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FEATURE ARTICLE

Enzymatic cleavage and formation of cyanohydrins: a reaction of biological and synthetic relevance

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Hydroxynitrile lyases (Hnls) catalyse enantioselectively both the cleavage and formation of cyanohydrins. Besides the well known (*R*)-selective enzyme from almonds, (*S*)-Hnls have recently become available in larger quantities by cloning and over-expression. The first three-dimensional structure of a Hnl has been established for the enzyme from *Hevea brasiliensis*. These results are the basis for a still broader application of Hnls for biocatalytic asymmetric syntheses on a preparative scale.

Introduction

Hydroxynitrile lyases (Hnls, EC 4.1.2.10, EC 4.1.2.11, EC 4.1.2.37, EC 4.1.2.39) are enzymes which catalyse the cleavage and formation of cyanohydrins.[1] Early work concentrated on the enzyme which is isolated easily from almond meal,[2] and which gives (*R*)-configured cyanohydrins from aromatic and aliphatic aldehydes. Although (*S*)-Hnls are also known (see below) their application for enantioselective biocatalytic syntheses has been hindered by the difficulty of obtaining sufficient amounts for larger scale preparations. Recently, the genes for the (*S*)-Hnls from *H. brasiliensis* and *Manihot esculenta* were cloned and over-expressed[3,4] and the first X-ray structure of a Hnl, the (*S*)-Hnl from *H. brasiliensis*, was elucidated.[5] Therefore, on the basis of suggestions on the mechanism of the enzyme catalysed cyanohydrin reaction (deduced from knowledge of the three-dimensional structure) and due to the availability of both (*R*)- and (*S*)-Hnls, the field now seems open to still broader synthetic applications. For this reason it is appropriate to summarise recent developments here. Although special emphasis is given to the Hnl from *H. brasiliensis* developed in this laboratory, a more general view is intended.

Natural function and biochemical characterization of hydroxynitrile lyases

Cyanogenesis, the release of hydrogen cyanide (HCN) from the tissue of plants or other organisms which occurs upon tissue damage, has been extensively described to date. This source of HCN has been identified in over 2000 plants as being cyanogenic glycosides or cyanolipids. One group of enzymes involved in this catabolism are the hydroxynitrile lyases. Their natural function is the catalysis of cyanohydrin cleavage from cyanogenic glycosides to give HCN and the corresponding aldehydes or ketones.

Cyanogenic glycosides have been found to possess two main functions in nature. (i) Plant defence. If the subcellular structures are destroyed by herbivoral, fungal or mechanical means, the catabolic enzymes can act on the cyanogenic glycosides. The catabolism is initiated by cleavage of the carbohydrate moiety by one or more β-glycosidases. After cleavage of the carbohydrate moiety the intermediates (cyanohydrins) may decompose either spontaneously or enzymatically, in the presence of a hydroxynitrile lyase, which considerably accelerates the reaction velocity. (ii) Nitrogen source. Cyanogenic glycosides may also be catabolised without release of hydrogen cyanide. HCN is refixed by the enzyme β-cyanoalanine synthetase and forms β-cyanoalanine with L-cysteine. β-Cyanoalanine is then hydrolysed by β-cyanoalanine hydrolase to L-asparagine. Consequently, HCN can also be considered as a nitrogen source for amino acid synthesis.

Eleven different Hnls from six different plant families have been isolated to date.[1] Interesting preparative applications in organic synthesis have been described only for four enzymes: (*R*)-hydroxynitrile lyase from *Prunus amygdalus* and (*S*)-hydroxynitrile lyases from *Sorghum bicolor*, *H. brasiliensis* and *M. esculenta* (Table 1). The differences between these four enzymes lies not only in their biochemical characteristics and enantiospecificities, but also in their substrate specificities. The Hnl of *P. amygdalus* (Pa-Hnl) accepts aromatic as well as aliphatic, unsaturated and heterocyclic aldehydes.[2] The Hnl of *S. bicolor* (Sb-Hnl) accepts only aromatic and heterocyclic aldehydes.[6] On the contrary, the Hnl of *H. brasiliensis* (Hb-Hnl) catalyses the formation of cyanohydrins from aliphatic, aromatic and heterocyclic aldehydes.[7] The Hnl of *M. esculenta* (Me-Hnl) is very similar to the *H. brasiliensis* enzyme, and therefore a very similar substrate specificity is to be expected.

Molecular cloning, characterization and overexpression of the *hnl*-gene from *Hevea brasiliensis*

Based on polyclonal antibodies and partial sequence information obtained from the Hnl which was purified to homogeneity from plant material,[11,12] the gene encoding for the Hb-Hnl enzyme could be cloned by antibody screening of a cDNA library prepared from leaves of the rubber tree.[4] Sequencing of the cDNA relevaed an open reading frame encoding for a polypeptide of 257 amino acids with a predicted molecular mass of 29 227 kDa (Fig. 1). This was in good agreement with the molecular weight of 30 kDa found for the natural Hnl, as determined by SDS polyacrylamide gel electrophoresis.

