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Applying Biocatalysis to the Synthesis of Diastereomerically Enriched Cyanohydrin Mannosides

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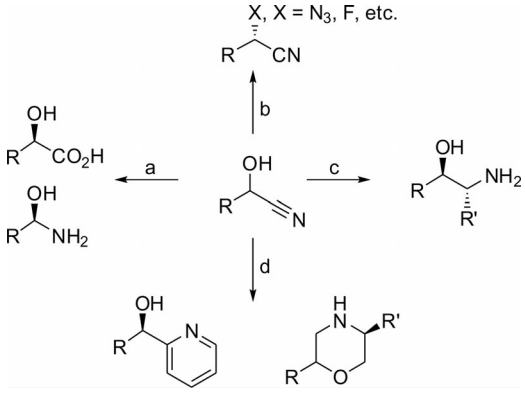
Fully acetylated D- and L-α-mannosylacetaldehydes have been prepared and used as substrates to produce the corresponding cyanohydrins or cyanohydrin acetates with (2*S*) or (2*R*) configuration, respectively, at the cyanohydrin moiety. The (*R*)-oxynitrilase-catalysed synthesis and lipase-catalysed diastereomeric kinetic and dynamic kinetic resolutions were investigated. Sequential catalysis with almond meal [an economic source of (*R*)-oxynitrilase] and *Burkholderia cepacia* lipase was shown to be a straightforward method that yielded the four diastereomeric target cyanohydrins, the absolute configurations of which were confirmed by ^{1}H NMR analysis.

Introduction

Glycosides, both natural and non-natural, currently have many applications in synthetic, medicinal and biological chemistry. Non-natural glycosides display significant potential in several developing areas, including asymmetric synthesis[1a] and carbohydrate-based drug discovery.[1b] They also serve as probes for chemical biology studies.[1c] A good monosaccharide building block for the synthesis of non-natural glycosides should bear an aglycon from which a large number of variations in structure and functional groups can be easily generated. It is also essential that the aglycon structure is accessible in its enantiomeric/diastereomeric forms. Cyanohydrin (α-hydroxy nitrile) glycosides can be considered to be versatile building blocks that fulfil many of the desired properties mentioned above (Scheme 1).[2]



Scheme 1. Funtional groups readily accessible from cyanohydrins: (a) α-hydroxy carboxylic acids and amides, (b) α-functionalized nitriles, (c) 1,2-amino alcohols, (d) N-heterocycles.

Traditional chemical catalysts for the asymmetric synthesis of cyanohydrins from hydrogen cyanide or, more often, from trimethylsilyl cyanide and an aldehyde or ketone include chiral complexes of transition metals (such as titanium alkoxides), other Lewis acids and organocatalysts (such as histidine-derived dioxopiperazines).[2a–2e] In addition, two enzymatic protocols have been thoroughly reviewed.[2f,2g] In more detail, these methods include the enantiofacial addition of hydrogen cyanide to aldehydes (Scheme 2a, routes 1 and 2) and ketones by oxynitrilases (also known as hydroxynitrile lyases, HNLs, E.C. 4.1.2.x) and the kinetic resolution of racemic cyanohydrins (Scheme 2b, route 3) by lipases (E.C. 3.1.1.3). Moreover, the reversible addition of cyanide to carbonyl compounds in the presence of a base has been exploited to convert enzymatic kinetic resolution by acylation into dynamic kinetic resolution (Scheme 2b, routes 3 + 4). In oxynitrilase-catalysed transcyanation (Scheme 2a), acetone cyanohydrin[3] can be used as the cyanide source in reversible reactions in which the enzyme first releases HCN from acetone cyanohydrin, the equilibrium being on the acetone side. This is followed by the enzyme-catalysed addition of HCN to the carbonyl group of the aldehyde. The whole process is based on the higher stability of aldehyde cyanohydrins over the ketone cyanohydrins, which, together with the initial excess of acetone cyanohydrin, drives the system towards the desired chiral product **3**. Accordingly, the use of highly hazardous hydrogen or trimethylsilyl cyanides used in chemical

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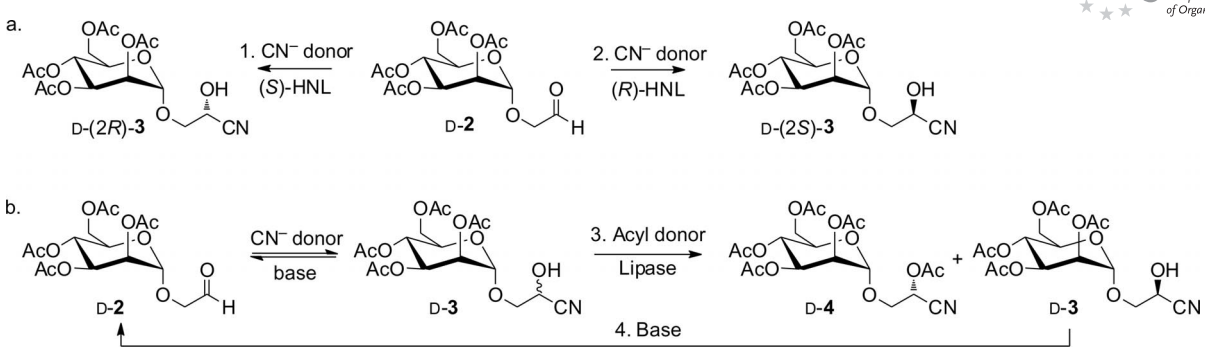


Scheme 2. Enzymatic strategies to diastereomerically enriched cyanohydrins (α-D-mannoside-based cyanohydrin formation shown): (a) oxynitrilase (HNL) catalysed asymmetric synthesis (routes 1 and 2), (b) lipase-catalysed diastereomeric kinetic (route 3) and dynamic kinetic resolution (routes 3 + 4). In this work, the CN^- donor is acetone cyanohydrin.

