Supporting Information

Enantioconvergent Biocatalytic Redox Isomerization
Yu-Chang Liu, Christian Merten, and Jan Deska*

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**General Remarks**

Alcohol dehydrogenases were purchased from Codexis (Codex® Ketoreductase Screening Kit) and from evocatal GmbH (evo 1.1.030 and evo 1.1.200). Nicotinamide cofactors were obtained from Carbolution Chemicals GmbH. Glucose oxidase (type II, *Aspergillus niger*) and chloroperoxidase (*Caldariomyces fumago*) were purchased from Sigma Aldrich.

All reactions that were carried out under argon atmosphere, were performed with dry solvents using anhydrous conditions. Dry solvents were taken from a solvent drying system MB-SPS-800 from M-Braun. Commercially available reagents were used without further purification. Enzymatic reactions were performed under non inert conditions on an orbital shaker in caped glass vials.

Column chromatography was performed with silica gel from Merck (Millipore 60, 40-60 µm, 240-400 mesh). Reactions were monitored by thin layer chromatography (TLC) carried out on Machery-Nagel precoated silica gel plates (TLC Silica gel 60 F$_{254}$). Visualisation of the TLC plates was done by using UV light and staining with a basic potassium permanganat solution.

$^1$H- and $^{13}$C-NMR-spectra were recorded on a Bruker AV-400 instrument at 20 °C. Chemical shifts are reported in parts per million (ppm) calibrated using residual non-deuterated solvents as internal reference [CHCl$_3$ at $\delta = 7.26$ ppm ($^1$H NMR) and 77.2 ppm ($^{13}$C NMR); MeOH-d$_4$ at $\delta = 3.31$ ppm ($^1$H NMR) and 49.2 ppm ($^{13}$C NMR)]. Infrared spectra were recorded on a Bruker ALPHA Eco-ATR spectrometer, absorption bands are reported in wave numbers [cm$^{-1}$]. High resolution mass spectrometry was performed on an Agilent 6530 (Q-TOF) mass spectrometer. Optical rotations were measured on an Autopol VI – automatic polarimeter from Rudolph Research Analytical. Gas chromatography was performed on a Hewlett Packard HP 6890 Series GC System using a Macherey-Nagel FS-Lipodex A and Macherey-Nagel FS-Lipodex E column (25 m x 0.25 mm), N$_2$, 1.0 ml/min; temperature program: 50 °C (1 min) / 5 °C-min$^{-1}$ (35 min) / 120 °C (15 min). High performance liquid chromatography analysis on an Agilent 1100 system with a G1312A binary pump and a G1312B diode array detector using analytical Daicel Chiralpak column (250 mm x 4.6 mm; ADH or AS-H).
Procedures and Analytical Data

Synthesis and Analytics of the Lactones

(S)-5-Hydroxy-1-oxaspiro[5.5]undec-3-en-2-one (2a)

Representative general procedure: In a 50 ml Erlenmeyer flask, rac-1a (10 mg, 55 µmol) was dissolved in Tris-HCl buffer (10 ml, 50 mM, pH 7.5), Codexis ADH P2-G03 (1.5 mg) and NADP+ (3.7 mg, 5 µmol) were added and the solution was incubated at 30 °C for 10 h. The reaction mixture was extracted with ethyl acetate (3×15 mL), the combined organic phases were dried over Na2SO4 and concentrated under reduced pressure. The residue was purified by column chromatography (SiO2, hexane:ethyl acetate, 1:1) yielding (S)-2a (7.5 mg, 41 µmol, 75%, 98% ee) as colorless oil.

[α]20D: +57.6 (c 0.49, CHCl3). Rf 0.36 (hexane/ethyl acetate 1/1). 1H-NMR (400 MHz, CDCl3): δ [ppm] = 6.83 (dd, J = 10.2 Hz, J = 4.5 Hz, 1H), 5.99 (dd, J = 9.9 Hz, J = 1.2 Hz, 1H), 4.12-4.15 (q, 1H), 3.48 (d, J = 8.7 Hz, 1H), 2.0-2.03 (m, 1H), 1.57-1.69 (m, 8H), 1.24-1.33 (m, 1H). 13C-NMR (100 MHz, CDCl3): δ [ppm] = 163.7, 145.5, 121.3, 84.5, 67.3, 34.3, 29.9, 25.1, 21.4, 20.7. FT-IR (ATR): ν [cm−1] = 3400 (s), 2934 (w), 2863 (w), 1694 (s), 1648 (w), 1241 (m), 1115 (m), 1033 (s), 1011 (s), 983 (s), 828 (s). HPLC (Chiralpak AS-H, hexane/isopropanol 9/1, 0.8 mL/min, 210 nm): tR ((S)-2a) = 30.1 min, tR ((R)-2a) = 40.4 min. HRMS (ESI+): m/z [M+H]+ calcd for C10H15O3: 183.1016; found: 183.1015.

(R)-10-Hydroxy-6-oxaspiro[4.5]dec-8-en-7-one (2b)

According to the general procedure, rac-1b (10 mg, 59 µmol) was reacted in presence of Codexis ADH P2-D12 (1.5 mg) and NADP+ (3.7 mg, 5 µmol). The residue was purified by column chromatography (SiO2, hexane:ethyl acetate, 1:1) yielding (R)-2b (8.7 mg, 53 µmol, 89%, 98% ee) as colorless oil.

[α]20D: −98.1 (c 0.44, CHCl3). Rf 0.36 (hexane/ethyl acetate 1/1). 1H-NMR (400 MHz, CDCl3): δ [ppm] = 6.95 (dd, J = 9.7 Hz, J = 4.5 Hz, 1H), 6.01 (dd, J = 9.7 Hz, J = 1.0 Hz, 1H), 4.16-4.19 (q, 1H), 3.21 (d, J = 8.6 Hz, 1H), 1.66-2.07 (m, 8H). 13C-NMR (100 MHz, CDCl3): δ [ppm] = 164.1, 146.5, 121.8, 94.6, 66.8, 37.6, 33.8, 24.4, 24.0. FT-IR (ATR): ν [cm−1] = 3399 (s), 2961 (w), 2875 (w), 1695 (s), 1628 (w), 1273 (m), 1133 (m), 1029 (m), 1002 (s), 826 (s). HPLC (Chiralpak
AS-H, hexane/isopropanol 9/1, 0.8 mL/min, 210 nm): $t_{R_s}$ ((S)-2b) = 35.4 min, $t_{R_r}$ ((R)-2f) = 45.1 min. HRMS (ESI+): $m/z$ [M+H]$^+$ calcd for $C_{9}H_{13}O_{3}$: 169.0859; found: 169.0849.

(S)-9-Hydroxy-5-oxaspiro[3.5]non-7-en-6-one (2c)

According to the general procedure, rac-1c (10 mg, 65 µmol) was reacted in presence of Codexis ADH P2-G03 (1.5 mg) and NADP$^+$ (3.7 mg, 5 µmol). The residue was purified by column chromatography (SiO$_2$, hexane:ethyl acetate, 1:1) yielding (S)-2c (6.7 mg, 44 µmol, 67%, 99% ee) as colorless oil.

