EurJOC European Journal of Organic Chemistry

FULL PAPER

DOI: 10.1002/ejoc.201200086

One-Pot Oxidation–Hydrocyanation Sequence Coupled to Lipase-Catalyzed Diastereoresolution in the Chemoenzymatic Synthesis of Sugar Cyanohydrin Esters

Ari Hietanen[a] and Liisa T. Kanerva*[a]

Keywords: Carbohydrates / Enzyme catalysis / Heterogeneous catalysis / Hydrolases / Chiral resolution / Oxidation

A three-step, one-pot synthesis and diastereoresolution sequence is described in anhydrous toluene starting from methyl α-D-2,3,4-tri-*O*-acetylgalacto- (**1a**), -manno- (**1b**) and -glucopyranosides (**1c**). The reaction sequence, including consecutive transformations through the aldehyde [$PhI(OAc)_2$, 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO)] and cyanohydrin [basic resin or (*R*)-oxynitrilase] into the (6*R*)-cyanohydrin ester (lipase) is shown to proceed in a one-pot cascade, except that the basic resin (when used) should be removed before the addition of the enzymatic acylation reagents. We have shown that the effective transformation of **1a** (75 % reaction yield) through labile intermediates gives the stable (6*R*)-cyanohydrin butanoate (85 % *de*). Further diastereomeric purification by chromatography is possible, although the product is already of high diastereopurity. (6*R*)-Cyanohydrin esters are obtained through acylation with *Burkholderia cepacia* lipase. The (6*S*)-ester (*de* 99 %) is produced by *Candida rugosa* lipase when the sequence is started from **1c** whereas the other sugar derivatives are less suited to the reaction with lipase.

Introduction

Modification of natural carbohydrates is a powerful approach both for synthetic objectives and to the elucidation of carbohydrate function in chemical biology studies.[1] When such modifications are required to generate new asymmetric centers, high stereochemical control, in addition to the control caused by the stereostructure of the carbohydrate itself, is necessary. The nonreducing end of a monosaccharide is an important site for synthetic modifications, for instance, for biological recognition processes. On the other hand, the C-6 position of aldohexoses is attractive for structural modifications that allow specific interactions with external substrates. For instance, cyclodextrins modified with cyanohydrin moieties at C-6 can function as efficient hydrolase-like catalysts.[2]

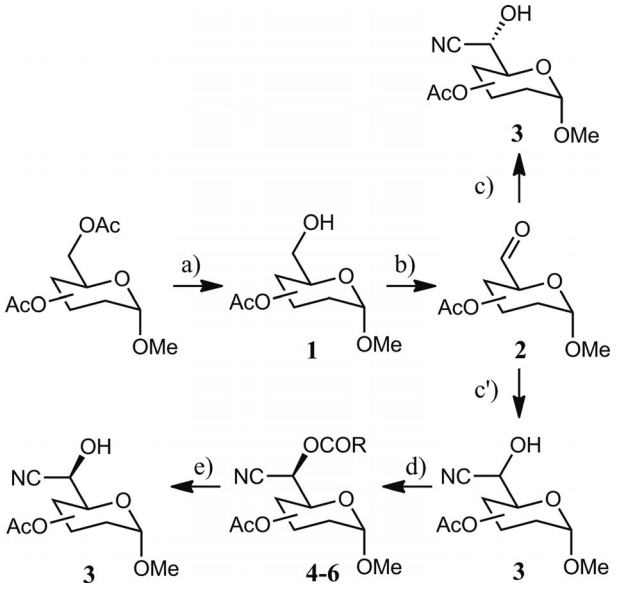
A strategy often employed in C-6 modifications of sugar derivatives consists of initial oxidation of the primary alcohol functionality to the corresponding aldehyde followed by the desired addition reaction. Examples of such reactions include a Staudinger reaction initiated process,[3] reductive amination,[4] Diels–Alder reaction,[5] and Grignard reaction.[6] The addition of formaldehyde dialkylhydrazones to sugar aldehydes followed by oxidative cleavage to yield free or benzyl-protected sugar cyanohydrins has also been reported.[7] Selectivity in these reactions is caused by internal asymmetric induction and leads to diastereomerically enriched products.

Cyanohydrins (α-hydroxynitriles) form a versatile class of compounds for synthetic chemistry as they allow easy access to important compounds, such as α-hydroxy acids and amides, α-functionalized nitriles, 1,2-amino alcohols, and N-heterocycles.[8] In our previous work, a cyanohydrin moiety was introduced into the aglycon part of peracetylated α-D- and α-L-mannosylacetaldehydes to afford novel mannosylglycosides.[9] Stereoselectivity was then achieved through lipase catalyzed acylation after a nonselective hydrocyanation step, affording the diastereomerically enriched epimers of the sugar cyanohydrin and cyanohydrin acetate. We now report the results of our studies on the introduction of a cyanohydrin moiety at the C-6 position of monosaccharides. The chemoenzymatic synthesis includes the deprotection of peracetylated methyl α-D-glycosides (formation of compound **1**, Step a), followed by a one-pot reaction sequence with oxidation (aldehyde **2** formation, Step b), hydrocyanation (C–C bond formation, Step c or c′), and acylation (Step d) at the C-6 position of a sugar pyranoside **3** under mild reaction conditions (Scheme 1). The synthesis was optimized for methyl α-D-galactopyranoside, and the method was then applied to the corresponding α-D-mannose- and α-D-glucose-based glycosides with ^1H NMR spectroscopic analysis of the reaction.

The absolute configurations of the cyanohydrin products in Scheme 1 are depicted as was expected on the basis of the models for (*R*)-oxynitrilase [(*R*)-HNL] and many lipase enzymes. Accordingly, the (6*S*)-cyanohydrin was expected

[a] Institute of Biomedicine/Department of Pharmacology, Drug Development and Therapeutics/Laboratory of Synthetic Drug Chemistry, University of Turku,
20014 Turku, Finland
E-mail: liisa.kanerva@utu.fi

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.201200086.



Scheme 1. Synthetic strategy from carbohydrates: (a) regioselective deprotection, (b) oxidation, (c) enzymatic hydrocyanation, (c′) chemical hydrocyanation, (d) enzymatic acylation, and (e) enzymatic deacylation.

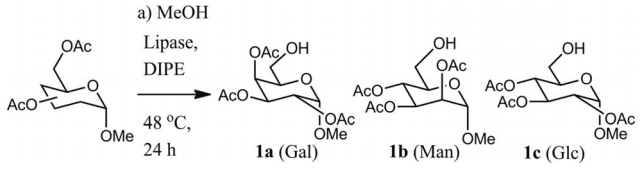
to be the major diastereomer obtained from the hydrocyanation reaction catalyzed by (*R*)-oxynitrilase (Scheme 2, a), whereas the formation of the (6*R*)-cyanohydrin ester was suggested to be the major diastereomer with lipase catalyzed *O*-acylation (Scheme 2, b).[10] NMR analysis was used to examine the presumed absolute configurations and it was found that these did not quite follow the expectations with (*R*)-oxynitrilases.