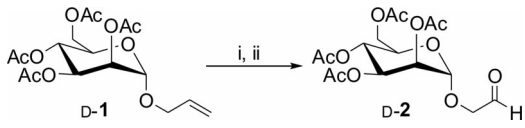
synthesis is avoided. These biocatalytic methods have been shown to have several attractive features, such as the commercial availability of the biocatalysts from different sources, high stereoselectivity and large variations in accepted substrates.

In this work (*R*)-oxynitrilases and lipases have been used to study the possibility of preparing the diastereomers of α-(2-cyano-2-hydroxy/acetoxy)mannosides (1′*S*,2′*S*,3′*S*,4′*R*,5′*R*,2*S*)-**3**, (1′*S*,2′*S*,3′*S*,4′*R*,5′*R*,2*R*)-**4**, (1′*R*,2′*R*,3′*R*,4′*S*,5′*S*,2*S*)-**3** and (1′*R*,2′*R*,3′*R*,4′*S*,5′*S*,2*R*)-**4** from the corresponding aldehydes D- and L-**2** (Scheme 2). An (*R*)-oxynitrilase [(*R*)-HNL] enzyme was expected to yield through route 2 (Scheme 2) the products D- and L-**3** possessing the absolute configuration (*S*) at C-2. Although stereochemically similar to (*R*)-mandelonitrile (the natural substrate of the oxynitrilases used),[2e,2g] the application of CIP rules now reverses the order of priority of the substituents and, accordingly, the notation of absolute configuration from (*R*) to (*S*). Lipases, on the other hand, show the opposite enantiopreference, and therefore the (*R*)-cyanohydrin acetates D- and L-(2*R*)-**4** were the expected products of route 3. These stereochemical predictions were experimentally confirmed in this work. Fully acetylated sugar aldehydes **2** were obtained in four steps with high yields from D- and L-mannose.

Results and Discussion

Preparation of Aldehydes 2

We have previously described the synthesis of allyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (**1**), and this substrate was used as the precursor to prepare aldehydes D- and L-**2** (Scheme 3).[4] The synthesis commenced by using slightly modified literature procedures.[5] In short, the alkene functionality was dihydroxylated with a catalytic amount of OsO_4 followed by fragmentation with $NaIO_4$ to give aldehyde D-**2** in almost quantitative yield over two steps. It was noticed that when following the procedure of Binder et al. the yield of the final fragmentation was only 50%.[5b] The main problem was identified as the extraction step for which the reported procedure used only Et_2O. When performing additional extractions with EtOAc and CH_2Cl_2 the isolated yield could be significantly improved. For the preparation of L-**2** a slightly modified glycosylation procedure was used to shorten the reaction time. It was observed that upon heating the reaction mixture at reflux the reaction is essentially complete within 3 h instead of the 24 h required in the absence of heating, and the product can be isolated in good yield (60%). The product was converted into L-**2** by the methods described above and in similar yields. Hence, both enantiomers of the substrate aldehyde were available for the hydrocyanation step.



Scheme 3. Synthesis of aldehydes **2** (α-D-mannoside-based synthesis shown here): Reagents and conditions: (i) OsO_4, NMO, acetone/H_2O (4:1; 91%); (ii) $NaIO_4$, CH_3CN/H_2O (2:1.2; quant.).

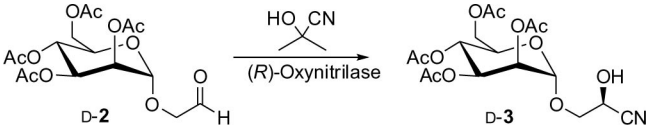
(*R*)-Oxynitrilase-Catalysed C–C Bond Formation

The transcyanation of D- and L-**2** with acetone cyanohydrin was investigated. Three (*R*)-oxynitrilase preparations, namely crude almond enzyme known as almond meal, covalently immobilized (*R*)-oxynitrilase from *Prunus amygdalus* (*Pa*HNL) and an aqueous solution of (*R*)-oxynitrilase from *Arabidopsis thaliana* (*At*HNL) were first studied by using D-**2** as substrate. The almond enzyme has a pH optimum at around 5.5–6.0 and has been successfully used at a pH as low as 3.75.[6,7a] Applications of *At*HNL, an α/β-hydrolase fold based (*R*)-oxynitrilase, have proceeded at pH = 5 and possibly enlarged the tolerated substrate scope compared with the almond enzyme alone.[8] Low pH and low water content are beneficial both for the stability of the free cyanohydrin and for the suppression of its chemical formation, which tends to take place in parallel with the enzymatic synthesis. However, the oxynitrilase enzymes are usually inactive under anhydrous conditions. In some cases, immobilization has been shown to render enzymatic activity at negligible water contents and even in neat organic sol-

vents.[9] *Pa*HNL specifically has been shown to retain its activity for extended periods at the water/diisopropyl ether (DIPE) interface.[10]

Our previous work on transcyanation reactions were based on the use of almond meal [a rich, readily available and inexpensive source of (*R*)-oxynitrilase] in DIPE containing just small amounts (usually 2% v/v) of added water.[7] Almond meal also turned out to be the preferred catalyst for the transcyanation of D-**2** with acetone cyanohydrin (Table 1). Owing to the low solubility of the present glycosides in DIPE, ethyl acetate and toluene were used as solvents. The transcyanation reactions were studied in four sets of reaction media by using two biphasic microaqueous media with 5 and 2 vol.-% of tartrate buffer (0.1 M, pH = 4.0–5.5), toluene saturated with buffer (0.03 vol.-% water) and anhydrous toluene (water content 20 ppm). Initial comparisons indicated much better cyanohydrin formation in toluene than in EtOAc. With regard to the two potential side-reactions under the employed reaction conditions, the α-glycosidic bonds were stable, whereas the spontaneous formation of D-**3** was significant in the absence of the enzyme. Lowering the amount of acetone cyanohydrin from 5 to 1 equiv. suppressed both the enzymatic and non-enzymatic reactions (Entry 5 vs. 4). As expected, the formation of D-**3** in the absence of almond meal considerably decreased when the water content was reduced at a given pH (e.g., Entries 3, 7 and 8) and when the pH was lowered at constant water content (e.g., Entries 5–7). Independent of the pH, cyanohydrin formation was completed in the presence of almond meal at a buffer content ≥2 vol.-% in 2 d. Under these conditions L-**2** behaved nearly identically to its D isomer. However, all the reactions proceeded with negligible diastereoselectivity (*de* ≤ 5%). Changing from a biphasic medium to water-saturated toluene (Entry 8) and further to anhydrous conditions (Entry 9) nearly completely suppressed the reactivity. The selectivity was not improved when almond meal was replaced by covalently immobilized *Pa*HNL or an aqueous solution of *At*HNL (Entries 10–12).