$[\alpha]_{20}^{D}$: +145.3 (c 0.36, CHCl$_3$). $R_f$ 0.36 (hexane/ethyl acetate 1/1). $^1$H-NMR (400 MHz, CDCl$_3$): δ [ppm] = 6.94 (dd, $J = 9.6$ Hz, $J = 4.9$ Hz, 1H), 6.02 (d, $J = 9.7$ Hz, 1H), 4.25 (dd, $J = 8.5$ Hz, $J = 5.0$ Hz, 1H), 3.01 (d, $J = 8.5$ Hz, 1H), 2.56-2.64 (m, 1H), 2.23-2.36 (m, 2H), 2.12-2.20 (m, 1H), 1.95-2.02 (m, 1H), 1.67-1.77 (m, 1H). $^{13}$C-NMR (100 MHz, CDCl$_3$): δ [ppm] = 163.3, 144.7, 122.2, 84.2, 65.3, 32.0, 29.2, 12.5. FT-IR (ATR): ν [cm$^{-1}$] = 3401 (s), 2995 (w), 2948 (w), 1698 (s), 1628 (w), 1236 (s), 1083 (s), 1052 (s), 825 (s). HPLC (Chiralpak AS-H, hexane/isopropanol 9/1, 0.8 mL/min, 210 nm): $t_{R_s}$ ((S)-2c) = 38.0 min, $t_{R_r}$ ((R)-2c) = 45.3 min. HRMS (ESI+): $m/z$ [M+H]$^+$ calcd for $C_{8}H_{11}O_{3}$: 155.0703; found: 155.0700.

(S)-8-Hydroxy-4-oxaspiro[2.5]oct-6-en-5-one (2d)

According to the general procedure, rac-1d (10 mg, 71 µmol) was reacted in presence of Codexis ADH P2-G03 (1.5 mg) and NADP$^+$ (3.7 mg, 5 µmol). The residue was purified by column chromatography (SiO$_2$, hexane:ethyl acetate, 1:1) yielding (S)-2d (1.5 mg, 11 µmol, 15%, 98% ee) as colorless oil.

$[\alpha]_{20}^{D}$: +172.2 (c 0.08, CHCl$_3$). $R_f$ 0.32 (hexane/ethyl acetate 1/1). $^1$H-NMR (400 MHz, CDCl$_3$): δ [ppm] = 7.05 (dd, $J = 9.9$ Hz, $J = 5.3$ Hz, 1H), 6.15 (d, $J = 9.6$ Hz, 1H), 3.84 (d, $J = 4.8$ Hz, 1H), 2.80 (br, 1H), 1.17-1.24 (m, 1H), 1.05-1.14 (m, 2H), 0.77-0.84 (m, 1H). $^{13}$C-NMR (100 MHz, CDCl$_3$): δ [ppm] = 164.5, 145.3, 122.2, 65.1, 9.0. FT-IR (ATR): ν [cm$^{-1}$] = 3397 (s), 3014 (s), 3014 (s), 1715 (s), 1630 (w), 1235 (s), 1130 (s), 1041 (s), 1029 (s), 989 (m), 825 (s). HPLC (Chiralpak AS-H, hexane/isopropanol 9/1, 0.8 mL/min, 210 nm): $t_{R_s}$ ((S)-2d) = 33.5 min, $t_{R_r}$ ((R)-2e) = 40.0 min. HRMS (ESI+): $m/z$ [M+H]$^+$ calcd for $C_{7}H_{9}O_{3}$: 141.0546; found: 141.0546.
(S)-9-Hydroxy-2,5-dioxaspiro[3.5]non-7-en-6-one (2e)

According to the general procedure, rac-1e (10 mg, 65 µmol) was reacted in presence of Codexis KRED-101 (1.5 mg) and NADP+ (3.7 mg, 5 µmol). The residue was purified by column chromatography (SiO₂, hexane:ethyl acetate, 1:1) yielding (S)-2e (2.3 mg, 15 µmol, 23%, 99% ee) as yellow oil.

[α]⁺²⁰: +20.9 (c 0.14, CHCl₃). Rf 0.15 (hexane/ethyl acetate 1/1). ¹H-NMR (400 MHz, CDCl₃): δ [ppm] = 6.98 (dd, J = 9.7 Hz, J = 4.2 Hz, 1H), 6.02 (d, J = 9.9 Hz, 1H), 4.93 (d, J = 7.7 Hz, 1H), 4.75 (d, J = 6.8 Hz, 1H), 4.70 (d, J = 8.5 Hz, 1H), 4.62-4.65 (q, 1H), 4.58 (d, J = 7.7 Hz, 1H), 4.09 (d, J = 7.7 Hz, 1H). ¹³C-NMR (100 MHz, CDCl₃): δ [ppm] = 162.1, 145.7, 121.7, 82.1, 78.6, 64.0. FT-IR (ATR): ν [cm⁻¹] = 3393 (s), 2952 (w), 2884 (w), 1705 (s), 1629 (w), 1229 (m), 1174 (m), 1105 (s), 1049 (s), 822 (s). HPLC (Chiralpak AS-H, hexane/isopropanol 85/15, 0.8 mL/min, 210 nm): tₘ (S)-2e = 42.9 min, tₘ (R)-2e = 50.2 min.

(S)-5-Hydroxy-6,6-dimethyl-5,6-dihydro-2H-pyran-2-one (2f)

According to the general procedure, rac-1f (10 mg, 70 µmol) was reacted in presence of Evocatal ADH evo 1.1.200 (1.5 mg) and NAD⁺ (3.3 mg, 5 µmol). The residue was purified by column chromatography (SiO₂, hexane:ethyl acetate, 1:1) yielding (S)-2f (7.3 mg, 51 µmol, 73%, 99% ee) as colorless oil.

[α]⁺²⁰: +92.6 (c 0.37, CHCl₃). Rf 0.34 (hexane/ethyl acetate 1/1). ¹H-NMR (400 MHz, CDCl₃): δ [ppm] = 6.83 (dd, J = 9.7 Hz, J = 4.1 Hz, 1H), 5.98 (dd, J = 9.8 Hz, J = 1.4 Hz, 1H), 4.19 (qd, J = 1.3 Hz, 1H), 3.73 (d, J = 7.8 Hz, 1H), 1.43 (s, 6H). ¹³C-NMR (100 MHz, CDCl₃): δ [ppm] = 163.9, 146.7, 120.8, 84.0, 68.2, 26.4, 21.7. FT-IR (ATR): ν [cm⁻¹] = 3393 (s), 2984 (w), 2940 (w), 1698 (s), 1630 (w), 1294 (m), 1108 (s), 987 (m), 829 (s). HPLC (Chiralpak AS-H, hexane/isopropanol 9/1, 0.8 mL/min, 210 nm): tₘ (S)-2f = 31.7 min, tₘ (R)-2f = 35.9 min. HRMS (ESI⁺): m/z [M+Na]⁺ calcd for C₇H₁₀O₃Na: 165.0522; found: 165.0523.
(S)-5-Hydroxy-6,6-diethyl-5,6-dihydro-2H-pyran-2-one (2g)

According to the general procedure, rac-1g (10 mg, 59 µmol) was reacted in presence of Codexis ADH P1-B05 (1.5 mg) and NADP⁺ (3.7 mg, 5 µmol). The residue was purified by column chromatography (SiO₂, hexane:ethyl acetate, 1:1) yielding (S)-2g (7.3 mg, 43 µmol, 73%, 99% ee) as colorless oil.

\[ \text{[α]}^20_\text{D}: +58.9 \text{ (c 0.37, CHCl}_3) \]. \text{R}_f 0.28 (hexane/ethyl acetate 1/1). \text{¹H-NMR} (400 MHz, CDCl₃): \( \delta \) [ppm] = 6.88 (dd, \( J = 9.8 \) Hz, \( J = 4.7 \) Hz, 1H), 6.03 (d, \( J = 9.7 \) Hz, 1H), 4.20-4.24 (q, 1H), 2.86 (d, \( J = 8.9 \) Hz, 1H), 1.89-1.95 (m, 2H), 1.64-1.79 (m, 2H), 0.93-0.99 (m, 6H).

