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Biocatalytic Approaches to the Henry (Nitroaldol) Reaction

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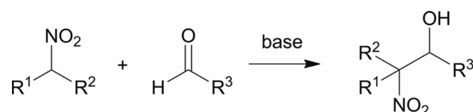
Enantiopure β-nitro alcohols are key chiral building blocks for the synthesis of bioactive pharmaceutical ingredients. The preparation of these target compounds in optically pure form has been the focus of much research and there has been an emergence of biocatalytic protocols in the past decade. For the first time, these biotransformations are the focus of this review. Herein, we describe two principal biocatalytic approaches to the Henry (nitroaldol) reaction. The first method is a direct enzyme-catalysed carbon–carbon bond formation resulting in either an enantio-enriched or enantiopure β-nitro alcohol. The second approach describes the Henry reaction without stereocontrol followed by a biocatalytic resolution to yield the enantiopure β-nitro alcohol.

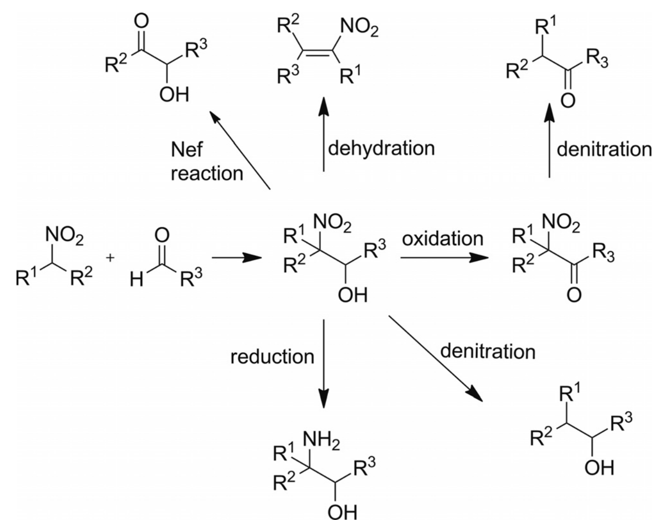
Introduction

The construction of carbon–carbon bonds is an essential element of synthetic organic chemistry. Among the various C–C bond forming reactions, the nitroaldol or Henry reaction[1] is one of the classical named reactions in organic synthesis. Essentially, this reaction describes the coupling of a nucleophilic nitro alkane with an electrophilic aldehyde or ketone to produce a synthetically useful β-nitro alcohol (Scheme 1).[2–5] Moreover, the Henry reaction facilitates the joining of two molecular fragments, under mild reaction conditions with the potential formation of two new stereogenic centres and a new C–C bond. The resulting β-nitro alcohols can undergo a variety of useful chemical transformations which lead to synthetically useful structural motifs, e.g. dehydration to conjugated nitro alkenes, reduction to 1,2-amino alcohols, denitration, oxidation to nitro carbonyl compounds and α-hydroxy carbonyl compounds via the Nef reaction (Scheme 2).[6–8]



Scheme 1. Henry reaction.

β-Nitro alkanols have been employed in the synthesis of many key intermediates to access biologically active compounds including natural products, insecticides, fungicides and antibiotics.[9–14] Furthermore, β-amino alcohols are constituents of many active pharmaceutical ingredients[15–19], e.g. sphingosine and ephedrine, which highlights the importance of the Henry reaction as a source of chiral building blocks (Scheme 3).[7]



Scheme 2. Synthetic utility of the Henry reaction.

The Henry reaction is usually performed at room temperature in the presence of typically about 10 mol-% base to give the desired β-nitro alcohol in good yields. A vast array of bases have been employed to perform this transformation; the most popular bases include carbonates, bicarbonates, alkali metal hydroxides, alkoxides and organic nitrogen bases. Unusual catalysts include the rare earth metal alkoxides, rare earth hexamethyldisilazides and binaphthol–rare earth metal complexes.[7] These reactions are often complicated by the formation of undesired side products, due to the ability of these strong bases to catalyse unwanted

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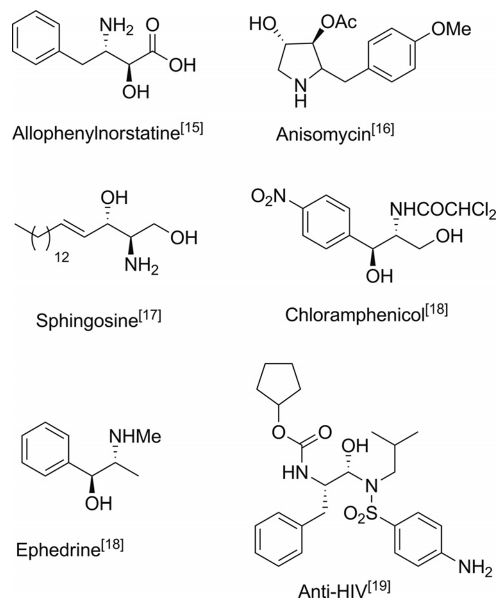
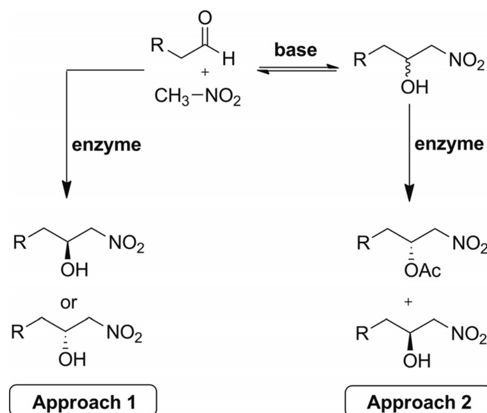


Scheme 3.

side reactions such as aldol, Cannizzaro and water elimination reactions.[5,20] More recent research has led to the development of mild reaction conditions which prevent the formation of unwanted side products, e.g. solvent free[21,22] or in aqueous media.[23]

Enzymatic Approaches to the Henry Reaction

Principal methods to the catalytic asymmetric Henry (nitroaldol) reaction include transition metal- and organocatalysed methods and these have been reviewed in detail.[3,24–26] In the past decade however, there has been an emergence of biocatalytic protocols. There are two biocatalytic approaches to enantio-enriched products of the Henry reaction reported in the literature; direct enzyme-catalysed nitroaldol reaction or initial chemical formation of the β-nitro alcohol product followed by enzymatic kinetic resolution of the stereoisomers (Scheme 4).