Table 1. Formation of D-**3** by (*R*)-oxynitrilase-catalysed transcyanation in toluene at room temp. after 2 d.



Entry	Biocatalyst	Buffer[a] vol.-%/pH	Yield[b] [%]	
			Enzyme present	Enzyme absent
1	meal	5/5.50	100	100
2	meal	5/4.75	100	100
3	meal	5/4.00	100	44
4[c]	meal	2/5.50	61	37
5	meal	2/5.50	100[d]	83
6	meal	2/4.75	100	77
7	meal	2/4.00	100	8
8[e]	meal	<1/4.00	7	0
9[f]	meal	0/–	11	0
10	*Pa*HNL	2/4.00	39	8
11	*Pa*HNL	0/–	0	0
12	*At*HNL	2/–	100	–

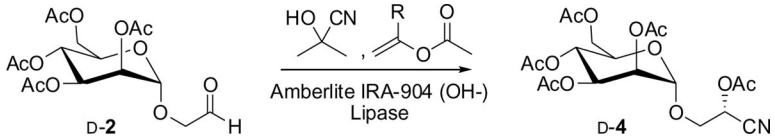
[a] 0.1 M Tartrate buffer. [b] Yields according to ^{1}H NMR spectroscopy by using the peak integrals of the α-H atom of the newly formed chiral centre at C-2. [c] 1 equiv. of acetone cyanohydrin, otherwise 5 equiv. [d] Yield already 100% after 1 d. [e] Toluene saturated with buffer. [f] Dry toluene (water content 20 ppm).

To summarize, although the (*R*)-oxynitrilases used accelerate the formation of cyanohydrins D- and L-**3**, the selectivity is negligible, and chemical transformations accompany the enzymatic reaction.

Lipase-Catalysed Acylation Under Dynamic Conditions

As (*R*)-oxynitrilase preparations were unable to give a sufficient selectivity for the synthesis of D- and L-**3**, attention was focused on the lipase-catalysed diastereomeric dynamic kinetic resolution in toluene (routes 3 + 4, Scheme 2b). Encouraged by the results of previous work,[11]

Table 2. Formation of D-**4** from D-**2** (0.05 M) by diastereomeric dynamic kinetic resolution with lipases in toluene at room temp. after 3 d.



Entry	R	Resin [mg mL^{-1}]	Acetone cyanohydrin [M]	Acyl donor [M]	Lipase	Yield[a] [%]	*de*[a] [%]
1	H	4	0.10	0.15	CAL-B	14	2
2	H	4	0.10	0.15	CAL-A[b]	14	48
3	H	4	0.10	0.15	CAL-A[c]	15	>99
4	H	4	0.10	0.15	Lipase PS C-II	27	>99
5	H	4	0.10	0.15	Lipase PS-D	34/17[d]	>99
6[e]	H	4	0.10	0.15	Lipase PS-D	18	>99
7	Me	4	0.10	0.15	Lipase PS-D	14	>99
8	H	4	0.20	0.30	Lipase PS-D	25	>99
9	H	20	0.20	0.30	Lipase PS-D	51	88

[a] Yield and *de* of D-**4** as determined from HPLC chromatograms. [b] Covalently immobilized CAL-A. [c] CAL-A adsorbed on Celite in the presence of sucrose.[12] [d] Isolated yield. [e] EtOAc instead of toluene.

reactions involving D-**2**, acetone cyanohydrin (the cyanide donor, 2 equiv.) and vinyl acetate (the acyl donor, 3 equiv.) were studied in toluene in the presence of Amberlite IRA-904 basic resin and one of the lipases from *Candida antarctica* (CAL-A and -B) and *Burcholderia cepacia* (lipase PS-D and PS C-II). In line with these works,[11] D-(2*R*)-**3** reacted with each lipase giving the product D-(2*R*)-**4** (route 3, Scheme 2b). The results are shown in Table 2. Lipase PS-D afforded the highest yields (Entry 5 vs. 1–4), and toluene was superior to EtOAc (Entry 5 vs. 6). Although the selectivity was excellent (*de* > 99%), except with CAL-B and covalently immobilized CAL-A (Entries 1 and 2), the general problem of low yields persisted. Neither replacement of vinyl acetate by isopropenyl acetate (Entry 7) nor increasing of the contents of acetone cyanohydrin and vinyl acetate (Entry 8) improved the yield. Only with a high resin content (20 instead of 4 mg mL^{-1}) was a clear effect on the yield detected but at the cost of selectivity (Entry 9). Separate epimerization experiments with diastereomerically pure D-(2*S*)-**3** indicated that the asymmetric centre at C-2 was completely transformed into a mixture of diastereomers D-**3**. Accordingly, slow epimerization did not explain the low yields observed. Rather the HPLC analysis implied the formation of base-induced condensation side-products.

To summarize, although the diastereomeric dynamic kinetic resolution method is able to produce D-(2*R*)-**4** in a diastereopure form, the need to use a base for racemization renders the yield impractical.