\text{¹³C-NMR} (100 MHz, CDCl₃): \( \delta \) [ppm] = 163.5, 145.3, 121.7, 88.0, 65.2, 26.8, 24.5, 7.8, 7.6. \text{FT-IR} (ATR): \( \nu \) [cm⁻¹] = 3400 (s), 2975 (w), 2945 (w), 1632 (w), 1700 (s), 1285 (w), 1108 (w), 985 (w), 828 (s). \text{HPLC} (Chiralpak AS-H, hexane/isopropanol 9/1, 0.8 mL/min, 210 nm): \( t_R \) ((S)-2g) = 24.7 min, \( t_R \) ((R)-2g) = 34.0 min. \text{HRMS} (ESI⁺): \( m/z \) [M+H]⁺ calcd for C₉H₁₅O₃: 171.1016; found: 171.1013.

(S)-5-Hydroxy-5,6-dihydro-2H-pyran-2-one (2h)

According to the general procedure, rac-1h (10 mg, 88 µmol) was reacted in presence of Codexis ADH P2-B02 (1.5 mg) and NADP⁺ (3.7 mg, 5 µmol). The residue was purified by column chromatography (SiO₂, hexane:ethyl acetate, 1:1) yielding (S)-2h (3.8 mg, 33 µmol, 38%, 95% ee) as colorless oil.

\[ \text{[α]}^20_\text{D}: +41.2 \text{ (c 0.23, CHCl}_3) \]. \text{R}_f 0.23 (hexane/ethyl acetate 1/1). \text{¹H-NMR} (400 MHz, CDCl₃): \( \delta \) [ppm] = 6.97 (dd, \( J = 9.9 \) Hz, \( J = 4.9 \) Hz, 1H), 6.03 (d, \( J = 9.8 \) Hz, 1H), 4.37-4.48 (m, 1H), 3.70-3.71 (d, \( J = 6.7 \) Hz, 1H). \text{¹³C-NMR} (100 MHz, CDCl₃): \( \delta \) [ppm] = 163.6, 146.9, 121.6, 72.0, 60.6. \text{FT-IR} (ATR): \( \nu \) [cm⁻¹] = 3382 (s), 2922 (w), 1698 (s), 1627 (w), 1237 (s), 1099 (s), 1082 (s), 1010 (s), 825 (s). \text{HPLC} (Chiralpak AD-H, hexane/isopropanol 9/1, 0.8 mL/min, 250 nm): \( t_R \) ((S)-2h) = 19.1 min, \( t_R \) ((R)-2h) = 25.9 min. \text{HRMS} (ESI⁺): \( m/z \) [M+H]⁺ calcd for C₅H₇O₃: 115.0390; found: 115.0389.
According to the general procedure, rac-5 (10 mg, 61 µmol) was reacted in presence of Codexis ADH P1-C01 (1.5 mg) and NADP+ (3.7 mg, 5 µmol). The residue was purified by column chromatography (SiO₂, hexane:ethyl acetate = 1:1) yielding (S)-6 (9.5 mg, 58 µmol, 95%, 97% ee) as colorless oil.

\[
\text{[a]}^{20} \beta: -15.2 (c 0.50, CHCl₃). \text{Rf} 0.36 (hexane/ethyl acetate 1/1). \text{H-NMR} (400 MHz, CDCl₃): \delta [ppm] = 8.07 (dd, J = 7.7 Hz, J = 1.4 Hz, 1H), 7.66 (dt, J = 1.5 Hz, 1H), 7.48-7.55 (m, 2H), 4.90-4.94 (m, 1H), 4.48-4.57 (m, 2H), 3.29 (d, J = 6.7 Hz, 1H).
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\text{C-NMR} (100 MHz, CDCl₃): \delta [ppm] = 164.7, 140.7, 134.5, 130.3, 129.4, 126.5, 123.7, 72.0, 64.1. \text{IR} (ATR): \nu [cm^{-1}] = 3393 (s), 2896 (w), 1704 (s), 1463 (s), 1392 (m), 1281 (s), 1240 (s), 1127 (s), 1075 (s), 766 (s), 716 (s), 698 (s).
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\text{HPLC} (Chiralpak AS-H, hexane/isopropanol 9/1, 0.8 mL/min, 210 nm): t_R((R)-6) = 35.0 min, t_R((S)-6) = 46.1 min. \text{HRMS} (ESI+): m/z [M+H]^+ calcd for C₉H₉O₃: 165.0546; found: 165.0547.
\]

(S)-6 (10 mg, 61 µmol) was dissolved in methanol (0.5 mL). At room temperature, aqueous NaOH (1 M, 1 mL) was added and the reaction mixture was stirred for 30 min. The solution was acidified by addition of aqueous HCl (1 M, 1.2 mL) and extracted with ethyl acetate (3 x 5 mL). The combined organic layers were dried over Na₂SO₄ and all volatiles were removed under reduced pressure. Purification by column chromatography (SiO₂, hexane:ethyl acetate, 2:1) yielded (S)-7 (8.2 mg, 50 µmol, 82%, 97% ee) as colorless oil.

\[
\text{[a]}^{20} \beta: +52.7 (c 0.21, CHCl₃). \text{Rf} 0.31 (hexane/ethyl acetate 1/1). \text{H-NMR} (400 MHz, CDCl₃): \delta [ppm] = 7.92 (d, J = 7.4 Hz, 1H), 7.60-7.68 (td, J = 1.0 Hz, 1H), 7.50-7.58 (m, 2H), 5.55-5.58 (q, 1H), 4.14 (dd, J = 12.3 Hz, J = 3.3 Hz, 1H), 3.91 (dd, J = 12.3 Hz, J = 5.7 Hz, 1H), 2.10 (bs, 1H).
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\text{C-NMR} (100 MHz, CDCl₃): \delta [ppm] = 146.5, 134.3, 129.6, 126.0, 122.1, 86.5, 81.6, 64.1. \text{IR} (ATR): \nu [cm^{-1}] = 3413 (s), 2927 (w), 1738 (s), 1615 (w), 1600 (w), 1467 (m), 1349 (m), 1287 (m), 1087 (s), 1065 (s), 1023 (s), 742 (s), 718 (m), 697 (m). \text{HRMS} (ESI+): m/z [M+H]^+ calcd for C₉H₉O₃: 165.0546; found: 165.0546.
Enzyme Sequences

Walterolactone B (2k)

In a 50 ml Erlenmeyer flask, 3-methylfurfuryl alcohol (10, 11 mg, 100 µmol) was dissolved in a mixture of citrate buffer (pH 6.0, 100 mM, 4.5 mL) and tert-butanol (0.5 mL). Chloroperoxidase (100 U), glucose oxidase (20 U) and D-glucose (120 µmol) were added and the reaction mixture was incubated at 30 °C under air for 5 h until TLC indicated full conversion. L-Methionine (1 mmol) was added and the solution was extracted with diethyl ether (3 x 10 mL). The combined organic phases were washed with brine and dried over Na₂SO₄. After removal of all volatiles in vacuo, the residue was redissolved in Tris-HCl buffer (10 ml, 50 mM, pH 7.5), Codexis ADH P2-D12 (1.5 mg) and NADP⁺ (3.7 mg, 5 µmol) were added and the solution was incubated at 30 °C for 10 h. The reaction mixture was extracted with ethyl acetate (3 x 15 mL), the combined organic phases were dried over Na₂SO₄ and concentrated under reduced pressure. The residue was purified by column chromatography (SiO₂, hexane:ethyl acetate, 1:1) yielding (S)-2k (4.9 mg, 38 µmol, 38%, 93% ee) as colorless oil.

