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Hydroxynitrile Lyase-Catalyzed Enzymatic Nitroaldol (Henry) Reaction

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Abstract: The hydroxynitrile lyase from *Hevea brasiliensis* not only catalyzes – according to the natural activity of this enzyme – the formation and cleavage of cyanohydrins but also the reaction of nitroalkanes with aldehydes (Henry reaction). This is the first example of an enzymatic nitroaldol reaction. With nitromethane and nitroethane a broad range of aldehydes can be transformed into the corresponding nitro alcohols in yields up to 77% and enantiomeric excess (*ee*) up to 99%.

Keywords: biotransformations; C–C bond formation; Henry reaction; hydroxynitrile lyase; β-nitro alcohols; stereoselectivity

Introduction

An important reaction in organic synthetic chemistry is the formation of carbon-carbon bonds. The most desired and challenging way is to obtain this C–C coupling stereoselectively. One possibility for enantioselective C–C bond formation is the use of enzymes.[1] Only few enzyme classes are capable of catalyzing C–C bond coupling reactions[1b], among them hydroxynitrile lyases (HNLs). In reversal of the *in vivo* reaction, HNLs catalyze the stereoselective addition of hydrocyanic acid to aldehydes or ketones to yield enantiopure α-hydroxy nitriles (Figure 1), which represent important building blocks for the synthesis of various pharmaceuticals and agrochemicals.[2]

In general, HNLs have been found to be rather wide-ranging with respect to the nature of the electrophile and a wide array of aliphatic, aromatic and heterocyclic carbonyl compounds are accepted.[2d] With respect to the nucleophile no other substrate except cyanide was recognized. However, for the hydroxynitrile lyase from *Hevea brasiliensis* (*Hb*HNL) (EC 4.1.2.39) we recently found a nitroaldolase activity. This enzyme is able to accept nitroalkanes as donors in a reaction with aldehydes to yield enantiomerically enriched β-nitro alcohols (Figure 2).[3] The observed activity represents the first example of a biocatalytic Henry reaction.

The nitroaldol reaction is an important transformation in preparative organic chemistry[4] and the thereby obtained nitro alcohols can be converted into a wide variety of synthetic intermediates.[5] Although the reaction has been known for more than a century[6], stereospecific protocols utilizing non-enzymatic chiral organocatalysts or metal catalysts have been developed only recently.[7] The development of these methods is impressive, but they still share a number of disadvantages, including long reaction times and sometimes harsh reaction conditions in the case of metal catalysts, or insufficient selectivities in the case of organocatalysts.

Herein we report on the scope and limitations at present and potential future developments of the enzyme-catalyzed nitroaldol reaction.

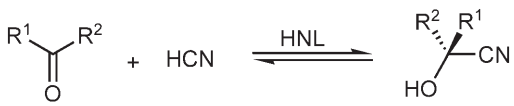


Figure 1. HNL-catalyzed asymmetric cyanohydrin formation.

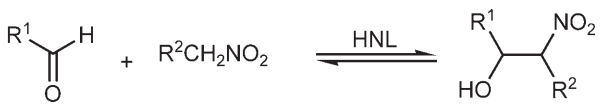


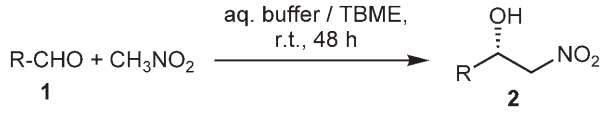
Figure 2. HNL-catalyzed asymmetric nitroaldol (Henry) reaction.

Results and Discussion

Initially, the *Hb*HNL-catalyzed nitroaldol reaction was investigated with aromatic, heteroaromatic and aliphatic aldehydes using nitromethane as the nucleophile. Examples for this transformation are given in Table 1. The reaction was performed at room temperature (r.t.) in a biphasic aqueous-organic system with *tert*-butyl methyl ether (TBME) as organic solvent. First experiments were carried out in a 50 mM phosphate buffer at pH 7.0. Presumably due to the fact that for the nitroaldol reaction a C–H acidic compound has to be deprotonated to form the nucleophile compared to the deprotonation of HCN in the cyanohydrin reaction, large amounts of enzyme and long reaction times are necessary. A similar effect has been recognized recently in another example of promiscuous enzyme activity: *Candida antarctica* lipase B catalyzes not only ester hydrolysis but also aldol reactions, however, in the latter case the reaction rate is much more slow.[8] The time course of the reaction is shown in Figure 3.