Detailed sequence homology studies were performed and revealed that Hb-Hnl is highly homologous to Hnl of cassava (*M. esculenta*)[10] but shows no significant homology to the (*R*)-hydroxynitrile lyase from *P. serotina*[13] or to the (*S*)-hydroxynitrile lyase from *S. bicolor*.[9a] Identification of distant relationships and homologies at the secondary structure level indicated that Hb-Hnl is a new member of the α/β-hydrolase fold family of enzymes.[14] The involvement of Ser_{80} and His_{235} in the catalytic action was clearly confirmed by replacing these amino acid residues of the predicted catalytic triad by site-specific mutagenesis. Both the $Ser_{80} \rightarrow Ala$ and the $His_{235} \rightarrow Ala$ mutant proteins were completely inactive.

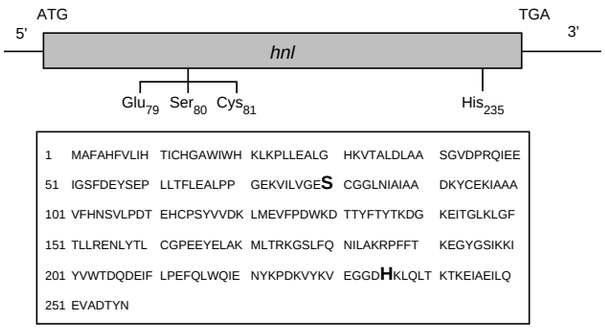


Fig. 1 The *hnl* gene from *Hevea brasiliensis* and deduced amino acid sequence of the Hnl polypeptide. The active site Ser_{80} and the His_{235} of the catalytic triade are highlighted in the sequence by bold letters.

Replacing the residues adjacent to the active site, Ser_{80}, Glu_{79} and Cys_{81}, resulted in low levels (1 and 3%) of the remaining specific Hnl activity of the mutant proteins $Glu_{79} \rightarrow$ Ala and $Cys_{81} \rightarrow$ Ser, which indicates that these residues may play an important role in enzyme function.[14]

The Hnl-encoding cDNA was used to construct expression vectors for overexpression of the *hnl* gene in different microorganisms. Overexpression in the bacterium *Escherichia coli* K12 resulted in efficient production of heterologous Hnl protein, however, the protein was intracellularly accumulated as insoluble inclusion bodies. Extraction of solubilized active Hnl enzyme was generally possible but resulted in low levels of specific activity. In contrast, expression in *Saccharomyces cerevisiae* resulted in efficient intracellular expression of soluble and highly active Hnl enzyme.[4] the specific activity of purified Hnl obtained by heterologous expression in *S. cerevisiae* (22 U mg^{-1}) was even slightly higher than the value of 18 U mg^{-1} found for the Hnl enzyme purified from plant leaves. The most successful heterologous expression could be achieved by the methanol-inducible *Pichia pastoris* (methylotrophic yeast) expression system. High levels (up to about 60% of total cellular proteins) of intracellularly produced soluble Hnl protein, exhibiting specific activity of around 40 U mg^{-1} (about twice the value found with the purified natural enzyme), were obtained. This expression system is also well developed for high cell density cultivation and yields more than 20 g of pure Hnl protein per litre of culture volume.[15] This high expression level represents an excellent basis for the establishment of efficient industrial processes for the production of recombinant *H. brasiliensis* Hnl enzyme.

Three-dimensional structure of the Hnl from *Hevea brasiliensis*

Knowledge of the three-dimensional structure is a prerequisite for understanding the enzyme mechanism as well as for any attempt to alter enzyme properties by chemical or genetic means. Therefore, efforts to crystallize Hnls with the goal of determining their three-dimensional structures by X-ray crystallography have been undertaken.[16] These efforts have recently led to the successful conclusion of the structure analysis of Hb-Hnl, yielding the first three-dimensional structure of an enzyme with hydroxynitrile lyase activity.[5] Preliminary suggestions pertaining to the reaction mechanism could be derived from these recent results, which also form the basis for continued mutagenesis experiments.

The Hnl from H. brasiliensis *belongs to the* α/β*-hydrolase fold family*

This is evident from the topology diagram [Fig. 2(*a*)] and from the three-dimensional structure [Fig. 2(*b*)]. The α/β-hydrolase fold is characterized by a well-conserved, large central β-sheet surrounded by α-helices in a less-well conserved arrangement, plus a variable 'cap' region. Other known members of this class of enzyme superfamily[17] have esterase, carboxypeptidase, dehalogenase and oxidoreductase activities, with somewhat related enzymatic mechanisms. Apart from their overall architecture, they also share a number of structurally conserved features in the vicinity of the active site, which can be used to identify the location of the active site as well as some of the residues involved in enzyme catalysis.

The active site is deeply buried inside the protein and connected to the surface by a narrow channel, as shown in Fig.3. This is consistent with the previously suggested ordered uni-bi mechanism[11,18] of hydroxynitrile lyases, which implies that substrates or products, depending on the reaction direction, access the active site in a sequential fashion. In the crystal structure, residual electron density interpreted as originating from a histidine molecule (presumably carried through from the buffer during enzyme purification) was observed within the active site region.