Combination of Almond Meal and Lipase PS-D Catalyses

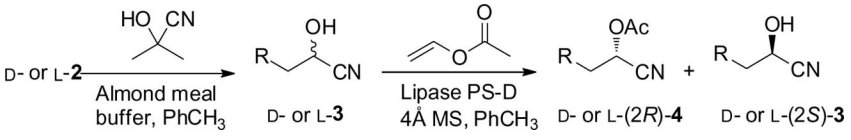
Because the synthesis of D- and L-**3** with (*R*)-oxynitrilase was high-yielding (but non-selective), whereas the acylation of D-**3** with lipase PS-D was highly diastereoselective (but low-yielding), a combination of the two methods was seen as a fascinating alternative to explore. Note that dry reaction conditions are necessary for lipase-catalysed acylation to prevent the enzymatic hydrolysis of the ester substrate and product and that – although the hydrocyanation step proceeds without an added catalyst – it is notably slower at low water content (e.g., conversion is 61% after 1 d and 83% after 2 d with 2 vol.-% of water: Entry 5, Table 1). Thus, almond meal, as a cheap source of (*R*)-oxynitrilase, provides kinetic rather than selectivity advantage over the uncatalysed alternative when working at this minimal water content. In practice, two methods were studied to achieve our goal. Cyanohydrin D-**3** was first isolated in 82% yield from the transcyanation mixture of D-**2**, acetone cyanohydrin and almond meal in toluene (buffer content 2 vol.-%, pH = 5.5) after 24 h. In this case, the cyanohydrin intermediate can well be prepared at some higher water content without an enzyme, unless one wants to avoid the isolation of the labile cyanohydrin. The isolated product was then subjected to classic kinetic diastereomeric resolution with vinyl acetate and lipase PS-D in toluene to yield cyanohydrin acetate D-(2*R*)-**4** (*de* 93%) and unreacted D-(2*S*)-**3** (*de* 92%). The diastereomeric excesses were somewhat lower than expected on the basis of the results in Table 2. When the resolution was performed in the presence of molecular sieves (4 Å), the *de* values increased to afford diastereomeric ratios *D* (as the analogue of enantiomeric ratio *E*) of over 100.

By another method, the procedure was simplified by leaving the intermediate D-**3** unpurified. Almond meal, in which most of the water (2 vol.-%, pH = 5.5 was used) was impregnated, was removed after completing the transcyanation, and the resulting reaction mixture was dried with Na_2SO_4 before it was subjected to lipase PS-D-catalysed acylation with vinyl acetate in the presence of molecular sieves. This method was applied to both D- and L-**2** and yielded the acetate products D- and L-(2*R*)-**4** and the unreacted cyanohydrins D- and L-(2*S*)-**3** with good stereopurity (Table 3). The L diastereomers gave lower selectivity and were more difficult to separate by conventional column chromatography than the D diastereomers, consistent with the reduced separation also observed on TLC. The isolated, seemingly low yields in Table 3 were calculated with respect to the achiral starting material **2**, but when considering the maximum theoretical yield of 50%, the yields obtained after two steps are more satisfactory.

Determination of Absolute Configurations

The absolute configurations of the unreacted cyanohydrin diastereomers D- and L-**3** were determined by the NMR method developed by Moon et al. using (*R*)- and (*S*)-man-

Table 3. Sequential synthesis and diastereomeric kinetic resolution for the formation of D- and L-(2*R*)-**4** and D- and L-(2*S*)-**3** at room temp. (R corresponds to the peracetylated α-mannoside moiety).



Substrate	Conversion [%][a]	Time [h]	*D*[b]	(2*R*)-**4** Yield [%][c]	(2*R*)-**4** Purity [%][d]	(2*R*)-**4** *de* [%]	(2*S*)-**3** Yield [%][c]	(2*S*)-**3** Purity [%][e]	(2*S*)-**3** *de* [%]
D-**2**	48	3	>100	35	>99	>99	34	>99	93
L-**2**	51	24	62	31	89	89	25	82	93

[a] Conversion was calculated as $c = de_3/(de_4 + de_3)$. [b] $D = \ln[(1 - c)(1 - de_3)]/\ln[(1 - c)(1 + de_3)]$. [c] Isolated yield as calculated from aldehyde **2**. [d] Contains (2*S*)-**3** as impurity. [e] Contains (2*R*)-**4** as impurity.

delic acids (in separate samples) in the presence of 4-(dimethylamino)pyridine (DMAP) as chiral shift reagent.[13] In the complex with (*R*)-mandelate the α-proton signal of both D- and L-**3** experiences a larger downfield shift than that of the complex with (*S*)-mandelate [$\Delta\delta^{RS}$ = +0.066 for D-**3** (a) and +0.063 for L-**3** (b), Figure 1]. The positive sign indicates that D- and L-**3** have a configuration similar to (*R*)-mandelonitrile. Bearing the CIP rules in mind, this leads to the assignment of D-(2*S*)-**3** and L-(2*S*)-**3**, respectively.

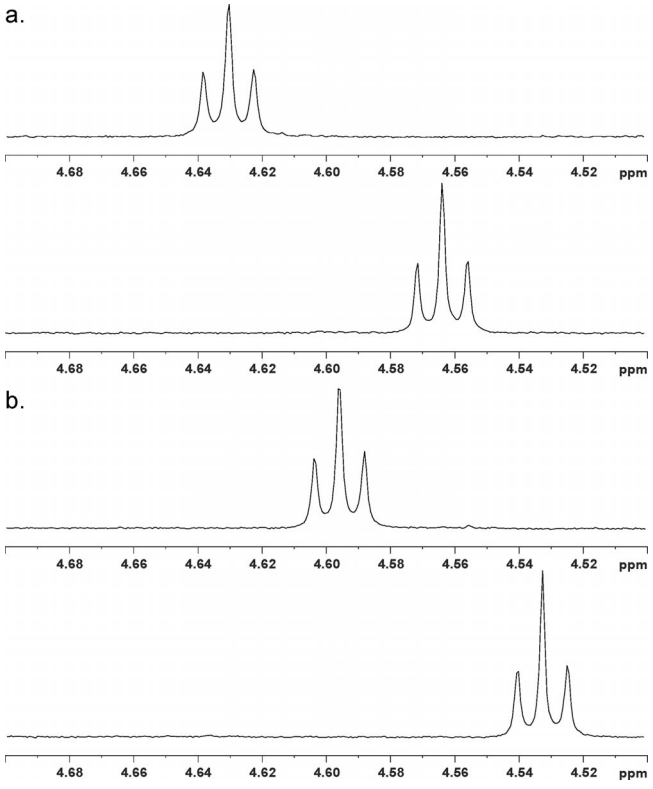


Figure 1. H NMR signals for the α-H of D-**3** (a) and L-**3** (b) complexed with (*R*)-mandelic acid (top) and (*S*)-mandelic acid (bottom) in the presence of DMAP (in $CDCl_3$, calibrated with TMS).

