2 µm	R [°C/min]	T [°C]	H [min]	
			-	60	1.0	DVL = 6.6
			50	160	2.0	
			50	185	4.0	1,5-PD = 8.3
			50	200	2.0	
			50	250	1.0	
4	 **4b**	GC[c] Chrialdex GTA, 50 m × 0.25 mm × 0.12 µm	R [°C/min]	T [°C]	H [min]	
			-	90	3.0	3-Me-DVL(*S*) = 23.8
			25	110	30.5	
			25	170	1.0	3-Me-DVL (*R*) = 24.9
						3-Me-1,5-PD = 18.8
	 **4b**	GC–MS[d] CPSil5 FactorFour VF 1-ms 25 m × 0.25 mm × 0.4 µm	R [°C/min]	T [°C]	H [min]	
			-	60	3.0	3-Me-DVL = 7.3
			15	315	2.0	
	 4-Methyltetrahydro-2*H*-pyran-2-ol	GC–MS[d] CPSil5 FactorFour VF 1-ms 25 m × 0.25 mm × 0.4 µm	R [°C/min]	T [°C]	H [min]	
			-	60	3.0	3-Me-DV-HA = 5.6
			15	315	2.0	
5	 **5b**	GC[a] CP-Wax 52 CB, 50 m × 0.53 mm × 2 µm	R [°C/min]	T [°C]	H [min]	
			-	60	1.0	ECL = 7.1
			50	160	2.0	
			50	190	4.0	
			50	200	2.0	1,6-HD = 9.7
			50	250	1.0	

[a] Entry 1,3,5: Total flow= 18 mL/min, N_2 as carrier gas.
[b] Entry 2 (GC Method): Pressure = 39.7 kPa, total flow = 24.4 mL/min, column flow = 1.02 mL/min, linear velocity = 22 cm/s, split = 20, He as carrier gas.
[c] Entry 4 (GC Method): Pressure = 130.3 kPa, total flow = 21.9 mL/min, column flow = 0.9 mL/min, linear velocity = 21.6 cm/s, split = 20, He as carrier gas.
[d] Entry 4 (GC–MS Method): Pressure = 33.2 kPa, total flow = 19 mL/min, column flow = 0.86 mL/min, linear velocity = 37cm/s, split = 20, He as carrier gas.
GC-MS equipment: Shimadzu GC-2010, Shimadzu QP-2010S mass detector
[e] Hemiacetal intermediate: 4-methyltetrahydro-2*H*-pyran-2-ol
[f] H: hold time (min), T: temperature, R: temperature ramp (°C/min). Entry 1, 3 and 5 (GC equipment): Shimadzu GC-2014. Entry 2 (GC equipment): Shimadzu GC-2010 Plus. Entry 4 (GC equipment): Shimadzu GC-2010
[i] No baseline separation

During the course of HLADH-catalyzed oxidation of diols (Figure S1) a significant pH-drop was observed. In order to concentrate reaction mixtures samples were evaporated under reduced pressure using SpeedVac (Savant). The concentrated fractions were dissolved in methanol and analyzed by GC–MS. The following table summarizes retention times of detected diols, lactones and acid products.

Table S6. Details for the GC-MS analytics to determine diols, lactones and the corresponding acid products.

Entry	Diol	Lactone	Acid	t_R[min]
1	 **1a**	 **1b**		Lactone = 4.7 Diol = 5.9 Acid= 7.6
2	 **2a**	 **2b**		Lactone = 5.3 Diol = 6.1 Acid= 7.7
3	 **3a**	 **3b**		Lactone = 6.7 Diol = 7.1 Acid= 8.7
4	 **4a**	 **4b**		Lactone = 7.3 Diol = 8.1 Acid= 9.0
5	 **5a**	 **5b**		Lactone = 7.9 Diol = 8.1 Acid= 9.4

GC–MS Method: Pressure = 33.2 kPa, total flow = 19 mL/min, column flow = 0.86 mL/min, linear velocity = 37 cm/s, split = 20, He as carrier gas. T = 60°C (3 min), R [°C/min] = 15 up to T = 315°C (2 min). GC-MS equipment: Shimadzu GC-2010, Shimadzu QP-2010S mass detector.

