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Supporting Information

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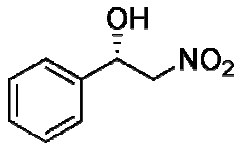
The first Example of a Biocatalytic Henry Reaction – the Hydroxynitrile Lyase from *Hevea brasiliensis* also catalyzes Nitroaldol Reactions

Thomas Purkarthofer, Karl Gruber, Mandana Gruber-Khadjawi, Kerstin Waich, Wolfgang Skranc, Daniel Mink, and Herfried Griengl

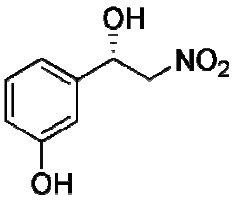
General Procedure for *Hb*HNL-catalyzed nitroaldol reaction: Wt-*Hb*HNL (4000 U / mmol aldehyde – activity determined for the cleavage of mandelonitrile, the enzyme was kindly provided by DSM) is stirred in phosphate buffer (pH=7, 50 mM) and TBME (1 / 1) until an emulsion is established. To the mixture freshly distilled aldehyde (1-10 mmol) is added. After stirring for 5 minutes the nitroalkane (10 mmol / mmol aldehyde) is added. The reaction is stirred for 48 h at room temperature. After centrifugation and separation of the layers, the aqueous phase is extracted with TBME. The combined organic phases are dried over Na_2SO_4 and concentrated *in vacuo*. The crude products were purified by column chromatography.

Molecular docking simulations: (*S*)- and (*R*)-2-nitro-1-phenylethanol (**6**) were docked into the active site of *Hb*HNL using the program AutoDock v3.0.[1] Molecular models of **6** were built and optimized using the program Sybyl v6.8 (Tripos Inc.), partial atomic charges for these compounds were calculated using the RESP protocol.[2] Protein coordinates were taken from the atomic resolution X-ray crystal structure of *Hb*HNL (PDB-entry: 1qj4).[3] Asp-, Glu-, Arg- and Lys-residues were treated as charged, protonation and tautomerization states of His-residues were chosen that resulted in sensible hydrogen bonding networks. The search for low energy binding modes (rigid protein, flexible ligands) employed a hybrid genetic algorithm with phenotypic local search (300 generations with a population size of 75).[1] The minimum energy structures from each of the 50 independent runs were clustered using an r.m.s.-tolerance of 1.5Å.

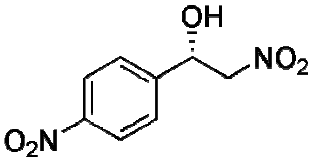


(*S*)-2-Nitro-1-phenylethanol[4] was purified by column chromatography (cyclohexane / ethyl acetate 16/1) to give a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 2.86 (br, 1H), 4.49 (dd, *J* = 13.2 Hz, *J* = 2.9 Hz, 1H), 4.58 (dd, *J* = 13.2 Hz, *J* = 9.8 Hz, 1H), 5.43 (dd, *J* = 9.8 Hz, *J* = 2.9 Hz, 1H), 7.33-7.39 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 71.2, 81.4, 126.2, 129.2, 129.3, 138.3. Enantiomeric excess was determined by HPLC with a Chiralcel OD-H column (*n*-

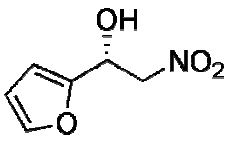
heptane / EtOH 9/1, 0.6 mL/min, 20°C, λ = 210nm): t_r = 18.5 min and 21.0 min (major); 97% e.e.; $[\alpha]_D^{20}$ +41.6 (c 1.0, CH_2Cl_2).



