

Supporting information: Enantioselective transfer hydrogenation of ketone catalysed by artificial metalloenzymes derived from bovine β -lactoglobulin

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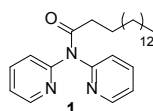
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Synthesis

General methods: ^1H NMR spectra were recorded either on 300 or 400 MHz spectrometers (Bruker) and coupling constants (J) are reported in Hz to ± 0.5 Hz. The following abbreviations were utilized to describe peak patterns when appropriate: br = broad, app = apparent, s = singlet, d = doublet, t = triplet, q = quartet and m = multiplet. Chemical shifts are quoted in parts per million (ppm) and are referenced to residual H signal of deuteriochloroform (7.26 ppm) or methylene chloride (5.32 ppm). ^{13}C NMR chemical shift are expressed in parts per million (ppm), referenced to the central peak of deuteriochloroform (77.00 ppm) or methylene chloride (54.00 ppm). When measured, DEPT signals are referred as (+) or (–) following the corresponding signals. Infrared spectra were recorded on a Tensor 27 FT-IR spectrometer (Bruker). High-resolution mass spectra were recorded by Electrospray (ESI) on a MStation JMS 700 (Jeol) mass spectrometer. UV spectra were recorded on a uv-mc² spectrometer (SAFAS). Analytical TLC was performed on plates coated with silica gel (Merck silica gel, 60 F254), which were visualized with UV fluorescence when applicable ($\lambda = 254$ nm) and by staining with vanillin sulphuric acid solutions followed by heating. Tetrahydrofuran (THF) and diethyl ether (Et_2O) were distilled from sodium–benzophenone. Dichloromethane (DCM) and triethylamine (TEA) were distilled from CaH_2 . All air and/or water sensitive reactions were carried out under a nitrogen atmosphere using a dual manifold high vacuum line with dry, freshly distilled solvents using standard syringe-cannula/septa and purge-and-refill techniques. Commercial 2,2-dipyridylamine was dried 2 times under azeotropic distillation with toluene before use. Bovine milk β -lactoglobulin (mixture of A and B variants, approximately 80% purity grade) was obtained from Sigma (catalog no. L2506) and used as supplied. Variants A and B were obtained from Sigma (catalog no. L7880 and L8005 respectively). Other chemicals were used as received. Dimer $(\text{Cp}^*\text{Rh}(\mu\text{-Cl})\text{Cl})_2$ was prepared as previously described.¹

Synthesis of N,N-di(pyridin-2-yl)palmitamide 1

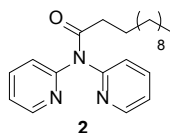


To a solution containing 2,2-dipyridylamine (250 mg, 1.46 mmol, 1.1 equiv.) in DCM (7 mL) was added palmitoyl chloride (403 μL , 1.31 mmol, 1.0 equiv.) under nitrogen at room temperature. To the reaction mixture

was added triethylamine (241 μL , 1.72 mmol, 1.3 equiv.) and the solution was stirred at room temperature overnight. Aqueous saturated NH_4Cl and DCM were added. Aqueous phase was extracted 3 times with DCM. The combined organic phases were washed 3 times with brine, dried over MgSO_4 , filtered and concentrated *in vacuo*. The crude product was purified by flash chromatography on silica gel (AcOEt/PE 50:50 to 70:30) to afford compound **1** (433 mg, 73%) as a white solid.

R_f 0.7 (9:1 AcOEt/PE). $^1\text{H NMR}$ (δ , ppm) CDCl_3 , 300 MHz: 8.45 (app dd, 2H, $J = 1.3, 4.7$ Hz), 7.76 (td, 2H, $J = 1.8, 7.8$ Hz), 7.51 (d, 2H, $J = 8.1$ Hz), 7.18 (dd, 2H, $J = 5.1, 7.1$ Hz), 2.29 (t, 2H, $J = 7.5$ Hz), 1.65 (m, 2H), 1.23 (br s, 23H), 0.87 (t, 3H, $J = 6.6$ Hz). $^{13}\text{C NMR}$ (δ , ppm) CDCl_3 : 75 MHz. 173.9, 154.8, 149.1 (+), 138.1 (+), 122.4 (+), 122.01 (+), 36.4 (-), 32.05 (-), 29.8 (-), 29.6 (-), 29.5 (-), 29.3 (-), 25.2 (-), 22.8 (-), 14.3 (+). **IR** (ν , cm^{-1}): 3052, 3003, 2917, 2849, 1698, 1584, 1568, 1464, 1436, 1408, 1378, 1349, 1315, 1302, 1287, 1266, 1250, 1235, 1179. **MS** (ICP, CINH_3): $m/z = 410.20$ $[\text{M}+\text{H}]^+$, **HRMS** (DI, EI): Calc for $[\text{C}_{26}\text{H}_{40}\text{N}_3\text{O}, \text{M}+\text{H}] = 410.3165$, found 410.3175. **UV** (λ_{max} , nm) EtOH = 234, 268.