The yields after 48 h are acceptable and the enantioselectivities ranged from moderate to high. To suppress a small contribution of a non-enzymatic deprotonation of nitromethane taking place at pH 7.0 McIlvaine buffer (citrate phosphate buffer) pH 5.5 was used in a second experimental set-up. While the yield decreased in most cases, the enantioselectivity (enantiomeric excess, *ee*) increased significantly for most substrates. *Hb*HNL was found to be *S*-selective.[3] A ketone (2-heptanone) was also found to be accepted by the enzyme in this particular transformation, but the conversion was extremely low. In further experiments more substrates were tested in McIlvaine buffer at pH 5.5 in order to prove the applicability of this method for preparative purposes and produce valuable chiral building blocks. The results are also listed in Table 1.

Table 1. *Hb*HNL-catalyzed Henry reactions of aldehydes with nitromethane.



1 2

2	R	pH 7.0		pH 5.5	
		Yield [%]	*ee* [%]	Yield [%]	*ee* [%]
a	Ph	63	92	32	97
b	3-HOC_6H_4	46	18		
c	4-$NO_2C_6H_4$	77	28	57	64
d	*n*-hexyl	25	89	34	96
e	$Ph(CH_2)_2$	9	66	13	66
f	2-furyl	57	72	43	88
g	$CH_3(CH_2)_4CHCH_3$	2	88		
h	2-thienyl			29	98
i	3-furyl			16	89
j	2-ClC_6H_4			23	95
k	3-ClC_6H_4			36	98
l	4-ClC_6H_4			25	97
m	4-$MeOC_6H_4$			20	99
n	cyclohexyl			18	99

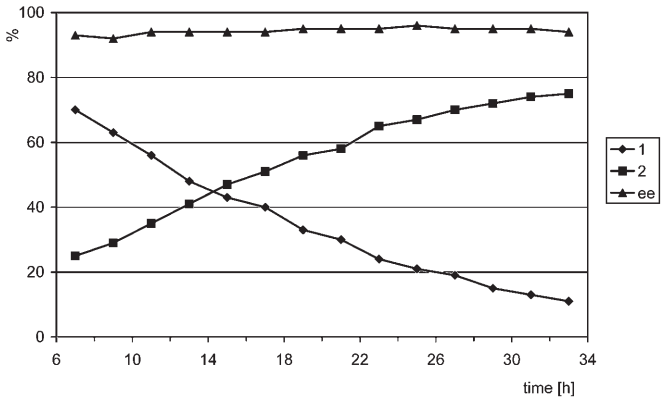


Figure 3. Time course of the reaction of benzaldehyde **1** with 10 equivalents nitromethane, phosphate buffer pH 7.0/TBME 1:1, 4000 U/mmol *Hb*HNL, room temperture.

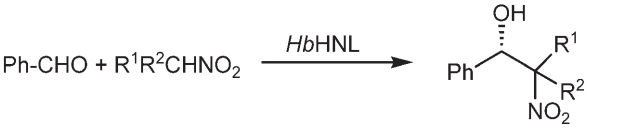
While no conversion for 2-furyl methyl ketone, methyl phenyl ketone, dodecanal and ferrocene-2-carbaldehyde could be detected, pyridine aldehydes gave high conversion but no selectivity, due to their enhanced intrinsic carbonyl activity (detectable in the blank) favoring the non-enzymatic unselective background reaction.

Despite the fact that nitroethane is about two orders of magnitude more acidic than nitromethane the reaction rates and conversions in the Henry reaction with benzaldehyde are comparable. This implicates that not only the *pKa* values but also the size of the carbanion nucleophile play a role in the mechanism of the *Hb*HNL-catalyzed Henry reaction. Therefore, we tested further nitroalkanes in order to gather more information about the scope and limitations of this reaction. The results are summarized in Table 2.