(*S*)-1-(3-Hydroxyphenyl)-2-nitro-ethanol[5] was purified by column chromatography (cyclohexane / ethyl acetate 10/1) to give a light yellow solid. mp: 64-69 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm) 4.48 (dd, *J* = 12.7 Hz, *J* = 10.3 Hz, 1H), 4.79 (dd, *J* = 13.2 Hz, *J* = 2.4 Hz, 1H), 5.16 (m, 1H), 6.01 (d, *J* = 4.9 Hz, 1H), 6.67-6.82 (m, 3H), 7.11-7.15 (m, 1H). 9.45 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm) 70.6, 82.7, 113.7, 115.5, 117.3, 130.1, 142.6, 158.1. Enantiomeric excess was determined by HPLC with a Chiralcel AD-H column (*n*-heptane / 2-propanol 95/5, 1.5 mL/min, 30°C, λ = 210nm): t_r = 47.4 min (major) and 50.8 min; 18% e.e.; $[\alpha]_D^{20}$ +1.2 (c 1.0, CH_3OH).

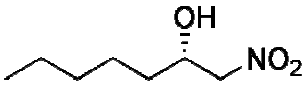


(*S*)-2-Nitro-1-(4-nitrophenyl)ethanol[4b] was purified by column chromatography (cyclohexane / ethyl acetate 8/1) to give a white solid. mp: 75-78 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm) 4.63 (dd, *J* = 12.7 Hz, *J* = 9.8 Hz, 1H), 4.94 (dd, *J* = 12.7 Hz, *J* = 2.9 Hz, 1H), 5.44 (m, 1H), 6.44 (d, *J* = 4.9 Hz, 1H), 7.73 (d, *J* = 8.3 Hz, 2H,) 8.21 (d, *J* = 8.8 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm) 69.8, 81.9, 124.1, 128.2, 147.8, 148.7. Enantiomeric excess was determined by HPLC with a Chiralcel AD-H column (*n*-heptane / 2-propanol 9/1, 1.3 mL/min, 20°C, λ = 210nm): t_r = 27.3 min and 37.2 min (major); 28% e.e.; $[\alpha]_D^{20}$ +6.1 (c 1.0, CH_2Cl_2).

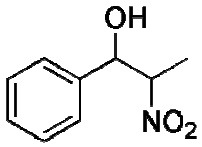


(*R*)-1-(2-Furyl)-2-nitroethanol[6] was purified by column chromatography (cyclohexane / ethyl acetate 16/1) to give a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 3.10 (br, 1H), 4.66 (dd, *J*

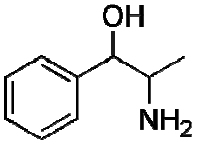
= 13.7 Hz, *J* = 3.4 Hz, 1H), 4.77 (dd, *J* = 13.2 Hz, *J* = 9.3 Hz, 1H), 5.46 (dd, *J* = 9.3 Hz, *J* = 3.4 Hz, 1H), 6.37-6.39 (m, 2H), 7.41 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 65.0, 78.6, 108.4, 110.9, 143.4, 150.9. Enantiomeric excess was determined by HPLC with a Chiralcel AD-H column (*n*-heptane / 2-propanol 98/2, 1.5 mL/min, 20°C, λ = 210nm): t_r = 41.6 min and 44.2 min (major); 72% e.e.; $[\alpha]_D^{20}$ +23.7 (c 1.0, CH_3OH).



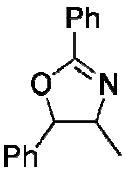
(*S*)-1-Nitro-2-heptanol[7] was purified by column chromatography (cyclohexane / ethyl acetate 16/1) to give a pale orange oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 0.89 (t, *J* = 6.8 Hz, 3H), 1.27-1.39 (m, 5H), 1.45-1.57 (m, 3H) 2.58 (br, 1H), 4.22-4.26 (m, 1H), 4.31 (dd, *J* = 13.2 Hz, *J* = 8.3 Hz, 1H), 4.37 (dd, *J* = 13.2 Hz, *J* = 2.9 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 14.2, 22.7, 25.1, 31.7, 33.9, 68.9, 80.9. Enantiomeric excess was determined by HPLC with a Chiralcel AD-H column (*n*-heptane / 2-propanol 9/1, 0.9 mL/min, 20°C, λ = 210nm): t_r = 8.8 min and 11.5 min (major); 89% e.e.; $[\alpha]_D^{20}$ +2.8 (c 1.0, CH_2Cl_2).



