DOI: 10.1002/ejoc.200500905

A Mild Chemo-Enzymatic Oxidation–Hydrocyanation Protocol

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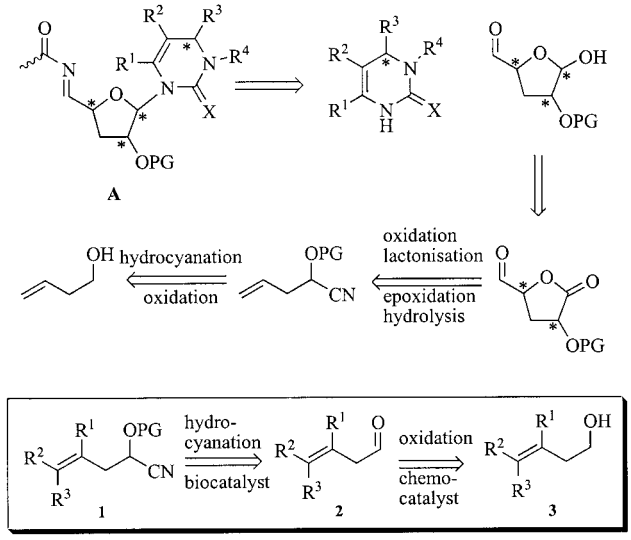
Keywords: TEMPO / Hydroxynitrile lyase / Oxidation / Oxynitrilase

Oxidation–hydrocyanation of γ,δ-unsaturated alcohols using (immobilised) TEMPO/PhI(OAc)$_2$ in combination with *Hb*HNL proceeds smoothly. After (in situ) protection, the resulting cyanohydrin derivatives were obtained in good overall yields and high *ee*'s. A mild TEMPO-catalysed oxidation protocol is described that yields β,γ-unsaturated aldehydes without isomerisation of the double bond and that is compatible with a subsequent *Hb*HNL-catalysed hydrocyanation performed in the same solvent system.

Introduction

Cyanohydrins are versatile chiral building blocks,[1–3] which can be transformed into α-aminonitriles,[4–6] α-hydroxy esters,[7] amino alcohols,[8] 2,3-disubstituted piperidines,[9] and 3-hydroxytetrahydropyridines.[10] The cyanohydrins are conveniently accessed by HNL-catalysed hydrocyanation of the corresponding aldehydes (HNL = hydroxynitrile lyase).[11] These biocatalysts are well established and both the (*R*)- and (*S*)-enantiomer of the cyanohydrins can be prepared in high yields and *ee* values.[12–14] During our studies towards a chemo-enzymatic *de novo* synthesis of non-natural nucleosides (**A**) containing a 3′-deoxy ribose moiety we required a series of differently substituted, optically pure, γ,δ-unsaturated cyanohydrins **1** (Scheme 1). For this, a biocatalytic hydrocyanation of β,γ-unsaturated aldehydes **2** was identified as a key-step.

Oxidation of γ,δ-unsaturated primary alcohols **3** to the corresponding aldehydes **2** is the most direct way to prepare the required substrates for the HNL-catalysed hydrocyanation. However, many of the most widely employed oxidations like, e.g., Swern oxidation or chromium(VI)-based reagents (PDC, PCC, etc) require relatively alkaline conditions. Such conditions are not suitable for the transformation of primary alcohols **3** to yield the desired β,γ-unsaturated aldehydes **2** because they promote the isomerisation of the double bond and thus yield the α,β-unsaturated isomers, which are difficult to separate from the required product.[15–19] Furthermore, the alkaline conditions would also promote the racemic chemical addition of HCN to the aldehydes **2** in the follow-up reaction, a reaction that should result in the desired enantiopure cyanohydrin derivatives **1**.



Scheme 1. Retro synthesis of non-natural nucleosides containing a 3′-deoxyribose moiety.

The work described in this paper aims at a mild oxidation–hydrocyanation protocol to produce optically pure γ,δ-unsaturated (*S*)-cyanohydrins **1** starting from readily available primary alcohols **3**. Since enzymatic redox reactions tend to be difficult due to problems with co-factor regeneration, a chemical reagent is envisaged for the oxidation step. The enantioselective C–C bond formation under close to neutral conditions, however, is difficult to perform with a chemical catalyst, therefore the efficient and highly enantioselective *Hb*HNL will be employed.[20]