Synthesis of N,N-di(pyridin-2-yl)dodecanamide **2**



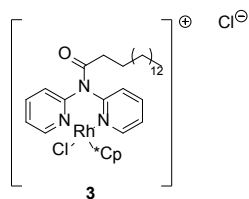
To a solution of lauric acid (447 mg, 2.23 mmol, 1 equiv.) in toluene (7 mL) was slowly added oxalyl chloride (375 μL , 4.46 mmol, 2 equiv.). The resulting reaction mixture was stirred for 2 h at room temperature followed by evaporation to dryness. The residual uncoloured oil was dissolved in DCM (10 mL) under nitrogen and 2,2-dipyridylamine (382 mg, 2.23 mmol, 1 equiv., diluted in DCM (1 mL) and TEA (403 μL , 2.90 mmol, 1.3 equiv.) were added. The reaction mixture was stirred overnight at room temperature. Saturated aqueous NH_4Cl was added. Aqueous phase was extracted 3 times with DCM. The combined organic phases were washed 3 times with brine, NaOH 0.1M and HCl 0.1M, dried over MgSO_4 , filtered and concentrated *in vacuo*. The crude product was purified by flash chromatography on silica gel (AcOEt/PE 50:50 to 90:10) to afford compound **2** (328 mg, 44%) as a light yellow solid.

$^1\text{H NMR}$ (δ , ppm) CDCl_3 , 300 MHz: 8.41 (m, 2H), 7.72 (td, 2H, $J = 2.0, 8.0$ Hz), 7.47 (d, 2H, $J = 8.1$ Hz), 7.14 (ddd, 2H, $J = 0.9, 4.9, 7.4$ Hz), 2.27 (t, 2H, $J = 7.7$ Hz), 1.64 (m, 2H), 1.21 (br s, 12H), 0.83 (t, 3H, $J = 6.7$ Hz). $^{13}\text{C NMR}$ (δ , ppm) CDCl_3 : 75 MHz. 173.8, 154.7, 149.0 (+), 138.0 (+), 122.2 (+), 121.9 (+), 36.2 (-), 31.9 (-), 29.6 (-), 29.5 (-), 29.4 (-), 29.3 (-), 29.2 (-), 25.1 (-), 22.7 (-), 14.1 (+). **IR** (ν , cm^{-1}): 3052, 3003, 2954, 2919, 2850, 1698, 1584, 1567, 1464, 1436, 1378, 1312, 1287, 1269, 1250, 1205, 1180, 1148. **HRMS** (DI, EI): Calc for $[\text{C}_{22}\text{H}_{32}\text{N}_3\text{O}, \text{M}+\text{H}] = 354.2539$, found 354.2540. **UV** (λ_{max} , nm) EtOH = 209, 235, 268.

General procedure for the synthesis of complexes 3-7:

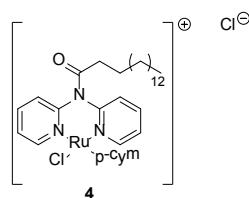
To a solution containing **1** or **2** (2 equiv.) in DCM (5 mL) was added the dimer (1 equiv.) at room temperature. The reaction mixture was stirred under argon for 16 h at room temperature. DCM was evaporated *in vacuo* and Et_2O was added to the resulting red oil. The solid residue was filtrated and washed several times with Et_2O and dried under vacuum.

Complex 3: From **1** (0.24 mmol, 100 mg) and $[\text{Cp}^*\text{Rh}(\mu\text{-Cl})\text{Cl}]_2$ (0.12 mmol, 72 mg). Red-orange solid (135 mg, 83%).



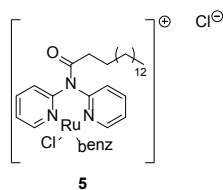
¹H NMR (δ, ppm) CD₂Cl₂, 400 MHz: 8.68 (d, 2H, *J* = 5.7 Hz), 8.21-8.13 (m, 4H), 7.56 (t, 2H, *J* = 5.7 Hz), 1.61 (s, 15H), 1.26 (br s, 28H), 0.87 (t, 3H, *J* = 6.7 Hz) **¹³C NMR** (δ, ppm) CD₂Cl₂, 100 MHz: 173.1, 152.6 (+), 151.3, 142.0 (+), 127.0 (+), 125.7 (+), 97.0, 96.9, 35.6 (-), 31.8 (-), 29.6 (-), 29.4 (-), 29.2 (-), 24.7 (-), 22.6 (-), 14.0 (+), 9.2 (+). **IR** (ν, cm⁻¹): 3063, 2923, 2852, 1700, 1598, 1478, 1464, 1376, 1326, 1250, 1186, 1160, 1027. **HRMS** (DI, EI): Calc for [C₃₆H₅₄ClN₃ORh (M-HCl)⁺] = 682.3005, found 682.3010. **UV** (λ_{max}, nm) EtOH = 233, 267, 360.

Complex 4: From **1** (0.26 mmol, 109 mg) and [(*p*-cym)Ru(μ-Cl)Cl]₂ (0.13 mmol, 82 mg). Red solid (147 mg, 84%).