Scheme 4.

Biocatalytic Carbon–Carbon Bond Formation by the Henry Reaction

Only a few enzyme classes are capable of catalysing carbon–carbon bond forming reactions, among these are the hydroxy nitrile lyases (HNL also referred to as oxynitrilases).[27] These enzymes were originally isolated from

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Anita Maguire undertook undergraduate and postgraduate studies at University College Cork (B.Sc. 1985, Ph.D. 1989), focusing during her Ph.D on asymmetric catalysis in reactions of α-diazo ketones. Following postdoctoral research in the Facultés Universitaires, Namur, Belgium and subsequently at the University of Exeter, she returned to Cork in 1991. Her research interests include development of new synthetic methodology including organosulfur chemistry, asymmetric synthesis including biocatalysis, and the design and synthesis of bioactive compounds with pharmaceutical applications.

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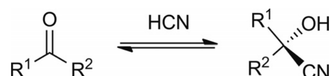
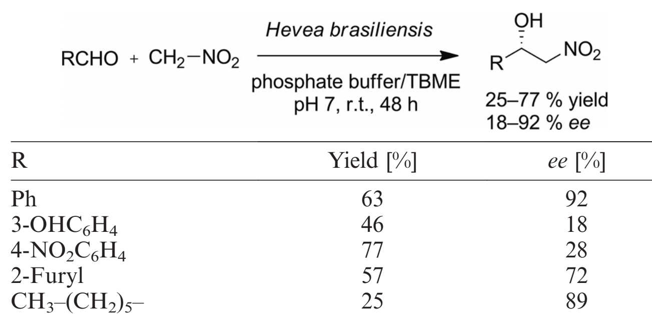
plants. The most common sources are almonds (*Prunus* sp.), rubber trees (*H. brasiliensis*) and flax (*Linum usitassimum*). Hydroxy nitrile lyases are traditionally known to catalyse the stereoselective addition of hydrocyanic acid to aldehydes or ketones to yield enantiomerically pure α-hydroxy nitriles (Scheme 5).[28,29] Moreover, these enzymes have been found to be tolerant of a wide array of electrophiles including a range of aliphatic, aromatic and heterocyclic carbonyl compounds. In contrast, until recently[30] the only accepted nucleophile of hydroxy nitrile lyases was hydrocyanic acid.[28,29]



Scheme 5.

In order to extend the applications of hydroxy nitrile lyases, Griengl et al. examined this enzyme class for nitroaldolase activity. The hydroxy nitrile lyase from *H. brasiliensis* (EC 4.1.2.39) was found to catalyse the reaction between a range of aromatic, heteroaromatic and aliphatic aldehydes with nitromethane to yield enantiomerically enriched β-nitro alcohols (Table 1). However, small amounts of the alkene elimination product (10–15%) were also observed. Furthermore, the biocatalysed reaction requires long reaction times and large amounts of enzyme, and also suffers from low conversions.[30] The kinetics of the *H. brasiliensis* hydroxy-nitrile-catalysed Henry reaction have been demonstrated to fit the classical Rapid Equilibrium Random Bi Uni model with independent substrate binding and it was concluded that the bottleneck of this enzymatic transformation is a very low turnover of the enzyme as opposed to substrate binding. The explanation for this has not yet been established.[31]

Table 1. *H. brasiliensis*-catalysed Henry reaction.[30]

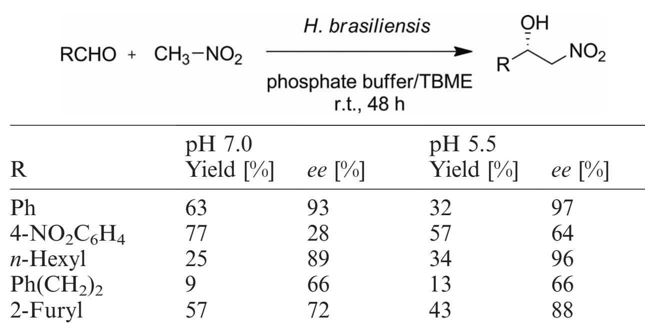


R	Yield [%]	*ee* [%]
Ph	63	92
3-OHC_6H_4	46	18
4-$NO_2C_6H_4$	77	28
2-Furyl	57	72
CH_3–$(CH_2)_5$–	25	89

The absolute configuration of the products is *S* which is in agreement with the known stereopreference of *H. brasiliensis* in cyanohydrin reactions. The hydroxy nitrile lyase from *Manihot esculenta* was also found to catalyse the nitroaldol reaction but with reduced activity and stereoselectivity. The *R*-selective hydroxy nitrile lyase from *Prunus amygdalus* was also examined and found to be inactive.[30]

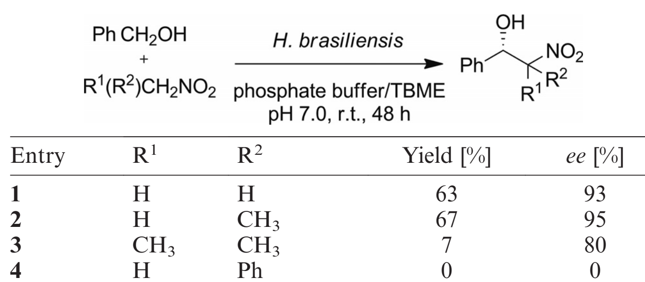
A number of reaction parameters were subsequently examined by the same research group in order to optimise this enzymatic transformation. It was found that the optimum reaction pH is 5.5 (Table 2). Furthermore, an aqueous/organic phase ratio of 1:2 was found to improve both the conversion and enantioselectivity of the reaction. Other nitro alkanes were also investigated; it was found with increase of steric bulk activity was decreased, for example substitution of nitromethane for (nitromethyl)benzene led to complete loss of enzymatic activity (Table 3).[32] Therefore, while oxynitrilases provide an attractive system for the asymmetric Henry reaction, the narrow substrate range is a significant limitation of this process. Additionally, although the oxynitrilase from *M. esculenta* is commercially available, the far more active analogue from *H. brasiliensis* is not currently available.