A (presumably) catalytic triad

A triad consisting of residues Ser_{80}, His_{235} and Asp_{207} was identified by sequence and structure comparison with other α/β-hydrolase fold enzymes, and its catalytic relevance was assessed by mutagenesis studies.[4,14] Other characteristic features of α/β-hydrolases were identified ('nucleophile elbow') or at least tentatively assigned ('oxyanion hole') by structural

Table 1 Biochemical characteristics of the hydroxynitrile lyases used for organic synthesis

Plants, Plant Family and Parts	Stereo-selectivity	Molecular weight/kDa		Optimum pH (co-enzyme)	Natural substrate (cyanogenic glycoside)
Prunus amygdalus Rosaceae almonds[a]	*R*	native: SDS-Page:	72 ± 2 72 ± 2	5–6 (FAD)	(*R*)-mandelonitrile (amygdalin, prunasin)
Sorghum bicolor Graminae seedlings	*S*	native: SDS-Page: reduced:	108 ± 3 55 ± 2 33 ± 1 (α) 23 ± 1(β)	n.d.[c] (none)	(*S*)-4-hydroxymandelonitrile (dhurrin)
Manihot esculenta Euphorbiaceae leaves[d]	*S*	native: SDS-Page:	92–124 28.5–30	5.4 (none)	acetone cyanohydrin, (*S*)-butan-2-one cyanohydrin, (linamarin, lotaustralin)
Hevea brasiliensis Euphorbiaceae leaves[e]	*S*	native: SDS-Page:	58 30 ± 1	5.5–6.0 (none)	acetone cyanohydrin (linamarin)

[a] Ref. 8. [b] Ref. 9. [c] n.d. = not determined. [d] Refs. 3 and 10. [e] Ref. 11.

comparison with other enzymes of the superfamily, most notably acetyl cholineesterase.[19] Each of the structurally conserved features of α/β-hydrolases is likely to play a similar function in the catalytic mechanism of the corresponding enzyme. Thus, the terminal residue of the catalytic triad (Ser_{80} in Hnl) is believed to act as an activated nucleophile, which typically attacks a carbonyl carbon atom (*e.g.* in serine carboxypeptidases[20]). The resulting negative charge on the oxygen of the thus formed tetrahedral intermediate is stabilized by accommodating this oxygen within what is frequently called the 'oxyanion hole'. The structural conservation of the catalytic triad in Hnl suggests a similar function. Therefore, it was proposed[5] that the catalytic cycle of Hnl also involves a tetrahedral intermediate formed by nucleophilic attack of the (activated) Ser_{80} oxygen on the carbonyl group of the cyanohydrin. For the natural substrate (acetone cyanohydrin) of the Hb-Hnl, the structure of the tentative intermediate (filled into the Hnl crystal structure by molecular modelling) is shown in Fig. 4. The Cys_{81} residue was proposed to be involved in proton transfer to and from cyanide[5] as deduced from the reduced catalytic activity of the $Cys_{81} \rightarrow$ Ser mutant.

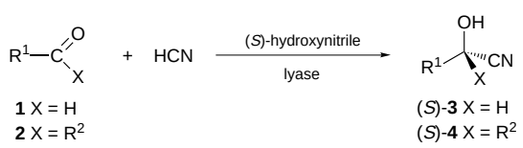
Enzyme catalysed synthesis of cyanohydrins

For preparative purposes, the (*R*)-Hnl from *P. amygdalus* can be easily isolated in sufficient quantities.[8] Its high stability made it possible to investigate its properties and substrate range. It was shown that aliphatic, aromatic and heteroaromatic aldehydes[21] as well as ketones[22] could be converted to the corresponding cyanohydrins in good yield and selectivity.

The first (*S*)-hydroxynitrile lyase employed for the synthesis of chiral cyanohydrins was isolated from four to eight day old shoots of *S. bicolor*.[6] For reactions on a larger scale this preparation is rather laborious. Broad application of this Hnl is not possible as only aromatic carbonyl compounds are accepted as substrates. In contrast to this, the hydroxynitrile lyase from *H. brasiliensis* was reported to be the first (*S*)-Hnl which also accepts aliphatic aldehydes as substrates.[7] This enzyme could be prepared in large quantities by over-expression in the yeast *P. pastoris*.[4] Recently, an (*S*)-Hnl has been isolated from *M. esculenta*,[10,23] which shows similar properties[24] to the enzyme from *H. brasiliensis*.[7,25] Regarding the (*S*)-cyanohydrins, the most thoroughly studied enzyme is Hb-Hnl. Therefore its use in organic synthesis will be concentrated on.