Results and Discussion

Chemoenzymatic Preparation of 1a–c (Step a)

Our approach to the target cyanohydrins and cyanohydrin esters required the secondary alcohol functionalities in the sugar moieties to be protected to block their reactivity later in the lipase catalyzed acylation and to promote solubility in organic solvents, whereas the primary alcohol group at C-6 was to remain unprotected so that the desired cyanohydrin functionality could be introduced (Scheme 1). After peracetylation by using literature methods,[11] a set of readily available lipase preparations was screened to assess their ability to deprotect position C-6 by regioselective methanolysis in diisopropyl ether (DIPE), affording compounds **1a**–**c** (Table 1). Lipase PS-D and PS-C II preparations (*Burkholderia cepacia* lipase immobilized on diatomaceous earth and ceramic, respectively) were effective with the galactoside substrate but displayed very low or no activity toward the other sugar acetates (Table 1, entries 1 and 2). Lipases A (CAL-A adsorbed on Celite) and B (CAL-B as a commercial Novozym 435 catalyst) from *Candida antarctica* and *Candida rugosa* lipase (CRL, adsorbed on Celite) gave generally low product yields for **1a**–**c** (Table 1, entries 3–5). *Thermomyces lanuginosus* lipase (Lipozyme TL IM) allowed highly efficient deprotection irrespective of which sugar was used (Table 1, entry 6), although the reaction was not perfectly selective for C-6, particularly with substrate **1c**. Nevertheless, Lipozyme TL IM was used for the preparative syntheses of **1a**–**c** in good isolated yields (64–84%).

Table 1. Formation of **1a**–**c** by lipase-catalyzed methanolysis of peracetylated methyl α-D-glycopyranosides.[a]

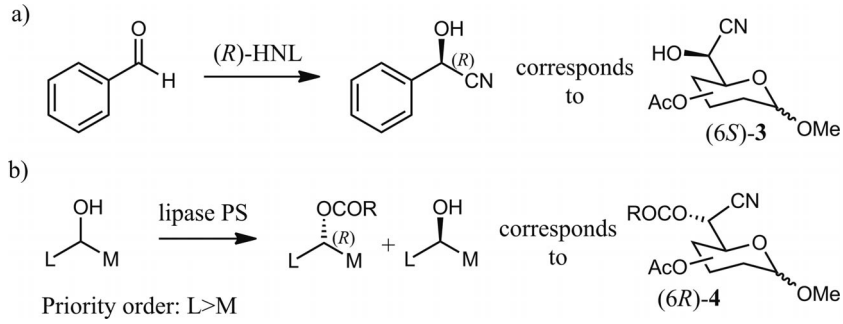


Entry	Lipase	**1a**[%]	**1b** [%]	**1c** [%]
1	Lipase PS-D	80	0	6
2	Lipase PS-C II	72	0	6
3	Novozym 435	14	36	0
4	CAL-A on Celite	10	0	17
5	CRL on Celite	15	28[b]	64
6	Lipozyme TL IM	95 (81[c])	92 (84[c])	69 (64[c])

[a] [Substrate] = 0.05 M, [MeOH] = 0.25 M, lipase content 50 mg mL^{-1} in DIPE. [b] Reaction time 72 h. [c] Isolated yields.

Oxidation of 1a (Step b)

Both biocatalytic and chemical oxidation are potential methods for the preparation of aldehydes **2a**–**c** from the corresponding alcohols **1a**–**c** (Step b of Scheme 1). The bio-

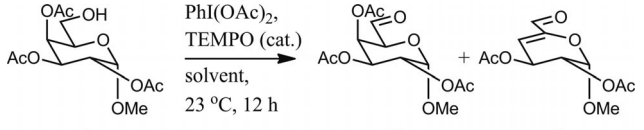


Scheme 2. Suggested configurations at C-6 based on (a) the benzaldehyde-mandelonitrile model for (*R*)-HNL and (b) the secondary alcohol model for acylation by lipase PS catalysis.

catalytic oxidation of the primary alcohol group of hexose sugars at C-6 reported in the literature largely rely on the use of galactose oxidase in aqueous solutions, restricting the studies to galactosyl moieties[12] and predisposing the product to side reactions.[13] Methods that rely on the oxidation of a primary alcohol to the aldehyde stage by chloroperoxidase from *Caldariomyces fumago* in organic solvents have been extensively studied,[14] but our efforts to use this approach failed with both unprotected methyl α-D-galactopyranoside and **1a** as substrates.

Literature reports concerning chemical oxidation can be be classified into three broad categories. Dimethyl sulfoxide-based oxidation of acetyl-protected sugars gave α,β-unsaturated aldehyde **7a** as the main product.[15] Catalytic 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO) coupled with a stoichiometric oxidant[16] or with a O_2/laccase system[17] for unprotected sugars carries the possibility of overoxidation, affording the carboxylic acid rather than the aldehyde, particularly when water or a base is present. On the other hand, Dess–Martin oxidation is an effective method for sugar oxidation, but the method is typically used in solvents such as dimethyl sulfoxide (DMSO), MeCN, or CH_2Cl_2 that are less suitable for subsequent enzymatic steps.[5,18] The method based on iodine(III) reagents with catalytic TEMPO, also related to the Dess–Martin oxidation, is particularly attractive because the isomerization of chirally labile aldehydes and overoxidation to carboxylic acids should be completely avoided.[18,19] For example, a diacetoxyiodobenzene/TEMPO oxidation of γ,δ-unsaturated primary alcohols coupled to enzymatic hydrocyanation is reported to provide advantages of better atom economy and a more facile work-up compared to the use of Dess–Martin periodinane alone.[20] Accordingly, the oxidation of **1a** with diacetoxyiodobenzene and catalytic TEMPO in dichloromethane resulted in 62% reaction yield for the desired aldehyde **2a** with one major impurity among other unidentified minor impurities (Table 2, entry 1). Whereas **2a** was unstable upon chromatographic purification, the main impurity was separated and shown to be the unsaturated aldehyde **7a**. Toluene and DIPE are solvents commonly used with oxynitrilases and lipases.[21] Oxidation in DIPE was not achieved, evidently due to the low solubility of the hypervalent iodine oxidant (Table 2, entry 3), whereas a yield of 75% for **2a** in toluene was accomplished without the formation of **7a** (Table 2, entry 2). Compared to soluble TEMPO, the use of TEMPO immobilized on polystyrene gave low product yields (15%); thus, soluble TEMPO was used throughout the work. In addition to purification by column chromatography, aqueous work-up was also deleterious for the produced aldehyde, causing product loss to the aqueous phase and the formation of impurities. Therefore, subsequent steps from **1a** in the reaction cascade were performed without isolating the aldehyde.

Table 2. Solvent effects on the oxidation of **1a** (0.050 M) with $PhI(OAc)_2$ (0.055 M)/TEMPO (0.05 M).



