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Straightforward Enzymatic Process Based on HNL CLEA-Catalysis towards Cyanohydrin Derivatives

Fabien L. Cabirol,[†,‡,§] Angela E. C. Lim,[‡] Ulf Hanefeld,[†] and Roger A. Sheldon*[,†]

Department of Biotechnology, Laboratory of Biocatalysis and Organic Chemistry, Delft University of Technology, Julianalaan 136, 2628 BL Delft, The Netherlands, and Biocatalysis Laboratory, Institute of Chemical and Engineering Sciences, 1 Pesek Road, Jurong Island S(627833), Singapore

Abstract:

An efficient enzymatic process based on HNL-CLEA catalysis in buffer-saturated organic media was developed to limit the amount of overall waste generated and the number of safety issues incurred by handling HCN-containing waste. Starting from benzaldehyde as a model substrate, (*R*)- and (*S*)-mandelonitrile can be obtained under these reaction conditions without an extraction step being required. The crude cyanohydrin intermediate was sufficiently pure to be used as starting material in a second step towards a range of derivatives.

Introduction

A century ago, in 1908, Rosenthaler reported the preparation of (*R*)-mandelonitrile from benzaldehyde and hydrogen cyanide using emulsin as catalyst.[1] This publication arguably marks the birth of asymmetric biocatalysis. Hydroxynitrile lyases (HNLs) and the preparation of enantioenriched cyanohydrins have been one of the key topics in this field of research ever since.[2−7]

Chiral cyanohydrins are mostly used as intermediates in synthetic chemistry.[2−4,8] One of the main drawbacks of cyanohydrins as commercial products is the possible release of HCN upon decomposition.[9] This potential source of hazard incurs additional costs in handling, shipping, and storage of bulk quantities. As a result, cyanohydrins are generally seen as in-house intermediates towards more stable and marketable chiral products such as α-hydroxy-amines, α-hydroxy-acids, and α-hydroxy-esters. *O*-Protected cyanohydrins are also of particular interest when further derivatization involves a basic medium that would otherwise lead to the decomposition of unprotected cyanohydrins.

The preparation of cyanohydrin esters, THP-protected, and trialkylsilyl derivatives by kinetic resolution of cyanohydrin acetates, followed by the corresponding downstream chemistry of the free cyanohydrin, has been reported.[10] However, in such kinetic resolutions yields are limited to 50%. Earlier attempts to combine a HNL with a lipase in one pot failed due to the hydrolysis of the acylating reagent. The acetic acid released then denatured the HNL.[11] The direct enantioselective synthesis of cyanohydrins from inexpensive HCN and readily available prochiral carbonyl compounds is nonetheless remarkably attractive in terms of atom economy. An integrated multistep synthetic strategy towards cyanohydrin derivatives would therefore answer a need for step economy[12,13] in order to "efficaciously deliver a meaningful supply of target" (a cyanohydrin derivative) as Wender described.[14]

Both (*R*)- and (*S*)-HNLs are naturally occurring, stable, and relatively inexpensive enzymes with a wide substrate range. The HNL-catalysed preparation of cyanohydrins is typically carried out in aqueous buffer or in a biphasic (buffer:organic) type medium. This type of medium is not suitable for the development of cost-effective multistep syntheses since the reagents required to derivatize the cyanohydrin would decompose in water. Purification of the cyanohydrin is therefore required and is usually carried out by extraction in organic media followed by distillation of the organic layer (Figure 1).

The extraction step was identified as a significant drawback in terms of waste generation since the aqueous layer needs to be disposed of and large amounts of organic solvent are usually required. Furthermore, each layer from the extraction step can potentially contain residual HCN from the reaction mixture and therefore requires appropriate handling and waste disposal procedures. In order to efficiently implement a multistep strategy towards cyanohydrin derivatives, the HNL-catalysed formation of cyanohydrins should be carried out in an organic medium (Figure 1).