The corresponding addition of nitroethane to benzaldehyde (Figure 4) produces two new stereo centers simultaneously and a diastereomeric mixture of 2-nitro-1-phenylpropanols was obtained. The *anti/syn* ratio was 9:1 and the enantiomeric excess of the *anti* isomer was 95%. Thus the product mixture contains about 90% of the main product (1*S*,2*R*)-**3**.[3]

For an evaluation of the synthetic value of the *Hb*HNL-catalyzed nitroaldol reaction several aspects have to be taken into consideration: (1) although at low yield in many cases excellent enantiopurities are obtained; (2) the access to the enzyme *Hb*HNL is easy since it is obtained by overexpression in *Pichia pastoris*; (3) to a certain extent (three times) the enzyme can be recycled although with loss of activity;

Table 2. Henry reactions of other nitroalkanes (benzaldehyde, *Hb*HNL, pH 7.0).



R^1	R^2	Nitroalkane	*pKa*[a]	Yield [%]	ee[%]
H	H	CH_3NO_2	10.2	63	92
H	CH_3	$CH_3CH_2NO_2$	8.6	67	95 (*anti*)[3]
CH_3	CH_3	$(CH_3)_2CHNO_2$	8.4	7	80
H	Ph	$PhCH_2NO_2$	6.9	0	0

[a] Calculated using Advanced Chemistry Development (ACD/Labs) Software V8.14 for Solaris (© 1994-2007 ACD/Labs).

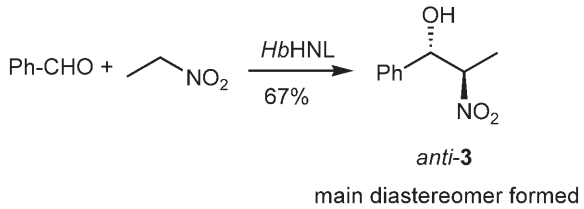


Figure 4. Stereoselective addition of nitroethane to benzaldehyde in the presence of *Hb*HNL at pH 7.0.

(4) enantiopure nitro alcohols are important synthetic intermediates, in some cases the follow-up chemistry can be performed more easily than from cyanohydrins which might be used in similar synthetic sequences.

Optimizations were undertaken to increase yield and selectivity, respectively. A phase ratio aqueous/organic 1:2 is beneficial for conversion and *ee*. The lower the amount of the aqueous phase the higher is the conversion, but a small quantity of water is necessary to keep the enzyme active. Without an aqueous phase (lyophilized enzyme powder in organic solvent) the reaction rate is very low, probably due to the denaturation of the enzyme. The presence of an organic solvent is beneficial for reaction rate, yield and *ee*. One reason is probably the increased solubility of the substrates. By performing the reaction even under inert gas atmosphere and with degassed buffer and freshly distilled benzaldehyde, a small amount of benzoic acid still could be detected. The larger the amount of the aqueous phase the larger is the amount of the benzoic acid. Benzoic acid was shown to be an inhibitor of *Hb*HNL.[9] The organic phase seems to protect benzaldehyde from oxidation to some extent. The use of water-miscible organic solvents like dimethylformamide (DMF), dimethyl sulfoxide (DMSO) and *N*-methyl-2-pyrrolidone (NMP) in order to enhance the solubility of the substrates showed no significant increase of conversion and *ee* by the use of up to 20% v/v. By using 30% v/v DMF, conversion decreased by an order of 3 and the stereoselectivity almost disappeared, probably because of enzyme denaturation.

Besides HNL from the tropical rubber tree *Hevea brasiliensis* also HNL from *Manihot esculenta* showed activity for the enzymatic Henry reaction. *Me*HNL was about 4 times less active and slightly less selective than *Hb*HNL. Both enzymes belong to the α,β-hydrolase fold enzyme family and show more than 75% homology.[10] We also tested an *R*-selective enzyme – HNL from *Prunus amygdalus* (almond) – but could not detect any activity under the conditions used.

Conclusions

We have shown that the hydroxynitrile lyase from the tropical rubber tree *Hevea brasiliensis* converts carbonyl compounds and nitroalkanes in an enzymatic Henry reaction with high selectivity to the corresponding chiral nitro alcohols. The HNL from *Manihot esculenta* is also able to catalyze this transformation but with lower activity and selectivity.

Enzyme-catalyzed Henry reactions show all the benefits and general advantages of biocatalysis - high selectivity, mild reaction conditions, low energy consumption and reduced dangerous waste. Experiments are in progress to obtain a better understanding of the reaction mechanism by kinetic investigations, by structural biology, by modeling and by the generation of enzyme mutants.