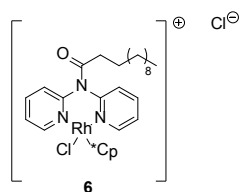
¹H NMR (δ, ppm) CDCl₃, 300 MHz: 8.85 (d, 2H, *J* = 5.4 Hz), 8.06 (d, 4H, *J* = 7.1 Hz), 7.49 (t, 2H, *J* = 5.6 Hz), 5.99 (d, 2H, *J* = 4.9 Hz), 5.64 (d, 2H, *J* = 5.6 Hz), 2.96 (br s, 2H), 2.84 (dt, 1H, *J* = 6.8, 13.5 Hz), 2.26 (br s, 2H), 1.95 (s, 3H), 1.76 (br s, 2H), 1.42 (br s, 2H), 1.25 (d, 6H, *J* = 6.9 Hz), 1.21 (br s, 20H), 0.84 (t, 3H, *J* = 6.6 Hz). **¹³C NMR** (δ, ppm) CDCl₃, 75 MHz: 172.9, 155.4 (-), 151.9, 141.8 (-), 126.2 (-), 125.6 (-), 105.3, 101.3, 86.2 (-), 83.6 (-), 35.3 (+), 31.9 (+), 30.6 (-), 29.7 (+), 29.6 (+), 29.4 (+), 29.3 (+), 25.0 (+), 22.7 (+), 22.6 (-), 18.3 (-), 14.2 (-). **IR** (ν, cm⁻¹): 3065, 3035, 3014, 2924, 2853, 1699, 1601, 1466, 1439, 1376, 1329, 1257, 1188, 1158, 1108. **ESI/MS** *m/z* (%) = 680.9 [M]⁺, **HRMS** (DI, EI): Calc for [C₃₆H₅₃ClN₃ORu] = 680.2915, found 680.2921. **UV** (λ_{max}, nm) EtOH = 211, 271.

Complex 5: From **1** (0.25 mmol, 103 mg) and [(benz)Ru(μ-Cl)Cl]₂ (0.12 mmol, 64 mg). Red solid (149 mg, 96%).



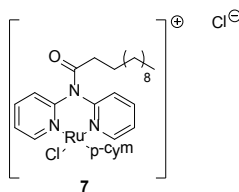
¹H NMR (δ, ppm) CD₂Cl₂, 400 MHz: 9.0 (br s, 2H), 8.08 (br s, 2H), 7.79 (d, 2H, *J* = 8.2 Hz), 7.55 (br s, 2H), 5.87 (s, 6H), 2.84 (br s, 2H), 1.96 (br s, 2H), 1.77 (br s, 2H), 1.35 (br s, 2H), 1.20 (br s, 20H), 0.87 (t, 3H, *J* = Hz). **¹³C NMR** (δ, ppm) CD₂Cl₂, 100 MHz: 173.2, 157.5 (-), 151.4, 141.9 (-), 128.3, 126.3 (-), 125.7 (-), 87.5 (-), 83.0 (-), 31.9 (+), 29.7 (+), 29.5 (+), 29.3 (+), 25.0 (+), 22.7 (+), 14.1 (-). **IR** (ν, cm⁻¹): 3071, 2923, 2852, 1696, 1602, 1467, 1436, 1376, 1329, 1259, 1189, 1156, 1107. **HRMS** (DI, EI): Calc for [C₃₂H₄₅ClN₃ORu] = 624.2289, found 624.2288. **UV** (λ_{max}, nm) EtOH = 219, 285, 338, 431.

Complex 6: From **2** (0.28 mmol, 100 mg) and $[\text{Cp}^*\text{Rh}(\mu\text{-Cl})\text{Cl}]_2$ (0.13 mmol, 83 mg). Red solid (150 mg, 89%).



$^1\text{H NMR}$ (δ , ppm) CD_2Cl_2 , 400 MHz: 8.71 (d, 2H, $J = 5.1$ Hz), 8.18-8.13 (m, 4H), 7.57 (t, 2H, $J = 6.1$ Hz), 1.61 (s, 15H), 1.27 (br s, 20H), 0.88 (t, 3H, $J = 6.7$ Hz). $^{13}\text{C NMR}$ (δ , ppm) CD_2Cl_2 , 100 MHz: 172.5, 153.0 (+), 151.5, 142.3 (+), 127.3 (+), 125.9 (+), 97.3, 97.2, 36.0 (-), 31.9 (-), 29.7 (-), 29.6 (-), 29.4 (-), 29.4 (-), 24.9 (-), 22.8 (-), 14.2 (+), 9.7 (+), 9.5 (+). **IR** (ν , cm^{-1}): 3063, 2924, 2853, 1700, 1598, 1478, 1463, 1376, 1326, 1249, 1187, 1159, 1107, 1063, 1025. **HRMS** (DI, EI): Calc for $[\text{C}_{32}\text{H}_{46}\text{ClN}_3\text{ORh}] = 626.2379$, found 626.2378. **UV** (λ_{max} , nm) EtOH = 235, 266, 360.

Complex 7: From **2** (0.28 mmol, 100 mg) and $[(p\text{-cym})\text{Ru}(\mu\text{-Cl})\text{Cl}]_2$ (0.13 mmol, 82 mg). Red solid (161 mg, 96%).