Engineering the reaction medium for the HNL-catalysed synthesis of cyanohydrins typically aims at limiting the contribution of the noncatalysed addition of HCN to the substrate which decreases the practical enantiopurity of the product.[15−21] To this end, the development of immobilized HNLs as cross-

* To whom correspondence should be addressed. Fax: (+31)-15-278-1415. E-mail: r.a.sheldon@tudelft.nl.

† Delft University of Technology, The Netherlands.

‡ Institute of Chemical and Engineering Sciences, Singapore.

§ Current Address: Codexis Laboratories Singapore, 61 Science Park Road, #03-15/24, The Galen, Singapore Science Park II S(117525), Singapore.

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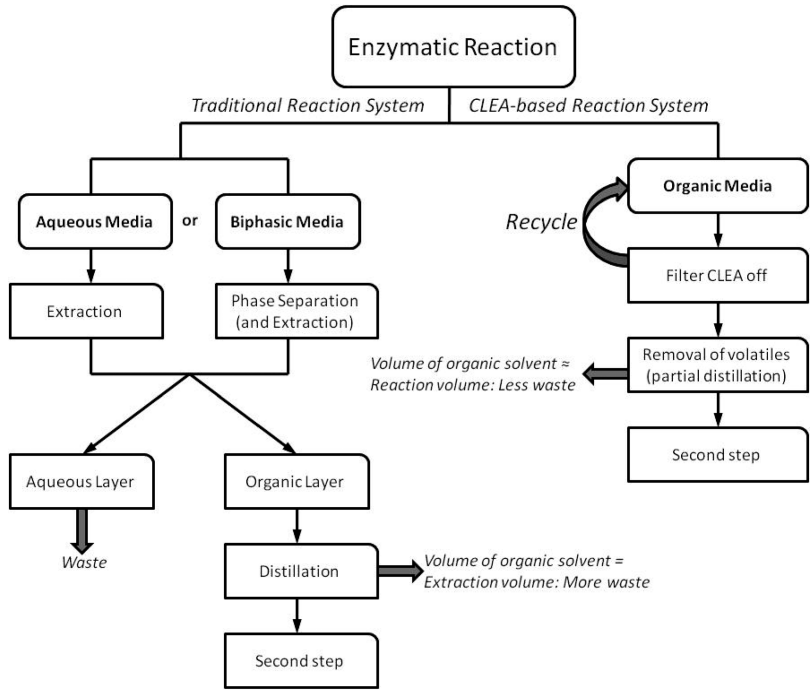

Figure 1. **Downstream processing of the reaction mixture depending on the choice of reaction media.**

linked-enzyme-aggregates (CLEA) was particularly successful.[21−24] CLEAs of the HNLs from *Linum usitatissimum* (*Lu*HNL),[25] *Manihot esculenta* (*Me*HNL),[21,26] and *Prunus amygdalus* (*Pa*HNL)[21,27,28] showed high volumetric activity and improved stability in organic media. Similar to earlier observations for *Pa*HNL[29] and other HNLs immobilized on different carriers[17,20,30] the HNL CLEAs were catalytically active in buffer-saturated organic solvents.

In order to illustrate the key advantages of a HNL CLEA-catalysed cyanohydrin synthesis in organic solvent as a part of multistep synthesis strategy towards cyanohydrin derivatives we selected benzaldehyde as model substrate and four subsequent mandelonitrile derivatization methods to be discussed herein.

Results and Discussion

We first established reaction conditions under which the enzymatic step reached more than 95% conversion with at least 97% ee in buffer-saturated diisopropyl ether (DIPE) within 4 h at room temperature. This could be achieved using 4 g/L CLEA at 52 g/L substrate loading (0.49 M) and 4 equiv HCN for both *Me*CLEA and *Pa*CLEA with (*S*)-mandelonitrile and (*R*)-mandelonitrile, respectively. Saturation of the organic solvent with aqueous buffer (50 mM citrate/phosphate pH = 5.5) was absolutely necessary for optimum catalytic performance since deactivation of the biocatalyst occurs under conditions where the medium can still remove water from the enzyme. Water plays an important part in the structure of HNLs and is present even in the active site.[31] The ability of the enzyme to retain structural water molecules therefore dictates the biocatalyst stability in the medium.[23]