The hydrogen cyanide required for the cyanohydrin formation (Scheme 1) can be added directly into an organic reaction medium or created *in situ* by the addition of acid to an aqueous solution of alkali cyanide. Alternatively the method of transhydrocyanation can be employed.[7a,26,27] Here, HCN is set free from acetone cyanohydrin in a preequilibrium step. To obtain chiral cyanohydrins in good enantiomeric purity a fundamental prerequisite is the suppression of the spontaneous addition of HCN to the carbonyl group. The extent of this non-enzymatic reaction strongly depends on the conditions. An aqueous reaction medium is only suitable if pH values below 4.0 are employed.[28] It is also possible to suppress the non-enzymatic addition of hydrogen cyanide by the use of an organic solvent. This reaction system has the additional advantage that the isolated yields of the corresponding cyanohydrins are in general higher than in aqueous medium. The first results were obtained mainly with aliphatic aldehydes[7] and ketones employing an aqueous, buffered reaction medium at pH 3.75 to 4.0 (Table 2). The majority of the substrates investigated can be converted into the corresponding cyanohydrins with good to excellent enantiomeric excess. By this means (*S*)-cyanohydrins of aryl-substituted aliphatic aldehydes became enzymatically accessible for the first time using hydroxynitrile lyases.[25] Also the excellent selectivity of this Hnl for thiophene and furane aldehydes in comparison to hydroxynitrile lyases from other plant sources is remarkable.

Using an aqueous reaction system, the isolated yields of the cyanohydrins are only moderate, probably due in part to the low solubility of several substrates in water. This can be circumvented by employing an organic solvent. It is not necessary to immobilise the enzyme. One can also apply a crude, concentrated aqueous enzyme solution together with an organic solvent as a second phase. Solvents of choice are ethyl acetate and some ethers. After performing the reaction, the aqueous phase containing the enzyme can be reisolated by a simple phase separation step. The activity of the enzyme used is unchanged.



Scheme 1

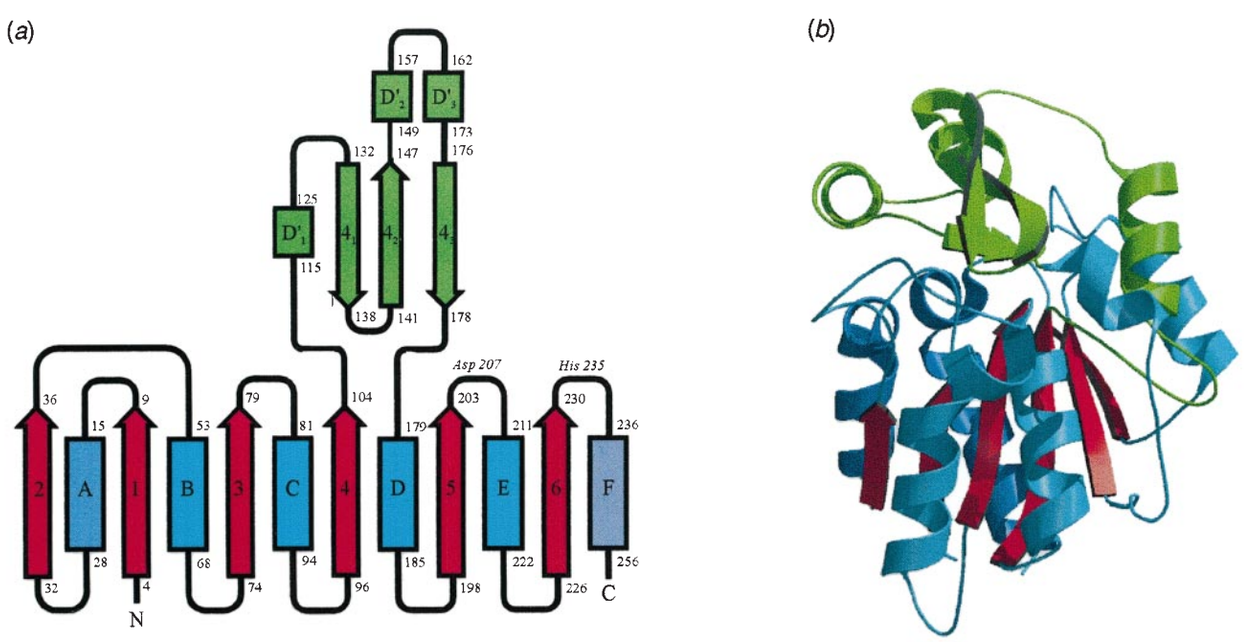


Fig. 2 (*a*) Topology diagram and (*b*) three-dimensional and structure of the hydroxynitrile lyase from *Hevea brasiliensis*. The 'cap' region is colour-coded in green, helices are blue and β-strands are red.

Therefore, the same batch of enzyme can be reused several times without significant reduction in the isolated yield and the enantiomeric excess of the product. This fact is of importance with respect to industrial applications in order to enhance the productivity of the desired process. The pH value of the reaction mixture can be adapted to a certain substrate by adding an aqueous buffer solution to the aqueous enzyme solution. In order to obtain high yields of the corresponding cyanohydrins in short reaction times, concentrated solutions of the starting material (1 to 1.5 M) in the desired organic solvent can be employed. Furthermore, the temperature has a great influence on the course of the reaction. The reaction rate of the spontaneous addition of HCN, which will reduce the enantiomeric purity of the product, can be suppressed with respect to the enzymatic reaction if the reaction temperature is kept below 5 °C. At this temperature, the stability of the enzyme against denaturation is also enhanced. In almost all cases a reaction time of approximately 1 h at 0–5 °C was sufficient to obtain the corresponding chiral cyanohydrin in good yield and enantiomeric purity (see Table 3).