$^1\text{H NMR}$ (δ , ppm) CD_2Cl_2 , 400 MHz: 8.86 (d, 2H, $J = 5.3$ Hz), 8.11 (2 t, 4H, $J = 7.7$ Hz), 7.52 (t, 2H, $J = 6.2$ Hz), 5.91 (d, 2H, $J = 5.6$ Hz), 5.57 (d, 2H, $J = 5.7$ Hz), 2.89-2.81 (m, 2H), 2.40-2.31 (m, 1H), 1.94 (s, 3H), 1.79 (br s, 2H), 1.43 (br s, 2H), 1.35-1.20 (m, 20H), 0.87 (t, 3H, $J = 6.9$ Hz). $^{13}\text{C NMR}$ (δ , ppm) CD_2Cl_2 , 100 MHz: 172.9, 155.9 (+), 152.6, 142.3 (+), 126.8 (+), 125.9 (+), 105.6, 102.0, 86.9 (+), 84.0 (+), 35.9 (-), 32.5 (-), 31.1 (+), 30.2 (-), 30.1 (-), 29.9 (-), 25.5 (-), 23.2, 22.8 (+), 18.7 (+), 14.4 (+). **IR** (ν , cm^{-1}): 3063, 2924, 2853, 1699, 1601, 1466, 1439, 1376, 1329, 1309, 1257, 1190, 1157. **HRMS** (DI, EI): Calc for $[\text{C}_{32}\text{H}_{45}\text{ClN}_3\text{ORu}] = 624.2289$, found 624.2288. **UV** (λ_{max} , nm) EtOH = 232, 271.

X-ray structure of β LG-palmitic acid and β LG-lauric acid complexes:

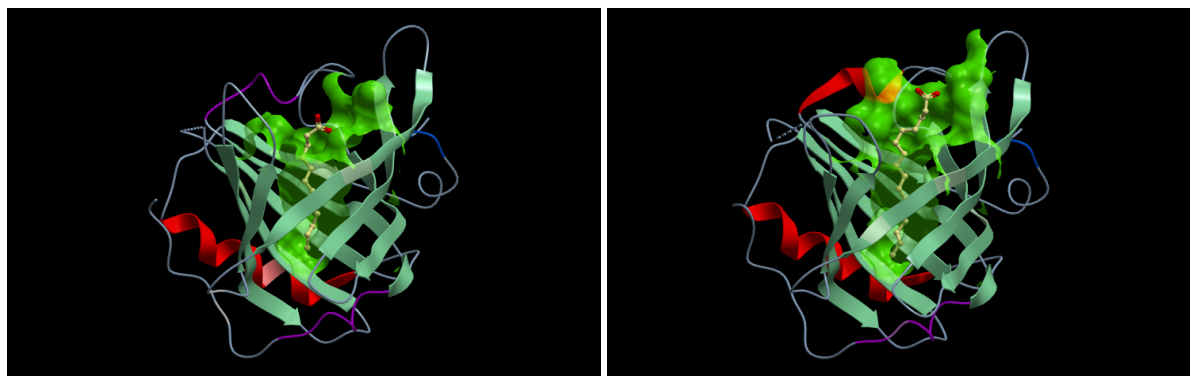


Figure S1 : Crystal structures of β LG in complex with lauric acid (left, PDB file: 3ueu) and palmitic acid (right, PDB file: 3uew). ² Figures generated with Molsoft browser

Catalytic runs with complexes – determination of conversion rates by HPLC:

Analytical reverse phase HPLC was performed on Beckman System Gold instrument using Jupiter Proteo C18 column, 4 μ m, 150 x 2 mm, 90A using an isocratic eluent containing 50% H₂O, 50% ACN for 10 min with a flow rate of 0.2 mL/min and detection set at 254 nm. Volume of injection: 20 μ L of a 1 mM solution in water. Retention time for **trifluoroacetophenone**: 3.88 min. Retention time for (+/-) **α -(trifluoromethyl)benzyl alcohol**: 5.97 min.

General procedure for the catalysis: To a solution of trifluoroacetophenone in water (10 mM, 1 ml) was added sodium formate (68.1 mg, 1 M) at room temperature and the pH was then adjusted with 0.1 M NaOH to reach 7.5. To this solution was added a solution of **3** in DMSO (10 mM, 20 μ L, 0.02 eq.). This resulting reaction mixture was stirred up to 72 h at 40°C. Aliquots (10 μ L) of the reaction mixture were taken periodically and diluted in 90 μ L of water and submitted to HPLC analysis. The pH was measured at the end of the reaction and was about 8.6.

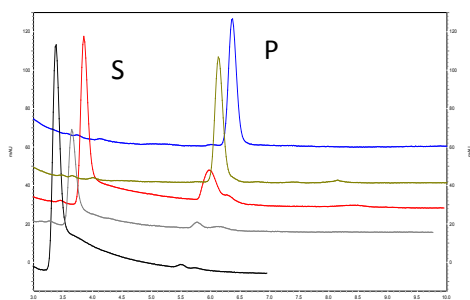


Figure S2 : HPLC traces of reaction mixtures at t = 24h. S = substrate, P = product - Conditions: 2% catalyst, 10 mM TFAP, 40°C, 1 M formate, pH 7.5. **Black**: catalyst **5**, **grey**: catalyst **7**, **red**: catalyst **4**, **green**: catalyst **3**, **blue**: catalyst **6**.