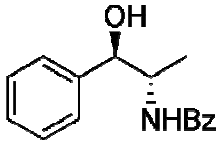
Compounds **1a** – **4a**[8] were purified by column chromatography (cyclohexane / ethyl acetate 16/1) to give a white solid. mp: 38 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) *anti*: 1.50 (d, *J* = 7 Hz, 3H), 2.65-2.70 (br, 1H), 4.70 (dq, *J* = 7 Hz, *J* = 3 Hz, 1H), 5.40 (d, *J* = 3 Hz, 1H), 7.35-7.45 (m, 5H); *syn*: 1.32 (d, *J* = 7 Hz, 3H), 2.60-2.65 (br, 1H), 4.78 (dq, *J* = 9 Hz, *J* = 7 Hz, 1H), 5.02 (d, *J* = 9 Hz, 1H), 7.35-7.45 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) *anti*: 12.3, 74.1, 87.7, 126.2, 128.8, 129.0, 138.7; *syn*: 16.7, 76.5, 88.7, 127.2, 129.3, 129.5, 138.5. Enantiomeric and diastereomeric excess was determined by HPLC (as well as NMR for determination of the diastereomeric composition) with a Chiralcel AD-H column (*n*-heptane / 2-propanol 9/1, 0.9 mL/min, 20°C, λ = 210nm): t_r = 9.4 min (1*S*,2*R*), 10.5 min (1*R*,2*S*), 12.2 min (1*S*,2*S*) and 13.6 min (1*R*,2*R*).



Compounds **1b** – **4b** were prepared according to a literature procedure.[9] ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) *anti*: 0.94 (d, *J* = 6Hz, 3H), 2.25-2.50 (br s, 3H), 3.14-3.20 (m, 1H), 4.51 (d, *J* = 5Hz, 1H), 7.25-7.36 (m, 5H) ; *syn*: 0.98 (d, *J* = 6Hz, 3H), 2.25-2.50 (br s, 3H), 3.00-3.09 (m, 1H), 4.24 (d, *J* = 7Hz, 1H), 7.21-7.38 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) *anti*: 18.2, 51.9, 77.5, 126.5, 127.4, 128.1, 141.3; *syn*: 20.4, 52.9, 78.6, 126.4, 127.4, 128.4, 142.6.



Compounds **5**, **6**, **7**[10] were purified by column chromatography (cyclohexane / ethyl acetate 2/1) to give a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) (4*S*,5*R*), (4*R*,5*S*): 0.90 (d, *J* = 7Hz, 3H), 4.68 (dq, *J* = 10Hz, *J* = 7Hz, 1H), 5.78 (d, *J* = 10Hz, 1H), 7.25-7.55 and 8.04-8.08 (m, 10H); (4*S*,5*S*), (4*R*,5*R*): 1.50 (d, *J* = 7Hz, 3H), 4.23 (dq, *J* = 8Hz, *J* = 7Hz, 1H), 5.12 (d, *J* = 8Hz, 1H), 7.30-7.55 and 8.04-8.08 (m, 10H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) (4*S*,5*R*), (4*R*,5*S*): 17.8, 65.5, 84.0, 126.1, 127.7, 127.8, 128.3, 128.3, 128.4, 131.4, 137.1, 163.0; (4*S*,5*S*), (4*R*,5*R*): 21.4, 70.9, 88.2, 125.6, 127.7, 128.3, 128.4, 128.5, 128.8, 131.4, 140.5, 162.7.



(1*R*,2*S*)-*N*-(2-Hydroxy-1-methyl-2-phenylethyl)benzamide was prepared from (1*R*,2*S*)-(-)-norephedrine and converted into (**7**) according to a literature procedure.[11]
^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm) 1.09 (d, *J* = 7Hz, 3H), 4.10-4.18 (m, 1H), 4.69 (d, *J* = 4Hz, 1H), 7.18-7.76 (m, 10H), 8.20 (d, *J* = 9Hz,1H);[12] ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm) 15.6, 51.8, 75.2, 126.9, 127.4, 127.9, 128.4, 128.8, 131.7, 135.4, 144.4, 166.2.

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