Table 2. *H. brasiliensis*-catalysed Henry reaction; pH investigation.[32]



R	pH 7.0 Yield [%]	*ee* [%]	pH 5.5 Yield [%]	*ee* [%]
Ph	63	93	32	97
4-$NO_2C_6H_4$	77	28	57	64
n-Hexyl	25	89	34	96
$Ph(CH_2)_2$	9	66	13	66
2-Furyl	57	72	43	88

Table 3. Henry reaction of other nitro alkanes.[32]



Entry	R^1	R^2	Yield [%]	*ee* [%]
1	H	H	63	93
2	H	CH_3	67	95
3	CH_3	CH_3	7	80
4	H	Ph	0	0

Recently Asano et al. reported for the first time an *R*-selective hydroxy nitrile lyase from *Arabidopsis thaliana*. As is evident from Table 4 moderate to excellent enantioselectivity was achieved. However, a maximum of 30% yield was achieved and the reaction is highly substrate dependent. The optimum reaction conditions are an aqueous-organic (*n*-butyl acetate) mixture (50:50), a large amount of enzyme (4000 U/mmol) and a pH of 7.[33] The hydroxy nitrile lyase from *H. brasiliensis* has also been described to catalyse the retro-Henry reaction (Scheme 6), however, this reaction suffered from low enantioselection due to product inhibition by benzaldehyde.[34] Liese et al. overcame this product inhibition by the conversion of benzaldehyde to (*S*)-mandelonitrile. Separation of (*S*)-mandelonitrile from (*R*)-2-nitro-1-phenylethanol proved cumbersome, therefore, a nitrile hydratase (NHase) was employed to catalyse the hydration and the resultant primary amide was separable by column chromatography (Scheme 6). Employment of this

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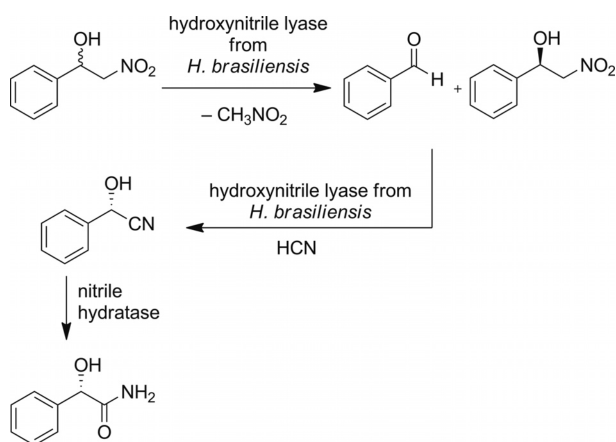
approach led to the formation of (*R*)-2-nitro-1-phenylethanol in good conversion and with enantioselectivities of up to 95% *ee* and 49% conversion in a one phase system.[34]

Table 4. *R*-selective hydroxy nitrile lyase-catalysed Henry reaction.[33]

R	Time [h]	Yield [%]	*ee* [%]
Ph	2	30	91
Ph	4	26	86
2-MeC_6H_4	2	12	95
3-MeC_6H_4	2	12	96
4-MeC_6H_4	2	11	94
2-$MeOC_6H_4$	2	13	90
3-$MeOC_6H_4$	2	17	91
4-$MeOC_6H_4$	2	2	79
2-ClC_6H_4	2	34	68
3-ClC_6H_4	2	17	91
4-ClC_6H_4	2	9	87
4-FC_6H_4	2	20	81
4-BrC_6H_4	2	9	82
2-Naphthyl	2	7	> 99.9
$Me(CH_2)_4$	2	trace	> 80
$Me(CH_2)_8$	2	no reaction	–



Scheme 6.

It should be noted at this point that a number of other enzyme systems have been reported to be effective catalysts for the Henry reaction, however, no enantioselectivity data has been reported thus far.[35–37] Zhu et al. examined the model reaction involving addition of nitromethane to *p*-nitrobenzaldehyde with a range of enzyme systems (Table 5). The highest level of activity was seen with a transglutaminase from *Streptoverticillium griseoverticillatum*.[35] Therefore, to generalise this, the addition of nitro alkanes with aliphatic, aromatic and heteroaromatic aldehydes was examined using this transglutaminase enzyme. Moderate to good yields were achieved and selected examples are described in Table 6. However, as is evident from both Tables 5 and 6, no enantioselectivity was reported.[35]

Table 5. Investigation of the catalytic activities of different enzymes in effecting the Henry reaction.[35]

Entry	Catalyst	Time [h]	Yield [%]
1	Transglutaminase from *Streptoverticillium griseoverticillatum*	32	93
2	Immobilized lipase from *Thermomyces lanuginosus*	48	64
3	Pancreatin from porcin pancreas	48	41
4	Papain from fruit jam from *chaenomeles*	48	15
5	Lysozyme from hen egg white	48	13
6	Chymosin from fruit jam from *chaenomeles*	48	13
7	Alkaline proteinase from *B. licheniformis* No. 2709	48	12
8	Nuclease from *Penicillium citrinum*	48	11
9	Acidic proteinase from *Aspergillus usamii* No. 537	48	trace
10	Neutral proteinase from *Bacillus subtilis* A.S.1.398	48	trace
11	Trypsin from porcin pancreas	48	trace
12	Bromelain from pineapple peduncle	48	trace
13	Cellulase from *Trichoderma*	48	trace
14	no enzyme	120	10
15	Bovine serum albumin (B.S.A.)	48	16
16	TGase denatured with EDTA	120	0
17	TGase inhibited with NBS	48	12