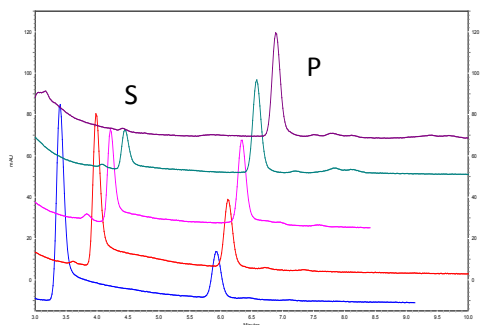


Figure S3 : HPLC traces of reaction mixtures at different times, S = substrate, P = product - Conditions: 2% catalyst **6**, 10 mM TFAP, 40°C, 1 M formate, pH 7.5. **Blue**: after 20 min. **Red**: after 40 min. **Pink**: after 60 min. **Grey**: after 90 min. **Purple**: after 145 min.

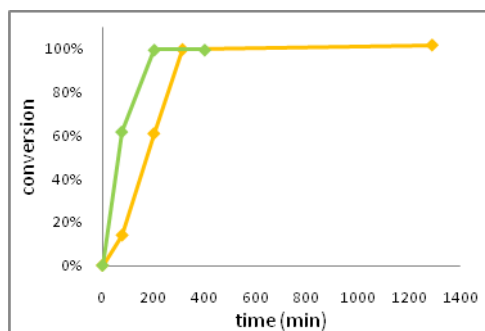


Figure S4 : kinetics of transfer hydrogenation of TFAP (10 mM) using 2% catalyst at 40°C with 1 M formate, pH 7.5. **Green**: catalyst **6**. **Yellow**: catalyst **3**.

Circular dichroism measurements:

General procedure for the near-UV CD measurements: Circular dichroism spectra were recorded on a J-815 UD spectrometer (JASCO) at 20°C in a quartz cuvette of 1 or 0.2 cm path length between 250-310 nm for ligands and ruthenium compounds and between 250-450 nm for rhodium compounds. Temperature control was provided by a Peltier thermostat. All spectra were accumulated 3 times (2 times for Rh compounds) with bandwidth of 1.0 nm and a resolution of 0.1 nm at a scan speed of 50 nm/min.

Preparation of β LG solutions: β LG (mixture of variants A and B) was dissolved in a 20 mM phosphate buffer (pH 7.5) at room temperature. Its concentration was determined using absorbance at 280 nm with an ϵ of 17600 M⁻¹cm⁻¹.³ Protein solutions were kept for up to 1 week at -20°C.

Preparation of ligand or complex solutions: compounds **1**, **2**, **4** and palmitic acid were dissolved in absolute ethanol to reach a 10 mM concentration. Compounds **3**, **5**, **6** and **7** were dissolved in DMSO to reach a 10 mM concentration. All these compounds are stable in solution when kept at 4°C.

CD measurements for association between ligands (or complexes) and β LG: A β LG solution (50 μ M in phosphate buffer, pH 7.5) was transferred in a 1 cm optical pathlength cuvette and small aliquots of ligand solutions (2 μ L) were added sequentially to achieve more than 1 equiv. of ligand. Alternatively, a 100 μ M β LG solution was transferred in a 0.2 cm optical path length cuvette and small aliquots of ligand solutions (2 μ L) were added sequentially to achieve more than 1 equiv. of ligand. In each case, the solutions were homogenized at each addition for 1 min before acquisition.

CD measurements for displacement studies: A β LG solution (100 μ M in phosphate buffer, pH 7.5) was transferred in a 0.2 cm optical pathlength cuvette and 0.8 equiv. of the first compound was added. The solution was homogenized for 1 min before acquisition. To this reaction mixture was then added sequentially small aliquots (2 μ L) of the second compound. The reaction mixture was homogenized for 1 min before each acquisition.

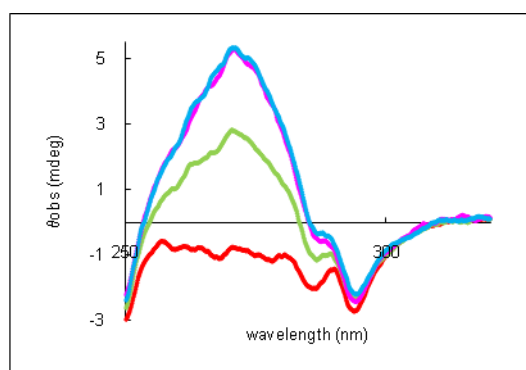


Figure S5 : Near UV-CD spectra of β LG in the absence or presence of **1** ($l = 0.2$ cm). **Red**: 88 μ M β LG. **Green**: addition of 0.4 equiv. of **1** (2.1 mM solution in EtOH). **Pink**: addition of 0.4 more equiv. of **1**. **Blue**: addition of 0.4 more equiv. of **1**.

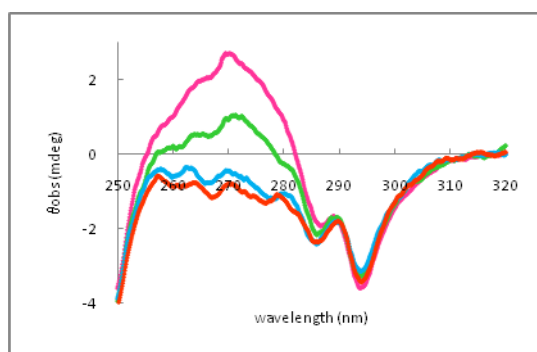


Figure S6 : Near UV-CD spectra of the displacement of **1** by palmitic acid ($l = 0.2$ cm). **Pink**: 126 μ M β LG with 0.8 equiv. of **1**. **Green**: addition of 2 equiv. of palmitic acid (8 μ L of a 15 mM solution in EtOH). **Blue**: addition of 2 more equiv. (8 μ L of a 15 mM solution in EtOH) of palmitic acid. **Red**: 126 μ M β LG.