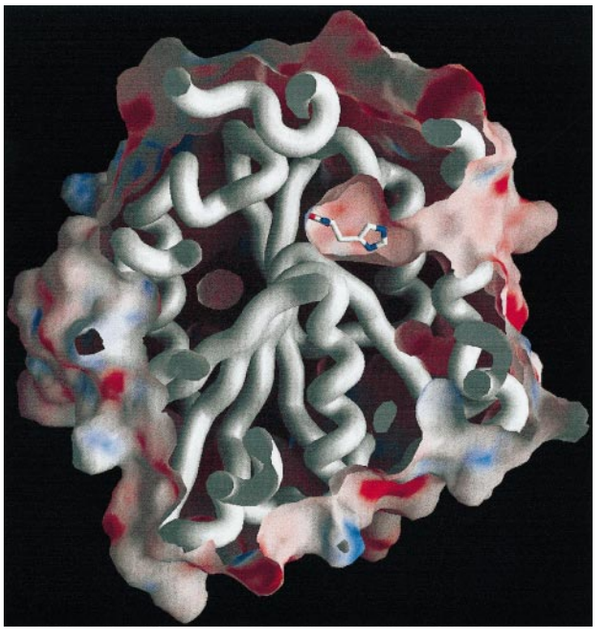


Fig. 3 Section through a surface representation of the Hnl crystal structure. The colour coding is according to the electrostatic potential. The section passes through the active site cavity and the tunnel connecting it with the surrounding solvent. Also shown is a histidine molecule observed within the active site.

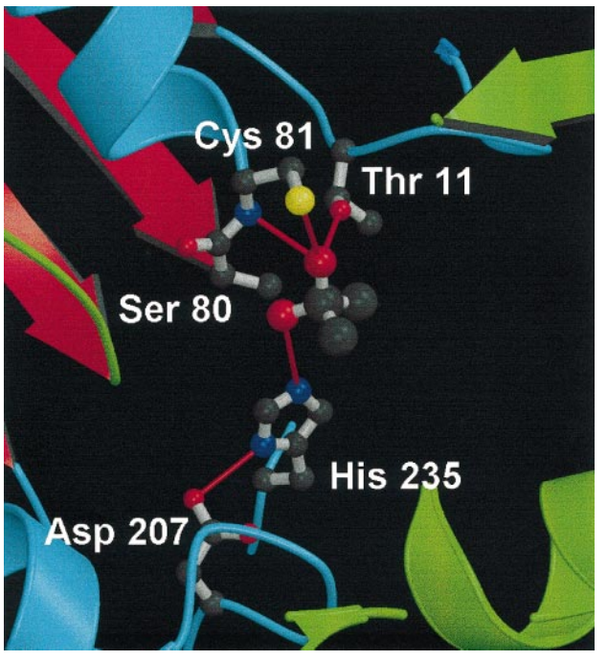


Fig. 4 Structure of the tentative tetrahedral intermediate formed by nucleophilic attack of the Ser_{80} oxygen on the carbonyl carbon of acetone. The structure of this intermediate was deduced by molecular modelling.

Synthetic transformations of chiral cyanohydrins

Cyanohydrins, in particular those derived from aldehydes, are functionalised chiral units that possess broad synthetic utility. Scheme 2 summarizes some recent developments. Several synthetic routes are shown, each of which describes an alternative transformation of either the hydroxy or cyano moiety. Thus following route A, it can be seen that conversion of the hydroxy functionality to the silyloxy ether allows for a range of amino alcohols and hydroxy ketones to be generated, *via* nucleophilic addition of a Grignard reagent (*e.g.* methylmagnesium iodide) to the nitrile followed by reduction[29] or acidic hydrolysis[30] of the intermediate imine. This addition reaction has been further exploited by developing a transimination reaction[31] which yields an *N*-derivatized amino alcohol. When the hydroxy group is suitably protected (usually a silyloxy ether), the cyanohydrin can be converted to an

Table 2 Preparation of (*S*)-cyanohydrins $R^1R^2CH(OH)CN$ in aqueous buffer using the (*S*)-Hnl from *H. brasiliensis*