Experimental Section

Chemicals

Wt-*Hb*HNL (4495 U/mL activity determined for the cleavage of mandelonitrile, protein content: 73 mg/mL, HNL purity > 99%) was kindly provided by DSM. *Me*HNL (1043 U/mL activity determined for the cleavage of mandelonitrile, protein content: 18.69 mg/mL, HNL purity unknown) was purchased from Jülich Fine Chemicals. Na_2HPO_4, KH_2PO_4, nitromethane, nitroethane and benzaldehyde were products of Fluka. All other chemicals were purchased from Aldrich. Except for benzaldehyde all the chemicals were used without further purification. Benzaldehyde was distilled prior to use. Melting points were measured on a MEL-TEMP® apparatus and are uncorrected. 500 MHz ^{1}H- and 125 MHz ^{13}C NMR spectra were recorded on a Varian Inova-500 (500 MHz) and are reported in ppm (δ) using solvent as an internal standard (s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet, br = broad). All coupling constants are given as *J* in Hz. For the determination of optical rotations a Perkin-Elmer 341 polarimeter with sodium lamp was used and reported as $[\alpha]_D^{20}$ (*c* g/100 mL, solvent). Flash chromatography was carried out at a pressure of *ca.* 1.5 bar, using Merck Kieselgel 60H. HPLC

analyses were performed on an Agilent 1100 with autosampler and multi wave detector (MWD).

General Procedure for *Hb*HNL-Catalyzed Nitroaldol Reaction (Products 1, 3–6, 8–14)

Wt-*Hb*HNL (2.0 mL), McIlvaine buffer (45 mM citrate buffer and 110 mM phosphate buffer) (pH 5.5, 100 μL) and *tert*-butyl methyl ether (4.2 mL) were stirred until an emulsion was established. To the mixture aldehyde (1.0 mmol) was added. After stirring for 5 min nitroalkane (10.0 mmol) was added. The reaction mixture was stirred at room temperature for 48 h. The reaction mixture was extracted three times with 20 mL of ethyl acetate. The combined extracts were dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The crude products were purified by column chromatography with cyclohexane/ethyl acetate 16:1 as eluent.

In the case of benzaldehyde the reaction was also performed on an up to 500-mmol scale in a biphasic system with phosphate buffer pH 7.0 using nitromethane and nitroethane, respectively. The yields and *ee*s were reproducible.

(*S*)-1-Phenyl-2-nitroethanol (2a):[7g] Colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$):[11a] δ = 2.86 (br, 1H, -O*H*), 4.49 (dd, ^2J = 13.2 Hz, ^3J = 2.9 Hz 1H, -C*H*HNO$_2$), 4.58 (dd, ^2J = 13.2 Hz, ^3J = 9.8 Hz, 1H, -CH*H*NO$_2$), 5.43 (dd, ^3J = 9.8 Hz, ^3J = 2.9 Hz, 1H, -C*H*OH-), 7.33–7.39 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$):[7c] δ = 71.2, 81.4, 126.2, 129.2, 129.3, 138.3. The enantiomeric excess was determined by HPLC with a Chiralcel OD-H column (heptane/ethanol 9:1, 0.7 mL min^{-1}, 20 °C, λ = 210 nm): t_r = 18.0 min and 21.0 min (major); 97 % *ee*; $[\alpha]_D^{20}$: +26.9 (*c* 1.2, CH_2Cl_2).[7h]