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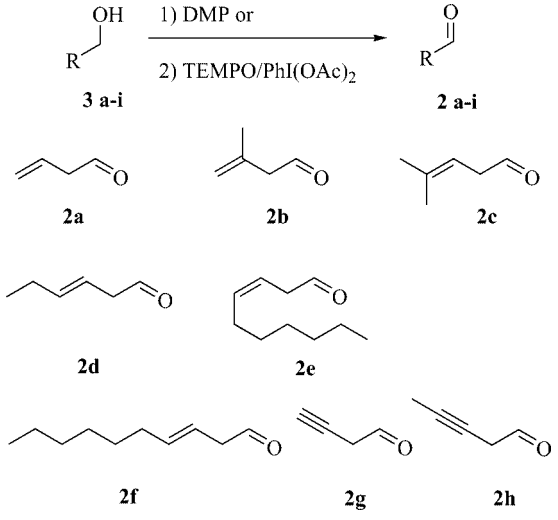
(*R*)-Cyanohydrins are accessible in good yields and *ee* values using the hydroxynitrile lyase *Prunus amygdalus* (*Pa*HNL) from almonds, which catalyses the hydrocyanation efficiently.[12] In general, (*S*)-cyanohydrins are readily available using the hydroxynitrile lyase from the rubber tree *Hevea brasiliensis* (*Hb*HNL).[13,14,20–27] However, reports on the enantioselective synthesis of γ,δ-unsaturated cyanohydrins **1** from β,γ-unsaturated aldehydes **2** are scarce.[15,28] Only two examples are known of (*R*)-**1** produced by *Pa*HNL, while no examples exist for the preparation of (*S*)-**1** using (*S*)-selective enzymes such as *Hb*HNL.

Results and Discussion

In initial experiments, the application of the very mild Dess–Martin periodinane (DMP) to oxidise **3** (Scheme 2) without isomerisation seemed feasible but following this procedure, we encountered a couple of practical disadvantages.[29,30] Commercial DMP is relatively expensive while at the same time its preparation is not entirely trivial and its storage can lead to degradation.[31] When oxidising primary alcohols of relatively low molecular weight, a large amount of DMP is needed to convert the alcohol to the aldehyde. In our case, one gram (13.9 mmol) of alcohol **3a** is converted into the corresponding aldehyde **2a** almost quantitatively, but this can only be achieved by using twelve grams (28.3 mmol) of DMP. Thus the Dess–Martin oxidation suffers from poor atom efficiency and the reaction work-up is troublesome. Consequently, the use of DMP in larger scale production of β,γ-unsaturated aldehydes becomes less convenient. Furthermore, we found that the filtration/evaporation procedure, which is necessary after the Dess–Martin oxidation, was accompanied by some isomerisation of the product aldehyde. Therefore we turned to another method to oxidise alcohols **3** to the corresponding aldehydes **2** (Scheme 2).



Scheme 2. Oxidation of γ,δ-unsaturated alcohols to β,γ-unsaturated aldehydes.

The use of relatively stable organic nitroxyl radicals, like 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO), as catalysts for the mild oxidation of alcohols has found widespread application.[32,33] Among the numerous described variants, the oxidation of sensitive primary alcohols that employs a catalytic amount of TEMPO together with $PhI(OAc)_2$ as the primary oxidant is particularly interesting.[34,35]

Initially the reaction was performed following the literature procedure[34] by using a 0.2 M solution of **3a** in CH_2Cl_2, 1.1 equiv. of $PhI(OAc)_2$, and 0.1 equiv. of TEMPO (entry 1, Table 1). After 2.5 h only 15% had been converted into the desired β,γ-unsaturated aldehyde **2a**. Although more oxidising agent improved the conversion, still only an unsatisfactory 38 or 50% conversion (entries 2 and 3, Table 1) was detected after 2.5 h. Prolonged reaction times did not result in a better conversion but instead isomerisation to the undesired α,β-unsaturated aldehyde occurred. Instead of using CH_2Cl_2, an attempt was made to change the solvent to a more environmentally friendly mixture of pentane and CH_2Cl_2. This also has the advantage that this mixture is more suitable for *Hb*HNL.[24] To our satisfaction, application of **3a** dissolved in a 9:1 mixture of pentane and CH_2Cl_2, respectively, together with 1.1 equiv. of $PhI(OAc)_2$, and 0.1 equiv. of TEMPO gave complete and selective conversion to **2a** after 2.5 h (entry 4, Table 1).

Table 1. Oxidation of **3a** with TEMPO/$PhI(OAc)_2$.

Entry	$PhI(OAc)_2$ [equiv.]	TEMPO [equiv.]	Solvent	Conversion[a] [%]
1	1.1	0.1	CH_2Cl_2	15
2	1.1	0.2	CH_2Cl_2	38
3	1.2	0.2	CH_2Cl_2	50
4	1.1	0.1	pentane/CH_2Cl_2, 9:1	100

[a] Conversion of **3a** after 2.5 h at room temperature determined by ^{1}H-NMR spectroscopy.