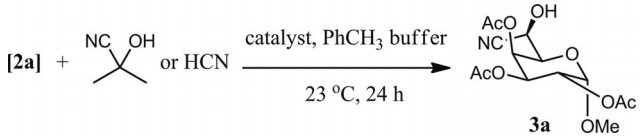
1a **2a** **7a**

Entry	Solvent	**1a** [%]	**2a** [%]	**7a** [%]
1	CH_2Cl_2	0	62	7
2	toluene	0	75	0
3	DIPE	81	4	0

Hydrocyanation of 2a–c (Steps c and c′)

A set of (*R*)-oxynitrilases were first studied for the trans-hydrocyanation between **2a** and acetone cyanohydrin in buffered microaqueous toluene containing all the reaction components from the previous oxidation Step b (Step c in Scheme 1). The reaction was performed (1) in a one-compartment reactor (all reagents and catalysts present in situ), and (2) in a two-compartment reactor (acetone cyanohydrin decomposes to HCN in the presence of Amberlite IRA-900 HO^- ion exchange resin in one of the compartments, thereafter diffusing freely into the enzymatic reaction mixture in the second compartment).[21] In Method 1, (*R*)-oxynitrilase in toluene containing tartrate buffer (4 vol.-%, 0.10 M, pH 5.4) was responsible for the decomposition of acetone cyanohydrin and for the formation of the sugar cyanohydrin **3a**. Low diastereomeric excess (27% *de*) and product yield (17%) for (6*R*)-**3a** obtained by almond meal catalysis (a rich source of (*R*)-oxynitrilase) were found to be drawbacks of the reaction (Table 3, entry 1). Similar results were obtained with commercial (*R*)-oxynitrilase catalysts from *Prunus amygdalis* (*Pa*HNL) and *Arabidopsis thaliana* (*At*HNL). Evidently, the rapid rate of HCN generation (and resulting in high HCN concentration) in Method 1 caused enzyme inhibition and resulted in low yield in the one-compartment reactor. In Method 2, the reaction yield of (6*R*)-**3a** increased considerably with a moderate improvement in the diastereomeric excess (Table 3, entry 2). Labile **3a** was isolated in 35% yield (37% *de*) by column chromatography, the rest of the product decomposing to

Table 3. Effect of catalyst and cyanide source on the (trans)hydrocyanation of **2a**.



3a

Entry	Catalyst (buffer vol.-%)	CN^- source	Yield [%]	*de* [%]
1	Almond meal[a] (4)	ACH[c]	17[d]	27
2	Almond meal[a] (4)	HCN[d]	74[d]	37
3	– (4)	ACH[c]	0	–
4	Almond meal[a] (0)	ACH[c]	0[d]	–
5	IRA-900 OH^-[b] (0)	ACH[c]	85	52

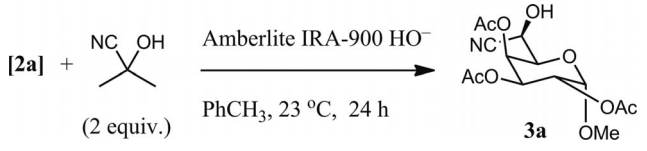
[a] 50 mg mL^{-1}. [b] 10 mg mL^{-1}. [c] 2 equiv. of acetone cyanohydrin (one-compartment reactor). [d] HCN formed from acetone cyanohydrin (5 equiv., two-compartment reactor).

aldehyde **7a** and other side products. Surprisingly, the prevailing diastereomer had the 6*R* rather than the originally expected 6*S* configuration (Scheme 2, a). There was no reaction in the absence of the enzyme under otherwise identical conditions, indicating low enzymatic diastereoselectivity (Table 3, entry 3). The stereostructure of the galactose ring evidently steers the stereochemical outcome of **3a**. The presence of some water is necessary for (*R*)-oxynitrilase catalysis (Table 3, entry 4) and, accordingly, the use of almond meal is preferred over other (*R*)-oxynitrilase catalysts because the water is mainly impregnated in the meal and thus easy to remove for the next step in the reaction cascade.[9]

In addition to the decomposition of acetone cyanohydrin (in the two-compartment reactor case above), the formation of cyanohydrins was also catalyzed by bases (Scheme 1, Step c′). Thus, Amberlite IRA-900 in the HO^- form was used to transform **2a** in the reaction mixture into diastereomerically enriched (6*R*)-**3a** (52% *de*) in high yield in 24 hours using acetone cyanohydrin as the cyanide source (Table 3, entry 5). Thus, asymmetric induction from the galactopyranose ring gave a diastereomeric mixture containing 76 mol-% (6*R*)-**3a** and 24 mol-% (6*S*)-**3a**.

Increasing the resin content from 5 to 20 mg mL^{-1} increased the yield of **3a** from 43 to 92% in 24 hours without affecting the diastereomeric excess (Table 4). On the other hand, the formation of **7a** (3% in 3 h and 20% in 24 h) was also clear in the absence of acetone cyanohydrin. Accordingly, the base catalyzes hydrocyanation considerably faster than elimination. When acetone cyanohydrin and the basic resin were both introduced after the oxidation step (add-on synthesis), hydrocyanation proceeded rapidly to 83% yield (2–3 h), and thereafter slowly increased to 92% (Figure 1, filled symbols). To explain the slight increase in yield at this point, we suggest that some condensation side-products might decompose back to the aldehyde when the aldehyde content in the reaction mixture is lowered due to hydrocyanation. However, when the oxidation and hydrocyanation reagents were introduced simultaneously into the toluene solution of **1a**, only a trace amount of aldehyde **2a** was detected and the reaction quickly halted at approximately 20% yield for **3a** (Figure 1, open circles). The reagents used in Steps b and c′ are clearly not fully compatible. Apparently, the base consumes active iodine oxidant and induces condensation reactions.

Table 4. Effect of Amberlite IRA-900 HO^- content on the trans-hydrocyanation of **2a**.



Entry	Resin [mg mL^{-1}]	**2a** [%]	**3a** [%]	*de* of **3a** [%]
1	0	75	0	–
2	5	22	43	52
3	10	0	85	52
4	20	0	92	52

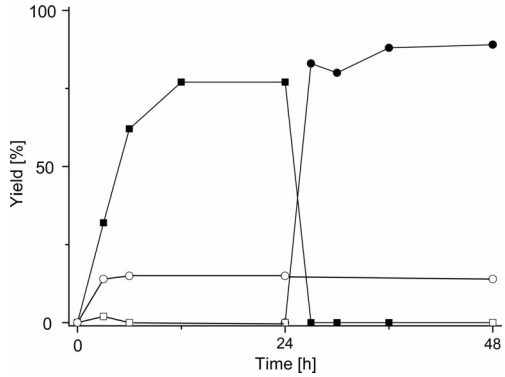


Figure 1. Yields of **2a** (■,□) and **3a** (●,○) with time scale; oxidation/hydrocyanation (20 mg mL^{-1} Amberlite IRA-900 HO^-) in one-pot by add-on (filled symbols) and at once (open symbols) reagent additions.

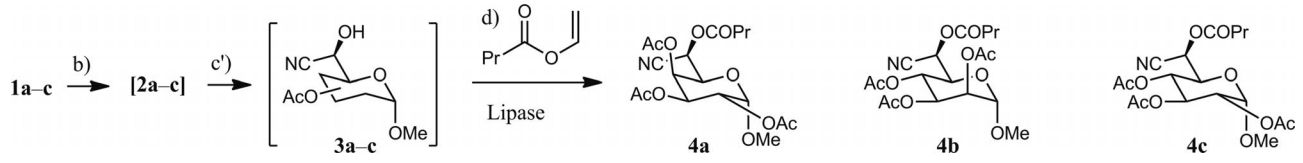
To conclude, chemical hydrocyanation under anhydrous conditions by the add-on principle is most suitable for the production of (6*R*)-**3a** (52% *de* in favor of the 6*R*-diastereomer), whereas the almond meal method gives the product with somewhat lower reaction yield (37% *de*). Pyranoside derivatives **1b** and **1c** were also subjected to the chemical oxidation/hydrocyanation conditions, but the reactions were prone to generate side reactions, producing the cyanohydrins **3b** and **3c** in 77 and 73% reaction yields, respectively, with negligible diastereoselectivity (19 and 5% *de*, respectively). These results indicate the importance of the axial substituent at the C-4 position of the carbohydrate scaffold as a stereocontrol element and the need to protect **2** against acetic acid elimination (formation of **7**).