Table 6. Transglutaminase-mediated Henry reaction.[35]

R^1	R^2	Time [h]	Yield [%]	*anti*/*syn*
Ph	H	96	58	–
2-$MeOC_6H_4$	H	120	51	–
3-$MeOC_6H_4$	H	120	59	–
4-$MeOC_6H_4$	H	120	50	–
2-Thienyl	H	120	21	–
2-Furyl	H	120	12	–
Ethyl	H	48	61	–
Isobutyl	H	48	74	–
4-$MeOC_6H_4$	CH_3	144	40	1:2.3
4-$CH_3C_6H_4$	CH_3	144	46	1:1.3
4-$NO_2C_6H_4$	CH_3	72	90	1:1.3
Isobutyl	CH_3	72	77	1:1

A number of hydrolases have exhibited nitroaldol activity, including the hydrolase from bovine serum albumin which catalysed the addition of both nitromethane and nitroethane to a range of aromatic and heteroaromatic aldehydes. Once again no enantioselectivity data was reported. Isolated yields were in the range of 46–91%. In this case negligible *anti*-selectivity was observed.[37] Other hydrolases reported to display nitroaldol activity include those from *Candida antarctica* [Lipase B (CALB), immobilised], *C. cylindracea*, hog pancreas, D-aminoacylase from *E. coli*, acylase "Amano" from *Aspergillus oryzae*, penicillin G acylase

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from *E. coli* (immobilised), Lipozyme immobilised from *Mucor meihei*, Lipase AK "Amano" and Amano Lipase M from *Mucor javanicus*.[36]

Lin et al. found D-aminoacylase from *E. coli* to be the most effective catalyst for the addition of either nitromethane or nitroethane to a range of aliphatic, aromatic and heteroaromatic aldehydes. The authors did comment in this instance that enantioselectivity was examined, but no stereoinduction was observed when examined by chiral stationary phase HPLC.[36] A rabbit muscle FDP (fructose 1,6-diphosphate) aldolase has also been reported to catalyse an intramolecular nitroaldol reaction in the synthesis of nitrocyclitols. Once again, no optical purity data was reported.[38]

Enzymatic Kinetic Resolution of the Products of the Henry Reaction

Another enzymatic route to enantiopure β-nitro alcohols is to employ an enzymatic kinetic resolution step in conjunction with the Henry reaction (approach 2, Scheme 4). Hydrolase-mediated kinetic resolution of secondary alcohols proceeds with excellent stereoselectivity across a wide range of substrates.[39,40] Early examples of biocatalytic resolution of nitro alcohols include those published by Kitayama et al.; this group reported the hydrolase-catalysed stereoselective preparation of four nitro alcohols. In addition, the effects of organic solvent over the course of hydrolase-mediated transesterification with Amano AK from *P. sp.* were examined; an increase in *E* value was reported with the use of *n*-propyl ether as solvent (Table 7).[41]

Table 7. Effect of solvent on the *E* value of hydrolase-mediated acetylation.

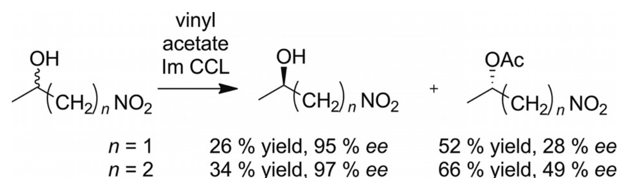
R	*E*					
	Dioxane	THF	Benzene	AcOEt	Hexane	*n*-Propyl ether
C_2H_9	4.8	4.1	5.6	21.9	9.6	20.9
C_3H_7	1.2	1.1	1.4	1.4	1.0	2.1
i-C_3H_7	1.3	1.9	2.4	1.7	6.7	12.5
C_4H_9	1.2	1.6	1.6	1.4	2.5	3.4

Barua et al.[42] were the first to examine a wide range of 2-nitro alcohols (12 in total) with a range of hydrolases including those from *Humicola lanuginose*, *C. antarctica*, *Rhizomucor meihei*, *C. rugosa* and *P. fluorescens*. The hydrolase from *P. fluorescens* was found to be the most effective for the transesterification of nitro alcohols at 30 °C. The elimination product was observed in the majority of aromatic systems; enantiopurities of the acetates ranged from 66–98% *ee*. Some examples of the aforementioned systems studied are depicted in Table 8.[42]

Table 8. Hydrolase-mediated kinetic resolution of a range of 2-nitro alcohols.

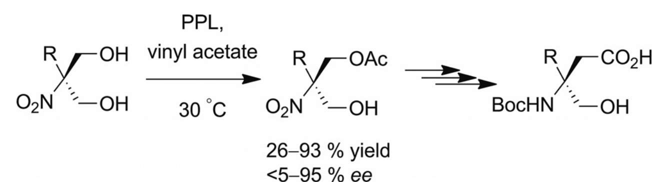
Substrate R^1	Reaction time	Alcohol *ee* [%]	Acetate *ee* [%]	*E*
Benzaldehyde	48	85 (*R*)	–	–
n-Butanal	24	85 (*R*)	83(*S*)	51
n-Hexanal	24	94 (*R*)	84(*S*)	53
n-Heptanal	24	88 (*R*)	90(*S*)	49

Hydrolase-mediated acylation and deacylation have been employed to gain access to enantio- and diastereomerically enriched β- and γ-nitro alcohols.[43–45] It is also worth noting that the hydrolysis of 2-nitrocyclohexyl butyrate with *C. cylindracea* Lipase (CCL) hydrolase in high enantioselectivity was achieved in 1989 by Honig et al. as a route to cyclic amino alcohol precursors.[46] Stereoselective transesterification of (*R*)-4-nitro-2-butanol and (*R*)-5-nitro-2-pentanol has been performed by *C. cylindracea* hydrolase immobilised on Celite® (Im CCL) with vinyl acetate as acyl donor. Only modest enantiomeric purities were achieved for the transformed acetate (28% *ee* in the case of the butyl ester and 49% *ee* for the pentyl ester) (Scheme 7).[47]



Scheme 7.