[α]²⁰D: +21.5 (c 0.23, CHCl₃). Rf 0.17 (hexane/ethyl acetate 1/1). ¹H-NMR (400 MHz, CDCl₃): δ [ppm] = 5.85-5.86 (m, 1H), 4.37-4.46 (m, 2H), 4.18-4.20 (m, 1H), 2.76 (d, J = 7.8 Hz, 1H), 2.12 (s, 3H). ¹³C-NMR (100 MHz, CDCl₃): δ [ppm] = 163.7, 158.0, 117.5, 71.5, 64.7, 19.9. FT-IR (ATR): v [cm⁻¹] = 3379 (s), 2987 (w), 2949 (w), 1695 (s), 1648 (m), 1267 (s), 1232 (s), 1106 (s), 1080 (s), 1004 (s), 859 (s). HPLC (Chiralpak AD-H, hexane/isopropanol 9/1, 0.8 mL/min, 210 nm): tᵣ ((S)-2k) = 14.4 min, tᵣ ((R)-2k) = 21.2 min. HRMS (ESI⁺): m/z [M+H]+ calcd for C₆H₉O₃: 129.0546; found: 129.0544.

(5S,6R)-trans-Osmundalactone (13)

In analogy to the Walterolactone B synthesis, (R)-furylethanol (11, 11 mg, 100 µmol, 99% ee) was treated in the two-step process using chloroperoxidase/glucose oxidase followed by Codexis P1-C01. Purification by column chromatography (SiO₂, hexane:ethyl acetate = 2:1) yielded (5S,6R)-trans-Osmundalactone 13 (7.0 mg, 55 µmol, 55%, 99% ee, 98% trans) as colorless oil.

[α]²⁰D: −6.9 (c 0.28, CHCl₃). Rf 0.26 (hexane/ethyl acetate 1/1). ¹H-NMR (400 MHz, CDCl₃): δ
[ppm] = 6.86 (dd, J = 10.2 Hz, J = 2.4 Hz, 1H), 6.01 (dd, J = 9.9 Hz, J = 1.9 Hz, 1H), 4.36-4.43 (m, 1H), 4.25-4.30 (m, 1H), 2.42(d, J = 7.0 Hz, 1H), 1.51(d, J = 6.3 Hz, 3H). \(^{13}C\)-NMR (100 MHz, CDCl\(_3\)): \(\delta\) [ppm] = 163.2, 148.6, 120.7, 79.0, 67.7, 18.2. FT-IR (ATR): \(\nu\) [cm\(^{-1}\)] = 3391 (s), 2990 (w), 2939 (w), 1687 (s), 1629 (w), 1256 (s), 1112 (m), 1054 (s), 827 (s). HRMS (ESI+): \(m/z\) [M+H]\(^+\) calcd for C\(_6\)H\(_9\)O\(_3\): 129.0546; found: 129.0546.

(5S,6S)-cis-Osmundalactone (14)

In analogy to the Walterolactone B synthesis, \((S)\)-furylethanol ((5)-11, 11 mg, 100 \(\mu\)mol, 99% ee) was treated in the two-step process using chloroperoxidase/glucose oxidase followed by Codexis P1-C01. Purification by column chromatography (SiO\(_2\), hexane:ethyl acetate = 2:1) yielded (5S,6S)-cis-Osmundalactone 13 (7.9 mg, 62 \(\mu\)mol, 62%, 99% ee, 98% cis) as a pale yellow oil.

\([\alpha]\)\(^{20}\)\(_D\): +131.7 (c 0.31, CHCl\(_3\)). R\(_f\) 0.22 (hexane/ethyl acetate 1/1). \(^1\)H-NMR (400 MHz, CDCl\(_3\)): \(\delta\) [ppm] = 7.03 (dd, J = 9.7 Hz, J = 5.7 Hz, 1H), 6.01 (d, J = 9.8 Hz, 1H), 4.53-4.55 (m, 1H), 4.03 (s, 1H), 2.96(d, J = 7.4 Hz, 1H), 1.51(d, J = 6.7 Hz, 3H). \(^{13}C\)-NMR (100 MHz, CDCl\(_3\)): \(\delta\) [ppm] = 164.3, 144.8, 122.5, 77.4, 62.9, 15.7. FT-IR (ATR): \(\nu\) [cm\(^{-1}\)] = 3391 (s), 2923 (w), 2855 (w), 1695 (s), 1627 (w), 1237 (m), 1053 (s), 1022 (s), 810 (s). HRMS (ESI+): \(m/z\) [M+H]\(^+\) calcd for C\(_6\)H\(_9\)O\(_3\): 129.0546; found: 129.0546.

**In vivo Isomerization**

Recombinant E. coli BL21(DE3) harboring a plasmid pET-15b-LkADH (encoding Lactobacillus kefir alcohol dehydrogenase)\(^1\) was cultivated overnight at 37 °C in Luria-Bertani (LB) medium containing kanamycin (50 \(\mu\)g/ml). Then 1 ml culture was inoculated into 100 ml of LB medium containing kanamycin (50 \(\mu\)g/ml), and incubated at 37 °C for 6 h. The expression was induced by the addition of 0.1 mM isopropyl-\(\beta\)-D-thiogalactopyranoside (IPTG), and the incubation was continued for 18 h at 30 °C. The cells were harvested by centrifugation, and washed twice with potassium phosphate buffer (0.1 M, pH 7.0).