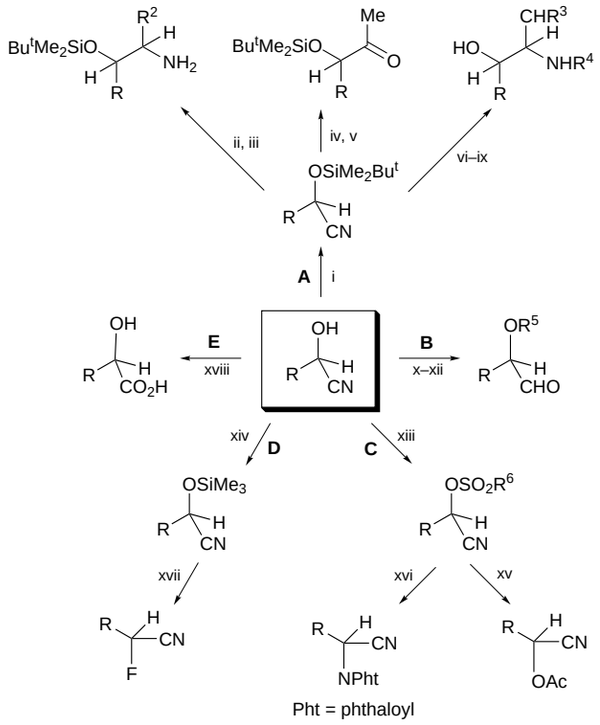
R^1	R^2	Yield (%)	Ee (%)
Ph	H	67	99
Bn	H	44	99
$Ph(CH_2)_2$	H	88	93
(*E*)-PhCH=CH	H	50	95
2-$MeOC_6H_4$	H	61	77
3-$MeOC_6H_4$	H	80	99
4-$MeOC_6H_4$	H	49	95
3-$PhOC_6H_4$	H	9	99
$PhOCH_2$	H	n.d.[a]	0
CH_2=CH	H	38	94
PrC≡C	H	43	80
Cyclohex-3-enyl	H	87	99
2-furyl	H	55	98
3-furyl	H	61	99
2-thienyl	H	52	99
3-thienyl	H	49	99
Pr^i	Me	23	52
Pr^i	Me	38	88
Pr	Me	51	75
Bu^t	Me	49	78
Bn	Me	74	95

[a] n.d. = not determined.

Table 3 Comparison of selected examples of conversions of aldehydes (R^1CHO) in Pr^i_2O and results obtained in aqueous reaction medium

	Aqueous buffer, pH 4.5		Pr^i_2O	
R^1	Yield (%)	Ee (%)	Yield (%)	Ee (%)
Ph	67	99	96	99
(*E*)-PhCH=CH	50	95	77	92
3-$PhOC_6H_4$	9	99	98	99
$BnOCH_2$	n.d.[a]	0	92	12
Cyclohexyl	94	99	95	99
2-furyl	55	98	95	94
3-furyl	61	99	96	97
C_5H_{11}	n.d.[a]	84	81	96
(*E*)-$Me(CH_2)$CH=CH	62	99	96	99

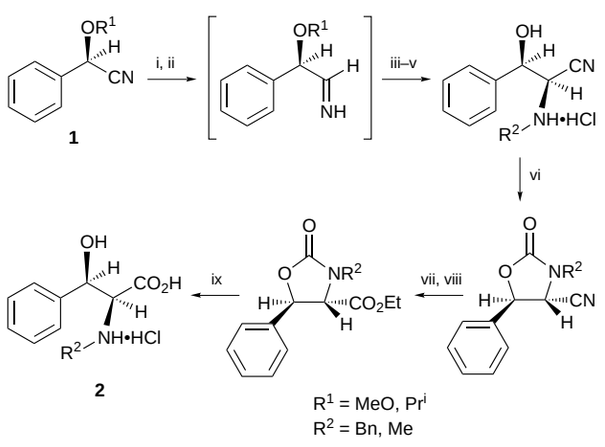
[a] n.d. = not determined.



Scheme 2 *Reagents*: i, Bu^tMe_2SiCl, imidazole; ii, R^2MgBr; iii, $NaBH_4$; iv, MeMgI; v, H_3O^+; vi, R^3CH_2MgBr; vii, R^4NH_2; viii, $NaBH_4$; ix, $LiAlH_4$; x, R^5X; xi, DIBAL-H; xii, H_3O^+; xiii, R^6SO_2Cl, pyridine; xiv, Me_3SiCl, pyridine; xv; KOAc, DMF; xvi, potassium phthalimide, DMF; xvii, Et_2NSF_3; xviii, conc. HCl

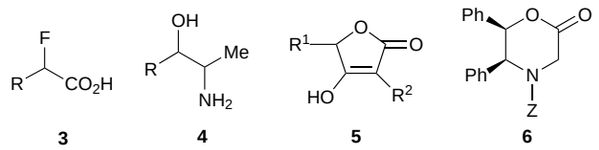
α-hydroxy aldehyde by treatment with excess diisobutylaluminium hydride followed by mild acidic hydrolysis (route B).[32] Alternatively, conversion of the hydroxy functionality into a suitable leaving group followed by nucleophilic displacement with various nucleophiles (*e.g.* acetate,[33] phthalimide[34] or fluoride[35]) introduces a new alternative functionality at the chiral centre with stereochemical inversion. The free cyanohydrins are particularly stable under anhydrous, strongly acidic conditions (*e.g.* dry hydrogen chloride), but treatment with an aqueous strong acid yields α-hydroxy acids.[6a] A more detailed coverage of the reactions presented in this scheme is given in recent reviews.[32,36]

A representative example of the synthetic potential of Hnls is shown in Scheme 3, where the cyanohydrin **1** (prepared from benzaldehyde) is elaborated[37] to the β-hydroxy-α-amino acid **2**,