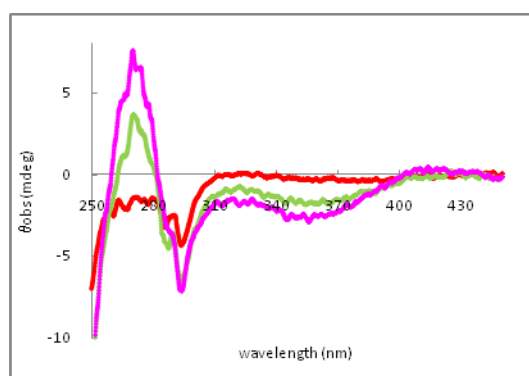


Figure S7 : Near UV-CD spectra of β LG in the presence or absence of **3** ($l = 1$ cm). **Red**: 51 μ M β LG. **Green**: addition of 0.4 equiv. (2 μ L) of **3**. **Pink**: addition of 0.4 more equiv. (2 μ L) of **3**.

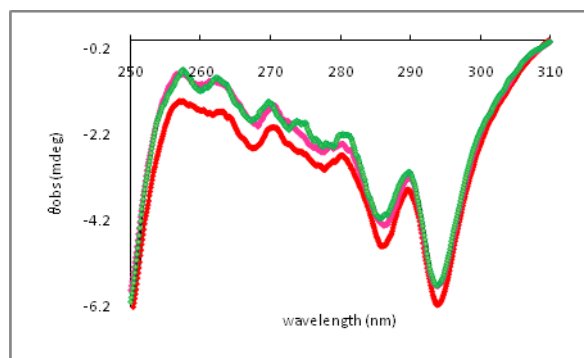


Figure S8 : Near UV-CD spectra of β LG in the presence or absence of **4** ($l = 0.2$ cm). **Red**: 184 μ M β LG. **Green**: addition of 0.6 equiv. (6 μ L of 9 mM solution in EtOH) of **4**. **Pink**: addition of 0.5 more equiv. (5 μ L of 9 mM solution in EtOH) of **4**.

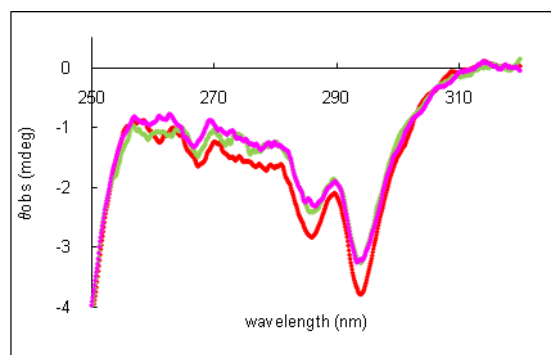


Figure S9 : Near UV-CD spectra of β LG in the presence or absence of **5** ($l = 0.2$ cm). **Red**: 126 μ M β LG. **Green**: addition of 0.5 equiv. (8 μ L of 4 mM solution in EtOH) of **5**. **Pink**: addition of 0.5 more equiv. (8 μ L of 4 mM solution in EtOH) of **5**.

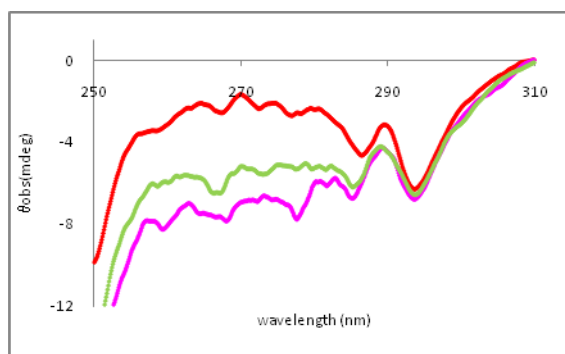


Figure S10 : Near UV-CD spectra of β LG in the presence or absence of **7** ($l = 1$ cm). **Red**: 51 μ M β LG. **Green**: addition of 0.4 equiv. (2 μ L) of **7**. **Pink**: addition of 0.4 more equiv. (2 μ L) of **7**.

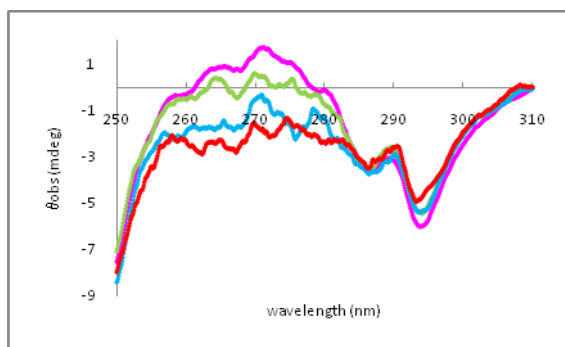


Figure S11 : Near UV-CD spectra of the displacement of **4** by **1** ($l = 0.2$ cm). **Pink**: 184 μ M β LG with 0.8 equiv. of **1** (11.8 mM solution in EtOH). **Green**: addition of 1 equiv. of **4** (12 μ L of 7.7 mM solution in EtOH). **Blue**: addition of 0.5 more equiv. (6 μ L) of **4**. **Red**: 184 μ M β LG.