The reaction was performed in potassium phosphate buffer (10 ml, 0.1 M, pH 7.0) containing recombinant E. coli cells (1g, fresh weight), and the substrate 1f (10 mg) at 30 °C, 200 rpm. After 4 hours, the reaction was extracted with ethyl acetate (3 \(\times\) 15ml). The combined organic layer was dried over anhydrous Na\(_2\)SO\(_4\) and concentrated under reduced pressure. The samples were analyzed with chiral GC, chiral HPLC and quantitative NMR.
Synthesis and Analytics of the Starting Materials

**rac-2-Hydroxy-1-oxaspiro[5.5]undec-3-en-5-one (1a)**

![rac-1a](image)

**Representative general procedure:** To a solution of furan (680 mg, 10.0 mmol) in anhydrous THF (20 mL) was added n-BuLi (2.5M, 1.5 equiv, 15.0 mmol) at −78 °C under argon, and the mixture was stirred for 4 h, allowing to warm to 0 °C. Cyclohexanone (1.5 equiv, 15.0 mmol) was added at −78 °C, and the reaction mixture was stirred for 10 h at 0 °C. The reaction was quenched with saturated aqueous NH₄Cl solution (10 mL), extracted with diethyl ether (2 x 20 mL), dried over Na₂SO₄, and concentrated under reduced pressure. The residue was purified by column chromatography (SiO₂, pentane: diethyl ether = 5:1). To a solution of the thus obtained 1-(2-furyl)cyclohexanol (830 mg, 5.0 mmol) in THF:H₂O (3:1, 12 mL), NaHCO₃ (2 equiv, 20.0 mmol), NaOAc·3H₂O (1 equiv, 5.0 mmol), and NBS (1 equiv, 5.0 mmol) were added at 0 °C. The reaction mixture was stirred for 1 h at 0 °C, then quenched with saturated aqueous NaHCO₃ (10 mL). The reaction mixture was extracted with ethyl acetate (3 x 15mL), dried over Na₂SO₄, and concentrated under reduced pressure. The residue was purified by flash column chromatography (SiO₂, hexane:ethyl acetate, 3:1) to yield the **rac-1a** (683 mg, 3.75 mmol, 75%) as colorless oil.

**¹H-NMR** (400 MHz, CDCl₃): δ [ppm] = 6.84 (dd, J = 10.2 Hz, J = 2.1 Hz, 1H), 6.02 (dd, J = 10.4 Hz, J = 1.3 Hz, 1H), 5.69 (dt, J = 7.2 Hz, 1H), 4.45 (d, J = 7.1 Hz, 1H), 1.86-1.90 (m, 1H), 1.49-1.81 (m, 8H), 1.21-1.25 (m, 1H).

**¹³C-NMR** (100 MHz, CDCl₃): δ [ppm] = 199.71, 146.0, 126.6, 87.4, 80.6, 33.4, 30.9, 26.9, 25.1, 21.0, 20.5.

**rac-7-Hydroxy-6-oxaspiro[4.5]dec-8-en-10-one (1b)**

![rac-1a](image)

According to the general procedure, **rac-1b** (581 mg, 3.46 mmol, 69%) was obtained from furan and cyclopentanone as colorless oil.

**¹H-NMR** (400 MHz, CDCl₃): δ [ppm] = 6.85 (dd, J = 10.3 Hz, J = 2.3 Hz, 1H), 6.08 (d, J = 9.8 Hz, 1H), 5.63 (d, J = 6.8 Hz, 1H), 4.68 (bs, 1H), 1.66-2.09 (m, 8H). **¹³C-NMR** (100 MHz, CDCl₃): δ [ppm] = 199.3, 146.3, 127.4, 89.5, 88.1, 38.7, 35.6, 24.6.
**rac-6-Hydroxy-5-oxaspiro[3.5]non-7-en-9-one (1c)**

![rac-1c](image)

According to the general procedure, rac-1c (494 mg, 3.21 mmol, 64%) was obtained from furan and cyclobutanone as colorless oil.

$^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ [ppm] = 6.86 (dd, $J = 10.1$ Hz, $J = 2.2$ Hz, 1H), 6.08 (d, $J = 10.2$ Hz, 1H), 5.63 (dq, $J = 6.7$ Hz, 1H), 4.69 (d, $J = 6.7$ Hz, 1H), 2.17-2.54 (m, 4H), 1.78-1.90 (m, 2H).

$^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta$ [ppm] = 197.6, 146.2, 126.6, 88.1, 80.2, 34.0, 31.0, 13.6.

**rac-5-Hydroxy-4-oxaspiro[2.5]oct-6-en-8-one (1d)**

![rac-1d](image)

According to the general procedure, rac-1d (143 mg, 1.02 mol, 50%) was obtained from furan and cyclopropanone as yellow oil.

$^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ [ppm] = 6.98 (dd, $J = 10.4$ Hz, $J = 2.5$ Hz, 1H), 6.23 (d, $J = 10.1$ Hz, 1H), 5.67 (s, 1H), 3.74 (bs, 1H), 1.42-1.48 (m, 1H), 1.33-1.38 (m, 1H), 1.17-1.28 (m, 2H).

$^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta$ [ppm] = 196.4, 146.0, 128.1, 89.5, 61.6, 19.7, 15.5.

**rac-6-Hydroxy-2,5-dioxaspiro[3.5]non-7-en-9-one (1c)**

![rac-1e](image)

According to the general procedure, rac-1e (432 mg, 2.77 mmol, 55%) was obtained from furan and oxetan-3-one as colorless solid.

$^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ [ppm] = 6.99 (dd, $J = 10.1$ Hz, $J = 3.3$ Hz, 1H), 6.20 (d, $J = 10.5$ Hz, 1H), 5.72 (m, $J = 3.4$ Hz, 1H), 5.06 (d, $J = 6.7$ Hz, 1H), 4.97 (d, $J = 6.8$ Hz, 2H), 4.83 (d, $J = 6.9$ Hz, 1H), 4.68 (d, $J = 6.7$ Hz, 1H).

$^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta$ [ppm] = 193.2, 145.9, 126.6, 88.0, 82.0, 78.7, 78.5.
**rac-2,2-Diethyl-6-hydroxy-2H-pyran-3(6H)-one (1g)**

![rac-1g]

According to the general procedure, rac-1g (550 mg, 3.24 mmol, 65%) was obtained from furan and 3-pentanone as colorless oil.

\[ ^1H\text{-NMR (400 MHz, CDCl}_3\text{)}: \delta [\text{ppm}] = 6.84 (dd, J = 10.3 \text{ Hz}, J = 2.2 \text{ Hz}, 1H), 6.04 (dd, J = 10.2 \text{ Hz}, J = 1.3 \text{ Hz}, 1H), 5.72 (dq, J = 7.0 \text{ Hz}, 1H), 4.41 (d, J = 6.5 \text{ Hz}, 1H), 1.55-1.94 (m, 4H), 0.83-0.88 (m, 6H). \]

\[ ^13C\text{-NMR (100 MHz, CDCl}_3\text{)}: \delta [\text{ppm}] = 199.6, 146.0, 127.2, 87.6, 85.0, 29.7, 27.7, 7.7, 7.6. \]

**rac-6-hydroxy-2H-pyran-3(6H)-one (1h)**

![rac-1h]

According to the general procedure, rac-1h (441 mg, 3.87 mmol, 77%) was obtained from commercial (2-furyl)methanol as colorless oil.

\[ ^1H\text{-NMR (400 MHz, CDCl}_3\text{)}: \delta [\text{ppm}] = 6.98 (dd, J = 10.3 \text{ Hz}, J = 3.1 \text{ Hz}, 1H), 6.18 (dd, J = 10.7 \text{ Hz}, J = 4.8 \text{ Hz}, 1H), 5.63-5.67 (m, 1H), 4.59 (dd, J = 16.2 \text{ Hz}, J = 7.6 \text{ Hz}, 1H), 4.15 (dd, J = 17.0 \text{ Hz}, J = 5.2 \text{ Hz}, 1H), 3.75 (br, 1H). \]

\[ ^13C\text{-NMR (100 MHz, CDCl}_3\text{)}: \delta [\text{ppm}] = 194.9, 146.1, 127.9, 88.2, 66.6. \]

**rac-2,2-Dimethyl-6-hydroxy-2H-pyran-3(6H)-one (1f)**

![rac-1f]