A number of γ,δ-unsaturated primary alcohols **3** were treated under similar conditions and in Table 2 the results are summarised. Conditions were optimised towards a minimum degree of isomerisation and optimum conversion. As can be seen, homo-allylic primary alcohols **3a**–**3f** were readily converted into the corresponding β,γ-olefinic aldehydes **2a**–**2f**. Also homo-propargylic primary alcohols can be oxidised to yield the corresponding aldehydes, however, 1-butynol **3g** proved to react sluggishly.

It should be noted that the above TEMPO/$PhI(OAc)_2$ oxidation protocol encounters some problems during reaction work-up. Even when the aldehyde was carefully co-distilled with ether under reduced pressure, a significant isomerisation of the aldehyde was observed. This provided an additional incentive to develop the planned reaction protocol. However, during the oxidation two equivalents of acetic acid are formed. As this acid would cause an immediate deactivation of the *Hb*HNL it had to be removed prior to the hydrocyanation reaction.[36] By washing the reaction mixture at the end of the TEMPO/$PhI(OAc)_2$ oxidation of **3a** with a saturated solution of $NaHCO_3$, all the acetic acid was removed completely. To our satisfaction no isomerisation was detected.

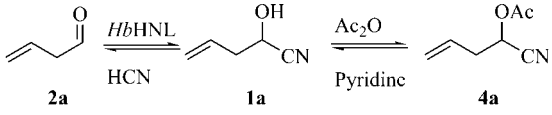
Table 2. Optimised TEMPO/$PhI(OAc)_2$ oxidation of γ,δ-unsaturated alcohols **3**.

Substrate	mmol of **3a**–**3i**	Conversion[a] [%]	$PhI(OAc)_2$ [equiv.]	TEMPO [equiv.]	Pentane/CH_2Cl_2	Conc. [mM]	Reaction time [min]
3a	27.5	quant.	1.1	0.1	9:1	0.20	150
3b	5	quant.	1.1	0.1	9:2	0.36	120
3c	2	quant.	1.1	0.1	9:2	0.36	40
3d	2	quant.	1.1	0.1	9:2	0.36	100
3e	4	quant.	1.2	0.2	2:1	0.66	30
3f	4	quant.	1.2	0.2	2:1	0.66	30
3g	0.25	0	1.2	0.2	1:1	1	–
3h	0.5	41	1.8	0.2	3:2	1	70

[a] Conversion of **3** determined by ^1H NMR.

Since both the formation of cyanohydrins and the isomerisation of the aldehyde **2a** are base-catalysed, the enzyme reaction should be performed in a mildly acidic buffer. A direct transfer of the conditions that were used in the *Pa*HNL-catalysed synthesis of the (*R*)-enantiomer of **1a** is not possible. *Pa*HNL and *Hb*HNL are structurally not related and their optimum reaction conditions are different.[37] Initially two different pH values (4.0 and 5.0) and two different temperatures (0° and 25 °C) were investigated for the *Hb*HNL-catalysed addition of HCN to the in-situ-generated aldehyde **2a** following a general procedure.[24] The results are summarised in Table 3.

Table 3. Selectivity at complete conversion of **2a** in the *Hb*HNL-catalysed hydrocyanation; various pH values and temperatures.[a]



2a **1a** **4a**

Entry	pH	*T* [°C]	*ee* of **4a** [%]
1	4.0	0	82
2	5.0	0	71
3	5.0	25	66

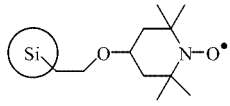
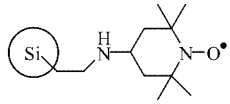
[a] Initially, *Hb*HNL and 3 equiv. of HCN were used but after 30 min the reaction stopped. Complete conversion was only achieved by the addition of extra *Hb*HNL and 1.5 equiv. HCN (see Exp. Sect.).

When the enzymatic hydrocyanation was performed at 0 °C and pH 4.0 the desired γ,δ-unsaturated cyanohydrin **1a** was formed with reasonable enantioselectivity (*ee* = 82% entry 1, Table 3). Even though this result is in line with *ee* values obtained previously with other short-chain aldehydes[1] it is still insufficient for synthetic use. The relatively low *ee* is not caused by chemical background reaction, as this is virtually absent under these conditions. Therefore, other reasons were investigated.