Lipase-Catalyzed Acylation of 3a–c (Step d)

The production of cyanohydrin esters under basic conditions from aldehydes in the presence of acetone cyanohydrin, a lipase, and an acyl donor is among the oldest dynamic kinetic resolution methods.[22,23a–23c] However, when acetone cyanohydrin, the basic resin, lipase PS-C II, and vinyl butanoate were introduced simultaneously into the reaction mixture obtained through Step b (Scheme 1), the formation of **4a** did not exceed 16%, with the main product being **7a**. The base-catalyzed formation of **7a** means that the dynamic equilibrium between **2a** and **3a** had turned towards the aldehyde. As a possible explanation, the so-called residual water introduced with the seemingly dry lipase favors hydrolysis of vinyl butanoate, disfavoring acylation,[23d] and reduces the stability of **3a**.

The one-pot reaction from **1a**–**c** to diastereomerically enriched **3a**–**c** through Steps a→b→c′ in anhydrous toluene was repeated according to the above principles at 23 °C to carry out the next lipase-catalyzed Step d (and e) of the reaction cascade (Scheme 1). On the basis of the diastereomeric excess values, diastereomerically enriched **3a**–**c** contained 76, 60 and 52 mol-% of galactosyl-, mannosyl- and glucosylpyranoside derivatives as the major dia-

Table 5. Lipase screening for the acylation of **3a**–**c** with vinyl butanoate in the reaction sequence starting from **1a**–**c**.[a]



Entry	Lipase	**4a** Yield [%]	*de* [%]	**4b** Yield [%]	*de* [%]	**4c** Yield [%]	*de* [%]
1	Lipase PS-D	42	87	65	30	69	19
2	Lipase PS-C II	75	85	65	33	56	42
3	Novozym 435	<1	–	76	12	71	15
4	CAL-A on Celite	10	83	69	25	5	–
5	CRL on Celite	0	–	77	16	27	99[b]
6	Lipozyme TL IM	4	–	<1	–	0	–

[a] Reagents and conditions: Step b: **1a**–**c** (0.05 M), $PhI(OAc)_2$ (1.1 equiv.), TEMPO (0.1 equiv.), toluene, 23 °C, 12 h; Step c′: acetone cyanohydrin (2 equiv.), Amberlite IRA-900 HO^- (20 mg mL^{-1}), one-pot reactor, 23 °C, 6 h, followed by decantation; Step d: vinyl butanoate (3 equiv.), lipase (50 mg mL^{-1}), 23 °C, 72 h, absolute configurations at C-6 of **4b** and **4c** unconfirmed. [b] Opposite diastereomeric preference to other lipases.

stereomers (evidently 6*R* also with **3b** and **3c**), respectively. A set of commonly employed microbial lipases and vinyl butanoate was studied next for the acylation of cyanohydrins **3a**–**c** in toluene in the one-pot reaction mixtures obtained after first removing the basic resin by filtration (Table 5, entries 1–6). Lipases PS-D and PS-C II accepted all three cyanohydrin pyranosides as substrates, although the diastereoselectivity was good only for **4a** (85–87% *de*, Table 5, entries 1 and 2). Novozym 435 did not accept galactoside **3a**, and the enzyme displayed very low diastereoselectivities with **3b** and **3c** (Table 5, entry 3). CAL-A (Table 5, entry 4) and CRL (Table 5, entry 5) were markedly active only with mannoside **3b**. The diastereoselectivity of CRL to produce **4c** was excellent (99% *de*) although opposite [evidently producing (*S*)-**4c**] to that of the other lipases studied. In acylation, the stereoselectivity of CRL is known to depend strongly on the structure of the racemic alcohol, with reversed enantioselectivity to common lipases (like lipase PS) being observed, for instance, with cyanohydrin substrates.[24] Lipozyme TL IM was completely inactive for the acylation of cyanohydrins **3a**–**c** (Table 5, entry 6). At this point, investigations with **3b** and **3c** were discontinued because of the disapointing results.

Next, optimization for the preparation of **4a** was continued by studying the effects of lipase PS-C II and vinyl butanoate concentration on the acylation of cyanohydrin **3a**. Acylation was faster when 100 mg mL^{-1} of enzyme was used than with 50 mg mL^{-1} with a set amount of the acyl donor, however, 80–90% yields were reached irrespective of the amount of acyl donor (Figure 2, a). At the same time, diastereomeric excess values depended only on conversion, allowing the formation of (6*R*)-**4a** at 89–92% diastereomeric excess up to a reaction yield of ca. 60% (Figure 2, b); at this point a sharp drop in the diastereomeric excess was observed as the less reactive (6*S*)-**3a** diastereomer became more active in product formation.

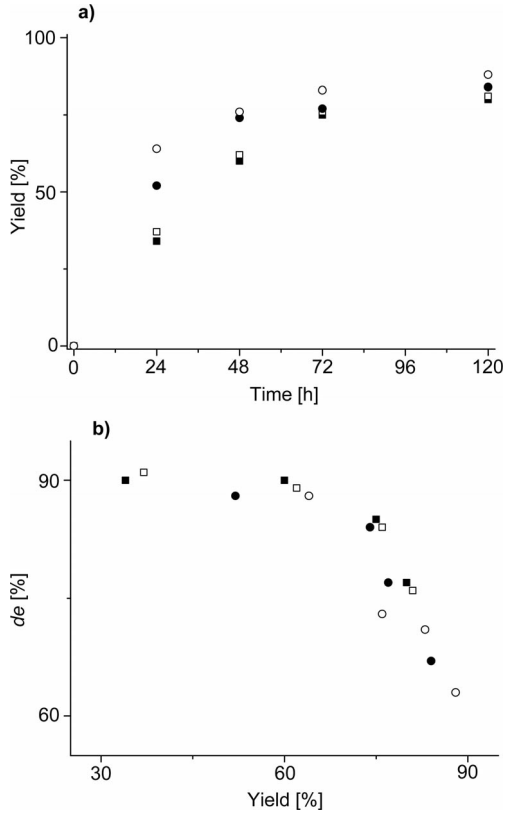
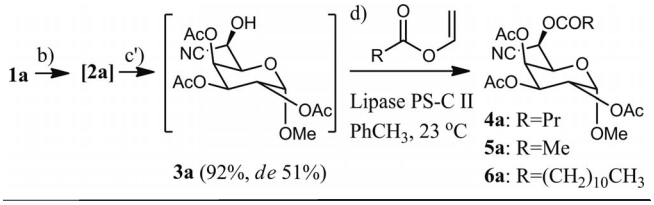


Figure 2. (a) Reaction yields of **4a** vs. time, and (b) diastereomeric excess vs. reaction yields for the lipase PS-C II-catalyzed acylation of **3a** (92%, 52% *de*) in toluene at 23 °C. (■) lipase (50 mg mL^{-1}) and vinyl butanoate (3 equiv.); (□) lipase (50 mg mL^{-1}) and vinyl butanoate (6 equiv.); (●) lipase (100 mg mL^{-1}) and vinyl butanoate (3 equiv.); (○) lipase (100 mg mL^{-1}) and vinyl butanoate (6 equiv.).