(*S*)-1-(3-Hydroxyphenyl)-2-nitroethanol (2b): Wt-*Hb*HNL (1.0 mL), phosphate buffer (pH 7, 50 mM, 0.67 mL) and *tert*-butyl methyl ether (1.3 mL) were stirred vigorously in a 10-mL round-bottom flask until an emulsion was established (10 min). To the mixture 3-hydroxybenzaldehyde (122 mg, 1.0 mmol) was added. After stirring for 5 min. nitromethane (0.51 mL, 10.0 mmol) was added in one portion. The reaction mixture was stirred at room temperature for 48 h and then extracted three times with 4 mL of diethyl ether. The combined extracts were dried over anhydrous Na_2SO_4 and the solvent removed under reduced pressure. The yellow oil was purified by column chromatography (cyclohexane/ethyl acetate 10:1) to afford a light yellow solid; yield:76.5 mg (46 %); mp 64–69 °C; ^{1}H NMR (500 MHz, DMSO): δ = 4.48 (dd, ^2J = 12.7 Hz, ^3J = 10.3 Hz, 1H, -C*H*HNO$_2$), 4.79 (dd, ^2J = 13.2 Hz, ^3J = 2.4 Hz 1H, -CH*H*NO$_2$), 5.16 (m, ^3J = 3.9 Hz, 1H, -C*H*OH-), 6.01 (d, 1H, -O*H*), 6.67–6.82 (m, 3H), 7.11–7.15 (m, 1H), 9.45 (s, 1H, Aryl-O*H*); ^{13}C NMR (125 MHz, DMSO): δ = 70.6, 82.7, 113.7, 115.5, 117.3, 130.1, 142.6, 158.1. the enantiomeric excess was determined by HPLC with a Chiralpack AD-H column (heptane/2-propanol, 95:5, 1.5 mL min^{-1}, 30 °C, λ = 210 nm): t_r = 47.4 min (major) and 50.8 min; 18 % *ee*; $[\alpha]_D^{20}$: +1.2 (*c* 1.0, CH_3OH).

(*S*)-2-Nitro-1-(4-nitrophenyl)ethanol (2c):[7d,g,h] Pale yellow solid; mp 79 °C; ^{1}H NMR[11a] (500 MHz, DMSO): δ = 4.62 (dd, ^2J = 12.7 Hz, ^3J = 9.8 Hz, 1H, -C*H*HNO$_2$), 4.94 (dd, ^2J = 12.7 Hz, ^3J = 2.9 Hz, 1H, -CH*H*NO$_2$), 5.43 (m, ^3J = 3.9 Hz, -C*H*OH-), 6.42 (d, ^3J = 4.9 Hz, 1H, -O*H*),7.72 (d, 2H) 8.21-(d, 2H); ^{13}C NMR (125 MHz, DMSO): δ = 69.7, 81.9, 124.1, 128.3, 147.8, 148.7. The enantiomeric excess was determined by HPLC with a Chiralpack AD-H column (heptane/2-propanol, 9:1, 1.3 mL min^{-1}, 20 °C, λ = 210 nm): t_r = 27.3 min and 37.2 min (major); 64 % *ee*; $[\alpha]_D^{20}$: +22.2 (*c* 1.0, CH_2Cl_2).[11a]

(*S*)-1-Nitroheptan-2-ol (2d):[12] Pale yellow oil; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.84 (t, ^3J = 6.8 Hz, 3H, -C*H*$_3$), 1.27–1.39 (m, 5H, $H_3CCH_2CH_2CH$H-), 1.45–1.57 (m, 3H, -CH*H*CH$_2$-) 2.76 (br, -CHO*H*-), 4.25 (br, 1H, -C*H*OH-), 4.32 (dd, ^2J=13.2 Hz, ^3J = 8.3 Hz, 1H, -C*H*HNO$_2$), 4.36 (dd, ^3J=13.2 Hz, ^3J = 2.9 Hz, 1H, -CH*H*NO$_2$); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.1, 22.7, 25.0, 31.7, 33.9, 68.9, 80.9. The enantiomeric excess (*ee*) was determined by HPLC with a Chiralpack AD-H column (heptane/2-propanol, 9:1, 0.9 mL min^{-1}, 20 °C, λ = 210 nm): t_r = 8.9 min and 11.6 min (major); 96 % *ee*; $[\alpha]_D^{20}$: +12.0 (*c* 1.1, CH_2Cl_2).

(*S*)-1-Nitro-4-phenylbutan-2-ol (2e):[7g,11c] Colorless needles; mp 96–98 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.80 (m, 1H, -CH_2C*H*HCHOH-), 1.85 (m, 1H, -CH_2C*H*HCHOH-), 2.75 (m, 1H, Aryl-CH*H*-CH_2-), 2.76 (s, -CHO*H*-), 2.87 (m, 1H, Aryl-C*H*HCH_2-), 4.31 (m, 1H, -C*H*OH-), 4.40 (m, 2H, -C*H*$_2$NO$_2$), 7.20–7.26 (m, 3H), 7.32 (t, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 31.6, 35.3, 68.0, 80.8, 126.6, 128.7, 128.9, 140.9. The enantiomeric excess was determined by HPLC with a Chiralpack AD-H column (heptane/2-propanol, 9:1, 0.9 mL min^{-1}, 20 °C, λ = 210 nm): t_r = 13.0 min and 16.2 min (major); 66 % *ee*; $[\alpha]_D^{20}$: −6.8 (*c* 0.8, CH_2Cl_2).[13a]