The *Hb*HNL-catalysed hydrocyanation of **2a** as described above stopped after 30 min (see Table 3). Apparently the enzyme was deactivated and additional *Hb*HNL and HCN were needed to allow the reaction to complete. After $NaHCO_3$ neutralisation of the crude reaction mixture that results from the oxidation, part of the highly reactive TEMPO is still present. To minimise a potentially harmful effect of TEMPO on the activity and the selectivity of the *Hb*HNL we investigated the influence of different immobilised variants of TEMPO in the oxidation–hydrocyanation protocol. This should allow for efficient removal of TEMPO from the reaction mixture just before addition of the *Hb*HNL. Furthermore, such a procedure opens the way to a more atom-efficient protocol that enables recycling of the TEMPO-catalyst.

TEMPO was immobilised on colloidal silica according to a known procedure[38] and compared with commercial TEMPO immobilised on silica gel. The TEMPO immobilisates were screened in the oxidation of **3a** for optimal conversion and minimal isomerisation of the aldehyde product **2a** (Table 4). The data in Table 4 (entry 1) show that oxidation of **3a** using TEMPO immobilised on colloidal silica proceeds about three times faster as compared to oxidation of **3a** using soluble TEMPO (Table 2). After 40 min at room temp. the reaction was complete and no isomerisation was observed. The TEMPO on colloidal silica could easily be recovered (by filtration) and re-used at least once without any loss of activity. The commercial TEMPO on silica (entry 2, Table 4) gave, under similar reaction conditions, only a few percent of 3-butenal **2a**. In the ^1H NMR of the reaction mixture, the un-reacted alcohol **3a** was clearly identified together with signals that could only be attributed to the undesired isomer of 3-butenal, crotonaldehyde.

Table 4. Oxidation of **3a** using TEMPO immobilised on colloidal silica and commercial TEMPO immobilised on silica.[a]

Entry	Structure	Loading [mmol/g]	Equiv.	**2a** [%][b]	Reaction time
1[c]		0.27	0.16	>98	40 min
2[d]		0.61	0.35	0[e]	3.5 h

[a] 1.2 Equiv. of $PhI(OAc)_2$ in a 0.2 M solution of **3a** in a 1:9 CH_2Cl_2/pentane mixture at room temperature. [b] Conversion determined by ^1H-NMR spectroscopy. [c] TEMPO immobilised on colloidal silica. [d] Commercial TEMPO immobilised on silica, available from Sigma–Aldrich. [e] The reaction yielded the unwanted isomer of **2a**, crotonaldehyde.

Finally, the oxidation of **3a** catalysed by immobilised TEMPO was combined with the *Hb*HNL-catalysed hydrocyanation to arrive at the desired cyanohydrin **1a**.[39] After the oxidation reaction, the immobilised TEMPO was removed by filtration and the resulting mixture neutralised with a saturated $NaHCO_3$ solution. The resulting solution is directly used in the *Hb*HNL-catalysed formation of γ,δ-unsaturated **1a**. **1a** (*ee* = 93%; entry 1, Table 5) could be isolated as its TBDMS-ether **5a** in 37% overall yield[40] starting from the γ,δ-unsaturated primary alcohol **3a**. Although, under these optimised conditions, the isolated yield of **1a** is 5–10% lower than the yield obtained after the oxidation–hydrocyanation sequence using soluble TEMPO, the optical purity of **1a** is considerably higher (93% vs. 82%; entry 1, Table 5 vs. entry 1, Table 3) when performing this protocol using immobilised TEMPO. The results indicate that TEMPO has a negative effect both on the activity and the selectivity of the enzyme.

Table 5. The three-step protocol oxidation–hydrocyanation and protection reaction starting from **3a**–**3d** using both homogeneous and heterogeneous TEMPO in combination with *Hb*HNL.

Entry	Substrate	Product	pH	Homogeneous Yield[a] (*ee*)[b] [%]	Heterogeneous Yield[a] (*ee*)[b] [%]
1	**3a**	**5a**	4.0	n.d. (82)	37 (93)
2	**3b**	**4b**	4.0	54 (92)	43 (97)
3	**3c**	**4c**	5.0	48 (78)	23 (95)
4	**3d**	**4d**	5.0	52 (61)	26 (87)

[a] Isolated yields over 3 steps. [b] *ee* determined by chiral GC.