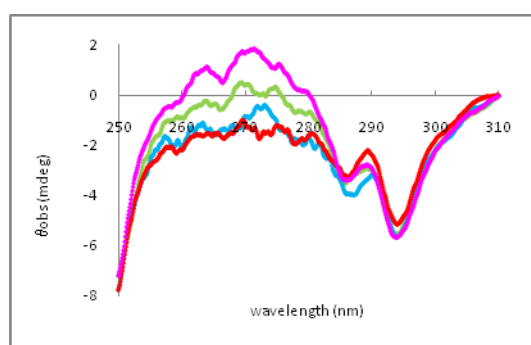


Figure S12 : Near UV-CD spectra of the displacement of **5** by **1** ($l = 0.2$ cm). **Pink**: 184 μ M β LG with 0.8 equiv. of **1** (11.8 mM solution in EtOH). **Green**: addition of 1 equiv. (12 μ L of 7.5 mM solution in EtOH) of **5**. **Blue**: addition of 0.5 more equiv. (6 μ L) of **5**. **Red**: 184 μ M β LG.

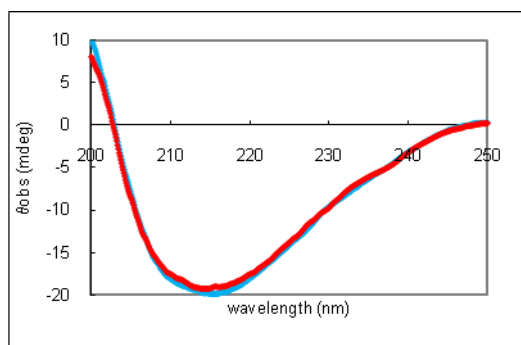


Figure S13 : Far UV-CD spectra of β LG (8.9 μ M) in the presence and absence of compound **1** ($l = 0.2$ cm). **Red**: 8.9 μ M β LG alone. **Blue**: addition of 1.2 equiv. of **1**.

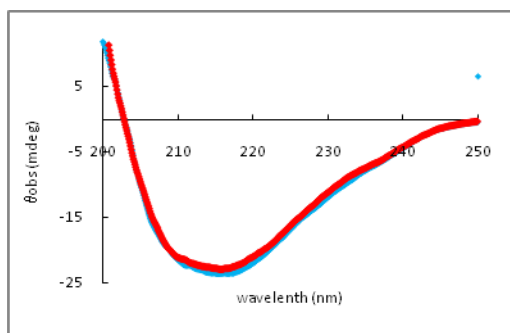


Figure S14 : Far UV-CD spectra of 8.9 μM of βLG in the presence and absence of **4** ($l = 0.2$ cm). **Red**: 8.9 μM βLG alone. **Blue**: addition of 1 equiv. (9 μL of 9 mM solution in EtOH) of **4**.

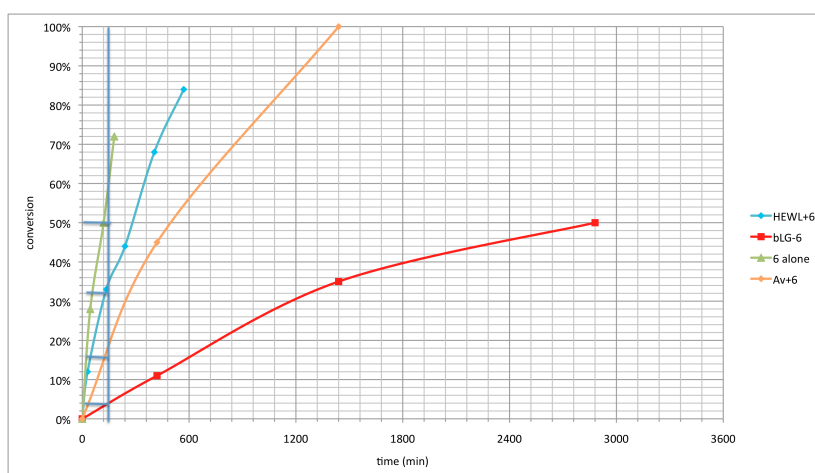


Figure S15 : Kinetics of transfer hydrogenation of TFAP (5 mM) using 2% of catalyst at 40°C with 1 M formate, pH 7.5. **Green**: **6** alone. **Blue**: HEWL+**6**. **Red**: βLG -**6**. **Orange**: Avidin+**6**.

Table S1. Turnover number (TON) and turnover frequency (TOF) of the transfer hydrogenation of TFAP (5mM) in the presence of 2% catalyst calculated at $t = 2$ h. Conditions: [formate] = 1 M (initial pH = 7.5), 40°C.