Scheme 3 *Reagents*: i, DIBAL-H; ii, NH_4Br, MeOH; iii, R^2NH_2; iv, HCN; v, HCl; vi, 1,1′-carbonylbis(1*H*-imidazole); vii, K_2CO_3, EtOH; viii, HCl; ix, 2 M KOH

which has the structural features of the amino acid side chain of Taxol. Therefore, in the chiral cyanohydrins the provision of a single asymmetric centre with at least two different functionalities is of significant practical synthetic value for differential and selective chemical elaboration to bioactive compounds and natural products. These products include α-fluoro carboxylic acids (**3**),[35] ethanol amines (**4**),[29] tetronic acids (**5**)[38] and the Williams glycine template (**6**).[39]



Unsaturated cyanohydrins from aldehydes

The synthetic utility of the chiral cyanohydrins can be extended by employing unsaturated aldehydes as substrates. The subsequent unsaturated cyanohydrins represent enzymatic products from enzyme catalysed reactions with an enhanced synthetic potential. Such opportunities include oxidative cleavage or epoxidation of the unsaturated functionality, nucleophilic substitution at the hydroxy centre and metal assisted addition to, or reduction of, the nitrile functionality (Fig. 5). This versatility makes them an important tool for organic chemists.

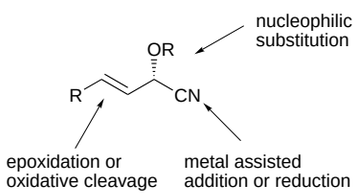


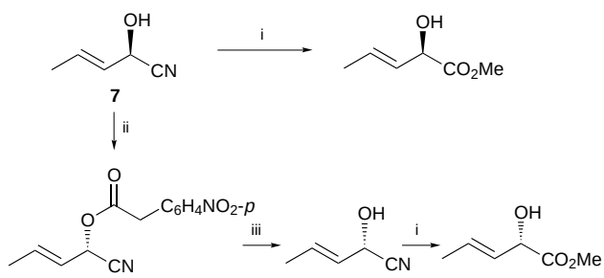
Fig. 5 Opportunities for further transformation of unsaturated *O*-protected cyanohydrins

Unsaturated hydroxy esters[40]

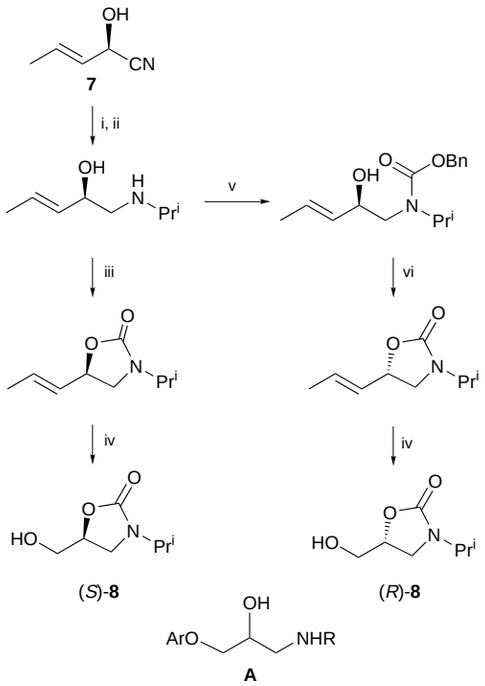
On treatment with MeOH–$HCl_{(g)}$, the unsaturated cyanohydrins (*e.g.* **7**) are easily converted into the corresponding esters (Scheme 4). These compounds are the multifunctional chiral starting materials for a number of organic transformations. Inherent in their structures are the properties of an allylic alcohol combined with those of α-hydroxy esters and β,γ-unsaturated carboxylic esters. The hydroxy group facilities directed epoxidation whilst the unsaturated ester (or carboxylic acid) may be applied to a variety of addition reactions. The enantiomeric counterpart of **7** can also be prepared by inversion of the chiral hydroxy group, under Mitsunobu conditions, followed by acidic hydrolysis of the *p*-nitrophenyl acetate and application of the Pinner reaction once more (Scheme 4).

Oxidative cleavage[41]

(*R*,*E*)-(−)-2-hydroxypent-3-enenitrile **7** has proven to be particularly useful in a synthesis in which, at an appropriate juncture,



Scheme 4 *Reagents*: i, HCl, MeOH–Et_2O, then H_2O, then H_2O; ii, $EtO_2CN{=}NCO_2Et$, PPh_3, *p*-nitrophenylacetic aicd; iii, MsOH, MeOH

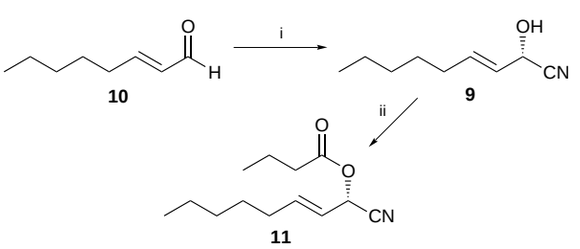


Scheme 5 *Reagents*: i, 2-methoxypropene; ii, DIBAI-H, then MeOH, NH_4Br, Pr^iNH_2; iii, 1,1′-carbonylbis(1*H*-imidazole); iv, O_3, then $NaBH_4$; v, benzyl chloroformate; vi, $SOCl_2$