The above procedures were used to prepare γ,δ-unsaturated cyanohydrins **1b**–**1d**, starting from the corresponding primary alcohols **3b**–**3d**. The resulting cyanohydrins were directly protected as acetates. The results are summarised in Table 5. A similar trend with regard to isolated yield and optical purity that was observed for the synthesis of **5a** is also found for the preparation of **4b**–**4d**. In general, the *ee* values of **4b**–**4d** (entries 2–4, Table 5) are significantly higher whereas their isolated yields (calculated from **3b**–**3d**) are somewhat lower if they are prepared by the immobilised TEMPO/*Hb*HNL protocol compared to the *ee* values and yields obtained by applying the same protocol by using soluble TEMPO. As the reactions were performed on a 100 mg scale and the compounds involved in this reaction are relatively polar and/or volatile some loss/low yields could not be avoided.

When testing both pH 4.0 and 5.0 for aldehydes **2c**–**2d** pH 5.0 proved to be favourable and was therefore utilized. Finally, the γ,δ-unsaturated primary alcohols **3e** and **3f** were readily oxidised by the TEMPO/$PhI(OAc)_2$ procedure to the corresponding aldehydes **2e** and **2f**. However, both aldehydes proved unreactive towards *Hb*HNL-catalysed hydrocyanation. This can be attributed to the length of the alkyl chain, which is known to be crucial for *Hb*HNL activity.[11–14]

Conclusion

In summary, we have developed an efficient oxidation–hydrocyanation protocol that produces optically enriched γ,δ-unsaturated cyanohydrins in good yields starting from the corresponding primary alcohols. The oxidation of the alcohols with TEMPO and $PhI(OAc)_2$ is a mild and selective method to prepare β,γ-unsaturated aldehydes, which are otherwise difficult to access as they readily undergo isomerisation to the α,β-unsaturated analogues. The thus generated aldehydes can be used directly in the subsequent *Hb*HNL-catalysed hydrocyanation to give the desired optically enriched γ,δ-unsaturated cyanohydrins. Moreover, when the *Hb*HNL catalyst was used in combination with the TEMPO catalyst immobilised on colloidal silica *ee* values up to 97% and overall yields up to 43% of the final cyanohydrin derivatives were obtained. The TEMPO-catalyst could be re-used at least once without any loss of catalytic activity.

Experimental Section

General Remarks: ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra are recorded in $CDCl_3$ on a Bruker Avance 250 (250.13 MHz and 62.90 MHz, respectively) or Bruker Avance 400 (400.13 MHz and 100.61 MHz, respectively) with chemical shifts (δ) reported in ppm downfield from tetramethylsilane. MS and HRMS data were measured at 70 eV with a Finnigan MAT900 spectrometer. To follow the course of the reactions, samples were taken directly from the reaction mixtures, dissolved in $CDCl_3$ and analysed by ^1H-NMR spectroscopy. For the oxidation and enzyme reaction, the conversion was determined by monitoring the H_2C–O (δ = 3.59–3.76) and the *H*C=O (δ = 9.66–9.74) signal, respectively. Flash column chromatography was performed with Baker 7024–02 silica gel (40 μ, 60 Å) solvents were petroleum ether (PE) with a boiling range between 40 °C and 60 °C, and ethyl acetate (EA). Thin-layer chromatography (TLC) was performed using silica plates from Merck (Kieselgel 60 F_{254} on aluminium with fluorescence indicator). Compounds on TLC were visualised by UV-detection or 5% (w/v) aqueous $KMnO_4$. The racemic cyanohydrin acetates were prepared from the corresponding aldehydes according to literature.[41] The enantiomeric excess of the acylated (*S*)-cyanohydrins **4** was determined on a Shimadzu GC-17A, equipped with a β-cyclodextrin column (CP-Chirasil-Dex CB 25 m × 0.32 mm ID), a FID detector, and a Shimadzu Auto-injector AOC-20i. The carrier gas was He with a linear gas velocity of 75 cm/s at 155kPa. The GC-retention times are summarised in Table 6. Optical rotations were measured on an AA-10 automatic polarimeter from Optical Activity Ltd. Pro analysis grade γ,δ-unsaturated primary alcohols **3a**–**3d**, **3g**, and **3h** were all commercially available and used without purification except for 3-butenol **3a**, which was distilled and stored under nitrogen and over 4-Å molecular sieves. Primary alcohols **3e** and **3f** were prepared by hydrogenation and LAH reduction, respectively, starting from 3-decynol following literature procedures.[42,43] TEMPO immobilised on silica gel (70–120 mesh) was purchased from Sigma–Aldrich. The loading of the TEMPO on colloidal silica was determined by elemental analysis on an Elementar Vario EL III analyzer. The hydroxynitrile lyase from *Hevea brasiliensis* (*Hb*HNL) was a generous gift from DSM (Wubbolts, NL). The activity of the *Hb*HNL (13.1 U/mg protein solution) was determined according to standard procedures.[44,45]

Table 6. Temperature program and retention times for the GC analysis of acetates **4a**–**4d**.