Selective acylation of substituted 2-nitropropane-1,3-diols mediated by porcine pancreatic hydrolase has been utilised as a new entry to the asymmetric synthesis of α-substituted serine analogues with high enantiopurity in some cases (Scheme 8).[48]



Scheme 8.

Use of hydrolases has been identified as a potential biocatalytic system for the resolution of the products of the Henry reaction. One of the first reports of hydrolase-mediated resolutions of a nitro aldol adduct describes an enantioselective saponification with pig liver esterase of a *meso*-nitroacetate substrate; high enantiopurity with moderate yields were obtained (Scheme 9).

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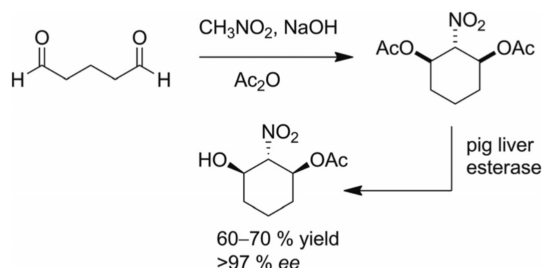
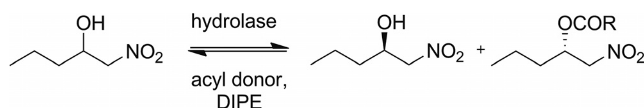


Scheme 9.

The first systematic study of a hydrolase-catalysed resolution of nitro aldol adducts in conjunction with the Henry reaction was reported in 2004.[49] The synthesis of a range of aliphatic β-nitro alcohols via the Henry reaction, followed by an enzymatic kinetic resolution was described (Scheme 10).



Scheme 10.

A number of hydrolases were examined for the enzymatic kinetic resolution of 1-nitro-2-pentanol including Novozym 435®, an immobilised preparation of *C. antractica* lipase B (CALB), two cross linked preparations of *C. antractica* hydrolase B, *C. antractica* hydrolase A, *Rhizopus meihei*, *C. rugosa*, *C. lipolytica* and *Burkholderia cepacia* in diisopropyl ether (DIPE) with succinic anhydride as acyl donor. CALB provided access to the best enantiospecificities. In order to optimise the process further, use of acyl donors succinic anhydride and vinyl acetate were investigated in a range of solvents as can be seen from Table 9.

Overall, CALB in diisopropyl ether and succinic anhydride led to the highest conversions and selectivities; however, this system was substrate-dependent (see Table 10).

Vongvilai et al. made two contributions to the biocatalytic resolution of β-nitro alcohols. Firstly, a dynamic combinatorial biocatalytic resolution process was described, whereby a range of aromatic aldehydes were reacted in the presence of triethylamine with 2-nitropropane and subsequently resolved with the hydrolase *P. cepacia* and *p*-chlorophenyl acetate as the acyl donor. A variety of substitutions were examined with enantioselectivities up to 99% *ee* reported.[50] This work was expanded to convert this kinetic bioresolution process to a one-pot reaction combining a nitroaldol Henry reaction and a dynamic kinetic enzyme-mediated resolution process. The first step required the investigation of a suitable catalyst for this enzymatic transformation (Table 11).

Table 9. Kinetic resolution of 1-nitro-2-pentanol in the presence of CALB; effect of solvent.

Solvent	Succinic anhydride % Conv. (24 h)	ee_s [%]	ee_p [%]	*E*	Vinyl acetate % Conv. (24 h)	ee_s [%]	ee_p [%]	*E*
$MeNO_2$	< 1	0	1	–	3	1	45	3
ACN	8	2	100	2	–	–	–	–
DME	4	3	76	7	37	38	52	5
TBME	42	70	95	100	46	41	35	3
DIPE	54	92	93	82	37	15	25	2

Table 11. Effect of hydrolase source on the kinetic bioresolution of 2-methyl-2-nitro-1-(4-nitrophenyl)propan-1-ol.

Enzyme source	Conversion	*ee* [%]	*E*
C. antarctica B	5	78	8
C. rugosa	0	0	0
P. cepacia	10	0	1
P. cepacia C1	11	99	> 200
P. cepacia C2	10	90	21
P. fluorescens	7	93	30

Table 10. Effect of substitution on reaction efficiency.

R	Succinic anhydride % Conv. (24 h)	*ee* [%]	*ee* [%]	*E*	Vinyl acetate % Conv. 24 h	*ee* [%]	*ee* [%]	*E*
CH_3	39	57	67	28 (*R*)	30	2	5	1 (*R*)
C_2H_5	47	44	43	4 (*R*)	72	1	–	1 (*R*)
C_3H_7	54	92	93	82 (*S*)	37	15	25	2 (*S*)
C_6H_5	4	3	75	7 (*S*)	13	13	100	20 (*S*)
$(C_6H_5)CH_2$	0	–	–	–	53	34	–	2 (*S*)
$(C_6H_5)C_2H_4$	42	71	97	96 (*S*)	56	21	100	2 (*S*)