To a solution of acetylfuran (1.10 g, 10.0 mmol) in anhydrous THF (20 mL) methylmagnesium bromide (2.0 equiv, 20.0 mmol) was added at 0°C under argon, and the mixture was allowed to warm to room temperature overnight. Saturated aqueous NH\textsubscript{4}Cl solution (10 mL) was added, the mixture was extracted with diethyl ether (2 x 20 mL), the combined organic layers dried over Na\textsubscript{2}SO\textsubscript{4}, and concentrated under reduced pressure. To a solution of the crude furfuryl alcohol 3 (630 mg, 5.0 mmol) in THF:H\textsubscript{2}O (3:1, 12 mL), NaHCO\textsubscript{3} (2 equiv, 20.0 mmol), NaOAc:3H\textsubscript{2}O (1 equiv, 5.0 mmol), and NBS (1 equiv, 5.0 mmol) were added at 0 °C. The reaction mixture was stirred for 1 h at 0 °C, then quenched with saturated aqueous NaHCO\textsubscript{3} (10 mL). The reaction mixture was extracted with ethyl acetate (3 x 15mL), dried over Na\textsubscript{2}SO\textsubscript{4},
and concentrated under reduced pressure. The residue was purified by flash column chromatography (SiO₂, hexane:ethyl acetate, 3:1) to yield the rac-1a (590 mg, 4.15 mmol, 83%) as colorless oil.

\[ ^1H\text{-NMR} \ (400 \text{ MHz, CDCl}_3): \delta \ [\text{ppm}] = 6.89 \ (\text{dd}, \ J = 10.5 \text{ Hz, } J = 2.4 \text{ Hz, } 1H), \ 6.07 \ (d, \ J = 10.5 \text{ Hz, } 1H), \ 5.69 \ (t, 1H), \ 4.31\ (br, 1H), \ 1.48\ (s, 1H), \ 1.38\ (s, 1H). \]

\[ ^{13}C\text{-NMR} \ (100 \text{ MHz, CDCl}_3): \delta \ [\text{ppm}] = 199.1, \ 146.1, \ 126.4, \ 87.8, \ 79.4, \ 26.5, \ 23.7. \]

**rac-1-Hydroxyisochroman-4-one (5)**

![img]

Phthal dialdehyde (268 mg, 2.0 mmol), paraformaldehyde (180 mg, 6.0 mmol) and 3-(2,6-diisopropylphenyl)-5,6,7,8-tetrahydro-4H-cycloheptathiazol-3-ium perchlorate (83 mg, 0.2 mmol) were suspended under argon in anhydrous THF (8 mL). N,N-Diisopropylethylamine (66 µL, 0.4 mmol) was added and the reaction mixture was heated to reflux overnight. The volatiles were removed under reduced pressure and purification of the residue by flash column chromatography (SiO₂, hexane:ethyl acetate, 3:1) yielded rac-5 (167 mg, 1.02 mmol, 51%) as yellow solid.

\[ ^1H\text{-NMR} \ (400 \text{ MHz, MeOH-d}_4): \delta \ [\text{ppm}] = 7.91 \ (\text{d}, \ J = 7.6 \text{ Hz, } 1H), \ 7.66 \ (\text{td}, \ J = 4 \text{ Hz, } J = 4 \text{ Hz, } 1H), \ 7.44-7.50 \ (\text{m, } 1H), \ 6.03 \ (s, 1H), \ 4.82 \ (s, 1H), \ 4.79 \ (d, \ J = 17.5 \text{ Hz, } 1H), \ 4.24 \ (d, \ J = 17.5 \text{ Hz, } 1H). \]

\[ ^{13}C\text{-NMR} \ (100 \text{ MHz, MeOH-d}_4): \delta \ [\text{ppm}] = 197.1, \ 144.9, \ 136.8, \ 131.1, \ 130.9, \ 128.2, \ 127.58, \ 94.1, \ 68.6. \]

**3-Methylfurfuryl alcohol (10)**

![img]

To a solution of methyl 3-methylfuran-2-carboxylate (1.40 g, 10.0 mmol) in anhydrous THF (20 mL) was added Red-Al (1.5 equiv, 15.0 mmol) at 0 °C under argon, and the reaction mixture was stirred for 2 h. The reaction was then quenched with saturated aqueous NH₄Cl solution, extracted with diethyl ether (2 x 20 mL), dried over Na₂SO₄, and concentrated under reduced pressure. The residue was purified by column chromatography (SiO₂, pentane:ethyl ether, 3:1) to yield 10 (0.91 g, 81%) as colorless liquid.
$^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ [ppm] = 6.68-6.70 (m, 1H), 5.69-5.61 (m, 1H), 4.55 (d, $J = 15.8$ Hz, 1H), 4.11 (d, $J = 15.8$ Hz, 1H), 4.11 (d, $J = 5.7$ Hz, 1H), 1.83-1.84 (t, 3H). $^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta$ [ppm] = 195.6, 141.2, 135.1, 89.1, 66.5, 14.3.

(R)-1-(Furan-2-yl)ethanol ((R)-11)

Alcohol dehydrogenase evo 1.1.200 (5.7 mg, 100 U) and 2-propanol (6 mL) were added to a solution of acetylfuran (110 mg, 1.0 mmol) and NADH (72 mg, 0.1 mmol) in phosphate buffer (54 mL, 50 mM, pH 7.0) and the reaction mixture was incubated at 25 °C (250 rpm) over night. After full conversion was reached, the reaction mixture was saturated with NaCl and extracted with ethyl acetate (3 x 10 mL). The combined organic layers were dried over MgSO$_4$, concentrated in vacuo and (R)-11 (67 mg, 0.60 mmol, 60%, 99% ee) was obtained after column chromatography (SiO$_2$, cyclohexane:ethyl acetate 4:1) as pale yellow oil.

[a]$^{20}$: +21.0 (c 1.0, CHCl$_3$). $R_f$ (cyclohexane:ethyl acetate, 2:1): 0.60. $^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ [ppm] = 7.39 (d, $J = 1.3$ Hz, 1H), 6.23 (dd, $J = 3.0$ Hz, $J = 1.7$ Hz, 1H), 6.32 (d, $J = 3.0$ Hz,1H), 4.92-4.84 (m, 1H), 1.99 (d, $J = 3.7$ Hz, 1H), 1.54 (d, $J = 6.5$ Hz, 3H). $^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta$ [ppm] = 157.7, 141.9, 110.1, 105.9, 63.6, 21.3. FT-IR (ATR): $\nu$ [cm$^{-1}$] = 3344 (br), 2980 (w), 2875 (w), 1450 (w), 1371 (w), 1328 (w), 1228 (w), 1149 (m), 1066 (m), 1008 (m), 991 (m), 927 (m), 877 (m), 810 (m), 734 (s). GC: $t_R$ ((S)-11) = 13.3 min, $t_R$ ((R)-11) = 13.9 min.

(S)-1-(Furan-2-yl)ethanol ((S)-11)

In analogy to the synthesis of (R)-11, using evo 1.1.030 instead of evo 1.1.200, alcohol (S)-11 (28 mg, 0.25 mmol, 25%, 99% ee) was obtained as pale yellow oil.