(*R*)-1-(2-Furyl)-2-nitroethanol (2f):[12a] Pale yellow oil; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.10 (br, -CHO*H*-), 4.66 (dd, ^2J=13.7 Hz, ^3J = 3.4 Hz, 1H, -C*H*HNO$_2$), 4.77 (dd, ^2J=13.2 Hz, ^3J = 9.3 Hz, 1H, -CH*H*NO$_2$), 5.46 (dd, ^3J = 9.3 Hz, ^3J = 3.4 Hz, 1H, -C*H*OH-), 6.38 (m, 2H), 7.41 (d, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 65.0, 78.6, 108.4, 110.9, 143.4, 150.9. The enantiomeric excess was determined by HPLC with a Chiralpack AD-H column (heptane/2-propanol, 98:2, 1.5 mL min^{-1}, 20 °C, λ = 210 nm): t_r = 45.4 min and 48.5 min (major); 88 % *ee*; $[\alpha]_D^{20}$: +27.7 (*c* 1.1, CH_3OH).

(*R*)-2-Nitro-1-thien-2-ylethanol (2h): Pale yellow oil; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.93 (d, -CHO*H*-), 4.58 (dd, ^2J = 13.2 Hz, ^3J = 3.4 Hz, 1H, -C*H*HNO$_2$), 4.69 (dd, ^2J = 13.7 Hz, ^3J = 9.3 Hz, 1H, -CH*H*NO$_2$), 5.70 (dd, ^3J = 9.3 Hz, ^3J = 4.4 Hz, 1H, -C*H*OH-), 6.99 (1H), 7.04 (1H), 7.31 (1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 67.4, 81.0, 125.4, 126.5, 127.5, 141.4. The enantiomeric excess was determined by HPLC with a Chiralpack AD-H column (heptane/2-propanol, 98:2, 1.5 mL min^{-1}, 30 °C, λ = 210 nm): t_r = 43.5 min and 45.4 min (major); 98 % *ee*; $[\alpha]_D^{20}$: +33.2 (*c* 1.1, CH_2Cl_2).

(*S*)-1-(3-Furyl)-2-nitroethanol (2i): Pale yellow oil; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.88 (s, -CHO*H*-), 4.50 (dd, ^2J = 13.7 Hz, ^3J = 3.4 Hz, 1H, -C*H*HNO$_2$), 4.59 (dd, ^2J = 13.2 Hz, ^3J = 9.3 Hz, 1H, -CH*H*NO$_2$), 5.40 (d, ^3J = 8.3 Hz, 1H, -C*H*OH-), 6.37 (1H), 7.40 (1H), 7.45 (1H); ^{13}C NMR (125 MHz, $CDCl_3$): d = 64.4, 80.5, 108.2, 123.6, 140.2, 144.4. The enantiomeric excess was determined by HPLC with a Chiralpack AD-H column (heptane/2-propanol, 9:1, 1.0 mL min^{-1}, 20 °C, λ = 210 nm): t_r = 14.9 min and 20.0 min (major); 89 % *ee*; $[\alpha]_D^{20}$: +17.5 (*c* 1.3, CH_2Cl_2).

(*S*)-1-(2-Chlorophenyl)-2-nitroethanol (2j):[11b] Colorless oil; ^{1}H NMR[11a] (500 MHz, $CDCl_3$): δ = 3.14 (br, 1H, -O*H*), 4.44 (dd, ^2J = 13.7 Hz, ^3J = 9.8 Hz, 1H, -C*H*HNO$_2$), 4.65 (dd, ^2J = 13.7 Hz, ^3J = 2.4 Hz, 1H, -CH*H*NO$_2$), 5.82 (br, -C*H*OH-), 7.28–7.38 (3H) 7.62(1H); ^{13}C NMR[13a] (125 MHz, $CDCl_3$): δ = 68.0, 79.5, 127.7, 127.8, 129.9, 130.1, 131.7, 135.7. The enantiomeric excess was determined by