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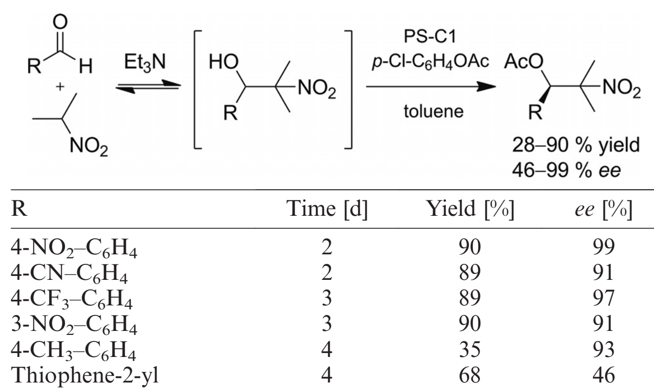
P. cepacia C1 was the most effective catalyst. Moreover, in combination with an increase of the amount of enzyme catalyst and temperature to 40 °C, the conversion was driven to 46% with conservation of enantiopurity. The next step examined a one-pot combined nitroaldol reaction with hydrolase-catalysed transesterification. A range of aldehydes were examined; it should be highlighted that the aliphatic aldehydes resulted in low enantiospecificities when compared with the aromatic aldehydes. Furthermore, the reactions required long reaction times (2–4 days). Some examples of these transformations are shown in Table 12.[51] While this paper clearly demonstrates the feasibility of a combination of the Henry reaction with enzyme-mediated dynamic resolution it is clear that there are significant limitations to the process as developed.

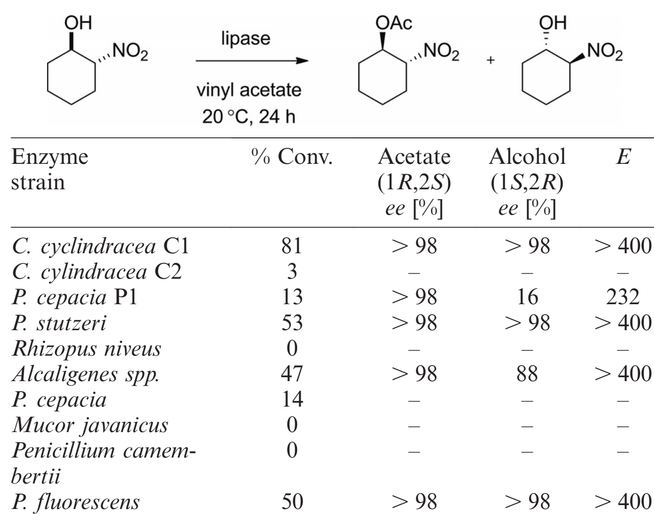
Table 12. Hydrolase-mediated dynamic kinetic resolution of β-nitro alcohols.



R	Time [d]	Yield [%]	*ee* [%]
$4\text{-}NO_2\text{–}C_6H_4$	2	90	99
$4\text{-}CN\text{–}C_6H_4$	2	89	91
$4\text{-}CF_3\text{–}C_6H_4$	3	89	97
$3\text{-}NO_2\text{–}C_6H_4$	3	90	91
$4\text{-}CH_3\text{–}C_6H_4$	4	35	93
Thiophene-2-yl	4	68	46

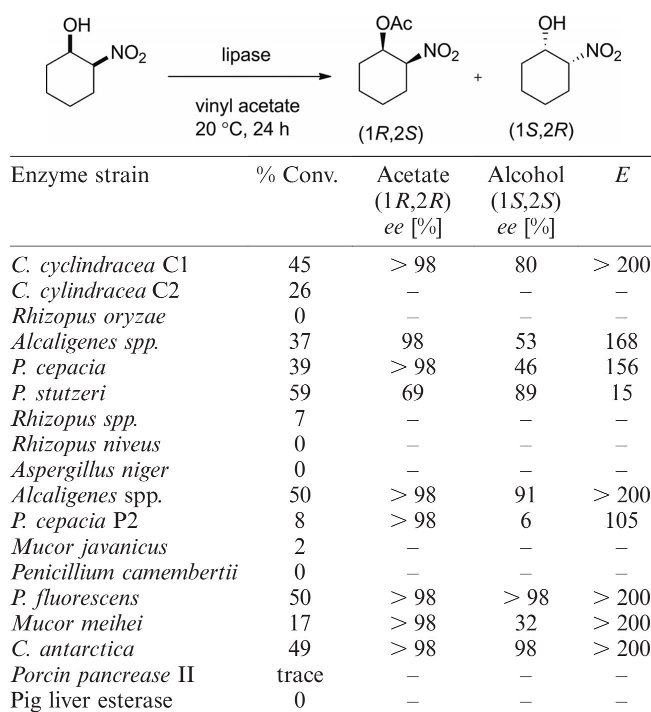
The enzyme-catalysed kinetic resolution of 2-nitrocyclohexanol was investigated by screening a range of hydrolases both for enantioselective transesterification and for enantioselective hydrolysis of the corresponding acetate. By appropriate choice of biocatalyst and conditions, both enantiomers of *cis* and *trans*-2-nitrocyclohexanol can be accessed in enantiopure form (Tables 13 and 14).[52]

Table 13. Hydrolase mediated transesterification of (±)-*trans*-2-nitrocyclohexanol in vinyl acetate as solvent and acyl donor.