3 Additional Schemes

3.1 Proposed mechanism of the acetosyringone-based LMS for NADH oxidation

The mechanism of the *Mt*laccase-acetosyringone regeneration system for oxidized nicotinamide cofactors remains to be elucidated. However, involvement of phenoxy radical species appears plausible.[6] In analogy to electrochemical mechanisms of NADH oxidation[7] we propose a sequence of H-atom transfer (HAT) and subsequent single electron transfer (SET) to occur:

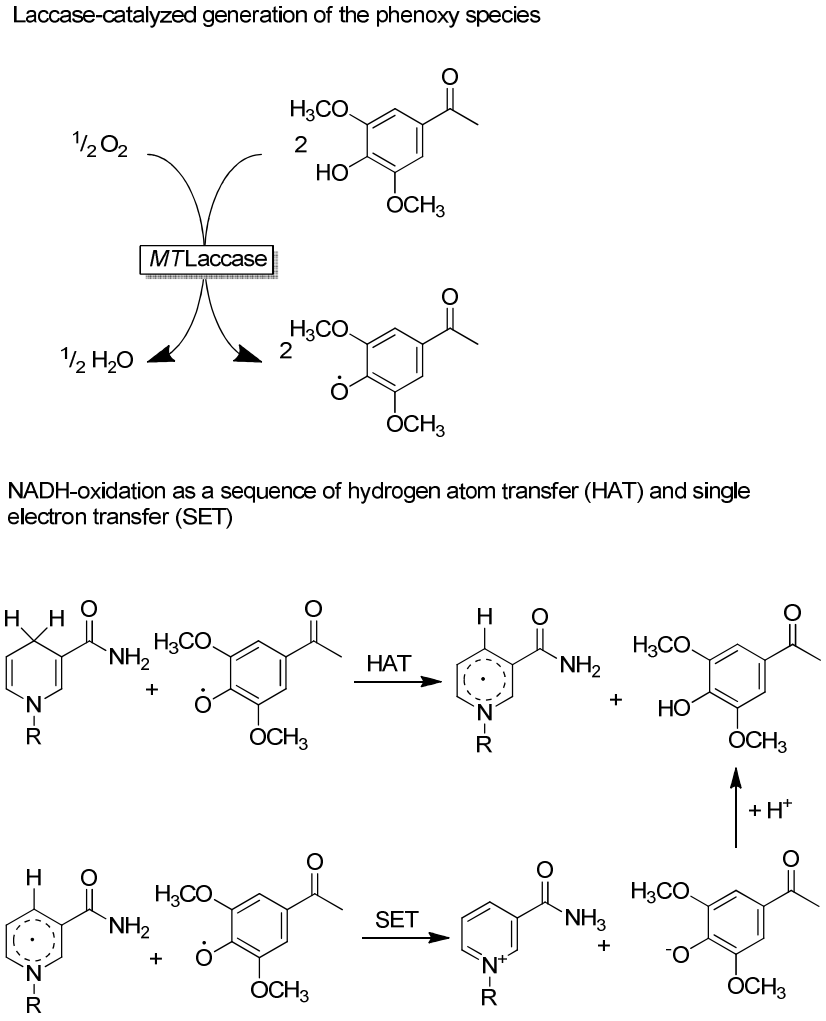


Figure S10: Proposed laccase-mediated formation of the acetosyringone-phenoxy radical (upper) as NADH oxidant (lower).

4 References

[1] Villela Filho, M., Stillger, T., Muller, M., Liese, A., Wandrey,C., *Angew. Chem. Int. Ed.* **2003**, *42*, 2993.

[2] Philipps, D. J., Graham, A. E., *Synlett*, **2008**, *5*, 649.

[3] Jones, J. B., Lok, K. P., *Can. J. Chem.* **1979**, *57*, 1025.

[4] Drueckhammer, D. G., Riddle, V. W., Wong, C.-H., *J. Org. Chem.* **1985**, *50*, 5387.

[5] Osa, T., Kashiwagi, Y., Yanagisawa, Y., *Chem. Lett.* **1994**, 367.

[6] Barneto, A.G., Aracri, E., Andreu, G., Vidal, T., *Bioresour. Technol.* **2012**, *112*, 327.

[7] a) Kochius, S., Magnusson, A., Hollmann, F., Schrader, J., Holtmann, D., *Appl. Microbiol. Biotechnol.* **2012**, *93*, 2251; b) Hollmann, F., Schmid, A., *Biocatal. Biotrans.* **2004**, *22*, 63.