Catalyst	Conversion (%)	[product] (mM)	TON ^a	TOF ^b (h^{-1})
6	50	2.5	25	12.5
6 + HEWL	32	1.6	16	8
6 + Av	16	0.8	8	4
6 \subset βLG	4	0.2	2	1

^a TON = [product] / [catalyst]

^b TOF = [product] / [catalyst]*time

Catalytic runs with metalloproteins – determination of conversion rates and enantiomeric excesses by HPLC:

Analytical chiral HPLC was performed with Beckman System Gold instrument using Nucleodex β -PM C18 column, 5 μm , 200 x 4 mm using a isocratic eluent containing 60% H_2O , 40% ACN at a flow rate of 0.4 mL/min and detection set at 254 nm. Volume of injection: 20 μL of a 1 mM solution diluted in water. Retention time for **trifluoroacetophenone**: 10.0 min. Retention time for (*S*) α -(trifluoromethyl)benzyl alcohol: 12.9 min. (*R*) α -(trifluoromethyl)benzyl alcohol: 13.8 min.

General procedure for catalysis with metalloproteins: To a solution of Na formate in water (1 M, pH adjusted to 7.5 with 0.1M NaOH, 1 mL) was added β LG (A, B or mixture, 2.7 mg, 0.028 equiv.) at room temperature and the mixture was stirred for 1 min. To this solution was added a solution of complex in DMSO (10 mM, 10 μ L, 0.02 equiv.) and the reaction mixture was stirred for 1 min at room temperature. Finally, TFAP (0.7 μ L) was added and the mixture was stirred up to 72 h at 40°C. Aliquots (10 μ L) of the reaction mixture were taken periodically, diluted in 90 μ L water and submitted to HPLC analysis.

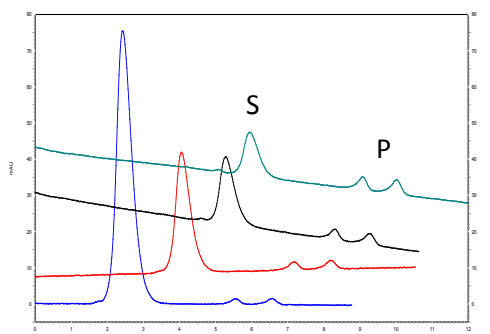


Figure S16 : HPLC traces for the determination of the conversion as well as the enantiomeric excesses - conditions: 1M formate, 5 mM TFAP, 2% β LG(A)-6. **Blue**: 7 h. **Red**: 24 h. **Black**: 48 h. **Green**: 72 h.

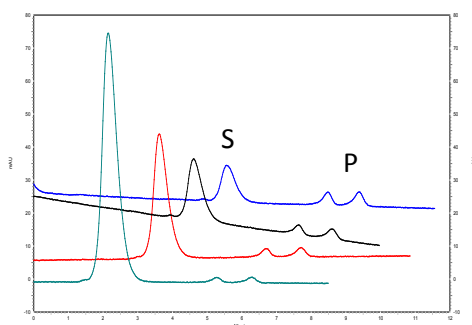


Figure S17 : HPLC traces for the determination of the conversion as well as the enantiomeric excesses - conditions: 1M formate, 5mM TFAP, 2% 6- β LG(B), pH 7.5, 40°C. **Green**: 7 h. **Red**: 24 h. **Black**: 48 h. **Blue**: 72 h.

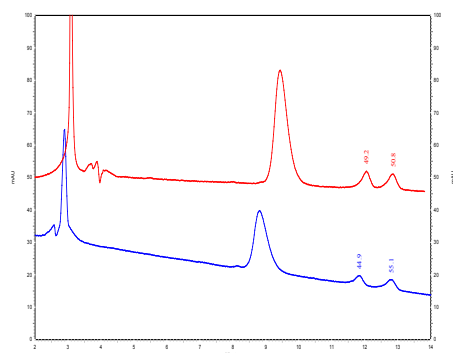


Figure S18 : comparison of the enantiomeric excess using β LG(A)-6 or avidin+6 – conditions: 1M formate, 5mM TFAP, 2% metalloprotein. **Blue**: β LG(A)-6. **Red**: avidin+6.

Determination of enantiomeric excesses by chiral GC analysis:

Preparation of solutions: after completion of catalysis, the reaction mixture was cooled to room temperature. Then, 900 μL of diisopropylether was added to the reaction mixture and was mixed for 1 min before injection.

Enantiomeric excesses were measured by GC on a CP-3880 gas chromatograph (Varian) equipped with a split/splitless injector ($T = 250\text{ }^{\circ}\text{C}$), a 25 m x 0.25 mm x 0.25 μm CP-Chirasil-DEX CB capillary column (Chrompack) and a FID detector ($T = 250^{\circ}\text{C}$). Volume of injection: 5 μL . The following programme was used for analysis: 50 $^{\circ}\text{C}$ (2 min), 50-110 $^{\circ}\text{C}$ (10 $^{\circ}\text{C}/\text{min}$), 110 $^{\circ}\text{C}$ (1 min), 110-140 $^{\circ}\text{C}$ (1.5 $^{\circ}\text{C}/\text{min}$), 140 $^{\circ}\text{C}$ (3 min), 140-200 $^{\circ}\text{C}$ (10 $^{\circ}\text{C}/\text{min}$), 200 $^{\circ}\text{C}$ (2 min). Retention times: **TFAP** 5.9 min; **α -(trifluoromethyl)benzyl alcohol** 16.6 min (*S*), 16.8 min (*R*)⁴

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