Downstream processing of the cyanohydrin was then conducted as described in Figure 1. The immobilized biocatalyst could be filtered easily through cotton wool and recycled directly for the next batch of cyanohydrin. We typically reused the catalyst three times without noticeable loss of activity. Distillation was avoided since cyanohydrins decompose upon heating and a distillation step would require additives such as citric acid to stabilize the product.[7] The volatiles were therefore removed under reduced pressure and recondensed carefully since the reaction mixture still contains excess HCN. This overall process fulfilled the target of limiting the amount of waste generated since no extraction step was required. Extraction would indeed

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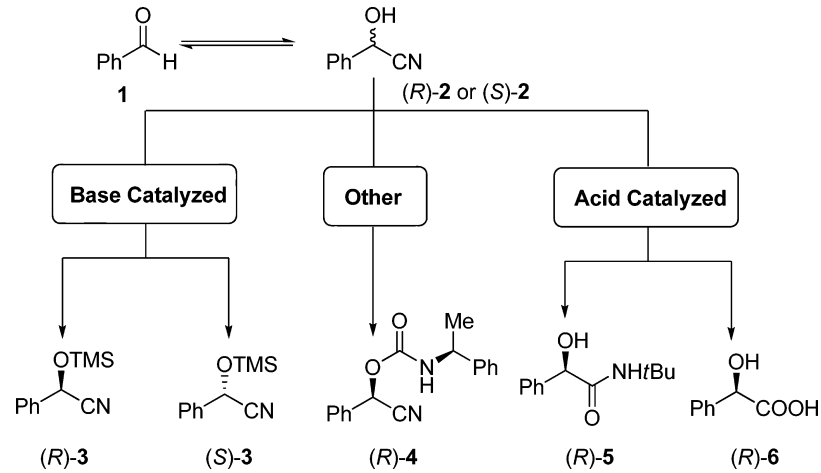

Figure 2. **Multistep syntheses investigated.**

require ~5 volumes of organic solvent with respect to the amount of water used as reaction media. This solvent is later removed during the distillation step (Figure 1), and its handling and possible recycling are difficult due to residual HCN. In the process described here only one volume of organic solvent is used (the reaction media). Moreover, we minimized potential hazard due to residual HCN by limited handling with a very straightforward downstream processing procedure.

Water from the media was the main impurity in the crude cyanohydrin obtained. This residual water can have a significant impact on the second step. We therefore investigated four subsequent derivatization methods to determine if the crude cyanohydrin obtained after this downstream process could be used without further purification (Figure 2).

As a first example of follow-up chemistry, we investigated the derivatization of the crude cyanohydrin obtained upon the catalysis of either *Pa*CLEA or *Me*CLEA into the TMS-derivatives (*R*)-**3** and (*S*)-**3**, respectively. Only a moderate excess (2.5 equiv) of chlorotrimethylsilane (TMSCl) and base (2 equiv) were needed to achieve good yields (>80%) and maintain the excellent enantiopurity (>99%) obtained in the enzymatic step. The deleterious effect of residual water was therefore not so significant as to preclude further reactions with water-sensitive reagents such as TMSCl. We also noticed that, when TMSCN was used as the sole protecting reagent, a slight drop in the enantiopurity of the protected cyanohydrin was observed. This effect was attributed to the racemic (noncatalysed) addition of TMSCN to residual benzaldehyde and could be suppressed completely when TMSCl was used as the protecting reagent.

The carbamoylation of chiral alcohols using isocyanates and copper bromide has been reported earlier for the determination of enantiopurity by NMR.[32] This derivatization method employs a different type of reagent to the protecting-group chemistry (typically base- or acid-catalysed) discussed earlier and was therefore suitable to further probe the robustness of our multistep approach toward cyanohydrin derivatives. Coupling of freshly prepared isocyanate[33] with (*R*)-mandelonitrile in THF proceeded smoothly toward the corresponding carbamate (*R*)-**4**. Only a slight excess of isocyanate (1.5 equiv) and $CuBr \cdot Me_2S$ (1.5 equiv) was necessary under these conditions. After purification by crystallisation good yields (82%) and excellent enantiopurity (99.5%) were obtained.