Conclusions

Four mannoside products [D- and L-(2*S*)-**3** and D- and L-(2*R*)-**4**] have been prepared from aldehydes D- and L-**2** by a sequential two-step method with almond meal as the source of (*R*)-oxynitrilase for the transcyanation reaction and lipase PS-D for diastereomeric kinetic resolution by acylation. Although exemplified here with mannosides, the synthetic methodology presented should be equally applicable to other carbohydrates. Kinetic resolution also has the advantage of providing both stereoisomers of the target compound, an important aspect considering its applications, for example, in the biological screening of new molecular entities. Conversion of the carbohydrate cyanohydrins prepared herein to biologically important building blocks by transformations analogous to those depicted in Scheme 1 will be investigated in future work.

Experimental Section

Materials and Methods: All reagents were purchased from commercial sources and used as received. Almond meal was acquired from Sigma as β-glucosidase from almonds, *Pa*HNL (170 U g^{-1}) and CAL-A, both covalently immobilized, were from ChiralVision, *At*HNL (aqueous solution, 39.5 U mg^{-1}, 32 mg mL^{-1} protein) from Evocatal, CAL-B (Novozym 435) and CAL-A powder from Novozymes and lipase PS-D and PS C-II from Amano Europe. Before use CAL-A powder was adsorbed on Celite in the presence of sucrose as described previously, the final lipase content in the preparation being 20% (w/w).[12] Amberlite IRA-904 was purchased from Acros and was conditioned as described previously.[14] HPLC analysis was performed with a Waters 2690 liquid chromatograph equipped with a Daicel Chiralcel OD-H column (0.46 × 25 cm at 23–24 °C) with *i*PrOH (10 vol.-%) in hexane (flow 0.8 mL min^{-1}) as eluent and with a Corona charged aerosol detector. NMR spectra were recorded with a Bruker Avance 600 MHz spectrometer and analysed with PERCH software by using spin simulation/iteration techniques.[15] HR mass spectra were measured in ESI$^+$ mode with a Bruker micro-TOF-Q quadrupole-TOF spectrometer. Optical rotations were obtained with a Perkin Elmer 241 polarimeter and $[a]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1} at 25 °C unless otherwise indicated. Enzymatic reactions were performed at room temperature (23–24 °C). Column chromatography was performed on silica gel (60 Å, Merck, 230–400 mesh, enriched with 0.1% Ca). All reactions with appreciable amounts of HCN or acetone cyanohydrin were performed in the presence of an HCN sensor and waste containing cyanides was treated with bleach at least overnight followed by acidic destruction of the residues.

Synthesis of Aldehydes D-2 and L-2: As a general procedure for the synthesis of aldehydes, the protected allyl glycoside **1** (1 equiv.) was dissolved in a mixture of acetone/H_2O (4:1, 2 mL/100 mg), and 4-methylmorpholine *N*-oxide (NMO, 2 equiv.) and a catalytic amount of OsO_4 (2.5 wt.-% solution) were added. The mixture was stirred for 4–7 h, diluted with CH_2Cl_2 (30 mL) and washed with brine (30 mL). The organic phase was separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic phases were dried with Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography with EtOAc as eluent to give a 1:1 mixture of diastereomers. The dihydroxylated glycoside was dissolved in a mixture of CH_3CN/H_2O (2:1.2, 6 mL/100 mg), and $NaIO_4$ (2 equiv.) was added. The reaction mixture was stirred for 1–3 h, diluted with Et_2O (30 mL) and washed with H_2O (30 mL). The aqueous layer was extracted with EtOAc (2 × 30 mL) and CH_2Cl_2 (2 × 30 mL). The combined organic phases were washed with brine (40 mL), dried with Na_2SO_4, filtered and concentrated to give pure aldehyde.

2-(2′,3′,4′,6′-Tetra-*O*-acetyl-α-D-mannopyranosyl)acetaldehyde (D-2): The compound was synthesized from allyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (**1**; 1.1 g, 2.9 mmol) according to the above general procedure for the synthesis of aldehydes. The dihydroxylated glycoside was obtained as a colourless oil (1.1 g, 91%). Part of this material (230 mg, 0.5 mmol) was used in the fragmentation step to provide the title compound as a colourless oil (210 mg, quant.). $[a]_D^{23}$ = +53 (*c* = 0.33, $CHCl_3$). ^{1}H NMR (600.13 MHz, $CDCl_3$, 25 °C): δ = 9.74 (dd, $J_{CHO,CH2a}$ = 0.7, $J_{CHO,CH2b}$ = 1.0 Hz, 1 H, C*H*O), 5.40 (dd, $J_{3,2}$ = 3.5, $J_{3,4}$ = 10.1 Hz, 1 H, 3-H), 5.38 (dd, $J_{2,1}$ = 1.8, $J_{2,3}$ = 3.5 Hz, 1 H, 2-H), 5.31 (dd, $J_{4,3}$ = 10.1, $J_{4,5}$ = 10.2 Hz, 1 H, 4-H), 4.28 (dd, $J_{6a,5}$ = 5.5, $J_{6a,6b}$ = –12.2 Hz, 1 H, 6a-H), 4.26 (dd, $J_{CH2a,CHO}$ = 0.7, $J_{CH2aCHO,CH2bCHO}$ = –17.7 Hz, 1 H, CH_{2a}CHO), 4.22 (dd, $J_{CH2b,CHO}$ = 1.0, $J_{CH2bCHO,CH2aCHO}$ = –17.7 Hz, 1 H, CH_{2b}CHO), 4.11 (dd, $J_{6b,6a}$ = –12.2, $J_{6b,5}$ = 2.6 Hz,