Compound	Temperature (°C)	R_t (*R*) **4a**–**d** (min)	R_t (*S*) **4a**–**d** (min)
4a	100	3.13	4.16
4b	135	1.13	1.20
4c	135	1.61	1.81
4d	135	1.63	1.83

Colloidal Silica: A mixture of tetramethoxysilane (25 mL) and acidic water (50 mL, pH adjusted to 2.8 by addition of HCl) was stirred until a homogeneous mixture was formed. After ageing at room temperature for 18 h, the gel was crushed to a fine powder and the water was removed by azeotropic distillation with toluene. The solid was collected by filtration and dried at 120 °C over-night to give a white glass like powder. The TEMPO was immobilised on the colloidal silica according to literature.[38]

General Oxidation–Hydrocyanation Procedure A, Using Homogeneous TEMPO and *Hb*HNL: To a solution of γ,δ-unsaturated primary alcohol **3a**–**d** in a pentane/CH_2Cl_2 mixture $PhI(OAc)_2$ and TEMPO were added, all quantities are according to Table 2. The reaction mixture was stirred at room temperature until full conversion of the alcohol was reached (according to ^1H NMR). Then, saturated $NaHCO_3$ solution was added at 0 °C to the reaction mixture until the CO_2 development ended. The aqueous layer was removed and hydroxynitrile lyase from *Hevea brasiliensis* (1.45 kU/mmol **3a**–**d**) dissolved in an equivolume of 0.1 M citrate buffer (pH 4.0 or pH 5.0) at 0 °C to generate a 1:1 mixture (v/v) of organic phase to buffer was added. The mixture was stirred vigorously until a stable emulsion was obtained, after which HCN dissolved in MTBE was added. [The HCN solution was prepared by dissolving sodium cyanide (3.0 equiv.) in water (10 mL) and adjusting the pH of the solution to 4.8 by addition of citric acid. This aqueous solution was extracted with MTBE (3 × 8 mL) at 0 °C]. After the hydrocyanation was complete (according to ^1H NMR) the organic layer was isolated and dried with $MgSO_4$. In the cases where the emulsion was too stable it was extracted with CH_2Cl_2 (5–10 mL). After evaporation of the solvents, the resulting oil was dissolved in CH_2Cl_2 (2 mL/mmol substrate) and acetic anhydride (3 equiv.), pyridine (2 equiv.) and 4-DMAP were added to the solutions of (*S*)-cyanohydrins **1b**–**d**. The reaction mixture was stirred overnight, washed with 1% HCl (2 × 10 mL), water (2 × 10 mL), followed by washing with saturated $NaHCO_3$ (2 × 10 mL) and water (2 × 10 mL). The organic layer was dried with Na_2SO_4 and concentrated under vacuum. The crude product was purified by column chromatography. In the case of **1a**, starting from 6 mmol, only an analytical sample was derivatised in the same manner. This showed an *ee* of 82%.

(2*S*)-2-Acetoxy-4-methyl-4-pentenenitrile (4b): The title compound was prepared from **3b** (430 mg, 5 mmol) according to general procedure A, using pH 4.0. **(*S*)-4b** was obtained as a clear oil (414 mg, 54% yield, 92% *ee*). For characterisation, see **(*S*)-4b** obtained from general procedure B.

(2*S*)-2-Acetoxy-5-methyl-4-hexenenitrile (4c): The title compound was prepared from **3c** (200 mg, 2 mmol) according to general procedure A, using pH 5.0. **(*S*)-4c** was obtained as a clear oil (161 mg, 48% yield, 78% *ee*). For characterisation, see **(*S*)-4c** obtained from general procedure B.

(2*S*,4*E*)-2-Acetoxy-4-heptenenitrile (4d): The title compound was prepared from **3d** (200 mg, 2 mmol) according to general procedure A, using pH 5.0. **(*S*)-4d** was obtained as a clear oil (174 mg, 52% yield, 61% *ee*). For characterisation, see **(*S*)-4d** obtained from general procedure B.