Derivatisation of the nitrile functionality in cyanohydrin chemistry leads to a range of very useful intermediates in organic synthesis such as α-hydroxy-acids, α-hydroxy-esters, and α-hydroxy-amides.[2−6]

The formation of α-hydroxy-*N*-*tert*-butyl-amide from cyanohydrins *via* the Ritter reaction has been reported earlier.[34] The reaction is performed in a mixture of 60% H_2SO_4 and *tert*-butanol as reagents/solvent. Starting from crude (*R*)-mandelonitrile prepared by our method, the reaction proceeded smoothly to afford the corresponding α-hydroxy-amide (*R*)-**5** in good yield (70%) and very good ee (96%).

As a final example of successful application of our HNL-CLEA-based multistep strategy toward cyanohydrin derivatives we selected the synthesis of (*R*)-mandelic acid from benzaldehyde on a multigram scale. α-Hydroxy-acids are indeed prepared industrially on a multiton scale from the corresponding aldehyde using *Pa*HNL as catalyst in a biphasic medium followed by acid hydrolysis.[7] Moreover, we recently reported the highly efficient synthesis of (*R*)-2-hydroxy-2-methyl-butyric acid from 2-butanone using a similar strategy based on CLEAs of the HNL from *Linum usitatissimum* (*Lu*HNL) as biocatalyst.[25] Using the *Pa*CLEA-catalysed approach developed here, recrystallized (*R*)-mandelic acid (*R*)-**6** was obtained in very good yields (80%) and excellent ee (99%).

Conclusions

The combination of immobilized biocatalysts and organic solvent as reaction media allowed the development of a straightforward overall process towards derivatives of either (*R*)- or (*S*)-mandelonitrile from benzaldehyde. In particular, downstream processing of the cyanohydrin intermediate did not require an extraction step, thereby reducing the amount of waste when compared to reactions in aqueous or biphasic media. Impurities in the crude cyanohydrin obtained, especially water and residual benzaldehyde, were minimal and did not limit a range of derivatization to be conducted successfully. These

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expedient procedures toward enantiopure cyanohydrin derivatives also facilitated the recycling of the catalyst and enabled high volumetric yields, which are all essential to the successful application in the laboratory and the implementation of cost-efficient industrial processes.

Experimental Section

CAUTION. All procedures involving hydrogen cyanide were performed in a well-ventilated fume hood equipped with a HCN detector. HCN-containing wastes were neutralized using commercial bleach and stored independently over a large excess of bleach for disposal.

General Methods. Chemicals and reagents were commercially available and used without further purification unless indicated otherwise. Benzaldehyde was distilled before use to remove traces of benzoic acid. Solutions of the hydroxynitrile lyases from *Prunus amygdalus* (*Pa*HnL, 690 U/mL) and *Manihot esculenta* (*Me*HnL, 2.23 kU/mL) were purchased from Codexis. The corresponding CLEAs were prepared from these solutions as previously reported.[21,26,27] ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 Avance Ultrashield spectrometer. Gas chromatography (GC) was performed using an Agilent Technologies 6890N chromatograph equipped with a HP-5 column or a β-Dex 225 chiral column and a FID detector. High performance liquid chromatography (HPLC) was performed on an Agilent Technologies 1100 series chromatograph equipped with a diode array detector. Optical rotations were measured at 589 nm in a 10 cm sample tube. A 2 M HCN stock solution in buffer-saturated DIPE was prepared as reported previously[21] and used without further purification for reaction.