The length of the acyl part of the acyl donor clearly affected reactivity, whereas the diastereomeric excess of **4a**–**5a** mainly depended on conversion (Table 6). Thus, al-

though the reaction was initially most effective with vinyl acetate (reaction yield 45% in 24 h, Table 6, entry 2), the reaction yield of 75% [approximately the theoretical yield for (6*R*)-**4a**] was reached only with vinyl butanoate in 72 h (Table 6, entry 1). Acylation with vinyl laurate (to give **6a**) was somewhat more selective but impractically slow for efficient application (Table 6, entry 3).

Table 6. Effect of an acyl donor (3 equiv.) on the acylation of **3a** with lipase PS-C II (50 mg mL^{-1}).



3a (92%, *de* 51%)

Entry	Vinyl ester	Product	24 h Yield [%]	*de* [%]	72 h Yield [%]	*de* [%]
1	butanoate	**4a**	34	90	75	85
2	acetate	**5a**	45	91	66	81
3	laurate	**6a**	13	96	34	96

Unlike free cyanohydrin **3a**, products **4a** and **5a** allowed the diastereomeric excess to be enhanced upon column chromatography. However, the recoverable yield of the pure diastereomer (de > 99%) remained low due to considerable tailing in the separation, even when highly diastereomerically enriched esters (*de* of the order of 85%) were purified. Thus, in the preparative synthesis, the fractions of both (6*R*)-**4a** and (6*R*)-**5a** were enriched to diastereomeric excesses of more than 99%, with isolated yields of 39–41% (reaction yield in the order of 75%) as calculated over the three steps from **1a**. The activity of lipase PS-C II was then used in the reverse direction by subjecting cyanohydrin acetate (6*R*)-**5a** to methanolysis (under the conditions detailed in Table 1, reaction time 24 h). Free cyanohydrin (6*R*)-**3a** was obtained by simple filtration of the enzyme without chromatography and epimerization in 73% yield.

Absolute Configuration

As described above, the (*R*)-oxynitrilase and lipase models predict different stereoisomeric preferences for the products **3** and **4** (Scheme 2). However, the same diastereomer preferentially forms in the base- (Step c′) as well as in the (*R*)-oxynitrilase-catalyzed (Step c) hydrocyanation, and this is also the preferentially reacting diastereomer in lipase PS-C II catalyzed acylation (Step d). Accordingly, NMR analysis of the diastereomeric mixture of **3a** could be used to confirm the absolute configuration of the obtained cyanohydrin esters.

First, both stereoisomers (6*R*)- and (6*S*)-**4a** have a minimum energy conformation in which H-5 and H-6 are in an *anti*-relationship along the C-5–C-6 bond. The coupling constants $^3J_{5,6}$ measured were 5.4 Hz for the minor and 8.7 Hz for the major diastereomer of **3a**. Thus, H-5 and H-6 of the major diastereomer are clearly in an *anti* orientation whereas there is more *gauche* character in the minor diastereomer. When the torsion angle energies were analyzed with MM2 molecular mechanics, the *gauche* conformations along the C-5–C-6 bond were found to be somewhat lower in energy with (6*S*)-**3a** (ca. 2 kcal mol^{-1} higher than *anti* conformation) than with (6*R*)-**3a** (ca. 5 kcal mol^{-1} higher than *anti* conformation), suggesting that (6*S*)-**3a** is the minor diastereomer.

Secondly, the diastereomeric mixture of **3a** (37% *de*, Table 3, entry 2) was dissolved with (*R*)- and (*S*)-mandelic acid, both in the presence of 4-(dimethylamino)pyridine (DMAP), and the ^{1}H NMR spectra were measured (Figure 3).[25] The downfield shift of H-6 of the minor diastereomer of **3a** in the complex with (*R*)-mandelate-DMAPH$^+$ compared to the shift with the (*S*)-mandelate-DMAPH$^+$ ($\Delta\delta^{RS}$ = +0.032 ppm) implies homochirality with (*R*)-mandelonitrile and, therefore, the assignment (6*S*)-**3a**; the major diastereomer of **3a** had nearly equal shift with both (*R*)- and (*S*)-mandelate-DMAPH$^+$. However, this was also observed with diastereomerically pure **3a** and full verification of the absolute configuration by this method could not be obtained.

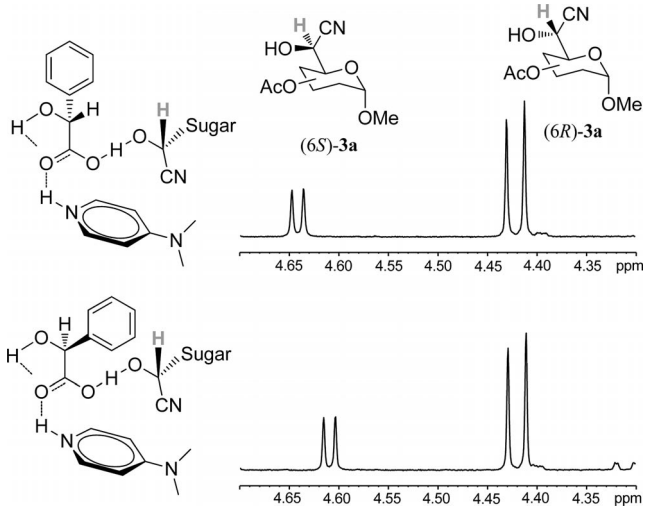


Figure 3. ^{1}H NMR signals of the minor (downfield) and major (upfield) diastereomer of **3a** (37% *de*) in complex with (*R*)-mandelic acid–DMAP (top) and (*S*)-mandelic acid–DMAP (bottom). The models for the respective complexes of (6*S*)-**3a** are shown on left.

Overall, the NMR experiments support, although do not unambiguously confirm, the assignment of (6*R*)-**3a** (contrary to the prediction of Scheme 2, a) and (6*R*)-**4a** configurations to be preferentially formed in the hydrocyanation and lipase PS-C II catalyzed acylation steps.

Conclusions

A straightforward, multistep synthesis and kinetic diastereoresolution sequence has been developed based on bio- and chemo-catalytic reaction steps, starting from peracetylated methyl α-D-glycosides. Essentially, three main reaction

steps, oxidation [$PhI(OAc)_2$, TEMPO], hydrocyanation (basic Amberlite IRA-900 ion exchange resin and acetone cyanohydrin), and acylation (lipase PS-C II and a vinyl ester) taking place through a cascade sequence in anhydrous toluene turned out to be most applicable. It is essential to note that the sugar aldehydes and cyanohydrins are unstable during chromatographic separation, emphasizing the importance of proceeding without purifying the intermediate products and obtaining stable cyanohydrin esters. The butanoate ester of galactosyl cyanohydrin was prepared with high diastereoselectivity (85% *de*) and reaction yield (75%). Further diastereomeric purification of the ester product by column chromatography afforded fractions of cyanohydrin acetate **5a** and butanoate **4a** as (6*R*)-diastereomers with diastereomeric excesses of more than 99%. However, the yield remained low (31–41%) due to imperfect diastereomeric separation. When coupled to enzymatic deacylation of (6*R*)-**4a** with methanol, the corresponding relatively labile free (6*R*)-cyanohydrin (*de* > 99%) was obtained through simple filtration of the lipase. It has been shown that the selectivity of the method depends on the sugar configuration because mannose- and glucose-derived substrates gave inferior results under the same conditions. In these cases, the method was hampered partly due to side reactions triggered by acetate elimination at the C-4 position of the acetylated sugar aldehyde. Changes in protective group strategy might enable improvements in synthesis yield and selectivity.