1 H, 6b-H), 4.11 (ddd, $J_{5,4}$ = 10.2, $J_{5,6a}$ = 5.5, $J_{5,6b}$ = 2.6 Hz, 1 H, 5-H), 2.17 (s, 3 H, 3-OCOCH_3), 2.10 (s, 3 H, 6-OCOCH_3), 2.06 (s, 3 H, 2-OCOCH_3), 2.01 (s, 3 H, 4-OCOCH_3) ppm. ^{13}C NMR (150.9 MHz, $CDCl_3$, 25 °C): δ = 198.2 (*C*HO), 170.6 (6-O*C*OCH$_3$), 169.9 (3-O*C*OCH$_3$, 2-O*C*OCH$_3$), 169.7 (4-O*C*OCH$_3$), 98.3 (C-1), 73.2 (*C*H$_2$CHO), 69.3 (C-5), 69.2 (C-2), 68.8 (C-3), 65.9 (C-4), 62.4 (C-6), 20.9 (3-OCO*C*H$_3$), 20.7 (2-OCO*C*H$_3$, 4-OCO*C*H$_3$, 6-OCO*C*H$_3$) ppm. HRMS: calcd. for $C_{16}H_{22}NO_{11}Na$ [M + Na]$^+$ 413.1054; found 413.1025.

2-(2′,3′,4′,6′-Tetra-*O*-acetyl-α-L-mannopyranosyl)acetaldehyde (L-2): The compound was synthesized from allyl 2,3,4,6-tetra-*O*-acetyl-α-L-mannopyranoside (**1**; 350 mg, 0.9 mmol) according to the general procedure for the synthesis of aldehydes to provide the title compound as a colourless oil (270 mg, 77%). $[\alpha]_D^{23}$ = –57 (*c* = 0.45, $CHCl_3$). The ^{1}H and ^{13}C NMR chemical shifts and coupling constants are identical to those of D-**2**. HRMS: calcd. for $C_{16}H_{22}NO_{11}Na$ [M + Na]$^+$ 413.1060; found 413.1061.

Synthesis of D-3 and L-3 with (*R*)-Oxynitrilase: Table 1. Almond meal or *Pa*HNL (20 mg mL^{-1}) was weighed into a reaction vial, tartrate buffer (0.1 M) was added, and the enzyme was allowed to wet for 15–30 min before one of the substrates D-**2** or L-**2** (0.05 M) in toluene or ethyl acetate was added. *At*HNL was added as an aqueous solution (2 vol.-%, 1500 units/mmol substrate). The addition of acetone cyanohydrin (0.25 M) initiated the reaction. The mixture was shaken at room temp. at 172 rpm. Samples (100 μL) were removed through a septum, concentrated and analysed in $CDCl_3$ by ^{1}H NMR spectroscopy.

Diastereomeric Dynamic Kinetic Resolution: Table 2. Amberlite IRA-904 (OH^-, 4 or 20 mg mL^{-1}) and an appropriate lipase (50 mg mL^{-1}) were weighed into a reaction vial, and D-**2** (0.05 M) in toluene was added. Vinyl acetate (0.15 or 0.30 M) or isopropenyl acetate (0.15 M) and acetone cyanohydrin (0.10 M or 0.20 M) were added. The reaction mixture was shaken at room temp. at 172 rpm. Samples were removed through a septum and analysed by HPLC without derivatization.

Synthesis and Diastereomeric Kinetic Resolution to Yield (2*R*)-4 and (2*S*)-3: Compound D-**3** was synthesized from D-**2** (821 mg, 2.10 mmol) in toluene (40.29 mL) containing tartrate buffer (0.1 M, 822 μL, 2 vol.-%, pH = 5.5), almond meal (840 mg, 20 mg mL^{-1}) and acetone cyanohydrin (959 μL, 10.50 mmol) by applying the above general procedure. The reaction mixture was shaken at room temp. at 172 rpm. After 24 h at 98% conversion, $NaSO_4$ (2.50 g) was added to dry the reaction mixture The enzyme was removed, washed with toluene (10 mL), and the combined organic phases were added to lipase PS-D (2.50 g, approximately 50 mg mL^{-1}) and molecular sieves (2.50 g, 4 Å). After 3 h (48% conversion), the lipase was filtered off and the filtrate concentrated and purified by column chromatography by using 60% EtOAc in hexane as eluent to yield the product D-(2*R*)-**4** (340 mg, 0.74 mmol, 35%, *de* >99%) and unreacted D-(2*S*)-**3** (296 mg, 0.71 mmol, 34%, *de* 93%). The L diastereomers were prepared similarly to the D isomers from L-**2** (124 mg, 0.32 mmol). Lipase PS-D catalysed diastereomeric kinetic resolution was stopped after 24 h at 51% conversion to yield L-(2*R*)-**4** (47 mg, 0.10 mmol, 31%, *de* 89%) and L-(2*S*)-**3** (34 mg, 0.08 mmol, 25%, *de* 93%).