[a]$^{20}$: –21.1 (c 1.0, CHCl$_3$). $R_f$ (cyclohexane:ethyl acetate, 2/1): 0.60. $^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ [ppm] = 7.39 (d, $J = 1.3$ Hz, 1H), 6.23 (dd, $J = 3.0$ Hz, $J = 1.7$ Hz, 1H), 6.32 (d, $J = 3.0$ Hz, 1H), 4.92-4.84 (m, 1H), 1.99 (d, $J = 3.7$ Hz, 1H), 1.54 (d, $J = 6.5$ Hz, 3H). $^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta$ [ppm] = 157.7, 141.9, 110.1, 105.9, 63.6, 21.3. FT-IR (ATR): $\nu$ [cm$^{-1}$] = 3344 (br), 2980 (w), 2875 (w), 1450 (w), 1371 (w), 1328 (w), 1228 (w), 1149 (m), 1066 (m), 1008 (m), 991 (m), 927 (m), 877 (m), 810 (m), 734 (s). GC: $t_R$ ((S)-11) = 13.3 min, $t_R$ ((R)-11) = 13.9 min.
**Circular Dichroism Spectroscopy**

The absolute configurations of the lactones 2h, 2f, 2a, and 6 (Suppl. Fig. 1) were determined by means of electronic and vibrational CD spectroscopy. Experimental IR and VCD spectra were obtained for solutions in CDCl₃ at 100 µm path length. The spectra were recorded on a Bruker Vertex 70 equipped with a PMA 50 module for VCD measurements accumulating about 35000 scans for the VCD spectrum (approx 8 hrs accumulation time). The baseline was corrected by subtraction of the solvent spectrum. The ECD spectra were recorded on a Chirascan CD spectrometer by Applied Photophysics in ACN.

Supplementary Figure 1. Structures and enantiomeric excess of the characterized lactones.

In order to analyse the experimental spectra, a conformational analysis was carried out at molecular force filed level (MMFF¹ using Spartan 14²) for the (S)-enantiomers all four lactones, and all conformers found in this analysis were subjected to further geometry optimization at DFT level using Gaussian 09³. At the B3LYP/6-311+G(2d,p)/IEFPCM level of theory, all conformers were confirmed to be local energy minima by inspections of the calculated vibrational frequencies. Spectra were simulated as Boltzmann-weighted average spectra using the relative Gibbs free energies. The vibrational frequencies were scaled by a factor of 0.985, while the wavelength axis of the predicted ECD spectra were linearly shifted by 15 nm to lower wavelength.

Supplementary Figure 2. Lowest energy conformers of the lactones 2h, 2f, 2a, and 6.

Supplementary Figures 3-6 provide a comparison of the experimentally obtained IR/VCD and ECD spectra with the spectra predicted for the (S)-enantiomers of the lactones. By themselves, the two techniques already suggest the assignment of an (S)-configuration to all experimental samples, although the agreement may not in all cases be considered perfect. Nevertheless, the combination of both techniques allows a clear assignment of the (S)-configuration to all four lactones.
Supplementary Figure 3. Experimental and calculated IR/VCD and ECD spectra of lactone 2h. IR/VCD spectra were recorded at a concentration of 0.6 M at 100 µm pathlength in CDCl$_3$; ECD spectra were recorded at 2.1 x 10$^{-4}$ M at 1 cm pathlength in ACN. Calculated spectra were obtained at the B3LYP/6-311+g(2d,p)//IEFPCM level of theory. Numbers in IR/VCD indicate some important band assignments.

Supplementary Figure 4. Experimental and calculated IR/VCD and ECD spectra of lactone 2f. IR/VCD spectra were recorded at a concentration of 0.65 M at 100 µm pathlength in CDCl$_3$; ECD spectra were recorded at 1.9 x 10$^{-4}$ M at 1 cm pathlength in ACN. Calculated spectra were obtained at the B3LYP/6-311+g(2d,p)//IEFPCM level of theory. Numbers in IR/VCD indicate some important band assignments.
Supplementary Figure 5. Experimental and calculated IR/VCD and ECD spectra of lactone 2a. IR/VCD spectra were recorded at a concentration of 0.3 M at 100 µm pathlength in CDCl₃; ECD spectra were recorded at 1.7 x 10⁻⁴ M at 1 cm pathlength in ACN. Calculated spectra were obtained at the B3LYP/6-311+g(2d,p)/IEFPCM level of theory. Numbers in IR/VCD indicate some important band assignments; carbonyl region in VCD left out due to total absorbance artefacts of the band.

Supplementary Figure 6. Experimental and calculated IR/VCD and ECD spectra of lactone 6. IR/VCD spectra were recorded at a concentration of 0.34 M at 100 µm pathlength in CDCl₃; ECD spectra were recorded at 2.2 x 10⁻⁴ M at 1 cm pathlength in ACN. Calculated spectra were obtained at the B3LYP/6-311+g(2d,p)/IEFPCM level of theory. Numbers in IR/VCD indicate some important band assignments.
Mechanistic Considerations

Potential involvement of glucose-active protein impurities

With the use of commercial enzyme preparations, reactivity by protein contaminations cannot be excluded entirely. Considering the structural similarity between the pyranone substrates and naturally occurring sugars, both glucose dehydrogenase and glucose oxidase were tested for their activity on model substrate rac-1f.

Glucose dehydrogenase: NADH generation was recorded in a spectrophotometrical assay (λ = 340 nm) by incubation of the enzyme with NAD⁺ (5 mM) and D-Glc (10 mM) or rac-1f (10 mM) at 37 °C in KPi buffer (pH 7.0). While in presence of the native substrate, rapid accumulation of the reduced cofactor was observed (yellow line), no activity could be detected during the incubation with 1f (Supplementary Fig. 7a). Hence, oxidation of 1f by GDH impurities in the enzymes used in this study can be ruled out.

Glucose oxidase: The native activity of glucose oxidase was visualized spectrophotometrically (λ = 420 nm) by incubation of the enzyme with ABTS (5 mM), horseradish peroxidase (10 U) and D-Glc (10 mM) at 37 °C in KPi buffer (pH 7.0) resulting in rapid consumption of the native substrate (green line, Supplementary Fig. 7b). In comparison, the stability of 1f (10 mM) in presence of glucose oxidase was recorded under identical conditions following the absorbance of the enone substrate (λ_{max} = 270 nm, orange line). Due to the absence of any decline in absorbance at λ_{max} under assay conditions, oxidation of 1f by potential GOx impurities in the enzymes used in this study can be ruled out.

a  Glucose dehydrogenase (Pseudomonas sp.)  b  Glucose oxidase (Aspergillus niger)

Supplementary Figure 7. a Time-resolved spectrophotometric detection of NADH (λ = 340 nm) formed by reduction of NAD⁺ (5 mM) through glucose dehydrogenase with D-glucose (yellow) and rac-1f (orange). b Dehydrogenation of D-glucose vs rac-1f by glucose oxidase (D-Glc: ABTS/HRP at 420 nm (green); 1f: no reporter, absorption of enone at λ_{max} (270 nm) due to interference with ABTS).
Potential involvement of other dehydrogenase impurities

In addition to sugar-active proteins catalyzing the lactol dehydrogenation, crude enzyme preparations might also provide other alcohol dehydrogenases as impurities that could be responsible for the initial ketolactone formation. In order to address this question, alcohol dehydrogenase from *L. kefir* was produced from recombinant *E. coli* and purified in analogy to the literature procedure by Weckbecker and Hummel.³