Experimental Section

Materials and Methods: Chemical reagents were purchased from commercial sources and used as received unless otherwise mentioned. Methyl-α-D-glycosides, $PhI(OAc)_2$, TEMPO (soluble and on polystyrene, 1.0 mmol g^{-1}), and acetone cyanohydrin were purchased from Aldrich; vinyl acetate, butanoate, and laurate were purchased from Fluka. Almond meal (β-glucosidase from almonds) was acquired from Sigma, immobilized *Pa*HNL from ChiralVision, aq. *At*HNL from Evocatal, lipase PS-D and PS-C II (*Burkholderia cepacia* lipase) from Amano Europe, and Novozym 435 (*Candida antarctica* lipase B) and Lipozyme TL IM (*Thermomyces lanuginosus* lipase) from Novozymes. CAL-A (*Candida antarctica* lipase A, from Novozymes) and CRL (*Candida rugosa* lipase, from Sigma) powders were immobilized on Celite in the presence of sucrose.[26] Chloroperoxidase from *Caldariomyces fumago* (lyophilized and immobilized preparations) were kindly obtained from Bio-Research Products, Inc. (Iowa, USA). Amberlite IRA-900 ion exchange resin (Aldrich) was conditioned to the HO^- form before use.[23] NMR spectra were measured with a Bruker Avance 500 MHz spectrometer, and high-resolution mass spectra were recorded with a Bruker micro-TOF-Q quadrupole-TOF spectrometer operating in the ESI+ mode. Optical rotations were measured with a Perkin–Elmer 241 polarimeter, and $[\alpha]_D^{25}$ values are given in units of 10^{-1} deg cm^2 g^{-1}. Analytical scale reactions were performed on 1–5 mL scale at room temp. (ca. 23 °C) or at 48 °C. Samples (100–200 μL) were analyzed by NMR (samples concentrated and redissolved in $CDCl_3$ with TMS as a calibrant). Whenever sampling resulted in more than ca. 10% loss in reaction volume, parallel reactions were run and sampled once each. Reactions were analyzed using assigned ^{1}H NMR signals according to Tables 7 and 8. Anomeric signals were assigned based on ^{13}C and HSQC spectra and 1-CH_3O signals correlated to those based on peak integrals. For galactoside derivatives (**Xa**) the signals were confirmed by full assignment of the prepared compounds, however, the assignments are tentative for mannosyl (**Xb**) and glucosyl (**Xc**) compounds. Column chromatography was performed on dried silica gel (60 Å, 230–400 mesh).

Table 7. Partial assignment and chemical shifts of the ^{1}H NMR signals used in the analysis of galactosides (**Xa**).

	H-1 [ppm]	H-6 [ppm]	OCH_3 [ppm]
1a	5.00	3.68, 3.52	3.42
2a	9.54	5.16	3.46
3a (*R*/*S*)	5.06/5.11	4.40/4.68	3.48/3.45
4a (*R*/*S*)	5.08/5.12	5.48/n.d.	3.49/3.43
5a (*R*/*S*)	5.08/5.12	5.46/n.d.	3.49/3.43
6a (*R*/*S*)	5.08/5.12	5.47/n.d.	3.49/3.43
7a	9.25	5.15	3.52

Table 8. Tentative assignment and chemical shifts of the ^{1}H NMR signals used in the analysis of mannosides (**Xb**) and glucosides (**Xc**).

	Mannosides (**Xb**)		Glucosides (**Xc**)	
	H-1 [ppm]	OCH_3 [ppm]	H-1 [ppm]	OCH_3 [ppm]
1	4.73	3.41	4.97	3.41
2	4.81	3.45	5.07	3.45
3 (*R*/*S*)	4.83/4.79	3.50/3.45	5.09/5.03	3.50/3.45
4 (*R*/*S*)	4.82/4.78	3.49/3.44	5.09/5.02	3.49/3.44

Syntheses of Alcohols 1a–c. Typical Procedure: Methyl α-D-galactopyranoside (1.01 g, 5.20 mmol) was stirred with I_2 (50 mg) in Ac_2O (5 mL) for 10 min at 23 °C. The reaction was diluted with EtOAc (10 mL) and washed with 10% aq. Na_2CO_3 (2 × 10 mL). The aqueous phases were combined and extracted with EtOAc (2 × 10 mL). The organic phases were combined, dried with Na_2SO_4, concentrated, and filtered through a silica pad (EtOAc/hexane, 4:1; R_f = 0.85) to collect the tetraacetylated product (1.79 g, 4.94 mmol, 95%).

One of the peracetylated sugars (450 mg, 1.24 mmol) and Lipozyme TL IM (620 mg) were shaken in diisopropyl ether (12.15 mL) and MeOH (251 μL, 6.20 mmol) at 48 °C. After 24 h, the enzyme was filtered off and the product was purified by column chromatography (EtOAc/hexane, 4:1; R_f = 0.55] to yield, for instance, **1a** (323 mg, 1.01 mmol, 81%).

The NMR spectra of **1a**–**c** are in accordance with those reported in ref.[27] and HRMS correspond to the sodium adducts of the molecular ion.

Almond Meal Catalyzed Synthesis of Cyanohydrin 3a: In a dried glass vessel with two compartments connected through a class tube between the headspace of the compartments, **1a** (141 mg, 0.440 mmol) was dissolved in anhydrous toluene (8.8 mL) in one of the compartments (compartment 1). $PhI(OAc)_2$ (156 mg, 0.484 mmol) and TEMPO (6.9 mg, 0.044 mmol) were added, and the reaction mixture was stirred at 23 °C. After 12 h, tartrate buffer (0.10 M, pH 5.4, 350 μL) and almond meal (435 mg) were added. For the production of HCN, in compartment 2, hexane (8.8 mL), Amberlite IRA-900 HO^- (88 mg) and acetone cyanohydrin (200 μL, 2.19 mmol) were introduced. The reaction mixtures in both compartments were stirred at 23 °C for 24 h. From the hydrocyanation mixture (compartment 1) the almond meal was filtered and the crude product (74%, 37% *de* by NMR analysis) was purified by column chromatography (EtOAc/hexane, 6:4; R_f = 0.50) to