CLEA-Catalysed Formation of (*R*)- and (*S*)-Mandelonitrile: General Method. The corresponding CLEA (~15 mg) was suspended in a 2 M stock solution of HCN in DIPE (4 mL, 8 mmol), and benzaldehyde (200 μL, 1.96 mmol) was added. After stirring gently (150 rpm) for 4 h at r.t. the CLEA was filtered off, and the volatiles were removed under reduced pressure to yield the crude cyanohydrin. Conversion of benzaldehyde into mandelonitrile was greater than 95%, and the ee of the corresponding cyanohydrin was greater than 97%. Conversion and ee were determined by chiral HPLC (Chiralcel OJ; Hex/iPA (90:10); 1.00 mL/min; R_t(benzaldehyde) = 3.76 min, $R_t(R)$ = 12.18 min, $R_t(S)$ = 15.62 min). ^{1}H and ^{13}C NMR spectra of the products were consistent with literature data: ^{1}H NMR[35] ($CDCl_3$, 400 MHz): δ = 3.46 (bs, 1H, OH), 5.54 (s, 1H, CH), 7.41−7.56 (m, 5H, Ph).^{13}C NMR[35] ($CDCl_3$, 100.65 MHz): δ = 63.3, 119.0, 126.8, 129.1, 129.8, 135.1.

TMS-Protected-(*R*)- and -(*S*)-Mandelonitrile. (*R*)-Mandelonitrile was prepared from benzaldehyde (206.2 mg, 1.94 mmol) using *Pa*CLEA (15.3 mg) and (*S*)-mandelonitrile from benzaldehyde (293.8 mg, 2.79 mmol) using *Me*CLEA (17.8 mg) as described in the general procedure. The crude cyanohydrin was cooled to 0 °C and TMSCl (635 μL, 2.5 equiv) was added to the residue at 0 °C under argon followed by a solution of pyridine (322 μL, 2 equiv) in methylene chloride (2 mL) dropwise. The mixture was stirred for 5 min at 0 °C and the ice water bath was removed. After 3 h at r.t., the volatiles were evaporated carefully and the crude product was filtered through silica using hexane as eluent. The solvent was removed under reduced pressure to give (*R*)-TMS-mandelonitrile (*R*)-**3** (339.3 mg, 1.65 mmol) in 85% yield (from benzaldehyde) and 99% ee and (*S*)-TMS-mandelonitrile (*S*)-**3** (475.3 mg, 2.31 mmol) in 83% yield (from benzaldehyde) and 99% ee respectively, as clear liquids. The *ee* of TMS-mandelonitrile was determined by HPLC (Chiralpak AD; Hex:iPA (99.8:0.2); 1.00 mL/min; $R_t(R)$ = 10.27 min, $R_t(S)$ = 12.17 min). ^{1}H and ^{13}C NMR spectra of the products were consistent with literature data: ^{1}H NMR[36] ($CDCl_3$, 400 MHz): δ = 0.24 (s, 9H, TMS), 5.50 (s, 1H, CH), 7.37−7.48 (m, 5H, Ph). ^{13}C NMR[36] ($CDCl_3$, 100.65 MHz): δ = −0.3, 64.0, 119.4, 126.8, 129.7, 129.8, 135.9.

(1-(*S*)-Phenyl-ethyl) Carbamic Acid (*R*)-Cyano-phenyl-methyl Ester. (*R*)-Mandelonitrile was prepared from benzaldehyde (255.7 mg, 2.41 mmol) using *Pa*CLEA (14.9 mg) as described in the general procedure. The crude cyanohydrin was cooled to 0 °C and THF (5 mL) was added. A solution of (*S*)-1-phenylethyl isocyanate[33] (534.2 mg, 1.5 equiv) in THF (5 mL) was added at this temperature followed by $CuBr\cdot Me_2S$ (736.2 mg, 1.5 equiv), and the mixture was allowed to warm up slowly to r.t.. After overnight stirring, the solids were filtered off and rinsed with diethyl ether (20 mL). The green solution was then concentrated *in vacuo*, and the residue was stirred vigorously in diethyl ether (20 mL). The insoluble materials were filtered off and rinsed with diethyl ether (20 mL); the volatiles were removed under reduced pressure to give the crude carbamate (722.4 mg). The pure carbamate derivative (555.5 mg, 1.98 mmol) was obtained after recrystallisation from pentane/iPA as a white solid in 82% yield (from benzaldehyde) and 99.5% ee. The ee of the carbamate was determined by HPLC (Chiralpak AD; Hex/iPA (70:30); 1.50 mL/min; $R_t(S)$ = 3.95 min, $R_t(R)$ = 5.13 min). ^{1}H NMR[37] ($CDCl_3$, 400 MHz): δ = 1.48, (d, 3H), 4.84 (m_c, 1H), 5.18 (bs, 1H), 6.41 (s, 1H), 7.29−7.53 (m, 10H). ^{13}C NMR[37] ($CDCl_3$, 100.65 MHz): δ = 22.1, 51.3, 63.6, 118.9, 125.6, 125.9, 127.7, 128.8, 129.1, 130.2, 132.1, 132.9, 154.4.