General Oxidation–Hydrocyanation Procedure B, Using TEMPO Immobilisates and *Hb*HNL: A 0.2 M solution of γ,δ-unsaturated primary alcohols **3** (1 equiv.) in a 9:1 pentane/CH_2Cl_2 mixture, $PhI(OAc)_2$ (1.1 equiv.) was mixed with immobilised TEMPO (TEMPO on colloidal silica: 0.27 mmol/g, 0.16 equiv. or commercial TEMPO on silica gel: 0.61 mmol/g, 0.35 equiv.) and stirred at room temperature until completion (according to ^1H NMR). After filtration of immobilised TEMPO, a saturated $NaHCO_3$ solution was added to the reaction mixture at 0 °C until the CO_2 development ended. The aqueous layer was removed and to the organic layer was added hydroxynitrile lyase from *Hevea brasiliensis* (1.45 kU/mmol **3a**–**d**) dissolved in an equivolume of 0.1 M citrate buffer (pH 4.0 or pH 5.0) at 0 °C to generate a 1:1 mixture (v/v) of organic phase to buffer. The mixture was stirred vigorously until a stable emulsion was obtained, after which HCN (3 equiv.) dissolved in MTBE was added. [The HCN solution was prepared by dissolving sodium cyanide (3.0 equiv.) in water (10 mL) and adjusting the pH of the solution to 4.8 by addition of citric acid. This aqueous solution was extracted with MTBE (3 × 8 mL) at 0 °C]. After the hydrocyanation was complete (according to ^1H NMR) the organic layer was separated and dried with $MgSO_4$. In the cases where the emulsion was too stable it was extracted with CH_2Cl_2 (5–10 mL). After evaporation of the solvents, the resulting oil was dissolved in CH_2Cl_2 (2 mL/mmol substrate) and acetic anhydride (3 equiv.), pyridine (2 equiv.) and 4-DMAP were added to the solutions of (*S*)-cyanohydrins **1b**–**d**. The reaction mixture was stirred overnight, washed with 1% HCl (2 × 10 mL), water (2 × 10 mL), followed by washing with saturated $NaHCO_3$ (2 × 10 mL) and water (2 × 10 mL). The organic layer was dried with Na_2SO_4 and concentrated under vacuum. The crude product was purified by column chromatography. In the case of **1a**, only an analytical sample was derivatised in the same manner to give the acetate with an *ee* of 95%. The remaining solution was derivatised as the corresponding TBDMS-ether **5a** (see below).

(2*S*)-2-Acetoxy-4-methyl-4-pentenenitrile (4b): The title compound was prepared from **3b** (431 mg, 5 mmol) according to general procedure B, using pH 4.0. **(*S*)-4b** was obtained as a clear oil (330 mg, 43% yield, 97% *ee*); R_f (PE/EA, 95:5) = 0.27. $[\alpha]_D^{22}$ = –74 (*c* = 1, $CHCl_3$). ^1H NMR (250.13 MHz): δ = 1.83 (s, 3 H, CH_3C=C), 2.16 (s, 3 H, CH_3C=O), 2.64 (d, *J* = 7.1 Hz, 2 H, CH_2CHCN), 4.98 (d, *J* = 18.9 Hz, 2 H, H_2C=CCH$_3$), 5.50 (t, *J* = 7.1 Hz, 1 H, CH–CN) ppm. ^{13}C NMR (62.90 MHz): δ = 20.3 (*C*H$_3$C=O), 22.3 (*C*H$_3$C=C), 40.4 (CH_2), 59.7 (CH–O), 116.10 (CH=*C*H$_2$), 116.7 (CN), 137.6 (*C*H=CH$_2$), 168.9 (C=O) ppm. MS ($C_8H_{11}O_2N$, *m*/*z*, relative intensity): 153 [M^+, 2], 111 (4), 93 (100), 66 (68), 55 (32).

(2*S*)-2-Acetoxy-5-methyl-4-hexenenitrile (4c): The title compound was prepared from **3c** (75 mg, 0.75 mmol) according to general procedure B, using pH 5.0. **(*S*)-4c** was obtained as a clear oil (29 mg, 23% yield, 95% *ee*); R_f (PE/EA, 95:5) = 0.27. $[\alpha]_D^{22}$ = –46 (*c* = 1, $CHCl_3$). ^1H NMR (250.13 MHz): δ = 1.71 (s, 3 H, CH_3C=C), 1.79 (s, 3 H, CH_3C=C), 2,18 (s, 3 H, CH_3C=O), 2.61–2.67 (m, 2 H, CH_2), 5.19 (t, *J* = 7.2 Hz, 1 H, C=C*H*), 5.30 (t, *J* = 6.9 Hz, 1 H, C*H*CN) ppm. ^{13}C NMR (100.61 MHz): δ = 17.9 (*cis*-*C*H$_3$), 20.3 (*C*H$_3$C=O), 25.7 (*trans*-*C*H$_3$), 31.1 (CH_2), 60.9 (CH–O), 114.9 (C=*C*H$_2$), 116.7 (CN), 138.5 (*C*=CH$_2$), 168.9 (CO) ppm. MS ($C_9H_{13}O_2N$, *m*/*z*, relative intensity): 167 [M^+, 16], 149 (16), 142 (64), 113 (40), 95 (40), 69 (100), 55 (48).