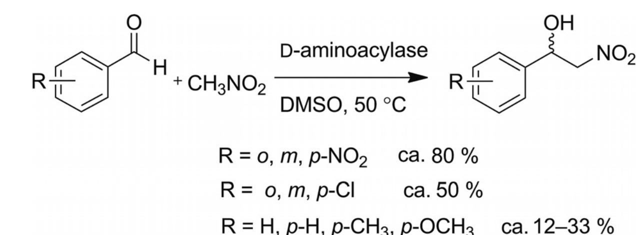
Enzyme strain	% Conv.	Acetate (1*R*,2*S*) *ee* [%]	Alcohol (1*S*,2*R*) *ee* [%]	*E*
C. cyclindracea C1	81	> 98	> 98	> 400
C. cylindracea C2	3	–	–	–
P. cepacia P1	13	> 98	16	232
P. stutzeri	53	> 98	> 98	> 400
Rhizopus niveus	0	–	–	–
Alcaligenes spp.	47	> 98	88	> 400
P. cepacia	14	–	–	–
Mucor javanicus	0	–	–	–
Penicillium camembertii	0	–	–	–
P. fluorescens	50	> 98	> 98	> 400

Table 14. Hydrolase-mediated transesterification of (±)-*cis*-2-nitrocyclohexanol in vinyl acetate as solvent and acyl donor.



Enzyme strain	% Conv.	Acetate (1*R*,2*R*) *ee* [%]	Alcohol (1*S*,2*S*) *ee* [%]	*E*
C. cyclindracea C1	45	> 98	80	> 200
C. cylindracea C2	26	–	–	–
Rhizopus oryzae	0	–	–	–
Alcaligenes spp.	37	98	53	168
P. cepacia	39	> 98	46	156
P. stutzeri	59	69	89	15
Rhizopus spp.	7	–	–	–
Rhizopus niveus	0	–	–	–
Aspergillus niger	0	–	–	–
Alcaligenes spp.	50	> 98	91	> 200
P. cepacia P2	8	> 98	6	105
Mucor javanicus	2	–	–	–
Penicillium camembertii	0	–	–	–
P. fluorescens	50	> 98	> 98	> 200
Mucor meihei	17	> 98	32	> 200
C. antarctica	49	> 98	98	> 200
Porcin pancrease II	trace	–	–	–
Pig liver esterase	0	–	–	–

The final example describes the employment of both the direct enzyme-mediated nitroaldol addition and subsequent kinetic resolution of the nitroaldol products. Initial formation of a series of racemic β-nitro alcohols was effected with D-aminoacylase as catalyst with conversions ranging from 50 to 80% yield (Scheme 11).[53]



Scheme 11.

A number of hydrolases were examined for the kinetic resolution step and it was found immobilised hydrolases from *B. cepacia* (PS-IM) showed the highest activity and enantioselectivtity. A summary of both the enantioselectivity and conversion data is shown in Table 15.[53]

Conclusions

Beyond doubt, the enzyme-catalysed kinetic resolution of the products of the Henry reaction is well developed and an efficient method to gain access to enantiopure β-nitro

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Table 15. Kinetic resolution of β-nitro alcohols.[53]

R	ee_s [%]	ee_p [%]	% Conversion	*E*
p-NO_2	97	> 99	48	> 200
m-NO_2	95	99	49	> 200
o-NO_2	–	–	< 1	–
p-Cl	97	98	46	> 200
m-Cl	91	98	47	> 200
o-Cl	–	–	< 1	–
H	95	96	47	155
p-CH_3	84	99	48	> 200
p-OCH_3	84	98	49	> 200

alcohols, albeit with a maximum of 50% yield. Initial reports of a dynamic biocatalytic resolution process do show promise, however, the narrow substrate range is a serious limitation and further investigation is warranted. The enzyme-catalysed production of enantiopure β-nitro alcohols is an attractive protocol, however, to date limited research has been performed in the area. This may be in part attributed to the absence of commercially available hydroxy nitrile lyases, although improvements in this area are being made. Further investigation into the use of genetic engineering is warranted to increase the substrate scope, operating conditions and general applicability of hydroxy nitrile lyase.