collect **3a** (54 mg, 0.156 mmol, 35%, 37% *de*). (6*R*)-**3a**: ^{1}H NMR (500.13 MHz, $CDCl_3$, 25 °C): δ = 5.51 (dd, $J_{4,3}$ = 3.4 Hz, $J_{4,5}$ = 1.0 Hz, 1 H, 4-H), 5.35 (dd, $J_{3,2}$ = 10.9 Hz, 1 H, 3-H), 5.18 (dd, $J_{2,1}$ = 3.7 Hz, 1 H, 2-H), 5.06 (d, 1 H, 1-H), 4.40 (d, $J_{6,5}$ = 8.7 Hz, 1 H, 6-H), 4.17 (dd, 1 H, 5-H), 3.7 (br., 1 H, OH), 3.48 (s, 3 H, OCH_3), 2.20 (s, 3 H, $COCH_3$), 2.10 (s, 3 H, $COCH_3$), 2.01 (s, 3 H, $COCH_3$) ppm. ^{13}C NMR (500.13 MHz, $CDCl_3$, 25 °C): δ = 170.5 (O*C*OCH_3), 170.3 (O*C*OCH_3), 170.1 (O*C*OCH_3), 118.2 (CN), 97.3 (1-C), 68.9 (5-C), 67.9 (2-C), 67.5 (4-C), 67.4 (3-C), 60.1 (6-C), 55.8 (OCH_3), 20.8 (OCO*C*H_3), 20.7 (OCO*C*H_3), 20.8 (OCO*C*H_3) ppm. (6*S*)-**3a**: ^{1}H NMR (500.13 MHz, $CDCl_3$, 25 °C): δ = 5.57 (dd, $J_{4,3}$ = 3.5 Hz, $J_{4,5}$ = 1.1 Hz, 1 H, 4-H), 5.37 (dd, $J_{3,2}$ = 10.9 Hz, 1 H, 3-H), 5.23 (dd, $J_{2,1}$ = 3.6 Hz, 1 H, 2-H), 5.11 (d, 1 H, 1-H), 4.68 (d, $J_{6,5}$ = 5.4 Hz, 1 H, 6-H), 4.17 (dd, 1 H, 5-H), 3.7 (br., 1 H, OH), 3.45 (s, 3 H, OCH_3), 2.23 (s, 3 H, $COCH_3$), 2.10 (s, 3 H, $COCH_3$), 2.03 (s, 3 H, $COCH_3$) ppm. ^{13}C NMR (125.77 MHz, $CDCl_3$, 25 °C): δ = 117.4 (CN), 97.5 (1-C), 67.8 (5-C), 67.8 (2-C), 67.8 (4-C), 67.0 (3-C), 60.4 (6-C), 56.1 (OCH_3), 21.1 (OCO*C*H_3), 20.9 (OCO*C*H_3) ppm (C=O signals too weak for detection). (6*R*/6*S*)-**3a**: HRMS: calcd. for $C_{14}H_{19}NO_9Na^+$ [M + Na]$^+$ 368.0952; found 368.0919.

One-Pot Synthesis of Cyanohydrin Butanoate (6*R*)-4a: Alcohol **1a** (438 mg, 1.37 mmol) was dissolved in anhydrous toluene (27.4 mL), $PhI(OAc)_2$ (485 mg, 1.51 mmol) and TEMPO (21.4 mg, 0.137 mmol) were added and **2a** was formed after 12 h as described above. Amberlite IRA-900 HO^- resin (540 mg) and acetone cyanohydrin (248 μL, 2.72 mmol) were added into the reaction mixture. After 6 h the solution contained (6*R*)-**3a** (86%, 55% *de*). The basic resin was removed by decanting the solution part into a second vessel loaded with lipase PS-C II (1.35 g) followed by the addition of vinyl butanoate (495 μL, 3.90 mmol). The mixture was shaken at 23 °C. After 72 h, the lipase was filtered off from the mixture containing (6*R*)-**4a** (75%, 85% *de*), and the filtrate was washed with EtOAc (3 × 30 mL). The combined filtrates were concentrated and purified by column chromatography (EtOAc/hexane, 6:4; R_f = 0.62) to collect (6*R*)-**4a** (233 mg, 0.56 mmol, 41%, *de* > 99%). $[\alpha]_D^{25}$ = +95 (*c* = 1.0, $CHCl_3$). ^{1}H NMR (500.13 MHz, $CDCl_3$, 25 °C): δ = 5.48 (d, $J_{6,5}$ = 9.5 Hz, 1 H, 6-H), 5.42 (dd, $J_{4,5}$ = 1.0, $J_{4,3}$ = 3.5 Hz, 1 H, 4-H), 5.35 (dd, $J_{3,2}$ = 11.0 Hz, 1 H, 3-H), 5.14 (dd, $J_{2,1}$ = 3.5 Hz, 1 H, 2-H), 5.08 (d, 1 H, 1-H), 4.32 (dd, 1 H, 5-H), 3.49 (s, 3 H, OCH_3), 2.29 (m, 2 H, $CH_2CH_2CH_3$), 2.11 (s, 3 H, $COCH_3$), 2.10 (s, 3 H, $COCH_3$), 1.98 (s, 3 H, $COCH_3$), 1.65 (m, 2 H, $CH_2CH_2H_3$), 0.95 (t, $J_{CH2-CH3}$ = 7.5 Hz, 3 H, $CH_2CH_2CH_3$) ppm. ^{13}C NMR (125.77 MHz, $CDCl_3$, 25 °C): δ = 170.9 (OCOPr), 170.3 (O*C*OCH_3), 170.0 (O*C*OCH_3), 169.8 (O*C*OCH_3), 115.4 (CN), 97.5 (1-C), 67.7 (2-C), 67.4 (5-C), 67.0 (3-C), 66.1 (4-C), 58.1 (6-C), 56.0 (OCH_3), 35.1 ($CH_2CH_2CH_3$), 20.8 (OCO*C*H_3), 20.6 (OCO*C*H_3), 20.5 (OCO*C*H_3), 17.9 ($CH_2CH_2CH_3$), 13.4 ($CH_2CH_2CH_3$) ppm. HRMS: calcd. for $C_{18}H_{25}NO_{10}Na^+$ [M + Na]$^+$ 438.1371; found 438.1402.

One-Pot Synthesis of Cyanohydrin Acetate (6*R*)-5a: Oxidation of **1a** and its hydrocyanation were performed as described above. For acylation, vinyl acetate (6 equiv.) and lipase PS-C II (100 mg mL^{-1}) were applied. After 24 h, a reaction mixture containing (6*R*)-**5a** (71%, 83% *de*) was obtained. Purification by column chromatography (EtOAc/hexane, 6:4; R_f = 0.71) gave (6*R*)-**5a** (39%, *de* > 99%). $[\alpha]_D^{25}$ = +128 (*c* = 1.0, $CHCl_3$). ^{1}H NMR (500.13 MHz, $CDCl_3$, 25 °C): δ = 5.46 (d, $J_{6,5}$ = 9.5 Hz, 1 H, 6-H), 5.44 (dd, $J_{4,5}$ = 1.3, $J_{4,3}$ = 3.4 Hz, 1 H, 4-H), 5.35 (dd, $J_{3,2}$ = 10.8 Hz, 1 H, 3-H), 5.15 (dd, $J_{2,1}$ = 3.6 Hz, 1 H, 2-H), 5.08 (d, 1 H, 1-H), 4.32 (dd, 1 H, 5-H), 3.49 (s, 3 H, OCH_3), 2.12 (s, 3 H, COCH_3), 2.11 (s, 3 H, COCH_3), 2.10 (s, 3 H, COCH_3), 1.98 (s, 3 H, COCH_3) ppm. ^{13}C NMR (125.77 MHz, $CDCl_3$, 25 °C): δ = 170.3 (O*C*OCH_3), 170.1 (O*C*OCH_3), 169.8 (O*C*OCH_3), 168.2 (O*C*OCH_3), 115.3 (CN), 97.5 (1-C), 67.7 (2-C), 67.4 (5-C), 67.0 (3-C), 66.1 (4-C), 58.3 (6-C), 56.0 (OCH_3), 21.0 (OCO*C*H_3), 20.6 (OCO*C*H_3), 20.5 (OCO*C*H_3), 20.0 (OCO*C*H_3) ppm (contains traces of **7a**). HRMS: calcd. for $C_{16}H_{21}NO_{10}Na^+$ [M + Na]$^+$ 410.1058; found 410.1074.