(2*S*,4*E*)-2-Acetoxy-4-heptenenitrile (4d): The title compound was prepared from **3d** (75 mg, 0.75 mmol) according to general procedure B, using pH 4.0. **(*S*)-4d** was obtained as a clear oil (32.6 mg,

26% yield, 87% *ee*); R_f (PE/EA, 95:5) = 0.35. $[\alpha]_D^{22}$ = –32 (*c* = 1, $CHCl_3$). ^{1}H NMR (250.13 MHz): δ = 1.02 (t, *J* = 7.4 Hz, 3 H, CH_3CH$_2$), 2.03–2.12 (m, 2 H, CH$_3$CH_2), 2.15 (s, 3 H, CH_3C=O), 2.56–2.62 (m, 2 H, CH_2CHCN), 5.30–5.45 (m, 2 H, CH=CH), 5.71–5.82 (m, 1 H, CHCN) ppm. ^{13}C NMR (100.61 MHz): δ = 13.2 (*C*H$_3$–CH$_2$), 20.1 (*C*H$_3$C=O), 25.4 (CH$_3$–*C*H$_2$), 35.4 (*C*H$_2$), 60.9 (*C*H–O), 116.4 (CN), 119.5 (*C*H–CH$_2$–CH), 139.0 (*C*H–CH$_2$–CH$_3$), 168.9 (C=O) ppm. MS ($C_9H_{13}O_2N$, *m*/*z*, relative intensity): 167 [M$^+$, 16], 149 (52), 142 (24), 106 (48), 83 (52), 69 (100).

(2*S*)-2-(*tert*-Butyldimethylsilanyloxy)-4-pentenenitrile (5a): A solution of recrystallised imidazole (0.87 g, 12.8 mmol) and *tert*-butyldimethylsilyl chloride (2.11 g, 14 mmol) in 70 mL DMF was stirred at 0 °C for 20 min. The crude solution of **1a**, prepared from **3a** (844 mg, 11.7 mmol) according to general procedure B using pH 4 was added, the mixture was warmed to room temp. and stirred overnight. The reaction mixture was diluted with 70 mL of water and extracted with diethyl ether (3 × 110 mL). The combined organic layers were washed with water (2 × 100 mL) and then with brine (1 × 100 mL). The organic layer was dried with Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography on silica (PE/EA, 98:2) yielding **5a** as a clear oil (659 mg, 37% yield, 95% *ee*); R_f (PE/EA, 95:5) = 0.74. $[\alpha]_D^{22}$ = –60 (*c* = 1, $CHCl_3$). ^{1}H NMR (250 MHz): δ = 0.17 (s, 3 H, CH_3Si), 0.22 (s, 3 H, CH_3Si), 0.94 (s, 9 H, *t*But), 2.53–2.59 (m, 2 H, CH_2CHCN), 4.47 (t, *J* = 6.5 Hz, 1 H, CHCN), 5.23–5.30 (m, 2 H, CH=CH_2), 5.84 (ddt, *J* = 17.4, 9.8 and 7.0 Hz, 1 H, CH=CH$_2$) ppm. ^{13}C NMR (62.90 MHz): δ = –4.93 (*C*H$_3$–Si), –4.73 (*C*H$_3$–Si), 18.49 [Si–C(CH$_3$)], 25.90 [Si–C(*C*H$_3$)], 41.11 (*C*H$_2$–CH–O), 62.31 (*C*H–O), 120.00 (CN), 120.51 (*C*H$_2$=CH), 131.40 (CH$_2$=*C*H) ppm. HRMS (EI) calculated for $C_{11}H_{21}NOSi$ (M$^+$) 211.1392 found 211.1409. MS ($C_{11}H_{21}NOSi$, *m*/*z*, relative intensity): 210 [M$^+$, 15], 156 (66), 126 (100), 73 (74).

Acknowledgments

We thank DSM (M. Wubbolts) for the *Hb*HNL. D. J. V. and L. V. thank NWO/JC and NWO/ACTS, respectively, for financial support. U. H. thanks the Royal Netherlands Academy of Arts and Sciences (KNAW) for a fellowship.

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Received: November 10, 2005
Published Online: January 26, 2006