(2*S*)-2-Hydroxy-3-(2′,3′,4′,6′-tetra-*O*-acetyl-α-D-mannopyranosyl)-propionitrile [D-(2*S*)-3]: Pale-yellow oil. $[\alpha]_D^{25}$ = +68 (*c* = 0.5, $CHCl_3$, *de* 93%). ^{1}H NMR (600.13 MHz, $CDCl_3$, 25 °C): δ = 5.33 (dd, $J_{2,1}$ = 1.7, $J_{2,3}$ = 3.0 Hz, 1 H, 2-H), 5.31 (dd, $J_{3,2}$ = 3.0, $J_{3,4}$ = 10.1 Hz, 1 H, 3-H), 5.31 (dd, $J_{4,3}$ = 10.1, $J_{4,5}$ = 10.1 Hz, 1 H, 4-H), 4.92 (d, $J_{1,2}$ = 1.7 Hz, 1 H, 1-H), 4.69 (dd, $J_{CHCN,CH2b}$ = 3.8, $J_{CHCN,CH2a}$ = 4.9 Hz, 1 H, C*H*CN), 4.28 (dd, $J_{6a,5}$ = 5.2, $J_{6a,6b}$ = –12.3 Hz, 1 H, 6a-H), 4.16 (dd, $J_{6b,6a}$ = –12.3, $J_{6b,5}$ = 2.2 Hz, 1 H, 6b-H), 4.12 (ddd, $J_{5,4}$ = 10.1, $J_{5,6a}$ = 5.2, $J_{5,6b}$ = 2.2 Hz, 1 H, 5-H), 3.95 (dd, $J_{CH2a,CH2b}$ = –10.6, $J_{CH2a,CHCN}$ = 4.9 Hz, 1 H, CH_{2a}), 3.89 (br., 1 H, OH), 3.84 (dd, $J_{CH2b,CH2a}$ = –10.6, $J_{CH2b,CHCN}$ = 3.8 Hz, 1 H, CH_{2b}), 2.18 (s, 3 H, 3-OCOCH_3), 2.13 (s, 3 H, 6-OCOCH_3), 2.07 (s, 3 H, 2-OCOCH_3), 2.00 (s, 3 H, 4-OCOCH_3) ppm. ^{13}C NMR (150.9 MHz, $CDCl_3$, 25 °C): δ = 170.7 (6-O*C*OCH$_3$), 170.3 (2-O*C*OCH$_3$), 170.1 (3-O*C*OCH$_3$), 169.8 (4-O*C*OCH$_3$), 117.6 (C≡N), 97.9 (C-1), 69.3 (C-5, OCH$_2$), 69.0 (C-2), 65.7 (C-3, C-4), 62.4 (C-6), 60.6 (*C*HCN), 20.8 (2×OCO*C*H$_3$), 20.7 (2×OCO*C*H$_3$) ppm. HRMS: calcd. for $C_{17}H_{23}NO_{11}Na$ [M + Na]$^+$ 440.1163; found 440.1202.

(2*R*)-2-Acetoxy-3-(2′,3′,4′,6′-tetra-*O*-acetyl-α-D-mannopyranosyl)-propionitrile [D-(2*R*)-4]: Colourless oil. $[\alpha]_D^{25}$ = +23 (*c* = 0.5, $CHCl_3$, *de* > 99%). ^{1}H NMR (600.13 MHz, $CDCl_3$, 25 °C): δ = 5.52 (dd, $J_{CHCN,OCH2b}$ = 4.7, $J_{CHCN,OCH2a}$ = 6.8 Hz, 1 H, C*H*CN), 5.30 (dd, $J_{3,2}$ = 3.2, $J_{3,4}$ = 10.2 Hz, 1 H, 3-H), 5.30 (dd, $J_{4,3}$ = 10.2, $J_{4,5}$ = 9.8 Hz, 1 H, 4-H), 5.27 (dd, $J_{2,1}$ = 1.8, $J_{2,3}$ = 3.2 Hz, 1 H, 2-H), 4.92 (d, $J_{1,2}$ = 1.8 Hz, 1 H, 1-H), 4.28 (dd, $J_{6a,5}$ = 5.5, $J_{6a,6b}$ = –12.3 Hz, 1 H, 6a-H), 4.13 (dd, $J_{6b,5}$ = 2.4, $J_{6b,6a}$ = –12.3 Hz, 1 H, 6b-H), 4.02 (ddd, $J_{5,4}$ = 9.8, $J_{5,6a}$ = 5.5, $J_{5,6b}$ = 2.4 Hz, 1 H, 5-H), 4.02 (dd, $J_{OCH2a,OCH2b}$ = –11.0, $J_{OCH2a,CHCN}$ = 6.8 Hz, 1 H, OCH_{2a}), 3.91 (dd, $J_{OCH2b,OCH2a}$ = –11.0, $J_{OCH2b,CHCN}$ = 4.7 Hz, 1 H, OCH_{2b}), 2.21 [s, 3 H, CH(CN)OCOCH_3], 2.17 (s, 3 H, 3-OCOCH_3), 2.11 (s, 3 H, 6-OCOCH_3), 2.06 (s, 3 H, 2-OCOCH_3), 2.00 (s, 3 H, 4-OCOCH_3) ppm. ^{13}C NMR (150.9 MHz, $CDCl_3$, 25 °C): δ = 170.6 (6-O*C*OCH$_3$), 170.0 (2-O*C*OCH$_3$), 169.8 (3-O*C*OCH$_3$), 169.7 (4-O*C*OCH$_3$), 168.8 [CH(CN)O*C*OCH$_3$], 114.7 (C≡N), 98.2 (C-1), 69.4 (C-5), 69.1 (C-2), 68.6 (C-4), 66.5 (O*C*H$_2$), 65.8 (C-3), 62.3 (C-6), 60.0 (*C*HCN), 20.8 (OCO*C*H$_3$), 20.7 (3×OCO*C*H$_3$), 20.3 (OCO*C*H$_3$) ppm. HRMS: calcd. for $C_{19}H_{25}NO_{12}Na$ [M + Na]$^+$ 482.1269; found 482.1316.