Enzymatic Methanolysis of (6*R*)-5a to give (6*R*)-3a: Cyanohydrin acetate (*R*)-**5a** (106 mg, 0.274 mmol, 99% *de*) and lipase PS-C II (273 mg) were shaken in diisopropyl ether (5.42 mL) at 48 °C, and MeOH (55 μL, 1.36 mmol) was added. After 24 h the lipase was filtered to yield (*R*)-**3a** (69 mg, 0.200 mmol, 73%, *de* > 99%). $[\alpha]_D^{25}$ = +117 (*c* = 1.0, $CHCl_3$). NMR spectra and HRMS correspond to those given for (6*R*)-**3a**.

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

Acknowledgments

The authors thank the Academy of Finland for financial support (research grant number 121983). This work was sponsored by the European Union (EU) in the frame of the COST Action CM0701.

[1] For example, see: *Carbohydrates in Chemistry and Biology*, 1st ed., vol. 1–4 (Eds.: B. Ernst, G. W. Hart, P. Sinaÿ), Wiley-VCH, Weinheim, Germany, **2000**.
[2] F. Ortega-Caballero, C. Rousseau, B. Christensen, T. E. Petersen, M. Bols, *J. Am. Chem. Soc.* **2005**, *127*, 3238–3239.
[3] X. Li, Q. Yin, L. Jiao, Z. Qin, J. Feng, H. Chen, J. Zhang, M. Meng, *Carbohydr. Res.* **2011**, *346*, 401–409.
[4] a) J. Neumann, J. Thiem, *Eur. J. Org. Chem.* **2010**, 900–908; b) F. Peri, J. Jiménez-Barbero, V. García-Aparicio, I. Tvaroška, F. Nicotra, *Chem. Eur. J.* **2004**, *10*, 1433–1444.
[5] P. A. Burland, D. Coisson, H. M. I. Osborn, *J. Org. Chem.* **2010**, *75*, 7210–7218.
[6] H. Stępowska, A. Zamojski, *Tetrahedron* **1999**, *55*, 5519–5538.
[7] a) R. Fernández, E. Martín-Zamora, C. Pareja, J. M. Lassaletta, *J. Org. Chem.* **2001**, *66*, 5201–5207; b) J. M. Lassaletta, R. Fernández, E. Martín-Zamora, C. Pareja, *Tetrahedron Lett.* **1996**, *37*, 5787–5790.
[8] J. Holt, U. Hanefeld, *Curr. Org. Synth.* **2009**, *6*, 15–37.
[9] A. Hietanen, F. S. Ekholm, R. Leino, L. T. Kanerva, *Eur. J. Org. Chem.* **2010**, 6974–6980.
[10] R. J. Kazlauskas, A. N. E. Weissfloch, A. T. Rappaport, L. A. Cuccia, *J. Org. Chem.* **1991**, *56*, 2656–2665.
[11] K. P. R. Kartha, R. A. Field, *Tetrahedron* **1997**, *53*, 11753–11766.
[12] a) R. Schoevaart, T. Kieboom, *Top. Catal.* **2004**, *27*, 3–9; b) V. Bonnet, R. Duval, C. Rabiller, *J. Mol. Catal. B* **2003**, *24–25*, 9–16.
[13] a) K. Parikka, M. Tenkanen, *Carbohydr. Res.* **2009**, *344*, 14–20; b) A. Maradufu, A. S. Perlin, *Carbohydr. Res.* **1974**, *32*, 127–136.
[14] See, for example: a) A. Zaks, D. R. Dodds, *J. Am. Chem. Soc.* **1995**, *117*, 10419–10424; b) E. Kiljunen, L. T. Kanerva, *Tetrahedron: Asymmetry* **1999**, *10*, 3529–3535; c) E. Kiljunen, L. T. Kanerva, *J. Mol. Catal. B* **2000**, *9*, 163–17; d) F. van de Velde, F. van Rantwijk, R. A. Sheldon, *Trends Biotechnol.* **2001**, *19*, 73–80.
[15] D. M. Mackie, A. S. Perlin, *Carbohydr. Res.* **1972**, *24*, 67–85.
[16] a) M. Angelin, M. Hermansson, H. Dong, O. Ramström, *Eur. J. Org. Chem.* **2006**, 4323–4326; b) T. Breton, G. Bashiardes, J.-M. Léger, K. B. Kokoh, *Eur. J. Org. Chem.* **2007**, 1567–1570.
[17] a) M. Marzorati, B. Danieli, D. Haltrich, S. Riva, *Green Chem.* **2005**, *7*, 310–315; b) L. Baratto, A. Candido, M. Marzorati, F. Sagui, S. Riva, B. Danieli, *J. Mol. Catal. B* **2006**, *39*, 3–8.
[18] T. Wirth, *Angew. Chem.* **2005**, *117*, 3722; *Angew. Chem. Int. Ed.* **2005**, *44*, 3656–3665.

[19] A. De Mico, R. Margarita, L. Parlanti, A. Vescovi, G. Piancatelli, *J. Org. Chem.* **1997**, *62*, 6974–6977.
[20] D. J. Vugts, L. Veum, K. al-Mafraji, R. Lemmens, R. F. Schmitz, F. J. J. de Kanter, M. B. Groen, U. Hanefeld, R. V. A. Orru, *Eur. J. Org. Chem.* **2006**, 1672–1677.
[21] a) E. Kiljunen, L. T. Kanerva, *Tetrahedron: Asymmetry* **1997**, *8*, 1225–1234; b) E. Kiljunen, L. T. Kanerva, *Tetrahedron: Asymmetry* **1996**, *7*, 1105–1116.
[22] L. T. Kanerva, A. Liljeblad, "Transesterification – Biological" in *Encyclopedia of Catalysis* (Ed.: I. T. Horváth), John Wiley & Sons, New York, **2010**; DOI: 10.1002/0471227617.
[23] a) M. Inagaki, J. Hiratake, T. Nishioka, J. Oda, *J. Am. Chem. Soc.* **1991**, *113*, 9360–9361; b) M. Inagaki, J. Hiratake, T. Nishioka, J. Oda, *J. Org. Chem.* **1992**, *57*, 5643–5649; c) L. T. Kanerva, K. Rahiala, O. Sundholm, *Biocatalysis* **1994**, *10*, 169–180; d) L. Veum, L. T. Kanerva, P. J. Halling, T. Maschmeyer, U. Hanefeld, *Adv. Synth. Catal.* **2005**, *347*, 1015–1021.
[24] See, for instance: K. Lundell, T. Raijola, L. T. Kanerva, *Enzyme Microb. Technol.* **1998**, *22*, 86–93.
[25] a) L. S. Moon, R. S. Jolly, Y. Kasetti, P. V. Bharatam, *Chem. Commun.* **2009**, 1067–1069; b) L. S. Moon, M. Pal, Y. Kasetti, P. V. Bharatam, R. S. Jolly, *J. Org. Chem.* **2010**, *75*, 5487–5498.
[26] O. Sundholm, L. T. Kanerva, *J. Chem. Soc. Perkin Trans. 1* **1993**, 2407–2410.
[27] A. R. Moen, T. Anthonsen, *Biocatal. Biotransform.* **2009**, *27*, 226–236.

Received: January 25, 2012
Published Online: March 19, 2012