Recombinant *E. coli* BL21(DE3) harboring a plasmid pET-15b-LkADH (encoding *Lactobacillus kefir* alcohol dehydrogenase) was cultivated overnight at 37 °C in Luria-Bertani (LB) medium containing kanamycin (50 μg/ml). Then 1 ml culture was inoculated into 100 ml of LB medium containing kanamycin (50 μg/ml), and incubated at 37 °C for 6 h. The expression was induced by the addition of 0.1 mM isopropyl-β-D-thiogalactopyranoside (IPTG), and the incubation was continued for 18 h at 30 °C. The cells were harvested by centrifugation, resuspended in Tris HCl buffer (100 mM, pH 7.2) and disrupted by sonication on ice for 10 min (Q500 ultrasonic oscillator, Qsonica). After removal of the cell debris (centrifugation at 12000 rpm, 20 min), the crude extract was subjected to enzyme purification over a Q-Sepharose FF column (1 x 15 cm). The column was equilibrated with triethanolamine buffer (50 mM, pH 7.0) containing MgCl₂ (1 mM) prior to elution of the enzyme with triethanolamine buffer (50 mM, pH 7.0) containing NaCl (1M) and MgCl₂ (1 mM). The active fractions were dialyzed against Tris HCl buffer (100 mM, pH 7.2) and stored at 4 °C.

As illustrated by SDS-page traces, the separation over Q-Sepharose FF resulted in fractions of high concentrations of the desired oxidoreductase (Supplementary Fig. 8), exhibiting significantly higher purities than some of the commercial dehydrogenases used in this work.

![Supplementary Figure 8.](image)

Supplementary Figure 8. Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis of crude alcohol dehydrogenase from *Lactobacillus kefir* (*E. coli* lysate) and fractions of (partially) purified LkADH after separation over Q-Sepharose FF, as well as selected commercial alcohol dehydrogenase preparations used during the studies.
The most pure fractions of the recombinant *L. kefir* dehydrogenase were subsequently utilized as biocatalyst in the redoxisomerization of 1f according to the general procedure (page 3). Gas chromatographic analysis of the crude reaction mixture indicated full conversion of the starting material rac-1f ($t_R = 45.4$ & $45.7$ min) after incubation with LkADH and NADP$^+$ and the selective formation of one major reaction product ($t_R = 50.4$ min). Comparison with the previously optimized system based on the commercial evo 1.1.200 unambiguously confirmed the formation of (S)-2f as redoxisomerization product by the purified dehydrogenase (Supplementary Fig. 9), thus strongly endorsing the hypothesis that single alcohol dehydrogenases can indeed act as a bifunctional catalyst to convert Achmatowicz-type pyranones to delta-lactones in a redox self-sufficient manner.

a 1f + NADP$^+$ + LkADH

b 1f + NAD$^+$ + evo 1.1.200

Supplementary Figure 9. Gas chromatographic traces of crude reaction mixtures after incubation with a purified alcohol dehydrogenase from *L. kefir* (7 h at 30 °C) and b evo 1.1.200 (20 h at 30 °C).
Isotopic studies

Experiments utilizing isotopologues of pyranone 1f (Supplementary Fig. 10) in combination with the alcohol dehydrogenase *evo 1.1.200* were used to shed light on the correlation between substrate, cofactor and potential organic mediators or reductants, as well as the role of diffusible intermediates vs. a fully protein-bound isomerization pathway.

Supplementary Figure 10. Isotopologues of 1f and 2f investigated in the studies.

Incubation of deuterated 1f-d₁, carrying the label at the oxidizable hemiacetal position, resulted in a productive dynamic redoxisomerization, yet taking place with a substantially reduced rate exhibiting the expected primary kinetic isotope effect ($k_H/k_D = 3.01$; Supplementary Fig. 11).

Supplementary Figure 11. Michaelis-Menten analysis for the enzymatic conversion of 1f and 1f-d₁ by *evo 1.1.200*. 

\[ \text{Supplementary Figure 11. Michaelis-Menten analysis for the enzymatic conversion of 1f and 1f-d₁ by *evo 1.1.200*.} \]
HRMS analysis of the products obtained through redoxisomerization of labelled and unlabelled 1f revealed an effective transfer of the deuterium label from the hemiacetal position to the newly generated secondary alcohol in 2f. Hence, any involvement of organic mediators, or external reductants and/or hydrogen acceptors, can be ruled out (Supplementary Fig. 12).

**Supplementary Figure 12.** Snapshots of HR mass spectra of the region of the sodiated molecular ion [M+Na]+ of 2f and 2f-d1 obtained via redoxisomerization from 1f (left) and 1f-d1 (right), respectively.

Coincubation of monodeuterated 1f-d1 with the hexadeuterated 1f-d6 with *evo 1.1.200* resulted in the formation of a mixture of isotopologues of 2f with a varying degree of deuteration (Supplementary Fig. 13). The extensive scrambling in this crossover experiment indicates that lactol dehydrogenation and ketone reduction are not necessarily linked in a concerted fashion, but that after formation of the ketolactone and NAD(P)H, dissociation and reassociation of either of the species is feasible.

**Supplementary Figure 13.** Snapshot of high resolution mass spectrum of the region of the protonated molecular ion [M+H]+ of 2f isotopologues obtained through coincubation of 1f-d1 and 1f-d6.
Proposed pathway

From the reported experiments, two distinct pathways appear to be potentially operational in the ADH-mediated redoxisomerization, initiated either by an oxidative lactol dehydrogenation or a reductive conversion of the enone ketone to a secondary alcohol (Supplementary Fig. 14). While both pathways offer a productive scenario to yield gamma-hydroxy-delta-lactones, we experience a significantly reduced turnover and the formation of various side products when performing the transformation of rac-1f under more reductive conditions, e.g. by supplementing the reaction mixture (1f, NAD+, TRIS buffer) with either isopropanol or glucose/glucose dehydrogenase to increase the effective concentrations of NADH (Supplementary Fig. 14). Thus it seems more likely that the reaction commences by a dehydrogenative event (Supplementary Fig. 14, left). Here, the configurational lability the lactol’s chirogenic centre would permit the oxidoreductase to conduct the initial step without the thread of enantiodiscrimination and convert the hydroxypyranone to the corresponding ketolactone under consumption of NAD(P)⁺. In case of 1f, the ketolactone intermediate could be synthesized and characterized and clearly be identified during kinetic studies as minor compound in GC traces. As suggested by the labelling studies, both ketolactone and the formed NAD(P)H are not bound to the enzyme throughout the entire process but are liberated to the solution. Re-association to and ketoreduction by the alcohol dehydrogenase furnishes the hydroxylactone and regenerates the NAD(P)⁺ to engage in the subsequent dehydrogenative initiation.

Supplementary Figure 14. Possible pathways for the alcohol dehydrogenase-catalyzed redoxisomerization of Achmatowicz pyranones
NMR Spectra of the Compounds
(S)-2f

OH

(S)-2f

OH
Supplementary References

1. Y.-C. Liu, C. Guo, Y. Liu, H.-B. Wang, Z.-L. Wu, *Org. Biomol. Chem.* **2017**, *15*, 2562-2568.
2. N. Kuhl, F. Glorius, *Chem. Commun.* **2011**, *47*, 573-575.
3. A. Weckbecker, W. Hummel, *Biocatal. Biotrans.* **2006**, *24*, 380-389.