(*R*)-*N*-*tert*-Butyl-2-hydroxy-2-phenylacetamide. (*R*)-Mandelonitrile was prepared from benzaldehyde (319.9 mg, 3.01 mmol) using *Pa*CLEA (15.4 mg) as described in the general procedure. The crude cyanohydrin was dissolved in *tert*-butanol (2 mL), then aqueous 60% H_2SO_4 (2 mL) was added slowly to the mixture at r.t., and stirring was continued for 24 h. The solution was diluted with 10 mL of water and extracted with 3 × 50 mL of methylene chloride. The combined organic layers were dried over $MgSO_4$ and evaporated under reduced pressure to give the crude product. Traces of *tert*-butanol were removed by stirring the crude product under high vacuum, and (*R*)-*N*-*tert*-butyl-2-hydroxy-2-phenylacetamide (436.1 mg, 2.10 mmol) was obtained as a white solid in 70% yield (from benzaldehyde) and 96% ee. Enantiopure product (ee > 99%) could be obtained by recrystallisation from a minimum amount of hexane. The ee of the product was determined by HPLC (Chiralpak AD; Hex/iPA (90:10); 1.00 mL/min; $R_t(R)$ = 6.35 min, $R_t(S)$ = 7.06 min). ^{1}H and ^{13}C NMR spectra of the products were consistent

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with literature data: ^{1}H NMR[38] ($CDCl_3$, 400 MHz): δ = 1.32 (s, 9H), 2.50 (bs, 1H), 4.90 (s, 1H), 5.83 (bs, 1H), 7.31−7.40 (m, 5H). ^{13}C NMR[38] ($CDCl_3$, 100.65 MHz): δ = 28.6, 51.9, 73.8, 127.0, 128.6, 129.0, 139.1, 170.4.

(*R*)-Mandelic Acid: Gram-Scale Preparation. (*R*)-Mandelonitrile was prepared from benzaldehyde (8.65 mL, 85 mmol) using *Pa*CLEA (677.8 mg) as described in the general procedure. A mixture (1:1) of conc. HCl/water (30 mL) was added to the crude cyanohydrin, the solution was heated to 65−70 °C, and the reaction was allowed to proceed upon vigorous stirring at this temperature for 40 h. The mixture was allowed to cool to r.t.; diethyl ether (200 mL) was added, and vigorous stirring was continued for 30 min. The insoluble materials were filtered off, and the phases were separated. The aqueous fraction was extracted with diethyl ether (2 × 200 mL), and the combined ethereal layers were evaporated under reduced pressure. The crude product (12.40 g) was recrystallised from toluene (150 mL) to give pure (*R*)-mandelic acid (10.34 g, 68 mmol) in 80% yield (from benzaldehyde) and 99% ee. The ee of the product was determined by comparison of the sample specific optical rotatory power to the literature data[39] $[\alpha]^{22}_D$ −151.9° (*c* 2.52, MeOH), (*lit.* $[\alpha]^{24}_D$ −152° (*c* 2.52, MeOH)). ^{1}H NMR spectrum of the product was consistent with literature data: ^{1}H NMR[40] ($CDCl_3$, 400 MHz): δ = 5.12 (s, 1H), 7.27−7.36 (m, 3H), 7.46−7.49 (m, 2H).

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