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[1] L. C. R. Henry, *Hebd. Seances Acad. Sci.* **1895**, 1265.
[2] H. Feuer, *The Chemistry of the Nitro and Nitroso Groups*, Wiley Interscience, New York, **1970**, vol. 2.
[3] F. A. Luzzio, *Tetrahedron* **2001**, *57*, 915–945.
[4] Steven V. Ley, in: *Comprehensive organic functional group transformations* (Ed.: A. R. Katritzky, O. Meth-Cohn, C. W. Rees), Cambridge University Press, Cambridge, UK, **1995**, vol. 2.
[5] G. Rosini, *Comprehensive Organic Synthesis*, Pergamon, New York, **1992**, vol. 2.
[6] R. S. Varma, R. Dahiya, S. Kumar, *Tetrahedron Lett.* **1997**, *38*, 5131–5134.
[7] N. Ono, *The Nitro Group in Organic Synthesis*, Wiley-VCH, New York, **2001**, pp. 30–69.
[8] W. E. Noland, *Chem. Rev.* **1955**, *55*, 137–155.
[9] P. B. Kisanga, J. G. Verkade, *J. Org. Chem.* **1999**, *64*, 4298–4303.
[10] R. J. Heffner, J. J. Jiang, M. M. Joullie, *J. Am. Chem. Soc.* **1992**, *114*, 10181–10189.
[11] R. Ballini, *J. Chem. Soc., Perkin Trans. 1* **1991**, 1419–1421.
[12] O. Sakanaka, T. Ohmori, S. Kozaki, T. Suami, *Bull. Chem. Soc. Jpn.* **1986**, *59*, 3523–3528.
[13] T. Suami, H. Sasai, K. Matsuno, *Chem. Lett.* **1983**, 819–822.
[14] G. Mikite, E. Jakucs, A. Kistamas, F. Darvas, A. Lopata, *Pestic. Sci.* **1982**, *13*, 557–562.
[15] M. E. Bunnage, S. G. Davies, C. J. Goodwin, O. Ichihara, *Tetrahedron* **1994**, *50*, 3975–3986.
[16] U. Veith, O. Schwardt, V. Jager, *Synlett* **1996**, 1181–1183.
[17] P. M. Koskinen, A. M. P. Koskinen, *Synthesis* **1998**, 1075–1091.
[18] D. A. Lednicer, *The Organic Chemistry of Drug Synthesis*, John Wiley & Sons, New York, **1975**.
[19] N. Shibata, T. Katoh, S. Terashima, *Tetrahedron Lett.* **1997**, *38*, 619–620.
[20] M. G. A. Shvekhgeimer, *Usp. Khim.* **1998**, *67*, 39–74.
[21] A. Majhi, S. T. Kadam, S. S. Kim, *Bull. Korean Chem. Soc.* **2009**, *30*, 1767–1770.
[22] R. Ballini, G. Bosica, M. Parrini, *Tetrahedron Lett.* **1998**, *39*, 7963–7964.
[23] R. Ballini, G. Bosica, *J. Org. Chem.* **1997**, *62*, 425–427.
[24] C. Palomo, M. Oiarbide, A. Laso, *Eur. J. Org. Chem.* **2007**, 2561–2574.
[25] C. S. Gan, J. Pan, *Chin. J. Org. Chem.* **2008**, *28*, 1193–1198.
[26] T. Marcelli, R. N. S. van der Haas, J. H. van Maarseveen, H. Hiemstra, *Angew. Chem.* **2006**, *118*, 943; *Angew. Chem. Int. Ed.* **2006**, *45*, 929–931.
[27] M. Breuer, B. Hauer, *Curr. Opin. Biotechnol.* **2003**, *14*, 570–576.
[28] M. Sharma, N. N. Sharma, T. C. Bhalla, *Enzyme Microb. Technol.* **2005**, *37*, 279–294.
[29] F. Effenberger, S. Forster, H. Wajant, *Curr. Opin. Biotechnol.* **2000**, *11*, 532–539.
[30] T. Purkarthofer, K. Gruber, M. Gruber-Khadjawi, K. Waich, W. Skranc, D. Mink, H. Griengl, *Angew. Chem.* **2006**, *118*, 3532; *Angew. Chem. Int. Ed.* **2006**, *45*, 3454–3456.
[31] R. Yuryev, T. Purkarthofer, M. Gruber, H. Griengl, A. Liese, *Biocatal. Biotransform.* **2010**, *28*, 348–356.
[32] M. Gruber-Khadjawi, T. Purkarthofer, W. Skranc, H. Griengl, *Adv. Synth. Catal.* **2007**, *349*, 1445–1450.
[33] K. Fuhshuku, Y. Asano, *J. Biotechnol.* **2011**, *153*, 153–159.
[34] R. Yuryev, S. Briechle, M. Gruber-Khadjawi, H. Griengl, A. Liese, *ChemCatChem* **2010**, *2*, 981–986.
[35] R. C. Tang, Z. Guan, Y. H. He, W. Zhu, *J. Mol. Catal. B: Enzym.* **2010**, *63*, 62–67.
[36] J.-L. Wang, X. Li, H.-Y. Xie, B.-K. Liu, X.-F. Lin, *J. Biotechnol.* **2010**, *145*, 240–243.
[37] E. Busto, V. Gotor-Fernandez, V. Gotor, *Org. Process Res. Dev.* **2011**, *15*, 236–240.
[38] W. C. Chou, C. Fotsch, C. H. Wong, *J. Org. Chem.* **1995**, *60*, 2916–2917.
[39] A. Ghanem, H. Y. Aboul-Enein, *Tetrahedron: Asymmetry* **2004**, *15*, 3331–3351.
[40] S. M. Roberts, *Preparative Biotransformations, Whole Cell and Isolated Enzymes in Organic Synthesis*, John Wiley & Sons, New York, **1997**.
[41] T. Kitayama, T. Rokutanzono, R. Nagao, Y. Kubo, M. Takatani, K. Nakamura, T. Okamoto, *J. Mol. Catal. B: Enzym.* **1999**, *7*, 291–297.
[42] J. C. Borah, J. Boruwa, B. Kalita, A. K. Hazarika, N. C. Barua, *Ind. J. Chem. B* **2005**, *44*, 1961–1965.
[43] B. Schwartz, D. G. Drueckhammer, *J. Am. Chem. Soc.* **1996**, *118*, 9826–9830.
[44] B. Morgan, B. R. Sarikonda, D. R. Dodds, M. J. Homann, R. Vail, *Tetrahedron: Asymmetry* **1999**, *10*, 3681–3690.
[45] G. Fantin, M. Fogagnolo, M. E. Guerzoni, E. Marotta, A. Medici, P. Pedrini, *Tetrahedron: Asymmetry* **1992**, *3*, 947–952.
[46] H. Honig, P. Seuferwasserthal, F. Fulop, *J. Chem. Soc., Perkin Trans. 1* **1989**, 2341–2345.
[47] K. Nakamura, Y. Inoue, T. Kitayama, A. Ohno, *Agricol. Biol. Chem.* **1990**, *54*, 1569–1570.

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[48] P. Tian, M. H. Xu, Z. Q. Wang, Z. Y. Li, G. Q. Lin, *Synlett* **2006**, 1201–1204.
[49] M. J. Sorgedrager, R. Malpique, F. van Rantwijk, R. A. Sheldon, *Tetrahedron: Asymmetry* **2004**, *15*, 1295–1299.
[50] P. Vongvilai, M. Angelin, R. Larsson, O. Ramstrom, *Angew. Chem.* **2007**, *119*, 966; *Angew. Chem. Int. Ed.* **2007**, *46*, 948–950.
[51] P. Vongvilai, R. Larsson, O. Ramstrom, *Adv. Synth. Catal.* **2008**, *350*, 448–452.
[52] S. E. Milner, M. Brossat, T. S. Moody, C. J. Elcoate, S. E. Lawrence, A. R. Maguire, *Tetrahedron: Asymmetry* **2010**, *21*, 1011–1016.
[53] F. Xu, J. L. Wang, B. K. Liu, Q. Wu, X. F. Lin, *Green Chem.* **2011**, *13*, 2359–2361.

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