HPLC with a Chiralcel OD-H column (heptane/2-propanol, 98:2, 0.9 mL min^{-1}, 20 °C, λ = 210 nm): t_r = 41.4 min and 45.3 min (major); 95 % *ee*; $[\alpha]_D^{20}$: +41.8 (*c* 0.9, CH_2Cl_2).[7d,13a]

(*S*)-1-(3-Chlorophenyl)-2-nitroethanol (2k):[14] Colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.05 (br, 1 H, -O*H*), 4.48–4.60 (m, 2 H, -C*H*$_2$NO$_2$), 5.44 (br, -C*H*OH-), 7.27 (1 H) 7.33 (2 H), 7.42 (1 H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 70.5, 81.1, 124.3, 126.4, 129.3, 130.5, 135.2, 140.2.[15] The enantiomeric excess was determined by HPLC with a Chiralcel OD-H column (heptane/ethanol, 97:3, 0.8 mL min^{-1}, 20 °C, λ = 210 nm): t_r = 39.7 min and 48.0 min (major); 98 % *ee*; $[\alpha]_D^{20}$: +41.1 (*c* 1.0, CH_2Cl_2).

(*S*)-1-(4-Chlorophenyl)-2-nitroethanol (2l):[11a] Colorless oil; ^{1}H NMR (500 MHz, DMSO): δ = 3.09 (d, 1 H, -O*H*), 4.45 (dd, ^2J = 13.2 Hz, ^3J = 2.9 Hz 1 H, -C*H*HNO$_2$), 4.53 (dd, ^2J = 13.7 Hz, ^3J = 9.3 Hz,1 H, -CH*H*NO$_2$),5.40 (dd, ^3J = 3.4 Hz, ^3J = 9.8 Hz 1 H, -C*H*OH-), 7.30–7.35 (4 H); ^{13}C NMR (125 MHz, DMSO):[13a] δ = 70.5, 81.2, 127.6, 129.4, 135.0, 136.8. The enantiomeric excess was determined by HPLC with a Chiralcel OD-H column (heptane/ethanol, 9:1, 0.7 mL min^{-1}, 20 °C, λ = 210 nm): t_r = 15.2 min and 17.7 min (major); 97 % ee; $[\alpha]_D^{20}$: +26.8 (*c* 1.0, CH_2Cl_2).

(*S*)-1-(4-Methoxyphenyl)-2-nitroethanol (2m):[11a] Yellow oil; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.92 (d, 1 H, -O*H*), 3.78 (s, 3 H, -OC*H*$_3$), 4.44 (dd, ^2J = 13.2 Hz, ^3J = 2.9 Hz 1 H, -C*H*HNO$_2$), 4.56 (dd, ^2J = 13.2 Hz, ^3J = 9.8 Hz, 1 H, -CH*H*NO$_2$), 5.36 (d, ^3J = 9.3 Hz, 1 H, -C*H*OH-), 6.89 (2 H), 7.28 (2 H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 55.6, 70.9, 81.4, 114.6, 127.5, 130.4, 160.2. The enantiomeric excess was determined by HPLC with a Chiralcel OD-H column (heptane/ethanol, 9:1, 0.7 mL min^{-1}, 20 °C, λ = 210 nm): t_r = 21.0 min and 23.3 min (major); 98 % *ee*; $[\alpha]_D^{20}$: +44.4 (*c* 1.0, CH_2Cl_2).[12a]

(*S*)-1-Cyclohexyl-2-nitroethanol (2n):[11a] Colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$):[13b] 0.94–1.45 (m, 5 H), 1.46–1.67 (m, 1 H), 1.67–1.88 (m, 5 H,), 2.75 (br, 1 H, -O*H*), 4.06 (ddd, ^3J = 11.7 Hz, ^3J = 5.9 Hz, ^3J = 2.2 Hz, 1 H, -C*H*OH-), 4.42 (d, ^3J = 10.3 Hz, 1 H, -CH*H*NO$_2$), 4.43 (d, ^3J = 1.5 Hz, 1 H, -C*H*NO$_2$); ^{13}C NMR (125 MHz, $CDCl_3$):[11c] δ = 26.0, 26.1, 26.3, 28.2, 29.0, 41.6, 73.1, 79.5. The enantiomeric excess was determined by HPLC with a Chiralpack AD-H column (heptane/2-propanol, 97:3 0.8 min, 20 °C, λ = 210 nm): t_r = 30.9 min and 33.5 min (major); 99 % *ee*; $[\alpha]_D^{20}$: +22.2 (*c* 1.0, CH_2Cl_2).[7d]