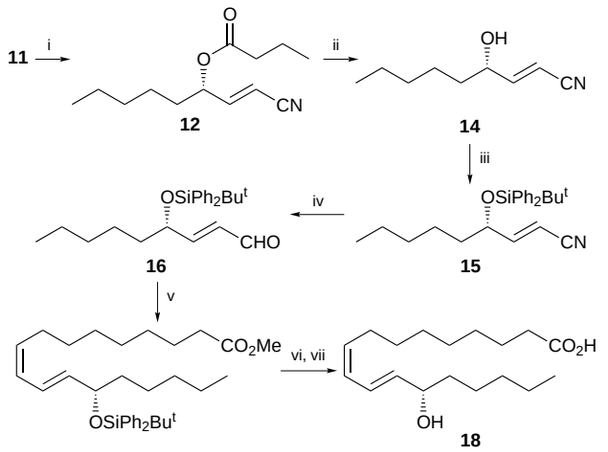
the carbon–carbon double bond is broken *via* oxidative cleavage. The (*R*)-cyanohydrin **7** was converted into both enantiomers of oxazolidinone **8**, which is a key intermediate in the synthesis of β-blockers of the general structure A (Scheme 5).

*Synthesis of (*9E,11E,13S*)*-13-*hydroxyoctadeca*-9,11-*dienoic acid by chirality transfer*[42,43]

The stereoselective addition of HCN to unsaturated aldehydes to yield unsaturated (*S*)-cyanohydrins using the hydroxynitrile lyase from *H. brasiliensis* was first reported here in Graz in 1995.[7b] This work showed that the enantiomeric excess of the subsequent cyanohydrin increased with the chain length of the aldehyde. With this in mind, the synthesis of a natural product, (9*Z*,11*E*,13*S*)-13-hydroxyoctadeca-9,11-dienoic acid, was established[42] in which the key chiral synthon **9** (Scheme 6) is generated from the Hnl catalysed addition of HCN to octenal **10**. The cyanohydrin product **9** was derivatized to the butyrate **11** which was shown to possess an enantiomeric excess of 99%. The butyrate was then easily converted into the unsaturated nitrile **12** using a [3,3]-sigmatropic rearrangement catalysed by bis(acetonitrile)dichloropalladium **13** (Scheme 7). This rearrangement proceeded, as previously described,[44,45] in a stereospecific manner with complete retention of configuration and enantiomeric excess. Hydrolysis of the butyrate yielded the alcohol **14**, which was then protected with the *tert*-butyldiphenylsilyloxy functionality to yield the nitrile **15**. Chiral analysis



Scheme 6 *Reagents*: i, Hnl from *Hevea brasiliensis*, HCN; ii, butyric anhydride, pyridine



Scheme 7 *Reagents*: i, $Pd(MeCN)_2Cl_2$, **13**, THF; ii, K_2CO_3, MeOH; iii, Bu^tPh_2SiCl, imidazole, DMF; iv, DIBAL-H, then H_3O^+; v, $Ph_3P^+Br^-(CH_2)_8CO_2Me$, **17**, $(Me_3Si)_2NLi$, THF, HMPA, −78 °C; vi, Bu_4NF, THF; vii, LiOH, H_2O–Pr^iOH, then acidification to pH 7

(high performance liquid chromatography) revealed that the nitrile **15** possessed an enantiomeric excess of 99%. The synthesis was then completed in a straightforward fashion. Reduction of **15** with DIBAL-H followed by acidic workup gave the unsaturated aldehyde **16**, which was coupled with the phosphonium salt **17** using a Wittig reaction. Deprotection of the hydroxy group and hydrolysis of the methyl ester then followed, which yielded the desired compound **18** (Scheme 7).

Conclusions and outlook

On the basis of the studies presented in this article it is expected that the enzyme catalysed cyanohydrin reaction will be further established as a versatile method to obtain enantiopure chiral intermediates. Investigations on the three-dimensional structure of Hnls from different source are in progress, together with genetic studies. The scope and limitations of the reaction are being clarified and the synthetic transformation of the enantiopure cyanohydrins to target structures of both scientific and industrial interest is in progress in several laboratories.

Taking into consideration the synthetic importance of enantiopure cyanohydrins, it is not surprising that methods other than the ones described above have been investigated to achieve this goal. With regard to enzymatic methods other than the use of hydroxynitrile lyases, the resolution of esters of racemic cyanohydrins has also been investigated.[46–48] Another approach uses cyclic dipeptides for asymmetric cyanohydrin synthesis,[36,49,50] or applies chiral Lewis acids.[36,50,51] However, regarding chemical yield, enantiopurity and substrate range accepted, the application of hydroxynitrile lyases at present seems to be the optimum choice.

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Footnote and References

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