(2*S*)-2-Hydroxy-3-(2′,3′,4′,6′-tetra-*O*-acetyl-α-L-mannopyranosyl)-propionitrile [L-(2*S*)-3]: Pale-yellow oil. $[\alpha]_D^{25}$ = –36 (*c* = 0.5, $CHCl_3$, *de* 93%). ^{1}H NMR (600.13 MHz, $CDCl_3$, 25 °C): δ = 5.34 (dd, $J_{3,2}$ = 3.5, $J_{3,4}$ = 9.8 Hz, 1 H, 3-H), 5.33 (dd, $J_{2,1}$ = 1.7, $J_{2,3}$ = 3.5 Hz, 1 H, 2-H), 5.28 (dd, $J_{4,3}$ = 9.8, $J_{4,5}$ = 10.5 Hz, 1 H, 4-H), 4.93 (d, $J_{1,2}$ = 1.7 Hz, 1 H, 1-H), 4.64 (dd, $J_{CHCN,OCH2b}$ = 3.4, $J_{CHCN,OCH2a}$ = 4.5 Hz, 1 H, C*H*CN), 4.23 (ddd, $J_{5,4}$ = 10.5, $J_{5,6b}$ = 3.9, $J_{5,6a}$ = 4.8 Hz, 1 H, 5-H), 4.22 (dd, $J_{6a,5}$ = 4.8, $J_{6a,6b}$ = –11.7 Hz, 1 H, 6a-H), 4.21 (dd, $J_{6b,5}$ = 3.9, $J_{6b,6a}$ = –11.7 Hz, 1 H, 6b-H), 4.04 (dd, $J_{OCH2a,CHCN}$ = 4.5, $J_{OCH2a,OCH2b}$ = –11.9 Hz, 1 H, OCH_{2a}), 4.00 (br., 1 H, OH), 3.92 (dd, $J_{OCH2b,CHCN}$ = 3.4, $J_{OCH2b,OCH2a}$ = –11.9 Hz, 1 H, OCH_{2b}), 2.17 (s, 3 H, 3-OCOCH_3), 2.13 (s, 3 H, 6-OCOCH_3), 2.07 (s, 3 H, 2-OCOCH_3), 2.01 (s, 3 H, 4-OCOCH_3) ppm. ^{13}C NMR (150.9 MHz, $CDCl_3$, 25 °C): δ = 170.6 (6-O*C*OCH$_3$, 2-O*C*OCH$_3$), 170.0 (3-O*C*OCH$_3$), 169.8 (4-O*C*OCH$_3$), 117.5 (C≡N), 99.2 (C-1), 71.8 (O*C*H$_2$), 69.8 (C-5), 69.2 (C-2), 68.6 (C-3), 65.8 (C-4), 62.6 (C-6), 61.1 (*C*HCN), 20.8 (2×OCO*C*H$_3$), 20.7 (2×OCO*C*H$_3$) ppm. HRMS: calcd. for $C_{17}H_{23}NO_{11}Na$ [M + Na]$^+$ 440.1163; found 440.1180.

(2*R*)-2-Acetoxy-3-(2′,3′,4′,6′-tetra-*O*-acetyl-α-L-mannopyranosyl)-propionitrile [L-(2*R*)-4]: Pale-yellow oil. $[\alpha]_D^{25}$ = –55 (*c* = 0.5, $CHCl_3$, *de* 89%). ^{1}H NMR (600.13 MHz, $CDCl_3$, 25 °C): δ = 5.57 (dd, $J_{CHCN,OCH2b}$ = 4.8, $J_{CHCN,OCH2a}$ = 5.5 Hz, 1 H, C*H*CN), 5.32 (dd, $J_{3,2}$ = 3.4, $J_{3,4}$ = 10.1 Hz, 1 H, 3-H), 5.30 (dd, $J_{2,1}$ = 1.8, $J_{2,3}$ = 3.4 Hz, 1 H, 2-H), 5.30 (dd, $J_{4,3}$ = 10.1, $J_{4,5}$ = 10.2 Hz, 1 H, 4-H), 4.92 (d, $J_{1,2}$ = 1.8 Hz, 1 H, 1-H), 4.27 (dd, $J_{6a,5}$ = 5.6, $J_{6a,6b}$ = –12.3 Hz, 1 H, 6a-H), 4.15 (dd, $J_{6b,5}$ = 2.3, $J_{6b,6a}$ = –12.3 Hz, 1 H, 6b-H), 4.07 (ddd, $J_{5,4}$ = 10.2, $J_{5,6a}$ = 5.6, $J_{5,6b}$ = 2.3 Hz, 1 H, 5-H), 4.00 (dd, $J_{OCH2a,CHCN}$ = 5.5, $J_{OCH2a,OCH2b}$ = –11.3 Hz, 1 H, OCH_{2a}), 3.93 (dd, $J_{OCH2b,CHCN}$ = 4.8, $J_{OCH2b,OCH2a}$ = –11.3 Hz, 1

H, OCH_{2b}), 2.20 [s, 3 H, CH(CN)OCOCH_3], 2.17 (s, 3 H, 3-OCOCH_3), 2.12 (s, 3 H, 6-OCOCH_3), 2.06 (s, 3 H, 2-OCOCH_3), 2.01 (s, 3 H, 4-OCOCH_3) ppm. ^{13}C NMR (150.9 MHz, $CDCl_3$, 25 °C): δ = 170.6 (6-O*C*OCH$_3$), 169.9 (2-O*C*OCH$_3$), 169.8 (3-O*C*OCH$_3$), 169.7 (4-O*C*OCH$_3$), 168.8 [CH(CN)O*C*OCH$_3$], 114.8 (C≡N), 98.0 (C-1), 69.5 (C-5), 69.1 (C-2), 68.7 (C-3), 66.5 (O*C*H$_2$), 65.8 (C-4), 62.4 (C-6), 59.7 (*C*HCN), 20.8 (OCO*C*H$_3$), 20.7 (3 × OCO*C*H$_3$), 20.2 (OCOCH$_3$) ppm. HRMS: calcd. for $C_{19}H_{25}NO_{12}Na$ [M + Na]$^+$ 482.1269; found 482.1240.

Supporting Information (see footnote on the first page of this article): ^{1}H and ^{13}C NMR spectra and HPLC chromatograms of the prepared compounds.

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