(*S*)-2-Methyl-2-nitro-1-phenylpropan-1-ol

To freshly distilled benzaldehyde (0.20 mL, 2.0 mmol) was added *tert*-butyl methyl ether TBME (2.7 mL). Wt-*Hb*HNL (2.0 mL) diluted with phosphate buffer (pH 7, 50 mM, 2.7 mL) was poured into the aldehyde solution. After stirring for 5 min, 2-nitropropane (1.8 mL, 20 mmol) was added. The mixture was stirred at room temperature for 48 hand then extracted 3× with 10 mL of TBME. The combined extracts were dried over anhydrous Na_2SO_4 and the solvent removed under reduced pressure. The pail yellow oil was purified by column chromatography (cyclohexane/ethyl acetate, 8:1) to afford a solid; yield: 26 mg (7 %); mp 66–67 °C;[15] ^{1}H NMR (500 MHz, $CDCl_3$):[16] δ = 1.45 (s, -C*H*$_3$), 1.58 (s, -C*H*$_3$), 5.31 (s, -C*H*OH-), 7.34–7.39 (m, 5 H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 19.2, 24.6, 78.3, 92.4, 127.8, 128.5, 128.9, 138.5.[17] The enantiomeric excess was determined by HPLC with a Chiralpack AD-H column (heptane/2-propanol, 9:1, 0.9 mL min^{-1}, 20 °C, λ = 210 nm): t_r = 8.2 min (major) and 9.8 min; 70 % *ee*; $[\alpha]_D^{20}$: +4.4 (*c* 0.8, CH_2Cl_2).

(1*S*,2*R*)-2-Nitro-1-phenylpropan-1-ol[11d,17] (3; Major Product)

Wt-*Hb*HNL (127 mL), phosphate buffer (pH 7, 50 mM, 85 mL) and *tert*-butyl methyl ether (164 mL) were stirred vigorously in a 3-necked flask until an emulsion was established (30 min). To the mixture freshly distilled benzaldehyde (12.9 mL, 127 mmol) was added. After stirring for 5 min, nitroethane (127 mL, 1.27 mol) was added dropwise over 3 h. The reaction mixture was stirred at room temperature for 48 h, then centrifuged. The aqueous layer was extracted twice with 250 mL of diethyl ether. The combined organic solutions were dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The yellow oil was purified by column chromatography (cyclohexane/ethyl acetate, 16:1) to afford colorless needles; yield: 15.0 g (65 %); mp 38 °C; ^{1}H NMR (500 MHz, $CDCl_3$):[18] δ = 1.31 (d, ^3J = 6.8 Hz, 3 H, -C*H*$_3$ in *syn*-isomer), 1.50 (d, ^3J = 6.8 Hz, 3 H, -C*H*$_3$ in *anti*-isomer), 2.85 (s, -CHO*H*-), 4.69 (dq, ^3J = 3.4 Hz, ^3J = 6.8 Hz, 1 H, -C*H*NO$_2$- in *anti*-isomer), 4.78 (dq, ^3J = 9.3 Hz, ^3J = 6.8 Hz, 1 H, -C*H*NO$_2$- in *syn*-isomer), 5.03 (d, ^3J = 9.3 Hz, 1 H, -C*H*OH- in *syn*-isomer), 5.40 (d, ^3J = 3.4 Hz, 1 H, -C*H*OH- in *anti*-isomer), 7.38–7.40 (m, 5 H); ^{13}C NMR (125 MHz, $CDCl_3$): *anti*: δ = 12.3, 74.2, 87.7, 126.2, 128.8, 129.0, 138.7. *syn*: 16.7, 76.5, 88.7, 127.2, 129.3, 129.5, 138.4. The enantiomeric excess was determined by HPLC with a Chiralpack AD-H column (heptane/2-propanol, 9:1, 0.9 mL min^{-1}, 20 °C, λ = 210 nm): t_1 = 9.4 min (1*S*,2*R*) (major), t_2 = 10.5 min (1*R*,2*S*), t_3 = 12.2 min (1*S*,2*S*), t_4 = 13.6 min (1*R*,2*R*); *ee anti*: 95 %, *ee syn*: 53 % , *anti*/*syn* 9:1.

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